Tetrahedron Letters,Vol.29,No.7,pp 807-810,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

THE IMPLICATION OF PHENYLACETALDEHYDES IN THE BIOSYNTHESIS OF THE PHENANTHROINDOLIZIDINE ALKALOID, TYLOPHORINE.

Stuart H. Hedges, Richard B. Herbert*, Eric Knagg, and Vinayagar Pasupathy†

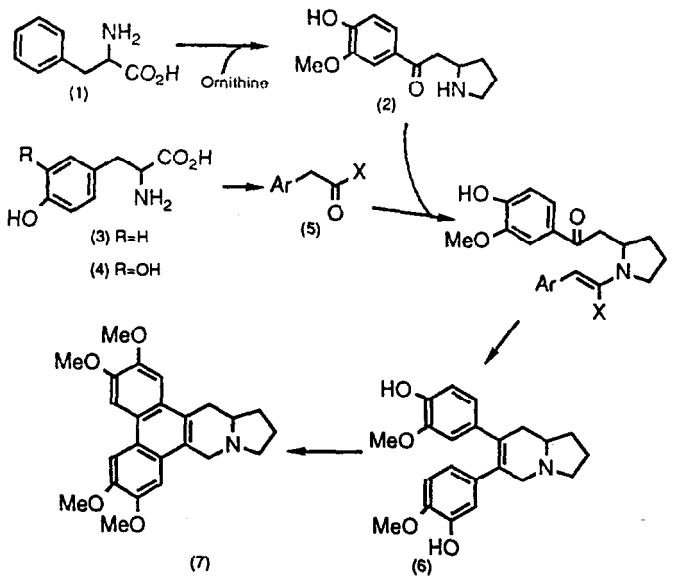
Department of Organic Chemistry, The University, Leeds LS2 9JT.

†On leave from the Department of Chemistry, The University of Jaffna, Jaffna, Sri Lanka.

Summary: 3,4-Dihydroxy[carbonyl-^{3}H]phenylacetaldehyde (12) and 3,4-dihydroxy[3'-^{14}C]-phenylpyruvic acid [as (10)] are both efficient precursors for tylophorine (7) in Tylophora asthmatica whereas none of the amino acids (13) - (15) are incorporated into Tylophora alkaloids, from which it is concluded that a key step in the biosynthesis of these alkaloids involves condensation of a phenylacetaldehyde derivative [as (11)] with the phenacylpyrrolidine (2) (Scheme, X=H).

The biosynthesis of phenanthroindolizidine alkaloids, e.g. tylophorine (7), in Tylophora asthmatica is from tyrosine (3)[1], phenylalanine (1)[2] (via cinnamic acid[3]), and probably ornithine[2] (Scheme). Dopa (4) is a better alkaloid precursor than tyrosine and it specifically provides the same part of tylophorine (7) as does tyrosine[4]. Key intermediates in the biosynthesis of these alkaloids are the phenacylpyrrolidine (2)[5] and the diarylhexahydroindolizine (6)[4,6]. An important question remains: what is the nature of the Dopa/tyrosine fragment (5) which condenses with (2) leading to intermediates like (6) (see Scheme)? By analogy with the biosynthesis of some alkaloids it could be a keto-acid[7] [as (10)] or, by analogy with the biosynthesis of others[8-10], an aldehyde [as (11)]. In biomimetic experiments (cf. Scheme) it has been found that phenacylpyrrolidines [as (2)] react with both aldehydes [as (11)][11] and keto-acids [as (10)] (see below) affording, respectively, compounds represented by structures (6) and (15). We report here experiments which distinguish between the involvement of such keto-acids and aldehydes in the biosynthesis of tylophorine (7).

3,4-Dihydroxyphenylacetaldehyde (11) was prepared with a tritium label on the aldehyde function so that any oxidation to the corresponding acid prior to use in biosynthesis would result in loss of label, i.e. incorporation of the acid would yield radioinactive alkaloid; significant reincorporation at a later stage is most unlikely. The labelled aldehyde (12) was prepared from 3,4-dibenzyloxybenzaldehyde (16) by a standard Darzens procedure in which the methyl chloroacetate used was exchanged with sodium

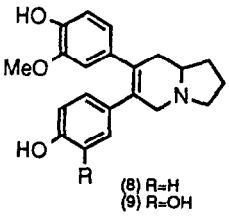


SCHEME X=H or COOH

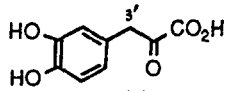
methoxide in methanol containing tritiated water prior to reaction with the aldehyde (16) to give the glycidic ester (17). The tritiated 3,4-dihydroxyphenylacetaldehyde (12) was obtained from (17) essentially as described elsewhere[12] for the preparation of phenylacetaldehydes; the benzyl protecting groups were removed by hydrogenolysis. The reaction sequence was also carried out with a deuterium label to check the location of the label. This involved the substitution of tetradeuteriomethanol for methanol in the first step involving exchange and condensation.

3,4-Dihydroxy[3'-^{14}C]phenylpyruvic acid [as (10)] was prepared by a very convenient procedure from [3'-^{14}C]Dopa[13]. The two labelled precursors were mixed (35 μCi ^{3}H; 9.1 μCi ^{14}C; ^{3}H/^{14}C 3.8) and fed to Tylophora asthmatica plants (feeding as a mixture provides the best comparison of incorporation efficiencies; feeding separately to different plants may give variable incorporations). The tylophorine (7) which was isolated (and recrystallized to constant radioactivity) showed very satisfactory and similar incorporation of both precursors (0.18% incorporation of ^{3}H; 0.29% incorporation of ^{14}C; ^{3}H/^{14}C = 2.4). The incorporation of labelled (10) correlates with that of its transamination product, dopa (4)[4]. The incorporation of tritium into (7) from the aldehyde (12) at a similar level to that of (10) indicated that the aldehyde is used directly in the biosynthesis of tylophorine (7) and without oxidation. Reasonably, (11) may be placed after (10) in the pathway.

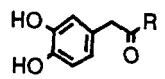
A decarboxylase which converts 4-hydroxyphenylpyruvate into 4-hydroxyphenylacetaldehyde, as a step in the biosynthesis of benzylisoquinoline alkaloids, has been isolated from Berberis stolonifera; the enzyme may also be active in decarboxylating 3,4-dihydroxyphenylpyruvate (10) to give the aldehyde (11)[14]. We conclude from our results that the biosynthesis of tylophorine (7) is from Dopa (4) → 3,4-dihydroxyphenylpyruvate (10)



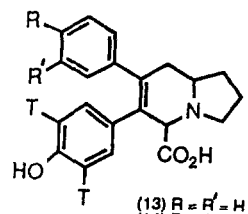
(8) R=H
(9) R=OH



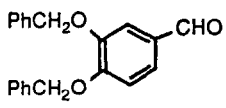
(10)



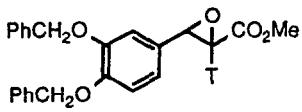
(11) R= H
(12) R= T



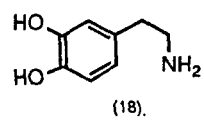
(13) R = R' = H,
(14) R = OH, R' = H
(15) R = OH, R' = OMe



(16)



(17)



(18).

→ 3,4-dihydroxyphenylacetaldehyde (11) → (7), *i.e.* condensation of (2) is with the aldehyde (11) rather than the keto-acid (10). Because of ready transamination an alternative (10) ⇄ (4) → (18) → (11) → (7) is also possible (*cf.* ref. 14). In overall support, the indolizine (9) has been shown[4] to be a precursor for *Tylophora* alkaloids. Further evidence, albeit of a negative kind, has been obtained which bears on these conclusions.

Condensation *in vivo* of acids of type (10) with phenacylpyrrolidines such as (2) would give as putative intermediates, amino acids of type (15). These reactions may be achieved in the laboratory (*cf.* Scheme) and by this means we have prepared the amino-acids (13) - (15), labelled with tritium as shown. [The labelling sites were checked using deuterium instead of tritium; the experimental procedures which were used followed essentially those developed for (2)[5], (6)[6], and (7)[11]. The product in each case was an inseparable mixture of two racemates, the minor racemate being present to the extent of 15-20%, as measured by ^{1}H n.m.r.]. None of (13) - (15) was incorporated into tylophorine (7), tylophorinine, or tylophorinidine in *T. asthmatica*. The amount of radioactivity fed in each case (0.2-0.4 mCi) was estimated to be sufficient to allow for loss of half the tritium on subsequent hydroxylation of the precursor and to allow for the possibility that the isomer involved in biosynthesis was one of the enantiomers of the minor component of the mixture fed.

The failure of (13) - (15) to act as precursors leads to the conclusion that neither (2) nor related phenacylpyrrolidines condense *in vivo* with 4-hydroxyphenylpyruvate *en route* to the *Tylophora* alkaloids. Since (8) is an intact and efficient precursor for these alkaloids[6] a normal pathway for biosynthesis may involve the condensation of (2) with 4-hydroxyphenylacetaldehyde (derived from tyrosine *via* 4-hydroxyphenylpyruvate) as an alternative to condensation involving 3,4-dihydroxyphenylacetaldehyde (11).

It may be difficult to distinguish between the normal involvement of 4-hydroxyphenylacetaldehyde and 3,4-dihydroxyphenylacetaldehyde (11) in the biosynthesis of these alkaloids as has proved to be the case for benzylisoquinoline alkaloids[8,14]. The

evidence here presented, however, indicates that a key step in the biosynthesis of Tylophora alkaloids involves the condensation of a phenacylpyrrolidine (2) with a hydroxylated phenylacetaldehyde and not a hydroxylated phenylpyruvate (Scheme, X=H). The involvement of an aldehyde in the biosynthesis of Tylophora alkaloids correlates with the involvement of aldehydes in the biosynthesis of the very large families of benzylisoquinoline[8] and terpenoid indole[9] alkaloids, also the phenethylisoquinoline alkaloid colchicine and others[10]. It is to be noted, however, that the biosynthesis of phenanthroindolizidine alkaloids apparently involves an enamine in ring-closure (cf. Scheme) whereas the biosynthesis of the other alkaloids involves an imine.

References

1. N. B. Mulchandani, S. S. Iyer, and L. P. Badheka, Phytochemistry, 1969, 8, 1931.
2. N. B. Mulchandani, S. S. Iyer, and L. P. Badheka, Phytochemistry, 1971, 10, 1047.
3. N. B. Mulchandani, S. S. Iyer, and L. P. Badheka, Phytochemistry, 1976, 15, 1697.
4. D. S. Bhakuni and V. K. Mangla, Tetrahedron, 1981, 37, 401.
5. R. B. Herbert, F. B. Jackson, and I. T. Nicolson, J. Chem. Soc., Perkin Trans. 1, 1984, 825; J. Chem. Soc., Chem. Commun., 1976, 865.
6. R. B. Herbert and . B. Jackson, J. Chem. Soc., Chem. Commun., 1977, 955.
7. R. B. Herbert and J. Mann, J. Chem. Soc., Perkin Trans. 1, 1982, 1523; G. J. Kapadia, G. S. Rao, E. Leete, M. B. E. Fayez, Y. N. Vaishnav, and H. M. Fales, J. Am. Chem. Soc., 1970, 92, 6943.
8. R. Stadler, T. M. Kutchan, S. Loeffler, N. Nagakura, B. Cassels, and M. H. Zenk, Tetrahedron Lett., 1987, 28, 1251.
9. R. B. Herbert, in "Indoles, Part 4, The Monoterpenoid Indole Alkaloids", ed. J. E. Saxton, Wiley, New York, 1983, p.1.
10. R. B. Herbert and E. Knagg, Tetrahedron Lett., 1986, 27, 1099; refs. cited.
11. J. E. Cragg, R. B. Herbert, F. B. Jackson, C. J. Moody, and I. T. Nicolson, J. Chem. Soc., Perkin Trans. 1, 1982, 2477.
12. J. E. Cragg and R. B. Herbert, J. Chem. Soc., Perkin Trans. 1, 1982, 2487; Y. Ban and T. Oishi, Chem. Pharm. Bull., 1958, 6, 574; M. Barash and J. M. Osbond, J. Chem. Soc., 1959, 2157.
13. M. L. Wilson and C. J. Coscia, J. Org. Chem., 1979, 44, 301.
14. M. Rueffer and M. H. Zenk, Z. Naturforsch., Part C, 1987, 42, 319.

(Received in UK 12 November 1987)