Phytochemistry, 1971, Vol. 10, pp. 1047 to 1050. Pergamon Press. Printed in England.

INCORPORATION OF PHENYLALANINE-2-^{14}C INTO TYLOPHORINE*

N. B. MULCHANDANI, S. S. IYER and L. P. BADHEKA
Biology Division, Bhabha Atomic Research Centre, Trombay, Bombay-85, India

(*Received* 12 *November* 1969, *in revised form* 30 *July* 1970)

Abstract—Administration of phenylalanine-2-^{14}C, sodium acetate-2-^{14}C and benzoic acid-1-^{14}C, to *Tylophora asthmatica* plants has revealed that ring A, carbon atoms −10 and −6′ of phenanthroindolizidine alkaloids like tylophorine are derived from phenylalanine. The shikimic acid-acetate pathway does not operate during this biosynthesis.

INTRODUCTION

THE BIOSYNTHESIS of tylophorine (III) and related compounds has been examined by us, in view of the suggested biogenetic pathways.[1,2] Using tyrosine-2-^{14}C, it has been shown[3] that it was efficiently incorporated into tylophorine and was a source of ring B and carbon atoms −9 and −7′.

In the present communication, the origin of ring A and carbon atoms −10 and −6′ has been explored. These could arise (Fig. 1) from 3,4-dihydroxybenzoylacetic acid (I). The same in turn could be formed from phenylalanine via cinnamic acid, *p*-coumaric acid and caffeic acid. Alternatively, the shikimic acid-acetate pathway could be operating.[2] With a view to resolving these possibilities, phenylalanine-2-^{14}C, benzoic acid-1-^{14}C and acetate-2-^{14}C were administered to *T. asthmatica* plants and tylophorine isolated in each case.

RESULTS

Phenylalanine-2-^{14}C was found to incorporate efficiently (Table 2). The degradation (Fig. 2) of labelled tylophorine via its methiodide[4] (IV), and Emde base[5,6] (V) to acetic acid (Table 1) revealed that acetic acid (sodium acetate) was inactive and carbon atom −7′ was devoid of any activity. This immediately suggested that transformation of phenylalanine to tyrosine had not taken place during the administration of the former precursor. On the other hand, the incorporation of phenylalanine via cinnamic acid would result in tylophorine with label at carbon atom −6′. This was confirmed by degrading labelled tylophorine to 2,3,6,7-tetramethoxyphenanthrene-9,10-dicarboxylic acid (VI), by mild $KMnO_4$ oxidation of tylophorinemethiodide (IV) in presence of pyridine. The acid was decarboxylated with copper chromite and the liberated carbon dioxide was absorbed in $Ba(OH)_2$ solution to get

* Part II in the projected series "Biosynthesis of Tylophorine".

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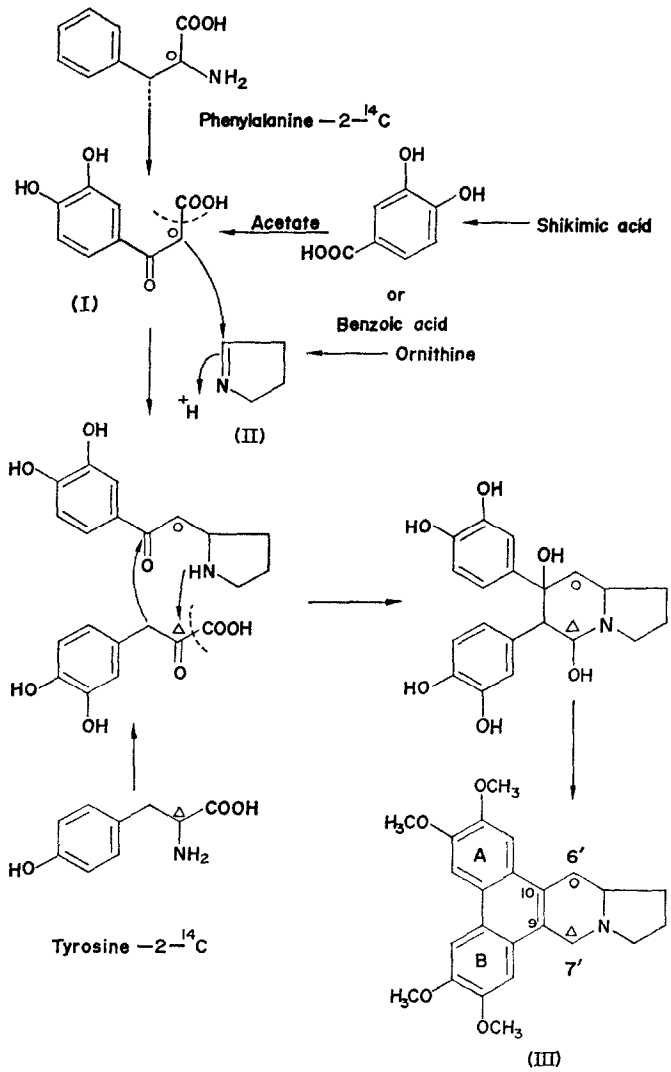
[4] T. R. GOVINDACHARI, B. R. PAI and K. NAGARAJAN, *J. Chem. Soc.* 2801 (1954).
[5] T. R. GOVINDACHARI, M. V. LAKSHMINATHAM, K. NAGARAJAN, B. R. PAI, *Tetrahedron* **4**, 311 (1958).
[6] T. R. GOVINDACHARI, M. V. LAKSHMINATHAM, B. R. PAI and S. RAJAPA, *Tetrahedron* **9**, 53 (1960).



○ △ } Location of ^{14}C.

FIG. 1. POSSIBLE MODE OF BIOGENESIS OF THE PHENANTHROINDOLIZIDINE ALKALOIDS.

$BaCO_3$. This possessed 50% of the activity as that of tylophorine, proving thereby that carbon atom —6′ was mainly active as carbon atom —7′ was already shown to be inactive.

The reaction mixture after decarboxylation was worked up to give 2,3,6,7-tetramethoxyphenanthrene (VII), which lacked any activity. This clearly establishes the relationship between phenylalanine and ring A and carbon atoms —10 and —6′ of tylophorine.

Benzoic acid-1-^{14}C did not incorporate into tylophorine. Acetate was found to be a poor precursor as it yielded tylophorine with low activity. Had these two precursors been directly involved, the degree of their incorporation into tylophorine, would have been comparable

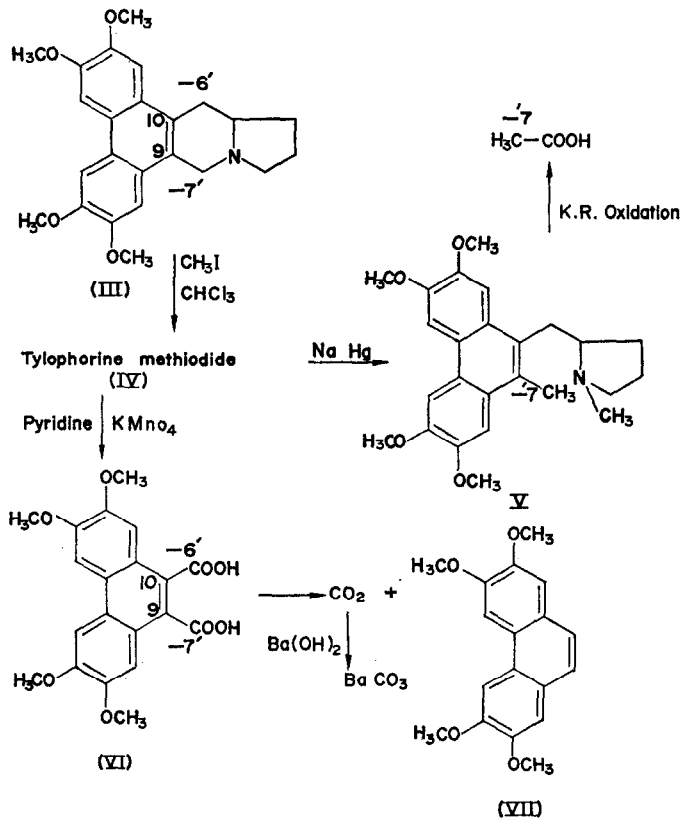


FIG. 2. DEGRADATION OF TYLOPHORINE.

TABLE 1. SPECIFIC ACTIVITIES OF UNDILUTED TYLOPHORINE OBTAINED FROM PHENYLALANINE-2-^{14}C AND ITS DEGRADATION PRODUCTS

	Activity in dis/min/m mole $\times 10^{-5}$
Tylophorine (III)	4·5
Tylophorine methiodide (IV)	4·5
Emde base of tylophorine (V)	4·4
Acetic acid (sodium acetate)	—
2,3,6,7-Tetramethoxyphenanthrenedicarboxylic acid (VI)	4·4
$BaCO_3$	2·2
2,3,6,7-Tetramethoxyphenanthrene (VII)	—

TABLE 2. SPECIFIC ACTIVITIES OF UNDILUTED TYLOPHORINE OBTAINED FROM OTHER PRECURSORS

	Tylophorine dis/min/mM	% Incorporation in tylophorine
Phenylalanine-2-^{14}C	$4·5 \times 10^5$	0·021
Sodium acetate-2-^{14}C	$3·5 \times 10^4$	0·001
Benzoic acid-1-^{14}C	—	—
Ornithine-5-^{14}C	$9·5 \times 10^5$	0·030

to that of phenylalanine, if not better. This was obviously not the case. The incorporation of acetate to the extent observed, could have easily resulted through the 'glyoxalate cycle', in which phosphoenolpyruvate, a known precursor of phenylalanine and tyrosine, is formed from acetate.

The above data therefore, offer strong support to phenylalanine being an important precursor in the biosynthesis of tylophorine and rule out the operation of shikimic acid-acetate pathway for this purpose.

Ornithine-5-^{14}C was also administered to *T. asthmatica* plants and found to be efficiently incorporated, thus suggesting its participation in tylophorine biosynthesis via Δ^1-pyrroline (II). However, since no degradations were carried out to determine the position of activity, its possible role as the precursor of the pyrrolidine ring in (III) is at present based on the analogy of results obtained in other plants (but see also Ref. 7).

EXPERIMENTAL

Phenylalanine-2-^{14}C (0·1 mc, 6·0 mg, 2·7 mc/mM), sodium acetate-2-^{14}C (0·1 mc, 4·1 mg, 2·0 mc/mM), benzoic acid-1-^{14}C (0·23 mc, 3·4 mg, 8·42 mc/mM), and ornithine-5-^{14}C (0·1 mc, 2·7 mg, in IN HCl, 4·9 mc/mM) were taken up in aqueous solution (4 ml) separately and administered to four 18-month-old *T. asthmatica* plants through a wick inserted into the stem in each case. The plants were harvested after 14 days and worked up to isolate tylophorine by the previously described procedure.[4]

To detect activity at carbon atom $-7'$, labelled tylophorine obtained from phenylalanine-2-^{14}C was degraded as reported in our earlier paper.[3]

Activity at Carbon Atom $-6'$

Tylophorinemethiodide. Tylophorine-^{14}C (10·0 mg, $4{\cdot}5 \times 10^5$ dis/mn/mmole) along with carrier tylophorine (90·0 mg) was converted into its methiodide.[4]

2,3,6,7-*Tetramethoxyphenanthrene*-9,10-*dicarboxylic acid.* The methiodide (100 mg) without further dilution was taken up in water (2 ml), pyridine (2·5 ml) and treated with $KMnO_4$ (N/10, 54 ml) solution dropwise first at room temp. and then at 30–40° with stirring for 3 hr till a faint pink colour persisted. The solution was then filtered and the filtrate concentrated under vacuum. The concentrate on acidification (HCl) and evaporation under vacuum yielded a brownish material which was taken up in methanol. The methanol soluble fraction was evaporated to dryness and treated with 10% aq. NaOH. The dicarboxylic acid was recovered from this solution by acidification (HCl), concentration under vacuum and repeated extraction with ether. The dried (Na_2SO_4) ethereal solution on evaporation gave acid (30·0 mg) m.p. 195° (uncorrected) which contained the same activity as that of tylophorine. The identity of the acid was confirmed by converting into its diester. Govindachari *et al.* reported this acid from tylophorineisomethohydroxide and isodihydrohomotylophorinemethine.[5] The formation of *m*-hemipinic acid under mild $KMnO_4$ oxidation of tylophorinemethiodide could not be expected to occur and the same was verified from u.v. spectrum of dicarboxylic acid, indicating phenanthrene chromophore and not an *m*-hemipinic acid system.

Decarboxylation. The acid (20·0 mg) was refluxed in freshly distilled quinoline (2·5 ml) containing copper chromite catalyst (30·0 mg) in a stream of CO_2 free nitrogen. The evolved CO_2 was passed into $Ba(OH)_2$ solution yielding $BaCO_3$ (8·0 mg) which possessed half the activity as that of tylophorine.

2,3,6,7-*Tetramethoxyphenanthrene.* The contents from decarboxylation reaction were poured into excess HCl (2N) and extracted with benzene. The extract was washed with dilute alkali and water. The dried Na_2SO_4 extract gave on evaporation and crystallization from methanol the required phenanthrene.[5] This did not possess any activity.

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