Cactaceae Alkaloids XVIII: Two New Alkaloids from *Coryphantha calipensis* H. Bravo

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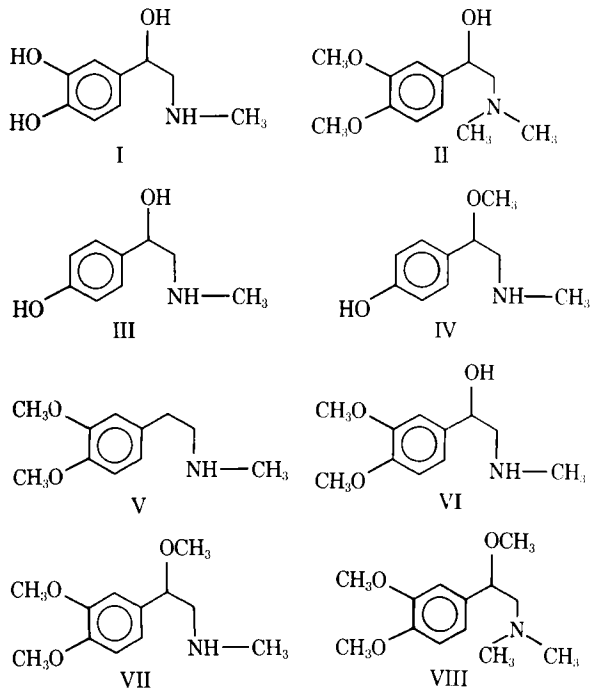
Abstract □ Two new alkaloids were isolated from the Mexican cactus *Coryphantha calipensis* H. Bravo. Based on chemical and spectroscopic data, their structures were determined as (−)-*N*-methyl-3,4-dimethoxy-β-methoxyphenethylamine [(−)-β-*O*-methylnormacromerine] and (−)-*N,N*-dimethyl-3,4-dimethoxy-β-methoxyphenethylamine [(−)-β-*O*-methylmacromerine]. The isolation of (−)-normacromerine and *N*-methyl-3,4-dimethoxyphenethylamine from this species is also reported.

Keyphrases □ Cactus alkaloids—isolation, identification of alkaloids from *Coryphantha calipensis* H. Bravo □ *Coryphantha calipensis* H. Bravo—isolation, identification of alkaloids □ (−)-*N*-Methyl-3,4-dimethoxy-β-methoxyphenethylamine [(−)-β-*O*-methylnormacromerine]—isolation, identification from *Coryphantha calipensis* H. Bravo □ (−)-*N,N*-Dimethyl-3,4-dimethoxy-β-methoxyphenethylamine [(−)-β-*O*-methylmacromerine]—isolation, identification from *Coryphantha calipensis* H. Bravo

Since the end of the 19th century Cactaceae species have been known to produce alkaloids, but not until recently has attention been drawn to the genus *Coryphantha* (2). From *Coryphantha macromeris* (Engelm.) Br. & R., Hodgkins *et al.* (3) isolated the new alkaloid macromerine (II), which was reported to cause hallucinations in test animals. The structure of macromerine is closely related to epinephrine (I) and the β-phenethylamine alkaloids found in several cacti (4, 5).

Further work on *Coryphantha* species showed the presence of macromerine in *C. runyonii* Br. & R. as well (6), and this cactus also contains several simpler phenethylamines, *e.g.*, *N*-methyl-3,4-dimethoxyphenethylamine (V) (2) and *N*-methyl-4-methoxyphenethylamine (7). Recently, the β-hydroxylated phenethylamines normacromerine (8), *N*-formylnormacromerine, metanephrine, *N*-methylmetanephrine, and synephrine (9) were found in *C. runyonii*[1].

In a field screening of cacti in Mexico (10), *C. calipensis* H. Bravo (11) was found to give a positive test for alkaloids. Plants were collected and the alkaloids were extracted and studied according to the principles already outlined (2). This paper describes the isolation of alkaloids from *C. calipensis* and the structure elucidation of these alkaloids.



EXPERIMENTAL[2]

Plant Material—*C. calipensis* H. Bravo was collected in Mexico[3]. Plants[4] were collected on April 19, 1971, not far from Calipan, on the road between Tehuacán and Ajalpan, Puebla (11).

Isolation and Separation of Alkaloids—The fresh plants (2.56 kg) were cut to pieces and immediately homogenized in twice their weight of ethanol. The slurry was left overnight at 4°. The ethanol solution was filtered and evaporated to dryness to give a crude extract. Portions (5 g) of the crude extract were dissolved in 100 ml acetic acid (3%), and the solution was extracted with 100 ml chloroform. The aqueous phase was basified to pH 10 with ammonia and extracted with 2 × 100 ml chloroform followed by 100 ml of chloroform–ethanol (3:1). The combined chloroform extracts were condensed to almost complete dryness. This alkaloid extract was dissolved in chloroform and purified by passing through an acid diatomaceous earth[5] column as earlier described (2). The total amount of crude alkaloids obtained in this manner was 3.6 g, 0.14% of the fresh plant material.

One gram of the alkaloid extract was fractionated on a 2 × 50-cm aluminum oxide column[6]. The solvents used are shown in Table I. A total of 200 ml of each solvent was passed through the column. Fractions of 10 ml each were collected and analyzed by TLC and GLC. The fractions richest in the respective alkaloids were combined, and the alkaloids were crystallized as the hydrochlorides from absolute ethanol–ether.

TLC and GLC—GLC was carried out using a flame-ionization detector[7] and glass columns [1.82 m × 0.31 cm (6 ft × 0.125 in.)] packed with 5% SE-30 and 5% XE-60 on Gas Chrom P (100–120 mesh). Mass spectra were recorded with a gas chromatograph–mass spectrometer[8]. For TLC, silica gel G plates were chromato-

[1] *C. runyonii* is sometimes considered a variety of *C. macromeris*, and these two species have also been grouped together in the genus *Lepidocoryphantha* (*cf.*, 7, 9).

[2] Melting points were determined with a Leitz Mikroskopheitztisch 350, using calibrated thermometers. IR spectra were recorded in KBr or chloroform using a Perkin-Elmer 237 IR spectrophotometer. NMR spectra were recorded with a Varian A-60 instrument in deuterochloroform or tetradeuteromethanol with tetramethylsilane as internal standard. Optical rotation was measured on a Perkin-Elmer 141 polarimeter.

[3] By J. G. Bruhn and Dr. Hernando Sánchez-Mejorada.

[4] The identification of the plant material was checked by Dra. Helia Bravo H., Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, where a herbarium specimen has been deposited.

[5] Celite.

[6] Merck, activity IV according to Brockmann.

[7] Varian 204 Aerograph.

[8] LKB 9000.

graphed with chloroform–ethanol–diethylamine (85:5:10) or chloroform–ethanol–concentrated ammonia (85:15:0.4) and sprayed with an iodoplatinate solution to locate alkaloids (12).

Characterization of (−)-*N*-Methyl-3,4-dimethoxy-β-methoxyphenethylamine (VII)—The major compound eluted from the column (Table I) was crystallized as the hydrochloride (yield 210 mg), mp 213–214°; $[\alpha]D^{25}$ − 91.7°; c 0.010 g/ml in absolute ethanol. The homogeneity of the alkaloid was shown by TLC and GLC. NMR and mass spectra indicated that the compound is VII. The mass spectrum showed major peaks at *m/e* (relative intensity): 44 (67), 151 (8), 166 (17), 181 (100), 182 (28), and 225 (M^+, 0.4). The NMR spectrum (60 MHz) of the free base in deuterochloroform showed a singlet at δ 6.91, indicating three aromatic hydrogens, and a doublet of doublets centered at δ 4.32 (J_1 = 8 Hz, J_2 = 5 Hz), indicating a benzylic proton. Two singlets at δ 3.91 and 3.88 were indicative of two methoxyl groups, and two three-proton singlets at δ 3.29 and 2.48 indicated an *O*-methyl and an *N*-methyl group, respectively.

Oxidation of VII—Alkaline permanganate oxidation of the alkaloid hydrochloride (50.0 mg) was performed as earlier described for 3,4-dimethoxyphenethylamine (13). Sublimation *in vacuo* afforded 11 mg of a crystalline derivative, mp 178–181°, which was identified as 3,4-dimethoxybenzoic acid by IR spectral comparison. The reported (13) melting point for 3,4-dimethoxybenzoic acid is 181°.

Acid Hydrolysis of VII—Compound VII-HCl (20 mg) was hydrolyzed overnight at room temperature in hydrochloric acid (6 *N*, 10 ml). The major product was purified by preparative TLC and identified as normacromerine (TLC, GLC, and IR) by comparison with an authentic sample.

Characterization of (−)-*N,N*-Dimethyl-3,4-dimethoxy-β-methoxyphenethylamine (VIII)—The hydrochloride of this alkaloid was recrystallized from absolute ethanol–ether (yield 40 mg), mp 178°; $[\alpha]D^{25}$ − 93.7°; c 0.010 g/ml in absolute ethanol. The isolated material was chromatographically homogeneous (TLC and GLC). The mass spectrum showed major peaks at *m/e* (relative intensity): 58 (100), 181 (6), 182 (0.7), and 239 (M^+, 0.2). This suggested that VIII was the *N*-methyl analog of VII. The NMR spectrum (60 MHz) of the free base in deuterochloroform confirmed this interpretation, showing a singlet at δ 6.90, indicating three aromatic protons, and a doublet of doublets centered at δ 4.30 (J_1 = 8 Hz, J_2 = 4 Hz), indicative of a benzylic hydrogen. Singlets at δ 3.92 and 3.90 indicated two methoxyl groups, and another *O*-methyl group was indicated by a three-proton singlet at δ 3.25. A six-proton singlet at δ 2.35 indicated two *N*-methyl groups.

Methylation of VII—Compound VII-HCl (50 mg) was dissolved in methanol (4 ml), and 0.2 ml of 36% formaldehyde solution was added. The mixture was stirred for 15 min and then reduced for 1 hr with sodium borohydride (0.3 g). The methanol was evaporated and the residue was taken up in 0.5 *N* HCl (3 ml). The solution was made alkaline (ammonia) and extracted with ether. The ether residue was crystallized as the hydrochloride from methanol–ether to yield VIII-HCl, 40 mg, mp 177–179°; $[\alpha]D^{25}$ − 83.7°; c 0.010 g/ml in absolute ethanol. Comparison with VIII showed the two compounds to be identical in all respects [NMR, IR (chloroform), mass spectroscopy, TLC, GLC, melting point, and mixed melting point]. (±)-*N,N*-Dimethyl-3,4-dimethoxy-β-methoxyphenethylamine was also prepared from (±)-macromerine (II) by refluxing a solution of the latter compound in 2 *N* HCl in methanol (14). The product, isolated by preparative TLC, was identical with VIII and the *N*-methyl derivative of VII [TLC, GLC, NMR, IR (chloroform), and mass spectroscopy].

Identification of (−)-Normacromerine (VI)—The chloroform–ethanol fractions (Table I) contained one major alkaloid, crystallized as the hydrochloride (yield 135 mg), mp 131–132°; $[\alpha]D^{25}$ −51.3°; c 0.010 g/ml in absolute ethanol. TLC and GLC analyses showed the homogeneity of the isolated alkaloid and also afforded a preliminary identification as normacromerine (VI). The reported (8) melting point of 132–133° and the reported (8) optical rotation of $[\alpha]D^{27}$ −47.5°, c 0.020 g/ml in absolute methanol, of natural (−)-normacromerine hydrochloride from *C. runyonii* also support this identification. Superimposable IR (chloroform) spectra were obtained with an authentic sample of (±)-normacromerine hydrochloride (8, synthetic) and the now isolated alkaloid. In addition, the melting point of VI-HCl (mp 131–132°) was not depressed by the addition of (−)-normacromerine hydrochloride isolated from *C. runyonii* (mixed mp 129–132°).

Table I—Column Chromatographic Separation of the Crude Alkaloid Extract of *C. calipensis* H. Bravo

Solvent[a]	Alkaloids Identified	Structure
Benzene	—	—
Chloroform–benzene		
1:2	—	—
1:1	(−)-*N,N*-Dimethyl-3,4-dimethoxy-β-methoxyphenethylamine	VIII
2:1	(−)-*N*-Methyl-3,4-dimethoxy-β-methoxyphenethylamine	VII
	N-Methyl-3,4-dimethoxyphenethylamine	V
Chloroform	—	—
Chloroform–ethanol		
4:1	(−)-Normacromerine	VI
Ethanol	—	—

[a] For details, see *Experimental*.

Identification of *N*-Methyl-3,4-dimethoxyphenethylamine (V)—In the fractions following VII (Table I), GLC analysis indicated the presence of small amounts of *N*-methyl-3,4-dimethoxyphenethylamine. After purification by preparative TLC in chloroform–ethanol–diethylamine (85:5:10), this compound could be crystallized as the hydrochloride from chloroform–ether (yield 3 mg), mp 134–137° [lit. (5) mp 134–136°]. The IR spectrum and chromatographic behavior (TLC and GLC) confirmed the identity of these crystals with reference V-HCl.

RESULTS AND DISCUSSION

As in previous investigations (2, 9, 15), the now isolated *Coryphantha* alkaloids are all β-phenethylamine derivatives. The major alkaloids were VII and VI. The percentage of alkaloids in fresh plants of *C. calipensis* H. Bravo was found to be 0.14%. Compounds VII and VIII have not previously been isolated from nature, although they are closely related to the known cactus alkaloids normacromerine (VI) and macromerine (II), being the β-*O*-methyl ethers of these compounds.

The identity of the alkaloids was established by spectroscopic data, chemical transformations, and comparison with authentic reference materials using TLC, GLC, IR, NMR, and mass spectroscopy. The two important fragment ions at *m/e* 44 and 181 in the mass spectrum of VII result from a benzylic cleavage.

(−)-Normacromerine earlier was isolated from *C. runyonii* (8) and recently from *Desmodium tiliaefolium* (Leguminosae) (16). *N*-Methyl-3,4-dimethoxyphenethylamine was first identified in *C. runyonii* (2) and is found in several cacti (1, 5).

The relationship of these alkaloids to human neural transmittor substances is of special interest. Compounds VI, VII, and VIII may be regarded as *O*-methylation products of epinephrine. Epinephrine has not been found in plants as yet, but its analog norepinephrine was reported in various species (17). The sympathomimetic drug (−)-synephrine (III), closely related to norepinephrine, has been isolated from citrus leaves (18) and various *Coryphantha* species (9, 15, 19). Similar in structure to the now isolated methyl ethers of macromerine and normacromerine is (−)-β-*O*-methylsynephrine (IV), found in tangerine leaves by Stewart and Wheaton (14). These researchers suggested that this compound could be an artifact from their extraction process. Compound IV was also identified together with synephrine in *C. cornifera* var. *echinus* (15) and in *C. ramillosa* (19).

The alkaloids of *C. calipensis*, except for V, are all levorotatory, as is naturally occurring macromerine[9] (20). The cold extraction of *C. calipensis* was carried out with absolute ethanol to avoid the easy formation of methyl ethers from β-hydroxylated compounds (14, *cf.*, 19). Therefore, VII and VIII are believed to be natural constituents of *C. calipensis*.

[9] Natural (−)-macromerine and natural epinephrine were recently shown to have the same absolute configuration, *R* (20).

REFERENCES

(1) J. G. Bruhn and C. Bruhn, *Econ. Bot.*, **27**, 241(1973).

(2) S. Agurell, *Lloydia*, **32**, 206(1969).

(3) J. E. Hodgkins, S. D. Brown, and J. L. Massingill, *Tetrahedron Lett.*, **14**, 1321(1967).

(4) S. Agurell, J. G. Bruhn, J. Lundström, and U. Svensson, *Lloydia*, **34**, 183(1971).

(5) S. Agurell, J. Lundström, and A. Masoud, *J. Pharm. Sci.*, **58**, 1413(1969).

(6) L. E. Below, A. Y. Leung, J. L. McLaughlin, and A. G. Paul, *ibid.*, **57**, 515(1968).

(7) S. Agurell, *Experientia*, **25**, 1132(1969).

(8) W. J. Keller and J. L. McLaughlin, *J. Pharm. Sci.*, **61**, 147(1972).

(9) W. J. Keller, J. L. McLaughlin, and L. R. Brady, *ibid.*, **62**, 408(1973).

(10) J. G. Bruhn, *Cactaceas Suculentas Mex.*, **16**, 51(1971).

(11) H. Bravo H., *ibid.*, **9**, 79(1964).

(12) J. Lundström and S. Agurell, *J. Chromatogr.*, **30**, 271(1967).

(13) J. Lundström, *Acta Pharm. Suecica*, **7**, 651(1970).

(14) I. Stewart and T. A. Wheaton, *J. Org. Chem.*, **33**, 471(1968).

(15) K. M. Kelley Hornemann, J. M. Neal, and J. L. McLaughlin, *J. Pharm. Sci.*, **61**, 41(1972).

(16) S. Ghosal and R. S. Srivastava, *Phytochemistry*, **12**, 193(1973).

(17) P. B. Applewhite, *ibid.*, **12**, 191(1973).

(18) I. Stewart, W. F. Newhall, and G. J. Edwards, *J. Biol. Chem.*, **239**, 930(1964).

(19) P. T. Sato, J. M. Neal, L. R. Brady, and J. L. McLaughlin, *J. Pharm. Sci.*, **62**, 411(1973).

(20) S. D. Brown, J. E. Hodgkins, and M. G. Reinecke, *J. Org. Chem.*, **37**, 773(1972).

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Entropy of Transfer of Molecular Benzoic Acid from a Pure Liquid to an Aqueous Solution

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Abstract □ The solubility of benzoic acid in distilled, deionized water was determined over a limited temperature range. The entropy of transfer of molecular benzoic acid from a pure liquid to an aqueous solution was calculated. Data also were analyzed in terms of the hypothetical partial molal entropy of transfer of molecular benzoic acid from an ideal or a regular solution to an aqueous solution. Interpretation of the data indicates that solute–solvent interactions result in a reduction of the number of independent molecules relative to an ideal or a regular solution.

Keyphrases □ Benzoic acid—transfer entropy from pure liquid to aqueous solution, calculation of partial molal entropy □ Solutes, semipolar nonelectrolyte (benzoic acid)—transfer entropy from pure liquid to aqueous solution, equations □ Entropy of transfer from pure liquid to aqueous solution—benzoic acid, calculation of partial molal entropy

Molecular interactions involving solute (nonelectrolyte)–solvent (water) components have attracted considerable research interest (1–10). Solute substances nearly insoluble in water have provided several hypotheses about the structure of water and interactions occurring in this condensed liquid phase (1–4). To date, no one hypothesis has gained universal acceptance (8, 11–13).

Bulk properties of water, such as surface tension and the dielectric constant, have been useful for interpreting solution behavior (14, 15). The importance of the entropy of solution as an interpretive quantity also has been discussed (16, 17).

This study was undertaken to investigate the behavior of a semipolar nonelectrolyte solute, benzoic acid, in aqueous systems. Chertkoff and Martin (18) mentioned that benzoic acid serves as a prototype of relatively polar pharmaceutical solids. Solubility data at several temperatures were analyzed in terms of the entropy consequence of transferring molecular benzoic acid from a pure liquid to an aqueous solution. Equations were used which allow the calculation of the partial molal entropy of transfer from an ideal or a regular solution to an aqueous solution.

THEORETICAL

The partial molal entropy of solution, $\Delta\bar{S}_2$, developed by Hilde-