Phytochemistry, 1976, Vol 15, pp. 1697–1699. Pergamon Press. Printed in England.

INCORPORATION OF CINNAMIC ACID-2-[^{14}C] INTO TYLOPHORINE

N. B. MULCHANDANI, S. S. IYER and L. P. BADHEKA
Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India

(*Received* 26 *February* 1976)

Key Word Index—*Tylophora indica*; Asclepiadaceae; phenanthroindolizidine alkaloids; tylophorine; biosynthesis.

Abstract—*Tylophora indica* plants have been shown to contain phenanthroindolizidine alkaloids of the tylophorine type. Cinnamic acid-[2-^{14}C]was incorporated efficiently into these alkaloids supporting the hypothesis that ring A and C-10 and C-6′ of tylophorine are derived from phenylalanine.

INTRODUCTION

The biosynthesis of tylophorine (**1**) and related compounds was reported earlier [1, 2] using tyrosine-[2-^{14}C] and phenylalanine-[2-^{14}C] as precursors. By degradative studies on the radioactive alkaloids isolated in each case, it was established that ring B and C-9 and C-7′ arise from tyrosine whereas ring A, C-10 and C-6′ are derived from phenylalanine. Transformation of phenylalanine to tyrosine did not take place during the administration of the former precursor. It was therefore suggested that phenylalanine could possibly be incorporated via cinnamic, *p*-coumaric and caffeic acids. In the present work cinnamic acid-[2-^{14}C] was administered to *Tylophora* plants, the tylophorine-[^{14}C] isolated and the labelling pattern determined.

RESULTS AND DISCUSSION

Cinnamic acid was found to be incorporated into tylophorine more efficiently than phenylalanine. Degradation (Fig. 1) of labelled tylophorine (**1**) via its methiodide (**2**) and Emde base (**3**) to HOAc revealed that the acid (NaOAc) was inactive and therefore C-7′ was unlabelled. However, the incorporation of cinnamic acid via hydroxylated cinnamic acids would result in tylophorine labelled at C-6′. This was confirmed by degrading labelled tylophorine to 2,3,6,7-tetramethoxyphenanthrenedicarboxylic acid (**4**) via oxidation of tylophorine methiodide by aq. $KMnO_4$. All the activity was located at C-6′ as assayed by $BaCO_3$ obtained by decarboxylation of labelled 2,3,6,7-tetramethoxyphenanthrenedicarboxylic acid. Tetramethoxyphenanthrene (**5**) was unlabelled as expected. The results are summarized in Table 1.

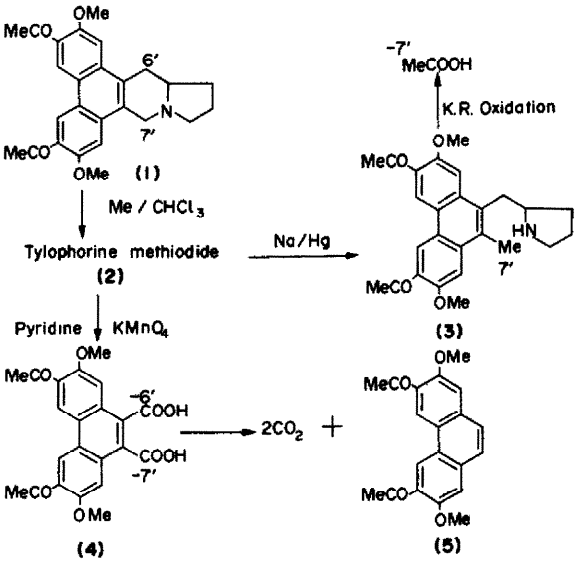
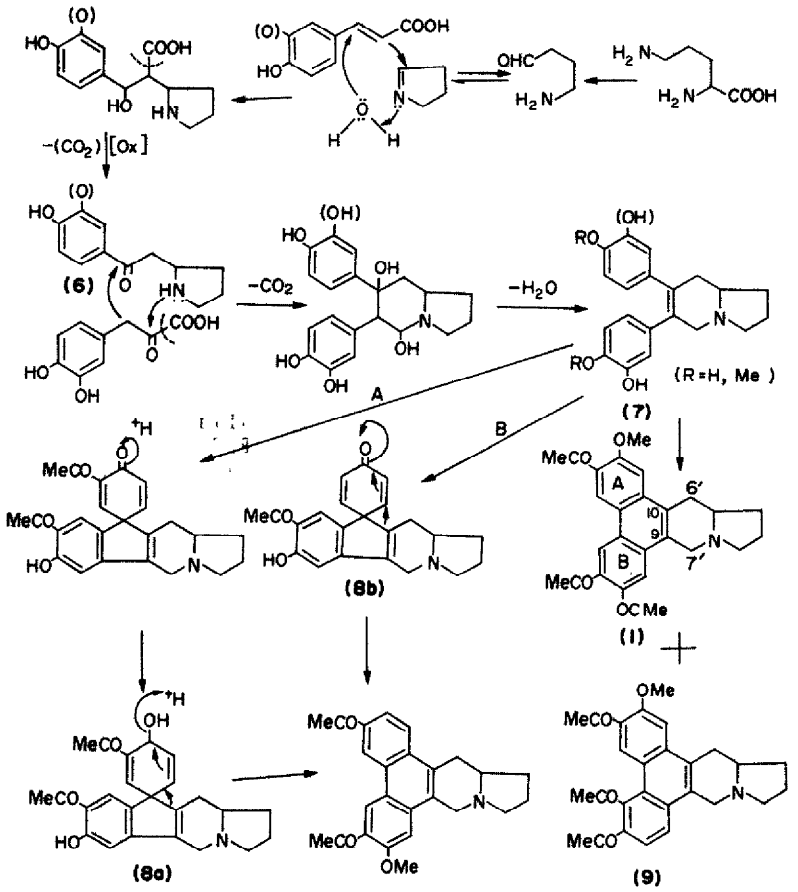


Fig. 1.

In a slightly modified biogenetic scheme it seems most reasonable that *p*-coumaric or caffeic acid could also provide precursors with an appropriate hydroxylation pattern as shown in Fig. 2. The precursor condenses with Δ′-pyrroline (derivable from ornithine) with the addition of one molecule of H_2O. Subsequent oxidation and decarboxylation gives (**6**). In our earlier experiments [2] ornithine was shown to be incorporated efficiently into tylophorine. Condensation of 3,4-dihydroxyphenylpyruvic acid with **6** followed by addition of the amine and then further oxidation and/or reduction of the carbinol amine affords the intermediate diaryl-$\Delta^{6,7}$-dehydroindolizidine (**7**). The final step involves phenol oxidative coupling [3, 4], a point not mentioned by other workers [5–9]. Here the formation of tylophorine (**1**) and

Table 1. Specific activities of undiluted tylophorine obtained from cinnamic acid-[2-^{14}C] and its degradation products

	Activity in dpm/mmol × 10^{-5}
Tylophorine	8.4
Tylophorine methiodide	8.3
Emde base of tylophorine	8.2
Acetic acid (sodium acetate)	—
2,3,6,7-Tetramethoxyphenanthrene-dicarboxylic acid	8.2
$BaCO_3$	4.1
2,3,6,7-Tetramethoxyphenanthrene	—



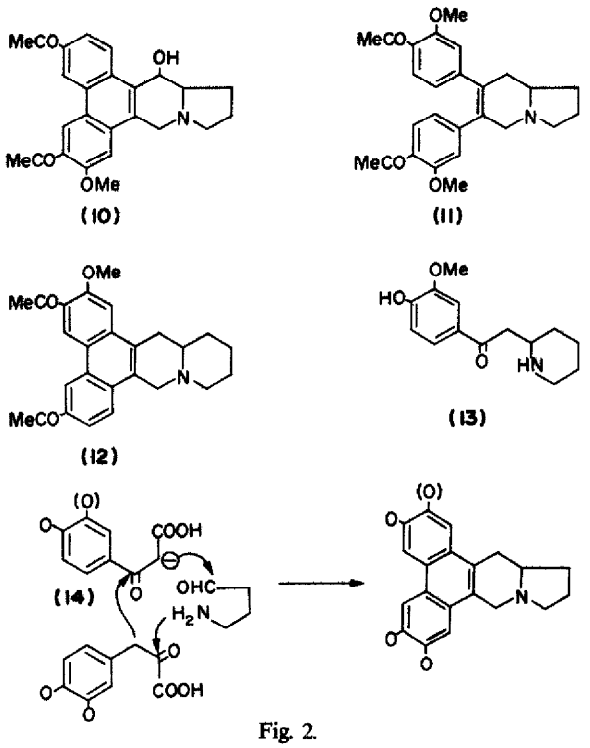


Fig. 2.

tylocrebrine (**9**) can be visualized by the *p*- and *o*-coupling, respectively, of the *m*-OH of ring B with the radical generated *p*- to the OH in parenthesis in **7**. This coupling could precede or be followed by methionine methoxylation of the alternate OH's with eventually complete methoxylation. The unusual hydroxylation pattern of tylophorinine (**10**) requires the occurrence of a dienone-phenol rearrangement following the phenol-phenol coupling. A most likely mechanism is depicted in **8a** (Path A) showing ring B already re-aromatized. An alternate mechanism (Path B) involving additional precursors, i.e. those without the parenthetical OH group, is depicted in **8b**. Credibility is given to this scheme by the recent isolation [10] of a new alkaloid septicine (**11**), occurring along with tylocrebrine (**9**) and tylophorine (**1**) in *Ficus septica* (family Moraceae). Septicine has also been isolated from *Tylophora indica* [11] and subsequently by us also. Septicine is transformed to a mixture of tylophorine and tylocrebrine by UV irradiation [10]. That this scheme is closely paralleled in the formation of cryptopleurine (**12**) can be deduced from the simultaneous occurrence of pleurospermine (**13**) [12] in *Cryptocarya pleurosperma*.

The intermediacy of a benzoyl acetic acid (**14**) in the biosynthesis of these alkaloids as suggested by Wenkert [6] and Leete [9] is yet to be proved in view of the lack of evidence for this type of *β*-keto acid as an intermediate in alkaloid biosynthesis. It was proposed

that such an acid could arise by reaction of shikimic and acetic acids; however again, shikimic acid metabolism is well known [13] and as yet this reaction has not been witnessed. Our experiments also indicated that acetate was a poor precursor for these alkaloids [2] and that the shikimic acid–acetate pathway did not operate.

EXPERIMENTAL

Cinnamic acid-[2-^{14}C] Na salt (0.1 mCi, 14.8 mg, 1 mCi/mM), was dissolved in H_2O (4 ml) and administered to four 18-month-old *T. asthmatica* plants through a wick inserted into the stem. The plants were harvested after 14 days and worked up to isolate tylophorine by the method of Ref. [14]. To detect activity at C-7′ and C-6′, labelled tylophorine obtained from cinnamic acid-[2-^{14}C] was degraded using our previous methods [1, 2].

Acknowledgements—We wish to express our thanks to Dr. D. S. Farrier, Siegfried, Aktiengesellschaft, Zofingen (Switzerland) for taking interest, regarding possible biogenesis of these alkaloids.

REFERENCES

1. Mulchandani, N. B., Iyer, S. S. and Badheka, L. P. (1969) *Phytochemistry* **8**, 1931.
2. Mulchandani, N. B., Iyer, S. S. and Badheka, L. P. (1971) *Phytochemistry* **10**, 1047.
3. Barton, D. H. R. (1963) *Proc. Chem. Soc.* 293.
4. Barton, D. H. R. and Cohen, T. (1957) in *Festschift A. Stoll. Birkhauser*, p. 117. Basel.
5. Govindachari, T. R., Lakshmikantham, M. V., Nagarajan, K. and Pai, B. R. (1958) *Tetrahedron* **4**, 311.
6. Wenkert, E. (1959) *Experientia* **15**, 165.
7. Robinson, R. (1955) in *The Structural Relations of Natural Products*, p. 78. Clarendon Press, London.
8. Marchini, P. and Belleau, B. (1958) *Can. J. Chem.* **36**, 581.
9. Leete, E. (1967) in *Biogenesis of Natural Compounds* (Bernfeld, P., ed.) p. 739. Macmillan, New York.
10. Russel, J. H. (1963) *Naturwissenschaften* **50**, 443.
11. Govindachari, T. R., Viswanathan, N., Radhakrishnan, J., Pai, B. R., Natarajan, S. and Subramanian, P. S. (1973) *Tetrahedron* **29**, 891.
12. Loder, J. W. (1962) *Australian J. Chem.* **15**, 296.
13. Bu'Lock, J. D. (1965) in *The Biosynthesis of Natural Products*, pp. 77 and 107. McGraw-Hill, New York.
14. Govindachari, T. R., Pai, B. R. and Nagarajan, K. (1954) *J. Chem. Soc.* 2801.