CHAPTER IV

RESULTS

4.1 The results from solvent extraction of *Croton oblongifolius* Roxb. stem bark.

Stem bark of *C. oblongifolius* (5.6 kg) was extracted with hexane (13 liters×3) at room temperature. After each filtration and evaporation of the solvent under reduced pressure. The plant material was subsequencently extracted by solvents including hexane (13 liters×3), ethyl acetate (13 liters×3), and methanol (13 liters×3), respectively. The result from the solvents extractions are shown in Table 3.

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

Solvent extract	Appearance	Weight(g)	%wt. By wt. Of the dried stem bark
Hexane	Dark-yellowed oil	155.3	2.77%
Ethyl acetate	Dark-yellowed oil	105.3	1.88%
Methanol	Dark-red gammy	12.1	0.22%

4.2 The results from the separation of crude hexane extract.

The crude hexane extract (155.3 g.) was separated by column chromatographic techniques, using hexane-ethyl acetate gradient in a stepwise fashion. The results from separation of crude hexane extract are summarized in Table 5.

Table 5. The results from the separation of crude hexane extract.

Fraction No.	Eluent (% by volume)	Remark
1-6	90%Hexane/EtOAc	White oil
7-8	90%Hexane/EtOAc	Yellow oil
9-16	90%Hexane/EtOAc	Solid in orange oil[1, 2]
17-29	80%Hexane/EtOAc	Yellow oil[3]
30-34	80%Hexane/EtOAc	Solid in dark yellow oil[4]
35-41	70%Hexane/EtOAc	Dark yellow oil
42-51	60%Hexane/EtOAc	Dark yellow oil
52-55	50%Hexane/EtOAc	Dark yellow oil
56-57	40%Hexane/EtOAc	Solid in dark yellow oil[5]
58	30%Hexane/EtOAc	Dark yellow oil
59-60	100%EtOAc	Dark yellow oil

[1] = Compound 1

[2] = Compound 2

[3] = Compound 3

[4] = Compound 4

[5] = Compound 5

4.3 Purification and properties of the isolated compounds from *Croton oblongifolius* Roxb.

4.3.1 Purification and properties of Compound 1

The crude hexane extract was separated by 10% ethyl acetate in hexane and was purified by re-crystallization with 40% chlorofrom in hexane. Similar fractions were combined, and the solvents were removed by evaporation to obtain the fraction number 9. After that it was re-columned (Merck's silica gel Art 1.09385.100) to obtain a mixture of Compounds 1 and 2. Compound 1 was crystallized from the mixture as a white solid crystal (1.5 g, 1.3% wt. by wt. of the dried stem bark). Compound 1 has m.p. 127-129 ^{0}C, $[\alpha]_D^{20}$ +3.2°($CHCl_3$; *c*1.0), and show the Rf value 0.53 on TLC plate using 20% ethyl acetate in hexane as the mobile phase.

This Compound was found to dissolve in organic solvent such as hexane, ethyl acetate, chloroform and methanol.

The spectral data, UV ($CHCl_3$) λ_{max} 242 sh(logε 2.9)

FT-IR spectrum (KBr) (Fig.5, Table 5), ν_{max} (cm^{-1}): 3500-2400 (br), 2954, 2933 and 2847 (m), 1682 (s), 1639 (m) and 1426 (m).

^{1}H-NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig.6, Table 6) δ (ppm): 6.89 (H-11, t, *J*= 8.0 Hz), 6.01 (H-2, d, *J*= 11.0 Hz), 5.91 (H-3, m), 5.14 (H-7, m), 2.40 (H-10, m), 2.37 (H-15, m), 2.36 (H-13, m), 2.26 (H-14, m), 2.24 (H-6, m), 2.21 (H-9, m), 2.17 (H-5, m), 1.71 (H-18, s), 1.69 (H-19, s), 1.07 (H-16, d, *J*= 6.79 Hz) and 1.03 (H-17, d, *J*= 6.79 Hz).

^{13}C-NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig.7, Table 7) δ (ppm): 173.4(s), 146.5 (s), 145.7 (d), 135.6 (s), 134.8 (s), 132.0 (s), 127.8 (d), 120.0 (d), 118.6 (d), 38.5 (t), 37.7 (t), 34.5 (d), 30.5 (t), 29.1 (t), 26.7 (t), 24.7 (t), 22.2 (q), 22.1 (q), 18.0 (q) and 17.4 (q).

DEPT-135 (Fig.8), DEPT-90 (Fig.9)

EI-MS spectrum (Fig.10) *m/z*: 302 [M^+, 23 %], 287 [M^+-CH_3, (2)], 259 (5), 189 (10), 152 (18), 136 (80), 121 (100), 107 (28) and 93 (93).

X-ray data (table 8-11).

4.3.2 Purification and properties of Compound 2

The hexane crude extract was separated by 10% ethyl acetate in hexane. Similar fractions were combined, and the solvents were removed by evaporation to obtain the fraction number 9. After that it was purified by re-columned chromatography with 40% chlorofrom (Merck's silica gel Art 1.09385.100) to obtain a mixture of Compounds **1** and **2**. Compound **2** was obtained from removing of crystal of Compound **1** from the mixture of Compound **1** and **2**. Compound **2** was yellowish viscous transparent oil (0.8 g, 0.7% wt. by wt. of the dried stem bark); $[\alpha]_D^{20}$ +12.2° ($CHCl_3$; *c*1.0), and show the Rf value 0.49 on TLC plate using 20% ethyl acetate in hexane as the mobile phase.

This Compound was found to dissolve in organic solvent such as hexane, ethyl acetate, chloroform and methanol.

The spectral data, UV ($CHCl_3$) λ_{max} 248 sh(logε 4.21)

FT-IR spectrum (KBr) (Fig.11, Table 14) ν_{max} (cm^{-1}): 3500-2400 (br), 2963 and 2847 (m), 1680 (s), 1635 (m) and 1443 (w).

^{1}H-NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig.12, Table 13) δ (ppm): 6.01 (H-2, m), 5.98 (H-11, m), 5.89 (H-3, m), 5.08 (H-7, m), 2.69 (H-10, m), 2.39 (H-13, m), 2.31 (H-14, m), 2.18 (H-5, m), 2.15 (H-9, m), 1.73 (H-18, s), 1.54 (H-19, s), 1.03 (H-16, d, *J*= 6.85 Hz) and 1.00 (H-17, d, *J*= 6.85 Hz).

^{13}C-NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig.13, Table 14) δ (ppm): 173.6(s), 146.7 (d), 146.3 (s), 135.2 (s), 134.0 (s), 130.9 (s), 125.7 (d), 121.6 (d), 118.7 (d), 39.2 (t), 38.5 (t), 33.7 (d), 33.6 (t), 28.7 (t), 26.4 (t), 25.1 (t), 22.1 (q), 22.1 (q), 17.0 (q) and 15.8 (q).

DEPT-135 (Fig.14), DEPT-90 (Fig.15)

EI-MS spectrum (Fig.16) *m/z*: 302 [M^+, 85 %], 287 [M^+-CH_3, (2)], 257 (5), 189 (20), 152 (78), 136 (80), 121 (100), 107 (40) and 93 (90).

4.3.3 Purification and properties of Compound 3

Compound **3** was obtained from the elution of siliga gel column chromatography with 20% ethyl acetate in hexane and was purified by re- column chromatography with 100% chloroform to obtain a yellowish oil (28 mg, 0.023% wt. by wt. of the dried stem bark) $[\alpha]_D^{20}$ +12.2° ($CHCl_3$; *c*1.0), Rf value 0.41 on TLC plate using 20% ethyl acetate in hexane as the mobile phase.

Compound **3** was found to dissolve in organic solvent such as hexane, ethyl acetate, chloroform and methanol.

FT-IR spectrum (KBr) (Fig.17, Table 15) ν_{max} (cm^{-1}): 3500 (br), 2945 (m), 1054 (s).

The spectral data, UV ($CHCl_3$) λ_{max} 221 sh(logε 3.39)

^{1}H-NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig.18, Table 16) δ (ppm): 5.39 (H-14, t, *J*= 6.76 Hz), 5.25 (H-7, s), 4.13 (H-15, d, *J*= 6.74 Hz), 1.66 (H-16, s), 1.52 (H-20, d, *J*= 1.5 Hz), 1.03 (H-19, s), 0.78 (H-17, d, *J*= 6.1 Hz), 0.72 (H-18, s).

^{13}C-NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig.19, Table 17) δ (ppm): 141.0(s), 139.9 (s), 123.1 (d), 122.8 (d), 59.4 (t), 44.6 (d), 40.1 (s), 37.8 (t), 37.3 (d), 36.9 (s), 36.5 (t), 33.1 (t), 32.7 (t), 28.8 (t), 24.1 (t), 19.8 (q), 17.7 (t), 17.3 (q), 16.5 (q) and 15.9 (q).

DEPT-135 (Fig.20), DEPT-90 (Fig.21)

EI-MS spectrum (Fig.22) *m/z*: 290 [M^+, 10 %], 257 (5), 189 (65), 121 (55), 109 (70), 107 (100), 95 (90) and 93 (40).

4.3.4 Purification and properties of Compound 4

Compound **4** was obtained from the elution of siliga gel column chromatography with 30% ethyl acetate in hexane and was purified by re-crystallization with 100% chloroform to obtain a white solid crystal (12.6 mg, 0.012% wt. by wt. of the dried stem bark). Compound **4** has m.p. 143-144 ^{0}C, $[\alpha]_D^{20}$ – 100.3°($CHCl_3$; *c*1.0), and show the Rf value 0.30 on TLC plate using 20% ethyl acetate in hexane as the mobile phase .

This Compound was found to dissolve in organic solvent such as hexane, ethyl acetate, chloroform and methanol.

The spectral data, UV ($CHCl_3$) λ_{max} 253 sh(logε 3.34)

FT-IR spectrum (KBr) (Fig.23, Table 18) ν_{max} (cm^{-1}): 3500-2400 (br), 2960 and 2865 (s), 1672 (s), 1629 and 1410 (w) and 1250 (s).

^{1}H-NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig.24, Table 19) δ (ppm): 7.33 (H-15, t, *J*= 1.5 Hz), 7.17 (H-14, s), 6.86 (H-3, s), 6.24 (H-16, s), 2.43 (H-6, m), 2.33 (H-2, m), 2.30 (H-12, m), 2.04 (H-1, m), 1.72 (H-11, m), 1.28(H-7, m), 1.27(H-19, s), 1.55 (H-8, m), 1.49 (H-10, m), 0.81 (H-17, d, *J*= 7 Hz), 0.80 (H-20, s).

^{13}C-NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig.25, Table 20) δ (ppm): 172.5(s), 142.7 (d), 141.5 (s), 140.3 (d), 138.4 (d), 125.6 (s), 110.0 (d), 46.7 (s), 38.8 (s), 38.7 (t), 37.6 (s), 36.3 (d), 35.8 (t), 27.5 (t), 27.3 (t), 20.5 (q), 18.3 (q), 18.2 (t), 17.5 (t) and 15.9 (q).

DEPT-135 (Fig.26), DEPT-90 (Fig.27)

EI-MS spectrum (Fig.28) *m/z*: 316 [M^+, 85 %], 299 [M^+-CH_3, (10)], 283 (6), 221 (18), 203 (23), 137 (22), 125 (100), 96 (53), 95(40) and 81 (55).

4.3.5 Purification and properties of Compound 5

Compound **5** was obtained from the elution of siliga gel column chromatography with 45% ethyl acetate in hexane and was purified by re-crystallization with 70% dichloromethane in ethyl acetate to obtain a white solid crystal (0.6 g, 0.4% wt. by wt. of the dried stem bark). Compound **5** has m.p. 145^{0}C, $[\alpha]_D^{20}$ –85°($CHCl_3$; *c*0.2), and Rf value 0.30 on TLC plate using 20% ethyl acetate in hexane as the mobile phase.

This Compound was found to dissolve in organic solvents such as hexane, ethyl acetate, chloroform and methanol.

The spectral data, UV (MeOH) λ_{max} (logε): 253(3.54),

FT-IR spectrum (Fig.29, Table 21) ν_{max} (cm^{-1}): 3500-2400 (br), 2960 and 2865 (s), 1784 (s), 1650 and 1250 (s).

^{1}H-NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig.30-34, Table 22) δ (ppm): 8.01 (H-16, s), 7.42 (H-15, d, *J*= 1.9 Hz), 6.74 (H-3, d, *J*= 8.0 Hz), 6.73 (H-14, d, *J*= 2.0 Hz), 4.33 (H-19, d, *J*= 7.9 Hz), 3.6 (COOMe, s), 3.21 (H-8, d, *J*= 13.1 Hz), 3.04 (H-11, d, *J*= 18.0 Hz), 2.73 (H-10, d, *J*= 10.9 Hz), 2.28 (H-2, m), 2.03 (H-7, d, *J*= 11.5 Hz), 1.98 (H-6, d, *J*= 13.4 Hz), 1.64 (H-1, d, *J*= 11.0 Hz), 0.82 (H-20, s).

^{13}C-NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig.35, Table 23) δ (ppm): 193.6(s), 174.0 (s), 169.0 (s), 147.1 (d), 144.3 (d), 137.8 (s), 136.2 (d), 128.6 (s), 108.5 (d), 71.4 (t), 51.4 (q), 48.7 (d), 46.7 (d), 46.5 (t), 45.1 (s), 39.6 (s), 33.2 (t), 27.3 (t), 22.1 (t), 20.1 (t), 19.2 (q).

DEPT-135 (Fig.36), DEPT-90 (Fig.37)

COSY-NMR spectrum (Fig.38-39), NOESY-NMR spectrum (Fig.40-41), HMQC-NMR spectrum (Fig.42-44) and HMBC-NMR spectrum (Fig.45-49).

EI-MS spectrum (Fig.50) *m/z*: 372 [M^+, 75 %], 341(10), 263 (38), 245 (53), 145 (78), 110 (60), 95 (100) and 91 (22).

X-ray data (table 24-27).