Biosynthesis of Phenanthroindolizidine Alkaloids *via* Derivatives of 2-Phenacylpyrrolidine and Benzoylacetic Acid

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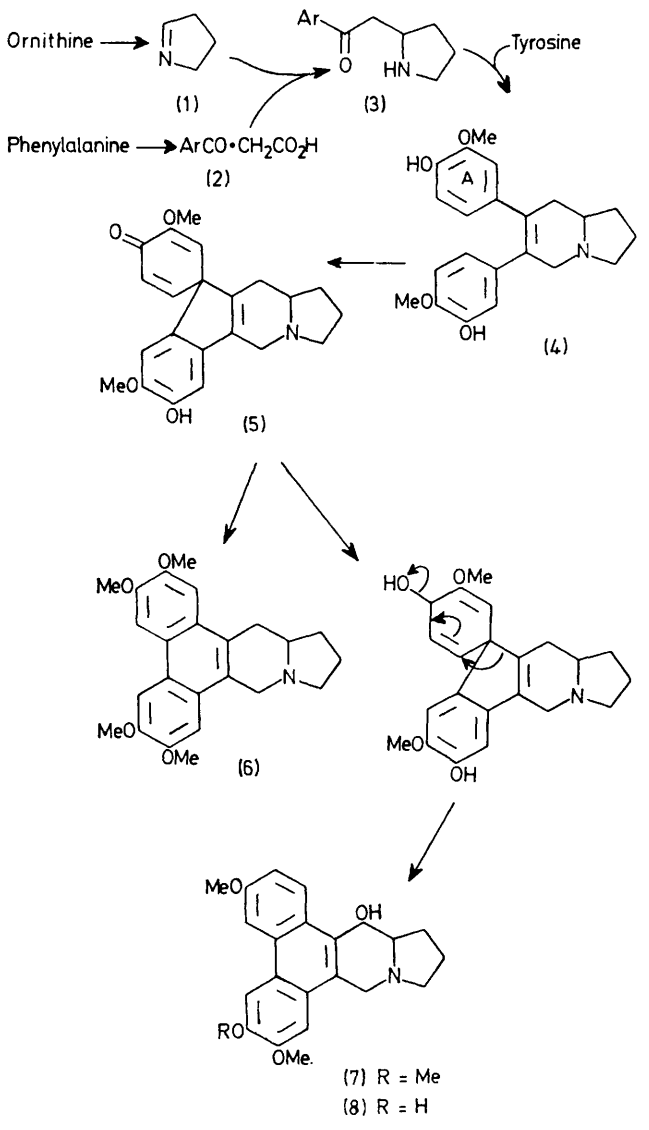
Summary The derivatives of 2-phenacylpyrrolidine, (**12**), (**13**), and (**14**), and of benzoylacetic acid, (**9**) and (**10**), are established as precursors of tylophorinine (**7**).

PREVIOUS results have shown that the phenanthroindolizidine alkaloid tylophorine (**6**) is derived from phenylalanine,[1] tyrosine,[2] and, probably, ornithine.[1,3] A hypo-

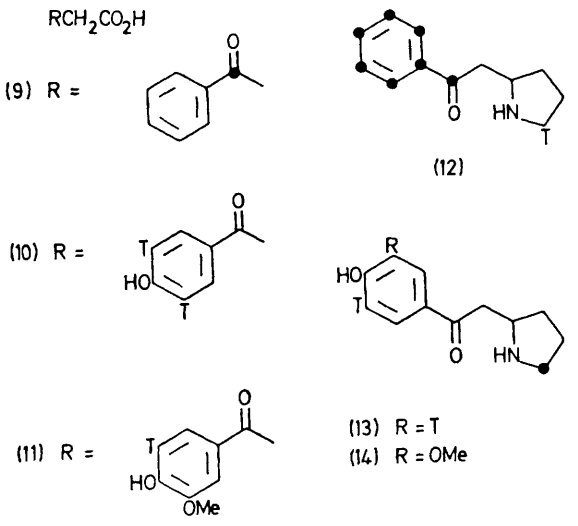
thetical biosynthetic pathway (see Scheme) can be proposed (*cf.* refs. 1, 2, and 4) consonant with these observations and taking into account the structures of the



SCHEME

naturally occurring bases,[5] represented by (**4**) and the six-membered-ring anologues of (**3**). Pivotal to such ideas are the 2-phenacylpyrrolidines (**3**) and we now report evidence that (**12**), (**13**), and (**14**) are intact precursors for tylophorinine (**7**) in *Tylophora asthmatica* Wight et Arn, thus establishing a biosynthetic pathway *via* these bases and giving substance to the previously held views. Further definition of tylophorinine biosynthesis was obtained by showing that (**9**) and (**10**) [*cf.* (**2**)] are also incorporated into (**7**).

2-Phenacylpyrrolidines (**3**) are conveniently prepared by condensation of Δ^1-pyrroline (**1**) with the appropriately substituted benzoylacetic acid.[6] In this way samples of (**12**), (**13**), and (**14**) were synthesized with labels as shown (^{14}C: ●). When they were administered to *T. asthmatica*, each of them was found to be a satisfactory precursor for tylophorinine (**7**) (Table). The amines (**12**) and (**14**) were incorporated without change in isotope ratio and are therefore utilized intact for biosynthesis. The incorporation of base (**13**) into tylophorinine (**7**) resulted in loss of essentially half the tritium. This was, however, expected since implication of (**13**) in the biosynthetic pathway logically involves entry of a hydroxy-group at one of the



tritiated sites. Thus (**13**) is also incorporated intact. These results strongly indicate that the biosynthesis of tylophorinine (**7**) proceeds by way of phenacylpyrrolidines (**3**) and involves in part the sequence represented as (**12**) → (**13**) → (**14**). Further, they imply that the biosynthesis of tylophorinine involves an intermediate (as **4**) with a dioxygenated ring A, which is consistent with a pathway involving a dienone (**5**) which gives either tylophorine (**6**) by rearrangement, or tylophorinine (**7**) after reduction followed by rearrangement (*cf.* ref. 4).

TABLE. Incorporation of precursors into tylophorinine (**7**)[a]

Precursor	Administered $^3H/^{14}C$	$^3H/^{14}C$	Isolated % Incorporation
(**9**)[b]	—	—	$1{\cdot}5 \times 10^{-3}$
(**10**)[b]	—	—	$5{\cdot}8 \times 10^{-3}$
(**11**)[b]	—	—	Inactive alkaloid
(**12**)[c]	12·6	13·0	$1{\cdot}7 \times 10^{-1}$
(**13**)[b]	6·1	2·6	$3{\cdot}8 \times 10^{-3}$
(**14**)[b]	2·7	3·3	$3{\cdot}5 \times 10^{-2}$

[a] Isolated as its acetate. [b] Absorption into excised stems. [c] Wick-feed to whole plants.

Further support for, and definition of, the early part of the pathway shown in the Scheme comes from the finding that the keto-acids (**9**) and (**10**), but not (**11**) (labels as shown; ^{14}C: ●) were satisfactory precursors for tylophorinine (Table). Although the sites of labelling in the derived alkaloid were not established the observation that there was a marked distinction in the incorporation of label from (**9**) and (**10**) compared to (**11**) and that the levels of

incorporation of (**9**) and (**10**) were simliar to those of (**13**) and (**14**) argues strongly that these keto-acids were incorporated intact. The incorporation of (**10**) indicates that hydroxylation of the aromatic nucleus can occur before formation of (**3**) as an alternative to hydroxylation of (**12**). However, the failure of (**11**) to act as a precursor suggests that only a single oxygen function may be introduced at the keto-acid level of biosynthesis.

A puzzling feature of our results was the isolation of inactive samples of tylophorine (**6**) and tylophorinidine (**8**) in all the experiments. In preliminary studies with tritiated samples of the precursors samples of (**6**), (**7**), and (**8**) were isolated with similar levels of radioactivity so we presume that at the time of our later experiments neither (**6**) nor (**8**) were being biosynthesized.

In conclusion, our results establish the importance of the keto-acids (**2**) and keto-amines (**3**) in the biosynthesis of phenanthroindolizidine alkaloids as exemplified by tylophorinine (**7**), and point the way to further experiments notably with compounds such as (**4**). Further, it can be expected that 2-phenacylpyrrolidines (**3**) will be found in plants.

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