The Cactus Alkaloids. I. Identification of *N*-Methylated Tyramine Derivatives in *Lophophora williamsii*

J. L. McLaughlin[1] and A. G. Paul

(College of Pharmacy, The University of Michigan, Ann Arbor 48104)

Anthropological studies (9, 21, 22, 24) point out that the peyote cactus, *Lophophora williamsii* (Lem.) Coult., has been used for centuries by the American Indians as a panacea, an amulet, and a hallucinogen. Early chemical investigations by Lewin (11) and Heffter (5, 6) demonstrated that the plant contained the alkaloids anhalonine, pellotine, anhalonidine, lophophorine, and mescaline. Since these initial investigations, anhalamine, anhalidine, anhalinine, *N*-methylmescaline, *N*-acetylmescaline, and *O*-methyl-*d*-anhalonidine have been isolated from the plant (7, 18, 19). Most of these alkaloids possess some physiological activity, but with the exception of mescaline, they are not hallucinogenic and are not currently used medicinally.

The goal of the present investigation was to analyze further the alkaloid content of *L. williamsii*. Spaeth and his coworkers (26–30), using classical isolation techniques, had found several new alkaloids by exhaustive analysis of the nonphenolic alkaloid fraction. The results of their studies suggested that the phenolic fraction should likewise be carefully analyzed. For such an investigation modern chromatographic techniques promised to be more useful than the techniques available to the previous workers.

MATERIAL AND METHODS

Plant Material.—Five kg of sliced and dried *L. williamsii*[2] was ground through a 1 mm sieve using a standard model no. 3 Wiley mill. Living specimens of *L. williamsii*,[3] obtained from Mexico, were maintained at The University of Michigan Botanical Gardens. Prior to extraction the living plants were sliced into thin sections, dried at 45° for 48 hr, and passed through a 20 mesh sieve using an intermediate model Wiley mill.

Reference Alkaloids.—Peyote alkaloids (*N*-acetylmescaline, anhalamine HCl, anhalonidine HCl, *O*-methylanhalonidine, *N*-methylmescaline, and pellotine) obtained from Dr. A. Brossi, (anhalamine HCl, anhalonidine HCl, anhalonine HCl, and lophophorine HCl) obtained from S. B. Penick and Co., anhalinine HCl isolated from *L. williamsii*, and mescaline HCl obtained from Mann Research Labs. were used in these studies. In addition *N*-methyltyramine (Dr. A. Brossi and Dr. L. Marion), hordenine (Mann Research Labs. and S. B. Penick and Co.), tyramine HCl, candicine I (obtained by methylating hordenine), pilocereine (Dr. C. Djerassi), betaine, and choline were used. Hordenine, candicine, and pilocereine are phenolic alkaloids which have been found in several cacti (35). Tryamine and *N*-methyltyramine were included because of their biogenetic relationship to hordenine and candicine (17). The common quaternary amines, betaine and choline, were included since they might complicate the identification of candicine.

Thin-Layer Chromatographic (TLC) Methods.—The preparation of aluminum oxide G (Al_2O_3G) and silica gel G (SGG) plates, application of test solutions, preparation of developing chambers, and one-dimensional development of chromatograms were carried out as previously described (15). To prepare SGG 0.1N

[1]Present address: School of Pharmacy, University of Missouri at Kansas City, Kansas City, Missouri 64110.

[2]Purchased from S. B. Penick and Co., New York, New York.

[3]Identifications confirmed by Dr. E. U. Clover, Botany Department, The University of Michigan.

NaOH plates, water was replaced with 0.1N NaOH. Silica gel H (SGH) *p*H 9.2 plates were prepared by replacing the water with a buffer composed of 48 g boric acid and 15 g sodium hydroxide per 1000 ml of solution. For two-dimensional development the alkaloid solutions were applied at a point 2 cm from the starting edge and 2 cm from the left edge of the plate. After development in the first dimension, the plates were air-dried 30 min and developed in the second dimension.

Preliminary screening of more than 25 different reagents led to the following choice of sprays used to visualize the location of the alkaloids on developed chromatograms:

1. Dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride)[4] (4, 34), 0.05% in acetone. After spraying 10 μg quantities of the peyote alkaloids with this reagent and examining the chromatogram under UV light, all, with the exception of *N*-acetylmescaline, exhibited a bright yellow-orange fluorescence on a light blue fluorescent background. Lophophorine and pellotine required about 30 min for the fluorescence to develop, but the other alkaloids produced fluorescent con-

TABLE 1. *Description of TLC solvent systems.*

Solvent system	Composition	Adsorbent	Approximate development time (min)
A	2-butanone–*N*,*N*-dimethylformamide–conc. ammonium hydroxide (13:1.9:0.1)	SGH *p*H 9.2	35
B	chloroform–acetone–diethylamine (5:4:1)	SGG	70
C	chloroform–diethylamine (9:1)	SGG	80
D	cyclohexane–chloroform–diethylamine (5:4:1)	SGG	80
E	benzene–ethyl acetate–diethylamine (7:2:1)	SGG 0.1*N* NaOH	60
F	1st dimension:benzene–methanol–5 per cent ammonium hydroxide (10:15:2) (10)		50
	2nd dimension: same as in solvent system A	SGH *p*H 9.2	35
G	1st dimension: same as in solvent system F		90
	2nd dimension: same as in solvent system A	SGG 0.1*N* NaOH	60
H	methanol–carbon tetrachloride–glacial acetic acid (28:12:1)	Al_2O_3G	60

jugates immediately. This reagent proved to be more sensitive than any of the alkaloid reagents tested, and it was used routinely to visualize the nonphenolic alkaloids.

2. Tetrazotized benzidine (25). This reagent differentiated between *p*-hydroxyphenols, which reacted to give a yellow color, and the 8-hydroxytetrahydroisoquinoline phenols of the known peyote alkaloids, which reacted to give a red color. It was used to locate the phenolic alkaloids and to aid in the determination of their structures.

3. Antimony pentachloride, 20% in $CHCl_3$ (12). *N*-Acetylmescaline, an amide, gave none of the typical alkaloid reactions. It was detected with this non-specific reagent which reacted to produce a pale gold color.

4. Wagner's reagent (iodine and potassium iodide T.S.) (32). This reagent was used to detect candicine, choline, and betaine. Betaine, however, was rela-

[4]California Corporation for Biochemical Research, Los Angeles, California.

tively insensitive to any of the reagents tested and could not be detected in amounts less than 20 μg.

Of 120 combinations of solvents and absorbants tested, those found to be most useful are shown in table 1. Solvent system A was the most effective. Provided that the phenolic and nonphenolic reference alkaloids were chromatographed separately, this system afforded a rapid and dependable method for the qualitative identification of each of the alkaloids in each group. Solvent systems B through E (33) were useful for further identification of the phenolic alkaloids. Solvent systems F and G are two-dimensional systems and were designed to identify tyramine in mixtures with *N*-methyltyramine and hordenine. Solvent system H (31) was used to separate the quaternary bases.

The order, magnitude, and precision of the separation of the alkaloids in these systems can be ascertained from the R_F values and standard deviations which are presented in tables 2–5. With the exception of pilocereine, the R_F values were

TABLE 2. R$_F$ *values and standard deviations of phenolic alkaloids in solvent systems A–E.*

Alkaloid	A	B	C	D	E
anhalamine HCl	0.08±0.01	0.04±0.00	0.03±0.00	0.01±0.00	0.02±0.00
anhalidine HCl	0.68±0.01	0.36±0.02	0.26±0.03	0.15±0.01	0.23±0.01
anhalonidine HCl	0.23±0.02	0.21±0.01	0.16±0.02	0.07±0.00	0.13±0.01
hordenine	0.56±0.02	0.42±0.01	0.30±0.02	0.14±0.00	0.23±0.01
N-methyltyramine HBr	0.10±0.01	0.19±0.01	0.13±0.01	0.06±0.00	0.10±0.01
pellotine	0.63±0.02	0.51±0.01	0.40±0.02	0.25±0.01	0.35±0.02
pilocereine	0.90±0.02	0.84±0.01	0.89±0.03	0.80±0.01	0.81±0.03
tyramine HCl	0.54±0.02	0.43±0.01	0.16±0.01	0.08±0.00	0.11±0.01

TABLE 3. R$_F$ *values and standard deviations of nonphenolic alkaloids in solvent system A.*

anhalinine HCl	0.22±0.02
anhalonine HCl	0.49±0.02
lophophorine HCl	0.82±0.02
mescaline HCl	0.55±0.02
N-acetylmescaline	0.72±0.01
N-methylmescaline	0.10±0.01
O-methylanhalonidine	0.34±0.01

TABLE 4. R$_F$ *values and standard deviations of the quaternary bases in solvent system H.*

betaine	0.37±0.04
candicine I	0.85±0.01
choline	0.77±0.02

TABLE 5. R$_F$ *values and standard deviations of hordenine, N-methyltyramine, and tyramine in solvent systems F and G.*

Alkaloid	F		G	
	Dimension		Dimension	
	1	2	1	2
hordenine	0.91±0.02	0.65±0.04	0.69±0.03	0.38±0.01
N-methyltyramine HBr	0.76±0.01	0.16±0.02	0.38±0.02	0.07±0.00
tyramine HCl	0.73±0.02	0.52±0.02	0.48±0.03	0.46±0.02

obtained by chromatographing 20 μg quantities of the reference alkaloids. As pilocereine was less sensitive to tetrazotized benzidine, 40 μg quantities were chromatographed. The R_F values for solvent systems A and H represent the averages of eight and three determinations, respectively; the values of systems B through G are the averages of four determinations.

Extraction Methods.—The extraction method of Spaeth and Becke (26) was used initially. TLC examination of the nonphenolic extracts revealed traces of the phenolic alkaloids, and the repeated macerations were time consuming. Consequently, new extraction and purification methods were developed.

Purification Method No. 1 (fig. 1)

Powdered drug was defatted with petroleum ether (30–60°) for 36 hr using a small (6.5×14 cm) Soxhlet extractor or for 72 hr using a large (9×30 cm) Soxhlet

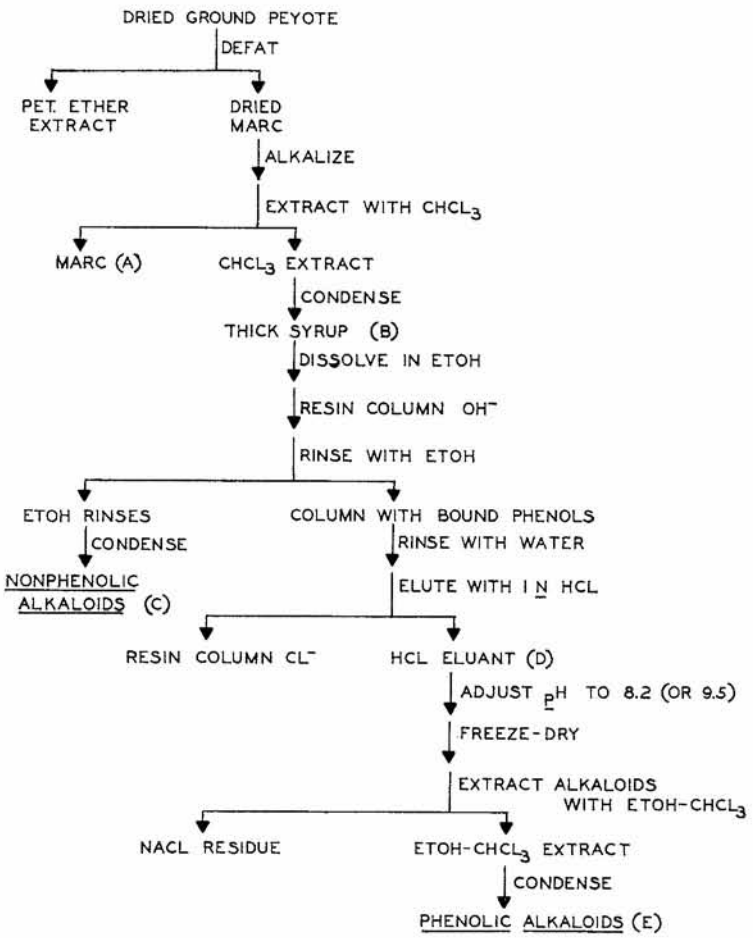

FIG. 1. *Purification method no. 1.*

extractor. Dried marc from this extraction was moistened with concentrated ammonium hydroxide–methanol–chloroform (1:2:2), macerated with concentrated ammonium hydroxide methanol–chloroform (0.1:0.9:9) and extracted with chloroform for 2 days in the small extractor or for 5 days in the large extractor.

To test the completeness of this extraction, a sample of the marc (A) was

extracted according to the method of Farnsworth and Euler (3). Analysis by TLC indicated that the material contained no alkaloids.

The filtered chloroform extract was condensed to a thick syrup (B) using a flash evaporator at 40–50° attached to a water pump. Syrup (B) was dissolved in ethanol using approximately 1 ml for every 15 g of powdered drug and filtered if necessary. The ethanol solution was added to a column of Amberlite IRA 401 (OH) ion-exchange resin (approx 20 g/100 g dried plant material) which had been rinsed with ethanol after preparation. Development of the ethanol extract proceeded at a flow rate of about 30 drops per min. The column was washed with ethanol, and the flow rate was increased to 150 drops per min. Washing was continued until the effluent fractions were colorless; the volume of ethanol required varied from 300–400 ml per 100 g of plant material. The combined ethanol rinses were evaporated to dryness on a steam bath with the aid of a current of air. Heat was discontinued during the final stage of the evaporation. The residue contained the nonphenolic alkaloids (C).

Excess ethanol was rinsed from the basic column with water, using approximately 200 ml per 20 g of resin. Phenolic alkaloids were eluted from the resin by washing the column with approximately 800 ml of 1N HCl per 100 g of plant material. The flow rate was adjusted to 30 drops per min and increased to 150 drops per min when a dark-brown zone reached the lower end of the column.

The *p*H of the combined 1N HCl eluants (D) was adjusted to 8.2 with 7.5N NaOH, and the solutions were freeze-dried. Phenolic alkaloids were extracted from the freeze-dried residue with an ethanol–chloroform (1:9) mixture. Residue representing 200 ml of solution was slurried with 40 ml of the solvent mixture, the suspension was filtered with suction, and the residue was rinsed twice with 20 ml of the solvent mixture. This procedure was repeated, usually two or three times, until the eluants were colorless. The combined extracts were filtered and evaporated to dryness on a steam bath under a current of air. Heat was discontinued during the latter stages of the evaporation. This residue contained the phenolic alkaloids (E).

Purification Method No. 2 (fig. 2)

In order to eliminate chloroform soluble nonalkaloid materials, method no. 1

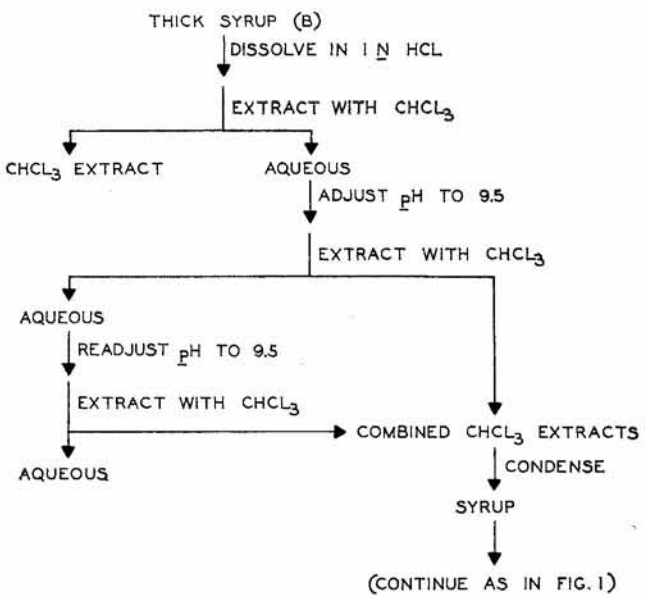

FIG. 2. *Procedures added to purification method no. 1 to form method no. 2.*

was modified. Figure 2 summarizes the added procedures. Syrup (B) was stirred with 1 ml of 1N HCl per g of dried plant material, and the suspension was filtered with suction. The acidic aqueous filtrate was extracted three times with equal volumes of chloroform, and the chloroform layers were discarded. The *p*H of the aqueous layer was adjusted to 9.5 with concentrated ammonium hydroxide using a *p*H meter. This solution was extracted three times with equal volumes of chloroform; the *p*H was readjusted to 9.5; and the aqueous solution was again extracted with chloroform. The combined chloroform layers were condensed to a thick syrup on a steam bath with the aid of a current of air, discontinuing the heat as evaporation neared completion.

This syrup was then treated in a manner identical with that described for the thick syrup (B) in method no. 1, with the exception that the *p*H of the combined HCl eluants (D) was adjusted to 9.5 rather than 8.2.

RESULTS

TLC Identification of Hordenine.—In a preliminary experiment the phenolic alkaloids (E) were isolated from 339 g of peyote from Penick using purification method no. 1. TLC analysis of the extract revealed a compound which reacted to produce a yellow color with tetrazotized benzidine and which chromatographed just below pellotine in solvent system A. A portion of the extract was chromatographed on a column of alumina (80–200 mesh, activated several hr at 105°) using increasing concentrations of ethanol in chloroform as the developer. Collected fractions were analyzed by TLC, and those which contained the yellow-reacting compound, relatively free of contaminants, were combined and assayed again using solvent systems A through E. In each system the yellow-reacting compound and samples of known hordenine chromatographed identically.

Isolation of Hordenine.[5]—Extract (E) obtained by purification method no. 1 from 633 g of material from Penick was adsorbed on approximately 1 g of silicic acid from chloroform. The dry powder was transferred to a column 137×3.3 cm containing 550 g of silicic acid (100 mesh activated several hr at 105°) which had been added to the column in suspension with ethanol–chloroform (1:9). The mixed zone was covered with a layer of silicic acid and a layer of sand.

The column was developed with ethanol–chloroform (1:9), using slight positive pressure at a flow rate of 1 ml per min. Fifteen-ml fractions were collected, and every third fraction was evaporated to dryness at 50° using a water pump. Residues were dissolved in 5 drops of methanol, and 10 μl quantities were analyzed by TLC using solvent system A and tetrazotized benzidine reagent. Fractions 42 through 2010 contained varying amounts of the four known phenolic alkaloids and the yellow-reacting compound had not been eluted.

At fraction 2011 the solvent mixture was changed to ethanol–chloroform (1:1). TLC analysis revealed that fractions 2157 through 3005 contained the unknown compound and that fractions 2179 through 2226 contained considerable amounts relatively free of contaminants. These fractions were combined, and the residue was subjected to sublimation using a 2×6 cm microsublimator attached to a water pump and heated at 140°. A total of 60.9 mg of crystalline sublimate (mp 178–180°)[6] was collected. Hordenine melts at 117–118° (16), while hordenine HCl melts at 177° (16). A positive Beilstein's test (23) indicated the presence of halogen in the unknown sublimate; since no report could be found stating that hordenine HCl sublimes, this salt was prepared and tested for sublimation in the microsublimator. Crystals melting at 178–180° were obtained, and they showed no melting point depression (mp 178–180°) when mixed with the unknown sublimate. In KBr pellets these two compounds exhibited identical infrared absorption spectra.

[5]The isolation of hordenine has been reported in a preliminary note (14).
[6]A calibrated Fisher-Johns melting point apparatus was used.

Ten mg of the unknown was placed in a microsublimator, dissolved in 0.5 ml of *p*H 9.2 borate buffer, and the *p*H was adjusted to 9.5 with 0.1N NaOH. The solution was freeze-dried and the residue was subjected to sublimation. This sublimate melted at 118° and showed no melting point depression (mp 116–118°) when mixed with hordenine (mp 116–118°).

Ten mg of the unknown base, 0.02 ml of methyl iodide, and 2 ml of ethyl ether were refluxed for 4 hr. The methiodide, recrystallized from methanol–ether, gave mp 234–235°, showed no melting point depression (mp 233–235°) with hordenine methiodide (mp 232–234°), and exhibited an infrared absorption sprectrum identical to that of hordenine methiodide.

To ascertain that the hordenine isolated from the material from Penick was not the result of contamination with one of the other hordenine-containing cacti, dried fresh *L. williamsii* was extracted by method no. 1, and the phenolic extract (E) was analyzed by TLC. A compound which cochromatographed with hordenine was present, confirming the presence of hordenine in authentic plant material.

TLC Identification of N-*Methyltyramine.*—In a preliminary experiment extract containing phenolic alkaloids (E) from material from Penick was chromatographed on a column of silicic acid using ethanol–chloroform (1:9). The initial mixed zone was removed and eluted with ethanol. TLC, using solvent systems A and E and tetrazotized benzidine reagent, indicated that the eluant was free of the four known phenolic alkaloids and that it contained hordenine and a second unknown compound. This unknown compound chromatographed slightly above anhalamine in solvent system A and reacted with tetrazotized benzidine to produce a yellow color. The unknown and *N*-methyltyramine chromatographed identically in both solvent systems A and E.

Isolation of N-*Methyltyramine.*—Extract containing phenolic alkaloids (E) obtained by purification method no. 1 from 750 g of the material from Penick was adsorbed from chloroform to approximately 1 g of silicic acid. The dry powder was transferred to a column 60×4.5 cm which was packed with activated silicic acid to a height of 50 cm. A layer of silicic acid and a layer of sand were added to the top of the mixed zone. The bottom of the column was fitted to a two-liter suction flask. Development was made with ethanol–chloroform (1:9), using a water pump to increase the flow rate.

A fast-moving brown band proceeded to a point 5 cm from the bottom of the column. Development was discontinued and 5 cm segments of the adsorbant were removed and numbered from the mixed zone downward. The first segment was eluted by suspending the adsorbant in 100 ml of ethanol, filtering with suction, and rinsing with 100 ml of ethanol; this elution was repeated five times. The remaining segments were eluted likewise, but the elution procedure was repeated only three times.

Combined eluants from each segment were partially evaporated on a steam bath under a current of air. Evaporation was completed using only air. Residues were dissolved in ethanol and analyzed by TLC using solvent system A and tetrazotized benzidine reagent. Eluants from the first segment contained hordenine and the desired unknown compound. The four known phenolic alkaloids were located in the second and third segments.

Residue from the eluants of the first segment was adsorbed from chloroform to approximately 1 g of activated alumina. The powder was transferred to a column 137×3.3 cm which had been packed to a height of 130 cm with the alumina. A layer of alumina and a layer of sand covered the mixed zone.

This column was developed with ethanol–chloroform (0.5:9.5) and 15 ml fractions were collected every 9 min. After 165 fractions the solvent mixture was changed to ethanol–chloroform (1:9). Every fifth fraction was evaporated to dryness at 50° using a water pump. Residues were dissolved in 5 drops of methanol, and 10 μl quantities were analyzed by TLC using solvent system A

and tetrazotized benzidine reagent. Only hordenine was present in fractions 550 through 710; fractions 715 through 855 contained hordenine plus the unknown compound; and fractions 860 through 1190 contained these two compounds plus unidentified contaminants.

Fractions 715 through 855 were combined, evaporated, dissolved in chloroform and adsorbed to approximately 0.5 g of silicic acid. The powder was transferred to a column 37×2.0 cm which had been packed with 80 g of activated silicic acid. A layer of silicic acid and a layer of sand covered the mixed zone.

Development was made with ethanol–chloroform (1:1), using slight positive pressure. Fifteen ml fractions were collected every 30 min. Every tenth fraction was evaporated to dryness on a steam bath under a current of air; heat was discontinued as the fraction neared dryness. The residues were dissolved in 5 drops of ethanol, and 10 μl quantities were assayed by TLC using solvent system A and tetrazotized benzidine reagent. Fractions 190 through 620 contained hordenine. Fractions 610 through 2300 contained the unknown compound, relatively free of contaminants.

Fractions 610 through 2300 were combined, filtered, and evaporated to dryness. The residue was dissolved in methanol, concentrated to 10 ml, and cooled at −23°. An amorphous precipitate formed and was removed by filtration. The filtrate was evaporated to dryness and extracted with four 5 ml portions of 1N HCl. After filtration this solution was extracted with three 10 ml portions of chloroform and adjusted to *p*H 9.5 with 7.5N NaOH. This solution was extracted with five 20 ml portions of ethyl ether, the combined extracts were filtered through anhydrous Na_2SO_4, and filtrate was evaporated to dryness.

The crystalline residue was dissolved in 5 ml of boiling anisole, filtered while hot, and condensed to 1 ml on a hot plate under a current of air (8). Crystallization was induced by cooling to −23° and scratching the sides of the container. The crystals were filtered with suction and dried overnight in a vacuum desiccator. A yield of 92.9 mg was obtained giving mp 126–128°.

N-methyltyramine (mp 127–129°; reported (8) 130–131°, (2) 127–128°) was regenerated from *N*-methyltyramine hydrobromide (mp 124–125°) and recrystallized from boiling anisole. The mp 127–129° was not depressed (mp 127–129°) when mixed with the isolated base. Reference *N*-methyltyramine and the unknown exhibited identical infrared absorption spectra.

Five mg of the unknown was dissolved in 0.1 ml of 5N HCl, and 0.1 ml of 5N HCl containing 50 mg of chloroplatinic acid was added. Under a current of air the solution was concentrated to 0.1 ml, filtered, and the crystals rinsed with 2 drops of 5N HCl (giving mp 192–196° decomp), showed no melting point depression (mp 192–195° decomp) when mixed with *N*-methyltyramine chloroplatinate (mp 194–197° decomp), and exhibited an infrared absorption spectrum identical to that of *N*-methyltyramine chloroplatinate.

Twelve mg of the unknown base was dissolved in 10 drops of ethanol and 3 ml of ethyl ether. The resulting solution was mixed with 3 ml of ethyl ether saturated with hydrogen bromide and cooled to −23° to induce crystallization. After filtration and desiccation this derivative melted at 124–125°, caused no melting point depression (mp 124–125°) when mixed with *N*-methyltyramine HBr (mp 124–125°), and exhibited an infrared absorption spectrum identical to that of known *N*-methyltyramine HBr.

TLC Identification of Tyramine.—During the isolation of *N*-methyltyramine, TLC assays of alumina column fractions 960 through 1145 indicated the presence of a yellow-reacting compound which chromatographed similarly to tyramine. The residue of fractions 951 through 1000 was dissolved in 2 ml of ethanol–chloroform (1.5:9.5), and 40-μl quantities were assayed by TLC using the two-dimensional systems F and G. In both systems the unknown compound cochromatographed with known tyramine.

To substantiate this finding, material prepared from fresh plants was examined, and material from Penick was reexamined.

Dried fresh plant material, weighing 89 g was extracted using purification method no. 2 to obtain extract (E). The extract was adsorbed from chloroform to approximately 200 mg of silicic acid. The powder was added to a column 40×2.0 cm which had been packed with activated silicic acid to a height of 35 cm. A layer of silicic acid and a layer of sand were added to the top of the mixed zone. The column was developed with ethanol–chloroform (1:9), using suction from a water pump. When approximately 1500 ml of eluant had been collected, development was discontinued.

Starting from the mixed zone and proceeding downward, the column was divided into 20 1-cm segments; below 20 cm the column was divided into 2-cm segments. Each of the 1-cm segments was eluted by mixing the adsorbent with 10 ml of ethanol, filtering with suction, and rinsing the residue twice with 10-ml portions of ethanol. The procedure was repeated. Each 2-cm segment was eluted in the same manner using 20-ml portions of ethanol.

After filtering, the eluants were evaporated to dryness on a stem bath under a current of air, discontinuing heat as evaporation neared completion. The residues were dissolved in 10 drops of ethanol, and 10-μl quantities were chromatographed using solvent system A and tetrazotized benzidine. The residue from the solvent which had eluted during development of the column was dissolved

TABLE 6. R$_F$ *values of tyramine in extracts.*

Extract		Solvent system			
		F		G	
		Dimension		Dimension	
		1	2	1	2
fresh plants column segments	alone	0.65	0.49	0.42	0.46
	added tyramine	0.62	0.49	0.40	0.47
material from Penick column segments	alone	0.51	0.52	0.40	0.44
	added tyramine	0.53	0.54	0.40	0.44
alumina column fractions	alone	0.55	0.56	0.43	0.45
	added tyramine	0.49	0.54	0.43	0.46

similarly and included in the TLC analysis. The analyses revealed that *N*-methyltyramine occurred in eluants of segments 1–4, hordenine in eluants of segments 5–10, and the tetrahydroisoquinoline phenolic alkaloids in eluants of segments 11–36 and in solvent that had been eluted during the development.

The combined eluants of segments 1–4 (fraction 1), of segments 5–10 (fraction 2), and of segments 11–36 plus solvent eluted during development (fraction 3) were treated separately. Each fraction was dissolved in 2 ml of ethanol–chloroform (1.5:8.5). Thirty-μl quantities of fraction 1 chromatographed in solvent systems F and G revealed the presence of a compound which cochromatographed with known tyramine. Tyramine could not be detected in fractions 2 or 3.

In order to estimate the quantity of tyramine in fraction 1, 10-, 20-, 30-, and 40-μl volumes were chromatographed using solvent system F and tetrazotized benzidine reagent. Similarly, 2-, 5-, 10-, 15-, 20-, 25-, 30-, and 40-μg quantities of tyramine were chromatographed. Visual comparison of the color intensities of the spots indicated that the concentration of tyramine in fraction 1 was approximately 0.5–0.7 μg per μl.

One hundred g of material from Penick was extracted, chromatographed, and assayed similarly. Visual extimation indicated that the concentration of tyramine in this fraction 1 solution was approximately 0.7 μg per μl.

Table 6 lists the R_F values of the tyramine found in the three extracts.

TLC Identification of Candicine.—Two hundred and fifty g of the material from Penick was refluxed with 1500 ml of 0.5% HCl in ethanol on a steam bath. At the end of 1.5 hr the supernatant was decanted, and the reflux was repeated twice using 800-ml portions of the solvent. The filtered extracts were combined and concentrated to a thick syrup by distillation.

This syrup was dissolved in 150 ml of water, and the acidic aqueous solution was extracted once with 200-ml and eight times with 100-ml portions of chloroform. The aqueous solution was filtered, the *p*H was adjusted to 9 with concentrated ammonia, and the basic solution was extracted nine times with 100-ml portions of chloroform.

After adjusting the *p*H to 4 with concentrated HCl, the solution was treated with Mayer's reagent until precipitation was complete (20). The precipitate was collected by suction filtration and suspended in 15 ml of hot water. Hydrogen sulfide was bubbled through the suspension until no further precipitation occurred. The black mercuric sulfide was removed by suction filtration, and the filtrate was freeze-dried.

The residue was suspended in 5 ml of methanol and filtered, and the resulting insoluble material was rinsed with an additional 5 ml of methanol. Fifteen ml of ethyl ether was added to precipitate the quaternary bases. The gray amorphous precipitate was filtered and rinsed with ether. Upon contact with air the precipitate started to become brown and sticky. The precipitate was quickly dissolved in 2 ml of methanol, but the solution continued to darken.

Examination with solvent system A, tetrazotized benzidine reagent, and dansyl chloride showed that the extract was free of the known peyote alkaloids. With solvent system H and Wagner's reagent, 10- and 40-μl quantities of the extract revealed the presence of a compound with an average R_F of 0.87 which cochromatographed with candicine iodide (table 4).

A second 250 g portion of the material from Penick, containing 25 mg of added candicine iodide, was extracted in an identical manner. TLC of this extract with solvent system H revealed a larger concentration of the compound that cochromatographed with candicine iodide. The color of the spots faded rapidly after spraying with Wagner's reagent; however, the initial color intensity indicated that during the extraction there was an estimated 50% loss of the candicine iodide.

This extract also contained a compound, having an average R_F of 0.75 in solvent system H (table 4), that chromatographed identically with choline.

Decomposition of the extracts prevented further TLC studies.

DISCUSSION AND CONCLUSIONS

TLC methods (tables 1–5) have been developed for the qualitative analysis of the nonphenolic and phenolic alkaloids of *L. williamsii*. Tetrazotized benzidine reagent was useful as a visualization aid for distinguishing *p*-hydroxyphenols and the 8-hydroxytetrahydroisoquinoline alkaloids of peyote. Dansyl chloride produces fluorescent conjugates with the alkaloids of *L. williamsii*, and these conjugates were useful for visualization on thin-layer chromatograms; this type of compound may be useful as an aid in chromatographic methods for the visualization of other alkaloids.

New purification methods (figs. 1–2) using a basic ion-exchange resin have been employed for the complete resolution of the alkaloids of *L. williamsii* into their respective nonphenolic and phenolic groups. Combining these purification methods with the TLC analyses, tyramine, *N*-methyltyramine, and hordenine have been identified in the phenolic extracts.

Hordenine has been isolated from these extracts as the hydrochloride by

sublimation of fractions collected from a column of silicic acid. The salt was converted to the free base, and the free base was converted to hordenine methiodide. The melting points, mixture melting points, and infrared absorption spectra of these compounds were essentially identical to those obtained with reference compounds. The approximate *p*Ka of the amine group of hordenine was determined by titration and found to be between 9.0 and 9.5. This finding indicated that the adjustment of the eluants from the resin column to *p*H 8.2 was not sufficient to convert hordenine HCl to the free base. Consequently, hordenine was isolated, perhaps inefficiently, as the hydrochloride. In this initial isolation 0.008% hordenine was obtained from the material from Penick.

N-Methyltyramine has also been isolated from the phenolic extracts of *L. williamsii*. The isolation procedure employed chromatography on successive columns of silicic acid, adsorption alumina, and silicic acid. The base was converted into the hydrobromide and the chloroplatinate. Melting points, mixture melting points, and infrared absorption spectra of these compounds were essentially identical to those obtained with reference compounds. The yield of *N*-methyltyramine from the material from Penick was 0.012%.

After the initial detection of tyramine in fractions encountered during the isolation of *N*-methyltyramine, the phenolic extracts of material from Penick and fresh plant material were closely examined for this compound. By TLC analysis of segments from a silicic acid column, traces of tyramine were found using two two-dimensional systems. Some of the resulting R_F values (table 6) are not in good agreement with those of reference tyramine (table 5). However, the discrepancies must be attributed to variations in the TLC techniques, since in each case the tyramine in the extracts cochromatographed with added tyramine. Visual comparison of the size and color intensity of the spots of tyramine in the extracts with standards indicated that both Penick and dried fresh plant material contained an estimated 0.001% tyramine.

TLC analyses of extracts of the quaternary bases of *L. williamsii* revealed a compound which cochromatographed with candicine. The small amounts of candicine present were not visually estimated by comparison with standards because the color produced with Wagner's reagent faded rapidly.

These results must be considered as only presumptive evidence for the presence of tyramine and candicine in *L. williamsii*. The isolation and chemical identification of these compounds are needed to confirm this evidence.

The concentrations of hordenine and *N*-methyltyramine in *L. williamsii* are perhaps sufficient to exert some physiological effect upon ingestion of the drug. These alkaloids possess a weak activity similar to epinephrine (18). Their concentrations in peyote may explain some of the primative uses in the treatment of ailments for which a sympathomimetic drug would be beneficial. The antiseptic action of hordenine could, in part, account for the effectiveness of the dried plant material in the treatment of wounds and infections. This action may also help to explain the bacteriostatic activity of peyocactin (13).

Precluding demethylation, hordenine and candicine would not be expected to be precursors of any of the previously known peyote alkaloids. Tyramine is a reported precursor of its *N*-methylated derivatives (17), and by *meta* oxidations and methylations, it is a plausible precursor of mescaline and its derivatives. *N*-Methyltyramine could conceivably be converted into *N*-methylmescaline, hordenine, and candicine.

Both tyramine and *N*-methyltyramine can be oxidized to their respective *m*-hydroxy derivatives (1). Under physiological conditions the resulting β-phenethylamines are capable of cyclization with an aldehyde to form tetrahydroisoquinoline structures (17). Through oxidations and methylations these tetrahydroisoquinolines could be precursors of some of the tetrahydroisoquinoline alkaloids of peyote.

L. williamsii is the fourth cactus in which hordenine and candicine have been

discovered (35), while the occurrence of tyramine and *N*-methyltyramine in members of the *Cactaceae* has never before been reported.

SUMMARY

Tyramine, *N*-methyltyramine, hordenine, and candicine have been identified by TLC as new alkaloids of the peyote cactus, *L. williamsii*. Hordenine and *N*-methyltyramine were isolated in crystalline form bringing the number of alkaloids isolated from peyote to thirteen.

ACKNOWLEDGMENTS

This investigation was supported, in part, by fellowship GF–14,694 from the Institute of General Medical Sciences, National Institutes of Health, U. S. Public Health Service, Bethesda, Maryland. The authors express gratitude to Dr. A. Brossi, Dr. L. Marion, and Dr. C. Djerassi for samples of reference alkaloids which were not available from commercial sources; to Dr. E. U. Clover for confirming the identifications of the living plants as *L. williamsii*; and to Mr. W. F. Kleinschmidt for maintaining the living plants at The University of Michigan Botanical Gardens.

Received 19 May, 1966.

LITERATURE CITED

1. **Correale, P.** and **E. Cortese.** 1954. The biogenesis of phenylalkylamines of *Sarothamnus scoparius*. *Naturwissenschaften* **41:** 457–458.
2. **Corti, U. A.** 1949. Über Ergebrisse der Methylierung von Tyrosin mit Dimethylsulfat und einige Derivate des N-Methyltyramins. *Helv. Chim. Acta* **32:** 681–686.
3. **Farnsworth, N. R.** and **K. L. Euler.** 1962. An alkaloid screening procedure utilizing thin-layer chromatography. *Lloydia* **25:** 186–195.
4. **Hartley, B. S.** and **V. Massey.** 1956. The active centre of chymotrypsin. I. Labelling with a fluorescent dye. *Biochem. Biophys. Acta.* **21:** 58–69.
5. **Heffter, A.** 1894. Ueber zwei Cacteenalkaloide. *Chem. Ber.* **27:** 2975–2976.
6. **Heffter, A.** 1896. Ueber Cacteenalkaloide (II. Mittheilung). *Chem. Ber.* **29:** 216–227.
7. **Henry, T. A.** 1949. *The plant alkaloids, 4th ed.* Blakiston, Philadelphia. pp. 154–162.
8. **Kirkwood, S.** and **L. Marion.** 1950. The biogenesis of alkaloids. I. The isolation of *N*-methyltyramine from barley. *J. Am. Chem. Soc.* **72:** 2522–2524.
9. **La Barre, W.** 1960. Twenty years of peyote studies. *Current Anthropology* **1:** 45–60.
10. **Leung, A. Y., A. H. Smith** and **A. G. Paul.** 1965. Production of psilocybin in *Psilocybe baeocystis* saprophytic culture. *J. Pharm. Sci.* **54:** 1576–1579.
11. **Lewin, L.** 1888. Ueber *Anhalonium Lewinii* und Andere Cacteen. *Arch. Exp. Pathol. Pharmakol.* **24:** 401–411.
12. **Marini-Bettolo, G. B.** 1964. *Thin layer chromatography.* Elsevier, New York. p. 81.
13. **McCleary, J. A., P. S. Sypherd** and **D. L. Walkington.** 1960. Antibiotic activity of an extract of peyote (*L. williamsii* (Lemaire) Coulter). *Econ. Botany* **14:** 247–249.
14. **McLaughlin, J. L.** and **A. G. Paul.** 1965. Presence of hordenine in *Lophophora williamsii*. *J. Pharm. Sci.* **54:** 661.
15. **McLaughlin, J. L., J. E. Goyan** and **A. G. Paul.** 1964. Thin-layer chromatography of ergot alkaloids. *J. Pharm. Sci.* **53:** 306–310.
16. Merck and Co., Inc. 1960. *The Merck index, 7th ed.* Merck and Co., Rahway, N. J. p. 524.
17. **Mothes, K.** and **H. R. Schutte.** 1963. The biosynthesis of alkaloids II. *Angew. Chem. Intern. Ed. Engl.* **2:** 441–458.
18. **Reti, L.** 1953. β-Phenethylamines. In **Manske, R. H. F.** and **H. L. Holmes,** *The alkaloids, chemistry and physiology, vol. 3.* Academic Press, New York. pp. 313–338.
19. **Reti, L.** 1954. Simple isoquinoline alkaloids. In **Manske, R. H. F.** and **H. L. Holmes,** *The alkaloids, chemistry and physiology, vol. 4.* Academic Press, New York. pp. 7–21.
20. **Reti, L.** 1933. Sur les Alcaloides de la Cactacee, *Trichocereus candicans* (Br. et Rose). *Comp. Rend. Soc. Biol.* **114:** 811–814.
21. **Safford, W. E.** 1917. Narcotic plants and stimulants of the ancient Americans. *Smithson. Rept. for 1916.* pp. 387–424.
22. **Schultes, R. E.** 1940. The aboriginal therapeutic uses of *Lophophora williamsii*. *Cact. Succ. Jour.* **12:** 177–181.
23. **Shriner, R. L., R. C. Fuson** and **D. Y. Curtin.** 1960. *The systematic identification of organic compounds, 4th ed.* John Wiley and Sons, New York. p. 60.

24. **Slotkin, J. S.** 1955. Peyotism, 1521–1891. *Am. Anthropologist* **57**: 202–230.
25. **Smith, I.** 1960. *Chromatographic and electrophoretic techniques, vol. 1.* Interscience Publishers, Inc., New York. p. 324.
26. **Spaeth, E.** and **F. Becke.** 1935. Über die Trennung der Anhaloniumbasen (15. Mitteilung über Kakteenalkaloide). *Monatsh. Chem.* **66**: 327–336.
27. **Spaeth, E.** and **J. Bruck.** 1935. Über ein neues Kakteenalkaloid, das Anhalinin, und zus Konstitution des Anhalonins (XIII. Mitteil. über Kakteen-alkaloide). *Chem. Ber.* **68**: 501–505.
28. **Spaeth, E.** and **J. Bruck.** 1937. Über ein neues Alkaloid aus den Mezcal buttons (XVIII. Mitteil. über Kakteen-alkaloide). *Chem. Ber.* **70**: 2446–2450.
29. **Spaeth, E.** and **J. Bruck.** 1938. *N*-Acetyl-mezcalin als Inhaltsstoff der Mezcal-buttons (XIX. Mitteil. über Kakteen-alkaloide). *Chem. Ber.* **71**: 1275–1276.
30. **Spaeth, E.** and **J. Bruck.** 1939. Über das *O*-Methyl-d-anhalonidin (XX. Mitteil. über Kakteen-Alkaloide). *Chem. Ber.* **72**: 334–338.
31. **Sullivan, G.** and **L. R. Brady.** 1965. Thin-layer chromatographic separation of betaine, choline, and muscarine. *Lloydia* **28**: 68–70.
32. United States Pharmacopeial Convention, Inc. 1960. *The Pharmacopoeia of the United States, 16th rev.* Mack Publishing Co., Easton, Pa. p. 1073.
33. **Waldi, D., K. Schnackerz** and **F. Munter.** 1961. Eine systematische Analyse von Alkaloiden auf Dünnschichtplatten. *J. Chromatog.* **6**: 61–73.
34. **Weber, G.** 1952. Polarization of the fluorescence of macromolecules. 2. Fluorescent conjugates of ovalbumin and bovine serum albumin. *Biochem. J.* **51**: 155–167.
35. **Willaman, J. J.** and **B. G. Schubert.** 1961. *Alkaloid bearing plants and their contained alkaloids.* U. S. Dept. of Agri. Tech. Bull. no. 1234, U. S. Government Printing Office, Washington, D. C. pp. 57–59, 89–91.