# CHAPTER III

# DISCUSSION

**Structural Elucidation of the Isolated Compounds from the bark of *Croton oblongifolius* Roxb.**

## 1. Structural Elucidation of Compound 1

The IR spectrum of compound 1 (Fig. 1) showed the presence of a carboxylic group as evidenced by the broad absorption band between 3000 and 3400 $cm^{-1}$ and the strong absorption band at 1690 $cm^{-1}$ due to the carboxylic acid carbonyl stretching. This latter adsorption band shifted to somewhat low frequencies probably due to conjugation with an unsaturated system. The band at 1640 $cm^{-1}$ indicated that it contained double bonds.

**Table 7** **The IR Absorption Band Assignments of Compound 1**

| Wavenumber ($cm^{-1}$) | Intensity | Tentative Assignments |
|---|---|---|
| 3400-3000 | broad | O-H stretching vibration of acid |
| 2980, 2900 | strong | C-H stretching vibration of $CH_3$-,-$CH_2$- |
| 1690 | strong | C=O stretching vibration of acid |
| 1640 | medium | C=C stretching vibration |

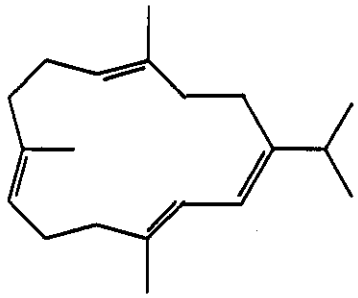
The $^1$H-NMR spectrum (Fig. 2) indicated that compound 1 possessed an isopropyl group (δ 1.04, 6H, d, $J$ = 7 Hz), two olefinic methyl groups attached to

double bonds (δ 1.54, 3H, s and 1.73, 3H, s ) and four olefinic protons (δ 5.10, 1H, t, $J$ = 6 Hz) and 5.85-6.04 , 3H, m).

The $^{13}$C-NMR spectrum (Fig. 3) suggested the presence of olefinic carbons according to the signals at 146.90 (s), 146.33 (d), 135.17 (s), 133.97 (s), 130.93 (s), 125.66 (d), 121.58 (d) and 118.74 (d) ppm. (4 doublets and 4 singlets). The signal at 174.12 ppm. should be the carbonyl group of carboxylic acid. There were 11 $sp^3$ carbons signals at 39.18 (t), 38.56 (t), 33.78 (d), 33.62 (t), 28.71 (t), 26.42 (t), 25.08 (t), 22.09 (2xq), 17.00 (q) and 15.78 (q) ppm. (4 quartets, 6 triplets and 1 doublet).

From DEPT-90 and DEPT-135 $^{13}$C-NMR (Fig. 4-5) indicated this compound possesses twenty carbon atoms and twenty nine protons. Assuming the compound may contain only carbon, proton and oxygen atoms. Thus, its molecular formula was established as $C_{20}H_{30}O_2$ which was confirmed by observing molecular ion at *m/z* 302 (Fig. 6). High resolution mass spectroscopy at 25,000 resolution gave *m/z* 302.212 which corresponded to $C_{20}H_{30}O_2$ ( calc. *m/z* 302.225).

The $^{13}$C NMR data revealed that the molecule possessed four double bonds (four $sp^2$ carbons: four singlets and four doublets). However, the DBE according to the molecular formula $C_{20}H_{30}O_2$ was 6 thus this compound must consist of a ring in addition to the 4 double bonds and a carbonyl group. Comparison of the characteristic $^1$H and $^{13}$C NMR in addition to the number of ring and double bonds required with those in the literature [17] suggested that this compound might possess a cembranoid structure which is a 14-membered-ring diterpene skeleton. (Table 8-9)



**Cembrene-C**

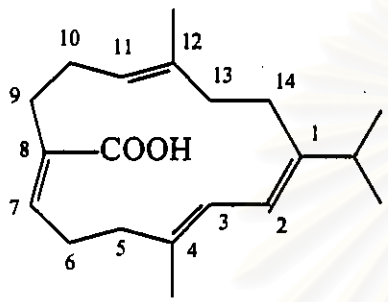
**Table 8** **$^1$H-NMR spectral data of Compound 1 compared with cembrene-C**

| Cembrene (ppm.) | Compound 1 (ppm.) |
|---|---|
| 1.04 (d) | 1.04 (d) |
| 1.50 (s) | - |
| 1.57 (s) | 1.54 (s) |
| 1.73 (s) | 1.73 (s) |
| 2.12 | 2.15 (m) |
| 2.26 (m) | 2.20-2.41 (m) |
| - | 2.70 (q) |
| 5.02 (br m) | 5.10 (t) |
| 5.98 (AB q) | 5.90 (m) |
| - | 6.00-6.03 (m) |

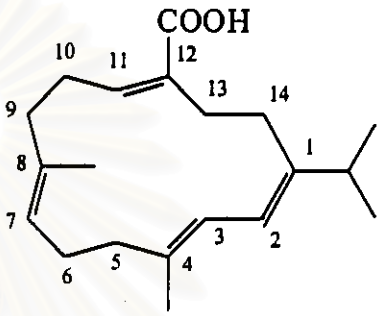
**Table 9** **$^{13}$C-NMR spectral data of Compound 1 compared with cembrene-C**

| Cembrene (ppm.) | Compound 1 (ppm.) |
|---|---|
| 15.0 (q) | 15.78 (q) |
| 17.1 (2q) | 17.00 (q) |
| 22.3 (2q) | 22.09 (2q) |
| 24.5 (t) | 25.08 (t) |
| 25.3 (t) | 26.42 (t) |
| 28.0 (t) | 28.71 (t) |
| - | 33.62 (t) |
| 33.9 (d) | 33.78 (d) |
| 38.7 (t) | - |
| 39.0 (t) | 38.56 (t) |
| 39.2 (t) | 39.18 (t) |
| 118.6 (d) | 118.74 (d) |
| 121.1 (d) | 121.58 (d) |
| 124.5 (d) | 125.66 (d) |
| 125.0 (d) | - |
| 134.1 (s) | 130.93 (s) |
| 134.3 (s) | 133.97 (s) |
| 134.6 (s) | 135.17 (s) |
| - | 146.33 (d) |
| 146.9 (s) | 146.90 (s) |
| - | 174.12 (s) |

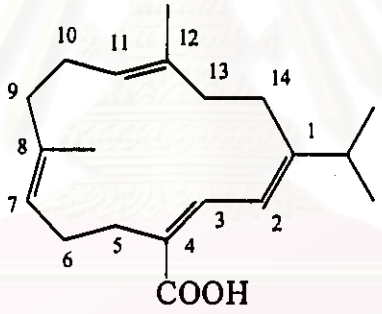
However, the molecular formula indicated that this molecule had a carboxylic group, an isolated isopropyl group (i.e. the carbon adjacent to it had no protons), a methyl group attached to an isolated double bond and a methyl group attached to the conjugated double bond. Based on the cembranoid skeleton and the data above, three possible structures were deduced.




I




II




III

Inspection of the $^{1}$H-NMR spectrum between δ 5.10 and 6.04 ppm. indicated that this compound had three olefinic CH groups at low field. From the structure **III**, there was only two olefinic CH groups at low field at positions 2 and 3. Thus, the compound 1 was either structure **I** or structure **II** which had three olefinic CH groups at low field at position 2, 3 (due to conjugation) and 7 (adjacent to the carboxyl group) in the structure **I** and position 2, 3 and 11 in the structure **II**.

Two dimensional NMR techniques were used for assisting the structure assignment. The protons directly attached to carbons of the compound 1 were resolved by an HMQC spectra (Fig. 7) as shown in Table 10.

**Table 10** **$^{1}$H attached to $^{13}$C-NMR spectral data of Compound 1**

| $^{13}$C-NMR (ppm.) | $^{1}$H-NMR (ppm.) |
|---|---|
| 15.78 q | 1.54 |
| 17.00 q | 1.73 |
| 22.09 2q | 1.04 |
| 25.08 t | 2.20 |
| 26.42 t | 2.70 |
| 28.71 t | 2.41 |
| 33.62 t | 2.41 |
| 33.78 d | 2.34 |
| 38.56 t | 2.15 |
| 39.18 t | 2.15 |
| 118.74 d | 6.00-6.03 |
| 121.58 d | 5.90 |
| 125.66 d | 5.10 |
| 130.93 s | - |
| 133.97 s | - |
| 135.17 s | - |
| 146.33 d | 6.01 |
| 146.90 s | - |
| 174.62 s | - |

Crucial long-range $^{1}H$-$^{13}C$ correlations by HMBC (Fig. 8) were : H (δ 1.54) with C (δ 133.97), CH (δ 125.66) and $CH_2$ (δ 38.56) (Fig. a) ; H (δ 1.73) with C (δ 135.17), CH (δ 121.58) and $CH_2$ (δ 39.18) (Fig. b) ; H (δ 1.04) with CH (δ 33.78) and C (δ 146.90) (Fig. c) ; H (δ 2.34) with $CH_2$ (δ 22.09), C (δ 146.90), CH (δ 118.74) and $CH_2$ (δ 28.71) (Fig. d).

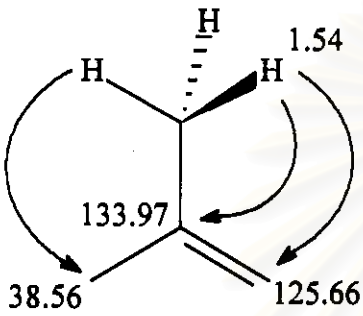



**Fig. a**

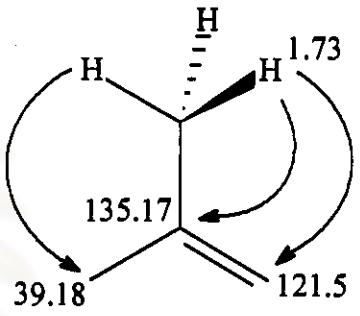



**Fig. b**

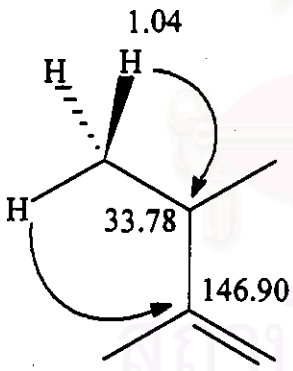



**Fig. c**

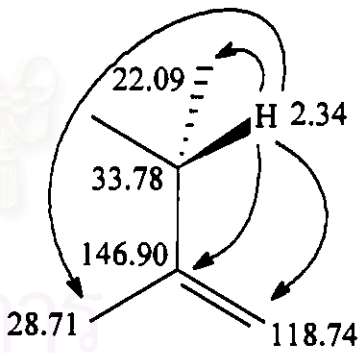



**Fig. d**

Because of the disappearance of the carboxylic proton, the long-range correlations between this proton and carbons were not shown. However, there was 2 $sp^2$ carbon atoms (δ 130.93 and δ 146.33) which did not attach to the methyl groups. They probably attached to the carboxylic group (Fig. e).

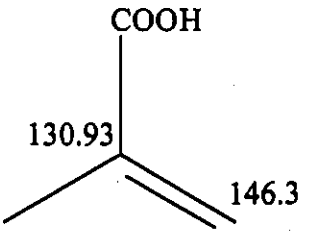



**Fig. e**

The COSY spectrum (Fig. 9) established the correlation between the proton at 5.90 ($\delta_C$ 121.58) and 6.03 ppm. ($\delta_C$ 118.74). Therefore, partial structure was obtained as follows (Fig. f).

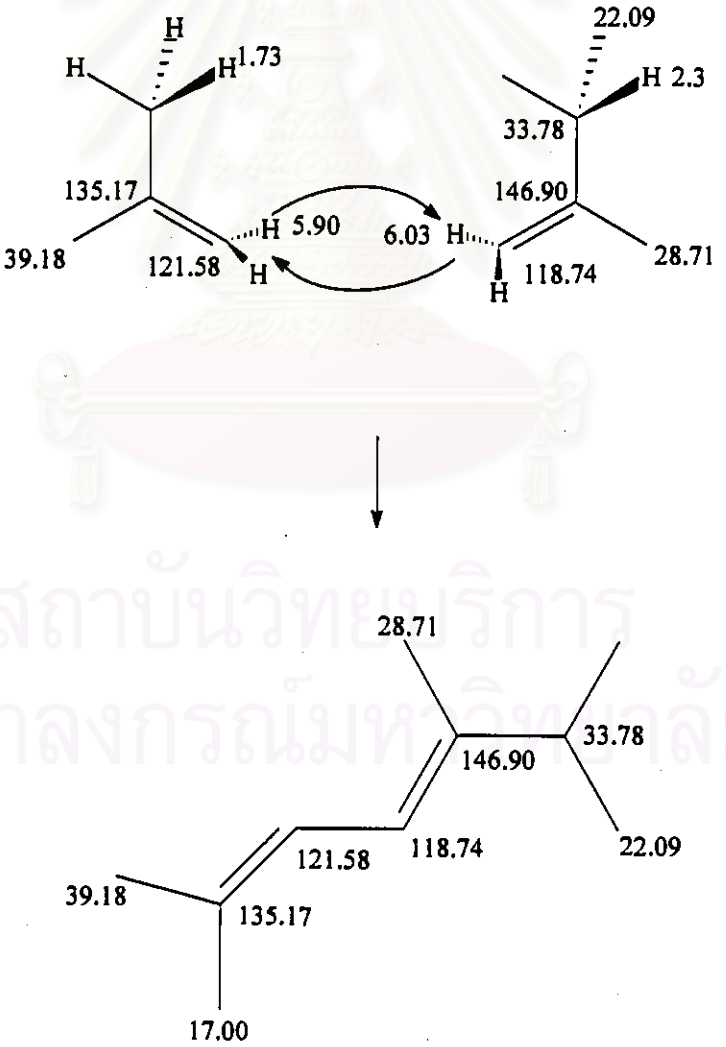



**Fig. f**

The $CH_2$ protons at 2.20 ppm. also showed long-range correlations with a $CH_2$ (δ 39.18) and a CH (δ 125.66) in the HMBC spectrum (Fig. g).

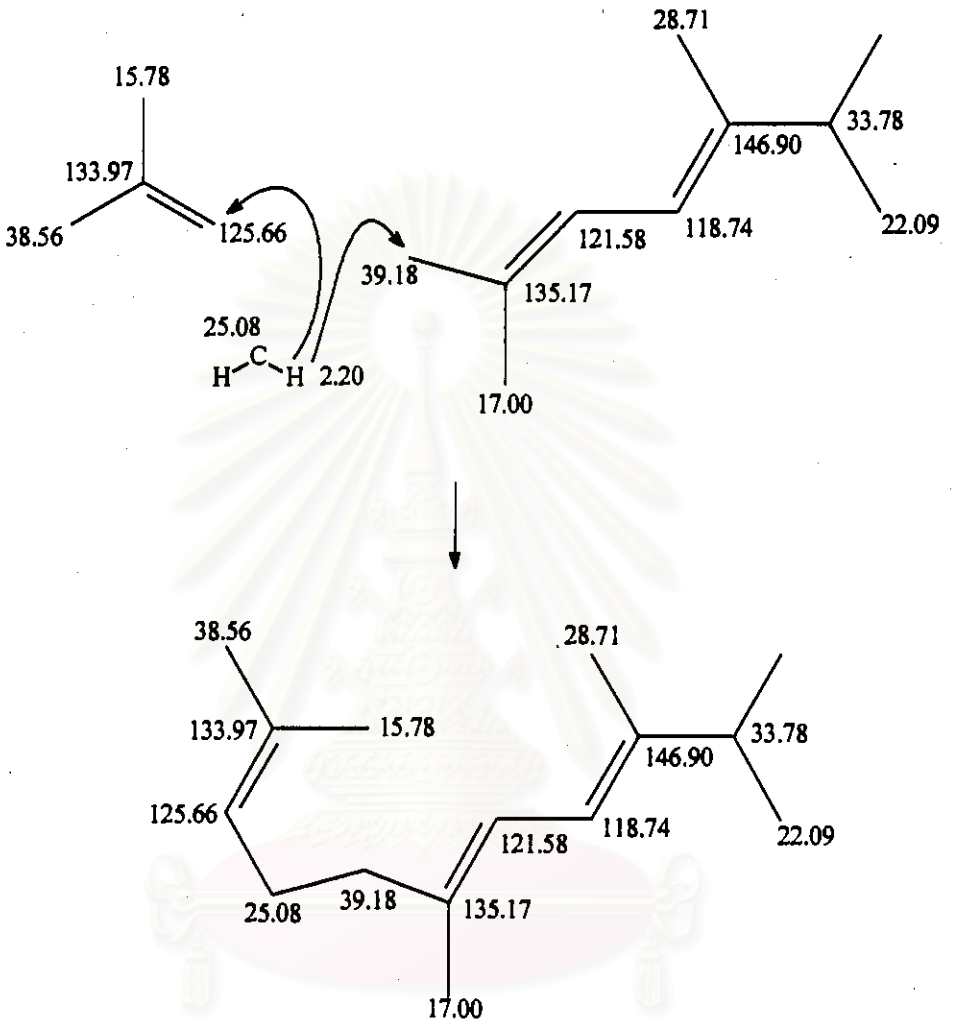



**Fig. g**

The protons at 2.70 ppm. showed long-range correlations with $CH_2$ (δ 38.56), C (δ 133.97), CH (δ 146.33) and C (δ 130.93). Then, the protons at 2.41 (δ 33.62) was correlated to $CH_2$ (δ 28.71). After connecting all possible fragments together, compound 1 must be the structure **II** as follows (Fig. h).

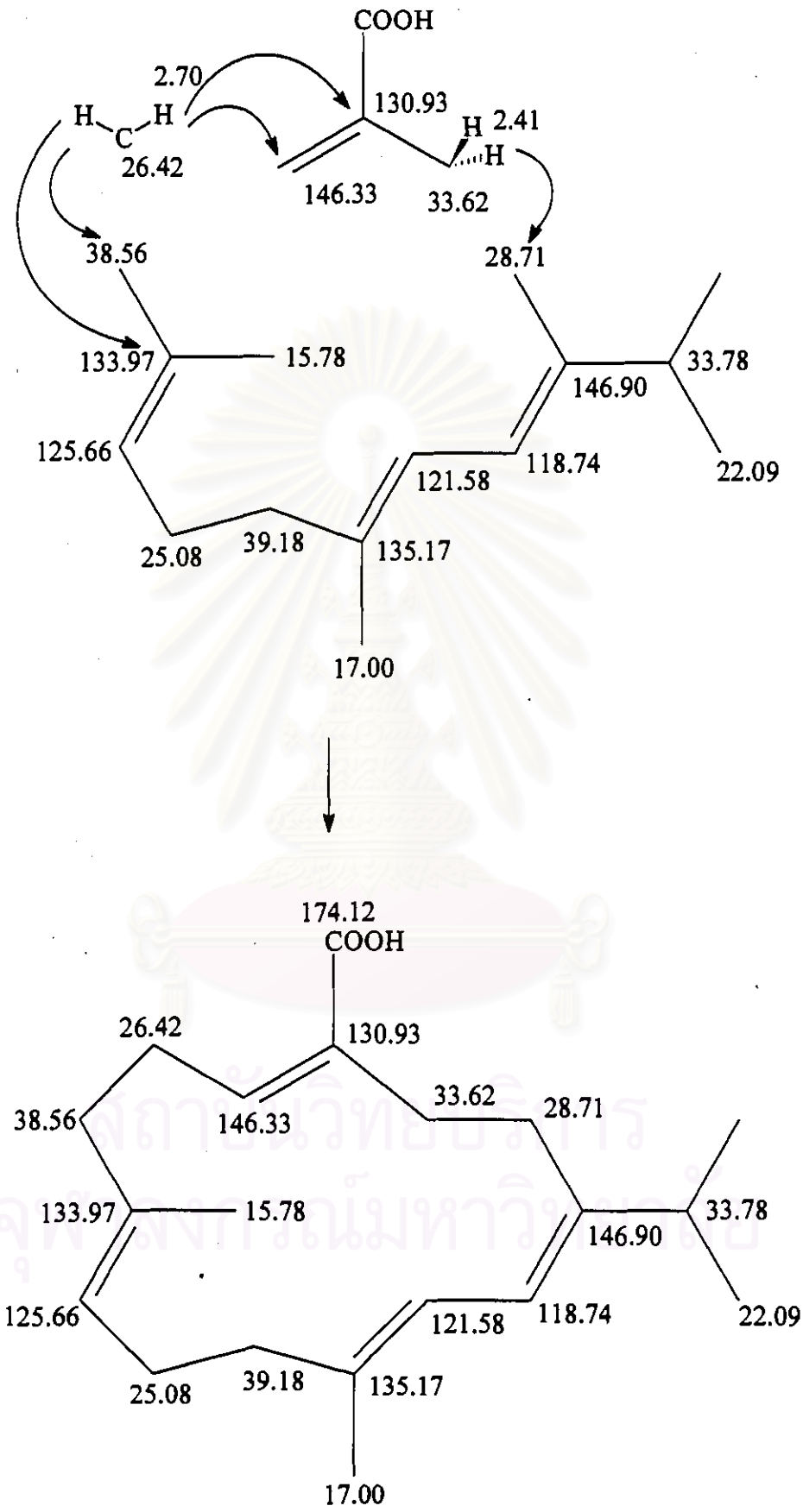

COOH
2.70
130.93
H
C
H
26.42
H 2.41
H
146.33
33.62
38.56
28.71
133.97
15.78
146.90
33.78
125.66
121.58
118.74
22.09
25.08
39.18
135.17
17.00
174.12
COOH
26.42
130.93
33.62
28.71
38.56
146.33
133.97
15.78
146.90
33.78
125.66
121.58
118.74
22.09
25.08
39.18
135.17
17.00

**Fig. h**

The long-range C-H correlations by HMBC spectrum were summarized in Table 11 and schematically shown as follows (Fig. i).

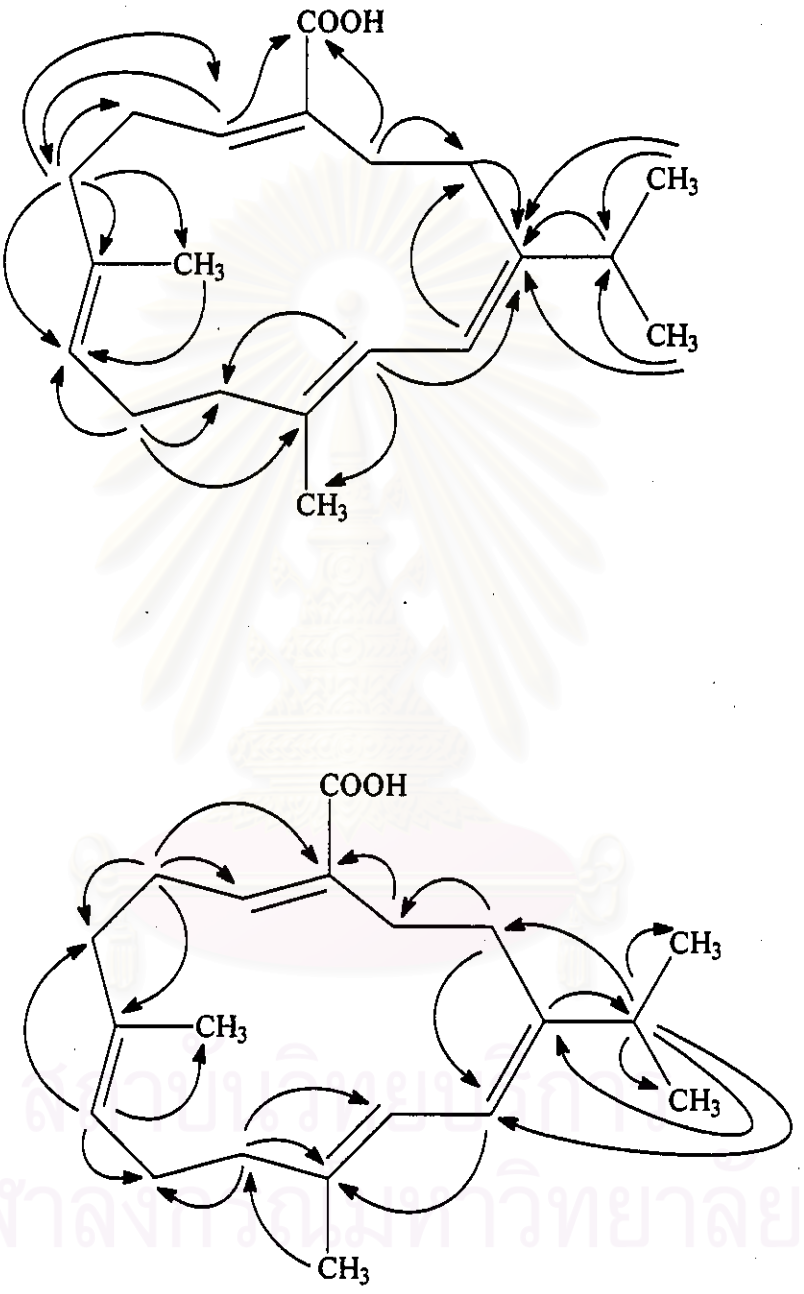
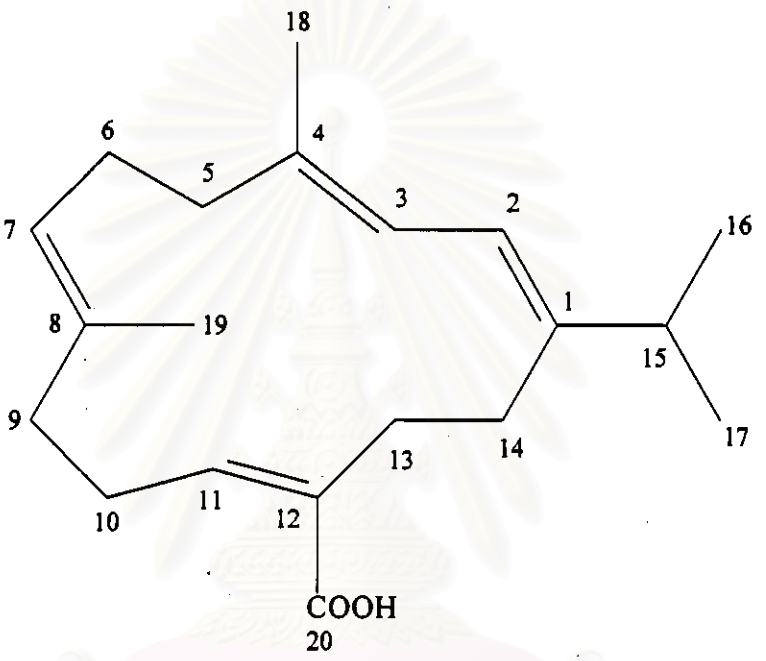



**Fig. i**

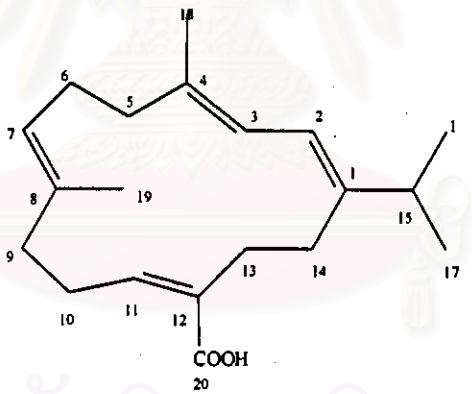



**Compound 1**

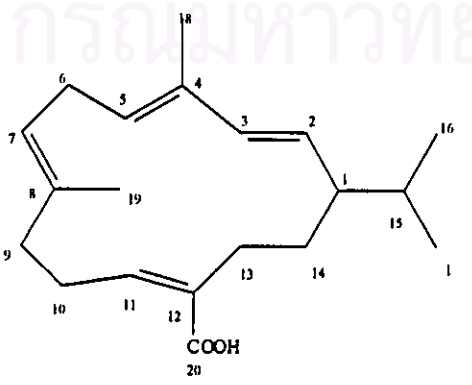
**Table 11** **The HMQC and HMBC data of Compound 1**

| Position | $\delta_C$ | $\delta_H$ | *J* correlations (HMBC) |
|---|---|---|---|
| 1 | 146.90 s | - | H-3; H-14; H-15; H-16,17 |
| 2 | 118.74 d | 6.00-6.03 (m) | H-14; H-15 |
| 3 | 121.58 d | 5.90 (d) | H-5; H-18 |
| 4 | 135.17 s | - | H-2; H-5; H-6; H-18 |
| 5 | 39.18 t | 2.15 (m) | H-3; H-6; H-18 |
| 6 | 25.08 t | 2.20 (m) | H-5; H-7 |
| 7 | 125.66 d | 5.10 (t) | H-6; H-9; H-19 |
| 8 | 133.97 s | - | H-9; H-10; H-19 |
| 9 | 38.56 t | 2.15 (m) | H-7; H-10;H-11; H-19 |
| 10 | 26.42 t | 2.70 (q) | H-9 |
| 11 | 146.33 d | 6.01 (m) | H-9; H-10 |
| 12 | 130.93 s | - | H-10; H-13 |
| 13 | 33.62 t | 2.41 (m) | H-14 |
| 14 | 28.71 t | 2.41 (m) | H-2; H-13; H-15 |
| 15 | 33.78 d | 2.34 (m) | H-2; H-16,17 |
| 16,17 | 22.09 2q | 1.04 (d) | H-15 |
| 18 | 17.00 q | 1.73 (s) | H-3 |
| 19 | 15.78 q | 1.54 (s) | H-7; H-9 |
| 20 | 174.12 s | - | H-11; H-13 |

Most of the cembranoids are found widely distributed in marine sources [17-23] and a variety of terrestrials [24]. Only recently that poilaneic acid, a cembranoid diterpene is isolated from *Croton poilanei* by Sato and co-workers [25]. It represents the first cembranoid isolated from *Croton* sp. Therefore, crotocembraneic acid is the second cembranoid diterpene ever isolated from *Croton* sp. The structural difference between the two compounds are the position of conjugated double bond inwhich crotocembraneic acid has conjugated double bonds at 1-2 and 3-4 positions, while poilaneic acid has them at 2-3 and 4-5 positions. Thus, poilaneic acid possessed a chiral centre at 1, while crotocembraneic acid possessed no chiral centre. Moreover, only four $sp^2$ CH (δ 146.33, 125.66, 121.58 and 118.74) are found in crotocembraneic acid while five $sp^2$ CH (δ 147.81, 131.30, 130.54, 128.04 and 125.73) are found in poilaneic acid.




**crotocembraneic acid**




**poilaneic acid**

## 2. Structural Elucidation of Compound 2

The IR spectrum (Fig. 10) revealed the presence of a β-substituted furan ring at 1560, 1510 and 880 $cm^{-1}$. The broad absorption band between 3080 and 3040 $cm^{-1}$ and a C=O absorption at 1690 $cm^{-1}$ in the IR indicated that the remaining oxygen atoms were present in a carboxyl group. The conjugation of the COOH and olefinic were inferred from the position of the C=O absorption at 1690 $cm^{-1}$ and the intensity of the C=C stretching in the IR spectra of the acid at 1640 $cm^{-1}$. The IR absorption band is shown in Table 12.

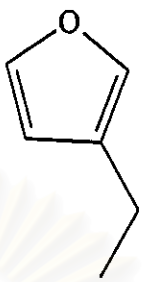
**Table 12** **The IR Absorption Band Assignments of Compound 2**

| Wavenumber ($cm^{-1}$) | Intensity | Tentative Assignments |
|---|---|---|
| 3080-3040 | medium | O-H stretching vibration of acid |
| 2880-2975 | strong | C-H stretching vibration of $CH_3$-,-$CH_2$- |
| 1690 | strong | C=O stretching vibration of acid |
| 1640 | medium | C=C stretching vibration |
| 1460, 1368 | medium | C-H bending vibration of -$CH_2$-, $CH_3$- |
| 1265 | medium | C-O stretching vibration |

The $^1$H-NMR spectrum (Fig. 11) showed the pattern of a β-monosubstitued furan at 7.33, 7.19 and 6.26 which agreed with $^{13}$C-NMR absorption at 142.65, 138.28 and 110.93, respectively.

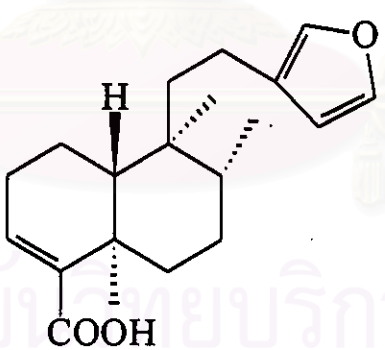
From DEPT-90, DEPT-135 and $^{13}$C-NMR spectrum (Fig. 12-14), there were twenty carbon atoms and twenty-seven protons. This compound probably contained carbon, hydrogen and oxygen atoms. The molecular formular, $C_{20}H_{28}O_3$, was determined from its mass spectrum (Fig. 13) which showed the molecular ion at *m/z* 316. The high resolution mass spectrometric analysis gave the accurate mass at

316.198 which corresponded to $C_{20}H_{28}O_3$ (calc. mass 316.204). Moreover, the prominent ion at *m/z* 221 (M-95) indicated that the Compound 2 probably contained a furano-ethyl side chain.




a furano-ethyl side chain

From the literature, these spectroscopic properties of Compound 2 data were consistent with (-)-hardwickiic acid, which was isolated from *Croton oblongifolius* in 1972 [4]. The $^1$H-NMR closely matched to those reported for (-)-hardwickiic acid (Table 13).




**(-)-hardwickiic acid**

**Table 13** **Comparison of $^{1}$H-NMR spectral data of Compound 2 and (-)-hardwickiic acid [26]**

| (-)-hardwickiic acid δH (ppm.) | Compound 2 |
|---|---|
| 7.20 | 7.33 |
| 7.05 | 7.19 |
| 6.77 | 6.85 |
| 6.12 | 6.26 |
| 1.27 | 1.26 |
| 0.85 | 0.84 |
| 0.80 | 0.76 |

Since there was no report on $^{13}$C-NMR chemical shift of (-)-hardwickiic acid in the literature, therefore further confirmation of the structure of (-)-hardwickiic acid was done using two dimensional NMR spectroscopy. The one bond $^{1}$H-$^{13}$C chemical shift correlations of compound 2 were determined by an HMQC experiment (Heteronuclear Multiple Quantum Coherence) (Fig. 16) as shown in Table 14.

**Table 14** **$^{1}H$ attached to $^{13}C$-NMR spectral data in Compound 2 according to HMQC experiment**

| $^{13}C$-NMR (ppm.) | $^{1}H$-NMR (ppm.) |
|---|---|
| 16.03 q | 0.85 |
| 17.48 t | 1.50, 1.70 |
| 18.21 t | 2.34, 2.18 |
| 18.36 q | 0.76 |
| 20.54 q | 1.25 |
| 27.30 t | 1.45 |
| 27.51 t | 2.20, 2.35 |
| 35.80 t | 1.18, 2.44 |
| 36.26 d | 1.60 |
| 37.60 s | - |
| 38.62 t | 1.50-1.70 |
| 38.81 s | - |
| 46.71 d | 1.40 |
| 110.93 d | 6.25 |
| 125.45 s | - |
| 138.28 d | 7.20 |
| 140.19 d | 6.85 |
| 141.54 s | - |
| 142.65 d | 7.35 |
| 172.81 s | - |

The long range $^{1}H$-$^{13}C$ correlations were determined by HMBC experiment(Heteronuclear Multiple Bond Correlation)(Fig. 17). The singlet carbon (δ 125.45) showed correlation with four protons, three of them were doublets and one was a triplet (δ 142.65, δ 110.93, δ 138.28 and δ 18.21)(Fig. j). Furthermore, the proton at 2.34 ppm. (δ 18.21) showed correlations with carbons at δ 125.45, δ 110.93, δ 138.28 and δ 17.48 ppm.(Fig. k). The COSY spectrum (Fig. 18) of compound 2 showed the connectivity with δ 7.20 and δ 7.35 (Fig. l). From the Figure j-l, the positions of a furano-ethyl side chain could be assigned in Figure m.

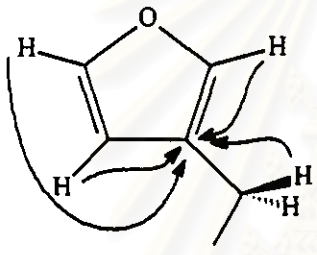



**Fig. j**

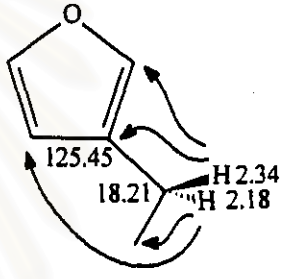



**Fig. k**

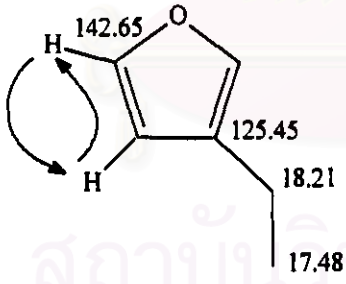



**Fig. l**

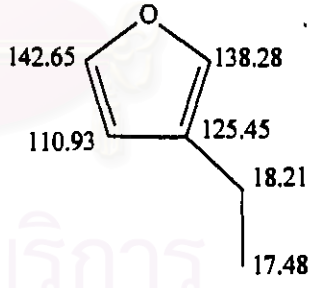



**Fig. m**

Considering the olefinic carbon atom region in $^{13}C$-NMR spectral data, there were four doublets and two singlets (three doublets and one singlet for furan ring and one doublet and one singlet for conjugated carbon atoms). Since the positions of furan ring had already been established the remaining carbons atoms were the conjugated carbons(Fig. n). Crucial long-range $^{1}H$-$^{13}C$ correlations were : H (δ 1.25)

with C (δ 141.54), CH (δ 46.71), C (δ 37.60) and $CH_2$ (δ 35.80) (Fig. o) ; H (δ 0.76) with CH (δ 46.71), C (δ 38.81) and CH (δ 36.26) (Fig. p); H (δ 0.85) with $CH_2$ (δ 38.81), CH (δ 36.26) and $CH_2$ (δ 27.30) (Fig. q).

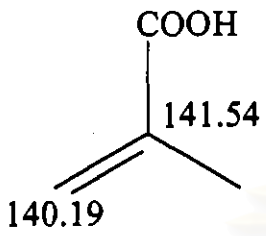



**Fig. n**

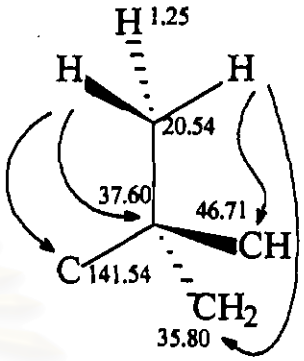



**Fig. o**

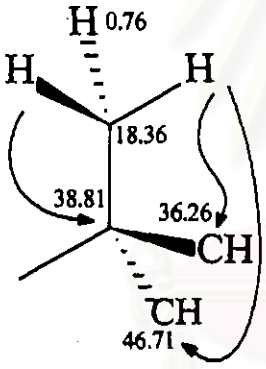



**Fig. p**

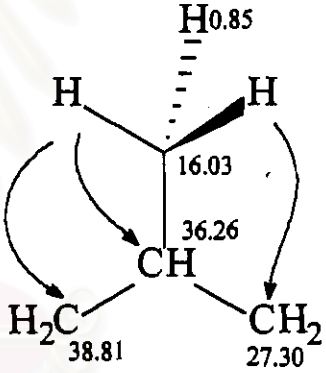



**Fig. q**

The structures in Figure n and o shared a common carbon atom resonating at 141.54 ppm. Thus the two structures could be joined as shown in Figure r. Furthermore, the structures in Figures p and q shared the same two carbon atoms at 36.26 and 38.81 ppm. The combined structure was shown in the Figure s.

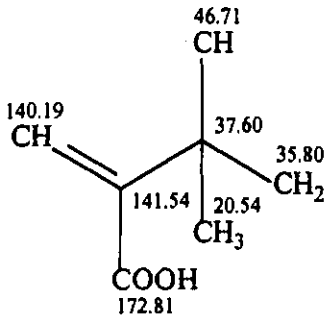



Fig. r

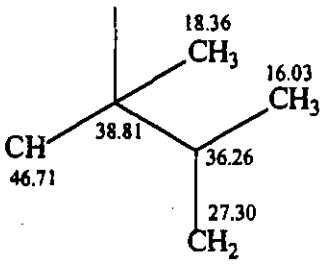



Fig. s

The structures in Figures r and s could be further joined via the common carbon atom resonating at δ 46.71 ppm. Then, the combined structure was shown below (Fig. t).

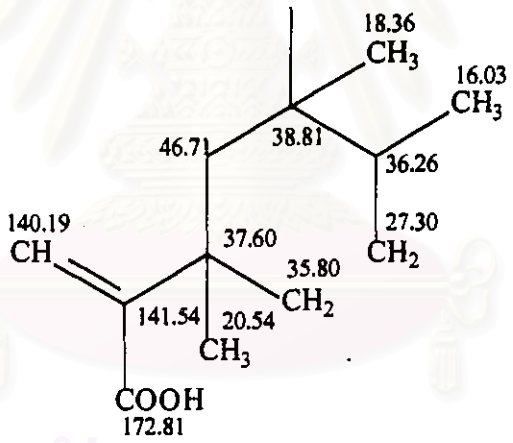



Fig. t

From the HMBC spectra (Fig. 17), the protons at 2.20, 2.35 ppm.(δ 27.51) showed long-range correlations with C (δ 141.54), the protons at 1.50-1.70 ppm.(δ 38.62) with a $CH_2$ (δ 38.62) and a quaternary C (δ 37.60) and the protons at 1.45 ppm. (δ 27.30) with a quaternary C (δ 37.60) as shown below (Fig. u).

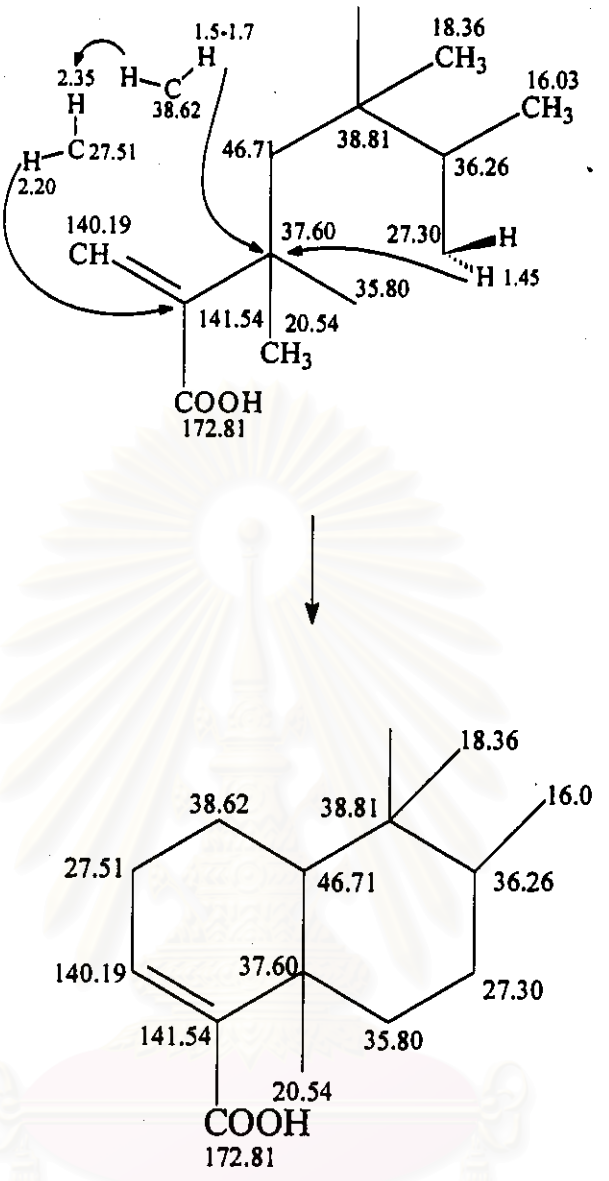



**Fig. u**

The protons at 1.50, 1.70 ppm. (δ 17.48) in a furano-ethyl side chain showed long-range correlation with C (δ 38.81) (Fig. v).

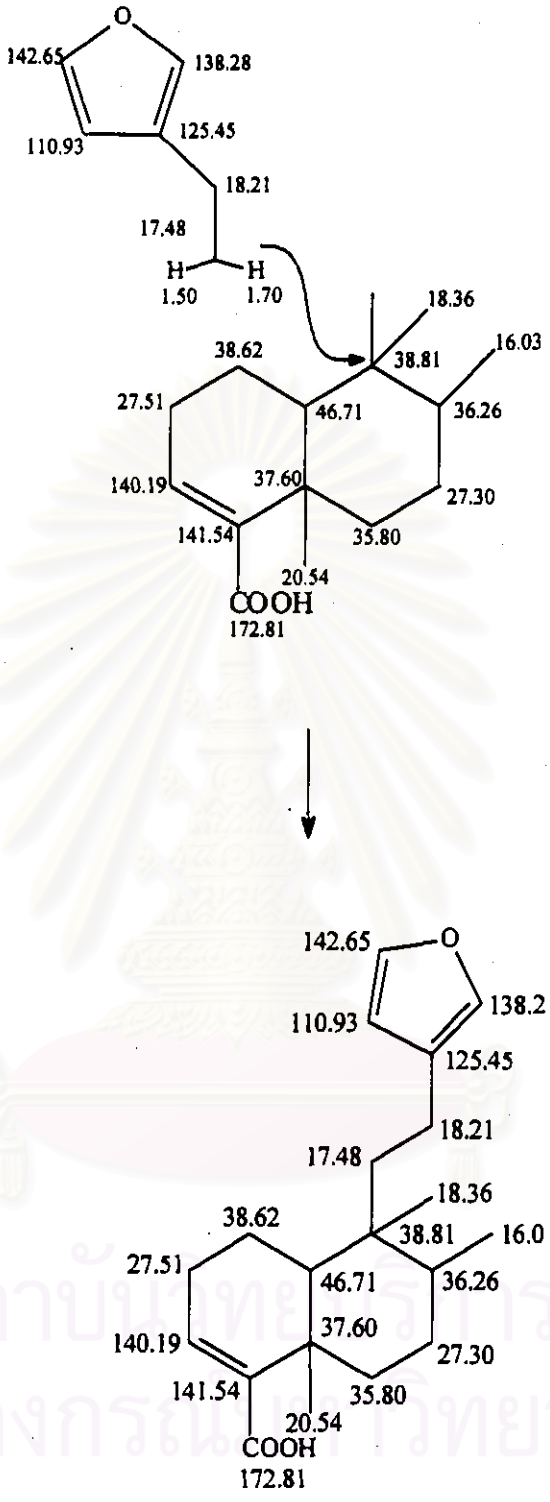



**Fig. v**

By the HMBC spectra (Fig. 17), the positions of the substituents of the compound 2 could be deduced as follows (Fig. w).

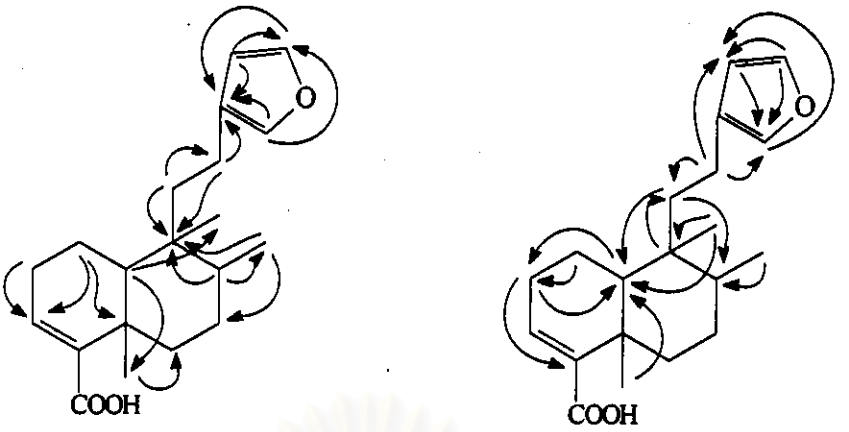
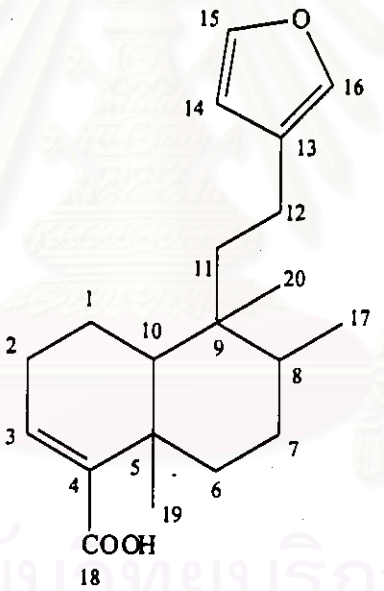



**Fig. w**

The correlation of HMQC and HMBC could be summarized in Table 15.




**Compound 2**

**Table 15** **The correlation of HMQC and HMBC Data of Compound 2**

| Position | $\delta_C$ | $\delta_H$ | *J* correlations (HMBC) |
|---|---|---|---|
| 1 | 38.62 t | 1.50-1.70 | - |
| 2 | 27.51 t | 2.20, 2.35 | H-1; H-10 |
| 3 | 140.19 d | 6.85 | H-1; H-2 |
| 4 | 141.54 s | - | H-2; H-19 |
| 5 | 37.60 s | - | H-1; H-7; H-10; H-19 |
| 6 | 35.80 t | 1.18, 2.44 | H-19 |
| 7 | 27.30 t | 1.45 | H-17 |
| 8 | 36.26 d | 1.60 | H-11; H-17; H-20 |
| 9 | 38.81 s | - | H-8; H-11; H-12; H-17; H-20 |
| 10 | 46.71 d | 1.40 | H-2; H-11; H-19; H-20 |
| 11 | 17.48 t | 1.50, 1.70 | H-10; H-12 |
| 12 | 18.21 t | 2.34, 2.18 | H-11 |
| 13 | 125.45 s | - | H-12; H-14; H-15; H-16 |
| 14 | 110.93 d | 6.25 | H-12; H-15; H-16 |
| 15 | 142.65 d | 7.35 | H-14; H-16 |
| 16 | 138.28 d | 7.20 | H-12; H-14; H-15 |
| 17 | 16.03 q | 0.85 | H-8 |
| 18 | 172.81 s | - | - |
| 19 | 20.54 q | 1.25 | H-10 |
| 20 | 18.36 q | 0.76 | H-10 |

### 3. Structural Elucidation of Mixture 3

The IR spectrum of Mixture 3 was shown in Fig. 19 which exhibited the absorption band of hydroxy group (-OH) at 3589-3271 $cm^{-1}$ , absorption band of unsaturation at 1645 $cm^{-1}$ and disubstituted and trisubstituted vinyl at 960 and 802 $cm^{-1}$ , respectively. The assignments of the IR absorption bands are shown in Table 16.

**Table 16 The IR Absorption Band Assignments of Mixture 3**

| Wavenumber ($cm^{-1}$) | Intensity | Tentative Assignments |
|---|---|---|
| 3589-3271 | medium | O-H stretching vibration |
| 2959-2868 | strong | C-H stretching vibration of $CH_3$-,-$CH_2$- |
| 1645 | weak | C=C stretching vibration |
| 1463, 1379 | medium | C-H bending vibration of -$CH_2$-, $CH_3$- |
| 1058 | medium | C-O stretching vibration |
| 960 | medium | C-H out of plane bending vibration of trans configuration |
| 802 | weak | C-H out of plane bending vibration of =$CH_2$ |

The EI mass spectrum of Mixture 3 (Fig. 20) showed the molecular ion peak at (*m/e*) 414,412 and 400 and peaks of relatively high relative abundance at *m/e* 396, 394, 255 and 213 respectively.

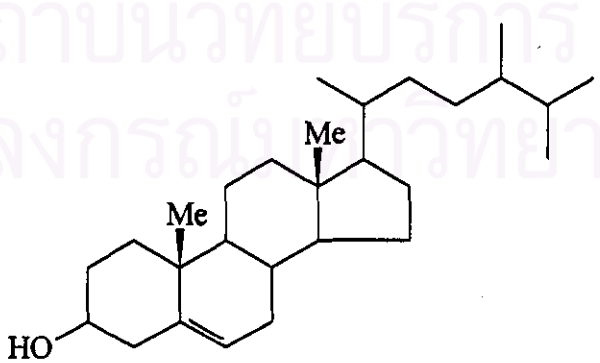
From the comparison of spectroscopic data (IR and Mass spectrum) of Mixture 3 with mixture of steroids [19], it was found that Mixture 3 should be a steroidal mixture.

To confirm the structure of mixture 3 , gas chromatography was used to compare the mixture 3 with a standard mixture of steroids (Fig. 21). The GLC analysis data showed three peaks at retention time 18.96, 19.80 and 22.41 corresponding to campesterol, stigmasterol and β-sitosterol (Fig. 22). The retention times of the mixture of three standard steroids and Mixture 3 are shown in Table 17.

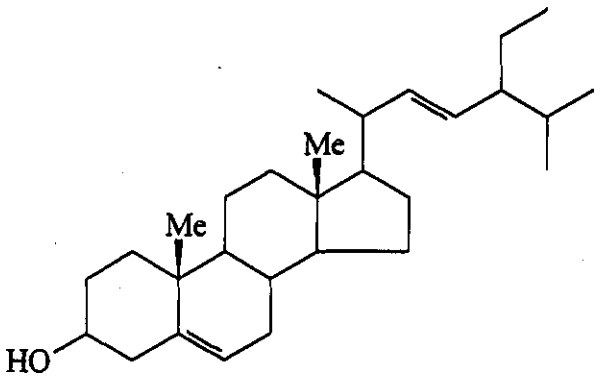
**Table 17** **Retention Times of The Mixture of Three Standard Steroids and Mixture 3**

| Name of Substances | Retention Time of Standard Steroids (min.) | Retention Time of Mixture 3 (min.) |
|---|---|---|
| campesterol | 18.74 | 18.96 |
| stigmasterol | 19.59 | 19.80 |
| β-sitosterol | 22.26 | 22.41 |

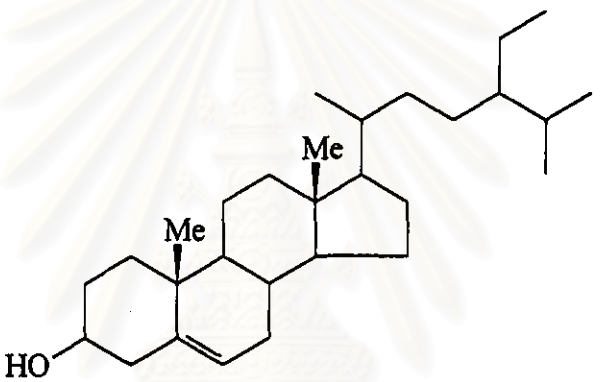
From all of the spectral data, it was concluded that Mixture 3 was a mixture of campesterol, stigmasterol and β-sitosterol.




**Campesterol**




**Stigmasterol**




**β-Sitosterol**

**4. Structural Elucidation of Mixture 4**

The IR spectrum of Mixture 4 (Fig 23) exhibited a broad band of hydroxy group (-OH) at 3340 $cm^{-1}$, the C-O stretching vibration of glycosidic linkage at 1025-1080 $cm^{-1}$ and an anomeric axial C-H deformation of β-sugar at 890 $cm^{-1}$ . The additional bands were consistent with a trisubstituted olefin and gem-dimethyl group at 1650 and 1380 $cm^{-1}$, respectively. The IR adsorption bands of Mixture 4 were shown in Table 18.

**Table 18 The IR Absorption Band Assignments of Mixture 4**

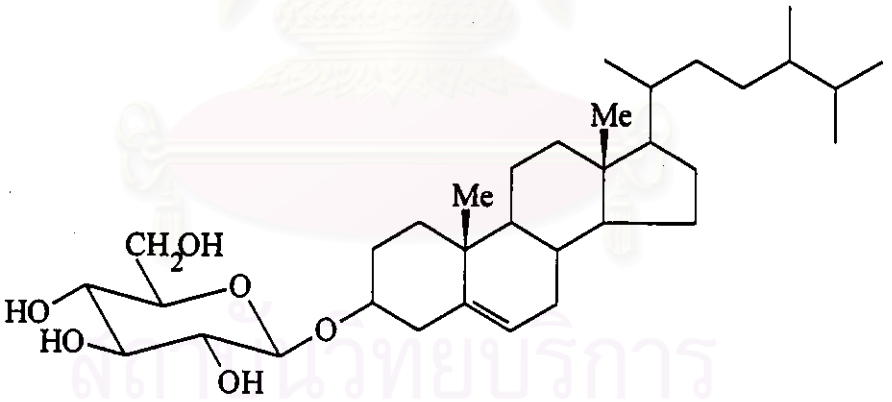
| Wavenumber ($cm^{-1}$) | Intensity | Tentative Assignments |
|---|---|---|
| 3340 | strong | O-H stretching vibration |
| 2880-2980 | strong | C-H stretching vibration of $CH_3$-, -$CH_2$- |
| 1650 | weak | C=C stretching vibration |
| 1470 | medium | C-H bending vibration of $CH_3$-, -$CH_2$- |
| 1380 | medium | C-H bending vibration of gem-dimethyl |
| 1025-1080 | strong | C-O stretching vibration of glycoside linkage |
| 890 | weak | anomeric axial C-H deformation of β-sugar |

The $^1$H-NMR spectrum (Fig. 24) showed signals which corresponded to steroidal glycosides. The signals at 0.63-2.69 ppm. appeared to be the methyl, methylene and methine protons of steroid while the broad multiplet at 3.69 ppm. could be assigned to the anomeric proton (-CH-O-sugar). The proton signal at 5.35 ppm. was the olefinic proton (-CH=C-) and the other sugar protons appeared as multiplets between 2.88-3.55 ppm.

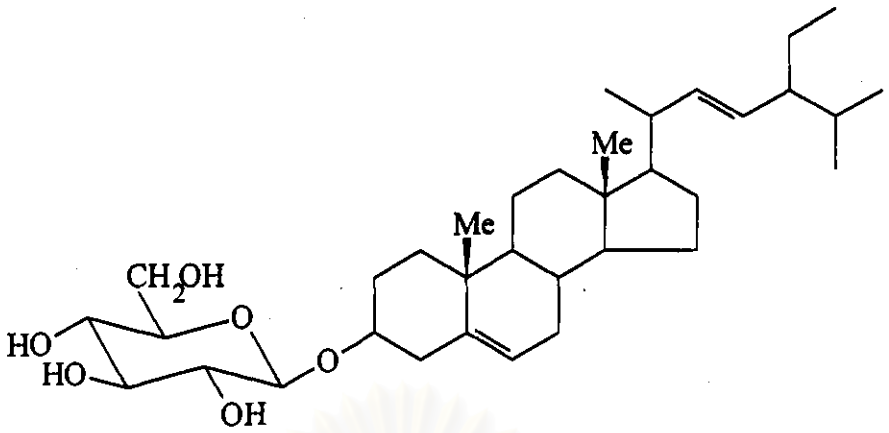
The $^{13}$C-NMR spectrum (Fig. 25) exhibited signals of carbon which showed the olefinic carbon signals at 121.19, 128.79, 138.01 and 140.41 and 6 carbon signals of sugar moiety at 61.05, 70.05, 73.43, 76.73, 76.86 and 100.75 ppm.. Other carbon signals between 11.66-56.15 ppm. were all $sp^3$ carbons of steroids.

The EI mass spectum did not give the molecular ion peak ($M^+$) but caused extensive fragmentation of the molecule. However, the dominant fragmentation ion peaks at (*m/e*) 414, 412 and 400 were similar to those of campesterol, stigmasterol and β-sitosterol (Fig. 26).

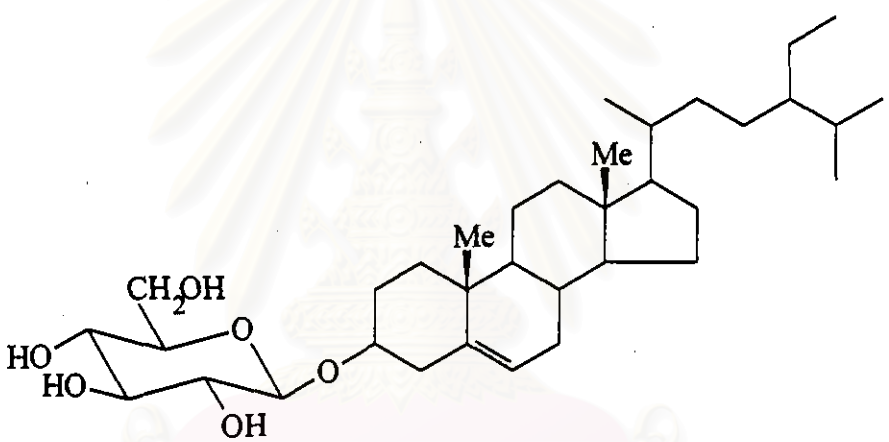
All the spectral data (IR, $^1$H and $^{13}$C-NMR spectrum and mass spectrum), were similar to the mixture of three steroidal glycosides [26] which suggested that the mixture 4 was of a mixture of campesteryl-3-*O*-β-D-glucopyranoside, stigmasteryl-3-*O*-β-D-glucopyranoside and β-sitosteryl-3-*O*-β-D-glucopyranoside.




**Campesteryl-3-*O*-β-D-glucopyranoside**




**Stigmasteryl-3-*O*-β-D-glucopyranoside**




**β-Sitosteryl-3-*O*-β-D-glucopyranoside**

**5. Structural Elucidation of Compound 5**

Reactions of Compound 5 were summarized in Table 6, this compound gave a purple-red colour to the flame test indicated that the cation was $K^+$. Furthermore, it reacted with sodium cobaltinitrite to give yellow precipitate of $K_3Co(NO_2)_6$ which was insoluble in dilute acetic acid as follows :

$$Na_3[Co(NO_2)_6]_{(aq)} + 3K^+_{(aq)} \rightarrow K_3[Co(NO_2)_6]_{(s)} + 3Na^+_{(aq)}$$

When the compound 5 reacted with $AgNO_3$, it gave white precipitate of AgCl. This precipitate was soluble in ammonia and then after addition of dilute $HNO_3$, the white precipitate of AgCl was recovered again which indicated that the anion was $Cl^-$ according to the equations below.

$$Cl^-_{(aq)} + Ag^+_{(aq)} \rightarrow AgCl_{(s)}$$

$$AgCl_{(s)} + 2NH_{3(aq)} \rightarrow [Ag(NH_3)_2]Cl_{(aq)}$$

$$[Ag(NH_3)_2]Cl_{(aq)} + 2HNO_{3(aq)} \rightarrow AgCl_{(s)} + 2NH_4NO_{3(aq)}$$

The results of the reactions of Compound 5 suggested that the Compound 5 was KCl.