CHAPTER III

RESULTS AND DISCUSSION

3.1 Isolated compounds from the roots and fruits of *H. perforata*

In the present study, the EtOAc crude extracts of the roots and fruits of *H. perforata* were subjected to column chromatography on siliga gel and Sephadex LH-20 leading to the isolation of a new chromone, harperamone (**8**), and two new rearranged limonoids, harperfolide (**2**) and harperforatin (**4**), along with six known compounds classified as limonoid, chromone, coumarin, diterpene and polyketide. These includes harrisonin (**1**), obacunone (**3**), (+)-vouacapenic acid (**5**), harrisonal A (**6**), peucenin-7-methyl ether (**7**) and braylin I (**9**). A coumarin braylin I (**9**) and a diterpene (+)-vouacapenic acid (**5**) were found to be first isolated from the genus *Harrisonia*. Structures of the isolated compounds are presented in Figure 3.1

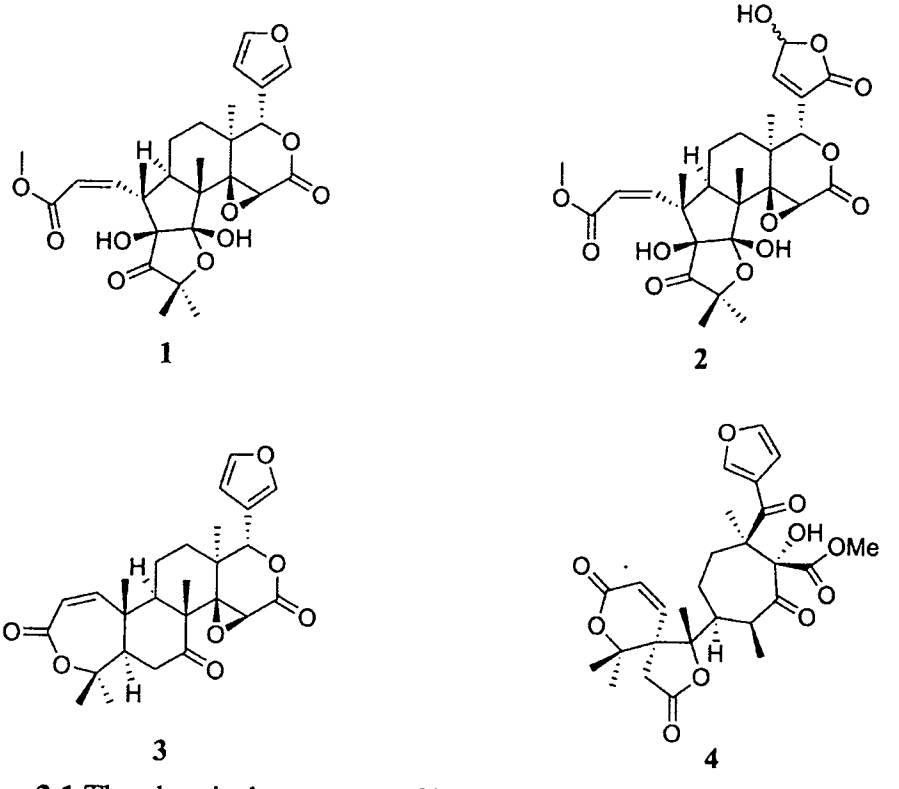


Figure 3.1 The chemical structures of isolated compounds from *H. perforata*

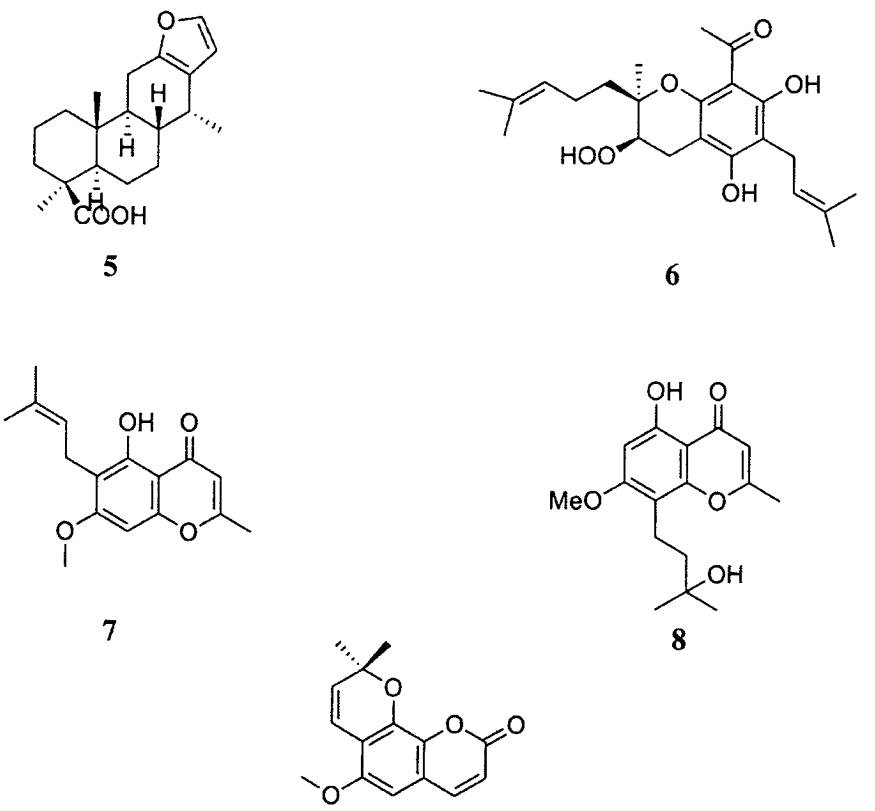


Figure 3.1 The chemical structures of isolated compounds from *H. perforata* (continued)

3.2 Structure elucidation of isolated compounds

3.2.1 Structure elucidation of compound 1

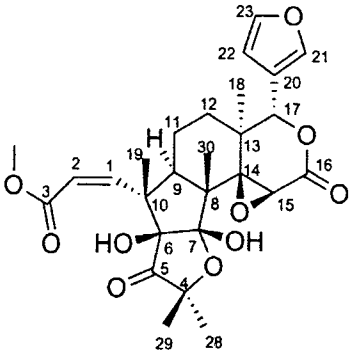


Figure 3.2 Compound **1**

Molecular formula	$C_{27}H_{32}O_{10}$
Appearance	Colorless needles
Melting point	158-160 °C
$[\alpha]_D^{20}$ (c 0.1 , MeOH)	+ 40
UV (MeOH) λ_{max} (log ε)	209 nm (3.76)
IR (KBr)	3443, 2979, 2947, 1739, 1715, 1637, 1435, 1385, 1268, 1208, 1180, 1017 and 875 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.1

Compound **1** was obtained as colorless needles and had the molecular formula $C_{27}H_{32}O_{10}$ based on the NMR data analysis. IR spectrum of **1** showed the typical bands at ν_{max} 3443 cm^{-1} of hydroxyl and a series of carbonyl bands at ν_{max} 1739, 1715 and 1637 cm^{-1}. The ^1H NMR spectrum of **1** displayed signals of five tertiary methyls (δ_H 1.14, 1.17, 1.26, 1.35 and 1.49), a furan ring (δ_H 6.32, 7.39 and 7.41), α,β unsaturated ketone/ester (δ_H 5.76 and 6.00), and a methoxy group (δ_H 3.78). In the ^{13}C NMR spectrum, signals for six olefinic carbons (δ_C 109.8, 120.9, 123.1, 141.1, 142.9 and 153.8), a hemiketal (δ_C 108.2) and epoxide carbons (δ_C 57.3 and 68.5) were observed. Based on the above data, it was suggested that the structure

of **1** was based on a rearranged limonoid, harrisonin, which had a five-membered ring B, Table 3.1 [8]. The structure was further confirmed by 2D NMR data. ^{1}H-^{1}H COSY correlations indicated the presence of –C(9)H–C(11)H_2–C(12)H_2– and –C(1)H=C(2)H– fragments. Observed HMBC correlations including between epoxide ring proton (δ_H 4.27) and quaternary carbons (δ_C 68.5, C-14) and carbonyl ester (δ_C 167.8, C-16), between two methyls (δ_C 1.17, 1.35) and oxygen-bearing carbon (δ_C 88.6, C-4) and ketone (δ_C 216.8, C-5) helped to establish the structure of **1** and to identify as harrisonin as shown in Figure 3.3. On the basis of the literature study, harrisonin has been reported from all three species belonging to the genus *Harrisonia.*

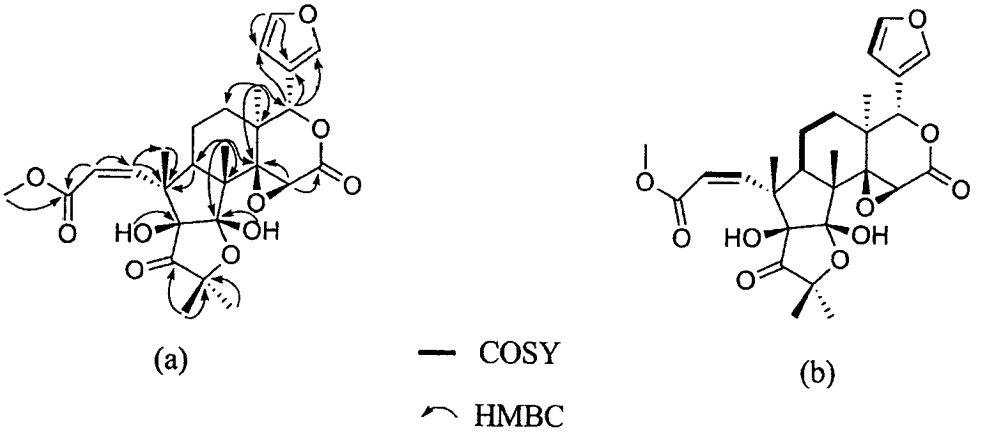


Figure 3.3 HMBC (a) and ^{1}H-^{1}H COSY (b) correlations of compound **1**

Table 3.1 The NMR data of compound **1** and harrisonin ($CDCl_3$)[a]

Position	Harrisonin[b]	Compound **1**[c]	
	δ_C	δ_H (mult, J in Hz)	δ_C
1	153.9	6.00 (d, J = 12 Hz, 1H)	153.8
2	123.1	5.76 (d, J = 12 Hz, 1H)	123.1
3	166.7		166.6
4	80.9		80.9
5	216.9		216.8
6	88.6		88.6
7	108.2		108.2
8	49.9		49.6
9	46.8	2.99/2.96 (m, 1H)	46.7
10	49.7		49.9
11	15.2	1.80 (m, 2H)	15.1
12	26.3	1.65 (m, 2H)	26.2
13	39.5		39.5
14	68.5		68.5
15	57.3	4.27 (s, 1H)	57.3
16	167.8		167.8
17	78.4	5.66 (s, 1H)	78.4
18	18.3	1.26 (s, 3H)	18.3
19	17.3	1.49 (s, 3H)	17.2
20	121.0		120.9
21	141.1	7.41 (brs, 1H)	141.1
22	109.9	6.32 (brs, 1H)	109.8
23	143.0	7.39 (brs, 1H)	142.9
28	24.1	1.17 (s, 3H)	24.0
29	27.4	1.35 (s, 3H)	27.3
30	14.7	1.14 (s, 3H)	14.6
3-OMe	52.0	3.78 (s, 3H)	52.0
6-OH		5.07 (brs, 1H)	
7-OH		3.68 (brs, 1H)	

[a] Spectra were recorded in $CDCl_3$, [b] 300 MHz, [c] 400 MHz

3.2.2 Structure elucidation of compound 2

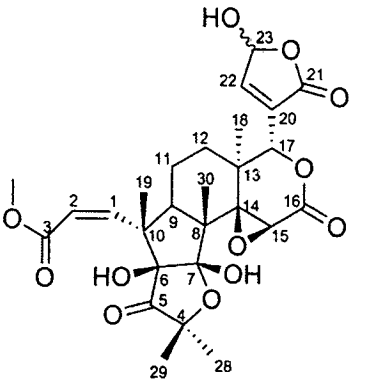


Figure 3.4 Compound **2**

Molecular formula	$C_{27}H_{32}O_{12}$
Appearance	Colorless crystals
Melting point	178-180 °C
$[\alpha]_D^{20}$ (*c* 0.1 , MeOH)	+ 11
UV (MeOH) λ_{max} (logε)	206 nm (4.21)
IR (KBr)	3454, 2976, 2947, 1764, 1725, 1630, 1438, 1382, 1268, 1208, 1020 and 935 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.2

Compound **2** was isolated as colorless crystals and had the molecular formula as $C_{27}H_{32}O_{12}$ by HR-ESI-MS (*m/z* 547.1886 [M - H]$^-$, Calcd 547.1810). The IR spectrum showed absorption bands at ν_{max} 3454 cm^{-1} for hydroxyl group, and at ν_{max} 1764, 1725 and 1630 cm^{-1} for a series of carbonyl groups. The ^1H NMR and ^{13}C NMR spectra displayed signals of an α,β-unsaturated methyl ester (δ_H 5.77, 6.00/6.01, 3.78 and δ_C 52.1 CH_3, 123.2/123.3 CH, 153.6/153.7 CH, 166.8), a γ-hydroxybutenolide ring (δ_H 6.18/6.22, 7.32/7.34 and δ_C 96.6/97.4, 134.3/134.6, 149.5/150.3, 169.3), and five tertiary methyls (δ_H 1.24/1.25, 1.15, 1.19, 1.24/1.25, 1.37 and δ_C 14.6, 17.3, 17.9, 24.1, 27.4). The existence of an α,β-epoxy-δ-lactone ring was confirmed by HMBC cross peaks from H-17 to both bridgehead carbons

(C-13 and C-14) and the C-16 ester carbonyl, from Me-18 to C-13, C-14 and C-17, and from H-15 to C-14 and C-15 (Figure 3.5). The NMR data of **2** were similar to those of harrisonin (**1**), a known rearranged limonoid isolated from this plant. This indicated they must share the same basic skeleton, except for the presence of a γ-hydroxybutenolide moiety instead of a furanyl ring in **1**. Moreover, observed HMBC correlations from H-17 to C-20, C-21 and C-22 of a butenolide group clarified the location of a γ-hydroxybutenolide at C-17 as shown in Figure 3.5. In addition, the appearance of pairs of most proton and carbon resonances in the NMR spectra of **2** (Table 3.2) suggested the presence of C-23 epimers, the same as those in moluccensin N [14]. Thus the compound **2** was found to be new, named as harperfolide.

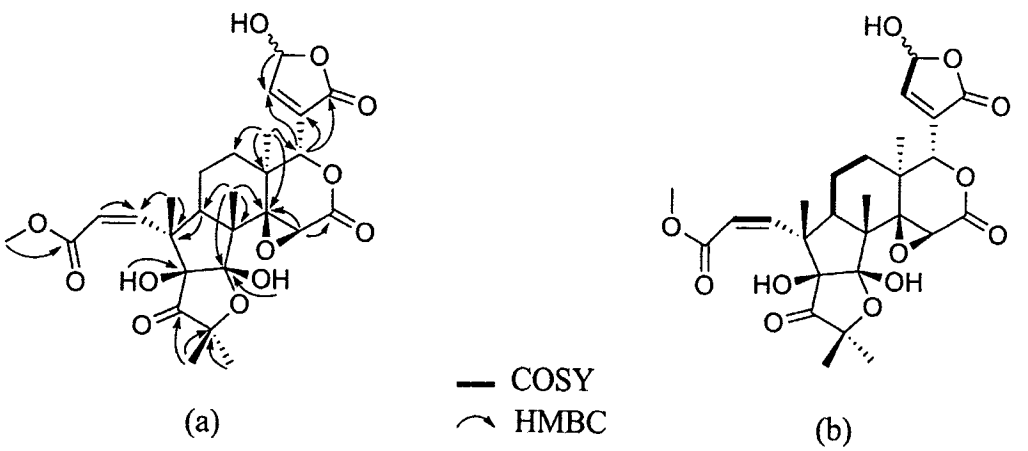


Figure 3.5 HMBC (a) and ^{1}H-^{1}H COSY (b) correlations of compound **2**

Table 3.2 The NMR data of compound **2** ($CDCl_3$, 400 MHz)

postition	δ_H (mult, J in Hz)	δ_C
1	6.00 (d, J = 12.4 Hz, 1H)	153.6
	6.01 (d, J = 12.4 Hz, 1H)	153.7
2	5.77 (d, J = 12.4 Hz, 1H)	123.2/123.3
3		166.8
4		81.0
5		216.6
6		88.5
7		108.3
8		49.6
9	2.97 (m, 1H)	46.7
10		50.0
11	1.75 (m, 2H)	15.1
12	1.80 (m, 2H)	25.8/25.9
13		40.1/40.2
14		68.4/68.6
15	4.28 (s, 1H)	57.0
16		167.1/167.6
17	5.59 br s/5.60 br s	76.2/76.6
18	1.24 (s, 3H)/1.25 (s, 3H)	17.9
19	1.50 (s, 3H)/1.51 (s, 3H)	17.3
20		134.6/134.6
21		169.3
22	7.32 (br s, 1H)/7.34 (br s, 1H)	149.5/150.3
23	6.18 (d, J = 4.0 Hz, 1H)	96.6
	6.22 (d, J = 12.0 Hz, 1H)	97.4
28	1.37 (s, 3H)	27.4
29	1.19 (s, 3H)	24.1
30	1.15 (s, 3H)	14.6
3-OMe	3.78 (s, 3H)	52.1
6-OH	5.10 (s, 1H)	
7-OH	3.64 (s, 1H)	
23-OH	3.66 (s, 1H)	

3.2.3 Structure elucidation of compound 3

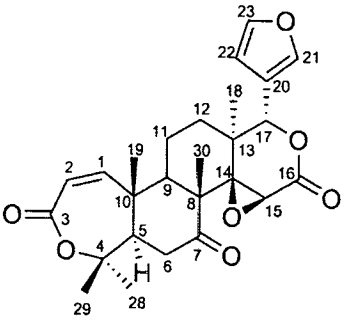


Figure 3.6 Compound **3**

Molecular formula	$C_{26}H_{30}O_7$
Appearance	Colorless crystals
Melting point	226-229 °C
UV (MeOH) λ_{max} (logε)	211 nm (4.11)
IR (KBr)	3447, 2969, 2944, 1739, 1704, 1622, 1452, 1392, 1282, 1165, 1027 and 804 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.3

Compound **3** was obtained as colorless crystals and its molecular formula $C_{26}H_{30}O_7$ was suggested by NMR data. IR and UV spectra showed absorption bands of carbonyls (ν_{max} 1739, 1704 and 1622 cm^{-1}) and β-substituted furan ring (λ_{max} 211 nm). The ^1H NMR spectrum presented typical signals of α,β unsaturated furan ring at δ_H 6.36, 7.39 and 7.41, α,β unsaturated 7-membered ring lactone at δ_H 5.95 and 6.51. These data and ^{13}C NMR spectrum indicated that **3** was a limonoid with five tertiary methyls (δ_C 16.4, 17.0, 21.1, 26.8 and 32.0), and three carbonyl carbons (δ_C 166.6 and 166.9 (lactones), and 207.4 (ketone)). Analysis of ^1H-^1H COSY correlations led to the establishment of four discrete spin systems, –C(9)H–C(11)H_2–C(12)H_2–, –C(5)H–C(6)H_2–, –C(1)H–C(2)H–, and –C(21)H–C(22)H– as shown in Figure 3.7(b). HMBC correlations from an epoxy ring (δ_H 3.65, H–15) to

C-14 and C-16 established the connectivity between an epoxide and a lactone carbonyl. In addition, correlations from H-5 (δ_H 2.59) to C-4 and from H_2-6 (δ_H 2.28 and 2.97) to C-10 resulted in the attachment of ring A to ring B at C-4, and the presence of a ketone at C-10, respectively. Further comparison of its NMR data with those in literature suggested compound **3** was obacunone, a limonoid previously isolated from plants in the genus *Harrisonia* and *Xylocarpus* as shown in Table 3.3 [15]. Moreover, the structure and relative configuration of **3** was subsequently confirmed by single crystal X-ray diffraction analysis as shown in Figure 3.8. In this study, the monoclinic form was obtained and its crystal data are presented in Table 3.4. An orthorhombic form of this compound was also previously reported [16].

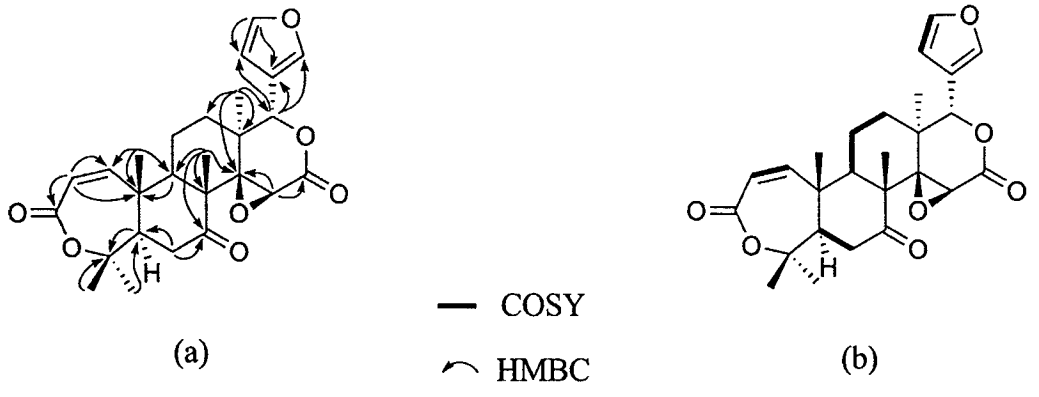


Figure 3.7 HMBC (a) and COSY (b) correlations of compound **3**

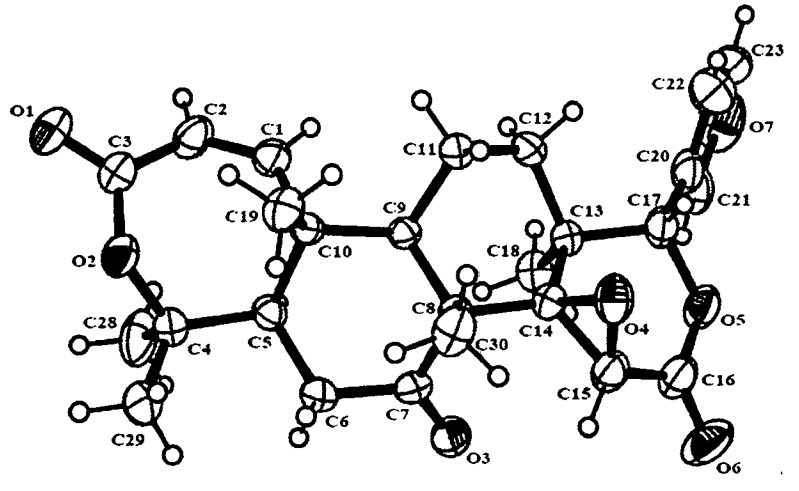


Figure 3.8 ORTEP diagram of compound **3**

Table 3.3 The NMR data of compound **3** ($CDCl_3$, 400 MHz)

Position	Obacunone	Compound **3**	
	δ_C	δ_H (mult, J in Hz)	δ_C
1	156.5	6.51 (d, J = 11.6 Hz, 1H)	156.7
2	122.4	5.95 (d, J = 11.6 Hz, 1H)	123.0
3	166.3		166.9
4	84.0		80.0
5	57.0	2.59 (m, 1H)	57.4
6	39.7	2.28 (d, J = 4.8 Hz, 1H)	39.9
		2.97 (d, J = 4.8 Hz, 1H)	
7	207.2		207.4
8	53.1		53.0
9	49.1	2.14 (m, 1H)	49.2
10	43.1		43.1
11	17.1	1.87 (m, 2H)	19.4
12	32.5	1.47 (m, 1H)	32.8
		1.89 (m, 1H)	
13	37.5		37.4
14	65.2		65.0
15	53.3	3.65 (s, 1H)	53.3
16	167.1		166.6
17	78.1	5.45 (s, 1H)	78.0
18	19.2	1.12 (s, 3H)	21.1
19	17.1	1.50 (s, 3H)	16.4
20	120.1		120.1
21	143.2	7.41 (brs, 1H)	141.0
22	109.5	6.36 (brs, 1H)	109.7
23	141.1	7.39 (brs, 1H)	143.2
28	21.1	1.50 (s, 3H)	26.8
29	32.1	1.45 (s, 3H)	32.0
30	26.1	1.24 (s, 3H)	17.0

Table 3.4 Crystal data and structure refinement for compound **3**

Formula	$C_{26}H_{30}O_7$
Molecular weight	454.50
Crystal size (mm)	0.7 × 0.26 × 0.24
Crystal system	monoclinic
Space group	$P2_12_12_1$
***a* (Å)**	7.8244(5)
***b* (Å)**	11.9408(10)
***c* (Å)**	12.5882(11)
***V* (Å^3)**	1172.38(16)
Z	2
D_{calc} **(g/cm^{-3})**	1.287
μ (mm^{-1})	0.093
***F*(000)**	484
Independent reflections/ Observed reflections [I > 4σ(I)], R_{int}	4129/3536, 0.0197
R_1	0.0557
wR_2 **[I > 2σ(I)]**	0.1614

3.2.4 Structure elucidation of compound 4

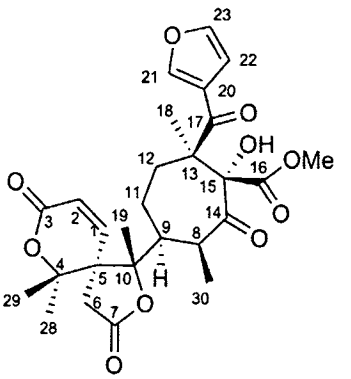


Figure 3.9 Compound **4**

Molecular formula	$C_{27}H_{32}O_{10}$
Appearance	Colorless crystals
Melting point	229-231 °C
$[\alpha]_D^{20}$ (c 0.1 , MeOH)	- 45
UV (MeOH) λ_{max} (logε)	203 nm (4.03)
IR (KBr)	3436, 2944, 2923, 1793, 1746, 1690, 1654, 1392, 1307, 1247, 1105, 1024 and 825 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.4

Compound **4** was obtained as colorless crystals and assigned the molecular formula $C_{27}H_{32}O_{10}$ from its HRESIMS (*m/z* 517.1947 $[M+H]^+$, cald 517.2068). The ^1H NMR spectrum of **4** (Table 3.5) displayed signals attributable to four tertiary methyls (δ_H 1.35, 1.51, 1.57, 1.59), one secondary methyl (δ_H 1.40, d, J = 8.0 Hz), one methoxy (δ_H 3.74), two olefinic protons (δ_H 6.30, 6.63, each d, J = 10.0 Hz), and a β-furanyl ring (δ_H 6.77, 7.40, 8.04, each br s). The ^{13}C NMR (Table 3.5) and HSQC data revealed the presence of five methyls (four tertiary, one secondary), three methylenes, eight methines (six olefinic), five quaternary carbons (three oxygenated), one methoxy, three ester and two ketone carbonyls. On the basis of the above NMR data, compound **4** had a tetracyclic skeleton due to eight units of the 12 unsaturations coming from five carbonyl groups and three carbon-carbon double

bonds. One ketone (δ_C 196.2) was connected to the C-20 of a β-furanyl ring because of the downfield shift of H-22 (δ_H 8.04), and its HMBC correlation with Me-18 (Figure 3.10). The other ketone moiety (δ_C 208.4) was assigned to C-14 by HMBC correlation between Me-30/C-14 and H-8/C-14. Observed HMBC correlations of Me-18/C-12, Me-18/C-13, Me-18/C-15, H-12/C-15 and Me-30/C-14, coupled with the connectivity of the partial structure $-CH_2(12)-CH_2(11)-CH(9)-CH(8)Me-$ by $^1H-^1H$ COSY correlations, suggested the existence o of seven-membered ring in **4** (Figure 3.10). In addition, the presence of two lactone rings connecting together through the C-5 spiro carbon were corroborated by HMBC cross peaks between H-1/C-5, Me-28/C-5, Me-29/C-9, H_2-6/C-7, H_2-6/C-10 and Me-19/C-10. This unit was further connected to a seven-membered ring at C-9 by a strong HMBC correlation from Me-19 to C-9. The complete structure and relative configuration of **4** was finally established by single-crystal X-ray diffraction analysis using Mo Kα radiation as shown in Figure 3.11 and its crystal data are presented in Table 3.6. To the best of our knowledge, the structure of **4** possesses a very unique two lactones connecting together through a spiro carbon. The biosynthetic pathway of **4** can plausibly be traced back to harperforin F, a highly rearranged limonoid also previously isolated from *H. perforata* [9]. Compound **4** might be a parent compound of harperforin F *via* Michael addition of C8 enolate to α,β-unsaturated ketone at C1 position as shown in Fig. 3.12.

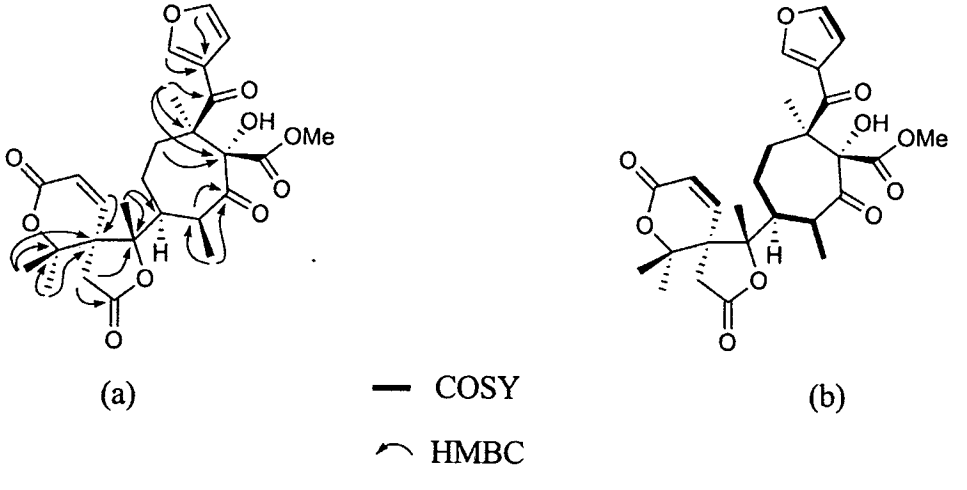


Figure 3.10 HMBC (a) and COSY (b) correlations of compound **4**

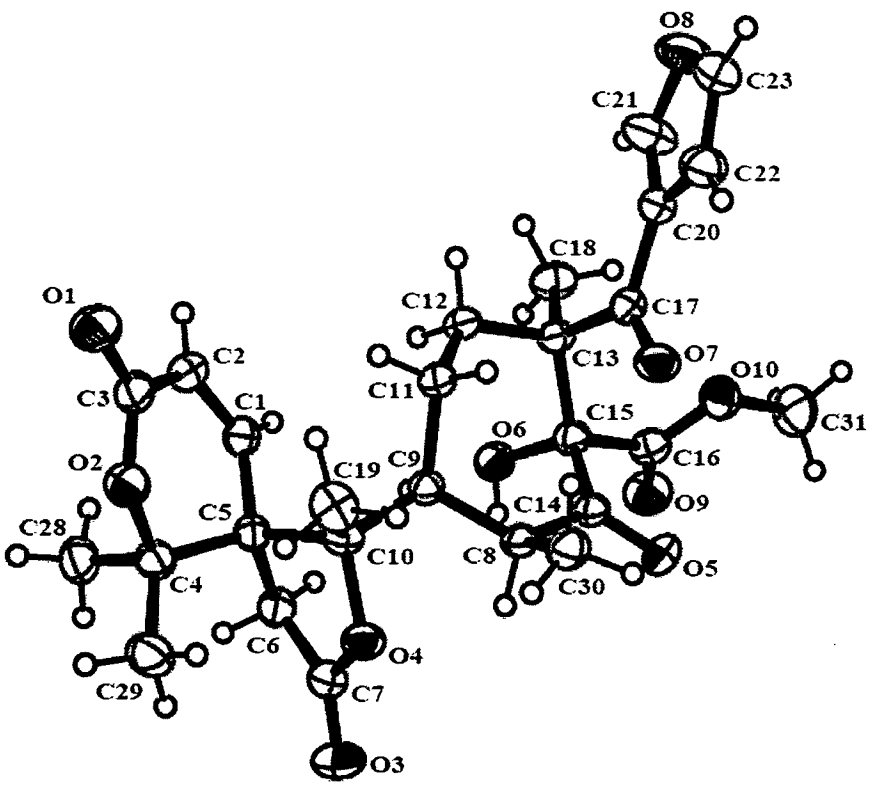
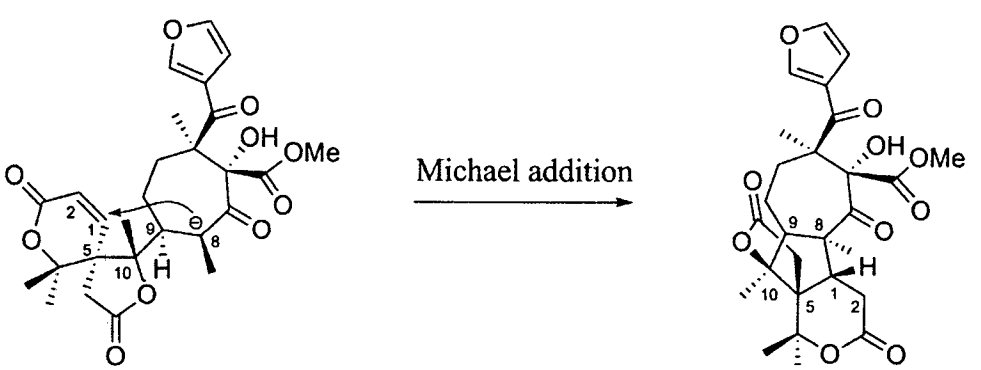


Figure 3.11 ORTEP diagram of compound **4**



Compound 4

Harperforin F

Figure 3.12 Proposed biosynthetic pathway between compound **4** and harperforin F

Table 3.5 The NMR data of compound **4** ($CDCl_3$, 400 MHz)

position	δ_H (mult, J in Hz)	δ_C
1	6.63 (d, J = 10.0 Hz, 1H)	146.9
2	6.30 (d, J = 10.0 Hz, 1H)	123.1
3		162.3
4		83.7
5		54.6
6	2.78 (d, J = 16.0 Hz, 1H)	38.4
	2.60 (d, J = 16.0 Hz, 1H)	
7		171.7
8	3.12 (m, 1H)	47.7
9	2.35 (m, 1H)	45.5
10		95.4
11	1.80 (m, 1H)	24.3
	1.62 (m, 1H)	
12	1.96 (m, 1H)	34.3
	2.02 (m, 1H)	
13		55.5
14		208.4
15		87.6
16		171.6
17		196.2
18	1.35 (s, 3H)	20.5
19	1.57 (s, 3H)	19.0
20		125.2
21	7.40 (br s, 1H)	143.1
22	8.04 (br s, 1H)	146.4
23	6.77 (br s, 1H)	110.6
28	1.59 (s, 3H)	25.6
29	1.51 (s, 3H)	28.8
30	1.40 (d, J = 8.0 Hz, 3H)	22.0
16-OMe	3.74 (s, 3H)	53.4

Table 3.6 Crystal data and structure refinement for compound **4**

Formula	$C_{27}H_{32}O_{10}$
Molecular weight	516.53
Crystal size (mm)	0.45 × 0.24 × 0.12
Crystal system	prism
Space group	$P2_12_12_1$
***a* (Å)**	8.3954(9)
***b* (Å)**	10.9498(11)
***c* (Å)**	14.3710(12)
***V* (Å^3)**	1266.8(2)
Z	2
D_{calc} **(g/cm^{-3})**	1.354
μ (mm^{-1})	0.103
***F*(000)**	548
Independent reflections/ Observed reflections [I > 4σ(I)], R_{int}	4185/3768, 0.0194
R_1	0.0319
wR_2 **[I > 2σ(I)]**	0.0793

3.2.5 Structure elucidation of compound 5

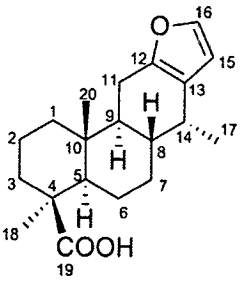


Figure 3.13 Compound **5**

Molecular formula	$C_{20}H_{28}O_3$
Appearance	pale yellow solid
Melting point	227-230 °C
UV (MeOH) λ_{max} (logε)	203 (3.68), 250 (3.27) and 355 nm (2.63)
IR (KBr)	3433, 2926, 2862, 1690, 1455, 1442, 1385, 1275 and 1013 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.7

Compound **5** was obtained as a pale yellow solid and established the molecular formula $C_{20}H_{28}O_3$ from NMR data analysis, suggesting seven degrees of unsaturation. Broad IR absorption at ν_{max} 3428 cm^{-1}, combined with absorption band at 1694 cm^{-1}, indicated the existence of a carboxylic acid group in the molecule. The ^1H and ^{13}C NMR spectra showed signals for a pair of aromatic protons (δ_H 6.18 and 7.22) and four carbon-carbon double bonds (δ_C 109.5, 122.5, 140.4 and 149.4), indicating the presence of a 2,3-disubstituted furan ring. Thus three remaining DBEs indicated that compound **5** had a tricarbocyclic skeleton. These features suggested that **5** was a cassane furanoditerpenoid. Doublet protons of a secondary methyl (δ_H 0.98, δ_C 17.6), showing HMBC correlations to C-8, C-13 and C-14, were assigned to Me-17. Singlet protons of a tertiary methyl (δ_H 1.24, δ_C 16.8), exhibiting HMBC correlations to C-3, C-4 and C-5, were identified as Me-18, while those of another tertiary methyl (δ_H 0.94, δ_C 14.6), showing HMBC correlations to C-1, C-5,

C-9 and C-10, were assigned to Me-20. The location of the carboxylic acid at C-19 was deduced from HMBC cross peak from Me-18 to a carboxyl carbon at δ_C 184.8. Based on the above data and 2D information (^{1}H-^{1}H COSY, HSQC and HMBC) studies could be concluded that compound **5** was (+)-vouacapenic acid. Comparison of its NMR data with those previously reported was also presented in Table 3.7, [17]. This is the first report for the isolation of (+)-vouacapenic acid from *Harrisonia* plant.

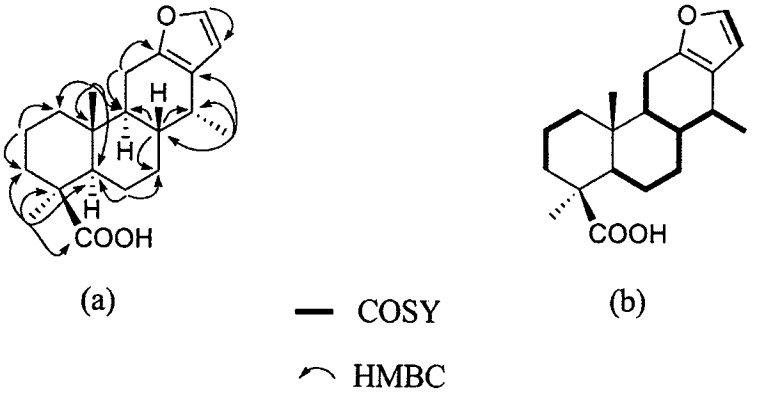


Figure 3.14 HMBC (a) and COSY (b) correlations of compound **5**

Table 3.7 The NMR data of compound **5** and (+)-vouacapenic acid

Position	(+)-vouacapenic acid[a]	Compound **5**[b]	
	δ_C	δ_H (mult, J in Hz)	δ_C
1	40.2	1.13 (m, 1H)	38.6
		1.74 (m, 1H)	
2	19.8	1.61 (m, 2H)	17.8
3	38.1	1.66 (m, 1H)	36.9
		1.78 (m, 1H)	
4	44.4		47.2
5	56.8	1.80 (m, 1H)	49.3
6	23.5	1.34 (m, 1H)	24.1
		1.51 (m, 1H)	
7	32.3	1.48 (m, 1H)	30.8
		1.66 (m, 1H)	
8	36.2	1.78 (m, 1H)	35.7
9	45.5	1.58 (m, 1H)	45.8
10	38.5		36.8
11	22.9	2.38 (m, 1H)	22.0
		2.57 (m, 1H)	
12	150.1		149.4
13	122.7		122.5
14	32.0	2.60 (m, 1H)	31.5
15	110.0	6.18 (d, J = 4.0 Hz, 1H)	109.5
16	140.8	7.22 (br s, 1H)	140.4
17	18.0	0.98 (d, J = 7.2 Hz, 3H)	17.6
18	29.6	1.24 (s, 3H)	16.8
19	185.0		184.8
20	13.9	0.94 (s, 3H)	14.6

[a] Spectra were recorded in $CDCl_3$, 500 MHz, [b] 400 MHz

3.2.6 Structure elucidation of compound 6

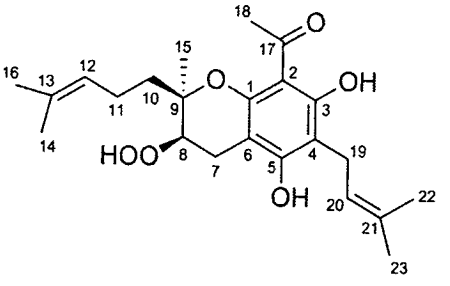


Figure 3.15 Compound **6**

Molecular formula	$C_{23}H_{32}O_6$
Appearance	yellow amorphous solid
Melting point	199-200 °C
UV (MeOH) λ_{max} (logε)	203 (3.95), 296 (3.72) and 334 nm (3.12)
IR (KBr)	3429, 2969, 2926, 1622, 1435, 1375, 1318, 1240 and 1077 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.8

Compound **6** was isolated as yellow amorphous solid, and its molecular formula was determined as $C_{23}H_{32}O_6$ (eight units of unsaturation) on the basis of NMR data analysis. UV absorption bands at 203, 296 and 334 nm and IR absorption bands at 1629 and 3429 cm^{-1} presented the presence of carbonyl and hydroxyl groups. The ^1H NMR data (Table 3.8) showed signals attributable to two olefinic protons at δ_H 5.12 and 5.24, and six tertiary methyl groups displaying as singlet at δ_H 1.28, 1.62, 1.69, 1.76, 1.81 and 2.63 (the last signal being due to methyl carbonyl group). The ^{13}C NMR spectrum displayed signals of a quaternary carbon bearing oxygen (δ_C 90.5), a chelating ketone carbonyl (δ_C 203.3), four unsaturated carbons (δ_C 121.3, 124.0, 132.3 and 135.5) and six aromatic carbons (Table 3.8). Thus the two remaining DBEs indicated the molecule of **6** was bicyclic. Studies on ^1H-^1H COSY correlations resulted in the construction of three subfragments, including –C(7)H_2–C(8)H–, –C(20)H=C(21)–, and –C(10)H_2–C(11)H_2–C(12)H= as shown in

Figure 3.16. Two tertiary methyls (δ_H 1.62 and 1.69) were assigned to Me-14 and Me-16 by the HMBC correlations of H_2-12/C-13, H_2-12/C-16 and Me-16/C-14, while the other two tertiary methyls (δ_H 1.77 and 1.82) were identified as Me-22 and Me-23 by those of Me-22/C-21 and Me-23/C-21. Singlet protons of a remaining tertiary methyl (δ_H 1:28), showing HMBC correlations to C-8, C-9 and C-10, were assigned to Me-15. A methyl carbonyl was located at C-2 of the aromatic ring due to its HMBC correlation to C-2. These data showed that compound **6** was a polyketide (Figure 3.16). Finally, Comparison of its NMR data with those in the literature (Table 3.8) helped to confirm that **6** was harrisonol A, which has been isolated from *H. perforata* in 2009 [18].

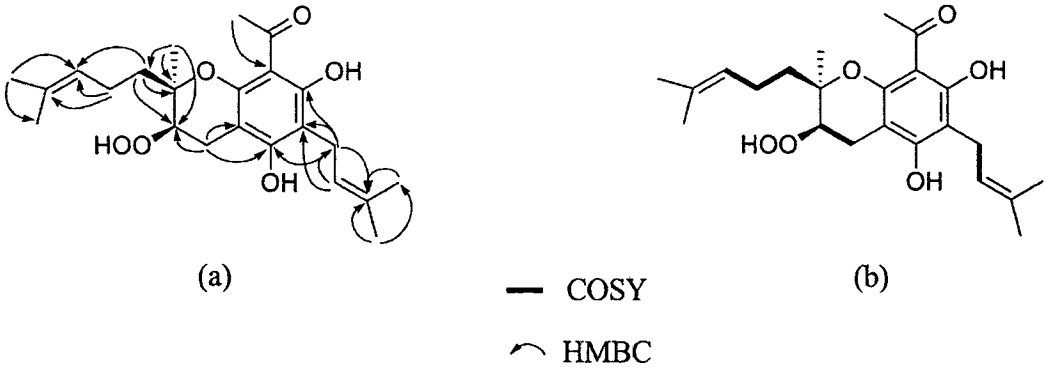


Figure 3.16 HMBC (a) and COSY (b) correlations of compound **6**

Table 3.8 The NMR data of compound **6** and harrisonol A ($CDCl_3$, 400 MHz)

Position	Harrisonol A		Compound **6**	
	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1		157.2		157.0
2		105.7		105.7
3		161.2		161.0
4		101.2		101.0
5		164.2		164.2
6		104.0		104.0
7	3.06 (dd, J = 8.3, 2.8 Hz , 2H)	26.9	3.06 (d, J = 8.0 Hz, 2H)	26.8
8	4.73 (t, J = 9.8 Hz, 1H)	90.5	4.73 (t, J = 8.0 Hz, 1H)	90.5
9		73.8		73.7
10	1.56 (m, 2H)	37.1	1.54 (m, 2H)	37.0
11	2.12 (m, 2H)	22.0	2.08 (m, 2H)	22.0
12	5.12 (br t, J = 5.6 Hz, 1H)	124.0	5.11 (br t, J = 8.0 Hz, 1H)	124.0
13		132.3		132.2
14	1.69 (s, 3H)	25.7	1.69 (s, 3H)	25.7
15	1.28 (s, 3H)	22.6	1.28 (s, 3H)	22.6
16	1.62 (s, 3H)	17.7	1.62 (s, 3H)	17.7
17		203.3		203.3
18	2.63 (s, 3H)	32.8	2.63 (s, 3H)	32.8
19	3.27 (d, J = 7.3 Hz, 2H)	22.4	3.27 (d, J = 8.0 Hz, 2H)	22.4
20	5.24 (br t, J = 6.0 Hz, 1H)	121.3	5.24 (br t, J = 8.0 Hz, 1H)	121.3
21		135.5		136.0
22	1.76 (s, 3H)	25.8	1.77 (s, 3H)	25.8
23	1.81 (s, 3H)	17.9	1.82 (s, 3H)	17.9

3.2.7 Structure elucidation of compound 7

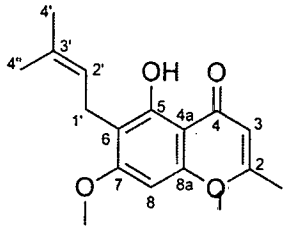


Figure 3.17 Compound 7

Molecular formula	$C_{16}H_{18}O_4$
Appearance	pale yellow needles
Melting point	105-106 °C
UV (MeOH) λ_{max} (logε)	235 (3.93), 259 (4.13), 298 (3.46) and 329 nm (3.40)
IR (KBr)	3436, 2993, 2912, 1665, 1615, 1591, 1417, 1378, 1328, 1275, 1212, 1080 and 861 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.9

Compound 7 was obtained as pale yellow needles, with a molecular formula $C_{16}H_{18}O_4$ (eight degrees of unsaturation). The UV absorption at 204, 230, 259 and 329 nm and the IR spectrum observed at ν_{max} 3436 and 1665 cm^{-1} suggested the presence of hydroxyl, carbonyl and aromatic groups. In addition, these data indicated that compound 7 was a chromone derivative. The ^1H NMR spectrum exhivited typical signals of phenolic proton chelated to carbonyl carbon (δ_H 12.77), three olefinic protons (δ_H 5.14, 5.99 and 6.35), three tertiary methyls bonded to double bonds (δ_H 1.67, 1.79 and 2.35) and one methoxy (δ_H 3.67). The ^{13}C NMR spectrum showed 10 unsaturated carbons (six for an aromatic) and an aryl ketone (δ_C 182.3). This compound was isolated as a major product from *H. perforata* roots, and its structure was confirmed by comparing its 1D NMR data to those previously

reported as shown in Table 3.9 [19]. Therefore compound **9** was identified as peucenin-7-methyl ether.

Table 3.9 The NMR data of compound **7** and peucenin-7-methyl ether ($CDCl_3$, 400 MHz)

Position	peucenin-7-methyl ether	Compound **7**	
	δ_C	δ_H (mult, J in Hz)	δ_C
2	105.3		104.6
3	112.7	5.99 (s, 1H)	107.6
4	182.3		182.9
4a	156.4		154.6
5	166.2		166.7
6	108.7		108.2
7	162.7		162.6
8	89.3	6.35 (s, 1H)	94.9
8a	158.3		160.4
1′	25.7	3.37 (d, J = 4.0 Hz, 2H)	25.7
2′	122.0	5.14 (t, J = 8.0 Hz, 1H)	122.0
3′	131.6		131.5
4′	20.2	1.79 (s, 3H)	21.5
4″	17.6	1.67 (s, 3H)	17.7
2-Me	21.4	2.35 (s, 3H)	20.5
7-OMe	55.7	3.67 (s, 3H)	56.0
5-OH		12.77 (s, 1H)	

3.2.8 Structure elucidation of compound 8

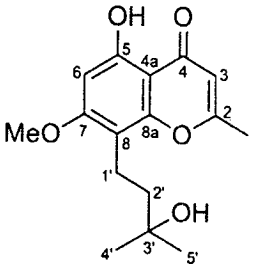


Figure 3.18 Compound **8**

Molecular formula	$C_{16}H_{20}O_5$
Appearance	light yellow solid
Melting point	108-110 °C
UV (MeOH) λ_{max} (logε)	232 (3.52), 258 (3.69), 292 (3.18) and 326 nm (3.04)
IR (KBr)	3449, 2969, 2930, 1654, 1619, 1591, 1424 1385, 1204, 1151 and 833 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.10

Compound **8** was obtained as a light yellow solid and the molecular formula $C_{16}H_{20}O_5$ was determined by HR-ESI-MS (*m/z* 293.1383 $[M + H]^+$, Calcd 293.1316). The UV absorption maxima showed at 232, 258, 292 and 326 nm and the IR spectrum observed at ν_{max} 3449, 1654, 1619 and 1591 cm^{-1} suggested the presence of hydroxyl, carbonyl and aromatic groups. The ^1H NMR spectrum displayed characteristic signals for three tertiary methyls (δ_H 1.30 (Me× 2), 2.36), one methoxy (δ_H 3.88), two olefinic protons (δ_H 6.02, 6.37), and one phenolic proton bonded to a carbonyl group (δ_H 12.75). Analysis of ^{13}C NMR and HSQC data further revealed the presence of three tertiary methyls, two methylenes, two olefinic methines, six olefinic quaternary carbons (three oxygenated), one oxygenated methine, and one ketone. These data indicated that **8** should be a chromone derivative. In addition, its 2D NMR spectra revealed the existence of a 3-hydroxy-4-methylbutyl

moiety due to the ^{1}H-^{1}H COSY correlation of $-CH_2(1')-CH_2(2')-$, combined with the HMBC correlations of Me-4'/C-2', Me-4'/C-3', Me-5'/C-2', and Me-5'/C-3' (Figure. 3.19). The attachment of this unit at C-8 of the chromone nucleus was confirmed by HMBC correlations from H_2-1' to C-7, C-8 and C-8a. The NMR data of **8** were similar to those of perforamone A [6], except for the presence of an additional methylene instead of an oxygenated methine. The position of the methoxy at C-7 and another tertiary methyl at C-2 was supported by HMBC correlations between methoxy methyl at δ_H 3.88 and C-7, and between the olefinic methyl at δ_H 2.36 and C-2, respectively. Thus, compound **8** was determined to be new and was named as harperamone.

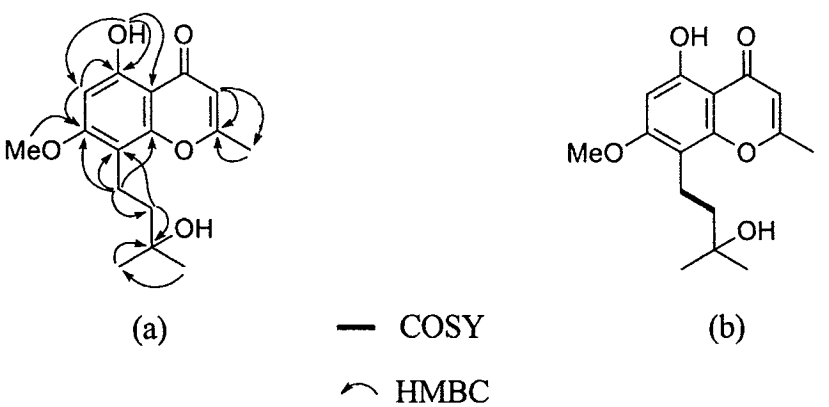


Figure 3.19 HMBC (a) and COSY (b) correlations of compound **8**

Table 3.10 The NMR data of compound **8** ($CDCl_3$, 400 MHz)

postition	δ_H (mult, J in HZ)	δ_C
2		166.7
3	6.02 s	108.3
4		183.0
4a		104.7
5		160.5
6	6.37 s	95.0
7		164.7
8		108.3
8a		154.7
1'	2.77 m	17.4
2'	1.64 m	42.9
3'		71.1
4'	1.30 s	29.0
5'	1.30 s	29.0
2-Me	2.36 s	20.5
7-OMe	3.88 s	56.0
5-OH	12.75 s	

3.2.9 Structure elucidation of compound 9

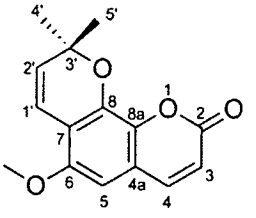


Figure 3.20 Compound **9**

Molecular formula	$C_{15}H_{14}O_4$
Appearance	light amorphous solid
Melting point	149-150 °C
UV (MeOH) λ_{max} (logε)	203 (3.38), 227 (3.62), 258 (3.23) and 349 nm (3.18)
IR (KBr)	2912, 1729, 1630, 1559 1467, 1297, and 1127 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.11

Compound **9** was isolated as a light amorphous solid and had the molecular formula as $C_{15}H_{14}O_4$ determined by HR-ESI-MS (*m/z* 259.0889 $[M + H]^+$, Calcd 259.0965). The IR and UV spectra showed absorptions for carbonyl at ν_{max} 1729 cm^{-1} and maxima absorptions at 203, 227, 258 and 349 nm, which was a characteristic of a coumarin. The ^1H and ^{13}C NMR spectra suggested the presence of one methoxy group (δ_H 3.88, δ_C 56.6) and a cyclic isopentenyl moiety. The attachment of the methoxy at C-6 was assigned by its HMBC correlation to C-6. An α,β-unsaturated lactone ring was corroborated by ^1H-^1H COSY correlation of -C(3)H=C(4)H- and their HMBC correlations of H-3/C-2, H-3/C-4a, H-4/C-2 and H-4/C-8a. The absence of any oxygenation at C-5 was confirmed by HMBC correlations of H-4 to its carbon (δ_C 108.8). Based on the above data, compound **9** was determined to be braylin I, and it was further confirmed by comparison of its NMR data with those in the literature as presented in Table 3.11. In addition, it was found that this is the first report of braylin I from *H. perforata.*

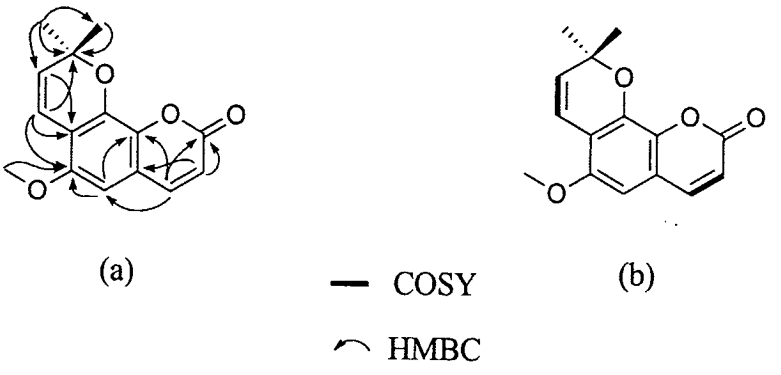


Figure 3.21 HMBC (a) and COSY (b) correlations of compound **9**

Table 3.11 The NMR data of compound **9** and braylin I ($CDCl_3$, 400 MHz)

position	δ_H (mult, J in HZ)	δ_C
2		161.2
3	6.25 (d, J = 8 Hz,1H)	113.2
4	7.57 (d, J = 8 Hz,1H)	143.7
4a		111.4
5	6.76 (s, 1H)	108.8
6		146.0
7		110.3
8		145.7
8a		145.0
1'	6.87 (d, J = 8 Hz, 1H)	115.2
2'	5.74 (d, J = 8 Hz, 1H)	130.8
3'		78.0
4'	1.52 (s, 3H)	28.0
5'	1.52 (s, 3H)	28.0
6-OMe	3.88 (s, 3H)	56.6

3.3 Anti-inflammatory activity of isolated compounds

Nitric oxide (NO) is one of the most important mediators in inflammatory processes. Upon inflammatory stimulation, macrophages are activated and produce nitric oxide and pro-inflammatory cytokines such as tumor necrosis factor (TNF)-alpha and interleukin (IL)-6. Overproduction of these mediators in macrophages causes many inflammatory diseases, including rheumatoid arthritis, atherosclerosis, and hepatitis [21-23]. Additionally, NO is mainly produced by inducible nitric oxide synthase (iNOS) [24], the inhibition of NO production by suppressing iNOS expression is thus an important target in the treatment of inflammatory diseases.

H. perforata root is considered to have antipyretic and anti-inflammatory effects, and it is utilized as a remedy for treatment of wound healing and diarrhea [3]. In the present study, the anti-inflammatory activity of isolated compounds from *H. perforata* fruits and roots was thus evaluated by monitoring the inhibition of nitric oxide (NO) production in LPS-activated murine macrophage J774.A1 cells, and the results expressed as $IC_{50} \pm SD$ are shown in Table 3.12. Among the tested compounds, the most potent activity was demonstrated by harperfolide (**2**), a new rearranged limonoid, with IC_{50} value of 6.51 ± 2.10 μM. Its activity was 20-fold greater than its analog harrisonin **3** ($IC_{50} = 134.54 \pm 5.66$ μM), indicating that the presence of a γ-hydroxybutenolide group may significantly enhance the NO production inhibitory activity of this type limonoid. Acute toxicity of compound **2** on unstimulated cell lines was further investigated. Cell viability, as measured by the MTT colorimetric method, displayed harperfolide (**2**) did not significant toxicity on macrophage J774.A1 cells at tested concentrations as shown in Figure 3.22. This result implied that harperfolide inhibited nitrite release without causing cell death.

Table 3.12 Inhibitory effect of isolated compounds on NO production in LPS-induced macrophages

Compound	IC_{50} (μM)
Harrisonin (**1**)	134.54 ± 12.66
Harperfolide (**2**)	6.51 ± 2.10
Obacunone (**3**)	83.61 ± 3.52
(+)-Vouacapenic acid (**5**)	131.81 ± 2.47
Harrisolanol A (**6**)	31.04 ± 0.72
Peucenin-7-methyl ether (**7**)	56.36 ± 1.45
Harperamone (**8**)	49.59 ± 2.58
Indomethacin	28.42 ± 3.51

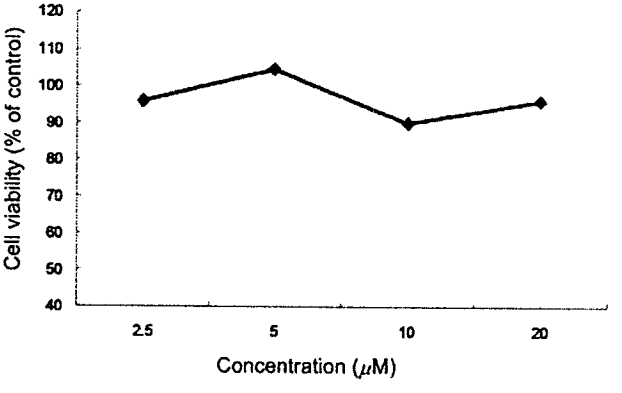


Figure 3.22 Effect of harperfolide (**2**) on cell viability

To investigate whether the inhibitory effect of harperfolide (**2**) on NO production was via the inhibition of the corresponding gene expression, the protein iNOS was evaluated by Western blot analysis. In unstimulated J774.A1 cells, the iNOS protein expression level was almost undetectable, while, after treatment with LPS, the protein expression level of iNOS was augmented markedly. Pretreatment of the cells with various concentrations of **2** attenuated LPS-induced iNOS protein expression in a concentration-dependent manner as shown in Figure 3.23. These data suggested harperfolide (**2**) can down regulate LPS-induced iNOS expression at the transcriptional level.

(a)

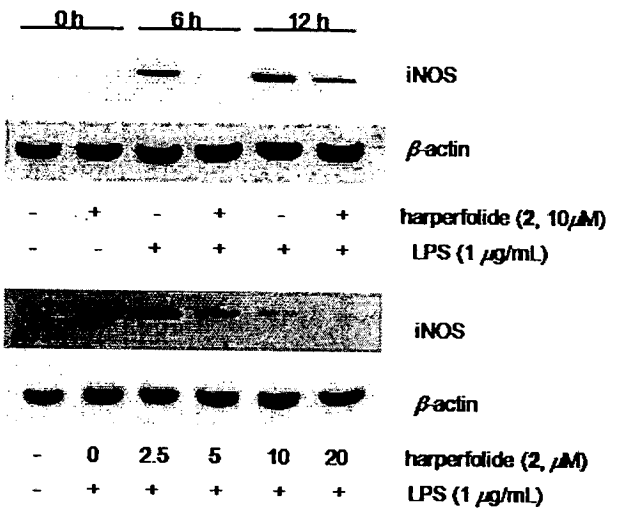


Figure 3.23 Effect of harperfolide (**2**) on the expression of iNOS protein in LPS-induced macrophages (a) at the indicated time (b) at various doses