QUINOLIZIDINE ALKALOIDS OF *CLATHROTROPIS BRACHYPETALA*

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Clathrotropis brachypetala (Tul.) Kleinh. (1) (Leguminosae) is a rain forest tree of Trinidad, W.I., and adjacent areas of South America (2). The plant was originally described as a species of *Diplotropis* and is still occasionally referred to as a member of that genus (3). The only previously reported phytochemical data for the genus concerns the silica content of *Clathrotropis* wood (4).

Although a decoction of *C. brachypetala* bark has reportedly been used by natives to destroy vermin (3), the property of the plant which led to this investigation was the very low level of predation of its rather large and fleshy seeds in communities where intense seed predation had been observed for other legume species with similar seeds (5). This indication of possible toxicity was confirmed when a suspension of the ground and defatted seeds was found to produce paralysis, convulsions, and respiratory arrest when administered orally to mice by intubation.

Fractionation of an ethanolic extract revealed that the toxic constituents resided in the alkaloid portion of the seeds. A number of alkaloid-containing legumes are closely related taxonomically to *C. brachypetala*. Hutchinson (3) places the genus *Clathrotropis* in the tribe Sophoreae of the subfamily Lotoideae, and almost all of the alkaloid containing genera of this tribe are known to contain quinolizidine derivatives (6).

Because of this taxonomic relationship, the alkaloid fraction of *C. brachypetala* was chromatographically (gc and tlc) screened for quinolizidine bases. A number of these compounds were detected and tentatively identified by their chromatographic and mass spectral properties by gc-ms. To confirm the identifications, the detected alkaloids were isolated by high pressure liquid chromatography and/or preparative tlc. Once isolated, each alkaloid was compared chromatographically with a standard in three tlc and two gc systems. The following compounds were identified from their chromatographic and mass spectral properties: anagyrine (1.29% of dry weight), cytisine (1.23%), Δ^5-dehydrolupanine (0.38%), rhombifoline (0.09%), 11-allylcytisine (0.07%), lupanine (0.05%) and *N*-methylcytisine (0.04%). Quantitative analysis was performed by use of a previously reported gc method (7).

Most of the alkaloids found in *C. brachypetala* seeds are widely distributed and have been reported from several members of the Leguminosae (6). An exception is 11-allylcytisine, which was only recently

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reported to be a trace constituent of unripe *Sophora secundiflora* fruits (8).

The alkaloid content of *C. brachypetala* seeds (1.26% of fresh weight) appears to explain their observed toxicity. The acute toxicity of cytisine is well documented (9), and this alkaloid probably contributes most to the lethal effects of the seeds. Little is known about the acute effects of anagyrine; however, this compound is suspected to be teratogenic (10). Because of their relatively low concentration, it is doubtful that the other alkaloids contribute significantly to the acute toxicity of the seeds. However, with the exception of *N*-methylcytisine (9), very little is known about the acute effects of the minor alkaloids.

The occurrence of toxic levels (1.26% fresh weight) of alkaloid in the seeds of *C. brachypetala* also appears to explain the low rate of seed loss due to predation by animals. Seed predation can be a significant factor influencing the reproductive success of some plants. By apparently conferring relative immunity against seed predation during the long post-seed-dispersal pre-germination period (up to 3 months), the alkaloid content of *C. brachypetala* seeds undoubtedly contributes to the reproductive success of this species. In addition, by protecting the large nutrient reserves of the seeds, the alkaloid content indirectly influences the competitive ability of the seedlings, which must establish themselves and grow under conditions of limited light and severe competition for soil nutrients (5).

EXPERIMENTAL

PLANT MATERIAL.—Mature *Clathrotropis brachypetala* seeds were collected in the Victoria-Mayaro Reserve, Trinidad, W.I., during 1976 and were stored frozen until freeze-dried for this study in June, 1977. The plant material was collected and identified by J. M. Rankin. A sample of the seeds has been deposited in the herbarium of the University of Michigan.

DETERMINATION OF TOXICITY.—Freeze-dried seeds were ground to a coarse powder in a Waring blender and defatted by Soxhlet extraction with petroleum ether (30–60°). The defatted seed material was then reduced to a fine powder in a ball mill. This powder was mixed with approximately 25% its weight of Tween 80, and water was added to give a suspension suitable for oral intubation of mice.

Five mice (female, Spartan) were anesthetized with ether and administered an oral dose of the suspension, equivalent to 10 g/kg of dried seed (8.8 g/kg of defatted seed). The mean dose volume was 0.8 ml. Five control animals received an equivalent dose of the Tween 80-water vehicle and exhibited no adverse effects. However, the effect of the *Clathrotropis* seed suspension was rapid and lethal. All five treated mice died within 10 minutes and exhibited convulsions, paralysis, and respiratory arrest. The petroleum ether extract residue (12.2% of the dried seed) was tested in a similar way and did not show any toxic effects during the 24-hour observation period.

In an effort to determine the nature of the toxic constituent(s), a portion of the defatted seed material was fractionated. The defatted seed powder (10 g) was exhaustively percolated with 95% ethanol, and the alcohol was removed by evaporation *in vacuo* to yield 1.6 g of residue. The residue was partitioned between 20 ml of 2% citric acid (pH 3) and dichloromethane. The dichloromethane extract was reduced to dryness, and the residue was found to be nontoxic when administered to mice as described above. The citric acid extract was basified (pH 10) and extracted with three 20-ml portions of dichloromethane. The residue from this dichloromethane extract was found to be toxic to mice and produced effects similar (stimulation followed by convulsions, paralysis, and in one of three animals tested, respiratory arrest) to those exhibited by the defatted seed. The pH of the citric acid extract was then adjusted to 6 and tested for toxicity. This extract did not exhibit toxic effects. Thus, the toxic constituent(s) of the seed appeared to reside in the alkaloid fraction of the seeds.

CHROMATOGRAPHIC SYSTEMS.—The following tlc systems utilized 0.25 mm silica gel G layers. Tlc system A: chloroform-methanol-ammonium hydroxide solution, 100:10:1. Tlc system B: ethyl ether-methanol-ammonium hydroxide solution, 100:10:1. Tlc system C: cyclohexane-diethylamine, 7:3. Alkaloids were detected with Dragendorff's reagent or 10% sulfuric acid followed by iodoplatinate reagent (11).

High pressure liquid chromatography (hplc) was conducted with two 3.9 mm x 30 cm μporasil® (10 μm silica, Waters Associates) columns in series. A Waters Associates 6000A solvent pump and 440A UV (254 nm) detector were employed. Two solvent

systems were used. Hplc system A: Chloroform-methanol-ammonium hydroxide solution, 100:10:1, flow rate 2.5 ml/min. Hplc system B: dichloromethane-methanol-ammonium hydroxide solution, 500:25:1, 3 ml/min.

The gas chromatography systems employed were as follows. Gc system A: 3% SP-2300 on Supelcoport, 100–120 mesh (36% cyanopropyl substituted silicone on acid washed, silanated diatomaceous earth), 2 mm id x 2 m glass, 250° isothermoal. Gc system B: 3% OV-17 on Gas Chrom Q, 100–120 mesh, 2 mm id x 2 m glass, 140° initial, 4°/min to 260°. Both systems used nitrogen as a carrier gas.

MASS SPECTRAL ANALYSIS.—Components of the alkaloid fraction were analyzed by combined gas chromatography-mass spectrometry. The effluent from gc system A or B entered the mass spectrometer through a glass jet separator maintained at 220°. The ion source temperature was 220° and the ionizing voltage was 70 ev. Spectra were recorded every 6 sec on a Du Pont 321 Dimaspec low-resolution mass spectrometer interfaced with a 320 data reduction system.

ALKALOID ISOLATION AND IDENTIFICATION.—Tentative identification of the alkaloids was made from gc retention times and mass spectral data. To confirm the identifications, the individual compounds were isolated and compared chromatographically with standards. The complexity of the alkaloid mixture, and the concentration differences between the alkaloids present, precluded direct determination of chromatographic characteristics using the alkaloid fraction. The alkaloids were isolated by preparative tlc (tlc system C, 1 mm layer) and/or hplc. The following is a summary of the data used to identify each alkaloid. In all cases the chromatographic and mass spectral data of the standard were identical to the isolated compound when obtained under the same conditions.

ANAGYRINE.—Isolated using hplc system B, retention time 3.8 min. Gc retention times: A 15.9 min, B 30.4 min. Tlc Rf values: A 0.55, B 0.27, C 0.23. Mass spectrum: M^+ *m/e* 244 (2%), 98 (100%).

CYTISINE.—Isolated using hplc system A, retention time 3.8 min. Gc retention times: A 7.9 min, B 23 min. Tlc Rf values: A 0.26, B 0.03, C 0.06. Mass spectrum: M^+ *m/e* 190 (28%), 146 (100%).

Δ^5-DEHYDROLUPANINE.—Isolated using hplc system B, retention time 3.4 min. Gc retention times: A 3.3 min, B 23 min. Tlc Rf values: A 0.65, B 0.36, C 0.48. Mass spectrum: M^+ *m/e* 246 (21%), 98 (100%).

RHOMBIFOLINE.—Isolated using hplc system B, retention time 2.6 min. Gc retention times: A 7.0 min, B 24.8 min. Tlc Rf values: A 0.66, B 0.51, C 0.25. Mass Spectrum: M^+ *m/e* 244 (2%), 203 (100%).

11-ALLYLCYTISINE.—Isolated using hplc system B, retention time 4.6 min, followed by ptlc (system C) to obtain separation from N-methylcytisine. Gc retention times: A 13.1 min., B 27.6 min. Tlc Rf values: A 0.46, B 0.15, C 0.16. Mass spectrum: M^+ *m/e* 230 (2%), 189 (100%).

LUPANINE.—Isolated using ptlc (system C). Gc retention times: A 4.5 min, B 24.0 min. Tlc Rf values: A 0.58, B 0.26, C 0.38. Mass spectrum: M^+ *m/e* 248 (36%), 136 (100%).

N-METHYLCYTISINE.—Isolated with 11-allylcytisine using hplc system B (retention time 4.6 min) followed by separation using ptlc (system C). Gc retention times: A 4.9 min, B 21.1 min. Tlc Rf values: A 0.46, B 0.17, C 0.19. Mass spectrum: M^+ *m/e* 204 (13%), 58 (100%).

QUANTITATION OF ALKALOIDS.—An internal standard (20 mg of *N,N*-dimethyl-3,4-dimethoxyphenethylamine) was added to 10 g of ground defatted seed material prior to percolation with 95% ethanol. The alkaloid fraction was obtained as previously described (7); the residue was analyzed using gc system A, which gives base-line separation between the components of the extract. The peak area-weight ratio obtained from analysis of standard solutions of the internal standard was used to determine the efficiency of extraction (89%).

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ERRATUM

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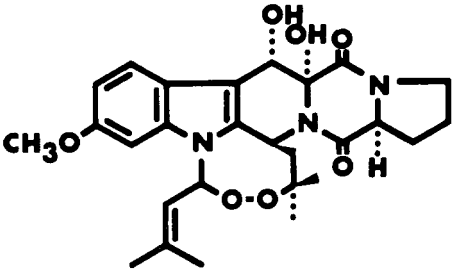

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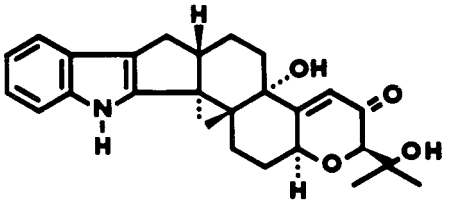

2. PAXILLINE