CHAPTER 2

EXPERIMENTAL

General Experimental Procedures

Melting points were measured using an Electrothermal melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP 1020 polarimeter. The IR spectra were obtained on a Perkin-Elmer 1760x FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AVANCE 400 MHz spectrometer. Chemical shifts are referenced to the residual solvent signals ($CDCl_3$: δ_H 7.24 and δ_C 77.0 ppm, pyridine-d_5: δ_H 7.19, 7.53, 8.71 and δ_C 12.5, 135.5, 149.9). HRESIMS was recorded on a Bruker Daltonics microTOF mass spectrometer. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use. Pre-coated TLC aluminium sheets of silica gel $60F_{254}$ (E. Merck) and pre-coated TLC aluminium sheets of DC-Alufolien RP-18 F_{254} (E. Merck) were used for analytical proposes and compounds were visualized under ultraviolet light (either at 254 or 365 nm) and by staining with anisaldehyde-H_2SO_4 solution and immidiate heat-up at 80-100 °C using hot plate to reveal spots. Column chromatography was performed by using silica gel 60 (70-230 mesh ASTM, or less than 230 mesh ASTM, E. Merck), Lichrolut RP-18 silica gel (40-63 μm,

E. Merck) and Sephadex LH-20 were applied. Analytical HPLC was performed on a TSP HPLC system equipped with a Spectra System UV2000 detector and Spectra System P2000 pump using LiChospher 100 RP-18 (5 μm, 250 x 4 mm, E, Merck) or RP-8 (5 μm, 250 x 4 mm, E, Merck) columns.

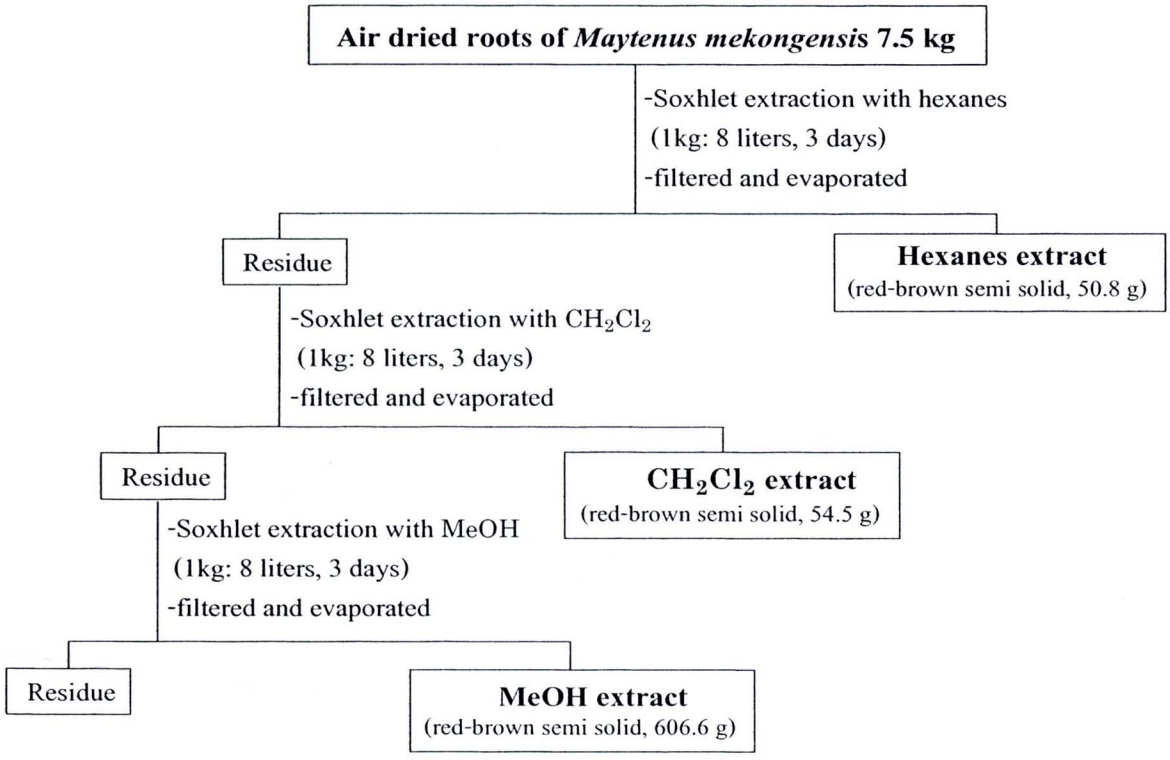
Source of Plant Material

The roots of *Maytenus mekongensis*, known in Thailand as "Naam Kaan Chaang", were collected from Don Muu, Kampeae subdistrict, Trakarnpoepon District, Ubonratchatani Province, Thailand, in June, 2004. The plant was identified by Assoc. Prof. Dr. Wongsatit Chuakul of the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. A voucher specimen (SSMMe/2004) is maintained at the Chemistry Department, Ramkhamhaeng University.

Extraction, Isolation and Characterization

Extraction

The air dried roots (7.5 kg) of *Maytenus mekongensis* were ground to small pieces and extracted successively using Soxhlet apparatus with hexanes, CH_2Cl_2 and CH_3OH, respectively. Removal of the solvent under reduced pressure on a rotary evaporator gave hexanes (50.8 g), dichloromethane (54.5 g) and methanol (606.6 g) extracts, respectively. The extraction sequence was as indicated in Scheme 1.



Scheme 1 Extraction of the air dried roots of *Maytenus mekongensis*

Isolation of Crude CH_2Cl_2 Extract of the Roots of *M. mekongensis*

The crude CH_2Cl_2 extract (54.5g) was fractionated by column chromatography on silica gel (300 g) by using gradient of hexanes-CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2-MeOH. Further repeated column chromatography of combined fractions using appropriate solvent systems yielded pure compounds as indicated in Schemes 2-4.

Isolation of Compound **I**

Compound **I** was obtained from fraction 11/4 (7.44 g) of the first column chromatography of the CH_2Cl_2 extract. Repeated silica gel column chromatography (hexanes-CH_2Cl_2 5:95 to CH_2Cl_2-MeOH 50:50) afforded

fraction 4-5/7 which after concentration and recrystallization, compound **I** (79.1 mg) was obtained as colorless needles (Scheme 2)

Abruslactone A (**I**): colorless needles m.p. 318-319 °C; $[\alpha]_D^{29}$ +67.41 (*c*0.64, $CHCl_3$); FT-IR (KBr) ν_{max} 3488, 2927, 1748, 1453, 1362, 1170, 1137, 1096, 783, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 1); HRESIMS *m/z* 455.3531 $[M + H]^+$ (calcd for $C_{30}H_{47}O_3$, 455.3525)

Isolation of Compound **II**

Compound **II** was obtained from fraction 11/4 (7.44 g) of the first column chromatography of the CH_2Cl_2 extract. Repeated silica gel column chromatography afforded fraction 6/7 (1.0062 g). Further purification by reversed phase column chromatography (MeOH-H_2O 50:50 to 100:0) gave fration 4/10, then recrystallized with CH_2Cl_2-MeOH gave fraction 3/21 which after washing with MeOH compound **II** (2.0 mg) was obtained as a colorless amorphous solid (Scheme 2).

3β,29-Dihydroxyolean-12-en (**II**): colorless amorphous solid; $[\alpha]_D^{31}$ +108.20 (*c* 0.10, $CHCl_3$); FT-IR (KBr) ν_{max} 3272, 2923, 2855, 1463, 1438, 1384, 1376, 1289, 1070, 1038, 995, 810 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$-MeOH-d_4 22:1 v/v) and ^{13}C NMR (100 MHz, $CDCl_3$-MeOH-d_4 22:1 v/v) and HMBC correlations (see Table 2); HRESIMS *m/z* 443.3881 $[M + H]^+$ (calcd for $C_{30}H_{51}O_2$, 443.3889).

Isolation of Compound **III**

Compound **III** was isolated using the same route as that of compound **II**. Combined fraction 7-8/7 purified by column chromatography (silica gel, CH_2Cl_2-MeOH 95:5 to 60:40) gave fraction 6-7/24 (1.9515 g). Further purification by silica gel column chromatography with hexanes-EtOAc 75:25 to 20:60 (Scheme 2) gave fraction 4/41. Fraction 4/41 solid was obtained after further purified by size exclusion column chromatography (Sephadex LH 20, CH_2Cl_2-MeOH 50:50) to give compound **III** (6.8 mg) as a colorless amorphous solid.

3β-Hydroxyolean-12-en-29-oic acid (**III**): colorless amorphous solid $[\alpha]_D^{31}$ +45.27 (*c* 0.20, $CHCl_3$); FT-IR (KBr) ν_{max} 3368, 2920, 2852, 1699, 1459, 1446, 1378, 1263, 1226, 1024, 993 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$-MeOH-d_4 32:1 v/v) and ^{13}C NMR (100 MHz, $CDCl_3$-MeOH-d_4 32:1 v/v) and HMBC correlations (see Table 3); HRESIMS *m/z* 457.3676 $[M + H]^+$ (calcd for $C_{30}H_{49}O_3$, 457.3682)

Isolation of Compound **IV**

Compound **IV** was obtained from fraction 11/4 (7.44 g) of the first column chromatography of the CH_2Cl_2 extract. Repeated silica gel column chromatography gave fraction 6/7 (1.0062 g). Further reversed phase column chromatography, eluting with MeOH-H_2O 50:50 to 100:0 afforded (fraction 5/10) as solid mixture. Final purification by silica gel column chromatography (CH_2Cl_2-MeOH 98.8:0.2 to 95:5) gave compound **IV** as a colorless amorphous solid (12.9 mg) (Scheme 2).

3-Oxofriedelan-29-oicacid, polpunonic acid (**IV**): colorless amorphous solid; m.p. 318-319 °C; $[\alpha]_D^{31}$-57.68 (*c* 0.13, $CHCl_3$); FT-IR (KBr) ν_{max} 2936, 2857, 1721, 1694, 1451, 1388, 1200, 1138, 1082, 1007, 914, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 4) ; HRESIMS *m/z* 479.3502 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3501)

Isolation of Compound **V**

Compound **V** was obtained from fraction 13/4 (11.2 g) of the first column chromatography of the CH_2Cl_2 extract. Further purification by silica gel column chromatography with CH_2Cl_2-MeOH 100:0 to 80:20 gave fraction 7/126 (1.8477 g). Repeated silica gel column chromatography (CH_2Cl_2-MeOH 100:0 to 60:40) to afford fraction 5-6/128 which after concentration and washing with MeOH, compound **V** (14.0 mg) was obtained as a colorless amorphous solid (Scheme 2).

Salaspermic acid (**V**): colorless amorphous solid; decomposed at 332 °C; $[\alpha]_D^{32}$ +10.45 (*c* 0.16, $CHCl_3$); FT-IR (KBr) ν_{max} 3401, 3295, 2920, 2852, 1742, 1688, 1447, 1381, 1222, 1137, 1011, 880 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) and HMBC correlations in pyridine-d_5 (see Table 5); HRESIMS *m/z* 495.3445 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 493.3450)

Isolation of Compound **VI**

Compound **VI** was isolated using the same route as that of compound **IV**. (Scheme 2) Fraction 3/10 was recrystallized from CH_2Cl_2-MeOH to give compound **VI** (8.6 mg) as colorless needles (Scheme 2).

Cangoronine (**VI**): colorless needles; m.p. 310-311 °C (decomposed at 270 °C); $[\alpha]_D^{32}$ +10.45 (*c* 0.16, $CHCl_3$); FT-IR (KBr) ν_{max} 2924, 1705, 1667, 1455, 1378, 1185, 1133, 1076, 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$-MeOH-d_4 15:1 v/v) and ^{13}C NMR (100 MHz, $CDCl_3$-MeOH-d_4 15:1 v/v) and HMBC correlations (see Table 6); HRESIMS *m/z* 519.2894 $[M + Cl]^+$ (calcd for $C_{30}H_{44}ClO_3$, 519.2883).

Isolation of Compound **VII**

Compound **VII** was isolated using the same route as that of compound **II**. Fraction 6-7/21 was further purified using Sephadex LH 20 column chromatography (CH_2Cl_2-MeOH 50:50) to give fraction 2/117. Compound **VII** (7.3 mg) was obtained as colorless needles after recrystallization of solid obtained from fraction 2/117 using CH_2Cl_2-MeOH (Scheme 2).

3-Hydroxy-2-oxofriedelan-3-ene-20α-carboxylic acid (**VII**): colorless needles; m.p. 330-332 °C; $[\alpha]_D^{32}$ +2.65 (*c* 0.17, $CHCl_3$); FT-IR (KBr) ν_{max} 3350, 2933, 2857, 1711, 1652, 1626, 1467, 1382, 1293, 1232, 1167, 1134, 1099, 1076, 1018, , 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$-MeOH-d_4 7:1 v/v) and ^{13}C NMR (100 MHz, $CDCl_3$-MeOH-d_4 7:1 v/v) and HMBC correlations in (see Table 7) HRESIMS *m/z* 493.3300 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3282).

Isolation of Compound **VIII**

Compound **VIII** was isolated using the same route as that of compound **IV**. Combined fractions 2/10 and 3/10 (filtrate) were fractionated by column chromatography (2×), silica gel, CH_2Cl_2-MeOH 95.5:0.5 to 60:40, then hexanes-EtOAc 90:10 to CH_2Cl_2-MeOH 50:50 gave fraction 4/16. Fraction 4/16 (29.3 mg) was purified by reversed column chromatography, eluting with MeOH-H_2O 70:30 to 100:0 to yield reddish brown amorphous solid (5.1 mg) of compound **VIII** (Scheme 2).

Tingenone (**VIII**): reddish brown amorphous solid; FT-IR (KBr) ν_{max} 3368, 2921, 2851, 1707, 1595, 1439, 1378, 1286, 1215, 1185, 1017, 868 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 8); HRESIMS *m/z* 421.2753 $[M + H]^+$ (calcd for $C_{28}H_{37}O_3$, 421.2743).

Isolation of Compound **IX**

Compound **IX** was obtained from fraction 11/4 (7.44 g) of the first column chromatography of the CH_2Cl_2 extract. Repeated silica gel column chromatography afforded fraction 9/7 (683 mg). Sephadex LH 20 column chromatography (CH_2Cl_2-MeOH 50:50) of fraction 9/7 gave fraction 2/68, further purification by reversed column chromatography using gradient of MeOH-H_2O 70:30 to 100:0 gave colorless amorphous solid (18.0 mg) of compound **IX** (Scheme 3).

Fraction 6-7/24 (1.9515 g) was purified by silica gel column chromatography with hexanes-EtOAc 75:25 to 20:60 and to give fraction 13/41

(Scheme 4). Purification of fraction 13/41 by Sephadex LH 20 and then reversed phase column chromatography gave compound **IX** (25.5 mg).

Euonymine (**IX**): colorless amorphous solid; $[\alpha]_D^{32}$ -17.67 (*c* 0.24, $CHCl_3$); FT-IR (KBr) ν_{max} 3500, 2977, 2942, 1747, 1566, 1371, 1254, 1232, 1118, 1046, 1012, 785cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 9); HRESIMS *m/z* 828.2690 $[M + Na]^+$ (calcd for $C_{38}H_{47}NO_{18}Na$, 828.2691).

Isolation of Compound **X**

Compound **X** was obtained from fraction 6-7/24 (1.9515 g) after further purification using silica gel column chromatography with hexanes-EtOAc (75:25 to 20:60) to give fraction 11/41 (Scheme 4). Further purification of fraction 11/41 by reversed phase column chromatography using C_{18} silica gel and appropriate solvent systems as eluents as indicated in Scheme 4, gave compound **X** as a colorless amorphous solid (5.3 mg).

Purification of fraction 12/41 (112.3 mg) using reversed-phase column chromatography gave compound **X** (4.4 mg).

7-*Epi*-euonymine (**X**): colorless, amorphous solid; $[\alpha]_D^{32}$ -18.57 (*c* 0.27, $CHCl_3$); FT-IR (KBr) ν_{max} 3487, 2930, 1755, 1566, 1370, 1316, 1251, 1228, 1119, 1092. 1060, 1039, 784 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 10); HRESIMS *m/z* 828.2685 $[M + Na]^+$ (calcd for $C_{38}H_{47}NO_{18}Na$, 828.2691).

Isolation of Compound **XI**

Compound **XI** was obtained from fraction 5/24 (685.0 mg) of the second column chromatography of the CH_2Cl_2 extract (Scheme 2). Further purification by Sephadex LH 20 column chromatography using appropriate solvent systems as eluents as indicated in Scheme 3 gave fraction 3/31. Fraction 3/31 was purified using reversed-phase column chromatography, eluting with MeOH-H_2O (70:30 to 100:0 and then 80:20 to 100:0) to afford colorless amorphous solid (33.3 mg) of compound **XI**.

Fraction 13/41 (516.9 mg) was further purified using Sephadex LH 20 column chromatography to eluting with MeOH to give fraction 2/44. Fraction 2/44 was column chromatographed, using C_{18} silica gel as adsorbents, and appropriate solvent systems as eluents as indicated in Scheme 4, gave compound **XI** (188.0 mg).

Mayteine (**XI**): colorless, amorphous solid; $[\alpha]_D^{32}$ -12.91 (*c* 0.22, $CHCl_3$); FT-IR (KBr) ν_{max} 3501, 2989, 2943, 1747, 1732, 1584, 1566, 1452, 1433, 1371, 1271, 1226, 1116, 1096, 1058, 713 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 11); HRESIMS *m/z* 868.3027 $[M + H]^+$ (calcd for $C_{43}H_{50}NO_{18}$, 868.3028).

Isolation of Compound **XII**

Compound **XII** was obtained from fraction 11/4 (7.44 g) of the first column chromatography of the CH_2Cl_2 extract (Scheme 2). Repeated silica gel column chromatography gave fraction 3/24 (475.2 mg). Further purification by size exclusion column chromatography, using Sephadex LH 20, eluting with

CH_2Cl_2-MeOH 50:50, gave fraction 2/27 (193.4 mg). Purification of fraction 2/27 by silica gel column chromatography with hexanes-EtOAc (75:25 to 50:50) gave fraction 7/30 which was finally purified by reversed phase column chromatography (C_{18}, MeOH-H_2O 70:30 to 100:0) to give a colorless amorphous solid of compound **XII** (8.2 mg) (Scheme 3).

Fraction 6-7/24 (1.9515 g) was further purified by silica gel column chromatography with hexanes-EtOAc (75:25 to 20:60) to give fraction 11/41 (Scheme 4). Reversed phase column chromatography of fraction 11/41 (C_{18}, MeOH-H_2O 80:20 to 100:0) gave fraction 2/56 and fraction 3/56. Fraction 2/56 was rechromatographed on C_{18} silica gel column using gradient of MeOH-H_2O (55:45 to 100:0) to give compound **XII**. Fraction 3/56 was purified by column chromatography (C_{18}, MeOH-H_2O 70:30 to 90:10) to give compound **XII** (20.2 mg).

7-Epi-Mayteine (**XII**): colorless amorphous solid; $[\alpha]_D^{31.0}$ -5.60 (*c* 0.25, $CHCl_3$); FT-IR (KBr) ν_{max} 3492, 2976, 1748, 1566, 1433, 1369, 1271, 1222, 1093, 1060, 1039, 712 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations in (see Table 12); HRESIMS *m/z* 890.2842 $[M + Na]^+$ (calcd for $C_{43}H_{49}NO_{18}Na$, 890.2847).

Isolation of Compound **XIII**

Compound **XIII** was obtained from fraction 11/4 (7.44 g) of the first column chromatography of the CH_2Cl_2 extract (Scheme 2). Repeated silica gel column chromatography gave fraction 2/24 (418.4 g). Purification of fraction 2/24 by Sephadex LH 20 column chromatography, eluting with CH_2Cl_2-MeOH

50:50, and subsequently on silica gel column using gradient of CH_2Cl_2-MeOH (100:0-20:80) afforded fraction 2/119. Final reversed phase column chromatography (C_{18}, MeOH-H_2O 70:30 to 100:0) gave compound **XIII** (4.0 mg) as a colorless amorphous solid (Scheme 3).

7-Epi-eujaponine A (**XIII**): colorless, amorphous solid; $[\alpha]_D^{32}$ +25.70 (*c* 0.20, $CHCl_3$); FT-IR (KBr) ν_{max} 3400, 2929, 1748, 1715, 1584, 1566, 1452, 1433, 1369, 1269, 1218, 1107, 1063, 712 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 13); HRESIMS *m/z* 848.2736 $[M + Na]^+$ (calcd for $C_{41}H_{47}NO_{17}Na$, 848.2742).

Isolation of Compound **XIV**

Compound **XIV** was isolated from fraction 11/4 (7.44 g) using the same route as that of compound **XIII** (Scheme 3) to afford compound **XIV** as a colorless amorphous solid (10.4 mg).

Compound **XIV** was also isolated from fraction 5/24 using the same route as that of compound **XI** (Scheme 3) to obtain a colorless amorphous solid (43.8 mg) after HPLC separation (LiChrospher RP-18, 250 × 4 mm, MeCN-H_2O 63:37, flow rate 1.0 mL/min at t_R = 16.14 min).

2-O-Benzoyl-2-deacetylmayteine (**XIV**): colorless amorphous solid; $[\alpha]_D^{28}$ +14.21 (*c* 0.52, $CHCl_3$); FT-IR (KBr) ν_{max} 3494, 2975, 1746, 1723, 1584, 1566, 1451, 1370, 1274, 1246, 1107, 1059, 1024, 883, 711, 603 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 14); HRESIMS *m/z* 930.3193 $[M + H]^+$ (calcd for $C_{48}H_{52}NO_{18}$, 930.3184).

Isolation of Compound **XV**

Compound **XV** was isolated from fractions 6-7/24 using the same route as that of compound **IX** in Scheme 4. Fraction 7/65 was purified by HPLC (LiChrospher RP-8, 250 × 4 mm, MeCN-H_2O 56:44, flow rate 1.0 mL/min) to give compound **XV** as a colorless amorphous solid (with t_R = 28.30 min, 3.9 mg).

7-epi-5-O-Benzoyl-5-deacetylperitassine A (**XV**): colorless amorphous solid; $[\alpha]_D^{32}$ -17.49 (*c* 0.20, $CHCl_3$); FT-IR (KBr) ν_{max} 3493, 2926, 2854, 1748, 1723, 1587, 1553, 1452, 1370, 1250, 1225, 1119, 1056, 715, 600 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 15); HRESIMS *m/z* 868.3049 $[M+H]^+$ (calcd for $C_{43}H_{50}NO_{18}$, 868.3028).

Isolation of Compound **XVI**

Compound **XVI** was isolated from fraction 11/4 (7.44 g) using the same route as that of compound **IX** (Scheme 3) to give fraction 6/68 (142.2 mg). Purification of fraction 6/68 by HPLC (LiChrospher RP-18, 250 × 4 mm, MeCN-H_2O 50:50, flow rate 1.0 mL/min) gave compound **XVI** as a colorless amorphous solid (t_R = 27.64 min, 44.6 mg) (Scheme 3).

Mekongensine (**XVI**): colorless, amorphous solid; $[\alpha]_D^{26}$ +11.80 (*c* 0.65, $CHCl_3$); FT-IR (KBr) ν_{max} 3542, 2945, 1748, 1585, 1568, 1451, 1434, 1372, 1254, 1237, 1133, 1098, 1050, 899, 763, 714cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 16); HRESIMS *m/z* 948.2902 $[M + Na]^+$ (calcd for $C_{45}H_{51}NO_{20}Na$, 948.2902).

Isolation of Compound **XVII**

Compound **XVII** was isolated from fractions 11/4 (7.44 g) using the same route as that of compound **XVI** (Scheme 3) as a colorless amorphous solid (t_R = 31.54 min, 21.3 mg) after HPLC separation (LiChrospher RP-18, 250 × 4 mm, MeCN-H_2O 50:50, flow rate 1.0 mL/min)

Compound **XVII** was also isolated from fractions 7/65 using the same route as that of compound **XV** (Scheme 4) as a colorless amorphous solid (6.8 mg) after HPLC separation (LiChrospher RP-8, 250 × 4 mm, MeCN-H_2O 56:44, flow rate 1.0 mL/min at t_R = 24.54 min).

7-Epi-mekongensine (**XVII**): colorless, amorphous solid; $[\alpha]_D^{26}$ +7.16 (*c* 0.49, $CHCl_3$); FT-IR (KBr) ν_{max} 3540, 2946, 1755, 1732, 1601, 1586, 1569, 1451, 1434, 1371, 1250, 1224, 1135, 1094, 1053, 905, 714 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 17); HRESIMS *m/z* 948.2885 $[M + Na]^+$ (calcd for $C_{45}H_{51}NO_{20}Na$, 948.2902).

Isolation of Compound **XVIII**

Compound **XVIII** was isolated from fractions 11/4 (7.44 g) using the same route as that of compound **XII** (Scheme 3). Fraction 5/30 was purified by HPLC separation (LiChrospher RP-18, 250 × 4 mm, MeCN-H_2O 63:37, flow rate 1.0 mL/min) to give a colorless amorphous solid (t_R = 15.64 min, 12.9 mg) of compound **XVIII**.

Purification of fraction 4/72 (Scheme 3) by HPLC (LiChrospher RP-8, 250 × 4 mm, MeCN-H_2O 56:44, flow rate 1.0 mL/min) gave compound **XVIII** (t_R = 31.04 min, 7.0 mg).

1-O-Benzoyl-1-deacetylmekongensine (**XVII**): colorless amorphous solid; $[\alpha]_D^{30}$ +22.33 (*c* 0.49, $CHCl_3$); FT-IR (KBr) ν_{max} 3543, 2926, 2854, 1747, 1732, 1602, 1585, 1451, 1372, 1247, 1107, 1057, 897, 712, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 17); HRESIMS *m/z* 1010.3015 $[M + Na]^+$ (calcd for $C_{50}H_{53}NO_{20}Na$, 1010.3059).

Isolation of Compound **XIX**

Compound **XIX** was isolated from fraction 11/4 (7.44 g) using the same route as that of compound **XVI** (Scheme 3) to obtain a colorless amorphous solid (21.3 mg) after HPLC separation (LiChrospher RP-18, 250 × 4 mm, MeCN-H_2O 50:50, flow rate 1.0 mL/min at t_R = 24.03 min).

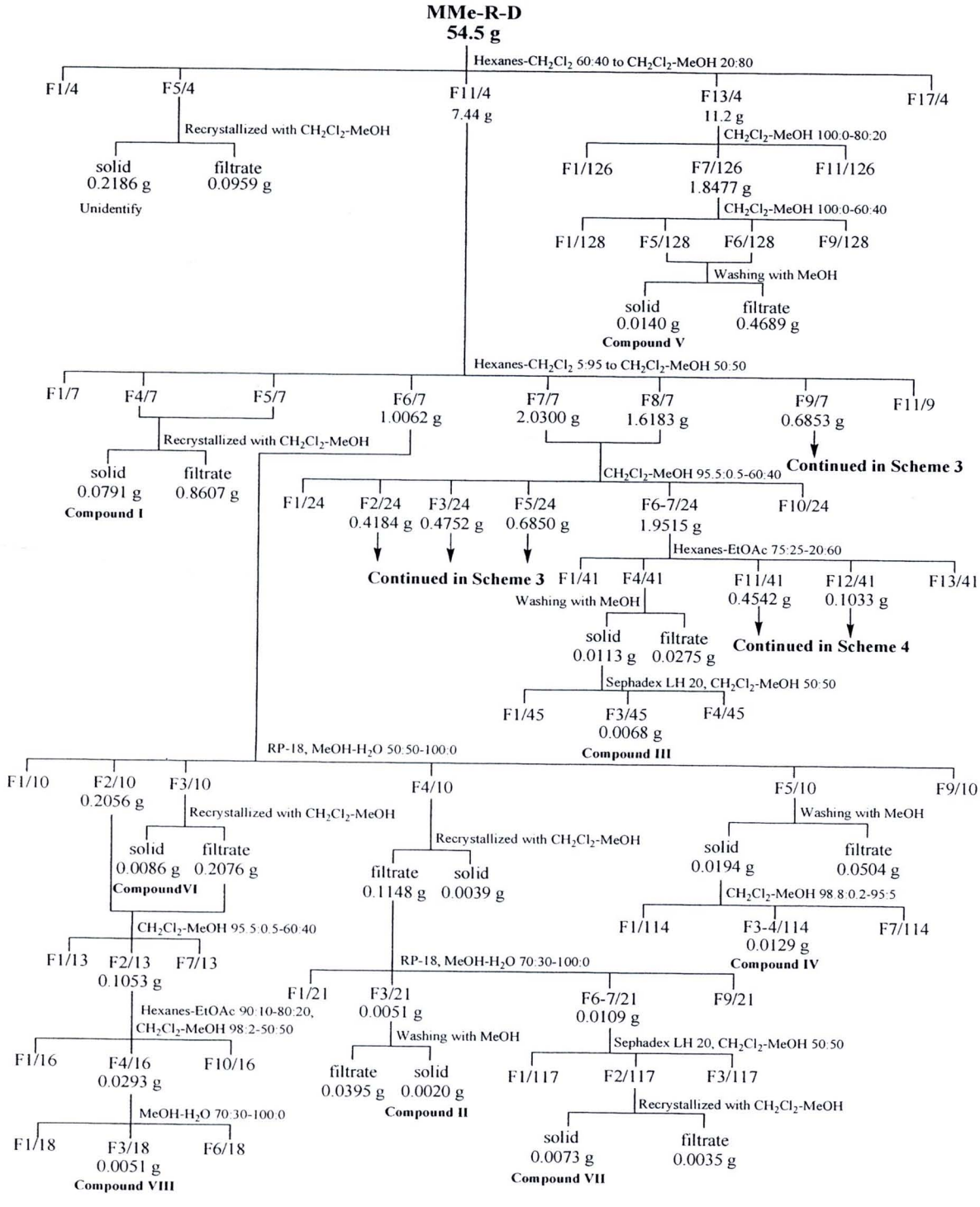
9′-Deacetoxymekongensine (**XIX**): colorless amorphous solid; $[\alpha]_D^{30}$ -7.09 (*c* 0.30, $CHCl_3$); FT-IR (KBr) ν_{max} 3568, 2930, 1748, 1723, 1585, 1568, 1451, 1371, 1254, 1231, 1096, 1047, 1007, 715 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 19); HRESIMS *m/z* 890.2856 $[M + Na]^+$ (calcd for $C_{43}H_{49}NO_{18}Na$, 890.2847).

Isolation of Compound **XX**

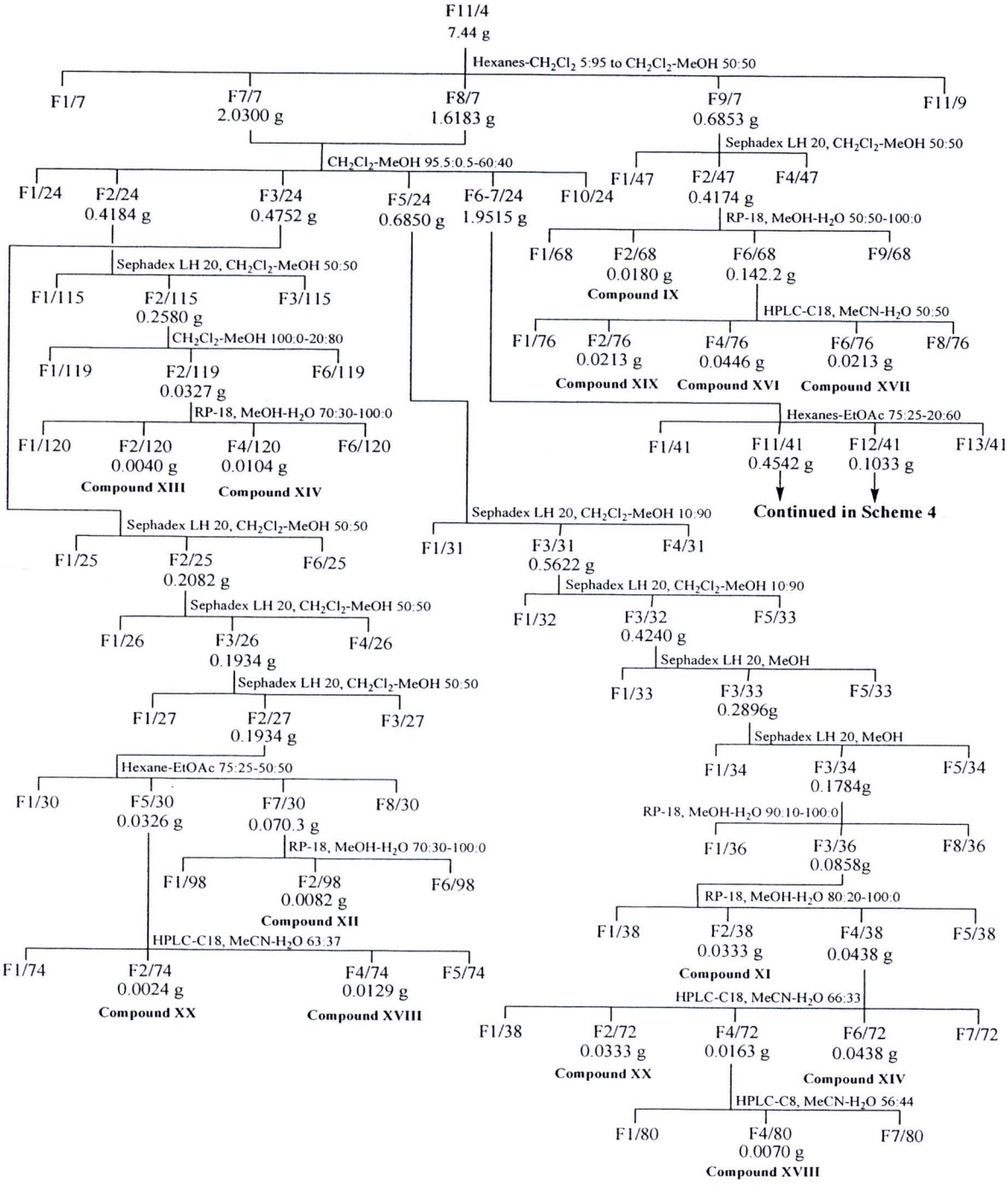
Compound **XX** was isolated from fraction 11/4 (7.44 g) using the same route as that of compound **XVIII** (Scheme 3) to obtain a colorless amorphous

solid (2.4 mg) after HPLC separation (LiChrospher RP-18, 250 × 4 mm, MeCN-H_2O 63:37, flow rate 1.0 mL/min at t_R = 13.94 min).

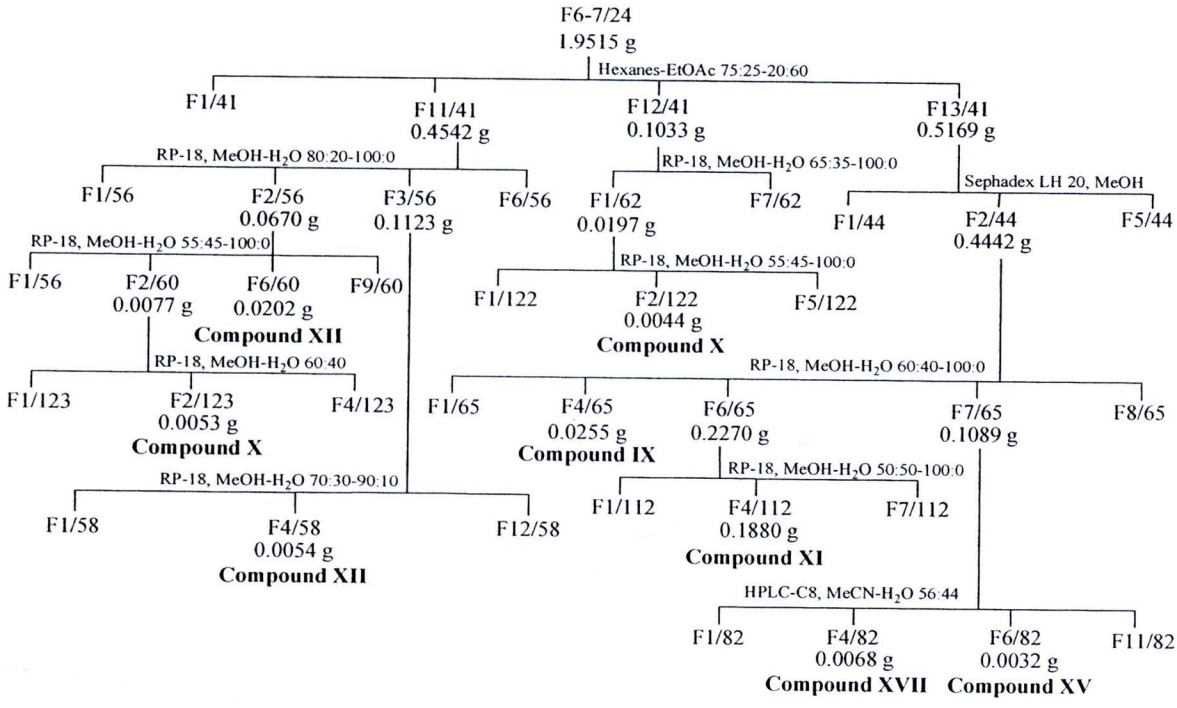
1-O-benzoyl-1-deacetyl-9'-deacetoxymekongensine (**XX**): colorless amorphous solid; $[\alpha]_D^{31}$ +3.39 (*c* 0.30, $CHCl_3$); FT-IR (KBr) ν_{max} 3467, 3068, 2935, 1747, 1723, 1602, 1585, 1567, 1451, 1371, 1255, 1224, 1097, 1048, 893, 713 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC correlations (see Table 20); HRESIMS *m/z* found: 952.3005 $[M + Na]^+$ (calcd for $C_{48}H_{51}NO_{18}Na$, 952.3004).



Scheme 2 Isolation of compounds **I-VIII** from the roots of *Maytenus mekongensis*



Scheme 3 Isolation of compounds **IX, XI-XIV** and **XVI-XX** from the roots of *Maytenus mekongensis*



Scheme 4 Isolation of compounds **IX-XII**, **XV** and **XVII** from the roots of *Maytenus mekongensis*

Bioactivity assays

The crude hexanes, CH_2Cl_2 and MeOH extract were submitted for cytotoxic assays. Some of the isolates (compounds **IX**, **XI**, **XII** and **XVI-XX**) were submitted for anti-TB, antimalarial and cytotoxic assays at the Bioassay Laboratory of the National Center for Genetic Engineering and Biotechnology, Pathumthani. Results were as shown in Table 21.

The cytotoxic activity assay was performed using the colorimetric method as of Skehan and co-workers.[45] Antiplasmodial activity was evaluated against *Plasmodium falciparum* (K1 multidrug-resistant strain) according to standard protocol.[46, 47]