CHAPTER II

EXPERIMENTS AND RESULTS

Plant Materials

The leaf of *Amoora gigantea* Pierre.* (Taa Suea Bai Lek) were collected at Bangbutr district, Rayong province, Thailand, in February 1995. They were air dried to avoid any chemical changes, ground and extracted with various solvents to separate and identify chemical constituents of leaf of *Amoora gigantea* Pierre.

Instruments and Equipments

1. Fourier Transform-Infrared Spectrophotometer (FT-IR)

The FT-IR spectra were recorded on a Perkin-Elmer Model IR-400 FT 1760X. Fourier Transform-Infrared Spectrophotometer. Solid sample were generally examined by incorporating the sample with potassium bromide (KBr) to from a pellet. Liquid samples were dropped on sodium chloride cell.

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* Identified as *Amoora gigantea* Pierre ex. Laness. by Miss Ampai Yongboongerd. Herbarium of Botany Section, Botany and Weed Science Division, Department of Agriculture, Ministry of Agriculture and Cooperative. Voucher specimen number is 57845.

2. Mass Spectrometer (MS)

The mass spectrometric analysis was performed on Fison Mass Spectrometer Model Trio 2000.

3. ^{1}H and ^{13}C-Nuclear Magnetic Resonance Spectrometer.

The ^{1}H-NMR and ^{13}C-NMR spectra were obtained by using a Bruker Model ACF 200 Spectrometer which operated at 200.13 MHz. for ^{1}H and 50.32 MHz. for ^{13}C nuclei. The 500 MHz. spectra were performed with a JNM 500 MHz. from Jeol. The chemical shift in δ (ppm) was assigned with reference to the signal from the residual proton in deuterated solvent. Accordingly, the signal due to deuterated chloroform was assigned to be 7.24 ppm. with reference to TMS.

4. Elemental Analyzer.

The elemental analysis was carried out using a Fisons Instruments model NA 2000.

5. Rotary Evaporator

Eyela rotary vacuum evaporator was used to evaporate the large amounts of volatile solvents.

6. Melting Points (m.p.)

The melting point was determined on a Fisher-John melting point apparatus and was uncorrected.

Chemical reagents

1. Solvents

All solvents used in this research such as hexane, chloroform, methanol were commercial grade. They were purified prior to use by distillation. Reagent grade used in recrystallization were used as received.

2. Other chemicals

2.1) Merck's silica gel 60 Art. 7734. (70-230 mesh ASTM) was used as adsorbents for column chromatography.

2.2) Merck's TLC aluminium sheets, silica gel 60F 254 precoated 25 sheets, 20x20 cm^2, layer 0.2 mm, was used to identify the identical fractions.

Physical Separation Techniques

1. Column Chromatography (CC)

A column chromatography was performed on a glass column using silica gel 60 Art. 7734 as an adsorbent. The size of the column used depended on the amount (weight) of the sample, normally, a sample consisted of 1-4% (wt./wt.) of the adsorbent and the amount of adsorbent was sufficient to make a maximum height to diameter ratio of 10:1.

Preparation :

The stopcock of the column was closed and then the glass rod was used to push the cotton wool to the bottom of column. The solvents was allowed to remain about half of the column. A slurry of the silica gel (25% wt./ v) in a suitable solvent was mixed and added to the chromatographic column. When the slurry of the silica gel was poured in the glass rod was pulled out

and the stopcock was let opened to release the solvent pouring down slowly until remained about 5 cm above the surface on the silica gel. The crude extracts were brought to incorporate with silica gel until friableness and then carefully added to the column and drown onto the adsorbent. A small amount of the solvents was then added and passes through the column. The dried silica gel was sprinkled on top of the column and then paper disc was placed on top of silica gel column prior to the addition of the eluent. The column was developed by the suitable solvents and the eluent was identified by thin layer chromatography.

2. Thin-Layer Chromatography (TLC)

In this research, Merck's TLC aluminium sheets, silica gel 60F 254 precoated sheets, 20x20 cm^2, 0.2 mm. thick was used. Two lined were drawn on each plate, the first was 1.0 cm from the lower edged, the another line was 8.0 cm above and parallel to the first line. The solution of substances to be identified was applied as small sports on the lower line of the plate. After the solvent had evaporated, the plate was placed in a closed glass utensil which had the eluting solvent to a depth lower than 1.0 cm. The eluting solvent moved up the plate immediately. When the solvent reached the upper line, the plate was removed, allowed the solvent to evaporate and the plate was detected with UV and I_2 .

Color Tests (80, 81, 83)

1. Liebermann-Burchard Test

This is a test for a steroidal or triterpenoidal nucleus. To a solution of solid or dry extract 2-3 mg in 0.5 cm^3 of chloroform, was added a few drops of acetic anhydride, followed by one drop of concentrated sulfuric acid. The color reaction obtained as blue green or blue indicated the presence of steroid

compound and that obtained as purple or pink indicated the presence of triterpenoid compounds.

2. Carbonyl Group Test

Add a solution of one or two drops of the compound to be tested in 2 cm^3 of 95% ethanol to 3 cm^3 of 2,4-dinitrophenylhydrazine reagent. Shake vigorously, and no precipitate form immediately, allow the solution to stand for 15 min.. A yellow precipitation indicates a positive test.

3. Unsaturated Compound Test

In this test 0.1 g. (0.2 cm^3 of a liquid) of the compound to be tested is added to 2 cm^3 of carbon tetrachloride, and a 5% solution of bromine in carbon tetrachloride is added drop by drop (with shaking) until the bromine color persist. A positive test for unsaturation is one in which the bromine color discharged *with the evolution* of hydrogen bromide.

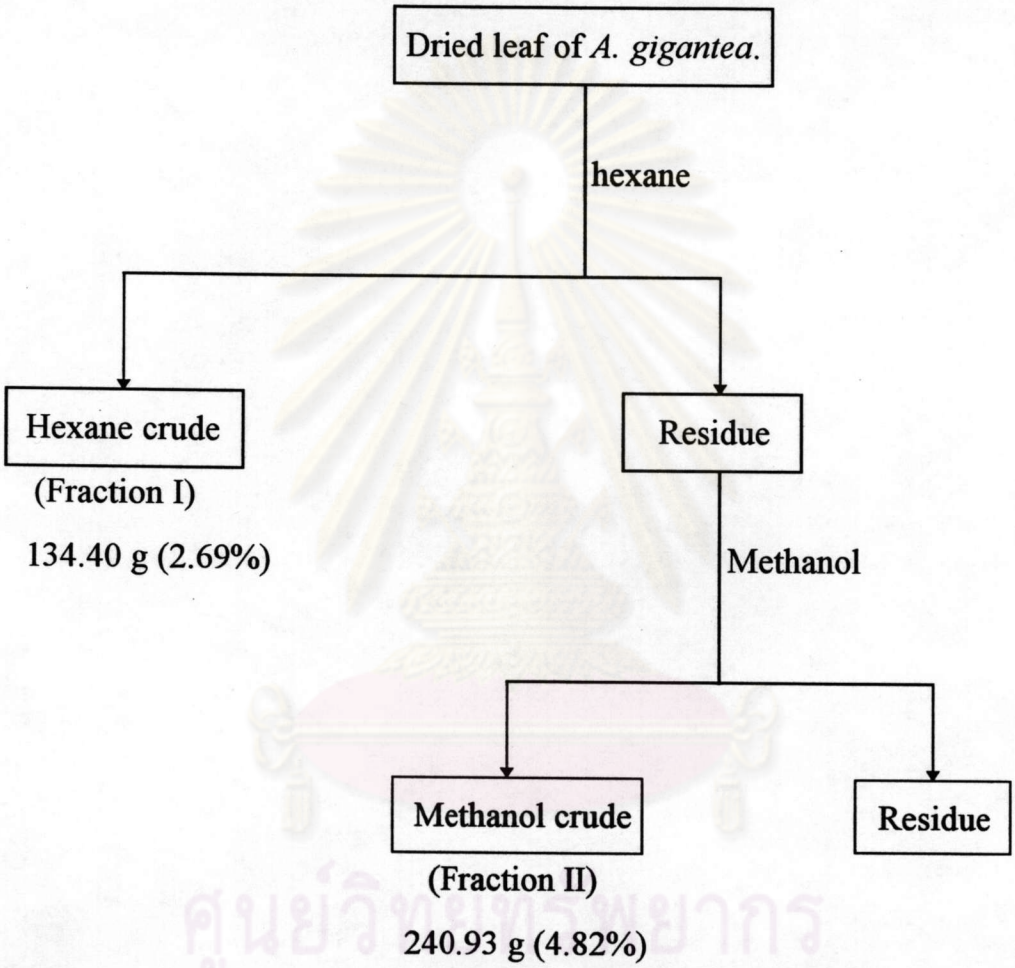
Extraction

The ground dried leaf of Taa Suea Bai Lek 5 kg were extracted with hexane three times for three day periods at room temperature. The solution was filtered and distilled by simple distillation. This distilled haxane was brought back to extract the leaf again until the solution was colorless. The crude extract of hexane, 134.40 g was obtained as a greenish-brown sticky liquid (Fraction I, 2.69%wt./wt. of the leaf).

The residue left after hexane extraction was reextracted by methanol until the solution was colorless. The filtrated solution was evaporated with rotary vacuum evaporator. The crude extract of methanol, 240.93 g was obtained as greenish-black crude (Fraction II, 4.82%wt./wt. of the leaf).

The procedure of the various extraction was shown in Scheme I.

Scheme I Extraction of the Leaf of *Amoora gigantea* Pierre.



Isolation of Chemical Constituents of the leaf of *Amoora gigantea* Pierre.

1. Separation of crude hexane extract by column chromatography

The crude hexane extract(Fraction I 79.95g) was chromatographed on a silica gel (800 g) column packed in hexane the eluent was changed stepwise from hexane, hexane-chloroform, chloroform, chloroform-methanol and methanol, respectively. The eluted solution was collected approximately 1000 cm^3 for each fraction. The solution in each fraction was concentrated by rotary evaporator and distillation to about 50 cm^3. They were further removed in a small flask. The elution was monitored by TLC. The identical fractions were combined together. The results of the column chromatographic separation of crude hexane extract (Fraction I) are presented in Table 4.

Table 4 The Results of the Column Chromatographic Separation of crude hexane extract (Fraction I)

Symbols	Eluents	Fraction No.	Remarks	Weight (g.)
H_1	Hexane	1-2	yellow oil+white ppt.	6.25
H_2	Hexane	3-27	yellow oil	5.09
H_3	Hexane-10%$CHCl_3$/Hexane	28-34	light yellow oil	1.24
H_4	10%$CHCl_3$/Hexane	35-40	solid in yellow oil	5.14
H_5	20%$CHCl_3$/Hexane	41-44	solid in orange oil	1.17
H_6	20%$CHCl_3$/Hexane	45-59	solid in orange oil	9.60
H_7	20%$CHCl_3$/Hexane	60-63	solid in yellow oil	1.39
H_8	20%$CHCl_3$/Hexane	64-68	solid in yellow oil	3.68
H_9	20%$CHCl_3$/Hexane	69-84	solid in yellow oil	0.92
H_{10}	20%$CHCl_3$/Hexane	85-90	solid in orange oil	1.46
H_{11}	20%$CHCl_3$/Hexane	91-98	solid in orange oil	4.36
H_{12}	30%$CHCl_3$/Hexane	99	yellow oil	0.22

Table 4 (continued)

Symbols	Eluents	Fraction No.	Remarks	Weight (g.)
H_{13}	30%$CHCl_3$/Hexane	100-103	solid in yellow-orange oil	1.71
H_{14}	30%$CHCl_3$/Hexane	104-113	solid in yellow-orange oil	4.83
H_{15}	40%$CHCl_3$/Hexane	114-137	yellow-orange oil	0.53
H_{16}	40%$CHCl_3$/Hexane	138-152	yellow-greenish oil	1.80
H_{17}	40-50%$CHCl_3$/Hexane	153-168	yellow-greenish oil	2.11
H_{18}	60-70%$CHCl_3$/Hexane	169-194	yellow-green oil	0.26
H_{19}	80%$CHCl_3$/Hexane	195-202	greenish-yellowoil	2.05
H_{20}	100%$CHCl_3$	203-209	greenish-yellow oil	1.97
H_{21}	100%$CHCl_3$	210-215	solid in green oil	1.24
H_{22}	2%MeOH/$CHCl_3$	219-225	greenish-yellow oil	1.67
H_{23}	2%MeOH/$CHCl_3$	226-228	light yellow-brown oil	1.10
H_{24}	5%MeOH/$CHCl_3$	229-233	yellow-green oil	0.74
H_{25}	10%MeOH/$CHCl_3$	234-239	light yellow oil	1.23
H_{26}	30%MeOH/$CHCl_3$	240-244	greenish-yellow oil	1.91
H_{27}	30-70%MeOH/$CHCl_3$	245-247	greenish-yellow oil	1.08
H_{28}	100%MeOH	248-250	brown tar	2.01

2. Separation of methanol crude extract by column chromatography

The crude methanol extract (Fraction II, 81.60 g) was separated in the same manner as crude hexane extract excepted that 1000 g of silica gel was used. The results of the column chromatographic separation of crude methanol extract (Fraction II) are presented in Table 5.

Table 5 The results of the column chromatographic separation of crude methanol extract (Fraction II)

Symbols	Eluents	Fraction No.	Remarks	Weight (g.)
M_1	Hexane	1-4	white ppt.	0.21
M_2	Hexane	5-7	light yellow oil	0.42
M_3	Hexane-10%$CHCl_3$/Hexane	8-17	light yellow oil	1.35
M_4	10%$CHCl_3$/Hexane	18-24	yellow oil	0.86
M_5	20%$CHCl_3$/Hexane	25-29	light yellow oil	0.73
M_6	30-40%$CHCl_3$/Hexane	30-35	yellow orange oil	1.83
M_7	50%$CHCl_3$/Hexane	36-39	light yellow-orange oil	2.17
M_8	50%$CHCl_3$/Hexane	40-44	solid in yellow-orange oil	5.20
M_9	50%$CHCl_3$/Hexane	45-48	solid in yellow oil	2.86
M_{10}	50%$CHCl_3$/Hexane	49-55	greenish-black oil	3.01
M_{11}	60%$CHCl_3$/Hexane	56-59	light yellow oil	1.89
M_{12}	60-80%$CHCl_3$/Hexane	60-87	yellow oil	1.05
M_{13}	80%$CHCl_3$/Hexane	88-94	greenish-brown oil	3.22
M_{14}	100%$CHCl_3$	95-115	solid in greenish-brown oil	2.94
M_{15}	100%$CHCl_3$	116-120	light greenish-brown oil	1.20
M_{16}	2-5%MeOH	121-131	light greenish-brown oil	1.76
M_{17}	5%MeOH	132-138	solid in greenish-brown oil	6.85
M_{18}	5%MeOH	139-141	solid in yellow-brown oil	2.61
M_{19}	10%MeOH	142-148	solid in yellow-brown oil	1.84
M_{20}	15%MeOH	149-168	yellow-brown oil	1.03

Table 5 (continued)

Symbols	Eluents	Fraction No.	Remarks	Weight (g.)
M_{21}	15-20%MeOH/$CHCl_3$	169-173	solid in brown oil	2.71
M_{22}	20%MeOH/$CHCl_3$	174-175	solid in brown oil	2.45
M_{23}	20%MeOH/$CHCl_3$	176-178	solid in brown oil	2.82
M_{24}	20-30%MeOH/$CHCl_3$	179-195	brown tar	6.08
M_{25}	40-100%MeOH/$CHCl_3$	196-215	brown tar	7.86

Purification and Properties of the Eluted Compounds by Column Chromatography

1. Purification and Properties of Compound 1

Compound 1 was composed of yellow oil and white solid which was obtained from the column chromatography of the hexane crude extract (Table 4) of the Fraction No.1-2 (H_1). It was eluted from the silica gel column with n-hexane. After washing yellow oil with methanol and recrystallization with hot methanol for several times. Compound 1 was obtained as white amorphous product, 3.54 g (2.63%wt/wt of hexane crude), m.p. 47-50°C, R_f value was 0.87 in 10%$CHCl_3$/hexane. This compound was soluble in hexane, chloroform, dichloromethane and hot methanol but slightly soluble in acetone, ethylacetate. It gave negative results to 2,4-DNP, Br_2 in CCl_4 and Liebermann-Burchard's reagent.

FT-IR spectrum (KBr), ν max($cm.^{-1}$) showed the absorption peak at 2956 and 2918(m, C-H stretching), 2489(s, C-H stretching), 1463 and 1384(m, C-H bending), 730 and 720(m, C-H rocking) (Fig. 10).

GC chromatography: (condition; column 2%OV-1, column temp. 250°C, injection temp. 290 °C, carrier gas N_2 50.0 ml/min. and FID detector) Chromatogram showed 10 peaks at retention time 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).

2. Purification and Properties of Compound 2

Compound 2 was a solid in yellow oil in Fraction No.35-40 (H_4) which was obtained from the column chromatography of hexane crude extract (Table 4). It was eluted from the silica gel column with 10%$CHCl_3$ in hexane. After washing yellow oil with methanol and recrystallization with hot methanol for several times, Compound 2 was obtained as white amorphous product, 3.37 g (2.51%wt/wt of hexane crude), m.p. 65-68 °C, R_f values 0.84 in 10% $CHCl_3$/hexane. This compound was soluble in chloroform, dichloromethane and hot methanol but slightly soluble in hexane. Compound 2 gave negative results to Br_2 in CCl_4, 2,4-DNP and Liebermann Burchard's reagents.

FT-IR spectrum (KBr), ν max($cm.^{-1}$) showed the absorption peak at 2919, 2849(s, C-H stretching), 1737(C=O stretching), 1473, 1464(m, C-H bending), 1174(C-O stretching), 730 and 720(m, C-H rocking) (Fig. 14).

^{1}H-NMR spectrum ($CDCl_3$) gave the proton signals at (δ) : 0.87(t), 1.24(s), 1.59(m), 2.26(t) and 4.02(t) ppm. (Fig. 15).

Mass spectrum(m/e) was shown in Fig. 16. Using library search software(NIST database), Compound 2 was found to be similar to mass spectrum of Octatadecyl eicosanoate($C_{38}H_{76}O_2$) (Fig. 17). The fragmentation ion peak at m/e 312 suggested that it was from Eicosanoic acid ($C_{20}H_{40}O_2$).

3. Purification and Properties of Compound 3

Compound 3 was a solid in orange oil in Fraction No. 45-59 (H_7) which was obtained from the column chromatography of hexane crude extract (Table 4). It was eluted from the silica gel column with 20%$CHCl_3$ in hexane and was also obtained from Fraction No. 40-44 (M_8) which was obtained from the column chromatography of methanol crude extract (Table 5). It was eluted from the silica gel column with 50%$CHCl_3$ in hexane. After washing orange oil with methanol and recrystallization with hot methanol for several times, bright white needle crystals were obtained 4.86 g (3.34%wt/wt of hexane crude and methanol crude), m.p. 75 °C. TLC revealed only one spot at R_f 0.56 (solvent system: 80%$CHCl_3$ in hexane). This compound was soluble in chloroform, dichloromethane and hot methanol but slightly soluble in hexane, acetone, ethylacetate and methanol. Compound 3 gave positive results (purple color) when it was treated with Liebermann-Burchard's reagent. It decolorized Br_2 in CCl_4 reagent and also gave a positive test to 2,4-DNP reagent.

FT-IR spectrum (KBr), ν max($cm.^{-1}$) showed the absorption peak at 3075(s, C-H stretching of alkene), 2969 and 2946(m, C-H stretching), 2853 (s, C-H stretching), 1704(s, C=O stretching), 1642(w, C=C stretching), 1449 and 1377(m, C-H bending), 879 and 826(m, C-H out of plane bending vibration of=CH_2) (Fig. 18).

^{1}H-NMR spectrum ($CDCl_3$) showed the proton signals at (δ): 0.88 (3H, s), 0.95(3H, s), 1.02(3H, s), 1.04(3H, s), 1.08(3H, s, 1.62(3H, s) and 1.69 (3H, bs. each), 1.05-2.54(26H, complex m), 4.72 and 4.75(1H, bs. each) and 5.13(1H, m) ppm. (Fig. 19-22).

^{13}C-NMR spectrum ($CDCl_3$) showed the chemical shift of 30 peaks at δ: 15.35, 15.83, 16.06, 17.72, 19.68, 21.03, 21.91, 24.99, 25.71, 26.77, 27.08, 28.87, 31.36, 34.10, 34.14, 34.76, 36.92, 39.96, 40.37, 45.41, 47.40, 47.74, 49.40, 50.31, 55.38, 107.61, 124.44, 131.40, 152.52 and 218.01 ppm. (Fig. 23-24).

DEPT-90 ^{13}C-NMR spectrum ($CDCl_3$) showed the chemical shift of CH signals 5 peaks at 45.41, 47.74, 50.31, 55.38 and 124.44 ppm.(Fig. 25-26).

DEPT-135 ^{13}C-NMR spectrum ($CDCl_3$) showed the chemical shift of CH_2 signals (down phase) 11 peaks at 19.68, 21.91, 24.99, 27.08, 28.87, 31.36, 34.10, 34.14, 34.76, 39.96 and 107.61 and showed the chemical shift of CH_3 and CH signals (up phase) 12 peaks at 15.35, 15.83, 16.06, 17.72, 21.03, 25.71, 26.77, 45.41, 47.74, 50.31, 55.38 and 124.44 ppm. (Fig. 25-26).

Mass spectrum showed the molecular ion peak (M^+) at m/e 424 ($C_{30}H_{48}O$) and other fragmentation ion peaks at m/e 409, 271, 245, 218, 206, 205, 190, 189 and 95 (Fig. 38).

Elemental analysis found : %C = 83.72 and %H = 11.36

Calcd. for $C_{30}H_{48}O$, MW. 424 : % C = 84.90 and %H = 11.32

4. Purification and Properties of Compound 4

Compound 4 was a solid in yellow oil in Fraction No. 64-68(H_8) which was obtained from the column chromatography of hexane crude extract (Table 4) and was eluted from silica gel with 20%$CHCl_3$ in hexane and was also obtained from Fraction No. 45-48(M_9) which was obtained from the column chromatography of methanol crude extract (Table 5) and was eluted from silica gel with 50%$CHCl_3$ in hexane. The precipitate was washed with methanol and recrystallized by hot acetone for several times, giving white amorphous solid 1.88 g (1.13%wt/wt of hexane crude), m.p. 78-79°C. R_f value was 0.61 (solvent system: 100% $CHCl_3$). This compound was soluble in hot acetone but slightly soluble in chloroform, dichloromethane and hexane and not soluble in acetone nor methanol. Compound 4 gave negative results to 2,4-DNP, Br_2 in CCl_4 and also Liebermann Burchard's reagent.

FT-IR spectrum (KBr), ν max($cm.^{-1}$) showed the absorption peak at 3502-3314(b, O-H stretching), 2918 and 2849(s, C-H stretching), 1473, 1463 1379(m, C-H stretching), 1059(m, C-O stretching), 730 and 720(m, C-H rocking) (Fig. 39).

^{1}H-NMR spectrum ($CDCl_3$ heat) gave the proton signals at chemical shift(δ) :0.87(t), 1.25(t), 1.55(m) and 3.62(t) ppm. (Fig. 40).

^{13}C-NMR spectrum ($CDCl_3$ heat) gave the proton signals at chemical shift(δ): 13.52, 22.27, 25.64, 29.28, 30.00, 31.83, 32.86 and 62.54 ppm. (Fig. 41).

GC Chromatography : (condition; column 2%OV-1, column temp. 250 °C, injection temp. 290 °C, carrier gas N_2 50.0 ml/min. and FID

detector), range 10^2, 10^3 and 1 µl. Chromatogram showed 2 peaks at retention time: 27.78 and 30.71 min., respectively (Fig. 43).

5. Purification and Properties of Compound 5

Compound 5 was a solid in orange oil in Fraction No. 91-98 (H_{12}) which was obtained from the column chromatography of hexane crude extract (Table 4). It was eluted from the silica gel with 20%$CHCl_3$ in hexane. After washing orange oil with methanol and recrystallization with hot methanol for several times, white plate 1.76 g (1.31%wt/wt of hexane crude) was obtained. Melting point was 128-129°C. TLC revealed only one spot at R_f 0.63 (solvent system: 5% MeOH in $CHCl_3$). This compound was soluble in chloroform, hot methanol and dichloromethane but slightly soluble in hexane, ethylacetate, acetone and methanol. Compound 5 gave positive result (purple color) when it was treated with Liebermann Burchard's reagent and also decolorized Br_2 in CCl_4 reagent but gave a negative result to 2,4-DNP reagent.

FT-IR spectrum (KBr), ν max($cm.^{-1}$) showed the absorption peak at 3376(b, O-H stretching), 3082(s, C-H stretching of alkene), 2927(m) and 2860(s, C-H stretching), 1643(w, C=C stretching) 1466, 1443, 1388 and 1375 (w, C-H bending), 1092, 1034(m, C-O stretching and O-H bending of R-OH), 985 and 887(s, C-H out of plane bending vibration of $=CH_2$) (Fig. 45).

^{1}H-NMR spectrum ($CDCl_3$) gave the proton signals (δ): 0.79(3H, s), 0.85(3H, s), 0.87(3H, s), 0.98(6H, s), 1.62 and 1.69(3H, bs. each), 1.04-2.34 (24H, complex m), 3.20(1H, m), 4.72 and 4.74(1H, bs. each) and 5.14 (1H, m) ppm.(Fig. 46-49).

^{13}C-NMR spectrum ($CDCl_3$) δ showed the chemical shift of 30 peaks at δ: 15.38, 15.66, 15.94, 16.24, 17.73, 18.31, 21.39, 24.99, 25.71, 27.10, 27.44, 28.03, 28.92, 31.41, 34.14, 35.45, 37.25, 38.99, 39.14, 40.49, 45.29, 47.86, 49.45, 50.97, 55.90, 78.97, 107.49, 124.49, 131.43 and 152.75 (Fig. 50).

DEPT-90 ^{13}C-NMR spectrum ($CDCl_3$) showed the chemical shift of CH signal 6 peaks at 45.29, 47.86, 50.97, 55.90, 78.97 and 124.49 ppm. (Fig. 51-53).

DEPT-135 ^{13}C-NMR spectrum ($CDCl_3$) showed the chemical shift of CH_3 and CH signals (up phase) 13 peaks at 15.38, 15.66, 15.94, 16.24, 17.73, 25.71, 28.03, 45.29, 47.86, 50.97, 55.90, 78.97 and 124.49. and showed the chemical shift of CH_2 signal (down phase) 11 peaks at 18.31, 21.39, 24.99, 27.10, 27.44, 28.92, 31.41, 34.14, 35.45, 39.14 and 107.49 ppm. (Fig. 51-53).

Mass spectrum showed the molecular ion peak (M^+) at m/e 426 ($C_{30}H_{50}O$) and other fragmentation ion peaks at m/e 408, 383, 257, 218, 207, and 189 (Fig. 69).

Elemental analysis found : %C = 84.59 and %H = 11.91

Calcd. for $C_{30}H_{50}O$, MW. 426 : % C = 84.51 and %H = 11.73

6. Purification and Properties of Compound 6

Compound 6 was a mixture of white needle crystal in a yellow-orange oil in Fraction No. 104-113 (H_{15}) which was obtained from the column chromatography of hexane crude extract (Table 4). It was eluted from the silica gel with 30%$CHCl_3$ in hexane. After yellow-orange oil was removed by washing with methanol, the remaining product was recrystallized from hot methanol for several times. It gave bright white needle crystal 2.41 g(1.79% wt/wt of hexane crude). Melting point was 130-132°C. TLC revealed only one spot at R_f 0.60 (solvent system: 5%MeOH in $CHCl_3$). This compound was soluble in chloroform, dichloromethane, ethylacetate, acetone and hot methanol but slightly soluble in hexane and methanol. Compound 6 gave a green color when it was treated with Liebermann Burchard's reagent and also decolorized Br_2 in CCl_4 reagent but gave negative result to 2,4-DNP reagent.

FT-IR spectrum (KBr), ν max($cm.^{-1}$) showed the absorption peak at 3600-3100(b, O-H stretching), 3061(s, C-H stretching of alkene) 2959, 2938, 2868(m, C-H stretching) 1648(w, C=C stretching), 1464 and 1380(m, C-H stretching), 1059,1023(m, C-O stretching and O-H bending of R-OH), 959(s, trans C-H bending out of plane $R_1R_2C=CHR_3$) (Fig. 70).

^{1}H-NMR spectrum ($CDCl_3$) gave the proton signals at (δ): 0.73-2.35(m), 3.55(m), 5.00-5.10(m) and 5.37(d) ppm.(Fig. 71-75).

^{13}C-NMR spectrum ($CDCl_3$) showed the chemical shift of 43 peaks at 11.74, 11.85, 11.92, 12.12, 18.65, 18.86, 18.91, 19.27, 19.68, 20.95, 21.08, 22.94, 24.18, 25.26, 25.95, 28.11, 28.78, 29.03, 31.48, 31.78, 33.82, 36.00, 36.40, 37.17, 39.56, 39.66, 39.89, 40.05, 40.35, 42.16, 42.19, 45.71, 50.02, 51.09, 55.83, 55.93, 56.63, 56.73, 71.46, 121.40, 129.13, 138.18 and 140.83 ppm.(Fig. 76-77).

DEPT-90 ^{13}C-NMR spectrum ($CDCl_3$) showed the chemical shift of CH signal 15 peaks at 29.03, 31.78, 36.00, 40.35, 45.71, 50.05, 51.09, 55.83, 55.93, 56.63, 56.73, 71.46, 121.40, 129.13 and 138.18 ppm.(Fig. 78-80).

DEPT-135 ^{13}C-NMR spectrum ($CDCl_3$) showed the chemical shift of CH_3 and CH signals (up phase) 24 peaks at 11.74, 11.85, 11.92, 12.12, 18.65, 18.86, 18.91, 19.68, 21.08, 29.03, 31.78, 36.00, 40.35, 45.71, 50.02, 51.09, 55.83, 55.93, 56.63, 56.73, 71.46, 121.40, 129.13 and 138.18 and showed the chemical shift of CH_2, signal (down phase) 13 peaks at 20.95, 22.94, 24.18, 25.26, 25.95, 28.11, 28.78, 31.48, 33.82, 37.17, 39.56, 39.66 and 42.16 ppm. (Fig. 78-80).

GC Chromatography : (condition; column 2%OV-1, column temp. 250 °C, injection temp. 290 °C, carrier gas N_2 50.0 ml/min. and FID detector), range 10^2, 10^3 and 1 μl. Chromatogram showed 2 peaks at retention time 15.84 and 17.28 min., respectively (Fig. 82).

Mass spectrum showed the 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, 382, 381, 329, 303, 275, 273, 255 and 213 (Fig. 83).

7. Purification and Properties of Compound 7

Compound 7 was a solid in greenish-brown oil which was obtained from the column chromatography of the methanol crude extract (Table 5) of the Fraction No.132-138 (M_{17}). It was eluted from the silica gel column with 5%MeOH/$CHCl_3$. After washing brown-yellowish oil with ethylacetate and the remaining material was recrystallized with hot ethanol for several times. Compound 7 was obtained as white amorphous solid, 0.18 g (0.07% wt/wt of methanol crude), m.p. 284°C (decmpose), R_f value was 0.23 in 10% MeOH/$CHCl_3$. This compound was soluble in hot ethanol and dimethylsulfoxide(DMSO) but not soluble in acetone, chloroform, dichloromethane, ethylacetate, methanol, hexane and ether. Compound 7 gave positive results (green color)when it was treated with Liebermann-Burchard's reagent and also decolorized Br_2 in CCl_4 reagent.

FT-IR spectrum (KBr), ν max($cm.^{-1}$) showed the absorption peak at 3423(b, O-H stretching), 2961-2874(s, C-H stretching), 1645(w, C=C stretching of alkene), 1463 and 1380(s, C-H bending), 1166(m, C-O stretching), 1074-1025(s, glycoside linkage) (Fig. 84).

^{1}H-NMR spectrum (DMSO) gave the proton signals at (δ) : 0.64-2.49(m), 2.89-3.41(m), 3.61(m), 4.18(d), 4.44(t), 4.91(m) and 5.32(m) ppm. (Fig. 85).

^{13}C-NMR spectrum (DMSO) showed the chemical shift of 36 peaks at 11.65, 11.76, 11.85, 18.59, 18.90, 19.08, 19.69, 20.50, 22.56, 23.05, 25.36, 27.80, 28.65, 29.23, 31.38, 33.31, 35.48, 36.18, 36.81, 38.20, 38.62, 39.04, 41.82, 45.10, 48.56, 49.56, 55.40, 56.16, 61.02, 70.02, 73.42, 76.72, 76.87, 100.76, 121.19 and 140.40 ppm.(Fig. 86).

Mass spectrum did not give the molecular ion peak (M^+), but it revealed the dominant mass fragment at m/e 414, 396, 381, 273, 255 and 213 (Fig. 87).

8. Purification and Properties of Compound 8

Compound 8 was a solid in brown oil in Fraction No.169-173 (M_{21}) which was obtained from the column chromatography of methanol crude extract (Table 5). It was eluted from the silica gel column with 15-20% MeOH/$CHCl_3$. After washing brown oil with ethylacetate and recrystallization with hot ethanol for several times, Compound 8 was obtained as ivory amorphous solid, 0.009 g (0.004%wt/wt of methanol crude), m.p. 86-88°C, R_f values 0.75 in 40% MeOH/$CHCl_3$. This compound was soluble in hot ethanol and slightly soluble in dimethylsulfoxide(DMSO) but not soluble in methanol, chloroform, dichloromethane, hexane, ethylacetate and acetone. Compound 8 gave negative results to Br_2 in CCl_4, 2,4-DNP and Liebermann Burchard's reagents.

FT-IR spectrum (KBr), ν max($cm.^{-1}$) showed the absorption peak at 3500-2400(very b., O-H stretching), 2918, 2849(s, C-H stretching), 1701 (C=O stretching), 1473, 1463(m, C-H bending), 1294(m, C-O stretching)and 720(m, C-H rocking) (Fig. 88).

Mass spectrum showed the molecular ion peak (M^+) at m/e 508 ($C_{34}H_{68}O_2$) and other fragmentation ion peaks at m/e 479, 465, 451, 423, 185, 171 and 129 which were the stepwise fragmentation of -CH_2- group. (Fig. 89).

9. Purification and Properties of Compound 9

Compound 9 was a solid in brown oil which was obtained from the column chromatography of the methanol crude extract (Table 5) of the Fraction No.174-175 (M_{22}). It was eluted from the silica gel column with 20% MeOH/$CHCl_3$. After washing brown-yellowish oil with ethylacetate and the remaining material was recrystallized with hot ethanol for several times. Compound 9 was obtained as ivory amorphous solid, 0.003 g (0.001%wt/wt of methanol crude), m.p.138-140°C, R_f value was 0.54 in 40%MeOH/$CHCl_3$. This compound was soluble in hot ethanol and dimethylsulfoxide(DMSO) but not soluble in acetone, chloroform, dichloromethane, ethylacetate, methanol, hexane and ether. Compound 9 gave negative results to Br_2 in CCl_4, Liebermann Burchard's and also 2,4-DNP reagents.

FT-IR spectrum (KBr), ν max($cm.^{-1}$) showed the absorption peak at 3500-2500(very b., O-H stretching), 2957, 2919 and 2849(s, C-H stretching), 1740(s, C=O stretching), 1470(s, C-H bending), 1228 to 1038 (m, C-O stretching), 721(m, C-H rocking) (Fig. 91).

Mass spectrum of Compound 9 revealed the dominant mass 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, 97, 83, 71 and 57 (Fig. 92).

10. Purification and Properties of Compound 10

Compound 10 was a solid in brown oil which was obtained from the column chromatography of the methanol crude extract (Table 5) of the Fraction No.176-178 (M_{22}). It was eluted from the silica gel column with 20% MeOH/$CHCl_3$. After washing brown-yellowish oil with ethylacetate and the remaining material was recrystallized with hot ethanol for several times. Compound 10 was obtained as ivory amorphous solid, 0.004 g (0.002%wt/wt of methanol crude), m.p.217-220°C, R_f value was 0.62 in 40%MeOH/$CHCl_3$. This compound was soluble in hot ethanol and dimethylsulfoxide(DMSO) but not soluble in acetone, chloroform, dichloromethane, ethylacetate methanol, hexane and ether. Compound 10 gave positive result to Br_2 in CCl_4 and 2,4-DNP reagents but gave negative result to Liebermann Burchard's reagent.

FT-IR spectrum (KBr), ν max($cm.^{-1}$) showed the absorption peaks at 3600-3050(b, O-H stretching), 2944(s,C-H stretching), 1736(m, C=O stretching), 1636(s, C=C stretching), 1460(s, C-H bending), 1148-1023 (s, C-O stretching), 755, 728(s, C-H rocking) (Fig. 93).

Mass spectrum did not give the molecular ion peak (M^+), but it revealed the dominant mass fragment at m/e 163, 149, 145, 133, 127, 115, 103, 91, 85, 74, 73(base peak) and 60 (Fig. 94).