CHAPTER III

EXPERIMENTAL

3.1 Plant materials

Fresh fruits of *Gymnema griffithii* and pods of *Holarrhena curtisii* were collected from collected from a dry dipterocarp forest at Nong Ya Plong, Phetchaburi Province, Thailand, in October 2010. The plant materials were identified by Professor Kasin Herbarium and deposited in the Professor Kasin Suvatabhandhu Herbarium, Department of Botany Herbarium, Faculty of Science, Chulalongkorn University, Thailand. (Herbarium No. BCU013532 for *holarrhena curtisii* and herbarium No. BCU013533 for *gymnema griffithii*)

3.2 General experimental procedures

3.2.1 Nuclear magnetic resonance spectrometer (NMR)

3.2.1.1

1D and 2D NMR spectra were recorded on a Varian Model Mercury (400 MHz) and Bruker Model AVANCE (400 MHz), at 400 and 100 MHz for ^{1}H and ^{13}C, respectively. The chemical shifts (δ) were reported in parts per million (ppm) and the coupling constants (*J*) in Hertz (Hz). Mestrelab Research – MestReNova NMR software version 6.0.6 – 5475 was used to analyze the spectral data. Chloroform-*d* ($CDCl_3$), methanol-d_4 (CD_3OD) and deuterium oxide (D_2O) were used in NMR experiments and chemical shifts were referenced to the signals of the residual solvent [$CDCl_3$ at δ_H 7.26 ppm, δ_C 77.16 ppm; CD_3OD at δ_H 3.31 ppm, δ_C 49.00 ppm; D_2O at δ_H 4.79 ppm].

3.2.2 Mass spectrometer (MS)

High resolution electrospray ionization mass spectrometry (HRESIMS) was recorded on Bruker Model micrOTOF spectrometer, Micromass UK Limited.

3.2.3 Fourier transforms infrared spectrophotometer (FTIR)

Attenuated total reflectance-Fourier transformed infrared spectroscopic (ATR-FTIR) spectra were obtained on Thermo Scientific Model Nicolet 6700 spectrometer.

3.2.4 Optical rotation

Optical rotations were determined at 589 nm on Perkin-Elmer Model 341 polarimeter.

3.2.5 Ultraviolet-visible spectrophotometer (UV-vis)

UV data were recorded on CARY 50 Probe UV-visible spectrophotometer.

3.2.6 Melting point

The melting points were measured on a MEL-TEMP melting point apparatus.

3.2.7 High-performance liquid chromatography (HPLC)

Semi-preparative HPLC was carried out on a Thermo Finnigan SpectraSYSTEM HPLC with a Thermo Scientific Hypersil ODS (C18) column (250 mm x 10 mm I.D., 10 μm) and UV detection at 220 nm.

3.2.8 Microplate spectrophotometer

UV data of chromogenic method were obtained from a PowerWave XS2 (Biotek Instrument Inc, USA) microplate reader.

3.3 Chemical

Thin layer chromatography (TLC) using 0.2 mm silicagel 60 F_{254} on an aluminium sheet (Merck) was used to monitor the products and reaction courses. Detection was visualized under UV light at wevelengths of 254 and 365 nm and stained with Ceric Ammonium Molybdate (CAM). For column chromatographic separations, silica gel 60 (230–400 mesh ASTM, Merck) and silica gel 60 RP-18 (40–60 μm) (Merck) were used as the adsorbent for normal CC and for reverse phase CC, respectively. All commercial grade solvents used in this research, such as hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and methanol (MeOH), were distilled prior to use. The reagent grade (AR) and HPLC grade solvents were purchased from Sigma-Aldrich, Burdick & Jackson and Merck® (Germany) used for thin layer chromatography, crystallization and HPLC experiments.

3.4 Extraction and isolation

3.4.1 Pericarps of *G. griffithii*

The fresh pericarps of *G. griffithii* fruits were cut into small pieces. The cut fresh pericarps (2 kg) were then extracted three times with MeOH (5 L x 3) and concentrated under vacuum. The entire crude extract (ca. ~100 g) was suspended in H_2O (1 L) and then extracted successively with CH_2Cl_2 (1 L x 3) and EtOAc (1 L x 3) to give the CH_2Cl_2–soluble extract (9.8 g) and EtOAc–soluble extract (150 mg), respectively. The CH_2Cl_2 extract was subjected to CC over silica gel [Silica gel, Merck (5 x 40 cm)] eluting with a stepwise gradient of CH_2Cl_2–MeOH (from 10:0 to 7:3). Fractions with a similar TLC profile after ceric ammonium molybdate staning were then combined resulting in eight fractions (Fr. A–H). To remove the green pigment from Fr. G (3.5 g), which had eluted with CH_2Cl_2–MeOH (1:9, v/v), it was subjected to Sephadex LH-20 CC (4 x 150 cm) and eluted with MeOH. The white solid (3.2 g) obtained was further separated by RP-18 CC (Silica gel RP-18; 4 x 20 cm), using a stepwise of MeOH–H_2O (6:4, 7:3 and 8:2, v/v) to give four subfractions (Fr. G1–G2, G3 and G4) eluting in the 6:4 (v/v), 7:3 (v/v) and 8:2 (v/v) MeOH–H2O fractions, respectively.

Fraction G3 (300 mg) was purified by semi-preparative RP-18 HPLC eluting with MeOH–H_2O (65:35, v/v) at 3 mL/min for 60 min to afford gymnemogriffithoside A (**61**) (116 mg, t_R 15.5 min), gymnemogriffithoside D (**64**) (40 mg, t_R 20.2 min), gymnemogriffithoside G (**67**) (22 mg, t_R 22.6 min) and gymnemogriffithoside H (**68**) (8 mg, t_R 24.6 min). Fraction G1 (40 mg) was subjected to semi-preparative RP-18 HPLC eluting with MeOH–H_2O (55:45, v/v) at 3 mL/min for 60 min to afford gymnemogriffithoside B (**62**) (3 mg, t_R 28.54 min). Fraction G2 (100 mg) was subjected to semi-preparative RP-18 HPLC eluting with MeOH–H_2O (60:40, v/v) at 3 mL/min for 60 min to afford gymnemogriffithoside E (**65**) (5 mg, t_R 19.22 min). Fraction G4 (580 mg) was further separated on silica gel (4 x 20 cm) CC eluting with CH_2Cl_2–MeOH (95:5, v/v) to give two subfractions (Fr. G4-1 and Fr. G4-2). Fraction G4-1 (160 mg) was subjected to semi-preparative RP-18 HPLC eluting with MeOH–H_2O (72.5:27.5, v/v) at 3 mL/min for 60 min to afford gymnemogriffithoside C (**63**) (7 mg, t_R 20.2 min). Fraction G4-2 (310 mg) was purified in the same manner to afford gymnemogriffithoside F (**66**) (9 mg, t_R 19.6 min).

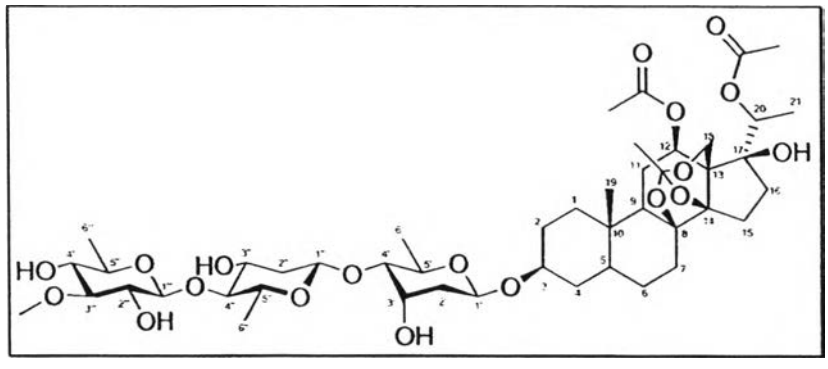
3.4.2 Pod of *H. curtisii*

The fresh pods of *H. curtisii* (700 g) were cut into small pieces and then extracted three times with MeOH (3 L x 3) at room temperature. After removing solvent under vacuum the entire crude extract (83.2 g) was suspended in H_2O (1 L) and then partitioned successively with CH_2Cl_2 (1 L x 3) and EtOAc (1 L x 3) to afford the CH_2Cl_2–soluble extract (12.6 g) and EtOAc–soluble extract (8.0 g), respectively. The CH_2Cl_2 extract was subjected to CC over silica gel 60 [Silica gel, Merck (5 x 40 cm)] eluting with a stepwise gradient of hexane–EtOAc (from 1:0 to 0:1) to give six fractions (Fr. A–F). Fraction B (1.2 g) was rechromatographed on silica gel column using a stepwise of hexane–CH_2Cl_2 (from 0:1 to 1:0) to give squalene (**83**) (20 mg) and four subfractions (Fr. B1–B4). Fr. B2 (80 mg) was further purified by semi-preparative RP-18 HPLC eluting with MeOH–H_2O (97:3, v/v) at 3 mL/min for 60 min to afford lupeol acetate (**86**) (8 mg, t_R 28.8 min), α-amyrin acetate (**85**) (10 mg, t_R 32.7 min) and β-amyrin acetate (**84**) (13 mg, t_R 34.5 min). Fraction B4 (900) was purified by semi-preparative RP-18 HPLC eluting with MeOH–H_2O (97:3, v/v) at 3 mL/min for 60 min to afford lupeol (**57**) (92 mg, t_R 21.3 min) and lanosta-7,24-dien-3β-ol (**87**) (110 mg, t_R 23.2 min). Fraction C (1.3 g) was repeatedly crystallized from CH_2Cl_2–MeOH (1:1) to give cycloeucalenol (**88**) (832 mg). The mother liquor was further separated by RP-18 CC eluting with MeOH to afford 24-methylenepollinastanol (**89**) (40 mg). Fraction E (930 mg) was subject to CC over silica gel using a stepwise of CH_2Cl_2–MeOH (from 20:1 to 10:1) to give two subfractions (Fr. E1 and E2). Fraction E1 (600 mg) was rechromatographed on silica gel column eluting with hexane–EtOAc–MeOH (10:2:1) to afford oleanolic acid (**90**) (30 mg), ursolic acid (**91**) (12 mg), and a mixture of oleanolic acid (**90**) and ursolic acid (**91**) (360 mg) (in 2:1 ratio based on the signal of olefinic proton H-12). Fraction E2 (40 mg) was purified by semi-preparative RP-18 HPLC eluting with MeOH–H_2O (77:23, v/v) at 3 mL/min for 60 min to afford 3β-hydroxy-11α-hydroperoxyolean-12-en-28-oic acid (**81**) (10 mg, t_R 19.4 min) and 3β-hydroxy-11α-hydroperoxyursan-12-en-28-oic acid (**82**) (11 mg, t_R 24.2 min).

The EtOAc extract was subjected to CC over silica gel 60 eluting with a stepwise gradient of EtOAc–MeOH (from 1:0 to 1:1) to give four subfractions (Fr. G–J). Fraction H was subjected to Sephadex LH-20 CC, eluting with MeOH to afford (–)-catechin (**92**) (510 mg) and (–)-gallocatechin (**93**) (40 mg).

3.5 Physical and spectral data of the isolated compounds from *G. griffithii*

3.5.1 Gymnemogriffithoside A (61)



Obtained as white amorphous powder.

mp. 178–180°C.

Optical Rotation: $[\alpha]_D^{25}$ = -10° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 202 and 217 nm.

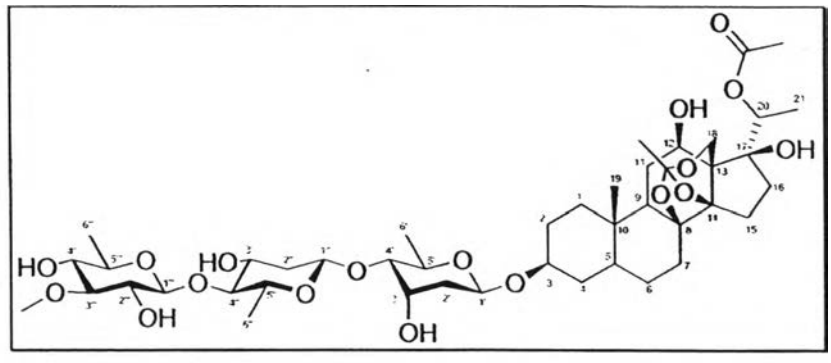
IR (ATR): ν_{max} 3447 br, 2934, 1731, 1372, 1252, 1064 and 1017 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.93 (1H, dd, *J* = 9.6, 1.7 Hz, H-1'), 4.59 (1H, dd, *J* = 10.7, 4.6 Hz, H-12), 4.57 (1H, dd, *J* = 9.7, 1.8 Hz, H-1''), 4.54 (1H, d, *J* = 12.0 Hz, H-18b), 4.45 (1H, br q, *J* = 6.1 Hz, H-20), 4.32 (1H, s, OH-3''), 4.29 (1H, d, *J* = 7.8 Hz, H-1'''), 4.28 (1H, br s, OH-17), 4.22 (1H, br s, H-3'), 4.16 (1H, d, *J* = 12.0 Hz, H-18a), 3.80 (1H, dq, *J* = 9.2, 6.2 Hz, H-5'), 3.66 (3H, s, 3'''-OCH_3), 3.65 – 3.55 (2H, m, H-3, H-3''), 3.53 – 3.44 (1H, m, H-2'''), 3.48 (1H, dq, *J* = 9.1, 6.1 Hz, H-5'''), 3.40 (1H, dq, *J* = 9.1, 6.1 Hz, H-5''), 3.23 (1H, td, *J* = 9.1, 2.2 Hz, H-4'''), 3.21 (1H, dd, *J* = 9.2, 3.2 Hz, H-4'), 3.12 (1H, t, *J* = 9.1 Hz, H-3'''), 2.99 (1H, t, *J* = 8.8 Hz, H-4''), 2.82 (1H, s, OH-3'), 2.39 (1H, s, OH-4'''), 2.35 (1H, s, OH-2'''), 2.26 (1H , ddd, *J* = 12.9, 5.2, 1.8 Hz, H-2b''), 2.06 (3H, s, 20-OAc), 1.94 (3H, s, 12-OAc), 1.56 (3H, s, CH_3-ortho), 1.35 (3H, d, *J* = 6.1 Hz, CH_3-6'''), 1.34 (3H, d, *J* = 6.1 Hz, CH_3-6''), 1.30 (3H , d, *J* = 6.1 Hz, CH_3-21), 1.24 (3H, d, *J* = 6.2 Hz, CH_3-6') and 0.96 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.4, 117.2, 103.8, 100.5, 95.6, 94.35, 88.2, 86.3, 85.5, 83.0, 82.3, 77.0, 74.7, 74.6, 74.4, 73.1, 72.4, 70.7, 69.5, 68.1, 66.9, 61.0, 60.2, 51.2, 46.8, 45.4, 38.5, 37.4, 37.4, 36.8, 34.0, 33.7, 32.2, 28.9, 25.9, 24.7, 24.3, 23.8, 21.7, 21.6, 18.3, 18.0, 17.6, 15.0 and 12.5.

HRESIMS: *m/z* 951.4549 $[M+Na]^+$ (951.4565 calcd for $C_{46}H_{72}NaO_{19}$).

3.5.2 Gymnemogriffithoside B (62)



Obtained as white amorphous powder.

mp. 176–178°C.

Optical Rotation: $[\alpha]_D^{25}$ = -7° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 202 nm.

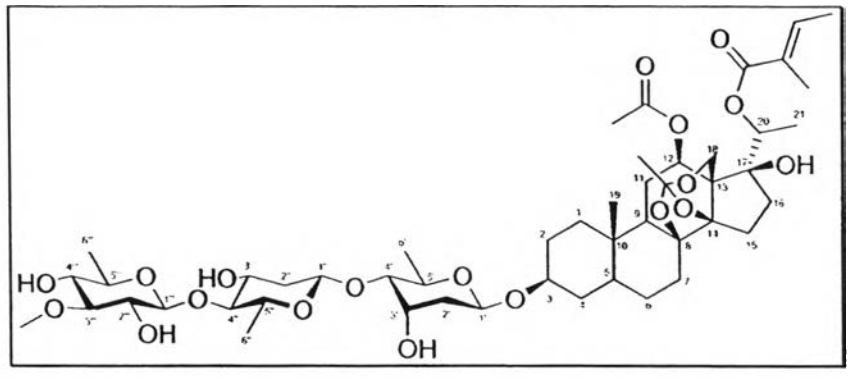
IR (ATR): ν_{max} 3419 br, 2933, 1719, 1372, 1253, 1064 and 1016 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.99 (1H, qd, *J* = 6.4, 1.2 Hz, H-20), 4.94 (1H, dd, *J* = 9.6, 1.6 Hz, H-1'), 4.57 (1H, dd, *J* = 9.8, 1.6 Hz, H-1''), 4.45 (1H, d, *J* = 12.0 Hz, H-18b), 4.35 (1H, s, OH-3''), 4.30 (1H, d, *J* = 7.8 Hz, H-1'''), 4.23 (1H, br s, H-3'), 4.14 (1H, br s, OH-17), 4.10 (1H, d, *J* = 12.0 Hz, H-18a), 3.80 (1H, dq, *J* = 9.4, 6.2 Hz, H-5'), 3.67 (3H, s, 3'''-OCH_3), 3.66 – 3.56 (2H, m, H-3, H-3''), 3.51 – 3.44 (3H, m, H-12, H-2''', H-5'''), 3.39 (1H, dd, *J* = 9.2, 6.1 Hz, H-5''), 3.24 (1H, t, *J* = 9.1 Hz, H-4'''), 3.22 (1H, dd, *J* = 9.4, 3.1 Hz, H-4'), 3.13 (1H, t, *J* = 9.1 Hz, H-3'''), 2.99 (1H, t, *J* = 8.8 Hz, H-4''), 2.84 (1H, s, OH-3'), 2.43 (1H, s, OH-4'''), 2.39 (1H, d, *J* = 2.5 Hz, OH-2'''), 2.26 (1H, ddd, *J* = 12.9, 5.1, 1.6 Hz, H-2b''), 2.00 (3H, s, 20-OAc), 1.55 (3H, s, CH_3-ortho), 1.35 (3H, d, *J* = 6.1 Hz, CH_3-6''), 1.34 (3H, d, *J* = 6.2 Hz, CH_3-6'''), 1.32 (3H, s, *J* = 6.4 Hz, CH_3-21), 1.24 (3H, d, *J* = 6.2 Hz, CH_3-6') and 0.98 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 117.0, 103.7, 100.5 95.6, 94.1, 88.1, 86.6, 85.5, 83.0, 82.2, 77.1, 74.7, 74.6, 74.5, 72.3, 70.7, 70.1, 69.5, 68.1, 66.9, 61.1, 59.9, 52.7, 47.2, 45.4, 38.5, 37.6, 37.3, 36.6, 34.0, 34.0, 32.2, 29.0, 27.9, 26.1, 24.7, 24.3, 21.5, 18.4, 18.0, 17.6, 15.1 and 12.6.

HRESIMS: *m/z* 909.4424 $[M+Na]^+$ (909.4460 calcd for $C_{44}H_{70}NaO_{18}$).

3.5.3 Gymnemogriffithoside C (63)



Obtaied as white amorphous powder.

mp. 174–176°C.

Optical Rotation: $[\alpha]_D^{25}$ = +2° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 203, 216, and 277 nm nm.

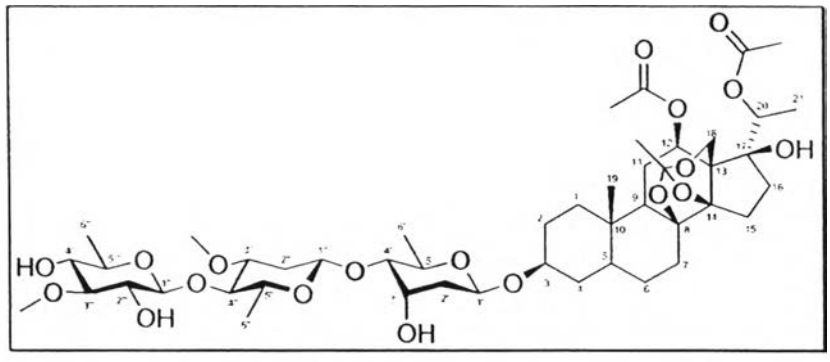
IR (ATR): ν_{max} 3446 br, 2933, 1736, 1703, 1371, 1264, 1065 and 1016 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.88 (1H, qd, *J* = 7.1, 1.0 Hz, CH-tig), 4.93 (1H, dd, *J* = 9.6, 1.6 Hz, H-1'), 4.59 (1H, dd, *J* = 10.8, 4.7 Hz, H-12), 4.57 (1H, dd, *J* = 9.7, 1.7 Hz, H-1''), 4.57 (1H, d, *J* = 11.8 Hz, H-18b), 4.51 (1H, qd, *J* = 6.2, 1.3 Hz, H-20), 4.34 (1H, s, OH-3''), 4.29 (1H, d, *J* = 7.8 Hz, H-1'''), 4.22 (1H, br s, H-3'), 4.16 (1H, d, *J* = 1.3 Hz, OH-17), 4.12 (1H, d, *J* = 11.8 Hz, H-18a), 3.79 (1H, dq, *J* = 9.3, 6.2 Hz, H-5'), 3.67 (3H, s, 3'''-OCH_3), 3.65 – 3.56 (2H, m, H-3, H-3''), 3.53 – 3.44 (1H, m, H-2'''), 3.49 (1H, dq, *J* = 9.1, 6.1, H-5'''), 3.40 (1H, dq, *J* = 9.3, 6.1 Hz, H-5''), 3.24 (1H, t, *J* = 9.1 Hz, H-4'''), 3.21 (1H, dd, *J* = 9.3, 3.1 Hz, H-4'), 3.12 (1H, t, *J* = 9.1 Hz, H-3'''), 2.99 (1H, t, *J* = 8.8 Hz, H-4''), 2.83 (1H, s, OH-3'), 2.40 (1H, s, OH-4'''), 2.35 (1H, d, *J* = 2.4 Hz, OH-2'''), 2.26 (1H , ddd, *J* = 12.8, 5.1, 1.7 Hz, H-2b''), 1.85 (3H, s, 12-OAc), 1.84 (3H, br s, CH_3-tig), 1.79 (3H, dd, *J* = 7.1, 1.0 Hz, CH_3-tig), 1.57 (3H, s, CH_3-ortho), 1.35 (3H, d, J = 6.1 Hz, CH3-6'''), 1.34 (3H, d, *J* = 6.1 Hz, CH_3-6''), 1.29 (3H , d, *J* = 6.2 Hz, CH_3-21), 1.24 (3H, d, *J* = 6.2 Hz, CH_3-6') and 0.94 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 167.3, 137.8, 129.1, 117.1, 103.7, 100.5, 95.6, 94.4, 88.1, 86.6, 85.5, 83.0, 82.3, 77.0, 74.7, 74.5, 74.1, 72.8, 72.3, 70.7, 69.5, 68.1, 66.9, 61.1, 60.5, 51.4, 46.7, 45.3, 38.5, 37.4, 37.3, 36.7, 34.0, 33.9, 32.1, 28.9, 26.0, 24.7, 24.3, 23.7, 21.7, 18.4, 18.0, 17.6, 15.1, 14.6, 12.5 and 12.3.

HRESIMS: *m/z* 991.4870 $[M+Na]^+$ (991.4878 calcd for $C_{49}H_{76}NaO_{19}$).

3.5.4 Gymnemogriffithoside D (64)



Obtained as white amorphous powder.

mp. 165–167°C.

Optical Rotation: $[\alpha]_D^{25}$ = -9° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 202 and 216 nm.

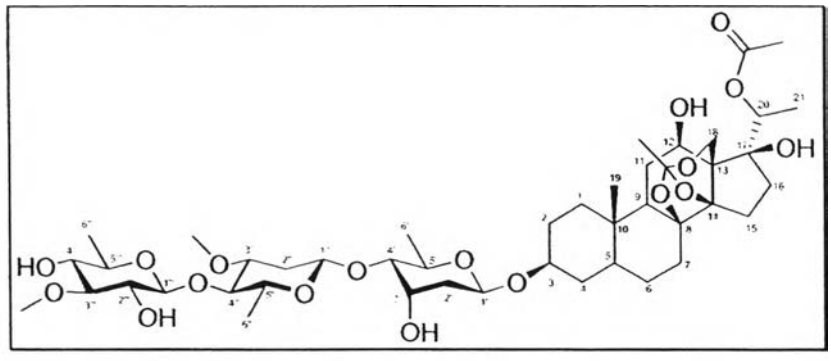
IR (ATR): ν_{max} 3508 br, 2933, 1730, 1372, 1253, 1063 and 989 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.93 (1H, dd, *J* = 9.6, 1.6 Hz, H-1'), 4.58 (1H, dd, *J* = 10.8, 4.6 Hz, H-12), 4.54 (1H, d, *J* = 12.0 Hz, H-18b), 4.52 (1H, dd, *J* = 9.6, 1.8 Hz, H-1''), 4.47 (1H, d, *J* = 8.0 Hz, H-1'''), 4.44 (1H, qd, *J* = 5.8, 1.3 Hz, H-20), 4.28 (1H, d, *J* = 1.3 Hz, OH-17), 4.21 (1H, br s, H-3'), 4.15 (1H, d, *J* = 12.0 Hz, H-18a), 3.79 (1H, dq, *J* = 9.3, 6.2 Hz, H-5'), 3.65 (3H, s, 3'''-OCH_3), 3.67 – 3.56 (1H, m, H-3), 3.45 (1H, td, *J* = 8.4, 2.3 Hz, H-2'''), 3.39 (3H, s, 3''-OCH_3), 3.40 – 3.31 (5H, m, H-3'', H-4'', H-5'', H-5''', OH-2'''), 3.20 (1H, dd, *J* = 9.3, 2.9 Hz, H-4'), 3.15 (1H, td, *J* = 8.8, 2.5 Hz, H-4'''), 3.08 (1H, t, *J* = 8.9 Hz, H-3'''), 2.86 (1H, s, OH-3'), 2.47 (1H, d, *J* = 2.5 Hz, OH-4'''), 2.36 (1H , ddd, *J* = 13.0, 4.2, 1.8 Hz, H-2b''), 2.05 (3H, s, 20-OAc), 1.93 (3H, s, 12-OAc), 1.55 (3H, s, CH_3-ortho), 1.34 (3H, d, *J* = 5.5 Hz, CH_3-6''), 1.30 (3H, d, *J* = 6.1 Hz, CH_3-6'''), 1.29 (3H , d, *J* = 5.8 Hz, CH_3-21), 1.24 (3H, d, *J* = 6.2 Hz, CH_3-6') and 0.95 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.4, 117.1, 101.9, 100.2, 95.6, 94.3, 86.2, 85.6, 83.1, 82.3, 79.4, 78.8, 77.0, 75.0, 74.4 73.4, 73.1, 72.1, 71.8, 68.0, 66.7, 60.8, 60.2, 56.2, 51.2, 46.7, 45.3, 37.4, 37.3, 36.7, 35.9, 33.9, 33.6, 32.1, 28.9, 25.8, 24.6, 24.3, 23.8, 21.7, 21.6, 18.7, 18.4, 17.9, 15.0 and 12.5.

HRESIMS: *m/z* 965.4712 $[M+Na]^+$ (965.4722 calcd for $C_{47}H_{74}NaO_{19}$).

3.5.5 Gymnemogriffithoside E (65)



Obtained as white amorphous powder.

mp. 166–168°C.

Optical Rotation: $[\alpha]_D^{25}$ = -6° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 203 nm.

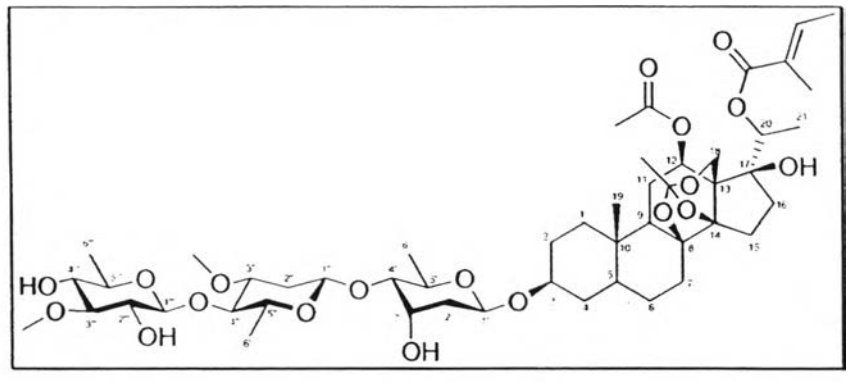
IR (ATR): ν_{max} 3449 br, 2934, 1719, 1372, 1254, 1060 and 989 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.99 (1H, dq, *J* = 6.4, 1.5 Hz, H-20), 4.94 (1H, dd, *J* = 9.6, 1.8 Hz, H-1'), 4.53 (1H, dd, *J* = 9.7, 1.9 Hz, H-1''), 4.48 (1H, d, *J* = 8.1 Hz, H-1'''), 4.45 (1H, d, *J* = 12.0 Hz, H-18b), 4.22 (1H, br s, H-3'), 4.13 (1H, br s, OH-17), 4.10 (1H, d, *J* = 12.0 Hz, H-18a), 3.80 (1H, dq, *J* = 9.3, 6.2 Hz, H-5'), 3.66 (3H, s, 3'''-OCH_3), 3.67 – 3.56 (1H, m, H-3), 3.51 – 3.43 (2H, m, H-12, H-2'''), 3.40 (3H, s, 3''-OCH_3), 3.40 – 3.31 (5H, m, H-3'', H-4'', H-5'', H-5''', OH-2'''), 3.21 (1H, dd, *J* = 9.3, 2.9 Hz, H-4'), 3.16 (1H, td, *J* = 8.9, 2.4 Hz, H-4'''), 3.09 (1H, t, *J* = 8.9 Hz, H-3'''), 2.86 (1H, s, OH-3'), 2.40 (1H, d, *J* = 2.6 Hz, OH-4'''), 2.37 (1H , ddd, *J* = 12.9, 5.2, 1.8 Hz, H-2b''), 2.00 (3H, s, 20-OAc), 1.55 (3H, s, CH_3-ortho), 1.34 (3H, d, *J* = 5.6 Hz, CH_3-6''), 1.32 (3H , d, *J* = 6.4 Hz, CH_3-21), 1.31 (3H, d, *J* = 6.2 Hz, CH_3-6'''), 1.24 (3H, d, *J* = 6.2 Hz, CH_3-6') and 0.98 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 117.0, 101.9, 100.2, 95.6, 94.1, 86.6, 85.6, 83.1, 82.1, 79.4, 78.8, 77.2, 75.0, 74.7, 73.4, 72.1, 71.8, 70.0, 68.0, 66.7, 60.8, 59.9, 56.2, 52.7, 47.2, 45.4, 37.6, 37.32, 36.6, 35.9, 34.0 (2), 32.2, 29.0, 27.9, 26.1, 24.7, 24.3, 21.5, 18.7, 18.4, 17.9, 15.1 and 12.6.

HRESIMS: *m/z* 923.4594 $[M+Na]^+$ (923.4616 calcd for $C_{45}H_{72}NaO_{18}$).

3.5.6 Gymnemogriffithoside F (66)



Obtained as white amorphous powder.

mp. 170–172°C.

Optical Rotation: $[\alpha]_D^{25}$ = +1° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 210 nm.

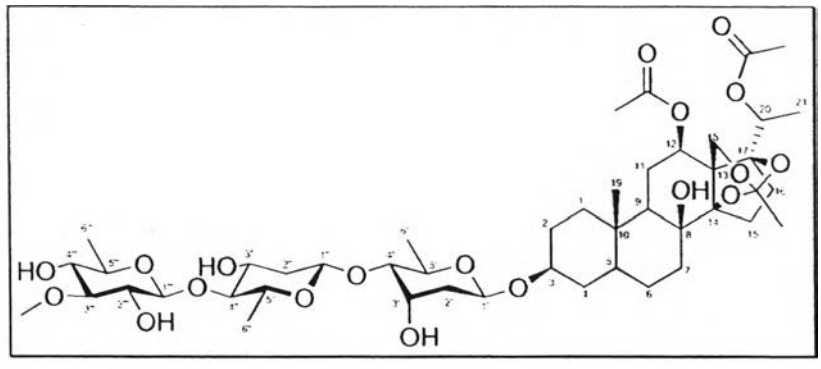
IR (ATR): ν_{max} 3486 br, 2932, 1731, 1703, 1371, 1263, 1230 and 1063 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.88 (1H, qd, *J* = 7.1, 1.0 Hz, CH-tig), 4.93 (1H, dd, *J* = 9.4, 1.4 Hz, H-1'), 4.56 – 4.50 (1H, m, H-20), 4.58 (1H, dd, *J* = 10.8, 4.6 Hz, H-12), 4.56 (1H, d, *J* = 11.8 Hz, H-18b), 4.53 (1H, dd, *J* = 9.6, 1.9 Hz, H-1''), 4.47 (1H, d, *J* = 8.1 Hz, H-1'''), 4.22 (1H, br s, H-3'), 4.16 (1H, d, *J* = 1.5 Hz, OH-17), 4.12 (1H, d, *J* = 11.8 Hz, H-18a), 3.79 (1H, dq, *J* = 9.3, 6.2 Hz, H-5'), 3.66 (3H, s, 3'''-OCH_3), 3.65 – 3.56 (1H, m, H-3), 3.46 (1H, td, *J* = 8.6, 2.2 Hz, H-2'''), 3.39 (3H, s, 3''-OCH_3), 3.40 – 3.33 (5H, m, H-3'', H-4'', H-5'', H-5''', OH-2'''), 3.21 (1H, dd, *J* = 9.3, 2.9 Hz, H-4'), 3.16 (1H, br t, *J* = 8.9 Hz, H-4'''), 3.09 (1H, t, *J* = 8.9 Hz, H-3'''), 2.85 (1H, s, OH-3'), 2.40 (1H, br s, OH-4'''), 2.37 (1H , ddd, *J* = 13.1, 4.5, 1.9 Hz, H-2b''), 1.85 (3H, s, 12-OAc), 1.84 (3H, br s, CH_3-tig), 1.79 (3H, dd, *J* = 7.1, 1.0 Hz, CH_3-tig), 1.57 (3H, s, CH_3-ortho), 1.34 (3H, d, *J* = 5.5 Hz, CH_3-6''), 1.31 (3H, d, *J* = 6.0 Hz, CH_3-6'''), 1.29 (3H , d, *J* = 5.5 Hz, CH_3-21), 1.24 (3H, d, *J* = 6.2 Hz, CH_3-6') and 0.94 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 167.3, 137.8, 129.1, 117.1, 101.9, 100.2, 95.6, 94.4, 86.6, 85.6, 83.1, 82.3, 79.4, 78.8, 77.0, 75.0, 74.1, 73.4, 72.8, 72.1, 71.8, 68.0, 66.8, 60.8, 60.5, 56.2, 51.4, 46.7, 45.3, 37.4, 37.3, 36.7, 35.9, 34.0, 33.9, 32.1, 28.9, 26.0, 24.6, 24.3, 23.7, 21.7, 18.7, 18.4, 17.9, 15.1, 14.6, 12.5 and 12.3.

HRESIMS: *m/z* 1005.5046 $[M+Na]^+$ (1005.5035 calcd for $C_{50}H_{78}NaO_{19}$).

3.5.7 Gymnemogriffithoside G (67)



Obtained as white amorphous powder.

mp. 178–180°C.

Optical Rotation: $[\alpha]_D^{25}$ = -8° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 203 nm.

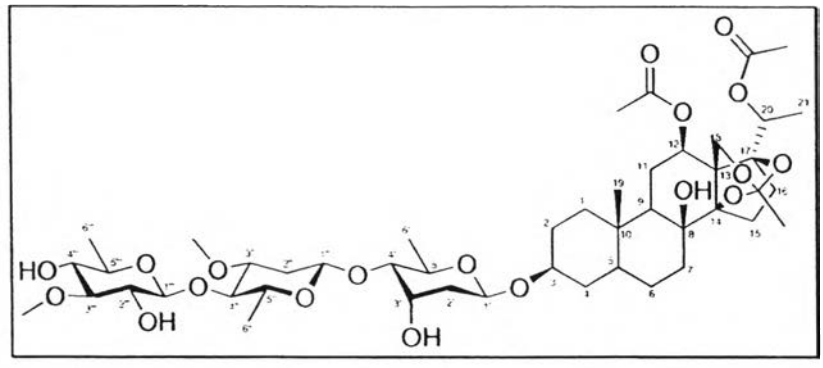
IR (ATR): ν_{max} 3452 br, 2934, 1734, 1375, 1249, 1066 and 1028 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.94 (1H, dd, *J* = 9.5, 1.7 Hz, H-1'), 4.77 (1H, q, *J* = 6.4 Hz, H-20), 4.75 (1H, d, *J* = 8.9 Hz, H-18a), 4.71 (1H, dd, *J* = 10.8, 5.8 Hz, H-12), 4.57 (1H, dd, *J* = 9.8, 1.7 Hz, H-1''), 4.46 (1H, d, *J* = 8.9 Hz, H-18b), 4.34 (1H, s, OH-3''), 4.29 (1H, d, *J* = 7.8 Hz, H-1'''), 4.22 (1H, br s, H-3'), 3.79 (1H, dq, *J* = 9.3, 6.2 Hz, H-5'), 3.67 (3H, s, 3'''-OCH_3), 3.65 – 3.55 (2H, m, H-3, H-3''), 3.51 – 3.43 (1H, m, H-2'''), 3.48 (1H, dq, *J* = 9.1, 6.1 Hz, H-5'''), 3.40 (1H, dq, *J* = 9.2, 6.1 Hz, H-5''), 3.23 (1H, td, *J* = 9.1, 2.9 Hz, H-4'''), 3.21 (1H, dd, *J* = 9.3, 3.4 Hz, H-4'), 3.12 (1H, t, *J* = 9.1 Hz, H-3'''), 2.99 (1H, t, *J* = 8.8 Hz, H-4''), 2.83 (1H, s, OH-3'), 2.57 (1H, d, *J* = 1.1 Hz, OH-8), 2.44 (1H, d, *J* = 2.9 Hz, OH-4'''), 2.39 (1H, d, *J* = 3.0 Hz, OH-2'''), 2.26 (1H , ddd, *J* = 12.9, 5.1, 1.7 Hz, H-2b''), 2.09 (3H, s, 20-OAc), 1.97 (3H, s, 12-OAc), 1.53 (3H, s, CH_3-ortho), 1.35 (3H, d, *J* = 6.1 Hz, CH_3-6'''), 1.34 (3H, d, *J* = 6.1 Hz, CH_3-6''), 1.29 (3H , d, *J* = 6.4 Hz, CH_3-21), 1.24 (3H, d, *J* = 6.2 Hz, CH_3-6') and 0.98 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.3, 108.3, 103.7, 100.5, 95.5, 90.4, 88.1, 87.9, 85.5, 83.1, 77.1, 74.7, 74.5, 73.6, 72.3, 72.1, 70.9, 70.7, 69.5, 68.1, 66.9, 61.7, 61.1, 46.9, 46.6, 45.5, 38.5, 38.1, 37.4, 36.6, 34.1, 34.0, 32.0, 31.8, 28.9, 24.4 (2), 23.7, 21.5, 21.4, 18.3, 18.0, 17.6, 15.1 and 12.6.

HRESIMS: *m/z* 951.4510 $[M+Na]^+$ (951.4565 calcd for $C_{46}H_{72}NaO_{19}$).

3.5.8 Gymnemogriffithoside H (68)



Obtained as white amorphous powder.

mp. 162–164°C.

Optical Rotation: $[\alpha]_D^{25}$ = -7° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 203 and 245 nm.

IR (ATR): ν_{max} 3480 br, 2934, 1731, 1373, 1249, 1064 and 989 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.94 (1H, dd, *J* = 9.6, 1.3 Hz, H-1'), 4.77 (1H, q, *J* = 5.4 Hz, H-20), 4.75 (1H, d, *J* = 8.8 Hz, H-18a), 4.71 (1H, dd, *J* = 10.8, 5.7 Hz, H-12), 4.53 (1H, dd, *J* = 9.6, 1.6 Hz, H-1''), 4.47 (1H, d, *J* = 8.0 Hz, H-1'''), 4.46 (1H, d, *J* = 8.8 Hz, H-18b), 4.22 (1H, br s, H-3'), 3.79 (1H, dq, *J* = 9.2, 6.2 Hz, H-5'), 3.66 (3H, s, 3'''-OCH_3), 3.62 (1H, tt, *J* = 11.2, 4.9 H-3), 3.46 (1H, br t, *J* = 8.5 Hz, H-2'''), 3.39 (3H, s, 3''-OCH_3), 3.38 – 3.32 (5H, m, H-3'', H-4'', H-5'', H-5''', OH-2'''), 3.21 (1H, dd, *J* = 9.2, 2.8 Hz, H-4'), 3.16 (1H, br t, *J* = 8.9 Hz, H-4'''), 3.09 (1H, t, *J* = 8.9 Hz, H-3'''), 2.84 (1H, s, OH-3'), 2.57 (1H, s, OH-8), 2.41 (1H, s, OH-4'''), 2.37 (1H , ddd, *J* = 12.7, 4.1, 1.6 Hz, H-2b''), 2.09 (3H, s, 20-OAc), 1.97 (3H, s, 12-OAc), 1.53 (3H, s, CH_3-ortho), 1.34 (3H, d, *J* = 5.3 Hz, CH_3-6''), 1.31 (3H, d, *J* = 6.1 Hz, CH_3-6'''), 1.29 (3H , d, *J* = 5.4 Hz, CH_3-21), 1.24 (3H, d, *J* = 6.2 Hz, CH_3-6') and 0.98 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.3, 108.3, 101.9, 100.3, 95.5, 90.3, 87.9, 85.6, 83.1, 79.4, 78.8, 77.2, 75.0, 73.6, 73.4, 72.1 (2), 71.8, 70.9, 68.0, 66.8, 61.7, 60.8, 56.2, 46.9, 46.6, 45.4, 38.1, 37.4, 36.6, 35.9, 34.1, 34.0, 32.0, 31.8, 28.9, 24.4 (2), 23.7, 21.5, 21.4, 18.7, 18.4, 17.9, 15.1 and 12.6.

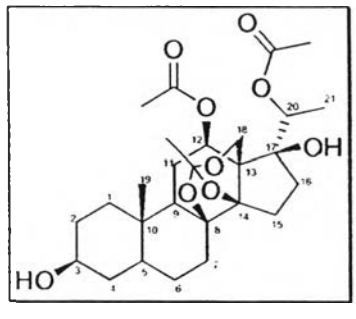
HRESIMS: *m/z* 965.4713 $[M+Na]^+$ (965.4722 calcd for $C_{47}H_{74}NaO_{19}$).

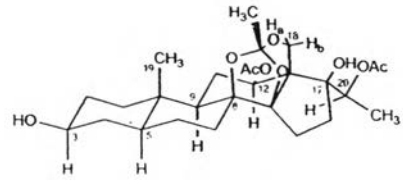
3.6 Acid hydrolysis and methanolysis of steroidal glycoside from *G. griffithii*

3.6.1 Acid hydrolysis of crude steroidal glycoside from *G. griffithii*

To a solution of crude steroidal glycoside (500 mg) in MeOH (50 mL), 0.05 M H_2SO_4 (50 mL) was added. The solution was heated at 60 °C for 5 h and then neutralized with saturated $Ba(OH)_2$ in H_2O. After the precipitate was filtered off, the filtrate was partitioned with CH_2Cl_2. The aqueous layer was concentrated and separated by silica gel CC eluting with CH_2Cl_2–MeOH (10:1, v/v) and then further fractionated by RP-18 CC eluting with a stepwise of MeOH–H_2O (6:4, 7:3 and 8:2, v/v) to give the two disaccharides, *β*-D-thevetopyranosyl-(1→4)-*O*-D-canaropyranoside (**73**) (20 mg) and *β*-D-thevetopyranosyl-(1→4)-*O*-D-oleandropyranoside (**74**) (13 mg), and four monosaccharides, D-digitoxose (**69**) (9 mg), D-canarose (**70**) (1 mg) D-oleandrose (**71**) (1.5 mg), and D-thevetose (**72**) (7 mg). The specific rotation of these sugars was determined and compared to the literature values for $[\alpha]^{D}_{25}$ of +43 (c 0.25 H_2O) for D-digitoxose (lit +45,[48]), -13 (c 0.15 H_2O) for D-oleandrose (lit -11.7, [48]), +25 (c 0.10 H_2O) for D-canarose (lit +22.8, [48]) and +55 (c 0.43 H_2O) for D-thevetose (lit +42.3, [49]). The CH_2Cl_2 layer was concentrated and subjected to silica gel CC, eluting with CH_2Cl_2–MeOH (20:1) to afford a mixture of **61a** and **67a**. This mixture was further separated by semi-preparative RP-18 HPLC eluting with MeOH–H_2O (68:32) at 3 mL/min for 40 min to afford **61a** (37 mg, t_R 8.5 min) and **67a** (12 mg, t_R 10.8 min).

3.6.1.1 Steroid 61a





Obtained as white amorphous powder.

mp. 168–170°C.

Optical Rotation: $[\alpha]_D^{25}$ = +4° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 202 nm.

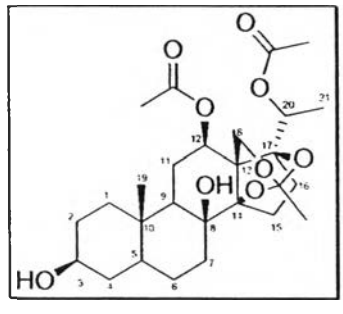
IR (ATR): ν_{max} 3518, 3289 br, 2931, 1726, 1373, 1252, 1170 and 1066 cm^{-1}.

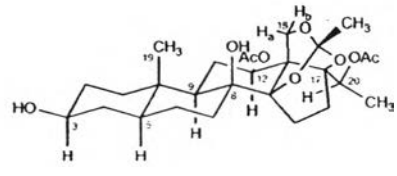
^{1}H NMR (400 MHz, $CDCl_3$): δ 4.59 (1H, dd, *J* = 10.9, 4.7 Hz, H-12), 4.55 (1H, d, *J* = 12.0 Hz, H-18b), 4.45 (1H, qd, *J* = 6.1, 1.3 Hz, H-20), 4.28 (1H, d, *J* = 1.3, OH-17), 4.16 (1H, d, *J* = 12.0 Hz, H-18a), 3.59 (1H, tt, *J* = 11.0, 4.7, H-3), 2.06 (3H, s, 20-OAc), 1.94 (3H, s, 12-OAc), 1.56 (3H, s, CH_3-ortho), 1.29 (3H , d, *J* = 6.1 Hz, CH_3-21) and 0.97 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.4, 117.2, 94.3, 86.2, 82.3, 74.4, 73.1, 71.1, 60.2, 51.2, 46.7, 45.4, 37.5, 37.3, 36.6, 33.6, 32.1, 31.0, 25.9, 24.6, 24.3, 23.8, 21.7, 21.6, 15.0 and 12.6.

HRESIMS: *m/z* 531.2506 $[M+Na]^+$ (531.2570 calcd for $C_{27}H_{40}NaO_9$).

3.6.1.2 Steroid 67a





Obtained as white amorphous powder.

mp. 178–180°C.

Optical Rotation: $[\alpha]_D^{25}$ = +5° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 203 nm.

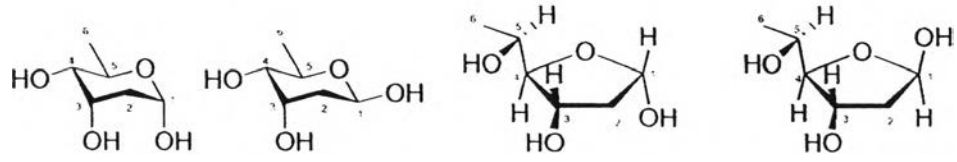
IR (ATR): ν_{max} 3533, 3283 br, 2927, 1731, 1374, 1255.9, 1088 and 1026 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.78 (1H, q, *J* = 6.5 Hz, H-20), 4.75 (1H, d, *J* = 8.8 Hz, H-18a), 4.72 (1H, dd, *J* = 10.9, 5.6 Hz, H-12), 4.47 (1H, d, *J* = 8.8 Hz, H-18b), 3.60 (1H, tt, *J* = 10.8, 4.8, H-3), 2.59 (1H, d, *J* = 1.2 Hz, OH-8), 2.09 (3H, s, 20-OAc), 1.98 (3H, s, 12-OAc), 1.53 (3H, s, CH_3-ortho), 1.30 (3H , d, *J* = 6.5 Hz, CH_3-21) and 1.00 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.3, 108.3, 90.34, 87.9, 73.6, 72.1, 71.3, 70.9, 61.7, 46.9, 46.6, 45.6, 38.0, 37.7, 36.4, 34.1, 32.0, 31.8, 31.0, 24.4, 24.3, 23.7, 21.5, 21.4, 15.1 and 12.7.

HRESIMS: m/z 531.2484 $[M+Na]^+$ (531.2570 calcd for C27H40NaO9).

3.6.1.3 D-digitoxose (69)



α-D-digitoxopyranose β-D-digitoxpyranose α-D-digitoxofuranse β-D-digitoxfuranose

The equilibrated aqueous solution of D-digitoxose usually appears as a mixture of four ring forms, α-,β-pyranoid and α-,β-furanoid ring forms [50]. The ratio of α-D-digitoxopyranose, β-D-digitoxopyranose, α-D-digitoxofuranose and β-D-digitoxofuranose was considered to be ca. 10:39:7:8 based on integrals of the anomeric signals:

Optical Rotation: $[\alpha]_D^{25}$ = +43° (c = 0.25, H_2O) (lit. +45°,[48]).

^{1}H NMR (400 MHz, D_2O) for α-D-digitoxopyranose: δ 5.19 (1H, br t, *J* = 3.1 Hz, H-1), 4.17 (1H, qd, *J* = 8.6, 6.4 Hz, H-5), 4.10 (1H, br t, *J* = 4.1 Hz, H-3), 3.41 (1H, dd, *J* = 8.6, 3.1 Hz, H-4), 2.00 (1H, dd, *J* = 4.8, 2.7 Hz, H-2b), 1.96 (1H, t, *J* = 3.6 Hz, H-2a) and 1.26 (1H, d, *J* = 6.4 Hz, CH_3-6)

^{1}H NMR. (400 MHz, D_2O) for β-D-digitoxopyranose: δ 5.12 (1H, dd, *J* = 10.0, 2.1 Hz, H-1), 4.13 (1H, br q, *J* = 3.1 Hz, H-3), 3.86 (1H, dq, *J* = 9.8 , 6.3 Hz, H-5), 3.33 (1H, dd, *J* = 9.8, 3.1 Hz, H-4), 2.08 (1H, ddd, *J* = 13.9, 3.6, 2.1 Hz, H-2b), 1.73 (1H, ddd, *J* = 13.9, 10.0, 2.8 Hz, H-2a) and 1.25 (3H, d *J* = 6.3 Hz, CH_3-6)

^{1}H NMR (400 MHz, D_2O) for α-D-digitoxofuranose: δ 5.60 (1H, t, *J* = 4.7 Hz, H-1), 4.49 (1H, br dt, *J* = 5.9, 3.9 Hz, H-3), 3.80 – 3.84 (1H, m, H-5), 3.71 (1H, dd, *J* = 5.9, 3.4 Hz, H-4), 2.20 –2.10 (2H, m, CH_2-2) and 1.22 (d, *J* = 6.4 Hz, CH_3-6).

^{1}H NMR (400 MHz, D_2O) for β-D-digitoxofuranose: δ 5.57 (1H, dd, *J* = 5.4, 1.5 Hz, H-1), 4.37 (1H, dt, *J* = 7.2, 2.7 Hz, H-3), 3.90 – 3.84 (1H, m, H-5), 4.01 (1H, t, *J* = 3.6 Hz, H-4), 2.30 (1H, ddd, *J* = 14.2, 7.2, 5.5 Hz, H-2b), 1.90 (1H, br ddd, *J* = 14.2, 2.3, 1.5 Hz, H-2a) and 1.19 (1H, d, *J* = 6.6 Hz, CH_3-6).

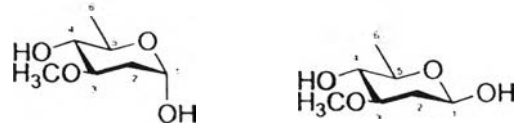
^{13}C NMR (100 MHz, D_2O) for α-D-digitoxopyranose: δ 90.9 (C-1), 71.9 (C-4), 66.8 (C-3), 65.2 (C-5), 35.0 (C-2) and 16.8 (C-6).

^{13}C NMR (100 MHz, D_2O) for β-D-digitoxopyranose: δ 91.5 (C-1), 72.1 (C-4), 69.5 (C-5), 67.5 (C-3), 38.4 (C-2) and 17.4 (C-6).

^{13}C NMR (100 MHz, D_2O) for α-D-digitoxofuranose: δ 98.0 (C-1), 89.3 (C-4), 71.0 (C-3), 67.7 (C-5), 41.3 (C-2) and 17.9 (C-6).

^{13}C NMR (100 MHz, D_2O) for β-D-digitoxopyranose: δ 98.2 (C-1), 89.2 (C-4), 70.2 (C-3), 67.1 (C-5), 41.6 (C-2) and 17.5 (C-6)

3.6.1.4 D-oleandose (71)



α-D-oleandose β-D-oleandose

The ratio of α,β-D-oleandose was considered to be ca. 8:10 based on integrals of the anomeric signals.

Optical Rotation: $[\alpha]_D^{25}$ = -13° (c = 0.15, H_2O) (lit. -11.7°, [48]).

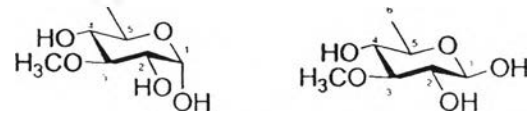
^{1}H NMR (400 MHz, D_2O) for α-D-oleandose: δ 5.37 (br d, *J* = 3.7 Hz, H-1), 3.91 (dq, *J* = 9.4, 6.3 Hz, H-5), 3.62 (1H, ddd, *J* = 11.8, 9.4, 4.9 Hz, H-3), 3.44 (3H, s, 3-OCH_3), 3.20 (1H, t, *J* = 9.4 Hz, H-4), 2.36 (1H, ddd, *J* = 13.4, 4.9, 1.2 Hz, H-2b), 1.60 (1H, ddd, *J* = 13.4, 11.8, 3.7 Hz, H-2a) and 1.28 (3H, d, *J* = 6.3 Hz, CH_3-6).

^{1}H NMR (400 MHz, D_2O) for β-D-oleandose: δ 4.92 (1H, dd, *J* = 9.8, 2.1 Hz, H-1), 3.45 (3H, s, 3-OCH_3), 3.49 – 3.39 (2H, m, H-3, H-5), 3.15 (1H, t, *J* = 9.3 Hz, H-4), 2.50 (1H, ddd, *J* = 12.3, 4.9, 2.1 Hz, H-2b), 1.39 (1H, td, *J* = 11.9, 9.8 Hz, H-2a) and 1.30 (3H, d, *J* = 6.2 Hz, CH_3-6).

^{13}C NMR (100 MHz, D_2O) for α-D-oleandose: δ 91.2 (C-1), 77.3 (C-3), 75.4 (C-4), 68.0 (C-5), 56.2 (3-OCH_3), 34.3 (C-2) and 17.0 (C-6).

^{13}C NMR (100 MHz, D_2O) for β-D-oleandose: δ 93.3 (C-1), 79.5 (C-3), 74.8 (C-4), 71.9 (C-5), 56.2 (3-OCH_3), 36.4 (C-2) and 17.0 (C-6).

3.6.1.5 D-thevetose (72)



α-D-thevetose β-D-thevetose

The ratio of α,β-D-thevetose was considered to be ca. 3:10 based on integrals of the anomeric signals.

Optical Rotation: $[\alpha]_D^{25}$ = +55° (c = 0.43, H_2O) (lit +42.3°, [49]).

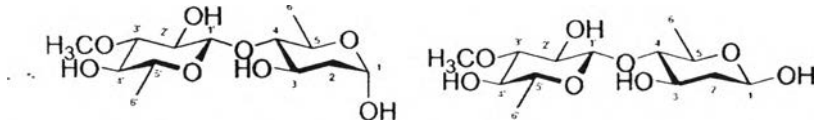
^{1}H NMR (400 MHz, D_2O) for α-D-thevetose: δ 5.17 (1H, br d, *J* = 3.8 Hz, H-1), 3.92 (1H, dq, *J* = 9.8, 6.2 Hz, H-5), 3.63 (3H, s, 3-OCH_3), 3.61 (1H, dd, *J* = 9.5, 3.8 Hz, H-2), 3.44 (1H, t, *J* = 9.5 Hz, H-3), 3.28 – 3.18 (1H, m, H-4) and 1.26 (3H, d, *J* = 6.2 Hz, CH_3-6).

^{1}H NMR (400 MHz, D_2O) for β-D-thevetose: δ 4.65 (1H, d, *J* = 7.8 Hz, H-1), 3.63 (3H, s, 3-OCH_3), 3.51 (1H, dq, *J* = 9.1, 6.2 Hz, H-5), 3.32 (1H, dt, *J* = 11.7, 7.7 Hz, H-2), .28 –3.18 (2H, m, H-3, H-4) and 1.29 (d, *J* = 6.2 Hz, CH_3-6).

^{13}C NMR (100 MHz, D_2O) for α-D-thevetose: δ 92.0 (C-1), 82.5 (C-3), 74.7 (C-4), 71.3 (C-2), 67.6 (C-5), 59.9 (3-OCH_3) and 16.7 (C-6).

^{13}C NMR (100 MHz, D_2O) for β-D-thevetose: δ 95.8 (C-1), 85.1 (C-3), 74.3 (C-4), 73.8 (C-2), 71.9 (C-5), 59.6 (3-OCH_3) and 16.7 (C-6).

3.6.1.6 β-D-thevetopyranosyl-(1→4)-O-D-canaropyranoside (73)



The ratio of β-D-thv-(1→4)-*O*-α-D-can and β-D-thv-(1→4)-*O*-β-D-can was considered to be ca. 2:3 based on integrals of the anomeric signals.

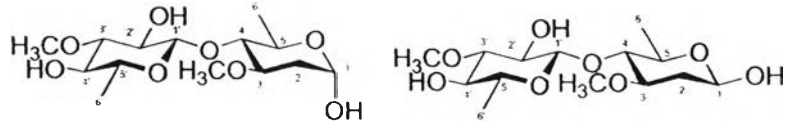
^{1}H NMR (400 MHz, D_2O for β-D-thevetopyranosyl-(1->4)-*O*-α-D-canaropyranoside: δ 5.34 (1H, br d, *J* = 2.9 Hz, H-1), 4.52 (dd, *J* = 7.9, 1.4 Hz, H-1'), 4.05 – 3.90 (2H, m, H-3, H-5), 3.62 (6H, s, 3'-OCH_3), 3.60 – 3.49 (1H, m, H-5'), 3.45 – 3.35 (1H, m, H-2'), 3.31 – 3.20 (3H, m, H-4, H-3', H-4'), 2.18 (1H, dd, *J* = 13.3, 5.3 Hz, H-2b), 1.71 (1H, br ddd, *J* = 13.3 12.0, 3.6 Hz, H-2a), 1.33 (3H, d, *J* = 6.3 Hz, CH_3-6) and 1.30 (6H, d, *J* = 6.1 Hz, CH_3-6').

^{1}H NMR (400 MHz, D_2O for β-D-thevetopyranosyl-(1->4)-*O*-β-D-canaropyranoside: δ 4.93 (dd, *J* = 9.8, 1.5 Hz, H-1), 4.52 (dd, *J* = 7.9, 1.4 Hz, H-1'), 3.75 (1H, ddd, *J* = 12.0, 8.8, 5.2 Hz, H-3), 3.62 (3H, s, 3'-OCH_3), 3.60 – 3.49 (2H, m, H-5, H-5'), 3.45 – 3.35 (1H, m, H-2'), 3.31 – 3.20 (3H, m, H-4, H-3', H-4'), 2.30 (1H, dd, *J* = 12.4, 5.0, 1.5 Hz, H-2b), 1.51 (1H, td, *J* = 12.0, 10.4 Hz, H-2a), 1.36 (3H, d, *J* = 6.3 Hz, CH_3-6) and 1.30 (6H, d, *J* = 6.1 Hz, CH_3-6').

^{13}C NMR (100 MHz, D_2O) for β-D-thevetopyranosyl-(1->4)-*O*-α-D-canaropyranoside: δ 102.7 (C-1'), 90.8 (C-1), 86.5 (C-4), 84.9 (C-3'), 74.1 (C-4'), 73.0 (C-2'), 71.8 (C-5'), 66.5 (2, C-3, C-5), 59.8 (3'-OCH_3), 36.7 (C-2), 16.8 (C-6) and 16.6 (C-6').

^{13}C NMR (100 MHz, D_2O) for β-D-thevetopyranosyl-(1->4)-*O*-β-D-canaropyranoside: δ 102.8 (C-1'), 93.0 (C-1), 85.9 (C-4), 84.9 (C-3'), 74.1 (C-4'), 73.0 (C-2'), 71.8 (C-5'), 70.7 (C-5), 69.0 (C-3), 59.9 (3'-OCH_3), 38.9 (C-2), 16.8 (C-6) and 16.6 (C-6').

3.6.1.7 β-D-thevetopyranosyl-(1→4)-O-D-oleandropyranoside (74)



The ratio of β-D-thv-(1→4)-*O*-α-D-ole and β-D-thv-(1→4)-*O*-β-D-ole was considered to be ca. 4:5 based on integrals of the anomeric signals.

^{1}H NMR (400 MHz, D_2O) for β-D-thevetopyranosyl-(1->4)-*O*-α-D-oleandopyranoside: δ 5.34 (1H, br dd, J = 2.8, 1.8 Hz, H-1), 4.54 1H, d, J = 7.9 Hz, H-1'), 3.99 (1H, dq, J = 9.3, 6.3 Hz, H-5), 3.69 (1H, ddd, J = 11.1, 8.5, 4.8 Hz, H-3), 3.61 (3H, s, 3'-OCH_3), 3.49 – 3.41 (1H, m, 3H, H-5'), 3.38 (3H, s, 3-OCH_3), 3.37 – 3.32 (2H, m, H-4, H-2'), 3.26 – 3.20 (2H, m, H-3', H-4'), 2.34 (1H, ddd, J = 13.7, 4.8, 1.8 Hz, H-2b), 1.64 (1H, ddd, J = 13.7, 11.1, 3.8 Hz, H-2a), 1.34 (3H, d, J = 6.3 Hz, CH_3-6) and 1.30 (3H, d, J = 6.1 Hz, CH_3-6').

^{1}H NMR (400 MHz, D_2O) for β-D-thevetopyranosyl-(1->4)-*O*-β-D-oleandopyranoside: δ 4.89 (1H, dd, J = 9.9, 1.8 Hz, H-1), 4.52 (1H, d, J = 7.9 Hz, H-1'), 3.61 (3H, s, 3'-OCH_3), 3.58 – 3.49 (m, 2H, H-3, H-5), 3.49 – 3.41 (1H, m, H-5'), 3.39 (3H, s, 3-OCH_3), 3.37 – 3.32 (1H, m, H-2'), 3.30 (1H, t, J = 9.2 Hz, H-4), 3.26 – 3.20 (2H, m, H-3', H-4'), 2.51 (1H, ddd, J = 12.4, 5.0, 1.8 Hz, H-2b), 1.39 (1H, td, J = 12.0, 9.9 Hz, H-2a), 1.37 (3H, d, J = 6.2 Hz, CH_3-6) and 1.30 (3H, d, J = 6.1 Hz, CH_3-6').

^{13}C NMR (100 MHz, D_2O) for β-D-thevetopyranosyl-(1->4)-*O*-α-D-oleandopyranoside: δ 102.8 (C-1'), 90.7 (C-1), 85.1 (C-3'), 83.2 (C-4), 76.0 (C-3), 74.3 (C-4'), 73.3 (C-2'), 71.7 (C-5'), 67.1 (C-5), 59.7 (3'-OCH_3), 55.7 (3-OCH_3), 33.8 (C-2), 17.2 (C-6) and 16.7 (C-6').

^{13}C NMR (100 MHz, D_2O) for β-D-thevetopyranosyl-(1->4)-*O*-β-D-oleandopyranoside: δ 102.9 (C-1'), 93.1 (C-1), 85.1 (C-3'), 82.6 (C-4), 78.1 (C-3), 74.3 (C-

4'), 73.3 (C-2'), 71.7 (C-5'), 71.1 (C-5), 59.8 (3'-OCH_3), 55.5 (3-OCH_3), 36.2 (C-2), 17.2 (C-6) and 16.7 (C-6').

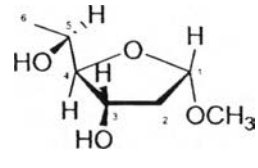
3.6.2 Acid hydrolysis of 61, 64 and 67

Pure compounds **61** (20 mg), **64** (10 mg) and **67** (10 mg) were hydrolyzed according to the same procedure as the crude steroidal glycoside. The products from all reactions in the CH_2Cl_2 layer were analyzed by ^{1}H NMR spectroscopy. The aglycones **61a** and **67a** were found as the products from the hydrolysis of **61**, **64** and **67**.

3.6.3 Methanolysis of crude steroidal glycoside from *G. griffithii*

To a solution of crude steroidal glycoside (500 mg) in MeOH (50 mL), 0.05 M H_2SO_4 (50 mL) was added. The solution was heated at 50 °C for 1 h and then neutralized with saturated $Ba(OH)_2$ in H_2O. After the precipitate was filtered off, the filtrate was partitioned with CH_2Cl_2. The aqueous layer was concentrated and separated by silica gel CC eluting with CH_2Cl_2–MeOH (20:1 and 10:1, v/v) and then further fractionated by RP-18 CC eluting with a stepwise of MeOH–H_2O (2:8, 3:7 and 4:6, v/v) to give two monosaccharides, *O*-methoxy-α-D-digitoxofuranose (**75**) (5 mg), *O*-methoxy-β-D-digitoxopyranose (**76**) (21 mg), and four disaccharides, β-D-thevetopyranosyl-(1→4)-*O*-methoxy-α-D-canaropyranoside (**77**) (37 mg), β-D-thevetopyranosyl-(1→4)-*O*-methoxy-β-D-canaropyranoside (**78**) (12 mg), β-D-thevetopyranosyl-(1→4)-*O*-methoxy-α-D-oleandropyranoside (**79**) (18 mg) and β-D-thevetopyranosyl-(1→4)-*O*-methoxy-β-D-oleandropyranoside (**80**) (8 mg).

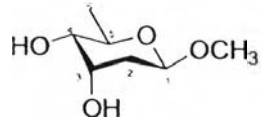
3.6.3.1 O-methoxy-α-D-digitoxofuranose (75)



^{1}H NMR (400 MHz, D_2O): δ 5.23 (1H, dd, *J* = 4.8, 3.7 Hz, H-1), 4.50 (1H, td, *J* = 6.0, 3.2 Hz, H-3), 3.83 – 3.73 (2H, m, H-4, H-5), 3.40 (3H, s, 1-OCH_3), 2.22 – 2.18 (2H, m, CH_2-2) and 1.25 (3H, d, *J* = 6.0 Hz, CH_3-6).

^{13}C NMR (100 MHz, D_2O): δ 105.4 (C-1), 89.7 (C-4), 71.3 (C-3), 67.8 (C-5), 55.2 (1-OCH_3), 40.3 (C-2) and 18.2 (C-6).

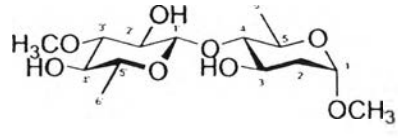
3.6.3.2 O-methoxy-β-D-digitoxopyranse (76)



^{1}H NMR (400 MHz, D_2O): δ 4.84 (1H, dd, *J* = 9.7, 1.6 Hz, H-1), 4.18 (1H, dd, *J* = 6.2, 3.0 Hz, H-3), 3.90 (1H, dq, *J* = 9.4, 6.3 Hz, H-5), 3.53 (3H, s, 1-OCH_3), 3.39 (1H, dd, *J* = 9.4, 3.0 Hz, H-4), 2.11 (1H, br dt, *J* = 13.9, 3.1 Hz, H-2b), 1.74 (1H, ddd, *J* = 13.9, 9.7, 2.8, Hz, H-2a) and 1.32 (3H, d, *J* = 6.3 Hz, CH_3-6).

^{13}C NMR (100 MHz, D_2O): δ 99.2 (C-1), 72.5 (C-4), 69.7 (C-5), 67.2 (C-3), 56.4 (1-OCH_3), 37.0 (C-2) and 17.2 (C-6).

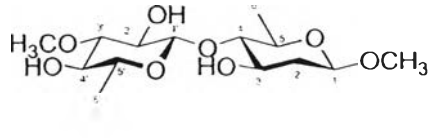
3.6.3.3 β-D-thevetopyranosyl-(1→4)-O-methoxy-α-D-canaropyranoside (77)



^{1}H NMR (400 MHz, D_2O): δ 4.88 (1H, br d, *J* = 3.1 Hz, H-1), 4.53 (1H, d, *J* = 7.9 Hz, H-1'), 3.89 (1H, ddd, *J* = 11.9, 8.9, 5.3 Hz, H-3), 3.81 (1H, dq, *J* = 9.7, 6.3 Hz, H-5), 3.64 (3H, s, 3'-OCH_3), 3.54 (1H, qd, *J* = 8.9, 6.2 Hz, H-5'), 3.42 (1H, br t, *J* = 8.9 Hz, H-2'), 3.37 (3H, s, 1-OCH_3), 3.33 – 3.22 (3H, m, H-4, H-3', H-4'), 2.21 (1H, dd, *J* = 13.6, 5.5 Hz, H-2b), 1.74 (1H, ddd, *J* = 13.6, 11.9, 3.7 Hz, H-2a), 1.38 (3H, d, *J* = 6.3 Hz, CH_3-6) and 1.31 (1H, d, *J* = 6.2 Hz, CH_3-6').

^{13}C NMR (100 MHz, D_2O): δ 102.8 (C-1'), 97.9 (C-1), 86.4 (C-4), 85.0 (C-3'), 74.2 (C-4'), 73.0 (C-3'), 71.9 (C-5'), 66.8 (C-3), 66.6 (C-5), 59.9 (3'-OCH_3), 54.5 (1-OCH_3), 36.0 (C-2) and 16.6 (2, C-6, C-6').

3.6.3.4 β-D-thevetopyranosyl-(1→4)-O-methoxy-β-D-canaropyranoside (78)

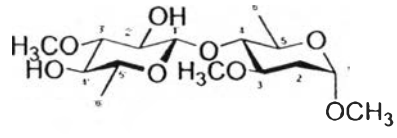


^{1}H NMR (400 MHz, D_2O): δ 4.64 (1H, dd, *J* = 9.8, 1.9 Hz, H-1), 4.54 (1H, d, *J* = 7.9 Hz, H-1'), 3.77 (1H, ddd, *J* = 11.8, 8.8, 5.2 Hz, H-3), 3.64 (3H, s, 3'-OCH_3), 3.58 (1H,

dq, *J* = 9.4, 6.3 Hz, H-5), 356 – 3.51 (1H, m, H-5') 3.53 (3H, s, 1-OCH_3), 3.41 (1H, br t, *J* = 9.2 Hz, H-2'), 3.33 – 3.23 (3H, m, H-4, H-3', H-4'), 2.32 (1H, ddd, *J* = 12.4, 5.2, 1.8 Hz, H-2b), 1.50 (1H, td, *J* = 12.1, 9.9 Hz, H-2a), 1.40 (3H, d, *J* = 6.2 Hz, CH_3-6) and 1.32 (d, *J* = 6.2 Hz, CH_3-6').

^{13}C NMR (100 MHz, D_2O): δ 102.8 (C-1'), 100.5 (C-1), 86.1 (C-4), 85.0 (C-3'), 74.2 (C-4'), 73.0 (C-3'), 71.9 (C-5'), 70.8 (C-5), 69.0 (C-3), 59.9 (3'-OCH_3), 56.6 (1-OCH_3), 37.6 (C-2), 16.8 (C-6) and 16.6 (C-6').

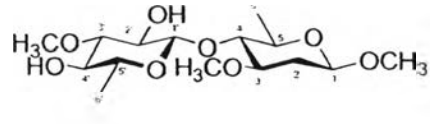
3.6.3.5 β-D-thevetopyranosyl-(1→4)-O-methoxy-α-D-oleandropyranoside (79)



^{1}H NMR (400 MHz, D_2O): 4.90 (1H, br d, *J* = 2.4 Hz, H-1), 4.54 (1H, d, *J* = 7.9 Hz, H-1'), 3.81 (1H, dq, *J* = 9.5, 6.2 Hz, H-5), 3.63 (3H, s, 3'-OCH_3), 3.66 – 3.60 (1H, m, H-3), 3.46 (1H, dq, *J* = 9.3, 6.2 Hz, H-5'), 3.38 (3H, s, 3-OCH_3), 3.37 (3H, s, 1-OCH_3), 3.42 – 3.34 (2H, m, H-4, H-2'), 3.29 – 3.20 (2H, m, H-3', H-4'), 2.38 (1H, ddd, *J* = 13.6, 5.0, 1.3 Hz, H-2b), 1.65 (1H, ddd, J = 13.6, 11.4, 3.8 Hz, H-2a), 1.39 (3H, d, *J* = 6.2 Hz, CH_3-6) and 1.31 (3H, d, *J* = 6.2 Hz, CH_3-6').

^{13}C NMR (100 MHz, D_2O): δ 102.9 (C-1'), 98.0 (C-1), 85.2 (C-3'), 83.1 (C-4), 76.2 (C-3), 74.3 (C-4'), 73.3 (C-2'), 71.7 (C-5'), 67.2 (C-5), 59.8 (3'-OCH_3), 55.8 (3-OCH_3), 54.5 (1-OCH_3), 33.3 (C-2), 17.1 (C-6) and 16.8 (C-6').

3.6.3.6 β-D-thevetopyranosyl-(1→4)-O-methoxy-β-D-oleandropyranoside (80)

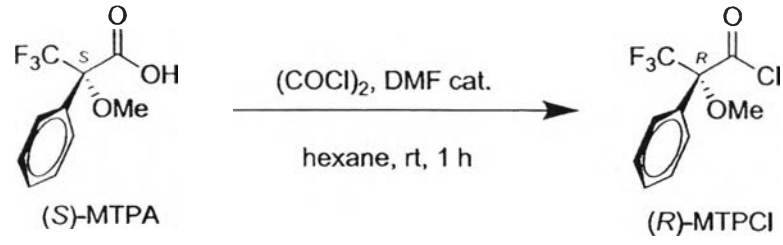


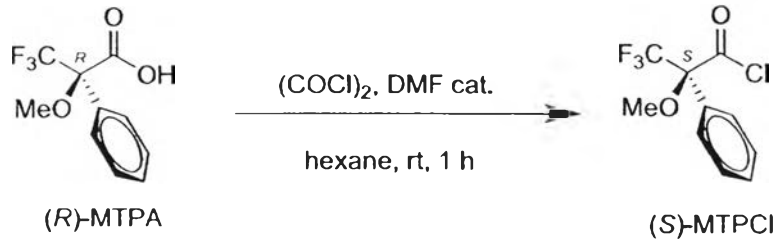
^{1}H NMR (400 MHz, D_2O): δ 4.61 (1H, dd, *J* = 9.7, 1.7 Hz, H-1), 4.54 (1H, d, *J* = 7.9 Hz, H-1'), 3.63 (3H, s, 3-OCH_3), 3.59 – 3.50 (2H, m, H-3, H-5), 3.52 (3H, s, 1-OCH_3), 3.46 (1H, dq, *J* = 9.2, 6.3 Hz, H-5'), 3.40 (3H, s, 3-OCH_3), 3.39 – 3.33 (1H, m, H-2'), 3.32 (1H, t, *J* = 9.1 Hz, H-4), 3.28 – 3.20 (2H, m, H-3', H-4'), 2.52 (1H, ddd, *J* = 12.3, 5.0, 1.7 Hz, H-2b), 1.41 (d, J = 6.2 Hz, 3H), 1.41 – 1.33 (1H, m, H-2a) and 1.31 (d, J = 6.2 Hz, 3H).

^{13}C NMR (100 MHz, D_2O): δ 102.9 (C-1'), 100.5 (C-1), 85.2 (C-3'), 83.0 (C-4), 78.1 (C-3), 74.4 (C-4'), 73.4 (C-2'), 71.7 (C-5'), 71.2 (C-5), 59.8 (3'-OCH_3), 56.6 (1-OCH_3), 55.7 (3-OCH_3), 34.9 (C-2), 17.2 (C-6) and 16.7 (C-6').

3.7 Preparation of the (*R*)- or (*S*)-Mosher acid ester of steroid 1a

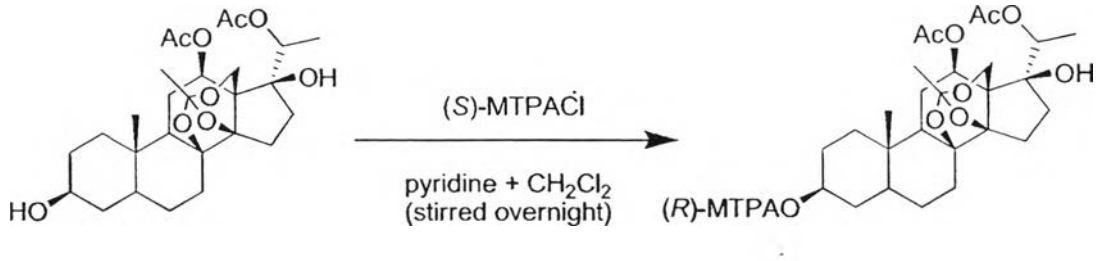
3.7.1 Preparation of the (*R*)- or (*S*)-MTPACl from (*R*)- or (*S*)-MTPA



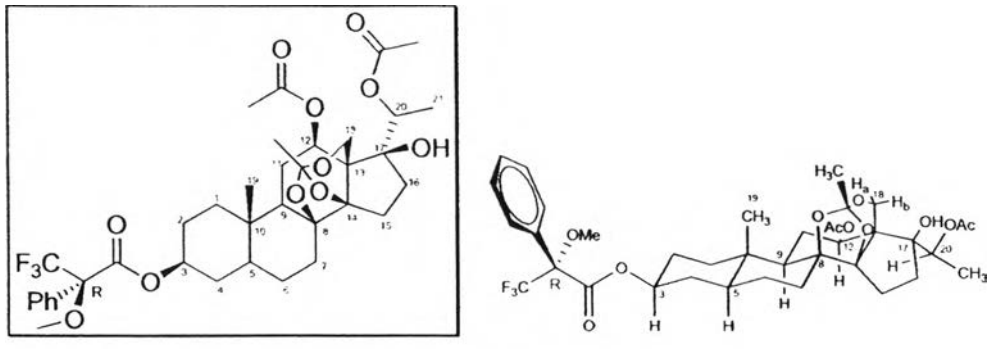


Oxalyl chloride (0.5 mL, excess) was added to a solution of (*R*)-MTPA (30 mg, 0.12 mmol) in hexane (5 mL) at room temperature. Then DMF (2 μL, 0.026 mmol) was added to the solution, a white precipitate formed immediately (DMFCl). After 1 h the mixture was filtered in order to remove DMFCl and concentrated under vacuum using water aspirator to remove excess oxalyl chloride to provide (*S*)-MTPACl (~30 mg, 0.12 mmol) [51]. (*R*)-MTPACl was prepared from (S)-MTPA by the same procedure of (*S*)-MTPACl.

3.7.2 (*R*)-MTPA ester derivative of Steroid 61a (61a$_R$)



To a solution of (*S*)-MTPACl (30 mg, 0.12 mmol) in dry CH_2Cl_2 (1 mL), compound **61a** (10 mg, 0.02 mmol) and pyridine (1 mL) were added, and the solution was allowed to stand overnight at room temperature. After the addition of 1 M HCl into the reaction mixture, the mixture was extracted with CH_2Cl_2 and washed with water. The CH_2Cl_2 layer was evaporated and then subjected to silica gel TLC using CH_2Cl_2–MeOH (50:1, v/v) as mobile phase to yield the (*R*)-Mosher's ester, **61a**$_R$ (3 mg, 0.004 mmol).



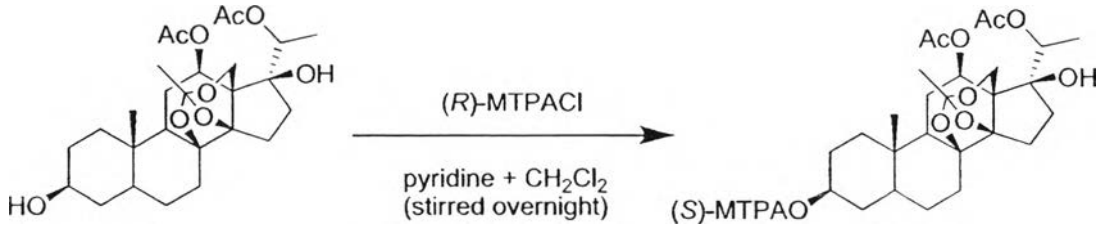
Optical Rotation: $[\alpha]_D^{25}$ = +22° (c = 0.1, MeOH).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55–7.48 (2H, m, Ph-H), 7.43–7.37 (3H, m, Ph-H), 4.96 (1H, m, H-3), 4.60 (1H, dd, *J* = 10.8, 4.6 Hz, H-12), 4.56 (1H, d, *J* = 12.0 Hz, H-18b), 4.46 (1H, q, *J* = 6.2 Hz, H-20), 4.26 (1H, s, OH-17), 4.15 (1H, d, *J* = 12.0 Hz, H-18a), 3.55 (3H, s, OCH_3), 2.07 (3H, s, 20-OAc), 1.95 (3H, s, 12-OAc), 1.56 (3H, s, CH_3-ortho), 1.30 (3H, d, *J* = 6.2 Hz, CH_3-21) and 0.98 (3H, s, CH_3-19).

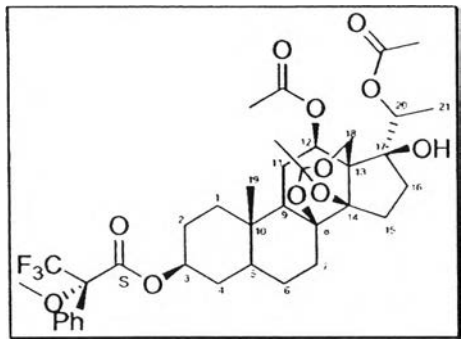
^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.4, 166.1, 132.6, 129.7, 128.5 (2), 127.5 (2), 123.5 (d, J_{CF} = 288.5 Hz), 117.2, 94.3, 86.2, 84.9, 82.1, 75.8, 74.4, 73.0, 60.2, 55.5, 51.2, 46.6, 45.2, 37.0, 36.6, 33.6, 32.9, 32.1, 26.8, 25.9, 24.4, 24.3, 23.7, 21.7, 21.6, 15.0 and 12.5.

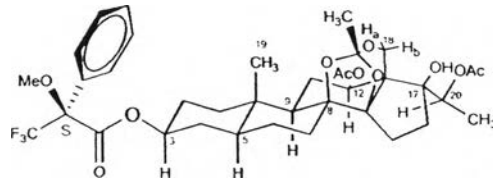
HRESIMS: *m/z* 747.2955 $[M+Na]^+$ (747.2968 calcd for $C_{37}H_{47}F_3NaO_{11}$).

3.7.3 (*S*)-MTPA ester derivative of Steroid 61a (61a$_S$)



Compound **61a** (10 mg, 0.02 mmol) was treated with (*R*)-MTPACl (30 mg, 0.12 mmol) as the same procedure of 3.7.2 to yield the (*S*)-Mosher's ester, **61a**$_S$ (4 mg, 0.005 mmol).





Optical Rotation: $[\alpha]_D^{25}$ = -8° (c = 0.1, MeOH).

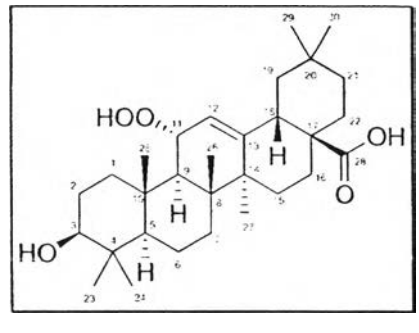
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 – 7.47 (2H, m, Ph-H), 7.42 – 7.38 (3H, m, Ph-H), 4.96 (1H, m, H-3), 4.60 (1H, dd, *J* = 10.9, 4.6 Hz, H-12), 4.56 (1H, d, *J* = 12.1 Hz, H-18b), 4.46 (1H, q, *J* = 6.0 Hz, H-20), 4.25 (1H, s, OH-17), 4.16 (1H, d, *J* = 12.1 Hz, H-18a), 3.55 (3H, s, OCH_3), 2.07 (3H, s, 20-OAc), 1.95 (3H, s, 12-OAc), 1.57 (3H, s, CH_3-ortho), 1.31 (3H, d, *J* = 6.0 Hz, CH_3-21) and 0.98 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.4, 166.1, 132.6, 129.7, 128.5 (2), 127.5 (2), 123.5 (d, J_{CF} = 288.3 Hz), 117.2, 94.3, 86.3, 84.8, 82.1, 75.8, 74.4, 72.9, 60.2, 55.5, 51.2, 46.6, 45.3, 36.9, 36.6, 33.6, 33.2, 32.1, 26.5, 25.9, 24.4, 24.3, 23.7, 21.7, 21.6, 15.0 and 12.5.

HRESIMS: *m/z* 747.2942 $[M+Na]^+$ (747.2968 calcd for $C_{37}H_{47}F_3NaO_{11}$).

3.8 Physical and spectral data of the isolated compounds from *H. curtisii*

3.8.1 3β-hydroxy-11α-hydroperoxyolean-12-en-28-oic acid (81)



Obtained as white solid.

mp. 184-186 °C.

Optical Rotation: $[\alpha]_D^{25}$ = +11° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 203 and 250 nm.

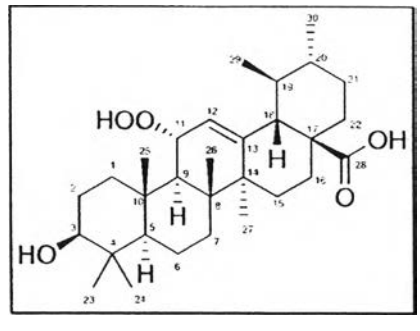
IR (ATR): ν_{max} 3385 br, 2938, 1687, 1397 and 1041 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 10:1): δ 5.52 (1H, d, *J* = 3.5 Hz, H-12), 4.38 (1H, dd, *J* = 8.5, 3.6 Hz, H-11), 3.14 (1H, t, *J* = 8.1 Hz, H-3), 2.83 (1H, dd, *J* = 14.3, 3.8 Hz, H-18), 1.15 (3H, s, CH_3-27), 0.94 (3H, s, CH_3-25), 0.91 (3H, s, CH_3-24), 0.88 (3H, s, CH_3-29), 0.84 (3H, s, CH_3-30), 0.73 (3H, s, CH_3-26) and 0.71 (3H, s, CH_3-23).

^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 10:1): δ 181.0, 150.7, 121.8, 81.2, 78.7, 55.2, 50.7, 46.1, 45.6, 42.8, 41.9, 40.9, 39.3, 39.0, 38.0, 33.9, 33.0 (2), 32.5, 30.7, 28.1, 28.0, 27.0, 24.7, 23.5, 22.9, 18.6, 18.4, 16.8 and 15.6.

HRESIMS: *m/z* 511.3377 $[M+Na]^+$ (511.3399 calcd for $C_{30}H_{48}NaO_5$) and 455.3512 $[M-OOH]^+$ (455.3525 calcd for $C_{30}H_{47}O_3$).

3.8.2 3β-hydroxy-11α-hydroperoxyursan-12-en-28-oic acid (82)



Obtained as white solid.

mp. 198-200 °C.

Optical Rotation: $[\alpha]_D^{25}$ = +8° (c = 0.1, MeOH).

UV (MeOH): λ_{max} 205 and 252 nm.

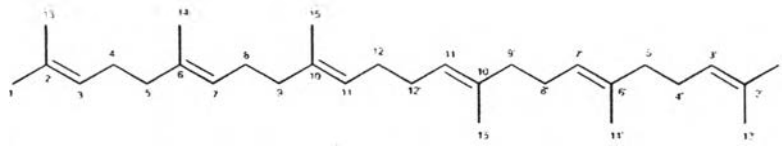
IR (ATR): ν_{max} 3366 br, 2926, 1686, 1457, 1388 and 1040 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 10:1): δ 5.42 (1H, br s, H-12), 4.36 (1H, d, *J* = 8.1 Hz, H-11), 3.09 (1H, t, *J* = 7.7 Hz, H-3), 2.16 (1H, d, *J* = 11.2 Hz, H-18), 1.05 (3H, s, CH_3-27), 0.93 (3H, s, CH_3-25), 0.88 (3H, d, *J* = 6.1 Hz, CH_3-29), 0.87 (3H, s, CH_3-24), 0.85 (3H, d, *J* = 5.9 Hz, CH_3-30), 0.74 (3H, s, CH_3-26), 0.67 (3H, s, CH_3-23).

^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 10:1): δ 180.8, 143.8, 125.6, 81.4, 78.5, 55.2, 52.3, 49.9, 47.4, 42.6, 42.1, 39.5, 38.9 (2), 38.8, 37.8, 36.7, 33.5, 30.5, 28.2, 28.1, 27.0, 24.1, 22.4, 21.0, 18.4, 18.3, 16.9, 16.5 and 15.5.

HRESIMS: *m/z* 511.3391 $[M+Na]^+$ (511.3399 calcd for $C_{30}H_{48}NaO_5$) and 455.3463 $[M-OOH]^+$ (455.3525 calcd for $C_{30}H_{47}O_3$).

3.8.3 Squalene (83)

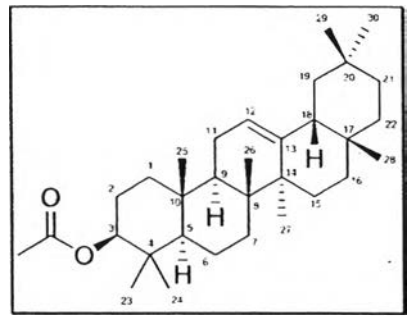


Obtained as yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.21 – 5.04 (6H, m, H-3, H-7, H-11, H-3', H-7', H-11'), 2.13 – 2.04 (8H, m, CH_2-4, CH_2-8, CH_2-4', CH_2-8'), 2.04 – 1.94 (12, m, CH_2-5, CH_2-9, CH_2-12, CH_2-5', CH_2-9', CH_2-12') 1.68 (6H, s, CH_3-1, CH_3-1') and 1.61 (18H, s, CH_3-13, CH_3-14, CH_3-15, CH_3-13', CH_3-14', CH_3-15').

^{13}C NMR (100 MHz, $CDCl_3$): δ 135.3 (2), 135.0 (2), 131.4 (2), 124.6 (2), 124.5 (2), 124.4 (2), 39.9 (4), 28.4 (2), 26.9 (2), 26.8 (2), 25.8 (2), 17.8 (2), 16.2 (2) and 16.1 (2).

3.8.4 β-Amyrin acetate (84)



Obtained as white pale solid.

mp. 214-216 °C (lit. 244-245 °C, [52]).

Optical Rotation: $[\alpha]_D^{25}$ = +83° (c = 0.1, $CHCl_3$) (lit. +81.4°, [52]).

UV ($CHCl_3$): λ_{max} 242 nm.

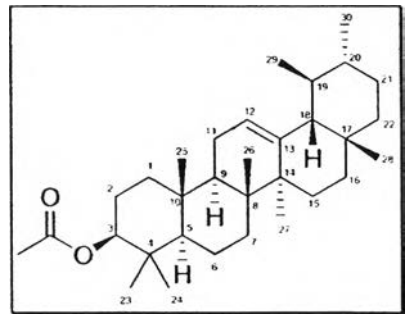
IR (ATR): ν_{max} 2913, 1731, 1460, 1364, 1247 and 1023 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.18 (1H, br s, H-12), 4.50 (1H, t, J = 7.5 Hz, H-3), 2.05 (3H, s, 3-OAc), 1.13 (3H, s, CH_3-27), 0.96 (6H, s, CH_3-26, CH_3-25), 0.87 (12H, s, CH_3-23, CH_3-24, CH_3-29, CH_3-30) and 0.83 (3H, s, CH_3-28).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 145.4, 121.8, 81.1, 55.4, 47.7, 47.4, 46.9, 41.9, 40.0, 38.4, 37.9, 37.3, 37.0, 34.9, 33.5, 32.7, 32.6, 31.2, 28.5, 28.2, 27.1, 26.3, 26.1, 23.8, 23.7 (2), 21.5, 18.4, 17.0, 16.8 and 15.7.

HRESIMS: *m/z* 937.7913 $[2M+H]^+$ (937.8013 calcd for $C_{64}H_{105}O_4$) and 409.3767 $[M-OAc]^+$ (409.3834 calcd for $C_{30}H_{49}$).

3.8.5 α-Amyrin acetate (85)



Obtained as white pale solid.

mp. 210-212 °C (lit. 243 °C, [53]).

Optical Rotation: $[\alpha]_D^{25}$ = +94° (c = 0.1, $CHCl_3$) (lit. +76°, [53]).

UV ($CHCl_3$): λ_{max} 241 nm.

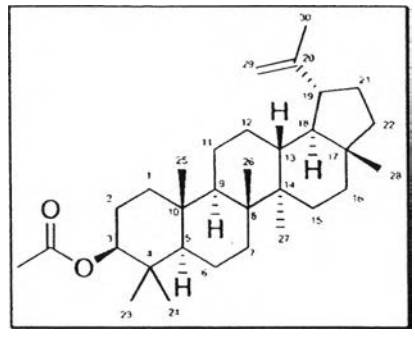
IR (ATR): ν_{max} 2906, 1733, 1447, 1367, 1242 and 1021 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.12 (1H, br s, H-12), 4.50 (1H, dd, J = 8.9, 7.1 Hz, H-3), 2.05 (3H, s, 3-OAc), 1.06 (3H, s, CH_3-27), 1.00 (3H, s, CH_3-26), 0.97 (3H, s, CH_3-25), 0.92 (3H, d, J = 5.0 Hz, CH_3-30), 0.87 (3H, s, CH_3-24), 0.86 (3H, s, CH_3-23) and 0.79 (6H, m, CH_3-28, CH_3-29).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 139.8, 124.5, 81.1, 59.3, 55.5, 47.8, 42.3, 41.7, 40.2, 39.8 (2), 38.7, 37.9, 37.0, 33.9, 33.1, 31.4, 28.9, 28.3, 28.2, 26.8, 23.8, 23.5, 23.4, 21.5, 21.4, 18.4, 17.7, 17.0, 16.9 and 15.9.

HRESIMS: *m/z* 937.7951 $[2M+H]^+$ (937.8013 calcd for $C_{64}H_{105}O_4$) and 409.3840 $[M-OAc]^+$ (409.3834 calcd for $C_{30}H_{49}$).

3.8.6 Lupeol acetate (86)



Obtained as white pale solid.

mp. 190-192 °C (lit. 210-212 °C, [54]).

Optical Rotation: $[\alpha]_D^{25}$ = +8° (c = 0.1, $CHCl_3$) (lit. +20°, [54]).

UV ($CHCl_3$): λ_{max} 242 nm.

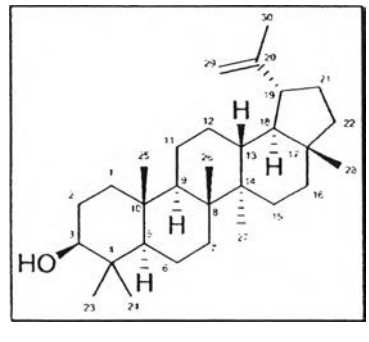
IR (ATR): ν_{max} 2940, 1732, 1637, 1444, 1365, 1247 and 1023 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.68 (1H, s, H-29a), 4.56 (1H, s, H-29b), 4.46 (1H, dd, *J* = 9.6, 6.6 Hz, H-3), 2.37 (1H, td, *J* = 10.9, 5.8 Hz, H-19), 2.04 (3H, s, 3-OAc), 1.68 (3H, s, CH_3-30), 1.02 (3H, s, CH_3-26), 0.93 (3H, s, CH_3-27), 0.85 (3H, s, CH_3-25,), 0.84 (3H, s, CH_3-24), 0.83 (3H, s, CH_3-23) and 0.78 (3H, s, CH_3-28).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 151.1, 109.5, 81.2, 55.6, 50.6, 48.5, 48.2, 43.2, 43.0, 41.1, 40.2, 38.6, 38.3, 38.0, 37.3, 35.8, 34.4, 30.0, 28.1, 27.6, 25.3, 23.9, 21.4, 21.1, 19.5, 18.4, 18.2, 16.6, 16.3, 16.2 and 14.7.

HRESIMS: *m/z* 937.8122 $[2M+H]^+$ (937.8013 calcd for $C_{64}H_{105}O_4$) and 409.3871 $[M-OAc]^+$ (409.3834 calcd for $C_{30}H_{49}$).

3.8.7 Lupeol (57)



Obtained as white crystalline solid.

mp. 192-194 °C (lit. 213 °C, [55]).

Optical Rotation: $[\alpha]_D^{25}$ = +16° (c = 0.1, $CHCl_3$) (lit. +25.7°, [55]).

UV ($CHCl_3$): λ_{max} 242 nm.

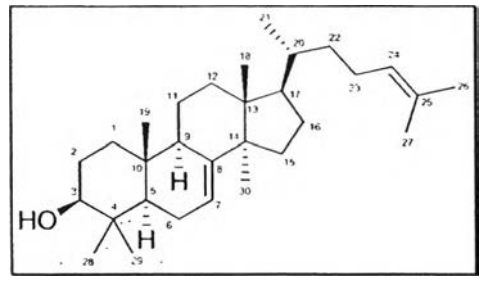
IR (ATR): ν_{max} 3329 br, 2942, 1639, 1452, 1379 and 1042 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.68 (1H, s, H-29a), 4.56 (1H, s, H-29b), 3.18 (1H, dd, *J* = 10.2, 5.0 Hz, H-3), 2.38 (1H, td, *J* = 11.2, 6.0 Hz, H-19), 1.68 (3H, s, CH_3-30), 1.02 (3H, s, CH_3-26), 0.96 (3H, s, CH_3-24), 0.94 (3H, s, CH_3-27), 0.82 (3H, s, CH_3-25), 0.78 (3H, s, CH_3-28) and 0.76 (3H, s, CH_3-23).

^{13}C NMR (100 MHz, $CDCl_3$): δ 151.1, 109.5, 79.2, 55.5, 50.6, 48.5, 48.2, 43.2, 43.0, 41.0, 40.2, 39.0, 38.9, 38.2, 37.3, 35.8, 34.5, 30.0, 28.2, 27.6 (2), 25.3, 21.1, 19.5, 18.5, 18.2, 16.3, 16.1, 15.5 and 14.7.

HRESIMS: *m/z* 853.7853 $[2M+H]^+$ (853.7802 calcd for $C_{60}H_{101}O_2$), 427.3784 $[M+H]^+$ (427.3940 calcd for $C_{30}H_{51}O$) and 409.3879 $[M-OH]^+$ (409.3834 calcd for $C_{30}H_{49}$).

3.8.8 Lanosta-7,24-dien-3β-ol (87)



Obtained as colorless solid.

mp. 104-106 °C.

Optical Rotation: $[\alpha]_D^{25}$ = -10° (c = 0.1, MeOH) (lit. -7.5°, [56]).

UV (MeOH): λ_{max} 205 nm.

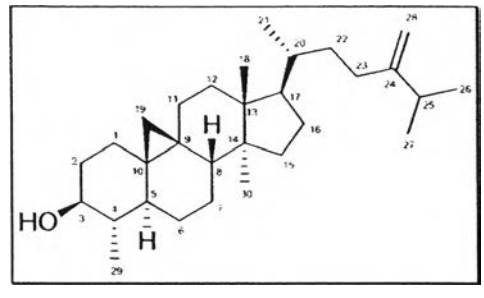
IR (ATR): ν_{max} 3391 br, 2927, 2850, 1455, 1374 and 1032 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.23 (1H, br s, H-7), 5.08 (1H, t, *J* = 7.0 Hz, H-24), 3.22 (1H, dd, *J* = 11.1, 4.2 Hz, H-3), 1.66 (3H, s, CH_3-26), 1.59 (3H, s, CH_3-27), 0.96 (3H, s, CH_3-30), 0.95 (3H, s, CH_3-29), 0.84 (3H, s, CH_3-28), 0.83 (3H, d, *J* = 5.9 Hz, CH_3-21), 0.79 (3H, s, CH_3-18) and 0.73 (3H, s, CH_3-19).

^{13}C NMR (100 MHz, $CDCl_3$): δ 145.9, 131.0, 125.2, 117.9, 79.3, 53.3, 51.3, 50.7, 48.9, 43.6, 39.0, 37.3, 35.9, 35.2, 35.0, 34.0, 33.9, 28.6, 27.7 (2), 27.4, 25.9, 25.4, 24.0, 22.2, 18.7, 18.2, 17.8, 14.9 and 13.2.

HRESIMS: *m/z* 427.3904 $[M+H]^+$ (427.3940 calcd for $C_{30}H_{51}O$) and 409.3830 $[M-OH]^+$ (409.3834 calcd for $C_{30}H_{49}$).

3.8.9 Cycloeucalenol (88)



Obtained as a white crystal needle.

mp. 116-118 °C (lit. 138-139 °C, [57]).

Optical Rotation: $[\alpha]_D^{25}$ = +52 ° (c = 0.1, MeOH) (lit. +45°, [57]).

UV (MeOH): λ_{max} 204 nm.

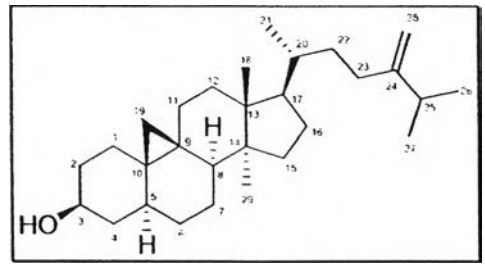
IR (ATR): ν_{max} 3295 br, 2934, 2869, 1701, 1636, 1454, 1373 and 1042 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.71 (1H, s, H-28a), 4.66 (1H, s, H-28b), 3.21 (1H, td, *J* = 10.5, 4.7 Hz, H-3), 2.23 (1H, sep, *J* = 6.5 Hz, H-25), 1.02 (6H, d, *J* = 6.8 Hz, CH_3-26, CH_3-27), 0.97 (3H, d, *J* = 7.2 Hz, CH_3-29), 0.96 (3H, s, CH_3-18), 0.89 (3H, d, *J* = 5.7 Hz, CH_3-21), 0.89 (3H, s, CH_3-30), 0.38 (1H, d, *J* = 3.8 Hz, H-19a) and 0.14 (1H, d, *J* = 3.8 Hz, H-19b).

^{13}C NMR (100 MHz, $CDCl_3$): δ 157.0, 106.0, 76.7, 52.3, 49.0, 47.0, 45.5, 44.7, 43.4, 36.3, 35.5, 35.1, 34.9, 33.9, 33.0, 31.4 30.9, 29.6 28.3, 27.4, 27.1, 25.3, 24.8, 23.7, 22.1, 22.0, 19.3, 18.5, 17.9 and 14.6.

HRESIMS: *m/z* 853.7860 $[2M+H]^+$ (853.7802 calcd for $C_{60}H_{101}O_2$) and 427.3924 $[M+H]^+$ (427.3940 calcd for $C_{30}H_{51}O$).

3.8.10 24-methylenepollinastanol (89)



Obtained as white pale solid.

mp. 94-96 °C (lit. 115-116 °C, [58]).

Optical Rotation: $[\alpha]_D^{25}$ = +28° (c = 0.1, MeOH) (lit. +47°, [58]).

UV (MeOH): λ_{max} 203 nm.

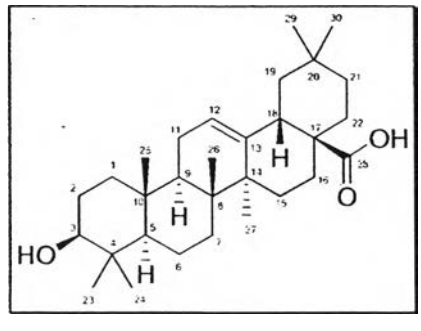
IR (ATR): ν_{max} 3370 br, 2920, 1707, 1456, 1370, 1050 and 1023 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.71 (1H, s, H-28a), 4.66 (1H, s, H-28b), 3.68 (1H, td, *J* = 10.6, 4.4 Hz, H-3), 2.23 (1H, sep, *J* = 6.8 Hz, H-25), 1.02 (6H, d, *J* = 6.8 Hz, CH_3-26, CH_3-27), 0.96 (3H, s, CH_3-18), 0.89 (3H, d, *J* = 5.0 Hz, CH_3-21), 0.88 (3H, s, CH_3-29), 0.42 (1H, d, *J* = 4.1 Hz, H-19a) and 0.06 (1H, d, *J* = 4.1 Hz, H-19b).

^{13}C NMR (100 MHz, $CDCl_3$): δ 157.0, 106.1, 71.3, 52.3, 49.2, 46.3, 45.6, 42.6, 37.3, 36.3, 35.4, 35.2 (2), 33.9, 33.0, 31.4, 30.7, 30.0, 28.2, 27.9, 27.2, 25.9, 24.8, 23.5, 22.1, 22.0, 19.1, 18.5 and 17.5.

HRESIMS: *m/z* 413.3752 $[M+H]^+$ (413.3783 calcd for $C_{29}H_{49}O$).

3.8.11 Oleanolic acid (90)



Obtained as white solid.

mp. 292-294 °C (lit. 306-308 °C, [59]).

Optical Rotation: $[\alpha]_D^{25}$ = +66° (c = 0.1, MeOH) (lit. +80°, [59]).

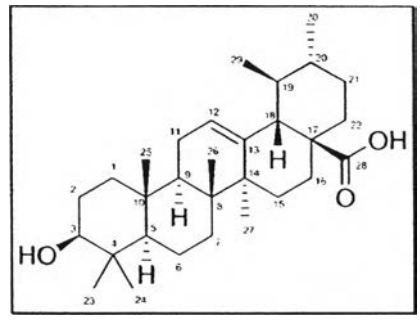
UV (MeOH): λ_{max} 206 nm.

IR (ATR): ν_{max} 3419 br, 2932, 2867, 1686, 1456, 1385 and 1027 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 10:1): δ 5.22 (1H, br s, H-12), 3.19 – 3.11 (1H, m, H-3), 2.77 (1H, d, *J* = 13.4 Hz, H-18), 1.08 (3H, s, CH_3-27), 0.93 (3H, s, CH_3-24), 0.87 (3H, s, CH_3-29), 0.85 (6H, s, CH_3-25, CH_3-30) and 0.72 (6H, s, CH_3-23, CH_3-26).

^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 10:1): δ 181.4, 143.9, 122.4, 79.0, 55.3, 47.7, 46.5, 46.0, 41.8, 41.2, 39.3, 38.8, 38.5, 37.1, 33.9, 33.1, 32.8, 32.6, 30.7, 28.1, 27.7, 26.9, 25.9, 23.6, 23.4, 23.1, 18.4, 16.9, 15.6 and 15.3.

3.8.12 Ursolic acid (91)



Obtained as white solid.

mp. 268-270 °C (lit. 281-282 °C, [60]).

Optical Rotation: $[\alpha]_D^{25}$ = +70° (c = 0.1, MeOH) (lit. +55.6°, [60]).

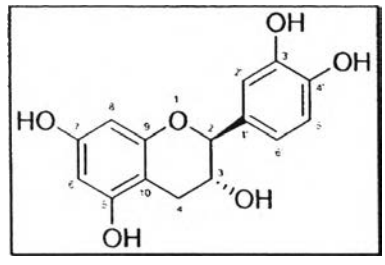
UV (MeOH): λ_{max} 205 nm.

IR (ATR): ν_{max} 3404 br, 2926, 1686, 1453 and 1029 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 10:1): δ 5.17 (1H, t, *J* = 3.1 Hz, H-12), 3.13 (1H, t, *J* = 7.9 Hz, H-3), 2.12 (1H, d, *J* = 11.2 Hz, H-18), 1.93 (1H, td, *J* = 13.2, 3.9 Hz, H-9), 1.02 (3H, s, CH_3-27), 0.91 (3H, s, CH_3-24), 0.87 (3H, d, *J* = 6.0 Hz, CH_3-30), 0.85 (3H, s, CH_3-25), 0.79 (3H, d, *J* = 6.4 Hz, CH_3-29), 0.74 (3H, s, CH_3-26) and 0.70 (3H, s, CH_3-23).

^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 10:1): δ 180.8, 138.2, 125.6, 78.9, 55.3, 52.8, 47.9, 47.6, 42.1, 39.5, 39.1, 38.9, 38.7 (2), 37.0, 36.8, 33.1, 30.7, 28.1 (2), 26.9, 24.2, 23.5, 23.3, 21.2, 18.3, 17.0, 16.9, 15.6 and 15.4.

3.8.13 (–)-Catechin (92)



Obtained as brown pale solid.

mp. 168-170 °C (lit. 172-174 °C, [61]).

Optical Rotation: $[\alpha]_D^{25}$ = -10° (c = 0.1, MeOH) (lit. -14.9°, [61]).

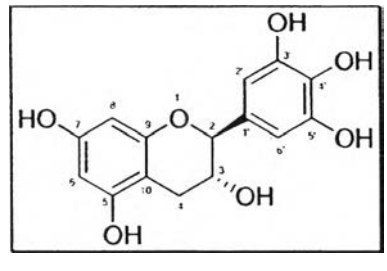
UV (MeOH): λ_{max} 214 and 279 nm.

IR (ATR): ν_{max} 3224 br, 1624, 1520, 1466, 1286, 1146 and 1028 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD): δ 6.84 (1H, s, H-2'), 6.77 (1H, d, *J* = 8.0 Hz, H-5'), 6.72 (1H, d, *J* = 8.0 Hz, H-6'), 5.94 (1H, s, H-8), 5.87 (1H, s, H-6), 4.57 (1H, d, *J* = 7.4 Hz, H-2), 3.98 (1H, br q, *J* = 6.4 Hz, H-3), 2.85 (1H, dd, *J* = 16.1, 5.0 Hz, H-4b) and 2.51 (1H, dd, *J* = 16.1, 8.0 Hz, H-4a).

^{13}C NMR (100 MHz, CD_3OD): δ 157.7, 157.5, 156.9, 146.2 (2), 132.2, 120.0, 116.1, 115.2, 100.8, 96.3, 95.5, 82.8, 68.8 and 28.4.

3.8.14 (–)-Gallocatechin (93)



Obtained as brown pale solid.

mp. 178-180 °C (lit. 185 °C, [61]).

Optical Rotation: $[\alpha]_D^{25}$ = -12° (c = 0.1, MeOH) (lit. -11.2°, [61]).

UV (MeOH): λ_{max} 210 and 272 nm.

IR (ATR): ν_{max} 3224 br, 1624, 1519, 1467, 1286, 1146 and 1028 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD): δ 6.51 (2H, s, H-2', H-6'), 6.06 (1H, s, H-8), 5.95 (1H, s, H-6), 4.66 (1H, d, *J* = 7.2 Hz, H-2), 4.14 (1H, br q, *J* = 6.5 Hz, H-3), 2.82 (1H, dd, *J* = 16.0, 4.5 Hz, H-4b), 2.51 (1H, dd, *J* = 16.0, 7.6 Hz, H-4a).

^{13}C NMR (100 MHz, CD_3OD): δ 156.3 (2), 155.8, 146.3 (2), 133.5, 131.1, 107.9 (2), 101.4, 96.8, 95.9, 81.9, 67.6 and 27.3.

3.9 MTT Cytotoxicity assay

The *in vitro* cytotoxicity was evaluated against five human tumor cell lines using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method by Institute of Biotechnology and Genetic Engineering, Chulalongkorn University [62]. All isolated compounds from *G. griffithii* were tested for their cytotoxicity against five human tumor cell lines, human breast ductal carcinoma ATCC No. HTB 20 (BT474), undifferentiated lung carcinoma (Chago), liver hepatoblastoma (Hep-G2), gastric carcinoma ATCC No. HTB 103 (KATO-3) and colon adenocarcinoma ATCC No. CCL 227 (SW-620).

Briefly, each of the five human cancer cell lines, 5 x 10^3 cells in 200 μL of RPMI 1640 medium containing 5% (v/v) fetal calf serum (FCS) were transferred to each well of a 96-well tissue culture plate, and incubated at 37°C in 5% (v/v) CO_2 for 24 h. Then, 2 μL of the serially diluted test compound in DMSO was transferred to each well of a 96-well microplate. The addition of 2 μL/well of DMSO alone was used as the control. Cells were then incubated as above for 72 h. The freshly prepared MTT reagent (10 μl) was added into each well and incubated for 4 h at 37°C in a 5% CO_2 incubator. After incubation, the culture medium supernatant was removed and then the formazan crystals was dissolved by aspiration in 150 μL of DMSO and 25 μL of 0.1 M glycine and the culture plate was shaken for 5 minutes. The cytotoxicity was determined by measuring the amount of purple formazan produced from yellow tetrazole at 540 nm by a microplate reader. Three replications of each trial were performed and doxorubicin was used a positive control.

3.10 α-glucosidase inhibition assay

Evaluation of the *in vitro* α-glucosidase inhibitory activity was performed according to the chromogenic method [63] in a 96-well microplate, using the yeast *Saccharomyces cerevisiae* (Sigma, G5003, Sigma-Aldrich Co. Ltd, USA.) as the working

enzyme and *p*-nitrophenyl-α-D-glucopyranoside (PNPG; Sigma, N1377, Sigma-Aldrich Co. Ltd., USA.) as the substrate. Briefly, 10 μL of the serially diluted test compound in DMSO was transferred to each well of a 96-well microplate. Then 30 μL of 0.1 M phosphate buffer (pH 6.8) and 10 μL α-glucosidase (1U/mL in 0.1 M phosphate buffer) were added and pre-incubated at 37 °C for 15 min. The enzyme reaction was initiated by adding 50 μL of PNPG (1 mM in 0.1 M phosphate buffer). After incubation at 37 °C for 20 min, 100 μL of 1 M Na_2CO_3 was added to stop the reaction. The α-glucosidase activity was determined by measuring the amount of released *p*-nitrophenol from the PNPG at 400 nm using a PowerWave XS2 (Biotek Instrument Inc, USA) microplate reader. The percent inhibition was calculated by $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, using 10 μL DMSO instead of sample, and A_1 is the absorbance of the test sample. Experiments were done in triplicate and acarbose was used as positive control.