Isolation of Flindersiamine, Isoflindersiamine, and a New Alkaloid, Heliparvifoline, from *Helietta parvifolia* (Rutaceae)

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Abstract □ A phytochemical investigation of the leaves and twigs of *Helietta parvifolia* (Rutaceae) resulted in the isolation of heliparvifoline, a new furoquinoline alkaloid, in addition to the known bases flindersiamine and isoflindersiamine.

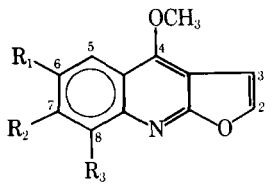
Keyphrases □ Alkaloids—flindersiamine, isoflindersiamine, and heliparvifoline isolated from *Helietta parvifolia* leaves and twigs, cytotoxicity evaluated □ *Helietta parvifolia*—leaves and twigs, alkaloids isolated, cytotoxicity evaluated □ Cytotoxicity—alkaloids from leaves and twigs of *Helietta parvifolia* isolated, evaluated

Helietta parvifolia (A. Gray) Benth (Rutaceae) is a tree native to Central and South America (1). Phytochemical studies on this plant have demonstrated the presence of the furoquinoline alkaloids flindersiamine (I) and kokusaginine (II) (2); the volatile oil components isosafrol, safrol, eugenol, and methyleugenol (2); an alkane, dotriacontane (3); and a straight-chain alcohol, tetracosanol (3).

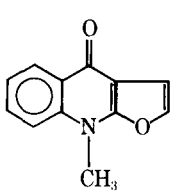
The only other species of this genus investigated for its constituents is *H. longifoliata*, which yielded the pentacyclic triterpene isobauerenol (4), maculine (III) (5), limonin (6), dictamnine (IV) (7), 6-methoxydictamnine (V) (7), kokusaginine (II) (7), flindersiamine (I) (7), skimmianine (VI) (7), isodictamnine (VII) (7), and heliettin (8).

No pharmacological study or folkloric information has been reported concerning *Helietta* species. However, preliminary investigation showed that a 50% ethanol extract of *H. parvifolia* exhibits activity against the P-388 lymphocytic leukemia (T/C = 133%) in DBA/2 mice at a dose of 66 mg/kg[1]. The chloroform-soluble fraction obtained after partitioning the concentrated methanol extract of the plant between chloroform and water showed marginal activity against the P-388 leukemia (T/C = 129%) at a dose of 400 mg/kg. An extract representing the total alkaloids of this plant showed cytotoxicity against Eagle's 9KB carcinoma of the nasopharynx in cell culture (ED_{50} = 5.0 μg/ml)[1].

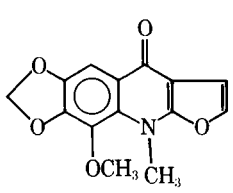
In view of the limited studies on the constituents of this plant and the preliminary results indicating activity in the 50% ethanol extract, the current study was initiated to isolate new potential antitumor agents from *H. parvifolia*.



I: R_{1-2} = —OCH_2O—, R_3 = OCH_3
II: R_1 = OCH_3, R_2 = OCH_3, R_3 = H
III: R_{1-2} = —OCH_2O—, R_3 = H
IV: R_1 = H, R_2 = H, R_3 = H
V: R_1 = OCH_3, R_2 = H, R_3 = H
VI: R_1 = H, R_2 = OCH_3, R_3 = OCH_3
VIII: R_1 = OCH_3, R_2 = OH, R_3 = H
IX: R_1 = H, R_2 = OCH_3, R_3 = H



VII



X

EXPERIMENTAL[2]

Plant Material—The plant material[3] was collected in Mexico during July 1972. The leaves and twigs were harvested, air dried, and milled to a coarse powder.

Preparation of Crude Alkaloid Fraction—Five kilograms of the plant material was extracted in a soxhlet apparatus for 36 hr with petroleum ether (bp 30–60°). After air drying, the defatted plant material was exhaustively extracted with methanol. The methanol extract yielded 502 g of residue after evaporation *in vacuo*.

A portion of the residue (450 g) was taken up in methanol (1 liter), and 2 *N* HCl (2 liters) was added. After thorough mixing, the methanol was removed *in vacuo*. The resulting acidic solution was made alkaline with 28% NH_4OH and extracted several times with chloroform until the final chloroform extract gave a negative alkaloid test with Mayer's reagent. The chloroform fractions were combined, dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to yield a residue (38 g).

Separation of Alkaloid Fraction—The major portion of the crude alkaloid fraction (33 g) was chromatographed on silica gel PE_{254}[4] (1.5 kg), employing benzene–chloroform–methanol (3:2:1) as the eluent. Fifty fractions (100 ml each) were collected (Column I). Fractions 14–26 were pooled and taken to dryness; then the residue was taken up in methanol, resulting in the formation of a crystalline compound (847 mg).

Based on the similarity of their TLC patterns on silica gel G

[1] The extracts were tested under the auspices of the Drug Research and Development branch of the National Cancer Institute. An extract is considered active if it causes a prolongation of life (T/C) in excess of 125% in the P-388 leukemia system and cytotoxic if it exhibits an $ED_{50} \leq 20$ μg/ml in the 9KB cell culture test system (9).

[2] All melting points were determined using a Köfler hot-stage instrument and are uncorrected. UV spectra were recorded on a Beckman model DB-G spectrophotometer using methanol as the solvent. IR spectra were recorded using a Beckman model 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1}. Intensities are reported as s (strong), m (medium), and w (weak). NMR spectra were recorded on a Varian Associates model T-60A instrument using tetramethylsilane as the internal standard. The chemical shifts are reported in parts per million (δ). Mass spectra were recorded using a Hitachi Perkin-Elmer model RMU-60 instrument.

[3] Voucher specimens were identified as *Helietta parvifolia* (Rutaceae) (CA-2022) by R. E. Perdue, Jr., and deposited at the Herbarium of the National Arboretum, Agricultural Research Service, U.S. Department of Agriculture, and at the Herbarium of the Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center.

[4] E. Merck, Darmstadt, Germany.

Table I—Chemical Shifts of Aromatic Protons in Selected Furoquinoline Alkaloids

	δ, Chemical Shift, ppm				δ, Chemical Shift, ppm			
	C-5	*ortho* Shift	*meta* Shift		C-8	*ortho* Shift	*meta* Shift	
6-Methoxydictamnine	7.50	0.61	0.06		7.90	0.60	0.02	0.05
7-Methoxydictamnine	8.10	0.66	0.01		7.25			
6,7-Dimethoxydictamnine	7.44				7.30	0.63		
Dictamnine	8.11				7.88			

plates[4], using Dragendorff's spray reagent and UV light as visualizing agents, the crystallization mother liquor (pooled fraction 14–26) was combined with fractions 27–41 and taken to dryness *in vacuo* to yield a residue (18 g). This residue was rechromatographed over a silica gel PF_{254} column (1.0 kg) (Column II), using chloroform as the eluent. A total of 303 fractions (100 ml each) was collected.

RESULTS AND DISCUSSION

Isolation and Identification of Flindersiamine (I)—The residue from pooled fractions 53–70, obtained from Column II, yielded a crystalline material (993 mg) from methanol. This material was shown to be identical to the compound isolated from fractions 14–26 (Column I) by mixed melting point and co-TLC. Recrystallization of one sample from methanol afforded an analytical sample, mp 205–206°; λ_{max} (methanol): 251 (log ε 4.91), 312 (4.17), and 325 (4.16) nm, indicating a 4-methoxyfuroquinoline moiety (10).

Mass spectral examination indicated a molecular ion at *m/e* 273 (100%). The fragmentation pattern, showing significant peaks at *m/e* 272 (55), 258 (9), 244 (44), 243 (27), 230 (40), 228 (47), 200 (15), and 172 (16), was identical with that reported for I (11). The NMR spectrum (dimethyl sulfoxide-d_6) showed two methoxy singlets at δ4.06 and 4.37; a singlet integrating for two protons at δ6.15, indicating the presence of a methylenedioxy group; two doublets (1H each) at δ7.38 (J = 2 Hz) and 7.97 (J = 2 Hz), indicating the presence of two furano protons; and a singlet (1H) at δ7.22, indicating the presence of an aromatic proton. The IR spectra of the isolate and reference flindersiamine[5] were superimposable, and the mixed melting point showed no depression, thus confirming the identity of the alkaloid.

Isolation and Characterization of Heliparvifoline (VIII)—The residue of pooled fractions 124–184, from Column II, yielded a crystalline material (239 mg) from methanol. This material, upon recrystallization from methanol, afforded an analytical sample, mp 245–247°. The UV spectrum exhibited absorption at λ_{max} (methanol): 247 (log ε 4.59), 313 (3.93), 324 (3.96), and 337 (3.89) nm, indicating a 4-methoxyfuroquinoline moiety (10). Upon addition of 0.1 *N* NaOH, the UV spectrum exhibited a bathochromic shift to 360 nm (log ε 3.99), suggesting the presence of a phenolic group. The original spectrum did not change upon the addition of 0.1 *N* HCl.

An IR spectrum (KBr) showed absorption bands at λ_{max} 3250–2500 (broad) (OH), 1625 (m), 1610 (s), 1550 (m), 1490 (s) (C=C), 1370 (s), 1270 (s), and 1050 (s) (=COC) cm^{-1}. Mass spectral examination indicated a molecular ion at *m/e* 245 (100%). Other significant peaks were observed at *m/e* 230 (55), 202 (19), 187 (10), 172 (15), and 159 (7). This fragmentation pattern is indicative of a dictamnine derivative (11).

The NMR spectrum (dimethyl sulfoxide-d_6) showed a broad singlet (1H) at δ3.37, which disappeared upon the addition of deuterium oxide. This signal was attributed to the presence of a phenolic proton. Two singlets (3H each), at δ3.90 and 4.40, suggested the presence of two methoxy groups, the downfield signal being due to the C-4 methoxy (12). Two singlets at δ7.20 and 7.43, each integrating for one proton, were assigned to two *para*-aromatic protons (at C-5 and C-8). Two doublets integrating for one proton each at δ7.92 and 7.38, with coupling constant J = 2 Hz, were assigned to C-2 and C-3 of the furan moiety, respectively. To determine the respective positions of the hydroxyl and methoxy groups on the aromatic ring, it was essential to assign the signals at δ7.20 and 7.43. Methylation with diazomethane at room temperature for 3 hr afforded kokusaginine (II) in high yield.

The NMR spectrum of II in dimethyl sulfoxide-d_6 showed three methoxy singlets at δ3.88, 3.92, and 4.40; two doublets integrating for one proton each at δ7.40 and 7.90 ppm (J = 2 Hz) for C-2 and C-3; and two singlets (1H each) at δ7.30 and 7.44, representing the two aromatic protons. To assign these resonances to the C-5 and C-8 protons of II, the aromatic regions of three additional compounds [6-methoxydictamnine (V), 7-methoxydictamnine (IX), and dictamnine (IV) itself] were examined.

The interpretation of the aromatic region in the NMR spectra of V and IX left little room for alternative explanations. In V, the two *ortho*-protons resonated at δ7.90 and 7.34; the latter was further coupled (J = 2 Hz) to a signal at δ7.50. In IX, the *ortho*-protons resonated at δ8.1 and 7.1; the latter was coupled (J = 2 Hz) to a signal at δ7.25.

There were two possible assignments for the δ7.30 and 7.44 resonances in II. It was felt that a decision could be made based on an examination of the effect of introducing an *ortho*-methoxy group on the chemical shift of an adjacent aromatic proton. In the absence of further structure modification, this shift would be expected to be reasonably consistent as a methoxy group is introduced at the 6- and 7-positions on the aromatic ring.

Table I shows the data for the four compounds under discussion, with a particular proton assignment selected for the aromatic protons of II. The shifts on introduction of a methoxy group *ortho* and *meta* to the C-5 and C-8 protons are also summarized in Table I. Introduction of a methoxy group consistently had the effect of shielding the adjacent *ortho*-proton by approximately δ0.62 and the *meta*-proton by approximately δ0.03. Therefore, the resonance at δ7.44 was assigned to the C-5 proton of II and that at δ7.30 was assigned to the C-8 proton, in agreement with a previous assignment (12).

The aromatic proton resonances remained to be assigned and thereby the position of the hydroxyl and methoxy groups in heliparvifoline.

In the methylation of heliparvifoline to II, the proton adjacent to the phenolic group was expected to experience the greatest shift. This proton, originally resonating at δ7.20, is deshielded by 0.1 ppm in II. This proton is at C-8 in II. Therefore, the phenolic group is located at C-7 in heliparvifoline.

An electron-releasing phenoxide ion is expected to cause increased shielding of the *ortho*-proton while affecting very little the *meta*-proton (13). Therefore, the resonance of the proton experiencing the greatest shift should be that due to the proton adjacent to the phenolic group. When the NMR spectrum of heliparvifoline was recorded in dimethyl sulfoxide-d_6 in the presence of a drop each of deuterium oxide and sodium deuteroxide, the resonance of the upfield aromatic proton was shifted from δ7.20 to 7.03 while the downfield proton was shifted from δ7.43 to 7.37. Thus, the hydroxyl group is located at the position adjacent to the proton resonating at δ7.20. This proton was previously assigned to the proton at C-8, thereby confirming the location of the hydroxyl group at C-7. Therefore, heliparvifoline has Structure VIII.

Isolation and Characterization of Isoflindersiamine (X)—Fractions 216–222, from Column II, were combined, taken to dryness, and dissolved in a minimum of hot methanol. From the cooled solution, 43 mg of a crystalline material was obtained. Recrystallization from methanol afforded an analytical sample, mp 212–213°; λ_{max} (methanol): 262 (log ε 4.32), 307 (3.60), 333 (3.73), and 348 (3.72) nm, indicating an *N*-methyl-4-furoquinoline moiety

[5] Supplied by Dr. E. Ritchie, Department of Organic Chemistry, University of Sidney, Sidney, N.S.W., Australia.

(14, 15). No change in the UV spectrum was observed upon the addition of either 0.1 *N* NaOH or 0.1 *N* HCl.

The IR spectrum (KBr) showed absorption bands at υ_{max} 3105 (w), 2950 (w), 2910 (w), 1610 (s), 1580 (w), 1450 (s) (C=C), 1470 (s) (CH_2), 1275 (s), 1050 (s) (=COC), and 930 (s) (CO) cm^{-1}. The NMR spectrum (dimethyl sulfoxide-d_6) showed two singlets (3H each) at δ4.00 and 4.10 ppm, representing one methoxy group at C-8 and one *N*-methyl group. A singlet integrating for two protons at δ6.23 ppm indicated the presence of a methylenedioxy group; two doublets at δ7.00 (J = 2 Hz) and 7.77 (J = 2 Hz) ppm, integrating for one proton each, were assigned to C-3 and C-2, respectively. A singlet integrating for one proton at δ7.50 ppm was assigned to a single aromatic proton.

Mass spectral examination indicated a molecular ion at *m/e* 273 (100%). The fragmentation pattern showed significant peaks at *m/e* 258 (42), 230 (9), 228 (12), 202 (5), 200 (6), and 172 (12). This fragmentation pattern differed from that of flindersiamine (I). The compound exhibited properties similar to those reported for isoflindersiamine (X) (16).

Previous work (16) had established a thermal isomerization of I to X. Repetition of this reaction[6] using the isolated I afforded X in 36% yield. The synthetic X exhibited identical UV, IR, mass, and NMR spectral data to the natural material, and a mixed melting-point determination showed no depression. The identity was therefore confirmed as X. Although not a new compound, this is the first reported natural occurrence of X.

Biological Activity—All three alkaloids isolated in this study were inactive in the 9KB cell culture and P-388 leukemia test systems.

SUMMARY

Separation of the chloroform-soluble alkaloid fraction of *H. parvifolia* by column chromatography resulted in the isolation of flindersiamine (I), isoflindersiamine (X), and a new furoquinoline alkaloid, heliparvifoline (VIII), whose structure was deduced by spectral means. The structure of X was confirmed by partial synthesis from I.

The copious yield of flindersiamine established it to be the major tertiary base in the aboveground parts of this plant. The quaternary alkaloid fraction of this plant is currently being investigated for the compound(s) responsible for the antileukemic and cytotoxic activities observed in the crude extracts.

[6] Flindersiamine (I) was heated in the presence of methyl iodide at 100° for 4 hr in a sealed tube. Crystallization from methanol afforded isoflindersiamine (X), mp 211–212°.

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