CHAPTER 4

RESULTS AND DISCUSSION

4.1 Structure elucidation of isolated compounds

In this investigation, some of biological activity of *O. paniculata* has been tested in which; dichloromethane and methanol extract were assay for their activity. The active dichloromethane extract was carried out for separation, purification and structure explanation (5 isolated compounds; eugenol (**22**), the mixture of *β*-sitosterol (**23**) and stigmasterol (**24**) along with, stigmastan-3,6-dione (**25**) and 3-acetyl aleuritolic acid (**26**)). The structures of all compounds were determined on the basis of spectral data such as ^{1}H NMR, ^{13}C NMR, COSY, DEPT, HMQC, HMBC, IR spectroscopy and mass spectrometry. The spectroscopic data were compared to the previously literature values.

Eugenol (**22**) (Figure 6), 4-allyl-2-methoxyphenol for a systematic name, which is brownish oil. It was isolated from fraction OPDF3 of the dichloromethane extract of *O. paniculata* twigs. The IR spectra data clearly indicated the appearance absorption of hydroxyl group at 3418 cm^{-1}, C–O–C linkage of ether around 1030 cm^{-1}, strong absorptions at 1610 and 1510 cm^{-1}were also found from eugenol due to terminal double bond and aromatic moiety. The estimation of molecular weight at *m/z* 165 [M^{+1}] which agreed with the molecular formula $C_{10}H_{12}O_2$.[22-24, 27] Fragmentation of eugenol (**22**) was indicated in Figure 12 compared with previous study.[30, 31]

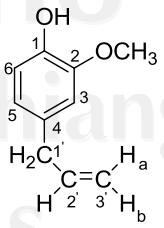


Figure 6 Labelling number of each carbon in structure of eugenol (**22**)

The structure of eugenol (**22**) was elucidated by spectroscopic techniques mainly ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC, MS and IR (Figure 7-12, Table 4 and 5). The 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) in $CDCl_3$ spectra of eugenol (**22**) showed the presence of 12 protons in the molecule.[25, 26] The presence of an aromatic protons at δ 6.88 (*d*, 1H, J = 8.5 Hz), 6.70-6.72 (*m*, 2H) could be assigned to be 1,2,4-trisubstituted benzene, together with the singlet of methoxy proton at δ 3.88 (*s*, 3H) and proton of hydroxyl group at δ 5.59 (*br*, 1H). The doublet pattern at δ 3.35 (*d*, 2H-1', J = 6.7 Hz) is attached to aromatic ring. The signal at δ5.09-5.14 (*m*, 2H-3') suggested that was methylene proton of terminal double bond and further confirmed by ^{1}H–^{1}H COSY spectrum (Figure 9) of the H-2' and H-3', indicating the location of terminal double bond. The ^{13}C NMR displayed of 10 carbon atoms which are corresponding to DEPT 90° and DEPT 135° (Figure 8).[25-28] It indicated three quaternary, four tertiary, two secondary and one methyl carbons. Notably, the chemical shift at 55.7 ppm was assigned to methoxy at *para* position of benzene ring. The downfield quaternary carbons at 143.84 and 146.39 ppm corresponded to aromatic carbon (C-1, C-2) bearing a hydroxyl and methoxy group while the upfield quaternary carbon (C-4) at 131.85 ppm was allylic group which confirmed by HMBC correlation (Figure 11). It showed the interactions between proton of methoxy group at 3.88 ppm to C-2, H-3 (δ 6.88) to C-1, C-2, C-4, C-5, C-6, H-1' (δ 3.55) to C-2', C-3', C-4, C-5, C-6 as shown in Table 5. Consequently, alkoxy group of 55.7 ppm and allylic group substantiated at C-2 (δ 146.3) and C-4 (δ 131.8). By further analysis of 2D data, HMQC correlation (Figure 10) showed the signals of 6.88 ppm (H-3), 5.93-6.04 ppm (H-2') and 3.88 ppm which correlated directly with C-3, C-2' and C-2, respectively. The structure of compound **22** was finally confirmed by directed comparison of ^{1}H and ^{13}C NMR with the value reported by Fujisawa *et al*.[28] and Toda *et al*.[29].

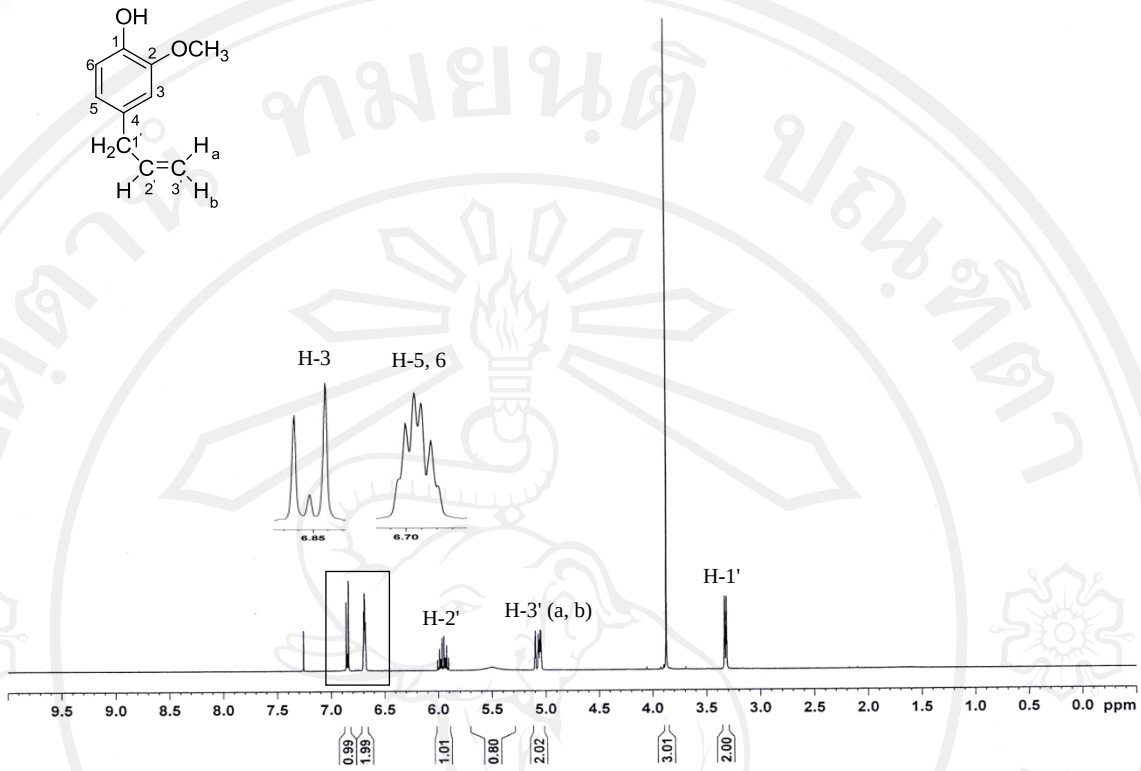


Figure 7 ^{1}H NMR spectrum of eugenol (**22**)

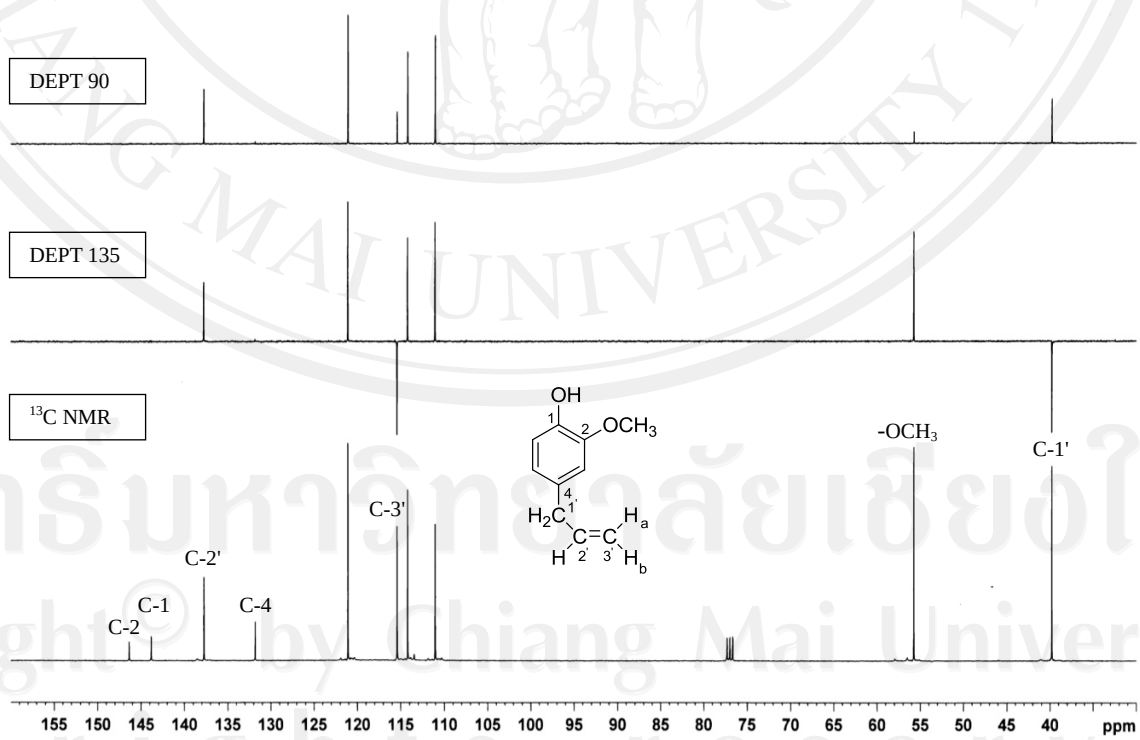


Figure 8 DEPT 90, DEPT 135 and ^{13}C NMR spectra of eugenol (**22**)

Table 3 ^{1}H and ^{13}C NMR data of eugenol (**22**) ($CDCl_3$)at 400 (^{1}H NMR) and 100 MHz (^{13}C NMR)

Position	Chemical shift (δ) in ppm	
	^{1}H NMR	^{13}C NMR
1	-	143.8
2	-	146.3
3	6.88 [*d*, 1H, *J* = 8.5 Hz]	114.2
4	-	131.8
5, 6	6.70-6.72 [*m*, 1H]	111.0, 121.1
1'	3.35 [*d*, 2H, *J* = 6.7 Hz]	39.8
2'	5.93-6.04 [*m*, 1H]	137.7
3'	5.09-5.14 [*m*, 2H],	115.4
O-C$\underline{H}_3$	3.88 [*s*, 3H]	55.7
OH	5.59 [*br*, 1H]	-

Table 4 ^{1}H–^{1}H COSY, HMQC, HMBC data of eugenol (**22**) ($CDCl_3$) at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR)

Proton position	^{1}H–^{1}H COSY (Coupling of H)	HMQC (Correlation of C)	HMBC (Correlation of C)
1	-	-	-
2	-	-	-
3	H-5, 6	C-3	C-1, 2, 4, 5, 6
4	-	-	-
5, 6	H-3	C-5, 6	C-1', 1, 2, 4
1'	H-3'	C-1'	C-3, 4, 5, 6, 2'
2'	H-1', 3'	C-2'	C-1', 4
3'	H-2'	C-3'	C-1', 2'
O-C$\underline{H}_3$	-	$\underline{C}H_3$-O	C-2
OH	-	-	-

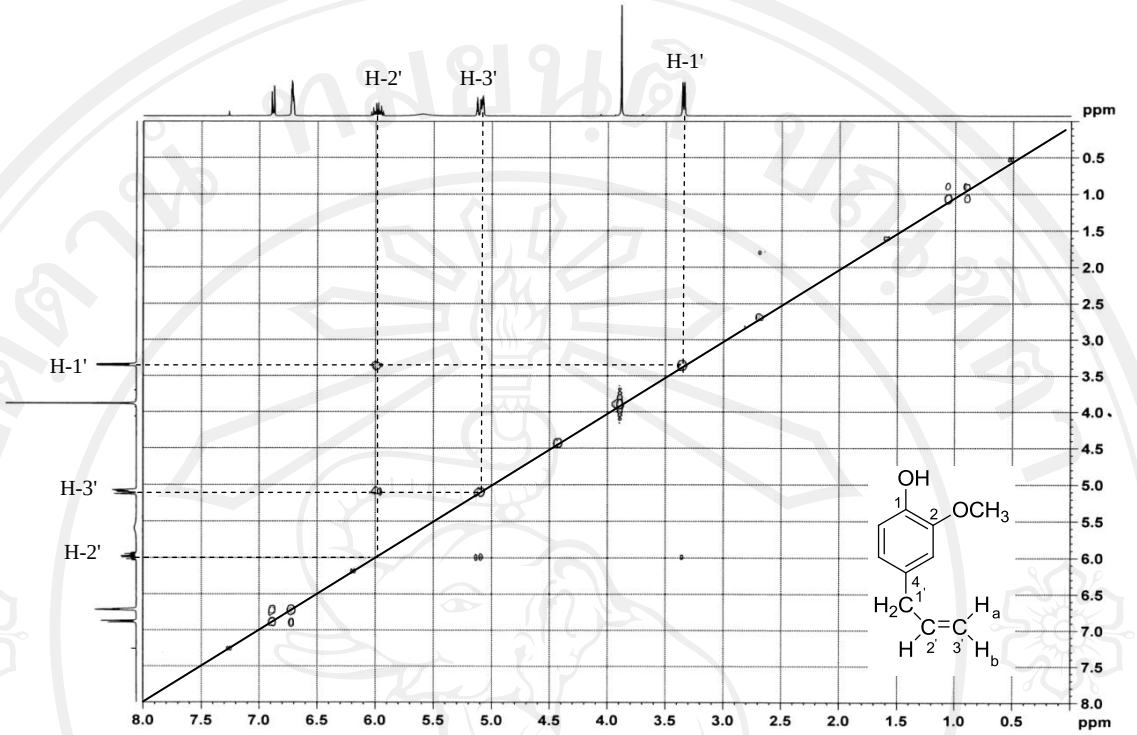


Figure 9 ^{1}H–^{1}H COSY spectrum of eugenol (**22**)

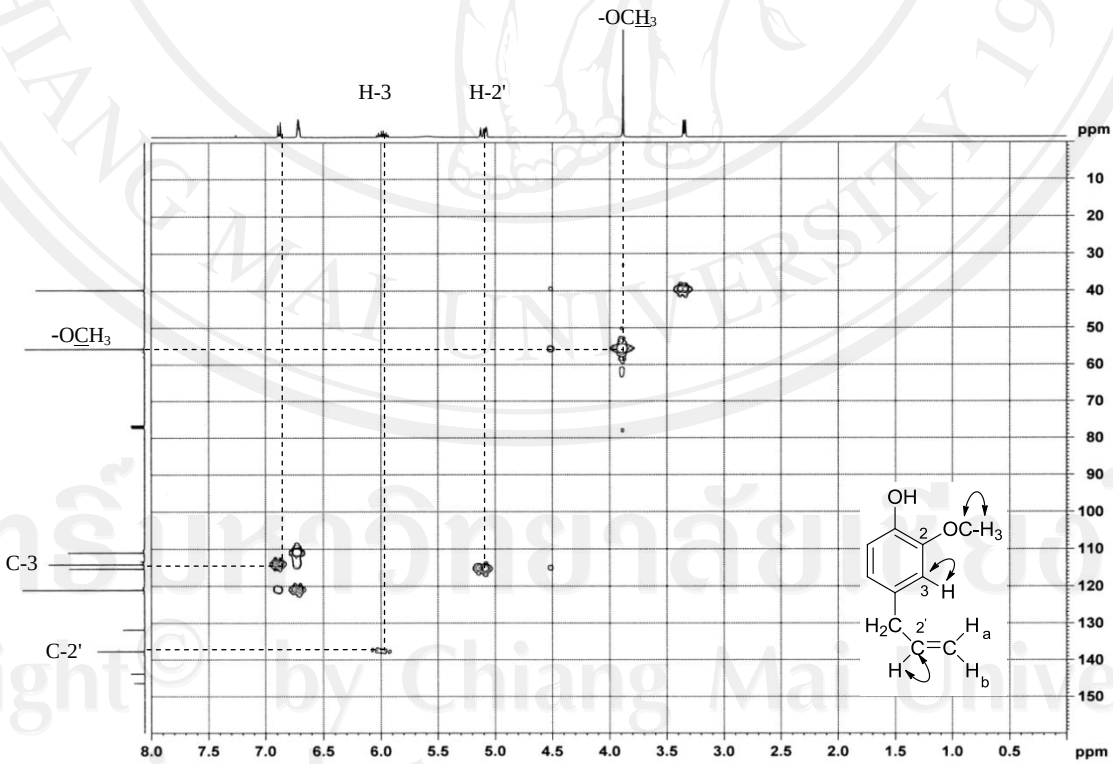


Figure 10 Selected HMQC correlation of eugenol (**22**)

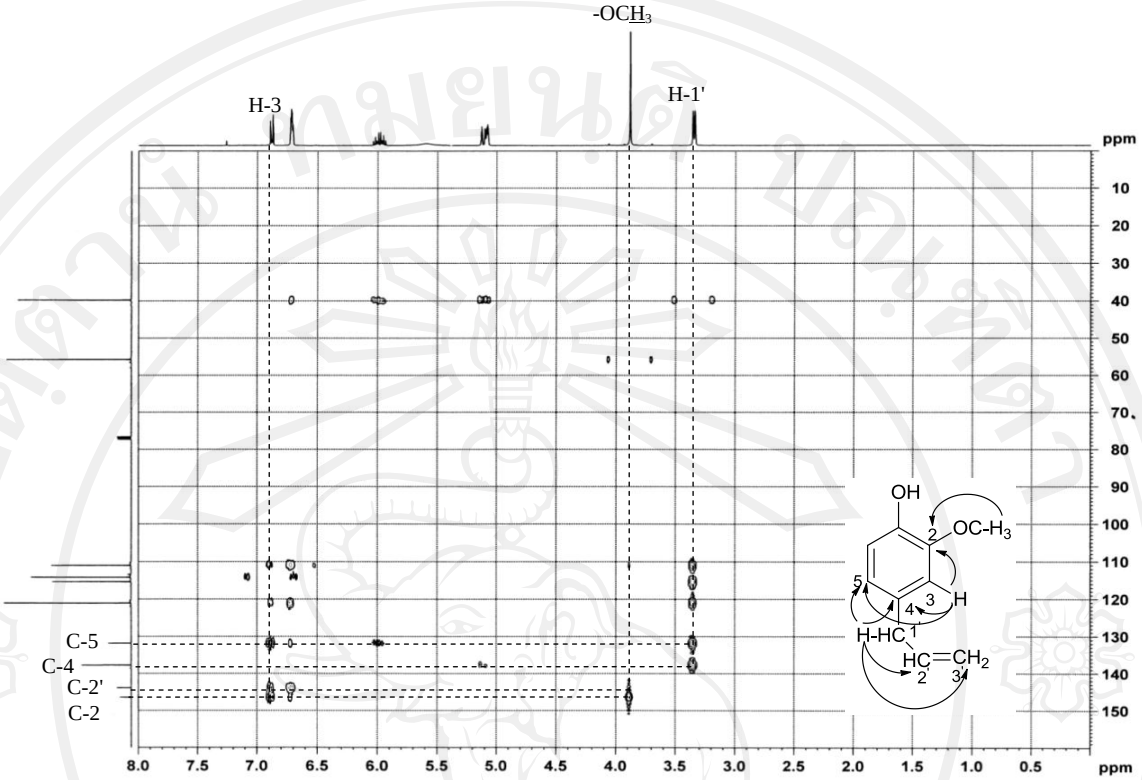


Figure 11 Selected HMBC correlation of eugenol (**22**)

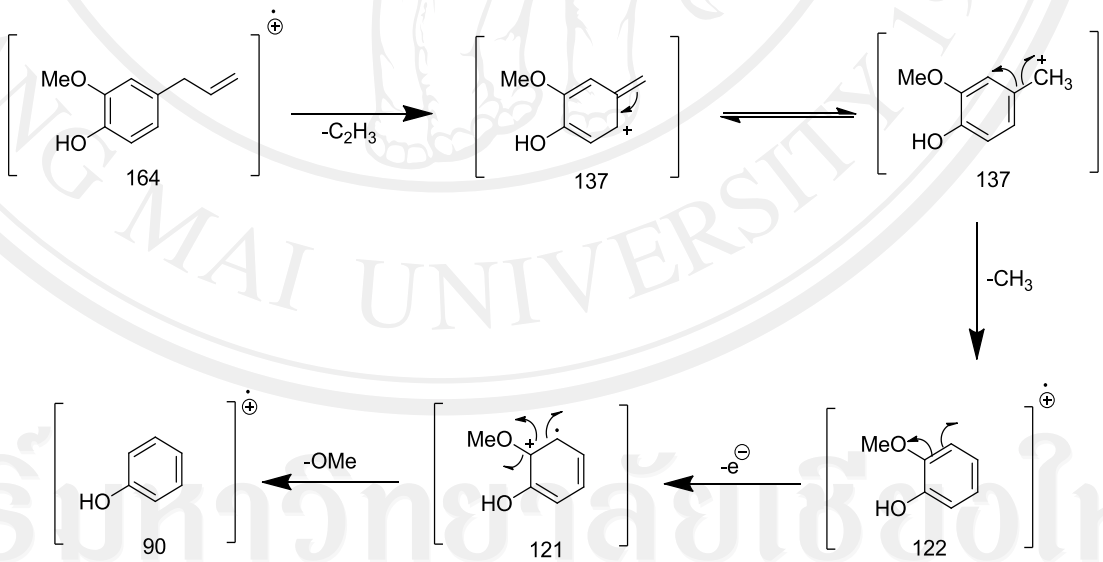


Figure 12 The fragmentation pathways for eugenol (**22**)

The structure of the mixture of phytosterol; *β*-sitosterol (**23**) and stigmasterol (**24**) were elucidated by spectroscopic techniques mainly ^{1}H NMR,^{13}C NMR, MS and IR (Figure 13-16, Table 6 and 7). The mixture of *β*-sitosterol (**23**) and stigmasterol

(**24**) obtained as colorless crystals. Melting point was found in a range of 133 – 136 °C.[34, 35] The EI-MS spectrum of these mixture compounds manifested a molecular ion peak at 412 and 414, subsequently which corresponds to $C_{29}H_{48}O$ and $C_{29}H_{50}O$. Explaining mechanism of their fragmentation is depicted in Figure 16.[33]

Its IR spectrum exhibited hydroxyl group around 3390-3430 $cm^{-1}$and olefinyl moieties at 1625 cm^{-1}.[31, 32] In the ^{1}H NMR spectrum (Figure 14), the major difference signals were the presence of olefinic protons at 5.06 (*dd*, 1H, *J* = 8.6, 8.7 Hz) and 5.19 (*dd*, 1H, *J* = 8.5, 8.6 Hz) which assigned to H-22 and H-23 corresponded to C-22 (138.3) and C-23 (129.7) of stigmasterol.[27, 28] The others signals 5.35 (*d*, 1H, *J* = 5.2 Hz) and 71.8 ppm were assigned to H-6 of both *β*-sitosterol and stigmasterol.[37-39] The intregation areas of H-6, H-22 and H-23 appeared to be in the ratio of 1.02:0.37:0.28. By directed comparison, of ^{1}H and ^{13}C NMR data to the previously reported compound.[33] They could be deduced that the mixture of *β*-sitosterol and stigmasterol was in ratio of 78:22.

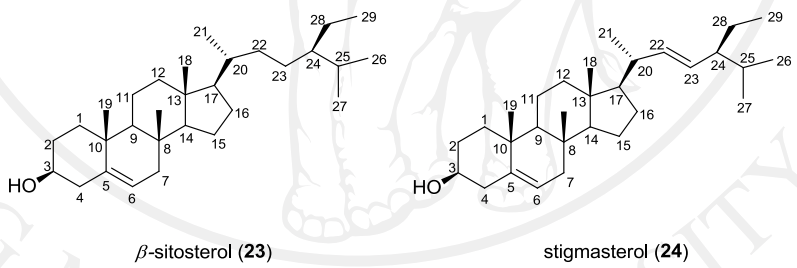


Figure 13 Labelling number of each carbon in structure of *β*-sitosterol (**23**) and stigmasterol (**24**)

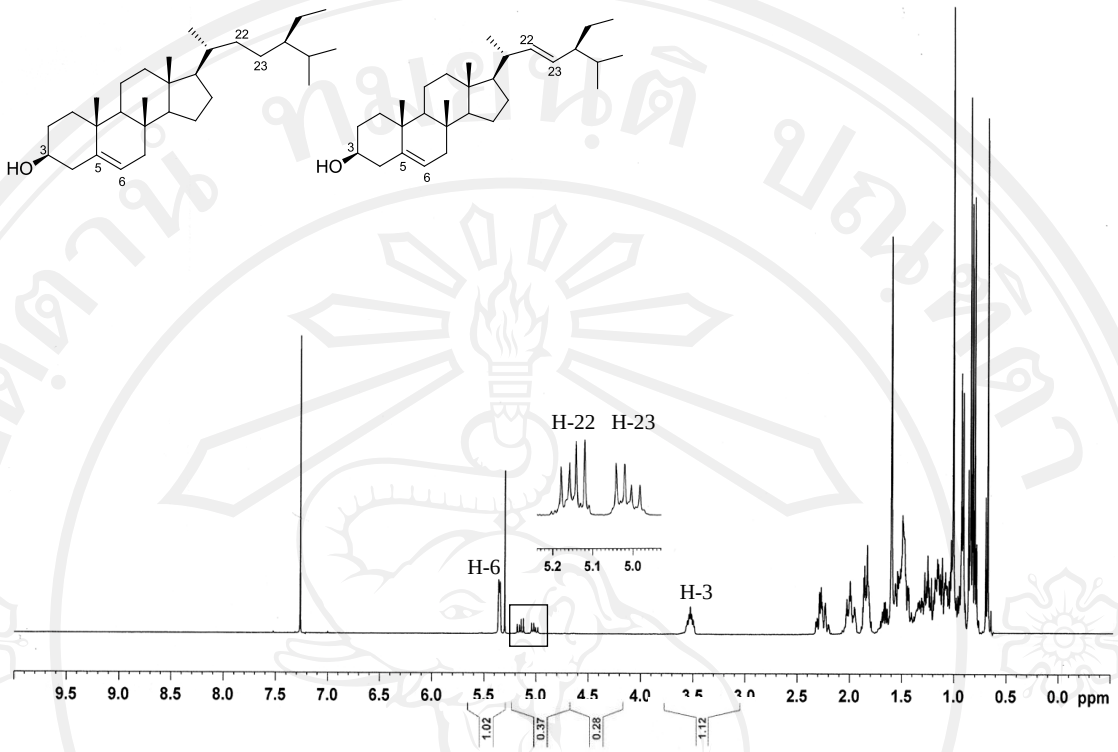


Figure 15 ^{1}H NMR spectrum of *β*-sitosterol (**23**) and stigmasterol (**24**)

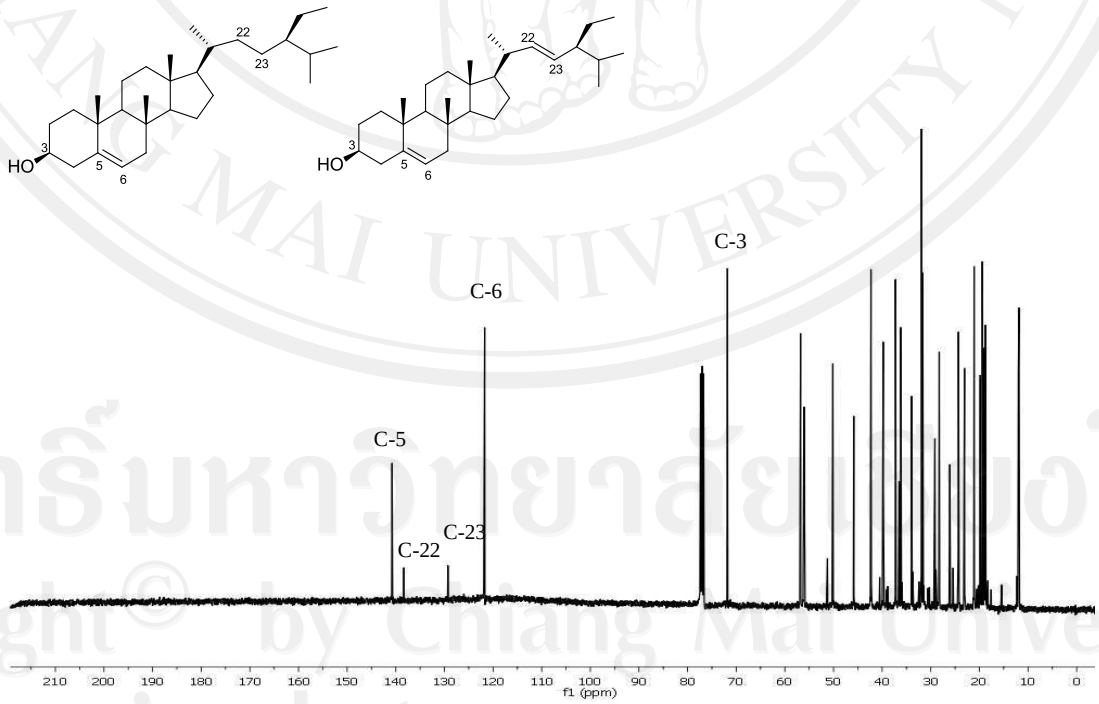


Figure 16 ^{13}C NMR spectrum of *β*-sitosterol (**23**) and stigmasterol (**24**)

Table 5 ^{1}H NMR data of β-sitosterol (**23**) and stigmasterol (**24**) ($CDCl_3$) at 400 MHz

Chemical shift (δ) in ppm		
^{1}H NMR (from experimental)	^{1}H NMR (from reference)[37]	$\lvert\Delta\delta_H\rvert$(ppm)
0.71 [*s*]	0.71 [*s*]	0.00
0.68 [*s*]	0.73 [*s*]	0.05
0.84 [*d*, *J* = 6.5 Hz]	0.85[*d*, *J* = 6.4 Hz]	0.01
0.86 [*d*, *J* = 1.5 Hz]	0.86[*d*, *J* = 6.8 Hz]	0.00
0.92 [*d*, *J* = 4.0 Hz]	0.88[*d*, *J* = 6.8 Hz]	0.04
1.02 [*s*]	1.04[*s*]	0.02
1.05 [*s*]	1.06[*s*]	0.01
1.21-1.30 [*m*]	1.21-1.30 [*m*]	0.00
1.40-1.65 [*m*]	1.45-1.65 [*m*]	0.05
1.64-1.71 [*m*]	1.67-1.70 [*m*]	0.01
1.80-1.89 [*m*]	1.84-1.90 [*m*]	0.03
2.24-2.33 [*m*]	2.24-2.30 [*m*]	0.03
3.48-3.55 [*m*]	3.53-3.59 [*m*]	0.01

Table 6 ^{13}C NMR data of β-sitosterol (**23**) and stigmasterol (**24**) ($CDCl_3$) at 100 MHz

Chemical shift (δ) in ppm		
^{13}C NMR (from experimental)	^{13}C NMR (from reference)[37]	$\lvert\Delta\delta_c\rvert$(ppm)
11.8-19.0	12.0-19.0	0.2
21.3-56.8	21.1- 56.8	0.2
71.9	71.8	0.1
121.7	121.7	0.0
129.7	129.1	0.6
138.3	138.2	0.1
140.7	140.8	0.1

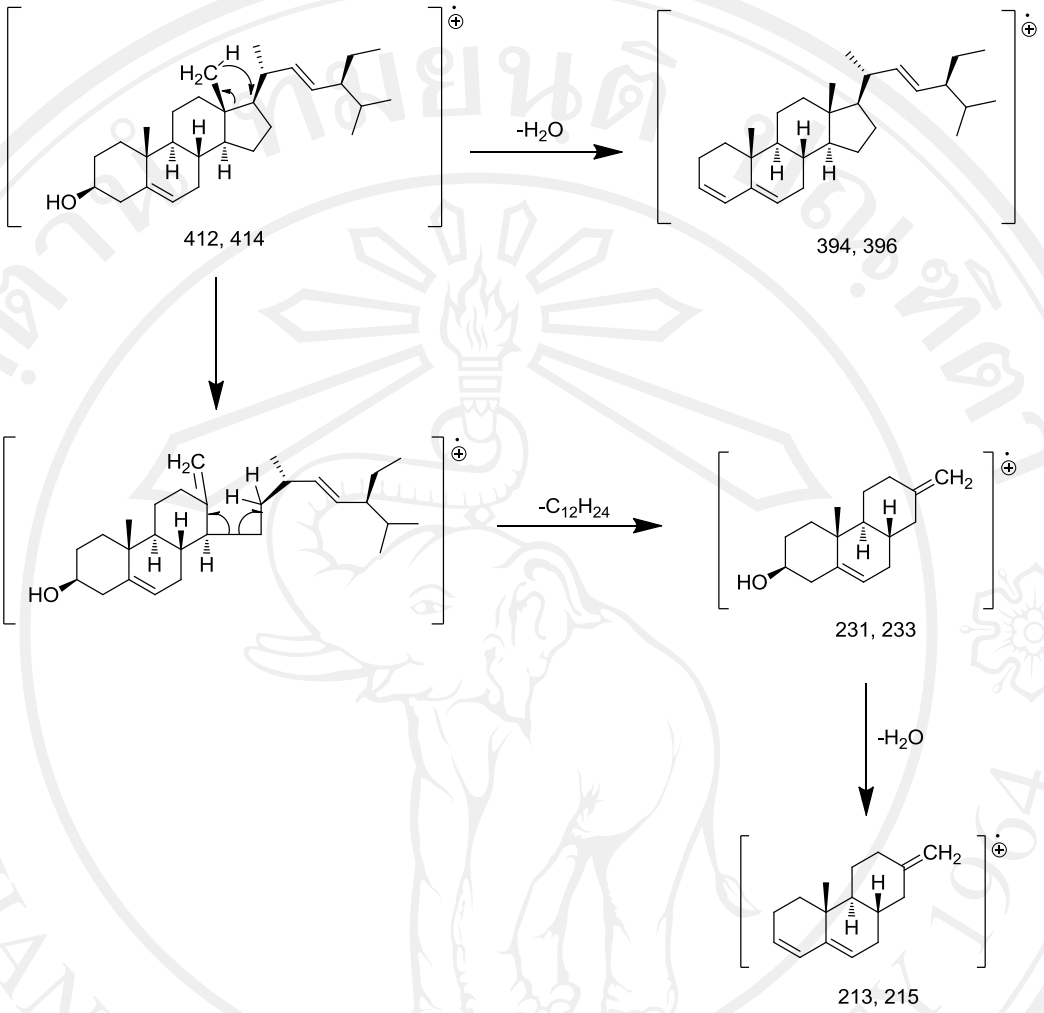
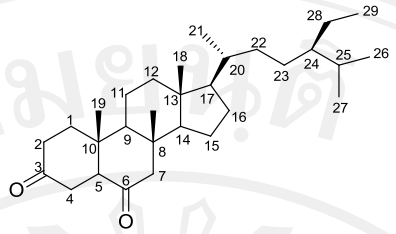


Figure 16 The fragmentation pathways for *β*-sitosterol (**23**) and stigmasterol (**24**)

The structure of stigmastan-3,6-dione (**25**) (Figure 17) was elucidated by spectroscopic techniques mainly ^{1}H NMR,^{13}C NMR, DEPT, COSY, HMQC, HMBC, MS and IR (Figure 18-23, Table 8 and 9). Stigmastan-3,6-dione (**25**); which called 5α-stigmastane-3,6-dione in the systemetic name. It was a white amorphous crystal, mp 192.0 – 194.0 °C, $[\alpha]^{29.3}_{D}$ +50.0° which corresponding to $C_{29}H_{48}O_2$ by means of EI-MS measurement on the $[M^+]$ 428.[39, 43] The probability of their fragmentation of compound **25** can describe with earlier report [40], as shown in Figure 23. Absorption bands of IR spectra associated with two ketonic in the region 1716 and 1708 cm^{-1}.[39, 40]



stigmastan-3,6-dione (**25**)

Figure 17 Labelling number of each carbon in structure of stigmastan-3,6-dione (**25**)

Additionally, the ^{1}H NMR (Figure 18) data are attributed to three active methylene groups of H-2, H-4, H-7 at 2.00-2.59 (*m*, 6H) and one methine proton at 2.59 (*m*, H-5) adjacent of two carbonyl groups which related with the interpretation of ^{1}H–^{1}H COSY spectrum (Figure 20). The signal at δ 2.33, 2.57 (H-2, 2H) showed correlation with signal proton at δ 2.36, 2.59 (H-4), 1.63 and 2.11 (H-1). At 2.59 ppm (H-5, 1H) showed the correlation with H-4 and H-7. In methyl region (0.69, 0.80, 0.84, 0.86, 0.92 and 0.95 ppm, 18H) assigned to the six methyl groups of two angular and four side-chain methyl.

The ^{13}C NMR and DEPT spectra (Figure 19) displayed 29 carbon signals. Two carbonyl carbon of ketone at 211.2 (C-3), 209.1 (C-6), which were confirmed by the presence of the HMBC spectra (Figure 22). It displayed that the signal of methylene proton (H-1) at (1.63, 2.11 ppm) with C-2, C-3 and C-19, H-2 (2.33, 2.57 ppm) with C-1 and C-3, H-4 (2.36, 2.59 ppm) with C-2, C-5 and also H-5 (2.59 ppm) with C-4 and C-7. In ^{1}H–^{13}C directed correlation observed in HMQC (Figure 21) experiment verified that protons on C-2 position at 2.33 and 2.57 ppm were correlated with C-2, two protons on C-4 at 2.36 and 2.59 ppm had relationship with C-4 and proton on C-5 position at 2.59 ppm was bonded with C-5 that adjoined with two quaternary carbons of ketone group. From above the evidence, compound **25** was determined as stigmastan-3,6-dione as reported previously.[40-44]

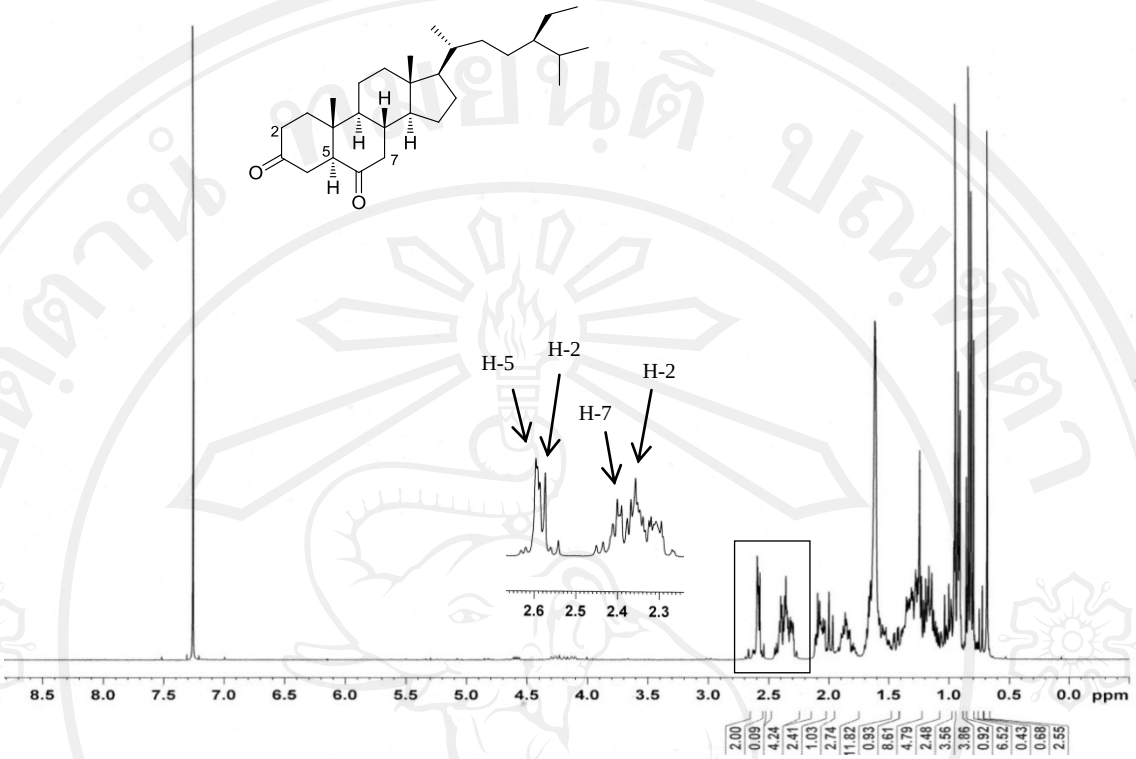


Figure 18 ^{1}H NMR spectrum of stigmastan-3,6-dione (**25**)

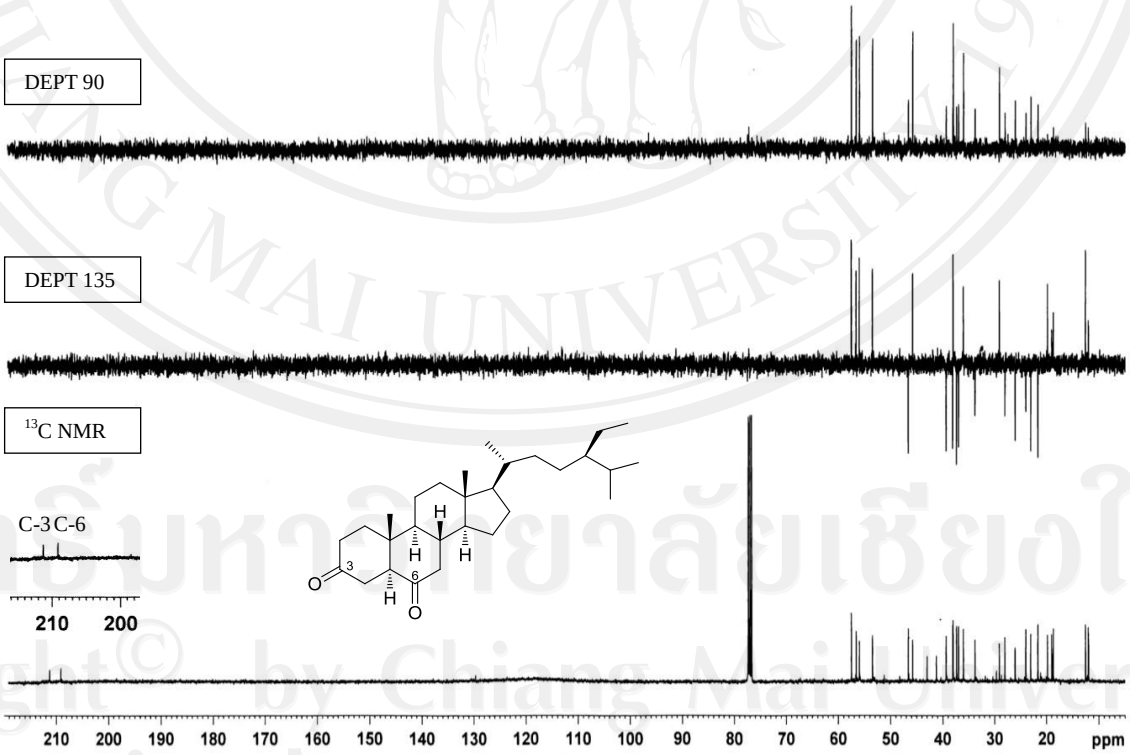


Figure 19 DEPT 90, DEPT 135 and ^{13}C NMR spectra of stigmastan-3,6-dione (**25**)

Table 7 ^{1}H and ^{13}C NMR data of stigmastan-3,6-dione (**25**) ($CDCl_3$) at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR)

Position	Chemical shift (δ) In ppm	
	^{1}H NMR	^{13}C NMR
1	1.63, 2.11 [*m*, 2H]	37.9
2	2.33, 2.57 [*m*, 2H]	37.0
3	-	211.3
4	2.36, 2.59 [*m*,2H]	38.1
5	2.59 [*m*, 1H]	57.5
6	-	209.3
7	2.00, 2.38 [*m*, 2H]	46.6
8	1.85 [*m*, 1H]	38.0
9	1.33 [*m*, 1H]	53.5
10	-	41.3
11	1.42, 1.65 [*m*, 2H]	21.7
12	1.25, 2.06 [*m*, 2H]	56.0
13	-	24.0
14	1.25 [*m*, 1H]	28.0
15	1.53 [*m*, 2H]	24.0
16	1.31 [*m*, 2H]	28.0
17	1.16 [*m*, 1H]	56.6
18	0.69 [*s*, 3H]	11.9
19	0.95 [*s*, 3H]	12.6
20	1.37 [*m*, 1H]	36.0
21	0.92 [*d*, 3H, *J* = 6.5 Hz]	18.7
22	1.02, 1.34 [*m*, 2H]	33.8
23	1.16 [*m*, 2H]	26.1
24	0.92 [*m*, 1H]	45.8
25	1.25 [*m*, 1H]	29.1
26	0.84 [*d*, 3H, *J* = 6.5 Hz]	19.8

Table 7 ^{1}H and ^{13}C NMR data of stigmastan-3,6-dione (**25**) ($CDCl_3$) at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) (Cont.)

Position	Chemical shift (δ) in ppm	
	^{1}H NMR	^{13}C NMR
27	0.84 [*d*, 3H, *J* = 7.1 Hz]	19.0
28	1.21, 1.28 [*m*, 2H]	23.1
29	0.86 [*t*, 3H, *J* = 5.8, 7.7 Hz]	12.0

Table 8 ^{1}H–^{1}H COSY, HMQC, HMBC data of stigmastan-3,6-dione (**25**) ($CDCl_3$) at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR)

Proton position	^{1}H–^{1}H COSY (Coupling of H)	HMQC (Correlation of C)	HMBC (Correlation of C)
1	H-2	C-1	C-2, 3, 19
2	H-1, 4	C-2	C-1, 3
3	-	-	-
4	H-2, 5	C-4	C-3, 4, 6, 10
5	H-4, 7	C-5	C-3, 6, 10
6	-	-	-
7	H-8	C-7	C-5, 6, 8, 9
8	H-7, 9, 14	C-8	C-7, 9, 11, 14
9	H-8, 11	C-9	C-8, 10, 11, 14
10	-	-	-
11	H-12	C-11	C-9, 12, 13, 19
12	H-11, 14	C-12	C-9, 11, 13
13	-	-	-
14	H-8, 12	C-14	C-8, 9, 13, 15
15	H-14, 17	C-15	C-14, 16, 17
16	-	-	-
17	H-15	C-17	C-16, 17, 18, 20
18	-	-	-

Table 8 ^{1}H–^{1}H COSY, HMQC, HMBC data of stigmastan-3,6-dione (**25**) ($CDCl_3$) at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) (Cont.)

Proton position	^{1}H–^{1}H COSY (Coupling of H)	HMQC (Correlation of C)	HMBC (Correlation of C)
19	-	-	-
20	H-21, 22	C-20	C-23
21	H-20, 22	C-21	C-17, 20, 22
22	H-20, 23	C-22	C-21, 23
23	H-28	C-23	C-22, 24
24	H-22, 23	C-24	C-23, 25, 28
25	H-26, 27	C-25	C-24, 26, 27
26	H-25, 26	C-26	C-24, 28
27	H-25, 26	C-27	C-24, 25, 26
28	H-21	C-28	C-23, 24, 25, 29
29	H-25	C-29	C-24, 28

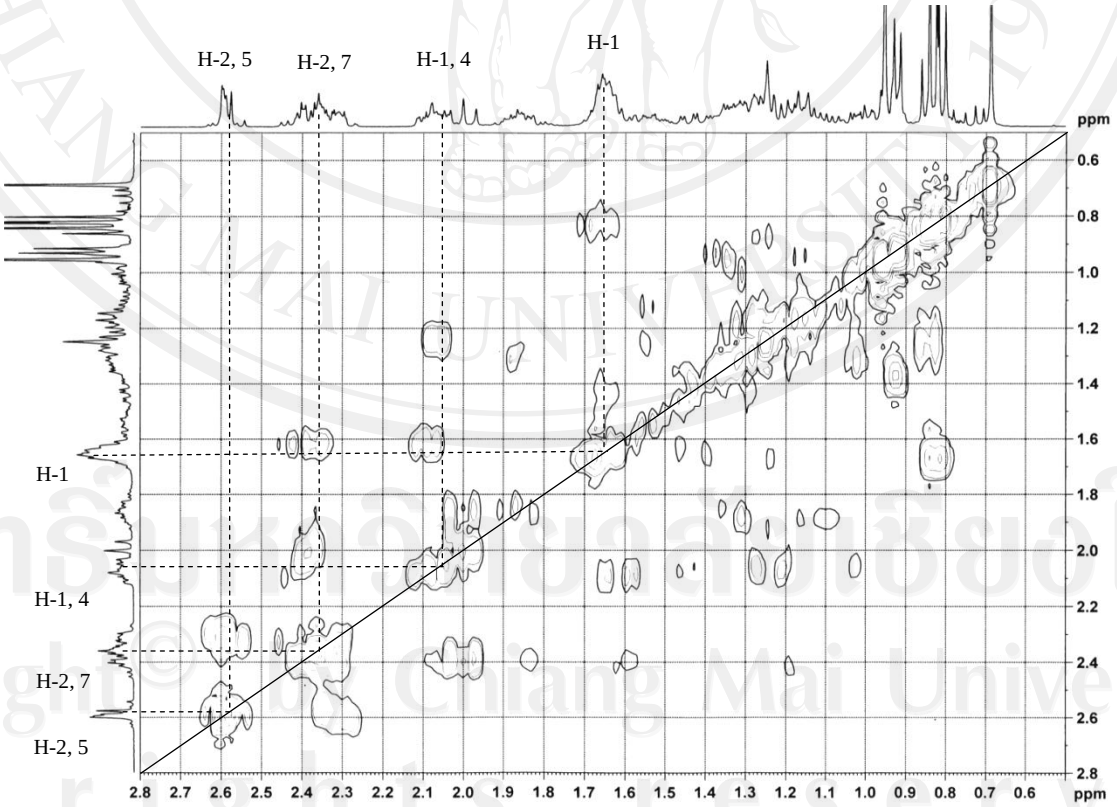


Figure 20 ^{1}H–^{1}H COSY spectrum of stigmastan-3,6-dione (**25**)

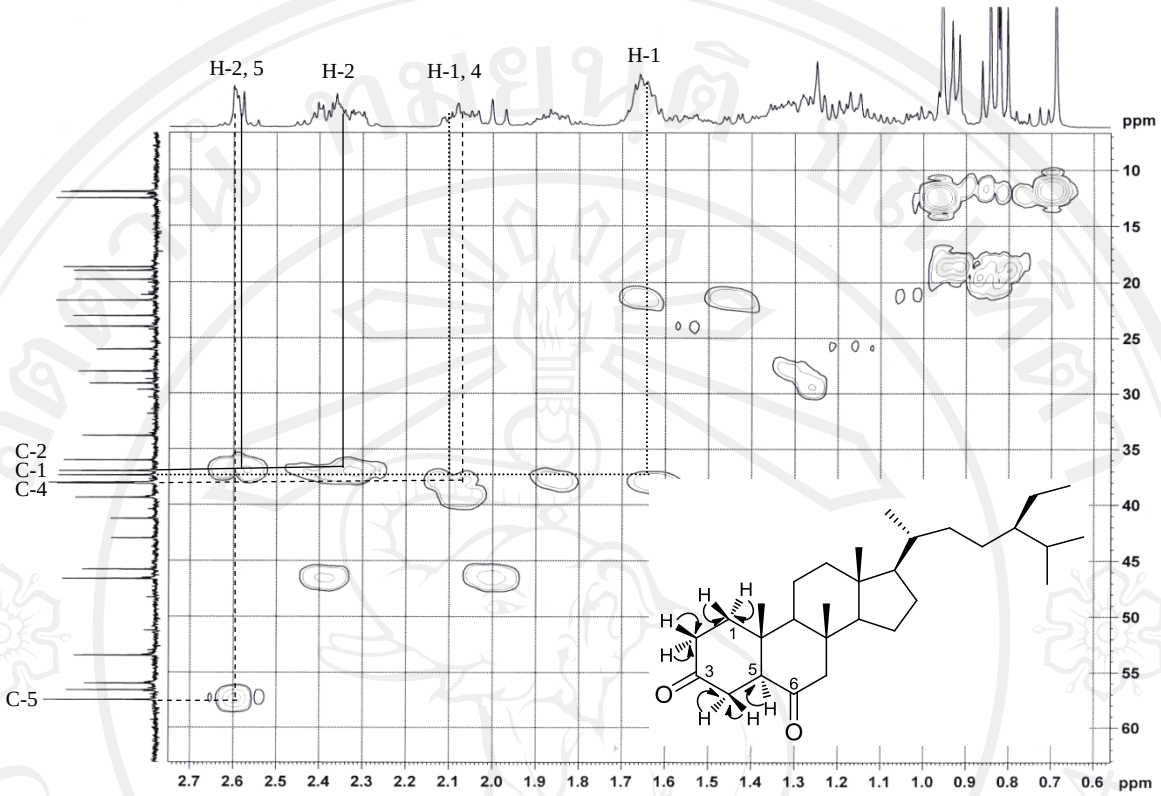


Figure 21 Selected HMQC correlation of stigmastan-3,6-dione (**25**)

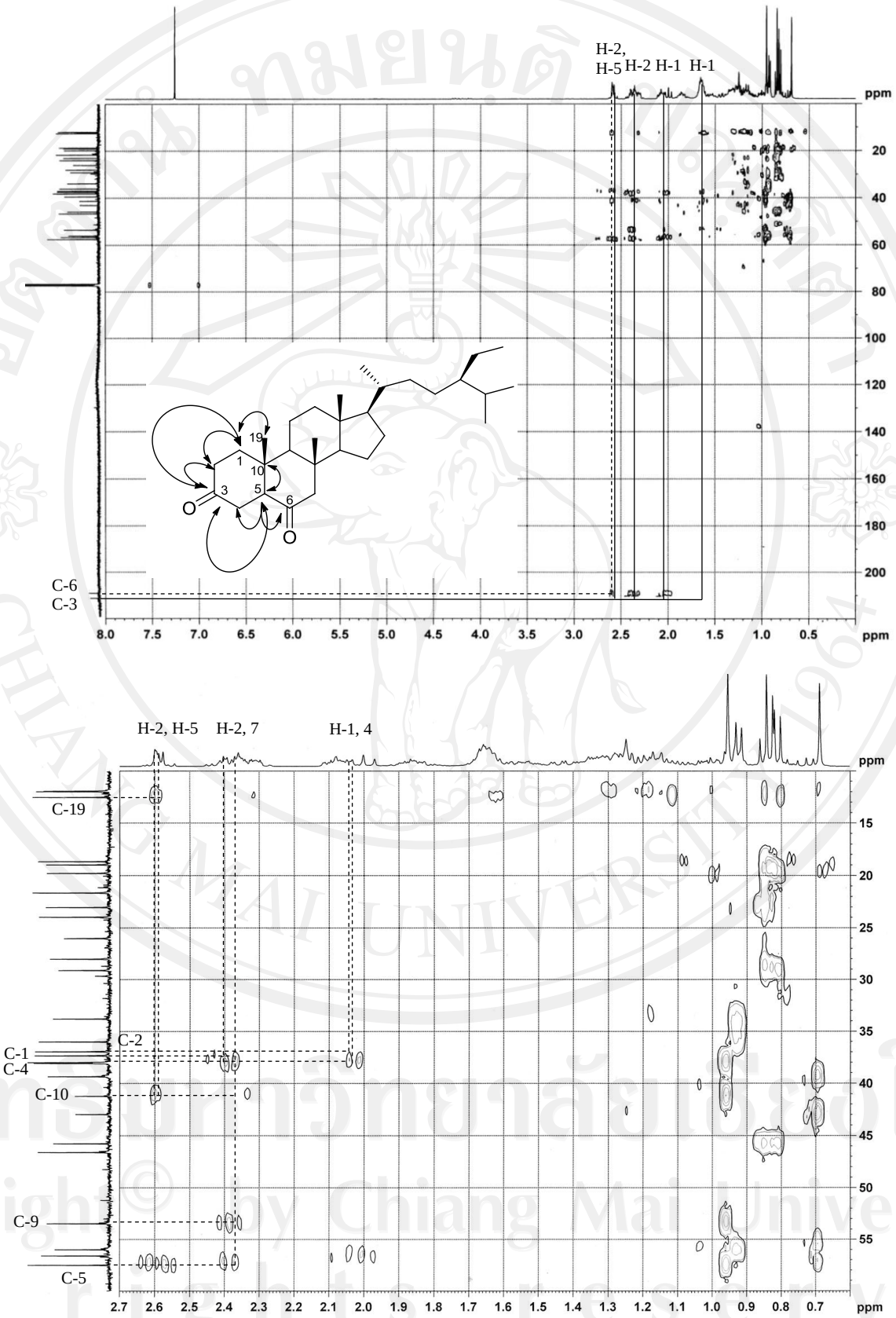


Figure 22 Selected HMBC correlation of stigmastan-3,6-dione (**25**)

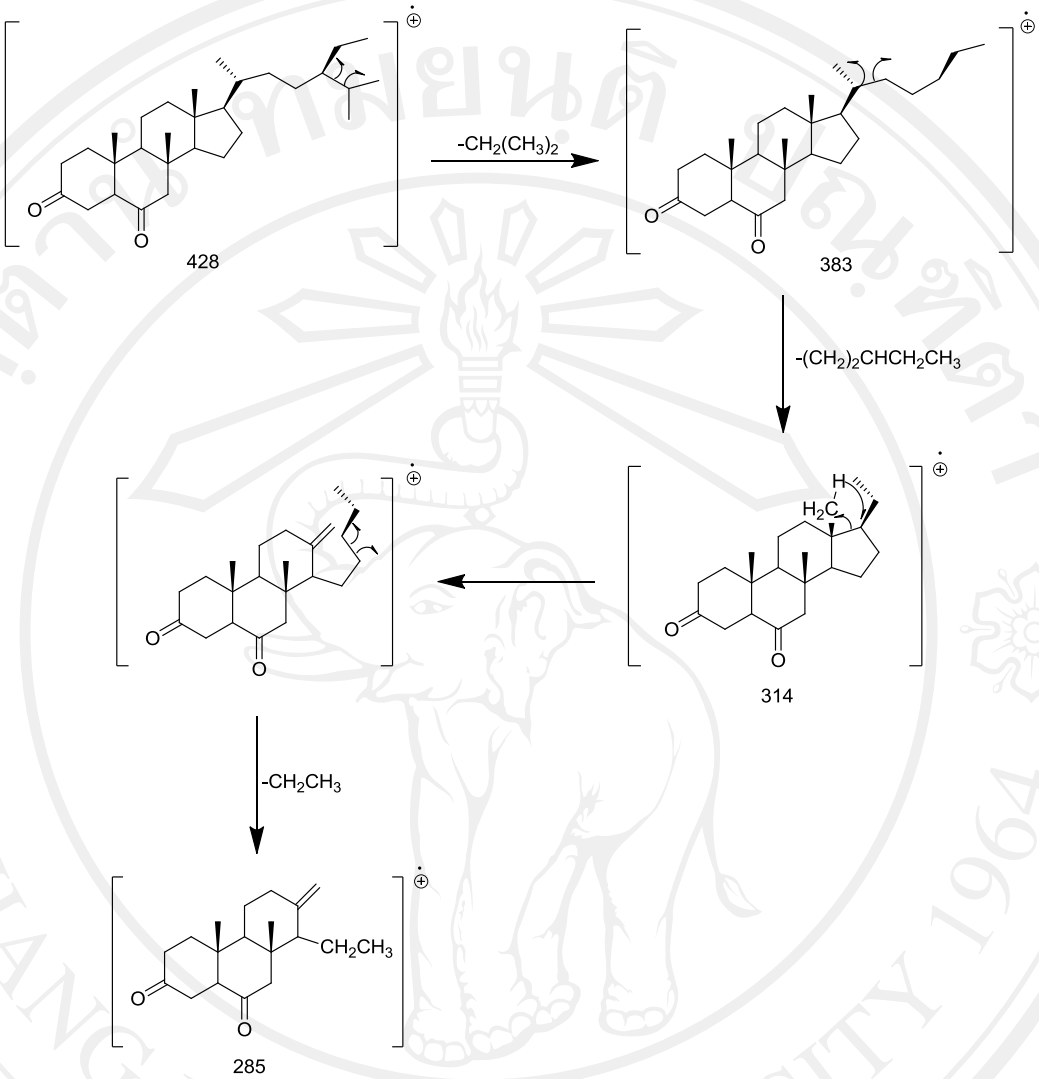
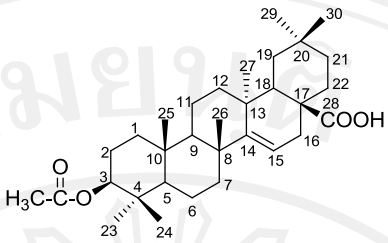


Figure 23 The fragmentation pathways for stigmastan-3,6-dione (**25**)

The structure of 3-acetyl aleuritolic acid (**26**) (Figure 24) was elucidated by spectroscopic techniques mainly ^{1}H NMR,^{13}C NMR, DEPT, COSY, HMQC, HMBC, MS and IR (Figure 25-30, Table 11 and 12). Compound **26**, as furnished white needles, mp 304 – 306°C, $[\alpha]^{29.3}_{D}$ +124.8°. The IR spectrum exhibited nearly identical absorption bands of carboxylic acid at 3434, 1733 and 1245 cm^{-1}attributable to acetoxy group, the C–O–C linkage of ether at 1026 cm^{-1} moieties and 1689 cm^{-1} for olefin group.[47, 49]

In the 400 MHz ^{1}H NMR (Figure 25), manifested a characteristics singlet at 2.04 ppm assigned to acetoxy protons, a doublet by doublet centered at δ 5.50 (*d*, H-15, *J* = 7.9, 3.3 Hz) indicating a proton on a trisubstituented double bond. Another doublet pattern downfield at δ 4.46 (*d*, H-3, *J* = 11.5, 6.3 Hz) assigned to the ubiquitous 3 α methane proton germinal to the acetoxyl group of triterpenes skeleton. In the region of 0.85-0.98 ppm, seven methyl signals appeared as singlet.

The ^{13}C NMR spectrum, in combination with DEPT experiments (Figure 26), demonstrated the presence of 32 carbon resonances which composed of seven methyl, ten methylene, five methine and seven quaternary carbons.[49] The signals at 171.0 and 183.6 ppm indicated the presence of carbonyl function of acetoxy and carboxylic acid. The acetyl group was assigned at 80.9 ppm (C-3) due to the presence of the HMBC correlations (Figure 29) between H-3 with carbonyl carbon of the acetyl group at 171.0 ppm and double bond was located at 116.8 and 160.6 ppm, as determined by cross-peaks at 5.50 ppm (H-15) with C-8, C-12 and C-17. Furthermore, the direct C-H bond correlation HMQC technique (Figure 28) was established. It revealed that the C-9, C-15 and C-18 were methine. The exploration of COSY spectrum (Figure 27) allowed us to confirm compound **26** by the observation of H-2, H-3, H-5, H-6 and H-7 in the A and B rings of pentacyclic skeketon. Full assignments of the ^{1}H and ^{13}C NMR signals were accomplished using; ^{1}H–^{1}H COSY, HMQC and HMBC experiments. This compound is a mono acetoxy pentacyclic triterpenes with mono carboxylic acid and appeared to be identical with the earlier report of 3-acetyl aleuritolic acid.[49-52]



3-acetyl aleuritolic acid (**26**)

Figure 24 Labelling number of each carbon in structure of 3-acetyl aleuritolic acid (**26**)

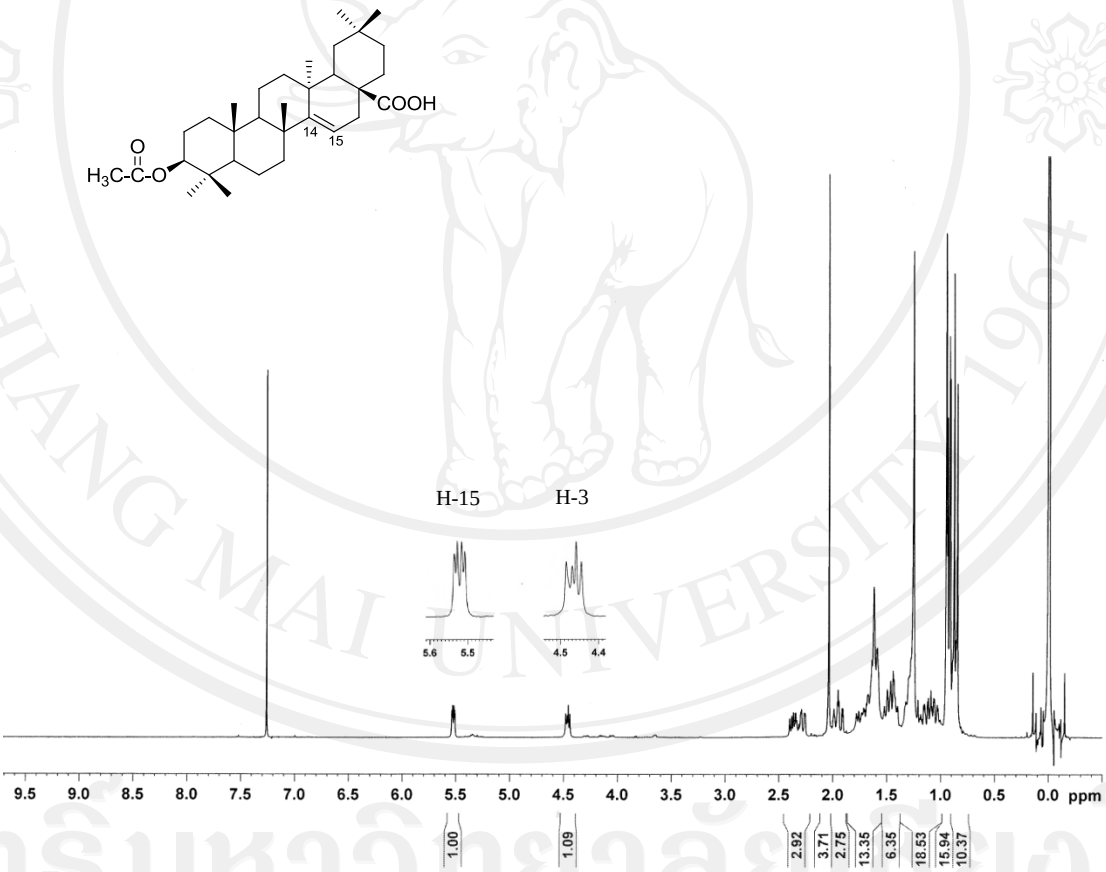


Figure 25 ^{1}H NMR spectrum of 3-acetyl aleuritolic acid (**26**)

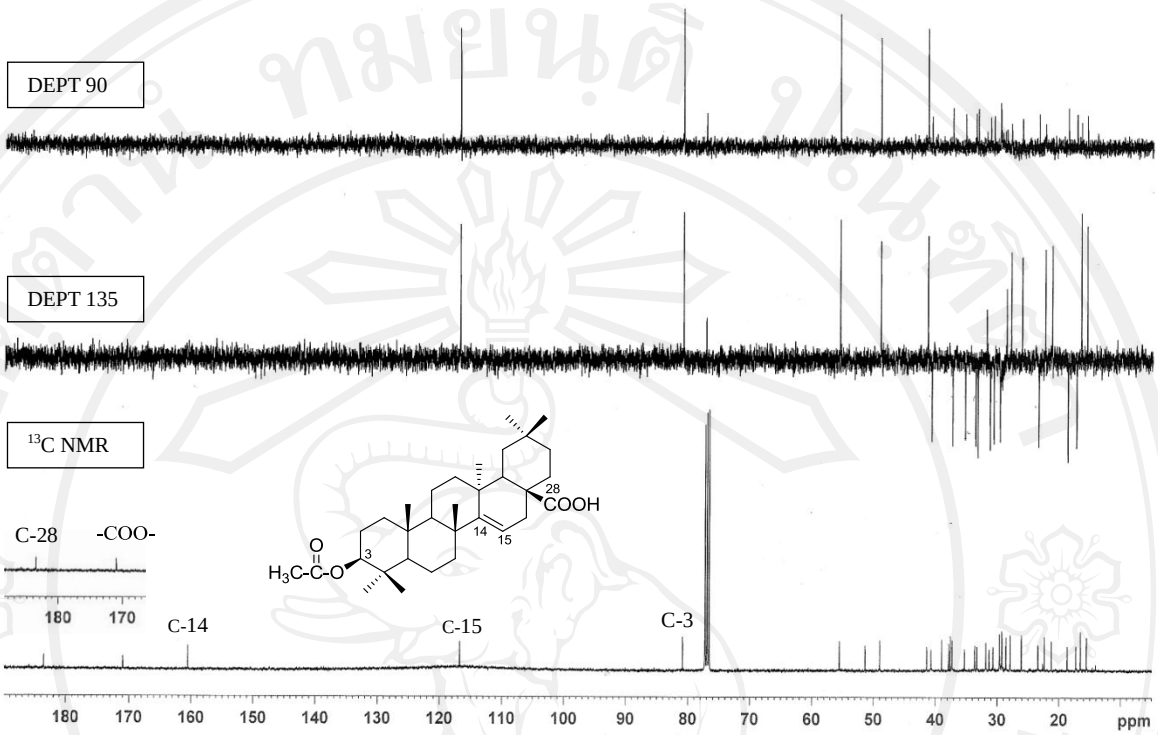


Figure 26 DEPT 90, DEPT 135 and ^{13}C NMR spectra of 3-acetyl aleuritolic acid (**26**)

Table 9 ^{1}H and ^{13}C NMR data of 3-acetyl aleuritolic acid (**26**) ($CDCl_3$) at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR)

Position	Chemical shift (δ) in ppm	
	^{1}H NMR	^{13}C NMR
1	0.99-1.03, 1.59-1.62 [*m*, 2H]	37.4
2	1.65, 1.74 [*obsc*, 2H]	23.5
3	4.46 [*dd*, 1H], J = 11.5, 6.3 Hz	80.9
4	-	37.7
5	0.85 [*m*, 1H]	55.6
6	0.97, 1.24 [*m*, 2H]	18.7
7	1.41-1.44 [*m*, 2H]	40.8
8	-	39.0

Table 9 ^{1}H and ^{13}C NMR data of 3-acetyl aleuritolic acid (**26**) ($CDCl_3$) at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) (Cont.)

Position	Chemical shift (δ), ppm	
	^{1}H NMR	^{13}C NMR
9	1.39-1.41 [*m*, 2H]	49.0
10	-	37.9
11	-	17.3
12	2.26-2.40 [*m*, 1H], 1.95 [*t*, 1H, *J* = 2.9, 3.4 Hz]	33.3
13	-	37.3
14	-	160.6
15	5.54 [*dd*, 1H, *J* = 7.9, 3.3 Hz]	116.8
16	1.69-1.72, 1.91-1.95 [*obsc*, 2H]	31.3
17	-	51.4
18	2.27 [*dd*, 1H], *J* = 2.2, 2.3 Hz	41.5
19	-	35.3
20	-	29.3
21	1.29 [*obsc*, 2H]	33.7
22	1.65 [*obsc*, 2H]	37.3
23	0.89 [*s*, 3H]	27.9
24	0.85 [*s*, 3H]	16.6
25	0.95 [*s*, 3H]	15.6
26	0.95 [*s*, 3H]	26.2
27	0.92 [*s*, 3H]	22.4
28	-	183.6
29	0.93 [*s*, 3H]	31.9
30	0.91 [*s*, 3H]	28.7
-O<u>C</u>OCH$_3$	-	171.0
-OCO<u>C</u>H$_3$	2.04 [*s*, 3H]	21.3

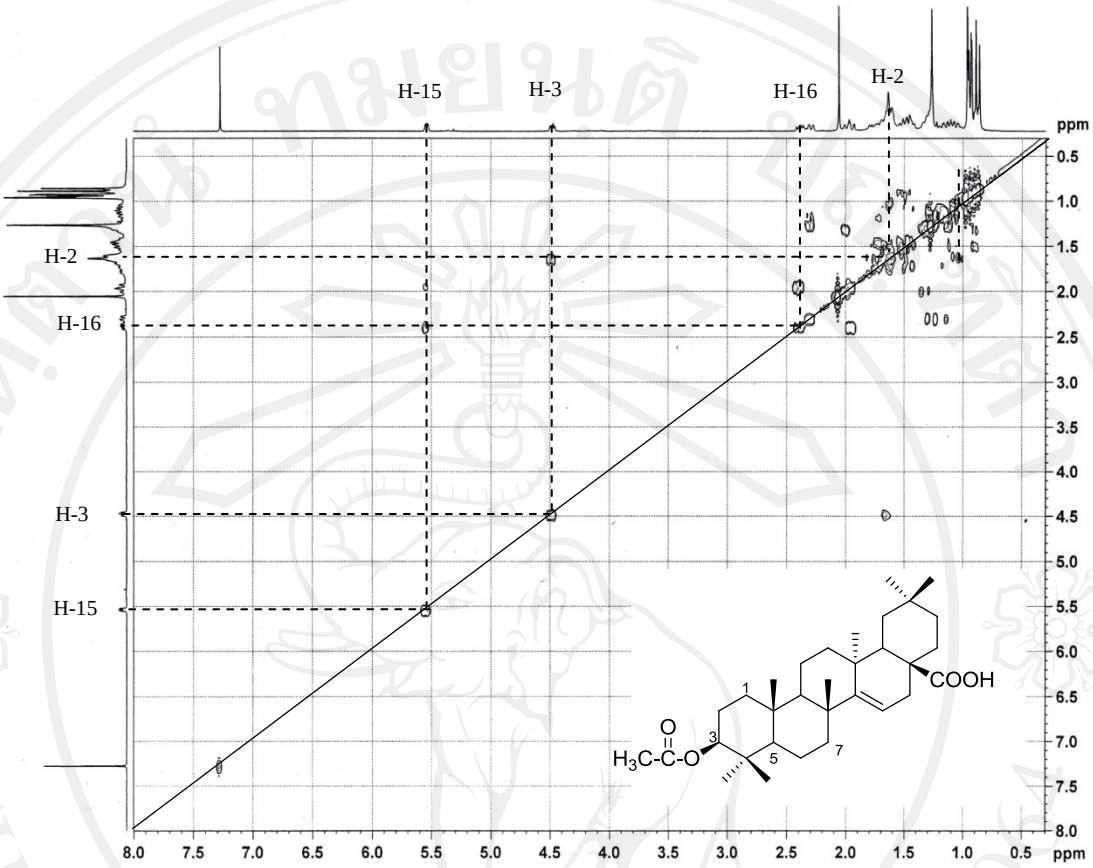


Figure 27 ^{1}H–^{1}H COSY spectrum of 3-acetyl aleuritolic acid (**26**)

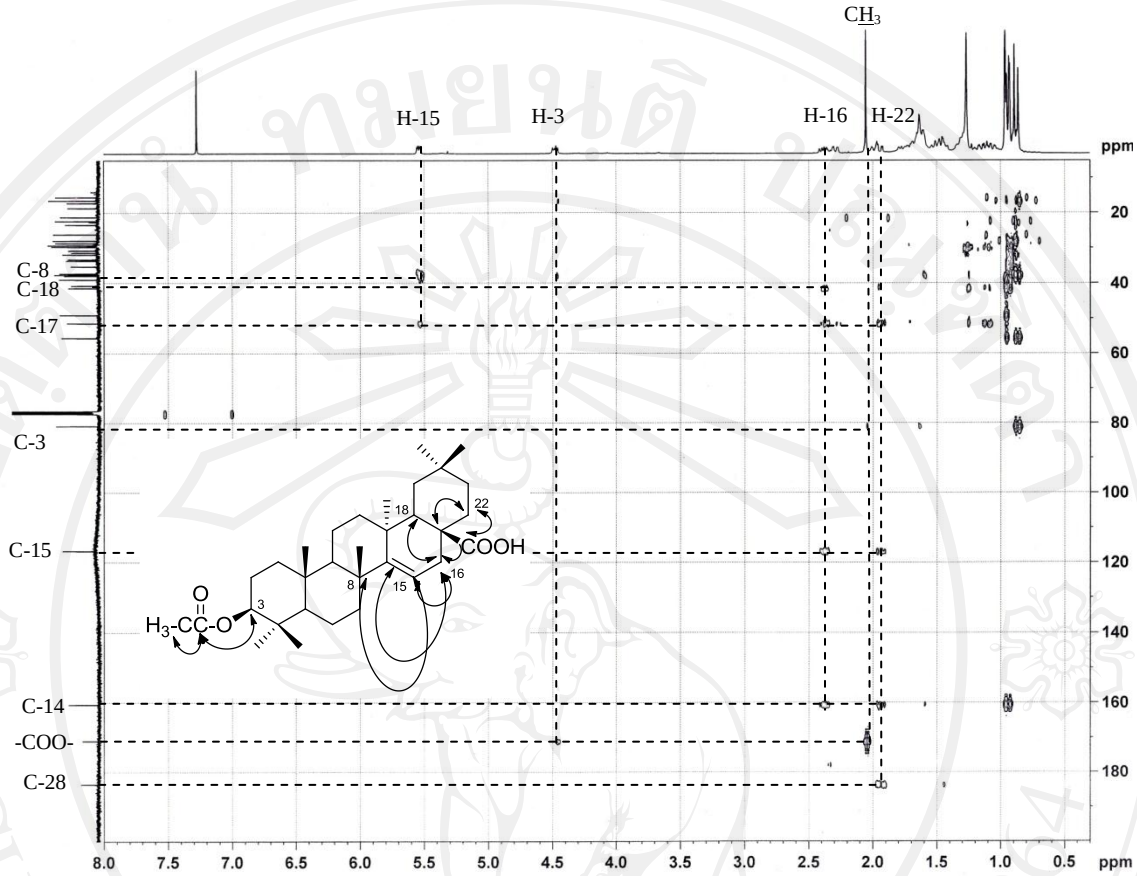


Figure 28 Selected HMQC correlation of 3-acetyl aleuritolic acid (**26**)

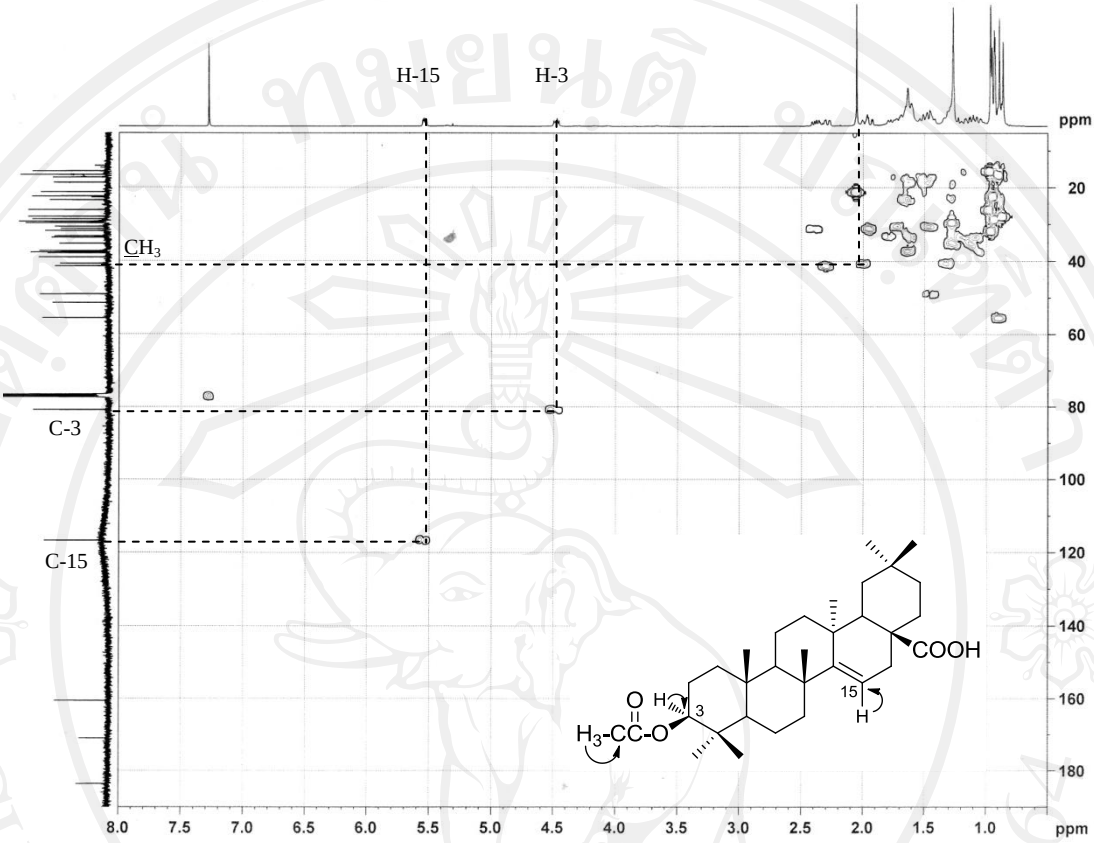


Figure 29 Selected HMBC correlation of 3-acetyl aleuritolic acid (**26**)

Table 10 ^{1}H–^{1}H COSY, HMQC, HMBC data of3-acetyl aleuritolic acid (**26**) ($CDCl_3$) at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR)

Proton position	^{1}H–^{1}H COSY (Coupling of H)	HMQC (Correlation of C)	HMBC (Correlation of C)
1	H-2, 5	C-1	C-3, 4
2	H-1, 3	C-2	C-3
3	H-2	C-3	C-2, 4, 6, -O<u>C</u>OCH$_3$
			-OCO<u>C</u>H$_3$
4	-	-	C-3, 6
5	H-6	C-5	C-7, 6, 9
6	H-5, 7	C-6	C-24
7	-	-	-
8	-	-	-

Table 10 $^{1}H–^{1}H$ COSY, HMQC, HMBC data of 3-acetyl aleuritolic acid (**26**) ($CDCl_3$) at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) (Cont.)

Proton position	$^{1}H–^{1}H$ COSY (Coupling of H)	HMQC (Correlation of C)	HMBC (Correlation of C)
9	-	-	-
10	-	-	-
11	-	-	-
12	H-15, 27	C-12	C-14, 15
13	-	-	-
14	-	-	-
15	H-12, 16	C-15	C-8, 12, 17
16	H-15	C-16	C-14, 15, 17, 18
17	-	-	-
18	H-27	C-18	C-13, 19
19	-	-	-
20	-	-	-
21	-	C-21	-
22	-	C-22	C-16, 17, 28
23	-	C-23	C-3, 4, 5
24	-	C-24	C-3, 4, 5
25	-	C-25	C-1, 5, 9, 10
26	-	C-26	C-14
27	-	C-27	C-13, 14, 18, 19
28	-	-	-
29	-	-OCO<u>C</u>H$_3$	C-19, 20, 21
30	-	C-30	C-19, 20, 21
-O<u>C</u>OCH$_3$	-	-	-
-OCO<u>C</u>H$_3$	-	-OCO<u>C</u>H$_3$	C-3, -O<u>C</u>OCH$_3$

From EI-MS method, the molecular ion peak [M^+] of this compound was not found in the experiment. However, the other key fragments were observed from mass spectrum at *m/z* 454, 249, 204 and 189 which was explained the mechanism in previous literature as indicated below Figure 27.[52]

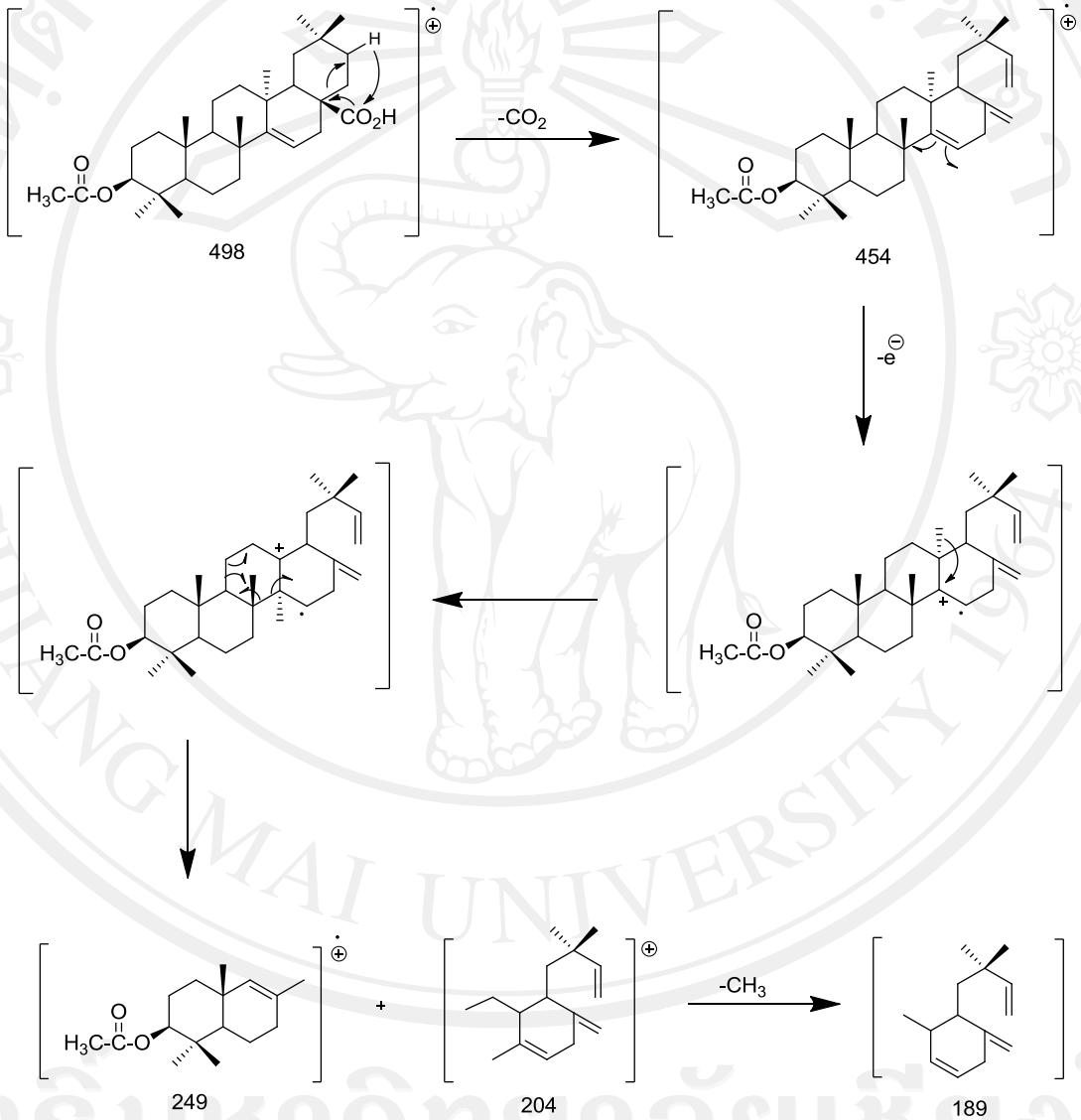


Figure 30 The fragmentation pathways for 3-acetyl aleuritolic acid (**26**)

4.2 Biosynthesis of isolated compounds

Eugenol (**22**) followed the pathway of shikimic acid pathway and synthesized from shikimic acid via the precursor of phenylalanine and coniferyl alcohol as depicted in Figure 31.[30,31]

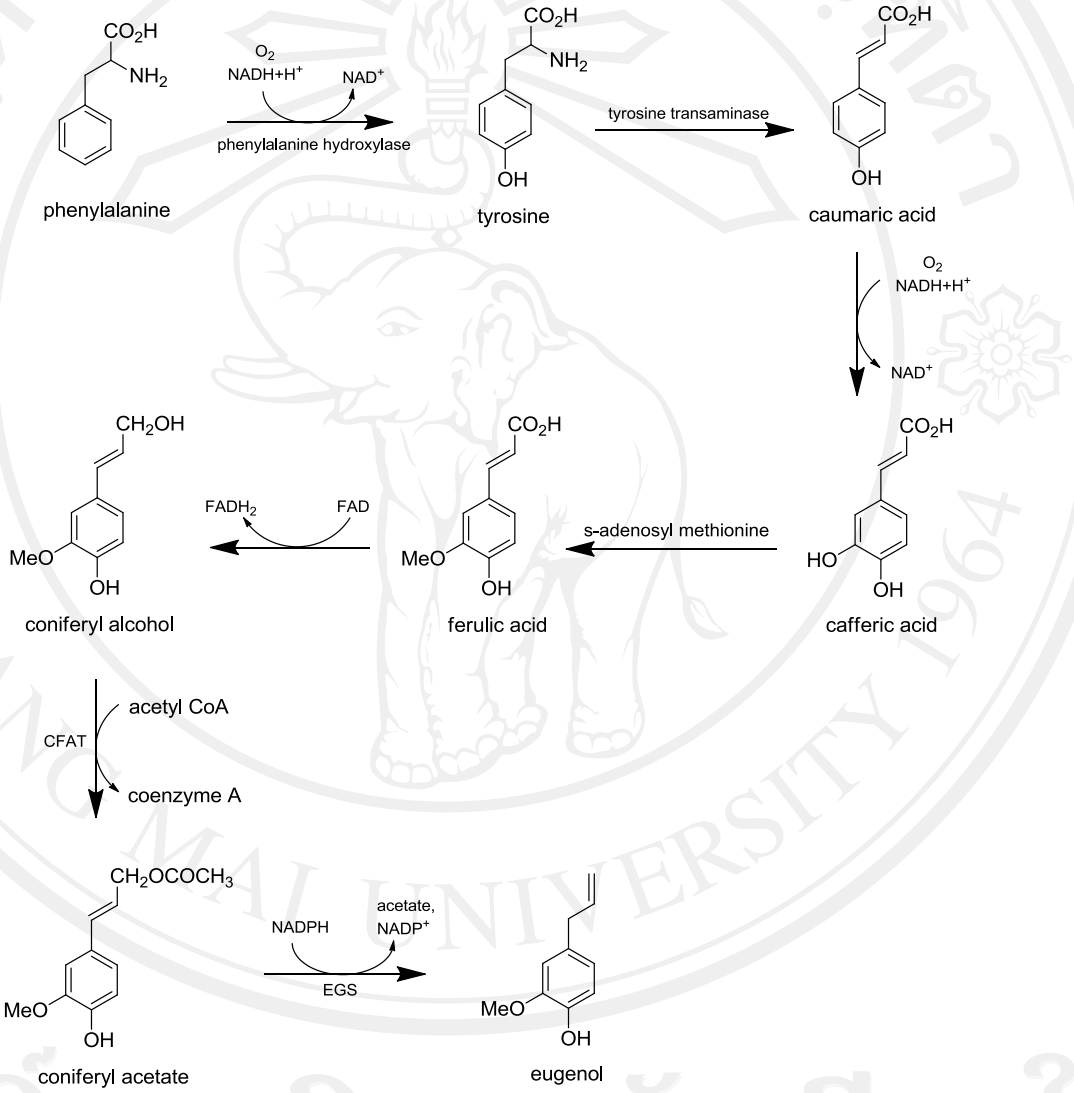


Figure 31 The biosynthesis of the eugenol (**22**)

β-sitosterol (**23**), stigmasterol (**24**) and stigmastan-3,6-dione (**26**) consist of six isoprene units. They derived from five-carbon of isoprenoids, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) to the formation of the C-30 and originated exclusively from the mevalonate pathway. They are mainly made up of cholesterol and their precursor are lanosterol as seen above in Figure 32.[20, 33, 40]

3-acetyl aleuritolic acid (**26**) was pentacyclic triterpenoids. They derived the acetate or mevalonate pathway. The pentacyclic system is generated from squalene in all-chair conformation. Squalene arises from an initial condensation of two molecules of farnesyl diphosphate (FPP) which undergoes a reductive rearrangement to squalene then converted to 2,3-squalene epoxide and by series of concerted cyclization as shown in Figure 33.[47, 51]

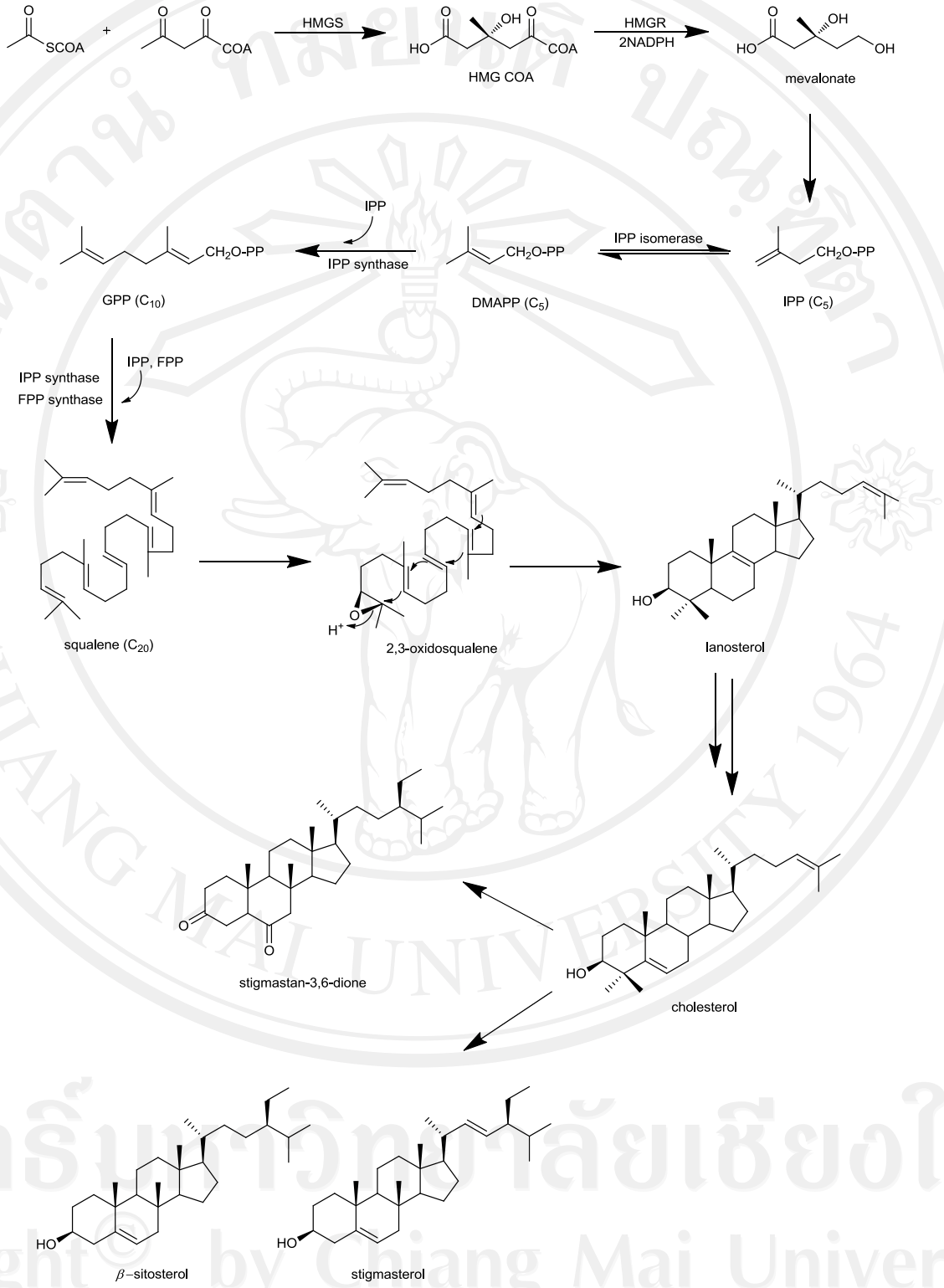


Figure 32 The biosynthesis of the *β*-sitosterol (**23**), stigmasterol (**24**) and stigmastan-3,6-dione (**26**)

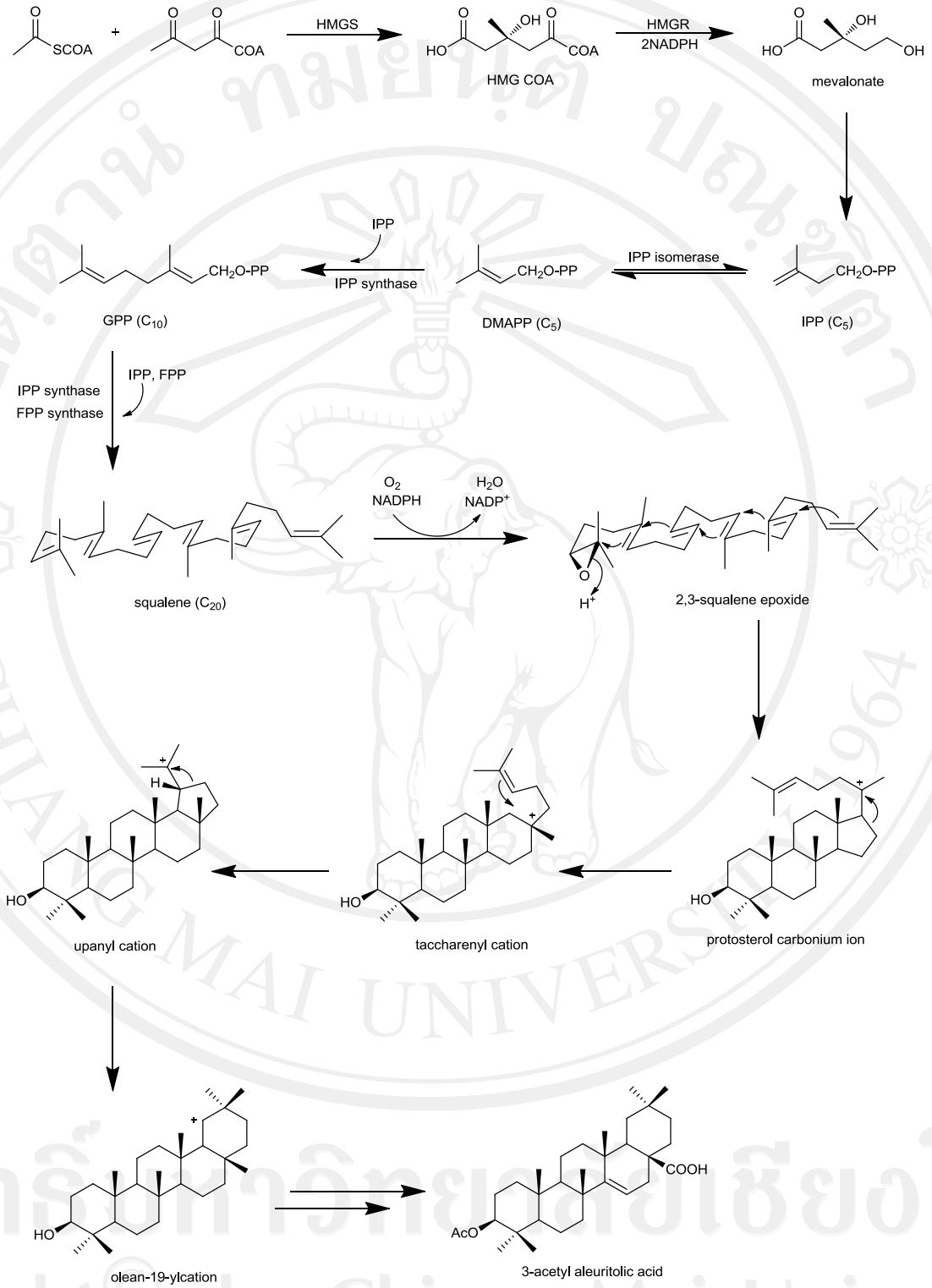


Figure 33 The biosynthesis of the 3-acetyl aleuritolic acid (**26**)