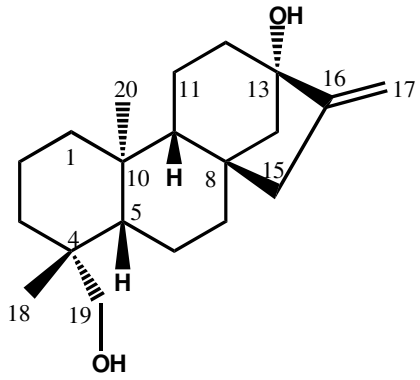
CHAPTER 3
RESULTS AND DISCUSSION

3.1 Structure elucidation of compounds from the roots of *B. cylindrica*

The air-dried and pulverized roots (4.0 kg) were exhaustively extracted with methylene chloride and acetone successively. The crude methylene chloride extract was subjected to quick column chromatography and/or crystallization to give ten diterpenoids as one new pimarane: **TK10** and one known: **TK8;** seven kauranes: **TK1**-**TK7** and a beyerane: **TK9**; seven known lupane triterpenoids: **TK11**-**TK17**; three known steroids: **TK18**-**TK20** and a ferulic acid ester: **TK21**.

Their structures were elucidated mainly by 1D and 2D NMR spectroscopic data: ^{1}H, ^{13}C NMR, DEPT 135°, DEPT 90°, HMQC, HMBC and ^{1}H-^{1}H COSY. Mass spectra were determined for a new compound **TK10** and a known **TK21**. The physical data of the known compounds were also compared with the reported values. In addition X-ray crystallographic structures were reported for compounds **TK1** and **TK5**.

3.1.1 Compound TK1



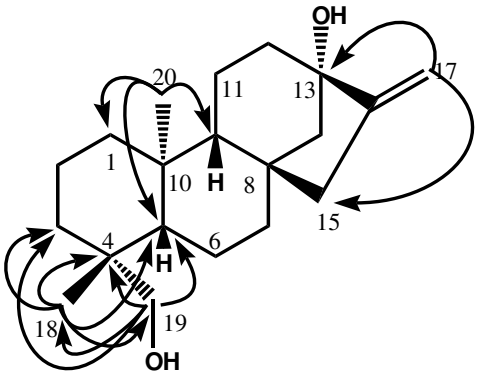
Compound **TK1** was obtained as a white amorphous solid, mp. 255-257 °C, $[\alpha]^{27}_{D}$: -22.7° (*c* = 0.30, $CHCl_3$). It exhibited hydroxyl (3292 cm^{-1}) and double bond (1620 cm^{-1}) absorptions in the IR spectrum (**Figure 4**). X-ray crystallographic analysis of **TK1** was carried out and gave ORTEP drawing as shown in **Figure 2** (Salae *et al*., 2007). The ^{13}C NMR spectrum and a DEPT experiment indicated that **TK1** has a total of 20 carbons, which is consistent with a diterpene skeleton.

The ^{1}H NMR spectral data of **TK1** (**Table 2, Figure 5**) revealed the presence of an exocyclic methylene protons (δ 4.74 *brs* and 4.91 *brt, J* = 2.2 Hz) and oxy-methylene protons (δ 3.35 *d*, *J* = 11.4 Hz and 3.65 *d*, *J* = 11.4 Hz). The latter formed an AB system, implying of their connection to a quaternary carbon.

The ^{13}C NMR spectral data of **TK1** (**Table 2, Figure 6**) showed all 20 carbon signals, whose DEPT spectrum enabled assignment as two methyl (δ 17.9 and 27.0), eleven methylene (δ 18.2, 20.2, 20.4, 35.5, 39.3, 40.4, 41.7, 47.0, 47.5, 65.5 and 102.8), two methine (δ 54.9 and 56.7) and five quaternary carbons (δ 38.6, 39.0, 41.6, 80.3 and 156.1). The ^{13}C NMR signals at δ 65.5, 102.8 and 156.5 confirmed the presence of oxy-methylene and exocyclic methylene carbons. Comparison of ^{13}C NMR chemical shifts with those of related kauranoid diterpenes (Subrahmanyam *et al*., 1999) and relative configurations from X-ray ORTEP diagram (**Figure 2**),

suggested that **TK1** possesses an *ent*-kaurane-type skeleton with oxy-methylene protons at C-19.

The position of oxy-methylene protons at C-19 was determined through an HMBC experiment (**Table 2, Figure 7**) in which the oxy-methylene protons at δ3.35 and 3.65 (H_2-19) showed correlations with C-3 (δ35.6), C-4 (δ39.0) and C-18 (δ27.0). The methyl protons at δ0.89 (H_3-18) showed correlations with C-3 (δ 35.6), C-4 (δ 39.0), C-5 (δ 56.7) and C-19 (δ 65.5). Thus on the basis of its spectroscopic data and comparison with the previous report [Subrahmanyam *et al*., 1999, $[\alpha]^{27}_{D}$: -47° (*c* = 0.10, CH_3OH)] (**Table 2**), compound **TK1** was assigned as *ent*-kaur-16-en-13,19-diol.



Selected HMBC correlation of **TK1**

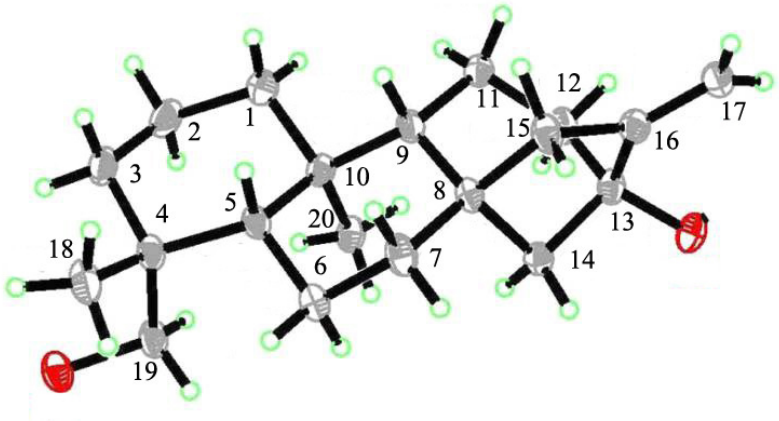


Figure 2 X-ray ORTEP diagram of compound **TK1**

Table 2 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **TK1** and *ent*-kaur-16-en-13,19-diol (**R**, C_5D_5N)

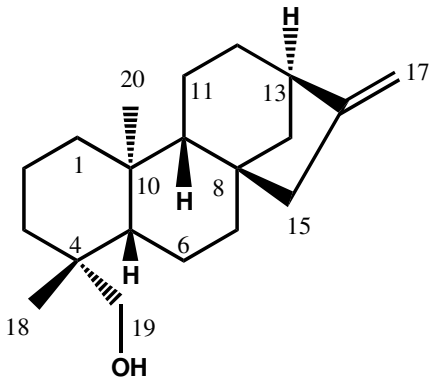
Position	Type of C	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK1	**R**	**TK1**	
1	CH_2	40.4	40.6	0.75 (*m*), 1.80 (*m*)[a]	-
2	CH_2	20.2	20.5	1.61 (*m*), 1.74 (*m*)[a]	-
3	CH_2	35.6	36.1	0.86 (*m*), 1.73 (*m*)[a]	-
4	C	39.0	39.2	-	-
5	CH	56.7	57.0	0.91 (*m*)[a]	-
6	CH_2	20.4	20.7	1.28 (*qd*, *J* = 12.4, 3.6 Hz)[a]	-
7	CH_2	41.7	42.2	1.40 (*m*), 1.46 (*m*)[a]	-
8	C	41.6	41.7	-	-
9	CH	54.9	55.2	0.88 (*m*)[a]	-
10	C	38.6	39.3	-	-
11	CH_2	18.2	18.7	1.35 (*m*), 1.50 (*m*)[a]	-
12	CH_2	39.3	40.6	1.49 (*m*), 1.76 (*m*)[a]	-
13	C	80.3	79.8	-	8, 14, 16, 17
14	CH_2	47.0	47.3	1.17 (*dd, J* = 10.8, 2.4 Hz),	-
				2.02 (*m*)[a]	-
15	CH_2	47.5	48.2	1.17 (*m*), 2.11 (*m*)[a]	-
16	C	156.1	157.4	-	-
17	CH_2	102.8	102.9	4.74 (*brs*),	13, 15
				4.91 (*brt , J* = 2.2 Hz)	
18	CH_3	27.0	28.0	0.89 (*s*)	3, 4, 5, 19
19	CH_2	65.5	64.0	3.35 (*d*, *J* = 11.4 Hz)[b]	}
				3.65 (*d*, *J* = 11.4 Hz)[b]	} 3, 4, 18
20	CH_3	17.9	18.2	0.93 (*s*)	1, 5, 9

* For **TK1**

[a] Deduced from HMQC experiment

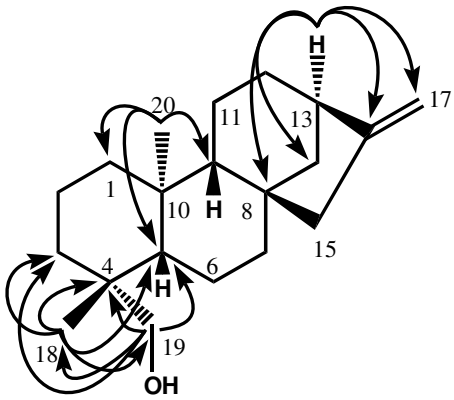
[b] May be interchanged

3.1.2 Compound TK2



Compound **TK2** was isolated as a white amorphous solid, mp. 140-141 $^{\circ}$C, $[\alpha]^{27}_{D}$: -75.0° (*c* = 0.34, $CHCl_3$). The IR spectrum showed absorption bands similar to those of compound **TK1**.

The ^{1}H and ^{13}C NMR spectral data of **TK2** (**Table 3, Figures 11** and **12**) were similar to those of **TK1** (**Table 2, Figures 5** and **6**). The difference in the spectrum of **TK2** was shown as an additional broad singlet methine proton signal at δ 2.64 and a methine carbon signal at δ 43.9 replaced an oxyquarternary carbon at δ 80.3 in **TK1,** thus suggesting a methine proton at C-13. By comparison of the ^{13}C NMR spectral data with the previously reported data [Antonio *et al*., 1981; Piozzi *et al*., 1971, $[\alpha]^{20}_{D}$: -82.0° (*c* = 0.42, $CHCl_3$)] (**Table 3**), therefore compound **TK2** was identified as *ent*-kaurenol.



Selected HMBC correlation of **TK2**

Table 3 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK2**, **TK1** and *ent*-kaurenol (**R**, $CDCl_3$)

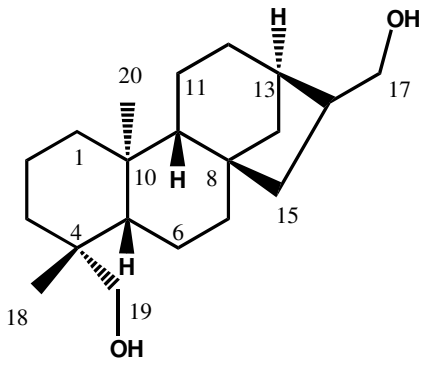
Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK2	**TK1**	**R**	**TK2**	
1	CH_2	40.4	40.4	40.5	0.81 (*m*), 1.87 (*m*)[a]	-
2	CH_2	18.3	20.2	18.3	1.40(*m*)[a]	-
3	CH_2	35.6	35.5	35.6	0.94 (*m*), 1.79 (*m*)[a]	-
4	C	38.6	39.0	38.7	-	-
5	CH	56.8	56.7	56.8	0.96 (*m*)[a]	-
6	CH_2	20.5	20.4	20.5	1.66 (*m*)[a]	-
7	CH_2	41.6	41.7	41.6	1.50 (*m*)[a]	-
8	C	44.1	41.6	44.2	-	-
9	CH	56.2	54.9	56.2	1.09 (*m*)[a]	-
10	C	39.2	38.6	39.2	-	-
11	CH_2	18.2	18.2	18.2	1.58 (*m*)[a]	-
12	CH_2	33.1	39.3	33.2	1.46 (*m*), 1.64 (*m*)[a]	-
13	CH	43.9	80.3	44.0	2.64 (*brs*)	-
14	CH_2	39.6	47.0	39.7	1.10 (*m*), 1.98 (*brd*)[a]	-
15	CH_2	49.1	47.5	49.1	2.07 (*dd*, *J* = 5.1, 2.4 Hz)	-
16	C	155.8	156.1	155.8	-	-
17	CH_2	102.9	102.8	103.0	4.73 (*brs*), 4.81 (*brs*)	-
18	CH_3	27.0	27.0	27.1	0.99 (*s*)	3, 4, 5, 19
19	CH_2	65.5	65.5	65.4	α 3.49 (*d*, *J* = 10.8 Hz)[b]	3, 4, 5, 18
					β 3.75 (*d*, *J* = 10.8 Hz)[b]	
20	CH_3	18.1	17.9	18.5	1.02 (*s*)	1, 5, 9

* For **TK2**

[a] Deduced from HMQC experiment

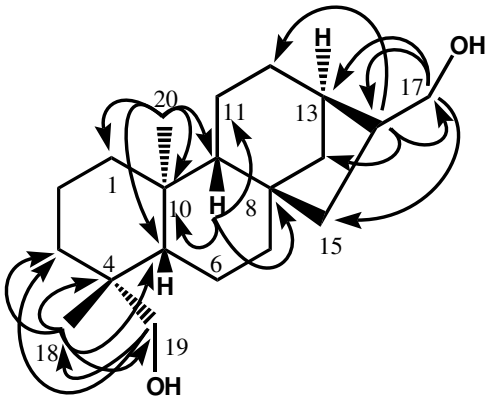
[b] May be interchanged

3.1.3 Compound TK3



Compound **TK3** was isolated as a white amorphous solid, mp. 112-114 °C, $[\alpha]^{27}_{D}$: -32.0° (*c* = 0.40, $CHCl_3$). It exhibited hydroxyl (3446 cm^{-1}) absorptions in the IR spectrum.

The ^{1}H and ^{13}C NMR spectral data of **TK3** (**Table 4, Figures 17** and **18**) were similar to those of **TK2** (**Table 3, Figures 11** and **12**). Difference in the spectrum of **TK3** was shown as the disappearance of an exocyclic methylene carbon at C-17 (δ 102.9 in **TK2**) and the appearance of oxymethylene carbon (δ 67.5) in the ^{13}C NMR spectrum of **TK3**. The ^{1}H NMR spectrum displayed a signal of oxymethylene protons at δ 3.40 instead of exocyclic methylene protons, thus suggesting oxymethylene protons at C-17 and a signal of a methine proton was shown at δ 1.93 (C-16). The position of oxymethylene protons at C-17 was determined through an HMBC experiment (**Table 4**) whose proton signals at δ 3.40 showed correlations with C-13 (δ 38.3), C-15 (δ 45.1) and C-16 (δ 43.4). Thus on the basis of its spectroscopic data and comparison with the previous report (Han *et al*., 2004; Bohlmann *et al*., 1981), compound **TK3** was assigned as 16α*H*-17,19-*ent*-kauranediol.



Selected HMBC correlation of **TK3**

Table 4 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK3** and **TK2**

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK3	**TK2**	**TK3**	
1	CH_2	40.5	40.4	0.80 (*m*), 1.87 (*m*)[a]	-
2	CH_2	18.6	18.3	1.59 (*m*)[a]	-
3	CH_2	35.6	35.6	0.97 (*m*), 1.75 (*m*)[a]	-
4	C	38.6	38.6	-	-
5	CH	56.8	56.8	0.94 (*m*)[a]	-
6	CH_2	20.9	20.5	1.34 (*m*), 1.67 (*m*)[a]	-
7	CH_2	42.0	41.6	1.45 (*m*)[a]	-
8	C	44.7	44.1	-	-
9	CH	56.4	56.2	1.03 (*m*)[a]	8, 12, 20
10	C	39.2	39.2	-	-
11	CH_2	18.3	18.2	1.42 (*m*)[a]	-
12	CH_2	31.5	33.1	1.46 (*m*), 1.59 (*m*)[a]	-
13	CH	38.3	43.9	2.08 (*brs*)[a]	15
14	CH_2	37.2	39.6	0.92 (*m*), 1.84 (*m*)[a]	-
15	CH_2	45.1	49.1	0.90 (*m*), 1.55 (*m*)[a]	-
16	CH	43.4	155.8	1.93 (*m*)[a]	12, 14, 17

Table 4 (continued)

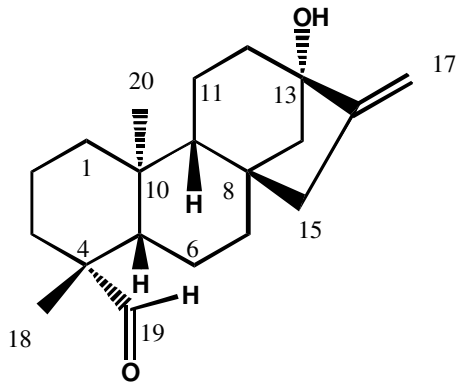
Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK3	**TK2**	**TK3**	
17	CH_2	67.5	102.9	3.40 (*m*)	13, 15, 16
18	CH_3	27.0	27.0	0.96 (*s*)	3, 4, 5, 19
19	CH_2	65.5	65.5	α 3.49 (*d*, *J* = 10.9 Hz)[b]	3, 18
				β 3.79 (*d*, *J* = 10.9 Hz)[b]	
20	CH_3	18.0	18.1	1.00 (*s*)	1, 5, 9, 10

* For **TK3**

[a] Deduced from HMQC experiment

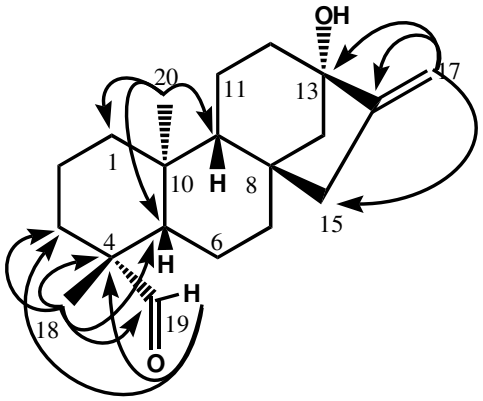
[b] May be interchanged

3.1.4 Compound TK4



Compound **TK4** was isolated as a white amorphous solid, mp. 118-119 $^{\circ}$C, $[\alpha]^{27}_{D}$: -56.9° (*c* = 1.00, $CHCl_3$). It exhibited hydroxyl (3340 cm^{-1}), carbonyl (1712 cm^{-1}) and double bond (1650 cm^{-1}) absorptions in the IR spectrum (**Figure 23**).

The ^{1}H and ^{13}C NMR spectral data of **TK4** (**Table 5, Figures 24** and **25**) were similar to those of **TK1** (**Table 2, Figures 5** and **6**). The difference was found in ring A, where the aldehydic proton at δ 9.70 (*s*, H-19) replaced signals of oxy-methylene protons at δ 3.35 and 3.65 in **TK1**. The aldehydic proton H-19 showed HMBC correlations (**Table 5**) with C-3 (δ 34.1) and C-4 (δ 48.4). The methyl protons at δ 1.01 (H_3-18) showed correlations with C-3 (δ 34.1), C-4 (δ 48.4), C-5 (δ 56.6) and C-19 (δ 205.7). Thus on the basis of its spectroscopic data and comparison with the previous report [Subrahmanyam *et al*., 1999, $[\alpha]^{30}_{D}$: -59.0° (*c* = 0.10, $CHCl_3$)], compound **TK4** was assigned as *ent*-kaur-16-en-13-hydroxy-19-al.



Selected HMBC correlation of **TK4**

Table 5 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK4**, **TK1** and *ent*-kaur-16-en-13-hydroxy-19-al (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK4	**TK1**	**R**	**TK4**	
1	CH_2	39.7	40.4	39.5	0.87 (*m*), 182 (*m*)[a]	-
2	CH_2	19.8	20.2	19.6	1.50 (*m*), 2.08 (*m*)[a]	-
3	CH_2	34.1	35.5	39.0	2.18 (*m*)[a]	-
4	C	48.4	39.0	48.2	-	-
5	CH	56.6	56.7	56.4	1.19 (*dd*, *J* = 12.6, 2.1 Hz)	-
6	CH_2	20.3	20.4	20.1	1.55 (*m*), 1.77 (*m*)[a]	-
7	CH_2	39.1	41.7	34.0	1.05 (*m*), 2.12 (*m*)[a]	-
8	C	41.5	41.6	41.3	-	-
9	CH	53.2	54.9	53.1	1.03 (*m*)[a]	-
10	C	39.1	38.6	39.2	-	-
11	CH_2	18.3	18.2	18.1	1.42 (*m*)[a]	-
12	CH_2	41.2	39.3	41.0	1.50 (*m*), 1.65 (*m*)[a]	-
13	C	80.1	80.3	79.9	-	-
14	CH_2	47.1	47.0	46.8	1.29 (*d*, *J* = 11.1 Hz),	-
					2.12 (*m*)[a]	-

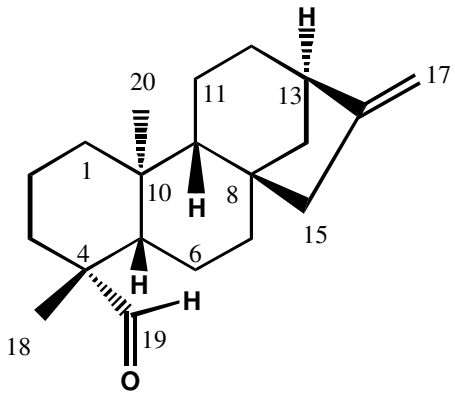
Table 5 (continued)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK4	**TK1**	**R**	**TK4**	
15	CH_2	47.4	47.5	47.3	1.31 (*d*, *J* = 10.8 Hz),	-
					2.08 (*m*)[a]	-
16	C	155.8	156.1	155.4	-	-
17	CH_2	103.1	102.8	103.0	4.82 (*brs*), 4.99 (*brs*)	13, 15, 16
18	CH_3	24.2	27.0	24.1	1.01 (*s*)	3, 4, 5, 19
19	CHO	205.7	65.5	205.6	9.70 (*s*)	3, 4
20	CH_3	16.2	17.9	16.1	0.87 (*s*)	1, 5, 9

* For **TK4**

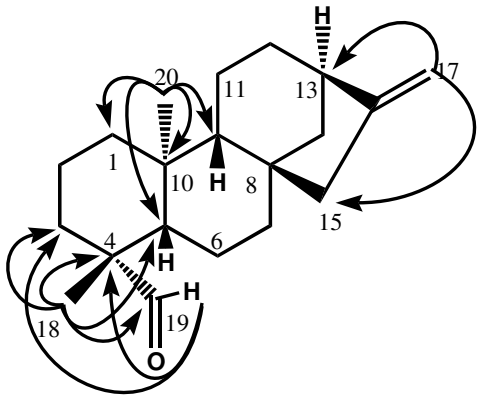
[a] Deduced from HMQC experiment

3.1.5 Compound TK5



Compound **TK5** was isolated as white needles, mp. 114-115 °C, $[\alpha]^{27}_{D}$: -76.0° (*c* = 0.43, $CHCl_3$). The IR spectrum was closely related to that of **TK4**.

The ^{1}H and ^{13}C NMR spectral data of **TK5** (**Table 6, Figures 30** and **31**) resembled those of **TK4** (**Table 5, Figures 24** and **25**). The difference was shown as the additional proton signal at δ 2.65 (*brs*) and the carbon signal at δ 43.7 in **TK5** replaced C-13 signal at δ 80.1 in **TK4**. The exocyclic methylene protons H_2-17 showed HMBC correlations with C-13 (δ 43.7) and C-15 (δ 49.0). Thus on the basis of its spectroscopic data and comparison with the previous report [Stefan *et al*., 2003; Piozzi *et al*., 1971, $[\alpha]^{20}_{D}$: -95.0° (*c* = 0.39, $CHCl_3$)], compound **TK5** was assigned as *ent*-kaurenal. X-ray crystallographic analysis of **TK5** (Chantrapromma *et al*., 2007) was also carried out and gave ORTEP drawing as shown in **Figure 3**.



Selected HMBC correlation of **TK5**

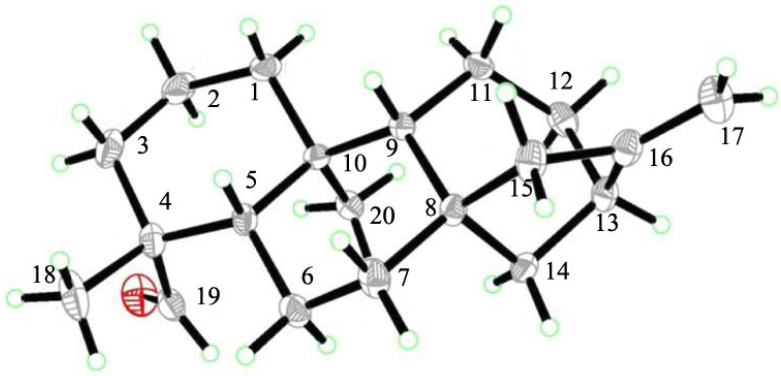


Figure 3 X-ray ORTEP diagram of compound **TK5**

Table 6 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK5** and **TK4**

Position	Type of C*	δ_C/ppm		δ_H/ppm (multiplicity, *J*/Hz)	HMBC*
		TK5	**TK4**	**TK5**	$^{1}H \rightarrow ^{13}C$
1	CH_2	39.9	39.7	0.80 (*m*)[a]	-
2	CH_2	18.4	19.8	1.68 (*m*)[a]	-
3	CH_2	34.2	34.1	1.01 (*m*), 2.16 (*m*)[a]	-
4	C	48.4	48.4	-	-
5	CH	56.7	56.6	1.17 (*m*)[a]	-
6	CH_2	19.8	20.3	1.72 (*m*), 1.91 (*m*)[a]	-
7	CH_2	41.1	39.1	1.60 (*m*)[a]	-
8	C	44.0	41.5	-	-
9	CH	54.5	53.2	1.11 (*m*)[a]	-
10	C	39.3	39.1	-	-
11	CH_2	18.3	18.3	1.50 (*m*)[a]	-
12	CH_2	32.9	41.2	1.64 (*m*)[a]	-
13	CH	43.7	80.1	2.65 (*brs*)[a]	-
14	CH_2	39.8	47.1	1.17 (*m*), 1.98 (*m*)[a]	-

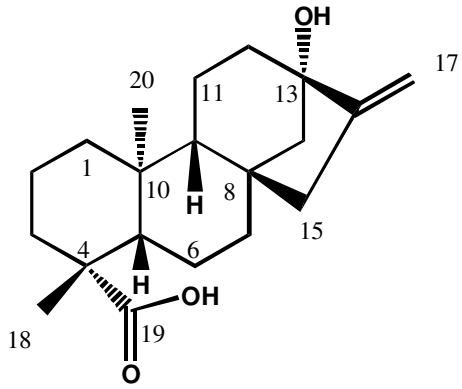
Table 5 (continued)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK5	**TK4**	**TK4**	
15	CH_2	49.0	47.4	2.09 (*m*)[a]	-
16	C	155.5	155.8	-	-
17	CH_2	103.2	103.1	4.75 (*brs*), 4.80 (*brs*)	13, 15
18	CH_3	24.2	24.2	1.00 (*s*)	3, 4, 5, 19
19	CHO	205.8	205.7	9.75 (*s*)	3, 4
20	CH_3	16.3	16.2	0.89 (*s*)	1, 5, 9, 10

* For **TK5**

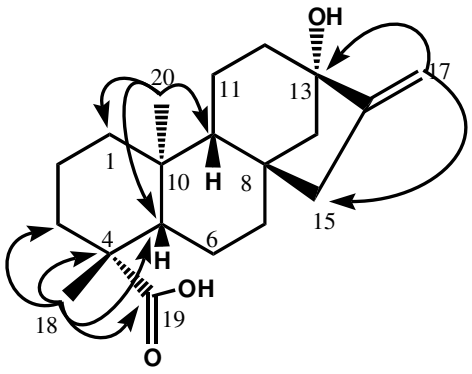
[a] Deduced from HMQC experiment

3.1.6 Compound TK6



Compound **TK6** was isolated as a white amorphous solid, mp. 199-201 $^{\circ}$C, $[\alpha]^{27}_{D}$: -58.1° (*c* = 2.00, $CHCl_3$). It exhibited hydroxyl (3396 cm^{-1}) and carboxyl (1687 cm^{-1}) absorptions in the IR spectrum (**Figure 36**).

The ^{1}H and ^{13}C NMR spectral data of **TK6** (**Table 7, Figures 37 and 38**) were similar to those of **TK4** (**Table 5, Figures 24** and **25**). The difference in the spectrum of **TK6** was shown as the disappearance of an aldehydic proton at δ 9.70 (H-19) in the ^{1}H NMR of **TK4** and the ^{13}C NMR spectrum of **TK6** displayed a signal of carboxyl carbon at δ 183.3 instead of an aldehydic carbon at δ 205.7, thus suggesting a carboxylic functionality at C-19. The location of the carboxyl group was confirmed by HMBC experiment (**Table 7**) in which the methyl protons at δ 1.21 (H_3-18) showed correlations with C-3 (δ 37.8), C-4 (δ 43.6), C-5 (δ 56.9) and C-19 (δ 183.3). NOESY correlation between H_3-20 and H_2-α14 (δ 2.10) supported the assignment. Thus on the basis of its spectroscopic data and comparison with the previous report [Subrahmanyam *et al*., 1999, $[\alpha]^{30}_{D}$: -69.0° (*c* = 0.06, $CHCl_3$); Yang *et al*., 2007], compound **TK6** was assigned as *ent*-kaur-16-en-13-hydroxy-19-oic acid (steviol).



Selected HMBC correlation of **TK6**

Table 7 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK6**, **TK4** and *ent*-kaur-16-en-13-hydroxy-19-oic acid (**R**, C_5D_5N)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK6	**TK4**	**R**	**TK6**	
1	CH_2	40.0	39.7	41.1	0.86 (*m*), 1.18 (*m*)[a]	-
2	CH_2	19.0	19.8	19.9	1.50 (*m*), 2.18 (*m*)[a]	-
3	CH_2	37.8	34.1	38.7	1.05 (*m*), 2.16 (*m*)[a]	-
4	C	43.6	48.4	44.0	-	-
5	CH	56.9	56.6	57.1	1.03 (*m*)[a]	-
6	CH_2	21.8	20.1	22.7	1.81 (*m*), 2.17 (*m*)[a]	-
7	CH_2	39.4	39.1	42.0	1.42 (*m*), 1.51 (*m*)[a]	-
8	C	41.7	41.3	41.9	-	-
9	CH	53.9	53.1	54.4	0.96 (*m*)[a]	-
10	C	39.5	39.2	39.9	-	-
11	CH_2	19.0	18.3	20.0	1.63 (*m*), 1.81 (*m*)[a]	-
12	CH_2	41.3	41.2	40.8	1.63 (*m*), 1.71 (*m*)[a]	-
13	C	80.4	80.1	79.9	-	-
14	CH_2	46.9	47.1	47.6	1.32 (*m*), 2.10 (*m*)[a]	-
15	CH_2	47.4	47.4	48.3	1.29 (*m*), 2.07 (*m*)[a]	-

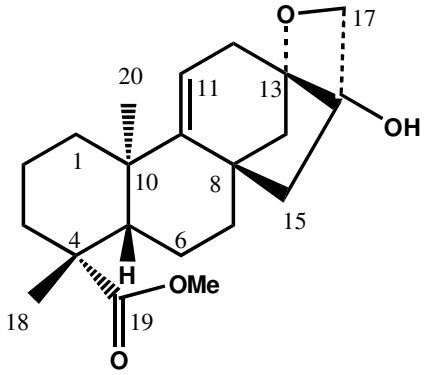
Table 7 (continued)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK6	**TK4**	**R**	**TK6**	
16	C	155.7	155.8	157.8	-	-
17	CH_2	103.0	103.1	103.0	4.97 (*brs*), 4.98 (*brs*)	13, 15
18	CH_3	28.8	24.2	29.4	1.21 (*s*)	3, 4, 5, 19
19	COO	183.3	205.7	180.2	-	-
20	CH_3	15.5	16.2	16.0	0.95 (*s*)	1, 5, 9

* **For TK6**

[a] Deduced from HMQC experiment

3.1.7 Compound TK7

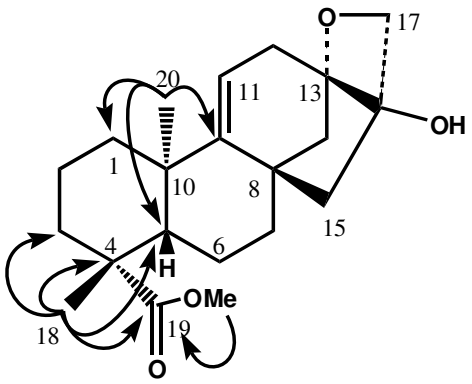


Compound **TK7** was isolated as a white amorphous solid, mp. 169-171 $^{\circ}$C, $[\alpha]^{27}_{D}$: +36.3° (*c* = 0.40, $CHCl_3$). It exhibited hydroxyl (3427 cm^{-1}), carbonyl (1728 cm^{-1}) and double bond (1649 cm^{-1}) absorptions in the IR spectrum (**Figure 43**).

The ^{13}C NMR and DEPT spectral data of **TK7** (**Table 8**, **Figures 45, 46 and 47**) showed the presence of 21 carbon signals of a diterpenoid with an acetoxyl group. The ^{13}C NMR signals were displayed as an ester carbonyl (δ 177.8), a trisubstituted double bond (δ 157.3), two quaternary oxygen bearing carbons (δ 78.6 and 80.1), an oxymethylene carbon (δ 67.8), a methoxyl carbon (δ 51.3), two methyl carbons (δ 23.3 and 28.0), eight methylene carbons (δ 17.9, 20.1, 30.0, 37.5, 38.2, 40.9, 49.2 and 52.9), one methine carbon (δ 46.5), one olefinic methine carbon (δ 114.7) and three quaternary carbons (δ 38.6 40.2 and 44.8).

The ^{1}H NMR spectral data of **TK7** (**Table 8, Figure 44**) showed a signal of a trisubstituted olefinic proton (δ 5.32 *brs*, H-11), carbomethoxyl protons (δ 3.61 *s*, H_3-21), two methyl groups (δ 0.91 *s*, H_3-20 and 1.16 *s*, H_3-18) and an AB system of oxymethylene protons (δ 3.53 *d*, *J* = 11.1 Hz and 3.57 *d*, *J* = 11.1 Hz, H_2-17). The coupling constant 11.4 Hz of oxymethylene protons of oxetane in **TK7** was in agreement with the reported value (11.4 Hz, Ammanamanchi *et al*., 2003). The location of the carbomethoxyl group was confirmed by HMBC experiment (**Table 8**) in which the methyl protons δ 1.16 (H_3-18) showed correlations with C-3 (δ 37.5), C-4 (δ 44.8), C-5 (δ 46.5) and C-19 (δ 177.8). Thus on the basis of its spectroscopic data

and comparison with the previous report [Subrahmanyam *et al*., 1999, $[\alpha]^{30}_{D}$: +22.0° (*c* = 0.10, CH_3OH); Ammanamanchi *et al*., 2003], compound **TK7** was assigned as methyl *ent*-kaur-9(11)-ene-13,17-epoxy-16-hydroxy-19-oate.



Selected HMBC correlation of **TK7**

Table 8 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **TK7** and methyl *ent*-kaur-9(11)-ene-13,17-epoxy-16-hydroxy-19-oate (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC*
		TK7	**R**	**TK7**	$^{1}H \rightarrow ^{13}C$
1	CH_2	40.9	41.0	1.18 (*m*), 1.80 (*m*)[a]	-
2	CH_2	20.1	20.1	1.46 (*m*), 1.76 (*m*)[a]	-
3	CH_2	37.5	37.6	2.46 (*m*), 2.52 (*m*)[a]	-
4	C	44.8	44.8	-	-
5	CH	46.5	46.5	1.52 (*m*)[a]	-
6	CH_2	17.9	18.0	2.44 (*m*)[a]	-
7	CH_2	30.0	30.0	1.45 (*m*), 1.99 (*m*)[a]	-
8	C	40.2	40.3	-	-
9	C	157.3	157.4	-	-
10	C	38.6	38.7	-	-
11	CH	114.7	114.7	5.32 (*brs*)	-

Table 8 (continued)

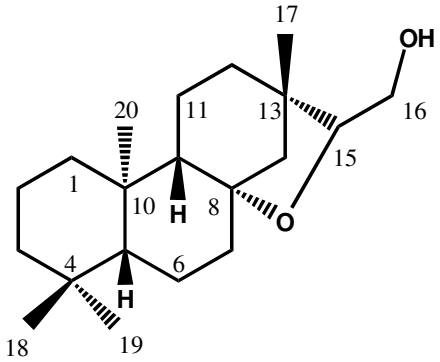
Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK7	**R**	**TK7**	
12	CH_2	38.2	38.3	1.01 (*m*), 2.13 (*brd*)[a]	-
13	C	80.1	80.0	-	-
14	CH_2	49.2	49.2	1.53 (*m*), 2.00 (*m*)[a]	-
15	CH_2	52.9	52.9	1.73 (*m*), 1.82 (m)[a]	-
16	C	78.6	78.7	-	-
17	CH_2	67.8	67.9	α 3.53 (*d*, *J* = 11.1 Hz)[b]	-
				β 3.57 (*d*, *J* = 11.1 Hz)[b]	-
18	CH_3	28.0	28.0	1.16 (*s*)	3, 4, 5, 19
19	COO	177.8	177.8	-	-
20	CH_3	23.3	23.4	0.91 (*s*)	1, 5, 9, 10
21	OCH_3	51.3	51.4	3.61 (*s*)	19

* For **TK7**

[a] Deduced from HMQC experiment

[b] May be interchanged

3.1.8 Compound TK8

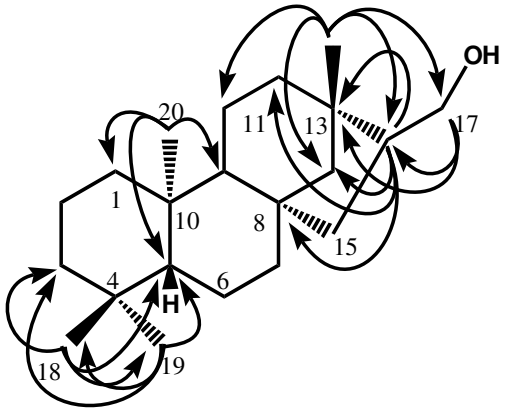


Compound **TK8** was isolated as a white amorphous solid, mp. 85-86°C, $[\alpha]^{27}_{D}$: -67.2° (*c* = 0.01, $CHCl_3$). It exhibited hydroxyl (3346 cm^{-1}) absorption in the IR spectrum.

The ^{13}C NMR spectral data of **TK8** (**Table 9**, **Figure 50**) showed all 20 carbon signals. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of four methyl (δ 14.8, 19.9, 22.2 and 33.9), nine methylene (δ 18.4, 19.3, 19.4, 38.5, 39.2, 40.2, 42.0, 52.2 and 64.7), three methine (δ 55.2, 55.3, and 84.7) and four quaternary carbons (δ 33.1, 37.0, 41.0 and 82.7).

The ^{1}H NMR spectral data of **TK8** (**Table 9, Figure 51**) showed oxymethylene protons (δ 3.38 *dd*, *J* = 11.1, 7.5 Hz and 3.49 *dd*, *J* = 11.1, 3.6 Hz, H_2-16), an oxymethine proton (δ 3.72 *dd*, *J* = 7.5, 3.6 Hz, H-15), four methyl singlets at δ 0.86 (x 2), 0.93, and 1.00. Comparison of the chemical shifts of all these groups with literature data (Herz *et al*., 1983) confirmed the presence of an *ent*-pimarane skeleton. The location of the oxymethylene and oxymethine protons was confirmed by HMBC experiment (**Table 8**) in which the oxymethylene protons at δ 3.38 and 3.49 (H_2-16) showed correlations with C-13 (δ 41.0) and C-15 (δ 84.7) and an oxymethine proton at δ 3.72 (H-15) showed correlations with C-12 (δ 39.2), C-13 (δ 41.0) and C-14 (δ 52.2). NOESY correlation between H_3-20 and H_3-19 supported the assignment. Thus on the basis of its spectroscopic data and comparison with the previous report (Herz *et*

al., 1983; Ammanamanchi *et al*., 2003), compound **TK8** was assigned as *ent*-8,15*R*-epoxypimaran-16-ol.



Selected HMBC correlation of **TK8**

Table 9 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **TK8** and *ent*-8,15*R*-epoxypimaran-16-ol (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK8	**R**	**TK8**	
1	CH_2	38.5	38.5	1.73 (*m*)[a]	-
2	CH_2	18.4	18.4	1.41 (*m*)[a]	-
3	CH_2	42.0	42.0	1.50 (*dd*, *J* = 12.9, 3.9 Hz),	-
4	C	33.1	33.1	1.40 (*m*)[a]	-
5	CH	55.2	55.2	-	-
6	CH_2	19.3	19.3	0.80 or 1.01 (*m*)[a]	-
7	CH_2	40.2	40.1	1.58 (*m*)[a]	-
8	C	82.7	82.7	0.81 (*m*), 1.65 (*m*)[a]	-
9	CH	55.3	55.4	-	-
10	C	37.0	37.1	0.80 or 1.01 (*m*)[a]	-
11	CH_2	19.4	19.5	-	-

Table 9 (continued)

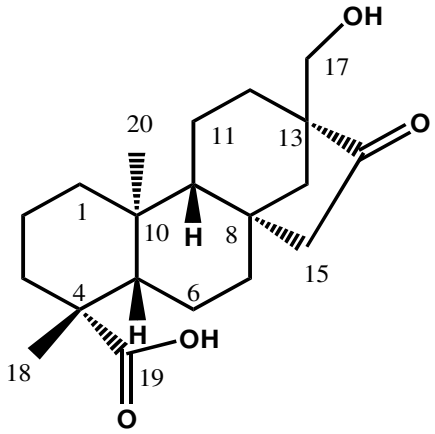
Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK8	**R**	**TK8**	
12	CH_2	39.2	39.2	1.49 (*m*)[a]	-
13	C	41.0	41.2	1.33 (*m*), 1.57 (*m*)[a]	-
14	CH_2	52.2	52.2	1.21 (*d*, *J* = 11.1 Hz)1.60 (*m*)[a]	-
15	CH	84.7	82.7	3.72 (*dd*, *J* = 7.5, 3.6 Hz)	8,12, 13, 14
16	CH_2	64.7	64.3	3.38 (*dd*, *J* = 11.1, 7.5 Hz)[b] 3.49 (*dd*, *J* = 11.1, 3.6 Hz)[b]	13, 15
17	CH_3	19.9	19.9	0.93 (*s*)	11, 14, 15, 16
18	CH_3	33.9	33.8	0.86 (*s*)	3, 5, 19
19	CH_3	22.2	22.2	0.86 (*s*)	3, 5, 18
20	CH_3	14.8	14.8	1.00 (*s*)	5, 9, 10

* For **TK8**

[a] Deduced from HMQC experiment

[b] May be interchanged

3.1.9 Compound TK9

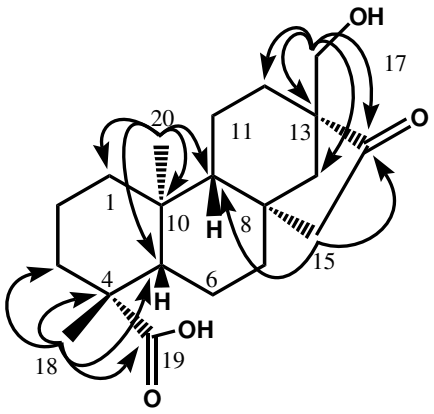


Compound **TK9** was isolated as a white amorphous solid, mp. 230-232 $^{\circ}$C, $[\alpha]^{27}_{D}$: -35.0° (*c* = 0.30, $CHCl_3$). It exhibited hydroxyl (3535 cm^{-1}), carbonyl (1719 cm^{-1}) and carboxyl (1650 cm^{-1}) absorptions in the IR spectrum.

The ^{13}C and DEPT spectral data of **TK9** (**Table 10**, **Figures 57, 59** and **60**) showed all 20 carbon signals as two methyl (δ 13.3 and δ 28.9), ten methylene (δ 18.8, 19.8, 21.6, 32.0, 37.6, 39.7, 41.3, 48.9, 49.0 and 65.0), two methine (δ 55.4 and 56.9) and six quaternary carbons (δ 38.2, 43.5, 43.6, 54.1, 182.8 and 223.7). The ^{13}C NMR signals at δ 65.0, 182.8 and 223.7 confirmed the presence of oxymethylene, carboxyl, and keto carbonyl functionalities, respectively.

The ^{1}H NMR spectral data of **TK9** (**Table 10**, **Figure 56**) showed signals for two tertiary methyl groups (δ 0.80 and 1.25). The two oxymethylene protons (δ 3.53 d, *J* = 11.4 Hz and 3.63 d, *J* = 11.4 Hz, H_2-17) formed an AB system, suggesting of their conection to a quaternary carbon. The HMBC correlations (**Table 10**) of these protons with carbon signals at δ 32.0 (C-12), 54.1 (C-13), 48.9 (C-14), and 223.7 (C-16) helped to locate the hydroxymethylene group at C-13 and the carbonyl group at C-16. One of the methylene proton (H-15) was displayed as a doublet of doublet 2.60 (*J* = 18.9, 3.6 Hz) which showed HMBC correlation with C-9 (δ 55.4) and C-16 (δ 223.7). The C-19 was taken as the carboxyl group in view of the presence of only one tertiary methyl carbon at δ 28.9 and H_3-18 showed correlations

with C-3 (δ 37.6), C-4 (δ 43.6), C-5 (δ 56.9) and C-19 (δ 182.8). The complete HMBC correlations were summarized in **Table 10**. The presence of a NOESY correlation between H-5 and H-9 suggested a *trans-trans* relationship between the junction C5-C10 and C10-C9. The NOESY correlation observed between H_3-20 and H_2-15 indicated that the bridge-head at C-8 and C-13 is *trans* to H_3-20 indicating the structure and relative stereochemistry of *ent*-17-hydroxy-16-keto-beyeran-19-oic acid. Thus on the basis of its spectroscopic data and comparison with the previous report (Oliveira *et al*., 1999), compound **TK9** was assigned as *ent*-17-hydroxy-16-keto-beyeran-19-oic acid.



Selected HMBC correlation of **TK9**

Table 10 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **TK9** and *ent*-17-hydroxy-16-keto-beyeran-19-oic acid (**R**, $CDCl_3$)

Position	Type of C*	δ_C/ppm		δ_H/ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK9	**R**	**TK9**	
1	CH_2	39.7	40.1	0.94 (*dd*, *J* = 13.5, 4.2 Hz), 1.78 (*m*)[a]	-
2	CH_2	18.8	19.2	1.41 (*m*), 1.83 (*m*)[a]	-
3	CH_2	37.6	38.1	1.04 (*dd*, *J* = 19.2, 3.9 Hz), 2.15 (*m*)[a]	-
4	C	43.6	44.0	-	-
5	CH	56.9	57.3	1.69 (*dd*, *J* = 11.4, 2.1 Hz)[a]	-
6	CH_2	21.6	22.0	1.79 (*m*), 1.89 (*m*)[a]	-

Table 10 (continued)

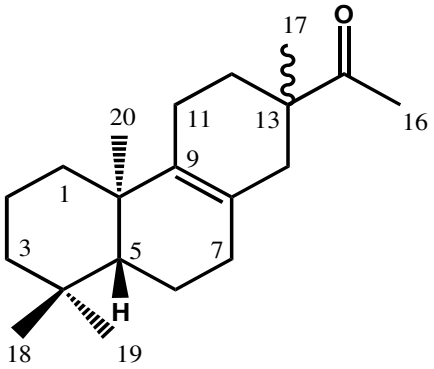
Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK9	**R**	**TK9**	
7	CH_2	41.3	41.7	1.55 (*dd*, *J* = 13.8, 4.2 Hz),	-
				1.73 (*m*)[a]	-
8	C	43.5	40.1	-	-
9	CH	55.4	55.8	1.25 (*m*)[a]	-
10	C	38.2	38.7	-	-
11	CH_2	19.8	20.2	1.28 (*m*)[a]	-
12	CH_2	32.0	32.5	1.39 (*m*), 1.87 (*m*)[a]	-
13	C	54.1	54.5	-	-
14	CH_2	48.9[b]	49.4[b]	1.31 (*dd*, *J* = 11.4, 3.6 Hz)[a,b]	-
				1.85(*m*)[a,b]	-
15	CH_2	49.0[b]	49.3[b]	1.85 (*m*)[a,b]	-
				2.60 (*dd*, *J* = 18.9, 3.6 Hz)[a]	9, 16
16	CO	223.7	223.7	-	-
17	CH_2	65.0	65.5	α 3.53 (*d*, *J* = 11.4 Hz)[b]	12, 13,
				β 3.63 (*d*, *J* = 11.4 Hz)[b]	14, 16
18	CH_3	28.9	29.3	1.25 (*s*)	3, 4, 5, 19
19	COOH	182.8	183.2	-	-
20	CH_3	13.3	13.7	0.80 (*s*)	1, 5, 9, 10

* For **TK9**

[a] Deduced from HMQC experiment

[b] May be interchanged

3.1.10 Compound TK10

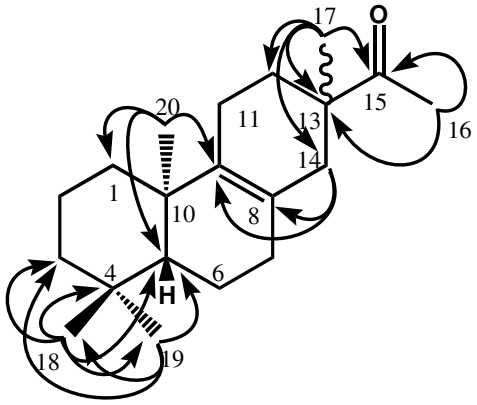


Compound **TK10** was obtained as pale yellow viscous oil, $[\alpha]^{27}_{D}$ +53.2° (*c* = 0.50, $CHCl_3$) and its molecular formula was assigned as $C_{20}H_{32}O$ from HREIMS (**Figure 70**). It exhibited carbonyl (1705 cm^{-1}) and double bond (1641 cm^{-1}) absorptions in the IR spectrum (**Figure 62**).

The ^{13}C NMR spectral data of **TK10** (**Table 11**, **Figure 64**) showed all 20 carbon signals. Analysis of DEPT 90° and 135° spectra of this compound suggested the presence of five methyl (δ 19.4, 20.8, 21.6, 24.7 and 33.2), eight methylene (δ 18.8, 18.9, 20.4, 30.6, 32.6, 36.7, 38.6 and 41.8), one methine (δ 51.7) and six quaternary carbons (δ 33.2, 37.5, 46.1, 123.7, 136.9 and 214.0). The ^{13}C NMR signals at δ 214.0, 123.7 and 136.9 confirmed the presence of keto and double bond functionalities, respectively.

The ^{1}H NMR spectral data of **TK10** (**Table 11**, **Figure 63**) displayed five singlet tertiary methyl groups at δ 0.82 (H_3-19), δ 0.88 (H_3-18), δ 0.94 (H_3- 20), δ 1.06 (H_3-17) and δ 2.03 (H_3-16). The position of methyl groups were determined through an HMBC experiment (**Table 11**) in which the methyl protons at δ 2.03 (H_3-16) showed correlations with C-13 (δ 46.1) and C-15 (δ 214.0), methyl protons at δ 1.06 (H_3-17) showed correlations with C-12 (δ 30.6), C-13 (δ 46.1), C-14 (δ 38.7) and C-15 (δ 214.0), methyl protons at δ 0.94 (H_3-20) showed correlations with C-1 (δ 36.6), C-5 (δ 51.7) and C-9 (δ 136.9), and methyl protons at δ 0.88 (H_3-18) showed correlations with C-3 (δ 41.8), C-4 (δ 33.2), C-5 (δ 51.7) and C-19 (δ 21.6). NOESY

correlation between H_3-20 and H_3-19 supported the assignment. Thus, compound **TK10** could be deduced as *ent*-8(9)-pimaren-15-one.



Selected HMBC correlation of **TK10**

Table 11 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **TK10**

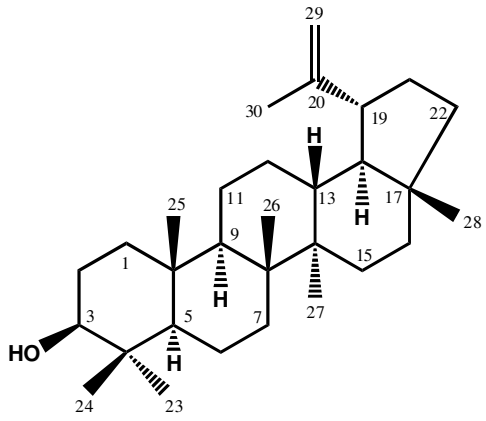
Position	Type of C	δ_C/ppm	δ_H/ppm (multiplicity, *J*/Hz)	HMBC $^{1}H \rightarrow ^{13}C$
		TK10	**TK10**	
1	CH_2	36.6	1.05 (*m*), 1.75 (*m*)[a]	-
2	CH_2	18.9	1.43 (*m*)[a]	-
3	CH_2	41.8	1.15 (*m*), 1.42 (*m*)[a]	-
4	C	33.2	-	-
5	CH	51.7	1.15 (*m*)[a]	-
6	CH_2	18.8	1.61 (*m*), 1.73 (*m*)[a]	-
7	CH_2	32.6	1.95 (*m*)[a]	-
8	C	123.7	-	-
9	C	136.9	-	-
10	C	37.5	-	-
11	CH_2	20.4	2.20 (*m*)[a]	8, 9
12	CH_2	30.6	1.62 (*m*)[a]	-
13	C	46.1	-	-
14	CH_2	38.7	1.67 (*m*), 2.20 (*m*)[a]	8, 9

Table 11 (continued)

Position	Type of C*	δ_C/ppm	δ_H/ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK10	**TK10**	
15	CO	214.0	-	-
16	CH_3	24.7	2.03 (*s*)	13, 15
17	CH_3	20.8	1.06 (*s*)	12, 13, 14, 15
18	CH_3	33.2	0.88 (*s*)	3, 4, 5, 19
19	CH_3	21.6	0.82 (*s*)	3, 4, 5, 18
20	CH_3	19.4	0.94 (*s*)	1, 5, 9

[a] Deduced from HMQC experiment

3.1.11 Compound TK11



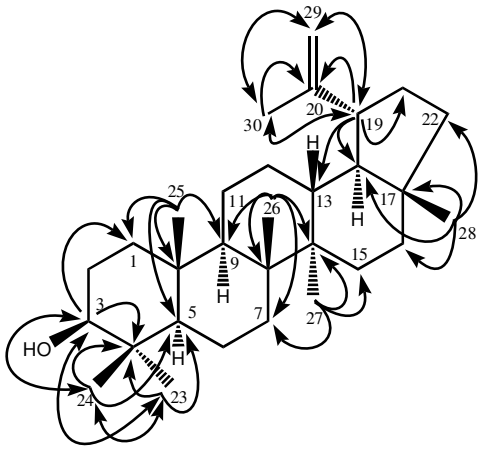
Compound **TK11** was obtained as a white solid, mp. 193-194 °C; $[\alpha]^{28}_{D}$: +25.0° (*c* = 0.20, $CHCl_3$). It exhibited hydroxyl (3343 cm^{-1}) and double bond (1638 cm^{-1}) absorptions in the IR spectrum (**Figure 71**) and gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^{13}C and DEPT spectral data of **TK11** (**Table12, Figures 73**, **74** and **75**) showed all 30 carbon signals as 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 of **TK11** (**Table12, Figure 72**) showed characteristic of lupane triterpenoid 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 splitting pattern together with a large coupling constant of H-3 with *Jax-ax* = 10.8 Hz and *Jax-aq* = 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 12**) 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) and C-30 (δ 19.3). Thus on the basis of its spectroscopic data and comparison with the previous report [Reyolds *et al*., 1986, $[\alpha]^{25}_{D}$: +23.0° (*c* = 0.50, EtOH); Thongdeeying 2005], compound **TK11** was assigned as 3β-lupeol.



Selected HMBC correlation of **TK11**

Table 12 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **TK11** and 3β-lupeol (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK11	**R**	**TK11**	
1	CH_2	38.7	38.7	0.91 (*m*)[a]	-
2	CH_2	27.4	27.4	1.56 (*m*)[a]	-
3	CH	79.0	79.0	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	1, 4, 23, 24
4	C	38.9	38.8	-	-
5	CH	55.3	55.3	0.69 (*m*)[a]	-
6	CH_2	18.3	18.3	1.40 (*m*), 1.55 (*m*)[a]	-
7	CH_2	34.3	34.2	1.40 (*m*)[a]	-
8	C	40.8	40.8	-	-

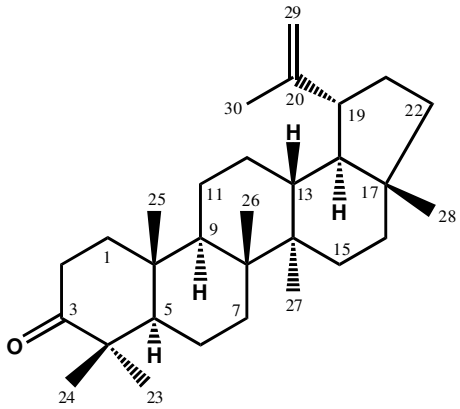
Table 12 (continued)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK11	**R**	**TK11**	
9	CH	50.5	50.4	1.28 (*m*)[a]	-
10	C	37.2	37.1	-	-
11	CH_2	20.9	20.9	1.22 (*m*), 1.45 (*m*)[a]	-
12	CH_2	25.2	25.1	1.08 (*m*)[a]	-
13	CH	38.1	38.0	1.67 (*m*)[a]	-
14	C	42.8	42.8	-	-
15	CH_2	27.5	27.4	1.56 (*m*)[a]	-
16	CH_2	35.6	35.5	1.51 (*m*)[a]	-
17	C	43.0	43.0	-	-
18	CH	48.3	48.2	1.38 (*m*)[a]	-
19	CH	48.0	47.9	2.38 (*dt*, *J* = 11.1, 5.7 Hz)	13, 18, 20, 21, 29, 30
20	C	151.0	150.9	-	-
21	CH_2	29.9	29.8	1.94 (*m*)[a]	-
22	CH_2	40.0	40.0	1.20 (*m*), 1.40 (*m*)[a]	-
23	CH_3	28.0	28.0	0.97 (*s*)	3, 4, 5, 24
24	CH_3	15.4	15.4	0.76 (*s*)	3, 4, 5, 23
25	CH_3	16.1	16.1	0.83 (*s*)	1, 5, 9, 10
26	CH_3	16.0	16.0	1.03 (*s*)	7, 8, 9, 14
27	CH_3	14.6	14.5	0.94 (*s*)	8, 14, 15
28	CH_3	18.0	18.0	0.79 (*s*)	16, 17, 18, 22
29	CH_2	109.3	109.3	4.56 (*m*), 4.68 (*d*, *J* = 2.1 Hz)	19, 30
30	CH_3	19.3	19.3	1.68 (*s*)	19, 30

* For **TK11**

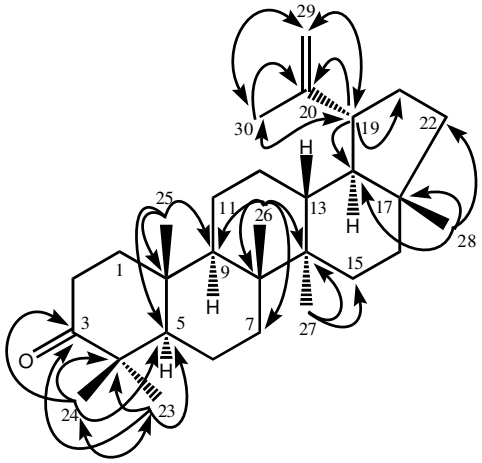
[a] Deduced from HMQC experiment

3.1.12 Compound TK12



Compound **TK12** was obtained as a white solid, mp. 163-165 °C; $[\alpha]^{28}_{D}$: +50.0° (*c* = 0.10, $CHCl_3$). It exhibited carbonyl (1704 cm^{-1}) and double bond (1642 cm^{-1}) absorptions in the IR spectrum (**Figure 78**) and gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^{1}H and ^{13}C NMR spectral data of **TK12** (**Table 13, Figures 79** and **80**) showed signals similar to **TK11** (**Table12, Figures 72** and **73**) except that in **TK12** a doublet of doublet signal of a methine proton H-3 disappeared and the carbon signal at C-3 (δ 217.0) was displayed as a carbonyl carbon instead of the oxy-methylene carbon at δ 79.0 in **TK11**. The location of the carbonyl group was confirmed by HMBC experiment (**Table 13**) in which both H_3-24 (δ 1.02) and H_3-23 (δ 1.07) showed long-range correlation with C-3 (δ 217.0), C-4 (δ 46.3) and C-5 (δ 54.3). Thus on the basis of its spectroscopic data and comparison with the previous report [Laphookhieo *et al*., 2004; Thongdeeying 2005; Razdan *et al*., 1988, $[\alpha]^{25}_{D}$: +61.0° ($CHCl_3$)], compound **TK12** was assigned as lupenone.



Selected HMBC correlation of **TK12**

Table 13 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK12**, **TK11** and lupenone (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK12	**TK11**	**R**	**TK12**	
1	CH_2	38.6	38.7	39.6	0.90 (*m*)[a]	-
2	CH_2	33.1	27.4	34.1	2.49 (*m*)[a]	-
3	C	217.0	79.0	217.9	-	-
4	C	46.3	38.9	47.2	-	-
5	CH	54.3	55.3	55.8	1.32 (*m*)[a]	-
6	CH_2	18.7	18.3	19.6	1.45 (*m*)[a]	-
7	CH_2	32.6	34.3	33.5	0.87 (*m*), 1.45 (*m*)[a]	-
8	C	39.8	40.8	40.7	-	-
9	CH	48.8	50.5	49.7	1.38 (*m*)[a]	-
10	C	35.9	37.2	36.8	-	-
11	CH_2	20.5	20.9	21.4	1.30 (*m*)[a]	-
12	CH_2	24.2	25.2	25.1	1.68 (*m*)[a]	-
13	CH	37.2	38.1	38.1	1.68 (*m*)[a]	-

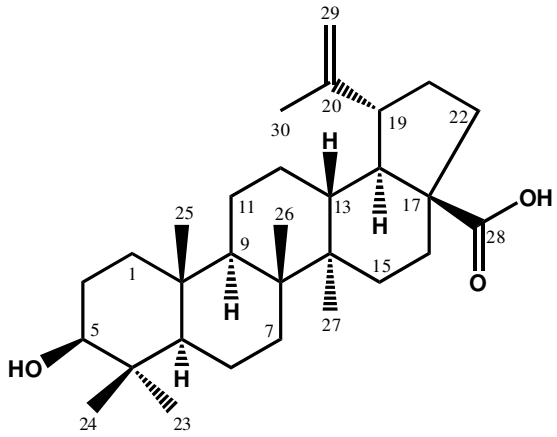
Table 13 (continued)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK12	**TK11**	**R**	**TK12**	
14	C	41.9	42.8	42.7	-	-
15	CH_2	26.4	27.5	27.4	0.82 (*m*)[a]	-
16	CH_2	34.5	35.6	35.6	1.37 (*m*), 1.50 (*m*)[a]	-
17	C	42.0	43.0	42.7	-	-
18	CH	47.3	48.3	48.2	1.38 (*m*)[a]	-
19	CH	47.0	48.0	47.8	2.40 (*m*)[a]	18, 20, 21, 29, 30
20	C	149.8	151.0	150.5	-	-
21	CH_2	28.8	29.9	29.8	1.26 (*m*), 1.92 (*m*)[a]	-
22	CH_2	39.0	40.0	39.9	1.19 (*m*), 1.41 (*m*)[a]	-
23	CH_3	25.7	28.0	26.6	1.07 (*s*)	3, 4, 5, 24
24	CH_3	20.0	15.4	21.0	1.02 (*s*)	3, 4, 5, 23
25	CH_3	15.0	16.1	15.8	0.93 (*s*)	5, 9, 10
26	CH_3	14.8	16.0	15.4	1.07 (*s*)	7, 8, 9, 14
27	CH_3	13.5	14.6	14.4	0.96 (*s*)	14, 15
28	CH_3	17.0	18.0	18.0	0.80 (*s*)	17, 18, 22
29	CH_2	108.1	109.3	109.2	4.57 (*m*), 4.69 (*d*, *J* = 2.1 Hz)	19, 30
30	CH_3	18.3	19.3	19.2.	1.68 (*s*)	19, 20, 29

* For **TK12**

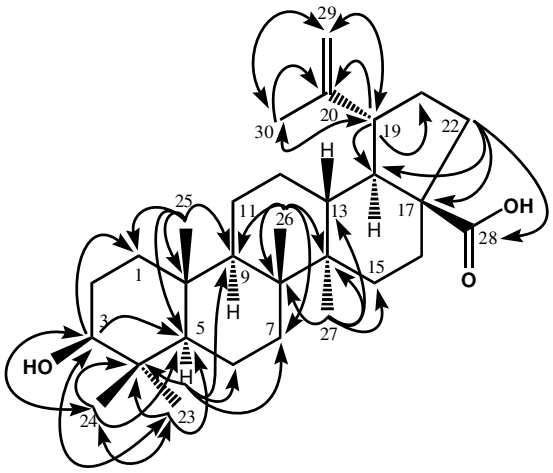
[a] Deduced from HMQC experiment

3.1.13 Compound TK13



Compound **TK13** was obtained as a white solid, mp. 279-280 °C; $[\alpha]^{28}_{D}$: +15.0° (*c* = 0.10, $CHCl_3$). It exhibited hydroxyl (3415 cm^{-1}) and a carboxyl (1686 cm^{-1}) absorptions in the IR spectrum (**Figure 81**). It also gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^{1}H and ^{13}C NMR spectral data of **TK13** (**Table 14, Figures 82** and **83**) were similar to those of **TK11** (**Table12, Figures 72** and **73**). The difference in the spectrum of **TK13** was shown as disappearance of a methyl signal at δ_H 0.79 (*s*, H_3-28, δ_C 18.0) in **TK11** and the appearance of a carboxyl signal at δ_C 179.6 (C-28) in **TK13**. The location of the carboxyl group was confirmed by HMBC experiment (**Table 14**) in which the methylene proton signals at δ 1.93 (1H, *m*, H-22a) and 1.40 (1H, *m*, H-22b) showed correlation with C-17 (δ 55.3), C-18 (δ 48.3) and C-28 (δ 179.6). Thus on the basis of its spectroscopic data and comparison with the previous report [Tinto *et al*., 1992, $[\alpha]^{28}_{D}$: +6.8° (*c* = 2.00, pyridine); Thongdeeying 2005; Pakhathirathien 2005], compound **TK13** was assigned as betulinic acid.



Selected HMBC correlation of **TK13**

Table 14 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK13**, **TK11** and betulinic acid (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK13	**TK11**	**R**	**TK13**	
1	CH_2	37.7	38.7	38.5	0.87 (*m*), 1.64 (*m*)[a]	-
2	CH_2	26.4	27.4	28.2	1.55 (*m*)[a]	-
3	CH	78.0	79.0	78.1	3.19 (*dd*, *J* = 10.8, 5.4 Hz)	1, , 23, 24
4	C	37.9	38.9	39.4	-	-
5	CH	54.4	55.3	55.9	0.69 (*m*)[a]	4, 6, 7, 9
6	CH_2	17.3	18.3	18.7	1.35 (*m*), 1.48 (*m*)[a]	-
7	CH_2	33.3	34.3	34.7	1.35 (*m*)[a]	-
8	C	39.7	40.8	41.0	-	-
9	CH	49.5	50.5	50.9	1.20 (*m*)[a]	-
10	C	36.2	37.2	37.5	-	-
11	CH_2	19.8	20.9	21.1	1.42 (*m*)[a]	-
12	CH_2	24.5	25.2	26.0	1.67 (*m*)[a]	-

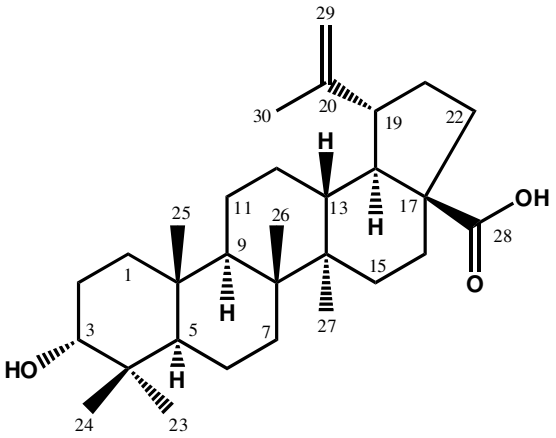
Table 14 (continued)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK13	**TK11**	**R**	**TK13**	
13	CH	37.4	38.1	39.2	2.20 (*m*)[a]	-
14	C	41.4	42.8	42.8	-	-
15	CH_2	28.7	27.5	30.2	1.14 (*m*), 1.23 (*m*)[a]	-
16	CH_2	31.2	35.6	32.8	2.22 (*m*)[a]	-
17	C	55.3	43.0	56.6	-	-
18	CH	48.3	48.3	49.7	1.55 (*m*)[a]	-
19	CH	45.9	48.0	47.7	3.00 (*m*)[a]	18, 20, 21, 29,30
20	C	149.4	151.0	151.4	-	-
21	CH_2	29.6	29.9	31.1	1.89 (*m*)[a]	-
22	CH_2	36.0	40.0	37.4	1.40 (*m*), 1.93, (*m*)[a]	17, 18, 28
23	CH_3	27.0	28.0	28.5	0.97 (*s*)	3, 4, 5, 24
24	CH_3	14.3	15.4	16.2	0.75 (*s*)	3, 4, 5, 23
25	CH_3	15.1	16.1	16.3	0.82 (*s*)	1, 5, 9,10
26	CH_3	15.0	16.0	16.2	0.94 (*s*)	7, 8, 9, 14
27	CH_3	13.7	14.6	14.8	0.98 (*s*)	8, 13,14, 15
28	COO	179.6	18.0	18.0	-	-
29	CH_2	108.7	109.3	109.2	4.74 (*brs*), 4.61 (*brs*)	19, 20, 30
30	CH_3	18.4	19.3	19.2	1.69 (*s*)	19, 20, 29

* For **TK13**

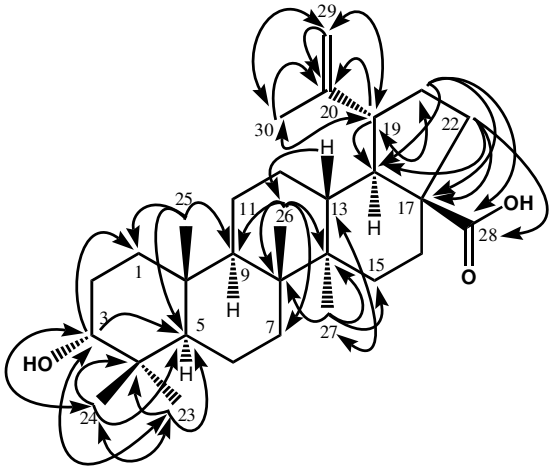
[a] Deduced from HMQC experiment

3.1.14 Compound TK14



Compound **TK14** was obtained as a white solid, mp. 257-259 °C; $[\alpha]^{28}_{D}$: -10.0° (*c* = 0.05, $CHCl_3$). The IR spectrum showed absorption bands similar to those of compound **TK13**. It gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^{1}H and ^{13}C NMR spectral data of **TK14** (**Table 15, Figures 88** and **89**) were similar to those of **TK13** (**Table 14, Figures 82** and **83**), except that the splitting pattern of H-3 in **TK14** at δ 3.38 was a triplet (*J* = 2.7 Hz) instead of a doublet of doublet (*J* = 10.8, 5.4 Hz) in **TK13**. The difference in the multiplicity with a small coupling constant of H-3 in compound **TK14** was in agreement with the respective coupling pattern (equatorial-equatorial and equatorial-axial) of H-3 and H-2, indicating that H-3 was situated in an equatorial position. The location of a hydroxyl group at C-3 was determined through an HMBC experiment (**Table 15**) in which the oxymethine proton signal at δ 3.38 (H-3) showed long-rang correlation with C-1 (δ 33.2) and C-5 (δ 49.0). Thus on the basis of its spectroscopic data and comparison with the previous report [Laphookhieo *et al*., 2004; Kitajima *et al*., 1990, $[\alpha]^{28}_{D}$: -12.0° (*c* = 1.28, $CHCl_3$); [Pakhathirathien, 2005], compound **TK14** was assigned as 3-*epi*-betulinic acid, an epimer of betulinic acid.



Selected HMBC correlation of **TK14**

Table 15 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK14**, **TK13** and 3-*epi*-betulinic acid (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK14	**TK13**	**R**	**TK14**	
1	CH_2	33.2	37.7	34.0	1.18 (*m*)[a]	-
2	CH_2	25.5	26.4	23.2	1.02 (*m*), 1.68 (*m*)[a]	-
3	CH	76.2	78.0	75.5	3.38 (*t*, *J* = 2.7 Hz)	1, 5, 23, 24
4	C	37.5	37.9	39.0	-	-
5	CH	49.0	54.4	49.3	1.18 (*m*)[a]	-
6	CH_2	18.2	17.3	18.6	1.34 (*m*), 1.38 (*m*)[a]	-
7	CH_2	34.1	33.3	34.8	1.30 (*m*)[a]	-
8	C	40.8	39.7	41.3	-	-
9	CH	50.3	49.5	50.7	1.40 (*m*)[a]	-
10	C	37.3	36.2	37.7	-	-
11	CH_2	20.7	19.8	21.0	1.42 (*m*)[a]	-
12	CH_2	25.3	24.5	26.1	1.52 (*m*), 1.82 (*m*)[a]	-

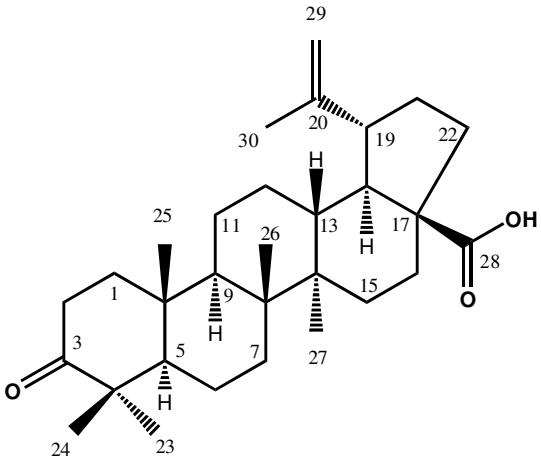
Table 15 (continued)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK14	**TK13**	**R**	**TK14**	
13	CH	38.2	37.4	38.5	2.21 (*m*)[a]	26, 27
14	C	42.5	41.4	42.9	-	-
15	CH_2	29.6	28.7	31.2	1.14 (*m*)[a]	-
16	CH_2	32.2	31.2	32.8	2.24 (*m*)[a]	-
17	C	56.2	55.3	56.6	-	-
18	CH	49.2	48.3	47.7	1.57 (*m*)[a]	-
19	CH	47.0	45.9	49.7	3.00 (*m*)[a]	-
20	C	150.7	149.4	151.2	-	-
21	CH_2	30.6	29.6	29.9	1.93 (*m*)[a]	17, 18, 19, 28
22	CH_2	37.1	36.0	37.5	1.95 (*m*)[a]	17, 18, 28
23	CH_3	28.2	27.0	29.2	0.93 (*s*)	3, 4, 5, 24
24	CH_3	22.1	14.3	22.5	0.82 (*s*)	3, 4, 5, 23
25	CH_3	15.9	15.1	16.4	0.94 (*s*)	1, 5, 9
26	CH_3	15.9	15.0	16.4	0.83 (*s*)	7, 8, 9, 14
27	CH_3	14.7	13.7	14.9	0.99 (*s*)	8, 13,14, 15
28	COOH	179.2	179.6	178.7	-	-
29	CH_2	109.5	108.7	109.8	4.73 (*d*, *J* = 1.8 Hz), 4.60 (*m*)	19, 20, 30
30	CH_3	19.3	18.4	19.4	1.69 (*s*)	19, 20, 29

* For **TK14**

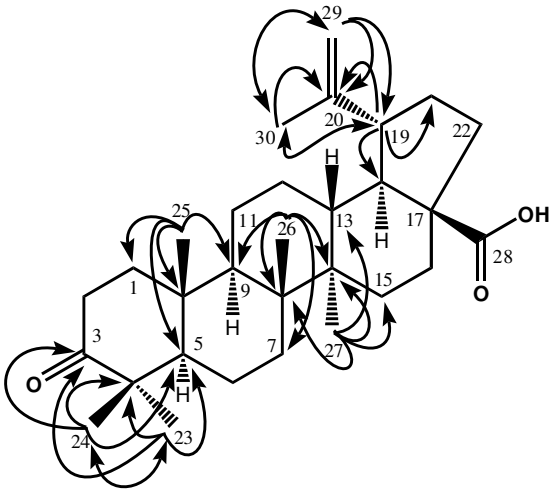
[a] Deduced from HMQC experiment

3.1.15 Compound TK15



Compound **TK15** was obtained as a white solid, mp. 250-254 °C; $[\alpha]^{28}_{D}$: +32.0° (*c* = 0.37, MeOH). It exhibited hydroxyl (3326 cm^{-1}), a carboxyl (1704 cm^{-1}) and double bond (1642 cm^{-1}) absorption in the IR spectrum. It gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^{1}H and ^{13}C NMR spectral data of **TK15** (**Table 16, Figures 90** and **91**) were closely related to compound **TK13** (**Table 14, Figures 82** and **83**), except the oxymethine proton (H-3) at δ 3.19 (*dd*, *J* = 10.8, 5.4 Hz) in **TK13** disappeared and the methylene protons (H_2-2) in **TK15** were shifted downfield to δ 2.45 (*m*) as compared to that of **TK13** at δ 1.55 (*m*). The ^{13}C NMR spectral data of **TK15** displayed a signal of a carbonyl group at δ 218.3 which was assigned to C-3 and no signal of oxymethine carbon C-3 (δ 78.0) as observed in **TK13**. The location of the carbonyl group was confirmed by HMBC experiment (**Table 16**) in which both H_3-24 (δ 1.02) and H_3-23 (δ 1.07) showed long-range correlation with C-3 (δ 218.3), C-4 (δ 47.3) and C-5 (δ 54.9). Thus on the basis of its spectroscopic data and comparison with the previous report [Pakhathirathien 2005; Gonzalez *et al*., 1983, $[\alpha]^{27}_{D}$: +27.0° (*c* = 0.28, MeOH)], compound **TK15** was assigned as betulonic acid.



Selected HMBC correlation of **TK15**

Table 16 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK15** and **TK13**

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK15	**TK13**	**TK15**	
1	CH_2	39.6	37.7	-	-
2	CH_2	34.1	26.4	2.45 (*m*)[a]	-
3	CH	218.3	78.0	-	-
4	C	47.3	37.9	-	-
5	CH	54.9	54.4	1.24 (*m*)[a]	-
6	CH_2	19.6	17.3	-	-
7	CH_2	33.6	33.3	-	-
8	C	40.6	39.7	-	-
9	CH	49.8	49.5	1.35 (*m*)[a]	-
10	C	36.9	36.2	-	-
11	CH_2	21.4	19.8	-	-

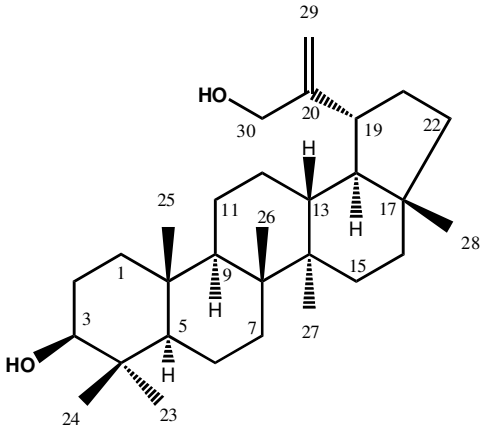
Table 16 (continued)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK15	**TK13**	**TK15**	
12	CH_2	25.5	24.5	-	-
13	CH	38.5	37.4	2.20 (*m*)[a]	-
14	C	42.5	41.4	-	-
15	CH_2	30.6	28.7	-	-
16	CH_2	32.1	31.2	-	-
17	C	56.4	55.3	-	-
18	CH	49.2	48.3	1.62 (*m*)[a]	-
19	CH	46.9	45.9	3.01 (*m*)[a]	18, 20, 21,30
20	C	150.3	149.4	-	-
21	CH_2	29.7	29.6	-	-
22	CH_2	37.0	36.0	-	-
23	CH_3	26.6	27.0	1.07 (*s*)	3, 4, 5, 24
24	CH_3	21.0	14.3	1.02 (*s*)	3, 4, 5, 23
25	CH_3	16.0	15.1	0.93 (*s*)	1, 5, 9,10
26	CH_3	15.8	15.0	0.98 (*s*)	7, 8, 9, 14
27	CH_3	14.6	13.7	0.99 (*s*)	8, 13,14, 15
28	CH_3	182.8	179.6	-	-
29	CH_2	109.8	108.7	4.74 (*brs*), 4.62 (*brs*)	19, 20, 30
30	CH_3	19.4	18.4	1.70 (*s*)	19, 20, 29

* For **TK15**

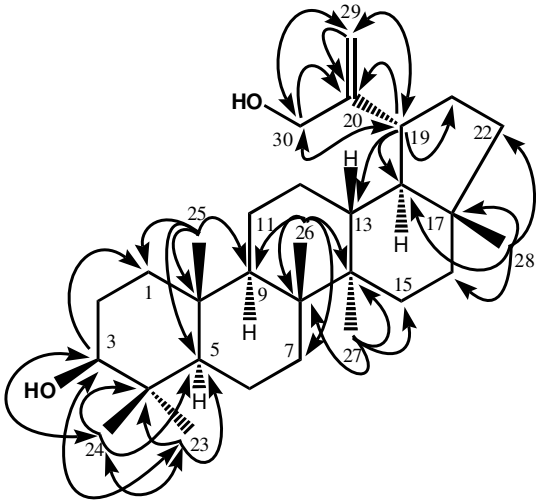
[a] Deduced from HMQC experiment

3.1.16 Compound TK16



Compound **TK16** was obtained as a white solid, mp. 203-204 °C; $[\alpha]^{28}_{D}$: -13.3° (*c* = 0.22, $CHCl_3$). The IR spectrum showed absorption bands similar to those of **TK11**. It gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^{1}H and ^{13}C NMR spectral data of **TK16** (**Table 17, Figures 92** and **93**) and **TK11** (**Table 12, Figures 73**, **74** and **75**) exhibited the same pattern, except that compound **TK16** 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 H_3-30 at δ 1.68 (*s*). The two signals of terminal olefinic protons of H_2-29 [δ 4.93 (*brs*) and 4.90 (*brs*)] were shown to be shifted more downfield than **TK11** (δ 4.56 and 4.68). In addition, the AB system of oxymethylene protons was shown at δ 4.14 and δ 4.09 with coupling constant 15.3 Hz which was assigned to H_2-30. Based on HMBC experiments (**Table 17**), the oxymethylene protons H_2-30 showed correlations with C-19 (δ 43.8), C-20 (δ 154.8) and C-29 (δ 106.8). Thus on the basis of its spectroscopic data and comparison with the previous report [Burns *et al.*, 2000, $[\alpha]^{25}_{D}$: -13.0° (*c* = 0.22, $CHCl_3$); Thongdeeying 2005], compound **TK16** was assigned as lup-20(29)-en-3β, 30-diol.



Selected HMBC correlation of **TK16**

Table 17 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK16**, **TK11** and lup-20(29)-en-3β, 30-diol (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK16	**TK11**	**R**	**TK16**	
1	CH_2	38.7	38.7	38.7	1.64 (*m*)[a]	-
2	CH_2	27.4	27.4	27.4	1.58 (*m*)[a]	-
3	CH	79.0	79.0	79.0	3.19 (*dd*, *J* = 10.8, 5.1 Hz)	1, 23, 24
4	C	38.9	38.9	38.9	-	-
5	CH	55.3	55.3	55.3	0.68 (*m*)[a]	-
6	CH_2	18.3	18.3	18.3	1.41 (*m*), 1.55 (*m*)[a]	-
7	CH_2	34.3	34.3	34.3	1.40 (*m*)[a]	-
8	C	40.9	40.8	40.9	-	-
9	CH	50.4	50.5	50.4	1.25 (*m*)[a]	-
10	C	37.2	37.2	37.2	-	-

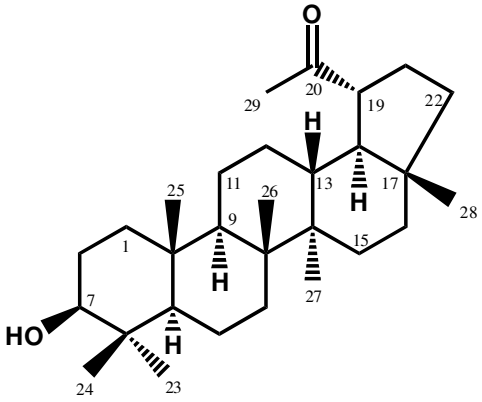
Table 17 (continued)

Position	Type of C*	δ_C /ppm			δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK16	**TK11**	**R**	**TK16**	
11	CH_2	21.1	20.9	21.0	1.25 (*m*), 1.44 (*m*)[a]	-
12	CH_2	26.7	25.2	26.7	1.65 (*m*)[a]	-
13	CH	38.0	38.1	38.0	1.71 (*m*)[a]	-
14	C	42.8	42.8	42.8	-	-
15	CH_2	27.4	27.5	27.4	1.62 (*m*)[a]	-
16	CH_2	35.5	35.6	35.5	1.55 (*m*)[a]	-
17	C	43.0	43.0	43.0	-	-
18	CH	48.9	48.3	48.9	1.46 (*m*)[a]	-
19	CH	43.8	48.0	43.8	2.28 (*dt*, *J* = 10.8, 4.8 Hz)	18, 20, 21,30
20	C	154.8	151.0	154.8	-	-
21	CH_2	31.8	29.9	31.8	2.06 (*m*)[a]	-
22	CH_2	39.9	40.0	39.9	1.24 (*m*), 1.41 (*m*)[a]	-
23	CH_3	28.0	28.0	28.0	0.97 (*s*)	3, 4, 5, 24
24	CH_3	15.4	15.4	15.4	0.76 (*s*)	3, 4, 5, 23
25	CH_3	16.1	16.1	16.1	0.83 (*s*)	1, 5, 9,10
26	CH_3	16.0	16.0	16.0	1.03 (*s*)	7, 8, 9, 14
27	CH_3	14.5	14.6	14.5	0.94 (*s*)	8,14, 15
28	CH_3	17.7	18.0	17.7	0.78 (*s*)	16, 17, 18,22
29	CH_2	106.8	109.3	106.8	4.90 (*brs*), 4.93 (*brs*)	19, 20, 30
30	CH_2	65.0	19.3	65.0	4.09 (*d*, *J* = 15.3 Hz) 4.14 (*d*, *J* = 15.3 Hz)	19, 20, 29

* For **TK16**

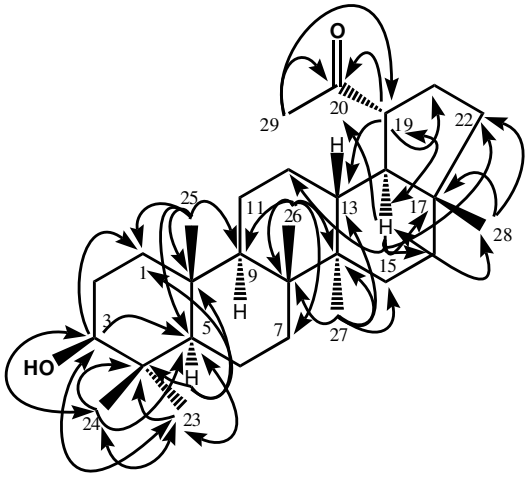
[a] Deduced from HMQC experiment

3.1.17 Compound TK17



Compound **TK17** was assigned as a white solid, mp. 234-235 °C; $[\alpha]^{28}_{D}$: -22.7° (*c* = 0.22, $CHCl_3$). It exhibited hydroxyl (3414 cm^{-1}) and carbonyl (1694 cm^{-1}) absorptions in the IR spectrum. It gave a blue vanillin-sulfuric acid test indicating a triterpene.

The ^{1}H and ^{13}C NMR spectral data of **TK17** (**Table 18, Figures 94** and **95**) and **TK11** (**Table12, Figures 73**, **74** and **75**) exhibited the same pattern, except that the two signals of terminal olefinic protons of H_2-29 at δ 4.68 (*d*, *J* = 2.1) and 4.56 (*m*) and vinylic methyl at δ 1.68 disappeared in **TK17**, whereas a singlet signal of acetyl protons was shown at δ 2.15 (H_3-29, *s*) which was not observed in **TK11**. In addition, the ^{13}C NMR spectral data showed carbonyl carbon at δ 212.9. The location of acetyl protons was assigned to be at C-29 on the basis of HMBC experiment (**Table 18**) of the protons at δ 2.15 (H_3-29) which showed long-range correlations with δ 52.6 (C-19) and δ 212.9 (C-20). Thus on the basis of its spectroscopic data and comparison with the previous report [Thongdeeying 2005; Koul *et al*., 2000, $[\alpha]^{25}_{D}$: -10.2° (*c* = 0.03, $CHCl_3$)], compound **TK17** was assigned as 30-nor-lupan-3β-ol-20-one.



Selected HMBC correlation of **TK17**

Table 18 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **TK17** ($CDCl_3$), **TK11** and 30-nor-lupan-3β-ol-20-one (**R**, $CDCl_3$)

Position	Type of C*	δ_C/ppm			δ_H/ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK17	**TK11**	**R**	**TK17**	
1	CH_2	38.7	38.7	39.2	0.89 (*m*),1.67 (*m*)[a]	-
2	CH_2	27.4	27.4	25.2	1.49 (*m*), 1.57 (*m*)[a]	-
3	CH	78.9	79.0	76.3	3.19 (*dd*, *J* = 11.1, 5.1 Hz)	1, 23, 24
4	C	38.9	38.9	38.4	-	-
5	CH	55.3	55.3	55.2	0.68 (*m*)[a]	1, 4, 10,23
6	CH_2	18.3	18.3	18.1	1.40 (*m*), 1.55 (*m*)[a]	-
7	CH_2	34.2	34.3	34.2	1.40 (*m*)[a]	-
8	C	40.7	40.8	41.1	-	-
9	CH	50.3	50.5	50.1	1.28 (*m*)[a]	-
10	C	37.2	37.2	36.3	-	-
11	CH_2	20.9	20.9	22.6	1.28 (*m*), 1.46 (*m*)[a]	-
12	CH_2	27.2	25.2	28.7	1.06 (*m*)[a]	-

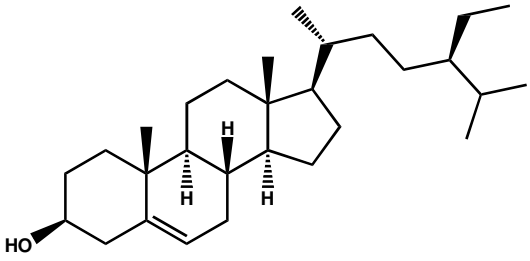
Table 18 (continued)

Position	Type of C*	δ_C/ppm			δ_H/ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK17	**TK11**	**R**	**TK17**	
13	CH	37.0	38.1	37.5	1.59 (*m*)[a]	-
14	C	42.7	42.8	43.6	-	-
15	CH_2	27.3	27.5	27.4	1.64 (*m*), 1.70 (*m*)[a]	-
16	CH_2	35.0	35.6	35.5	1.49 (*m*)[a]	-
17	C	43.1	43.0	42.9	-	-
18	CH	49.7	48.3	48.2	1.81 (*t*, *J* = 11.4 Hz)	12, 16, 17, 19, 20, 22, 28
19	CH	52.6	48.0	47.9	2.58 (*dt*, *J* = 11.4, 5.7 Hz)	13, 18, 20, 21
20	C	212.9	151.0	207.3	-	-
21	CH_2	27.6	29.9	31.0	2.05 (*m*)[a]	-
22	CH_2	39.9	40.0	40.1	1.35 (*m*), 1.49 (*m*)[a]	-
23	CH_3	28.0	28.0	28.5	0.97 (*s*)	3, 4, 5, 24
24	CH_3	15.4	15.4	15.4	0.76 (*s*)	3, 4, 5, 23
25	CH_3	15.9	16.1	16.2	0.82 (*s*)	1, 5, 9, 10
26	CH_3	16.1	16.0	15.9	1.01 (*s*)	7, 8, 9, 14
27	CH_3	14.5	14.6	14.5	0.97 (*s*)	8, 13,14, 15
28	CH_3	18.0	18.0	18.4	0.77 (*s*)	16, 17, 18, 22
29	CH_3	29.2	109.3	23.5	2.15 (*s*)	19, 20
30	-	-	19.3	-	-	-

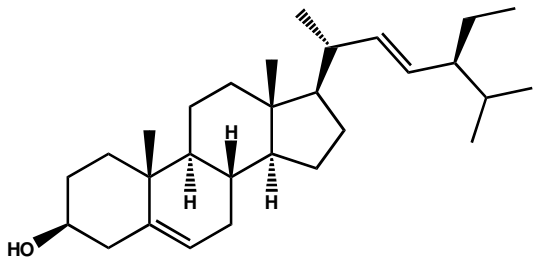
* For **TK17**

[a] Deduced from HMQC experiment

3.1.18 Compounds **TK18** and **TK19**



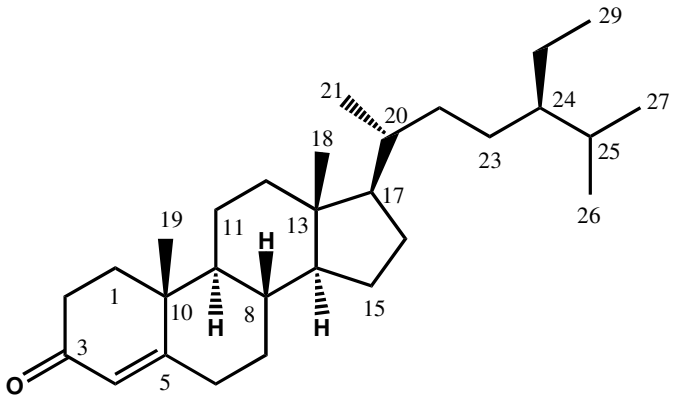
TK18



TK19

The mixture of **TK18** and **TK19** was isolated as a white solid. Its IR spectrum showed absorption bands at 3425 (hydroxyl) and 1642 cm^{-1} (double bond). The ^1H NMR (**Figure 40**) spectral data contained an oxymethine proton at δ 3.57-3.47 (*m*), three olefinic protons at δ 5.36-5.34 (*d*, *J* = 5.1 Hz), 5.16 (*dd*, *J* = 15.1, 8.4 Hz) and 5.01 (*dd*, *J* = 15.1, 8.4 Hz). The ^1H NMR data was corresponded to previous reported data of β-sitosterol and stigmasterol. Thus, this mixture was identified as β-sitosterol (**TK18**) and stigmasterol (**TK19**) (Cheenpracha, 2004).

3.1.19 Compound TK20

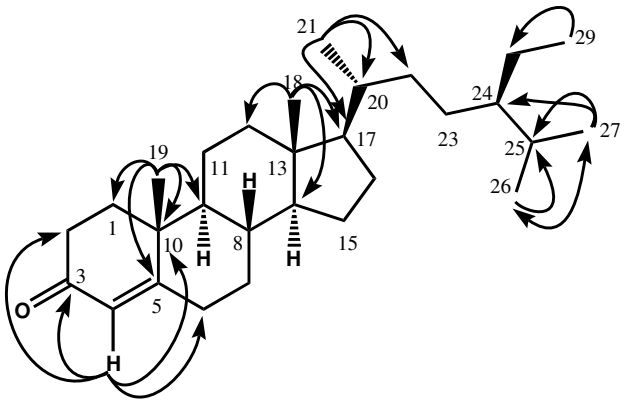


Compound **TK20** was isolated as colorless viscous oil, $[\alpha]^{28}_{D}$: +66.4° (*c* = 0.40, $CHCl_3$). Its IR spectrum showed absorption bands for α, β- unsaturated carboxyl group at (1674 cm^{-1}) and double bond (1616 cm^{-1}) (**Figure 98**). The UV absorption was shown at 241 nm (**Figure 97**).

The ^{13}C NMR and DEPT spectral data of **TK20** (**Table 19**, **Figure 100**) showed all 29 carbon signals, six methyl (δ 11.9, 12.0, 17.4, 18.7, 19.0 and 19.8), eleven methylene (δ 21.0, 23.1, 24.2, 26.1, 28.2, 32.1, 32.9, 33.9, 34.0, 35.7 and 39.6), eight methine (δ 29.2, 35.6, 36.1, 45.8, 53.8, 55.9, 56.1 and 123.7) and four quaternary carbons (δ 38.6, 42.4, 171.6 and 199.6).

The ^{1}H NMR spectral data of **TK20** (**Table 19**, **Figure 99**) and the mixture of **TK18** and **TK19** (**Figure 96**) exhibited the same pattern, except that an oxymethine proton signal between δ 3.57-3.47 in **TK18** and **TK19** was not evidenced in **TK20** and **TK20** displayed a more downfield olefinic proton at δ 5.72 (H-4). The ^{13}C NMR spectrum confirmed the presence of a carbon - carbon double bond at δ 123.7 (C-4) and 171.6 (C-5) and the downfield chemical shift of C-5 (δ 171.6) also indicated the presence of the conjugated carbonyl function. On the basis of HMBC (**Table 19**) the olefinic proton H-4 (δ 5.72) showed correlation with C-2 (δ 33.9), C-3 (δ 199.6), C-6 (δ 32.9) and C-10 (δ 38.6) suggesting the presence of a double bond between C-4 and C-5 and a carbonyl carbon at C-3. On the basis of its spectroscopic

data and comparison with previously reported data (Daengrot, 2006; Della Greca *et al*., 1990), compound **TK20** was identified as stigmast-4-en-3-one.



Selected HMBC correlation of **TK20**

Table 19 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **TK20** and stigmast-4-en-3-one (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC*
		TK19	**R**	**TK19**	$^{1}H \rightarrow ^{13}C$
1	CH_2	35.7	35.7	1.54 (*m*), 1.67 (*m*)	-
2	CH_2	33.9	33.9	2.28 (*m*), 2.20 (*m*)	-
3	C	199.6	198.9	-	-
4	CH	123.7	123.6	5.72 (*brs*)	2, 3, 6, 10
5	C	171.6	171.0	-	-
6	CH_2	32.9	32.9	2.25 (*m*), 2.40 (*m*)	-
7	CH_2	32.1	32.1	1.01 (*m*), 1.85 (*m*)	-
8	CH	35.6	35.7	1.71 (*m*)	-
9	CH	53.8	53.8	0.92 (*m*)	-
10	C	38.6	38.6	-	-
11	CH_2	21.0	21.0	1.40 (*m*), 1.50 (*m*)	-
12	CH_2	39.6	39.5	1.15 (*m*), 2.04 (*m*)	-

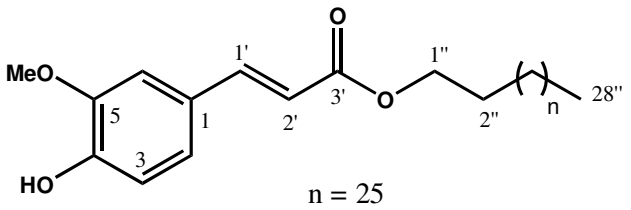
Table 19 (continued)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^1H \rightarrow ^{13}C$
		TK19	**R**	**TK19**	
13	C	42.4	42.4	-	-
14	CH	55.9	55.9	1.00 (*m*)	-
15	CH_2	24.2	24.1	1.23 (*m*), 1.29 (*m*)	-
16	CH_2	28.2	28.1	1.27 (*m*), 1.32 (*m*)	-
17	CH	56.1	56.1	1.11 (*m*)	-
18	CH_3	12.0	12.0	0.71 (*m*)	12, 14, 17
19	CH_3	17.4	17.4	1.18 (*m*)	1, 5, 9, 10
20	CH	36.1	36.1	2.01 (*m*)	-
21	CH_3	18.7	18.7	0.92 (*d*, *J* = 6.3 Hz)	17, 20, 22
22	CH_2	34.0	34.0	2.39 (*m*)	-
23	CH_2	26.1	26.0	1.17 (*m*)	-
24	CH	45.8	45.8	0.93 (*m*)	-
25	CH	29.2	29.1	1.26 (*m*)	-
26	CH_3	19.8	19.8	0.85 (*d*, *J* = 6.9 Hz)	24, 25, 27
27	CH_3	19.0	19.2	0.84 (*d*, *J* = 6.6 Hz)	24, 25, 26
28	CH_2	23.1	23.1	1.29 (*m*)	-
29	CH_3	11.9	11.4	0.83 (*d*, *J* = 6.6 Hz)	24, 28

* For **TK20**

[a] Deduced from HMQC experiment

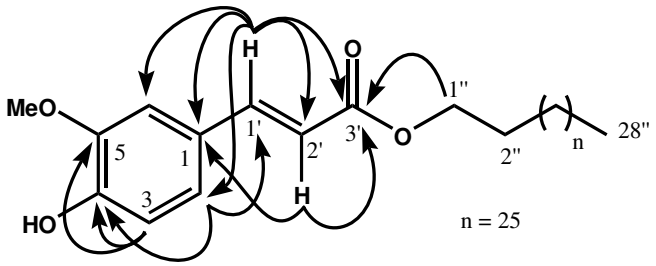
3.1.20 Compound TK21



Compound **TK21** was isolated as a colorless viscous oil, It exhibited hydroxyl (3375 cm^{-1}), conjugate ester (1695 cm^{-1}) and double bond (1635 cm^{-1}) absorptions in the IR spectrum. The UV spectrum showed absorption bands at λ_{max} : 234, 297 and 325 nm (**Figure 105**), again suggesting the presence of conjugation in the molecule. Its molecular formula, $C_{38}H_{66}O_4$ ($[M]^+$ 586.6, calcd 586.5), was deduced by EI mass spectrum.

In the ^1H NMR spectral data of **TK21** (**Table 20**, **Figure 106**), the presence of a *trans* double bond was evidenced by two doublet signals at δ 6.30 and 7.60 ppm with a coupling constant of 16.2 Hz. ^1H NMR signals at δ 6.93 (*d*, *J* = 8.1 Hz), δ 7.06 (*dd*, *J* = 8.1 and 2.1 Hz) and δ 7.03 (*d*, *J* = 2.1 Hz) established the presence of three aromatic protons with *ortho*, *ortho*/*meta* and *meta* coupling, respectively. The presence of one methoxyl group was also shown by a three-proton singlet at δ 3.92 ppm. Furthermore, the calculated MW of 586.5 was in agreement with molecular formula, $C_{38}H_{66}O_4$ as deduced by EI mass spectrum. The ^1H NMR spectrum showed signal of methylene protons at δ 4.20 (H_2-1″), a triplet at δ 0.89 (H_3-28″) and a broad signal at δ 1.12-1.14 which could be deduced from molecular formula to be those of 50H. Therefore, compound **TK21** should be a long chain ester of ferulic acid. The ^{13}C NMR spectral data of **TK21** (**Table 20**, **Figure 107**) showed signals at δ 167.3 (C-3′) due to the carbonyl group of an ester function and δ 144.6 (C-1′) and δ 115.7 (C-2′) due to a side chain C-C double bond. Further confirmation of this skeleton came from the mass spectrum of **TK21** which showed, besides the molecular ion, significant fragment peaks at m/z 177 and 194, both characteristic of a methoxy and hydroxyl substituted cinamic moiety. HMBC correlations were

summarized in **Table 20.** On the basis of its spectroscopic data and comparison with previously reported data (Ruan et *al*., 2007), compound **TK21** was identified as erythrinassinate A.



Selected HMBC correlation of **TK21**

Table 20 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **TK21** and erythrinassinate A (**R**, $CDCl_3$)

Position	Type of C*	δ_C /ppm		δ_H /ppm (multiplicity, *J*/Hz)	HMBC* $^{1}H \rightarrow ^{13}C$
		TK21	**R**	**TK21**	
1	C	127.0	127.0	-	-
2	CH	123.4	123.0	7.06 (*dd,* 8.1, 2.1 Hz)	1', 4
3	CH	114.6	114.6	6.93 (*d*, 8.1 Hz)	4, 5
4	C	147.8	147.8	-	-
5	C	146.7	146.7	-	-
6	CH	109.2	109.2	7.03 (*d*, 2.1 Hz)	-
1'	CH	144.6	144.6	7.60 (*d*, 16.2 Hz)	1, 2, 6, 2', 3'
2'	CH	115.7	115.7	6.30 (*d*, 16.2 Hz)	1, 3'
3'	COO	167.3	167.3	-	-
1"	CH_2	64.2	64.6	4.20 (*t*, 6.6 Hz)	3'
2"	CH_2	28.7	31.9	1.70 (*m*)	-
28"	CH_3	14.1	14.0	0.89 (*t*, 6.3 Hz)	-
OMe	CH_3	55.9	55.9	3.92 (*s*)	-

* For **TK21**