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BIOSYNTHESIS OF PELLOTINE

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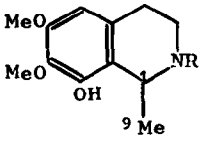
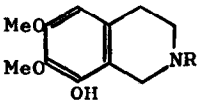
Earlier research (1-4) on the biosynthesis of isoquinoline alkaloids occuring in the peyote cactus Lophophora williamsii proved that the phenethylamine unit of these systems, e.g. of pellotine (I), is derived in vivo from tyrosine and 3,4-dihydroxyphenylalanine and also from corresponding amines, tyramine and dopamine. It was further shown that the two-carbon unit [C-1 and C-9 of (I)] is approximately evenly labelled, and to a high level, when [1-^{14}C] acetate is administered to the cacti (2). The methyl group of methionine did not serve as a source of the two-carbon unit (2). We now outline further results bearing upon the origin of both portions of the pellotine molecule.



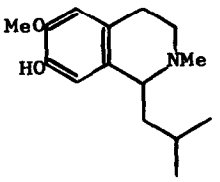
(I) R = Me

(II) R = H



(III) R = Me

(IV) R = H



(V)

Pellotine (I) underwent acid-catalysed nuclear tritiation and the product was used to determine the pH range and conditions over which there was no significant loss of tritium by exchange. These conditions were then used in the extraction of pellotine from cacti fed with the tritiated amines listed in Table 1 (Expt. 1-3). The amines were prepared by routes to be described in our full Paper and were labelled by acid-catalysed exchange at 100°; the ^{14}C -labelled amines (Expt. 4-6) were synthesised by standard methods. Expts. 1-6 were carried out at different times of the year and therefore too much

Table 1. Incorporation Experiments on Lophophora williamsii.

Expt. No. and Month	Substance fed (^3H : ^{14}C ratio)	% Incorp. into pellotine (I) (^3H : ^{14}C ratio)
1, December	[aryl-^{3}H]3,4-Dimethoxyphenethylamine	0.29
2, December	[aryl-^{3}H]3,4-Dihydroxy-5-methoxyphenethylamine	0.35
3, September	[aryl-^{3}H]3,4-Dimethoxy-5-hydroxyphenethylamine	0.028
4, March	[1'-^{14}C]3,4-Dimethoxy-5-hydroxyphenethylamine	0.028
5, August	[1'-^{14}C]3-Methoxy-4-hydroxyphenethylamine	1.5
6, June	[1'-^{14}C]Mescaline	0.34
7, September	[aryl-^{3}H]Anhalonidine	1.5
8, September	[methyl-^{3}H, ^{14}C]Methionine (Ratio 4.45)	0.59 (Ratio 4.33)
9, December	Sodium [2-^{14}C]acetate	0.045
10, February	Sodium [2-^{14}C]acetate	0.043
11, February	Sodium [1-^{14}C]acetate	0.043
12, April	Sodium ^{14}C-bicarbonate	0.002
13, December	Sodium ^{14}C-formate	0.13
14, April	Sodium [^{3}H, ^{14}C]formate (Ratio 12.5)	0.006 (Ratio 45.1)
15, April	Sodium [2-^{3}H, ^{14}C]acetate (Ratio 22.6)	0.021 (Ratio 14.4)

significance must not be read into small differences of incorporation. However, compared with that found (2) for dopamine (7.5%), all the incorporations are low. This suggests that none of these amines lies on the direct pathway to pellotine and that possibly they are entering as a result of O-demethylation. The precise agreement for Expts. 3 and 4 is fortuitous but strengthens confidence in the results from the other ^{3}H-labelled substances. Expt. 7 shows that N-methylation of anhalonidine (II) occurs to give pellotine (I) and the reverse step was also observed when [aryl-^{3}H]pellotine

Table 2. Kuhn-Roth Degradation of Doubly-labelled Pellotine

	SUBSTANCE FED	
	Sodium [^{3}H, ^{14}C]formate	Sodium [2-^{3}H, ^{14}C]acetate
Expt. No.	14	15
Ratio ^{3}H : ^{14}C in precursor	12.5	22.6
Ratio ^{3}H : ^{14}C in pellotine	45.1	14.4
% of total ^{14}C (and ^{3}H : ^{14}C ratio) in derived HOAc (C-1 + C-9)	35 (28.3)	84 (6.5)
% of total ^{14}C (and ^{3}H : ^{14}C ratio) in derived $MeNH_2$ (C-9)	18 (54.3)	41 (13.2)
% of total ^{14}C (and ^{3}H : ^{14}C ratio) in O-methyl groups	47 (38.5)	4 (14.7)

led to radioactive anhalonidine (II ; 4.9% incorp.)

The recent work of Lundström and Agurell (5) on the peyote cactus is in broad agreement with our results; in particular, they found that 3,4,5-trihydroxyphenethylamine is incorporated into anhalamine (IV) as effectively as dopamine. The combined evidence points to a stepwise introduction of hydroxyl groups leading to dopamine and on to 3,4,5-trihydroxyphenethylamine with O-methylation mainly taking place after ring-closure to the isoquinoline system. Further work will be necessary, however, to establish the sequence rigorously.

The earlier experiments (2) with [1-^{14}C]acetate and [methyl-^{14}C]methionine have now been considerably extended. Pellotine from Expt. 8 contained 63% of the ^{14}C activity in the O-methyl groups and 35% in the N-methyl group. The ^{3}H-retention shows that the S-methyl group of methionine is transferred intact in agreement (6) with work on nicotine. Anhalidine (III) isolated from this experiment was highly radioactive (0.42% incorp.) and showed a ^{3}H : ^{14}C ratio of 4.13. Sodium [2-^{14}C]acetate was incorporated into pellotine (duplicate Expts. 9 and 10) and degradation (2) showed that C-1 and C-9 together carried 87% and 94%, respectively, of the total activity and that C-9 alone carried 50% and 47%, respectively. It was established by using synthetic materials of

known labelling pattern, that the degradative routes are accurate and reliable. [1-^{14}C]Acetate again led to heavy labelling of the two-carbon unit (Expt. 11), 59% of the total activity being found at C-1 and 26% at C-9. Acetic acid is thus able to act as an effective precursor of the two-carbon unit but is undergoing degradation *in vivo*. Expts. 12 and 13 were carried out to gain information about the degradative process. Whereas ^{14}C-bicarbonate is clearly an ineffective precursor (Expt. 12), sodium ^{14}C-formate (Expt. 13) was more efficiently used than sodium acetate. Degradation (2) of the formate-derived pellotine showed that 37% of the total activity was present at C-1 and 39% at C-9; the two O-methyl groups together carried only 17% of the total. Thus formate has in this case labelled the two-carbon unit more than four times as heavily as it labels the O-methyl groups. When sodium [^{3}H, ^{14}C]formate acted as precursor in a short feeding experiment (Expt. 14), there was a sharp rise in ^{3}H:^{14}C ratio in the pellotine isolated. This is interpreted as being due to preferential oxidative loss of ^{14}C-labelled formate relative to the ^{3}H-labelled species because of the large ^{3}H-isotope effect. The two-carbon unit was again strongly labelled (Table 2) and the ^{3}H:^{14}C results show that the carbon of formate is incorporated together with the hydrogen it originally carried into the O-methyl and C-methyl groups. Similarly, sodium [2-^{3}H, ^{14}C]acetate gave valuable information (Expt. 15). Here, a fall in ^{3}H:^{14}C ratio was observed and the values found (Table 2) for this ratio at the C-methyl and O-methyl groups were roughly equal.

The foregoing results allow the following conclusions to be drawn concerning the biosynthetic origin of the two-carbon unit (a) it is derived from a precursor to which both atoms of acetate and that of formate can donate but is not derivable from the S-methyl groups of methionine (b) the incorporation of formate does not occur via carbon dioxide (c) the methyl group of acetate undergoes partial loss of hydrogen in the course of biological transformation but incorporation is not via unbound formate (d) it is improbable that a symmetrical precursor is involved.

These conclusions suggest that glycine, serine and pyruvate (■ alanine) should be tested as precursors of the two-carbon unit. A biosynthesis

involving degradation of a C_5 side-chain [cf. structure (7) of lophocerine (V)] derived from mevalonate could also be in keeping with our results and deserves further study. Finally, there is still a possibility that acetate is incorporated intact but that the expected labelling pattern is obscured by some effective 'scrambling' mechanism; this possibility is, however, now a remote one.

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