

CHAPTER 2

EXPERIMENTAL

2.1 Instruments and Chemicals

Melting point was recorded in °C and was measured on an Electrothermal Melting Point Apparatus. Infrared spectra were recorded using FTS FT-IR spectrophotometer and major bands (ν) were recorded in wave number (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded as wavelengths (nm) and $\log \epsilon$ in chloroform solution. Nuclear magnetic resonance spectra were recorded on 500 MHz Varian UNITY INOVA spectrometer. Spectra were recorded in deuteriochloroform solution and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). Single-crystal X-ray diffraction measurements were collected using SMART 1-K CCD diffractometer with monochromated Mo-K α radiation ($\lambda = 0.71073 \text{ \AA}$) using ω -scan mode and SHELXTL for structure solution and refinement. Optical rotation was measured in chloroform solution with sodium D line (590 nm) on an AUTOPOL^R II automatic polarimeter. Solvent for extraction and chromatography were distilled at their boiling point ranges prior to use except diethyl ether was analytical grade reagent. Quick column chromatography was performed on silica gel 60 GF₂₅₄ (Merck). Column chromatography was performed on silica gel (Merck) type 100 (0.063 – 0.200). Precoated plates of silica gel 60 GF₂₅₄ or reversed-phase C₁₈ were used for analytical purposes.

2.2 Plant material

Seeds of *C. odollam* were collected from Penang, Malaysia. Barks and latex of *C. odollam* were collected from Trang Province, Thailand. The plant was identified by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and the voucher specimen was deposited in the herbarium.

2.3 Extraction

2.3.1 Fresh seeds (940 g) of *C. odollam* were extracted twice with methylene chloride (2.5 l), over the period of 5 days each at room temperature. The mixture was filtered and concentrated under reduced pressure, some white solid (0.3085 g) precipitated which was filtered. The filtrate was further evaporated to dryness to afford crude methylene chloride extract (105.50 g) as a yellow oil.

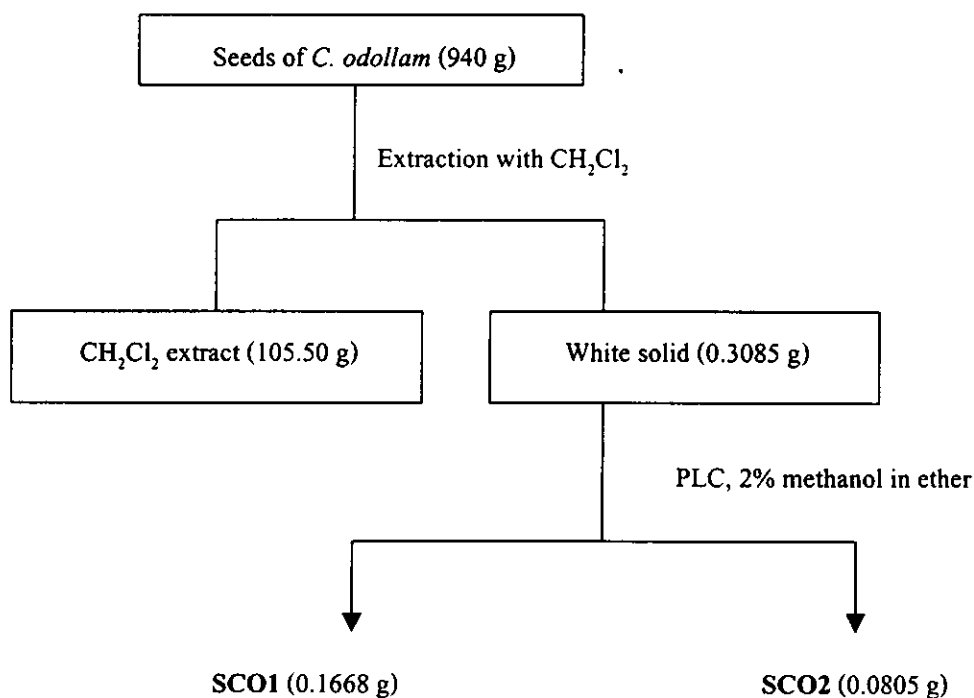
2.3.2 Fresh latex of *C. odollam* was stirred in acetone at room temperature for 1-2 hours and then filtered, evaporated under reduced pressure and finally partition with H₂O:EtOAc to give ethyl acetate extract (6 g).

2.3.3 Air-dried barks (1520 g) of *C. odollam* were extracted twice with methylene chloride (12.5 l), over the period of 5 days each at room temperature. The filtered solution was then evaporated to dryness under reduced pressure to afford crude methylene chloride extract (6 g).

2.4 Isolation and Chemical Investigation

2.4.1 Investigation of the crude methylene chloride extract from the seeds of *C. odollam*

The white solid from the methylene chloride extract (0.3085 g) was purified by PLC using 2% methanol in ether as eluent to yield two compounds, **SCO1**, as a white solid (0.1668 g, $R_f = 0.19$, 30% acetone: hexane) and **SCO2**, as a white solid (0.0805 g, $R_f = 0.38$, 30% acetone: hexane), as shown in **Scheme 1**.



Scheme 1 Extraction and isolation of compounds **SCO1** and **SCO2** from the seeds of *C. odollam*

Compound SCO1 (White solid) $[\alpha]_D^{26}$: -48.78° ($c = 0.0041$, CHCl_3)

mp: 220-224 °C

UV (CHCl_3) λ_{max} (nm) ($\log \epsilon$): 244 (3.22)IR (KBr) $\nu(\text{cm}^{-1}) = 3481$ (O-H stretching), 2927 (C-H stretching), 1728 (C=O stretching), 1679 (C=C stretching) $^1\text{H NMR}$ (CDCl_3) (δ ppm) (500 MHz): 5.87 (1H, *s*), 4.97 (1H, *dd*, $J = 18.5, 1.5$ Hz), 4.83 (1H, *d*, $J = 3.5$ Hz), 4.79 (1H, *dd*, $J = 18.5, 1.5$ Hz), 3.95 (1H, *br s*), 3.71 (1H, *dq*, $J = 6, 9$ Hz), 3.66 (3H, *s*), 3.53 (1H, *dd*, $J = 3.5, 9$ Hz), 3.24 (1H, *t*, $J = 9$ Hz), 3.10 (1H, *t*, $J = 9$ Hz), 2.77 (1H, *dd*, $J = 5, 9$ Hz), 1.22 (3H, *d*, $J = 6$ Hz), 0.94 (3H, *s*), 0.86 (3H, *s*) $^{13}\text{C NMR}$ (CDCl_3) (δ ppm) (125 MHz): 175.06, 175.04, 117.48, 97.15, 85.24, 84.55, 74.68, 73.60, 73.39, 72.66, 67.60, 60.54, 50.90, 49.65, 41.65, 39.95, 36.85, 35.66, 35.19, 32.91, 30.55, 29.91, 26.89, 26.55, 26.44, 23.78, 21.29, 21.14, 17.40, 15.71**DEPT-135° (CDCl_3)**

CH: 117.48, 97.15, 85.24, 74.68, 73.39, 72.66, 67.60, 50.90, 41.65, 36.85, 35.66

CH_2 : 73.60, 39.95, 32.91, 30.55, 29.91, 26.89, 26.55, 26.44, 21.29, 21.14

CH_3 : 60.54, 23.78, 17.40, 15.71

Compound SCO2 (White solid)

$[\alpha]_D^{26}$: -90.90° ($c = 0.0044$, CHCl_3)

mp: 204-206 $^\circ\text{C}$

UV (CHCl_3) λ_{max} (nm) ($\log \epsilon$): 242 (3.32)

IR (KBr) ν (cm^{-1}): 3473 (O-H stretching), 2943 (C-H stretching), 1746 (C=O stretching), 1717 (C=O stretching), 1632 (C=C stretching)

^1H NMR (CDCl_3) (δ ppm) (500 MHz): 5.88 (1H, *s*), 5.05 (1H, *d*, $J = 4$ Hz), 5.00 (1H, *dd*, $J = 18.5, 2$ Hz), 4.82 (1H, *dd*, $J = 18.5, 2$ Hz), 4.63 (1H, *dd*, $J = 4, 9$ Hz), 3.87 (1H, *br s*), 3.79 (1H, *dq*, $J = 6, 9$ Hz), 3.58 (3H, *s*), 3.58 (1H, *t*, $J = 9$ Hz), 3.21 (1H, *t*, $J = 9$ Hz), 2.78 (1H, *dd*, $J = 5, 9$ Hz), 2.07 (3H, *s*), 1.25 (3H, *d*, $J = 6$ Hz), 0.95 (3H, *s*), 0.87 (3H, *s*)

^{13}C NMR (CDCl_3) (δ ppm) (125 MHz): 174.69, 174.68, 170.27, 117.61, 93.73, 85.49, 80.93, 75.33, 74.30, 73.48, 72.25, 67.03, 60.49, 50.92, 49.62, 41.82, 40.00, 36.47, 35.65, 35.19, 33.12, 30.36, 29.60, 26.88, 26.62, 26.59, 23.89, 21.34, 21.19, 20.89, 17.54, 15.76

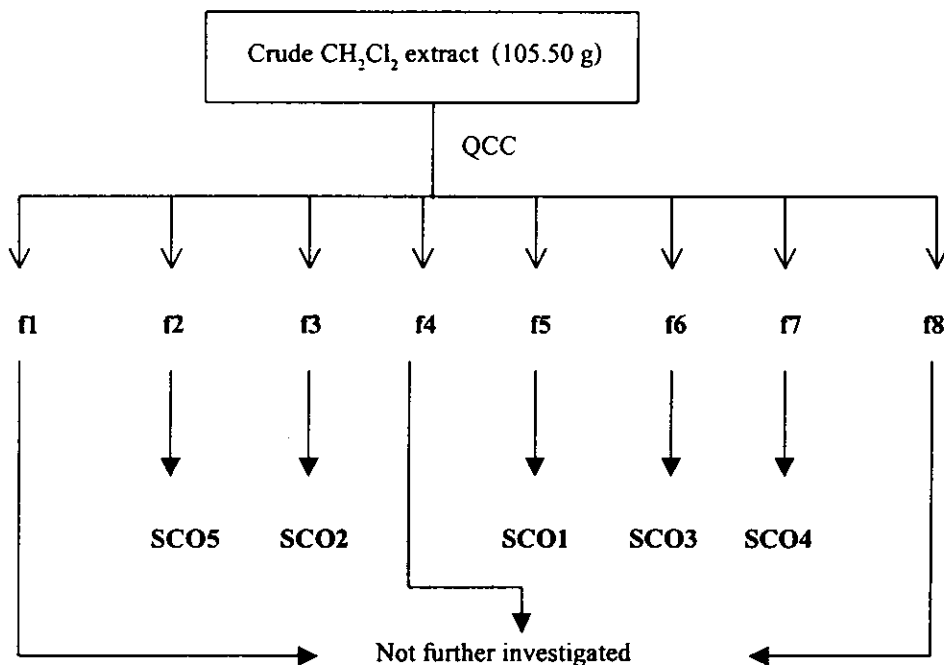
DEPT-135° (CDCl₃)

CH: 117.61, 93.73, 80.93, 75.33, 74.30, 72.25, 67.03, 50.92, 41.82, 36.47, 35.65

CH₂: 73.48, 40.00, 33.12, 30.36, 29.60, 26.88, 26.62, 26.59, 21.34, 21.19

CH₃: 60.49, 23.89, 20.89, 17.54, 15.76

The methylene chloride extract of seeds of *C. odollam* (yellow oil, 105.50 g) was subjected to chromatography on silica gel (QCC) using hexane as eluent and increasing polarity with ether and methanol, respectively, to give 8 fractions.



Scheme 2 Isolation of compounds SCO1, SCO2, SCO3, SCO4 and SCO5

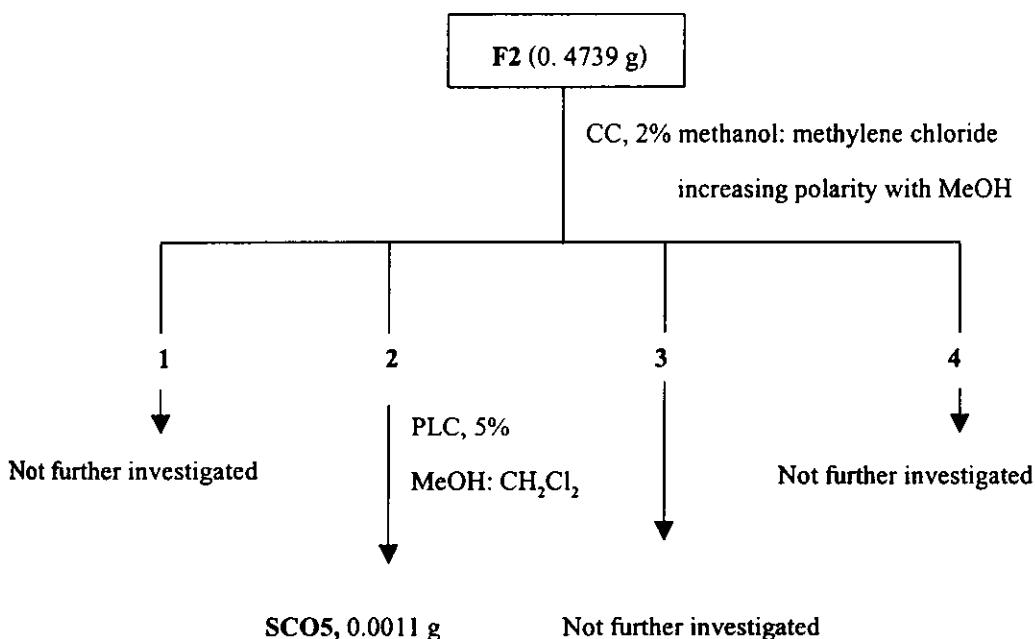
Fraction f3, as a solid, upon crystallization with methanol: methylene chloride gives **SCO2** (0.3357 g).

Fraction f5, as a solid, upon crystallization with methanol: methylene chloride gives **SCO1** (0.0335 g).

Fraction f6 (0.1222 g), as a solid, was purified by PLC using 1% methanol in ether as eluent to give **SCO3** (0.005 g) as a white solid ($R_f = 0.25$, 80% diethyl ether: methylene chloride).

Fraction f7, as a solid (0.1203 g), was purified by PLC using 50% ether in methylene chloride to give **SCO4** (0.0101 g) as a white solid ($R_f = 0.24$, 3% methanol: methylene chloride).

Fraction f2 (0.4739 g) as a solid was subjected to repeated chromatography (CC and PLC) to give compound **SCO5** (0.0011 g) as a white solid ($R_f = 0.24$, 3% methanol: methylene chloride), as shown in **Scheme 3**.



Scheme 3 Isolation of compounds **SCO5**

Compound SCO3 (White solid)

$[\alpha]_D^{26}$: -111.11° ($c = 0.0044$, CHCl₃)

mp: 215-218 °C

UV (CHCl₃) λ_{max} (nm) (log ϵ): 242 (3.04)

IR (KBr) $\nu(\text{cm}^{-1})$: 3415 (O-H stretching), 2926 (C-H stretching), 1735 (C=O stretching), 1638 (C=C stretching)

¹H NMR (CDCl₃) (δ ppm) (500 MHz): 5.89 (1H, *t*, $J = 1.5$ Hz), 4.98 (1H, *dd*, $J = 18.5, 1.5$ Hz), 4.86 (1H, *d*, $J = 4$ Hz), 4.80 (1H, *dd*, $J = 18.5, 1.5$ Hz), 3.97 (1H, *br s*), 3.75 (1H, *dq*, $J = 6, 9$ Hz), 3.69 (3H, *s*), 3.59 (1H, *dt*, $J = 4, 9$ Hz), 3.25 (1H, *t*, $J = 9$ Hz), 3.15 (1H, *t*, $J = 9$ Hz), 2.79 (1H, *dd*, $J = 5, 9$ Hz), 1.25 (3H, *d*, $J = 6$ Hz), 0.96 (3H, *s*), 0.88 (3H, *s*)

¹³C NMR (CDCl₃) (δ ppm) (125 MHz): 174.46, 174.40, 117.76, 97.18, 85.55, 84.62, 74.70, 73.42, 73.28, 72.94, 67.48, 60.63, 50.87, 49.57, 41.81, 39.98, 36.90, 35.69, 35.24, 33.17, 30.61, 29.92, 26.86, 26.55, 26.50, 23.89, 21.34, 21.17, 17.52, 15.76

DEPT -135°(CDCl₃)

CH: 117.76, 97.18, 84.62, 74.70, 73.28, 72.94, 67.48, 50.87, 41.81, 36.90, 35.69

CH₂: 73.42, 39.98, 33.17, 30.61, 29.92, 26.86, 26.55, 26.50, 21.34, 21.17

CH_3 : 60.63, 23.89, 17.52, 15.76

Compound SCO4 (White solid)

$[\alpha]_D^{26}$: -17.85° ($c = 0.0056$, CHCl_3)

mp: 196-198 $^\circ\text{C}$

UV (CHCl_3) λ_{max} (nm) ($\log \mathcal{E}$): 244 (3.18)

IR (KBr) ν (cm^{-1}): 3415 (O-H stretching), 2922 (C-H stretching), 1720 (C=O stretching), 1638 (C=C stretching)

^1H NMR (CDCl_3) (δ ppm) (500 MHz): 5.70 (1H, *t*, $J = 1.5$ Hz), 4.84 (1H, *d*, $J = 3.5$ Hz), 4.70 (1H, *dd*, $J = 16.5, 1.5$ Hz), 4.55 (1H, *dd*, $J = 16.5, 1.5$ Hz), 3.96 (1H, *br m*), 3.73 (1H, *dq*, $J = 6, 9$ Hz), 3.69 (3H, *s*), 3.58 (1H, *dt*, $J = 4, 9$ Hz), 3.24 (1H, *t*, $J = 9$ Hz), 3.15 (1H, *t*, $J = 9$ Hz), 3.09 (1H, *d*, $J = 7$ Hz), 2.86 (1H, *m*), 1.25 (3H, *d*, $J = 6$ Hz), 0.95 (3H, *s*), 0.80 (3H, *s*)

^{13}C NMR (CDCl_3) (δ ppm) (125 MHz): 220.90, 173.68, 170.55, 116.75, 97.48, 84.68, 74.79, 73.44, 73.03, 72.89, 67.61, 60.77, 53.21, 48.81, 47.44, 45.91, 44.16, 42.63, 37.46, 37.37, 32.00, 30.28, 29.01, 27.03, 26.78, 26.59, 24.10, 23.43, 21.41, 17.62

DEPT -135°(CDCl₃)

CH: 116.75, 97.48, 84.68, 74.79, 73.44, 73.03, 67.61, 53.21, 45.91, 37.46

CH₂: 72.89, 44.16, 42.63, 32.00, 30.28, 29.01, 27.03, 26.78, 24.10, 21.41

CH₃: 60.77, 26.59, 23.43, 17.62

Compound SCO5 (White solid)

$[\alpha]_D^{26}$: -62.50° ($c = 0.0016$, CHCl₃)

mp: 209-211 °C

UV (CHCl₃) λ_{max} (nm) (log ϵ): 243 (3.22)

IR (KBr) ν (cm⁻¹): 3414 (O-H stretching), 2922 (C-H stretching), 1744 (C=O stretching), 1733 (C=O stretching), 1638 (C=C stretching)

¹H NMR (CDCl₃) (δ ppm) (500 MHz): 5.69 (1H, *t*, $J = 1.5$ Hz), 5.05 (1H, *d*, $J = 2$ Hz), 4.69 (1H, *dd*, $J = 18, 1.5$ Hz), 4.65 (1H, *dd*, $J = 4, 9$ Hz), 4.57 (1H, *dd*, $J = 18, 1.5$ Hz), 3.88 (1H, *br s*), 3.79 (1H, *dq*, $J = 6, 9$ Hz), 3.58 (3H, *s*), 3.57 (1H, *t*, $J = 9$ Hz), 3.21 (1H, *t*, $J = 9$ Hz), 3.10 (1H, *d*, $J = 7$ Hz), 2.85 (1H, *m*), 1.27 (3H, *d*, $J = 6$ Hz), 0.97 (3H, *s*), 0.89 (3H, *s*)

^{13}C NMR (CDCl_3) (δ ppm) (125 MHz): 220.93, 173.56, 170.45, 170.25, 116.63, 93.85, 80.83, 75.28, 74.26, 72.77, 72.15, 66.98, 60.55, 53.10, 48.75, 47.35, 45.81, 44.04, 42.57, 37.26, 36.88, 31.65, 29.80, 28.96, 26.92, 26.82, 26.52, 24.03, 23.33, 21.31, 20.90, 17.58

DEPT -135°(CDCl_3)

CH: 116.63, 93.85, 80.83, 75.28, 74.26, 72.15, 66.98, 53.10, 45.81, 36.88

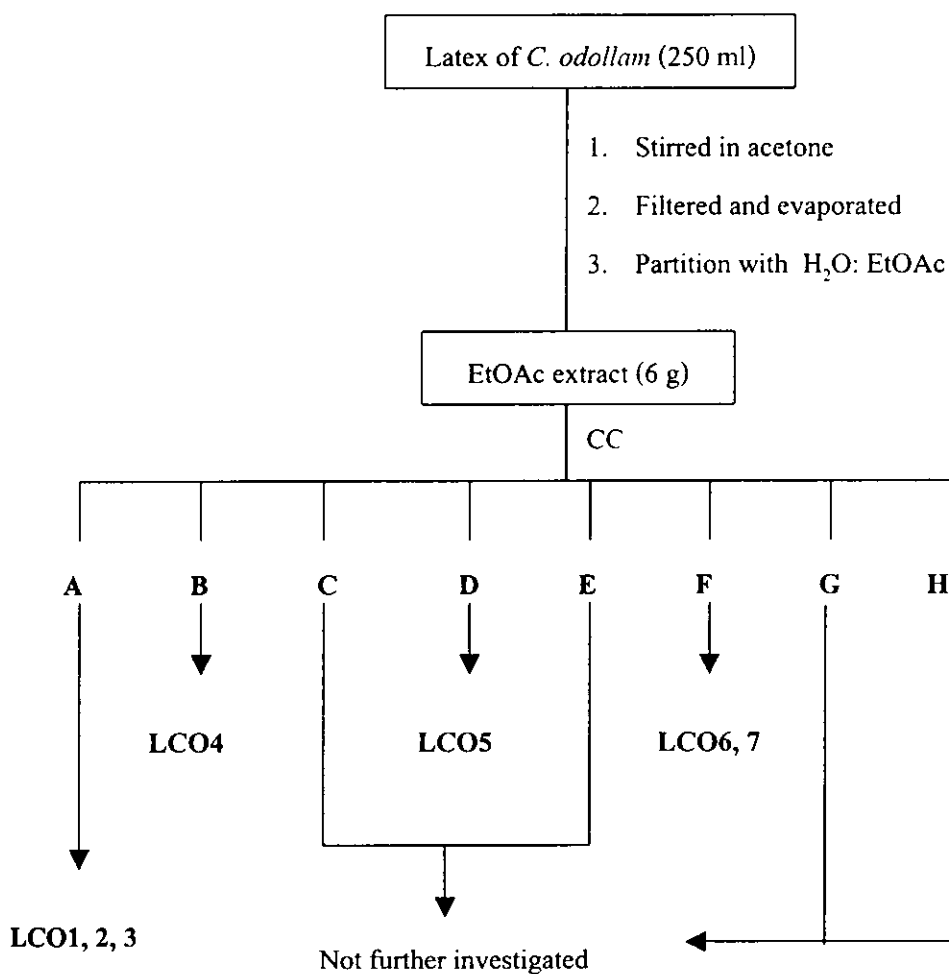
CH₂: 72.77, 44.04, 42.57, 31.65, 29.80, 28.96, 26.92, 26.82, 24.03, 21.31

CH₃: 60.55, 26.52, 23.33, 20.90, 17.58

2.4.2 Investigation of the crude ethyl acetate extract from latex of

C. odollam

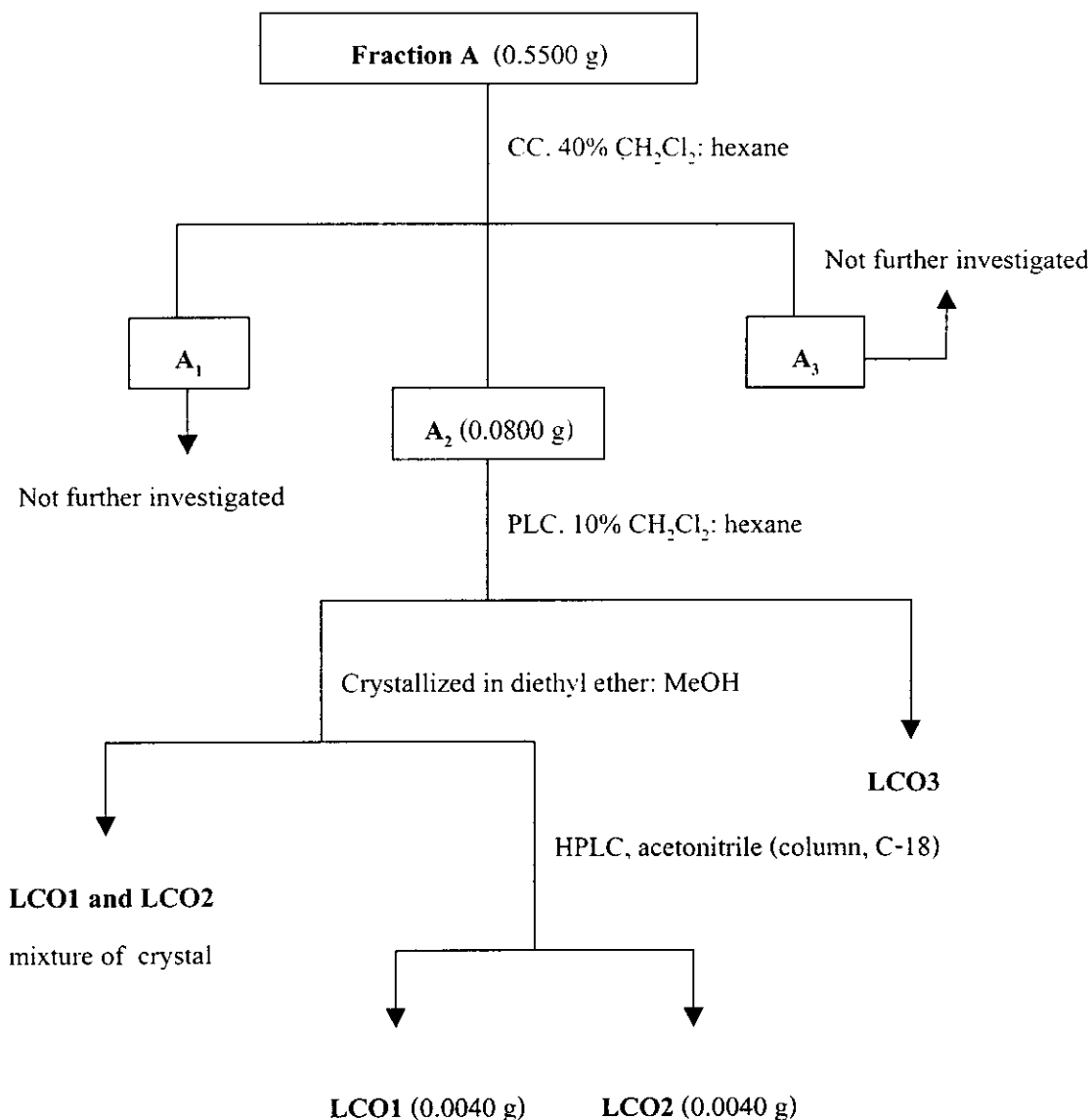
The ethyl acetate extract (6 g) was chromatographed on silica gel using hexane as eluent and increasing polarity with acetone to give 8 fractions. Fractions **A**, **B**, **D**, **F** and **G** were further purified by chromatography and crystallization. Fractions **C**, **E** and **H** were not further investigated, as shown in **Scheme 4**.



Scheme 4 Extraction and isolation of compounds LCO1- LCO9 from latex of

C. odollam

Fraction A (0.5500 g) was rechromatographed on silica gel using 40% methylene chloride:hexane as eluent to give 3 subfractions, as shown in **Scheme 5**.



Scheme 5 Isolation of compounds **LCO1-LCO3**

Subfraction A₂ (0.0800 g) was purified using 10% methylene chloride:hexane as eluent to give compound **LCO3**, as a colorless needles (0.0120 g, $R_f = 0.12$,

15% methylene chloride:hexane) and the mixture of compounds **LCO1** and **LCO2** (0.0300 g). The mixture of compounds **LCO1** and **LCO2** were further purified by HPLC using Hypersil ODS column with RI detector and acetonitrile as mobile phase (flow rate 1.5 ml/min) to give compound **LCO1** (0.0040 g, retention time =14.63 min) and compound **LCO2** (0.0040 g, retention time = 15.68 min).

Compound LCO1 (White solid)

$[\alpha]_D^{29}$: -71.42° ($c = 0.0014$, CHCl_3)

mp: 214-217 °C

IR (KBr) $\nu(\text{cm}^{-1})$: 2945 (C-H stretching), 1733 (C=O stretching)

$^1\text{H NMR}$ (CDCl_3) (δ ppm) (500 MHz): 5.13 (1H, *t*, $J = 3.5$ Hz), 4.51 (1H, *dd*, $J = 6, 10.5$ Hz), 2.05 (3H, *s*), 1.06 (3H, *s*), 1.01 (3H, *s*), 0.98 (3H, *s*), 0.95 (3H, *d*, 6.0 Hz), 0.87 (3H, *s*), 0.86 (3H, *s*), 0.80 (3H, *s*), 0.80 (3H, *d*, $J = 5.5$ Hz)

$^{13}\text{C NMR}$ (CDCl_3) (δ ppm) (125 MHz): 171.01, 139.62, 124.30, 80.94, 59.04, 55.24, 47.62, 42.05, 41.52, 40.01, 39.63, 39.59, 38.44, 37.70, 36.77, 33.73, 32.85, 31.23, 28.73, 28.07, 28.05, 26.58, 23.59, 23.35, 23.21, 21.38, 21.31, 18.22, 17.49, 16.85, 16.72, 15.72

DEPT -135° (CDCl₃)

CH: 124.30, 80.94, 59.04, 55.24, 47.62, 39.63, 39.59

CH₂: 41.52, 38.44, 32.85, 31.23, 28.07, 26.58, 23.59, 23.35, 18.22

CH₃: 28.73, 28.05, 23.21, 21.38, 21.31, 17.49, 16.85, 16.72, 15.72

Compound LCO2 (White solid)

$[\alpha]_D^{29}$: +69.76° ($c = 0.0043$, CHCl₃)

mp: 194-196 °C

IR (KBr) ν (cm⁻¹): 2945 (C-H stretching), 1733 (C=O stretching)

¹H NMR (CDCl₃) (δ ppm) (500 MHz): 5.19 (1H, *t*, $J = 3.5$ Hz), 4.50 (1H, *dd*, $J = 6, 10$ Hz), 2.05 (3H, *s*), 1.13(3H, *s*), 0.97 (3H, *s*), 0.96 (3H, *s*), 0.87 (4 x CH₃, each *s*), 0.83 (3H, *s*)

¹³C NMR (CDCl₃) (δ ppm) (125 MHz): 170.99, 145.24, 121.68, 80.97, 55.30, 47.60, 47.28, 46.83, 41.75, 39.85, 38.31, 37.74, 37.18, 36.88, 34.77, 33.34, 32.63, 32.51, 31.61, 28.41, 28.06, 26.97, 26.17, 25.96, 23.71, 23.60, 23.56, 21.31, 18.30, 16.84, 16.71, 15.57

DEPT -135° (CDCl₃)**CH:** 121.68, 80.97, 55.30, 47.60, 47.28**CH₂:** 46.83, 38.31, 37.18, 34.77, 32.63, 26.97, 26.17, 23.60, 23.56, 18.30**CH₃:** 33.34, 28.41, 28.06, 25.96, 23.71, 21.31, 16.84, 16.71, 15.57**Compound LCO3 (White solid)****[α]_D²⁹:** +31.9 ° (*c* = 0.0094, CHCl₃)**mp:** 184-187 °C**IR (KBr) ν(cm⁻¹):** 2930 (C-H stretching), 1733 (C=O stretching)**¹H NMR (CDCl₃) (δ ppm) (500 MHz):** 4.68 (1H, *d*, *J* = 2.5 Hz), 4.57 (1H, *m*), 4.47 (1H, *dd*, *J* = 6, 10 Hz), 2.37 (H, *dt*, *J* = 6, 11.5 Hz), 2.04 (3H, *s*), 1.68 (3H, *s*), 1.03(3H, *s*), 0.93 (3H, *s*), 0.85 (3H, *s*), 0.84 (3H, *s*) 0.83 (3H, *s*), 0.78 (3H, *s*)**¹³C NMR (CDCl₃) (δ ppm) (125 MHz):** 170.99, 150.94, 109.34, 80.95, 55.36, 50.32, 48.26, 47.98, 42.97, 42.80, 40.82, 39.97, 38.37, 38.01, 37.78, 37.06, 35.55, 34.19, 29.81, 27.92, 27.41, 25.07, 23.69, 21.31, 20.92, 19.27, 18.18, 17.98, 16.47, 16.16, 15.95, 14.48

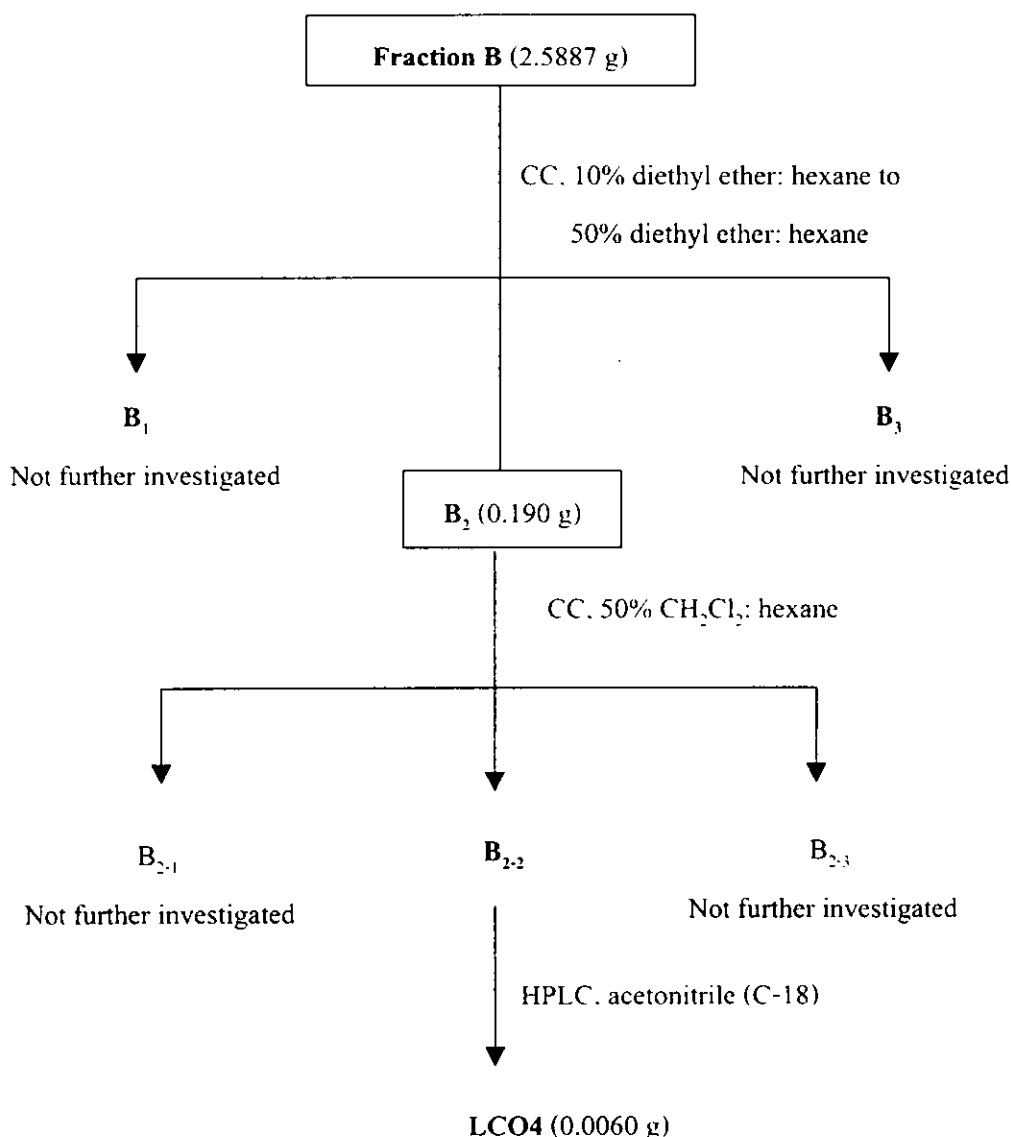
DEPT -135° (CDCl₃)

CH: 80.95, 55.36, 50.32, 48.26, 47.98, 38.01

CH₂: 109.34, 39.97, 38.37, 35.55, 34.19, 29.81, 27.41, 25.07, 23.69, 20.92, 18.18

CH₃: 27.92, 21.31, 19.27, 17.98, 16.47, 16.16, 15.95, 14.48

Fraction B (2.5887 g) was rechromatographed on silica gel using 10% diethyl ether: hexane as eluent and increasing polarity with diethyl ether to 50% diethyl ether: hexane to afford 3 subfractions. Subfraction **B₂** (0.1900 g) was rechromatographed on silica gel using 50% methylene chloride: hexane to give 3 subfractions. Subfraction **B₂₋₂** was purified by HPLC using Hypersil ODS column with RI detector and acetonitrile as mobile phase (flow rate 1.5 ml/min) to give compound **LCO4** (0.0060 g) as a viscous liquid ($R_f = 0.18$, 60% methylene chloride: hexane), as shown in **Scheme 6**.



Scheme 6 Isolation of compound LCO4

Compound LCO4 (White solid)

$[\alpha]_D^{25}$: +11.23° ($c = 0.0089$, CHCl₃)

IR (Neat) ν (cm⁻¹): 3402 (O-H stretching), 2924 (C-H stretching)

^1H NMR (CDCl_3) (δ ppm) (500 MHz): 5.25 (1H, *dd*, $J = 3.5, 7$ Hz), 5.10 (1H, *m*), 3.52 (1H, *dd*, $J = 4, 11$ Hz), 1.68 (3H, *s*), 1.60 (3H, *s*), 0.97 (6H, *s*), 0.86 (3H, *s*), 0.85 (3H, *d*, $J = 6.5$ Hz), 0.80 (3H, *s*), 0.74 (3H, *s*)

^{13}C NMR (CDCl_3) (δ ppm) (125 MHz): 146.92, 131.99, 126.15, 118.81, 80.29, 54.25, 52.30, 51.63, 49.92, 44.54, 39.99, 38.22, 36.82, 36.18, 35.96, 34.97, 34.84, 29.50, 28.71, 28.63, 28.35, 26.78, 26.39, 24.97, 23.11, 19.62, 19.17, 18.71, 15.76, 14.13

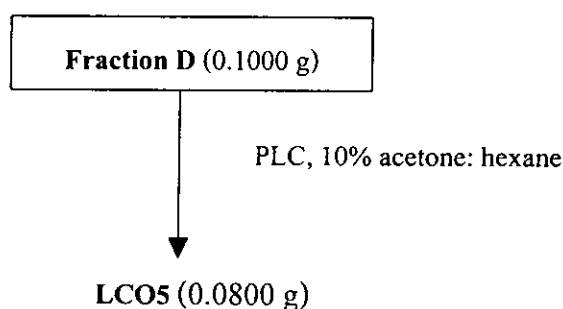
DEPT -135° (CDCl_3)

CH: 126.15, 118.81, 80.29, 54.25, 52.30, 51.63, 36.82

CH₂: 38.22, 36.18, 34.97, 34.84, 29.50, 28.63, 26.39, 24.97, 19.17

CH₃: 28.71, 28.35, 26.78, 23.11, 19.62, 18.71, 15.76, 14.13

Fraction D (0.1000 g) was purified by repeated PLC using 10% acetone: hexane as eluent to afford compound LCO5 (0.0800 g) as a white powder ($R_f = 0.20$, 10% acetone: hexane), as shown in Scheme 7.



Scheme 7 Isolation of compound LCO5

Compound LCO5 (White solid)

$[\alpha]_D^{26}$: +49.97° ($c = 0.0087$, CHCl_3)

mp: 140-142 °C

IR (KBr) $\nu(\text{cm}^{-1})$: 3364 (O-H stretching), 2953 (C-H stretching)

$^1\text{H NMR}$ (CDCl_3) (δ ppm) (500 MHz): 4.72 (1H, *s*), 4.67 (1H, *d*, $J = 1.5$ Hz), 3.11 (1H, *m*), 2.23 (1H, *septet*, $J = 6.5$ Hz), 1.03 (3H, *d*, $J = 7$ Hz), 1.02 (3H, *d*, $J = 7$ Hz), 1.00 (3H, *d*, $J = 6.5$ Hz), 0.97 (3H, *s*), 0.92 (3H, *d*, $J = 6.5$ Hz), 0.89 (3H, *s*), 0.71 (3H, *s*)

$^{13}\text{C NMR}$ (CDCl_3) (δ ppm) (125 MHz): 156.90, 134.61, 133.57, 105.91, 76.52, 50.37, 49.83, 47.03, 44.50, 39.19, 36.47, 36.31, 34.98, 34.97, 33.78, 31.27, 31.14, 31.04, 30.76, 28.17, 25.52, 24.41, 21.98, 21.85, 21.74, 20.70, 18.71, 18.20, 15.71, 15.05

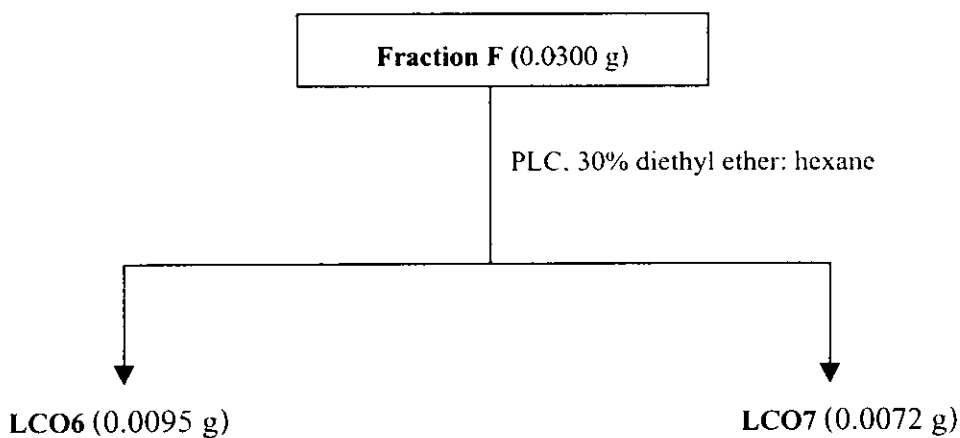
DEPT -135° (CDCl_3)

CH: 76.52, 50.37, 47.03, 39.19, 36.47, 33.78

CH₂: 105.91, 34.97, 31.27, 31.14, 31.04, 30.76, 28.17, 25.52, 21.74, 20.70

CH₃: 24.41, 21.98, 21.85, 18.71, 18.20, 15.71, 15.05

Fraction F (0.0300 g) was purified by PLC using 30% diethyl ether: hexane as eluent to afford compound **LCO6** (0.0095 g), as a white solid ($R_f = 0.55$, 30% diethyl ether: hexane, 8 times) and compound **LCO7** (0.0072 g), as a white solid ($R_f = 0.61$, 30% diethyl ether: hexane, 8 times), as shown in **Scheme 8**.



Scheme 8 Isolation of compounds **LCO6** and **LCO7**

Compound **LCO6** (White solid)

$[\alpha]_D^{25}$: -22.47° ($c = 0.0089$, CHCl_3)

mp: 118-120 °C

IR (KBr) ν (cm^{-1}): 3415 (O-H stretching), 2938 (C-H stretching), 1638 (C=C stretching)

^1H NMR (CDCl_3) (δ ppm) (500 MHz): 5.36 (1H, *m*), 5.11 (1H, *q*, $J = 7$ Hz), 3.53 (1H, *m*), 2.83 (1H, *septet*, $J = 7$ Hz), 1.58 (3H, *d*, $J = 7$ Hz), 1.01 (3H, *s*), 0.97 (6H, *d*, $J = 7$ Hz), 0.94 (3H, *d*, $J = 6.5$ Hz), 0.68 (3H, *s*)

^{13}C NMR (CDCl_3) (δ ppm) (125 MHz): 145.86, 140.74, 121.70, 116.43, 71.79, 56.74, 55.99, 50.10, 42.32, 42.28, 39.75, 37.23, 36.49, 36.13, 35.94, 31.89, 31.88, 31.64, 28.59, 28.22, 27.88, 24.28, 21.07, 21.06, 20.99, 19.38, 18.78, 12.74, 11.84

DEPT -135° (CDCl_3)

CH: 121.70, 116.43, 71.79, 56.74, 55.99, 50.10, 36.13, 31.88, 28.59

CH₂: 42.32, 39.75, 37.23, 35.94, 31.89, 31.64, 28.22, 27.88, 24.28, 21.06

CH₃: 21.07, 20.99, 19.38, 18.78, 12.74, 11.84

Compound LCO7 (White solid)

$[\alpha]_D^{26}$: +14.70° ($c = 0.0068$, CHCl_3)

mp: 120-122 °C

IR (KBr) ν (cm^{-1}): 3415 (O-H stretching), 2938 (C-H stretching), 1638 (C=C stretching)

^1H NMR (CDCl_3) (δ ppm) (500 MHz): 5.16 (1H, *m*), 5.12 (1H, *q*, $J = 7$ Hz), 3.60 (1H, *m*), 2.83 (1H, *septet*, $J = 7$ Hz), 1.59 (3H, *d*, $J = 7$ Hz), 0.98 (6H, *d*, $J = 7$ Hz), 0.95 (3H, *d*, $J = 6.5$ Hz), 0.79 (3H, *s*), 0.54 (3H, *s*)

^{13}C NMR (CDCl_3) (δ ppm) (125 MHz): 145.83, 139.58, 117.44, 116.46, 71.05, 56.01, 55.02, 49.43, 43.39, 40.24, 39.55, 37.98, 37.13, 36.57, 35.90, 34.19, 31.47, 29.64, 28.59, 28.00, 27.94, 22.95, 21.54, 21.07, 21.00, 18.92, 13.03, 12.74, 11.84

DEPT -135 $^\circ$ (CDCl_3)

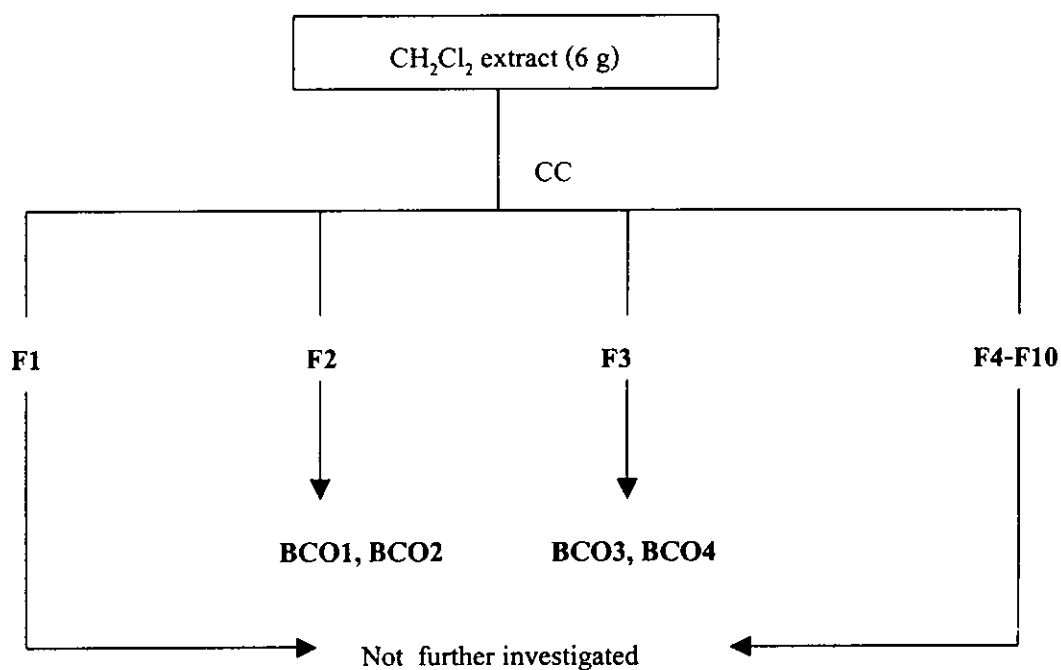
CH: 117.44, 116.46, 71.05, 56.01, 55.02, 49.43, 40.24, 36.57, 28.59

CH₂: 39.55, 37.98, 37.13, 35.90, 31.47, 29.64, 28.00, 27.94, 22.95, 21.54

CH₃: 21.07, 21.00, 18.92, 13.03, 12.74, 11.84

2.4.3 Investigation of the crude methylene chloride extract from the barks of *C. odollam*

The crude methylene chloride extract (6 g) was chromatographed on silica gel using gradient elution of hexane-chloroform, chloroform-ethyl acetate and ethyl acetate-methanol to give 10 fractions, as shown in **Scheme 9**.



Scheme 9 Isolation of compounds **BCO1 – BCO4** from the barks of *C. odollam*

Fraction F2 (0.3500 g), a solid, was washed with hexane to afford compound **BCO1** (0.1500 g) and recrystallized in chloroform to give yellow needles ($R_f = 0.18$, 60% chloroform: hexane). The solution of hexane was further purified by

PLC using 50% diethyl ether: hexane to give compound **BCO2** (0.0036 g, $R_f = 0.32$, 20% ethyl acetate: hexane).

Compound BCO1 (Yellow needles)

mp: 184-186 °C

UV (MeOH) λ_{max} (nm): 429, 331, 291, 253, 209

IR (KBr) ν (cm⁻¹): 2830 (C-H stretching for formyl group), 1724 (C=O stretching), 1639 (C=O stretching), 1556 (C=C stretching)

¹H NMR (CDCl₃) (δ ppm) (500 MHz): 9.95 (1H, *s*), 6.16 (1H, *s*), 8.50 (1H, *s*), 7.98 (1H, *d*, $J = 3.5$ Hz), 7.12 (1H, *d*, $J = 3.5$ Hz), 4.00 (3H, *s*)

¹³C NMR (CDCl₃) (δ ppm) (125 MHz): 185.66, 165.17, 150.08, 148.56, 148.39, 130.56, 125.30, 124.05, 115.20, 113.64, 52.25

DEPT -135° (CDCl₃)

CH: 185.66, 150.08, 148.56, 148.39, 113.47

CH₃: 52.25

Compound BCO2 (White solid)

mp: 128-130 °C

IR (KBr) ν (cm^{-1}): 34155 (O-H stretching), 2937 (C-H stretching), 1618 (C=C stretching)

^1H NMR (CDCl_3) (δ ppm) (500 MHz): 5.36 (1H, *m*), 3.53 (1H, *m*), 2.30 (1H, *ddd*, $J = 2, 5, 13$ Hz), 2.27-2.21 (1H, *m*), 2.03-1.94 (2H, *m*), 1.88-1.80 (3H, *m*), 1.01 (3H, *s*), 9.22 (3H, *d*, $J = 6.5$ Hz), 0.84 (3H, *s*), 0.84 (3H, *s*)

^{13}C NMR (CDCl_3) (δ ppm) (125 MHz): 140.75, 121.73, 71.81, 56.75, 56.03, 50.11, 45.81, 42.31, 42.29, 39.76, 37.24, 36.49, 36.14, 33.93, 31.91, 31.89, 31.66, 29.70, 29.12, 28.25, 26.03, 24.30, 23.05, 21.07, 19.82, 19.40, 19.02, 18.77, 11.98, 11.86

Fraction F3 (0.2500 g) was crystallized with chloroform to give yellow solid and recrystallized with acetone to give compound **BCO3** (0.0130 g) as a yellow needles ($R_f = 0.25$, 50% ethyl acetate: hexane). The mother liquor of fraction **F3** was rechromatographed on silica gel using 10% ethyl acetate: hexane as eluent to give compound **BCO4** (0.0050 g, $R_f = 0.25$ 40% ethyl acetate: hexane).

Compound BCO3 (Yellow solid)

mp: 230-240 °C

UV (MeOH) λ_{max} (nm) : 205, 287

IR (KBr) ν (cm⁻¹): 1696 (C=O stretching)

¹H NMR (CDCl₃) (δ ppm) (500 MHz): 5.82 (2H, s), 3.81 (6H, s)

¹³C NMR (CDCl₃) (δ ppm) (125 MHz): 187.50, 172.00, 157.75, 107.50, 56.20

Compound BCO4 (Yellow solid)

mp: 104-105 °C

UV (MeOH) λ_{max} (nm): 280

IR (KBr) ν (cm⁻¹): 3438 (O-H stretching), 2848 (C-H stretching for formyl group) and 1673 (C=O stretching)

¹H NMR (CDCl₃) (δ ppm) (500 MHz): 9.82 (1H, s), 7.15 (2H, s), 6.05 (1H, s), 3.97 (6H, s)

¹³C NMR (CDCl₃) (δ ppm) (125 MHz): 190.72, 147.33, 140.78, 128.40, 106.67, 56.46