Chemical Constituents of *Legnephora moorei* Miers (Menispermaceae)

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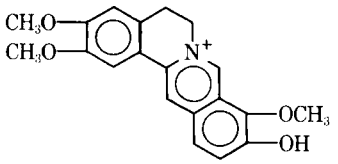
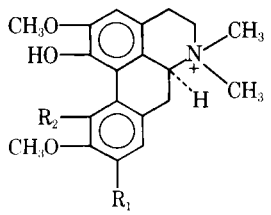
Abstract □ A phytochemical investigation of an ethanolic extract of the whole plant of *Legnephora moorei* Miers (Menispermaceae) resulted in the isolation and characterization of the alkaloids dehydrocorydalmine, magnoflorine, laurifoline, and stepharine; the pentacyclic triterpene friedelin; and the sterol mixture campesterol, stigmasterol, and sitosterol.

Keyphrases □ *Legnephora moorei* Miers (Menispermaceae)—isolation and identification of dehydrocorydalmine, magnoflorine, laurifoline, stepharine, friedelin, campesterol, stigmasterol, and sitosterol ■ Column chromatography—separation of *Legnephora moorei* constituents ■ GLC-mass spectrometry—identification of campesterol, stigmasterol, and sitosterol mixture from *Legnephora moorei*

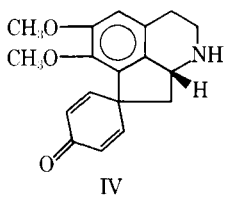
Legnephora moorei is a vine indigenous to Australia and New Guinea. An initial survey showed the presence of alkaloids in the genus (1) and, in particular, in the species *L. moorei* (2). A preliminary phytochemical study (3) resulted in the isolation of the cyclitol (+)-quercitol and the quaternary aporphine alkaloid isocorydine methiodide. The relative paucity of knowledge of the constituents of *Legnephora* species plus the established toxicity of many Menispermaceous species prompted the phytochemical investigation of this plant. The isolation and identification of the alkaloids dehydrocorydalmine (I), magnoflorine (II), laurifoline (III), and stepharine (IV); the pentacyclic triterpene friedelin; and the sterol mixture campesterol, stigmasterol, and sitosterol are reported.



I



II: R_1 = H, R_2 = OH
III: R_1 = OH, R_2 = H



IV

DISCUSSION

The plant material was moistened with dilute ammonium hydroxide and extracted by percolation to exhaustion with ethanol. The extract residue was partitioned between dilute hydrochloric acid and ether. The ether extract containing the nonbasic substances was designated Fraction A. Chromatography of Fraction A over neutral alumina afforded the pentacyclic triterpene friedelin and the sterol mixture campesterol, stigmasterol, and sitosterol. The acid extract was basified with ammonium hydroxide and extracted with ether. The ether extract, containing the nonquaternary bases, was separated into nonphenolic (Fraction B) and phenolic (Fraction C) portions by treatment with dilute sodium hydroxide. Chromatography of Fraction B over neutral alumina afforded the proaporphine alkaloid stepharine. Fraction C was reserved for future investigation. The ammonium hydroxide layer was acidified with dilute hydrochloric acid, and the quaternary bases were precipitated by treatment with ammonium reineckate solution. Treatment of the reineckate complex with an anion-exchange resin in the chloride form afforded a quaternary alkaloid chloride fraction (Fraction D). Chromatography of Fraction D over silicic acid afforded the quaternary alkaloids dehydrocorydalmine (I), magnoflorine (II), and laurifoline (III).

The occurrence of proaporphine, aporphine, and protoberberine alkaloids in members of the plant family Menispermaceae has been firmly established (4). However, this is the first reported occurrence of dehydrocorydalmine, magnoflorine, laurifoline, and stepharine in the genus *Legnephora*. Since the occurrence of sterols and triterpenes in higher plants is rather common (5), the isolation of the pentacyclic triterpene friedelin and the sterol mixture campesterol, stigmasterol, and sitosterol was not unexpected.

EXPERIMENTAL[1]

Plant Material—*Legnephora moorei* Miers (Menispermaceae)[2] was received from Australia after identification at the point of collection. The whole plant was air dried and ground to a fine powder.

Extraction and Initial Fractionation—The plant material (10 kg) was moistened with aqueous ammonium hydroxide (10%) and extracted by percolation to exhaustion with ethanol. The ethanol extract was concentrated *in vacuo* at 40° to a brown syrup (464 g) which was stirred with 1% hydrochloric acid (1.5 liters). The acid solution was filtered and extracted with ether (3 × 1.5 liters). The combined ether extracts were dried (anhydrous sodium sulfate) and evaporated to leave a residue (44.8 g) of acidic and neutral compounds (Fraction A). The aqueous acidic solution was basified to pH 8 with concentrated ammonium hydroxide and extracted with ether (3 × 1.5 liters). The combined ether extracts were dried (anhydrous sodium sulfate) and evaporated to leave a residue (9.5 g) of crude bases. This residue was dissolved in ether

[1] Melting points were taken on a Thomas-Hoover Uni-Melt capillary apparatus and are corrected. The IR spectra were determined on a Perkin-Elmer model 257 or 137 spectrometer in KBr pellets. The UV spectra were obtained on a Perkin-Elmer model 202 recording spectrometer. Optical rotations were measured in a Rudolph polarimeter. Mass spectra were taken with a LKB-9000 or DuPont 21-492 mass spectrometer. For GLC analysis, an F&M Biomedical gas chromatograph, model 400, was operated isothermally at 255°. The column was 0.31 cm (0.125 in.), 1.84 m (6 ft) stainless steel, packed with 0.8% OV-17 (phenyl methyl silicone) on 80–100 mesh Gas Chrom Q. Helium was the carrier gas at a flow of 30 ml/min. Peak area calculations were made by the method of peak height × width at half-height.

[2] Collected in Casino Forestry District by the Forestry Commission of New South Wales, Australia (Lot 43653). Voucher specimens are deposited in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, University of Mississippi.

(500 ml) and extracted with 2% sodium hydroxide (3 × 500 ml). The ether layer was washed with water, dried, and evaporated to afford a residue (1.4 g) of nonphenolic bases (Fraction B). The sodium hydroxide solution was acidified to pH 5 with 1% hydrochloric acid, alkalinized with ammonium hydroxide to pH 9, extracted with ether (3 × 1.5 liters), and evaporated to yield a residue (1.7 g) of crude phenolic bases (Fraction C).

The ammoniacal solution from which the bases had been extracted was reacidified to pH 3 with 1% hydrochloric acid and treated with a 2% ammonium reineckate solution until precipitation was complete. The precipitate was filtered by suction, suspended in dilute hydrochloric acid solution (pH 5), and stirred for 12 hr with 200 g of anion-exchange resin[3]. The resulting suspension was filtered and the filtrate was evaporated *in vacuo* to afford a mixture of crude quaternary chlorides (20 g) (Fraction D).

Chromatography of Quaternary Chloride Salts (Fraction D)—Fraction D (20 g) was chromatographed over a column (6.3 × 45 cm) of silicic acid[4]-diatomaceous earth[5] (4:1) (1.3 kg) with chloroform (1 liter) and chloroform–methanol mixtures (1 liter) of increasing polarity (1, 2, 4, 8, 16, and 32% methanol).

Isolation of Dehydrocorydalmine (I)—Elution with 16% methanol in chloroform afforded a fraction (200 mg), a portion of which (80 mg) was rechromatographed on preparative silica gel G plates[6] [methanol–ammonium hydroxide–water (8:1:3)]. Elution with methanol and addition of saturated methanolic potassium iodide (0.1 ml) afforded needles of dehydrocorydalmine iodide (I) (13 mg); mp 239–241° dec. [lit. (6) mp 228–230° dec.]; λ_{max} (ethanol): 228 (log ϵ 4.51), 278 (4.44), 348 (4.40), and 435 nm (3.65). The alkaloid was identified as I by direct comparison (IR, UV, and melting point) with an authentic sample.

Isolation of Magnoflorine (II)—An aliquot (1 g) of the fraction eluted with 32% methanol in chloroform was rechromatographed over a column (2.5 × 60 cm) of polyamide[7] (90 g) in ammonium hydroxide–water (1:10) (1 liter) and then over a column (3.5 × 60 cm) of acidic alumina[7] (Grade IV) (195 g). Elution with 32% methanol in chloroform (500 ml) followed by treatment with methanolic potassium iodide yielded magnoflorine iodide (II) (30 mg), mp 248–249° dec.; [lit. (7) mp 248–249° dec.]; $[\alpha]_D^{25}$ + 202.0° (c 0.70, methanol), λ_{max} (ethanol): 220 (log ϵ 4.55), 273 (3.90), and 311 nm (3.99). A direct comparison (IR, UV, melting point, and mixed melting point) with an authentic sample of II confirmed the identity.

Isolation of Laurifoline (III)—Another aliquot of the fraction eluted with 32% methanol in chloroform (1 g) was chromatographed over a column (2.5 × 60 cm) of acidic alumina[7] (Grade V) (140 g). Elution with 16% methanol in chloroform (250 ml) followed by treatment with methanolic potassium iodide (0.1 ml) afforded laurifoline iodide (III) (15 mg), mp 241.5–242° dec.; λ_{max} (ethanol): 224 (log ϵ 4.60), 280 (4.12), and 305 nm (4.22). A direct comparison (IR, UV, and melting point) with an authentic sample of III confirmed the identity. Although the optical rotation of the alkaloid was positive (confirming the configuration as *S*), a sufficient quantity of base was not available to determine accurately the specific rotation.

Isolation of Stepharine (IV)—Chromatography of a portion of Fraction B (1.1 g) over a column (2.5 × 60 cm) of neutral alumina (Grade V) (106 g) and elution with chloroform (500 ml) afforded IV (12 mg), mp 177–179° [lit. (8) mp 179–181°]; $[\alpha]_D^{25}$ + 110° (c, 0.10, chloroform); λ_{max} (ethanol): 218 (log ϵ 4.37), 233 (4.30), and 286 nm (3.52). The mass spectrum showed a molecular ion at *m/e* 297 (100%) corresponding to $C_{18}H_{19}NO_3$ and significant peaks at *m/e* 296 (43), 268 (65), 253 (15), 237 (13), and 225 (13). The alkaloid was identical with an authentic sample by direct comparison (IR, UV, mass spectroscopy, melting point, and mixed melting point).

Chromatography of Fraction A—An aliquot of Fraction A (6.0 g) was chromatographed over a column (3.75 × 60 cm) over neutral alumina[7] (Grade I) (500 g) in petroleum ether.

Isolation of Friedelin—Elution with petroleum ether–benzene (4:1) (1 liter) gave a fraction (605 mg) which was further purified by preparative TLC on silica gel G utilizing a solvent system of methanol–ammonium hydroxide–water (8:1:3). Elution of the band at R_f 0.72 with 2% methanol in chloroform afforded a residue, which on crystallization from ether gave friedelin (6 mg), mp 251–253° [lit. (9) mp 248–252°]; ν_{max} (KBr): 2920, 1718, and 1390 cm^{-1}. The mass spectrum showed a molecular ion at *m/e* 426 (35%) for $C_{30}H_{50}O$ and important fragment ions at *m/e* 411 (12), 341 (7), 302 (23), and 273 (40). The compound was identical with an authentic sample of friedelin by direct comparison (IR, mass spectroscopy, melting point, and mixed melting point).

Isolation of Sterol Mixture—Continued elution with petroleum ether–benzene (4:1) (1 liter) afforded a fraction (820 mg) which gave a positive Liebermann-Burchard test. This fraction was rechromatographed on a column (2.5 × 60 cm) of silicic acid[4] –diatomaceous earth[5] (4:1) (150 g). Elution with petroleum ether–benzene (1:1) (500 ml) afforded a sterol mixture which crystallized from ethanol (14 mg); ν_{max} (KBr): 3410, 2940, 1380, and 1360 cm^{-1}. GLC[1] showed the mixture to be composed of sitosterol (48%), stigmasterol (39%), and campesterol (13%). The identity was confirmed by GLC–mass spectroscopy. The spectra were consistent with those of authentic samples.

[3] IRA-400 (Cl), Mallinckrodt Chemical Works, St. Louis, Mo.
[4] Mallinckrodt Chemical Works, St. Louis, Mo.
[5] Celite, supplied as Celite Analytical Filter Aid by Johns-Manville Co., New York, N.Y.
[6] PF-254, Brinkmann Instruments, Westbury, N.Y.
[7] Woelm, Alupharm Chemicals, New Orleans, La.

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