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Biosynthesis of Mescaline and Tetrahydroisoquinoline Alkaloids in *Lophophora williamsii* (Lem.) Coult. Occurrence and Biosynthesis of Catecholamine and Other Intermediates

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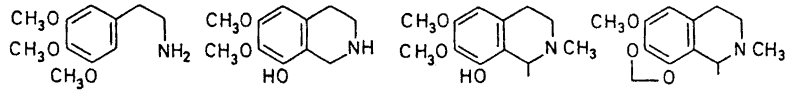
The occurrence and biosynthesis in peyote of some di- and tri-substituted phenethylamines, which are plausible precursors of mescaline and tetrahydroisoquinoline alkaloids, has been investigated in "trapping experiments". The natural occurrence of dopamine, epinine, 4-hydroxy-3-methoxyphenethylamine, and 3,4-dihydroxy-5-methoxyphenethylamine is now established. The presence in peyote of dopa and 3,4,5-trihydroxyphenethylamine is uncertain.

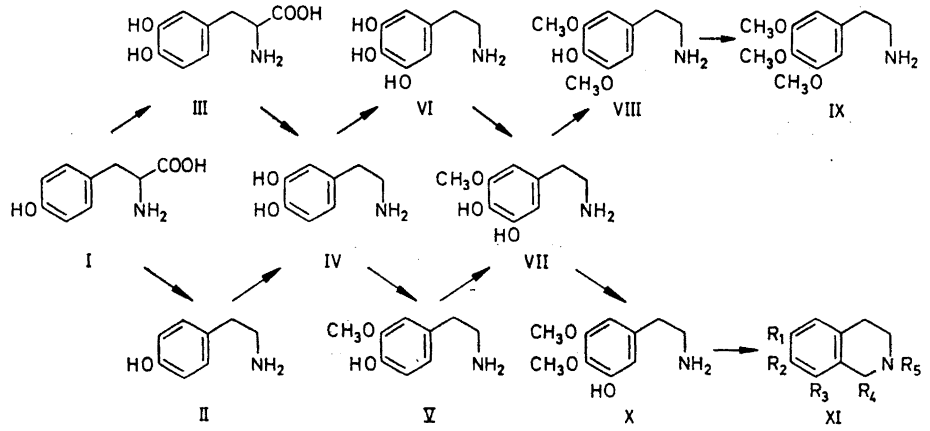
The results indicate a major biosynthetic pathway: tyrosine → tyramine → dopamine with the possible alternative sequence: tyrosine → dopa → dopamine being only of minor importance. The main route from dopamine to the alkaloids involves first a *meta-O*-methylation followed by hydroxylation to 3,4-dihydroxy-5-methoxyphenethylamine. The following alternative sequence is at most of minor importance: dopamine → 3,4,5-trihydroxyphenethylamine → 3,4-dihydroxy-5-methoxyphenethylamine. The latter compound is previously shown to be the common precursor of mescaline and tetrahydroisoquinoline alkaloids. There is a high preference in peyote for the *meta-O*-methylation of dopamine.

The well known Mexican peyote cactus, *Lophophora williamsii* (Lem.) Coult., contains[1] apart from the hallucinogenic mescaline (IX), a number of tetrahydroisoquinoline alkaloids (XI) such as anhalamine (XII), pellotine (XIII) and lophophorine (XIV). Our identification of three plausible intermediates (V, VIII, X; Scheme 1) in peyote and other mescaline producing



IX XII XIII XIV

cacti [2,3] suggested a biosynthetic pathway for the formation of mescaline and related tetrahydroisoquinolines.[2,3] As reported in our earlier preliminary accounts (*cf.* Ref. 4) and also by others (*cf.* Ref. 5) these assumptions were



Scheme 1.

supported by biosynthetic experiments using labelled precursors. The results of incorporation experiments in the mescaline producing cactus *T. pachanoi* have recently been published in detail,[4] and a biosynthetic sequence as shown in Scheme 1 was proposed.

The corresponding detailed account of the incorporation of labelled precursors into mescaline and tetrahydroisoquinoline alkaloids in peyote is published separately.[6] However, it is necessary to establish the natural occurrence of an incorporated precursor, since a not naturally occurring but theoretically plausible compound accidentally might be metabolized to the proper alkaloid. 3-Hydroxy-4,5-dimethoxyphenethylamine (X), a precursor of tetrahydroisoquinoline alkaloids,[6,7] and *N*-methyl-4-hydroxy-3-methoxyphenethylamine (XX; Scheme 2) were previously shown to occur in peyote [2,8] whereas the two mescaline precursors (Scheme 1) 4-hydroxy-3-methoxyphenethylamine (V) and 4-hydroxy-3,5-dimethoxyphenethylamine (VIII) so far, are known only from *T. pachanoi*.[2]

The purpose of the present study was to show the natural occurrence of some crucial intermediates, particularly catecholamine intermediates, *e.g.* IV, VI, and VII in the biosynthesis of mescaline and tetrahydroisoquinoline alkaloids in peyote. This paper is a report of this study.

Catecholamines are difficult to isolate and identify. After preliminary trials with paper chromatography,[9] ion pair extraction,[10] dansylation [11] and gas chromatography,[12] the presence of the proper phenolic intermediates in peyote was ascertained by "inverse isotope dilution" technique which is usually in biosynthetic experiments referred to as "trapping experiment".[3] In principle, this involves the feeding of a plausible radioactive precursor of the compound

in question. After a suitable time lapse, non-labelled compound is added to the plant extract, reisolated and rigorously purified. If the reisolated compound retains a constant specific activity, this shows the presence of the compound in the plant and by the same token also its formation from the administered precursor.

RESULTS

Tyrosine (I), dopa (III) and dopamine (IV) are well established precursors in the early steps of the biosynthesis of mescaline and tetrahydroisoquinoline alkaloids. The results obtained with these ^{14}C-labelled compounds are presented for each precursor separately.

Tyrosine-^{14}C. The results of two experiments using DL-tyrosine-^{14}C as precursor are summarized in Table 1. In the first experiment the extractable

Table 1. Incorporations of tyrosine-α-^{14}C into mono- and disubstituted phenolic amines in *L. williamsii*.

10 days exposure 10 μC/0.22 mg		5 days exposure 10 μC/0.22 mg	
Recovery of radioactivity	%	Recovery of radioactivity	%
Phenolic alkaloid fraction	7.2	Polar amine fraction	4.2
GLC fraction 1 – 5 (Fig. 2)	6.3	Dopamine (IV)	0.07
Tyramine (II)	3.2[a]	Epinine (XVII)	0.09
N-Methyltyramine (XV)	1.7[a]	Dopa (III)	< 0.005
4-Hydroxy-3-methoxy-phenethylamine (V)	0.5[a]		
N-Methyl-4-hydroxy-3-methoxyphenethylamine (XX)	0.4[a]		

[a] Calculated from scan B, Fig. 1.

phenolic amine fraction (see Experimental) was investigated. By a combination of preparative gas chromatography and paper chromatogram scanning (Figs. 1 and 2) the major part of the radioactivity of this fraction could be assigned to tyramine (II) and *N*-methyltyramine (XV). It was also shown in this experiment that tyrosine-^{14}C rendered 4-hydroxy-3-methoxyphenethylamine (V) and *N*-methyl-4-hydroxy-3-methoxyphenethylamine (XX) radioactive.

In a second experiment using shorter exposure time (Table 1), after extraction of alkaloids, the polar amine fraction was isolated with a cation exchange column. Addition of carrier dopamine and epinine (*N*-methyldopamine; XVII) showed after reisolation and purification by paper chromatography followed by repeated crystallizations, rather small but yet reliable incorporations into dopamine and epinine. A similar trapping using dopa (III) as carrier failed to yield a preparation of constant radioactivity.

In summary, this experiment using tyrosine-^{14}C and inverse isotope dilution technique, has shown the natural occurrence (Scheme 2) of dopamine (IV) and epinine (XVII). The metabolic conversion of tyrosine to tyramine

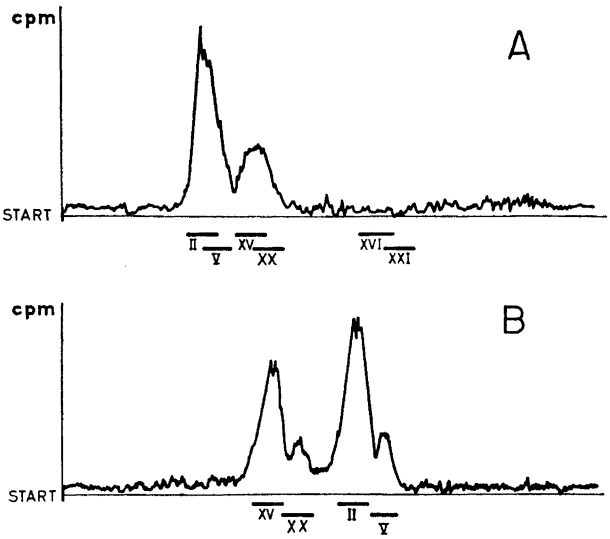


Fig 1. Radio chromatogram scans of phenolic peyote alkaloids, GLC fraction 1 – 5 (Fig. 2), obtained from tyrosine-^{14}C experiment. A. Formamide-treated paper eluted (descending) with pyridine: chloroform (1:5). B. Silica gel loaded paper, Whatman SG, eluted with acetone:chloroform:diethylamine (5:5:1). GLC fraction 1 – 5 contains the compounds II, XV, XVI, V, XX, and XXI. The two combined scans show incorporations into tyramine (II) and 4-hydroxy-3-methoxyphenethylamine (V) and the *N*-methylderivatives of these compounds (XV), (XX). (Radioactivities in the *N*,*N*-dimethyl homologues XVI, XXI are not detectable here.)

and its *N*-methyl derivatives, and to dopamine, epinine and their 3-*O*-methyl derivatives (V, XX) is also established.

Dopa-^{14}C. The administration of DL-dopa-^{14}C (10 μCi; 0.02 mg: 5 days exposure) and isolation of the polar amine fraction showed a 1.5 % incorporation. After addition of carrier dopamine and epinine and reisolation, an incorporation of 0.09 % into dopamine and 0.06 % into epinine was found. This established the formation of dopamine from dopa.

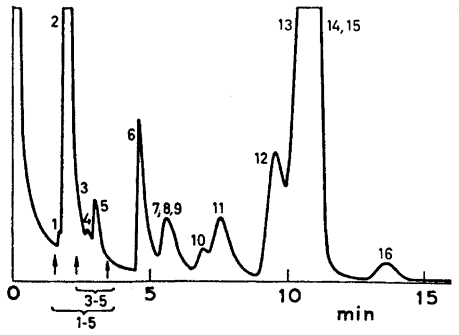


Fig. 2. Gas chromatogram (5 % SE – 30, preparative column [30]) of phenolic alkaloid fraction of *L. williamsii*. The fractions 1 – 5 and 3 – 5 were investigated in this report. The figures correspond to retention times of: 1. Tyramine (II). 2. *N*-Methyltyramine (XV) and (major part) hordenine (XVI). 3. 4-Hydroxy-3-methoxyphenethylamine (V). 4. *N*-Methyl-4-hydroxy-3-methoxyphenethylamine (XX). 5. *N*,*N*-Dimethyl-4-hydroxy-3-methoxyphenethylamine (XXI) 6. (X). 7. (IX). 8 – 11. Unknowns. 12 – 15. (XI). 16. Unknown.

Dopamine-^{14}C. In a first experiment, after feeding DL-dopamine-^{14}C to the peyote cactus, the phenolic alkaloid fraction was isolated and in part separated by preparative GLC (Fig. 2). The fraction 3 – 5 corresponding to 4-hydroxy-3-methoxyphenethylamine (V), its *N*-methyl (XX) and *N*,*N*-dimethyl (XXI) homologues were collected. Inactive carrier material (25 mg) of each of these substances was added to the GLC fraction and then by a combination of TLC-systems each compound was reisolated free from the other and crystallized. The results, summarized in Table 2 show significant incorporations into all three compounds V, XX, and XXI.

Another GLC fraction 3 – 5 were added 3-hydroxy-4-methoxyphenethylamine (XXII) and *N*-methyl-3-hydroxy-4-methoxyphenethylamine (XXIII; Scheme 3), the isomers of V and XX, which have very close retention times on SE – 30. A similar work up, however, failed to demonstrate any activity in XXII and XXIII.

Table 2. Incorporations of dopamine-α-^{14}C into di- and trisubstituted phenolic amines in *L. williamsii*.

10 days exposure 10 μC/0.07 mg		5 days exposure 20 μC/0.14 mg	
Recovery of radioactivity	%	Recovery of radioactivity	%
Phenolic alkaloid fraction	26.8		
4-Hydroxy-3-methoxy-phenethylamine (V)	13.5	Polar amine fraction	5.0
N-Methyl-4-hydroxy-3-methoxyphenethylamine (XX)	3.2	3,4-Dihydroxy-5-methoxy-phenethylamine (VII)	0.29
N,*N*-Dimethyl-4-hydroxy-3-methoxyphenethylamine (XXI)	1.7	3,4,5-Trihydroxy-phenethylamine (VI)	< 0.01

In a second dopamine-^{14}C experiment (Table 2), inactive carrier of 3,4-dihydroxy-5-methoxyphenethylamine (VII) and 3,4,5-trihydroxyphenethylamine (VI) were added to the aqueous extract before the ion exchange step. Reisolation of these compounds including two paper chromatographic procedures and repeated recrystallizations gave a 3,4-dihydroxy-5-methoxyphenethylamine (VII) of constant specific activity while the specific activity of the 3,4,5-trihydroxyphenəthylamine (VI) fell below values which could be accurately determined.

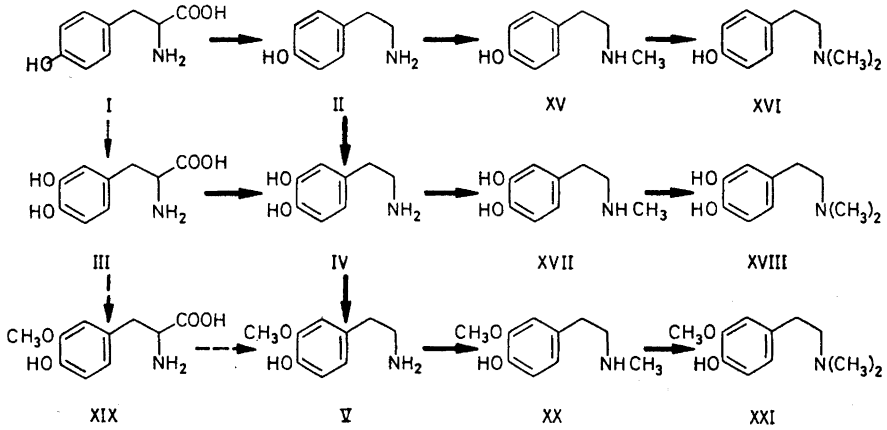
The location of the label in V from dopamine-α-^{14}C was determined by degradation. After methylation with diazomethane to 3,4-dimethoxyphenethylamine and oxidation to 3,4-dimethoxybenzoic acid,[4] only 1.1 % of the label remained thereby proving a direct conversion. *N*-Methyl-4-hydroxy-3-methoxyphenethylamine (XX) and *N*,*N*-dimethyl-4-hydroxy-3-methoxyphenethylamine (XXI) were degraded with similar results. Diazomethane methylation of 3,4-dihydroxy-5-methoxy-phenethylamine followed by oxidation to 3,4,5-trimethoxybenzoic acid [4] confined the label to the α-carbon of the side chain (96 %).

These results show the natural occurrence in peyote of 4-hydroxy-3-methoxyphenethylamine, which previously is known from *Trichocereus* species,[13,14] and its *N*-methyl and *N*,*N*-dimethylderivatives. Also the natural occurrence of 3,4-dihydroxy-5-methoxyphenethylamine in peyote is ascertained.

DISCUSSION

The following discussion is divided into two sections dealing with the occurrence and formation of disubstituted and trisubstituted phenethylamines, respectively.

Disubstituted phenethylamines. Based on incorporation experiments [4,7] a likely biosynthetic sequence for the formation of mescaline and tetrahydroisoquinoline alkaloids was suggested (Scheme 1). In Scheme 2 are depicted the



Scheme 2.

hypothetical interrelations of a large number of phenolic phenethylamines in peyote. The non-phenolic compounds are omitted since they serve as poor precursors and then apparently first after *p*-*O*-demethylation to a phenol.[4,15]

The natural occurrence of compounds II, XV, XVI, and XX in peyote is known previously.[1,14,16] We have now by "trapping experiments" established the presence of IV, V, XVII, XX, and XXI (Scheme 2). Tyrosine (I) was converted to alkaloids II and XV; IV and XVII; and V and XX. Dopa (III) was metabolized to IV and XVII and finally, dopamine (IV) to V, XX, and XXI. These results taken together indicate the biosynthetic patways shown with solid arrows in Scheme 2.

The biosynthetic sequence: tyrosine (I) → tyramine (II) → *N*-methyltyramine (XV) → hordenine (XVI) was established by Leete *et al.*[17] in barley over a decade ago. The validity of this metabolic route also for the peyote cactus was suggested by the results of McLaughlin.[16] This is now verified in our experiments (Fig. 1).

Of special interest are the alternative formations of dopamine from tyrosine, either *via* tyramine or *via* dopa, as have been indicated by precursor experiments (Scheme 1). While tyramine has been identified in peyote and also was formed in good yield from tyrosine-^{14}C, dopa could not be clearly identified in the cactus. This may signify either that there is a very minute metabolic pool of dopa, or that it does not occur in peyote. Dopa is, however, known to occur in plants.[18]

The amount of radioactivity trapped in a "trapping experiment" is, barring "compartmentation",[19,36] related to the "pool" size of the compound in question. The size of the "pool" depends upon the rate of formation and the rate of metabolism. A high turn-over of dopa, *e.g.* to IV and V (Scheme 2), or perhaps preferably to non-alkaloidal compounds, would make it hard to detect by "trapping", but still it could be a true intermediate.

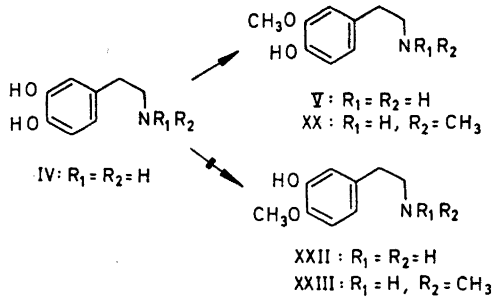
However, dopa itself can, as found by precursor experiments, on the whole hardly be considered to be a more efficient precursor of mescaline than tyramine [4,7] (although, penetration and transport may be different for an amino acid and an amine). A comparison of the "pool" sizes of tyramine and dopa, based upon the amount of radioactivity from tyrosine-^{14}C in respective compounds (Table 1), would suggest the amount of tyramine to be at least a thousand times larger than that of dopa. For these reasons it is assumed that the contribution of the alternative pathway I → III → IV will be minimal.

The relatively good incorporation of dopa into mescaline [7] may be explained by the ease at which dopa can be decarboxylated by an unspecific L-aromatic amino acid decarboxylase.[20] In fact, even the non-enzymatic decarboxylation of dopa in neutral solution has been shown to proceed at a considerable rate.[21]

A participation in the biosynthesis of mescaline of substituted amino acids, *e.g.* XIX, seems unlikely for above mentioned reasons. In fact, a special search for compounds like XIX by Kapadia *et al.*[22] gave no evidence for their presence in peyote. The decarboxylation of XIX have been shown to be slower than that of dopa in animal tissues.[23]

The *N*-methyl derivative of dopamine, epinine (XVII), was shown to originate in peyote both from tyrosine-^{14}C and dopa-^{14}C. Epinine is previously known from Leguminosae.[24] It is quite plausible that also the *N*,*N*-dimethyl derivative of dopamine (XVIII; Scheme 2) is formed in peyote, in analogy to the formation of XVI and XXI. Whether the *N*-methylated derivatives as such or after demethylation can be hydroxylated, *e.g.* XV → XVII, is at present not known.

We have recently identified 3-hydroxy-4-methoxyphenethylamine (XXII; Scheme 3) in the tetrahydroisoquinoline alkaloid producing cactus *Pachycereus pecten-aboriginum*.[14] The *N*,*N*,*N*-trimethyl derivative of this compound is frequent in *Magnolia* species.[25,26] Incorporation experiments both in *L. williamsii* [6,7] and *T. pachanoi* [4] have shown that only one isomer, 4-hydroxy-3-methoxyphenethylamine (V), is a precursor of mescaline. The results from previous experiments in *T. pachanoi* [4] and the present data likewise agree in that there is a high preference for a methylation of dopamine on the *meta*-hydroxy group (Scheme 3). This preference for *meta*-*O*-methylation for catecholamines is also pronounced in the animal organism *in vivo* (*cf.* Refs. 27, 28). Recently, Creveling *et al.*[27] have presented results that this is also true for the

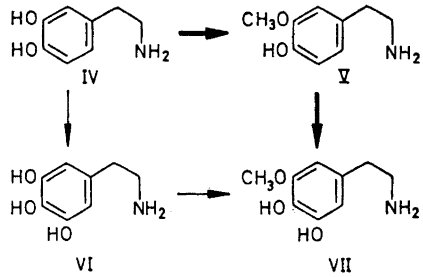


Scheme 3.

in vitro methylation of catecholamines with purified catechol-*O*-methyltransferase.

How does Scheme 2 agree with the biosynthesis and metabolism of the catecholamines in the animal organism? The main route of dopamine formation in the mammal [28] is tyrosine → dopa → dopamine whereas in peyote this is at most a minor pathway. On the other hand, the conversion of tyramine to dopamine by a rather non-specific "tyramine hydroxylase" in mammals [28] is of minor importance, but apparently the main route in peyote. The formation of epinine from dopamine has been demonstrated in the rabbit.[28] However, as discussed, the preference for *meta*-*O*-methylation of catechols is high both in animals and in the biosynthesis of alkaloids in peyote (Scheme 3).

Trisubstituted phenethylamines. Previous results [7,15] show that both 3,4,5-trihydroxyphenethylamine (VI) and 3,4-dihydroxy-5-methoxyphenethylamine (VII) can serve as precursors of mescaline and tetrahydroisoquinoline alkaloids. Neither of these compounds have been identified in plants although VII has been implicated [29] as an enzymic product of V. There are, as indicated in Schemes 1 and 4, alternative biosynthetic routes available, *viz.* IV → V → VII or IV → VI → VII. The presence in peyote of VII, but not VI, was indicated in the "trapping experiment" using dopamine-^{14}C as precursor. Provided the metabolism of VI, *e.g.* to VII, was rapid, VI still could be an important intermediate, since the small "pool" would make it hard to detect. However, since compound V is incorporated about three times more efficiently than VI into mescaline in peyote,[7] it would appear that the major pathway



Scheme 4.

(Scheme 4) is IV → V → VII and that IV → VI → VII is probably a minor path for the biosynthesis of alkaloids in peyote.

Detailed results of the incorporation of labelled precursors into mescaline and other peyote alkaloids and the relation of these results to this paper will be published separately.[6]

EXPERIMENTAL

Melting points were determined with a Leitz Mikroskopheiztisch 350 using calibrated thermometers (Leitz AG). NMR spectra were recorded with a Varian A-60 instrument with tetramethylsilane as internal reference $\delta = 0$ ppm. Mass spectra (70 eV) were obtained with the LKB-9000 gas chromatograph-mass spectrometer. Varian Aerograph 202 or Aerograph 204 gas chromatographs were used (30). Radioactivities were measured with a Packard Tri-Carb Model 3375 liquid scintillation spectrometer, in a solvent system consisting of 2 ml absolute ethanol and 10 ml "TPP" (0.5 % PPO and 0.03 % POPOP in toluene). External standard or the channel ratio method was used for efficiency determination. For paper and thin layer chromatogram scanning a Nuclear Chicago, Model 1032, chromatogram scanner was used.

Plant material. Lophophora williamsii (Lem.) Coult., was purchased from K. Edelman, Reeuwijk, the Netherlands. Plants weighing 70 – 90 g were used.

Labelled compounds. The ^{14}C-labelled compounds used were purchased from The Radiochemical Centre, Amersham, England. The radioactive compounds were injected in small volumes of water into the cactus stems.

Reference compounds. The compounds I, II, III, IV, XVI, were of commercial source. Synthesized according to reported procedures were the compounds XV,[31] XVII,[32] VI,[4] VII and XXII (*cf.* Ref. 33).

N-*Methyl-4-hydroxy-3-methoxyphenethylamine (XX).* A general procedure for *N*-monomethylation [37] was used with slight modifications. 4-Benzyloxy-3-methoxyphenethylamine HCl [33] (100 mg) was suspended in dichloromethane (5 ml) and to the suspension was added 0.5 ml trifluoroacetic acid anhydride. The resulting solution was stirred for 30 min and then the solvent and reagent were evaporated. The solid residue was dissolved in dry acetone (2 ml), methyl iodide (0.09 ml) and powdered potassium hydroxide (28 mg) were added and the mixture was heated under reflux for 5 min. Excess of methyl iodide and acetone was removed, water (3 ml) was added and the mixture was heated under reflux for 10 min to hydrolyze the trifluoroacetamide. After a usual work up, a yield of 60 mg (57 %) *N*-methyl-4-benzyloxy-3-methoxyphenethylamine HCl was obtained. M.p. 157 – 59°.

Debenzylation of this compound was carried out by hydrogenation at atmospheric pressure in acetic acid solution over palladium on charcoal (10 %) catalyst. The yield, of *N*-methyl-4-hydroxy-3-methoxyphenethylamine HCl was 35 mg (82 %) after recrystallization from ethanol-ether. M.p. 154 – 155°. Mass spectrum, major peaks: *m/e* 44 (100 %) *m/e* 137 (8 %), *m/e* 138 (25 %), *m/e* 181 (M^+, 1 %).

N,N-*Dimethyl-4-hydroxy-3-methoxyphenethylamine (XXI).* *N*-methyl-4-benzyloxy-3-methoxyphenethylamine HCl (200 mg) was dissolved in methanol (6 ml) and 0.2 ml of 36 % formaldehyde solution was added. The mixture was stirred for 15 min and then reduced during 1 h with sodium borohydride (0.3 g). The yield of *N,N*-dimethyl-4-benzyloxy-3-methoxyphenethylamine HCl was 175 mg (85 %). M.p. 153 – 156°. NMR ($CDCl_3$): $\delta = 7.42$ (5H, C_6H_5); 6.82 (3H, ArH –2,5,6); 5.17 (2H, $O-CH_2-$); 3.87 (3H, OCH_3); 3.20 (4H, $-CH_2-CH_2-$); 2.80/2.87 (3H/3H, $N(CH_3)_2$).

Debenzylation of this compound yielded 110 mg (87 %) of *N,N*-dimethyl-4-hydroxy-3-methoxyphenethylamine HCl. M.p. 190 – 191°. Mass spectrum, major peaks: *m/e* 58 (100 %), *m/e* 137 (4 %), *m/e* 195 (M^+ 0.3 %).

N-*Methyl-3-hydroxy-4-methoxyphenethylamine (XXIII).* This compound was prepared from 3-benzyloxy-4-methoxyphenethylamine HCl [33] (100 mg) as described for (XX). Yield of *N*-methyl-3-hydroxy-4-methoxyphenethylamine HCl was 42 mg (56 %). M.p. 176 – 180°. Mass spectrum, major peaks: *m/e* 44 (100 %), *m/e* 137 (7 %), *m/e* 138 (28 %), *m/e* 181 (M^+, 1 %).

Paper chromatography. For the purification of dopamine (IV) and epinine (XVII) two solvent systems were used: (i) butanol:acetic acid:water (5:1:4) and (ii) butanol,

saturated with 3 N HCl.[8] Dopa, 3,4-dihydroxy-5-methoxyphenethylamine, and 3,4,5-trihydroxyphenethylamine were purified in system (i).[34] Whatman 1 and Whatman 3MM papers were used.

Thin layer chromatography. Silica gel F 0.25 mm, precoated aluminium plates (Merck AG) were used with the following solvents: (a) chloroform:ethanol:diethylamine (85:5:10), (b) chloroform:acetone:diethylamine (5:3:1), and (c) chloroform:ethanol:conc. NH_3 (85:20:1).

R_F-values × 100 of compounds purified

	Solvent system		
	a	*b*	*c*
4-Hydroxy-3-methoxy-phenethylamine (V)	26	37	20
N-Methyl-4-hydroxy-3-methoxy-phenethylamine (XX)	29	19	18
N,*N*-Dimethyl-4-hydroxy-3-metho xy-phenethylamine (XXI)	48	36	50
3-Hydroxy-4-methoxy-phenethylamine (XXII)	13	27	17
N-Methyl-3-hydroxy-4-methoxy-ph enethylamine (XXIII)	18	8	16

Isolation of amine fractions. Detailed methods for the extraction and separation of alkaloids from cacti are described previously [4] and separately.[4] A modification of this procedure suitable for the separation of non-extractable amines is described below.

An extract of the frozen plant was obtained by homogenization in methanol (500 ml) and filtration. The extract was added 0.5 g ascorbic acid and evaporated under reduced pressure to a volume of 20 – 30 ml. After addition of water (50 ml) the solution was extracted with chloroform (2 × 70 ml). The water phase was adjusted to pH 8 and again extracted with chloroform (3 × 75 ml). The alkaloids contained in the combined chloroform extracts were purified and divided in non-phenolic and phenolic fractions as earlier reported.[4]

The water phase was acidified by addition of ascorbic acid and then passed through a strong cation exchange column (Dowex 50W – X4, 1 cm × 8 cm) to adsorb the polar amines. The column was washed with water (50 ml) and 1 M HCl (50 ml). Model experiments showed that none of the diphenolic amines investigated here were eluted in these washings. The polar amines were recovered by elution with 5 N HCl (50 ml) and evaporation under reduced pressure at room temperature.

Trapping of catecholamine derivatives. A suitable amount (20 – 50 mg) of inactive catecholamine derivative was added as carrier to the polar amine fraction before or after the ion exchange step. The substances were reisolated and purified by paper chromatography followed by repeated crystallizations as their hydrochlorides in methanol-ether until constant specific activity.

Dopa (100 mg) was crystallized from 1 N HCl-saturated sodium acetate solution.[35]

Trapping of V, XX and XXI. The phenolic alkaloid fraction was in part (1/10) subjected to preparative GLC and the fraction corresponding to 4-hydroxy-3-methoxy-phenethylamine and its *N*-methyl and *N*,*N*-dimethyl homologues was collected (Fig. 3, fraction 3 – 5). To the fraction 3 – 5 was added 20 mg each of inactive 4-hydroxy-3-methoxyphenethylamine, *N*-methyl-4-hydroxy-3-methoxyphenethylamine and *N*,*N*-dimethyl-4-hydroxy-3-methoxyphenethylamine. The compounds were reisolated by preparative chromatography in TLC systems *b* and *c* (see R_F-values) and crystallized as hydrochlorides in methanol-ether to constant specific activity.

To another fraction 3 – 5 was added 20 mg of each 3-hydroxy-4-methoxyphenethylamine and *N*-methyl-3-hydroxy-4-methoxyphenethylamine. Repeated purifications by TLC systems *a* and *b* and crystallizations from methanol-ether were carried out.

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