Cactus Alkaloids XLII: 3,4-Dimethoxy-β-phenethylamine and Heliamine from the Mexican Cereoid *Backebergia militaris*

RACHEL MATA and JERRY L. McLAUGHLIN ˣ

Received May 31, 1979, from the *Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.* Accepted for publication July 16, 1979.

Abstract □ *Backebergia militaris* (Andot) Bravo ex Sánchez Mejorada yielded alkaloid crystals from a fractionated ethanol extract of only 20 g of plant material. The alkaloid was identified as heliamine (6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) hydrochloride. A second alkaloid, 3,4-dimethoxy-β-phenethylamine hydrochloride, was crystallized after preparative TLC of the mother liquors. Both compounds were isolated previously from other cactus species.

Keyphrases □ Alkaloids, cactus—heliamine, 3,4-dimethoxy-β-phenethylamine, isolation, identification □ Heliamine—isolation from cacti, identification, 3,4-dimethoxy-β-phenethylamine □ 3,4-Dimethoxy-β-phenethylamine—isolation from cacti, identification, heliamine □ Cactus alkaloids—heliamine, 3,4-dimethoxy-β-phenethylamine, isolation, identification

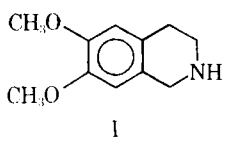
In a chemotaxonomic analysis of several giant Mexican cereoid species of cacti, the new technique of mass-analyzed, ion kinetic energy spectrometry was used to detect and identify alkaloids in ethanol extracts of small (1-g) samples of dried cactus material (1). This survey included *Backebergia militaris* (Andot) Bravo ex Sánchez Mejorada, in which alkaloids at *m/e* 194 and 208 were detected; the major components at these peaks were identified on the basis of fragmentation patterns as heliamine (6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) and *N*-methylheliamine (oxymethylcorypalline). Both alkaloids were identified previously in other cactus species (2–4). Alkaloid ions at *m/e* less than 194 were not examined.

DISCUSSION

In a previous TLC screening of this species as part of a continuing search for cactus alkaloids, two major compounds (a primary amine and a secondary amine) were detected in the screening extracts. Subsequently, an ethanol extract of only 20 g of plant material was subjected to anion exchange chromatography to resolve phenolic and nonphenolic alkaloids (5). Analytical TLC of the phenolic fraction was negative for any major alkaloids, but analytical TLC of the nonphenolic fraction detected two major and at least two minor alkaloid components. After acid–base partitioning of the nonphenolic fraction, one of the major alkaloids (I) crystallized directly as the hydrochloride. Preparative TLC of the mother liquor permitted resolution of the second major alkaloid from I and a third unidentified minor alkaloid; the second major alkaloid (II) then crystallized as the hydrochloride.

Analytical TLC of I with a series of reference cactus alkaloids suggested that it was heliamine (6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline); this identification was confirmed by melting-point comparisons, IR spectroscopy, and electron-impact mass spectrometry. TLC of II tentatively identified it as 3,4-dimethoxy-β-phenethylamine, and this identification was confirmed by its melting point and its IR and electron-impact mass spectra. Heliamine was reported initially as a natural product from the related cereoid cactus, *Pachycereus pecten-aboriginum* (Engelm.) Br. and R. (2); concurrently, it was crystallized from another Mexican cereoid, *Lemaireocereus weberi* (Coult.) Br. and R. (3). 3,4-Dimethoxy-β-phenethylamine was detected or isolated previously from several cactus species representing all three major cactus tribes (6–8).

Heliamine biogenesis would likely involve cyclization of a primary amine such as 3,4-dimethoxy-β-phenethylamine (or, perhaps, its 3-*O*-demethylated derivative) (9); consequently, the cooccurrence of heliamine and 3,4-dimethoxy-β-phenethylamine is not surprising. Heliamine reportedly has inhibitory activity against sarcoma 45 in rats (10), while 3,4-dimethoxy-β-phenethylamine has questionable involvement in certain mental processes (11).



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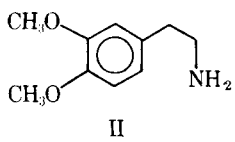

II

EXPERIMENTAL

Plant Material—Cuttings of fresh *B. militaris* (2.8 kg) conforming to published taxonomic descriptions (12) were obtained[1]. The fresh sections were sliced, frozen, freeze dried, and reduced to a powder through a 2-mm screen in a Wiley mill.

Alkaloid Extraction and Fractionation—Twenty grams of the powdered plant material was macerated with stirring in 1 liter of ethanol for 24 hr. After filtration to remove the marc, the ethanol extract was concentrated and subjected to anion-exchange chromatography on 20 g of resin[2] prepared in the hydroxide form (5). Upon analytical TLC using solvent systems and procedures as described previously (14), the phenolic fraction was devoid of any major alkaloids while the nonphenolic fraction contained two major and at least three minor compounds.

The residue from the nonphenolic extract was dissolved in 100 ml of 1 *N* HCl and partitioned with chloroform and ether (three times with 100 ml of each solvent). The aqueous solution pH was adjusted to 9.5 with sodium hydroxide, and the partitions were repeated. The combined chloroform–ether extracts from the basic solutions were dried over anhydrous sodium sulfate, concentrated, and acidified with 5% HCl in ethanol.

Isolation and Identification—*Heliamine (I)*—Upon further concentration of the acidic solution and addition of ether, a precipitate (300 mg) of alkaloid hydrochlorides formed. This precipitate was recrystallized (ethanol–ether) to produce 150 mg (0.75% yield) of the hydrochloride of I (mp 255°)[3]. Analytical TLC with Systems A, B, F, and G (14) identified I as heliamine; when the plate was sprayed with fluorescamine, the purple chromophore observed under UV light was diagnostic for the secondary amine function (15). IR[4] and electron-impact mass[5] spectra were essentially identical to those observed with previously isolated (mp 248°) and synthesized (mp 252°) heliamine hydrochloride (3).

3,4-Dimethoxy-β-phenethylamine (II)—The mother liquors from the crystallization of I were subjected to preparative TLC [eight plates, Solvent A (14)]. Three bands were scraped from the plates and eluted with ethanol: one was additional heliamine; a second was an unidentified compound which failed to crystallize; and the third, II, formed a crystalline hydrochloride (8 mg). Recrystallizations (ethanol–ether) raised the melting point to 149° (5 mg, 0.025% yield). Analytical TLC (14) identified II as 3,4-dimethoxy-β-phenethylamine; when the plate was sprayed with fluorescamine, the bright-yellow fluorescence observed under UV light was diagnostic for the primary amine function (15). IR and electron-impact mass spectra were indistinguishable from those of reference 3,4-dimethoxy-β-phenethylamine hydrochloride[6] (mp 150°).

[1] From Dr. Arthur C. Gibson, Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, who recently studied the phylogeny of the Mexican columnar cacti (13). The samples were collected June 25, 1978, 10 km west of Apatzingan on Highway 120, state of Michoacán, Mexico, from plants approximately 8 m in height. A voucher specimen is filed at the University of Arizona Herbarium: Gibson 3433 (ARIZ).

[2] Amberlite IRA 401S, Mallinckrodt Chemical Co.

[3] Mel-Temp apparatus, uncorrected.

[4] Beckman IR 33, potassium bromide pellets.

[5] DuPont 21-492B mass spectrometer (low resolution).

[6] Calbiochem.

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Determination of Two Fenamates in Plasma by High-Performance Liquid Chromatography

CHIEN K. LIN, CHING S. LEE ˣ, and JOHN H. PERRIN

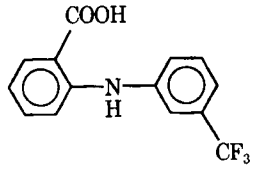
Received March 6, 1979, from the *Department of Pharmacy, College of Pharmacy, University of Florida, Gainesville, FL 32610.* Accepted for publication July 2, 1979.

Abstract □ A high-performance liquid chromatographic determination of two fenamates in human plasma is described. Plasma samples, 1.0 ml, to which 4 μg of internal standard had been added, were extracted with carbon tetrachloride under acidic conditions. Portions of the organic layer were transferred and evaporated to dryness under nitrogen. Residues were dissolved in methanol, and an aliquot was injected into the liquid chromatograph. An intermediate polarity, bonded cyanopropylsilane column was used with a mobile phase of water–acetonitrile–acetic acid (60:30:10 v/v/v). The flow rate was 1 ml/min, and the effluent was monitored at 254 nm. Flufenamic acid and mefenamic acid had retention times of 10.4 and 9.2 min, respectively. In the 1–10-μg range, the mean flufenamic acid recovery from control plasma was 100.7 ± 3.4% (n = 18). A typical calibration curve had a regression equation of $y = 0.132x - 0.04$ with $\gamma^2 = 0.99$. Preliminary stability tests showed that flufenamic acid is stable for at least 2 weeks in plasma after freezing.

Keyphrases □ Flufenamic acid—analysis, high-performance liquid chromatography, plasma, rats, humans □ Mefenamic acid—analysis, high-performance liquid chromatography, plasma, rats, humans □ High-performance liquid chromatography—analysis, flufenamic acid, mefenamic acid, plasma, rats, humans

Flufenamic acid[1] (I) and mefenamic acid[1] (II) are potent nonsteroidal analgesic and anti-inflammatory agents used in the management of rheumatoid arthritis. Spectrophotometric (1, 2), colorimetric (3), and fluorometric (2, 4, 5) methods have been applied for fenamate analysis in aqueous solution and in biological samples such as plasma, urine, and milk. A fluorometric method for the determination of I in the nanogram range used a chamber paper analysis apparatus (6). A convenient TLC technique was reported for screening three fenamates and their metabolites (7).

Only one GLC method (8) utilizing electron-capture detection has been reported. Although it was described for II, it could be adapted to the analysis of I in blood and urine. However, details of the assay were not provided (8).



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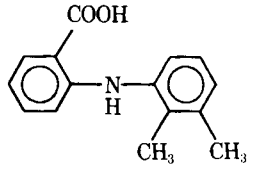

II

This article describes the high-performance liquid chromatographic (HPLC) determination of plasma fenamate levels. A single extraction step is followed by reversed-phase chromatography, eliminating the tedious and time-consuming procedures required by the previously reported methods (3, 7). Flufenamic acid and mefenamic acid can be internal standards for each other during either assay. The use of an internal standard improves both the precision and the accuracy of plasma level determination.

EXPERIMENTAL

Apparatus—Fenamate analyses were carried out on a liquid chromatograph[2] equipped with dual-delivery pumps[3], a single injector[4], and a single-chamber UV absorbance detector[5].

Reagents—Carbon tetrachloride[6], acetic acid[6], and sulfuric acid[7] were analytical reagent grade. Methanol[8] and acetonitrile[8] were distilled in glass. Solvents including distilled, deionized water were filtered routinely through 0.45-μm filters[9] prior to use in the liquid chromatograph.

[1] Provided by Parke-Davis and Co., Detroit, Mich.

[2] Model 204, Waters Associates, Milford, Mass.
[3] Model 6000A, Waters Associates, Milford, Mass.
[4] U6K, Waters Associates, Milford, Mass.
[5] Model 440, Waters Associates, Milford, Mass.
[6] Mallinckrodt, St. Louis, Mo.
[7] Eastman Kodak, Rochester, N.Y.
[8] Burdick & Jackson, Muskegon, Mich.
[9] Millipore Corp., Bedford, Mass.

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