# **924.** *Studies in Relation to Biosynthesis. Part XVIII.* Penicillic Acid.*

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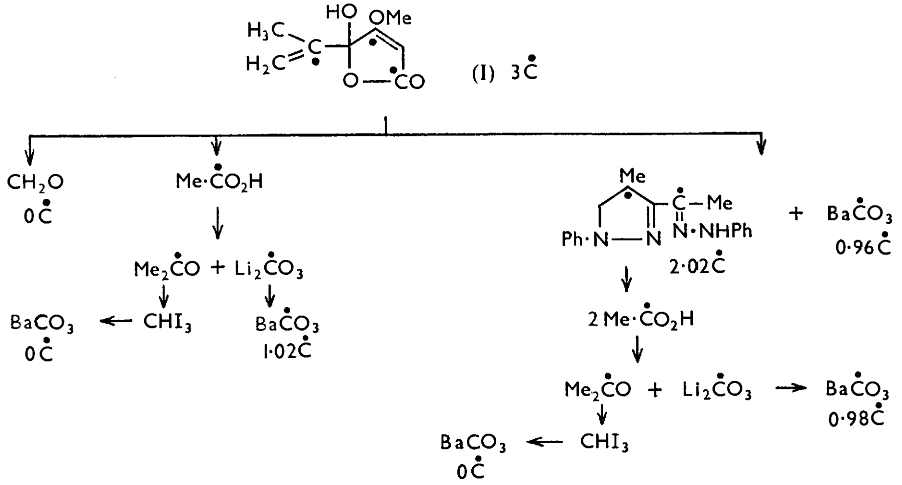
The biosynthesis of penicillic acid (I) by *Penicillium cyclopium* Westling has been studied by feeding experiments with [2-$^{14}$C]mevalonic lactone and $CH_3 \cdot {}^{14}CO_2H$.

The classical mould metabolite penicillic "acid" has been shown[1,2] to possess the branched-chain lactone structure (I). Our interest in the biosynthesis arose from consideration of the possible ways for the production of the branched $C_7$ chain. If mevalonic acid[3] is involved as an intermediate, the biosynthesis would result either from the degradation of a $C_{10}$ terpenoid chain or from the addition of the $C_5$ to a $C_2$ unit (probably acetic acid). In either case the process would be of considerable interest for the study of early stages in terpene biosynthesis. Other methods are potentially available for the production of branched carbon chains in Nature, notably those involving the introduction of $C_1$ units on to "methyl"-carbon atoms of straight chains derived by head-to-tail-linkage of acetic acid units. However, in penicillic acid the point of branching does not correspond to such an origin.

When *Penicillium cyclopium* Westling was grown on a Raulin–Thom medium containing [2-$^{14}$C]mevalonic lactone,[4] an insignificant amount (0·03%) of the radioactivity was incorporated into the penicillic acid and this radioactivity was found to be generally distributed along the carbon chain. The possibility of a biosynthetic relation between the terpenes and penicillic acid was thus discounted. Radioactivity from $CH_3 \cdot {}^{14}CO_2H$ was, however, incorporated to an appreciable extent (2·3%) and degradation of the labelled metabolite as illustrated in the scheme, showed this activity to be located to an equal extent upon carbon atoms 1, 3, and 5.



It will be seen that the distribution of radioactivity in the *iso*pentane portion parallels that encountered when $CH_3 \cdot {}^{14}CO_2H$ is biologically incorporated into terpenoid compounds. The above is therefore an important illustration of a route to a substance containing an "isoprene" unit which does not involve mevalonic acid. The exact route is not

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* Part XVII, preceding paper.

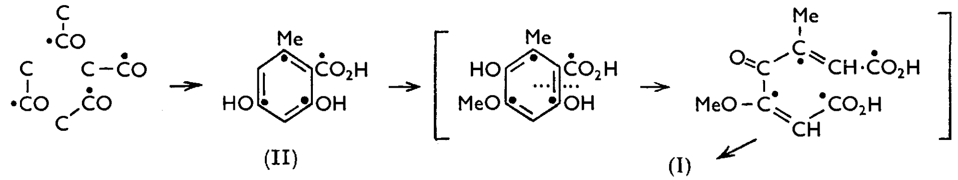
[1] Birkinshaw, Oxford, and Raistrick, *Biochem. J.*, 1936, **30**, 394.

[2] Shaw, *J. Amer. Chem. Soc.*, 1946, **68**, 2510; Raphael, *J.*, 1947, 805.

[3] Wolf, Hoffman, Aldrich, Skeggs, Wright, and Folkers, *J. Amer. Chem. Soc.*, 1957, **79**, 1486.

[4] Cornforth, Cornforth, and Youhotsky-Gore, *Biochem. J.*, 1957, **66**, 10P.

clear: the possibility that it is akin to the biosynthesis of valine would require [5] all the radioactivity from $CH_3\cdot{}^{14}CO_2H$ to be located on the carbon atom [1] of the *iso*valeric acid skeleton and is excluded by the " isoprenoid " labelling pattern. One possibility in accord with the above evidence is that the substance could be formed by oxidation and ring cleavage of a precursor related to orsellinic acid (II), possibly the 4-methyl ether, itself derived from a straight acetic-acid-based chain.* Recently Bassett and Tanenbaum [6] have presented



(II) (I)

indirect evidence of a similar cleavage of gentisic acid in the production of patulin by *P. patulum,* confirming a suggestion first made by Birkinshaw.[7] In this organism the gentisic acid appears to come from 6-methylsalicylic acid [7] which we have shown to arise by head-to-tail linkage of four acetic acid units.[8]

## EXPERIMENTAL

General directions are as for Part XVII. Series (a) and (b) refer to materials derived from $CH_3\cdot{}^{14}CO_2H$ and [2-$^{14}$C]mevalonic lactone respectively.

[$^{14}$C]*Penicillic Acid.—Penicillium cyclopium* Westling was cultured as previously described, except that on the seventh day $CH_3\cdot{}^{14}CO_2Na$ (0·2 mc) or [2-$^{14}$C]mevalonic lactone (0·07 mc) was added. The cultures were harvested and the penicillic acid was isolated in the known manner. Additional purification could be effected by chromatography in light petroleum (b. p. 60—80°)–benzene on Florisil. The penicillic acid had m. p. 84° [Found: r.m.a., (a) $1{\cdot}07 \times 10^5$; (b) $4{\cdot}88 \times 10^3$].

*Degradations of* [$^{14}$C]*Penicillic Acid.*—Treatment of penicillic acid with phenylhydrazine in acetic acid as described earlier gave 3-acetyl-4 : 5-dihydro-4-methyl-1-phenylpyrazole phenylhydrazone, m. p. 175—176° (lit.,[1] m. p. 175—176°) [Found: r.m.a. (a) $7{\cdot}24 \times 10^4$ (2C, $7{\cdot}13 \times 10^4$)], and barium carbonate [Found: r.m.a., (a) $3{\cdot}45 \times 10^4$ (1C, $3{\cdot}57 \times 10^4$); (b) $5{\cdot}12 \times 10^2$]. Ozonolysis of penicillic acid in glacial acetic acid gave formaldehyde, examined as the dimedone derivative, m. p. 189° [Found: r.m.a. (a) 0; (b) $6{\cdot}72 \times 10^2$]. Kuhn–Roth oxidation of penicillic acid gave acetic acid (0·8 mol.), the lithium salt of which was pyrolysed,[9] giving $BaCO_3$(Me) [Found: r.m.a. (a) 0; (b) $5{\cdot}28 \times 10^2$], and $BaCO_3(CO_2H)$ [Found: r.m.a. (a) $3{\cdot}66 \times 10^4$ (1C, $3{\cdot}57 \times 10^4$); (b) $4{\cdot}93 \times 10^2$]. Kuhn–Roth oxidation of the phenylhydrazone gave acetic acid (1·6 mol.), the lithium salt of which was pyrolysed as before, to $BaCO_3$(Me) [Found: r.m.a. (a) $2{\cdot}36 \times 10^2$], and $BaCO_3(CO_2H)$ [Found: r.m.a. (a) $3{\cdot}49 \times 10^4$ (1C, $3{\cdot}57 \times 10^4$)].

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* Professor Ehrensvärd (Lund) has kindly informed us that he is engaged in tracer work to test this hypothesis independently considered by him.

[5] Strassman, Thomas, and Weinhouse, *J. Amer. Chem. Soc.*, 1955, **77**, 1261.
[6] Bassett and Tanenbaum, *Experientia*, 1958, **14**, 38.
[7] Birkinshaw, *Ann. Rev. Biochem.*, 1953, **22**, 379.
[8] Birch, Massy-Westropp, and Moye, *Austral. J. Chem.*, 1955, **8**, 539.
[9] Cornforth, Hunter, and Popjak, *Biochem. J.*, 1953, **54**, 597.