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INCORPORATION OF TYROSINE-2-W INTO TYLOPHORINE*

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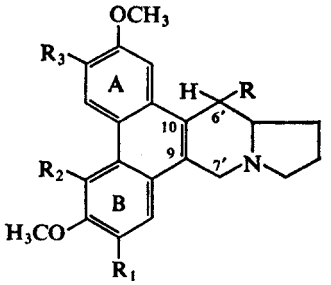
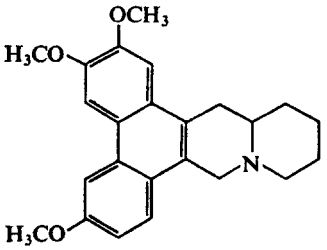
Abstract—Tyrosine-2-^{14}C administered to *Tylophora asthmatica* plants, is found to be efficiently incorporated into tylophorine and tylophorinine. Degradation of tylophorine-^{14}C showed that carbon atom 7' was derived from tyrosine.

TYLOPHORINE (I) and tylophorinine (II) were first isolated from *Tylophora asthmatica* (Asclepiadaceae).[1,2] Tylocrebrine[3] (III) and antofine,[4,5] containing three methoxyl groups, are the two other known alkaloids possessing the same phenanthroindolizidine structure. Cryptopleurine[6] (IV) is related to tylophorine but has the pyrrolizidine ring replaced by a quinolizidine moiety. Some of these alkaloids have been shown to inhibit protein synthesis' in Ehrlich ascites-tumour cells, while tylocrebrine shows high antileukaemic activity.[8]



(I) $R = R_2 = -H$; $R_1 = R_3 = -OCH_3$
(II) $R = -OH$; $R_2 = R_3 = -H$; $R_1 = -OCH_3$
(III) $R = R_1 = -H$; $R_2 = R_3 = -OCH_3$



(IV)

Since the biogenesis of such alkaloids is of great interest, a possible hypothetical scheme is given in Fig. 1. 3,4-Dihydroxybenzoylacetic acid (V) by reaction with Δ^1-pyrroline from ornithine could yield compound VI which on condensation with 3,4-dihydroxyphenylpyruvic

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

1 A. N. RATNAGIRISWARAN and K. VENKATACHALAM, *Indian J. Med. Res. 22,433 (1935).*
2 T. R. GOVINDACHARI, B. R. PAI and K. NAGARAJAN, *J. Chem. Soc.* 2801 (1954).
3 E. GELLERT, T. R. GOVINDACHARI, M. V. LAKSHMINATHAM, I. S. RAGADE, R. RUDZATS and N. VISWANATHAN, *J. Chem. Soc. 1008 (1962).*
4 T. F. PLATONOVA, A. D. KUZOVKOV and P. S. MASSAGETOW, *Zh.* Obsch. Khim. 28, 3131 (1958); *Chem. Abs.* 53, 7506d (1959).
5 M. PAILER and W. STREICHER, *Monatsh. Chem.* 96, 1094 *(1965).*
6 E. GELLERT, *Australian J. Chem. 7,* 113 (1954).
7 G. R. DONALDSON, M. R. ATKINSON and A. W. MURRAY, *Biochem. Biophys. Res. Commun. 31,104* (1968).
8 E. GELLERT and R. RUDZATS, *J. Med.* Chem. 7,361 (1964).

acid (VIII), would be expected to give compound VII and the latter by oxidative coupling could yield tylophorine (I).

Accordingly, if tyrosine-2-^{14}C is the precursor of 3,4-dihydroxyphenylpyruvic acid, the tylophorine produced should be labelled at C-7'. 3,4-Dihydroxybenzoylacetic acid could

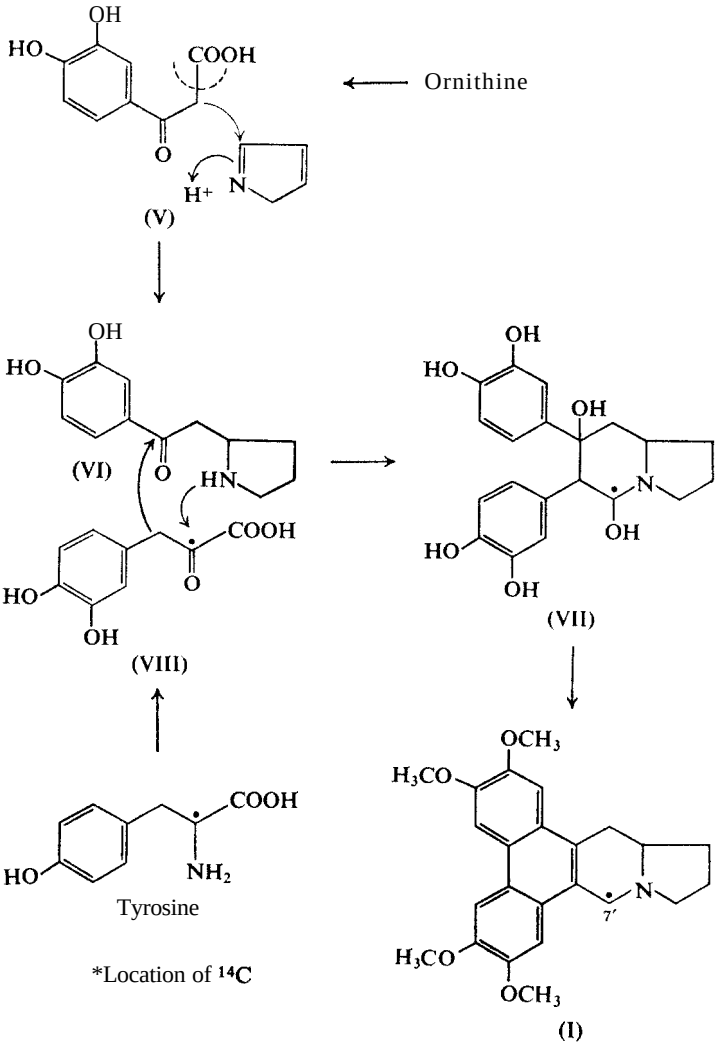
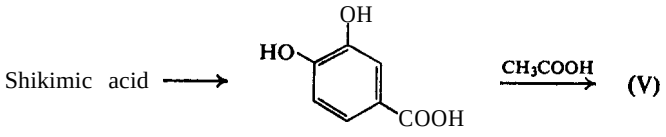


FIG. 1. POSSIBLE MODE OF BIOGENESIS OF THE PHENANTHROINDOLIZIDINE ALKALOIDS.

arise either from prephenate via cinnamic acid[9] or protocatechuic acid by way of aromatization of shikimic acid and condensation with an acetate unit.[10] Such aromatization of shikimic acid does occur in ***Neurospora crassa***[11] but has yet to be demonstrated in higher plants.

[9] E. WENKERT, ***Experientia*** **15, *165 (1959).***
[10] E. LEETE, ***Biogenesis of*** *Natural Products*, revised edition, p. 974, Pergamon Press, Oxford (1967).
[11] S. R. GROSS, ***J. Biol. Chem. 233,*** 1147 (1958).



Cryptopleurine (IV) is presumably biogenetically related to tylophorine and tylophorinine, where Δ^1-piperidine participates instead of Δ^1-pyrroline in the original condensation (Fig. 1).

The present communication deals with the incorporation of tyrosine-2-^{14}C into tylophorine. It has been found that a major part of the activity from this precursor is located at C-7'. The results are presented in Table 1.

TABLE 1. SPECIFIC ACTIVITIES OF UNDILUTED TYLOPHORINE AND ITS DEGRADATION PRODUCTS*

	Activity in dpm/m mole x 10^{-6}
Tylophorine (I)	2.3
Tylophorine methiodide (IX)	2.1
Emde base of tylophorine (X)	2·0
Acetic acid (sodium acetate)	2·0
N-Methylbenzamide (XI)	1.8
Barium carbonate	—

* Radioactive samples werecounted on a Packard Model 314 EX TRI-Carb Liquid Scintillation Spectrometer. The scintillation mixture was prepared by dissolving 4 g of BBOT: 2,5-bis-[2-(5-tert butyl benzoxazoyl)] thiophene, in 1 1. of toluene. Toluene-^{14}C was added as an internal standard to correct for quenching.

Tyrosine-2-^{14}C (0·1 mc, 13.25 mg, 1·36 mc/mM) in aqueous solution (4 ml) was administered to four 1½-yr-old *T. asthmatica* plants cultivated at Trombay Experimental Field Station using the wick technique. The plants were harvested after 14 days and worked up in usual manner.[3] The alkaloids I and II were separated and recrystallized to constant radioactivity. The active samples were also shown to be homogeneous by TLC (Alumina G (Merck) using $CHCl_3$ as solvent). Tylophorine (32·0 mg, $2·3 \times 10^6$ dpm/mM, incorporation 0·1 per cent) and tylophorinine (25·0 mg, $1·5 \times 10^6$ dpm/mM, incorporation 0·05 per cent) were obtained.

In order to establish the position of the label, tylophorine was degraded as shown in Fig. 2. Carrier tylophorine was added to the active sample.

EXPERIMENTAL

Degradation of Tylophorine-^{14}C

Tylophorine methiodide. Tylophorine-^{14}C (14.0 mg, $2·3 \times 10^6$ dpm/m mole) along with carrier tylophorine (126.0 mg) was converted into its methiodide.[2]

Emde base. Tylophorine methiodide (130.0 mg), without further dilution, was dissolved in hot water and few drops of methanol. Freshly prepared Na amalgam (5 per cent, 13 g) was added to the above solution at 100°, and the solution left at room temperature overnight. A colourless solid separated which was extracted with benzene. The benzene solution was washed with water, dried (Na_2SO_4), and evaporated under vacuum, to yield an amorphous mass crystallizing from benezene-light petroleum (40–60°), as colourless needles

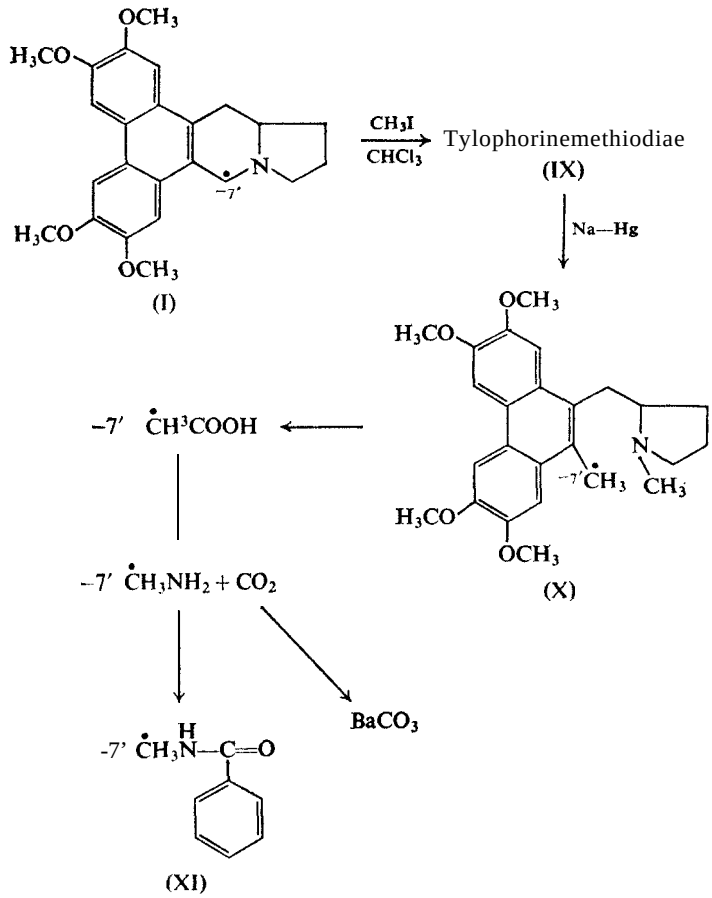


FIG. 2. DEGRADATION OF LABELLED TYLOPHORINE.

identical with isodihydrohomotylophorine (X, Emde base). Govindachari et al. reported this base from tylophorine isomethiodide[12] and tylophorine methochloride.[13]

Kuhn Roth oxidation. CrO_3 (Analar, 5·0 g) was dissolved in 2 N H_2SO_4 (10 ml) by warming, and to this was added a solution of Emde base (40·0 mg) in 2 N H_2SO_4 (2 ml). The reaction mixture was then distilled carefully at 125–130°, water being added from a dropping funnel to maintain the volume. Digestion and distillation werecontinued for 3 hr until the distillate was no longer acidic (about 50 ml). The distillate was redistilled and neutralized with NaOH (0·008 N) to the phenolphthalein end-point and evaporated to dryness. The residue was crystallized from absolute EtOH–Et_2O mixture, affording sodium acetate (5.5 mg) as colourless needles. TLC[14] of the acid generated from this material on silica gel G, using solvent systems (1) pyridine/light petroleum (1 : 2) and (2) EtOH/NH_4OH/H_2O (80 : 4 : 16) revealed a single spot corresponding to acetic acid (R_f 0·58 and 0·66 for the two solvent systems respectively). The labelled sodium acetate (5·0 mg) was further diluted with carrier (45.00 mg) and recrystallized.

Schmidt reaction. Sodium acetate (40·0 mg) was dissolved in warm conc. H_2SO_4 (0·75 ml) and cooled to 0". NaN_3 (100.0 mg) was added, and thereaction vessel was swept through with CO,-free N_2 which was then passed through a freshly prepared solution of $KMnO_4$ in 5 per cent H_2SO_4 (to remove any volatilized hydrazoic acid) and then into 0·2 N $Ba(OH)_2$. The temperature was slowly raised and maintained at 60–70° for 1 hr during which time $BaCO_3$ was precipitated in $Ba(OH)_2$ solution, which was rapidly filtered, washed with distilled

[12] T. R. GOVINDACHARI, M. V. LAKSHMINATHAM, K. NAGARAJAN and B. R. PAI, *Tetrahedron 4,311* (1958).
[13] T. R. GOVINDACHARI, M. V. LAKSHMINATHAM, B. R. PAI, and S. RAJAPA, *Tetrahedron* **9**, 53 (1960).
[14] K. RANDERATH, *Thin Layer Chromatography,* second edition, p. 218, Verlag Chemie/Academic Press (1964).

water, EtOH and then Et_2O. After drying at 60° the $BaCo_3$ (15.0 mg) was assayed as suspension in Packard Gel Powder (CAB-0-SIL) in a liquid scintillation counter and showed no activity.

The contents of the reaction mixture were added to a 100-ml flask containingalittleiceandmade basicwith 10 ml of 10 per cent NaOH and the solution distilled into a cooled conical flask containing 2 N HCl (10 ml), distilled water (20 ml) being added during the distillation. The aqueous distillate was evaporated giving a white crystalline substance to which was added benzoyl chloride (0·2 ml) and 1 N NaOH (8 ml). After standing overnight the contents of the flask were extracted with Et_2O, the extract dried (Na_2SO_4) and evaporated. The residue was sublimed *in vacuo* to yield *N*-methylbenzamide, (10.0 mg), m.p. 78-79" which was crystallized from light petroleum, b.p. 60-80".