Cactus Alkaloids. XI. Isolation of Tyramine, N-Methyltyramine, and Hordenine from *Obregonia denegrii*[1]

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Introduction

Obregonia denegrii Fric is one of several small Mexican cacti commonly classed as "peyotes," perhaps indicating some relationship to *Lophophora* (1). In several reviews, Schultes (2–5) has emphasized the need for phytochemical analysis of these plants. Previous reports of the chemistry of *O. denegrii* are minimal. Reko (6) noted that the species is toxic and speculated that the simple alkaloid hordenine (anhaline, N,N-dimethyltyramine) might be present. In a preliminary screening of 31 cactus species, Dominguez et al. (7) tentatively identified β-sitosterol in petroleum ether extracts of *O. denegrii* and detected three Dragendorff-positive spots (alkaloids) on thin-layer chromatograms of ethanolic extracts. While undertaking a taxonomic re-evaluation of the monotypic genus *Obregonia*, Anderson (8) obtained microchemical precipitates indicative of alkaloids and also suggested that hordenine might be present. This current investigation was undertaken to further characterize the alkaloids of *O. denegrii*.

Experimental

Plant Material. Approximately 100 living plants of *O. denegrii* were purchased,[3] and representative plants are being maintained as greenhouse specimens.[4] The remaining plants were sliced, dried (69% moisture), and ground to a powder as previously described (9).

Extraction of Crude Alkaloids. A total of 3.1 kg of the powdered plant material was defatted, basified, and extracted with chloroform, using a 15- × 40-cm percolator with methods as previously reported (9). The chloroform extract was condensed under rotary vacuum to a thin syrup, and the syrup was extracted by shaking vigorously with 150 ml portions of 1 N HCl. Resulting emulsions were broken by rotary vacuum evaporation, and the aqueous portions were then decanted. After each decantation small amounts of chloroform were re-added to the residue, and the emulsification and extraction process was repeated until the aqueous washings were colorless. A total of 600 ml of acidic aqueous solution was thus obtained.

This solution was extracted twice with equal volumes of ethyl ether and twice with equal volumes of chloroform to remove non-alkaloidal, organic-soluble material. The pH of the aqueous solution was then adjusted to 10.5 with sodium hydroxide. The basic solution was extracted three times with equal volumes of chloroform and two times with equal volumes of ethyl ether. The organic extracts were dried over anhydrous sodium sulfate, filtered, combined, and condensed under rotary vacuum to a residue (crude alkaloid fraction A). After removal of traces of the organic solvents under rotary vacuum, the extracted, basic, aqueous solution was freeze-dried, and the residue from lyophilization was extracted three times with 40-ml portions of 10% absolute ethanol in chloroform. Rotary vacuum evaporation was then used to condense these combined washings to a residue (crude alkaloid fraction B). Crude alkaloid fractions A and B were each dissolved in 40 ml of ethanol and subjected to ion-exchange chromatography on 100-gm

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[3] Purchased from Homer Jones, Southwest Cactus Co., P. O. Box 851, Alpine, Texas.

[4] The identification was confirmed by Dr. Edward F. Anderson, Department of Biology, Whitman College, Walla Walla, Washington.

columns of Amberlite IRA 401[5] as previously described (10), to separate phenolic and nonphenolic alkaloids.

Thin-Layer Chromatography (TLC). TLC assays, using solvent systems, adsorbants, and visualization reagents as previously reported (9–11), revealed only minute quantities of unidentified alkaloids in the nonphenolic fractions of extracts A and B. The phenolic fractions of A and B, however, were shown to contain isolable quantities of tyramine, N-methyltyramine, and hordenine. For these identifications two different solvent systems [(1) ethyl acetate-methanol-conc. ammonium hydroxide (17:2:1) and (2) ethyl ether-acetone-methanol-conc. ammonium hydroxide (9:8:2:1)] were necessary. The concentrations of tyramine and N-methyltyramine were relatively higher than hordenine in fraction B, while hordenine predominated in fraction A.

Using phenolic extracts A and B, solvent system 1 was employed with four preparative TLC plates, utilizing methods as previously reported (9, 12), to separate the upper hordenine-containing band from a lower one containing a mixture of tyramine and N-methyltyramine. The contents of the lower band were cocrystallized as the hydrochloride salts (175 mg) (12), redissolved in ethanol, applied to five additional preparative TLC plates, and resolved by developing in solvent system 2.

Confirmation of Alkaloid Identification. The crystalline hydrochloride derivatives of the separated alkaloids were prepared as previously described (12).

From the upper band of the first series of plates, 64 mg of hordenine HCl was isolated. Synthetic hordenine HCl[6] and the isolated salt melted at 180–181° C,[7] while the mixed material melted at 179.5–181° C. The IR spectra[8] of the reference and isolated compounds were essentially identical.

The upper band of the second series of plates provided a total of 93 mg of tyramine HCl, which melted in the range of 269–276° C. After three recrystallizations from absolute ethanol-ethyl ether the resulting 62 mg melted at 272–275° C. Reference tyramine HCl[9] alone and upon admixture with the isolated compound melted at 273–275° C. IR spectra of the reference and isolated compounds were indistinguishable.

Elution of the lower band of the second series of plates yielded 5.2 mg of yellowish hydrochloride crystals melting at 142–147° C. A single recrystallization from absolute ethanol-ethyl ether gave 1.4 mg of white plates that melted at 149–152° C. Authentic N-methyltyramine HCl[10] melted at 150–152° C, and a mixture melting point was at the same temperature. Again IR spectra of the authentic and isolated compounds were indistinguishable.

Discussion

Tyramine HCl (0.003% yield) and hordenine HCl (0.002% yield) were crystallized from alkaloid extracts of *O. denegrii*. N-methyltyramine was isolated in a much lower concentration (0.0002% yield). Consequently, this species is unlike some other "peyote" cacti (*Lophophora* and *Ariocarpus* species) that contain only traces of, if any, tyramine (9–11, 13). Loss of appreciable tyramine and N-methyltyramine during the usual extraction procedures (10, 13) was prevented by further manipulations to produce crude alkaloid fraction B.

All three of these simple β-phenethylamines have been previously identified in other cacti (14, 15). Hordenine and N-methyltyramine have previously been crystallized from extracts of cacti; however, this is apparently the first report of the isolation of crystalline tyramine from a cactus species (9–11, 13). All three alkaloids are known pharmacologically to be sympathomimetics (16–18), and their presence might account for some physiological effects upon ingestion of the plant.

Acknowledgments

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[5] Mallinckrodt Chemical Works.

[6] Prepared from hordenine sulfate obtained from Mann Research Labs.

[7] Fisher-Johns melting point apparatus, uncorrected.

[8] KBr pellets, Beckman IR5A.

[9] Purchased from Calbiochem.

[10] Prepared from N-methyltyramine HBr, generously supplied by Dr. A. Brossi, Hoffmann-LaRoche, Inc.

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