CHAPTER IV

RESULTS

4.1 Results of stem bark of *Croton oblongifolius* Roxb. extraction.

The stem bark (2.5 kg) of *Croton oblongifolius* Roxb. was extracted by hexane, ethyl acetate and methanol to obtain the hexane crude extract (85 g), ethyl acetate crude extract (132 g) and the methanol crude extract (35 g), respectively. The results from the extraction of the stem bark of *Croton oblongifolius* is shown in Table 3.

Table 3 The results from the extraction of the stem bark of *Croton oblongifolius*

Solvent extract	Appearence	Weight(g)	%wt/wt of the dried stem bark
Hexane	Yellowish brown oil	85	3.40
Ethyl acetate	Dark brown oil	132	5.28
Methanol	Dark red oil	35	1.40

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4.2 Results of hexane crude extraction.

The hexane crude extract (50g) was separated by column chromatographic techniques using hexane–ethyl acetate gradient in a stepwise fashion. The results from separation of hexane crude extract are summarized in Table 4.

Table 4 Results from separation of hexane crude extract

Fraction Number	Eluents	Appearance	Weight (g)
1	100% Hexane	Colorless transparent liquid	0.39
2	5% EtOAc in Hexane	Yellowish transparent liquid	0.60
3	10% EtOAc in Hexane	Yellowish viscous liquid	4.71
4	10% EtOAc in Hexane	Yellowish viscous liquid	5.33
5	20% EtOAc in Hexane	Yellowish solid	15.41
6	20% EtOAc in Hexane	Yellowish liquid	1.67
7	30% EtOAc in Hexane	Yellowish-green viscous liquid	1.54
8	30% EtOAc in Hexane	Greenish viscous liquid	1.32
9	40% EtOAc in Hexane	Yellowish viscous liquid	0.63
10	70% EtOAc in Hexane	Brown viscous liquid	1.66
11	80% EtOAc in Hexane	Dark brown gummy	1.14
12	100% EtOAc in Hexane	Dark brown gummy	2.88

The fractions from hexane crude extract of the stem bark of *C. oblongifolius* were separated by silica gel column chromatography. The results of purified compounds are summarized in Table 5.

Table 5 Results from the separation of the hexane crude extract of *C. oblongifolius* by column chromatography

Compounds	Physical appearance	Weight (g)	% wt/wt from dried stem bark
1	White needle crystal	0.282	0.0113
2	Yellowish viscous transparent oil	0.115	0.0046
3	White solid	8.780	0.3512
4	Yellowish viscous transparent oil	0.512	0.0200
5	Greenish viscous transparent oil	0.257	0.0100

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4.3 Purification and properties of the compounds from *Croton oblongifolius*

4.3.1 Purification and properties of the compound 1

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 4. After that it was re-columned by column chromatography (Merck's silica gel Art 1.09385.100) to obtain the mixture of compounds 1 and 2. Compound 1 was crystallized from fraction of the mixture, and it was recrystallized in hexane to obtain the needle–like white crystalline solid (282 mg, 0.564 % yield from crude hexane and 0.0113 % yield from dried stem bark); mp. 108–110 °C; $[\alpha]_D^{20}$ +12.3°(*c* 0.18, $CHCl_3$), UV ($CHCl_3$) λ_{max} 248 sh (log ε 4.21)

This compound was found to dissolve in chloroform, ethyl acetate, methanol and ethanol. The R_f value was found to be 0.45 (10 % EtOAc in hexane).

FT-IR spectrum (KBr) (Fig. 12 and Table 6), ν_{max} (cm^{-1}): 3500–2400 (br), 2963 and 2911 (m), 1680 (s), 1635 (m) and 1443 (w).

^{1}H–NMR spectrum ($CDCl_3$, 200.13 MHz) (Fig. 13 and Table 7) δ (ppm): 6.01 (1H, m), 5.98 (1H, m), 5.89 (1H, m), 5.08 (1H, m), 2.69 (2H, m), 2.39 (4H, m), 2.31 (1H, m), 2.18 (2H, m), 2.15 (4H, m), 1.73 (3H, s), 1.54 (3H, s), 1.03 (3H, d, *J*=6.85 Hz) and 1.00(3H, d, *J*=6.85 Hz)

^{13}C–NMR spectrum ($CDCl_3$, 200.13 MHz) (Fig. 14 and Table 8) 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 (2xq), 17.0 (q) and 15.8 (q).

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).

4.3.2 Purification and properties of the 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 4. After that it was re-columned by column chromatography (Merck's silica gel Art 1.09385.100) to obtain the mixture of compounds 1 and 2. Compound 2 was obtained from mother liquor after removal of crystal of compound 1 from the mixture of compound 1 and 2. Compound 2 was yellowish viscous transparent oil (115 mg, 0.230 % yield from crude hexane and 0.0046 % yield from dried stem bark); $[\alpha]_D^{20}$ +3.2^O(*c* 0.30, $CHCl_3$), UV($CHCl_3$) λ_{max} 248 sh (log ε 4.31).

This compound was found to dissolve in chloroform, ethyl acetate, methanol and ethanol. The R_f value was found to be 0.40 (10 % EtOAc in hexane).

FT-IR spectrum (KBr) (Fig. 17 and Table 9), 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. 18 and Table 10) δ (ppm): 6.89 (1H, t, *J*=8.0 Hz), 6.01 (1H, d, *J*=11.0 Hz), 5.91 (1H, m), 5.14 (1H, m), 2.40 (1H, m), 2.37 (2H, m), 2.36 (2H, m), 2.26 (2H, m), 2.24 (2H, m), 2.21 (2H, m), 2.17 (2H, m), 1.71 (3H, s), 1.69 (3H, s), 1.07 (3H, d, *J*=6.79 Hz).and 1.03 (3H, d, *J*=6.79 Hz).

^{13}C–NMR spectrum ($CDCl_3$, 200.13 MHz) (Fig. 19 and Table 11) 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).

EI-MS spectrum (Fig. 21) *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).

4.3.3 Purification and properties of the compound 3

The hexane crude extract was separated by 20 % ethyl acetate in hexane. Similar fractions were combined and the solvents were removed by evaporation to obtain the fraction number 5 and it was further purified by column chromatography (Merck's silica gel Art 1.09385.100). The column was eluted with 10–20 % ethyl acetate in hexane. The white solid of compound 3 was obtained. (8.78 g, 17.56 % yield from crude hexane and 0.3512 % yield from dried stem bark); mp. 93–95 ^{O}C; $[\alpha]_D^{20}$ –45.9^{O}(*c* 0.333, $CHCl_3$), UV ($CHCl_3$) λ_{max} 242 sh (log ε 2.89), Calcd. C 76.02%, H 8.93%

This compound is soluble in chloroform, ethyl acetate, hot hexane, methanol and ethanol. The R_f value was found to be 0.575 (25 % EtOAc in hexane).

FT-IR spectrum (KBr) (Fig. 22 and Table 12),ν_{max} (cm^{-1}): 3500–2400 (br), 2966 2945 and 2875 (s), 1708 (s) and 1465 (m).

^{1}H–NMR spectrum ($CDCl_3$, 500.00 MHz) (Fig. 23 and Table 13) δ (ppm): 7.32 (1H, dd, *J*=1.53, 1.83 Hz), 7.19 (1H, dd, *J*=0.92, 1.53 Hz), 6.25 (1H, dd, *J*= 0.91, 1.83 Hz), 2.35 (1H, m), 2.12 (1H, m), 2.07 (1H, m), 2.00 (1H, m), 1.95 (2H, m), 1.90 (1H, m), 1.78 (1H, m), 1.72 (1H, m), 1.65 (2H, m), 1.62 (1H, m), 1.53 (1H, m), 1.50 (1H, m), 1.37 (1H, m), 1.28 (3H, s), 0.86 (3H, d, *J*= 7.02 Hz) and 0.84 (3H,s).

^{13}C–NMR spectrum ($CDCl_3$, 500.00 MHz) (Fig. 24 and Table 13) δ (ppm): 184.7 (s), 142.6 (d), 138.4 (d), 135.9 (s), 131.0 (s), 125.8 (s), 111.0 (d), 47.4 (s), 40.8 (s), 36.4 (t), 35.4 (t), 33.2 (d), 26.7 (t), 25.9 (t), 25.1 (t), 22.8 (q), 20.8 (q), 19.4 (2xt) and 16.0 (q).

EI-MS spectrum (Fig. 30) *m/z*: 316 [M^+, 1 %], 221 [M^+-$C_6H_7O^+$, (70)], 175 (45), 119 (40), 105 (42), 91 (50) and 81 (100).

4.3.4 Purification and properties of the compound 4

The hexane crude extract was separated by 30 % ethyl acetate in hexane. Similar fractions were combined, and the solvents were removed by rotary evaporation to obtain the fraction number 6 and 7. Then, both fractions were further purified several time by column chromatography (Merck's silica gel Art 1.09385.100). The column was eluted with 28 % ethyl acetate in hexane to obtain the yellowish viscous transparent oil (512 mg, 1.024 % yield from crude hexane and 0.02% yield from dried stem bark); $[\alpha]_D^{20}$ –75.8^O(*c* 0.333, $CHCl_3$), UV($CHCl_3$) λ_{max} 242 sh (log ε 3.97).

This compound dissolved in chloroform, ethyl acetate, methanol and ethanol. The R_f value was found to be 0.50 (30 % EtOAc in hexane).

FT-IR spectrum (KBr) (Fig. 31 and Table 14), ν_{max} (cm^{-1}): 3600–3100 (br), 2933 (s), 1715 and 1683 (s), 1632 (m) and 1272 (s).

^{1}H–NMR spectrum ($CDCl_3$, 200.13 MHz) (Fig. 32 and Table 15) δ (ppm): 8.00 (2H, d, *J*=1.63 Hz), 7.55 (1H, dd, *J*=5.90, 5.90 Hz), 7.45 (2H, d, *J*=7.61, 7.61 Hz), 7.34 (1H, d, *J*=1.53 Hz), 7.21 (1H, s), 6.91 (1H, m), 6.26 (1H, d, *J*=1.53 Hz), 4.50 (1H, d, *J*=11.78 Hz), 4.31 (1H, d, *J*=11.78 Hz), 2.53–1.65 (11H, m), 1.58 (1H, m), 1.52 (1H, m), 1.30 (3H, s), 1.23 (1H, m), 1.00 (3H, d, *J*=6.71 Hz)

^{13}C–NMR spectrum ($CDCl_3$, 200.13 MHz) (Fig. 33 and Table 16) δ (ppm): 172.3 (s), 166.9 (s), 142.9 (d), 141.0 (s), 140.6 (d),138.5 (d), 133.0 (s), 130.3 (s), 129.5 (2xd), 128.5 (2xd), 125.2 (s), 111.0 (d), 67.8 (t), 47.4 (d), 42.3 (s), 37.7 (s), 36.4 (d), 36.0 (t), 32.4 (t), 28.1 (t), 27.2 (t), 20.2 (q), 19.2 (t), 17.9 (t) and 17.0 (q).

EI-MS spectrum (Fig. 35) *m/z*: 436 [M^+, 2 %], 341 [M^+-$C_6H_7O^+$, (6)], 314 (10), 219 (10), 175 [219-COO^+, (8)], 125 (13), 105 [$PhCO^+$, (100)], 95 (45), 77 [Ph^+, (30)].

4.3.5 Purification and properties of the compound 5

The hexane crud extract was separated by 30-40 % ethyl acetate in hexane. Similar fractions were combined and the solvents were removed by rotary evaporation to obtain the fraction number 8 and 9. Then, the both fractions were further purified several time by column chromatography (Merck's silica gel Art 1.09385.100). The column was eluted with 30 % ethyl acetate in hexane to obtain the greenish viscous oil.(257 mg, 0.514 % yield from crude hexane and 0.01% yield from dried stem bark); $[\alpha]_D^{20}$ –10.5^O(*c* 0.333, $CHCl_3$), UV($CHCl_3$) λ_{max} 242 sh (log ε 4.38), Calcd. C 68.42 %, H 6.38 %.

This compound was found to dissolve in chloroform, ethyl acetate, methanol and ethanol. The R_f value was found to be 0.40 (30 % EtOAc in hexane).

FT-IR spectrum (KBr) (Fig. 36 and Table 17), ν_{max} (cm^{-1}): 3452 (m), 2938 (s), 1714 (s), 1613 (m), 1457 (m), 1384 (m) and 1276 (s).

^{1}H–NMR spectrum ($CDCl_3$, 200.13 MHz) (Fig. 37 and Table 18) δ (ppm): 8.04 (1H, d, *J*=1.50 Hz), 8.00 (1H, d, *J*=1.60 Hz), 7.56-7.38 (3H, m), 6.41 (2H, s), 4.33 (2H, t, *J*=6.40 Hz), 3.83 (6H, s), 2.70 (2H, t, *J*=7.5 Hz) and 2.10 (2H, m)

^{13}C–NMR spectrum ($CDCl_3$, 200.13 MHz.) (Fig. 38 and Table 18) δ (ppm): 166.6 (s)147.0 (2xs), 133.0 (d), 132.2 (2xs), 130.3 (s), 129.5 (2xd), 128.3 (2xd), 105 (2xd), 63.4 (t), 56.2 (2xq), 32.5 (t) and 30.5 (t).

EI-MS spectrum (Fig. 40) *m/z*: 316 [M^+, 67 %], 211 (5), 194 (80), 167 (70), 163 (50), 105 [$PhCO^+$, (100)], 91 (22), 77 [Ph^+, (60)].