

CHAPTER III

RESULTS AND DISCUSSION

Structural Elucidation of the Isolated Compounds from the Leaf of *Amoora gigantea* Pierre.

1. Structural Elucidation of Compound 1

Compound 1 was white amorphous solid, 3.54 g (2.63%wt/wt of hexane crude), m.p. 47-50°C. R_f value 0.87 (solvent system: 10%CHCl₃ in hexane). This compound gave negative results to Liebermann-Burchard's, Br₂ in CCl₄ and 2,4-DNP reagents which indicated that it was not composed of steroidal or triterpenoidal skeleton, unsaturated part, and (as ketone or aldehyde) carbonyl as functional groups, respectively.

The IR spectrum of compound 1 in Fig. 10 suggested that this compound should be saturated long chain aliphatic hydrocarbons. The IR absorption bands are shown in Table 6.

Table 6 The IR Absorption Band Assignments of Compound 1

Wavenumber (cm ⁻¹)	Intensity	Tentative Assignments
2956	high	C-H stretching vibration of CH ₃ -
2918, 2849	high	C-H stretching vibration of CH ₃ -, -CH ₂ -
1463	medium	C-H asym. bending vibration of CH ₃ -, -CH ₂ -
1384	low	C-H sym. bending vibration of CH ₃ -
730,720	medium	C-H rocking vibration of (-CH ₂ -) _n , n>4

When Compound 1 was analyzed by GLC technique, the chromatogram showed 10 peaks at retention times: 1.67, 2.64, 3.02, 6.88, 8.81, 10.76, 14.10, 18.43, 23.52 and 30.37 min., respectively (Fig. 12). Therefore, Compound 1 was a mixture of 10 long chain hydrocarbons. A standard curve between log retention time and number of carbon atom (Fig. 13) from standard long chain aliphatic hydrocarbons (C_{24} - C_{26} , C_{28} - C_{33}) (Fig. 11) indicated that number of carbon of Compound 1 as 22, 23, 24, 27, 28, 29, 30, 31, 32 and 33, respectively. The chromatogram in Fig. 12 revealed that the peak at retention time 18.43 min.(C=31) was the major component of this mixture. The retention time of standard long chain hydrocarbons(C_{24} - C_{26} , C_{28} - C_{33}) and compound 1 is shown in Table 7.



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Table 7 Retention Time of Standard Long Chain Aliphatic Hydrocarbons (C₂₄-C₂₆, C₂₈-C₃₃) and compound 1

Name of Substances	Retention Time (min.)	Log Retention Time	Number of Carbon	% Composition
Tetracosane	3.16	0.50	24	5.71
Pentacosane	4.02	0.60	25	7.84
Hexacosane	5.21	0.72	26	4.78
Octacosane	8.57	0.93	28	9.58
Nonacosane	11.04	1.04	29	13.21
Triacontane	14.04	1.17	30	5.42
Hentriacontane	18.16	1.25	31	43.14
Dotriacontane	24.85	1.39	32	6.19
Trtriacontane	32.66	1.51	33	4.13
Compound <u>1</u>	1.67	0.22	22	20.73
	2.64	0.42	23	0.09
	3.02	0.48	24	0.53
	6.88	0.84	27	0.09
	8.81	0.94	28	0.14
	10.76	1.03	29	9.67
	14.10	1.15	30	2.25
	18.43	1.27	31	46.47
	23.52	1.37	32	5.01
30.37	1.48	33	15.02	

The composition of saturated long chain aliphatic hydrocarbons contained in Compound 1 is shown in Table 8.

Table 8 Various Type of Saturated Long Chain Aliphatic Hydrocarbons Found in Compound 1

Name of Substances	Molecular Formula	Structural Formula	Molecular Weight	%Composition
Docosane	C ₂₂ H ₄₆	CH ₃ -(CH ₂) ₂₀ -CH ₃	310	20.73
Tricosane	C ₂₃ H ₄₈	CH ₃ -(CH ₂) ₂₁ -CH ₃	324	0.09
Tetracosane	C ₂₄ H ₅₀	CH ₃ -(CH ₂) ₂₂ -CH ₃	338	0.53
Heptacosane	C ₂₇ H ₅₆	CH ₃ -(CH ₂) ₂₅ -CH ₃	380	0.09
Octacosane	C ₂₈ H ₅₈	CH ₃ -(CH ₂) ₂₆ -CH ₃	394	0.14
Nonacosane	C ₂₉ H ₆₀	CH ₃ -(CH ₂) ₂₇ -CH ₃	408	9.67
Triacontane	C ₃₀ H ₆₂	CH ₃ -(CH ₂) ₂₈ -CH ₃	422	2.25
Hentriacontane	C ₃₁ H ₆₄	CH ₃ -(CH ₂) ₂₉ -CH ₃	436	46.47
Dotriacontane	C ₃₂ H ₆₆	CH ₃ -(CH ₂) ₃₀ -CH ₃	450	5.01
Tritriacontane	C ₃₃ H ₆₈	CH ₃ -(CH ₂) ₃₁ -CH ₃	464	15.02

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2. Structural Elucidation of Compound 2

Compound 2 was white amorphous solid, 3.37 g (2.51%wt/wt of hexane crude), m.p. 65-68°C. R_f value 0.84 (solvent system:10%CHCl₃ in hexane). This compound gave negative results to Liebermann-Burchard's, 2,4-DNP and Br₂ in CCl₄ which indicated that it was not composed of steroidal or triterpenoidal skeleton, unsaturated part and carbonyl functional groups of ketone or aldehyde in its structure.

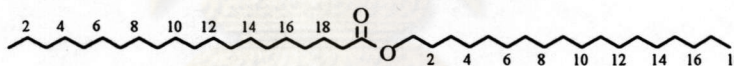
The IR spectrum of compound 2 was shown in Fig. 14 which indicated that this compound contained an ester functional group at 1737cm⁻¹ (C=O stretching), and 1174 cm⁻¹ (C-O stretching). Other absorption bands were observed at 2919, 2849, 1473, 730 and 720 cm⁻¹ corresponded to long chain aliphatic. The moiety IR absorption bands are tabulated in Table 9.

Table 9 The IR Absorption Band Assignments of Compound 2

Wavenumber (cm ⁻¹)	Intensity	Tentative Assignments
2919, 2849	high	C-H stretching vibration of CH ₃ -, -CH ₂ -
1737	high	C=O stretching vibration of ester
1473, 1464	medium	C-H asym. bending vibration of CH ₃ -, -CH ₂ -
1174	medium	C-O stretching vibration of ester
730, 720	medium	C-H rocking vibration of (-CH ₂ -) _n , n>4

The $^1\text{H-NMR}$ spectrum (Fig. 15) exhibited proton signals of $-\text{CH}_3$ at chemical shift (δ) 0.87 ppm. The signal at chemical shift (δ) 1.24, 1.59 ppm. exhibited proton signals of $(-\text{CH}_2-)_n$. Two signals were observed and could be assigned to be protons of ester as follow: 2.26 ppm. ($-\text{CH}_2-\text{C}=\text{O}$) and 4.02 ppm. ($-\text{CH}_2-\text{O}-\text{C}=\text{O}$).

The mass spectrum of Compound 2 exhibited the important fragmentation ion peaks at m/e 312. The fragmentation ion pattern of mass spectrum of Compound 2 was found to be similar to mass spectrum of Octadecyl eicosanoate ($\text{C}_{38}\text{H}_{76}\text{O}_2$) (Fig. 17). The fragmentation ion peaks at m/e 312 suggested that it belonged to the partial of Eicosanoic acid ($\text{C}_{20}\text{H}_{40}\text{O}_2$) part cleaved from the parent compound. The structure of Compound 2 is shown below.



Octadecyl eicosanoate ($\text{C}_{38}\text{H}_{76}\text{O}_2$)

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3. Structural Elucidation of Compound 3

Compound 3 was bright white needle crystal 4.86 g (3.34%wt/wt of hexane crude and methanol crude), m.p. 75°C. R_f value 0.56 (solvent system: 80%CHCl₃ in hexane). This compound gave positive results (purple color) with Liebermann-Burchard's, decolourized Br₂ in CCl₄ and also gave a positive result to 2,4-DNP reagents which indicated that it was composed of triterpenoidal skeleton, unsaturated part and carbonyl functional groups as ketone or aldehyde in its structure.

The IR spectrum of compound 3 (Fig. 18) which is assigned in Table 10, indicated that this compound contained a carbonyl function group (at 1704 cm⁻¹), addition bands of C=C stretching vibration (at 1642 cm⁻¹) and C-H bending vibration of gem-dimethyl group (at 1377 cm⁻¹). According to the IR spectrum and color tests, Compound 3 should be an unsaturated triterpenoidal ketone.

Table 10 The IR Absorption Band Assignments of Compound 3

Wavenumber (cm ⁻¹)	Intensity	Tentative Assignments
3075	high	C-H stretching vibration of alkene
2969-2946	high	C-H stretching vibration of CH ₃ -, -CH ₂ -
2853	high	C-H stretching vibration of -CH ₂ -
1704	high	C=O stretching vibration of ketone 1
1642	medium	non conjugated C=C stretching vibration
1481,1449	medium	C-H asym. bending vibration of CH ₃ -, -CH ₂ -
1377	high	C-H sym. bending vibration of gem-dimethyl
879	medium	C-H out of plane bending vibration of =CH ₂
826	low	C-H out of plane bending vibration of =CH ₂

The $^1\text{H-NMR}$ spectrum of Compound 3 (Fig. 19-22) showed the the singlet signals of seven methyl protons at 0.88, 0.95, 1.02, 1.04, 1.08, 1.62 and 1.69 ppm.(3H each). Other signals could be assigned as follows; 1.05-2.54 ppm. corresponded to the proton signals of methylene and methine protons(26 H), the two signals at 4.72 and 4.75 ppm.(2H, $J= 1.53, 0.93$ Hz.) showed the characteristic of terminal olefinic proton($\text{CH}_2=\text{C-}$) in agreement with the IR spectrum and a multiplet signal at 5.13 ppm.(1H) was corresponded to the vinylic proton.

The $^{13}\text{C-NMR}$ spectrum (Fig. 23-24) showed the olefinic carbon signals at 107.61, 124.44, 131.40 and 152.52 ppm. The signal at 218.01 ppm. should be the carbonyl functional group of ketone. Other signals around 15.35 to 55.38 ppm. ought to be methyl, methylene, methine and quaternary carbons.

Mass spectrum of Compound 3 (Fig. 38) showed the molecular ion peak at (m/e) 424 and other fragmentation peaks at m/e 409, 218, 206, 205, 190, 189, 163, 109, 95 and 81.

The molecular formula of Compound 3 was proposed as $\text{C}_{30}\text{H}_{48}\text{O}$ (MW 424) supported by the mass spectrum data and the elemental analysis result.

From data comparison (IR, ^1H , $^{13}\text{C-NMR}$ and Mass spectrum), of Compound 3 with many triterpenoidal compounds it indicated that Compound 3 should be 5α -dammara-20(21),24-dien-3-one. However, the $^{13}\text{C-NMR}$ and $^1\text{H-NMR}$ chemical shift assignments of 5α -dammara-20(21),24-dien-3-one has not been reported(64-65). Therefore, the tentative assignments of proton and carbon chemical shift of Compound 3 are proposed by comparing to those of the reported 5α -dammara-20(21),24-dien-3 β -ol which are shown in Table 11 and Table 12, respectively.

Table 11 The Data Comparison of $^1\text{H-NMR}$ Spectrum of 5α -dammara-20(21),
24-dien-3 β -ol and Compound 3

Position of Proton	Chemical Shift (ppm)	
	Compound <u>3</u>	5α -dammara-20(21),24-dien-3 β -ol (66-67)
H ₁₈	0.95	1.00
H ₁₉	1.04	0.86
H ₂₁	4.72-4.75	4.75
H ₂₄	5.13	5.16
H ₂₆	1.62	1.62
H ₂₇	1.69	1.70
H ₂₈	1.08	1.00
H ₂₉	1.02	0.79
H ₃₀	0.88	0.88

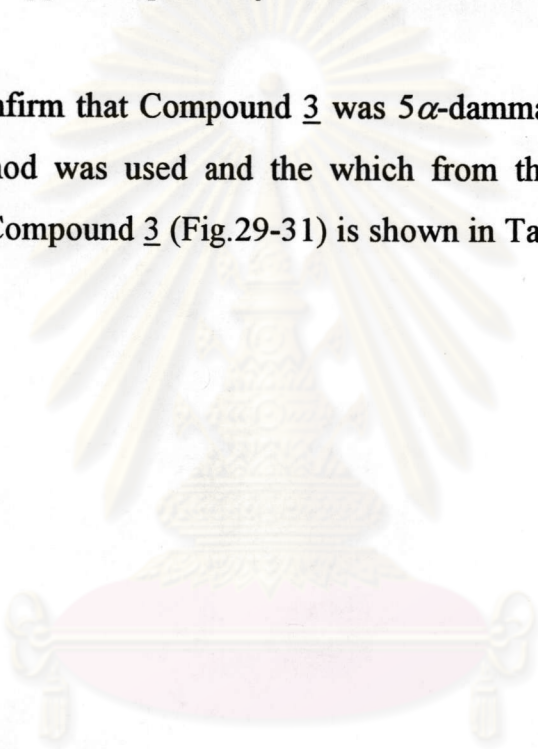
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Table 12 The Data Comparison of ^{13}C -NMR Spectrum of 5α -dammara-20(21),
24-dien-3 β -ol and Compound 3

Position of Carbon	Chemical Shift (ppm)	
	Compound <u>3</u>	5α -dammara-20(21),24-dien-3 β -ol (68-70)
C ₁	39.96	39.2
C ₂	34.10	27.4
C ₃	218.01	78.9
C ₄	47.40	39.0
C ₅	55.38	55.9
C ₆	19.68	18.3
C ₇	34.14	34.2
C ₈	40.37	40.5
C ₉	50.31	51.0
C ₁₀	36.92	37.2
C ₁₁	21.91	21.4
C ₁₂	28.87	28.9
C ₁₃	45.41	45.3
C ₁₄	49.40	49.5
C ₁₅	27.08	27.1
C ₁₆	34.76	35.5
C ₁₇	47.74	47.8
C ₁₈	16.06	15.7
C ₁₉	21.03	16.2
C ₂₀	152.52	152.6
C ₂₁	107.61	107.5
C ₂₂	31.36	31.4
C ₂₃	24.99	25.0
C ₂₄	124.44	124.4
C ₂₅	131.40	131.3
C ₂₆	17.72	17.7
C ₂₇	25.71	25.7
C ₂₈	26.77	28.1
C ₂₉	15.36	15.4
C ₃₀	15.83	16.0

From Table 11 and 12, they were found that proton and carbon of Compound 3 were close resemblance to those of with 5α -dammara-20(21),24-dien-3 β -ol. Therefore, Compound 3 should be 5α -dammara-20(21),24-dien-3-one which was a triterpenoid containing ketone group at C-3. The C-1, C-2 and C-4 adjacent to the C-3 in Compound 3 was appeared downfield at 39.96, 34.10 and 47.40 ppm, respectively but the other carbon was appeared at usual shift.

To confirm that Compound 3 was 5α -dammara-20(21),24-dien-3-one, 2D-NMR method was used and the which from the information of ^{13}C - ^1H correlation of Compound 3 (Fig.29-31) is shown in Table 13.



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Table 13 The Chemical Shift of ^{13}C - ^1H Correlation Assignment of
Compound **3**

Position		Chemical Shift Correlation	
Proton	Carbon	Proton	Carbon
H ₁	C ₁	1.95	39.96
H ₂	C ₂	2.44-2.50	34.10
H ₅	C ₅	1.38	55.38
H ₆	C ₆	1.55	19.68
H ₇	C ₇	1.97	34.14
H ₉	C ₉	1.42	50.31
H ₁₁	C ₁₁	1.52	21.91
H ₁₂	C ₁₂	1.91	28.87
H ₁₃	C ₁₃	1.68	45.41
H ₁₅	C ₁₅	2.12	27.08
H ₁₆	C ₁₆	1.34	34.76
H ₁₇	C ₁₇	2.20	47.74
H ₁₈	C ₁₈	0.95	16.06
H ₁₉	C ₁₉	1.04	21.03
H ₂₁	C ₂₁	4.72-4.75	107.61
H ₂₂	C ₂₂	1.23	31.36
H ₂₃	C ₂₃	1.60	24.99
H ₂₄	C ₂₄	5.13	124.44
H ₂₆	C ₂₆	1.62	17.72
H ₂₇	C ₂₇	1.69	25.71
H ₂₈	C ₂₈	1.08	26.77
H ₂₉	C ₂₉	1.02	15.36
H ₃₀	C ₃₀	0.88	15.83

From the information of ^1H - ^1H COSY analysis of Compound 3 (Fig. 27-28) the proton signal at δ 2.12 ppm. was linked to the proton signal at δ 1.97 ppm., the proton signal at δ 2.44-2.50 ppm. was linked to the proton signal at δ 1.42 and 1.95 ppm., the proton signal at δ 2.20 ppm. was linked to proton signal at δ 1.69 and 1.42 ppm., the proton signal at δ 1.62 ppm. was linked to with the proton signal at δ 1.08 and 1.34 ppm. and the proton signal at δ 1.42 ppm. was linked to the proton signal at δ 1.95 ppm..

The information of ^1H - ^1H NOESY analysis of Compound 3 (Fig. 32-34) indicated that the proton signal at δ 5.13 ppm. was linked to the proton signal at δ 1.69 ppm., the proton signal at δ 2.44-2.50 ppm was linked to the proton signal at δ 0.95 ppm., the signal proton at δ 2.20 ppm. was linked to the proton signal at δ 0.88 ppm., the signal proton at δ 1.97 ppm. adjoins with the signal proton at δ 2.12 ppm., the proton signal at δ 1.91 ppm. was linked to the proton signal at δ 0.88 ppm. and the proton signal at δ 1.95 ppm. was linked to the proton signal at δ 1.42 ppm..

The ^{13}C - ^1H COLOC of Compound 3 (Fig. 35-37) is assigned in Table 14.

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Table 14 The Chemical Shift of ^{13}C - ^1H COLOC Assignment of Compound 3

Position		Chemical Shift Correlation	
Proton	Carbon	Proton	Carbon
H ₁₉ , H ₁₈	C ₁	1.04, 0.95	39.96
H ₁	C ₂	1.95	34.10
H ₂₈ , H ₁₉ , H ₂₉	C ₄	1.08, 1.04, 1.02	47.40
H ₂₈ , H ₅	C ₅	1.08, 1.38	55.38
H ₅ , H ₆ , H ₂₆	C ₆	1.38, 1.55, 1.62	19.68
H ₁₅	C ₇	2.12	34.14
H ₃₀ , H ₁₇ , H ₁₁	C ₈	0.88, 1.34, 1.52	40.37
H ₁₈ , H ₂₉ , H ₉ , H ₂₃	C ₉	0.95, 1.02, 1.42, 1.60	50.31
H ₁₈ , H ₅ , H ₉	C ₁₀	0.95, 1.38, 1.42	36.92
H ₂₂ , H ₉ , H ₂₃	C ₁₁	1.23, 1.42, 1.60	21.91
H ₂₃	C ₁₂	1.60	28.87
H ₃₀ , H ₁₁ , H ₂₃ , H ₁₃	C ₁₃	0.88, 1.52, 1.60, 1.68	45.41
H ₃₀ , H ₂₃ , H ₁₃	C ₁₄	0.88, 1.60, 1.68	49.40
H ₁₅	C ₁₅	2.12	27.08
H ₁₆	C ₁₆	1.34	34.76
H ₁₃	C ₁₇	1.68	47.74
H ₁₈ , H ₉	C ₁₈	0.95, 1.42	16.06
H ₉	C ₁₉	1.42	21.03
H ₁ , H ₂₇ , H ₉	C ₂₀	1.95, 1.69, 1.42	152.52
H ₂₁	C ₂₁	4.72-4.75	107.61
H ₃₀	C ₂₂	0.88	31.36
H ₁₃	C ₂₃	1.68	24.99
H ₂₆ , H ₂₇	C ₂₄	1.62, 1.69	124.44
H ₂₆ , H ₂₇	C ₂₅	1.62, 1.69	131.40
H ₂₆ , H ₂₇	C ₂₆	1.62, 1.69	17.72
H ₂₆ , H ₂₇	C ₂₇	1.62, 1.69	25.71
H ₁₉	C ₂₈	1.04, 1.38	26.77
H ₃₀ , H ₂₉ , H ₉ , H ₂₆	C ₂₉	0.88, 1.02, 1.42, 1.62	15.36
H ₃₀ , H ₂₇	C ₃₀	0.88, 1.69	15.83

Literature surveys on 5α -dammarane-20(21),24-dien-3-one indicated that only IR spectra and melting point were available but no information on $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and 2D-NMR. The comparison of melting point data of 5α -dammarane-20(21),24-dien-3-one with Compound 3 is shown in Table 15.

Table 15. The Comparison of Melting Points Between 5α -dammarane-20(21),24-dien-3-one With Compound 3

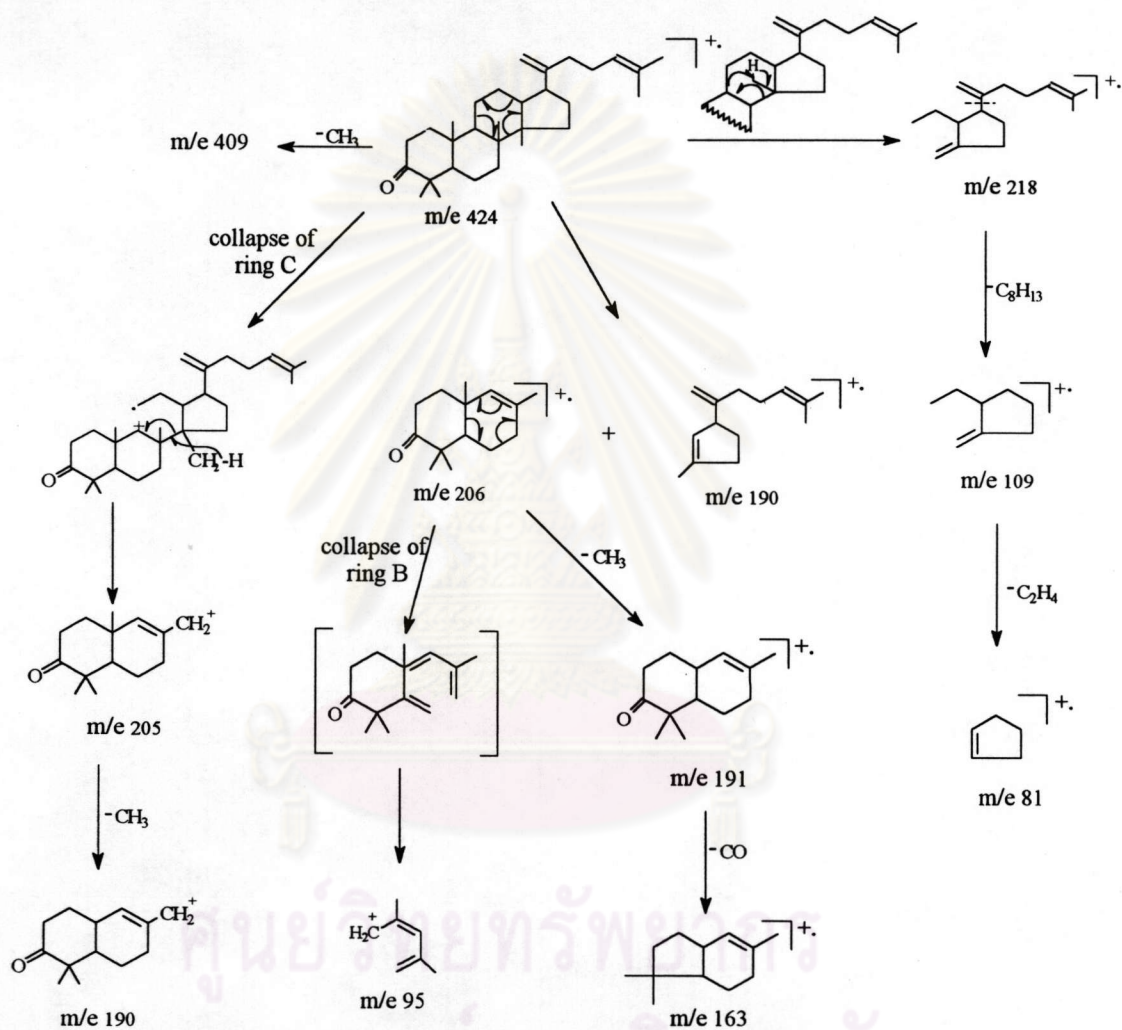
Compound	Melting Point ($^{\circ}\text{C}$)	Ref.
5α -dammarane-20(21),24-dien-3-one	78-79	64, 71-72
Compound <u>3</u>	75*	-

*The melting point of Compound 3 is uncorrected.

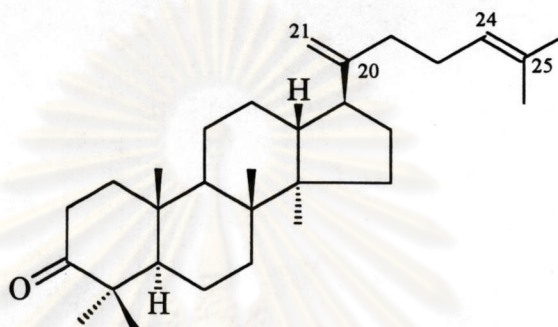
The possible mass fragmentation pattern of Compound 3 (66) is presented in Scheme 2.

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Scheme 2 The Possible Mass Fragmentation Pattern of Compound 3



According to all of spectral evidences and color test, Compound 3 was 5α -dammara-20(21),24-dien-3-one. The structure of this compound is shown below.



5α -dammara-20(21),24-dien-3-one

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4. Structural Elucidation of Compound 4

Compound 4 was white amorphous solid, 1.88 g (1.13%wt/wt of hexane crude and methanol crude), m.p.78-79°C. R_f value was 0.61 (solvent system: 100%CHCl₃ in hexane). Compound 4 gave negative results to Liebermann-Burchard's, 2,4-DNP and Br₂ in CCl₄ reagents which indicated that it was not composed of steroidal or triterpenoidal skeleton, unsaturated part and carbonyl functional groups of ketone or aldehyde in its structure.

The IR spectrum of compound 4 was shown in Fig. 39 which exhibited the absorption band at 3502-3314 cm⁻¹ indicated that Compound 4 should contain OH group as its functional group. Other absorption bands were similar to Compound 1. Thus, this compound ought to be a saturated long chain aliphatic primary alcohol. The IR absorption band assignments of compound 4 is shown in Table 16.

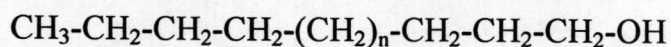
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Table 16 The IR Absorption Band Assignments of Compound 4

Wavenumber (cm ⁻¹)	Intensity	Tentative Assignments
3502-3314	medium	O-H stretching vibration
2918, 2849	high	C-H stretching vibration of CH ₃ -, -CH ₂ -
1470, 1463	medium	C-H asym. bending vibration of CH ₃ -, -CH ₂ -
1379	low	C-H sym. bending vibration of CH ₃ -
1059	high	C-O stretching vibration of 1° ROH
730, 720	medium	C-H rocking vibration of (-CH ₂) _n , n>4

The ¹H-NMR spectrum (Fig. 40) showed the important triplet signals at 3.62 ppm. which was the signal of the proton on the carbon attaching to oxygen atom (-CH₂-OH). The high intensity singlet signal at 1.25 ppm. revealed that there were several interlinking of methylene groups in the molecule of this compound. The singlet signal at 0.87 ppm. was corresponded to the methyl group signal.

The ¹³C-NMR spectrum (Fig. 41) showed the signal of carbon at chemical shift (δ) 13.52-32.86 ppm. (CH₃, -CH₂-) and the signal of carbon at 62.54 ppm. which was the carbon adjacent to OH. The other signal of carbon at 30.00 ppm. was the signal of (-CH₂)_n which its structure could be assigned below.



13.52 22.27 31.83 29.28 30.00 25.64 32.86 62.54

The GLC data analysis indicated that Compound 4 was not a single compound. The chromatogram (Fig. 43) showed 2 retention times at 27.78, 30.71 min. The standard chromatogram of long chain aliphatic alcohols (C = 14, 16, 18, 20, 22) (Fig 43) and calibration curve of log retention times and number of carbons of standard long chain aliphatic primary alcohols (Fig. 44). indicated that Compound 4 was a mixture of 2 saturated long chain aliphatic primary alcohols with 32 and 33 carbon atoms. The retention time of standard long chain aliphatic primary alcohols (C = 14, 16, 18, 20, 22) and Compound 4 are shown in Table 17.

Table 17 Retention Time of Standard Long Chain Aliphatic Primary Alcohols (C = 14, 16, 18, 20, 22) and Compound 4

Name of Substances	Retention Time (min.)	Log Retention Time	Number of Carbon	% Composition
CH ₃ (CH ₂) ₁₂ CH ₂ OH	0.81	-0.09	14	11.28
CH ₃ (CH ₂) ₁₄ CH ₂ OH	1.06	0.03	16	32.06
CH ₃ (CH ₂) ₁₆ CH ₂ OH	1.52	0.18	18	32.86
CH ₃ (CH ₂) ₁₈ CH ₂ OH	2.42	0.38	20	9.26
CH ₃ (CH ₂) ₂₀ CH ₂ OH	3.82	0.58	22	14.57
Compound <u>4</u>	27.78	1.44	32	0.99
	30.71	1.49	33	99.01

Various type of saturated long chain aliphatic primary alcohols in Compound 4 is shown in Table 18.

Table 18 Various Type of Saturated Long Chain Aliphatic Primary Alcohols Found in Compound 4

Name of Substances	Molecular Formula	Structural Formula	Molecular Weight	%Composition
Dotriacontanol	C ₃₂ H ₆₆ O	CH ₃ -(CH ₂) ₃₀ -CH ₂ OH	466	0.99
Tritriacontanol	C ₃₃ H ₆₈ O	CH ₃ -(CH ₂) ₃₁ -CH ₂ OH	480	99.01

5. Structural Elucidation of Compound 5

Compound 5 was white platet solid 1.76 g (1.31%wt/wt of hexane crude), m.p. 128-129°C. R_f value 0.63 (solvent system: 5%MeOH in CHCl₃). This compound gave positive results (purple color) to Liebermann-Burchard's reagent and also decolorized Br₂ in CCl₄ reagent but gave negative result to 2,4-DNP reagent which indicated that it was composed of triterpenoidal skeleton and unsaturated part in its structure.

The IR spectrum of compound 5 (Fig. 45) gave good agreements to color tests. Its IR spectrum gave the characteristic absorption band of secondary alcohol at 3376 cm⁻¹(O-H stretching vibration) and at 1092 and 1034 cm⁻¹ (C-O stretching vibration), addition bands of C=C stretching vibration at 1643 cm⁻¹ and C-H bending vibration of gem-dimethyl group at 1388 and 1375 cm⁻¹. According to the IR spectrum and color tests, Compound 5 should be an unsaturated triterpenoidal alcohol. The IR absorption band assignments of compound 5 is shown in Table 19.

Table 19 The IR Absorption Band Assignments of Compound 5

Wavenumber (cm^{-1})	Intensity	Tentative Assignments
3376	high	O-H stretching vibration
3082	low	C-H stretching vibration of asym. $\text{R}_1\text{R}_2\text{C}=\text{CH}_2$
2927, 2860	high	C-H stretching vibration of CH_3 -, $-\text{CH}_2$ -
1643	medium	non conjugated $\text{C}=\text{C}$ stretching vibration
1466, 1443	high	C-H bending vibration of $-\text{CH}_2$ -, CH_3 -
1388, 1375	medium	C-H bending vibration of gem-dimethyl
1092, 1034	medium	C-O stretching vibration of 3β -OH (equatorial)
985	low	C-H out of plane bending vibration of $=\text{CH}_2$
887	medium	C-H out of plane bending vibration of $=\text{CH}_2$

The ^1H -NMR spectrum (Fig. 46-49) showed the the singlet signals of seven methyl protons at 0.78, 0.85, 0.87, 0.98, 1.62 and 1.69 ppm. (3H each). Other signals could be assigned as follows; 1.04-2.24 ppm. corresponded to the proton signals of methylene and methine protons(24H), a broad multiplet at 3.20 ppm. was typical for a hydrogen geminal to a hydroxy group at C-3 ($-\text{CHOH}$), the two signals at 4.72 and 4.74 ppm.(2H, $J=1.53, 0.92$ Hz.) showed the characteristic of terminal olefinic proton($\text{CH}_2=\text{C}-$) in agreement with the IR spectrum and a multiplet signal at 5.14 ppm.(1H) was corresponded to the vinylic proton.

The ^{13}C -NMR spectrum (Fig. 50) showed the olefinic carbon signals at 107.49, 124.49, 131.43 and 152.74 ppm.. The signal at 78.97 ppm. should be the carbon signal adjacent to oxygen atom bearing one proton ($-\text{CHOH}$). Other signals from 15.38 to 55.90 ppm. ought to be methyl, methylene, methine and quaternary carbons.

Mass spectrum of Compound 5 (Fig. 69) showed the molecular ion peak at (m/e) 426 and other fragmentation peaks at m/e 408, 218, 208, 207, 190, 189 and 95.

The molecular formula of Compound 5 was proposed as $C_{30}H_{50}O$ which was supported by the mass spectrum data and the elemental analysis result.

From the comparison of spectroscopic data (IR, 1H , ^{13}C -NMR and Mass spectrum) of Compound 5 with many triterpenoidal compounds it was found that Compound 5 should belong to 5α dammarane triterpenoid series(64-65) with one equatorial hydroxy group at C-3, seven methyl group and two vinylidene groups. It was assigned that it should be 5α -dammara-20(21),24-dien-3 β -ol. The 1H -NMR and ^{13}C -NMR spectra of this compound were very closely to 5α -dammara-20(21),24-dien-3 β -ol. The tentative assignments of proton and carbon chemical shift of Compound 5 were compared with those reported for 5α -dammara-20(21),24-dien-3 β -ol in Table 20 and Table 21, respectively.

Table 20 The Comparison of 1H -NMR Spectrum of 5α -dammara-20(21), 24-dien-3 β -ol and Compound 5

Position of Proton	Chemical Shift (ppm)	
	Compound 5	5α -dammara-20(21),24-dien-3 β -ol (66-67)
H ₃	3.20	3.24
H ₁₈	0.98	1.00
H ₁₉	0.85	0.86
H ₂₁	4.72-4.74	4.75
H ₂₄	5.14	5.16
H ₂₆	1.62	1.62
H ₂₇	1.69	1.70
H ₂₈	0.98	1.00
H ₂₉	0.78	0.79
H ₃₀	0.87	0.88

Table 21 The Comparison of ^{13}C -NMR Spectrum of 5α -dammara-20(21),24-dien-3 β -ol and Compound 5

Position of Carbon	Chemical Shift (ppm)	
	Compound <u>5</u>	5α -dammara-20(21),24-dien-3 β -ol (68-70)
C ₁	39.14	39.2
C ₂	27.44	27.4
C ₃	78.97	78.9
C ₄	38.99	39.0
C ₅	55.90	55.9
C ₆	18.31	18.3
C ₇	34.14	34.2
C ₈	40.49	40.5
C ₉	50.97	51.0
C ₁₀	37.25	37.2
C ₁₁	21.39	21.4
C ₁₂	28.92	28.9
C ₁₃	45.29	45.3
C ₁₄	49.45	49.5
C ₁₅	27.10	27.1
C ₁₆	35.45	35.5
C ₁₇	47.86	47.8
C ₁₈	15.66	15.7
C ₁₉	16.24	16.2
C ₂₀	152.75	152.6
C ₂₁	107.49	107.5
C ₂₂	31.41	31.4
C ₂₃	24.99	25.0
C ₂₄	124.49	124.4
C ₂₅	131.43	131.3
C ₂₆	17.73	17.7
C ₂₇	25.71	25.7
C ₂₈	28.03	28.1
C ₂₉	15.38	15.4
C ₃₀	15.94	16.0

From Table 20 and 21, they were found that the proton and carbon carbon of Compound 5 were very close resemblance with those of 5α -dammara-20(21),24-dien-3 β -ol. Therefore, Compound 5 should be 5α -dammara-20(21),24-dien-3 β -ol which was a triterpenoid containing hydroxy group at C-3.

To confirm that Compound 5 was 5α -dammara-20(21),24-dien-3 β -ol by 2D-NMR method which the information on ^{13}C - ^1H correlation of Compound 5 (Fig. 59-60) is carried out and the data is assigned in Table 22.

Table 22 The Chemical Shift of ^{13}C - ^1H Correlation Assignment of Compound 5

Position		Chemical Shift Correlation	
Proton	Carbon	Proton	Carbon
H ₁	C ₁	1.72	39.14
H ₂	C ₂	1.58	27.44
H ₃	C ₃	3.20	78.97
H ₅	C ₅	0.72	55.90
H ₆	C ₆	1.53	18.31
H ₇	C ₇	1.96	34.14
H ₉	C ₉	1.30	50.97
H ₁₁	C ₁₁	1.51	21.39
H ₁₂	C ₁₂	1.89	28.92
H ₁₃	C ₁₃	1.66	45.29
H ₁₅	C ₁₅	2.12	27.10
H ₁₆	C ₁₆	1.27	35.45
H ₁₇	C ₁₇	2.18	47.86
H ₁₈	C ₁₈	0.98	15.66
H ₁₉	C ₁₉	0.85	16.24
H ₂₁	C ₂₁	4.72-4.74	107.49

Table 22 (continued)

Position		Chemical Shift Correlation	
Proton	Carbon	Proton	Carbon
H ₂₂	C ₂₂	1.10	31.41
H ₂₃	C ₂₃	1.54	24.99
H ₂₄	C ₂₄	5.14	124.49
H ₂₆	C ₂₆	1.62	17.73
H ₂₇	C ₂₇	1.69,	25.71
H ₂₈	C ₂₈	0.98	28.03
H ₂₉	C ₂₉	0.78	15.38
H ₃₀	C ₃₀	0.87	15.94

The information on ¹H-¹H COSY analysis of Compound 5 (Fig. 54-58) indicated that the proton signal at δ 5.14 ppm. was linked to the proton signal at δ 2.12, 1.62 and 1.69 ppm., the proton signal at δ 3.20 ppm was linked to the proton signal at δ 1.58 ppm., the proton signal at δ 2.18 ppm. was linked to the proton signal at δ 1.27 ppm., the proton signal at δ 2.12 ppm. was linked to adjoins with the proton signal at δ 1.96 ppm., the proton signal at δ 1.89 ppm. was linked to the proton signal at δ 1.51 ppm. and the proton signal at δ 1.69 ppm. was linked to the proton signal at δ 0.98 ppm.

The information on ¹H-¹H NOESY analysis of Compound 5 (Fig. 61-65) indicated that the proton signal at δ 5.14 ppm. was linked to the proton signal at δ 1.69 ppm., the proton signal at δ 4.72-7.74 ppm was linked to the proton signal at δ 1.62 ppm., the proton signal at δ 3.20 ppm. was linked to the proton signal at δ 0.78 and 0.98 ppm, the proton signal at δ 2.18 ppm. was

linked to the proton signal at δ 0.87 ppm. and the proton signal at δ 2.12 ppm. was linked to the proton signal at δ 1.96 ppm.

For the result of ^{13}C - ^1H COLOC of Compound 5 (Fig. 66-68) is assigned in Table 23.

Table 23 The Chemical Shift of ^{13}C - ^1H COLOC Assignment of Compound 5

Position		Chemical Shift Correlation	
Proton	Carbon	Proton	Carbon
H ₁₉	C ₁	0.85	39.14
H ₁	C ₂	1.72	27.44
H ₂₈	C ₃	0.98	78.97
H ₂₉ , H ₂₈	C ₄	0.78, 0.98	38.99
H ₂₈ , H ₁₉ , H ₅	C ₅	0.98, 0.85, 0.72	55.90
H ₃₀ , H ₁₈ , H ₁₁	C ₈	0.87, 0.98, 1.51	40.49
H ₁₈ , H ₁₉	C ₉	1.27, 0.89, 0.85	50.97
H ₁₉ , H ₉ , H ₆	C ₁₀	0.85, 1.30, 1.53	37.25
H ₃₀	C ₁₃	0.87	45.29
H ₁₈ , H ₃₀	C ₁₄	0.98, 0.87	49.45
H ₁₈	C ₁₆	0.98	35.45
H ₁₈	C ₁₈	0.98	15.66
H ₁₉ , H ₅ , H ₁₈	C ₁₉	0.85, 0.72, 0.98	16.24
H ₂₁	C ₂₁	4.72-4.74	107.49
H ₃₀	C ₂₂	0.87	31.41
H ₂₇ , H ₂₆	C ₂₄	1.69, 1.62	124.49
H ₂₆ , H ₂₇	C ₂₅	1.62, 1.69	131.43
H ₂₇ , H ₂₆	C ₂₆	1.69, 1.62	17.73
H ₂₆ , H ₂₇	C ₂₇	1.62, 1.69	25.71
H ₂₉ , H ₂₈	C ₂₈	0.78, 0.98	28.03
H ₂₉ , H ₅ , H ₂₈	C ₂₉	0.78, 0.72, 0.98	15.38
H ₃₀	C ₃₀	0.87	15.94

Literature surveys on 5α -dammara-20(21),24-dien- 3β -ol found that there was report on IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ spectra and melting point but no previously reported on 2D-NMR. The melting point data of 5α -dammara-20(21),24-dien- 3β -ol and Compound 5 is shown in Table 24.

Table 24 The Comparison of Melting Point Between of 5α -dammara-20(21), 24-dien- β -ol With Compound 5

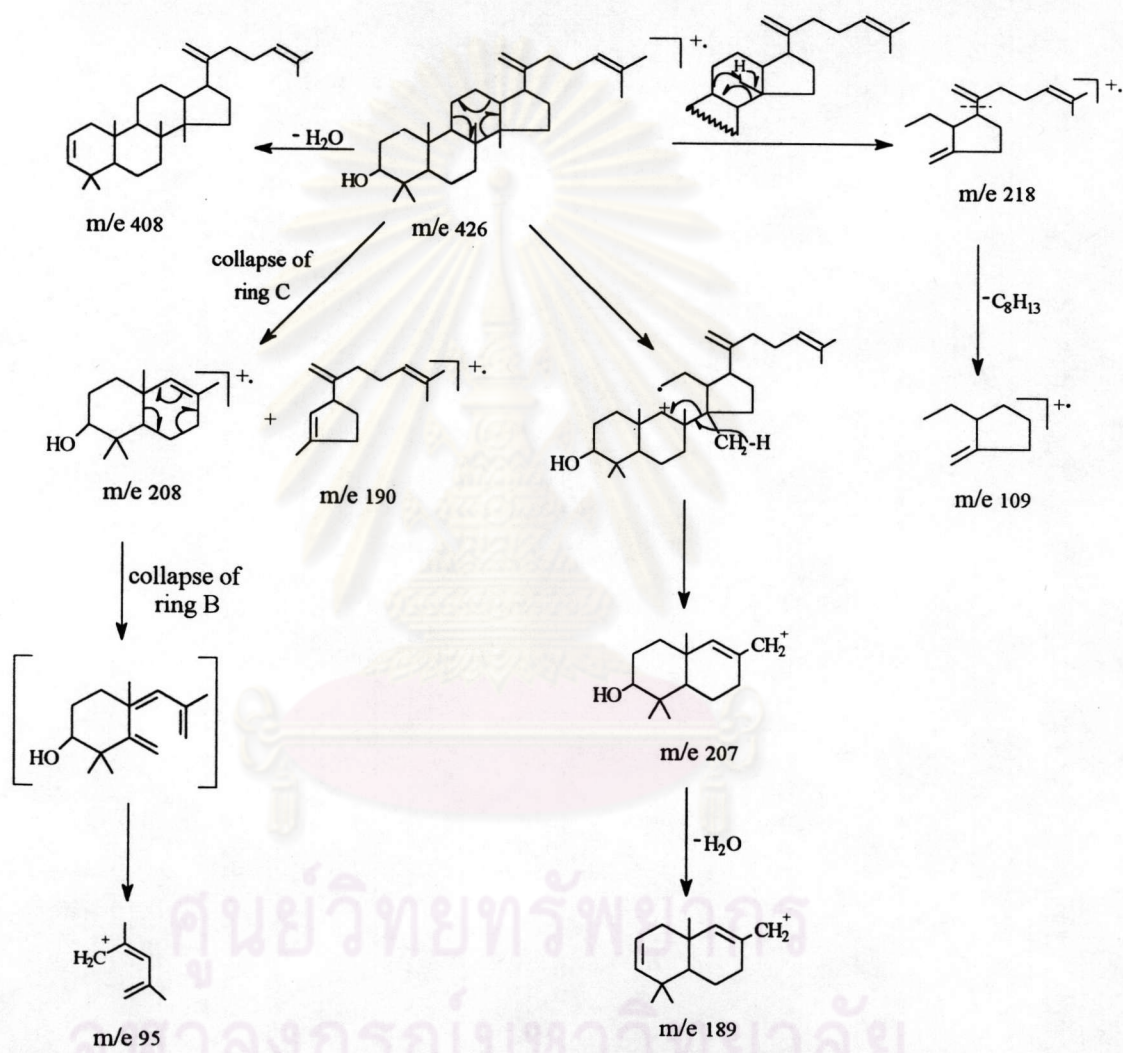
Compound	Melting Point ($^{\circ}\text{C}$)	Ref.
5α -dammara-20(21),24-dien- 3β -ol	136-138	64, 71-72
Compound <u>5</u>	128-129*	-

*The melting point of Compound 5 is uncorrected.

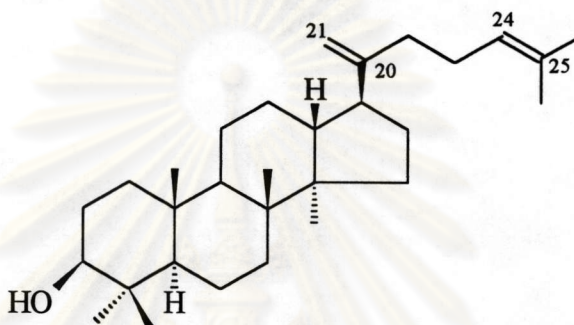
The possible mass fragmentation pattern of Compound 5 is presented in Scheme 3.

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Scheme 3 The Possible Mass Fragmentation Pattern of Compound 5



According to all of spectral evidences, and color test, it was concluded that Compound 5 was 5α -dammara-20(21),24-dien-3 β -ol. The structure of this compound is shown below.



5α -dammara-20(21),24-dien-3 β -ol

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6. Structural Elucidation of Compound 6

Compound 6 was bright white needle crystal 2.41 g (1.79%wt/wt. of hexane crude), m.p. 130-132°C. R_f value 0.60 (solvent system: 5%MeOH in CHCl_3). This compound gave positive results (green color) with Liebermann-Burchard's reagent and also decolorized Br_2 in CCl_4 reagent but gave a negative result to 2,4-DNP reagent which indicated that it was composed of steroidal skeleton and unsaturated part in its structure.

The IR spectrum of compound 6 was shown in Fig. 70 which exhibited the characteristic absorption band of hydroxy group (OH) at 3430 cm^{-1} , absorption band of unsaturation at 1646 cm^{-1} and disubstituted and trisubstituted vinyl at 959 and 802 cm^{-1} , respectively. The IR absorption band is shown in Table 25.

Table 25 The IR Absorption Band Assignments of Compound 6

Wavenumber (cm^{-1})	Intensity	Tentative Assignments
3430	high	O-H stretching vibration
2960-2868	high	C-H stretching vibration of CH_3 -, $-\text{CH}_2$ -
1646	low	C=C stretching vibration
1464, 1380	medium	C-H bending vibration of $-\text{CH}_2$ -, CH_3 -
1059, 1023	medium	C-O stretching vibration of 3β -OH (equatorial)
959	medium	C-H out of plane bending vibration of trans configuration
802	low	C-H out of plane bending vibration of $=\text{CH}_2$

The ^1H -NMR spectrum (Fig. 71-75) showed that the signals of protons at chemical shift 0.73-2.35 ppm. corresponded to the proton signals of methyl, methylene and methine protons, a broad multiplet at 3.55 ppm. exhibited the proton linked to carbon having hydroxy group at C-3(- CHOH). The signal at 5.00-5.10 ppm. indicated - $\text{CH}=\text{CH}$ - group and the proton signals at 5.37 ppm. was assigned to be the signal of vinylic proton (- $\text{CH}=\text{C}$ -).

The ^{13}C -NMR spectrum (Fig. 76-77), DEPT-135 and DEPT-90 ^{13}C -NMR (Fig. 78-80) exhibited the carbon signals of 43 peaks which were the olefinic carbon signals at 121.40, 129.13, 138.18 and 140.83 ppm. The signal at 71.46 ppm. should be the carbon signal adjacent to oxygen atom (- CHOH). Other signals at 11.74 to 56.73 ppm. ought to be methyl, methylene, methine and quaternary carbons.

Mass spectrum of Compound 6 (Fig. 83) showed the molecular ion peak at (m/e) 414 and 412 and other fragmentation peaks at m/e 396, 394, 275, 273, 255 and 213, respectively.

The formation obtained from all spectral evidences and color test of Compound 6 suggested that it might be a steroidal compound.

The structure of Compound 6 was confirmed by GLC technique by comparison of its chromatogram with the chromatogram of the mixture of three standard steroids (Fig. 81). The GLC analysis data showed that Compound 6 was not a single compound. The 2 peaks at retention time 15.84 and 17.28 min. (Fig. 82) which indicated that Compound 6 was a mixture of stigmasterol and β -sitosterol, respectively. Retention time of the mixture of three standard steroids and Compound 6 is shown in Table 26.

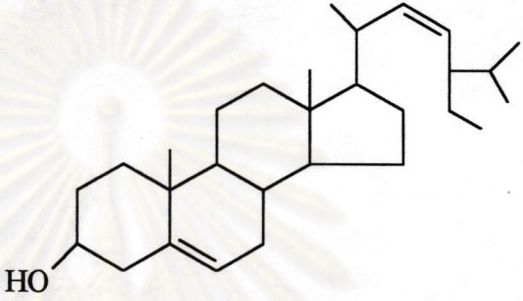
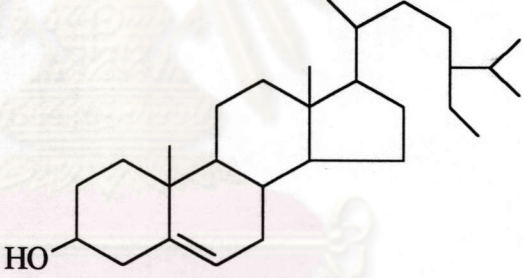
Table 26 Retention Time of the Mixture of Three Standard Steroids and Compound 6

Name of substances	Retention time (min.)	Log Retention time	% Composition
campesterol	14.80	1.17	10.86
stigmasterol	15.64	1.19	26.10
β -sitosterol	17.30	1.23	63.04
Compound <u>6</u>	15.84	1.19	22.63
	17.28	1.23	77.37

From all of the spectral data (IR, ^1H and ^{13}C -NMR spectrum, GLC chromatogram and Mass spectrum), it was concluded that Compound 6 was a mixture of stigmasterol and β -sitosterol. The structure of steroids is shown in Table 27.

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Table 27 Various Type of Steroids Found in Compound 6

Name of substance	Molecular formula	Structural formula	Molecular weight
stigmasterol	$C_{29}H_{48}O$		412
β -sitosterol	$C_{29}H_{50}O$		414

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7. Structural Elucidation of Compound 7

Compound 7 was white amorphous solid, 0.18g (0.07%wt/wt of methanol crude), m.p. 284°C (decompose). R_f value 0.23 (solvent system: 10% MeOH in CHCl_3). This compound gave positive results (green color) with Liebermann-Burchard's reagent and decolourized Br_2 in CCl_4 reagent but gave negative result to 2,4-DNP reagent which indicated that it was composed of steroidal skeleton and unsaturated part in its structure.

The IR spectrum of compound 7 (Fig. 84) was exhibited the characteristic absorption band of hydroxy group (OH) at 3423 cm^{-1} , C-O stretching vibration of glycosidic linkage at $1074\text{-}1025\text{ cm}^{-1}$ and an anomeric axial C-H deformation of β -sugar at 890 cm^{-1} . The additional bands were compatible with a trisubstituted olefin and gem-dimethyl group at 1645 and 1380 cm^{-1} , respectively. The IR absorption band of Compound 7 is assigned in Table 28.

Table 28 The IR Absorption Band Assignments of Compound 7

Wavenumber (cm^{-1})	Intensity	Tentative Assignments
3423	high	O-H stretching vibration
2961-2874	high	C-H stretching vibration of CH_3 -, $-\text{CH}_2$ -
1645	low	C=C stretching vibration
1463	medium	C-H bending vibration of CH_3 -, $-\text{CH}_2$ -
1380	medium	C-H bending vibration of gem-dimethyl
1074-1025	medium	C-O stretching vibration of glycosidic linkage
890	low	anomeric axial C-H deformation of β -sugar

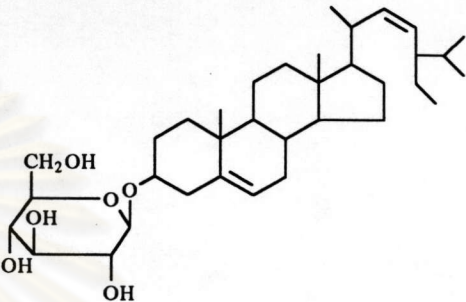
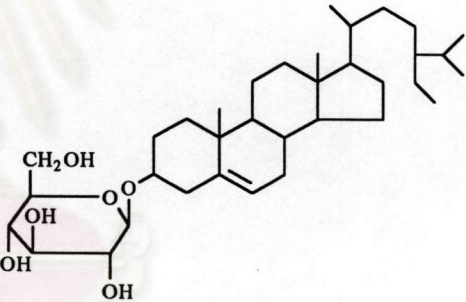
The $^1\text{H-NMR}$ spectrum (Fig. 85) showed that the signals of protons at chemical shift 0.64-2.49 ppm. was corresponded to the proton signals of methyl, methylene and methine protons of steroid. A broad multiplet at 3.61 ppm. was assigned for the proton on sugar (-CH-O-sugar). The proton signal at 5.32 ppm. should be an olefinic proton (-CH=C-) and the signal at 2.89-3.41 ppm. was assigned for the proton of sugar moiety.

The $^{13}\text{C-NMR}$ spectrum (Fig. 86) showed 36 signal of carbon which the carbon signals 36 peaks which showed the olefinic carbon signals at 121.19 and 140.40 ppm and 6 carbon signals of sugar moiety at 61.02, 70.02, 73.42, 76.72, 76.87 and 100.76 ppm.. Other carbon signals of this compound were similar to those of Compound 6 (a mixture of β -sitosterol and stigmasterol) which suggested that it might to be a glycoside of Compound 6.

Mass spectrum of Compound 7 (Fig. 87) did not give the molecular ion peak, but it exhibited the dominant fragmentation ion peaks at (m/e) 414 and 412. The series of fragmentation ion peaks was similar to those of glycosides of β -sitosterol and stigmasterol(75) which suggested that this compound was a mixture of sterol glycosides of β -sitosterol and stigmasterol.

Literature studies suggested that the carbohydrate which was linked to both of these sterols was D-glucose. Thus, Compound 7 consisted of a mixture of β -sitosterol-3-O- β -D-glucopyranoside and stigmasterol-3-O- β -D-glucopyranoside. The structure of steroids glycoside is shown in Table 29.

Table 29 Various Type of Steroid Glycosides Found in Compound 7

Name of substance	Structural formula
stigmasterol-3-O- β -D-glucopyranoside	
β -sitosterol-3-O- β -D-glucopyranoside	

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8. Structural Elucidation of Compound 8

Compound 8 was ivory amorphous solid, 0.009 g (0.004%wt/wt of methanol crude), m.p. 86-88°C. R_f value 0.75 (solvent system: 40% MeOH in CHCl_3). This compound gave negative results with Liebermann-Burchard's, 2,4-DNP and also decolourized Br_2 in CCl_4 reagents which indicated that it was not composed of steroidal skeleton, carbonyl functional group and unsaturated part in its structure.

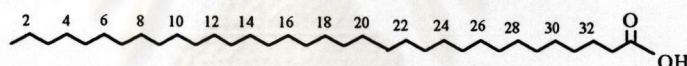
The IR spectrum of compound 8 was shown in Fig. 88 which exhibited the characteristic absorption band of O-H stretching vibration peak of carboxylic group (very broad) at 3500-2400 cm^{-1} . The absorption bands at 1701 and 1294 cm^{-1} ought to be C=O stretching vibration of carboxylic acid and C-O stretching vibration mode of carboxylic group, respectively. The C-H stretching vibration peaks of an aliphatic compound were observed at 2918 and 2849 cm^{-1} and the absorption peak at 720 cm^{-1} revealed the presence of saturated long chain of $(-\text{CH}_2)_n$ group. The IR absorption band is shown in Table 30.

Table 30 The IR Absorption Band Assignments of Compound 8

Wavenumber(cm^{-1})	Intensity	Tentative Assignments
3500-2400	high	O-H stretching vibration of acid
2918, 2849	high	C-H stretching vibration of CH_3 -, $-\text{CH}_2$ -
1701	low	C=O stretching vibration of acid
1473, 1463	medium	C-H bending vibration of $-\text{CH}_2$ -, CH_3 -
1294	medium	C-O stretching vibration of coupled with O-H deformation
720	medium	C-H rocking vibration of $-(\text{CH}_2)_n$, $n > 4$

Mass spectrum of Compound 8 (Fig. 89) showed the molecular ion peak at (m/e) 508 and other fragmentation peaks at m/e 480, 479, 466, 465, 451, 437, 423, 409, 185, 129, 111, 97, and 57, respectively which were corresponded to be lost $-\text{CH}_2-$ (m/e 14) step by step.

The information obtained from the IR spectrum suggested that Compound 8 should be long chain carboxylic acid and when compound the fragmentation ion pattern of mass spectra of Compound 8 with those of reference substances through library search (NIST database) of MS spectrometer. Compound 8 matched to that of the fragmentation of mass spectra of Tetratriacontanoic acid ($\text{C}_{34}\text{H}_{68}\text{O}_2$) very well (Fig. 90). Its structure is shown below:

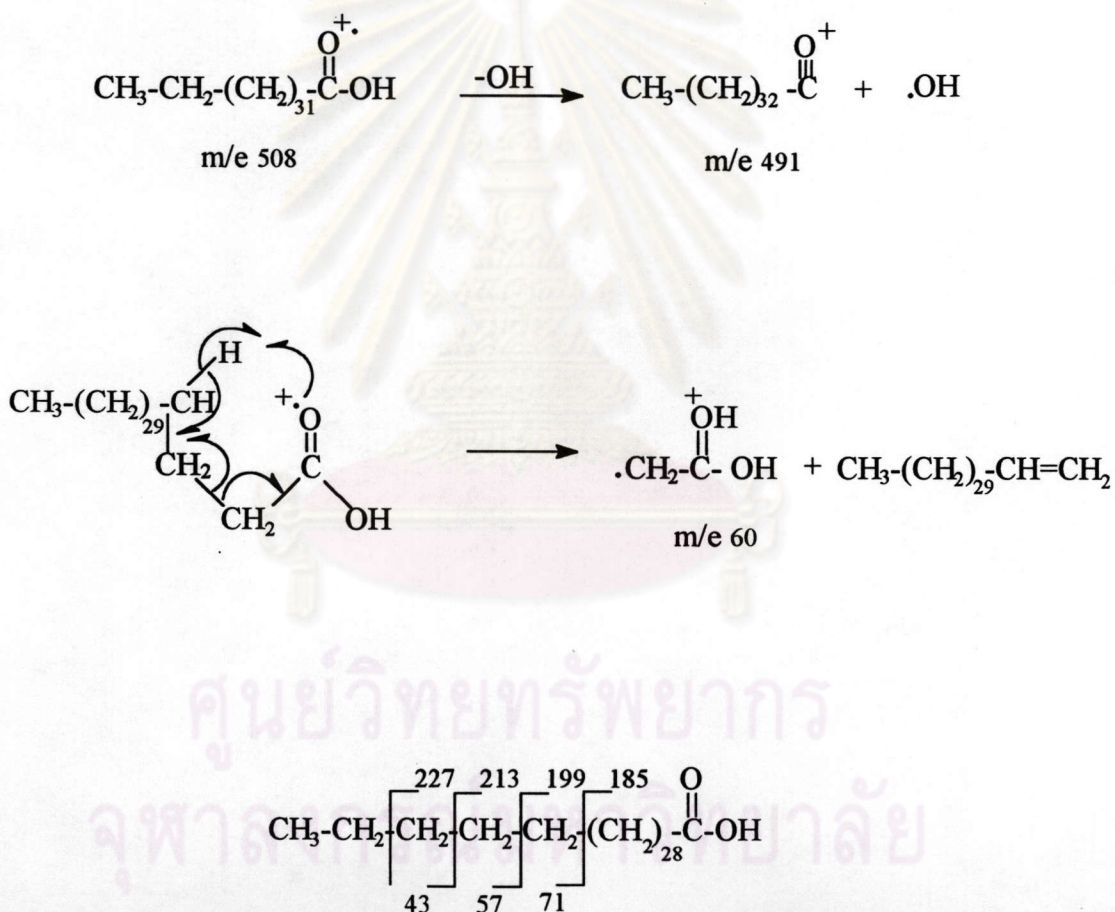


Tetratriacontanoic acid ($\text{C}_{34}\text{H}_{68}\text{O}_2$)

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The possible mass fragmentation pattern of Compound 8 is presented in Scheme 4.

Scheme 4 The Possible Mass Fragmentation Pattern of Compound 8



9. Structural Elucidation of Compound 9

Compound 9 was ivory amorphous solid, 0.003g (0.001%wt/wt of methanol crude), m.p. 138-140°C. R_f value 0.54 (solvent system: 40% MeOH in CHCl_3). This compound gave negative results with Liebermann-Burchard's reagent and also decolourized Br_2 in CCl_4 reagent which indicated that it was not composed of steroidal skeleton and unsaturated part in its structure.


The IR spectrum of compound 9 was shown in Fig. 91 which exhibited the characteristic absorption band of O-H stretching vibration peak of carboxylic group (very broad) at 3500-2500 cm^{-1} . The absorption bands at 1740 and 1288-1038 cm^{-1} ought to be C=O stretching vibration of carboxylic acid and C-O stretching vibration mode of carboxylic group, respectively. The C-H stretching vibration peaks of an aliphatic compound were observed at 2919 and 2849 cm^{-1} and the absorption peak at 721 cm^{-1} revealed the presence of saturated long chain of $(-\text{CH}_2-)_n$. The IR absorption band is shown in Table 31.

Table 31 The IR Absorption Band Assignments of Compound 9

Wavenumber(cm^{-1})	Intensity	Tentative Assignments
3500-2500	medium	O-H stretching vibration of acid
2919, 2849	high	C-H stretching vibration of CH_3- , $-\text{CH}_2-$
1740	medium	C=O stretching vibration of acid
1470	low	C-H bending vibration of CH_2- CH_3-
1228-1038	medium	C-O stretching vibration of coupled with O-H deformation
721	low	C-H rocking vibration of $-(\text{CH}_2)_n$, $n > 4$

Mass spectrum of Compound 9 (Fig. 92) showed the important fragmentation ion peaks at m/e 578, 550, 507, 479, 465, 451, 423, 367, 354, 341, 331, 313(base peak), 299, 267, 256, 240, 239, 213, 185, 171, 157, 134, 129, 116, 98, 83, 71 and 57, respectively.

From information obtained above it could be assigned that Compound 9 should have carboxyl as its functional group. Unfortunately, this compound was obtained in such a limited amount that its structure cannot be further elucidated.



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10. Structural Elucidation of Compound 10

Compound 10 was ivory amorphous solid, 0.004g (0.002%wt/wt of methanol crude), m.p.217-220°C. R_f value 0.62 (solvent system: 40%MeOH in CHCl_3). This compound gave negative result with Liebermann-Burchard's reagent but gave positive result to Br_2 in CCl_4 and 2,4-DNP reagents which indicated that it was not composed of steroidal skeleton but composed unsaturated part and carbonyl functional group in its structure.


The IR spectrum of compound 10 was shown in Fig.93 which exhibited the characteristic absorption band of O-H stretching vibration peak of carboxylic group (very broad) at $3600\text{-}3050\text{ cm}^{-1}$, absorption band of carbonyl group (C=O) at 1736 cm^{-1} and C-O stretching vibration mode of carboxylic group at $1148\text{ to }1023\text{ cm}^{-1}$. The C=C stretching vibration peaks was observed at 1636 cm^{-1} and the absorption peak at $966, 775$ and 728 cm^{-1} supported the existence of $(-\text{CH}_2-)_n$ group. The IR absorption band is shown in Table 32.

Table 32 The IR Absorption Band Assignments of Compound 10

Wavenumber(cm^{-1})	Intensity	Tentative Assignments
3600-3050	medium	O-H stretching vibration
2944	high	C-H stretching vibration of CH_3- , $-\text{CH}_2-$
1736	low	C=O stretching vibration of acid
1636	low	C=C stretching vibration
1460	low	C-H bending vibration of CH_2- CH_3-
1148-1038	medium	C-O stretching vibration of glycosidic linkage and O-H bending vibration
996, 755, 728		C-H rocking vibration of $(-\text{CH}_2)_n$, $n > 4$

Mass spectrum of Compound 10 (Fig. 94) showed the important fragmentation ion peaks at m/e 163, 149, 145, 133, 127, 115, 103, 91, 85, 74, 73(base peak) and 60, respectively.

From information obtained above it could be assigned that Compound 10 should have hydroxyl, carbonyl($C=O$), $C=C$ stretching and $C-O$ stretching vibration of glycosidic linkage as its functional group. Unfortunately, this compound was obtained in such a limited amount that its structure cannot be further elucidated.



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