J. Sci. Soc. Thailand 14 (1988) 73 - 76

CONSTITUENTS OF THE STEM BARK OF MICHELIA LONGIFOLIA*

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* *Part VII in the series of "Studies on Thai Medicinal Plants." For Part VI see ref. (1).*

(Received 4 February 1988)

Abstract

The bark of Michelia longifolia *Blume (Magnoliaceae) yielded four compounds: parthenolide, ß-sitosterol, costunolide and liriodenine.*

Michelia longifolia Blume *(M. alba* DC.*)* is a member of the Magnoliaceae family and has been used in the traditional medicine of South-east Asia. In the Malay Peninsula an infusion of the flowering buds is given to women for sapremia following a miscarriage.[2] Previously reported constituents of the essential oil were linalool, nerol, limonene, benzyl acetate, hydroxycitronellal, benzaldehyde, benzyl benzoate, methyl eugenol and eugenol[3] along with the alkaloids ushinsunine, oxoushinsunine (liriodenine), salicifoline, and michelalbine.[4] We report herein the isolation and identification of four constituents of the bark of *M. longifolia.*

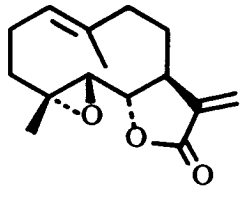
The residue from the chloroform extract of the fresh bark was separated into four pure components by column chromatography as described in the experimental section. The first component eluted was a white solid which showed a molecular ion at m/z 248 corresponding to the molecular formula $C_{15}H_{20}O_3$. Infrared bands at 1770 and 1650 cm^{-1} suggested the presence of an α-methylene-γ-lactone moiety and the 400 MHz ^1H NMR spectrum of this component was in excellent agreement with that of the germacranolide epoxide, parthenolide (1), isolated previously in our laboratory from *Paramichelia baillonii.*[5]

The IR spectrum of the second component revealed the presence of hydroxy and olefinic functions and the electron impact mass spectrum (MS EI) exhibited a weak parent ion for $C_{29}H_{50}O$ and an intense M-H_2O peak which is characteristic of Δ^5-3-ß-sterols.[6] The ^1H and ^{13}C NMR spectra were identical to those obtained for a sample of

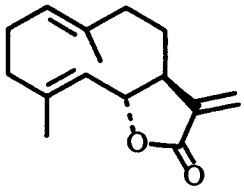
ß-sitosterol, which had been isolated from *Typha elephantina,*[7] and also were in accord with other spectra published previously for this sterol.[8-10] Thus, the second component is ß-sitosterol, 2.

The third component, a white solid, displayed in its MS EI a parent peak (m/z 232) corresponding to the molecular formula $C_{15}H_{20}O_2$. IR bands at 1772 and 1650 cm^{-1} suggested the presence of an α-methylene-γ-lactone moiety and ^1H and ^{13}C NMR spectra were in complete agreement with those reported previously[11,12] for the germacranolide costunolide, **3.**

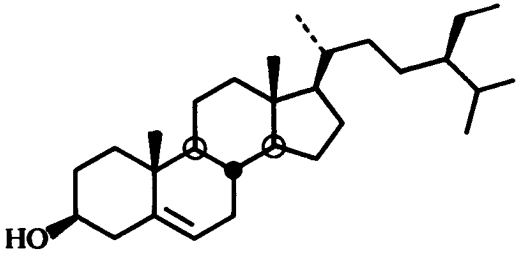
The fourth and most polar compound was a high-melting, yellow, crystalline solid. Its MS EI exhibited a strong molecular ion at m/z 217 ($C_{17}H_9NO_3$) and a fragmentation pattern similar to that reported for the alkaloid liriodenine, **4.**[13] In a previous paper[5] we had assigned completely the ^1H and ^{13}C NMR spectra of **4** and comparison of these spectra unambiguously established that this fourth component was the oxoaporphinoid liriodenine, **4.** This alkaloid has previously been reported to be present in a number of different Magnoliaceae genera[14, 15] as well as in the buds of *M. longifolia.*[4]



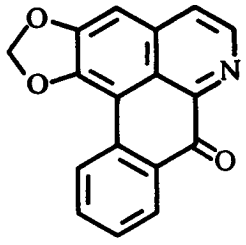
1



3



2



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As *Michelia longifolia* has been used for medicinal purposes, the biological activities of the constituents isolated from the bark deserve some comment. Parthenolide, **1,** displayed significant activity against the human laryngeal epidermoid carcinoma (ED_{50} = 0.76)[16] and the 9KB cell culture system (ED_{50} = 0.45)[17] while costunolide, **3,** showed reproducible inhibitory activity against the KB cell culture of a human carcinoma of the nasopharynx.[12] The anticarcinogenic activity of ß-sitosterol, 2, against N-methyl-N-nitrosourea in colon carcinogenesis of animals has been documented also.[18]

Instrumentation: Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra were obtained on a Nicolet Model 20 SX/C FT-IR spectrometer and mass spectra on a VG Micromass 7070F or a ZAB-E spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WH-400 spectrometer and optical rotations were obtained on a Bendix-NPL automatic polarimeter in chloroform.

Plant Material: Stem bark of *M. longifolia* was collected from Uttaradit province, Thailand, during July, 1986. The plant was identified by comparison with a known specimen from the medicinal plant garden of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Isolation of 1-4: Fresh stem bark of *M. longifolia* (2.5 kg) was blended with 95% ethanol (10 l) and filtered. The filtrate was evaporated *in vacuo* to yield a syrupy mass (81.2 g) which was then suspended in water (150 ml), extracted with chloroform (5 × 300 ml), dried over anhydrous sodium sulfate and evaporated to yield 10 g of a crude extract. The extract was chromatographed using silica gel and chloroform/acetone (9:1) and 25 ml fractions were collected. Fractions 12-35 afforded a crude mixture (0.32 g) which upon further purification with silica gel and benzene/ethyl acetate (9:1, 25 ml fractions) gave parthenolide (106 mg, fractions 22-26), ß-sitosterol (29 mg, fractions 30-32) and costunolide (50 mg, fractions 35-40). Fractions 80-95 from the initial separation afforded a crude yellow residue which was rechromatographed using chloroform/acetone (7:3) to give liriodenine (14 mg).

(-)-Parthenolide, **1.** M.p. 112-115°C [lit.[19] 115°C]; $[\alpha]_D^{20}$ - 78°($CHCl_3$) [lit.[19] - 81.4]; MS EI m/z (rel. int.) 248 (M^+, 2), 230(9), 191(25), 190(61), 119(100); IR(CCl_4) 3020, 2920, 1770, 1650, 1281 cm^{-1}; ^{1}H and ^{13}C NMR, see ref. (5).

ß-Sitosterol, **2.** M.p. 134-135°C [lit.[16] 136-137°C]; IR, MS EI, ^{1}H and ^{13}C NMR, see ref. (7).

(+)-Costunolide, **3.** M.p. 105-107°C [lit.[12] 105-106°C]; $[\alpha]_D^{20}$ + 125° ($CHCl_3$) [lit.[12],$[\alpha]_D^{29}$ + 131° ($CHCl_3$)]; IR and MS EI, see ref. (12); ^{1}H and ^{13}C NMR, see ref. (20).

Liriodenine, **4.** M.p. 278-282°C (dec.) [lit.[17] 282°C]; IR, ^{1}H and ^{13}C NMR, see ref. (5).

Acknowledgements

G.L.L. acknowledges the Natural Sciences and Engineering Research Council of Canada (N.S.E.R.C.) for support in the form of an operating grant and C.P.D. acknowledges N.S.E.R.C. for support in the form of a post-graduate scholarship.

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บทคัดย่อ

จากการสกัดและตรวจหาสูตรโครงสร้างของสารในเปลือกต้นจำปี *(Michelia longifolia* Blume) วงศ์ Magnoliaceae พบสารเคมี 4 ชนิดคือ parthenolide, ß-sitosterol, costunolide และ liriodenine

Supplementary material: Spectroscopic data of compounds described in this article are available upon request.