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THE ROLE OF *N*-ACETYL AMINES IN TETRAHYDRO-β-CARBOLINE AND TETRAHYDROISOQUINOLINE BIOSYNTHESIS

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Abstract—Three labelled *N*-acetyl amines have been synthesized and tested as precursors of tetrahydro-β-carboline and tetrahydroisoquinoline alkaloids These are *N*-1^{14}C-acetyltryptamine, *N*-1-^{14}C-acetyl-3-hydroxy-4-methoxyphenylethylamine and *N*-1-^{14}C-acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine which were tested as precursors for eleagnine in *Elaeagnus angustifolia*, salsoline in *Echinocereus merkeri* and anhalonidine in *Lophophora williamsii*, respectively Under conditions when the plants were synthesizing these alkaloids from other precursors none of these amides was incorporated

INTRODUCTION

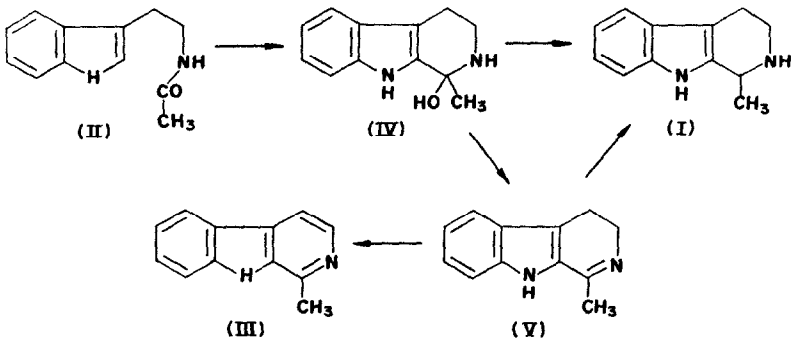
A PREVIOUS paper[1] from this laboratory suggested that *N*-acetyltryptamine is a precursor of the β-carboline alkaloid harman An implication of this result is that other simple alkaloids presumed to be formed from tryptophan or tyrosine and a C-2 fragment could arise from the *N*-acetyl derivative of the appropriate amines. Such alkaloids are the 1-methyl tetrahydro-β-carbolines, and the 1-methyl-tetrahydroisoquinolines This hypothesis has been tested in the present paper by synthesizing and feeding radioactive *N*-acetyl amines to plants which produce these alkaloids.

RESULTS AND DISCUSSION

There are about 30 alkaloids of known structure in the β-carboline and the tetrahydroisoquinoline series which can be formally derived from either a tryptamine or a tyramine unit and a C-2 fragment How the C-2 fragment combines with the amines to form these alkaloids has been the subject of continuing speculation mainly because the great metabolic activity of C_2 and C_3 compounds such as acetate, acetaldehyde and pyruvate makes it very difficult to obtain unambiguous results from classical feeding experiments Acetate-1-^{14}C fed to *Elaeagnus angustifolia* led to specific incorporation of the label into the C-1 of eleagnine (I) [2] In contrast, when radioactive acetate was fed to *Peganum harmala*[3] and *Lophophora williamsii*[4,5] it led to apparent randomization of the label in harmine and pellotine respectively The feeding of pyruvate to these plants was claimed[3,6] to lead to significant specific incorporation of the label. Finally the spontaneous condensation and cyclization of acetaldehyde with the aromatic amines has made *per se* feeding experiments worthless It is the comparatively restricted metabolism of tyrosine and tryptophan which has enabled the

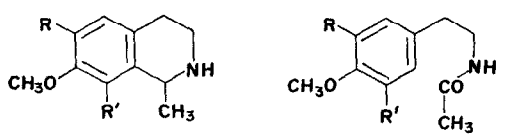
[1] M SLAYTOR and I J MCFARLANE, *Phytochem* **7**, 605 (1968)
[2] D G O'DONOVAN and M F. KENNEALLY, *J Chem Soc* 1109 (1967)
[3] K STOLLE and D GROGER, *Archiv Pharm* **301**, 561 (1968)
[4] A R BATTERSBY, R BINKS and R HUXTABLE, *Tetrahedron Letters* 563 (1967)
[5] A R BATTERSBY, R BINKS and R. HUXTABLE, *Tetrahedron Letters* 6111 (1968).
[6] E LEETE and J D BRAUNSTEIN, *Tetrahedron Letters* 451 (1969)

unambiguous interpretation of the incorporation of these amino acids into alkaloids. Accordingly, it was reasoned that if the C-2 unit could be removed from general metabolism by combining it with the aromatic amine unit, then such a compound could be tested as a potential precursor of the alkaloids *N*-2-^{14}C-acetyltryptamine (II) is an example and this has been shown[1] to be an efficient precursor of harman (III) in *Passiflora edulis* Further, labelled *N*-acetyltryptamine was trapped after feeding either radioactive tryptophan or tryptamine to plants which had previously been fed unlabelled *N*-acetyltryptamine This result suggested that *N*-acetyl amines may be precursors for the 1,2,3,4-tetrahydro-β-carboline and the 1,2,3,4-tetrahydroisoquinoline alkaloids



For example, eleagnine (I) could rise from the carbinol (IV) formed on cyclization of the amide by dehydration to harmalan (V) and then reduction Alternatively the carbinol, assumed as a transient intermediate, could be converted directly to eleagnine by a reductive elimination of OH These same considerations could also apply to the biosynthesis of the tetrahydroisoquinolines

Three alkaloids were chosen for biosynthetic study These were the tetrahydro-β-carboline eleagnine (I) in *Elaeagnus angustifolia*, and the tetrahydroisoquinolines, salsoline (VI) in *Echinocereus merkeri* and anhalonidine (VII) in *Lophophora williamsii* The availability of plant material was the determining factor in the choice of alkaloids



VI (R=OH, R'=H)
VII (R=OCH3, R'=OH)
VIII (R=OH, R'=H)
IX (R=OCH3, R'=OH)

In the case of harman and tetrahydroharman, there is only one amine, viz tryptamine, but with salsoline and anhalonidine the introduction of hydroxy and methoxy groups into the benzene ring means that there are a number of possible *N*-acetyl amines which have to be considered as potential precursors for the tetrahydroisoquinoline skeleton The incorporation of dopa and dopamine into a number of tetrahydroisoquinolines as well as the unreported occurrence of any mono-oxygenated tetrahydroisoquinolines strongly suggests that the oxygen pattern in salsoline is formed prior to the tetrahydroisoquinoline ring and may be necessary for the cyclization The amine precursor involving the least number of steps from dopamine to salsoline is 3-hydroxy-4-methoxyphenylethylamine and this has been shown

to be efficiently incorporated into salsoline under similar conditions.[7] The introduction of the third oxygen into anhalonidine will obviously result in a larger number of possibilities for the normal phenylethylamine. 3,4-Dimethoxy-5-hydroxyphenylethylamine has been shown to be incorporated into pellotine (*N*-methyl anhalonidine)[8] and anhalamine[9] in *L. williamsii* though the confusing results from feeding experiments of variously methylated trioxygenated phenylethylamines makes it difficult to designate this amine as the normal ultimate phenylethylamine precursor of anhalonidine. Based on these considerations *N*-1-^{14}C-acetyltryptamine (II) *N*-1-^{14}C-acetyl-3-hydroxy-4-methoxyphenylethylamine (VIII) and *N*-1-^{14}C-acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine (IX) were synthesized and tested as precursors for eleagnine, salsoline and anhalonidine respectively. The results of feeding the *N*-acetyl amines are in Table 1 and are uniformly negative.

TABLE 1 FEEDING EXPERIMENTS WITH *E angustifolia*, *E merkeri* AND *L. williamsii*

Plant	Feeding method	Substrate	Specific activity mc/m-mole
E angustifolia	Hydroponic	L-methylene-^{14}C-tryptophan	54 5
E angustifolia	Wick	α-^{14}C-tryptamine HCl	0 315
E angustifolia	Hydroponic	α-^{14}C-tryptamine HCl	0 315
E angustifolia	Hydroponic	β-^{14}C-tryptamine HCl ring-(G)-^{3}H-tryptamine HCl } *	0 018 49 1
E. angustifolia	Hydroponic	*N*-1-^{14}C-acetyltryptamine	0 018
E angustifolia	Hydroponic	*N*-1-^{14}C-acetyltryptamine	0 018
E angustifolia	Hydroponic	ring (G)-^{3}H-*N*-1-^{14}C-acetyltryptamines §	
E angustifolia	Hydroponic	1-^{14}C-harmalan-HCl	14 3
E merkeri	Injection	*N*-1-^{14}C-acetyl-3-hydroxy-4-methoxyphenylethylamine	0 018
L williamsii	Injection	*N*-1-^{14}C-acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine	1 33

Plant	μmoles absorbed	Expected product	μmoles formed
E angustifolia	0 037	Eleagnine	4 3 × 10^{-6}
E angustifolia	1 1	Eleagnine	0 05
E. angustifolia	13 6	Eleagnine	0 17
E angustifolia	46 0†	Eleagnine	0 21†‡
E angustifolia	6 95	Eleagnine	0 00
E angustifolia	3 65	Eleagnine	0 00
E angustifolia	17 2	Eleagnine	0 00
E angustifolia	8 62	Eleagnine	0 06
E merkeri	13 4	Salsoline	0 00
L. williamsii	26 47	Anhalonidine	0 00

* ^{3}H ^{14}C, 20 8 1. † based on ^{14}C ‡ ^{3}H ^{14}C, 11 4 1 § ^{3}H ^{14}C, 42 5 1

[7] I J MCFARLANE and M SLAYTOR, *Phytochem* **11**, 235 (1972)
[8] K L KHANNA, M TAKIDO, H ROSENBERG and A G. PAUL, *Phytochem* **9**, 1811 (1970)
[9] J LUNDSTRÖM and S AGURELL, *Tetrahedron Letters* 3371 (1969)

Although there always remains the possibility that the amides are not reaching the site of synthesis, we prefer, because of the incorporation of *N*-acetyltryptamine into harman in *P edulis*, to regard our results as valid, i e in the biosynthesis of tetrahydro-β-carbolines and the tetrahydroisoquinolines *N*-acetyl amines are not involved

As controls for these experiments alkaloid synthesis was demonstrated in each case in similar plant material from at least one precursor Both tryptophan and tryptamine were incorporated into eleagnine in *E angustifolia*, 3-hydroxy-4-methoxyphenylethylamine was incorporated into salsoline in *E merkeri* and dopamine was incorporated into anhalonidine in *L williamsii* In addition, under conditions whereby *N*-acetyltryptamine in *P edulis* could be trapped[1] after feeding either tryptophan or tryptamine, no such result could be demonstrated in *E angustifolia* (see Table 2)

Table 2 Attempted double label trapping experiments with *E. angustifolia*

Feeding method	Trap	μmoles added	Radioactive L-tryptophan added (dis/min × 10^{-6})	μmoles of radioactivity trapped
Hydroponic (shoots)	*N*-acetyltryptamine	2 00	^{3}H-(G)* 7 64 ^{14}C-(β)† 0 222	0 00 0 00
Wick	*N*-acetyltryptamine	5 55	^{3}H-(G)* 7·64 ^{14}C-(β)† 0 222	0 00 0 00

* Specific activity 500 mc/m-mole
† Specific activity 54 5 mc/m-mole

If *N*-acetyltryptamine is not a precursor of eleagnine then the observed incorporation of harmalan, the expected cyclization product of *N*-acetyltryptamine into eleagnine must be regarded as incidental to the biosynthesis of eleagnine and merely a reaction the plant is capable of carrying out Similarly the incorporation of eleagnine into harman in *P edulis*[1] is probably incidental to the biosynthesis of harman

Harman is exceptional among the alkaloids under discussion in being fully aromatic, and it is possible that *N*-acetyl amines are involved only in the biosynthesis of the rarely occurring β-carbolines This means that separate pathways are operating in those plants where the β-carbolines and the tetrahydro-β-carbolines occur together An alternate explanation for the co-occurrence is that the β-carbolines are autoxidation products of the tetrahydro-β-carbolines and are artifacts of extraction In support of this the tetrahydroisoquinolines are more stable to autoxidation and there are no reported co-occurrences of tetrahydroisoquinolines and isoquinolines Further there are no known isoquinolines derived from a phenylethylamine unit and a C-2 fragment

EXPERIMENTAL

General M ps were determined on a Kofler block and are uncorrected. Autoradiograms were prepared using Kodirex Medical X-ray film (Kodak (Australasia) Pty Ltd) IR spectra were obtained (Nujol mull) using a Unicam SP 200 spectrophotometer NMR spectra were run in D_2O using Na 3-(trimethylsilyl)-1-propane sulphonate as an internal standard on a Varian Associates A-60 spectrometer

Plant material Seed of *Elaeagnus angustifolia* was purchased from Thompson and Morgan Ltd., Ipswich, England *Lophophora williamsii* and *Echinocereus merkeri* plants were obtained from members of the N S W Cactus and Succulent Society

All plant material was grown in a temperature controlled green house at 21–24°, daylight being supplemented with fluorescent lighting set on a 16-hr day

E angustifolia plants were obtained by germinating the seeds in sand Two types of plant material from *E angustifolia* were used (a) growing shoots from a mature plant, (a) young seedlings 4–6 in high

L williamsii and *E merkeri* plants were used for feeding experiments after being allowed to grow in the greenhouse for at least 4 weeks

Chromatography Descending paper chromatography was performed on Whatman No 3 paper using *n*-BuOH–toluene–HOAc–H_2O (20 10 3·9) Before applying the sample the paper was equilibrated by dipping in the aq phase with acetone (3 7) and allowing the acetone to evaporate

TLC was carried out on plates (1 mm) prepared in the usual way (neutral plates) as was described previously (basic plates) [1] If required activated they were heated at 110° for 60 min The following solvent systems (v/v) were used (A) MeOH, (B) $CHCl_3$–MeOH (19 1), (C) $CHCl_3$–EtOH–Et_2NH (85·10 5), (D) Et_2O

Administration of radioactive substrates Where possible radioactive substrates were added in aq solution *N*-acetyltryptamine was dispersed in 0 2% Tween 80 and (VIII) and (IX) in 10% Me_2SO_2 After wick feeding, the plants were grown for 2–3 weeks With hydroponic feeding the shoots were grown in water for 3–9 days while after injection the cacti were grown for 3 weeks

Isolation and detection of alkaloids E angustifolia material was homogenized in a Ten Broeck homogenizer or Waring Blendor with carrier alkaloid (2 mg) and 90% EtOH and allowed to stand for 2 days at 0° After filtration the extract was washed through a cellulose phosphate ion-exchange column (1 × 10 cm) (H^+ form) with 90% EtOH solution The basic fraction was eluted with 90% EtOH made 0 5 N with respect to HCl or EtOH–conc NH_4OH (9 1), then separated by TLC (basic activated plates, solvent A) and the alkaloid eluted with MeOH The alkaloid was recrystallized to constant specific activity after addition of unlabelled material from either MeOH EtOAc (eleagnine-HCl) or EtOAc (eleagnine)

Alkaloids from cacti were isolated by a combination of the methods of Leete[10] and Lundstrom and Agurell [11] After separation on preparative TLC (neutral plates, solvent C) and elution with EtOH, the alkaloid HCl-ide was recrystallized to constant specific activity after addition of further unlabelled material from MeOH–EtOAc

Eleagnine was detected on TLC either by its purple fluorescence under UV light or its magenta colour with 1% xanthydrol in EtOH–conc HCl (95 5), salsoline and anhalonidine by their orange and crimson colours respectively with 0 5% *o*-dianisidine in 1 M HCl and 10% $NaNO_2$

Trapping experiments *N*-acetyltryptamine was added 72 hr before the radioactive tryptophan and the shoots were then allowed to grow for 7 days Plant material was homogenized in a Waring Blendor with carrier (*ca* 30–40 mg) in 90% EtOH and the homogenate allowed to stand for 2 days at 0° After filtration the extract was washed through a cellulose phosphate ion-exchange column (1 × 10 cm) (H^+ form) with 90% EtOH solution The eluate was evaporated to dryness and the residue dissolved in Et_2O This solution was twice extracted with 0 01 M NaOH, dried (Na_2SO_4) and the solvent removed The residue was sublimed (160°, 0 15 mm Hg), the sublimate purified by preparative TLC (basic plates, solvent D) and the *N*-acetyltryptamine eluted with MeOH

Counting of radioactivity ^{14}C and ^{3}H containing compounds were counted on a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3375) in 15 ml of scintillant (containing 4 g of PPO and 100 mg POPOP/l of toluene), 1 ml Soluene TM 100 and mercaptoethanol (20 μl) All vials were internally standardized using ^{14}C or ^{3}H-toluene

For samples containing both ^{3}H and ^{14}C, conditions for counting were determined using pure samples of the two isotopes The isotope of higher energy (^{14}C) was counted under conditions where less than 0 001% of the low energy (^{3}H) isotope counts were detected For the isotope of lower energy the percentage of the high energy counts detected under the conditions used was determined and the appropriate correction made

Chemical syntheses *Ring-(G)-^{3}H-tryptamine* Tryptamine (70 mg) was heated at 100° in $^{3}H_2O$ (0 17 ml, 26 4 mc/m-mole) containing oleum (2% SO_3 in H_2SO_4, 0 03 ml) for 4 hr in a sealed tube The cooled solution was diluted with MeOH (4 ml), basified with 10 M NaOH and the solvents removed *in vacuo* Extraction of the residue with hot benzene and evaporation of the benzene yielded a pale brown solid This was sublimed (100–120°, 0 1 mm Hg) and crystallized from benzene to give ^{3}H-tryptamine (45 6 mg), m p 114–116° Single spot on autoradiography of TLC (basic plates with solvent system A) Specific activity 49 1 mc/m-mole In a parallel experiment in D_2O it was shown by NMR that 84% of the aromatic hydrogens had exchanged

N-1-^{14}C-*acetyl-ring-(G)-^{3}H-tryptamine* (II) Acetic-1-^{14}C anhydride (0 1 mc, 0 34 mg) was transferred by heating under vacuum to a solution of unlabelled Ac_2O (0 22 ml), ring-(G)-^{3}H-tryptamine (0 88 mc, 178 5 mg) and anhydrous NaOAc (6 2 mg) frozen in liquid N_2 The reaction mixture was then heated at 100° for 2 5 hr when the excess Ac_2O was decomposed with 5 M HCl (10 ml) and the solution extracted with Et_2O (4×) Extraction of the combined Et_2O fractions with dilute Na_2CO_3 solution and removal of the solvent under N_2 gave an oily residue which crystallized on standing Sublimination at 160–180° (0 6 mm Hg) and recrystallization from Et_2O–light petroleum (62–76°) gave ^{3}H ^{14}C-*N*-acetyltryptamine as long

[10] E Leete, *J Am Chem Soc* **88**, 4218 (1966)
[11] J Lundström and S Agurell, *J Chromatog* **30**, 271 (1967)

colourless needles Yield 157 mg (70%), m p 78–80° Single spot on autoradiography of TLC (basic plates with solvent system A)

N-1-^{14}C-*acetyltryptamine* Similarly prepared from 1-^{14}C-Ac_2O and tryptamine Specific activity of *N*-1-^{14}C-acetyltryptamine 0 018 mc/m-mole Single spot on autoradiography of TLC (basic plates with solvent system A)

N-1-^{14}C-*acetyl*-3-*hydroxy*-4-*methoxyphenylethylamine* (VIII) 3-Hydroxy-4-methoxyphenylethylamine was converted to the diacetate (IR spectrum 1690 cm^{-1} (CONHR) and 1750 cm^{-1} (CH_2COOR) as described for (II)) The diacetate was heated at 100° in 1 M $NaHCO_3$ (2 ml) for 3 hr in a sealed tube The cooled solution was adjusted to pH 6 and extracted with $CHCl_3$ Evaporation of the $CHCl_3$ gave a colourless, partially crystalline oil A solution of this in 30% aq MeOH (50 ml) was slowly applied to an Amberlite IRA 400 (OH^- form) ion-exchange column (1 × 20 cm) and the non-phenolic material washed through with 30% aq MeOH 200 ml) The *N*-acetyl-3-hydroxy-4-methoxyphenylethylamine was eluted with a solution of MeOH–H_2O–HOAc (6 3 1) (200 ml) Sublimation (120–140°, 0 2–0 3 mm Hg) followed by crystallization from EtOH–heptane of the residue after removal of the solvent gave (VIII) Yield 116 mg (55%), m p 125° Specific activity 0 018 mc/m-mole Single spot on autoradiography of TLC (neutral plates with solvent system B)

N-1-^{14}C-*acetyl*-3,4-*dimethoxy* -5-*hydroxyphenylethylamine* (IX) Prepared from 3,4-dimethoxy-5-hydroxyphenylethylamine and acetic-1-^{14}C-anhydride as described for the synthesis of (VIII) Colourless crystals of (IX) were obtained from EtOH–heptane, m p 102–105° Specific activity 1 33 mc/m-mole Single spot on autoradiography of TLC (neutral plates with solvent system B)

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Key Word Index—*Eleagnus angustifolia*, *Echinocereus merkeri*, *Lophophora williamsii*, tetrahydro-β-carbolines, tetrahydroisoquinolines, alkaloids, biosynthesis.