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STUDIES ON WEST AFRICAN MEDICINAL PLANTS—I.
BIOGENESIS OF CARPAINE IN *CARICA PAPAYA* LINN.

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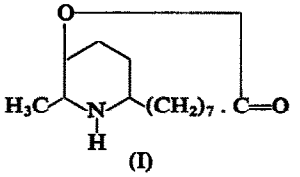
Abstract—The incorporation of [2-^{14}C]-acetate, [U-^{14}C]-lysine and [2-^{14}C]-mevalonate into the alkaloid, carpaine, by excised shoots of *Carica papaya* Linn. has been investigated. It was found that acetate was, by far, more efficiently incorporated than the other two compounds; and it is suggested that this finding could be consistent with a fatty acid or "polyketide" type of mechanism for the biosynthesis of carpaine.

CARPAINE from the leaves of *Carica papaya* Linn. has the structure (I).[1] Inspection of the formula suggests a possible biogenetic relation to the fatty acid or "polyketide" routes;[2] but the detailed origin of the piperidine ring cannot be assigned with certainty. In view of the interest in the origins of piperidine rings in alkaloids[3] we have examined the incorporation of [^{14}C]-labelled acetate, lysine and mevalonate into carpaine by excised shoots of *C. papaya*. We report here the preliminary conclusions: the detailed chemical degradations of the labelled alkaloid obtained are still under investigation.



(I)

RESULTS

Since the quantity of each radioactive compound administered per shoot was always less than 400 μg, and never more than 10 μc, it is assumed that it would not greatly alter the normal pool size of the compound in the plant; and should not have any significant deleterious effect on the metabolism of the plant.[4] It is assumed further that no significant permeability differences exist in the absorption of the three compounds into the cells of the plant, and also that the chemical recovery of labelled carpaine was equally efficient in the three sets of experiments. With these provisos in mind the relative efficiencies of the administered compounds as precursors for carpaine can be compared, as in Table 1, by making use of an arbitrarily defined incorporation factor given by:

$$\frac{\text{Total counts per sec}}{\text{Administered radioactivity } (\mu c \times \text{wt. of plant tissue, g})}$$

[1] G. BARGER, R. ROBINSON and T. S. WORK, *J. Chem. Soc.* 711 (1937); H. RAPOPORT, H. D. BALDRIDGE and E. J. VOLCHEK, *J. Amer. Chem. Soc.* **75**, 5290 (1953); T. R. GOVINDACHARI and N. S. NARASIMHAN, *J. Chem. Soc.* 2635 (1953), 1563 (1955); M. TICHY and J. SICHER, *Tetrahedron Letters* 511 (1962).
[2] A. J. BIRCH, *Proc. Chem. Soc.* 3 (1962).
[3] K. MOTHES and H. R. SCHÜTTE, *Angew. Chem.* (*Internat. Ed.*) **2**, 341 (1963).
[4] E. NOWACKI and R. U. BYERRUM, *Biochem. Biophys. Res. Comm.* 7, 58 (1962).

TABLE 1. RELATIVE EFFICIENCIES OF ACETATE, LYSINE AND MEVALONATE AS PRECURSORS FOR CARPAINE

	[2-^{14}C]-Acetate	[U-^{14}C]-Lysine	[2-^{14}C]-Mevalonate
Activity in carpaine (cps) (c)	126,500	1991	4028
Administered activity (μc) (a)	40	8	8
wt. of plant tissue (g) (w)	100·3	170·6	195·7
Incorporation factor $\left(\frac{a \times w}{c}\right)$	31·5	1·46	2·51

DISCUSSION

The results indicate that acetate is incorporated far more efficiently than lysine or mevalonate into carpaine. From structural considerations it seems unlikely that mevalonate could be involved directly in the biosynthesis of carpaine. Moreover if mevalonate were involved directly, then it should have proved more efficient than acetate as a precursor. There is some indication, however, that mevalonate could undergo biological degradation to yield products which may then be channelled into "non-polyprenoid" synthetic pathways.[5] It is probable, therefore, that the slight incorporation observed with mevalonate arose via some such degradation; and therefore is not biogenetically significant.

Lysine is known to be the precursor of piperidine-2-carboxylic acid in the rat, in plants and in *Neurospora crassa*.[6] Among the alkaloids proper there is much evidence that lysine and its decarboxylation product, cadaverine, are the precursors of the piperidine rings of anabasine, the lupin alkaloids, matrine, etc.[3] But the finding from the present investigation that lysine is even less efficiently incorporated than mevalonate indicates that it is improbable as a direct natural intermediate in the biogenesis of carpaine.

Carpaine contains an intact carbon chain of the same length as myristic acid (which, incidentally, occurs in the seed-oil of *Carica papaya*[7]). It could therefore arise by the acetate–malonate pathway characteristic of both the fatty acids and polyketides. Although the terminal unit in such cases (i.e. acetyl coenzyme A) differs from the other units (i.e. malonyl coenzyme A) it might be expected that under the prolonged conditions of the experiment, complete equilibration should occur.

Leete[8] has recently shown that the related piperidine alkaloid, coniine, very probably arises by a "polyacetate" route, and not from lysine as had previously been postulated.[9]

EXPERIMENTAL

Apparatus and Materials

Radioactive chemicals were supplied by the Radiochemical Centre, Amersham, England. The ordinary carpaine used to dilute the biosynthesized [^{14}C]-carpaine was prepared as described by Govindachari *et al.*[10] Liquid scintillation counting was done in re-distilled

[5] M. J. COON, F. P. KUPIECKI, E. E. DECKER, M. J. SCHLESINGER and A. DEL CAMPILLO, in *The Biosynthesis of Terpenes and Sterols: Ciba Foundation Symposium, London*, (1958) (Edited by G. E. W. WOLSTENHOLME and M. O'CONNOR), p. 62, J. & A. Churchill, London (1959); T. RAMASARMA and T. RAMAKRISHNAN, *Biochem. J.* **81**, 303 (1961).

[6] L. FOWDEN, in *Annual Reports Progr. Chem.* (1959) **56**, 359, The Chemical Society, London (1960).

[7] J. M. WATT and M. G. BREYER-BRANDWIJK, *Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd Ed., p. 173, E. & S. Livingstone Ltd., Edinburgh and London (1962).

[8] E. LEETE, *J. Amer. Chem. Soc.* **85**, 3522 (1963).

[9] U. SCHIEDT and H. G. HÖSS, *Z. Naturforsch.* **13b**, 691 (1958); *Hoppe-Seyler's. Z.* **330**, 74 (1962).

[10] T. R. GOVINDACHARI, B. R. PAI and N. S. NARASIMHAN, *J. Chem. Soc.* 1847 (1954).

sulphur-free toluene, as described by Stitch,[11] using an Ecko Type N621A counter connected to a stable power source (a.c. voltage stabilizer Type AC2 Mk IIB, Servomex Controls Ltd.), and an automatic scaler (Ecko Type N 530 G). The organic phosphor was a 0·3% (w/v) solution of 2,5-diphenyloxazole (i.e. "PPO") in toluene.[12] A constant total volume of 3 ml of phosphor was used for each count.[11]

Since piperidine itself had been reported to exhibit considerable "quenching" property with regard to "PPO" as phosphor,[12] the "self-quenching" by carpaine was determined beforehand, using 3 ml of phosphor solution containing about 0·06 μc of carrier-free [2-^{14}C]-mevalonate, as the standard scintillator. From the results it was deduced that a suitable amount of [^{14}C]-carpaine to be used for each count was 10 mg (−12% quenching).

General Incorporation and Extraction Procedure

Eight and a half months old *C. papaya* seedlings were cut under water, about 40–50 cm from the crown, and each excised shoot was transferred quickly into a large test-tube containing a solution of the radioactive compound in 2 ml of water. The assembly was placed inside an illumination chamber made of wire-netting, and illuminated with two 80W fluorescent tubes and four ordinary 60W "pearl" bulbs. After most of the solution had been absorbed the test-tube was rinsed down thrice with small amounts of water; the plant being allowed to absorb almost all of each rinse before the washing was repeated. Finally 25 ml of water was added, and the shoot was illuminated continuously for 14 days; its cut end being maintained under water throughout.

By the above method a set of four excised shoots were given 10 μc (ca. 113 μg) of sodium [2-^{14}C]-acetate per shoot; a second set of four shoots were given 2 μc (ca. 394 μg) of [2-^{14}C]-mevalonolactone per shoot; and a third set 2 μc (ca. 31 μg) of [U-^{14}C]-lysine monohydrochloride per shoot. The shoots all remained generally healthy and produced several new leaves during the experiment. After 14 days they were harvested, cut up together in appropriate sets, weighed, and each set of wet tissue was macerated in a Waring blendor, together with 600 ml of chloroform and 60 ml of 14 N ammonia.[13] The marc was stood for 48 hr, with occasional stirring; and the liquid was then drawn off, and the residue washed with 400 ml of chloroform. The extract and washing were combined, transferred into a separatory funnel, and the organic phase was run off. The aqueous phase was extracted further with chloroform (3 × 100 ml) and then discarded. The combined organic extract was shaken with 2 N HCl (4 × 50 ml), and the acidic extract was washed with chloroform, to remove non-basic oils. All the chloroform fractions were then discarded.

To the acidic extract was added inactive carpaine; and the solution was cooled in ice, treated with excess ammonia, and extracted with chloroform or ether (4 × 100 ml). The organic phase was washed with water, dried over Na_2SO_4 and the solvent removed on a water-bath. The residue was next digested with sulphur-free toluene (3 × 50 ml); and the insoluble resin (which, in each case, gave an orange-brown colour with Dragendorff's reagent) was discarded. The toluene-soluble fraction always gave a single, elongated, orange-pink spot on paper chromatograms run in *n*-BuOH:acetic acid:H_2O (4:1:5, top phase), and sprayed with Dragendorff's reagent.[14] The toluene was removed on a water-bath, under nitrogen; and, after prolonged drying of the residue *in vacuo* over P_2O_5, 10 mg of it was counted.

[11] R. STITCH, *Biochem. J.* **73**, 287 (1959).
[12] F. N. HAYES, R. D. HIEBERT and R. L. SCHUCH, *Science*, **116**, 140 (1952).
[13] E. LEETE, *J. Amer. Chem. Soc.* **78**, 3520 (1956); **80**, 4393 (1958).
[14] C. W. L. BEVAN and A. U. OGAN, *W. African Sci. J.* (in press).

After dilution with 85 mg of crude, inactive alkaloid as described above, the final product from the experiment with [^{14}C]-acetate was chromatographically pure, semi-crystalline carpaine (72 mg) with activity of 126,500 cps (extrapolated from an observed value of 1757 cps on 10 mg). The product from the experiment with lysine was diluted with 41 mg of inactive, crystalline carpaine, and finally yielded 41 mg of semi-crystalline, chromatographically pure base with a total extrapolated activity of 1991 cps. Similarly the [^{14}C]-carpaine from the mevalonate experiment was diluted with 30 mg of inactive, crystalline carpaine; and the semi-crystalline, chromatographically pure alkaloid isolated finally (33 mg) had a total extrapolated activity of 4028 cps.

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