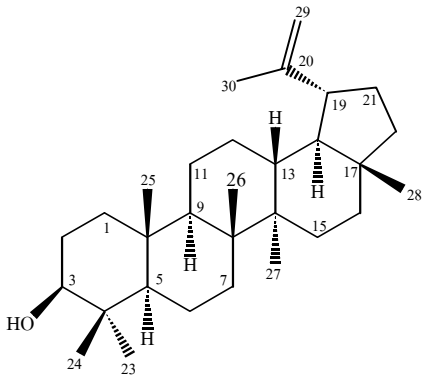
CHAPTER 2.3

RESULTS AND DISCUSSION

2.3.1 Structural Elucidation of Compounds from the Twigs of M. kunstleri

The crude methanol extract from the twigs of *M. kunstleri* was dissolved with chloroform. The chloroform soluble portion was purified by chromatographic methods to yield thirteen compounds; eight pentacyclic triterpenes (**MKT1**, **MKT3**, **MKT5**, **MKT6**, **MKT7**, **MKT8**, **MKT10** and **MKT12**), two xanthones (**MKT9** and **MKT13**), one benzoic acid derivative (**MKT11**), a mixture of two steroids (**MKT2**) and a mixture of two steroid glucosides (**MKT4**). All structures were determined by 1D and/or 2D NMR spectroscopic data and/or comparison of ^{1}H and/or ^{13}C spectral data from those reported in the literature. The ^{13}C NMR signals were assigned from ^{13}C, DEPT, HMQC and HMBC spectra.

2.3.1.1 Compound MKT1: Lup-20(29)-en-3β-ol (lupeol)



Compound **MKT1** was obtained as a white solid, melting at 204.5-205.7 °C (ref. 212.0-214.0 °C); $[\alpha]_D^{30}$ +19.36°, c = 0.16, EtOH (ref. $[\alpha]_D^{25}$ +23.00°, EtOH). The IR spectrum (**Figure 37**) exhibited an absorption band for a hydroxyl group at 3417 cm^{-1}. The ^1H NMR spectrum (**Figure 38**) (**Table 34**) of **MKT1** indicated the presence of seven tertiary methyls [δ_H 1.68, 1.03, 0.97, 0.95, 0.83, 0.79 and 0.76], one oxymethine proton (δ_H 3.19, *dd*, *J* = 10.8 and 5.4 Hz) and a methine proton (δ_H 2.38, *dt*, *J* = 11.1 and 5.7 Hz). The above informations were regarded as being due to a pentacyclic triterpene having a 3β-hydroxyl group. The signal of terminal olefinic protons and one vinylic methyl proton at δ_H 4.69 (1H, *d*, *J* = 2.1 Hz) and δ_H 4.57 (1H, *brqd*, *J* = 2.1 and 1.2 Hz) and δ_H 1.68 (3H, *s*), respectively, established the presence of an isopropenyl unit (-C(CH_3)=CH_2). It was in agreement with the HMBC correlation data (**Figure 42**) (**Table 34**). The ^{13}C NMR spectrum (**Figure 39**) (**Table 34**) exhibited six quaternary carbons (δ_C 150.95, 43.01, 42.84, 40.84, 38.86 and 37.18), six methine carbons (δ_C 78.99, 55.32, 50.45, 48.32, 47.99 and 38.07), eleven methylene carbons (δ_C 109.33, 40.01, 38.73, 35.60, 34.30, 29.86, 27.46, 27.42, 25.16, 20.94 and 18.33) and seven methyl carbons (δ_C 28.00, 19.32, 18.01, 16.12, 15.99, 15.38 and 14.56).

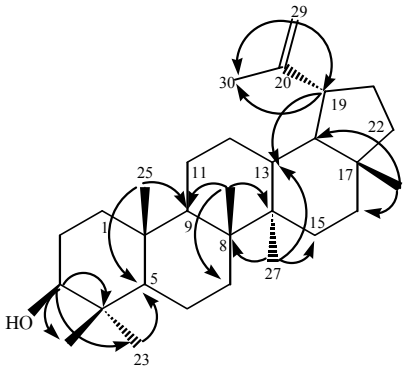
Table 34 The NMR spectral data of compound **MKT1**

Position	δ_H, *mult.*, *J* (Hz)	Type of C	δ_C	HMBC
1	0.91 (*m*, 2H)	CH_2	38.73	
2	1.55 (*m*, 2H)	CH_2	27.46	
3	3.19 (*dd*, 10.8, 5.4, 1H)	CH	78.99	C-4, C-23, C-24
4	-	C	38.86	
5	0.69 (*brd*, 9.0, 1H)	CH	55.32	C-6, C-7, C-9, C-23
6	1.53 (*m*, 1H); 1.40 (*m*, 1H)	CH_2	18.33	C-5, C-8, C-9, C-26
7	1.40 (*m*, 2H)	CH_2	34.30	

Table 34 (continued)

Position	δ_H, *mult.*, *J* (Hz)	Type of C	δ_C	HMBC
8	-	C	40.84	
9	1.27 (*m*, 1H)	CH	50.45	C-5, C-10, C-11, C-26
10	-	C	37.18	
11	1.45 (*m*, 1H); 1.26 (*m*, 1H)	CH_2	20.94	
12	1.69 (*m*, 2H)	CH_2	25.16	
13	1.68 (*m*, 1H)	CH	38.07	
14	-	C	42.84	
15	1.67 (*m*, 2H)	CH_2	27.42	
16	1.50 (*m*, 1H); 1.35 (*m*, 1H)	CH_2	35.60	
17	-	C	43.01	
18	1.37 (*m*, 1H)	CH	48.32	C-17, C-19
19	2.38 (*dt*, 11.1, 5.7, 1H)	CH	47.99	C-13, C-18, C-21, C-30
20	-	C	150.95	
21	1.94 (*m*, 1H); 1.27 (*m*, 1H)	CH_2	29.86	
22	1.41 (*m*, 1H); 1.19 (*m*, 1H)	CH_2	40.01	
23	0.97 (*s*, 3H)	CH_3	28.00	C-3, C-4, C-5, C-24
24	0.76 (*s*, 3H)	CH_3	15.38	C-3, C-5, C-23
25	0.83 (*s*, 3H)	CH_3	16.12	C-5, C-9, C-10
26	1.03 (*s*, 3H)	CH_3	15.99	C-7, C-8, C-9, C-14
27	0.95 (*s*, 3H)	CH_3	14.56	C-8, C-13, C-14, C-15
28	0.79 (*s*, 3H)	CH_3	18.01	C-16, C-17, C-18
29	4.69 (*d*, 2.1, 1H); 4.57 (*brqd*, 2.1, 1.2, 1H)	CH_2	109.33	C-19, C-20, C-21, C-30
30	1.68 (*s*, 3H)	CH_3	19.32	C-19, C-20, C-29

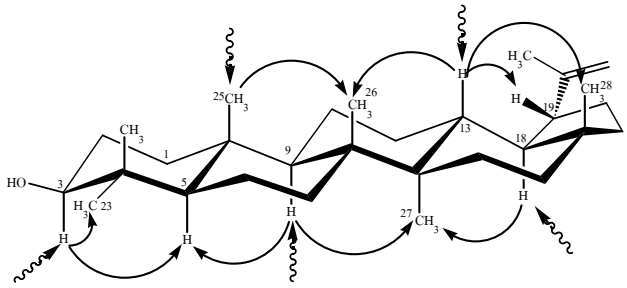
In the HMBC correlation spectrum (**Figure 42**) (**Table 34**), an oxymethine proton at δ_H 3.19 (*dd*, *J* = 10.8 and 5.4 Hz) exhibited correlation peaks with C-4 (δ_c 38.86), C-23 (δ_C 28.00) and C-24 (δ_C 15.38). These results confirmed the position of oxymethine proton at C-3 of ring A. The methine proton at C-19 (δ_H 2.38) showed correlations with C-13 (δ_C 38.07), C-18 (δ_C 48.32), C-21 (δ_C 29.86) and C-30 (δ_C 19.32), suggesting that the isopropenyl moiety was linked at the position 19 of ring E as shown. Furthermore, HMBC correlation spectrum showed the correlations of Me-25 (δ_H 0.83) with C-5 (δ_C 55.32), C-9 (δ_C 50.45) and C-10 (δ_C 37.18); Me-26 (δ_H 1.03) with C-7 (δ_C 34.30), C-8 (δ_C 40.84), C-9 (δ_C 50.45) and C-14 (δ_C 42.84); Me-27 (δ_H 0.95) with C-8 (δ_C 40.84), C-13 (δ_C 38.07), C-14 (δ_C 42.84) and C-15 (δ_C 27.42) and Me-28 (δ_H 0.79) with C-16 (δ_C 35.60), C-17 (δ_C 43.01) and C-18 (δ_C 48.32). These results confirmed the locations of Me-25, Me-26, Me-27 and Me-28, respectively.



HMBC correlations

The relative stereochemistry of **MKT1** was established by NOE difference results. Firstly, signals of Me-23 (δ_H 0.97) and H-5 (δ_H 0.69) were enhanced when the oxymethine proton H-3 (δ_H 3.19) was irradiated (**Figure 47**), H-5 (δ_H 0.69) and Me-27 (δ_H 0.95) were enhanced when irradiated at H-9 (δ_H 1.27) (**Figure 44**) and Me-27 (δ_H 0.95) was enhanced when methine proton H-18 (δ_H 1.37) was irradiated (**Figure 45**).

These results indicated that H-5, H-9, Me-23, Me-27 and H-18 were located on the same side of the molecule as H-3, α-side. Secondly, Me-26 (δ_H 1.03), Me-28 (δ_H 0.79) and H-19 (δ_H 2.38) were enhanced when H-13 (δ_H 1.68) was irradiated (**Figure 46**) and Me-26 (δ_H 1.03) was enhanced after irradiated at Me-25 (δ_H 0.83) (**Figure 43**), indicating that the Me-26, Me-28, H-19, H-13 and Me-25 were located on the same side, β–side.



NOE of **MKT1**

Comparison of the ^{13}C NMR spectral data of compounds **MKT1** with those of **lupeol** (Reynolds, *et al*., 1986) (**Table 35**) showed similarity. Thus, compound **MKT1** was identified as **lupeol**.

Table 35 Comparison of ^{13}C NMR spectral data of compounds **MKT1** with **lupeol**

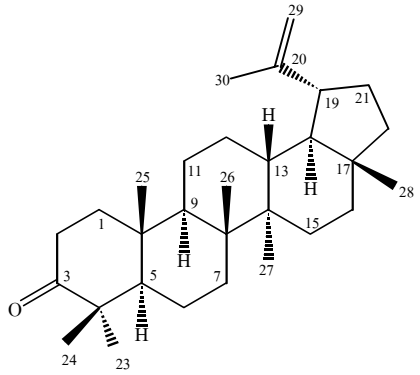
Position	**MKT1**[a], δ_C (ppm)	**lupeol**[b], δ_C (ppm)
1	38.73	38.67
2	27.46	27.35
3	78.99	78.94
4	38.86	38.81
5	55.32	55.25
6	18.33	18.28
7	34.30	34.23
8	40.84	40.78

Table 35 (continued)

Position	**MKT1**[a], δ_C (ppm)	**lupeol**[b], δ_C (ppm)
9	50.45	50.38
10	37.18	37.11
11	20.94	20.89
12	25.16	25.08
13	38.07	38.00
14	42.84	42.78
15	27.42	27.41
16	35.60	35.54
17	43.01	42.95
18	48.32	48.24
19	47.99	47.94
20	150.95	150.88
21	29.86	29.80
22	40.01	39.96
23	28.00	27.95
24	15.38	15.35
25	16.12	16.09
26	15.99	15.94
27	14.56	14.51
28	18.01	17.97
29	109.33	109.31
30	19.32	19.28

[a]300 MHz, in $CDCl_3$; [b]400 MHz, in $CDCl_3$

2.3.1.2 Compound MKT6: Lup-20(29)-en-3-one (lupenone)



Compound **MKT6** was obtained as a yellow viscous-liquid, $[\alpha]_D^{30}$ +44.22°, c = 0.19, $CHCl_3$ (ref. $[\alpha]_D$ +61.00°). The IR spectrum (**Figure 48**) showed the presence of a carbonyl group (1704 cm^{-1}). The ^1H NMR spectrum (**Figure 49**) (**Table 36**) was similar to that of **MKT1** (**lupeol**), but an oxymethine proton [δ_H 3.19 (*dd*, *J* = 10.8 and 5.4 Hz)] disappeared in **MKT6**. These results suggested that the hydroxyl group at C-3 was replaced with the carbonyl group. The ^{13}C NMR spectrum (**Figure 50**) (**Table 36**) confirmed the above conclusion by absence of oxymethine proton and the presence of one carbonyl carbon, which was supported by the correlation of Me-23 (δ_H 1.08) and Me-24 (δ_H 1.04) to the carbonyl carbon in HMBC experiment (**Figure 53**). Comparison of its NMR data with **MKT1** (**lupeol**) and the previously reported data of **lupenone** (Razden, *et al*., 1988) (**Table 37**) indicated that **MKT6** had the same structure as **lupenone**.

Table 36 The NMR data of compounds **MKT6** and **MKT1**

Position	**MKT6**		**MKT1**	
	δ_H, *mult*., *J* (Hz)	δ_C (C-Type)	δ_H, *mult*., *J* (Hz)	δ_C (C-Type)
1	1.89 (*m*, 1H); 1.42 (*m*, 1H)	39.62 (CH_2)	0.91 (*m*, 1H)	38.73 (CH_2)
2	2.45 (*m*, 2H)	34.15 (CH_2)	1.55 (*m*, 2H)	27.46 (CH_2)
3	-	218.15 (C)	3.19 (*dd*, 10.8, 5.4, 1H)	78.99 (CH)
4	-	47.33 (C)	-	38.86 (C)
5	1.32 (*m*, 1H)	54.93 (CH)	0.69 (*brd,* 9.0, 1H)	55.32 (CH)
6	1.46 (*m*, 2H)	19.69 (CH_2)	1.53 (*m*, 1H); 1.40 (*m*, 1H)	18.33 (CH_2)
7	1.46 (*m*, 2H)	33.57 (CH_2)	1.40 (*m*, 2H)	34.30 (CH_2)
8	-	40.78 (C)	-	40.84 (C)
9	1.37 (*m*, 1H)	49.79 (CH)	1.27 (*m*, 1H)	50.45 (CH)
10	-	36.88 (C)	-	37.18 (C)
11	1.47 (*m*, 2H)	21.48 (CH_2)	1.45 (*m*, 1H); 1.26 (*m*, 1H)	20.94 (CH_2)
12	1.71 (*m*, 2H)	25.16 (CH_2)	1.69 (*m*, 2H)	25.16 (CH_2)
13	1.68 (*m*, 1H)	38.17 (CH)	1.68 (*m*, 1H)	38.07 (CH)
14	-	42.90 (C)	-	42.84 (C)
15	1.71 (*m*, 1H); 1.00 (*m*, 1H)	27.44 (CH_2)	1.67 (*m*, 2H)	27.42 (CH_2)
16	1.50 (*m*, 1H); 1.37 (*m*, 1H)	35.53 (CH_2)	1.50 (*m*, 1H); 1.35 (*m*, 1H)	35.60 (CH_2)
17	-	42.99 (C)	-	43.01 (C)
18	1.38 (*m*, 1H)	48.25 (CH)	1.37 (*m*, 1H)	48.32 (CH)
19	2.39 (*m*, 1H)	47.96 (CH)	2.38 (*dt*, 11.1, 5.7, 1H)	47.99 (CH)
20	-	150.84 (C)	-	150.95 (C)
21	1.93 (*m*, 1H); 1.27 (*m*, 1H)	29.84 (CH_2)	1.94 (*m*, 1H); 1.27 (*m*, 1H)	29.86 (CH_2)
22	1.19 (*m*, 2H)	39.98 (CH_2)	1.41 (*m*, 1H); 1.19 (*m*, 1H)	40.01 (CH_2)
23	1.08 (*s*, 3H)	26.66 (CH_3)	0.97 (*s*, 3H)	28.00 (CH_3)
24	1.04 (*s*, 3H)	21.04 (CH_3)	0.76 (*s*, 3H)	15.38 (CH_3)

Table 36 (continued)

Position	**MKT6**		**MKT1**	
	δ_H, *mult., J* (Hz)	δ_C (C-Type)	δ_H, *mult., J* (Hz)	δ_C (C-Type)
25	0.94 (*s*, 3H)	15.97 (CH_3)	0.83 (*s*, 3H)	16.21 (CH_3)
26	1.08 (*s*, 3H)	15.79 (CH_3)	1.03 (*s*, 3H)	15.99 (CH_3)
27	0.97 (*s*, 3H)	14.49 (CH_3)	0.95 (*s*, 3H)	14.56 (CH_3)
28	0.81 (*s*, 3H)	18.02 (CH_3)	0.79 (*s*, 3H)	18.01 (CH_3)
29	4.70 (*brd*, 2.1, 1H);	109.41 (CH_2)	4.69 (*d*, 2.1, 1H);	109.3(CH_2)
	4.58 (*brqd,* 2.1, 1.2, 1H)		4.57 (*brqd,* 2.1, 1.2, 1H)	
30	1.69 (*brs*, 3H)	19.32 (CH_3)	1.68 (*s*, 3H)	19.32 (CH_3)

Table 37 Comparison of ^{13}C NMR spectral data of compounds **MKT6** and **lupenone**

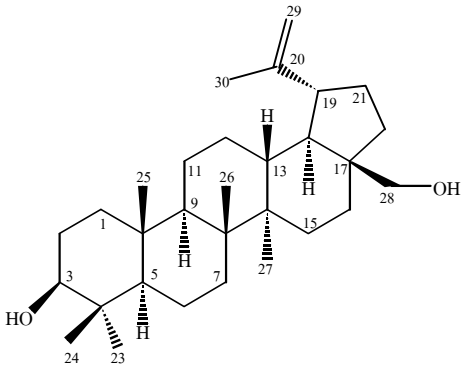
Position	**MKT6**[a], δ_C (ppm)	**lupenone**[b], δ_C (ppm)
1	39.62	39.6
2	34.15	34.1
3	218.15	217.9
4	47.33	47.2
5	54.93	55.8
6	19.69	19.6
7	33.57	33.5
8	40.78	40.7
9	49.79	49.7
10	36.88	36.8
11	21.48	21.4
12	25.16	25.1
13	38.17	38.1
14	42.90	42.7

Table 37 (continued)

Position	**MKT6**[a], δ_C (ppm)	**lupenone**[b], δ_C (ppm)
15	27.44	27.4
16	35.53	35.6
17	42.99	42.7
18	48.25	48.2
19	47.96	47.8
20	150.84	150.5
21	29.84	29.8
22	39.98	39.9
23	26.66	26.6
24	21.04	21.0
25	15.97	15.8
26	15.79	15.4
27	14.49	14.4
28	18.02	18.0
29	109.41	109.2
30	19.32	19.2

[a] 75 MHz, in $CDCl_3$; [b] 63 MHz, in $CDCl_3$

2.3.1.3 Compound MKT5: Lup-20(29)-en-3β,28-diol (betulin)



Compound **MKT5** was obtained as a white solid, melting at 248.4-248.8 °C (ref. 236.0-238.0 °C); $[\alpha]_D^{28}$ +22.67°, c = 0.16, pyridine (ref. $[\alpha]_D$ +24.00°, pyridine). The IR spectrum (**Figure 54**) exhibited an absorption band for a hydroxyl group at 3424 cm^{-1}. The ^1H NMR spectrum (**Figure 55**) (**Table 38**) exhibited signals similar to those of **MKT1** (3β-hydroxyl group, isopropenyl group) except for the replacement of Me-28 [δ_H 0.79 (*s*, 3H)] with oxymethylene proton signals [δ_H 3.80 (1H, *dd*, *J* = 10.8, 1.5 Hz) and δ_H 3.33 (1H, *d*, *J* = 10.8 Hz)]. In the HMBC correlation spectrum (**Figure 59**), oxymethylene protons exhibited a correlation peaks with C-16 (δ_C 29.18), C-17 (δ_C 47.79) and C-22 (δ_C 33.97). The ^{13}C NMR spectrum (**Figure 56**) (**Table 38**) confirmed the above conclusion by the absence of one methyl group and the presence of an additional oxymethylene carbon. Furthermore, the NMR data were compared with those of **betulin** (Tinto, *et al*., 1992) and **MKT1**, suggesting that **MKT5** was **betulin**.

Table 38 The NMR spectral data of compounds **MKT5**, **MKT1** and **betulin**

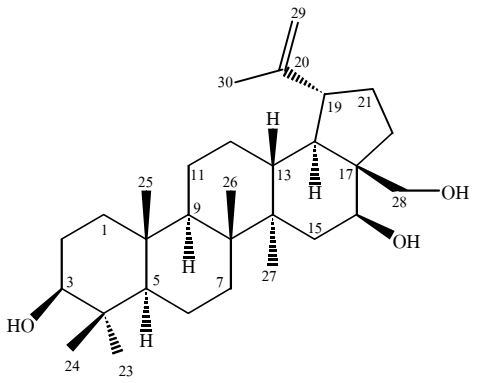
Position	**MKT5**[a]		**MKT1**[a]		**betulin**[b]	
	δ_H, *mult.*, *J* (Hz)	δ_C	δ_H, *mult.*, *J* (Hz)	δ_C	δ_H	δ_C
1	1.62 (*m*, 1H); 0.89 (*m*, 1H)	38.70	0.91 (*m*, 2H)	38.73	1.65; 0.89	38.76
2	1.55 (*m*, 2H)	27.39	1.55 (*m*, 2H)	27.46	1.58	27.19
3	3.19 (*dd*, 10.9, 5.2, 1H)	78.98	3.19 (*dd*, 10.8, 5.4, 1H)	78.99	3.18	78.92
4	-	38.84	-	38.86	-	38.86
5	0.68 (*brd*, 9.3, 1H)	55.29	0.69 (*brd,* 9.0, 1H)	55.32	0.67	55.34
6	1.52 (*m*, 1H); 1.39 (*m*, 1H)	18.30	1.53 (*m*, 1H); 1.40 (*m*, 1H)	18.33	1.52; 1.38	18.33
7	1.38 (*m*, 2H)	34.24	1.40 (*m*, 2H)	34.30	1.39	34.26
8	-	40.92	-	40.84	-	40.94
9	1.24 (*m*, 1H)	50.40	1.27 (*m*, 1H)	50.45	1.27	50.43
10	-	37.16	-	37.18	-	37.17
11	1.43 (*m*, 1H); 1.22 (*m*, 1H)	20.83	1.45 (*m*, 1H); 1.26 (*m*, 1H)	20.94	1.41; 1.19	20.87
12	1.66 (*m*, 2H)	25.22	1.69 (*m*, 2H)	25.16	1.63; 1.03	25.25
13	1.66 (*m*, 1H)	37.31	1.68 (*m*, 1H)	38.07	1.64	37.34
14	-	42.72	-	42.84	-	42.73
15	1.67 (*m*, 1H); 1.07 (*m*, 1H)	27.05	1.67 (*m*, 2H)	27.42	1.70; 1.04	27.04
16	1.92 (*m*, 1H); 1.20 (*m*, 1H)	29.18	1.50 (*m*, 1H); 1.35 (*m*, 1H)	35.60	1.93; 1.20	29.20
17	-	47.79	-	43.01	-	47.76
18	1.58 (*m*, 1H)	48.77	1.37 (*m*, 1H)	48.32	1.57	48.81
19	2.39 (*dt*, 10.5, 5.8, 1H)	47.79	2.38 (*dt*, 11.1, 5.7, 1H)	47.99	2.38	47.83

Table 38 (continued)

Position	**MKT5**[a]		**MKT1**[a]		**betulin**[b]	
	δ_H, *mult.*, *J* (Hz)	δ_C	δ_H, *mult.*, *J* (Hz)	δ_C	δ_H	δ_C
20	-	150.48	-	150.95	-	150.60
21	2.00 (*m*, 2H)	29.76	1.94 (*m*, 1H); 1.27 (*m*, 1H)	29.86	1.95; 1.40	29.78
22	1.90 (*m*, 1H); 1.02 (*m*, 1H)	33.97	1.41 (*m*, 1H); 1.19 (*m*, 1H)	40.01	1.86; 1.02	34.00
23	0.96 (*s*, 3H)	27.98	0.97 (*s*, 3H)	28.00	0.96	27.98
24	0.76 (*s*, 3H)	15.35	0.76 (*s*, 3H)	15.38	0.76	15.40
25	0.82 (*s*, 3H)	16.10	0.83 (*s*, 3H)	16.21	0.82	16.12
26	1.02 (*s*, 3H)	15.98	1.03 (*s*, 3H)	15.99	1.02	15.97
27	0.98 (*s*, 3H)	14.76	0.95 (*s*, 3H)	14.56	0.98	14.77
28	3.80 (*dd*, 10.8, 1.5, 1H) 3.33 (*d*, 10.8, 1H)	60.56	0.79 (*s*, 3H)	18.01	3.77; 3.31	60.21
29	4.68 (*d*, 1.8, 1H); 4.58 (*brs*, 1H)	109.68	4.69 (*d*, 2.1, 1H); 4.57 (*brqd*, 2.1, 1.2, 1H)	109.33	4.68; 4.58	109.64
30	1.68 (*s*, 3H)	19.09	1.68 (*brs*, 3H)	19.32	1.68	19.11

[a] 300 MHz, in $CDCl_3$; [b]400 MHz, in $CDCl_3$+CD_3OD

2.3.1.4 Compound MKT3: Lup-20(29)-en-3β,16β, 28-triol



Compound **MKT3** was obtained as colourless needles, melting at 277.3-279.5 °C, $[\alpha]_D^{27}$ +15.00°, c = 0.12, acetone. It exhibited an IR absorption band at 3449 cm^{-1} (**Figure 60**) for a hydroxyl group. The ^{1}H NMR spectrum (**Figure 61**) (**Table 39**) was similar to that of **MKT5** (an oxymethylene proton, a 3β-hydroxyl group, an isopropenyl group and six tertiary methyl groups) except for the fact that **MKT3** showed an additional oxymethine proton at δ_H 3.84 (*dd*, *J* = 11.2 and 5.0 Hz) which was located at C-16 evidenced by HMBC correlations (**Figure 65**) (**Table 39**). The large coupling constant (*J* = 11.2 Hz) between H-16 and one of H-15 pointed to their axial disposition, indicating that the 16-hydroxyl group was equatorially oriented. The ^{13}C NMR spectrum (**Figure 62**) (**Table 39**) confirmed the above conclusion by the absence of one methylene carbon and the presence of one additional oxymethine carbon. On the basis of the evidence described above and the comparison of its NMR data with **MKT5** (**betulin**) (**Table 40**), the structure of **MKT3** was assigned as **lup-20 (29)-en-3β,16β, 28-triol**.

Table 39 The NMR spectral data of compound **MKT3**

Position	δ_H, *mult*., *J* (Hz)	Type of C	δ_C	HMBC
1	1.64 (*m*, 1H); 0.89 (*m*, 1H)	CH_2	38.70	
2	1.60 (*m*, 2H)	CH_2	26.99	
3	3.20 (*dd*, 10.9, 5.3, 1H)	CH	78.74	C-4, C-23, C-24
4	-	C	38.77	
5	0.68 (*brd*, 10.2, 1H)	CH	55.29	C-4, C-6, C-7, C-10
6	1.55 (*m*, 1H); 1.43 (*m*, 1H)	CH_2	18.21	
7	1.42 (*m*, 2H)	CH_2	34.15	
8	-	C	40.91	
9	1.24 (*m*, 1H)	CH	49.86	C-7, C-11, C-26
10	-	C	37.03	
11	1.45 (*m*, 1H); 1.21 (*m*, 1H)	CH_2	20.68	
12	1.62 (*m*, 2H)	CH_2	24.84	
13	1.54 (*m*, 1H)	CH	36.61	
14	-	C	44.47	
15	1.84 (*m*, 1H); 1.44 (*m*, 1H)	CH_2	37.22	C-8, C-14
16	3.84 (*dd*, 11.2, 5.0, 1H)	CH	78.58	C-22, C-28
17	-	C	51.02	
18	1.53 (*m*, 1H)	CH	47.88	
19	2.43 (*m*, 1H)	CH	47.60	C-13, C-17, C-18, C-20, C-21, C-22, C-22, C-30
20	-	C	149.59	
21	2.05 (*m*, 2H)	CH_2	29.74	
22	2.36 (*m*, 1H); 1.18 (*m*, 1H)	CH_2	32.08	
23	0.99 (*s*, 3H)	CH_3	27.83	C-3, C-4, C-5, C-24
24	0.78 (*s*, 3H)	CH_3	15.27	C-4, C-5, C-23
25	0.85 (*s*, 3H)	CH_3	16.00	C-1, C-5, C-9
26	1.07 (*s*, 3H)	CH_3	15.88	C-7, C-8, C-9, C-14

Table 39 (continued)

Position	δ_H, *mult.*, *J* (Hz)	Type of C	δ_C	HMBC
27	1.01 (*s*, 3H)	CH_3	15.88	C-14, C-15
28	4.19 (*brd*, 11.6, 1H);	CH_2	61.00	C-16, C-17, C-22
	3.41 (*brd*, 11.6, 1H)			
29	4.71 (*brd*, 1.5, 1H);	CH_2	109.91	C-19, C-20, C-21, C-30
	4.62 (*brs*, 1H)			
30	1.70 (*s*, 3H)	CH_3	19.11	C-19, C-20, C-29

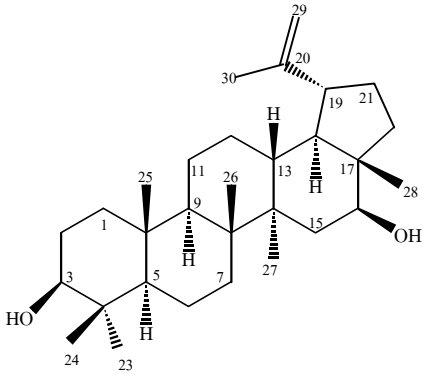
Table 40 The NMR spectral data of compounds **MKT3** and **MKT5**

Position	**MKT3**		**MKT5**	
	δ_H, *mult.*, *J* (Hz)	δ_C	δ_H, *mult.*, *J* (Hz)	δ_C
1	1.64 (*m*, 1H); 0.89 (*m*, 1H)	38.70	1.62 (*m*, 1H); 0.89 (*m*, 1H)	38.70
2	1.60 (*m*, 2H)	26.99	1.55 (*m*, 2H)	27.39
3	3.20 (*dd*, 10.9, 5.3, 1H)	78.74	3.19 (*dd*, 10.9, 5.2, 1H)	78.98
4	-	38.77	-	38.84
5	0.68 (*brd*, 10.2, 1H)	55.29	0.68 (*brd*, 9.3, 1H)	55.29
6	1.55 (*m*, 1H); 1.43 (*m*, 1H)	18.21	1.52 (*m*, 1H); 1.39 (*m*, 1H)	18.30
7	1.42 (*m*, 2H)	34.15	1.38 (*m*, 2H)	34.24
8	-	40.91	-	40.92
9	1.24 (*m*, 1H)	49.86	1.24 (*m*, 1H)	50.40
10	-	37.03	-	37.16
11	1.45 (*m*, 1H); 1.21 (*m*, 1H)	20.68	1.43 (*m*, 1H); 1.22 (*m*, 1H)	20.83
12	1.62 (*m*, 2H)	24.84	1.66 (*m*, 2H)	25.22
13	1.54 (*m*, 1H)	36.61	1.66 (*m*, 1H)	37.31
14	-	44.47	-	42.72
15	1.84 (*m*, 1H); 1.44 (*m*, 1H)	37.22	1.67 (*m*, 1H); 1.07 (*m*, 1H)	27.05

Table 40 (continued)

Position	**MKT3**		**MKT5**	
	δ_H, *mult.*, *J* (Hz)	δ_C	δ_H, *mult.*, *J* (Hz)	δ_C
16	3.84 (*dd*, 11.2, 5.0, 1H)	78.58	1.92 (*m*, 1H); 1.20 (*m*, 1H)	29.18
17	-	51.02	-	47.79
18	1.53 (*m*, 1H)	47.88	1.58 (*m*, 1H)	48.77
19	2.43 (*m*, 1H)	47.60	2.39 (*dt*, 10.5, 5.8, 1H)	47.79
20	-	149.59	-	150.48
21	2.05 (*m*, 2H)	29.74	2.00 (*m*, 2H)	29.76
22	2.36 (*m*, 1H), 1.18 (*m*, 1H)	32.08	1.90 (*m*, 1H); 1.02 (*m*, 1H)	33.97
23	0.99 (*s*, 3H)	27.83	0.96 (*s*, 3H)	27.98
24	0.78 (*s*, 3H)	15.27	0.76 (*s*, 3H)	15.35
25	0.85 (*s*, 3H)	16.00	0.82 (*s*, 3H)	16.10
26	1.07 (*s*, 3H)	15.88	1.02 (*s*, 3H)	15.98
27	1.01 (*s*, 3H)	15.88	0.98 (*s*, 3H)	14.76
28	4.19 (*brd*, 11.6, 1H);	61.00	3.80 (*dd*, 10.8, 1.5, 1H)	60.56
	3.41 (*brd*, 11.6, 1H)		3.33 (*d*, 10.8, 1H)	
29	4.71 (*brd*, 1.5, 1H);	109.91	4.68 (*d*, 1.8, 1H);	109.68
	4.62 (*brs*, 1H)		4.58 (*brs*, 1H)	
30	1.70 (*s*, 3H)	19.11	1.68 (*s*, 3H)	19.09

2.3.1.5 Compound MKT7: Lup-20(29)-en-3β,16β-diol



Compound **MKT7** was obtained as a white solid, melting at 195.5-197.5 °C; $[\alpha]_D^{28}$ +15.61°, c = 0.21, $CHCl_3$ (ref. $[\alpha]_D$ +14.00°, $CHCl_3$). Its IR spectrum (**Figure 66**) exhibited absorption band at 3386 cm^{-1} for a hydroxyl group. Its ^1H NMR spectrum (**Figure 67**) (**Table 41**) was similar to that of **MKT1** (**lupeol**) except for the replacement of signal of the methylene proton with a signal of an oxymethine proton [δ_H 3.61 (1H, *dd, J* = 11.1 and 4.8 Hz)] and which correlated to C-22 (δ_C 37.72) and C-28 (δ_C 11.68). Moreover, methyl protons at δ_H 0.80 (Me-28) showed a correlation with an oxymethine carbon at C-16 (δ_C 77.09) in the HMBC experiment (**Figure 71**) (**Table 41**). These results indicated that the hydroxyl group was located at C-16. Thus, the structure of **MKT7** could be elucidated as **lup-20(29)-en-3β,16β-diol**.

Table 41 The NMR spectral data of compound **MKT7**

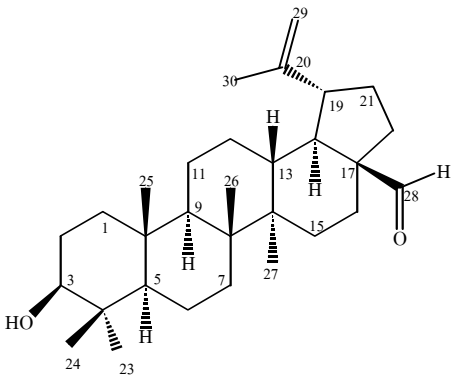
Position	δ_H, *mult.*, *J* (Hz)	Type of C	δ_C	HMBC
1	1.64 (*m*, 1H); 0.88 (*m*, 1H)	CH_2	38.75	
2	1.60 (*m*, 2H)	CH_2	27.38	
3	3.19 (*dd*, 10.8, 5.1, 1H)	CH	78.95	C-23, C-24
4	-	C	38.87	
5	0.68 (*brd*, 9.0, 1H)	CH	55.33	C-23

Table 41 (continued)

Position	δ_H, *mult.*, *J* (Hz)	Type of C	δ_C	HMBC
6	1.53 (*m*, 1H); 1.39 (*m*, 1H)	CH_2	18.30	
7	1.39 (*m*, 2H)	CH_2	34.25	
8	-	C	40.94	
9	1.23 (*m*, 1H)	CH	50.02	C-8, C-11
10	-	C	37.13	
11	1.45 (*m*, 1H); 1.23 (*m*, 1H)	CH_2	20.88	
12	1.78 (*m*, 1H); 1.01 (*m*, 1H)	CH_2	24.79	
13	1.51 (*m*, 1H)	CH	37.26	
14	-	C	44.08	
15	1.53 (*m*, 2H)	CH_2	36.90	
16	3.61 (*dd*, 11.1, 4.8, 1H)	CH	77.09	C-22, C-28
17	-	C	48.60	
18	1.37 (*m*, 1H)	CH	47.60	
19	2.50 (*dt*, 10.8, 5.7, 1H)	CH	47.72	C-21, C-29, C-30, C-22, C-30
20	-	C	149.98	
21	1.97 (*m*, 2H)	CH_2	29.92	C-17, C-19
22	1.57 (*m*, 1H); 1.26 (*m*, 1H)	CH_2	37.72	C-28
23	0.97 (*s*, 3H)	CH_3	27.99	C-3, C-4, C-5, C-24
24	0.76 (*s*, 3H)	CH_3	15.36	C-3, C-4, C-5, C-23
25	0.83 (*s*, 3H)	CH_3	16.18[a]	C-1, C-5
26	1.04 (*s*, 3H)	CH_3	15.99	C-7, C-8, C-9, C-14, C-27
27	0.99 (*s*, 3H)	CH_3	16.12[a]	C-10
28	0.80 (*s*, 3H)	CH_3	11.68	C-16, C-17
29	4.71 (*brd*, 2.1, 1H); 4.60 (*brqd*, 2.1, 1.2, 1H)	CH_2	109.80	C-19, C-20, C-30
30	1.68 (*s*, 3H)	CH_3	19.34	C-20, C-29

[a] maybe interchanged.

2.3.1.6 Compound MKT8: 3β-Hydroxylup-20(29)-en-28-al (betulinaldehyde)



Compound **MKT8** was isolated as white needles, melting at 141.9-143.7 °C (ref. 192.0-193.0 °C); $[\alpha]_D^{30}$ +21.17°, c = 0.34, $CHCl_3$ (ref. $[\alpha]_D$ +19.00°). The IR spectrum (**Figure 72**) exhibited absorption bands at 3417 (a hydroxyl group) and 1701 cm^{-1} (a carbonyl group). The ^1H NMR spectrum (**Figure 73**) (**Table 42**) showed five methyl groups, appearing as *singlets*, bonded to quaternary carbons. In addition, the presence of an isopropenyl group was evident by a lowfield methyl signal at δ_H 1.70 (*s*, 3H), and two vinylic protons at δ_H 4.76 (*brd, J* = 2.1 Hz, 1H) and δ_H 4.62 (*brdd*, 2.1, 1.5 Hz, 1H). These data supported that **MKT8** belonged to the lupane family. An oxymethine proton signal at δ_H 3.18 (*dd, J* = 11.0 and 5.3 Hz, 1H), was attributed to H-3 due to HMBC data (**Figure 77**) (**Table 42**). The large coupling constant (*J* = 11.0 Hz) between H-3 and H-2 indicating that the 3-hydroxyl group was at β-face. These ^1H NMR data were similar to **MKT1** except for the presence of aldehydic proton signal at δ_H 9.68 (*d, J* = 1.5 Hz, 1H) instead of Me-28. The presence of a signal at δ_C 206.73 for a carbonyl group in the ^{13}C NMR spectrum of **MKT8** confirmed an aldehyde functionality. Its location was confirmed by the correlations of H-16 (δ_H 2.12), H-18 (δ_H 1.73) and H-22 (δ_H 1.78) to the carbonyl carbon in the HMBC experiment. The

structure of **MKT8** was assigned as **betulinaldehyde** by comparison of its spectral dada with those of **betulinaldehyde** (Monaco, *et al*., 1984) (**Table 43**).

Table 42 The NMR spectral data of compound **MKT8**

Position	δ_H, *mult*., *J* (Hz)	Type of C	δ_C	HMBC
1	1.68 (*m*, 1H); 0.91 (*m*, 1H)	CH_2	38.71	
2	1.63 (*m*, 2H)	CH_2	27.39	
3	3.18 (*dd*, 11.0, 5.3, 1H)	CH	78.99	C-23, C-24
4	-	C	38.71	
5	0.67 (*m*, 1H)	CH	55.32	C-7, C-10
6	1.51 (*m*, 1H); 1.38 (*m*, 1H)	CH_2	18.27	
7	1.38 (*m*, 2H)	CH_2	34.33	
8	-	C	40.83	
9	1.26 (*m*, 1H)	CH	50.47	C-8, C-10, C-25
10	-	C	37.16	
11	1.45 (*m*, 1H); 1.38 (*m*, 1H)	CH_2	20.75	
12	1.80 (*m*, 2H)	CH_2	25.54	
13	2.04 (*m*, 1H)	CH	38.71	
14	-	C	42.56	
15	2.02 (*m*, 1H); 1.20 (*m*, 1H)	CH_2	29.26	C-13, C-14, C-17, C-27
16	2.12 (*m*, 2H)	CH_2	28.81	C-14, C-17, C-18, C-28
17	-	C	59.33	
18	1.73 (*m*, 1H)	CH	48.07	C-17, C-28
19	2.86 (*dt,* 11.1, 7.5, 1H)	CH	47.54	C-18, C-20, C-21, C-29, C-30
20	-	C	149.73	
21	1.49 (*m*, 2H)	CH_2	29.87	
22	1.78 (*m*, 1H); 1.36 (*m*, 1H)	CH_2	33.23	C-28

Table 42 (continued)

Position	δ_H, *mult.*, *J* (Hz)	Type of C	δ_C	HMBC
23	0.96 (*s*, 3H)	CH_3	27.98	C-3, C-5, C-24
24	0.75 (*s*, 3H)	CH_3	15.35	C-3, C-5, C-23
25	0.82 (*s*, 3H)	CH_3	15.90	C-5, C-9, C-10
26	0.91 (*s*, 3H)	CH_3	16.13	C-7, C-9
27	0.97 (*s*, 3H)	CH_3	14.27	C-8, C-13, C-14, C-16
28	9.68 (*d*, 1.5, 1H)	CH	206.73	C-17, C-18
29	4.76 (*brd*, 2.1, 1H);	CH_2	110.16	C-19, C-30
	4.62 (*brqd*, 2.1, 1.5, 1H)			
30	1.70 (*s*, 3H)	CH_3	19.00	C-19, C-20, C-29

Table 43 Comparison of ^{13}C NMR spectral data of compounds **MKT8** and **betulinaldehyde**

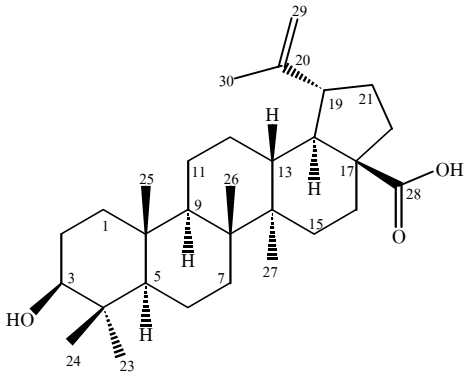
Position	**MKT8**[a], δ_C (ppm)	**betulinaldehyde**[b], δ_C (ppm)
1	38.71	38.7
2	27.39	27.3
3	78.99	78.9
4	38.71	38.8
5	55.32	55.5
6	18.27	18.2
7	34.33	34.3
8	40.83	40.8
9	50.47	50.4
10	37.16	37.0
11	20.75	20.7

Table 43 (continued)

Position	**MKT8** [a], δ_C (ppm)	**betulinaldehyde** [b], δ_C (ppm)
12	25.54	25.5
13	38.71	38.7
14	42.56	42.5
15	29.26	29.2
16	28.81	28.8
17	59.33	59.3
18	48.07	48.0
19	47.54	47.5
20	149.73	149.7
21	29.87	29.8
22	33.23	33.2
23	27.98	27.9
24	15.35	15.4
25	15.90	15.9
26	16.13	16.1
27	14.27	14.2
28	206.73	205.6
29	110.16	110.1
30	19.00	19.0

[a]75 MHz, in $CDCl_3$; [b]68 MHz, in $CDCl_3$

2.3.1.7 Compound MKT10: 3β-Hydroxylup-20(29)-en-oic acid (betulinic acid)



Compound **MKT10** was obtained as a white solid, melting at 284.0-286.0 °C; $[\alpha]_D^{27}$ +9.23°, c = 0.07, acetone. Its IR spectrum (**Figure 78**) exhibited characteristic absorption bands of carboxylic acid at 3417 (a hydroxyl group) and 1687 cm^{-1} (a carbonyl group). The ^{1}H NMR spectrum (**Figure 79**) (**Table 44**) showed the presence of five methyl singlets (δ_H 0.77, 0.83, 0.94, 0.97 and 0.99), and isopropenyl chain [δ_H 4.59 (1H, *brqd,* 2.1, 1.2 Hz), δ_H 4.73 (1H, *brd, J* = 2.1 Hz) and δ_H 1.69 (3H, *s*)] and an oxymethine proton at δ_H 3.18 (1H, *dd*, *J* = 10.2 and 6.0 Hz). These signals were similar to those of **MKT8** (**betulinaldehyde**), but aldehydic proton signal at δ_H 9.68 (1H, *d, J* = 2.0 Hz) disappeared in **MKT10**. The absence of aldehyde signal in the ^{1}H NMR spectrum and the presence of a signal at δ_C 177.67 for a carbonyl group in the ^{13}C NMR spectrum (**Figure 80**) (**Table 44**) suggested that the aldehyde group in **MKT8** was replaced by a carboxylic group in **MKT10**. Base on this fact and the HMBC correlations (**Figure 83**) (**Table 44**) from H-18 (δ_H 1.60) and H-22 (δ_H 1.99) to C-28 (δ_C 177.67), the carbonyl group was assigned at C-28. Comparison of its NMR data with those of **MKT8** and **betulinic acid** (Francisco, *et. Al*., 1994) (**Table 45**) indicated that **MKT10** had the same structure as **betulinic acid**.

Table 44 The NMR spectral data of compound **MKT10**

Position	δ_H , *mult*., *J* (Hz)	Type of C	δ_C	HMBC
1	1.68 (*m*, 1H); 0.95 (*m*, 1H)	CH_2	38.71	
2	1.60 (*m*, 2H)	CH_2	27.23	
3	3.18 (*dd*, 10.2, 6.0, 1H)	CH	78.41	C-4, C-23, C-24
4	-	C	38.76	
5	0.70 (*brd*, 10.5, 1H)	CH	55.34	C-4, C-6, C-7, C-10, C-23
6	1.55 (*m*, 1H); 1.40 (*m*, 1H)	CH_2	18.23	
7	1.40 (*m*, 2H)	CH_2	34.30	
8	-	C	40.65	
9	1.28 (*m*, 1H)	CH	50.50	C-8, C-10, C-25
10	-	C	37.11	
11	1.47 (*m*, 1H); 1.24 (*m*, 1H)	CH_2	20.81	
12	1.72 (*m*, 2H)	CH_2	25.48	
13	2.28 (*m*, 1H)	CH	38.18	C-11, C-27
14	-	C	42.36	
15	1.54 (*m*, 1H); 1.19 (*m*, 1H)	CH_2	29.63	
16	2.21 (*m*, 2H)	CH_2	32.12	C-14, C-17, C-18
17	-	C	55.98	
18	1.60 (*m*, 1H)	CH	49.17	C-13, C-14, C-16, C-17, C-19, C-20, C-28
19	3.04 (*dt,* 11.1, 4.5, 1H)	CH	46.83	C-18, C-20, C-29, C-30
20	-	C	150.58	
21	1.98 (*m*, 2H)	CH_2	30.51	C-30
22	1.99 (*m*, 1H); 1.43 (*m*, 1H)	CH_2	36.94	C-28
23	0.97 (*s*, 3H)	CH_3	27.89	C-3, C-4, C-5, C-24
24	0.77 (*s*, 3H)	CH_3	15.33	C-3, C-4, C-5, C-23
25	0.83 (*s*, 3H)	CH_3	16.01[a]	C-1, C-5, C-9
26	0.94 (*s*, 3H)	CH_3	15.90[a]	C-7, C-8, C-9, C-14

Table44 (continued)

Position	δ_H, *mult.*, *J* (Hz)	Type of C	δ_C	HMBC
27	0.99 (*s*, 3H)	CH_3	14.56	C-8, C-14
28	-	C	177.67	C-19, C-30
29	4.73 (*brd*, 2.1, 1H);	CH_2	109.36	C-19, C-20, C-30
	4.59 (*brqd*, 2.1, 1.2, 1H)			
30	1.69 (*s*, 3H)	CH_3	19.20	C-19, C-20, C-29

[a]maybe interchanged.

Table 45 Comparison of ^{13}C NMR spectral data of compounds **MKT10**, **MKT8** and **betulinic acid**

Position	**compound MKT10**[a], δ_C (ppm)	**compound MKT8**[b], δ_C (ppm)	**betulinic acid**[c], δ_C (ppm)
1	38.71	38.71	38.5
2	27.23	27.39	28.2
3	78.99	78.99	78.1
4	38.76	38.71	39.4
5	55.34	55.32	55.9
6	18.23	18.27	18.7
7	34.30	34.33	34.7
8	40.65	40.83	41.0
9	50.50	50.47	50.9
10	37.11	37.16	37.5
11	20.81	20.75	21.1
12	25.48	25.54	26.0
13	38.18	38.71	39.2
14	42.36	42.56	42.8

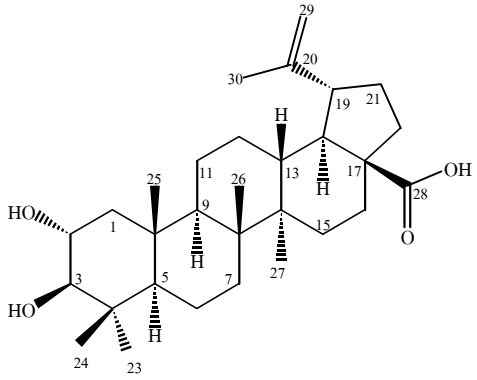
Table 45 (continued)

Position	**compound MKT10,** δ_C (ppm)	**compound MKT8,** δ_C (ppm)	**betulinic acid,** δ_C (ppm)
15	29.63	29.26	30.2
16	32.12	28.81	32.8
17	55.98	59.33	56.6
18	49.17	47.07	49.7
19	46.83	47.54	47.7
20	150.58	149.73	151.4
21	30.51	29.87	31.1
22	36.94	33.23	37.4
23	27.89	27.98	28.5
24	15.33	15.35	16.2
25	16.01[d]	15.90	16.3
26	15.90[d]	16.13	16.2
27	14.56	14.27	14.8
28	177.67	206.73	179.0
29	109.36	110.16	110.0
30	19.20	19.00	19.4

[a] 75 MHz, in Acetone-d_6+$CDCl_3$; [b] 75 MHz, in $CDCl_3$; [c] 100 MHz, in $CDCl_3$;

[d] maybe interchanged.

2.3.1.8 Compound MKT12: 2□, 3□-Dihydroxylupe-20(29)-en-28-oic acid



Compound **MKT12** was obtained as a white solid, melting at 278.1-279.0 °C; $[\alpha]_D^{27}$ –32.00°, c = 0.03, $CHCl_3$. The IR spectrum (**Figure 84**) exhibited similar absorption bands to **MKT10**, indicating the presence of a carbonyl group. The ^{1}H NMR spectrum (**Figure 85**) (**Table 46**) was similar to that of **MKT10** except for the fact that the oxymethine proton H-3 [δ_H 2.98 (1H, *d*, *J* = 9.6 Hz)] was shifted to upper field along with an additional oxymethine proton at δ_H 3.68 (1H, *ddd*, *J* = 11.1, 9.6 and 4.5 Hz). From the multiplicity and the coupling constant of this proton, the second oxymethine proton was located at C-2 position. The coupling constant (*J* = 9.6 Hz) between H-3 and H-2 pointed to their axial disposition, indicating that the hydroxyl groups at the C-2 and C-3 were equatorial oriented. The ^{13}C NMR spectrum (**Figure 86**) (**Table 46**) confirmed the above conclusion by the absence of one methylene carbon and the presence of one additional oxymethine carbon. These were supported by HMBC correlations (**Figure 89) (Table 46**). One of methylene proton, H-1 (δ_H 2.05) and oxymethine proton, H-3 (δ_H 2.98) showed correlations to C-2 (δ_C 69.25) which confirmed the location of the second hydroxyl group. Therefore, the structure of **MKT12** was assigned as **2□,3□-dihydroxylupe-20(29)-en-28-oic acid**.

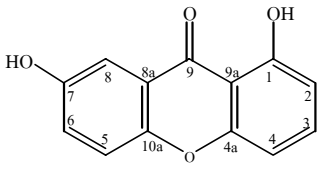
Table 46 The NMR spectral data of compound **MKT12**

Position	δ_H, *mult.*, *J* (Hz)	Type of C	δ_C	HMBC
1	2.05 (*m*, 1H); 0.85 (*m*, 1H)	CH_2	46.73	C-2, C-3, C-4
2	3.68 (*ddd*, 11.1, 9.6, 4.5, 1H)	CH	69.25	
3	2.98 (*d*, 9.6, 1H)	CH	83.91	C-2, C-10, C-23, C-24
4	-	C	38.58	
5	0.83 (*m*, 1H)	CH	55.45	
6	1.55 (*m*, 1H); 1.42 (*m*, 1H)	CH_2	18.27	
7	1.40 (*m*, 2H)	CH_2	34.22	
8	-	C	40.77	
9	1.36 (*m*, 1H)	CH	50.47	
10	-	C	39.18	
11	1.35 (*m*, 2H)	CH_2	20.97	
12	1.72 (*m*, 2H)	CH_2	25.37	
13	2.21 (*m*, 1H)	CH	38.31	
14	-	C	42.50	
15	1.55 (*m*, 1H); 1.19 (*m*, 1H)	CH_2	29.64	
16	2.30 (*m*, 1H); 1.43 (*m*, 1H)	CH_2	32.13	
17	-	C	56.28	
18	1.60 (*t*, 10.4, 1H)	CH	49.24	C-13, C-16, C-17, C-19, C-20, C-28
19	3.01 (*m*, 1H)	CH	46.88	
20	-	C	150.25	
21	2.00 (*m*, 2H)	CH_2	30.54	
22	1.97 (*m*, 1H); 1.44 (*m*, 1H)	CH_2	37.00	
23	1.01 (*s*, 3H)	CH_3	28.46	C-3, C-5, C-10, C-24
24	0.80 (*s*, 3H)	CH_3	16.50	C-3, C-5, C-10, C-23
25	0.90 (*s*, 3H)	CH_3	17.37	C-1, C-4, C-5, C-9
26	0.93 (*s*, 3H)	CH_3	16.06	C-7, C-8, C-9, C-14

Table 46 (continued)

Position	δ_H, *mult.*, *J* (Hz)	Type of C	δ_C	HMBC
27	0.98 (*s*, 3H)	CH_3	14.66	C-8, C-13, C-14, C-15
28	-	C=O	180.22	
29	4.74 (*brd*, 2.1, 1H);	CH_2	109.79	C-19, C-30
	4.61 (*brs*, 1H)			
30	1.69 (*s*, 3H)	CH_3	19.37	C-19, C-20, C-29

2.3.1.9 Compound MKT9: 1,7-Dihydroxyxanthone (euxanthone)



Compound **MKT9** was obtained as yellow needles, melting at 239.8-240.8 °C (ref. 226-229 °C). Its UV spectrum (**Figure 90**) showed the occurrence of a xanthone nucleus (λ_{max} 235, 259, 287 and 389 nm). In the IR spectrum (**Figure 91**), absorption characteristic of xanthone were observed at 1639 cm^{-1} (conjugated carbonyl) as well as at 1606 and 1580 cm^{-1} (aromatic moieties) and 3306 cm^{-1} (hydroxyl group). The ^1H NMR spectrum (**Figure 92**) (**Table 47**) exhibited a resonance of a chelated hydroxyl group (1-OH) at δ_H 12.71 and a *singlet* signal of a hydroxyl at δ_H 9.01, which was assigned to 7-OH. The ABM system of aromatic protons: δ_H 6.74 (*dd*, *J* = 8.4 and 0.9 Hz, 1H), δ_H 6.95 (*dd*, *J* = 8.4 and 0.9 Hz, 1H) and δ_H 7.64 (*t*, *J* = 8.4 Hz, 1H), were assigned as H-2, H-4 and H-3 of the xanthone skeleton, respectively. The signals at δ_H 7.46 (*dd*, *J* = 9.0 and 0.4 Hz, 1H), δ_H 7.39 (*dd*, *J* = 9.0 and 3.0 Hz, 1H) and δ_H 7.61 (*dd*, *J* = 3.0 and 0.4 Hz, 1H) were assigned as H-5, H-6 and H-8, respectively.

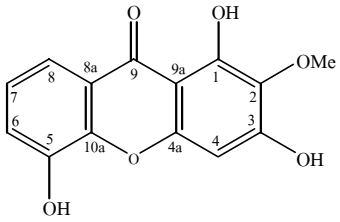
Therefore, the structure of **MKT9** was assigned as **1,7-dihydroxyxanthone**. To confirm the conclusion, the ^{1}H NMR data of **MKT9** was compared with the previously reported data of **1,7-dihydroxyxanthone** (**euxanthone**) (Fujita, *et al*., 1992).

Table 47 The NMR spectral data of compounds **MKT9** and **euxanthone**

Position	**MKT9**[a]			HMBC correlation	**euxanthone**[b]
	δ_H, *mult*., *J* (Hz)	Type of C	δ_C		δ_H, *mult*., *J* (Hz)
1	-	C	161.86		
2	6.74 (*dd*, 8.4, 0.9, 1H)	CH	109.61	C-1	6.77 (*dd*, 8.4, 0.5, 1H)
3	7.64 (*t*, 8.4, 1H)	CH	136.61	C-1, C-4a	7.59 (*t*, 8.8, 1H)
4	6.95 (*dd*, 8.4, 0.9, 1H)	CH	106.81	C-2, C-4a, C-9	6.95 (*dd*, 8.4, 0.5, 1H)
4a	-	C	156.40		
5	7.46 (*dd*, 9.0, 0.4, 1H)	CH	119.09	C-7, C-8a, C-9	7.41 (*d*, 9.3, 1H)
6	7.39 (*dd*, 9.0, 3.0, 1H)	CH	125.17	C-8, C-10a	7.33 (*dd*, 9.3, 2.9, 1H)
7	-	C	153.98		
8	7.61 (*dd*, 3.0, 0.4, 1H)	CH	108.50	C-9, C-10a	7.63 (*d*, 2.9, 1H)
8a	-	C	120.97		
9	-	C	182.06		
9a	-	C	108.50		
10a	-	C	150.13		
1-OH	12.71 (*s*, 1H)			C-1, C-2, C-3	12.62 (*s*, 1H)
7-OH	9.01 (*s*, 1H)				

[a] 300 MHz, in Acetone-d_6+$CDCl_3$; [b] 200 MHz, in $CDCl_3$

2.3.1.10 Compound MKT13: 1,3,5-Trihydroxy-2-methoxyxanthone



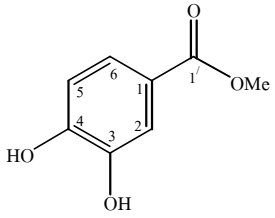
Compound **MKT13** was isolated as a yellow solid, melting at 175.6-177.0 °C. The UV spectrum (**Figure 97**) showed maximum absorption bands at 220, 244, 312, and 358 nm. The IR spectrum (**Figure 98**) showed the absorption band of O-H stretching at 3469 cm^{-1} and C=O stretching at 1653 cm^{-1}. The ^{1}H NMR spectrum (**Figure 99**) (**Table 48**) exhibited a sharp *singlet* signal of chelated hydroxy proton (C-1-OH) at δ_H13.13 and a *broad singlet* signal of two phenolic hydroxyl groups at δ_H 9.35 (2H). A *singlet* signal of aromatic proton, δ_H 6.54, was observed and was assigned for a signal of H-4 according to the correlations to C-2, C-3, C-4a, C-9a and C-9 on the HMBC experiment (**Figure 103**) (**Table 48**). The ABM pattern in aromatic region, δ_H 7.67 (*dd*, *J* = 7.8 and 1.8 Hz, 1H), δ_H 7.35 (*dd*, *J* = 7.8 and 1.8 Hz, 1H) and δ_H 7.28 (*t*, *J* = 7.8 Hz, 1H) were present in the spectrum and were proposed for the characteristic signals of H-8, H-6 and H-7, respectively. The most deshielded aromatic proton signal was assigned for H-8 according to an anisotropic effect of the carbonyl group. The assignment of three aromatic protons H-6, H-7 and H-8 were supported by ^{3}J coupling of H-6 to C-8 and C-10a; H-7 to C-5 and C-8a and H-8 to C-6, C-9 and C-10a on the HMBC experiment. The remaining methoxyl group appeared at δ_H 3.89 as a *singlet* resonance and it was located at C-2, which was supported by the correlation to C-2 in HMBC experiment. Therefore, the structure of **MKT13** was assigned as **1,3,5-trihydroxy-2-methoxyxanthone**. The spectral data were in agreement with the previously reported data of **1,3,5-trihydroxy-2-methoxyxanthone** (Pinto, *et al*., 1994).

Table 48 The NMR spectral data of compounds **MKT13** and **1,3,5-trihydroxy-2-methoxyxanthone**

Position	**MKT13**[a]				**1,3,5-trihydroxy-2-methoxyxanthone**[b]	
	δ_H, *mult*., *J* (Hz)	Type of C	δ_C	HMBC	δ_H, *mult*., *J* (Hz)	δ_C
1	-	C	155.34		-	153.9
2	-	C	131.57		-	130.7
3	-	C	153.85		-	159.3
4	6.54 (*s*, 1H)	CH	94.78	C-2, C-3, C-4a, C-9, C-9a	6.50 (*s*, 1H)	94.1
4a	-	C	159.29		-	152.3
5	-	C	146.94		-	146.0
6	7.35 (*dd*, 7.8, 1.8, 1H)	CH	121.31	C-8, C-10a	7.28 (*dd*, 7.9, 2.2, 1H)	120.3
7	7.28 (*t*, 7.8, 1H)	CH	124.81	C-5, C-8a	7.21 (*dd*, 7.9, 7.4, 1H)	123.8
8	7.67 (*dd*, 7.8, 1.8, 1H)	CH	116.14	C-6, C-9, C-10a	7.60 (*dd*, 7.4, 2.2, 1H)	114.3
8a	-	C	121.75		-	120.4
9	-	C	182.14		-	180.4
9a	-	C	103.96		-	144.8
10a	-	C	146.11		-	102.2
1-OH	13.13 (*s*, 1H)	-	-	C-1, C-2, C-9a	12.91 (*s*, 1H)	
3,5-OH	9.35 (*brs*, 2H)					
OMe	3.89 (*s*, 3H)	CH_3	60.76	C-2	3.75 (*s*, 3H)	59.8

[a] 300 MHz, in Acetone-d_6; [b] 200 MHz, in DMSO-d_6

2.3.1.11 Compound MKT11: Methyl-3,4-dihydroxybenzoate

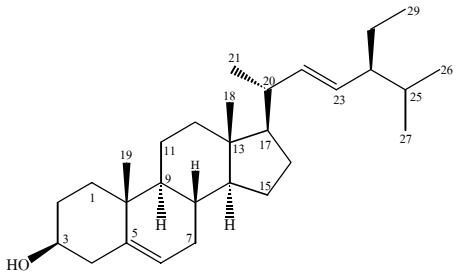


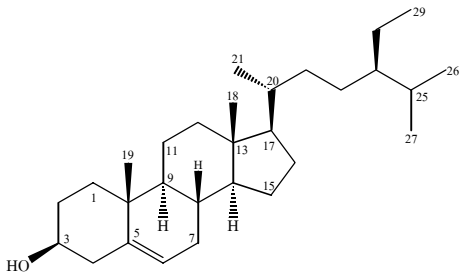
Compound **MKT11** was isolated as yellow needles, melting at 115.5-127.6°C. The UV spectrum (**Figure 104**) showed the maximum absorption bands at 219, 261 and 295 nm. The IR spectrum (**Figure 105**) showed the absorption bands of O-H stretching at 3454 and 3255 cm^{-1} and C=O stretching at 1690 cm^{-1}. The ^{1}H NMR spectrum (**Figure 106**) (**Table 49**) showed the *singlet* signal of methoxy protons at δ_H 3.81. In addition, the correlation of the methoxy proton to the carbonyl carbon on the HMBC experiment (**Figure 110**) (**Table 49**). This results was confirmed the presence of ester group. The aromatic proton signals at δ_H 7.49 (*d*, 1.8 Hz, 1H) and δ_H 7.44 (*dd*, 8.1 and 1.8 Hz, 1H) which were assigned to the proton at C-2 and C-6, respectively, according to the correlation to the carbonyl carbon on the HMBC experiment (**Figure 110**) (**Table 49**). The remaining signal at δ_H 6.90 (*d*, 8.1 Hz, 1H) was ascribable to the proton at position 5, which was supported by the correlation to C-1 (δ_C 121.91), C-3 (δ_C 144.28) and C-4 (δ_C 149.95). On the basis of the evidence described above, the structure of **MKT11** was assigned as **methyl-3,4-dihydroxybenzoate**.

Table 49 The NMR spectral data of compound **MKT11**

Position	**MKT11**			
	δ_H, *mult.*, *J* (Hz)	Type of C	δ_C	HMBC
1	-	C	121.91	
2	7.49 (*d*, 1.8, 1H)	CH	116.26	C-3, C-4, C-6, C-1′
3	-	C	144.79	
4	-	C	149.95	
5	6.90 (*d*, 8.1, 1H)	CH	114.86	C-1, C-3, C-4
6	7.44 (*dd*, 8.1, 1.8, 1H)	CH	122.36	C-2, C-4, C-1′
1′		C=O	166.21	
OMe	3.81 (*s*, 3H)	CH_3	50.96	C-1′

2.3.1.12 Compound MKT2

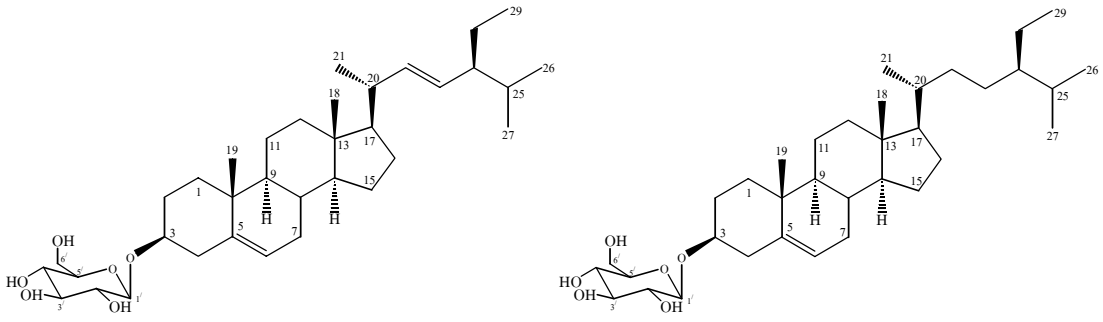




Compound **MKT2** was obtained as a white solid, melting at 133.0-135.5 °C; $[\alpha]_D^{28}$ –28.75°, c = 0.16, $CHCl_3$. The IR spectrum (**Figure 111**) exhibited the absorption band at 3439 cm^{-1} for a hydroxyl group. The ^1H NMR spectrum (**Figure 112**) showed a low-intensity characteristic signals of olefinic proton of sitosterol [δ_H 5.08 (*dd*, *J* = 15.0 and 8.4 Hz); δ_H 4.94 (*dd*, *J* = 15.0 and 8.4 Hz)]. Additionally, the presence of an olefinic proton [δ_H 5.28 (*d*, *J* = 5.1 Hz) indicating that it contained one

trans-disubstituted double bond and one trisubstituted double bond. From the ratio of relative integral of their olefinic proton (δ_H 5.08 (*dd*, *J* = 15.0 and 8.4 Hz); 4.94 (*dd*, *J* = 15.0 and 8.4 Hz; δ_H 5.28 (*d*, *J* = 5.1 Hz), 1:1:2, suggested that **MKT2** was a mixture of **β-sitosterol** and **β-stigmasterol** in a ratio of 1:1.

2.3.1.13 Compound MKT4



Compound **MKT4** was isolated as a white solid, melting at 277.0-280.0 °C; $[\alpha]_D^{29}$ –51.43°, c = 0.10, pyridine. The IR spectrum (**Figure 113**) showed the presence of a hydroxyl group at 3410 cm^{-1}. It had the similar spectral data as **MKT2** except for the additional signals at δ_H 5.33-3.87 of a sugar moiety. These data were identical to those of the mixture of **β-stigmasterol** and **β-sitosterol** glucoside (Hiranrat, 2001).