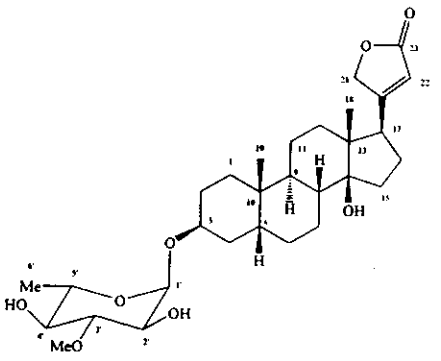
CHAPTER 3

RESULTS AND DISCUSSION

3.1 Structural elucidation of compounds from the seeds of C. odollam

The white solid precipitate was purified by PLC to yield two compounds, **SCO1** and **SCO2**. The methylene chloride extract of seeds of *C. odollam* was subjected to chromatography and/or crystallization and/or PLC to give five cardenolide glycosides, **SCO1, SCO2, SCO3, SCO4** and **SCO5**. One of them is a new compound (**SCO5**). Their structures were determined using 1D and 2D NMR spectroscopic data. All carbon of aglycone unit were assigned by ^{13}C NMR, HMQC and HMBC data. The chemical shift of sugar moiety were determined using 1D ^{1}H NMR and ^{1}H-^{1}H COSY spectroscopic data. In addition, the structure of **SCO1** and **SCO2** were confirmed by X-ray diffraction.

3.1.1 Compound SCO1

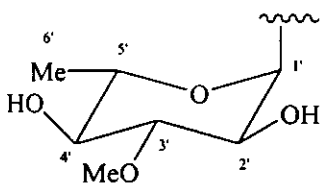


Compound **SCO1** was obtained as a white solid mp = 220-224 °C, $[\alpha]_D^{26}$= -48.78° (c = 0.0041, $CHCl_3$). The IR spectrum (**Fig. 8**) showed absorption bands at 3491cm^{-1} and 1728 cm^{-1} corresponding to a hydroxy group and carbonyl group, respectively. The presence of carbonyl carbon at δ 175.06 from ^{13}C NMR spectrum supported the above conclusion. The UV spectrum showed maximum at 242 nm (**Fig. 7**)

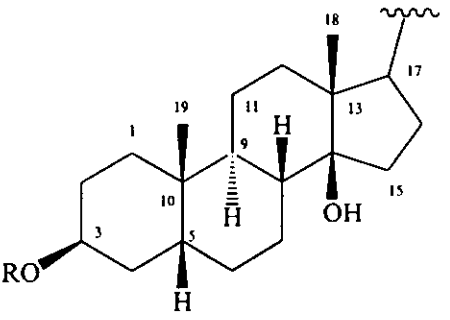
The ^{13}C NMR spectrum (see **Table 5, Fig. 10**) showed 30 signals for 30 carbon atoms. Analysis of the DEPT-45°, DEPT-90° and DEPT-135° (see **Table 2, Fig. 11**) spectra of this compound suggested the presence of four methyl carbon atoms (δ60.54, 23.78, 17.40 and 15.71), ten methylene carbon atoms (δ73.60, 39.95, 32.91, 30.55, 29.21, 26.89, 26.55, 26.44, 21.29 and 21.14), eleven methine carbon atoms (δ 117.48, 97.15, 85.24, 74.68, 73.39, 72.66, 67.60, 50.90, 41.65, 36.85 and 35.66) and five signals for quaternary carbon atoms (δ175.06, 175.04, 84.55, 49.65 and 35.19).

The ^{1}H NMR spectrum (see **Table 5, Fig. 9**) recorded in $CDCl_3$ was a typical of cardenolide glycoside. The *singlet* signal at δ5.87 (1H, H-22) accompanied by AB system at δ4.79 (1H, *dd, J* = 18.5 and 1.5 Hz, H-21b) and 4.97 (1H, *dd, J* = 18.5 and 1.5 Hz, H-21a), were characteristic peaks of a butenolide ring (Srivatava, *et.al.,* 1993),

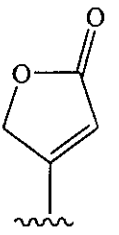
together with the sugar protons at δ4.83 (1H, *d*, J = 3.5 Hz), 3.71 (1H, *dq*, J = 6 and 9 Hz), 3.66 (3H, *s*), 3.53 (1H, *dd*, J = 3.5 and 9 Hz), 3.24 (1H, *t*, J = 9 Hz), 3.10 (1H, *t*, J = 9 Hz), and 1.22 (3H, *d*, J = 6 Hz) which were assigned to H-1′, 5′, 3′-OMe, 2′, 3′, 4′ and 6′, respectively. The sugar unit was identified as L-thevetose by comparison with the previously reported data (Yamauchi, *et.al.*,1987). The two methyl protons, one oxymethine and one methine protons of the steroidal ring were shown at δ0.86 (3H, *s*), 0.94 (3H, *s*), 3.95 (1H, *br s*) and 2.77 (1H, *dd*, J = 5 and 9 Hz), which could be assigned to CH_3-18, CH_3-19, H-3 and H-17, respectively. The remaining methylene protons appeared at δ 1.21 to 2.21. Thus, this compound exhibited tetracyclic of steroidal skeleton, butenolide ring and the sugar moiety as indicated by ^{1}H NMR.



Sugar unit



Steroid unit



Butenolide unit

The complete assignment of ^{13}C and ^{1}H NMR (see **Table 5, Fig. 9** and **10**) signals were made with the information from ^{1}H-^{1}H COSY (see **Table 3, Fig. 12**), HMQC (**Fig. 13**) and HMBC spectrum (see **Table 4, Fig. 14**). In the HMBC spectrum the carbon signals at δ 67.60 (C-5′), 73.39 (C-3) and 85.24 (C-3′) showed the correlation peaks with the H-1′(4.83), indicating that the glycosidic linkage was formed between sugar moiety and the steroid at C-3 (73.39). The carbon signals at δ39.95 (C-12), 49.65 (C-13), 73.60 (C-21), 117.48 (C-22) and 175.04 (C-20) showed

the correlation peaks with the H-17 (2.77), confirming that the butenolide ring was attached to the C-17 (50.90) of the steroidal structure at ring D. In NOE experiment (**Fig. 15**), irradiation of methine proton at δ2.77 (H-17) resulted in the enhancement of the signals at 5.87 (H-22), 4.97 (H-21a) and 4.79 (H-21b) while the signal at δ0.86 (CH_3-18) has not changed. These observations suggested that CH_3-18 and H-17 are opposite. Thus, this compound should be β-butenolide at C-17.

Table 2 ^{13}C and DEPT spectral data of compound **SCO1**

δ_C	**DEPT-45°**	**DEPT-90°**	**DEPT-135°**	**Type of Ccarbon**
175.06				C
175.04				C
117.48	117.48	117.48	117.48	= CH
97.15	97.15	97.15	97.15	CH
85.24	85.24	85.24	85.24	CH
84.55				C
74.68	74.68	74.68	74.68	CH
73.60	73.60		73.60	CH_2
73.39	73.39	73.39	73.39	CH
72.66	72.66	72.66	72.66	CH
67.60	67.60	67.60	67.60	CH
60.54	60.54		60.54	CH_3
50.90	50.90	50.90	50.90	CH
49.65				C
41.65	41.65	41.65	41.65	CH
39.95	39.95		39.95	CH_2
36.85	36.85	36.85	36.85	CH
35.66	35.66	35.66	35.66	CH

Table 2 (Continued)

δ_C	DEPT-45°	DEPT-90°	DEPT-135°	Type of Ccarbon
35.19				C
32.91	32.91		32.91	CH_2
30.55	30.55		30.55	CH_2
29.91	29.91		29.91	CH_2
26.89	26.89		26.89	CH_2
26.55	26.55		26.55	CH_2
26.44	26.44		26.44	CH_2
23.78	23.78		23.78	CH_3
21.29	21.29		21.29	CH_2
21.14	21.14		21.14	CH_2
17.40	17.40		17.40	CH_3
15.71	15.71		15.71	CH_3

Table 3 500 MHz COSY Correlation of some protons of **SCO1**

δ_H (ppm)	Proton Correlation with δ_H (ppm)
H-21a (4.97)	H-21b
H-21b (4.79)	H-21a
H-1′ (4.83)	H-2′
H-2′ (3.53)	H-1′ and H-3′
H-3′ (3.24)	H-2′ and H-4′
H-4′ (3.10)	H-3′ and H-5′
H-5′ (3.71)	H-4′ and H-6′
H-6′ (1.22)	H-5′

Table 4 Major HMBC Correlation of **SCO1**

Position	δ_H (ppm)	δ_C (ppm)
3	3.95 (1H, *br s*)	C-5 (36.82)
8	1.55 (1H, *m*)	C-6 (26.89), C-13 (49.65) and C-14 (84.55)
17	2.77 (1H, *dd, J* = 5, 9 Hz)	C-12 (39.95), C-13 (49.65), C-20 (175.04), C-21 (73.60) and C-22 (117.48)
18	0.86 (3H, *s*)	C-12 (39.95), C-13 (49.65) C-14 (84.55) and C-17 (50.90)
19	0.94 (3H, *s*)	C-1 (29.91), C-5 (36.82) and C-9 (35.66)
21	4.97 (1H, *dd, J* = 18.5, 1.5 Hz) 4.79 (1H, *dd, J* = 18.5, 1.5 Hz)	C-20 (175.04), C-22 (117.48) and C-23 (175.06)
22	5.87 (1H, *s*)	C-17 (50.90), C-20 (175.04), C-21 (73.60) and C-23 (175.06)
$1'$	4.83 (1H, *d, J* = 4 Hz)	C-3 (73.39), C-$3'$ (85.24) and C-$5'$(67.60)
$3'$	3.24 (1H, *t, J* = 9 Hz)	C-$2'$ (72.66), C-$4'$ (74.68) and C-$3'$OMe (60.54)

Table 4 (Continued)

Position	δ_H (ppm)	δ_C (ppm)
4′	3.10 (1H, *t*, *J* = 9 Hz)	C-3′ (85.24), C-5′ (67.60) and C-6′ (17.40)
5′	3.71 (1H, *dq*, *J* = 6, 9 Hz)	C-3′ (85.24)
6′	1.22 (1H, *d*, *J* = 6 Hz)	C-4′ (74.68) and C-5′ (67.60)

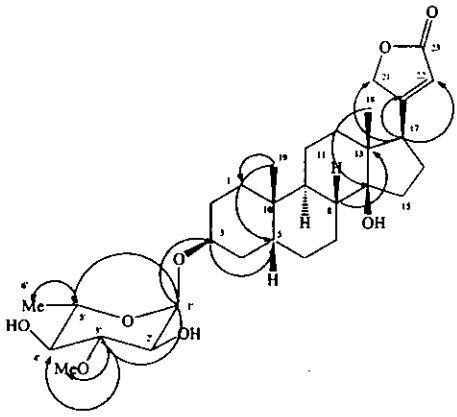
Table 5 ^{1}H and ^{13}C spectral data of compound **SCO1**

Position	δ_C^*(ppm)		δ_H (ppm)
1	29.91	CH_2	1.76 (1H, *m*), 1.45 (1H, *m*)
2	26.55[a]	CH_2	**
3	73.39	CH	3.95 (1H, *br s*)
4	30.55	CH_2	1.54 (1H, m), 1.37 (1H, *m*)
5	36.52	CH	1.62 (1H, *m*)
6	26.28[a]	CH_2	**
7	21.14[b]	CH_2	***
8	41.64	CH	1.55 (1H, *m*)
9	35.66	CH	1.60 (1H, *m*)
10	35.19	C	-
11	21.29[b]	CH_2	***
12	39.95	CH_2	1.50 (1H, *m*), 1.37 (1H, *m*)
13	49.63	C	-
14	84.55	C	-

Table 5 (Continued)

Position	δ_C[#](ppm)		δ_H(ppm)
15	32.91	CH_2	2.08 (1H, *m*), 1.68 (1H, *m*)
16	26.44[a]	CH_2	**
17	50.90	CH	2.77 (1H, *dd*, *J* = 5, 9 Hz)
18	15.71	CH_3	0.86 (3H, *s*)
19	23.78	CH_3	0.94 (3H, *s*)
20	175.04[c]	C	-
21	73.60	CH_2	4.97 (1H, *dd*, *J* = 18.5, 1.5 Hz)
			4.79 (1H, *dd*, *J* = 18.5, 1.5 Hz)
22	117.48	= CH	5.87 (1H, *s*)
23	175.06[c]	C	-
1′	97.15	CH	4.83 (1H, *d*, *J* = 3.5 Hz)
2′	72.66	CH	3.53 (1H, *dd*, *J* = 3.5, 9 Hz)
3′	85.24	CH	3.24 (1H, *t*, *J* = 9 Hz)
4′	74.68	CH	3.10 (1H, *t*, *J* = 9 Hz)
5′	67.60	CH	3.71 (1H, *dq*, *J* = 6, 9 Hz)
6′	17.40	CH_3	1.22 (3H, *d*, *J* = 6 Hz)
3′-OMe	60.54	CH_3	3.66 (3H, *s*)

[a, b, c] Assignment with the same superscripts maybe interchanged, [#] Carbon type deduced from DEPT experiment, [**] The chemical shift of protons resonated at δ 1.25- 2.15, [***] The chemical shift of protons resonated at δ 1.22- 1.68.



Selected HMBC correlation of **SCO1**

Comparison of ^{1}H and ^{13}C NMR spectrum between compound **SCO1** and 17β-neriifolin, showed similarity. Thus compound **SCO1** was identified as 3β-*O*-(L-thevetosyl)-14β-hydroxy-5β-card-20(22)-enolide (17β-neriifolin) which was previously isolated from the leaves of *C. odollam* and *C. manghas.* (Yamauhi, *et.al.*, 1987). The structure of this compound was finally confirmed by X-ray diffraction (**Fig. 2**).

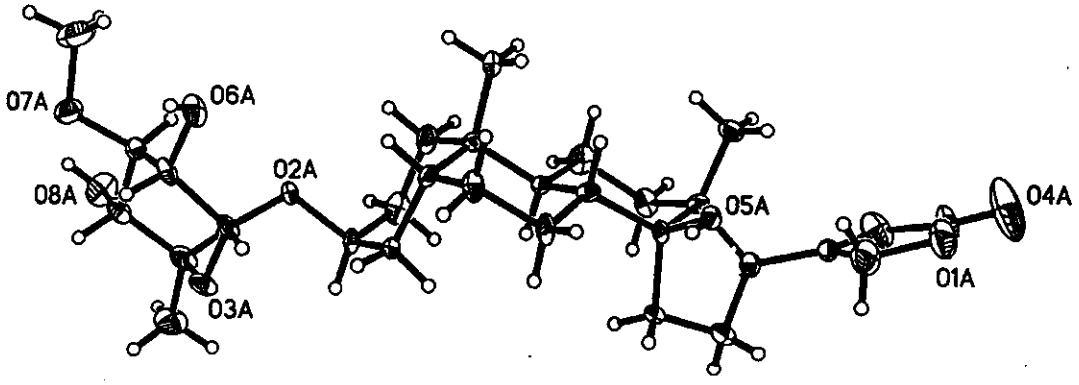


Figure 2 X-ray ORTEP diagram of compound **SCO1**

Table 6 Comparison of ^{1}H NMR spectral data between 17β-neriifolin and compound **SCO1**

Position	17β-neriifolin δ_H (ppm) (Recorded in pyridine)	Compound SCO1 δ_H (ppm) (Recorded in $CDCl_3$)
3	4.18 (1H, *br s*)	3.95 (1H, *br s*)
17	2.80 (1H, *dd*, *J* = 6, 9 Hz)	2.77 (1H, *dd*, *J* = 5, 9 Hz)
18	0.83 (3H, *s*)	0.86 (3H, *s*)
19	1.02 (3H, *s*)	0.94 (3H, *s*)
21	5.03 (1H, *dd*, *J* = 18, 1 Hz)	4.97 (1H, *dd*, *J* = 18.5, 1.5 Hz)
	5.31 (1H, *dd*, *J* = 18, 1 Hz)	4.79 (1H, *dd*, *J* = 18.5, 1.5 Hz)
22	6.13 (1H, *br s*)	5.87 (1H, *s*)
1′	5.24 (1H, *d*, *J* = 4 Hz)	4.83 (1H, *d*, *J* = 3.5 Hz)
2′		3.53 (1H, *dd*, *J* = 3.5, 9 Hz)
3′	4.00 (1H, *t*, *J* = 9 Hz)	3.24 (1H, *t*, *J* = 9 Hz)
4′	3.66 (1H, *t*, *J* = 9 Hz)	3.10 (1H, *t*, *J* = 9 Hz)
5′	4.31 (1H, *m*)	3.71 (1H, *dq*, *J* = 6, 9 Hz)
6′	1.41 (1H, *d*, *J* = 6 Hz)	1.22 (1H, *d*, *J* = 6 Hz)
3′-OMe	3.84 (3H, *s*)	3.66 (3H, *s*)

Table 7 Comparison of ^{13}C NMR spectral data between 17β-neriifolin and compound **SCO1**

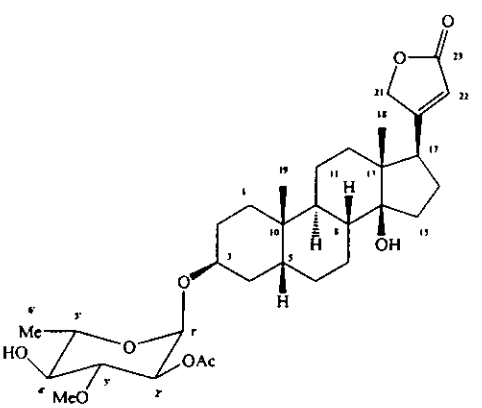
Position	17β-neriifolin, δ_C (ppm) (Recorded in pyridine)	Compound SCO1, δ_C (ppm) (Recorded in $CDCl_3$)
1	30.3	29.91
2	26.9[a]	26.55[a]
3	73.7	73.39
4	31.0	30.55
5	36.8	36.52
6	27.1[a]	26.28[a]
7	21.5[b]	21.14[b]
8	41.9	41.64
9	35.8	35.66
10	35.5	35.19
11	21.9[b]	21.29[b]
12	39.8	39.95
13	50.1	49.63
17	51.5	50.90
18	16.2	15.71
19	23.8	23.78
20	175.9	175.04[c]
21	73.6	73.60
22	117.6	117.48
23	174.4	175.06[c]
1′	98.8	97.15
2′	73.4	72.66
3′	85.4	85.24
4′	76.6	74.68

Table 7 (Continued)

Position	17β-neriifolin, δ_C (ppm) (Recorded in pyridine)	Compound SCO1, δ_C (ppm) (Recorded in $CDCl_3$)
5′	68.9	67.60
6′	18.5	17.40
3′-OMe	60.5	60.54

[a, b, c] Assignment with the same superscripts maybe interchanged.

3.1.2 Compound SCO2



Compound **SCO2** was isolated as a white solid, mp = 202-206 °C, $[\alpha]_D^{26}$= -90.90° (c = 0.0044, $CHCl_3$). The IR spectrum (**Fig. 17**) showed absorption bands which were ascribed to O—H stretching of hydroxy group (3473 cm^{-1}) and C=O stretching of carbonyl (1781 and 1746 cm^{-1}). The UV spectrum (**Fig. 16**) showed maximum at 242 nm.

The complete analysis of ^{13}C and ^{1}H NMR spectral data of compound **SCO2** (see **Table 11**, **Fig. 18** and **19**) were assigned with information provided from ^{1}H-^{1}H COSY (see **Table 9**, **Fig. 21**), ^{1}H-^{13}C correlation (HMQC) (**Fig. 22**) and ^{1}H-^{13}C correlation by long-range coupling (HMBC) (see **Table 10**, **Fig. 23**), along with comparison of ^{1}H NMR spectral data to compound **SCO1** (see **Table 12**). The ^{13}C NMR (see **Table 11**, **Fig. 18**) spectrum of compound **SCO2** recorded in $CDCl_3$ showed 32 signals for 32 carbon atoms. Analysis of the DEPT-45°, DEPT-90° and DEPT-135° spectra of this compound (see **Table 8**, **Fig. 20**) suggested the presence of five methyl carbon atoms (δ 60.49, 23.89, 20.89, 17.54 and 15.76), ten methylene carbon atoms (δ 73.48, 40.00, 33.12, 30.36, 29.60, 26.88, 26.62, 26.59, 21.34 and 21.19), eleven methine carbon atoms (δ 117.61, 93.73, 80.93, 75.33, 74.30, 72.25, 67.03, 50.92, 41.82, 36.47 and 35.65) and six quaternary carbon atoms (δ 174.69, 174.68, 170.27, 85.49, 49.62 and 35.19).

Compound **SCO2**, a derivative of compound **SCO1**, showed similar characteristic bands in IR and UV spectrum of **SCO1**. Comparison of the ^{1}H NMR spectral data (see **Table 12**) of the two compounds revealed close structural similarity. Difference in the spectrum of compound **SCO2** was shown as a signal of acetoxy proton at δ 2.07 (3H, *s*) which was not observed in compound **SCO1**. The characteristic of butenolide ring resonated at δ5.88 (1H, *s*) and *doublet of doublet* AB system at δ5.00 (J = 18.5, 2 Hz) and 4.82 (J = 18.5, 2 Hz) which were assigned to H-22 and 21a,b, respectively. The moiety of sugar appeared at δ5.05 (1H, *d*, J = 4 Hz), 4.63 (1H, *dd*, J = 4, 9 Hz), 3.79 (1H, *dq*, J = 6, 9 Hz), 3.58 (1H, *t*, J = 9 Hz), 3.58 (-OCH_3), 3.21 (1H, *t*, J = 9 Hz), 1.27 (3H, *d*, J = 6 Hz), which could be assigned to H-1′, 2′, 5′, 3′, 4′, 3′-OCH_3 and 6′, respectively. The H-2′ in compound **SCO2** (4.63, 1H, *dd*, J = 4, 9 Hz) appeared at the lower field than H-2′ in compound **SCO1** (3.53, 1H,

dd, J = 3.5, 9 Hz). These observation indicated that the position 2′ of compound **SCO2** should be connected with the acetyl group.

The HMBC correlation of compound **SCO2** (see **Table 10**, **Fig. 23**) showed the same correlation with compound **SCO1** (see **Table 4, 10**) except the proton signal at C-2′ (δ4.53, 1H, *dd*, J = 4, 9 Hz). This signal gave correlation peaks with carbonyl group (170.27) and C-3′ (80.93), thus confirming the position of the acetoxy group at C-2′ in the sugar moiety. Compound **SCO2** was identified as 3β-*O*-(L-2′-*O*-acetyl thevetosyl) -14β-hydroxy-5β-card-20(22)-enolide (cerberin) which was the compound previously isolated from the leaves of *C. odollam* and *C. manghas*. (Yamauhi, *et.al.*, 1987). The structure of this compound was finally confirmed by X-ray diffraction (**Fig. 3**).

Table 8 ^{13}C and DEPT spectral data of compound **SCO2**

δ_C	DEPT-45°	DEPT-90°	DEPT-135°	Type of Carbon
174.69				C
174.68				C
170.27				C
117.61	117.61	117.61	117.61	= CH
93.73	93.73	93.73	93.73	CH
85.49				C
80.93	80.93	80.93	80.93	CH
75.33	75.33	75.33	75.33	CH
74.30	74.30	74.30	74.30	CH
73.48	73.48		73.48	CH_2
72.25	72.25	72.25	72.25	CH
67.03	67.03	67.03	67.03	CH

Table 8 (Continued)

δ_C	DEPT-45°	DEPT-90°	DEPT-135°	Type of Carbon
60.49	60.49		60.49	CH_3
50.92	50.92	50.92	50.92	CH
49.62				C
41.82	41.82	41.82	41.82	CH
40.00	40.00		40.00	CH_2
36.47	36.47	36.47	36.47	CH
35.65	35.65	35.65	35.65	CH
35.19				C
33.12	33.12		33.12	CH_2
30.36	30.36		30.36	CH_2
29.60	29.60		29.60	CH_2
26.88	26.88		26.88	CH_2
26.62	26.62		26.62	CH_2
26.59	26.59		26.59	CH_2
23.89	23.89		23.89	CH_3
21.34	21.34		21.34	CH_2
21.19	21.19		21.19	CH_2
20.89	20.89		20.89	CH_3
17.54	17.54		17.54	CH_3
15.76	15.76		15.76	CH_3

Table 9 500 MHz COSY Correlation of some protons of **SCO2**

δ_H (ppm)	Proton Correlation with δ_H (ppm)
H-21a (5.00)	H-21b
H-21b (4.82)	H-21a
H-1′ (5.05)	H-2′
H-2′ (4.63)	H-1′ and H-3′
H-3′ (3.58)	H-2′ and H-4′
H-4′ (3.21)	H-3′ and H-5′
H-5′ (3.79)	H-4′ and H-6′
H-6′ (1.27)	H-5′

Table 10 Major HMBC Correlation of **SCO2**

Position	δ_H (ppm)	δ_C (ppm)
3	3.87 (1H, *br s*)	C-1 (30.36), C-5 (36.47) and C-1′(93.73)
17	2.78 (1H, *dd*, J = 5, 9 Hz)	C-12 (40.00), C-13 (49.62), C-14 (85.49), C-16 (26.59), C-20 (174.69), C-21 (73.48) and C-22 (117.61)
18	0.87 (3H, *s*)	C-12 (40.00), C-13 (49.62), C-14 (85.49) and C-17 (50.92)
19	0.95 (3H, *s*)	C-1 (30.36) and C-5 (36.47)

Table 10 (Continued)

Position	δ_H (ppm)	δ_C (ppm)
21	5.00 (1H, *dd*, *J* = 18.5, 2 Hz) 4.80 (1H, *dd*, *J* = 18.5, 2 Hz)	C-17 (50.92), C-20 (174.69) and C-23 (174.68)
22	5.88 (1H, *s*)	C-17 (50.92), C-20 (174.69), C-21 (73.48), C-20 (174.69) and C-23 (174.68)
1′	5.05 (1H, *d*, *J* = 4 Hz)	C-3 (72.25), C-3′ (80.93) and C-5′ (67.08)
2′	4.63 (1H, *dd*, *J* = 4, 9 Hz)	C-3′ (80.93) and 2′-C=O (170.27)
3′	3.58 (1H, *t*, *J* = 9 Hz)	C-2′ (74.30), C-4′ (75.33) and 3′- OMe (60.49)
4′	3.21 (1H, *t*, *J* = 9 Hz)	C-3′ (80.93), C-5′ (67.08) and C-6′ (17.54)
5′	3.79 (1H, *dq*, *J* = 6, 9 Hz)	C-4′ (75.33)
6′	1.27 (1H, *d*, *J* = 6 Hz)	C-4′ (75.33) and C-5′ (67.08)

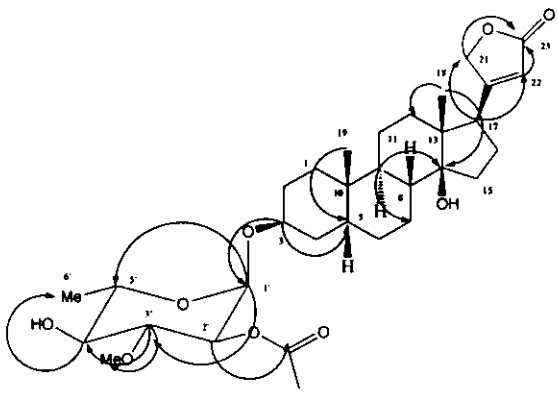
Table 11 ^{1}H and ^{13}C spectral data of compound **SCO2**

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
1	30.36	CH_2	1.48 (2H, *m*)
2	26.88[a]	CH_2	**
3	72.25	CH	3.87 (1H, *br s*)
4	29.90	CH_2	1.68 (1H, *m*), 1.31 (1H, *m*)
5	36.47	CH	1.64 (1H, *m*)
6	26.62[a]	CH_2	**
7	21.19[b]	CH_2	***
8	41.82	CH	1.55 (1H, *m*)
9	35.65	CH	1.60 (1H, *m*)
10	35.19	C	-
11	21.34[b]	CH_2	***
12	40.00	CH_2	1.51 (1H, *m*), 1.38 (1H, *m*)
13	49.62	C	-
14	85.49	C	-
15	33.12	CH_2	1.68 (1H, *m*), 2.10 (1H, *m*)
16	26.59[a]	CH_2	**
17	50.92	CH	2.78 (1H, *dd*, J = 5, 9 Hz)
18	15.76	CH_3	0.87 (3H, *s*)
19	23.89	CH_3	0.95 (3H, *s*)
20	174.69[c]	C	-
21	73.48	CH_2	5.00 (1H, *dd*, J = 18.5, 2 Hz)
			4.82 (1H, *dd*, J = 18.5, 2 Hz)
22	117.61	= CH	5.88 (1H, *s*)
23	174.68[c]	C	-
1′	93.73	CH	5.05 (1H, *d*, J = 3.5 Hz)
2′	74.30	CH	4.63 (1H, *dd*, J = 3.5, 9 Hz)
3′	80.93	CH	3.66 (1H, *t*, J = 9 Hz)

Table 11 ^{1}H and ^{13}C spectral data of compound **SCO2**

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
4′	75.33	CH	3.21 (1H, *t*, *J* = 9 Hz)
5′	67.08	CH	3.79 (1H, *dq*, *J* = 6, 9 Hz)
6′	17.54	CH_3	1.27 (3H, *d*, *J* = 6 Hz)
2′-OAc	20.89	CH_3	2.07 (3H, *s*)
2′-C=O	170.27	C	-
3′-OMe	60.49	CH_3	3.66 (3H, *s*)

[a, b, c] Assignment with the same superscripts maybe interchanged, [#] Type of carbon deduced by DEPT, [**] The chemical shifts of proton resonated at δ 1.25-2.15, [***] The chemical shifts of proton resonated at δ 1.22-1.68.



Selected HMBC correlation of **SCO2**

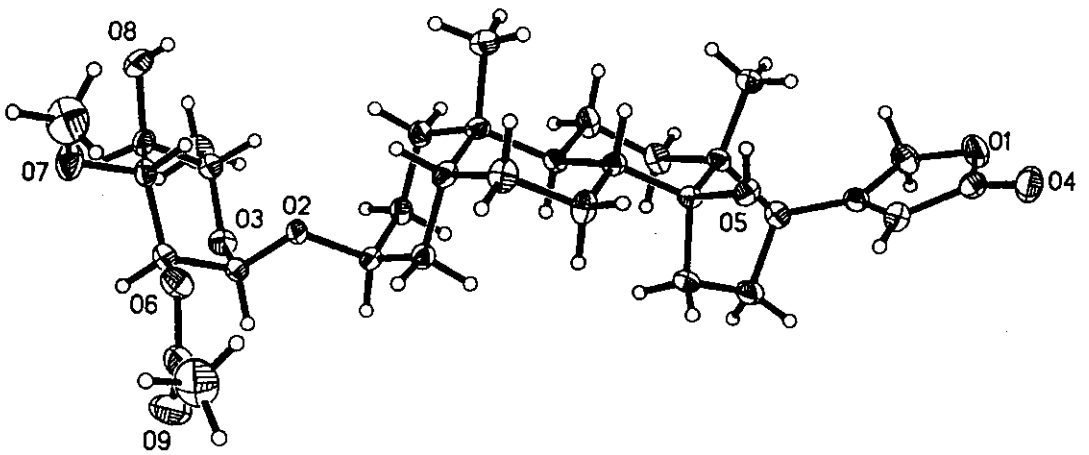


Figure 3 X-ray ORTEP diagram of compound **SCO2**

Table 12 Comparison of ^{1}H NMR spectral data between compound **SCO2** and **SCO1**

Position	Compound SCO 2, δ_H (ppm)	Compound SCO 1, δ_H (ppm)
1	1.48 (2H, *m*)	1.76 (1H, *m*), 1.45 (1H, *m*)
3	3.87 (1H, *br s*)	3.95 (1H, *br s*)
4	1.68 (1H, *m*), 1.31 (1H, *m*)	1.54 (1H, *m*), 1.37 (1H, *m*)
5	1.64 (1H, *m*)	1.62 (1H, *m*)
8	1.55 (1H, *m*)	1.55 (1H, *m*)
9	1.60 (1H, *m*)	1.60 (1H, *m*)
12	1.51 (1H, *m*), 1.38 (1H, *m*)	1.50 (1H, *m*), 1.37 (1H, *m*)
15	2.10 (1H, *m*), 1.68 (1H, *m*)	2.08 (1H, *m*), 1.68 (1H, *m*)
17	2.78 (1H, *dd*, J = 5, 9 Hz)	2.77 (1H, *dd*, J = 5, 9 Hz)
18	0.87 (3H, *s*)	0.86 (3H, *s*)
19	0.95 (3H, *s*)	0.94 (3H, *s*)

Table 12 (Continued)

Position	Compound SCO 2, δ_H (ppm)	Compound SCO 1, δ_H (ppm)
21	5.00 (1H, *dd*, *J* = 18.5, 2 Hz)	4.97 (1H, *dd*, *J* = 18.5, 1.5 Hz)
	4.80 (1H, *dd*, *J* = 18.5, 2 Hz)	4.79 (1H, *dd*, *J* = 18.5, 1.5 Hz)
22	5.88 (1H, *s*)	5.87 (1H, *s*)
1′	5.05 (1H, *d*, *J* = 4 Hz)	4.83 (1H, *d*, *J* = 3.5 Hz)
2′	4.63 (1H, *dd*, *J* = 4, 9 Hz)	3.53 (1H, dd, *J* = 3.5, 9 Hz)
3′	3.58 (1H, *t*, *J* = 9 Hz)	3.24 (1H, *t*, *J* = 9 Hz)
4′	3.21 (1H, *t*, *J* = 9 Hz)	3.10 (1H, *t*, *J* = 9 Hz)
5′	3.79 (1H, *dq*, *J* = 6, 9 Hz)	3.71 (1H, *dq*, *J* = 6, 9 Hz)
6′	1.27 (3H, *d*, *J* = 6 Hz)	1.22 (3H, *d*, *J* = 6 Hz)
2′-OAc	2.07 (3H, *s*)	-
3′-OMe	3.58 (3H, *s*)	3.66 (3H, *s*)

Table 13 Comparison of ^{13}C NMR spectral data between compound **SCO2** and **SCO1**

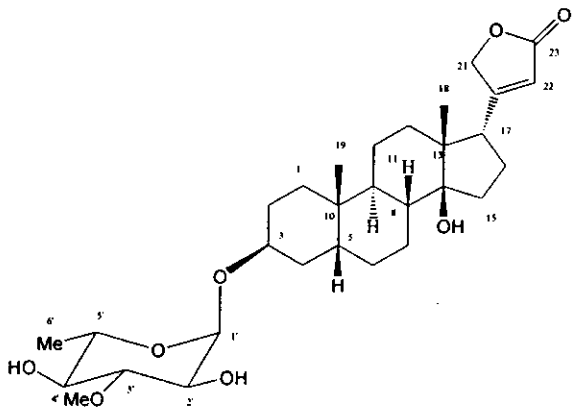
Position	Compound SCO2, δ_C (ppm)	Compound SCO1, δ_C (ppm)
1	30.36	29.91
2	26.88[a]	26.55[a]
3	72.25	73.39
4	29.90	30.55
5	36.47	36.52
6	26.62[a]	26.28[a]
7	21.19[b]	21.14[b]
8	41.82	41.64
9	35.65	35.66
10	35.19	35.19

Table 13 (Continued)

Position	Compound SCO2, δ_C (ppm)	Compound SCO1, δ_C (ppm)
11	21.34[b]	21.29[b]
12	40.00	39.95
13	49.62	49.44
14	85.49	84.55
15	33.12	32.91
16	26.59	26.44[a]
17	50.92	50.90
18	15.76	15.71
19	23.89	23.78
20	174.69[c]	175.04[c]
21	73.48	73.60
22	117.61	117.48
23	174.69[c]	175.06[c]
1′	93.73	97.15
2′	74.30	72.66
3′	80.93	85.24
4′	75.33	74.68
5′	67.08	67.60
6′	17.54	17.40
2′-C=O	170.27	-
2′-OAc	20.89	-
3′-OMe	60.49	60.54

[a, b, c] Assignment with the same superscripts maybe interchanged.

3.1.3 Compound SCO3



Compound **SCO3** was isolated as a white solid, mp = 215-218 °C, $[\alpha]_D^{26}$ = -111.11° (c = 0.0018, $CHCl_3$). The IR spectrum of this compound (**Fig. 25**) showed absorption of hydroxy group (3415 cm^{-1}) and carbonyl group (1735 cm^{-1}). The UV spectrum (**Fig. 24**) showed maximum at 242 nm.

The complete analysis of ^{13}C and ^{1}H NMR spectral data of compound **SCO3** (see **Table 17, Fig. 26** and **27**) were assigned with the information provided from ^{1}H-^{1}H COSY NMR (see **Table 15, Fig. 29**), HMQC (**Fig. 30**), HMBC (see **Table 16, Fig. 31**) and NOE experiments (**Fig. 26** and **27**). The ^{13}C NMR spectrum of compound **SCO3** (see **Table 17, Fig. 27**) recorded in $CDCl_3$ showed 30 signals for 30 carbon atoms. Analysis of the DEPT-90° and DEPT-135° spectrum of this compound (see **Table 14, Fig. 28**) suggested the presence of four methyl carbon atoms (δ 60.63, 23.89, 17.52 and 15.76), ten methylene carbon atoms (δ 73.42, 39.98, 33.17, 30.61, 29.92, 26.86, 26.55, 26.50, 21.34 and 21.17), eleven methine carbon atoms (δ 117.76, 97.18, 84.62, 74.70, 73.28, 72.94, 67.48, 50.87, 41.81, 36.90 and 35.69) and five signals for quaternary carbon atoms (δ 174.46, 174.40, 85.55, 49.57 and 35.24).

Compound **SCO3**, an isomer of compound **SCO1**, showed the same characteristic bands in the IR spectrum and the similar maximum UV spectrum as compound **SCO1**. Comparison of the ^{1}H NMR spectral data (see **Table 18**) of the two compounds revealed their close structural similarity. The proton signals of butenolide ring appeared at δ 5.89 (1H, *t*, *J* = 1.5 Hz), 4.98 (1H, *dd*, *J* = 18.5, 1.5 Hz) and 4.82 (1H, *dd*, *J* = 18.5, 1.5 Hz). The moiety of sugar protons resonated at 4.86 (1H, *d*, *J* = 4 Hz), 3.75 (1H, *dq*, *J* = 6, 9 Hz), 3.69 (3H, *s*), 3.59 (1H, *dt*, *J* = 4, 9 Hz), 3.25 (1H, *t*, *J* = 9 Hz), 3.15 (1H, *t*, *J* = 9 Hz) and 1.25 (1H, *d*, *J* = 6 Hz) could be assigned to H-1′, 5′, 3′-OMe, 2′, 3′, 4′ and 6′, respectively.

In the HMBC correlation of compound **SCO3** (see **Table 16**, **4**, **Fig. 25**), the carbon signals at C-4 (30.61), C-5 (36.90) and C-1′ (97.18) showed correlation peaks with the H-3 (3.97) and the carbon signals at C-3 (73.28), C-3′ (84.62) and C-5′ (67.48) showed correlation peaks with the H-1′(4.86), indicating that the glycosidic linkage was formed between sugar moiety and the steroid at C-3 (73.28). The carbon signals at C-12 (39.98), C-13 (49.57), C-14 (85.55), C-16 (26.55), C-20 (174.40) and C-21 (73.42) showed the correlation peaks with the H-17 (2.79), confirming the position of the butenolide ring at C-17.

In the NOE experiment (**Fig. 32** and **33**), irradiation of methyl group at δ 0.88 (CH_3-18) enhanced the signal at δ 5.89 (H-22), 4.98 and 4.82 (H-21a, b) and 2.79 (H-17). Irradiation of the methine proton at δ 2.79 (H-17) enhanced the signals at δ 0.88 (CH_3-18), 5.89 (H-22) and 4.98 and 4.82 (H-21a, b). These observations indicated that this compound should be α-butenolide at C-17.

Table 14 ^{13}C and DEPT spectral data of compound **SCO3**

δ_C	DEPT-90°	DEPT-135°	Type of Carbon
174.46			C
174.40			C
117.76	117.76	117.76	= CH
97.18	97.18	97.18	CH
85.55			C
84.62	84.62	84.62	CH
74.70	74.70	74.70	CH
73.42		73.42	CH_2
73.28	73.28	73.28	CH
72.94	72.94	72.94	CH
67.48	67.48	67.48	CH
60.63		60.63	CH_3
50.87	50.87	50.87	CH
49.57			C
41.81	41.81	41.81	CH
39.98		39.98	CH_2
36.90	36.90	36.90	CH
35.69	35.69	35.69	CH
35.24			C
33.17		33.17	CH_2
30.61		30.61	CH_2
29.92		29.92	CH_2
26.86		26.86	CH_2
26.55		26.55	CH_2
26.50		26.50	CH_2
23.89		23.89	CH_3
21.34		21.34	CH_2

Table 14 (Continued)

δ_C	DEPT-90°	DEPT-135°	Type of Carbon
21.17		21.17	CH_2
17.52		17.52	CH_3
15.76		15.76	CH_3

Table 15 500 MHz COSY Correlation of some protons of **SCO3**

δ_H (ppm)	Proton Correlation with δ_H(ppm)
H-21a (4.98)	H-21b
H-21b (4.82)	H-21a
H-1′ (4.86)	H-2′
H-2′ (3.59)	H-1′ and H-3′
H-3′ (3.25)	H-2′ and H-4′
H-4′ (3.15)	H-3′ and H-5′
H-5′ (3.75)	H-4′ and H-6′
H-6′ (1.25)	H-5′

Table 16 Major HMBC Correlation of **SCO3**

Position	δ_H (ppm)	δ_C (ppm)
3	3.97 (1H, *br m*)	C-4 (30.61), C-5 (36.90) and C-1′ (97.18)
8	1.55 (1H, *m*)	C-5 (36.90), C-7 (21.34), C-13 (49.57) and C-14 (85.55)

Table 16 (Continued)

Position	δ_H (ppm)	δ_C (ppm)
17	2.79 (1H, *dd*, *J* = 5, 9 Hz)	C-12 (39.98), C-13 (49.57), C-14 (85.55),C-16 (26.55), C-20 (174.40), C-21 (73.42) and C-22 (117.76)
18	0.88 (3H, *s*)	C-12 (39.98), C-13 (49.57), C-14 (85.55) and C-17 (50.87)
19	0.96 (3H, *s*)	C-4 (30.61), C-6 (26.89) and C-9 (35.69)
21	4.98 (1H, *dd*, *J* = 18.5, 1.5 Hz) 4.82 (1H, *dd*, *J* = 18.5, 1.5 Hz)	C-20 (174.40), C-22 (117.76) and C-23 (174.46)
22	5.89 (1H, *t*, *J* = 1.5 Hz)	C-17 (50.87), C-20 (174.40), C-21 (73.42) and C-23 (174.46)
1′	4.86, (1H, *d*, *J* = 4 Hz)	C-3 (73.28), C-3′ (84.64) and C-5′ (67.48)
3′	3.25 (1H, *t*, *J* = 9 Hz)	C-2′ (72.94), C-3′ -OMe (60.63) and C-4′ (74.70)
4′	3.15 (1H, *t*, *J* = 9 Hz)	C-3′ (84.62), C-5′ (67.48) and C-6′ (17.52)
5′	3.75 (1H, *dq*, *J* = 6, 9 Hz)	C-3′ (84.62)
6′	1.25 (1H, *d*, *J* = 6 Hz)	C-4′ (74.70) and C-5′ (67.48)

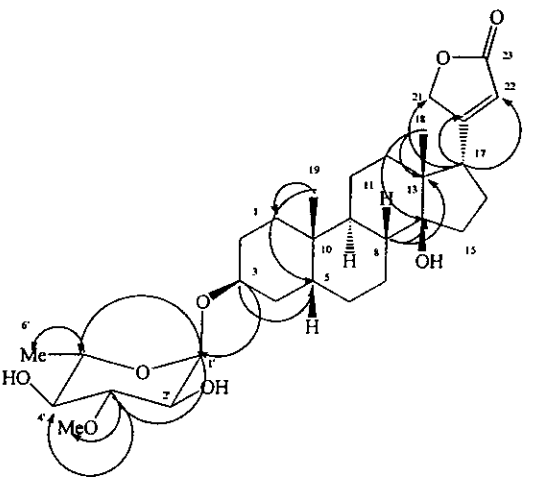
Table 17 ^{1}H and ^{13}C spectral data of compound **SCO3**

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
1	29.92	CH_2	1.80 (1H, *m*), 1.26 (1H, *m*)
2	26.50[a]	CH_2	**
3	73.28	CH	3.97 (1H, br *m*)
4	30.61	CH_2	1.58 (2H, *m*)
5	36.90	CH	1.66 (1H, *m*)
6	26.86[a]	CH_2	**
7	21.34[b]	CH_2	***
8	41.81	CH	1.55 (1H, *m*)
9	35.69	CH	1.60 (1H, *m*)
10	35.24	C	-
11	21.17[b]	CH_2	***
12	39.98	CH_2	1.55 (1H, *m*), 1.40 (1H, *m*)
13	49.57	C	-
14	85.55	C	-
15	33.17	CH_2	2.16 (1H, *m*), 1.70 (1H, *m*)
16	26.55[a]	CH_2	**
17	50.87	CH	2.79 (1H, *dd*, J = 5, 9 Hz)
18	15.73	CH_3	0.88 (3H, *s*)
19	23.89	CH_3	0.96 (3H, *s*)
20	174.40	C	-
21	73.42	CH_2	4.98 (1H, *dd*, J = 18.5, 1.5 Hz)
			4.82 (1H, *dd*, J = 18.5, 1.5 Hz)
22	117.76	= CH	5.89 (1H, *t*, J = 1.5 Hz)
23	174.46	C	-
1′	97.18	CH	4.86, (1H, *d*, J = 4 Hz)
2′	72.94	CH	3.59, (1H, *dt*, J = 4, 9 Hz)
3′	84.62	CH	3.25 (1H, *t*, J = 9 Hz)

Table 17 (Continued)

Position	δ_C (ppm)		δ_H (ppm)
4′	74.70	CH	3.15 (1H, *t*, *J* = 9 Hz)
5′	67.48	CH	3.75 (1H, *dq*, *J* = 6, 9 Hz)
6′	17.52	CH_3	1.25 (1H, *d*, *J* = 6 Hz)
3′-OMe	60.63	CH_3	3.69 (3H, *s*)

[a, b] [a, b, c] Assignment with the same superscripts maybe interchanged, [#] Carbon type deduced from DEPT experiment, [**] The chemical shift of proton resonated at δ 1.30-2.16, [***] The chemical shift of proton resonated at δ 1.26-1.70.



Selected HMBC correlation of **SCO3**

Table 18 Comparison of ^{1}H NMR spectral data between compound **SCO3** and **SCO1**

Position	Compound SCO 3, δ_H (ppm)	Compound SCO 1, δ_H (ppm)
1	1.80 (1H, *m*), 1.26 (1H, *m*)	1.76 (1H, *m*), 1.45 (1H, *m*)
3	3.97 (1H, *br m*)	3.95 (1H, *br s*)
4	1.58 (2H, *m*)	1.54 (1H, *m*), 1.37 (1H, *m*)
5	1.66 (1H, *m*)	1.62 (1H, *m*)
8	1.55 (1H, *m*)	1.55 (1H, *m*)
9	1.60 (1H, *m*)	1.60 (1H, *m*)
12	1.55 (1H, *m*), 1.40 (1H, *m*)	1.50 (1H, *m*), 1.37 (1H, *m*)
15	2.16 (1H, *m*), 1.70 (1H, *m*)	2.08 (1H, *m*), 1.68 (1H, *m*)
17	2.79 (1H, *dd*, *J* = 5, 9 Hz)	2.77 (1H, *dd*, *J* = 5, 9 Hz)
18	0.88 (3H, *s*)	0.86 (3H, *s*)
19	0.96 (3H, *s*)	0.94 (3H, *s*)
21	4.98 (1H, *dd*, *J* = 18.5, 1.5 Hz)	4.97 (1H, *dd*, *J* = 18.5, 1.5 Hz)
	4.82 (1H,dd, *J* = 18.5, 1.5 Hz)	4.79 (1H, *dd*, *J* = 18.5, 1.5 Hz)
22	5.89 (1H, *t*, *J* = 1.5 Hz)	5.87 (1H, *s*)
1′	4.86 (1H, *d*, *J* = 4 Hz)	4.83 (1H, *d*, *J* = 3.5 Hz)
2′	3.59 (1H, *dt*, *J* = 4, 9 Hz)	3.53 (1H, *dd*, *J* = 3.5, 9 Hz)
3′	3.24 (1H, *t*, *J* = 9 Hz)	3.24 (1H, *t*, *J* = 9 Hz)
4′	3.15 (1H, *t*, *J* = 9 Hz)	3.10 (1H, *t*, *J* = 9 Hz)
5′	3.75 (1H, *dq*, *J* = 6, 9 Hz)	3.71 (1H, *dq*, *J* = 6, 9 Hz)
6′	1.25 (3H, *d*, *J* = 6 Hz)	1.22 (3H, *d*, *J* = 6 Hz)
3′-OMe	3.69 (3H, *s*)	3.66 (3H, *s*)

Table 19 Comparison of ^{13}C NMR spectral data between compound **SCO3** and **SCO1**

Position	Compound SCO3, δ_C (ppm)	Compound SCO1, δ_C (ppm)
1	29.92[a]	29.91
2	26.50	26.55[a]
3	73.28	73.39
4	30.61	30.55
5	36.90	36.52
6	26.86[a]	26.28[a]
7	21.34[b]	21.14[b]
8	41.81	41.64
9	35.69	35.66
10	35.24	35.19
11	21.17[b]	21.29[b]
12	39.98	39.95
13	49.57	49.44
14	85.55	84.55
15	33.17	32.91
16	26.55[a]	26.44[a]
17	50.87	50.90
18	15.73	15.71
19	23.89	23.78
20	174.40	175.04[c]
21	73.42	73.60
22	117.76	117.48
23	174.46	175.06[c]
1′	97.18	97.15
2′	72.94	72.66
3′	84.62	85.24
4′	74.70	74.68

Table 19 (Continued)

Position	Compound SCO3, δ_C (ppm)	Compound SCO1, δ_C (ppm)
5′	67.48	67.60
6′	17.52	17.40
3′-OMe	60.63	60.54

a, b, c Assignment with the same superscripts maybe interchanged.

By comparison of ^{1}H and ^{13}C NMR spectral data between compound **SCO3** and 17α-neriifolin, both compounds showed similarlity. Thus compound **SCO3** was identified as 3β-*O*-(L-thevetosyl)-14β-hydroxy-5β-17β-card-20(22)-enolide (17α-neriifolin) which was the compound previously isolated from the leaves of *C.odollam* and *C. manghas* (Yamauhi, *et.al.*, 1987).

Table 20 Comparison of ^{1}H NMR spectral data between compound **SCO3** and 17α-neriifolin

Position	Compound SCO3, δ_H (ppm) (Recorded in $CDCl_3$)	17α-Neriifolin, δ_H (ppm) (Recorded in pyridine)
3	3.97 (1H, *br m*)	4.11 (1H, *br s*)
17	2.79 (1H, *dd*, *J* = 5, 9 Hz)	2.81 (1H, *dd*, *J* = 5, 9 Hz)
18	0.88 (3H, *s*)	0.99 (3H, *s*)
19	0.96 (3H, *s*)	1.04 (3H, *s*)
21	4.98 (1H, *dd*, *J* = 18.5, 1.5 Hz)	4.99 (1H, *dd*, *J* = 18, 1 Hz)
	4.82 (1H, *dd*, *J* = 18.5, 1.5 Hz)	5.20 (1H, *dd*, *J* = 18, 1 Hz)
22	5.89 (1H, *t*, *J* = 1.5 Hz)	6.13 (1H, *br s*)

Table 20 (Continued)

Position	Compound SCO3, δ_H (ppm) (Recorded in $CDCl_3$)	17α-Neriifolin, δ_H (ppm) (Recorded in pyridine)
1′	4.86 (1H, *d*, *J* = 4 Hz)	5.22 (1H, *d*, *J* = 4 Hz)
2′	3.53 (1H, *dt*, *J* = 4, 9 Hz)	4.08 (1H, *dd*, *J* = 4, 9 Hz)
3′	3.25 (1H, *t*, *J* = 9 Hz)	4.02 (1H, *t*, *J* = 9 Hz)
4′	3.15 (1H, *t*, *J* = 9 Hz)	3.66 (1H, *t*, *J* = 9 Hz)
5′	3.75 (1H, *dq*, *J* = 6, 9 Hz)	4.29 (1H, *m*)
6′	1.25 (3H, *d*, *J* = 6 Hz)	1.62 (3H, *d*, *J* = 6 Hz)
3′-OMe	3.69 (3H, *s*)	3.83 (3H, *s*)

Table 21 Comparison of ^{13}C NMR spectral data between compound **SCO3** and 17α-neriifolin

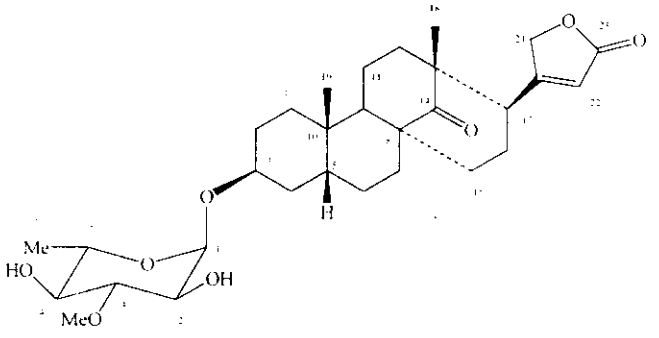
Position	Compound SCO3, δ_C (ppm) (Recorded in $CDCl_3$)	17α-Neriifolin, δ_C (ppm) (Recorded in pyridine)
1	29.92[a]	32.0[a]
2	26.50	27.3
3	73.28	73.0
4	30.61	32.9[a]
5	36.90	34.2
6	26.86[a]	28.2
7	21.34[b]	50.9
8	41.81	64.4
9	35.69	31.9
10	35.24	33.8
11	21.17[b]	20.9
12	39.98	40.8

Table 21 (Continued)

Position	Compound SCO2, δ_C (ppm) (Recorded in $CDCl_3$)	17α-Neriifolin, δ_C (ppm) (Recorded in pyridine)
13	49.57	52.6
14	85.55	81.7
15	33.17	35.2
16	26.55[a]	28.7
17	50.87	50.9
18	15.73	17.3
19	23.89	24.4
20	174.40	175.0
21	73.42	73.6
22	117.76	117.7
23	174.46	174.3
1′	97.18	98.9
2′	72.94	73.3
3′	84.62	85.3
4′	74.70	76.5
5′	67.48	69.0
6′	17.52	18.5
3′-OMe	60.63	60.5

[a, b] Assignment with the same superscripts maybe interchanged.

3.1.4 Compound SCO4



Compound **SCO4** was isolated as a white solid, mp = 196-198 °C, $[\alpha]_D^{26}$ = -17.85° (*c* = 0.0056, $CHCl_3$). Its UV absorption spectrum (**Fig. 34**) showed maximum at 244 nm. The IR spectrum of compound **SCO4** (**Fig. 35**) showed absorption bands at 3415 cm^{-1} (hydroxy group) and 1720 cm^{-1} (carbonyl group).

The ^{13}C NMR spectrum of compound **SCO4** (see **Table 25**, **Fig. 37**) in $CDCl_3$ showed 30 signals for 30 carbon atoms, however, no C-14 carbinol carbon peak was observed at δ 83 to 85 when compared with compound **SCO1** (see **Table 27**). Analysis of the DEPT-90° and DEPT-135° spectrum of this compound (see **Table 22**, **Fig. 38**) suggested the prescence of four methyl carbon atoms (δ 60.77, 26.59, 23.43 and 17.62), eleven methylene carbon atoms (δ 72.89, 44.16, 42.63, 32.00, 30.28, 29.01, 27.03, 26.78, 24.10 and 21.41), ten methine carbon atoms (δ 116.75, 97.48, 84.68, 74.79, 73.44, 73.03, 67.61, 53.21, 45.91 and 37.46) and five signals for quaternary carbon atoms (δ 220.90 (C=O), 173.68 (C=O), 170.55, 48.81, 47.44 and 37.37).

The ^1H NMR spectrum of compound **SCO4** (see **Table 25**, **Fig. 36**) was a typical of cardenolide glycoside (similar to compound **SCO1**, see **Table 26**). The

normal typical characteristic peaks were shown at δ 5.70 (1H, *t*, *J* = 1.5 Hz) accompanied by AB system at δ 4.70 (1H, *dd*, *J* = 16.5, 1.5 Hz) and 4.55 (1H, *dd*, *J* = 16.5, 1.5 Hz) which were attributed to a butenolide ring and these protons were assigned to H-22 and H-21a, b, respectively. The sugar protons appeared at δ 4.84 (1H, *d*, *J* = 3.5 Hz), 3.73 (1H, *dq*, *J* = 6, 9 Hz), 3.69 (3H, *s*), 3.58 (1H, *dt*, *J* = 3.5, 9 Hz), 3.24 (1H, *t*, *J* = 9 Hz), 3.15 (1H, *t*, *J* = 9 Hz) and 1.25 (3H, *d*, *J* = 6 Hz) which were assigned to H-1′, 5′, 3′-OMe, 2′, 3′, 4′ and 6′, respectively. The two methyl protons, one oxymethine and one methine proton of steroidal ring appeared at δ 0.81 (3H-19, *s*), 0.95 (3H-18, *s*), 3.09 (1H-17, *d*, *J* = 7 Hz) and 3.96 (1H-3, *br m*), respectively, one proton resonated at δ 2.81(1H, *m*) and the remaining protons at δ 1.04 to 2.49.

The structure of compound **SCO4** was deduced from its ^{13}C and ^{1}H spectral data (see **Table 25**, **Fig. 36** and **37**) together with the results of ^{1}H-^{1}H correlation (COSY, see **Table 23**, **Fig. 39**), HMQC (**Fig. 40**) and HMBC (see **Table 24**, **Fig. 41**).

The correlation peaks in the HMBC spectra (see **Table 24**, **Fig. 41**) between H-3 (δ 3.96) with the carbons at δ 32.00 (C-1), 37.46 (C-5) and 97.48 (C-1′); and H-1′ (δ 4.84) with the carbons at 67.61 (C-5′), 73.44 (C-3) and 84.68 (C-3′), indicating that the glycosidic linkage was formed between sugar moiety and the steroid at C-3 (73.44). The proton signal at δ 3.09 (H-17) showed the correlation by long-range coupling with the carbon signals at 26.78 (C-16), 42.63 (C-12), 44.16 (C-15), 47.44 (C-13), 72.89 (C-21), 116.75 (C-22), 170.55 (C-20) and 220.90 (C-14) and proton at δ 2.86 (1H, *m*) showed correlation peaks with 53.21 (C-17) and 170.55 (C-20), thus the position of the butenolide ring at C-17 was confirmed. The proton at δ 2.86 (1H, *m*) should be assigned to H-16a.

Table 22 ^{13}C and DEPT spectral data of compound **SCO4**

δ_C	DEPT-90°	DEPT-135°	Type of Carbon
220.90			C
173.68			C
170.55			C
116.75	116.75	116.75	= CH
97.48	97.48	97.48	CH
84.68	84.68	84.68	CH
74.79	74.79	74.79	CH
73.44	73.44	73.44	CH
73.03	73.03	73.03	CH
72.89		72.89	CH_2
67.61	67.61	67.61	CH
60.77		60.77	CH_3
53.21	53.21	53.21	CH
48.81			C
47.44			C
45.91	45.91	45.91	CH
44.16		44.16	CH_2
42.63		42.63	CH_2
37.46	37.46	37.46	CH
37.37			C
32.00		32.00	CH_2
30.28		30.28	CH_2
29.01		29.07	CH_2
27.03		27.03	CH_2
26.78		26.78	CH_2
26.59		26.59	CH_3
24.10		24.10	CH_2

Table 22 (Continued)

δ_C	DEPT-90°	DEPT-135°	Type of Carbon
23.43		23.43	CH_3
21.41		21.41	CH_2
17.62		17.62	CH_3

Table 23 500 MHz COSY Correlation of some protons of **SCO4**

δ_H (ppm)	Proton Correlation with δ_H(ppm)
H-17 (3.09)	H-16a (2.86) , H-16b (1.56)
H-21a (4.67)	H-21b
H-21b (4.55)	H-21a
H-1′(4.84)	H-2′
H-2′ (3.58)	H-1′ and H-3′
H-3′ (3.24)	H-2′ and H-4′
H-4′ (3.15)	H-3′ and H-5′
H-5′ (3.73)	H-4′ and H-6′
H-6′ (1.25)	H-5′

Table 24 Major HMBC Correlation of compound **SCO4**

Position	δ_H (ppm)	δ_C (ppm)
3	3.96 (1H, *br m*)	C-1 (32.00), C-5 (37.46) and C-1′ (97.48)
9	2.48 (1H, *m*)	C-5 (37.46), C-8 (48.81) C-11 (21.41), C-12 (42.63) and C-19 (26.59)
16	2.86 (1H, m), 1.56 (1H, *m*)	C-15 (44.16), C-17 (53.21) and C-20 (170.55)
17	3.09 (1H, *d*, J = 7 Hz)	C-12 (42.63), C-13 (47.44), C-14 (220.90), C-15 (44.16), C-16 (26.78), C-20 (170.55), C-21 (72.89) and C-22 (116.75)
18	0.98 (3H, *s*)	C-13 (47.44), C-14 (220.90), C-12 (42.63) and C-17 (53.21)
19	0.81 (3H, s)	C-1 (32.00), C-5 (37.46) and C-9 (45.91)
21	4.67 (1H,dd, J = 16.5, 1.5 Hz) 4.55 (1H,dd, J = 16.5, 1.5 Hz)	C-20 (170.55), C-22 (116.75) and C-23 (170.66)
22	5.70 (1H, *t*, J = 1.5 Hz)	C-17 (53.21), C-20 (170.55), C-21 (72.89) and C-23 (170.66)
1′	4.87 (1H, d, J = 3.5 Hz)	C-3 (73.44), C-3′ (84.68) and C-5′ (67.61)
3′	3.24 (1H, *t*, J = 9 Hz)	C-2′ (73.03) and C-3′-OMe (60.77)

Table 24 (Continued)

Position	δ_H (ppm)	δ_C (ppm)
4′	3.15 (1H, *t*, *J* = 9 Hz)	C-3′ (84.68), C-5′ (67.61) and C-6′ (17.63)
5′	3.73 (1H, *dq*, *J* = 6, 9 Hz)	C-3′ (84.64)
6′	1.25 (1H, *d*, *J* = 6 Hz)	C-4′ (74.79) and C-5′ (97.61)

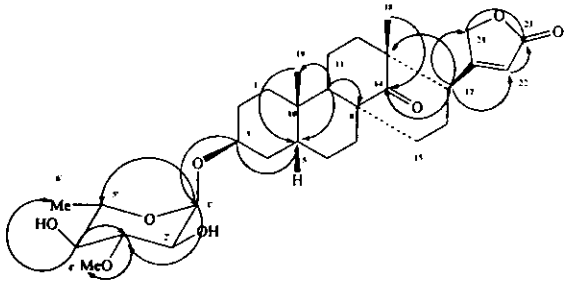
Table 25 ^{1}H and ^{13}C NMR spectral data of compound **SCO4**

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
1	32.00	CH_2	1.64 (1H, *m*), 1.38 (1H, *m*)
2	30.28	CH_2	1.68 (1H, m), 1.46 (1H, *m*)
3	73.44	CH	3.96 (1H, *br m*)
4	29.01	CH_2	1.25 (1H, *m*)
5	37.46	CH	1.61 (1H, *m*)
6	27.03	CH_2	1.97 (1H, *m*), 1.09 (1H, *m*)
7	24.10	CH_2	2.19 (1H, *m*), 1.14 (1H, *m*)
8	48.81	C	-
9	45.91	CH	2.48 (1H, *m*)
10	37.37	C	-
11	21.41	CH_2	2.48 (1H, *m*), 1.82 (1H, *m*)
12	42.63	CH_2	2.04 (1H, *m*), 1.80 (1H, *m*)
13	47.44	C	-
14	220.90	C	-
15	44.16	CH_2	2.10 (1H, *m*)
16	26.78	CH_2	2.86 (1H, *m*), 1.56 (1H, *m*)

Table 25 (Continued)

Position	δ_C [#] (ppm)		δ_H (ppm)
17	53.21	CH	3.09 (1H, *d*, *J* =∇ Hz)
18	23.43	CH_3	0.95 (3H, *s*)
19	26.59	CH_3	0.81 (3H, *s*)
20	170.55	C	-
21	72.89	CH_2	4.70 (1H, *dd*, *J* = 16.5, 1 Hz)
			4.55 (1H, *dd*, *J* = 16.5, 1 Hz)
22	116.75	= CH	5.70 (1H, *t*, *J* = 1.5 Hz)
23	173.66	C	-
1′	97.48	CH	4.84, (1H, *d*, *J* = 3.5 Hz)
2′	73.03	CH	3.58, (1H, *dt*, *J* =3.5, 9 Hz)
3′	84.68	CH	3.24 (1H, *t*, *J* = 9 Hz)
4′	74.79	CH	3.15 (1H, *t*, *J* = 9 Hz)
5′	67.61	CH	3.73 (1H, *dq*, *J* = 6, 9 Hz)
6′	17.63	CH_3	1.25 (1H, *d*, *J* = 6 Hz)
3′-OMe	60.77	CH_3	3.69 (3H, *s*)

[#] Carbon type deduced from DEPT experiment.



Selected HMBC correlation of **SCO4**

Table 26 Comparison of ^{1}H NMR spectral data between compound **SCO4** and **SCO1**

Position	δ_H (ppm), Compound SCO4	δ_H (ppm), Compound SCO1
1	1.64 (1H, *m*), 1.38 (1H, *m*)	1.76 (1H, *m*), 1.45 (1H, *m*)
2	1.68 (1H, m), 1.46 (1H, *m*)	\ **
3	3.96 (1H, *br m*)	3.95 (1H, *br m*)
4	1.25 (1H, *m*)	1.54 (1H, m), 1.371H, *m*)
5	1.61 (1H, *m*)	1.62 (1H, *m*)
6	1.97 (1H, *m*), 1.09 (1H, *m*)	**
7	2.19 (1H, *m*), 1.14 (1H, *m*)	***
8	-	1.55 (1H, *m*)
9	2.48 (1H, *m*)	1.60 (1H, *m*)
11	2.48 (1H, *m*), 1.82 (1H, *m*)	***
12	2.04 (1H, *m*), 1.80 (1H, *m*)	1.50 (1H, *m*), 1.37 (1H, *m*)
15	2.10 (1H, *m*)	2.08 (1H, *m*), 1.68 (1H, *m*)
16	2.86 (1H, *m*), 1.56 (1H, *m*)	**
17	3.09 (1H, *d*, J = 7 Hz)	2.77 (1H, *dd*, J = 5, 9 Hz)
18	0.95 (3H, *s*)	0.86 (3H, *s*)
19	0.81 (3H, *s*)	0.94 (3H, *s*)
21	4.70 (1H, *dd*, J = 16.5, 1.5 Hz)	4.97 (1H, *dd*, J = 18.5, 1.5 Hz)
	4.55 (1H, *dd*, J = 16.5, 1.5 Hz)	4.79 (1H, *dd*, J = 18.5, 1.5 Hz)
22	5.70 (1H, *t*, J = 1.5 Hz)	5.87 (1H, *s*)
1′	4.84, (1H, *d*, J = 3.5 Hz)	4.83 (1H, *d*, J = 3.5 Hz)
2′	3.58, (1H, *dt*, J =3.5, 9 Hz)	3.53 (1H, *dd*, J = 3.5, 9 Hz)
3′	3.24 (1H, *t*, J = 9 Hz)	3.24 (1H, *t*, J = 9 Hz)
4′	3.15 (1H, *t*, J = 9 Hz)	3.10 (1H, *t*, J = 9 Hz)
5′	3.73 (1H, *dq*, J = 6, 9 Hz)	3.71 (1H, *dq*, J = 6, 9 Hz)
6′	1.25 (1H, *d*, J = 6 Hz)	1.22 (3H, *d*, J = 6 Hz)
3′-OMe	3.69 (3H, *s*)	3.66 (3H, *s*)

* The chemical shifts of proton resonated at δ 1.25- 2.15, *** The chemical shifts of proton resonated at δ 1.22- 1.68.

Table 27 Comparison of ^{13}C NMR spectral data between compound **SCO4** and **SCO1**

Position	δ_H (ppm), Compound SCO4	δ_H (ppm), Compound SCO1
1	32.00	29.91
2	30.28	26.55[a]
3	73.44	73.39
4	29.01	30.55
5	37.46	36.82
6	27.03	26.28[a]
7	24.10	21.89[b]
8	48.81	41.65
9	45.91	35.66
10	37.37	35.19
11	21.41	21.29[b]
12	42.63	39.95
13	47.44	49.65
14	220.90	84.55
15	44.16	32.91
16	26.78	26.44[a]
17	53.21	50.90
18	23.43	15.71
19	26.59	23.78
20	170.55	175.06[c]
21	72.89	73.60
22	116.75	117.48
23	173.66	175.04[c]
1′	97.48	97.15
2′	73.03	72.66
3′	84.68	85.24

Table 27 (Continued)

Position	δ_H (ppm), Compound SCO4	δ_H (ppm), Compound SCO1
4′	74.79	74.68
5′	67.61	67.60
6′	17.63	17.40
3′-OMe	60.77	60.54

[a, b, c] Assignment with the same superscripts maybe interchanged.

Comparison of ^{1}H and ^{13}C NMR spectral data between compound **SCO4** and 17β-cerleaside A (see **Table 28 and 29**) showed similarlity. Thus compound **SCO4** was identified as 3β-*O*-(L-thevetosyl)-15(8⟶14)-*abeo*-5β-(8R)-14-oxo-card-20(22)-enolide (17β-cerleaside A) which was the compound previously isolated from the leaves of *C.odollam* and *C. manghas* (Yamauchi, *et.al.*, 1987).

Table 28 Comparison of ^{1}H NMR spectral data between compound **SCO4** and cerleaside A

Position	δ_H (ppm), Compound SCO4 (Recorded in $CDCl_3$)	δ_H (ppm), Cerleaside A (Recorded in pyridine)
3	3.96 (*br m*)	4.20 (*br s*)
17	3.09 (1H, *d*, J = 7 Hz)	2.98 (1H, br d, J = 7 Hz)
18	0.95 (3H, *s*)	0.71 (3H, *s*)
19	0.81 (3H, *s*)	0.90 (3H, *s*)
21	4.70 (1H, *dd*, J = 16.5, 1.5 Hz)	4.83 (1H, *dd*, J = 18, 1 Hz)
	4.55 (1H, *dd*, J = 16.5, 1.5 Hz)	4.73 (1H, *dd*, J = 18, 1 Hz)

Table 28 (Continued)

Position	δ_H (ppm), Compound SCO4 (Recorded in $CDCl_3$)	δ_H (ppm), Cerleaside A (Recorded in pyridine)
22	5.70 (1H, *t*, *J* = 1.5 Hz)	5.88 (1H, *br s*)
1′	4.87 (1H, *d*, *J* = 3.5 Hz)	5.23 (1H, *d*, *J* = 3 Hz)
2′	3.58 (1H, *dt*, *J* = 3.5, 9 Hz)	4.06 (1H, *dd*, *J* = 3, 9 Hz)
3′	3.24 (1H, *t*, *J* = 9 Hz)	3.99 (1H, *t*, *J* = 9 Hz)
4′	3.15 (1H, *t*, *J* = 9 Hz)	3.66 (1H, *t*, *J* = 9 Hz)
5′	3.73 (1H, *dq*, *J* = 6, 9 Hz)	4.29 (1H, *m*)
6′	1.25 (1H, *d*, *J* = 6 Hz)	1.61 (1H, *d*, *J* = 6 Hz)
3′-OMe	3.69 (3H, *s*)	3.83 (3H, *s*)

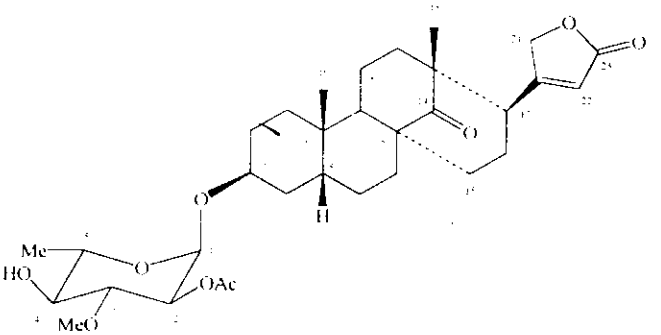
Table 29 Comparison of ^{13}C NMR spectral data between compound **SCO4** and cerleaside A

Position	δ_H (ppm), Compound SCO4 (recorded in $CDCl_3$)	δ_H (ppm), Cerleaside A (recorded in pyridine)
1	32.00	30.3
2	30.28	26.8
3	73.44	73.3
4	29.01	29.1
5	37.46	37.0
6	27.03	27.0
7	24.10	24.4
8	48.81	47.4
9	45.91	45.9

Table 29 (Continued)

Position	δ_H (ppm), Compound SCO4 (recorded in $CDCl_3$)	δ_H (ppm), Cerleaside A (recorded in pyridine)
10	37.37	37.5
11	21.41	21.2
12	42.63	43.9
13	47.44	48.8
14	220.90	221.2
15	44.16	42.5
16	26.78	32.2
17	53.21	52.8
18	23.43	23.3
19	26.59	26.2
20	170.55	171.7
21	72.89	73.3
22	116.75	116.2
23	173.66	173.7
1′	97.48	98.9
2′	73.03	73.4
3′	84.68	85.3
4′	74.79	76.5
5′	67.61	68.9
6′	17.63	18.5
3′-OMe	60.77	60.5

3.3.5 Compound SCO5



Compound **SCO5** was isolated as a white solid, mp = 209-211 °C, $[\alpha]_D^{26}$ = -62.5° (*c* = 0.0016, $CHCl_3$). Its UV absorption spectrum (**Fig. 42**) showed maximum at 243 nm. The IR spectrum of compound **SCO5** (**Fig. 43**) showed absorption bands at 3414 cm^{-1} (hydroxy group) and 1744 and 1722 cm^{-1} (carbonyl groups).

The complete analysis of ^{13}C and ^{1}H NMR spectrum of compound **SCO5** (see **Table 33**, **Fig. 44** and **45**) were assigned with the information provided from ^{1}H-^{1}H COSY (see **Table 31**, **Fig. 47**), HMQC (**Fig. 48**), and HMBC, (see **Table 32**, **Fig. 49**). The ^{13}C NMR spectrum of compound **SCO5** recorded in $CDCl_3$ (see **Table 32**, **Fig. 45**) showed 32 signals for 32 carbon atoms. The DEPT-90° and DEPT-135° (see **Table 30**, **Fig. 46**) indicating the existence of five methyl carbon atoms (δ 60.55, 26.53, 23.33, 20.90 and 17.58), ten methylene carbon atoms (δ 72.77, 44.04, 42.57, 31.65, 29.80, 28.96, 26.92, 26.82, 24.03 and 21.31), ten methine carbon atoms (δ 116.63, 93.85, 80.83, 75.28, 74.26, 72.15, 66.98, 53.10, 45.81 and 36.88) and seven quaternary carbon atoms (δ 220.93, 173.60, 170.45, 170.25, 48.75, 47.35 and 37.26).

Compound **SCO5**, a derivative of compound **SCO4**, showed the same characteristic peak in the IR and UV spectrum of compound **SCO4**. Comparison of the ^{1}H NMR spectrum (see **Table 34**) of the two compounds revealed their close structural similarity. Differences in the spectrum of compound **SCO5** was shown as acetoxy

proton at δ 2.08 (3H, s) which was not observed in compound **SCO4** (see **Table 35, 36**). The characteristic signals of butenolide ring resonated at δ 5.71 (1H, *t*, J = 1.5 Hz) and *doublet of doublet* AB system at δ 4.69 (J = 18, 1.5 Hz) and 4.57 (J = 18, 1.5 Hz) which were assigned to H-22 and 21a,b, respectively. The sugar moiety signals appearing at δ 5.07 (1H, *d*, J = 2 Hz), 4.65 (1H, *dd*, J = 4, 9 Hz), 3.81 (1H, *dq*, J = 6, 9 Hz), 3.59 (-OCH_3, *s*), 3.57 (1H, *t*, J = 9 Hz), 3.23 (1H, *t*, J = 9 Hz), 1.27 (3H, *d*, J = 6 Hz) could be assigned to H-1′, 2′, 5′, 3′-OCH_3, 3′, 4′, and 6′, respectively. The H-2′ in compound **SCO5** (4.65, 1H, *dd*, J = 4, 9 Hz) appeared at the lower field than H-2′ in compound **SCO4** (3.58, 1H, *t*, J = 9 Hz). These observations indicated that the 2′ position should be connected with the acetyl group.

The HMBC correlation of compound **SCO5** (see **Table 32, Fig. 49**) were similar to compound **SCO4** except at H-2′ of **SCO5** (4.65, 1H, *dd*, J = 4, 9 Hz) showed correlation peaks with C-2′-C=O (173.56) and C-3′ (80.83), thus confirmed the position of the acetoxy group at C-2′ (73.03). These observations indicated that both compounds are derivatives. Thus compound **SCO5** was identified as 3β-*O*-(L-2′-*O*-acetyl thevetosyl)-15(8⟶14)-*abeo*-5β-(8R)-14-oxo-card-20(22)-enolide (2′-acetoxy cerleaside A). This compound has not been reported before.

Table 30 ^{13}C and DEPT spectral data of compound **SCO5**

δ_C	**DEPT-90°**	**DEPT-135°**	**Type of Carbon**
220.93			C
173.56			C
170.45			C
170.25			C
116.63	116.63	116.63	= CH

Table 30 (Continued)

δ_C	DEPT-90°	DEPT-135°	Type of Carbon
93.85	93.85	93.85	CH
80.83	80.83	80.83	CH
75.28	75.28	75.28	CH
74.26	74.26	74.26	CH
72.77		72.77	CH_2
72.15	72.15	72.15	CH
66.98	66.98	66.98	CH
60.55		60.55	CH_3
53.10	53.10	53.10	CH
48.75			C
47.35			C
45.81	45.81	45.81	CH
44.04		44.04	CH_2
42.57		42.57	CH_2
37.26			C
36.88	36.88	36.88	CH
31.65		31.65	CH_2
29.80		29.80	CH_2
28.96		28.96	CH_2
26.92		26.92	CH_2
26.82		26.82	CH_2
26.52		26.52	CH_3
24.03		24.08	CH_2
23.33		23.33	CH_3
21.31		21.31	CH_2
20.90		20.90	CH_3
17.58		17.58	CH_3

Table 31 500 MHz COSY Correlation of some protons of **SCO5**

δ_H (ppm)	Proton Correlation with δ_H (ppm)
H-17 (3.10)	**H-16a (2.87) and 16b (1.58)**
H-21a (4.69)	H-21b
H-21b (4.57)	H-21a
H-1′(5.07)	H-2′
H-2′ (4.65)	H-1′ and H-3′
H-3′ (3.59)	H-2′ and H-4′
H-4′ (3.23)	H-3′ and H-5′
H-5′ (3.81)	H-4′ and H-6′
H-6′ (1.27)	H-5′

Table 32 Major HMBC Correlation of compound **SCO5**

Position	δ_H (ppm)	δ_C (ppm)
3	3.88 (1H, *br m*)	C-1 (31.65), C-5 (36.87) and C-1′ (93.85)
9	2.50 (1H, *d*, *J* = 9 Hz)	C-1 (31.65), C-5 (36.87), C-8 (48.75), C-12 (42.57), C-14 (220.93), C-15 (44.04) and C-19 (26.52)
16	2.87 (1H, *m*), 1.58 (1H, *m*)	C-15 (44.04), C-17 (53.10) and C-20 (170.24)
17	3.10 (1H, *d*, *J* = 7 Hz)	C-12 (42.57), C-13 (47.35), C-14 (220.93), C-15 (44.04), C-20 (170.24), C-21 (72.77) and C-22 (116.63)

Table 32 (Continued)

Position	δ_H (ppm)	δ_C (ppm)
18	0.97 (3H, *s*)	C-12 (42.57), C-13 (47.35), C-14 (220.93) and C-17 (53.10)
19	0.89 (3H, *s*)	C-1 (31.65), C-5 (36.87) and C-9 (45.80)
21	4.69 (1H, *dd*, *J* = 18, 1.5 Hz) 4.57 (1H, *dd*, *J* = 18, 1.5 Hz)	C-20 (170.24), C-22 (116.63) and C-23 (170.45)
22	5.71 (1H, *t*, *J* = 1.5 Hz)	20 (170.24), C-21 (72.77), and C-23 (170.45)
1′	5.07 (1H, *d*, *J* = 2 Hz)	C-3(72.15), C-3′(80.83) and C-5′(66.98)
2′	4.65 (1H, *dd*, *J* = 4, 9 Hz)	C-3′(80.83) and C-2′-C=O (173.56)
3′	3.59 (1H, *t*, *J* = 9 Hz)	C-2′ (74.26) and C-3′-OMe (60.55)
4′	3.23 (1H, *t*, *J* = 9 Hz)	C-3′ (80.83), C-5′ (66.98) and 6′ (17.58)
5′	3.81 (1H, *dq*, *J* = 9, 6 Hz)	C-3′ (80.83), C-4′ (75.28) and 6′ (17.58)
6′	1.27 (3H, *d*, *J* = 6Hz)	C-4′ (75.28) and C-5′ (66.98)

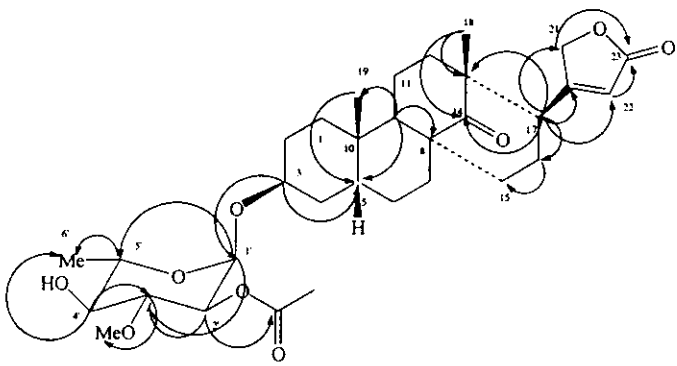
Table 33 ^{1}H and ^{13}C NMR spectral data of compound **SCO5**

Position	δ_C (ppm)		δ_H (ppm)
1	31.65	CH_2	1.59 (1H, *m*), 1.45 (1H, *m*)
2	29.80	CH_2	1.61 (1H, *m*), 1.33 (1H, *m*)
3	72.15	CH	3.88 (1H, *br m*)
4	28.96	CH_2	1.96 (1H, *m*), 1.09 (1H, *m*)
5	36.87	CH	1.60 (1H, *m*)
6	24.02	CH_2	2.29 (1H, *m*), 1.11 (1H, *m*)
7	28.96	CH_2	1.97 (1H, *m*)
8	48.75	C	-
9	45.80	CH	2.50 (1H, *m*)
10	37.26	C	-
11	21.30	CH_2	2.47 (1H, *m*), 1.84 (1H, *m*)
12	42.57	CH_2	2.10 (1H, *m*)
13	47.35	C	-
14	220.93	C	-
15	44.04	CH_2	2.17 (1H, *m*)
16	26.92	CH_2	2.87 (1H, *m*), 1.57 (1H, *m*)
17	53.10	CH	3.10 (1H, *d*, J = 7 Hz)
18	26.52	CH_3	0.97 (3H, *s*)
19	23.33	CH_3	0.89 (3H, *s*)
20	170.24	C	-
21	72.77	CH_2	4.69 (1H, *dd*, J = 18, 1.5 Hz)
			4.57 (1H, *dd*, J = 18, 1.5 Hz)
22	116.63	= CH	5.71 (1H, *t*, J = 1.5 Hz)
23	170.45	C	-
1′	93.85	CH	5.07 (1H, *d*, J = 2 Hz)
2′	74.26	CH	4.65 (1H, *dd*, J = 4, 9 Hz)
3′	80.83	CH	3.59 (1H, *t*, J = 9 Hz)

Table 33 (Continued)

Position	δ_C [#] (ppm)		δ_H (ppm)
4′	75.28	CH	3.23 (1H, *t*, *J* = 9 Hz)
5′	66.98	CH	3.81 (1H, *dq*, *J* = 6, 9 Hz)
6′	17.58	CH_3	1.27 (3H, *d*, *J* = 6 Hz)
2′-OAc	20.89	CH_3	2.08 (3H, *s*)
2′-C=O	173.56	C	-
3′-OMe	60.55	CH_3	3.59 (3H, *s*)

[#] Carbon type deduced from DEPT experiment.



Selected HMBC correlation of **SCO5**

Table 34 Comparison of ^{1}H NMR spectral data between compound **SCO5** and **SCO4**

Position	δ_H (ppm), compound SCO5	δ_C (ppm), compound SCO4
1	1.59 (1H, *m*), 1.45 (1H, *m*)	1.64 (1H, m), 1.38 (1H, *m*)
2	1.61 (1H, *m*), 1.33 (1H, *m*)	1.68 (1H, m), 1.46 (1H, *m*)
3	3.88 (1H, *br m*)	3.97 (1H, *br m*)

Table 34 (Continued)

Position	δ_H (ppm), compound SCO5	δ_C (ppm), compound SCO4
4	1.96 (1H, *m*), 1.09 (1H, *m*)	1.25 (1H, *m*)
5	1.60 (1H, *m*)	1.61 (1H, *m*)
6	2.29 (1H, *m*), 1.11 (1H, *m*)	1.97 (1H, *m*), 1.09 (1H, *m*)
7	1.97 (1H, *m*)	2.19 (1H, *m*), 1.14 (1H, *m*)
9	2.50 (1H, *m*)	2.48 (1H, *m*)
11	2.47 (1H, *m*), 1.84 (1H, *m*)	2.48 (1H, *m*), 1.82 (1H, *m*)
12	2.10 (1H, *m*)	2.04 (1H, *m*), 1.80 (1H, *m*)
15	2.17 (1H, *m*)	2.10 (1H, *m*)
16	2.87 (1H, m), 1.57 (1H, *m*)	2.86 (1H, *m*), 1.56 (1H, *m*)
17	3.10 (1H, *d*, J = 7 Hz)	3.09 (1H, *d*, J = 7 Hz)
18	0.97 (3H, *s*)	0.95 (3H, *s*)
19	0.89 (3H, *s*)	0.81 (3H, *s*)
21	4.69 (1H, *dd*, J = 18, 1.5 Hz)	4.70 (1H, *dd*, J = 16.5, 1.5 Hz)
	4.57 (1H, *dd*, J = 18, 1.5 Hz)	4.55 (1H, *dd*, J = 16.5, 1.5 Hz)
22	5.71 (1H, *t*, J = 1.5 Hz)	5.70 (1H, *t*, J = 1.5 Hz)
1′	5.07 (1H, *d*, J = 2 Hz)	4.84, (1H, *d*, J = 3.5 Hz)
2′	4.65 (1H, *dd*, J = 4, 9 Hz)	3.58, (1H, *dt*, J = 3.5, 9 Hz)
3′	3.59 (1H, *t*, J = 9 Hz)	3.24 (1H, *t*, J = 9 Hz)
4′	3.23 (1H, *t*, J = 9 Hz)	3.15 (1H, *t*, J = 9 Hz)
5′	3.81 (1H, *dq*, J = 6, 9 Hz)	3.73 (1H, *dq*, J = 6, 9 Hz)
6′	1.27 (3H, *d*, J = 6 Hz)	1.25 (3H, *d*, J = 6 Hz)
2′-OAc	2.08 (3H, *s*)	-
3′-OMe	3.59 (3H, *s*)	3.69 (3H, *s*)

Table 35 Comparison of ^{13}C NMR spectral data between compound **SCO5** and **SCO4**

Position	δ_H (ppm), compound SCO5	δ_C (ppm), compound SCO4
1	31.65	32.00
2	29.80	30.28
3	72.15	73.44
4	28.96	29.01
5	36.87	37.46
6	24.02	27.03
7	28.96	24.10
8	48.75	48.81
9	45.80	45.91
10	37.26	37.37
11	21.30	21.41
12	42.57	44.16
13	47.35	47.44
14	220.93	220.90
15	44.04	42.63
16	26.92	26.78
17	53.10	53.21
18	23.33	23.43
19	26.52	26.59
20	170.24	170.55
21	72.77	72.89
22	116.63	116.75
23	170.45	173.66
1′	93.85	97.48
2′	74.26	73.03
3′	80.83	84.68
4′	75.28	74.79

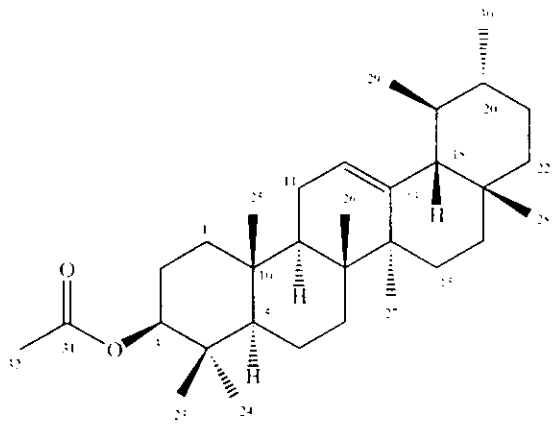
Table 35 (Continued)

Position	δ_H (ppm), compound SCO5	δ_C (ppm), compound SCO4
5′	66.98	67.61
6′	17.58	17.63
2′-OAc	20.89	
2′-C=O	173.56	
3′-OMe	60.55	60.77

3.2 Structural elucidation of compounds isolated from latex of C. odollam

The ethyl acetate extract (6 g) was purified by chromatographic technique and crystallization to give seven compounds, **LCO1, LCO2, LCO3, LCO4, LCO5, LCO6** and **LCO7**. Their structures were determined using 1D and 2D NMR spectroscopic data. In addition, the structures of **LCO1** and **LCO2** were confirmed by X-ray diffraction.

3.2.1 Compound LCO1



Compound **LCO1** was isolated as a white solid mp = 214-217 °C, $[\alpha]^{29}_{D}$= -71.42 ° (c = 0.0014, $CHCl_3$), this compound showed the characteristic of triterpene by giving a purple spot in vanillin sulfuric acid reagent . Its IR spectrum **(Fig. 50)** showed the presence of carbonyl group at 1733 cm^{-1}.

The ^{13}C NMR spectrum of compound **LCO1** (see **Table 38, Fig. 52**) recorded in $CDCl_3$ showed 32 signals for 32 carbon atoms. The DEPT 90° and DEPT 135° (see **Table 36, Fig. 53**) indicated the existence of nine methyl carbon atoms (δ 28.73, 28.05, 23.21, 21.38, 21.31, 17.49, 16.85, 16.72 and 15.72), nine methylene carbon atoms (δ 41.52, 38.44, 32.85, 31.23, 28.07, 26.58, 23.59, 23.35 and 18.22),

seven methine carbon atoms (δ 124.30, 80.94, 59.04, 55.24, 47.62, 39.63 and 39.59) and seven quaternary carbon atoms (δ 171.01 (C=O), 139.62, 42.05, 41.01, 37.70, 36.77 and 33.73). The mass spectrum of this compound (**Fig. 57**) showed the molecular ion peak at 468.4 *m/z*, thus this compound had molecular formula $C_{32}H_{52}O_2$.

The ^{1}H NMR spectrum of compound **LCO1** (see **Table 38, Fig. 51**), with the characteristic peaks of triterpene, demonstrated the presence of six methyl singlets at δ0.80, 0.86, 0.87, 0.98 1.01 and 106 and two methyl *doublets* at δ0.80 (J = 5.5 Hz) and 0.92 (J = 6 Hz), one acetoxy proton at δ 2.05 (*s*). The olefinic proton and the oxymethine proton were resonated at δ 5.13 (1H, *t*, J = 3.5 Hz) and 4.59 (1H, *dd*, J = 6, 10 Hz), respectively, which could be assigned to the position C-12 (124.30) and C-3 (80.94), respectively.

The structure of compound **LCO1** was deduced from its ^{13}C and ^{1}H spectral data (see **Table 38, Fig. 51, 52**) together with the results of ^{1}H -^{1}H COSY (**Fig. 54**), HMQC (**Fig. 55**) and HMBC (see **Table 37, Fig. 56**). The correlation peaks in the HMBC spectrum (see **Table 37, Fig. 50**) between H-3 (δ 4.51) and the carbon at 171.01 (C-31, C=O), 37.70 (C-4), 28.05 (C-23) and 16.72 (C-24) confirmed the position of the acetoxy group at C-3 position.

Table 36 ^{13}C and DEPT spectral data of compound **LCO1**

δ_C (ppm)	DEPT 45°	DEPT 90 °	DEPT 135 °	Type of Carbon
171.01				C
139.62				C
124.30	124.30	124.30	124.30	= CH
80.94	80.94	80.94	80.94	CH
59.04	59.04	59.04	59.04	CH

Table 36 (Continued)

δ_C (ppm)	DEPT 45 °	DEPT 90 °	DEPT 135 °	Type of Carbon
55.24	55.24	55.24	55.24	CH
47.62	47.62	47.62	47.62	CH
42.05				C
41.52	41.52		41.52	CH_2
40.01				C
39.63	39.63	39.63	39.63	CH
39.59	39.59	39.59	39.59	CH
38.44	38.44		38.44	CH_2
37.70				C
36.77				C
33.73				C
32.85	32.85		32.85	CH_2
31.23	31.23		31.23	CH_2
28.73	28.73		28.73	CH_3
28.07	28.07		28.07	CH_2
28.05	28.05		28.05	CH_3
26.58	26.58		26.58	CH_2
23.59	23.59		23.59	CH_2
23.35	23.35		23.35	CH_2
23.21	23.21		23.21	CH_3
21.38	21.38		21.38	CH_3
21.31	CH_3		21.31	CH_3
18.22	CH_2		18.22	CH_2
17.49	CH_3		17.49	CH_3
16.85	CH_3		16.85	CH_3
16.72	CH_3		16.72	CH_3
15.72	CH_3		15.72	CH_3

Table 37 Major HMBC correlation of **LCO1**

Position	δ_H (ppm)	HMBC correlation
1	1.66 (2H, *m*)	C-3 (80.94), C-5 (55.24) and C-9 (47.62)
3	4.51 (1H, *dd*, *J* = 6, 10.5 Hz)	C-31 (171.01), C-4 (37.70), C-23 (28.05) and C-24 (16.72)
5	0.85 (1H, *m*)	C-3 (80.94), C-4 (37.70) and C-10 (36.77)
9	1.55 (1H, *m*)	C-1 (38.44), C-5 (55.24), C-8 (40.01), C-10 (36.77), C-11 (23.59), C-14 (42.05) and C-25 (15.72)
12	5.13 (1H, *t*, *J* = 3.5 Hz)	C-9 (47.62), C-14 (42.05) and C-18 (59.04)
18	1.30 (1H, *m*)	C-12 (124.30) and C-13 (139.62)
19	1.30 (1H, *m*)	C-12 (124.30), C-13 (139.62) and C-20 (39.59)
22	1.42 (1H, *m*)	C-18 (59.04) and C-19 (39.63)
23	0.87 (3H, *s*)	C-3 (80.94), C-4 (37.70), C-5 (55.24) and C-24 (16.72)
24	0.86 (3H, *s*)	C-4 (37.70), C-5 (55.24) and C-23 (28.05)
25	0.98 (3H, *s*)	C-1 (38.44), C-5 (55.24), C-9 (47.62) and C-10 (36.77)

Table 37 (Continued)

Position	δ_H (ppm)	HMBC correlation
26	1.01 (3H, *s*)	C-8 (40.01), C-9 (47.62) and C-14 (42.05)
27	1.06 (3H, *s*)	C-8 (40.41), C-13 (139.62) and C-14 (42.05)
28	0.80 (3H, *s*)	C-17 (33.73), C-18 (59.04) and C-22 (41.52)
29	0.80 (3H, *d*, J = 5.5 Hz)	C-18 (59.04) and C-30 (21.31)
30	0.95 (3H, *d*, J = 6 Hz)	C-21 (32.85) and C-21 (41.52)

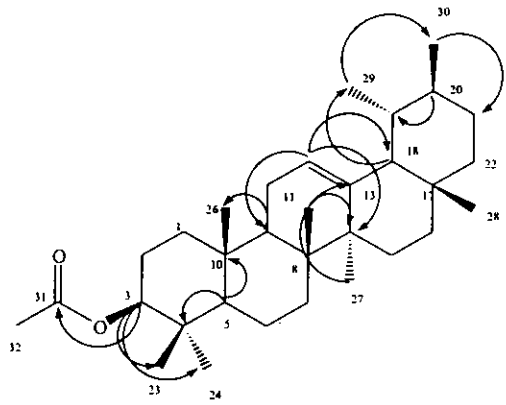
Table 38 ^{1}H and ^{13}C NMR spectral data of compound **LCO1**

Position	δ_C^* (ppm)		δ_H (ppm)
1	38.44	CH_2	1.66 (2H, *m*)
2	26.58	CH_2	1.84 (2H, *m*)
3	80.94	CH	4.51 (1H, *dd*, J = 6, 10.5 Hz)
4	37.70	C	-
5	55.24	CH	1.85 (1H, *m*)
6	18.22	CH_2	1.54 (1H, *m*), 1.40 (1H, *m*)
7	31.23	CH_2	1.40 (1H, *m*), 1.27 (1H, *m*)
8	40.01	C	-
9	47.62	CH	1.55 (1H, *m*)
10	36.77	C	-

Table 38 (Continued)

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
11	23.59	CH_2	1.90 (1H, *m*)[a]
12	124.30	= CH	5.13 (1H, *t*, *J* = 3.5 Hz)
13	139.62	C	-
14	42.05	C	-
15	28.07	CH_2	2.20 (2H, *m*)
16	23.35	CH_2	1.64 (1H, *m*)[a]
17	33.73	C	-
18	59.04	CH	1.30 (1H, *m*)
19	39.63	CH	1.30 (1H, *m*)
20	39.59	CH	0.92 (1H, *m*)
21	32.85	CH_2	1.58 (1H, *m*), 1.36 (1H, *m*)
22	41.52	CH_2	1.42 (1H, *m*), 1.30 (1H, *m*)
23	28.05	CH_3	0.87 (3H, *s*)[b]
24	16.72	CH_3	0.86 (3H, *s*)[b]
25	15.72	CH_3	0.98 (3H, *s*)
26	16.85	CH_3	1.01 (3H, *s*)
27	23.21	CH_3	1.06 (3H, *s*)
28	28.73	CH_3	0.80 (3H, *s*)
29	17.49	CH_3	0.80 (3H, *d*, *J* = 5.5 Hz)
30	21.31	CH_3	0.95 (3H, *d*, *J* = 6 Hz)
31	171.01	C	-
32	21.38	CH_3	2.05 (3H, *s*)

[a, b] Assignment with the same superscripts maybe interchange, [#] Carbon type deduced from DEPT experiment.



Selected HMBC correlation of compound **LCO1**

Comparison of ^{13}C NMR spectral data between compound **LCO1** and α-amyrin (Itokawa, *et.al.*, 1981) (see **Table 39**) showed similarity. Thus compound **LCO1** was identified as urs-12-ene-3β-acetate. The structure of this compound was finally confirmed by X-ray diffraction (**Fig. 4**).

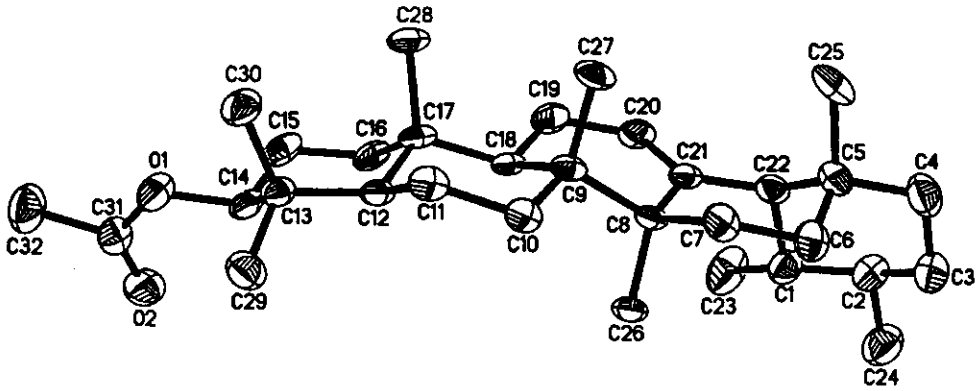


Figure 4 X-ray ORTEP diagram of compound **LCO1**

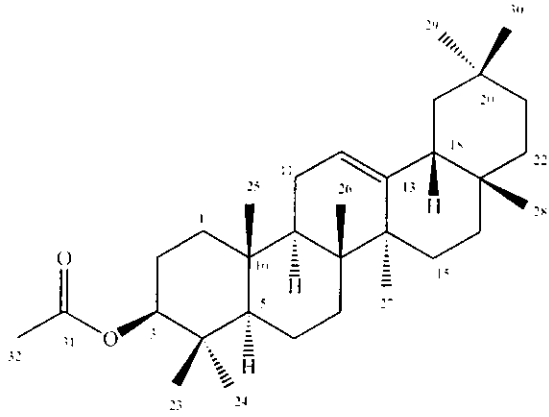
Table 39 Comparison of ^{13}C NMR spectral data between compound **LCO1** and α-amyrin

Position	Compound LCO1	α-amyrin
1	38.44	38.7
2	26.58	27.2
3	80.94	73.8
4	37.70	38.7
5	55.21	55.2
6	18.22	18.3
7	31.23	32.9
8	40.01	40.0
9	47.62	47.7
10	36.77	36.9
11	23.59	23.3
12	124.30	124.3
13	139.62	139.3
14	42.05	42.0
15	28.07	28.7
16	23.35	26.6
17	33.37	33.7
18	59.04	58.9
19	39.63	39.6
20	39.59	39.6
21	32.88	31.2
22	41.52	41.5
23	28.05	28.1
24	16.72	15.6
25	15.72	15.6
26	16.85	16.8

Table 39 (Continued)

Position	Compound LCO1	α-amyrin
27	23.21	23.3
28	28.73	28.1
29	17.49	17.4
30	21.31	21.3
31	170.99	-
32	21.38	-

3.2.2 Compound LCO2



Compound **LCO2** was obtained as a white needle, mp = 214-217 °C, $[\alpha]_D^{29}$= -71.42 ° (c = 0.0014, $CHCl_3$), compound **LCO2** showed the characteristic of triterpene by giving a purple spot in vanillin sulfuric acid reagent. The IR spectrum of **LCO2 (Fig. 58)** exhibited carbonyl (1733 cm^{-1}) absorption.

The complete analysis of ^{13}C and ^{1}H NMR spectral data of compound **LCO2** (see **Table 40, Fig. 59, 60**) were assigned with the information provided from COSY (**Fig. 62**), HMQC correlation (**Fig. 63**) and HMBC correlation (see **Table 41, Fig. 64**). The ^{13}C NMR spectrum (see **Table 42** and **40, Fig. 60**) together with data from DEPT (see **Table 40, Fig. 55**) of compound **LCO2** showed nine methyl carbons (δ 15.57, 16.71, 16.84, 21.31 (acetoxy carbon), 23.71, 25.96, 28.06, 28.41 and 33.34), ten methylene carbons (δ 18.29, 23.56, 23.60, 26.17, 26.97, 32.63, 34.77,37.18, 38.31 and 46.83), five methine carbons (δ 47.28, 47.60, 55.30, 80.97, and 121.68) and eight quaternary carbons (31.10, 32.51, 36.88, 37.74, 39.85, 41.75, 145.24 and 170.99). The mass spectrum of this compound (**Fig. 65**) showed the molecular ion peak at 468.4 *m/z*, thus compound **LCO2** had molecular formula $C_{32}H_{52}O_2$.

The ^{1}H NMR spectrum of this compound revealed signals for eight *singet* methyls at δ 0.83, 0.87 (4 x CH_3), 0.96, 0.97 and 1.13. The signals at δ 2.05 (3H, *s*) and 5.19 (1H, *t*, J = 3.5 Hz) showed the presence of an acetoxy proton and a vinylic proton, respectively. The vinylic proton could be assigned to H-12. A one proton *doublet of doublet* observed at δ 4.50 (J = 6 and 10 Hz) could be assigned to the position C-3. These signals were regarded as being due to a pentacyclic triterpene (Mahato, *et.al.*, 1994).

In ^{2}J and ^{3}J HMBC experiments (see **Table 41, Fig. 64**), the methine proton at position C-3 (δ 4.50) showed correlation with two methyl carbons and two quaternary carbons at C-23 (28.06), C-24 (15.57), C-4 (37.74) and carbonyl group at δ 170.99 (C-31). The olefinic proton at δ 5.19 showed correlation with quarternary carbon at C-14 (41.75).

Table 40 ^{13}C and DEPT spectral data of compound **LCO2**

δ_C (ppm)	DEPT 90°	DEPT 135°	Type of Carbon
170.99			C
145.24			C
121.68	121.68	121.68	= CH
80.97	80.97	80.97	CH
55.30	55.30	55.30	CH
47.60	47.60	47.60	CH
47.28	47.28	47.28	CH
46.83		46.83	CH_2
41.75			C
39.85			C
38.31		38.31	CH_2
37.74			C
37.18		37.18	CH_2
36.88			C
34.77		34.77	CH_2
33.34		33.34	CH_3
32.63		32.63	CH_2
32.51			C
31.10			C
28.41		28.41	CH_3
28.06		28.06	CH_3
26.97		26.97	CH_2
26.17		26.17	CH_2
25.96		25.96	CH_3
23.71		23.71	CH_3
23.60		23.60	CH_2
23.56		23.56	CH_2

Table 40 (Continued)

δ_C (ppm)	DEPT 90°	DEPT 135°	Type of Carbon
21.31		21.31	CH_3
18.30		18.30	CH_2
16.84		16.84	CH_3
16.71		16.71	CH_3
15.57		15.57	CH_3

Table 41 Major HMBC correlation of compound **LCO2**

Position	δ_H (ppm)	HMBC correlation
1	1.62 (2H, *m*)	C-3 (80.98), C-5 (55.30) and C-10 (36.88)
3	4.50 (1H, *dd*, *J* = 6, 10 Hz)	C-4 (37.74), C-23 (28.06), C-24 (15.57) and C-31 (170.99)
5	0.82 (1H, *m*)	C-3 (80.97), C-4 (37.74) and C-6 (18.30)
7	1.51 (1H, *m*), 1.31 (1H, *m*)	C-5 (55.30), C-6 (18.30), C-8 (39.85), C-9 (47.60) and C-10 (36.88)
9	1.57 (1H, *m*)	C-5 (55.30), C-8 (39.85), C-10 (36.88) C-11 (23.56) and C-25 (16.71)
12	5.19 (1H, *t*, *J* = 3.5 Hz)	C-14 (41.75)
18	1.83 (1H, *m*)	C-12 (121.68) and C-13 (145.24)

Table 41 (Continued)

Position	δ_H (ppm)	HMBC correlation
23	0.87 (3H, *s*)	C-3 (80.97), C-4 (37.74) and C-5 (55.30)
24	0.87 (3H, *s*)	C-3 (80.97), C-4 (37.74) and C-5 (55.30)
25	0.97 (3H, *s*)	C-1 (38.31), C-5 (55.30), C-9 (47.60) and C-10 (36.88)
26	0.96 (3H, *s*)	C-7 (32.63), C-8 (39.85) and C-14 (41.75)
27	1.13 (3H, *s*)	C-8 (39.85), C-13 (145.24), C-14 (41.75) and C-15 (26.17)
28	0.83 (3H, *s*)	C-16 (26.97) and C-17 (32.51)
29	0.87 (3H, *s*)	C-19 (46.83), C-20 (31.10) and C-21 (34.77)
30	0.87 (3H, *s*)	C-19 (46.83), C-20 (31.10) and C-21 (34.77)

Table 42 ^{1}H and ^{13}C spectral data of compound **LCO2**

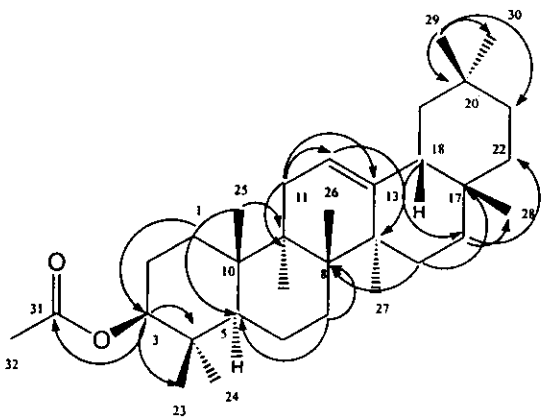
Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
1	38.31	CH_2	1.62 (2H, *m*)
2	23.60	CH_2	160 (2H, *m*)[a]
3	80.97	CH	4.50 (1H, *dd*, *J* = 6, 10 Hz)
4	37.74	C	-
5	55.30	CH	0.82 (1H, *m*)

Table 42 (Continued)

Position	δ_C [#] (ppm)		δ_H (ppm)
6	18.30	CH_2	1.23 (1H, *m*)
7	32.63	CH_2	1.53 (1H, *m*), 1.31 (1H, *m*)
8	39.85	C	-
9	47.60	CH	1.57 (1H, *s*)
10	36.88	C	-
11	23.56	CH_2	1.86 (2H, *m*)[a]
12	121.68	= CH	5.19 (1H, *t*, *J* = 3.5 Hz)
13	145.24	C	-
14	41.75	C	-
15	26.17	CH_2	1.76 (2H, *m*)[b]
16	26.97	CH_2	2.00 (2H, *m*)[b]
17	32.51	C	-
18	47.28	CH	1.94 (1H, *m*)[c]
19	46.83	CH_2	1.02 (1H, *m*)[c]
20	31.10	C	-
21	34.77	CH_2	1.31 (1H, *m*), 1.10 (1H, *m*)
22	37.18	CH_2	1.42 (1H, *m*), 1.20 (1H, *m*)
23	28.06	CH_3	0.87 (3H, *s*)
24	15.57	CH_3	0.87 (3H, *s*)
25	16.71	CH_3	0.97 (3H, *s*)
26	16.84	CH_3	0.96 (3H, *s*)
27	25.96	CH_3	1.13 (3H, *s*)
28	28.41	CH_3	0.83 (3H, *s*)
29	33.34	CH_3	0.87 (3H, *s*)
30	23.71	CH_3	0.87 (3H, *s*)
31	170.99	C	-
32	21.31	CH_3	2.05 (3H, *s*)

[a, b] Assignment with the same superscripts maybe interchanged, [#] Carbon type deduced from DEPT experiment.

Comparison of ^{13}C NMR spectral data between compound **LCO2** and β-amyrin (Gonzales, *et.al.*, 1988) (see **Table 43**) showed similarity. Thus compound **LCO2** was identified as olean-12-ene-3β-acetate and its structure was also confirmed by X-ray diffraction (**Fig. 5**).



Selected HMBC correlation of compound **LCO2**

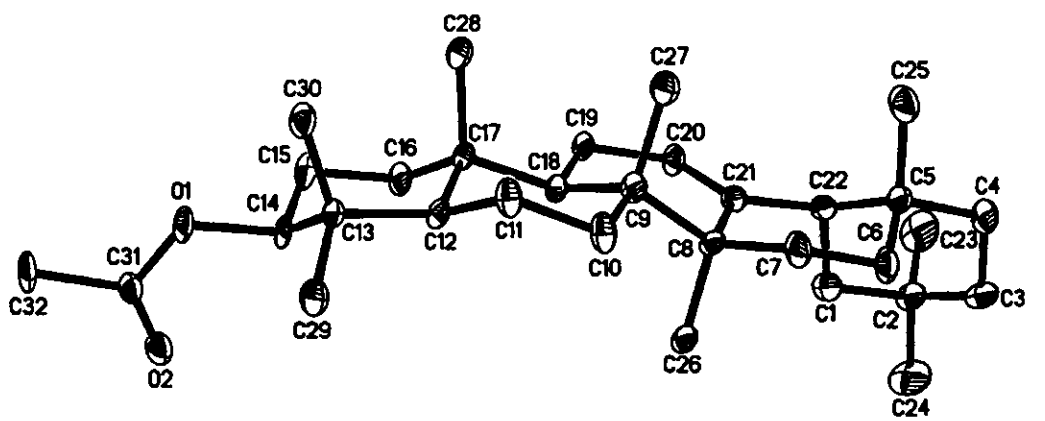


Figure 5 X-ray ORTEP diagram of compound **LCO2**

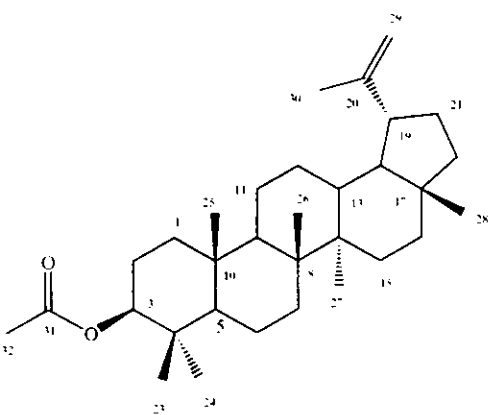
Table 43 Comparison of ^{13}C NMR spectral data of compound **LCO2** and β-amyrin

Position	Compound LCO2	β-amyrin
1	38.31	38.7
2	23.60	27.3
3	80.98	79.0
4	37.74	38.8
5	55.30	55.3
6	18.30	18.5
7	32.63	32.8
8	39.85	38.8
9	47.60	47.7
10	36.88	37.6
11	23.56	23.6
12	121.68	121.8
13	145.24	145.1
14	41.75	41.8
15	26.17	26.2
16	26.97	27.0
17	32.51	32.5
18	47.28	47.4
19	46.83	46.9
20	31.10	31.1
21	34.77	34.8
22	37.18	37.2
23	28.06	28.2
24	15.57	15.5
25	16.71	15.6
26	16.84	16.9
27	25.96	26.0

Table 43 (Continued)

Position	Compound LCO2	β-amyrin
28	28.41	28.4
29	33.34	33.3
30	23.71	23.7
31	170.99	-
32	21.31	-

3.2.3 Compound LCO3



Compound **LCO3** was obtained as white needles, mp = 184-187 °C, $[\alpha]_D^{29}$ = +31.9 (c = 0.0094 , $CHCl_3$). This compound showed the characteristic of triterpene by visualizing as a purple spot with vanillin sulfuric acid reagent. Its IR spectrum (**Fig. 66**) exhibited carbonyl ester (1733 cm^{-1}) absorption.

The complete analysis of ^{13}C and ^{1}H NMR spectral data of compound **LCO3** (see **Table 44, Fig 67, 68**) were assigned with the information provided from COSY (**Fig. 70**), HMQC correlation (**Fig. 71**) and HMBC correlation (see **Table 45, Fig. 72**). The ^{13}C NMR spectra of compound **LCO3** (see **Table 46, Fig 68**) revealed 32 signals,

which were assigned by DEPT (see **Table 44, Fig. 69**) as eight methyl carbons (δ 14.48, 15.95, 16.16, 16.47, 17.98, 19.27, 21.31 and 27.92), eleven methylene carbons (δ18.18, 20.92, 23.69, 25.07, 27.41, 29.81, 34.19, 35.55, 38.37, 39.97 and 109.34), six methine carbons (δ38.01, 47.98, 48.26, 50.32, 55.36 and 80.95) and seven quaternary carbons (δ37.06, 37.78, 40.82, 42.80, 42.97, 150.94 and 170.99).

The ^{1}H NMR spectrum (see **Table 46, Fig. 67**) showed six *singlet* methyls (δ 0.78, 0.83, 0.84, 0.85, 0.93 and 1.03), one acetoxymethyl (δ 2.04, *s*), one oxymethine proton at δ4.47 (1H, *dd, J* =6, 10 Hz) and one methine proton at δ2.37 (*dt, J* = 6, 11.5 Hz). The above information were regarded as being due to a pentacyclic triterpene. The signals of two olefinic and one vinylic methyl protons at δ 4.68 (1H, *d, J* = 2.5 Hz) and 4.57 (1H, *m*) and 1.68 (*s*), respectively, suggested the structure of isopropenyl unit [-C(CH_3)=CH_2]. It was in agreement with the ^{1}H-^{1}H COSY (**Fig. 70**) and HMBC correlation data (see **Table 45, Fig. 72**).

From HMBC correlation spectrum (see **Table 45, Fig. 72**), methine proton at δ4.48 (*dd, J* = 6, 10 Hz) exhibited correlation peaks with C-1 (38.37), C-2 (23.69), C-4 (37.06), C-23 (27.92), C-24 (16.47) and C-31 (170.99), thus confirmed the position of the acetoxy at C-3 of ring A. The methine proton at C-19 (δ 2.38) showed correlation with C-17 (37.78), C-18 (48.26), C-20 (150.94), C-21 (29.81) and C-29 (109.34), thus confirmed that an isopropenyl moiety was linked at position 19 of ring E.

The relative stereochemistry of isopropenyl unit was established by the NOE experiment (**Fig. 73, 74**). The enhancement of the methyl proton at δ 1.68 was observed by the irradiation at δ4.57 the and not observed when irradiated at δ4.68, indicating that the methyl proton at 1.68 and the olefinic proton at δ4.57 were *cis*. Thus, the olefinic protons at δ 4.68 and 4.57 were assigned to H29a and 29b, respectively, and the methyl proton at 1.68 could be assigned to H-30.

Table 44 ^{13}C and DEPT spectral data of compound **LCO3**

δ_C (ppm)	DEPT 45°	DEPT 90°	DEPT 135°	Type of Carbon
170.99				C
150.94				C
109.34	109.34		109.34	$=CH_2$
80.95	80.95	80.95	80.95	CH
55.36	55.36	55.36	55.36	CH
50.32	50.32	50.32	50.32	CH
48.26	48.26	48.26	48.26	CH
47.98	47.98	47.98	47.98	CH
42.97				C
42.80				C
40.82				C
39.97	39.97		39.97	CH_2
38.37	38.37		38.37	CH_2
38.01	38.01	38.01	38.01	CH
37.78				C
37.06				C
35.55	35.55		35.55	CH_2
34.19	34.19		34.19	CH_2
29.81	29.81		29.81	CH_2
27.92	27.92		27.92	CH_3
27.41	27.41		27.41	CH_2
25.07	25.07		25.07	CH_2
23.69	23.69		23.69	CH_2
21.31	21.31		21.31	CH_3
20.92	20.92		20.92	CH_2
19.27	19.27		19.27	CH_3
18.18	18.18		18.18	CH_2

Table 44 (Continued)

δ_C (ppm)	DEPT 45°	DEPT 90°	DEPT 135°	Type of Carbon
17.98	17.98		17.98	CH_3
16.47	16.47		16.47	CH_3
16.16	16.16		16.16	CH_3
15.95	15.95		15.95	CH_3
14.48	14.48		14.48	CH_3

Table 45 Major HMBC correlation of compound **LCO3**

Position	δ_C (ppm)	HMBC Correlation
3	4.48 (1H, *dd*, *J* = 6, 10 Hz)	C-2 (23.69), C-31 (170.99), C-4 (37.06), C-23 (27.92) and C-24 (16.47)
5	0.78 (1H, *m*)	C-3 (80.95), C-10 (42.80), C-23 (27.92) and C-24 (16.47)
9	1.30 (1H, *m*)	C-5 (55.36) and C-10 (42.80)
18	1.38 (1H, *m*)	C-14 (42.97), C-19 (47.98) and C-20 (150.94)
19	2.38 (1H, *dt*, *J* = 6, 11.5 Hz)	C-17 (37.78), C-18 (48.26), C-20 (150.94), C-21 (29.81), C-29 (109.34) and C-30 (19.27)

Table 45 (Continued)

Position	δ_C (ppm)	HMBC Correlation
23	0.84 (3H, *s*)[a]	C-4 (37.06), C-5 (55.36) and C-24 (16.47)
24	0.83 (3H, *s*)[a]	C-4 (37.06), C-5 (55.36) and C-23 (27.92)
25	0.85 (3H, *s*)[a]	C-5 (55.36) and C-9 (50.32)
26	1.03 (3H, *s*)	C-7 (34.19), C-8 (40.82), C-9 (50.32) and C-14 (42.97)
27	0.93 (3H, *s*)	C-8 (40.82), C-13 (38.01), C-14 (42.97) and C-15 (27.41)
28	0.78 (3H, *s*)	C-16 (35.55), C-18 (48.26) and C-22 (39.97)
29	4.68 (1H, *d*, *J* = 2.5 Hz) 4.57 (1H, *m*)	C-19 (47.98), C-20 (150.94) and C-30 (19.27)
30	1.68 (3H, *d*, *J* = 0.5 Hz)	C-19 (47.98), C-20 (150.94) and C-29 (109.34)

[a] Assignment with the same superscripts maybe interchanged.

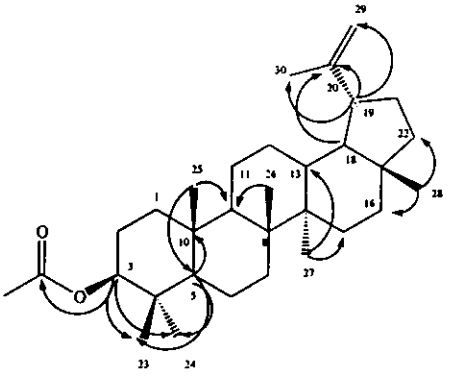
Table 46 ^{1}H and ^{13}C NMR spectrum of compound **LCO3**

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
1	38.37	CH_2	1.65 (2H, *m*)
2	23.69	CH_2	1.62 (2H, *m*)
3	80.95	CH	4.48 (1H, *dd*, J = 6, 10 Hz)
4	37.06	C	-
5	55.36	CH	0.78 (1H, *m*)
6	18.18	CH_2	1.50 (1H, *m*), 1.40 (1H, *m*)
7	34.19	CH_2	1.40 (1H, *m*)
8	40.82	C	-
9	50.32	CH	1.30 (1H, *m*)
10	42.80[a]	C	-
11	20.92	CH_2	1.40 (1H, *m*), 1.22 (1H, *m*)
12	25.07	CH_2	1.66 (1H, *m*), 1.08 (1H, *m*)
13	38.01	CH	0.98 (1H, *m*)
14	42.97[a]	C	-
15	27.41	CH_2	1.58 (1H, *m*), 1.00 (1H, *m*)
16	35.55	CH_2	1.49 (1H, *m*), 1.36 (1H, *m*)
17	37.78	C	-
18	48.26	CH	1.38 (1H, *m*)
19	47.98	CH	2.38 (1H, *dt*, J = 6, 11.5 Hz)
20	150.94	C	-
21	29.81	CH_2	1.92 (1H, *m*), 1.26 (1H, *m*)
22	39.97	CH_2	1.38 (1H, *m*), 1.20 (1H, *m*)
23	27.92	CH_3	0.84 (3H, *s*)[c]
24	15.95[b]	CH_3	0.83 (3H, *s*)[c]
25	16.47[b]	CH_3	0.85 (3H, *s*)[c]
26	16.16	CH_3	1.03 (3H, *s*)
27	14.48	CH_3	0.93 (3H, *s*)

Table 46 (Continued)

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
28	17.98	CH_3	0.78 (3H, *s*)
29	109.34	$=CH_2$	4.68 (1H, *d*, J = 2.5 Hz)
			4.57 (1H, *m*)
30	19.27	CH_3	1.68 (3H, *s*)
31	170.99	C	-
32	21.31	CH_3	2.04 (3H, *s*)

[#] Carbon type deduced from DEPT experiment, [a, b, c] Assignment with the same superscripts maybe interchanged.



Selected HMBC correlation of compound **LCO3**

Comparison of ^{13}C NMR spectral data of compound **LCO3** and 3β-lupeol (Tsuda, *et.al.*, 1984) (see **Table 47**) showed similarity. Thus compound **LCO3**, lupeol derivative, was identified as lup-20(29)-ene-3β-acetate.

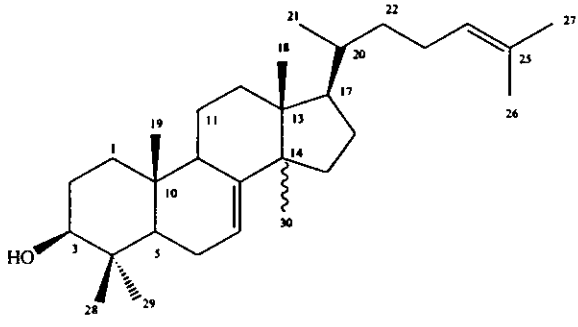
Table 47 Comparison of ^{13}C NMR spectral data of compound **LCO3** and 3β-lupeol

Position	Compound LCO3	3β-lupeol
1	38.37	38.7
2	23.69	27.4
3	80.95	78.9
4	37.06	38.8
5	55.36	55.3
6	18.18	18.3
7	34.19	34.2
8	40.82	40.8
9	50.32	50.4
10	42.80	37.1
11	20.92	20.9
12	25.07	25.1
13	38.01	38.0
14	42.97	42.8
15	27.41	27.4
16	35.55	35.5
17	37.78	43.0
18	48.26	48.2
19	47.98	47.9
20	150.94	150.9
21	29.81	29.8
22	39.97	40
23	27.92	28.0
24	15.95	15.4
25	16.47	16.1
26	16.16	15.9
27	14.48	14.5

Table 47 (Continued)

Position	Compound LCO3	3β-lupeol
28	17.98	18.0
29	109.34	109.2
30	19.27	19.3
31	170.99	-
32	21.31	-

Compound LCO4



Compound **LCO4** was isolated as a colorless viscous liquid, $[\alpha]_D^{25}$= +11.23° (c = 0.0089, $CHCl_3$), this compound showed purple spot with vanillin sulfuric acid reagent indicating the triterpene characteristic. Its IR spectrum (**Fig. 75**) showed absorption band at 3402 cm^{-1} for hydroxy group.

The complete analysis of ^{13}C and ^{1}H NMR spectral data of compound **LCO4** (see **Table 50, Fig. 76** and **77**) were assigned with the information provided from COSY (**Fig 79**), HMQC (**Fig. 80**) and HMBC (see **Table 49, Fig 81**). The ^{13}C NMR spectral data of compound **LCO4** (see **Table 49, Fig. 77**) recorded in $CDCl_3$ showed

30 signals for 30 carbon atoms. The DEPT 45°, 90° and 135° (see **Table 48, Fig. 78**) indicated the existence of eight methyl carbon atoms (δ 28.71, 28.35, 26.78, 23.11, 19.62, 18.71, 15.76, 14.13), nine methylene carbon atoms (δ 38.22, 36.18, 34.97, 34.84, 29.50, 28.63, 26.39, 24.97, 19.17), seven methine carbon atoms (δ 126.15, 118.81, 80.29, 54.25, 52.30, 51.63 and 36.82) and six quaternary carbon atoms (δ 146.92, 131.99, 49.92, 44.54, 39.99 and 35.96).

The ^{1}H NMR spectrum of compound **LCO4** (see **Table 50, Fig. 76**) revealed seven methyl *singlet* signals at δ 1.68, 1.60 .0.97 (2 x CH_3), 0.86, 0.80 and 0.74, one *doublet* at δ 0.85 (J = 6.5 Hz), one oxymethine showed signal at δ 3.52 (*dd*, J = 4, 11 Hz) and two olefinic protons resonated at δ 5.26 (*dd*, J = 3, 7 Hz) and 5.10 (*m*). The above information were regarded as being due to tetracyclic triterpene. The signals of olefinic proton at δ 5.26 (*dd*, J = 3, 7 Hz) together with two methyl protons at δ 1.60 (*s*), 1.68 (*s*) and one *doublet* at δ 0.85 (J = 6.5 Hz) suggested the structure of the side chain to be [-C(H)$CH_3CH_2CH_2$CH=C$(CH_3)_2$]. It was in agreement with the ^{1}H-^{1}H COSY (**Fig. 79**) and HMBC correlation data (see **Table 49, Fig. 81**). Therefore the other vinylic proton at δ 5.10 was expected to be in the tetracyclic system.

The HMBC correlation of compound **LCO4** (see **Table 49, Fig 81**), H-3 (δ 3.52) showed correlation peaks with carbon signals at δ 15.76 (C-29) and 28.35 (C-28), H-6 (δ 2.18 and 1.96) with δ 118.81 (C-7) and 131.99 (C-8). A cross peak between H-17 (δ 1.30) and C-20 (36.82), C-18 (14.13) and C-14 (49.92) together with H-21 (δ 0.85) which showed correlation peaks with C-17 (52.30) and C-20 (36.82) confirmed that the position of the side chain was linked at C-17 (52.30). The methyl signal at δ 0.97 showed correlation with C-8 (146.92), C-13 (44.54) and C-14 (49.92), indicated that this methyl group was attached to C-14. Hence the double bond should be located in ring B at position 7 and 8. Thus compound **LCO4** was identified as lanosta-7,24-dien-3β-ol.

Table 48 ^{13}C and DEPT spectral data of compound **LCO4**

δ_C (ppm)	DEPT 45°	DEPT 90°	DEPT 135°	Type of Carbon
146.92				C
131.99				C
126.15	126.15	126.15	126.15	= CH
118.81	118.81	118.81	118.81	= CH
80.29	80.29	80.29	80.29	CH
54.25	54.25	54.25	54.25	CH
52.30	52.30	52.30	52.30	CH
51.63	51.63	51.63	51.63	CH
49.92				C
44.54				C
39.99				C
38.22	38.22		38.22	CH_2
36.82	36.82	36.82	36.82	CH
36.18	36.18		36.18	CH_2
35.96				C
34.97	34.97		34.97	CH_2
34.84	34.84		34.84	CH_2
29.50	29.50		29.50	CH_2
28.71	28.71		28.71	CH_3
28.63	28.63		28.63	CH_2
28.35	28.35		28.35	CH_3
26.78	26.78		26.78	CH_3
26.39	26.39		26.39	CH_2
24.97	24.97		24.97	CH_2
23.11	23.11		23.11	CH_3
19.62	19.62		19.62	CH_3
19.17	19.17		19.17	CH_2

Table 48 (Continued)

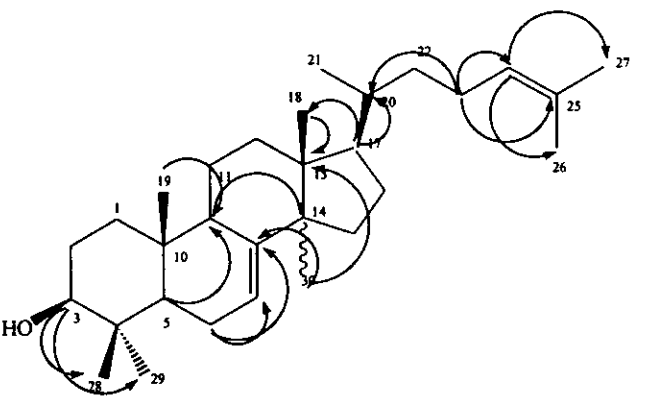
δ_C (ppm)	DEPT 45°	DEPT 90°	DEPT 135°	Type of Carbon
18.71	18.71		18.71	CH_3
15.76	15.76		15.76	CH_3
14.13	14.13		14.13	CH_3

Table 49 HMBC correlation of compound **LCO4**

Position	δ_H (ppm)	HMBC Correlation
2	1.65 (1H, *m*), 1.12 (1H, *m*)	C-3 (80.29) and C-10 (35.96)
3	3.25 (1H, *dd*, *J* = 4, 11 Hz)	C-28 (28.35) and C-29 (15.76)
5	0.72 (1H, *m*)	C-9 (51.63), C-10 (35.96) and C-29 (15.76)
9	1.30 (1H, *m*)	C-4 (39.99), C-10 (35.96) and C-14 (49.92)
17	1.30 (1H, *m*)	C-14 (49.92), C-18 (14.13) and C-20 (36.82)
18	0.80 (3H, *s*)	C-13 (44.54), C-17 (52.30) and C-20 (36.82)
19	0.74 (3H, *s*)	C-9 (51.63)
21	0.85 (3H, *d*, *J* = 6.5 Hz)	C-17 (52.30) and C-20 (36.82)
23	1.80 (1H, *m*), 1.44 (1H, *m*)	C-20 (36.82), C-24 (126.15) and C-25 (131.99)
24	5.10 (1H, *m*)	C-26 (18.71) and C-27 (26.78)

Table 49 (Continued)

Position	δ_H (ppm)	HMBC Correlation
26	1.60 (3H, *s*)	C-24 (126.15), C-25 (131.99) and C-27 (26.78)
27	1.68 (3H, *s*)	C-24 (126.15), C-25 (131.99) and C-26 (18.71)
28	0.97 (3H, *s*)	C-3 (80.29), C-4 (39.99), C-5 (54.25) and C-29 (15.76)
29	0.86 (3H, *s*)	C-3 (80.29), C-4 (39.99), C-5 (54.25) and C-28 (28.35)
30	0.97 (3H, *s*)	C-8 (146.92), C-13 (44.54) and C-14 (49.92)



Selected HMBC correlation of compound **LCO4**

Table 50 ^{1}H and ^{13}C NMR spectral data of compound **LCO4**

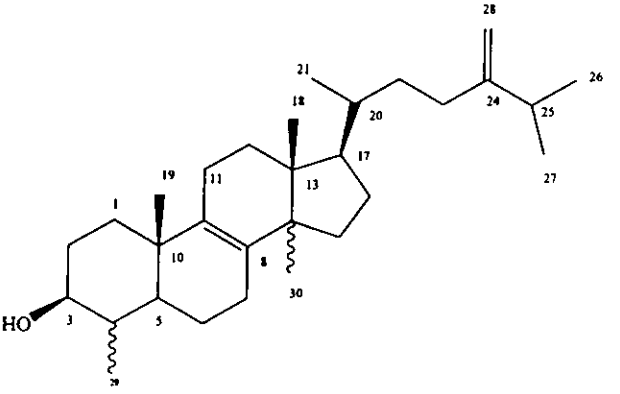
Position	δ_C^* (ppm)		δ_H (ppm)
1	36.18	CH_2	
2	38.22	CH_2	1.65 (1H, *m*), 1.12 (1H, *m*)
3	80.29	CH	3.25 (1H, *dd*, *J* = 4, 11 Hz)
4	39.99	C	-
5	54.25	CH	0.72 (1H, *m*)
6	24.97	CH_2	2.15 (1H, *m*), 1.97 (1H, *m*)
7	118.81	= CH	5.25 (1H, *dd*, *J* = 3.5, 7 Hz)
8	146.92	C	-
9	51.63	CH	1.30 (1H, *m*)
10	35.96	C	-
11	19.17	CH_2	1.50 (2H, *m*)
12	26.39	CH_2	1.68 (2H, *m*)
13	44.54	C	-
14	49.92	C	-
15	29.50	CH_2	1.24 (2H, *m*)
16	28.63	CH_2	1.27 (2H, *m*)
17	52.30	CH	1.30 (1H, *m*)
18	14.13	CH_3	0.80 (3H, *s*)
19	23.11	CH_3	0.74 (3H, *s*)
20	36.82	CH	
21	19.62	CH_3	0.85 (3H, *d*, *J* = 6.5 Hz)
22	34.84	CH_2	1.64 (2H, *m*)
23	34.97	CH_2	1.80 (1H, *m*), 1.44 (1H, *m*)
24	126.15	CH	5.10 (1H, *m*)
25	131.99	C	-
26	18.71	CH_3	1.60 (3H, *s*)
27	26.78	CH_3	1.68 (3H, *s*)

Table 50 (Continued)

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
28	28.35	CH_3	0.97 (3H, *s*)
29	15.76	CH_3	0.86 (3H, *s*)
30	28.71	CH_3	0.97 (3H, *s*)

[#] Carbon type deduced from DEPT experiment.

Compound LCO5



Compound **LCO5** was isolated as a white solid, mp = 140-142 °C, $[\alpha]_D^{26}$ = +49.97° (*c* = 0.0087, $CHCl_3$), this compound showed the characteristic of triterpene by giving a purple spot with vanillin sulfuric acid reagent. The IR spectrum of compound **LCO5 (Fig. 82)** showed the presence of hydroxy group at 3360 cm^{-1}.

The ^{13}C NMR spectrum (see **Table 53, Fig. 84**) recorded in $CDCl_3$ revealed 30 signals for 30 carbon atoms. Analysis of DEPT 45°, 90° and 135° spectra of this compound (see **Table 51, Fig. 85**) suggested the presence of seven methyl carbon

atoms (δ24.41, 21.98, 21.85, 18.71, 18.20, 15.75 and 15.05), eleven methylene cabon atoms (δ 105.91, 34.98, 34.97, 31.27, 31.14, 31.04, 30.76, 28.17, 25.52, 21.74 and 20.70), six methine carbon atoms (δ76.52, 50.37, 47.03, 39.19, 36.47 and 33.78) and six quaternary carbon atoms (δ156.90, 134.61, 133.57, 49.83, 44.50, 36.31).

The ^{1}H NMR spectrum (see **Table 53, Fig. 83**) showed three *singlets* of methyl protons at δ0.71, 0.89 and 0.97, four *doublets* of methyl protons at δ0.92 (J = 6.5 Hz), 1.00 (J = 6.5 Hz) and 1.03 (2 x CH_3, J = 7 Hz), the methylene protons were shown at δ4.67 (1H, d, J = 1.5 Hz) and 4.72 (1H, *s*), one oxymethine proton at δ3.11 (*m*) and the methine proton at 2.23. The above information were regarded as being due to tetracyclic triterpene which have one hydroxy, one tetrasubstituted and one disubstituted double bond. The signals of olefinic protons at δ4.67 (1H, d, J = 1.5 Hz) and 4.72 (1H, *s*) and three *doublet* methyl protons at δ0.92 (J = 6.5 Hz), 1.02 (J = 7 Hz) and1.03 (J = 7 Hz) suggested the structure of the side chain to be [-C(H)$CH_3CH_2CH_2C$=$CH_2CH(CH_3)_2$]. It was in agreement with the ^{1}H-^{1}H COSY (**Fig. 86**) and HMBC correlation data (see **Table 52, Fig. 88**). Therefore the tetrasubstituted was expected to be in the tetracyclic system.

The complete assignment of ^{13}C and ^{1}H NMR (see **Table 53, Fig. 83, 84**) signals were made with the information from ^{1}H-^{1}H COSY (**Fig. 86**), HMQC (**Fig. 87**) and HMBC (see **Table 52, Fig. 88**) spectrum. In the HMBC spectrum (see **Table 52, Fig. 88**) the carbon signals at δ44.50 (C-13), 36.47 (C-20), 28.17 (C-16) and 15.71 (C-18) showed the correlation peaks with the proton 17 (δ 1.50) and H-21 showed correlation peak with the carbon signal at δ50.37 (C-17), indicating that the side chain of this compound was linked to the C-17 (δ 50.37). The methylene proton H-28 (δ 4.67 and 4.72) showed the correlation peaks with δ 156.90 (C-24), 33.78 (C-25) and 31.14 (C-23). Thus this compound was identified as ergosta-8,24(28)-dien-3β-ol.

Table 51 ^{13}C and DEPT spectral data of compound **LCO5**

δ_c (ppm)	DEPT 45°	DEPT 90°	DEPT 135°	Type of Carbon
156.90				C
134.61				C
133.57				C
105.91	105.91		105.91	$=CH_2$
76.52	76.52	76.52	76.52	CH
50.37	50.37	50.37	50.37	CH
49.83				C
47.03	47.06	47.06	47.06	CH
44.50				C
39.19	39.19	39.19	39.19	CH
36.47	36.47	36.47	36.47	CH
36.31				C
34.98	34.98		34.98	CH_2
34.97	34.97		34.97	CH_2
33.78	33.78	33.78	33.78	CH
31.27	31.27		31.27	CH_2
31.14	31.14		31.14	CH_2
31.04	31.04		31.04	CH_2
30.76	30.76		30.76	CH_2
28.17	28.17		28.17	CH_2
25.52	25.52		25.52	CH_2
24.41	24.41		24.41	CH_3
21.98	21.98		21.98	CH_3
21.85	21.85		21.85	CH_3
21.74	21.74		21.74	CH_2
20.70	20.70		20.70	CH_2
18.71	18.71		18.71	CH_3

Table 51 (Continued)

δ_C (ppm)	DEPT 45°	DEPT 90°	DEPT 135°	Type of Carbon
18.20	18.20		18.20	CH_3
15.71	15.71		15.71	CH_3
15.05	15.05		15.05	CH_3

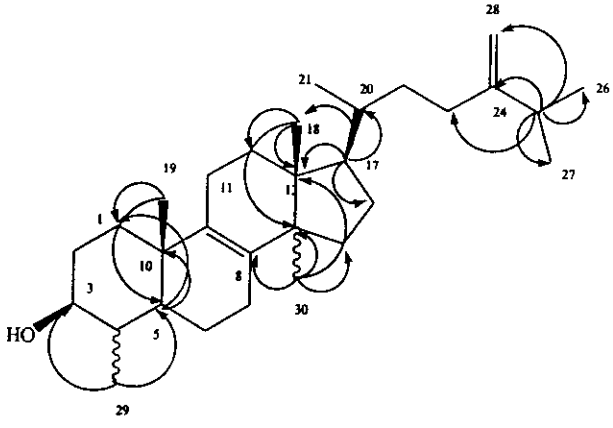
Table 52 HMBC correlation of compound **LCO5**

Position	δ_H (ppm)	HMBC Correlation
5	0.96 (1H, *m*)	C-1 (34.98) and C-10 (36.31)
17	1.50 (1H, *m*)	C-13 (44.50), C-16 (28.17), C-18 (15.71) and C-20 (36.47)
18	0.71 (3H, *s*)	C-12 (30.76), C-13 (44.50) and C-14 (49.83)
19	0.97 (3H, *s*)	C-1 (34.98) and C-5 (47.03)
21	0.92 (3H, *d*, J = 6.5 Hz)	C-17 (50.37)
25	2.23 (1H, *septet*, J = 6.5 Hz)	C-23 (31.14), C-24 (156.90), C-26 (21.85), C-27 (21.98) and C-28 (105.91)
26	1.02 (3H, *d*, J = 7 Hz)[a]	C-24 (156.90), C-25 (33.78) and C-27 (21.98)

Table 52 HMBC correlation of compound **LCO5**

Position	δ_H(ppm)	HMBC Correlation
27	1.03 (3H, *d*, *J* = 7 Hz)[a]	C-24 (156.90), C-25 (33.78) and C-26 (21.85)
28	4.67 (1H, *d*, *J* = 1.5 Hz) 4.72 (1H, *s*)	C-23 (31.14), C-24 (156.90) and C-25 (33.78)
29	1.00 (3H, *d*, *J* = 6.5 Hz)	C-3 (76.52), C-4 (39.19) and C-5 (47.03)
30	0.88 (3H, *s*)	C-8 (134.61), C-13 (44.50), C-14 (49.83) and C-15 (31.27)

[a] Assignment with the same superscripts maybe interchanged.



Selected HMBC correlation of compound **LCO5**

Table 53 ^{1}H and ^{13}C spectral data of compound **LCO5**

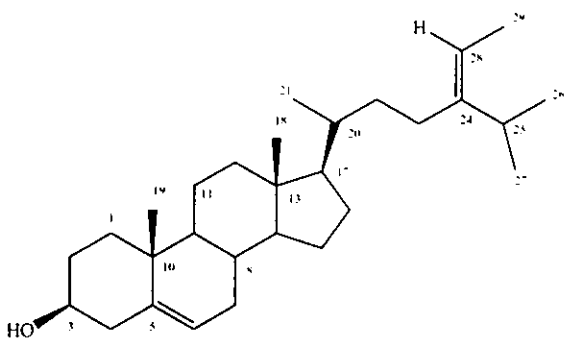
Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
1	34.98	CH_2	1.78 (1H, *m*), 1.22 (1H, *m*)[a]
2	31.04[b]	CH_2	**
3	76.52	CH	3.11 (1H, *m*)
4	39.19	CH	1.34 (1H, *m*)
5	47.03	CH	0.96 (1H, *m*)
6	20.70	CH_2	} 2.06 (2H, *m*)1.78 (1H, *m*), 1.28 (1H, *m*)
7	21.74	CH_2	
8	134.61	C	-
9	133.57	C	-
10	36.31	C	-
11	25.52	CH_2	2.02 (1H, *m*)
12	30.76[b]	CH_2	**
13	44.50	C	-
14	49.83	C	-
15	31.27[b]	CH_2	**
16	28.17	CH_2	1.94 (1H, *m*), 1.38 (1H, *m*)
17	50.37	CH	1.50 (1H, *m*)
18	15.71	CH_3	0.71 (3H, *s*)
19	18.20	CH_3	0.97 (3H, *s*)
20	36.47	CH	**
21	18.71	CH_3	0.92 (3H, *d*, J = 6.5 Hz)
22	34.97	CH_2	1.58 (1H, *m*), 1.14 (1H, *m*)[a]
23	31.14[b]	CH_2	**
24	156.90	C	-
25	33.78	CH	2.23 (1H, *septet*, J = 6.5 Hz)
26	21.85	CH_3	1.02 (3H, *d*, J = 7 Hz)[c]
27	21.98	CH_3	1.03 (3H, *d*, J = 7 Hz)[c]

Table 53 (Continued)

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
28	105.91	$=CH_2$	4.67 (1H, *d*, *J* = 1.5 Hz)
			4.72 (1H, *s*)
29	15.05	CH_3	1.00 (3H, *d*, *J* = 6.5 Hz)
30	24.41	CH_3	$CH_3$0.89 (3H, *s*)

[a, b, c] Assignment with the same superscripts maybe interchanged, [**] The chemical shift of proton resonated at δ 1.20- 2.12, [=] Carbon type deduced from DEPT experiment.

Compound LCO6



Compound **LCO6** was isolated as a white solid, mp = 118-120 °C, $[\alpha]_D^{26}$= -22.47° (*c* = 0.0089, $CHCl_3$). This compound showed green color with Lieberman-Berchard reagent, indicating the steroid characteristic. Its IR spectrum (**Fig. 89**) showed absorption band at 3314 cm^{-1} for hydroxy group.

The complete analysis of ^{13}C and ^{1}H NMR spectrum of compound **LCO6** (see **Table 56, Fig. 90, 91**), were assigned with the information provided from COSY

(**Fig. 93**), HMQC (**Fig. 94**), HMBC (see **Table 55**, **Fig. 95**) and NOE (**Fig. 96, 97**) spectral data.

The ^{13}C NMR spectrum (see **Table 56, Fig. 92**) recorded in $CDCl_3$ showed 29 signals for 29 carbon atoms. Analysis of the DEPT spectrum (see **Table 54, Fig. 92**) revealed six methyl carbon atoms (δ 21.07, 20.99, 19.38, 18.78, 12.74 and 11.84), ten methylene carbon atoms (δ 42.32, 39.75, 37.23, 35.94, 31.89, 31.64, 28.22, 27.88, 24.28 and 21.06), nine methine carbon atoms (δ 121.70, 116.43, 71.79, 56.74, 55.99, 50.10, 36.13, 31.88 and 28.59) and four quaternary carbon atoms (δ 145.86, 140.74, 42.28 and 36.49).

The ^{1}H NMR spectrum (see **Table 56, Fig. 90**) revealed six methyl groups, two of them showed *singlets* at δ 0.68 and 1.01 which could be assigned to H-18 and H-19, respectively. The other ones resonated as *doublet* at δ 0.94 (J = 6.5 Hz), 0.97 (2 x CH3, J = 7 Hz) and 1.58 (J = 7 Hz), which were assigned to H-21, H-26, H-27 and H-29, respectively. The two olefinic protons at δ 5.36 (m) and 5.15 (q, J = 7 Hz) and one oxymethine proton at δ 3.53 (m) were assigned to H-6, H-28 and H-3, respectively. One methine proton resonated at δ 2.83 (*septet*, J = 7 Hz), could be assigned to H-25. The remaining methylene protons resonated at δ 1.38-1.90 for fourteen protons except 2H-4, 2H-22 and 2H-23 were shown at δ 2.20-2.32 (3H, 2H-4 and 1H-22) and δ 1.94-2.03 (3H, 2H-23 and 1H-22). From the above information the possible structure should have a steroid skeleton with two trisubstituted double bond in the steroid skeleton at the alkyl side chain and at either C-5 or C-9. The side chain of this compound was suggested to be [-C(H)$CH_3CH_2CH_2$C=(CHCH_3)CH(CH_3)$_2$]. It was in agreement with the ^{1}H-^{1}H COSY (**Fig. 93**) and HMBC correlation data (see **Table 55, Fig. 95**).

In the HMBC spectrum (see **Table 55, Fig. 95**), the H-17 (δ 1.13) showed correlation peaks between carbon signals at δ 36.13 (C-20), 39.75 (C-12), 42.28 (C-13), 56.74 (C-14) and 11.84 (C-18), H-21 (δ 0.94) with δ 55.99 (C-17) and 36.13 (C-

20), indicating that the side chain in compound **LCO6** was linked to the C-17 (δ 55.99) of the main skeleton. In addition, H-19 (δ 1.01) showed correlation with C-5 (140.74), C-9 (50.10) and C-10 (36.49) and H-4 (δ 2.04-2.32) showed correlation peaks with C-3 (71.79), C-5 (140.74) and C-6 (121.70). Finally, as confirmed by the ^{1}H-^{1}H COSY, the H-3 (δ 3.53) showed correlation with H-2 (δ 1.52-1.86) and H-4 (δ 2.05-2.32) and H-4 (δ 2.05-2.32) showed allylic coupling with H-6 (δ 5.36), indicating that the double bond should be located at C-5.

The geometry of this compound at C-24 and C-28 should be *E* geometry because in the NOE experiment (**Fig. 96, 97**), the irradiation of methyl group at 1.58 (H-29) enhanced the signal at δ 2.83 (H-25) and the irradiation of methine proton at δ 2.83 (H-25) enhanced the signal at δ 1.58 (H-29). These observations led us to conclude that this compound is *E* geometry. Thus, this compound was identified as 5, 24(28)-stigmastadien-3β-ol (fucosterol) which was a known compound (Idler, *et.al.*, 1953).

Table 54 ^{13}C and DEPT spectral data of compound **LCO6**

δ_C (ppm)	DEPT 45°	DEPT 90°	DEPT 135°	Type of Carbon
145.86				C
140.74				C
121.70	121.70	121.70	121.70	= CH
116.43	116.43	116.43	116.43	= CH
71.79	71.79	71.79	71.79	CH
56.74	56.74	56.74	56.74	CH
55.99	55.99	55.99	55.99	CH
50.10	50.10	50.10	50.10	CH
42.32	42.32		42.32	CH_2
42.28				C

Table 54 (Continued)

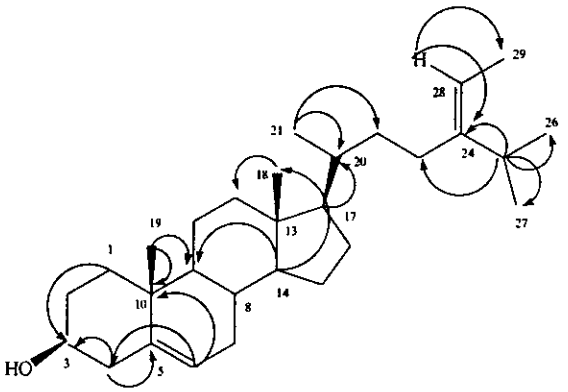
δ_C (ppm)	DEPT 45°	DEPT 90°	DEPT 135°	Type of Carbon
39.75	39.75		39.75	CH_2
37.23	37.23		37.23	CH_2
36.49				C
36.13	36.13	36.13	36.13	CH
35.94	35.94		35.94	CH_2
31.89	31.89		31.89	CH_2
31.88	31.88	31.88	31.88	CH
31.64	31.64		31.64	CH_2
28.59	28.59	28.59	28.59	CH
28.22	28.22		28.22	CH_2
27.88	27.88		27.88	CH_2
24.28	24.28		24.28	CH_2
21.07	21.07		21.07	CH_3
21.06	21.06		21.06	CH_2
20.99	20.99		20.99	CH_3
19.38	19.38		19.38	CH_3
18.78	18.78		18.78	CH_3
12.74	12.74		12.74	CH_3
11.84	11.84		11.84	CH_3

Table 54 HMBC correlation of compound **LCO6**

Position	δ_H (ppm)	HMBC Correlation
1	1.52 (2H, *m*)	C-2 (31.89), C-3 (71.79), C-5 (140.74), C-6 (121.70) and C-9 (50.10)
2	1.86 (1H, *m*), 1.52 (1H, *m*)	C-1 (35.94), C-3 (71.79), C-4 (42.32) and C-5 (140.74)
4	2.05-2.32 (2H, *m*)	C-1 (35.94), C-2 (31.89), C-3 (71.79), C-5 (140.74), C-6 (121.70) and C-10 (36.49)
6	5.36 (1H, *m*)	C-4 (42.32), C-8 (31.88) and C-10 (36.49)
17	1.10 (1H, *m*)	C-12 (39.75), C-13 (42.28) C-14 (56.78), C-18 (11.84) and C-20 (36.13)
18	0.68 (3H, *s*)	C-12 (39.75) and C-13 (42.28)
19	1.01 (3H, *s*)	C-5 (140.74), C-9 (50.10) and C-10 (36.49)
21	0.94 (3H, *d*, J = 6.5 Hz)	C-20 (36.13) and C-17 (55.99)
25	2.83 (1H, *septet*, J = 7 Hz)	C-23 (27.88), C-24 (145.86), C-26 (19.38), C-27 (18.78) and C-28 (116.43)
26	0.97 (3H, *d*, J = 7 Hz)	C-24 (145.86) and C-25 (28.59)

Table 55 (Continued)

Position	δ_H (ppm)	HMBC Correlation
27	0.97 (3H, *d*, *J* = 7 Hz)	C-24 (145.86) and C-25 (28.59)
28	5.10 (1H, *q*, *J* = 7 Hz)	C-25 (28.59) and C-29 (12.74)
29	1.58 (3H, *d*, *J* = 7 Hz)	C-24 (145.86) and C-28 (116.43)



Selected HMBC correlation of compound **LCO6**

Table 56 ^{1}H and ^{13}C spectral data of compound **LCO6**

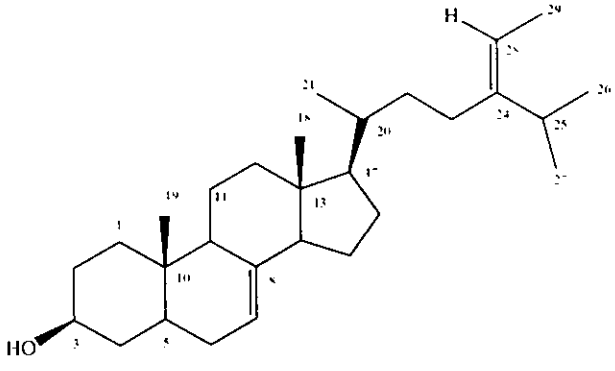
Position	δ_C (ppm)		δ_H (ppm)
1	35.94	CH_2	1.52 (2H, *m*)
2	31.89	CH_2	1.86 (1H, *m*), 1.52 (1H, *m*)
3	71.79	CH	3.53 (1H, *m*)
4	42.32	CH_2	2.05-2.32 (2H, *m*)
5	140.74	C	-
6	121.70	= CH	5.36 (1H, *m*)
7	31.64	CH_2	1.50 (2H, *m*)
8	31.88	CH	1.98 (1H, *m*)
9	50.10	CH	1.94 (1H, *m*)
10	36.49	C	-
11	21.06	CH_2	1.52 (2H, *m*)
12	39.75	CH_2	2.22 (1H, *m*), 1.98 (1H, *m*)
13	42.28	C	-
14	56.74	CH	1.14 (1H, *m*)
15	24.28	CH_2	1.60 (2H, *m*)[b]
16	28.22	CH_2	1.28 (2H, *m*)[b]
17	55.99	CH	1.10 (1H, *m*)
18	11.84	CH_3	0.68 (3H, *s*)
19	21.07	CH_3	1.01 (3H, *s*)
20	36.13	CH	1.40 (1H, *m*)
21	20.99	CH_3	0.94 (3H, *dd*, J = 6.5 Hz)
22	37.23	CH_2	1.86 (1H, *m*), 1.20 (1H, *m*)
23	27.88	CH_2	1.94-2.03 (2H, *m*)
24	145.86	C	-
25	28.59	CH	2.83 (1H, *septet*, J = 7 Hz)
26	19.38[a]	CH_3	0.97 (3H, *d*, J = 7 Hz)
27	18.78[a]	CH_3	0.97 (3H, *d*, J = 7 Hz)

Table 56 (Continued)

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
28	116.43	= CH	5.15 (1H, *q*, *J* = 7 Hz)
29	12.74	CH_3	1.58 (3H, *d*, *J* = 7 Hz)

[a, b] Assignments with the same superscripts maybe interchanged, [=] Carbon type deduce from DEPT experiment.

Compound LCO7



Compound **LCO7** was isolated as a white solid mp = 120-122 °C, $[\alpha]_D^{26}$ = +14.70° (*c* = 0.0068, $CHCl_3$). This compound showed green color with Lieberman-Berchard reagent, indicating the steroid characteristic. The IR spectrum of compound **LCO7** (**Fig. 98**) showed absorption band of hydroxy group at 3415 cm^{-1}.

The structure of compound **LCO7** was deduced from its ^{13}C and ^{1}H NMR spectral data (see **Table 59, Fig. 99, 100**) together with the results of ^{1}H-^{1}H COSY (**Fig. 102**), HMQC (**Fig. 103**) and HMBC (see, **Table 58, Fig. 104**) spectral data.

The ^{13}C NMR spectrum of compound **LCO7** (see **Table 59, Fig. 100**) recorded in $CDCl_3$ revealed 29 signals for 29 carbon atoms. Analysis of the DEPT 45°, 90° and 135° spectra of this compound (see **Table 57, Fig. 101**) suggested the presence of six methyl carbon atoms (δ 21.07, 21.00, 18.92, 13.03, 12.74,11.84), ten methylene carbon atoms (δ39.55, 37.98, 37.13, 35.90, 31.47, 29.64, 28.00, 27.94, 22.95, 21.54), nine methine carbon atoms (δ 117.44, 116.46, 71.05, 56.01, 55.02, 49.43, 40.24, 36.57, 28.59) and four quaternary carbon atoms (δ145.83, 139.58, 43.39 and 34.19).

The ^{1}H NMR spectrum of compound **LCO7** (see **Table 59, Fig. 99**) showed six methyl groups, two of them are *singlets* which resonated at δ 0.54 and 0.79 and could be assigned to H-18 and H-19, respectively. The other ones showed *doublets* at δ0.95 (J = 6.5 Hz), 0.97 (J = 7 Hz, 2 x CH_3) and 1.59 (J = 7 Hz) were assigned to H-21, H-26, H-27 and H-29, respectively. The two olefinic protons at δ5.16 (m) and 5.12 (q, J = 7 Hz) and the oxymethine proton at δ3.60 (*m*), could be assigned to H-7, H-28 and H-3, respectively. One methine proton at δ2.83 (*septet*, J = 7 Hz) was assigned to H-25. The proton NMR spectral data of this compound was similar to compound **LCO6** except the olefinic proton in the tetracyclic system, in compound **LCO6** resonated at δ5.36 while at δ5.12 in compound **LCO7**. Their the unsaturated unit should be located either at C-7 or C-9.

The HMBC correlation of compound **LCO7** (see, **Table 56, Fig. 104**) showed correlation peaks between H-19 (δ0.79) and carbon signals at δC-10 (34.19), C-1 (37.98), C-5 (49.43) and C-9 (40.24). The chemical shift of C-9 at δ40.24 was a value for sp^3 hybrid carbon indicating the impossiblility of a double bond. Thus this compound was identified as 7, 24(28)-stigmastadien-3β-ol (avenasterol).

Table 57 ^{13}C and DEPT spectral data of compound **LCO7**

δ_c (ppm)	DEPT 45°	DEPT 90°	DEPT 135°	Type of Carbon
145.83				C
139.58				C
117.44	117.44	117.44	117.44	= CH
116.46	116.46	116.46	116.46	= CH
71.05	71.05	71.05	71.05	CH
56.01	56.01	56.01	56.01	CH
55.02	55.02	55.02	55.02	CH
49.43	49.43	49.43	49.43	CH
43.39				C
40.24	40.24	40.24	40.24	CH
39.55	39.55		39.55	CH_2
37.98	37.98		37.98	CH_2
37.13	37.13		37.13	CH_2
36.57	36.57	36.57	36.57	CH
35.90	35.90		35.90	CH_2
34.19				C
31.47	31.47		31.47	CH_2
29.64	29.64		29.64	CH_2
28.59	28.59	28.59	28.59	CH
28.00	28.00		28.00	CH_2
27.94	27.94		27.94	CH_2
22.95	22.95		22.95	CH_2
21.54	21.54		21.54	CH_2
21.07	21.07		21.07	CH_3
21.00	21.00		21.00	CH_3
18.92	18.92		18.92	CH_3
13.03	13.03		13.03	CH_3

Table 57 (Continued)

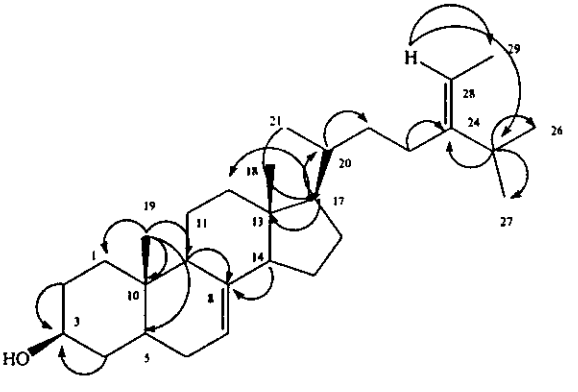
δ_C (ppm)	DEPT 45°	DEPT 90°	DEPT 135°	Type of Carbon
12.74	12.74		12.74	CH_3
11.84	11.84		11.84	CH_3

Table 58 HMBC correlation of compound **LCO7**

Position	δ_H (ppm)	HMBC Correlation
2	1.74 (1H, *m*), 1.26 (1H, *m*)	C-3 (71.05)
4	1.82 (1H, *m*), 1.10 (1H, *m*)	C-3 (71.05)
6	1.80 (1H, *m*), 1.40 (1H, *m*)	C-8 (139.58)
9	1.60 (1H, *m*)	C-7 (117.44) and C-8 (139.58)
14	1.80 (1H, *m*)	C-8 (139.58)
17	1.24 (1H, *m*)	C-12 (39.55), C-13 (43.39) and C-20 (36.57)
18	0.54 (3H, *s*)	C-12 (39.55), C-13 (43.39), C-14 (55.02) and C-17 (56.01)
19	0.79 (3H, *s*)	C-1 (37.98), C-5 (49.43), C-9 (40.24) and C-10 (34.19)
20	1.50 (1H, *m*)	C-13 (43.39), C-17 (56.01) and C-21 (18.92)
21	0.95 (3H, *d*, J = 6.5 Hz)	C-17 (56.01) and C-20 (36.57)

Table 58 (Continued)

Position	δ_H(ppm)	HMBC Correlation
23	2.00 (1H, *m*), 1.75 (1H, *m*)	C-24 (145.83) and C-28 (116.46)
25	2.83 (1H, *septet*, J = 7 Hz)	C-23 (27.94), C-24 (145.83), C-26 (21.07), C-27 (21.00) and C-28 (116.46)
26	0.97 (3H, *d*, J = 7 Hz)	C-24 (145.83), C-25 (28.59) and C-27 (21.00)
27	0.97 (3H, *d*, J = 7 Hz)	C-24 (145.83), C-25 (28.59) and C-26 (21.07)
28	5.12 (1H, *q*, J = 7 Hz)	C-25 (28.59) and C-29 (13.03)
29	1.59 (3H, *d*, J = 7 Hz)	C-24 (145.83) and C-28 (116.46)



Selected HMBC correlation of compound **LCO7**

Table 59 ^{1}H and ^{13}C spectral data of compound **LCO7**

Position	$\delta_C^{\#}$ (ppm)		δ_H (ppm)
1	37.98	CH_2	1.72 (1H, *m*), 1.28 (1H, *m*)
2	29.64	CH_2	1.74 (1H, *m*), 1.26 (1H, *m*)
3	71.05	CH	3.60 (1H, *m*)
4	37.13	CH_2	1.82 (1H, *m*), 1.10 (1H, *m*)
5	49.43	CH	1.65 (1H, *m*)
6	31.47	CH_2	1.80 (1H, *m*), 1.40 (1H, *m*)
7	117.44	= CH	5.16 (1H, *m*)
8	139.58	C	-
9	40.24	CH	1.60 (1H, *m*)*
10	34.19	C	-
11	21.54	CH_2	1.40 (1H, *m*), 1.48 (1H, *m*)
12	39.55	CH_2	2.20 (1H, *m*), 1.24 (1H, *m*)
13	43.39	C	-
14	55.02	CH	1.80 (1H, *m*)
15	28.00	CH_2	1.30 (2H, *m*)
16	22.95	CH_2	1.56 (1H, *m*), 1.40 (1H, *m*)
17	56.01	CH	1.24 (1H, *m*)
18	11.84	CH_3	0.54 (3H, *s*)
19	12.74	CH_3	0.79 (3H, *s*)
20	36.57	CH	1.50 (1H, *m*)
21	18.92	CH_3	0.95 (3H, *d*, J = 6.5 Hz)
22	35.90	CH_2	1.12 (2H, *m*)
23	27.94	CH_2	2.00 (1H, *m*), 1.75 (1H, *m*)
24	145.83	C	-
25	28.59	CH	2.83 (1H, *septet*, J = 7 Hz)
26	21.07	CH_3	0.97 (3H, *d*, J = 7 Hz)
27	21.00	CH_3	0.97 (3H, *d*, J = 7 Hz)

Table 59 ^{1}H and ^{13}C spectral data of compound **LCO7**

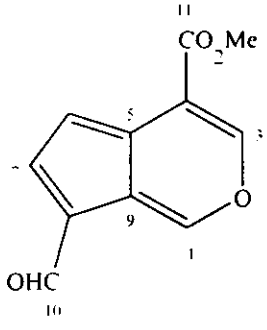
Position	$\delta_C^{\#}$ **(ppm)**		δ_H **(ppm)**
28	116.46	= CH	5.12 (1H, *q*, *J* = 7 Hz)
29	13.03	CH_3	1.59 (3H, *d*, *J* = 7 Hz)

$^{\#}$ Carbon type deduced from DEPT experiment, * Assignment by HMBC.

3.3 Structural elucidation of compounds isolated from the barks of C. odollam

The crude methylene chloride extract (6 g) was further purified by chromatographic technique and crystallization to give four compounds; iridoid monoterpene, **BCO1**, steroid, **BCO2**, quinone derivative, **BCO3** and benzaldehyde derivative, **BCO4**. Their structures were determined using 1D and 2D NMR spectroscopic data. In addition, the structure of **BCO1** was confirmed by X-ray diffraction.

Compound BCO1



Compound **BCO1** was isolated as a yellow needles, mp = 184-186 °C. Its molecular formula was $C_{11}H_8O_4$ as indicated by EIMS. The EIMS of compound **BCO1** (**Fig. 112**) showed the molecular ion peak at 204 *m/z* and fragment ion peaks at *m/z* 203 ($[M-H]^+$), 175 ($[M-CHO]^+$) and 145 ($[M-CO_2Me]^+$). The IR spectrum of compound **BCO1** (**Fig. 106**) showed absorption bands at 2830 cm^{-1} (C-H stretching of formyl group), and 1724 and 1639 cm^{-1}(carbonyl groups). The UV spectrum (**Fig. 105**) showed maxima at 209, 253, 291, 331 and 429 nm.

The ^{13}C NMR spectrum of compound **BCO1** (see **Table 60, Fig. 108**) recorded in $CDCl_3$ showed 11 signals for 11 carbon atoms. The DEPT-135° spectrum (see **Table 60, Fig. 109**) indicated the existence of one methoxy carbon atom (δ 52.25), five methine carbon atoms (δ 185.66, 150.08, 148.56, 148.39 and 133.64) and five quaternary carbon atoms (δ165.17, 130.56, 125.30, 124.05 and 115.20). The ^{1}H NMR spectrum of compound **BCO1** (see **Table 60, Fig. 107**) showed the methoxy proton at δ4.00 (3H, *s*) four olefinic protons at δ9.10 (1H, *s*), 8.50 (1H, *s*), 7.98 (1H, *d*, J = 3.5 Hz) and 7.12 (1H, *d*, J = 3.5 Hz) and one formyl proton at δ9.95 (1H, *s*).

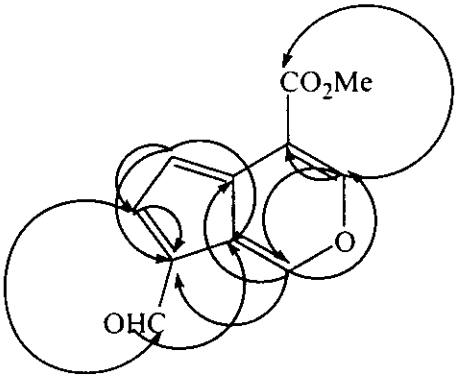
The structure of compound **BCO1** was deduced from its ^{13}C and ^{1}H spectral data (see **Table 68, Fig. 107, 108**) together with the results of HMQC (**Fig. 110**) and HMBC (see **Table 60, Fig. 111**) spectral data. The HMBC correlation of compound **BCO1** (see **Table 60, Fig. 111**) showed correlation peaks between H-7 (7.98) and C-10 (185.66), C-8 (130.56), C-5 (125.04) and C-6 (113.64), thus confirmed the position of formyl group at C-8 (130.56). The carbon signals at C-1 (150.80), C-4 (115.20) and C-11 (165.17) gave a correlation peak with the proton at δ8.50 (H-3) which confirmed the position of carbomethoxy group at C-4 (115.20).

Based on the data described above, compound **BCO1** was determined to be cerbinal, as a known compound previously isolated from the bark of *C.manghas* (Abe, *et.al.*,1977). The structure of this compound was finally confirmed by X-ray diffraction (Laphookhieo, *et.al.*,2002).

Table 60. ^{1}H, ^{13}C NMR and HMBC correlation of **BCO1**

Position	δ_H	δ_C	Type of Carbon[#]	HMBC
1	9.16 (1H, *s*)	150.08	CH	C-3, C-8, C-5
2	-	-	-	-
3	8.50 (1H, *s*)	148.56	CH	C-11, C-1, C-4
4	-	115.20	C	-
5	-	124.00	C	-
6	7.12 (1H, *dd*, J = 3.5, 1 Hz)	113.64	CH	C-7, C-8, C-9
7	7.98 (1H, *d*, J = 1, 3.5Hz)	148.39	CH	C-8, C-10, C-5, C-6
8	-	130.56	C	-
9	-	125.30	C	-
10	9.95 (*s*)	185.66	CH	C-9
11	-	165.17	C	-
12	4.00 (*s*)	52.25	CH_3	-

[#] Carbon type deduced from DEPT experiment.



HMBC correlation of **BCO1**

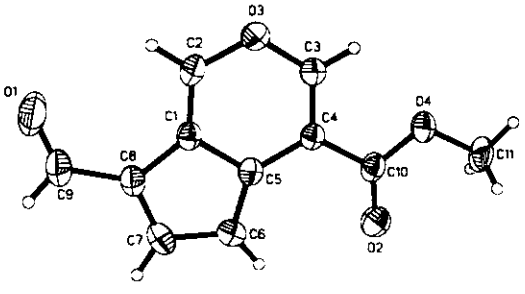
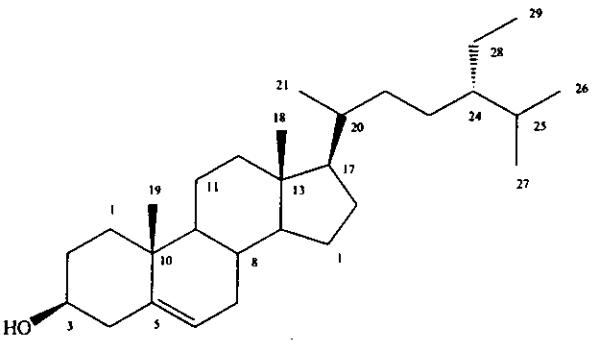


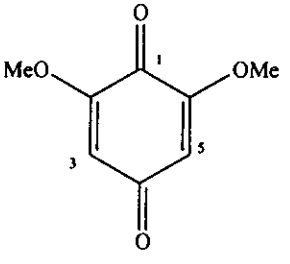
Figure 6 X-ray ORTEP diagram of compound **BCO1**

Compound BCO2



Compound **BCO2** was isolated as a white solid, mp = 128-130 °C. The IR spectrum (**Fig. 113**) showed absorption band at 3415 cm^{-1} (O-H stretching). The ^{1}H and ^{13}C NMR spectrum of this compound (**Fig. 114 and 115**) were identical to those of β-sitosterol (Sukpondma, 2001). Thus compound **BCO2** was identified as β-sitosterol.

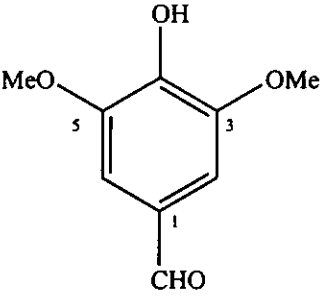
Compound BCO3



Compound **BCO3** was isolated as a yellow needles. The UV spectrum (**Fig.116**) showed an absorption band at 288 nm. The IR spectrum (**Fig. 117**) showed an absorption band at 1696 cm^{-1} (C=O stretching). The ^{1}H NMR spectrum of this compound (**Fig. 118**) showed a *singlet* of methoxy proton at δ3.81 (6H) together with a *singlet* olefinic proton at δ5.82 (2H). The ^{13}C NMR spectrum (**Fig. 119**) revealed 4

signals for 8 carbon atoms at δ187.50 (C=O), 172.00 (C=O) 157.33 (2 x C), 107.42 (2 x C) and 56.45 (2 x C). Comparison this compound with 2, 6-dimethoxybenzoquinone (the authentic sample) showed the identical of the ^{1}H, ^{13}C, IR and melting point. Thus compound **BCO3** was identified as 2, 6-dimethoxybenzoquinone.

Compound BCO4



Compound **BCO4** was isolated as a white solid, mp = 104-105 °C. The UV spectrum (**Fig. 120**) showed an absorption band at 280 nm. The IR spectrum (**Fig. 121**) showed absorptions at 3438 (O-H stretching), 2848 (C-H stretching of formyl group), 1673 cm^{-1} (C=O stretching). The ^{1}H NMR spectrum of this compound (**Fig. 122**) showed the characteristic signal of aldehyde proton at δ 9.80 (*s*) together with two aromatic protons superimposed at δ7.15 (2H) and two methoxy proton at δ3.97 (6H, *s*). The ^{13}C NMR spectrum (**Fig. 123**) showed 6 signals for 9 carbon atoms at δ190.72 (C=O), 147.33, 140.78, 128.40 (2 x C), 106.67 (2 x C) and 56.46 (2 x C). Thus compound **BCO4** was identified as 3,5-dimethoxy- 4-hydroxybenzaldehyde.

3.4 Biological activities of the crude extract and the pure compounds from C. odollam

The biological activity of the crude extract of the seeds exhibited strong activity against both KB and BC cells. It also showed activity against *Mycobacterium tuberculosis* H37Ra but no antimalarial and antifungal activity. The pure compounds (**SCO1**, **SCO2**, **SCO3**, and **SCO5**) exhibited strong activity against KB, BC and NCI-H187 cells except compound **SCO5** showed only strong activity against BC and moderate activity against KB and NCI-H187 cells. Compounds **SCO1** and **SCO2** exhibited no activity against *Mycobacterium tuberculosis* H37Ra. The crude extract of latex showed only *in vitro* antituberculous activity against *Mycobacterium tuberculosis* H37Ra while no activity was observed with the pure compounds (**LCO1**, **LCO2**, **LCO3**, **LCO5**, **LCO6** and **LCO7**). The crude extract of the barks and the pure compound (**BCO1**) exhibited weak and moderate activity against KB and BC cells, respectively and active against *Mycobacterium tuberculosis* H37Ra. The results were summarized in **Table 61**.

Table 61 Biological activities of the crude extract and the pure compounds from *C. odollam*

Sample	KB [a]		BC [b]		NCI-H187 [c]		Antituberculous		Antimalarial	Antifungal
	Cytotoxicity	ED_{50}	Cytotoxicity	ED_{50}	Cytotoxicity	ED_{50}	Inactive/ Active	(MIC, µg/ml) TB [d]		
A	Moderate	6.1	Strong	3.8	-	-	Active	200	Inactive	Inactive
B	Inactive	-	Inactive	-	Inactive	-	Active	100	Inactive	Inactive
C	Weak	11.5	Moderate	7.9	Moderate		Active	200	Inactive	Inactive
D	-	-	-	-	-	-	Inactive	-	-	-
SCO1	Strong	0.017	Strong	0.048	Strong	0.076	-	Inactive	-	-
SCO2	Strong	1.92	Strong	1.63	Strong	1.24	-	Inactive	-	-
SCO3	Strong	0.078	Strong	0.049	Strong	0.032	-	-	-	-
SCO5	Moderate	7.56	Strong	4.62	Moderate	7.42	-	-	-	-
BCO1	Weak	14.1	Moderate	6.4	-	-	Active	200	-	-

[a] Oral human epidermoid carcinoma, [b] Human breast cancer cells, [c] Human, small cells lung cancer, [d] *In vitro* antituberculous activity against *Mycobacterium tuberculosis* H37Ra, **A** = The methylene chloride extract of the seeds, **B** = The ethyl acetate extract of latex, **C** = The methylene chloride extract of the barks, **D** = the pure compounds from the ethyl acetate extract of latex (**LCO1**, **LCO2**, **LCO3**, **LCO5**, **LCO6** and **LCO7**), **ED_{50}** = Effective Dose (µg/ml), **MIC** = Minimum Inhibitory Concentration.