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STUDIES ON THE MODE OF INCORPORATION OF MEVALONIC ACID INTO ERGOT ALKALOIDS

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RECENT reports[1,2,3] have shown (2-^{14}C) DL-mevalonic acid to be an active precursor of lysergic acid and clavine type alkaloids. Degradation studies of the radioactive agroclavine and elymoclavine isolated from C. purpurea fed with (2-^{14}C) DL-mevalonic by Birch et al.[1] have shown that about 30% of the radioactivity was present at the $C_{(17)}$ in agroclavine and about 90% in the case of elymoclavine*. These experiments supplemented with those concerned with the incorporation of (1-^{14}C) and (2-^{14}C) acetic acid are consistent with the incorporation of mevalonate but do not present a clear picture of the distribution of the radioactive carbon of mevalonic acid in the ergot alkaloids. Further speculation respecting the mode of incorporation of mevalonic acid requires a more detailed picture.

Groger et al.[2] have shown that the specific percentage incorporation of (2-^{14}C), (2-T), (4-T) DL-mevalonic acids into the ergot alkaloids are

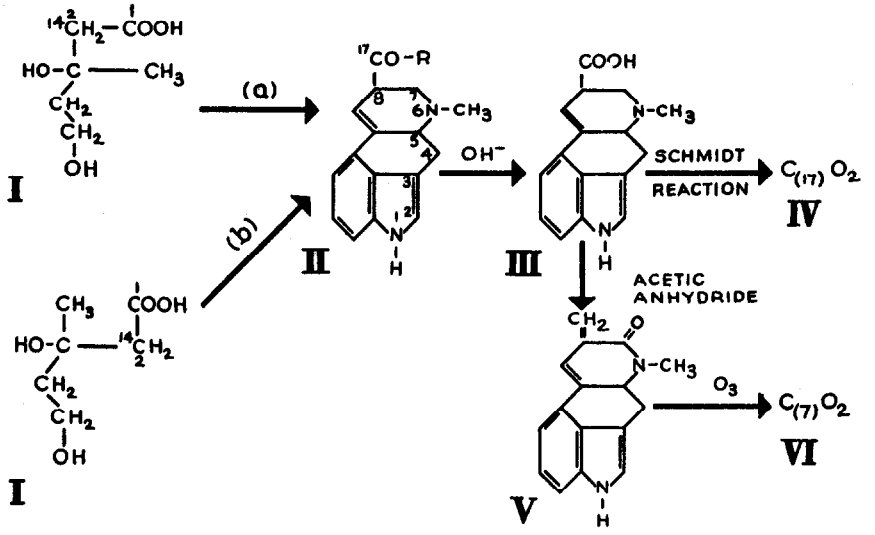
* A very recent personal communication from Dr. Birch indicates that subsequent data has revealed 90% of the radioactivity (2-^{14}C) mevalonic lactone at $C_{(17)}$ for agroclavine as well.

1 A.J. Birch, D.J. McLoughlin and H. Smith, Tetrahedron Letters No. 7, 1 (1960).

2 K. Mothes, H. Simon, H.G. Floss and F. Weygand, Z. Naturf. 15b, 141 (1960).

3 E.H. Taylor and E. Ramstad, Nature,Lond. 188, 494 (1960).

very close to each other suggesting the incorporation of mevalonic acid as a unit. Assuming therefore, the incorporation of mevalonic acid as a unit with the loss of the carboxyl carbon prior or subsequent to incorporation, the (2-^{14}C) of the (2-^{14}C) DL-mevalonic acid should appear at $C_{(17)}$ (I $\xrightarrow{(a)}$ II) or at $C_{(7)}$ (I $\xrightarrow{(b)}$ II) of the ergot alkaloids. The distribution of the (2-^{14}C) between the $C_{(17)}$ and $C_{(7)}$ might also occur to some degree and the randomization of this radioactive carbon is conceivable either prior to or after incorporation.



In order that more definite information respecting the relative degree of localization of the (2-^{14}C) carbon of mevalonate might be obtained a degradation was devised whereby the $C_{(7)}$ and $C_{(17)}$ of lysergic acid was isolated individually as carbon dioxide. The radioactive ergosine (II, R=peptide side chain of ergosine) isolated from the cultures of Claviceps purpurea, PRL 1578, to which had been added 200 μc of (2-^{14}C) DL-mevalonic acid (I), was diluted with an appropriate quantity of unlabelled ergosine

and was converted[4] to lysergic acid (III). The Schmidt reaction of (III) afforded the $C_{(17)}$ as carbon dioxide (IV). Lysergic acid treated[5] with acetic anhydride yielded the lactam (V) which yielded formaldehyde on ozonization. The formaldehyde so obtained was converted into carbon dioxide (VI) derived from the $C_{(7)}$ of lysergic acid.

Preliminary experiments were also carried out concerning the pathway of incorporation of mevalonate. Assuming a pathway similar to cholesterol[6], (1-^{14}C) DL-mevalonic acid was added to a clavine producing strain of C. purpurea to obtain data respecting the fate of the carboxyl group of mevalonic acid. The inhibitory effect of unlabelled isopentenylpyrophosphate and dimethylallylpyrophosphate on the incorporation of (2-^{14}C) mevalonate was also investigated. (Dimethylallylpyrophosphate was prepared from dimethylallyl alcohol obtained by the reduction of dimethylacrylic acid with lithium aluminium hydride by phosphorylation[7] in a manner similar to that reported by Yuan and Bloch[8] for the preparation of isopentenylpyrophosphate.)

Table 1

	Lysergic acid	Lactam	$C_{(17)}O_2$	$C_{(7)}O_2$
d.p.m./mM	2.38×10^4	2.38×10^4	2.39×10^4	1.51×10^3
Recovery %			100.4	6.3

[4] W.A. Jacobs and L.C. Craig, J.Biol.Chem. 104, 547 (1934).

[5] A. Stoll, A. Hofmann and F. Troxler, Helv.Chim.Acta 32, 506 (1948).

[6] T.T. Tchen, in Metabolic Pathways (Edited by D.M. Greenberg) Vol. I, p.392. Academic Press, New York (1960).

[7] R.M. Baxter, S.I. Kandel and K.L. Tam, unpublished data.

[8] C. Yuan and K. Bloch, J.Biol.Chem. 234, 2605 (1959).

In Table 1 are shown the specific activities of lysergic acid, the lactam and its degradation products ($C_{(17)}O_2$ and $C_{(7)}O_2$). These data indicate that nearly all the activity is localized in the carboxyl carbon $C_{(17)}$ of lysergic acid with only a small percentage in the $C_{(7)}$ and constitute strong evidence that the (2-^{14}C) of mevalonic acid becomes the $C_{(17)}$ of lysergic acid (I $\xrightarrow{(a)}$ II). It would appear that the randomization of mevalonate occurs to a small extent. Whether this occurs during its conversion to more immediate precursors (isopentenylpyrophosphate ⟶ dimethylallylpyrophosphate) or at a later stage remains to be established.

Table 2

Additives		Specific activity c.p.m./mM	Inhibition %
^{14}C	Unlabelled		
(1-^{14}C) DL-Mevalonic acid (2 μc)	-	-	
(2-^{14}C) DL-Mevalonic acid (0.73 μc)	-	7.12 x 10^5	
(2-^{14}C) DL-Mevalonic acid (0.73 μc)	IsPP*, 0.500 mg	7.0 x 10^5	-
(2-^{14}C) DL-Mevalonic acid (0.73 μc)	DMPP**, 0.500 mg	6.6 x 10^5	7.3
(2-^{14}C) DL-Mevalonic acid (0.73 μc)	DMPP**, 2.5 mg	4.47 x 10^5	38.6
(2-^{14}C) DL-Mevalonic acid (0.73 μc)	-	4.73 x 10^5	
(2-^{14}C) DL-Mevalonic acid (0.73 μc)	IsPP*, 0.642 mg	3.73 x 10^5	21
(2-^{14}C) DL-Mevalonic acid (0.73 μc)	IsPP*, 1.284 mg	2.83 x 10^5	32
(2-^{14}C) DL-Mevalonic acid (0.73 μc)	DMPP**, 0.642 mg	2.40 x 10^5	50

* IsPP = Isopentenylpyrophosphate cyclohexylammonium salt.
** DMPP = Dimethylallylpyrophosphate cyclohexylammonium salt.

The data in Table 2 indicate that none of the radioactivity from (1-^{14}C) DL-mevalonate was incorporated into the alkaloids isolated (pyroclavine and festuclavine) and that both of the assumed precursors inhibit the incorporation of (2-^{14}C) DL-mevalonate. Since it is not possible to assume with complete certainty that the pool size of the assumed precursors and that the rate of formation of the alkaloids are the same in each case it is not possible to unequivocably state which of the two mevalonic acid metabolites is the more immediate precursor. The randomization of mevalonate however indicates a preference for the dimethylallylpyrophosphate. The inhibitory effect of isopentenylpyrophosphate would be an indirect one since it is converted into dimethylallylpyrophosphate, a fact which is consistent with published data[9]. The incorporation of deuterated isopentenylpyrophosphate into clavine alkaloids was reported[10] during the course of this work. It is anticipated that more direct information will be obtained from experiments which are in progress using labelled dimethylallylpyrophosphate.

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[9] B.W. Agranoff, H. Eggerer, U. Henning and F. Lynen, J.Biol.Chem. 235, 326 (1960).

[10] H. Plieninger, R. Fischer, G. Keilich and H.D. Orth, Ann. 642, 213 (1961).