

CHAPTER III

RESULTS AND DISCUSSION

Structural elucidation of the isolated compounds from the Branch of *Amoora gigantea* Pierre ex. Laness.

1. Structural elucidation of mixture I

Mixture I was obtained as an yellow oil 1.66 g. (5.51% wt./wt. of the hexane crude extract). The IR spectrum, Fig.3, indicated that this mixture contained one or more ester groups (C=O stretching vibration peak at 1740 cm^{-1} and C-O stretching vibration peak of ester group at 1171 cm^{-1}). Other absorption peaks were observed at 2927 , 2855 , 1740 , 1459 , 1378 and 725 cm^{-1} corresponded to long chain aliphatic.

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Table 8 The IR absorption band assignments of mixture I

Wavenumber (cm^{-1})	Intensity	Tentative Assignments
2927, 2855	strong	C-H stretching vibration of CH_2 , CH_3
1740	strong	C=O stretching vibration of ester
1459	strong	C-H asymmetric bending vibration of CH_2 , CH_3
1378	weak	C-H symmetric bending vibration of CH_3
1171	medium	C-O stretching vibration of ester
725	medium	C-H rocking vibration of $(-\text{CH}_2-)_n$, $n > 4$

The $^1\text{H-NMR}$ spectrum, Fig.4, exhibited proton signals of $-\text{CH}_3$ at chemical shift 0.84 ppm. The high intensity singlet peak at 1.29 ppm. was consistent with several interlinking methylene protons, $(-\text{CH}_2-)_n$, in this type of compound. The signal of α -proton in the acidic portion, $-\text{CH}_2-\text{C}=\text{O}$, was observed at 2.19 ppm. The peak at 3.58 ppm. indicated the presence of a methoxy group. The protons of olefinic group appeared as the signal at 5.30 ppm.

The $^{13}\text{C-NMR}$ spectra, Fig.5, showed the carbonyl carbon of ester group at 173.9 ppm. The olefinic carbons of olefinic group appeared as the signals at 121.5-131.1 ppm. The signal of interlinking methylene carbons were observed around 29.0-29.7 ppm. The α - and β - carbons next to the carbonyl group were located at 32.0 and 33.8 ppm. The signal at 13.7 ppm. belonged to a methyl group of the carboxylic part of an ester molecule.

The spectroscopic data of compound I indicated that it is composed of a mixture of saturated and unsaturated of methyl esters. To study the chemical constituents of the mixture, it was analyzed by GC-MS spectrometer and GC chromatogram, Fig.6, was obtained. GC chromatogram displayed peaks at retention times (R_t) 7.74, 13.85, 15.99, 16.18, 16.69 and 23.19 min., respectively. Each peak was analyzed by MS spectrometer and comparing the fragmentation ion pattern of MS with those substances through library search. The composition of mixture I was presented in Table 9

Table 9 The composition of esters contained in mixture I

Retention time (min.)	Name of substance	Molecular formular	Molecular weight
7.74	10-Methyl-methylundecanoate	$C_{13}H_{26}O_2$	214
13.85	14-Methyl-methylpentadecanoate	$C_{17}H_{34}O_2$	270
15.99	Methyl-9,12-octadecadienoate	$C_{19}H_{34}O_2$	294
16.18	Methyl-9-octadecenoate	$C_{19}H_{36}O_2$	296
16.69	15-Methyl-methylheptadecanoate	$C_{19}H_{38}O_2$	298

However, 10-Methyl-methylundecanoate and 14-Methyl-methylpentadecanoate were the main components of this mixture. The identity of this components oughts to be confirmed by comparison with authentic samples. In addition, These esters in Table 9 may be generated by methylation as while methanol solvent was removed by

distillation from the methanol crude extract. Then, the composition of long chain carboxylic acids contained in mixture I are summarized in Table 10.

Table 10. The composition of long chain carboxylic acids contained in mixture I

Retention time (min.)	Name of substance	Molecular formular	Molecular weight
7.74	Methylundecanoic acid	$C_{12}H_{24}O_2$	200
13.85	Methylpentadecanoic acid	$C_{16}H_{32}O_2$	256
15.99	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280
16.18	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282
16.69	Methylheptadecanoic acid	$C_{18}H_{36}O_2$	284

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2. Structural elucidation of compound II

The bright solid (30.27 mg., 0.10% wt./wt. of the hexane crude extract) was obtained from the crude hexane extract separation. The melting point was 76-77°C and the R_f value was 0.7 (silica gel : CHCl_3). This compound was tested with Liebermann-Burchard reaction and showed a purple colour.

The IR spectrum of compound II was shown in Fig.11 which exhibited the absorption band of a carbonyl group at 1705 cm^{-1} , bands of C=C stretching vibration at 1641 cm^{-1} and band of C-H bending vibration at 1381 cm^{-1} . The IR spectrum is assigned in Table 11.

Table 11 The IR absorption band assignments of compound II

Wavenumber (cm^{-1})	Intensity	Tentative Assignments
3070	strong	C-H stretching vibration of alkene
2947, 2875	strong	C-H stretching vibration of CH_2 , CH_3
1705	strong	C=O stretching vibration of ketone
1641	weak	non conjugated C=C stretching vibration
1452	medium	C-H bending vibration of CH_2 , CH_3
1381	medium	C-H bending vibration of gem-dimethyl
881, 836	weak	C-H out of plane bending vibration of $=\text{CH}_2$

The $^1\text{H-NMR}$ spectrum in Fig.12 exhibited the signals at chemical shift 0.88, 0.95, 1.02, 1.04, 1.08, 1.62 and 1.69 ppm. which indicated the methyl protons. The signals at chemical shift 0.98-2.54 ppm. corresponded to the methylene and methine protons. The signal at 4.75 ppm. corresponded to terminal olefin protons and the signal at 5.13 ppm. corresponded to protons of $-\text{CH}=\text{C}-$ group.

The $^{13}\text{C-NMR}$ spectrum in Fig.13 exhibited the carbon signals of 30 peaks which were the olefinic carbon signals at 107.57, 124.41, 131.44 and 152.58 ppm.. The signal at 218.12 ppm. indicated carbonyl group of ketone. Other signals at chemical shift 15.34-55.37 should be the carbon signals of methyl, methylene, methine and quaternary carbons.

The DEPT-135 and DEPT-90 $^{13}\text{C-NMR}$ spectrum (Fig.14) exhibited CH_2 signals 11 peaks at chemical shift 19.65, 21.89, 24.96, 27.05, 28.86, 31.33, 34.10, 34.74, 34.85, 39.95 and 107.57 ppm., CH signals 5 peaks at chemical shift 45.40, 47.73, 50.28, 55.37 and 124.41 ppm. and CH_3 signals 7 peaks at chemical shift 15.34, 15.82, 16.03, 17.69, 21.00, 25.67 and 26.74 ppm.

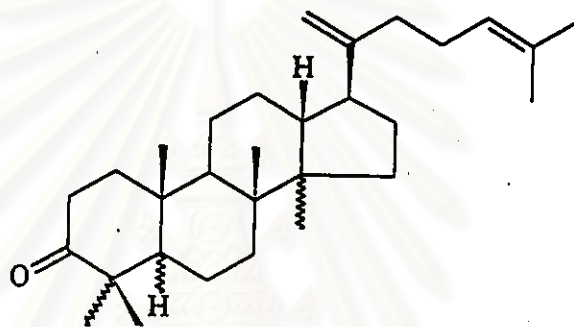
The mass spectrum of compound II (Fig.15) showed the molecular ion peak at m/e 424 and other fragmentation peaks at m/e 205, 109, 189 and 95 respectively. The molecular formula should be $\text{C}_{30}\text{H}_{48}\text{O}$.

Previous data indicated that this compound was a triterpenoid. The comparison of melting point of compound II with many triterpenoidal compounds ($\text{C}_{30}\text{H}_{48}\text{O}$) indicated that it might be 5α -dammara-20(21), 24 -diene -3-one. This compound was previously isolated from leaves of *Amoora gigantea* Pierre ex. Laness. by Nisakorn[5]. To confirm the structure, the comparison of $^{13}\text{C-NMR}$ was used.

Table 12 The comparison of ^{13}C -NMR spectrum of 5α -dammar-20(21), 24 -diene -3-one from Ref.5 and compound II

Position of carbon	Chemical shift (ppm.)	
	5α -dammar-20(21), 24 -diene -3-one	Compound II
C ₁	39.96	39.95
C ₂	34.10	34.10
C ₃	218.01	218.12
C ₄	47.40	47.39
C ₅	55.38	55.37
C ₆	19.68	19.65
C ₇	34.14	34.74
C ₈	40.37	40.36
C ₉	50.31	50.28
C ₁₀	36.92	36.91
C ₁₁	21.91	21.89
C ₁₂	28.87	28.86
C ₁₃	45.41	45.40
C ₁₄	49.40	49.39
C ₁₅	27.08	27.05
C ₁₆	34.76	34.85
C ₁₇	47.74	47.73
C ₁₈	16.06	16.03
C ₁₉	21.03	21.00
C ₂₀	152.52	152.58
C ₂₁	107.61	107.57
C ₂₂	31.36	31.33
C ₂₃	24.99	24.96
C ₂₄	124.44	124.41
C ₂₅	131.40	131.44
C ₂₆	17.72	17.69
C ₂₇	25.71	25.67
C ₂₈	26.77	26.74
C ₂₉	15.36	15.34
C ₃₀	15.83	15.82

All of the spectral data, physical properties and chemical properties indicated that compound II was 5 α -dammar-20(21), 24 -diene -3-one. The structure is shown below :



5 α -dammar-20(21), 24-diene-3-one

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3. Structural elucidation of mixture III

Mixture III was obtained as a white needle crystal (0.98 g., 3.25% wt./wt. of the hexane crude extract). The melting point was 131-132°C and the R_f value was 0.6 (silica gel : 5% MeOH in CHCl_3). This compound gave green colour with Libermann-Burchard's reagent which indicated that it had a steroidal skeleton.

The IR spectrum of compound III, Fig.16, was exhibited the absorption band of hydroxy group (OH) at 3608-3219 cm^{-1} and the absorption band of unsaturation at 1640 cm^{-1} . The C-O stretching vibration was observed at 1058 cm^{-1} . The absorption peaks at 970 and 960 corresponded to disubstituted alkene and the absorption peaks at 839 and 802 corresponded to trisubstituted alkene.

Table 13 The IR absorption band assignments of mixture III

Wavenumber (cm^{-1})	Intensity	Tentative Assignments
3608-3219	strong	O-H stretching vibration
2958-2870	strong	C-H stretching vibration of CH_2 , CH_3
1640	medium	C=C stretching vibration
1465, 1381	strong	C-H bending vibration of CH_2 , CH_3
1058	strong	C-O stretching vibration
970, 960	medium	C-H out of plane bending vibration of $\text{R}_1\text{CH}=\text{CHR}_2$
839, 802	medium	C-H out of plane bending vibration of $\text{R}_1\text{R}_2\text{C}=\text{CHR}_3$

The $^1\text{H-NMR}$ spectrum, Fig.17, exhibited the signals at chemical shift 0.66-2.26 ppm. which indicated the protons of methyl, methylene and methine protons. The signal at chemical shift 3.55 ppm. corresponded to the proton linked to carbon having hydroxy group. The double of doublet signal at 5.10 ppm. indicated disubstituted vinylic protons ($-\text{CH}=\text{CH}-$) and the proton signal at 5.34 ppm. corresponded to the proton of $-\text{CH}=\text{C}-$ group.

The $^{13}\text{C-NMR}$ spectrum in Fig.18 exhibited the olefin carbon signals at 121.71, 129.25, 138.32 and 140.74 ppm. The signal at chemical shift 71.79 ppm. should be the carbon signal adjacent to oxygen atom. Other signals at 11.85-56.85 ppm. should be the carbon signal of methyl, methylene, methine and quaternary carbons.

Mass spectrum of mixture III in Fig.21 showed the molecular ion peaks at 414, 412 and 400 respectively. Other fragmentation peaks exhibited at 396, 381, 329, 255 and 213 respectively.

Because the results above indicated that this mixture might be a mixture of steroidal compound. Then, the GLC technique was used to confirm the structure. GC chromatogram (Fig.19, 20) showed retention times at 19.93, 21.01 and 23.82 min., respectively. The retention time of each peak was compared with those of a standard mixture of steroid. The results is shown in Table 14.

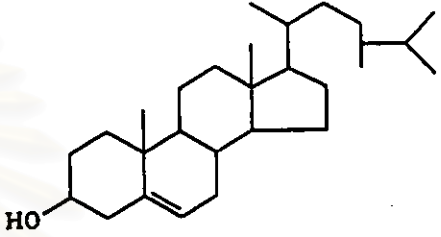
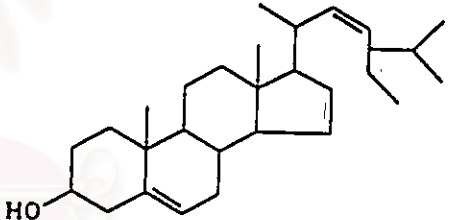
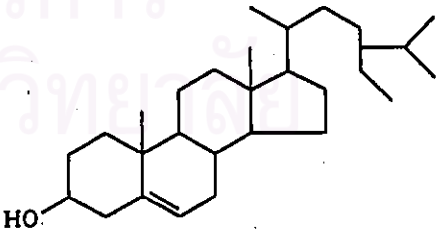
Table 14 Retention time of the mixture of standard steroid and mixture III

Name of substance	Retention time of standard steroid (min.)	Retention time of mixture III (min.)
campesterol	19.93	19.93
stigmasterol	21.01	21.01
β -sitosterol	23.82	23.82

All of spectral data, physical and chemical properties and GC chromatogram indicated that mixture III was a mixture of three steroids. The molecular formula, molecular weight and structural formula is shown in Table 15.

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Table 15 The composition of steroids contained in mixture III

Name of substance	Molecular formular	Molecular weight	Structural formular
campesterol	$C_{28}H_{48}O$	400	
stigmasterol	$C_{29}H_{48}O$	412	
β -sitosterol	$C_{29}H_{50}O$	414	

4. Structural elucidation of mixture IV

The white solid, mixture IV, was obtained (0.27 g., 0.90% wt./wt. of the hexane crude extract) from the separation of Fraction I. The melting point was 75-77°C and the R_f value was 0.7 (silica gel : 5% MeOH in CHCl_3).

The IR spectrum of mixture IV in Fig.22 showed the absorption band of hydroxy group at 3629-3010 cm^{-1} , carbonyl group at 1706 cm^{-1} , C-O stretching vibration at 1069 cm^{-1} and saturated long chain aliphatic at 2918, 2850, 1464, 1377 and 720 cm^{-1} .

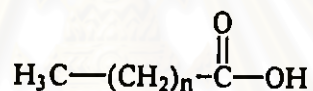
Table 16 The IR absorption band assignments of mixture IV

Wavenumber (cm^{-1})	Intensity	Tentative Assignments
3629-3010	strong	O-H stretching vibration of acid
2918, 2850	strong	C-H stretching vibration of CH_2 , CH_3
1706	medium	C=O stretching vibration of acid
1464	strong	C-H bending vibration of CH_2 , CH_3
1377	strong	C-H bending vibration of CH_3
724	medium	C-H rocking vibration of $(-\text{CH}_2-)_n$, $n > 4$

The $^1\text{H-NMR}$ spectrum in Fig.23 showed the triplet signal at chemical shift 2.33 ppm. which was the signal of proton on the carbon attaching to carbonyl group. The high intensity singlet signal at 1.23 ppm. indicated several interlinking methylene protons. The triplet signal at 0.86 ppm. corresponded to the methyl group signal.

The $^{13}\text{C-NMR}$ spectrum in Fig.24 showed the carboxyl carbon at 179.53 ppm. The signals of interlinking methylene carbons was observed at 14.14-29.70 ppm., while the signal of methyl group was located at 14.14 ppm. The α - and β - carbons connected to carboxyl group were observed at 33.95 and 31.93 ppm.

The spectral data above indicated that the structure could be assigned below :



This mixture was converted to methyl ester by diazomethane and analyzed by GC-MS technique. The GC chromatogram, Fig.26, showed 9 retention times at 16.65, 17.77, 20.34, 21.85, 23.22, 24.44, 25.56, 26.56 and 27.51 min., respectively. Each peak was analyzed by MS spectrometer and compared the fragmentation ion pattern of mass spectra with those of the substances in library search of GC-MS spectrometer.

The physical properties and all of the spectral data indicated that mixture IV was a mixture of saturated long chain carboxylic acids.

Table 17 The composition of saturated long chain carboxylic acids contained in mixture IV

Retention time	Name of substance	Molecular formular	Molecular weight
16.65	Octadecanoic acid	$C_{18}H_{36}O_2$	284
17.77	Nonadecanoic acid	$C_{19}H_{38}O_2$	298
20.34	Eicosanoic acid	$C_{20}H_{40}O_2$	312
21.85	Heneicosanoic acid	$C_{21}H_{42}O_2$	326
23.22	Docosanoic acid	$C_{22}H_{44}O_2$	340
24.44	Tricosanoic acid	$C_{23}H_{46}O_2$	354
25.56	Tetracosanoic acid	$C_{24}H_{48}O_2$	368
26.56	Pentacosanoic acid	$C_{25}H_{50}O_2$	382
27.51	Hexacosanoic acid	$C_{26}H_{52}O_2$	396

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5. Structural elucidation of mixture V

Mixture V was obtained as an amorphous white solid (1.15 g.) from CHCl_3 and ethylacetate crude extract separation. The melting point was $270\text{-}275^\circ\text{C}$ and the R_f value was 0.6 (silica gel : 20% MeOH in CHCl_3). This mixture was tested with Liebermann-Burchard reagent and showed a green colour which indicated that it had steroidal structure.

The IR spectrum was shown in Fig.36 which exhibited the absorption band of hydroxy group (-OH) at $3655\text{-}3149\text{ cm}^{-1}$, C-O stretching vibration of glycosidic linkage at $1163\text{-}1027\text{ cm}^{-1}$ and anomeric axial C-H deformation of β -sugar at 892 cm^{-1} .

Table 18 The IR absorption band assignments of mixture V

Wavenumber (cm^{-1})	Intensity	Tentative Assignments
3655-3149	strong	O-H stretching vibration
3026	weak	C-H stretching vibration of alkene
2960, 2934	strong	C-H stretching vibration of CH_2 , CH_3
1640	strong	C=C stretching vibration
1465	strong	C-H bending vibration of CH_2 , CH_3
1379	strong	C-H bending vibration of CH_3
1163-1027	strong	C-O stretching vibration of glycosidic linkage
892	weak	anomeric axial C-H deformation of β -sugar
840, 800	weak	C-H out of plane bending vibration of $\text{R}_1\text{R}_2\text{C}=\text{CHR}_3$

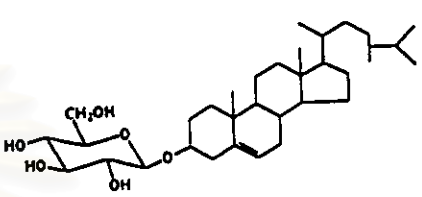
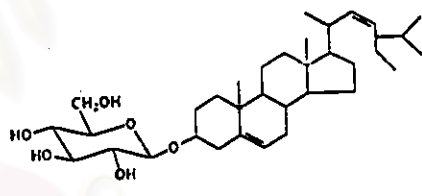
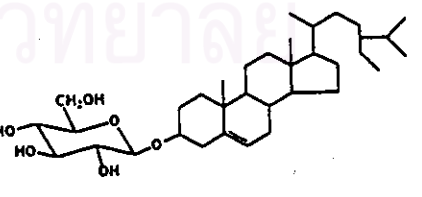
The $^1\text{H-NMR}$ spectrum in Fig.37 showed the signals at chemical shift 0.55-2.40 ppm. was present for the protons of methyl, methylene and methine. The proton signals at 5.10 and 5.29 ppm. corresponded to the olefinic protons. A doublet at 4.20 ppm. was assigned for anomeric protons of $\beta\text{-D-glucose}$ and a broad multiplet at 3.62 ppm. was assigned for the proton on sugar.

The $^{13}\text{C-NMR}$ spectrum in Fig.38 showed the olefinic carbon signals at 121.71, 128.80, 138.02 and 140.41 ppm. and the signals of sugar moiety at 61.04, 70.02, 73.42, 76.72, 76.90 and 100.79 ppm. Other signals were similar to a mixture of $\beta\text{-sitosterol}$, stigmasterol and campesterol.

The EI-MS, Fig.39 did not give the molecular ion peak. The spectrum showed the dominant fragmentation ion peaks at (m/e) 414, 412 and 400. The other fragmentation ion peak was similar to the fragmentation pattern of steroid (mixture III).

All of the spectral data, physical properties, chemical properties and literature comparison suggested that mixture V should be the mixture of campesterol-3-O- $\beta\text{-D-glucopyranoside}$, stigmasterol-3-O- $\beta\text{-D-glucopyranoside}$ and $\beta\text{-sitosterol-3-O-}\beta\text{-D-glucopyranoside}$.

Table 19 The composition of steroidal glycosides contained in mixture V

Name of substance	Structural formular
campesterol-3 o- β -D-glucopyranoside	
stigmasterol-3-O- β -D-glucopyranoside	
β -sitosterol-3-O- β -D-glucopyranoside	

6. Structural elucidation of mixture VI

Compound VI (0.11 g., 0.14% wt./wt. of the MeOH crude extract) was obtained as colorless crystals. The melting point was 192°C and the R_f value was 0.4 (silica gel : 20% MeOH in CHCl_3)

The IR spectrum was shown in Fig.40 which exhibited the characteristic absorption band of O-H group at 3530-3014 cm^{-1} and absorption band of C-O stretching vibration at 1161-1006 cm^{-1} . The IR spectrum of compound VI was similar to IR spectrum of sucrose.

Table 20 The IR absorption band assignments of compound VI

Wavenumber (cm^{-1})	Intensity	Tentative Assignments
3530-3014	strong	O-H stretching vibration
2994, 2982	weak	C-H stretching vibration of CH, CH_2
1476, 1386	strong	C-H bending vibration of CH, CH_2
1161-1006	strong	C-O stretching vibration and O-H bending vibration

The $^1\text{H-NMR}$ spectrum in Fig.41 showed the signals at chemical shifts 3.53-3.56 ppm. were presented for the protons of methylene and methine which linked to hydroxy group.

The ^{13}C -NMR spectrum in Fig.42 showed the signals of sugar moiety at chemical shifts 61.98, 62.09, 69.77, 71.58, 72.78, 74.22, 76.94, 82.51, 91.72 and 1.3.98 ppm. All of carbon signals were similar to those of sucrose.

Mass spectrum of compound VI, Fig.43, did not give the molecular ion peak, but it exhibited the fragmentation ion peaks at 149, 131, 103, 86, and 73. Literature studies suggested that this compound might be sucrose.

From information obtained above it could be assigned that compound VI was sucrose.



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7. Structural elucidation of compound VII

Mixture VII was obtained from separation of Fraction IV as colorless needle crystal (0.65 g., 0.81% wt./wt. of the MeOH crude extract). The melting point was 223-224°C and the R_f value was 0.2 (silica gel : 20% MeOH in CHCl_3).

The IR spectrum was shown in Fig.44 which exhibited the characteristic absorption band of O-H group at 3500-3010 cm^{-1} and absorption band of C-O stretching vibration at 1171-1000 cm^{-1} . The IR spectrum of compound VII was similar to IR spectrum of inositol.

Table 21 The IR absorption band assignments of compound VII

Wavenumber (cm^{-1})	Intensity	Tentative Assignments
3500-3010	strong	O-H stretching vibration
2994, 2982	weak	C-H stretching vibration of CH, CH_2
1476, 1386	strong	C-H bending vibration of CH, CH_2
1171-1000	strong	C-O stretching vibration and O-H bending vibration

The $^1\text{H-NMR}$ spectrum in Fig.45 showed the signals at chemical shifts 2.49-4.65 ppm. were presented for the protons of methine which linked to hydroxy group.

The ^{13}C -NMR spectrum in Fig.46 showed four signals of carbons which linked to hydroxy group at 71.22, 72.41, 72.83 and 75.10 ppm. The peaks at 71.22 and 72.83 ppm. had higher intensity than the others. Then, this compound should have 6 carbons in the molecule.

Mass spectrum of compound VII, Fig.47, did not give the molecular ion peak, but it exhibited the fragmentation ion peaks at 144, 126, 109, 102, 89, 71 and 60. Literature studies suggested that this compound might be inositol.

From previous data, compound VII was inositol. It might be myo-inositol or epi-inositol because both of them are symmetric molecule. To confirm the structural isomer, the comparison of ^{13}C -NMR was used.

Table 22 The comparison of ^{13}C -NMR of compound VII, myo-inositol and epi-inositol .

Chemical shift (ppm.)		
compound VII	myo-inositol	epi-inositol
71.2(high intensity)	71.1(high intensity)	66.8
72.4	72.2	70.1
72.8(high intensity)	72.4(high intensity)	71.7(high intensity)
75.1	74.3	74.5(high intensity)

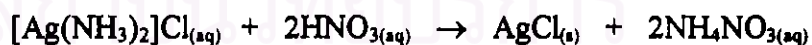
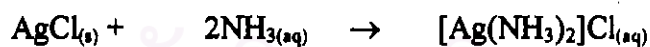
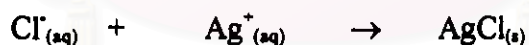
The comparison of ^{13}C -NMR and all of spectral evidences indicated that compound VII was myo-inositol.

8. Structural elucidation of compound VIII

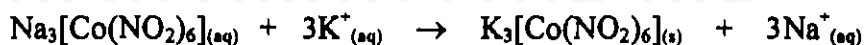
The compound VIII was obtained from separation of Fraction IV as needle crystal (2.75 g., 3.37% wt./wt. of the MeOH crude extract) with melting point above 280°C. It did not absorb IR at wavenumber 4000-450 cm⁻¹. Then, this compound might not be an organic compound. It was soluble in distilled water. When the content in the solution was reacted with AgNO₃ solution, it gave white precipitate. This precipitate was soluble in ammonia solution. This result indicated that the anion should be Cl⁻. The flame test gave purple-red colour. Then, the cation should be K⁺. To confirm this result, the solution of this compound was reacted with sodium hexanitrocobaltate(III) and obtained the yellow precipitate of K₃Co(NO₂)₆.

Equations :

Anion



Cation



From the results above, compound VIII was KCl.