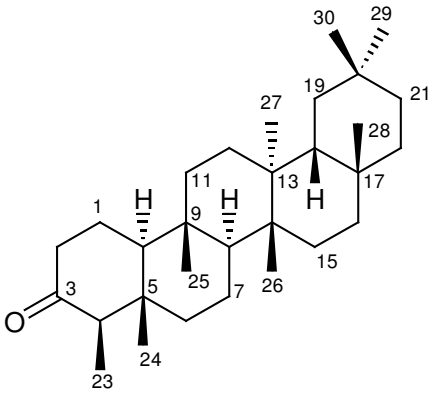
CHAPTER 3
RESULTS AND DISCUSSION

3.1 Structure elucidation of compounds from the bark of *H. littoralis*

The air-dried bark of *H. littoralis* (6.0 kg) was extracted with hexane, dichloromethane and acetone, successively. The crude hexane extract was subjected to chromatography and/or crystallization to give three triterpenes: **CD1, CD2** and **CD5**, five steroids: **CD7**-**CD11**, one anthraquinone: **CD14** and one benzoic acid derivative: **CD15**. The crude dichloromethane extract was purified by chromatography and/or crystallization to yield three triterpenes: **CD3, CD4** and **CD6**, one steroid: **CD13** and one sesquiterpene: **CD16**. The acetone extract was separated by chromatography and/or crystallization to afford one steroid: **CD12**, one resorcinol derivative: **CD17** and one flavanol monomer (catechin): **CD18**.

Their structures were elucidated by 1D and 2D NMR spectroscopic data. All carbons were assigned by ^{13}C NMR, DEPT 135^{o}, DEPT 90^{o}, HMQC and HMBC data.

3.1.1 Compound CD1

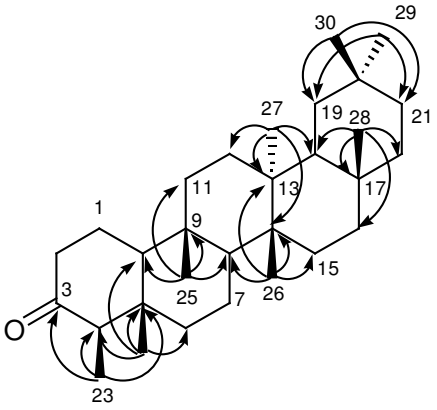


Compound **CD1** was obtained as a white solid, mp 245-247 ^{o}C, $[\alpha]_{D}^{28}$: -22.3^{o} (c = 0.54, $CHCl_3$). The IR spectrum (**Figure 8**) showed absorption bands for carbonyl group at 1715 cm^{-1}. It gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^{13}C NMR spectral data (**Table 2, Figure 10**) recorded in $CDCl_3$ showed 30 signals for 30 carbons. Analysis of DEPT 90^{o} and DEPT 135^{o} spectra of this compound suggested the presence of eight methyl (δ 6.8, 14.7, 17.9, 18.5, 20.3, 31.8, 32.1 and 35.0), eleven methylene (δ 18.2, 22.3, 30.5, 32.4, 32.8, 35.3, 35.6, 36.0, 39.3, 41.3 and 41.5), four methine (δ 42.8, 53.1, 58.2 and 59.5) and seven quaternary carbons (δ 28.2, 30.0, 37.4, 38.3, 39.7, 42.2 and 213.3).

The ^{1}H NMR spectral data (**Table 2, Figure 9**) showed characteristic of friedelan triterpenes as one methyl doublet at δ 0.89 (3H-23, *d*, *J* = 6.3 Hz) and seven methyl singlets at δ 0.72, 0.87, 0.95, 1.00, 1.01, 1.05 and 1.18.

The position of methyl group at C-23 was determined through an HMBC experiment (**Table 2, Figure 15**) in which the methyl proton at δ 0.89 (3H-23) showed correlations with C-3 (δ 213.3), C-4 (δ 58.2) and C-5 (δ 42.2). Thus on the basis of its spectroscopic data (**Table 2**) and comparison with the previously reported data of friedelin (Ahad *et al*., 1991), compound **CD1** was, therefore, assigned as friedelin.



Selected HMBC correlation of **CD1**

Table 2 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD1** ($CDCl_3$) and friedelin (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD1**) $^{1}H \rightarrow ^{13}C$
		CD1	**R**	**CD1**	
1	CH_2	22.3	22.3	1.64 (*m*)[a], 1.69 (*m*)[a]	-
2	CH_2	41.5	41.5	2.36 (*m*)[a], 2.23 (*m*)[a]	-
3	C	213.3	21.32	-	-
4	CH	58.2	58.2	2.24 (*m*)[a]	-
5	C	42.2	42.2	-	-
6	CH_2	41.3	41.3	2.44 (*m*)[a], 1.78 (*m*)[a]	-
7	CH_2	18.2	18.2	1.52 (*m*)[a], 1.39 (*m*)[a]	-
8	CH	53.1	53.1	1.42 (*m*)[a]	-
9	C	37.4	37.5	-	-
10	CH	59.5	59.5	1.56 (*m*)[a]	-
11	CH_2	35.6	35.6	1.61 (*m*)[a], 1.43 (*m*)[a]	-
12	CH_2	30.5	30.5	1.46 (*m*)[a], 1.34 (*m*)[a]	-
13	C	39.7	39.7	-	-
14	C	38.3	38.3	-	-
15	CH_2	32.4	32.4	1.51 (*m*)[a], 1.29 (*m*)[a]	-
16	CH_2	36.0	36.0	1.61 (*m*)[a], 1.36 (*m*)[a]	-
17	C	30.0	30.0	-	-
18	CH	42.8	42.8	1.53 (*m*)[a]	-

Table 2 (Continued)

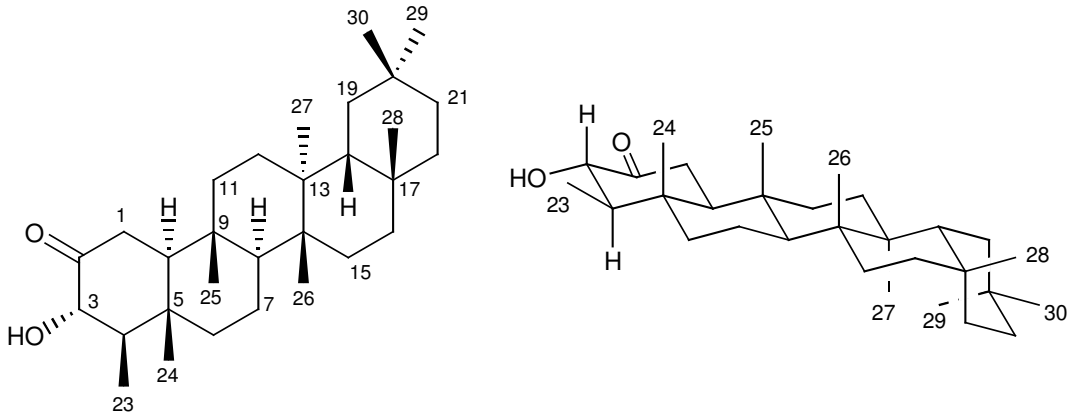
Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD1**) $^1H \rightarrow ^{13}C$
		CD1	**R**	**CD1**	
19	CH_2	35.3	35.4	1.62 (*m*)[a], 1.49 (*m*)[a]	-
20	C	28.2	28.1	-	-
21	CH_2	39.3	39.3	1.48 (*m*)[a], 0.93 (*m*)[a]	-
22	CH_2	32.8	32.8	1.50 (*m*)[a], 1.26 (*m*)[a]	-
23	CH_3	6.8	6.8	0.89 (*d*, 6.3)	3, 4, 5
24	CH_3	14.7	14.7	0.72 (*s*)	4, 5, 6, 10
25	CH_3	17.9	18.0	0.87 (*s*)	8, 9, 10. 11
26	CH_3	20.3[1]	18.7	1.01 (*s*)	8, 13, 14, 15
27	CH_3	18.5[1]	20.3	1.05 (*s*)	12, 13, 14, 18
28	CH_3	32.1	32.1	1.18 (*s*)	16, 17, 18, 22
29	CH_3	31.8	31.8	1.00 (*s*)	19, 20, 21
30	CH_3	35.0	35.0	0.95 (*s*)	19,20, 21

* For **CD1**

[a] Deduced from HMQC experiment

[1] Values bearing the same notation are interchangeable

3.1.2 Compound CD2

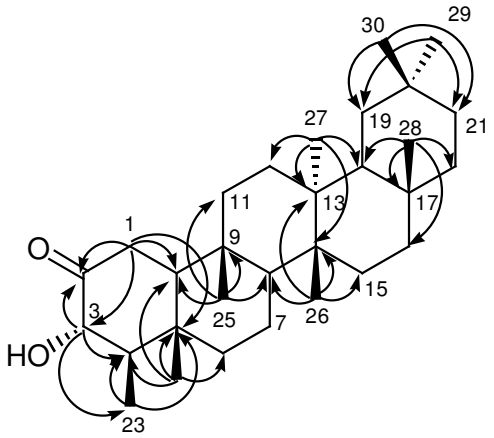


Compound **CD2** was obtained as a white solid, mp 254-256 ^{o}C, $[\alpha]_{D}^{28}$: -28.4^{o} (c = 0.31, $CHCl_3$). The IR spectrum exhibited absorption at 3431 cm^{-1} (hydroxyl) and 1633 cm^{-1}(carbonyl). It gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^{1}H NMR spectral data (**Table 3**, **Figure 16**) were closely related to compound **CD1** (**Table 2, Figure 9**), except that the signals of methylene protons (2H-2) at δ 2.23 (*m*) and 2.36 (*m*) of **CD1** disappeared and those of the methylene protons (2H-1) were shifted downfield to δ 2.39 (1H, *d*, *J* = 13.5 Hz) and δ 2.53 (1H, *dd*, *J* = 13.5, 3.6 Hz) as compared to those of **CD1** at δ 1.64 (*m*) and 1.69 (*m*). In addition, the oxymethine proton (H-3) was shown at δ 3.82 (*dd*, *J* = 11.7, 3.3 Hz), which was not observed in compound **CD1**. The doublet of doublet splitting pattern with a large coupling constant of H-3 with $J_{ax\text{-}ax}$ = 11.7 Hz (coupled with H-4) and J_{vic} = 3.3 Hz (coupled with the hydroxyl group at δ 3.54, *J* = 3.3 Hz) indicated an axial (β) orientation of H-3. A doublet signal at δ 3.54 exchangeable with D_2O corresponded to a hydroxyl group.

The ^{13}C NMR spectral data (**Table 3, Figure 17**) of compound **CD2** displayed a signal of an oxymethine carbon at δ 76.9 which was assigned to C-3 and a signal at δ 211.9 which was assigned to C-2. The location of the oxymethine carbon and carbonyl group was confirmed by HMBC experiment (**Table 3**) in which both α H-1 (δ 2.53) and β H-1 (δ 2.39) showed long-range correlation with C-2 (δ 211.9),

C-3 (δ 76.9), C-5 (δ 38.1) and C-10 (δ 60.4). By comparison of the ^{13}C NMR spectral data with the previously reported data of 3α-hydroxy friedelan-2-one (Castola *et al*., 2002) (**Table 3**), compound **CD2** was, therefore, assigned as 3α-hydroxy friedelan-2-one.



Selected HMBC correlation of **CD2**

Table 3 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CD2**, **CD1** ($CDCl_3$) and 3α-hydroxy friedelan-2-one (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD2**) $^{1}H \rightarrow {}^{13}C$
		CD2	**CD1**	**R**	**CD2**	
1	CH_2	36.1	22.3	36.0	2.39 (*t,* 13.5), 2.53 (*dd,* 13.5, 3.6)	2, 3, 5, 10
2	C	211.9	41.5	211.9	-	-
3	CH	76.9	213.3	77.0	3.82 (*dd,* 11.7, 3.3)	2, 4, 23
4	CH	54.1	58.2	54.6	1.80 (*m*)[a]	-
5	C	38.1	42.2	38.1	-	-
6	CH_2	40.6	41.3	40.6	1.08 (*m*)[a] 1.85 (*dd,* 12.9, 3.3)	5, 7, 10, 24
7	CH_2	17.6	18.2	17.6	1.43 (*m*)[a], 1.53 (*m*)[a]	-
8	CH	53.1	53.1	53.2	1.31 (*m*)[a]	-
9	C	37.6	37.4	37.7	-	-

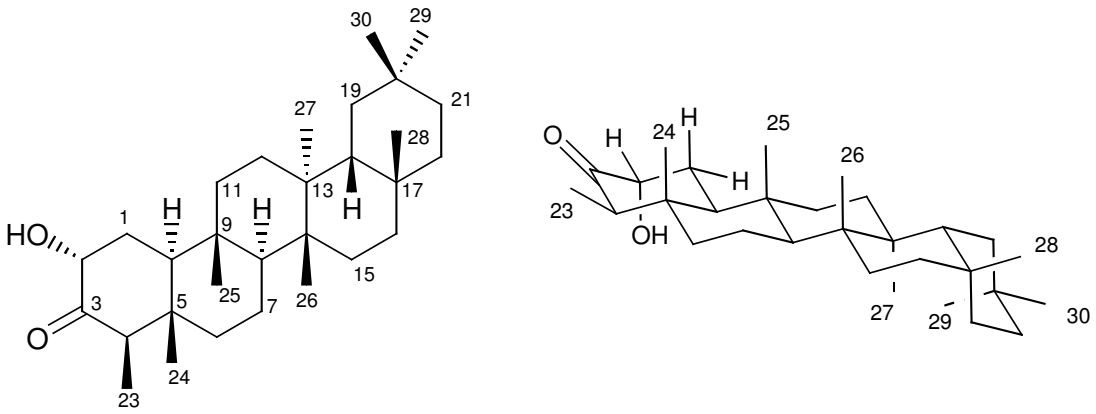
Table 3 (Continued)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD2**) $^1H \rightarrow ^{13}C$
		CD2	**CD1**	**R**	**CD2**	
10	CH	60.4	59.5	60.5	1.33 (*m*)[a]	-
11	CH_2	35.3	35.6	35.1	1.18 (*m*)[a], 159 (*m*)[a]	-
12	CH_2	35.1	30.5	35.0	1.12 (*m*)[a], 1.38 (*m*)[a]	-
13	C	39.7	39.7	39.7	-	-
14	C	38.3	38.3	38.4	-	-
15	CH_2	32.7	32.4	32.8	1.22 (*m*)[a], 1.48 (*m*)[a]	-
16	CH_2	35.9	36.0	36.0	1.17 (*m*)[a], 1.58 (*m*)[a]	-
17	C	30.0	30.0	30.0	-	-
18	CH	42.8	42.8	42.8	1.58 (*m*)[a]	-
19	CH_2	39.2	35.3	39.3	0.90 (*m*)[a], 1.44 (*m*)[a]	-
20	C	28.2	28.2	28.7	-	-
21	CH_2	32.4	39.3	32.4	1.18 (*m*)[a], 1.52 (*m*)[a]	-
22	CH_2	30.3	32.8	30.4	1.26 (*m*)[a], 1.34 (*m*)[a]	-
23	CH_3	10.8	6.8	10.8	1.05 (*d*, 6.6)	3, 4, 5
24	CH_3	14.2	14.7	14.7	1.03 (*s*)	4, 5, 6, 10
25	CH_3	17.4	17.9	17.4	0.89 (*s*)	8, 9, 10. 11
26	CH_3	20.2	20.3	20.2	1.01 (*s*)	8, 13, 14, 15
27	CH_3	18.6	18.5	18.6	0.98 (*s*)	12, 13, 14, 18
28	CH_3	32.1	32.1	32.1	1.17 (*s*)	16, 17, 18, 22
29	CH_3	31.7	31.8	31.7	0.99 (*s*)	19, 20, 21,30
30	CH_3	35.0	35.0	34.9	0.94 (*s*)	19,20, 21, 29
3-OH	-	-	-	-	3.54 (*d,* 3.3)	2, 3, 4

* For **CD2**

[a] Deduced from HMQC experiment

3.1.3 Compound CD3

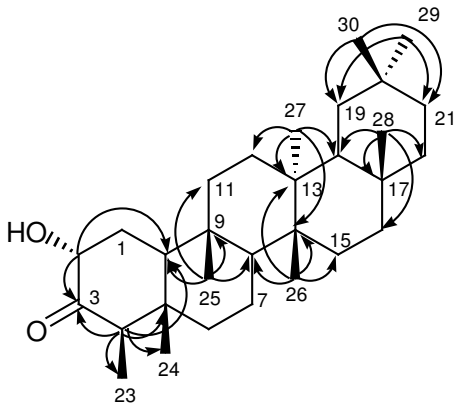


Compound **CD3** was obtained as a white solid, mp 256-257 ^{o}C, $[\alpha]_{D}^{28}$: -121.7^{o} (c = 0.11, $CHCl_3$). It gave a positive vanillin-sulfuric acid test. The IR spectrum showed absorption bands similar to those of compound **CD2**.

The ^{1}H and ^{13}C NMR spectral data (**Table 4**, **Figures 18** and **19**) of compound **CD3** were similar to those of **CD2** (**Table 4, Figures 16** and **17**) except for the change of the position of oxymethine proton H-3 (δ 3.82) and carbonyl group C-2 (δ 211.9) of compound **CD2** to oxymethine proton H-2 (δ 3.96) and carbonyl group C-3 (δ 214.0) of compound **CD3**. The configuration of the oxymethine proton H-2 was derived from its ^{1}H NMR splitting pattern which was displayed as a broad triplet at δ 3.96, thus indicating an equatorial (β) orientation.

The position of the hydroxyl group at C-2 was determined through an HMBC experiment (**Table 4**) in which the oxymethine proton at δ 3.96 (H-2) showed correlation with C-3 (δ 214.0) and C-10 (δ 52.3). The location of the carbonyl group was confirmed by HMBC experiment (**Table 4**) in which the methine proton at δ 2.88 (H-4) showed long-range correlation with C-3 (δ 214.0), C-5 (δ 43.4), C-10 (δ 52.3), C-23 (δ 6.6) and C-24 (δ 14.1).

By comparison of the ^{13}C NMR spectral data (**Table 4**) with the previously reported data of cerin (Moileiro *et al*., 2001), compound **CD3** was, therefore, assigned as cerin.



Selected HMBC correlation of **CD3**

Table 4 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CD3** ($CDCl_3$ +CD_3OD), **CD2** ($CDCl_3$) and **Cerin** ($CDCl_3$)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD3**) $^{1}H \rightarrow ^{13}C$
		CD3	**CD2**	**Cerin**	**CD3**	
1	CH_2	30.6	36.1	32.4	1.69 (*m*)[a], 1.19 (*m*)[a]	-
2	CH	73.6	211.9	76.9	3.96 (*br t*)	3, 10
3	C	214.0[b]	76.9	212.0	-	-
4	CH	53.1	54.1	52.7	2.88 (*q,* 6.6)	3, 5, 10, 23, 24
5	C	43.4	38.1	42.7	-	-
6	CH_2	41.3	40.6	40.8	1.33 (*m*)[a], 1.75 (*m*)[a]	-
7	CH_2	18.4	17.6	17.5	1.31 (*m*)[a], 1.46 (*m*)[a]	-
8	CH	53.3	53.1	52.7	1.96 (*m*)[a]	-
9	C	37.0	37.6	38.0	-	-
10	CH	52.3	60.4	52.7	1.46 (*m*)[a]	-
11	CH_2	35.5	35.3	35.6	1.36 (*m*)[a], 1.40 (*m*)[a]	-
12	CH_2	30.4	35.1	30.2	1.79 (*m*)[a], 1.98 (*m*)[a]	-
13	C	39.9	39.7	39.4	-	-
14	C	38.5	38.3	38.9	-	-
15	CH_2	32.9	32.7	32.7	1.23 (*m*)[a], 1.52 (*m*)[a]	-
16	CH_2	35.5	35.9	36.5	1.35 (*m*)[a], 1.42 (*m*)[a]	-
17	C	30.1	30.0	29.8	-	-
18	CH	42.9	42.8	42.4	1.50 (*m*)[a]	-

Table 4 (Continued)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD3**) $^1H \rightarrow ^{13}C$
		CD3	**CD2**	Cerin	**CD3**	
19	CH_2	39.4	39.2	35.0	0.93 (*m*)[a], 1.46 (*m*)[a]	-
20	C	28.3	28.2	27.8	-	-
21	CH_2	32.6	32.4	32.0	1.49 (*m*)[a], 1.55 (*m*)[a]	-
22	CH_2	36.2	30.3	40.8	0.95 (*m*)[a], 1.51 (*m*)[a]	-
23	CH_3	6.6	10.8	6.3	0.87 (*d*, 6.3)	3, 4, 5
24	CH_3	14.1	14.2	13.8	0.70 (*s*)	4, 5, 6, 10
25	CH_3	18.0	17.4	17.9	0.85 (*s*)	8, 9, 10. 11
26	CH_3	18.8[1]	20.2	18.4	1.01 (*s*)	8, 13, 14, 15
27	CH_3	20.4[1]	18.6	19.9	1.05 (*s*)	12, 13, 14, 18
28	CH_3	32.2	32.1	32.1	1.18 (*s*)	16, 17, 18, 22
29	CH_3	31.9	31.7	31.7	1.00 (*s*)	19, 20, 21,30
30	CH_3	35.1	35.0	34.7	0.95 (*s*)	19,20, 21, 29

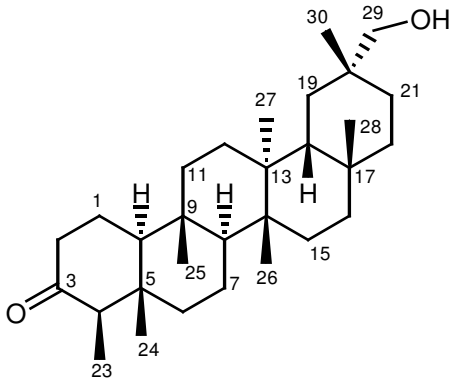
* For **CD3**

[a] Deduced from HMQC experiment

[b] Deduced from HMBC experiment

[1] Values bearing the same notation are interchangeable

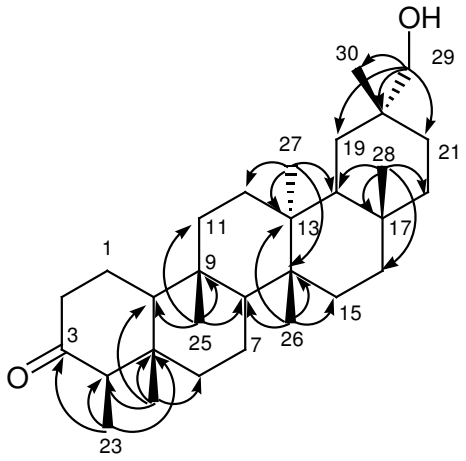
3.1.4 Compound CD4



Compound **CD4** was obtained as a white solid, mp 230-232 ^{o}C, $[\alpha]_{D}^{28}$: -63.5^{o} (c = 0.22, $CHCl_3$). It gave a purple vanillin-sulfuric acid test. The IR spectrum showed absorption at 3368 cm^{-1} (hydroxyl) and 1705 cm^{-1} (carbonyl).

Comparison of ^{1}H and ^{13}C NMR spectral data (**Table 5**, **Figure 20** and **21**) of compound **CD4** and **CD1** (**Table 2, Figures 9** and **10**) revealed close structural similarity. Difference in the spectrum of compound **CD4** was shown as only six singlet signals of methyl group at δ 0.73, 0.87, 1.03 (x 2), 1.05 and 1.22. In addition, the AB system of oxymethylene protons (2H-29) was shown at δ 3.24 (1H, *d*, *J* = 10.5 Hz) and 3.29 (1H, *d*, *J* = 10.5 Hz) which was not observed in compound **CD1**. The oxymethylene protons were assigned at C-29 position from HMBC experiment (**Table 5**) in which the oxymethylene protons (2H-29) showed long-range correlation with C-19 (δ 39.5), C-20 (δ 33.1), C-21 (δ 29.8) and C-30 (δ 25.8). Additional confirmation of the hydroxyl group on C-29 was established through NOESY spectral analysis which showed spatial connectivities of 3H-27 (δ 0.98) with 2H-29 (δ 3.24 and 3.29).

By comparison of the ^{13}C NMR spectral data (**Table 5**) with the previously reported data of friedelan-3-one-29-ol (Martinez *et al*., 1988). Compound **CD4** was, therefore, assigned as friedelan-3-one-29-ol.



Selected HMBC correlation of **CD4**

Table 5 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CD4**, **CD1** ($CDCl_3$) and friedelan-3-one-29-ol (**R**, $CDCl_3$)

Posi-tion	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD4**) $^{1}H \rightarrow ^{13}C$
		CD4	**CD1**	**R**	**CD4**	
1	CH_2	22.3	22.3	22.3	1.71 (*m*)[a], 1.55 (*m*)[a]	-
2	CH_2	41.3	41.5	41.6	2.28 (*m*)[a], 2.40 (*m*)[a]	-
3	C	213.2	213.3	212.9	-	-
4	CH	58.2	58.2	58.4	2.25 (*m*)[a]	-
5	C	42.2	42.2	42.1	-	-
6	CH_2	41.5	41.3	41.5	1.75 (*m*)[a], 1.79 (*m*)[a]	-
7	CH_2	18.2	18.2	18.3	1.50 (*m*)[a], 1.41 (*m*)[a]	-
8	CH	53.4	53.1	53.5	1.40 (*m*)[a]	-
9	C	37.4	37.4	37.6	-	-
10	CH	59.5	59.5	59.7	1.54 (*m*)[a]	-
11	CH_2	35.6	35.6	35.8	1.28 (*m*)[a], 0.96 (*m*)[a]	-
12	CH_2	30.4	30.5	30.7	1.36 (*m*)[a], 1.34 (*m*)[a]	-
13	C	38.2	39.7	38.4	-	-
14	C	40.0	38.3	40.1	-	-
15	CH_2	32.7	32.4	32.9	1.58 (*m*)[a], 1.52 (*m*)[a]	-
16	CH_2	35.9	36.0	36.0	1.48 (*m*)[a], 1.64 (*m*)[a]	-
17	C	30.5	30.0	30.6	-	-

Table 5 (Continued)

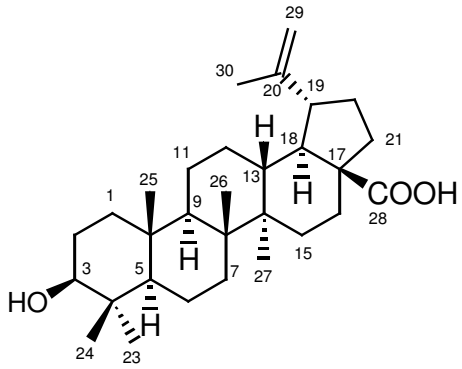
Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD4**) $^1H \rightarrow ^{13}C$
		CD4	**CD1**	**R**	**CD4**	
18	CH	41.9	42.8	42.2	1.63 (*m*)[a]	-
19	CH_2	39.5	35.3	39.6	0.99 (*m*)[a], 1.38 (*m*)[a]	-
20	C	33.1	28.2	33.2	-	-
21	CH_2	29.8	39.3	29.9	1.48 (*m*)[a], 1.52 (*m*)[a]	-
22	CH_2	27.8	32.8	27.9	1.34 (*m*)[a], 1.39 (*m*)[a]	-
23	CH_3	6.8	6.8	6.8	0.88 (*d*, 6.3)	3, 4, 5
24	CH_3	14.7	14.7	14.7	0.73 (*s*)	4, 5, 6, 10
25	CH_3	17.9	17.9	17.9	0.87 (*s*)	8, 9, 10, 11
26	CH_3	20.8[1]	20.3	18.5	1.03 (*s*)	8, 13, 14, 15
27	CH_3	18.4[1]	18.5	20.8	1.05 (*s*)	12, 13, 14, 18
28	CH_3	32.1	32.1	32.2	1.22 (*s*)	16, 17, 18, 22
29	CH_2	74.8	31.8	74.8	3.24 (*d*, 10.5) 3.29 (*d*, 10.5)	19, 20, 21,30
30	CH_3	25.8	35.0	25.9	1.03 (*s*)	19,20, 21, 29

* For **CD4**

[a] Deduced from HMQC experiment

[1] Values bearing the same notation are interchangeable

3.1.5 Compound CD5

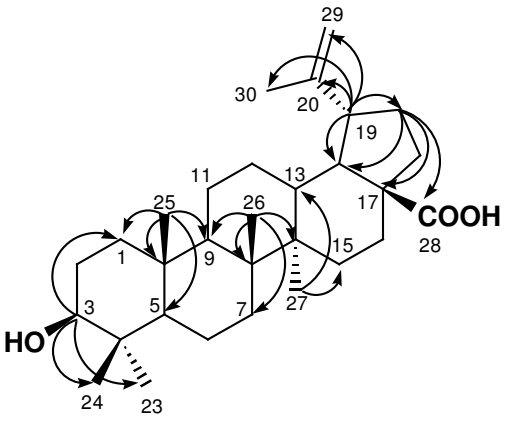


Compound **CD5** was obtained as a white solid, mp. 280-282 ^{o}C, $[\alpha]_{D}^{28}$: +17.7^{o} (c = 0.03, $CHCl_3$). It gave a purple vanillin-sulfuric acid test. The IR spectrum showed absorption band of a hydroxyl group at 3415 cm^{-1} and a carbonyl group at 1686 cm^{-1}.

The ^{13}C NMR spectral data (**Table 6**, **Figure 24**) recorded in $CDCl_3$ +CD_3OD showed 30 signals for 30 carbons. Analysis of DEPT 90^{o} and DEPT 135^{o} spectra of this compound suggested the presence of six methyl (δ 14.5, 15.2, 15.6, 15.9, 19.1 and 27.6), eleven methylene (δ 18.2, 20.8, 25.4, 26.9, 29.6, 30.5, 32.2, 34.2, 37.1, 38.7 and 109.3), six methine (δ 38.2, 46.9, 49.1, 50.5, 55.3 and 78.7) and seven quaternary carbons (δ 37.1, 38.7, 40.6, 42.3, 56.1, 150.7 and 179.1).

The ^{1}H NMR spectral data (**Table 6**, **Figure 23**) showed characteristic of lupane triterpenoids as one vinylic methyl at δ 1.69, two protons of an isopropenyl moiety at δ 4.61 (*br s*) and 4.78 (*br s*) and a typical lupine βH-19 proton at δ 3.01 (*m*). An oxymethine proton was shown at δ 3.19 (*dd*, *J* = 10.8, 5.4 Hz). The doublet of doublet splitting pattern together with a large coupling constant of H-3 with $J_{ax\text{-}ax}$ = 10.8 Hz and $J_{ax\text{-}eq}$ = 5.4 Hz indicated an axial (α) orientation of H-3. The ^{13}C NMR spectrum displayed a signal of a carboxyl carbon at δ 179.1, thus suggesting a carboxylic functionality at C-28. The location of the carboxyl group was confirmed by HMBC experiment (**Table 6**, **Figure 29**) in which the methylene protons 2H-22 (δ 1.41 and 1.93) showed correlations with C-17 (δ 56.1), C-18 (δ 49.1) and C-28 (δ 179.1). Thus on the basis of its spectroscopic data and comparison with those reported

in the literature (Macias *et al*., 1994 and Thongdeeying, 2005) (**Table 6**), compound **CD5** was therefore assigned as betulinic acid.



Selected HMBC correlation of **CD5**

Table 6 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD5** ($CDCl_3$+CD_3OD) and betulinic acid (**R**, pyridine-d_5)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD5**) $^{1}H \rightarrow ^{13}C$
		CD5	**R**	**CD5**	**R**	
1	CH_2	38.7	38.5	0.88 (*m*)[a], 1.65 (*m*)[a]	0.95, 1.70	-
2	CH_2	26.9	28.2	1.57 (*m*)[a], 1.61 (*m*)[a]	1.57, 1.62	-
3	CH	78.7	78.1	3.19 (*dd*, 10.8, 5.4)	3.13 (*dd*, 11.5, 4.9)	1, 23, 24
4	C	38.7	39.4	-		
5	CH	55.3	55.9	0.69 (*m*)[a]	0.71	4, 6, 7, 9
6	CH_2	18.2	18.7	1.36 (*m*)[a], 1.51 (*m*)[a]	1.45, 1.55	-
7	CH_2	34.2	34.7	1.38 (*m*)[a]	1.42	-
8	C	40.6	41.0	-	-	-
9	CH	50.5	50.9	1.26 (*m*)[a]	1.33	-
10	C	37.1	37.5	-	-	-
11	CH_2	20.8	21.1	1.23 (*m*)[a], 1.43 (*m*)[a]	1.25, 1.45	-
12	CH_2	25.4	26.0	1.69 (*m*)[a]	1.07, 1.73	-
13	CH	38.2	39.2	2.22 (*m*)[a]	2.30	-
14	C	42.3	42.8	-	-	-
15	CH_2	29.6	30.2	1.15 (*m*)[a], 1.51 (*m*)[a]	1.18, 1.53	-

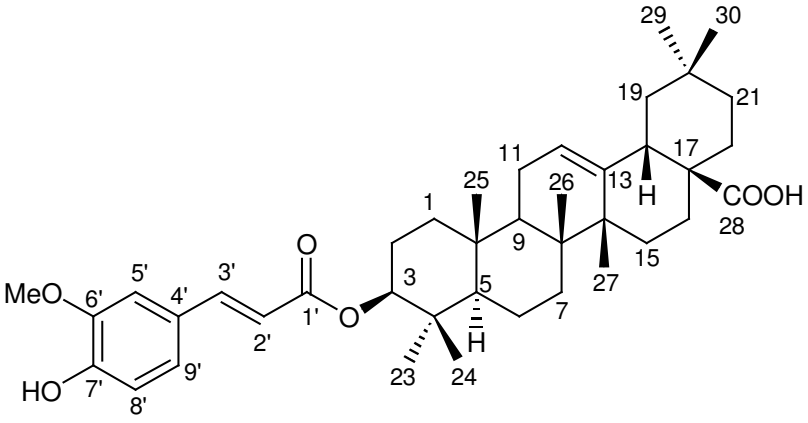
Table 6 (Continued)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD5**) $^1H \rightarrow ^{13}C$
		CD5	**R**	**CD5**	**R**	
16	CH_2	32.2	32.8	1.40 (*m*)[a], 2.25 (*m*)[a]	1.43, 2.23	-
17	C	56.1	56.6	-	-	-
18	CH	49.1	49.7	1.58 (*m*)[a]	1.63	-
19	CH	46.9	47.7	3.01(*m*)	3.02	18, 20, 21, 29, 30
20	C	150.7	151.4	-	-	-
21	CH_2	30.5	31.1	1.42 (*m*)[a], 1.91 (*m*)[a]	1.40, 1.93	-
22	CH_2	37.1	37.4	1.41 (*m*)[a], 1.93 (*m*)[a]	1.43, 1.91	17, 18, 28
23	CH_3	27.6	28.5	0.97 (*s*)	0.95	3, 4, 5, 24
24	CH_3	15.2	16.2	0.75 (*s*)	0.75	3, 4, 5, 23
25	CH_3	15.9	16.3	0.82 (*s*)	0.86	1, 5, 9, 10
26	CH_3	15.6	16.2	0.94 (*s*)	0.97	7, 8, 9, 14
27	CH_3	14.5	14.8	0.98 (*s*)	1.01	8, 13, 14, 15
28	C	179.1	179.0	-	-	-
29	CH_2	109.3	110.0	4.61 (*br s*) 4.74 (*br s*)	4.59 (*dd*, 2.2, 1.0) 4.71 (*d*, 2.2)	19, 30
30	CH_3	19.1	19.4	1.69 (*s*)	1.69 (*d*, 1.0)	19, 20, 29

* For **CD5**

[a] Deduced from HMQC experiment

3.1.6 Compound CD6

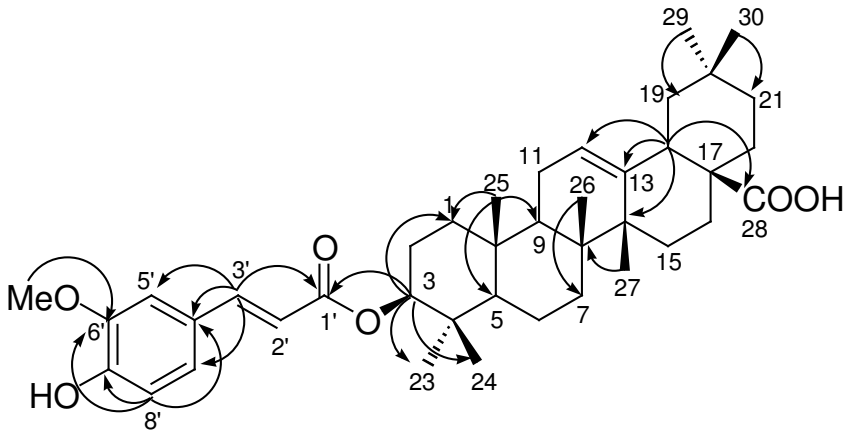


Compound **CD6** was obtained as pale yellow viscous oil, $[\alpha]_D^{28}$: +25.5° (c = 0.23, $CHCl_3$). The IR spectrum (**Figure 31**) suggested hydroxyl (3375 cm^{-1}), conjugated ester (1695 cm^{-1}) and double bond (1602 cm^{-1}) functionalities. The UV spectrum showed absorption bands at λ_{max}: 204, 235, 304 and 325 nm (**Figure 30**), again suggesting the presence of conjugation in the molecule. It gave a purple vanillin-sulfuric acid test.

The ^{13}C NMR spectral data (**Table 7**, **Figure 33**) recorded in $CDCl_3$ showed 40 signals for 40 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of eight methyl (δ 15.4, 16.9, 17.1, 23.6, 25.9, 28.1, 33.0 and 56.0), ten methylene (δ 18.2, 23.0, 23.4, 27.7, 29.7, 32.4, 32.6, 33.8, 38.1 and 45.9), ten methine (δ 41.1, 47.6, 55.4, 80.8, 109.3, 114.7, 116.2, 122.6, 123.0, and 144.4) and twelve quaternary carbons (δ 30.7, 37.0, 38.0, 39.3, 41.7, 46.5, 127.1, 143.6, 146.8, 147.8, 167.1 and 182.8).

The ^{1}H NMR spectrum (**Table 7**, **Figure 32**) displayed seven methyl singlets [δ 0.78, 0.92 (x 2), 0.94 (x 2), 0.97 and 1.15] and a broad singlet at δ 5.29 characteristic of a typical Δ^{12}- oleanan skeleton. This was confirmed in the ^{13}C NMR spectrum with the signals in the region of δ 15.4-55.4, at δ 122.6 and 143.6 attributable respectively to seven methyl groups, to carbons 12 and 13 of the Δ^{12}-oleanan skeleton. The oxymethine proton (H-3) was shown to be shifted more downfield to δ 4.64 (*t*, *J* = 7.8 Hz) as compared to that of oleanolic acid (δ 3.22, *dd*, *J*

= 10.8, 4.5) as a result of the ester substituent at C-3 (Pakhathirathien, 2006). The ^{1}H NMR spectral data exhibited the presence of a trans double bond by two doublet signals at δ 6.29 (H-2′) and 7.59 (H-3′) with a coupling constant of 15.9 Hz, a typical ABX spin system in the aromatic ring at δ 6.91 (H-8′, *d*, *J* = 8.1 Hz), 7.04 (H-5′, *d*, *J* = 1.5 Hz) and 7.07 (H-9′, *dd*, *J* = 8.1, 1.5 Hz) with *ortho*, *meta* and *ortho*/*meta* coupling constant respectively, a singlet of three protons at δ 3.93 corresponding to a methoxyl group. This indicated the presence of a feruloyl or isoferuloyl unit in compound **CD6**. This was supported by ^{13}C NMR spectrum which showed signal at δ 167.1 due to the carbonyl group at an ester function. The positions occupied by the hydroxyl and methoxyl groups on aromatic ring were established through NOESY spectral (**Figure 39**) analysis which showed spatial connectivities of H-5′ at δ 7.04 with methoxyl at δ 3.93. This indicated that H-5′ was in close spatial proximity with respect to methoxyl group. Thus the methoxyl was attached at the C-6′ position and the hydroxyl group at the C-7′ position in the aromatic ring. On the basis of HMBC (**Table 7**), the *E* - feruloyl moiety was located at C-3 by correlation of H-3 signal at δ 4.64 with C-1′ (δ 167.1), C-1 (δ 38.1), C-23 (δ 28.1) and C-24 (δ 16.9). Thus compound **CD6** was identified as 3β-*O*-*E*-feruloyl oleanolic acid by comparison of its spectral data with those reported data (David *et al*., 2004 and Vardamides *et al*., 2003).



Selected HMBC correlation of **CD6**

Table 7 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD6** ($CDCl_3$) and 3β-*O*-*E*-feruloyl oleanolic acid (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD6**) $^{1}H \rightarrow ^{13}C$
		CD6	**R**	**CD6**	**R**	
1	CH_2	38.1	38.1	0.78 (*m*)[a], 1.66 (*m*)[a]	-	-
2	CH_2	27.7	26.1	1.64 (*m*)[a],1.73 (*m*)[a]	-	-
3	CH	80.8	80.6	4.64 (*t*, 7.8)	4.70 (*t*, 8.7)	1, 23, 24, 1′
4	C	37.0	37.4	-	-	-
5	CH	55.4	55.7	0.90 (*m*)[a]	-	-
6	CH_2	18.2	18.3	1.60 (*m*)[a]	-	-
7	CH_2	32.4	32.5	1.57 (*m*)[a], 1.80 (*m*)[a]	-	-
8	C	39.3	39.6	-	-	-
9	CH	47.6	47.8	1.60 (*m*)[a]	-	-
10	C	38.0	37.4	-	-	-
11	CH_2	23.4	24.1	1.68 (*m*)[a], 1.88 (*m*)[a]	-	-
12	CH	122.6	122.4	5.29 (*br s*)	5.40 (*br s*)	9, 11, 14, 18
13	C	143.6	144.3	-	-	-
14	C	41.7	41.8	-	-	-
15	CH_2	29.7	28.0	1.25 (*m*)[a]	-	-
16	CH_2	23.0	24.2	1.60 (*m*)[a], 1.97 (*m*)[a]	-	-
17	C	46.5	41.6	-	-	-
18	CH	41.1	41.4	2.83 (*br d*, 9.9)	2.97 (*dd*, 14.0, 4.0)	12, 14, 16, 17, 19, 28
19	CH_2	45.9	47.5	1.16 (*m*)[a], 1.63 (*m*)[a]	-	-
20	C	30.7	30.8	-	-	-
21	CH_2	33.8	35.5	1.19 (*m*)[a], 1.35 (*m*)[a]	-	-
22	CH_2	32.6	33.2	1.57 (*m*)[a], 1.80 (*m*)[a]	-	-
23	CH_3	28.1	28.4	0.92 (*s*)		3, 5, 24
24	CH_3	16.9	17.1	0.94 (*s*)		3, 5, 23
25	CH_3	15.4	15.6	0.97 (*s*)		1, 5, 9
26	CH_3	17.1	17.5	0.78 (*s*)		8, 9, 14
27	CH_3	25.9	26.1	1.15 (*s*)	0.86-1.31 (*s*)	8, 13, 14
28	C	182.8[b]	180.5	-	-	-
29	CH_3	33.0	33.8	0.92 (*s*)		19, 21, 30
30	CH_3	23.6	23.7	0.94 (*s*)		19, 20, 29

Table 7 (Continued)

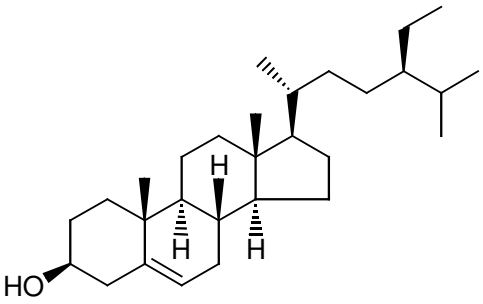
Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD6**) $^1H \rightarrow ^{13}C$
		CD6	**R**	**CD6**	**R**	
1′	C	167.1	168.6	-	-	-
2′	CH	116.2	110.0	6.29 (*d*, 15.9)	6.38 (*d*, 16.0)	1′, 3′, 4′
3′	CH	144.4	144.0	7.59 (*d*, 15.9)	7.65, (*d*, 16.0)	1′, 2′, 4′, 5′, 9′
4′	C	127.1	127.6	-	-	-
5′	CH	109.3	115.4	7.04 (*br s*)	7.10 (*br s*)	3′, 4′, 6′, 7′, 9′
6′	C	147.8	147.9	-	-	-
7′	C	146.8	147.1	-	-	-
8′	CH	114.7	116.2	6.91 (*d*, 8.1)	6.97 (*d*, 8.5)	4′, 6′, 7′
9′	CH	123.0	122.8	7.07 (*dd*, 8.1, 1.5)	7.18 (*dd*, 8.5, 2.1)	3′, 5′, 7′
6′-OMe	CH_3	56.0	56.0	3.93 (*s*)	3.98 (*s*)	6′
7′-OH	-	-	-	5.84 (*br s*)	5.85 (*br s*)	-

* For **CD6**

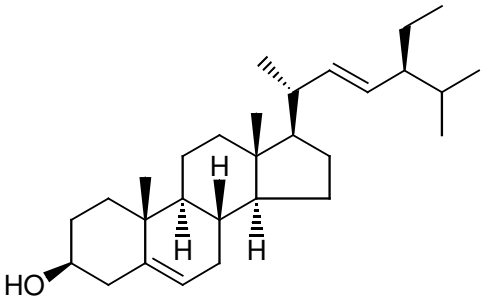
[a] Deduced from HMQC experiment

[b] Deduced from HMBC experiment

3.1.7 Compounds CD7 and CD8



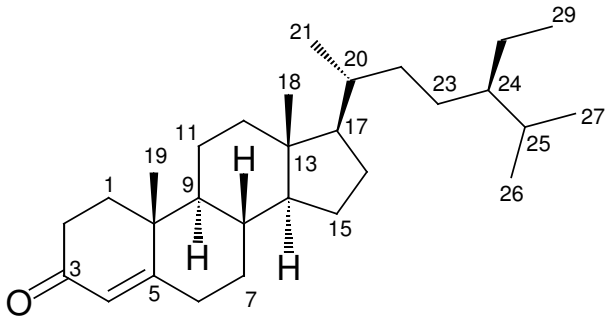
CD7



CD8

The mixture of **CD7** and **CD8** was isolated as a white solid. Its IR spectrum showed absorption bands at 3425 (hydroxyl) and 1642 cm^{-1} (double bond). The ^1H NMR (**Figure 40**) spectral data contained an oxymethine proton at δ 3.57-3.47 (*m*), three olefinic protons at δ 5.36-5.34 (*d*, *J* = 5.1 Hz), 5.16 (*dd*, *J* = 15.1, 8.4 Hz) and 5.01 (*dd*, *J* = 15.1, 8.4 Hz). The ^1H NMR data was corresponded to previous reported data of β-sitosterol and stigmasterol. Thus, this mixture was identified as β-sitosterol (**CD7**) and stigmasterol (**CD8**) (Cheenpracha, 2004).

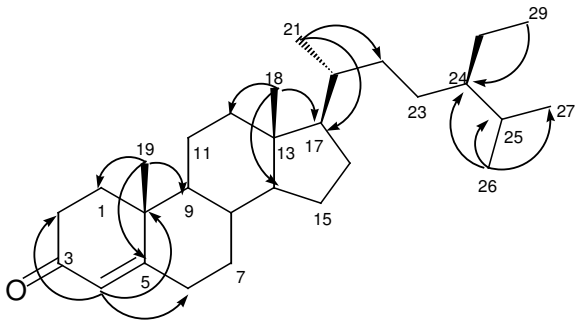
3.1.8 Compound CD9



Compound **CD9** was isolated as a colorless viscous oil; $[\alpha]_D^{28}$: +66.4^o (c = 0.40, $CHCl_3$). Its IR spectrum (**Figure 42**) showed absorption bands for α, β - unsaturated carbonyl group at 1674 cm^{-1} and double bond at 1616 cm^{-1}. The UV absorption was shown at 241 nm.

The ^{13}C NMR spectral data (**Table 8**, **Figure 44**) recorded in $CDCl_3$ showed 29 signals for 29 carbons. Analysis of DEPT 90^o and DEPT 135^o spectra of this compound suggested a presence of six methyl (δ 11.9, 12.0, 17.4, 18.7, 19.0 and 19.8), eleven methylene (δ 21.0, 23.1, 24.2, 26.1, 28.2, 32.1, 32.9, 33.9, 34.0, 35.7 and 39.6), eight methine (δ 29.2, 35.6, 36.1, 45.8, 53.8, 55.9, 56.1 and 123.7) and four quaternary carbons (δ 38.6, 42.4,171.6 and 199.6).

The ^1H NMR spectral data of compound **CD9** (**Figure 43**) and the mixture of **CD7** and **CD8** (**Figure 40**) exhibited the same pattern (**Table 8**), except that compound **CD9** displayed a more downfield vinyl proton at δ 5.72 (H-4). The ^{13}C NMR spectrum confirmed the presence of a carbon - carbon double bond at δ 123.7 (C-4) and 171.6 (C-5) and the downfield chemical shift of C-5 (δ 171.6) also indicated the presence of the conjugated carbonyl function. On the basis of HMBC (**Table 8**, **Figure 49**) the vinyl proton (δ 5.72) showed correlation with C-2 (δ 33.9), C-3 (δ 199.6), C-6 (δ 32.9) and C-10 (δ 38.6) suggesting the presence of a double bond between C-4 and C-5. On the basis of its spectroscopic data and comparison with previously reported data (Della Greca *et al*., 1990), (**Table 8**), compound **CD9** was identified as stigmast-4-en-3-one.



Selected HMBC correlation of **CD9**

Table 8 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD9** ($CDCl_3$) and stigmast-4-en-3-one (**R**, $CDCl_3$)

Posi-tion	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD9**) $^{1}H \rightarrow ^{13}C$
		CD9	**R**	**CD9**	**R**	
1	CH_2	35.7	35.7	1.54 (*m*)[a], 1.67 (*m*)[a]	-	-
2	CH_2	33.9	33.9	2.28 (*m*)[a], 2.50 (*m*)[a]	-	-
3	C	199.6	198.9	-	-	-
4	CH	123.7	123.6	5.72 (*br s*)	5.74 (*d*, 2.2)	2, 3, 6, 10
5	C	171.6	171.0	-	-	-
6	CH_2	32.9	32.9	2.25 (*m*)[a], 2.40 (*m*)[a]	-	-
7	CH_2	32.1	32.1	1.01 (*m*)[a], 1.85 (*m*)[a]	-	-
8	CH	35.6	35.7	1.71 (*m*)[a]	-	-
9	CH	53.8	53.8	0.92 (*m*)[a]	-	-
10	C	38.6	38.6	-	-	-
11	CH_2	21.0	21.0	1.40 (*m*)[a], 1.50 (*m*)[a]	-	-
12	CH_2	39.6	39.5	1.15 (*m*)[a], 2.04 (*m*)[a]	-	-
13	C	42.4	42.4	-	-	-
14	CH	55.9	55.9	1.00 (*m*)[a]	-	-
15	CH_2	24.2	24.1	1.23 (*m*)[a], 1.29 (*m*)[a]	-	-
16	CH_2	28.2	28.1	1.27 (*m*)[a], 1.32 (*m*)[a]	-	-
17	CH	56.1	56.1	1.11 (*m*)[a]	-	-
18	CH_3	12.0	12.0	0.71 (*s*)	0.72 (*s*)	12, 14, 17
19	CH_3	17.4	17.4	1.18 (s)	1.19 (s)	1, 5, 9, 10
20	CH	36.1	36.1	2.01 (*m*)[a]	-	-
21	CH_3	18.7	18.7	0.92 (*d,* 6.3)	0.93 (*d,* 6.6)	17, 20, 22

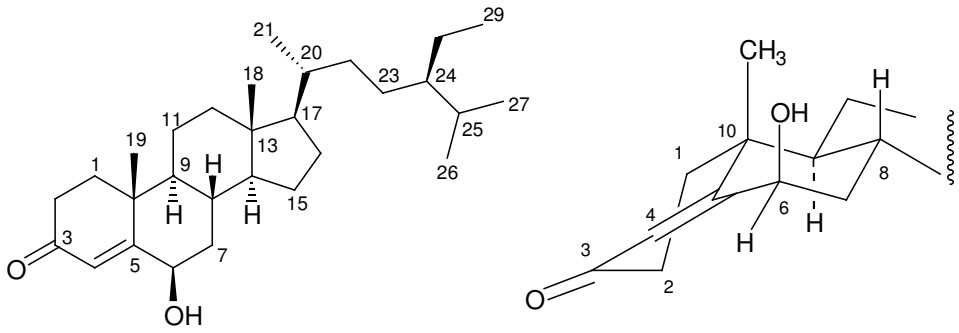
Table 8 (Continued)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD9**) $^1H \rightarrow ^{13}C$
		CD9	**R**	**CD9**	**R**	
22	CH_2	34.0	34.0	2.39 (*m*)[a]	-	-
23	CH_2	26.1	26.0	1.17 (*m*)[a]	-	-
24	CH	45.8	45.8	0.93 (*m*)[a]	-	-
25	CH	29.2	29.1	1.26 (*m*)[a]	-	-
26	CH_3	19.8	19.8	0.85 (*d,* 6.9)	0.84 (*d,* 6.8)	24, 25, 27
27	CH_3	19.0	19.2	0.84 (*d,* 6.6)	0.82 (*d,* 6.8)	24, 25, 26
28	CH_2	23.1	23.1	1.29 (*m*)[a]	-	-
29	CH_3	11.9	11.4	0.83 (*d,* 6.6)	0.85 (*d,* 7.2)	24, 28

* For **CD9**

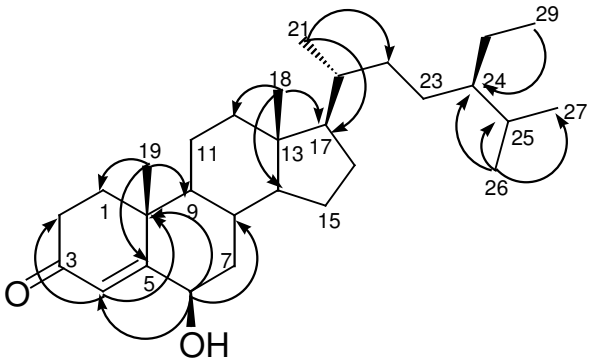
[a] Deduced from HMQC experiment

3.1.9 Compound CD10



Compound **CD10** was isolated as a colorless viscous oil; $[\alpha]_D^{28}$: +10.7° (c = 0.63, $CHCl_3$). The absorption bands for IR and UV spectrum were similar to compound **CD9** with additional IR hydroxyl absorption at 3446 cm^{-1}.

The ^1H NMR and ^{13}C NMR spectral data of compound **CD10** (**Table 9**, **Figures 50** and **51**) and **CD9** (**Table 8**, **Figures 43** and **44**) showed structural similarity, except for additional signal for an oxymethine proton at δ 4.35 (H-6) in **CD10**. The multiplicity of the oxymethine proton signal as a broad triplet (J = 3.0 Hz) indicated its equatorial (α) orientation. The location of the oxymethine proton was assigned to be at C-6 on the basis of HMBC experiment (**Table 9**) of the oxymethine proton at δ 4.35 (H-6) which showed long-range correlations with C-4 (δ 126.3), C-8 (δ 29.7) and C-10 (δ 38.0). Therefore, based on the above evidence and comparison with previously reported data, the structure of **CD10** was, therefore, assigned as 6β-hydroxystigmast-4-en-3-one (Arai *et al*., 1998) (**Table 9**).



Selected HMBC correlation of **CD10**

Table 9 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CD10**, **CD9** ($CDCl_3$) and 6β-hydroxystigmast-4-en-3-one (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD10**) $^{1}H \rightarrow {}^{13}C$
		CD10	**CD9**	**R**	**CD10**	
1	CH_2	37.1	35.7	37.1	1.72 (*m*)[a], 2.26 (*m*)[a]	-
2	CH_2	34.3	33.9	34.3	2.38 (βH *dt,* 16.8, 3.3) 2.52 (αH *td,* 15.0, 5.1)	3, 4, 10
3	C	200.5	199.6	200.5	-	-
4	CH	126.3	123.7	126.3	5.82 (*br s*)	2, 3, 5, 10
5	C	168.6	171.6	168.5	-	-
6	CH	73.3	32.9	73.3	4.35 (*br t*, 3.0)	4, 8, 10
7	CH_2	38.6	32.1	38.6	1.27 (*m*)[a], 2.04 (*m*)[a]	-
8	CH	29.7	35.6	29.8	1.28 (*m*)[a], 2.00 (*m*)[a]	-
9	CH	53.5	53.8	53.6	0.92 (*m*)[a]	-
10	C	38.0	38.6	38.0	-	-
11	CH_2	21.0	21.0	21.0	1.13 (*m*)[a], 1.67 (*m*)[a]	-
12	CH_2	39.6	39.6	39.6	1.14 (*m*)[a], 1.54 (*m*)[a]	-
13	C	42.5	42.4	42.5	-	-
14	CH	56.1	55.9	56.1	1.03 (*m*)[a]	-
15	CH_2	24.2	24.2	24.5	1.65 (*m*)[a], 1.77 (*m*)[a]	-
16	CH_2	28.2	28.2	28.2	1.31 (*m*)[a], 1.85 (*m*)[a]	-
17	CH	55.9	56.1	55.9	1.15 (*m*)[a]	-
18	CH_3	12.0	12.0	12.0	0.74 (*s*)	12, 13, 14, 17

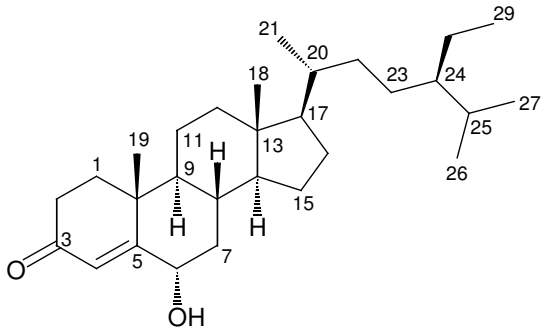
Table 9 (Continued)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD10**) $^1H \rightarrow ^{13}C$
		CD10	**CD9**	**R**	**CD10**	
19	CH_3	19.5	17.4	19.5	1.38 (s)	1, 5, 9
20	CH	36.1	36.1	36.1	1.41 (*m*)[a]	-
21	CH_3	18.7	18.7	18.7	0.92 (*d,* 6.6)	17, 20, 22
22	CH_2	33.9	34.0	33.9	1.18 (*m*)[a]	-
23	CH_2	26.1	26.1	26.0	1.17 (*m*)[a]	-
24	CH	45.9	45.8	45.8	0.91 (*m*)[a]	-
25	CH	29.2	29.2	29.2	1.69 (*m*)[a]	-
26	CH_3	19.8	19.8	19.8	0.81 (*d,* 6.9)	24, 25, 27
27	CH_3	19.1	19.0	19.0	0.84 (*d,* 6.6)	24, 25, 26
28	CH_2	23.1	23.1	23.1	1.26 (*m*)[a]	-
29	CH_3	12.0	11.9	12.0	0.86 (*t,* 6.6)	24, 28

* For **CD10**

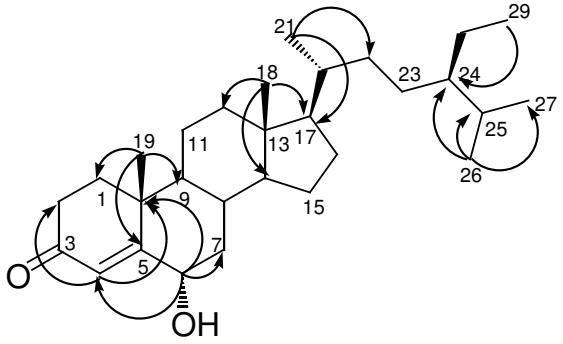
[a] Deduced from HMQC experiment

3.1.10 Compound CD11



Compound **CD11** was obtained as a colorless viscous oil; $[\alpha]_D^{28}$: +16.0° (c = 0.39, $CHCl_3$). The IR and UV spectrum showed absorption bands similar to those of compound **CD10**.

The ^1H and ^{13}C NMR spectral data (**Table 10**, **Figures 52** and **53**) of compound **CD11** were similar to those of compound **CD10** (**Table 9**, **Figures 50** and **51**), except that the splitting pattern of H-6 in **CD11** at δ 4.33 was a doublet of doublet of doublet (J = 17.7, 5.7, 1.2 Hz) instead of a broad triplet in **CD10**. The difference in the multiplicity with a large coupling constant of H-6 in compound **CD11** was in agreement with the respective coupling pattern ($J_{ax\text{-}ax}$ = 17.7, $J_{ax\text{-}eq}$ = 5.7 and $J_{allylic}$ = 1.2 Hz) of H-6 with 2H-7 and H-4, indicating that H-6 was situated in an axial (β) position. The location of a hydroxyl group at C-6 was determined through an HMBC experiment (**Table 10**) in which the oxymethine proton signal at δ 4.33 (H-6) showed long-range correlations with C-3 (δ 198.5), C-5 (δ 170.6), C-7 (δ 40.5) and C-10 (δ 38.0). Thus on the basis of its spectroscopic data and comparison with previously reported data (Della Greca *et al*., 1990) (**Table 10**), compound **CD11** was assigned as 6α-hydroxystigmast-4-en-3-one.



Selected HMBC correlation of **CD11**

Table 10 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CD11**, **CD10** ($CDCl_3$) and 6α-hydroxystigmast-4-en-3-one (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD11**) $^{1}H \rightarrow ^{13}C$
		CD11	**CD10**	**R**	**CD11**	
1	CH_2	35.3	37.1	36.3	1.74 (*m*)[a], 1.79 (*m*)[a]	-
2	CH_2	32.9	34.3	34.1	2.32 (*m*)[a], 2.38 (*m*)[a]	-
3	C	198.5	200.5	202.9	-	-
4	CH	118.7	126.3	119.4	6.17 (*d,* 1.2)	2, 5, 6, 10
5	C	170.6	168.6	157.8	-	-
6	CH	67.7	73.3	68.7	4.33 (*ddd,* 17.7, 5.7, 1.2)	4, 5, 7, 10
7	CH_2	40.5	38.6	39.4	1.08 (*m*)[a], 2.15 (*m*)[a]	-
8	CH	33.2	29.7	33.8	1.63 (*m*)[a]	-
9	CH	52.8	53.5	53.7	0.95 (*m*)[a]	-
10	C	38.0	38.0	39.3	-	-
11	CH_2	20.0	21.0	21.0	1.51 (*m*)[a], 1.55 (*m*)[a]	-
12	CH_2	38.5	39.6	39.4	2.02 (*m*)[a], 2.06 (*m*)[a]	-
13	C	41.5	42.5	41.5	-	-
14	CH	54.7	56.1	55.5	1.12 (*m*)[a]	-
15	CH_2	23.2	24.2	24.4	1.12 (*m*)[a], 1.64 (*m*)[a]	-
16	CH_2	28.7	28.2	28.1	1.28 (*m*)[a], 1.71 (*m*)[a]	-
17	CH	55.0	55.9	55.9	1.16 (*m*)[a]	-
18	CH_3	10.9	12.0	11.9	0.71 (*s*)	12, 13, 14, 17
19	CH_3	17.3	19.5	17.9	1.18 (s)	1, 5, 9, 10

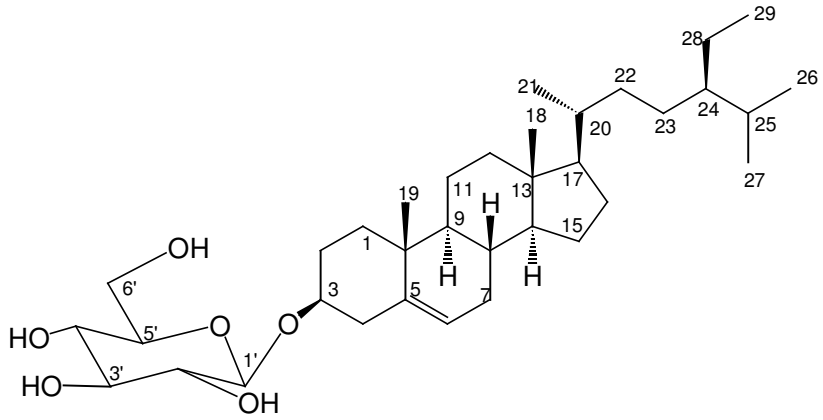
Table 10 (Continued)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, J/Hz)	HMBC (**CD11**) $^1H \rightarrow ^{13}C$
		CD11	**CD10**	**R**	**CD11**	
20	CH	35.1	36.1	36.1	2.05 (*m*)[a]	-
21	CH_3	17.7	18.7	18.7	0.92 (*d,* 6.3)	17, 20, 22
22	CH_2	32.8	33.9	33.9	2.48 (*m*)[a]	-
23	CH_2	27.1	26.1	26.1	0.88 (*m*)[a]	-
24	CH	44.8	45.9	45.8	0.97 (*m*)[a]	-
25	CH	28.2	29.2	29.2	1.62 (*m*)[a]	-
26	CH_3	18.8	19.8	19.7	0.84 (*d,* 6.6)	24, 25, 27
27	CH_3	18.0	19.1	19.0	0.81 (*d,* 6.6)	24, 25, 26
28	CH_2	22.1	23.1	23.1	1.18 (*m*)[a]	-
29	CH_3	11.0	12.0	11.9	0.85 (*t,*6.9)	24, 28

* For **CD11**

[a] Deduced from HMQC experiment

3.1.11 Compound CD12



Compound **CD12** was obtained as a white solid: mp 278-280 ^{o}C, $[\alpha]_{D}^{28}$: -50.0^{o} (*c* = 0.100, MeOH). It gave a purple vanillin-sulfuric acid test. The IR spectrum showed absorption band for hydroxyl (3414 cm^{-1}).

The ^{13}C NMR spectral data (**Table 11**, **Figure 55**) showed the existence of 35 signals for 35 carbon atoms in the molecule. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of six methyl (δ 11.9, 12.0, 18.9, 19.1, 19.4 and 19.9), twelve methylene (δ 21.2, 23.2, 24.4, 26.2, 28.4, 29.8, 32.0, 34.1, 37.4, 38.9, 39.9 and 62.0), fourteen methine (δ 29.3, 32.0, 36.3, 46.0, 50.3, 56.2, 56.9, 70.3, 73.7, 75.9, 76.5, 79.3, 122.3, including one anomeric carbon at δ 101.2) and three quaternary carbons (δ 36.8, 42.4 and 140.4).

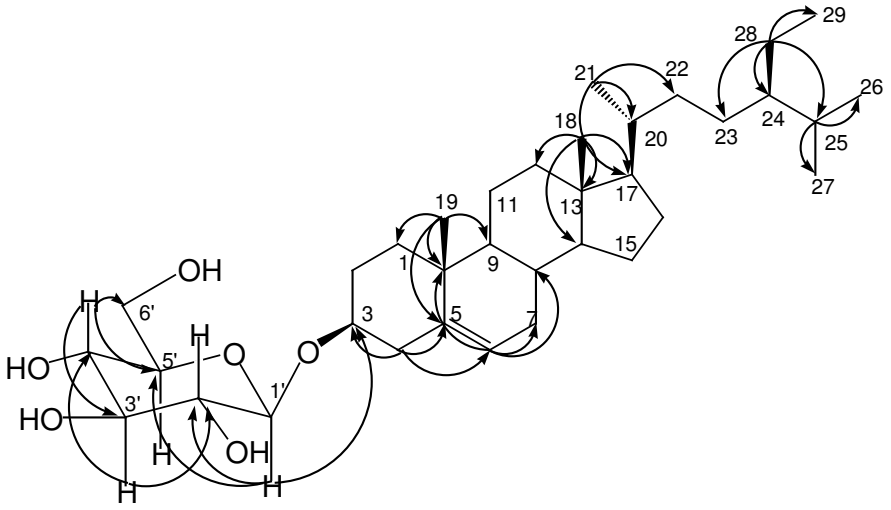
The ^{1}H NMR spectral data (**Table 11**, **Figure 54**) recorded in $CDCl_3$ + CD_3OD displayed a characteristic signal of sitosterol and a sugar unit. The sitosterol unit was shown as two methyl singlet signals at δ 0.69 (3H-18) and 1.01 (3H-19), three methyl doublets at δ 0.93 (*d*, *J* = 6.3 Hz, 3H-21), 0.84 (3H-26) and 0.82 (3H-27) [each *d*, *J* = 6.6 Hz], one methyl triplet at δ 0.85 (*t*, *J* = 6.6 Hz, 3H-29), one olefinic proton at δ 5.37 (*br d*, *J* = 5.1 Hz, H-6) and one oxymethine proton at δ 3.60 (1H, *m*, H-3). The four methine protons in the sugar unit were shown as multiplet signals at δ 3.24 (H-2′), 3.30 (H-5′), 3.41 (H-3′) and 3.44 (H-4′), one anomeric proton at δ 4.41 (*d*, *J* = 7.5 Hz, H-1′) and the oxymethylene protons AB system were shown

at δ3.84 (*dd*, *J* = 12.0, 3.0 Hz) and 3.75 (*dd*, *J* = 12.0, 4.5 Hz) which were assigned to H-6′.

The complete assignment of ^{13}C and ^{1}H NMR (**Table 11**) signals were made with the information from ^{1}H-^{1}H COSY, HMQC and HMBC spectrum (**Table 11**). In the HMBC spectrum the carbon signals at δ73.7 (C-2′), 75.8 (C-5′) and 79.3 (C-3) showed the correlation peaks with the H-1′ (δ 4.41), indicating that the glycosidic linkage was formed between sugar moiety and the steroid at C-3 (δ79.3).

In the NOESY experiment, the anomeric proton at δ 4.41 (H-1′) showed cross peaks with δ3.30 (H-5′), 3.41 (H-3′) and 3.60 (H-3) while the signal of δ3.24 (H-2′) showed cross peak with H-4′ (δ3.44). These observations suggested that H-2′ and H-4′ are opposite to H-3, H-1′, H-3′ and H-5′. Thus this sugar should be β-glucopyranoside attached at C-3.

By comparison of the ^{1}H and ^{13}C NMR spectral data (**Table 11**) with those of atroside (**Figure 6**), (Ali *et al*., 2001 and Thongdeeying, 2005), compound **CD12** was identified as β-sitosterol glucopyranoside.



Selected HMBC correlation of **CD12**

Table 11 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD12** ($CDCl_3$ + CD_3OD) and atroside (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD12**) $^{1}H \rightarrow ^{13}C$
		CD12	**R**	**CD12**	**R**	
1	CH_2	37.4	37.2	1.87 (*m*) [a], 1.08 (*m*) [a]	-	-
2	CH_2	29.8	29.4	1.91 (*m*) [a], 1.63 (*m*) [a]	-	-
3	CH	79.3	79.6	3.60 (*m*)	3.14 (*m*)	1′
4	CH_2	38.8	38.9	2.41 (*m*) [a], 2.27 (*m*) [a]	-	2, 3, 5, 6, 10
5	C	140.4	140.2	-	-	-
6	CH	122.3	122.1	5.37 (*br d*, 5.1)	5.31 (*dist t*)	4, 7, 8, 10
7	CH_2	32.0	31.8	1.68 (*m*) [a], 1.43 (*m*) [a]	-	-
8	CH	32.0	31.9	2.00 (*m*) [a]	-	-
9	CH	50.3	50.1	0.93 (*m*) [a]	-	-
10	C	36.8	36.7	-	-	-
11	CH_2	21.2	21.1	1.54 (*m*) [a], 1.48 (*m*) [a]	-	-
12	CH_2	39.9	39.7	2.03 (*m*) [a], 1.18 (*m*) [a]	-	-
13	C	42.4	42.3	-	-	-
14	CH	56.9	56.7	1.03 (*m*) [a]	-	-
15	CH_2	24.4	24.3	1.56 (*m*) [a]	-	-
16	CH_2	28.4	28.2	1.31 (*m*) [a]	-	-
17	CH	56.2	56.1	1.11 (*m*) [a]	-	-
18	CH_3	11.9	11.9	0.69 (*s*)	0.66 (*s*)	12, 13, 14, 17
19	CH_3	19.4	19.4	1.01 (s)	0.99 (*s*)	1, 5, 9, 10
20	CH	36.3	36.1	1.38 (*m*) [a]	-	-
21	CH_3	18.9	18.8	0.93 (*d*, 6.3)	0.90 (*d*, 6.4)	17, 20, 22
22	CH_2	34.1	33.9	1.31 (*m*) [a], 1.08 (*m*) [a]	-	-
23	CH_2	26.2	26.0	1.16 (*m*) [a]	-	-
24	CH	46.0	48.5	0.93 (*m*) [a]	-	-
25	CH	29.3	29.1	1.26 (*m*) [a]	-	23, 24, 26, 27, 28
26	CH_3	19.9	19.8	0.84 (*d*, 6.6)	0.79 (*d*, 6.5)	24, 25, 27
27	CH_3	19.1	19.0	0.82 (*d*, 6.6)	0.79 (*d*, 6.5)	24, 25, 26
28	CH_2	23.2	23.0	1.25 (*m*) [a]	-	23, 25, 29
29	CH_3	12.0	12.0	0.85 (*t*, 6.6)	0.81 (*t*, 6.5)	24, 28
1′	CH	101.2	101.1	4.41 (*d*, 7.5)	4.31 (*d*, 7.6)	3, 2′,5′
2′	CH	73.7	73.5	3.24 (*m*) [a]	3.61-3.33 (*m*)	4′, 3′

Table 11 (Continued)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD12**) $^1H \rightarrow ^{13}C$
		CD12	**R**	**CD12**	**R**	
3′	CH	76.5	75.9	3.41 (*m*) [a]	3.61-3.33 (*m*)	2′, 4′
4′	CH	70.3	70.1	3.44 (*m*) [a]	3.61-3.33 (*m*)	2′, 3′, 5′, 6′
5′	CH	75.8	73.9	3.30 (*m*) [a]	3.61-3.33 (*m*)	1′, 4′
6′	CH_2	62.0	63.2	3.75 (*dd*, 12.0, 4.5)	4.12 (*br d*, 12.1)	3′, 4′, 5′
				3.84 (*dd*, 12.0, 3.0)	4.42 (*dd*, 12.0, 4.3)	
1″	-	-	174.6	-	-	-
2″	-	-	34.2	-	-	-
3″	-	-	25.0	-	-	-
4″-7″	-	-	29.3	-	-	-
8″	-	-	31.8	-	-	-
9″	-	-	22.7	-	-	-
10″	-	-	14.1	-	0.83 (*s*)	-

* For **CD12**

[a] Deduced from HMQC experiment

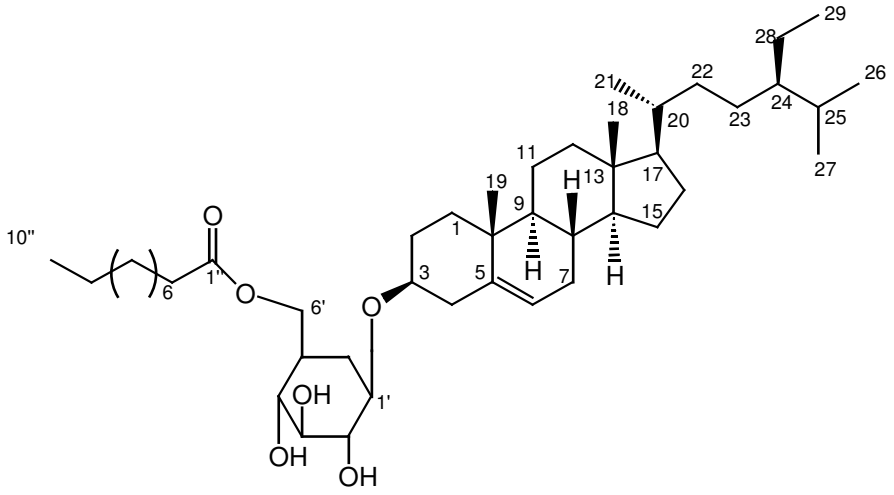
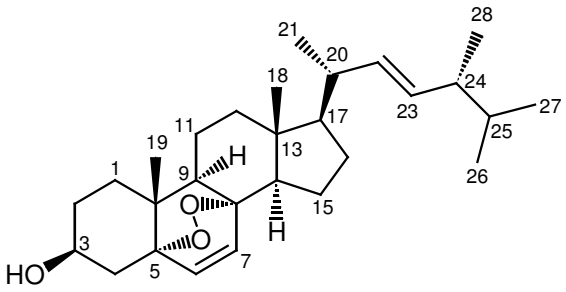


Figure 6 The structure of atroside

3.1.12 Compound CD13

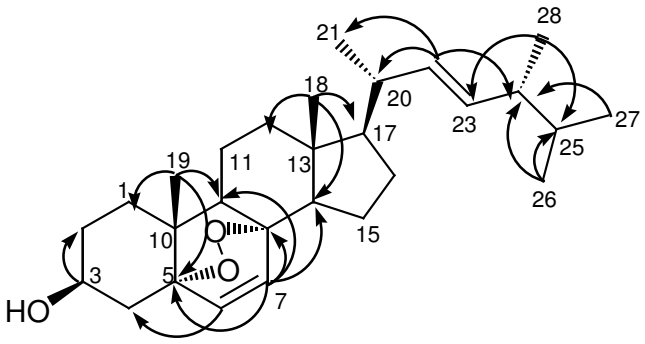


Compound **CD13** was obtained as colorless viscous oil; $[\alpha]_D^{28}$: -12.8° (c = 0.42, $CHCl_3$). The absorption bands for IR spectrum were similar to compound **CD12**. The low resolution EIMS (%) of **CD13** showed major fragment ions at *m/z* 428 $[M]^+$ (3.0), 410 $[M - H_2O]^+$ (5.5), 396 $[M - O_2]^+$ (100), 285 $[M - H_2O - (C_9H_{17})]^+$ (5.5), 253 $[M - O_2 - H_2O\ (C_9H_{17})]^+$ (15.0).

The ^{13}C NMR spectral data (**Table 12**, **Figure 57**) recorded in $CDCl_3$ showed 28 signals for 28 carbons. Analysis of the DEPT 90° and DEPT 135° spectra of this compound suggested the presence of six methyl (δ 12.9, 17.6, 18.2, 19.6, 19.9 and 20.9), seven methylene (δ 20.6, 23.4, 28.6, 30.1, 34.7, 37.0 and 39.4) eleven methine (δ 33.1, 39.7, 42.8, 51.1, 51.7, 56.2, 66.5, 130.8, 132.3, 135.2 and 135.4) and four quaternary carbons (δ 36.9, 44.6, 79.4 and 82.2). Two quaternary carbon signals at δ 82.2 and 79.4 were, respectively, assignable to C-5 and C-8 bearing a 5α, 8α - peroxide bond.

The ^1H NMR spectral data (**Table 12**, **Figure 56**) showed characteristic of ergostane-type sterol as four methyl doublets at δ 0.82 (3H-26, *J* = 6.6 Hz), 0.83 (3H-27, *J* = 6.9 Hz), 0.91 (3H-28, *J* = 6.9 Hz) and 1.01 (3H-21, *J* = 6.6 Hz) and two methyl singlets at δ 0.82 (3H-18) and 0.88 (3H-19). Two parts of olefinic proton signals at δ 6.27 (H-6) and 6.50 (H-7) (each 1H, *d*, *J* = 8.7 Hz) and 5.14 (H-22) and 5.23 (H-23) (each 1H, *dd*, *J* = 15.3, 7.8 Hz) were attributable to Δ^6 and Δ^{22} double bonds, respectively. The oxymethine proton signal at δ 3.97 (H-3, *m*) was assigned to H-3α. The configuration of H-3 was confirmed by NOESY correlations, from which the signal of H-3 (δ 3.97) did not show cross peak with 3H-19 (δ 0.88).

The location of the peroxide bond was confirmed by HMBC experiment (**Table 12**) in which the olefinic proton H-6 (δ 6.27) showed correlations with C-4 (δ 39.4), C-5 (δ 82.2) and C-8 (δ 79.4). The olefinic proton H-7 (δ 6.50) showed long-range correlations with C-5 (δ 82.2), C-8 (δ 79.4), C-9 (δ 51.1) and C-14 (δ 51.7). Thus on the basis of its spectroscopic data and comparison with those reported in the literature (Yue *et al*., 2001 and Rosecke *et al*., 2000) (**Table 12**), compound **CD13** was, therefore, assigned as ergosterol peroxide.



Selected HMBC correlation of **CD13**

Table 12 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD13** ($CDCl_3$) and ergosterol peroxide (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD13**) $^{1}H \rightarrow ^{13}C$
		CD13	**R**	**CD13**	**R**	
1	CH_2	30.1	30.2	1.56 (*m*)[a], 1.85 (*m*)[a]	-	-
2	CH_2	34.7	34.8	1.71 (*m*)[a], 1.98 (*m*)[a]	-	-
3	CH	66.5	66.5	3.97 (*m*)	3.97 (*m*)	2
4	CH_2	39.4	39.4	1.25 (*m*)[a], 1.96 (*m*)[a]	-	-
5	C	82.2	82.2	-	-	-
6	CH	135.4	135.2	6.27 (*d,* 8.7)	6.24 (*d,* 8.7)	4, 5, 8
7	CH	130.8	130.7	6.50 (*d,* 8.7)	6.51(*d,* 8.7)	5, 8, 9, 14
8	C	79.4	79.4	-	-	-
9	CH	51.1	51.3	1.51 (*m*)[a]	-	-
10	C	36.9	37.0	-	-	-

Table 12 (Continued)

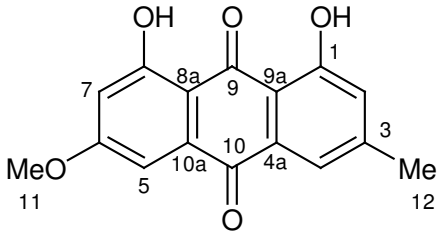
Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD13**) $^1H \rightarrow ^{13}C$
		CD13	**R**	**CD13**	**R**	
11	CH_2	20.6	20.7	1.42 (*m*)[a], 1.61 (*m*)[a]	-	-
12	CH_2	37.0	37.0	1.91 (*m*)[a], 2.13 (*m*)[a]	-	-
13	C	44.6	44.6	-	-	-
14	CH	51.7	51.8	1.61 (*m*)[a]	-	-
15	CH_2	23.4	23.4	1.24 (*m*)[a], 1.53 (*m*)[a]	-	-
16	CH_2	28.6	28.6	1.40 (*m*)[a], 1.76 (*m*)[a]	-	-
17	CH	56.2	56.3	1.24 (*m*)[a]	-	-
18	CH_3	12.9	12.9	0.82 (*s*)	0.82 (*s*)	12, 14, 17
19	CH_3	18.2	18.2	0.88 (s)	0.88 (s)	1, 5, 9
20	CH	39.7	39.6	2.04 (*m*)[a]	-	-
21	CH_3	20.9	20.9	1.01[1] (*d,* 6.6)	0.91 (*d,* 6.6)	17, 20, 22
22	CH	135.2	135.5	5.14[2] (*dd,* 15.3, 7.8)	5.22 (*dd,* 15.3, 8.2)	20, 21, 24
23	CH_2	132.3	132.4	5.23[2] (*dd,* 15.3, 7.8)	5.14 (*dd,* 15.3, 7.6)	20, 24, 28
24	CH	42.8	42.8	1.87 (*m*)[a]	-	-
25	CH	33.1	33.1	1.49 (*m*)[a]	-	-
26	CH_3	19.6	19.6	0.82 (*d,* 6.6)	0.82 (*d,* 12.6)	24, 25, 27
27	CH_3	19.9	19.9	0.83 (*d,* 6.6)	0.83 (*d,* 17.1)	24, 25, 26
28	CH_2	17.6	17.6	0.91[1] (*d,* 6.9)	1.00 (*d,* 6.6)	23, 25

* For **CD13**

[a] Deduced from HMQC experiment

[1, 2] Values bearing the same notation are interchangeable

3.1.13 Compound CD14

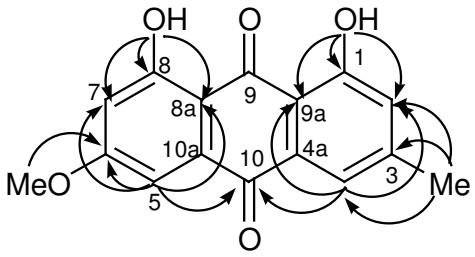


Compound **CD14** was isolated as an orange solid; mp 208-209°C. The IR spectrum (**Figure 59**) showed absorption bands for the hydroxyl group at 3446 cm^{-1} and carbonyl group at 1630 cm^{-1}. The UV spectrum (**Figure 58**) showed absorption bands at λ_{max}: 221, 248, 264, 286 and 434 nm.

The ^{13}C NMR spectral data (**Table 13**, **Figure 61**) recorded in $CDCl_3$ showed 16 signals for 16 carbons. Analysis of the DEPT 90° and DEPT 135° spectra of this compound suggested the presence of two methyl carbons (δ 21.2 and 55.1), four aromatic methine carbons (δ 105.7, 107.2, 120.3 and 123.5) and ten quaternary aromatic carbons (δ 109.0, 112.8, 134.3, 139.0, 147.4, 161.5, 164.2, 165.5, 182.0 and 190.8).

The ^{1}H NMR spectral data (**Table 13**, **Figure 60**) consisted of the singlet signal of two chelated hydroxyl protons at δ 12.26 (8-OH) and 12.06 (1-OH). The signals of all four aromatic protons had small coupling constants, with the protons *ortho* to the phenolic groups appearing at δ 6.62 (H-7, *d*, *J* = 2.7 Hz) and 7.02 (H-2, *br d*, *J* = 1.2 Hz). The protons *para* to the phenolic groups were more deshielded with the H-4 being the most lowfield signal at δ 7.56 (*br d*, *J* = 1.2 Hz), leaving H-5 at δ 7.30 (*d*, *J* = 2.7 Hz). The most highfield signal was methyl proton singlet at δ 2.39 (3-CH_3), with the methoxyl protons (6-OCH_3) further downfield at δ 3.87, due to their association with an oxygen atom. The location of a methyl and a methoxyl group was confirmed by HMBC experiment (**Table 13**, **Figure 66**) in which the methyl protons 3-CH_3 (δ 2.39) showed correlations with C-4 (δ 120.3), C-3 (δ 147.4) and C-2 (δ 123.5) and the methoxyl protons 6-OCH_3 (δ 3.87) showed correlations with C-6 (δ 165.5) suggesting that the methyl and methoxyl groups were linked to C-3 and C-6,

respectively. Thus on the basis of its spectroscopic data and comparison with previously reported data of physcion (Chu *et al*., 2005), (**Table 13**), compound **CD14** was assigned as physcion.



Selected HMBC correlation of **CD14**

Table 13 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD14** ($CDCl_3$) and physcion (**R**, DMSO+$CDCl_3$)

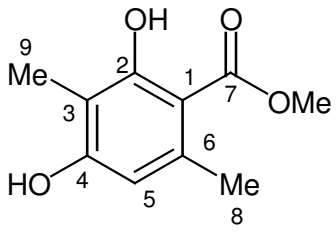
Posi-tion	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD14**) $^{1}H \rightarrow {}^{13}C$
		CD14	**R**	**CD14**	**R**	
1	C	161.5	164.7	-	-	-
2	CH	123.5	120.7	7.02 (*br d*, 1.2)	7.09 (*s*)	1, 4, 9a, 3-Me
3	C	147.4	148.0	-	-	-
4	CH	120.3	124.0	7.56 (*br d*, 1.2)	7.64 (*s*)	2, 10, 9a, 3-Me
5	CH	107.2	107.8	7.30 (*d,* 2.7)	7.32 (*s*)	6, 7, 10, 8a
6	C	165.5	161.9	-	-	-
7	CH	105.7	106.1	6.62 (*d*, 2.7)	6.74 (*s*)	5, 8, 8a
8	C	164.2	166.1	-	-	-
9	C	190.8	190.9	-	-	-
10	C	182.0	181.6	-	-	-
4a	C	139.0	132.7	-	-	-
8a	C	109.0	110.2	-	-	-
9a	C	112.8	113.1	-	-	-
10a	C	134.3	134.7	-	-	-
6-OMe	CH_3	55.1	55.7	3.87 (*s*)	3.92 (*s*)	6
3-Me	CH_3	21.2	21.7	2.39 (*s*)	2.42 (*s*)	2, 3, 4

Table 13 (Continued)

Posi-tion	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD14**) $^1H \rightarrow ^{13}C$
		CD14	**R**	**CD14**	**R**	
8-OH	-	-	-	12.26 (*s*)	12.19 (*s*)	7, 8, 8a
1-OH	-	-	-	12.06 (*s*)	12.06 (*s*)	1, 2, 9a

* For **CD14**

3.1.14 Compound CD15



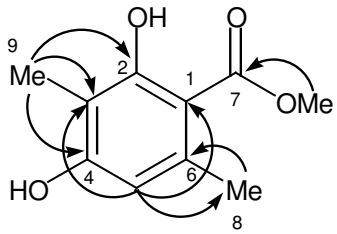
Compound **CD15** was isolated as pale yellow viscous oil. The IR spectrum (**Figure 68**) suggested hydroxyl (3393 cm^{-1}) and carbonyl (1625 cm^{-1}) functionalities. The UV spectrum (**Figure 67**) showed absorption bands at λ_{max}: 267 and 302 nm.

The ^{13}C NMR spectral data (**Table 14**, **Figure 70**) recorded in $CDCl_3$ showed 10 signals for 10 carbons. Analysis of the DEPT 90° and DEPT 135° spectra of this compound suggested the presence of three methyl carbons (δ 6.6, 23.8 and 50.8), one aromatic methine carbon (δ 109.5) and six quaternary aromatic carbons (δ 104.2, 107.5, 139.1, 154.1, 162.2 and 171.6).

The ^1H NMR spectral data (**Table 14**, **Figure 69**) demonstrated the resonances of a chelated hydroxyl group at δ 11.80 (2-OH, *s*) and an aromatic proton at δ 6.14 (H-5, *s*). The ^1H NMR spectrum also gave a singlet signal for a methoxyl group at δ 3.85 and two methyl groups at δ 2.03 (3H-9) and 2.39 (3H-8). On the basis of HMBC (**Table 14**, **Figure 75**), the methyl groups were located at C-3 and C-6 by

correlation of 3H-9 signal (δ 2.03) with C-2 (δ 162.2), C-3 (δ 107.5) and C-4 (δ 154.1) and 3H-8 (δ 2.39) with C-1 (δ 104.2), C-5 (δ 109.5) and C-6 (δ 139.1).

By comparison of the ^{1}H and ^{13}C NMR spectral data with previously reported data (Ahad *et al*., 1991). (**Table 14**), compound **CD15** was identified as methyl β-orcinolcarboxylate.



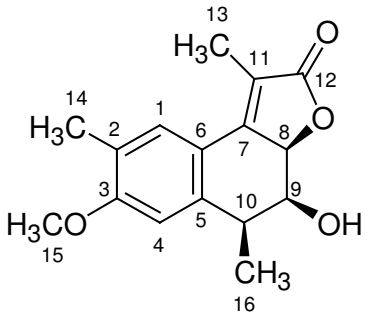
Selected HMBC correlation of **CD15**

Table 14 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD15** ($CDCl_3$) and methyl β-orcinolcarboxylate (**R**, $CDCl_3$)

Posi-tion	Type of C*	δ_C /ppm	δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD15**) $^{1}H \rightarrow ^{13}C$
		CD15	**CD15**	**R**	
1	C	104.2	-	-	-
2	C	162.2	-	-	-
3	C	107.5	-	-	-
4	C	154.1	-	-	-
5	CH	109.5	6.14 (*s*)	6.18 (*s*)	1, 3, 8
6	C	139.1	-	-	-
7	C	171.6	-	-	-
8	CH_3	23.8	2.39 (*s*)	2.42 (*s*)	1, 5, 6
9	CH_3	6.6	2.03 (*s*)	2.10(*s*)	2, 3, 4
7-OCH_3	CH_3	50.8	3.85 (*s*)	3.90 (*s*)	7
2-OH	-	-	11.80 (*s*)	12.02 (*s*)	1, 2, 3

* For **CD15**

3.1.15 Compound CD16



Compound **CD16** was isolated as pale yellow viscous oil; $[\alpha]_D^{28}$: -142.7° (c = 0.72, $CHCl_3$). The IR spectrum (**Figure 77**) showed absorption band of hydroxyl group at 3432 cm^{-1} and carbonyl (lactone) at 1744 cm^{-1}. The UV spectrum (**Figure 76**) showed absorption bands at λ_{max} = 233 nm again suggesting the presence of enone group of an α, β-unsaturated γ-lactone and λ_{max} : 217, 297 and 308 nm suggesting an aromatic ring in the molecule.

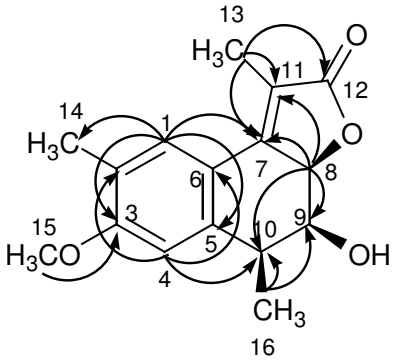
The ^{13}C NMR spectral data (**Table 15**, **Figure 79**) recorded in $CDCl_3$ showed 16 signals for 16 carbons. Analysis of the DEPT 90° and DEPT 135° spectra of this compound suggested the presence of four methyl (δ 8.9, 15.0, 16.4 and 54.4), five methine (δ 38.5, 76.4, 82.3, 107.8 and 128.2) and seven quaternary carbons (δ 116.4, 119.0, 125.1, 140.2, 153.0, 158.9 and 174.4).

The ^1H NMR spectral data (**Table 15**, **Figure 78**) suggested the presence of two aromatic protons at δ 6.79 (H-4, *br s*) and 7.31 (H-1, *br s*). The broad singlet signal of H-4 and H-1 indicated that the two aromatic protons were *para* with no vicinal protons. A further study of the ^1H NMR spectrum showed evidence of three nonequivalent methyl resonances. The first one displayed a doublet at δ 1.47 (J = 6.9 Hz) which was assigned as 3H-16. The second one was also a doublet with a small coupling constant (J = 1.5 Hz) at δ 2.06 which was assigned as 3H-13 coupling with H-8 whose signal appeared at δ 4.75 (*dq, J* = 9.9, 1.5). The third one showed a singlet at δ 2.18 indicating attachment to quaternary carbon (C-2) which could be assigned as 3H-14. The ^1H NMR also gave signals for three methines at δ 2.94 (H-10, *m*), a

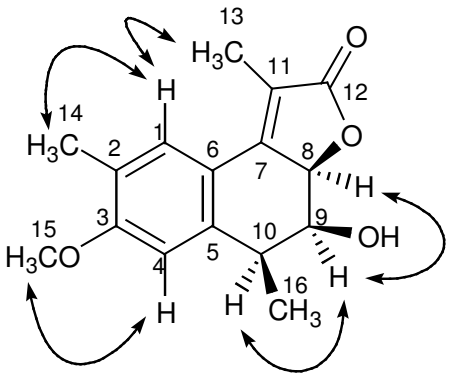
proton on carbon bearing oxygen at δ 4.75 (H-8, *dq*, *J* = 9.9 and 1.5 Hz), and a proton at δ 3.41 (H-9, *t*, *J* = 9.9 Hz) and a methoxyl group at δ 3.83 (3H-15, *s*). On the basis of HMBC (**Table 15**, **Figure 84**), the methoxyl group was located at C-3 by correlation of 3H-15 signal (δ 3.83) with C-3 (δ 158.9).

The relative stereochemistry of **CD16** was supported by NOESY (**Figure 85**) correlations. Proton H-8 (δ 4.75) showed cross peak with H-9 (δ 3.41) and H-10 (δ 2.94). These observations suggested that H-8, H-9 and H-10 were on the same side.

Thus compound **CD16** was established as vallapin by comparison of its spectroscopic data with those reported in the literature (Miles *et al*., 1991), (**Table 15**).



Selected HMBC correlation of **CD16**



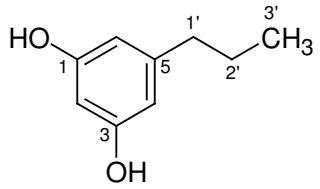
NOESY correlation of some protons of **CD16**

Table 15 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD16** ($CDCl_3$) and vallapin (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm	δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD16**) $^{1}H \rightarrow ^{13}C$
		CD16	**CD16**	**R**	
1	CH	128.2	7.31 (*br s*)	7.48 (*s*)	3, 5, 7, 14
2	C	119.0	-	-	-
3	C	158.9	-	-	-
4	CH	107.8	6.79 (*br s*)	6.74 (*s*)	2, 6, 10
5	C	140.2	-	-	-
6	C	125.1	-	-	-
7	C	153.0	-	-	-
8	CH	82.3	4.75 (*dq*, 9.9, 1.5)	5.22 (*s*)	7, 9, 10, 11
9	CH	76.4	3.41 (*t,* 9.9)	4.42 (*s*)	7, 16
10	CH	38.5	2.94 (*m*)	3.06 (*m*)	5, 9, 16
11	C	116.4	-	-	-
12	C	174.4	-	-	-
13	CH_3	8.9	2.06 (*d,* 1.5)	2.20 (*s*)	7, 11, 12
14	CH_3	15.0	2.18 (*s*)	2.31 (*s*)	1, 2, 3
15	CH_3	54.4	3.83 (*s*)	3.95 (*s*)	3
16	CH_3	16.4	1.47 (*d,* 6.9)	1.45 (*d,* 10.0)	5, 9, 10

* For **CD16**

3.1.16 Compound CD17

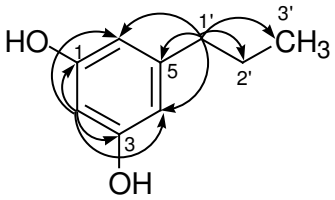


Compound **CD17** was obtained as pale yellow viscous oil. The IR spectrum (**Figure 87**) exhibited absorption bands at 3335 cm^{-1} (hydroxyl group) and 1600 cm^{-1} (aromatic ring). The UV spectrum (**Figure 86**) showed absorption maxima at 206, 227 and 278 nm.

The ^{13}C NMR spectral data (**Table 16**, **Figure 89**) recorded in $CDCl_3$ showed 7 signals for 9 carbons. Analysis of the DEPT 90° and DEPT 135° spectra of this compound suggested the presence of a methyl (δ 13.8), two methylene (δ 24.1 and 37.9), three methine (δ 100.2 and 2 X 108.1) and three quaternary carbons (δ 145.2 and 2 X 156.6).

The ^{1}H NMR spectral data (**Table 16**, **Figure 88**) displayed three aromatic doublet signals with coupling constant 2.1 Hz at δ 6.18 (H-2) and 6.24 (H-4 and H-6). The characteristic signals of a propyl unit were displayed at δ 2.44 (2H-1′, *t*, *J* = 7.5 Hz), 1.57 (2H-2′, *sextet*, *J* = 7.5 Hz) and 0.91 (3H-3′, *t*, *J* = 7.5 Hz). The propyl unit was located at C-5 by HMBC correlation of 2H-1′ (δ 2.44) with C-4 (δ 108.1), C-5 (δ 145.2), C-6 (δ 108.1), C- 2′ (δ 24.1) and C-3′ (δ 13.8).

By comparison of the ^{1}H NMR spectral data (**Table 16**) with those of 3-methoxy-5-propylphenol (**Figure 7**), (Elix *et al*., 1997), compound **CD17** was identified as 5-propylresorcinol.



Selected HMBC correlation of **CD17**

Table 16 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD17** ($CDCl_3$) and 3-methoxy-5-propylphenol (**R**, $CDCl_3$)

Posi-tion	Type of C*	δ_C /ppm	δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD17**) $^{1}H \rightarrow ^{13}C$
		CD17	**CD17**	**R**	
1	C	156.6	-	-	-
2	CH	100.2	6.18 (*d,* 2.1)	6.24 (*d,* 2.1)	1, 3, 4, 6
3	C	156.6	-	-	-
4	CH	108.1	6.24 (*d,* 2.1)	6.26 (*br s*)	2, 3, 6, 1′
5	C	145.2	-	-	-
6	CH	108.1	6.24 (*d,* 2.1)	6.33 (*br s*)	1, 2, 4, 1′
1′	CH_2	37.9	2.44 (*t,* 7.5)	2.50 (*t,* 7.6)	4, 5, 6, 2′, 3′
2′	CH_2	24.1	1.57 (*sextet,* 7.5)	1.61 (*sextet,* 7.5)	5, 1′, 3′
3′	CH_3	13.8	0.91(*t,* 7.5)	0.93 (*t,* 7.4)	1′, 2′
3-OMe	-	-	-	3.77 (*s*)	-
OH	-	-	-	4.74 (*s*)	-

* For **CD17**

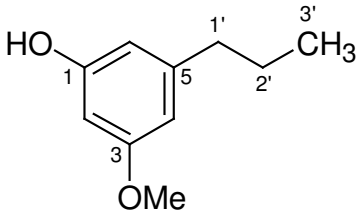
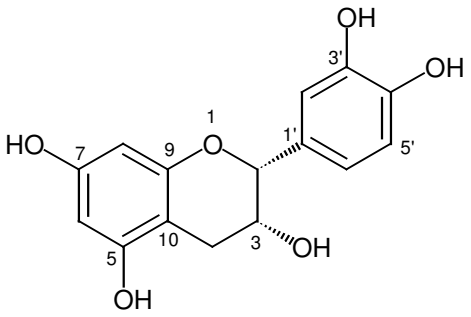


Figure 7 The structure of 3-methoxy-5-propylphenol

3.1.17 Compound CD18



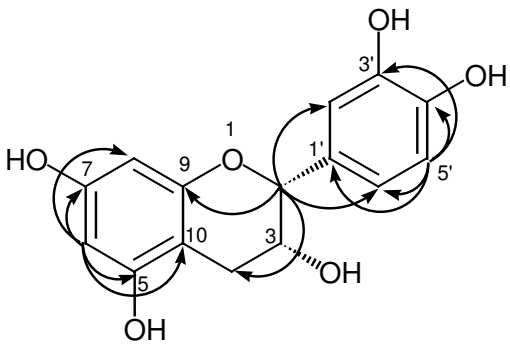
Compound **CD18** was isolated as pale yellow solid; mp 240-243 ^{o}C; $[\alpha]_{D}^{28}$: - 25.1^{o} (c = 0.23, MeOH). The IR spectrum (**Figure 96**) showed absorption bands for hydroxyl (3453 cm^{-1}). The UV spectrum (**Figure 95**) showed absorption maxima at 208, 226 and 280 nm.

The ^{13}C NMR spectral data (**Table 17**, **Figure 98**) recorded in acetone-d_6 showed 15 signals for 15 carbons. Analysis of the DEPT 90^{o} and DEPT 135^{o} spectra of this compound suggested the presence of five aromatic methine carbons (δ 95.7, 96.2, 115.2, 115.6 and 119.4), two oxymethine carbons (δ 66.9 and 79.4), a methylene carbon (δ 28.9) and seven quaternary aromatic carbons [δ 99.8, 132.2, 145.2, 145.4, 157.1 and 157.5 (x 2)].

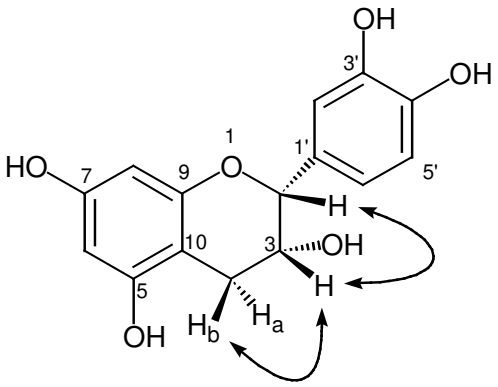
The ^{1}H NMR spectral data (**Table 17**, **Figure 97**) suggested the presence of five aromatic protons (δ 5.93, 6.03, 6.79, 6.84 and 7.06), two methine protons (δ 4.22 and 4.89), two methylene protons (δ 2.75 and 2.88) and five hydroxyl groups at δ 3.68, 7.82, 7.86, 8.04 and 8.18 (each 1H, *s*, -OH, disappeared on D_2O exchange). Two doublet resonances at δ 5.93 and 6.03 with the coupling constant of 2.4 Hz corresponded to the resonances of *mata* protons H-8 and H-6, respectively. A doublet at δ 6.79 (J = 8.4 Hz), a doublet of doublet at δ 6.84 (J = 8.4 and 1.8 Hz) and a doublet at δ 7.06 (J = 1.8 Hz) were assigned for the resonances of H-5′, H-6′ and H-2′, respectively. The spectra further showed the resonances of H-2 (δ 4.89, *br s*), H-3 (δ 4.22, *br d*, J = 4.8 Hz) and 2H-4 (δ 2.75, *dd*, J = 16.5, 2.7 Hz and 2.88, *dd*, J = 16.5, 5.1 Hz). The downfield chemical shift of H-2 and H-3 indicated that these two protons were next to oxygen-bearing carbons. In addition, the broad singlet of H-2

and broad doublet of H-3 (J = 4.8 Hz) suggested that the stereochemistry of H-2 and H-3 were *cis*. From NOESY experiments (**Figure 104**), the methine proton at δ 4.89 (H-2) showed cross peak with δ 4.22 (H-3), 6.84 (H-6′) and 7.06 (H-2′), indicating that H-2 and H-3 were *cis*.

On the basis of HMBC (**Table 17**, **Figure 103**), the aromatic ring moiety was located at C-2 by correlation of H-2 signal (δ 4.89) with C-4 (δ 28.9), C-9 (δ 157.6), C-2′ (δ 115.2) and C-6′ (δ 119.4). Consequently, compound **CD18** was proposed to be (-) epicatechin of which the (-) isomer was indicated from optical rotation ($[\alpha]_D^{28}$: -25.1°). The assignment was in agreement with the previous data of (-) epicatechin (Deachathai, 2005).



Selected HMBC correlation of **CD18**



NOESY correlation of some protons of **CD18**

Table 17 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CD18** (acetone-d_6) and (-) epicatechin (**R**, $CDCl_3$+DMSO-d_6)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, J/Hz)		HMBC (**CD18**) $^{1}H \rightarrow ^{13}C$
		CD18	**R**	**CD18**	**R**	
2	CH	79.4	78.3	4.89 (*br s*)	4.78 (s)	4, 9, 2′, 6′
3	CH	66.9	65.6	4.22 (*br d,* 4.8)	4.13 (br s)	2, 4, 10, 1′
4	CH_2	28.9	28.1	H_a: 2.75 (*dd,* 16.5, 2.7) H_b: 2.88 (*dd,* 16.5, 5.1)	2.66 (*dd*, 16.8, 4.5) 2.79 (*dd*, 16.8, 2.5)	2, 3, 6, 10
5	C	157.1	156.6	-	-	-
6	CH	96.2	95.7	6.03 (*d,* 2.4)	5.99 (*d*, 1.8)	5, 7, 8, 10
7	C	157.5	156.3	-	-	-
8	CH	95.7	94.7	5.93 (*d,* 2.4)	5.87 (*d*, 1.8)	6, 7. 9, 10
9	C	157.5	155.7	-	-	-
10	C	99.8	98.7	-	-	-
1′	C	132.2	130.6	-	-	-
2′	CH	115.2	114.5	7.06 (*d,* 1.8)	6.99 (*d*, 1.8)	2, 3′, 6′
3′	C	145.2	144.5	-	-	-
4′	C	145.4	144.5	-	-	-
5′	CH	115.6	115.1	6.79 (*d,* 8.4)	6.77 (*d*, 7.2)	1′, 3′, 4′, 6′
6′	CH	119.4	118.1	6.84 (*dd,* 8.4, 1.8)	(*dd*, 7.2, 1.8)	2, 2′, 6′
3-OH	-	-	-	3.68 (*d,* 5.1)	4.26 (*br s*)	-
5-OH	-	-	-	8.18 (*s*)	8.99 (*br s*)	5, 6, 10
7-OH	-	-	-	8.04 (*s*)	8.84 (*br s*)	6, 7, 8
3′-OH	-	-	-	7.86 (*s*)	8.64 (*br s*)	2′, 3′, 4′
4′-OH	-	-	-	7.82 (*s*)	8.50 (*br s*)	3′, 4′, 5′

* For **CD18**

3.2 Biological activities of the pure compounds from *H. littoralis*

The crude hexane, dicloromethane and acetone extracts from the bark of *H. littoralis* were tested for various biological activities: anti-allergic, antimicrobial (*Staphylococcus aureus* and methicillin–resistant *Staphylococcus aureus*), anticancer (MCF-7, Hela, HT-29 and KB cell lines), anti-oxidant and antiprotozoa activities (*Entamoeba histolytica* and *Giardia intestinalis*). The results indicated that the crude extracts showed no activity for all tests, except the anti-allergic activity which exhibited strong activity. Therefore the pure compounds from *H. littoralis* were tested only for anti-allergic activity (Inhibitory effect of pure compounds from *H. littoralis* on the release of β-hexosaminidase from RBL-2H3 cells).

Hypersensitivity type I or allergy is caused by certain types of antigens such as dust, mites, medicines, foods, pollens, spores, and cosmetics. This class of antigens induces the production of antigen-specific IgE antibodies that bind to receptors on mast cells or basophiles; and finally cause mast cells or basophiles degranulate and secrete mediators that induce vasodilation, mucous secretion and bronchoconstriction. β-hexosaminidase is the enzyme that stores in the secretory granules of mast cells and basophiles, and releases along with histamine when mast cells and basophiles are activated. Thus, this enzyme is used as the marker of mast cell or basophile degranulation (Cheong *et al*., 1998).

Table 18 Anti-allergic activity of the crude extracts from the bark of *H. littoralis* [a]

Compound	% Inhibition at various concentration (μg/mL)	
	0	100
CDH	0.0 ± 5.2	96.1 ± 3.0**
CDM	0.0 ± 5.2	98.5 ± 3.8**
CDA	0.0 ± 5.2	101.3 ± 2.0**

[a]Each value represents mean ± S.E.M. of four determinations.

Statistical significance, * $p<0.05$, ** $p<0.01$

CDH = crude hexane extract

CDM = crude dichloromethane extract

CDA = crude acetone extract

Table 19 Anti-allergic activity of compounds from the bark of *H. littoralis* [a]

Compound	% Inhibition at various concentration (μM)					IC_{50} (μM)	Enzyme inhibition at 100 μ M
	0	3	10	30	100		
CD1	0.0 ± 4.7	-	5.0 ± 1.5	21.4 ± 2.2	57.1 ± 2.8**	86.1	14.3
CD2	0.0 ± 5.2	-	4.8 ± 1.1	49.4 ± 3.7**	70.3 ± 5.0**	41.9	15.6
CD3	0.0 ± 4.9	-	-6.4 ± 3.4	44.1 ± 1.3**	93.3 ± 3.7**	35.9	15.8
CD4	0.0 ± 4.1	-	-	-	16.7 ± 2.8	>100	-
CD5	0.0 ± 4.1	-	-	-	49.1 ± 6.4**	>100	-
CD6	0.0 ± 4.7	-	-	-	17.1 ± 4.5	>100	-
CD9	0.0 ± 6.5	-	1.7 ± 2.4	15.7 ± 2.3	55.5 ± 4.1**	90.2	16.2
CD10	0.0 ± 4.8	-	12.8 ± 4.4	58.2 ± 2.6**	93.6 ± 1.7**	27.0	12.4
CD11	0.0 ± 4.9	-	-5.7 ± 4.7	46.3 ± 2.4**	96.5 ± 3.3**	34.2	16.1
CD12	0.0 ± 6.6	-	2.5 ± 2.1	18.2 ± 3.1	55.3 ± 4.7**	89.8	13.7
CD13	0.0 ± 5.5	-	5.1 ± 2.7	16.2 ± 2.5	53.5 ± 3.1**	93.4	15.5
CD14	0.0 ± 3.3	-	13.8 ± 5.9	49.4 ± 3.5**	62.7 ± 2.5**	45.4	15.8
CD15	0.0 ± 4.7	-	-0.3 ± 3.5	39.5 ± 2.4**	99.1 ± 1.8**	34.0	15.1
CD16	0.0 ± 1.4	30.4 ± 4.1**	68.9 ± 2.6**	87.4 ± 1.8**	98.1 ± 1.4**	5.7	12.2
CD17	0.0 ± 5.3	-	-	11.2 ± 2.6	35.5 ± 1.8**	>100	-
CD18	0.0 ± 5.2	-	16.0 ± 6.0	21.3 ± 3.6*	86.3 ± 3.7**	57.4	15.4
Ketotifen fumarate	0.0 ± 4.6	-	12.8 ± 0.5	38.3 ± 3.2**	68.2 ± 1.5**	47.5	15.8

[a]Each value represents mean ± S.E.M. of four determinations.

Statistical significance, * $p<0.05$, ** $p<0.01$

As shown in **Table 19**, **CD16** exhibited the most potent activity with an IC_{50} value of 5.7 μM, followed by **CD10** (IC_{50} = 27.0 μM), **CD15** (IC_{50} = 34.0 μ M), **CD11** (IC_{50} = 34.2 μM), **CD3** (IC_{50} = 35.9 μM), **CD2** (IC_{50} = 41.9 μM) and **CD14** (IC_{50} = 45.4 μM). All these compunds possessed stronger anti-allergic activity than ketotifen fumarate, a positive control (IC_{50} = 47.5 μM). This is the first report of their anti-allergic activity. **CD16** exhibited strong activity, so it has potential to be developed as an anti-allergic drug. Whereas, compounds **CD1**, **CD4-6**, **CD9**, **CD12**, **CD13**, **CD17** and **CD18** showed weak activity. The active compounds **CD2-3**, **CD10-11** and **CD14-16** were also examined on the enzyme activity of β-hexosaminidase

whose results indicated that these compounds inhibited the antigen-induced degranulation but not enzyme activity of β-hexosaminidase.