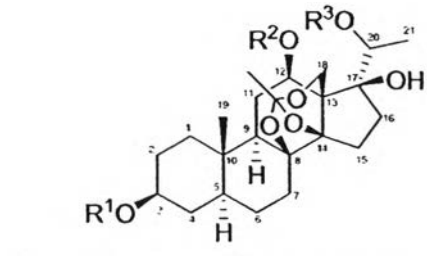
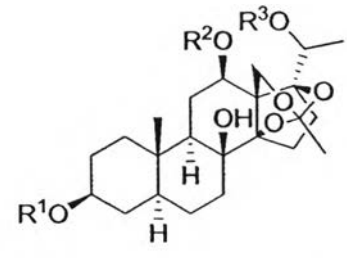
CHAPTER IV

RESULTS AND DISCUSSION

4.1 Isolation and structure elucidation of the isolated compounds from *G. griffithii*

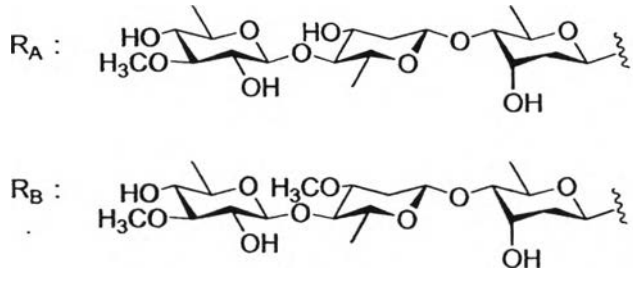
After extraction of the cut fresh pericarp *of G. griffithii* fruits with methanol, the residue was suspended in H_2O and successively partitioned with (CH_2Cl_2) and ethyl acetate (EtOAc). The CH_2Cl_2-soluble extract was fractionated by successive chromatographic techniques to give eight new steroidal glycosides, gymnemogriffithoside A–H (**61** – **68**) (Figure 4.1).



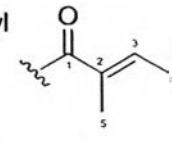


61 $R^1 = R_A$ R^2 = Ac R^3 = Ac
62 $R^1 = R_A$ R^2 = H R^3 = Ac
63 $R^1 = R_A$ R^2 = Ac R^3 = Tig
64 $R^1 = R_B$ R^2 = Ac R^3 = Ac
65 $R^1 = R_B$ R^2 = H R^3 = Ac
66 $R^1 = R_B$ R^2 = Ac R^3 = Tig
61a R^1 = H R^2 = Ac R^3 = Ac

67 $R^1 = R_A$ R^2 = Ac R^3 = Ac
68 $R^1 = R_B$ R^2 = Ac R^3 = Ac
67a R^1 = H R^2 = Ac R^3 = Ac



Tig : Tigloyl



Ac : Acetyl

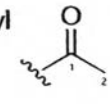


Figure 4.1 Isolated compounds from pericarp of *G. Griffithii* fruits.

Gymnemogriffithoside A (**61**) was obtained as white amorphous powder. The molecular formula was established as $C_{46}H_{72}O_{19}$, based on its high resolution electrospray ionization mass spectrometry (HRESIMS) data (*m/z* 951.4549 $[M+Na]^+$, calcd 951.4560), suggesting eleven degrees of unsaturation. The ATR-FTIR spectrum of **61** showed absorption bands for hydroxy (3,447 cm^{-1}) and carbonyl (1,731 cm^{-1})

groups. Analysis of the ^{13}C NMR and HSQC spectra revealed that **61** contained eight methyl carbons, one methoxy carbon, eleven sp^3 methylene carbons (one oxygenated carbon at δ_C 60.2), eighteen sp^3 methine carbons (three anomeric carbons at δ_C 95.6, 100.5 and 103.8 and thirteen oxygenated carbons at δ_C 77.0, 73.1, 74.4, 66.9, 83.0, 68.1, 69.5, 88.2, 70.7, 74.6, 85.5, 74.7 and 72.4), six sp^3 quaternary carbons (one orthoacetate carbon at δ_C 117.2 and three oxygenated carbons at δ_C 82.3, 94.4 and 86.3) and two carbonyls (Table 4.1). Analysis of the ^1H NMR spectrum indicated the presence of two acetyl signals at δ_H 1.94 (s) and 2.06 (s), two tertiary methyl signals at δ_H 0.96 (s) and 1.56 (s), four secondary methyl signals at δ_H 1.30 (d, J = 6.1 Hz, CH_3-21), 1.24 (d, J = 6.2 Hz, CH_3-6'), 1.34 (d, J = 6.1 Hz, CH_3-6") and 1.35 (d, J = 6.1 Hz, CH_3-6'"), a methoxy group at δ_H 3.66 (s), three anomeric proton signals at δ_H 4.93 (dd, J = 9.6 and 1.7 Hz, H-1'), 4.57 (dd, J = 9.7 and 1.8 Hz, H-1"), and 4.29 (d, J = 7.8 Hz, H-1'"), three oxygenated methine signals at δ_H 3.62 (m, H-3), 4.59 (dd, J = 10.7 and 4.5 Hz, H-12) and 4.45 (q, J = 6.3 Hz, H-20), two signals for CH_2-18 at δ_H 4.16 (d, J = 12.0 Hz, H-18a) and 4.54 (d, J = 12.0 Hz, H-18b) and five hydroxy signals at δ_H 4.28, 2.82, 4.32, 2.35 and 2.39. The spectroscopic data of the protons and carbons suggested that compound **61** was a steroidal glycoside. In total, 21 of the 46 carbons were assigned to the steroidal skeleton, while of the remainder four were assigned to two acylated moieties, two to one orthoacetate and nineteen to a trisaccharide moiety. By detailed analyses of the NMR spectroscopic data (HSQC, COSY, HMBC and NOESY) and comparison with the published data, the steroidal skeleton was deduced to be a dihydrosarcostin substituted with one orthoacetate and two acetyl groups [64].

HMBC correlations of the acetyl carbonyl signals at δ_C 170.8 and δ_C 170.4 with H-12 at δ_H 4.59 and with H-20 at δ_H 4.45, respectively, established the acylation substitution positions at C-12 and C-20. The HMBC correlations from the hydroxy group (δ_H 4.28) to the methine C-20 and quaternary C-17 indicated that this hydroxy group was located at C-17. The HMBC correlation from the methyl group (δ_H 0.98) to C-1, C-5, C-9 and C-10 indicated that this methyl group was located at C-10. The observed HMBC correlations between the resonance for the hydrogens of the methylene C-18 at δ_H 4.16 and 4.54 and those for the carbon signals of orthoacetate (δ_C 117.2), C-13 (δ_C 51.4), C-12 (δ_C 73.1), C-14 (δ_C 94.4) and C-17 (δ_C 86.3) suggested that the orthoacetate was substituted at C-8, C-14 and C-18. The three anomeric protons at δ_H 4.93, 4.57 and 4.29 and the three corresponding carbons at δ_C 95.6,

100.5 and 103.8 indicated a trisaccharide moiety. Since acid hydrolysis of **61** provided a low yield of monosaccharides, the crude steroidal glycoside was used instead of the high purity sample of **61**. The acid hydrolysis furnished four monosaccharides, which were identified as D-digitoxose, D-canarose, D-oleandrose and D-thevetose by comparison of the specific rotation with previous reports [48, 49]. Analysis of the 2D NMR spectroscopic data and the spin–spin couplings in the ^{1}H NMR of **61** allowed the identification of β-D-digitoxose (Dig), β-D-canarose (Can) and β-D-thevetose (Thv) moieties (Table 4.1). The anomeric configurations of the digitoxo pyranosyl, canaropyranosyl and thevetopyranosyl moieties were defined as β, according to their $^3J_{H1,H2}$ (9.6, 9.7 and 7.8 Hz, respectively), and supported by the NOESY correlation of the proton signals on the six-membered sugar rings. The linkage of the digitoxopyranosyl, canaropyranosyl and thevetopyranosyl were established by analysis of their HMBC correlations between: δ_H 3.62 (H-3 of aglycon) and δ_C 95.6 (C-1' of Dig), δ_H 4.93 (H-1' of Dig) and δ_C 77.0 (C-3 of aglycone), δ_H 4.29 (H-1''' of Thv (1→4)) and δ_C 88.2 (C-4" of Can), and δ_H 4.57 (H-1" of Can (1→4)) and δ_C 83.0 (C-4' of Dig). The same conclusion of the sugar sequence was derived from the NOESY correlation between: δ_H 3.62 (H-3 of aglycon) and δ_H 4.93 (H-1' of Dig), δ_H 3.21 (H-4' of Dig) and δ_H 4.57 (H-1" of Can), and δ_H 2.99 (H-4" of Can) and δ_H 4.29 (H-1''' of Thv) (Figure 4.2).

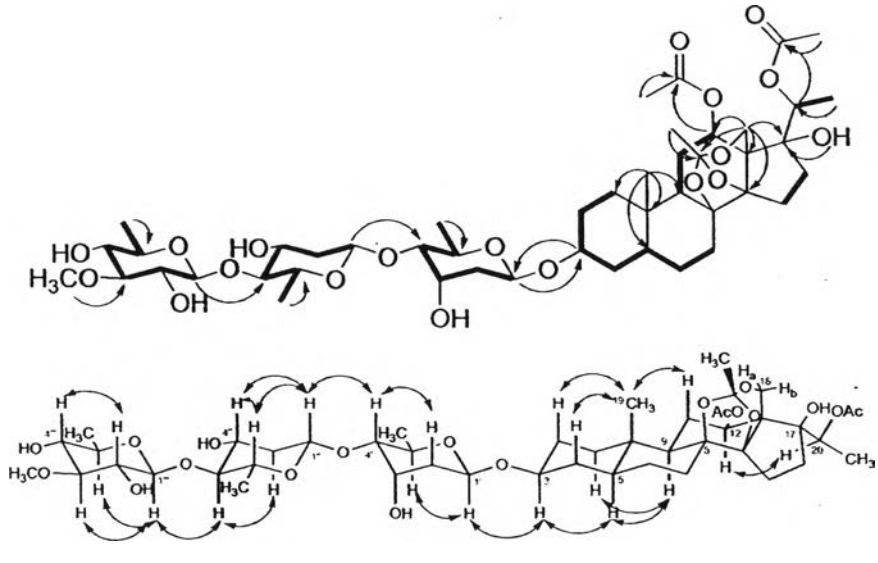


Figure 4.2 Key COSY (—), HMBC (⌒) and NOESY (⌒) correlations of **61**.

Since the relative configurations of **61** were not fully assigned, it was essential to characterize its aglycone. After acid hydrolysis of **61**, the CH_2Cl_2 extract of the acid

hydrolysate was isolated by silica gel column chromatography (CC) eluting with 20:1 (v/v) CH_2Cl_2:MeOH, and followed by semi-preparative RP-18 HPLC eluting with 68:32 (v/v) MeOH:H_2O to afford **61a** and a small amount of **67a**. Detailed analysis of the 1D and 2D NMR spectroscopic data was performed to assign all the proton signals of **61a** and **67a** and to confirm their relative configurations (Figure 4.3).

Compound **61a** was obtained as white amorphous powder. The molecular formula of **61a** was established as $C_{27}H_{40}O_9$, based on its HRESIMS data (*m/z* 531.2538 $[M+Na]^+$, calcd 531.2570). The large coupling constant ($J \approx 12$ Hz) of H-3, H-9 and H-12 and the observed NOEs in the NOESY data between: H-3 and H-5, H-5 and H-9, and H-9 and H-12 suggested that those protons of **61a** were axially oriented. As there was no NOESY correlation between H-5 and CH_3-19, suggested that the CH_3-19 was on the opposite face to H-5. The observed NOEs between: CH_3-19 and H_{ax}-11, H_{ax}-11 and H-18a, and H-18b and the proton of 17-OH in the NOESY data revealed that the orthoacetate and 17-OH occupied the same face as the CH_3-19. Due to the NOESY correlation between: H-12 and H-20, H-20 and H_α-16, CH_3-21 and 17-OH, CH_3-21 and H_β-16, 17-OH and H_β-16, and 20-OAc and H-18b, the configuration of 1a at C-20 was assigned as shown in Figure 4.3.

Compound **67a** was assigned the same molecular formula of $C_{27}H_{40}O_9$ as **1a** from its HRESIMS spectrum (*m/z* 531.2484 $[M+Na]^+$, calcd 531.2570). Comparison of the ^1H and ^{13}C NMR spectra of **67a** with that of **61a** revealed that the chemical shifts of C-8 of **67a** were were upfield-shifted up-field while the chemical shifts of C-17 of **67a** were shifted relatively down-field. These suggested the isomer position of the orthoacetate moiety in **67a**. By analysis of the COSY, HSQC, and HMBC spectra of **67a**, two hydroxy groups were at C-3 and C-8 and the orthoacetate was substituted at C-14, C-17 and C-18. Additionally, the relative stereochemical configurations (3, 5, 8, 9, 10, 12, 13, 14 and 20) were assigned from the NOESY data and the large coupling constant of the axial protons to be the same as **61a** (Figure 4.3).

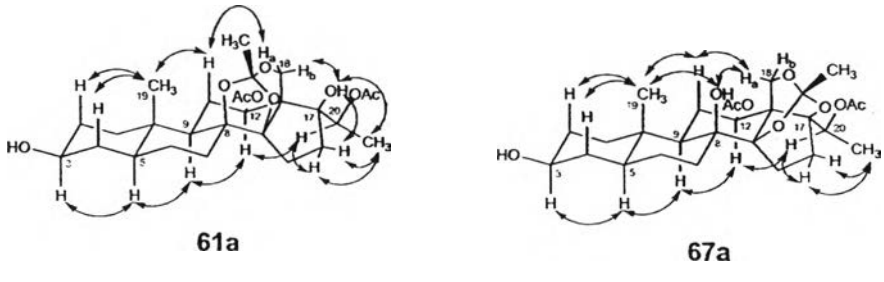


Figure 4.3 Key NOESY (↷) correlations of **61a** and **67a**.

Furthermore, the absolute stereochemistry of **61a** was examined by the ^{1}H NMR analysis on the corresponding α-methoxy-α-trifluoromethyl phenylacetic (MTPA) esters [65, 66]. The (*R*)- and (*S*)-MTPA esters **61a**$_R$ and **61a**$_S$ from **61a** were prepared by using the corresponding (*S*)- and (*R*)-MTPACl, respectively. The values of $\Delta\delta_{SR}$ (δ_S–δ_R) of H-1 and H-2 were negative, while the values of $\Delta\delta_{SR}$ for H-4, H-5 and H-6 were positive, letting us to determine that **61a** possessed a 3*S* configuration (Table 4.4) (Figure 4.4). Consequently, the stereochemistry of **61a** was confirmed as 3*S*, 5*S*, 8*S*, 9*R*, 10*S*, 12*R*, 13*R*, 14*R*, 17*S* and 20*S*. Therefore, gymnemogriffithoside A (**61**) was established as (3*S*,5*S*,8*S*,9*R*,10*S*,12*R*,13*R*,14*R*,17*S*,20*S*)-12,20-di-*O*-acetyl-(8,14,18-ortho acetate)-dihydrosarcostin 3-*O*-β-D-thevetopyranosyl-(1→4)-*O*-β-D-canaropyranosyl-(1→4)-*O*-β-D-digi toxopyranoside.

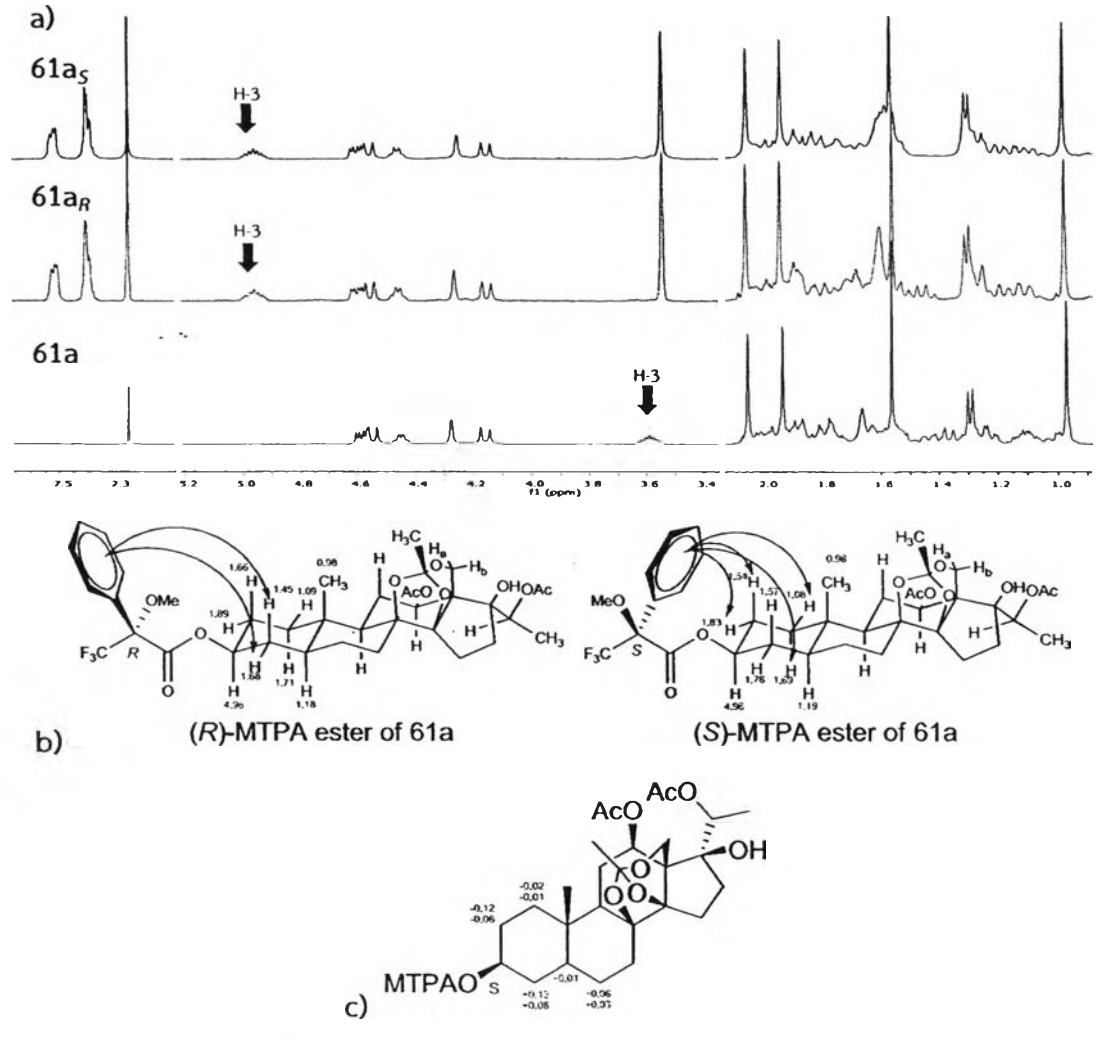


Figure 4.4 a) ^{1}H NMR spectra of **61a**, **61a**$_R$ and **61a**$_S$, b) Significant anisotropic chemical shift shielding effects of **61a**$_R$ and **61a**$_S$, and c) Values of $\Delta\delta_{SR}$ (δ_S–δ_R) obtained from **61a**$_R$ and **61a**$_S$.

Table 4.1 NMR data of aglycone moiety of compound **61** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	37.4 *t*	0.99 *m*, 1.66 *m*	-
2	28.9 *t*	1.87 *m*, 1.51 *m*	-
3	77.0 *d*	3.62 *m*	C-1'
4	34.0 *t*	1.67 *m*, 1.30 *m*	C-3
5	45.4 *d*	1.06 *m*	-
6	24.7 *t*	1.24 *m*, 1.55 *m*	-
7	32.2 *t*	1.10 *m*, 1.79 *m*	-
8	82.3 *s*	-	-
9	46.8 *d*	1.21 *m*	C-10
10	36.8 *s*	-	-
11	23.8 *t*	1.76 *m*, 1.86 *m*	C-9, C-12
12	73.1 *d*	4.59 *dd* (10.7, 4.6)	C-18, 12-OAc
13	51.2 *s*	-	-
14	94.4 *s*	-	-
15	25.9 *t*	1.86 *m*, 1.97 *m*	C-14
16	33.7 *t*	1.89 *m*, 2.02 *m*	-
17	86.3 *s*	-	-
18	60.2 *t*	4.16 *d* (12.0), 4.54 *d* (12.0)	C-12, C-13, C-14, C-17, C-ortho
19	12.5 *q*	0.96 *s*	C-1, C-5, C-9, C-10
20	74.4 *d*	4.45 *br q* (6.1)	20-OAc
21	15.0 *q*	1.30 *d* (6.1)	C-17, C-20
Ortho acetate			
1	117.2 *s*	-	-
2	24.3 *q*	1.56 *s*	C-ortho
12-OAc			
1	170.8 *s*	-	-
2	21.7 *q*	1.94 *s*	12-OAc
20-OAc			
1	170.4 *s*	-	-
2	21.6 *q*	2.06 *s*	20-OAc
OH-17		4.28 *br s*	C-17, C-20

Table 4.1 (Cont.) NMR data of sugar moiety of compound **61** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
D-Dig			
1'	95.6 *d*	4.93 *dd* (9.6, 1.7)	C-3
2'	37.4 *t*	1.68 *m*, 2.07 *m*	-
3'	66.9 *d*	4.22 *br s*	-
4'	83.0 *d*	3.21 *dd* (9.2, 3.2)	C-3'
5'	68.1 *d*	3.80 *dq* (9.2, 6.2)	C-4'
6'	18.3 *q*	1.24 *d* (6.2)	C-4', C-5'
D-Can			
1"	100.5 *d*	4.57 *dd* (9.7, 1.8)	C-4'
2"	38.5 *t*	1.61 *m*, 2.26 *ddd* (12.9, 5.2, 1.8)	C-1", C-3", C-4"
3"	69.5 *d*	3.60 *m*	C-4"
4"	88.2 *d*	2.99 *t* (8.8)	C-3", C-5", C-6", C-1""
5"	70.7 *d*	3.40 *dq* (9.1, 6.1)	-
6"	18.0 *q*	1.34 *d* (6.1)	C-4", C-5"
D-Thv			
1""	103.8 *d*	4.29 *d* (7.8)	C-4"
2""	74.6 *d*	3.47 *m*	C-1""
3""	85.5 *d*	3.12 *t* (9.1)	C-2"", C-4"", 3""-OCH_3
4""	74.7 *d*	3.23 *td* (9.1, 2.2)	C-3""
5""	72.4 *d*	3.48 *dq* (9.1, 6.1)	-
6""	17.6 *q*	1.35 *d* (6.1)	C-4"", C-5""
3""-OCH_3	61.0 *q*	3.66 *s*	C-3""

Table 4.2 NMR data of compound **61a** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	37.5 *t*	1.00 *td* (13.0, 4.2), 1.65 *m*	C-2, C-3, C-19
2	31.0 *t*	1.79 *m*, 1.44 *ddd* (13.5, 11.0, 4.0)	C-1, C-4, C-5
3	71.1 *d*	3.59 *tt* (11.0, 4.7)	-
4	37.3 *t*	1.62 *m*, 1.36 *dt* (12.5, 11.9)	C-3, C-5
5	45.4 *d*	1.09 *m*	-
6	24.6 *t*	1.26 *m*, 1.55 *m*	-
7	32.1 *t*	1.11 *m*, 1.79 *m*	C-5, C-8
8	82.3 *s*	-	-
9	46.7 *d*	1.22 *br dd* (13.2, 3.7)	C-5, C-8, C-10, C-11, C-19
10	36.6 *s*	-	-
11	23.8 *t*	1.76 *m*, 1.89 *m*	C-7, C-8, C-9, C-12
12	73.1 *d*	4.59 *dd* (10.9, 4.7)	C-11, C-18, 12-OAc
13	51.2 *s*	-	-
14	94.4 *s*	-	-
15	25.9 *t*	1.88 *m*, 1.96 *m*	C-14, C-16
16	33.6 *t*	1.89 *m*, 2.01 *m*	C-15, C-17
17	86.2 *s*	-	-
18	60.2 *t*	4.16 *d* (12.0), 4.55 *d* (12.0)	C-12, C-13, C-14, C-17, C-ortho
19	12.6 *q*	0.97 *s*	C-1, C-5, C-9, C-10
20	74.4 *d*	4.45 *qd* (6.1, 1.3)	C-21, 20-OAc
21	15.0 *q*	1.29 *d* (6.1)	C-17, C-20
Ortho acetate			
1	117.2 *s*	-	-
2	24.3 *q*	1.56 *s*	C-18, C-ortho
12-OAc			
1	170.9 *s*	-	-
2	21.7 *q*	1.94 *s*	12-OAc
20-OAc			
1	170.4 *s*	-	-
2	21.6 *q*	2.06 s	20-OAc
OH-17		4.28 *d* (1.3)	C-13, C-17, C-20

Table 4.3 NMR data of compound **67a** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	38.0 *t*	0.99 *m*, 1.68 *m*	C-2, C-3, C-19
2	31.0 *t*	1.79 *m*, 1.46 *m*	C-1, C-4, C-5
3	71.3 *d*	3.60 *tt* (10.8, 4.8)	-
4	37.7 *t*	1.61 *m*, 1.38 *dt* (12.5, 11.9)	C-2, C-3, C-5, C-10
5	45.6 *d*	1.08 *tt* (12.5, 1.1)	-
6	24.3 *t*	1.19 *m*, 1.68 *m*	-
7	34.1 *t*	1.31 *m*, 1.79 *m*	C-5, C-8
8	73.6 *s*	-	-
9	46.9 *d*	1.22 *br dd* (13.2, 3.9)	C-8, C-10, C-11, C-12, C-19
10	36.4 *s*	-	-
11	23.7 *t*	1.75 *m*, 1.76 *m*	C-8, C-9, C-12
12	70.9 *d*	4.72 *dd* (10.9, 5.6)	C-11, C-13, C-17, C-18, 12-OAc
13	46.6 *s*	-	-
14	90.3 *s*	-	-
15	32.0 *t*	1.81 *m*, 1.91 *m*	C-14, C-16, C-17
16	31.8 *t*	1.80 *m*, 1.80 *m*	C-14, C-15, C-17
17	87.9 *s*	-	-
18	61.7 *t*	4.47 *d* (8.8), 4.75 *d* (8.8)	C-12, C-13, C-14, C-17, C-ortho
19	12.7 *q*	1.00 *s*	C-1, C-5, C-9, C-10
20	72.1 *d*	4.78 *q* (6.5)	C-21, 20-OAc
21	15.1 *q*	1.30 *d* (6.5)	C-17, C-20
Ortho acetate			
1	108.3 *s*	-	-
2	24.4 *q*	1.53 *s*	C-14, C-17, C-18, C-ortho
12-OAc			
1	170.6 *s*	-	-
2	21.4 *q*	2.09 *s*	C-12, 12-OAc
20-OAc			
1	170.3 *s*	-	-
2	21.5 *q*	1.98 *s*	C-20, 20-OAc
OH-8		2.59 *d* (1.2)	C-8, C-9, C-14

Table 4.4 NMR data of compounds **61a**, **61a$_R$** and **61a$_S$** in $CDCl_3$

Position	1a		1a$_R$		1a$_S$	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	37.5 *t*	1.00 *td* (13.0, 4.2)	37.0 *t*	1.09 *m*	36.9 *t*	1.08 *m*
		1.65 *m*		1.71 *m*		1.69 *m*
2	31.0 *t*	1.79 *m*	26.8 *t*	1.89 *m*	26.5 *t*	1.83 *m*
		1.44 *ddd* (13.5, 11.0, 4.0)		1.66 *m*		1.54 *m*
3	71.1 *d*	3.59 *tt* (11.0, 4.7)	75.8 *d*	4.96 *m*	75.8 *d*	4.96 *m*
4	37.3 *t*	1.62 *m*	32.9 *t*	1.68 *m*	33.2 *t*	1.76 *m*
		1.36 *dt* (12.5, 11.9)		1.45 *m*		1.57 *m*
5	45.4 *d*	1.09 *m*	45.2 *d*	1.18 *m*	45.3 *d*	1.19 *m*
6	24.6 *t*	1.26 *m*	24.4 *t*	1.26 *m*	24.4 *t*	1.29 *m*
		1.55 *m*		1.54 *m*		1.58 *m*
7	32.1 *t*	1.11 *m*	32.1 *t*	1.11 *m*	32.1 *t*	1.13 *m*
		1.79 *m*		1.81 *m*		1.82 *m*
8	82.3 *s*	-	82.1 *s*	-	82.1 *s*	-
9	46.7 *d*	1.22 *br dd* (13.2, 3.7)	46.6 *d*	1.26 *m*	46.6 *d*	1.26 *m*
10	36.6 *s*	-	36.6 *s*	-	36.6 *s*	-
11	23.8 *t*	1.76 *m*	23.7 *t*	1.74 *m*	23.7 *t*	1.74 *m*
		1.89 *m*		1.88 *m*		1.88 *m*
12	73.1 *d*	4.59 *dd* (10.9, 4.7)	73.0 *d*	4.60 *dd* (10.8, 4.6)	72.9 *d*	4.60 *dd* (10.9, 4.6)
13	51.2 *s*	-	51.2 *s*	-	51.2 *s*	-
14	94.4 *s*	-	94.3 *s*	-	94.3 *s*	-
15	25.9 *t*	1.88*m*	25.9 *t*	1.89 *m*	25.9 *t*	1.90 *m*
		1.96 *m*		1.99 *m*		2.00 *m*
16	33.6 *t*	1.89 *m*	33.6 *t*	1.90 *m*	33.6 *t*	1.90 *m*
		2.01 *m*		2.04 *m*		2.04 *m*
17	86.2 *s*	-	86.3 *s*	-	86.3 *s*	-
18	60.2 *t*	4.16 *d* (12.0)	60.2 *t*	4.15 *d* (12.0)	60.2 *t*	4.15 *d* (12.1)
		4.55 *d* (12.0)		4.56 *d* (12.0)		4.56 *d* (12.1)
19	12.6 *q*	0.97 *s*	12.5 *q*	0.98 *s*	12.5 *q*	0.98 *s*
20	74.4 *d*	4.45 *qd* (6.1, 1.3)	74.4 *d*	4.46 *q* (6.2)	74.4 *d*	4.46 *q* (6.0)
21	15.0 *q*	1.29 *d* (6.1)	15.0 *q*	1.31 *d* (6.2)	15.0 *q*	1.31 *d* (6.0)
Ortho acetate						
	117.2 *s*	-	117.2 *s*	-	117.2 *s*	-
	24.3 *q*	1.56 *s*	24.3 *q*	1.56 *s*	24.3 *q*	1.57 *s*
12-OAc						
	170.9 *s*	-	170.9 *s*	-	170.8 *s*	-
	21.7 *q*	1.94 *s*	21.7 *q*	1.95 *s*	21.7 *q*	1.95 *s*
20-OAc						
	170.4 *s*	-	170.4 *s*	-	170.4 *s*	-
	21.6 *q*	2.06 *s*	21.6 *q*	2.07 *s*	21.6 *q*	2.07 *s*
17-OH						
		4.28 *d* (1.3)		4.26 *s*		4.25 *s*

Gymnemogriffithoside B (**62**) was obtained as white amorphous powder. The molecular formula of **62** was assigned as $C_{44}H_{70}O_{18}$, based on its HRESIMS data (*m/z* 909.4424 $[M+Na]^+$, calcd 909.4454), as well as its ^{13}C NMR spectroscopic data (Table 4.5). Comparison of the NMR spectroscopic data of **62** with that of **61** indicated no difference between the two compounds except for replacement of the 12β-acetyl group by a 12β-hydroxy group. The relatively upfield-shift of H-12 at δ_H 3.48 and C-12 at δ_C 70.1 was consistent with no acylation at C-12. HMBC correlations of an acetyl carbonyl signal at δ_C 171.2 with H-20 at δ_H 4.99 established an acylation substitution position at C-20. Careful comparison of the sugar signals in the HSQC and COSY data indicated that 2 had an identical trisaccharide moiety to **61**. Similar to **61**, the configuration of **62** was determined from the NOESY data. Full examination of the NMR spectroscopic data further confirmed the structure of **62** as 20-*O*-acetyl-(8,14,18-orthoacetate)-dihydrosarcostin 3-*O*-β-D-thevetopyranosyl-(1→4)-*O*-β-D-cana ropyranosyl-(1→4)-*O*-β-D-digitoxopyranoside (Figure 4.5).

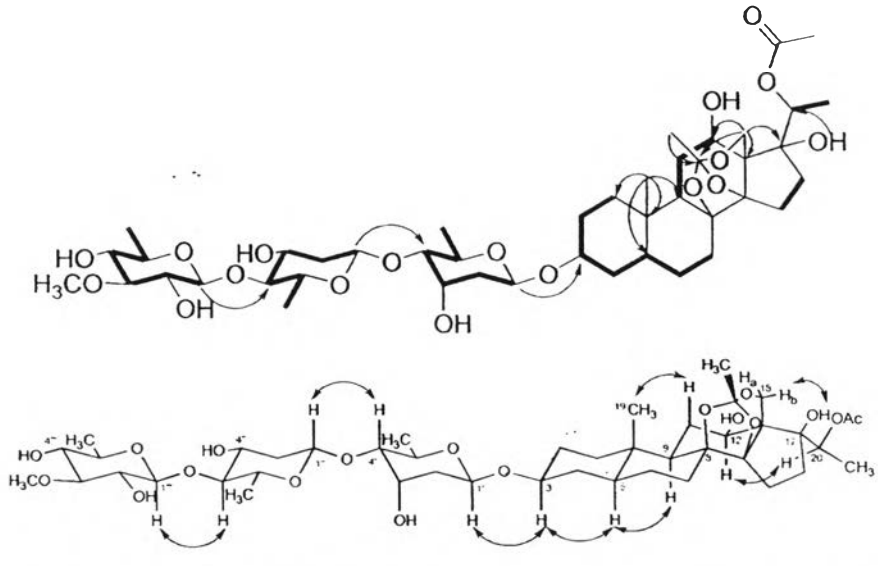


Figure 4.5 Key COSY (—), HMBC ( ) and NOESY ( ) correlations of **62**.

Table 4.5 NMR data of aglycone moiety of compound **62** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	37.6 *t*	0.97 *m*, 1.70 *m*	-
2	29.0 *t*	1.89 *m*, 1.54 *m*	-
3	77.1 *d*	3.63 *m*	-
4	34.0 *t*	1.68 *m*, 1.33 *m*	-
5	45.4 *d*	1.06 *m*	-
6	24.7 *t*	1.25 *m*, 1.52 *m*	-
7	32.2 *t*	1.08 *m*, 1.79 *m*	-
8	82.2 *s*	-	-
9	47.2 *d*	1.15 *br dd* (13.2, 3.4)	-
10	36.6 *s*	-	-
11	27.9 *t*	1.58 *m*, 2.01 *m*	-
12	70.1 *d*	3.48 *m*	-
13	52.7 *s*	-	-
14	94.1 *s*	-	-
15	26.1 *t*	1.81 *m*, 1.97 *m*	-
16	34.0 *t*	1.90 *m*, 2.03 *m*	-
17	86.6 *s*	-	-
18	59.9 *t*	4.10 *d* (12.0), 4.45 *d* (12.0)	C-12, C-13, C-17, C-ortho
19	12.6 *q*	0.98 *s*	C-1, C-5, C-9, C-10
20	74.6 *d*	4.99 *qd* (6.4, 1.2)	20-OAc
21	15.1 *q*	1.32 *d* (6.4)	C-17, C-20
Ortho acetate			
1	117.0 *s*	-	-
2	24.3 *q*	1.56 *s*	C-ortho
20-OAc			
1	171.2 *s*	-	-
2	21.5 *q*	2.00 *s*	20-OAc
OH-17		4.14 *br s*	C-20

Table 4.5 (Cont.) NMR data of sugar moiety of compound **62** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
D-Dig			
1'	95.6 *d*	4.94 *dd* (9.6, 1.6)	C-3
2'	37.3 *t*	1.72 *m*, 2.08 *m*	-
3'	66.9 *d*	4.23 *br s*	-
4'	83.0 *d*	3.22 *dd* (9.4, 3.1)	C-5', C-6'
5'	68.1 *d*	3.80 *dq* (9.4, 6.2)	-
6'	18.4 *q*	1.24 *d* (6.2)	C-4', C-5'
D-Can			
1"	100.5 *d*	4.57 *dd* (9.8, 1.6)	C-4'
2"	38.5 *t*	1.62 *m*, 2.26 *ddd* (12.9, 5.1, 1.6)	C-1", C-3", C-4"
3"	69.5 *d*	3.60 *m*	-
4"	88.1 *d*	2.99 *t* (8.8)	C-5", C-6", C-1""
5"	70.7 *d*	3.39 *dq* (9.2, 6.1)	-
6"	18.0 *q*	1.35 *d* (6.1)	C-4", C-5"
D-Thv			
1""	103.7 *d*	4.30 *d* (7.8)	C-4"
2""	74.5 *d*	3.48 *m*	C-1"", C-3""
3""	85.5 *d*	3.13 *t* (9.1)	C-2"", C-4"", 3""-OCH_3
4""	74.7 *d*	3.24 *t* (9.1)	-
5""	72.3 *d*	3.49 *m*	-
6""	17.6 *q*	1.34 *d* (6.2)	C-4"", C-5""
3""-OCH_3	61.1 *q*	3.67 *s*	C-3""

Gymnemogriffithoside C (**63**) was obtained as white amorphous powder. It had a molecular formula of $C_{49}H_{76}O_{19}$, as determined by analysis of its HRESIMS (*m/z* 991.4870 $[M+Na]^+$, calcd 991.4873) and ^{13}C NMR spectroscopic data (Table 4.6). The ^1H and ^{13}C NMR of **63** showed similar signals as to that of **61**, except for replacement of an acetyl group by a tigloyl group. HMBC correlations of an acetyl carbonyl signal at δ_C 171.0 with proton resonances of H-12 at δ_H 4.59 and a tigloyl carbonyl signal at δ_C 167.3 with H-20 at δ_H 4.51 established the acylated substitutions at C-12 and C-20. The analysis of the acylated groups of **63** at C-12 and C-20 established the partial structure of 12β-acetyl-20-tigloyl. Comparison of the sugar signals in the HSQC and COSY data indicated that **63** had an identical trisaccharide moiety to **61** and the stereochemical configurations were confirmed by NOESY analysis. Full examination of the NMR spectroscopic data further confirmed the structure of **63** as 12-*O*-acetyl-20-*O*-tigloyl-(8,14,18-orthoacetate)-dihydrosarcostin 3-*O*-β-D-thevetopyranosyl-(1→4)-*O*-β-D-canaropyranosyl-(1→4)-O-β-D-digitoxopyrano side (Figure 4.6).

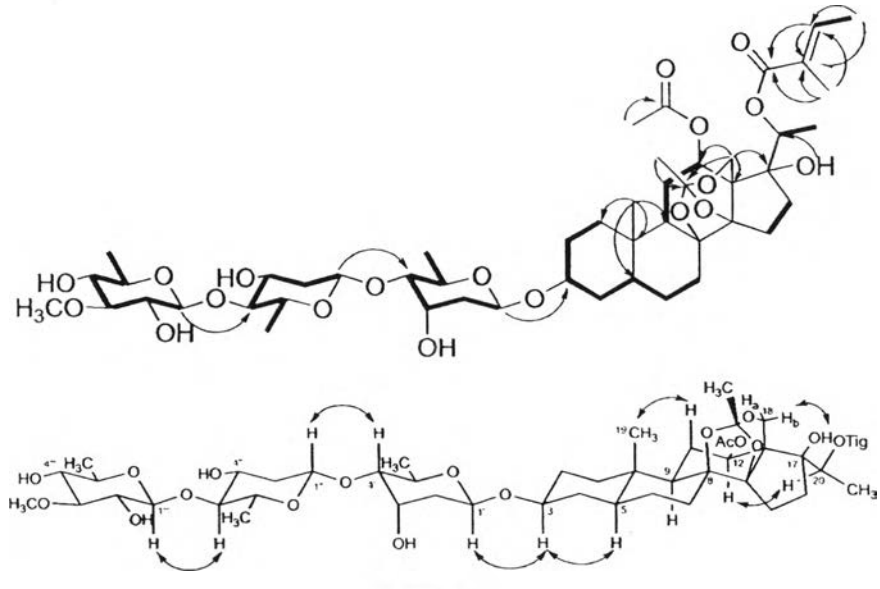


Figure 4.6 Key COSY (—), HMBC ( ) and NOESY ( ) correlations of **63**.

Table 4.6 NMR data of aglycone moiety of compound **63** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	37.4 *t*	0.98 *m*, 1.71 *m*	-
2	28.9 *t*	1.87 *m*, 1.51 *m*	-
3	77.0 *d*	3.63 *m*	-
4	33.9 *t*	1.69 *m*, 1.33 *m*	C-3
5	45.3 *d*	1.07 *m*	-
6	24.7 *t*	1.25*m*, 1.53 *m*	-
7	32.1 *t*	1.11 *m*, 1.80 *m*	-
8	82.3 *s*	-	-
9	46.7 *d*	1.20 *m*	-
10	36.7 *s*	-	-
11	23.7 *t*	1.75 *m*, 1.85 *m*	-
12	72.8 *d*	4.59 *dd* (10.8, 4.7)	-
13	51.4 *s*	-	-
14	94.4 *s*	-	-
15	26.0 *t*	1.86 *m*, 1.99 *m*	-
16	34.0 *t*	1.93 *m*, 2.04 *m*	-
17	86.6 *s*	-	-
18	60.5 *t*	4.12 *d* (11.8), 4.57 *d* (11.8)	C-12, C-13, C-14, C-17, C-ortho
19	12.5 *q*	0.94 *s*	C-1, C-5, C-9, C-10
20	74.1 *d*	4.51 *qd* (6.2, 1.3)	20-OTig
21	15.1 *q*	1.29 *d* (6.2)	C-17, C-20
Ortho acetate			
1	117.1 *s*	-	-
2	24.3 *q*	1.57 *s*	C-ortho
12-OAc			
1	171.0 *s*	-	-
2	21.7 *q*	1.85 *s*	12-OAc
20-OTig			
1	167.3 *s*	-	-
2	129.1 *s*	-	-
3	137.8 *d*	6.88 *qd* (7.1, 1.0)	20-OTig, Tig-4, Tig-5
4	14.6 *q*	1.79 *dd* (7.1, 1.0)	Tig-2, Tig-3
5	12.3 *q*	1.84 *br s*	20-OTig, Tig-2, Tig-3
OH-17		4.16 *d* (1.3)	C-20

Table 4.6 (Cont.) NMR data of sugar moiety of compound **63** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
D-Dig			
1'	95.6 *d*	4.93 *dd* (9.6, 1.7)	C-3
2'	37.3 *t*	1.68 *m*, 2.07 *m*	-
3'	66.9 *d*	4.22 *br s*	-
4'	83.0 *d*	3.21 *dd* (9.2, 3.2)	-
5'	68.1 *d*	3.80 *dq* (9.2, 6.2)	-
6'	18.3 *q*	1.24 *d* (6.2)	C-4', C-5'
D-Can			
1"	100.5 *d*	4.57 *dd* (9.7, 1.8)	C-4'
2"	38.5 *t*	1.61 *m*, 2.26 *ddd* (12.9, 5.2, 1.8)	C-1", C-3", C-4"
3"	69.5 *d*	3.60 *m*	C-4"
4"	88.1 *d*	2.99 *t* (8.8)	C-3, C-5", C-1"'
5"	70.7 *d*	3.40 *dq* (9.1, 6.1)	-
6"	18.1 *q*	1.34 *d* (6.1)	C-4", C-5"
D-Thv			
1"'	103.7 *d*	4.29 *d* (7.8)	C-4"
2"'	74.6 *d*	3.47 *m*	C-1"', C-3"'
3"'	85.5 *d*	3.12 *t* (9.1)	C-2"', C-4"', 3"'-OCH_3
4"'	74.7 *d*	3.23 *td* (9.1, 2.2)	C-3"'
5"'	72.4 *d*	3.48 *dq* (9.1, 6.1)	-
6"'	17.6 *q*	1.35 *d* (6.1)	C-4"', C-5"'
3"'-OCH_3	61.1 *q*	3.66 *s*	C-3"'

Gymnemogriffithoside D (**64**) was obtained as white amorphous powder. The molecular formula of **64** was established as $C_{47}H_{74}O_{19}$, based on its HRESIMS data (m/z 965.4712 $[M+Na]^+$, calcd 965.4716). The signals of the aglycone and the sugar moiety were almost identical to those in **61** except for the presence of a methoxy signal of the trisaccharide. Analysis of the 2D NMR spectroscopic data and spin–spin couplings in the ^{1}H NMR of **64** allowed the identification of *β*-D-digitoxose (Dig), *β*-D-oleandrose (Ole) and *β*-D-thevetose (Thv) moieties (Table 4.7). The anomeric configurations of the digitoxopyranosyl, oleandropyranosyl and thevetopyranosyl moieties were defined as *β*, according to their $^3J_{H1,H2}$ (9.6, 9.6 and 8.1 Hz, respectively). The NOESY correlation of proton signals on the six-membered sugar rings also supported the digitoxopyranosyl, oleandropyranosyl and thevetopyranosyl configurations. The linkage of the digitoxopyranosyl, olean dropyranosyl and thevetopyranosyl were established by analysis of their HMBC correlations between: δ_H 3.61 (H-3 of aglycon) and δ_C 95.6 (C-1' of Dig), δ_H 4.93 (H-1' of Dig) and δ_C 77.0 (C-3 of aglycone), δ_H 4.47 (H-1''' of Thv (1→4)), and δ_C 79.4 (C-4'' of Ole), and δ_H 4.52 (H-1'' of Ole (1→4)) and δ_C 83.1 (C-4' of Dig). Consequently, the chemical structure of 4 was concluded to be 12,20-di-*O*-acetyl-(8,14,18-ortho acetate)-dihydrosarcostin 3-*O*-*β*-D-thevetopyranosyl-(1→4)-*O*-*β*-D-oleandropyranosyl-(1→4)-*O*-*β*-D-digitoxopyranoside (Figure 4.7).

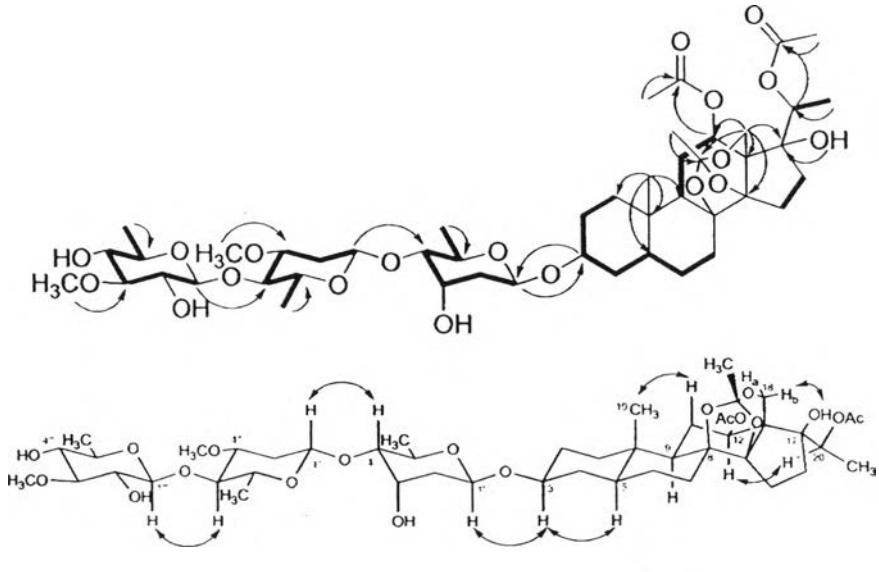


Figure 4.7 Key COSY (—), HMBC ( ) and NOESY ( ) correlations of **64**.

Table 4.7 NMR data of aglycone moiety of compound **64** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	37.4 *t*	0.97 *m*, 1.67 *m*	-
2	28.9 *t*	1.87 *m*, 1.59 *m*	-
3	77.0 *d*	3.61 *m*	C-1'
4	33.9 *t*	1.67 *m*, 1.32 *m*	C-3, C-5
5	45.3 *d*	1.06 *m*	-
6	24.6 *t*	1.24 *m*, 1.54 *m*	-
7	32.1 *t*	1.09 *m*, 1.79 *m*	-
8	82.3 *s*	-	-
9	46.7 *d*	1.20 *m*	-
10	36.7 *s*	-	-
11	23.8 *t*	1.75 *m*, 1.87 *m*	C-9, C-12
12	73.1 *d*	4.58 *dd* (10.8, 4.6)	C-18, 12-Ac-1
13	51.2 *s*	-	-
14	94.3 *s*	-	-
15	25.8 *t*	1.86 *m*, 1.97 *m*	C-14, C-15
16	33.6 *t*	1.89 *m*, 2.02 *m*	-
17	86.2 *s*	-	-
18	60.2 *t*	4.15 *d* (12.0), 4.54 *d* (12.0)	C-12, C-13, C-14, C-17, C-ortho
19	12.5 *q*	0.95 *s*	C-1, C-5, C-9, C-10
20	74.4 *d*	4.44 *qd* (5.8, 1.3)	C-21, 20-OAc
21	15.0 *q*	1.29 *d* (5.8)	C-17, C-20
Ortho acetate			
1	117.1 *s*	-	-
2	24.3 *q*	1.55 *s*	C-ortho
12-OAc			
1	170.9 *s*	-	-
2	21.7 *q*	1.93 *s*	12-OAc
20-OAc			
1	170.4 *s*	-	-
2	21.6 *q*	2.05 *s*	20-OAc
OH-17		4.28 *d* (1.3)	C-17, C-20

Table 4.7 (Cont.) NMR data of sugar moiety of compound **64** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
D-Dig			
1'	95.6 *d*	4.93 *dd* (9.6, 1.6)	C-3
2'	37.3 *t*	1.68 *m*, 2.07 *m*	-
3'	66.7 *d*	4.21 *br s*	-
4'	83.1 *d*	3.20 *dd* (9.3, 2.9)	C-3', C-5', C-1'
5'	68.0 *d*	3.79 *dq* (9.3, 6.2)	C-4'
6'	18.4 *q*	1.24 *d* (6.2)	C-4', C-5'
D-Ole			
1"	100.2 *d*	4.52 *dd* (9.6, 1.8)	C-4'
2"	35.9 *t*	1.51 *m*, 2.36 *ddd* (13.0, 4.2, 1.8)	C-1", C-3", C-4"
3"	78.8 *d*	3.36 *m*	-
4"	79.4 *d*	3.33 *m*	C-5"
5"	71.8 *d*	3.36 *m*	C-4"
6"	18.7 *q*	1.34 *d* (5.5)	C-4", C-5"
3"-OCH_3	56.2 *q*	3.39 *s*	C-3"
D-Thv			
1"'	101.9 *d*	4.47 *d* (8.0)	C-4"
2"'	73.4 *d*	3.45 *td* (8.4, 2.3)	C-1"'
3"'	85.6 *d*	3.08 *t* (8.9)	C-2"', C-4"', 3"'-OCH_3
4"'	75.0 *d*	3.15 *td* (8.9, 2.5)	C-3"'
5"'	72.1 *d*	3.36 *m*	-
6"'	17.9 *q*	1.30 *d* (6.1)	C-4"', C-5"'
3"'-OCH_3	60.8 *q*	3.65 *s*	C-3"'

Gymnemogriffithoside E (**65**) was obtained as white amorphous powder. The molecular formula of **65** was established as $C_{45}H_{72}O_{18}$, based on its HRESIMS data (*m/z* 923.4594 $[M+Na]^+$, calcd 923.4611), as well as its ^{13}C NMR spectroscopic data (Table 4.8). Analysis of the ^{1}H and ^{13}C NMR spectra of **65** showed the presence of signals corresponding to the presence of the same aglycone moiety as in **62** and of the same sugar moiety as in **64**. The same conclusion was derived from analysis of the 2D NMR spectroscopic data of **65**. Therefore, the structure of **65** was determined to be 20-*O*-acetyl-(8,14,18-orthoacetate)-dihydrosarcostin 3-*O*-*β*-D-thevetopyranosyl-(1→4)-*O*-*β*-D-oleandropyranosyl-(1→4)-*O*-*β*-D-digitoxopyranoside (Figure 4.8).

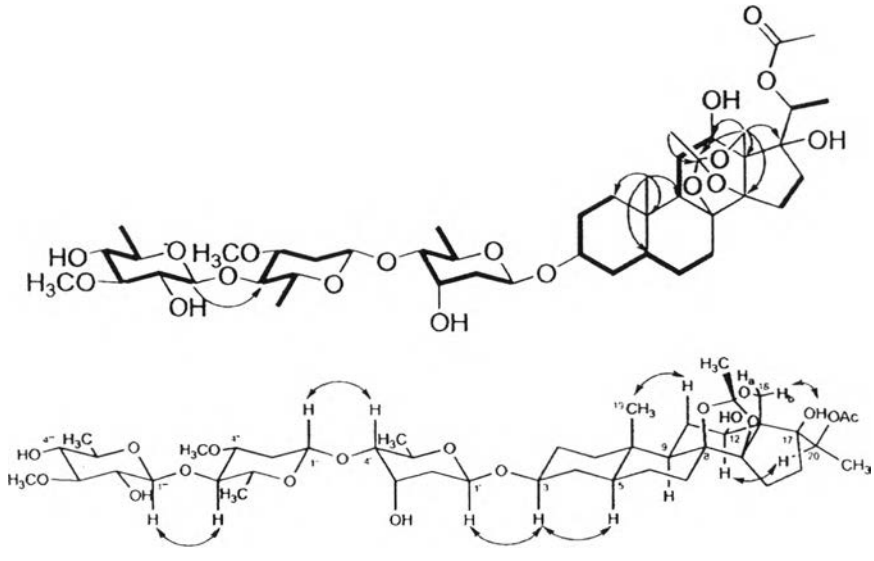


Figure 4.8 Key COSY (—), HMBC (⌒) and NOESY (⌒) correlations of **65**.

Table 4.8 NMR data of aglycone moiety of compound **65** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	37.6 *t*	0.94 *m*, 1.68 *m*	-
2	29.0 *t*	1.88 *m*, 1.53 *m*	-
3	77.2 *d*	3.61 *m*	-
4	34.0 *t*	1.67 *m*, 1.32 *m*	-
5	45.4 *d*	1.03 *m*	-
6	24.7 *t*	1.23 *m*, 1.58 *m*	-
7	32.2 *t*	1.08 *m*, 1.79 *m*	-
8	82.1 *s*	-	-
9	47.2 *d*	1.13 *br dd* (13.2, 3.5)	-
10	36.6 *s*	-	-
11	27.9 *t*	1.57 *m*, 2.00 *m*	-
12	70.0 *d*	3.47 *m*	-
13	52.7 *s*	-	-
14	94.1 *s*	-	-
15	26.1 *t*	1.77 *m*, 1.93 *m*	-
16	34.0 *t*	1.88 *m*, 2.01 *m*	-
17	86.6 *s*	-	-
18	59.9 *t*	4.10 *d* (12.0), 4.45 *d* (12.0)	C-12, C-13, C-17, C-ortho
19	12.6 *q*	0.98 *s*	C-1, C-5, C-9, C-10
20	74.7 *d*	4.99 *qd* (6.4, 1.5)	-
21	15.1 *q*	1.32 *d* (6.4)	C-17, C-20
Ortho acetate			
1	117.0 *s*	-	-
2	24.3 *q*	1.56 *s*	C-ortho
20-OAc			
1	171.1 *s*	-	-
2	21.5 *q*	2.00 *s*	20-OAc
OH-17		4.13 *br s*	C-20

Table 4.8 (Cont.) NMR data of sugar moiety of compound **65** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
D-Dig			
1'	95.6 *d*	4.94 *dd* (9.6, 1.8)	-
2'	37.3 *t*	1718 *m*, 2.07 *m*	-
3'	66.7 *d*	4.22 *br s*	-
4'	83.1 *d*	3.21 *dd* (9.3, 2.9)	-
5'	68.0 *d*	3.80 *dq* (9.3, 6.2)	-
6'	18.4 *q*	1.24 *d* (6.2)	C-4', C-5'
D-Ole			
1"	100.2 *d*	4.53 *dd* (9.7, 1.9)	-
2"	35.9 *t*	1.51 *m*, 2.37 *ddd* (13.2, 4.7, 1.9)	C-3"
3"	78.8 *d*	3.37 *m*	-
4"	79.4 *d*	3.35 *m*	-
5"	71.8 *d*	3.36 *m*	-
6"	18.7 *q*	1.34 *d* (5.6)	C-4", C-5"
3"-OCH_3	56.2 *q*	3.40 *s*	C-3"
D-Thv			
1""	101.9 *d*	4.48 *d* (8.1)	C-4"
2""	73.4 *d*	3.45 *m*	-
3""	85.6 *d*	3.09 *t* (8.9)	C-2"", C-4"", 3""-OCH_3
4""	75.0 *d*	3.16 *td* (8.9, 2.4)	-
5""	72.1 *d*	3.36 *m*	-
6""	17.9 *q*	1.31 *d* (6.2)	C-4"", C-5""
3""-OCH_3	60.8 *q*	3.66 *s*	C-3""

Gymnemogriffithoside F (**66**) was obtained as white amorphous powder. The molecular formula of **66** was established as $C_{50}H_{78}O_{18}$, based on its HRESIMS data (*m/z* 1005.5046 $[M+Na]^+$, calcd 1005.5030), as well as its ^{13}C NMR spectroscopic data (Table 4.9). Comparison of the ^{1}H and ^{13}C NMR spectra of **66** with those from **63**, **64** and **65** indicated that the aglycone moiety of **66** was 12-*O*-acetyl-20-*O*-tigloyl-dihydrosarcostin and the sugar moiety was the same as in **64** and **65**. Further 2D NMR spectroscopic analysis supported this conclusion. Thus, the structure of **66** was determined to be 12-*O*-acetyl-20-*O*-tigloyl-(8,14,18-orthoacetate)-dihydrosarcostin 3-*O*-*β*-D-thevetopyranosyl-(1→4)-*O*-*β*-D-oleandropyranosyl-(1→4)-*O*-*β*-D-digitoxopyranoside (Figure 4.9).

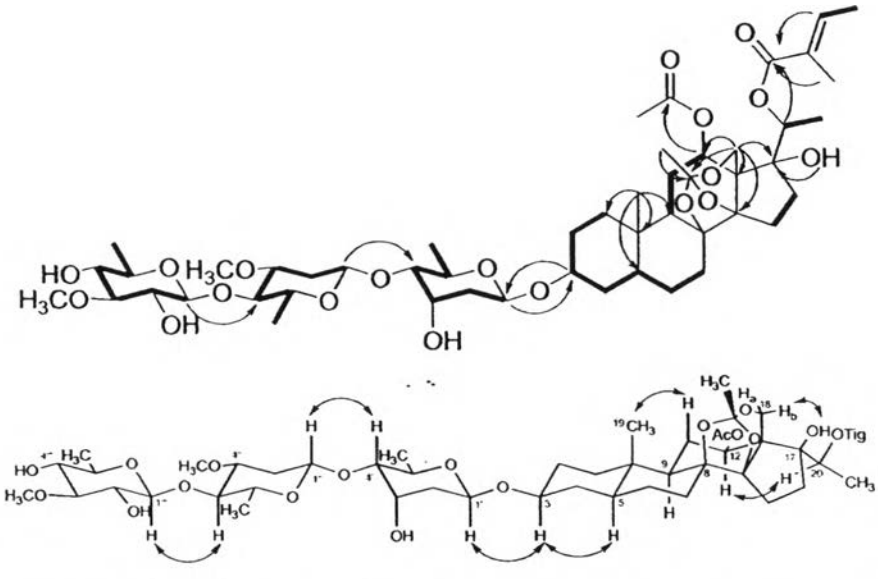


Figure 4.9 Key COSY (—), HMBC (⌒) and NOESY (⌒) correlations of **66**.

Table 4.9 NMR data of aglycone moiety of compound **66** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	37.4 *t*	0.98 *m*, 1.64 *m*	-
2	28.9 *t*	1.87 *m*, 1.51 *m*	-
3	77.0 *d*	3.61 *m*	C-1'
4	33.9 *t*	1.67 *m*, 1.32 *m*	C-3, C-5
5	45.3 *d*	1.06 *m*	-
6	24.6 *t*	1.25*m*, 1.55 *m*	-
7	32.1 *t*	1.10 *m*, 1.80 *m*	-
8	82.3 *s*	-	-
9	46.7 *d*	1.20 *m*	-
10	36.7 *s*	-	-
11	23.7 *t*	1.74 *m*, 1.85 *m*	C-9, C-12
12	72.8 *d*	4.58 *dd* (10.8, 4.6)	C-18, 12-OAc
13	51.4 *s*	-	-
14	94.4 *s*	-	-
15	26.0 *t*	1.86 *m*, 1.99 *m*	C-14
16	34.0 *t*	1.94 *m*, 2.03 *m*	C-13
17	86.6 *s*	-	-
18	60.5 *t*	4.12 *d* (11.8), 4.56 *d* (11.8)	C-12, C-13, C-14, C-17, C-ortho
19	12.5 *q*	0.94 *s*	C-1, C-5, C-9, C-10
20	74.1 *d*	4.51 *m*	20-OTig
21	15.1 *q*	1.29 *d* (5.5)	C-17, C-20
Ortho acetate			
1	117.1 *s*	-	-
2	24.3 *q*	1.57 *s*	C-ortho
12-OAc			
1	171.0 *s*	-	-
2	21.7 *q*	1.85 *s*	C-12, 12-Ac-1
20-OTig			
1	167.3 *s*	-	-
2	129.1 *s*	-	-
3	137.8 *d*	6.88 *qd* (7.1, 1.0)	20-OTig, Tig-4, Tig-5
4	14.6 *q*	1.79 *dd* (7.1, 1.0)	Tig-2, Tig-3
5	12.3 *q*	1.84 *br s*	20-OTig, Tig-2, Tig-3
OH-17		1.84 *br s*	C-17, C-20

Table 4.9 (Cont.) NMR data of sugar moiety of compound **66** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
D-Dig			
1'	95.6 *d*	4.93 *dd* (9.4, 1.4)	C-3
2'	37.3 *t*	168 *m*, 2.07 *m*	-
3'	66.8 *d*	4.22 *br s*	-
4'	83.1 *d*	3.21 *dd* (9.3, 2.9)	C-5'
5'	68.0 *d*	3.79 *dq* (9.3, 6.2)	C-4'
6'	18.4 *q*	1.24 *d* (6.2)	C-4', C-5'
D-Ole			
1"	100.2 *d*	4.53 *dd* (9.6, 1.9)	C-4'
2"	35.9 *t*	1.51 *m*, 2.37 *ddd* (13.1, 4.5, 1.9)	C-3", C-4"
3"	78.8 *d*	3.38 *m*	-
4"	79.4 *d*	3.34 *m*	C-5"
5"	71.8 *d*	3.37 *m*	C-4"
6"	18.7 *q*	1.34 *d* (5.5)	C-4", C-5"
3"-OCH_3	56.2 *q*	3.39 *s*	C-3"
D-Thv			
1"'	101.9 *d*	4.47 *d* (8.1)	C-4"
2"'	73.4 *d*	3.46 *td* (8.6, 2.2)	C-1"', C-3"'
3"'	85.6 *d*	3.09 *t* (8.9)	C-2"', C-4"', 3"'-OCH_3
4"'	75.0 *d*	3.16 *br t* (8.9)	C-3"'
5"'	72.1 *d*	3.36 *m*	-
6"'	17.9 *q*	1.31 *d* (6.0)	C-5"'
3"'-OCH_3	60.8 *q*	3.66 *s*	C-3"'

Gymnemogriffithoside G (**67**) was obtained as white amorphous powder. The HRESIMS spectrum of **67** showed a $[M+Na]^+$ peak at *m/z* 951.4510 (calcd 951.4560) indicating the same molecular formula ($C_{46}H_{72}O_{19}$) as that for **61**. Comparison of the NMR spectroscopic data of **67** with that of **61** indicated no difference between the two compounds except for the relatively upfield-shift of C-8 and the downfield-shift of C-17, suggesting an isomer position for the orthoacetate group (Table 4.10). HMBC correlations of the C-8 signal at δ_C 73.6 with OH at δ_H 2.57 indicated that C-8 was substituted with a hydroxy group instead of the orthoacetate group. The observed HMBC correlations between the resonance for the hydrogens of the methylene C-18 at δ_H 4.46 and 4.75 and those for the carbon signals of C-12 (δ_C 70.9), C-13 (δ_C 46.6), C-14 (δ_C 90.4), C-17 (δ_C 87.9), and orthoacetate (δ_C 108.3), revealed that the orthoacetate was substituted at C-14, C-17 and C-18. The HMBC correlations of an acetyl carbonyl signal at δ_C 170.5 with H-12 at δ_H 4.71 and of an acetyl carbonyl signal at δ_C 170.3 with H-20 at δ_H 4.77 established the acylation substitution positions at C-12 and C-20 as in **61**. Full examination of the spin–spin couplings in the ^{1}H NMR and 2D NMR spectroscopic data elucidated that the steroidal skeleton of **67** was the same structure as **67a** and further confirmed the structure of **67** as 12,20-di-*O*-acetyl-(14,17,18-orthoacetate)-dihydrosarcostin 3-*O*-*β*-D-thevetopyranosyl-(1→4)-*O*-*β*-D-canaropyranosyl-(1→4)-*O*-*β*-D-digitoxopyranoside (Figure 4.10).

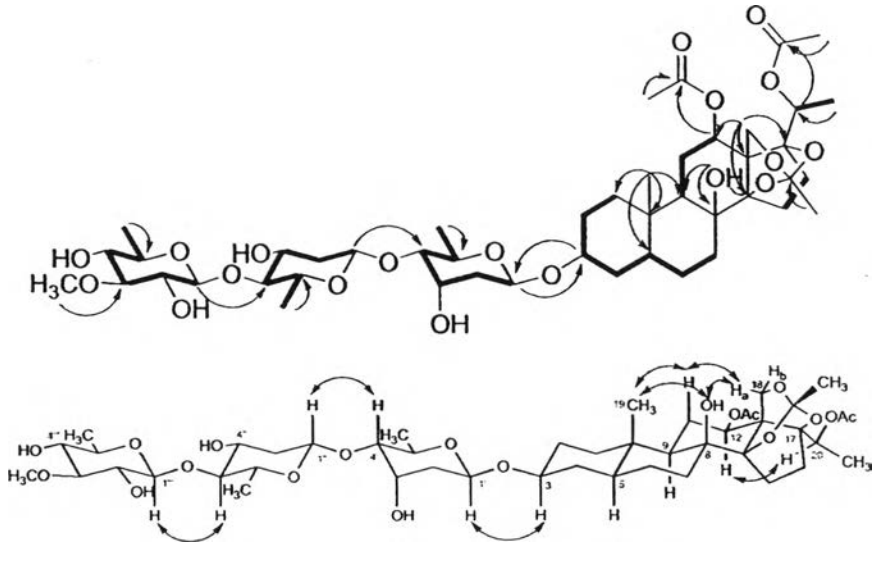


Figure 4.10 Key COSY (—), HMBC (⌒) and NOESY (⌒) correlations of **67**.

Table 4.10 NMR data of aglycone moiety of compound **67** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	38.1 *t*	0.97 *m*, 1.64 *m*	-
2	28.9 *t*	1.85 *m*, 1.52 *m*	-
3	77.1 *d*	3.63 *m*	-
4	34.0 *t*	1.67 *m*, 1.32 *m*	C-3, C-5
5	45.5 *d*	1.04 *br t* (12.4)	-
6	24.4 *t*	1.17 *m*, 1.63 *m*	-
7	34.1 *t*	1.32 *m*, 1.74 *m*	-
8	73.6 *s*	-	-
9	46.9 *d*	1.21 *m*	-
10	36.6 *s*	-	-
11	23.7 *t*	1.74 *m*, 1.74 *m*	C-12
12	70.9 *d*	4.71 *dd* (10.8, 5.8)	C-18, 12-OAc
13	46.6 *s*	-	-
14	90.4 *s*	-	-
15	32.0 *t*	1.80 *m*, 1.90 *m*	-
16	31.8 *t*	1.80 *m*, 1.80 *m*	-
17	87.9 *s*	-	-
18	61.7 *t*	4.75 *d* (8.9), 4.46 *d* (8.9)	C-13, C-14, C-17
19	12.6 *q*	0.98 *s*	C-1, C-5, C-9, C-10
20	72.1 *d*	4.77 *q* (6.4)	20-OAc
21	15.1 *q*	1.29 *d* (6.4)	C-17, C-20
Ortho acetate			
1	108.3 *s*	-	-
2	24.4 *q*	1.53 *s*	C-ortho
12-OAc			
1	170.5 *s*	-	-
2	21.4 *q*	1.97 *s*	12-OAc
20-OAc			
1	170.3 *s*	-	-
2	21.5 *q*	2.09 *s*	20-OAc
OH-8		2.57 *d* (1.1)	C-8, C-9, C-14

Table 4.10 (Cont.) NMR data of sugar moiety of compound **67** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
D-Dig			
1'	95.5 *d*	4.94 *dd* (9.5, 1.7)	C-3
2'	37.4 *t*	1.69 *m*, 2.06 *m*	-
3'	66.9 *d*	4.22 *br s*	-
4'	83.1 *d*	4.22 *br s*	C-3'
5'	68.1 *d*	3.21 *dd* (9.3, 3.4)	-
6'	18.3 *q*	3.79 *dq* (9.3, 6.2)	C-4', C-5'
D-Can			
1"	100.5 *d*	4.57 *dd* (9.8, 1.7)	C-4'
2"	38.5 *t*	1.62 *m*, 2.26 *ddd* (12.9, 5.1, 1.7)	C-1", C-3", C-4"
3"	69.5 *d*	3.60 *m*	-
4"	88.1 *d*	2.99 *t* (8.8)	C-3", C-5", C-1""
5"	70.7 *d*	3.40 *dq* (9.2, 6.1)	-
6"	18.0 *q*	1.34 *d* (6.1)	C-4", C-5"
D-Thv			
1""	103.7 *d*	4.29 *d* (7.8)	C-4"
2""	74.5 *d*	3.47 *m*	C-1""
3""	85.5 *d*	3.12 *t* (9.1)	C-2"", C-4"", 3""-OCH_3
4""	74.7 *d*	3.23 *td* (9.1, 2.9)	-
5""	72.3 *d*	3.48 *dq* (9.1, 6.1)	-
6""	17.6 *q*	1.35 *d* (6.1)	C-4"", C-5""
3""-OCH_3	61.1 *q*	3.67 *s*	C-3""

Gymnemogriffithoside H (68) was obtained as a white amorphous powder. The molecular formula of **68** was established as $C_{47}H_{74}O_{19}$, based on HRESIMS (*m/z* 965.4713 $[M+Na]^+$, calcd 965.4716). The ^1H and ^{13}C NMR signals of the aglycone and the sugar moiety of the aglycone were almost identical to those in **67** except for the presence of a methoxy signal of the trisaccharide (Table 4.11). Analysis of the 2D NMR spectroscopic data and the spin–spin couplings in the ^1H NMR elucidated the chemical structure of **68** as 12,20-di-*O*-acetyl-(14,17,18-orthoacetate)-dihydrosarcostin 3-*O*-*β*-D-thevetopyranosyl-(1→4)-*O*-*β*-D-oleandropyranosyl-(1→4)-*O*-*β*-D-digitoxopyran oside (Figure 4.11).

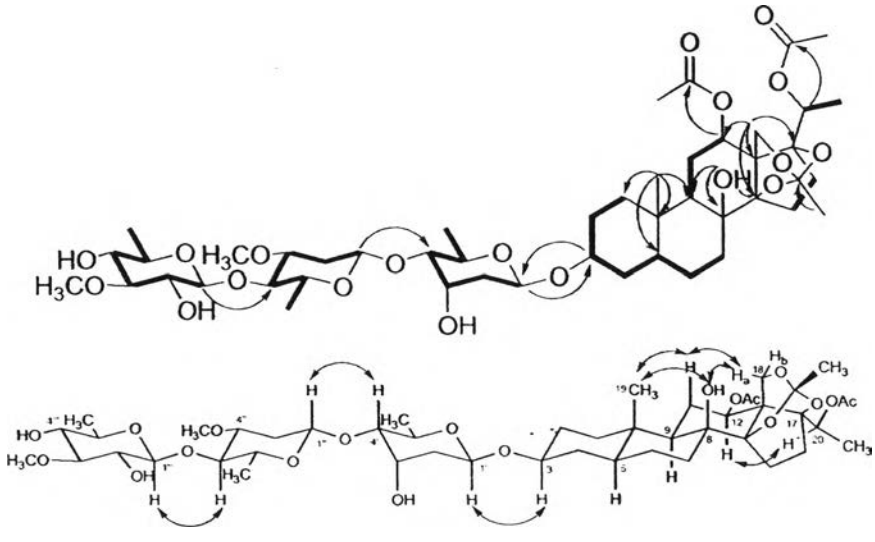


Figure 4.11 Key COSY (—), HMBC ( ) and NOESY ( ) correlations of **68**.

Table 4.11 NMR data of aglycone moiety of compound **68** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	38.1 *t*	0.95 *m*, 1.65 *m*	C-19
2	28.9 *t*	1.85 *m*, 1.55 *m*	-
3	77.2 *d*	3.62 *tt* (11.2, 4.9)	C-2, C-1'
4	34.0 *t*	1.66 *m*, 1.32 *m*	C-2, C-3, C-4
5	45.4 *d*	1.02 *br t* (12.5)	-
6	24.4 *t*	1.17 *m*, 1.66 *m*	-
7	34.1 *t*	1.32 *m*, 1.74 *m*	-
8	73.6 *s*	-	-
9	46.9 *d*	1.19 *m*	C-8, C-10, C-11, C-19
10	36.6 *s*	-	-
11	23.7 *t*	1.75 *m*, 1.75 *m*	C-9, C-12
12	70.9 *d*	4.71 *dd* (10.8, 5.7)	C-11, C-13, C-14 12-OAc
13	46.6 *s*	-	-
14	90.3 *s*	-	-
15	32.0 *t*	1.80 *m*, 1.90 *m*	C-14, C-16
16	31.8 *t*	1.80 *m*, 1.80 *m*	C-15, C-17
17	87.9 *s*	-	-
18	61.7 *t*	4.75 *d* (8.8), 4.46 *d* (8.8)	C-12, C-13, C-14, C-17, C-ortho
19	12.6 *q*	0.98 *s*	C-1, C-5, C-9, C-10
20	72.1 *d*	4.77 *q* (5.4)	C-21, 20-OAc
21	15.1 *q*	1.29 *d* (5.4)	C-17, C-20
Ortho acetate			
1	108.3 *s*	-	-
2	24.4 *q*	1.53 *s*	C-ortho
12-OAc			
1	170.5 *s*	-	-
2	21.4 *q*	1.97 *s*	12-OAc
20-OAc			
1	170.3 *s*	-	-
2	21.5 *q*	2.09 *s*	20-OAc
OH-8		2.57 *s*	C-8,C-9, C-13, C-14

Table 4.11 (Cont.) NMR data of sugar moiety of compound **68** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
D-Dig			
1'	95.5 *d*	4.94 *dd* (9.6, 1.3)	C-3
2'	37.4 *t*	168 *m*, 2.07 *m*	C-3', C-4'
3'	66.8 *d*	4.22 *br s*	C-1', C-4', C-5'
4'	83.1 *d*	3.21 *dd* (9.2, 2.8)	C-3', C-4', C-5', C-6', C-1"
5'	68.0 *d*	3.79 *dq* (9.2, 6.2)	C-3', C-4', C-6'
6'	18.4 *q*	1.24 *d* (6.2)	C-4', C-5'
D-Ole			
1"	100.3 *d*	4.53 *dd* (9.6, 1.6)	C-4', C-2"
2"	35.9 *t*	1.52 *m*, 2.37 *ddd* (12.7, 4.1, 1.6)	C-1", C-3", C-4"
3"	78.8 *d*	3.38 *m*	3"-OCH_3
4"	79.4 *d*	3.34 *m*	C-5", C-1""
5"	71.8 *d*	3.36 *m*	C-4", C-6""
6"	18.7 *q*	1.34 *d* (5.3)	C-4", C-5"
3"-OCH_3	56.2 *q*	3.39 *s*	C-3"
D-Thv			
1""	101.9 *d*	4.47 *d* (8.0)	C-4"
2""	73.4 *d*	3.46 *br t* (8.5)	C-1"", C-3""
3""	85.6 *d*	3.09 *t* (8.9)	C-1"", C-2"", C-4"", 3""-OCH_3
4""	75.0 *d*	3.16 *br t* (8.9)	C-2"", C-3"", C-4"", C-5""
5""	72.1 *d*	3.36 *m*	C-6""
6""	17.9 *q*	1.31 *d* (6.1)	C-4"", C-5""
3""-OCH_3	60.8 *q*	3.66 *s*	C-3""

The presence of the aglycones **61a** and **67a** in the CH_2Cl_2 layer from the acid hydrolysis of **61** suggested the occurrence of the acid catalyzed isomerization-cyclization of the orthoacetate group. The acid hydrolysis of the 8,14,18-orthoacetate substituted steroidal glycoside (compound **64**) and 14,17,18-orthoacetate substituted steroidal glycoside (compound **67**) were, therefore, further investigated. It was found that aglycones **61a** and **67a** were detected from both acid hydrolysates. This indicated that the acid catalyzed isomerization-cyclization of the orthoacetate substituent had arisen during the acid hydrolysis. The proposed mechanism is shown in Figure 4.12. Upon the steroidal sugar side chain cleavage by 0.05 M H_2SO_4, protonation of the alkoxy group at C-8 of **61** and **64** and C-17 of **67** occurred. The carbocation intermediate of C-orthoacetate was formed and stabilized by the intramolecular facilitation of the hydroxy group at C-8 and C-17, which was then further deprotonated to obtain both **61a** and **67a** as the final products.

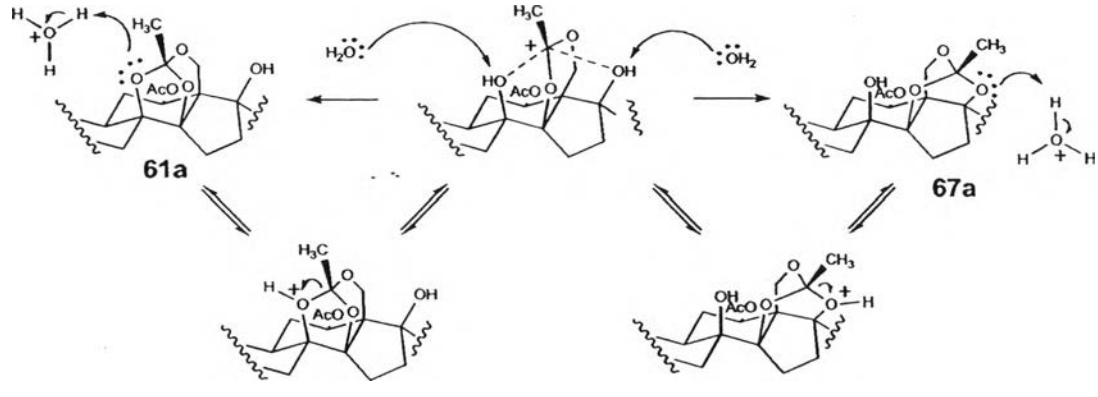


Figure 4.12 Proposed acid catalyzed isomerization-cyclization mechanism of **61a** and **67a**.

4.2 Isolation and structure elucidation of the isolated compounds from *H. curtisii*

Preliminary study of an α-glucosidase inhibitor from the pods of *H. curtisii* indicated that the methanolic extract exhibited against yeast α-glucosidase (*Saccharomyces cerevisiae*) with IC_{50} value of 423.8 μg/mL. The methanolic extract of fresh pods *of H. curtisii* was suspended in water and partitioned with CH_2Cl_2 and EtOAc, successively. The CH_2Cl_2-soluble extract was subjected to several chromatographic separation steps to give two new triterpenoids, 3β-hydroxy-11α-hydroperoxyolean-12-en-28-oic acid (**81**) and 3β-hydroxy-11α-hydroperoxyursan-12-en-28-oic acid (**82**), together with ten known triterpenoids, squalene (**83**), β-amyrin acetate (**84**), α-amyrin acetate (**85**), lupeol acetate (**86**), lupeol (**57**), lanosta-7,24-dien-3β-ol (**87**), cycloeucalenol (**88**), 24-methylenepollinastanol (**89**), oleanolic acid (**90**) and ursolic acid (**91**), while the chromatographic separation of EtOAc-soluble extract led to isolation of two known flavanols, (–)-catechin (**92**) and (–)-gallocatechin (**93**) (Figure 4.13).

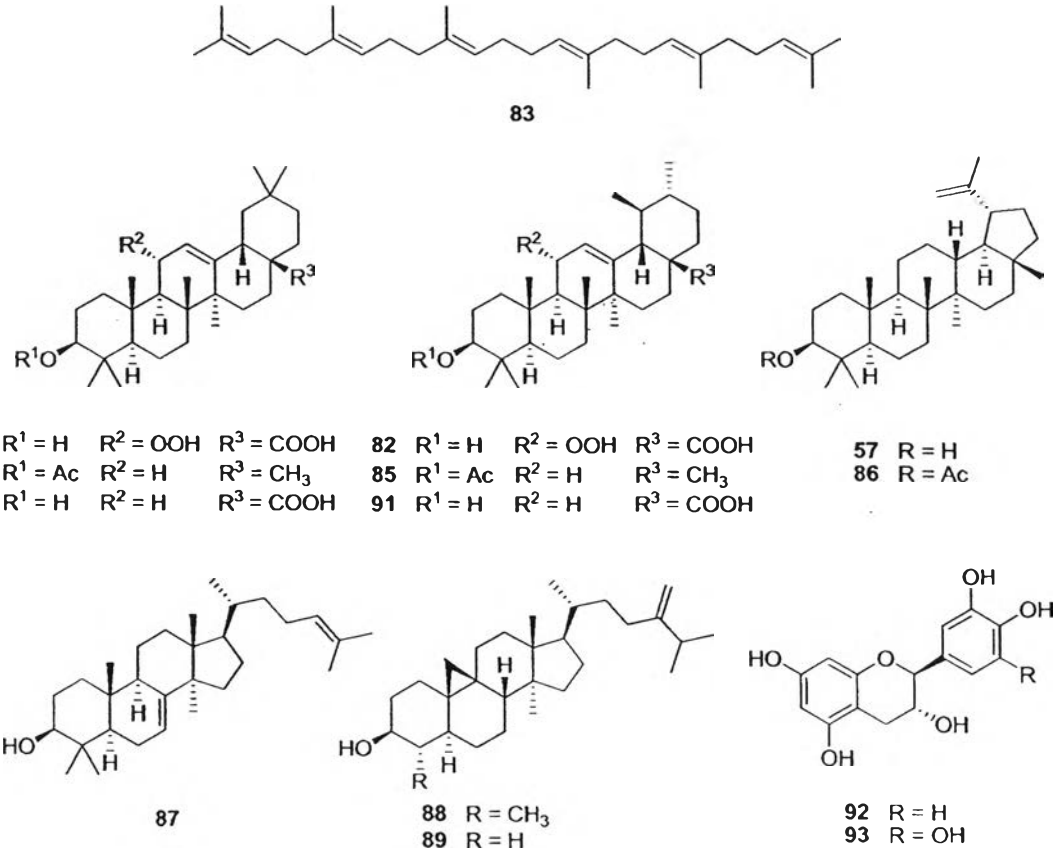


Figure 4.13 Isolated compounds from pods of *H. curtisii*.

Compound **81** was obtained as white solid. The molecular formula was established as $C_{30}H_{48}O_5$, based on its HRESIMS data (*m/z* 511.3377 $[M+Na]^+$, calcd 511.3399), suggesting seven degrees of unsaturation. The ATR-FTIR spectrum of **81** showed absorption bands for hydroxy (3385 br cm^{-1}) and carbonyl (1687 cm^{-1}) groups. The ^{13}C NMR spectrum of **81** exhibited thirty carbon signals, including seven methyl carbons, nine sp^3 methylene carbons, five sp^3 methine carbons (two oxygenated carbon at δ_C 78.7 and 81.2), six sp^3 quaternary carbons, a carbonyl carbon, and two olefinic carbons (one methine carbons at δ_C 121.8, and one quaternary carbons at δ_C 150.7) (Table 4.12). The ^1H NMR spectrum displayed, seven tertiary methyl signals at δ_H 0.71 (s, CH_3-23), 0.73 (s, CH_3-26), 0.84 (s, CH_3-30), 0.88 (s, CH_3-29), 0.91 (s, CH_3-24), 0.94 (s, CH_3-25) and 1.15 (s, CH_3-27), two oxygenated methine signals at δ_H 3.14 (t, *J* = 8.1 Hz, H-3) and 4.38 (dd, *J* = 8.5, 3.5 Hz, H-11), an olefinic proton of the Δ^{12} proton signal of pentacyclic triterpenoid at δ_H 5.52 (d, *J* = 3.5 Hz, H-12), and a doublet of doublet for a methine signal at δ_H 2.83 (dd, *J* = 14.3, 3.8 Hz, H-18) ascribed to H-18 of Δ^{12} proton signal of oleanane skeleton [67]. By detailed analyses of the NMR spectroscopic data (HSQC, COSY, HMBC and NOESY) and comparison of the ^1H and ^{13}C NMR spectra of **81** with oleanolic acid (**91**) indicated no difference between two compounds except the presence of oxygenated methine signal instead of those of the methylene signal in oleanolic acid (**91**). The oxygenated methine was assigned to C-11 position due to the observed HMBC correlations of olefinic proton at δ_H 5.52 (H-12) to oxygenated methine at δ_C 81.2 (C-11), and oxygenated methine signal δ_H 4.38 (H-11) to carbon signals at δ_C 50.7 (C-9), 38.0 (C-10), 121.8 (C-12) and 150.7 (C-13). The position of oxygenated methine C-11 was confirmed by the presence of correlations in COSY spectrum between; δ_H 1.65 (H-9) and δ_H 4.38 (H-11), and δ_H 4.38 (H-11) and δ_H 5.52 (H-12). Thus the structure of **81** was established (Figure 4.14). The presence of downfield-shift of the H-11 signal at δ_H 4.38 and δ_C 81.2 of compound **81** when comparing to 3β,11α-dihydroxyurs-12-en-28-oic acid at δ_H 3.99 (H-11) and δ_C 65.8 (C-11) [68] and 11α-hydroxy-β-amyrin at δ_H 4.19 (H-11) and δ_C 67.6 (C-11) [69] in the previous reports, suggested the occurrence of hydroperoxy group instead of hydroxyl group [59, 60]. Also, the presence of 11α-hydroperoxy group was supported by ^1H and ^{13}C chemical shift of **81** in accordance with H-11 (δ_H 4.48) and C-11 (δ_C 81.0) of 11α-hydroperoxy-diacetyl-hederagenin [61] and by a loss of hydroperoxy group (*m/z* 455.3512 $[M-OOH]^+$) in ESIMS data. Since the large coupling constant ($J \approx 8$ Hz) of H-11 suggested an axial orientation, the hydroperoxy was therefore assigned in an

equatorial position [70]. The relative configuration of **81** was demonstrated by NOESY experiment. The observed NOEs between: CH_3-23 and CH_3-25, CH_3-25 and CH_3-26, CH_3-25 and H-11, CH_3-26 and H-11, H-11 and H-12, and H-12 and H-18 indicated a β-orientation of these protons. Additionally, the observed NOEs between: H-3 and H-5, H-5 and H-9, H-9 and CH_3-27 indicated an α-orientation of these protons. Base on spectroscopic data above, compound **81** was identified as 3β-hydroxy-11α-hydroperoxyolean-12-en-28-oic acid (Figure 4.14).

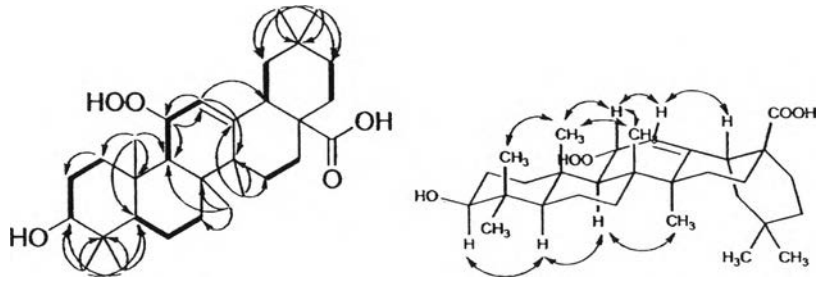


Figure 4.14 Key COSY (—), HMBC (⌒) and NOESY (⌒) correlations of **81**.

Table 4.12 NMR data of compound **81** in $CDCl_3$:CD_3OD (10:1)

Position	^{13}C	^{1}H	HMBC
1	39.3 *t*	1.16 *m*, 1.89 *m*	C-2, C-3, C-5, C-25
2	27.0 *t*	1.54 *m*, 1.54 *m*	-
3	78.7 *d*	3.14 *t* (8.1)	C-4, C-23
4	39.0 *s*	-	-
5	55.2 *d*	0.70 *m*	-
6	18.4 *t*	1.31 *m*, 1.51 *m*	-
7	33.0 *t*	1.19 *m*, 1.35 *m*	-
8	41.9 *s*	-	-
9	50.7 *d*	1.65 *m*	C-5, C-8, C-10, C-14, C-25, C26
10	38.0 *s*	-	-
11	81.2 *d*	4.38 *dd* (8.5, 3.5)	C-9, C-10, C-12, C-13
12	121.8 *d*	5.52 *d* (3.5)	C-9, C-11, C-13, C-14, C-18
13	150.7 *s*	-	-
14	42.8 *s*	-	-
15	28.0 *t*	1.02 *m*, 1.59 *m*	-
16	22.9 *t*	1.57 *m*, 1.92 *m*	-
17	46.1 *s*	-	-
18	40.9 *d*	2.83 *dd* (14.3, 3.8)	-
19	45.6 *t*	1.20 *m*, 1.55 *m*	C-18
20	30.7 *s*	-	-
21	33.9 *t*	1.15 *m*, 1.29 *m*	-
22	32.5 *t*	1.51 *m*, 1.68 *td* (13.8, 4.2)	-
23	15.6 *q*	0.71 *s*	C-3, C-4, C-5, C-24
24	28.1 *q*	0.91 *s*	C-3, C-4, C-5, C-23
25	16.8 *q*	0.94 *s*	C-1, C-4, C-5, C-9, C-10
26	18.6 *q*	0.73 *s*	C-7, C-8, C-9, C-14
27	24.7 *q*	1.15 *s*	C-8, C-13, C-14, C-15
28	181.0 *s*	-	-
29	23.5 *q*	0.88 *s*	C-19, C-20, C-21, C-30
30	33.0 *q*	0.84 *s*	C-18, C-19, C-20, C-21, C-29

Compound **82** was obtained as white solid. The molecular formula was established as $C_{30}H_{48}O_5$, based on its HRESIMS data (*m/z* 511.3391 $[M+Na]^+$, calcd 511.3399, and *m/z* 455.3463 $[M-OOH]^+$, calcd 455.3525), indicating the same molecular formula as that for **81**. The ATR-FTIR spectrum of **82** showed absorption bands for hydroxy (3366 br cm^{-1}) and carbonyl (1686 cm^{-1}) groups. The ^{13}C NMR spectrum of **82** exhibited thirty carbon signals, including seven methyl carbons, eight sp^3 methylene carbons, seven sp^3 methine carbons (two oxygenated carbon at δ_C 78.5 and 81.4), five sp^3 quaternary carbons, a carbonyl carbon, and two olefinic carbons (one methine carbons at δ_C 125.6, and one quaternary carbons at δ_C 143.8) (Table 4.13). The ^1H NMR spectrum displayed, five tertiary methyl signals at δ_H 0.67 (s, CH_3-23), 0.74 (s, CH_3-26), 0.87 (s, CH_3-24), 0.93 (s, CH_3-25) and 1.05 (s, CH_3-27), two secondary methyl signals at δ_H 0.85 (d, J = 5.9 Hz, CH_3-30) and 0.88 (d, J = 6.1 Hz, CH_3-29), two oxygenated methine signals at δ_H 3.09 (t, *J* = 7.7 Hz, H-3) and 4.36 (d, *J* = 8.1 Hz, H-11), broad singlet for an olefinic proton at δ_H 5.42 (br s, H-12), and a doublet signal at δ_H 2.16 (d, *J* = 11.2 Hz, H-18) for a methine signal of H-18 of Δ^{12} ursane skeleton [67]. Also comparison of the ^1H and ^{13}C NMR spectra of **82** with **81** revealed that **82** had the same degree of unsaturation as **81**, and **82** consisted of five tertiary methyl signals and two secondary methyl signals while **81** consisted of seven tertiary methyl signals, suggesting that **82** have the ursane skeleton. Additionally, comparison of ^1H and ^{13}C NMR spectra of **82** with those previously reported of 3β-acetoxy-11α-hydroperoxyursan-12-en-28-oic acid [71] indicated no difference between two compounds except for replacement of the 3β-acetyl group by a 3β-hydroxy group. The relatively upfield-shift of H-3 at δ_H 3.09 and C-3 at δ_C 78.5 of compound **82** instead of H-3 at δ_H 4.52 and C-3 at δ_C 80.5 was consistent with no acylation at C-3. Full examination of the NMR spectroscopic data further confirmed the structure of **82** as 3β-hydroxy-11α-hydroperoxyursan-12-en-28-oic acid (Figure 4.15).

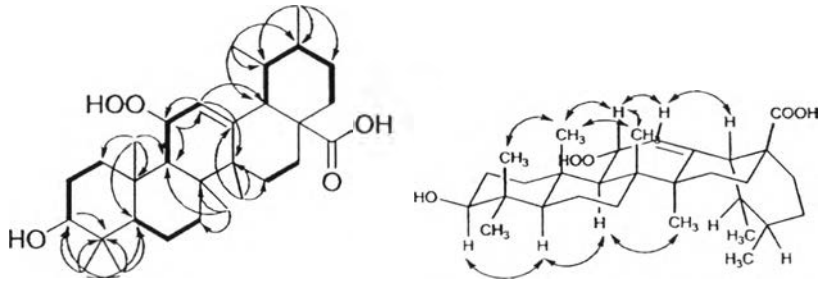


Figure 4.15 Key COSY (—), HMBC (⌒) and NOESY (⌒) correlations of **82**.

Table 4.13 NMR data of compound **82** in $CDCl_3$:CD_3OD (10:1)

Position	^{13}C	^{1}H	HMBC
1	39.5 *t*	1.11 *m*, 1.96 *m*	C-2, C-3, C-5, C-10, C-25
2	27.0 *t*	1.49 *m*, 1.49 *m*	-
3	78.5 *d*	3.09 *t* (7.7)	C-23, C-25
4	38.9 *s*	-	-
5	55.2 *d*	0.65 *m*	-
6	18.3 *t*	1.24 *m*, 1.45 *m*	-
7	33.5 *t*	1.17 *m*, 1.34 *m*	-
8	42.1 *s*	-	-
9	49.9 *d*	1.60 *m*	C-1, C-5, C-8, C-10, C-25
10	37.8 *s*	-	-
11	81.4 *d*	4.36 *d* (8.1)	C-9,C-10, C-12, C-13
12	125.6 *d*	5.42 *br s*	C-11, C-13, C-18, C-27
13	143.8 *s*	-	-
14	42.6 *s*	-	-
15	28.2 *t*	0.98 *m*, 1.72 *m*	-
16	24.1 *t*	1.56 *m*, 1.91 *m*	-
17	47.4 *s*	-	-
18	52.3 *d*	2.16 *d* (11.2)	-
19	38.8 *d*	1.22 *m*	-
20	38.9 *d*	0.92 *m*	-
21	30.5 *t*	1.21 *m*, 1.40 *m*	-
22	36.7 *t*	1.52 *m*, 1.61 *m*	-
23	15.5 *q*	0.67 *s*	C-3, C-4, C-5, C-24
24	28.1 *q*	0.87 *s*	C-3, C-4, C-5, C-23
25	16.5 *q*	0.93 *s*	C-1, C-5, C-9, C-10
26	18.4 *q*	0.74 *s*	C-7, C-8, C-9
27	22.4 *q*	1.05 *s*	C-9, C-13
28	180.8 *s*	-	-
29	16.9 *q*	0.88 *d* (6.1)	C-18, C-19, C-20
30	21.0 *q*	0.85 *d* (5.9)	C-19, C-20, C-21

Compound **83** was obtained as yellow oil. The ^{13}C NMR spectrum of **83** exhibited fifteen carbon signals, including four methyl carbons, five sp^3 methylene carbons and six olefinic carbons (three methine carbons at δ_C 124.4, 124.5 and 124.6, and three quaternary carbons at δ_C 131.4, 135.0 and 135.5) (Table 4.14). The ^1H NMR spectrum showed four tertiary methyl signals at δ_H 1.61 (br s, *cis*-allylic-CH_3, CH_3-13, CH_3-14, CH_3-15) and 1.68 (br s, *tran*-allylic-CH_3, CH_3-1), three olefinic proton at δ_H 5.15 (m, H-11) and 5.11 (m, H-3, H-7), and three methylene signals at δ_H 2.08 (m, CH_2-4, CH_2-8) and 2.02 08 (m, CH_2-2). The integral ratio of *tran*- to *cis*-allylic methyls was closely to 3.00, suggested the presence of all-*tran*-isomer squalene. Based on spectroscopic data above, compound **83** was identified as squalene. The structure of compound **83** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Pogliani and co-worker in 1994 [72] (Figure 4.16).

Figure 4.16 Key COSY (—) and HMBC (⌒) correlations of **83**.

Table 4.14 NMR data of compound **83** in $CDCl_3$

Position	^{13}C	^1H	HMBC
1	25.8 *q*	1.68 *br s*	C-2, C-3, C-13
2	131.4 *s*	-	-
3	124.6 *d*	5.11 *m*	C-1, C-4, C-5, C-13
4	26.9 *t*	2.08 *m* 2.08 *m*	C-2, C-3, C-5, C-6
5	39.9 *t*	1.98 *m*, 1.98 *m*	C-3, C-4, C-6, C-7, C-14
6	135.3 *s*	-	-
7	124.5 *d*	5.11 *m*	C-5, C-8, C-9, C-14
8	26.8 *t*	2.08 *m* 2.08 *m*	C-6, C-7, C-9, C-10
9	39.9 *t*	1.98 *m*, 1.98 *m*	C-7, C-8, C-10, C-11, C-15
10	135.0 *s*	-	-
11	124.4 *d*	5.15 *m*	C-12, C-15
12	28.4 *t*	2.02 *m*, 2.02 *m*	C-10, C-11, C-12'
13	17.8 *q*	1.61 *br s*	C-1, C-2, C-3
14	16.2 *q*	1.61 *br s*	C-5, C-6, C-7
15	16.1 *q*	1.61 *br s*	C-9, C-10, C-11

Compound **84** was obtained as white pale solid. The ^{13}C NMR spectrum of **84** exhibited thirty two carbon signals, including nine methyl carbons, ten sp^3 methylene carbons, four sp^3 methine carbons (one oxygenated carbon at δ_C 81.1), six sp^3 quaternary carbons, a carbonyl carbon, and two olefinic carbons (one methine carbons at δ_C 121.8, and one quaternary carbons at δ_C 145.4) (Table 4.15). The ^1H NMR spectrum displayed, acetyl signals at δ_H 2.05 (s, 3-OAc), eight tertiary methyl signals at δ_H 0.83 (m, CH_3-28), 0.87 (s, CH_3-23, CH_3-24, CH_3-29, CH_3-30), 0.96 (s, CH_3-25, CH_3-26), and 1.13 (s, CH_3-27), one oxygenated methine signal at δ_H 4.50 (t, J = 7.5 Hz, H-3), and broad singlet for an olefinic proton at δ_H 5.18 (br s, H-12), characteristic of the Δ^{12} proton signal of pentacyclic triterpenoid skeleton [67]. The HMBC correlations of the acetyl carbonyl signals at δ_C 171.2 with H-3 at δ_H 4.50 established the acylation substitution position at C-3. Based on spectroscopic data above, compound **84** was identified as β-amyrin acetate. The structure of compound **84** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Xue and co-workers in 2010 [73] (Figure 4.17).

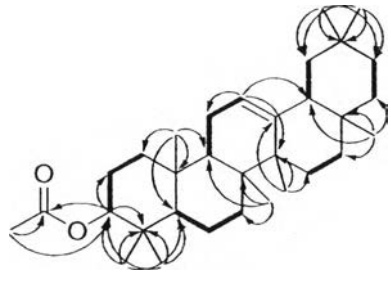


Figure 4.17 Key COSY (—) and HMBC (⌒) correlations of **84**.

Table 4.15 NMR data of compound **84** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	38.4 *t*	1.05 *m*, 1.63 *m*	C-2, C-5, C-10
2	23.7 *t*	1.62 *m*, 1.62 *m*	C-3
3	81.1 *d*	4.50 *t* (7.5)	C-2, C-4, C-23, C-24, 3-OAc
4	37.9 *s*	-	-
5	55.4 *d*	0.85 *m*	C-4, C-10, C-23, C-25
6	18.4 *t*	1.41 *m*, 1.53 *m*	-
7	32.6 *t*	1.33 *m*, 1.51 *m*	-
8	40.0 *s*	-	-
9	47.7 *d*	1.57 *m*	C-8, C-10, C-11, C-25
10	37.0 *s*	-	-
11	23.7 *t*	1.86 *m*, 1.86 *m*	C-9, C-12
12	121.8 *d*	5.18 *br s*	C11,C14,C18
13	145.4 *s*	-	-
14	41.9 *s*	-	-
15	26.3 *t*	0.94 *m*, 1.76 *m*	-
16	27.1 *t*	0.79 *m*, 1.98 *m*	C-17, C-28
17	32.7 *s*	-	
18	47.4 *d*	1.94 *m*	C-17
19	46.9 *t*	1.02 *m*, 1.66 *m*	-
20	31.2 *s*	-	-
21	34.9 *t*	1.11 *m*, 1.31 *m*	-
22	37.3 *t*	1.22 *m*, 1.41 *m*	-
23	16.8 *q*	0.86 *s*	C-2, C-3, C-4, C-5, C-24
24	28.2 *q*	0.87 *s*	C-2, C-3, C-4, C-5, C-23
25	15.7 *q*	0.96 *s*	C-1, C-5, C-9, C-10
26	17.0 *q*	0.97 *s*	C-7, C-8, C-9, C-14
27	26.1 *q*	1.13 *s*	C-8, C-13, C-14, C-15
28	28.5 *q*	0.82 *s*	C-16, C-17, C-18, C-22
29	23.8 *q*	0.87 *s*	C-19, C-20, C-21, C-30
30	33.5 *q*	0.87 *s*	C-19, C-20, C-21, C-29
3-OAc			
1	171.2 *s*	-	-
2	21.5 *q*	2.05 *s*	C-3, 3-OAc

Compound **85** was obtained as white pale solid. The ^{13}C NMR spectrum of **85** exhibited thirty two carbon signals, including nine methyl carbons, nine sp^3 methylene carbons, six sp^3 methine carbons (one oxygenated carbon at δ_C 81.1), five sp^3 quaternary carbons, a carbonyl carbon, and two olefinic carbons (one methine carbons at δ_C 124.5, and one quaternary carbons at δ_C 139.8) (Table 4.16). The ^1H NMR spectrum displayed, acetyl signals at δ_H 2.05 (s, 3-OAc), six tertiary methyl signals at δ_H 0.79 (m, CH_3-28), 0.86 (s, CH_3-23), 0.87 (s, CH_3-24), 0.97 (s, CH_3-25), 1.00 (s, CH_3-26), and 1.06 (s, CH_3-27), two secondary methyl signals at δ_H 0.79 (m, CH_3-29), 0.91 (d, J = 5 Hz, CH_3-30), one oxygenated methine signal at δ_H 4.50 (dd, J = 8.9, 7.1 Hz, H-3), and broad singlet for an olefinic proton at δ_H 5.12 (br s, H-12), characteristic of the Δ^{12} proton signal of pentacyclic triterpenoid skeleton [67]. The HMBC correlations of the acetyl carbonyl signals at δ_C 171.1 with H-3 at δ_H 4.50 established the acylation substitution position at C-3. Based on spectroscopic data above, compound **85** was identified as α-amyrin acetate. The structure of compound **85** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Niaz Ali in 2013 [53] (Figure 4.18).

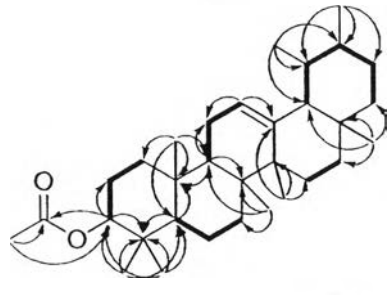


Figure 4.18 Key COSY (—) and HMBC (⌒) correlations of **85**.

Table 4.16 NMR data of compound **85** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	38.7 *t*	1.09 *m*, 1.66 *m*	C-2, C-5, C-10
2	23.8 *t*	1.63 *m*, 1.63 *m*	C-3
3	81.1 *d*	4.5 0 *dd* (8.9, 7.1)	C-2, C-4, C-23, C-24, 3-OAc
4	37.9 *s*	-	-
5	55.5 *d*	0.84 *m*	C-4, C-10, C-23, C-25
6	18.4 *t*	1.40 *m*, 1.53 *m*	-
7	33.1 *t*	1.35 *m*, 1.54 *m*	-
8	40.2 *s*	-	-
9	47.8 *d*	1.54 *m*	C-5, C-8, C-10, C-11, C-25
10	37.0 *s*	-	-
11	23.5 *t*	1.91 *m*, 1.91 *m*	C-9, C-12
12	124.5 *d*	5.12 *br s*	C-9, C-11, C-13, C-14, C-18
13	139.8 *s*	-	-
14	42.3 *s*	-	-
15	26.8 *t*	0.97 *m*, 1.82 *td* (13.5, 5.1)	C-8, C-14, C16
16	28.2 *t*	0.84 *m*, 2.00 *m*	C-15, C-17, C-28
17	33.9 *s*	-	-
18	59.3 *d*	1.31 *m*	C-12, C-13, C-19
19	39.8 *d*	0.87 *m*	-
20	39.8 *d*	1.31 *m*	-
21	31.4 *t*	1.38 *m*, 1.26 *m*	-
22	41.7 *t*	1.27 *m*, 1.42 *m*	-
23	16.9 *q*	0.86 *s*	C-2, C-3, C-4, C-5, C-24
24	28.3 *q*	0.87 *s*	C-2, C-3, C-4, C-5, C-23
25	15.9 *q*	0.97 *s*	C-1, C-5, C-9, C-10
26	17.0 *q*	1.00 *s*	C-7, C-8, C-9, C-14
27	23.4 *q*	1.06 *s*	C-8, C-13, C-14, C-15
28	28.9 *q*	0.79 *m*	C-16, C-17, C-18, C-22
29	17.7 *q*	0.79 *m*	C-18, C-19, C-20
30	21.5 *q*	0.92 *d* (5.0)	C-19, C-20, C-21
3-OAc			
1	171.1 *s*	-	-
2	21.4 *q*	2.05 *s*	C-3, 3-OAc

Compound **86** was obtained as white pale solid. The ^{13}C NMR spectrum of **86** exhibited thirty two carbon signals, including eight methyl carbons, ten sp^3 methylene carbons, six sp^3 methine carbons (one oxygenated carbon at δ_C 81.2), five sp^3 quaternary carbons, a carbonyl carbon, and two vinylic carbons (one exomethylene carbons at δ_C 109.5, and one quaternary carbons at δ_C 151.1) (Table 4.17). The ^{1}H NMR spectrum displayed, acetyl signals at δ_H 2.04 (s, 3-OAc), six tertiary methyl signals at δ_H 0.78 (s, CH_3-28), 0.83 (s, CH_3-23), 0.84 (s, CH_3-24), 0.85 (s, CH_3-25), 0.93 (s, CH_3-27) and 1.02 (s, CH_3-26), vinylic methyl signal at δ_H 1.68 (s, CH_3-30), one oxygenated methine proton at δ_H 4.46 (dd, J = 9.6, 6.6 Hz, H-3), and two exomethylene signals at δ_H 4.68 (s, H-29a) and 4.56 (s, H-29b), characteristic of the lupane triterpenoid skeleton [67]. The HMBC correlations of the acetyl carbonyl signals at δ_C 171.1 with H-3 at δ_H 4.46 established the acylation substitution position at C-3. Based on spectroscopic data above, compound **86** was identified as lupeol acetate. The structure of compound **86** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Prachayasittikul and co-workers in 2010 [74] (Figure 4.19).

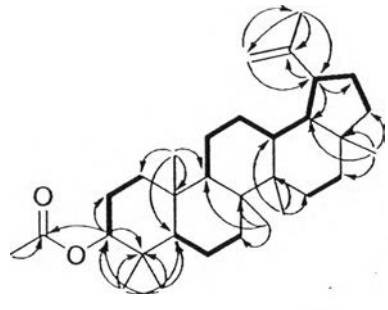


Figure 4.19 Key COSY (—) and HMBC (⌒) correlations of **86**.

Table 4.17 NMR data of compound **86** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	38.6 *t*	0.98 *m*, 1.67 *m*	-
2	23.9 *t*	1.61 *m*, 1.61 *m*	-
3	81.2 *d*	4.46 *dd* (9.6, 6.6)	C-2, C-3, C-23, C-24, 3-OAc
4	38.0 *s*	-	-
5	55.6 *d*	0.79 *qd* (12.6, 4.7)	-
6	18.4 *t*	1.41 *m*, 1.50 *m*	-
7	34.4 *t*	1.39 *m*, *1.41 m*	-
8	41.1 *s*	-	-
9	50.6 *d*	1.30 *m*	-
10	37.3 *s*	-	-
11	21.1 *t*	1.23 *m*, 1.41 *m*	-
12	25.3 *t*	1.07 *m*, 1.67 *m*	-
13	38.3 *d*	1.64 *m*	C-14
14	43.0 *s*	-	-
15	27.6 *t*	1.00 *m*, 1.68 *m*	-
16	35.8 *t*	1.48 *m*	-
17	43.2 *s*	-	-
18	48.5 *d*	1.37 *m*	-
19	48.2 *d*	2.37 *td* (10.9)	C-18, C-20, C-21, C-22, C-29, C-30
20	151.1 *s*	-	-
21	30.0 *t*	1.33 *m*, 1.92 *m*	-
22	40.2 *t*	1.21 *m*, 1.38 *m*	-
23	16.6 *q*	0.83 *s*	C-3, C-4, C-5, C-24
24	28.1 *q*	0.84 *s*	C-3, C-4, C-5, C-23
25	16.3 *q*	0.85 *s*	C-1, C-5, C-9, C-10
26	16.2 *q*	1.02 *s*	C-7, C-8, C-9, C-14
27	14.7 *q*	0.93 *s*	C-8, C-13, C-14, C-15
28	18.2 *q*	0.78 *s*	C-16, C-17, C-18, C-22
29	109.5 *t*	4.56 *s*, 4.68 *s*	C-19, C-30
30	19.5 *q*	1.68 *s*	C-19, C-20, C-29
3-OAc			
1	171.1 *s*	-	-
2	21.4 *q*	2.04 *s*	3-OAc

Compound **57** was obtained as white crystalline solid. The ^{13}C NMR spectrum of **57** exhibited thirty carbon signals, including seven methyl carbons, ten sp^3 methylene carbons, six sp^3 methine carbons (one oxygenated carbon at δ_C 79.2), five sp^3 quaternary carbons, and two vinylic carbons (one exomethylene carbons at δ_C 109.5, and one quaternary carbons at δ_C 151.1) (Table 4.18). The ^1H NMR spectrum displayed six tertiary methyl signals at δ_H 0.76 (s, CH_3-23), 0.78 (s, CH_3-28), 0.82 (s, CH_3-25), 0.94 (s, CH_3-27), 0.96 (3H, s, CH_3-24) and 1.02 (s, CH_3-26), vinylic methyl signal at δ_H 1.68 (s, CH_3-30), one oxygenated methine signal at δ_H 3.18 (dd, J = 10.2, 5.0 Hz, H-3), and two exomethylene signals at δ_H 4.68 (s, H-29a) and 4.56 (s, H-29b), characteristic of the lupane triterpenoid skeleton [67]. Based on spectroscopic data above, compound **57** was identified as lupeol. The structure of compound **57** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Fotie and co-workers in 2006 [55] (Figure 4.20).

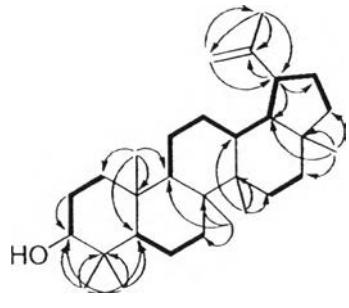


Figure 4.20 Key COSY (—) and HMBC (⌒) correlations of **57**.

Table 4.18 NMR data of compound **57** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	38.9 *t*	0.91 *m*, 1.66 *m*	-
2	27.6 *t*	0.98 *m*, 1.59 *m*	C-3
3	79.2 *d*	3.18 *dd* (10.2, 5.0)	C-23, C-24
4	39.0 *s*	-	-
5	55.5 *d*	0.68 *m*	C-4, C-6, C-7, C-10, C-23,
6	18.5 *t*	1.39 *m*, 1.52 *m*	-
7	34.5 *t*	1.38 *m*, 1.38 *m*	-
8	41.0 *d*	-	-
9	50.6 *d*	1.27 *m*	C-1, C-8, C-10, C-25
10	37.3 *s*	-	-
11	21.1 *t*	1.23 *m*, 1.41 *m*	-
12	25.3 *t*	1.07 *m*, 1.66 *m*	C-18
13	38.2 *d*	1.65 *m*	-
14	43.0 *s*	-	-
15	27.6 *t*	1.02 *m*, 1.71 *m*	-
16	35.8 *t*	1.38 *m*, 1.48 *m*	C-28
17	43.2 *s*	-	-
18	48.5 *d*	1.36 *m*	-
19	48.2 *d*	2.38 *td* (11.2, 6.0)	C-13, C-18, C-20, C-21, C-29, C-30
20	151.1 *s*	-	-
21	30.0 *t*	1.33 *m*, 1.91 *m*	-
22	40.2 *t*	1.20 *m*, 1.38 *m*	-
23	15.5 *q*	0.76 *s*	C-3, C-4, C-5, C-24
24	28.2 *q*	0.96 *s*	C-3, C-4, C-5, C-23
25	16.3 *q*	0.82 *s*	C-1, C-5, C-9, C-10
26	16.1 *q*	1.02 *s*	C-7, C-8, C-9, C-13
27	14.7 *q*	0.94 *s*	C-8, C-13, C-14, C-15
28	18.2 *q*	0.78 *s*	C-16, C-17, C-18, C-21
29	109.5 *t*	4.56 *s*, 4.68 *s*	C-19, C-30
30	19.5 *q*	1.68 *s*	C-19, C-29, C-20

Compound **87** was obtained as colorless gum. The molecular formula was established as $C_{30}H_{50}O$, based on its HRESIMS data (*m/z* 427.3904 $[M+H]^+$, calcd 427.3940), suggesting six degrees of unsaturation. The ^{13}C NMR spectrum of **87** exhibited thirty carbon signals, including eight methyl carbons, nine sp^3 methylene carbons, five sp^3 methine carbons (one oxygenated carbon at δ_C 79.3), four sp^3 quaternary carbons, and four olefinic carbons (two methine carbons at δ_C 117.9 and 125.2, and two quaternary carbons at δ_C 145.9 and 131.0) (Table 4.19). The ^1H NMR spectrum displayed seven tertiary methyl signals at δ_H 0.73 (s, CH_3-19), 0.79 (s, CH_3-18), 0.84 (s, CH_3-28), 0.95 (s, CH_3-29), 0.96 (s, CH_3-30), 1.59 (s, CH_3-27) and 1.66 (s, CH_3-26), one secondary methyl signal at δ_H 0.83 (d, J = 5.9 Hz, CH_3-21), one oxygenated methine signal at δ_H 3.22 (dd, J = 11.1, 4.2 Hz, H-3), and two olefinic proton at δ_H 5.23 (br s, H-7) and 5.08 (t, J = 7.0 Hz, H-24). Based on spectroscopic data above, compound **87** was identified as lanosta-7,24-dien-3β-ol. The structure of compound **87** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Furukawa and co-workers in 2002 [56] (Figure 4.21).

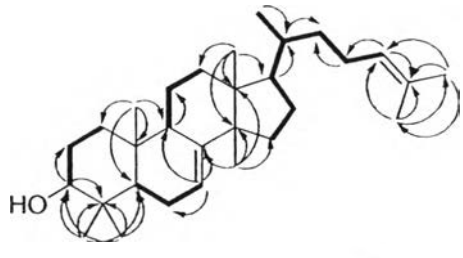


Figure 4.21 Key COSY (—) and HMBC ( ) correlations of **87**.

Table 4.19 NMR data of compound **87** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	37.3 *t*	1.12 *td* (12.5, 4.3), 1.66 *m*	C-2, C-10, C-11, C-19
2	27.7 *t*	1.64 *m*, 1.64 *m*	C-1, C-3
3	79.3 *d*	3.22 *dd* (11.1, 4.2)	C-1, C-4, C-23, C-24
4	39.0 *s*	-	-
5	50.7 *d*	1.31 *dd* (5.8, 12.3)	C-3, C-4, C-5, C-6, C-9, C-10, C-19, C-29, C-30
6	24.0 *t*	1.98 *m* , 2.14 *m*	C-5, C-7, C-8, C-9, C-10
7	117.9 *d*	5.23 *br s*	C-5, C-6, C-7, C-14
8	145.9 *s*	-	-
9	48.9 *d*	2.19 *m*	C19, C11
10	35.0 *s*	-	-
11	18.2 *t*	1.51 *m*, *1.51 m*	C-8, C-9, C-14
12	33.9 *t*	1.64 *m*, 1.79 *m*	C-11, C-13, C-14, C-17, C-18
13	51.3 *s*	-	-
14	43.6 *s*	-	-
15	34.0 *t*	1.44 *m*, *1.44 m*	C-13, C-14, C-30
16	25.4 *t*	2.03 *m*, 1.87 *m*	-
17	53.3 *d*	1.48 *m*	C-12, C-13, C-14, C-18, C-20
18	22.2 *q*	0.79 *s*	C-11, C-12, C-13, C-16
19	13.2 *q*	0.73 *s*	C-1, C-5, C-9, C-10
20	35.9 *d*	1.40 *m*	-
21	18.7 *q*	0.83 *s*	C-17, C-20
22	35.2 *t*	0.99 *m*, 1.58 *m*	-
23	28.6 *t*	1.26 *m*, 1.93 *m*	-
24	125.2 *d*	5.08 *t* (7.0)	C-22, C-23, C-26, C-27
25	131.0 *s*	-	-
26	25.9 *q*	1.66 *s*	C-24, C-25, C-27
27	17.8 *q*	1.59 *s*	C-24, C-25, C-26
28	14.9 *q*	0.84 *s*	C-3, C-4, C-5, C-29
29	27.7 *q*	0.96 *s*	C-3, C-4, C-5, C-28
30	27.4 *q*	0.97 *s*	C-8, C-13, C-14, C-15

Compound **88** was obtained as colorless needle crystals. The molecular formula was established as $C_{30}H_{50}O$, based on its HRESIMS data (*m/z* 427.3924 $[M+H]^+$, calcd 427.3940), suggesting six degrees of unsaturation. The ^{13}C NMR spectrum of **87** exhibited thirty carbon signals, including six methyl carbons, eleven sp^3 methylene carbons, seven sp^3 methine carbons (one oxygenated carbon at δ_C 76.7), four sp^3 quaternary carbons, and two olefinic carbons (one exomethylene carbons at δ_C 106.0, one one quaternary carbons at δ_C 157.0) (Table 4.20). The ^1H NMR spectrum displayed two tertiary methyl signals at δ_H 0.89 (s, CH_3-30) and 0.96 (s, CH_3-18), four secondary methyl signal at δ_H 0.89 (d, *J* = 5.7 Hz, CH_3-21), 0.97 (d, *J* = 7.2 Hz, CH_3-29) and 1.02 (d, *J* = 6.8 Hz, CH_3-26, CH_3-27), one oxygenated methine signal at δ_H 3.21 (td, *J* = 10.5, 4.7 Hz, H-3), two exomethylene signals at δ_H 4.71 (s, H-28a) and 4.66 (s, H-28b), and two methylene proton signals at δ_H 0.38 (d, *J* = 3.8 Hz, H-19a) and 0.14 (1H, d, *J* = 3.8 Hz, H-19b), characteristic of the C-19 methylene protons of cyclopropane ring of a cycloartane triterpenoid skeleton [75]. Based on spectroscopic data above, compound **88** was identified as cycloeucalenol. The structure of compound **88** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Song and co-workers in 2007 [57] (Figure 4.22).

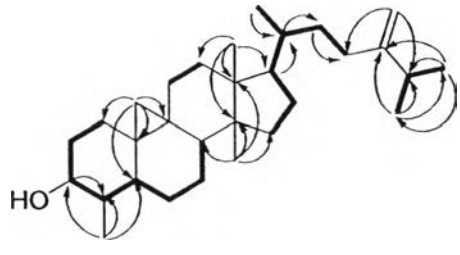


Figure 4.22 Key COSY (—) and HMBC (⌒) correlations of **88**.

Table 4.20 NMR data of compound **88** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	30.9 *t*	1.27 *m*, 1.54 *m*	C-2, C-10, C-18
2	34.9 *t*	1.40 *m*, 1.98 *m*	C-3
3	76.7 *d*	3.21 *td* (10.5, 4.7)	C-4,C-28
4	44.7 *d*	1.15 *m*	-
5	43.4 *d*	1.18 *m*	-
6	24.8 *t*	0.57 *m*, 1.67 *m*	-
7	25.3 *t*	1.04 *m*, 1.30 *m*	-
8	47.0 *d*	1.57 *m*	C-7, C-9, C-13, C-30
9	23.7 *s*	-	-
10	29.6 *s*	-	-
11	27.1 *t*	1.18 *m*, 1.98 *m*	C-9, C-12
12	33.0 *t*	1.62 *m*, 1.62 *m*	-
13	45.5 *s*	-	-
14	49.0 *s*	-	-
15	35.5 *t*	1.27 *m*, 1.27 *m*	-
16	28.3 *t*	1.29 *m*, 1.92 *m*	-
17	52.3 *d*	1.59 *m*	-
18	17.9 *q*	0.96 *s*	C-12, C-13, C-17
19	27.4 *t*	0.14 *d* (3.8), 0.38 *d* (3.8)	C-1, C-5, C-8, C-9, C-10, C-11
20	36.3 *d*	1.39 *m*	
21	18.5 *q*	0.89 *d* (5.7)	C-17, C-20, C-21
22	35.1 *t*	1.13 *m*, 1.55 *m*	-
23	31.4 *t*	1.88 *m*, 2.12 *m*	C-22, C-24, C-28
24	157.0 *s*	-	-
25	33.9 *d*	2.23 sep (6.5)	C-24, C-26, C-27, C-28
26	22.0 *q*	1.02 *d* (6.8)	C-24, C-25, C-27
27	22.1 *q*	1.02 *d* (6.8)	C-24, C-25, C-26
28	106.0 *t*	4.66 *s*, 4.71 *s*	C-23, C-24, C-25
29	14.6 *q*	0.97 *d* (7.2)	C-3, C-5
30	19.3 *q*	0.89 *s*	C-8, C-11, C-12

Compound **89** was obtained as white pale solid. The molecular formula was established as $C_{29}H_{48}O$, based on its HRESIMS data (*m/z* 413.3752 $[M+H]^+$, calcd 413.3783), suggesting six degrees of unsaturation. The ^{13}C NMR spectrum of **89** exhibited twenty nine carbon signals, including five methyl carbons, twelve sp^3 methylene carbons, six sp^3 methine carbons (one oxygenated carbon at δ_C 71.3), four sp^3 quaternary carbons, and two olefinic carbons (one exomethylene carbons at δ_C 106.1, one one quaternary carbons at δ_C 157.0) (Table 4.21). The ^1H NMR spectrum displayed two tertiary methyl signals at δ_H 0.88 (s, CH_3-29) and 0.96 (s, CH_3-18), three secondary methyl signal at δ_H 0.89 (d, J = 5.0 Hz, CH_3-21) and 1.02 (d, J = 6.8 Hz, CH_3-26, CH_3-27), one oxygenated methine signal at δ_H 3.68 (td, J = 10.6, 4.4 Hz, H-3), two exomethylene signals at δ_H 4.71 (s, H-28a) and 4.66 (s, H-28b), and two methylene proton signals at δ_H 0.42 (d, J = 4.1 Hz, H-19a) and 0.06 (d, J = 4.1 Hz, H-19b), characteristic of the C-19 methylene protons of cyclopropane ring of a cycloartane triterpenoid skeleton [75]. Based on spectroscopic data above, compound **89** was identified as 24-methylene pollinastanol. The structure of compound **89** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Thompson and co-workers in 1978 [58] (Figure 4.23).

Figure 4.23 Key COSY (—) and HMBC (⌒) correlations of **89**.

Table 4.21 NMR data of compound **89** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	30.7 *t*	1.29 *m*, 1.53 *m*	C-2, C-10, C-18
2	35.4 *t*	1.35 *m*, 1.98 *m*	C-3
3	71.3 *d*	3.68 *td* (10.6, 4.4)	-
4	42.6 *t*	1.11 *m*, 1.82 *m*	C-3
5	37.3 *d*	1.53 *m*	C-3, C-10
6	27.9 *t*	0.78 *m*, 1.37 *m*	C-4, C-5, C-7, C-8
7	24.8 *t*	1.12 *m*, 1.30 *m*	-
8	46.3 *d*	1.71 *m*	C-9, C-10, C-13, C-14, C-15, C-19, C-29
9	23.5 *s*	-	-
10	30.0 *s*	-	-
11	27.2 *t*	1.28 *m*, 1.89 *m*	-
12	33.0 *t*	1.60 *m*, 1.60 *m*	C-9, C-11, C-13, C-14, C-17, C-18
13	45.6 *s*	-	-
14	49.2 *s*	-	-
15	35.2 *t*	1.28 *m*, 1.28 *m*	-
16	28.2 *t*	1.28 *m*, 1.91 *m*	-
17	52.3 *d*	1.60 *m*	-
18	17.5 *q*	0.96 *s*	C-12, C-13, C-14, C-17
19	25.9 *t*	0.42 *d* (4.1), 0.06 *d* (4.1)	C-1, C-8, C-5, C-9 ,C-10, C-11
20	36.3 *d*	1.41 *m*	-
21	18.5 *q*	0.89 *d* (5.0)	C-17, C-20, C-22
22	35.2 *t*	1.13 *m*, 1.57 *m*	-
23	31.4 *t*	1.88 *m*, 2.12 *m*	C-22, C-24, C-25, C-28
24	157.0 *s*	-	-
25	33.9 *d*	2.23 *sep* (6.8)	C-23, C-24, C-26, C-27, C-28
26	22.0 *q*	1.02 *d* (6.8)	C-24, C-25, C-27
27	22.1 *q*	1.02 *d* (6.8)	C-24, C-25, C-26
28	106.1 *t*	4.66 *s*, 4.71 *s*	C-23, C-24, C-25
29	19.1 *q*	0.88 *s*	C-8, C-13, C-14, C-15

Compound **90** was obtained as white amorphous powder. The ^{13}C NMR spectrum of **90** exhibited thirty carbon signals, including seven methyl carbons, ten sp^3 methylene carbons, four sp^3 methine carbons (one oxygenated carbon at δ_C 79.0), six sp^3 quaternary carbons, a carbonyl carbon, and two olefinic carbons (one methine carbons at δ_C 122.4, and one quaternary carbons at δ_C 143.9) (Table 4.22). The ^1H NMR spectrum displayed, seven tertiary methyl signals at δ_H 0.72 (s, CH_3-23, CH_3-26), 0.85 (s, CH_3-25, CH_3-30), 0.87 (s, CH_3-29), 0.93 (s, CH_3-24) and 1.08 (s, CH_3-27), one oxygenated methine signal at δ_H 3.15 (m, H-3), broad singlet for an olefinic proton at δ_H 5.22 (br s, H-12), and broad doublet for a methine signal at δ_H 2.77 (d, J = 13.4 Hz, H-18), characteristic of the Δ^{12} proton signal of pentacyclic triterpenoid skeleton [67]. Based on spectroscopic data above, compound **90** was identified as oleanolic acid. The structure of compound **90** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Seebacher and co-workers in 2003 [76] (Figure 4.24).

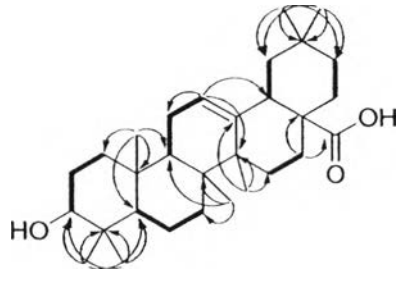


Figure 4.24 Key COSY (—) and HMBC (⌒) correlations of **90**.

Table 4.22 NMR data of compound **90** in $CDCl_3$:CD_3OD (10:1)

Position	^{13}C	^{1}H	HMBC
1	38.5 *t*	0.91 *m*, 1.56 *m*	-
2	26.9 *t*	1.54 *m*, 1.54 *m*	C-3
3	79.0 *d*	3.15 *m*	C-23, C-24
4	38.8 *s*	-	-
5	55.3 *d*	0.68 *m*	C-1, C-6, C-10, C-25
6	18.4 *t*	1.33 *m*, 1.49 *m*	-
7	32.8 *t*	1.24 *m*, 1.39 *m*	-
8	39.3 *s*	-	-
9	47.7 *d*	1.49 *m*	-
10	37.1 *s*	-	-
11	23.4 *t*	1.82 *m*, 1.82 *m*	-
12	122.4 *d*	5.22 *br s*	C-9, C-11, C-14, C-18
13	143.9 *s*	-	-
14	41.8 *s*	-	-
15	27.7 *t*	1.02 *m*, 1.66 *m*	-
16	23.1 *t*	1.55 *m*, 1.92 *m*	C-17, C-28
17	46.5 *s*	-	-
18	41.2 *d*	2.77 *d* (13.4)	C-12, C-13, C-14
19	46.0 *t*	1.09 *m*, 1.59 *m*	-
20	30.7 *s*	-	-
21	33.9 *t*	1.16 *m*, 1.29 *m*	-
22	32.6 *t*	1.50 *m*, 1.70 *m*	-
23	15.6 *q*	0.72 *s*	C-3, C-4, C-5, C-24
24	28.1 *q*	0.93 *s*	C-3, C-4, C-5, C-23
25	15.3 *q*	0.85 *s*	C-1, C-5, C-9, C-10
26	16.9 *q*	0.72 *s*	C-7, C-8, C-9, C-14
27	25.9 *q*	1.08 *s*	C-8, C-13, C-14, C-15
28	181.4 *s*	-	-
29	23.6 *q*	0.87 *s*	C-19, C-20, C-21, C-30
30	33.1 *q*	0.85 *s*	C-19, C-20, C-21 C-29

Compound **91** was obtained as white amorphous powder. The ^{13}C NMR spectrum of **91** exhibited thirty carbon signals, including seven methyl carbons, nine sp^3 methylene carbons, six sp^3 methine carbons (one oxygenated carbon at δ_C 78.9), five sp^3 quaternary carbons, a carbonyl carbon, and two olefinic carbons (one methine carbons at δ_C 125.6, and one quaternary carbons at δ_C 138.2) (Table 4.23). The ^1H NMR spectrum displayed, five tertiary methyl signals at δ_H 0.70 (s, CH_3-23), 0.74 (s, CH_3-26), 0.85 (s, CH_3-25), 0.91 (s, CH_3-24) and 1.02 (s, CH_3-27), two secondary methyl signals at δ_H 0.79 (d, J = 6.4 Hz, CH_3-29) and 0.87 (d, J = 6.0 Hz, CH_3-30), one oxygenated methine signal at δ_H 3.13 (t, J = 7.9 Hz, H-3), broad triplet for an olefinic proton at δ_H 5.17 (t, J = 3.1 Hz, H-12), and broad doublet for a methine signal at δ_H 2.12 (d, J = 11.2 Hz, H-18 characteristic of the Δ^{12} proton signal of pentacyclic triterpenoid skeleton. Based on spectroscopic data above, compound **91** was identified as ursolic acid. The structure of compound **91** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Seebacher and co-workers in 2003 [76] (Figure 4.25).

Figure 4.25 Key COSY (—) and HMBC (⌒) correlations of **91**.

Table 4.23 NMR data of compound **91** in $CDCl_3$:CD_3OD (10:1)

Position	^{13}C	^{1}H	HMBC
1	38.7 *t*	0.88 *m*, 1.57 *m*	-
2	26.9 *t*	1.52 *m*, 1.52 *m*	C-3
3	78.9 *d*	3.13 *t* (7.9)	C-2,C-23,C-24
4	38.7 *s*	-	-
5	55.3 *d*	0.65 d (11.2)	C-4, C-6, C-9, C-10, C-23, C-24
6	18.3 *t*	1.29 *m*, 1.46 *m*	-
7	33.1 *t*	1.25 *m*, 1.41 *m*	-
8	39.5 *s*	-	-
9	47.6 *d*	1.43 *m*	C-5, C-8, C-10, C-11, C-14, C-26
10	37.0 *s*	-	-
11	23.3 *t*	1.84 *m*, 1.84 *m*	-
12	125.6 *d*	5.17 *t* (3.1)	C-9, C-11, C-13, C-18
13	138.2 *s*	-	-
14	42.1 *s*	-	-
15	28.1 *t*	1.02 *m*, 1.80 *tt* (13.2, 3.9)	-
16	24.2 *t*	1.58 *m*, 1.93 *td* (13.2, 3.9)	-
17	47.9 *s*	-	-
18	52.8 *d*	2.12 *d* (11.2)	C-12, C-13, C-14, C-16, C-17, C-19, C-27, C-28
19	39.1 *d*	1.27 *m*	-
20	38.9 *d*	0.90 *m*	-
21	30.7 *t*	1.24 *m*, 1.43 *m*	-
22	36.8 *t*	1.57 *m*, 1.63 *td* (13.1, 3.4)	C-28
23	15.6 *q*	0.70 *s*	C-3, C-4, C-5, C-24
24	28.1 *q*	0.91 *s*	C-3, C-4, C-5, C-23
25	15.4 *q*	0.85 *s*	C-1, C-5, C-9, C-10
26	16.9 *q*	0.74 *s*	C-7, C-8, C-9, C-13
27	23.5 *q*	1.02 *s*	C-8, C-12, C-13, C-14
28	180.8 *s*	-	-
29	17.0 *q*	0.79 *d* (6.4)	C-18, C-19, C-20
30	21.2 *q*	0.87 *d* (6.0)	C-19, C-20, C-21

Compound **92** was obtained as pale brown solid. The ^{13}C NMR spectrum of **92** exhibited fifteen carbon signals, including one sp^3 methylene carbon, two oxygenated sp^3 methine carbons, and twelve aromatic carbons (Table 4.24). The ^1H NMR spectrum displayed, two methylene signals at δ_H 2.51 (dd, J = 16.1, 8.0 Hz, H-4a) and 2.85 (dd, J = 16.1, 5.0 Hz, H-**4**b), two oxygenated methine signal at δ_H 3.98 (br q, J = 6.4 Hz, H-3) and 4.57 (d, J = 7.4 Hz, H-2), and five aromatic signals at δ_H 6.84 (s, H-2'), 6.77 (d, J = 8.0 Hz, H-5'), 6.72 (d, J = 8.0 Hz, H-6'), 5.94 (s, H-8), 5.87 (s, H-6). The optical rotation was -10° in methanol. Based on spectroscopic data above, compound **92** was identified as (–)-catechin. The structure of compound **92** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Seto and co-workers in 1997 [61] (Figure 4.26).

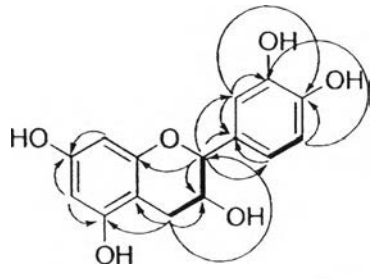


Figure 4.26 Key COSY (—) and HMBC (⌒) correlations of **92**.

Table 4.24 NMR data of compound **92** in CD_3OD

Position	^{13}C	^1H	HMBC
2	82.8 *d*	4.57 *d* (7.4)	C-3, C-9, C-1', C-2', C-3 '
3	68.8 *d*	3.98 *br q* (6.4)	-
4	28.4 *t*	2.51 *dd* (16.1, 8.0), 2.85 *dd* (16.1, 5.0)	C-2, C-3, C-5, C-10
5	157.5 *s*	-	-
6	95.5 *d*	5.87 *s*	C-5, C-7, C-8, C-10
7	157.7 *s*	-	-
8	96.3 *d*	5.94 *s*	C-6, C-7, C-10
9	156.9 *s*	-	-
10	100.8 *s*	-	-
1'	132.2 *s*	-	-
2'	115.2 *d*	6.84 *s*	C-2, C-1', C-3', C-4', C-6'
3'	146.2 *s*	-	-
4'	146.2 *s*	-	-
5'	116.1 *d*	6.77 *d* (8.0)	C-1', C-2', C-3', C-4', C-6'
6'	120.0 *d*	6.72 *d* (8.0)	C-2, C-1' C-2', C-3', C-4', C-5'

Compound **93** was obtained as pale brown solid. The ^{13}C NMR spectrum of **93** exhibited thirteen carbon signals (chemical equivalent at δ_C 107.9 and 146.3), including one sp^3 methylene carbon, two oxygenated sp^3 methine carbons, and ten aromatic carbons (Table 4.25). The ^1H NMR spectrum displayed, two methylene signals at δ_H 2.51 (dd, *J* = 16.0, 7.6 Hz, H-4a) and 2.82 (dd, *J* = 16.0, 4.5 Hz, H-4b), two oxygenated methine signal at δ_H 4.14 (br q, *J* = 6.5 Hz, H-3) and 4.66 (d, *J* = 7.2 Hz, H-2), and three aromatic signals at δ_H 6.51 (2H, s, H-2', H-6'), 6.06 (s, H-8), 5.99 (s, H-6). The optical rotation was -12° in methanol. Based on spectroscopic data above, compound **93** was identified as (–)-gallocatechin (**93**). The structure of compound **93** was finally confirmed by analysis of 2D NMR spectra and comparison of spectral data with those previously reported by Seto and co-workers in 1997 [61] (Figure 4.27).

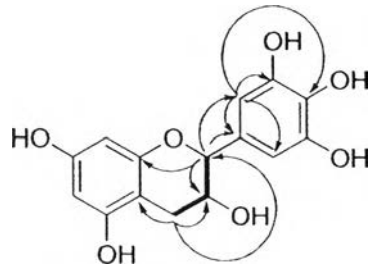


Figure 4.27 Key COSY (—) and HMBC (⌒) correlations of **93**.

Table 4.25 NMR data of compound **93** in CD_3OD

Position	^{13}C	^1H	HMBC
2	81.9 *d*	4.66 *d* (7.2)	C-2, C-3, C-9, C-1', C-2', C-3 '
3	67.6 *d*	4.14 *br q* (6.5)	-
4	27.3 *t*	2.51 *dd* (16.0, 7.6), 2.82 *dd* (16.0, 4.5)	C-2, C-3, C-10
5	156.3 *s*	-	-
6	95.9 *d*	5.99 *s*	-
7	156.3 *s*	-	-
8	96.8 *d*	6.06 *s*	C-7, C-10
9	155.8 *s*	-	-
10	101.4 *s*	-	-
1'	131.1 *s*	-	-
2'	107.9 *d*	6.51 *s*	C-2, C-3', C-4', C-5', C-6'
3'	146.3 *s*	-	-
4'	133.5 *s*	-	-
5'	146.3 *s*	-	-
6'	107.9 *d*	6.47 *s*	C-2, C-2', C-3', C-4', C-5'

4.3 Cytotoxic activity of the isolated compounds from *G. griffithii*

All isolated compounds from *G. griffithii*, steroidal glycosides (**61-68**) and the two derived aglycones (**61a** and **67a**) were tested for their *in vitro* cytotoxicity against five human tumor cell lines (BT 474, Chago, Hep-G2, KATO-III and SW620), using the MTT colorimetric assay (Table 4.26). Doxorubicin was used as positive control. Compounds **61**, **61a**, **62-67**, **67a**, and **68** did not show apparent cytotoxicity against the tumor cell lines. Compounds **63** and **66**, containing a tigloyl moiety at C-20, showed a slight cytotoxicity against all tested cell lines and exhibited a more potent cytotoxicity than the other tested compounds, suggesting that the presence of the tigloyl moiety influenced the cytotoxic activity of the compounds in this type.

Table 4.26 *In vitro* cytotoxicity data for compounds **61** – **68**, **61a** and **67a**

Compounds	IC_{50} (μM)				
	BT474	Chago	Hep-G2	KATO-III	SW620
61	>100	>100	>100	>100	>100
62	>100	>100	83.1	>100	77.2
63	63.8	45.1	62.8	79.7	50.7
64	>100	>100	>100	>100	>100
65	>100	82.1	98.1	83.2	59.5
66	66.0	49.1	56.5	73.0	54.5
67	>100	>100	>100	>100	>100
68	75.6	86.2	>100	>100	54.6
61a	>100	>100	>100	>100	91.2
67a	>100	>100	>100	>100	>100
Doxorubicin	1.31	0.86	0.20	1.31	0.13

4.4 Anti α-glucosidase activity of the isolated compounds from *G. griffithii* and *H. curtisii*

All isolated compounds, except squalene (**83**), were tested for their *in vitro* α-glucosidase inhibitory activity against yeast *Saccharomyces cerevisiae*. Acarbose was used as positive control (Table 4.27). Based on their structure, these compounds can be classified in to four groups including steroidal glycoside, steroid, triterpenoid and flavanoid. According to structurally diverse of the isolated triterpenoids and their α-glucosidase inhibitory activity, various types of triterpenoid nucleus and the position of functional groups are significant for their α-glucosidase inhibitory activity. Many molecular docking studies of pentacyclic triterpenoids, oleanane- and ursane-type triterpenoids, showed that the hydroxy group at C-3 position [77] and carboxylic group at C-28 position [78] play an important role in inhibiting enzyme α-glucosidase activity. Compound **86**, containing acetyl group at C-3 position, showed less potent activity (IC_{50} = 127.1 μM) than that of compound **57** (IC_{50} = 115.2 μM) with hydroxy group. Previous report of α-glucosidase inhibitory activity of oleanolic acid (IC_{50} = 11.2 μM) and oleanolic acid-3-acetate (IC_{50} = 55.1 μM) also shown that the replacement of hydroxy group by acetyl group lead to slightly decrease their potency[79]. In addition, the presence of hydroperoxy group at C-11 of **81** (IC_{50} = 79.3 μM) and 82 (IC_{50} = 49.7 μM) instead of methylene carbon of **90** (IC_{50} = 14.7 μM) and **91** (IC_{50} = 46.3 μM) slightly decrease their activity. The large difference in activity between compound **90** (IC_{50} = 14.7 μM) and compound **84** (IC_{50} > 200 μM) might come from carboxylic group at C-28 position. Apparently, carboxylic group at C-28 of compounds **81**, 82, **90** and **91**, oleanane- and ursane-type triterpenoids, were key role for α-glucosidase inhibitory activity. Compounds **61**–**68** processed with steroidal skeleton conjugated with three sugar unit at C-3 position were considered to be inactive (IC_{50} > 1000 μM), while their steroidal aglycone **61a** (IC_{50} = 888.1 μM) and **67a** (IC_{50} = 514.7 μM) showed a moderate α-glucosidase inhibitory activity, suggesting that the presence of sugar moiety decreased the α-glucosidase inhibitory activity of these compounds.

Table 4.27 *In vitro* anti α-glucosidase activity data for compounds **57**, **61–68**, **61a**, **67a**, **81**, **82** and **84–93**

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
61	>1000	**84**	>200*
62	>1000	**85**	173.2
63	>1000	**86**	127.1
64	>1000	**57**	115.2
65	>1000	**87**	131.6
66	>1000	**88**	>1000
67	>1000	**89**	145.0
68	>1000	**90**	14.7
61a	888.1	**91**	46.3
67a	514.7	**92**	>1000
81	79.3	**93**	397.8
82	49.7	**Acarbose**	884.6

*The maximum concentration of **84** was tested at 200 μM due to its solubility in DMSO.