

## The Biosynthesis of Eburicoic Acid

By W. LAWRIE, J. McLEAN, P. L. PAUSON, and J. WATSON

(Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, C.1)

EBURICOIC acid (I;  $R = R' = H$ ) has previously been obtained by Dauben<sup>1</sup> using labelled acetate in a culture medium of *Polyporus sulphureus*.

The use of larger precursors in terpene biosynthesis is exemplified by the work of Popjak *et al.*,<sup>2</sup> who have shown that rat liver enzyme preparations

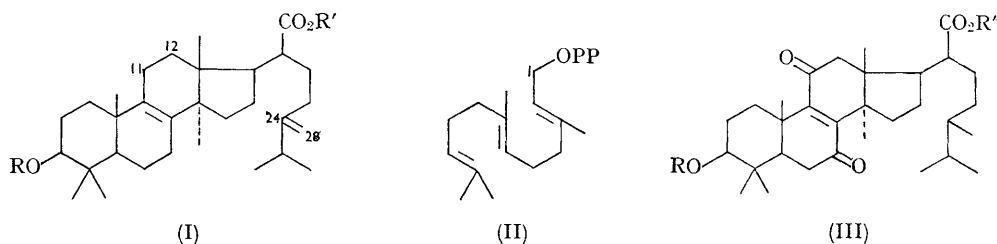
<sup>1</sup> W. G. Dauben and J. H. Richards, *J. Amer. Chem. Soc.*, 1956, **78**, 5329.

<sup>2</sup> G. Popjak, J. W. Cornforth, R. Ryhage, and De W. S. Goodman, *J. Biol. Chem.*, 1962, **237**, 56.

promote the conversion of labelled farnesyl pyrophosphate into squalene, while Bloch *et al.*<sup>3</sup> using the same precursor and enzyme preparations from yeast have also obtained labelled squalene. It was of interest to us to ascertain if the fungus *P. sulphureus* would tolerate the C<sub>15</sub> farnesyl

specific activity of  $6.1 \times 10^4$  dis./sec./mmole equivalent to an incorporation of 0.4%.

The radioactive ester was diluted with inactive ester to a specific activity of  $5.08 \times 10^4$  dis./sec./mmole and subsequent reactions were conducted on this material.



unit as a precursor of eburicoic acid, and accordingly we introduced *trans,trans*-farnesyl pyrophosphate (II), tritiated at C-1 (specific activity  $1.492 \times 10^7$  dis./sec./mmole) into a culture of *P. sulphureus* growing in a potato broth medium containing glucose.

If the fungus is capable of utilising the C<sub>15</sub> unit, and if we accept the biogenetic mechanism proposed by Eschenmoser *et al.*,<sup>4</sup> the eburicoic acid should contain tritium atoms distributed between C-11 and C-12.<sup>5</sup> We have confirmed this experimentally.

After a growth period of three months, the dried mycelium was extracted with light petroleum to remove fats, and the eburicoic acid, obtained by subsequent extraction with ether, was converted into methyl acetyl[11,12-<sup>3</sup>H]eburicoate (I, R = Ac, R' = Me). The latter was purified by repeated chromatography on alumina and by repeated recrystallisation from methanol to a constant

The tritiated acetoxy-ester was hydrogenated over platinum in ethyl acetate to the 24,28-dihydro-ester, which showed a specific activity of  $4.98 \times 10^4$  dis./sec./mmole. The dihydro-ester was oxidised with chromic acid to the enedione (III, R = Ac, R' = Me), of specific activity  $2.49 \times 10^4$  dis./sec./mmole. Equilibration of the tritiated enedione with refluxing methanolic potassium hydroxide gave methyl 3-hydroxy-7,11-dioxoeburic-8-en-21-oate of specific activity  $3.5 \times 10^3$  dis./sec./mmole.

The relative activities in passing from the dihydro-ester to the enedione (III; R = Ac, R' = Me) and then to the equilibrated hydroxy-ester (III, R = OH, R' = Me) are 1:0.5:0.06. This confirms the presence of tritium at C-11 and C-12 in the eburicoic acid and proves that the basidiomycete, *P. sulphureus*, is capable of absorbing a C<sub>15</sub> unit and utilising it in biosynthetic processes.

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<sup>3</sup> C. R. Childs and K. Bloch, *J. Biol. Chem.*, 1962, **237**, 62.

<sup>4</sup> A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, 1955, **38**, 1890.

<sup>5</sup> R. B. Clayton, *Quart. Rev.*, 1965, **19**, 168, and references cited therein.