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SHORT COMMUNICATION

ALKALOID BIOSYNTHESIS IN *ECHINOCEREUS MERKERI*

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Abstract—The biosynthesis of 3,4-dimethoxyphenylethylamine and salsoline in *Echinocereus merkeri* has been studied using D,L-2′-^{14}C-tyrosine, 1′,2′-^{3}H-dopamine Ring-(G)-^{3}H-3-hydroxy-4-methoxyphenylethylamine and 1-methyl-1-^{14}C-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid The first three were incorporated into 3,4-dimethoxyphenylethylamine but only the last two were incorporated into salsoline

INTRODUCTION

THE ALKALOIDS produced by *Echinocereus merkeri* fall into three groups, tyramine derivatives, dopamine derivatives and the 1,2,3,4-tetrahydroisoquinoline, salsoline [1] This diversity must mean that their biosynthesis involves problems common to many more complex systems but the simple structures of the *E merkeri* alkaloids makes this plant a good system for investigation 2′-^{14}C-tyrosine, 1′,2′-^{3}H-dopamine, ring-(G)-^{3}H-3-hydroxy-4-methoxyphenylethylamine and 1-^{14}C-1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid were available, and the present paper gives the information obtained about alkaloid biosynthesis in *E merkeri* using these substrates in classical feeding experiments

RESULTS AND DISCUSSION

Table 1 provides a summary of the feeding experiments and Table 2 records the results of degradation experiments carried out to confirm the incorporation of precursors The results of feeding and degradation experiments are consistent with the scheme presented in Fig 1

The essentials of this scheme are that tyramine (II) is a branch point for hordenine (III) and all the other alkaloids, and dopamine (IV) is a branch point for 3,4-dimethoxyphenylethylamine (VI) and salsoline (IX). Further, the methyltransferases necessary for the conversion of tyramine to hordenine and for the conversion of dopamine to 3-hydroxy-4-methoxyphenylethylamine (VII) appear to be blocked The main conclusion from the feeding experiments is that alkaloid synthesis is directed to 3,4-dimethoxyphenylethylamine and not to salsoline or hordenine The incorporation of tyrosine into tyramine and of tyrosine and dopamine into 3,4-dimethoxyphenylethylamine does not give any information on the role of dopa Thus there remains the possibility that the reaction sequence tyrosine → dopa → dopamine is operating The biosynthesis of hordenine (III) in barley from tyrosine and tyramine is well established [2] In *E. merkeri*, tyrosine is efficiently incorporated into tyramine but not into hordenine This can only be explained by the non-availability of the *N*-methylating enzymes at this stage of the plant's development The non-incorporation of both

[1] S AGURELL, J LUNDSTROM and A MASOUD, *J Pharm Sci* **58**, 1413 (1969)
[2] E LEETE, S KIRKWOOD and L MARION, *Can J Chem* **30**, 749 (1952)

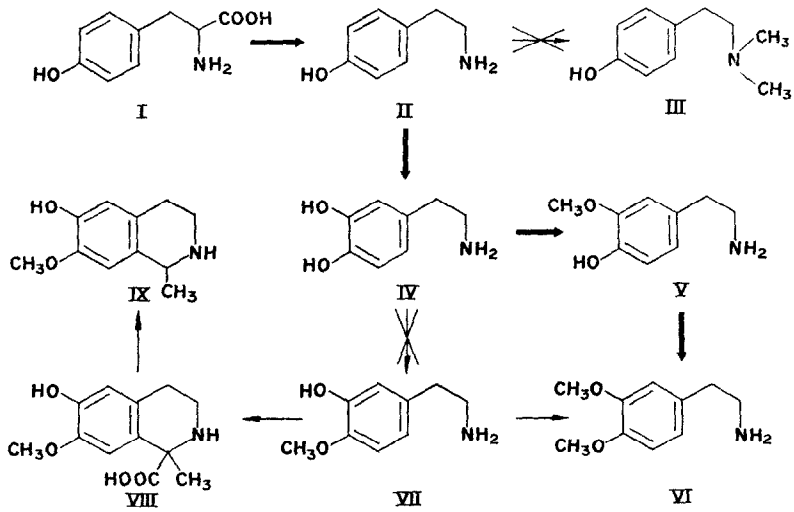

FIG. 1

tyrosine and dopamine into salsoline (IX) is surprising particularly as 3-hydroxy-4-methoxyphenylethylamine (VII) is incorporated. These results point to a blockage in the pathway, specifically to the methylating enzyme which converts dopamine into (VII) There are a number of possible intermediates between (VII) and salsoline which differ in the origin of the C-2 unit Two of these possible intermediates are suitable for incorporation studies. One is the *N*-acetyl amine from (VII), namely *N*-acetyl-3-hydroxy-4-methoxyphenylethylamine which could be formed from acetyl CoA and (VII) This amide is not incorporated into salsoline in *E merkeri* [3] A second possibility is 1-methyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid, (VIII) which could be synthesized in the plant from (VII) and pyruvate This acid is incorporated into salsoline in *E merkeri*. An analogue of this acid, 1-methyl-6,7-dimethoxy-8-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid has been found[4] in *Lophophora williamsii* and proposed

Table 1 Feeding experiments to *E merkeri*

Substrate	Specific activity (mc/m-mole)	μmoles injected	μmoles ^{14}C-product formed: 3,4-Dimethoxyphenylethylamine	μmoles ^{14}C-product formed: Salsoline
D,L-2′-^{14}C-Tyrosine*	50	0 74	0 004	0 000
1′,2′-^{3}H-Dopamine HCl	390	0 2	0 0015	0 000
Ring-(G)-^{3}H-3-hydroxy-4-methoxyphenylethylamine HCl	13 6	9 34	0 84	0 28
1-Methyl-1-^{14}C-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid	0 068	4 4	—	0 006

* Tyrosine gave 0 036 μmoles of tyramine but no detectable amount of hordenine

[3] I J McFarlane and M Slaytor, *Phytochem* **11**, 229 (1972)
[4] G J Kapadia, M B E Fayez, Y S Vaishnav, H M Fales and G Subba Rao, *Lloydia* **32**, 525 (1969)

TABLE 2 PERMANGANATE OXIDATION OF PHENYLETHYLAMINES FROM *E merkeri*

Substrate	Product	Specific activity (dpm/μmole)	3,4-Dimethoxybenzoic acid*	*p*-Methoxybenzoic acid*
D,L-2′-^{14}C-Tyrosine	Tyramine HCl	119	—	0 8
D,L-2′-^{14}C-Tyrosine	3,4-Dimethoxyphenylethylamine HCl	154	1 1	—
1′,2′-^{3}H-Dopamine HCl	3,4-Dimethoxyphenylethylamine HCl	314	1 8	—
Ring-(G)-^{3}H-3-hydroxy-4-methoxyphenylethylamine HCl	3,4-Dimethoxyphenylethylamine HCl	6182	95 0	—
				—

* Per cent of product activity

as an immediate precursor of anhalonidine The major alkaloids (80%) in *E merkeri* are 3,4-dimethoxyphenylethylamine and its *N*-methyl derivatives[1] and it is perhaps not surprising that in these feeding experiments alkaloid synthesis is directed towards their production That is, both tyrosine and dopamine are incorporated into 3,4-dimethoxyphenylethylamine (VI) In addition (VII) was also incorporated into (VI) It is suggested that (VII) is not a normal intermediate in the biosynthesis of (VI) and that (VI) is being produced from it by non-specific methylation Rather, the true intermediate is 3-methoxy-4-hydroxyphenylethylamine (V) which has been found in *E merkeri* [1] This means that the direction of methylation of dopamine determines whether the plant makes (VI) and its derivatives or whether it makes salsoline It is interesting to note that in *Trichocereus pachanoi* which produces (VI) a detailed gas chromatography mass spectrometric analysis of the phenolic fraction could only detect (V) and not (VII) [5] The importance of methylation is alkaloid synthesis in *L williamsii* has been noted [6–8] The methylation of 3-methoxy-4,5-dihydroxyphenylethylamine apparently determines whether mescaline or the tetrahydroisoquinolines are synthesized Thus 3-methoxy-4,5-dihydroxyphenylethylamine is a precursor for both alkaloids[6,7] while the 3,4-dimethoxy-5-hydroxy derivative is an intermediate for the tetrahydroisoquinolines[6] and the 3,5-dimethoxy-4-hydroxy derivatives is essentially only an intermediate in mescaline synthesis [8]

The results illustrate the caution which must be used both with positive and negative results from feeding experiments For example it is unlikely that tyrosine is not a precursor of all the alkaloids under discussion but reports of such non-incorporation of basic precursors are too rare in the literature

EXPERIMENTAL

General Plant material, chromatography, autoradiography, m p determinations and radioactive counting were as described previously [3]

Administration of radioactive substrates All radioactive substrates were injected in aq solution The tetrahydroisoquinoline carboxylic acid (VIII) was converted to the HCl-ide by cautious addition of 0 01 M HCl After injection the cacti were grown for 3 weeks

Isolation and detection of alkaloids This was as described previously [3] In addition, tyramine HCl, hordenine sulphate and 3,4-dimethoxyphenylethylamine HCl were recrystallized from MeOH–EtOAc They

[5] S AGURELL, *Lloydia* **32,** 40 (1969)
[6] K L KHANNA, M TAKIDO and H ROSENBERG, *Phytochem* **9,** 1811 (1970)
[7] K L KHANNA, H ROSENBERG and A G PAUL, *Chem Commun* 315 (1969)
[8] A G PAUL, K L KHANNA, H ROSENBERG and M TAKIDO, *Chem Commun* 838 (1969)

were detected on TLC with 0 5% *o*-dianisidine in 1 M HCl and 10% $NaNO_2$ (tyramine and hordenine) or iodoplatinate (tyramine and 3,4-dimethoxyphenylethylamine)

Chemical syntheses 1-*Methyl*-1-^{14}C-1,2,3,4-*tetrahydroisoquinoline*-1-*carboxylic acid* (VIII) This was synthesized by a modification of the method of Hahn and Rumpf [9] Sodium 2-^{14}C-pyruvate (0 05 mc, 82 5 mg) and 3-hydroxy-4-methoxyphenylethylamine HCl (102 mg) were dissolved in H_2O (0 5 ml) and the pH adjusted to 6 with dil NH_4OH Incubation for 4 days at 20–25° gave white crystals of (VIII) It was recrystallized from H_2O (100°) yield 53 mg (45%), m p 252° (reported 254°) [5] Specific activity 0 068 mc/m-mole For *in vivo* experiments (VIII) was further purified on TLC (neutral plates with solvent system A)

Ring-(G)-^{3}H-3-*hydroxy*-4-*methoxyphenylethylamine* HCl Prepared from 3-hydroxy-4-methoxyphenylethylamine HCl and $(CF_3)_2CO$ and ^3H_2O The exchange was effected by allowing the reactants to stand for 7 days at −15° in a sealed tube M p 203–205° (reported 206–207°) Specific activity 13 6 mc/m-mole Single spot on autoradiography of TLC (neutral plates with solvent system C) In a parallel experiment in D_2O it was shown by NMR that 31 per cent of the aromatic protons had exchanged

O-*Methylhordenine methiodide* Tyramine HCl-ide (211 mg) was added to EtOH (10 ml) in which Na (100 mg) had been dissolved The mixture was refluxed for 5 min in N_2 MeI (3 ml) was then added and the refluxing continued for 2 hr $NaHCO_3$ (0 5 g) and more MeI (3 ml) were then added and the mixture was refluxed for 3 hr The residue obtained on evaporation was extracted with hot $CHCl_3$ which was then filtered and evaporated The residue was crystallized from EtOH affording colourless plates of *O*-methylhordenine methiodide m p 230–231° (reported 230–231°) [10]

Permanganate oxidation of phenylethylamines The phenylethylamine (or *o*-methylhordenine methiodide) (c 100 mg) was dissolved in 5 ml of H_2O and NaOH (0 05 ml, 20%) $KMnO_4$ (16 ml, 3%) was added and the solution gently boiled under reflux for 30 min While still boiling, excess $KMnO_4$ was destroyed by the addition of a few drops of EtOH The solution was filtered, the filtrate acidified with HCl and extracted with $CHCl_3$ (4×) The combined $CHCl_3$ extracts were dried ($CaSO_4$) and the solvent removed under N_2 The crystalline residue was recrystallized 2× boiling H_2O

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[9] G Hahn and F Rumpf, *Chem Ber* **71**, 2141 (1938)
[10] G Barger, *J Chem Soc* **95**, 2197 (1909)

Key Word Index—*Echinocereus merkeri*, Cactaceae, alkaloids, biosynthesis, tyramines, isoquinolines