OXOAPORPHINE ALKALOIDS FROM *SIPARUNA GILGIANA*

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Siparuna species (Monimiaceae) are trees, shrubs, or climbers indigenous to tropical and subtropical regions of the Southern Hemisphere (1–3). There are no reported ethnomedical uses of *Siparuna gilgiana* Aubl. However, the leaves of an unidentified Ecuadorian *Siparuna* species known locally as "Guayusa" were used to treat stomach pain and female sterility, the leaves of *S. guianensis* Subl. were used in Brazil to treat mange and other skin disease, and *S. nicaraguensis* Hemsl. was reported to have unspecified medicinal uses (4).

Although *Siparuna* is the largest genus of the Monimiaceae (5), only two species have been phytochemically investigated. Screening of *S. pauciflora* D.C. fruit revealed the presence of alkaloids (6). Two oxoaporphine alkaloids, liriodenine and cassamedine, as well as β-sitosterol and stigmasterol were isolated from *S. guianensis* (7). This communication reports the isolation of the oxoaporphines liriodinine and oxonantenine from a methanolic extract of *S. gilgiana* roots. The alkaloids were identified by a comparison of their mp, uv, ir, pmr, and ms with those of reference compounds.

This represents the first phytochemical investigation of *S. gilgiana* as well as the first reported isolation of oxonantenine from the genus *Siparuna*.

EXPERIMENTAL[2]

Plant material.—Roots of *Siparuna gilgiana* Aubl. were collected in Peru during June, 1974, and were provided through the auspices of the United States Department of Agriculture under contract from the National Cancer Institute. The plant was identified by Dr. R. E. Perdue, Jr., formerly of the Economic Botany Laboratory, Agricultural Research Service, U.S.D.A., Beltsville, Maryland. A voucher specimen was deposited in the Herbarium of the National Arboretum, Agricultural Research Service, U.S.D.A., Washington, D.C.

Extraction and fractionation.—The dried, coarsely milled roots (1.49 kg) were continuously extracted for 36 hr with methanol in a Lloyd-type extractor, and the extract was evaporated *in vacuo* at 40°. The residue was dissolved in methanol (1 liter), an equal volume of 2N HCl was added, and the methanol was removed *in vacuo*. Following filtration of the slurry, the process was repeated three times and the acid extracts were combined and made alkaline with 28% NH_4OH. Extraction with chloroform (1 liter each time) was continued until the final extract gave a negative alkaloid test with modified Dragendorff's reagent (8). The chloroform extracts were combined, dried (Na_2SO_4), filtered, and evaporated to dryness *in vacuo* to yield Fraction A (56.2 g). Fraction A was dissolved in methanol (700 ml), 10% acetic acid (700 ml) was added, and the methanol was removed *in vacuo*. The resulting suspension was filtered, and the process was repeated for the insoluble residue. The combined acetic acid filtrates were extracted four times with petroleum ether (bp 30–60°; 700 ml), and the aqueous fraction was basified with 28% NH_4OH (*p*H 9–10) and extracted five times with chloroform (700 ml each). The chloroform extracts were combined, dried (Na_2SO_4), filtered, and evaporated to dryness *in vacuo* to yield Fraction B (20.36 g).

Fraction B was chromatographed over a column of Merck Silica gel PF_{254} (2 kg). Elution was initiated with chloroform; the polarity of the eluting solvent was gradually increased by the addition of increasing amounts of methanol to chloroform. Frac-

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[2]Melting points were determined by means of a Kofler hot-stage microscope and are uncorrected. Uv spectra were obtained with a Beckman model DB-G grating spectrophotometer. Ir spectra were recorded on a Beckman model 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1}. Pmr spectra were obtained with a Varian model T-60A instrument with Nicolet TT-7 Fourier transform attachment; tetramethylsilane was used as an internal standard. Mass spectra were obtained with a Hitachi Perkin-Elmer model RMU-60 single focussing spectrometer or a Varian MAT 112S double focussing spectrometer. Hplc separations were carried out with Waters Associates model 6000-A liquid chromatographs equipped with a Rheodyne model 7120 syringe-loaded sample injector, Waters Assoc. model 660 solvent programmer, Waters Assoc. model 450 variable wavelength uv spectrophotometer, Texas Instruments Servo-Riter II portable recorder, and Waters Assoc. 3.9 mm I.D. x 30 cm μPorasil column.

tions (100 ml each) were collected and combined on the basis of similar tlc patterns in an appropriate system.

IDENTIFICATION OF LIRIODENINE.—Chromatographic fractions 100–140 (chloroform eluant) were combined, evaporated to dryness *in vacuo*, and the residue (1.22 g) dissolved in warm chloroform. After cooling to room temperature, yellowish green needles were deposited, collected, and recrystallized from chloroform to give liriodenine (0.2 g, 0.014%), mp 252–258° (dec.). The uv, ir, pmr and ms data are in agreement with those reported for liriodenine (9), and the identity was confirmed (mmp, tlc, ir, pmr, ms) by comparison with an authentic sample.

IDENTIFICATION OF OXONANTENINE.—Chromatographic fractions 1034–1181 (chloroform-methanol, 9:1 eluant; 1.01 g) were combined, evaporated to dryness *in vacuo*, dissolved in methanol (20 ml), and an equal volume of 1N HCl was added. The methanol was evaporated *in vacuo* and the mixture was filtered. This process was repeated until the acid-insoluble residue gave a negative alkaloid test with modified Dragendorff's reagent. Combined acid extracts were made alkaline with 1N NaOH (*p*H 13) and extracted five times with chloroform (40 ml). The pooled, dried chloroform extracts (0.2 g) were combined with chromatographic fraction 1019–1033 (chloroform-methanol, 24:1; 0.47 g) since both were similar by tlc. Preparative tlc of the combined material on Silica gel PF_{254} and ethyl acetate-methanol (9:1) yielded a major component (0.14 g), which was subsequently chromatographed on a μPorasil column by gradient elution hplc. The gradient (curve 10 of Waters Assoc. solvent programmer) was conducted over 20 min with 5% methanol in chloroform changing to 20% methanol in chloroform. The solvent flow rate was 0.3 ml/min with uv detection at 275 nm. The major hplc fraction was collected and reinjected onto the column under identical conditions to give 0.114 g of residue which, when crystallized and recrystallized from warm ethanol, yielded yellow-brown crystals (27.0 mg, 0.0019%), mp 225–227° (dec.); uv, λ max (MeOH) 228 (log ε 4.72), 242 (4.57), 270 (0.60), 285 (4.05), 318 (3.41), and 360 nm (3.64); ir, ν max (KBr) 1650 cm^{-1} (s, highly conjugated C=O); pmr, δ ($CHCl_3$) 3.99 (s, 3H), 4.08 (s, 3H), 6.12 (s, 2H), 7.16 (s, 1H), 7.73 (d, J=5.1 Hz, 1H), 7.97 (s, 1H), 8.64 (s, 1H), and 8.85 (d, J=5.1 Hz, 1H); ms, *m/e*, M^+ 335 (45.0%), 320 (33.7), 292 (7.4), 290 (7.6), 277 (5.2), 262 (7.2), 249 (2.4), and 247 (1.4).

Although reference oxonantenine was unavailable, these physical data (ir, pmr) were identical with reference spectra for authentic compound.

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