CHAPTER IV

RESULTS AND DISCUSSION

Three compounds were isolated from the root of *Siphonodon celastrineus* Griff. The identification and structure elucidation of the isolated compound were established by analysis of the spectroscopic data (UV , IR , MS, and NMR) , as well as comparison with the data of other related compounds.

1. Structure elucidation of compound SC1

Compound SC1 was obtained as colorless needles. Its IR spectrum (Figure 7, page 86) suggested the presence of hydroxyl (3468 cm^{-1}), and carbonyl (1716 cm^{-1}) group. The mass spectrum (Figure 8, page 87) of compound SC1 exhibited the $[M + 1]^+$ peak at m/z 621 , in agreement with the molecular formula $C_{39}H_{56}O_6$.

The ^1H – NMR spectra showed signals for an aromatic moiety [δ 8.07 (2 H , d, $J = 7.4$ Hz), 7.57 (1 H , t , $J = 7.4$ Hz) and 7.45 (2 H, t , $J = 7.4$ Hz)] , and for two oxygen bearing protons, at δ 4.48 and 6.44. Furthermore , the presence of 9 methyl singlets [$\delta = 2.02$, 1.49 , 1.23 , 1.04 , 1.03 , 0.90 , 0.87 , 0.86 , 0.84] were observed.

The ^{13}C - NMR spectrum revealed the presence of 39 resolved signals in the (Figure 10, page 91 – 92). From DEPT 135 spectrum (Figure 11 , page 93) revealed 9 methylene carbons [δ 40.3 , 39.1 , 38.3 , 34.5 , 34.0 , 30.0 , 23.9 , 22.9 , 17.7] , 11 quaternary carbons [δ 202.2 , 171.2 , 165.6 , 133.2 , 83.1 , 44.8 , 44.1 , 39.3 , 38.4 , 33.5 , 31.4] , 10 methine carbons [δ 133.2 , 130.1 (2 C), 128.6 (2 C) , 80.2 , 75.1 , 55.2 , 54.2 , 49.1] and 9 methyl carbons [δ 32.3 , 31.5 , 28.2 , 24.5 , 21.4 , 20.9 , 18.9 , 16.4 (2 C)]. From 11 methine carbons , the two of them at δ 80.2 and 75.1 were assigned to be oxygenated carbons and the three of them at δ 133.2 , 130.1 and 128.6 were assigned to be the carbons of benzene ring. From 10 quaternary carbon , the signal at δ 202.2 indicated the presence of keto carbonyl and the signals at δ 171.2 and 165.6 implied the presence of two ester carbonyl whereas the signal at δ 83.1 in indicated the presence of an oxygen bearing quaternary carbon in the molecule. In the HMBC spectra , the protons at δ 8.07 and 2.02 showed correlation to both ester carbonyl at δ 165.6 and 171.2 , respectively . This observation suggested the presence of benzoyl and acetoxyl as the

substitution groups. This suggestion were supported by the fragment ion at m/z 77 , 105 , and 121 together with at m/z 43 in the FAB - MS (Scheme 6). Thus , the remaining 30 carbons were assigned to be the carbons of the triterpene skeleton.

The main skeleton of SC1 was determine to be oleanane type since the analysis of overall correlations of its protons , especially , 8 methyl protons to their 30 carbons skeleton were carried out , in HMBC spectra .

The position of benzoyl and acetoxyl groups were proven as at carbon at δ 75.1 and 80.2 , respectively , through the HMBC correlation of the oxymethine proton signal [δ 6.44] to the carbonyl carbon of the benzoyl group [δ 165.6] and of the oxymethine proton signal [δ 4.48] to the carbonyl carbon of acetoxyl group [δ 171.2]. To be agreed with the molecular formula and molecular weight , the hydroxyl group could be assigned at oxygen bearing quaternary carbon at δ 83.1. This suggestion was supported by the presence of fragment ion peak at m/z 208.

The stereochemistry of 3-acetoxyl and 11-benzoyl were determined on the basis of NOE difference spectrum (Figure 15, page 101) and the coupling constant. Irradiation at the resonance frequency of methyl proton at C - 25 and C - 23 produced strong enhancements of methine proton at C - 11 and C - 3 , and *trans* diaxial coupling constant[137] of H - 11(J = 12.6 Hz) and H - 3 (J = 11.8 Hz) suggested the α and β configurations of benzoyl at C - 11 and acetoxyl at C - 3, respectively , and the 13-hydroxyl automatically should be β to minimize the ring strain of the triterpene skeleton.

The structure of compound SC1 could be elucidated as , 3β- acetoxy-11α-benzoyl-13β-hydroxyolean-12-one, and was named as siphonodone (36), a new naturally occurring oleanane triterpene , which its structure was very similar to 3β-acetoxy-11α,13β-dihydroxyolean-12-one (37) and 3β,11α-diacetoxy-13β-hydroxyolean-12-one (38), two oleanane triterpenes from *Gordonia ceylanica* in family Theaceae.[138] The ^{13}C - NMR data for (36), (37) and (38) were summarized in Table 6.
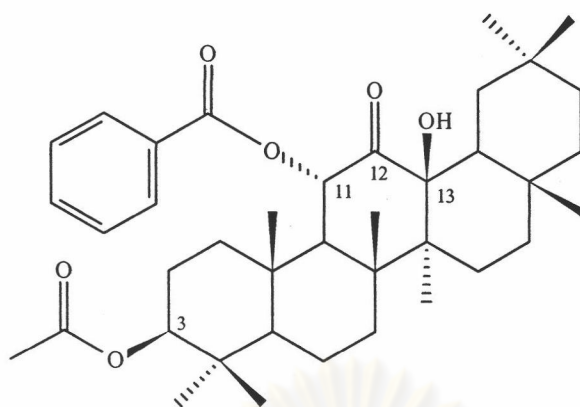
(36)
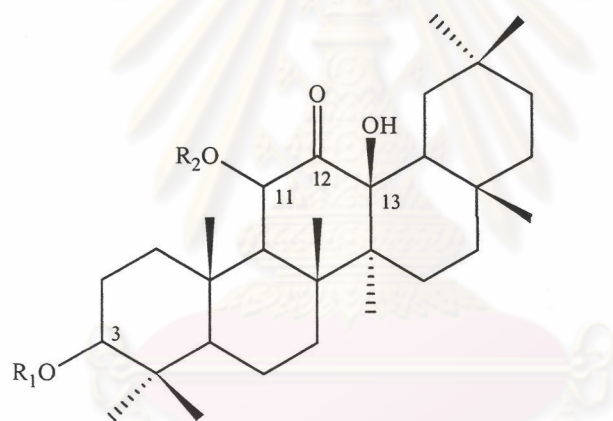
(37) $R_1 = COCH_3$, $R_2 = H$

(38) $R_1 = R_2 = COCH_3$
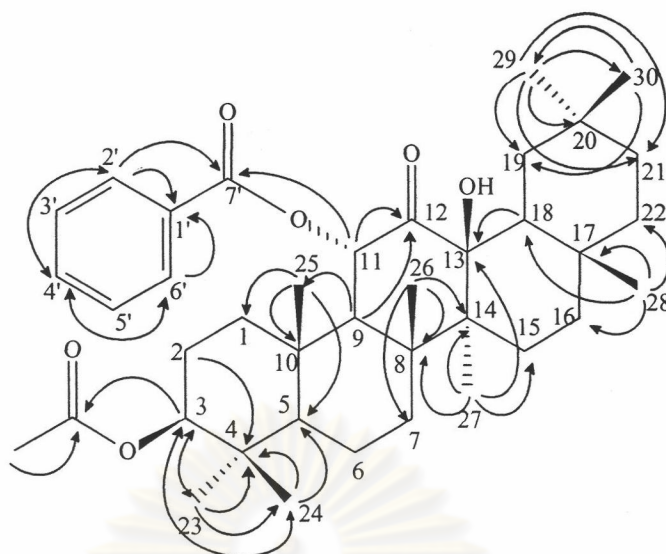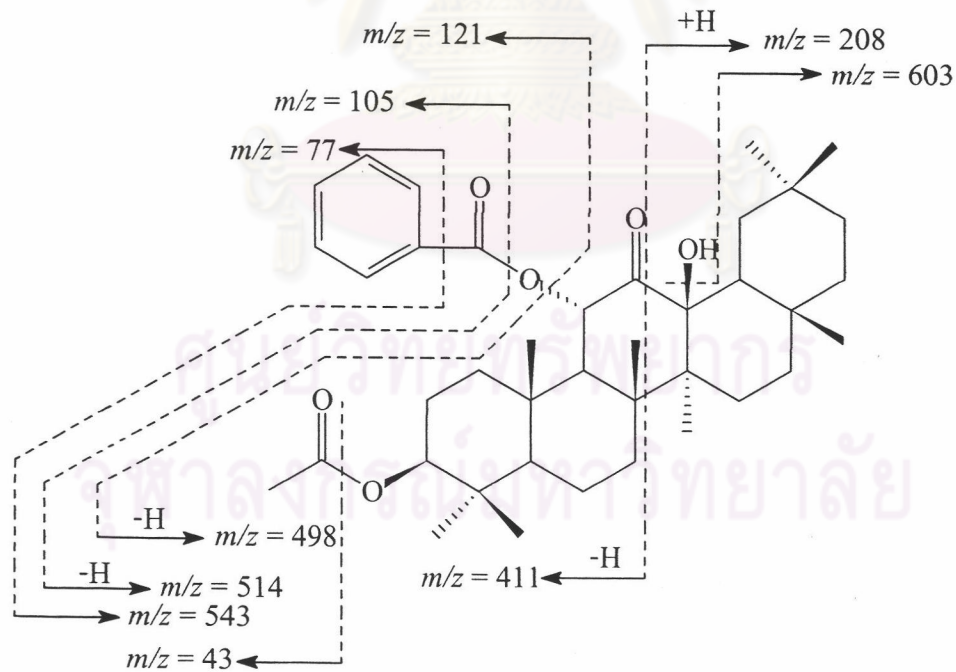
Figure 4. Some important long – range correlations from HMBC ($^n J_{CH}$ = 8 Hz) spectral data of compound SC1

Scheme 6 . Mass fragmentation of compound SC1

Table 5 . NMR data of compound SC1

Position	^{1}H	^{13}C	HMBC (H to C)
1	2.14 m	40.3 (t)	
	1.34 m		
2	1.58 m	23.9 (t)	
	1.51 m		C - 4
3	4.48 dd (J =11.8, 4.6 Hz)	80.2 (d)	C - 4 , O$\underline{C}$OCH$_3$
4	-	38.4 (s)	
5	0.88 m	55.2 (d)	C - 6
6	1.63 m	17.7 (t)	
7	1.56 m	34.0 (t)	
	1.43 m		
8	-	44.1 (s)	
9	2.06 d (J = 12.6 Hz)	54.2 (d)	C - 1, C-10, C -11,C-12 ,C -25 , C-26
10	-	39.3 (s)	
11	6.44 d (J = 12.6 Hz)	75.1 (d)	C - 8, C - 9 , C-10 , C-12 , C- 7'
12	-	202.2 (s)	
13	3.50 br s	83.1 (s)	
14	-	44.8 (s)	
15	2.16 m	22.9 (t)	
	1.12 m		C - 17
16	1.88 m	30.0 (t)	C - 17
	1.13 m		
17	-	33.5 (s)	
18	1.52 m	49.1 (d)	C - 13, C - 19
19	1.98 m	38.3 (t)	
	1.22 m		
20	-	31.4 (s)	
21	1.20 m	34.5 (t)	
22	1.41 m	39.1 (t)	
	1.21 m		
23	0.90 s	28.2 (q)	C - 3, C - 4 , C- 5 , C - 24
24	0.87 s	16.4 (q)	C - 3, C - 4 , C - 5
25	1.04 s	16.4 (q)	C - 1, C - 5 , C - 9 , C -10
26	1.49 s	20.9 (q)	C - 7, C - 8 , C - 9 , C - 14
27	1.03 s	18.9 (q)	C - 8, C -13, C - 14 , C - 15
28	1.23 s	31.5 (q)	C - 16, C -17, C - 18 , C - 22
29	0.84 s	32.3 (q)	C - 19, C-20, C- 21 , C- 30
30	0.86 s	24.5 (q)	C - 19, C-29
1'	-	133.2 (s)	
2', 6'	8.07 d (J = 7.4 Hz)	130.1 (d)	C - 1', C - 4', C - 7'
3', 5'	7.45 t (J = 7.4 Hz)	128.6 (d)	C - 2', C - 6'
4'	7.57 t (J = 7.4 Hz)	133.2 (d)	C - 2', C - 6'
7'		165.6 (s)	
O$\underline{C}$OCH$_3$	-	171.2 (s)	
OCO$\underline{C}$H$_3$	2.02 s	21.4 (q)	O$\underline{C}$OCH$_3$

Table 6. Comparison of ^{13}C NMR data for compound SC1 (36), 37 and 38

	(36)	(37)[138]	(38)[138]
1	40.3	38.1	38.4
2	23.9	24.7	23.8
3	80.2	80.8	80.0
4	38.4	38.4	38.3
5	55.2	55.2	55.0
6	17.7	17.9	17.6
7	34.0	33.2	33.9
8	44.1	43.1	43.8
9	54.2	56.7	53.8
10	39.3	39.6	39.7
11	75.1	71.9	74.2
12	202.2	201.2	202.4
13	83.1	81.5	82.8
14	44.8	44.9	44.6
15	22.9	20.7	22.7
16	30.0	39.1	39.2
17	33.5	31.4	33.5
18	49.1	44.9	48.8
19	38.3	37.9	39.0
20	31.4	27.7	33.4
21	34.5	33.7	34.3
22	39.1	30.4	30.3
23	28.2	27.7	28.1
24	16.4	15.9	16.4
25	16.4	17.2	16.2
26	20.9	20.2	20.7
27	18.9	23.4	18.6
28	31.5	29.3	31.3
29	32.3	29.3	32.0
30	24.5	31.0	24.7
1'	133.2	-	-
2', 6'	130.1	-	-
3', 5'	128.6	-	-
4'	133.1	-	-
7'	165.6	-	-
O$\underline{C}$OCH$_3$	171.2	171.5	170.9
OCO$\underline{C}$H$_3$	21.4	22.3	21.3
O$\underline{C}$OCH$_3$	-	-	170.1
OCO$\underline{C}$H$_3$	-	-	21.4

1.2 Identification of compound SC2

Compound SC2 obtained as white needles. It gave green color to Liebermann Burchard's test. Thus, it appear to be the steroidal compound.
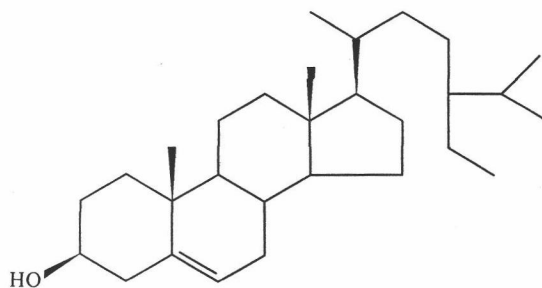
Its IR spectrum suggested the presence of hydroxyl group (3500 cm^{-1}), methyl and methylene groups (3000 and 1470 cm^{-1}) . (Figure 17 , page 103)

Its EI - MS spectrum (Figure 18, page 104) exhibited molecular ion peak at m/z 414 , which was in agreement with the molecular formula $C_{29}H_{50}O$, and major fragmentation peaks at m/z 145 , 107 , 95 , 91 , 81 and 55 .

SC2 could be assigned as the known plant sterol , β- sitosterol , through analysis of its ^{1}H - and ^{13}C - NMR spectra. The ^{1}H - NMR spectrum (Figure 19 , pages 105 – 106) of SC2 showed the signal at δ 0.70 - 1.00 ppm , which were the signals of methyl protons of the positions 18 , 19 and in the side chain of the steroidal compound. The signals at δ 1.1-2.3 ppm were those of methylene and methine protons. The signal at δ 5.35 ppm could be assigned to the olefinic H-6 , whereas the signal at δ 3.52 ppm was the signal of H- 3.

The ^{13}C-NMR spectrum (Figure 20 , pages 107 – 108) showed the signals of 3 quaternary, 9 methine, 11 methylene and 6 methyl carbons. The carbon assignments of SC2 are shown in table 8. The ^{1}H- and ^{13}C-NMR data and mass fragmentation pattern of SC2 were in full agreement with the published values of β- sitosterol . [139-140] Therefore, it was concluded that compound SC2 was β- sitosterol (39), a common phytosterol widely distributed in the plant kingdom.

(39)

Table 7. Comparison of ^{13}C - NMR data for compound SC2 and β - sitosterol.

Position	SC2	β-sitosterol[140]	Position	SC2	β-sitosterol[140]
1	37.2	37.2	16	28.2	28.3
2	31.7	31.7	17	56.0	56.0
3	71.8	72.0	18	12.0	12.0
4	42.3	42.3	19	19.4	19.4
5	140.8	140.5	20	36.1	36.2
6	121.7	122.0	21	18.8	18.8
7	31.9	32.0	22	33.9	33.9
8	31.9	32.0	23	26.1	26.1
9	50.1	50.1	24	45.8	45.9
10	36.5	36.4	25	29.1	29.1
11	21.1	21.0	26	19.8	19.8
12	39.8	39.8	27	19.0	19.0
13	42.3	42.3	28	23.1	23.0
14	56.8	56.8	29	11.9	11.9
15	24.3	24.3			

- H$_2$O

m/z 399 $\longrightarrow$ m/z 381

- CH$_3$

-side chain $\quad$ -H$_2$O

m/z 273 $\qquad$ M$^+$ 414 $\qquad$ m/z 396

-ring D $\qquad\qquad$ -side chain

m/z 231 $\qquad$ m/z 329 $\qquad$ m/z 255

-ring D

m/z 173 $\qquad\qquad$ m/z 213

- ring C

m/z 147

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Scheme 7. Mass fragmentation of SC2

1.3) Identification of compound SC3

Compound SC3, obtained as orange - red crystals. Its IR spectrum (Figure 22, page 110) contained absorption band due to hydroxyl (3414 cm^{-1}) and carbonyl (1727 cm^{-1}) group which were further supported by the ^{13}C - NMR data (Figure 25, pages 115 - 116) at δ 146.0 , 178.3 , and 178.7 , respectively.

The ^{1}H - NMR spectrum (Figure 24 , pages 112 - 114) revealed the presence of six tertiary methyls [δ 2.20 , 1.45 , 1.26 , 1.17 , 1.10 and 0.53 (each 3 H, s)] , one methoxy proton [δ 3.55 (3 H , s)] , three olefinic protons [δ 7.01 (1 H , dd , $J = 7.0$, 1.5 Hz) , 6.53 (1 H , d , $J = 1.5$ Hz) and 6.34 (1 H , d , $J = 7.0$ Hz)] and one methine proton [δ 6.96 (1 H , br s)] attached to an oxygen function. In the ^{13}C-NMR spectrum , seven methyl carbon signals [δ 51.6 , 38.3 , 32.7 , 31.6 , 21.6 , 18.3 and 10.2] , seven methylene carbon signals [δ 36.4 , 34.8 , 33.6 , 30.9 , 29.9 , 29.6 and 28.6] , four methine carbon signals [δ 134.0 , 119.6 , 118.1 and 44.3] , and twelve quaternary carbon signals [δ 178.7 , 178.3 , 170.0 , 164.8 , 146.0 , 127.4 , 117.1 , 45.0 , 42.9 , 40.4 , 39.4 and 30.5] were differentiated by DEPT (Figure 26 , pages 117 - 118) experiment. The assignments of the proton resonances were achieved by the HMQC experiment (Figure 28 , pages 122 – 124)

The FAB - MS (Figure 23 , page 111) showed a [M + H]$^{+}$ ion peak at m / z 465, consistent with a molecular formula of $C_{30}H_{40}O_4$. The FAB - MS showed two important fragment ions at m / z 201 and 202 (Scheme 8) , characteristics for the cleavage of C and D rings of typical quinone - methide triterpenes , with the quinone- methide chromophore on ring A / B and a hydroxyl group at C - 3. [113] In the HMBC spectra (Figure 29 , pages 125 - 130) of compound SC3 , correlation between H - 1 to C - 3, C – 9 and C - 5, OH -3 to C - 2, H - 23 to C - 3, C – 4 and C - 5 , H - 6 to C -10 , C - 4 and C - 8, H - 7 to C - 5 and C - 9 also supported this suggestion.

The correlation between the methoxy proton to the ester carbonyl at δ 178.3 suggested the presence of methyl ester in the molecule and its position was proven as at C – 20 of ring E through the HMBC correlation.

The overall correlations in HMBC spectra suggested the main structure of compound SC3 being pristimerin type.

By comparison with previous report , compound SC3 could be identified as pristimerin (**16**) , a well - known quinone - methide triterpene , first isolated from *Pristimera indica* (Willd.) A. C. Smith. in 1951.[53]
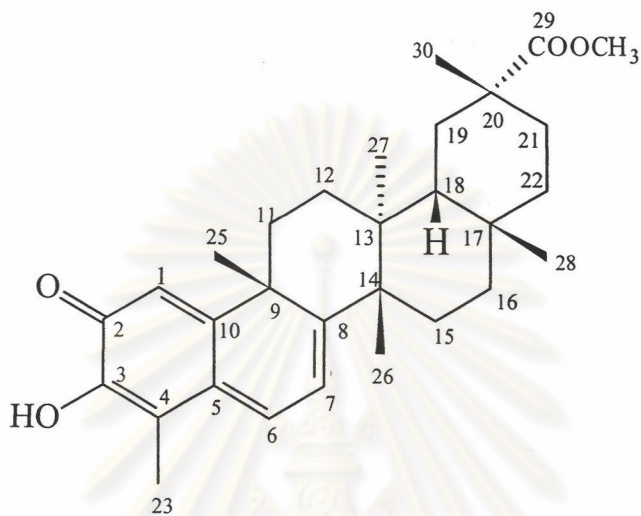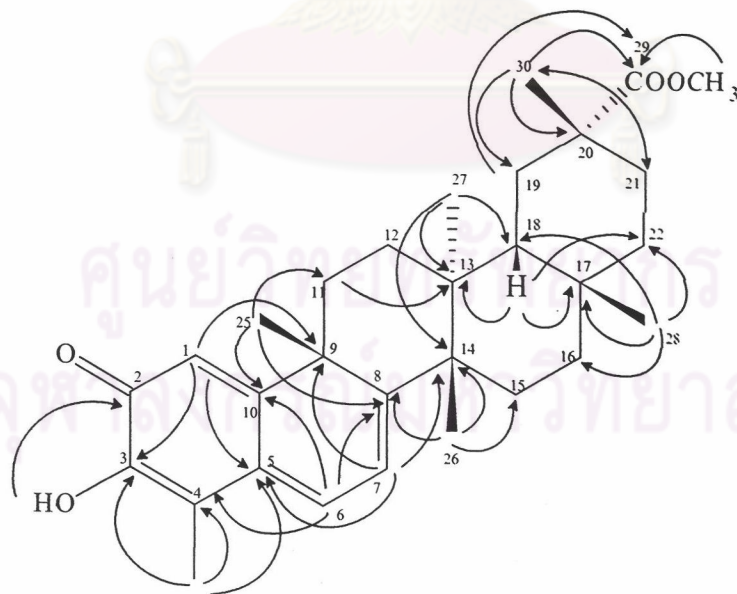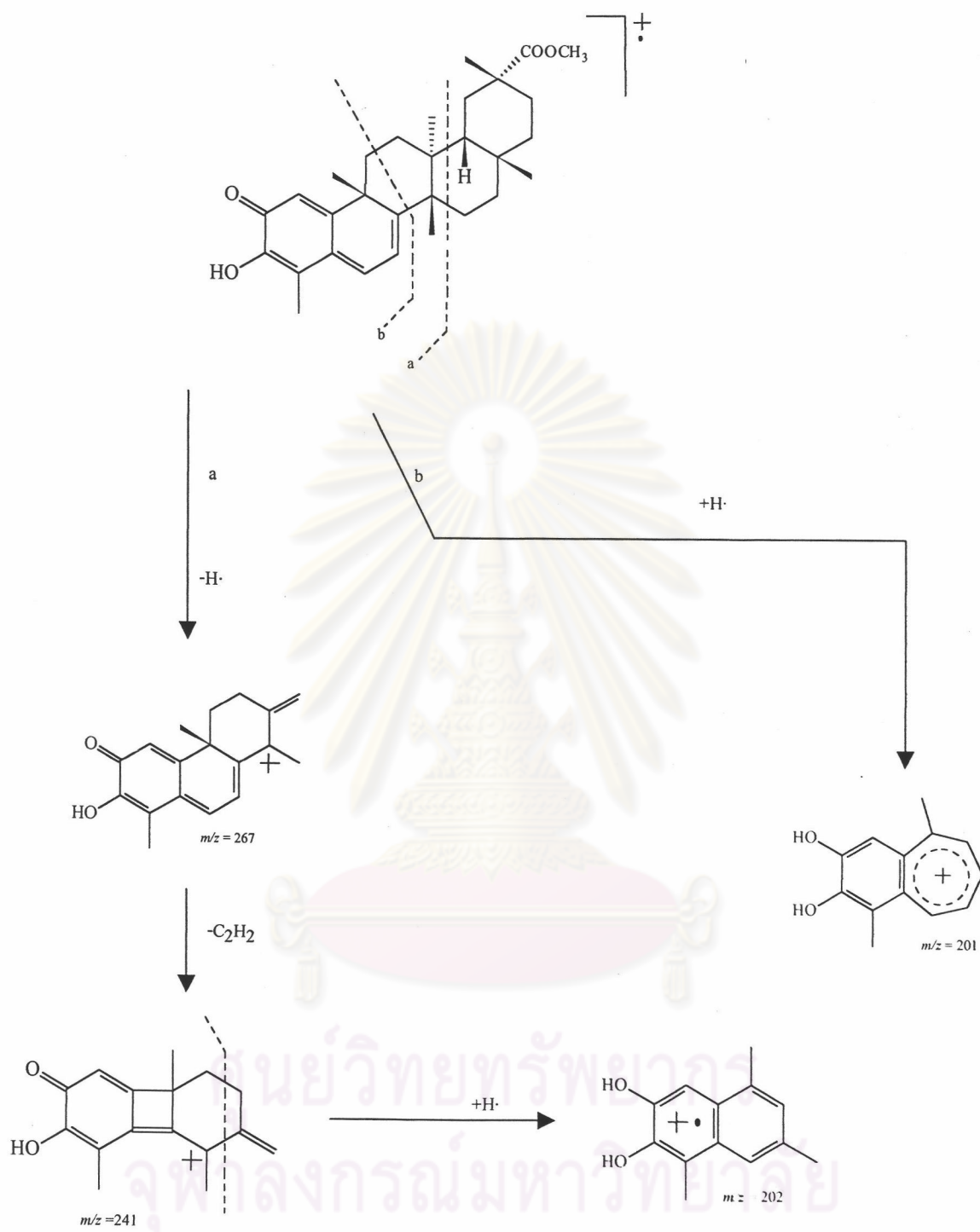

(16)

Figure 5. Some important long – range correlations from HMBC ($^nJ_{CH}$ = 8 Hz) spectral data of compound SC3.

Scheme 8. Mass fragmentation of compound SC3

Table 8. NMR data of compound SC3

Position	^{1}H	^{13}C	HMBC
1	6.53 d (J = 1.5 Hz)	119.6 (d)	C-3 , C-5
2	-	178.7 (s)	
3	6.96 br s	146.0 (s)	
4	-	117.1 (s)	
5	-	127.4 (s)	
6	7.01 dd (J = 7.0, 1.5 Hz)	134.0 (d)	C-4 , C-8 , C-10
7	6.34 d (J = 7.0 Hz)	118.1 (d)	C-5 , C-9 , C-14
8	-	170.0 (s)	
9	-	42.9 (s)	
10	-	164.8 (s)	
11	2.14 m	33.6 (t)	
	1.87 td (J = 14.0 , 6.1 Hz)		C-25 , C-12
12	1.82 dd (J = 12.8 , 5.5 Hz)	29.6 (t)	C-9 , C-13 , C-14
	1.78 m		
13	-	39.4 (s)	
14	-	45.0 (s)	
15	1.65 m	28.6 (t)	
	1.55 m		
16	1.53 m	36.4 (t)	
	1.50 m		
17	-	30.5 (s)	
18	1.57 m	44.3 (d)	C-13 , C-17 , C-22 , C-27
19	2.45 d (J = 15.6 Hz)	30.9 (t)	C-13 , C-18 ,C-21, C- 29
	1.70 d (J = 15.6 Hz)		C-13, C-30, C-29
20	-	40.4 (s)	
21	2.15 dd (J = 11.6, 4.6 Hz)	29.9 (t)	
	1.38 td (J = 14.0, 4.6 Hz)		C-20 , C-22, C- 30 , C-29
22	2.05 td (J = 14.3 ,10.1Hz)	34.8 (t)	
	0.99 dt (J = 14.3 ,4.5 Hz)		
23	2.20 s	10.2 (q)	C-3 , C-4 , C-5
25	1.45 s	38.3 (q)	C-8 , C-10 , C-11
26	1.26 s	21.6 (q)	C-8 , C-14 , C-15
27	0.53 s	18.3 (q)	C-13 , C-14 , C-18
28	1.10 s	31.6 (q)	C-16 , C-17 , C-18 , C-22
29	-	178.3 (s)	
30	1.17 s	32.7 (q)	C-19 , C-20 , C-29
COOC$\underline{\text{H}}_3$	3.55 s	51.6 (q)	C-29

Table 9. Comparison of ^{13}C – NMR data for compound SC 3 and pristimerin

	SC3	Pristimerin[53]
1	119.6	120.2
2	178.7	178.2
3	146.0	145.9
4	117.1	117.8
5	127.4	127.4
6	134.0	135.2
7	118.1	118.2
8	170.0	169.8
9	42.9	43.1
10	164.8	165.0
11	33.6	33.6
12	29.6	29.5
13	39.4	39.3
14	45.0	45.3
15	28.6	28.7
16	36.4	36.4
17	30.5	30.6
18	44.3	44.2
19	30.9	30.9
20	40.4	39.9
21	29.9	29.7
22	34.8	34.9
23	10.2	10.4
25	38.3	38.3
26	21.6	21.6
27	18.3	18.3
28	31.6	31.5
29	178.3	178.7
30	32.7	32.3
COOCH₃	51.6	51.3