described in this report is utilized for the screening of molluscicide activity.

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Peyote Alkaloids IX: Identification and Synthesis of 3-Demethylmescaline, a Plausible Intermediate in the Biosynthesis of the Cactus Alkaloids

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Abstract □ The presence of 3-demethylmescaline in the alkaloidal mixture of peyote was proven by GLC-mass spectrometry through direct comparison. A new synthesis of this compound was realized by elaborating the ethylamine side chain onto the appropriately substituted benzene residue (with benzyl-etherification of the phenol group). The sequence involved transformation of the acid chloride successively into the diazoketone, the acid amide, and the ethylamine. A discussion is given of the possible role of 3-demethylmescaline and its congeners in the biosynthesis of peyote alkaloids.

Keyphrases □ Peyote alkaloids □ 3-Demethylmescaline—isolation, identification □ Synthesis, 3-demethylmescaline—structure confirmation □ GLC—separation, identity □ Mass spectroscopy—identity, structure

The role of partially methylated phenolic intermediates in the biosynthesis of the phenethylamine and tetrahydroisoquinoline alkaloids of peyote (*Lophophora williamsii*) may well be anticipated in view of the proven (1) involvement of the hydroxylic rather than the methoxylic phenethylamines. Such partially methylated intermediates of the trioxygenated phenethylamine may be envisaged to transform *in vivo* into products such as mescaline (the principal alkaloid in the plant) by *O*-methylation and also into several possible tetrahydroisoquinolines by cyclization. A consideration of the nature and distribution of substituents in the latter type of peyote alkaloids, where several of them carry a hydroxyl group on C-8, suggests that a likely precursor is specifically 3-hydroxy-4,5-dimethoxy-β-phenethylamine (3-demethylmescaline) (I). The authors (2) have earlier identified the *N*-formyl and *N*-acetyl derivatives of I in the nonbasic fraction of peyote, a fact which prompted a search for this relatively simple alkaloid in the basic fraction of the plant.

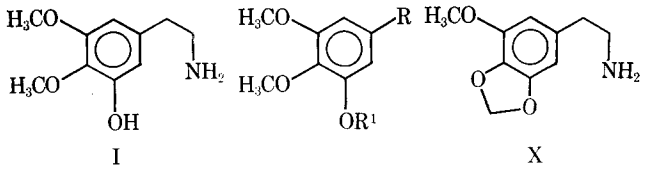
Inspection by GLC of the phenolic-basic fraction obtained from peyote revealed the presence of no less than ten products which include the previously reported (3) hordenine, pellotine, anhalonidine, and anhalamine. The presence[1] of I in the mixture was demonstrated unequivocally by direct comparison in the gas chromatograph and from a consideration of its mass spectrum. The latter contained ions at *m/e* 197 (M^+), 168 (100%, loss of CH_2NH), 167 (hydroxydimethoxybenzyl cation), 153 (loss of CH_3 from ion *m/e* 168), 137 (loss of CH_3O from ion *m/e* 168), 125 (loss of CO from ion *m/e* 153), and 123 (loss of CH_2O from ion *m/e* 153).

In order to obtain experimental evidence for the role of I in the biogenetic pattern of peyote alkaloids, it was necessary to secure a convenient method for its synthesis.[2] Several approaches were tried all starting with, methyl gallate followed by partial methylation to the 3,4-dimethyl ether (II). The complexities arising from the unprotected phenol group rendered abortive the route (6) proceeding by lithium aluminum hydride reduction of the methyl ester of II to give the benzyl alcohol III. The latter was intended to lead successively to the corresponding benzyl chloride and cyanide derivatives and ultimately to I by reduction. For the same reason the diazoketone IV—obtained from II *via* the corresponding *O*-carbomethoxy derivative and acid chloride—failed to give an acid amide upon treatment with silver nitrate in ammoniacal solution, in which the phenol group was doubtless regenerated. These difficulties were overcome only by initial benzyl etherification of the phenol group in II. This product (V) was trans-

[1] After the present work was completed, Agurell and Lundström (4) reported the detection of I in peyote using a similar technique. However, the authors identification of 3-demethylmescaline herein reported has previously been presented before the Organic Chemistry Discussion Group, National Institutes of Health, on October 15, 1968.

[2] A different synthesis of this compound has much earlier been reported by Späth and Röder (5). However, due to the difficulties met in preparing the starting material (II) and in purifying the aldehyde (R = CHO, R_1 = $COOC_2H_5$) intermediate in this synthesis in addition to the low overall yield of the final product, it was decided that an alternative route was desirable.

formed into the acid chloride VI and subsequently into the diazoketone VII, the acid amide VIII, the amine IX, and finally into the desired product (I) (see Scheme I).



Scheme I

II; R = COOH, R_1 = H;
III; R = CH_2OH, R_1 = H;
IV; R = $COCHN_2$, R_1 = $COOCH_3$;
V; R = COOH, R_1 = $CH_2C_6H_5$;
VI; R = COCl, R_1 = $CH_2C_6H_5$;
VII; R = $COCHN_2$, R_1 = $CH_2C_6H_5$;
VIII; R = CH_2CONH_2, R_1 = $CH_2C_6H_5$;
IX; R = $CH_2CH_2NH_2$, R_1 = $CH_2C_6H_5$.

It is possible that 3-demethylmescaline (I) represents an important phase in the biogenesis of peyote alkaloids and that its turnover takes place with abundance. However, its rapid transformation—essentially by cyclization and/or *O*-methylation—may be the reason for its detection in minute amounts in the plant tissue. The authors (7) have recently suggested that the *N*-formyl and *N*-acetyl derivatives of peyote phenethylamines (including those of I) may be involved in the pathway leading to tetrahydroisoquinolines. More recently, Leete and Braunstein (8) also suggested that the *N*-acetyl derivative of I may be an intermediate in the elaboration of anhalonidine. It may also be conjectured that the methylenedioxy phenethylamine analog (homomyristicylamine, X) of the anhalonine and lophophorine alkaloids may likewise exist in the natural mixture and that it may be biogenetically related to them. It does not appear too unlikely that X may arise from I—inasmuch as anhalonine and lophophorine may arise from anhalonidine and pellotine, respectively—since such transformation of aromatic substituents is a proven (9) biosynthetic event. Currently a special search is being conducted for such possible intermediates and also evidence for their role.

EXPERIMENTAL

Isolation of the Phenolic Alkaloids from Peyote—One individual plant (196 g.) was homogenized and then exhausted with methanol. The residue (5 g.) from the extract was defatted with petroleum ether then extracted with 100 ml. of 0.5% hydrochloric acid (centrifugation). After washing successively with benzene and chloroform, the acid extract was basified with ammonia solution, then extracted with chloroform. The residue (0.82 g.) from the latter extract was percolated in ethanolic solution through an ion-exchange resin (Amberlite IRA 400) (OH) which was subsequently washed with ethanol. A stream of carbon dioxide was then passed through a mixture of the resin and ethanol–water and the residue (0.183 g.) remaining from the solution was examined by GLC using a 1.8-m. × 0.62-cm. (6-ft. × 0.125-in.) column containing 1% methyl-phenyl silicone (1:1) on diatomaceous earth[3] at 200° with an argon pressure of 18 p.s.i. and hydrogen flame-ionization detector. Hordenine, pellotine, anhalonidine, and anhalamine were detected in this fraction by consideration of their retention times (1.03, 4.31, 4.87, and 5.25 min., respectively) in comparison with authentic samples. In addition, peaks appeared at 1.5, 2.35, 2.81, 3.28, 3.75, and 6.56 min. of which the second[4] was identical with that of authentic 3-hydroxy-4,5-dimethoxy-β-phenethylamine. The mass spectrum (determined with an LKB-Producter instrument at 70 ev., 100 amp. trap current at 3.5 kv. accelerating voltage) of this peak was determined and compared with that measured under the same conditions for authentic material.

3-Hydroxy-4,5-dimethoxybenzoic Acid (II)—A solution of methyl gallate (120 g.) in 10% sodium hydroxide solution (550 ml.) was treated at 5° with dimethyl sulfate (238 g.) added dropwise over 1.5 hr. (nitrogen atmosphere). After basification with sodium hydroxide and heating to 60° for 10 min., a deposit (43 g.) was formed which was shown to be trimethyl gallic acid methyl ester, m.p. 81–82° (undepressed); reported (10) m.p. 82.5°. The alkaline filtrate was washed with ether, acidified with hydrochloric acid, then extracted with chloroform. The residue from the latter extract was methylated with methanol–sulfuric acid (20-hr. reflux) and, after neutralization with sodium bicarbonate solution, methanol was removed and the aqueous concentrate treated with sodium hydroxide solution, then extracted with ether to remove trimethyl gallic acid methyl ester. A stream of carbon dioxide was passed through the alkaline solution followed by extraction with chloroform; this extract was shown (by GLC) to contain essentially methyl 4-hydroxy-3,5-dimethoxybenzoate. Acidification of the aqueous layer with hydrochloric acid gave 3-hydroxy-4,5-dimethoxybenzoic acid as a precipitate (44 g., with an additional 1.5 g. by extraction of the filtrate), m.p. 189–191°. Reported (5) m.p. 193–194°.

3-Benzyloxy-4,5-dimethoxybenzoic Acid (V)—A solution of 16.36 g. of Compound II in 270 ml. of acetone containing 24 g. of anhydrous potassium carbonate was treated with benzyl bromide (32.7 g.) under reflux for 24 hr. After the usual work-up, the product (benzyl ester-ether) was saponified with 6% methanolic potash (7-hr. reflux) and the reaction mixture washed with chloroform. The material (20.8 g.) which deposited upon acidification of the aqueous layer was crystallized from methanol to give 17.6 g. (an additional 1 g. was obtained by chloroform extraction), m.p. 176°. Reported (5) m.p. 170–172°.

Anal.—Calcd. for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 66.60; H, 5.61.

3-Benzyloxy-4,5-dimethoxybenzoyl Chloride (VI)—A solution of V (2.25 g.) in dry benzene (20 ml.) was briefly heated with oxalyl chloride (11 ml.) and the mixture then left at room temperature for 16 hr. The residue remaining after evaporation *in vacuo* was crystallized from benzene-light petroleum to give fine needles (2.21 g.), m.p. 69–70°. A pure sample had m.p. 83–84°.

Anal.—Calcd. for $C_{16}H_{15}ClO_4$: C, 62.65; H, 4.92. Found: C, 62.84; H, 4.93.

3-Benzyloxy-4,5-dimethoxyphenylacetamide (VIII)—A solution of the acid chloride VI (1.83 g.) in ether (100 ml.) was treated with an ethereal solution (50 ml.) of diazomethane (about 1 g.). After standing at room temperature overnight and evaporation, an oily product (1.9 g.) was obtained which could not be induced to crystallize. This material (presumably the diazoketone VII) was directly treated in a dioxane solution (20 ml.) with concentrated ammonia solution (20 ml.) and 10% aqueous silver nitrate solution (2.5 ml.) under reflux (steam bath) for 17 hr. After filtration the reaction mixture was decolorized with activated carbon (Norite) then concentrated to a small volume. The crude product which deposited was repeatedly crystallized from methanol–water then from benzene to give prisms (0.82 g.), m.p. 117–118°.

Anal.—Calcd. for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.83; H, 6.35; N, 4.70.

3-Benzyloxy-4,5-dimethoxy-β-phenethylamine Hydrochloride (IX)—To a stirred suspension of 1 g. of lithium aluminum hydride in ether (70 ml.) was added portionwise a solution of the acid amide (VIII, 0.8 g.) in benzene (25 ml.). After 3.5-hr. reflux and standing overnight at room temperature, the mixture was worked up in the usual manner. The crude product was treated with a benzene solution of hydrogen chloride and the material which deposited (0.41 g.) was crystallized from benzene to give matted needles, m.p. 150–152°.

Anal.—Calcd. for $C_{17}H_{21}NO_3.HCl$: C, 63.05; H, 6.84; N, 4.32. Found: C, 63.13; H, 6.83; N, 4.29.

[3] Gas Chrom Q, 100 mesh, Applied Science Laboratories, Inc., State College, Pa.

[4] This peak is different from that given by 4-demethylmescaline (4-hydroxy-3,5-dimethoxy-β-phenethylamine).

3-Hydroxy-4,5-dimethoxy-β-phenethylamine Hydrochloride (I)—A solution of the benzyl ether IX (0.3 g.) in acetic acid (15 ml.) containing 0.1 g. of 10% palladized charcoal was shaken with hydrogen (26 p.s.i.) at room temperature for 90 min. After removal of the catalyst, the solution was evaporated and the residue crystallized from ethanol–benzene to give flat needles (0.195 g.), m.p. and mixed m.p. with an authentic sample[5] 178–179°; identical IR spectra.

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Structure and Conformation of the Cortisone Side Chain

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Abstract □ The results of a recent crystal structure analysis of the 4-chloro-derivative of cortisone have been used to describe the exact conformation of the C-17 side chain in this molecule. The results of this analysis are compared with those obtained from molecular orbital calculations, and from optical rotatory dispersion, circular dichroism, infrared and nuclear magnetic resonance measurements. The C-17 side chain shows no evidence of internal hydrogen bonding, either with the C-17 or with the C-21 hydroxyl groups, and it is shown that such hydrogen bonding is unfeasible, both in the crystal and in solution. Evidence regarding the preferred conformation of C-17 acyl side chains in general, is also presented.

Keyphrases □ Cortisone side chain—structure, conformation □ C-17 side chain conformation—4-chloro-derivative, cortisone □ Crystalline structure data—cortisone side chain conformation

The conformation and relative positions of the functional groups on the steroid molecules are of particular interest in steroid structure studies, in that they are thought to be the key to understanding the way in which steroids act upon their target organs (1). The configuration of the 17-side chain is less restricted than the rest of the steroid structure and is open to greater speculation. The authors have recently completed a detailed crystallographic structure analysis of the 4-chloro-derivative of cortisone (2) and are now able to accurately describe the geometry of the side chain of this corticosteroid.

EXPERIMENTAL

Conformation of the C-17 Side Chain—From molecular orbital calculations on cortisol, Kier (3) has predicted a side-chain conformation in which the carbonyl oxygen atom (O-20) is situated over the D-ring, and is equidistant from both C-13 and C-16 (Fig. 1*a*). Also, he predicted that the entire side chain, including C-17 and the α-oriented C-17 hydroxyl group, is planar.

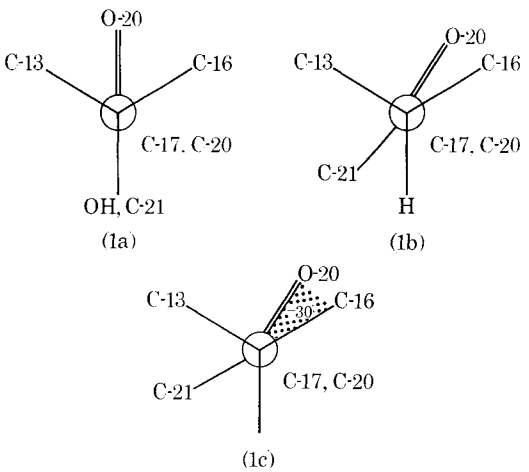

Figure 1—*Conformation of the C-17 side chain, showing Newman projections along the C-17–C-20 bond;* (a) *according to Kier (3);* (b) *according to Wellman and Djerassi (4);* (c) *the preferred conformation for the C-17 acyl side chains (6).*