STYRYLDEHYDROPYRONE AND CLERODANE-TYPE DITERPENE FROM CROTON ARGYRATUS

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Keywords: Croton argyratus, Euphorbiaceae, goniothalamin, junceic acid.

Abstract
Phytochemical study on the roots of Croton argyratus (Euphorbiaceae) has been carried out. Isolation and purification of the methanolic extract afforded a styryldehydropyrone, (+)-goniothalamin and a clerodane-type diterpene, (-)-junceic acid. The structures of these compounds were determined by spectroscopy methods such as mass spectrometry (MS), 1H and 13C NMR and by comparison with those of previously reported data. This paper reports the isolation and elucidation of the above compounds.

Introduction
Croton is a very large genus of trees, shrubs and herbs of the family Euphorbiaceae, found throughout the tropics and subtropics area (Burkill 1966). There are 750 species found throughout the warmer parts of earth. In Malaysia, there are 11 species (Corner, 1988). Croton argyratus, known locally as cheret budak, semelit sayor or akar cheret budak is a tree of about 18 m tall and 90 cm girth, which is common throughout Malaysia, Burma to Moluccas and Bali (Burkill 1966). The leaves and stems of C. argyratus were used by the locals to stop purging. This plant are also known as ‘Silver Croton’ as the undersides of the leaves are silvery white or silvery brown. The tree is a primary forest, forest edges and lowlands of up to 600 m (Whitmore, 1972).

Literature search has shown that Croton exhibited a wide range of biological and pharmacological activities such as anti-inflammatory (Suarez et al. 2006; Habashy et al., 2005), anticancer (Sylvestre et al., 2006) and cytotoxicity (Morales et al., 2005). Cytotoxicity test carried out by Horgen et al., (2001) on the methanol extracts of the leaves/twigs, roots and stem bark of C. argyratus showed that extracts tested on human lung cancer cell line exhibited IC50 values of < 5.0 µg/ml . All extracts displayed selectivity of >10-fold against Lu-1 cell line compared with other cell lines tested.

Previous reports showed that the genus of Croton contained diterpenes such as clerodanes and labdanes (Maciel at al., 2000; Silva et al., 2005; Melo et al., 2003; Roengsumran et al., 1999; Sutthivaiyakit et al., 2001). Detailed chemical studies on the roots of Croton argyratus yielded a styryldehydropyran, (+)-goniothalamin and a clerodane-type diterpene, (-)-junceic acid.

Experimental
General
Melting point was measured on Gallenkamp apparatus and were uncorrected. Ultra-violet (UV) spectra were recorded on Shimadzu UV-160 while Infra-red (IR) spectra were obtained in MeOH on a Perkin-Elmer FT-IR 1725-X. 1H Nuclear Magnetic Resonance (NMR) (400 MHz) and 13C NMR (100.56 MHz) measurements were carried out on a JEOL ECP-400 spectrometer. Chemical shifts are reported in ppm (part per million) and the
coupling constants are given in Hz. Gas chromatography-mass spectrometer (GC-MS) were obtained on a Macromass LCT.

**Extraction and isolation**

The roots of *C. argyratus* were collected from Ulu Muda, Kedah. The roots (279 g) were air-dried, ground and soaked in MeOH for 48 hours. The resulting extract was filtered and concentrated under reduced pressure to give 18.56 g of crude extract. The crude extract was subjected to vacuum liquid chromatography (VLC) to produce six fractions. Fraction 5 was chromatographed over repeated radial chromatography to yield compound 1 (30 mg). Fraction 6 was subjected to radial chromatography and eluted with hexane with increasing amount of ethyl acetate to yield compound 2 (45 mg).

**Goniothalamin**

C₁₉H₁₂O₂, white needle crystal. m.p.: 86-88°C. [α]₀ +125.32° (c, 1.38 CHCl₃). **UV** λₘₐₓ nm (CHCl₃): 207, 255, 284. IR νₘₐₓ cm⁻¹ (CHCl₃): 1720, 1494, 1380, 1245, 1055, 1020, 965, 752. MS: 200 [M⁺], 172, 131, 115, 104, 91, 77, 68. ¹H NMR ppm: d7.35 (5H, m, H-2', H-3', H-4', H-5', H-6'), 6.90 (1H, dt, J = 9.8, 4.0 Hz, H-4), 6.71 (1H, d, J = 16.0 Hz, H-2'), 6.26 (1H, dd, J = 16.0, 6.8 Hz, H-1'). 6.06 (1H, dt, J = 9.8, 1.7 Hz, H-3'), 5.08 (1H, qd, J = 6.8, 1.7 Hz, H-6), 2.45 (2H, m, H-5). ¹³C NMR: δ183.3 (C-20), 143.6 (C-4), 143.0 (C-16), 138.7 (C-15), 124.8 (C-1'), 122.3 (C-3), 78.6 (C-6), 30.5 (C-5).

**Juncieic acid**

C₁₉H₂₂O₃, yellowish oil. [α]₀ -19.72° (c, 8.25 CHCl₃). UV λₘₐₓ nm (CHCl₃): 257, 262, IR νₘₐₓ cm⁻¹ (CHCl₃): 3395, 2915, 2850, 1710, 1255, 1020. MS: 316 [M⁺]. ¹H NMR ppm: d7.35 (1H, t, J = 1.8 Hz, H-16), 7.23 (1H, s, H-15), 6.27 (1H, d, J = 1.8 Hz, H-14), 5.25 (1H, br s, H-3), 2.35 (1H, m, H-1-1), 2.17 (1H, m, H-17), 2.08 (1H, m, H-1-2), 1.92 (1H, m, H-1-1), 1.79 (1H, m, H-1-2), 1.66 (1H, m, H-1-1), 1.63 - 2.37 (5H, m, H-10, H-11, H-12), 1.62 (1H, m, H-8), 1.58 (3H, s, H-17), 1.45 (1H, m, H-7), 1.14 (3H, d, J = 6.6 Hz, H-19), 0.95 (3H, s, H-18). ¹³C NMR: δ183.3 (C-20), 143.6 (C-4), 143.0 (C-16), 138.7 (C-15), 124.8 (C-13), 121.2 (C-3), 111.0 (C-14), 50.1 (C-9), 48.5 (C-10), 39.0 (C-5), 37.6 (C-6), 37.2 (C-8), 33.9 (C-11), 27.5 (C-7), 27.4 (C-2), 20.8 (C-12), 18.3 (C-19), 18.0 (C-1), 17.7 (C-18), 16.8 (C-17).

**Results and Discussion**

Two compounds were successfully isolated from the roots of *C. argyratus*. Compound 1 was obtained as white needle crystals with m.p. 83-84°C. The IR spectrum showed strong bands at 1720, 1245 and 752 cm⁻¹ assignable to the resonance of a,b-unsaturated ?-lactone moiety. Additional bands at 1494 and 965 cm⁻¹ were typical of styryl functionality. The mass spectrum (MS) exhibited a molecular ion peak at m/z 200 which consistent with the formula molecule C₁₉H₂₂O₃. The base peak was observed at m/z 68, corresponding to the ionized furan. The optical rotation was done in chloroform and calculated as +125.32°.

![Image](1)

The ¹H NMR spectrum displayed a multiplet at d7.33 which was referring to five aromatic protons from a mono-substituted phenyl ring (H-2" to H-6"). Two olefinic protons of a trans configuration were observed at d6.71 (d, J = 16.0 Hz) and d6.26 (dd, J = 16.0 and 6.8 Hz) ascribable to H-2" and H-1" respectively. Resonances at d6.90 (dt, J = 9.8 and 4.0 Hz) and d6.06 (dt, J = 9.8 and 1.7 Hz) were assigned to H-3 and H-4 of a,b-unsaturated ?-lactone moiety. A multiplet was observed at d2.45 corresponding to an allylic methylene (H-5) and a proton on a carbon bearing the oxygen of the lactone group appeared as a quadruple doublet at d5.08 (H-6).

The APT (Attached Proton Test) NMR spectrum of compound 1 exhibited 11 signals corresponding to 13 carbon resonances. Signal at d164.5 was ascribable to carbonyl carbon attached to oxygen (C-2), while signal at...
d136.4 was assigned to a quarternary carbon C-1”. Monosubstituted ring system that gave equivalent peaks at d129.3 and d127.3 were attributed to C-3’/5” and C-2’/4” respectively. Four olefinic carbons were observed at d126.3, 133.7, 122.3 and 145.3 corresponding to C-1’, C-2’, C-3 and C-4 respectively. The rest of the signals were assigned to a methylene carbon (d30.5) and a deshielded methine carbon at d78.6. Based on the spectral data and comparison with literature, compound 1 was assigned as goniothalamin (Cavallheiro and Yoshida, 2000; Sundby et al., 2004). The optical rotation was done in chloroform and calculated as +125.32°, hence confirmed the conformation of 1 as (+)-goniothalamin.

Goniothalamin was a major compound found in most of the Goniothalamus species (Hisham et al., 2003; Hasan et al., 1994; Ahmad et al., 1991; Sam et al., 1987). Goniothalamin has been reported to occur in Trypanosoma cruzi (de Fatima et al., 2006), Cryptocarya moschata (Cavallheiro and Yoshida, 2000), and Bryonopsis laciniosa (Mosaddik et al., 2000). This compound has been shown to have anti-cancer and apoptosis-inducing properties against various human tumor and animal cell lines (Umar-Tsafe et al., 2004). Goniothalamin was also found as a potential genotoxic or clastogenic substance (Chan et al., 2006).

Compound 2 was obtained as an oil. The IR spectrum showed bands at 1710, 1255, 1020 which were characteristic of furanoditerpenic acid. The MS displayed a molecular ion peak at 158 which consistent with the formula molecule C_{20}H_{32}O_{3}. 1H NMR spectrum exhibited a deshielded triplet at d7.35 which corresponded to proton of H-16. A singlet was observed at d7.23 which was assignable to H-15. Signal at d6.27 (d, J = 1.8 Hz) was assigned to H-14, while a singlet at d5.25 was referring to olefinic proton at H-3. The remaining of the signals were up field signals (d2.37 to 0.95) corresponding to six methylene (H-1, H-2, H-6, H-7, H-11, H-12), three methyl (H-17, H-18, H-19) and two methine protons (H-8, H-10). The assignment of these protons to their respective carbons were determined via HMOC experiment.

The APT NMR spectrum showed 20 signals, which include the most deshielded signal for carboxylic acid carbon at d183.3. Signals for two quaternary aromatic carbons were observed at d143.6 (C-4) and d124.8 (C-13). Resonance for three aromatic carbons of the furanoic ring (C-14, C-15, C-16) and an olefinic carbon (C-3) appeared at d111.0, 138.7, 143.0 and 121.2 respectively. Two signals at d39.0 and 50.1 were assigned to quaternary aliphatic carbons of C-5 and C-9 while signals at d37.2 and 48.5 were referring to methine aliphatic carbons (C-8 and C-10). Six methylene carbons (C-1, C-2, C-6, C-7, C-11, C-12) exhibited signals in the aliphatic region at d18.0, 27.4, 37.6, 27.5, 33.9 and 20.8 respectively. Signal for three methyl carbons (C-17, C-18, C-19) could be seen in the most upfield region of the NMR spectrum at d16.8, 17.7 and 18.3 respectively. On the basis of spectral evidence and comparison with those of literature, compound 2 was deduced as junceic acid (De Heluani et al. 1998; Asakawa et al. 1990). The optical rotation done in chloroform was calculated as -19.72°, thus giving the conformation of 2 as (-)-juncieic acid.
Junceic acid has been reported to occur in the roots of *Croton sarcopetalus* (de Heluani et al., 1998). The stereoisomer of 2, (+)-juncieic acid has been isolated from the liverworts, *Heteroscyphus bescherellei* (Asakawa et al., 1990).

**Acknowledgements**

This work was supported by a grant from Malaysian Government under the IRPA scheme (Grant No. 09-02-02-0086-EA 227). The authors wish to thank UiTM for sponsoring one of the authors for her Ph.D study.

**References**