

CHAPTER III

DISCUSSION

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

1. Structural Elucidation of Mixture 1

Mixture 1 was a white amorphous solid, m.p. 58-60°C. The R_f value was 0.6 (stationary phase: silica, solvent system: hexane).

The IR spectrum of Mixture 1 in Fig.3 suggested that this Mixture should be a mixture of saturated long chain aliphatic hydrocarbons. The important IR absorption bands and their assignments are shown in Table 8.

Table 8 The IR Absorption Band Assignments of Mixture 1

| Wavenumber (cm ⁻¹) | Intensity | Tentative Assignments |
|--------------------------------|-----------|---|
| 2918, 2849 | high | C-H stretching vibration of CH ₃ -, -CH ₂ - |
| 1473, 1463 | medium | C-H bending vibration of CH ₃ -, -CH ₂ - |
| 721 | low | C-H rocking vibration of (-CH ₂) _n |

When Mixture 1 was analyzed by GLC, the chromatogram showed 7 peaks at retention times: 6.37, 8.19, 10.58, 13.63, 17.69, 22.78 and 29.41 min., respectively (Fig.5). Therefore, Mixture 1 was a mixture of 7 long chain aliphatic hydrocarbons. The calibration curve between log retention time and number of carbon atom from standard long chain aliphatic hydrocarbons (C₂₄-C₃₃) (Fig.6) was created and comparison with the retention times indicated that the numbers of carbon atoms of hydrocarbons in Mixture 1 are 27, 28, 29, 30, 31, 32 and 33, respectively. The

retention time of standard long chain hydrocarbons (C₂₄-C₃₃) and Mixture 1 is shown in Table 9.

Table 9 Retention Time of Standard Long Chain Aliphatic Hydrocarbons and Mixture 1

| Substances | Retention Time (min.) | Log Retention Time | Number of Carbon |
|----------------|--------------------------|--------------------|---------------------|
| Tetracosane | 3.03 | 0.48 | 24 |
| Pentacosane | 3.84 | 0.58 | 25 |
| Hexacosane | 4.91 | 0.69 | 26 |
| Heptacosane | 6.32 | 0.80 | 27 |
| Octacosane | 8.11 | 0.91 | 28 |
| Nonacosane | 10.45 | 1.02 | 29 |
| Triacontane | 13.52 | 1.13 | 30 |
| Hentriacontane | 17.48 | 1.24 | 31 |
| Dotriacontane | 22.62 | 1.35 | 32 |
| Tritriacontane | 29.32 | 1.47 | 33 |
| Mixture 1 | 6.37 | 0.80 | 27 |
| | 8.19 | 0.91 | 28 |
| | 10.58 | 1.02 | 29 |
| | 13.63 | 1.13 | 30 |
| | 17.69 | 1.25 | 31 |
| | 22.78 | 1.36 | 32 |
| | 29.41 | 1.47 | 33 |

So the structure of saturated long chain aliphatic hydrocarbons found in Mixture 1 can be assigned as shown in Table 10.

Table 10 Various types of Long Chain Aliphatic Hydrocarbons is founded in Mixture 1

| Substances | MW Formula | Structure Formula | MW | % Composition |
|----------------|---------------------------------|--|-----|---------------|
| Heptacosane | C ₂₇ H ₅₆ | CH ₃ -CH ₂ -(CH ₂) ₂₃ -CH ₂ -CH ₃ | 380 | 0.53 |
| Octacosane | C ₂₈ H ₅₈ | CH ₃ -CH ₂ -(CH ₂) ₂₄ -CH ₂ -CH ₃ | 394 | 1.31 |
| Nonacosane | C ₂₉ H ₆₀ | CH ₃ -CH ₂ -(CH ₂) ₂₅ -CH ₂ -CH ₃ | 408 | 39.39 |
| Triacontane | C ₃₀ H ₆₂ | CH ₃ -CH ₂ -(CH ₂) ₂₆ -CH ₂ -CH ₃ | 422 | 4.50 |
| Hentriacontane | C ₃₁ H ₆₄ | CH ₃ -CH ₂ -(CH ₂) ₂₇ -CH ₂ -CH ₃ | 436 | 37.67 |
| Dotriacontane | C ₃₂ H ₆₆ | CH ₃ -CH ₂ -(CH ₂) ₂₈ -CH ₂ -CH ₃ | 450 | 4.84 |
| Tritriacontane | C ₃₃ H ₆₈ | CH ₃ -CH ₂ -(CH ₂) ₂₉ -CH ₂ -CH ₃ | 464 | 11.75 |

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2. Structural Elucidation of Compound 2

Compound 2 was a pale yellow oil. The R_f value was 0.42 (stationary phase: silica, solvent system: 50%CHCl₃/hexane).

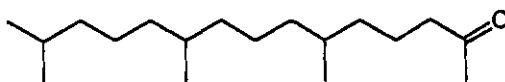
The IR spectrum of Compound 2 was shown in Fig.7 which indicated that this compound possessed carbonyl functional group, probably a ketone, at 1718 cm⁻¹ (C=O stretching). Other absorption bands were observed at 2953, 2928, 2870, 1461 and 1378 cm⁻¹ and these corresponded to aliphatic hydrocarbon. The IR absorption bands and their assignments are shown in Table 11.

Table 11 The IR Absorption Band Assignments of Compound 2

| Wavenumber (cm ⁻¹) | Intensity | Tentative Assignments |
|--------------------------------|-----------|---|
| 2953,2928,2870 | high | C-H stretching vibration of CH ₃ -, -CH ₂ - |
| 1718 | medium | C=O stretching vibration |
| 1461,1378 | medium | C-H bending vibration of CH ₃ -, -CH ₂ - |

The EI mass spectrum of Compound 2 (Fig.8) showed important fragmentation ion peaks at m/e 268, 250, 210, 179, 165, 137, 123 and 109. From Library Search (NIST) the fragmentation ion pattern of mass spectrum of this compound was found to be similar to 6,10,14-trimethyl-2-pentadecanone (C₁₈H₃₆O) (Fig. 9). In addition, the CI mass spectrum of this compound in Fig.10 showed the quasimolecular ion (MH)⁺ peak at m/e 269.

On the basis of IR and MS spectra, it was concluded that Compound 2 was 6,10,14-trimethyl-2-pentadecanone. The structure of Compound 2 is shown below.



6,10,14-trimethyl-2-pentadecanone (C₁₈H₃₆O)

3. Structural Elucidation of Mixture 3

Mixture 3 was a white amorphous solid, m.p. 85-87°C. The R_f value was 0.65 (stationary phase: silica, solvent system: CHCl_3).

The IR spectrum of Mixture 3 (Fig.11) suggested that this Mixture should be a mixture of saturated long chain aliphatic alcohols. The IR absorption bands and their assignments are shown in Table 12.

Table 12 The IR Absorption Band Assignments of Mixture 3

| Wavenumber (cm^{-1}) | Intensity | Tentative Assignments |
|---------------------------------|-----------|---|
| 3504-3230 | medium | O-H stretching vibration |
| 2919, 2849 | high | C-H stretching vibration of CH_3 -, $-\text{CH}_2$ - |
| 1473, 1463 | medium | C-H bending vibration of CH_3 -, $-\text{CH}_2$ - |
| 1065 | low | C-O stretching vibration |
| 722 | low | C-H rocking vibration of $(-\text{CH}_2)_n$ |

When Mixture 3 was analyzed by GLC, the chromatogram showed 5 peaks at retention times: 0.79, 1.05, 1.52, 2.37 and 3.78 min., respectively (Fig.13). Hence, Mixture 3 was a mixture of 5 long chain aliphatic alcohols. Comparison of the retention times with a calibration curve between log retention time and number of carbon atom of standard long chain aliphatic alcohols ($\text{C} = 14, 16, 18, 20$ and 22) (Fig.14) indicated that the numbers of carbon of Mixture 3 are 28, 29, 31, 32 and 34, respectively. The retention time of standard long chain aliphatic alcohols ($\text{C} = 14, 16, 18, 20$ and 22) and Mixture 3 is shown in Table 13.

Table 13 Retention Times of Standard Long Chain Aliphatic Alcohols and Mixture 3

| Substances | Retention Time (min.) | Log Retention Time | Number of Carbon |
|--------------|--------------------------|--------------------|------------------|
| Tetradecanol | 0.79 | -0.10 | 14 |
| Hexadecanol | 1.05 | 0.02 | 16 |
| Octadecanol | 1.52 | 0.18 | 18 |
| Eicosanol | 2.37 | 0.37 | 20 |
| Docosanol | 3.78 | 0.58 | 22 |
| Mixture 3 | 10.62 | 1.03 | 28 |
| | 13.72 | 1.14 | 29 |
| | 17.67 | 1.25 | 31 |
| | 22.98 | 1.36 | 32 |
| | 30.51 | 1.48 | 34 |

Thus the structure of saturated long chain aliphatic alcohols found in Mixture 3 can be assigned as shown in Table 14.

Table 14 Various types of Long Chain Aliphatic Alcohols is founded in Mixture 3

| Substances | MW Formula | Structure Formula | MW | % Composition |
|-------------------|-----------------------------------|---|-----|---------------|
| Octacosanol | C ₂₈ H ₅₆ O | CH ₃ -CH ₂ -(CH ₂) ₂₅ -CH ₂ -OH | 410 | 7.86 |
| Nonacosanol | C ₂₉ H ₆₀ O | CH ₃ -CH ₂ -(CH ₂) ₂₆ -CH ₂ -OH | 424 | 3.44 |
| Hentriacosanol | C ₃₁ H ₆₂ O | CH ₃ -CH ₂ -(CH ₂) ₂₈ -CH ₂ -OH | 452 | 24.57 |
| Dotriacontanol | C ₃₂ H ₆₆ O | CH ₃ -CH ₂ -(CH ₂) ₂₉ -CH ₂ -OH | 466 | 3.78 |
| Tetratriacontanol | C ₃₄ H ₆₈ O | CH ₃ -CH ₂ -(CH ₂) ₃₁ -CH ₂ -OH | 494 | 60.35 |

4. Structural Elucidation of Mixture 4

Mixture 4 was bright white needle like crystals, m.p. 139-141°C. The R_f value was 0.65 (stationary phase: silica, solvent system: 5%MeOH in CHCl_3)

The IR spectrum of Mixture 4 in Fig.15 exhibited the characteristic absorption band of hydroxy group (OH) at 3525-3198 cm^{-1} , absorption band of unsaturation at 1645 cm^{-1} and disubstituted and trisubstituted vinyl at 960 and 802 cm^{-1} , respectively. The IR absorption bands and their assignments are as shown in Table 15.

Table 15 The IR Absorption Band Assignments of Mixture 4

| Wavenumber (cm^{-1}) | Intensity | Tentative Assignments |
|---------------------------------|-----------|---|
| 3525-3198 | medium | O-H stretching vibration |
| 2940, 2869 | high | C-H stretching vibration of CH_3 -, $-\text{CH}_2$ - |
| 1645 | low | C=C stretching vibration |
| 1462, 1380 | medium | C-H bending vibration of CH_3 -, $-\text{CH}_2$ - |
| 1061 | medium | C-O stretching vibration |
| 960 | low | C-H out of plane bending vibration of trans configuration |
| 802 | low | C-H out of plane bending vibration of $=\text{CH}_2$ |

Chromatogram of Mixture 4 in Fig.17 showed 2 peaks at retention time 18.84 and 21.46. By comparison of its chromatogram with the chromatogram of the mixture of standard steroids (Fig.16), Mixture 4 was shown to be a mixture of stigmasterol and β -sitosterol, respectively. The Retention times of standard steroids and Mixture 4 are shown in Table 16.

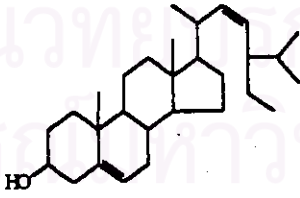
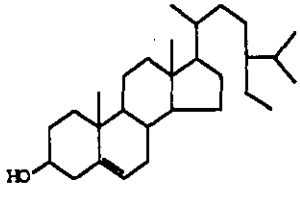
Table 16 Retention Time of Standard Steroids and Mixture 4

| Substances | Retention Time (min.) | % Composition |
|---------------------|--------------------------|----------------|
| Stigmasterol | 18.33 | 42.97 |
| β -sitosterol | 20.78 | 57.03 |
| Mixture 4 | 18.84 21.46 | 45.02 54.98 |

The EI mass spectrum of Mixture 4 in Fig.18 showed important fragmentation ion peaks at m/e 414 ($C_{29}H_{50}O$) and 412 ($C_{29}H_{48}O$) and other fragmentation ion peaks at m/e 396, 394, 275, 273, 255 and 213.

From all of the data (IR, GLC chromatogram and Mass spectrum), it was concluded that Mixture 4 was a mixture of stigmasterol ($C_{29}H_{50}O$, MW= 414) and β -sitosterol ($C_{29}H_{48}O$, MW= 412). The structure of these steroids are shown in Table 17.

Table 17 Various types of Steroids is founded in Mixture 4

| Substances | MW Formula | Structure Formula | MW | % Composition |
|---------------------|-----------------|---|-----|---------------|
| Stigmasterol | $C_{29}H_{48}O$ |  | 412 | 45.02 |
| β -sitosterol | $C_{29}H_{50}O$ |  | 414 | 54.98 |

5. Structural Elucidation of Compound 5

Compound 5 was a colourless solid, m.p. 127-129°C. The R_f value was 0.15 (stationary phase: silica, solvent system : chloroform).

The IR spectrum of compound 5 (Fig. 19) exhibited the characteristic absorption band of a hydroxy group at 3400-3050 cm^{-1} , the carboxylic acid carbonyl group at 1684 cm^{-1} and the unsaturation system at 1635 cm^{-1} . The IR absorption bands and their assignments are as shown in Table 18.

Table 18 The IR Absorption Band Assignments of Compound 5

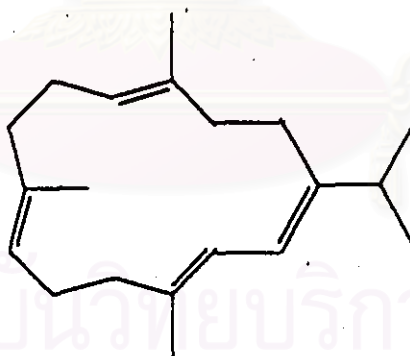
| Wavenumber (cm^{-1}) | Intensity | Tentative Assignments |
|---------------------------------|-----------|---|
| 3400-3050 | broad | O-H stretching vibration of acid |
| 2956, 2929 | strong | C-H stretching vibration of CH_3 -, $-\text{CH}_2$ - |
| 1684 | strong | C=O stretching vibration of acid |
| 1635 | medium | C=C stretching vibration |

The $^1\text{H-NMR}$ spectrum (Fig. 20) showed the proton signals of an isopropyl group, $-\text{CH}-(\text{CH}_3)_2$, ($\delta = 1.05$, 6H, d, $J = 7$ Hz), two methyl groups attached to double bonds, $-\text{C}=\text{C}-\text{CH}_3$, ($\delta = 1.68$, 3H, s and $\delta = 1.71$, 3H, s), thirteen sp^3 protons, $-\text{CH}_2-$, ($\delta = 2.2$, 8H, m and $\delta = 2.38$, 5H, m) and four olefinic protons, $-\text{C}=\text{CH}$, ($\delta = 5.14$, 1H, t, $J = 5.5$ Hz; $\delta = 5.91$, 1H, d, $J = 11$ Hz; $\delta = 6.01$, 1H, d, $J = 10.7$ Hz and $\delta = 6.89$, 1H, t, $J = 8$ Hz).

The $^{13}\text{C-NMR}$ spectrum (Fig. 21), DEPT-90 and DEPT-135 $^{13}\text{C-NMR}$ spectrum (Fig. 22) showed the signals of eleven sp^3 carbons ($\delta = 17.38$ (q); 17.96 (q); 22.09 (2xq); 24.70 (t); 26.74 (t); 29.14 (t); 30.54 (t); 34.56 (d); 37.71 (t) and 38.54 (t), (4 quartets, 6 triplets and 1 doublet), eight sp^2 carbons ($\delta = 118.61$ (d), 120.02 (d), 127.77 (d), 132.08 (s), 134.76 (s), 135.55 (s), 145.70 (d) and 146.49 (s), (4 doublets and 4 singlets) and one sp carbon of the carbonyl group of carboxylic acid ($\delta = 173.49$ (s)).

From the above NMR spectrums, this compound has 20 carbons and 29 protons (plus one for carboxylic acid = 30 protons). In addition, the EI mass spectrum (Fig. 23) showed the molecular ion peak at m/e 302. If it is assumed that this compound contain only carbons, protons and oxygens. A molecular formula of $C_{20}H_{30}O_2$ (calc. $m/e = 302.225$) can be established.

Double bond equivalent (DBE) of this compound, $C_{20}H_{30}O_2$, was 6 while the spectral data showed that this compound has four double bonds and one carbonyl group. Thus, it might consist of four double bonds, one carbonyl group and one ring. Comparison of the characteristic 1H and ^{13}C NMR in addition to the number of ring and double bonds required with literature [20] suggested that this compound might consist of a cembranoid structure, 14-membered-ring diterpene skeleton. Although there are many cembrenes appeared in the literature, the structure of Istoneocembrene-A seemed to fit all the number and type of bonds and required in Compound 5. (see Table 19-20)



Istoneocembrene-A

Table 19 Comparison the $^1\text{H-NMR}$ spectral data of Compound 5 with Istoneocembrene-A

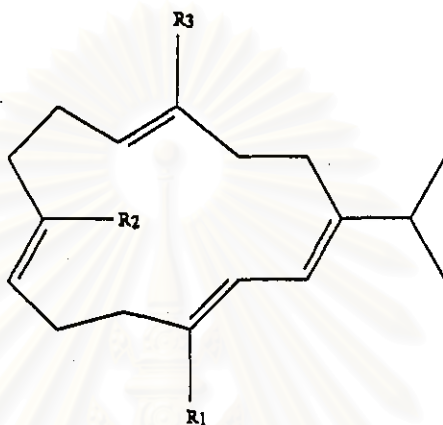
| Istoneocembrene-A (ppm.) | Compound <u>5</u> (ppm.) |
|--------------------------|--------------------------|
| 1.04 (d) | 1.05 (d) |
| 1.50 (s) | - |
| 1.57 (s) | 1.68 (s) |
| 1.73 (s) | 1.71 (s) |
| 2.12 (m) | 2.21 (m) |
| 2.26 (m) | 2.38 (m) |
| 5.02 (br m) | 5.14 (t) |
| - | 5.91 (d) |
| 5.98 (AB q) | 6.01 (d) |
| - | 6.89 (t) |

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Table 20 Comparison the ^{13}C -NMR spectral data of Compound 5 with Isonocembrene-A

| Isonocembrene-A (ppm.) | Compound <u>5</u> (ppm.) |
|------------------------|--------------------------|
| 15.0 (q) | 17.38 (q) |
| 17.1 (2xq) | 17.96 (q) |
| 22.3 (2xq) | 22.09 (2xq) |
| 24.5 (t) | 24.70 (t) |
| 25.3 (t) | 26.74 (t) |
| 28.0 (t) | 29.14 (t) |
| - | 30.54 (t) |
| 33.9 (d) | 34.56 (d) |
| 38.7 (t) | - |
| 39.0 (t) | 37.71 (t) |
| 39.2 (t) | 38.54 (t) |
| 118.6 (d) | 118.61 (d) |
| 121.1 (d) | 120.02 (d) |
| 124.5 (d) | 127.77 (d) |
| 125.0 (d) | - |
| 134.1 (s) | 132.08 (s) |
| 134.3 (s) | 134.76 (s) |
| 134.6 (s) | 135.55 (s) |
| - | 145.70 (d) |
| 146.9 (s) | 146.49 (s) |
| - | 173.49 (s) |

Based on the cembranoid structure and the data above, this compound has a carboxylic group which substituted a methyl group attached to double bond, three possible structures were presented as follows.



Structure I : $R_1 = \text{COOH}$, $R_2 = \text{CH}_3$, $R_3 = \text{CH}_3$

II : $R_1 = \text{CH}_3$, $R_2 = \text{COOH}$, $R_3 = \text{CH}_3$

III : $R_1 = \text{CH}_3$, $R_2 = \text{CH}_3$, $R_3 = \text{COOH}$

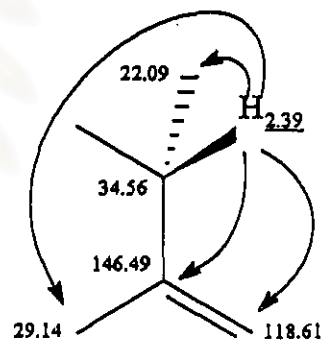
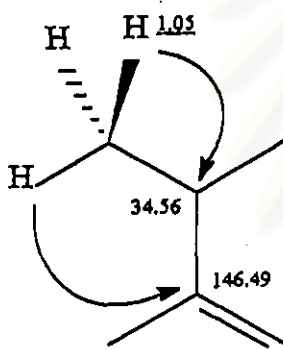
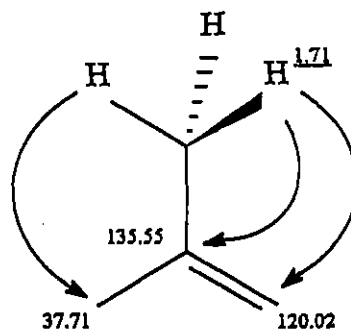
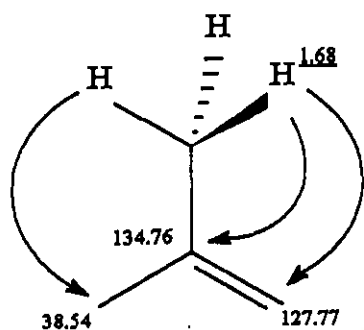
Two dimensional NMR techniques were used for assisting the structure assignment. The protons directly attached to carbons of the compound 5 were assigned by HMQC spectra (Fig. 24) as shown in Table 21.

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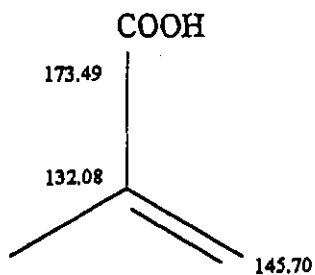
Table 21 ^1H attached to ^{13}C -NMR spectral data, HMQC, of Compound **5**

| ^{13}C -NMR (ppm.) | ^1H -NMR (ppm.) |
|-----------------------------|--------------------------|
| 17.38 (q) | 1.68 |
| 17.96 (q) | 1.71 |
| 22.09 (2xq) | 1.05 |
| 24.70 (t) | 2.23 |
| 26.74 (t) | 2.36 |
| 29.14 (t) | 2.26 |
| 30.54 (t) | 2.38 |
| 34.56 (d) | 2.39 |
| 37.71 (t) | 2.15 |
| 38.54 (t) | 2.20 |
| 118.61 (d) | 6.01 |
| 120.02 (d) | 5.91 |
| 127.77 (d) | 5.14 |
| 132.08 (s) | - |
| 134.76 (s) | - |
| 135.55 (s) | - |
| 145.70 (d) | 6.89 |
| 146.49 (s) | - |
| 173.49 (s) | - |

Crucial long-range ^1H - ^{13}C correlations as established by an HMBC experiment (Fig. 25) were : H ($\delta = 1.68$) with C ($\delta = 134.76$), CH ($\delta = 127.77$) and CH_2 ($\delta = 38.54$) (Fig. a) ; H ($\delta = 1.71$) with C ($\delta = 135.55$), CH ($\delta = 120.02$) and CH_2 ($\delta = 37.71$) (Fig. b) ; H ($\delta = 1.05$) with CH ($\delta = 34.56$) and C ($\delta = 146.49$) (Fig. c) ; H ($\delta = 2.39$) with CH_3 ($\delta = 22.09$), C ($\delta = 146.49$), CH ($\delta = 118.61$) and CH_2 ($\delta = 29.14$) (Fig. d).



Because of the disappearance of the carboxylic proton, long-range correlation between this proton and carbons nearby were not observed but as there is only one position left for it to attach, which is the quaternary olefinic carbon at $\delta = 132.08$ ppm. (Fig. e).



The COSY spectrum (Fig. 26) established the one bond correlation between the proton at 5.91 ($\delta_c = 120.02$) and 6.01 ppm. ($\delta_c = 118.61$). Therefore, partial structure was obtained as follow (Fig. f).

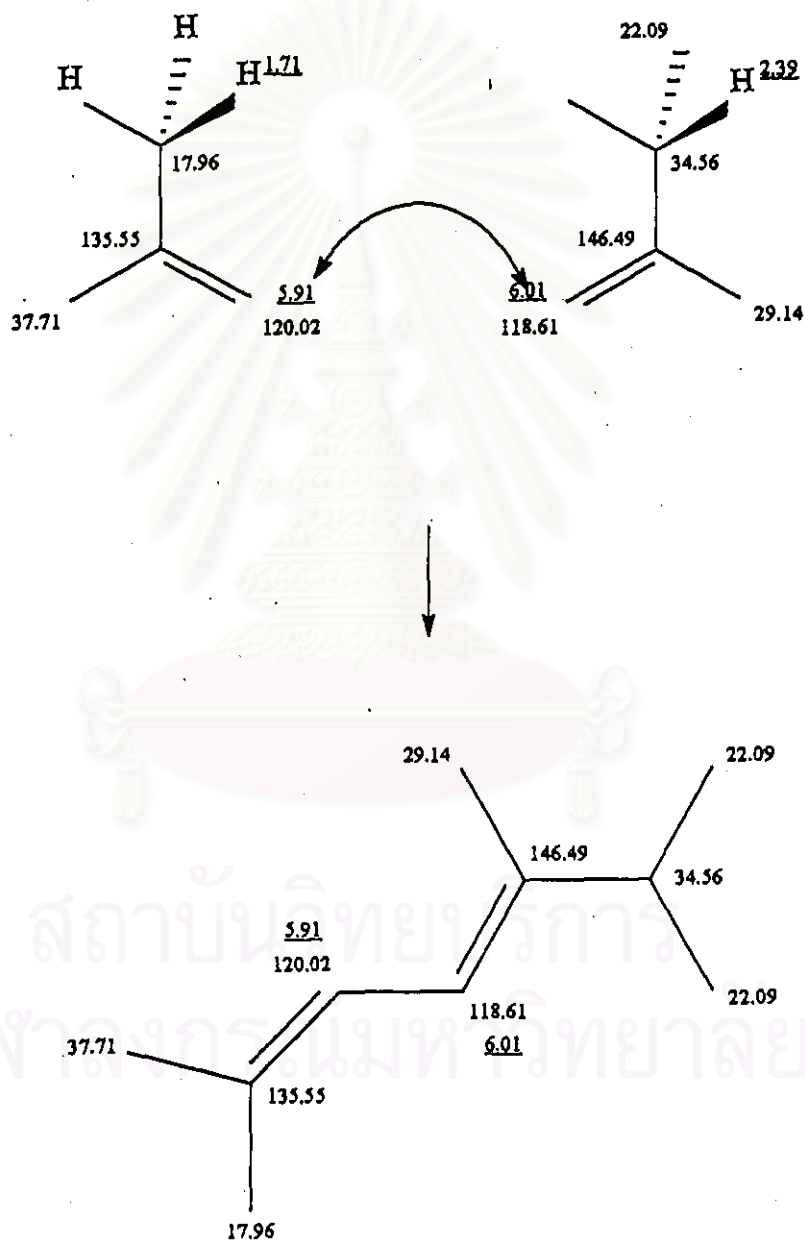


Fig. f

The CH₂ protons at 2.23 ($\delta_c = 24.70$) ppm. also showed long-range correlations with CH₂ ($\delta = 37.71$) and CH ($\delta = 127.77$) in the HMBC spectrum (Fig. g).

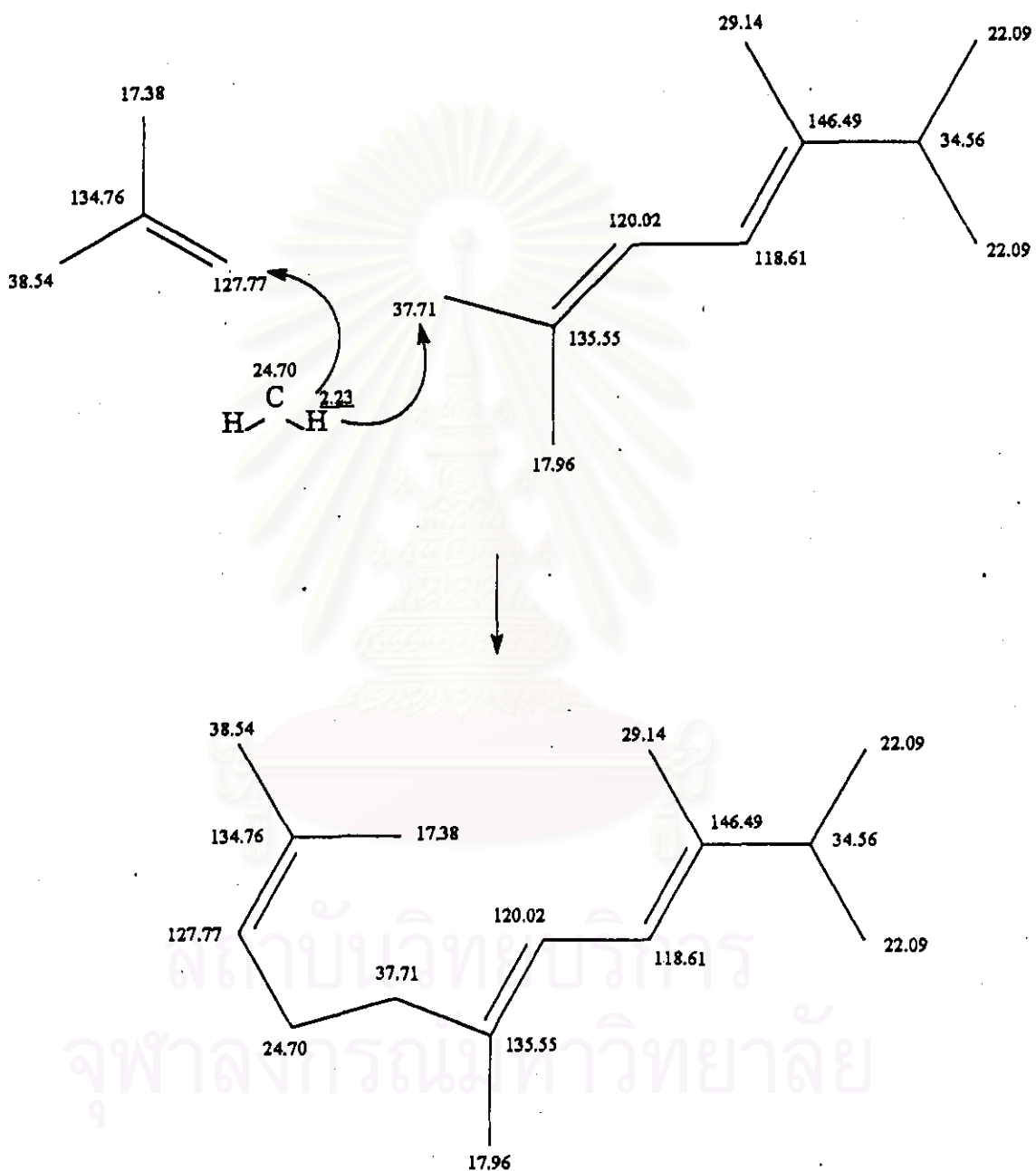


Fig. g

The protons at 2.26 ($\delta_c = 29.14$) ppm. showed long-range correlation with CH_2 ($\delta = 26.74$), C ($\delta = 132.08$), CH ($\delta = 34.56$) and CH ($\delta = 118.61$). In addition, the protons at 2.38 ($\delta_c = 30.54$) ppm. was correlated to CH ($\delta = 145.70$). After connecting all possible fragments together, Compound 5 must be structure III as follow (Fig. h).

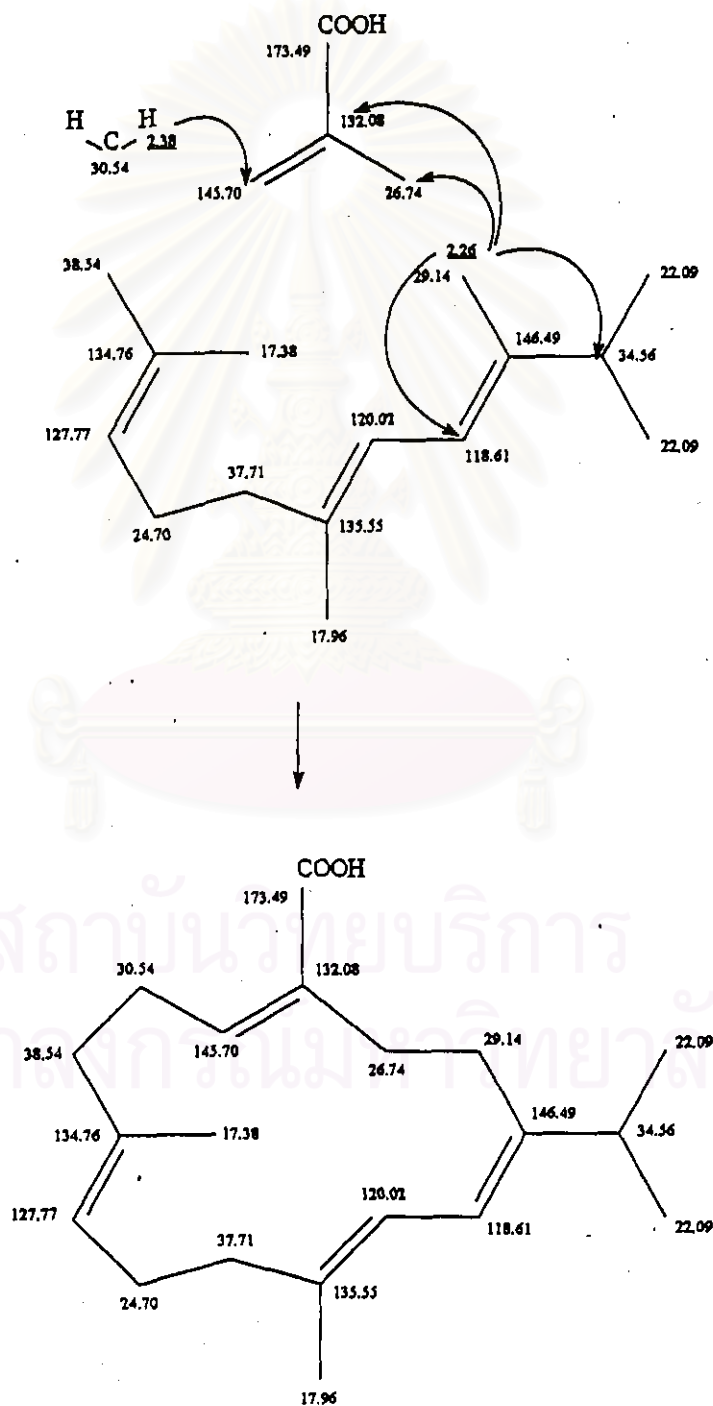


Fig. h

The long-range C-H correlations by HMBC spectrum were summarized in Table 22 and schematically shown as follows (Fig. i).

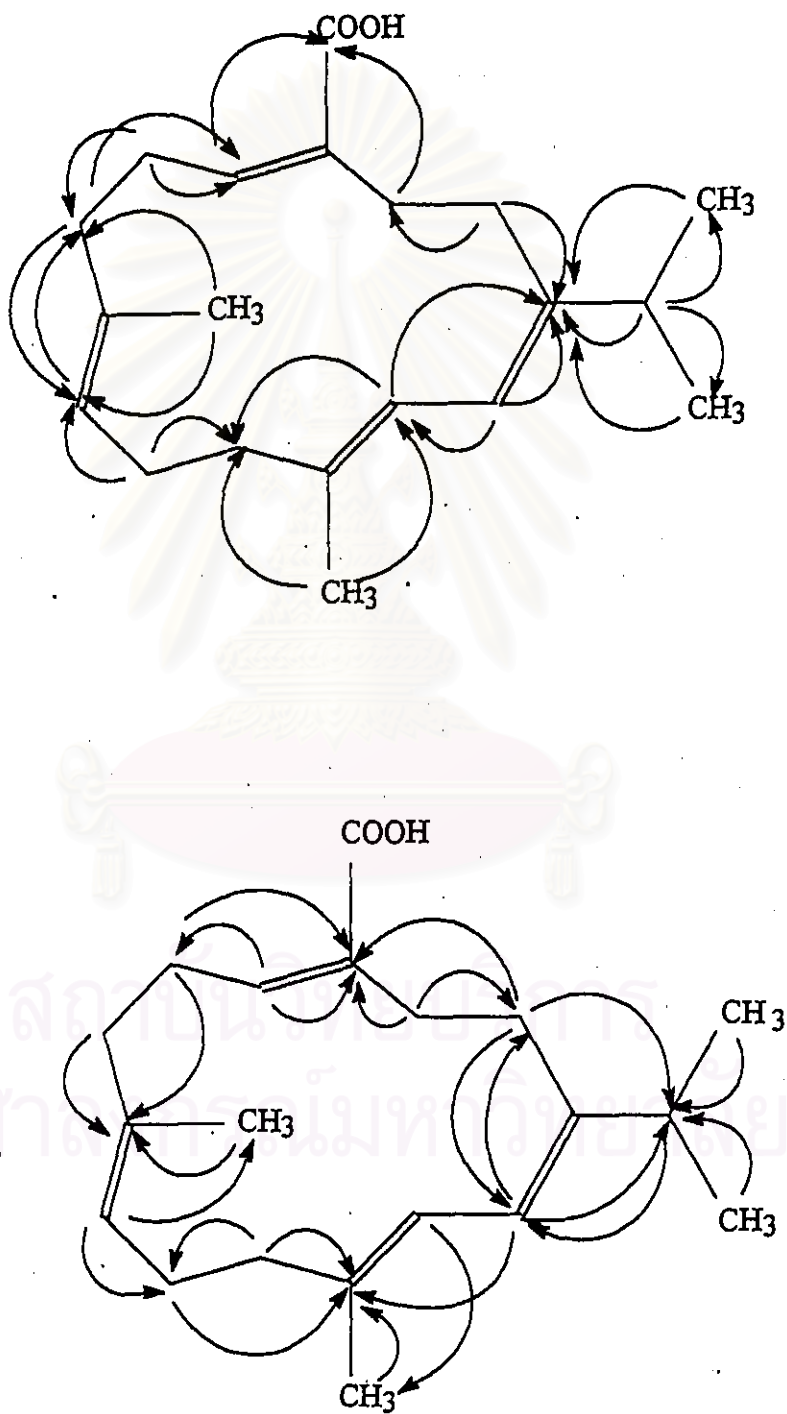


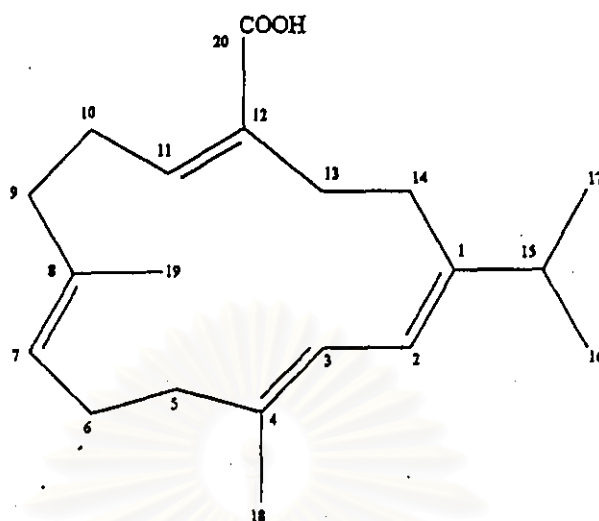
Fig. i

Table 22 The HMQC and HMBC data of Compound 5

| Position | δ_C | δ_H | J correlations (HMBC) |
|----------|------------|------------|-------------------------------|
| 1 | 146.49 s | - | H-2; H-3; H-14; H-15; H-16,17 |
| 2 | 118.61 d | 6.01 (d) | H-14; H-15 |
| 3 | 120.02 d | 5.91 (d) | H-2; H-18 |
| 4 | 135.55 s | - | H-2; H-5; H-6; H-18 |
| 5 | 37.71 t | 2.15 (m) | H-3; H-6; H-18 |
| 6 | 24.70 t | 2.23 (m) | H-5; H-7 |
| 7 | 127.77 d | 5.14 (t) | H-6; H-9; H-19 |
| 8 | 134.76 s | - | H-9; H-10; H-19 |
| 9 | 38.54 t | 2.20 (m) | H-7; H-10; H-19 |
| 10 | 30.54 t | 2.38 (m) | H-11 |
| 11 | 145.70 d | 6.89 (t) | H-9; H-10 |
| 12 | 132.08 s | - | H-10; H-11; H-13; H-14 |
| 13 | 26.74 t | 2.36 (m) | H-14 |
| 14 | 29.14 t | 2.26 (m) | H-2; H-13 |
| 15 | 34.56 d | 2.39 (m) | H-2; H-14; H-16,17 |
| 16,17 | 22.09 2xq | 1.05 (d) | H-15 |
| 18 | 17.96 q | 1.71 (s) | H-3 |
| 19 | 17.38 q | 1.68 (s) | H-7 |
| 20 | 173.49 s | - | H-11; H-13 |

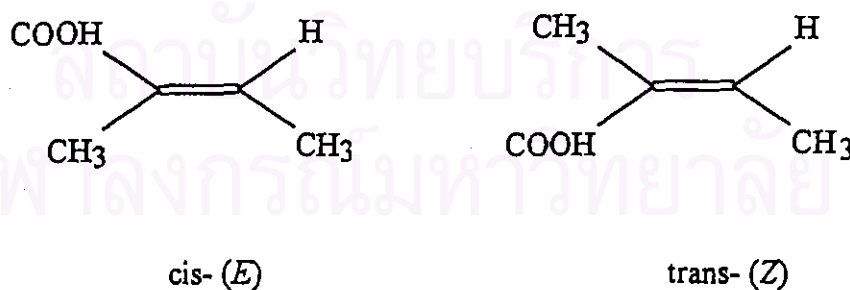
The data above suggested that the structure of Compound 5 is identical with crotoembraneic acid isolated from the stem bark of *Croton oblongifolius* Roxb.

[21,22]



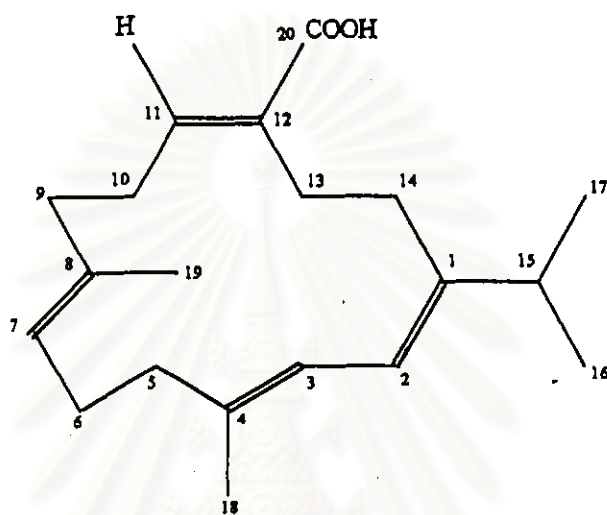
Crotoembraneic acid

However, there is a marked difference between their ^1H and ^{13}C NMR spectra in the region near the carboxylic acid group. Compound 5 could relate to crotoembraneic acid in that they are the two possible geometrical isomers around C=C bond conjugating to a carboxylic acid group i.e. cis- (*E*) or trans- (*Z*), assuming the rest of the molecule have the same configuration according to the similar ^{13}C chemical shifts at other positions.



Since the all ring-trans configuration was assigned to crotoembraneic acid based on ^{13}C chemical shift and NOESY data [22], Compound 5 must be the C₁₁-C₁₂-cis- (*E*) isomer. This conclusion is supported by the fact that the chemical shift of H₁₁ is rather down field ($\delta_{\text{H}}=6.89$ ppm. compared with $\delta_{\text{H}}=6.01$ ppm. of crotoembraneic

acid) which is in accord with the calculated value [23] (calculation; $\delta_{H, cis} = 6.83$ ppm. and $\delta_{H, trans} = 6.19$ ppm.). Thus, Compound 5 is (1*E*,3*E*,7*E*,11*E*)-1-isopropyl-4,8-dimethylcyclotetradeca-1,3,7,11-tetraene-12-carboxylic acid or neo-Crotoembraneic acid.



Compound 5

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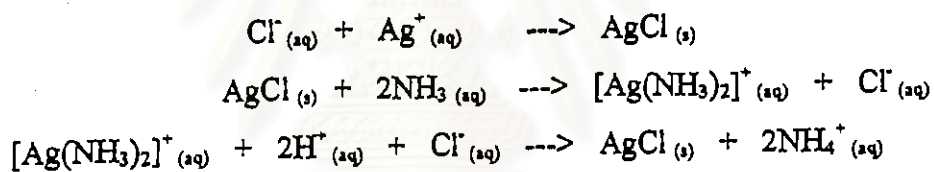
6. Structural Elucidation of Compound 6

Compound 6 was a colourless crystal, m.p. above 300 °C.

The results of inorganic ion tests of Compound 6 were shown in Table 7. This compound gave characteristic purple-red flame of potassium ion. In addition, it reacted with $\text{Na}_3[\text{Co}(\text{NO}_2)_6]$, it gave a yellow precipitate of $\text{K}_2\text{Na}[\text{Co}(\text{NO}_2)_6]$. These indicated that the cation was potassium ion (K^+).



When this compound reacted with AgNO_3 , it gave a white precipitate which was soluble in ammonia and precipitated back on addition of nitric acid. These indicated that the anion was chloride ion (Cl^-).



From the results of the reactions of Compound 6, it suggested that this compound was potassium chloride (KCl).

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