# RESEARCH OUTPUT

## Patent

Ruchirawat, S. Synthesis of Lamellarins and Their Intermediates. No. 1063 Granted on August 8, 2003 by the Department of Intellectual Property, Ministry of Commerce, Thailand

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# RESEARCH OUTPUT

อนุสิทธิบัตร 1063

อสป/200 - ข

# อนุสิทธิบัตร

## อาศัยอำนาจตามความในพระราชบัญญัติสิทธิบัตร พ.ศ. 2522 แก้ไขเพิ่มเติมโดยพระราชบัญญัติสิทธิบัตร (ฉบับที่ 3) พ.ศ. 2542 อธิบดีกรมทรัพย์สินทางปัญญาออกอนุสิทธิบัตรฉบับนี้ให้แก่

สถาบันวิจัยจุฬาภรณ์

มหาวิทยาลัยมหิดล

สำนักงานกองทุนสนับสนุนการวิจัย

**สำหรับการประดิษฐ์ตามรายละเอียดการประดิษฐ์ ข้อถือสิทธิ และรูปเขียน (ถ้ามี) ที่ปรากฏในอนุสิทธิบัตรนี้**

| | |
|---|---|
| เลขที่คำขอ | 0203000988 |
| วันขอรับอนุสิทธิบัตร | 25 ตุลาคม 2545 |
| ผู้ประดิษฐ์ | รศ.ดร.สมศักดิ์ รุจิรวัฒน์ และคณะ |
| ข้อที่แสดงถึงการประดิษฐ์ | กรรมวิธีการสังเคราะห์สารลาเมลลารินและสารมัธยันต์ที่ได้จากกรรมวิธีดังกล่าว |

ให้ผู้ทรงอนุสิทธิบัตรมีสิทธิและหน้าที่ตามกฎหมายว่าด้วยสิทธิบัตรทุกประการ

ออกให้ ณ วันที่ 8 เดือน สิงหาคม พ.ศ. 2546

หมดอายุ ณ วันที่ 24 เดือน ตุลาคม พ.ศ. 2551

(ลงชื่อ) .....

( นายยรรยง พวงราช )

อธิบดีกรมทรัพย์สินทางปัญญา

ผู้ออกอนุสิทธิบัตร

พนักงานเจ้าหน้าที่

หมายเหตุ 1. ผู้ทรงอนุสิทธิบัตรต้องชำระค่าธรรมเนียมรายปีเริ่มแต่ปีที่ 5 ของอายุอนุสิทธิบัตร มิฉะนั้น อนุสิทธิบัตรจะสิ้นอายุ

2. ผู้ทรงอนุสิทธิบัตรจะขอชำระค่าธรรมเนียมรายปีล่วงหน้าโดยชำระทั้งหมดในคราวเดียวก็ได้

3. ภายใน 90 วันก่อนวันสิ้นอายุอนุสิทธิบัตร ผู้ทรงสิทธิบัตรมีสิทธิขอต่ออายุอนุสิทธิบัตรได้ 2 คราว มีกำหนดคราวละ 2 ปี โดยยื่นคำขอต่ออายุต่อพนักงานเจ้าหน้าที่

4. การอนุญาตให้ใช้สิทธิตามอนุสิทธิบัตรและการโอนอนุสิทธิบัตรต้องทำเป็นหนังสือและจดทะเบียนต่อพนักงานเจ้าหน้าที่

**รายละเอียดการประดิษฐ์**

**ชื่อที่แสดงถึงการประดิษฐ์**

กรรมวิธีการสังเคราะห์สารลาเมลลารินและสารมัธยันต์ที่ได้จากกรรมวิธีดังกล่าว

**สาขาวิทยาการที่เกี่ยวข้องกับการประดิษฐ์**

เคมีอินทรีย์และเภสัชศาสตร์

**ลักษณะและความมุ่งหมายของการประดิษฐ์**

การประดิษฐ์นี้เกี่ยวข้องกับสารประกอบไพโรลไอโซควิโนลิน**ชนิดใหม่** รวมทั้งกรรมวิธีการสังเคราะห์สารเหล่านี้และสารลาเมลลาริน**ที่ได้จากสารเหล่านี้**

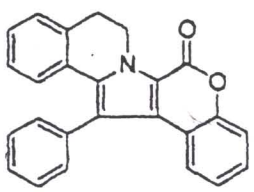
**การประดิษฐ์นี้เกี่ยวข้องกับการสังเคราะห์สารมัธยันต์ที่ได้ระหว่างการสังเคราะห์สารลาเมลลาริน ซึ่งสารมัธยันต์เหล่านี้ก็เป็นสารใหม่ และเป็นจุดมุ่งหมายของการประดิษฐ์นี้ด้วยเช่นกัน**

**จุดมุ่งหมายต่อมาของการประดิษฐ์นี้คือกรรมวิธีการสังเคราะห์สารลาเมลลารินที่มีประสิทธิภาพและไม่ยุ่งยากทำให้ประหยัดทั้งเวลาและค่าใช้จ่ายในการเตรียมสารลาเมลลาริน**

**ภูมิหลังของศิลปะหรือวิทยาการที่เกี่ยวข้อง**

นักวิทยาศาสตร์ได้ทำการศึกษาเกี่ยวกับผลิตภัณฑ์ธรรมชาติจากทะเลมาเป็นเวลานานแล้ว ในการศึกษานี้ได้พบสารใหม่ ๆ หลายชนิด ปัจจุบันการศึกษาทางด้านนี้ยิ่งได้รับความสนใจมากขึ้นเนื่องจากสารผลิตภัณฑ์เหล่านี้แสดงฤทธิ์ทางชีวภาพที่น่าสนใจ เช่น สามารถยับยั้งการเจริญเติบโตของเชื้อไวรัสของเซลล์มะเร็ง เป็นต้น

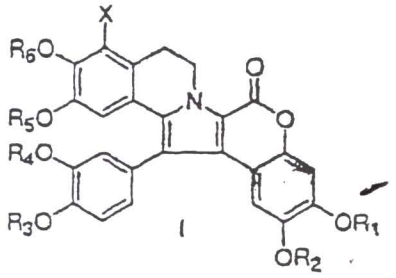
ลาเมลลาริน เป็นสารผลิตภัณฑ์ธรรมชาติจากทะเลชนิดหนึ่ง ซึ่งมีสูตรโครงสร้างโดยทั่วไปดังนี้



ในปี ค.ศ. 1985 กลุ่มวิจัยของ Faulker ได้ทำการสกัดสารผลิตภัณฑ์ธรรมชาติจาก *Lamellaria sp.* และค้นพบสารลาเมลลาริน A – D ทางกลุ่มวิจัยของ Fenical สามารถแยกสารลาเมลลารินอีก 4 ชนิด จาก *Didemnum cartaceum* ในปี ค.ศ. 1988 และเมื่อไม่นานมานี้ กลุ่มวิจัยชาวออสเตรเลียได้พบสารลาเมลลารินเพิ่มอีก 6 ชนิด ลาเมลลาริน I – N ในปัจจุบันมีการค้นพบสารกลุ่มลาเมลลารินมากกว่า 35 ชนิด สำหรับฤทธิ์ทางชีวภาพของสารลาเมลลารินนั้น ในการทดสอบเบื้องต้นพบว่า สารลาเมลลาริน C และ D สามารถยับยั้งเซลล์ในไข่ ปัจจุบันได้มีการนำสารลาเมลลาริน I , K และ L มาทดสอบในห้องปฏิบัติการกับเซลล์มะเร็ง ปรากฏว่า สารเหล่านี้มีฤทธิ์ในการยับยั้งการเจริญเติบโตของเซลล์มะเร็ง รวมทั้งยังสามารถทำให้เซลล์มะเร็งที่มีการดื้อต่อยาหลายชนิด เช่น vinblastine , vincristine , etoposide , teniposide , doxorubin , daunorubicin , plicamycin และ actinomycin D กลับมาตอบสนองต่อยาดังกล่าวที่ใช้ในการรักษาได้ดี และปราศจากความเป็นพิษ (non-toxic) จากคุณสมบัติดังกล่าว ทำให้สารลาเมลลารินเป็นที่น่าสนใจมาก ในปัจจุบันมีกลุ่มวิจัยหลายกลุ่มได้ศึกษาเพื่อหาวิธีสังเคราะห์สารลาเมลลาริน ทางห้องปฏิบัติการของสถาบันวิจัยจุฬาภรณ์ได้ประสบผลสำเร็จในการสังเคราะห์สารลาเมลลารินด้วยวิธีที่สั้นและมีประสิทธิภาพ

<u>การเปิดเผยการประดิษฐ์โดยสมบูรณ์</u>

การประดิษฐ์นี้จะให้สารประกอบสูตร I



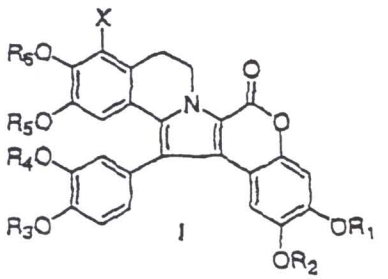

I

ซึ่ง

$R_1$ , $R_2$ , $R_3$ , $R_4$ , $R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ ไฮโดรเจนอะตอม หรือหมู่เมทิล

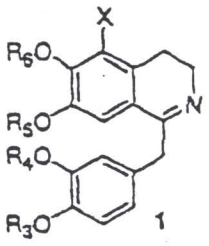
X คือ ไฮโดรเจนอะตอม , หมู่ไฮดรอกซี หรือหมู่เมทอกซี

กรรมวิธีสำหรับเตรียมสารประกอบสูตร I ตามการประดิษฐ์นี้มีดังต่อไปนี้



ซึ่ง $R_1$ , $R_2$ , $R_3$ , $R_4$ , $R_5$ , $R_6$ และ X มีความหมายดังข้างต้น ซึ่งประกอบรวมด้วยขั้นตอนของ

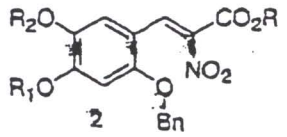
ก. การทำปฏิกิริยาระหว่างสารประกอบสูตร 1



ซึ่ง

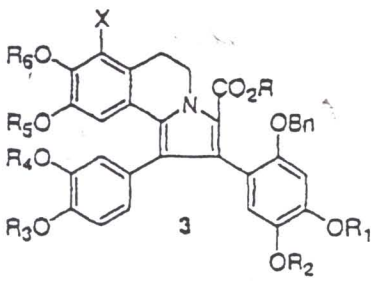
$R_3$ , $R_4$ , $R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ ไฮโดรเจนอะตอม , หมู่เมทิล หรือหมู่เบนซิล

X คือ ไฮโดรเจนอะตอม , หมู่ไฮดรอกซี หรือหมู่เบนซิลออกซี กับสารประกอบสูตร 2



ซึ่ง

R , $R_1$ และ $R_2$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ ไฮโดรเจนอะตอม , หมู่เมทิล หรือ หมู่เบนซิลภายใต้สภาวะที่มีเบสและการกลั่นไหลกลับ จะได้สสารประกอบไพโรลไอโซควิโนลินที่มีสูตร 3

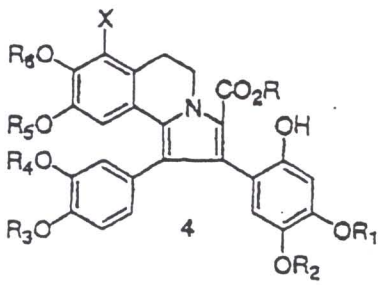


ซึ่ง

$R, R_1, R_2, R_3, R_4, R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ ไฮโดรเจนอะตอม , หมู่เมทิล หรือหมู่เบนซิล

X คือ ไฮโดรเจนอะตอม , หมู่ไฮดรอกซี , หมู่เมทอกซี หรือหมู่เบนซิลออกซี

ข. การแทนที่หมู่เบนซิลด้วยไฮโดรเจนของสารประกอบไพโรลไอโซควิโนลินสูตร 3 โดยใช้แก๊สไฮโดรเจน และแพลเลเดียมบนผงถ่านเป็นตัวคะตะลิสต์ จะได้สารประกอบไพโรลไอโซควิโนลินที่มีสูตร 4




ซึ่ง

$R, R_1, R_2, R_3, R_4, R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ ไฮโดรเจนอะตอม หรือหมู่เมทิล

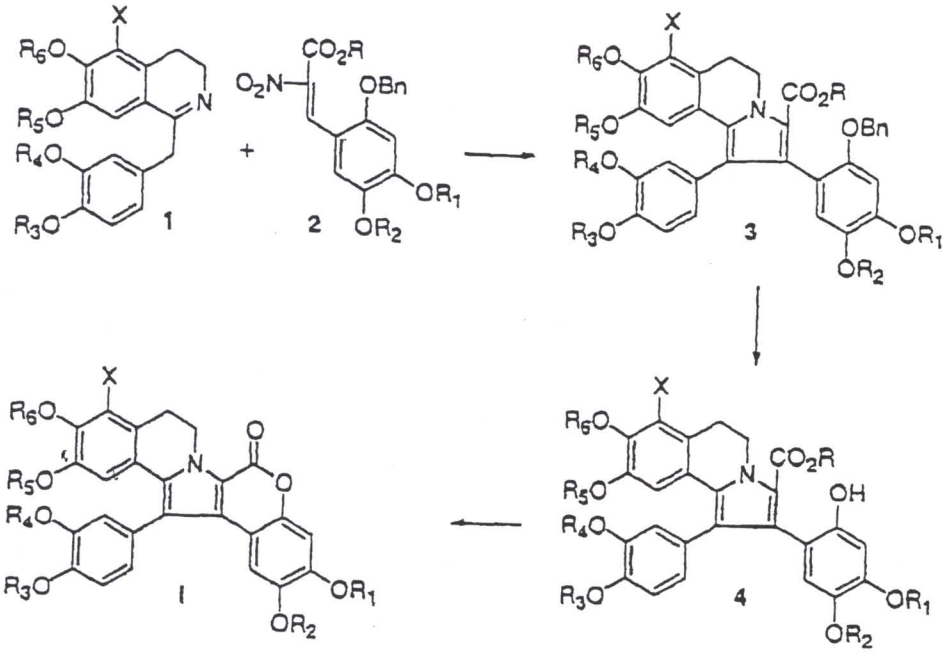
X คือ ไฮโดรเจนอะตอม , หมู่ไฮดรอกซี หรือหมู่เมทอกซี

ค. การปิดวงแหวนของสารประกอบไพโรลไอโซควิโนลินสูตร 4 โดยใช้โซเดียมไฮไดรด์เป็นเบส ในเตตระไฮโดรฟิวแรน

ซึ่งสารประกอบที่ได้จากการประดิษฐ์นี้อาจอยู่ในรูปของไอโซเมอร์เชิงแสง (optical isomer) ที่บริสุทธิ์หรือสารผสมของไอโซเมอร์เชิงแสง (optical isomer) ในอัตราส่วนต่าง ๆ หรือในรูปของราซีเมท (racemate)

<u>ตัวอย่างการสังเคราะห์ลาเมลลาริน</u>

การสังเคราะห์สารลาเมลลารินนั้นสามารถเกิดผ่านปฏิกิริยาหลายขั้นตอน ซึ่งในแต่ละขั้นตอนจะให้สารมัธยันต์ที่นำไปสู่สารลาเมลลาริน ดังนี้



ซึ่ง

R , $R_1$ , $R_2$ , $R_3$ , $R_4$ , $R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ ไฮโดรเจนอะตอม , หมู่เมทิล หรือหมู่เบนซิล

X คือ ไฮโดรเจนอะตอม , หมู่ไฮดรอกซี , หมู่เมทอกซี หรือหมู่เบนซิลออกซี

<u>แผนภาพที่ 1</u>

1. การเกิดปฏิกิริยาระหว่างสารเริ่มต้นที่มีสูตร 1 และ 2 ภายใต้สภาวะที่เป็นเบสและการกลั่นไหลกลับจะให้สารมัธยันต์ คือ สารประกอบไพโรลไอโซควิโนลินสูตร 3

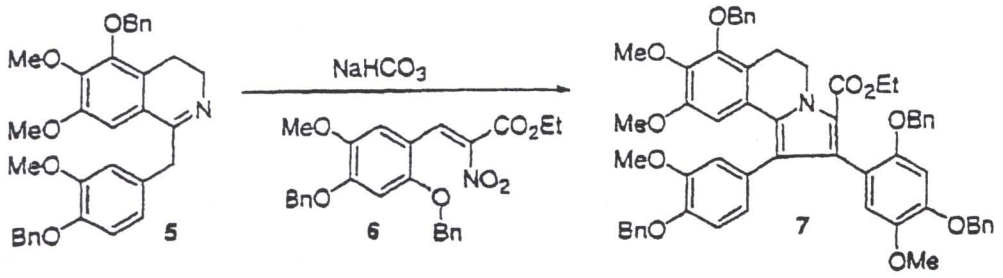
2. การแทนที่หมู่เบนซิลด้วยไฮโดรเจนของสารมัธยันต์ที่เป็นสารประกอบไพโรลไอโซควิโนลินสูตร 3 โดยใช้แก๊สไฮโดนเจน และแพลเลเดียมบนผงถ่านเป็นตัวเร่งปฏิกิริยา (คะตะลิสต์) จะได้สารมัธยันต์ที่เป็นสารประกอบไพโรลไอโซควิโนลินสูตร 4

3. การปิวงแหวนของสารมัธยันต์ที่เป็นสารประกอบไพโรลไอโซควิโนลินสูตร 4 ภายใต้สภาวะที่เป็นเบส จะได้สารลาเมลลารินที่มีสูตร I

4. ในแต่ละขั้นตอนได้พิสูจน์โครงสร้างของสารที่ได้มาด้วยเครื่องมือที่ใช้วิเคราะห์หาโครงสร้างต่าง ๆ เช่น เครื่องอินฟราเรดสเปกโทรโฟโตมิเตอร์ (IR) เครื่องนิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโทรมิเตอร์ (NMR) เครื่องแมสสเปกโทรมิเตอร์ (MS) เป็นต้น

ตัวอย่างของกรรมวิธีในการเตรียมอนุพันธ์ของลาเมลลาริน

1. การสังเคราะห์ลาเมลลาริน K; $R_1 = R_3 = X = H$ , $R_2 = R_4 = R_5 = R_6 = Me$




นำของผสมระหว่างไดไฮโดรพาพาเวอรีน 5 (770 มิลลิกรัม) โซเดียมไบคาร์บอเนต (120 มิลลิกรัม) และไนโตร-เอสเทอร์สไตรีน 6 (450 มิลลิกรัม) ในตัวทำละลาย อะซีโตไนไตรล์ (15 มิลลิลิตร) ไปกลั่นไหลกลับ 15 ชั่วโมง เมื่อทิ้งไว้ให้เย็นลง ณ อุณหภูมิห้อง จึงเติมน้ำและสกัดด้วยเอทิลอะซีเตต ดูดความชื้นด้วยโซเดียมซัลเฟต กรอง แล้วทำให้แห้งด้วยเครื่องระเหยภายใต้ความดันต่ำ จากนั้นแยกสารให้บริสุทธิ์ด้วยคอลัมน์โครมาโทกราฟี โดยใช้ซิลิกาเจลเป็นเฟสคงที่และใช้ 20% เอทิลอะซีเตตในเฮกเซนเป็นตัวชะ ได้ผลิตผล 7 เป็นของแข็งเหนียวสีขาวขุ่น (640 มิลลิกรัม) คิดเป็นเปอร์เซ็นต์ของผลผลิตได้ 70 เปอร์เซ็นต์ ผลิตภัณฑ์นี้มีคุณสมบัติทางกายภาพดังนี้

FTIR (KBr): $\nu_{max}$ 2934, 1685, 1508, 1457, 1417, 1216, 1206 $cm^{-1}$

$^1H$ NMR (ดิวเทอริโอคลอโรฟอร์ม) : δ

0.83 (t, 3H, $J$ = 7.1 Hz)

2.91 (br s, 2 H)

3.22 (s, 3 H)

3.54 (s, 3 H)

3.63 (s, 3 H)

3.87 (s, 3 H)

3.99 (br q, 2 H, $J$ = 7.1 Hz)

4.40 (br s, 2 H)

4.73 (s, 2 H)

5.03 (s, 2 H)

5.08 (s, 2 H)

5.13 (s, 2 H)

6.43 (s, 1 H)

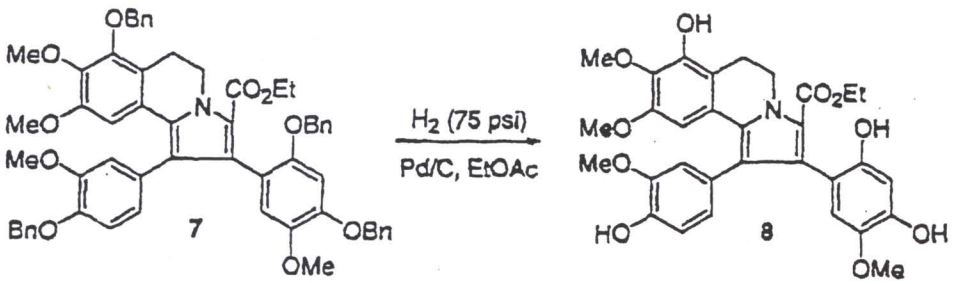
6.53 (s, 1 H)

6.56 – 6.76 (m, 4 H)

7.07 – 7.44 (m, 20 H)

$^{13}$C NMR (ดิวเทอริโอคลอโรฟอร์ม): δ 13.71, 22.60, 29.68, 42.48, 55.20, 55.77, 56.49, 59.61, 60.97, 70.68, 71.18, 71.63, 75.36, 103.0, 105.2, 113.5, 114.5, 116.1, 118.6, 119.4, 122.4, 123.1, 126.7, 127.0, 127.2, 127.4, 127.77, 127.79, 128.2, 128.47, 128.5, 128.6, 128.8, 130.3, 137.1, 137.2, 137.3, 137.8, 141.2, 143.5, 146.5, 147.1, 148.8, 149.1, 150.6, 151.5, 161.9.

แมสสเปคตรัม : m/z (เปอร์เซ็นต์ของปริมาณสัมพัทธ์) 938 ($M^+$, 4), 573(92), 91 (100.0), 42 (90)




นำสารละลายไพโรลเอสเทอร์ 7 (1.15 กรัม) ในเอทิลอะซีเตต (100 มิลลิลิตร) มาใส่ในเครื่องอัดความดันสูงพาร์และเติมพัลลาเดียมบนผงถ่าน (ประมาณ 100 มิลลิกรัม) ณ อุณหภูมิห้อง นำของผสมที่ได้ไปอัดไว้ภายใต้บรรยากาศของแก๊สไฮโดรเจน (75 พีเอสไอ) เป็นเวลา 15 ชั่วโมง นำของผสมไปกรองและทำให้แห้งด้วยเครื่องระเหยภายใต้ความดันต่ำได้เป็นผลิตผล 8 (700 มิลลิกรัม) ซึ่งเป็นของแข็งหนืด คิดเป็นเปอร์เซ็นต์ของผลผลิตได้ 99 เปอร์เซ็นต์ นำผลิตภัณฑ์ที่ได้ไปใช้ในขั้นตอนต่อไปโดยไม่ผ่านการทำให้บริสุทธิ์ ผลิตภัณฑ์ที่ได้มีคุณสมบัติทางกายภาพดังต่อไปนี้

FTIR (KBr): $\nu_{max}$ 3420 (br) , 2939, 1683, 1422, 1247 cm$^{-1}$

$^1$H NMR (ดิวเทอริโอคลอโรฟอร์ม) : δ

1.08 (t, 3H, $J$ = 7.1 Hz)

3.04 – 3.16 (br m, 2 H)

3.35 (s, 3 H)

3.55 (s, 3 H)

3.64 (s, 3 H)

3.88 (s, 3 H)

4.17 (q, 2 H, $J$ = 7.1 Hz)

4.20 (m, 1 H)

5.00 ms, 1 H)

5.47, 5.57, 5.98 (3br s, 4 H)

6.32 (s, 1 H)

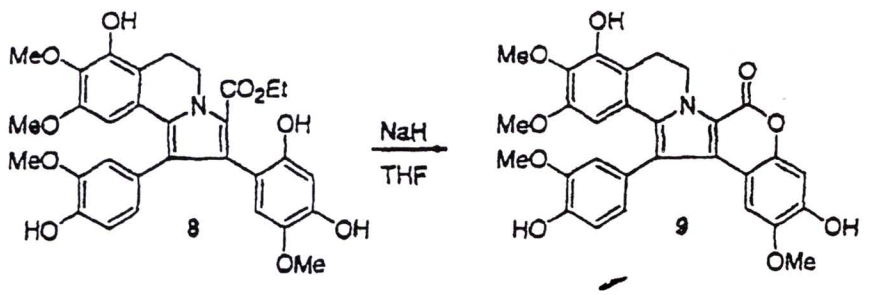
6.40 (s, 1 H)

6.54 (s, 1 H)

6.55 – 6.83 (m, 3 H)

$^{13}$C NMR (ดิวเทอริโอคลอโรฟอร์ม): δ 13.77, 21.70, 42.67, 55.16, 55.90, 56.43, 60.44, 61.00, 101.8, 102.5, 112.4, 113.3, 114.0, 114.2, 119.6, 123.5, 123.6, 123.8, 126.8, 131.6, 134.7, 140.1, 144.4, 145.7, 145.9, 146.3, 148.7, 150.2, 161.9.

แมสสเปคตรัม : m/z (เปอร์เซ็นต์ของปริมาณสัมพัทธ์) 576 ($M^+$, 4), 91 (100.0)




นำไพโรลเตตระออล 8 (190 มิลลิกรัม) ไปละลายในเตตระไฮโดรฟิวแรน (THF) (25 มิลลิลิตร) ที่อุณหภูมิ 0 องศาเซลเซียสภายใต้บรรยากาศของแก๊สอาร์กอน จานั้นเติมโซเดียมไฮไดรด์ (50 มิลลิกรัม) ที่ 0 องศาเซลเซียสและคนของผสมที่ได้ทิ้งไว้ที่อุณหภูมิห้องเป็นเวลา 2 ชั่วโมง หลังจากนั้นเติมน้ำ สกัดด้วยเอทิลอะซีเตต ดูดความชื้นด้วยโซเดียมซัลเฟต กรอง แล้วทำให้แห้งด้วยเครื่องระเหยความดันต่ำ จากนั้นแยกสารให้บริสุทธิ์ด้วยโครมาโทกราฟีโดยใช้ซิลิกาเจลเป็นเฟสคงที่และใช้ 1.5 % เมททานอลในไดคลอโรมีเทนเป็นตัวชะ หลังจากนั้น นำสารที่ได้ไปตกผลึกด้วยเมททานอล ได้ผลิตผล 9 (163 มิลลิกรัม) คิดเป็นเปอร์เซ็นต์ของผลผลิตได้ 93 เปอร์เซ็นต์ ของแข็งนี้มีคุณสมบัติทางกายภาพดังนี้

จุดหลอมเหลว (เมททานอล) : > 250 °ซ

FTIR (KBr): $\nu_{max}$ 3404 (br) , 2936, 1712, 1544, 1510, 1457, 1265, 1118 $cm^{-1}$

$^1$H NMR (ดิวเทอริโอคลอโรฟอร์ม) : δ

3.04 (m, 2 H)

3.28 (s, 3 H)

3.39 (s, 3 H)

3.75 (s, 3 H)

3.78 (s, 3 H)

4.08 (br s, 3 H)

4.55 (m, 1 H)

4.73 (m, 1H)

6.33 (s, 1H)

6.55 (s, 1H)

6.79 (s, 1H)

6.91 (dd, 1 H, $J$ = 1.8, 8.0 Hz)

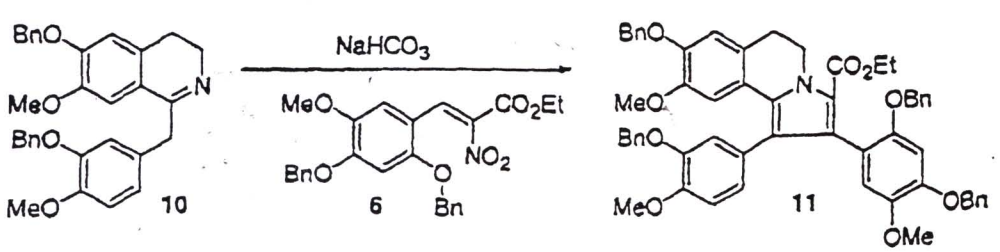
6.94 (d, 1 H, $J$ = 1.8 Hz)

7.00 (d, 1 H, $J$ = 8.0 Hz)

$^{13}$C NMR (ดิวเทอริโอคลอโรฟอร์ม): δ 21.39, 42.01, 54.90, 55.27, 55.96, 60.66, 101.5, 103.5, 104.5, 109.8, 113.3, 113.75, 113.84, 115.6, 123.1, 123.9, 126.8, 128.7, 135.9, 136.1, 144.4, 145.9, 146.0, 146.1, 146.7, 148.0, 150.7, 156.2

แมสสเปคตรัม : m/z (เปอร์เซ็นต์ของปริมาณสัมพัทธ์) 532 ($M^+$+1, 38), 531 ($M^+$, 100.0), 516 (52), 484 (23)

2. การสังเคราะห์ลาเมลลาริน L; $R_1 = R_4 = R_6 = X = H$ , $R_2 = R_3 = R_5 = Me$



นำของผสมระหว่าง ไดไฮโดรพาพาเวอรีน 10 (430 มิลลิกรัม) โซเดียมไบคาร์บอเนต (70 มิลิกรัม) และไนไตร-เอสเทอร์สไตรีน 6 (270 มิลลิกรัม) ในตัวทำละลาย อะซีโตไนไตรล์ (9 มิลลิลิตร) ไปกลั่นไหลกลับ 15 ชั่วโมง เมื่อทิ้งไว้ให้เย็นลง ณ อุณหภูมิห้อง จึงเติมน้ำและสกัดด้วย เอธิลอะซีเตต ดูดความชื้นด้วยโซเดียมซัลเฟต กรอง แล้วทำให้แห้งด้วยเครื่องระเหยภายใต้ความดันต่ำ จากนั้นแยกสารให้บริสุทธิ์ด้วยคอลัมน์โครมาโทกราฟี โดยใช้ซิลิกาเจล เป็นเฟสคงที่และใช้ 20% เอทิลอะซีเตต ในเฮกเซนเป็นตัวชะ ได้ผลิตผล 11 เป็นของแข็งเหนียว สีขาวขุ่น (370 มิลลิกรัม) คิดเป็นเปอร์เซนของผลผลิตได้ 70 เปอร์เซ็นต์ ผลิตภัณฑ์นี้มีคุณสมบัติทางกายภาพดังนี้

FTIR (KBr): $\nu_{max}$ 2933, 1685, 1498, 1381, 1254, 1212, 1174 $cm^{-1}$

$^1H$ NMR (ดิวเทอริโอคลอโรฟอร์ม) : δ

0.82 (t, 3H, $J$ = 7.1 Hz)

2.99 (br s, 2H)

3.31 (s, 3H)

3.64 (s, 3H)

3.82 (s, 3 H)

3.99 (br q, 2 H, $J$ = 7.1 Hz )

4.59 (br s, 2H)

4.72 (s, 2 H)

4.77 (s, 2 H)

5.01 (s, 2 H)

5.14 (s, 2 H)

6.44 (s, 1 H)

6.57 (s, 1 H)

6.65 (s, 1 H)

6.74 (s, 1 H)

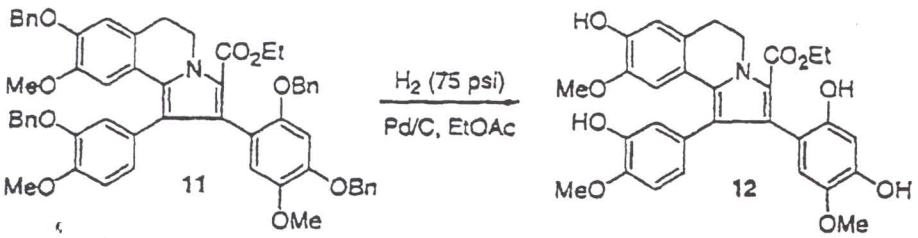
6.76 (s, 1 H)

7.06-7.46 (m, 20H)

$^{13}C$ NMR (ดิวเทอริโอคลอโรฟอร์ม): δ13.73, 29.61, 42.78, 55.20, 56.02, 56.46, 59.56, 70.84, 70.97, 71.16, 71.72, 103.1, 109.1, 111.5, 113.1, 116.1, 116.5, 118.8, 119.1, 121.5, 121.7,

123.7, 125.6, 126.8, 127.2, 127.3, 127.4, 127.7, 127.8, 127.9, 128.2, 128.4, 128.5, 128.6, 130.9, 136.95, 137.01, 137.1, 137.9, 143.5, 147.1, 147.8, 147.9, 148.2, 150.6, 162.0

แมสสเปคตรัม : m/z (เปอร์เซ็นต์ของปริมาณสัมพัทธ์) 907 ($M^+$, 6), 615 (100.0), 573 (84), 84 (38)




นำสารละลายไพโรลเอสเทอร์ 11 (1.1 กรัม) ในเอทิลอะซีเตต (100 มิลลิลิตร) มาใส่ในเครื่องอัดความดันสูงพาร์และเติมพัลลาเดียมบนผงถ่าน (ประมาณ 100 มิลลิกรัม) ณ อุณหภูมิห้อง นำของผสมที่ได้ไปอัดไว้ภายใต้บรรยากาศของแก๊สไฮโดรเจน (75 พีเอสไอ) เป็นเวลา 15 ชั่วโมง นำของผสมไปกรองและทำให้แห้งด้วยเครื่องระเหยภายใต้ความดันต่ำได้เป็นผลิตผล 12 (660 มิลลิกรัม) ซึ่งเป็นของแข็งหนืด คิดเป็นเปอร์เซ็นต์ของผลผลิตได้ 100 เปอร์เซ็นต์ นำผลิตภัณฑ์ที่ได้ไปใช้ในขั้นตอนต่อไปโดยไม่ผ่านการทำให้บริสุทธิ์ ผลิตภัณฑ์ที่ได้มีคุณสมบัติทางกายภาพดังนี้

FTIR (KBr): $\nu_{max}$ 3410 (br) , 2935, 1675, 1546, 1481, 1413, 1327, 1245 $cm^{-1}$

$^1$H NMR (ดิวเทอริโอคลอโรฟอร์ม): δ

1.06 (t, 3H, $J$ = 7.1 Hz)

2.90-3.10 (br m, 2H)

3.38 (s, 3H)

3.60 (s, 3H)

3.83 (s, 3H)

4.13 (q, 2 H, $J$ = 7.1 Hz)

4.32 (m, 1 H)

4.87 (m, 1 H)

5.38, 5.57, 5.58, 5.65 (4br s, 4 H)

6.40 (s, 1 H)

6.52 (s, 1 H)

6.62 (s, 1 H)

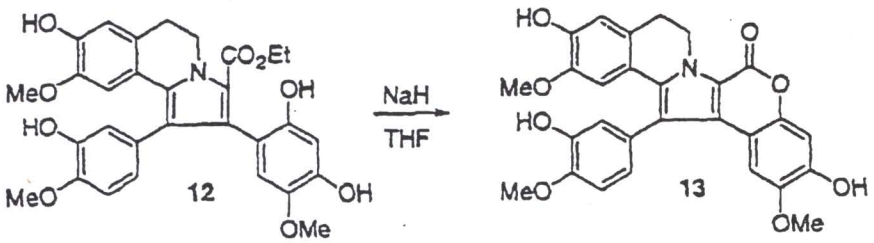
6.62 (m, 1 H)

6.71 (s, 1 H)

6.77 (m, 1 H)

$^{13}$C NMR (ดิวเทอริโอคลอโรฟอร์ม): δ 13.76, 28.70, 42.94, 55.30, 55.91, 56.48, 60.26, 108.5, 110.6, 113.7, 114.3, 117.0, 119.0, 120.0, 122.2, 122.6, 126.8, 128.3, 132.3, 140.0, 144.9, 145.0, 145.3, 145.5, 145.6, 148.6, 161.9

แมสสเปคตรัม : m/z (เปอร์เซ็นต์ของปริมาณสัมพัทธ์) 547 ($M^+$, 11), 531 (50), 470 (40), 178 (44), 91 (100.0)




นำไพโรลเตตระออล 12 (600 มิลลิกรัม) ไปละลายในเตตระไฮโดรฟิวแรน (THF) (70 มิลลิลิตร) ที่อุณหภูมิ 0 องศาเซลเซียสภายใต้บรรยากาศของแก๊สอาร์กอน จากนั้นเติมโซเดียมไฮไดรด์ (160 มิลลิกรัม) ที่ 0 องศาเซลเซียสและคนของผสมที่ได้ทิ้งไว้ที่อุณหภูมิห้องเป็นเวลา 2 ชั่วโมง หลังจากนั้นเติมน้ำ สกัดด้วยเอทิลอะซีเตต ดูดความชื้นด้วยโซเดียมซัลเฟต กรอง แล้วทำให้แห้งด้วยเครื่องระเหยความดันต่ำ จากนั้นแยกสารให้บริสุทธิ์ด้วยโครมาโทกราฟีโดยใช้ซิลิกาเจลเป็นเฟสคงที่และใช้ 1.5% เมททานอลในไดคลอโรมีเทนเป็นตัวชะ หลังจากนั้นนำสารที่ได้ไปตกผลึกด้วยเมททานอล ได้ผลิตผล 13 (495 มิลลิกรัม) คิดเป็นเปอร์เซ็นต์ของผลผลิตได้ 90 เปอร์เซ็นต์ ของแข็งนี้มีคุณสมบัติทางกายภาพดังนี้

จุดหลอมเหลว (เมททานอล):>250 °C

FTIR (KBr): $\nu_{max}$ 3629, 3473, 3266 (br), 2957, 1672, 1589, 1485, 1421, 1278 cm$^{-1}$

$^1$H NMR (ดิวเทอริโอคลอโรฟอร์ม): δ

30.5 (apparent t, 2 H, $J$ = 6.5 Hz)

3.41 (s, 3 H)

3.52 (s, 3 H)

3.96 (s, 3 H)

4.64-4.80 (m, 2 H)

6.72 (s, 2 H)

6.77 (s, 1 H)

6.89 (s, 1 H)

6.98 (dd, 1 H, $J$ = 2.0, 8.2 Hz)

7.05 (d, 1 H, $J$ = 8.2 Hz)

7.08 (d, 1 H, $J$ = 2.0 Hz)

$^{13}$C NMR (ดิวเทอริโอคลอโรฟอร์ม): δ 28.32, 42.25, 55.11, 55.39, 56.15, 103.4, 104.5, 108.8, 109.9, 111.7, 113.2, 114.4, 117.6, 119.3, 122.8, 127.3, 128.5, 136.4, 143.9, 145.4, 145.8, 145.9, 146.1, 146.6, 146.8, 156.0

แมสสเปคตรัม : m/z (เปอร์เซ็นต์ของปริมาณสัมพัทธ์) 501 ($M^+$, 0.55), 251 (51), 42 (100.0)

<u>รายการสัญลักษณ์ประกอบ</u>

| | | |
|---|---|---|
| $NaHCO_3$ | = | โซเดียมไบคาร์บอเนต |
| Bn | = | หมู่เบนซิล ($CH_2Ph$) |
| Et | = | หมู่เอทิล ($CH_2CH_3$) |
| Me | = | หมู่เมทิล ($CH_3$) |
| $NO_2$ | = | หมู่ไนโตร |
| Pd/C | = | แพลเลเดียมบนผงถ่าน |
| EtOAc | = | เอทิลอะซิเตต |
| H | = | แก๊สไฮโดรเจน |
| psi | = | หน่วยวัดค่าความดันแก๊ส |
| NaH | = | โซเดียมไฮไดรด์ |
| THF | = | เตตระไฮโดรฟิวแรน |

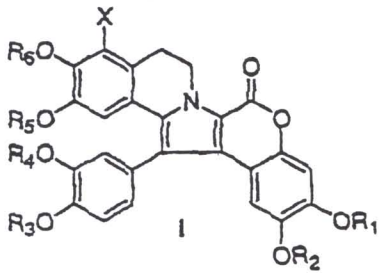
| | | | |
|---|---|---|---|
| | °ซ | = | องศาเซลเซียส |
| | FTIR | = | ฟูริเออร์ทรานสฟอร์ม อินฟาเรดสเปกโทรมิเตอร์ |
| | KBr | = | โพแตสเซียมโบรไมด์ |
| | $\nu_{max}$ | = | จำนวนคลื่นที่ดูดกลืนสูงสุด |
| | $cm^{-1}$ | = | ต่อเซนติเมตร |
| | br | = | การดูดกลืนคลื่นที่มีช่วงการดูดกลืนกว้าง |
| | $^{1}H$ NMR | = | โปรตอนนิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโทรมิเตอร์ |
| | $^{13}C$ NMR | = | คาร์บอน-13 นิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโทรมิเตอร์ |
| | δ | = | เดลตา แสดงตำแหน่งการเกิดเรโซแนนซ์ของโปรตอน |
| | s | = | 1 พีก |
| | t | = | 3 พีก |
| | q | = | 4 พีก |
| | m | = | หลายพีก |
| | br s | = | 1 พีกกว้าง |
| | br q | = | 4 พีกกว้าง |
| | br m | = | หลายพีกกว้าง |
| | J | = | ค่าคงที่ของคู่ควบ |
| | Hz | = | เฮิรทซ์ หน่วยของค่าคงที่ของคู่ควบ |
| | m/z | = | อัตราส่วนมวลต่อประจุ |
| | $M^{+}$ | = | โมเลกุลไอออน |

<u>วิธีการในการประดิษฐ์ที่ดีที่สุด</u>

เหมือนกับที่บรรยายไว้ในการเปิดเผยการประดิษฐ์โดยสมบูรณ์

**ข้อถือสิทธิ**

1. กรรมวิธีสำหรับเตรียมสารประกอบสูตร I



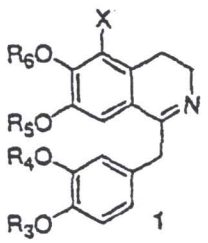
ซึ่ง

**$R_1$, $R_2$, $R_3$, $R_4$, $R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ ไฮโดรเจนอะตอม หรือหมู่เมทิล**

**X คือ ไฮโดรเจนอะตอม , หมู่ไฮดรอกซี หรือหมู่เมทอกซี**

ซึ่งกรรมวิธีประกอบด้วย

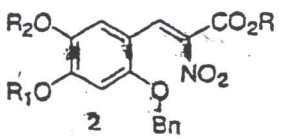
ก. การทำปฏิกิริยาระหว่าง สารประกอบสูตร 1



ซึ่ง

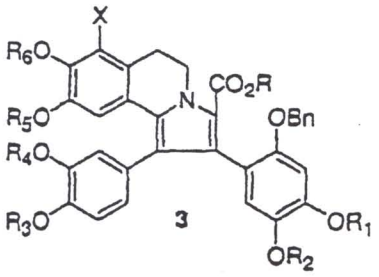
$R_3$, $R_4$, $R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ **ไฮโดรเจนอะตอม** , หมู่เมทิล หรือหมู่เบนซิล

X คือ **ไฮโดรเจนอะตอม** , หมู่ไฮดรอกซี , หมู่เมทอกซี หรือ หมู่เบนซิลออกซี กับสารประกอบสูตร 2



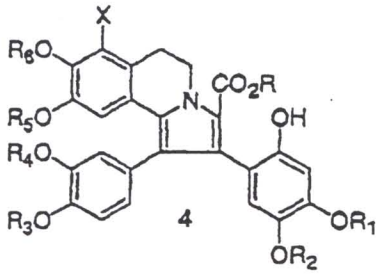
ซึ่ง

$R$, $R_1$ และ $R_2$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ **ไฮโดรเจนอะตอม** , หมู่เมทิล หรือหมู่เบนซิลภายใต้สภาวะที่มีเบสและการกลั่นไหลกลับ จะได้สารประกอบไพโรลไอโซควิโนลิน สูตร 3




ซึ่ง R และ $R_1$ ถึง $R_6$ และ X มีความหมายดังข้างต้น

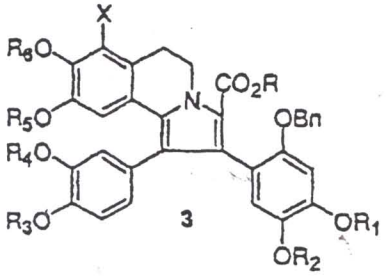
ข. การแทนที่หมู่เบนซิลด้วยไฮโดรเจนของสาร**ประกอบไพโรลไอโซควิโนลิน**สูตร 3 โดยใช้แก๊สไฮโดรเจน และแพลเลเดียมบนผงถ่านเป็นตัวคะตะลิสต์ จะได้สาร**ประกอบไพโรลไอโซควิโนลิน**สูตร 4




ซึ่ง R และ $R_1$ ถึง $R_6$ และ X มีความหมายดังข้างต้น

ค. การปิดวงแหวนของสาร**ประกอบไพโรลไอโซควิโนลิน**สูตร 4 โดยใช้โซเดียมไฮไดรด์เป็นเบส ในเตตระไฮโดรฟิวแรน

2. สารประกอบไพโรลไอโซควิโนลิน สูตร 3



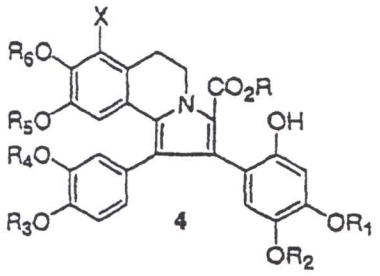

ซึ่ง

R, $R_1$, $R_2$, $R_3$, $R_4$, $R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ **ไฮโดรเจนอะตอม** , หมู่เมทิล หรือ หมู่เบนซิล

X คือ **ไฮโดรเจนอะตอม** , หมู่ไฮดรอกซี , หมู่เมทอกซี หรือ หมู่เบนซิลออกซี

3. สารประกอบของข้อถือสิทธิ 2 ซึ่งอาจอยู่ในรูปของไอโซเมอร์เชิงแสง (optical isomer) ที่บริสุทธิ์หรือสารผสมของไอโซเมอร์เชิงแสง (optical isomer) ในอัตราส่วนต่าง ๆ หรือในรูปของราซีเมท (racemate)

4. สารประกอบไพโรลไอโซควิโนลิน สูตร 4




4

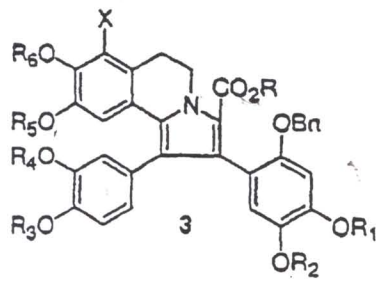
ซึ่ง

R, $R_1$, $R_2$, $R_3$, $R_4$, $R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ **ไฮโดรเจนอะตอม** หรือหมู่เมทิล

X คือ **ไฮโดรเจนอะตอม** , หมู่ไฮดรอกซี หรือหมู่เมทอกซี

5. สารประกอบของข้อถือสิทธิ 4 ซึ่งอาจอยู่ในรูปของไอโซเมอร์เชิงแสง (optical isomer) ที่บริสุทธิ์หรือสารผสมของไอโซเมอร์เชิงแสง (optical isomer) ในอัตราส่วนต่าง ๆ หรือในรูปของราซีเมท (racemate)

6. กรรมวิธีสำหรับเตรียมสารประกอบไพโรลไอโซควิโนลิน สูตร 3



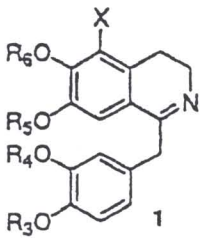

3

ซึ่ง

**$R, R_1, R_2, R_3, R_4, R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนหรือต่างกัน คือ ไฮโดรเจนอะตอม หมู่เมทิล หรือ หมู่เบนซิล**

**X คือ ไฮโดรเจนอะตอม , หมู่ไฮดรอกซี หรือหมู่เบนซิลออกซี**

ซึ่งกรรมวิธีประกอบด้วยการทำปฏิกิริยาระหว่างสารประกอบสูตร 1

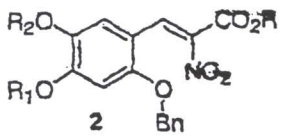



1

ซึ่ง

$R_3, R_4, R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ **ไฮโดรเจนอะตอม** หรือ หมู่เบนซิล

X คือ **ไฮโดรเจนอะตอม** , หมู่ไฮดรอกซี , หมู่เมทอกซี หรือ หมู่เบนซิลออกซี กับสารประกอบสูตร 2

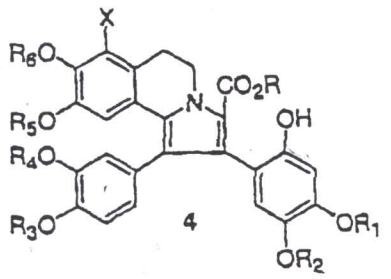



2

ซึ่ง

$R, R_1$ และ $R_2$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ **ไฮโดรเจนอะตอม** , หมู่เมทิล หรือ หมู่เบนซิลภายใต้สภาวะที่มีเบสและการกลั่นไหลกลับ

7. กรรมวิธีสำหรับเตรียมสารประกอบไพโรลไอโซควิโนลิน สูตร 4




4

ซึ่ง

**$R$, $R_1$, $R_2$, $R_3$, $R_4$, $R_5$ และ $R_6$ เป็นอิสระต่อกัน ซึ่งอาจเหมือนกันหรือต่างกัน คือ ไฮโดรเจนอะตอม หรือหมู่เมทิล**

**X คือ ไฮโดรเจนอะตอม , หมู่ไฮดรอกซี หรือหมู่เมทอกซี**

ซึ่งกรรมวิธีประกอบด้วยการทำปฏิกิริยาระหว่างสารประกอบ**ไพโรลไอโซควิโนลิน** สูตร 3 ดังในข้อถือสิทธิ 2 กับแก๊สไฮโดรเจน โดยมีแพลเลเดียมบนผงถ่านเป็นตัวคะตะลิสต์

Pharmaceutical Biology
2000, Vol. 38, Supplement, pp. 6–15

1388-0209/00/380S-0006$15.00


# CYTOTOXIC NATURAL PRODUCTS FROM THAI PLANTS: A RECENT STUDY

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*ABSTRACT*

*Natural products will continue to be the most prolific source of bioactive compounds. Natural products exhibiting antitumor activity continue to be the subject of extensive research aimed at the development of drugs for the treatment of different human tumors. It is generally accepted that natural products offer a diversity and complexity of structure unmatched by even the most active imaginations of synthetic organic chemists. This paper reviews the research of selected Thai plants for the discovery of therapeutic agents. Attention will be focused on our recent research on Thai plants that possess cytotoxic properties. Synthetic modification and reaction of some of these compounds aimed at enhancing their potency will also be presented.*

## INTRODUCTION

Due to the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovery of potential new drugs and biological entities (Shu, 1998). However, only approximately 5,000 of the world's estimated 250,000–400,000 flowering plants have as yet been analysed for their possible medicinal uses (Balandrin et al., 1993). Moreover, in developing countries, medicinal plants continue to be the main source of medication. In China alone, 7,295 plant species are utilized as medicinal agents. The World Health Organization has estimated that for some 3.4 billion people in the developing world, plants represent the primary source of medicine. This represents about 88% of the world's inhabitants who rely mainly on traditional medicine for their primary health care. Farnsworth (1988) reported that at least 119 compounds derived from 90 plant species can be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine. The importance of natural products is also evidenced by the fact that in 1991 nearly half of the best selling drugs were either natural products or their derivatives (Farnsworth & Bingel, 1977; Farnsworth et al., 1985; Farnsworth, 1988). It is thus a matter of utmost concern to public health and indeed to human life that urgent action is taken to prevent further diminution of actual and potential availability of medicinal and biological agents (O'Neill et al., 1993). Cragg et al. (1997) of the NCI, USA have reported some interesting statistics that for new drug applications, anticancer drugs and drugs for infectious diseases show a high number of compounds from natural sources indicating the increasing importance of natural products for the treatment of these two types of diseases.

*Keywords:* Anticancer, bioactive natural products, cholangiocarcinoma, colchicine analogues, *Derris dericulata, Gloriosa superba.*

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### Natural Products as Anticancer Agents

Apart from being an excellent source of anti-infectious drugs (Phillipson & Wright, 1991; Mahidol et al., 1997a), plants are also a good source of anticancer agents (Cordell et al., 1993; Hamburger & Hostettmann, 1991; Hostettmann et al., 1998; King-

horn et al., 1995; Mahidol et al., 1998; Pezzuto, 1997; Suffness et al., 1995; Valeriote et al., 1995). The National Cancer Institute (NCI), USA has launched an extensive program for the development of natural products for the treatment of various forms of cancer. Many clinically useful drugs have been discovered from various plants.

Vinblastine (**1**) and vincristine (**2**) (Neuss et al., 1964; Rahman et al., 1994), derivatives of the periwinkle plant (*Catharanthus roseus*), have proved to be active against tumor growth. Vinblastine has been shown to be a very effective treatment for Hodgkin's disease, and has also been used to treat breast cancer, Kaposi's sarcoma, and other diseases. Vincristine is well known for its 90% success rate in treating different types of childhood leukemia.

Other anticancer agents include the semisynthetic epipodophyllotoxin, etoposide (**3**) (Issell, 1982), as well as the recently discovered taxol (**4**) and its semisynthetic derivative, taxotere (**5**) (Rowinsky, 1997; DeFuria, 1997). Taxol (paclitaxel, **4**) has been isolated from the bark of the Pacific or American yew tree, *Taxus brevifolia*. The discovery of taxol has indeed heightened the interest in plant-derived anticancer drugs, and more than 350 taxane diterpenoids have now been identified (Baloglu & Kingston, 1999). Discovered in 1971 (Wani et al., 1971), taxol appears to be an exceptionally promising drug. It exhibits a very broad spectrum of activity against leukemias and solid tumors and taxol has been found to be beneficial in treatment of refractory ovarian, breast, and other cancers, and the drug was recently approved for marketing. It is estimated that four mature yew trees which take about 100 years to reach full maturity are needed to produce enough taxol to treat a single case of ovarian cancer. At present, although the synthesis of this extremely complicated taxol molecule has been accomplished, it has not yet become commercially feasible. It is likely that taxol will be produced semisynthetically for commercial production using intermediates related to baccatin III.

The addition of taxol to the list of anticancer drugs is testimony to the synergism of broadly based contributions from multidisciplinary scientific endeavour. The isolation and structure elucidation led the way to pharmacological and toxicological testing. The finding of baccatin III from other phytochemical sources coupled with synthetic organic chemistry make the drug available for human trials.

The finding that taxoids act through the stabilization of microtubules has led to the search for new agents that function by a comparable mechanism. As a result, new compounds have been discovered. Epothilones (**6** and **7**) are a new class of macrocyclic natural products which were first isolated from myxobacteria (Gerth et al., 1996). Epothilones (Finlay, 1997) are more potent than taxol in some cell lines and they hold great promise for further investigation. Like taxol, epothilones have captured the interest of many synthetic organic chemists and the syntheses of these compounds have recently been accomplished (Harris & Danishefsky, 1999).

In addition to the above-mentioned clinically approved drugs and promising drug candidates, some other plant-derived compounds show a great deal of potential for future use as anticancer agents. Camptothecin (**8**) (Wall et al., 1966) was originally isolated from the Chinese tree *Camptotheca acuminata* and a number of camptothecin analogues (Wall & Wani, 1993) are currently being developed as anticancer agents. Camptothecin was established as having *in vivo* activity against murine leukemia and rat Walker carcinosarcoma 256 models. While early clinical trials on the parent alkaloid and camptothecin sodium were not particularly successful due to toxicity problems, interest in camptothecin intensified once it was discovered that it exhibits a novel mechanism of action by inhibiting the enzyme DNA topoisomerase I. Accordingly, a number of camptothecin analogues have been developed in an attempt to reduce toxicity, optimize efficacy, and improve water solubility without opening the lactone ring present in the parent molecule, and topotecan (**9**) and irinotecan are two interesting camptothecin analogues (Henegar et al., 1997; Cao et al., 1998).

Another plant-derived alkaloid which is also under clinical trial is homoharringtonine (**10**), a cephalotaxine alkaloid (Powell et al., 1972; Feldman et al., 1992). The compound was originally isolated from *Cephalotaxus harringtonia*; it shows antineoplastic activity, especially against murine lymphocytic leukemias. It was found to be more active than vincristine against mouse leukemias and melanomas.

We have investigated the plant *Phyllanthus amarus* Schum. & Thonn. (Euphorbiaceae), locally known as Look Tai Bai, for cytotoxic activity. *Phyllanthus amarus* (Somanabandhu et al., 1993) has been traditionally used for the treatment of jaundice and other hepatic diseases. Although the antihepatotoxic potential of the plant has been controversial, the major chemical components were known to be phyllanthin (**11**) and hypophyllanthin (**12**), with structures as shown. Apart from the structural study, the biological activities of these compounds have also been investigated.

In addition to *Phyllanthus amarus*, *Gloriosa superba* L. was investigated for anticancer activity. *Gloriosa superba* is known in Thai as "Dong Dueng" or "Dao Dueng", a climber plant in the family Colchicaceae, which is widely distributed in the tropical parts of Asia and Africa, with many varieties present in Thailand. The active principle of *Gloriosa superba* is the alkaloid colchicine (**13**) which is obtained from the dried tuber of the plant. Colchicine has long been used for the treatment of arthritis. From the dried tuber of Thai *Gloriosa superba*, four tropolone alkaloids (**13–16**) (Engprasert, 1995; Capraro & Brossi, 1984; Bentley, 1998) were isolated and the structures are as shown.

The structures were elucidated using various spectroscopic techniques, such as UV, IR, MS, one- and two-dimensional NMR. The cytotoxicity data of various colchicines with various cell lines have also been established.

Another cancer cell line of interest to us is the cholangiocarcinoma cell line. Cholangiocarcinoma, a form of bile duct cancer, is a rare type of cancer in the Western world, but it is highly prevalent in Thailand and in many other Asian countries. The cause of the disease is believed to be associated with infestation of *Opisthorchis viverrini* or liver fluke and exposure to a chemical carcinogen in food or in the environment, presumably, dimethylnitrosamine (DMN). The effectiveness of some new anticancer agents against cholangiocarcinoma was evaluated. This process was carried out by *in vitro* testing with the cholangiocarcinoma (HuCCA-1) cell line (Sirisinha et al., 1991, 1993). Some colchicine derivatives have been subjected to cytotoxicity testing, using a microculture protein assay (Table 1).

The $ED_{50}$ for the cholangiocarcinoma cell line with the methanol extract of *Gloriosa superba* was found to be 2.5 μg/ml, while the $ED_{50}$ of 3-demethyl-*N*-formyl-*N*-deacetylcolchicine was found to be 0.0625 micrograms per ml, in contrast with the $ED_{50}$ of about 0.02 μg/ml for colchicine. These values were approximately two-times higher than the $ED_{50}$ values for the KB cell line. These results showed that the cholangiocarcinoma cell line is highly susceptible to derivatives of the tropolone alkaloids, at least when tested *in vitro*; whether or not these agents will be effective *in vivo* remains to be determined in further experiments.

Various analogues of colchicine have been synthesized with the aim of improving the therapeutic index of the target compound by enhancing the potency of these analogues. The modification of the aromatic ring of colchicine was first studied.

Demethylation at the C-2 position of colchicine (Scheme 1) could be performed by the action of concentrated sulfuric acid at 45 °C for 7 h (Rösner et al., 1981). The 2-demethylated product was alkylated with $C_{16}$ alkyl iodide to give the corresponding ether (Scheme 2).

Acetylation of the C-2 demethylated product with acetic anhydride in dry pyridine gave colchicine acetate (Scheme 3). Esterification with longer chain fatty acids could also be effected, for example, acylation with palmitic acid chloride in pyridine gave the corresponding colchicine palmitate (Scheme 4). The results of the biological testing indicated that the longer chain of the alkyl or ester group attached to the aromatic ring of colchicine did not improve the biological activity in the cholangiocarcinoma cell line.

The Seitz procedure was also applied to modify the tropolone ring by using the Diels-Alder reaction of cholchicine with various dienophiles (Brecht et al., 1997). Reaction of singlet oxygen with colchicine produced the cycloaddition product of colchicine peroxide (Scheme 5).

*N*-Phenyl-1,2,4-triazolinedione (PTAD), the highly reactive nitrogen dienophile, reacted with colchicine. When the two reactants were heated in toluene at 110 °C for half an hour, only the endo adduct was formed. Under relatively drastic conditions, colchicine gave the maleimide adduct. When colchicine was heated with *N*-methyl maleimide in mesitylene at 166 °C for 14 h, two stereoisomers of endo and exo adducts were obtained in a (1:1) ratio (Scheme 6).

The modification of the peripheral functional group of the tropolone ring was also studied according a the known procedure (Cavazza & Pietra, 1998). Reaction of colchicine with guanidine in methanol led to the guanidyl colchicine derivative (Scheme 7). Also, reac-

Table 1. $ED_{50}$ values against KB and HuCCA-1 cell lines.

| *Gloriosa superba* | KB (μg/ml) | HuCCA-1 (μg/ml) |
|---|---|---|
| Methanol extract | 0.5 | 2.5 |
| Colchicine (**13**) | 0.01 | 0.02 |
| 3-Demethyl-*N*-formyl-*N*-deacetylcolchicine (**15**) | 0.03125 | 0.0625 |

HuCCA-1 = Human cholangiocarcinoma cell line

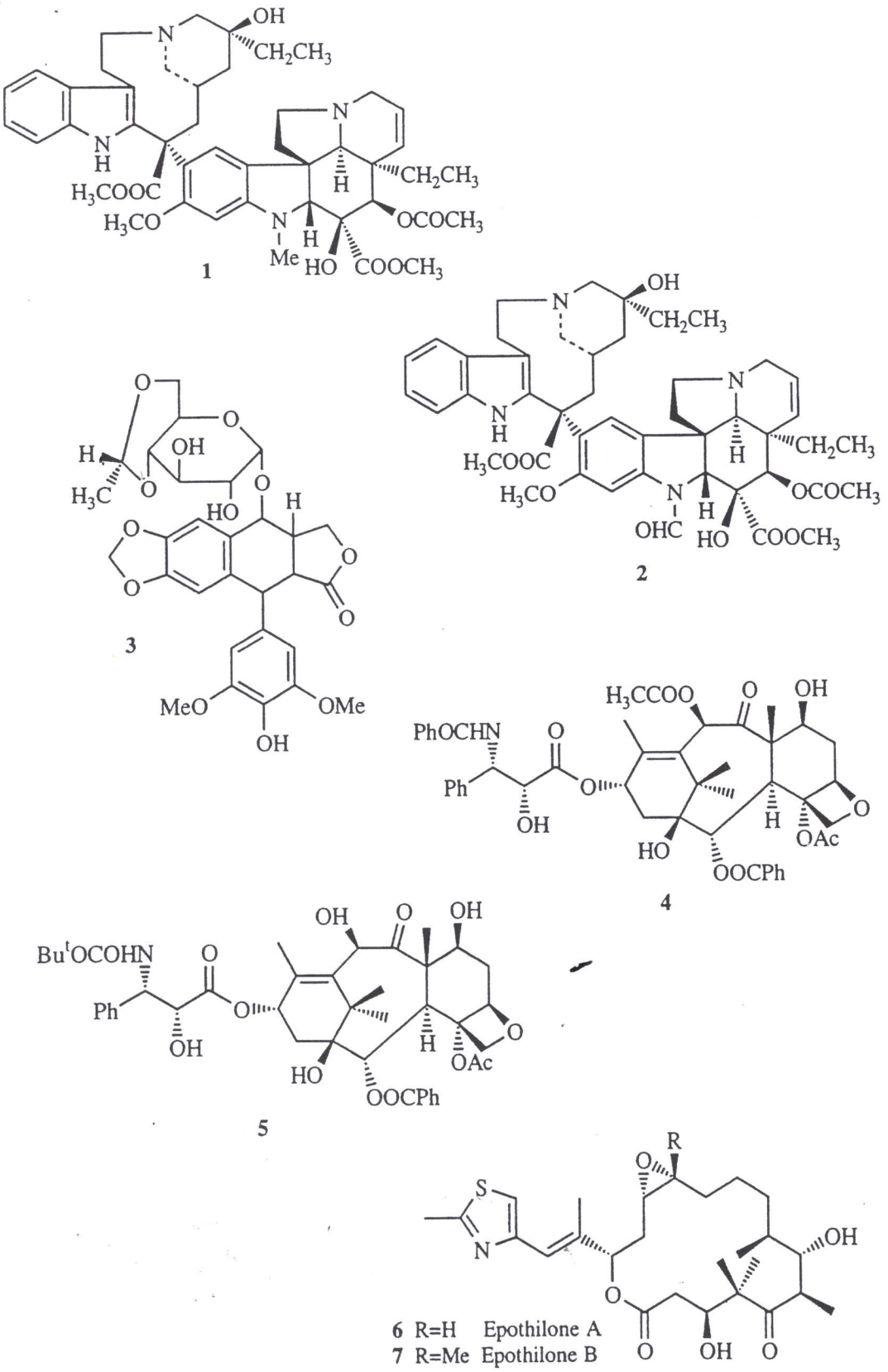
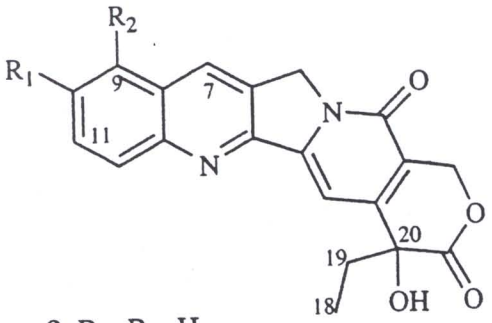
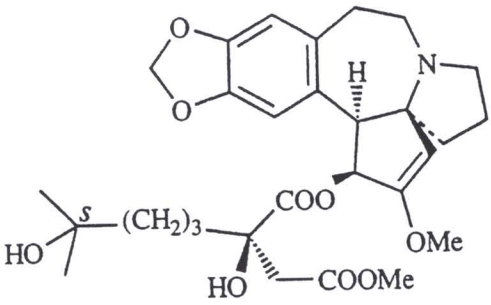


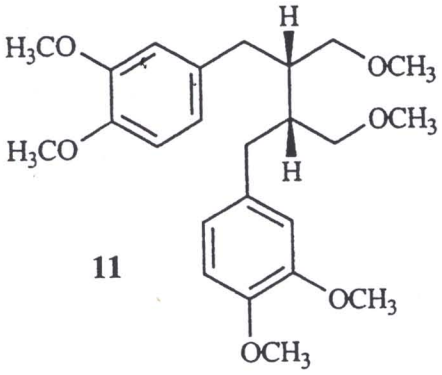

Fig. 1. Structures of compounds 1–7.



**8** $R_1=R_2=H$
**9** $R_1=OH$, $R_2=-CH_2-NMe_2$



**10**



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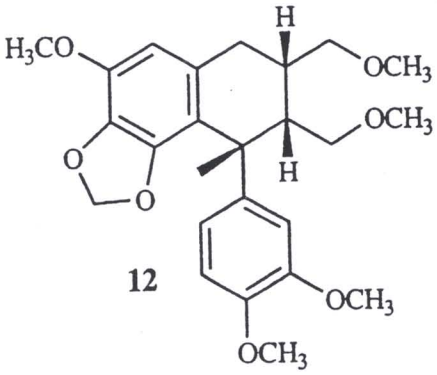

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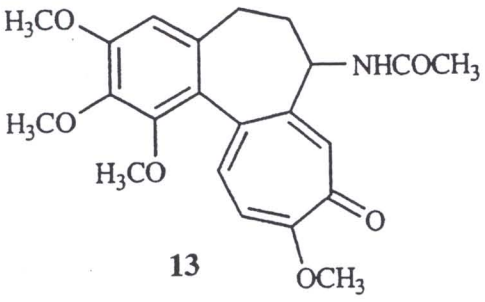

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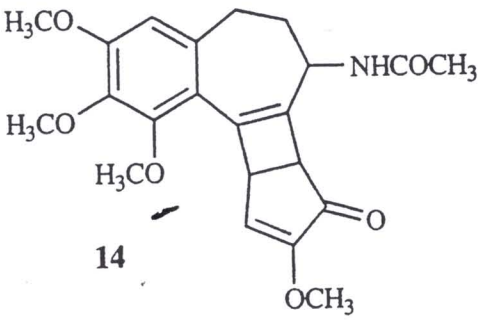

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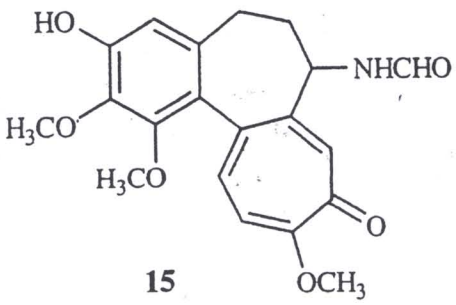

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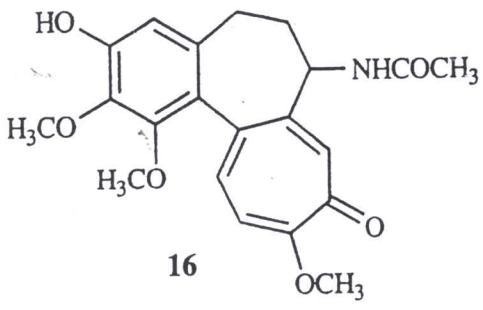
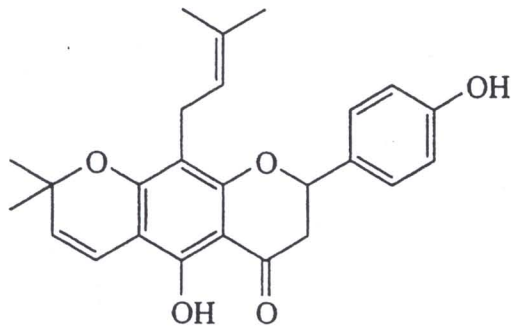


**16**

Fig. 2. Structures of compounds **8–16**.

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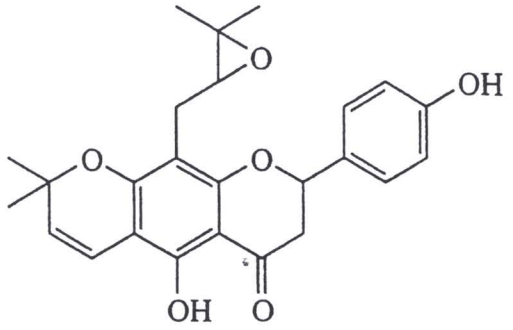


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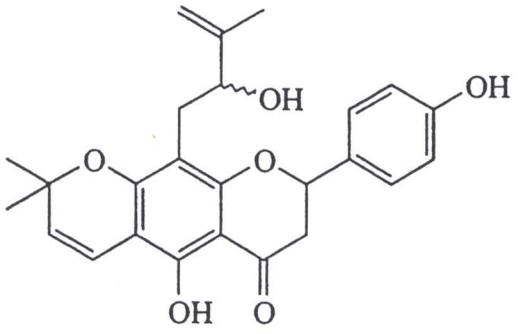


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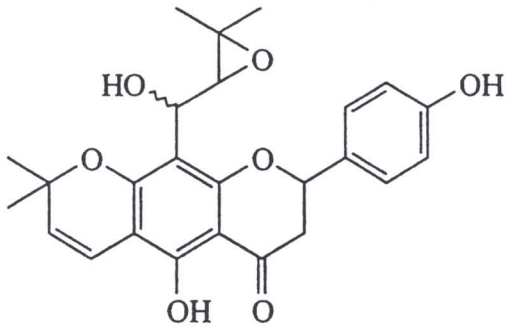



20

Fig. 3. Structures of compounds **17–20**.

tion of colchicine with benzamidine in dry benzene gave the benzamidyl colchicine derivative (Scheme 8).

The results of biological testing of these compounds using the cholangiocarcinoma cell line showed very low biological activities as compared to colchicine; these results clearly illustrated the importance of the tropolone ring in the activity.

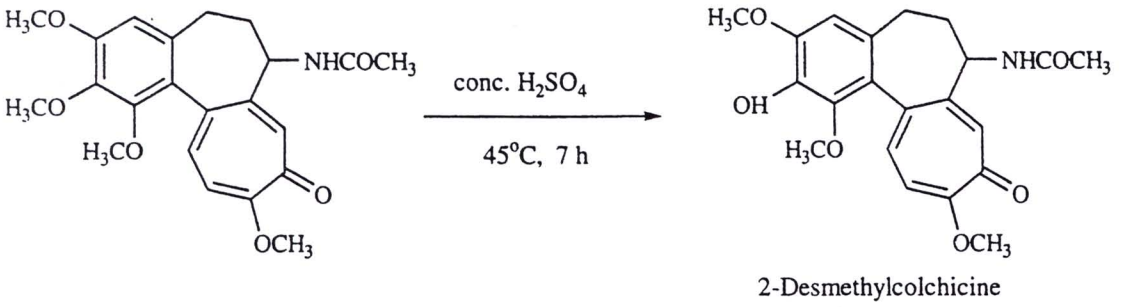
We have recently investigated another plant, *Derris reticulata*, locally known as Cha-aim thai (Mahidol et al., 1997b). Cha-aim thai is a medicinal plant of Thailand used for the relief of thirst and as an expectorant. Our studies led to the isolation of three new pyranoflavanone compounds. Lupinifolin (**17**), a known flavanone, was isolated as the major constituent of this plant. Its structure was confirmed by detailed analysis of NMR spectra such as COSY, NOESY, APT, HETCOR and selective INEPT.

The first unknown isolate, named epoxylupinifolin (**18**), was shown to be the 2‴,3‴-epoxide of lupinifolin by spectroscopic methods. The structure of the epoxide was further confirmed by successful epoxidation of lupinifolin with magnesium monoperoxyphthalate hexahydrate (MMPP)

Two other new compounds isolated were named dereticulatin (**19**) and hydroxy-epoxylupinifolin (**20**) and the structures were found to be hydroxy derivatives through the analysis of the NMR spectra of these compounds and the corresponding derivatives.

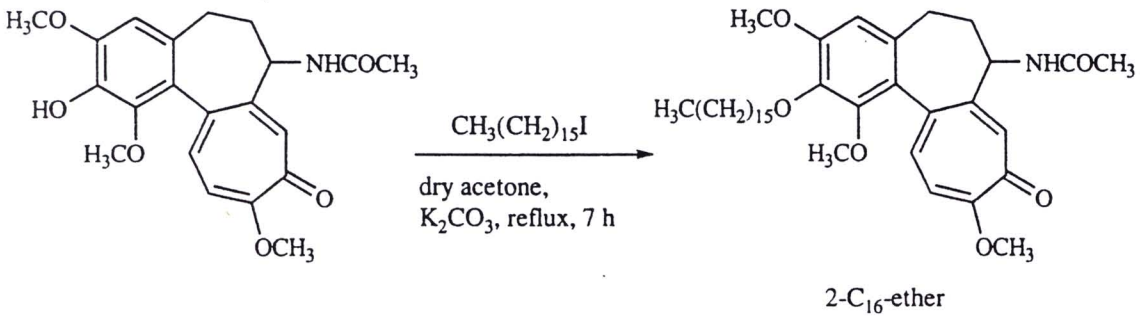
The *in vitro* bioassay evaluation of lupinifolin, epoxylupinifolin and dereticulatin triacetate was also carried out. The results showed that each of them inhibited the P-388 cell line at 0.4–0.5 μg/ml, but were inactive against the KB cell line.

In conclusion, it is our conviction that research on



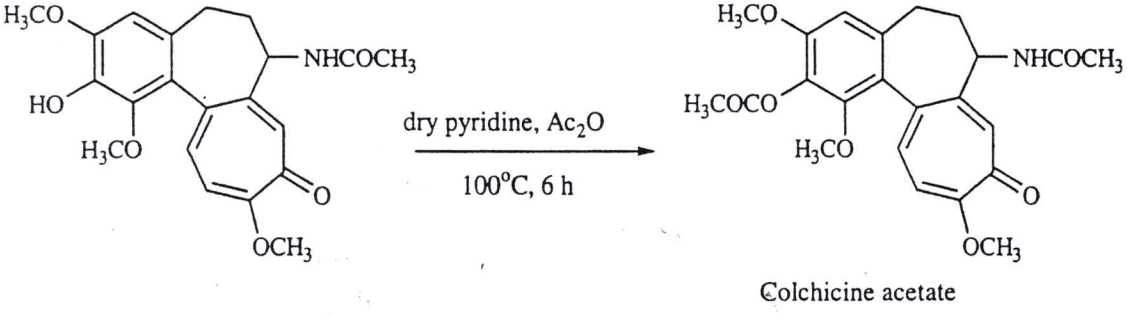

2-Desmethylcolchicine

**Scheme 1**




2-$C_{16}$-ether

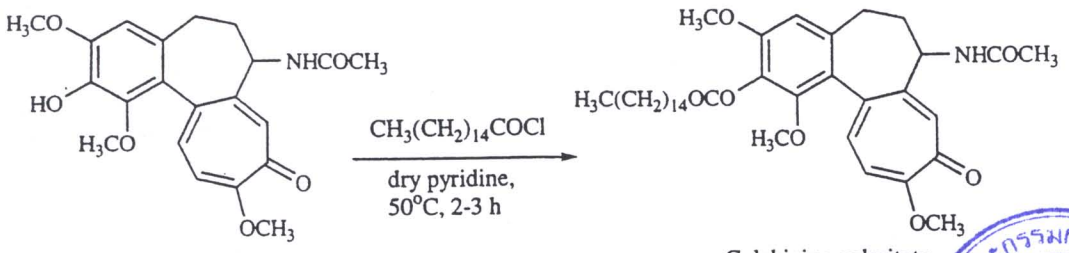
**Scheme 2**




Colchicine acetate

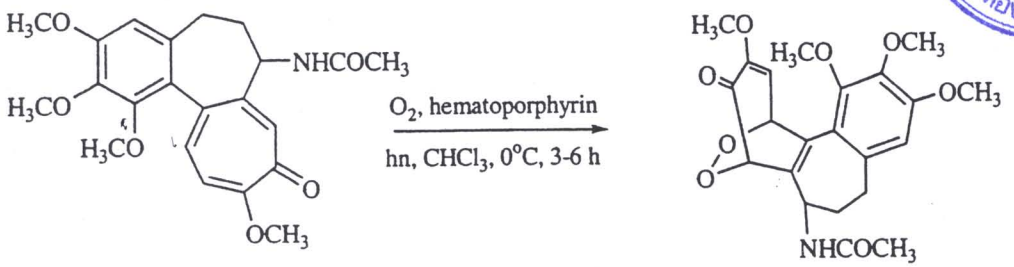
**Scheme 3**

natural products is most worthwhile despite some signs that the interest in natural product research is waning with the explosive growth of combinatorial chemistry.



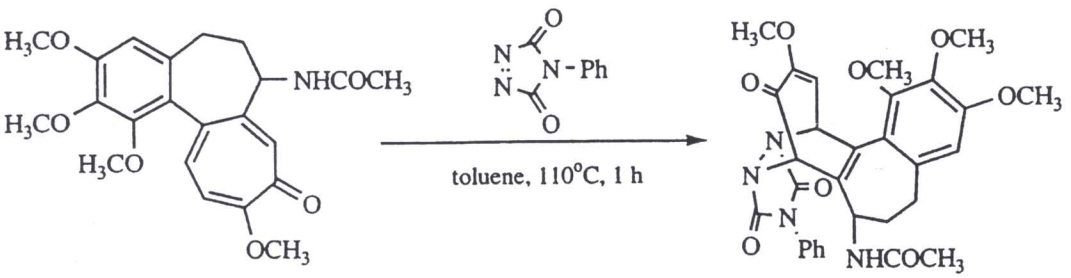

Colchicine palmitate

**Scheme 4**

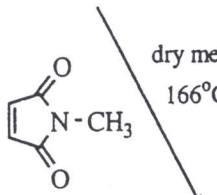


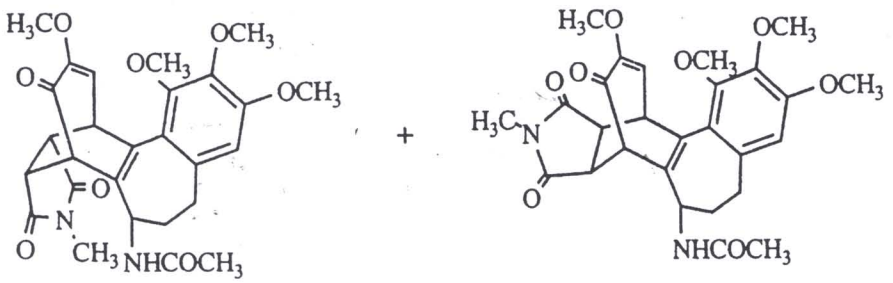

Colchicine peroxide

**Scheme 5**




Triazolinedione adduct

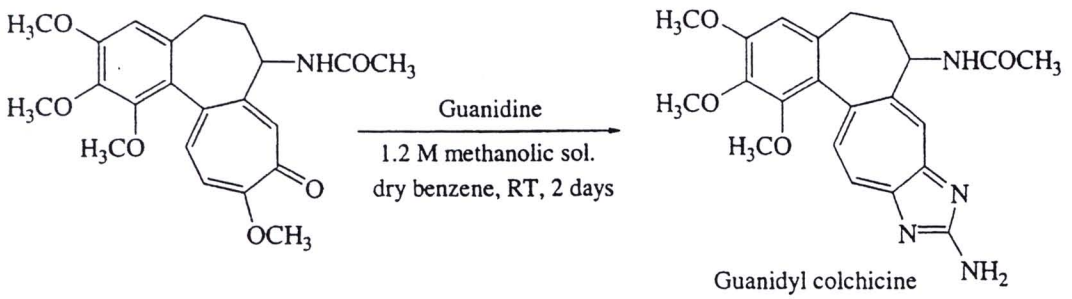


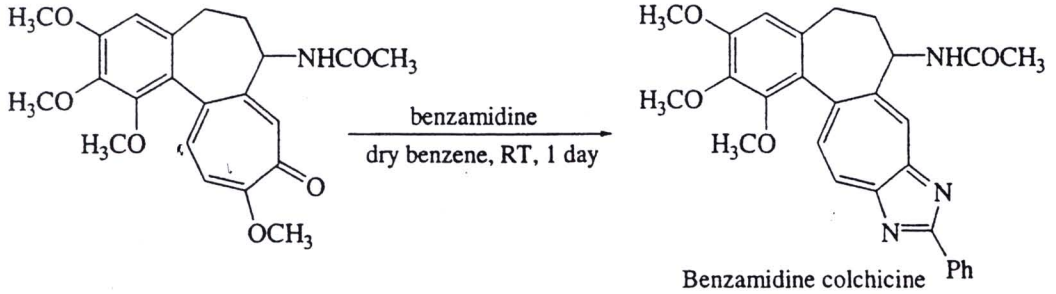

Endo-Maleimide adduct

Exo-Maleimide adduct

**Scheme 6**




**Scheme 7**




**Scheme 8**

ACKNOWLEDGEMENTS

One of the authors (SR) acknowledges the generous support of Thailand Research Fund (TRF). All the biological testing was conducted by Dr. Tengchaisri and Dr. Sirisinha.

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Accepted: August 11, 2000

VOLUME 38 • SUPPLEMENT • 2000 • ISSN 1388-0209 • CODEN BHPIFC

# PHARMACEUTICAL BIOLOGY



Guest Editors: Chulabhorn Mahidol and Kurt Hostettmann

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## IOCD – INTERNATIONAL SYMPOSIUM

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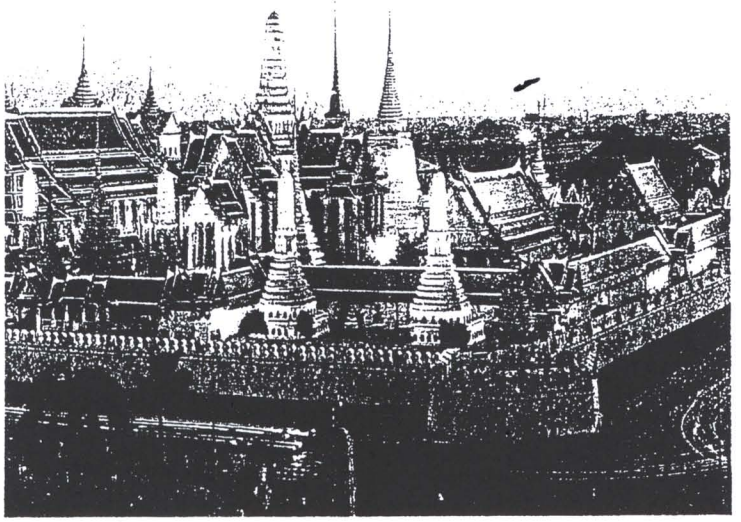
# CHEMISTRY AND PHARMACOLOGY OF ASIAN PLANTS AND VALIDATION OF PHYTOPHARMACEUTICALS



1–2 December 1999, Bangkok, Thailand

Pharmaceutical Biology
2000, Vol. 38, Supplement, pp. 63–67

1388-0209/00/380S-0063$15.00


# REINVESTIGATION OF *DERRIS RETICULATA*

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*ABSTRACT*

*The novel compound 1'''-hydroxy-2''',3'''-epoxylupinifolin (1), together with three known prenylated flavanones were identified from the stem of* Derris reticulata *during our reinvestigation of the plant. The structures were determined by spectroscopic methods including detailed study of NMR spectral data (DEPT, 2D-COSY, HMQC and HMBC) as well as by chemical derivatizations.*

## INTRODUCTION

*Derris reticulata* Benth. (Leguminosae) is a Thai medicinal plant used for the relief of thirst and as an expectorant. We have recently further investigated this plant and the novel 1'''-hydroxy-2''',3'''-epoxylupinifolin (**1**), along with three previously identified pyranoflavanones, lupinifolin, 2''',3'''-epoxylupinifolin and dereticulatin (Mahidol et al., 1997) have now been isolated and identified from the dichloromethane extract of dry powdered stems of this plant. The structural determination of compound **1** is reported here.

## MATERIALS AND METHODS

Melting points (uncorr.) were determined on a Buchi 535 apparatus. IR ($CHCl_3$ or KBr pellets) spectra were measured on a Perkin-Elmer system 2000 FT-IR infrared spectrometer. UV (MeOH) spectra were measured on a Shimadzu UV-2100S spectrophotometer. Mass spectra were determined on Finnigan Mat 90 or INCOS 50 mass spectrometers. NMR data were recorded on a Bruker AM 400 (400 MHz for $^1H$ and 100 MHz for $^{13}C$) spectrometer, using TMS as internal standard. Precoated Si gel 60 $PF_{254}$ plates (Merck, layer thickness 0.2 mm) were used for analytical TLC while preparative TLC was performed on Si gel $PF_{254}$ plates (Merck, thickness 1 mm). Spots and bands were revealed under UV light (254 nm). HPLC was carried out on a Thermal Separation Product, UV-VIS, λ 280 nm (UV6000LP for analytical measurements).

### Plant Material

Dried stems of *Derris reticulata* were purchased from a local traditional drug store in Bangkok, Thailand. Botanical identification was achieved through comparison with the specimen provided by Prof. Nijsiri Ruangrungsi. A herbarium voucher specimen is retained at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

### Extraction and Isolation

The dried stems (2 kg) of *Derris reticulata* were exhaustively extracted with $CH_2Cl_2$ at room temperature. After filtration, the extract was evaporated *in vacuo* to dryness (88 g). The $CH_2Cl_2$ extract (13 g) was separated by vacuum liquid chromatography (VLC) over silica gel using gradient elution from hexane to hexane-EtOAc (35–65) to yield 8 fractions. VLC $F_6$ (429 mg) was separated by preparative TLC using a mixture of methanol: acetone: hexane (2: 3: 64) to give compound **1** (150 mg).

1'''-Hydroxy-2''',3'''-epoxylupinifolin (**1**): yellow crystalline solid, mp 137–140 °C; IR(KBr) $\nu_{max}$ 3386 (OH), 2975, 2928, 1647, 1520, 1448, 1380, 1244, 1198, 1162, 1130, 1011, 895, 835, 747 $cm^{-1}$. UV

*Keywords: Derris reticulata*, Leguminosae, 1D-NMR, 2D-NMR, prenylated flavanone.

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(MeOH) $\lambda_{max}$ 225, 273, 296, 307, 362 nm. EIMS *m/z* 420 ($M^{+\cdot}$-$H_2O$, 28), 405 ($M^+$-$H_2O$ -$CH_3$, 29), 387 (11), 347 (13), 303 (3), 285 (60), 267 (34), 227 (34), 215 (5), 120 (10), 43 (100). FABMS (positive mode) *m/z* 439 ($[M+H]^+$, 40), 421 ($[M+H-H_2O]^+$, 100).

*Acetylation of compound* **1**: A solution of compound **1** (12.5 mg) in pyridine (5 drops) and acetic anhydride was stirred at 15 °C for 3 h. After general work-up, the product was chromatographed on preparative TLC (silica gel) using 2% MeOH in $CH_2Cl_2$ as developing solvent to give triacetate **2** (3.0 mg; 31 %) and methoxy-monoacetate **3** (5.5 mg; 50%). Triacetate **2**: colorless solid, FABMS (positive mode) 565 ($[M+H]^+$, 8). $^1H$ NMR ($CDCl_3$) δ: 5.69 (*m*, H-2), 2.95 (*dd*, *J* = 16.7, 13.2 Hz, H-3α), 2.82, 2.78 (*dd*, *J* = 16.7, 3.2 Hz, H-3β), 7.51, 7.50 (*d*, *J* = 8.6 Hz, H-2', 6'), 7.16, 7.15 (*d*, *J* = 8.6 Hz, H-3', 5'), 5.68 (*d*, *J* = 10.0 Hz, H-3"), 6.39, 6.38 (*d*, *J* = 10.0 Hz, H-4"), 1.50, 1.49, 1.49, 1.485 (*s*, $CH_3$-5", 6"), 6.09, 5.68 (*d*, *J* = 8.2 Hz, H-1'"), 3.69, 3.66 (*d*, *J* = 8.2 Hz, H-2'"), 1.28, 1.27, 1.25, 1.22 (*s*, $CH_3$-4'", 5'"), 2.43 (*s*, $OCOCH_3$-5), 2.32 (*s*, $OCOCH_3$-4'), 2.07, 2.05 (*s*, $OCOCH_3$-1'"). Methoxy-monoacetate **3**: yellow crystalline solid, mp 183–184 °C, IR ($CHCl_3$) $\nu_{max}$ 3009, 1754, 1645, 1628, 1579, 1509, 1447, 1371 $cm^{-1}$. FABMS (positive mode) 495 ($[M+H]^+$, 20). EIMS *m/z* 494 ($M^{+\cdot}$, 13), 479 (18), 423 (100), 405 (5), 389 (18), 347 (2), 305 (1), 285 (3), 261 (57), 243 (19), 227 (48), 215 (4), 120 (5), 43 (88). $^1H$ NMR ($CDCl_3$) δ: 5.42, 5.40 (*dd*, *J* = 12.7, 3.2 Hz, H-2), 3.05, 3.04 (*dd*, *J* = 17.2, 12.7 Hz, H-3α), 2.89, 2.86 (*dd*, *J* = 17.2, 3.2 Hz, H-3β), 7.46, 7.44 (*d*, *J* = 8.2 Hz, H-2', 6'), 7.15 (*d*, *J* = 8.2 Hz, H-3', 5'), 5.54 (*d*, *J* = 10.0 Hz, H-3"), 6.65, 6.64 (*d*, *J* = 10.0 Hz, H-4"), 1.462, 1.458, 1.45, 1.447 (*s*, $CH_3$-5", 6"), 4.52 (*d*, *J* = 7.2 Hz, H-1'"), 3.64, 3.62 (*d*, *J* = 7.2 Hz, H-2'"), 1.24, 1.19, 1.15, 1.13 (*s*, $CH_3$-4'", 5'"), 12.44, 12.42 (*s*, O*H*-5), 2.32 (*s*, $OCOCH_3$-4'), 3.35, 3.33 (*s*, $OCH_3$-1'").

*Transformation of compound 1 to chalcone derivatives 4 and 5 by pyridine*: Compound **1** (25 mg) was dissolved in pyridine at room temperature for several weeks. After general work-up, the product was chromatographed by preparative TLC using Si gel and 7% MeOH in $CH_2Cl_2$ as developing solvents to give chalcones **4** and **5**. $^1H$ NMR ($CDCl_3$ + acetone-$d_6$) (**4**) δ: 7.81, 7.80 (*d*, *J* = 15.5 Hz, H-α), 7.98 (*d*, *J* = 15.5 Hz, H-β), 7.52 (*d*, *J* = 8.6 Hz, H-2, 6), 6.89 (*d*, *J* = 8.6 Hz, H-3, 5), 5.47, 5.469 (*d*, *J* = 10.1 Hz, H-3"), 6.65 (*d*, *J* = 10.1 Hz, H-4"), 1.49, 1.46 (*s*, $CH_3$-5", 6"), 5.51 (*brd*, H-1'"), 4.57 (*d*, *J* = 3.7 Hz, H-2'"), 1.44,1.39 (*s*, $CH_3$-4'", 5'"), 8.88 (*s*, OH-4), 14.68 (*s*, OH-6'). $^1H$ NMR ($CDCl_3$ + acetone-$d_6$) (**5**) δ: 7.93 (*d*, *J* = 15.4 Hz, H-α), 8.18 (*d*, *J* = 15.4 Hz, H-β), 7.66 (*d*, *J* = 8.6 Hz, H-2, 6), 6.97 (*d*, *J* = 8.6 Hz, H-3, 5), 5.58 (*d*, *J* = 10.1 Hz, H-3"), 6.77 (*d*, *J* = 10.1 Hz, H-4"), 1.51 (*s*, $CH_3$-5", 6"), 6.65 (*s*, H-1'"), 1.76 (*s*, $CH_3$-4'", 5'"), 8.95 (*s*, OH-4), 14.76 (*s*, OH-6').

## RESULTS AND DISCUSSION

During reinvestigation of the dichloromethane extract of the stems of *Derris reticulata*, the new 1'"-hydroxy-2'",3'"-epoxylupinifolin (**1**) has been isolated. Compound **1** was obtained as a yellow crystalline solid, mp 137–140 °C. The molecular formula was determined as $C_{25}H_{26}O_7$ on the basis of the ion peak at *m/z* 439 $[M+H]^+$ in the positive FABMS and $^1H$ and $^{13}C$ NMR (Tables 1 and 2) spectral data. The IR (KBr) spectrum showed the presence of an hydroxyl group (3386 $cm^{-1}$). The UV absorptions at 225, 273, 296, 307, 362 nm were indicative of a pyranoflavanone chromophore (Smalberger et al., 1974). This compound showed one spot on TLC and one peak on reversed-phase HPLC (Luna $C_8$ and $C_{18}$, Hichrom $C_{18}$) with several solvent systems. However, $^1H$ and $^{13}C$ NMR spectra (Tables 1 and 2) exhibited clearly two sets of signals with partial overlapping. The $^1H$ and $^{13}C$ NMR spectra of flavanone 1 were nearly identical with those of 2'",3'"-epoxylupinifolin previously isolated from this plant (Mahidol et al., 1997). The only difference appeared in the prenyl group. Compound **1** showed the presence of an extra hydroxyl group at the benzylic position at δ 5.47, 5.46 (*d*, *J* = 7.2 Hz) in the $^1H$ NMR and at δ 66.42, 66.37 in the $^{13}C$ NMR. The structure of compound **1** was further confirmed by various other 2D-NMR techniques including HMBC and COSY experiments as shown in Figure 1. Acetylation of flavanone **1** with acetic anhydride in pyridine for 3 h was attempted. The product was chromatographed on silica gel preparative TLC to give triacetate **2** (31%) and methoxy-monoacetate **3** (51%), respectively. Examination of the $^1H$ NMR spectrum of **2** revealed the signals of H-3" and H-4" of the 2,2-dimethylpyran ring at δ 5.68 and 6.38, 6.39, respectively. The diamagnetic shift of the H-4" resonance required its placement *peri* to the 5-$OCOCH_3$ group (Arnone et al., 1967), thereby locating the pyran ring between C-6 and C-7 of ring A.

The formation of the abnormal methoxy derivative **3** under these acetylation conditions can be explained by the mechanism shown in Scheme 1. Acetylation of the hydroxyl group led to the acetoxy derivative. Loss of the acetic acid with the help of the phenolic hydroxyl

Table 1. $^1$H NMR spectroscopic assignments of compound **1**.

| Proton | Compound **1** ($CD_3OD$ + acetone-$d_6$) | Compound **1** (pyridine-$d_5$) |
|---|---|---|
| 2 | 5.43, 5.41 (*dd*, *J* = 13.2, 3.0) | 5.48, 5.41 (*dd*, 13.0, 2.9) |
| 3α | 3.18, 3.15 (*dd*, 17.2, 13.2) | 3.24, 3.237 (*dd*, 17.1, 13.0) |
| 3β | 2.74, 2.72 (*dd*, 17.2, 3.0) | 2.91, 2.89 (*dd*, 17.1, 2.9) |
| 2', 6' | 7.34, 7.33 (*d*, 8.6) | 7.51, 7.48 (*d*, 8.6) |
| 3', 5' | 6.80 (*d*, 8.6) | 7.18 (*d*, 8.6) |
| 3" | 5.61 (*d*, 10.1) | 5.58 (*d*, 10.0) |
| 4" | 6.56 (*d*, 10.1) | 6.85 (*d*, 10.0) |
| 5", 6" | 1.44, 1.434, 1.427, 1.42 (*s*) | 1.46, 1.43, 1.41, 1.40 (*s*) |
| 1"" | 4.75, 4.74 (*d*, 7.3) | 5.47, 5.46 (*d*, 7.2) |
| 2"" | 3.50, 3.49 (*d*, 7.3) | 4.16, 4.15 (*d*, 7.2) |
| 4"", 5"" | 1.14, 1.12, 1.09, 1.08 (*s*) | 1.36, 13.6,1.34, 1.32 (*s*) |
| OH-5 | – | 13.06 (*s*) |
| OH-4' | – | 11.81, 11.80 (*s*) |

Table 2. $^{13}$C NMR spectroscopic assignments of compound **1**.

| Carbon | Compound **1** (pyridine-$d_5$) |
|---|---|
| 2 | 80.09, 79.98 |
| 3 | 43.61, 43.13 |
| 4 | 197.85 |
| 4a | 103.47* |
| 5 | 158.47 |
| 6 | 103.32* |
| 7 | 160.44, 160.30# |
| 8 | 110.36 |
| 8a | 160.82, 160.57# |
| 1' | 129.51, 129.38 |
| 2', 6' | 128.72, 128.60 |
| 3', 5' | 116.56 |
| 4' | 159.65, 159.59 |
| 2" | 79.08 |
| 3" | 126.79 |
| 4" | 115.85 |
| 5", 6" | 28.49, 28.34, 28.26, 28.12 |
| 1"" | 66.42, 66.37 |
| 2"" | 68.30, 68.07 |
| 3"" | 58.09, 58.02 |
| 4"", 5"" | 25.36, 25.36, 20.04, 19.80 |

*, # Interchangeable assignments.

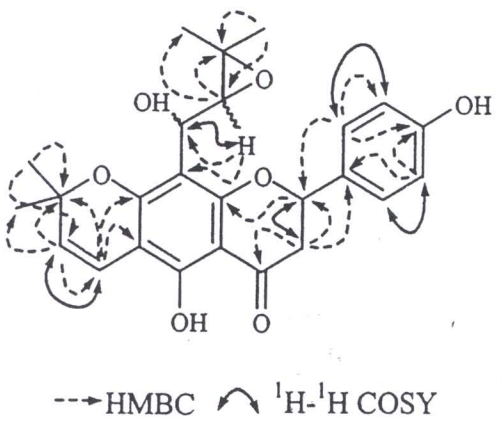
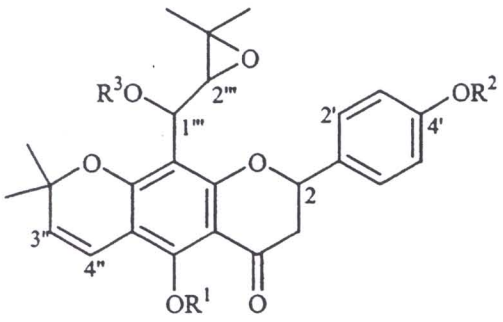


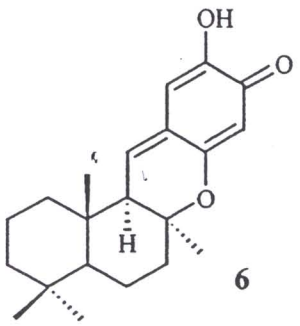

Fig. 1. Summary of important connectivities observed in compound **1** by HMBC and $^1$H-$^1$H COSY.

group led to the quinone methide intermediate which could then react with methanol to give the methoxy compound **3**. Apparently the compound was formed during preparative TLC purification when methanol was used as developing solvent. Interestingly, a methanol adduct **6** was very recently isolated as an artefact resulting from the addition of methanol on quinone methide **7** (Rourguet-Kondracki et al., 1999).

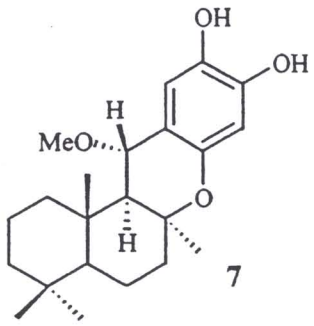
The structure of **1** was further supported by chemical transformation (Scheme 2), revealing the labile characteristic of the epoxide. When compound **1** was dissolved in pyridine at room temperature for several weeks, two chalcone derivatives **4** and **5** were obtained. From the above evidence, the structure of compound **1** was proposed as 1'"-hydroxy-2'",3'"-epoxylupinifolin.



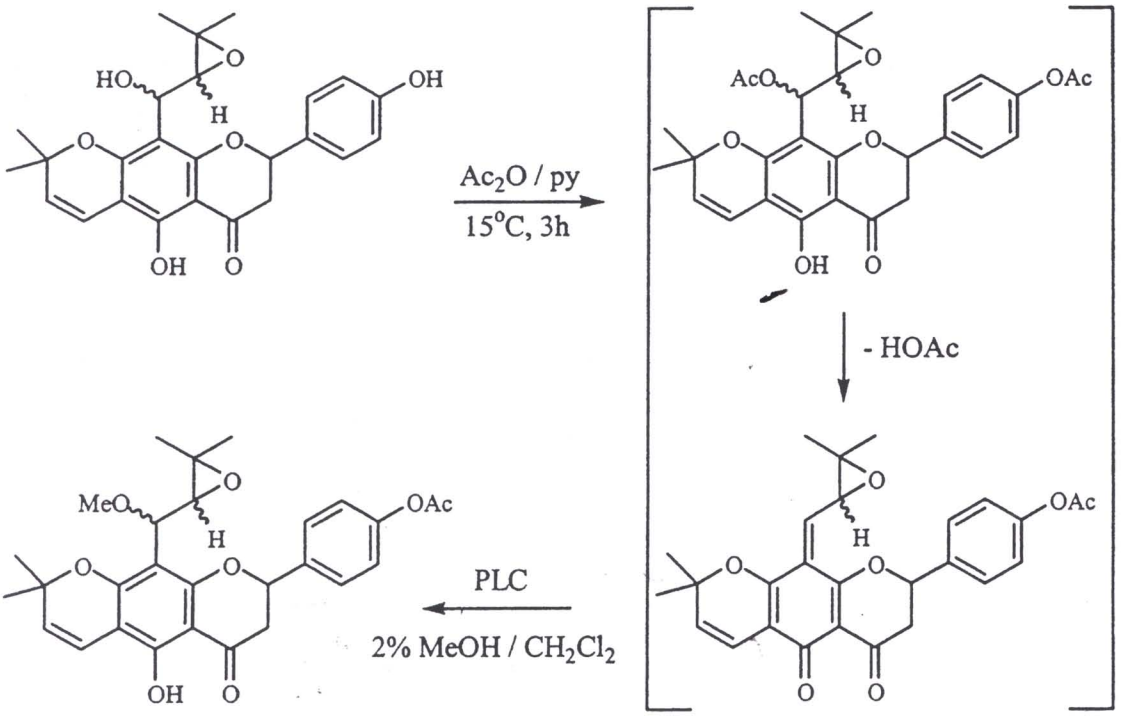
**1** $R^1 = R^2 = R^3 = H$
**2** $R^1 = R^2 = R^3 = COCH_3$
**3** $R^1 = H$, $R^2 = COCH_3$, $R^3 = CH_3$



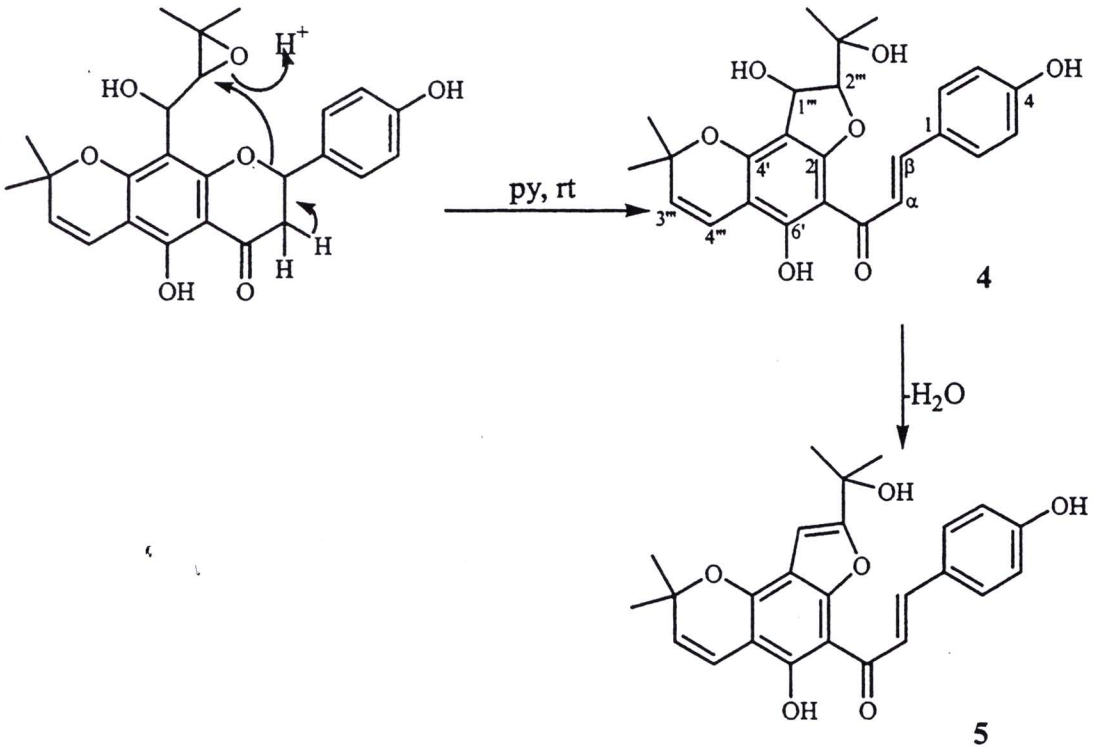
**6**



**7**




Scheme 1




Scheme 2

ACKNOWLEDGMENT

Financial support from the Thailand Research Fund (TRF) is gratefully acknowledged.

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Accepted: August 11, 2000

VOLUME 38 • SUPPLEMENT • 2000 • ISSN 1388-0209 • CODEN BHPIFC

# PHARMACEUTICAL BIOLOGY

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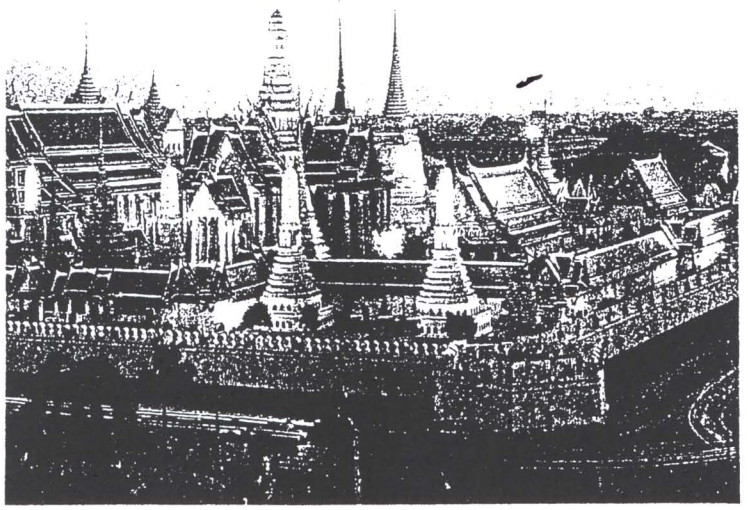

1–2 December 1999, Bangkok, Thailand

Pharmaceutical Biology
2000, Vol. 38, Supplement, pp. 58–62

1388-0209/00/380S-0058$15.00


# NMR Study of Seven Coumarins from *Mammea siamensis*

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*ABSTRACT*

*Seven known mammea coumarins, mammea A/AA cyclo D (1), mammea A/AD cyclo D (2), mammea A/AB cyclo D (3), mammea A/AC cyclo F (4), mammea A/AB cyclo F (5), mammea A/AA cyclo F (6), mammea B/AC cyclo F (7), were isolated for the first time from the hexane extract of* Mammea siamensis. *A detailed analysis of both 1D and 2D NMR spectral data of these compounds was made.*

## INTRODUCTION

*Mammea siamensis* (Miq.) T. Anders, locally known in Thailand as 'sarapee', is a member of the tribe Calophylleae, subfamily Calophylloideae, family Guttiferae, which is distributed in Thailand, Myanmar, Laos, Cambodia and Vietnam. The flowers of this plant have been used in traditional Thai medicine as a heart tonic. Previous phytochemical investigations of the flowers, twigs and leaves of *M. siamensis* have demonstrated that it contains coumarins (Thebtaranonth et al., 1981), xanthones (Poobrasert et al., 1998) and proanthocyanidine polymers (Balza et al., 1989). Continuing our investigation on the twigs of *M. siamensis*, we have isolated and identified coumarins in addition to other previously reported constituents.

*Keywords:* Coumarins, *Mammea siamensis*, Guttiferae, NMR.

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## MATERIALS AND METHODS

Melting points (uncorr.) were determined on an electrothermal melting point apparatus (Electrothermal 9100). $^1$H-, $^{13}$C- and 2D-NMR spectra were recorded on a Bruker-AM 400 MHz and a Bruker DPX-300 with TMS as the internal standard. IR spectra were obtained on a Perkin Elmer System 2000 FT-IR spectrometer. Mass spectra were determined using Finnigan INCOS 50 and MAT 90 mass spectrometers. UV spectra were measured with Milton Roy Spectronic 3000 Array and Jasco UVIDEC-650 double beam spectrophotometers. Optical rotations were measured using JASCO DIP-370 digital polarimeter in $CHCl_3$. Column chromatography was carried out by using silica gel 60 (70–230 mesh ASTM, ≤ 230 mesh, ASTM) and silica gel 60 $PF_{254}$ (Merck) for TLC. Semipreparative HPLC was performed using an ODS column (HICHROM Exsil 100–10 ODS, 20 mm i.d. × 250 mm; detector UV 280 nm).

### Plant Material

Twigs of *Mammea siamensis* (Miq.) T. Anders. were obtained from a specimen growing in a botanical garden in Saraburi province, Thailand in November 1996.

### Extraction and Isolation

The air-dried twigs (6.5 kg) of *Mammea siamensis* were ground and extracted with hexane (16 L × 7 days × 3 times). The filtrate was concentrated to give a crude extract (74 g) that was chromatographed over silica gel and eluted with solvents of increasing polarity (hexane, ethyl acetate, methanol) to give 17 fractions. Fractions **10** and **11**, which were eluted by 5–15% hexane-ethyl acetate (10 g), were further separated by flash column chromatography over silica gel using a gradient mixture of hexane and ethyl acetate as eluents

to give subfractions A-G. Subfraction C was purified by preparative TLC on silica gel using hexane:EtOAc:$Et_3N$ (30:1:3) as mobile phase to afford two isolated bands. The higher $R_f$ band was purified by preparative TLC on silica gel using hexane:diisopropylamine (9:1) as an eluent to yield compound **1** (12 mg). The lower $R_f$ band was separated and purified by RP-prep. HPLC (ODS 20 × 250 mm, flow rate 10 mL $min^{-1}$, UV detector operating at 280 nm) using MeOH-$H_2O$ (9:1) as an eluent to give compounds **2** (5 mg, $R_t$ 18.01 min) and **3** (3 mg, $R_t$ 21.20 min). Subfraction G was separated using RP-prep HPLC [ODS 20 × 250 mm, flow rate 10 mL $min^{-1}$, UV detector operating at 280 nm] with MeOH-$H_2O$ (8:3), yielding (in order) **4** (16.6 mg, $R_t$ 51.76 min), **5** (20 mg, $R_t$ 66.67 min), and **6**, **7** as a mixture (82 mg, $R_t$ 72.71 min). The mixture was further separated by preparative TLC, eluting with $CH_2Cl_2$-MeOH (99:1) to give compounds **6** (58.5 mg) and **7** (12.8 mg).

*Mammea A/AA cyclo D (1):* yellow needles, mp 149–150 °C; UV (EtOH) $\lambda_{max}$: 205 (4.57), 234 (4.62), 286 (4.73) nm; FTIR (KBr) $\nu_{max}$: 3400, 2956, 2869, 1744, 1641, 1611, 1467, 1422, 1375, 1257, 1190, 1137, 1120, 850, 770, 705 $cm^{-1}$; $^{13}C$ NMR (100 MHz, $CDCl_3$), Table 2; $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.00 (1H, *s*, H-3), 14.81 (1H, *s*, 5-OH), 7.32 (2H, *m*, H-2', 6'), 7.41 (2H, *m*, H-3', 5'), 7.41 (1H, *m*, H-4'), 2.96 (2H, *d*, *J* = 6.7 Hz, H-2"), 2.23 (1H, *m*, H-3"), 0.96 (6H, *d*, *J* = 6.7 Hz, H-4", 5"), 5.63 (1H, *d*, *J* = 10 Hz, H-3'"), 6.90 (1H, *d*, *J* = 10 Hz, H-4'"), 1.58 (6H, *s*, H-5'", 6'"); EIMS *m/z*: 404 ($M^+$, 43), 389 (100), 371 (18), 347 (16), 115 (16), 77 (17), 43 (53), 41 (39).

*Mammea A/AD cyclo D (2)*: yellow needles, mp 153-154 °C; UV (EtOH) $\lambda_{max}$: 205 (4.61), 234 (4.64), 286 (4.72) nm; FTIR (KBr) $\nu_{max}$: 3350, 2925, 2852, 1735, 1611, 1645, 1582, 1464, 1379, 1257, 1193, 1134, 1115, 854, 746, 705, 693 $cm^{-1}$; $^{13}C$ NMR (100 MHz, $CDCl_3$), Table 2; $^1H$ NMR (400 MHz, $CDCl_3$) δ 5.99 (1H, *s*, H-3), 14.64 (1H, *s*, 5-OH), 7.31 (2H, *m*, H-2', 6'), 7.40 (2H, *m*, H-3', 5'), 7.40 (1H, *m*, H-4'), 3.83 (1H, *sept*, *J* = 6.7 Hz, H-2"), 1.18 (6H, *d*, *J* = 6.7 Hz, H-3", 4"), 5.63 (1H, *d*, *J* = 10 Hz, H-3'"), 6.90 (1H, *d*, *J* = 10 Hz, H-4'"), 1.57 (6H, *s*, H-5'", 6'"); EIMS *m/z*: 390 ($M^+$, 41), 375 (82), 357 (42), 347 (100), 149 (16), 115 (15), 81 (25), 69 (52), 57 (25), 43 (54).

*Mammea A/AB cyclo D (3)*: yellow needles, mp 98-99 °C; UV (EtOH) $\lambda_{max}$: 204 (4.39), 234 (4.41), 286 (4.52) nm; FTIR (KBr) $\nu_{max}$: 3300, 2397, 2925, 2362, 1733, 1648, 1609, 1466, 1421, 1380, 1258, 1195, 1136, 1119, 911, 861, 766, 703, 574 $cm^{-1}$; $^{13}C$ NMR (100 MHz, $CDCl_3$), Table 2; $^1H$ NMR (400 MHz, $CDCl_3$) δ 5.99 (1H, *s*, H-3), 14.69 (1H, *s*, 5-OH), 7.32 (2H, *m*, H-2', 6'), 7.40 (2H, *m*, H-3', 5'), 7.40 (1H, *m*, H-4'), 3.71 (1H, *sext*, *J* = 6.7 Hz, H-2"), 1.83 (1H, *m*, H-3a"), 1.41 (1H, *m*, H-3b"), 0.90 (3H, *t*, *J* = 7.4 Hz, H-4"), 1.16 (3H, *d*, *J* = 6.7 Hz, H-5"), 5.64 (1H, *d*, *J* = 10 Hz, H-3'"), 6.90 (1H, *d*, *J* = 10 Hz, H-4'"), 1.58 (6H, *s*, H-5'", 6'"); EIMS *m/z*: 404 ($M^+$, 25), 389 (59), 371 (31), 347 (100), 115 (30), 105 (19), 77 (32), 57 (25), 43 (29), 41 (31), 29 (24); $[\alpha]_D^{27}$ –8.21° (*c* 0.195, $CHCl_3$).

*Mammea A/AC cyclo F (4)*: yellow needles, mp 176-177.5 °C; UV (EtOH) $\lambda_{max}$: 226 (4.19), 279 (4.43), 351 (4.07) nm; FTIR (KBr) $\nu_{max}$: 3462, 2925, 1707, 1610, 1438, 1384, 1235, 1197, 1146, 1035, 927, 867, 764, 704 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data ($CDCl_3$), in good agreement with published data (Morel et al., 1999); EIMS *m/z*: 408 ($M^+$, 96), 375 (23), 365 (25), 347 (24), 349 (77), 337 (26), 321 (55), 307 (100), 293 (89), 279 (28), 165 (31), 115 (38), 59 (92), 43 (69); $[\alpha]_D^{29}$ –2.45° (*c* 0.245, $CHCl_3$).

*Mammea A/AB cyclo F (5)*: yellow needles; FTIR (KBr) $\nu_{max}$: 3423, 2973, 2935, 1738, 1713, 1618, 1434, 1388, 1234, 1139, 1114, 916, 850 $cm^{-1}$; $^1H$ and $^{13}C$ NMR ($CDCl_3$), Table 1; EIMS *m/z*: 422 ($M^+$,10), 365 (19), 347 (17), 349 (3), 307 (29), 293 (97), 171 (40), 152 (23), 115 (57), 105 (36), 77 (34), 59 (100).

*Mammea A/AA cyclo F (6)*: yellow needles, mp 110–112 °C; UV (EtOH) $\lambda_{max}$: 205 (4.43), 231 (4.10), 282 (4.39), 350 (3.98) nm; FTIR (KBr) $\nu_{max}$: 3476, 3220, 2926, 1712, 1610, 1440, 1382, 1233, 1195, 1153, 1117, 1036, 926, 868, 767, 702 $cm^{-1}$; $^{13}C$ NMR (75 MHz, $CDCl_3$): see Table 2; $^1H$ NMR (300 MHz, $CDCl_3$) δ 5.86 (1H, *s*, H-3), 14.47 (1H, *s*, 5-OH), 7.25 (2H, *m*, H-2', 6'), 7.38 (2H, *m*, H-3', 5'), 7.38 (1H, *m*, H-4'), 2.84 (1H, *dd*, *J* = 15.5, 7.0 Hz, H-2a"), 3.00 (1H, *dd*, *J* = 15.5, 7.0, Hz, H-2b"), 2.21 (1H, *m*, H-3"), 0.97 (3H, *d*, *J* = 6.5 Hz, H-4"), 0.96 (3H, *d*, *J* = 6.5 Hz, H-5"), 4.91 (1H, *t*, *J* = 9.0 Hz, H-2'"), 3.26 (1H, *dd*, *J* = 15.4, 9.4 Hz, H-3a'"), 3.28 (1H, *dd*, *J* = 15.4, 8.3 Hz, H-3b'"), 2.25 (1H,*br s*, OH, H-4'"), 1.32 (3H, s, H-5'"), 1.44 (3H, *s*, H-6'"); EIMS *m/z*: 422 ($M^+$, 47), 389 (16), 365 (18), 363 (31), 349 (19), 347 (15), 335 (24), 307 (39), 293 (57), 279 (18), 165 (30), 152 (20),115 (43), 105 (27), 69 (24), 59 (100); $[\alpha]_D^{29}$ –7.32° (*c* 0.41, $CHCl_3$).

*Mammea B/AC cyclo F (7)*: yellow needles, mp 121–122.5 °C; UV (EtOH) $\lambda_{max}$: 223 (4.18), 282 (4.41), 335 (4.00) nm; FTIR (KBr) $\nu_{max}$: 3455, 3223, 2957, 2917, 2849, 1715, 1615, 1443, 1401, 1248, 1176, 1135, 1109, 1082, 907, 832 $cm^{-1}$; $^{13}C$ NMR (100 MHz, $CDCl_3$), Table 2; $^1H$ NMR (400 MHz, $CDCl_3$) δ 5.87 (1H, *s*, H-3), 14.96 (1H, *s*, 5-OH), 2.88 (1H, *dt*, *J*

Table 1. $^{13}C$ (75 MHz) and $^{1}H$ NMR (300 MHz) data for compound 5[a].

| Position | $^{13}C$[b] | $^{1}H$ (*J*, in Hz) | COLOC |
|---|---|---|---|
| 2 | 159.77 (s) | | |
| 3 | 111.94 (d) | 5.91 (*s*) | C-1', C-2, C-4a |
| 4 | 156.65 (s) | | |
| 4a | 102.48 (s) | | |
| 5 | 164.88/164.85 (s) | 14.60/14.61 (OH) | C-5, C-4a |
| 6 | 102.83/102.74 (s) | | |
| 7 | 163.85 (s) | | |
| 8 | 105.10 (s) | | |
| 8a | 155.53 (s) | | |
| 1' | 139.05 (s) | | |
| 2', 6' | 127.16 (d) | 7.28 (*m*) | C-4 |
| 3', 5' | 127.56 (d) | 7.37 (*m*) | C-1' |
| 4' | 128.19 (d) | 7.37 (*m*) | |
| 1" | 209.50/209.39 (s) | | |
| 2" | 45.76/45.56 (d) | 3.62 (*m*) | |
| 3" | 26.41/26.29 (t) | 1.44 (*m*) | |
| | | 1.80 (*m*) | |
| 4" | 11.60/11.80 (q) | 0.91 (*t*, *J* = 7.4) | |
| 5" | 16.44/16.03 (t) | 1.15/1.17 (*d*, *J* = 6.6) | C-1" |
| 2"' | 92.86/92.75 (d) | 4.91/4.92 (*t*, *J* = 9) | |
| 3"' | 26.68/26.74 (t) | 3.31/3.32 (*d*, *J* = 9) | C-8 |
| 4"' | 71.52/71.56 (s) | | |
| 5"' | 24.87/24.81 (q) | 1.32(*s*) | C-4"', C-6"' |
| 6"' | 26.07/26.19 (q) | 1.42/1.43(*s*) | C-2"' |

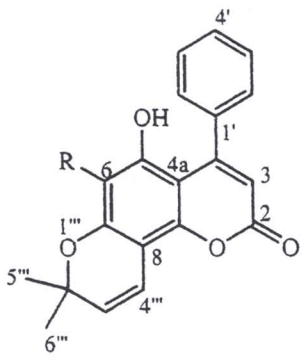
[a]Data were recorded in $CDCl_3$ at 300 MHz for $^{1}H$ and 75 MHz for $^{13}C$ NMR.
[b]Multiplicity deduced from DEPT spectroscopy.

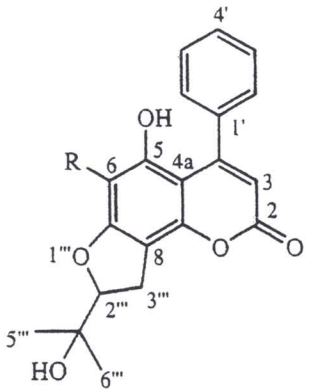
Table 2. $^{13}C$ NMR data for compounds **1**, **2**, **3**, **6**, **7** in $CDCl_3$ ($\delta_C$).

| Position | 1[a] | 2[a] | 3[a] | 6[b] | 7[a] |
|---|---|---|---|---|---|
| 2 | 159.63 | 159.67 | 159.67 | 159.83 | 160.19 |
| 3 | 112.70 | 112.67 | 112.72 | 111.68 | 109.55 |
| 4 | 156.37 | 156.41 | 156.44 | 156.70 | 159.64 |
| 4a | 102.50 | 102.27 | 102.50 | 102.19 | 103.36 |
| 5 | 164.44 | 164.54 | 164.46 | 164.30* | 164.91 |
| 6 | 107.18 | 106.38 | 107.10 | 103.27 | 103.07 |
| 7 | 158.11 | 157.79 | 157.85 | 164.21* | 163.63* |
| 8 | 101.51 | 101.44 | 101.56 | 105.15 | 105.12 |
| 8a | 154.81 | 154.77 | 154.80 | 155.40 | 155.82* |
| 1' | 139.25 | 139.27 | 139.29 | 138.90 | 38.24 |
| 2', 6' | 127.17 | 127.15 | 127.17 | 127.15 | 22.66 |
| 3', 5' | 127.61 | 127.61 | 127.62 | 127.15 | 13.94* |
| 4' | 128.21 | 128.20 | 128.21 | 128.16 | |
| 1" | 206.74 | 211.46 | 211.43 | 205.19 | 205.77 |
| 2" | 53.60 | 39.92 | 46.67 | 51.87 | 45.23 |
| 3" | 25.10 | 19.28 | 26.68 | 24.99 | 17.84 |
| 4" | 22.65 | 19.28 | 11.84 | 22.54* | 13.84* |
| 5" | 22.65 | | 16.69 | 22.65* | |
| 2"' | 79.88 | 79.87 | 79.85 | 92.92 | 92.80 |
| 3"' | 126.31 | 126.26 | 126.33 | 26.57 | 26.74 |
| 4"' | 115.56 | 115.52 | 115.59 | 71.31 | 71.51 |
| 5"' | 28.27 | 28.20 | 28.17 | 24.94 | 24.79 |
| 6"' | 28.27 | 28.20 | 28.17 | 26.24 | 26.22 |

[a]The $^{13}C$ NMR spectra were recorded at 100 MHz in $CDCl_3$. [b]75 MHz in $CDCl_3$.
*Chemical shifts within a given column are interchangeable.

= 14.4, 7.5 Hz, H-1a'), 2.93 (1H, *dt*, *J* = 14.4, 7.5 Hz, H-1b'), 1.62 (2H, *sext*, *J* = 7.5 Hz, H-2'), 1.01* (3H, *t*, *J* = 7.5 Hz, H-3'), 3.02 (1H, *dt*, *J* = 16.5, 7.4 Hz, H-2a"), 3.07 (1H, *dt*, *J* = 16.5, 7.4 Hz, H-2b"), 1.76 (2H, *sext*, *J* = 7.4 Hz, H-3"), 1.02* (3H, *t*, *J* = 7.4 Hz, H-4"), 4.88 (1H, *t*, *J* = 9 Hz, H-2"'), 3.23 (1H, *dd*, *J* = 15.5, 9.5 Hz, H-3a"'), 3.28 (1H, *dd*, *J* = 15.5, 8.6 Hz, H-3b"'), 1.29 (3H, s, H-5"'), 1.42 (3H, *s*, H-6"'); EIMS





**1** : R = $COCH_2CH(CH_3)_2$

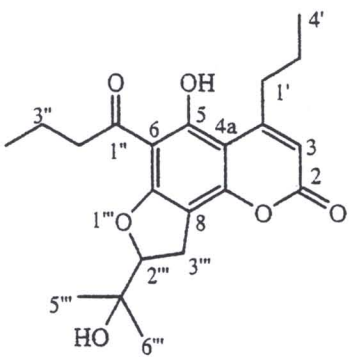
**2** : R = $COCH(CH_3)_2$

**3** : R = $COCH(CH_3)CH_2CH_3$

**4** : R = $COCH_2CH_2CH_3$

**5** : R = $COCH(CH_3)CH_2CH_3$

**6** : R = $COCH_2CH(CH_3)_2$



**7**

Fig. 1. Structures of compounds **1–7**.

*m/z*: 374($M^+$,100), 359 (7), 346 (9), 341 (25), 331 (11), 315 (20), 313 (14), 303 (59), 287 (14), 273 (28), 259 (23), 245 (13), 59 (34); $[\alpha]_D^{28}$ +1.90° (*c* 0.42, $CHCl_3$). *These values may be interchanged.

## RESULTS AND DISCUSSION

Dried twigs of *M. siamensis* were extracted with hexane to give a crude extract that was separated by column chromatography, preparative TLC, semipreparative HPLC on a reverse-phase column to afford seven coumarins (**1–7**) (Fig. 1).

Compounds **1–7**, which have never been previously isolated from *M. siamensis*, were identified as mammea A/AA cyclo D (**1**) (Chakraborty et al., 1969; Crombie et al., 1967), mammea A/AD cyclo D (**2**) (Chakraborty et al., 1969), mammea A/AB cyclo D (**3**) (Carpenter et al., 1971), mammea A/AC cyclo F (**4**) (Morel et al., 1999), mammea A/AB cyclo F (**5**) (Crombie et al., 1972), mammea A/AA cyclo F (**6**) (Crombie et al., 1972), and mammea B/AC cyclo F (**7**) (Crombie et al., 1987). The detailed assignments of the NMR spectral data of all of these coumarins, except for compound **4**, have not been reported. Using $^1H$ and $^{13}C$ NMR spectra with the aid of $^1H$-$^1H$ COSY, $^1H$-$^{13}C$ COSY, $^1H$-$^{13}C$ COLOC and by comparison with existing data from the literature, we have confirmed the structures of these coumarins. Among them, mammea A/AB cyclo F (**5**), was isolated as an inseparable mixture. Both $^1H$ and $^{13}C$ NMR (Table 1) showed two sets of resonances of nearly equal intensity and virtually identical chemical

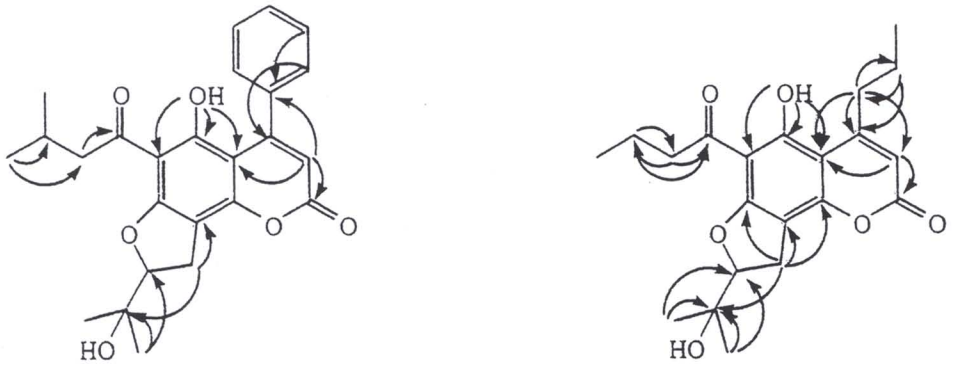


Fig. 2. COLOC correlations of compounds **6** and **7**.

shifts due to the presence of a diastereomeric mixture. Furthermore, the position of the chelated OH group was confirmed by COLOC experiment. In the COLOC spectrum, the chelated OH showed cross peaks with the carbons C-4a and C-5, demonstrating that the coumarin nucleus was substituted by the acyl chain at C-6. The COLOC correlations of compounds **6** and **7** are illustrated in Figure 2. Interpretations of their $^{13}C$ NMR spectra, except for mammea A/AC cyclo F (**4**), are given in Table 2, and the assignments were based on the analysis of HETCOR, DEPT and COLOC spectra.

ACKNOWLEDGEMENT

Financial support from the Thailand Research Fund (TRF) is gratefully acknowledged.

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Accepted: August 11, 2000

Pharmaceutical Biology
2000, Vol. 38, Supplement, pp. 55–57

1388-0209/00/380S-0055$15.00


# CHEMICAL INVESTIGATION OF *MAMMEA SIAMENSIS*

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*ABSTRACT*

*A new 4-alkylcoumarin, mammea B/AC cyclo D (1), together with a 4-phenylcoumarin, mammea A/AC cyclo D (2), were isolated from the hexane extract of the dried flower of* Mammea siamensis. *Their structures were determined on the basis of spectroscopic evidence.*

## INTRODUCTION

*Mammea siamensis* T. Anders. belongs to the Guttiferae and the tribe Calophylleae of the subfamily Calophylloideae. The plant was previously known as *Ochrocarpus siamensis* and is widely distributed in Thailand, Myanmar, Laos, Cambodia and Vietnam. Plants of this subfamily are known to be rich sources of xanthones (Bandaranayake et al.,1980), triterpenes (Bandaranayake et al., 1980), flavonoids (Tosa et al., 1997) and coumarins (Games et al., 1972). The Thai name for the plant is 'sarapee' and its flower is a well known ingredient in traditional Thai medicine, especially used as a cardiac stimulant.

## MATERIALS AND METHODS

Melting points were determined on an electrothermal melting point apparatus (Electrothermal 9100) and reported without correction. $^1$H NMR spectra were recorded on a Bruker AM 400 in deuterochloroform using tetramethylsilane as an internal standard. Infrared spectra (IR) were obtained on Perkin Elmer System 2000 FT-IR and JASCO A-302 spectrometers. Mass spectra were determined using GC-MS Finnigan INCOS 50 and GC-MS MAT 90 instruments. UV spectra were measured with a Shimadzu UV-VIS 2001S spectrophotometer. Column chromatography was carried out using silica gel 60 (0.063–0.200 mm) and silica gel 60 (particle size less than 0.063 mm). Thin-layer chromatography (TLC) and preparative thin-layer chromatography (preparative TLC) were carried out on silica gel 60 $PF_{254}$ (cat. No. 7747E, Merck).

### Plant Material

Dried flowers of *Mammea siamensis* T. Anders. were purchased from a local traditional drug store in Bangkok, Thailand.

### Extraction and Isolation

The air-dried flowers (8.5 kg) of *Mammea siamensis* were ground and extracted with hexane (12 L × 2 days × 7 times) at room temperature for 14 days, followed by filtration. The filtrates were combined and evaporated under reduced pressure to give a dark brown gum (428 g). A portion of this gum (300 g) was first subjected to coarse separation by column chromatography over silica gel using gradient elution of ethyl acetate in hexane with increasing polarity of ethyl acetate. Successive fractions were combined on the basis of their behavior on TLC and GC-MS and evaporated to give six fractions. Fraction **5** (62.5 mg) was purified further by preparative TLC on silica gel using 7% ethyl acetate-hexane to give a new mammea B/AC cyclo D, **1** (20 mg), together with the known mammea A/AC cyclo D or 6-butyryl-5-hydroxy-4-phenylseselin, **2** (27 mg).

*Keywords: Mammea siamensis*, Guttiferae, 4-substituted coumarins.

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Table 1. NMR spectral data of mammea B/AC cyclo D, **1**.

| $^1H$ and $^{13}C$ No. (group)* | $^{13}C$ δ ppm | HETCOR correlates with $^1H$-No. δ ppm | Multiplicity *J* in Hz | COSY correlates with | COLOC correlates with |
|---|---|---|---|---|---|
| 2(C) | 160.06 | – | – | – | – |
| 3(CH) | 110.32 | H-3, 5.93 | *s* | – | C-4a |
| 4(C) | 159.49 | – | – | – | – |
| 4a(C) | 103.22 | – | – | – | – |
| 5(C) | 165.11 | OH-5, 15.34 | *s* | – | C-4a, C-6 |
| 6(C) | 106.99 | – | – | – | – |
| 7(C) | 157.67 | – | – | – | – |
| 8(C) | 101.50 | – | – | – | – |
| 8a(C) | 155.09 | – | – | – | – |
| 1'($CH_2$) | 38.45 | H-1', 2.92 | *dd*, 7.6, 7.5 | H-2' | C-3, C-4, C-4a |
| 2'($CH_2$) | 22.74 | H-2', 1.63 | br sextet | H-1', H-3' | – |
| 3'($CH_3$) | 13.98 | H-3', 0.99 | *t*, 7.3 | H-2' | – |
| 1"(C) | 207.47 | – | – | – | – |
| 2"($CH_2$) | 46.88 | H-2", 3.06 | *t*, 7.4 | H-3" | C-1" |
| 3"($CH_2$) | 18.28 | H-3", 1.72 | sextet, 7.4 | H-2", H-4" | – |
| 4"($CH_3$) | 13.90 | H-4", 1.00 | *t*, 7.4 | H-3" | – |
| 2'''(C) | 79.65 | – | – | – | – |
| 3'''(CH) | 126.20 | H-3''', 5.57 | *d*, 10.0 | H-4''' | C-2''', C-8 |
| 4'''(CH) | 115.67 | H-4''', 6.81 | *d*, 10.0 | H-3''' | – |
| 5'''($CH_3$) | 28.69 | H-5''', 1.52 | *s* | – | C-2''', C-3''' |
| 6'''($CH_3$) | 29.69 | H-6''', 1.52 | *s* | – | C-2''', C-3''' |

*Determined from DEPT spectra

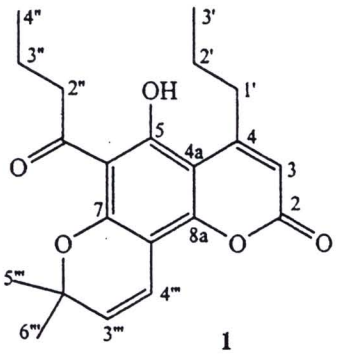
Mammea B/AC cyclo D (**1**) was recrystallized from dichloromethane-hexane to give yellow needles, m.p. 105–106 °C. IR $\nu_{max}CHCl_3$: 3028, 2928, 2855, 1731, 1643, 1615, 1583, 1465, 1426, 1384, 1267, 1189, 1152, 1115 $cm^{-1}$. UV (MeOH) $\lambda_{max}$ nm (log ε): 227 (4.245), 285 (4.446), 335 (3.732), 375 (3.586). High resolution FABMS (positive mode) obs. 357.1697 calcd. for $C_{21}H_{24}O_5$+H 357.1701. EIMS *m/z* (rel. int.): 356 ($M^{+\cdot}$, 32), 341 ([M-$CH_3$]$^+$, 100), 269 ([341-$OCCH_2CH_2CH_3$]$^+$, 2), 227 ([269-$CH_2CH_2CH_3$]$^+$).

Mammea A/AC cyclo D or 6-butyryl-5-hydroxy-4-phenylseselin (**2**) was recrystallized from ethyl acetate-hexane to give yellow crystals, m.p. 133–134.7 °C. (138–139 °C; Thebtaranonth et al., 1981). IR $\nu_{max}$ $CHCl_3$: 3500, 2969, 1725, 1642, 1610, 1582, 1465, 1380, 1140, 1118, 860, 700 $cm^{-1}$ UV (EtOH) $\lambda_{max}$ nm (log ε): 234 (4.75), 285 (4.66). FABMS *m/z* (rel. int.) 391 ([M+H]$^+$, 100). EIMS *m/z* (rel. int): 390 ($M^{+\cdot}$, 30), 375 ([M-$CH_3$]$^+$, 100), 357 (20), 347 (14). $^1H$ NMR (400 MHz, $CDCl_3$) δ: 5.96 (*s*, H-3), 14.73 (*s*, OH-5), 7.29 (*m*, H-2', H-6'), 7.38 (*m*, H-3', H-4', H-5'), 3.02 (*t*, *J* = 7.3 Hz, H-2"), 1.67 (sextet, *J* = 7.3 Hz, H-3"), 0.97 (*t*, *J* = 7.3 Hz, H-4"), 5.60 (*d*, *J* = 10.0 Hz, H-3'''), 6.86 (*d*, *J* = 10.0 Hz, H-4'''), 1.55 (*s*, H-5''', H-6'''). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 159.63 (C-2), 112.66 (C-3), 156.38 (C-4), 102.15 (C-4a), 164.37 (C-5), 106.97 (C-6), 158.20 (C-7), 101.48 (C-8), 154.79 (C-8a), 139.21 (C-1'), 127.15 (C-2', C-6'), 127.60 (C-3', C-5'), 128.21 (C-4'), 207.20 (C-1"), 46.79 (C-2"), 18.19 (C-3"), 13.07 (C-4"), 79.84 (C-2'''), 126.31 (C-3'''), 115.51 (C-4'''), 28.26 (C-5''', C-6''').

## RESULTS AND DISCUSSION

Dried flowers of *Mammea siamensis* were extracted with hexane and chromatographic separation on silica gel led to the isolation of the new mammea B/AC cyclo D (**1**) and known mammea A/AC cyclo D (**2**).

Mammea B/AC cyclo D, **1**, was obtained as yellow needles, m.p. 105–106 °C. The IR spectrum of **1** showed two carbonyl group absorptions at 1731 (unsaturated δ-lactone) $cm^{-1}$ and at 1643 (aryl ketone) $cm^{-1}$. The UV spectrum of compound **1** exhibited the absorption maxima at 227, 285, 335, 375 nm. The molecular formula of **1** was determined to be $C_{21}H_{24}O_5$ from ($M^{+\cdot}$ + H) at *m/z* 357.1701 in the high resolution FABMS (positive mode). Its EI mass spectrum displayed the molecular ion peak at *m/z* 356 ($M^{+\cdot}$) and the base peak for ($M^+$ - $CH_3$) at *m/z* 341 (100%). Mammea **1** has been detected by GC-MS in *Mammea america*, and tentatively assigned the structure without $^1H$ and $^{13}C$ spectral data (Games et al., 1972). We have carried out the detailed assignment of the chemical shifts of protons and carbons in **1** using COSY, HETCOR and COLOC experiments as shown in Table 1. The $^1H$ NMR spectrum of compound **1** showed the presence of a 2,2-dimethylchromene ring system [δ

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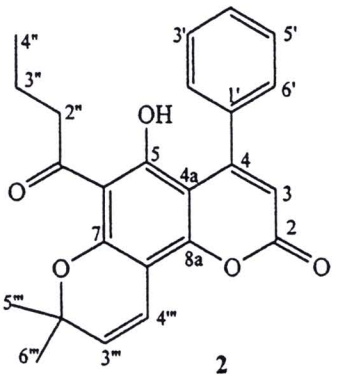


2

5.57 (H-3'''), 6.81 (H-4'''), 2H, AB system $J_{AB}$ = 10.0 Hz; 1.52 (H-5''', H-6''', 6H, *s*], hydroxyl group at C-5 (δ 15.34, 1H, *s*), propyl group [δ 2.92 (H-1'), 2H, *dd*, *J* = 7.6, 7.5 Hz; 1.63 (H-2'), 2H, br sextet; and 0.99 (H-3'), 3H, *t*, *J* = 7.3 Hz]. Substitution at C-4 of the coumarin was apparent from the C-3 proton singlet at δ 5.93, and the nature of the substituent at C-6 was deduced as a butyryl chain from the signals at δ 1.0 (H-4", 3H, *dd*, *J* = 7.4 Hz); 1.72 (H-3", 2H, *sextet*, *J* = 7.4 Hz) and 3.06 (H-2", 2H, *t*, *J* = 7.4 Hz). The $^{13}C$ proton decoupling NMR spectrum of **1** (Table 1) showed 21 signals. Analysis of the DEPT spectra of this compound suggested the presence of ten quaternary carbon atoms at δ 160.06 (C-2), 159.49 (C-4), 103.22 (C-4a), 165.11 (C-5), 106.99 (C-6), 157.67 (C-7), 101.50 (C-8), 155.09 (C-8a), 79.65 (C-2'''), 207.47 (C=O of butyryl group), three olefinic methine carbon atoms at δ 110.32 (C-3), 126.20 (C-3'''), 115.67 (C-4'''), four methyl carbon atoms at δ 13.98 (Me-3'), 13.90 (Me-4"), 28.69 (Me-5'''), 29.69 (Me-6'''). All the connectivities were supported by the COLOC spectrum (Table 1). The proton signal of *OH*-5 at δ 15.34 ppm showed cross peaks with the carbon signals of C-4a (δ 103.22) and C-6 (δ 106.99), the proton signal of H-3 at δ 5.93 ppm showed a cross peak with the carbon signal C-4a (δ 103.22) and the proton signal of H-1' at δ 2.92 showed cross peaks with the carbon signals of C-3 (δ 110.32), C-4 (δ 159.49) and C-4a (δ 103.22).

The known mammea A/AC cycloD **2** or 6-butyryl-5-hydroxy-4-phenylseselin was identified by comparing its physical and spectroscopic data with literature values (Morel et al., 1999; Thebtaranonth et al., 1981).

ACKNOWLEDGEMENT

Financial support from the Thailand Research Fund (TRF) is gratefully acknowledged.

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Accepted: August 11, 2000

VOLUME 38 • SUPPLEMENT • 2000 • ISSN 1388-0209 • CODEN BHPIFC

# PHARMACEUTICAL BIOLOGY

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## Proceedings of the
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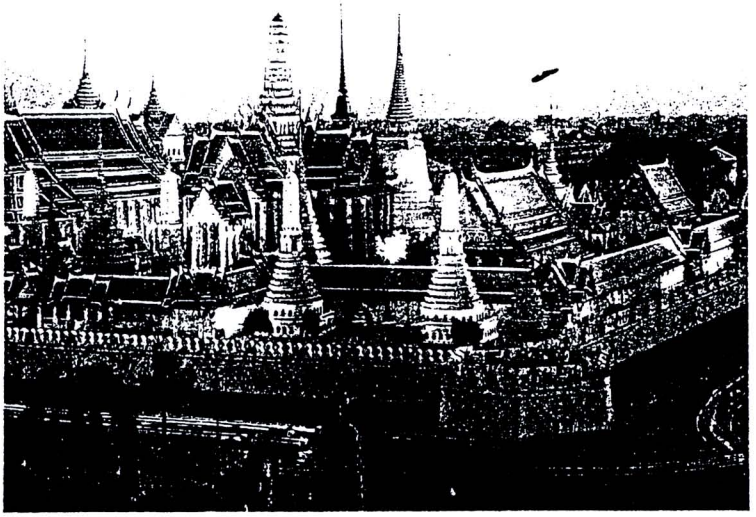
# CHEMISTRY AND PHARMACOLOGY OF ASIAN PLANTS AND VALIDATION OF PHYTOPHARMACEUTICALS



1–2 December 1999, Bangkok, Thailand

Pergamon

Tetrahedron Letters 41 (2000) 8007–8010

TETRAHEDRON LETTERS

# A novel synthesis of isoindolobenzazepine alkaloids: application to the synthesis of lennoxamine

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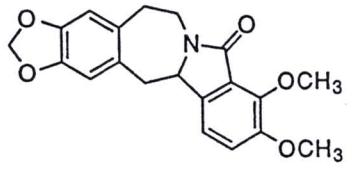
Received 5 June 2000; revised 9 August 2000; accepted 16 August 2000

**Abstract**

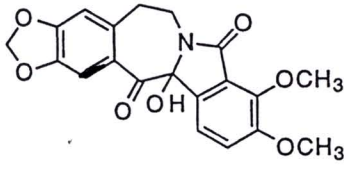
A novel, highly efficient synthesis of lennoxamine, a representative of isoindolobenzazepine alkaloid, is described. 

*Keywords:* lennoxamine; isoindolobenzazepine.

Lennoxamine **1**[1] and chilenine **2**[2] are two representatives of a class of isoindolobenzazepine alkaloids.[3] Both compounds were first found in the plants of the Chilean *Berberis* species, lennoxamine was isolated from *Berberis darwinii* Hook while chilenine was found in *Berberis empetrifolia* Lam. Due to their unique structural features, the isoindolobenzazepine alkaloids[4] in general and chilenine[5,6] and lennoxamine[6,7] in particular, have captured the interest of many groups of synthetic chemists.




**1**




**2**

We have previously reported[4b] the synthesis of the isoindolobenzazepine (aporhoeadane) skeleton by using the route suggested by retrosynthetic analysis as shown in route A. However, attempts to apply this route to the synthesis of more complex oxygenated natural products

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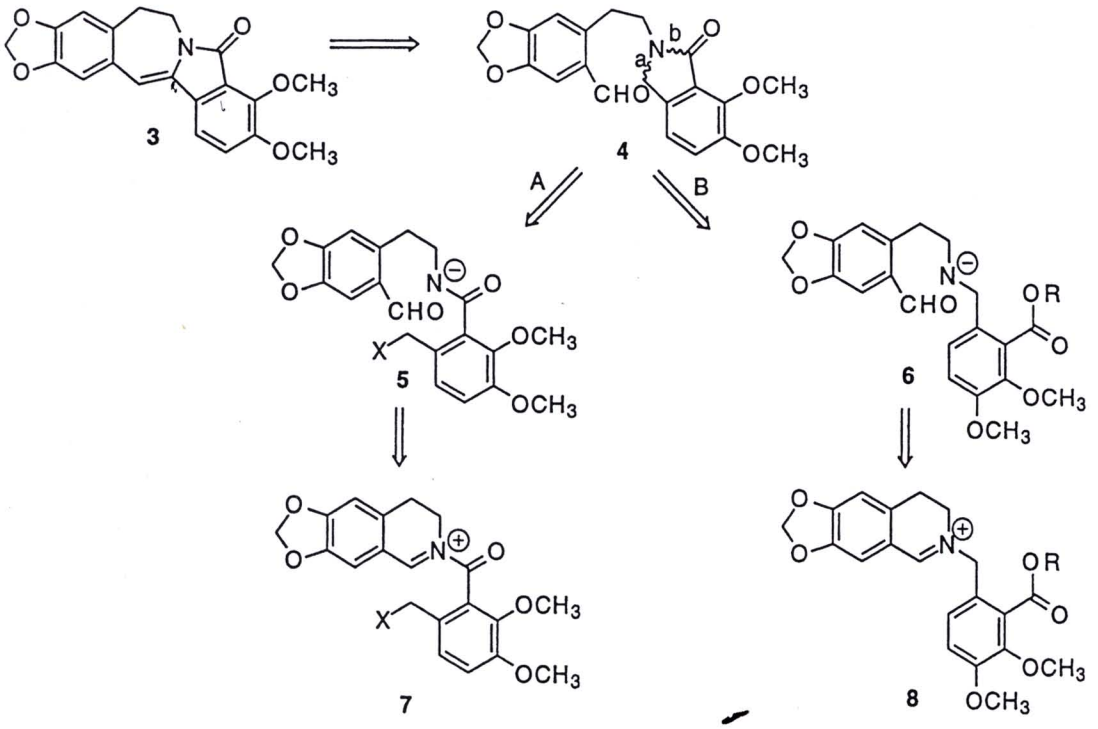
PII: S0040-4039(00)01390-3

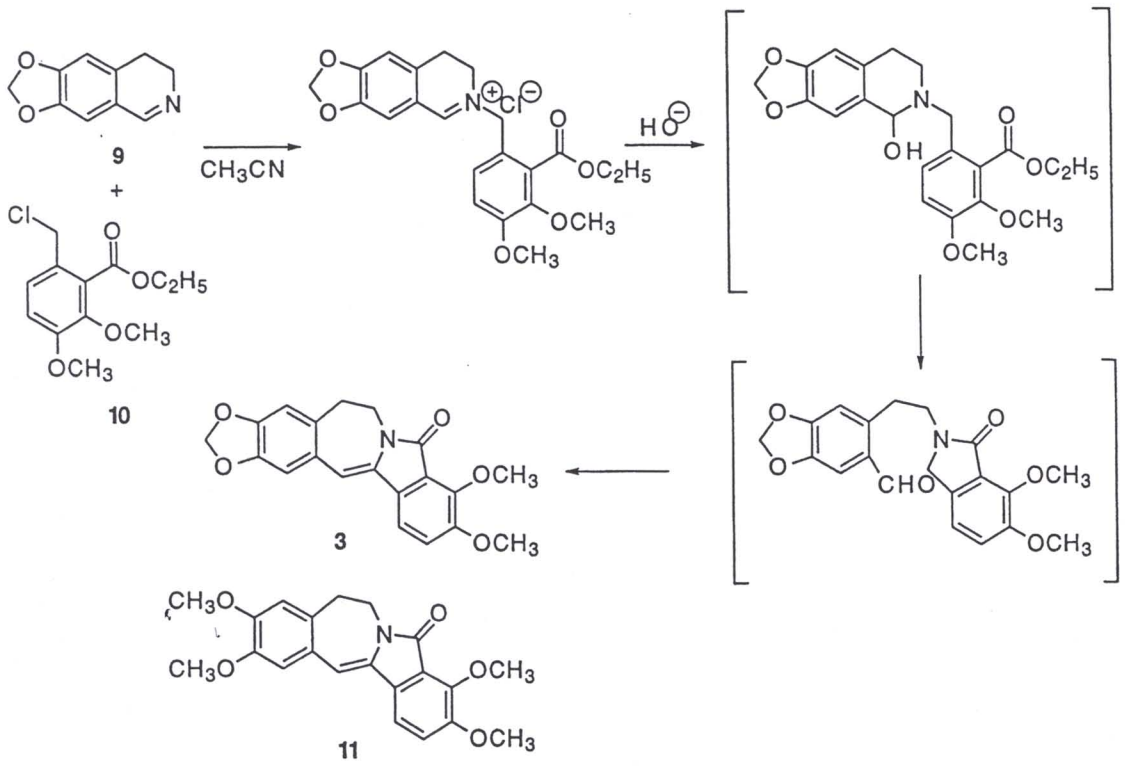
required the synthesis of not readily accessible halomethylbenzoyl chlorides. We have also found that when 6,7-methylenedioxy-3,4-dihydroisoquinoline was used as one of the components in the reaction with chloromethylbenzoyl chloride, the required lactam was obtained in disappointing yield. We have now solved the above drawbacks and successfully applied a new approach to the synthesis of lennoxamine.

Our retrosynthetic analysis is shown in Scheme 1. Breaking of the carbon–carbon double bond of the benzazepine skeleton in dehydrobenzazepine **3** leads to the lactam intermediate **4**. In our previous analysis, this lactam intermediate would be formed by the intramolecular alkylation of the amide intermediate **5** in route A. However, the alternative breaking of bond b in route B leads to the amino aldehyde intermediate **6**, i.e. the formation of the lactam would involve the reaction of an amine and an ester. It was expected that formation of the amide bond via route B would be more facile than the alkylation in route A due to the basicity of the nitrogen which is an amine in route B and an amide moiety in route A. Intermediates **5** and **6** could be obtained from acyliminium salt **7** or benzyliminium salt **8**, respectively.




Scheme 1.

In order to test the validity of the above mentioned idea, lennoxamine was synthesized as shown in Scheme 2. Alkylation of the 6,7-methylenedioxy-3,4-dihydroisoquinoline **9** with the readily available ethyl 6-chloromethyl-2,3-dimethoxybenzoate[8] **10** in acetonitrile gave the required iminium chloride. The iminium chloride so obtained was not isolated but was treated with potassium hydroxide or sodium hydroxide. It was indeed gratifying to find that the iminium salt was smoothly converted directly to dehydrolennoxamine **3** in 73% yield by potassium hydroxide and in 58% yield by sodium hydroxide. The presumed pseudobase and the aldehydic lactam intermediates were not isolated in the reaction.



Scheme 2.

By replacement of 6,7-methylenedioxy-3,4-dihydroisoquinoline with 6,7-dimethoxy-3,4-dihydroisoquinoline in the above reaction, the analogue of the dehydrolennoxamine **11** was successfully synthesized in 75% overall yield. Dehydrolennoxamine and its analogue were hydrogenated with 10% palladium on carbon in ethyl acetate to give lennoxamine and its analogue[9] in 76 and 80% yields, respectively.

## Acknowledgements

We are grateful for the generous support of the Thailand Research Fund.

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9. All compounds have been fully characterized. Dehydrolennoxamine **3**: m.p. 208–209°C; IR (nujol) 1690, 1642 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 3.0 (t, 2H, $J=4.9$ Hz), 3.95 (s, 3H), 4.08 (s, 3H), 6.00 (s, 2H), 6.35 (s, 1H), 6.68 (s, 1H), 6.80 (s, 1H), 7.12 (d, 1H, $J=8$ Hz), 7.48 (d, 1H, $J=8$ Hz). $^{13}C$ NMR (100 MHz) $\delta$ 35.39, 41.72, 56.62, 62.38, 101.19, 104.87, 110.06, 110.18, 114.28, 116.24, 120.22, 127.71, 130.99, 133.16, 133.83, 146.49, 146.72, 146.82, 152.82, 163.58. EIMS 351(100), 336(20), 322(10), 175(7). Anal. calcd for $C_{20}H_{17}NO_5$: C, 68.37; H, 4.84; N, 3.98. Found: C, 68.50; H, 4.80; N, 3.95. Dehydrolennoxamine analogue **11**: m.p. 185–189°C; IR (nujol) 1690, 1638 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 3.03 (t, 2H, $J=4.6$ Hz), 3.87 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 4.08 (s, 3H), 6.35 (s, 1H), 6.65 (s, 1H), 6.81 (s, 1H), 7.09 (d, 1H, $J=8.4$Hz), 7.39 (d, 1H, $J=8.4$ Hz). $^{13}C$ NMR (100 MHz) $\delta$ 35.18, 41.62, 55.87, 56.62, 62.35, 104.97, 113.02, 113.70, 114.21, 116.27, 120.25, 126.44, 131.05, 132.50, 133.17, 146.70, 147.44, 148.00, 152.74, 163.64. EIMS 368(31), 367(100), 353(16), 352(65), 308(9), 184(8). Anal. calcd for $C_{21}H_{21}NO_5$: C, 68.66; H, 5.72; N, 3.81. Found: C, 68.60; H, 5.72; N, 3.83. Lennoxamine **1**: m.p. 226–227°C; Lit[1], 225°C; Lit[7a], 228–229°C; IR (nujol) 1688 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 2.83 (m, 2H), 2.93 (m, 2H), 3.10 (dd, 1H, $J=14.7$, 1.7 Hz), 3.92 (s, 3H), 4.10 (s, 3H), 4.30 (dd, 1H, $J=10.5$, 1.3 Hz), 4.74 (m, 1H), 5.95 (d, 1H, $J=1.47$), 5.96 (d, 1H, $J=1.47$), 6.71 (s, 1H), 6.78 (s, 1H), 7.13 (d, 1H, $J=8.2$ Hz), 7.18 (dd, 1H, $J=8.8$, 0.4 Hz). $^{13}C$ NMR (100 MHz) $\delta$ 35.93, 41.11 42.71, 56.75, 60.17, 62.54, 101.04, 110.34, 110.34, 116.26, 117.05, 124.18, 130.94, 134.85, 138.22, 146.08, 146.35, 147.26, 152.63, 165.18. EIMS 354(29), 353(96), 352(20), 338(51), 335(27), 162(69), 161(100), 160(29), 149(27), 131(47). Anal. calcd for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.38; N, 3.96. Found: C, 67.83; H, 5.42; N, 3.97. Lennoxamine analogue: m.p. 213–214°C; IR (nujol) 1680 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 2.84 (m, 2H), 2.95 (m, 2H), 3.13 (dd, 1H, $J=14.5$, 1.6 Hz), 3.90 (s, 3H), 3.92 (s, 3H), 3.915 (s, 3H), 4.11 (s, 3H), 4.32 (dd, 1H, $J=10.7$, 0.5 Hz), 4.76 (m, 1H), 6.74 (s, 1H), 6.82 (s, 1H), 7.14 (d, 1H, $J=8.2$ Hz), 7.21 (dd, 1H, $J=8.2$, 0.5 Hz). $^{13}C$ NMR (100 MHz) $\delta$ 35.80 41.19, 42.64, 55.95, 56.06, 56.66, 62.44, 113.46, 113.68, 116.15, 117.02, 124.10, 129.72, 133.67, 138.28, 146.70, 147.14, 147.14, 147.46, 152.53, 165.11. EIMS 370(20), 369(85), 353(44), 351(29), 177(100). Anal. calcd for $C_{21}H_{23}NO_5$: C, 68.29; H, 6.23; N, 3.79. Found: C, 68.38; H, 6.32; N, 3.77.

PERGAMON

Phytochemistry 56 (2001) 811–814

PHYTOCHEMISTRY

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# Labdane and pimarane diterpenes from *Croton joufra*☆

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Received 8 March 2000; received in revised form 25 July 2000

**Abstract**

From the chloroform extract of the leaves of *Croton joufra*, the diterpenes 2α,3α-dihydroxy-labda-8(17),12(13),14(15)-triene and 3β-hydroxy-19-*O*-acetyl-pimara-8(9),15-dien-7-one, were isolated. Their structures were establised by spectroscopic methods. One of the compounds showed weak lethality in the brine shrimp assay. 

*Keywords:* *Croton joufra*; Euphorbiaceae; Labdane; 2,3-Dihydroxy-labda-8(17),12(13),14(15)-triene; Pimarane; 3-Hydroxy-19-*O*-acetyl-pimara-8(9),15-dien-7-one; Brine shrimp lethality test

## 1. Introduction

*Croton joufra* Roxb. (Euphorbiaceae) is a medium size shrub commonly named in Thai as "Plau noi", the same name as that used for *Croton sublyratus*.

A decoction of the leaves and bark have been used as an antidysentery and peptic promotor and a decoction of the flowers as an anthelmintic (Phupattanapong and Wongprasart, 1987). The heartwood and stems have been used as a blood tonic and antipyretic (Mokkhasmit et al., 1971). In previous studies, two furanoditerpenes, plaunol A and plaunol C, were reported from the methanolic extract of the stems by TLC (Ogiso et al., 1981). Subsequently, Roengsumran et al. (1982) isolated another furanoditerpene swassin from the stems of this plant. We report here on the isolation and structural characterization of a labdane and a pimarane diterpene from the leaves of this plant.

## 2. Results and discussion

In our search for bioactive compounds from plants of the Euphorbiaceae, we used the *Artemia salina* (brine shrimp) toxicity test as an in-house bioassay method (Meyer et al., 1982). Among the hexane, chloroform and methanol extracts, the chloroform extract, which showed $LC_{50} = 62.8$ μg/ml, was chosen for further purification. Two novel compounds were obtained from the chloroform extract after several chromatographic separations. Identification of these compounds was based on spectral data.

Compound **1** was obtained as a pale yellow powder, mp 72–74°C. The EIMS gave a molecular ion at *m/z* 304, corresponding to the molecular formula $C_{20}H_{32}O_2$. The FT–IR spectrum indicated secondary alcohol absorptions ($\nu_{max}$ 3405 and 1054 $cm^{-1}$) and an olefinic double bond ($\nu_{max}$ 1645 and 891 $cm^{-1}$). The $^1H$ NMR spectrum showed three methyl group singlets at δ 0.78, 0.80 and 1.02, in addition to a downfield methyl group signal at δ 1.76 H(*s*) assignable to $CH_3$–C=C. The spectrum also exhibited olefinic protons at δ 5.08 (1H, *d*, $J = 10.8$ Hz), 5.17 (1H, *d*, $J = 17.2$ Hz), 5.26 (1H, *t*, $J = 6.4$ Hz) and 6.73 (1H, *dd*, $J = 10.8$, 17.3 Hz), together with additional exocyclic methylene group signals as two broad one-proton singlets at δ 4.50 and 4.86. These signals, particularly the olefinic proton signals, resemble those reported in 12,13*E*-biformen (Bohlmann and

☆ Part of this work was presented at the Fourth Princess Chulabhorn International Science Congress, Chemicals in the 21st Century, 28 November–2 December 1999, Bangkok, Thailand.

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PII: S0031-9422(00)00382-4

Czerson, 1979). Two sets of additional one-proton signals at $\delta$ 3.02 (*d*, $J$=9.6 Hz) and 3.70 (*ddd*, $J$=4.1, 9.8 and 11.2 Hz) were characterized as two vicinal oxymethine protons of which the former carbinolic carbon was bonded to a tertiary carbon; these two protons were assigned to H-3 and H-2, respectively. The $^1$H–$^1$H COSY spectrum further indicated that the one-proton signal at $\delta$ 3.70 (H-2) was coupled to the one-proton doublet signal at $\delta$ 3.02 (H-3) and also to two other signals at $\delta$ 1.21 (1H, *dd*, $J$=4.3 and 12.4 Hz, H-1e) and 2.11 (1H, *dd*, $J$=3.0 and 3.6 Hz, H-1a). The $^{13}$C NMR spectrum showed 20 carbons comprising four quaternary (of which two were olefinic), and six methine [including two oxymethine carbons at $\delta$ 69.1(*d*) and 83.4 (*d*)]. The compound was proposed as a 2,3-dihydroxylabda-8 (17),12(13),14(15)-triene (**1**). Extensive use of NMR spectroscopic techniques, including $^1$H–$^1$H COSY, HETCOR, COLOC, led to the complete assignments of the $^1$H and $^{13}$C shift values, as shown in Table 1. The important long-range- $^1$H–$^{13}$C (COLOC) correlations are shown in Fig. 1.

The relative stereochemistry of **1** was deduced from the NOESY spectrum. The key NOE effects observed between H-2/H-3, H-2/C-19-Me, H-2/C-20-Me, H-3/C-18-Me and H-5/C-18-Me suggested that the two hydroxyl groups at C-2 and C-3 are both α oriented (Fig. 1).

Compound **2** was obtained as a colorless gum. The HR-FABMS (glycerol matrix) gave an $[M+H]^+$ ion at *m/z* 361.23804 corresponding to the molecular formula $C_{22}H_{32}O_4+H$. The FT-IR spectrum indicated a hydroxyl group ($\nu_{max}$ 3460 cm$^{-1}$), an ester group ($\nu_{max}$ 1730 cm$^{-1}$), an α/β-unsaturated ketone ($\nu_{max}$ 1650 cm$^{-1}$), and a vinylidene group ($\nu_{max}$ 3090 and 908 cm$^{-1}$). The $^1$H NMR spectrum showed three methyl group singlets at $\delta$ 1.02, 1.11 and 1.15, together with a low field acetate methyl group signal at $\delta$ 2.08 (*s*). Three

Table 1
$^1$H and $^{13}$C NMR spectral data for **1** and **2** ($CDCl_3$)[a]

| | **1** | | **2** | |
|---|---|---|---|---|
| Position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 1.21 (*m*) | 45.0 (*t*) | 1.92 (*m*) | 34.0 (*t*) |
| 1′ | 2.11 (*dd*, 4.3, 12.4) | | | |
| 2 | 3.70 (*ddd*, 4.2, 9.8, 11.3) | 69.1 (*d*) | 1.36 (*m*) | 27.2 (*t*) |
| 2′ | | | 1.85 (*m*) | |
| 3 | 3.02 (*d*, 9.6) | 83.4 (*d*) | 3.35 (*dd*, 4.8, 11.5) | 78.0 (*d*) |
| 4 | | 39.4 (*s*) | | 41.8 (*s*) |
| 5 | 1.19 (*br d*, 12.3) | 54.5 (*d*) | 1.73 (*dd*, 3.9, 14.0) | 49.4 (*d*) |
| 6 | 1.38 (*dddd*, 4.1, 12.8, 12.8 12.9) | 23.6 (*t*) | 2.53 (*dd*, 14.0, 17.6) | 35.6 (*t*) |
| 6′ | 1.69 (*m*) | | 2.63 (*dd*, 3.9, 17.6) | |
| 7 | 1.99 (*ddd*, 4.7, 12.8, 12.9) | 37.7 (*t*) | | 199.5 (*s*) |
| 7′ | 2.39 (*m*) | | | |
| 8 | | 147.2 (*s*) | | 129.0 (*s*) |
| 9 | 1.73 (*m*) | 56.9 (*d*) | | 164.3 (*s*) |
| 10 | | 40.2 (*s*) | | 39.3 (*s*) |
| 11 | 2.19 (*dd*, 7.0, 10.9) | 22.3 (*t*) | 2.00 (*m*)[b] | 23.1 (*t*) |
| 11′ | 2.37 (*m*) | | 2.18 (*m*) | |
| 12 | 5.26 (*t*, 6.4) | 130.9 (*d*) | 1.28 (*m*) | 33.5 (*t*) |
| 12′ | | | 1.62 (*m*) | |
| 13 | | 131.9 (*s*) | | 34.4 (*s*) |
| 14 | 6.73 (*dd*, 10.8, 17.3) | 133.7 (*d*) | 2.00 (*m*)[b] | 33.2 (*t*) |
| 14′ | | | 2.38 (*dd*, 1.5, 17.8) | |
| 15 | 5.08 (*d*, 10.8) | 113.5 (*t*) | 5.66 (*dd*, 10.8, 17.5) | 144.9 (*d*) |
| 15′ | 5.17 (*d*, 17.3) | | | |
| 16 | 1.76 (*s*) | 19.7 (*q*) | 4.83 (*dd*, 1.2, 17.5) | 111.7 (*t*) |
| 16′ | | | 4.93 (*dd*, 1.2, 10.8) | |
| 17 | 4.50 (*br s*) | 108.7 (*t*) | 1.02 (*s*) | 28.2 (*q*) |
| 17′ | 4.86 (*br s*) | | | |
| 18 | 1.02 (*s*) | 28.8 (*q*) | 1.15 (*s*) | 21.9 (*q*) |
| 19 | 0.80 (*s*) | 16.6 (*q*) | 4.23 (*d*, 11.8) | 65.0 (*t*) |
| 19′ | | | 4.41 (*d*, 11.8) | |
| 20 | 0.78 (*s*) | 15.5 (*q*) | 1.11 (*s*) | 17.5 (*q*) |
| CO | | | | 171.1 (*s*) |
| $CH_3$ | | | 2.08 (*s*) | 21.0 (*q*) |

[a] $\delta$ in ppm and $J$ (in parentheses) in Hz.
[b] These signals overlapped.

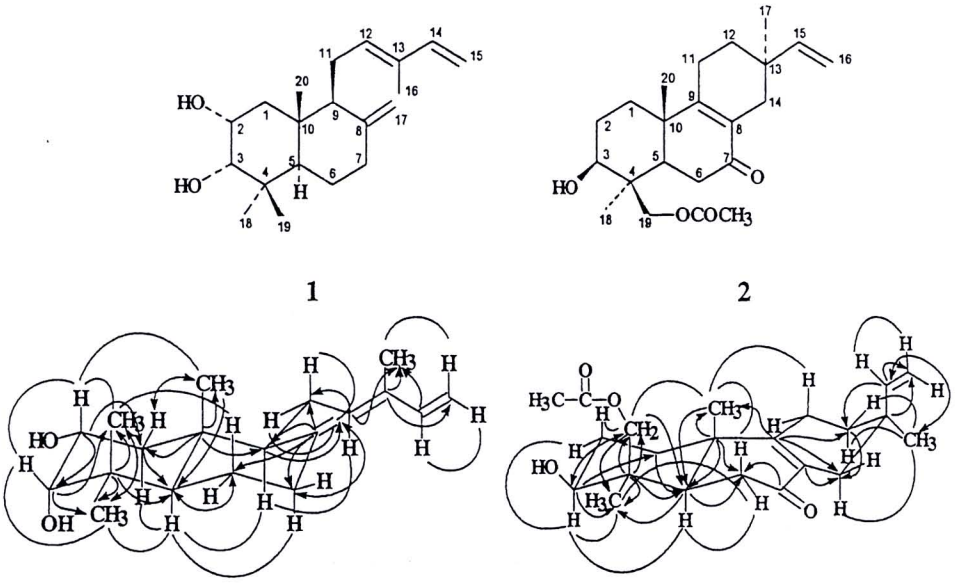

Fig. 1. Structures: key long-range $^{1}H$–$^{13}C$ (C H) and NOESY (H H) correlations of **1** and **2**.

sets of one proton signals $\delta$ 4.83 (1H, *dd*, $J=1.2$ and 17.5 Hz), 4.93 (1H, *dd*, $J=1.2$ and 10.8 Hz) and 5.66 (1H, *dd*, $J=10.8$ and 17.5 Hz), in addition to the $^{13}C$ NMR spectroscopic signals at $\delta$ 111.7 (*t*) and 144.9 (*d*), indicated a vinylidene group. The key $^{1}H$–$^{13}C$ long range correlation (HMBC) between a methyl proton signal at $\delta$ 1.02 and the $^{13}C$ signal at $\delta$ 144.9 (*d*), in addition to correlations of the two vinylidene proton signals at either $\delta$ 4.83 or 4.93 to the $^{13}C$ signal at $\delta$ 34.4 (*s*), indicated a pimarane diterpene with a vinyl group bonded to C-13 (Rao et al., 1968). The one-proton signals at $\delta$ 4.23 (*d*, $J=11.8$ Hz) and 4.41 (*d*, $J=11.8$ Hz) and the $^{13}C$ signal at $\delta$ 65.0 (*t*) implied an oxymethylene moiety bonded to a $COCH_3$ group. The signals of two non-equivalent protons at $\delta$ 2.53 (*dd*, $J=14.0$ and 17.6 Hz) and 2.63 (*dd*, $J=3.9$ and 17.6 Hz), which were coupled to a methine proton at $\delta$ 1.73 (*dd*, $J=3.9$ and 14.0 Hz) indicated a $CH–CH_2–CO$ moiety. The absence of either an $\alpha$- or $\beta$-proton signal of an $\alpha,\beta$-unsaturated carbonyl group, in addition to the $^{13}C$ signals at $\delta$ 129.0 (*s*), 164.3 (*s*), and 199.0 (*s*), suggested a fully substituted olefinic double bond at C-8(9) and a keto group at C-7. The $^{3}J$ coupling between a one-proton signal at $\delta$ 3.35 (*dd*, $J=4.8$ and 11.5 Hz) and the $^{13}C$ signals at $\delta$ 21.9 (*q*), 65.0 (*t*) and 34.0 (*t*) indicated the location of a hydroxyl group at C-3. Another $^{3}J$ correlation between the oxymethylene proton signals at $\delta$ 4.23 and 4.41 and the $^{13}C$ signal at $\delta$ 49.4 (*d*, assigned to C-5) implied the link of an $OCOCH_3$ group at either C-18 or C-19. The NOEs obtainable from the NOESY spectrum indicated the bonding between the $OCOCH_3$ group and C-19 from the interactions between C-20-Me/C-19-$CH_2O$-$COCH_3$ and H-2a/C-19-$CH_2OCOCH_3$. Furthermore, the NOE correlations between H-3/H-5, H-3/C-18-Me and H-3/H-2e also suggested the stereochemistry of C-3-OH as $\beta$ (equatorial). Compound **2** was therefore proposed to be 3$\beta$-hydroxy-19-*O*-acetyl-pimara-8(9),15-dien-7-one. Complete $^{1}H$ and $^{13}C$ chemical shifts (Table 1) were obtained from $^{1}H$–$^{1}H$ COSY, HMQC and HMBC spectra. The key HMBC and NOESY correlations are illustrated in Fig. 1.

The brine shrimp assay of **1** and **2** indicated weak toxicity in comparison to colchicine. The percent deaths at 204.2, 204.2 and 1.0 μg/ml of **1**, **2** and colchicine after 24 h were 53.3, 20.0 and 50, respectively. The $LC_{50}$ were found to be 197.1, > 204.0 and 1.0 μg/ml, respectively.

## 3. Experimental

### 3.1. General

Mps uncorrected; $^{1}H$ and $^{13}C$ NMR spectra were acquired at 400 and 100 MHz, respectively. The solvent signals were employed for reference purposes.

### 3.2. Plant material

The leaves of *Croton joufra* were collected from Kalasin Province, in the Northeast of Thailand, during December, 1995. The plant was kindly identified by Nijsiri Ruangrangsi. Voucher specimens (SSCJ/1995) are deposited at the Department of Chemistry, Faculty of Science, Ramkhamhaeng University and at the

Herbarium, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

### 3.3. Extraction and isolation

The dried leaves of *Croton joufra* were milled to obtain a fine powder (624 g) which were extracted successively with *n*-hexane, chloroform and methanol in a Soxhlet extraction apparatus. After evaporation of the solvents under reduced pressure, dark green gums of *n*-hexane (21.52 g), chloroform (31.70 g) and methanol (91.80 g) extracts were obtained.

The chloroform extract was fractionated by silica gel column chromatography using a gradient of *n*-hexane–chloroform (1:1 to 0:1) followed by chloroform–MeOH (10:0 to 1:1) to yield eight major frs. after combination of similar frs. as judged by TLC. Fr. 6 was subjected to additional silica gel cc ($CHCl_3$–MeOH (1:0 to 1:1) to give eight subfrs. (subfrs. 6.1–6.8). Subfr. 6.4 was subjected to chromatography (2×, silica gel cc, $CH_2Cl_2$–MeOH 19:1 to 1:1 and *n*-hexane–EtOAc 17:3) to yield **2** (5.5 mg). Subfr. 6.5 was subjected to chromatography (2×, silica gel cc, $CHCl_3$–MeOH 10:0 to 4:1 and *n*-hexane ($CHCl_3$ 3:7 to 1:4) to yield **1** (103.6 mg).

### 3.4. Compound 1 (2α, 3α-dihydroxy-labda-8(17),12(13),14(15)-triene)

Pale yellow powder, mp 72–74°C; $[\alpha]_D^{25}$ −18.24° ($CHCl_3$, *c* 0.34); HR–EIMS *m/z*: 304.24150, $[M]^+$, $C_{20}H_{32}O_2$ requires 304.24023; EI–MS 70 eV *m/z* (rel. int.): 304 ($[M]^+$, 14.5), 248 (50.5), 187 (25.0), 135 (100), 93 (98.5), 81 (36.5), 55 (99.5), 43 (67); IR (film, $cm^{-1}$): 3405, 2941, 1715, 1645, 1456, 1385, 1054, 954, 891; $^1H$ and $^{13}C$ NMR spectral data are shown in Table 1.

### 3.5. Compound 2 (3β-hydroxy-19-O-acetyl-pimara-8(9),15-dien-7-one)

Colorless gum; $[\alpha]_D^{25}$ −127.62° ($CHCl_3$, *c* 0.105); HR–FABMS (glycerol) *m/z*: 361.23804, $[M+H]^+$, $C_{22}H_{32}O_4+H$ requires 361.23788; IR (film, $cm^{-1}$): 3460, 3090, 2931, 2863, 1731, 1648, 1373, 1241, 1094, 1036, 908, 756; $^1H$ and $^{13}C$ NMR spectral data are shown in Table 1.

## Acknowledgements

We are grateful to Ramkhamhaeng University and the Thailand Research Fund for financial support. Thanks are due to Ms. I. Klaiber, Institut für Chemie, Universität Hohenheim, FRG. for HR-EIMS, Mr. N. Chimnoi, Chulabhorn Research Institute, for HR-FABMS, Department of Chemistry, Kasetsart University for FT–IR measurements, Ms. S. Unganont and Ms. S. Prommas for some technical assistances.

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# A SYNTHESIS OF BIPOWINE AND BIPOWINONE

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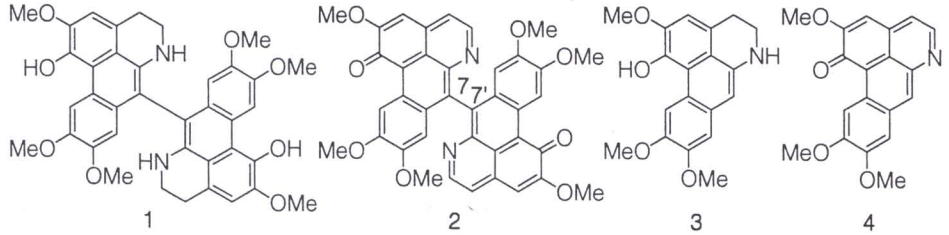
**Abstract-** Various oxidizing agents were investigated for the synthesis of bipowine (**1**) and bipowinone (**2**), two symmetrical dimeric aporphine alkaloids, from the oxidative coupling of dehydroaporphine (**3**).

Dimeric aporphine alkaloids[1-5] are a small group of natural alkaloids isolated from plants of the family Annonaceae. There are two types of linkages between the two aporphine units i.e. C4-C7 and C7-C7. Bipowine (**1**) and bipowinone (**2**) are the two representative of the symmetrical 7,7'-bisaporphine alkaloids isolated from the Indonesian annonaceous plant *Popowia pisocarpa.*[2]

The dimerization of aporphine and dehydroaporphine alkaloids to the corresponding 7,7'-bisaporphines has been achieved. Various reagents utilized to effect such dimerization include $I_2/C_2H_5OH$,[6] $Hg(NO_3)_2$ /$CH_3CN$,[7] $Hg(OAc)_2/CH_3OH$,[7] NCS/base and air.[2,8] Bipowinone (**2**) has been previously obtained from the oxidation of wilsonirine (dihydro derivative of **3**) with NCS followed by reaction with sodium ethoxide, but in a very low yield (1.6%)[2] the main product was later found to be pancoridine (**4**).[8] However, no satisfactory methods for the synthesis of **2** have so far been reported. In this study various oxidizing agents were investigated for the oxidative dimerization of dehydroaporphine alkaloid. In this paper we report the synthesis of bipowine (**1**) and bipowinone (**2**).




6a,7-Dehydronorthaliporphine (**3**) was used in our study of the oxidative coupling reaction. This dehydroaporphine alkaloid was obtained[9] from hydrogenation of pancoridine (**4**)[9,10] using $PtO_2$ as catalyst.

The reaction of dehydroaporphine (**3**) with various oxidizing agents is shown in Table I.

**Table I** "The reaction of **3** with various oxidizing agents[a)]"

| Entry | Oxidizing agents (equivalent) | Reaction Time | Products (%) | |
|---|---|---|---|---|
| | | | **1** | **2** |
| 1 | $Hg(OAc)_2$ (0.6) | 15 min | 60 | trace |
| 2 | $Hg(OAc)_2$ (2.7) | 3 h | - | 68 |
| 3 | $PhI(OAc)_2$ (0.6) | 5 min | 53 | trace |
| 4 | $PhI(OAc)_2$ (2.6) | 5 min | - | 74 |
| 5 | air | 48 h | 58 | - |

"a) The reaction was carried out in dichloromethane at rt"

$Hg(OAc)_2$ could be used to oxidise **3** to the dimeric bipowine (**1**) and bipowinone (**2**) depending on the ratio of the oxidizing agent and the duration of the reaction. More significantly, we have found that the above dimerization process can be conveniently effected by (diacetoxyiodo)benzene [$PhI(OAc)_2$]. Hypervalent iodine compounds[11-13] have recently been utilized in many organic functional group transformations. The relatively low toxicity of hypervalent iodine compounds[11i] as compared to the mercury compounds makes the above finding very attractive.

**Table II** "Formation of **2** by the oxidation of **1**[a)]"

| Entry | Oxidizing agent (equivalent) | Reaction time | Product (**2**) (%) |
|---|---|---|---|
| 1 | $Hg(OAc)_2$ (4.2) | 3 h | quantitative yield |
| 2 | $PhI(OAc)_2$ (4.1) | 5 min | 73 |
| 3 | $Ag_2O$ (4.3) | 10 min | 90 |

"a) The reaction was carried out in dichloromethane at rt"

Furthermore, we have also found that bipowine (**1**) was easily oxidized to bipowinone (**2**) by excess amount of $Hg(OAc)_2$, $PhI(OAc)_2$, and $Ag_2O$. The results are summarized in Table II. Reaction of **3** with $Ag_2O$[14] gave pancoridine (**4**) in 53% yield and bipowinone (**2**) in 28% yield. In addition, when [bis (trifluoroacetoxy)iodo]benzene was used as oxidizing agent, only 12% of **2** was obtained together with 12% of pancoridine (**4**).

In conclusion, the formation of bipowine and bipowinone could be adjusted according to the experimental procedure and we have introduced the use of $PhI(OAc)_2$ in the oxidative dimerization of the dehydroaporphine alkaloid to the corresponding bisaporphine alkaloid.

## EXPERIMENTAL

Melting points are uncorrected. $^1H$ NMR spectra were taken at 300 or 400 MHz as specified and $^{13}C$ NMR at 100 MHz. Tetramethylsilane was used as the internal standard, and chemical shifts are reported as $\delta_H$ (ppm) or $\delta_C$ (ppm). MS spectra were obtained by electron impact technique (EI).

**6a,7-Dehydronorthaliporphine** (**3**) was prepared according to Cava's procedure[9] and exhibited the

following data. mp 197°C (decomp) (ether) (lit.,[9] mp 198°-199°C); IR (KBr): $\nu_{max}$ 3460 (NH), 3327 $cm^{-1}$ (OH); $^1$H NMR (300 MHz, acetone $d_6$): δ (ppm) 3.13-3.17 (m, 2H, C-4, $CH_2$-C$H_2$-NH), 3.38-3.42 (m, 2H, C-5, C$H_2$-$CH_2$-NH), 3.88, 3.89, 4.01 (3s, 9H, 3xOC$H_3$), 6.63 (s, 1H, C-8-Ar*H* ), 7.01 (s, 1H, C-7-=C*H* ), 7.11 (t, 1H, J = 0.9 Hz, C-3-Ar*H* ), 9.27 (s, 1H, C-11-Ar*H* ); MS: m/z 325 ($M^+$,100), 310 (54).

**Formation of bipowine (1) by oxidation of 6a,7-dehydronorthaliporphine (3)**

a) By air oxidation:

A solution of **3** (140 mg, 0.43 mmol) in dichloromethane-methanol (9:1, 150 mL) was oxidized by bubbling air at rt until starting materials were consumed (2 days, monitored by TLC). The solution was evaporated to dryness and the crude product so obtained was purified by column chromatography ($Al_2O_3$, neutral) using dichloromethane as eluting solvent to give bipowine as a pale yellow solid, which was recrystallized from chloroform-acetone to yield **1** (80 mg, 58%). mp 250-252°C (decomp) (lit.,[2] mp > 249 °C); IR (KBr): $\nu_{max}$ 3467 (NH), 3388 $cm^{-1}$ (OH); UV: $\lambda_{max}$ MeOH (log ε) 207 (4.40), 266 (4.82), 335 (4.16), 389 (3.90) nm, $\lambda_{max}$ MeOH+NaOH 216, 263, 355, 398, 496 nm, $\lambda_{max}$ MeOH+HCl 205, 263, 293, 338, 356, 374 nm; $^1$H NMR (400 MHz, $CDCl_3$): δ 3.09-3.34 (m, 4H, C$H_2$-C$H_2$NH), 3.49, 4.05, 4.07 (3s, 9H, 3xOC$H_3$), 6.65 (s, 1H, C-8-Ar*H*), 6.86 (s, 1H, O*H*), 7.03 (s, 1H, C-3-Ar*H*), 9.33 (s, 1H, C-11-Ar*H*), $^{13}$C NMR (100 MHz, $CDCl_3$-$CD_3OD$) : δ 143.6 (C-1), 119.8 (C-1a), 117.8 (C-1b), 148.9 (C-2), 110.6 (C-3), 125.1 (C-3a), 30.7 (C-4), 49.2 (C-5), 141.1 (C-6a), 128.4 (C-7), 138.7 (C-7a), 104.1 (C-8), 145.4 (C-9), 143.6 (C-10), 109.2 (C-11), 119.5 (C-11a), 55.3, 55.8, 56.9 ($OCH_3$-2, 3, 10); MS : m/z 648 ($M^+$, 89), 633 (33), 325 (92), 324 (100), 310 (68), 292 (39), 290 (69).

b) By oxidation with mercuric acetate:

$Hg(OAc)_2$ (16.0 mg, 0.05 mmol) was added to a solution of **3** (28.9 mg, 0.09 mmol) in dichloromethane (3 mL). The mixture was allowed to stir at rt for 15 min, then filtered. Removal of dichloromethane gave a residue, which was chromatographed on aluminum oxide (neutral) and eluted with dichloromethane to give **1** (17.2 mg, 60%).

c) By oxidation with diacetoxyiodobenzene:

Diacetoxyiodobenzene (17.7 mg, 0.05 mmol) was added to a solution of **3** (29.3 mg, 0.09 mmol) in dichloromethane (3 mL). The mixture was stirred for 5 min at rt, then water was added and the mixture was extracted with dichloromethane (2x20 mL). The dichloromethane extract was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography ($Al_2O_3$, neutral) using dichloromethane as eluting solvent to give **1** (15.5 mg, 53%).

**Bipowinone (2) from 6a,7-dehydronorthaliporphine (3)**

a) By oxidation with mercuric acetate:

$Hg(OAc)_2$ (70.0 mg, 0.22 mmol) was added to a solution of **3** (26.8 mg, 0.08 mmol) in dichloromethane (3 mL). The mixture was allowed to stir for 3 h at rt. Removal of dichloromethane gave the residue which was chromatographed on aluminum oxide (neutral) and eluted with dichloromethane to afford an orange-red solid (18 mg, 68%), which was crystallized from dichloromethane-acetone to give **2**. mp 292 °C (decomp) (lit.,[2] mp > 295 °C); IR (KBr): $\nu_{max}$ 1626 $cm^{-1}$ (C=O); UV: $\lambda_{max}$ MeOH (log ε) 237 (4.72), 278 (4.42), 288 (4.37), 300 (4.29), 411 (4.26), 476 (4.23), 502 (4.20) nm, $\lambda_{max}$ MeOH+HCl 204, 250, 432, 496 nm; $^1H$ NMR (400 MHz, $CDCl_3$) : δ 3.36, 4.05, 4.20 (3s, 9H, 3xOC*H*$_3$-9, 2, 10) 6.44 (s, 1H, C-8-Ar*H*), 6.89 (s, 1H, C-3-Ar*H*), 7.45, 8.66 (AB, $J_{AB}$ = 4.4 Hz, 2H, C*H*=C*H*N), 9.91 (s, 1H, C-11-Ar*H*), $^{13}C$ NMR ($CDCl_3$-$CD_3OD$) : δ 180.8 (C-1), 131.8 (C-1a), 120.5 (C-1b), 156.1 (C-2), 105.4 (C-3), 141.8 (C-3a), 121.1 (C-4), 149.7 (C-5), 156.3 (C-6a), 131.6 (C-7), 135.1 (C-7a), 105.3 (C-8), 150.5 (C-9), 144.4 (C-10), 107.5 (C-11), 120.3 (C-11a), 55.4, 56.0, 56.4 ($OCH_3$-2, 3, 10); MS: m/z 640 ($M^+$, 17), 625 (25), 609 (15), 321 (57), 320 (18), 307 (23), 290 (100).

b) By oxidation with diacetoxyiodobenzene:

Diacetoxyiodobenzene (69.8 mg, 0.22 mmol) was added to a solution of **3** (27.4 mg, 0.08 mmol) in dichloromethane (3 mL). The mixture was stirred for 5 min at rt. Water was added and the mixture was extracted with dichloromethane (2x20 mL). The dichloromethane extract was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography ($Al_2O_3$; neutral ) using dichloromethane as eluting solvent to give **2** as an orange-red solid (20.1 mg, 74%).

**Oxidation of bipowine (1) to bipowinone (2)**

a) By oxidation with mercuric acetate:

$Hg(OAc)_2$ (36.7 mg, 0.12 mmol) was added to a solution of **1** (17.7 mg, 0.03 mmol) in dichloromethane (3 mL). The mixture was allowed to stir for 3 h at rt, then filtered. Removal of dichloromethane gave a residue which was chromatographed on aluminum oxide (neutral) and eluted with 1% methanol-dichloromethane to give **2** (quantitative yield).

b) By oxidation with diacetoxyiodobenzene:

Diacetoxyiodobenzene (36.1 mg, 0.11 mmol) was added to a solution of **1** (17.4 mg, 0.03 mmol) in dichloromethane (3 mL). The mixture was stirred for 5 min at rt. Water was added and the mixture was extracted with dichloromethane (2x20 mL). The dichloromethane extract was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography ($Al_2O_3$; neutral) using 1% methanol-dichloromethane as eluting solvent to give **2** (12.5 mg, 73%).

c) By oxidation with silver oxide:

Silver(I) oxide (27.1 mg, 0.12 mmol) was added to a solution of **1** (17.7 mg, 0.03 mmol) in dichloromethane (3 mL). The solution was allowed to stir for 10 min at rt, then filtered. Removal of

dichloromethane gave a residue which was chromatographed on aluminum oxide (neutral) and eluted with 1% methanol-dichloromethane to give **2** (15.8 mg, 90%).

Pancoridine (**4**)

Silver(I) oxide (53.3 mg, 0.23 mmol) was added to a solution of **3** (28.8 mg, 0.09 mmol) in dichloromethane (3 mL). The mixture was allowed to stir for 30 min at rt, then filtered. Removal of dichloromethane gave a residue which was chromatographed on aluminum oxide (neutral) and eluted with dichloromethane to give **4** (15.2 mg, 53%) and **2** (8.0 mg, 28% ). Pancoridine (**4**); mp (dichloromethane-hexane) 233-234 °C (decomp)(lit.,[9] 214-215°C, lit.,[10] 236-238 °C); FTIR (KBr) : $\nu_{max}$ 1626 $cm^{-1}$ (C=O); UV: $\lambda_{max}$ MeOH (log ε), 203(4.09), 232(4.59), 243(4.52), 285(4.15), 402(4.02), 466(3.92) nm, $\lambda_{max}$ MeOH+HCl, 203, 243, 277, 295, 419, 485 nm; $^1$H NMR (400 MHz, $CDCl_3$) : δ 4.03, 4.09, 4.15 (3s, 9H, 3xOC*H*$_3$), 6.78 (s, 1H, C-3-Ar*H* ), 6.89 (s, 1H, C-8-Ar*H*), 7.45, 8.66 (AB, $J_{ab}$= 4.3Hz, 2H,C*H* =C*H* N), 8.70 (s, 1H, C-7-Ar*H* ), 9.51 (s, 1H, C-11-Ar*H* ), $^{13}$C NMR ($CDCl_3$-$CD_3OD$), δ 180.4(C-1), 132.0(C-1a),135.1(C-1b),156.3(C-2), 106.7(C-3), 141.4(C-3a), 121.0(C-4), 150.0(C-5), 156.5(C-6a),104.8(C-7), 135.1(C-7a), 107.1(C-8), 150.3(C-9), 144.6(C-10), 106.7(C-11), 131.7(C-11a), 56.4, 55.9, 55.86. (OC*H*$_3$-2,3,10); MS: m/z 321($M^+$,73.80), 290(100.00).

**ACKNOWLEDGEMENT**

We are grateful to the Thailand Research Fund (TRF) for the generous support of our research programme. We also acknowledge the facilities provided by the PERCH programme.

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Received, 11st October, 2000

Pergamon

Tetrahedron Letters 42 (2001) 1359–1361

TETRAHEDRON LETTERS

# An efficient synthesis of argemonine, a pavine alkaloid†

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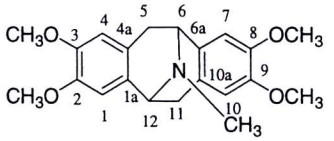
Received 30 November 2000; accepted 5 December 2000

**Abstract**—A method for the synthesis of 1,2-dihydroisoquinoline derivatives is described and the conversion of the 1,2-dihydroisoquinoline intermediate to a pavine alkaloid via palladium-induced intramolecular hydroarylation reaction and radical cyclization is presented. 

Argemonine is a prototypical member of the pavine alkaloids, a small group of tetracyclic natural products, and contains the tetrahydroisoquinoline core embedded in its skeleton.[1] Recent findings of biological activities of the pavine alkaloids include inhibition of *herpes simplex* virus type 1[2] and inhibitory activity against tumor necrosis factor-α (TNF-α) production.[3]




Argemonine

There are a number of syntheses[1,4] of pavine alkaloids reported, but most involve an acid-catalyzed intramolecular cyclization of the activated aromatic ring with an iminium salt as in the Pictet–Spengler reaction. The drawback of such a procedure is the failure with nonactivated aromatic compounds and the lack of chemoselectivity in the cyclization of unsymmetrical compounds.

We have developed a new synthesis of pavine alkaloids based on the retrosynthetic analysis shown in Scheme 1. The key steps involve a palladium-induced intramolecular hydroarylation[5] and a radical cyclization[6] of the key halo derivatives of 1-benzyl-*N*-carboethoxy-1,2-dihydroisoquinolines. The intramolecular hydroarylation involves the reduction of the organopalladium complex formed during the carbon–carbon bond formation in the much exploited Heck reaction.[7] We have also developed a new method for the synthesis of the key 1,2-dihydroisoquinoline derivatives. During our investigation a method for the synthesis[8] of this type of compound was reported involving the addition of a benzylstannane to an isoquinoline in the presence of methyl chloroformate in dichloromethane. We found that 1-benzyl-*N*-carboethoxy-1,2-dihydroisoquinolines could be conveniently obtained by the reaction of 1-benzylisoquinoline derivatives with tributyltin hydride. For example, treatment of papaverine **4a** with tributyltin hydride in dichloromethane at room temperature under a nitrogen atmosphere, followed by addition of ethyl chloroformate at −78°C and warming to room temperature gave a white solid which, after recrystallization from methanol–dichloromethane, provided compound **3a** in 79% yield. Having found a method for the synthesis of 1-benzyl-*N*-carboethoxy-1,2-dihydroisoquinolines, we then began the synthesis of our key intermediate. The synthesis started with bromination of commercially available papaverine **4a** with a solution of bromine in acetic acid at room temperature for 2 h to give 2′-bromopapaverine[9] **4b** in 75% yield.

Application of the tin hydride reduction gave the required product which was recrystallized from methanol–dichloromethane to give compound **3b** as a white solid in 85% yield. It is interesting to note the

*Keywords*: pavine alkaloid; intramolecular hydroarylation reaction; radical cyclization reaction.

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† This work has been taken in part from Namsa-aid, A., M.Sc. Thesis, Mahidol University 1997, and was presented at the 8th Belgian Organic Synthesis Symposium, July 10–14, 2000, Gent, Belgium.

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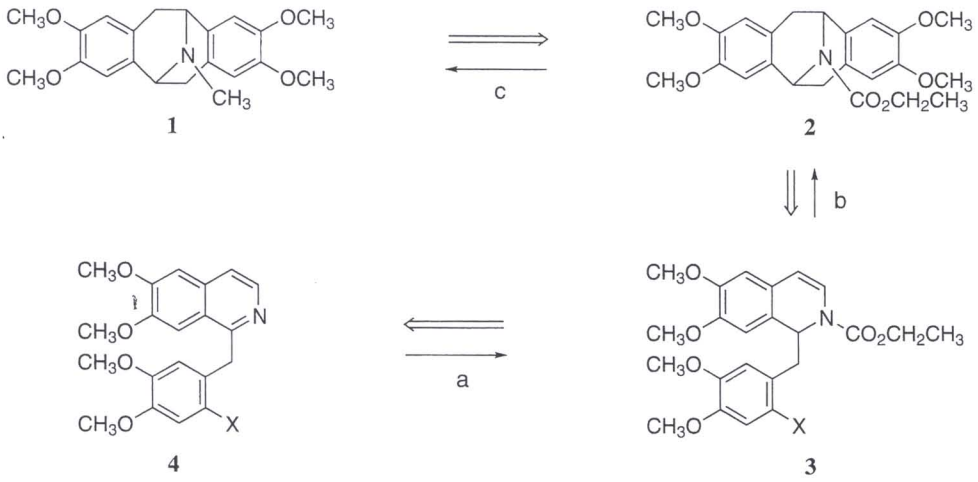


a) X = H, b) X = Br, c) X = I

**Scheme 1.** *Reagents and conditions*: (a) i. $Bu_3SnH/CH_2Cl_2$, ii. $EtOCOCl/CH_2Cl_2$, −78°C→rt (**3a**, 79%; **3b**, 85%; **3c**, 85%); (b) See Table 1; (c) LAH/THF, reflux 4 h (**1**, 87%).

chemoselective reduction of the iminium intermediate with tributyltin hydride in the presence of the aryl bromide group. Once the key compound **3b** was obtained, we applied the intramolecular hydroarylation cyclization to effect pavine ring formation. The cyclization of **3b** was accomplished using 10–20 mol% of a reactive catalyst formed from $Pd(PPh)_4$ in DMF at 80–90°C in the presence of sodium formate as a reducing agent. After purification by PLC, *N*-ethoxycarbonylpavine **2**[10] was obtained in 44% yield together with the corresponding reduction product **3a** in 34% yield as shown in entry 1 of Table 1. The yield of the *N*-ethoxycarbonylpavine **2** was increased to 56% when the starting iodo compound **3c** was used instead of the bromo compound **3b** under similar conditions (entry 3, Table 1). The 2′-iodopapaverine **4c** could be conveniently obtained by the reaction of papaverine with iodine and silver trifluoroacetate.[11] The appearance of four aromatic protons as singlets in the NMR spectrum indicated that 2′-iodopapaverine was obtained.

In addition, the radical cyclization using tributyltin hydride and AIBN of compounds **3b** and **3c** was investigated. It was found that the yield of *N*-ethoxycarbonylpavine **2** was only 30% from the cyclization of the bromo compound **3b** under the tributyltin hydride/AIBN conditions. The corresponding reduction product **3a** was obtained from the reaction in 5% yield. Similarly, the iodo compound **3b** underwent cyclization with tributyltin hydride to afford the pavine **2** in 42% yield together with 10% of the reduction product **6**. The *N*-ethoxycarbonylpavine **2** was reduced by LAH to form the *N*-methylpavine in 87% yield. The physical and spectroscopic data of our synthetic compound are in full agreement with those of argemonine **1**.[4b]

In conclusion, we have devised a new method for the synthesis of pavine alkaloids as illustrated in the synthesis of natural (±)-argemonine. With appropriate introduction of the iodo group, the approach could be used to synthesize both symmetrical and unsymmetrical pavine alkaloids. We found that the palladium-catalyzed reductive intramolecular arylation reaction is very useful and gives better yields than radical cyclization.

## Acknowledgements

We are grateful to the Thailand Research Fund (TRF) for the generous financial support. We acknowledge the facilities in the Department of Chemistry provided by the PERCH programme.

**Table 1.** Intramolecular arylation of 3,4-dihydroisoquinoline derivatives **3a** and **3b** to pavine (**2**)

| Entry | Starting material | Conditions | Yield% | Yield% |
|---|---|---|---|---|
| | | | Comp. **2** | Comp. **3a** |
| 1 | Compound **3b** | $Pd(PPh_3)_4/DMF/HCO_2Na$/reflux 24 h | 44 | 34 |
| 2 | Compound **3b** | $Bu_3SnH$/AIBN, benzene, reflux 10 h | 30 | 5 |
| 3 | Compound **3c** | $Pd(PPh_3)_4/DMF/HCO_2Na$/reflux 24 h | 56 | 15 |
| 4 | Compound **3c** | $Bu_3SnH$/AIBN, benzene reflux 10 h | 42 | 10 |

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All compounds have been fully characterized. Spectroscopic data of some selected compounds, 1-(2-iodo-4,5-dimethoxybenzyl)-2-ethoxycarbonyl-6,7-dimethoxy-1,2-dihydroisoquinoline **3c**: mp 154–156°C; FT-IR (Nujol) 2926, 1708, 1633, 1227 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 1.15 (t, 3H, $J=7.1$ Hz), 1.25 (t, 3H, $J=7.1$ Hz), 2.82–3.06 (m, 4H), 3.66 (s, 3H), 3.72 (s, 3H), 3.73 (s, 3H), 3.77 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.87 (s, 3H), 3.89 (s, 3H), 4.02 (m, 2H), 4.17 (q, 2H, $J=7.1$ Hz), 5.45 (dd, 1H, $J=8.4$, 5.6 Hz), 5.55 (t, 1H, $J=7.1$ Hz), 5.79, 6.79 (AB q, 2H, $J$ab $=7.1$ Hz), 5.94, 6.96 (AB q, 2H, $J$ab $=7.1$ Hz), 6.23 (s, 1H), 6.34 (s, 1H), 6.40 (s, 1H), 6.48 (s, 1H), 6.60 (s, 1H), 6.64 (s, 1H), 7.17 (s, 1H), 7.23 (s, 1H). $^{13}C$ NMR (100 MHz) $\delta$ 14.28, 14.53, 43.78, 44.23, 55.20, 55.71, 55.82, 55.92, 55.97, 56.05, 56.24, 61.99, 62.19, 88.89, 89.60, 107.90, 108.03, 108.39, 108.95, 109.85, 110.23, 113.42, 113.69, 121.24, 121.34, 122.70, 123.15, 123.53, 123.56, 123.75, 124.30, 132.51, 132.61, 147.70, 147.78, 148.07, 148.52, 148.75, 148.96, 152.81, 153.54. FABMS 540 ($M^+$+1, 2.24), 413 (8.16), 262 (100.00). Anal. calcd for $C_{23}H_{26}NO_6I$: C, 51.19; H, 4.86; N, 2.63; Found: C, 50.99: H, 4.82; N, 2.41. *N*-Ethoxycarbonylpavine **2**: mp 190–192°C (lit.[10] mp 183–184°C); FT-IR (KBr) 1686, 1519, 1463, 1254 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 1.28 (t, 3H, $J=7.1$ Hz), 2.76 (d, 2H, $J=15.9$ Hz), 3.38 (dd, 1H, $J=15.9$, 5.6 Hz), 3.42 (dd, 1H, $J=15.9$, 5.6 Hz), 3.77 (s, 3H), 3.78 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 4.11–4.25 (m, 2H), 5.42 (d, 1H, $J=5.6$ Hz), 5.52 (d, 1H, $J=5.6$ Hz), 6.45 (s, 1H), 6.48 (s, 1H), 6.66 (s, 1H), 6.67 (s, 1H). $^{13}C$ NMR (100 MHz) $\delta$ 14.65, 35.78, 36.04, 48.97, 49.70, 55.64, 55.89, 61.38, 108.97, 109.20, 111.47, 111.65, 123.95, 124.48, 128.78, 129.08, 147.45, 147.96, 148.05, 154.21. EI-MS 413 ($M^+$, 17.50), 412 (4.89), 340 (8.25), 278 (6.84), 262 (52.36), 28 (100.00). Anal. calcd for $C_{26}H_{22}N_2O_3$: C, 66.40; H, 6.59; N, 3.48. Found: C, 66.24; H, 6.46; N, 3.73.

Pergamon

Tetrahedron Letters 42 (2001) 1205–1208

TETRAHEDRON LETTERS

# An efficient synthesis of lamellarin alkaloids: synthesis of lamellarin G trimethyl ether

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Received 30 November 2000; accepted 4 December 2000

**Abstract**—A general and efficient synthesis of lamellarin alkaloids is described. The synthesis involves the formation of the core pyrrolo[2,1-*a*]isoquinoline, followed by the formation of the lactone ring. © 2001 Published by Elsevier Science Ltd.

Lamellarins are a group of marine natural products which were isolated from the prosobranch mollusc *Lamellaria* sp and the ascidians.[1] The first four lamellarins were isolated by Faulkner et al. in 1985 and named lamellarins A, B, C, D. The structure of lamellarin A was determined by X-ray crystallographic analysis and the structures of the remaining compounds were derived from spectroscopic data.[2] At present 35 lamellarins have so far been isolated and identified.[2,3] Some of these lamellarins and related compounds exhibit interesting biological activities[4] including cell division inhibition, cytotoxicity and immunomodulatory activity and the recently discovered multidrug-resistant (MDR) reversal[5] and HIV-1 integrase inhibition.[6] Due to this impressive array of biological activity profiles, ever increasing elegant synthetic routes

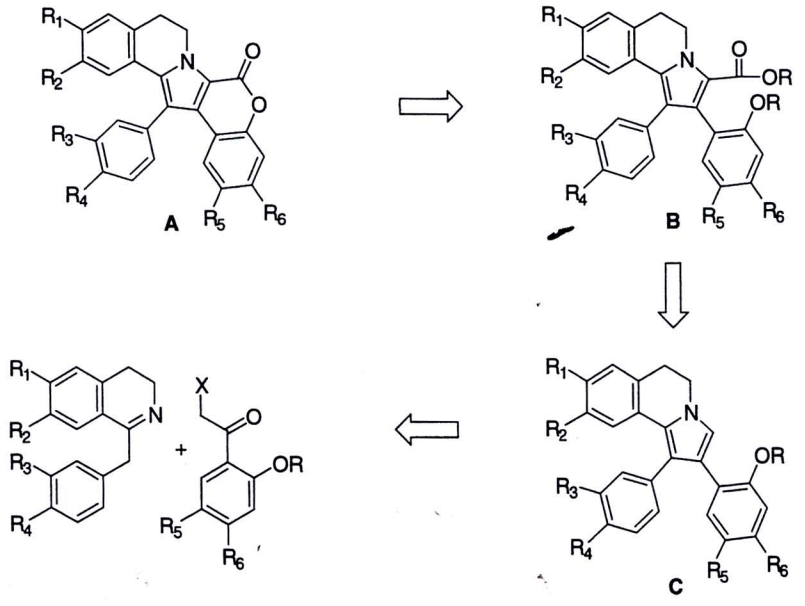


**Figure 1.**

*Keywords*: lamellarin alkaloid.

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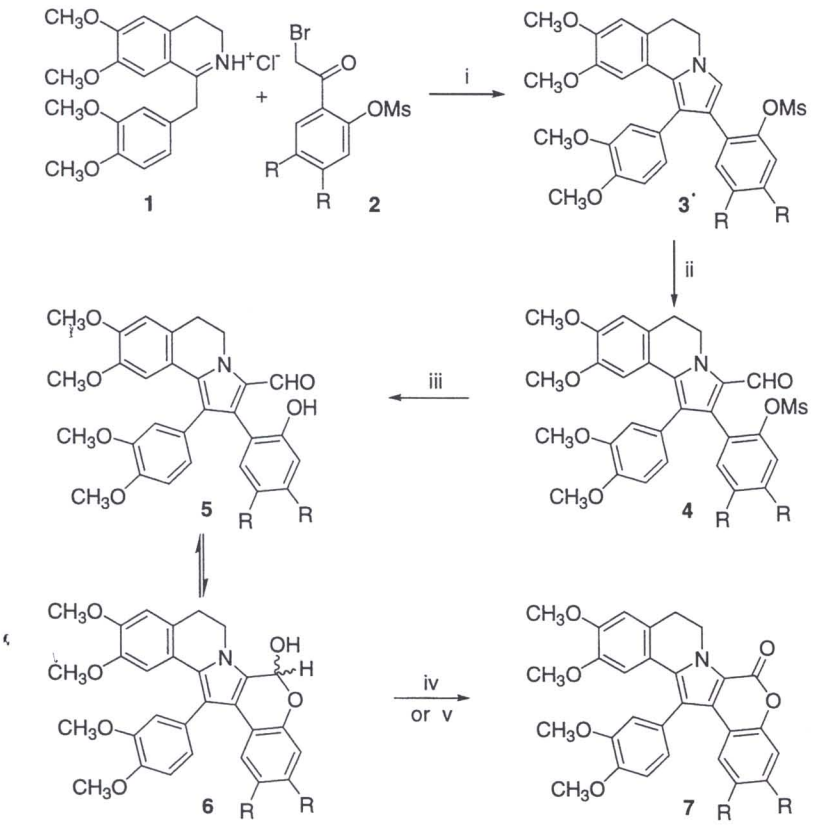
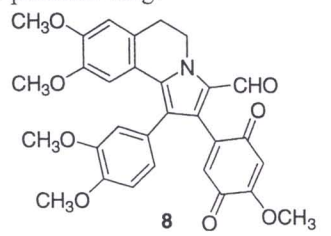


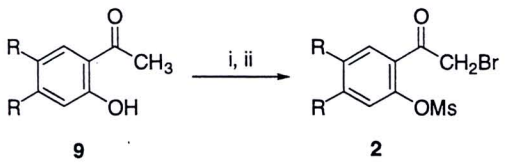

**Scheme 1.** *Reagents and conditions*: (i) $K_2CO_3$, $CH_3CN$, reflux (**3a**, 63%; **3b**, 63%); (ii) DMF, $POCl_3$, rt (**4a**, 80%; **4b**, 82%); (iii) KOH, EtOH, reflux (**5a**, 77%; **5b**, 81%); (iv) $MnO_2$, $CH_2Cl_2$, rt (**7a**, 54%; **7b**, 20% and **8**, 37%); (v) $Pd(OAc)_2$, $PPh_3$, $K_2CO_3$, DMF, PhBr, 120°C, 12 h (**7a**, 80%; **7b**, 80%).

have been developed for the synthesis of lamellarins[7] and related 3,4-diaryl pyrrole derivatives,[8] notably by Steglich and Banwell. The core skeleton of these lamellarins **A** can be viewed as the fusion of the pyrrolo[2,1-*a*]isoquinoline with the lactone unit. Our retrosynthetic analysis as shown in Fig. 1 involves the lactonization of the appropriate pyrrolo[2,1-*a*]isoquinoline derivative **B**. Pyrrolo[2,1-*a*]isoquinoline **C** can be synthesized from the reaction of 3,4-dihydroisoquinoline with a phenacyl bromide derivative.

In practice, the condensation of 3,4-dihydropapaverine hydrochloride **1** with *o*-mesyloxyphenacyl bromide **2a**[9] in the presence of potassium carbonate in acetonitrile gave the expected mesyloxy pyrrolo[2,1-*a*]isoquinoline analogue **3a** in 63% yield. The reaction presumably involves the intramolecular reaction of the derived enamine from the isoquinolinium salt and the ketone as found in the Knorr pyrrole synthesis.[10] The introduction of the formyl group on the pyrrole ring was accomplished by the Vilsmeier reaction.[11] The reaction was carried out using dimethylformamide in phosphorus oxychloride as a formylating agent at room temperature. The expected product **4a** was obtained in 85% yield after purification by preparative thin layer chromatography. The mesyl protecting group in the derived aldehyde intermediate was easily removed by heating with potassium hydroxide in ethanol. The phenol **5a** was produced in 77% yield after purification by preparative thin layer chromatography. We found that manganese dioxide in dichloromethane could be used to oxidize the phenolic aldehyde **5a** to the corresponding lamellarin derivative **7a** in 54% yield, presumably via the hemiacetal intermediate **6a**.

The above approach has also been applied to the synthesis of lamellarin G trimethyl ether as shown in series b of Scheme 1. The first three steps used in the synthesis proceeded well as planned. The condensation of 3,4-dihydroisoquinoline **1** with the phenacyl bromide derivative **2b**[9] gave the corresponding pyrrolo[2,1-*a*]isoquinoline **3b** in 63% yield. The introduction of the formyl group and the removal of mesyloxy protecting group could be accomplished in 82 and 81% yield, respectively. However, the oxidation of compound **5b** with manganese dioxide gave lamellarin G trimethyl ether **7b** in disappointing yield (20%). The byproduct was found to be the quinone derivative **8** formed by the preferred oxidation of the electron rich phenolic ring.



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Series a R = H
Series b R = OMe

**Scheme 2.** *Reagents and conditions*: Series a. (i) $MeSO_2Cl$, $Et_3N$, $CH_2Cl_2$ (97%); (ii) $BnN^+Me_3Br_3^-$, $CH_2Cl_2$ (**2a**, 81%): Series b. (i) $MeSO_2Cl$, $Et_3N$, $CH_2Cl_2$ (97%); (ii) $BnN^+Me_3Br_3^-$, $CH_2Cl_2$ (**2b**, 83%).

After some experimentation, we found that the above conversion could be conveniently carried out by oxidation with bromobenzene, palladium acetate and triphenylphosphine using DMF as the solvent and potassium carbonate as the base in the reaction.[12] The product **7b** was formed in 80% yield. The physical and spectroscopic data of the product **7b** are in good agreement with that reported for lamellarin G trimethyl ether.[7] Tetrakis(triphenylphosphine)palladium(0) could be used in place of palladium acetate and the reaction proceeded in the same yield. The oxidation of unsubstituted analogue **5a** with the above system also gave the required lactone **7a** in 80% yield.

## Acknowledgements

We acknowledge the financial contribution from the Thailand Research Fund (TRF) for the generous support of the research program. We also acknowledge the facilities in the Department of Chemistry provided by the PERCH program.

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(s, 1H, C-22Ar*H*), 6.99 (s, 1H, C-3Ar*H*). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 148.9, 147.8, 147.4, 147.2, 147.1, 147.0, 139.9, 128.8, 125.9, 124.0, 123.0, 121.8, 121.0, 120.0, 119.2, 117.8, 114.1, 113.7, 112.3, 111.1, 107.4, 106.9, 56.1, 56.0, 55.9 (2×C), 55.6, 55.2, 44.8, 38.0, 29.4. MS: 595 ($M^+$, 44.78), 516 (100.0), 499 (11.61), 485 (29.25), 470 (10.22), 442 (6.65), 426 (5.39), 410 (5.53), 243 (28.77). Anal. calcd for $C_{31}H_{33}NO_9S$: C, 62.49; H, 5.59; N, 2.35. Found: C, 62.46; H, 5.71; N, 2.07. 3-Formyl-2-(3″,4″-dimethoxy-2″-mesyloxyphenyl)-1-(3′,4′-dimethoxyphenyl)-8,9-dimethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline (**4b**) mp (MeOH): 192–193°C; FTIR ($CHCl_3$): $\nu_{max}$ 3026, 2939, 2839, 1643, 1531, 1483, 1465, 1426, 1263 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 2.89 (s, 3H, $OSO_2CH_3$), 3.00–3.20 (m, 2H, $CH_2CH_2N$), 4.51 (m, 1H, $CH_2CHHN$), 4.95 (m, 1H, $CH_2CHHN$), 3.35, 3.60, 3.68, 3.85, 3.89, 3.90 (6s, 18H, C-9, C-21, C-8, C-20, C-13 and C-14, 6×$OCH_3$), 6.57 (s, 1H, C-10Ar*H*), 6.76 (s, 2H, C-19, C-22Ar*H*), 6.82 (m, 3H, C-12, C-15, C-16Ar*H*), 6.87 (s, 1H, C-7Ar*H*), 9.45 (s, 1H, C-23C*H*O). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 180.2, 149.0 (2×C), 148.9, 148.0, 147.3, 147.2, 140.2, 133.8, 132.4, 126.8, 126.7, 125.7, 122.9, 122.3, 119.6, 118.0, 114.7, 114.0, 111.0, 110.7, 109.1, 106.5, 56.1, 55.9 (3×C), 55.8, 55.2, 42.3, 38.4, 28.7. MS: 623 ($M^+$, 20.55), 528 (100.0), 516 (18.48), 485 (9.73), 470 (6.23), 454 (3.81), 440 (3.73), 410 (3.57), 272 (35.41), 264 (61.22), 243 (38.99). Anal. calcd for $C_{32}H_{33}NO_{10}S$: C, 61.61; H, 5.34; N, 2.25. Found: C, 61.56; H, 5.28; N, 2.01.

# SYNTHESIS OF 4-ARYLTETRAHYDROISOQUINOLINES: APPLICATION TO THE SYNTHESIS OF CHERYLLINE

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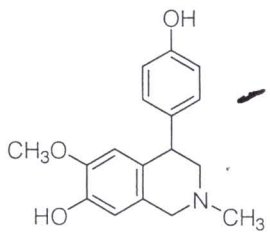
**Abstract-** A concise route for the synthesis of 4-aryltetrahydroisoquinolines was developed using the addition of Grignard reagents to nitrostyrene derivatives as the key step. The application to the synthesis of cherylline was described.

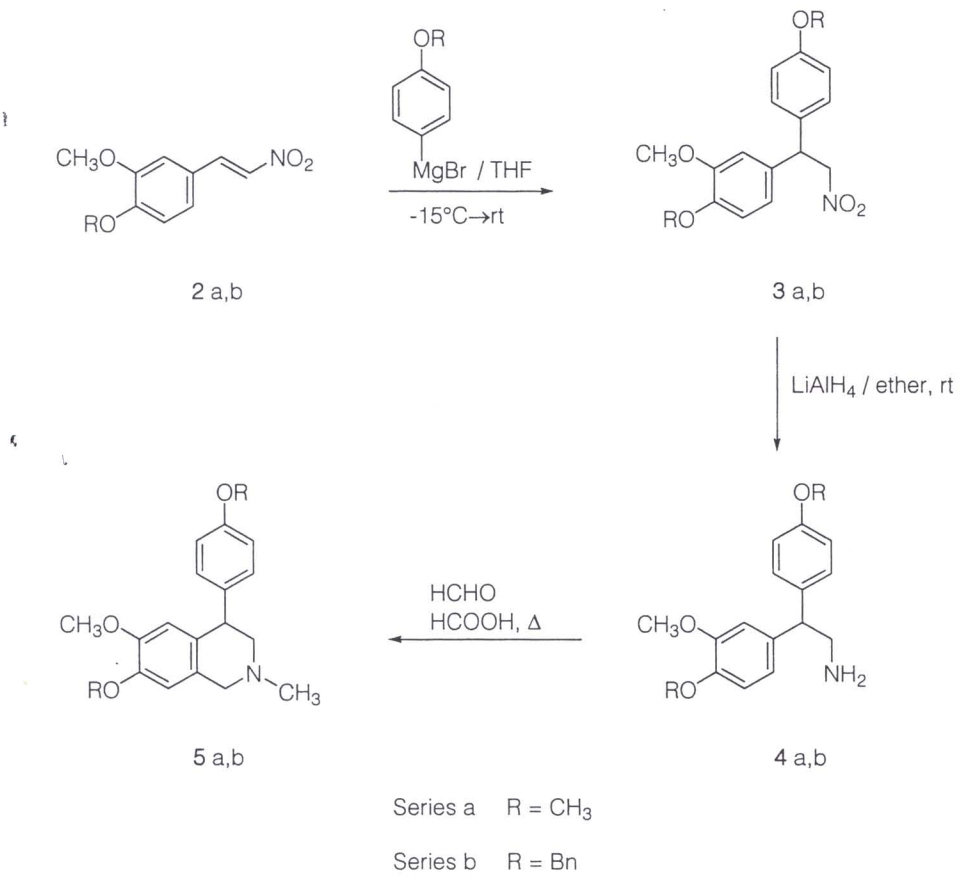
Cherylline has been isolated from *Crinum powellii* var. *alba* and other *Crinum* species.[1] It is a representative of the very rare natural 4-aryl-1,2,3,4-tetrahydroisoquinoline alkaloids. Due to the uniqueness of the structure and potential medicinal properties of the 4-arylisoquinoline derivatives,[2] many synthetic routes for these compounds[3] and especially cherylline (**1**)[4] have been reported. In the course of our synthetic work on alkaloids, we have successfully developed a new approach for the synthesis of cherylline.




Cherylline **1**

The key step of our approach involved conjugate addition of the Grignard reagents to the appropriate nitrostyrene derivative. Nitroalkene chemistry[5] has been exploited for various synthetic operations, particularly the potential usefulness of the conjugate addition of various organometallic reagents to the nitroalkenes as a means of forming carbon-carbon bonds, which may be followed by further

transformation of the derived nitroalkane products to various nitrogen heterocycles.[6] Recent application of the nitroalkene chemistry has been extended to the asymmetric synthesis[7] of various compounds as well as the solid phase synthesis.[8] The strategy of our approach is illustrated for the synthesis of (±)-dimethylcherylline and cherylline as shown (Scheme 1).




**Scheme 1**

We have found that by control of the exothermic reaction, 1,4-addition of Grignard reagent to nitrostyrene occurs smoothly at below room temperature in satisfactory yield. The Grignard reagent was prepared by addition of a solution of 4-bromoanisole in dry tetrahydrofuran to a stirred suspension of magnesium turning in dry tetrahydrofuran while maintaining a gentle reflux under nitrogen atmosphere. A solution of 3,4-dimethoxynitrostyrene in dry tetrahydrofuran was then added dropwise to the Grignard reagent which was maintained at –15 °C. The product (**3a**) was obtained in 69% yield after purification by column chromatography on silica gel. The nitro compound so obtained could be conveniently reduced by lithium aluminium hydride in ether at room temperature to give the amine (**4a**) in 75% yield. The amine was purified by preparative layer chromatography on silica gel and could be further purified by recrystallization of the derived oxalate salt. Treatment of the amine derivative with 37% formaldehyde

and formic acid at 100 °C for 3 h gave directly (±)-*O,O*-dimethylcherylline (**5a**) in 93% yield according to our previous finding that formaldehyde and formic acid promoted the cyclization and *N*-methylation of activated phenylethylamine in a "one pot" reaction.[9]

The successful preparation of (±)-*O,O*-dimethylcherylline prompted us to further investigate the generality of this method for the preparation of (±)-cherylline. Reaction of nitrostyrene (**2b**) with the Grignard reagent generated from 4-bromo-*O*-benzylphenol furnished nitro compound (**3b**) in 74% yield. Reduction of the addition product (**3b**) with LAH gave amine (**4b**) in 70% yield. The reaction of the amine with formaldehyde and formic acid gave the expected isoquinoline compound (**5b**) in 89% yield. The final step was accomplished by hydrogenolytic removal of the benzyl protecting groups of (±)-*O,O*-dibenzylcherylline in ethyl acetate-ethanol (1:1 by volume) containing 10 % palladium on charcoal at 1 atm. The reaction proceeded in 94 % yield to give(±)-cherylline (**1**). All the spectral data of the synthetic (±)-cherylline exhibited good correlation with those of (±)-cherylline reported in the literature.[4d] All new compounds exhibit satisfactory analytical and spectroscopic data.[10]

In conclusion, we have developed an efficient synthesis of (±)-*O,O*-dimethylcherylline (**5a**) and (±)-cherylline (**1**) from readily available starting materials and the reactions involved are operationally simple which we view as a very attractive process. The key step employed in the synthesis, *i.e.*, the addition of Grignard reagents to the nitrostyrenes works well under the employed conditions. The application of this approach to the synthesis of other alkaloids is under further investigation.

## ACKNOWLEDGEMENTS

We are grateful to the generous financial support of the Thailand Research Fund (TRF). We also acknowledge the facilities provided by the PERCH programme.

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10. All compounds have been fully characterized : Compound (**3a**), oil, IR (neat) 1605, 1590, 1545, 1510, 1460, 1375, 1250, 1140, 1025 $cm^{-1}$. NMR (400 MHz, $CDCl_3$) δ 3.78 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 4.80 (br t, 2H), 4.915 (d, 1H, *J* = 7.2 Hz), 4.918 (d, 1H, *J* = 9.4 Hz), 6.70 (d, 1H, *J* = 2.0 Hz), 6.77 (dd, 1H, *J* = 8.0, 2.0 Hz), 6.82 (d, 1H, *J* = 8.0 Hz), 6.86, 7.15 (AA'BB', 2H each, *J* = 8.7 Hz). $^{13}C$ (100 MHz) δ 47.83, 55.22, 55.86, 79.61, 111.14, 111.34, 114.33, 119.27, 128.58, 131.35, 131.92, 148.35, 149.23, 158.86. MS 317($M^+$, 55.43), 271(22.44), 270(100), 257(53.92), 239(15.35). Anal. Calcd for $C_{17}H_{19}NO_5$ : C, 64.34; H, 6.03; N, 4.41. Found : C, 64.21; H, 6.17; N, 4.29. Compound (**3b**), oil, IR (neat) 1600, 1580, 1545, 1510, 1500, 1450, 1375, 1235, 1140, 1020 $cm^{-1}$. NMR (400 MHz, $CDCl_3$) δ 3.83 (s, 3H), 4.78 (br t, 1H), 4.888 (d, 1H, *J* = 7.5 Hz), 4.890 (d, 1H, *J* = 8.7 Hz), 5.02 (s, 2H), 5.11 (s, 2H), 6.69 (dd, 1H, *J* = 8.0, 2.0 Hz), 6.72 (d, 1H, *J* = 2.0 Hz), 6.82 (d, 1H, *J* = 8.0 Hz), 6.92, 7.13 (AA'BB', 2H each, *J* = 8.7 Hz), 7.26-7.43 (m, 10H). $^{13}C$ (100 MHz) δ 47.86, 56.03, 70.02, 70.99, 79.57, 111.72, 114.02, 115.24, 119.30, 127.21, 127.45, 127.86, 128.02, 128.54, 128.59, 128.65, 131.58, 132.41,136.76, 136.98, 147.57, 149.86, 158.10. MS 469($M^+$, 5.90), 422(2.20), 331 (6.13), 92(8.67), 91(100). Anal. Calcd for $C_{29}H_{27}NO_5$ : C, 74.18; H, 5.80; N, 2.98. Found : C, 74.01; H, 6.10; N, 2.65. Compound (**4a**), m.p. (oxalate salt, MeOH-ether) 108-109 °C. IR (KBr) 2930(br), 1610, 1590, 1515, 1250, 1150, 1025 $cm^{-1}$. NMR (400 MHz, $CDCl_3$) δ 1.56 (br s, 2H), 3.27 (br s, 2H), 3.77 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 3.88 (t, 2H, *J* = 7.5 Hz), 6.73 (d, 1H, *J* = 1.6 Hz), 6.79 (dd, 1H, *J* = 8.0, 1.7 Hz), 6.82 (d, 1H, *J* = 8.3 Hz), 6.85, 7.16 (AA'BB', 2H each, *J* = 8.7 Hz). $^{13}C$ (100 MHz) δ 7.16, 53.77, 55.19, 55.81, 55.84, 111.24, 111.53, 113.97, 119.67, 128.84, 135.01, 135.59, 147.59, 148.99, 158.14. MS 287($M^+$, 4.49), 258(26.96), 257(100). Anal. Calcd for $C_{19}H_{23}NO_7 \cdot 1/2\ H_2O$ : C, 59.06; H, 6.26; N, 3.62. Found : C, 59.40; H, 6.18; N, 3.61. Compound (**4b**), m.p. (oxalate salt, MeOH-ether) 114-116 °C. IR (KBr) 3050-2950(br), 1605, 1580, 1505, 1250, 1225, 1140, 1020 $cm^{-1}$. NMR (400 MHz, $CDCl_3$) δ 1.4 (br s, 2H), 3.23 (d, 2H, *J* = 7.7 Hz), 3.86 (t, 1H, *J* = 7.6 Hz), 3.84 (s, 3H), 5.03 (s, 2H), 5.12 (s, 2H), 6.72 (dd, 1H, *J* = 8.0, 2.0 Hz), 6.75 (d, 1H, *J* = 2.0 Hz), 6.82 (d, 1H, *J* = 8.0 Hz), 6.92, 7.15 (AA'BB', 2H each, *J* = 8.7 Hz), 7.26-7.44 (m, 10H). $^{13}C$ (100 MHz) δ 7.26, 53.85, 55.99, 69.99, 71.16, 112.13, 114.03, 114.89, 119.72, 127.21, 127.44, 127.74, 127.91, 128.48, 128.54, 128.90, 135.29, 136.17, 137.28, 146.81, 149.63, 157.39. MS 439($M^+$, 1.47), 410 (15.02), 409(38.12), 92(8.33), 91(100), 44(11.28). Anal. Calcd for $C_{31}H_{31}NO_7 \cdot 1/2\ H_2O$: C, 69.13; H, 5.99; N, 2.60. Found : C, 68.73; H, 5.90; N, 2.82. Compound (**5a**), m.p. (ether-hexane) 97-99 °C (lit.,[4d] 97-99 °C). IR ($CHCl_3$) 1610, 1580, 1515, 1460, 1250, 1140, 1035 $cm^{-1}$. NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3H), 2.48 (dd, 1H, *J* = 8.0, 11.4 Hz), 2.97 (ddd, 1H, *J* = 11.6, 5.5, 1.0 Hz), 3.54 (d, 1H, *J* = 14.3 Hz), 3.66 (d, 1H, *J* = 14.3 Hz), 3.64 (s, 3H), 3.79 (s, 3H), 3.85 (s, 3H), 4.16 (br t, 1H),

6.35 (s, 1H), 6.56 (s, 1H), 6.83, 7.10 (AA'BB', 2H each, $J$ = 8.7 Hz). $^{13}$C (100 MHz) δ 44.64, 45.86, 55.14, 55.78, 58.07, 62.10, 108.70, 111.89, 113.65, 127.27, 129.15, 129.81, 136.82, 147.40, 147.49, 158.11. MS 313($M^+$, 35.43), 270(52.86), 269(20.66), 240(19.76), 239(100), 135(11.42). Compound (**5b**), m.p. (ethyl acetate) 141-142 °C(lit.,[4c,4g] 144-145 °C). IR ($CHCl_3$) 1610, 1580, 1505, 1255, 1240, 1140 $cm^{-1}$. NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3H), 2.47 (dd, 1H, $J$ = 11.0, 8.0 Hz), 2.96 (dd, 1H, $J$ = 11.0, 5.0 Hz), 3.48 (d, 1H, $J$ = 14.5 Hz), 3.59 (d, 1H, $J$ = 14.5 Hz), 3.65 (s, 3H), 4.15 (dd, 1H, $J$ = 8, 6 Hz), 5.04 (s, 2H), 5.11 (s, 2H), 6.37 (s, 1H), 6.58 (s, 1H), 6.91, 7.10 (AA'BB', $J$ = 8.7 Hz), 7.27-7.46 (m, 10H). $^{13}$C (100 MHz) δ 44.69, 45.84, 55.99, 57.99, 62.04, 70.01, 71.06, 111.58, 112.59, 114.64, 127.27, 127.50, 127.74, 127.91, 128.48, 128.53, 129.82, 129.90, 137.12, 137.25, 146.66, 148.23, 157.42. MS 465($M^+$, 7.28), 422(10.06), 331(15.52), 91(100).

Received, 29th January, 2001

SYNTHETIC COMMUNICATIONS, 31(11), 1765–1769 (2001)

# THE FIRST SYNTHESIS OF WRIGHTIADIONE

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## ABSTRACT

The first synthesis of tetracyclic isoflavone wrightiadione 1 was achieved through the benzylic oxidation of the key intermediate isoflavone 2 which in turn could be obtained by condensation of 2-indanone with methyl salicylate and LDA.

Wrightiadione 1, a novel isoflavone, was isolated from the bark of *Wrightia tomentosa* which has been used as a medicinal plant in Thailand.[1] This compound contains a unique tetracyclic ring system which represents the first natural tetracyclic isoflavone.

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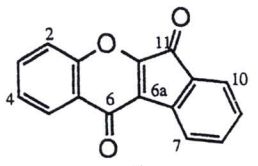
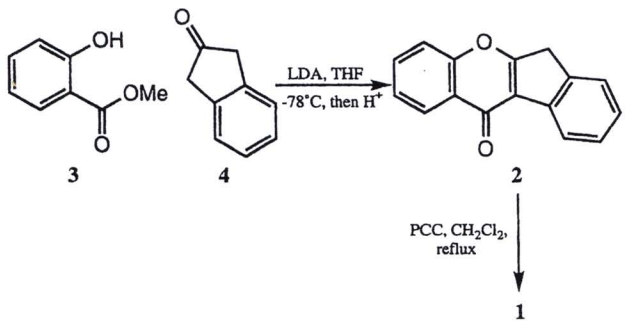


1

The retrosynthetic analysis of compound **1** suggested isoflavone **2** as the key intermediate as methylene position of this compound **2** could be oxidised to the target molecule **1**. Compound **2** could conceivably be prepared from the condensation of methyl salicylate **3** with 2-indanone **4**.

It was found that isoflavone **2** could be synthesised from the reaction of 2-indanone **4** and the unprotected methyl salicylate **3** using 2.2 equivalents of LDA as the base in THF at–78 °C in moderate yield (49 %) as shown in the scheme. The condensation of the 2-indanone **4** with O-benzyl methyl salicylate failed to give the required product. The formation of phenolate apparently activates methoxide as a leaving group. The use of unprotected methyl salicylate in the condensation with the enolate has recently been reported.[2]




3 4 2 1

***Scheme.***

In order to obtain the final product, the key benzylic oxidation had to be performed. The benzylic oxidation to the corresponding carbonyl group is a well known process. We have investigated many oxidising agents for the above transformation and the results are shown in the table. We found that oxidation with PCC in methylene chloride gave the best result yielding wrightiadione **1** in 68% yield.

There are some discrepancies between the NMR spectral data of our synthetic compound and those reported for the natural product.[14] In the $^{13}C$ NMR spectrum of the natural product the carbonyl group absorption at

*Table.* Yield of Wrightiadione **1** from Various Oxidation Methods

| Entry | Conditions | time(h) | % yield **1** |
|---|---|---|---|
| 1 | PDC, celite 535, benzene, reflux[3] | 8 | 39 |
| 2 | PDC, celite 535, benzene, reflux[3] | 18 | 44 |
| 3 | $SeO_2$.EtOH, $H_2O$, reflux[4,5] | 7 | 8 |
| 4 | $CuBr_2$, EtOAc, reflux[6,7] | 4 | 33 |
| 5 | 50 % HBr, DMSO, reflux[8] | 8 | 31 |
| 6 | CAN, MeOH, RT[9] | 15 | 17 |
| 7 | CAN, AcOH, reflux[10] | 2 | 22 |
| 8 | PCC, $CH_2Cl_2$, reflux[11,12] | 20 | 68 |
| 9 | NHPI*,$CH_3CN$, reflux[13] | 20 | 25 |

*NHPI=N-Hydroxyphthalimide

C-11 was not observed, while in the $^{13}C$ NMR of the synthetic compound the C-6a absorption was not detected. We have confirmed the structure of compounds **1** and **2** by 1D and 2D NMR techniques. The structure of our synthetic compound was confirmed by X-ray crystallography and found to be identical to that reported for natural wrightiadione.

## EXPERIMENTAL

Melting points are uncorrected. Nuclear magnetic resonance (NMR) data for $^1H$ NMR were taken at 400 MHz and $^{13}C$ NMR at 100 MHz. Tetramethylsilane was used as the internal standard, and chemical shifts are reported as $\delta_H$ (ppm) or $\delta_C$ (ppm). Mass spectra (MS) were obtained by electron impact technique (EI). Elemental analyses were performed at the Faculty of Science, Mahidol University.

### Benz[b]indeno[1,2-e]pyran-6-one (2): Reaction of 2-Indanone 4 with Methyl Salicylate 3

A solution of LDA in dry THF (150 mL) was prepared by adding diisopropylamine (16.8 mL, 0.12 mol) dropwise into 1.29 M of *n*-BuLi (85 mL, 0.11 mol) in hexane under nitrogen atmosphere at 0 °C. The ice-water bath was replaced by a dry ice/acetone bath. The stirring was continued for 30 min at–78 °C, then 2-indanone **4**(6.60 g, 0.05 mol)in dry THF (50 mL) was added. The pale yellow solution turned deep red, indicating anion formation. The reaction mixture was allowed to warm to 0°C by

ice-water bath, stirred for 10 min and the ice-water bath was replaced by a dry ice/acetone bath. The solution of methyl salicylate **3** (7.60 g, 0.05 mol) in dry THF(50 mL) was added slowly by syringe to the above mixture. The stirring at this temperature was continued for 2 h and then the reaction mixture was warmed to room temperature, then 2 N HCl was added and stirred for 1 h. Removal of the solvent under vacum gave a residue which was extracted with methylene chloride. The combined organic extracts were washed with aqueous sodium carbonate solution, water and brine solution, and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was chromatographed on silica gel column to provide starting material, methyl salicylate **3** and benz[b]indeno[1,2-e]pyran-6-one **2** as adduct, respectively. After recrystallization of the adduct from ethanol benz[b]indeno[1,2-e]pyran-6-one **2** (5.71 g, 49 %) was obtained as a colorless crystal: mp 188-189 °C(lit.[15] 176-177 °C,); IR(Nujol) 1640(C = 0), 1615 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 3.91 (s, 2H, *H* 11), 7.26(ddd, 1H, J = 8.0 Hz, 1.1, *H*,7.6, 9), 7.38(t, 1H, J = 7.6, 7.6 Hz, *H* 8), 7.42(d, 1H, J = 8.0 Hz, *H* 10), 7.45(ddd,1H, J = 0.8, 7.7, 7.9 Hz, *H* 4), 7.52(dd, 1H, J = 0.8, 8.4 Hz, *H* 2), 7.66(ddd, 1H, J = 1.6, 7.7, 8.4 Hz, *H* 3), 8.23 (dd, 1H, J = 1.1, 7.6 Hz, *H* 7), 8.34 (dd, 1H, J = 1.6, 7.9 Hz, *H* 5); $^{13}C$ NMR (100 MHz, $CDCl_3$) ppm 36.7(C-11), 118.0(C-2), 121.1(C-6a), 122.6(C-7), 123.7(C-10), 124.8(C-5a), 125.1 (C-4), 125.8 (C-9), 126.1 (C-5), 127.4 (C-8), 132.9 (C-3), 134.1 (C-10a), 138.2 (C-6b), 156.3 (C-1a), 171.8 (C-11a), 174.2 (C-6); MS(EI) *m/z* 234($M^+$, 100), 205(29), 176(15), 76(20). Anal.calcd for $C_{16}H_{10}O_2$: C, 82.04; H, 4.30. Found: C, 82.17; H,4.07.

### Wrightiadione (1): Oxidation of Benz[b]indeno[1,2-e]pyran-6-one 2 with PCC in Methylene Chloride

The mixture of benz[b]indeno[1,2-e]pyran-6-one 2(0.24 g, 1.0 mmol) and pyridinium chlorochromate (0.2 g) was heated under reflux with string in methylene chloride (20 mL) for 3 h. After the precipitate was filtered and washed thoroughly with methylene chloride, the residue was obtained after evaporation of the solvent under reduced pressure. The orange residue was recrystallized from ethanol to give orange crystals of 1(0.18 g, 68%): mp 244–246 °C (lit.[1] mp 228-230 °C); IR (Nujol) 1727 (C=0), 1641 (C=0), 1614, 1605, 1483, 1459, 1371 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.30(ddd, 1H, J=0.9, 7.3, 7.6 Hz, *H* 9), 7.53 (ddd, 1H, J=1.1, 7.3, 7.6 Hz, *H* 8), 7.54 (ddd, 1H, J=1.1, 7.1, 8.0 Hz, *H* 4), 7.61 (bd, 1H, J=7.3 Hz, *H* 7), 7.69 (dd,1H, J=1.1, 8.5 Hz, *H* 2), 7.80 (ddd, 1H, J=1.6, 7.1, 8.5 Hz, *H* 3), 7.96 (dd, 1H, J=0.9, 7.3 Hz, *H* 10), 8.30 (dd, 1H, J=1.6, 8.0 Hz, *H* 5) and $^{13}C$ NMR (100 MHz, $CDCl_3$) ppm120.7(C-2), 125.1(C-10), 126.1 (C-7), 127.4(C-5),

127.4 (C-5a), 128.3 (C-11a), 130.1 (C-9), 136.1 (C-3), 137.4 (C-8), 141.4(C-10a), 157.3 (C-6b), 157..6 (C-1a), 176.7 (C-6), 189.67 (C-11), C-6a absorption was not observed; MS(EI) *m/z* 248 ($M^+$, 100), 220(24), 164(12).

## ACKNOWLEDGMENTS

Financial support from Thailand Research Fund (TRF) is gratefully acknowledged. The X-ray crystallographic analysis was kindly carried out by Dr. Palangpon Kongseri of Mahidol University.

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Received in the UK June 19, 2000

# A NEW 12a-HYDROXYELLIPTONE FROM THE STEMS OF *DERRIS MALACCENSIS*

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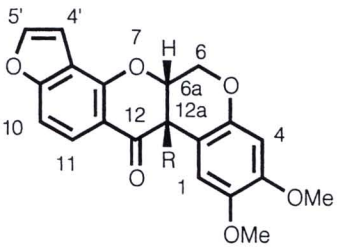
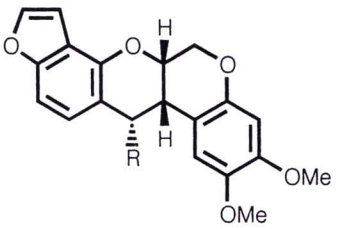
**Abstract-** A new rotenoid, 12a-hydroxyelliptone **(1)** and the known rotenoid 12-deoxo-12α-acetoxyelliptone **(2)** were isolated from the stems of *Derris malaccensis*. The structures were established by spectroscopic and chemical methods as well as by comparison with published data.

Many plants in the family Leguminosae, especially in the genera *Derris*, *Lonchocarpans*, *Millettia*, *Mundulea* and *Tephrosia* have been used as fish poison and insecticides.[1,2] These plants were often used by the native population to treat infestations of insect parasites and some other pests.[1] *Derris* plants are found throughout the tropical regions of Asia and East Africa and are widely used in cattle and sheep dips for the control of ticks and other ectoparasites.[1] It is currently used in horticulture against aphids, caterpillars, sawflies, wasps, raspberry beetles and red spiders.[1]

*Derris malaccensis* is a Thai plant locally known as "haang lai kaow". The plant is used for pest control and fish poison[2,3] and has not been previously studied chemically. In this paper, we report the isolation and structural elucidation of a new rotenoid, 12a-hydroxyelliptone **(1)**, and the known rotenoid 12-deoxo-12α-acetoxyelliptone **(2)**.[4] A number of 12a-hydroxyrotenoid derivatives have recently been isolated.[5-11] Compound **(1)** was isolated from the hexane extract of the plant after preparative TLC. The molecular formula of $C_{20}H_{16}O_7$ was established from the elemental analysis and MS spectrum exhibiting the molecular peak at *m/z* 368. It showed UV maxima ($CHCl_3$) at 281, 242 and 233 nm, and IR absorptions

($CHCl_3$) at 3450 (br, OH) and 1675 $cm^{-1}$ (chelated C=O). The $^1H$ NMR spectrum showed a hydroxyl signal at δ 4.53, two aromatic singlets at δ 6.56 and 6.45, four aromatic proton signals at δ 7.87, 7.56, 7.17 and 6.95 which were reminiscent of 4,5-disubstituted benzofuran ring, and two methoxy singlets at δ 3.78 and 3.70. A pair of nonequivalent methylene proton signals at δ 4.70 and 4.56 and a methine proton doublet at δ 4.74 are similar to ABC system (δ 4.56, 1H, d, $J$ = 12.0 Hz; δ 4.70, 1H dd, $J$ = 12.0, 2.3 Hz; δ 4.74, 1H, d, $J$ = 2.3 Hz) corresponding to a O-$CH_2$-CH-O segment. In agreement with the latter assignment, $^{13}C$ NMR spectrum of **1** showed methylene and methine carbon resonances at δ 64.0 and 76.8, respectively. The $^1H$ NMR spectrum of **1** is similar to that of elliptone **(5)** [12] except for a hydroxy group in place of the α-hydrogen at C-12a. The structure of **1** was further supported by the $^{13}C$ NMR spectrum which showed a quaternary carbon of C-12a at δ 67.9 as compared with δ 44.0 of **5**.[13] The aforementioned data suggested the structure of **1** as 12a-hydroxyrotenoid, the hydroxyl group could be acetylated by acetic anhydride in pyridine to give the corresponding acetate derivative **(3)**, $C_{22}H_{18}O_8$, which showed in its $^1H$ NMR spectrum the signal of an aliphatic acetyl group (δ 1.80), assigned to the group attached at 12a position.




**1** 12a-hydroxyelliptone, R = OH
**3** 12a-acetoxyelliptone, R = OAc
**5** elliptone, R = H

**2** 12-deoxo-12α-acetoxyelliptone, R = OAc
**4** elliptinol, R = OH

In the MS spectrum of **1**, two fragment ion peaks at *m/z* 160 and 208 resulting from cleavage between the B/C rings of 6a, 12a-saturated rotenoids revealing that ring D possessed benzofuran ring (*m/z* 160), and rings A and B had two methoxyl and one hydroxyl groups (*m/z* 208). Similar fragmentation was observed for acetate derivative **3** giving rise to two corresponding fragments at *m/z* 160 and *m/z* 250. The H-1 chemical shift value (δ 6.56) indicated that the B/C ring junction in **1** was *cis*.[14-15] The *cis* stereochemistry at 6a and 12a positions was also supported from the acetylation product of 12a-hydroxyroteniods. In the NMR spectrum, the 6a-H of 12a-acetoxyelliptone **(3)** was significantly shifted to lower field as compared to 6a-H of 12a-hydroxyroteniods **(1)**. This anisotropic effect is possible when 6a-H and acetoxy group are in the *cis* relationship, but we cannot conclude whether the above formula or its enantiomeric form represents its actual absolute configuration.[16,17] Compound **(2)** was identified to be 12-deoxo-12α-acetoxyelliptone **(2)** by comparison of its mp, $[\alpha]^{20}_D$, UV, IR, $^1H$ and $^{13}C$ NMR, and

MS data with published values.[4] The $^1H$ and $^{13}C$ NMR assignments of **2** were obtained through analysis of the $^1H$-$^1H$ and $^1H$-$^{13}C$ COSY spectra. Hydrolysis of **2** was accomplished by 50% NaOH in methanol to give elliptinol **(4)**.[4]

## EXPERIMENTAL

**General:** MPs were determined on an electrothermal melting point apparatus (Electothermal 9100) and are uncorrected. Optical rotations were measured with a JASCO DIP-370 Digital Polarimeter. UV spectra were taken in EtOH on a JASCO Uvidex-650 double beam spectrometer. IR spectra were recorded in a chloroform solution on a JASCO A-302 spectrometer. MS spectra were measured on AEI-MS-902 instrument. $^1H$, $^{13}C$ NMR spectra were recorded on Bruker AM400 at 400 or 100 MHz and Bruker ACF 200 at 200 or 50 MHz using TMS as internal standard.

**Plant material:** Stems of *D. malaccensis* were collected from Ubonradchathanee Province during 1990. Botanical identification was achieved through comparison with a specimen from the Forest Herbarium, Bangkok, Thailand. A herbarium voucher specimen is retained at the Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand.

**Extraction and Isolation:** Dried powdered stems of *D. malaccensis* (1 kg) were extracted with hexane (7L) at room temperature for 1 week. After filtration the extract was evaporated to give the residue (13.1 g). The stems left after hexane extraction were further extracted with EtOAc (6.5L) and MeOH (7L) each at room temperature for 1 week to give the residues (17.6 g and 104.0 g respectively). The residues from hexane extract and MeOH extract were each chromatographed on silica gel using hexane, $CHCl_3$ and increasing polarity of MeOH giving combined 12a-hydroxyelliptone **(1)** (209 mg) and 12-deoxo-12α-acetoxyelliptone **(2)** (313 mg).

**12a-Hydroxyelliptone (1):** Colorless needles (EtOH): mp >200°C (decomp); $[\alpha]^{20}_D$ +24.6° (*c* = 0.25, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ $cm^{-1}$ 3450 (OH), 1675 (C=O), 1617 (C=C); UV $\lambda_{max}$ ($CHCl_3$) (log $\varepsilon$) 233 (4.6), 242 (2.3), 281 (0.7) nm; $^1H$-NMR (400 MHz, $CDCl_3$): δ 3.70 (3H, *s*, $OCH_3$-2), 3.78 (3H, *s*, $OCH_3$-3), 4.53 (1H, *s*, 12a-OH, $D_2O$ exchangeable), 4.56 (1H, *d*, *J* = 12.0 Hz, H-6β), 4.70 (1H, *dd*, *J* = 12.0, 2.3 Hz, H-6α), 4.74 (1H, *d*, *J* = 2.3 Hz, H-6a), 6.45 (1H, *s*, H-4), 6.56 (1H, *s*, H-1), 6.95 (1H, *dd*, *J* = 2.3, 1.1 Hz, H-4'), 7.17 (1H, *dd*, *J* = 8.6, 1.1 Hz, H-10), 7.56 (1H, *d*, *J* = 2.3 Hz, H-5'), 7.87 (1H, *d*, *J* = 8.6 Hz, H-11); $^{13}C$-NMR (100 MHz, $CDCl_3$): δ 56.0 ($OCH_3$-3), 56.4 ($OCH_3$-2), 64.0 (C-6), 67.9 (C-12a), 76.8 (C-6a), 101.2 (C-4), 104.8 (C-4'), 107.1 (C-10), 108.7 (C-12b), 109.4 (C-1), 112.0 (C-8), 117.5 (C-11a), 124.0 (C-11), 144.0 (C-2), 145.0 (C-5'), 148.4 (C-4a), 151.2 (C-3), 156.0 (C-7a), 160.7 (C-9), 192.1 (C-12); EIMS *m/z* (rel. int.): 368 $[M]^+$ (11), 208 $[C_{11}H_{12}O_4]^+$ (100), 207 $[C_{11}H_{11}O_4]^+$ (44), 193 $[C_{10}H_9O_3]^+$ (10), 160 $[C_9H_4O_3]^+$ (8); Anal. Calcd for $C_{20}H_{16}O_7$: C, 65.24; H, 4.38. Found: C, 65.01; H, 4.19.

**12a-Acetoxyelliptone (3):** Colorless needles (EtOH): mp >230°C (decomp); $[\alpha]^{20}_D$ +11.3° (*c* = 0.25, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ $cm^{-1}$ 1736 (C=O), 1680 (C=C), 1617 (C=C); UV $\lambda_{max}$ ($CHCl_3$) (log $\varepsilon$) 235 (3.9),

238 (3.1), 242 (2.4), 280 (0.7) nm; $^1$H-NMR (200 MHz, $CDCl_3$): δ 2.16 (3H, *s*, 12a-$OCOCH_3$), 3.75 (3H, *s*, $OCH_3$-2), 3.80 (3H, *s*, $OCH_3$-3), 4.39 (1H, *dd*, *J* = 12.0, 1.0 Hz, H-6β), 4.68 (1H, *dd*, *J* = 12.0, 2.3 Hz, H-6α), 5.59 (1H, *m*, H-6a), 6.47 (1H, *s*, H-4), 6.85 (1H, *s*, H-1), 6.88 (1H, *dd*, *J* = 2.3, 1.0 Hz, H-4'), 7.16 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-10), 7.55 (1H, *d*, *J* = 2.3 Hz, H-5'), 7.92 (1H, *d*, *J* = 8.0 Hz, H-11); $^{13}$C-NMR (50 MHz, $CDCl_3$): δ 21.4 ($OCOCH_3$), 55.9 ($OCH_3$-3), 56.3 ($OCH_3$-2), 63.8 (C-6), 73.2 (C-6a), 74.8 (C-12a), 100.8 (C-4), 103.2 (C-12b), 104.8 (C-4'), 107.1 (C-10), 110.8 (C-1), 113.6 (C-8), 117.2 (C-11a), 124.4 (C-11), 144.2 (C-2), 145.1 (C-5'), 149.5 (C-4a), 151.9 (C-3), 154.8 (C-7a), 160.6 (C-9), 170.0 (COO), 186.1 (C-12); EIMS *m/z* (rel. int.): 410 $[M]^+$ (11), 250 $[C_{13}C_{14}O_5]^+$ (43), 208 $[C_{11}H_{12}O_4]^+$ (100), 207 $[C_{11}H_{11}O_4]^+$ (43), 160 $[C_9H_4O_3]^+$ (6); Anal. Calcd for $C_{22}H_{18}O_8$: C, 64.39; H, 4.42. Found: C, 64.13; H, 4.52.

**12-Deoxo-12α-acetoxyelliptone (2):** Colorless needles (EtOH): mp 153-155°C (decomp) (lit.,[4] mp 150-152°C); $[\alpha]^{20}_D$ –320.5° (*c* = 0.5, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ $cm^{-1}$ 1726 (O-C=O), 1620 (C=C); UV $\lambda_{max}$ ($CHCl_3$) (lgg ε) 250 (3.07), 258 (2.80), 292 (1.76) nm; $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.80 (3H, *s*, 12-$OCOCH_3$), 3.67 (1H, *dd*, *J* = 4.9, 4.7 Hz, H-12a), 3.84 (6H, *s*, $OCH_3$-2 and $OCH_3$-3), 4.33 (1H, *dd*, *J* = 10.5, 4.9 Hz, H-6β), 4.53 (1H, *t*, *J* = 10.5 Hz, H-6α), 5.00 (1H, *m*, H-6a), 6.43 (1H, *s*, H-4), 6.70 (1H, *s*, H-1), 6.87 (1H, *dd*, *J* = 2.2, 1.0 Hz, H-4'), 7.11 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-10), 7.21 (1H, *d*, *J* = 8.0 Hz, H-11), 7.55 (1H, *d*, *J* = 2.2 Hz, H-5'); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 21.0 ($OCOCH_3$), 37.0 (C-12a), 56.0 ($OCH_3$-3), 57.0 ($OCH_3$-2), 64.5 (C-6), 67.0 (C-12), 69.5 (C-6a), 101.0 (C-4), 104.0 (C-4'), 105.5 (C-10), 109.0 (C-12b), 111.5 (C-8), 112.5 (C-1), 117.5 (C-11a), 127.0 (C-11), 144.0 (C-2), 144.5 (C-5'), 147.0 (C-4a), 149.0 (C-3), 149.5 (C-7a), 157.0 (C-9), 170.0 (COO); EIMS *m/z* (rel. int.): 396 $[M]^+$ (7), 192 $[C_{11}H_{12}O_3]^+$ (100), 191 $[C_{11}H_{11}O_3]^+$ (23); Anal. Calcd for $C_{22}H_{20}O_7$: C, 66.66; H, 5.09. Found: C, 66.74; H, 4.95.

**ACKNOWLEDGEMENT**

We are grateful for the Thailand Research Fund (TRF) for the generous support of our research program. One of the authors (NT) acknowledges the scholarship from National Science and Technology Development Agency (NSTDA). We also acknowledge the facilities in the Department of Chemistry provided by the PERCH programme.

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Received, 22nd February, 2001

Pergamon

Tetrahedron Letters 43 (2002) 4515–4517

TETRAHEDRON LETTERS

# The application of the Baker–Venkataraman rearrangement to the synthesis of benz[*b*]indeno[2,1-*e*]pyran-10,11-dione

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Received 4 March 2002; revised 15 April 2002; accepted 25 April 2002

Abstract—A tetracyclic benzocyclopentabenzopyran-4-one was synthesized via a domino reaction involving an initial aroyl transfer as in the Baker–Venkataraman rearrangement. The derived 1,3 diketone underwent the intramolecular acylation followed by cyclization to give the product. © 2002 Elsevier Science Ltd. All rights reserved.

Benz[*b*]indeno[2,1-*e*]pyran-10,11-dione **1a** and analogues have been prepared and studied for enhancing the biosynthesis of erythropoietin (Epo), a hematopoietic growth factor which stimulates differentiation and supports the survival of cells of the erythroid lineage, by Williams's group.[1] Their synthetic route to **1b** involved sulfoxide chemistry and a symmetrical dialdehyde followed by further manipulation which led to benz[*b*]indeno[2,1-*e*]pyran-10,11-dione **1a** (Fig. 1).[1] Similarly, a multi-step synthesis of coniochaetone A **2**, an antifungal cyclopentabenzopyran-4-one from a coprophilous fungus *Coniochaeta sarcardoi*,[2] involving sulfoxide chemistry was reported by Mori.[3]

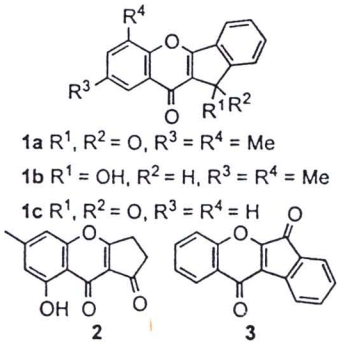


Figure 1.

We have recently reported a successful synthesis of wrightiadione **3**,[4] a rare and unusual oxygen heterocycle isolated from the bark of *Wrightia tomentosa*,[5] a medicinal plant of Thailand. The methanol extract of the dried leaves and stems of this plant showed weak activity against human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT). With this finding and the reported interesting biological activity of benz[*b*]indeno[2,1-*e*]pyran-10,11-dione, we undertook a study of the synthesis of these compounds.

Herein, we present our strategy leading to a short synthesis of **1c** and 2-benzyl benzofuran-3-one **11**. Compound **1c** has been previously synthesized by two routes, however the use of symmetrical intermediates in both of these reports[1,6] creates difficulties when the indenone moiety is to be substituted.

We envisaged that benz[*b*]indeno[2,1-*e*]pyran-10,11-dione **1c** could be obtained via two synthetic routes as shown in the retrosynthetic analysis in Scheme 1. It was expected that ring closure of the chalcone intermediate[7] **5** could lead to the flavanone **6** which could undergo further intramolecular acylation and oxidation to give the product. Alternatively, the flavone **7** could be obtained by application of the Baker–Venkataraman rearrangement (BK–VK).[8,9] Further cyclization either through flavanone **6** or directly could then lead to the required compound.

Having the above ideas in mind, we studied the first route. We attempted to prepare chalcone **6** using a

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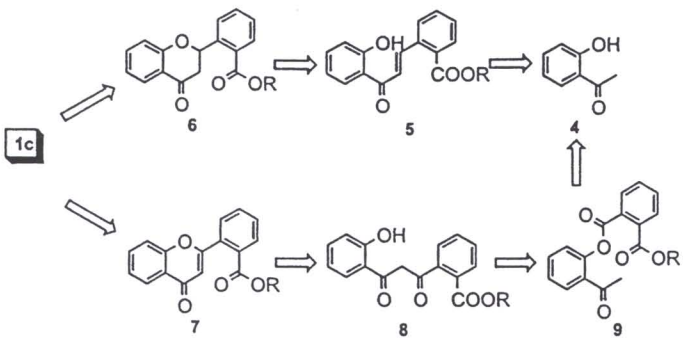
PII: S0040-4039(02)00818-3




Scheme 1.

general procedure by treatment of 2-hydroxy acetophenone **4** and phthaldehydic acid **10** with KOH in MeOH under reflux for 8 h. Acidification (2N HCl) of the mixture yielded not the expected chalcone **5** but the 2-benzyl benzofuran-3-one **11** in 76% yield after recrystallization from ethanol (Scheme 2). The structure of **11** was confirmed using IR and $^1$H NMR.[10] The IR spectrum of **11** exhibited a band at 1634 cm$^{-1}$ (C=O of carboxylic group) and a band at 1764 cm$^{-1}$ (C=O of furanone system). The $^1$H NMR spectrum of **11** showed a triplet at $\delta_H$ 6.14 for the methine C-2 proton. The formation of the 2-benzyl benzofuran-3-one **11** can be explained through the preferred 5-*exo* trig cyclization of chalcone **5** (R = H) induced by the electron withdrawing group at the C-2′ position rather than the 6-*endo* trig cyclization.

To investigate the second route, acylation of **4** with *mono* methyl phthalate **12** using Steglich esterification[11] gave *o*-benzoylacetophenone **9** in 68% yield (Scheme 2).

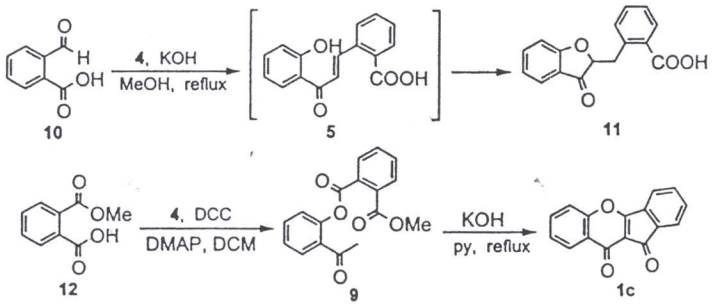
The BK–VK rearrangement of **9** was carried out with potassium hydroxide in pyridine, under reflux for 30 min. The mixture was then poured into 2N hydrochloric acid solution which led to the precipitation of a yellow solid.

After recrystallization from ethanol, yellow crystals were obtained in 72% yield. The structure of the product obtained under these conditions was elucidated by IR, NMR and MS and it was gratifying to find that the product was *not* the expected flavanone **7** (R = Me) but the ultimate target compound **1c**.[12] The IR spectrum of **1c** showed a band at 1620 cm$^{-1}$ (C=O of chromone system) and a band at 1710 cm$^{-1}$ (C=O of indenone system) which corresponded with two peaks due to the ketone carbonyls in the $^{13}$C NMR at $\delta$ 179.2 and 187.6. The mechanism of formation of **1c** could be rationalized as involving formation of the 1,3-diketone intermediate **8** through the BK–VK rearrangement. Intramolecular cyclization of diketone **8** could lead to the 1,3-indanedione **13**. Formation of hemiketal **14** followed by dehydration could then give the product as shown in Scheme 3.

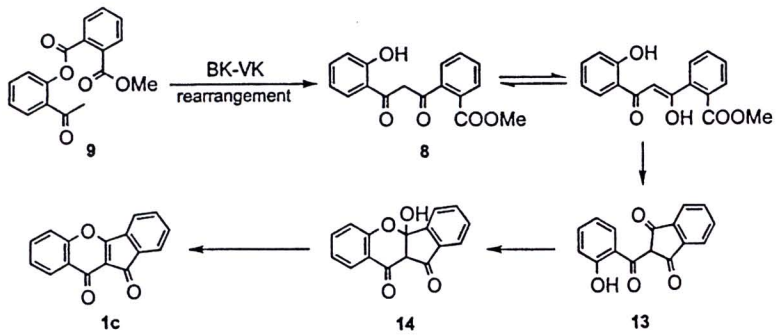
In conclusion, we have developed an operationally simple, highly efficient domino reaction for the synthesis of benz[*b*]indeno[2,1-*e*]pyran-10,11-diones. The method should be amenable to other complex analogues.

## Acknowledgements

We acknowledge generous financial support from the Thailand Research Fund (TRF) and the award of Senior Research Scholar to S.R. as well as the award of the Royal Golden Jubilee Scholarship to N.T. We also acknowledge the facilities in the Department of Chemistry provided by the Postgraduate Education and Research in Chemistry programme (PERCH).




Scheme 2.




Scheme 3.

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10. 2-Benzyl benzofuran-3-one (**11**) mp (EtOH): 148–150°C; FTIR (KBr): $\nu_{max}$ 3042 (OH), 1764 (C=O), 1634 (C=O), 1470, 1443, 1376 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 3.44 (dd, 1H, $J$=6.4, 17.4 Hz, *H* β), 3.75 (dd, 1H, $J$=6.2, 18.0 Hz, *H* β), 6.14 (t, 1H, $J$=6.2, 6.6 Hz, *H* 2), 6.88 (t, 1H, $J$=7.4, 8.0 Hz, *H* 5), 7.00 (d, 1H, $J$=8.6 Hz, *H* 7), 7.59 (m, 5H, Ar*H*), 7.90 (d, 1H, $J$=7.6 Hz, *H* 6'), 12.00 (s, 1H, O*H*); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 201.7, 170.0, 162.5, 149.3, 137.2, 134.4, 129.8, 129.6, 125.8, 122.6, 119.3, 119.0, 118.7, 76.5, 43.1; MS (EI) $m/z$ 268 ($M^+$, 23), 250 (19), 223 (18), 147 (100), 132 (38), 121 (60). HRMS calcd for $C_{16}H_{12}O_4$: 269.0814. Found: 269.0816.
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12. Isowrightiadione (**1c**) mp (EtOH): >260°C; FTIR (KBr): $\nu_{max}$ 1710 (C=O), 1620, 1591, 1464, 1385, 1260 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.45 (ddd, 1H, $J$=1.3, 6.8, 7.8 Hz, *H* 8), 7.53–7.74 (m, 6H, Ar*H*), 8.29 (dd, 1H, $J$=1.2, 7.6 Hz, *H* 9); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 187.6, 179.2, 171.6, 155.8, 134.8, 134.1, 133.9, 133.7, 133.0, 127.1, 126.7, 126.5, 123.7, 121.3, 118.6, 111.4; MS (EI) $m/z$ 248 ($M^+$, 88), 220 (100), 192 (7), 164 (20), 163 (49). HRMS calcd for $C_{16}H_8O_3$: 248.0473. Found: 248.0474. Anal calcd for $C_{16}H_8O_3$: C, 77.42; H, 3.25. Found: C, 77.43; H, 3.24%.

# Mammea Coumarins from the Flowers of *Mammea siamensis*

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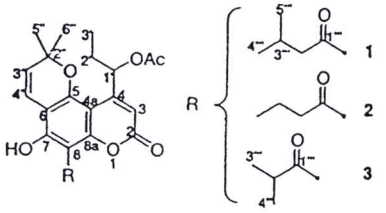
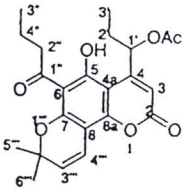
*Received November 21, 2001*

Four new mammea coumarins, mammea E/BA cyclo D (**1**), mammea E/BC cyclo D (**2**), mammea E/BD cyclo D (**3**), and mammea E/AC cyclo D (**4**), were isolated from the flowers of *Mammea siamensis*, along with six known coumarins. Extensive 1D and 2D NMR experiments and other spectroscopic studies, as well as chemical transformations, were employed to determine the structures of **1**–**4**.

*Mammea siamensis* (Miq.) T. Anders. is a Thai medicinal plant in the family Guttiferae, locally known as "Sa rapi" and used as a heart tonic. Plants of the genus mammea are known to be rich sources of various coumarins[1–8] and xanthones.[9,10] In 1981, the initial isolation of 4-phenylcoumarins was reported from petroleum extracts of flowers of *M. siamensis*.[5] Coumarins are reported to exhibit diverse biological activities, and their occurrence in the plant kingdom is widespread.[11]

In a continuation of our study on the flowers of this plant,[7] we now report the isolation and structure elucidation of four new mammea coumarins (**1**–**4**). The structures of these new coumarins were determined using 1D and 2D NMR techniques ($^1$H, $^{13}$C NMR, COSY, HETCOR or HMQC, and COLOC or HMBC).





Ten compounds were isolated from fraction E-2 of a hexane extract of the flowers of *M. siamensis* by successive silica gel column chromatography, preparative TLC, and HPLC. Four new compounds, mammea E/BA cyclo D (**1**), mammea E/BC cyclo D (**2**), mammea E/BD cyclo D (**3**), and mammea E/AC cyclo D (**4**), were identified by means of spectroscopic studies and confirmed by chemical transformations. Six known coumarins, mammea A/BC,[12] mammea B/AC cyclo D,[4,7] mammea A/AC cyclo D,[5–7] mammea B/AC cyclo F,[3,8,13] mammea A/AA cyclo F,[1,3,4,8] and mammea A/AC cyclo F,[3,8,9] were also isolated and established by comparison of their spectral data with those described in the literature.

Coumarin **1** was isolated as a yellow semisolid, which was shown to be optically active ($[\alpha]^{26}_D$ −68.8°, *c* 0.07). The compound gave a parent ion by HRFABMS (negative ion) at *m/z* 427.1753 [M − H]$^-$, corresponding to a molecular formula $C_{24}H_{28}O_7$. The EIMS showed the molecular ion at *m/z* 428 and fragment ions at *m/z* 413 ([M − $CH_3$])$^+$, 385, 371, and 353. Its IR spectrum showed absorption bands corresponding to the carbonyl groups of an ester and an aryl ketone at 1732 and 1645 cm$^{-1}$, respectively. The NMR spectrum (Table 1) revealed signals at $\delta$ 6.60 (1H, dd, $J$ = 8.8, 2.8 Hz), 1.99 (1H, ddq, $J$ = 14.5, 7.3, 2.8 Hz), 1.67 (1H, ddq, $J$ = 14.5, 7.3, 8.8 Hz), 1.05 (3H, t, $J$ = 7.3 Hz), and 2.18 (3H, s), which are due to the presence of a 1-acetoxypropyl group. The signal at $\delta$ 14.54 (1H, s) was ascribed to a phenolic group hydrogen bonded to an acyl group. Two singlets of three hydrogens each at $\delta$ 1.56 and 1.59 and the presence of two doublets of one hydrogen each at $\delta$ 5.61 ($J$ = 10.0 Hz) and 6.74 ($J$ = 10.0 Hz) established the presence of a 2,2-dimethyl-$\Delta^3$-pyran ring.[5] Substitution at C-4 of the coumarin nucleus was apparent from the C-3 proton singlet at $\delta$ 6.30 (1H). The nature of the substituent at C-8 was deduced to be a 3-methylbutyryl chain from the doublet of doublets of one hydrogen each at $\delta$ 3.12 and 3.15 with coupling constants of 15.5 and 6.6 Hz, a multiplet of one proton at $\delta$ 2.27, and two doublets of three hydrogens each at $\delta$ 1.03 and 1.026 with a coupling constant of 6.7 Hz. From the proton-decoupled $^{13}$C NMR spectrum of **1** (Table 2), 24 signals were observed. The DEPT spectra (DEPT 90 and 135) of **1** exhibited six methyl carbon atoms at $\delta$ 10.0 (C-3′), 28.5 (C-5″), 27.8 (C-6″), 22.6 (C-4‴ and C-5‴), and 21.0 (methyl carbon atom of acetoxyl group), two methylene carbon atoms at $\delta$ 53.6 (C-2‴) and 28.7 (C-2′), three olefinic methine carbon atoms at $\delta$ 106.6 (C-3), 126.8 (C-3″), and 115.8 (C-4″), two methine carbon atoms at $\delta$ 25.5 (C-3‴) and 73.0 (C-1′), and 11 quaternary carbon atoms at $\delta$ 159.2 (C-2), 157.3 (C-4), 100.9 (C-4a), 155.8 (C-5), 106.5 (C-6), 163.3 (C-7), 104.7 (C-8), 157.1 (C-8a), 170.3 ($OCOCH_3$), 80.3 (C-2″), and 206.2 (C-1‴).

The position of the phenolic group at C-7 in **1** was established by the COLOC NMR spectral data (Figure 1) of the phenolic proton O*H*-7 to C-7, C-6, and C-8 as well as the NOE interaction with H-4″, thereby locating the pyran ring of **1** between C-5 and C-6. Additionally, the proton signal of H-3 at $\delta$ 6.30 showed a cross-peak with the carbon signals of C-2, C-4a, and C-1′, and a cross-peak

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10.1021/np010579u CCC: $22.00 
Published on Web 04/12/2002

Table 1. $^1$H NMR Spectral Data of Compounds **1**–**3** in $CDCl_3$ (400 MHz, *J* in Hz)

| position | 1 | 2 | 3 |
|---|---|---|---|
| 3 | 6.30, s | 6.30, s | 6.31, s |
| O*H*-7 | 14.54, s | 14.51, s | 14.44, s |
| 1′ | 6.60, dd (8.8, 2.8) | 6.59, dd (8.9, 2.9) | 6.61, dd (8.8, 2.7) |
| COO*CH*$_3$ | 2.18, s | 2.18, s | 2.18, s |
| 2′a | 1.99, ddq (14.5, 7.3, 2.8) | 1.99, ddq (14.5, 7.1, 2.9) | 2.00, ddq (14.5, 7.4, 2.7) |
| 2′b | 1.67, ddq (14.5, 7.3, 8.8) | 1.65, ddq (14.5, 7.1, 8.9) | 1.66, ddq (14.5, 7.4, 8.8) |
| 3′ | 1.05, t (7.3) | 1.06, t (7.1) | 1.06, t (7.4) |
| chromene moiety | | | |
| 3″ | 5.61, d (10.0) | 5.61, d (10.0) | 5.61, d (10.0) |
| 4″ | 6.74, d (10.0) | 6.73, d (10.0) | 6.74, d (10.0) |
| 5″ | 1.56, s | 1.56, s | 1.57, s |
| 6″ | 1.59, s | 1.59, s | 1.60, s |
| 8-acyl moiety | | | |
| 2‴ | 3.12, dd (15.5, 6.6)<br>3.15, dd (15.5, 6.6) | 3.26, t (7.3) | 4.03, septet (6.7) |
| 3‴ | 2.27, m | 1.78, sextet (7.3) | 1.27, d (6.7) |
| 4‴ | 1.03, d (6.7) | 1.03, t (7.3) | 1.27, d (6.7) |
| 5‴ | 1.026, d (6.7) | | |

Table 2. $^{13}$C NMR Spectral Data of Compounds **1**–**3** in $CDCl_3$ (100 MHz)[a]

| carbon | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 159.2 (s) | 159.3 (s) | 159.3 (s) |
| 3 | 106.6 (d) | 106.5 (d) | 106.5 (d) |
| 4 | 157.3 (s) | 157.4 (s) | 155.5 (s) |
| 4a | 100.9 (s) | 100.9 (s) | 101.7 (s) |
| 5 | 155.8 (s) | 156.0 (s) | 155.7 (s) |
| 6 | 106.5 (s) | 106.5 (s) | 106.6 (s) |
| 7 | 163.3 (s) | 163.2 (s) | 163.5 (s) |
| 8 | 104.7 (s) | 104.5 (s) | 103.8 (s) |
| 8a | 157.1 (s) | 157.1 (s) | 156.8 (s) |
| 1′ | 73.0 (d) | 73.1 (d) | 73.0 (d) |
| $OCOCH_3$ | 170.3 (s) | 170.3 (s) | 170.3 (s) |
| $OCOCH_3$ | 21.0 (q) | 21.0 (q) | 20.2 (q) |
| 2′ | 28.7 (t) | 28.7 (t) | 28.7 (t) |
| 3′ | 10.0 (q) | 10.0 (q) | 10.0 (q) |
| chromene moiety | | | |
| 2″ | 80.3 (s) | 80.3 (s) | 80.2 (s) |
| 3″ | 126.8 (d) | 126.8 (d) | 126.8 (d) |
| 4″ | 115.8 (d) | 115.8 (d) | 115.9 (d) |
| 5″ | 28.5 (q) | 28.5 (q) | 28.4 (q) |
| 6″ | 27.8 (q) | 27.8 (q) | 27.8 (q) |
| 8-acyl moiety | | | |
| 1‴ | 206.2 (s) | 206.4 (s) | 210.8 (s) |
| 2‴ | 53.6 (t) | 46.7 (t) | 40.4 (d) |
| 3‴ | 25.5 (d) | 18.0 (t) | 19.2 (q) |
| 4‴ | 22.6 (q) | 13.8 (q) | 19.2 (q) |
| 5‴ | 22.6 (q) | | |

[a] Multiplicities were determined by the DEPT pulse sequence.

of the H-1′ signal at $\delta$ 6.60 with the C-4 carbon signal was also observed. These results clearly indicated that the 1-acetoxypropyl substituent was attached to C-4. The bathochromic shift (372 nm to 390 nm) with alkali of its UV spectrum suggested that **1** contains an 8-acylcoumarin chromophore.[2,13] On the basis of the above evidence, therefore, compound **1** was characterized as mammea E/BA cyclo D.

Coumarin **2** was isolated as a yellow solid and recrystallized from a mixture of dichloromethane–hexane as yellow needles. Compound **2** has a molecular formula of $C_{23}H_{26}O_7$ determined from its positive-ion HRFABMS. The UV ($\lambda_{max}$ 269, 305, 373; in base 208, 250, 391 nm), IR ($\nu_{max}$ 1732, 1644 cm$^{-1}$), and $^1$H (Table 1) and $^{13}$C NMR (Table 2) spectra of **2** were almost identical with those of compound **1**. However, coumarin **2** showed different $^1$H and $^{13}$C NMR spectral data from those of **1** only in the signals of the 8-acyl group. In compound **2**, proton signals appeared at $\delta$ 3.26 (2H, t, $J$ = 7.3 Hz), 1.78 (2H, sextet, $J$ = 7.3 Hz), and 1.03 (3H, t, $J$ = 7.3 Hz) and carbon signals at $\delta$ 206.3 (C-1‴), 46.7 (C-2‴), 18.0 (C-3‴), and 13.8 (C-4‴) due to the presence of a butyryl group. The positions of the butyryl group and the pyran ring in **2** were confirmed by acetylation, which resulted in the appropriate NMR upfield shift of 0.42 ppm of H-4″ in the chromene ring. The diamagnetic shift of the H-4″ resonance required its placement peri to the OAc-7 group, thereby locating the pyran ring between C-5 and C-6 in **2**.[14] The COLOC spectrum (Figure 1) revealed three- and two-bond correlations between the O*H*-7 proton with C-6, C-7, and C-8. On the basis of the above evidence, therefore, compound **2** was assigned as mammea E/BC cyclo D.

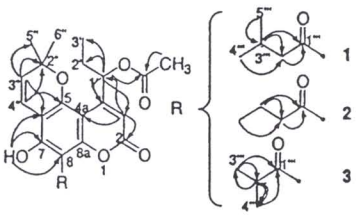
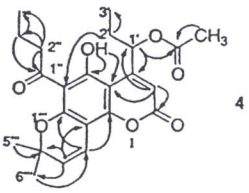



**Figure 1.** COLOC correlations for **1** and **2** and HMBC correlations for **3**.

Compound **3** was recrystallized from dichloromethane as yellow crystals. The UV, IR, HRFABMS, and EIMS data for compound **3** closely resembled those for compound **2**. The $^1$H (Table 1) and $^{13}$C NMR (Table 2) spectra of **3** were almost identical with those of compounds **1** and **2**. However, compound **3** showed $^1$H and $^{13}$C NMR spectral data that were different from those of **1** and **2** only in the signal of the 8-acyl group. Compound **3**, which has a 2-methylpropionyl group, showed proton signals at $\delta$ 4.03 (septet, $J$ = 6.7 Hz, H-2‴) and 1.27 (d, $J$ = 6.7 Hz, H-3‴ and H-4‴) and carbon signals at $\delta$ 210.8 (C-1‴), 40.4 (C-2‴), and 19.2 (C-3‴ and C-4‴). The HMBC (Figure 1) and UV spectra supported the position of the acyl substituent in compound **3** at C-8. On the basis of the above evidence, therefore, compound **3** was characterized as mammea E/BD cyclo D.

Compound **4** was isolated as a yellow gum which was shown to be optically active ($[\alpha]^{31}_D$ +8°, *c* 0.12). The IR spectrum of **4** showed a band that was ascribed to an $\alpha,\beta$-unsaturated $\delta$-lactone (1729 cm$^{-1}$) group. The molecular formula of **4** was determined to be $C_{23}H_{26}O_7$ from the positive-ion HRFABMS (calcd *m/z* 415.1757 for $C_{23}H_{27}O_7$, found 415.1755). In addition, the EIMS of **4** showed a fragmentation pattern similar to those of compounds **2** and **3**. Extensive NMR analysis of **4** showed that this coumarin has the same substituents as **2** since an 1-acetoxypropyl,



4

Figure 2. HMBC correlations for compound **4**.

a butyryl, and a 2,2-dimethyl $\Delta^3$ pyran ring were revealed from its $^1$H and $^{13}$C NMR spectral data. However, **2** and **4** exhibited quite different shifts with alkaline reagents in their UV spectral data. It was therefore deduced that **4** is a regioisomer of compound **2**.

The HMBC spectral data of **4** (Figure 2) revealed three- and two-bond correlations between the O*H*-5 proton with the C-4a (101.5), C-5 (164.4), and C-6 (107.1) signals, and the UV spectral data supported the position of the acyl substituent in compound **4** at C-6.[2,13] The angular fusion of the pyran ring was confirmed by acetylation of **4** to the corresponding acetate derivative. The $^1$H NMR of the acetate derivative of **4** showed downfield shifts of 0.14 ppm for H-3‴ and 0.06 ppm for H-4‴ in the chromene ring.[14] On the basis of the above evidence, therefore, compound **4** was characterized as mammea E/AC cyclo D.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on an electrothermal melting point apparatus (Electrothermal 9100) and are reported without correction. Optical rotations were measured in chloroform solution at the sodium D line (589 nm) on a JASCO DIP-370 digital polarimeter. UV spectra were measured with Shimadzu UV–vis 2001S spectrophotometer. Infrared spectra were obtained from Perkin-Elmer System 2000 FT-IR or JASCO A-302 spectrometers. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AM 400 and a Varian Gemini 2000; $CDCl_3$ was used as the solvent and TMS as an internal standard. Chemical shifts are given in parts per million downfield from TMS, and coupling constants are measured in Hz. DEPT, HETCOR/HMQC, COSY, COLOC/HMBC, NOE, and COSY NMR experiments were obtained using standard Bruker software. Mass spectra were determined using GC–MS Finnigan INCOS 50 and GC–MS MAT 90 instruments. HPLC was performed on a Thermo Separation Products system (San Jose, CA) (pump, P4000; detecter, UV6000LP for analysis, UV2000 for preparative). The HPLC conditions were as follows: (a) LUNA 5 $\mu$m $C_8$ stainless steel column, 150 × 4.60 mm, cat. no. 00F-4040-E0 for analytical applications; (b) LUNA 10 $\mu$m $C_8$ 100 A stainless steel column, 250 × 21.20 mm, cat. no. 00G-4093-P0 for preparative applications. Compounds were purified by isocratic separation using $H_2O$–MeOH as mobile phase; scanning wavelengths were from 190 to 420 nm. Column chromatography was carried out using Si gel 60 (0.063–0.200 mm) and Si gel 60 (particle size less than 0.063 mm). TLC and preparative TLC were carried out on Si gel 60 $F_{254}$ plates (cat. no. 7747 E. Merck). Compounds were detected by their UV absorbance at 254 and 366 nm. All commercial grade solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements.

**Plant Material.** Dried flowers of *Mammea siamensis* were purchased from a local traditional drug store in Bangkok, in October 1995. The plant materials were further identified by Dr. Wongsatit Chuakul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. A voucher specimen (PBM3231) is deposited in the Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

**Extraction and Isolation.** The dried flowers (8.5 kg) of *Mammea siamensis* were extracted exhaustively with hexane at room temperature, followed by filtration. The filtrates were combined and evaporated under reduced pressure to afford a dark brown gum (428 g). The dried extract (300 g) was submitted to Si gel column chromatography and eluted with a gradient of hexane–EtOAc (0%–100%) to afford six fractions (A–F). A portion of fraction E (131 g) was then separated by column chromatography over Si gel with mixtures of EtOAc in hexane of increasing polarity to give eight fractions (E-1–E-8). Fraction E-2 was further separated by column chromatography on a Si gel column with a hexane–EtOAc gradient and produced eight further fractions (f-1–f-8). Fraction f-3 was subjected to column chromatography on Si gel with hexane–EtOAc (7%) and further purified by preparative TLC with hexane–EtOAc (7%) as developing solvent (five developments), affording 41.9 mg of **1** ($R_f$ 0.39), 53.2 mg of **2** ($R_f$ 0.36), 23.7 mg of **3** ($R_f$ 0.33), 20.8 mg of mammea B/AC cyclo D ($R_f$ 0.49), and 27.9 mg of mammea A/AC cyclo D ($R_f$ 0.43). Fraction f-4 was subjected to column chromatography on Si gel with hexane–EtOAc (15%) and further purified by preparative TLC with hexane–EtOAc (18%; three developments) to afford 13.5 mg of mammea B/AC ($R_f$ 0.49). Fraction f-5 was chromatographed on a Si gel column with hexane–EtOAc (20–25%) and then purified by preparative reversed-phase HPLC, run isocratically using 83.5% MeOH–$H_2O$ with UV detection at 280 nm, with a flow rate of 8 mL/min, affording 6.6 mg of mammea B/AC cyclo F ($t_R$ 22.18 min), 7.9 mg of mammea A/AA cyclo F ($t_R$ 20.73 min), and 12.4 mg of mammea A/AC cyclo F ($t_R$ 17.79 min). Fraction f-6 was chromatographed on a Si gel column with a gradient of hexane–EtOAc (25–30%) and then purified by preparative reversed-phase HPLC run isocratically using 85% MeOH–$H_2O$ with UV detection at $\lambda$ 280 nm, flow rate 8 mL/min, to afford 4.9 mg of **4**, $t_R$ 22.56 min.

**Mammea E/BA cyclo D (1):** yellow semisolid; $[\alpha]^{26}_D$ −68.8° (*c* 0.07, $CHCl_3$); UV $\lambda_{max}$ EtOH (log $\epsilon$) 270 (4.29), 307 (4.17), 372 (3.73), and $\lambda_{max}$ EtOH + 0.01 N NaOH (log $\epsilon$) 209 (4.90), 250 (4.17), 390 (4.21) nm; IR ($CHCl_3$) $\nu_{max}$ 3027, 2965, 2874, 1732, 1645, 1610, 1584, 1464, 1397, 1291, 1238, 1194, 1129, 1045, 971, 884, 668 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR, see Tables 1 and 2, respectively; EIMS *m/z* 428 $[M]^+$ (40), 413 $[M - CH_3]^+$ (100), 385 (27), 371 (51), 353 (35), 300 (53); HRFABMS (negative ion) *m/z* 427.1753 (calcd for $C_{24}H_{27}O_7$, 427.1757).

**Mammea E/BC cyclo D (2):** yellow needles; mp 139–140 °C; $[\alpha]^{26}_D$ −48.6° (*c* 0.205, $CHCl_3$); UV $\lambda_{max}$ EtOH (log $\epsilon$) 269 (4.39), 305 (4.43), 373 (4.02), and $\lambda_{max}$ EtOH + 0.01 N NaOH (log $\epsilon$) 208 (5.32), 250 (sh), 391 (4.48) nm; IR ($CHCl_3$) $\nu_{max}$ 3026, 2974, 1732, 1644, 1610, 1584, 1463, 1396, 1209, 1193, 1151, 1122, 1101, 1045, 970, 884 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR, see Tables 1 and 2, respectively; EIMS *m/z* 414 $[M]^+$ (47), 399 $[M - CH_3]^+$ (100), 371 (33), 357 (48), 339 (20); HRFABMS (positive ion) *m/z* 415.1762 (calcd for $C_{23}H_{27}O_7$, 415.1757).

**Acetylation of 2.** Treatment of compound **2** (10 mg) with acetic anhydride (1 mL), 4-*N*,*N*-(dimethylamino)pyridine (0.5 mg), and pyridine (1 mL) at room temperature for 2 h gave the acetate derivative of **2** (9.5 mg, 86%): $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 6.40 (1H, s, H-3), 2.32 (3H, s, OCO*CH*$_3$-7), 6.58 (1H, dd, *J* = 8.5, 3.0 Hz, H-1′), 2.19 (3H, s, OCO*CH*$_3$-1′), 2.0 (1H, m, H-2′a), 1.66 (1H, m, H-2′b), 0.98 (3H, t, *J* = 7.2 Hz, H-3′), 5.72 (1H, d, *J* = 10.0 Hz, H-3″), 6.31 (1H, d, *J* = 10.0 Hz, H-4″), 1.59 (3H, s, H-5″), 1.56 (3H, s, H-6″), 2.94 (2H, t, *J* = 7.2 Hz, H-2‴), 1.70 (2H, m, H-3‴), 1.05 (2H, t, *J* = 7.1 Hz, H-4‴).

**Mammea E/BD cyclo D (3):** yellow crystals; mp 82–83 °C; $[\alpha]^{31}_D$ −24.2° (*c* 0.16, $CHCl_3$); UV $\lambda_{max}$ EtOH (log $\epsilon$) 269 (4.29), 305 (4.24), 385 (3.97), and $\lambda_{max}$ EtOH + 0.01 N NaOH (log $\epsilon$) 207 (5.28), 250 (sh), 390 (4.28) nm; IR ($CHCl_3$) $\nu_{max}$ 2929, 1733, 1608, 1386, 1230, 1144 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR, see Tables 1 and 2, respectively; EIMS *m/z* 414 $[M]^+$ (25), 399 $[M - CH_3]^+$ (100), 371 $[M - CH_3CO]^+$ (20), 357 (49), 339 (27); HRFABMS (positive ion) *m/z* 415.1751 (calcd for $C_{23}H_{27}O_7$, 415.1757).

**Mammea E/AC cyclo D (4):** yellow gum; $[\alpha]^{31}_D$ +8° (*c* 0.12, $CHCl_3$); UV $\lambda_{max}$ EtOH (log $\epsilon$) 226 (4.19), 285 (4.35), and $\lambda_{max}$ EtOH + 0.01 N NaOH (log $\epsilon$) 209 (5.19), 309 (4.29), 422 (3.88) nm; IR ($CHCl_3$) $\nu_{max}$ 2929, 2857, 1729, 1608, 1477, 1391, 1230, 1123 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 6.20 (1H, br s, H-3),

15.60 (1H, s, OH-5), 6.53 (1H, dd, $J$ = 8.4, 2.6 Hz, H-1′), 2.16 (3H, s, COO$CH_3$), 2.01 (1H, ddq, $J$ = 14.4, 7.3, 2.6 Hz, H-2′), 1.65 (1H, ddq, $J$ = 14.4, 7.3, 8.4 Hz, H-2′), 1.04 (3H, t, $J$ = 7.3 Hz, H-3′), 3.09 (2H, br t, $J$ = 7.4 Hz, H-2″), 1.75 (2H, sextet, $J$ = 7.4 Hz, H-3″), 1.03 (3H, t, $J$ = 7.4 Hz, H-4″), 5.61 (1H, d, $J$ = 10.0 Hz, H-3‴), 6.82 (1H, d, $J$ = 10.0 Hz, H-4‴), 1.56 (3H, s, H-5‴), 1.55 (3H, s, H-6‴); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 160.0 (s, C-2), 106.3 (d, C-3), 158.0 (s, C-4), 101.5 (s, C-4a), 164.4 (s, C-5), 107.1 (s, C-6), 158.0 (s, C-7), 101.8 (s, C-8), 154.6 (s, C-8a), 73.8 (d, C-1′), 170.3 (s, O$COCH_3$), 21.0 (q, OCO$CH_3$), 28.6 (t, C-2′), 10.3 (q, C-3′), 207.5 (s, C-1″), 46.8 (t, C-2″), 18.2 (t, C-3″), 13.9 (q, C-4″), 79.9 (s, C-2‴), 126.5 (d, C-3‴), 115.5 (d, C-4‴), 208.3 (q, C-5‴), 28.2 (q, C-6‴); EIMS *m/z* 414 $[M]^+$ (29), 399 $[M - CH_3]^+$ (100), 371 $[M - CH_3CO]^+$ (20), 357 (49), 339 (27); HRFABMS (positive ion) *m/z* 415.1755 (calcd for $C_{23}H_{27}O_7$, 415.1757).

**Acetylation of Compound 4.** Compound **4** (4.9 mg) was dissolved in 0.5 mL of pyridine and 1 mL of $Ac_2O$ using *N,N*-(dimethylamino)pyridine as a catalyst. The reaction mixture was stirred at room temperature for 2 h. After the usual workup, the product was isolated by preparative TLC using 18% ethyl acetate in hexane to give the acetate derivative of **4** (2.8 mg, 52%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.41 (1H, s, H-3), 2.33 (3H, s, OCO$CH_3$-5), 6.23 (1H, br d, $J$ = 5.71 Hz, H-1′), 2.16 (3H, s, OCO$CH_3$-1′), 1.95 (1H, m, H-2′a), 1.66 (1H, m, H-2′b), 0.98 (6H, t, $J$ = 7.3 Hz, H-3′ and H-4″), 2.83 (1H, t, $J$ = 7.3 Hz, H-2″a), 2.82 (1H, t, $J$ = 7.3 Hz, H-2″b), 1.70 (2H, m, H-3″), 5.75 (1H, d, $J$ = 10.0 Hz, H-3‴), 6.88 (1H, d, $J$ = 10.0 Hz, H-4‴), 1.51 (1H, s, H-5‴), 1.52 (3H, s, H-6‴).

**Acknowledgment.** We are grateful to the generous financial support of the Thailand Research Fund (TRF Grant No RTA/07/2544). Thanks are also due to Dr. Wongsatit Chuakul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Thailand, for identification of the plant material. We also acknowledge the facilities provided by the PERCH program.

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NP010579U

HETEROCYCLES, Vol. 57, No. 7, 2002, pp. 1287 - 1292, Received, 28th March, 2002

# TWO NEW PYRANOFLAVANONES FROM THE STEMS OF *DERRIS RETICULATA*

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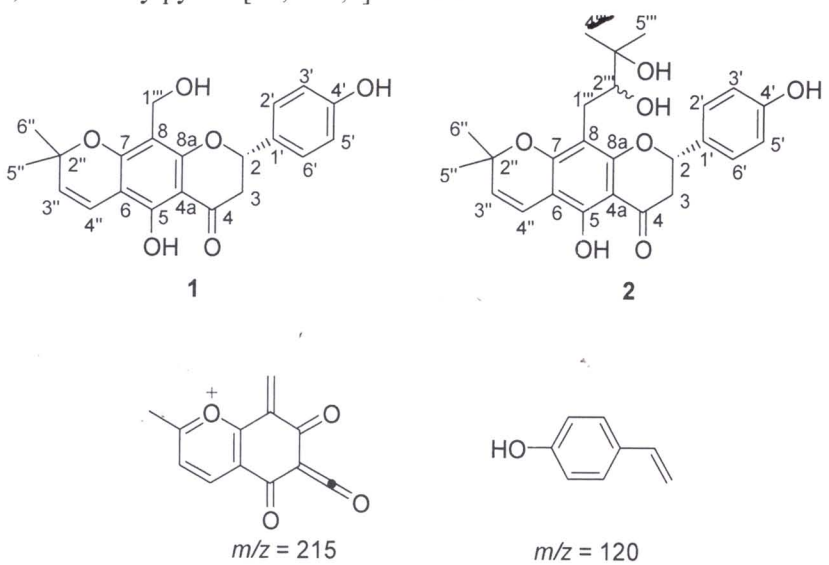
**Abstract-** Two new flavonoids, 4′,5-dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone (**1**), and 2″,3″-dihydroxylupinifolin (**2**) were isolated from the stems of *Derris reticulata*. Both compounds showed cytotoxic activity in the P-388 cell line. The structures were established by spectroscopic analysis and chemical transformations. The biosynthetic origin and the mechanism of formation of the hydroxymethyl group in compound (**1**) are proposed.

The Leguminosae is known to be a rich source of flavonoids and most of the prenyl derivatives have been found in this family.[1,2] The flavonoids exhibit diverse biological activities and recent interest has been focussed on their medicinal and nutritional values.[3] Recently, some biologically active prenylated flavonoids have been reported[4-6] and, significantly, it was found that the prenyl groups on the flavonoid skeleton play an important role in anti-HIV activity.[7] *Derris reticulata* (Leguminosae) is a well known Thai herbal medicine used for the relief of thirst and as an expectorant. Previously, we have reported the isolation and structural characterization of four prenylated flavanones, lupinifolin, 2‴,3‴-epoxylupinifolin, dereticulatin,[8] and 1‴-hydroxy-2‴,3‴-epoxylupinifolin from the stems *of Derris reticulata*.[9] Further investigation of this herb has led to the isolation of two new cytotoxic flavonoids, 4′,5-dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone (**1**), and 2″,3″-dihydroxy-lupinifolin (**2**). In this report we present the isolation, structure determination, and biological evaluation of these two new compounds.

Compound (**1**) was obtained as a yellow solid, mp 141-142 °C, $[\alpha]_D$ –18.2°(*c* 0.12, $CHCl_3$). The UV

spectrum ($\lambda_{max}$ 226, 273, 298 nm) suggested the presence of a pyranoflavanone chromophore.[10] HREIMS of **1** gave a molecular ion at *m/z* 368.1260, with the calculated value for $C_{21}H_{20}O_6$ being 368.1260. Its IR spectrum (EXPERIMENTAL) showed absorptions typical of hydroxyl, substituted aromatic ring, and carbonyl groups. The $^1$H NMR spectrum of **1** showed the typical pattern of protons at C-2 and C-3 in a flavanone skeleton as three one-proton doublet of doublets at δ 5.34 ($J$ = 12.9, 3.0 Hz), 3.07 ($J$ = 17.2, 12.9 Hz), and 2.77 ($J$ = 17.2, 3.0 Hz). In the aromatic region of the $^1$H NMR spectrum of **1**, two doublets appearing at δ 7.27 and 6.83 (each 2H, $J$ = 8.6 Hz) were assigned to the protons of *para*-substituted ring B. The signal at δ 12.32 (1H, s) was ascribed to a phenolic group hydrogen bonded to an acyl group and the signals at δ 4.54 and 4.59 (each 1H, d, $^2J$ = 11.7 Hz) were assigned to methylene protons of the benzylic alcohol. The signals that could be assigned to the 2,2-dimethylpyran group were at δ 1.43 and 1.45 (each 3H, s, 2 × Me), δ 5.51 (d, $J$ = 10.0 Hz), and 6.58 (d, $J$ = 10.0 Hz). No more protons in the aromatic region was observed in the NMR spectrum, suggesting the absence of aromatic protons on ring A. Therefore, the three groups mentioned above were linked to ring A. The $^{13}$C NMR spectrum of **1** showed signals of four oxygenated aromatic carbons (160.1, 160.2, 157.2, 157.4) and a carbonyl carbon (196.6). The $^1$H and $^{13}$C NMR (Table 1) spectra of **1** showed that the phenolic groups were located at C-5, and C-4′. HMBC (Figure 1) experiments showed long-range C-H correlation in which two methylene protons of the benzylic alcohol (δ 3.07 and 2.77) correlated with C-7, C-8, and C-8a. Furthermore, the structure of compound (**1**) was confirmed by the EIMS spectrum which showed ions at *m/z* 215, and 120, resulting from the retro Diels-Alder cleavage of the ion at *m/z* 353 [$M^+$-$CH_3$], 335 [$M^+$-$CH_3$ -$H_2O$] (Scheme 1). The presence of an ion at *m/z* 120 confirmed that one phenolic group was located in ring B. Based on the above spectroscopic evidence, the structure of **1** is proposed to be 4′,5-dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone.[11]




Scheme 1. Mass fragmentation of compound (**1**).

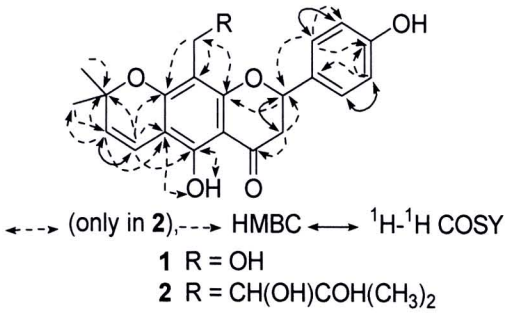
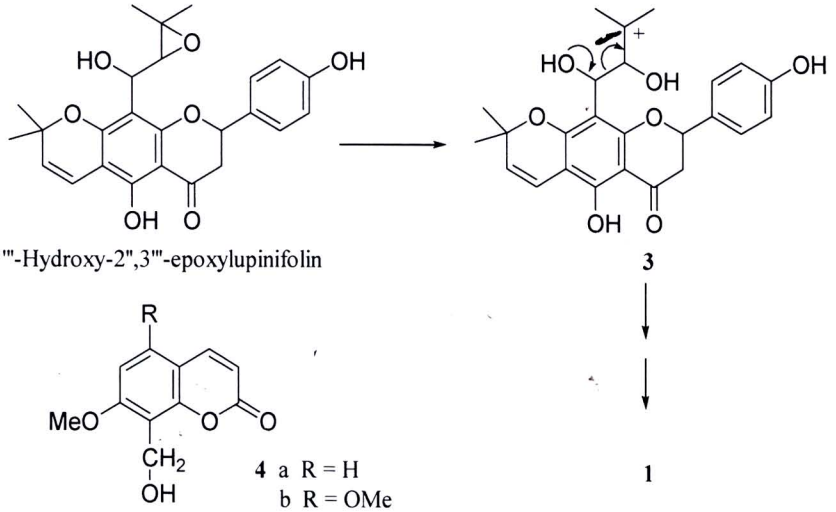



Figure 1. Summary of important connectivities observed in **1** and **2** by HMBC and COSY.

Compound (**2**), $[\alpha]_D$ –26.89°(*c* 0.10, $CHCl_3$) and the UV absorptions at 225, 266, 274, 300, 312, 363 nm were indicative of a pyranoflavanone chromophore.[5] HRFABMS (positive mode) exhibited [$M^+$+1] *m/z* 441.1913 corresponding to a molecular formula of $C_{25}H_{28}O_7$, The $^1$H and $^{13}$C NMR spectral data of **2** (Table 1) exhibited two sets of signals with partial overlapping suggesting the presence of two forms of the vicinal diol. The $^1$H and $^{13}$C NMR spectra of flavanone (**2**) were nearly identical with those of 2‴,3‴-epoxylupinifolin previously isolated from this plant.[8] Comparison of the molecular formula of **2** and 2‴,3‴-epoxylupinifolin suggested an additional $H_2O$ molecule for **2**. The structure was also confirmed by reaction of epoxylupinifolin with 1% sulfuric acid in THF at room temperature for 24 h. From this reaction compound (**2**) was isolated as yellow solid. The structure of this compound was further confirmed by various spectroscopic methods including the correlations of the $^{13}$C-$^1$H and $^1$H-$^1$H in HMBC and COSY respectively as shown in Figure 1. Thus, from the spectroscopic studies and from semisynthesis, **2** was deduced to be 2‴,3‴-dihydroxylupinifolin. The stereochemistry at C-2 in compounds (**1**) and (**2**) was assigned to be *S* configuration by correlation with other related derivatives[8,10] isolated from this plant.




Scheme 2. The proposed biosynthesis of compound (**1**).

The co-occurrence of compound (**1**) with 1‴-hydroxy-2‴,3‴-epoxylupinifolin and compound (**2**) lends support to the proposal in Scheme 2 that the hydroxymethyl group in compound (**1**) could be derived

from this compound by acid catalyzed opening of the epoxide ring or protonation of the hydroxyl group in compound (**2**) to give the corresponding stable carbonium ion followed by carbon-carbon bond cleavage as shown in **3** to give the aldehyde, an immediate precursor to compound (**1**). This biosynthetic route apparently could be applied to rationalize the biosynthesis of the extremely rare hydroxymethyl coumarins, murrayacarpin-A and –B (**4a** and **4b**) which also co-occur with the prenyl derivatives.[12]

*In vitro* bioassay evaluation of compounds (**1**) and (**2**) showed cytoxic activity in the P-388 cell line with $IC_{50}$ values of 6.4 and 1.3 µg/mL respectively, but were inactive against the KB cell line.

Table 1: $^{1}H$ (400 MHz, $CDCl_3$) and $^{13}C$ (100 MHz, $CDCl_3$) NMR Spectral Data for **1** and **2**.

| position | **1** | | **2** | |
|---|---|---|---|---|
| | δH (mult., *J* in Hz) | δC (mult.) | δH (mult., *J* in Hz) | δC (mult.) |
| 2 | 5.34 (dd, *J* = 12.9, 3.0) | 78.8 (d) | 5.27, 5.33 (dd, *J* = 13.1, 3.0) | 78.9, 79.0[a] (d) |
| 3 | 3.07 (dd, *J* = 17.2, 12.9) | 42.5 (t) | 2.99, 2.97 (dd, *J* = 17.1, 13.1) | 43.0, 43.1 (t) |
| | 2.77 (dd, *J* = 17.2, 3.0) | | 2.73, 2.71 (dd, *J* = 17.1, 3.0) | |
| 4 | | 196.6 (s) | | 196.4 (s) |
| 4a | | 102.4 (s) | | 102.7, 102.8 (s) |
| 5 | OH 12.32 (s) | 157.2[a] (s) | OH 12.18 (s) | 157.0, 157.1[b] (s) |
| 6 | | 102.2 (s) | | 102.9 (s) |
| 7 | | 160.2[b] (s) | | 159.8, 159.7[c] (s) |
| 8 | | 107.4 (s) | | 105.6 (s) |
| 8a | | 160.1[b] (s) | | 159.6, 159.5[c] (s) |
| 1′ | | 128.8 (s) | | 129.9, 129.8 (s) |
| 2′, 6′ | 7.27 (d, *J* = 8.5) | 127.4 (d) | 7.19 (d, *J* = 8.3) | 127.6, 127.7 (d) |
| 3′, 5′ | 6.83 (d, *J* = 8.5) | 115.1 (d) | 6.76, 6.75 (d, *J* = 8.3) | 115.8, 115.7 (d) |
| 4′ | | 157.4[a] (s) | | 156.7, 156.6[b] (s) |
| 2″ | | 78.4 (s) | | 78.8 (s) |
| 3″ | 5.51 (d, *J* = 10) | 126.0 (d) | 5.44 (d, *J* = 10.0) | 125.9, 125.8 (d) |
| 4″ | 6.58 (d, *J* = 10) | 114.9 (d) | 6.57 (d, *J* = 10.0) | 115.6, 115.5 (d) |
| 5″ | 1.43 (s) | 27.8 (q) | 1.40, 1.38[a] (all s) | 28.6, 28.5[d] (all s) |
| 6″ | 1.45 (s) | 27.6 (q) | 1.37, 1.36[a] (all s) | 28.4, 28.3[d] (all s) |
| 1‴ | 4.59 (d, *J* = 11.7) | 52.3 (t) | 2.76, 2.75 (dd, *J* = 14.0, 1.9) | 25.3, 25.2 (t) |
| | 4.54 (d, *J* = 11.7) | | 2.53, 2.51 (dd, *J* = 14.0, 10.4) | |
| 2‴ | | | 3.45, 3.42 (dd, *J* = 10.4, 1.9) | 79.2, 79.1[a] (d) |
| 3‴ | | | | 73.2 (s) |
| 4‴, 5‴ | - | | 1.14, 1.13, 1.23, 1.12 (all s) | 25.9, 23.4 (all s) |

[a-d] Assignments may be interchangeable

## EXPERIMENTAL

**General:** Melting points were determined on a Buchi 535 and are uncorrected. Optical rotations were measured with a JASCO P-1020 Digital Polarimeter. UV (MeOH) spectra were measured on a Shimadzu UV-2100S spectrophotometer. IR (KBr) spectra were recorded on a Perkin-Elmer system 2000 FT-IR spectrophotometer. The NMR spectra were recorded on Bruker AM 400 (400 MHz for $^{1}H$ and 100 MHz for $^{13}C$) spectrometer and chemical shifts were recorded in δ (ppm) using TMS as internal standard. MS spectra were determined on Finnigan Mat 90 and Finnigan Polaris instruments. HPLC was performed on the Thermo Separation Products, San Jose, CA, U. S. A. (pump, P4000; detector, UV6000LP for analysis, UV2000 for preparative isolation).

**Plant material:** Dry stems of *Derris reticulata* were purchased from a local traditional drug store in Bangkok, Thailand (June 1998). Botanical identification was achieved through comparison by Prof. Nijsiri Ruangrungsi with the authentic specimen in the Bangkok Herbarium (BK 36776), Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

**Extraction and Isolation:** The dichloromethane extract (20 g) of dry powdered stems of *Derris reticulata* was separated by VLC (Vacuum Liquid Chromatography) using hexane and increasing amounts of ethyl acetate to give 8 fractions. Fraction 5 (200 mg) was resolved by preparative HPLC using Luna $C_8$ and UV detection at 274 nm. Elution with MeOH: $H_2O$ (77:23), flow rate 8 mL/min, afforded compound (**1**) (3 mg); and 1‴-hydroxy-2‴,3‴-epoxylupinifolin (15 mg). Fraction 6 (429 mg) was further purified by repeated preparative TLC developed twice with a mixture of methanol:acetone:hexane (1:16:33) as eluent to yield 2 bands. The final purification of band 1 was achieved by preparative HPLC using Luna $C_8$ and UV detection at 280 nm, eluted with MeOH:$H_2O$ (62.5:37.5) to furnish compound (**2**) (10 mg).

**4′,5-Dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone (1):** yellow solid (3 mg); mp 141-142 °C; $[\alpha]^{26}_{D}$ -18.2° (*c* 0.12, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 226 (4.16), 267 (4.41), 273 (4.47), 298 (3.94), 312 (3.92), 360 (3.29) nm; IR (KBr) $\nu_{max}$ 3300 (OH), 2925, 1647 (C=O), 1600, 1520, 1460, 1380, 1133, 830 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR ($CDCl_3$), see Table 1; EIMS *m/z* 368 $[M]^+$ (73), 353 $[M-CH_3]^+$ (37), 350 $[M-H_2O]^+$ (36), 335 $[M-CH_3, -H_2O]^+$ (87), 215 (100), 120 (12); HREIMS *m/z* 368.1260 (calcd for $C_{21}H_{20}O_6$, 368.1260).

**2″,3″-Dihydroxylupinifolin (2):** as yellow solid, $[\alpha]_D$ –26.89° (*c* 0.10, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 225 (4.14), 266 (4.48), 274 (4.56), 300 (3.91), 312 (3.93), 363 (3.36) nm; IR (KBr) $\nu_{max}$ 3411, 3244, 2974, 1620, 1521, 1451, 1382, 832 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR ($CDCl_3$), see Table 1; EIMS *m/z* 440 $[M]^+$ (50), 425

$[M\text{-}CH_3]^+$ (70), 407 (12), 381 (60), 351 (78), 335 (20), 305 (5), 287 (23), 261 (83), 231 (100), 215 (48), 120 (13); HRFABMS (positive ion) *m/z* 441.1913 (calcd for $C_{25}H_{29}O_7$, 441.1913).

**Preparation of 2‴,3‴-dihydroxylupinifolin from 2‴,3‴-epoxylupinifolin:** A solution of 2‴,3‴-epoxylupinifolin (100 mg) in 1% $H_2SO_4$ in THF (5 mL) was stirred at rt for 24 h. The reaction mixture was poured into water (25 mL) and extracted with $CH_2Cl_2$ (3 × 20 mL). The organic layer was evaporated to give crude product which was separated by silica gel preparative TLC using 1% MeOH in $CH_2Cl_2$ to give 2‴,3‴-dihydroxylupinifolin (53%).


## ACKNOWLEDGEMENT

We acknowledge the Thailand Research Fund (TRF) ( Grant No. RTA/07/2544 and PDF/81/2544) for generous support of our research program and the award of Senior Research Scholar to S.R.. We also acknowledge the facilities in the Department of Chemistry, Mahidol University provided by the Postgraduate Education and Research Program in Chemistry (PERCH). We are grateful to Miss Pongpan Siripong, National Cancer Institute, Bangkok, Thailand, for cytotoxicity test.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# Efficient Synthesis of Ningalin C

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*Organic* LETTERS

Reprinted from
Volume 4, Number 16, Pages 2633–2635

ORGANIC LETTERS

2002
Vol. 4, No. 16
2633−2635

# Efficient Synthesis of Ningalin C

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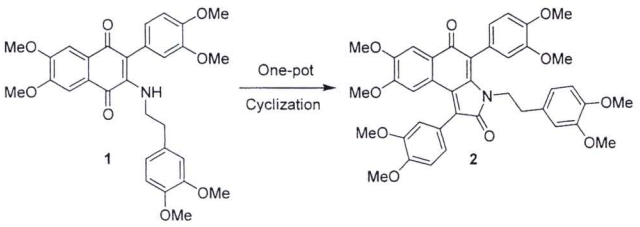
**Received April 24, 2002** *(Revised Manuscript Received July 10, 2002)*

## ABSTRACT




**A concise and efficient synthesis of the permethyl derivative of the marine alkaloid ningalin C (2) has been accomplished. The key step involves the formation of a pyrrolinone from an aminoquinone in one pot. An efficient route for the synthesis of the key aminoquinone has also been developed.**

In 1997, Kang and Fenical[1] reported the isolation of four novel aromatic alkaloids, ningalin A−D (Figure 1), from an unidentified ascidian of the genus *Didemnum*[2] collected in ascidia-rich habitats near Ningaloo Reef region at the northwest cape of western Australia. These ningalin derivatives, as well as lukianols, polycitrins, and lamellarins, appeared to be derived from the condensation of 3,4-dihydroxyphenylalanine (DOPA) in the biosynthetic pathway.[3]

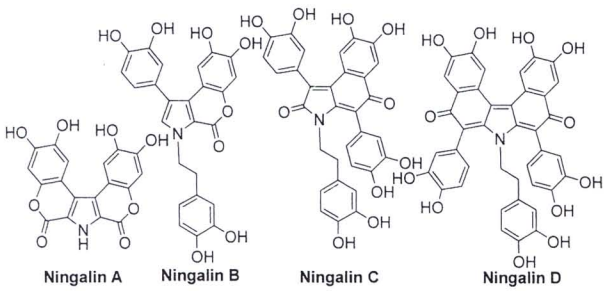



**Figure 1.** Structures of ningalin A, B, C, and D.

The first total syntheses of ningalin A and B were reported by Boger et al. in 1999, utilizing the application of the versatile heteroaromatic azadiene Diels−Alder reaction.[4] Steglich has recently reported an efficient synthesis of ningalin C using an intramolecular Friedel−Crafts acylation.[5]

In this paper, we report a new and efficient method for the synthesis of ningalin C as a continuation of our synthetic

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10.1021/ol026074s CCC: $22.00 
**Published on Web 07/18/2002**

(1) Kang, H.; Fenical, W. *J. Org. Chem.* **1997**, *62*, 3254.

(2) (a) Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clady, J. *J. Org. Chem.* **1988**, *53*, 4570. (b) Carrol, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 489. (c) Urban, S.; Hobbs, L.; Hooper, J. N. A.; Capon, R. J. *Aust. J. Chem.* **1996**, *49*, 711. (d) Urban, S.; Capon, R. J. *Aust. J. Chem.* **1995**, *48*, 1491.

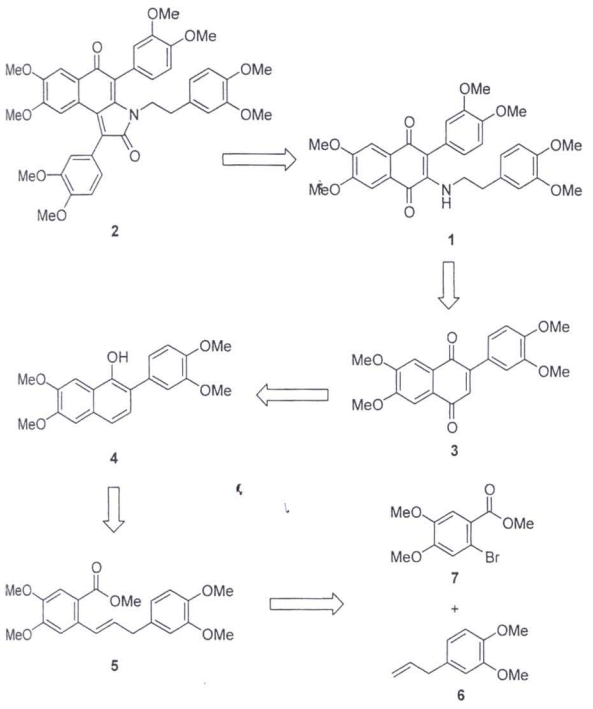
(3) For a review see: Bowden, B. F. In *Studies in Natural Products Chemistry*; Rahman, A., Ed.; Elsevier Science: New York, 2000; Vol. 23, pp 233−283.

(4) (a) Boger, D. L.; Boyce, C. W.; Labroli, M. A.; Sehon, C. A.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 54. (b) Boger, D. L.; Soenen, D. R.; Boyce, C. W.; Hedrick, M. P., Jin, Q. *J. Org. Chem.* **2000**, *65*, 2479.

(5) Peschko, C.; Steglich, W. *Tetrahedron Lett.* **2000**, *41*, 9477.

program on lamellarins and other bioactive pyrrole alkaloids.[6] Our retrosynthetic analysis of this compound is outlined in Scheme 1.

**Scheme 1**

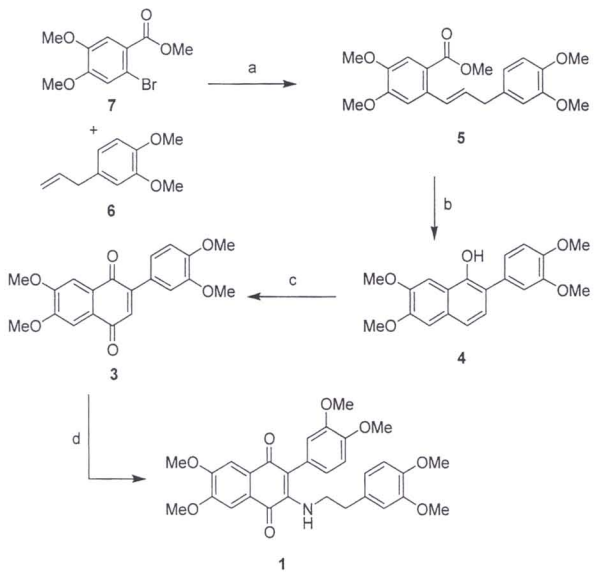


It was planned that the pyrrolinone system of the target ningalin compound **2** could be synthesized from naphthoquinone **1** via addition of the enolate derived from methyl homoveratrate and subsequent lactam bond formation. This aminoquinone could be synthesized by addition of an amine to quinone **3** derived from the oxidation of naphthol derivative **4**. It was expected that an allyl carbanion prepared from **5** would undergo intramolecular acylation to give compound **4**. The alkene **5** could conceivably be prepared by the reaction of eugenol methyl ether **6** with bromo compound **7** via the Heck reaction.[7]

In practice, the palladium-catalyzed coupling reaction[8] between the methyl 2-bromoveratrate **7** and commercially available eugenol methyl ether **6** gave ester **5** in 65% yield. (Scheme 2).

Further cyclization[9] of methyl ester **5** was accomplished by treatment with 2 equiv of lithium diisopropylamide in

**Scheme 2**[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, $NaHCO_3$, DMF, reflux, 24 h, 65%; (b) 2 equiv of LDA, THF, −78 °C, 2 h, then rt 2 h, 76%; (c) $H_2O_2$, $I_2$, $H_2SO_4$, MeOH, 62%; (d) homoveratrylamine (**9**), EtOH, rt, 24 h, 80%

THF at −78 °C to give naphthol **4** (76%), which has recently been obtained by a different route.[10]

Oxidation[11] of naphthol **4** with 30% hydrogen peroxide containing a trace amount of iodine and sulfuric acid in methanol afforded the desired naphthoquinone **3** in 62% yield as a red solid.

On the basis of the synthetic plan proposed in Scheme 1, the key aminoquinone intermediate could be prepared via the nucleophilic addition of amine to naphthoquinone derivative **3**. Indeed, it was found that treatment of naphthoquinone **3** with homoveratrylamine **9** in ethanol[12] at room temperature furnished the homologous amide, aminonaphthoquinone **1**, as a deep red solid in 80% yield (Scheme 2).

Similarly, the aminonaphthoquinone **10** was readily prepared by the addition of homoveratrylamine **9** to commercially available menadione **8** in ethanol at room temperature for 24 h. The aminoquinone derivatives have recently been shown to be versatile intermediates in the synthesis of various natural products.[13]

The construction of the pyrrolinone moiety in the ningalin skeleton was initially investigated using this readily available aminoquinone derivative **10**.

It was reasoned that the presence of the amino group would render the C-1 carbonyl less electrophilic and thus the

(6) Ruchirawat, S.; Mutarapat, T. *Tetrahedron Lett.* **2001**, *42*, 1205.

(7) For some recent reviews, see: (a) Ikeda, M.; El Bialy, S. A. A.; Yakura, T. *Heterocycles* **1999**, *51*, 1957. (b) Amatore, C.; Jutand, A. *Acc. Chem. Res.* **2000**, *33*, 314. (c) Beletskaya, I. P.; Cheprakov, A. V. *Chem. Rev.* **2000**, *100*, 3009. (d) Poli, G.; Giambastiani, G.; Heumann, A. *Tetrahedron* **2000**, *56*, 5959.

(8) Amorese, A.; Arcadi, A.; Bernocchi, E.; Cacchi, S.; Cerini, S.; Fedili, W.; Ortar, G. *Tetrahedron* **1989**, *45*, 813.

(9) (a) Sibi, M. P.; Dankwardt, J. W.; Snieckus, V. *J. Org. Chem.* **1986**, *51*, 273. (b) de Koning, C. B.; Michael, J. P.; Rosseau, A. L. *Tetrahedron Lett.* **1997**, *38*, 893. (c) Hattori, T.; Takeda, A.; Suzuki, K.; Koike, N.; Koshiishi, E.; Miyano, S. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3661.

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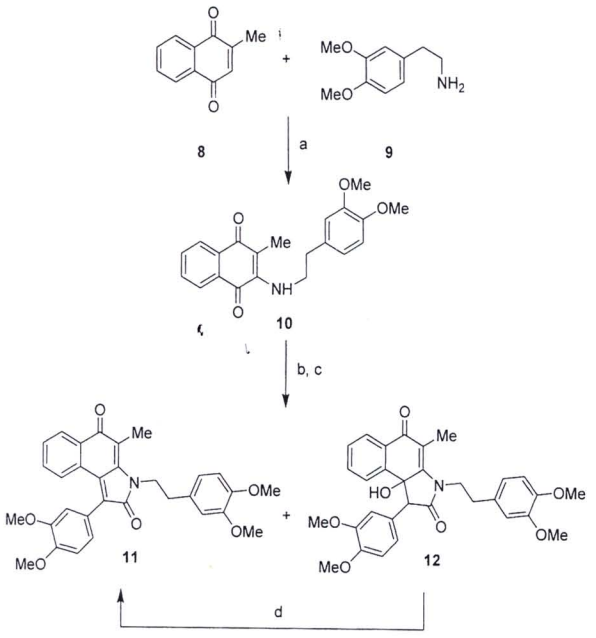
(11) Minisci, F.; Citterio, A.; Vsaimara, E.; Fontana, F.; De Bernardinis, S. *J. Org. Chem.* **1989**, *54*, 728.

(12) (a) Barret, R.; Roue, N. *Tetrahedron Lett.* **1999**, *40*, 3889. (b) Tohma, H.; Harayama, Y.; Hashizumi, M.; Iwata, M.; Egi, M.; Kita, Y. *Angew. Chem., Int. Ed.* **2002**, *41*, 348.

(13) Nicolaou, K. C.; Sugita, K.; Baran, P. S.; Zhong, Y.-L. *Angew. Chem., Int. Ed.* **2001**, *40*, 207 and references therein.

nucleophile would preferentially attack C-4 carbonyl group.[14] This prediction was indeed found to be the case. When aminonaphthoquinone **10** was treated with the carbanion derived from the reaction of methyl homoveratrate and 2 equiv of lithium diisopropylamide in tetrahydrofuran at −78 °C, the desired ningalin C skeleton **11** was obtained (69%) as an orange solid together with hydrated product **12**, isolated as a white solid in 9% yield (Scheme 3). It was gratifying

**Scheme 3**[a]

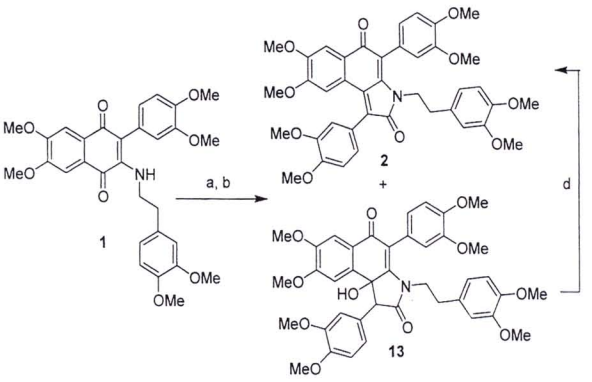



[a] Reagents and conditions: (a) EtOH, rt, 24 h, 82%; (b) 2 equiv of LDA, THF, −78 °C; (c) 1 equiv of methyl homoveratrate, THF, −78 °C, 2 h then rt 2 h, 69%(**11**), 9%(**12**); (d) 2 M HCl, $CH_2Cl_2$, rt, 5 h

to see that not only was the addition of the anionic species to the right ketone group of compound **10** but also the lactam cyclization took place to generate the ultimate pyrrolinone system in one pot. We were unable to carry out the *intermolecular* amide bond formation by the reaction of aminonaphthoquinone **10** with homoveratroyl chloride in the presence of various bases ($Na_2CO_3$, $K_2CO_3$, NaH, $Et_3N$). This is presumably due to the fact that the lone pair of electrons on nitrogen is not readily available because the nitrogen is part of a vinylogous amide. It was thus concluded that in the key formation of the pyrrolinone the formation of the carbon−carbon bond preceded the *intramolecular* amide bond formation.

With the starting material **1** and an efficient method for the synthesis of pyrrolinones in hand, the synthesis of permethyl ningalin C **2** was then investigated. The reaction of aminoquinone **1** with methyl homoveratrate and 2 equiv of LDA gave the required permethyl ningalin C **2** (73%) and the hydrated compound **13** (15%) in a straightforward manner (Scheme 4). The hydrated compounds **12** and **13**

**Scheme 4**[a]




[a] Reagents and conditions: (a) 2 equiv of LDA, THF, −78 °C; (b) 1 equiv of methyl homoveratrate, THF, −78 °C, 2 h then rt 2 h, 73% (**2**), 15%(**13**); (d) 2 M HCl, $CH_2Cl_2$, rt, 5 h.

could be readily dehydrated by treatment with 2 M hydrochloric acid in dichloromethane at room temperature for 5 h to give the ningalin compound **11** and permethyl ningalin C **2** in quantitative yield. Permethyl ningalin C **2** was demethylated[5] by $BBr_3$ in methylene chloride to give ningalin C in 72% yield. The spectral data (IR, UV, $^1H$ NMR, $^{13}C$ NMR, MS) of our synthetic ningalin C are in full agreement with the published data for the natural product.[1,5]

In conclusion, our synthetic approach to ningalin C demonstrates the utility of the one-pot reaction for the preparation of the pyrrolinone system, the core moiety of ningalin C. The synthesis of the quinone and aminoquinone also shows great promise for application in the synthesis of related quinone derivatives.

**Acknowledgment.** We gratefully thank the Thailand Research Fund (TRF) for generous financial support and the award of Senior Research Scholar to S.R. and the Golden Jubilee Scholarship to A.N. We acknowledge the facilities in the Department of Chemistry, Mahidol University, provided by the Postgraduate Education and Research in Chemistry (PERCH) program. We are grateful to Professor W. Steglich for copies of NMR spectra of permethyl ningalin C and ningalin C and the procedure for the demethylation.

**Supporting Information Available:** Detailed experimental procedures and characterization data for new compounds and spectra (IR, UV, $^1H$ NMR, $^{13}C$ NMR, MS) of the synthetic ningalin C. This material is available free of charge via the Internet at http://pubs.acs.org.

OL026074S

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*Phytochemistry Reviews* **1:** 287–297, 2002.



# Investigation of some bioactive Thai medicinal plants

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*Key words:* anticancer, anti-HIV, anti-infectious diseases, anti-inflammatory agents, antimalarial, antiulcer, Thai medicinal plants

**Abstract**

It has been estimated that plants are the most important source of medicine for more than 80% of the world's population. Medicinal plants are a vital source of medication in developing countries. Despite the wealth of human experience and folklore concerning the medicinal uses of plants, proper scientific investigation has only been applied to a small fraction of the world's plants. This is a cause of grave concern as plant species continue to disappear. A rapid response to this situation is urgently needed to prevent the disappearance of the plant species and the ethnopharmacological knowledge that accompanies them. In this review, recent work on the investigation of selected bioactive Thai medicinal plants is presented. Their biological activities against infectious diseases including antimalarial and anti-HIV, are highlighted, as well as their anticancer, antiulcer and anti-inflammatory properties. The chemical transformations of some selected compounds are discussed.

## Introduction

Throughout the ages humans have relied on nature for their basic needs for the production of foodstuffs, shelter, clothing, fertilizers, flavors and fragrances, and, not least, medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years. In industrialized nations at the present time, some fifty percent of all prescribed drugs are derived or synthesized from natural products, the only available sources for which are animals, marine species, plants, and microorganisms (Farnsworth and Morris, 1976, p. 46). The importance of natural products is also evidenced by the fact that in 1991 nearly half of the best selling drugs were either natural products or their derivatives (O'Neill and Lewis, 1993, p. 48). It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovery of potential new drugs and biological entities (Balandrin et al., 1985; Hamburger et al., 1991; Cox and Balick, 1994; Cordell, 1995; Clark, 1996; Hostettmann et al., 1998; Cordell, 2000). However, only a small percentage of the world's estimated 250,000–400,000 flowering plants have as yet been analysed for their possible medicinal uses. Moreover, in developing countries, medicinal plants continue to be the main source of medication. It has been estimated that approximately 80% of the world's inhabitants and 88% of the inhabitants of underdeveloped countries rely mainly on traditional medicine for their primary health care (Farnsworth et al., 1985; Pezzuto, 1997).

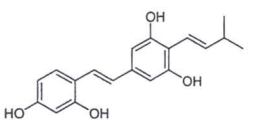
Our country, Thailand, due to its unique geographical location has long enjoyed the luxury of an innumerable variety of plants. Evergreen forest is found in the southern part of Thailand, while the northern mountains have been penetrated with a number of the eastern Himalaya temperate taxa thus making this area one of the richest floristic regions of the world. Thailand is endowed with a great diversity of indigenous medicinal plants. The Thais have a long tradition of

folklore medicine, utilizing alleged medicinal herbs and plants. Herbal drugs have been used in Thailand for centuries as an integral part of Thai culture. A great potential is foreseen for indigenous plants to be used as a source of new drugs. However, many of the claimed curative properties have neither been scientifically proved nor properly investigated. When screening for biologically active plant constituents, the selection of the plant species to be studied is obviously a crucial factor for the ultimate success of the investigation. Besides random collection of plant material, targeted collection based on consideration of chemotaxonomic relationships and exploitation of ethnomedical information is currently performed. Plants used in traditional medicine are more likely to yield pharmacologically active compounds. Research work on medicinal plants in Thailand mainly uses folklore medicine as a guideline in the selection of the plants for study.

In 1997, Cragg and Newman of NCI reported some interesting statistics. They found that during the period of 1983 to 1994, among new approved drugs for all disease types, those derived from synthesis are approximately twice the number of those derived from natural products, both modified and unmodified. However, of the 93 newly approved anti-infective drugs, 7 are unmodified natural products and 45 are modified natural products, giving 63% of drugs derived from natural sources. The same trend is observed in the cancer area where the number of anticancer drugs derived from natural products both modified and unmodified is higher than synthetic drugs, i.e., 62% of the 87 approved anticancer drugs. It is very interesting to note that for the pre-new drug application anticancer drugs, 50 compounds are from natural sources indicating the increasing importance of natural products as anticancer agents. It is obvious from the above statistics that natural products play a major role as drugs for the treatment of infectious diseases and cancer. In this review, phytochemical and biological investigations of some recent *selected* Thai medicinal plants will be discussed.

## Anti-infectious diseases

New and reemerging infectious diseases for which no effective therapy is available and the development of resistance of many pathogens to currently used drugs are of utmost concern. In the developing countries, malaria, TB and HIV are the three major infectious disease threats. They account for approximately half of mortality caused by infectious diseases which is almost half of the mortality in developing countries. It is not at all an exaggeration to say that malaria has been responsible for much of the human suffering and misery accompanying the process of social and economic development. There are more than 300 million cases of malaria in the world every year and malaria kills more than one million people every year. Over the last ten years, the malaria situation has been worsening in many areas of the world. The need to find new antimalarials is pressing, due to the discovery of the resistance of the human malarial parasite, *Plasmodium falciparum* to the presently available common antimalarial drugs. Treatment has thus become both less effective and much more expensive. The problem is further aggravated by the resistance of vector anopheline mosquitoes to the most effective and least toxic insecticides which were used to kill them. The potential of natural products as therapeutic agents in the treatment of malaria is enormous and the research work in this area has been the subject of some recent reviews (Mahidol et al., 1997a; Ekthawatchai et al., 1999). Some further investigations include the isolation of an antimalarial stilbene from *Artocarpus integer*. Stilbene (**1**) exhibited *in vitro* antimalarial activity against *P. falciparum* with the $EC_{50}$ value of 1.7 $\mu$g/ml (Boonlaksiri et al., 2000, p. 415). From *Artocarpus gomezianus*, a dimeric stilbene was isolated and this compound was found to have tyrosinase inhibitory activity (Likhitwitayawuid and Sritularak 2001, p. 1457).

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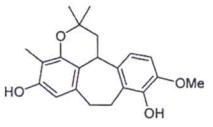


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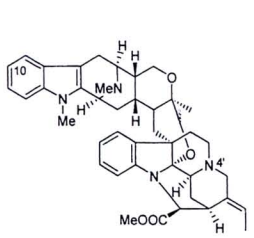
Racemosol (**2**) has been previously isolated from *Bauhinia racemosa* (Anjaneyulu et al., 1986, p. 2417) and *Bauhinia rufescens* (Maillard et al., 1991, p. 791). The compound has also been isolated from *Bauhinia malabarica* Roxb. and found to have antimalarial activity with $EC_{50}$ of 0.9 $\mu$g/ml (Kittakoop et al., 2000, p. 349). Plants in the *Alstonia* species have been used as antimalarial remedies in traditional medicine. Investigations of three Thai *Alstonia* species, *A. scholaris*, *A. macrophylla* and *A. glaucescens*, have resulted in the isolation of thirteen indole alkaloids. Villalstonine (**3**) and macrocarpamine (**4**), the

macroline-pleiocarpamine bisindoles, exhibited significant antimalarial activity with $IC_{50}$ values of 0.27 and 0.36 $\mu$M, respectively (Keawpradub et al., 1999, p. 690). Some coumarin and carbazole derivatives from *Clausena harmandiana* also exhibited antimalarial activity (Yenjai et al., 2000, p. 277).



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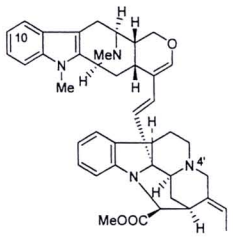
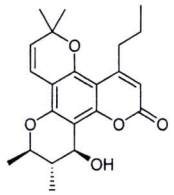


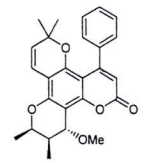
4

Anti-HIV drug discovery and development has been the subject of intensive study. The search for anti-HIV drugs, especially natural product-based anti-HIV reverse transcriptase inhibitors, has been the theme of recent reviews (Matthée et al., 1999; Yang et al., 2001). Many natural products have been found to be inhibitors of HIV-1-RT and these compounds are derived from various sources including terrestrial and marine plants, microorganisms, and marine animals. These compounds belong to diverse structural classes e.g. coumarins, flavonoids, tannins, alkaloids, lignans, terpenes and quinones. One of the most prominent compounds is calanolide A (**5**), a coumarin isolated from the tropical rainforest tree, *Calophyllum lanigerum* (Guttiferae) first collected from Sarawak, Malaysia (Galinis et al., 1996, p. 4507). Calophyllum species from Sri Lanka have recently been investigated (Dharmaratne et al., 2002, p. 86).



5

We have also undertaken the phytochemical investigation of Thai *Calophyllum inophyllum*. The methanol/dichloromethane (1:1) extract of the dried leaves of the plant was fractionated by vacuum liquid column chromatography and/or column chromatography and/or PTLC procedures to provide eight compounds **6–13** which were known and were identified by spectroscopic and/or chemical examinations as well as comparison with the published data (Harborne et al., 1965; Kawazu et al., 1972; Patil et al., 1993). These isolated compounds were identified as 12-methoxyinophyllum D (**6**), calophyllolide (**7**), inophyllum C (**8**), inophyllum E (**9**), calophyllic acid (**10**), isocalophyllic acid (**11**), kaempferol-3-*O*-$\alpha$-L-rhamnoside (**12**), and quercetin-3-*O*-$\alpha$-L-rhamnoside (**13**). Four new mammea coumarins as well as six known coumarins have recently been isolated from the flowers of *Mammea siamensis* (Mahidol et al., 2002a, p. 757).



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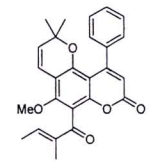

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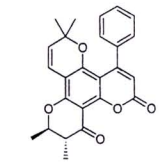

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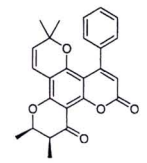

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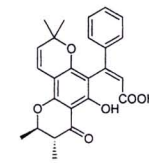

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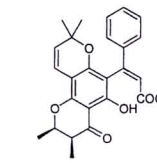

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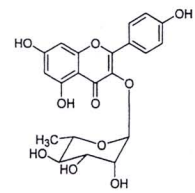

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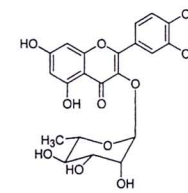

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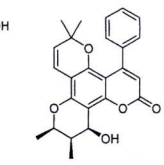
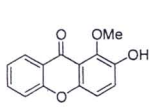


14

In addition, a hexane extract of the dried leaves of *C. inophyllum* was separated using vacuum liquid column chromatography. Further purification of the resulting fractions was carried out by PTLC leading to the isolation of known compound **14** which was identified by spectroscopic methods and comparison with the published data as inophyllum A. Furthermore, the dichloromethane extract was subjected to chromatography to give compounds **15–17**, which have already been isolated earlier from the same plant, and compounds **18–20** isolated from other plants. Structure elucidation of these compounds was accomplished by the use of 2D NMR technology and comparison with the literature data.



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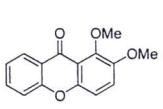

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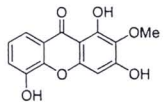

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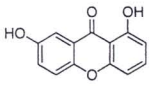

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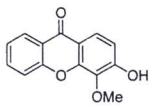

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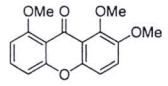
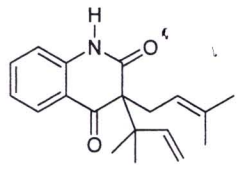


20

Buchapine (**21**) and a quinolone derivative (**22**) were isolated from *Euodia roxburghiana* collected in Surat Thani, the southern province of Thailand. These compounds were found to be active against infectious HIV-1 as well as inhibiting HIV-1-RT (McCormick et al., 1996, p. 469).



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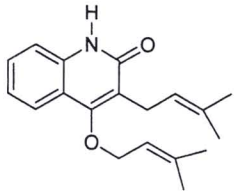
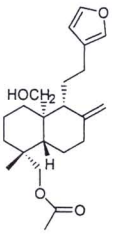


**22**

The fruits of *Momordica charantia* have been shown to contain many proteins with varying molecular weights and these proteins exhibit a ribosome-inactivating property. MAP30, a ribosomal inactivating protein, was isolated from this fruit and found to inhibit HIV-1 reverse transcription, viral core protein synthesis and syncytium formation between the infected and the new white blood cells. From the ripe fruit and seed of Thai *Momordica charantia*, a protein (MRK29) of molecular weight 28.6 kD was isolated and purified. MRK29 was found to inhibit HIV-1 reverse transcriptase with 50% of inhibitory ratio (IR) at a concentration of 18 $\mu$g/ml. The protein increased TNF activity 3-fold suggesting that the compound might have a modulatory role on immune cells (Jiratchariyakul et al., 2001, p. 350). *Potamogeton malaianus* is a high salt-tolerant water plant found in the northeast of Thailand. From this plant, potamogetonol (**23**), a furanoid labdane diterpene, was isolated and identified. The compound and other related compounds were found to exhibit antiviral (HSV-1) activity (Kittakoop et al., 2001, p. 385). Sclerocarpic acid (**24**), a sesquiterpene, and various quinone-methide triterpene derivatives (**25**) were isolated from the stem bark of *Glyptopetalum sclerocarpum* (Sotanaphun et al., 1998, 1999a). Sclerocarpic acid was also shown to exhibit antiviral (HSV-1 and 2) and antimicrobial activity while the triterpene derivatives were effective against *Bacillus cereus*, *B. subtilis*, *Sarcina lutea*, *Staphylococcus aureus*, *Microsporum gypseum* and a Gram-negative bacterium, *Klebsiella pneumoniae*. The quinone methide moiety was crucial for the biological activity (Sotanaphun et al., 1999b, p. 450). Some diterpenes, *ent*-abietadienolides, from *Euphorbia sessiliflora* also showed moderate antibacterial activities (Sutthivaiyakit et al., 2000, p. 947).



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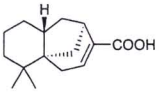

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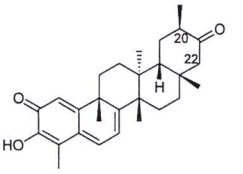

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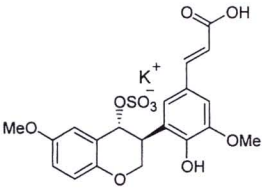


**26**

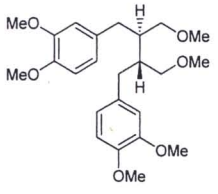
An antiviral isoflavonoid sulfate, torvanol (**26**), was isolated from the MeOH extract of the fruits of *Solanum torvum* (Arthan et al., 2002, p. 459). The compound exhibited activity against the herpes simplex virus type 1 with the $IC_{50}$ value of 9.6 $\mu$g/ml. Water extracts of the plants *Maclura cochinchinensis* and *Mangifera indica* have been found to exhibit activity against herpes simplex viruses (HSV-1 and -2) in the plaque inhibition assay (Yoosook et al., 2000, p. 411).

## Anticancer agents

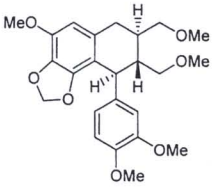
As mentioned earlier, apart from being an excellent source of anti-infectious drugs, plants are also a good source of anticancer agents. These include paclitaxel (Taxol®) from *Taxus brevifolia*, vincristine (Oncovin®) from *Catharanthus roseus*, podophyl-

lotoxin, the natural product precursor of etoposide from *Podophyllum peltatum* and camptothecin from *Camptotheca acuminata*. The finding that taxoids act through the stabilization of microtubules has led to the search for new agents that function by a comparable mechanism. Towards this end, new compounds have been discovered. Epothilones are a new class of macrocyclic natural products which were first isolated from myxobacteria (Höfle et al., 1996, p. 1567). Epothilones are more potent than taxol in some cell lines and they hold great promise for further investigation (Bollag et al., 1995; Gerth et al., 1996; Finlay et al., 1997).

We have been interested in the screening of Thai medicinal plants for anticancer properties. The plant *Phyllanthus amarus* Schum. & Thonn. of the Euphorbiaceae family, locally known as *Look Tai Bai*, has been investigated for cytotoxicity activity. *Phyllanthus amarus* has been traditionally used for the treatment of jaundice and other hepatic diseases. Two major components, phyllanthin (**27**) and hypophyllanthin (**28**), were isolated from this plant (Somanabandhu et al., 1993, p. 233).

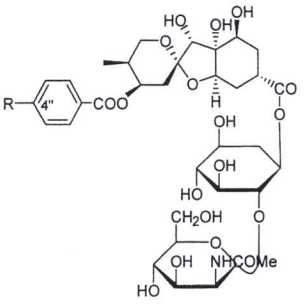


**27**

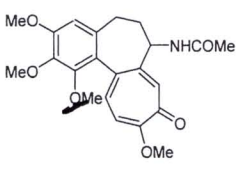


**28**

Apart from structural studies, the biological activities of these compounds have also been investigated. Evaluation of the cytotoxic potential of phyllanthin and hypophyllanthin was conducted with a battery of human tumor cell lines including P-388, BCA-1, HT-1080, LUC-1, MEL-2, COL-2, A-431, LNCaP, and ZR-75-1 cell lines. The $ED_{50}$ values of both compounds exceeded the highest concentration tested which is 20 $\mu$g/ml. However, phyllanthin (**27**) demonstrated an $ED_{50}$ value of 9.0 $\mu$g/ml with the drug-resistant cell line, KB-V1 in the absence of vinblastine, and very interestingly, this value was decreased to 2.1 $\mu$g/ml in the presence of vinblastine. Hypophyllanthin (**28**) did not mediate a cytotoxic response in the absence of vinblastine, but upon addition of this substance, an $ED_{50}$ value of 3.8 $\mu$g/ml was obtained. However, neither compound demonstrated activity with the drug-sensitive cell line, KB-3. Recently another Thai phyllanthus plant, *Phyllanthus acidus*, was investigated (Vongvanich et al., 2000, p. 5420). The spirit extract of this plant has been reported to reduce the craving for alcohol. Two cytotoxic water-soluble norbisabolane glycosides, phyllanthusol A (**29**) and phyllanthusol B (**30**), were isolated from the methanol extract of the roots of this plant. Phyllanthusols A and B exhibited cytotoxicity against BC ($EC_{50}$ at 4.2 and 4.0 $\mu$g/ml) and KB ($EC_{50}$ at 14.6 and 8.9 $\mu$g/ml) cell lines. Apart from *Phyllanthus amarus*, we have also investigated *Gloriosa superba* Linn. for anticancer activity. *Gloriosa superba* Linn. is known in Thai as '*Dong Dueng*' or '*Dao Dueng*', a climber plant in the family 'Colchicaceae', which is widely distributed in the tropical parts of Asia and Africa, with many varieties present in Thailand. The active principle of *Gloriosa superba* is the alkaloid colchicine which is isolated from the dried tubers of the plant. Colchicine has long been used for the treatment of arthritis. From the dried tubers of Thai *Gloriosa superba*, four tropolone alkaloids, colchicine (**31**), lumicolchicine (**32**), 3-demethyl-N-formyl-N-deacetylcolchicine (**33**), and 3-demethylcolchicine (**34**) were isolated (Mahidol et al., 2000, p. 6).



**29** R = OH; **30** R = H



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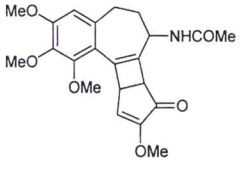

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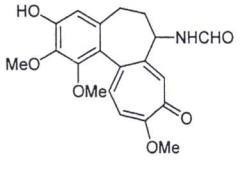

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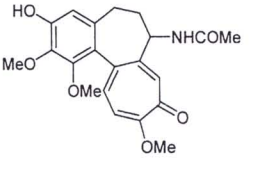
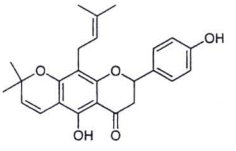


**34**

Another cancer cell line of interest to us is the cholangiocarcinoma cell line. Cholangiocarcinoma, a form of bile duct cancer, is a rare type of cancer in the Western world but it is highly prevalent in Thailand and in many other Asian countries. The cause of the disease is believed to be associated with infestation of *Opisthorchis viverrini* (O.V.) or liver fluke and exposure to a chemical carcinogen in food or in the environment, presumably, dimethylnitrosamine (DMN). We have conducted the evaluation of the effectiveness of some new anticancer agents against cholangiocarcinoma.

The $ED_{50}$ for the cholangiocarcinoma cell line for 3-demethyl-*N*-formyl-*N*-deacetylcolchicine (**33**) was found to be 0.0625 $\mu$g/ml, in contrast with the $ED_{50}$ of about 0.02 $\mu$g/ml for colchicine (**31**) itself. These values were approximately two times higher than the $ED_{50}$ values for the KB cell line. These results showed that the cholangiocarcinoma cell line is highly susceptible to the derivatives of the tropolone alkaloids, at least when testing *in vitro*, whether or not these agents will be effective *in vivo* remains to be determined in further experiments. We have synthesized various analogues of colchicine with the goal of improving the therapeutic index of the target compound by enhancing the potency of these analogues. Modification of the aromatic ring of colchicine was first studied. Colchicine could be selectively demethylated at C-2 by the action of sulfuric acid. Ester and ether analogues of 2-desmethylcolchicine were synthesized. The biological testing indicated that the longer chain of the alkyl or ester group attached to the aromatic ring of colchcine did not improve the biological activity in the cholangiocarcinoma cell line. Modifications of the tropolone ring and the peripheral functional groups of the tropolone ring have also been studied. The results of the biological testings of these compounds using the cholangiocarcinoma cell line showed very low biological activities as compared to colchicine; these results clearly illustrated the importance of the tropolone ring in the activity. Even though the results of the above biological testing have been very discouraging, the results clearly indicated to us that modifications of the aromatic ring and the tropolone ring of colchicine molecule will not yield any compound with higher biological activity than colchicine itself. At this point there is only one alternative left, that is the modification of the nitrogen side chain. Many colchicine derivatives with modification at the nitrogen atom have been synthesized and these compounds have been subjected to biological testing for anti-cholangiocarcinoma activity and found to be more potent than colchicine itself. Some derivatives exhibited very impressive activity, 20–30 times more potent than colchicine.

We have also investigated a plant named *Derris reticulata* Benth. (Leguminosae), it is a well known Thai herbal medicine used for the relief of thirst and as an expectorant. The Leguminosae is known to be a rich source of flavonoids and most of the prenyl derivatives have been found in this family (Harborne and Williams, 1998, 2001). The flavonoids exhibit diverse biological activities and recent interest has been focussed on their medicinal and nutritional values (Harborne and Williams, 2000, p. 481). Recently, some biologically active prenylated flavonoids have been reported (Manfredi et al., 2001; Sekine et al., 1999; Tseng et al., 2001) and, significantly, it was found that the prenyl groups on the flavonoid skeleton play an important role in anti-HIV activity (Meragelman et al., 2001, p. 546). Initially, we reported the isolation and structural characterization of four prenylated flavanones, lupinifolin (**35**), 2‴,3‴-epoxylupinifolin (**36**), dereticulatin (**37**), and 1‴-hydroxy-2‴,3‴-epoxylupinifolin (**38**) from the stems of *Derris reticulata* (Mahidol et al., 1997b; Prawat et al., 2000). The structures of these compounds were deduced from various spectroscopic analyses, especially 1D and 2D NMR as well as chemical transformations.

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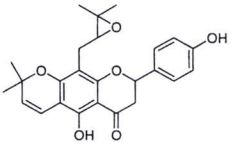


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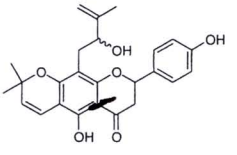


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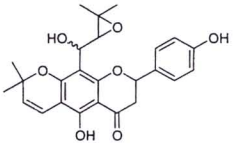
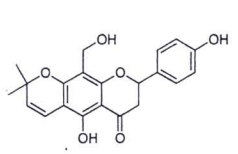



**38**

Further investigation of this herb has led to the isolation of two new cytotoxic flavonoids, 4′,5-dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano [2″,3″:7,6]flavanone (**39**), and 2‴,3‴-dihydroxylupinifolin (**40**) (Mahidol et al., 2002b, p. 1287).



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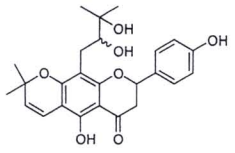
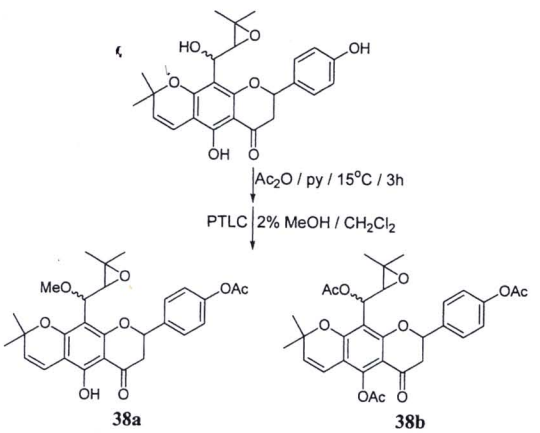


40

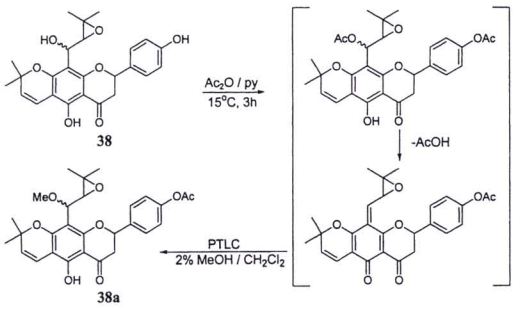
We have also investigated the chemistry of some of these compounds. For example, epoxidation of lupinifolin (**35**) with magnesium monoperoxy-phthalate hexahydrate (MMPP) gave epoxylupinifolin (**36**). Acetylation of 1‴-hydroxy-2‴,3‴-epoxylupinifolin (**38**) with acetic anhydride in pyridine was attempted. After general workup, the crude was chromatographed on silica gel by PTLC using 2% MeOH in $CH_2Cl_2$ as developing solvent to give two compounds **38a** and **38b** in 50% and 31% yields respectively as shown in Scheme 1.




38a 38b

*Scheme 1.* Acetylation of compound **38**.

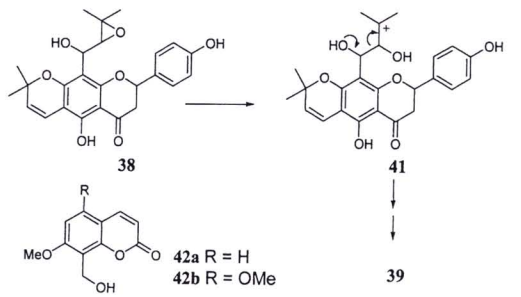
The formation of the abnormal methoxy derivative under these acetylation conditions can be explained by the mechanism as shown in Scheme 2. Partial acetylation of the hydroxyl group leads to the acetoxy derivative. Loss of the acetic acid with the help of the hydroxyl group can lead to the quinone methide intermediate which could then react with methanol to give the methoxy compound. Apparently, the compound was formed during PTLC purification when methanol was used as developing solvent.




38 38a

*Scheme 2.* Proposed mechanism for the formation of methoxy derivative **38a**.

The co-occurrence of hydroxymethyl compound **39** with 1‴-hydroxy-2‴, 3‴-epoxylupinifolin (**38**) and compound **40** lends support to the proposal in Scheme 3 that the hydroxymethyl group in compound **39** could be derived from this compound by acid catalyzed opening of the epoxide ring or protonation of the hydroxyl group in compound **40** to give the corresponding stable carbonium ion followed by carbon-carbon bond cleavage as shown in **41** to give the aldehyde, an immediate precursor to compound **39**. This biosynthetic route apparently could be applied to rationalize the biosynthesis of the extremely rare hydroxymethyl coumarins, murrayacarpin-A and -B, **42a** and **42b**, which also co-occur with the prenyl derivatives (Wu et al., 1989, p. 293).

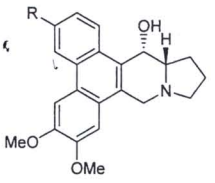



38 41 39

*Scheme 3.* The proposed biosynthesis of compound **39**.

We have also carried out the *in vitro* bioassay evaluation of lupinifolin, epoxylupinifolin and dereticulatin triacetate. They inhibited the P-388 cell line at 0.4–0.5 $\mu$g/ml while hydroxymethyl compound (**39**) and 2‴,3‴-dihydroxylupinifolin (**40**) inhibited at 6.4 and 1.3 $\mu$g/ml. All compounds were inactive against the KB cell line. Other flavonoid compounds including the calycopterones, a new class of biflavonoids, which exhibited novel cytotoxicity in a diverse panel of human tumor cell lines (Wall et al., 1994, p. 1465). Labdane

diterpenes from *Croton joufra* (Sutthivaiyakit et al., 2001, p. 811) and *Croton oblongifolius* (Roengsumran et al., 2001, 2002) collected from various parts of Thailand have found to possess cytotoxicity which is also found in clerodane derivatives from the same plant. *O*-methyltylophorinidine (**43**), a phenanthroindolizidine alkaloid, has been reisolated from *Ficus hispida* collected in Chiang Rai in the northern part of Thailand (Peraza-Sánchez et al., 2002, p. 186). The compound was shown to be highly cytotoxic in many cell lines tested (Col2, $ED_{50}$ = 0.02 $\mu$g/ml; Lu1, $ED_{50}$ = 0.018 $\mu$g/ml; KB, $ED_{50}$ = 0.02 $\mu$g/ml; and LNCaP, $ED_{50}$ = 0.03 $\mu$g/ml). Interestingly, a related phenanthroindolizidine (**44**) was isolated from the Danaid butterfly, *Ideopsis similis*. The compound was found to have a potent cytotoxic property against a human gastric cancer cell line, TMK-1 ($IC_{50}$ = 0.5 ng/ml) (Komatsu et al., 2001, p. 1833).

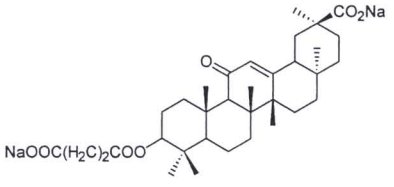



**43** R = OMe

**44** R = OH

## Antiulcer agents

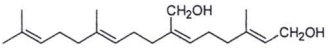
It has been estimated that about 10–20% of people in the West suffer from a peptic ulcer at some stage of their lives, and the treatment of this condition has long been of interest to the medical community.




**45**

Plants are also a good source of antiulcer drugs. The modern treatment of peptic ulcers started in the 1960s with the use of a drug called carbenoxolone (**45**) which is a sodium salt of a triterpenic acid. The drug was discovered after the intensive investigation of the roots and rhizomes of liquorice (*Glycyrrhiza glabra*). It is a transformation product of glycyrrhizic acid, a natural product found in this plant.

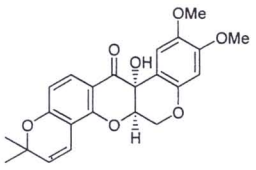
As far as the discovery of antiulcer drugs is concerned, the highlight must be the discovery of plaunotol (**46**) from the plant from Thailand called *Plao-Noi*, *Croton sublyratus* Kurz. (Euphorbiaceae) by Japanese scientists at the Sankyo company.



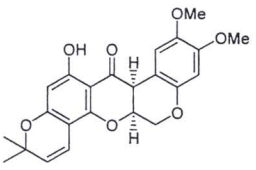

**46**

The structure of compound **46** was proved to be the diterpene as shown by spectroscopic methods and synthesis (Ogiso et al., 1978, p. 3117). The compound was proved to be a potent antiulcer drug. The mechanism of the action of this drug is probably that it raises the defensive factors since it enhances prostaglandin levels in gastric mucosa. Prostaglandins are known to inhibit acid secretion and stimulate the secretion of mucus, and of bicarbonate. This compound is now commercially available under the trade name of Kelnec. The antiulcerative effect of Thai bananas of different varieties have also been recently investigated (Pannangpetch et al., 2001, p. 407).

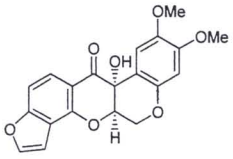
It is now generally accepted that *Helicobacter pylori* infection is the major cause of chronic active gastritis and peptic ulcer disease. It was found that rotenoids from the roots of *Derris malaccensis* exhibited selective activity against *Helicobacter pylori*. Tephrosin (**47**) and toxicarol (**48**) gave the best result with minimum inhibitory concentrations (MIC) of 0.3 mg/ml. The toxicity of the rotenoids as insecticides and piscicides is caused by the inhibition of NADH oxidation in the respiratory chain. It is thus likely that the selective anti-*H. pylori* activity in these rotenoid compounds might be due to the inhibition of NADH oxidation (Takashima et al., 2002, p. 611). *Derris malaccensis* also grows in Thailand and is locally known as '*haang lai kaow*'. The plant is used for pest control and as a fish poison. We have recently reported the isolation and structural elucidation of a new rotenoid, 12a-hydroxyelliptone (**49**), and the known rotenoid, 12-deoxo-12$\alpha$-acetoxyelliptone (**50**), from this plant (Thasana et al., 2001, p. 1121).



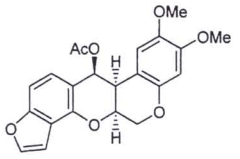
47



48



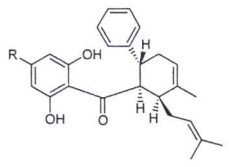
49



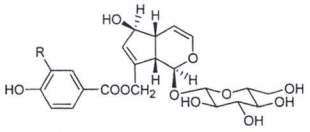
50

## Anti-inflammatory agents

Some Thai medicinal plants have recently been investigated for anti-inflammatory activity. (–) Hydroxypanduratin A (**51**) and panduratin A (**52**) have been isolated from the chloroform extract of a red rhizome variety of *Boesenbergia pandurata*. Both compounds showed significant topical anti-inflammatory activity in the TPA-induced ear edema assay in rats (Tuchinda et al., 2002, p. 169). Iridoids (**53**) and (**54**) were isolated from the polar fractions of the butanol extract of *Vitex peduncularis* Wall. (Verbenaceae) and were tested for inhibition of cyclooxygenase (COX, prostaglandin H synthase)-1 and COX-2 regulated prostaglandin biosynthesis using COX deficient murine cell lines. Iridoid (**53**) showed preferential inhibition of COX-2 over COX-1 (COX-2 $IC_{50} = 0.026 \pm 0.015$ mg/ml and less than 10% inhibition of COX-1 at this concentration). Similarly, iridoid (**54**) had a COX-2 $IC_{50}$ value of $0.15 \pm 0.21$ mg/ml while having almost no effect on COX-1 activity as indicated by less than 10% inhibition at this concentration (Suksamrarn et al., 2002, p. 72).



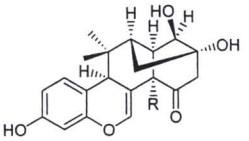
**51** R = OH; **52** R = OMe



**53** R = H; **54** R = OMe

## Miscellaneous agents

The plant '*Kwao Keur*', *Pueraria mirifica*, has captured the interest of local newspapers during the past years because of its rejuvenating properties. Miroestrol (**55**) was previously isolated and found to exhibit potent estrogenic activity. It was regarded as the compound with the highest estrogenic potency among the known phytoestrogens. Recent investigation resulted in the isolation of deoxymiroestrol (**56**) and it was found that deoxymiroestrol was 10 times more potent than the previously isolated miroestrol in terms of their growth-promoting effects on MCF-7 human breast cancer cells in the presence of an estrogen antagonist, toremifene. Due to the facile aerial oxidation of deoxymiroestrol to miroestrol, it is likely that miroestrol was an artifact (Chansakaow et al., 2000, p. 173).



**55** R = OH

**56** R = H

8-Isopentenylnaringenin, a prenylflavonoid, isolated from a methanol extract of the heartwood of *Anaxagorea luzonensis*, has been found to exhibit estrogen agonist activity (Kitaoka et al., 1998, p. 511). Similar agonist activity has also been reported for retrodihydrochalcone derivatives isolated from *Dracaena loureiri* (Ichikawa et al., 1997, p. 540).

In conclusion, at present there are a large number of chronically debilitating or life-threatening diseases that urgently require improved or new medical treatments. Chemotherapy is a well-established approach for the remedy of these diseases. With new diseases and increasing resistance to existing drugs, there is a pressing need to discover and develop new innovative drugs with diminished side-effects to combat cancer cells, viruses and other threats. The research on natural products will be essential for the discovery of lead compounds in the future because of the incredible diversity of chemical structures that are produced by living organisms.

## Acknowledgements

We thank the Thailand Research Fund (TRF) for generous financial support and the award of Senior Research Scholar to S.R. We also acknowledge the facilities in the Department of Chemistry, Mahidol University, provided by the Postgraduate Education and Research in Chemistry (PERCH) program.

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# Anti-invasive effects of curcuminoid compounds from *Curcuma aromatica* Salisb. on murine colon 26-L5 carcinoma cells

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Reprinted from : Journal of Traditional Medicines (J. Trad. Med.) **19** (6), 209－215, 2002
Medical and Pharmaceutical Society for WAKAN-YAKU
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# Anti-invasive effects of curcuminoid compounds from *Curcuma aromatica* Salisb. on murine colon 26-L5 carcinoma cells

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*(Received October 16, 2002. Accepted November 22, 2002.)*

## Abstract

Bioassay-directed fractionation of the active chloroform extract from the rhizomes of *Curcuma aromatica* Salisb. (Zingiberaceae) led to the isolation of four main curcuminoid constituents: curcumin (CA-1), demethoxycurcumin (CA-2), 5'-methoxycurcumin (CA-3) and bisdemethoxycurcumin (CA-4). This is the first report to describe the isolation of CA-3 from *C. aromatica*. The chemical structures of these compounds were determined on the basis of spectral analysis and their inhibitory effects on the proliferation, invasion and migration of murine colon 26-L5 adenocarcinoma cells were evaluated *in vitro*. Curcumin and its analogues (CA-2, 3 and 4), at the non-cytotoxic concentration of 10 $\mu$M, inhibited the invasive ability of colon 26-L5 cells to the ranges of 22.8, 28.9, 10.3 and 62.0%, respectively. A similar effect of these constituents on the migration of colon 26-L5 cells was also observed. Among these curcuminoids, CA-4 showed the strongest activities, inhibiting both tumor cell invasion and migration in a concentration-dependent manner.

**Key words** *Curcuma aromatica* Salisb., Zingiberaceae, curcuminoid analogues, metastasis, invasiveness, colon 26-L5 carcinoma cells.

**Abbreviations** UV, ultra-violet; IR, infra-red; $^1$H-NMR, $^1$H-nuclear magnetic resonance; $^{13}$C-NMR, $^{13}$C-nuclear magnetic resonance; TLC, thin layer chromatrography; AcOEt, ethyl acetate; $CHCl_3$, chloroform; MeOH, methanol; m.p., melting point; MS, mass spectrometry; WST-1, 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzenedisulfonate; BSA, bovine serum albumine; DMSO, dimethylsulfoxide.

## Introduction

Metastasis is the major cause of morbidity and death for cancer patients. The majority of the patients in the treatment failure group succumb to the direct effect of metastasis or to complications associated with treatment of metastasis.[1] Thus, the development of new drugs for optimal treatment with less resistance and furthermore less complication is required.

Wild turmeric or yellow zedoary (*Curcuma aromatica* Salisb., family Zingiberaceae) is a perennial herb indigenous to and cultivated in Tropical Asia. This plant has no value as a spice but is used in cosmetic formulation and medicines. For medical purposes, the crude drug prepared from its rhizomes has been used as a cholagogue, stomachic, carminative, chloretic, analgesic and sedative product, and also for the treatment of hepatitis, menstrual disorders, epilepsy and skin disorders. It has been recently used as a health food in Japan.[2] Moreover, in Thai and Chinese traditional medicines, it is used for the treatment of several types of cancers such as cervical cancer and liver cancer.[3] This plant is well known as the source of monoterpenoids,[4] sesquiterpenoids[4-8]

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and curcuminoids[9,10] that have been reported to possess antimicrobial,[11,12] antifungal, antioxidant and antitumor activities.[13,14] A previous work reported that $\beta$-elemene, a sesquiterpene isolated from *C. aromatica*, markedly prolonged the survival of mice with Ehrlich's ascites tumor and ascitic hepatoma cells *in vitro*.[15,16] However, no information regarding the inhibitory activity of tumor cell invasion and the structure-activity relationship of chemical compounds isolated from this plant has been reported yet.

Several mechanisms for the anti-metastatic effect of curcumin have been reported,[17-22] such as the suppression of invasion of B16F-10 melanoma cells by inhibition of metalloproteinase production[17] or its anti-invasive effect on SK-Hep-1 cells (human hepatocellular carcinoma) that was associated with MMP-9 expression.[18] Curcuminoids also inhibited the angiogenic response stimulated by fibroblast growth factor-2 and the expression of matrix metalloproteinase gelatinase B.[22] In addition, our recent study showed that the combined treatment with curcumin and the anti-cancer drug cis-diamine-dichloroplatinum (CDDP) resulted in a marked inhibition of mediastinal lymph node metastasis of orthotopically implanted LLC (Lewis lung cancer) cells, in addition to the inhibition of tumor growth at the implanted site.[23] Therefore, to extend our investigations on the anti-metastasis activity of curcumin and related compounds, we focused our attention on the anti-invasive effect of curcuminoids isolated from the rhizomes *of C. aromatica* and examined their activities on the *in vitro* proliferation, invasion and migration of the murine colon 26-L5 adenocarcinoma cells.

## Materials and Methods

*General experimental procedures*: All melting points were determined on a Buchi 512 melting point apparatus and are uncorrected. UV absorption spectra were recorded on the double beam spectrophotometer, Hitachi 220 A. IR absorption spectra were obtained using KBr disc on a Shimadzu 440 spectrometer. $^1$H and $^{13}$C-NMR spectra were recorded on a JEOL JNM-FX 500 MHz spectrophotometer in $CDCl_3$/$CD_3OD$ using tetramethylsilane as an internal standard ( $\delta$ value, ppm). Mass spectra were measured on a JEOL DX-300/JMA 2000 operating at 70 eV. TLC was performed on pre-coated silica gel 60 $F_{254}$ plate (Merck). Column chromatography was carried out on silica gel (Kieselgel 60 (70-230 mesh), Merck).

*Plant materials*: Rhizomes of *Curcuma aromatica* were collected in Chiang Mai Province, Thailand in 2000. A voucher specimen was deposited at the Herbarium of the Natural Products Research Section, Research Division, National Cancer Institute, Bangkok, Thailand.

*Extraction and isolation*: Dried coarse powder of rhizomes (1.5 kg) of *C. aromatica* was extracted successively in a Soxhlet apparatus with *n*-hexane, followed by chloroform and methanol. Concentration of the extracts under reduced pressure afforded crude extracts of *n*-hexane (35.85 g), chloroform (23.79 g) and methanol (32.8 g). All crude extracts were determined for the antiproliferative activity against KB and P388 tumor cells *in vitro*, and the chloroform extract showed the strongest activity.

*Purification and identification of curcuminoid compounds*: The active chloroform extract (23 g) was subjected to column chromatography on silica gel and eluted with chloroform and methanol by gradient system. Fractions of 75 ml were collected and then combined (*t.l.c.*) to provide 6 fractions (A-F). Fractions B-E, which showed a significant anti-proliferative activity against tumor cell lines, were further purified by repeated column chromatography. Fraction B (5.1 g) was chromatographed on a silica gel column and eluted with chloroform and methanol by gradient system. After recrystallization from *n*-hexane, compound 1 was obtained (orange needles, 1.16 g). Fraction C (4.3 g) was rechromatographed on silica gel column, eluted with chloroform: methanol (19:1) and two compounds were isolated. Re-crystallization of these compounds with *n*-hexane afforded compound 1 (0.907g) and compound 2 (yellow needles, 0.56 g). Fraction D (6.9 g) was rechromatographed on silica gel column, eluted with *n*-hexane: AcOEt (1:1) to give compound 1 (0.21 g), compound 2 (0.92 g) and compound 3 (red powder, 0.14 g). Fraction E (5.8 g) was chromatographed on a silica gel column, eluted with $CHCl_3$: MeOH (9:1) and recrystallized with chloroform to give compound 2 (0.08 g) and compound 4 (yellow needles, 0.82 g).

The identification of these active compounds was confirmed by mixed m.p. and comparison of the spectral

data ( UV, IR, $^1H$ and $^{13}C$-NMR and MS).

*Compound 1: curcumin (CA-1)*; orange needles from *n*-hexane, m.p. 178-180℃ (lit. 182-183[24]); $C_{21}H_{20}O_6$; MW 368; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 261 and 420; IR $\nu_{max}$ (KBr) $cm^{-1}$ ; 3450, 2950, 1650, 1600, 1505, 1422, 1275, 1150, 1110, 950, 850 and 800. $^1H$-NMR ($CDCl_3$) $\delta$: 7.57 (2H, d, J=15.87 Hz, H-1 and H-7), 7.11 (2H, dd, J = 8.24, 1.83 Hz, H-6'and H-6"), 7.03 (2H, d, J=1.83 Hz, H2'and H-2"), 6.92(2H, d, J=8.24 Hz, H-5'and H-5"), 6.46 (2H, d, J=15.87 Hz, H-2 and H-6), 5.78 (1H, s, H-4), 3.93 (6H, s, OMe-3' and 0Me-3"); $^{13}C$-NMR ($CDCl_3$) $\delta$: 140.5 (C-1), 121.7 (C-2), 183.2 (C-3), 101.2 (C-4), 183.2 (C-5), 121.7 (C-6), 140.5 (C-7), 127.7 (C-1' and C-1"), 109.6 (C-2' and C-2"), 146.8 (C-3' and C-3"), 147.8 (C-4' and C-4"), 114.8 (C-5' and C-5"), 122.9 (C-6' and C-6"), 55.9 (OMe-3' and OMe-3"); MS m/z (rel. int. %): 368 ($M^+$, 44), 350 ($M^+$-$H_2O$, 40), 177(100).[24-27]

*Compound 2: demethoxycurcumin (CA-2)*; yellow needles from chloroform, m.p. 181-182℃ (lit. 175-177[24]); $C_{20}H_{18}O_5$; MW 338; UV $\lambda_{max}$ (MeOH) nm(log $\varepsilon$): 250 and 418[1]; IR $\nu_{max}$ (KBr) $cm^{-1}$ : 3450, 2820, 1622, 1580, 1560, 1440, 1260, 1150, 1020, 960 and 890; $^1H$-NMR ($CDCl_3$) $\delta$: 7.52 (1H, d, J=15.87 Hz , H-1), 7.50 (1H, d, J=15.87 Hz , H-7), 7.39 (2H, dd, J=8.54, 1.83 Hz, H-2" and H-6"), 7.04 (1H, d, J=8.54 Hz, H-2'), 7.03 (1H, dd, J=8.54, 1.83 Hz, H-6'), 6.82 (1H, d, J=8.85 Hz, H-5'), 6.79 (2H, dd, J=8.54, 1.83 Hz, H-3"and H-5"), 6.44 (1H, d, J=15.87 Hz, H-2), 6.43 (1H, d, J=15.87 Hz, H-6), 5.80 (1H, s, H-4), 3.87 (3H, s, OMe); $^{13}C$ NMR ($CDCl_3$) $\delta$: 141.1 (C-1), 121.1 (C-2), 183.1 (C-3), 101.6 (C-4), 183.6 (C-5), 121.4 (C-6), 141.1 (C-7), 127.5 (C-1'), 126.9 (C-1"), 110.5 (C-2'), 130.3 (C-2"), 147.9 (C-3'), 116.1 (C-3"), 148.9 (C-4'), 159.6 (C-4"), 115.6 (C-5'), 116.1 (C-5"), 123.2 (C-6'), 130.3 (C-6"), 55.9 (OMe-3'); MS m/e (% rel. int): 338 ($M^+$, 29), 320 ($M^+$-$H_2O$, 30), 147(100).[24-27]

*Compound 3: 5'-methoxycurcumin (CA-3)*; red powder from chloroform, m.p. 145-146℃; $C_{22}H_{22}O_7$; MW 398; $^1H$-NMR ($CDCl_3$) $\delta$: 7.58 (1H, d, J=15.87 Hz., H-1), 7.55 (1H, d, J=15.87 Hz, H-7), 7.11 (1H, dd, J=8.00 and 1.83 Hz, H-6"), 7.03 (1H, d, J=1.83 Hz, H-2"), 6.91 (1H, d, J=8.00 Hz, H-5"), 6.78 (1H, s, H-2'), 6.46 (1H, d, J=15.87 Hz, H-2), 6.47 (1H, d, J=15.87 Hz, H-6), 5.79 (1H, s, H-4), 5.86 (1H, brs, OH-4'), 5.76 (1H, brs, OH-4"), 3.93 (3H, s, OMe-3"), 3.92 (6H, s, OMe-3'and OMe-5'); $^{13}C$ NMR ($CDCl_3$) $\delta$: 140.7 (C-1), 122.0 (C-2), 183.5 (C-3), 101.2 (C-4), 182.8 (C-5), 121.8 (C-6), 140.7 (C-7), 126.6 (C-1'), 127.6(C-1"), 105.1 (C-2'), 109.6 (C-2"), 147.2 (C-3'), 146.8 (C-3"), 137.0 (C-4'), 147.9 (C-4"), 147.2 (C-5'), 114.8 (C-5"), 105.1 (C-6'), 122.8 (C-6"), 55.9 (OMe-3"); 56.3 (2OMe-3' and 5'); MS m/e (% rel. int): 398[28-29]

*Compound 4: bisdemethoxycurcumin (CA-4)*; yellow needles from chloroform, m.p. 222-223℃ (lit. 232-234℃[24]); $C_{19}H_{16}O_4$; MW 308; UV $\lambda_{max}$ (MeOH) nm: 250 and 480; IR $\nu_{max}$ (KBr) $cm^{-1}$ ; 3200-3300, 1622, 1600, 1560, 1510, 1430, 1275, 1231, 1165, 1140, 975 and 830; $^1H$-NMR ($Me_2CO$-$d_6$) $\delta$: 7.61 (d, 2H, J=15.9 Hz, H-1 and H-7), 7.57(d, 4H, J=8.6 Hz, H-2', H-6', H-2" and H-6"), 6.91 (d, 4H, J=8.6 Hz, H-3', H-5', H-3" and H-5"), 5.98 (s, 1H, H-4); $^{13}C$-NMR ($CDCl_3$) $\delta$: 141.1 (C-1), 122.3 (C-2), 184.5 (C-3), 101.6 (C-4), 184.5 (C-5), 122.3 (C-6), 141.1 (C-7), 128.2 (C-1' and C-1"), 130.9 (C-2' and C-2"), 116.2 (C-3' and C-3"), 160.9 (C-4' and C-4"), 116.2 (C-5' and C-5"), 130.9 (C-6' and C-6"); MS m/e (rel.int. %): 308 ($M^+$, 35), 290 ($M^+$-$H_2O$, 50), 147 (100).[24-27]

*Assay for the anti-metastatic activity against Colon 26-L5 cells*

*Cell Culture*: The murine colon 26-L5 adenocarcinoma cells (colon 26-L5) were maintained as monolayer cultures in RPMI-1640 medium (Nissui Pharm Co. Ltd., Tokyo, Japan) supplemented with 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY.), 0.1% sodium bicarbonate and 2 mM glutamine (Wako Pure Chemical Ind., Ltd., Kyoto, Japan) and were incubated at 37℃ in a humidified atmosphere containing 5% $CO_2$ in air.

*Cell Proliferation Assay*: Cellular viability in the presence or absence of experimental agents was determined using a WST-1 Cell Counting Kit (Wako Pure Chemicals Ind., Ltd., Japan). Briefly, Colon 26-L5 cells ($1x10^4$) suspended in 100 $\mu$l of RPMI-1640 medium containing 0.03% BSA were seeded onto a 96-well culture plate (Costar, Cambridge, MA, U.S.A.). After 24 h of pre-incubation, various concentrations of the isolated compounds were added and then incubated for a further 24 h. At the end of incubation, 10 $\mu$l of WST-1 solution were added to each well and then incubated for 4 h. The amount of formazan formed was measured spectrophotometrically at 450 nm using an Immuno Mini NJ-2300

plate reader. Each assay was performed in triplicate. Test compounds were dissolved in DMSO and then diluted with medium. Solutions containing less than 0.1% of DMSO had no cytotoxic effect on the cells. The $IC_{50}$ values were calculated from the mean values ± S.D. of absorbances.

*In Vitro Invasion and Migration Assays*: Invasion of tumor cell through the reconstituted basement membrane Matrigel was assayed as described previously.[30] Firstly, Transwell cell culture chambers (Costar 3422, Cambridge, MA, U.S.A.) were set up with polyvinylpyrrolidone-free carbonate filters of 8.0 μm pore size (Nucleopore, Pleasanton, U.S.A). The lower surface of the filters was coated with 2 μg of fibronectin (Iwaki Glass Co., Ltd., Japan) and the upper surface was coated with 10 μg of Matrigel (Collaborative Research Inc, Bedford, MA, USA). Colon 26-L5 cells ($2x10^5$ cells/chamber) were suspended in RPMI-1640 (100 μl/chamber) containing 0.1% of bovine serum albumin (BSA) and distinct concentrations of curcuminoid compounds (0, 1, 3 and 10 μM). The cell suspension was then applied to the upper compartment of the chambers and incubated in a 24-well culture plate containing 600 μl of 0.1% BSA-containing medium at 37°C for 24 h. The filters were finally fixed with 30% methanol and then stained with 0.5% crystal violet for 5 min. After gentle rinsing, the cells on the upper surface of the filter were removed by wiping with a cotton swab. The cells that had invaded through the Matrigel and filter were extracted with 30% acetic acid and colorimetrically assessed by measuring its absorbance at 590 nm using an Immuno Mini NJ-2300 plate reader. Each experiment was done in quaduplicate.

The migration assay was performed in a similar procedure to that of the invasion assay, but differed on the non-coating of the filters with Matrigel.

*Statistical analysis*: All data are expressed as mean values ±S.D. Student's *t*- test for unpaired samples (2-tailed) was used to determine statistic differences that were accepted to be significant when *p* values were lower than 0.05.

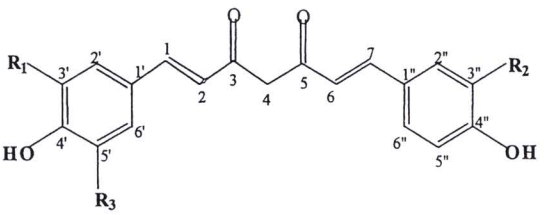
## Results

*Chemical structures of Curcuminoids isolated from the rhizomes of Curcuma aromatica Salisb.*

The chloroform extract, which exhibited a potent anti-proliferative activity against KB and P388 tumor cells *in vitro*, was purified by silica gel column and four curcuminoid compounds were isolated. By the comparison of the spectral data (UV, IR, $^1H$ and $^{13}C$-NMR and MS), these compounds were found to belong to the diarylheptanoids group and were identified as curcumin (CA-1), demethoxycurcumin (CA-2), 5'-methoxycurcumin (CA-3) and bisdemethoxycurcumin (CA-4) (Fig. 1).

*Effect of Curcuminoids on Tumor Cell Proliferation*

The antiproliferative activity of CA-1, CA-2, CA-3 and CA-4 against colon 26-L5 cells was determined *in vitro* by the WST-1 cytotoxicity assay. As shown in Fig. 2, all compounds at concentrations of less than 10 μM




CA-1: $R_1$=$R_2$=$OCH_3$; $R_3$=H

CA-2: $R_1$=$OCH_3$, $R_2$=$R_3$=H

CA-3 : $R_1$=$R_2$=$R_3$=$OCH_3$

CA-4: $R_1$=$R_2$=$R_3$=H

Fig. 1. Chemical structures of four curcuminoid compounds isolated from *Curcuma aromatica*.

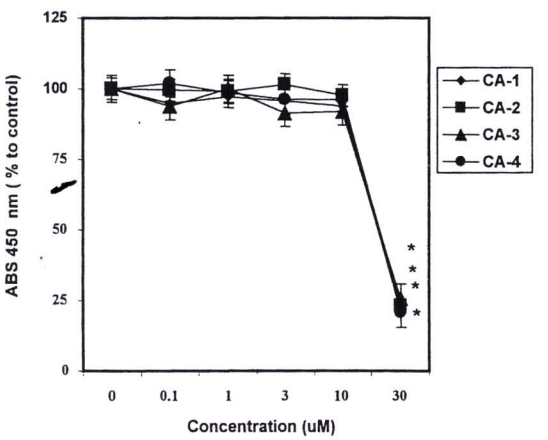



Fig. 2. Effect of curcuminoid compounds on the proliferation of Colon 26-L5 cells.
Colon 26-L5 cells ($1x10^4$ cells/well) were seeded into a 96-well plate in 0.1% BSA-RPMI 1640 medium. After 24 h of incubation, various concentrations of four curcuminoid compounds (◆CA-1, ■CA-2, ▲CA-3, ●CA-4) were added to the cultures and then incubated for an additional 24 h. WST-1 solution (10 μl/well) was added to each well and the plate was incubated at 37°C for 4 h. before termination of the assay. Proliferation was assessed by measuring absorbance of the culture at 450 nm. The data are expressed as mean ± S.D. of triplicate wells. *$p$<0.01.

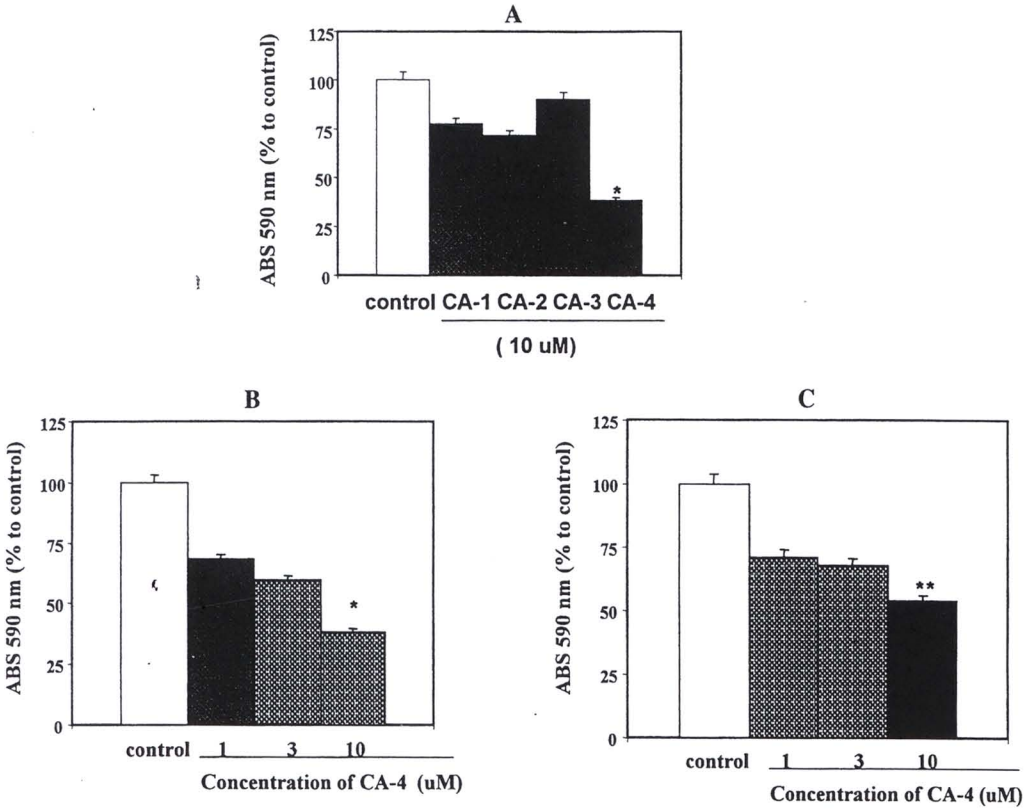



Fig. 3. Effect of curcuminoid compounds on the invasive and migrating properties of Colon 26-L5 cells.
For the invasion assay, Colon 26-L5 cells ($2x10^5$ cells/chamber) were seeded onto filters pre-coated with 10 $\mu$g of Matrigel on the upper surface and 2 $\mu$g of fibronectin on the lower surface in Transwell chambers in the presence and absence of curcuminoid compounds (10 $\mu$M). After 24 h. of incubation, the number of cells that invaded the lower surface was determined by crystal violet staining and colorimetrically assessed by measuring the absorbance at 590 nm. For the migration assay, the filters were not coated with Matrigel. A, Four curcuminoid compounds were tested by the invasion assay; B, Dose-response of CA-4 was determined by the invasion assay; C, Dose-response of CA-4 was determined by the migration assay. The data were expressed as mean $\pm$ S.D. of four repeat chambers. *$p$<0.01, **$p$<0.05.

did not show any direct cytotoxicity against tumor cells *in vitro*.

*Effect of Curcuminoids on Tumor Cell Invasion and Migration*

The effect of curcumin (CA-1) and its analogues (CA-2, 3, 4) on the inhibition of invasion of colon 26-L5 cells through Matrigel/fibronectin- and fibronectin-coated filters were evaluated. Among the compounds, CA-4 at the non-cytotoxic concentration of 10 $\mu$M, significantly inhibited the invasion of colon 26-L5 cells as compared with the control (Fig. 3A, P<0.01). As shown in Fig 3B and 3C, CA-4 inhibited tumor cell invasion and migration in a concentration-dependent manner.

## Discussion

The rhizomes of *Curcuma aromatica* Salisb. were extracted in a Soxhlet apparatus with *n*-hexane, chloroform and methanol as described in the experiment section. The chloroform extract from rhizomes of *C. aromatica* was found to show anti-proliferative activity against KB and P388 cells at $IC_{50}$ values corresponding to 6.8 and 9.0 $\mu$g/ml, respectively. The fractionation of this extract by repeated silica gel column chromatography and recrystallization afforded four main curcuminoid constituents: curcumin (CA-1), demethoxycurcumin (CA-2), 5'-methoxycurcumin (CA-3) and bisdemethoxycurcumin (CA-4) (Fig. 1). CA-3 has been found in *Curcuma xanthorrhiza*[28] and *C. zedoaria*,[29] but this is the first report to describe its isolation from *C. aromatica*.

We next investigated the inhibitory effects of CA compounds on the *in vitro* proliferation, invasion and migration of murine colon 26-L5 adenocarcinoma cells, and found that curcumin and its analogues (CA-2 and CA-4) significantly showed antiproliferative activity against colon 26-L5 cells at the concentration of 30 $\mu$M (Fig. 2). Moreover, CA-4 at the non-cytotoxic concentration of 10 $\mu$M was markedly effective at inhibiting the tumor cell

invasion among the compounds (Fig. 3A). CA-4 also inhibited the migration of tumor cells to fibronectin-coated substrates as well as tumor invasion in a concentration-dependent manner (Fig. 3B and 3C).

Taking into account the inhibitory effect of CA-4 on the invasion assay of colon 26-L5 cells, we suggest that the anti-invasive effect of the curcuminoids investigated in this study might be related with the number of substituted methoxyl or hydroxyl groups in their benzene rings. The greater number of hydroxyl substituents (as seen in CA-4) may contribute to increase this effect, while the greater number of methoxyl substituents (as seen in CA-3) may be associated with a decreased effect. It seems to be in agreement with the known antitumor and antioxidant effects of curcumin that are mainly due to the presence of phenolic groups essential for the free radical scavenging activity, but that are decreased with the presence of methoxyl groups.[28] Some studies on the structure-activity relationships (SAR) of curcuminoid compounds reported that the presence of diketone moiety relates with the inhibitory effect of curcuminoids on the proliferation of human breast cancer cells,[31] or still that hydroxyl groups present at ortho-position on the aromatic rings of curcumin analogs are associated with their inducing activity on Phase 2 detoxification enzymes.[32] To further establish the SAR of compounds CA-1, CA-2, CA-3 and CA-4, the synthesis of derivatives compounds is currently under consideration.

The present study showed that CA-4 had inhibitory effects on the invasion and migration of colon 26-L5 cells without affecting cell growth. Bisdemethoxycurcumin (CA-4) isolated from *C. aromatica* rhizomes may be considered as an inhibitor for tumor invasion and useful for the prevention of liver metastasis of colon cancer without causing toxic effects.

## Acknowledgments

This study was supported in part by a Grant-in Aid for Leading Regional Utilizing Potential of Regional Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology, Japan, by the Thai Government Grant of the National Cancer Institute, Ministry of Public Health, Thailand, and by the Japanese-Thai Collaborative Scientific Research Fellowship (JSPS-NRCT), Japan in 2001. The authors are sincerely grateful to Professor Dr. Hiroshi Watanabe, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan, the representative of the JSPS fellowship of Japan for his kindness on providing this grant for continuing some part of the research work in Japan.

## 和文抄録

ショウガ科に属する *Curcuma aromatica* Salisb. の根茎のクロロホルム抽出エキスから，化学構造の明らかな4種のクルクミンおよびその関連化合物：curcumin (CA-1)，demethoxycurcumin (CA-2)，5'-methoxycurcumin (CA-3)，bisdemethoxycurcumin (CA-4) を分離した。これらの化合物を用いてマウス結腸癌細胞 (colon 26-L5) に対する増殖，基底膜への浸潤，細胞運動に及ぼす効果について検討した。クルクミン (CA-1) とその関連化合物 (CA-2, 3 および 4) は，細胞に対して傷害性を示さない10 μMの濃度において，マウス結腸癌細胞の基底膜への浸潤を抑制した（それぞれ22.8, 28.9, 10.3 および 62.0% の抑制率)。この癌細胞の運動能に対しても同様の抑制効果が観察された。これらのクルクミン関連化合物の中で，CA-4 は強い抑制活性を持ち，癌細胞の浸潤および運動能に対して濃度依存的な抑制効果を示した。このように，クルクミン関連化合物の芳香族環の hydroxyl 基および methoxyl 基が癌細胞の浸潤活性の発現と関係している可能性が示唆された。

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# The effects of Kampo herbal medicines (Scutellariae Radix, Carthami Flos, Linderae Radix) on the atherosclerosis mouse model introduced with heat shock protein (Hsp) 60 and high cholesterol diet

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*(Received September 30, 2002. Accepted December 12, 2002.)*

## Abstract

In the process of the formation of atherosclerosis, many immune factors, such as cytokines and chemokines are involved. On the other hand, heat shock proteins (Hsps) work as a Chaperon and are considered to have an effect that protects the cell from protein damage by restoration of the degenerated proteins. We were successful in establishing an atherosclerosis mouse model in C57BL/6NJc mice immunized with Hsp60 and simultaneously treated with a high cholesterol diet (HCD). At this time, using this model, we verified the effects of Kampo herbal medicines, Scutellariae Radix (SR), Carthami Flos (CF) and Linderae Radix (LR), on the pathological atherosclerositic change in the aorta, change in body weight, and alteration of serum cytokine levels. At first, compared with the control group, the reduction in the body weight of the groups that was administered with SR and CF were suppressed significantly ($p<0.05$). On the other hand, the production of IFN-$\gamma$ of the groups that were administered with SR and CF were suppressed significantly, but the LR group only showed a tendency of suppression. The lipid deposits that we observed have a tendency to increase the volume and area gradually from the aortic valve to the root of ascending aorta. The deposits were observed in each mouse of the control group and SR group, but only 20 to 60% of the mice in the remaining groups (CF group and LR group) exhibited lipid deposition. Consequently we found that the herbal medicines reduced the adjuvant function of Hsp60, and simultaneously reduced the progression of the atheroscleosis.

**Key words** Hsps, Hsp60, Hsp70, atherosclerosis, Kampo herbal medicines, model mouse, anti-Hsp antibody, Scutellariae Radix, Carthami Flos, Linderae Radix.

**Abbreviations** CF, Carthami Flos; Hsps, heat shock proteins; HCD, high cholesterol diet; LR, Linderae Radix; SR, Scutellariae Radix.

## Introduction

Atherosclerosis develops easily in medium and large-sized arteries, and the best-known mechanism of the onset of atherosclerosis is the response-to- injury hypothesis by R. Ross, 1973.[1,2)]

On the other hand, heat shock proteins (Hsps, or Chaperonins[3)]) are a group of proteins that are not only introduced and released from cells by many stimuli, but also exist within the cell without any stimuli. Hsps work as Chaperons that inhibit the over-expression of heteroproteins produced by external microorganisms, or the production of toxins, and the degeneration and aggression of proteins in the host cells while the dysbolism is caused by injury that was introduced by infection.[4)] Furthermore hsps are also considered to protect the cell from protein injury by restoring degenerated proteins.[5)]

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Tetrahedron Letters 44 (2003) 1019–1021

TETRAHEDRON LETTERS

# Synthesis of aryl α-keto esters via the rearrangement of aryl cyanohydrin carbonate esters

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Received 8 October 2002; revised 18 November 2002; accepted 29 November 2002

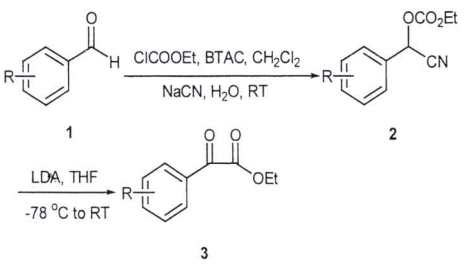
**Abstract**—A facile synthesis of aryl α-keto esters is reported involving the rearrangement of aryl cyanohydrin carbonate esters induced by the α-carbanion to the nitrile group generated by LDA. However, under similar conditions, an *o*-benzyloxycyanohydrin carbonate ester rearranged via a domino reaction leading to 2-phenylbenzofuran-3-carboxylic acid. 

α-Keto acids play a key role in biosynthesis as important substrates and products in the pyridoxal phosphate dependent transaminase enzymatic reaction.[1] Aryl α-keto esters have also been shown to be antisunburn compounds.[2] Interestingly, methyl and butyl 2-(4-methoxyphenyl)-2-oxoacetates have recently been isolated from the hydrophilic extract of the ascidian *Polycarpa aurata*.[3]

Aryl α-keto esters have been described as important intermediates in the synthesis of a variety of oxygenated heterocycles, such as furan derivatives,[4] and in the asymmetric synthesis of biologically active compounds[5] as well as other synthetic compounds.[6] Due to the importance of these α-keto acid derivatives, various methods have been reported for the synthesis of these compounds.[7]

Herein, we present our strategy leading to a short synthesis of aryl α-keto esters **3** via the rearrangement of aromatic cyanohydrin carbonate esters **2**. The aromatic cyanohydrin carbonate esters **2** were prepared by the reaction of aromatic aldehydes **1**, ethyl chloroformate, aq. KCN, and benzyltrimethylammonium chloride (BTAC) in dichloromethane. The reaction is initiated by the attack of cyanide ion on the aldehyde to generate a transient cyanohydrin anion **4** which is then trapped by the chloroformate to give the corresponding cyanohydrin carbonate ester **2** (Scheme 1). Recently, it was found that the preparation of cyanohydrin esters can be effected by the reaction of acylals and KCN in DMSO at room temperature in good to excellent yields.[8] Moreover, when acylals were treated with a mixture of trimethylsilyl cyanide and titanium(IV) chloride, cyanohydrin esters were obtained in good yield from both aliphatic and aromatic acylals.[8]

We found that when the aromatic cyanohydrin carbonate esters **2** were treated with LDA in dry THF at −78°C for an hour and at room temperature for 2 hours, they rearranged smoothly to the aryl α-keto esters **3**. The mechanism of the rearrangement of the aromatic cyanohydrin carbonate ester **2** presumably involved the α-carbanion **5** which could react with the carbonyl function to give the alkoxy epoxide intermedi-




**Scheme 1.**

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0040-4039/03/$ - see front matter 
PII: S0040-4039(02)02725-9

## Acknowledgements

We acknowledge the financial contribution from the Thailand Research Fund (TRF) for the generous support of the research program and the award of Senior Research Scholar to S.R. as well as the award of the Royal Golden Jubilee Scholarship to N.T. We also acknowledge the facilities in the Department of Chemistry provided by the PERCH program.

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10. *A typical procedure for the preparation of aromatic cyanohydrin carbonate ester*: To a stirred solution of 2-benzyloxy benzaldehyde **1h** (5.3 g, 25.0 mmol), ethyl chloroformate (3.0 g, 27.5 mmol), and benzyltrimethyl ammonium chloride (0.3 g, 1.6 mmol), in dichloromethane (40 mL) cooled in an ice-bath were slowly added a solution of potassium cyanide (2.45 g, 50.0 mmol), in distilled water (40 mL). The mixture was stirred overnight at room temperature. The organic layer was washed with water, saturated sodium hydrogen carbonate solution, dried ($Na_2SO_4$), and evaporated to give a yellow oil. Purification by distillation under reduced pressure yielded 2-benzyloxyphenyl cyanohydrin carbonate ester **2h** (6.03 g, 78%).
11. Compound **2h**: oil; IR (neat): $\nu_{max}$ 1757 (C=O), 1603, 1494, 1455, 1372, 1251, 1008 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 1.28 (t, 3H, $J=7.0$ Hz, $CH_3$), 4.21 (dq, 2H, $J=7.0$ Hz, $CH_2$), 5.12 (s, 2H, $CH_2$), 6.65 (s, 1H, $CH$), 6.97 (dd, 1H, $J=1.0$, 8.0 Hz, Ar$H$), 7.02 (t, 1H, $J=7.4$ Hz, Ar$H$), 7.37 (m, 6H, Ar$H$), 7.59 (dd, 1H, $J=1.8$, 7.6 Hz, Ar$H$); $^{13}C$ NMR (50 MHz, $CDCl_3$) ppm 14.0, 61.8, 65.3, 70.3, 112.2, 115.8, 119.7, 121.1, 127.1, 128.0, 128.5, 128.7, 131.9, 135.9, 155.3, 155.7; MS (EI) $m/z$ 311 ($M^+$, 14), 310 (37), 245 (16), 220 (16), 91 (100). HRMS calcd for $C_{18}H_{17}NO_4$: 311.1158. Found: 311.1159.
12. *A typical procedure for the aromatic cyanohydrin carbonate ester rearrangement*: A solution of lithium diisopropyl amide (LDA) (1.2 mmol) was prepared at 0°C from diisopropyl amine (0.2 mL, 1.2 mmol) in dry THF (3 mL) and 1.3 M *n*-butyllithium (1 mL, 1.2 mmol). After 30 min, the solution was cooled to −78°C and a solution of 2-benzyloxyphenyl cyanohydrin carbonate ester **2h** (0.17 g, 0.5 mmol) in dry THF (2 mL) was added dropwise. The solution was stirred at −78°C for an hour and allowed to reach room temperature overnight. The mixture was quenched with saturated aq. ammonium chloride and extracted with dichloromethane. The organic layer was washed with water, brine, dried ($Na_2SO_4$), and evaporated to give a yellow solid. Recrystallization using ethyl acetate and hexane yielded 2-phenylbenzofuran-3-carboxylic acid **11** (0.077 g, 65%) as yellowish crystals.
13. Compound **11**: mp 167–168°C; IR (KBr): $\nu_{max}$ 3216 (OH), 1607 (C=O), 1562, 1482, 1418, 1288, 1213, 1131 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.06 (bs, 1H, O$H$), 7.51 (m, 5H, Ar$H$), 7.72 (dt, 1H, $J=1.4$, 8.6 Hz, Ar$H$), 8.27 (m, 3H, Ar$H$); $^{13}C$ NMR (100 MHz, $CDCl_3$) ppm 118.3, 120.7, 124.5, 125.5, 127.8, 128.6, 130.2, 131.1, 133.6, 138.5, 144.9, 155.5, 173.5; MS (EI) $m/z$ 238 ($M^+$, 100). HRMS calcd for $C_{15}H_{10}O_3$: 238.0629. Found: 238.0625.

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Tetrahedron Letters 44 (2003) 1363–1366

TETRAHEDRON LETTERS

# Further developments in the synthesis of lamellarin alkaloids via direct metal–halogen exchange

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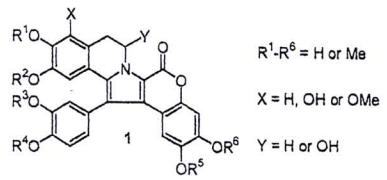
Received 28 October 2002; revised 11 December 2002; accepted 20 December 2002

**Abstract**—Direct metal–halogen exchange of 2-bromopyrrole carbonate derivatives with *tert*-butyllithium followed by the intramolecular lactonization of the resulting 2-pyrrole anion onto the carbonate provided the corresponding lamellarins in moderate to good yield. The lamellarin framework could be obtained from the direct metal–halogen exchange strategy in a 26–33% overall yield over 5–6 steps. 

Lamellarins **1**, whose structures contain polyoxygenated aromatics on their periphery and can be classified as 3,4-diarylpyrroloisoquinoline lactones, are a group of marine natural products isolated from the prosobranch mollusc *Lamellaria* sp. and also from the ascidians.[1,2] Including the first four lamellarins isolated by Faulkner in 1985, a total of 35 lamellarins have been isolated and identified thus far.[3,4]




Some of the lamellarins have been found to exhibit a wide array of interesting and significant biological activities including cell division inhibition, cytotoxicity, HIV-1 integrase inhibition and immunomodulatory activity.[5,6] Lamellarin K (X=OH; $R^1$, $R^2$, $R^3$ and $R^5$=Me; $R^4$ and $R^6$=H; Y=H) and lamellarin L (X=H; $R^1$, $R^3$, and $R^6$=H; $R^2$, $R^4$ and $R^5$=Me; Y=H), for example, exhibited significant cytotoxicity against P388 and A549 cultured cancer cell lines with the mean $IC_{50}$s of 0.7 μg/mL (0.06 μM) and 0.4 μg/mL (0.04 μM), respectively.[3] A recent study by Faulkner also showed that the presence of sulfate groups on the periphery could greatly influence the selectivity of HIV-1 integrase inhibition.[7] More importantly, lamellarins also act as non-toxic inhibitors of acquired multi-drug resistance (MDR).[8] Lamellarin I (X=OMe; $R^1$, $R^2$, $R^3$, $R^4$ and $R^5$=Me; $R^6$=H; Y=H) showed sensitizing effects in multidrug-resistant P388/Schabel cells to doxorubicin.[3,9]

Up to now, several studies directed towards the total synthesis of these marine natural products have been reported,[10] notably by Steglich,[11,12] Banwell,[13,14] Boger[9] and Ishibashi.[15,16] Previously, our research group reported an efficient synthesis of lamellarin derivatives, as shown in Scheme 1.[17] Synthesis of the lamellarin skeleton was achieved by first condensing the appropriately substituted benzylisoquinoline **2** with the phenacyl bromide mesylate **3**. The resulting 2*H*-3,4-disubstituted pyrrole intermediate **4** was smoothly formylated under Vilsmeier conditions. Following the removal of the mesyl group, the cyclic hemiacetal (lactol) **6** was oxidized to give the desired lamellarin skeleton **7**.

One drawback, albeit a minor one, in our previous Scheme was the use of a mesyl protecting group, which added two steps to the synthesis. It occurred to us that a better approach could be realized by using a hydroxy protecting group on the phenacyl bromide synthon that can act as a directing group for the remote deprotona-

*Keywords*: lamellarin alkaloid; metal–halogen exchange; DreM; natural products; pyrrole.

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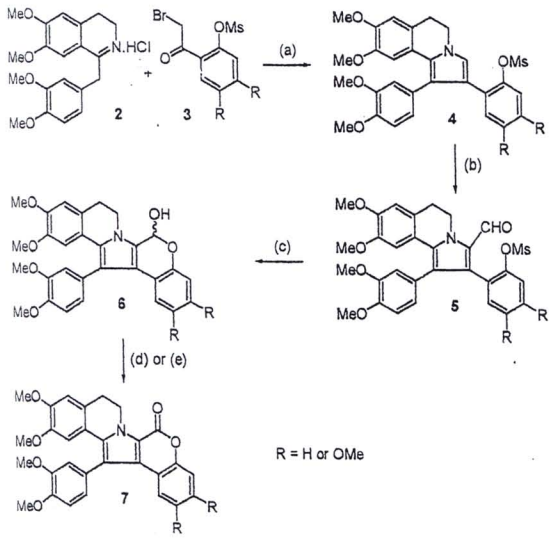
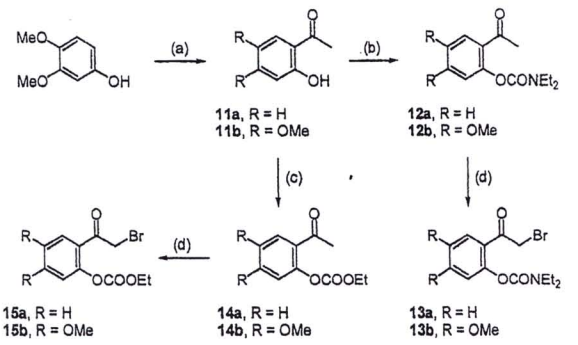
PII: S0040-4039(02)02887-3




Scheme 1. *Reagents and conditions*: (a) $K_2CO_3$, $CH_3CN$, reflux, 63%; (b) DMF, $POCl_3$, rt, 80–82%; (c) KOH, EtOH, reflux, 77–81%; (d) $MnO_2$, $CH_2Cl_2$, rt, 20–54%; (e) $Pd(OAc)_2$, $PPh_3$, $K_2CO_3$, DMF, PhBr, 120°C, 12 h, 80%.

tion at the C-2 position of the pyrrole as well as being the source of the lactone group in the subsequent lactonization of the resulting anion without the need for a separate formyl group equivalent. This strategy was pioneered by Snieckus and termed DreM (for directed remote metalation).[18] The directing group is typically a carbonate or a carbamate group, as depicted in Scheme 2. Alternatively, the 2*H*-pyrrole intermediate 9 could be selectively brominated at the 2-position[8] of the pyrrole to give the corresponding bromo compound 10 which could undergo metal–halogen exchange to provide an anion similar to that from the DreM strategy after initial remote deprotonation.

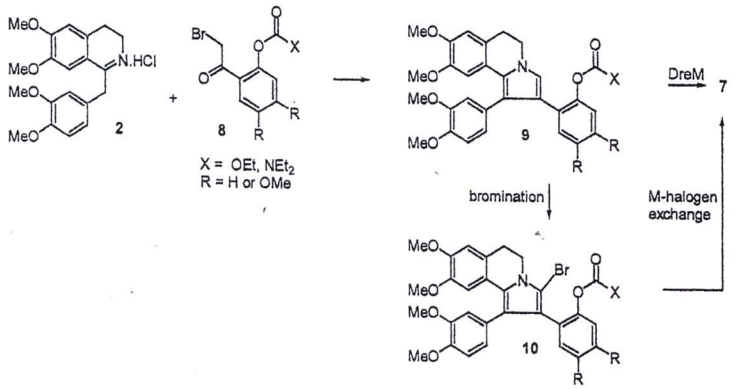
Both synthetic strategies required the benzylisoquinoline **2** and the carbonate or carbamate phenacyl bromide derivatives **8**. Our synthesis commenced with the preparation of **8** starting from commercially available 2-hydroxyacetophenone **11a** (R = H) and 2-hydroxy-4,5-dimethoxyacetophenone **11b** (R = OMe) which was




Scheme 3. *Reagents and conditions*: (a) $BF_3{\cdot}Et_2O$, $Ac_2O$, 80–90°C, 90%; (b) $Et_2NC(O)Cl$, DMAP (cat.), $Et_3N$, $CH_2Cl_2$, rt, 82% (**12a**) and 79% (**12b**); (c) NaH, EtOCOCl, THF, rt, 96% (**14a**) and 83% (**14b**); (d) $BnMe_3NBr_3$, $CH_2Cl_2$, 0°C to rt, 82% (**13a**), 70% (**13b**), 70% (**15a**) and 90% (**15b**).

synthesized in 90% yield from acetylation of 3,4-dimethoxyphenol with acetic anhydride and $BF_3{\cdot}Et_2O$, as shown in Scheme 3. Use of DMAP, $Et_3N$ and *N*,*N*-diethylcarbamoyl chloride smoothly converted **11a** and **11b** into their corresponding carbamate derivatives **12a** and **12b** in 82 and 79% yields, respectively. However, when similar reaction conditions were used for carbonating **11a** and **11b**, the desired products **14a** and **14b** were produced in only 66 and 49% yields, respectively, since the product was often obtained as an inseparable mixture with remaining starting material. The use of a stronger base such as NaH in place of $Et_3N$ and ethyl chloroformate yielded the desired carbonate derivatives **14a** and **14b** in 96 and 83% yields, respectively, with no starting material remaining. Subsequent bromination of **12a**, **12b**, **14a** and **14b** with $BnMe_3NBr_3$ effectively provided the desired phenacyl bromide derivatives **13a**, **13b**, **15a** and **15b** in 82, 70, 70 and 90% yields along with the dibrominated products in approximately 8% yield.

When benzylisoquinoline **2** was reacted with the carbamate derivatives **13a** and **13b** in the presence of $NaHCO_3$ in refluxing acetonitrile,[17] the corresponding pyrrole carbamates **16a** and **16b** were obtained in 91 and 81% yields, respectively (Scheme 4). The carbonate



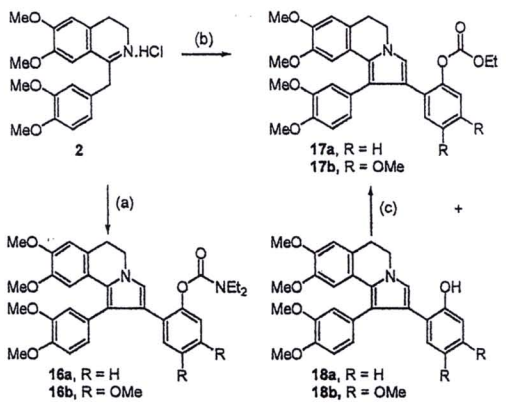

Scheme 2. Directed remote metalation (DreM) and metal–halogen exchange strategies for the synthesis of lamellarin skeleton **7**.

derivatives **15a** and **15b** were also coupled with **2** under similar conditions to give the pyrrole carbonates 17a and **17b** in 72 and 60% overall yields after subjecting the inseparable mixture of the desired carbonate product and the pyrrole phenols **18a** and **18b** (the decarbonated products) obtained from the coupling reaction to the carbonation conditions with DMAP, $Et_3N$ and ethyl chloroformate.

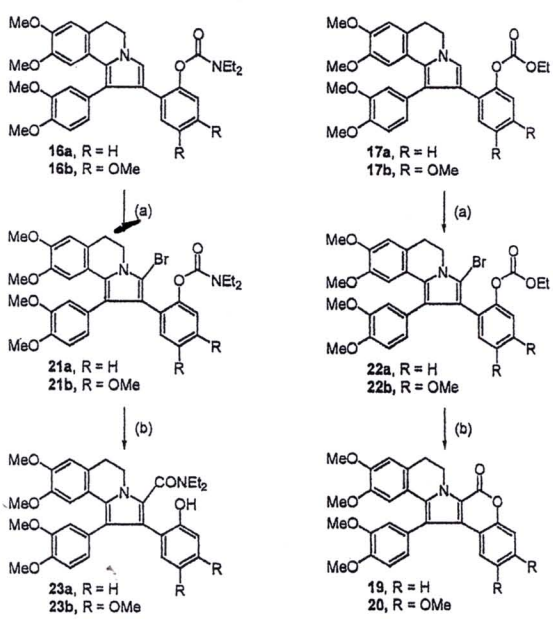
With the required carbamates **16a** and **16b** and carbonates **17a** and **17b** in our hands, we then performed a study of the DreM methodology of these compounds. After some exploratory work, we found that refluxing the carbonate **17a** with 7 equiv. of LDA in THF for 36 h gave the desired lamellarins **19** but in only 35% yield. In addition to the low yields, in our hands, the DreM/cyclization reactions were not highly reproducible and partial deprotonation of the starting material was frequently encountered. These problems together with the seemingly required prolonged reaction time have prompted us to consider another approach. The alternative approach ideally would feature a more effective means of generating the C-2 pyrrole anion as well as of facilitating the cyclization of the resulting anion onto the carbonate or carbamate at lower temperature and with a shorter reaction time.

We then considered a more direct way to generate the C-2 pyrrole anion via metal–halogen exchange, this would require the corresponding C-2 halo pyrrole. As shown in Scheme 5, the C-2 position of the pyrroloisoquinolines **16a**, **16b**, **17a** and **17b** could be selectively brominated with *N*-bromosuccinimide (NBS) to give the corresponding bromo pyrroles **21a**, **21b**, **22a**[19] and **22b** in excellent yields (>95%). Subsequent lithium–halogen exchange of carbamates **21a** and **21b** using *tert*-BuLi gave only the corresponding 2-(*N*,*N*-diethyl)amido-pyrroles **23a** and **23b** in virtually quantitative yield. Various attempts to affect the ring closure of these amido-pyrroles failed.[18] Lithium–halogen exchange of carbonates **22a** and **22b** with *tert*-BuLi,[20] on the other hand, proceeded smoothly to give the desired lamellarins **19**[17] and **20**[17] in 72 and 67% yields, respectively. From the isolation of **23a** and **23b** as the product, it appears that cyclization of the C-2 pyrrole anion may proceed via the intermediacy of the corresponding 2-amido and 2-alkoxycarbonyl pyrroles.[18]

In conclusion, two approaches towards the total synthesis of the lamellarin skeleton have been developed. Both DreM and metal–halogen exchange strategies share a similar C-2 pyrrole anion intermediate which, upon cyclization onto a carbonate or carbamate, gives the desired lamellarin framework. Results from both DreM and metal–halogen exchange are summarized in Table 1. From Table 1, the synthesis of lamellarin **20**, with two methoxy groups on the periphery, is less efficient than that of lamellarin **19**. These two strategies are relatively short (only 4–6 steps) and more efficient than our previously reported one which provided lamellarin **19** only in 25% overall yield in six steps and lamellarin **20** in 15% overall yield in seven steps. Between the two strategies, the direct metal–halogen exchange provided lamellarins more efficiently. The two best overall yields for the synthesis of **19** and **20** from DreM and metal–halogen exchange strategies are 33% in five steps and 26% in six steps, respectively.




**Scheme 4.** *Reagents and conditions*: (a) $NaHCO_3$, $CH_3CN$, reflux, **13a**, 91% (**16a**), or **13b**, 81% (**16b**); (b) $NaHCO_3$, $CH_3CN$, reflux, **15a** or **15b**; (c) DMAP, $Et_3N$, $CH_2Cl_2$, ClC(O)OEt, 72% (**17a**), 60% (**17b**).




**Scheme 5.** *Reagents and conditions*: (a) NBS, $CH_2Cl_2$, rt, 99% (**21a**), 99% (**21b**), 99% (**22a**), 95% (**22b**); (b) *tert*-BuLi, THF, −78°C to rt, 99% (**23a**), 98% (**23b**), 72% (**19**), 67% (**20**).

Table 1. Summary of total syntheses of lamellarins **19** and **20**

| Lamellarins | DreM | Metal–halogen exchange | |
|---|---|---|---|
| | Carbonate yield (%) | Carbonate yield (%) | Carbamate yield (%) |
| 19 | 17 | 33[b] | –[d] |
| 20 | –[a] | 26[c] | –[d] |

[a] The reaction was not performed.
[b] The overall yield of five steps.
[c] The overall yield of six steps.
[d] The reaction gave only the amido-pyrrole intermediate.

## Acknowledgements

We acknowledge the financial contribution from the Thailand Research Fund (TRF; Grant No. RTA/07/2544 for S.R. and PDF/82/2544 for P.P.) for the generous support of the research program and the award of Senior Research Scholar to S.R. We also acknowledge the facilities in the Department of Chemistry, Mahidol University provided by the Postgraduate Education and Research Program in Chemistry (PERCH).

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19. **22a**: Mp 88–89°C; IR (KBr): $\nu_{max}$ 2937, 1759, 1495, 1466, 1248, 1135, 1029 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 1.22 (t, 3H, $J$=7.2 Hz, $OCH_2CH_3$), 3.00–3.11 (m, 2H, $NCH_2CH_2Ar$), 3.40, 3.60, 3.83, 3.87 (4s, 12H, $OCH_3$), 4.09–4.26 (m, 4H, $OCH_2CH_3$ and $NCH_2CH_2Ar$), 6.71–6.78 (m, 4H, Ar*H*), 6.83–6.89 (m, 1H, Ar*H*), 7.08–7.11 (m, 2H, Ar*H*), 7.14–7.18 (m, 1H, Ar*H*), 7.22–7.27 (m, 1H, Ar*H*); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 14.02, 29.02, 43.33, 55.22, 55.65, 55.76, 55.83, 64.25, 102.9, 107.5, 110.9, 111.0, 114.2, 119.5, 121.1, 121.7, 121.8, 122.7, 123.9, 125.4, 126.6, 127.2, 127.8, 127.9, 132.9, 147.2, 147.4, 147.6, 148.6, 149.2, 152.9; LRMS (EI) *m/z* (rel. intensity) 609 ($M^+$+2, 37), 607 ($M^+$, 43), 529 (100), 483 (23), 481 (23); HRMS (FAB) ($C_{31}H_{30}BrNO_7$+H) calcd 608.1284, found 608.1282.
20. A typical procedure is as follows: To a mixture of **22a** (0.30 g, 0.49 mmol) in THF (10 mL) at −78°C was added *tert*-butyllithium (0.73 mL, 1.23 mmol, $c$=1.7 M in pentane). The mixture turned dark red immediately. The mixture was allowed to stir at −78°C and slowly warmed up to room temperature at which the mixture was stirred for 16 h. The reaction was then quenched with water (5 mL) and diluted with EtOAc (5 mL). The two layers were separated and the aqueous phase was extracted with EtOAc (2×10 mL). The organic layers were combined, dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure to give the crude product which was further purified by column chromatography on silica (50% EtOAc/hexanes) to give the desired lamellarin **19** as a solid (0.17 g, 0.35 mmol, 72%). Spectroscopic data of **19** were identical to those of the compound synthesized by a different approach previously reported in Ref. 17.

SYNTHETIC COMMUNICATIONS®
Vol. 33, No. 4, pp. 621–625, 2003

# A One-Pot Synthesis of (±) Cryptostylines I, II, III

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## ABSTRACT

A one-pot synthesis of cryptostylines I, II, III, via the Pictet–Spengler reaction is reported.

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DOI: 10.1081/SCC-120015817
0039-7911 (Print); 1532-2432 (Online)

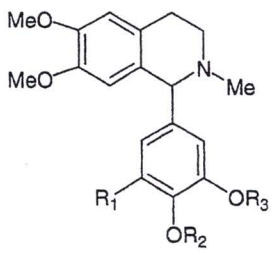
www.dekker.com

*Key Words:* 1-Aryltetrahydroisoquinoline alkaloids; Pictet-Spengler reaction.

Cryptostylines I, II, III, are rare 1-aryltetrahydroisoquinoline alkaloids which have been isolated from *Cryptostylis fulva*[1] and *Cryptostylis erythroglosa*.[2] Several asymmetric syntheses[3] and racemic syntheses[1,4] of the cryptostylines have been reported, all involving multi-steps with discreet operations. In continuation with our interest in the application of the Pictet–Spengler reaction for the synthesis of alkaloids,[5] we report our work on the application of this reaction to the one-pot synthesis of cryptostyline I, cryptostyline II, and cryptostyline III in this article.

The Pictet–Spengler reaction[6] is a classical reaction involving the electrophilic substitution of activated aromatic compounds with an iminium intermediate. The reaction is commonly used for the synthesis of 1,2,3,4-tetrahydroisoquinoline and carboline alkaloids. Recent investigations have applied the reaction to the stereospecific synthesis[7] of these compounds as well as to solid phase synthesis.[8] The reaction has also recently been extended to the synthesis of other related compounds.[9] Normally the Pictet–Spengler reaction requires acids as the condensing agent, but in some special cases the reaction proceeds under neutral conditions. Recently it was shown that cyclization reactions for carboline formation can also be performed with hypervalent iodine compounds.[10]

The Pictet–Spengler reaction has also been applied to the synthesis of a 1-phenyltetrahydroisoquinoline intermediate, in which dopamine can be condensed with benzaldehyde in the presence of dilute hydrochloric acid, whereas for a similar cyclization in the case of homoveratrylamine, phosphoric acid was required.[11]




Cryptostyline I $R_1$ = H, $R_2+R_3 = CH_2$
Cryptostyline II $R_1$ = H, $R_2=R_3$ = Me
Cryptostyline III $R_1$ = OMe, $R_2=R_3$ = Me

We found that formic acid could conveniently be employed as both acid and solvent for the condensation of 3,4-dimethoxyphenylethylamine (homoveratrylamine) with aromatic aldehydes to yield the corresponding 1-aryltetrahydroisoquinoline derivatives. Condensation of homoveratrylamine with piperonal in formic acid at an oil bath temperature of 110°C for 7 h gave the required product, norcryptostyline I, in 58% yield after purification by crystallization of the oxalate salt. Having successfully effected the required cyclization by the action of formic acid, we then investigated the "one pot" synthesis of cryptostylines I, II, III. Since cyclization could be effected by **formic acid** and *N*-methylation by formaldehyde and **formic acid** is well documented[12] we anticipated that by adding formaldehyde to the above cyclization reaction we would be able to effect both the cyclization and *N*-methylation in sequence to produce the required compound in one pot.

The above prediction was realized by heating homoveratrylamine with piperonal in formic acid at 110°C for 7 h then adding 37% formaldehyde and continuing heating the reaction mixture for a further 4 h. After work-up and purification by preparative layer chromatography, cryptostyline I was isolated in 61% yield. Similarly, cryptostylines II and III could be synthesized by heating homoveratrylamine with veratraldehyde and 3,4,5-trimethoxybenzaldehyde in formic acid followed by *N*-methylation with formaldehyde in 69 and 79% yield respectively after purification by preparative layer chromatography.

In conclusion, the above synthesis fits with one of the criteria of the ideal synthesis[13] requiring that consecutive reactions should be carried out in the same medium. Due to environmental concerns, use of less solvent is very much favored. We have found that formic acid, as the solvent, serves as an excellent acid for the Pictet–Spengler reaction and also acts as a good reducing agent in the further methylation of the intermediate so obtained.

## EXPERIMENTAL

### Representative Procedure

A mixture of 2-(3,4-dimethoxyphenyl) ethylamine (0.362 g, 2.00 mmol) and 3,4-methylenedioxybenzaldehyde (0.360 g, 2.40 mmol) in 99% formic acid (4 mL) was heated in an oil bath at 110°C for 7 h,

then 2 mL of 37% formaldehyde was added and the mixture was further heated for 4 h. The excess of formic acid and formaldehyde was removed by distillation and the mixture was then cooled and made basic with 1 M sodium carbonate. The solution was extracted twice with methylene chloride. The organic extracts were combined, washed with water, dried over anhydrous sodium sulfate, filtered and evaporated to dryness to give brown solid which was recrystallized from ether to give the product (0.399 g) as a white solid.

**Cryptostyline I (61%):** M.p. 117–118°C (ether) (Lit.[1] 117–118°C); IR (KBr) 2949, 1609, 1511, 1369, 1245, 1217, 1140, 1036 $cm^{-1}$; $^1H$ NMR (400 MHz, CDCl3) $\delta$ 2.25 (s, 3H, $NCH_3$), 2.59 (dt, 1H, $J = 4.0$, 11.0 Hz), 2.72 (dt, 1H, $J = 3.3$, 15.6 Hz), 3.12 (m, 2H), 3.62, 3.85 (s, 3H, $OCH_3$), 4.12 (s, 1H, C*H*), 5.93 (d, 2H, $J_{AB} = 0.7$ Hz, $OCH_2O$), 6.17 (s, 3H, Ar*H*), 6.59 (s, 3H, Ar*H*), 6.71 (s, 3H, Ar*H*), 6.76 (m, 2H, Ar*H*); $^{13}C$ NMR (100 MHz, $CDCl_3$) ppm 28.9, 44.1, 52.1, 55.7, 55.8, 70.6, 100.9, 107.4, 109.3, 110.7, 111.4, 122.8, 126.5, 130.2, 137.7, 146.8, 147.0, 147.4, 147.8; MS (EI) *m/z* 327 ($M^+$, 28), 206 (100).

**Cryptostyline II (69%):** M.p. 101–102°C (ether) (Lit.[1] 103–104°C); IR (KBr) 2894, 1465, 1355, 1220, 1190, 824 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 2.24 (s, 3H, N*H*), 2.60 (dt, 1H, $J = 4.0$, 11.1 Hz), 2.76 (m, 1H), 3.14 (m, 2H), 3.58 (s, 3H, $OCH_3$), 3.82 (s, 3H, $OCH_3$), 3.85 (s, 3H, $OCH_3$), 3.89 (s, 3H, $OCH_3$), 4.11 (s, 1H, C*H*), 6.14 (s, 1H, Ar*H*), 6.60 (s, 1H, Ar*H*), 6.76 (d, 1H, $J = 1.7$ Hz), 6.82 (s, 1H, Ar*H*), 6.83 (d, 1H, $J = 1.7$ Hz); $^{13}C$ NMR (100 MHz, $CDCl_3$) ppm 28.8, 44.2, 52.4, 55.6, 55.7, 55.8, 70.9, 110.3, 110.6, 111.4, 122.0, 126.3, 130.4, 136.0, 146.9, 147.4, 148.3, 149.0; MS (EI) *m/z* 343 ($M^+$, 17 ), 206 (100).

**Cryptostyline III (79%):** M.p. 140–141°C (EtOH) (Lit.[1] 141–142°C); IR (KBr) 2948, 1463, 1360, 1220, 1182, 812 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 2.25 (s, 3H, $NCH_3$), 2.59 (m, 1H), 2.72 (m, 1H), 3.12 (m, 2H), 3.61 (s, 3H, $OCH_3$), 3.82 (s, 6H, $2 \times OCH_3$), 3.85 (s, 6H, $2 \times OCH_3$), 4.06 (s, 1H, C*H*), 6.17 (s, 1H, Ar*H*), 6.59 (s, 1H, Ar*H*), 4.50 (s, 2H, Ar*H*); $^{13}C$ NMR (100 MHz, $CDCl_3$) ppm 28.8, 44.4, 52.5, 55.7, 55.9, 56.1, 60.8, 71.6, 106.4, 110.7, 111.4, 126.4, 130.1, 137.2, 139.4, 146.9, 147.5, 153.0; MS (EI) *m/z* 373 ($M^+$, 13), 206 (100).

## ACKNOWLEDGMENTS

We acknowledge financial support from the Thailand Research Fund (TRF) and the facilities at the Department of Chemistry, Mahidol University, provided by the PERCH programme.

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Received in the UK January 25, 2002

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Tetrahedron Letters 44 (2003) 3637–3640

TETRAHEDRON LETTERS

# A novel 8,9-*seco*-rhamnofolane and a new rhamnofolane endoperoxide from *Jatropha integerrima* roots

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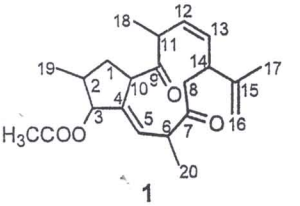
Received 13 January 2003; revised 3 March 2003; accepted 14 March 2003

**Abstract**—Integerrimene, a possible biogenetic precusor of the rhamnofolane diterpenes and a new rhamnofolane endoperoxide 2-epicaniojane together with caniojane and 1,11-bisepicaniojane were isolated from *J. integerrima* roots. Their structures were elucidated by spectroscopic methods. The X-ray structure of 2-epicaniojane is also presented. © 2003 Elsevier Science Ltd. All rights reserved.

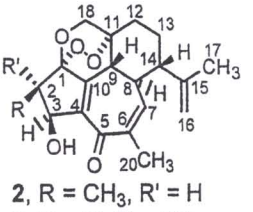
In our ongoing investigation of bioactive compounds from the Euphorbiaceae plants we have studied the roots of *Jatropha integerrima* (synonymous name *J. pandurifolia* Andr.) known in Thai as 'Pattavia'.[1] No medicinal use of *J. integerrima* has been reported but its latex is known to be toxic. Leaves, if accidentally chewed, can cause squeamish, stomachalgia and can be very purgative.[2] Jatropha species are known to be abundant sources of diterpenes with various skeletons. Previously reported diterpene constituents from the species of this genus comprise the macrocyclic diterpene jatrophone,[3,4] jatrophatrione,[5] jatropholone A–B,[6] riolozatrione,[7] curcusones A–D,[8] rhamnofolane,[9] lathyrane,[10] 12-deoxy-16-hydroxyphorbol esters[11] and the cleistanthane[12] series of diterpenes.

We herein report the isolation and structural determination of a macrocyclic diterpene integerrimene **1** with a novel 8,9-*seco*-rhamnofolane skeleton and a new rhamnofalane endoperoxide 2-epicaniojane **2** together with caniojane **3**[9] and 1,11-bisepicaniojane **4**[9] from the roots of *J. integerrima*.

Roots of *J. integerrima* were collected within the Ramkhamhaeng University area in May 2000. The chloroform extract obtained was fractionated on a silica gel column with a solvent gradient. The moderately polar fraction was further purified by successive column chromatography to yield **1** (7.5 mg, $1.5\times10^{-4}$ % based on dry wt),[13] **2** (4.9 mg, $9.8\times10^{-5}$ %)[14] and **3** (13.2 mg, $2.64\times10^{-4}$ %)[15] and **4** (3.4 mg, $6.8\times10^{-5}$ %).[16] Compound **1** was obtained as colorless liquid. The HREIMS gave a molecular formula of $C_{22}H_{30}O_4$. The FT-IR spectrum showed the presence of carbonyl groups at 1732 and 1715 $cm^{-1}$ as well as olefinic functions at 1646 $cm^{-1}$. The $^1H$ NMR spectrum showed three secondary methyl group signals at $\delta_H$ 1.00, 1.15 and 1.16 in addition to a less shielded methyl group signal at $\delta_H$ 1.71 (s) assignable to $CH_3$–C=C. The spectrum also exhibited olefinic proton signals at $\delta_H$ 5.10, 5.58 and 5.77, together with additional exocyclic methylene group signals ($H_2$-16) as two broad one proton singlets at $\delta_H$ 4.73 and 4.80. The $^1H$–$^1H$ COSY spectrum indicated correlations between signals at $\delta_H$ 1.16 (H-18)/$\delta_H$ 3.29 (H-11); H-11/$\delta_H$ 5.10 (H-12); H-12/$\delta_H$ 5.58 (H-13); H-13/$\delta_H$ 2.85 (H-14); H-14/$\delta_H$ 1.71 ($H_3$-17), 2.73 (H-8) and 4.73 (H-16). Further correlations were also observed between $H_3$-20 ($\delta_H$ 1.15)/H-6 ($\delta_H$ 3.36); H-6/H-5 ($\delta_H$ 5.77); H-5/H-10 ($\delta_H$ 3.63); H-10/H-1 ($\delta_H$ 1.83 and 2.09); H-1/H-2 ($\delta_H$ 2.01); H-2/



**1**



**2**, R = $CH_3$, R' = H
**3**, R = H, R' = $CH_3$
**4**, **3** with 1,11 bis *epi*

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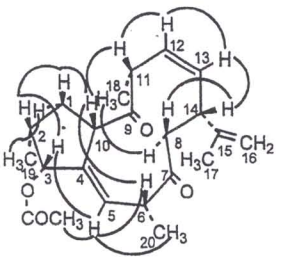
doi:10.1016/S0040-4039(03)00704-4

$H_3$-19 ($\delta_H$ 1.00) and H-3 ($\delta_H$ 5.25). The placement of the two keto functions at C-7 and C-9 was established through long range $^1H$–$^{13}C$ correlations particularly of H-1, H-11, H-12 and H-18 to C-9 ($\delta_C$ 210.4) and of H-6, H-8 and H-20 to C-7 ($\delta_C$ 211.7). The location of the acetoxy group at C-3 was suggested by the HMBC correlations between H-3 and carbon signals at $\delta_C$ 37.2 (C-1), 51.6 (C-10) and 131.5 (C-5). The trisubstituted double bond was established at C-4 (5) through correlations of H-3, H-6, H-10 and H-20 to C-5. Detailed $^1H$ and $^{13}C$ NMR chemical shifts are shown in Table 1.

The relative stereochemistry of **1** was obtained from NOESY and NOE difference spectra (Fig. 1). Compound **1** was concluded to be 3-*O*-acetyl-8,9-*seco*-rhamnofola-4(5),12(13),15(16)-trien-7,9-dione. This macrocyclic diterpene appears to be a possible biogenetic precusor of rhamnofolane by a further condensation. It may be postulated that this compound arises biogenetically in the plant either from a lathyrane type diterpene by ring opening of the cyclopropane ring or from a cembrane diterpene via cyclization (Scheme 1).[17]

Compound **2** was obtained as a crystalline solid. The EIMS gave a molecular ion at $m/z$ 344 corresponding to the formula $C_{20}H_{24}O_5$. The IR spectrum indicated absorptions for hydroxy (3509 $cm^{-1}$) and carbonyl (1685 $cm^{-1}$) functionalities. The $^1H$ NMR signals at $\delta_H$ 6.37, 4.91 and 4.84 with a less shielded methyl proton signal at $\delta_H$ 1.63 as well as $^{13}C$ NMR signals at $\delta_C$ 150.0 (s), 145.8 (s), 141.6 (d), 138.2 (s), 135.4 (s), 114.1 (t) indicated the presence of one tetrasubstituted and one trisubstituted double bond and an isopropenyl group. The $^{13}C$ NMR spectrum also showed signals for one dioxygenated ($\delta_C$ 108.0, s, C-1), one oxygenated quaternary ($\delta_C$ 75.4, s, C-11), one oxymethine ($\delta_C$ 74.9, d, C-3) and one oxymethylene carbon ($\delta_C$ 73.7, t, H-18) in addition to a keto carbonyl carbon ($\delta_C$ 192.0, s). The



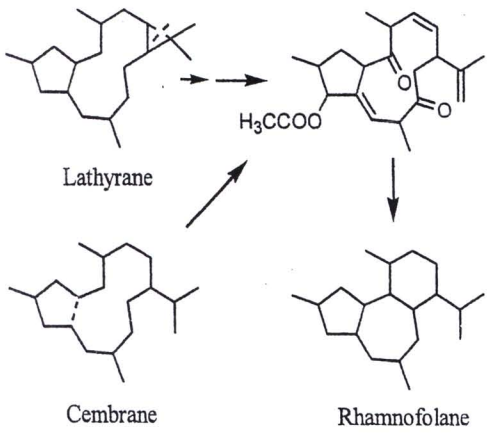
**1**

**Figure 1.** Selected NOE interactions and configuration of compound **1**.

**Table 1.** $^1H$ and $^{13}C$ NMR spectral data of **1** and **2** ($CDCl_3$, $\delta$ in ppm and $J$ in Hz)[a]

| Compound | 1 | | | 2 | | |
|---|---|---|---|---|---|---|
| Position | $\delta_H$ | $\delta_C$ | HMBC (H to C) | $\delta_H$ | $\delta_C$ | HMBC (H to C) |
| 1 | 2.09 (α-H, m),* 1.83 (β-H, m) | 37.2 (t) | C-2, 3, 4, 9, 10, 19 | – | 108.0 (s) | – |
| 2 | 2.01 (m) | 37.8 (d) | C-1, 19, 3-O*CO*$CH_3$ | 2.54 (quin, 7.6) | 40.8 (d) | C-1, 4, 19 |
| 3 | 5.25 (d, 4.6) | 81.4 (d) | C-1, 2, 5, 10, 3-O*CO*$CH_3$ | 5.14 (dd, 7.4, 2.3) | 74.9 (d) | C-4, 10 |
| 4 | – | 140.1 (s) | – | – | 135.4 (s) | – |
| 5 | 5.77 (dd, 10.3, 1.8) | 131.5 (d) | C-3, 10, 20 | – | 192.0 (s) | – |
| 6 | 3.36 (dq, 10.3, 7.2) | 49.3 (d) | C-4, 5, 7, 20 | – | 138.2 (s) | – |
| 7 | – | 211.7 (s) | – | 6.37 (dq, 3.5, 1.5) | 141.6 (d) | C-5, 20 |
| 8 | 2.73 (α-H, dd, 12.2, 10.5), 2.38 (β-H, dd, 10.4, 2.9) | 46.6 (t) | C-6, 7, 13, 14, 15 | 2.92 (dddq, 13.2, 1.7, 3.5, 1.8) | 37.2 (d) | – |
| 9 | – | 210.4 (s) | – | 2.77 (dd, 13.0, 2.3) | 43.5 (d) | C-4, 8, 10 |
| 10 | 3.63 (dt, 6.6, 1.8) | 51.6 (d) | C-1, 2, 3, 4, 5, 9 | – | 150.1 (s) | – |
| 11 | 3.29 (dq, 9.2, 6.7) | 53.7 (d) | C-9, 12, 13, 18 | – | 75.4 (s) | – |
| 12 | 5.10 (dd, 15.2, 9.4) | 131.9 (d) | C-9, 11, 14, 18 | 1.57 (ddd, 11.6, 5.3, 3.2) | 25.6 (t) | C-13, 14 |
| 13 | 5.58 (dd, 15.2, 9.6) | 134.6 (d) | C-8, 11, 14, 15 | 1.85 (dt, 13.9, 3.2), (m) 1.45 (ddd, 13.7, 6.8, 5.3) | 28.8 (t) | C-11, 12 |
| 14 | 2.85 (br dt, 9.6, 2.9) | 48.9 (d) | C-7, 8, 13, 15, 16 | 2.05 (ddd, 11.6, 11.4, 3.2) | 49.0 (d) | C-16 |
| 15 | – | 146.3 (s) | – | – | 145.8 (s) | – |
| 16 | 4.80 (br s), 4.73 (br s) | 110.5 (t) | C-14, 15, 17 | 4.91 (t, 1.6), 4.84 (s) | 114.1 (t) | C-14, 17 |
| 17 | 1.71 (s) | 21.7 (q) | C-14, 15, 16 | 1.63 (s) | 19.0 (q) | C-15, 16 |
| 18 | 1.16 (d, 6.7) | 17.1 (q) | C-9, 11, 12 | 4.29 (d, 9.7), 3.91 (d, 9.7) | 73.7 (t) | C-1, 9, 11 |
| 19 | 1.00 (d, 6.6) | 14.0 (q) | C-1, 2, 3 | 1.01 (d, 7.6) | 7.7 (q) | C-1, 2, 3 |
| 20 | 1.15 (d, 7.2) | 18.1 (q) | C-5, 6, 7 | 1.90 (br t, 1.7) | 20.5 (q) | C-5, 6, 7 |
| 3-*CO*$CH_3$ | – | 171.2 (s) | – | 3-OH, 3.63 (br s) | | |
| 3-CO*CH*$_3$ | 2.08 (s)* | 21.6 (q) | 3-O*CO*$CH_3$ | | | |

[a] Data recorded on a 400 MHz spectrometer with reference to the solvent signals ($\delta_H$ 7.24 ppm/$\delta_C$ 77.0 ppm).
* Overlapped signals.




**Scheme 1.** Possible biogenesis and transformation of **1**.

$^1$H–$^1$H COSY spectrum indicated sequential correlations from H-20/H-7, H-7/H-8, H-8/H-9, H-8/H-14, H-14/H-13, H-13/H-12, H-16/H-17, H-19/H-2, H-2/H-3 and H-18/H-18′. The α-methyl substituted α,β-unsaturated carbonyl function was revealed from the long range $^1$H–$^{13}$C correlations between $H_3$-20 and H-7/C-5. The positions of tetra-substituted double bond at C-4 (10) and three oxygenated carbons were established from $^1$H–$^{13}$C correlations particularly between H-3/C-4, C-10; H-9/C-4, C-8, C-10; H-2/C-1, C-4, C-19; H-18/C-1, C-9, C-11 and H-13/C-11. The $^1$H and $^{13}$C NMR spectral data (Table 1) indicated that **2** was structurally related to caniojane **3**, firstly isolated from *J. Grossidentata*[9] and also isolated in the present study. The distinctive difference was the signal at $\delta_H$ 5.14 (H-3) found to resonate at a less shielded position than that of **3**. The stereochemistry of **2** was obtained from X-ray diffraction analysis (Fig. 2)[18] and therefore unambiguously proved to be 2-epicaniojane.

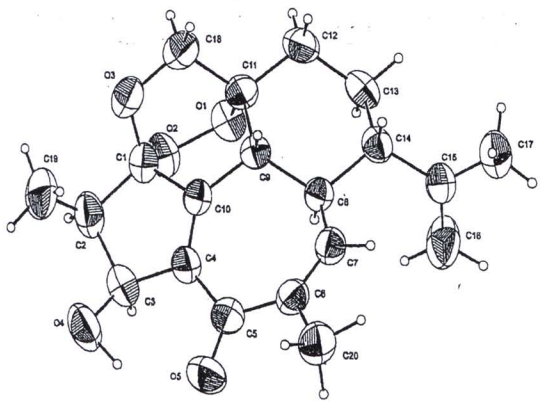



**Figure 2.** ORTEP structure of **2**. The absolute configuration shown is arbitrary.

**Acknowledgements**

We are grateful to the Thailand Research Fund and Ramkhamhaeng University for financial support. We thank Mr. N. Chimnoi, CRI for HRMS measurements. S.R. and P.K. thank the Postgraduate Education and Research in Chemistry (PERCH) for facilities at the Department of Chemistry, Faculty of Science, Mahidol University.

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13. **Integerrimene** (**1**). $[\alpha]_D$ −21.60 (*c* 0.100, $CHCl_3$); IR (film) $\nu_{max}$ 3445, 3083, 2963, 2927, 2854, 1732, 1715, 1646, 1455, 1373, 1245, 1131, 1054, 1020, 984, 894, 611, 556 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 1; EI-MS *m*/*z* (%) 358 ($M^+$, 17), 340 (5), 299 (8), 198 (30), 283 (10), 270 (5), 255 (9), 227 (6), 213 (5), 199 (6), 161 (5), 149 (16), 131 (6), 121 (12), 105 (13), 91 (26), 79 (17), 55 (12), 43 (100) HRFABMS calcd for $C_{22}H_{30}O_4$ 358.2144, found 358.2151.
14. **2-Epicaniojane** (**2**). $[\alpha]_D$ −286.75 (*c* 0.080, $CHCl_3$); IR (film) $\nu_{max}$ 3509, 3079, 2924, 2882, 2848, 1685, 1645, 1623, 1606, 1455, 1403, 1376, 1310, 1252, 1225, 1195, 1130, 1079, 1058, 1026, 951, 923, 890, 817, 484 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 1; EI-MS *m*/*z* (%) 344 ($M^+$, 44), 328 (24), 314 (27) 312 (41), 299 (19), 297 (22), 296 (65), 284 (32), 281 (21), 271 (29), 253 (31), 241 (22), 240 (19), 227 (21), 225 (21), 218 (25), 204 (28), 203 (66), 189 (42), 187 (100), 185 (48), 176 (24); HRFABMS calcd for $C_{20}H_{24}O_5$ $[M]^+$ 344.1624, found 344.1621.

15. **Caniojane** (**3**). $[\alpha]_D$ −233.44 (*c* 0.090, $CHCl_3$).
16. **1,11-Bisepicaniojane** (**4**) $[\alpha]_D$ −22.1544 (*c* 0.065, $CHCl_3$); $^{13}C$ NMR (in $CDCl_3$): C-1-20 $\delta_C$: 104.0, 45.9, 80.3, C-4 not observed, 191.5, 138.0, 140.1, 38.4, 44.6, 149.0, 75.7, 28.7, 27.8, 49.5, 146.0, 114.5, 19.2, 68.8, 10.1, 20.8.
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18. X-Ray crystal structure analysis of **2**: Crystal data: $C_{20}H_{24}O_5$, monoclinic, *C*2, $a=19.3150(6)$, $b=5.4100(2)$, $c=19.4330(8)$ Å, $\beta=118.220(2)°$, $V=1789.3(1)$ Å$^3$, $Z=4$, crystal size: 0.2×0.2×0.1 mm. A total of 2,274 unique reflections were collected using graphite monochromated Mo Kα radiation ($\lambda=0.71073$ Å) on a Bruker-Nonius Kappa CCD diffractometer. The structure was solved by direct methods (SIR-97) refined by full matrix least-squares techniques based on $F^2$ to give $R_1=0.0544$, $wR_2=0.1697$. Additional crystallographic details, CCDC 206849 (atomic coordinates and equivalent isotropic displacement coefficients) have been deposited at the Cambridge Crytallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

# The Synthesis of Wrightiadione via Directed Remote Metalation

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*Received 27 February 2003*

**Abstract:** The application of directed remote metalation (DreM) and electrophilic substitution is reported for the synthesis of wrightiadione using *N,N*-diethylcarboxamide as a directed metalation group (DMG) and electrophilic group. The lithiation of *N,N*-diethylisoflavone-2′-carboxamide with LDA gave a carbanion at C-2 which further cyclized to wrightiadione.

**Key words:** directed remote metalation, wrightiadione, *N,N*-diethylisoflavone-2′-carboxamide, directed metalation group, electrophilic substitution

Wrightiadione **1** is a rare and unusual oxygen heterocycle isolated from the bark of *Wrightia tomentosa*, a medicinal plant of Thailand, and it has been shown to exhibit cytotoxicity against a cultured murine P388 lymphocytic leukemia cell line ($ED_{50}$ 1.1 $\mu gmL^{-1}$). The methanol extract of the dried leaves and stems of this plant also exhibited weak activity against HIV-1 reverse transcriptase.[1] Recently we have disclosed a successful synthesis of wrightiadione[2] **1** and also isowrightiadione[3] **2**, an isomeric compound.

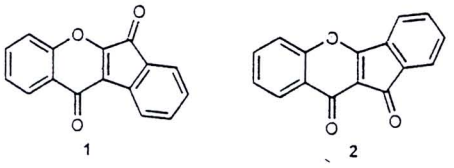
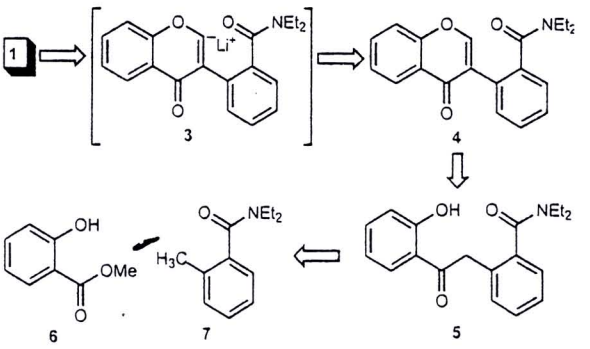


**Figure 1**

We wish to report another efficient route for the synthesis of wrightiadione **1** by directed remote metalation (DreM).[4] Heteroatom-facilitated metalation has become an increasingly important strategy in organic synthesis and has been widely adopted in the functionalization of both aromatic and heterocyclic systems.[4,5]

The lithiation of flavones and isoflavones has been successfully used to introduce substituents by Dean's group.[6] Isoflavone was lithiated at position 2 but it did not react easily; after carboxylation the yield of the 2-carboxylic acid was low.[6] In addition, it appeared not to react at all with the lithium bistrimethylsilylamide reagent. Chromones unsubstituted at C-2 are susceptible to ring cleavage and consequently C-2 lithiation is difficult to achieve. Although the evidence is not strong, it does support a preference for 3- over 2-lithiation. Lithiation at both positions can be improved by appropriate substituents. With an acetal at position 3, chromone derivatives were readily lithiated at position 2 and the anion could be captured with electrophiles.[7]

On the basis of this pioneering work, we have developed a strategy for the synthesis of wrightiadione **1**. We envisaged that the carbanion at C-2 of *N,N*-diethylisoflavone-2′-carboxamide **4** could be generated under remote metalation conditions using *N,N*-diethylcarboxamide as a directed metalation group (DMG). The resulting carbanion **3** could counter attack with the *N,N*-diethylcarboxamide functioning as an electrophile to give the product **1**. The key intermediate isoflavone **4** could be prepared from 2-hydroxybenzoin **5** using a $C_1$ addition procedure as shown in a general method for the synthesis of isoflavones.[8] Compound **5** could be prepared from the lateral lithiation reaction[9] of toluamide **7** with methyl salicylate **6** as shown in Scheme 1.



**Scheme 1**

The lateral lithiation reaction[10] of toluamide **7** using LDA as base in THF at –78 °C followed by reaction with methyl salicylate (**6**) provided 2-hydroxybenzoin **5**[11] in 58% yield as shown in Scheme 2. However, the tolyl anion **8** could also react with another molecule of **7** to give the deoxybenzoin intermediate **9** which further lactonized to isocoumarin[12] **10** (11%). Similar coupling of the anion of methyl *ortho*-toluate to the corresponding isocoumarin has been reported by Hauser.[13]

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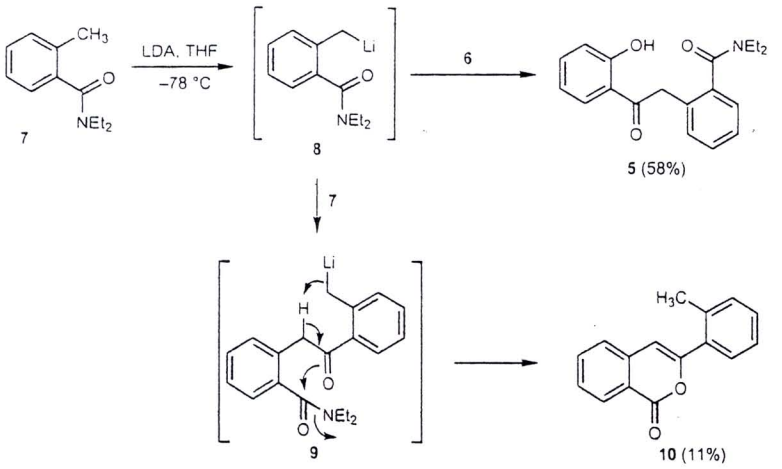


Scheme 2

Compound **5** was converted to the desired isoflavone[15] **4** in 48% yield accompanied by the spiro compound[16] **12** in 14% yield by reaction with a mixture of DMF–$MeSO_2Cl$ and $BF_3{\cdot}Et_2O$ at 70 °C.[14]

The spiro lactone **12** was probably formed by autooxidation at the benzylic position of deoxybenzoin **5** to produce intermediate benzoin **11** which could further cyclize in a tandem fashion to compound **12** as shown in Scheme 3.[17] The lithiation of isoflavone **4** was carried out by using LDA (5 equiv) in THF at –78 °C.[18] The carbanion intermediate **3** thus formed then reacted with the carboxamide group to give the desired wrightiadione **1** in moderate yield (49%). The identity of wrightiadione was proved by comparison of the spectroscopic data with that of the previously synthesized product.[2]

In conclusion, we have described an alternative approach to the synthesis of wrightiadione **1** via a basic directed remote metalation strategy. The synthesis is highly concise and employs the rarely used isoflavone metalation.

## Acknowledgment

We acknowledge the Thailand Research Fund (TRF) for the generous support of our research program and the award of Senior Research Scholar to S. R. as well as the award of the Royal Golden Jubilee Scholarship to N. T. We also acknowledge the facilities in the Department of Chemistry provided by the PERCH program.

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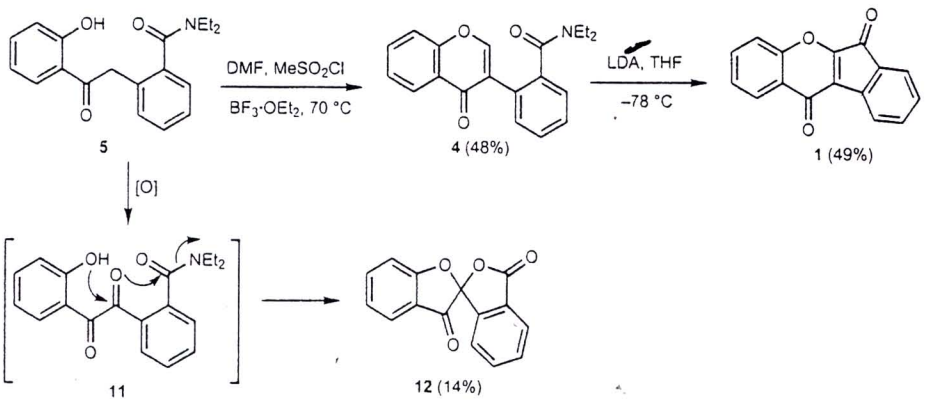


Scheme 3

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(10) Reaction of *N,N*-diethyl-*O*-toluamide (**7**) with methyl salicylate (**6**) in the presence of LDA: A solution of LDA in dry THF (200 mL) was prepared by adding diisopropylamine (14.7 mL, 0.10 mol) dropwise to a 0.95 M solution of *n*-BuLi (95 mL, 0.10 mol) in hexane under argon at 0 °C. The ice-water bath was replaced by a dry ice/acetone bath. The stirring was continued for 30 min at –78 °C, and then *N,N*-diethyl-*O*-toluamide (**7**, 14.4 g, 75.0 mmol) in dry THF (30 mL) was added. The pale yellow solution turned to deep red, indicating anion formation. The reaction mixture was allowed to warm to 0 °C with an ice-water bath, stirred for 10 min and the ice-water bath was replaced by a dry ice/acetone bath. The stirring was continued for 1 h. A solution of methyl salicylate (**6**, 11.4 g, 75.0 mmol) in dry THF (30 mL) was added slowly via syringe to the above mixture. Stirring at this temperature was continued for 2 h and then the reaction mixture was warmed to room temperature. 2 N HCl was added and the entire mixture was stirred for 1 h. Removal of solvent under reduced pressure gave a residue which was extracted with $CH_2Cl_2$. The combined organic extracts were washed with aqueous sodium carbonate solution, water and brine solution, and dried over anhydrous sodium sulfate. After removal of solvent, the residue was column chromatographed on silica gel using EtOAc and hexane as eluents to provide the desired 2-(2-*N,N*-diethylcarboxamidephenyl)-1-(2-hydroxyphenyl)ethan-1-one (**5**) as the major adduct (13.5 g, 58%) and 3-(2-methylphenyl) isochromen-1-one (**10**) as the minor adduct (1.9 g, 11%).
(11) Compound **5**: colorless crystals, mp 168–169 °C (EtOAc:hexane); IR (KBr) 3061 (OH), 1744 (C=O), 1648 (C=O), 1628, 1603, 1486, 1272, 1215 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) δ 1.03 (t, 3 H, *J* = 7.2 Hz), 1.09 (t, 3 H, *J* = 7.2 Hz), 3.15 (q, 2 H, *J* = 7.2 Hz), 3.45 (m, 2 H), 4.44 (br s, 2 H), 6.95 (m, 2 H), 7.30 (m, 4 H), 7.47 (dd, 1 H, *J* = 1.2 Hz, 7.6 Hz), 7.90 (dd, 1 H, *J* = 1.0 Hz, 8.1 Hz), 12.09 (s, 1 H); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 12.1, 13.7, 38.5, 42.0, 42.8, 118.3, 119.0, 119.2, 125.6, 127.0, 128.9, 130.3, 131.2, 131.3, 136.6, 137.3, 162.4, 170.3 (CON), CO not observed; MS (EI) *m/z* 311 ($M^+$, 0), 238 (68), 210 (85), 181 (100), 152 (21). HRMS (FAB) calcd for $C_{19}H_{21}NO_3$ [$MH^+$]: 312.1560; found 312.1560.
(12) Compound **10**: white solid: mp 80–82 °C (EtOH); IR (KBr) 1719 (C=O), 1648 (C=O) $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) δ 2.46 (s, 3 H), 6.56 (s, 1 H), 7.26 (m, 3 H), 7.46 (m, 3 H), 7.69 (dd, 1 H, *J* = 1.2 Hz, 7.2 Hz), 8.28 (dd, 1 H, *J* = 0.8 Hz, 8.0 Hz); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 20.7, 105.9, 120.3, 125.8, 126.0, 128.2, 129.2, 129.5, 129.8, 131.0, 132.7, 134.8, 136.7, 137.5, 155.5, 162.6; MS (EI) *m/z* 236 ($M^+$, 100), 208 (83), 207 (39), 193 (27), 179 (43) Anal. calcd. for $C_{16}H_{12}O_2$: C, 81.34; H 5.12. Found: C, 80.94; H 4.87.
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(14) 3-(2-*N,N*-Diethylcarboxamidephenyl)-4*H*-chromen-4-one (**4**). 2-Hydroxydeoxybenzoin (**6**, 0.62 g. 2.0 mmol) was dissolved in distilled $BF_3{\cdot}Et_2O$ (5 mL) under argon. A solution of methanesulfonyl chloride (3 mL) in dry DMF (15 mL) was slowly added and the mixture was heated at 70 °C for 2 h. The reaction was cooled to room temperature and poured into an ice-cold aq sodium acetate. The mixture was extracted with ethyl acetate. The combined organic extracts were washed with water and dried over anhydrous sodium sulfate. Solvent was removed under vacuum and the residue was purified by preparative layer chromatography on silica gel (elution with $CH_2Cl_2$) to give isoflavone **4** as a white solid (0.30 g, 48%).
(15) Compound **4**: mp 173–175 °C (EtOH); IR (KBr) 1639 (C=O), 1595, 1471, 1386, 1359, 1299, 1228 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) δ 0.89 (m, 6 H), 3.04 (m, 4 H), 7.35 (m, 6 H), 7.63 (ddd, 1 H, *J* = 0.6 Hz, 1.2 Hz, 7.2 Hz), 7.57 (dd, 1 H, *J* = 1.2 Hz, 7.2 Hz), 7.61 (dd, 1 H, *J* = 1.6 Hz, 7.0 Hz, 8.6 Hz), 8.06 (s, 1 H), 8.18 (ddd, 1 H, *J* = 0.4 Hz, 1.6 Hz, 8.0 Hz); $^{13}C$ NMR [50(16) MHz, $CDCl_3$] δ 12.1, 13.7, 38.5, 42.8, 118.2, 123.5, 124.2, 125.3, 126.0, 126.1, 128.3, 128.7, 131.4, 133.8, 137.5, 154.7, 156.3, 170.2, 176.2; MS (EI) *m/z* 321 ($M^+$, 13), 320 (24), 249 (100), 221 (55), 192 (6), 165 (25) Anal. calcd. for $C_{20}H_{19}NO_3$: C, 74.75; H 5.96 N, 4.36. Found: C, 74.95; H 5.54 N, 4.07.
(16) Compound **12**: white solid (14%): mp 183–185 °C (EtOH); IR (KBr) 1719 (C=O), 1649 (C=O) $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) δ 7.19 (m, 2 H), 7.33 (m, 1 H), 7.70 (m, 2 H), 7.78 (m, 2 H), 7.92 (m, 1 H); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 104.2, 113.7, 118.6, 122.6, 123.6, 125.8, 126.2, 127.1, 132.0, 135.2, 139.8, 142.3, 167.0, 171.3, 192.6; MS (EI) *m/z* 252 ($M^+$, 62), 224 (41), 223 (23), 196 (26), 195 (15), 180 (100), 168 (37) Anal. calcd. for $C_{15}H_8O_4$: C, 71.43; H 3.20. Found: C, 71.74; H 3.10.
(17) Letcher, R. M.; Kwok, N.-C.; Lo, W.-H.; Ng, K.-W. *J. Chem. Soc. Perkin Trans. 1* **1998**, 1715.
(18) Wrightiadione **1**: A solution of LDA in dry THF (10 mL) was prepared as in ref.[10] using diisopropylamine (0.4 mL, 2.6 mmol) and 0.7 M solution of *n*-BuLi (3.0 mL, 2.5 mmol) in hexane. The LDA was stirred for 30 min at –78 °C, and then isoflavone **4** (0.15 g, 0.5 mmol) in dry THF (5 mL) was added. Stirring at this temperature was continued for 2 h and then the reaction mixture was warmed to room temperature. Water was added and the mixture was stirred for 30 min. The organic layer was separated and washed again with water, dried over $Na_2SO_4$, filtered, and evaporated under reduced pressure. The resulting orange residue was purified by preparative layer chromatography on silica gel using $CH_2Cl_2$ as eluent to give crystals of wrightiadione **1** (0.07 g, 49%).

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Journal of Ethnopharmacology 86 (2003) 253–256
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Short communication

# Anti-metastatic effects of aqueous extract of *Helixanthera parasitica*

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(Received 20 November 2002; received in revised form 17 February 2003; accepted 19 February 2003

**Abstract**

Metastasis, the spread of cancer in body, is a major cause of death. We have screened anti-metastatic activity of aqueous and dichloromethane extracts of several not previously studied Thai herbs, using an in vitro invasion test. This involves the in vitro invasion of HCC-S102, a hepatocellular carcinoma cell line derived from a Thai patient, through a reconstituted-basement membrane (Matrigel). The aqueous extract of a plant (*Helixanthera parasitica*) revealed a significant inhibitory effect on the cancer cell invasion, and showed antioxidant activity. The aqueous extract was partially purified by silica gel column chromatography, and the highest anti-metastatic activity fraction showed 83% inhibition of invasion with low cytotoxic effect. However, anti-metastatic activity was not associated with the antioxidant activity of the aqueous extract.


*Keywords:* Metastasis; Invasion; Liver cancer; Medicinal plants; Thai herbs; Antioxidant

## 1. Introduction

Liver cancer (hepatocellular carcinoma, HCC) is one of the most common causes of death from cancer in Thailand, and ranked the fourth in terms of cancer mortality throughout the world. The high rate of recurrence, together with the mainly intrahepatic metastasis, often leads to an unsatisfactory outcome, as shown by the very low relative 5-year survival rate of 5% (Tang, 2001).

A new approach to cancer therapy focuses on metastasis, and requires anti-metastatic agents, with little or no cytotoxic activity, which can be used for long-term treatment, in combination with conventional short-term treatment with cytotoxic anti-cancer drugs (Kohn and Liotta, 1995).

Several anti-metastatic compounds have been found as natural products of plant origin (Ogasawara et al., 2001a). Interestingly, some of them such as (−)-epigallocatechin gallate (EGCG), a tea compound, also possess antioxidant activity (Zang et al., 2000), which is of interest as a beneficial property in chemoprevention such as in anti-carcinogenesis and anti-angiogenesis (Cao and Cao, 1999).

Tumor invasion is a critical step in metastasis, involving cell motility, attachment and digestion of the basement membrane. In this study, we have screened the anti-invasive activity of several Thai plant extracts on a human HCC cell line using an in vitro invasion assay.

## 2. Materials and methods

### 2.1. Plant material

Thai medicinal plants were collected from Ubonratchathani Province in August 2001. The plants of *Psychotria monticola* (RUBIACEAE; CRINP0844), *Euonymus cochinchinensis* (CELASTRACEAE; CRINP1344), *Uraria acaulis* (FABACEAE; CRINP1444), *Dendrophthoe varians* (LORANTHACEAE; CRINP1644), *Helixanthera parasitica* (LORANTHACEAE; CRINP1744) were kindly identified by Dr. Wongsatit Chuakul of the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. Voucher specimens are kept at

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0378-8741/03/$ – see front matter 
doi:10.1016/S0378-8741(03)00072-2

the Laboratory of Natural Products, Chulabhorn Research Institute, Bangkok, Thailand.

### 2.2. Preparation of plant extracts

The air dried plants were ground and macerated with MeOH three times at room temperature. The MeOH extract was partitioned between $CH_2Cl_2$ and $H_2O$. After evaporation of the solvent under reduced pressure, the aqueous and dichloromethane extracts were evaluated for cytotoxic and anti-invasion activity.

The dried whole plant of *P. monticola* (420 g) yielded 3.91 g of dichloromethane extract and 36.62 g of aqueous extract. The dried bark of *E. cochinchinensis* (224.86 g) yielded 2.45 g of dichloromethane extract and 17.35 g of aqueous extract. The dried whole plant of *U. acaulis* (51 g) yielded 0.48 g of dichloromethane extract and 2 g of aqueous extract. The dried whole plant of *D. varians* (715.2 g) yielded 14.09 g of dichloromethane extract and 36.16 g of aqueous extract. The dried whole plant of *H. parasitica* (2.97 kg) yielded 38.43 g of dichloromethane extract and 339.83 g of aqueous extract.

The aqueous extract of *H. parasitica* (60 g) was subjected to chromatography on a silica gel column (15 cm × 15 cm), eluted with a step gradient of EtOAc–MeOH–$H_2O$ to yield eight fractions as follows: F-1 (0.05 g), F-2 (14.15 g), F-3 (8.20 g), F-4 (4.96 g), F-5 (4.28 g), F-6 (2.39 g), F-7 (15.75 g), and F-8 (3.47 g).

### 2.3. Cell culture

Human HCC cell line (HCC-S102) was established from a Thai patient (Laohathai and Bhamarapravati, 1985), and kindly provided by Dr. Sumalee Tungpradabkul of the Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand. The cells were grown in RPMI 1640 (Gibco, Grand Island, NY) containing 10% fetal bovine serum (FBS; Hyclone, UT), in humidified atmosphere, 95% air, 5% $CO_2$ at 37 °C. Human fetal lung fibroblast cell line (MRC-5) was kindly provided by the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. The cells were grown in DMEM (Gibco, Grand Island, NY) containing 10% FBS, in humidified atmosphere, 95% air, 5% $CO_2$ at 37 °C. MRC-5-conditioned media were collected after 3 days incubation and sterilized by filtration through 0.2 μm membrane filter. The conditioned media were used as chemoattractant in the invasion assay. *Cis*-platin (Sigma, St. Louis, MO) was used as a reference anti-cancer drug.

### 2.4. Invasion assay

The invasion assay was performed as previously described (Albini et al., 1987; Ogasawara et al., 2001b), using a Transwell cell culture chamber with 8 μm pore size (Costar, Cambridge, MA). Briefly, the filter (of the upper chamber), coated with 30 μg of Matrigel (BD Bioscience, Bedford, MA) on the upper surface, was placed in the chamber. After trypsinization, a cell suspension of HCC-S102 in RPMI 1640 containing 10% FBS and the test compound (final 50 μg/ml, 0.2% DMSO) was incubated for 30 min, at 37 °C, and then seeded into the upper chamber at $1 \times 10^5$ cells (200 μl) per filter. The MRC-5-conditioned medium (600 μl) containing the same concentration of the test compound and vehicle was added into the lower chamber as chemoattractant. The cells, which invaded through the Matrigel-coated filter, attached to the undersurface of the upper chamber. After incubation for 18 h in humidified atmosphere, 95% air, 5% $CO_2$ at 37 °C, the cells on the upper surface were removed by wiping with a cotton swab, and the filter was fixed with 25% MeOH and stained with 0.5% (w/v) crystal violet solution. The number of invaded cells was counted over five fields under a microscope (magnification 100×). Data was expressed as percent invasion compared with control.

### 2.5. Cytotoxic assay

Cytotoxic assay was performed as previously described (Tengchaisri et al., 1998). Briefly, HCC-S102 cells suspended in RPMI 1640 containing 10% FBS were seeded at $1 \times 10^4$ cells (100 μl) per well in 96-well plate, and incubated in humidified atmosphere, 95% air, 5% $CO_2$ at 37 °C. After 24 h, additional medium (100 μl) containing the test compound and vehicle was added to a final concentration of 50 μg/ml, 0.2% DMSO, and further incubated for 3 days. After that, the cells were fixed with 95% EtOH, stained with crystal violet solution, and lysed with a solution of 0.1N HCl in MeOH, after which absorbance was measured at 550 nm. The number of surviving cells was determined from the absorbance. Results were expressed as percent survival compared with control.

### 2.6. 1,1-Diphenyl-picrylhydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging activity was measured according to the procedure described by Hatano et al. (1989). Briefly, the sample dissolved in EtOH (500 μl) was mixed with an equal volume of DPPH solution (60 μM). The resulting solution was thoroughly mixed with a vortex mixer, and absorbance was measured at 517 nm after 30 min. Residual DPPH free radicals were determined from the absorbance. The $IC_{50}$ value is the concentration of sample required to scavenge 50% DPPH free radicals.

## 3. Results and discussion

Several Thai herbs were collected from forests in Thailand. Both aqueous and dichloromethane extracts were previously tested for cytotoxicity with an oral cancer cell line and a cholangiocarcinoma cell line. Extracts with low

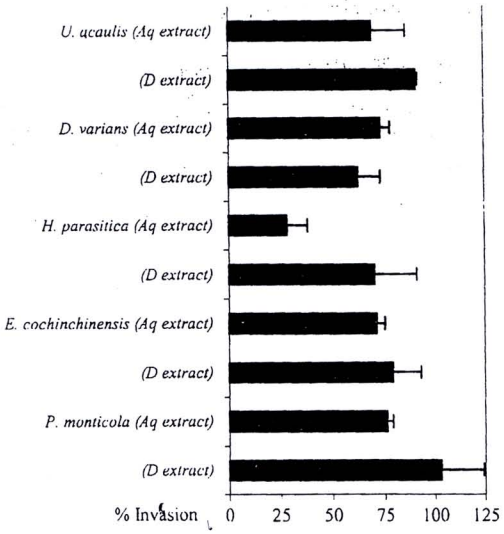


Fig. 1. Anti-invasion activity of aqueous (Aq) and dichloromethane (D) extracts of several Thai herbs. All samples were assayed at 50 μg/ml. Data represent mean with S.E.M. for three experiments. Data were expressed as percent invasion compared with control.

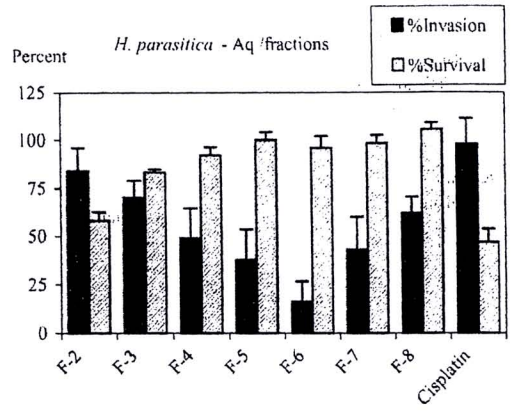


Fig. 2. Anti-invasion and cytotoxic activity of partially purified fractions from an aqueous extract of *H. parasitica*. The fractions were assayed at 50 μg/ml, in comparison with *cis*-platin (10 μg/ml). Data represent mean with S.D. of an experiment. The data were expressed as percent invasion and percent surviving cells compared with control.

cytotoxic activity ($IC_{50}$ > 100 μg/ml) were chosen for further study. The extracts were tested for inhibitory effect on the invasion of cancer cells at a non-lethal concentration, 50 μg/ml (Fig. 1). An aqueous extract of *H. parasitica* exhibited 72% inhibition.

The aqueous extract of *H. parasitica* was chromatographed in a silica gel column. Anti-invasion and cytotoxic activity of the eluted fractions were assayed at 50 μg/ml, in comparison to *cis*-platin (10 μg/ml). The highest anti-metastatic activity (83% inhibition of invasion) was found in a fraction (F-6), which had low cytotoxic activity (96% survival; Fig. 2). In comparison, the anti-cancer drug *cis*-platin, which causes interstrand cross-links in DNA, did not affect the cancer cell invasion, at a concentration having substantial cytotoxic activity (47% survival).

The aqueous extract of *H. parasitica* also showed interesting antioxidant activity, $IC_{50}$ for radical scavenging was 4.80 μg/ml, compared to 2.25 and 3.04 μg/ml for well-known antioxidant compounds, caffeic acid and ascorbic acid, respectively. Therefore, antioxidant activity of the eluted fractions were determined with the following results: F-1 (24.85 μg/ml), F-2 (1.81 μg/ml), F-3 (2.88 μg/ml), F-4 (3.18 μg/ml), F-5 (3.95 μg/ml), F-6 (5.41 μg/ml), F-7 (7.48 μg/ml), and F-8 (25.26 μg/ml), respectively. The highest antioxidant activity was found in fraction (F-2), which had low anti-metastatic activity.

*H. parasitica* is an interesting plant, since its aqueous extract possesses both anti-metastatic and antioxidant activity. Unlike compounds of tea plant which suppressed cancer cell invasion through antioxidant activity (Zang et al., 2000), our results indicate that the anti-metastatic activity of the aqueous extract of *H. parasitica* is not associated with the antioxidant activity, and resides in ingredients of the plant having low cytotoxicity. The active compound is being purified and will be further studied to elucidate its mechanism.

## Acknowledgements

This work was supported by the Chulabhorn Research Institute. Dr. Somsak Ruchirawat and Dr. Jisnuson Svasti are Senior Research Fellows of the Thailand Research Fund. We would like to thank Dr. Mammen Mammen and Dr. Ananda Nisalak (Department of Virology, the Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand) for kindly providing cell lines.

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TETRAHEDRON LETTERS

# Highly efficient synthesis of buflavine: a unique *Amaryllidaceae* alkaloid

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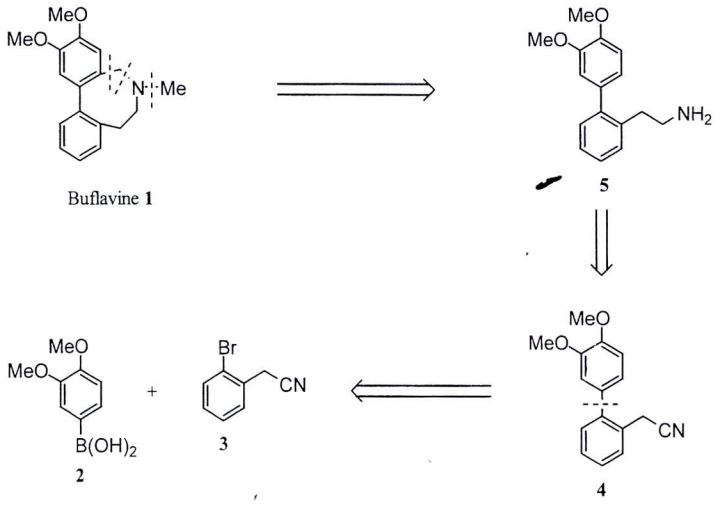
Received 10 April 2003; revised 6 May 2003; accepted 16 May 2003

**Abstract**—The *Amaryllidaceae* alkaloid buflavine **1** has been synthesized in three steps by Suzuki–Miyaura cross coupling, reduction and the cascade reactions of Pictet–Spengler type and Eschweiler–Clarke *N*-methylation. 

Buflavine **1** belongs to a group of natural *Amaryllidaceae* alkaloids isolated from *Boophane flava* bulbs.[1] It possesses a very rare 5,6,7,8-tetrahydrodibenz[*c*,*e*]azocine skeleton composed of a biaryl ring system linked via an eight-membered *N*-heterocyclic ring. Such compounds have been shown to exhibit potential α-adrenolytic and anti-serotonin activities.[2] The unique structure of buflavine and its interesting biological activities have prompted our efforts to synthesize this alkaloid. All previous synthetic routes[3,4] including the most recent publication[5] firstly involve formation of the biaryl ring by three different standard procedures (Ullmann, Suzuki–Miyaura and Meyer's biaryl coupling) followed by construction of the eight-membered *N*-heterocyclic ring via condensation of a $C_1$ side-chain with the remaining side-chain. Herein, we are pleased to report an efficient three-step total synthesis of buflavine **1** based on the retrosynthetic analysis outlined in Scheme 1.




**Scheme 1.** Retrosynthetic analysis of buflavine **1**.

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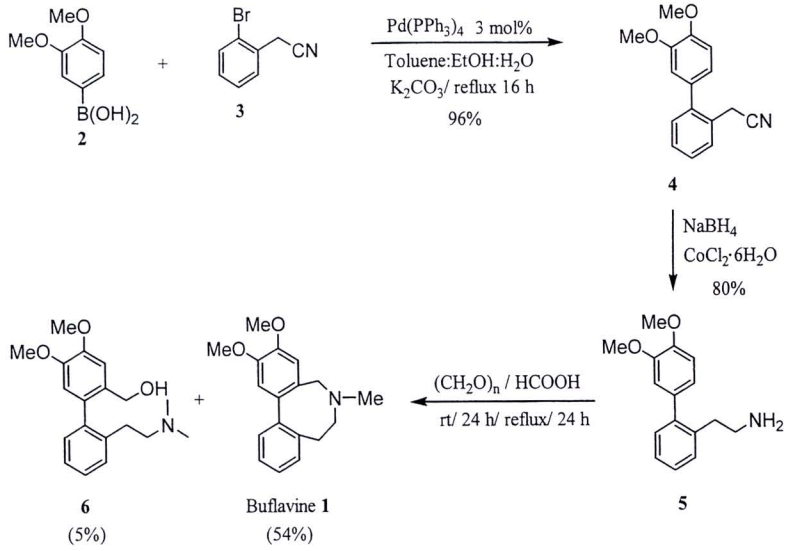
0040-4039/03/$ - see front matter 
doi:10.1016/S0040-4039(03)01207-3




**Scheme 2.** Synthetic route of buflavine **1**.

The eight-membered *N*-heterocyclic ring could be simply formed by a cascade of Pictet–Spengler type reaction then *N*-methylation under the reaction conditions employed. The biarylethylamine precursor **5** is derived from biarylacetonitrile **4** which is easily prepared via Suzuki–Miyaura cross coupling of the commercially available 3,4-dimethoxyphenylboronic acid **2** and *o*-bromophenyl acetonitrile **3** (Scheme 2).

Suzuki–Miyaura cross coupling[6] of **2** and **3** using 3 mol% $Pd(PPh_3)_4$ and $K_2CO_3$ in a mixture of toluene, ethanol and $H_2O$ (3:3:2) gave the expected biarylacetonitrile **4** in 96% yield. Conversion of biarylacetonitrile **4** to the key intermediate, biarylethylamine **5**, was accomplished by reduction with $NaBH_4$ in the presence of cobalt chloride hexahydrate[7] in 80% yield. We have previously exploited the tandem reaction of the Pictet–Spengler reaction[8a–d] and Eschweiler–Clarke reaction[9] in the synthesis of various alkaloids by performing the reaction in formic acid.[10a–c] It was gratifying to find that the formation of the eight-membered *N*-heterocyclic ring and *N*-methylation could indeed be carried out by reacting the biarylethylamine with paraformaldehyde in formic acid. To complete the ring formation and suppress the non-cyclized *N*,*N*-dimethylbiarylethylamine product, the mixture of biarylethylamine **5** and 5 equiv. of paraformaldehyde in formic acid was first stirred at room temperature for 24 h and subsequently an additional 5 equiv. of paraformaldehyde was added and the mixture was then heated at reflux for another 24 h to give buflavine **1** as a viscous oil in 54% yield accompanied by biarylalcohol-amine **6**[11] in 5% yield after preparative thin layer chromatography (10% MeOH–$CH_2Cl_2$). The biarylalcohol-amine **6** was presumably derived from electrophilic substitution of the aryl ring with paraformaldehyde in parallel with *N*,*N*-dimethylation. The spectroscopic data of our synthetic compound corresponded well with those reported for the natural product.[1] As far as we are aware, this is the first report on the successful application of the Pictet–Spengler type reaction in the formation of an eight-membered heterocyclic ring.

In conclusion, we have developed a concise and highly efficient route for the synthesis of buflavine **1** in three steps in 44% overall yield from commercially available starting materials using a Suzuki–Miyaura cross coupling to form a biaryl ring in conjunction with a cascade of Pictet–Spengler then *N*-methylation of the biarylethylamine in a one-pot reaction to construct the eight-membered heterocyclic ring.

## Acknowledgements

We are grateful to the Thailand Research Fund (TRF) for the generous support of our research program and the award of Senior Research Scholar to S.R. We also acknowledge the facilities in the Department of Chemistry, Mahidol University, provided by the Postgraduate Education and Research Program in Chemistry (PERCH).

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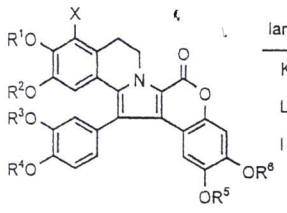
11. All compounds were fully characterized. Biarylacetonitrile (**4**) pale yellow crystals, mp (EtOAc) 87.0–87.5°C. IR (KBr) 2250 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 3.64 (s, 2H), 3.90, 3.94 (2s, 2×3H), 6.85 (broad s, 2H), 6.95 (d, 1H, $J=8.8$ Hz), 7.26–7.44 (m, 3H), 7.46–7.56 (m, 1H). $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 22.02, 55.92, 111.31, 112.30, 118.34, 121.11, 127.94, 128.11, 128.98, 130.45, 132.51, 141.75, 148.66, 148.93. EIMS (*m/z*, % relative intensity) 254 ($M^+$+1, 25), 253 ($M^+$, 100), 210 (54), 192 (16), 182 (15), 180 (11), 166 (11), 165 (17). HRMS (FAB+) calcd for $C_{16}H_{16}NO_2$, 254.1181. Found 254.1179. Anal. calcd for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.51; H, 5.74; N, 5.26%. Biarylethylamine (**5**) viscous oil, mp (HCl salt) 209–210°C, mp (oxalate salt) 148–149°C. IR (neat) 3345, 2934, 1605, 1585, 1520, 1485, 1246, 1027 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 1.43 (s, 2H), 2.78 (s, 4H), 3.88, 3.93 (2s, 2×3H), 6.80–6.95 (m, 3H), 7.2–7.4 (m, 4H). $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 55.43, 110.62, 112.33, 120.88, 125.59, 126.79, 129.09, 129.81, 134.0, 136.59, 141.60, 147.62, 148.09. EIMS (*m/z*, % relative intensity) 258 ($M^+$+1, 6), 257 ($M^+$, 24), 240 (45), 228 (100), 213 (17), 209 (12). HRMS (FAB+) calcd for $C_{16}H_{20}NO_2$, 258.1494. Found 258.1496. Buflavine **1** viscous oil, mp (oxalate salt) 194.0–194.5°C. IR (neat) 2931, 1606, 1520, 1452, 1357 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 2.49 (s, 3H), 2.49–2.80 (m, 3H), 3.07 (d, 1H, $J=13.5$ Hz), 3.26 (t, 1H, $J=9.5$ Hz), 3.54 (d, 1H, $J=13.5$ Hz), 3.90, 3.96 (2s, 2×3H), 6.80 (s, 1H), 6.91 (s, 1H), 7.31 (m, 4H). $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 32.45, 45.82, 55.89, 58.24, 58.62, 112.06, 113.51, 126.08, 127.88, 129.01, 129.43, 129.58, 132.92, 139.96, 141.19, 147.84, 148.34. EIMS (*m/z*, % relative intensity) 284 ($M^+$+1, 37), 283 ($M^+$, 99.8), 268 (100), 240 (67), 225 (71), 197 (76), 179 (47), 178 (28), 165 (38), 153 (22), 152 (27). HRMS (FAB+) calcd for $C_{18}H_{22}NO_2$, 284.1651. Found 284.1649. Biarylalcohol-amine **6** viscous oil, IR (neat) 3364, 2936, 1607, 1515, 1465 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 2.00 (s, 6H), 2.40–2.9 (m, 4H), 3.20 (broad s, 1H), 3.85, 3.95 (2s, 2×3H), 4.24 (d, 1H, $J=12$ Hz),), 4.48 (d, 1H, $J=12$ Hz), 6.67 (s, 1H), 7.08 (s, 1H), 7.20–7.38 (m, 4H). $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 30.15, 44.74, 55.95, 56.01, 60.81, 62.49, 112.37, 112.94, 125.94, 127.73, 129.57, 130.29, 131.92, 132.54, 138.52, 140.01, 147.76, 148.35. EIMS (*m/z*, % relative intensity) 316 ($M^+$+1, 26), 315 ($M^+$, 14), 270 (56), 239 (100), 240 (28), 238 (23), 211 (23), 196 (27), 195 (24), 181 (33), 165 (48), 153 (33), 152 (44), 58 (88). HRMS (FAB+) calcd for $C_{19}H_{26}NO_3$, 316.1913. Found 316.1909.

**Marine Alkaloid Synthesis**

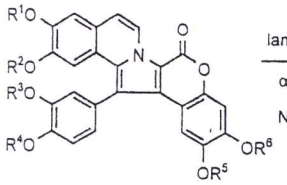
# A Highly Efficient Synthesis of Lamellarins K and L by the Michael Addition/Ring-Closure Reaction of Benzyldihydroisoquinoline Derivatives with Ethoxycarbonyl-β-nitrostyrenes**

*Poonsakdi Ploypradith, Chulabhorn Mahidol, Poolsak Sahakitpichan, Siriporn Wongbundit, and Somsak Ruchirawat**

Among the recently discovered marine natural products isolated from the prosobranch mollusc *Lamellaria* sp. and also from the ascidians is a group of 3,4-diarylpyrroloisoquinoline lactone derivatives known as lamellarins, whose structures contain different patterns of polyoxygenated aromatics on their periphery (Scheme 1). Since the first four of these alkaloids were isolated by Faulkner and co-workers in 1985, a total of 35 lamellarins have been identified thus far.[1] Our research group has been particularly interested in lamellarins K (**1a**) and L (**1b**) for their reported biological activities which include cytotoxicity, HIV-1 integrase inhibition, and multidrug-resistance (MDR) reversal.[2,3] A recent biological evaluation by Faulkner and co-workers of lamellarin α (**2a**) and its 20-sulfate and 13,20-disulfate derivatives for inhibition of HIV-1 integrase showed that the presence of sulfate groups on the periphery could greatly influence selectivity in HIV-1 integrase inhibition.[4] It has also been found that lamellarins act as nontoxic inhibitors of acquired MDR. Lamellarin I (**1c**) showed sensitizing effects to doxorubicin in multidrug-resistant P388/Schabel cells at concentrations as low as 0.2 μM and showed full potentiation at a concentration 10-times lower than that of the prototype MDR inhibitor verapamil.[5] Fürstner and co-workers have recently shown that cytotoxicity and MDR reversal of lamellarins can be uncoupled.[6] The exact molecular mechanism of action of lamellarins and their related compounds is currently under extensive investigation.



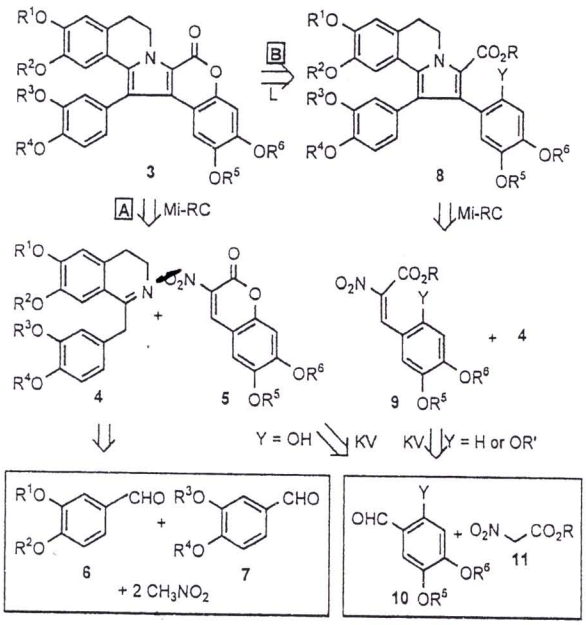
| lamellarin | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ | X |
|---|---|---|---|---|---|---|---|
| K (**1a**) | Me | Me | Me | H | Me | H | OH |
| L (**1b**) | H | Me | H | Me | Me | H | H |
| I (**1c**) | Me | Me | Me | Me | Me | H | OMe |



| lamellarin | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ |
|---|---|---|---|---|---|---|
| α (**2a**) | Me | Me | H | Me | Me | H |
| N (**2b**) | H | Me | H | Me | Me | H |

*Scheme 1.* Structures of lamellarins K (**1a**), L (**1b**), I (**1c**), α (**2a**), and N (**2b**).

There have been several studies[7] directed toward the total synthesis of lamellarins, notably by the research groups of Steglich,[8] Banwell,[9] Boger,[10] Ishibashi,[11] and ourselves.[12] Previously, we reported two synthetic approaches to the lamellarin skeleton, both of which involved the key condensation of the appropriately substituted benzyldihydroisoquinoline with phenacyl bromide derivatives to form the pyrrole core.[12] We now envisioned that the lamellarin skeleton **3** could arise from condensation of the benzyldihydroisoquinoline **4** with a Michael acceptor, such as **5** or **9**, which essentially would install the lactone or ester group on the 2-position (Scheme 2). This synthetic approach would prove highly convergent since, in a single step, it would form



*Scheme 2.* Retrosynthetic analysis and strategies for the synthesis of the lamellarin skeleton **3**. L = lactonization, Mi-RC = Michael addition/ring-closure reaction, KV = Knoevenagel reaction.

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[**] We acknowledge financial contributions from the Thailand Research Fund to S.R. (RTA/07/2544 and Senior Research Scholar Award) and to P.P., and facilities provided by the Postgraduate Education and Research Program in Chemistry (PERCH), Department of Chemistry, Mahidol University.

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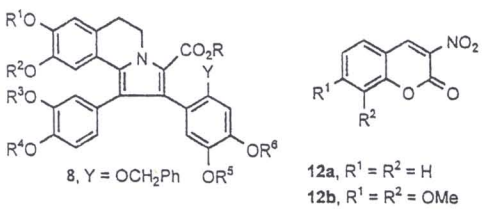
  DOI: 10.1002/anie.200352043 

the pyrrole as well as providing the lactone directly or the ester group for subsequent lactonization. Since imines, which exist in equilibrium with their enamines, have been shown to react with $\beta$-nitrostyrene to give the corresponding pyrroles,[13] it was expected that Michael addition of an enamine derived from benzyldihydroisoquinoline with a powerful Michael acceptor, such as **5** or **9**, followed by ring closure and aromatization could provide a more direct route to the lamellarin alkaloids than previous methods.

Modeling the Michael addition/ring-closure reaction between simple $\beta$-nitrostyrenes and 3,4-dihydropapavarine hydrochloride under basic conditions resulted in complete consumption of both starting materials but gave no desired product. We then examined the use of ester nitrostyrenes in place of the simple nitrostyrenes in a similar Michael addition/ring-closure reaction. The ester nitrostyrenes are more powerful Michael acceptors than the simple nitrostyrenes due to the additional electron-withdrawing effect provided by the ester group; this allows the ester nitrostyrenes to react under milder reaction conditions than those required for the simple nitrostyrenes.

We turned our attention to the coumarin derivatives **12a** and **12b** (Scheme 3) as the ester nitrostyrenes for the reaction under basic conditions (pathway A in Scheme 2). These

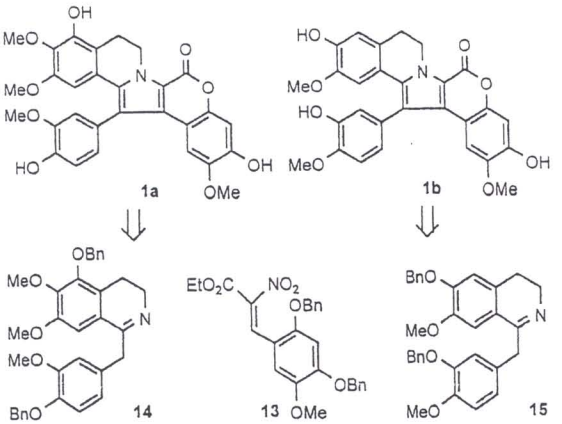



*Scheme 3.* Structures of intermediate pyrrole ester **8** and nitrocoumarins **12a** and **12b**.

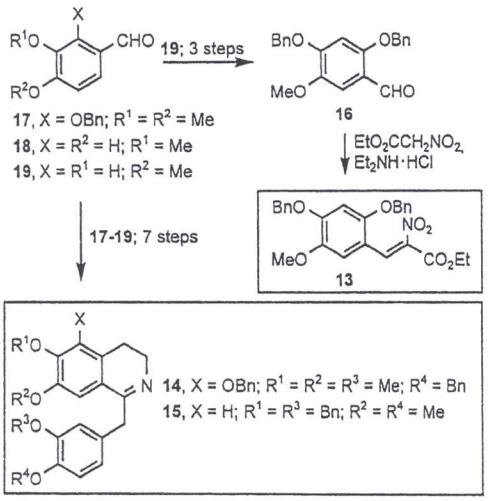
coumarin derivatives offered a significant advantage in that their structures already contained the lactone moiety. We anticipated that, if the reaction occurred with these coumarins, all of the lamellarin skeleton, in particular the pyrrole and lactone moieties, would be successfully installed in one chemical operation. Unfortunately, such a reaction with these coumarins gave the desired lamellarins in only 5–6% yields.

The poor yield resulting from the reaction with nitrocoumarins **12a** and **12b** prompted us to examine the use of acyclic ester nitrostyrenes like **9** (pathway B in Scheme 2). Despite adding one step of lactonization into the synthesis, this alternative synthetic route would probably allow for a more effective preparation of the lamellarin framework. Our required intermediate would then assume the structure of compound **8** (Scheme 3).

From the structure of compound **8**, it was apparent that the desired lactone moiety could be formed by unmasking the benzyloxy-protected phenol by hydrogenolysis and subsequently initiating base-mediated lactonization. Retrosynthetic analysis (Scheme 4) revealed that our target lamellarins K (**1a**) and L (**1b**) would require the same ester nitrostyrene, **13**, which could be prepared in 56% overall yield in four steps from **19** including the Knoevenagel condensation of aldehyde **16**[14] with ethyl nitroacetate (Scheme 5). As an alternative to the procedure depicted in Scheme 5, aldehyde




*Scheme 4.* Retrosynthetic analysis for the synthesis of lamellarins K (1a) and L (1b). Bn = benzyl.



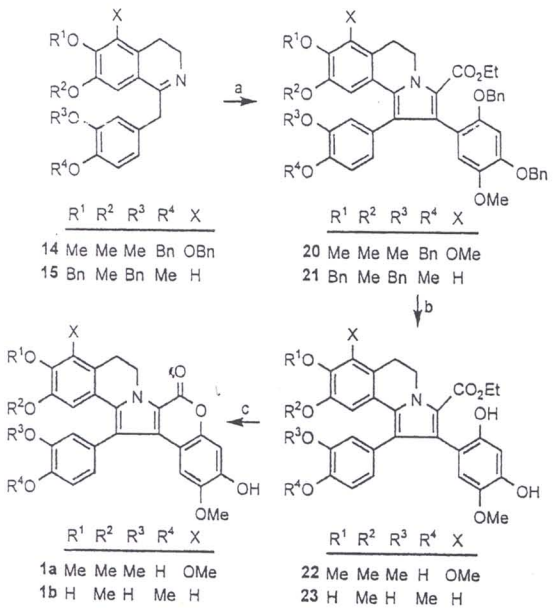

*Scheme 5.* Synthesis of ester nitrostyrene **13** and benzyldihydroisoquinoline derivatives **14** and **15**.

**16** could be prepared in 79% overall yield by selective bisdemethylation of 2,4,5-trimethoxybenzaldehyde with $AlCl_3$[15] followed by benzylation. Synthesis of the substituted benzyldihydroisoquinolines **14** and **15** is well known in the literature[16–24] and both compounds were synthesized in seven steps by Bischler–Napieralski reactions of the appropriate aryl ethylamines and aryl acetic acids, which were readily prepared from three common starting materials, **17–19** (Scheme 5).

The Michael addition/ring-closure reaction of the imines **14** and **15** with the ester nitrostyrene **13** proceeded smoothly in refluxing anhydrous acetonitrile in the presence of

$NaHCO_3$ to give the desired pyrroles **20** and **21**, both in 70% yield (Scheme 6). The syntheses were completed by subjecting pyrroles **20** and **21** to hydrogenolysis to give compounds **22** and **23** quantitatively, followed by base-mediated lactonization with sodium hydride in dry THF to produce lamellarin K (**1a**) in 93% yield and lamellarin L (**1b**) in 87% yield over two steps.




*Scheme 6.* Synthesis of lamellarins K (**1a**) and L (**1b**). a) $NaHCO_3$, **13**, $CH_3CN$, reflux, 70% (**20**), 70% (**21**); b) $H_2$, Pd/C, EtOAc; c) NaH, THF, 93% (**1a**, over two steps), 87% (**1b**, over two steps).

In summary, lamellarins K and L were successfully synthesized in three steps from benzyldihydroisoquinolines **14** and **15** with ester nitrostyrene **13** in 65% and 61% overall yields, respectively. The key step was the Michael addition/ring-closure reaction which proceeded in 70% yield for both lamellarins. The basic building blocks for the lamellarins are the simple and easily prepared substituted-benzaldehyde derivatives. Each lamellarin could be analyzed to consist of three such building blocks, two in the benzyldihydroisoquinoline derivative and the other in the ester nitrostyrene. Our convergent synthetic approach offers a significant improvement over others reported thus far in that it allows easy incorporation of all aryl groups on the lamellarin skeleton without the need for complex protecting-group strategies. The benzyl group was chosen as the only necessary hydroxy-protecting group since all of the benzyl groups could be removed in the same step by simple palladium-catalyzed hydrogenolysis. The syntheses of other lamellarins employing this similar approach will be reported in due course.

Keywords: alkaloids · cyclization · Michael addition · nitrostyrenes · total synthesis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Received: June 3, 2003
Revised: November 14, 2003 [Z52043]

  

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Tetrahedron 60 (2004) 4169–4172

# A practical and highly efficient synthesis of lennoxamine and related isoindolobenzazepines

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Received 12 January 2004; revised 27 February 2004; accepted 18 March 2004

**Abstract**—Lennoxamine and related isoindolobenzazepines were prepared in high yield by intramolecular condensation of aldehyde isoindolones under basic conditions followed by catalytic hydrogenation of the resulting dehydroisoindolobenzazepines.


## 1. Introduction

Lennoxamine (**1a**), an isoindolobenzazepine, was isolated from the Chilean plant *Berberis darwinii*.[1] Even though no important pharmacological activity of lennoxamine has been reported as normally found in the benzazepine derivatives,[2a–e] its unique structural features have captured the interest of many synthetic groups over the past 20 years.

The previous syntheses of lennoxamine and other isoindolobenzazepine derivatives could be classified into various approaches depending on the order of bond formation as shown in Figure 1.

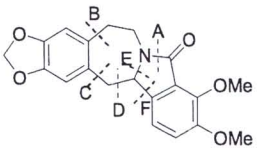
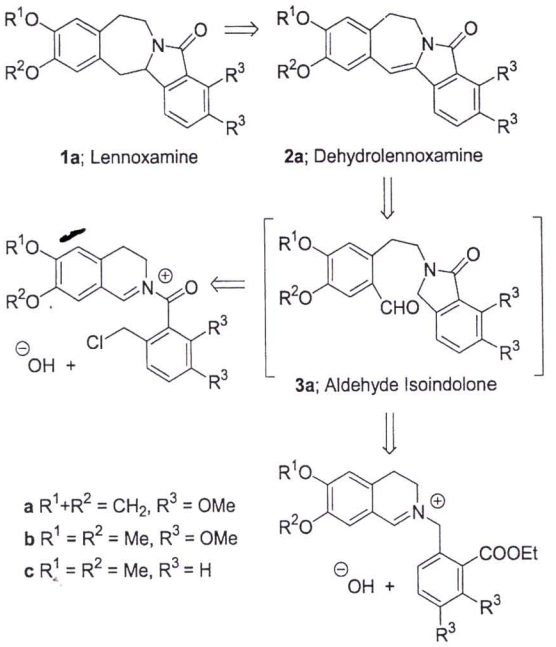



**Figure 1.**

The first approach involved prior construction of the benzazepine skeleton followed by isoindolone ring formaton via bond A[3a,b] or bond F.[4] The second approach concentrated on first the isoindolone ring formation which could then be manipulated to form the benzazepine ring via formation of bond B[5a–c] or bond C.[6a–c] Bond D formation of various phthalimide derivatives[7a–d] has been exploited in the third approach. The simultaneous formation of the isoindolone and the benzazepine skeleton was the focus of the fourth approach.[6c,8] The fifth approach utilized the rearrangement of various isoquinoline derivatives as a means to synthesize the isoindoloisoquinolines.[9a–e]

## 2. Results and discussion

In our previous synthetic routes (Scheme 1), the aldehyde isoindolone **3a** was the common unisolated intermediate which further cyclized smoothly to the dehydrolennoxamine




**Scheme 1.** Synthetic route.

*Keywords*: Lennoxamine; Isoindolobenzazepine alkaloids.
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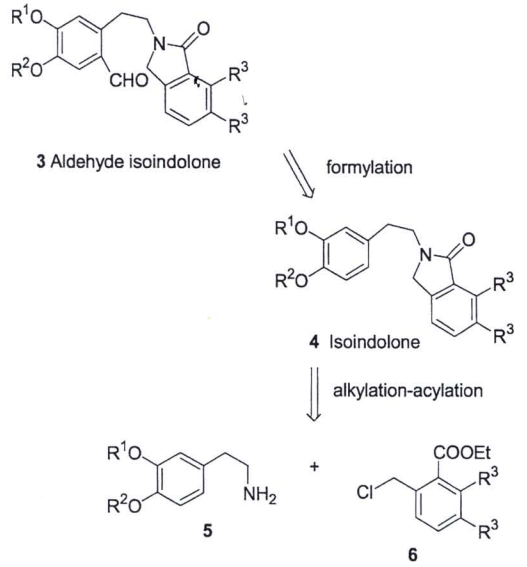
doi:10.1016/j.tet.2004.03.049

2a in high yield. The former pathway provided the desired isoindolone via hydroxide addition to the iminium salt to give the carbinolamine derivative which was opened to give the amide anion followed by intramolecular alkylation. The above pathway worked well only when the sixth position of the isoquinoline derivative was substituted with a methoxy group. The methoxy group apparently facilitated the breaking of the carbon–nitrogen bond to give the aldehyde and amide anion. Alternatively, in the other pathway, the methoxy group *ortho* to the carboethoxy group is required for the success of the synthesis. The methoxy group presumably activates the leaving group ability of the ester.[10] In this paper, we report a highly efficient, direct synthesis of aldehyde isoindolone which could be successfully cyclized to the isoindolobenzazepine derivatives irrespective of the oxygenation pattern on the aromatic ring.

The aldehyde isoindolone was synthesized by the route suggested by retrosynthetic analysis as shown in Scheme 2.




Scheme 2. Retrosynthetic analysis.

The aldehyde isoindolone **3** could be synthesized by formylation of the isoindolone precursor **4** which could conceivably be prepared by alkylation–acylation of the arylethylamine derivatives with ethyl 2-chloromethylbenzoates **6**.

To test the above idea, homoveratrylamine **5** was heated at reflux with ethyl 2-chloromethylbenzoate **6** in acetonitrile in the presence of triethylamine to give the expected isoindolone **4c** in 74% yield. Similarly, the other two isoindolones, **4a**, **4b** were obtained in 81 and 76% yields respectively from the reaction of the appropriate arylethylamines and ethyl 2,3-dimethoxy-6-chloromethylbenzoate.[11]

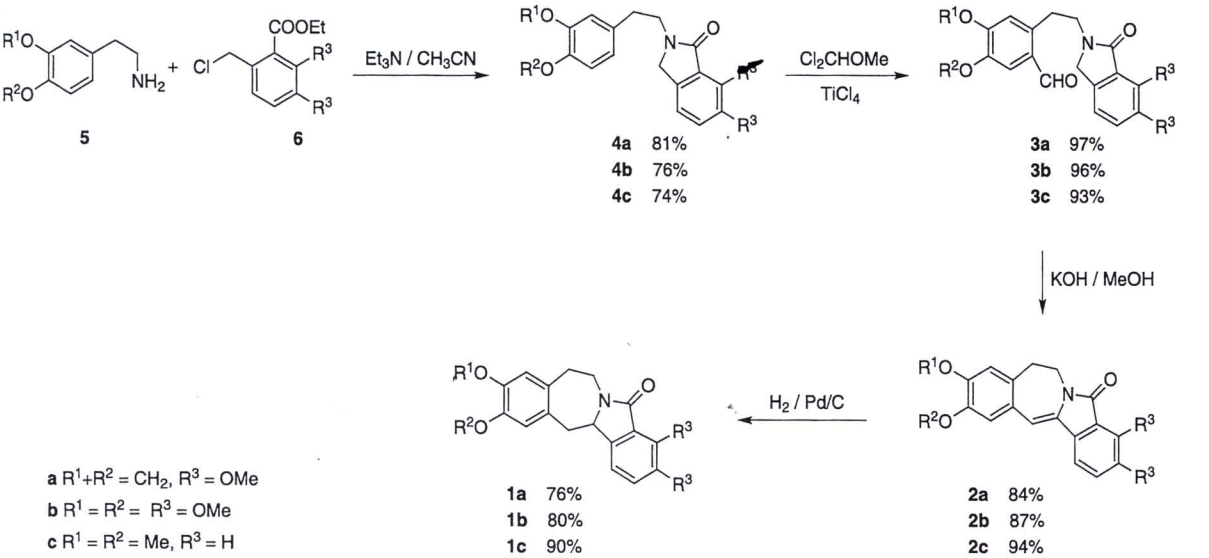
Formylation of the resulting isoindolones were carried out conveniently using dichloromethyl methyl ether and titanium tetrachloride in dichloromethane[12a–c] to give the aldehyde isoindolones, **3a**, **3b**, **3c** in excellent yields

The derived aldehyde isoindolones were cyclized smoothly in refluxing methanolic KOH to give the required dehydroisoindolobenzazepines, **2a**, **2b**, and **2c**, in excellent yields.

Lennoxamine, **1a** as well as other isoindolobenzazepines, **1b**, **1c**, could be readily obtained by catalytic hydrogenation of the dehydro intermediates **2a**, **2b**, and **2c** in 76, 80 and 90% yields, respectively (Scheme 3).

## 3. Conclusion

We have successfully developed a practical and highly efficient synthetic route for lennoxamine and other related isoindolobenzazepines. The route involved condensing of aldehyde isoindolones under basic conditions followed by catalytic hydrogenation of the resulting dehydroisoindolobenzazepines. The key aldehyde isoindolones were derived in two steps from alkylation–acylation of arylethylamines with ethyl 2-chloromethylbenzoate derivatives and insertion




Scheme 3. Preparation of lennoxamine and its derivatives.

of the C-1 aldehyde onto the aromatic ring using dichloromethyl methyl ether and $TiCl_4$.

## 4. Experimental

### 4.1. General

$^1H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ at 400 and 100 MHz respectively using TMS as an internal standard. Mass spectra were determined at an ionizing voltage of 70 eV. Column chromatographic purifications were carried out using silica gel (70–230 mesh).

### 4.2. General procedure for the synthesis of isoindolones

A solution of arylethylamine derivatives (1 mmol), ethyl 2-chloromethylbenzoates (1 mmol) and triethylamine (1.2 mmol) in $CH_3CN$ (5 mL) was heated under reflux for 3 h under nitrogen atmosphere. $CH_3CN$ was removed and the crude product was extracted with $CH_2Cl_2$ and washed with water. The organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated to dryness to give the crude amide as yellow solid, the product was further purified by column chromatography using 2% MeOH/$CH_2Cl_2$ as eluting solvent to give the isoindolones as pale yellow solid.

**4.2.1. 2-(3,4-Methylenedioxyphenethyl)-6,7-dimethoxyphthalimidine (4a).** Pale yellow crystals (81%), mp (EtOAc) 99–100 °C; IR (nujol) 1678 $cm^{-1}$; $^1H$ NMR $\delta$ 2.88 (t, 2H, $J$=7.5 Hz), 3.75 (t, 2H, $J$=7.5 Hz), 3.88 (s, 3H), 4.08 (s, 3H), 4.13 (s, 3H), 5.91 (s, 2H), 6.67 (dd, 1H, $J$=8.0, 1.6 Hz), 6.71 (d, 1H, $J$=8.0 Hz), 6.73 (d, 1H, $J$=1.6 Hz), 7.02, 7.06 (AB, 1H each, $J$=8.0 Hz). $^{13}C$ NMR $\delta$ 34.4, 44.3, 49.6, 56.7, 62.5, 100.8, 108.3, 109.0, 116.3, 117.6, 121.5, 125.0, 132.6, 134.5, 146.1, 147.1, 147.7, 152.2, 166.6. EIMS 341($M^+$, 23), 206(96), 194(67), 193(16), 162(13), 149(16), 148(100), 135(13). Anal. Calcd for $C_{19}H_{19}NO_5$: C, 66.85; H, 5.61; N, 4.10. Found: C, 66.90; H, 5.80; N, 3.92.

**4.2.2. 2-(3,4-Dimethoxyphenethyl)-6,7-dimethoxyphthalimidine (4b).** Pale yellow crystals (76%), mp (EtOAc) 120–120.5 °C; IR (nujol) 1670 $cm^{-1}$; $^1H$ NMR $\delta$ 2.94 (t, 2H, $J$=7.5 Hz), 3.80 (t, 2H, $J$=7.5 Hz), 3.81 (s, 3H), 3.86 (s, 3H), 3.89 (s, 3H), 4.10 (s, 3H), 4.11 (s, 2H), 6.77 (d, 1H, $J$=8.5 Hz), 6.80 (dd, 1H, $J$=8.0, 0.4 Hz), 7.01, 7.07 (AB, 1H each, $J$=8.0 Hz). $^{13}C$ NMR $\delta$ 34.1, 44.2, 49.7, 55.72, 55.74, 55.81, 62.5, 111.3, 111.8, 116.3, 117.6, 120.5, 125.1, 131.4, 134.5, 147.1, 147.6, 148.9, 152.21, 166.6. EIMS 357($M^+$, 13), 206(34), 165(14), 164(100). Anal. Calcd for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92. Found: C, 66.83; H, 6.60; N, 3.82.

**4.2.3. 2-(3,4-Dimethoxyphenylethyl)phthalimidine (4c).** Pale yellow crystals (74%), mp (EtOAc–Hexane) 98–99 °C; IR (nujol) 1670 $cm^{-1}$; $^1H$ NMR $\delta$ 2.95 (t, 2H, $J$=7.0 Hz), 3.78 (s, 3H), 3.85 (s, 3H), 3.86 (t, 2H, $J$=7.0 Hz), 4.19 (s, 2H), 6.74 (s, 1H), 6.77 (d, 1H, $J$=8.0 Hz), 6.78 (d, 1H, $J$=8.0 Hz), 7.37 (d, 1H, $J$=7.0 Hz), 7.45 (t, 1H, $J$=7.0 Hz), 7.51 (td, 1H, $J$=7.0, 1.0 Hz), 7.85 (d, 1H, $J$=7.0 Hz). $^{13}C$ NMR $\delta$ 34.3, 44.2, 50.7, 55.74, 55.82, 111.3, 111.8, 120.5, 122.6, 123.5, 127.9, 131.11, 131.28, 132.8, 141.1, 147.6, 148.9, 168.4. EIMS 297($M^+$, 13), 165(12), 164(100), 146(42), 91(15). Anal. Calcd for $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.59; H, 6.28; N, 4.76.

### 4.3. General procedure for the synthesis of aldehyde isoindolones

A solution of isoindolones (2.18 mmol) in 25 mL of dry $CH_2Cl_2$ was cooled in an ice bath, and 0.3 mL of dichloromethyl methyl ether was added. While the solution was stirred and cooled, 1.2 mL (10.91 mmol) of $TiCl_4$ was added. After the addition was complete, the mixture was stirred for 5 min in an ice bath and for 3 h at room temperature. The reaction mixture was then poured into a flask containing crushed ice and was shaken thoroughly. The organic layer was separated, and the aqueous solution was extracted with $CH_2Cl_2$. The combined organic solution was washed with water and dried over anhydrous $Na_2SO_4$. After evaporation of the solvent, the white solid so obtained was further purified by column chromatography using 2% MeOH/$CH_2Cl_2$ as eluting solvent to give the aldehyde isoindolones as white solid.

**4.3.1. 2-(2-Formyl-4,5-methylenedioxyphenethyl)-6,7-dimethoxyphthalimidine (3a).** White crystals (97%), mp (EtOH) 175.5–176.5 °C; IR (nujol) 1670 (broad), 1748 $cm^{-1}$; $^1H$ NMR $\delta$ 3.32 (t, 2H, $J$=7.0 Hz), 3.74 (t, 2H, $J$=7.0 Hz), 3.89 (s, 3H), 4.08 (s, 3H), 4.28 (s, 2H), 6.05 (s, 2H), 6.82 (s, 1H), 7.06, 7.09 (AB, 1H each, $J$=8.0 Hz), 7.27 (s, 1H), 10.09 (s, 1H). $^{13}C$ NMR $\delta$ 31.4, 44.4, 49.6, 56.7, 62.5, 102.0, 111.1, 111.3, 116.4, 117.7, 124.9, 128.4, 134.5, 138.5, 147.1, 152.25, 152.29, 166.8, 190.1. EIMS 369 ($M^+$, 27), 206(100), 194(8), 162(9), 148(9). Anal. Calcd for $C_{20}H_{19}NO_6$: C, 65.03; H, 5.18; N, 3.79. Found: C, 65.11; H, 5.14; N, 3.79.

**4.3.2. 2-(2-Formyl-4,5-dimethoxyphenylethyl)-6,7-dimethoxyphthalimidine (3b).** White crystals (96%), mp (EtOH) 157–158 °C; IR (nujol) 1675 (broad), 1748 $cm^{-1}$; $^1H$ NMR $\delta$ 3.37 (t, 2H, $J$=7.0 Hz), 3.78 (t, 2H, $J$=7.0 Hz), 3.87 (s, 3H), 3.90 (s, 3H), 3.94 (s, 3H), 4.09 (s, 3H), 4.22 (s, 2H), 6.81 (s, 1H), 7.04, 7.08 (AB, 1H each, $J$=8.0 Hz), 7.33 (s, 1H), 10.15 (s, 1H). $^{13}C$ NMR $\delta$ 30.7, 44.2, 49.7, 56.0, 56.2, 56.8, 62.5, 113.6, 114.0, 116.3, 117.7, 124.9, 126.8, 134.5, 136.2, 147.1, 147.8, 152.2, 153.6, 166.8, 190.5. EIMS 385($M^+$, 31), 207(13), 206(100), 193(14), 192(19), 164(31). Anal. Calcd for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.31; H, 6.05; N, 3.80.

**4.3.3. 2-(2-Formyl-4,5-dimethoxyphenylethyl)phthalimidine (3c).** White crystals (93%), mp (EtOAc–hexane) 144–145 °C, lit.[9a] 148–150 °C; IR (nujol) 1680 (broad), 1748 $cm^{-1}$; $^1H$ NMR $\delta$ 3.39 (t, 2H, $J$=7.0 Hz), 3.84 (s, 3H), 3.85 (t, 2H, $J$=7.0 Hz), 3.94 (s, 3H), 4.32 (s, 2H), 6.80 (s, 1H), 7.32 (s, 1H), 7.41 (d, 1H, $J$=7.0 Hz), 7.46 (t, 1H, $J$=7.0 Hz), 7.52 (td, 1H, $J$=7.0, 1.0 Hz), 7.85 (d, 1H, $J$=7.0 Hz), 10.14 (s, 1H). $^{13}C$ NMR $\delta$ 30.9, 44.1, 50.6, 56.0, 56.2, 113.7, 114.3, 122.7, 123.5, 126.8, 128.0, 131.3, 132.7, 136.1, 141.2, 147.8, 153.7, 168.5, 190.7. EIMS 325($M^+$, 10), 192(38), 191(48), 146(80), 105(100), 77(55), 51(20). Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31. Found: C, 69.81; H, 6.11; N, 4.31.

### 4.4. General procedure for the synthesis of dehydroisoindolobenzazepine derivatives

Aldehyde (1 mmol) was dissolved in a solution of KOH (500 mg) in MeOH (25 mL) and the mixture was heated at reflux for 1 h. MeOH was removed by evaporation and water was added. The aqueous mixture was extracted with $CH_2Cl_2$ and dried. The organic extracts were evaporated to dryness under reduced pressure. The crude product was recrystallized from MeOH to give the required dehydroisoindolobenzazepines.

**4.4.1. 3,4-Dimethoxy-7,8-dihydro-10,11-methylenedioxy-5*H*-isoindolo[1,2-*b*][3]benzazepine-5-one (2a).** Yellow crystals (84%), mp (MeOH) 208–209 °C, lit.[6b] 209–211 °C, lit.[9e] 213–214 °C. The spectroscopic data of compounds **2a**, **2b**, and **2c** are the same as those previously published.[9c]

**4.4.2. 3,4-Dimethoxy-7,8-dihydro-10,11-dimethoxy-5*H*-isoindolo[1,2-*b*][3]benzazepine-5-one (2b).** Yellow crystals (87%), mp (MeOH) 185–189 °C, lit.[9c] 185–189 °C.

**4.4.3. 7,8-Dihydro-10,11-dimethoxy-5*H*-isoindolo[1,2-*b*][3]benzazepine-5-one (2c).** Yellow crystals (94%), mp (MeOH) 192–194 °C, lit.[6a] 195–196 °C, lit.[9a] 190–192 °C.

### 4.5. General procedure for the synthesis of isoindolobenzazepine derivatives

To a stirred solution of dehydroisoindolobenzazepines (250 mg) in EtOAc (15 mL), 10% Pd on carbon (51 mg) was slowly added. The mixture was hydrogenated (1 atm, balloon) and when the reaction was complete (TLC showed the absence of the highly fluorescent spot of the starting material), catalyst residue was removed by filtration, washed with EtOAc, and evaporated to dryness to give the required isoindolobenzazepines.

**4.5.1. 3,4-Dimethoxy-13,13a-tetrahydro-10,11-methylenedioxy-5*H*-isoindolo[1,2-*b*][3]benzazepine-5-one (1a).** White crystals (76%), mp (MeOH) 226–227 °C, lit.[1] 225 °C, lit.[3a] 228–229 °C. lit.[9e] 235–235.5 °C.

**4.5.2. 3,4-Dimethoxy-13,13a-tetrahydro-10,11-dimethoxy-5*H*-isoindolo[1,2-*b*][3]benzazepine-5-one (1b).** White crystals (80%), mp (MeOH) 213–214 °C, lit.[9c] 213–214 °C.

**4.5.3. 7,8,13,13a-Tetrahydro-10,11-dimethoxy-5*H*-isoindolo[1,2-*b*][3]benzazepine-5-one (1c).** White crystals (90%), mp (EtOAc) 178–179 °C, lit.[5c] 178–179 °C, lit.[6c] 179 °C.

## Acknowledgements

We are grateful to the Thailand Research Fund (TRF) for the generous support of our research program and the award of Senior Research Scholar to S.R. We also acknowledge the facilities in the Department of Chemistry, Mahidol University, provided by the Postgraduate Education and Research Program in Chemistry (PERCH).

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# The pharmacodynamic study of a potent new antimalarial ($MC_1$)

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**Abstract**

2,3-bis(Trifluoromethyl)-4-(3-hydroxyquinuclidinylquinoline) or $MC_1$ is a new synthetic compound with potent antimalarial activity in vitro and in vivo studies. The $IC_{50}$ values of $MC_1$ and chloroquine in in vitro culture of *Plasmodium falciparum* are $7.0 \times 10^{-8}$ and $6.06 \times 10^{-7}$ M, respectively. In an in vivo study using *Plasmodium berghei* infected mice as the test model, the survival time of the infected mice without drug treatment was $6.00 + 0.58$ days. Chloroquine and $MC_1$ at an equal dose of 7.5 mg/kg, orally administered once daily for 4 days, prolonged the survival time of the infected mice from 6 to 14 days, and more than 28 days, respectively. At the doses that exhibit potent antimalarial activity in vivo, there are no observable toxic effects. Preliminary studies of the pharmacodynamic activity of this newly synthesized compound revealed that at the doses which exhibit potent antimalarial activity, there is no alteration in motor activity such as distance traveled, rotational behavior, and stereotypic activity. The blood glucose was not significantly altered. In the spontaneous beating, isolated right atria of mice, $MC_1$ exhibits direct negative chronotropism at high concentrations ($10^{-4}$ M). This effect is augmented in hyper-$K^+$ bathing solution. A direct negative chronotropic effect was also observed when mefloquine at $5 \times 10^{-5}$ M was used. Preliminary pharmacodynamic study suggested that $MC_1$ is a potential new antimalarial drug that should be studied further.


*Keywords:* Chloroquine; Chronotropic effect; Stereotypic activity

## 1. Introduction

Resistance to antimalarial drugs has become a major problem. Chloroquine used to be very effective in curing all forms of malaria, with few side effects. Unfortunately, most strains of *falciparum* malaria are now resistant to chloroquine. Mefloquine was first introduced in 1971. This quinoline methanol derivative is related structurally to quinine which is used as an antiarrhythmic drug. Mefloquine was effective against malaria which was resistant to other forms of treatment when first introduced, but widespread resistance has now developed in southeast Asia. Furthermore,

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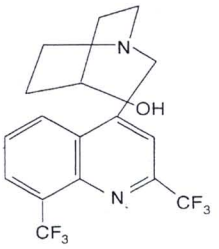
doi:10.1016/j.actatropica.2003.10.007

side effects of currently useful antimalarial drugs have been reported, for example, asymptomatic sinus bradycardia and sinus arrhythmia have been reported in malarial patients receiving mefloquine (Laothavorn et al., 1992). These cardiovascular side effects become of greater concern when it is anticipated that higher doses of mefloquine may be needed for resistant strains of *Plasmodium falciparum*. In addition, it was reported that insomnia was more commonly encountered during use of mefloquine than proguanil (Van Riemsdijk et al., 1997). Neuropsychiatric effects of mefloquine occurred more frequently in females than males; these effects were more common in first-time users than in individuals who had used mefloquine before (Van Riemsdijk et al., 2002). Recently, there have been reports about the potential neurotoxicity of artemisinin derivatives in experimental animals (Nontprasert et al., 1998, 2000, 2002a,b). However, the focal damage to brain stem nuclei involving auditory processing could not be detected in patients treated previously with artemether or artesunate (Van Vugt et al., 2000); artemisinin or artesunate (Kissinger et al., 2000). The problems of drug resistance together with the emergence of cardiovascular and neurological side effects of presently available antimalarial drugs indicates that new and more effective drugs are needed.

Preliminary study in our laboratories revealed that $MC_1$ is a new and potent synthetic antimalarial in vitro and in vivo rodent malaria model. Since its chemical structure shares a common structure with mefloquine (Fig. 1), in our preliminary pharmacological study, we studied particularly its cardiovascular and neurological effects.




$(C_{18}H_{16}F_6N_2O)$

Fig. 1. 2,8-bis(Trifluoromethyl)-4-[3-hydroxyquinuclidinyl]quinoline ($MC_1$).

## 2. Materials and methods

### 2.1. *In vitro: antimalarial activity of $MC_1$ on Plasmodium falciparum ($K_1$)*

#### 2.1.1. *Cell culture*

Human erythrocytes (type O) were infected with T9/94 (chloroquine resistant), of *P. falciparum* maintained in continuous culture, following the method described by Trager and Jensen (1976). RPMI 1640 culture medium (Gibco, USA) supplemented with 25 mM of HEPES (Sigma, USA), 40 mg/l gentamicin sulfate (Government Pharmaceutical Organization, Thailand) and 10% human serum was used in continuous culture.

Before starting the experiment, *P. falciparum* culture was synchronized by sorbitol hemolysis (Lambros and Vanderberg, 1979) to get only ring-infected cells and then incubated for 48 h to avoid the effect of sorbitol. Parasitemia was estimated from methanol-fixed Geimsa stain smears.

#### 2.1.2. *Drug sensitivity test*

The experiments were started with synchronized suspensions of ring-infected red blood cells (0.5–1% parasitemia). Parasitized red blood cells were suspended with culture medium supplemented with 15% human serum to get 10% cell suspension. The parasite suspension was transferred into a 96 well microtitrator plate with 50 μl in each well that already contained 50 μl of drug sample. After that, the experimental plate was incubated in an atmosphere of 5% $CO_2$, 94% $N_2$, and 1% $O_2$ at 37 °C for 48 h. The percent of parasitemia in each well was examined using microscopy and methanol-fixed Geimsa stained thin smears.

### 2.2. *In vivo: effect of $MC_1$ on survival time in mice infected with P. berghei*

Each mouse was infected with *Plasmodium berghei* intraperitoneally, 50 μl of infected blood containing approximately $4 \times 10^6$ parasitized erythrocytes and then divided into five groups.

1. Control, distilled water.
2. 7.5 mg/kg BW: chloroquine dissolved in water.
3. Control 10% DMSO.
4. 7.5 mg/kg BW: $MC_1$ dissolved in 10% DMSO.
5. 15 mg/kg BW: $MC_1$ dissolved in 10% DMSO.

All drugs and solvents were administered orally, once daily starting on day 3 (*P. berghei* infected day) till day 6.

Cumulative death, mean survival time, and percentage parasitemia were determined.

### 2.3. Preliminary study of the effects of $MC_1$ on selected pharmacological activities

#### 2.3.1. Effect of $MC_1$ on motor activities

Male Swiss albino mice, weight 27–32 g were used in this experiment. Animals were divided into three groups ($n = 5$): control (distilled water, 10% DMSO (0.1 ml/10 g BW), $MC_1$ 7.5 mg/kg BW. The animals were treated orally, once daily for a period of 4 days.

The motor activities were measured 3 h after treatment. The duration of measurement was 10 min for each animal using a Columbus Animal Activities Cage, Opto-Varimex TM (Columbus Instruments, USA) and autotract system. The methods were the same as reported by Satayavivad et al. (1997).

Mice were acclimatized individually in a transparent plastic cage (42 cm × 42 cm × 20 cm) for 10 min before experiments. The Opto-Varimex TM was equipped with four horizontal sensors in the surrounding base and two vertical sensors placed perpendicular to each other on the sides. Each sensor consisted of 15-infrared beam. Three major groups of motor activity; distance traveled, stereotypic, and rotational behaviors were recorded.

#### 2.3.2. Effect of $MC_1$ on serum glucose level

Three hours after treatment at day 5, each mouse was anesthesized with diethylether, and blood was collected by heart puncture. Serum was separated from blood by centrifugation at 12,000 × *g* for 30 min. Then the serum glucose level of each animal was determined by quantitative, enzymatic (glucose oxidase) determination method using Sigma diagnostic kit.

#### 2.3.3. Effect of $MC_1$ on spontaneously beating right artria

Male Swiss albino mice, weight 25–30 g were used in this study. They were anesthesized with diethylether. The isolated atria were incubated in Kreb's solution aerated with 95% $O_2$ and 5% $CO_2$ (the method was slightly modified from Sadavongvivad and Satayavivad, 1974).

The pH of Kreb's solution was kept between 7.4 and 7.5 and the temperature of the solution was maintained at 35 °C. Kreb's solution was also prepared as hypo- and hyper-$K^+$ solution; the concentration of $K^+$ in these solutions were 2 and 8 mM, respectively. These solutions were used to study the influence of extracellular $K^+$ concentration on the effect of 0.1 mM $MC_1$. The atria were preloaded with 1 g and the equilibrium time before starting experiment was 20 min.

## 3. Results

### 3.1. Antimalarial activity of $MC_1$ on Plasmodium falciparum ($K_1$)

The results showed that the $IC_{50}$ of $MC_1$ and chloroquine in in vitro culture of *P. falciparum* ($K_1$) are $7.0 \times 10^{-8}$ and $6.06 \times 10^{-7}$ M, respectively. Antimalarial activity of $MC_1$ is about 16.3 times more potent than chloroquine in chloroquine-resistant *P. falciparum* ($K_1$).

### 3.2. Effect of $MC_1$ on the survival time in mice infected with P. berghei

Chloroquine and $MC_1$ at an equal dose of 7.5 mg/kg prolonged the survival time of infected mice from 6 to 14 days, and more than 28 days, respectively. These results correlated well with percentage parasitemia as shown in Fig. 2. At the dose that exhibited potent antimalarial activity in vivo, there were no observable toxic effects in the infected mice.

### 3.3. Effect of $MC_1$ on motor activity

At the effective dose of $MC_1$ which exhibited antimalarial activity (7.5 mg/kg) a slight reduction in distance traveled was observed in days 1, 2, 3, and 4 but this reduction was not statistically significant from control group exception day 1. The stereotypic time was also reduced and was statistically significant from control group in day 2. However, these two parameters were not statistically significant from the10% DMSO group. This suggests that the observed effect of $MC_1$

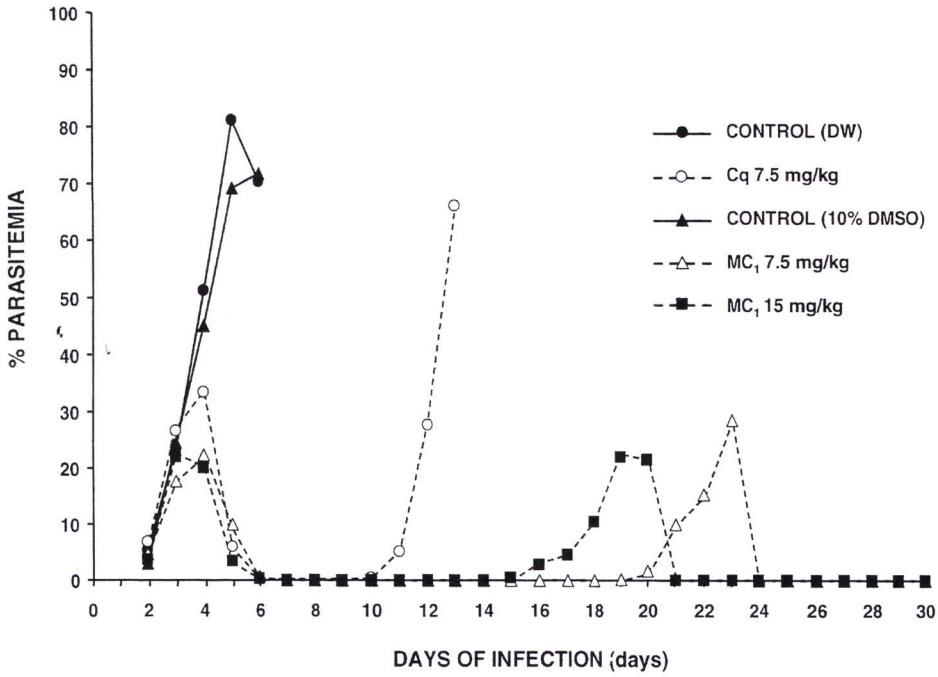



Fig. 2. Effect of $MC_1$ on percentage parasitemia in *P. berghei* infected mice. Chloroquine and $MC_1$ were given orally on day 3, once daily for 4 days. Percent parasitemia was detected daily for 30 days.

was probably due to the effect of DMSO, which was used as the solvent (Fig. 3).

### 3.4. Effect of $MC_1$ on serum glucose level

The results showed that 10% DMSO increased serum glucose level significantly. $MC_1$ increased the serum glucose level but this increase is less than that observed when 10% DMSO was used alone (Table 1).

Table 1
Effects of $MC_1$ on serum glucose level

| Groups | Serum glucose (mg/dl) |
|---|---|
| Control | 338.826 ± 23.38 |
| 10% DMSO | 402.698 ± 26.38* |
| $MC_1$ (7.5 mg/kg) | 364.286 ± 29.47 |

The serum glucose levels were determined 3 h after the last $MC_1$ treatment on day 5. Serum glucose level of each animal was determined by quantitative, enzymatic (glucose oxidase) determination method using Sigma diagnostic kit.

* Statistically significant difference from control at $P < 0.05$.

### 3.5. Effect of $MC_1$ on spontaneously beating right atria

The effects of $MC_1$ on spontaneously beating right atria were studied in normal $K^+$ (5.8 mM), hyper-$K^+$ (8 mM) and hypo-$K^+$ (2 mM). The results showed that $MC_1$ exhibits direct negative chronotropism at high concentration ($10^{-4}$ M). This effect was augmented in hyper-$K^+$ condition (Fig. 4). The direct negative chronotropic effect was also observed when mefloquine at $5 \times 10^{-5}$ M was used.

## 4. Discussion

$MC_1$ is a new synthetic compound which exhibits more potent antimalarial activity than chloroquine both in in vitro (chloroquine-resistant *P. falciparum*) and in vivo (*P. berghei*) rodent model. A part of its chemical structure resembles mefloquine; therefore, in this preliminary pharmacodynamic study, attempts have been made to study pharmacological effects

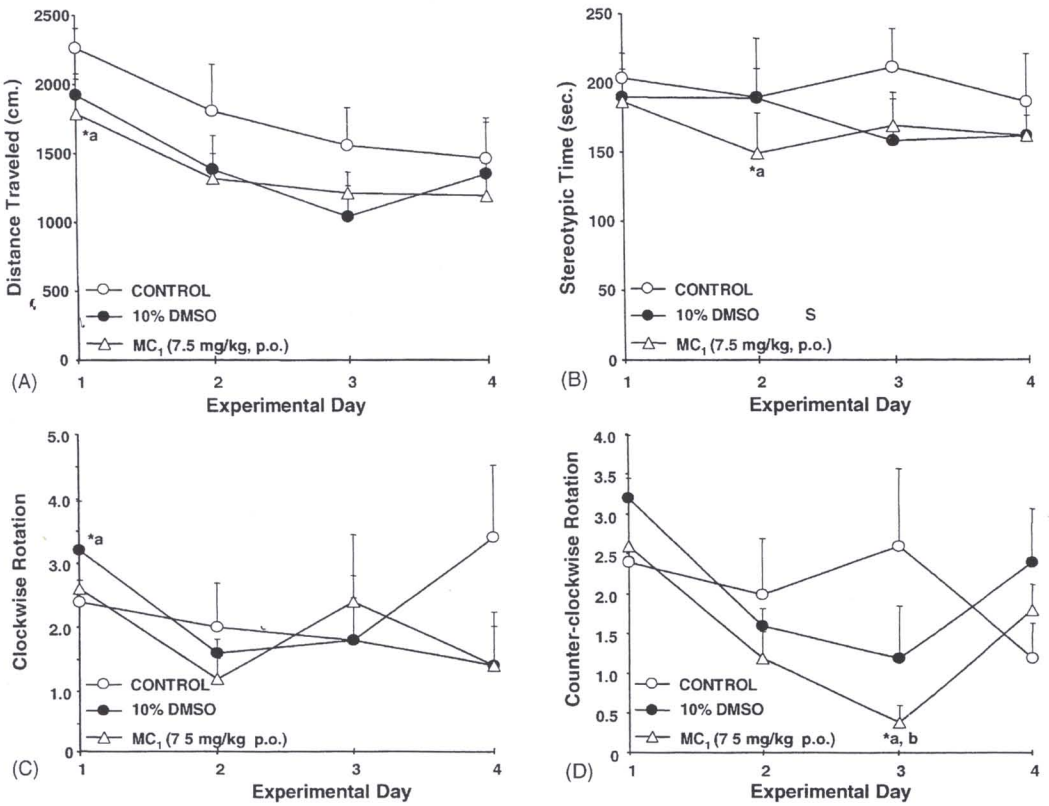



Fig. 3. Effects of $MC_1$ on motor activities in mice. $MC_1$ 7.5 mg/kg was administered orally for 4 days. Motor activities of mice were measured 3 h after drug administration. The observation time was 10 min on motor activities. (A) Distance traveled, (B) stereotypic time, (C) clockwise rotation, (D) counter-clockwise rotation. (*) Statistically significant difference at $P < 0.05$. (a) Represents a significant difference from control group and (b) represents a significant difference from 10% DMSO group.

which are related to the undesirable side effects of mefloquine. Neurologic and psychiatric adverse effects were reported in association with mefloquine prophylaxis (Weinke et al., 1991; Bem et al., 1992). The precise mechanism of serious neurologic and psychiatric reactions is unknown. Patients with a history of seizures or manic-depressive illness had an increased risk of these adverse reactions.

In this study, the effect of $MC_1$ at the dose exhibited potent antimalarial activity had been studied to detect neurological adverse effects of $MC_1$. Three major motor activities, distance traveled, stereotypic and rotational behaviors were observed. Hyperactive test animals will travel (move horizontally from one place to the other) more than normal animals. Animals treated with central nervous system depressant drugs or drugs producing muscle weakness will not move freely. $MC_1$ produced a significant reduction in distance traveled when compared with the day 1 control group receiving distilled water; however, it was not significantly different from the DMSO-treated group. The stereotypic time of the $MC_1$-treated group was significantly decreased when compared with control group on day 2; however, it was not significantly different from the DMSO-treated group. These results suggest that the effects of $MC_1$ on these two parameters of motor activity is probably due to the effect of DMSO which was used as the solvent to dissolve $MC_1$.

$MC_1$ produced a significant reduction in counter-clockwise rotation in day 3. DMSO is a solvent used to dissolve $MC_1$, and in this study 10% solution was used. In a recent study, Cavaletti et al. (2000)

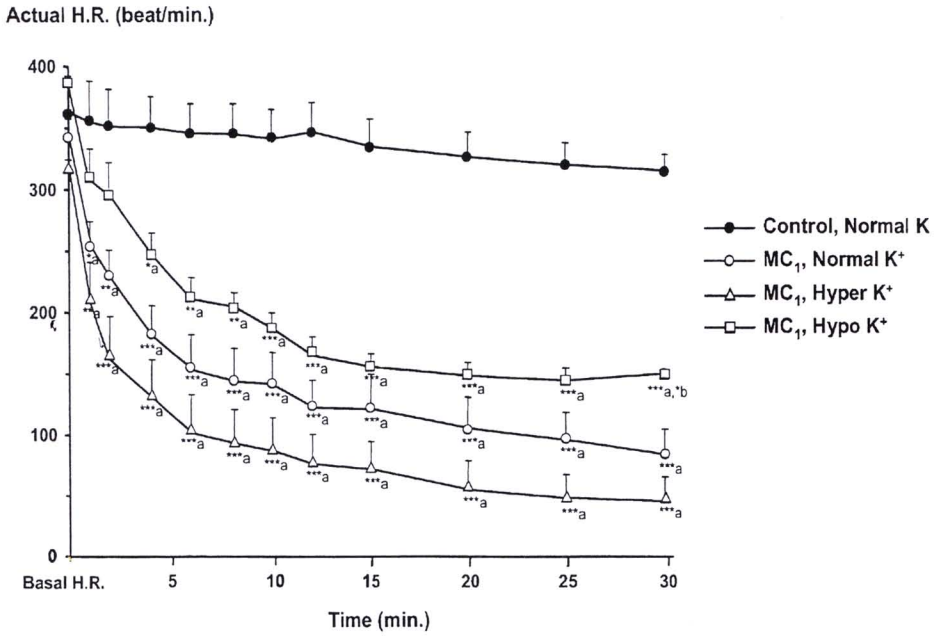



Fig. 4. The direct negative chronotropism of $MC_1$. $MC_1$ $10^{-4}$ M was added in the bathing solution. The rates of isolated spontaneously beating atria were recorded for 30 min there were three different concentrations of $K^+$ in the bathing physiological solutions (normal $K^+$ = 5.8 mM; hypo-$K^+$ = 2 mM and hyper-$K^+$ = 8 mM). (*, **, ***) Represent a statistically significant difference at $P < 0.05$, 0.01, 0.001. (a) Represents a significant difference from control normal $K^+$ group and (b) represents a significant difference from $MC_1$ normal $K^+$ group.

demonstrated that repeated intraperitoneal administration of DMSO in rats, at commonly used concentrations, produced a marked and dose-dependent reduction in nerve conduction velocity in rats. Authier et al. (2002) reported that repeated intraperitoneal administration of DMSO (1.8–7.2%) for 10 days showed no motor deficits in DMSO-treated rats. In this study, a higher concentration of DMSO (10%) administered orally for 4 days, and there was a trend to decrease motor activity; however, it was not significantly different from the control group receiving distilled water. Further study is needed to clarify whether DMSO could contribute to the observed transient neurotoxic effect of $MC_1$.

Quinine and mefloquine were reported to stimulate insulin release from pancreatic islets in vitro. Inhibition of the beta-cell ATP-sensitive potassium channels is suggested to be responsible for its increased insulin secretion leading to hypoglycemia (Gribbe et al., 2000). At the dose used, $MC_1$ did not produce hypoglycemia in the present study.

Mefloquine has been reported to produce sinus bradycardia. Our unpublished data showed that this bradycardia could not be antagonized by atropine, which is the antagonist of cardiac muscarinic receptor. High doses of $MC_1$ ($10^{-4}$ M) produce a significant reduction of spontaneously beating atria. This negative chronotropism of $MC_1$ is influenced by the external concentration of $K^+$. It was found that the negative chronotropism is augmented in hyper-$K^+$ condition and is attenuated in hypo-$K^+$ conditions. These results suggest the involvement of $K^+$ channels in mediating the direct negative chronotropism of $MC_1$.

The results from this study revealed that $MC_1$ at high doses exhibited a direct negative chronotropism in isolated spontaneously beating atria. However, this effect may not appear in intact animals at therapeutic doses. Further study is needed to detect its cardiovascular effects in malarial infected animals.

The neurological effects of $MC_1$ observed in this study seemed to be related to its solvent, DMSO.

In summary, $MC_1$ is a potent synthetic antimalarial compound. Preliminary pharmacological study revealed that at doses exhibiting antimalarial activity its effects on motor behaviors are not significant, the negative chronotropism was less than mefloqine, and the hypoglycemic effect was not observed. Further study is needed to observe its subchronic toxicity with special emphasis on the cardiovascular and neurological systems.

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# Aliphatic Alcohol and Iridoid Glycosides from *Asystasia intrusa*

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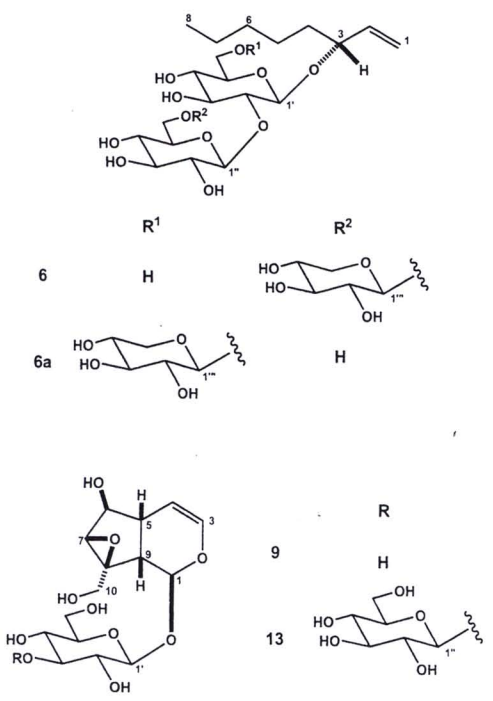
**An aliphatic alcohol glycoside (asystoside) and an iridoid diglucoside (3′-*O*-*β*-D-glucopyranosyl-catalpol) were isolated from the aerial part of *Asystasia intrusa* along with benzyl *β*-D-glucopyranoside, zizybeoside I, (6*S*,9*R*)-roseoside, verbascoside, ehrenoside, 6*β*-hydroxyantirrhide, angeloside, catalpol, ajugol, 6-deoxycatalpol, and scutellarioside II. The structural elucidations were based on analyses of physical and spectroscopic data.**

**Key words** *Asystasia intrusa*; Acanthaceae; aliphatic alcohol; iridoid diglucoside; asystoside

As part of our ongoing studies of Acanthaceous plants,[1—4] we investigated the constituents of *Asystasia intrusa* BLUME (Thai name: *Ya-Yaa*), collected from the Botanical Gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. The aerial part of this plant has been used as forage, althrough there is no mention of its medicinal uses in Thai traditional medicine. Phytochemical investigation has not been carried out in this species. Previous studies on plants in this genus reported iridoid glycosides from *Asystasia bella*.[5,6] The present study deals with the isolation and structural determinations of a new aliphatic glycoside (**6**) and a new iridoid diglucoside (**13**), together with 11 known compounds (**1**—**5**, **7**—**12**) from the aerial part of this plant.

## Results and Discussion

The methanolic extract was suspended in $H_2O$ and defatted with $Et_2O$. The aqueous layer was subjected to a Diaion HP-20 column, using $H_2O$, MeOH and $Me_2CO$ successively. The portion eluted with MeOH was repeatedly chromatographed on columns of silica gel, RP-18, and preparative HPLC-ODS to afford 13 compounds. Eleven were identified as the known compounds benzyl *β*-D-glucopyranoside (**1**),[7] zizybeoside I (**2**),[8] (6*S*,9*R*)-roseoside (**3**),[9] verbascoside (**4**),[10] ehrenoside (**5**),[11] 6*β*-hydroxyantirrhide (**7**),[12] angeloside (**8**),[13] catalpol (**9**),[14] ajugol (**10**),[15] 6-deoxycatalpol (**11**),[16] and scutellarioside II (**12**)[17] by comparison of physical data with values reported in the literature and from spectroscopic evidence.




The molecular formula of compound **6** was determined to be $C_{25}H_{44}O_{15}$ by negative high-resolution (HR)-FAB mass spectrometry. The $^1$H- and $^{13}$C-NMR spectra revealed the presence of three sugar units from the anomeric proton signals at $\delta$ 4.32 (d, $J$=7.6 Hz), 4.43 (d, $J$=7.6 Hz), and 4.62 (d, $J$=7.8 Hz), and from the carbon signals at $\delta$ 101.8, 104.9, and 105.3. Acid hydrolysis gave D-xylose and D-glucose, identified by TLC and comparison of the optical rotation with that of authentic samples. The negative FAB-MS exhibited the characteristic fragment ions of a linear sugar chain at *m/z* 451 [M−pentose]$^-$ and 289 [M−pentose−hexose]$^-$, indicating that xylose is a terminal sugar connected to an inner glucose. The distortionless enhancement by polarization transfer (DEPT) experiments indicated the presence of one methyl ($\delta$ 14.5), five methylenes ($\delta$ 23.7, 25.6, 33.1, 35.8, 116.9), as well as two methines ($\delta$ 83.7, 140.6) of the aglycone moiety, which could be assigned to (3*R*)-1-octen-3-ol (matsutake alcohol) by comparing the spectral data with those in the literature.[2,18] The chemical shifts of compound **6** were almost the same as those of ebracteatoside B (**6a**), previously isolated from *Acanthus ebracteatus*,[2] except for the difference in the chemical shifts of the sugar chain (Table 1). The sugar moiety was identified as a *β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl unit by heteronuclear multiple-bond connectivity (HMBC), in which long-range correlations were found between i) H-1‴ ($\delta$ 4.32, d, $J$=7.6 Hz) and C-6″ ($\delta$ 69.5), and C-5‴ ($\delta$ 66.8); ii) H-1″ ($\delta$ 4.62, d, $J$=7.8 Hz) and C-2′ ($\delta$ 82.4); and iii) H-1′ ($\delta$ 4.43, d, $J$=7.6 Hz) and C-3 ($\delta$ 83.7) and C-2′ ($\delta$ 82.4), as shown in Fig. 1. Consequently, the structure of compound **6** was concluded to be (3*R*)-1-octen-3-ol-3-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, namely asystoside.

The aliphatic alcohol glycosides, which have the aglycone (3*R*)-1-octen-3-ol or its derivatives, are rarely found from plant sources. Only a few studies identified (3*R*)-1-octen-3-

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Table 1. $^{13}$C-NMR Spectral Data of **6a** and **6** (100 MHz, $CD_3OD$)

| No. | 6a | 6 |
|---|---|---|
| Aglycone | | |
| 1 | 116.9 | 116.9 |
| 2 | 140.5 | 140.6 |
| 3 | 83.8 | 83.7 |
| 4 | 35.7 | 35.8 |
| 5 | 25.5 | 25.6 |
| 6 | 32.9 | 33.1 |
| 7 | 23.6 | 23.7 |
| 8 | 14.5 | 14.5 |
| Glc-1′ | 101.7 | 101.8 |
| 2′ | 82.3 | 82.4 |
| 3′ | 77.5[a)] | 77.6[a)] |
| 4′ | 71.0 | 71.1[b)] |
| 5′ | 77.5[a)] | 78.2[a)] |
| 6′ | 69.3 | 62.9 |
| Glc-1″ | 104.8 | 104.9 |
| 2″ | 75.9 | 76.1 |
| 3″ | 77.8[a)] | 78.0[a)] |
| 4″ | 71.4 | 71.1[b)] |
| 5″ | 78.0[a)] | 76.8 |
| 6″ | 62.7 | 69.5 |
| Xyl-1‴ | 105.1 | 105.3 |
| 2‴ | 74.7 | 74.8 |
| 3‴ | 77.5[a)] | 77.7[a)] |
| 4‴ | 71.4 | 71.6[b)] |
| 5‴ | 66.7 | 66.8 |

*a, b*) Assignments may be interchanged in each column.

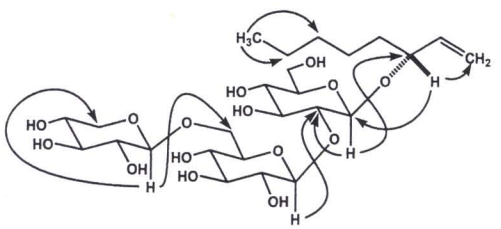


Fig. 1. Significant HMBC Correlations of Compound **6**

ol-3-*O*-$\beta$-D-glucopyranoside from *Mentha spicata*,[18] (3*R*)-1-octen-3-ol-3-*O*-$\beta$-D-xylopyranosyl-(1→6)-$\beta$-D-glucopyranoside from *M. spicata*,[18] *Barleria lupulina*,[1] and *Barleria strigosa*,[4] ebracteatosides B—D from *Acanthus ebracteatus*,[2] and ilicifolioside B from *Acanthus ilicifolius*.[19]

The molecular formula of compound **13** was determined to be $C_{21}H_{32}O_{15}$ by negative HR-FAB mass spectrometry. Analysis of the $^{13}$C-NMR spectral data revealed the presence of two $\beta$-glucopyranosyl units in addition to nine carbon signals in the aglycone moiety. Acid hydrolysis provided D-glucose, identified by TLC and comparison of the optical rotation with that of an authentic sample. DEPT experiments indicated that compound **13** contains one methylene ($\delta$ 61.4), seven methines ($\delta$ 39.0, 43.5, 62.5, 79.5, 95.3, 104.0, 141.7), and one quaternary carbon ($\delta$ 66.2) in the aglycone part, corresponding to an iridoid. The chemical shift at $\delta$ 95.3 was characteristic of an acetal group at C-1. The methine signals at $\delta$ 141.7 and 104.0 were assigned to a disubstituted olefin group at C-3 and C-4. The chemical shifts at $\delta$ 62.5 and 66.2 belonged to an epoxyl group on C-7 and C-8 of the cyclopentanopyran ring. The $^{13}$C-NMR spectral data were very similar to those of catalpol (**9**) except that the signals of one more $\beta$-D-glucopyranosyl unit were observed (Table 2). This additional unit was located at C-3′ since the chemical shifts of C-3′, C-2′, and C-4′ were changed by +8.4, −0.7, and −1.7, respectively. The chemical shifts of the sugar moiety were also in agreement with the reported data for the $\beta$-D-glucopyranosyl-(1→3)-$\beta$-D-glucopyranosyl unit.[3] Therefore the structure of compound **13** was elucidated to be 3′-*O*-$\beta$-D-glucopyranosyl-catalpol.

Table 2. $^{13}$C-NMR Spectral Data of **9** and **13** (100 MHz, $CD_3OD$)

| No. | 9 | 13 |
|---|---|---|
| Aglycone | | |
| 1 | 95.3 | 95.3 |
| 3 | 141.8 | 141.7 |
| 4 | 104.0 | 104.0 |
| 5 | 39.1 | 39.0 |
| 6 | 79.6 | 79.5 |
| 7 | 62.6 | 62.5 |
| 8 | 66.2 | 66.2 |
| 9 | 43.6 | 43.5 |
| 10 | 61.7 | 61.4 |
| Glc-1′ | 99.7 | 99.4 |
| 2′ | 74.9 | 74.2 |
| 3′ | 78.7 | 87.1 |
| 4′ | 71.8 | 70.1 |
| 5′ | 77.7 | 77.7[a)] |
| 6′ | 63.0 | 62.7[b)] |
| Glc-1″ | | 105.1 |
| 2″ | | 75.5 |
| 3″ | | 78.0[a)] |
| 4″ | | 71.5 |
| 5″ | | 78.1[a)] |
| 6″ | | 62.6[b)] |

*a, b*) Assignments may be interchanged in each column.

The biological activities of the isolated compounds have not been investigated. However, the biological and pharmacologiced activities of phenylethanoids and naturally occurring iridoids have been reviewed.[20,21] Further investigations of the isolated compounds are in progress.

## Experimental

**General Procedures** NMR spectra were recorded in $CD_3OD$ using a JEOL JNM $\alpha$-400 spectrometer (400 MHz for $^1$H-NMR and 100 MHz for $^{13}$C-NMR). MS values were obtained on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a union PM-1 digital polarimeter. For column chromatography, silica gel 60 (70—230 mesh, GE0049, Scharlau Chemie S. A.), RP-18 (50 $\mu$m, YMC), and Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.) were used. Preparative HPLC was carried out on an ODS column (150×20 mm i.d., YMC) with a Shimadzu RID-6A refractive index detector. The flow rate was 6 ml/min. The solvent systems were: I) EtOAc–MeOH (9:1); II) EtOAc–MeOH–$H_2O$ (40:10:1); III) EtOAc–MeOH–$H_2O$ (70:30:3); IV) 10—50% aqueous MeOH; V) 2.5% aqueous MeCN; VI) 4% aqueous MeCN; VII) 5% aqueous MeCN; VIII) 10% aqueous MeCN; IX) 15% aqueous MeCN; and X) 20% aqueous MeCN. The spraying reagent used for TLC was 10% $H_2SO_4$ in 50% EtOH.

**Plant Material** *A. intrusa* BLUME was cultivated and collected in August 2003 from the Botanical Garden, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand. The plant was identified by Mr. Bamrung Thavinchiua, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher sample (KKU 0046) is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

**Extraction and Isolation** Dried whole *A. intrusa* (1.3 kg) was extracted three times with hot MeOH (5 l for each extraction, under reflux). The solvent was concentrated *in vacuo* to give a greenish powder (143.3 g). This residue was suspended in $H_2O$ and defatted with $Et_2O$ three times (1 l each). The aqueous layer was applied to a column of Diaion HP-20 and eluted with

$H_2O$, MeOH, and $Me_2CO$ successively. The fraction eluted with MeOH (20.2 g) was concentrated to dryness and subjected to a silica gel column using solvent systems I, II, and III. Six fractions were collected. Fraction 2 (2.3 g) was applied to a column of RP-18 using solvent system IV to give 10 fractions. Fractions 2-3 and 2-4 were combined and purified by preparative HPLC-ODS with solvent system VIII to afford compounds **1** (34 mg) and **3** (97 mg). Fraction 2-5 was subjected to preparative HPLC-ODS with solvent system IX to give compound **12** (41 mg). Fraction 2-6 was purified by HPLC-ODS with solvent system X to provide compound **4** (73 mg). Fraction 3 (4.2 g) was subjected to a column of RP-18 using solvent system IV, affording nine fractions. Fraction 3-1 was purified by preparative HPLC-ODS with solvent system V to give compounds **7** (32 mg), **8** (49 mg), and **9** (124 mg). Fraction 3-2 was further purified by preparative HPLC-ODS with solvent system VI to provide compound **10** (124 mg). Fraction 3-3 was purified by preparative HPLC-ODS with solvent system VII to obtain compound **11** (23 mg). Fraction 3-4 was subjected to preparative HPLC-ODS with solvent system VIII to afford compound **2** (52 mg), and fraction 3-7 was purified by preparative HPLC-ODS with solvent system IX to provide compound **5** (108 mg). Similarly, fraction 4 (3.9 g) was separated on a column of RP-18 using solvent system IV to give 12 fractions. Fraction 4-1 was purified by preparative HPLC-ODS with solvent system V to obtain compound **13** (55 mg). Finally, fraction 4-8 was purified by preparative HPLC-ODS using solvent system X to provide compound **6** (38 mg).

Compound **6:** Amorphous powder, $[\alpha]_D^{27}$ +7.7° ($c$=1.56, MeOH); $^1$H-NMR ($CD_3OD$): $\delta$:5.86 (1H, ddd, $J$=17.1, 10.2, 7.3 Hz, H-2), 5.22 (1H, br d, $J$=17.1 Hz, H-1), 5.13 (1H, br d, $J$=10.2 Hz, H-1), 4.62 (1H, d, $J$=7.8 Hz, H-1″ Glc), 4.43 (1H, d, $J$=7.6 Hz, H-1′ Glc), 4.32 (1H, d, $J$=7.6 Hz, H-1‴ Xyl), 4.12 (1H, dd, $J$=13.4, 6.8 Hz, H-3), 1.66 (1H, *m*, H-4), 1.49 (1H, *m*, H-4), 1.22—1.39 (6H, *m*, H-5, 6, 7), 0.90 (3H, t, $J$=6.8 Hz, H-8); $^{13}$C-NMR ($CD_3OD$): Table 1. Negative FAB-MS *m/z* 583 $[M-H]^-$, 451 $[M-Xyl]^-$, 289 $[M-Xyl-Glc]^-$. Negative HR-FAB-MS, *m/z*: 583.2589 ($C_{25}H_{43}O_{15}$ required 583.2601).

Compound **13**: Amorphous powder, $[\alpha]_D^{27}$ −74.9° ($c$=3.41, MeOH); $^1$H-NMR ($CD_3OD$): $\delta$: 6.34 (1H, d, $J$=5.4Hz, H-3), 5.07 (1H, dd, $J$=5.4, 4.6 Hz, H-4), 5.02 (1H, d, $J$=9.8 Hz, H-1), 4.77 (1H, d, $J$=7.8 Hz, H-1′ Glc), 4.59 (d, $J$=7.8 Hz, H-1″ Glc), 4.11 (1H, d, $J$=13.1 Hz, H-10a), 3.92 (1H, br d, $J$=8.0 Hz, H-6), 3.80 (1H, d, $J$=13.1 Hz, H-10b), 3.45 (1H, br s, H-7), 2.53 (1H, dd, $J$=9.8, 8.3 Hz, H-9), 2.27 (1H, m, H-5); $^{13}$C-NMR ($CD_3OD$): Table 2. Negative HR-FAB-MS, *m/z*: 523.1677 ($C_{21}H_{31}O_{15}$ required 523.1663).

**Acid Hydrolysis of Compounds 6 and 13** Compound **6** (25 mg) was dissolved in 5% HCl and heated at 90°C for 2 h. After cooling, the reaction mixture was extracted with $Et_2O$. The aqueous layer was neutralized with saturated $NaHCO_3$ and concentrated to dryness. The residue was applied to a silica gel column, using solvent system II, to obtain D-xylose (4 mg, $[\alpha]_D^{27}$ +20.0°) and D-glucose (7 mg, $[\alpha]_D^{27}$+49.5°) in comparison with authentic samples. By the same method, compound **13** (22 mg) gave D-glucose (5 mg, $[\alpha]_D^{27}$+51.2°).

**Acknowledgments** This study was financially supported by a Grant-in-Aid for Scientific Research (No. MRG-4680108) from the Thailand Research Fund and Ministry of University Affairs. We also thank Mr. Bunlert Khamya of the Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, for help in obtaining the plant material.

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# Triterpenoidal glycosides from *Justicia betonica*

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Received 15 January 2004; received in revised form 16 April 2004
Available online 31 July 2004

**Abstract**

From the aerial portion of *Justicia betonica* L., four triterpenoidal glycosides (justiciosides A–D) were isolated. Their structures were established through chemical and NMR spectroscopic analyses as olean-12-ene-1β,3β,11α,28-tetraol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, olean-12-ene-1β,3β,11α,28-tetraol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, 11α-methoxy-olean-12-ene-1β,3β,28-triol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, 11α-methoxy-olean-12-ene-1β,3β,28-triol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, respectively.


*Keywords:* *Justicia betonica*; Acanthaceae; Justiciosides A–D; Triterpenoidal glycoside; Olean-12-ene-1β; 3β; 11α; 28-tetraol; 11α-Methoxy-olean-12-ene-1β, 3β; 28-triol

## 1. Introduction

*Justicia betonica* L. (Acanthaceae; Thai name: Tri-Cha-Va, Hang-Kra-Rok) is an ornamental plant, commonly grown in Northeast of Thailand, but for which there is no ethnopharmacological use in Thai traditional medicine. However, its aerial parts are used in Indian traditional medicine as an anti-diarrhea medicine as well as an anti-inflammatory agent. Preliminary studies on plants in the genus *Justicia* have led to the isolation of several compounds such as lignans (Okigawa et al., 1970; Ghosal et al., 1979, 1980; Trujillo et al., 1990; Asano et al., 1996; Rajasekhar et al., 1998; Rajasekhar and Subbaraju, 2000) and an amide (Lorenz et al., 1999). However, there have been no reports on triterpenoidal glycosides of *Justicia* species, although a number of triterpenoidal glycosides were isolated from other genera in the family Acanthaceae. In our continuing studies on the chemical constituents of Acanthaceous plants (Kanchanapoom et al., 2001, 2002), we isolated four new triterpenoidal glycosides, justiciosides A–D (**1**–**4**, Scheme 1), from the aerial portion of this plant. The present paper deals with the isolation and structural elucidation of these compounds.

## 2. Results and discussion

Justicioside A (**1**) was obtained as an amorphous powder and determined as $C_{42}H_{70}O_{14}$ by HR-FAB mass spectrometry. Inspection of the $^{13}C$ NMR spectral data revealed the presence of two sugar units (anomeric carbons at $\delta$ 103.5 and 105.9) in addition to 30 carbon signals for the aglycone moiety. The appearance of seven tertiary methyl groups ($\delta$ 0.79, 0.80, 1.01, 1.05, 1.21,

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doi:10.1016/j.phytochem.2004.06.029

1.26 and 1.33) and one trisubstituted olefinic proton ($\delta$ 5.51) of the aglycone moiety in the $^1$H NMR spectrum, together with information from the $^{13}$C NMR spectrum (seven sp$^3$ carbon at $\delta$ 13.6, 16.0, 18.2, 23.7, 25.7, 28.6 and 33.2, and two sp$^2$ olefinic carbons at $\delta$ 126.1 and 147.0) indicated that the aglycone has an olean-12-ene skeleton (Doddrell et al., 1974). Enzymatic hydrolysis of **1** with crude hesperidinase afforded a new aglycone (**1a**) with a molecular formula $C_{30}H_{50}O_4$, and D-glucose which was identified by TLC and comparison of its optical rotation with an authentic sample. The structure of **1a** was established by analyzing the 1D- and 2D-NMR spectra (including HSQC and HMBC) in addition to the coupling constants in the $^1$H NMR spectrum. In the $^1$H NMR spectrum of **1a**, the signals due to seven methyls, three oxymethine protons, two oxymethylene protons, and one olefinic proton were clearly observed. The $^{13}$C NMR spectrum coupled with the DEPT experiments indicated the presence of seven methyls, nine methylenes, seven methines and seven quaternary carbons. All protonated carbons were determined by HSQC spectral analyses (Table 1). The oxymethylene protons at $\delta$ 3.56 and 3.81 (each *d*, $J$=10.7 Hz) were assigned to H-28 on the basis of correlations with C-16 ($\delta$ 22.7), C-17 ($\delta$ 37.3) and C-18 ($\delta$ 41.8) in the HMBC spectrum (Fig. 1). The oxymethine proton at $\delta$ 3.94, which showed long-range correlations to C-25 ($\delta$ 13.6) and C-9 ($\delta$ 57.3) could be assigned to H-1. The proton signal at $\delta$ 3.58 was diagnostic for H-3, deducing from an HMBC correlation between H-23 ($\delta$ 1.24), H-24 ($\delta$ 1.08) and C-3 ($\delta$ 75.4). The methine proton signal at $\delta$ 4.56, which had the significant correlations to C-9 ($\delta$ 57.3), C-12 ($\delta$ 126.1) and C-13 ($\delta$ 147.4), was assigned to H-11. The occurrence of H-1 as a doublet of doublets having coupling constants with H-2$_{ax}$ ($J$=11.5 Hz) and H-2$_{eq}$ ($J$=4.4 Hz), indicated that H-1 was in the axial position; this in turn suggested a β-configuration of the hydroxyl group at C-1. For the same reason, the hydroxyl group at C-3 was also determined to have a β-configuration, this being supported by the splitting pattern of H-3 (*dd*, $J$=12.9, 4.4 Hz). The hydroxyl group at C-11 was concluded to be in the α-configuration from the coupling constant between H-11 ($\delta$ 4.56) and H-9 ($\delta$ 2.05) with $J$=8.1 Hz, and also from H-11 ($\delta$ 4.56) and H-12 ($\delta$ 5.58) with $J$=3.7 Hz (Calis et al., 1993). Therefore, structure **1a** was assigned as olean-12-ene-1β,3β,11α,28-tetraol. Comparison of the $^{13}$C NMR spectral data of **1** with those of **1a** revealed glycosylation shifts for C-28 ($\Delta\delta$+7.8) and C-17 ($\Delta\delta$−0.6) on going from **1a** to **1**, demonstrating that the sugar moiety was linked to C-28. The sugar sequence was identified to be a β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl unit by comparison of the chemical shifts with that of reported data (Kasai et al., 1988). Moreover, negative FAB-MS of **1** exhibited significant fragment ions at *m/z* 635 [M–162]$^-$ and 473 [M–162–162]$^-$. Consequently, structure **1** was olean-12-ene-1β,3β,11α,28-tetraol28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Table 1
$^1$H and $^{13}$C NMR spectral data for aglycone (**1a**, in $C_5D_5N$)

| Position | DEPT | $\delta_C$ | $\delta_H$ |
|---|---|---|---|
| 1 | CH | 77.7 | 3.94 (1H, *dd*, $J$=11.5, 4.4 Hz) |
| 2 | $CH_2$ | 37.3 | 2.40 (1H, *ddd*, $J$=12.9, 4.4, 4.4 Hz) |
| | | | 2.34 (1H, *m*) |
| 3 | CH | 75.4 | 3.58 (1H, *dd*, $J$=12.9, 4.4 Hz) |
| 4 | C | 39.6 | |
| 5 | CH | 52.9 | 0.86 (1H, *m*) |
| 6 | $CH_2$ | 18.3 | 1.65 (1H, *m*) |
| | | | 1.55 (1H, *m*) |
| 7 | $CH_2$ | 31.7 | 1.94 (1H, *m*) |
| | | | 1.65 (1H, *m*) |
| 8 | C | 41.9 | |
| 9 | CH | 57.3 | 2.05 (1H, *d*, $J$=8.1 Hz) |
| 10 | C | 44.8 | |
| 11 | CH | 66.3 | 4.56 (1H, *dd*, $J$=8.1, 3.7 Hz) |
| 12 | CH | 126.1 | 5.58 (1H, *d*, $J$=3.7 Hz) |
| 13 | C | 147.4 | |
| 14 | C | 44.1 | |
| 15 | $CH_2$ | 26.3 | 1.88 (1H, *m*) |
| | | | 1.01 (1H, *m*) |
| 16 | $CH_2$ | 22.7 | 1.99 (1H, *m*) |
| | | | 1.51 (1H, *m*) |
| 17 | C | 37.3 | |
| 18 | CH | 41.8 | 2.31 (1H, *br d*, $J$=12.4 Hz) |
| 19 | $CH_2$ | 46.4 | 1.75 (1H, *dd*, $J$=13.6, 12.4 Hz) |
| | | | 1.14 (1H, *dd*, $J$=13.6, 4.2 Hz) |
| 20 | C | 31.1 | |
| 21 | $CH_2$ | 34.5 | 1.44 (1H, *m*) |
| | | | 1.22 (1H, *m*) |
| 22 | $CH_2$ | 33.4 | 1.53 (1H, *m*) |
| | | | 1.22 (1H, *m*) |
| 23 | $CH_3$ | 28.8 | 1.24 (3H, *s*) |
| 24 | $CH_3$ | 16.0 | 1.08 (3H, *s*) |
| 25 | $CH_3$ | 13.6 | 1.29 (3H, *s*) |
| 26 | $CH_3$ | 18.2 | 1.04 (3H, *s*) |
| 27 | $CH_3$ | 25.8 | 1.39 (3H, *s*) |
| 28 | $CH_2$ | 68.7 | 3.81 (1H, *d*, $J$=10.7 Hz) |
| | | | 3.56 (1H, *d*, $J$=10.7 Hz) |
| 29 | $CH_3$ | 33.2 | 0.83 (3H, *s*) |
| 30 | $CH_3$ | 23.6 | 0.88 (3H, *s*) |

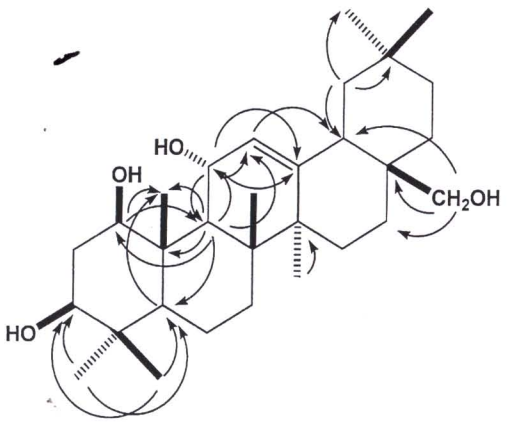
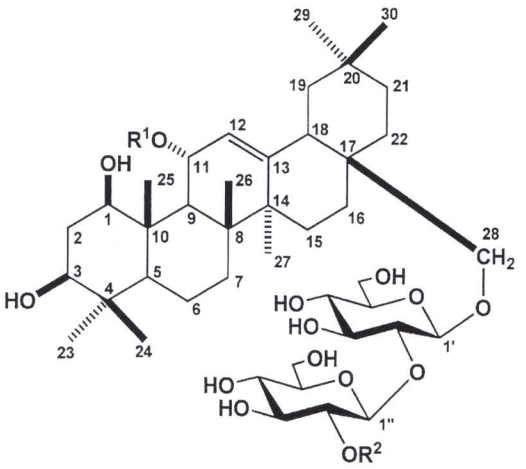



Fig. 1. Significant HMBC correlations for aglycone (**1a**).

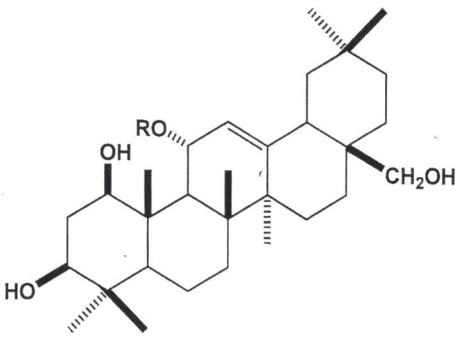
Justicioside B (**2**) was isolated as an amorphous powder. Its molecular formula was determined as $C_{48}H_{80}O_{19}$ by HR-FAB mass spectrometric analyses. The $^1H$ and $^{13}C$ NMR spectra showed the presence of three sugar units from the anomeric proton signals at $\delta$ 4.48 (*d*, $J$=7.6 Hz), 5.27 (*d*, $J$=7.6 Hz) and 5.43 (*d*, $J$=7.6 Hz), and from carbon signals at $\delta$ 103.0, 103.4 and 106.1. Enzymatic hydrolysis of **2** with crude hesperidinase gave **1a** and D-glucose. The negative FAB-MS displayed characteristic fragment ions of a linear sugar unit at *m/z* 797 $[M-162]^-$, 635 $[M-162-162]^-$, 473 $[M-162-162-162]^-$. The chemical shifts of **2** were closely related to those of justicioside A (**1**), except for a set of additional signals arising from a β-D-glucopyranosyl unit. This additional unit was assigned to be attached to C-2″ of the inner sugar because the chemical shifts of C-2″ ($\delta$ 85.3), C-1″ ($\delta$ 103.0) and C-3″ ($\delta$ 77.6) changed by +8.4, −2.9 and −0.6, respectively. Thus, **2** was elucidated as olean-12-ene-1β,3β,11α,28-tetraol 28-*O*-β-D-glucopyran osyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Justicioside C (**3**) was obtained as an amorphous powder. Its molecular formula, $C_{43}H_{72}O_{14}$, was determined by HR-FAB mass spectrometric analyses. Negative FAB-MS exhibited fragment ions at *m/z* 811 $[M-H]^-$, 649 $[M-162]^-$, 487 $[M-162-162]^-$. The $^1H$ and $^{13}C$ NMR spectral data revealed that **3** contains the same sugar moiety as justicioside A (**1**) with a different aglycone. Enzymatic hydrolysis of **3** provided D-glucose and an aglycone (**3a**) with a molecular formula $C_{31}H_{52}O_4$. The structure of **3a** was established by comparison of its chemical shifts to those of **1a**, in which the additional signal due to a methoxyl group was observed in the spectra ($\delta$ 3.27 in the $^1H$ NMR spectrum and $\delta$ 51.3 in the $^{13}C$ NMR spectrum). This additional group was located at C-11 of the aglycone since the chemical shifts of C-9, C-11, C-12 and C-13 were significantly changed by −7.1, +8.0, −4.2 and +4.6, respectively, when compared to **1a** (Calis et al., 1993). Thus, **3a** was concluded to be an 11α-methoxy derivative of **1a**. Accordingly, **3** was elucidated as 11α-methoxy-olean-12-ene-1β,3β,28-triol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Justicioside D (**4**) was obtained as an amorphous powder and determined as $C_{49}H_{82}O_{19}$ by HR-FAB mass spectrometric analyses. The $^1H$ and $^{13}C$ NMR spectra indicated the presence of three sugar units, one methoxyl group and signals for the aglycone moiety. The chemical shifts of the aglycone moiety were superimposable with those of **3**, as well as the chemical shifts of three sugar units were the same as those of **2**. Enzymatic hydrolysis of **4** afforded **3a** and D-glucose. Besides, negative




| | $R^1$ | $R^2$ |
|---|---|---|
| 1 | H | H |
| 2 | H | β-D-Glc |
| 3 | Me | H |
| 4 | Me | β-D-Glc |




| | R |
|---|---|
| 1a | H |
| 3a | Me |

Scheme 1.

FAB-MS showed the significant fragment ions at *m/z* 973 $[M-H]^-$, 811 $[M-162]^-$, 649 $[M-162-162]^-$, 487 $[M-162-162-162]^-$. Consequently, **4** was identified to 11α-methoxy-olean-12-ene-1β, 3β, 28-triol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (see Scheme 1).

## 3. Experimental

### 3.1. General

NMR spectra were recorded in $C_5D_5N$ using a JEOL JNM α-400 spectrometer (400 MHz for $^1H$ NMR and 100 MHz for $^{13}C$ NMR) with tetramethylsilane (TMS) as internal standard, whereas MS obtained using a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Union PM-1 digital polarimeter. For CC, silica gel G (Scharlau GE0049, 70-230 mesh ASTM), YMC-gel ODS (50 μm, YMC) and highly porous copolymer resin of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co. Ltd.) were used. HPLC (Waters 515 HPLC pump) was carried on a column of ODS (150×20 mm i.d., YMC) with a Shimadzu refractive index (RID-6A) detector.

### 3.2. Plant material

The aerial portion of *J. betonica* L. was collected from Kalasin Province, Thailand, in November, 2002, and identified by Mr. Bamrung Tavinchiua of the Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. A voucher sample (KKU-0045) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

### 3.3. Extraction and isolation

The dried aerial portion (1.8 kg) of *J. betonica* was extracted with hot EtOH-$H_2O$ (95:5,v/v) under reflux 4 times (each 8 l, 3 h, 70 °C). The EtOH extract was concentrated to dryness (256.6 g) and partitioned between $Et_2O$ and $H_2O$, with the aqueous soluble applied to a column of highly porous copolymer resin of styrene and divinylbenzene, and eluted with $H_2O$, MeOH–$H_2O$ (3:2), MeOH and $Me_2CO$, successively. The fraction eluted with MeOH (17.5 g) was applied to a silica gel column using solvent systems EtOAc–MeOH (9:1, 5 l), EtOAc–MeOH–$H_2O$ (40:10:1, 5 l) and EtOAc–MeOH–$H_2O$ (70:30:3, 3.5 l) to give ten fractions. Fraction 6 (2.9 g) was applied to a column of ODS using a gradient system [MeOH–$H_2O$ (2:3, 1 l) to MeOH (1 l)] to afford six fractions. Fraction 6-3 was purified by prep. HPLC-ODS (37% MeCN in $H_2O$) to provide compounds **1** (113 mg) and **3** (42 mg). Fraction 7 (3.8 g) was similarly applied to a column of ODS using a gradient system [MeOH–$H_2O$ (2:3, 1 l) to MeOH (1 l)] to give eight fractions. Fraction 7-2 was purified by prep. HPLC-ODS (33% MeCN in $H_2O$) to afford compound **2** (589 mg) and **4** (330 mg).

### 3.4. Justicioside A (1)

Amorphous powder, $[\alpha]_D^{27}$ – 1.9° (MeOH, *c* 2.65); $^1H$ NMR ($C_5D_5N$): aglycone moiety δ 5.51 (1H, *d*, *J*=3.0 Hz, H-12), 4.53 (1H, *dd*, *J*=8.1, 3.0 Hz, H-11), 3.80 (1H, *d*, *J*=9.3 Hz, H-28a), 3.71 (1H, *d*, *J*=9.3 Hz, H-28b), 3.55 (1H, *dd*, *J*=11.7, 3.9 Hz, H-3), 1.33 (3H, *s*, H-27), 1.26 (3H, *s*, H-25), 1.21 (3H, *s*, H-23), 1.05 (3H, *s*, H-26), 1.01 (3H, *s*, H-24), 0.80 (3H, *s*, H-30), 0.79 (3H, *s*, H-29), sugar moiety δ 5.37 (1H, *d*, *J*=7.6 Hz, H-1″), 4.89 (1H, *d*, *J*=7.3 Hz, H-1′); $^{13}C$ NMR ($C_5D_5N$): Table 3; Negative FAB-MS *m/z* 797 $[M-H]^-$, 635 $[M-Glc]^-$, 473 $[M-Glc-Glc]^-$; Negative HR-FAB-MS, *m/z*: 797.4681 $[M-H]^-$ (calcd for $C_{42}H_{69}O_{14}$, 797.4687).

### 3.5. Olean-12-ene-1β,3β,11α,28-tetraol (1a)

Amorphous powder, $[\alpha]_D^{27}$ + 37.5° (MeOH, *c* 0.13); $^1H$ and $^{13}C$ NMR ($CD_3OD$): Table 1; Negative HR-FAB-MS, *m/z* : 476.3639 $[M-H]^-$ (calcd for $C_{30}H_{49}O_4$, 473.3630).

### 3.6. Justicioside B (2)

Amorphous powder, $[\alpha]_D^{27}$ – 4.2° (MeOH, *c* 2.61); $^1H$ NMR ($C_5D_5N$): aglycone moiety δ 5.54 (1H, *d*, *J*=3.2 Hz, H-12), 4.51 (1H, *dd*, *J*=8.1, 3.2 Hz, H-11), 3.76 (1H, *d*, *J*=9.5 Hz, H-28a), 3.71 (1H, *d*, *J*=9.5 Hz, H-28b), 3.51 (1H, *dd*, *J*=11.7, 3.9 Hz, H-3), 2.15 (1H, *dd*, *J*=12.9, 2.9 Hz, H-18), 1.99 (1H, *d*, *J*=8.1 Hz, H-9), 1.33 (3H, *s*, H-27), 1.29 (3H, *s*, H-25), 1.15 (3H, *s*, H-23), 1.08 (3H, *s*, H-26), 1.02 (3H, *s*, H-24), 0.78 (3H, *s*, H-30), 0.75 (3H, *s*, H-29), sugar moiety δ 5.43 (1H, *d*, *J*=7.6 Hz, H-1″), 5.27 (1H, *d*, *J*=7.6 Hz, H-1″), 4.48 (1H, *d*, *J*=7.6 Hz, H-1′); $^{13}C$ NMR ($C_5D_5N$): Table 3; Negative FAB-MS *m/z* 959 $[M-H]^-$, 797 $[M-Glc]^-$, 635 $[M-Glc-Glc]^-$, 473 $[M-Glc-Glc-Glc]^-$ ; Negative HR-FAB-MS, *m/z*: 959.5224 $[M-H]^-$ (calcd for $C_{48}H_{79}O_{19}$, 959.5215).

### 3.7. Justicioside C (3)

Amorphous powder, $[\alpha]_D^{27}$ + 10.1° (MeOH, *c* 3.57); $^1H$ NMR ($C_5D_5N$): aglycone moiety δ 5.27 (1H, *d*, *J*=3.2 Hz, H-12), 3.82 (1H, *d*, *J*=10.0 Hz, H-28a), 3.63 (1H, *d*, *J*=10.0 Hz, H-28b), 3.52 (1H, *dd*, *J*=11.7, 3.4 Hz, H-3), 1.95 (1H, *d*, *J*=9.0 Hz, H-9), 1.26 (3H, *s*, H-27), 1.20 (3H, *s*, H-23), 1.16 (3H, *s*, H-25), 1.02 (3H, *s*, H-24), 0.94 (3H, *s*, H-26), 0.89 (6H, *s*, H-29, 30), sugar

moiety $\delta$ 5.38 (1H, *d*, $J$=7.6 Hz, H-1″), 4.88 (1H, *d*, $J$=7.6 Hz, H-1′); $^{13}$C NMR ($C_5D_5N$): Table 3; Negative FAB-MS *m/z* 811 $[M-H]^-$, 649 $[M-Glc]^-$, 487 $[M-Glc-Glc]^-$; Negative HR-FAB-MS, *m/z*: 811.4851 $[M-H]^-$ (calcd for $C_{43}H_{71}O_{14}$, 811.4843).

### 3.8. 11α-Methoxy-olean-12-ene-1β,3β,28-triol (3a)

Amorphous powder, $[\alpha]_D^{27}$ + 46.7° (MeOH, *c* 0.71); $^1$H and $^{13}$C NMR ($CD_3OD$): Table 2; Negative HR-FAB-MS, *m/z*: 487.3796 $[M-H]^-$ (calcd for $C_{31}H_{51}O_4$, 487.3787).

### 3.9. Justicioside D (4)

Amorphous powder, $[\alpha]_D^{27}$ + 13.6° (MeOH, *c* 7.79); $^1$H NMR ($C_5D_5N$): aglycone moiety $\delta$ 5.27 (1H, *d*, $J$=3.2 Hz, H-12), 3.77 (1H, *d*, $J$=9.5 Hz, H-28a), 3.65 (1H, *d*, $J$=9.5 Hz, H-28b), 3.49 (1H, *dd*, $J$=12.0, 3.8 Hz, H-3), 1.94 (1H, *d*, $J$=803 Hz, H-9), 1.26 (3H, *s*, H-27), 1.16 (3H, *s*, H-23), 1.15 (3H, *s*, H-25), 1.00 (3H, *s*, H-24), 0.99 (3H, *s*, H-26), 0.86 (6H, *s*, H-30), 0.82 (3H, *s*, H-29), sugar moiety $\delta$ 5.42 (1H, *d*, $J$=7.6 Hz, H-1″), 5.32 (1H, *d*, $J$=7.6 Hz, H-1‴), 4.88 (1H, *d*, $J$=7.6 Hz, H-1′); $^{13}$C NMR ($CD_3OD$) spectra: Table 1; Negative FAB-MS *m/z* 973 $[M-H]^-$, 811 $[M-Glc]^-$, 649 $[M-Glc-Glc]^-$, 487$[M-Glc-Glc-Glc]^-$; Negative HR-FAB-MS, *m/z*: 973.5267 $[M-H]^-$ (calcd for $C_{49}H_{81}O_{19}$, 973.5371).

Table 2
$^1$H and $^{13}$C NMR spectral data for aglycone (**3a**, in $C_5D_5N$)

| Position | DEPT | $\delta_C$ | $\delta_H$ |
|---|---|---|---|
| 1 | CH | 77.2 | 3.63 (1H, *dd*, $J$=11.6, 4.4 Hz) |
| 2 | $CH_2$ | 37.2 | 2.34 (1H, *ddd*, $J$=14.2, 4.4, 4.4 Hz) |
| | | | 2.23 (1H, *ddd*, $J$=14.2, 12.2, 11.6 Hz) |
| 3 | CH | 75.2 | 3.56 (1H, *dd*, $J$=12.2, 4.4 Hz) |
| 4 | C | 39.6 | |
| 5 | CH | 52.8 | 0.78 (1H, *br d*, $J$=9.7 Hz) |
| 6 | $CH_2$ | 18.2 | 1.67 (1H, *m*) |
| | | | 1.50 (1H, *m*) |
| 7 | $CH_2$ | 31.6 | 1.99 (1H, *m*) |
| | | | 1.67 (1H, *br d*, $J$=15.4 Hz) |
| 8 | C | 41.7 | |
| 9 | CH | 50.2 | 2.00 (1H, *d*, $J$=8.5 Hz) |
| 10 | C | 45.1 | |
| 11 | CH | 74.3 | 4.41 (1H, *dd*, $J$=8.5, 3.7 Hz) |
| 12 | CH | 121.9 | 5.31 (1H, *d*, $J$=3.7 Hz) |
| 13 | C | 152.0 | |
| 14 | C | 44.1 | |
| 15 | $CH_2$ | 26.5 | 1.86 (1H, *br d*, $J$=13.2 Hz) |
| | | | 1.01 (1H, *m*) |
| 16 | $CH_2$ | 22.7 | 2.01 (1H, *m*) |
| | | | 1.50 (1H, *m*) |
| 17 | C | 37.3 | |
| 18 | CH | 42.4 | 2.40 (1H, *dd*, $J$=14.1, 4.4 Hz) |
| 19 | $CH_2$ | 47.0 | 1.88 (1H, *dd*, $J$=13.7, 13.2 Hz) |
| | | | 1.14 (1H, *dd*, $J$=13.7, 4.2 Hz) |
| 20 | C | 31.3 | |
| 21 | $CH_2$ | 34.5 | 1.41 (1H, *br dd*, $J$=13.7, 3.7 Hz) |
| | | | 1.26 (1H, *m*) |
| 22 | $CH_2$ | 33.3 | 1.48 (1H, *br dd*, $J$=12.7, 3.2 Hz) |
| | | | 1.25 (1H, *m*) |
| 23 | $CH_3$ | 28.6 | 1.23 (3H, *s*) |
| 24 | $CH_3$ | 15.9 | 1.05 (3H, *s*) |
| 25 | $CH_3$ | 13.5 | 1.18 (3H, *s*) |
| 26 | $CH_3$ | 18.2 | 0.96 (3H, *s*) |
| 27 | $CH_3$ | 24.5 | 1.33 (3H, *s*) |
| 28 | $CH_2$ | 68.6 | 3.76 (1H, *d*, $J$=10.5 Hz) |
| | | | 3.55 (1H, *d*, $J$=10.5 Hz) |
| 29 | $CH_3$ | 33.3 | 0.94 (3H, *s*) |
| 30 | $CH_3$ | 23.7 | 0.97 (3H, *s*) |
| MeO-11 | $CH_3$ | 51.3 | 3.27 (3H, *s*) |

Table 3
$^{13}$C NMR spectral data for justiciosides A–D (**1–4**, in $C_5D_5N$)

| Position | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 77.8 | 77.8 | 77.2 | 77.1 |
| 2 | 37.3 | 37.0 | 37.1 | 36.8 |
| 3 | 75.4 | 75.3 | 75.2 | 75.1 |
| 4 | 39.6 | 39.4 | 39.5 | 39.4 |
| 5 | 52.9 | 52.9 | 52.8 | 52.7 |
| 6 | 18.2 | 18.2 | 18.1 | 18.1 |
| 7 | 32.3 | 32.0 | 32.2 | 31.8 |
| 8 | 41.8 | 41.7 | 41.5 | 41.2 |
| 9 | 57.2 | 57.1 | 50.2 | 50.1 |
| 10 | 44.8 | 44.6 | 45.0 | 44.9 |
| 11 | 66.2 | 66.2 | 74.3 | 74.2 |
| 12 | 126.1 | 126.1 | 122.0 | 121.9 |
| 13 | 147.0 | 147.0 | 151.6 | 151.5 |
| 14 | 44.1 | 44.1 | 44.2 | 44.1 |
| 15 | 26.6 | 26.5 | 26.9 | 26.7 |
| 16 | 21.8 | 22.0 | 21.9 | 22.0 |
| 17 | 36.7 | 36.6 | 36.7 | 36.6 |
| 18 | 42.2 | 41.9 | 42.7 | 42.3 |
| 19 | 46.0 | 45.9 | 46.6 | 46.5 |
| 20 | 30.9 | 30.8 | 31.1 | 31.0 |
| 21 | 34.3 | 34.2 | 34.3 | 34.2 |
| 22 | 33.2 | 33.2 | 33.1 | 33.2 |
| 23 | 28.6 | 28.5 | 28.6 | 28.5 |
| 24 | 16.0 | 15.9 | 15.9 | 15.8 |
| 25 | 13.6 | 13.5 | 13.5 | 13.4 |
| 26 | 18.2 | 18.2 | 18.2 | 18.2 |
| 27 | 25.7 | 25.8 | 24.6 | 24.5 |
| 28 | 76.5 | 76.8 | 76.4 | 76.6 |
| 29 | 33.2 | 33.1 | 33.2 | 33.2 |
| 30 | 23.7 | 23.7 | 23.8 | 23.8 |
| MeO-11 | | | 51.2 | 51.2 |
| Glc-1′ | 103.5 | 103.4 | 103.5 | 103.3 |
| 2′ | 82.7 | 82.6 | 82.7 | 82.6 |
| 3′ | 77.6 | 77.5 | 77.9 | 77.5 |
| 4′ | 71.6 | 71.0 | 71.6 | 71.0 |
| 5′ | 78.1 | 77.8 | 78.1 | 77.8 |
| 6′ | 62.6 | 62.4 | 62.6 | 62.4 |
| Glc-1″ | 105.9 | 103.0 | 105.9 | 103.0 |
| 2″ | 76.9 | 85.3 | 76.9 | 85.2 |
| 3″ | 78.2 | 77.6 | 78.2 | 77.6 |
| 4″ | 71.5 | 70.6 | 71.5 | 70.6 |
| 5″ | 78.4 | 77.6 | 78.4 | 77.8 |
| 6″ | 62.6 | 62.3 | 62.7 | 62.3 |
| Glc-1‴ | | 106.1 | | 106.1 |
| 2‴ | | 76.2 | | 76.2 |
| 3‴ | | 78.7 | | 78.2 |
| 4‴ | | 71.3 | | 71.3 |
| 5‴ | | 78.9 | | 78.9 |
| 6‴ | | 62.7 | | 62.7 |

### 3.10. Enzymatic hydrolysis of justiciosides A–D

Each sample of justicioside A (**1**, 25 mg) and B (**2**, 65 mg) was dissolved in 0.5 ml of MeOH. A solution of crude hesperidinase (Kohda and Tanaka, 1975) (100 mg in 20 ml of $H_2O$) was added in each experiment. After stirring at 37 °C for 1 week, the mixtures were extracted with EtOAc, concentrated to dryness, and then purified by prep. HPLC-ODS using MeOH–$H_2O$ (9:1) as solvent system to give **1a** (8 and 22 mg, respectively). The aqueous layer of each sample was concentrated to dryness and applied to a silica gel column [EtOAc–MeOH–$H_2O$ (40:10:1)], affording D-glucose (7 and 18 mg, respectively) in comparison of its optical rotation with an authentic sample. By the same method, justicioside C (**3**, 20 mg) and D (**4**, 50 mg) provided **3a** (4 and 17 mg, respectively) and D-glucose (5 mg and 9 mg, respectively).

## Acknowledgements

The authors thank the Thailand Research Fund (TRF) for financial support of this work. Also, we are grateful to the Research Center for Molecular Medicine, Hiroshima University, Japan for the provision of NMR used in this investigation.

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# Phenylethanoid and Iridoid Glycosides from the Thai Medicinal Plant, *Barleria strigosa*

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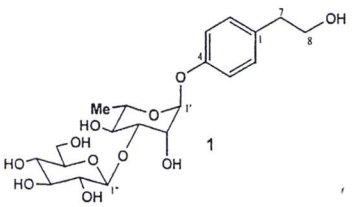
**A phenylethanoid (4-hydroxyphenylethyl 4-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside) and an iridoid (10-*O*-*trans*-coumaroyl-eranthemoside) were isolated from an entire *Barleria strigosa* plant together with verbascoside, isoverbascoside, decaffeoylverbascoside, (+)-lyoniresinol 3α-*O*-β-D-glucoside, apigenin 7-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucoside, 7-*O*-acetyl-8-*epi*-loganic acid and (3*R*)-1-octen-3-ol-3-*O*-β-D-xylosyl-(1→6)-β-D-glucoside. The structural elucidations were based on analyses of physical and spectroscopic data.**

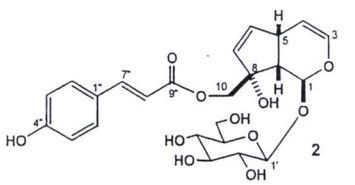
**Key words** *Barleria strigosa*; Acanthaceae; phenylethanoid; iridoid; strigoside; 10-*O*-*trans*-coumaroyl-eranthemoside

*Barleria strigosa* WILLD. (Acanthaceae, Thai name: Sang-Ko-Ra-Ni) is a shrub native to tropical regions of Asia. The leaves are used in Thai traditional medicine as an antipyretic as well as an antidote for detoxification of poisons. In our continuing studies on Thai medicinal plants, the constituents of this plant were investigated, following plant collection from the Botanical Gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. In the preliminary study, a quaternary amine (betaine) was reported.[1] The present study deals with the isolation and structural determinations of a new phenylethanoid glycoside (**1**) and a new iridoid glucoside (**2**), together with seven known compounds (**3**—**9**) from an entire plant.

## Results and Discussion

The methanolic extract was suspended in $H_2O$ and defatted with $Et_2O$. The aqueous layer was subjected to a column of Diaion HP-20, using $H_2O$, MeOH and $Me_2CO$, successively. The portion eluted with MeOH was repeatedly chromatographed on columns of silica gel, RP-18 and prep. HPLC-ODS to afford nine compounds. Seven were identified as known compounds: verbascoside (**3**), isoverbascoside (**4**), decaffeoylverbascoside (**5**),[2] (+)-lyoniresinol 3α-*O*-β-D-glucoside (**6**),[3] apigenin 7-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucoside (**7**),[4] 7-*O*-acetyl-8-*epi*-loganic acid (**8**)[5] and (3*R*)-1-octen-3-ol-3-*O*-β-D-xylosyl-(1→6)-β-D-glucoside (**9**)[6] by comparison of physical data with literature values and from spectroscopic evidence.




The molecular formula of compound **1** was determined as $C_{20}H_{30}O_{11}$ by negative high-resolution (HR)-FAB mass spectrometry. The $^1$H-NMR spectrum revealed the presence of the signals of a *para*-disubstituted aromatic ring at $\delta$ 6.98 (2H, d, $J$=8.5 Hz) and $\delta$ 7.15 (2H, d, $J$=8.5 Hz), two sets of methylene protons at $\delta$ 2.76 (2H, t, $J$=6.9 Hz) and $\delta$ 3.71 (2H, t, $J$=6.9 Hz) in addition to two anomeric signals at $\delta$ 4.59 (1H, d, $J$=7.8 Hz) and $\delta$ 5.40 (1H, d, $J$=1.8 Hz). The $^{13}$C-NMR spectrum showed 20 carbon signals (Table 1), of which eight were assignable to the aglycone moiety, and the remaining belonging to the sugar part. All protonated carbons were assigned by heteronuclear single quantum coherence (HSQC). From these spectral data, compound **1** was a glycoside of 4-hydroxyphenylethyl alcohol. Acid hydrolysis

Table 1. NMR Spectral Data of **1** ($CD_3OD$, $^1$H-NMR 400 MHz, $^{13}$C-NMR 100 MHz)

| Position | $\delta_C$ | $\delta_H$ |
|---|---|---|
| 1 | 134.2 | |
| 2, 6 | 131.0 | 7.15 (2H, d, $J$=8.5 Hz) |
| 3, 5 | 117.5 | 6.98 (2H, d, $J$=8.5 Hz) |
| 4 | 156.1 | |
| 7 | 39.3 | 2.76 (2H, t, $J$=6.9 Hz) |
| 8 | 64.3 | 3.71 (2H, t, $J$=6.9 Hz) |
| Rha | | |
| 1′ | 99.6 | 5.40 (1H, d, $J$=1.8 Hz) |
| 2′ | 71.4 | 4.28 (1H, dd, $J$=3.0, 1.8 Hz) |
| 3′ | 82.7 | 3.94 (1H, dd, $J$=9.3, 3.0 Hz) |
| 4′ | 72.6 | 3.63 (1H, dd, $J$=9.5, 9.3 Hz) |
| 5′ | 70.2 | 3.70 (1H, m) |
| 6′ | 18.1 | 1.22 (3H, d, $J$=5.9 Hz) |
| Glc | | |
| 1″ | 105.8 | 4.59 (1H, d, $J$=7.8 Hz) |
| 2″ | 75.4 | 3.34 (1H, dd, $J$=8.8, 7.8 Hz) |
| 3″ | 77.7 | 3.40 (1H, dd, $J$=9.3, 8.8 Hz) |
| 4″ | 71.0 | 3.39 (1H, dd, $J$=9.3, 8.1 Hz) |
| 5″ | 77.6 | 3.32 (1H, m) |
| 6″ | 62.2 | 3.84 (1H, dd, $J$=12.0, 2.0 Hz) |
| | | 3.73 (1H, dd, $J$=12.0, 4.4 Hz) |

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Table 2. NMR Spectral Data of **2** ($CD_3OD$, $^1$H-NMR 400 MHz, $^{13}$C-NMR 100 MHz)

| Position | $\delta_C$ | $\delta_H$ |
|---|---|---|
| 1 | 94.4 | 5.37 (1H, d, $J$=3.4 Hz) |
| 3 | 139.9 | 6.11 (1H, dd, $J$=6.2, 2.0 Hz) |
| 4 | 105.9 | 4.90 (1H, dd, $J$=6.2, 3.4 Hz) |
| 5 | 40.0 | 3.25 (1H, m) |
| 6 | 138.2 | 5.89 (1H, dd, $J$=5.6, 2.4 Hz) |
| 7 | 132.4 | 5.56 (1H, dd, $J$=5.6, 1.9 Hz) |
| 8 | 85.0 | |
| 9 | 46.9 | 2.52 (1H, dd, $J$=8.3, 3.4 Hz) |
| 10 | 70.1 | 4.20 (1H, d, $J$=11.2 Hz) |
| | | 4.09 (1H, d, $J$=11.2 Hz) |
| Glc | | |
| 1′ | 99.7 | 4.61 (1H, d, $J$=7.8 Hz) |
| 2′ | 74.7 | 3.17 (1H, dd, $J$=8.8, 7.8 Hz) |
| 3′ | 77.9 | 3.30 (1H, dd, $J$=8.8, 8.5 Hz) |
| 4′ | 71.4 | 3.23 (1H, dd, $J$=8.5, 8.1 Hz) |
| 5′ | 78.2 | 3.24 (1H, m) |
| 6′ | 62.5 | 3.74 (1H, dd, $J$=12.0, 2.0 Hz) |
| | | 3.56 (1H, dd, $J$=12.0, 5.1 Hz) |
| Coumaroyl moiety | | |
| 1″ | 127.1 | |
| 2″, 6″ | 131.3 | 7.37 (2H, d, $J$=8.8 Hz) |
| 3″, 5″ | 116.8 | 6.72 (2H, d, $J$=8.8 Hz) |
| 4″ | 161.3 | |
| 7″ | 147.0 | 7.54 (1H, d, $J$=15.9 Hz) |
| 8″ | 114.9 | 6.25 (1H, d, $J$=15.9 Hz) |
| 9″ | 169.1 | |

afforded D-glucose and L-rhamnose by comparison of the optical rotation with authentic samples. The appearance of the methylene signal (C-8) at $\delta$ 64.3 suggested that this position was unsubstituted, demonstrating that the sugar moiety was located at C-4. Negative FAB-MS of compound **1** exhibited a significant fragment ion at $m/z$ 283 $[M-Glc]^-$, indicating that glucose is the terminal sugar. This sugar was assigned to be at C-3′ of the rhamnosyl unit based on the downfield shift of C-3′ (+10.6) together with the upfield shift of C-2′ (−0.8) and C-4′ (−1.3), when compared to the rhamnosyl carbon signals of compound **5**. The assignment was supported by heteronuclear multiple bond connectivity (HMBC), in which long-range correlations were found between (i) H-1′ ($\delta$ 5.40, $J$=1.8 Hz) and C-4 ($\delta$ 156.1), C-5′ ($\delta$ 70.2), C-3′ ($\delta$ 82.7), and (ii) H-1″ ($\delta$ 4.59, $J$=7.8 Hz) and C-3′ ($\delta$ 82.7) as shown in Fig. 1. Consequently, the structure of compound **1** was concluded to be 4-hydroxyphenylethyl 4-*O*-$\beta$-D-glucopyranosyl-(1→3)-*O*-$\alpha$-L-rhamnopyranoside, namely strigoside.

The molecular formula of compound **2** was determined as $C_{24}H_{28}O_{11}$ by negative HR-FAB mass spectrometry. Inspection of the $^{13}$C-NMR spectral data revealed the presence of one $\beta$-glucosyl unit and one coumaroyl moiety in addition to nine carbon signals for the aglycone moiety. The distortionless enhancement by polarization transfer (DEPT) experiments indicated that compound **2** contained one methylene ($\delta$ 70.1), seven methines ($\delta$ 40.0, 46.9, 94.4, 105.9, 132.4, 138.2 and 139.9) and one quaternary carbon ($\delta$ 85.0) for an aglycone part, consistent with an iridoid skeleton. The chemical shift at $\delta$ 94.4 was characteristic of an acetal group of C-1. The methine signals at $\delta$ 139.9, 105.9, 138.2 and 132.4 were assigned to two disubstituted olefin groups, locating at C-3, C-4, C-6 and C-7, respectively. The coumaroyl moiety

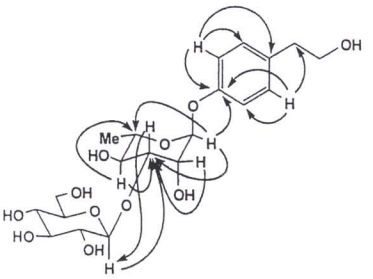

Fig. 1. Significant HMBC Correlation of Compound **1**

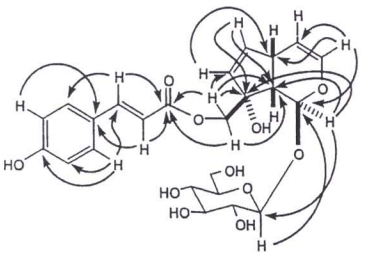

Fig. 2. Significant HMBC Correlation of Compound **2**

was assigned as *trans* by the coupling constant of the signals at $\delta$ 7.54 and 6.25 with $J$=15.9 Hz from the $^1$H-NMR spectrum. The $^1$H-NMR spectrum showed methine signals at $\delta$ 5.37 (d, $J$=3.4 Hz), 6.11 (dd, $J$=6.2, 2.0 Hz), 4.90 (dd, $J$=6.2, 3.4 Hz), 3.50 (m), 5.89 (dd, $J$=5.6, 2.4 Hz), 5.56 (dd, $J$=5.6, 1.9 Hz) and 2.52 (dd, $J$=8.3, 3.4 Hz) assignable to H-1, H-3, H-4, H-5, H-6, H-7 and H-9, respectively. Also, it showed an AB type of methylene signals at $\delta$ 4.20 and 4.09 (each d, $J$=11.2 Hz) attributable to H-10a and H-10b. The $^1$H- and $^{13}$C-NMR spectral data were closely related to those of eranthemoside,[7] except for lacking the coumaroyl moiety. The complete assignments were established by analyzing the 2D-NMR spectra including HSQC and HMBC in addition to the coupling constants in the $^1$H-NMR spectrum. In the HMBC spectrum (Fig. 2), the long-range correlations were observed between H-10a, H-10b and C-9″, indicating that the coumaroyl moiety linked to C-10. The glucosyl moiety attached to C-1 from the chemical shift value of this carbon at $\delta$ 94.4, and this was confirmed by the HMBC correlations (Fig. 2). The coupling constant between H-1 and H-9 ($J$=3.4 Hz), H-5 and H-9 ($J$=8.3 Hz) led to the conclusion that the position of the protons at C-1, C-5 and C-9 were in $\alpha$, $\beta$ and $\beta$-orientations, respectively. Therefore, the structure of compound **2** was elucidated as 10-*O*-*trans*-coumaroyl-eranthemoside.

## Experimental

**General Procedures** NMR spectra were recorded in $CD_3OD$ using a JEOL JNM $\alpha$-400 spectrometer (400 MHz for $^1$H-NMR and 100 MHz for $^{13}$C-NMR). MS values were obtained on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a union PM-1 digital polarimeter. For column chromatography, silica gel 60 (70—230 mesh, GE0049, Scharlau Chemie S.A.), RP-18 (50 $\mu$m, YMC) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used. Preparative HPLC was carried out on an ODS column (150×20 mm i.d., YMC) with a Shimadzu RID-6A refractive index detector. The flow rate was 6 ml/min. The solvent systems were: (I) EtOAc–MeOH (9 : 1), (II) EtOAc–MeOH–$H_2O$ (40 : 10 : 1), (III) EtOAc–MeOH–$H_2O$ (70 : 30 : 3), (IV) 10—50% aq. MeOH. (V) 6% aq. MeCN, (VI)

10% aq. MeCN, (VII) 15% aq. MeCN and (VIII) 18% MeCN, The spraying reagent used for TLC was 10% $H_2SO_4$ in 50% EtOH.

**Plant Material** *Barleria strigosa* WILLD. was cultivated and collected in July 2003 from the Botanical Garden, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand. The plant was identified by Mr. Bamrung Thavinchiua, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences of Khon Kaen University. A voucher sample (KKU 0043) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences at the university.

**Extraction and Isolation** The dried intact *B. strigosa* (1.9 kg) was extracted with MeOH (20 l) under reflux for 15 h. The MeOH extract (246.5 g) was concentrated to dryness and partitioned between $Et_2O$ and $H_2O$. The aqueous layer was applied to a column of Diaion HP-20 and eluted successively with $H_2O$, MeOH and $Me_2CO$. The fraction eluted with MeOH (39.6 g) was subjected to a silica gel column using solvent systems I, II and III. Six fractions were collected. Fraction 3 (7.2 g) was applied to a column of RP-18 using solvent system IV to provide eight fractions. Fraction 3—6 was purified by prep. HPLC-ODS with solvent system VIII to afford compounds **2** (90 mg), **3** (85 mg) and **4** (47 mg). Fraction 4 (3.7 g) was subjected to a column of RP-18 using solvent system IV, affording eleven fractions. Fraction 4-2 was purified by prep. HPLC-ODS with solvent system V to give compound **5** (116 mg). Fraction 4-4 was further purified by prep. HPLC-ODS with solvent system VII to provide compound **6** (79 mg). Compound **7** (309 mg) was crystallized from fraction 4-7. Fraction 4-9 was dried to give an amorphous powder of compound **8** (105 mg). Fraction 5 (6.3 g) was similarly separated on a column of RP-18 using solvent system IV to give seven fractions. Fraction 5-2 was purified by prep. HPLC-ODS with solvent system VI to obtain compound **1** (73 mg). Finally, fraction 5-4 was purified by prep. HPLC-ODS using solvent system VII to provide compound **9** (24 mg).

Compound **1**: Amorphous powder, $[\alpha]_D^{27}$ −25.1° ($c$=3.07, MeOH); $^1$H- and $^{13}$C-NMR ($CD_3OD$) spectra: Table 1; Negative HR-FAB-MS, *m/z*: 445.1717 ($C_{20}H_{29}O_{11}$ required 445.1709).

Compound **2**: Amorphous powder, $[\alpha]_D^{27}$ −33.3° ($c$=1.35, MeOH); $^1$H- and $^{13}$C-NMR ($CD_3OD$) spectra: Table 2; Negative HR-FAB-MS, *m/z*: 491.1545 ($C_{24}H_{27}O_{11}$ required 491.1553).

**Acid Hydrolysis of Compound 1** Compound **1** (34 mg) was dissolved in 5% HCl and heated at 90 °C for 2 h. After cooling, the reaction mixture was extracted with $Et_2O$. The aqueous layer was neutralized with saturated $NaHCO_3$ and concentrated to dryness. The residue was applied to a silica gel column using solvent system II to give D-glucose (9 mg, $[\alpha]_D^{27}$ +50.2°) and L-rhamnose (7 mg, $[\alpha]_D^{27}$ +8.3°) in comparison with authentic samples.

**Acknowledgements** This study was financially supported by a Grant-in-Aid for Scientific Research (No. MRG-4680108) from the Thailand Research Fund (TRF) and Ministry of University Affairs. The authors also thank Mr. Bunlert Khamya of the Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University for help in obtaining the plant material.

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PHYTOCHEMICAL ANALYSIS
*Phytochem. Anal.* 15, 365–371 (2004)
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002.pca.789

# Determination and Variation of Three Active Diterpenoids in *Andrographis paniculata* (Burm.f.) Nees

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**Simple and rapid methods have been developed for the extraction and simultaneous determination of the three active diterpenoids, andrographolide ($AP_1$), 14-deoxy-11,12-didehydroandrographolide ($AP_3$) and neoandrographolide ($AP_4$), from various samples of *Andrographis paniculata* (Burm.f.) Nees. Methanol extracts from the dried leaves, stems and crude products were analysed by isocratic HPLC using a methanol and water mobile phase with monitoring at 220 nm. There was a large variation of the three active diterpenoids in different *A. paniculata* products obtained from Thai markets. The results indicated that the amounts of these active compounds consumed, based on the recommended daily doses, from materials obtained from the different suppliers will be different. In addition, the stability of these three active compounds was also examined in dry herbs stored at room temperature. The results showed that andrographolide was more stable than the others. In contrast, the content of 14-deoxy-11,12-didehydroandrographolide increased and the neoandrographolide content fluctuated during storage time. The combination of different levels of these compounds in the source materials and the changes during storage could have a significant effect on the efficacy of this traditional herbal medicine in clinical treatment.** 

*Keywords:* HPLC; diterpenoids; variation; stability; *Andrographis paniculata*.

## INTRODUCTION

Although herbal products are extensively used in traditional treatments, especially in Asian countries, many problems relating to the quality control of the active compounds in medicinal herbs need to be addressed, particularly by the pharmaceutical industry. Identification and determination of the active components in herbs to serve as markers for their analysis is a valuable technique which could be used to improve the quality of herbal products.

*Andrographis paniculata* (Burm.f.) Nees (Acanthaceae), commonly known as *Fa Tha Lai*, has been widely used in Thailand and other countries for the treatment of the common cold, fever and non-infectious diarrhoea (Thanagkul and Chaichantipayut, 1985; Laorpaksa *et al.*, 1988; Vedavathy and Rao, 1991; Gupta *et al.*, 1993; Caceres *et al.*, 1999). In addition, *A. paniculata* is also used for treating animal diseases, e.g. respiratory infection and diarrhoea, as an alternative to antibiotics (Tipakorn, 2002). In Thailand, this plant was selected by the Ministry of Public Health as one of the medicinal plants to be included in 'The National List of Essential Drugs A.D. 1999' (List of Herbal Medicinal Products).

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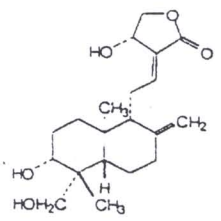
Contract/grant sponsor: UNDP/RAS/97/001 Project.
Contract/grant sponsor: Thailand Research Fund.



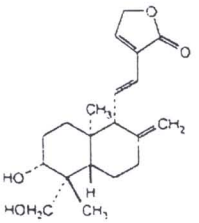
*A. paniculata* has various pharmacological activities, including anti-inflammatory (Shen *et al.*, 2000), anti-diarrhoeal (Gupta *et al.*, 1993), immune-stimulatory (Puri *et al.*, 1993), anti-HIV (Otake *et al.*, 1995; Calabrese *et al.*, 2000), anti-malarial (Misra *et al.*, 1992; Najila *et al.*, 2002), hepatoprotective (Handa and Sharma, 1990; Kapil *et al.*, 1993; Clander *et al.*, 1995), and cardiovascular (Zhang and Tan, 1997; Zhang *et al.*, 1998). The therapeutic activities of this plant are attributed to andrographolide ($AP_1$) and related diterpenes, i.e. deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide ($AP_3$) and neoandrographolide ($AP_4$; Fig. 1). However, each of these constituents exhibit subtly different therapeutic activities. Thus $AP_1$ shows higher anti-inflammatory (Shen *et al.*, 2000) and hepatoprotective activities against galactosamine and paracetamol intoxication (Clander *et al.*, 1995), $AP_3$ produces a more potent hypotensive effect in anaesthetised rats and isolated right atria (Zhang *et al.*, 1998), whilst $AP_4$ shows greater activity against malaria (Misra *et al.*, 1992) and is hepatoprotective against carbon tetrachloride (Kapil *et al.*, 1993). The difference in the potency of these three compounds indicates that qualitative and quantitative control of the active principles in medicinal herbs is very important in order to ensure maximal therapeutic value for a particular treatment.

The active diterpenoids in *A. paniculata* have been previously analysed by various techniques including titrimetric (Jewvachdamrongkul *et al.*, 1990; Ministry of Public Health, 1995), TLC (Rajani *et al.*, 2000), HPTLC (Chauhan *et al.*, 1999; Saxena *et al.*, 2000), micellar

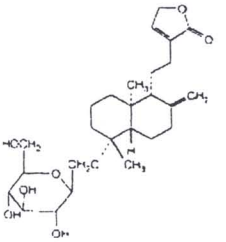
*Received 10 July 2003*
*Revised 28 October 2003*
*Accepted 28 October 2003*



Andrographolide-$AP_1$



14-Deoxy-11,12-didehydroandrographolide-$AP_3$



Neoandrographolide-$AP_4$

**Figure 1.** Structures of andrographolide ($AP_1$), 14-deoxy-11,12-didehydroandrographolide ($AP_3$) and neoandrographolide ($AP_4$).

electrokinetic chromatography (MEKC; Cheung *et al.*, 2001; Zhao *et al.*, 2002), and a more common HPLC method (Jain *et al.*, 2000; Li and Fitzloff, 2002; Du *et al.*, 2003). The TLC method normally takes 1–3 h to separate the components in herbal extracts with comparatively poor sensitivity. There are a few reports demonstrating the simultaneous determination of $AP_1$, $AP_3$ and $AP_4$ by HPLC (Jain *et al.*, 2000) and MEKC (Cheung *et al.*, 2001; Zhao *et al.*, 2002); however, the extraction processes in these reports are time consuming (3–12 h). Li and Fitzloff (2002) determined only the content of $AP_1$ in three commercial *A. paniculata* products using HPLC with an evaporative light scattering detector (HPLC-ELSD).

Since there is an increase in the use of *A. paniculata* herb as an alternative medicine, it is becoming even more important to determine the content of these active compounds in the plant. Given that the three main active diterpenoids have different pharmacological potencies, it is anticipated that the variation in the amount of these compounds will affect the efficacy and safety of the plant preparation. Thus, the objectives of the present study were to develop a simple method for the extraction and quantification of these active compounds in plant materials, and also to examine the variation in the concentrations of the three diterpenoids within *A. paniculata* products available in markets in Thailand.

Moreover, there is minimal information concerning the stability of these active compounds in dried tissues during storage under ambient conditions. Lomlim *et al.* (2003) studied only the stability of pure andrographolide (crystal and amorphous forms) under heat-accelerated conditions over a 3 month period. Hence, included in the present study was an investigation of the stability of these diterpenoids during storage.

## EXPERIMENTAL

### Chemicals

The diterpenoids $AP_1$, $AP_3$ and $AP_4$ used as reference standards in this study were purified by TLC and identified using UV, IR and NMR spectral data by the Laboratory of Natural Products, Chulabhorn Research Institute, Thailand, following the published method of Matsuda *et al.* (1994). $AP_2$ is a mixture of $AP_1$ and $AP_4$, therefore it was not used in this study. HPLC grade methanol was obtained from Merck (Darmstadt, FR Germany). Milli-Q deionised water (Branstead, Newton, USA) was used throughout this experiment.

### Plant materials and *A. paniculata* products

Material of *A. paniculata* was obtained from the project site of Chulabhorn Research Institute at Sakaew province (Thailand) and air-dried and powdered for use in determining the total lactone content and the amount of the three active diterpenoids in leaves and stems. Plants of *A. paniculata* were kindly identified by Dr Wongsatit Chuakul and a voucher specimen is deposited at the Pharmaceutical Botany Mahidol Herbarium, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand (PBM 3760). Twenty samples of commercial *A. paniculata* were purchased from different markets in Thailand. Plant materials were kept at room temperature until extraction and analysis.

### Chromatographic conditions

Crude extracts were analysed by HPLC using a Hewlett Packard (Palo Alto, CA, USA) model HP1100 instrument, with thermostatically controlled column oven and a photodiode array detector (PAD), equipped with a LiChrospher (Macherey-Nagal, Duren, Germany) $RP_{18}$ reverse phase column (125 × 4 mm i.d.) protected by a LiChrospher $RP_{18}$ guard column (4 × 4 mm i.d.). The column was equilibrated with the mobile phase, consisting of 50.5% methanol in water, at a flow rate of 1.2 mL/min for 30 min before analysis. An aliquot (1 μL) of sample was injected onto the column and UV detection was set at 220 nm. The temperature of the column was 25°C. A standard mixture containing $AP_1$, $AP_3$ and $AP_4$ was freshly prepared at five concentrations in the range 0.025–1.0 μg. The peak area for each compound was plotted against as a function of concentration. The calibration curves were determined each day of analysis. The percentage recovery of each compound was measured by adding known amounts of reference standards (0.5, 1 and 2 mg) to the crude extracts.

### Sample preparation and analysis

**Determination of total lactone content.** Total lactones were determined by using the standard titrimetric

method from the Thai Herbal Pharmacopoeia Vol. 1 (Ministry of Public Health, 1995). Air-dried leaves of *A. paniculata* harvested in Sakaew province 25 July 2001 (sample $S_2$) were ground through a no. 180 sieve, and a 1.0 g sample was accurately weighed into a 100 mL round-bottomed flask. The sample was extracted with 50 mL 85% (v/v) ethanol, refluxed in a water bath for 2 h and filtered through Whatman no. 1 filter paper into a flask. The marc was washed with 85% ethanol until the last washing was almost colourless. The solvent extracts were combined and allowed to cool to room temperature. Basic lead acetate (1.0 mL) was added to the extracts and they were set aside for 15 min. The extracts were filtered through Whatman no. 1 filter paper and the precipitate was then washed with ethanol until there was no green colour in the last washing. The solvent extracts were combined, 1.0 mL of 25% (w/v) of sodium sulphate was added drop-wise with gentle swirling, and the whole mixed well. The extracts were set aside for 1 h at room temperature after which 500 mg of decolourising charcoal was added and the mixture refluxed in a water bath for 10 min. The mixture was filtered through a Buckner funnel containing 500 mg of decolourising charcoal and washed three times with 2.0 mL hot ethanol. Distilled water (20 mL) was added, the extracts were allowed to cool at room temperature and then neutralised with 0.1 M sodium hydroxide using phenolphthalein as indicator. An aliquot (5.0 mL) of 0.1 M sodium hydroxide was added, the solution was refluxed on a water-bath for 30 min and then allowed to cool at room temperature. The solution was titrated with 0.05 M hydrochloric acid: each 1.0 mL volume of 0.1 M sodium hydroxide used in titration was equivalent to 35.05 mg of $AP_1$.

**Preparation of basic lead acetate solution.** Lead (II) oxide (14 *g*) was dissolved in 10 mL distilled water, a further 10 mL of distilled water was added and the mixture transferred to a 100 mL flask. Lead (II) acetate trihydrate (22 *g*) was dissolved in 70 mL distilled water and the solution added to the lead (II) oxide solution. The solutions were mixed and set aside for 1 week. After this time the solution was filtered through Whatman no. 1 filter paper into a 100 mL volumetric flask and diluted to 100 mL with boiling water.

**Analysis of the three diterpenoids in *A. paniculata* products.** Methanol (4.0 mL) was added to accurately weighed amounts (300 mg each) of dried, powdered leaves or stems of *A. paniculata* (Chulabhorn Research Institute), and of each *A. paniculata* product obtained from the market (three replicates per product). Samples were agitated for 5 min and then centrifuged at 2000 *g* for 10 min. The supernatant was transferred to a glass tube and the marc was re-extracted twice more with 3.0 mL methanol. All extracts were combined and filtered through a 0.45 μM nylon membrane (13 mm dia.; Orange Scientific, Braine-l'Alleud, Belgium) prior to HPLC analysis.

**Stability of the three diterpenoids in dry leaves of *A. paniculata*.** Dried, powdered leaves of *A. paniculata* harvested at different times in 2001 (sample $S_1$, 18 July; $S_2$, 25 July; $S_3$, 26 July; $S_4$, 8 August) were kept in dry plastic bags and stored at room temperature. Samples (300 mg each) were extracted and analysed for the contents of $AP_1$, $AP_3$ and $AP_4$ at 0, 3, 6, 11, 12 and 15 months using the HPLC method previously described.

## RESULTS AND DISCUSSION

### Development of chromatographic conditions

A simple and reliable method was developed to determine the contents of the three diterpenoids, $AP_1$, $AP_3$ and $AP_4$, extracted from leaves of *A. paniculata*. High-resolution and sharp symmetrical peaks for the three diterpenoids were obtained [Fig. 2(A, B)] using a LiChrospher $RP_{18}$ reverse-phase column (125 × 4 mm i.d.) eluted isocratically with 50.5% methanol in water at a flow rate of 1.2 mL/min. The retention times of $AP_1$, $AP_3$ and $AP_4$ were 3.2, 10.5 and 11.6 min, respectively; the $r^2$ coefficients of $AP_1$, $AP_3$ and $AP_4$ were 0.99998, 0.99996 and 0.99999, respectively; the percentage recoveries of these compounds ranged between 97 and 104%. The detection limits of $AP_1$, $AP_3$ and $AP_4$ were 0.5, 2 and 2 ng, respectively.

The method described here was effective and consistent for the separation of these three compounds, either pure or as part of complex plant extracts. Jain *et al.* (2000) have described an HPLC method for the determination of these compounds in extracts of *A. paniculata*; however, the present method is more sensitive since the recoveries of the three compounds were higher and the detection limit was lower than in the previous study. In

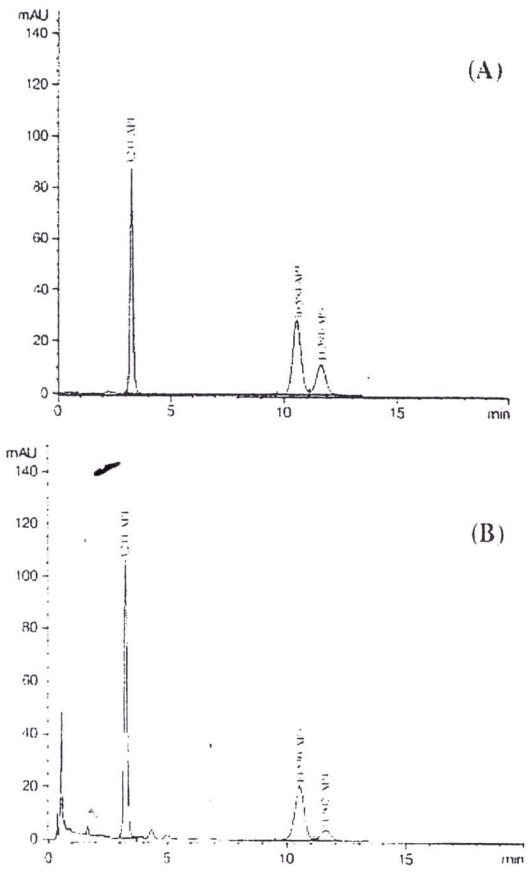



Figure 2. HPLC chromatograms of (A) a reference standard solution containing 500 μg/mL of $AP_1$, $AP_3$ and $AP_4$, and (B) a typical product made from *A. paniculata*. (For chromatographic protocol see Experimental section.)

comparison with a MEKC method (Cheung *et al.*, 2001), HPLC was found to be more sensitive. Furthermore, MEKC used a higher temperature (35°C) to operate the system, which could lead to the degradation of some compounds, and the compounds needed to be dissolved in buffer. Another comparison between HPLC and MEKC methods for the determination of these diterpenoids indicated that HPLC showed better reproducibility in retention time (Zhao *et al.*, 2002), but the HPLC method considered employed a high temperature (35°C) and produced poorer peak shapes compared with MECK.

The extraction and HPLC system described in the present study could also be used for high-throughput determination of these diterpenoids in large numbers of samples because the extraction procedure is simple and the HPLC run time is short (less than 13 min).

### Variation of $AP_1$, $AP_3$ and $AP_4$ in *A. paniculata* products

There was a large variation in the contents of $AP_1$, $AP_3$ and $AP_4$ among *A. paniculata* products purchased from different markets in Thailand (Fig. 3). The content of $AP_1$ was higher than $AP_3$ and $AP_4$, whilst the content of $AP_4$ was the lowest in all of the *A. paniculata* leaf products except for product 03 where the content of $AP_4$ was higher than $AP_3$. The highest content of $AP_1$ was found in product 13 (61.62 mg/g dry weight), whilst product 12 had the lowest content (5.27 mg/g) of this diterpene. Product 01 contained the highest levels of $AP_3$ (31.36 mg/g) and of $AP_4$ (13.43 mg/g); product 12 had the lowest content of $AP_3$ (2.14 mg/g) and the lowest content of $AP_4$ was found in product 17 (1.05 mg/g). The variation of active compounds in *A. paniculata* products may be due to differences in raw materials (genotypes, growing environment, time of harvesting, age of leaves at harvest etc.). In addition, the active compounds may degrade during processing or the raw materials may have been stored in unsuitable conditions before processing or final delivery to the consumer. The variation in raw materials indicated that the amounts of $AP_1$, $AP_3$ and $AP_4$ present in the recommended daily doses (Fig. 4) suggested by different suppliers were also different. The recommended daily doses of product 13 contained the highest levels of $AP_1$ and $AP_3$, and the daily doses of product 07 had the highest contents of $AP_4$. The lowest contents of $AP_1$ and $AP_3$ in daily doses were found in product 12, and the daily dose of product 17 had the lowest contents of $AP_4$. Since the three diterpenoids have different pharmacological activities, the variation of these compounds in commercial products may lead to differences in the efficacy of this plant for the treatment of specific disorders/diseases. Moreover, this variation may give rise to different degrees of adverse effect, for example, products having a high content of $AP_3$ (Fig. 4; products 01, 02, 05, 07, 08 and 13) will have more potential to produce cardiovascular side effects than the others when they are used in the treatment of common cold (Zhang *et al.*, 1998; Sahasitiwat, 2002). Studies performed using MECK also showed variations of the content of andrographolides in some phytomedicinal tablets produced mainly from *A. paniculata* (Zhao *et al.*, 2002). In contrast to the present results, the tablets previously analysed contained the highest levels of $AP_3$ and lowest levels of $AP_1$.

The contents of $AP_1$, $AP_3$ and $AP_4$ in leaf material of *A. paniculata* used in the present study were more than twice as high than those found in the stem (Table 1). However, Cheung *et al.* (2001) found that the stem of *A. paniculata* contained the highest levels of these

**Table 1. The contents of andrographolide ($AP_1$), 14-deoxy-11,12-didehydroandrographolide ($AP_3$) and neoandrographolide ($AP_4$) in leaves and stems of *A. paniculata***

| | Contents (mg/g dry weight)[a] | | |
|---|---|---|---|
| Sample | $AP_1$ | $AP_3$ | $AP_4$ |
| Leaves | 17.45 ± 0.16 | 17.38 ± 0.11 | 6.14 ± 0.07 |
| Stems | 8.37 ± 0.12 | 2.64 ± 0.06 | 0.37 ± 0.02 |

[a] Values represent the mean ± standard error ($n$ = 3).

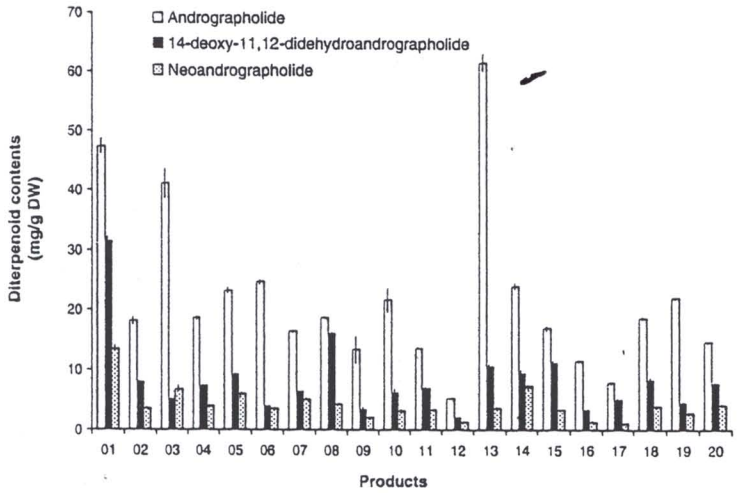



**Figure 3.** Variation in the contents of $AP_1$, $AP_3$ and $AP_4$ in products of *A. paniculata* from different suppliers in Thailand. Values shown are means ($n$ = 3) and standard error bars.

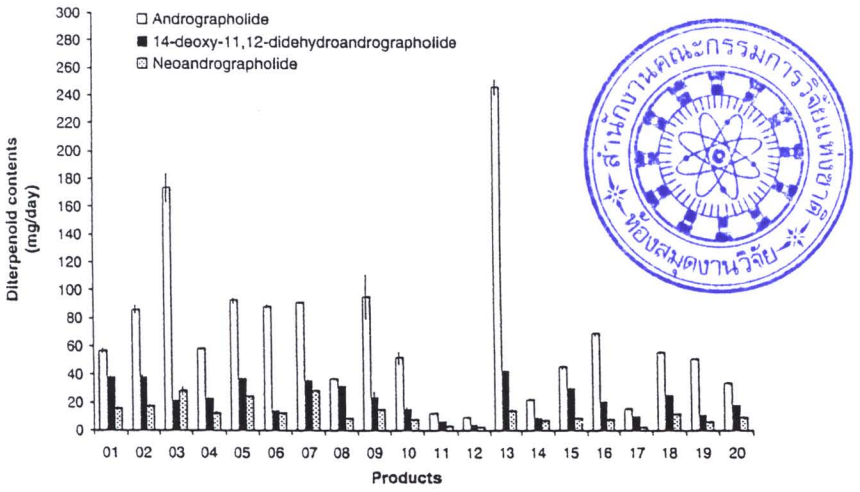





**Figure 4.** The amounts of $AP_1$, $AP_3$ and $AP_4$ in recommended daily doses of products of *A. paniculata* from different suppliers in Thailand. Values shown are means ($n = 3$) and standard error bars.

compounds. The divergence between these two studies may be due to the difference in genotypes used, including the effects of different environments where the plants grow and the harvesting time of this plant, which affects the content of the active compounds in the plant. Therefore, good agricultural practice (GAP) also plays an important role in the quality control of plant materials and the production of herbal medicinal products.

### Total lactone contents

Before storage, the total lactone content in dried leaves from Sakaew province (sample $S_2$) was 10.67% ($n = 3$). After storage of the dry herb at room temperature, the total lactone content decreased over time (Fig. 5) by 5.3% after 3 months, 7.6% after 6 months, and 32.6% after 9 months. These results agree with the previous results from Dechatiwonse Na Ayudhya *et al.* (1993), who reported that the total lactone content in dried, powdered aerial parts of *A. paniculata* decreased by 26% after storage of this herb under ambient conditions for up to 1 year. However, the raw materials from Sakaew province analysed in the present study still possessed large quantities of the active compounds according to the standard specification of this plant in Thailand, which indicated that the total lactone content calculated as andrographolide should not be less than 6%.

### Stability of three diterpenoids in dry herb of *A. paniculata*

In contrast to the total lactone content, the total content of the diterpenoids $AP_1 + AP_3 + AP_4$ decreased slightly during the first 3 months and then increased over storage time in all samples tested (Fig. 6). At the end of experiment, the content of these compounds increased by 19.9, 8.1, 30.3 and 7.9% for $S_1$, $S_2$, $S_3$ and $S_4$, respectively. These results indicate that the content of these diterpenoids accounts for only a portion of the total lactone contents.

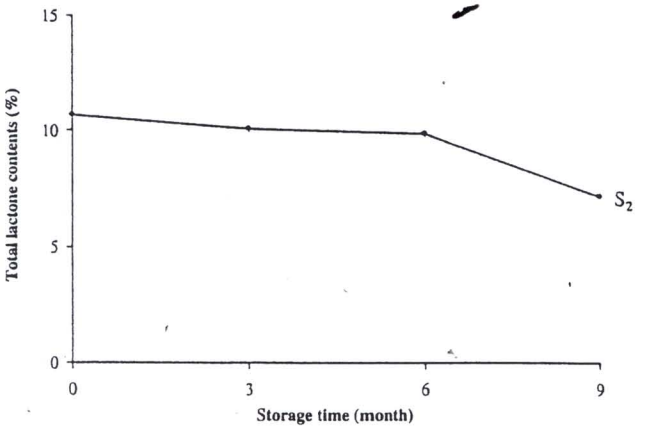



**Figure 5.** Stability of the total lactone content of leaves of *A. paniculata* (sample $S_2$ harvested on 25 July, 2001). Values shown are means ($n = 2$) and standard error bars.

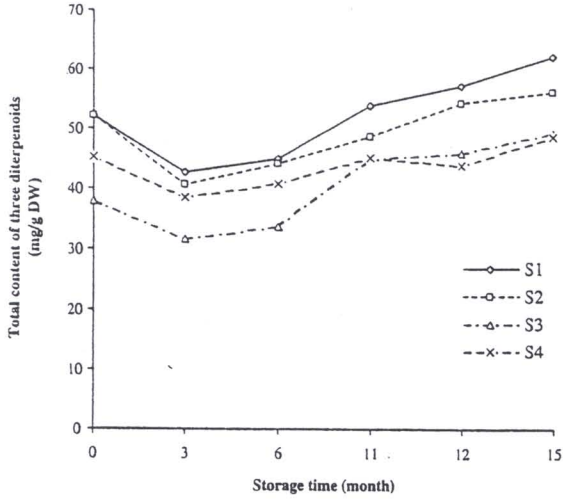



**Figure 6.** Changes in the total contents of the diterpenoids $AP_1$ + $AP_3$ + $AP_4$ in leaves of *A. paniculata* during storage (samples $S_1$, $S_2$, $S_3$ and $S_4$ harvested on 18 July, 25 July, 26 July and 8 August 2001, respectively).

The content of $AP_1$ in all samples decreased during the first 3 months and then remained constant for up to 15 months (Fig. 7). The maximum decrease in $AP_1$ was found in sample $S_3$ (26.1%) followed by sample $S_2$ (20.5%), sample $S_4$ (12.5%) and sample $S_1$ (11.6%). In contrast, the content of $AP_3$ increased with storage time after a slight decrease at the beginning (3 months). After 15 months, the content of $AP_3$ in all samples increased by more than 50%. The maximum increase was found in sample $S_3$ where the content of $AP_3$ increased more than 100%. This may indicate release of this compound from precursor or inter-conversion of other diterpenoids. The content of $AP_4$ fluctuated during the storage time: in samples $S_1$, $S_2$ and $S_3$ $AP_4$ levels remained constant over the first 3 months while the content in sample $S_4$ decreased. After 6 months, $AP_4$ content decreased in all samples except for $S_3$ where the content increased after 11 months followed by a decrease until the end of experiment (Fig. 7).

Lomlim *et al.* (2003) studied the stability of pure $AP_1$ (crystal and amorphous forms) at 70°C (75% relative humidity) for 3 months and found that crystalline andrographolide was highly stable over a period of 3 months whilst its amorphous form degraded rapidly during 2 months' storage under the same conditions. The major degradation product of $AP_1$ was $AP_3$. In the present study using *A. paniculata* dry herb, the $AP_3$ content increased over a period of time while the levels of $AP_1$ and $AP_4$ decreased. These results would be partly explained if $AP_1$ was transformed during storage to $AP_3$ as the major decomposition product. However, the increase of $AP_3$ was greater than the decrease of $AP_1$, which indicated that there may be other compounds (including $AP_4$) in this herb that could also degrade to yield $AP_3$. In fact $AP_4$ was only present in small amounts in this herb and hence its transformation would have little effect on the increase of $AP_3$. The stability of the pure compounds $AP_1$, $AP_3$ and $AP_4$ need to be studied further at both room temperature and under accelerated conditions, and in relation to the other lactones in the samples. The changes in the contents

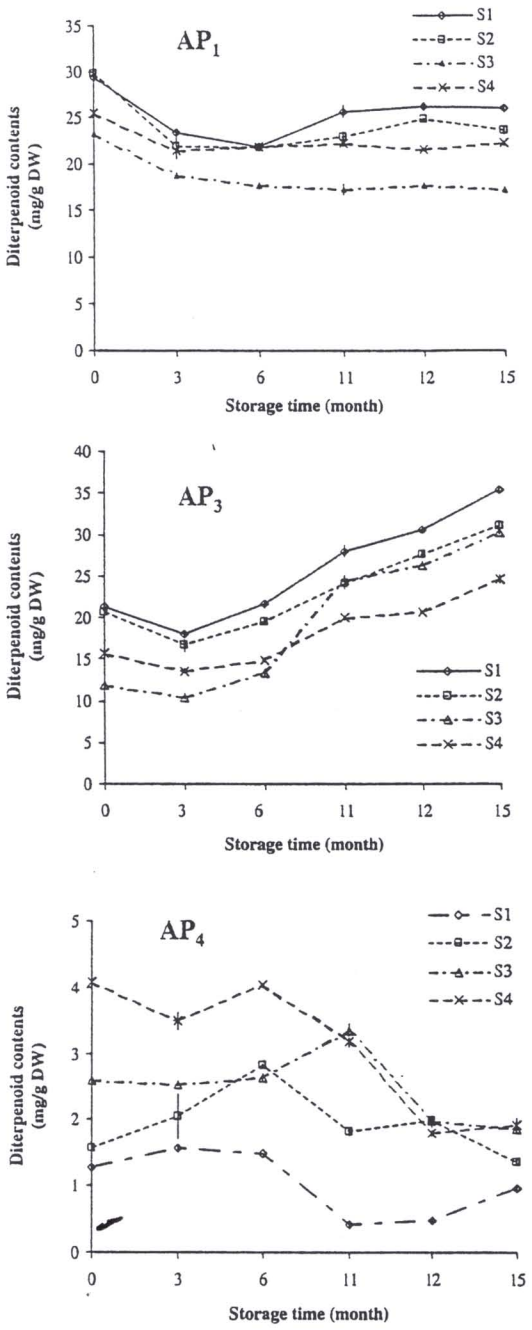



**Figure 7.** Changes in the contents of $AP_1$, $AP_3$ and $AP_4$ in dry herb of *A. paniculata* stored at room temperature for up to 15 months (samples $S_1$, $S_2$, $S_3$ and $S_4$ harvested on 18 July, 25 July, 26 July and 8 August 2001, respectively). Values shown are means ($n$ = 3) and standard error bars.

of these three active compounds in *A. paniculata* during storage will also affect the clinical treatment and safety of this plant.

There are many *A. paniculata* products in Thailand, and there are large variations in the contents of three active diterpenoids in the products; therefore, it is important to have a reliable and accurate method to control the quality of the raw materials, the production

processes and the finished products. The simple and reproducible extraction and HPLC method developed in this study provided stable retention times, a low detection limit, and good separation of the analytes in complex plant extracts. The method is rapid and can be used to analyse a large number of samples per day. The contents of the three diterpenoids ($AP_1 + AP_3 + AP_4$) and the total lactone content of the dry herb of *A. paniculata* varied during storage. Therefore, the stability of these active compounds should be taken into account when this herb is stored for a period of time to ensure the efficacy and safety of these compounds.

## Acknowledgements

We gratefully acknowledge Jittra Saehun and Tawit Suriyo (Laboratory of Pharmacology, Chulabhorn Research Institute) for their technical assistance in HPLC and the total lactone analysis in this study. This work was partially supported by UNDP/RAS/97/001 Project and Thailand Research Fund (S.R.).

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# Ziziphine N, O, P and Q, new antiplasmodial cyclopeptide alkaloids from *Ziziphus oenoplia* var. *brunoniana*

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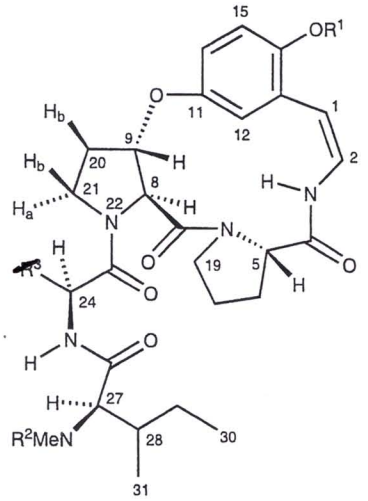
Received 21 May 2004; revised 1 November 2004; accepted 18 November 2004

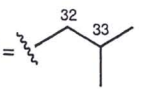
Available online 13 December 2004

**Abstract**—Bioassay-guided fractionation of the EtOAc extract of the roots of Thai *Ziziphus oenoplia* var. *brunoniana* resulted in the isolation of four new 13-membered cyclopeptide alkaloids of the 5(13) type, ziziphine N–Q. The structures of the new metabolites were elucidated on the basis of spectroscopic analyses and the stereochemical assignments were established by comparison with other related compounds of known stereochemistry. Ziziphine N and Q exhibited significant antiplasmodial activity against the parasite *Plasmodium falciparum* with the inhibitory concentration ($IC_{50}$) values of 3.92 and 3.5 μg/mL, respectively. Ziziphine N and Q also demonstrated weak antimycobacterial activity against *Mycobacterium tuberculosis* with the same MIC value of 200 μg/mL.


## 1. Introduction

The Rhamnaceous *Ziziphus* (formerly known as *Zizyphus*) species have been investigated due to the rich source of new and/or bioactive cyclopeptides.[1–3] To date over 170 cyclopeptides have been published[1–3] which can be classified into five groups of the 4(13)-, 5(13)-, 4(14)-, 5(14)- and 4(15)-type of compounds.[3] Among these, 81 cyclopeptide alkaloids have been reported from various *Ziziphus* species and these include 35 13-membered, 39 14-membered and seven 15-membered ring cyclopeptides.[2] Some *Ziziphus* plants have been found to possess biological activities, for example sedative,[4] hypoglycemic,[5] antibacterial and antifungal activities.[6] *Ziziphus oenoplia* (L.) Mill. is a thorny sprawling bush, widely spread and used traditionally as a folk medicine in Thailand for its antiinfectious, antidiabetic and diuretic activities.[7–8] Previous phytochemical studies of this plant species resulted in the isolation of cyclopeptides of the 5(13)-zizyphine-A type (zizyphine A–C,[9] F,[10] I[11] and K),[12] 4(14)-amphibine-B type (zizyphine H),[2,13] 4(14)-amphibine-F type (zizyphine G),[10]




**1** Ziziphine N: $R^1 = R^2 = Me$, $R^3 =$

**2** Ziziphine O: $R^1 = Me$, $R^2 = H$, $R^3 = CH_2CH(CH_3)_2$
**3** Ziziphine P: $R^1 = H$, $R^2 = Me$, $R^3 = CH_2CH(CH_3)_2$
**4** Ziziphine Q: $R^1 = R^2 = Me$, $R^3 = CH(CH_3)_2$
**5** Zizyphine A: $R^1 = R^2 = Me$, $R^3 = CH(CH_3)CH_2CH_3$

*Keywords*: *Ziziphus oenoplia* var. *brunoniana*; Rhamnaceae; Cyclopeptide alkaloid; Ziziphine N–Q; Antiplasmodial activity; Antimycobacterial activity.
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doi:10.1016/j.tet.2004.11.053

4(14)-frangulanine-type (frangufoline),[14] 4(15)-mucronine-A type (abyssinine A–B and zizyphine D–E)[9] and 5(14)-amphibine-B type (amphibine B and mauritine D).[14] It should be noted that the structures of zizyphine J, L and M have not been presented.[1–3,12] In continuation of the search for bioactive substances of new structural type from Thai natural resources,[15–17] we have found that the root extract of the Thai *Z. oenoplia* (L.) Mill. var. *brunoniana* (Cl. ex Brand.) Tard exhibited a significant in vitro antimalarial potential against *Plasmodium falciparum*. In this paper we describe the isolation and structure elucidation of four new 13-membered cyclopeptide alkaloids of the 5(13)-type, ziziphine N–Q (**1–4**) from the roots of this plant species.

## 2. Results and discussion

The pulverized, dried root of *Z. oenoplia* var. *brunoniana* was successively extracted with *n*-hexane, EtOAc and MeOH. The resulting fractions were tested for antiplasmodial activity and the EtOAc extract, which exhibited antiplasmodial activity, was subjected to further investigation. Bioassay-directed fractionation and chromatographic separation of this extract resulted in the isolation of four new 13-membered cyclopeptide alkaloids of the 5(13) type, ziziphine N–Q (**1–4**).

The major metabolite, ziziphine N (**1**), a colorless solid, mp 117–119 °C, displayed a pseudomolecular ion at *m/z* 612 $[M+H]^+$ in the EIMS, and the molecular formula $C_{33}H_{49}N_5O_6$ was established by HRFABMS (*m/z* 612.3769 $[M+H]^+$, $\Delta+0.8$ mmu). Compound **1**, as well as the metabolites **2–4**, gave a very faint positive coloration with Dragendorff's reagent and a blue coloration with anisaldehyde-$H_2SO_4$ reagent. The $^1H$ NMR spectrum of **1** (Table 1) displayed signals corresponding to two olefinic protons, three aromatic protons, one methoxyl and a number of methine and methylene protons. While the $^{13}C$ NMR spectrum showed 33 carbon signals and DEPT analysis provided signals for four methyls, one *N,N*-dimethyl, one methoxyl, seven methylenes, 12 (including one oxygenated and two olefinic) methines and seven quaternary carbons,

Table 1. $^1H$ and $^{13}C$ NMR spectral data for compounds **1–4** in $CDCl_3$

| | $\delta_H$* | | | | $\delta_C$ | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 1 | 5.82 d (8.9) | 5.92 d (8.8) | 5.89 d (8.6) | 5.94 d (8.7) | 106.6 | 106.6 | 105.9 | 106.6 |
| 2 | 6.79 dd (11.9, 8.9) | 6.92 dd (11.5, 8.8) | 6.90 dd (11.5, 8.6) | 6.94 dd (8.7, 11.5) | 121.3 | 121.6 | 121.5 | 121.6 |
| 3 | 8.22 d (11.9) | 8.33 d (11.5) | 8.33 d (11.5) | 8.34 d (11.5) | | | | |
| 4 | | | | | 167.5 | 167.7 | 168.0 | 167.7 |
| 5 | 4.39 dd (8.9, 3.5) | 4.53 dd (9.1, 3.8) | 4.52 dd (9.0, 3.8) | 4.51 m | 61.8 | 62.0 | 62.0 | 62.1 |
| 7 | | | | | 171.3 | 171.6 | 171.7 | 171.5 |
| 8 | 4.26 d (5.4) | 4.38 d (5.7) | 4.34 d (5.7) | 4.39 d (5.7) | 62.5 | 62.7 | 62.8 | 62.6 |
| 9 | 5.09 ddd (8.8, 7.4, 5.4) | 5.24 ddd (8.9, 7.1, 5.7) | 5.18 ddd (7.8, 7.5, 5.7) | 5.25 dt (6.7, 5.7) | 78.3 | 78.5 | 78.4 | 78.5 |
| 11 | | | | | 150.6 | 151.2 | 150.7 | 150.9 |
| 12 | 6.65 br s | 6.73 br s | 6.68 d (2.0) | 6.83 br s | 110.4 | 110.7 | 110.2 | 110.7 |
| 13 | | | | | 123.7 | 124.0 | 122.6 | 124.0 |
| 14 | | | | | 151.0 | 150.8 | 148.0 | 151.2 |
| 15 | 6.75 d (9.6) | 6.83 d (8.1) | 6.85 d (8.8) | 6.85 m | 113.6 | 113.7 | 117.7[a] | 113.5 |
| 16 | 6.70 d (9.6) | 6.80 d (8.1) | 6.73 dd (8.8, 2.0) | 6.85 m | 116.8 | 116.9 | 118.8[a] | 116.9 |
| 17a | 2.10 m | 2.20 m | 2.20 m | 2.25 m | 28.8 | 29.0 | 29.0 | 29.0 |
| 17b | 1.83 m | 1.95 m | 1.95 m | 2.00 m | | | | |
| 18a | 1.83 m | 1.95 m | 1.95 m | 2.00 m | 24.6 | 24.9 | 24.9 | 24.9 |
| 18b | 1.68 m | 1.60 m | 1.95 m | 1.85 m | | | | |
| 19a | 4.12 m | 4.25 m | 4.20 m | 4.21 m | 47.6 | 47.8 | 47.9 | 47.8 |
| 19b | 3.17 m | 3.25 m | 3.23 m | 3.32 m | | | | |
| 20a | 2.25 m | 2.50 m | 2.45 m | 2.45 m | 32.4 | 32.7 | 32.6 | 32.6 |
| 20b | 2.33 m | 2.35 m | 2.35 m | 2.35 m | | | | |
| 21a | 4.12 m | 4.25 m | 4.20 m | 4.21 m | 45.1 | 45.3 | 45.3 | 45.5 |
| 21b | 3.49 m | 3.60 m | 3.60 m | 3.63 m | | | | |
| 23 | | | | | 171.0 | 171.2 | 171.3 | 170.8 |
| 24 | 4.66 dt (8.1, 7.8) | 4.47 dt (8.6, 7.4) | 4.74 q (8.5) | 4.51 m | 47.6 | 47.8 | 48.0 | 54.9 |
| 25 | 6.81 d (8.1) | 7.50 d (8.6) | 6.95[a] | 6.90[b] | | | | |
| 26 | | | | | 171.6 | 173.4 | 172.2 | 172.1 |
| 27 | 2.45 d (5.4) | 2.83 d (4.6) | 2.55 d (5.3) | 2.58 d (5.5) | 74.0 | 69.8 | 74.4 | 74.5 |
| 28 | 1.68 m | 1.75 m | 1.80 m | 1.85 m | 34.0 | 38.2 | 34.3 | 34.3 |
| 29a | 1.39 m | 1.50 m | 1.50 m | 1.55 m | 26.6 | 25.3 | 27.0 | 26.9 |
| 29b | 1.08 m | 1.15 m | 1.20 m | 1.20 m | | | | |
| 30 | 0.80 t (6.7) | 0.90 d (6.5) | 0.90 t (6.8) | 0.93 t (6.8) | 11.7 | 11.7 | 12.0 | 11.9 |
| 31 | 0.75 d (6.5) | 0.86 d (6.5) | 0.85 d (6.6) | 0.95 d (6.2) | 14.1 | 15.8 | 14.5 | 14.7 |
| 32a | 1.42 m | 1.50 m | 1.45 m | 2.00 m | 40.5 | 40.9 | 40.6 | 30.8 |
| 32b | 1.39 m | 1.50 m | 1.45 m | | | | | |
| 33 | 1.52 m | 1.75 m | 1.65 m | 0.93 d (6.6) | 24.4 | 24.6 | 24.7 | 18.5[f] |
| 34 | 0.80 d (6.5) | 0.90 d (6.5) | 0.89 d (6.8) | 0.90 d (6.6) | 22.9[c] | 23.0[d] | 23.1[e] | 19.0[f] |
| 35 | 0.80 d (6.5) | 0.90 d (6.5) | 0.89 d (6.8) | | 21.4[c] | 21.8[d] | 21.6[e] | |
| OMe | 3.66 s | 3.77 s | | 3.79 s | 55.7 | 55.9 | | 55.9 |
| NMe | 2.12 s | 2.36 s | 2.22 s | 2.24 s | 42.8 | 36.0 | 43.2 | 43.0 |

*The *J* values are in Hz in parentheses. [a,b]Partially obscured signal. [c–f]Signals under the same superscript may be reversed.

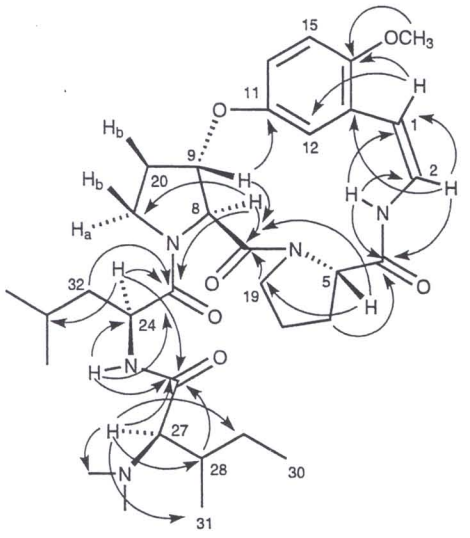


**Figure 1.** Selected HMBC correlations for **1**.

four of which corresponded to the carbonyl groups (Table 1).

Analysis of the $^{1}H$–$^{1}H$ COSY and $^{1}H$–$^{13}C$ HMQC spectra of **1** and comparison with the reported value[3,18] led to the assignments of the spin systems for amino acid units of proline, 3-oxygenated proline, leucine and *N*,*N*-dimethylisoleucine. The presence of a *meta*-oxygenated *Z*-styrylamino group suggested that **1** was a 13-membered cyclopeptide alkaloid which was further supported by its UV spectrum (266 and 319 nm).[19] Its IR spectrum showed the presence of amino (3399 $cm^{-1}$), amide (1693, 1653, 1641 $cm^{-1}$), styryl double bond (1625 $cm^{-1}$) and phenol ether (1223 and 1054 $cm^{-1}$) functionality. Connections among these subgroups were provided by analysis of HMBC and NOESY spectra (Figs. 1 and 2, respectively). Thus the resonance at $\delta$ 8.22 of *m*-oxystyrylamino-NH at the 3-position showed HMBC cross peaks with C-1, C-2 and C-4. Correlations of H-2 to C-1, C-4 and C-13 and H-1 to C-12 were also observed. The correlations of H-5, H-8 and H-9 to C-7 revealed the connection between the proline and β-oxyproline amino acids. Furthermore, HMBC correlation from H-9 to C-11 confirmed that the β-oxyproline unit was attached to the aryl group. The cross-peak of the methoxyl signal at $\delta$ 3.66 with a quaternary aromatic carbon signal of C-14 at $\delta$ 151.0 in HMBC spectrum confirmed that the methoxyl group was placed at C-14 of the 13-membered cyclopeptide feature. The overlapping of the aromatic proton signals in the $^{1}H$ NMR spectroscopic data recorded in $CDCl_3$ were clearly resolved when the spectrum was recorded in DMSO-$d_6$ (see Section 3). The strong NOE effect displayed between the methoxyl group and H-15 in the NOESY experiment (Fig. 2) in DMSO-$d_6$ further supported the placement of the methoxyl group at C-14. The H-8 proton and the leucyl protons H-24 showed connectivities with C-23 in which the latter signal proton also exhibited cross-peaks with C-26 and C-33 in HMBC spectrum. NOE interactions observed between H-24 and H-21 in the NOESY spectrum further revealed that the position of the leucine unit was attached to the hydroxyproline amino acid. The HMBC correlations of NH-25 to C-24 and C-26, and of the isoleucyl proton H-27 to C-26, C-29, C-31 and *N*,*N*-dimethyl carbon were also observed. Thus the connections between the leucyl group and the hydroxyprolyl *N* as well as the isoleucyl *N* were established.

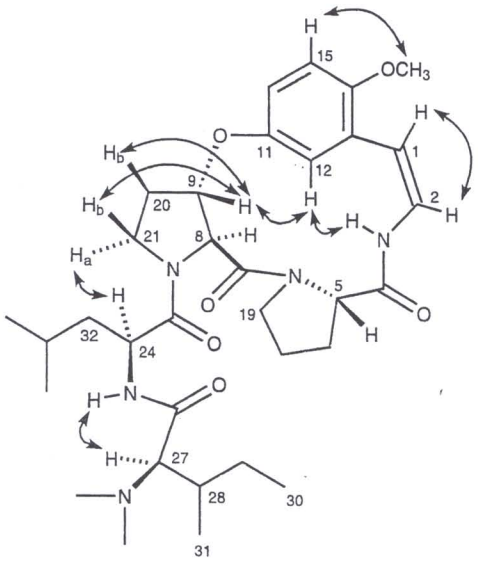


**Figure 2.** Key NOESY correlations for **1**.

The mass spectrum of compound **1** followed the typical fragmentation pattern of a zizyphine A-type 13-membered cyclopeptide alkaloid.[1] The base peak at *m*/*z* 114 represents the amine fragment which indicates that the end amino acid is *N*,*N*-dimethylisoleucine. Compound **1** and zizyphine A (**5**)[3,20] showed similar mass fragmentation patterns indicating their gross structural similarity. Based on these findings the structural framework of ziziphine N was proved to be **1**, which differs from that of zizyphine A (**5**) in having a leucine instead of an isoleucine amino acid attached to the hydroxyproline unit of the macrocyclic ring system.

The *Z*-geometry of the 1,2-double bond was established on the basis of the coupling constant value of 8.9 Hz for H-1 and H-2. The relative stereochemistry at the C-5 position of the amino acid (proline) and the C-8 and C-9 positions of the β-hydroxyproline units were determined by analysis of $^{1}H$ NMR coupling constants and NOESY interactions (Fig. 2). The small vicinal coupling constant value 5.4 Hz of the methine protons H-8 and H-9 indicated a *trans* configuration.[21,22] No significant NOE observed in NOESY spectrum between these two protons also supported the *trans* relationship between H-8 and H-9.[21] Strong NOE enhancements between NH-3 and the aromatic proton H-12 and between the later proton and H-9 were observed. Moreover the coupling constant value between H-2 and NH-3 ($J$=11.9 Hz) implied that they were in *trans* coplanar position.[21] No NOE interaction shown between H-5 and NH-3 revealed the opposite orientation of these two protons. Strong correlations were observed between H-9 and both H-20b and H-21b, but not with H-20a and H-21a, indicating

that H-9, H-20b and H-21b were on the same side of the pyrrolidine ring. The $^{1}H$ and $^{13}C$ NMR spectroscopic data of **1** were very similar to those of the natural zizyphine A (**5**),[3,23] except for those of the leucine moiety in the former and the isoleucine in the latter, suggesting the relative stereochemistry of these two compounds to be the same. Since the total synthesis of **5** was achieved and it was identical to the natural zizyphine A in all respects,[24] and since the amino acid units of the acyclic part attached to N-22 were those of L-isoleucine and L-*N*,*N*-dimethylisoleucine, the configuration of the corresponding amino acid units (leucine and *N*,*N*-dimethylisoleucine) of **1** could possibly be L. Furthermore, the $^{1}H$ and $^{13}C$ NMR spectroscopic data of the leucine unit (C-23, C-24 and C-32 to C-35) of **1** were very similar to those of mucronine D, the structurally related 13-membered cyclopeptide alkaloid isolated from *Z. mucronata* and the absolute configuration of the amino acids of which was determined as L by degradation of the alkaloid and analysis of the amino acids.[25] In addition, the $^{1}H$ and $^{13}C$ NMR spectroscopic data of the *N*,*N*-dimethylisoleucine moiety (C-26 to C-31 and $N(CH_3)_2$) in **1** were also very similar to those of compound **5**.[3,23] The stereochemistry of the two acyclic amino acid moieties was thus tentatively inferred to be L. Therefore, the absolute configuration of ziziphine N is as shown in **1**.

Ziziphine O (**2**) was obtained as a colorless solid, mp 106–108 °C. On the basis of its HRFABMS (*m/z* 598.3604, $[M+H]^+$, $\Delta$ −0.1 mmu) the molecular formula of **2** was established as $C_{32}H_{47}N_5O_6$. The UV and IR absorption spectra of **2** were similar to those of **1** suggesting the presence of styrylamine chromophore of 13-membered cyclopeptide alkaloid.[19] The $^{1}H$ NMR spectrum of **2** (Table 1) were almost identical to that of **1**; the different features are the signals at $\delta$ 7.50 (H-25), $\delta$ 2.83 (H-27) and $\delta$ 1.75 (H-28). Furthermore, the singlet signal at $\delta$ 2.36, integrating for three protons, corresponded to one *N*-methyl group instead of two as found in **1**. The main differences between the $^{13}C$ NMR spectra of **1** and **2** (Table 1) are those of C-26, C-27, C-28 and the *N*-methyl carbon which are in agreement with a structural change in the region of the isoleucine residue. The HMBC and NOESY information revealed that the two compounds possessed the same molecular framework. α-Cleavage at the terminal amino acid led to the base peak at *m/z* 100 in EIMS data also provided the evidence for the presence of a *N*-methylisoleucine unit. Thus the structure of **2** was deduced to be the *N*-desmethyl analogue of ziziphine N.

The minor cyclopeptide alkaloid, ziziphine P (**3**), was obtained as a colorless solid, mp 127–129 °C. The molecular formula $C_{32}H_{47}N_5O_6$ was derived from the HRFABMS where the $[M+H]^+$ ion was observed at *m/z* 598.3590 ($\Delta$ −1.5 mmu). Its 1D ($^{1}H$ and $^{13}C$ NMR (Table 1), DEPT and 2D (COSY, HMQC, HMBC and NOESY) spectral data were very similar to those of **1** except for the absence of the methoxyl signal, indicating that **3** was another ziziphine N analogue. The IR and molecular composition revealed that **3** contained a phenolic group as compared with **1**. The structure of **3** was, therefore, established as the *O*-desmethyl analogue of ziziphine N.

Ziziphine Q (**4**), isolated as a colorless solid, was found to have a molecular formula of $C_{32}H_{47}N_5O_6$ by HRFABMS (*m/z* 598.3607, $[M+H]^+$, $\Delta$ +0.2 mmu). Its UV and IR spectroscopic data suggested that **4** also possessed a 13-membered cyclopeptide. The $^{1}H$ and $^{13}C$ NMR spectroscopic data (Table 1) of **4** closely resembled to those of ziziphine N (**1**) except for the presence of a valine unit [$\delta_H$ 4.51 (H-24), $\delta_C$ 54.9 (C-24); $\delta_H$ 2.00 (H-32), $\delta_C$ 30.8 (C-32); $\delta_H$ 0.93 (H-33), $\delta_C$ 18.5 (C-33) and $\delta_H$ 0.90 (H-34), $\delta_C$ 19.0 (C-34)] instead of leucine amino acid residue. The absence of one methylene unit observed in the DEPT spectrum of **4** as compared with **1** also supported that compound **4** possessed a valine group. The identical HMBC and NOESY data of **4** in comparison with those of **1** confirmed that the valinyl group in **4** was located in the same position as those found for the leucine moiety in compound **1**. Thus ziziphine Q possessed the structure **4** with valine as the amino acid residue bound to the hydroxyproline of the macrocyclic ring and connected to the isoleucyl residue. In fact ziziphine **4** was a derivative of zizyphine K, a compound obtained from a Pakistani *Z. oenoplia*.[12] The only difference was that the hydroxyl group in zizyphine K was replaced by a methoxyl group at C-14 to give **4**. The structure of **4** was, therefore, concluded to be *O*-methyl zizyphine K.

Rhamnaceous cyclopeptide alkaloids are generally composed of L-amino acids and *trans*-β-hydroxy-L-proline, including zizyphine A-type alkaloid.[3] Due to the similarity of the $^{1}H$ and $^{13}C$ NMR spectral data and levorotatory optical rotations of compounds **2**–**4** as compared with **1**, it is thus concluded that ziziphine O–Q share the same stereochemistry as that of ziziphine N and zizyphine A.

Many 13-membered cyclopeptides constructed with a variation of amino acid residues have been reported.[1–3] However, to our knowledge those with leucine and isoleucine residues are rare. Ziziphine N–Q (**1**–**4**) are additional members of the 5(13)-membered cyclopeptides which belong to the zizyphine A-type. It should be noted that while ziziphine N (**1**) was the major alkaloid of the Thai *Z. oenoplia* var. *brunoniana*, zizyphine A and its analogues were not detected.

All isolates **1**–**4** were tested in vitro for antimalarial potential against *P. falciparum*.[26,27] No activity was observed in ziziphine O (**2**) and P (**3**). On the other hand, ziziphine N (**1**) and Q (**4**) demonstrated significant antiplasmodial activity with the $IC_{50}$ values of 3.92 and 3.5 μg/mL, respectively. The cyclopeptides **1** and **4** also exhibited weak antituberculosis activity against *Mycobacterium tuberculosis*[28] with the same MIC value of 200 μg/mL, whilst cyclopeptides **2** and **3** were found inactive in the same test. All metabolites did not show cytotoxicity to KB and BC cell lines[29] at $IC_{50}$ value of 20 μg/mL. Based on these observations, the preliminary structure–activity relationship regarding these alkaloids was tentatively summarized that both the methoxyl and the *N*,*N*-dimethylamino groups in **1** and **4** are crucial for the activity. In addition, substitution of leucine unit in **1** with valine moiety in **4** showed similar trend of biological activities. To the best of our knowledge, this is the first report of in vitro antiplasmodial and antimycobacterial activities from the Rhamnaceous plants.

## 3. Experimental

### 3.1. General experimental procedures

Melting points were determined using a Griffin melting point apparatus. Optical rotations were measured on a Jasco digital polarimeter. UV spectra were obtained on a Shimadzu UV-2401 PC spectrophotometer. IR spectra were recorded on a Perkin Elmer FT-IR Spectrum BX spectrophotometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AVANCE 300 FT-NMR spectrometer, operating at 300 MHz ($^1$H) and 75 MHz ($^{13}$C). For the spectra taken in $CDCl_3$ and DMSO-$d_6$, the residual nondeuterated solvent signals at $\delta$ 7.24 and $\delta$ 2.49 and the solvent signals at $\delta$ 77.00 and $\delta$ 39.50 were used as references for $^1$H and $^{13}$C NMR spectra, respectively. EI and FAB mass spectra were run on a Thermo Finnigan Polaris Q and a Finnigan MAT 90 instruments. Column chromatography and TLC were carried out using Merck silica gel 60 (<0.063 mm) and precoated silica gel 60 $F_{254}$ plates, respectively. Plates of silica gel $PF_{254}$, thickness 1.25 mm, were used for preparative TLC. Spots on TLC were visualized under UV light and by spraying with anisaldehyde-$H_2SO_4$ followed by heating.

### 3.2. Materials and methods

The roots of *Z. oenoplia* (L.) Mill. var. *brunoniana* (Cl. ex Brand.) Tard was collected from Chanae District, Naratiwat Province, Thailand, in April 1999 and a voucher specimen, (Mayuso Kuno 002) is deposited at the CMU Herbarium, Faculty of Science, Chiang Mai University, Thailand.

### 3.3. Extraction and separation

Pulverized, dry root (5.16 kg) of *Z. oenoplia* var. *brunoniana* was defatted with hexane and then extracted successively with EtOAc and MeOH at 50 °C for 48 h and the solvents were evaporated to yield the EtOAc (58.2 g) and MeOH (40.2 g) extracts, respectively. The EtOAc extract exhibited antiplasmodial activity, whereas the MeOH extract was found inactive. Thus, the EtOAc soluble fraction (44.7 g) was investigated extensively through serial fractionations by quick column chromatography,[30] eluted with a gradient system, to provide eight major fractions. Fraction seven (7.10 g) which was active to the antiplasmodial test was subjected to column chromatography employing solvent gradient $CHCl_3$–MeOH and eight subfractions (1–8) were collected. A portion of subfraction 2 (87 mg) was further chromatographed, eluting with EtOAc, to give ziziphine N (**1**, 43 mg) and ziziphine Q (**4**, 10 mg). Ziziphine O (**2**, 29 mg) and ziziphine P (**3**, 25 mg) were obtained after repeated column chromatography of subfractions 3 (110 mg, eluting with $CHCl_3$–MeOH, 98:2) and subfraction 4 (132 mg, eluting with $CHCl_3$–MeOH, 97:3), respectively. Other fractions contained small quantities of the isolated cyclopeptide alkaloids **1–4** and other type of compounds.

**3.3.1. Ziziphine N (1).** Colorless solid, mp 117–119 °C; $[\alpha]_D^{30} = -326.6$ (*c* 0.18, $CHCl_3$); UV (EtOH) $\lambda_{max}$ 266 (log $\varepsilon$ 4.40), 319 nm (log $\varepsilon$ 4.28); IR $\nu_{max}$ (KBr) 3399, 2960, 2926, 2826, 2783, 1693, 1653, 1641, 1625, 1511, 1223, 1054, 772 cm$^{-1}$; $^1$H (300 MHz, $CDCl_3$) and $^{13}$C NMR (75 MHz, $CDCl_3$), see Table 1; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.44 (1H, d, $J$=10.6 Hz, NH-3), 8.11 (1H, d, $J$=8.0 Hz, NH-25), 6.98 (1H, d, $J$=9.0 Hz, H-15), 6.81 (1H, dd, $J$=9.0, ca. 2.0 Hz, H-16), 6.77 (1H, dd, $J$=10.6, 7.6 Hz, H-2), 6.71 (1H, d, ca. 2.0 Hz, H-12), 5.86 (1H, d, $J$=7.6 Hz, H-1), 5.03 (1H, ddd, $J$=8.5, 7.3, 6.1 Hz, H-9), 4.62 (1H, dt, $J$=8.0, 6.0 Hz, H-24), 4.27 (1H, d, $J$=6.1 Hz, H-8), 4.15 (1H, m, H-19a), 4.03 (2H, m, H-5 and H-21a), 3.71 (3H, s, $OCH_3$), ca. 3.40 (1H, m, H-19b), ca. 3.30 (1H, m, H-21b), 2.70 (1H, d, $J$=10.2 Hz, H-27), 2.50 and 2.20 (2×1H, each m, H-20), 2.15 (2×3H, s, $N(CH_3)_2$), 2.10 and 1.75 (2×1H, each m, H-17), 1.80 (3H, m, H-18 and H-28), 1.60 and 1.15 (2×1H, each m, H-29), 1.40 and 1.20 (2×1H, each m, H-32), 1.10 (1H, m, H-33), 0.86 (3H, d, $J$=6.2 Hz, H-35),[a] 0.85 (3H, d, $J$=6.4 Hz, H-34),[a] 0.80 (3H, t, $J$=7.3 Hz, H-30), 0.71 (3H, d, $J$=6.4 Hz, H-31), ('a' stands for the assignments may be reversed for signals with the same superscript); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 171.0 (C-7 and C-23), 169.8 (C-26), 167.8 (C-4), 150.9 (C-11), 150.6 (C-14), 123.8 (C-13), 121.9 (C-2), 116.5 (C-16), 113.6 (C-15), 111.6 (C-12), 107.5 (C-1), 79.1 (C-9), 70.6 (C-27), 62.4 (C-8), 62.2 (C-5), 55.7 ($OCH_3$), 47.8 (C-19), 47.5 (C-24), 44.7 (C-21), 41.3 (N-$CH_3$), 39.5 (C-32), 32.3 (C-28 and C-20), 28.6 (C-17), 24.9 (C-29), 24.6 (C-18), 24.2 (C-33), 23.1 (C-34), 21.1 (C-35), 15.5 (C-31), 10.3 (C-30); EIMS *m/z* 612 $[M+H]^+$ (60), 596 (15), 582 (95), 568 (65), 554 (90), 497 (45), 496 (45), 454 (55), 358 (2), 216 (2), 114 (100), 86 (5), 70 (10); HRFABMS (positive ion mode) *m/z* 612.3769 $[M+H]^+$ (calcd for $C_{33}H_{49}N_5O_6$+H, 612.3761).

**3.3.2. Ziziphine O (2).** Colorless solid, mp 106–108 °C; $[\alpha]_D^{31} = -380.2$ (*c* 0.15, $CHCl_3$); UV (EtOH) $\lambda_{max}$ 268 (log $\varepsilon$ 4.01), 319 nm (log $\varepsilon$ 3.89); IR (KBr) $\nu_{max}$ 3399, 2964, 2935, 2879, 2797, 1686, 1654, 1639, 1625, 1509, 1418, 1223, 1053, 771 cm$^{-1}$; $^1$H (300 MHz, $CDCl_3$) and $^{13}$C NMR (75 MHz, $CDCl_3$), see Table 1; EIMS *m/z* 598 $[M+H]^+$ (0.3), 597 (0.6), 554 (7), 511 (1), 455 (5), 412 (8), 385 (2), 358 (6), 100 (100), 70 (9); HRFABMS (positive ion mode) *m/z* 598.3604 $[M+H]^+$ (calcd for $C_{32}H_{47}N_5O_6$+H, 598.3605).

**3.3.3. Ziziphine P (3).** Colorless solid, mp 127–129 °C; $[\alpha]_D^{31} = -385.4$ (*c* 0.15, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 265 (log $\varepsilon$ 4.73), 321 nm (log $\varepsilon$ 4.59); IR (KBr) $\nu_{max}$ 3450, 3394, 2960, 2935, 2877, 2783, 1686, 1642, 1513, 1429, 1210, 1053, 786 cm$^{-1}$; $^1$H (300 MHz, $CDCl_3$) and $^{13}$C NMR (75 MHz, $CDCl_3$), see Table 1; EIMS *m/z* 598 $[M+H]^+$ (0.1), 554 (0.1), 540 (0.2), 397 (0.1), 365 (0.1), 344 (0.1), 114 (100), 57 (7); HRFABMS (positive ion mode) *m/z* 598.3590 $[M+H]^+$ (calcd for $C_{32}H_{47}N_5O_6$+H, 598.3605).

**3.3.4. Ziziphine Q (4).** Colorless solid, mp 140–142 °C; $[\alpha]_D^{29} = -345.0$ (*c* 0.16, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 265 (log $\varepsilon$ 3.64), 319 nm (log $\varepsilon$ 3.50); IR (KBr) $\nu_{max}$ 3586, 3396, 2962, 2927, 2782, 1689, 1640, 1509, 1418, 1222, 1054 cm$^{-1}$; $^1$H (300 MHz, $CDCl_3$) and $^{13}$C NMR (75 MHz, $CDCl_3$), see Table 1; FABMS (positive ion mode) *m/z* 598 $[M+H]^+$ (38), 358 (4), 310 (1), 241 (1), 193 (6), 165 (2), 114 (100), 70 (6); HRFABMS (positive ion mode) *m/z* 598.3607 $[M+H]^+$ (calcd for $C_{32}H_{47}N_5O_6$+H, 598.3605).

### 3.4. Bioassay procedure

Antiplasmodial activity was evaluated against the parasite *P. falciparum* (K1, multidrug resistant strain), which was cultured continuously according to the method of Targer and Jensen.[26] Quantitative assessment of antiplasmodial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins.[27] The inhibitory concentration which causes 50% reduction in parasite growth as indicated by the in vitro uptake of 3[*H*]-hypoxanthine by *P. falciparum*. An $IC_{50}$ value of 1 ng/mL was observed for the standard compound, artemisinine, in the same test system. The antimycobacterial activity was assessed against *M. tuberculosis* $H_{37}Ra$ using the Microplate Alamar Blue Assay.[28] Standard drugs, isoniazid and kanamycin sulfate, the reference compounds for the antimycobacterial assay, showed MIC of 0.6 and 2.5 μg/mL, respectively. The cytotoxicity of compounds **1–4** was determined, employing the colorimetric method as described by Skehan et al.[29] The reference substance, ellipticine, exhibited activities towards BC and KB cells with $IC_{50}$ of 1.33 and 1.46 μg/mL, respectively.


## Acknowledgements

This work was supported by the Thailand Toray Science Foundation. Partial support from the Biodiversity Research and Training Program (BRT) is gratefully acknowledged. We are indebted to the Bioassay Research Facility of BIOTEC for bioactivity tests and to James F. Maxwell, Department of Biology, Faculty of Science, Chiang Mai University, for identifying the plant species. S.R. is the recipient of the TRF senior research scholar award.


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# A New Cytotoxic Daphnane Diterpenoid, Rediocide G, from *Trigonostemon reidioides*

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Received January 28, 2005; accepted April 18, 2005

**Rediocide G (7), a new daphnane diterpenoid, was isolated from the roots of *Trigonostemon reidioides* (Euphorbiaceae), together with two congeners, rediocide A and rediocide B, (+)-syringaresinol, scopoletin, tomentin and stigmasterol. The structure of the new natural product was elucidated by comparison of its NMR and mass spectral data with those of previously known rediocides and confirmed by extensive 2D NMR spectral analysis. Rediocide G (7) was found to be cytotoxic to various cancer cell lines.**

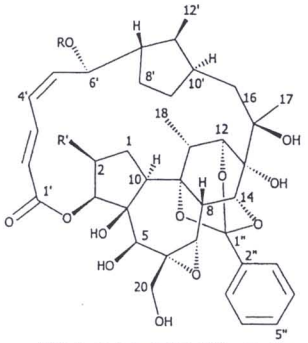
**Key words** *Trigonostemon reidioides*; Euphorbiaceae; daphnane diterpenoid; rediocide G

During our research for bioactive compounds from Thai Medicinal Plants, we have investigated the *in vitro* cytotoxicity of 39 extracts from 11 Thai plants of 10 families.[1] One of the active extracts from this investigation was the dichloromethane extract of *Trigonostemon reidioides* (KURZ) CRAIB. The plant is known as *Lot thanong* in Thai and belongs to the Euphorbiaceae family. The roots when ground with water have been used in traditional Thai medicine as an emetic for food poisoning, especially from toxic mushrooms and shells, as well as being used as a laxative and antiasthmatic.[2] Previous studies on the chemical constituents of *T. reidioides* have led to the isolation of several classes of compounds such as trigonostemone, a phenanthrenone,[3] afzelechin-(4→8)-afzelechin and lotthanongine, a novel flavonoidal indole alkaloid.[4] In 2000 the first daphnane diterpenoid, rediocide A (**1**), was isolated from the roots of *T. reidioides*.[5] Very recently, five novel daphnane diterpenes, rediocides B—F (**2**—**6**) were isolated from *T. reidioides*[6,7] and these compounds showed very potent anti-flea activity.[6] All rediocides isolated have been found to be among the most potent groups of anti-flea compounds.[6]

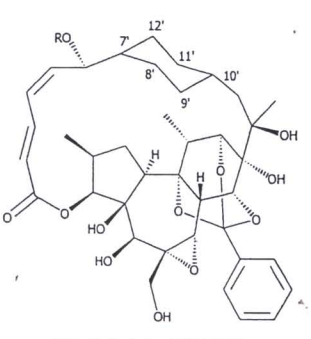
Many daphnane types of diterpenes, known as phorbol esters, have been found in plants of the families Thymelaeceae and Euphorbiaceae. Their bioactivities have indicated a much wider and still largely untapped biological potential. Most daphnanes can produce severe irritant effects, especially on mucous membranes and the eye.[8] These compounds were the first tumor-promoting agents isolated from natural sources, and were known to be powerful activators of protein kinase C.[9] However, some of these diterpenes are not tumor promoters and instead are very effective antiviral (HIV-1) agents.[9] Other biological activity and molecular pharmacology of daphnanes, such as antileukemia, piscicidal, toxicity, anticancer, abortion (birth and fertility regulation) and neurotrophy have been reported.[8] Moreover, some phorbol derivatives, recently isolated from *Sapium indicum* L. (Euphorbiaceae), were found to exhibit antimycobacterial activity.[10]

## Results and Discussion

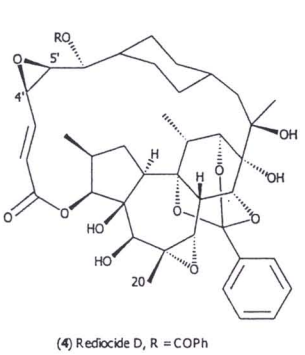
Sequential extraction of the plant material was carried out in *n*-hexane, dichloromethane and 95% ethanol, respectively. The dichloromethane extract was subjected to further purification processes to ultimately give a white powder of three daphnane diterpenoids, rediocide A, rediocide B and the new



(**1**) Rediocide A, R = $COCH_2CHMe_2$, R' = Me
(**3**) Rediocide C, R = COPh, R' = Me
(**5**) Rediocide E, R = $COCH_2CHMe_2$, R' = H
(**6**) Rediocide F, R = COPh, R' = H



(**2**) Rediocide B, R = $COCH_2CHMe_2$
(**7**) Rediocide G, R = COPh



(**4**) Rediocide D, R = COPh

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rediocide G. The HR-FAB-MS (negative) of **7** established a molecular formula of $C_{46}H_{53}O_{13}$ ($[M-H]^-$ *m/z* 813.3484, calculated 813.3486), which was 20 amu more than rediocide A (**1**) and was isomeric with rediocide C (**3**).[6] Comparison of carbon multiplicities in the DEPT spectrum of rediocide A (**1**) and C (**3**) with rediocide G (**7**) revealed that rediocide G (**7**) possesses one less $CH_3$ and a CH group, and has two additional $CH_2$ groups, suggesting the presence of a cyclohexyl instead of methylcyclopentyl ring. The COSY spectrum showed the correlations between both $H_2$-8′ with $H_2$-9′ and $H_2$-11′ with $H_2$-12′. The strong COSY correlations of H-7′ with H-6′, and H-10′ with $H_2$-16 indicated that the linkage of this cyclohexyl ring is a 1,4-diequatorial configuration at C-7′ and C-10′, while the aromatic region of $^1$H- and $^{13}$C-NMR spectra of rediocide G (**7**) showed the presence of an additional aromatic moiety and the signals for the isobutyl moiety were absent when compared to the NMR spectra of rediocide A (**1**). This indicated that the isobutyl moiety was replaced by the aromatic moiety which was substantiated by the differences in the number of carbons. Some of the stereochemical features of rediocide G (**7**) were obtained from the measurement of *J* couplings and NOESY correlations (Fig. 1, Table 1). The $^1$H-NMR, COSY, and HMBC spectra re-

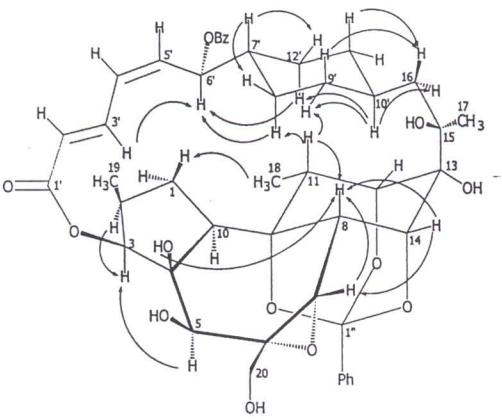


Fig. 1. Significant NOESY Data

Table 1. Rediocide G (**7**) $^1$H- and $^{13}$C-NMR, HMBC, and NOESY Spectral Summary

| No. | $^{13}$C[a] | $^1$H (mult, *J* in Hz)[a] | HMBC[a] (H→C) | NOESY[b] |
|---|---|---|---|---|
| 1 | 35.3 | 1.72, m; 2.12, m | H-3, 10, $H_3$-19 | H-2, 3, 10, $H_3$-18 |
| 2 | 35.4 | 1.72, m | H-3, 10, $H_3$-19 | H-1, 3, 10 |
| 3[b] | 80.3 | 5.15, br d, 3.5 | H-1, 5, 10, $H_3$-19, OH-4 | H-1, 2, 5, OH-5 |
| 4 | 80.9 | — | H-1, 10, OH-4, OH-5 | — |
| 5 | 70.6 | 3.78, d, 7.5 | — | H-3, 10, OH-5 |
| 6 | 62.0 | — | H-5, 7, 8, $H_2$-20, OH-5 | — |
| 7 | 64.0 | 3.28, br s | H-5, 8, 14, $H_2$-20 | H-8, $H_2$-20 |
| 8 | 35.1 | 4.72, br s | H-7, 11, 10, 14 | H-7, 11, 14, OH-4 |
| 9 | 76.9 | — | H-7, 8, 10, 11, 12, $H_3$-18 | — |
| 10 | 46.2 | 3.10, m | H-1, 3 | H-1, 2, 5 |
| 11 | 37.0 | 3.10, m | H-8, 10, $H_3$-18 | H-8, 8′, 9′ |
| 12 | 84.6 | 3.54, br d, 1.8 | H-14, $H_3$-18, OH-13 | H-16 |
| 13 | 72.0 | — | H-12, 14, $H_3$-17, OH-13, OH-15 | — |
| 14 | 80.1 | 4.18, br d, 1.6 | H-7, 8, 12 | H-7, 8 |
| 15 | 76.0 | — | $H_3$-17, OH-13, OH-15 | — |
| 16 | 42.9 | 1.12, m; 1.80, m | $H_3$-17, OH-15 | H-12, 9′, 10′ |
| 17 | 28.1 | 1.28, s | OH-15 | OH-13 |
| 18 | 19.3 | 1.49, d, 6.5 | H-11, 12 | H-1 |
| 19 | 13.4 | 0.9, d, 6.2 | — | — |
| 20 | 63.2 | 3.38, m; 3.82, dd, 12.8, 5.7 | H-5, OH-20 | — |
| 1′ | 164.8 | — | H-3, 2′, 3′ | — |
| 2′ | 124.9 | 6.04, d, 15.2 | H-3′, 4′ | H-4′ |
| 3′ | 136.6 | 7.49, dd, 15.2, 11.1 | H-2′, 4′, 5′ | H-6′ |
| 4′ | 129.6 | 6.42, t, 11.1, 11.1 | H-2′, 6′ | H-2′, 5′ |
| 5′ | 135.3 | 5.74, dd, 11.1, 9.6 | H-3′, 6′ | H-4′ |
| 6′ | 78.1 | 5.49, t, 9.6, 9.6 | H-4′ | H-3′, 8′, 12′ |
| 7′ | 36.2 | 1.85, m | H-8′, 12′ | H-8′, 12′ |
| 8′ | 29.9 | 0.90, m; 1.50, m | — | H-6′, 7′, 9′,10′, 12′ |
| 9′ | 31.2 | 1.12, m; 2.10, m | — | H-11, 8′, 10′ |
| 10′ | 35.7 | 1.85, m | H-16, 9′ | H-16, 8′, 9′, 12′ |
| 11′ | 33.3 | 0.76, m; 2.20, m | — | H-16, 9′, 12′ |
| 12′ | 32.4 | 1.12, m; 1.47, m | H-7′ | H-6′, 7′, 8′, 9′, 11′ |
| 1″ | 107.6 | — | H-12, 14, 3″, 7″ | — |
| 2″ | 139.2 | — | H-3″, 4″, 5″, 6″, 7″ | — |
| 3″, 7″ | 125.2 | 7.59, m | H-4″, 5″, 6″ | H-4″, 5″, 6″ |
| 4″, 6″ | 127.5 | 7.36, m | H-5″ | H-3″, 7″ |
| 5″ | 128.7 | 7.36, m | H-3″, 4″, 6″, 7″ | H-3″, 7″ |
| 1‴ | 165.2 | — | H-6′, 3‴, 7‴ | — |
| 2‴ | 129.5 | — | H-3‴, 4‴, 6‴, 7‴ | — |
| 3‴, 7‴ | 129.1 | 7.97, dd, 8.5, 1.2 | H-4‴, 5‴, 6‴ | H-5′, 4‴, 6‴ |
| 4‴, 6‴ | 128.7 | 7.52, t, 7.5 | H-3‴, 7‴ | H-3‴, 5‴, 7‴ |
| 5‴ | 133.4 | 7.65, t, 7.5 | H-3‴, 4‴, 6‴, 7‴ | H-4‴, 6‴ |

Hydroxyl groups of rediocide G (**7**) are 4-OH ($\delta_H$, 2.7, s), 5-OH ($\delta_H$, 5.51, d, 7.7), 13-OH ($\delta_H$ 4.11, s), 15-OH ($\delta_H$, 4.55, s) and 20-OH ($\delta_H$, 4.46, t, 6.2). *a*) Recorded at 125 MHz for $^{13}$C-NMR and 500 MHz for $^1$H-NMR in DMSO-$d_6$. *b*) Recorded at 100 MHz for $^{13}$C-NMR and 400 MHz for $^1$H-NMR in $CDCl_3$.

vealed an unusual 12-carbon polyketide to be 2*E*,4*Z*-dodecadienolate, which was confirmed by NOESY correlations. The 2*E* and 4*Z* stereochemistry was evident from the coupling constants of 15.2 Hz and 11.1 Hz for the 2′,3′ and 4′,5′ double bond, respectively. The relative stereochemistry of **7** was deduced using NOESY spectrum. The six-membered ring of daphnane is locked to chair conformation by a 1,3,5-triaxially connected *ortho*-ester group at C-9, C-12, and C-14, which is $\alpha$-oriented. In the NOESY spectrum of **7**, H-5 was found to exhibit correlations with H-3 and H-10, H-3 to H-2, and H-10 to H-1$\alpha$ thus these protons should be directed to the $\alpha$-position. The $\beta$-oriented proton, H-8, showed a 1,3-diaxial relationship with H-11 and correlation to 4-OH. H-8 appeared as broad singlet due to a 90 degree dihedral angle with the two vicinal protons H-7 and H-14, and to H-11 showing no coupling with H-12, thus placing H-12 and H-14 in the equatorial position. Moreover, H-11 showed a NOESY correlation to H-9′$\alpha$ indicating that the cyclohexyl ring of C-12 polyketide was close and over the cyclohexyl ring of daphnane diterpene. All other observed NOESY correlations are depicted in Fig. 1 and Table 1.

We have also isolated rediocide A[1] (**1**) and rediocide B (**2**) from *T. reidioides*, together with (+) syringaresinol,[11,12] scopoletin,[13] tomentin[14] and stigmasterol. We propose that the methylcyclopentyl ring is the biogenetic precursor of the cyclohexyl ring. The biosynthesis of the cyclohexyl ring presumably passes first through hydroxylation of the methyl group to give the hydroxymethylcyclopentyl ring. Activation of the leaving group *via* phosphorylation of the alcohol, followed by Wagner–Meerwein type rearrangement, can then lead to the cyclohexene ring which can then be converted to the cyclohexyl ring.

We evaluated the cytotoxicity[1] of rediocide G (**7**) against various cell lines and found that the compound exhibited cytotoxicity against GepGII, HeLa, HuCCA-1 and KB cell lines with $ED_{50}$ of 6.4, 4.8, 5.0, and 5.0 $\mu$g/ml, respectively. We have also found that rediocide A (**1**) exhibited cytotoxic activity against HeLa ($ED_{50}$ 5.0 $\mu$g/ml) and HepG2 ($ED_{50}$ 6.7 $\mu$g/ml).[1] The procedure for cytotoxic evaluation was reported in previously published literature.[1]

## Experimental

**General Procedures** Melting points were determined on a melting point apparatus (Buchi 535) and are uncorrected. UV spectra were taken in $CHCl_3$ on a SHIMADZU UV-VIS 2100S spectrometer. IR spectra were recorded in a chloroform solution on a 1760X Perkin-Elmer spectrometer. Mass spectra were measured on Finnigan INCOS 50 and MAT 90. NMR spectra were recorded on Bruker AM 400 at 400 MHz and BRUKER AVANCE 500 at 500 MHz for $^1H$, and at 100 and 125 MHz for $^{13}C$ nuclei, respectively, using TMS as an internal standard. HPLC was performed on a Thermo Separation Products system (San Jose, CA, U.S.A.) (pump, P4000; detector, UV6000LP for analysis, UV2000 for preparative mode).

**Plant Material** The roots of *T. reidioides* were purchased from Loei Province, northern Thailand, in February 2001. Root of *Trigonostemon reidioides* Craib was identified by comparison with the authentic specimen at Forest Herbarium (BKF 36612), National Park, Wildlife and Plant Conservation Department, Ministry of National Resources and Environment, Bangkok, Thailand.

**Extraction and Isolation** The bioassay-guided fractionation of a dichloromethane extract from the roots of *T. reidioides* using cytotoxicity on KB and HuCCA-1 cell lines as a viability model allowed the isolation of bioactive components from the extracts of plant materials which was carried out with *n*-hexane, dichloromethane and 95% ethanol. Dichloromethane extract (12.3 g) was first submitted to column chromatography on silica gel G (230—400 mesh). The eluent was from 2% dichloromethane in 95% ethanol to pure 95% ethanol to afford 42 mg of the tenth fraction, which was further purified by chromatography on a column of silica gel G (230—400 mesh) to provide the rediocide-containing fractions. Subsequent purification by prep HPLC-ODS with 82% MeOH/$H_2O$, using a 260 nm UV detector, afforded a white powder consisting of three daphnane diterpenoids, rediocide A (**1**) (14.1 mg), rediocide B (**2**) (1.6 mg) and rediocide G (**7**) (8.3 mg) as a new congener.

Rediocide A (**1**): Colorless solid, mp >230 °C [lit.[5] 213—215 °C, lit.[7] 193—195 °C]; IR ($CHCl_3$) $\nu_{max}$: 3566, 1717, 1690. UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$): 260 (0.6). HR-FAB-MS (negative) *m/z*=793.3793 $[M-H]^-$ (Calcd for $C_{46}H_{53}O_{13}$ 793.3799 $[M-H]^-$).

Rediocide B (**2**): White powder, mp >230 °C [lit.[6] pale yellow gum]; IR ($CHCl_3$) $\nu_{max}$: 3749, 1717, 1542, 1508. UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$): 262 (0.4). HR-FAB-MS (negative) *m/z*=793.3799 $[M-H]^-$ (Calcd for $C_{46}H_{53}O_{13}$ 793.3799 $[M-H]^-$).

Rediocide G (**7**): White powder, mp >230 °C (decomp.); IR ($CHCl_3$) $\nu_{max}$: 3562, 1717, 1683, 1463, 1334, 1276, 1111, 1072. UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$): 262 (0.4). $^1H$-NMR (500 MHz, DMSO-$d_6$) and $^{13}C$-NMR (125 MHz, DMSO-$d_6$) see Table 1. HR-FAB-MS (negative) *m/z*=813.3484 $[M-H]^-$ (Calcd for $C_{46}H_{53}O_{13}$ 813.3486 $[M-H]^-$).

**Acknowledgements** We are grateful for the generous financial support of the Thailand Research Fund (TRF). We also acknowledge the facilities provided by the PERCH program.

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**SYNTHESIS** Journal of Synthetic Organic Chemistry **REPRINT**

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Thieme

# Efficient Synthesis of Diospyrol via Suzuki–Miyaura and Modified in situ Cross-Coupling

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*Received 5 March 2005; revised 18 May 2005*

**Abstract:** Tetramethoxydiospyrol was synthesized via Suzuki–Miyaura cross-coupling of the two key intermediates, halonaphthalene and naphthaleneboronic acid derivatives, which were derived from the same naphthol. Moreover, the product could be conveniently obtained by a one-pot modified in situ Suzuki coupling. The naphthol was synthesized via the cyclization of *ortho*-allylbenzamide intermediate.

**Key words:** biaryls, Suzuki–Miyaura cross-coupling, metalation, diospyrol

Diospyrol[1] (**1a**), a symmetrical dimeric naphthol, was isolated from *Diospyros mollis* berries widely used in Thailand as an anthelmintic.[2] Over the years, the synthesis of this interesting structural motif has challenged many synthetic groups.[3,4] The interest in this molecule was intensified by the recent isolation of the michellamine alkaloids reported to exhibit potent anti-HIV activity.[5] The structure of michellamine, typified by michellamine B, composed of two important structural units, i.e. 1,3-dimethyltetrahydroisoquinoline and the core binaphthol, which is structurally similar to diospyrol (Figure 1).

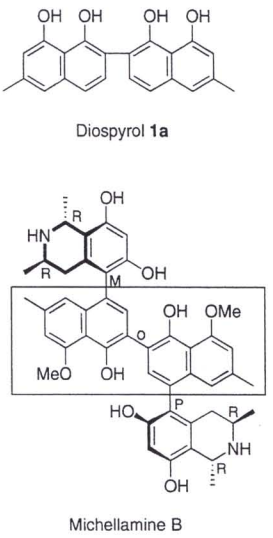
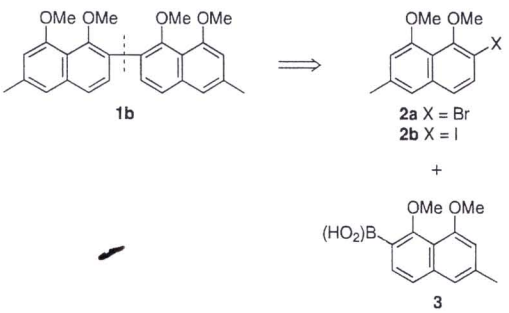



**Figure 1** Diospyrol (**1a**) and michellamine B

Retrosynthetic analysis suggested that breaking the $C_2$ symmetric bond can form two naphthalene units as shown in Scheme 1. In our approach, we planned to utilize the Suzuki–Miyaura cross-coupling[6] of naphthalene derivatives, i.e. halonaphthalene **2** and naphthaleneboronic acid **3**, for the synthesis of this compound. Herein we report both the classical and modified in situ Suzuki cross-coupling for the synthesis of diospyrol.

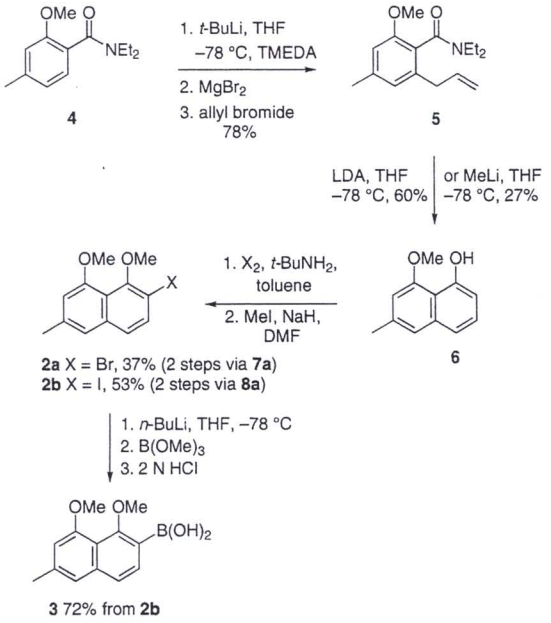



**Scheme 1** Retrosynthetic analysis of tetramethoxydiospyrol (**1b**)

The naphthol precursor **6** was required for the synthesis of the first key intermediate, halonaphthalene **2**. Many synthetic methodologies have been devised for synthesis of the naphthol derivatives.[7] We adopted the procedure developed by Snieckus et al.[7d] for the synthesis of our naphthol derivative. The naphthol **6** was synthesized in 60% yield by cyclization of the *o*-allylbenzamide **5** in the presence of excess LDA. The use of methyllithium as a base led also to the cyclized adduct **6** but in lower yield (27%).[7g] The precursor allylbenzamide **5** was synthesized in one pot by selective *ortho* metalation[8] of benzamide **4**[9] with *t*-BuLi followed by transmetalation with $MgBr_2$ and the resulting organomagnesium intermediate was trapped with allyl bromide to give the product in 78% yield (Scheme 2).

The first key intermediate, halonaphthalene **2**, could be synthesized using selective *ortho* halogenation[10] of naphthol precursor **6** followed by methylation. The selective *ortho* halogenation of naphthol **6** with bromine or iodine in the presence of *tert*-butylamine and further methylation gave bromonaphthalene **2a** (37%, two steps) and io-

**SYNTHESIS** 2005, No. 17, pp 2934–2938
Advanced online publication: 17.08.2005
DOI: 10.1055/s-2005-872176; Art ID: Z04805SS

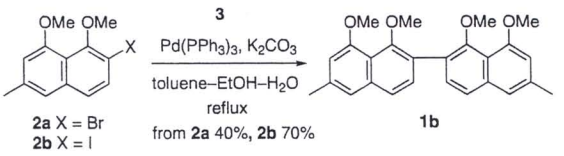



**Scheme 2** Synthesis of key intermediate halonaphthalene **2** and naphthaleneboronic acid **3**

donaphthalene **2b** (53%, two steps), respectively.[11] The other key intermediate, naphthaleneboronic acid **3**, could be prepared in 72% yield from iodonaphthalene **2b** under metal–halogen exchange condition[12] followed by quenching with $B(OMe)_3$ and hydrolysis with 2 N HCl.

With both key intermediates in hand, the Suzuki–Miyaura cross-coupling was studied.[6,13] The classical Suzuki–Miyaura cross-coupling was carried out by refluxing naphthaleneboronic acid **3** with both bromonaphthalene **2a** and iodonaphthalene **2b** in the presence of 3 mol% $Pd(PPh_3)_4$ and $K_2CO_3$ in a mixed solvent system (toluene–EtOH–$H_2O$ = 3:3:2) at 115–120 °C for 19 hours to obtain tetramethoxydiospyrol (**1b**) in 40 and 70% yield, respectively (Scheme 3). The tetramethoxydiospyrol (**1b**) could be converted to the natural diospyrol **1a** by a known method.[3a,c]

The modified one-pot, in situ Suzuki cross-coupling was developed by Keay[13] and Bräse's[14] groups. Both protocols involved the preparation of 0.5 equivalent of arylboronic




**Scheme 3** Classical Suzuki–Miyaura cross-coupling in the synthesis of tetramethoxydiospyrol (**1b**)

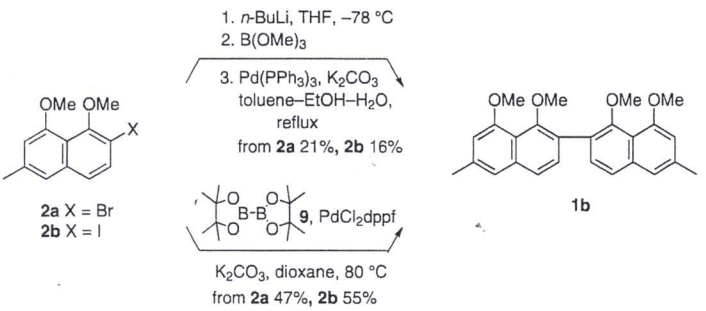
compound in situ from 1.0 equivalent of haloarene followed by Suzuki–Miyaura cross-coupling in the same flask. In the first protocol (Method A)[13] arylboronic ester was prepared by metal–halogen exchange with *n*-BuLi followed by quenching with $B(OMe)_3$ whereas in the second protocol (Method B)[14] the arylboronic ester was prepared by reacting haloarene directly with bis(pinacolato)diboron (**9**) under palladium catalyst. We have utilized both protocols for the in situ cross-coupling of both bromonaphthalene **2a** and iodonaphthalene **2b** as shown in Scheme 4. By using method A, the product **1b** was obtained in 21 and 16% yield when bromo compound and iodo compound were used respectively and the product was obtained in 47 and 55% yield when method B was employed.

In summary, we have successfully synthesized tetramethoxydiospyrol using classical and modified Suzuki–Miyaura cross-coupling reaction of naphthalene derivatives which were prepared from the same common naphthol intermediate. The iodonaphthalene was found to react more efficiently than bromonaphthalene in the cross-coupling reaction.

All commercial solvents and reagents were used without purification prior to use. THF was distilled from benzophenone ketyl under argon. Column chromatography purifications were carried out using silica gel (70–30 mesh).

**2-Allyl-*N*,*N*-diethyl-6-methoxy-4-methylbenzamide (5)**

To a stirred solution of *N*,*N*-diethyl-6-methoxy-4-methylbenzamide (**4**; 2.0 g, 9.05 mmol) and TMEDA (1.5 mL, 10.0 mmol) in anhyd THF (50 mL) at −78 °C was slowly added *t*-BuLi (1.0 M, 10.85 mL, 10.85 mmol) and the mixture was further stirred for 1 h. $MgBr_2$ etherate (4 mL) was added and the solution was warmed to r.t. The mixture was recooled to −78 °C and the stirring was continued for 40 min. Allyl bromide (1.5 mL, 17.96 mmol) was then added and the mixture was warmed to r.t. and stirred overnight. Aq sat. $NH_4Cl$




**Scheme 4** The modified in situ Suzuki cross-coupling for the synthesis of tetramethoxydiospyrol (**1b**)

was added and the mixture was extracted with $CH_2Cl_2$ (2 × 40 mL). The combined organic layers were washed with $H_2O$, brine and dried ($Na_2SO_4$). $CH_2Cl_2$ was evaporated to dryness to give a crude viscous oil (2.5 g). Further purification by column chromatography ($SiO_2$, 25% EtOAc–hexane) gave the required allylamide **5** as a viscous oil (1.85 g, 78%) together with the starting compound (230 mg).

IR ($CHCl_3$): 1631, 1578 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 1.02 (t, *J* = 7 Hz, 3 H, $CH_3$), 1.24 (t, *J* = 7 Hz, 3 H, $CH_3$), 2.32 (s, 3 H, $CH_3$), 3.05 (m, 2 H, $NCH_2$), 3.30 (d, *J* = 7 Hz, 2 H, $CH_2$), 3.40 (m, 1 H, NCH), 3.77 (m, 1 H, NCH), 3.77 (s, 3 H, $OCH_3$), 5.07 (m, 2 H, $=CH_2$), 5.93 (m, 1 H, =CH), 6.56 (s, 1 H, H-5), 6.66 (s, 1 H, H-3).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 12.7, 13.7, 21.6, 36.9, 38.3, 42.6, 55.4, 109.4, 116.0, 122.3, 123.5, 136.7, 137.6, 139.23, 155.4, 168.2.

MS (EI, 70 eV): *m/z* (%) = 105 (23), 143 (27), 161 (88), 188 (76), 189 (100), 190 (24), 261 (32, [$M^+$]), 262 (42, [M + $H^+$]).

HRMS-FAB: *m/z* [M + $H^+$] calcd for $C_{16}H_{23}NO_2$: 262.1807; found: 262.1806.

### 8-Methoxy-6-methyl-1-naphthol (6)

Diisopropylamine (2.4 mL, 16.86 mmol) was added by syringe to anhyd THF (50 mL). *n*-BuLi (1.2 M, 13.4 mL) was added at –78 °C and the mixture was warmed to 0 °C and further stirred for 20 min. The mixture was then cooled to –78 °C and a solution of allylamide **5** (2.0 g, 7.66 mmol) in anhyd THF (20 mL) was slowly added. The mixture was stirred at –78 °C for 3 h and then warmed to r.t. overnight. Aq sat. $NH_4Cl$ was added and the mixture was extracted with $CH_2Cl_2$ (2 × 40 mL). The combined organic layers were washed with $H_2O$, brine and dried ($Na_2SO_4$). Further purification by column chromatography ($SiO_2$, $CH_2Cl_2$) gave the required naphthol **6** as a pale brown viscous oil (860.0 mg, 60%).[15]

IR ($CHCl_3$): 3404 (s) $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.42 (s, 3 H, $CH_3$), 3.99 (s, 3 H, $OCH_3$), 6.56 (d, *J* = 1.2 Hz, 1 H, H-7), 6.79 (dd, *J* = 7.6, 1.2 Hz, 1 H, H-2), 7.16 (s, 1 H, H-5), 7.18 (dd, *J* = 7.6, 1.2 Hz, 1 H, H-4), 7.30 (t, *J* = 8 Hz, 1 H, H-3), 9.25 (s, 1 H, OH).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.8, 56.0, 106.1, 109.5, 113.3, 118.2, 120.8, 127.7, 135.5, 136.9, 154.4, 155.9.

MS (EI, 70 eV): *m/z* (%) = 115 (7), 145 (9), 188 (100, [$M^+$]), 189 (16, [M + $H^+$]).

HRMS-FAB: *m/z* [M – $H^-$] calcd for $C_{12}H_{12}O_2$: 187.0759; found: 187.0753.

### 2-Iodo-8-methoxy-6-methyl-1-naphthol (8a) and 2,4-Diiodo-8-methoxy-6-methyl-1-naphthol (8b)

To a stirred solution of *tert*-butylamine (2.14 mL, 20.27 mmol) in anhyd toluene (20 mL) was added a solution of $I_2$ (2.58 g, 10.16 mmol) in anhyd toluene (35 mL) at r.t. and the mixture was further stirred for 1 h. The resulting mixture was then transferred to a stirred solution of naphthol **6** (1.91 g, 10.16 mmol) in anhyd toluene (25 mL) at 0 °C via cannula. After the addition was complete, the reaction was further stirred for 10 min. Aq sat. $Na_2S_2O_3$ (30 mL) was added and the mixture was extracted with EtOAc (30 mL), and the $Et_2O$ layer was washed with $H_2O$ and brine. The combined extracts were dried ($Na_2SO_4$) and evaporated to dryness. Further purification was carried out by column chromatography ($SiO_2$, 5% EtOAc–hexane) to obtain *o*-iodonaphthol **8a** (1.87 g, 58%) and *o*,*p*-diiodonaphthol **8b** (603.6 mg, 14%).[11]

#### 8a

Mp 116–116.5 °C ($CH_2Cl_2$–hexane).

IR (KBr): 3320, 1626, 1603, 1579, 1495, 1403, 1370 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.42 (s, 3 H, $CH_3$), 4.01 (s, 3 H, $OCH_3$), 6.62 (s, 1 H, H-7), 6.95 (d, *J* = 8.8 Hz, 1 H, H-4), 7.14 (s, 1 H, H-5), 7.64 (d, *J* = 8.8 Hz, 1 H, H-3), 10.16 (s, 1 H, OH).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.9, 56.3, 107.0, 113.1, 119.7, 120.8, 136.4, 136.5, 153.2, 154.8.

MS (EI, 70 eV): *m/z* (%) = 172 (34), 188 (26), 299 (34), 314 (100, [$M^+$]), 315 (12, [M + $H^+$]).

HRMS-FAB: *m/z* [M – $H^-$] calcd for $C_{12}H_{11}IO_2$: 312.9724; found: 312.9726.

#### 8b

Mp 110 °C (dec.) ($CH_2Cl_2$–hexane).

IR (KBr): 3266, 1623, 1604, 1557, 1449, 1407, 1355 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.49 (s, 3 H, $CH_3$), 4.05 (s, 3 H, $OCH_3$), 6.71 (s, 1 H, H-7), 7.43 (s, 1 H, H-5), 8.25 (s, 1 H, H-3), 10.38 (s, 1 H, OH).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 22.2, 56.7, 85.8, 107.8, 113.4, 125.7, 136.3, 138.2, 146.2, 154.4, 154.8.

MS (EI, 70 eV): *m/z* (%) = 298 (25), 425 (27), 440 (100, [$M^+$]), 441 (14, [M + $H^+$]).

Anal. Calcd for $C_{12}H_{10}I_2O_2$: C, 32.76; H, 2.29. Found: C, 33.01; H, 2.12.

### Methylation of 1-Hydroxy-2-iodo-8-methoxy-6-methylnaphthalene (8a): 2-Iodo-1,8-dimethoxy-6-methylnaphthalene (2b); Typical Procedure

To a stirred suspension of NaH (80% in oil, 179 mg, 5.97 mmol) in DMF (5 mL) was added a solution of iodonaphthol **8a** (1.25 g, 3.98 mmol) in DMF (10 mL) at r.t. The mixture was stirred for 1 h and MeI (0.5 mL, 8 mmol) was then added and the mixture was stirred overnight. $H_2O$ was slowly added and the mixture was extracted with EtOAc (2 × 25 mL). The combined EtOAc extracts were washed with $H_2O$, brine and dried ($Na_2SO_4$) and evaporated to dryness. The crude product was purified by column chromatography ($SiO_2$, 2–5% EtOAc–hexane) to obtain **2b** as a viscous oil (1.19 g, 91%).

IR (neat): 2929, 1625, 1568, 1454, 1330 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.45 (s, 3 H, $CH_3$), 3.84 (s, 3 H, $OCH_3$), 3.99 (s, 3 H, $OCH_3$), 6.72 (s, 1 H, H-7), 7.17 (s, 1 H, H-5), 7.20 (d, *J* = 8.8 Hz, 1 H, H-4), 7.72 (d, *J* = 8.8 Hz, 1 H, H-3).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.8, 56.3, 61.7, 88.5, 108.9, 119.0, 120.1, 125.2, 135.9, 136.8, 137.6, 155.0, 155.9.

MS (EI, 70 eV): *m/z* (%) = 328 (100, [$M^+$]), 329 (19, [M + $H^+$]).

HRMS-FAB: *m/z* [M + $H^+$] calcd for $C_{13}H_{13}IO_2$: 329.0037; found: 329.0030.

### 2-Bromo-1,8-dimethoxy-6-methylnaphthalene (2a)

Viscous oil.

IR (neat): 2940, 2840, 1597, 1462, 1355, 1264, 1079, 747 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.39 (s, 3 H, $CH_3$), 3.82 (s, 3 H, $OCH_3$), 3.93 (s, 3 H, $OCH_3$), 6.67 (s, 1 H, H-7), 7.10 (s, 1 H, H-5), 7.23 (d, *J* = 8.8 Hz, 1 H, H-4), 7.47 (d, *J* = 8.8 Hz, 1 H, H-3).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.8, 56.3, 61.6, 109.1, 113.9, 119.6, 120.1, 124.6, 130.6, 136.6, 136.7, 153.1, 155.3.

MS (GC, 70 eV): *m/z* (%) = 115 (56), 128 (81), 139 (30), 158 (91), 186 (83), 207 (33), 209 (28), 280 (97, [$M^+$]), 282 (100, [$M^+$ + 2]).

### 1,8-Dimethoxy-6-methylnaphthalene-2-boronic Acid (3)

To a stirred solution of iodonaphthalene **2b** (301.7 mg, 0.92 mmol) in THF (7 mL) at –78 °C under argon was added *n*-BuLi (1.12 mL, 1.84 mmol) followed immediately by $B(OMe)_3$ (200 μL, 1.78

mmol). After stirring at –78 °C for 30 min, the mixture was warmed to r.t. and stirred for 1 h. The resulting mixture was quenched with 2 N HCl and extracted with $CH_2Cl_2$ (2 × 20 mL). The combined extracts were washed with $H_2O$ and brine and dried ($Na_2SO_4$). The solvent was removed to obtain the crude boronic acid which was purified by preparative layer chromatography (PLC) ($SiO_2$, 1% MeOH–$CH_2Cl_2$) to give boronic acid **3** as a white solid (163 mg, 72%); mp 157.5–158 °C ($CH_2Cl_2$–hexane).

IR (KBr): 2937(br), 1610, 1605, 1572, 1467, 1376 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.49 (s, 3 H, $CH_3$), 3.9 (s, 3 H, $OCH_3$), 4.04 (s, 3 H, $OCH_3$), 6.71 (s, 2 H, 2 OH), 6.74 (s, 1 H, H-7), 7.24 (s, 1 H, H-5), 7.52 (d, *J* = 16.4 Hz, 1 H, H-4), 7.83 (d, *J* = 16.8 Hz, 1 H, H-3).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.9, 56.1, 63.8, 108.4, 117.3, 120.4, 123.9, 132.0, 137.7, 139.9, 155.7, 163.8.

MS (EI, 70 eV): *m/z* (%) = 191 (22), 201 (22), 202 (15), 204 (75), 231 (14), 245 (27), 246 (100, $[M^+]$), 247 (14, $[M + H^+]$).

Anal. Calcd for $C_{13}H_{15}BO_4$: C, 63.45; H, 6.14. Found: C, 63.32; H, 5.77.

### Tetramethoxydiospyrol (1b) by Classical Suzuki–Miyaura Cross-Coupling Reaction

A mixture of iodonaphthalene **2b** (158.5 mg, 0.48 mmol), naphthaleneboronic acid **3**[4] (118.9 mg, 0.48 mmol), $Pd(PPh_3)_4$ (11 mg, 3 mol%) and $K_2CO_3$ (133 mg, 0.96 mmol) in a mixture of toluene–EtOH–$H_2O$ (3:3:2, 8 mL) was refluxed at 115–120 °C for 19 h. After cooling the mixture, $H_2O$ was added and extracted with EtOAc (2 × 20 mL). The combined EtOAc extracts were washed with $H_2O$, brine and dried ($Na_2SO_4$). Further purification was carried out by PLC ($SiO_2$, 0.5% MeOH–$CH_2Cl_2$) to give tetramethoxydiospyrol (**1b**) which was recrystallized from benzene to afford **1b** as white crystals (136.2 mg, 70%).

### Tetramethoxydiospyrol (1b) Modified in situ Suzuki Cross-Coupling

*Method A*:[13] To a solution of bromonaphthalene (150 mg, 0.5 mmol) in THF (10 mL) at –78 °C was added 0.5 equiv of 0.77 M of *n*-BuLi (0.4 mL) followed by 6 equiv of $B(OMe)_3$ (0.8 mL). The resulting solution was warmed to r.t. for 4 h and subsequently stirred overnight under argon. To the solution were then added toluene (6 mL), EtOH (6 mL), $H_2O$ (4 mL), $K_2CO_3$ (130 mg, 1.0 mmol), and $Pd(PPh_3)_4$ (2 mg, 10 mol%). The resulting mixture was refluxed under argon for 10 h. The reaction was allowed to warm to r.t. and extracted with $CH_2Cl_2$ (3 × 30 mL). The organic phases were combined, washed with $H_2O$, dried ($Na_2SO_4$), and concentrated under reduced pressure to afford the crude binaphthalene which was purified on PLC to yield recovered bromonaphthalene **2a** (60 mg, 37%), tetramethoxydiospyrol (**1b**) (40 mg, 21%) and debromonaphthalene (30 mg, 26%).[16]

*Method B*:[14] A mixture of iodonaphthalene **2b** (165.4 mg, 0.5 mmol), bis(pinacolato)diboron (**9**; 63.5 mg, 0.25 mmol), $PdCl_2$dppf (14.6 mg, 4 mol%), and $K_2CO_3$ (207 mg, 1.5 mmol) in dioxane (5 mL) was heated at 80 °C for 16 h. After cooling the mixture, $H_2O$ was added and extracted with $CH_2Cl_2$. The combined $CH_2Cl_2$ extracts were washed with $H_2O$, 20% aq NaOH, and dried ($Na_2SO_4$). Further purification was carried out by column chromatography ($SiO_2$, 0.5% MeOH–$CH_2Cl_2$) to give tetramethoxydiospyrol (**1b**) (54.9 mg, 55%) as a white solid; mp (benzene) 239–239.5 °C (Lit.[1a] mp 232 °C, Lit.[1d] mp 243 °C).

IR (KBr): 1625, 1563, 1451, 1338, 1262 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.50 (s, 6 H, 2 $CH_3$), 3.55 (s, 6 H, 2 $OCH_3$), 4.01 (s, 6 H, 2 $OCH_3$), 6.73 (s, 2 H, H-7,7′), 7.26 (s, 2 H, H-5,5′), 7.52 (d, *J* = 16.4 Hz, 2 H, H-4,4′), 7.83 (d, *J* = 16.8 Hz, 2 H, H-3,3′).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.8, 56.1, 61.4, 108.0, 118.6, 120.0, 122.7, 128.5, 130.8, 135.0, 137.2, 153.6, 156.2.

MS (EI, 70 eV): *m/z* (%) = 298 (8), 341 (25), 357 (21), 402 (100, $[M^+]$), 403 (29, $[M + H^+]$).

## Acknowledgment

We are grateful to the Thailand Research Fund (TRF) for the generous support of our research program (TRG468008 to NT) and for the award of Senior Research Scholar to S.R.

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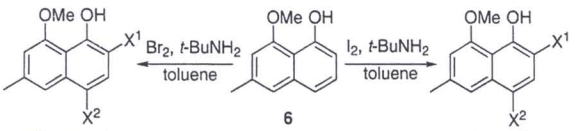
(11) The bromination and iodination of naphthol **6** also gave *para* halogenated products (Scheme 5). Compounds **7a** and **7b** were further methylated without purification.




**7a** $X^1$ = Br, $X^2$ = H
**7b** $X^1$ = H, $X^2$ = Br

**8a** $X^1$ = I, $X^2$ = H
**8b** $X^1$ = $X^2$ = I

**Scheme 5**

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(16) 1,8-Dimethoxy-3-methylnaphthalene[17] obtained as dehalogenation adduct from half-Suzuki cross-coupling was a white solid; mp 89–90 °C (MeOH). $^1$H NMR (200 MHz, $CDCl_3$): δ = 2.47 (s, 3 H, $CH_3$), 3.98 (s, 6 H, 2 $OCH_3$), 6.69 (d, *J* = 1.0 Hz, 1 H), 6.78 (dd, *J* = 6.2, 8.4 Hz, 1 H), 7.19 (br s, 1 H), 7.35 (m, 2 H). $^{13}$C NMR (50 MHz, $CDCl_3$): δ = 21.7, 56.2, 56.3, 105.2, 108.2, 115.6, 120.0, 120.2, 126.4, 136.1, 137.5, 156.8, 157.0. MS (EI, 70 eV): *m/z* (%) = 128 (71), 129 (99), 159 (58), 201 (100) 202 (50, [M$^+$]).

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**SYNTHESIS** Journal of Synthetic Organic Chemistry **REPRINT**

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Thieme

# Efficient Synthesis of Diospyrol via Suzuki–Miyaura and Modified in situ Cross-Coupling

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*Received 5 March 2005; revised 18 May 2005*

**Abstract:** Tetramethoxydiospyrol was synthesized via Suzuki–Miyaura cross-coupling of the two key intermediates, halonaphthalene and naphthaleneboronic acid derivatives, which were derived from the same naphthol. Moreover, the product could be conveniently obtained by a one-pot modified in situ Suzuki coupling. The naphthol was synthesized via the cyclization of *ortho*-allylbenzamide intermediate.

**Key words:** biaryls, Suzuki–Miyaura cross-coupling, metalation, diospyrol

Diospyrol[1] (**1a**), a symmetrical dimeric naphthol, was isolated from *Diospyros mollis* berries widely used in Thailand as an anthelmintic.[2] Over the years, the synthesis of this interesting structural motif has challenged many synthetic groups.[3,4] The interest in this molecule was intensified by the recent isolation of the michellamine alkaloids reported to exhibit potent anti-HIV activity.[5] The structure of michellamine, typified by michellamine B, composed of two important structural units, i.e. 1,3-dimethyltetrahydroisoquinoline and the core binaphthol, which is structurally similar to diospyrol (Figure 1).

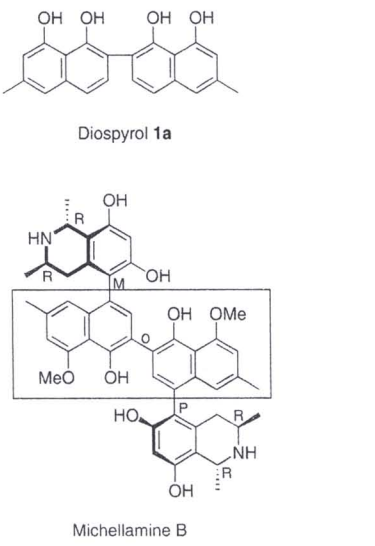
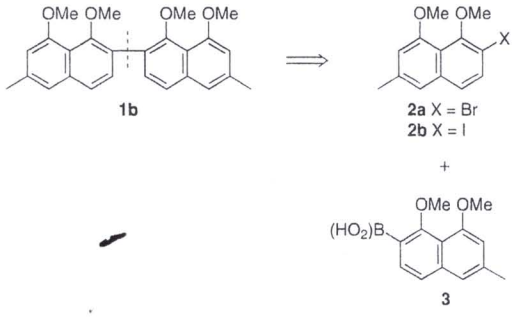



**Figure 1** Diospyrol (**1a**) and michellamine B

Retrosynthetic analysis suggested that breaking the $C_2$ symmetric bond can form two naphthalene units as shown in Scheme 1. In our approach, we planned to utilize the Suzuki–Miyaura cross-coupling[6] of naphthalene derivatives, i.e. halonaphthalene **2** and naphthaleneboronic acid **3**, for the synthesis of this compound. Herein we report both the classical and modified in situ Suzuki cross-coupling for the synthesis of diospyrol.

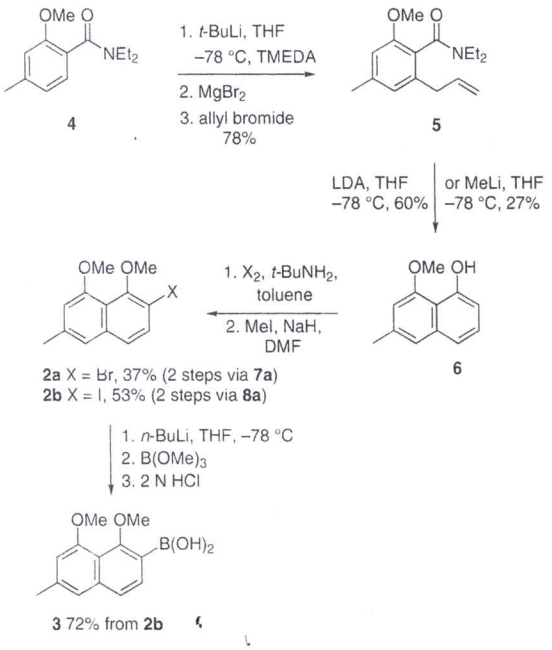



**Scheme 1** Retrosynthetic analysis of tetramethoxydiospyrol (**1b**)

The naphthol precursor **6** was required for the synthesis of the first key intermediate, halonaphthalene **2**. Many synthetic methodologies have been devised for synthesis of the naphthol derivatives.[7] We adopted the procedure developed by Snieckus et al.[7d] for the synthesis of our naphthol derivative. The naphthol **6** was synthesized in 60% yield by cyclization of the *o*-allylbenzamide **5** in the presence of excess LDA. The use of methyllithium as a base led also to the cyclized adduct **6** but in lower yield (27%).[7g] The precursor allylbenzamide **5** was synthesized in one pot by selective *ortho* metalation[8] of benzamide **4**[9] with *t*-BuLi followed by transmetalation with $MgBr_2$ and the resulting organomagnesium intermediate was trapped with allyl bromide to give the product in 78% yield (Scheme 2).

The first key intermediate, halonaphthalene **2**, could be synthesized using selective *ortho* halogenation[10] of naphthol precursor **6** followed by methylation. The selective *ortho* halogenation of naphthol **6** with bromine or iodine in the presence of *tert*-butylamine and further methylation gave bromonaphthalene **2a** (37%, two steps) and io-

**SYNTHESIS** 2005, No. 17, pp 2934–2938
Advanced online publication: 17.08.2005
DOI: 10.1055/s-2005-872176; Art ID: Z04805SS

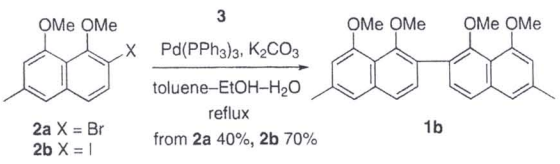



**Scheme 2** Synthesis of key intermediate halonaphthalene **2** and naphthaleneboronic acid **3**

donaphthalene **2b** (53%, two steps), respectively.[11] The other key intermediate, naphthaleneboronic acid **3**, could be prepared in 72% yield from iodonaphthalene **2b** under metal–halogen exchange condition[12] followed by quenching with $B(OMe)_3$ and hydrolysis with 2 N HCl.

With both key intermediates in hand, the Suzuki–Miyaura cross-coupling was studied.[6,13] The classical Suzuki–Miyaura cross-coupling was carried out by refluxing naphthaleneboronic acid **3** with both bromonaphthalene **2a** and iodonaphthalene **2b** in the presence of 3 mol% $Pd(PPh_3)_4$ and $K_2CO_3$ in a mixed solvent system (toluene–EtOH–$H_2O$ = 3:3:2) at 115–120 °C for 19 hours to obtain tetramethoxydiospyrol (**1b**) in 40 and 70% yield, respectively (Scheme 3). The tetramethoxydiospyrol (**1b**) could be converted to the natural diospyrol **1a** by a known method.[3a,c]

The modified one-pot, in situ Suzuki cross-coupling was developed by Keay[13] and Bräse's[14] groups. Both protocols involved the preparation of 0.5 equivalent of arylboronic




**Scheme 3** Classical Suzuki–Miyaura cross-coupling in the synthesis of tetramethoxydiospyrol (**1b**)

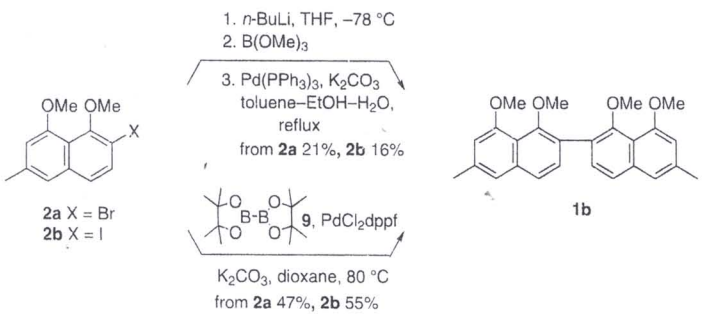
compound in situ from 1.0 equivalent of haloarene followed by Suzuki–Miyaura cross-coupling in the same flask. In the first protocol (Method A)[13] arylboronic ester was prepared by metal–halogen exchange with *n*-BuLi followed by quenching with $B(OMe)_3$ whereas in the second protocol (Method B)[14] the arylboronic ester was prepared by reacting haloarene directly with bis(pinacolato)diboron (**9**) under palladium catalyst. We have utilized both protocols for the in situ cross-coupling of both bromonaphthalene **2a** and iodonaphthalene **2b** as shown in Scheme 4. By using method A, the product **1b** was obtained in 21 and 16% yield when bromo compound and iodo compound were used respectively and the product was obtained in 47 and 55% yield when method B was employed.

In summary, we have successfully synthesized tetramethoxydiospyrol using classical and modified Suzuki–Miyaura cross-coupling reaction of naphthalene derivatives which were prepared from the same common naphthol intermediate. The iodonaphthalene was found to react more efficiently than bromonaphthalene in the cross-coupling reaction.

All commercial solvents and reagents were used without purification prior to use. THF was distilled from benzophenone ketyl under argon. Column chromatography purifications were carried out using silica gel (70–30 mesh).

**2-Allyl-*N*,*N*-diethyl-6-methoxy-4-methylbenzamide (5)**

To a stirred solution of *N*,*N*-diethyl-6-methoxy-4-methylbenzamide (**4**; 2.0 g, 9.05 mmol) and TMEDA (1.5 mL, 10.0 mmol) in anhyd THF (50 mL) at –78 °C was slowly added *t*-BuLi (1.0 M, 10.85 mL, 10.85 mmol) and the mixture was further stirred for 1 h. $MgBr_2$ etherate (4 mL) was added and the solution was warmed to r.t. The mixture was recooled to –78 °C and the stirring was continued for 40 min. Allyl bromide (1.5 mL, 17.96 mmol) was then added and the mixture was warmed to r.t. and stirred overnight. Aq sat. $NH_4Cl$




**Scheme 4** The modified in situ Suzuki cross-coupling for the synthesis of tetramethoxydiospyrol (**1b**)

was added and the mixture was extracted with $CH_2Cl_2$ (2 × 40 mL). The combined organic layers were washed with $H_2O$, brine and dried ($Na_2SO_4$). $CH_2Cl_2$ was evaporated to dryness to give a crude viscous oil (2.5 g). Further purification by column chromatography ($SiO_2$, 25% EtOAc–hexane) gave the required allylamide **5** as a viscous oil (1.85 g, 78%) together with the starting compound (230 mg).

IR ($CHCl_3$): 1631, 1578 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 1.02 (t, *J* = 7 Hz, 3 H, $CH_3$), 1.24 (t, *J* = 7 Hz, 3 H, $CH_3$), 2.32 (s, 3 H, $CH_3$), 3.05 (m, 2 H, $NCH_2$), 3.30 (d, *J* = 7 Hz, 2 H, $CH_2$), 3.40 (m, 1 H, NCH), 3.77 (m, 1 H, NCH), 3.77 (s, 3 H, $OCH_3$), 5.07 (m, 2 H, $=CH_2$), 5.93 (m, 1 H, =CH), 6.56 (s, 1 H, H-5), 6.66 (s, 1 H, H-3).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 12.7, 13.7, 21.6, 36.9, 38.3, 42.6, 55.4, 109.4, 116.0, 122.3, 123.5, 136.7, 137.6, 139.23, 155.4, 168.2.

MS (EI, 70 eV): *m/z* (%) = 105 (23), 143 (27), 161 (88), 188 (76), 189 (100), 190 (24), 261 (32, [$M^+$]), 262 (42, [M + $H^+$]).

HRMS-FAB: *m/z* [M + $H^+$] calcd for $C_{16}H_{23}NO_2$: 262.1807; found: 262.1806.

**8-Methoxy-6-methyl-1-naphthol (6)**

Diisopropylamine (2.4 mL, 16.86 mmol) was added by syringe to anhyd THF (50 mL). *n*-BuLi (1.2 M, 13.4 mL) was added at –78 °C and the mixture was warmed to 0 °C and further stirred for 20 min. The mixture was then cooled to –78 °C and a solution of allylamide **5** (2.0 g, 7.66 mmol) in anhyd THF (20 mL) was slowly added. The mixture was stirred at –78 °C for 3 h and then warmed to r.t. overnight. Aq sat. $NH_4Cl$ was added and the mixture was extracted with $CH_2Cl_2$ (2 × 40 mL). The combined organic layers were washed with $H_2O$, brine and dried ($Na_2SO_4$). Further purification by column chromatography ($SiO_2$, $CH_2Cl_2$) gave the required naphthol **6** as a pale brown viscous oil (860.0 mg, 60%).[15]

IR ($CHCl_3$): 3404 (s) $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.42 (s, 3 H, $CH_3$), 3.99 (s, 3 H, $OCH_3$), 6.56 (d, *J* = 1.2 Hz, 1 H, H-7), 6.79 (dd, *J* = 7.6, 1.2 Hz, 1 H, H-2), 7.16 (s, 1 H, H-5), 7.18 (dd, *J* = 7.6, 1.2 Hz, 1 H, H-4), 7.30 (t, *J* = 8 Hz, 1 H, H-3), 9.25 (s, 1 H, OH).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.8, 56.0, 106.1, 109.5, 113.3, 118.2, 120.8, 127.7, 135.5, 136.9, 154.4, 155.9.

MS (EI, 70 eV): *m/z* (%) = 115 (7), 145 (9), 188 (100, [$M^+$]), 189 (16, [M + $H^+$]).

HRMS-FAB: *m/z* [M – $H^-$] calcd for $C_{12}H_{12}O_2$: 187.0759; found: 187.0753.

**2-Iodo-8-methoxy-6-methyl-1-naphthol (8a) and 2,4-Diiodo-8-methoxy-6-methyl-1-naphthol (8b)**

To a stirred solution of *tert*-butylamine (2.14 mL, 20.27 mmol) in anhyd toluene (20 mL) was added a solution of $I_2$ (2.58 g, 10.16 mmol) in anhyd toluene (35 mL) at r.t. and the mixture was further stirred for 1 h. The resulting mixture was then transferred to a stirred solution of naphthol **6** (1.91 g, 10.16 mmol) in anhyd toluene (25 mL) at 0 °C via cannula. After the addition was complete, the reaction was further stirred for 10 min. Aq sat. $Na_2S_2O_3$ (30 mL) was added and the mixture was extracted with EtOAc (30 mL), and the $Et_2O$ layer was washed with $H_2O$ and brine. The combined extracts were dried ($Na_2SO_4$) and evaporated to dryness. Further purification was carried out by column chromatography ($SiO_2$, 5% EtOAc–hexane) to obtain *o*-iodonaphthol **8a** (1.87 g, 58%) and *o*,*p*-diiodonaphthol **8b** (603.6 mg, 14%).[11]

**8a**

Mp 116–116.5 °C ($CH_2Cl_2$–hexane).

IR (KBr): 3320, 1626, 1603, 1579, 1495, 1403, 1370 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.42 (s, 3 H, $CH_3$), 4.01 (s, 3 H, $OCH_3$), 6.62 (s, 1 H, H-7), 6.95 (d, *J* = 8.8 Hz, 1 H, H-4), 7.14 (s, 1 H, H-5), 7.64 (d, *J* = 8.8 Hz, 1 H, H-3), 10.16 (s, 1 H, OH).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.9, 56.3, 107.0, 113.1, 119.7, 120.8, 136.4, 136.5, 153.2, 154.8.

MS (EI, 70 eV): *m/z* (%) = 172 (34), 188 (26), 299 (34), 314 (100, [$M^+$]), 315 (12, [M + $H^+$]).

HRMS-FAB: *m/z* [M – $H^-$] calcd for $C_{12}H_{11}IO_2$: 312.9724; found: 312.9726.

**8b**

Mp 110 °C (dec.) ($CH_2Cl_2$–hexane).

IR (KBr): 3266, 1623, 1604, 1557, 1449, 1407, 1355 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.49 (s, 3 H, $CH_3$), 4.05 (s, 3 H, $OCH_3$), 6.71 (s, 1 H, H-7), 7.43 (s, 1 H, H-5), 8.25 (s, 1 H, H-3), 10.38 (s, 1 H, OH).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 22.2, 56.7, 85.8, 107.8, 113.4, 125.7, 136.3, 138.2, 146.2, 154.4, 154.8.

MS (EI, 70 eV): *m/z* (%) = 298 (25), 425 (27), 440 (100, [$M^+$]), 441 (14, [M + $H^+$]).

Anal. Calcd for $C_{12}H_{10}I_2O_2$: C, 32.76; H, 2.29. Found: C, 33.01; H, 2.12.

**Methylation of 1-Hydroxy-2-iodo-8-methoxy-6-methylnaphthalene (8a): 2-Iodo-1,8-dimethoxy-6-methylnaphthalene (2b); Typical Procedure**

To a stirred suspension of NaH (80% in oil, 179 mg, 5.97 mmol) in DMF (5 mL) was added a solution of iodonaphthol **8a** (1.25 g, 3.98 mmol) in DMF (10 mL) at r.t. The mixture was stirred for 1 h and MeI (0.5 mL, 8 mmol) was then added and the mixture was stirred overnight. $H_2O$ was slowly added and the mixture was extracted with EtOAc (2 × 25 mL). The combined EtOAc extracts were washed with $H_2O$, brine and dried ($Na_2SO_4$) and evaporated to dryness. The crude product was purified by column chromatography ($SiO_2$, 2–5% EtOAc–hexane) to obtain **2b** as a viscous oil (1.19 g, 91%).

IR (neat): 2929, 1625, 1568, 1454, 1330 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.45 (s, 3 H, $CH_3$), 3.84 (s, 3 H, $OCH_3$), 3.99 (s, 3 H, $OCH_3$), 6.72 (s, 1 H, H-7), 7.17 (s, 1 H, H-5), 7.20 (d, *J* = 8.8 Hz, 1 H, H-4), 7.72 (d, *J* = 8.8 Hz, 1 H, H-3).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.8, 56.3, 61.7, 88.5, 108.9, 119.0, 120.1, 125.2, 135.9, 136.8, 137.6, 155.0, 155.9.

MS (EI, 70 eV): *m/z* (%) = 328 (100, [$M^+$]), 329 (19, [M + $H^+$]).

HRMS-FAB: *m/z* [M + $H^+$] calcd for $C_{13}H_{13}IO_2$: 329.0037; found: 329.0030.

**2-Bromo-1,8-dimethoxy-6-methylnaphthalene (2a)**

Viscous oil.

IR (neat): 2940, 2840, 1597, 1462, 1355, 1264, 1079, 747 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.39 (s, 3 H, $CH_3$), 3.82 (s, 3 H, $OCH_3$), 3.93 (s, 3 H, $OCH_3$), 6.67 (s, 1 H, H-7), 7.10 (s, 1 H, H-5), 7.23 (d, *J* = 8.8 Hz, 1 H, H-4), 7.47 (d, *J* = 8.8 Hz, 1 H, H-3).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.8, 56.3, 61.6, 109.1, 113.9, 119.6, 120.1, 124.6, 130.6, 136.6, 136.7, 153.1, 155.3.

MS (GC, 70 eV): *m/z* (%) = 115 (56), 128 (81), 139 (30), 158 (91), 186 (83), 207 (33), 209 (28), 280 (97, [$M^+$]), 282 (100, [$M^+$ + 2]).

**1,8-Dimethoxy-6-methylnaphthalene-2-boronic Acid (3)**

To a stirred solution of iodonaphthalene **2b** (301.7 mg, 0.92 mmol) in THF (7 mL) at –78 °C under argon was added *n*-BuLi (1.12 mL, 1.84 mmol) followed immediately by $B(OMe)_3$ (200 μL, 1.78

mmol). After stirring at –78 °C for 30 min, the mixture was warmed to r.t. and stirred for 1 h. The resulting mixture was quenched with 2 N HCl and extracted with $CH_2Cl_2$ (2 × 20 mL). The combined extracts were washed with $H_2O$ and brine and dried ($Na_2SO_4$). The solvent was removed to obtain the crude boronic acid which was purified by preparative layer chromatography (PLC) ($SiO_2$, 1% MeOH–$CH_2Cl_2$) to give boronic acid **3** as a white solid (163 mg, 72%); mp 157.5–158 °C ($CH_2Cl_2$–hexane).

IR (KBr): 2937(br), 1610, 1605, 1572, 1467, 1376 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.49 (s, 3 H, $CH_3$), 3.9 (s, 3 H, $OCH_3$), 4.04 (s, 3 H, $OCH_3$), 6.71 (s, 2 H, 2 OH), 6.74 (s, 1 H, H-7), 7.24 (s, 1 H, H-5), 7.52 (d, *J* = 16.4 Hz, 1 H, H-4), 7.83 (d, *J* = 16.8 Hz, 1 H, H-3).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.9, 56.1, 63.8, 108.4, 117.3, 120.4, 123.9, 132.0, 137.7, 139.9, 155.7, 163.8.

MS (EI, 70 eV): *m/z* (%) = 191 (22), 201 (22), 202 (15), 204 (75), 231 (14), 245 (27), 246 (100, [$M^+$]), 247 (14, [M + $H^+$]).

Anal. Calcd for $C_{13}H_{15}BO_4$: C, 63.45; H, 6.14. Found: C, 63.32; H, 5.77.

### Tetramethoxydiospyrol (1b) by Classical Suzuki–Miyaura Cross-Coupling Reaction

A mixture of iodonaphthalene **2b** (158.5 mg, 0.48 mmol), naphthaleneboronic acid **3** (118.9 mg, 0.48 mmol), $Pd(PPh_3)_4$ (11 mg, 3 mol%) and $K_2CO_3$ (133 mg, 0.96 mmol) in a mixture of toluene–EtOH–$H_2O$ (3:3:2, 8 mL) was refluxed at 115–120 °C for 19 h. After cooling the mixture, $H_2O$ was added and extracted with EtOAc (2 × 20 mL). The combined EtOAc extracts were washed with $H_2O$, brine and dried ($Na_2SO_4$). Further purification was carried out by PLC ($SiO_2$, 0.5% MeOH–$CH_2Cl_2$) to give tetramethoxydiospyrol (**1b**) which was recrystallized from benzene to afford **1b** as white crystals (136.2 mg, 70%).

### Tetramethoxydiospyrol (1b) Modified in situ Suzuki Cross-Coupling

*Method A*:[13] To a solution of bromonaphthalene (150 mg, 0.5 mmol) in THF (10 mL) at –78 °C was added 0.5 equiv of 0.77 M of *n*-BuLi (0.4 mL) followed by 6 equiv of $B(OMe)_3$ (0.8 mL). The resulting solution was warmed to r.t. for 4 h and subsequently stirred overnight under argon. To the solution were then added toluene (6 mL), EtOH (6 mL), $H_2O$ (4 mL), $K_2CO_3$ (130 mg, 1.0 mmol), and $Pd(PPh_3)_4$ (2 mg, 10 mol%). The resulting mixture was refluxed under argon for 10 h. The reaction was allowed to warm to r.t. and extracted with $CH_2Cl_2$ (3 × 30 mL). The organic phases were combined, washed with $H_2O$, dried ($Na_2SO_4$), and concentrated under reduced pressure to afford the crude binaphthalene which was purified on PLC to yield recovered bromonaphthalene **2a** (60 mg, 37%), tetramethoxydiospyrol (**1b**) (40 mg, 21%) and debromonaphthalene (30 mg, 26%).[16]

*Method B*:[14] A mixture of iodonaphthalene **2b** (165.4 mg, 0.5 mmol), bis(pinacolato)diboron (**9**; 63.5 mg, 0.25 mmol), $PdCl_2$dppf (14.6 mg, 4 mol%), and $K_2CO_3$ (207 mg, 1.5 mmol) in dioxane (5 mL) was heated at 80 °C for 16 h. After cooling the mixture, $H_2O$ was added and extracted with $CH_2Cl_2$. The combined $CH_2Cl_2$ extracts were washed with $H_2O$, 20% aq NaOH, and dried ($Na_2SO_4$). Further purification was carried out by column chromatography ($SiO_2$, 0.5% MeOH–$CH_2Cl_2$) to give tetramethoxydiospyrol (**1b**) (54.9 mg, 55%) as a white solid; mp (benzene) 239–239.5 °C (Lit.[1a] mp 232 °C, Lit.[1d] mp 243 °C).

IR (KBr): 1625, 1563, 1451, 1338, 1262 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.50 (s, 6 H, 2 $CH_3$), 3.55 (s, 6 H, 2 $OCH_3$), 4.01 (s, 6 H, 2 $OCH_3$), 6.73 (s, 2 H, H-7,7′), 7.26 (s, 2 H, H-5,5′), 7.52 (d, *J* = 16.4 Hz, 2 H, H-4,4′), 7.83 (d, *J* = 16.8 Hz, 2 H, H-3,3′).

$^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 21.8, 56.1, 61.4, 108.0, 118.6, 120.0, 122.7, 128.5, 130.8, 135.0, 137.2, 153.6, 156.2.

MS (EI, 70 eV): *m/z* (%) = 298 (8), 341 (25), 357 (21), 402 (100, [$M^+$]), 403 (29, [M + $H^+$]).

## Acknowledgment

We are grateful to the Thailand Research Fund (TRF) for the generous support of our research program (TRG468008 to NT) and for the award of Senior Research Scholar to S.R.

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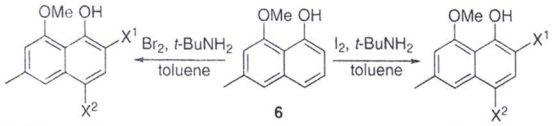
(11) The bromination and iodination of naphthol **6** also gave *para* halogenated products (Scheme 5). Compounds **7a** and **7b** were further methylated without purification.




**7a** $X^1$ = Br, $X^2$ = H
**7b** $X^1$ = H, $X^2$ = Br

**8a** $X^1$ = I, $X^2$ = H
**8b** $X^1$ = $X^2$ = I

**Scheme 5**

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(16) 1,8-Dimethoxy-3-methylnaphthalene[17] obtained as dehalogenation adduct from half-Suzuki cross-coupling was a white solid; mp 89–90 °C (MeOH). $^1$H NMR (200 MHz, $CDCl_3$): δ = 2.47 (s, 3 H, $CH_3$), 3.98 (s, 6 H, 2 $OCH_3$), 6.69 (d, *J* = 1.0 Hz, 1 H), 6.78 (dd, *J* = 6.2, 8.4 Hz, 1 H), 7.19 (br s, 1 H), 7.35 (m, 2 H). $^{13}$C NMR (50 MHz, $CDCl_3$): δ = 21.7, 56.2, 56.3, 105.2, 108.2, 115.6, 120.0, 120.2, 126.4, 136.1, 137.5, 156.8, 157.0. MS (EI, 70 eV): *m/z* (%) = 128 (71), 129 (99), 159 (58), 201 (100) 202 (50, [M$^+$]).

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# Two Protocols for the Conversion of Biphenol to Binaphthol: Synthesis of Diospyrol

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*Received 11 July 2005*

**Abstract:** The application of directed orthometallation (DoM), Fries rearrangement and transmetallation followed by allylation and cyclization is reported for the conversion of biphenol to binaphthol as a means for the synthesis of diospyrol. Furthermore, the same transformation can be accomplished by the reaction of the dienolate anion of an α,β-unsaturated amide with an aryne intermediate.

**Key words:** arynes, biaryls, ring closure, directed orthometallation, Fries rearrangement

The regiospecific preparation and modification of polysubstituted aromatic molecules has remained a fundamental problem in organic synthesis in both industrial and academic laboratories.[1,2] The directed orthometallation (DoM) reaction, discovered 70 years ago by Gilman[3] and Wittig,[4] has been extensively studied and exploited for the regioselective construction of polysubstituted aromatics and heteroaromatics.

Diospyrol (**1**) has been isolated from *Diospyros mollis*, a tree distributed throughout Thailand. It has a dimeric naphthalene skeleton with a C-2–C-2′ linkage between the 1-naphthol ring systems.[5] The fresh berries of this plant have long been used especially as anthelmintics.[6] Recently, michellamine alkaloids have been isolated and reported to exhibit potent anti-HIV activity.[7] Their structures are composed of two important units, a 1,3-dimethyltetrahydroisoquinoline and the core binaphthol. The structure of the core binaphthol is also similar to diospyrol. Michellamine B (Figure 1), the most studied compound of this group, showed interesting activity to protect MT-2 cells from both AZT-resistant and pyridone-resistant strains of HIV-1.[8] Several strategies have been evolved for the construction of this unit.[9,10]

We envisaged two pathways for the synthesis of the binaphthol, both starting from biphenol as shown in Scheme 1. In route A, the binaphthol could be derived from the double cyclization of the allyl carbanion onto the adjacent carboxamide group in compound **3**. Compound **3** could be synthesized from the *o*-allylation of carboxamide **4** which could be obtained from the biphenol **5**. In the second pathway as shown in route B it was planned that binaphthol could be directly generated via aryne annulation. It was anticipated that the trapping of bisaryne **8**, derived from tetrabromobiphenyl ether **6**, with diene **9**, generated from unsaturated amide **7**, could lead directly to the binaphthol **2**. An alternative mechanism for the formation of the same binaphthol **2** could involve the sequential reactions of the monoaryne derived from compound **6** with diene **9** followed by the reaction of another monoaryne and diene **9**. The tetrabromobiphenyl ether **6** could be easily obtained from the same biphenol **5** as used in the first pathway.

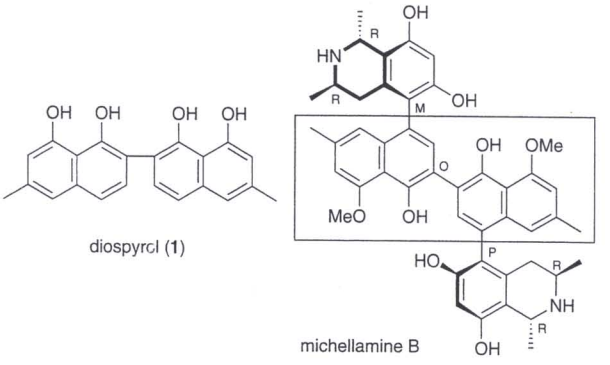
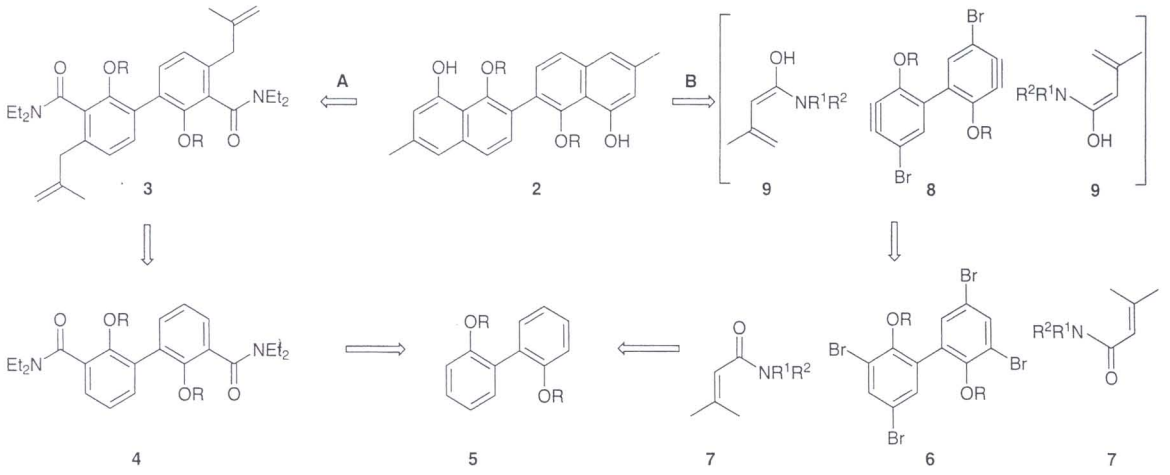



**Figure 1** Structure of diospyrol (**1**) and michellamine B

Herein, harnessing Snieckus chemistry, we report the application of directed orthometallation (DoM),[11] Fries rearrangement,[12] transmetallation–bis-allylation,[13] and double cyclization to the synthesis of binaphthol **2**. We also report the aryne cycloaddition reaction[10] of dienolate anion **9** of α,β-unsaturated amide **7** with tetrabromobiphenyl ether **6** in the presence of strong base affording diospyrol derivative **2**.

Commercially available biphenol **5** was first reacted with *N*,*N*-diethylcarbamoyl chloride and NaH in DMF to yield dicarbamate **10** (75%) as shown in Scheme 1.[11] *N*,*N*-Diethyl-2,2′-dihydroxy-1,1′-biphenyl-3,3′-dicarboxamide **4** (R = H) was obtained in good yield (80%) by double anionic *ortho*-Fries rearrangement[12] of 2,2′-dicarbamate-1,1′-biphenyl **10** using *t*-BuLi and TMEDA.[14] Compound **4** (R = H) was then protected as its methyl ether, isopropyl ether and methylenedioxy ether by reaction with MeI, 2-bromopropane or dibromomethane to give *N*,*N*-diethyl-2,2′-dialkoxyl-1,1′-biphenyl-3,3′-dicarboxamides

*SYNLETT* 2006, No. 7, pp 1080–1084
Advanced online publication: 24.04.2006
DOI: 10.1055/s-2006-939075; Art ID: D19405ST



**Scheme 1** Synthetic plans for the conversion of biphenol to binaphthol.

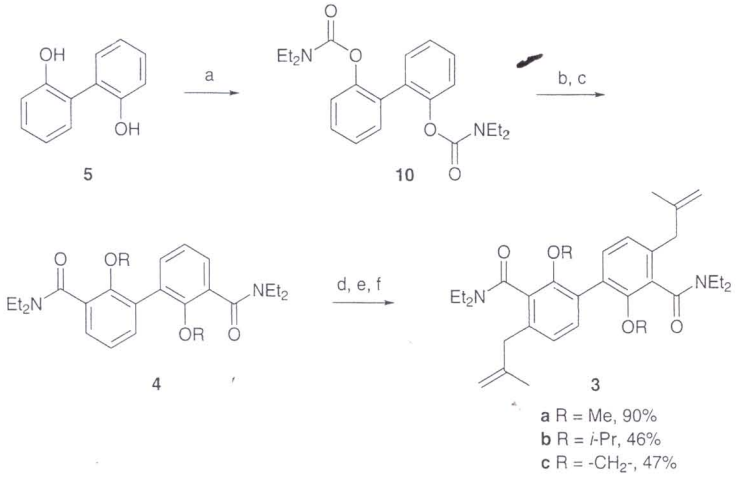
**4a–c** in excellent yields (94–100%). These were ortholithiated using *t*-BuLi/TMEDA in THF at –78 °C, transmetallated with CuCN/LiCl[13] and trapped with β-methallyl chloride in a one-pot reaction.

The reaction was allowed to warm to room temperature and stirred overnight to give the required compounds **3a–c** in moderate to good yields as shown in Scheme 2.

We have investigated various bases and conditions for the base-induced double cyclization of compounds **3**. When compound **3a** was treated with five or ten equivalents of LDA in THF, complex mixtures of products were obtained. Treatment of compound **3b** with five equivalents of LDA gave the desired product **2b** in 21% yield together with the half-cyclized product **11b** in 33% yield (Table 1, entry 3). Increasing the amount of LDA to ten equivalents gave lower yields of both compounds **2b** and **11b** (entry 4). It was gratifying to find that compound **3a** could be induced to cyclize to the corresponding binaphthol by using MeLi.[16] The desired binaphthol **2a** (R = Me) was isolated in good yield (75%) when six equivalents of MeLi (entry 6)[17] were used but in lower yield (67%) when only four equivalents of MeLi were employed (entry 5). When the MeLi-induced cyclization (6 equiv of MeLi) was also applied to compound **3b**, the required product **2b** was obtained in 52% (entry 7).

Compound **3c** gave a complex mixture on treatment with LDA and MeLi.[16] Attempts to activate the carboxamide group of compound **3a** with $Tf_2O$ in the presence of pyridine[18] to induce cyclization also failed.

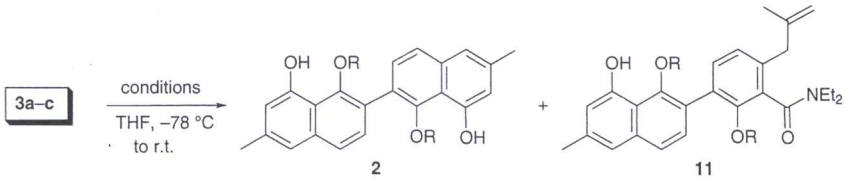
The two-step double cyclization and methylation of the intermediate **3a** to tetramethoxydiospyrol was also examined in a one-pot process and provided a good yield (75%) of the product. The reaction was carried out using six equivalents MeLi for double cyclization and the crude product was used in the next step without purification by methylation with MeI in presence of NaH in DMF. The spectroscopic data of the compound obtained were identical with those derived from tetramethoxydiospyrol



**a** R = Me, 90%
**b** R = *i*-Pr, 46%
**c** R = -$CH_2$-, 47%

**Scheme 2** *Reagents and conditions*: a) $ClCONEt_2$, NaH, DMF (75%); b) *t*-BuLi, TMEDA, THF, –78 °C (80%);[14] c) RX, NaH, DMF (**4a**: R = Me, 94%, **4b**: R = *i*-Pr, 100%, **4c**: R = -$CH_2$-, 98%); d) *t*-BuLi, TMEDA, THF, –78 °C; e) CuCN, LiCl, THF; f) β-methallyl chloride (**3a**: 90% over 3 steps, **3b**: 46% over 3 steps,[15] **3c**: 47% over 3 steps).

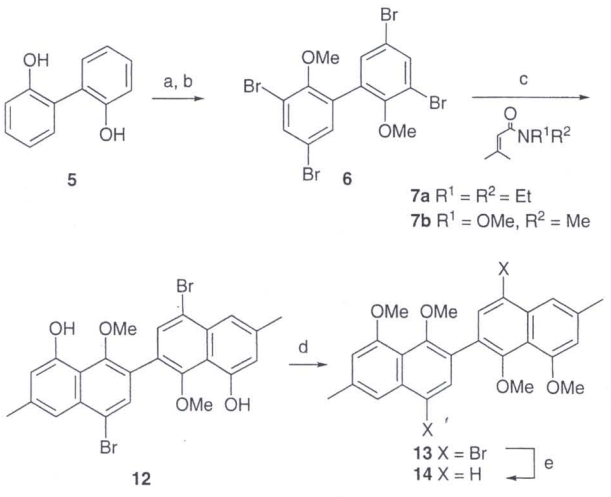
**Table 1** Double Ring Closure of Key Intermediates **3a–c**




| Entry | R | Conditions | Yield of **2** (%) | Yield of **11** (%) |
|---|---|---|---|---|
| 1 | Me | LDA (5 equiv) | Complex mixture | |
| 2 | Me | LDA (10 equiv) | Complex mixture | |
| 3 | *i*-Pr | LDA (5 equiv) | 21 | 33 |
| 4 | *i*-Pr | LDA (10 equiv) | 19 | 29 |
| 5 | Me | MeLi (4 equiv) | 67 | – |
| 6 | Me | MeLi (6 equiv)[17] | 75 | – |
| 7 | *i*-Pr | MeLi (6 equiv) | 52 | – |

synthesized by another route.[9] The tetramethoxydiospyrol could be demethylated to diospyrol by the previously published procedure.[9b,c]

The remarkable regiospecificity of the aryne annulation reaction[10] has been extensively used for the synthesis of naphthols. As an extension of this type of synthetically useful cycloaddition reaction, we were interested in the application of this approach for the synthesis of binaphthols in general and the synthesis of diospyrol in particular. With this idea in mind, biphenol **5** was converted to tetrabromo-2,2′-dihydroxybiphenyl by bromination with bromine in AcOH in quantitative yield. The tetrabromophenol was methylated using dimethylsulfate and $K_2CO_3$ in refluxing acetone to give tetrabromo-2,2′-dimethoxybiphenyl **6** in 67% yield.




**Scheme 3** *Reagents and conditions*: a) $Br_2$, AcOH; b) $Me_2SO_4$, $K_2CO_3$, acetone, reflux (67%, 2 steps); c) LTMP, THF, –78 °C (20% from **7a**,[19] 14% from **7b**); d) $Me_2SO_4$, $K_2CO_3$, acetone, reflux (96%); e) *n*-$Bu_3SnH$, AIBN, reflux (81%).

Having the tetrabromobiphenyl ether **6** in hand, the aryne annulation was then investigated. *N*,*N*-Diethylsenecio amide **7a** was treated with an excess of LDA at –78 °C in THF in order to generate the lithiated amide. The tetrabromobiphenyl ether **6** was added to the solution to generate the aryne and the mixture was allowed to warm to room temperature. After purification, the undesired LDA addition products were obtained.

To overcome this problem, the more hindered base, LTMP, was used. Treatment of tetrabromobiphenoldimethyl ether **6** with an excess of LTMP and *N*,*N*-diethylsenecio amide **7a** gave dibromodiospyrol adduct **12** directly in 20% yield together with other unidentified products.[19] Using the Weinreb amide **7b**, a lower yield (14%) of binaphthol **12** was obtained.

Methylation of 4,4′-dibromodiospyrol **12** with dimethylsulfate in the presence of $K_2CO_3$ in refluxing acetone gave 4,4′-dibromodiospyrol tetramethyl ether **13** in high yield (96%) which was converted to diospyrol tetramethyl ether **14** by debromination with tributyltin hydride in good yield (81%).[20] The dibromodiospyrol derivative was recently synthesized by a different approach.[21] Significantly, the remaining bromine group can be used as a handle for further coupling.

In summary, we have successfully developed two direct approaches for the conversion of biphenol to binaphthol and applied to the synthesis of diospyrol. The methodology should be applicable to the synthesis of related oxygen heterocycles.

## Acknowledgment

We acknowledge the Thailand Research Fund (TRF) for the generous support of our research programme and the award of Senior Research Scholar to SR as well as the award of a research grant (TRG4680008) to N.T.

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(14) **Directed Orthometallation (DoM) and Fries Rearrangement.**
To a solution *t*-BuLi (1.7 M, 30 mL, 50 mmol) and TMEDA (7.5 mL, 50 mmol) in dry THF (100 mL) was slowly added a solution of 2,2′-*N*,*N*-diethylcarbamoyl-1,1′-biphenyl (**10**, 7.68 g, 20 mmol) in THF (50 mL) at –78 °C under $N_2$ atmosphere. The stirred reaction mixture was allowed to attain r.t. overnight and treated with a sat. $NH_4Cl$ solution. The organic solvent was removed in vacuo and the remaining solution was extracted with $CH_2Cl_2$. The combined organic layer was washed with $H_2O$, brine, dried (anhyd $Na_2SO_4$), and evaporated to give a viscous oil. After purification by flash column chromatography using EtOAc–hexane as eluent, 2,2′-dihydroxybiphenyl-3,3′-dicarboxylic acid bisdiethyl amide(**4**) was obtained (6.14 g, 80%) as a white solid; mp 140–141 °C (EtOAc–hexane). IR (KBr): 3428, 2981, 1600, 1572, 1488, 1450, 1408, 1353, 1311, 1259, 1141 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$): δ = 1.27 (t, *J* = 7.0 Hz, 12 H), 3.53 (q, *J* = 7.0 Hz, 8 H), 6.99 (t, *J* = 7.2, 7.8 Hz, 2 H), 7.31 (dd, *J* = 1.8, 7.8 Hz, 2 H), 7.38 (dd, *J* = 2.0, 7.7 Hz, 2 H). $^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 13.4, 41.2, 119.3, 120.8, 127.1, 127.2, 133.7, 149.0, 171.0. MS (EI): *m/z* (%) = 385 (20) [$M^+$ + 1], 384 (72) [$M^+$], 383 (51), 313 (78), 312 (46), 311 (82), 310 (45), 295 (21), 285 (72), 283 (38), 240 (27), 239 (100). HRMS (FAB): *m/z* calcd for $C_{22}H_{28}N_2O_4$ [M + $H^+$]: 385.2127; found: 385.2128.

(15) Yield of **3b** was improved to 53% using CuBr·$SMe_2$.

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(17) **Representative Procedure for the Double Ring Closure.**
To a stirred THF (5 mL) solution of 2,2′-dimethoxy-4,4′-bis(2-methylallyl)biphenyl-3,3′-dicarboxylic acid bisdiethyl amide (**3a**, 0.1316 g, 0.25 mmol) at –78 °C under Ar was added a solution of MeLi (1.4 M, 1.1 mL, 1.5 mmol) in $Et_2O$. The solution turned deep violet and was allowed to warm to r.t. and stirred at this temperature overnight. The reaction was quenched by the addition of 20 mL of sat. $NH_4Cl$ and extracted with $CH_2Cl_2$. The organic layer was combined, washed with $H_2O$, brine and dried (anhyd $Na_2SO_4$). The crude product obtained after evaporation of $CH_2Cl_2$ was purified by PLC using $CH_2Cl_2$–hexane (2:1) as eluent to give white solid as 1,1′-dimethoxy-6,6′-dimethyl-2,2′-binaphthalenyl-8,8-diol (**1a**, 0.0699 g, 75%); mp 234–236 °C (EtOAc–hexane). IR (KBr): 3321, 2926, 1637, 1573, 1460, 1378, 1354, 1058 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): δ = 2.47 (s, 6 H), 3.58 (s, 6 H), 6.82 (d, *J* = 1.4 Hz, 2 H), 7.17 (br s, 2 H), 7.50 (d, *J* = 8.5 Hz, 2 H), 7.58 (d, *J* = 8.5 Hz, 2 H). $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 21.7, 61.8, 112.9, 115.5, 118.4, 123.7, 124.3, 129.1, 136.5, 138.7, 153.4, 154.1. MS (EI): *m/z* (%) = 374 (61) [$M^+$], 356 (15), 343 (23), 342 (81), 329 (36), 328 (100). HRMS (micro-TOF, ESI): *m/z* calcd for $C_{24}H_{22}O_4$ [M + $H^+$]: 375.1591; found: 375.1584.

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(19) **Representative Procedure for the Aryne Annulation.** A solution of lithium 2,2,6,6-tetramethylpiperidine (LTMP, 6.4 mmol) was prepared at 0 °C from 2,2,6,6-tetramethylpiperidine (1.1 mL) and *n*-BuLi (4.95 mL) in dry THF (20 mL). After 30 min, the solution was cooled to –78 °C and a solution of *N*,*N*-diethyl seneciomide (**7a**, 0.66 g, 4.3 mmol) in dry THF (5 mL) was added and stirred at this temperature for 1 h. A solution of tetrabromo-2,2′-dimethoxybiphenyl (**6**, 0.57 g, 1.1 mmol) in dry THF (15 mL) was added dropwise, the reaction turned to dark red. After the addition was complete, the reaction was slowly warmed to r.t. and stirred overnight. Then, sat. $NH_4Cl$ was added and the mixture was extracted with $CH_2Cl_2$. The combined organic layer was washed with $H_2O$, brine and dried ($Na_2SO_4$) and evaporated to dryness. Further purification was carried out by PLC ($SiO_2$, 4% EtOAc–hexane) to obtain binaphthol **12** (0.117 g, 20%).

Compound **12**: mp 249–250 °C (EtOAc–hexane). IR ($CHCl_3$): 3344 (OH), 3010, 1636, 1369, 803 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$): δ = 2.55 (s, 6 H), 3.61 (s, 6 H), 6.91 (d, *J* = 1.3 Hz, 2 H), 7.56 (d, *J* = 0.9 Hz, 2 H), 7.84 (s, 2 H), 9.76 (s, 2 H). $^{13}C$ NMR (50 MHz, $CDCl_3$): δ = 22.0, 62.3, 114.3, 116.3, 118.2, 118.3, 123.1, 132.1, 134.4, 140.4, 153.4, 154.4. MS (EI): *m/z* (%) = 534 (50) [$M^+$ + 2], 532 (100) [$M^+$], 530 (48), 488 (41), 486 (84), 484 (42). Anal. Calcd for $C_{24}H_{20}Br_2O_4$: C, 54.16; H, 3.79. Found: C, 54.27; H, 3.76.

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