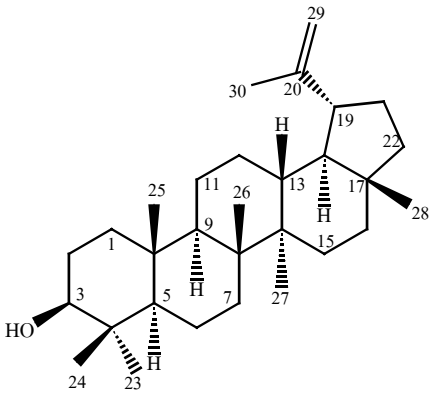
3. RESULT AND DISCUSSION

3.1 Structure elucidation of compounds from the leaves of C. decandra

The air-dried ground leaves of *C. decandra* (3.9 kg) were extracted with hexane, methylene chloride and acetone, successively. The white-green solid and the crude hexane extract were subjected to chromatography and/or crystallization to give nineteen triterpenoids: **PTH1**-**PTH19** and a mixture of two steroids: **PTH20** and **PTH21**. Three triterpenoids are new compounds: **PTH13**, **PTH14** and **PTH15**. The crude methylene chloride extract was purified by chromatography and/or crystallization to yield one triterpenoid (**PTM1**), two norsesquiterpenoids (**PTM2** and **PTM3**), one lignan (**PTM4**) and one steroid glucoside (**PTM5**).

Their structures were elucidated by 1D and 2D NMR spectroscopic data. All carbons were assigned by ^{13}C NMR, DEPT 135°, DEPT 90°, HMQC and HMBC data. The structure of **PTH9** was additionally confirmed by X-ray diffraction.

3.1.1 Compound PTH1

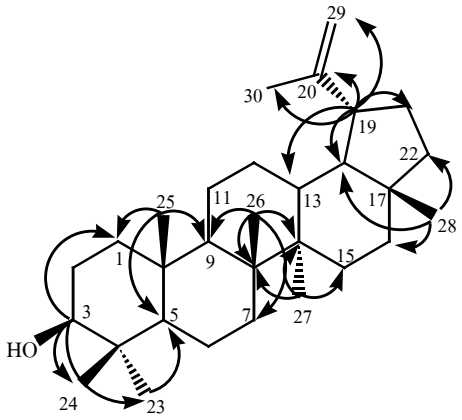


Compound **PTH1** was obtained as a white solid, mp 193-194 °C, $[\alpha]_D^{28}$: +25.0° (*c* = 0.200, $CHCl_3$). The IR spectrum (**Figure 4**) showed absorption bands for hydroxyl group at 3343 cm^{-1} and double bond at 1638 cm^{-1}. It gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^{13}C NMR spectral data (**Table 2**, **Figure 6**) recorded in $CDCl_3$ showed 30 signals for 30 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of seven methyl (δ 14.6, 15.4, 16.0, 16.1, 18.0, 19.3 and 28.0), eleven methylene (δ 18.3, 20.9, 25.2, 27.4, 27.5, 29.9, 34.3, 35.6, 38.7, 40.0 and 109.3), six methine (δ 38.1, 48.0, 48.3, 50.5, 55.3 and 79.0) and six quaternary carbons (δ 37.2, 38.9, 40.8, 42.8, 43.0 and 151.0).

The ^{1}H NMR spectral data (**Table 2**, **Figure 5**) showed characteristic of lupane triterpenoids as seven methyl singlet signals at δ 0.76, 0.79, 0.83, 0.94, 0.97 and 1.03 including one vinylic methyl at δ 1.68, two protons of an isopropenyl moiety at δ 4.68 (1H, *d*, *J* = 2.1 Hz) and 4.56 (1H, *m*) and a typical lupane H_β-19 proton at δ 2.38 (*dt*, *J* = 11.1, 5.7 Hz). An oxymethine proton was shown at δ 3.19 (1H, *dd*, *J* = 10.8, 5.1 Hz, H-3). The doublet of doublet splitting pattern together with a large coupling constant of H-3 with *Jax-ax* = 10.8 Hz and *Jax-eq* = 5.1 Hz indicated an axial (α) orientation of H-3.

The position of the hydroxyl group at C-3 was determined through an HMBC experiment (**Table 2**, **Figure 11**) in which the oxymethine proton at δ 3.19 (H-3) showed correlations with C-1 (δ 38.7), C-4 (δ 38.9), C-23 (δ 28.0) and C-24 (δ 15.4). The position of a methine proton at C-19 was determined from HMBC correlation of H-19 (δ 2.38) with C-18 (δ 48.3), C-20 (δ 151.0), C-21 (δ 29.9), C-29 (δ 109.3) and C-30 (δ 19.3). Thus on the basis of its spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectral data (**Table 3** and **4**, respectively) with the previously reported data of lupeol (Reynolds *et al*., 1986), compound **PTH1** was assigned as lupeol.



Selected HMBC correlation of **PTH1**

Table 2 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH1**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.7	CH_2	0.91 (*m*) [a]	-
2	27.4	CH_2	1.56 (*m*) [a]	-
3	79.0	CH	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	1, 4, 23, 24
4	38.9	C	-	-
5	55.3	CH	0.69 (*m*) [a]	-
6	18.3	CH_2	1.40 (*m*), 1.55 (*m*) [a]	-
7	34.3	CH_2	1.40 (*m*) [a]	-
8	40.8	C	-	-
9	50.5	CH	1.28 (*m*) [a]	-
10	37.2	C	-	-
11	20.9	CH_2	1.22 (*m*), 1.45 (*m*) [a]	-
12	25.2	CH_2	1.08 (*m*) [a]	-
13	38.1	CH	1.67 (*m*) [a]	-
14	42.8	C	-	-
15	27.5	CH_2	1.56 (*m*) [a]	-
16	35.6	CH_2	1.51 (*m*) [a]	-
17	43.0	C	-	-
18	48.3	CH	1.38 (*m*) [a]	-
19	48.0	CH	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	13, 18, 20, 21, 29, 30
20	151.0	C	-	-
21	29.9	CH_2	1.94 (*m*) [a]	-
22	40.0	CH_2	1.20 (*m*), 1.40 (*m*) [a]	-
23	28.0	CH_3	0.97 (*s*)	3, 4, 5, 24
24	15.4	CH_3	0.76 (*s*)	3, 4, 5, 23
25	16.1	CH_3	0.83 (*s*)	1, 5, 9,10

[a] Deduced from HMQC experiment

Table 2 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	16.0	CH_3	1.03 (*s*)	7, 8, 9, 14
27	14.6	CH_3	0.94 (*s*)	8, 14, 15
28	18.0	CH_3	0.79 (*s*)	16, 17, 18, 22
29	109.3	CH_2	4.56 (*m*), 4.68 (*d*, *J* = 2.1 Hz)	19, 30
30	19.3	CH_3	1.68 (*s*)	19, 20, 29

Table 3 Comparison of ^{1}H NMR spectral data between lupeol and compound **PTH1** (recorded in $CDCl_3$)

Position	lupeol, δ_H (ppm)	Compound PTH1, δ_H (ppm)
1	0.91(*t*), 1.68 (*d*)	0.91 (*m*) [a]
2	1.54 (*q*), 1.61 (*d*)	1.56 (*m*) [a]
3	3.18 (*dd*)	3.19 (*dd*, *J* = 10.8, 5.1 Hz)
5	0.69 (*d*)	0.69 (*m*) [a]
6	1.39 (*q*), 1.54 (*d*)	1.40 (*m*), 1.55 (*m*) [a]
7	1.41 (*m*)	1.40 (*m*) [a]
9	1.28 (*d*)	1.28 (*m*) [a]
11	1.25 (*q*), 1.42 (*d*)	1.22 (*m*), 1.45 (*m*) [a]
12	1.07 (*q*), 1.68(*d*)	1.08 (*m*) [a]
13	1.67 (*t*)	1.67 (*m*) [a]
15	1.01 (*d*), 1.71 (*t*)	1.56 (*m*) [a]
16	1.38 (*t*), 1.49 (*d*)	1.51 (*m*) [a]
18	1.37 (*t*)	1.38 (*m*) [a]
19	2.39 (*m*)	2.38 (*dt*, *J* = 11.1, 5.7 Hz)
21	1.33 (*m*), 1.93 (*m*)	1.94 (*m*) [a]

Table 3 (Continued)

Position	lupeol, δ_H (ppm)	Compound PTH1, δ_H (ppm)
22	1.20 (*m*), 1.42 (*m*)	1.20 (*m*), 1.40 (*m*) [a]
23	0.98 (*s*)	0.97 (*s*)
24	0.77 (*s*)	0.76 (*s*)
25	0.84 (*s*)	0.83 (*s*)
26	1.04 (*s*)	1.03 (*s*)
27	0.97 (*s*)	0.94 (*s*)
28	0.79 (*s*)	0.79 (*s*)
29	4.56 (*m*), 4.69 (*m*)	4.56 (*m*), 4.68 (*d*, J = 2.1 Hz)
30	1.69 (*s*)	1.68 (*s*)

[a] Deduced from HMQC experiment

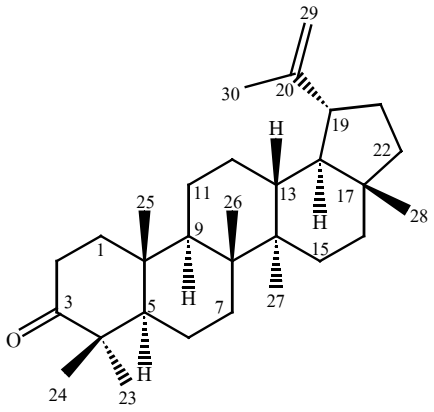
Table 4 Comparison of ^{13}C NMR spectral data between lupeol and compound **PTH1** (recorded in $CDCl_3$)

Position	lupeol, δ_C (ppm)	Compound PTH1, δ_C (ppm)
1	38.7	38.7
2	27.4	27.4
3	79.0	79.0
4	38.8	38.9
5	55.3	55.3
6	18.3	18.3
7	34.2	34.3
8	40.8	40.8
9	50.4	50.5
10	37.1	37.2
11	20.9	20.9

Table 4 (Continued)

Position	lupeol, δ_C (ppm)	Compound PTH1, δ_C(ppm)
12	25.1	25.2
13	38.0	38.1
14	42.8	42.8
15	27.4	27.5
16	35.5	35.6
17	43.0	43.0
18	48.2	48.3
19	47.9	48.0
20	150.9	151.0
21	29.8	29.9
22	40.0	40.0
23	28.0	28.0
24	15.4	15.4
25	16.1	16.1
26	16.0	16.0
27	14.5	14.6
28	18.0	18.0
29	109.3	109.3
30	19.3	19.3

3.1.2 Compound PTH2



Compound **PTH2** was obtained as a white solid, mp. 163-165°C, $[\alpha]_D^{28}$: +50.0° (*c* = 0.100, $CHCl_3$). The IR spectrum (**Figure 12**) exhibited absorption band of a carbonyl group at 1704 cm^{-1}. It gave a purple vanillin- sulfuric acid test indicating a triterpene.

The ^1H and ^{13}C NMR spectral data (**Table 5, Figure 13** and **14**) were closely related to compound **PTH1** (**Table 6** and **7**), except the oxymethine proton (H-3) at δ 3.19 (*dd*, *J* = 10.8, 5.1 Hz) disappeared and the methylene proton (H-2) was shifted downfield to δ 2.49 (*m*) as compared to that of **PTH1** at δ 1.56 (*m*). The ^{13}C NMR spectral data (**Table 5**, **Figure 14**) of compound **PTH2** displayed a signal of a carbonyl group at δ 217.0 which was assigned to C-3 and no signal of an oxymethine carbon at δ 79.0 was observed. The location of the carbonyl group was confirmed by HMBC experiment (**Table 5**) in which both 3H-24 (δ 1.02) and 3H-23 (δ 1.07) showed long-range correlation with C-3 (δ 217.0), C-4 (δ 46.3) and C-5 (δ 54.3). By comparison of the ^{13}C NMR spectral data with the previously reported data of lupenone (Razdan *et al.*, 1988) (**Table 7**), compound **PTH2** was assigned as lupenone.

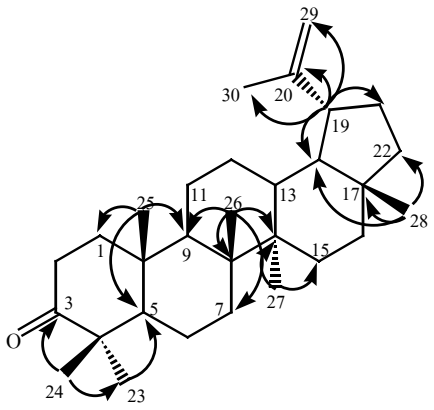
Table 5 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH2**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.6	CH_2	1.90 (*m*) [a]	-
2	33.1	CH_2	2.49 (*m*) [a]	-
3	217.0	C	-	-
4	46.3	C	-	-
5	54.3	CH	1.32 (*m*) [a]	-
6	18.7	CH_2	1.45 (*m*) [a]	-
7	32.6	CH_2	0.87 (*m*), 1.45 (*m*) [a]	-
8	39.8	C	-	-
9	48.8	CH	1.38 (*m*) [a]	-
10	35.9	C	-	-
11	20.5	CH_2	1.30 (*m*) [a]	-
12	24.2	CH_2	1.68 (*m*) [a]	-
13	37.2	CH	1.68 (*m*) [a]	-
14	41.9	C	-	-
15	26.4	CH_2	0.82 (*m*) [a]	-
16	34.5	CH_2	1.37 (*m*), 1.50 (*m*) [a]	-
17	42.0	C	-	-
18	47.3	CH	1.38 (*m*) [a]	-
19	47.0	CH	2.40 (*m*)	18, 20, 21, 29, 30
20	149.8	C	-	-
21	28.8	CH_2	1.26 (*m*), 1.92 (*m*) [a]	-
22	39.0	CH2	1.19 (*m*), 1.41 (*m*) [a]	-
23	25.7	CH_3	1.07 (*s*)	3, 4, 5, 24
24	20.0	CH_3	1.02 (*s*)	3, 4, 5, 23

[a] Deduced from HMQC experiment

Table 5 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
25	15.0	CH_3	0.93 (*s*)	5, 9, 10
26	14.8	CH_3	1.07 (*s*)	7, 8, 9, 14
27	13.5	CH_3	0.96 (*s*)	14, 15
28	17.0	CH_3	0.80 (*s*)	17, 18, 22
29	108.1	CH_2	4.57 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	19, 30
30	18.3	CH_3	1.68 (*s*)	19, 20, 29



Selected HMBC correlation of **PTH2**

Table 6 Comparison of ^{1}H NMR spectral data between compounds **PTH1** and **PTH2** (recorded in $CDCl_3$)

Position	Compound PTH1, δ_H (ppm)	Compound PTH2, δ_H (ppm)
1	0.91 (*m*) [a]	1.90 (*m*) [a]
2	1.56 (*m*) [a]	2.49 (*m*) [a]
3	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	-
5	0.69 (*m*) [a]	1.32 (*m*) [a]
6	1.40 (*m*), 1.55 (*m*) [a]	1.45 (*m*) [a]
7	1.40 (*m*) [a]	0.87 (*m*), 1.45 (*m*) [a]
9	1.28 (*m*) [a]	1.38 (*m*) [a]
11	1.22 (*m*), 1.45 (*m*) [a]	1.30 (*m*) [a]
12	1.08 (*m*) [a]	1.68 (*m*) [a]
13	1.67 (*m*) [a]	1.68 (*m*) [a]
15	1.56 (*m*) [a]	0.82 (*m*) [a]
16	1.51 (*m*) [a]	1.37 (*m*), 1.50 (*m*) [a]
18	1.38 (*m*) [a]	1.38 (*m*) [a]
19	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	2.40 (*m*)
21	1.94 (*m*) [a]	1.26 (*m*), 1.92 (*m*) [a]
22	1.20 (*m*), 1.40 (*m*) [a]	1.19 (*m*), 1.41 (*m*) [a]
23	0.97 (*s*)	1.07 (*s*)
24	0.76 (*s*)	1.02 (*s*)
25	0.83 (*s*)	0.93 (*s*)
26	1.03 (*s*)	1.07 (*s*)
27	0.94 (*s*)	0.96 (*s*)
28	0.79 (*s*)	0.80 (*s*)
29	4.56 (*m*), 4.68 (*d*, *J* = 2.1 Hz)	4.57 (*m*), 4.69 (*d*, *J* = 2.1 Hz)
30	1.68 (*s*)	1.68 (*s*)

[a] Deduced from HMQC experiment

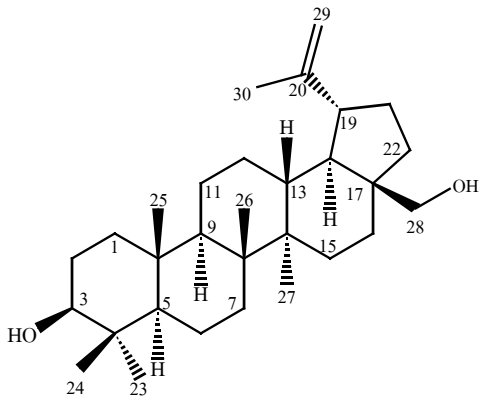
Table 7 Comparison of ^{13}C NMR spectral data of lupenone, compounds **PTH1** and **PTH2** (recorded in $CDCl_3$)

Position	lupenone, δ_C (ppm)	PTH1, δ_C (ppm)	PTH2, δ_C (ppm)
1	39.6	38.7	38.6
2	34.1	27.4	33.1
3	217.9	79.0	217.0
4	47.2	38.9	46.3
5	55.8	55.3	54.3
6	19.6	18.3	18.7
7	33.5	34.3	32.6
8	40.7	40.8	39.8
9	49.7	50.5	48.8
10	36.8	37.2	35.9
11	21.4	20.9	20.5
12	25.1	25.2	24.2
13	38.1	38.1	37.2
14	42.7	42.8	41.9
15	27.4	27.5	26.4
16	35.6	35.6	34.5
17	42.7	43.0	42.0
18	48.2	48.3	47.3
19	47.8	48.0	47.0
20	150.5	151.0	149.8
21	29.8	29.9	28.8
22	39.9	40.0	39.0
23	26.6	28.0	25.7
24	21.0	15.4	20.0
25	15.8	16.1	15.0

Table 7 (Continued)

Position	lupenone, δ_C (ppm)	PTH1, δ_C (ppm)	PTH2, δ_C (ppm)
26	15.4	16.0	14.8
27	14.4	14.6	13.5
28	18.0	18.0	17.0
29	109.2	109.3	108.1
30	19.2	19.3	18.3

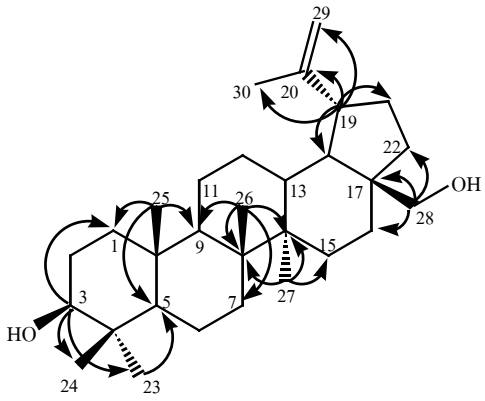
3.1.3 Compound PTH3



Compound **PTH3** was obtained as a white solid, mp. 230-231°C, $[\alpha]_D^{28}$: +16.7° (*c* = 0.150, $CHCl_3$). It gave a purple vanillin-sulfuric acid test. The IR spectrum showed similar characteristic bands to those of **PTH1**.

Comparison of ^1H and ^{13}C NMR spectral data (**Table 9** and **10**, respectively) of compound **PTH3** (**Table 8**, **Figure 15** and **16**) and **PTH1** (**Figure 5** and **6**) revealed close structural similarity. Difference in the spectrum of compound **PTH3** was shown as six singlet signals of methyl groups at δ 0.76, 0.82, 0.97, 0.98, 1.02 and 1.68. In addition, the AB system of oxymethylene protons was shown at δ 3.80 (1H, *dd*, *J* =

10.8, 1.5 Hz) and 3.33 (1H, *d*, *J* = 10.8 Hz) which was not observed in compound **PTH1**. On the basis of HMBC experiment (**Table 8**), the oxymethylene protons (2H-28) showed long-range correlation with C-16 (δ 29.2), C-17 (δ 47.8) and C-22 (δ 34.0), thus the oxymethylene protons were located at C-28 (δ 60.6). This compound was established as betulin by comparison of its spectral data (**Table 9** and **10**) with those reported in the literature (Tinto *et al*., 1992).



Selected HMBC correlation of **PTH3**

Table 8 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH3**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.7	CH_2	0.90 (*m*), 1.70 (*m*) [a]	-
2	27.4	CH_2	1.59 (*m*) [a]	-
3	79.0	CH	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	1, 4, 23, 24
4	38.9	C	-	-
5	55.3	CH	0.68 (*m*) [a]	-
6	18.3	CH_2	1.41 (*m*) [a]	-
7	34.2	CH_2	1.04 (*m*), 1.40 (*m*) [a]	-
8	40.9	C	-	-
9	50.4	CH	1.27 (*m*) [a]	-
10	37.2	C	-	-
11	20.8	CH_2	1.28 (*m*), 1.46 (*m*) [a]	-
12	25.2	CH_2	1.68 (*m*) [a]	-
13	37.3	CH	1.67 (*m*) [a]	-
14	42.7	C	-	-
15	27.0	CH_2	1.11 (*m*), 1.66 (*m*) [a]	-
16	29.2	CH_2	1.20 (*m*), 1.98 (*m*) [a]	-
17	47.5	C	-	-
18	48.8	CH	1.60 (*m*) [a]	-
19	47.5	CH	2.38 (*dt*, *J* = 10.5, 5.7 Hz)	13, 18, 20, 21, 29, 30
20	150.5	C	-	-
21	29.8	CH_2	1.91 (*m*) [a]	-
22	34.0	CH_2	1.80 (*m*), 1.88 (*m*) [a]	-
23	28.0	CH_3	0.97 (*s*)	3, 4, 5, 24
24	15.4	CH_3	0.76 (*s*)	3, 4, 5, 23

[a] Deduced from HMQC experiment

Table 8 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
25	16.1	CH_3	0.82 (*s*)	1, 5, 9, 10
26	16.0	CH_3	1.02 (*s*)	7, 8, 9, 14
27	14.8	CH_3	0.98 (*s*)	8, 13, 14, 15
28	60.6	CH_2	3.33 (*d*, *J* = 10.8 Hz),	
16, 17, 22			3.80 (*dd*, *J* = 10.8, 1.5 Hz)	
29	109.7	CH_2	4.68 (*d*, *J* = 2.1 Hz), 4.58 (*m*)	19, 20, 30
30	19.1	CH_3	1.68 (*s*)	19, 20, 29

Table 9 Comparison of ^{1}H NMR spectral data of betulin, compounds **PTH1** and **PTH3** (recorded in $CDCl_3$)

Position	betulin, δ_H (ppm)	PTH1, δ_H (ppm)	PTH3, δ_C (ppm)
1	0.89, 1.65	0.91 (*m*) [a]	0.90 (*m*), 1.70 (*m*) [a]
2	1.58	1.56 (*m*) [a]	1.59 (*m*) [a]
3	3.18	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	3.19 (*dd*, *J* = 10.8, 5.1 Hz)
5	0.67	0.69 (*m*) [a]	0.68 (*m*) [a]
6	1.38, 1.52	1.40 (*m*), 1.55 (*m*) [a]	1.41 (*m*) [a]
7	1.39	1.40 (*m*) [a]	1.04 (*m*), 1.40 (*m*) [a]
9	1.27	1.28 (*m*) [a]	1.27 (*m*) [a]
11	1.19, 1.41	1.22 (*m*), 1.45 (*m*) [a]	1.28 (*m*), 1.46 (*m*) [a]
12	1.03, 1.63	1.08 (*m*) [a]	1.68 (*m*) [a]
13	1.64	1.67 (*m*) [a]	1.67 (*m*) [a]
15	1.04, 1.70	1.56 (*m*) [a]	1.11 (*m*), 1.66 (*m*) [a]
16	1.20, 1.93	1.51 (*m*) [a]	1.20 (*m*), 1.98 (*m*) [a]
18	1.57	1.38 (*m*) [a]	1.60 (*m*) [a]
19	2.38	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	2.38 (*dt*, *J* = 10.5, 5.7 Hz)

Table 9 (Continued)

Position	betulin, δ_H (ppm)	PTH1, δ_H (ppm)	PTH3, δ_C (ppm)
21	1.40, 1.95	1.94 (*m*) [a]	1.91 (*m*) [a]
22	1.02, 1.86	1.20 (*m*), 1.40 (*m*) [a]	1.80 (*m*), 1.88 (*m*) [a]
23	0.96	0.97 (*s*)	0.97 (*s*)
24	0.76	0.76 (*s*)	0.76 (*s*)
25	0.82	0.83 (*s*)	0.82 (*s*)
26	1.02	1.03 (*s*)	1.02 (*s*)
27	0.98	0.94 (*s*)	0.98 (*s*)
28	3.31, 3.77	0.79 (*s*)	3.33 (*d*, *J* = 10.8 Hz), 3.80 (*dd*, *J* = 10.8, 1.5 Hz)
29	4.58, 4.68	4.56 (*m*), 4.68 (*d*, *J* = 2.1 Hz)	4.58 (*m*), 4.68 (*d*, *J* = 2.1 Hz)
30	1.68	1.68 (*s*)	1.68 (*s*)

[a] Deduced from HMQC experiment

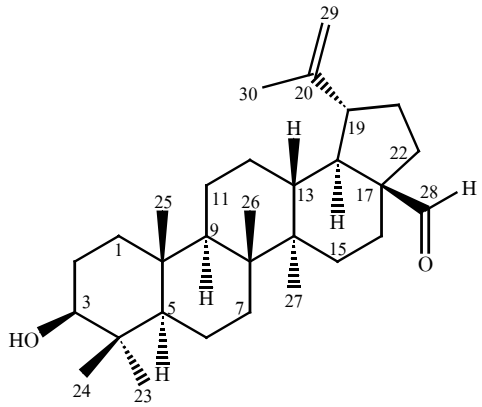
Table 10 Comparison of ^{13}C NMR spectral data of betulin, compounds **PTH1** and **PTH3** (recorded in $CDCl_3$)

Position	betulin, δ_C (ppm)	PTH1, δ_C (ppm)	PTH3, δ_C (ppm)
1	38.8	38.7	38.7
2	27.2	27.4	27.4
3	78.9	79.0	79.0
4	38.9	38.9	38.9
5	55.3	55.3	55.3
6	18.3	18.3	18.3
7	34.3	34.3	34.2
8	40.9	40.8	40.9

Table 10 (Continued)

Position	betulin, δ_C (ppm)	PTH1, δ_C (ppm)	PTH3, δ_C (ppm)
9	50.4	50.5	50.4
10	37.2	37.2	37.2
11	20.9	20.9	20.8
12	25.3	25.2	25.2
13	37.3	38.1	37.3
14	42.7	42.8	42.7
15	27.0	27.5	27.0
16	29.2	35.6	29.2
17	47.8	43.0	47.5
18	48.8	48.3	48.8
19	47.8	48.0	47.5
20	150.6	151.0	150.5
21	29.8	29.9	29.8
22	34.0	40.0	34.0
23	28.0	28.0	28.0
24	15.4	15.4	15.4
25	16.1	16.1	16.1
26	16.0	16.0	16.0
27	14.8	14.6	14.8
28	60.2	18.0	60.6
29	109.6	109.3	109.7
30	19.1	19.3	19.1

3.1.4 Compound PTH4



Compound **PTH4** was obtained as a colorless viscous oil. It gave a purple vanillin-sulfuric acid test. Due to its instability, no IR spectrum was obtained.

The ^{1}H and ^{13}C NMR spectral data of **PTH4** (**Table 11**, **Figure 17** and **18**) were similar to those of **PTH1** (**Table 12** and **13**, **Figure 5** and **6**), excert that **PTH4** had only six methyl singlets at δ 0.75, 0.82, 0.92, 0.96, 0.98 and 1.70 and showed additional signal of aldehydic proton at δ 9.68 (1H, *d*, *J* = 1.5 Hz). The signal of a methine proton (H-19, δ 2.86) was shifted more downfield than **PTH1** (δ 2.38). On the basis of HMBC (**Table 11**), the aldehyde group was located at C-28 (δ 206.7) from correlation of H-28 (δ 9.68) with C-17 (δ 59.3) and C-18 (δ 48.1). Compound **PTH4** was established as betulinaldehyde by comparison of its spectral data with those reported in the literature (Macias *et al*., 1994) (**Table 12** and **13**).

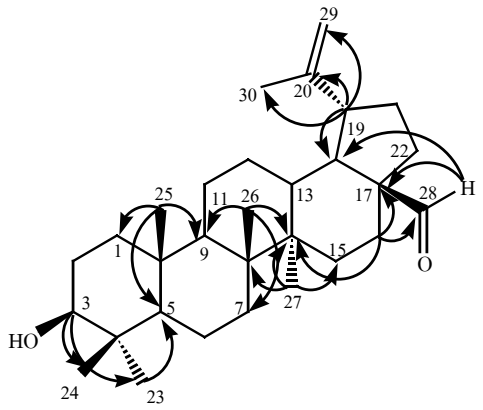
Table 11 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH4**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.7	CH_2	0.91 (*m*), 1.67 (*m*) [a]	-
2	27.4	CH_2	1.58 (*m*), 1.66 (*m*) [a]	-
3	79.0	CH	3.18 (*dd*, *J* = 10.8, 5.1 Hz)	23, 24
4	38.8	C	-	-
5	55.3	CH	0.67 (*m*) [a]	-
6	18.3	CH_2	1.40 (*m*), 1.55 (*m*) [a]	-
7	34.3	CH_2	1.38 (*m*), 1.44 (*m*) [a]	-
8	40.8	C	-	-
9	50.5	CH	1.26 (*m*) [a]	-
10	37.2	C	-	-
11	20.7	CH_2	1.27 (*m*), 1.46 (*m*) [a]	-
12	25.2	CH_2	1.75 (*m*) [a]	-
13	38.7	CH	2.03 (*m*) [a]	-
14	42.6	C	-	-
15	29.3	CH_2	1.46 (*m*) [a]	-
16	28.8	CH_2	1.17 (*m*), 2.12 (*m*) [a]	14, 17, 18, 28
17	59.3	C	-	-
18	48.1	CH	1.73 (*m*) [a]	-
19	47.5	CH	2.86 (*dt*, *J* = 10.8, 5.7 Hz)	18, 21, 30
20	149.7	C	-	-
21	29.9	CH_2	1.26 (*m*), 1.89 (*m*) [a]	-
22	33.2	CH_2	1.34 (*m*), 1.80 (*m*) [a]	-
23	28.0	CH_3	0.96 (*s*)	3, 4, 5, 24
24	15.3	CH_3	0.75 (*s*)	3, 4, 5, 23

[a] Deduced from HMQC experiment

Table 11 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
25	16.1	CH_3	0.82 (*s*)	1, 5, 9, 10
26	15.9	CH_3	0.92 (*s*)	7, 8, 9, 14
27	14.3	CH_3	0.98 (*s*)	8, 14, 15
28	206.7	CH	9.68 (*d*, *J* = 1.5 Hz)	17, 18
29	110.2	CH_2	4.63 (*m*), 4.76 (*m*)	19, 30
30	19.0	CH_3	1.70 (*s*)	19, 20, 29



Selected HMBC correlation of **PTH4**

Table 12 Comparison of ^{1}H NMR spectral data of betulinaldehyde, compounds **PTH1** and **PTH4** (recorded in $CDCl_3$)

Position	betulinaldehyde, δ_H (ppm)	Compound PTH1, δ_H (ppm)	Compound PTH4, δ_H (ppm)
1	0.90, 1.65	0.91 (*m*) [a]	0.91 (*m*), 1.67 (*m*) [a]
2	1.54, 1.59	1.56 (*m*) [a]	1.58 (*m*), 1.66 (*m*) [a]
3	3.17	3.19	3.18
	(*dd*, *J* = 11.1, 5.0 Hz)	(*dd*, *J* = 10.8, 5.1 Hz)	(*dd*, *J* = 10.8, 5.1 Hz)
5	0.67	0.69 (*m*) [a]	0.67 (*m*) [a]
6	1.36, 1.49	1.40 (*m*), 1.55 (*m*) [a]	1.40 (*m*), 1.55 (*m*) [a]
7	-	1.40 (*m*) [a]	1.38 (*m*), 1.44 (*m*) [a]
9	1.16	1.28 (*m*) [a]	1.26 (*m*) [a]
11	1.24, 1.42	1.22 (*m*), 1.45 (*m*) [a]	1.27 (*m*), 1.46 (*m*) [a]
12	1.02, 1.74	1.08 (*m*) [a]	1.75 (*m*) [a]
13	2.01	1.67 (*m*) [a]	2.03 (*m*) [a]
15	1.17	1.56 (*m*) [a]	1.46 (*m*) [a]
16	1.42, 2.06	1.51 (*m*) [a]	1.17 (*m*), 2.12 (*m*) [a]
18	1.71	1.38 (*m*) [a]	1.73 (*m*) [a]
19	2.85	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	2.86 (*dt*, *J* = 10.8, 5.7 Hz)
21	1.45, 1.87	1.94 (*m*) [a]	1.26 (*m*), 1.89 (*m*) [a]
22	1.33, 1.74	1.20 (*m*), 1.40 (*m*) [a]	1.34 (*m*), 1.80 (*m*) [a]
23	0.95	0.97 (*s*)	0.96 (*s*)
24	0.74	0.76 (*s*)	0.75 (*s*)
25	0.80	0.83 (*s*)	0.82 (*s*)
26	0.90	1.03 (*s*)	0.92 (*s*)
27	0.96	0.94 (*s*)	0.98 (*s*)
28	9.66 (*d*)	0.79 (*s*)	9.68 (*d*, *J* = 1.5 Hz)

[a] Deduced from HMQC experiment

Table 12 (Continued)

Position	betulinaldehyde, δ_H (ppm)	Compound PTH1, δ_H (ppm)	Compound PTH4, δ_H (ppm)
29	4.62,	4.56 (*m*),	4.63 (*m*),
	4.74	4.68 (*d*, *J* = 2.1 Hz)	4.76 (*m*)
30	1.68	1.68 (*s*)	1.70 (*s*)

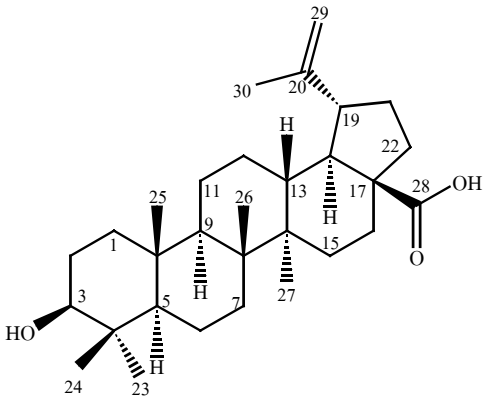
Table 13 Comparison of ^{13}C NMR spectral data of betulinaldehyde, compounds **PTH1** and **PTH4** (recorded in $CDCl_3$)

Position	betulinaldehyde, δ_C (ppm)	Compound PTH1, δ_C (ppm)	Compound PTH4, δ_C (ppm)
1	38.7	38.7	38.7
2	27.3	27.4	27.4
3	78.9	79.0	79.0
4	38.8	38.9	38.8
5	55.5	55.3	55.3
6	18.2	18.3	18.3
7	34.3	34.3	34.3
8	40.8	40.8	40.8
9	50.4	50.5	50.5
10	37.1	37.2	37.2
11	20.7	20.9	20.7
12	25.5	25.2	25.2
13	38.7	38.1	38.7
14	42.5	42.8	42.6
15	29.2	27.5	29.3
16	28.8	35.6	28.8

Table 13 (Continued)

Position	betulinaldehyde, δ_C (ppm)	Compound PTH1, δ_C (ppm)	Compound PTH4, δ_C (ppm)
17	59.3	43.0	59.3
18	48.0	48.3	48.1
19	47.5	48.0	47.5
20	149.7	151.0	149.7
21	29.8	29.9	29.9
22	33.2	40.0	33.2
23	27.9	28.0	28.0
24	15.4	15.4	15.3
25	15.9	16.1	16.1
26	16.1	16.0	16.0
27	14.2	14.6	14.3
28	205.6	18.0	206.7
29	110.1	109.3	110.2
30	19.0	19.3	19.0

3.1.5 Compound PTH5



Compound **PTH5** was obtained as a white solid, mp. 279-280°C, $[\alpha]_D^{28}$: +15.0° (*c* = 0.100, $CHCl_3$). It gave a purple vanillin-sulfuric acid test. The IR spectrum (**Figure 19**) showed absorption band of a hydroxyl group at 3415 cm^{-1} and a carbonyl group at 1686 cm^{-1}.

The ^{1}H and ^{13}C NMR spectral data of **PTH5** (**Table 14, Figure 20** and **21**) were similar to those of **PTH4** (**Table 15 and 16, Figure 17** and **18**). Difference in the spectrum of **PTH5** was shown as the disappearance of an aldehydic proton at δ 9.68 (H-28) in the ^{1}H NMR and the ^{13}C NMR spectrum displayed a signal of a carboxyl carbon at δ 179.1 instead of an aldehydic carbon at δ 206.7, thus suggesting a carboxylic functionality at C-28. The location of the carboxyl group was confirmed by HMBC experiment (**Table 14**) in which the methylene protons 2H-22 (δ 1.41 and 1.93) showed correlations with C-17 (δ 56.1) 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) (**Table 15** and **16**), compound **PTH5** was assigned as betulinic acid.

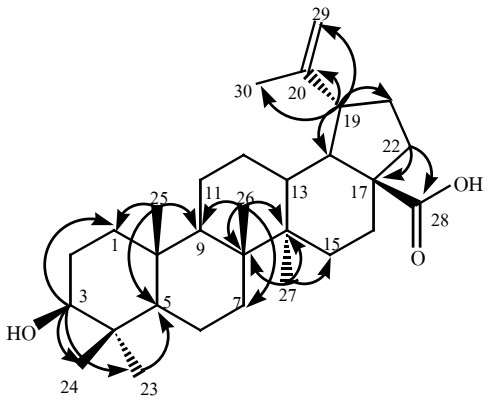
Table 14 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH5**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.7	CH_2	0.88 (*m*), 1.65 (*m*)[a]	-
2	26.9	CH_2	1.57 (*m*), 1.61 (*m*)[a]	-
3	78.7	CH	3.19 (*dd*, *J* = 10.8, 5.4 Hz)	1, 23, 24
4	38.7	C	-	-
5	55.3	CH	0.69 (*m*)[a]	4, 6, 7, 9
6	18.2	CH_2	1.36 (*m*), 1.51 (*m*)[a]	-
7	34.2	CH_2	1.38 (*m*)[a]	-
8	40.6	C	-	-
9	50.5	CH	1.26 (*m*)[a]	-
10	37.1	C	-	-
11	20.8	CH_2	1.23 (*m*), 1.43 (*m*)[a]	-
12	25.4	CH_2	1.69 (*m*)[a]	-
13	38.2	CH	2.22 (*m*)[a]	-
14	42.3	C	-	-
15	29.6	CH_2	1.15 (*m*), 1.51 (*m*)[a]	-
16	32.2	CH_2	1.40 (*m*), 2.25 (*m*)[a]	-
17	56.1	C	-	-
18	49.1	CH	1.58 (*m*)[a]	-
19	46.9	CH	3.01 (*m*)	18, 20, 21, 29, 30
20	150.7	C	-	-
21	30.5	CH_2	1.42 (*m*), 1.91 (*m*)[a]	-
22	37.1	CH_2	1.41 (*m*), 1.93 (*m*)[a]	17, 18, 28
23	27.6	CH_3	0.97 (*s*)	3, 4, 5, 24
24	15.2	CH_3	0.75 (*s*)	3, 4, 5, 23

[a] Deduced from HMQC experiment

Table 14 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
25	15.9	CH_3	0.82 (*s*)	1, 5, 9, 10
26	15.6	CH_3	0.94 (*s*)	7, 8, 9, 14
27	14.5	CH_3	0.98 (*s*)	8, 13, 14, 15
28	179.1	C	-	-
29	109.3	CH_2	4.61 (*br s*), 4.74 (*br s*)	19, 30
30	19.1	CH_3	1.69 (*s*)	19, 20, 29



Selected HMBC correlation of **PTH5**

Table 15 Comparison of ^{1}H NMR spectral data between betulinic acid, compounds **PTH4** and **PTH5** (recorded in $CDCl_3$)

Position	betulinic acid, δ_H (ppm)	Compound PTH4, δ_H (ppm)	Compound PTH5, δ_H (ppm)
1	0.95, 1.70	0.91 (*m*), 1.67 (*m*) [a]	0.88 (*m*), 1.65 (*m*) [a]
2	1.57, 1.62	1.58 (*m*), 1.66 (*m*) [a]	1.57 (*m*), 1.61 (*m*) [a]
3	3.13	3.18	3.19
	(*dd*, *J* = 11.5, 4.9 Hz)	(*dd*, *J* = 10.8, 5.1 Hz)	(*dd*, *J* = 10.8, 5.4 Hz)
5	0.71	0.67 (*m*) [a]	0.69 (*m*) [a]
6	1.45, 1.55	1.40 (*m*), 1.55 (*m*) [a]	1.36 (*m*), 1.51 (*m*) [a]
7	1.42	1.38 (*m*), 1.44 (*m*) [a]	1.38 (*m*) [a]
9	1.33	1.26 (*m*) [a]	1.26 (*m*) [a]
11	1.25, 1.45	1.27 (*m*), 1.46 (*m*) [a]	1.23 (*m*), 1.43 (*m*) [a]
12	1.07, 1.73	1.75 (*m*) [a]	1.69 (*m*) [a]
13	2.30	2.03 (*m*) [a]	2.22 (*m*) [a]
15	1.18, 1.53	1.46 (*m*) [a]	1.15 (*m*), 1.51 (*m*) [a]
16	1.43, 2.23	1.17 (*m*), 2.12 (*m*) [a]	1.40 (*m*), 2.25 (*m*) [a]
18	1.63	1.73 (*m*) [a]	1.58 (*m*) [a]
19	3.02	2.86 (*dt*, *J* = 10.8, 5.7 Hz)	3.01 (*m*)
21	1.40, 1.93	1.26 (*m*), 1.89 (*m*) [a]	1.42 (*m*), 1.91 (*m*) [a]
22	1.43, 1.91	1.34 (*m*), 1.80 (*m*) [a]	1.41 (*m*), 1.93 (*m*) [a]
23	0.95	0.96 (*s*)	0.97 (*s*)
24	0.75	0.75 (*s*)	0.75 (*s*)
25	0.86	0.82 (*s*)	0.82 (*s*)
26	0.97	0.92 (*s*)	0.94 (*s*)
27	1.01	0.98 (*s*)	0.98 (*s*)

[a] Deduced from HMQC experiment

Table 15 (Continued)

Position	betulinic acid, δ_H (ppm)	Compound PTH4, δ_H (ppm)	Compound PTH5, δ_H (ppm)
28	-	9.68 (*d*, *J* = 1.5 Hz)	-
29	4.59 (*dd*, *J* = 2.2, 1.0 Hz), 4.71 (*d*, *J* = 2.2 Hz)	4.63 (*m*), 4.76 (*m*)	4.61 (*br s*), 4.74 (*br s*)
30	1.69 (*d*, *J* = 1.0 Hz)	1.70 (*s*)	1.69 (*s*)

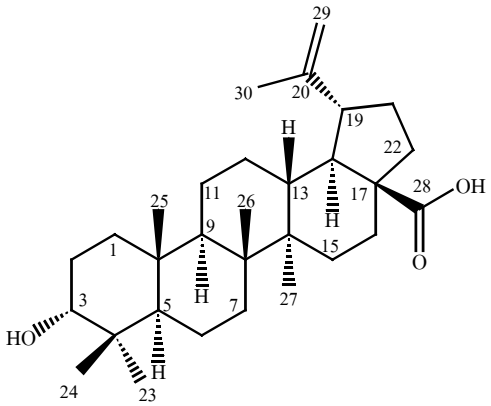
Table 16 Comparison of ^{13}C NMR spectral data of betulinic acid (recorded in pyridine-d_5), compounds **PTH4** and **PTH5** (recorded in $CDCl_3$+CD_3OD)

Position	betulinic acid, δ_C (ppm)	Compound PTH4, δ_C (ppm)	Compound PTH5, δ_C (ppm)
1	38.5	38.7	38.7
2	28.2	27.4	26.9
3	78.1	79.0	78.7
4	39.4	38.8	38.7
5	55.9	55.3	55.3
6	18.7	18.3	18.2
7	34.7	34.3	34.2
8	41.0	40.8	40.6
9	50.9	50.5	50.5
10	37.5	37.2	37.1
11	21.1	20.7	20.8
12	26.0	25.2	25.4
13	39.2	38.7	38.2
14	42.8	42.6	42.3
15	30.2	29.3	29.6

Table 16 (Continued)

Position	betulinic acid, δ_C (ppm)	Compound PTH4, δ_C (ppm)	Compound PTH5, δ_C (ppm)
16	32.8	28.8	32.2
17	56.6	59.3	56.1
18	49.7	48.1	49.1
19	47.7	47.5	46.9
20	151.4	149.7	150.7
21	31.1	29.9	30.5
22	37.4	33.2	37.1
23	28.5	28.0	27.6
24	16.2	15.3	15.2
25	16.3	16.1	15.9
26	16.2	16.0	15.6
27	14.8	14.3	14.5
28	179.0	206.7	179.1
29	110.0	110.2	109.3
30	19.4	19.0	19.1

3.1.6 Compound PTH6



Compound **PTH6** was obtained as a white solid, mp. 257-259°C, $[\alpha]_D^{28}$: -10.0° (*c* = 0.050, $CHCl_3$). It gave a positive vanillin-sulfuric acid test. The IR spectrum showed absorption bands similar to those of compound **PTH5**.

The ^{1}H and ^{13}C NMR spectral data (**Table 17**, **Figure 22** and **23**) of compound **PTH6** were similar to those of compound **PTH5** (**Table 18** and **19**, **Figure 20** and **21**), except that the splitting pattern of H-3 in **PTH6** at δ 3.38 was a triplet (*J* = 2.7 Hz) instead of a doublet of doublet (*J* = 10.8, 5.4 Hz) in **PTH5**. The difference in the multiplicity with a small coupling constant of H-3 in compound **PTH6** was in agreement with the respective coupling pattern (equatorial-equatorial and equatorial-axial) of H-3 and 2H-2, indicating that H-3 is situated in an equatorial position. The location of a hydroxyl group at C-3 was determined through an HMBC experiment (**Table 17**) in which the oxymethine proton signal at δ 3.38 (H-3) showed long-range correlations with C-1 (δ 33.2), C-5 (δ 49.0), C-23 (δ 28.2) and C-24 (δ 22.1). Thus on the basis of its spectroscopic data and comparison with previously reported compound (Sung *et al*., 1991 and Kitajima *et al*.,1990) (**Table 18** and **19**), compound **PTH6** was assigned as 3-*epi*-betulinic acid, an epimer of betulinic acid (**PTH5**).

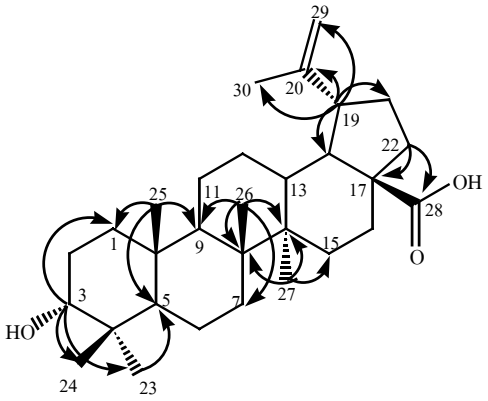
Table 17 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH6**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	33.2	CH_2	1.18 (*m*)[a]	-
2	25.5	CH_2	1.02 (*m*), 1.68 (*m*)[a]	-
3	76.2	CH	3.38 (*t*, *J* = 2.7 Hz)	1, 5, 23, 24
4	37.5	C	-	-
5	49.0	CH	1.18 (*m*)[a]	-
6	18.2	CH_2	1.34 (*m*), 1.38 (*m*)[a]	-
7	34.1	CH_2	1.30 (*m*)[a]	-
8	40.8	C	-	-
9	50.3	CH	1.40 (*m*)[a]	-
10	37.3	C	-	-
11	20.7	CH_2	1.42 (*m*)[a]	-
12	25.3	CH_2	1.52 (*m*), 1.82 (*m*)[a]	-
13	38.2	CH	2.21 (*m*)[a]	26, 27
14	42.5	C	-	-
15	29.6	CH_2	1.14 (*m*)[a]	-
16	32.2	CH_2	2.24 (*m*)[a]	-
17	56.2	C	-	-
18	49.2	CH	1.57 (*m*)[a]	-
19	47.0	CH	3.00 (*m*)	-
20	150.7	C	-	-
21	30.6	CH_2	1.93 (*m*)[a]	17, 18, 19, 28
22	37.1	CH_2	1.95 (*m*)[a]	17, 18, 28
23	28.2	CH_3	0.93 (*s*)	3, 4, 5, 24
24	22.1	CH_3	0.82 (*s*)	3, 4, 5, 23
25	15.9	CH_3	0.94 (*s*)	1, 5, 9

[a] Deduced from HMQC experiment

Table 17 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	15.9	CH_3	0.83 (*s*)	7, 8, 9, 14
27	14.7	CH_3	0.99 (*s*)	8, 13, 14, 15
28	179.2	C	-	-
29	109.5	CH_2	4.73 (*d*, *J* = 1.8 Hz), 4.60 (*m*)	19, 20, 30
30	19.3	CH_3	1.69 (*s*)	19, 20, 29



Selected HMBC correlation of **PTH6**

Table 18 Comparison of ^{1}H NMR spectral data of 3-*epi*-betulinic acid (recorded in $CDCl_3$), compounds **PTH5** (recorded in $CDCl_3$) and **PTH6** (recorded in $CDCl_3$+ CD_3OD)

Position	3-*epi*-betulinic acid, δ_H (ppm)	Compound PTH5, δ_H (ppm)	Compound PTH6, δ_C (ppm)
1	-	0.88 (*m*), 1.65 (*m*)[a]	1.18 (*m*)[a]
2	-	1.57 (*m*), 1.61 (*m*)[a]	1.02 (*m*), 1.68 (*m*)[a]
3	3.39 (*t*, *J* = 2.7 Hz)	3.19 (*dd*, *J* = 10.8, 5.4 Hz)	3.38 (*t*, *J* = 2.7 Hz)
5	-	0.69 (*m*)[a]	1.18 (*m*)[a]
6	-	1.36 (*m*), 1.51 (*m*)[a]	1.34 (*m*), 1.38 (*m*)[a]
7	-	1.38 (*m*)[a]	1.30 (*m*)[a]
9	-	1.26 (*m*)[a]	1.40 (*m*)[a]
11	-	1.23 (*m*), 1.43 (*m*)[a]	1.42 (*m*)[a]
12	-	1.69 (*m*)[a]	1.52 (*m*), 1.82 (*m*)[a]
13	-	2.22 (*m*)[a]	2.21 (*m*)[a]
15	-	1.15 (*m*), 1.51 (*m*)[a]	1.14 (*m*)[a]
16	-	1.40 (*m*), 2.25 (*m*)[a]	2.24 (*m*)[a]
18	-	1.58 (*m*)[a]	1.57 (*m*)[a]
19	3.00 (*dt*, *J* = 11.0, 4.5 Hz)	3.01 (*m*)	3.00 (*m*)
21	-	1.42 (*m*), 1.91 (*m*)[a]	1.93 (*m*)[a]
22	-	1.41 (*m*), 1.93 (*m*)[a]	1.95 (*m*)[a]
23	0.92 (*s*)	0.97 (*s*)	0.93 (*s*)
24	0.80 (*s*)	0.75 (*s*)	0.82 (*s*)
25	0.92 (*s*)	0.82 (*s*)	0.94 (*s*)

[a] Deduced from HMQC experiment

Table 18 (Continued)

Position	3-*epi*-betulinic acid, δ_H (ppm)	Compound PTH5, δ_H (ppm)	Compound PTH6, δ_C (ppm)
26	0.81 (*s*)	0.94 (*s*)	0.83 (*s*)
27	0.98 (*s*)	0.98 (*s*)	0.99 (*s*)
29	4.59 (*m*),	4.61 (*br s*),	4.60 (*m*),
	4.72 (*m*)	4.74 (*br s*)	4.73 (*d*, *J* = 1.8 Hz)
30	1.68 (*s*)	1.69 (*s*)	1.69 (*s*)

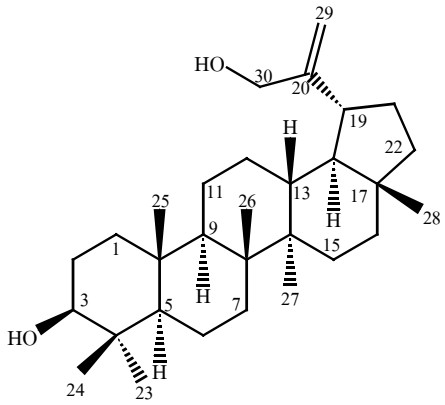
Table 19 Comparison of ^{13}C NMR spectral data of 3-*epi*-betulinic acid, compounds **PTH5** and **PTH6** (recorded in $CDCl_3$+CD_3OD)

Position	3-*epi*-betulinic acid, δ_C (ppm)	Compound PTH5, δ_C (ppm)	Compound PTH6, δ_C (ppm)
1	34.0	37.7	33.2
2	23.2	26.4	25.5
3	75.5	78.0	76.2
4	39.0	37.9	37.5
5	49.3	54.4	49.0
6	18.6	17.3	18.2
7	34.8	33.3	34.1
8	41.3	39.7	40.8
9	50.7	49.5	50.3
10	37.7	36.2	37.3
11	21.0	19.8	20.7
12	26.1	24.5	25.3
13	38.5	37.4	38.2
14	42.9	41.4	42.5

Table 19 (Continued)

Position	3-*epi*-betulinic acid, δ_C (ppm)	Compound PTH5, δ_C (ppm)	Compound PTH6, δ_C (ppm)
15	31.2	28.7	29.6
16	32.8	31.2	32.2
17	56.6	55.3	56.2
18	47.7	48.3	49.2
19	49.7	45.9	47.0
20	151.2	149.4	150.7
21	29.9	29.6	30.6
22	37.5	36.0	37.1
23	29.2	27.0	28.2
24	22.5	14.3	22.1
25	16.4	15.1	15.9
26	16.4	15.0	15.9
27	14.9	13.7	14.7
28	178.7	179.6	179.2
29	109.8	108.7	109.5
30	19.4	18.4	19.3

3.1.7 Compound PTH7



Compound **PTH7** was obtained as a white solid, mp. 203-204°C, $[\alpha]_D^{28}$ -22.7° (*c* = 0.220, $CHCl_3$). It gave a purple vanillin-sulfuric acid test. Its IR spectrum showed absorption bands similar to those of **PTH1**.

The ^1H and ^{13}C NMR spectral data of compound **PTH7** (**Table 20**, **Figure 24** and **25**) and **PTH1** (**Table 21** and **22**, **Figure 5** and **6**) exhibited the same pattern, except that compound **PTH7** displayed only six methyl singlets (δ 0.76, 0.78, 0.83, 0.94, 0.97 and 1.03) with disappearance of a vinylic methyl group of 3H-30 at δ 1.68 (*s*). The two signals of terminal olefinic protons of 2H-29 [δ 4.93 (*br s*) and 4.90 (*br s*)] were shown to be shifted more downfield than **PTH1** [δ 4.68 (*d*, *J* = 2.1 Hz) and 4.56 (*m*)]. In addition, the AB system of oxymethylene protons was shown at δ 4.14 and 4.09 with coupling constant 15.3 Hz which was assigned to 2H-30. Based on HMBC experiments (**Table 20**), the oxymethylene protons 2H-30 showed correlations with C-19 (δ 43.8), C-20 (δ 154.8) and C-29 (δ 106.8). Thus compound **PTH7** was established as lup-20(29)-en-3β, 30-diol by comparison of its spectral data with previously reported data (Burns *et al*., 2000), (**Table 21** and **22**).

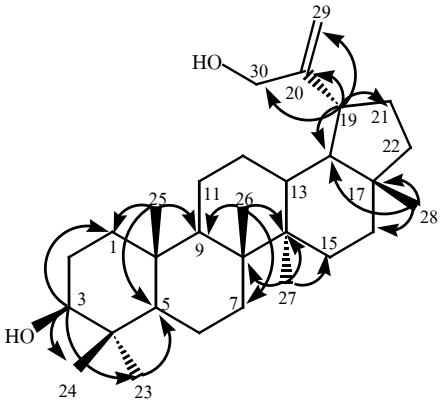
Table 20 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH7**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.7	CH_2	1.64 (*m*)[a]	-
2	27.4	CH_2	1.58 (*m*)[a]	-
3	79.0	CH	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	1, 23, 24
4	38.9	C	-	-
5	55.3	CH	0.68 (*m*)[a]	-
6	18.3	CH_2	1.41 (*m*), 1.55 (*m*)[a]	-
7	34.3	CH_2	1.40 (*m*)[a]	-
8	40.9	C	-	-
9	50.4	CH	1.25 (*m*)[a]	-
10	37.2	C	-	-
11	21.1	CH_2	1.25 (*m*), 1.44 (*m*)[a]	-
12	26.7	CH_2	1.65 (*m*)[a]	-
13	38.0	CH	1.71 (*m*)[a]	-
14	42.8	C	-	-
15	27.4	CH_2	1.62 (*m*)[a]	-
16	35.5	CH_2	1.55 (*m*)[a]	-
17	43.0	C	-	-
18	48.9	CH	1.46 (*m*)[a]	-
19	43.8	CH	2.28 (*dt*, *J* = 10.8, 4.8 Hz)	18, 19, 20, 21, 30
20	154.8	C	-	-
21	31.8	CH_2	2.06 (*m*)[a]	-
22	39.9	CH_2	1.24 (*m*), 1.41 (*m*)[a]	-
23	28.0	CH_3	0.97 (*s*)	3, 4, 5, 24
24	15.4	CH_3	0.76 (*s*)	3, 4, 5, 23
25	16.1	CH_3	0.83 (*s*)	1, 5, 9, 10

[a] Deduced from HMQC experiment

Table 20 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	16.0	CH_3	1.03 (*s*)	7, 8, 9, 14
27	14.5	CH_3	0.94 (*s*)	8, 14, 15
28	17.7	CH_3	0.78 (*s*)	16, 17, 18, 22
29	106.8	CH_2	4.90 (*br s*), 4.93 (*br s*)	19, 20, 21, 30
30	65.0	CH_2	4.09 (*d*, *J* = 15.3 Hz),	
19, 20, 29			4.14 (*d*, *J* = 15.3 Hz)	



Selected HMBC correlation of **PTH7**

Table 21 Comparison of ^{1}H NMR spectral data of lup-20(29)-en-3β, 30-diol, compounds **PTH1** and **PTH7** (recorded in $CDCl_3$)

Position	lup-20(29)-en-3β, 30-diol, δ_H (ppm)	Compound PTH1, δ_H (ppm)	Compound PTH7, δ_H (ppm)
1	0.89, 1.66	0.91 (*m*)[a]	1.64 (*m*)[a]
2	1.56, 1.61	1.56 (*m*)[a]	1.58 (*m*)[a]
3	3.19	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	3.19 (*dd*, *J* = 10.8, 5.1 Hz)
5	0.68	0.69 (*m*)[a]	0.68 (*m*)[a]
6	1.39, 1.52	1.40 (*m*), 1.55 (*m*)	1.41 (*m*), 1.55 (*m*)
7	1.39	1.40 (*m*)[a]	1.40 (*m*)[a]
9	1.26	1.28 (*m*)[a]	1.25 (*m*)[a]
11	1.22, 1.42	1.22 (*m*), 1.45 (*m*)[a]	1.25 (*m*), 1.44 (*m*)[a]
12	1.09, 1.42	1.08 (*m*)[a]	1.65 (*m*)[a]
13	1.65	1.67 (*m*)[a]	1.71 (*m*)[a]
15	1.02, 1.69	1.56 (*m*)[a]	1.62 (*m*)[a]
16	1.39, 1.49	1.51 (*m*)[a]	1.55 (*m*)[a]
18	1.45	1.38 (*m*)[a]	1.46 (*m*)[a]
19	2.28	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	2.28 (*dt, J* = 10.8, 4.8 Hz)
21	1.33, 2.06	1.94 (*m*)[a]	1.26 (*m*), 2.06 (*m*)[a]
22	1.25, 1.40	1.20 (*m*), 1.40 (*m*)[a]	1.24 (*m*), 1.41 (*m*)[a]
23	0.97	0.97 (*s*)	0.97 (*s*)
24	0.76	0.76 (*s*)	0.76 (*s*)
25	0.83	0.83 (*s*)	0.83 (*s*)
26	1.03	1.03 (*s*)	1.03 (*s*)
27	0.95	0.94 (*s*)	0.94 (*s*)

[a] Deduced from HMQC experiment

Table 21 (Continued)

Position	lup-20(29)-en-3β, 30-diol, δ_H (ppm)	Compound PTH1, δ_H (ppm)	Compound PTH7, δ_H (ppm)
28	0.78	0.79 (*s*)	0.78 (*s*)
29	4.91, 4.94	4.56 (*m*), 4.68 (*d*, *J* = 2.1 Hz)	4.90 (*br s*), 4.93 (*br s*)
30	4.11, 4.13	1.68 (*s*)	4.09 (*d*, *J* = 15.3 Hz), 4.14 (*d*, *J* = 15.3 Hz)

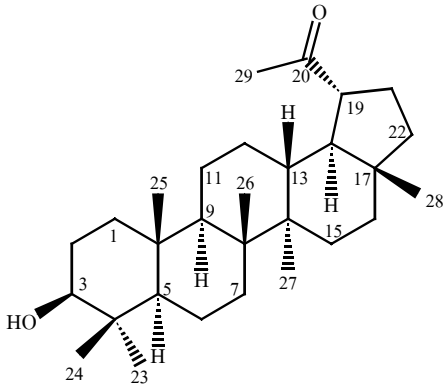
Table 22 Comparison of ^{13}C NMR spectral data of lup-20(29)-en-3β, 30-diol, compounds **PTH1** and **PTH7** (recorded in $CDCl_3$)

Position	lup-20(29)-en-3β, 30-diol, δ_C (ppm)	Compound PTH1, δ_C (ppm)	Compound PTH7, δ_C (ppm)
1	38.7	38.7	38.7
2	27.4	27.4	27.4
3	79.0	79.0	79.0
4	38.9	38.9	38.9
5	55.3	55.3	55.3
6	18.3	18.3	18.3
7	34.3	34.3	34.3
8	40.9	40.8	40.9
9	50.4	50.5	50.4
10	37.2	37.2	37.2
11	21.0	20.9	21.1
12	26.7	25.2	26.7
13	38.0	38.1	38.0
14	42.8	42.8	42.8

Table 22 (Continued)

Position	lup-20(29)-en- 3β, 30-diol, δ_C (ppm)	Compound PTH1, δ_C (ppm)	Compound PTH7, δ_C (ppm)
15	27.4	27.5	27.4
16	35.5	35.6	35.5
17	43.0	43.0	43.0
18	48.9	48.3	48.9
19	43.8	48.0	43.8
20	154.8	151.0	154.8
21	31.8	29.9	31.8
22	39.9	40.0	39.9
23	28.0	28.0	28.0
24	15.4	15.4	15.4
25	16.1	16.1	16.1
26	16.0	16.0	16.0
27	14.5	14.6	14.5
28	17.7	18.0	17.7
29	106.8	109.3	106.8
30	65.0	19.3	65.0

3.1.8 Compound PTH8



Compound **PTH8** was assigned as a white solid, mp. 234-235°C, $[\alpha]_D^{28}$: -22.7 (*c* = 0.220, $CHCl_3$). It gave a blue vanillin-sulfuric acid test. The IR spectrum showed absorption bands for hydroxyl (3414 cm^{-1}) and carbonyl (1694 cm^{-1}) functionalities.

The ^{1}H and ^{13}C NMR spectral data (**Table 24** and **25**) of compound **PTH8** (**Figure 26** and **27**) and **PTH1** (**Figure 5** and **6**) exhibited the same pattern, except that the two signals of terminal olefinic protons of 2H-29 at δ 4.68 (*d*, *J* = 2.1 Hz) and 4.56 (*m*) and vinylic methyl at δ 1.68 disappeared in **PTH8**. A singlet signal of acetoxy protons was shown at δ 2.15 (3H, *s*) which was not observed in compound **PTH1**. In addition, the ^{13}C NMR spectral data showed carbonyl carbon at δ 212.9. The location of acetoxy protons was assigned to be at C-29 on the basis of HMBC experiment (**Table 23**) of the protons at δ 2.15 (3H-29) which showed long-range correlations with δ 52.6 (C-19) and δ 212.9 (C-20). Therefore, based on the above evidence and comparison with previously reported data, the structure of **PTH8** was assigned as 30-nor-lupan-3β-ol-20-one (Koul *et al*., 2000), (**Table 24 and 25**).

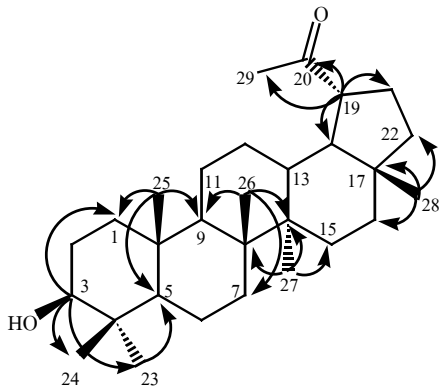
Table 23 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH8**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.7	CH_2	0.89 (*m*), 1.67 (*m*)[a]	-
2	27.4	CH_2	1.49 (*m*), 1.57 (*m*)[a]	-
3	78.9	CH	3.19 (*dd*, *J* = 11.1, 5.1 Hz)	1, 23, 24
4	38.9	C	-	-
5	55.3	CH	0.68 (*m*)[a]	1, 4, 10, 23
6	18.3	CH_2	1.40 (*m*), 1.55 (*m*)[a]	-
7	34.2	CH_2	1.40 (*m*)[a]	-
8	40.7	C	-	-
9	50.3	CH	1.28 (*m*)[a]	-
10	37.2	C	-	-
11	20.9	CH_2	1.28 (*m*), 1.46 (*m*)[a]	-
12	27.2	CH_2	1.06 (*m*)[a]	-
13	37.0	CH	1.59 (*m*)[a]	-
14	42.7	C	-	-
15	27.3	CH_2	1.64 (*m*), 1.70 (*m*)[a]	-
16	35.0	CH_2	1.49 (*m*)[a]	-
17	43.1	C	-	-
18	49.7	CH	1.81 (*t*, *J* = 11.4 Hz)	12, 16, 17, 19, 20, 22, 28
19	52.6	CH	2.58 (*dt*, *J* = 11.4, 5.7 Hz)	13, 18, 20, 21
20	212.9	C	-	-
21	27.6	CH_2	2.05 (*m*)[a]	-
22	39.9	CH_2	1.35 (*m*), 1.49 (*m*)[a]	-
23	28.0	CH_3	0.97 (*s*)	3, 4, 5, 24
24	15.4	CH_3	0.76 (*s*)	3, 4, 5, 23
25	15.9	CH_3	0.82 (*s*)	1, 5, 9, 10

[a] Deduced from HMQC experiment

Table 23 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	16.1	CH_3	1.01 (*s*)	7, 8, 9, 14
27	14.5	CH_3	0.97 (*s*)	8, 13, 14, 15
28	18.0	CH_3	0.77 (*s*)	16, 17, 18, 22
29	29.2	CH_3	2.15 (*s*)	19, 20



Selected HMBC correlation of **PTH8**

Table 24 Comparison of ^{1}H NMR spectral data of 30-nor-lupan-3β-ol-20-one, compounds **PTH1** and **PTH8** (recorded in $CDCl_3$)

Position	30-nor-lupan-3β-ol-20-one, δ_H (ppm)	Compound PTH1, δ_H (ppm)	Compound PTH8, δ_H (ppm)
1	-	0.91 (*m*)[a]	0.89 (*m*), 1.67 (*m*)[a]
2	-	1.56 (*m*)[a]	1.49 (*m*), 1.57 (*m*)[a]
3	3.20 (*dd*, *J* = 7.6, 4.0 Hz)	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	3.19 (*dd*, *J* = 11.1, 5.1 Hz)
5	-	0.69 (*m*)[a]	0.68 (*m*)[a]
6	-	1.40 (*m*), 1.55 (*m*)[a]	1.40 (*m*), 1.55 (*m*)[a]
7	-	1.40 (*m*)[a]	1.40 (*m*)[a]
9	-	1.28 (*m*)[a]	1.28 (*m*)[a]
11	-	1.22 (*m*), 1.45 (*m*)[a]	1.28 (*m*), 1.46 (*m*)[a]
12	-	1.08 (*m*)[a]	1.06 (*m*)[a]
13	-	1.67 (*m*)[a]	1.59 (*m*)[a]
15	-	1.56 (*m*)[a]	1.64 (*m*), 1.70 (*m*)[a]
16	-	1.51 (*m*)[a]	1.49 (*m*)[a]
18	-	1.38 (*m*)[a]	1.81 (*t*, *J* = 11.4 Hz)
19	-	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	2.58 (*dt*, *J* = 11.4, 5.7 Hz)
21	-	1.94 (*m*)[a]	2.05 (*m*)[a]
22	-	1.20 (*m*), 1.40 (*m*)[a]	1.35 (*m*), 1.49 (*m*)[a]
23	0.80 (*s*)	0.97 (*s*)	0.97 (*s*)
24	0.84 (*s*)	0.76 (*s*)	0.76 (*s*)
25	0.88 (*s*)	0.83 (*s*)	0.82 (*s*)
26	1.02 (*s*)	1.03 (*s*)	1.01 (*s*)
27	0.97 (*s*)	0.94 (*s*)	0.97 (*s*)

[a] Deduced from HMQC experiment

Table 24 (Continued)

Position	30-nor-lupan-3β-ol-20-one, δ_H (ppm)	Compound PTH1, δ_H (ppm)	Compound PTH8, δ_H (ppm)
28	0.95 (*s*)	0.79 (*s*)	0.77 (*s*)
29	2.15 (*d*, *J* = 4.6 Hz)	4.56 (*m*), 4.68 (*d*, *J* = 2.1 Hz)	2.15 (*s*)
30	-	1.68 (*s*)	-

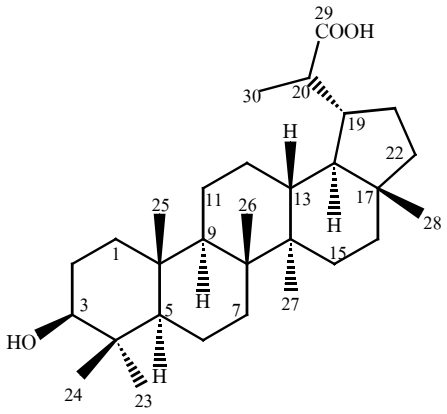
Table 25 Comparison of ^{13}C NMR spectral data of 30-nor-lupan-3β-ol-20-one, compounds **PTH1** and **PTH8** (recorded in $CDCl_3$)

Position	30-nor-lupan-3β-ol-20-one, δ_C (ppm)	Compound PTH1, δ_C (ppm)	Compound PTH8, δ_C (ppm)
1	39.2	38.7	38.7
2	25.2	27.4	27.4
3	76.3	79.0	78.9
4	38.4	38.9	38.9
5	55.2	55.3	55.3
6	18.1	18.3	18.3
7	34.2	34.3	34.2
8	41.1	40.8	40.7
9	50.1	50.5	50.3
10	36.3	37.2	37.2
11	22.6	20.9	20.9
12	28.7	25.2	27.2
13	37.5	38.1	37.0
14	43.6	42.8	42.7
15	27.4	27.5	27.3

Table 25 (Continued)

Position	30-nor-lupan-3β-ol-20-one, δ_C (ppm)	Compound PTH1, δ_C (ppm)	Compound PTH8, δ_C (ppm)
16	35.5	35.6	35.0
17	42.9	43.0	43.1
18	48.2	48.3	49.7
19	47.9	48.0	52.6
20	207.3	151.0	212.9
21	31.0	29.9	27.6
22	40.1	40.0	39.9
23	28.5	28.0	28.0
24	15.4	15.4	15.4
25	16.2	16.1	15.9
26	15.9	16.0	16.1
27	14.5	14.6	14.5
28	18.4	18.0	18.0
29	23.5	109.3	29.2
30	-	19.3	-

3.1.9 Compound PTH9



Compound **PTH9** was isolated as a colorless crystal, mp. 245-247°C, $[\alpha]_D^{28}$: -45.6° (*c* = 0.125, MeOH). It gave a purple vanillin-sulfuric acid test. The IR spectrum showed absorption bands for hydroxyl (3413 cm^{-1}) and carbonyl (1697 cm^{-1}) functionalities.

By comparison of the ^{1}H and ^{13}C NMR spectral data (**Table 27** and **28**) of compounds **PTH9** (**Figure 28** and **29**) and **PTH1** (**Figure 5** amd **6**), the two signals of two terminal olefinic protons at δ 4.68 and 4.56 ppm disappeared in **PTH9** whereas additional signal of H-20 at δ 2.76 ppm and a signal of methyl doublet were displayed at δ 1.13 (3H, *d, J* = 6.9 Hz, 3H-30). The ^{13}C NMR spectral data showed the presence of a carboxyl carbon at δ 180.0 ppm which was assigned to C-29. The location of the carboxyl group was confirmed by HMBC experiment (**Table 26**), from which the methyl protons (3H-30) showed long-range correlation with C-19 (δ 43.4), C-20 (δ 41.8) and C-29 (δ 180.0). Compound **PTH9** was identified as 3β-hydroxylupan-29-oic acid which was previously isolated from *Gymnosporia wallichiana* (Kulshreshtha 1977). The structure of **PTH9** was additionally confirmed by X-ray diffraction (**Figure 2**), (Thongdeeying *et al*., 2005).

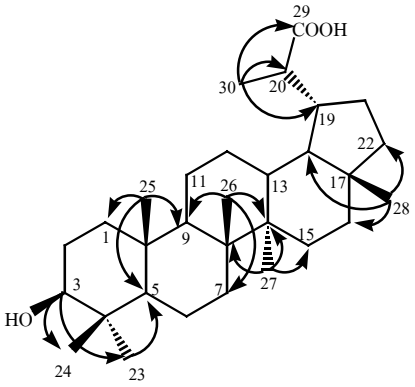
Table 26 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH9**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.7	CH_2	1.70 (*m*)[a]	-
2	27.0	CH_2	1.49 (*m*)[a]	-
3	78.9	CH	3.20 (*dd*, *J* = 10.5, 5.7 Hz)	23, 24
4	38.8	C	-	-
5	55.2	CH	0.69 (*m*)[a]	-
6	18.3	CH_2	1.51 (*m*), 1.38 (*m*)[a]	-
7	34.3	CH_2	1.70 (*m*)[a]	-
8	40.8	C	-	-
9	50.0	CH	1.30 (*m*)[a]	-
10	37.1	C	-	-
11	20.9	CH_2	1.28 (*m*)[a]	-
12	23.7	CH_2	1.76 (*m*), 1.71(*m*)[a]	-
13	37.7	CH	1.71 (*m*)[a]	-
14	43.0	C	-	-
15	27.3	CH_2	1.66 (*m*)[a]	-
16	35.4	CH_2	1.29 (*m*)[a]	-
17	43.0	C	-	-
18	48.5	CH	1.41 (*m*)[a]	-
19	43.4	CH	1.75 (*m*)[a]	-
20	41.8	CH	2.76 (*m*)	-
21	27.1	CH_2	1.55 (*m*)[a]	-
22	39.6	CH_2	1.30 (*m*)[a]	-
23	27.9	CH_3	0.97 (*s*)	3, 4, 5, 24
24	15.3	CH_3	0.77 (*s*)	3, 4, 5, 23
25	16.0	CH_3	0.84 (*s*)	1, 5, 9, 10

[a] Deduced from HMQC experiment

Table 26 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	16.0	CH_3	1.04 (*s*)	7, 8, 9, 14
27	14.3	CH_3	0.92 (*s*)	8, 13, 14, 15
28	17.7	CH_3	0.75 (*s*)	16, 17, 18, 22
29	180.0	C	-	-
30	17.3	CH_3	1.13 (*d*, *J* = 6.9 Hz)	19, 20, 29



Selected HMBC correlation of **PTH9**

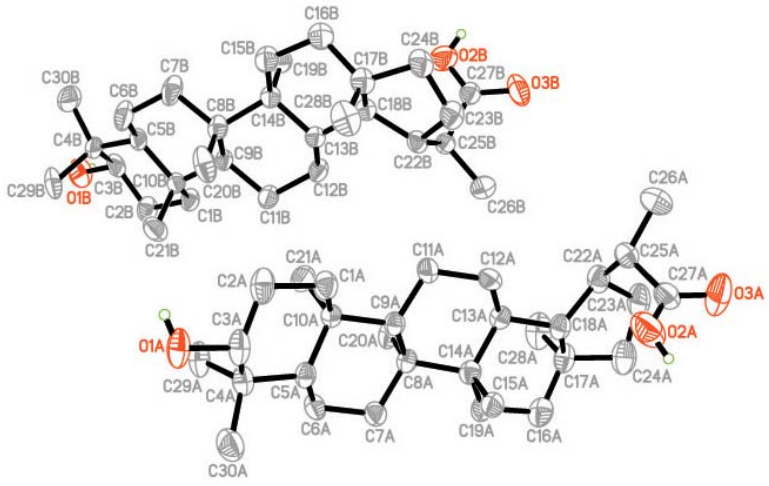


Figure 2 X-ray ORTEP diagram of compound **PTH9**. For clarity, H atoms have been omitted, except hydroxyl H atoms.

Table 27 Comparison of ^{1}H NMR spectral data between compounds **PTH1** and **PTH9**

Position	**Compound PTH1, δ_H (ppm)** (recorded in $CDCl_3$)	**Compound PTH9, δ_H (ppm)** (recorded in $CDCl_3$+ CD_3OD)
1	0.91 (*m*)[a]	1.70 (*m*)[a]
2	1.56 (*m*)[a]	1.49 (*m*)[a]
3	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	3.20 (*dd*, *J* = 10.5, 5.7 Hz)
5	0.69 (*m*)[a]	0.69 (*m*)[a]
6	1.40 (*m*), 1.55 (*m*)[a]	1.51 (*m*), 1.38 (*m*)[a]
7	1.40 (*m*)[a]	1.70 (*m*)[a]
9	1.28 (*m*)[a]	1.30 (*m*)[a]
11	1.22 (*m*), 1.45 (*m*)[a]	1.28 (*m*)[a]
12	1.08 (*m*)[a]	1.76 (*m*), 1.71(*m*)[a]
13	1.67 (*m*)[a]	1.71 (*m*)[a]
15	1.56 (*m*)[a]	1.66 (*m*)[a]
16	1.51 (*m*)[a]	1.29 (*m*)[a]
18	1.38 (*m*)[a]	1.41 (*m*)[a]
19	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	1.75 (*m*)[a]
20	-	2.76 (*m*)
21	1.94 (*m*)[a]	1.55 (*m*)[a]
22	1.20 (*m*), 1.40 (*m*)[a]	1.30 (*m*)[a]
23	0.97 (*s*)	0.97 (*s*)
24	0.76 (*s*)	0.77 (*s*)
25	0.83 (*s*)	0.84 (*s*)
26	1.03 (*s*)	1.04 (*s*)
27	0.94 (*s*)	0.92 (*s*)

[a] Deduced from HMQC experiment

Table 27 (Continued)

Position	Compound PTH1, δ_H (ppm) (recorded in $CDCl_3$)	Compound PTH9, δ_H (ppm) (recorded in $CDCl_3$+ CD_3OD)
28	0.79 (*s*)	0.75 (*s*)
29	4.56 (*m*), 4.68 (*d*, *J* = 2.1 Hz)	-
30	1.68 (*s*)	1.13 (*d*, *J* = 6.9 Hz)

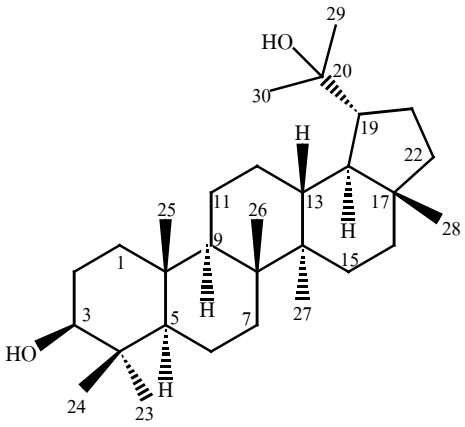
Table 28 Comparison of ^{13}C NMR spectral data between compounds **PTH1** and **PTH9**

Position	Compound PTH1, δ_C (ppm) (recorded in $CDCl_3$)	Compound PTH9, δ_C (ppm) (recorded in $CDCl_3$+CD_3OD)
1	38.7	38.7
2	27.4	27.0
3	79.0	78.9
4	38.9	38.8
5	55.3	55.2
6	18.3	18.3
7	34.3	34.3
8	40.8	40.8
9	50.5	50.0
10	37.2	37.1
11	20.9	20.9
12	25.2	23.7
13	38.1	37.7
14	42.8	43.0
15	27.5	27.3
16	35.6	35.4

Table 28 (Continued)

Position	**Compound PTH1, δ_C (ppm)** (recorded in $CDCl_3$)	**Compound PTH9, δ_C (ppm)** (recorded in $CDCl_3$+CD_3OD)
17	43.0	43.0
18	48.3	48.5
19	48.0	43.4
20	151.0	41.8
21	29.9	27.1
22	40.0	39.6
23	28.0	27.9
24	15.4	15.3
25	16.1	16.0
26	16.0	16.0
27	14.6	14.3
28	18.0	17.7
29	109.3	180.0
30	19.3	17.3

3.1.10 Compound PTH10



Compound **PTH10** was isolated as a white solid, mp.: 210-212°C, $[\alpha]_D^{28}$: +6.4° (*c* = 0.078, $CHCl_3$). It gave a purple vanillin-sulfuric acid test. The IR spectrum showed absorption bands similar to compound **PTH1**.

By comparison of the ^1H and ^{13}C NMR spectral data (**Table 30** and **31**) of compound **PTH10** (**Figure 30** and **31**) and **PTH1** (**Figure 5** and **6**), the signals of the two terminal olefinic protons at δ 4.68 (*br d*, *J* = 2.1 Hz) and 4.56 (*m*) were not observed in **PTH10** and eight methyl singlets were displayed at δ 0.76, 0.81, 0.84, 0.96, 0.97, 1.06, 1.12 and 1.23. The ^{13}C NMR spectral data (**Table 29**, **Figure 31**) showed an additional signal of an oxyquaternary carbon at δ 73.5 which was located at C-20 based on HMBC experiment (**Table 29**) in which 3H-30 (δ 1.23) showed long-range correlation with C-20 (δ 73.5), C-19 (δ 50.0) and C-29 (δ 24.8). On the basis of its spectroscopic data and comparison with those reported in the literature (Yuruker *et al*., 1998) (**Table 30** and **31**), compound **PTH10** was assigned to be 3β, 20-dihydroxylupane.

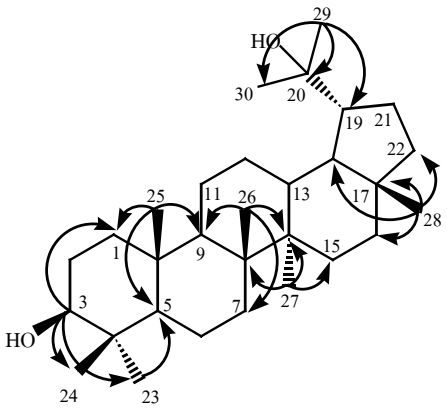
Table 29 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH10**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.7	CH_2	1.70 (*m*)[a]	-
2	27.6	CH_2	1.59 (*m*)[a]	-
3	79.0	CH	3.20 (*dd*, *J* = 10.8, 5.4 Hz)	1, 4, 23, 24
4	38.9	C	-	-
5	55.2	CH	0.70 (*m*)[a]	-
6	18.4	CH_2	1.42 (*m*), 1.56 (*m*)[a]	-
7	34.6	CH_2	1.42 (*m*)[a]	-
8	41.4	C	-	-
9	50.3	CH	1.28 (*m*)[a]	-
10	37.1	C	-	-
11	21.4	CH_2	1.27 (*m*), 1.49 (*m*)[a]	-
12	27.4	CH_2	1.79 (*m*)[a]	-
13	37.5	CH	1.75 (*m*)[a]	-
14	43.5	C	-	-
15	28.8	CH_2	1.94 (*m*)[a]	-
16	35.6	CH_2	1.55 (*m*)[a]	-
17	44.7	C	-	-
18	48.3	CH	1.35 (*m*)[a]	-
19	50.0	CH	1.81 (*m*)[a]	-
20	73.5	C	-	-
21	29.1	CH_2	1.83 (*m*), 1.89 (*m*)[a]	-
22	40.2	CH_2	1.11 (*m*), 1.36 (*m*)[a]	-
23	28.0	CH_3	0.97 (*s*)	3, 4, 5, 24
24	15.4	CH_3	0.76 (*s*)	3, 4, 5, 23
25	16.2	CH_3	0.84 (*s*)	1, 5, 9, 10

[a] Deduced from HMQC experiment

Table 29 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	16.2	CH_3	1.06 (*s*)	7, 8, 9, 14
27	14.9	CH_3	0.96 (*s*)	8, 13, 14, 15
28	19.2	CH_3	0.81 (*s*)	16, 17, 18, 22
29	24.8	CH_3	1.12 (s)	19, 20, 30
30	31.6	CH_3	1.23 (*s*)	19, 20, 29



Selected HMBC correlation of **PTH10**

Table 30 Comparison of ^{1}H NMR spectral data of compounds **PTH1** and **PTH10** (recorded in $CDCl_3$)

Position	Compound PTH1, δ_H (ppm)	Compound PTH10, δ_H (ppm)
1	0.91 (*m*)[a]	1.70 (*m*)[a]
2	1.56 (*m*)[a]	1.59 (*m*)[a]
3	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	3.20 (*dd*, *J* = 10.8, 5.4 Hz)
5	0.69 (*m*)[a]	0.70 (*m*)[a]
6	1.40 (*m*), 1.55 (*m*)[a]	1.42 (*m*), 1.56 (*m*)[a]
7	1.40 (*m*)[a]	1.42 (*m*)[a]
9	1.28 (*m*)[a]	1.28 (*m*)[a]
11	1.22 (*m*), 1.45 (*m*)[a]	1.27 (*m*), 1.49 (*m*)[a]
12	1.08 (*m*)[a]	1.79 (*m*)[a]
13	1.67 (*m*)[a]	1.75 (*m*)[a]
15	1.56 (*m*)[a]	1.94 (*m*)[a]
16	1.51 (*m*)[a]	1.55 (*m*)[a]
18	1.38 (*m*)[a]	1.35 (*m*)[a]
19	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	1.81 (*m*)[a]
21	1.94 (*m*)[a]	1.83 (*m*), 1.89 (*m*)[a]
22	1.20 (*m*), 1.40 (*m*)[a]	1.11 (*m*), 1.33 (*m*)[a]
23	0.97 (*s*)	0.97 (*s*)
24	0.76 (*s*)	0.76 (*s*)
25	0.83 (*s*)	0.84 (*s*)
26	1.03 (*s*)	1.06 (*s*)
27	0.94 (*s*)	0.96 (*s*)
28	0.79 (*s*)	0.81 (*s*)
29	4.56 (*m*), 4.68 (*d*, *J* = 2.1 Hz)	1.12 (s)
30	1.68 (*s*)	1.23 (*s*)

[a] Deduced from HMQC experiment

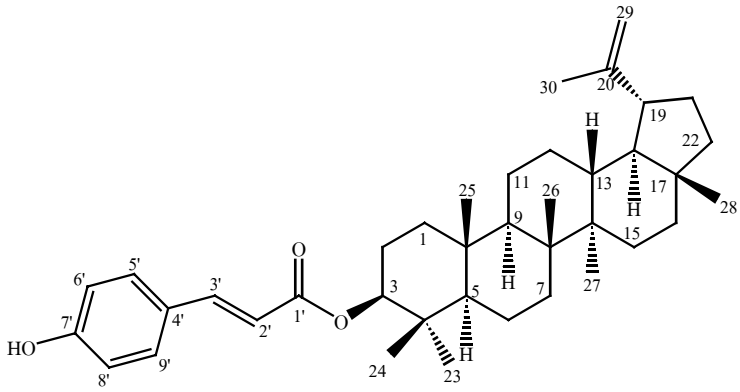
Table 31 Comparison of ^{13}C NMR spectral data of 3β, 20-dihydroxylupane, compounds **PTH1** and **PTH10** (recorded in $CDCl_3$)

Position	3β, 20-dihydroxy-lupane, δ_C (ppm)	Compound PTH1, δ_C (ppm)	Compound PTH10, δ_C (ppm)
1	38.7	38.7	38.7
2	27.6	27.4	27.6
3	79.0	79.0	79.0
4	38.9	38.9	38.9
5	55.2	55.3	55.2
6	18.3	18.3	18.4
7	34.6	34.3	34.6
8	41.4	40.8	41.4
9	50.3	50.5	50.3
10	37.1	37.2	37.1
11	21.4	20.9	21.4
12	27.4	25.2	27.4
13	37.5	38.1	37.5
14	43.6	42.8	43.5
15	28.8	27.5	28.8
16	35.6	35.6	35.6
17	44.7	43.0	44.7
18	48.3	48.3	48.3
19	50.0	48.0	50.0
20	73.5	151.0	73.5
21	29.1	29.9	29.1
22	40.2	40.0	40.2
23	28.0	28.0	28.0
24	16.2	15.4	15.4

Table 31 (Continued)

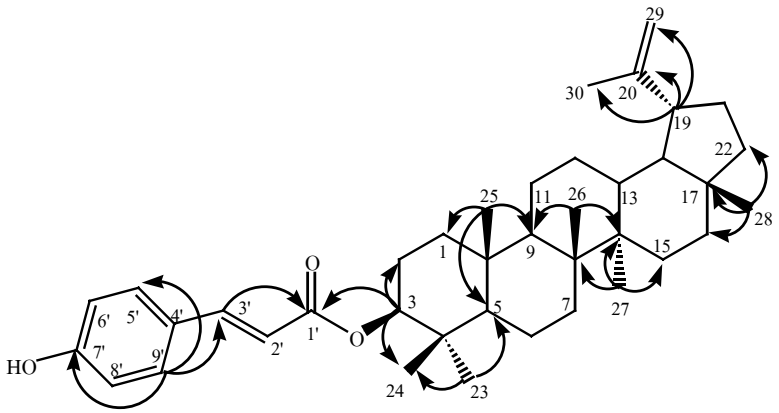
Position	3β, 20-dihydroxy-lupane, δ_C (ppm)	Compound PTH1, δ_C (ppm)	Compound PTH10, δ_C (ppm)
25	15.4	16.1	16.2
26	16.3	16.0	16.2
27	14.9	14.6	14.9
28	19.2	18.0	19.2
29	24.8	109.3	24.8
30	31.6	19.3	31.6

3.1.11 Compound PTH11



Compound **PTH11** was isolated as a white solid, mp. 166-167°C, $[\alpha]_D^{28}$: +200.0° (*c* = 0.050, $CHCl_3$). The IR spectrum (**Figure 33**) suggested hydroxyl (3397 cm^{-1}), conjugated ester (1670 cm^{-1}) and double bond (1602 cm^{-1}) functionalities. The UV absorption maxima at 227 and 313 nm (**Figure 32**), again suggested the presence of conjugation in the molecule. It gave a purple vanillin-sulfuric acid test.

The ^{1}H and ^{13}C NMR spectral data of compound **PTH11** (**Figure 34** and **35**) and **PTH1** (**Figure 5** and **6**) exhibited the same pattern (**Table 33** and **34**). The difference was shown in the compound **PTH11** which displayed additional signals due to the presence of *trans*-coumaroyl substituent as two *para*-disubstituted aromatic protons at δ 7.41 and 6.85 (each *d*, *J* = 8.7 Hz, H-5′, H-9′ and H-6′, H-8′, respectively) and two *trans* olefinic protons at δ 7.61 (H-3′) and 6.29 (H-2′) as a doublet with coupling constant 15.9 Hz. The oxymethine proton (H-3) was shown at δ 4.62 (*m*) which was shifted more downfield than compound **PTH1** as a result of the ester substituent at C-3. The ^{13}C NMR spectral data of compound **PTH11** (**Table 32**, **Figure 35**) suggested the presence of an ester group as a signal at δ 167.8, which was confirmed by HMBC experiment (**Table 32**), from which the oxymethine proton H-3 showed long-range correlation with C-1′ (δ 167.8), C-4 (δ 38.1), C-23 (δ 28.0) and C-24 (δ 16.2). Thus compound **PTH11** was identified as 3β-*E*-coumaroyllupeol by comparison of its spectral data with those reported data (Ali *et al*., 1997), (**Table 33** and **34**).



Selected HMBC correlation of **PTH11**

Table 32 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH11**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.4	CH_2	1.70 (*m*)[a]	-
2	23.9	CH_2	1.70 (*m*)[a]	-
3	81.2	CH	4.62 (*m*)	1′, 2, 23, 24
4	38.1	C	-	-
5	55.4	CH	0.82 (*m*)[a]	-
6	18.2	CH_2	1.42 (*m*), 1.55 (*m*)[a]	-
7	34.2	CH_2	1.42 (*m*)[a]	-
8	40.9	C	-	-
9	50.4	CH	1.31 (*m*)[a]	-
10	37.1	C	-	-
11	21.0	CH_2	1.47 (*m*)[a]	-
12	25.1	CH_2	1.71 (*m*)[a]	-
13	38.1	CH	1.63 (*m*)[a]	-
14	42.9	C	-	-
15	27.5	CH_2	1.04 (*m*)[a]	-
16	35.6	CH_2	1.48 (*m*), 1.53 (*m*)[a]	-
17	43.0	C	-	-
18	48.3	CH	1.38 (*m*)[a]	-
19	48.0	CH	2.38 (*dt*, J = 10.5, 5.4 Hz)	20, 29, 30
20	151.0	C	-	-
21	29.9	CH_2	1.89 (*m*), 1.95 (*m*)[a]	-
22	40.0	CH_2	1.20 (*m*), 1.41 (*m*)[a]	-
23	28.0	CH_3	0.89 (*s*)	3, 4, 5, 24
24	16.7	CH_3	0.92 (*s*)	3, 4, 5, 23
25	16.2	CH_3	0.88 (*s*)	1, 5, 9, 10
26	16.0	CH_3	1.04 (*s*)	7, 8, 9, 14

[a] Deduced from HMQC experiment

Table 32 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
27	14.6	CH_3	0.95 (*s*)	8, 13, 14, 15
28	18.0	CH_3	0.79 (*s*)	16, 17, 18, 22
29	109.4	CH_2	4.58 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	19, 30
30	19.3	CH_3	1.69 (*s*)	19, 20, 29
1′	167.8	C	-	-
2′	115.9	CH	6.29 (*d*, *J* = 15.9 Hz)	1′, 3′, 4′
3′	144.4	CH	7.61 (*d*, *J* = 15.9 Hz)	1′, 2′, 5′, 9′
4′	127.0	C	-	-
5′	130.0	CH	7.41 (*d*, *J* = 8.7 Hz)	3′, 7′, 9′
6′	116.0	CH	6.85 (*d*, *J* = 8.7 Hz)	4′, 7′, 8′
7′	158.1	C	-	-
8′	116.0	CH	6.85 (*d*, *J* = 8.7 Hz)	4′, 6′, 7′
9′	130.0	CH	7.41 (*d*, *J* = 8.7 Hz)	3′, 5′, 7′

Table 33 Comparison of ^{1}H NMR spectral data of 3β-*E*-coumaroyllupeol, compounds **PTH1** and **PTH11** (recorded in $CDCl_3$)

Position	3β-*E*-coumaroyl-lupeol, δ_H (ppm)	Compound PTH1, δ_H (ppm)	Compound PTH11, δ_H (ppm)
1	1.00 (*m*), 1.66 (*m*)	0.91 (*m*) [a]	1.70 (*m*) [a]
2	1.59 (*m*), 1.67 (*m*)	1.56 (*m*) [a]	1.70 (*m*) [a]
3	4.56 (*m*)	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	4.62 (*m*) [a]
5	0.81 (*m*)	0.69 (*m*) [a]	0.82 (*m*) [a]
6	0.74 (*m*), 1.38 (*m*)	1.40 (*m*), 1.55 (*m*) [a]	1.42 (*m*), 1.55 (*m*) [a]
7	1.38 (*m*), 1.42 (*m*)	1.40 (*m*) [a]	1.42 (*m*) [a]
9	1.25 (*m*)	1.28 (*m*) [a]	1.31 (*m*) [a]

Table 33 (Continued)

Position	3β-*E*-coumaroyl-lupeol, δ_H (ppm)	Compound PTH1, δ_H (ppm)	Compound PTH11, δ_H (ppm)
11	1.28 (*m*), 1.40 (*m*)	1.22 (*m*), 1.45 (*m*) [a]	1.47 (*m*) [a]
12	1.08 (*m*), 1.66 (*m*)	1.08 (*m*) [a]	1.71 (*m*) [a]
13	1.35 (*m*)	1.67 (*m*) [a]	1.63 (*m*) [a]
15	0.85 (*m*), 0.90 (*m*)	1.56 (*m*) [a]	1.04 (*m*) [a]
16	1.32 (*m*), 1.40 (*m*)	1.51 (*m*) [a]	1.48 (*m*), 1.53 (*m*) [a]
18	1.39 (*m*)	1.38 (*m*) [a]	1.38 (*m*) [a]
19	2.36 (*m*)	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	2.38 (*dt*, *J* = 10.5, 5.4 Hz)
21	1.23 (*m*), 1.30 (*m*)	1.94 (*m*) [a]	1.89 (*m*), 1.95 (*m*) [a]
22	1.15 (*m*), 1.36 (*m*)	1.20 (*m*), 1.40 (*m*) [a]	1.20 (*m*), 1.41 (*m*) [a]
23	0.94 (*s*)	0.97 (*s*)	0.89 (*s*)
24	1.03 (*s*)	0.76 (*s*)	0.92 (*s*)
25	0.87 (*s*)	0.83 (*s*)	0.88 (*s*)
26	0.94 (*s*)	1.03 (*s*)	1.04 (*s*)
27	0.90 (*s*)	0.94 (*s*)	0.95 (*s*)
28	0.76 (*s*)	0.79 (*s*)	0.79 (*s*)
30	1.67 (*s*)	1.68 (*s*)	1.69 (*s*)
2′	6.29 (*d*, *J* = 16.2 Hz)	-	6.29 (*d*, *J* = 15.9 Hz)
3′	7.57 (*d*, *J* = 16.2 Hz)	-	7.61 (*d*, *J* = 15.9 Hz)
5′, 9′	7.42 (*d* , *J* = 8.7 Hz)	-	7.41 (*d*, *J* = 8.7 Hz)
6′, 8′	6.82 (*d*, *J* = 8.7 Hz)	-	6.85 (*d*, *J* = 8.7 Hz)

[a] Deduced from HMQC experiment

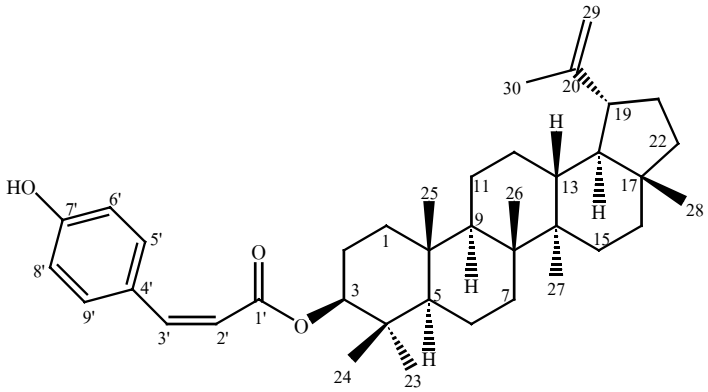
Table 34 Comparison of ^{13}C NMR spectral data of 3β-*E*-coumaroyllupeol, compounds **PTH1** and **PTH11** (recorded in $CDCl_3$)

Position	3β-*E*-coumaroyl-lupeol, δ_C (ppm)	Compound PTH1, δ_C (ppm)	Compound PTH11, δ_C (ppm)
1	38.5	38.7	38.4
2	23.9	27.4	23.9
3	80.9	79.0	81.2
4	38.1	38.9	38.1
5	55.5	55.3	55.4
6	18.3	18.3	18.2
7	34.3	34.3	34.2
8	40.9	40.8	40.9
9	50.4	50.5	50.4
10	37.2	37.2	37.1
11	21.0	20.9	21.0
12	25.2	25.2	25.1
13	38.1	38.1	38.1
14	42.9	42.8	42.9
15	27.5	27.5	27.5
16	35.6	35.6	35.6
17	43.0	43.0	43.0
18	48.3	48.3	48.3
19	48.0	48.0	48.0
20	151.0	151.0	151.0
21	29.9	29.9	29.9
22	40.0	40.0	40.0
23	28.0	28.0	28.0
24	16.0	15.4	16.7

Table 34 (Continued)

Position	3β-*E*-coumaroyl-lupeol, δ_C (ppm)	Compound PTH1, δ_C (ppm)	Compound PTH11, δ_C (ppm)
25	16.7	16.1	16.2
26	16.2	16.0	16.0
27	14.6	14.6	14.6
28	18.0	18.0	18.0
29	109.4	109.3	109.4
30	19.3	19.3	19.3
1′	167.2	-	167.8
2′	116.5	-	115.9
3′	143.8	-	144.4
4′	127.6	-	127.0
5′, 9′	129.9	-	130.0
6′, 8′	115.8	-	116.0
7′	157.4	-	158.1

3.1.12 Compound PTH12



Compound **PTH12** was obtained as a colorless viscous oil. $[\alpha]_D^{28}$: +38.5° (*c* = 0.052, $CHCl_3$). The absorption bands for UV and IR spectrum were similar to compound **PTH11**.

The ^{1}H NMR spectral data (**Table 36**) of compound **PTH12** (**Figure 36**) and **PTH11** (**Figure 34**) showed structural similarity, except for the olefinic proton signals at δ 6.82 and 5.83 which were shown as a doublet with small *J* value (12.9 Hz), suggesting that the double bond should have a *Z* geometry. An oxymethine proton H-3 was shown as a doublet of doublet with *J* = 11.1 and 4.8 Hz indicating it to be an α-proton. On the basis of HMBC (**Table 35**), the *Z*- coumaroyl moiety was located at C-3 by correlation of H-3 signal at δ 4.52 with C-1′ (δ 166.5), C-23 (δ 28.0) and C-24 (δ 16.5). Thus compound **PTH12** was assigned as 3β-*Z*-coumaroyllupeol by comparison of its spectral data with those of **PTH11** (**Table 36** and **37**).

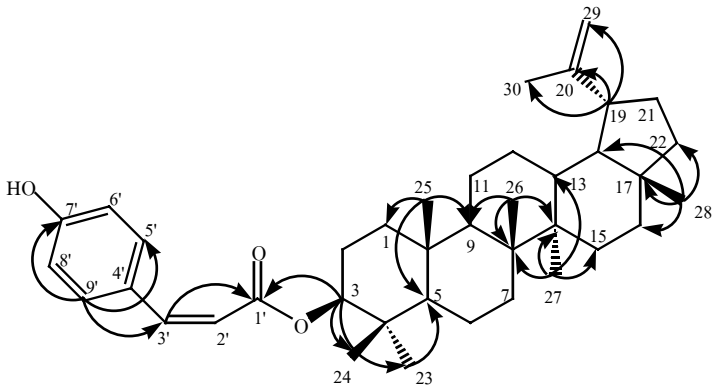
Table 35 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH12**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.4	CH_2	1.01 (*m*) [a]	-
2	23.7	CH_2	1.42 (*m*) [a]	-
3	81.1	CH	4.52 (*dd*, *J* = 11.1, 4.8 Hz)	1′, 23, 24
4	37.9	C	-	-
5	55.5	CH	0.82 (*m*) [a]	-
6	18.2	CH_2	1.40 (*m*), 1.54 (*m*) [a]	-
7	34.2	CH_2	1.41 (*m*) [a]	-
8	40.9	C	-	-
9	50.4	CH	1.31 (*m*) [a]	-
10	37.1	C	-	-
11	21.0	CH_2	1.15 (*m*) [a]	-
12	25.1	CH_2	1.10 (*m*), 1.67 (*m*) [a]	-
13	38.1	CH	1.70 (*m*) [a]	-
14	42.8	C	-	-
15	27.5	CH_2	1.03 (*m*) [a]	-
16	35.6	CH_2	1.55 (*m*) [a]	-
17	43.0	C	-	-
18	48.3	CH	1.38 (*m*) [a]	-
19	48.0	CH	2.37 (*dt*, *J* = 11.1, 5.7 Hz)	20, 29, 30
20	151.0	C	-	-
21	29.9	CH_2	1.27 (*m*) [a]	19, 30
22	40.0	CH_2	1.20 (*m*), 1.40 (*m*) [a]	-
23	28.0	CH_3	0.86 (*s*)	3, 4, 5, 24
24	16.5	CH_3	0.80 (*s*)	3, 4, 5, 23

[a] Deduced from HMQC experiment

Table 35 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
25	16.0	CH_3	0.86 (*s*)	1, 5, 9, 10
26	16.2	CH_3	1.03 (*s*)	7, 8, 9, 14
27	14.5	CH_3	0.94 (*s*)	8, 13, 14, 15
28	18.0	CH_3	0.79 (*s*)	16, 17, 18, 22
29	109.3	CH_2	4.57 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	19, 30
30	19.3	CH_3	1.69 (*s*)	19, 20, 29
1′	166.5	C	-	-
2′	117.9	CH	5.83 (*d*, *J* = 12.6 Hz)	1′, 3′, 4′
3′	143.1	CH	6.82 (*d*, *J* = 12.6 Hz)	1′, 5′, 9′
4′	127.9	C	-	-
5′	132.3	CH	7.62 (*d*, *J* = 8.7 Hz)	3′, 7′, 9′
6′	115.0	CH	6.78 (*d*, *J* = 8.7 Hz)	4′, 7′, 8′
7′	156.6	C	-	-
8′	115.0	CH	6.78 (*d*, *J* = 8.7 Hz)	4′, 6′, 7′
9′	132.3	CH	7.62 (*d*, *J* = 8.7 Hz)	3′, 5′, 7′



Selected HMBC correlation of **PTH12**

Table 36 Comparison of ^{1}H NMR spectral data between compounds **PTH11** and **PTH12** (recorded in $CDCl_3$)

Position	Compound PTH11, δ_H (ppm)	Compound PTH12, δ_H (ppm)
1	1.70 (*m*)[a]	1.01 (*m*)[a]
2	1.70 (*m*)[a]	1.42 (*m*)[a]
3	4.62 (*m*)	4.52 (*dd*, *J* = 11.1, 4.8 Hz)
5	0.82 (*m*)[a]	0.82 (*m*)[a]
6	1.42 (*m*), 1.55 (*m*)[a]	1.40 (*m*), 1.54 (*m*)[a]
7	1.42 (*m*)[a]	1.41 (*m*)[a]
9	1.31 (*m*)[a]	1.31 (*m*)[a]
11	1.47 (*m*)[a]	1.15 (*m*)[a]
12	1.71 (*m*)[a]	1.10 (*m*), 1.67 (*m*)[a]
13	1.63 (*m*)[a]	1.70 (*m*)[a]
15	1.04 (*m*)[a]	1.03 (*m*)[a]
16	1.48 (*m*), 1.53 (*m*)[a]	1.55 (*m*)[a]
18	1.38 (*m*)[a]	1.38 (*m*)[a]
19	2.38 (*dt*, *J* = 10.5, 5.4 Hz)	2.37 (*dt*, *J* = 11.1, 5.7 Hz)
21	1.89 (*m*), 1.95 (*m*)[a]	1.27 (*m*)[a]
22	1.20 (*m*), 1.41 (*m*)[a]	1.20 (*m*), 1.40 (*m*)[a]
23	0.89 (*s*)	0.86 (*s*)
24	0.92 (*s*)	0.80 (*s*)
25	0.88 (*s*)	0.86 (*s*)
26	1.04 (*s*)	1.03 (*s*)
27	0.95 (*s*)	0.94 (*s*)
28	0.79 (*s*)	0.79 (*s*)
29	4.58 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	4.57 (*m*), 4.69 (*d*, *J* = 2.1 Hz)

[a] Deduced from HMQC experiment

Table 36 (Continued)

Position	Compound PTH11, δ_H (ppm)	Compound PTH12, δ_H (ppm)
30	1.69 (*s*)	1.69 (*s*)
2′	6.29 (*d*, *J* = 15.9 Hz)	5.83 (*d*, *J* = 12.6 Hz)
3′	7.61 (*d*, *J* = 15.9 Hz)	6.82 (*d*, *J* = 12.6 Hz)
5′, 9′	7.41 (*d*, *J* = 8.7 Hz)	7.62 (*d*, *J* = 8.7 Hz)
6′, 8′	6.85 (*d*, *J* = 8.7 Hz)	6.78 (*d*, *J* = 8.7 Hz)

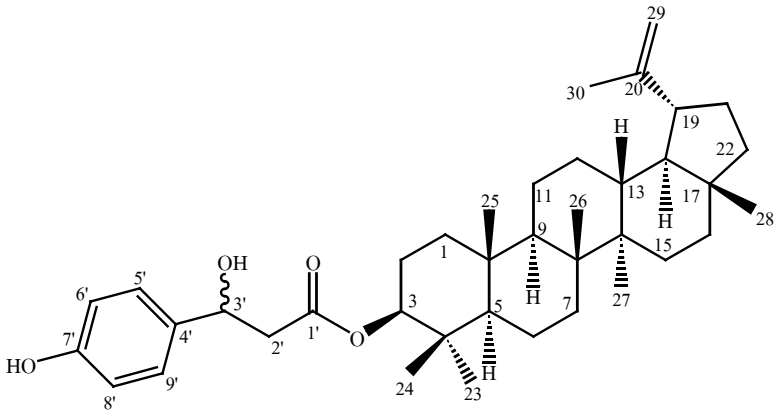
Table 37 Comparison of ^{13}C NMR spectral data between compounds **PTH11** and **PTH12** (recorded in $CDCl_3$)

Position	Compound PTH11, δ_C (ppm)	Compound PTH12, δ_C (ppm)
1	38.4	38.4
2	23.9	23.7
3	81.2	81.1
4	38.1	37.9
5	55.4	55.5
6	18.2	18.2
7	34.2	34.2
8	40.9	40.9
9	50.4	50.4
10	37.1	37.1
11	21.0	21.0
12	25.1	25.1
13	38.1	38.1
14	42.9	42.8
15	27.5	27.5

Table 37 (Continued)

Position	Compound PTH11, δ_C (ppm)	Compound PTH12, δ_C (ppm)
16	35.6	35.6
17	43.0	43.0
18	48.3	48.3
19	48.0	48.0
20	151.0	151.0
21	29.9	29.9
22	40.0	40.0
23	28.0	28.0
24	16.7	16.5
25	16.2	16.0
26	16.0	16.2
27	14.6	14.5
28	18.0	18.0
29	109.4	109.3
30	19.3	19.3
1′	167.8	166.5
2′	115.9	117.9
3′	144.4	143.1
4′	127.0	127.9
5′, 9′	130.0	132.3
6′, 8′	116.0	115.0
7′	158.1	156.6

3.1.13 Compound PTH13



Compound **PTH13** was isolated as a white solid. It gave a purple vanillin-sulfuric acid test. Its EI-MS mass spectrum (**Figure 43**) showed the $[M\text{-}H_2O]^+$ ion peak at *m/z* 572.4187, corresponding to the molecular formula $C_{39}H_{56}O_3$. The melting point was not reported due to decomposition of the compound.

The ^{1}H NMR and DEPT spectra (**Table 39** and **40**, **Figure 38**, **39** and **40**) were similar to those of **PTH12** (**Figure 36** and **37**), except for the *para*-disubstituted aromatic protons at δ 7.25 (2H, *d*, *J* = 8.7 Hz, H-5′, H-9′) of **PTH13** were shifted upfield than **PTH12** (δ 7.62). The two *trans*-olefinic protons at δ 5.83 (H-2′) and 6.82 (H-3′) were replaced by an oxymethine proton at δ 5.07 (1H, *m*, H-3′) and two methylene protons at δ 2.73 (2H, *m*, H-2′). The assignment was confirmed by COSY spectrum. The connectivities of coumaroyl moiety was assigned by HMBC experiment (**Table 38**, **Figure 42**) in which the oxymethine proton H-3 (δ 4.53, *m*) was located at C-3 (δ 81.7) by correlation with C-1′ (δ 172.2), C-23 (δ 27.9) and C-24 (δ 16.2) and the oxymethine proton H-3′ (δ 5.07) showed long-range correlations with C-1′ (δ 172.2), C-2′ (δ 43.6), C-4′ (δ 135.0) and C-5′, C-9′ (δ 127.3). Compound **PTH13** was postulated as 3β-(3′,7′-dihydroxy)dihydrocinnamoyl lupeol, a new compound.

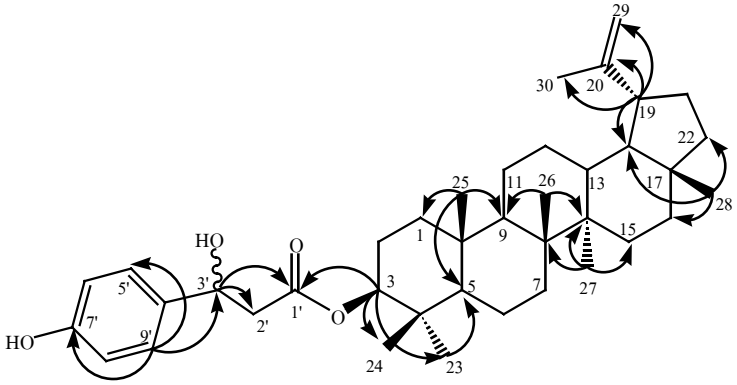
Table 38 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH13**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.4	CH_2	-	-
2	23.7	CH_2	-	-
3	81.7	CH	4.53 (*m*)	1′, 23, 24
4	38.0[b]	C	-	-
5	55.4	CH	-	-
6	18.2	CH_2	-	-
7	34.2	CH_2	-	-
8	41.0[b]	C	-	-
9	50.3	CH	-	-
10	37.4[b]	C	-	-
11	21.0	CH_2	-	-
12	25.1	CH_2	-	-
13	38.0	CH	-	-
14	43.0[b]	C	-	-
15	27.4	CH_2	-	-
16	35.6	CH_2	-	-
17	43.0[b]	C	-	-
18	48.3	CH	-	-
19	48.0	CH	2.38 (*m*)	13, 18, 20, 29
20	151.0[b]	C	-	-
21	29.8	CH_2	-	-
22	40.0	CH_2	-	-
23	27.9	CH_3	0.81 (*s*)	3, 4, 5, 24
24	16.2	CH_3	0.81 (*s*)	3, 4, 5, 23
25	16.6	CH_3	0.85 (*s*)	1, 5, 9, 10
26	16.0	CH_3	1.03 (*s*)	7, 8, 9, 14
27	14.5	CH_3	0.94 (*s*)	8, 13, 14, 15

Table 38 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
28	18.0	CH_3	0.79 (*s*)	16, 17, 18, 22
29	109.4	CH_2	4.57 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	19
30	19.3	CH_3	1.69 (*s*)	19, 20, 29
1′	172.0 [b]	C	-	-
2′	43.6	CH_2	2.73 (*m*)	1′, 3′, 4′
3′	70.1	CH	5.07 (*m*)	1′, 2′, 5′, 9′
4′	135.0 [b]	C	-	-
5′	127.3	CH	7.25 (*d*, *J* = 8.7 Hz)	3′, 7′, 9′
6′	115.3	CH	6.80 (*d*, *J* = 8.7 Hz)	4′, 7′, 8′
7′	155.0 [b]	C	-	-
8′	115.3	CH	6.80 (*d*, *J* = 8.7 Hz)	4′, 6′, 7′
9′	127.3	CH	7.25 (*d*, *J* = 8.7 Hz)	3′, 5′, 7′

[b] Quaternary carbons deduced from DEPT 90°, 135° and HMBC experiments



Selected HMBC correlation of **PTH13**

Table 39 Comparison of ^{1}H NMR spectral data between compounds **PTH12** and **PTH13** (recorded in $CDCl_3$)

Position	Compound PTH12, δ_H (ppm)	Compound PTH13, δ_H (ppm)
3	4.52 (*dd*, *J* = 11.1, 4.8 Hz)	4.53 (*m*)
19	2.37 (*dt*, *J* = 11.1, 5.7 Hz)	2.38 (*m*)
23	0.86 (*s*)	0.81 (*s*)
24	0.80 (*s*)	0.81 (*s*)
25	0.86 (*s*)	0.85 (*s*)
26	1.03 (*s*)	1.03 (*s*)
27	0.94 (*s*)	0.94 (*s*)
28	0.79 (*s*)	0.79 (*s*)
29	4.57 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	4.57 (*m*), 4.69 (*d*, *J* = 2.1 Hz)
30	1.69 (*s*)	1.69 (*s*)
2′	5.83 (*d*, *J* = 12.6 Hz)	2.73 (*m*)
3′	6.82 (*d*, *J* = 12.6 Hz)	5.07 (*m*)
5′, 9′	7.62 (*d*, *J* = 8.7 Hz)	7.25 (*d*, *J* = 8.7 Hz)
6′, 8′	6.78 (*d*, *J* = 8.7 Hz)	6.80 (*d*, *J* = 8.7 Hz)

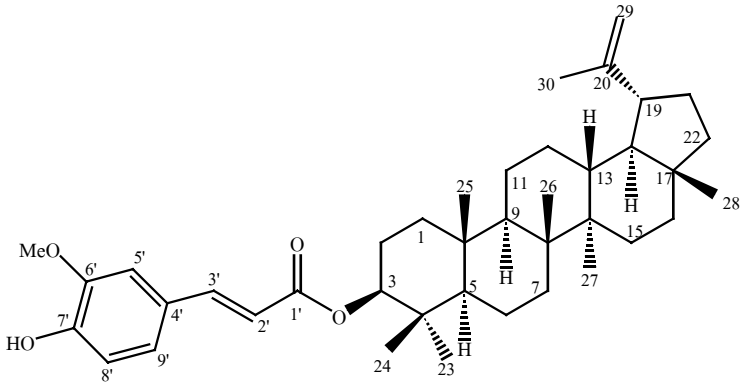
Table 40 Comparison of ^{13}C NMR spectral data between compounds **PTH12** and **PTH13** (recorded in $CDCl_3$)

Position	Compound PTH12, δ_C (ppm)	Compound PTH13, δ_C (ppm)
1	38.4	38.4
2	23.7	23.7
3	81.1	81.7
4	37.9	38.0
5	55.5	55.4
6	18.2	18.2
7	34.2	34.2
8	40.9	41.0
9	50.4	50.3
10	37.1	37.4
11	21.0	21.0
12	25.1	25.1
13	38.1	38.0
14	42.8	43.0
15	27.5	27.4
16	35.6	35.6
17	43.0	43.0
18	48.3	48.3
19	48.0	48.0
20	151.0	151.0
21	29.9	29.8
22	40.0	40.0
23	28.0	27.9
24	16.5	16.2
25	16.0	16.6
26	16.2	16.0

Table 40 (Continued)

Position	Compound PTH12, δ_C (ppm)	Compound PTH13, δ_C (ppm)
27	14.5	14.5
28	18.0	18.0
29	109.3	109.4
30	19.3	19.3
1′	166.5	172.0
2′	117.9	43.6
3′	143.1	70.1
4′	127.9	135.0
5′, 9′	132.3	127.3
6′, 8′	115.0	115.3
7′	156.6	155.0

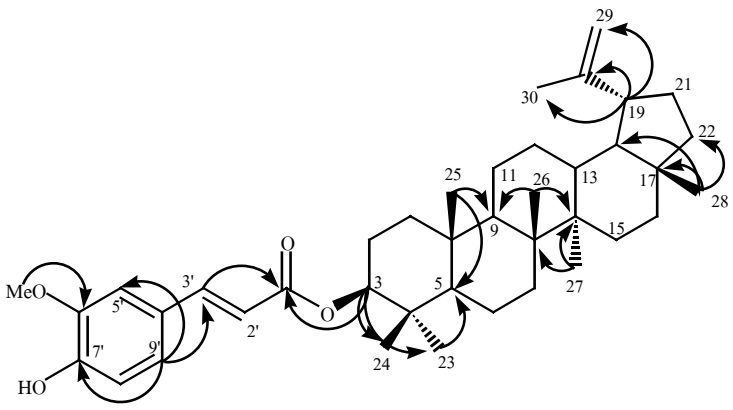
3.1.14 Compound PTH14



Compound **PTH14** was obtained as a white solid, mp:167-169°C, $[\alpha]_D^{27}$: +140° (*c* = 0.003, $CHCl_3$). Its ESI-TOF-MS mass spectrum (**Figure 53**) showed the $[M-H]^-$ ion peak at *m/z* 601.4244, corresponding to the molecular formula $C_{40}H_{58}O_4$. The IR spectrum (**Figure 45**) suggested hydroxyl (3534 cm^{-1}), double bond (1635, 1604 cm^{-1}), and conjugated ester (1703 cm^{-1}) functionalities. This compound exhibited UV absorption maxima at 234, 298, and 325 nm (**Figure 44**), again suggesting the presence of conjugation in the molecule. It gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^1H and ^{13}C NMR spectra of **PTH14** (**Figure 46** and **47**) and **PTH11** (**Figure 34** and **35**) exhibited the same pattern (**Table 42** and **43**). The difference was shown in the ^1H NMR spectra of substituent group which supported the presence of a *trans*-feruloyl as three 1,2,4-trisubstituted aromatic protons at δ 6.91 (1H, *d*, *J* = 8.1 Hz, H-8′), 7.03 (1H, *d*, *J* = 1.8 Hz, H-5′), and 7.07 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-9′), two *trans*-oriented vinyl protons at δ 6.29 and 7.59 (each *d*, *J* = 15.9 Hz, H-2′, H-3′, respectively), and aromatic methoxy protons at δ 3.93 (3H, *s*). A signal of a hydroxyl proton (disappeared on D_2O exchange) was shown at δ 5.85 (1H, s). A cross peak between H-5′ and the aromatic OMe in the NOESY spectrum located the latter at position C-6′. Lupane triterpenoid skeleton was evident from the following ^1H NMR

signals : six methyls at δ 0.79, 0.88, 0.89, 0.92, 0.95, 1.04 (3H, *s*, each), an isopropenyl group [δ 1.69 (3H, *s*), 4.60 (1H, *m*), 4.69 (1H, *d*, *J* = 2.1 Hz], and a typical lupane H$_\beta$-19 proton at δ 2.37 (1H, *m*). An oxymethine proton in proximity to an ester moiety was shown at δ 4.62 (*dd*, *J* = 9.0, 5.4 Hz, H-3). The doublet of doublet splitting pattern together with large coupling constant of H-3 with *Jax-ax* = 9.0 Hz and *Jax-eq* = 5.4 Hz indicated an axial (α) orientation of H-3. The ester carbonyl was also confirmed by ^{13}C NMR signal at δ 167.1. The ester substituent was placed at C-3 as a result of downfield shift observed for H-3 and C-3 in the proton and ^{13}C NMR spectra, respectively, compared with an analogous data of lupeol , and from the correlations between H-3 (δ 4.62) and C-23 (δ 28.0), C-24 (δ 16.2), and C-1′ (δ 167.1) observed in the HMBC spectrum (**Table 41**, **Figure 52**). The ^{13}C NMR signals (**Table 41**, **Figure 47**) for sp^2 methine carbons were shown at δ 116.3 (C-2′), δ 144.3 (C-3′), δ 109.3 (C-5′), δ 114.7 (C-8′), and δ 123.1 (C-9′), and one olefinic methylene carbon at δ 109.4 (C-29). In addition, seven methyl, one methoxy, eleven methylene, eleven methine and ten quaternary carbon signals were characterized by a DEPT experiment (**Figure 48 and 49**). Therefore compound **PTH14** was assigned as 3β-*E*-feruloyllupeol, a new compound (Ponglimanont and Thongdeeying, 2005).



Selected HMBC correlation of **PTH14**

Table 41 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH14**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.5	CH_2	1.63 (*m*)[a]	-
2	23.9	CH_2	1.71 (*m*)[a]	-
3	80.9	CH	4.62 (*dd*, *J* = 9.0, 5.4 Hz)	1′, 23, 24
4	38.1	C	-	-
5	55.5	CH	0.86 (*m*)[a]	-
6	18.3	CH_2	1.42 (*m*), 1.57 (*m*)[a]	-
7	34.3	CH_2	1.43 (*m*)[a]	-
8	40.9	C	-	-
9	50.4	CH	1.33 (*m*)[a]	-
10	37.2	C	-	-
11	21.0	CH_2	1.16 (*m*)[a]	-
12	25.2	CH_2	1.71 (*m*)[a]	-
13	38.1	CH	1.64 (*m*)[a]	-
14	42.9	C	-	-
15	27.5	CH_2	1.05 (*m*)[a]	-
16	35.6	CH_2	1.54 (*m*)[a]	-
17	43.0	C	-	-
18	48.3	CH	1.37 (*m*)[a]	-
19	48.0	CH	2.37 (*m*)	20, 30, 29, 21
20	151.0	C	-	-
21	29.9	CH_2	1.28 (*m*)[a]	-
22	40.0	CH_2	1.21 (*m*), 1.40 (*m*)[a]	-
23	28.0	CH_3	0.88 (*s*)	3, 4, 5, 24
24	16.2	CH_3	0.89 (*s*)	3, 4, 5, 23
25	16.7	CH_3	0.92 (*s*)	1, 5, 9, 10

[a] Deduced from HMQC experiment

Table 41 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	16.0	CH_3	1.04 (*s*)	7, 8 , 9, 14
27	14.6	CH_3	0.95 (*s*)	8, 13, 14, 15
28	18.0	CH_3	0.79 (*s*)	16, 17, 18, 22
29	109.4	CH_2	4.60 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	18, 30
30	19.3	CH_3	1.69 (*s*)	19, 20, 29
1′	167.1	C	-	-
2′	116.3	CH	6.29 (*d*, *J* = 15.9 Hz)	1′, 4′
3′	144.3	CH	7.59 (*d*, *J* = 15.9 Hz)	1′, 2′, 4′, 5′, 9′
4′	127.2	C	-	-
5′	109.3	CH	7.03 (*d*, *J* = 1.8 Hz)	3′, 4′, 7′, 9′
6′	146.8	C	-	-
7′	147.8	C	-	-
8′	114.7	CH	6.91 (*d*, *J* = 8.1 Hz)	4′, 6′
9′	123.1	CH	7.07 (*dd*, *J* = 8.1, 1.8 Hz)	3′, 5′, 7′
OMe	56.0	CH_3	3.93 (*s*)	6′
OH	-	-	5.85 (*s*)	7′, 8′

Table 42 Comparison of ^{13}C NMR spectral data between compounds **PTH11** and **PTH14** (recorded in $CDCl_3$)

Position	Compound PTH11, δ_H (ppm)	Compound PTH14, δ_H (ppm)
3	4.62 (*m*)	4.62 (*dd*, *J* = 9.0, 5.4 Hz)
19	2.38 (*dt*, *J* = 10.5, 5.4 Hz)	2.37 (*m*)
23	0.89 (*s*)	0.88 (*s*)
24	0.92 (*s*)	0.89 (*s*)
25	0.88 (*s*)	0.92 (*s*)

Table 42 (Continued)

Position	Compound PTH11, δ_H (ppm)	Compound PTH14, δ_H (ppm)
26	1.04 (*s*)	1.04 (*s*)
27	0.95 (*s*)	0.95 (*s*)
28	0.79 (*s*)	0.79 (*s*)
29	4.58 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	4.60 (*m*), 4.69 (*d*, *J* = 2.1 Hz)
30	1.69 (*s*)	1.69 (*s*)
2′	6.29 (*d*, *J* = 15.9 Hz)	6.29 (*d*, *J* = 15.9 Hz)
3′	7.61 (*d*, *J* = 15.9 Hz)	7.59 (*d*, *J* = 15.9 Hz)
5′	7.41 (*d*, *J* = 8.7 Hz)	7.03 (*d*, *J* = 1.8 Hz)
6′	6.85 (*d*, *J* = 8.7 Hz)	-
8′	6.85 (*d*, *J* = 8.7 Hz)	6.91 (*d*, *J* = 8.1 Hz)
9′	7.41 (*d*, *J* = 8.7 Hz)	7.07 (*dd*, *J* = 8.1, 1.8 Hz)
OMe	-	3.93 (*s*)
OH	-	5.85 (*s*)

Table 43 Comparison of ^{13}C NMR spectral data between compounds **PTH11** and **PTH14** (recorded in $CDCl_3$)

Position	Compound PTH11, δ_C (ppm)	Compound PTH14, δ_C (ppm)
1	38.4	38.5
2	23.9	23.9
3	81.2	80.9
4	38.1	38.1
5	55.4	55.5
6	18.2	18.3
7	34.2	34.3
8	40.9	40.9

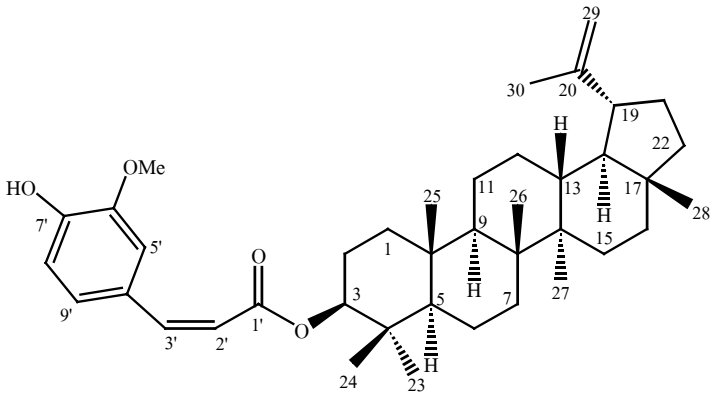
Table 43 (Continued)

Position	Compound PTH11, δ_C (ppm)	Compound PTH14, δ_C (ppm)
9	50.4	50.4
10	37.1	37.2
11	21.0	21.0
12	25.1	25.2
13	38.1	38.1
14	42.9	42.9
15	27.5	27.5
16	35.6	35.6
17	43.0	43.0
18	48.3	48.3
19	48.0	48.0
20	151.0	151.0
21	29.9	29.9
22	40.0	40.0
23	28.0	28.0
24	16.7	16.2
25	16.2	16.7
26	16.0	16.0
27	14.6	14.6
28	18.0	18.0
29	109.4	109.4
30	19.3	19.3
1′	167.8	167.1
2′	115.9	116.3
3′	144.4	144.3
4′	127.0	127.2
5′	130.0	109.3

Table 43 (Continued)

Position	Compound PTH11, δ_C (ppm)	Compound PTH14, δ_C (ppm)
6′	116.0	146.8
7′	158.1	147.8
8′	116.0	114.7
9′	130.0	123.1
OMe	-	56.0

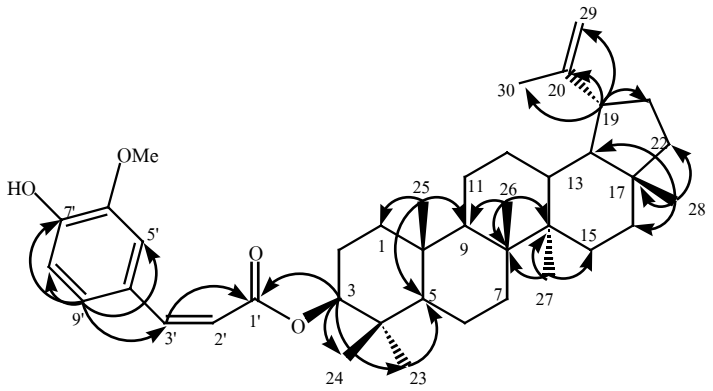
3.1.15 Compound PTH15



Compound **PTH15** was obtained as a white solid, mp: 195-197°C, $[\alpha]_D^{27}$: +41.66° (*c* = 0.060, $CHCl_3$). Its ESI-TOF-MS mass spectrum (**Figure 63**) showed the $[M-H]^-$ ion peak at *m/z* 601.4260, corresponding to the molecular formula $C_{40}H_{58}O_4$. The IR and UV spectrum showed absorption bands similar to those of **PTH14**.

The ^1H and ^{13}C NMR spectral data (**Table 45** and **46**, **Figure 56** and **57**) were closely related to those of **PTH14** (**Figure 46** and **47**), except for the olefinic proton signals at δ 5.81 (1H, *d*, *J* = 12.9 Hz) and 6.77 (1H, *d*, *J* = 12.9 Hz) assignable, respectively to H-2′ and H-3′ on the feruloyl group. Judging from the small *J* value

(12.9 Hz), the double bond should have a *Z* geometry. These spectral data implied a lupeol bearing a *Z*-feruloyl group. On the basis of HMBC (**Table 44**, **Figure 62**), the *Z*-feruloyl moiety was located at C-3 by correlation of H-3 signal (δ 4.54) with C-1′ (δ 166.4), C-23 (δ 28.0), and C-24 (δ 16.2). The coupling constant and splitting pattern of H-3 (*dd*, *J* = 11.1, 5.4 Hz) indicated an α-orientation of H-3. Thus compound **PTH15** was assigned as 3β-*Z*-feruloyllupeol, a new compound (Ponglimanont and Thongdeeying, 2005).



Selected HMBC correlation of **PTH15**

Table 44 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH15**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.5	CH_2	1.00 (*m*), 1.61 (*m*)[a]	-
2	23.9	CH_2	1.64 (*m*), 1.72 (*m*)[a]	-
3	80.9	CH	4.54 (*dd*, *J* = 11.1, 5.4 Hz)	1′, 2, 4, 23, 24
4	38.1	C	-	-
5	55.5	CH	0.80 (*m*)[a]	-
6	18.3	CH_2	1.40 (*m*), 1.52 (*m*)[a]	-
7	34.3	CH_2	1.34 (*m*), 1.44 (*m*)[a]	-
8	40.9	C	-	-
9	50.4	CH	1.33 (*m*)[a]	-
10	37.2	C	-	-
11	21.0	CH_2	1.29 (*m*), 1.46 (*m*)[a]	-
12	25.2	CH_2	1.10 (*m*), 1.61 (*m*)[a]	-
13	38.1	CH	1.66 (*m*)[a]	-
14	42.9	C	-	-
15	27.5	CH_2	1.02 (*m*), 1.68 (*m*)[a]	-
16	35.6	CH_2	1.37 (*m*), 1.52 (*m*)[a]	-
17	43.0	C	-	-
18	48.3	CH	1.37 (*m*)[a]	-
19	48.0	CH	2.38 (*m*)	13, 20, 21, 29, 30
20	151.0	C	-	-
21	29.9	CH_2	1.90 (*m*)[a]	-
22	40.0	CH_2	1.19 (*m*), 1.40 (*m*)[a]	-
23	28.0	CH_3	0.86 (*s*)	3, 4, 5, 24
24	16.2	CH_3	0.81 (*s*)	3, 4, 5, 23
25	16.7	CH_3	0.86 (*s*)	1, 5, 9, 10

[a] Deduced from HMQC experiment

Table 44 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	16.0	CH_3	1.03 (*s*)	7, 8, 9, 14
27	14.5	CH_3	0.94 (*s*)	8, 13, 14, 15
28	18.0	CH_3	0.79 (*s*)	16, 17, 18, 22
29	109.4	CH_2	4.69 (*d*, *J* = 2.1 Hz), 4.57 (*m*)	19, 20, 30
30	19.4	CH_3	1.69 (*s*)	19, 20, 29
1′	166.4	C	-	-
2′	117.4	CH	5.81 (*d*, *J* = 12.9 Hz)	1′, 3′, 4′
3′	143.5	CH	6.77 (*d*, *J* = 12.9 Hz)	1′, 2′, 5′, 9′
4′	127.3	C	-	-
5′	112.9	CH	7.78 (*d*, *J* = 1.8 Hz)	3′, 4′, 6′, 7′, 9′
6′	146.0	C	-	-
7′	147.0	C	-	-
8′	113.9	CH	6.87 (*d*, *J* = 8.4 Hz)	4′, 6′, 7′
9′	125.6	CH	7.10 (*dd*, *J* = 8.4, 1.8 Hz)	3′, 5′, 7′, 8′
OMe	56.0	CH_3	3.91 (*s*)	6′
OH	-	-	5.88 (*s*)	6′, 7′, 8′

Table 45 Comparison of ^{1}H NMR spectral data between compounds **PTH14** and **PTH15** (recorded in $CDCl_3$)

Position	Compound PTH14, δ_H (ppm)	Compound PTH15, δ_H (ppm)
1	1.63 (*m*) [a]	1.00 (*m*), 1.61 (*m*) [a]
2	1.71 (*m*) [a]	1.64 (*m*), 1.72 (*m*) [a]
3	4.62 (*dd*, *J* = 9.0, 5.4 Hz)	4.54 (*dd*, *J* = 11.1, 5.4 Hz)
5	0.86 (*m*) [a]	0.80 (*m*) [a]
6	1.42 (*m*), 1.57 (*m*) [a]	1.40 (*m*), 1.52 (*m*) [a]

Table 45 (Continued)

Position	Compound PTH14, δ_H (ppm)	Compound PTH15, δ_H (ppm)
7	1.43 (*m*)[a]	1.34 (*m*), 1.44 (*m*)[a]
9	1.33 (*m*)[a]	1.33 (*m*)[a]
11	1.16 (*m*)[a]	1.29 (*m*), 1.46 (*m*)[a]
12	1.71 (*m*)[a]	1.10 (*m*), 1.61 (*m*)[a]
13	1.64 (*m*)[a]	1.66 (*m*)[a]
15	1.05 (*m*)[a]	1.02 (*m*), 1.68 (*m*)[a]
16	1.54 (*m*)[a]	1.37 (*m*), 1.52 (*m*)[a]
18	1.37 (*m*)[a]	1.37 (*m*)[a]
19	2.37 (*m*)	2.38 (*m*)
21	1.28 (*m*)[a]	1.90 (*m*)[a]
22	1.21 (*m*), 1.40 (*m*)[a]	1.19 (*m*), 1.40 (*m*)[a]
23	0.88 (*s*)	0.86 (*s*)
24	0.89 (*s*)	0.81 (*s*)
25	0.92 (*s*)	0.86 (*s*)
26	1.04 (*s*)	1.03 (*s*)
27	0.95 (*s*)	0.94 (*s*)
28	0.79 (*s*)	0.79 (*s*)
29	4.60 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	4.57 (*m*), 4.69 (*d*, *J* = 2.1 Hz)
30	1.69 (*s*)	1.69 (*s*)
2′	6.29 (*d*, *J* = 15.9 Hz)	5.81 (*d*, *J* = 12.9 Hz)
3′	7.59 (*d*, *J* = 15.9 Hz)	6.77 (*d*, *J* = 12.9 Hz)
5′	7.03 (*d*, *J* = 1.8 Hz)	7.78 (*d*, *J* = 1.8 Hz)
8′	6.91 (*d*, *J* = 8.1 Hz)	6.87 (*d*, *J* = 8.4 Hz)
9′	7.07 (*dd*, *J* = 8.1, 1.8 Hz)	7.10 (*dd*, *J* = 8.4,1.8 Hz)
OMe	3.93 (*s*)	3.91 (*s*)
OH	5.85 (*s*)	5.88 (*s*)

[a] Deduced from HMQC experiment

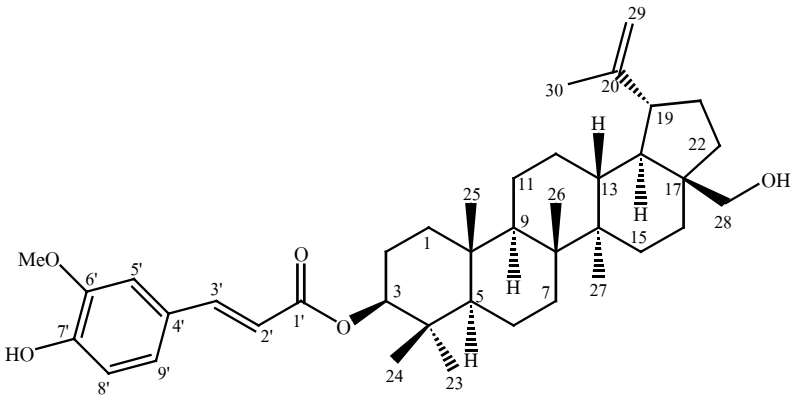
Table 46 Comparison of ^{13}C NMR spectral data between compounds **PTH14** and **PTH15** (recorded in $CDCl_3$)

Position	Compound PTH14, δ_C (ppm)	Compound PTH15, δ_C (ppm)
1	38.5	38.5
2	23.9	23.8
3	80.9	80.7
4	38.1	37.1
5	55.5	55.5
6	18.3	18.3
7	34.3	34.3
8	40.9	40.9
9	50.4	50.4
10	37.2	37.9
11	21.0	21.0
12	25.2	25.1
13	38.1	38.1
14	42.9	43.0
15	27.5	27.5
16	35.6	35.6
17	43.0	42.8
18	48.3	48.3
19	48.0	48.0
20	151.0	150.9
21	29.9	29.9
22	40.0	40.0
23	28.0	28.0
24	16.2	16.2
25	16.7	16.5

Table 46 (Continued)

Position	Compound PTH14, δ_C (ppm)	Compound PTH15, δ_C (ppm)
26	16.0	16.0
27	14.6	14.5
28	18.0	18.0
29	109.4	109.4
30	19.3	19.4
1′	167.1	166.4
2′	116.3	117.4
3′	144.3	143.5
4′	127.2	127.3
5′	109.3	112.9
6′	146.8	146.0
7′	147.8	147.0
8′	114.7	113.9
9′	123.1	125.6
OMe	56.0	56.0

3.1.16 Compound PTH16



Compound **PTH16** was isolated as a colorless viscous oil. $[\alpha]_D^{28}$: +15.0° (*c* = 0.020, $CHCl_3$). Its IR and UV spectrum showed absorption bands similar to compound **PTH14**.

The ^{1}H and ^{13}C NMR spectral data of compound **PTH16** (**Figure 64** and **65**) and **PTH14** (**Figure 46** and **47**) exhibited the same pattern (**Table 48** and **49**), except that compound **PTH16** displayed only six methyl singlets (δ 0.88, 0.89, 0.92, 0.99, 1.03 and 1.71). It appeared that a singlet signal of 3H-28 was replaced with the AB system of oxymethylene protons at δ 3.80 and 3.34 (each *d*, *J* = 10.5 Hz). The parent triterpene structure was identified as betulin by a combination of HMQC and HMBC experiments (**Table 47**). Thus on the basis of its spectroscopic data and comparison of the NMR spectral data with previously reported data (Kuo *et al*., 1997), (**Table 48** and **49**), compound **PTH16** was assigned as 3β-*E*-feruloylbetulin.

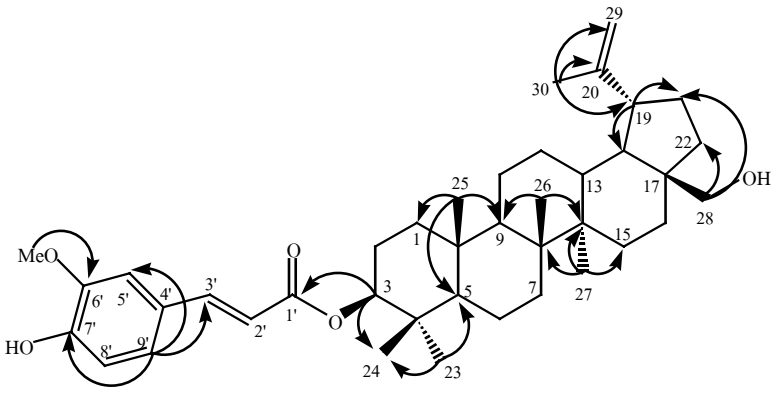
Table 47 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH16**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.4	CH_2	1.70 (*m*)[a]	-
2	23.8	CH_2	1.71 (*m*)[a]	-
3	80.8	CH	4.62 (*m*)	1′, 24
4	38.1	C	-	-
5	55.4	CH	0.85 (*m*)[a]	-
6	18.2	CH_2	1.57 (*m*)[a]	-
7	34.0	CH_2	1.06 (*m*)[a]	-
8	41.0	C	-	-
9	50.3	CH	1.30 (*m*)[a]	-
10	37.1	C	-	-
11	20.9	CH_2	1.22 (*m*)[a]	-
12	25.2	CH_2	1.71 (*m*)[a]	-
13	37.3	CH	1.65 (*m*)[a]	-
14	42.7	C	-	-
15	27.1	CH_2	1.05 (*m*)[a]	-
16	29.2	CH_2	1.96 (*m*)[a]	-
17	47.8	C	-	-
18	48.8	CH	1.63 (*m*)[a]	-
19	47.8	CH	2.39 (*m*)	18, 20, 21
20	150.5	C	-	-
21	29.8	CH_2	1.93 (*m*)[a]	-
22	34.2	CH_2	1.43 (*m*), 1.90 (*m*)[a]	-
23	28.0	CH_3	0.89 (*s*)	3, 4, 5, 24
24	16.7	CH_3	0.92 (*s*)	3, 4, 5, 23
25	16.2	CH_3	0.88 (*s*)	1, 5, 9, 10

[a] Deduced from HMQC experiment

Table 47 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	16.0	CH_3	1.03 (*s*)	7, 8, 9, 14
27	14.7	CH_3	0.99 (*s*)	8, 13, 14, 15
28	60.6	CH_2	3.34 (*d*, *J* = 10.5 Hz),	
16, 22			3.80 (*d*, *J* = 10.5 Hz)	
29	109.7	CH_2	4.59 (*m*), 4.68 (*d*, *J* = 1.8 Hz)	19, 20, 30
30	19.1	CH_3	1.71 (*s*)	19, 20, 29
1′	167.1	C	-	-
2′	116.3	CH	6.28 (*d*, *J* = 15.9 Hz)	1′, 4′
3′	144.3	CH	7.59 (*d*, *J* = 15.9 Hz)	1′, 2′, 4′, 5′, 9′
4′	127.2	C	-	-
5′	109.3	CH	7.03 (*d*, *J* = 1.5 Hz)	3′, 7′, 9′
6′	146.8	C	-	-
7′	147.8	C	-	-
8′	114.7	CH	6.91 (*d*, *J* = 8.1 Hz)	4′,6′
9′	123.0	CH	7.07 (*dd*, *J* = 8.1, 1.5 Hz)	3′, 5′, 7′
OMe	56.0	CH_3	3.85 (*s*)	6′
OH	-	-	5.89 (*br s*)	7′,8′



Selected HMBC correlation of **PTH16**

Table 48 Comparison of ^{1}H NMR spectral data of 3β-*E*-feruloylbetulin, compounds **PTH14** and **PTH16** (recorded in $CDCl_3$)

Position	3β-*E*-feruloylbetulin, δ_H (ppm)	Compound PTH14, δ_H (ppm)	Compound PTH16, δ_H (ppm)
3	4.61 (*m*)	4.62 (*dd*, *J* = 9.0, 5.4 Hz)	4.62 (*m*)
19	2.37 (*m*)	2.37 (*m*)	2.39 (*m*)
23	0.86 (*s*)	0.88 (*s*)	0.89 (*s*)
24	1.01 (*s*)	0.89 (*s*)	0.92 (*s*)
25	0.85 (*s*)	0.92 (*s*)	0.88 (*s*)
26	0.87 (*s*)	1.04 (*s*)	1.03 (*s*)
27	0.97 (*s*)	0.95 (*s*)	0.99 (*s*)
28	3.31 (*d*, *J* = 10.7 Hz), 3.78 (*d*, *J* = 10.7 Hz)	0.79 (*s*)	3.34 (*d*, *J* = 10.5 Hz), 3.80 (*d*, *J* = 10.5 Hz)
29	4.57 (*d*, *J* = 2.0 Hz), 4.67 (*d*, *J* = 2.0 Hz)	4.60 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	4.59 (*m*), 4.68 (*d*, *J* = 1.8 Hz)
30	1.67 (*s*)	1.69 (*s*)	1.71 (*s*)
2′	6.26 (*d*, *J* = 16.0 Hz)	6.29 (*d*, *J* = 15.9 Hz)	6.28 (*d*, *J* = 15.9 Hz)
3′	7.56 (*d*, *J* = 16.0 Hz)	7.59 (*d*, *J* = 15.9 Hz)	7.59 (*d*, *J* = 15.9 Hz)
5′	7.01 (*d*, *J* = 1.6 Hz)	7.03 (*d*, *J* = 1.8 Hz)	7.03 (*d*, *J* = 1.5 Hz)
8′	6.88 (*d*, *J* = 8.2 Hz)	6.91 (*d*, *J* = 8.1 Hz)	6.91 (*d*, *J* = 8.1 Hz)
9′	7.04 (*dd*, *J* = 8.2, 1.6 Hz)	7.07 (*dd*, *J* = 8.1, 1.8 Hz)	7.07 (*dd*, *J* = 8.1, 1.5 Hz)
OMe	3.91 (*s*)	3.93 (*s*)	3.85 (*s*)
OH	-	5.85 (*s*)	5.89 (*br s*)

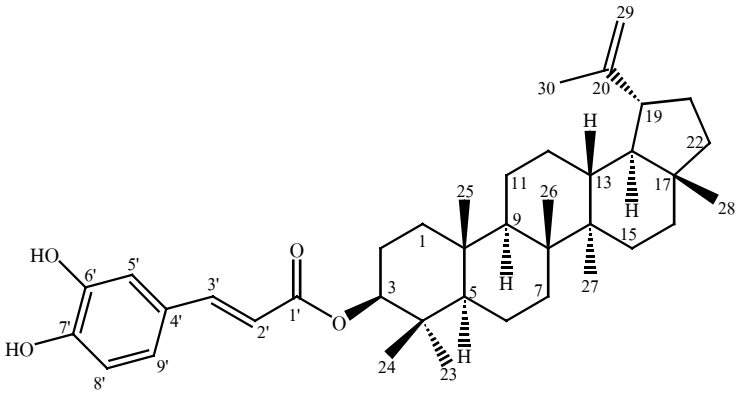
Table 49 Comparison of ^{13}C NMR spectral data of 3β-*E*-feruloylbetulin, compounds **PTH14** and **PTH16** (recorded in $CDCl_3$)

Position	3β-*E*-feruloylbetulin, δ_C (ppm)	Compound PTH14, δ_C (ppm)	Compound PTH16, δ_C (ppm)
1	38.4	38.5	38.4
2	23.7	23.9	23.8
3	80.8	80.9	80.8
4	38.1	38.1	38.1
5	55.4	55.5	55.4
6	18.2	18.3	18.2
7	34.0	34.3	34.0
8	40.9	40.9	41.0
9	50.3	50.4	50.3
10	37.1	37.2	37.1
11	20.9	21.0	20.9
12	25.2	25.2	25.2
13	37.3	38.1	37.3
14	42.7	42.9	42.7
15	27.0	27.5	27.1
16	29.2	35.6	29.2
17	47.8	43.0	47.8
18	48.7	48.3	48.8
19	47.8	48.0	47.8
20	150.5	151.0	150.5
21	29.7	29.9	29.8
22	34.2	40.0	34.2
23	28.0	28.0	28.0

Table 49 (Continued)

Position	3β-*E*-feruloylbetulin, δ_C (ppm)	Compound PTH14, δ_C (ppm)	Compound PTH16, δ_C (ppm)
24	16.0	16.2	16.7
25	16.2	16.7	16.2
26	16.6	16.0	16.0
27	14.7	14.6	14.7
28	60.7	18.0	60.6
29	109.7	109.4	109.7
30	19.1	19.3	19.1
1′	167.1	167.1	167.1
2′	114.6	116.3	116.3
3′	144.3	144.3	144.3
4′	127.1	127.2	127.2
5′	109.2	109.3	109.3
6′	146.7	146.8	146.8
7′	147.8	147.8	147.8
8′	116.2	114.7	114.7
9′	123.0	123.1	123.0
OMe	56.0	56.0	56.0

3.1.17 Compound PTH17



Compound **PTH17** was isolated as a white solid, mp. 147-149°C, $[\alpha]_D^{28}$:+10.6° (c = 0.047, $CHCl_3$). Its IR spectrum suggested hydroxyl (3413 cm^{-1}), conjugated ester (1671 cm^{-1}) and double bond (1616 cm^{-1}) functionalities. This compound exhibited UV absorption similar to compound **PTH14**.

Comparison of the ^{1}H and ^{13}C NMR spectral data (**Table 51** and **52**) of compound **PTH17** (**Figure 66** and **67**) and **PTH14** (**Figure 46** and **47**) revealed close structural similarity. The difference was shown in the absence of the aromatic methoxy protons at δ 3.93 (3H, *s*, OMe-6′) which was confirmed by HMBC experiment (**Table 50**) in which H-8′ [δ 6.87 (*d*, J = 8.1 Hz)] showed correlation with C-4′ (δ 127.4), C-6′ (δ 144.0), C-7′ (δ 147.0) and C-9′ (δ 122.3). Thus on the basis of its spectroscopic data and comparison with previously reported data of 3β-*E*-caffeoyllupeol (Alvarenga *et al*., 2000), (**Table 51** and **52**), compound **PTH17** was assigned as 3β-*E*-caffeoyl-lupeol.

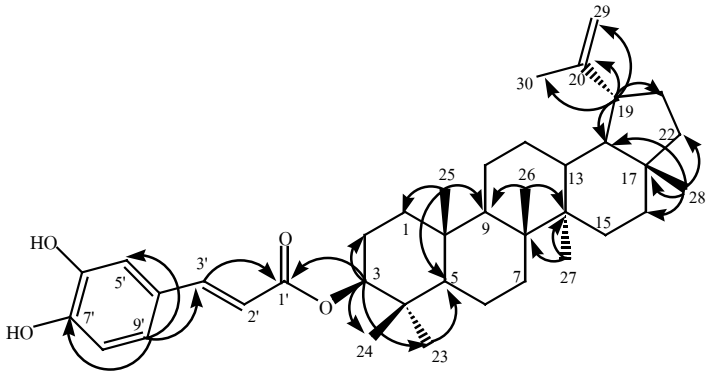
Table 50 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTH17**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.4	CH_2	1.63 (*m*), 1.68 (*m*)[a]	-
2	23.8	CH_2	1.69 (*m*), 1.74 (*m*)[a]	-
3	81.5	CH	4.60 (*m*)	1′, 2, 4, 23, 24
4	38.1	C	-	-
5	55.4	CH	0.84 (*m*)[a]	-
6	18.2	CH_2	1.42 (*m*), 1.54 (*m*)[a]	-
7	34.2	CH_2	1.42 (*m*)[a]	-
8	40.9	C	-	-
9	50.4	CH	1.30 (*m*)[a]	-
10	37.1	C	-	-
11	21.0	CH_2	1.21 (*m*), 1.46 (*m*)[a]	-
12	25.1	CH_2	1.16 (*m*)[a]	-
13	38.1	CH	1.74 (*m*)[a]	-
14	42.9	C	-	-
15	27.5	CH_2	1.92 (*m*)[a]	-
16	35.6	CH_2	1.53 (*m*)[a]	-
17	43.0	C	-	-
18	48.3	CH	1.38 (*m*)[a]	-
19	48.0	CH	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	13, 18, 20, 21, 29, 30
20	151.0	C	-	-
21	29.9	CH_2	1.93 (*m*)[a]	-
22	40.0	CH_2	1.20 (*m*), 1.40 (*m*)[a]	-
23	28.0	CH_3	0.88 (*s*)	3, 4, 5, 24
24	16.7	CH_3	0.91 (*s*)	3, 4, 5, 23
25	16.2	CH_3	0.88 (*s*)	1, 5, 9, 10

[a] Deduced from HMQC experiment

Table 50 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	16.0	CH_3	1.04 (*s*)	7, 8, 9, 14
27	14.6	CH_3	0.95 (*s*)	8, 13, 14, 15
28	18.0	CH_3	0.79 (*s*)	16, 17, 18, 22
29	109.4	CH_2	4.57 (*m*), 4.69 (*d*, *J* = 2.4 Hz)	19, 20, 30
30	19.3	CH_3	1.69 (*s*)	19, 20, 29
1′	168.0	C	-	-
2′	116.0	CH	6.26 (*d*, *J* = 15.9 Hz)	1′, 3′, 4′
3′	144.9	CH	7.56 (*d*, *J* = 15.9 Hz)	1′, 2′, 4′, 5′, 9′
4′	127.4	C	-	-
5′	114.4	CH	7.11 (*d*, *J* = 1.8 Hz)	3′, 4′, 6′, 7′, 9′
6′	144.0	C	-	-
7′	146.6	C	-	-
8′	115.4	CH	6.87 (*d*, *J* = 8.1 Hz)	4′, 7′, 9′
9′	122.3	CH	6.99 (*dd*, *J* = 8.1, 1.8 Hz)	3′, 5′, 7′



Selected HMBC correlation of **PTH17**

Table 51 Comparison of ^{1}H NMR spectral data of 3β-*E*-caffeoyllupeol, compounds **PTH14** and **PTH17** (recorded in $CDCl_3$)

Position	**3β-*E*-caffeoyllupeol, δ_H (ppm)**	**Compound PTH14, δ_H (ppm)**	**Compound PTH17, δ_H (ppm)**
3	4.60 (*t*)	4.62 (*dd*, *J* = 9.0, 5.4 Hz)	4.60 (*m*)
19	2.38 (*m*)	2.37 (*m*)	2.38 (*dt*, *J* = 11.1, 5.7 Hz)
23	0.89 (*s*)	0.88 (*s*)	0.88 (*s*)
24	0.91 (*s*)	0.89 (*s*)	0.91 (*s*)
25	0.89 (*s*)	0.92 (*s*)	0.88 (*s*)
26	1.04 (*s*)	1.04 (*s*)	1.04 (*s*)
27	0.96 (*s*)	0.95 (*s*)	0.95 (*s*)
28	0.79 (*s*)	0.79 (*s*)	0.79 (*s*)
29	4.58 (*br s*), 4.70 (*br s*)	4.60 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	4.57 (*m*), 4.69 (*d*, *J* = 2.4 Hz)
30	1.69 (*s*)	1.69 (*s*)	1.69 (*s*)
2′	6.26 (*d*)	6.29 (*d*, *J* = 15.9 Hz)	6.26 (*d*, *J* = 15.9 Hz)
3′	7.56 (*d*)	7.59 (*d*, *J* = 15.9 Hz)	7.56 (*d*, *J* = 15.9 Hz)
5′	7.12 (*s*)	7.03 (*d*, *J* = 1.8 Hz)	7.11 (*d*, *J* = 1.8 Hz)
8′	6.87 (*d*)	6.91 (*d*, *J* = 8.1 Hz)	6.87 (*d*, *J* = 8.1 Hz)
9′	7.00 (*d*)	7.07 (*dd*, *J* = 8.1, 1.8 Hz)	6.99 (*dd*, *J* = 8.1, 1.8 Hz)
OMe	-	3.93 (*s*)	-
OH	-	5.85 (*s*)	-

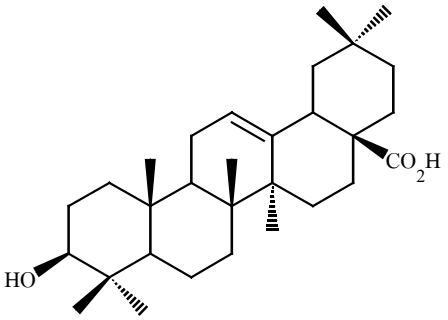
Table 52 Comparison of ^{13}C NMR spectral data of 3β-*E*-caffeoyllupeol, compounds **PTH14** and **PTH17** (recorded in $CDCl_3$)

Position	3β-*E*-caffeoyllupeol, δ_C (ppm)	Compound PTH14, δ_C (ppm)	Compound PTH17, δ_C (ppm)
1	38.4	38.5	38.4
2	23.8	23.9	23.8
3	81.2	80.9	81.5
4	38.0	38.1	38.1
5	55.4	55.5	55.4
6	18.2	18.3	18.2
7	34.2	34.3	34.2
8	40.9	40.9	40.9
9	50.3	50.4	50.4
10	37.1	37.2	37.1
11	27.4	21.0	21.0
12	25.1	25.2	25.1
13	38.0	38.1	38.1
14	42.8	42.9	42.9
15	20.9	27.5	27.5
16	35.6	35.6	35.6
17	43.0	43.0	43.0
18	48.3	48.3	48.3
19	48.0	48.0	48.0
20	150.9	151.0	151.0
21	29.8	29.9	29.9
22	40.4	40.0	40.0
23	28.0	28.0	28.0

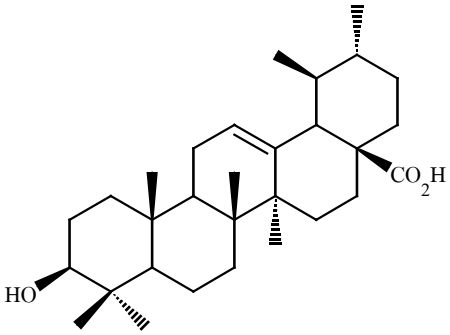
Table 52 (Continued)

Position	3β-*E*-caffeoyllupeol, δ_C (ppm)	Compound PTH14, δ_C (ppm)	Compound PTH17, δ_C (ppm)
24	16.6	16.2	16.7
25	16.2	16.7	16.2
26	16.0	16.0	16.0
27	14.5	14.6	14.6
28	18.0	18.0	18.0
29	109.3	109.4	109.4
30	19.3	19.3	19.3
1′	167.5	167.1	168.0
2′	116.3	116.3	116.0
3′	144.4	144.3	144.9
4′	127.6	127.2	127.4
5′	115.4	109.3	114.4
6′	143.8	146.8	144.0
7′	146.2	147.8	146.6
8′	114.3	114.7	115.4
9′	122.3	123.1	122.3
OMe	-	56.0	-

3.1.18 Compounds PTH18 and PTH19



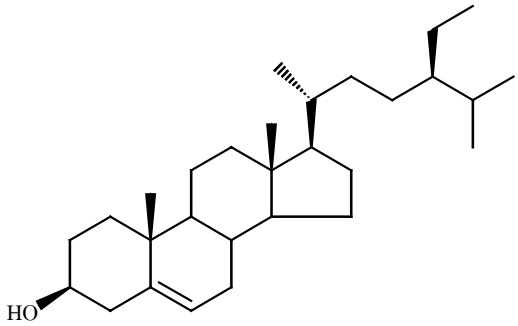
PTH18



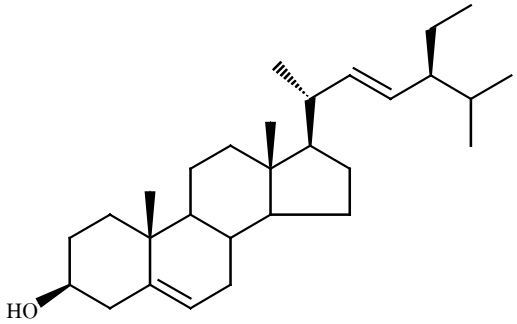
PTH19

The mixture of **PTH18** and **PTH19** was isolated as a white solid. It gave a purple vanillin-sulfuric acid test. The IR spectrum exhibited absorption bands for hydroxyl (3414 cm^{-1}) and carbonyl (1680 cm^{-1}) functionalities. The ^{1}H NMR spectral data contained an olefinic proton at δ 5.29-5.23 (*m*), a signal of oxymethine proton at δ 3.20 (*dd*, *J* = 8.7, 6.9 Hz) and a methine proton at δ 2.83 (*dd*, *J* = 13.8, 3.6 Hz). The ^{1}H and ^{13}C NMR data of **PTH18** and **PTH19** were corresponded to previous reported data of oleanolic and ursolic acid. Thus, this mixture was identified as oleanolic and ursolic acid (Seebacher *et al*., 2003 and Lin *et al*., 1987).

3.1.19 Compounds PTH20 and PTH21



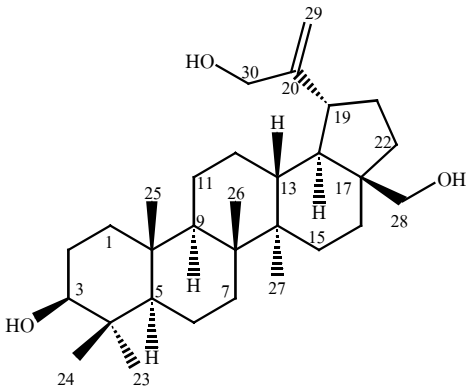
PTH20



PTH21

The mixture of **PTH20** and **PTH21** was isolated as a white solid. Its IR spectrum showed absorption bands at 3425 (hydroxy) and 1642 cm^{-1} (double bond). The ^{1}H NMR 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 ^{1}H NMR data was corresponded to previous reported data of β-sitosterol and stigmasterol. Thus, this mixture was identified as β-sitosterol (**PTH20**) and stigmasterol (**PTH21**) (Cheenpracha, 2004).

3.1.20 Compound PTM1



Compound **PTM1** was isolated as a white solid, It gave a purple vanillin-sulfuric acid test. Its IR spectrum showed absorption bands similar to compound **PTH3**. The melting point was not reported due to insufficient amount and instability of the compound.

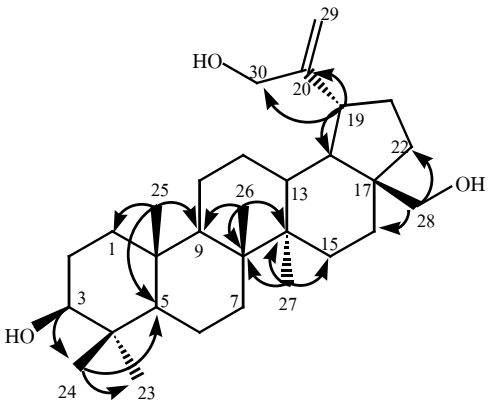
The ^{1}H and ^{13}C NMR spectral data of compound **PTM1** (**Table 53**, **Figure 70** and **71**) revealed close structural similarity to compounds **PTH3** (**Figure 15** and **16**) and **PTH7** (**Figure 24** and **25**) (**Table 54** and **55**), except that compound **PTM1** displayed only five methyl singlets at δ 0.76, 0.82, 0.97, 0.98 and 1.02. The two signals of terminal olefinic protons of 2H-29 were shown at δ 4.95 (*m*) and 4.91 (*br s*) which were shifted more downfield than compound **PTH3** (δ 4.68 and 4.58). In addition, the three signals of oxymethylene protons were shown [two signals of the AB system at δ 3.30 and 3.80 (each *d*, *J* = 11.4 Hz, 2H-28) and a *broad singlet* signal at δ 4.12 (2H, *br s*, 2H-30)]. On the basis of HMBC experiment (**Table 53**) the oxymethylene protons of 2H-30 showed long-range correlations with C-20 (δ 152.0) and C-29 (δ 107.2) and 2H-28 showed correlations with C-16 (δ 29.2) and C-22 (δ 33.8). Thus compound **PTM1** was identified as 3β, 28, 30-lup-20(29)-en-triol by comparison of its spectral data (**Table 56** and **57**) with those reported in the literature (Gonzalez *et al*., 1992).

Table 53 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTM1**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	38.7	CH_2	-	-
2	27.4	CH_2	-	-
3	80.0	CH	3.18 (*dd*, *J* = 10.8, 5.1 Hz)	24
4	38.9	C	-	-
5	55.3	CH	0.68 (*m*)	-
6	18.3	CH_2	-	-
7	34.3	CH_2	-	-
8	40.9	C	-	-
9	50.4	CH	-	-
10	37.2	C	-	-
11	21.9	CH_2	-	-
12	26.8	CH_2	-	-
13	37.2	CH	-	-
14	42.7	C	-	-
15	27.0	CH_2	-	-
16	29.2	CH_2	-	-
17	47.8	C	-	-
18	49.5	CH	-	-
19	43.5	CH	2.30 (*m*)	18, 20, 29, 30
20	152.0	C	-	-
21	31.6	CH_2	-	-
22	33.8	CH_2	-	-
23	28.0	CH_3	0.97 (*s*)	3, 4, 5, 24
24	15.4	CH_3	0.76 (*s*)	3, 4, 5, 23
25	16.1	CH_3	0.82 (*s*)	1, 5, 9, 10

Table 53 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
26	16.0	CH_3	1.02 (*s*)	7, 8, 9, 14
27	14.8	CH_3	0.98 (*s*)	8, 13, 14, 15
28	60.3	CH_2	3.30 (*d*, *J* = 11.4 Hz), 3.80 (*d*, *J* = 11.4 Hz)	16, 22
29	107.2	CH_2	4.95 (*m*), 4.91 (*br s*)	19, 20, 30
30	65.1	CH_2	4.12 (*br s*)	20, 29



Selected HMBC correlation of **PTM1**

Table 54 Comparison of ^{1}H NMR spectral data of compounds **PTH3**, **PTH7**, and **PTM1** (recorded in $CDCl_3$)

Position	PTH3, δ_H (ppm)	PTH7, δ_H (ppm)	PTM1, δ_C (ppm)
3	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	3.18 (*dd*, *J* = 10.8, 5.1 Hz)
5	0.68 (*m*)	0.68 (*m*)	0.68 (*m*)
19	2.38 (*m*)	2.28 (*m*)	2.30 (*m*)
23	0.97 (*s*)	0.97 (*s*)	0.97 (*s*)
24	0.76 (*s*)	0.76 (*s*)	0.76 (*s*)
25	0.82 (*s*)	0.83 (*s*)	0.82 (*s*)
26	1.02 (*s*)	1.03 (*s*)	1.02 (*s*)
27	0.98 (*s*)	0.94 (*s*)	0.98 (*s*)
28	3.33 (*d*, *J* = 10.8 Hz), 3.80 (*dd*, *J* = 10.8, 1.5 Hz)	0.78 (*s*)	3.30 (*d*, *J* = 11.4 Hz), 3.80 (*d*, *J* = 11.4 Hz)
29	4.58 (*m*), 4.68 (*d*, *J* = 2.1 Hz)	4.90 (*br s*), 4.93 (*br s*)	4.91 (*br s*), 4.95 (*d*, *J* = 1.2 Hz)
30	1.68 (*s*)	4.09 (*d*, *J* = 15.3 Hz), 4.14 (*d*, *J* = 15.3 Hz)	4.12 (*br s*)

Table 55 Comparison of ^{13}C NMR spectral data of compounds **PTH3**, **PTH7** and **PTM1** (recorded in $CDCl_3$)

Position	PTH3, δ_C (ppm)	PTH7, δ_C (ppm)	PTM1, δ_C (ppm)
1	38.7	38.7	38.7
2	27.4	27.4	27.4
3	79.0	79.0	80.0
4	38.9	38.9	38.9
5	55.3	55.3	55.3
6	18.3	18.3	18.3
7	34.2	34.3	34.3
8	40.9	40.9	40.9
9	50.4	50.4	50.4
10	37.2	37.2	37.2
11	20.8	21.1	21.9
12	25.2	26.7	26.8
13	37.3	38.0	37.2
14	42.7	42.8	42.7
15	27.0	27.4	27.0
16	29.2	35.5	29.2
17	47.5	43.0	47.8
18	48.8	48.9	49.5
19	47.5	43.8	43.5
20	150.5	154.8	152.0
21	29.8	31.8	31.6
22	34.0	39.9	33.8
23	28.0	28.0	28.0
24	15.4	15.4	15.4

Table 55 (Continued)

Position	PTH3, δ_C (ppm)	PTH7, δ_C (ppm)	PTM1, δ_C (ppm)
25	16.1	16.1	16.1
26	16.0	16.0	16.0
27	14.8	14.5	14.8
28	60.6	17.7	60.3
29	109.7	106.8	107.2
30	19.1	65.0	65.1

Table 56 Comparison of ^{1}H NMR spectral data between 3β, 28, 30-lup-20(29)-en-triol and **PTM1** (recorded in $CDCl_3$)

Position	3β, 28, 30-lup-20(29)-en-triol, δ_H (ppm)	Compound PTM1, δ_H (ppm)
3	3.15, 3.21 (*dd*, *J* = 10.9, 5.6 Hz)	3.18 (*dd*, *J* = 10.8, 5.1 Hz)
23	0.96 (*s*)	0.97 (*s*)
24	0.75 (*s*)	0.76 (*s*)
25	0.81 (*s*)	0.82 (*s*)
26	1.01 (*s*)	1.02 (*s*)
27	0.97 (*s*)	0.98 (*s*)
28	3.31 (*d*, *J* = 10.6 Hz),	3.30 (*d*, *J* = 11.4 Hz),
	3.79 (*d*, *J* = 10.6 Hz)	3.80 (*d*, *J* = 11.4 Hz)
29	4.89 (*s*), 4.94 (*s*)	4.91 (*br s*), 4.95 (*d*, *J* = 1.2 Hz)
30	4.12 (*s*)	4.12 (*br s*)

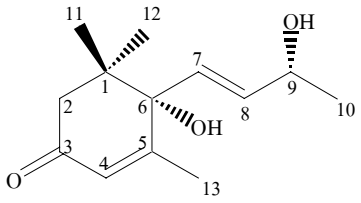
Table 57 Comparison of ^{13}C NMR spectral data between 3β, 28, 30-lup-20(29)-en-triol and compound **PTM1**

Position	**3β, 28, 30-lup-20(29)-en-triol, δ_C (ppm)** (recorded in CD_3OD)	**Compound PTM1, δ_C (ppm)** (recorded in $CDCl_3$)
1	38.2	38.7
2	30.6	27.4
3	82.3	80.0
4	40.9	38.9
5	59.5	55.3
6	22.0	18.3
7	37.5	34.3
8	42.7	40.9
9	54.5	50.4
10	32.2	37.2
11	24.7	21.9
12	30.8	26.8
13	41.3	37.2
14	44.8	42.7
15	33.0	27.0
16	38.0	29.2
17	46.4	47.8
18	53.3	49.5
19	47.4	43.5
20	150.7	152.0
21	35.4	31.6
22	33.1	33.8
23	31.2	28.0

Table 57 (Continued)

Position	3β, 28, 30-lup-20(29)-en-triol, δ_C (ppm) (recorded in CD_3OD)	Compound PTM1, δ_C(ppm) (recorded in $CDCl_3$)
24	19.3	15.4
25	18.6	16.1
26	19.2	16.0
27	17.8	14.8
28	62.9	60.3
29	109.8	107.2
30	67.8	65.1

3.1.21 Compound PTM2

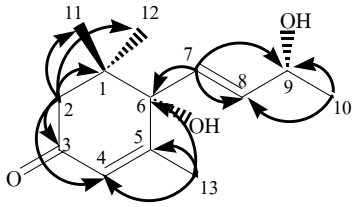


Compound **PTM2** was obtained as a colorless viscous oil, $[\alpha]_D^{28}$: +36.6 (*c* = 0.041, $CHCl_3$). The UV spectrum (λ_{max} 235 nm) (**Figure 72**) and the IR absorption (ν_{max} 1660 cm^{-1}) (**Figure 73**) indicated the presence of an α, β-unsaturated ketone.

Its ^{13}C NMR spectral data (**Table 58**, **Figure 75**) recorded in $CDCl_3$ showed 13 signals for 13 carbons. Analysis of the DEPT 90° and DEPT 135° spectra of this compound suggested the presence of four methyl carbons (δ 18.9, 22.9, 23.8 and 24.1),

one methylene carbon (δ 49.7), four methine carbons (δ 68.0, 126.9, 129.0 and 135.8), three quaternary carbons (δ 41.2, 79.1 and 162.6) and one carbonyl carbon at δ 197.9.

The ^{1}H NMR spectral data (**Table 58**, **Figure 74**) displayed typical signals of norsesquiterpenoids as four methyl groups at δ 1.01 (*s*), 1.08 (*s*), 1.31 (*d*, *J* = 6.6 Hz) and 1.90 (*d*, *J* = 1.2 Hz). The two olefinic methine protons were shown at δ 5.78 (1H, *dd*, *J* = 15.6, 0.6 Hz, H-7) and δ 5.85 (1H, *dd*, *J* = 15.6, 5.1 Hz, H-8) indicating them to have trans configuration. In addition, the methine proton at δ 5.91 (*m*) was assigned to H-4 and one oxymethine proton at δ 4.42 (1H, *qn*, *J* = 6.6 Hz) was assigned to H-9. The signals of AB system of oxymethylene protons were displayed at δ 2.46 (1H, *d*, *J* = 17.1 Hz) and 2.25 (1H, *dd*, *J* = 17.1 Hz) which were assigned to H-2a and H-2b, respectively. On the basis of HMBC (**Table 58**, **Figure 80**), the AB system of oxymethylene protons was located at C-2 by correlation of 2H-2 signal (δ 2.46 and 2.25) with C-1 (δ 41.2), C-3 (δ 197.9), C-4 (δ 126.9), C-6 (δ 79.1), C-11 (δ 24.1) and C-12 (δ 22.9), the vinylic methyl proton at δ 1.90 (3H-13) showed correlation with C-4 (δ 126.9), C-5 (δ 162.6) and C-6 (δ 79.1) suggesting the presence of a double bond between C-4 and C-5 and *trans* olefinic proton (H-8) showed long-range correlation with C-6 (δ 79.1), C-7 (δ 129.0) and C-9 (δ 68.0). Thus on the basis of its spectroscopic data and comparison with previously reported data (Kisiel *et al*., 2004), (**Table 59** and **60**), compound **PTM2** was identified as blumenol A.



Selected HMBC correlation of **PTM2**

Table 58 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTM2**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	41.2	C	-	-
1, 3, 4, 6, 11, 12		CH_2	2.25 (*d*, *J* = 17.1 Hz), 2.46 (*d*, *J* = 17.1 Hz)	
3	197.9	C	-	-
4	126.9	CH	5.91 (*m*)	2, 6, 13
5	162.6	C	-	-
6	79.1	C	-	-
7	129.0	CH	5.78 (*dd*, *J* = 15.6, 0.6 Hz)	6, 8, 9
8	135.8	CH	5.85 (*dd*, *J* = 15.6, 5.1 Hz)	5, 6, 7, 9
9	68.0	CH	4.42 (*qn*, *J* = 6.6 Hz)	7, 8, 10
10	23.8	CH_3	1.31 (*d*, *J* = 6.6 Hz)	8, 9
11	24.1	CH_3	1.08 (*s*)	1, 2, 6, 12
12	22.9	CH_3	1.01 (*s*)	1, 2, 3, 6, 11
13	18.9	CH_3	1.90 (*d*, *J* = 1.2 Hz)	4, 5, 6

Table 59 Comparison of ^{1}H NMR spectral data between blumenol A and compound **PTM2** (recorded in $CDCl_3$)

Position	blumenol A, δ_H (ppm)	Compound PTM2, δ_H (ppm)
2a	2.25 (*d*, *J* = 16.8 Hz)	2.25 (*d*, *J* = 17.1 Hz),
2b	2.45 (*d*, *J* = 16.8 Hz)	2.46 (*d*, *J* = 17.1 Hz)
4	5.91 (*br s*)	5.91 (*m*)
7	5.79 (*d*, *J* = 15.7 Hz)	5.78 (*dd*, *J* = 15.6, 0.6 Hz)
8	5.87 (*dd*, *J* = 15.7, 5.1Hz)	5.85 (*dd*, *J* = 15.6, 5.1 Hz)
9	4.42 (*m*)	4.42 (*qn*, *J* = 6.6 Hz)
10	1.30 (*d*, *J* = 6.3 Hz)	1.31 (*d*, *J* = 6.6 Hz)
11	1.02 (*s*)	1.01 (*s*)
12	1.11 (*s*)	1.08 (*s*)
13	1.90 (*br s*)	1.90 (*d*, *J* = 1.2 Hz)

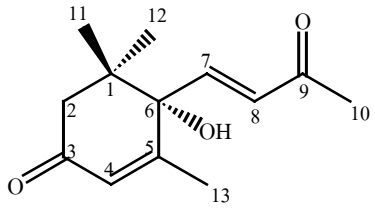
Table 60 Comparison of ^{13}C NMR spectral data between blumenol A and compound **PTM2** (recorded in $CDCl_3$)

Position	blumenol A, δ_C (ppm)	Compound PTM2, δ_C (ppm)
1	41.1	41.2
2	49.7	49.7
3	197.9	197.9
4	127.0	126.9
5	162.6	162.6
6	79.1	79.1
7	135.7	129.0
8	129.0	135.8
9	68.1	68.0
10	23.8	23.8

Table 60 (Continued)

Position	blumenol A, δ_C (ppm)	Compound PTM2, δ_C (ppm)
11	22.9	24.1
12	24.0	22.9
13	18.9	18.9

3.1.22 Compound PTM3



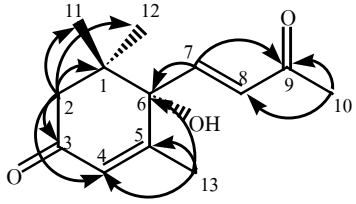
Compound **PTM3** was isolated as a colorless viscous oil, $[\alpha]_D^{28}$: +125.0 (*c* = 0.032, $CHCl_3$). The UV and IR spectra were similar to those of compound **PTM2**.

Its ^1H and ^{13}C NMR spectral data (**Table 61**, **Figure 81** and **82**) were similar to those of **PTM2** (**Figure 74** and **75**), except the oxymethine proton at δ 4.42 (1H, *qn*, *J* = 6.6 Hz, H-9) and a doublet signal of 3H-10 at δ 1.31 disappeared and the acetoxy protons were shown at δ 2.24 (3H, *s*). For this compound, assignments of carbon and proton signals (**Table 61**) were confirmed by means of HMQC and HMBC experiments. In the HMBC spectrum (**Table 61**) the methyl proton (3H-10) showed correlations with C-7 (δ 143.9), C-8 (δ 129.4) and C-9 (δ 197.0). Thus compound **PTM3** was assigned to be dehydrovomifoliol by comparison of its spectral data with previously reported data (Gonzalez *et al*., 1994), (**Table 62** and **63**).

Table 61 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTM3**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	40.1	C	-	-
2	48.6	CH_2	2.44 (*d*, *J* = 17.7 Hz)	
1, 3, 4, 6, 11, 12			2.27 (*d*, *J* = 17.7 Hz)	
3	197.0[c]	C	-	-
4	126.8	CH	5.89 (*br s*)	13
5	159.1	C	-	-
6	79.0	C	-	-
7	143.9	CH	6.76 (*d*, *J* = 15.9 Hz)	6, 9
8	129.4	CH	6.40 (*d*, *J* = 15.9 Hz)	6, 7, 9
9	197.0[c]	C	-	-
10	27.4	CH_3	2.24 (*s*)	7, 8, 9
11	23.3	CH_3	0.96 (*s*)	1, 2, 3, 6, 12
12	21.9	CH_3	1.04 (*s*)	1, 2, 6, 11
13	17.6	CH_3	1.82 (*d*, *J* = 1.5 Hz)	4, 5, 6, 7

[c] Deduced from HMBC experiment



Selected HMBC correlation of **PTM3**

Table 62 Comparison of ^{1}H NMR spectral data of dehydrovomifoliol, compounds **PTM2** and **PTM3** (recorded in $CDCl_3$)

Position	dehydrovomifoliol, δ_H (ppm)	Compound PTM2, δ_H (ppm)	Compound PTM3, δ_H (ppm)
2	2.33 (*d*, *J* = 17.2 Hz), 2.49 (*d*, *J* = 17.2 Hz)	2.25 (*dd*, *J* = 17.1 Hz), 2.46 (*d*, *J* = 17.1 Hz)	2.27 (*d*, *J* = 17.7 Hz), 2.44 (*d*, *J* = 17.7 Hz)
4	5.95 (*t*-like)	5.91 (*m*)	5.89 (*br s*)
7	6.82 (*d*, *J* = 15.7 Hz)	5.78 (*dd*, *J* = 15.6, 0.6 Hz)	6.76 (*d*, *J* = 15.9 Hz)
8	6.45 (*d*, *J* = 15.7 Hz)	5.85 (*dd*, *J* = 15.6, 5.1 Hz)	6.40 (*d*, *J* = 15.9 Hz)
9	-	4.42 (*dt*, *J* = 11.7, 6 Hz)	-
10	2.30 (*s*)	1.31 (*d*, *J* = 6.6 Hz)	2.24 (*s*)
11	1.10 (*s*)	1.01 (*s*)	0.96 (*s*)
12	1.02 (*s*)	1.08 (*s*)	1.04 (*s*)
13	1.88 (*d*, *J* = 1.4 Hz)	1.90 (*d*, *J* = 1.2 Hz)	1.82 (*d*, *J* = 1.5 Hz)

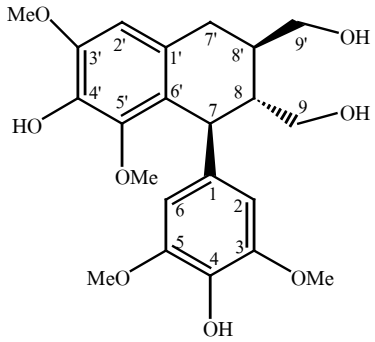
Table 63 Comparison of ^{13}C NMR spectral data of dehydrovomifoliol, compounds **PTM2** and **PTM3** (recorded in $CDCl_3$)

Position	dehydrovomifoliol, δ_C (ppm)	Compound PTM2, δ_C (ppm)	Compound PTM3, δ_C (ppm)
1	41.4	41.2	40.1
2	49.6	49.7	48.6
3	197.2	197.9	197.0
4	127.9	126.9	126.8
5	160.1	162.6	159.1
6	79.3	79.1	79.0
7	144.9	129.0	143.9
8	130.4	135.8	129.4

Table 63 (Continued)

Position	dehydrovomifoliol, δ_C (ppm)	Compound PTM2, δ_C (ppm)	Compound PTM3, δ_C (ppm)
9	196.8	68.0	197.0
10	28.4	23.8	27.4
11	24.3	24.1	23.3
12	22.9	22.9	21.9
13	18.6	18.9	17.6

3.1.23 Compound PTM4



Compound **PTM4** was obtained as a colorless viscous oil, $[\alpha]_D^{28}$ + 34.5° (*c* = 0.220, MeOH). Its IR spectrum (**Figure 84**) exhibited absorption bands at 3401 cm^{-1} (hydroxyl group) and 1612, 1500 cm^{-1} (aromatic ring). The UV spectrum (**Figure 83**) showed absorption maxima at 236 and 280 nm.

The ^{13}C NMR spectral data (**Table 66**, **Figure 86**) recorded in $CDCl_3$ showed 19 signals for 22 carbons. Analysis of the DEPT 90° and DEPT 135° spectra of this compound suggested the presence of four methoxy carbons (δ 56.1, 56.4, 56.4 and

59.5), three methylene carbons (δ 33.5, 64.0 and 66.8), six methine carbons (δ 40.4, 43.1, 49.5, 105.4, 105.4 and 105.9) and nine quaternary carbons (δ 125.2, 128.6, 133.0, 137.0, 138.3, 145.5, 146.0, 146.8 and 146.8).

The ^{1}H, ^{13}C NMR and DEPT spectra of compound **PTM4** possessed signals characteristic of an aryl-tetralin type lignan (**Table 66**).

The ^{1}H NMR spectral data (**Table 66**, **Figure 85**) displayed two hydroxyl groups at δ 5.37 and 5.40 (each 1H, *s*, -OH, disappeared on D_2O exchange) and four aromatic methoxyl singlet signals at δ 3.30, 3.80, 3.80 and 3.89. The two aromatic methine protons exhibited signals at δ 6.35 (2H, *s*, H-2 and H-6) and one aromatic methine proton at δ 6.45 (1H, *s*, H-2′). In addition, two signals of oxymethylene protons were shown as multiplets at δ 3.58 and 3.82 which were assigned to 2H-9 and δ 3.78 and 3.64 which were assigned to 2H-9′. The complete assignments of ^{13}C and ^{1}H NMR (**Table 66**, **Figure 85** and **86**) were made with the information from ^{1}H-^{1}H COSY (**Table 64**, **Figure 89**), NOESY (**Table 65**), HMQC and HMBC experiments (**Table 66**, **Figure 90** and **91**). In the HMBC experiment the methine proton at δ 4.02 (H-7) showed long-range correlation with C-1 (δ 138.3), C-2 (δ 105.4), C-6 (δ 105.4), C-8 (δ 49.5), C-1′ (δ 128.6), C-6′ (δ 125.2) and C-8′ (δ 40.4). The locations of oxymethylene protons of 2H-9 and 2H-9′ were determined from COSY experiment which were summarized in **Table 64**.

The relative stereochemistry of **PTM4** was supported by NOESY correlations. Proton H-7 (δ 4.02) showed cross peak with H-8′ (δ 1.75), while the signal of H-8 (δ 1.93) was not observed. These observations suggested that H-7 and H-8′ were on the same side and opposite to H-8.

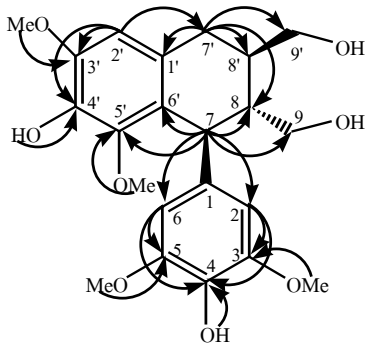
Thus compound **PTM4** was established as lyoniresinol by comparison of its spectroscopic data with those reported in the literature (Zhang *et al*., 1999), (**Table 67** and **68**).

Table 64 300 MHz COSY Correlation of some protons of compound **PTM4**

δ_H (ppm)	Proton correlation with δ_H (ppm)
H-7	H-8
H-8	H-7, H-9, H-8′
H-9	H-8
H-7′	H-8′
H-8′	H-8, H-7′, H-9′
H-9′	H-8′

Table 65 300 MHz NOESY Correlation of some protons of compound **PTM4**

δ_H (ppm)	Proton correlation with δ_H (ppm)
H-2	H-8, OMe-3
H-6	H-7, OMe-5, OMe-5′
H-7	H-2, H-6, H-8′, H-9
H-8	H-2, H-7′, H-9
H-2′	H-7′, OMe-3′
H-8′	H-7′, H-9′



Selected HMBC correlation of **PTM4**

Table 66 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTM4**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	138.3	C	-	-
2	105.4	CH	6.35 (*s*)	1, 3, 4, 6, 7
3	146.8	C	-	-
4	133.0	C	-	-
5	146.8	C	-	-
6	105.4	CH	6.35 (*s*)	1, 2, 4, 5, 7
7	43.1	CH	4.02 (*d*, *J* = 7.8 Hz)	1, 2, 6, 8, 9, 1′, 5′, 6′, 8′
8	49.5	CH	1.93 (*m*)	-
9	64.0	CH_2	3.58 (*m*), 3.82 (*m*)[a]	8′, 7
1′	128.6	C	-	-
2′	105.9	CH	6.45 (*s*)	3′, 4′, 6′, 7′
3′	146.0	C	-	-
4′	137.0	C	-	-
5′	145.5	C	-	-
6′	125.2	C	-	-
7′	33.5	CH_2	2.68 (*dd*, *J* = 15.3, 11.4 Hz)	
8, 1′, 2′, 6′, 8′, 9′			2.59 (*dd*, *J* = 15.3, 4.5 Hz)	
8′	40.4	CH	1.75 (*m*)	-
9′	66.8	CH_2	3.64 (*m*), 3.78 (*m*)[a]	8, 7′
OMe-3	56.4	CH_3	3.80 (*s*)	3
OMe-5	56.4	CH_3	3.80 (*s*)	5
OMe-3′	56.1	CH_3	3.89 (*s*)	3′
OMe-5′	59.5	CH_3	3.30 (*s*)	5′
OH-4	-	-	5.40 (*s*)	3, 4, 5
OH-4′	-	-	5.37 (s)	3′, 4′, 5′

[a] Deduced from HMQC experiment

Table 67 Comparison of ^{1}H NMR spectral data between lyoniresinol and compound **PTM4**

Position	**lyoniresinol, δ_H (ppm)** (recorded in acetone-d_6)[d]	**Compound PTM4, δ_H (ppm)** (recorded in $CDCl_3$)
2	6.29 (*s*)	6.35 (*s*)
6	6.29 (*s*)	6.35 (*s*)
7	4.23 (*d*, *J* = 5.8 Hz)	4.02 (*d*, *J* = 7.8 Hz)
8	1.86 (*m*)	1.93 (*m*)
9	3.26 (*m*)	3.58 (*m*), 3.82 (*m*)[a]
2′	6.54 (*s*)	6.45 (*s*)
7′	2.38 (*dd*, *J* = 14.0, 11.8 Hz),	2.59 (*dd*, *J* = 15.3, 4.5 Hz),
	2.58 (*dd*, *J* = 14.8, 4.6 Hz)	2.68 (*dd*, *J* = 15.3, 11.4 Hz)
8′	1.44 (*m*)	1.75 (*m*)
9′	3.45 (*m*), 3.85 (*m*)	3.64 (*m*), 3.78 (*m*)[a]
OMe-3	3.64 (*s*)	3.80 (*s*)
OMe-5	3.64 (*s*)	3.80 (*s*)
OMe-3′	3.77 (*s*)	3.89 (*s*)
OMe-5′	3.35 (*s*)	3.30 (*s*)
OH-4	7.39 (*s*)	5.40 (*s*)
OH-4′	7.16 (*s*)	5.37 (s)

[a] Deduced from HMQC experiment

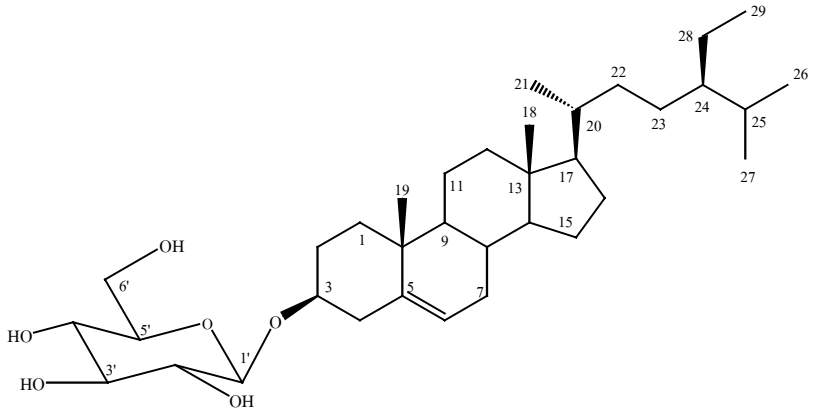
[d] The assignment was based upon COSY and HMQC experiments.

Table 68 Comparison of ^{13}C NMR spectral data between lyoniresinol and compound **PTM4**

Position	**lyoniresinol, δ_C (ppm)** (recorded in acetone-d_6)[d]	**Compound PTM4, δ_C (ppm)** (recorded in $CDCl_3$)
1	137.8	138.3
2	106.5	105.4
3	147.7	146.8
4	134.8	133.0
5	147.7	146.8
6	106.5	105.4
7	40.4	43.1
8	46.8	49.5
9	62.7	64.0
1′	128.8	128.6
2′	107.0	105.9
3′	147.0	146.0
4′	137.3	137.0
5′	146.6	145.5
6′	125.1	125.2
7′	32.3	33.5
8′	39.3	40.4
9′	64.9	66.8
OMe-3	56.4	56.4
OMe-5	56.4	56.4
OMe-3′	55.9	56.1
OMe-5′	59.2	59.5

[d] The assignment was based upon COSY and HMQC experiments.

3.1.24 Compound PTM5



Compound **PTM5** was obtained as a white solid: mp 278-280°C $[\alpha]_D^{28}$: -50° (*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 69**, **Figure 93**) 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 11.93, 12.03, 18.85, 19.09, 19.40 and 19.87), twelve methylene (δ 21.17, 23.18, 24.39, 26.19, 28.35, 29.80, 32.04, 34.06, 37.37, 38.83, 39.87 and 62.02), fourteen methine (δ 29.27, 32.00, 36.26, 45.98, 50.30, 56.18, 56.87, 70.28, 73.66, 75.83, 76.50, 79.31, 122.30, including one anomeric carbon at δ 101.21) and three quaternary carbons (δ 36.83, 42.44 and 140.39).

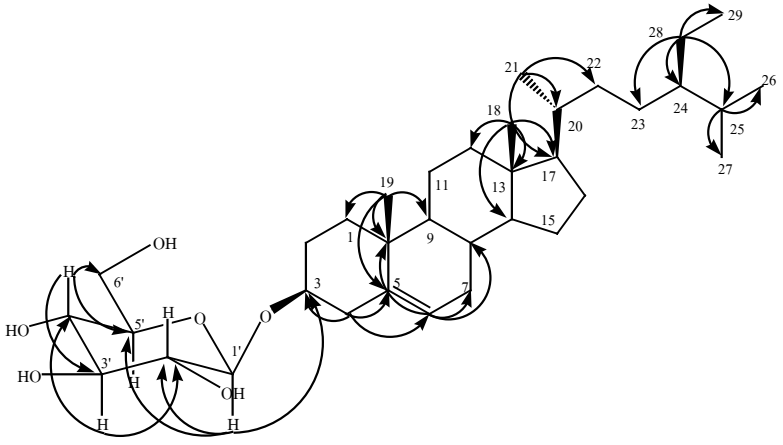
The ^{1}H NMR spectral data (**Table 69**, **Figure 92**) recorded in $CDCl_3$+CD_3OD displayed a characteristic signal of sitosterol and sugar unit. The sitosterol unit was shown as two methyl singlet signals at δ0.69 (3H-18) and 1.01 (3H-19), three methyl doublets at δ0.93 (*d*, *J* = 6.3 Hz, 3H-21), 0.84 (3H-26) and 0.82 (3H-27) [each *d*, *J* = 6.6 Hz], one methyl triplet at δ0.85 (*t*, *J* = 7.2 Hz, 3H-29), one olefinic proton at δ5.37 (*br d*, *J* = 5.1 Hz, H-6) and one oxymethine proton at δ3.60 (1H, *m*, H-3). The four

methine protons in the sugar unit were shown as multiplet signals at δ3.24 (H-2′), 3.30 (H-5′), 3.41 (H-3′) and 3.44 (H-4′), one anomeric proton at δ4.41 (*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 69**) signals were made with the information from ^{1}H-^{1}H COSY, HMQC and HMBC spectrum (**Table 69**). 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 δ4.41 (H-1′) showed cross peak 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 at C-3.

By comparison of the ^{1}H and ^{13}C NMR spectral data (**Table 70** and **71**) with those of atroside (**Figure 3**), (Ali *et al*., 2001), compound **PTM5** was identified as β-sitosterol glucopyranoside.



Selected HMBC correlation of **PTM5**

Table 69 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PTM5**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	37.4	CH_2	1.87 (*m*), 1.08 (*m*)[a]	-
2	29.8	CH_2	1.91 (*m*), 1.63 (*m*)[a]	-
3	79.3	CH	3.60 (*m*)	1′
4	38.8	CH_2	2.41 (*m*), 2.27 (*m*)[a]	2, 3, 5, 6, 10
5	140.4	C	-	-
6	122.3	CH	5.37 (*br d*, *J* = 5.1 Hz)	4, 7, 8, 10
7	32.0	CH_2	1.68 (*m*), 1.43 (*m*)[a]	-
8	32.0	CH	2.00 (*m*)[a]	-
9	50.3	CH	0.93 (*m*)[a]	-
10	36.8	C	-	-
11	21.2	CH_2	1.54 (*m*), 1.48 (*m*)[a]	-
12	39.9	CH_2	2.03 (*m*), 1.18 (*m*)[a]	-
13	42.4	C	-	-
14	56.9	CH	1.03 (*m*)[a]	-
15	24.4	CH_2	1.56 (*m*)[a]	-
16	28.4	CH_2	1.31 (*m*)[a]	-
17	56.2	CH	1.11 (*m*)[a]	-
18	11.9	CH_3	0.69 (*s*)	12, 13, 14, 17
19	19.4	CH_3	1.01 (s)	1, 5, 9, 10
20	36.3	CH	1.38 (*m*)[a]	-
21	18.9	CH_3	0.93 (*d*, *J* = 6.3 Hz)	17, 20, 22
22	34.1	CH_2	1.31 (*m*), 1.08 (*m*)[a]	-
23	26.2	CH_2	1.16 (*m*)[a]	-
24	46.0	CH	0.93 (*m*)[a]	-
25	29.3	CH	1.26 (*m*)[a]	23, 24, 26, 27, 28
26	19.9	CH_3	0.84 (*d*, *J* = 6.6 Hz)	24, 25, 27
27	19.1	CH_3	0.82 (*d*, *J* = 6.6 Hz)	24, 25, 26

Table 69 (Continued)

Position	δ_C (ppm)		δ_H (ppm)	HMBC
28	23.2	CH_2	1.25 (*m*) [a]	23, 24, 25, 29
29	12.0	CH_3	0.85 (*t*, *J* = 6.6 Hz)	24, 28
1′	101.2	CH	4.41 (*d*, *J* = 7.5 Hz)	3, 2′,5′
2′	73.7	CH	3.24 (*m*) [a]	4′, 3′
3′	76.5	CH	3.41 (*m*) [a]	2′, 4′
4′	70.3	CH	3.44 (*m*) [a]	2′, 3′, 5′, 6′
5′	75.8	CH	3.30 (*m*) [a]	1′, 4′
6′	62.0	CH_2	3.75 (*dd*, *J* = 12.0, 4.5 Hz)	
3′, 4′, 5′			3.84 (*dd*, *J* = 12.0, 3.0 Hz)	

[a] Deduced from HMQC experiment

Table 70 Comparison of ^{1}H NMR spectral data between atroside and compound **PTM5**

Position	atroside, δ_H (ppm) (recorded in $CDCl_3$)	Compound PTM5, δ_H (ppm) (recorded in $CDCl_3$+CD_3OD)
3	3.14 (*m*)	3.60 (*m*)
6	5.31 (*dist t*)	5.37 (*br d*, *J* = 5.1 Hz)
18	0.66 (*s*)	0.69 (*s*)
19	0.99 (*s*)	1.01 (s)
21	0.90 (*d*, *J* = 6.4 Hz)	0.93 (*d*, *J* = 6.3 Hz)
26	0.79 (*d*, *J* = 6.5 Hz)	0.84 (*d*, *J* = 6.6 Hz)
27	0.79 (*d*, *J* = 6.5 Hz)	0.82 (*d*, *J* = 6.6 Hz)
29	0.81 (*t*, *J* = 6.5 Hz)	0.85 (*t*, *J* = 6.6 Hz)
1′	4.31 (*d*, *J* = 7.6 Hz)	4.41 (*d*, *J* = 7.5 Hz)
2′	3.61-3.33 (*m*)	3.24 (*m*) [a]

Table 70 (Continued)

Position	atroside, δ_H (ppm) (recorded in $CDCl_3$)	Compound PTM5, δ_H (ppm) (recorded in $CDCl_3$+CD_3OD)
3′	3.61-3.33 (*m*)	3.41 (*m*) [a]
4′	3.61-3.33 (*m*)	3.44 (*m*) [a]
5′	3.61-3.33 (*m*)	3.30 (*m*) [a]
6′a	4.12 (*br d*, *J* = 12.1Hz)	3.84 (*dd*, *J* = 12.0, 3.0 Hz)
b	4.42 (*dd*, *J* = 12.0, 4.3Hz)	3.75 (*dd*, *J* = 12.0, 4.5 Hz)
chain	1.24 (*br s*)	-
10′′	0.83 (*s*)	-

[a] Deduced from HMQC experiment

Table 71 Comparison of ^{13}C NMR spectral data between atroside and compound **PTM5**

Position	atroside, δ_C(ppm) (recorded in $CDCl_3$)	Compound PTM5, $\delta_C$ppm) (recorded in $CDCl_3$+CD_3OD)
1	37.2	37.4
2	29.4	29.8
3	79.6	79.3
4	38.9	38.8
5	140.2	140.4
6	122.1	122.3
7	31.8	32.0
8	31.9	32.0
9	50.1	50.3
10	36.7	36.8
11	21.1	21.2

Table 71 (Continued)

Position	atroside, δ_H (ppm) (recorded in $CDCl_3$)	Compound PTM5, δ_H (ppm) (recorded in $CDCl_3$+CD_3OD)
12	39.7	39.9
13	42.3	42.4
14	56.7	56.9
15	24.3	24.4
16	28.2	28.4
17	56.1	56.2
18	11.9	11.9
19	19.4	19.4
20	36.1	36.3
21	18.8	18.9
22	33.9	34.1
23	26.0	26.2
24	48.5	46.0
25	29.1	29.3
26	19.8	19.9
27	19.0	19.1
28	23.0	23.2
29	12.0	12.0
1′	101.1	101.2
2′	73.5	73.7
3′	75.9	76.5
4′	70.1	70.3
5′	73.9	75.8
6′	63.2	62.0
1′′	174.6	-
2′′	34.2	-

Table 71 (Continued)

Position	atroside, δ_H (ppm) (recorded in $CDCl_3$)[a]	Compound PTM5, δ_H (ppm) (recorded in $CDCl_3$+CD_3OD)
3′′	25.0	-
4′′-7′′	29.3	-
8′′	31.8	-
9′′	22.7	-
10′′	14.1	-

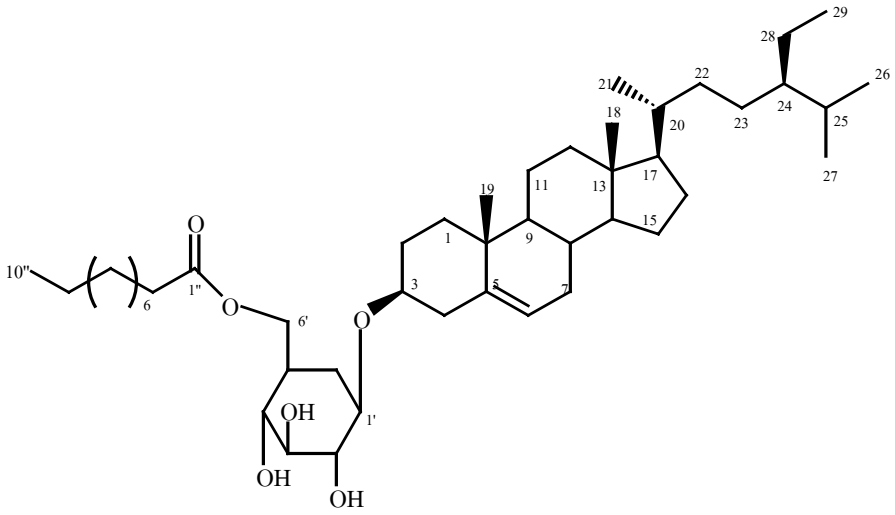


Figure 3 The structure of atroside

3.2 Biological activities of the pure compounds from C. decandra

The biological activities of the pure compounds (**PTH1**-**PTH19** and **PTM1**-**PTM5**) from *C. decandra* were tested only against NCI-H187 cell lines (Human small cell lung cancer) because the crude methylene chloride extract exhibited weak activity against NCI-H187 cell lines but the crude hexane extract showed no activity. Only one compound (**PTH5**) exhibited strong activity (IC_{50} 2.90 μg/mL), two compounds (**PTH6** and **PTH16**) exhibited moderate activity (IC_{50} 8.48 and 6.20 μg/mL, respectively) whereas the rest of the pure compounds showed no activity.