

CHAPTER V

DISCUSSION

5.1 Structure elucidation of compound 1

Compound 1 was obtained by column chromatography using 10% ethyl acetate-hexane and recrystallization technique. The structure of compound 1 was elucidated by FT-IR, NMR and mass spectroscopic data as follows.

IR spectrum of compound 1 is shown in Fig. 9 and the details of absorption peaks are summarized in Table 6.

Table 6 IR absorption bands of compound 1

Wave number (cm ⁻¹)	Intensity	Tentative assignment
3500-2400	Broad	O-H stretching of acid
2963, 2911	Medium	C-H stretching of CH ₃ , -CH ₂ -
1680	Strong	C=O stretching of carboxylic group
1635	Weak	C=C stretching olefin

The ¹H-NMR spectrum (Fig.10 and Table 7) of compound 1 indicated that it possesses an isopropyl group which showed doublet signals of two methyl groups attaching to saturated methine carbon (C-15) at δ_H 1.03 and 1.00 ppm and two singlet signals of methyl groups attaching to quaternary carbon (C-8, C-4) at δ_H 1.54 and 1.73 ppm, respectively. There were four olefinic protons (H-7, H-3, H-11 and H-2) at δ_H 5.08, 5.89, 5.98 and 6.01 ppm, respectively.

The ^{13}C -NMR spectrum (Fig. 11 and Table 8) showed 19 signals which the carbonyl group of carboxylic acid corresponding to the signal at 173.6 ppm. Eight signals of olefinic carbons appeared at 146.7, 146.3, 135.2, 134.0, 130.9, 125.7, 121.6, and 118.7 ppm. There were sp^3 eleven carbon signals at 39.2 (t), 38.5 (t), 33.7 (d), 33.6 (t), 28.7 (t), 26.4 (t), 25.1 (t), 22.1 (2xq), 17.0 (q) and 15.8 (q) ppm.

From DEPT-90 and DEPT-135 (Fig. 12), compound 1 possesses twenty carbon atoms and twenty nine protons. Assuming that compound 1 contains only carbon, hydrogen and oxygen atoms. Thus, its molecular formula was established as $\text{C}_{20}\text{H}_{30}\text{O}_2$ which was confirmed by a molecular ion of m/z 302 (Fig 13) and indicated double bond equivalent of six. Therefore, this compound should consist of one ring in addition to the four double bonds and one carboxylic group. From data analysis, compound 1 could be a cembranoid diterpene which is a 14-membered ring diterpene skeleton.

It could be concluded that compound 1 exhibited the ^{13}C -NMR chemical shifts similar to that of (1*E*, 3*E*, 7*E*,11*Z*)-1-isopropyl-4,8-dimethyl-cyclotetradeca-1,3,7,11-tetraene-12-carboxylic acid or crotocebraneic acid. The spectroscopic and physical data of compound 1 was identical to those reported for crotocebraneic acid obtained from *C. oblongifolius* from Petchaboon province [37] and the molecular structure of compound 1 was showed as Fig 3.

The ^1H and ^{13}C -NMR chemical shifts of compound 1 and crotocebraneic acid were compared as showed in Tables 7 and 8, respectively.

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Table 7 ^1H -NMR chemical shifts of compound **1** and crotoembraneic acid

Carbon position	Chemical shifts (ppm)	
	Compound 1	Crotoembraneic acid [37]
1	-	-
2	6.01 (1H, d, m)	6.03 (1H, d, $J=11.0$ Hz)
3	5.89 (1H, dd, m)	5.90 (1H, dd, $J=11.0, 0.9$ Hz)
4	-	-
5	2.15 (2H, m)	2.15 (2H, m)
6	2.18 (2H, m)	2.20 (2H, m)
7	5.08 (1H, dt, m)	5.10 (1H, dt, $J=6.4, 1.2$ Hz)
8	-	-
9	2.15 (2H, m)	2.15 (2H, m)
10	2.69 (2H, m)	2.70 (2H, m)
11	5.98 (1H, m)	6.01 (1H, t, $J=6.5$ Hz)
12	-	-
13	2.39 (2H, m)	2.41 (2H, m)
14	2.39 (2H, m)	2.41 (2H, m)
15	2.31 (1H, m)	2.34 (1H, m)
16	1.03 (3H, d, $J=6.85$ Hz)	1.04 (3H, d, $J=6.7$ Hz)
17	1.00 (3H, d, $J=6.85$ Hz)	1.04 (3H, d, $J=6.7$ Hz)
18	1.73 (3H, s)	1.73 (3H, d, $J=0.9$ Hz)
19	1.54 (3H, s)	1.54 (3H, s)
20	-	-

Table 8 ^{13}C -NMR chemical shifts of compound 1 and crotoembraneic acid

Carbon position	Chemical shifts (ppm)	
	Compound <u>1</u>	Crotoembraneic acid [37]
1	146.3 s	146.9 s
2	118.7 d	118.7 d
3	121.6 d	121.6 d
4	135.2 s	135.2 s
5	39.2 t	39.2 t
6	25.1 t	25.1 t
7	125.7 d	125.7 d
8	134.0 s	134.0 s
9	38.5 t	38.6 t
10	26.4 t	26.4 t
11	146.7 d	146.3 d
12	130.9 s	130.9 s
13	33.6 t	33.6 t
14	28.7 t	28.7 t
15	33.7 d	33.8 d
16	22.1 q	22.1 q
17	22.1 q	22.1 q
18	17.0 q	17.0 q
19	15.8 q	15.8 q
20	173.6 s	174.1 s

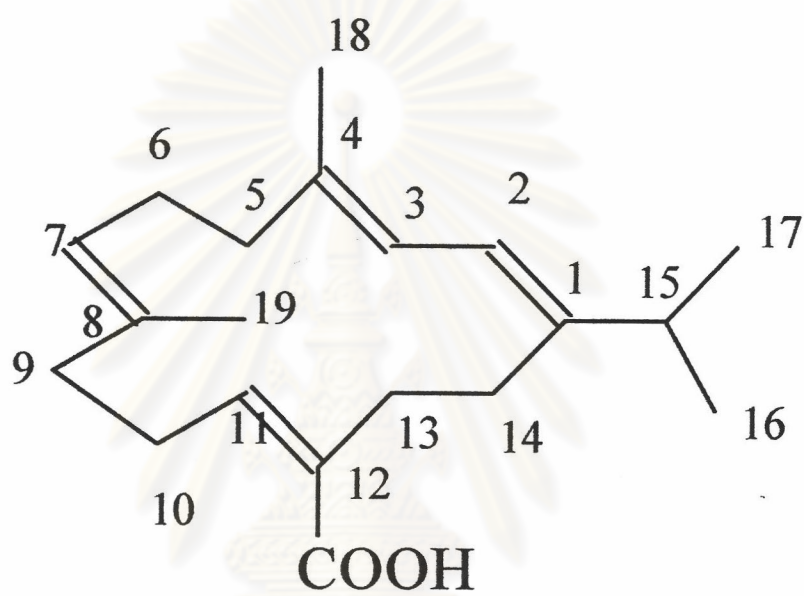


Figure 3 Structure of Compound 1

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5.2 Structure elucidation of compound 2

Compound 2 was obtained by column chromatography using 10% ethyl acetate-hexane. The structure of this compound was elucidated by using FT-IR, NMR and mass spectroscopic data as follows.

IR spectrum of compound 2 is shown in Fig. 14 and details of absorption peaks are shown in Table 9.

Table 9 IR absorption bands of compound 2

Wave number (cm ⁻¹)	Intensity	Tentative assignment
3500-2400	Broad	O-H stretching of acid
2954, 2933, 2874	Medium	C-H stretching of CH ₃ , -CH ₂ -
1682	Strong	C=O stretching of carboxylic group
1639	Weak	C=C stretching olefin

The ¹H-NMR spectrum (Fig. 15 and Table 10) of compound 2 indicated that it possesses an isopropyl group which showed doublet signals of two methyl groups attaching to saturated methine carbon (C-15) at δ_H 1.07 and 1.03 ppm and two singlet signals of methyl groups attaching to quaternary carbons (C-8, C-4) at δ_H 1.69 and 1.71 ppm, respectively. There were four olefinic protons (H-7, H-3, H-2 and H-11) at δ_H 5.14, 5.91, 6.01 and 6.89 ppm, respectively.

The ¹³C-NMR spectrum (Fig. 16 and Table 11) showed 19 signals which the carbonyl group of carboxylic acid corresponding to the signal at 173.4 ppm. Eight signals of olefinic carbons appeared at 146.5, 145.7, 135.6, 134.8, 132.0, 127.8, 120.0, and 118.6 ppm. There were sp³ eleven carbon signals at 38.5 (t), 37.7 (t), 34.5 (d), 30.5 (t), 29.1 (t), 26.7 (t), 24.7 (t), 22.2(q), 22.1 (q), 18.0 (q) and 17.4 (q) ppm.

From DEPT-90 and DEPT-135 (Fig.17), compound 2 possesses twenty carbon atoms and twenty nine protons. Assuming that compound 2 contained only carbon, hydrogen and oxygen atoms. Its molecular formula was established as $C_{20}H_{30}O_2$ which was confirmed by observing the molecular ion of m/z 302 (Fig 18). The molecular formula ($C_{20}H_{30}O_2$) of this compound indicated six double bond equivalent. Therefore, this compound should consist of one ring in addition to the four double bonds and one carbonyl group. Results suggest that compound 2 could be a cembranoid diterpene which is a 14-membered ring diterpene skeleton.

It could be concluded that compound 2 exhibited the ^{13}C -NMR chemical shifts similar to that of (1*E*, 3*E*, 7*E*, 11*E*)-1-isopropyl-4,8-dimethyl-cyclotetradeca-1,3,7,11-tetraene-12-carboxylic acid or neocrotocembraneic acid. The spectroscopic and physical data of compound 2 was identical to those reported for neocrotocembraneic acid obtained from *C. oblongifolius* from Petchaboon province [37] and the molecular structure of compound 2 was showed as Fig 4.

A comparison of the 1H and ^{13}C -NMR chemical shifts of compound 2 and neocrotocembraneic acid are shown in Tables 10 and 11, respectively.

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Table 10 ^1H -NMR chemical shifts of compound 2 and neocrotocembraneic acid

Carbon position	Chemical shifts (ppm)	
	Compound <u>2</u>	Neocrotocembraneic acid [37]
1	-	-
2	6.01 (1H,d, $J=11.0$ Hz)	6.01 (1H, d, $J=11.0$ Hz)
3	5.91 (1H, m)	5.91 (1H, dd, $J=11.0$)
4	-	-
5	2.17 (2H, m)	2.15 (2H, m)
6	2.24 (2H, m)	2.23 (2H, m)
7	5.14 (1H, m)	5.14 (1H, dt, $J=8.0, 2.2$ Hz)
8	-	-
9	2.21 (2H, m)	2.20 (2H, m)
10	2.37 (2H, m)	2.38 (2H, m)
11	6.89 (1H,t, $J=8.0$ Hz)	6.89 (1H, t, $J=8.0$ Hz)
12	-	-
13	2.36 (2H,m)	2.36 (2H, m)
14	2.26 (2H,m)	2.26 (2H, m)
15	2.40 (1H,m)	2.39 (1H, m)
16	1.07 (3H, d, $J=6.79$ Hz)	1.05 (3H, d, $J=7.0$ Hz)
17	1.03 (3H, d, $J=6.79$ Hz)	1.05 (3H, d, $J=7.0$ Hz)
18	1.71 (3H,s)	1.71 (3H, s)
19	1.69 (3H,s)	1.68 (3H, s)
20	-	-

Table 11 ^{13}C -NMR chemical shifts of compound 2 and neocrotocembraneic acid

Carbon position	Chemical shifts (ppm)	
	Compound <u>2</u>	Neocrotocembraneic acid [37]
1	146.5 s	146.5 s
2	118.6 d	118.6 d
3	120.0 d	120.0 d
4	135.6 s	135.6 s
5	37.7 t	37.7 t
6	24.7 t	24.7 t
7	127.8 d	127.8 d
8	134.8 s	134.8 s
9	38.5 t	38.5 t
10	30.5 t	30.5 t
11	145.7 d	145.7 d
12	132.0 s	132.1 s
13	26.7 t	26.7 t
14	29.1 t	29.1 t
15	34.5 d	34.6 d
16	22.2 q	22.1 q
17	22.1 q	22.1 q
18	18.0 q	18.0 q
19	17.4 q	17.4 q
20	173.4 s	173.5 s

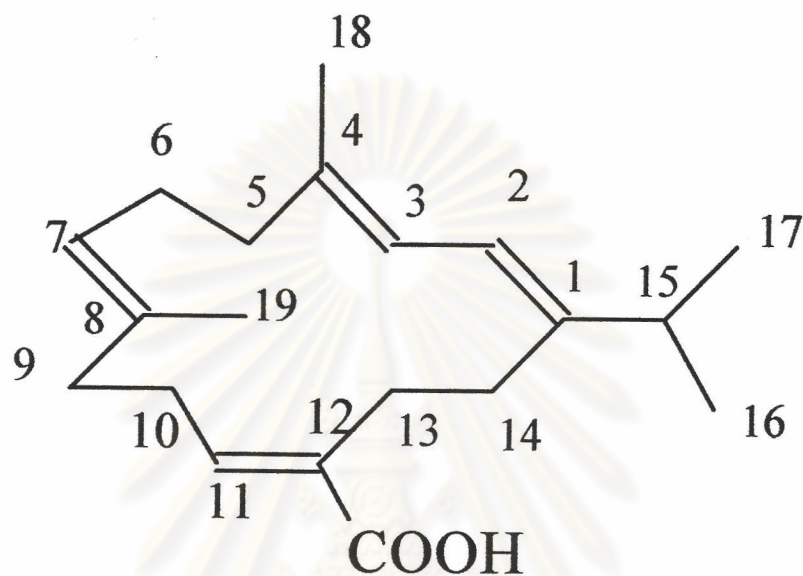


Figure 4 Structure of Compound 2

Crotoembraneic acid and Neocrotoembraneic acid were found for first time in 1998 [32]. They were obtained from *Croton oblongifolius*, from Petchaboon province. According to literature reviews, cembranoid diterpene compounds have been found in soft coral and marine organisms, and in tobacco (*Nicotina tabacum* L.) [48-51]. Moreover, they have been found in other plants such as pine tree (*Haploxyton sp.*) [52], frankincense (*Boswellia carteri*) [53], Termite soldier (*Isoptera termitidae*) [54], *Cleome viscosa* [55], leather hat (*Echinodorus grandiflorus*) [56] and *Croton poilanei* [57].

5.3 Structure elucidation of compound 3

Compound 3 was obtained by column chromatography using 20% ethyl acetate-hexane. The structure of this compound was elucidated using FT-IR, mass, 1D and 2D NMR spectroscopic data as follows

The IR spectrum of compound 3 is shown in Fig. 19 and the details of absorption peaks are shown in Table 12.

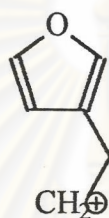
Table 12 The IR absorption bands assignment of compound 3

Wave number (cm ⁻¹)	Intensity	Tentative assignment
3500-2400	Broad	O-H stretching of acid
2980, 2945, 2875	Strong	C-H stretching of CH ₃ , -CH ₂ -
1708	Strong	C=O stretching of carboxylic group

The ¹H-NMR spectrum (Fig. 20 and Table 13) of compound 3 indicated that it possesses three methyl groups at δ_{H} 0.84, 0.86 and 1.28 ppm, respectively. There were three olefinic protons of furanoid group at δ_{H} 6.25 (dd, $J=0.91, 1.83$ Hz), 7.19 (dd, $J=0.91, 1.83$ Hz) and 7.32 (dd, $J=0.91, 1.83$ Hz) ppm, respectively.

The ¹³C-NMR, DEPT-90 and DEPT-135 spectrum (Figs. 21 and 22, Table 13) showed 19 signals which the carbonyl group of carboxylic acid corresponding to the signal at 184.7 ppm. Six signals of olefinic carbons appeared at 142.6, 138.4, 135.9, 131.0, 125.8 and 111.0 ppm. There were thirteen sp³ carbon signals at 47.4 (s), 40.8 (s), 36.4 (t), 35.4 (t), 33.2 (d), 26.7 (t), 25.9 (t), 25.1 (t), 22.8 (q), 20.8 (q), 19.4 (2xt) and 16.0 (q) ppm.

Molecular formula of compound 3 was established as $C_{20}H_{28}O_3$ which was confirmed by mass spectroscopy with molecular ion of m/z 316 (Fig 27). The molecular formula, $C_{20}H_{28}O_3$, of this compound defined a double bond equivalent of seven. Therefore, this compound should consist of one ring of furan (DBE = 3) in addition to one double bond, two rings and one carbonyl group of carboxylic acid. Moreover, the prominent ion at m/z 221 [$M^+ - C_6H_7O^+$] indicated that compound 3 probably contained a furano-ethyl side chain group.



A furano-ethyl side chain

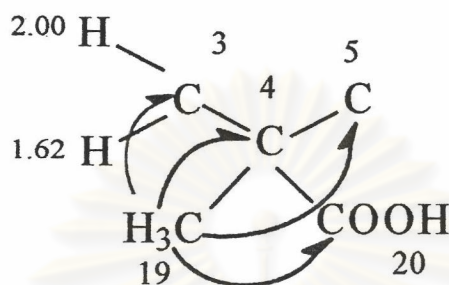
From these data indicated that compound 3 should be a halimane diterpene group. According to the comparison with the NMR-spectrum of crotohalimanic acid [37], compound 3 should have the same basic skeleton. The 1H and ^{13}C -NMR chemical shifts of crotohalimanic acid are shown in Table 13 and 1H , ^{13}C -NMR and 2D long range 1H - ^{13}C correlation in the HMBC spectrum data of compound 3 are shown in Table 14.

Table 13 ^1H , ^{13}C -NMR chemical shifts of Crotohalimaneic acid

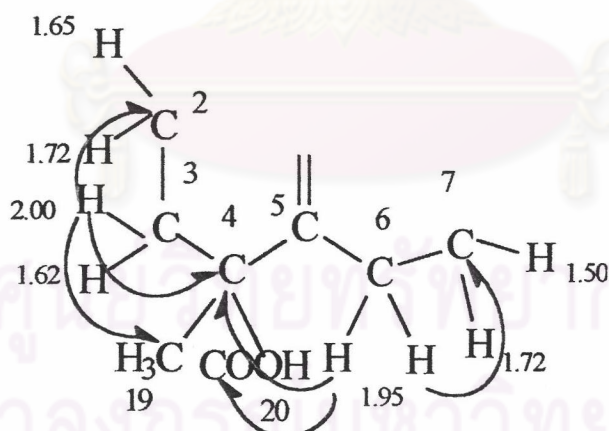
Position	Chemical shifts (ppm)	
	^1H -NMR	^{13}C -NMR
1	1.84 (1H, m)	25.1 (t)
	2.10 (1H, m)	
2	1.64 (2H, m)	20.0 (t)
	1.63 (1H, m)	
3	2.04 (1H, m)	36.4 (t)
	-	
4	-	47.5 (s)
5	-	131.3 (s)
6	1.82 (1H, m)	27.7 (t)
	2.10 (1H, m)	
7	1.46 (2H, m)	26.9 (t)
8	1.70 (1H, m)	33.6 (d)
9	-	41.1 (s)
10	-	136.3 (s)
11	1.48 (1H, m)	36.5 (t)
	1.63 (1H, m)	
12	2.00 (1H, m)	19.5 (t)
	2.32 (1H, m)	
13	-	125.7 (t)
14	6.24 (1H, m, br)	111.0 (d)
15	7.32 (1H, t, $J=1.5$ Hz)	142.7 (d)
16	7.18 (1H, t, $J=1.5$ Hz)	138.4 (d)
17	0.87 (3H, d, $J=7.0$ Hz)	16.1 (q)
18	0.88 (3H, s)	20.8 (q)
19	1.27 (3H, s)	24.3 (q)
20-COOH	-	184.0 (s)

Crucial ^1H - ^{13}C long range correlations (HMBC) of compound **3** (Fig 26) including protons of the C-19 methyl group (H_3 -19, $\delta = 1.28$) were coupled to C-3 (δ 35.4), C-4 (δ 47.4), C-5 (δ 131.0) and C-20 (δ 184.7), HMQC spectrum (Fig.23) showed that the C-3 was connected to protons at 1.62 and 2.00 ppm (see scheme 3).

Scheme 3 Correlations of protons and carbons at carbon position 3-5 and 19-20.

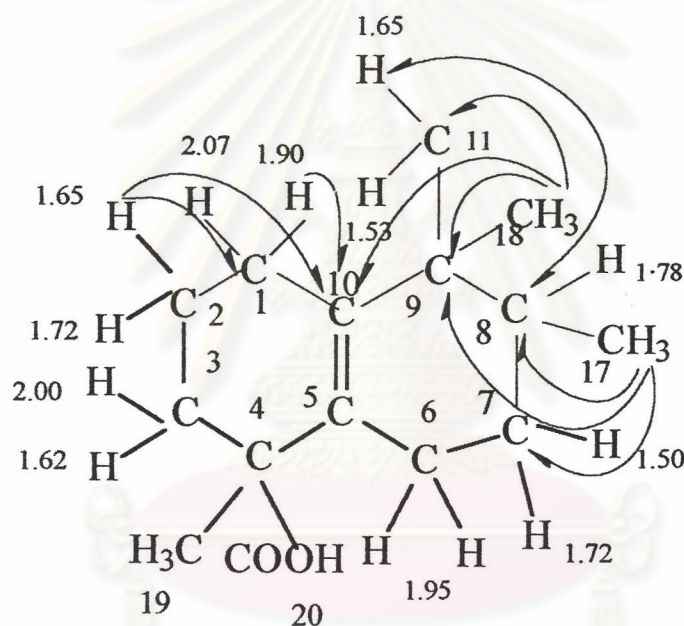


HMBC spectrum showed that the proton of C-3 (H -3, δ 2.00) was coupled to C-19 (δ 22.8), C-4 (δ 47.4) and C-2 (δ 19.4), HMQC spectrum showed that C-2 was connected with protons at 1.65 and 1.72 ppm. Moreover, HMQC showed that the C-6 was connected with the proton at 1.95 ppm (2H) and it was coupled to C-4 (δ 47.4), C-7 (δ 26.7) and C-20 (δ 184.7). According to HMQC, the C-7 (δ 26.7) was connected to protons at 1.37 and 1.50 ppm (see scheme 4).



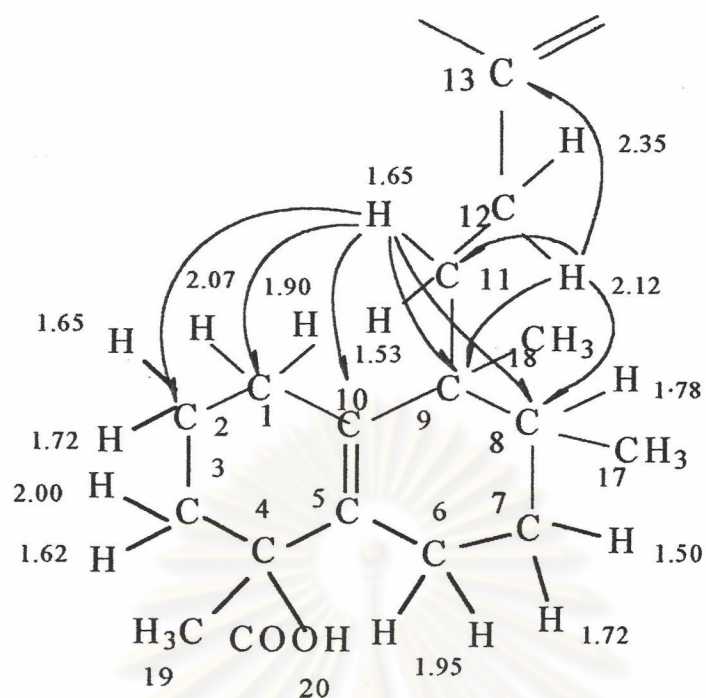
Scheme 4 Correlations of protons and carbons at carbon position 2-7 and 19-20.

The data from HMBC showed that proton of C-2 (δ 19.4) at 1.90 ppm was coupled to C-1 (δ 25.1) and C-10 (δ 135.9), protons of C-1 (δ 25.1) at 1.90 and 2.07 was connected to C-10 (δ 135.9). HMQC and ^1H -spectrum data showed that protons of C-18 ($\text{H}_3\text{-18}$, δ 0.84) was singlet and the quaternary carbon was next to C-18, and H-18 were coupled to C-9 (δ 40.8), C-10 (δ 135.9) and C-11 (δ 36.4). According to COSY and HMQC, C-8 (δ 33.2) which was between C-9 and C-7 (δ 26.7), was coupled by methyl carbon (C-17, δ 0.86) and the proton at 1.78 ppm. HMBC showed that protons of C-17 (H-17, δ 0.86) was connected to C-7 (δ 26.7) and C-8 (δ 33.2) (see scheme 5).



Scheme 5 Correlations of protons and carbons at carbon position 1-11 and 17-20.

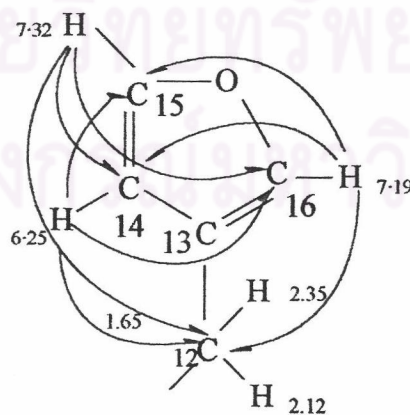
HMBC showed that proton of C-11 (H-11, δ 1.65) was coupled with C-1 (δ 25.1), C-2 (δ 19.5), C-8 (δ 33.2), C-9 (δ 40.8), C-10 (δ 135.9) and C-13 (δ 125.8). HMQC showed that proton of C-12 (δ 19.4) at 2.12 was coupled to C-8 (δ 33.2), C-9 (δ 40.8), C-11 (δ 36.4) and C-13 (δ 125.8) (see scheme 6).



Scheme 6 Correlations of protons and carbons

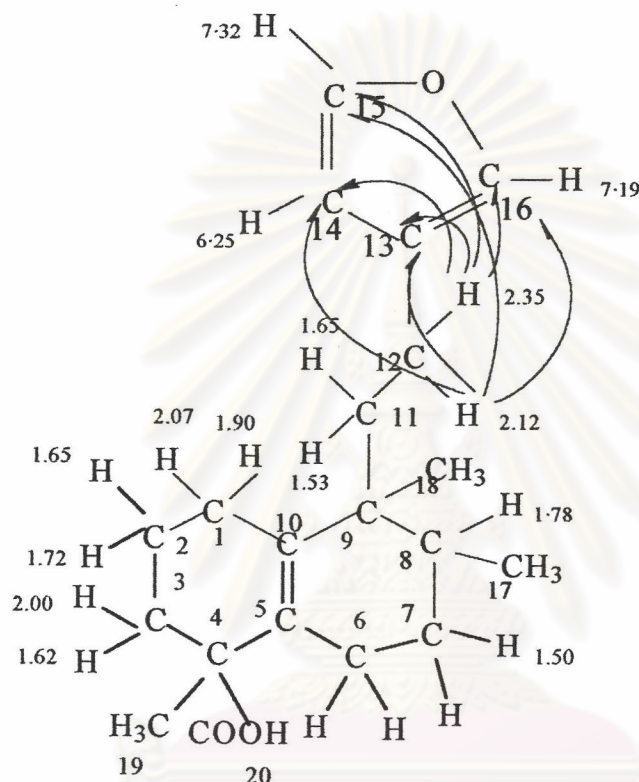
at carbon position 1-13 and 17-20.

The carbon, DEPT-135 and 90 NMR spectral data showed that C-13 was quaternary carbon and HMBC showed that proton of C-14 (δ 111.0) at 6.25 ppm was coupled with C-12 (δ 19.4), C-15 (δ 142.6) and C-16 (δ 138.4), the proton of C-15 (H-15, δ 7.32) was coupled to C-12 (δ 19.4), C-14 (δ 111.0) and C-16 (δ 138.4), the proton of C-16 (H-16, δ 7.19) was coupled to C-12 (δ 19.4), C-14 (δ 111.0) and C-15 (δ 142.6) (see scheme 7).



Scheme 7 Correlations of protons and carbons at carbon position 12-16.

The furanoid fragment was joint with others at C-12. The HMBC showed that both protons of C-12 (H-12, δ 2.12 and 2.35) were coupled to C-13 (δ 125.8), C-14 (δ 111.0), C-15 (δ 142.6) and C-16 (δ 138.4) (see scheme 8).



Scheme 8 Correlations of protons and carbons at carbon position 1-20.

From the above data the structure of compound 3 was assigned as crotohalimaneic acid [37]. However the spectral data showed a little bit different in chemical shift of protons and carbons. It could be concluded that compound 3 was the stereoisomer of crotohalimaneic acid. The NOESY-spectrum data was not clear, so the exact structure of compound 3 could not be identified. Thus, compound 3 was assigned as crotohalimaneic acid and its molecular structure was showed as Fig 5.

The ^1H , ^{13}C -NMR and 2D long range ^1H - ^{13}C correlation in the HMBC spectrum data of compound 3 are shown in Table 13.

Table 14 ^1H , ^{13}C -NMR and 2D long range ^1H - ^{13}C correlation in the HMBC spectrum data of compound **3**.

Carbon position	Chemical shifts (ppm)		Correlated hydrogen
	^1H -NMR	^{13}C -NMR	
1	1.90 (1H, m) 2.07 (1H, m)	25.1 (t)	H-2, H-11
2	1.65 (1H, m) 1.72 (1H, m)	19.4 (t)	H-3, H-11
3	1.62 (1H, m) 2.00 (1H, m)	35.4 (t)	H-19
4	-	47.4 (s)	H-3, H-6, H-19
5	-	131.0 (s)	H-19
6	1.95 (2H, m)	25.9 (t)	
7	1.37 (1H, m) 1.50 (1H, m)	26.7 (t)	H-6, H-17
8	1.78 (1H, m)	33.2 (d)	H-11, H-12, H-17
9	-	40.8 (s)	H-11, H-12, H-17, H-18
10	-	135.9 (s)	H-1, H-2, H-17, H-18
11	1.53 (1H, m) 1.65 (1H, m)	36.4 (t)	H-12, H-18
12	2.12 (1H, m) 2.35 (1H, m)	19.4 (t)	
13	-	125.8 (t)	H-11, H-12, H-14, H-15, H-16
14	6.25 (1H, dd, $J=0.91, 1.83$ Hz)	111.0 (d)	H-12, H-15, H-16
15	7.32 (1H, dd, $J=1.53, 1.83$ Hz)	142.6 (d)	H-12, H-14, H-16
16	7.19 (1H, dd, $J=0.92, 1.53$ Hz)	138.4 (d)	H-12, H-14, H-15
17	0.86 (3H, d, $J=7.02$ Hz)	16.0 (q)	
18	0.84 (3H, s)	20.8 (q)	
19	1.28 (3H, s)	22.8 (q)	H-3
20-COOH	-	184.7 (s)	H-6, H-19

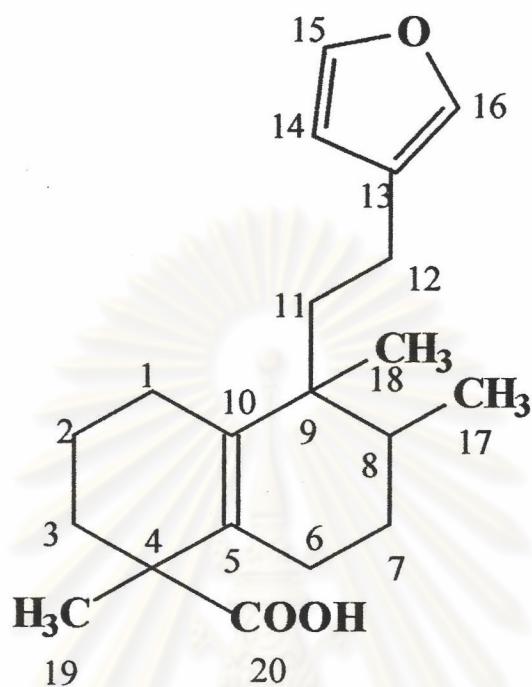


Figure 5 Structure of Compound 3

Crotohalimaneic acid was firstly found from *Croton oblongifolius*, from Nakornrachasima Province [37]. According to the literature reviews, isolation of this compound from other plants has not been reported.

The structure of halimane diterpene group was established from constituents of *Halimum viscosum* (Cistaceae) in 1987 [58]. Halimane compounds were found as its derivatives from this plant again [59-63]. Moreover, they were found from *Ophryspor us charrua* [64], *Jungermannia infusca* [65] and *Polyalthia longifolia* [66,67].

5.4 Structure elucidation of compound 4

Compound 4 was obtained by column chromatography using 28 % ethyl acetate in hexane. The structure of compound 4 was elucidated by FT-IR, NMR, mass spectroscopic data as follows.

IR spectrum of compound 4 is shown in Fig. 28 and details of absorption peaks are shown in Table 15.

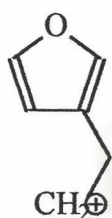
Table 15 IR absorption bands of compound 4

Wave number (cm ⁻¹)	Intensity	Tentative assignment
3600-3100	Broad	O-H stretching of acid
2933	Strong	C-H stretching of CH ₃ , -CH ₂ -
1715,1683	Strong	C=O stretching of carbonyl group
1632	Medium	C=C stretching olefin
1272	Strong	C-O stretching

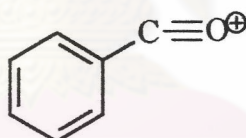
The ¹H-NMR spectrum (Fig. 29 and Table15) of compound 4 indicated that it possesses two methyl groups attaching to quaternary carbons (C-5 and C-8) at δ_H 1.30 and 1.00 ppm, respectively and three olefinic protons of furanoid group at δ_H 6.26, 7.21 and 7.34 ppm, five olefinic protons of a benzoyl group attaching to C-20 at δ_H 7.45(2H), 7.55 (1H) and 8.00 (2H) ppm. There were two protons (H-20) at δ_H 4.31 (d, $J=11.78$ Hz) and 4.50 (d, $J=11.78$ Hz) ppm and one olefinic proton attaching to unsaturated methine carbon (C-3) at δ_H 6.91 ppm.

The ^{13}C -NMR, DEPT-90 and DEPT-135 spectrum (Figs.30 and 31 and Table 16) showed 25 signals. Twelve signals of olefinic carbons appeared at δ 142.9, 141.0, 140.6, 138.5, 133.0, 130.3, 129.5 (2xd), 128.5 (2xd), 125.2 and 111.0 ppm. The chemical shift at 172.3 ppm should be the carbonyl of carboxylic acid attaching to a quaternary carbon (C-4). The signal at 166.8 ppm should be a carbonyl of benzoyl group. There were thirteen sp^3 carbon signals at 67.8 (t), 47.4 (d), 42.3 (s), 37.7 (s), 36.4 (d), 36.0 (t), 32.4 (t), 28.1 (t), 27.2 (t), 20.2 (q), 19.2 (t), 17.9 (t) and 17.0 (q) ppm.

Compound 4 showed a molecular ion of m/z 436 ($\text{C}_{27}\text{H}_{32}\text{O}_5$) (Fig 32.) which indicated double bond equivalent of twelve. Moreover, the prominent ion at m/z 341 [$\text{M}^+ - \text{C}_6\text{H}_7\text{O}^+$], 175 [$219 - \text{COO}^+$] and 105 [PhCO^+] indicated that compound 4 probably contained a furano-ethyl side chain, carboxylic group and benzoyl group, respectively. Therefore, compound 4 should consist of one ring of furan (DBE=3) in addition to one double bond (DBE=1), two rings (DBE=2), one carbonyl of carboxylic acid (DBE=1) and one benzoyl group (DBE=5).



A furano-ethyl side chain



A benzoyl group

Comparison of spectral data including ^1H -NMR, ^{13}C -NMR, DEPT-90 and DEPT-135 of this compound indicated that compound 4 should be identical to (-)-20-benzyloxyhardwickiic acid. The spectroscopic and physical data of compound 4 were reported for benzyloxyhardwickiic acid which obtained from *C. oblongifolius* from Udonthani Province [39] and its molecular structure was showed as Fig 6.

The ^1H and ^{13}C -NMR chemical shifts of compound 4 and (-)-20-benzyloxy hardwickiic acid are compared as showed in Table 16 and 17, respectively.

Table 16 ^1H -NMR chemical shifts of compound **4** and (-)-20-benzyloxyhardwickiic acid

Carbon position	Chemical shifts (ppm)	
	Compound 4	(-)-20-benzyloxyhardwickiic acid [39]
1	2.53-1.65(1H, m)	1.72 (1H, m)
	2.53-1.65(1H, m)	1.95 (1H, m)
	2.53-1.65(1H, m)	2.20 (1H, m)
2	2.53-1.65(1H, m)	2.35 (1H, m)
3	6.91 (1H, m)	6.92 (1H, dd, $J=2.45, 4.58\text{Hz}$)
4	-	-
5	-	-
6	2.53-1.65(1H, m)	1.24 (1H, m)
	2.53-1.65(1H, m)	2.53 (1H, ddd, $J=3.05, 3.05, 12.82\text{Hz}$)
	1.53 (1H, m)	1.53 (1H, m)
7	2.53-1.65(1H, m)	1.65 (1H, m)
8	2.53-1.65(1H, m)	1.78 (1H, m)
9	-	-
10	1.58 (1H, m)	1.58 (1H, d, $J=12.51\text{Hz}$)
11	2.53-1.65(1H, m)	1.93 (1H, m)
	2.53-1.65(1H, m)	2.08 (1H, m)
	2.53-1.65(1H, m)	2.25 (1H, m)
12	2.53-1.65(1H, m)	2.40 (1H, m)
13	-	-
14	6.26 (1H, d, $J=1.53\text{Hz}$)	6.28 (1H, d, $J=1.53\text{Hz}$)
15	7.34 (1H, d, $J=1.53\text{Hz}$)	7.35 (1H, d, $J=1.53\text{Hz}$)
16	7.21 (1H, s)	7.24 (1H, s)
17	1.00 (3H, d, $J=6.71$)	1.02 (3H, d, $J=6.71$)
18	-	-
19	1.30 (3H, s)	1.32 (3H, s)
20	4.31 (1H, d, $J=11.78\text{Hz}$)	4.30 (1H, d, $J=11.9\text{Hz}$)
	4.50 (1H, d, $J=11.78\text{Hz}$)	4.50 (1H, d, $J=11.9\text{Hz}$)
21	-	-
22	-	-
23	8.00(1H, d, $J=1.63\text{Hz}$)	8.01(1H, d, $J=1.22\text{Hz}$)
24	7.45 (1H, dd, $J=7.61, 7.61\text{Hz}$)	7.45 (1H, dd, $J=7.63, 7.63\text{Hz}$)
25	7.55(1H, dd, $J=5.90, 5.90\text{Hz}$)	7.55(1H, dd, $J=7.63, 7.63\text{Hz}$)
26	7.45 (1H, dd, $J=7.61, 7.61\text{Hz}$)	7.45 (1H, dd, $J=7.63, 7.63\text{Hz}$)
27	8.00(1H, d, $J=1.63\text{Hz}$)	8.01(1H, d, $J=1.22\text{Hz}$)

Table 17 ^{13}C -NMR chemical shifts of compound **4** and (-)-20-benzyloxyhardwickiic acid

Carbon position	Chemical shifts (ppm)	
	Compound 4	(-)-20-benzyloxyhardwickiic acid [39]
1	19.2 t	19.2 t
2	28.1 t	28.1 t
3	140.6 d	140.5 d
4	141.0 s	140.9 s
5	37.7 s	37.7 s
6	36.0 t	36.0 t
7	27.2 t	27.2 t
8	36.4 d	36.3 d
9	42.3 s	42.3 s
10	47.4 d	47.4 d
11	32.4 t	32.4 t
12	17.9 t	17.9 t
13	125.2 s	125.1 s
14	111.0 d	110.9 d
15	142.9 d	142.9 d
16	138.5 d	138.5 d
17	17.0 q	16.9 q
18	172.3 s	172.0 s
19	20.2 q	20.2 q
20	67.8 t	67.7 t
21	166.9 s	166.8 s
22	130.3 s	130.4 s
23	129.5 d	129.5 d
24	128.5 d	128.5 d
25	133.0 d	132.9 d
26	128.5 d	128.5 d
27	129.5 d	129.5 d

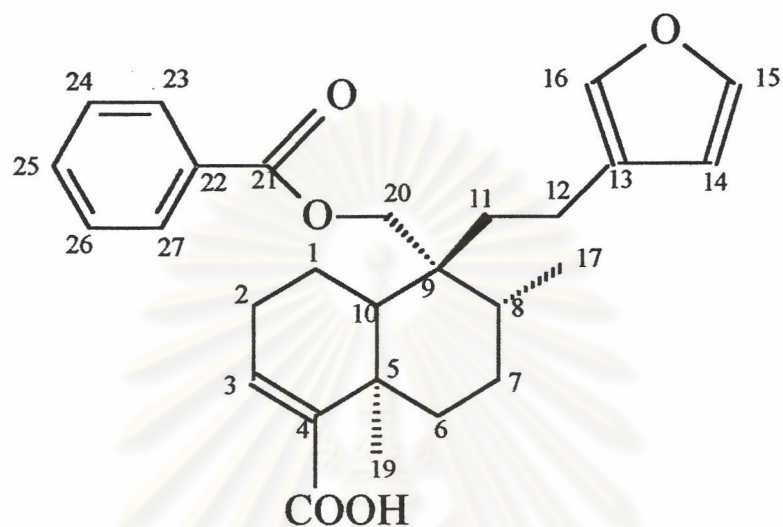


Figure 6 Structure of Compound 4

Benzyloxyhardwikiic acid was firstly found in *Croton oblongifolius*, Udonthani Province [39]. Additionally, it was found again from *Croton oblongifolius*, Chachoengsao Province [44].

ศูนย์วิทยทรัพยากร
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5.5 Structure elucidation of compound 5

Compound 5 was obtained by column chromatography using 30 % ethyl acetate in hexane. The structure of compound 5 was elucidated by using FT-IR, NMR, mass spectroscopic data as follows.

The IR spectrum of compound 5 is shown in Fig. 33 and the details of absorption peaks are shown in Table 18.

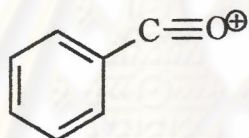
Table 18 IR absorption bands of compound 5

Wave number (cm ⁻¹)	Intensity	Tentative assignment
3452	Medium	O-H stretching of acid
2938	Strong	C-H stretching of CH ₃ , -CH ₂ -
1714	Strong	C=O stretching of carbonyl group
1613	Medium	C=C stretching of olefin
1457,1384	Medium	-CH ₂ ,-CH ₃ bending
1276	Strong	C-O stretching

The ¹H-NMR spectrum (Fig.34 and Table 18) of compound 5 indicated two methoxy groups attaching to quaternary carbons (C-3' and C-5') at δ_{H} 3.83 ppm, one proton on hydroxy group at δ_{H} 5.40 ppm, two olefinic protons attaching to sp² methine carbons (H-2' and H-6') at δ_{H} 6.41 ppm. There were six protons of a propanyl group (H-2, H-1 and H-3) at δ_{H} 2.10 (2H, m), 4.33 (2H, t, $J=6.4$ Hz) and 2.70 (2H, t, $J=7.5$ Hz) ppm, respectively, and five olefinic protons of a benzoyl group which attached to C-3 at 7.38–7.56 (3H, m), 8.00 (1H, d, $J=1.6$ Hz) and 8.04 (1H, d, $J=1.5$ Hz) ppm.

The ^{13}C -NMR, DEPT-90 and DEPT-135 spectrum (Figs. 35 and 36 and Table 18) showed 12 signals. The signals of olefinic carbons appeared at δ 147.0 (2xs), 133.0 (d), 132.2 (2xs), 130.3 (s), 129.5 (2xd), 128.3 (2xd), 105 (2xd) ppm. The signal at 166.6 ppm should be the carbonyl of a benzoyl group. There were two sp^3 carbon signals at 32.5 (t) and 30.5 (t) ppm and three signals at 63.4 (t) and 56.2 (2xq) of methylene and methyl carbon connected to an O atom.

Mass spectrum of compound 5 (Fig 37.) indicated a molecular ion of m/z 316 ($\text{C}_{18}\text{H}_{20}\text{O}_5$) with double bond equivalent of nine. Moreover, the prominent ion at m/z 105 [PhCO^+ , (100)] and 77 [Ph^+ , (60)] indicated that this compound probably contained a benzoyl group and phenol ring. Therefore, compound 5 should consist of one ring in addition to three double bond (DBE=4), and one benzoyl group. (DBE=5).



A benzoyl group

From IR, NMR and MS spectrum data, compound 5 was considered to be benzoate ester of dihydrosyringenin [46].

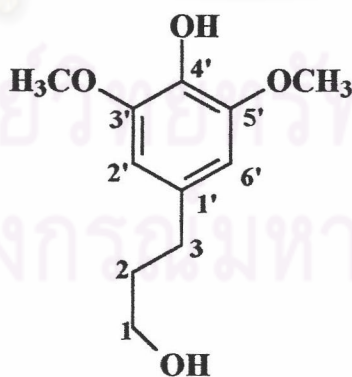
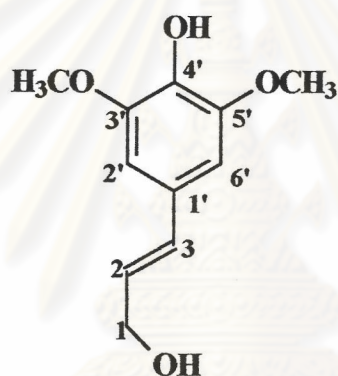


Fig.7 Structure of dihydrosyringenin.

The ^1H and ^{13}C -NMR spectrum of compound 5 are shown in Table 20, and a comparison of ^1H -NMR of dihydrosyringenin and compound 5 is shown in Table 19. The chemical shift of compound 5 were found to correspond to dihydrosyringenin. The benzoyl group of compound 5 was considerably attached to sp^3 carbon (C-3). Therefore, compound 5 was assigned as 3-(3,5 dimethoxy-4-hydroxy phenyl) propanyl benzoate or benzoyl 2,3-dihydrosyringenin and its molecular structure was showed as Fig 8.

From structure elucidation, compound 5 could be a new compound in the group of sinapyl alcohol or syringenin group [45]. It is monolignol unit, which was the intermediate compound of lignin biosynthesis pathway. Sinapyl alcohol was found from *Solidago decurrens* [68].



Sinapyl alcohol or Syringenin

Syringin or syringoside is 1 or 4'-glycoside derivative of sinapyl alcohol that found in various plants such as *Osmanthus asiaticus* [69], *Zantadeschia aethiopica* [70], *Ligularia intermedia* [71] and *Stellera chamaejasme* [72].

Furthermore, other derivatives of sinapyl alcohol were found in many kind of plants as follow; *Odontocline glabra* [73], *Selaginella doederleinii* [74] and pineapple (*Ananus comosus*) [75].

Table 19 ^1H -NMR chemical shifts of compound 5 and dihydrosyringenin

Carbon position	Chemical shifts (ppm)	
	compound <u>5</u>	dihydrosyringenin
1	4.33 (2H, t, $J=6.4$ Hz)	3.69 (2H, t, $J=6.5$ Hz)
2	2.10 (2H, m)	1.89 (2H, tt, $J=6.5, 7.5$ Hz)
3	2.70 (2H, t, $J=7.5$ Hz)	2.64 (2H, t, $J=7.5$ Hz)
1'	-	-
2'	6.41 (1H, s)	6.43 (1H, s)
3'	-	-
4'	-	-
5'	-	-
6'	6.41 (1H, s)	6.43 (1H, s)
2XOCH ₃	3.83 (6H, s)	3.87 (6H, s)
1''	-	-
2''	8.00 (1H, d, $J=1.60$ Hz)	-
3''	7.56–7.38 (1H, m)	-
4''	7.56–7.38 (1H, m)	-
5''	7.56–7.38 (1H, m)	-
6''	8.04 (1H, d, $J=1.50$ Hz)	-
C=O	-	-

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Table 20 ^1H , ^{13}C -NMR spectrum data of compound 5

Carbon position	Chemical shifts (ppm)	
	^1H -NMR	^{13}C -NMR
1	4.33 (2H, t, 6.4 Hz)	64.3 t
2	2.10 (2H, m)	30.5 t
3	2.70 (2H, t, 7.5 Hz)	32.5 t
1'	-	132.2 s
2'	6.41 (1H, s)	105.0 d
3'	-	147.0 s
4'	-	132.2 s
5'	-	147.0 s
6'	6.41 (1H, s)	105.0 d
2XOCH ₃	3.83 (6H, s)	56.2 q
1''	-	130.3 s
2''	8.00 (1H, d, 1.60 Hz)	129.5 d
3''	7.56 – 7.38 (1H, m)	128.3 d
4''	7.56 – 7.38 (1H, m)	133.0 d
5''	7.56 – 7.38 (1H, m)	128.3 d
6''	8.04 (1H, d, 1.50 Hz)	129.5 d
C=O	-	166.5 s

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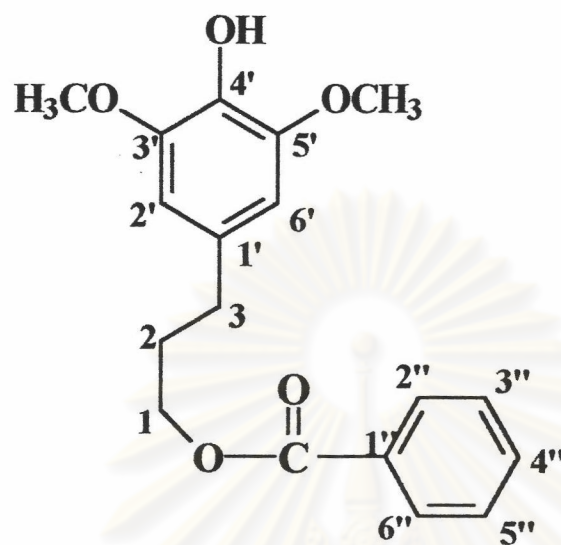


Figure 8 Structure of 3-(3,5 dimethoxy-4-hydroxy phenyl) propanyl benzoate or benzoyl 2,3-dihydroxyringenin.

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