equilibrium between myocardial release and decay kinetics. The downslope is predominantly associated with decay phenomena (21).

Animals yielding the upper limits of enzyme release were experiencing serious ventricular arrhythmias and death.

Figure 2 demonstrates the relationship between myocardial damage (in grams) and serum creatine phosphokinase activity. These results agree with a previous conclusion that there is a direct relationship between infarct size and serial enzyme release (22). The pharmacological significance of this model is that it allows study of the dynamic changes of the infarct and also institution of possible therapeutic regimens.

This work also showed that there is a relationship among ECG T-wave elevation, grams of damage, and death in addition to the correlation with enzyme data. Of the 54 experimentally infarcted animals, 51 showed T-wave elevation indicating a hypoxic state. Furthermore, each of the 14 animals experiencing ventricular fibrillation and death was in the group displaying a T-wave elevation. This work demonstrates the sensitivity of enzyme analysis with respect to ECG wave alterations in assessing myocardial infarction.

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Cactus Alkaloids XXXIII: β-Phenethylamines from the Guatemalan Cactus *Pilosocereus maxonii*

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Abstract □ TLC analysis of extracts of *Pilosocereus maxonii* (Rose) Byles and Rowley detected six identifiable alkaloids. Preparative TLC aided in the crystallization of the hydrochlorides of *N*-methyl-3,4-dimethoxyphenethylamine, *N*-methyl-3-methoxytyramine, and *N,N*-dimethyl-3-methoxytyramine. Traces of 3,4-dimethoxyphenethylamine (TLC and mass spectrometry), tyramine (TLC), and *N*-methyltyramine (TLC) were identified. While all of these compounds were isolated and/or detected previously in other cactus species, this study is the first reported crystallization of *N*-methyl- and *N,N*-dimethyl-3-methoxytyramine from a natural source.

Keyphrases □ *Pilosocereus maxonii*—whole plant extract, six alkaloids isolated and identified □ Alkaloids—isolated from whole plant extract of *Pilosocereus maxonii* □ β-Phenethylamines, various—isolated from whole plant extract of *Pilosocereus maxonii*

The cactus genus *Pilosocereus* comprises some 50–60 species, ranging from Florida, the Carribean, and Mexico through Central America into northern South America (1–3). These plants are large, columnar, "giant cacti," and dense wooly hairs or bristles characteristically cover the areoles at the apex of the shoots. Throughout their natural range, species of *Pilosocereus* are used for hedges; a few have edible fruit, but their greatest value in the United States stems from their sale as ornamentals (4, 5). No previous reports have been made regarding the alkaloid content of members of this genus.

In a continuing survey of the Cactaceae for new alkaloid-containing species, extracts of *Pilosocereus maxonii* (Rose) Byles and Rowley were screened. This attractive species is indigenous to Guatemala (3), but propagated plants are available in the United States from commercial cactus dealers. Six β-phenethylamine alkaloids were identified by TLC, and three were successfully crystallized as their hydrochlorides. This paper describes the extraction, isolation, structure elucidation, identification, and synthesis procedures for these alkaloids.

EXPERIMENTAL

Plant Material—Terminal cuttings, both rooted and unrooted, of the plants were received on July 10, 1975, February 5, 1976, and March 5,

1976; rooted commercial stock remains available from the supplier[1]. Reference photographs are on file. The plants conformed to the description of *Cephalocereus maxonii* Rose as described by Britton and Rose (6). The name *Pilosocereus maxonii* (Rose) Byles and Rowley represents the latest taxonomic revision of the species (3); additional names include *Cereus maxonii* Vaupel and *Pilocereus maxonii* (Rose) Knuth (4, 6). The fresh plants were sliced, frozen, freeze dried at temperatures not exceeding 30°, and ground to a powder using a 2-mm sieve in a laboratory mill[2].

Preparation of Crude Alkaloid Fractions—The powdered plant material (500 g) was defatted, basified, and extracted with 16 liters of chloroform *via* percolation (7). The chloroform extract was filtered and condensed to produce a thick syrup, which was extracted with nine successive 300-ml portions of 1 *N* HCl. The filtered aqueous extracts were combined and processed, essentially as previously described (8, 9), to yield crude Fractions A (alkaloids), B (nonalkaloidal materials), and C (water-soluble alkaloids).

Analytical TLC detected an abundance of alkaloids in Fractions A and C, while Fraction B was devoid of alkaloids. Fractions A and C were combined in ethanol and resolved into phenolic and nonphenolic portions using a basic anion-exchange resin as previously reported (10).

TLC—Analytical TLC procedures (8, 9) provided tentative identification of four alkaloids in the extracts. Preparative TLC methods (8, 9) were used to resolve and isolate the major alkaloids in the phenolic and nonphenolic fractions. The TLC systems employed were Solvents A–G as previously described (8, 9); the adsorbants were silica gel plates[3] (analytical) and 1-mm layers of silica gel PF_{254} on 20 × 20-cm plates (preparative). A combination of UV light, fluorescamine[4], dansyl chloride, and tetrazotized benzidine as visualization aids (10, 11) served to distinguish between primary and secondary amines and to provide chromophores distinctive for various structures.

Resolution of Nonphenolic Alkaloids—TLC analysis of this fraction in Solvent E indicated the presence of one major alkaloid (R_f 0.34, secondary amine) and one minor alkaloid (R_f 0.62, primary amine). A 0.30-g portion (from the 2.50-g total) of the nonphenolic alkaloid fraction was resolved *via* preparative TLC (eight plates, one development each) in Solvent E. After detection under UV light (shortwave), the bands were removed and eluted with ethanol followed by methanol.

Identification of Nonphenolic Alkaloids—Synthetic *N*-methyl-3,4-dimethoxyphenethylamine (12) cochromatographed in five TLC solvent systems (A, C, and E–G) with the eluates of the alkaloid having R_f 0.34 in Solvent E. Visualization reactions (dark purple under UV with fluorescamine and a white chromophore with tetrazotized benzidine) were likewise identical with the reference compound. The hydrochloride was prepared as previously described (9) and was crystallized from ethanol–ether. The yield was 56.5 mg (0.094%), mp 135–137° [lit. (12) mp 135–137°], mixed melting point 135–137°; mass spectrum[5]: *m/e* 44 (base peak). The IR[6] spectrum was indistinguishable from the spectrum for reference *N*-methyl-3,4-dimethoxyphenethylamine hydrochloride.

With the same five TLC systems, the alkaloid having R_f 0.62 in Solvent E consistently cochromatographed with reference 3,4-dimethoxyphenethylamine[7]. The visualization reactions (bright yellow under UV with fluorescamine and faint yellow with tetrazotized benzidine) were identical with the reference compound. The trace of alkaloid in the eluate failed to produce a crystalline hydrochloride even after additional preparative TLC. A mass spectral determination of the eluate revealed a molecular ion peak at *m/e* 181 with a base peak at *m/e* 152, consistent with the mass spectrum of reference 3,4-dimethoxyphenethylamine hydrochloride.

Resolution of Phenolic Alkaloids—The phenolic alkaloid fraction (0.88 g) revealed two major alkaloids and two minor alkaloids upon TLC analysis in Solvent G. Preparative TLC (18 plates, three developments each) in Solvent G was used to resolve these compounds into two bands, each containing one major and one minor alkaloid.

The major alkaloid in the lower band gave TLC visualization reactions indicative of a secondary amine (dark purple under UV with fluorescamine and golden under UV after overspraying with dansyl chloride). Tetrazotized benzidine produced a yellow chromophore typical of 4-hydroxyphenethylamines (10). The hydrochloride of the major alkaloid crystallized from methylene chloride–ether (9.5 mg, 0.002% yield).

The major alkaloid in the upper band gave no reaction with fluorescamine, indicating a tertiary amine. Yellow fluorescence under UV after spraying with dansyl chloride confirmed the presence of a phenolic hydroxyl, and a yellow chromophore with tetrazotized benzidine again indicated a 4-hydroxyphenethylamine. The hydrochloride of this major alkaloid also crystallized from methylene chloride–ether (23.4 mg, 0.004% yield).

Crystallizations of the two minor alkaloids from the respective mother liquors of these crystallizations were not attempted.

Identification of *N*-Methyl-3-methoxytyramine—The unknown crystalline hydrochloride from the lower phenolic band gave UV spectra[8] [λ_{max} 228 (ϵ 5510) and 281 (ϵ 2594), 3.2×10^{-5} g/ml in methanol] that were similar to those of 3-methoxytyramine (13). Mass spectral data revealed a molecular ion peak at *m/e* 181 (1.1%, $C_{10}H_{15}NO_2$) with a base peak at *m/e* 44 (100%, C_2H_6N) and another peak at *m/e* 137 (7.4%, $C_8H_9O_2$), indicating β-cleavage of an *N*-methylphenethylamine (9). These data, combined with the TLC visualization reactions, were consistent with the identification of this alkaloid as *N*-methyl-3-methoxytyramine hydrochloride. The observed melting point of 150–153° was close to the literature (14) melting point of 154–155°, and the crystalline compound cochromatographed with a reference sample[9] of *N*-methyl-3-methoxytyramine hydrochloride in five TLC solvents (A, C, and E–G).

Identification of *N,N*-Dimethyl-3-methoxytyramine—The unknown crystalline hydrochloride from the upper phenolic band also gave UV spectra [λ_{max} 228 (ϵ 5590) and 281 (ϵ 2549), 4.0×10^{-5} g/ml in methanol] that were similar to those of 3-methoxytyramine (13). Mass spectral data showed a molecular ion peak at *m/e* 195 (1.2%, $C_{11}H_{17}NO_2$) with a base peak at *m/e* 58 (100%, C_3H_8N) and another peak at *m/e* 137 (3.6%, $C_8H_9O_2$), indicating β-cleavage of an *N,N*-dimethylphenethylamine (15). The data were consistent with the identification of this alkaloid as *N,N*-dimethyl-3-methoxytyramine hydrochloride. The observed melting point of 188–192° was comparable with the literature (14) melting point of 190–191°, and the crystalline alkaloid cochromatographed with a reference sample[10] of *N,N*-dimethyl-3-methoxytyramine hydrochloride in the usual five TLC systems (A, C, and E–G).

Identification of Tyramine and *N*-Methyltyramine—The minor alkaloids in the mother liquors from the upper and lower phenolic bands were identified as tyramine and *N*-methyltyramine, respectively. These compounds consistently cochromatographed (Solvents A, C, and E–G) with reference tyramine hydrochloride[11] and reference *N*-methyltyramine hydrochloride[12], and the TLC visualization reactions were identical with the reference compounds (11).

Synthesis of Reference *N,N*-Dimethyl-3-methoxytyramine Hydrochloride—The method of Cherayil (16) was followed with slight modifications (the suggested chromatographic purification of the product was not necessary). 3-Methoxytyramine hydrochloride[13] (290 mg, 1.5 mmoles) was mixed with sodium bicarbonate (42 mg, 1.5 mmoles), 88% (w/w) formic acid (131 mg, 7.5 mmoles), 37% (w/w) formaldehyde (203 mg, 7.5 mmoles), and 15 ml of dimethylformamide. The mixture was refluxed for 5 hr, cooled, added to a mixture of 15 ml of water and 20 ml of 0.5 *M* pH 10 sodium borate, extracted three times with 50-ml portions of ethyl acetate, and dried over anhydrous sodium sulfate. The extract was then evaporated under reduced pressure to obtain 0.21 g of slightly yellow liquid. The hydrochloride was prepared and crystallized from ethanol–ether to yield white crystals (mp 191–192°, 156 mg, 53.8% yield). Cochromatographic, mixed melting-point, IR, and mass spectral data identified the synthetic and isolated materials as *N,N*-dimethyl-3-methoxytyramine hydrochloride.

Synthesis of Reference *N*-Methyl-3-methoxytyramine Hydrochloride—Nitromethane was condensed with 4-benzyloxy-3-methoxybenzaldehyde[13] (15 g) *via* the method of Gairaud and Lappin (17), using ammonium acetate and acetic acid to yield 4-benzyloxy-3-methoxy-β-nitrostyrene [10.95 g, 73% yield, mp 121–123°, lit. (18) mp 122–123°]. The reduction of this compound (2.85 g) was performed using standard procedures with lithium aluminum hydride in anhydrous ether (19); the ether extract was dried over anhydrous sodium sulfate and saturated with anhydrous hydrogen chloride. The white precipitate of 4-benzyloxy-3-methoxy-β-phenethylamine hydrochloride was recrystallized from ethanol [2.13 g, 75% yield, mp 175–178°, lit. (18) mp 173–175°].

[1] Abbey Garden, Carpinteria, Calif.
[2] Model 4, Thomas-Wiley.
[3] Bakerflex-IB2.
[4] Fluram, Roche.
[5] Hitachi RMU-6 mass spectrometer.
[6] Obtained using potassium bromide pellets and a Beckman IR 33 spectrophotometer.
[7] Calbiochem.
[8] Cary model 17 recording spectrophotometer.
[9] Supplied by Dr. J. Lundström, Astra Pharmaceuticals AB, Sodertalje, Sweden.
[10] Supplied by Dr. G. D. Cherayil, Medical College of Milwaukee, Milwaukee, Wis.
[11] Eastman.
[12] Isolated from *Opuntia clavata* Eng.
[13] Aldrich Chemical Co.

The hydrochloride (1.2 g) was dissolved in 25 ml of water and made alkaline with 1 *N* NaOH. The alkaline solution was extracted three times with equal portions of ether. The ether extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield the free base as an oily liquid. This oil was dissolved in 50 ml of methyl formate and transferred to a high pressure vessel. The vessel was purged with nitrogen, sealed, and heated for 12 hr on a steam bath. After cooling and removing the solvent under reduced pressure, the *N*-formyl compound was obtained as a yellow oil, which could not be induced to crystallize. The IR spectrum showed expected strong peaks for amide NH and carbonyl. TLC in chloroform–methanol (3:4) and visualization with fluorescamine showed complete absence of the original primary amine.

The entire yield of *N*-formyl-4-benzyloxy-3-methoxy-β-phenethylamine was dissolved in 50 ml of ether and added dropwise into a mixture of 1 g of lithium aluminum hydride and 25 ml of ether. After addition, the mixture was refluxed for 10 hr; the excess reducing agent was destroyed by the careful addition of water and 20% sodium hydroxide. After stirring for 15 min, the ether layer was decanted. The aqueous residue was washed three times with 40-ml portions of ether. After drying over anhydrous sodium sulfate, the combined ether portions were saturated with anhydrous hydrogen chloride. Recrystallization of the precipitate from ethanol yielded *N*-methyl-4-benzyloxy-3-methoxy-β-phenethylamine hydrochloride [580 mg, 50% overall yield, mp 155–156°, lit. (14) mp 154–155°].

Debenzylation of this compound (500 mg) was performed by hydrogenation for 3 hr at 40–50 psi in 20 ml of ethanol over 125 mg of 10% palladium-on-charcoal. The reaction mixture was filtered (diatomaceous earth[14]), and the filtrate was concentrated. Crystallization from ethanol–ether yielded *N*-methyl-3-methoxytyramine hydrochloride [210 mg, 42% yield, mp 152°, lit. (14) mp 154–155°]. Cochromatographic, mixed melting-point, IR, and mass spectral data confirmed the identity of the synthetic product and the isolated alkaloid.

DISCUSSION

By using previously described techniques, an alkaloid extract was obtained from defatted *P. maxonii* and resolved into nonphenolic and phenolic fractions. TLC analyses revealed the presence of one major alkaloid (*N*-methyl-3,4-dimethoxyphenethylamine) and one minor alkaloid (3,4-dimethoxyphenethylamine) in the nonphenolic fraction. Preparative TLC aided in the crystallization of the hydrochloride of the major nonphenolic alkaloid, and comparisons of physical and spectral data (melting point, mixed melting point, and mass and IR spectra) of the isolated alkaloid and reference *N*-methyl-3,4-dimethoxyphenethylamine confirmed the original TLC identification. The trace of 3,4-dimethoxyphenethylamine would not crystallize, but the TLC identification (five solvent systems) was confirmed by mass spectral analysis using concentrated eluates from preparative TLC.

Analysis of the phenolic alkaloid fraction by TLC revealed two major alkaloids (unknowns) and two minor alkaloids (tyramine and *N*-methyltyramine). After preparative TLC, the hydrochlorides of the major alkaloids crystallized and were identified (TLC visualization reactions and UV and mass spectral data) as *N*-methyl-3-methoxytyramine and *N,N*-dimethyl-3-methoxytyramine hydrochlorides. To confirm these identifications, the two compounds were synthesized following established methods; the synthetic compounds were identical in all respects (TLC, melting point, mixed melting point, IR, and mass spectra) to the isolated alkaloids. The mother liquors from the crystallizations of the two major phenolic alkaloids contained traces of tyramine and *N*-methyltyramine, which were identified by TLC (five solvent systems).

[14] Celite.

All six of these alkaloids were detected and/or isolated from other cactus species. Tyramine, *N*-methyltyramine, 3,4-dimethoxyphenethylamine, and *N*-methyl-3,4-dimethoxyphenethylamine are fairly common in alkaloidiferous cacti. However, the two major phenolic alkaloids, *N*-methyl- and *N,N*-dimethyl-3-methoxytyramine, are much less common; this paper is the first report of their crystallization from a natural source. Agurell *et al.* (19) detected *N*-methyl-3-methoxytyramine in *Trichocereus courantii* (K. Sch.) Backb. using TLC, GLC, and GLC–mass spectrometry, and Bruhn and Bruhn (20) used these same methods to detect a trace of *N,N*-dimethyl-3-methoxytyramine in *Ariocarpus agavoides* (Castaneda) E. F. Anderson. Lundström (14) used "trapping experiments" to detect traces of both compounds in peyote, *Lophophora williamsii* (Lem.) Coult. The pharmacological activity and especially the psychoactive effects of these alkaloids remain to be explored.

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