CHAPTER III

EXPERIMENTAL

3.1 Plant material

The stem barks of *Croton oblongifolius* Roxb. (Plao Yai) were collected from Sakolnakorn province, Thailand, in October 1999. Botanical identification was claimed through comparison with voucher specimen No. BKF 084729 in the herbarium of the Royal Forest Department, Ministry of Agricultural and Cooperatives, 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 Spectrometer. Spectra of solid samples were recorded as KBr pellets and liquid samples were recorded as thin film on KBr cells.

3.2.2. Ultraviolet-Visible Spectrophotometer (UV-Vis)

The UV-Vis spectra were recorded on a Hewlett packard 8452 A diode array spectrophotometer, using chloroform as a solvent.

3.2.3 Mass Spectrometer (MS)

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

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

The ^{1}H and ^{13}C spectra were recorded on a Bruker Model AC-F 200 spectrometer operated at 200.13 MHz and a JEOL JNM-A500 spectrometer operated at 500.13 MHz.

3.2.4 Optical Rotation

The optical rotation values were measured on a Perkin Elmer instruments model 341 polarimeter, using chloroform as a solvent.

3.3 Chemical Reagents

3.3.1 Solvents

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

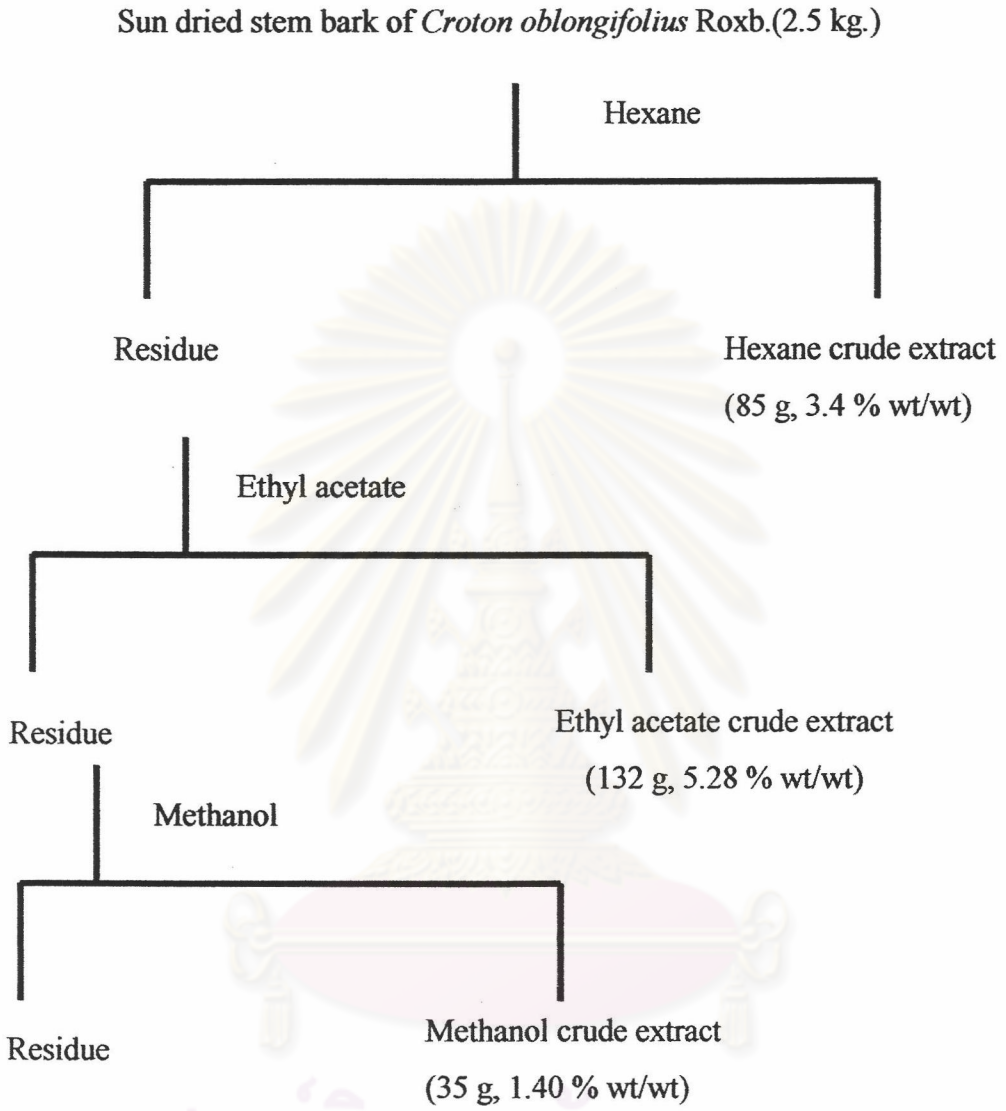
3.3.2 Packing material

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 and flash column chromatography

3.3.2.2 Merck's TLC aluminum sheets, 20 X 20 cm^2, layer 0.2 mm were used to identify the identical fraction.

3.4 Extraction and Isolation

The sun-dried and ground stem bark (2.5 kg) of *Croton oblongifolius* Roxb. was soaked in hexane at room temperature for a week. The hexane solution was filtered and then evaporated under reduced pressure to dryness, giving the hexane crude extract (85 g). The residue was further extracted with ethyl acetate and methanol, respectively, giving the ethyl acetate crude extract (132 g) and the methanol crude extract (35 g), respectively. The extraction procedure is shown in Scheme 1.



Scheme 1 Extraction procedure of the stem bark of *Croton oblongifolius* Roxb.

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

3.5.1 Separation of hexane crude extract

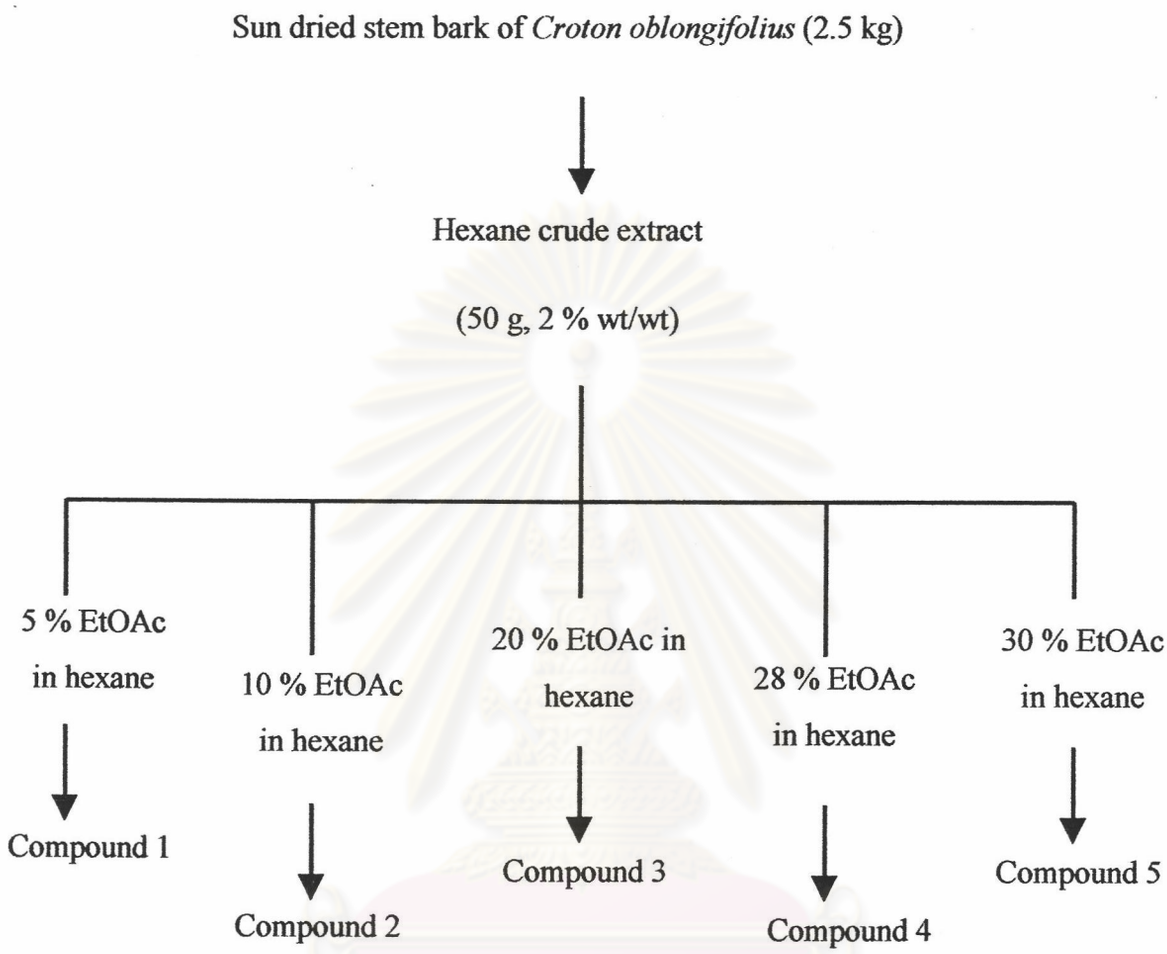
The hexane crude extract (50g) was separated by column chromatography packed with silica gel 60 Art. 7734 (70 –230 mesh ASTM). The crude extract was dissolved in a small amount of suitable solvent and mixed with silica gel (1:1) to afford the extract paste and was placed on top of the column. The column was eluted with hexane, hexane–ethyl acetate gradient in a stepwise fashion. Each fraction was collected, and then checked by thin layer chromatography (TLC) to combine the fractions which had the same components. The results from separation of hexane crude extract are summarized in Table 4. The fractions were further purified by column chromatography or crystallization. The isolation of compounds from hexane crude extracts is shown in Scheme 2. The results of purified compounds are shown in Table 5.

3.5.2 Separation of ethyl acetate crude extract

The ethyl acetate crude extract (30 g) was separated by column chromatography on siliga gel 60 Art. (70-230 mesh ASTM). The column was eluted with hexane, hexane–ethyl acetate, ethyl acetate and ethyl acetate–methanol, respectively. Each fraction was collected and then checked by TLC to combine the fraction which had the same components. The compounds in ethyl acetate and hexane extract were found to be the same.

3.5.3 Separation of methanol crude extract

The methanol crude extract was a residue gummy that was insoluble in methanol, and not separated by column chromatography.



Scheme2 Isolation procedure of hexane crude extract.