CHAPTER III

EXPERIMENTS

3.1 Plant material

The stem barks of *C. oblongifolius* Roxb. were collected from Chachoengsao Province, Thailand in June 1999. Botanical identification was claimed through comparison with voucher specimen no. BKF 084729 in the herbarium of the Royal Forest Department, Ministry of Agriculture and Co-operatives, Bangkok, Thailand.

3.2 Instrument and Equipments

3.2.1 Fourier Transform Infrared Spectrophotometer (FT-IR)

The Infrared spectra were recorded on a Nicolet Impact 410 Fourier Transform Infrared Spectrophotometer. Spectra of solid samples were recorded as KBr pellets and liquid samples were recored as thin film on KBr cells.

3.2.2 Mass Spectrometer (MS)

The mass spectra were recorded on a Fisons Instruments Mass Spectrometer model Trio 2000 GC-MS in Electron Impact (EI) mode at 70 eV.

3.2.3 Ultraviolet-Visible Spectrophotometer (UV-Vis)

The UV-Vis spectra were recorded on a Hewlett Packard 8452 A diode array spectrophotometer in chloroform.

3.2.4 ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectrometer (NMR)

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Model AC-F 200 spectrorneter operated at 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C nuclei.

3.2.5 Optical Rotation

The optical rotation were measured on a Perkin-Elmer 341 polarimeter in $CHCl_3$.

3.3 Chemical Regents

3.3.1 Solvents

All solvents used in this research such as hexane, chloroform, ethyl acetate and methanol were commercial grade and were purified prior to use by distillation.

3.3.2 Packing material

3.3.2.1 Merck's silica gel 60 G Art. 7734 (70-230 mesh ASTM) and 9385 (230-400 mesh ASTM) were used as adsorbents for normal column chromatography and flash column chromatography.

3.3.2.2 Merck's SiO_2 aluminum sheets TLC, 20×20 cm^3, layer thickness 0.2 mm was used to monitor identical fractions.

3.4 Extraction and Isolation

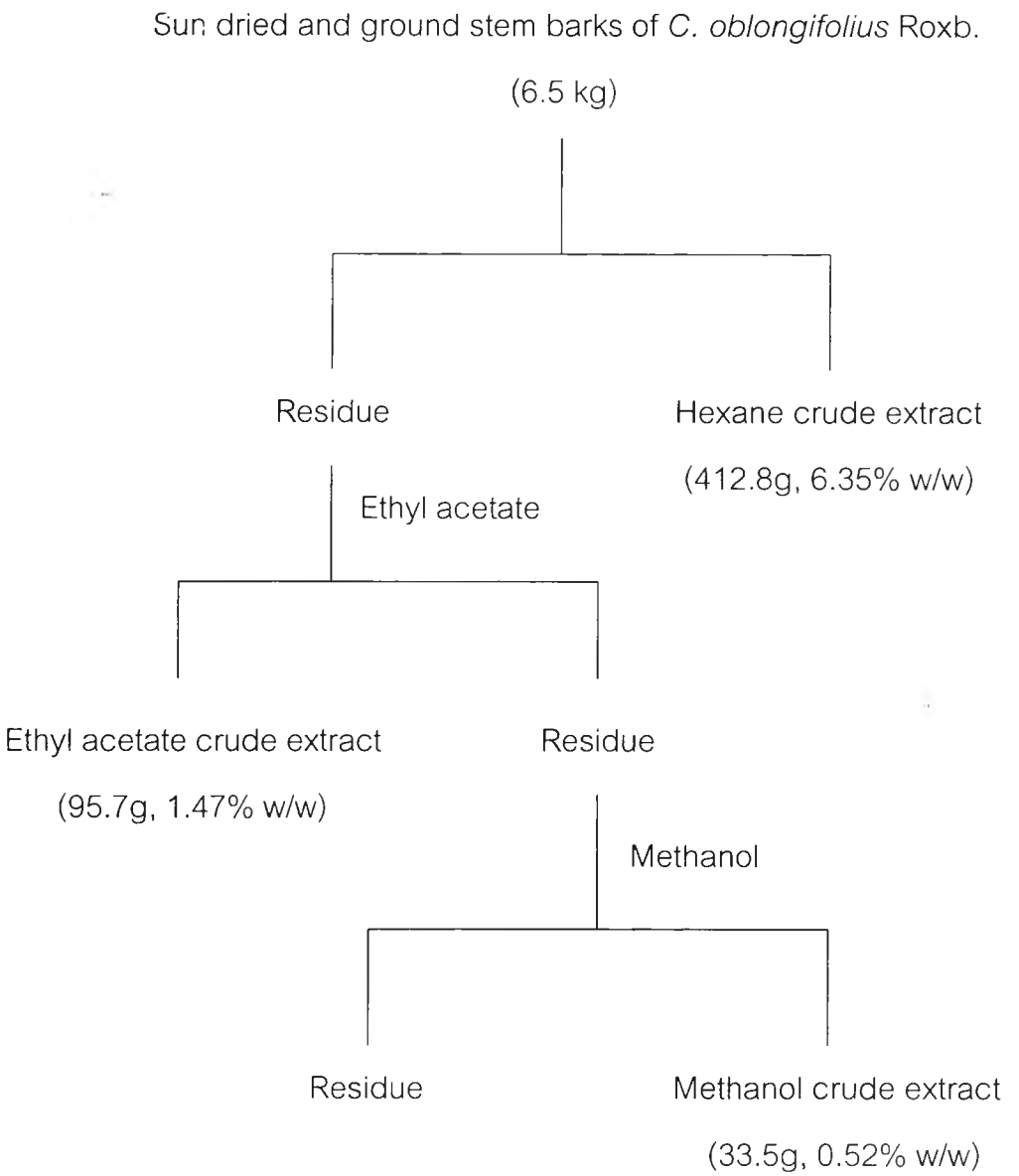
The sun-dried and ground stem barks (6.5 kg) of *C. oblongifolius* Roxb. was soaked in hexane (3×10 liters) at room temperature for 2 weeks. The hexane solution was filtered and evaporated under reduced pressure to

dryness, yielding the hexane crude extract. The residue was extracted again with ethyl acetate (3×10 liters) and methanol (2×10 liters), then the two extracts were filter and evaporated to obtain ethyl acetate and methanol crude extracts, respectively.

Three different crude extracts of the stem barks of *C. oblongifolius* are shown in Table 2 and the extraction procedure are shown in scheme 1.

Table 3 The various extracts of the stem bark of *C. oblongifolius* Roxb.

Solvent extract	Appearance	Weight (g)	%wt/wt of the dried stem bark
Hexane	Yellowish green oil	412.8	6.35
Ethyl acetate	Dark brown oil	95.7	1.47
Methanol	Dark red oil	33.5	0.52

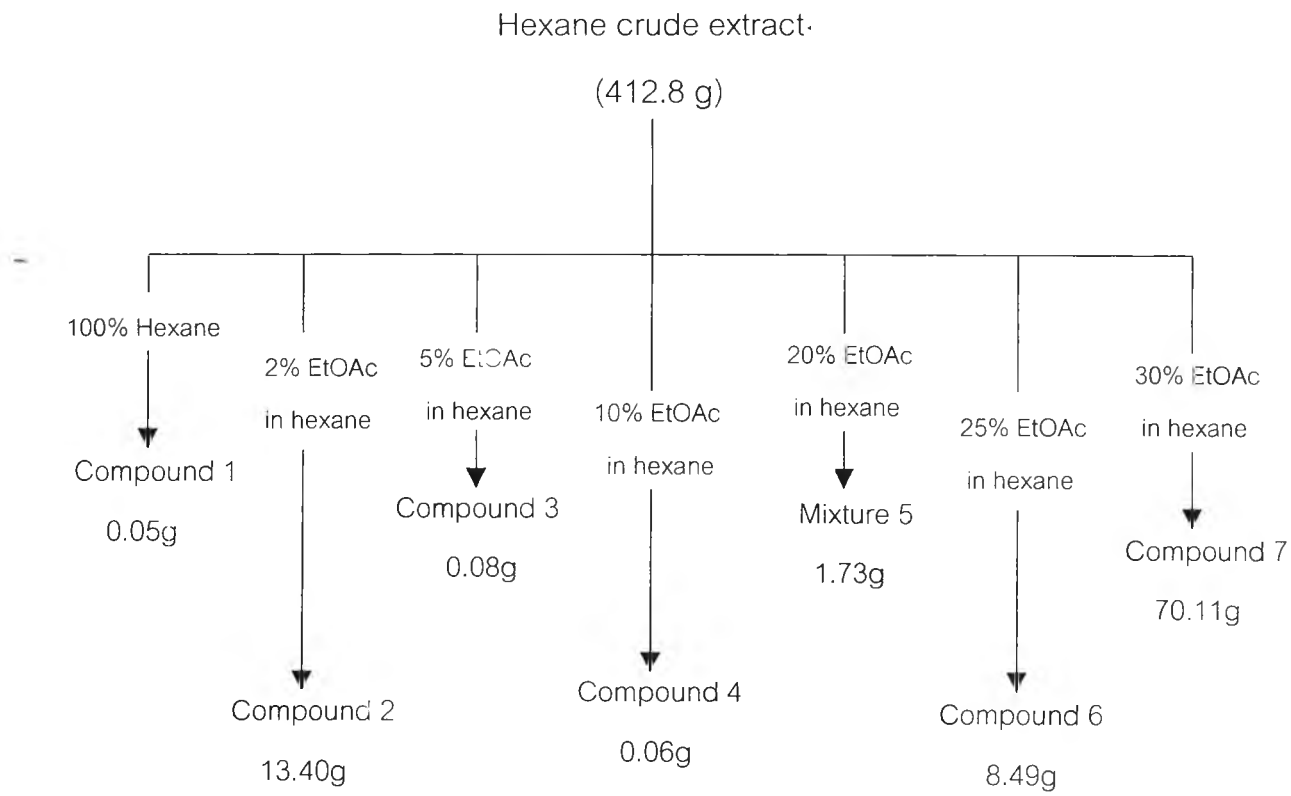


Scheme 1 The extraction procedure of the stem barks of *C. oblongifolius* Roxb.

3.5 Isolation of crude extract of *C. oblongifolius* Roxb.

3.5.1 Separation of hexane crude extract

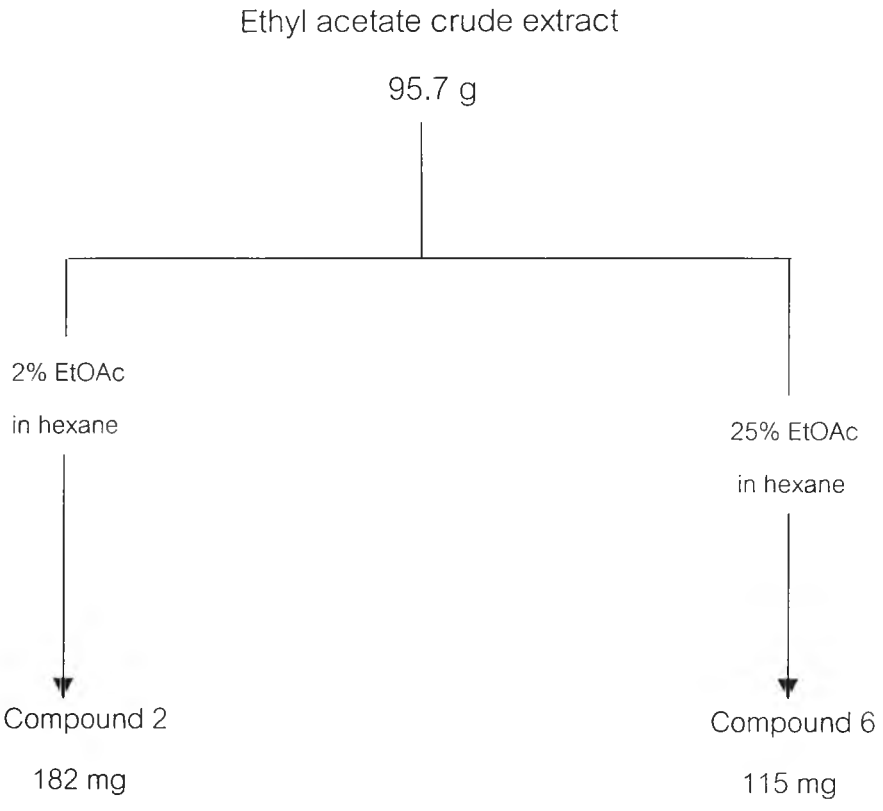
The hexane crude extract (400g) was separated by open column chromatographic techniques. The column was packed with silica gel 60 Art. 7734 (70-230 mesh ASTM, 400g) and the crude extract was dissolved in a small amount of a suitable solvent and mixed with silica gel (1:1) to afford the extract paste. The paste was evaporated to dryness under reduced pressure before being placed on top of column. The column was eluted with hexane, hexane-ethyl acetate gradient in a stepwise fashion. Each fraction was collected (500 ml), concentrated to a small volume (30 ml) and then checked by TLC in order to combine the fractions which had the same TLC pattern. The fraction which contained UV active components were further purified by column chromatography or crystallization. The isolations of the mixtures and compounds from hexane crude extract was briefly summarized in scheme 2.



Scheme 2 The isolation procedure of hexane crude extract

3.5.2 Separation of ethyl acetate crude extract

The ethyl acetate crude extract (10g) was separated by column chromatography on silica gel 60 Art. 7734 (70-230 mesh ASTM, 10g). The column was eluted with hexane, hexane-ethyl acetate, ethyl acetate and ethyl acetate-methanol, respectively. About 125 ml of each fraction was collected and was evaporated to give about 30 ml, then it was analyzed by TLC in order to combine the fractions which had the same TLC pattern.



Scheme 3 The isolation procedure of ethyl acetate crude extract

3.5.3 Separation of methanol crude extract

The methanol crude extract was gummy residue which was insoluble in all solvent. Therefore, it was not separated by column chromatography.

3.6 Purification and properties of the compounds eluted from column chromatography of hexane crude extract

3.6.1 Purification and properties of Compound 1

Compound 1 was eluted with n-hexane. The solvent was removed by rotary evaporation and the residue was purified by column chromatography (Merck's silica gel Art. 1.09385.1000). This compound is soluble in chloroform, ether, ethyl acetate and methanol.

Compound 1 is colourless needle-like crystals (50 mg, 0.0121% yield from crude hexane and 0.0008% yield from starting material), $[\alpha]_D^{20}$ –15.93° ($CHCl_3$, *c*=1.0), R_f; 0.30 (pure chloroform), m.p. 118-120°C, UV (EtOH) λ_{max} 232sh (log ε 4.47).

FT-IR spectrum (KBr) (Fig.11, Table 5) ν_{max} (cm^{-1}): 3421-2626(br.,s), 2946(s), 1707(s), 1652(m), 1604(w), 1459(w), 1430(m), 1383(m), 1345(w), 1208(s).

^{1}H-NMR spectrum ($CDCl_3$) (Fig.12) δ (ppm): 6.90(1H, dt,*J*=2.1,6.2), 6.31(1H, dd,*J*=10.7,17.4), 5.47(1H, t,*J*=6.7), 5.00(1H, d,*J*=17.4), 4.84(1H, d,*J*=11.0), 2.58(1H, m), 2.37(1H, m), 2.32(1H, dd,*J*=6.1,16.8), 2.19(1H, m), 1.87(1H, m), 1.67(3H, s), 1.40(2H, d,*J*=2.5), 1.18(1H, d,*J*=4.3), 0.89(3H, s), 0.86(3H, s), 0.82(3H, s).

^{13}C-NMR spectrum ($CDCl_3$) (Fig.13, Table 6) δ (ppm): 174.3(s), 141.8 (d), 140.6(d), 133.6(s), 133.5(d), 133.1(s), 110.0(t), 49.9(d), 49.3(d), 41.9(t), 40.1(t), 36.9(s), 33.3(q), 32.8(s), 26.0(t), 24.0(t), 22.2(q), 18.6(t), 14.8(q), 11.7 (q).

EIMS (Fig.15) *m/z* (rel. int.): 302[M^+](45), 284[M^+-H_2O](9), 221(17), 220 (13), 205(9), 203(14), 178(8), 175(29), 165(15), 151(38), 139(84), 137(29), 133 (19), 125(7), 119(21), 109(72), 105(32), 97(38), 91(53), 81(100), 79(49), 77 (28), 69(46), 55(30).

3.6.2 Purification and properties of Compound 2

Compound 2 was eluted with n-hexane. The solvent was removed by rotary evaporation and the residue was purified by column chromatography (Merck's silica gel Art. 1.09385.1000; eluting with 2% ethyl acetate in hexane). It is soluble in hexane, dichloromethane, chloroform, ethyl acetate, diethyl ether and methanol.

Compound 2 is a white solid (13.21 g, 3.20 % yield from crude hexane and 0.20 % yield from starting material), $[\alpha]_D^{20}$ –125.28° ($CHCl_3$, *c*=1.0), R_f; 0.29 (pure chloroform), m.p. 103-104°C, UV ($CHCl_3$) λ_{max} 242sh (log ε 3.21).

FT-IR spectrum (KBr) (Fig.16, Table 7) ν_{max} (cm^{-1}): 3600-3400(br.,s), 2960, 2930 and 2868(s), 1675(s), 1634(m), 1460(m), 1396(m), 1381(m), 1280 (m), 893(m).

^{1}H-NMR spectrum ($CDCl_3$) (Fig. 17) δ (ppm): 7.33(1H, br.,s), 7.19(1H, s), 6.85(1H, t,*J*=3.0), 6.26(1H, br.,s), 2.50-2.16(6H, m), 1.73-1.40(8H, m), 1.26 (3H, s), 0.84(3H, d,*J*=6.0), 0.76(3H, s).

^{13}C-NMR ($CDCl_3$) (Fig.18, Table 8) δ (ppm): 173.1(s), 142.7(d), 141.5 (s), 140.4(d), 138.4(d), 125.6(s), 110.9(d), 46.7(d), 38.8(s), 38.6(t), 37.6(s), 36.2(d), 35.8(t), 27.5(t), 27.3(t), 20.5(q), 18.3(q), 18.2(t), 17.4(t), 16.0(q).

EIMS (Fig.20) *m/z* (rel. int.): 316[M^+] (7), 299(20), 283(13), 221(96), 203 (77), 175(16), 151(23), 137(37), 125(100), 107(12), 105(13), 96(49), 95(41), 81 (38), 79(8).

3.6.3 Purification and properties of Compound 3

Compound 3 was obtained from 10% chloroform in hexane. Similar fractions were combined and the solvents were removed by rotary evaporation and further purified by column chromatography (Merck's silica gel Art. 1.09385.1000; eluting with 5% ethyl acetate in hexane). This compound is soluble in chloroform, ethyl acetate, diethyl ether and methanol.

Compound 3 is white solid (85 mg, 0.02% yield from crude hexane and 0.0013 % yield from starting material), $[\alpha]_D^{20}$ +51.74° ($CHCl_3$, *c* =1.0), R_f; 0.34 (20% EtOAc in hexane), m.p. 102-103°C, UV ($CHCl_3$) λ_{max} 244sh (log ε 4.45).

FT-IR spectrum (KBr) (Fig.21, Table 9) ν_{max} (cm^{-1}): 3465(br.,m), 2982, 2940 and 2862(s), 1730(s), 1642(m), 1441, 1373 and 1252(m), 1036(m), 758 (m).

^{1}H-NMR spectrum ($CDCl_3$) (Fig.22) δ (ppm): 6.31(1H,dd,*J*=11.0,17.4), 5.35(1H,dd,*J*=6.4,6.4), 5.05(1H,d,*J*=17.4), 4.96(1H,ddd,*J*=4.3,10.0,11.7), 4.89 (1H,d,*J*=11.0), 4.87(1H,br.,d,*J*=1.5), 4.49(1H,br.,d,*J*=1.5), 3.23 (1H,dd,*J*=5.2,10.0), 2.42(1H,ddd,*J*=2.4,4.3,13.1), 2.29(1H,br.,dd,*J*=4.3,15.0), 2.19(1H,dd,*J*=6.4,10.6), 2.12(1H,dd,*J*=4.3,11.7), 2.10(3H,s), 2.00 (1H,dd,*J*=5.2,13.1), 1.79(1H,br.,d,*J*=11.6), 1.74(3H,d,*J*=0.9), 1.74 (1H,d,*J*=1.0), 1.41(1H,dddd,*J*=4.3,12.6,12.6,12.6), 1.25(1H,dd,*J*=11.7,11.7), 1.22(1H,dd,2.7,12.6), 1.06(3H,s), 0.87(3H,s), 0.85(3H,s).

^{13}C-NMR spectrum ($CDCl_3$) (Fig.23, Table 10) δ (ppm): 171.6(s), 146.9 (s), 141.4(d), 133.7(s), 133.1(d), 110.1(t), 108.8(t), 80.4(d), 73.2(d), 56.6(d), 54.4(d), 42.3(t), 40.1(s), 39.9(s), 37.6(t), 28.7(q), 23.5(t), 23.4(t), 21.4(q), 16.5 (q), 15.2(q), 11.9(q).

EIMS (Fig.25) *m/z* (rel. int.): 346[M^+] (24), 331[M^+-CH_3](6), 328[M^+-H_2O] (4), 317(7), 304(8), 290(15), 286[M^+-CH_3COOH](75), 272(16), 271(72), 268

(63), 255(25), 253(100), 243(49), 230(28), 229(43), 225(34), 213(33), 191(30), 187(46), 185(32), 173(37), 171(27), 147(31), 145(41), 133(32), 131(40), 119 (40), 105(57), 91(70), 79(42), 77(66).

3.6.4 Purification and properties of Compound 4

Compound 4 was eluted with 10% chloroform in hexane. Similar fractions were combined and the solvents were removed by rotary evaporation and further purified by column chromatography (Merck's silica gel Art.1.09385.1000; eluting with 10% ethyl acetate in hexane). This compound is soluble in dichloromethane, chloroform, ethyl acetate, diethyl ether and methanol.

Compound 4 is a white solid (60 mg, 0.01% yield from crude hexane and 0.0009 % yield from starting material), $[\alpha]_D^{20}$ +9.46° ($CHCl_3$, *c* =1.0), R_f; 0.35 (20% EtOAc in hexane), m.p. 99-101°, UV ($CHCl_3$) λ_{max} 244sh (log ε 4.53).

FT-IR spectrum (KBr) (Fig.26, Table 11) ν_{max} (cm^{-1}): 3439 (br.,m), 2940, 2848 and 1722 (s), 1644(m), 1460, 1373 and 1250 (m).

^{1}H-NMR spectrum ($CDCl_3$)(Fig. 27) δ(ppm): 6.32 (H,dd,*J*=11.0,17.4), 5.40 (H,dd,*J*=6.4,6.4), 5.05 (1H,d,*J*=17.4), 4.89 (1H,d,*J*=11.0), 4.87 (1H,br.,d,*J*=1.5), 4.55 (1H,d,*J*=10.1), 4.51 (1H,br.,d, *J*=1.5), 3.81 (1H,ddd,*J*=4.3,10.1,11.6), 2.41 (1H,ddd,*J*=2.4,4.3,12.1), 2.36 (1H,m), 2.21 (1H,dd,*J*=4.3,12.1), 2.19 (1H,m), 2.15 (3H,s), 2.02 (1H,ddd,*J*=4.9,13.1,13.1), 1.80 (1H,br.,d,*J*=10.4), 1.75 (3H,d,*J*=0.9), 1.74(1H,dddd,*J*=2.6,5.2,5.2,12.8), 1.41 (1H,dddd,*J*=4.3,12.8,12.8,12.8), 1.29 (1H,dd,*J*=12.1,12.1), 1.09 (1H,dd,*J*=2.4,12.8), 0.90 (3H,s), 0.87 (3H,s) 0.80 (3H,s).

^{13}C-NMR spectrum ($CDCl_3$) (Fig.28, Table 12) δ (ppm): 172.4 (s), 146.9 (t), 141.4 (d), 133.8 (s), 133.0 (d), 110.1 (t), 108.8 (t), 84.5 (d), 67.8 (d), 56.6 (d), 54.4 (d), 46.3 (t), 40.0 (s), 39.3 (s), 37.6 (t), 28.7 (q), 23.5 (t), 23.3 (t), 21.1 (q), 17.5 (q), 15.4 (q), 11.9 (q).

EIMS (Fig.30) *m/z* (rel. int.):346[M^+] (9), 328[M^+-H_2O] (4), 313 (1), 286 [M^+-CH_3COOH] (19), 271 (45), 268 (32), 255 (22), 253 (78), 243 (39), 229 (44), 213 (32), 203 (27), 187 (52), 173 (67), 159 (56), 147 (68), 145 (60), 135 (73), 133 (87), 121 (80), 119 (95), 107 (82), 105 (100), 95 (53), 93 (68), 91 (70), 81 (67), 79 (89).

3.6.5 Purification and properties of Mixture 5

Mixture 5 was eluted with 20% chloroform in hexane. The solvent was removed by rotary evaporation and the eluted material contained white solid together with yellow oil. After removal of the yellow oil by methanol, the solid was recrystallized from hot hexane for several times. These compounds were soluble in chloroform, acetone, ethyl acetate, diethyl ether, hot methanol and slightly soluble in n-hexane and methanol. Mixture 5 was white needle crystals (1.73 g, 0.42% yield from crude hexane and 0.03 % yield from starting material), m.p. 143-145^{o}C. The R_f value was 0.45 using 20% EtOAc in hexane as a developing solvent.

FT-IR spectrum (Fig.31) ν_{max} (cm-1): 3430(br.,s), 2937(s), 1641(w), 1464(m), 1381(m), 1059(m), 950(w),800(w).

^{1}H-NMR spectrum ($CDCl_3$) (Fig.32) δ (ppm): 5.35(1H,d), 5.09(1H,m), 3.50(1H,m), 1.08-2.30(m), 1.68(3H,s), 1.25(3H,s), 0.98(6H,s), 0.82(3H,s), 0.68 (3H,s).

^{13}C-NMR spectrum ($CDCl_3$) (Fig.33) δ (ppm): 140.8(s), 138.3(d), 129.4 (d), 121.7(d), 71.8(d), 56.9(d), 56.0(d), 51.3(q), 50.2(d), 42.4(t), 42.4(s), 39.8 (t), 37.3(t), 36.6(s), 36.2(s), 36.2(d), 33.9(d), 32.0(t), 32.0(d), 31.7(t), 29.2(q), 28.2(t), 26.2(t), 24.3(t), 21.1(t), 21.1(q), 19.7(q), 19.3(d), 19.0(d), 12.2(d), 11.9 (q).

Mass spectrum *m/z* (Fig.34): 414[M^+](85), 412(47), 400(12), 399(22), 396[M^+-H_2O](37), 381(21), 369[M^+-CH_3], 351(17), 329(28), 303(29), 300(17), 273(24), 271(27), 256(10), 255(45), 231(18), 215(12), 213(39), 199(16), 185 (13), 173(19), 163(27), 161(32), 159(43), 147(35), 145(52), 133(48), 121(34), 119(45), 107(61), 105(64), 95(66), 91(61), 81(88), 79(56), 69(73), 67(45), 57 (57), 55(100), 44(89).

GLC analysis (Fig.35) (DB1 capillary column, column temperature isothermal 290°C, injection temperature 250°C, mass detector, flow rate of He 50 cm^3/sec): gave 3 peaks on gas chromatogram at retention time 20.12, 20.99 and 23.96 min, respectively. The results of GLC analysis of standard steroids namely campesterol, stigmasterol and β-sitosterol showed three peaks at retention time 19.77, 20.91 and 24.15 min, respectively.

3.6.6 Purification and properties of Compound 6

Compound 6 was eluted with 30% chloroform in hexane. The solvent was removed by rotary evaporation and the residue was purified by column chromatography (Merck's silica gel Art.1.09385.1000; eluting with 30% ethyl acetate in hexane). It is soluble in dichloromethane, chloroform, ethyl acetate, diethyl ether and methanol.

Compound 6 is a white solid (8.49 g, 2.06% yield from crude hexane and 0.13% yield from starting material), $[\alpha]_D^{20}$ +6.96° ($CHCl_3$,*c*=1.0), R_f; 0.45 (30% EtOAc in hexane), m.p. 69-70°C, UV ($CHCl_3$) λ_{max} 245.5sh (log ε 4.14).

FT-IR spectrum (KBr) (Fig.36, Table 16) ν_{max} (cm^{-1}): 3374(br.,s), 2975, 2945 and 2854(s), 1645 and 1610(m), 1446 and 1388(m), 1218(m).

^{1}H-NMR spectrum ($CDCl_3$) (Fig.37) δ (ppm): 6.29 (1H,dd,*J*=11.0,17.4), 5.38 (1H,dd,*J*=6.1,6.1), 5.02 (1H,d,*J*=17.4), 4.86 (1H,d,*J*=11.0), 4.85 (1H,br.,d,*J*=1.2), 4.47 (1H,br.,d,*J*=1.2), 3.69 (1H,ddd,*J*=4.3,9.6,11.7), 3.02 (1H,d,*J*=9.6), 2.39 (1H,ddd,*J*=2.4,4.0,12.8), 2.34 (1H,br.,dd,*J*=5.5,11.0), 2.17 (1H,dd,*J*=6.7,11.0), 2.10 (1H,dd,*J*=4.6,12.5), 1.99 (1H,m), 1.76 (1H,br.,d,*J*=10.7), 1.72 (3H,d,*J*=0.9), 1.71 (1H,m), 1.40 (1H,dddd,*J*=4.3,12.5,12.5,12.5), 1.19 (1H,dd,*J*=2.7,12.5), 1.18 (1H,dd,*J*=11.7,12.5), 1.01 (3H,s), 0.80 (3H,s), 0.78 (3H,s).

^{13}C-NMR spectrum ($CDCl_3$) (Fig.38, Table 17) δ(ppm): 147.2 (s), 141.5 (d), 133.7 (s), 133.2 (d), 110.1 (t), 108.6 (t), 83.5 (d), 69.1 (d), 56.7 (d), 54.6 (d), 45.0 (t), 40.1 (s), 39.4 (s), 37.7 (t), 28.8 (q), 23.6 (t), 23.3 (t), 16.6 (q), 15.4 (q), 11.9 (q).

EIMS (Fig.40) *m/z* (rel. int.): 304[M^+] (27), 289[M^+-CH_3] (21), 286[M^+-CH_3COOH] (6), 271 (27), 253(20), 248 (51), 243(14), 233 (27), 229(15), 215 (19), 201 (23), 191(30), 187 (65), 175(30), 173 (47), 161(43), 159 (45), 147 (61), 145 (65), 135(75), 133 (73), 131(55), 121(74), 119 (74), 109(63), 107(81), 105 (74), 95(55), 93(70), 91(70) 81(72), 79 (100), 77(70), 69(64), 67(77), 65 (46), 59(22), 57(31), 55(100).

3.6.7 Purification and properties of Compound 7

Compound 7 was obtained from 50% chloroform in hexane. Similar fractions were combined and the solvents were removed by rotary evaporation and further purified by column chromatography (Merck's silica gel Art. 1.09385.1000; eluting with 30% ethyl acetate in hexane). This compound is soluble in chloroform, ethyl acetate, diethyl ether and methanol.

Compound 7 is a viscous transparent oil (108 mg, 0.03% yield from crude hexane and 0.0017% yield from starting material), $[\alpha]^{20}_{D}$ -46.79° ($CHCl_3$, *c*=1.0), R_f; 0.42 (30% EtOAc in hexane), UV (EtOH) λ_{max} 243 sh (log ε 3.59).

FT-IR spectrum (neat) (Fig.41, Table 18) ν_{max} (cm^{-1}): 3500-3100 (br.,s), 1718(s), 1675(s), 1243(m).

^{1}H-NMR spectrum ($CDCl_3$) (Fig.42) δ (ppm): 8.01(2H, d,*J*=1.2), 7.55 (1H, dd,*J*=7.6,7.6), 7.45(2H, dd,*J*=7.6,7.6), 7.35(1H, d,*J*=1.5), 7.26(1H, s), 6.92(1H, dd,*J*=2.5,4.6), 6.28(1H, d,*J*=1.5), 4.50(1H, d,*J*=11.9), 4.30(1H, d,*J*=11.9), 2.53(1H, ddd,*J*=3.1,3.1,12.8), 2.40(1H, m), 2.35(1H, m), 2.25(1H, m), 2.20(1H, m), 2.08(1H, m), 1.95(1H, m), 1.93(1H, m), 1.78(1H, m), 1.72(1H, m), 1.65(1H, m), 1.58(1H, d,*J*=12.5), 1.53(1H, m), 1.32(3H, s), 1.24(1H, m), 1.02(3H, d,*J*=6.7).

^{13}C-NMR spectrum ($CDCl_3$) (Fig.43, Table 19) δ (ppm): 173.0(s), 167.4 (s), 143.4(d), 141.5(s), 141.0(d), 139.0(d), 133.5(d), 130.9(s), 130.0(d), 130.0 (d), 129.0(d), 129.0(d), 125.7(s), 111.5(d), 68.3(t), 47.9(d), 42.8(s), 38.2(s), 36.8(d), 36.5(t), 32.9(t), 28.6(t), 27.7(t), 20.7(q), 19.7(t), 18.4(t), 17.5(q).

EIMS (Fig.45) *m/z* (rel. int.): 436[M^+](16), 418(5), 414(5), 403(9), 342(8), 341(29), 331(36), 321(8), 315(13), 314(45), 300(8), 299(27), 296(18), 287(10), 281(16), 269(7), 267(7), 232(11), 220(25), 219(38), 205(24), 201(30), 189(22),

175(25), 173(33), 159(25), 149(36), 135(29), 133(33), 125(44), 121(39), 109 (34), 105(100).

3.7 Cytotoxicity Test [26-27]

Bioassay of cytotoxic activity against 6 tumor cell lines, which were Hs 27 (fibroblast), Kato-3 (gastric), BT 474 (breast), Chago (lung), SW 620 (colon) and HEP-G2 (hepatoma) culture *in vitro* was performed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method [26-27]. In principle, the viable cell number/ well is directly proportional to the production of formazan, which follow solubilization, can be measured spectrophotometrically.

This experiment was performed by Mrs. Songchan Phuthong at the institute of Biotechnology and Genetic Engineering.