The Biosynthesis of Mycophenolic Acid

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Summary The biosynthesis of mycophenolic acid (I) in *Penicillium brevi-compactum* occurs through introduction and a subsequent partial removal of a farnesyl side-chain into 5,7-dihydroxy-4-methylphthalide (II); the methyl group of (II) is introduced prior to the formation of the aromatic system.

IN a previous paper[1] on the biosynthesis of mycophenolic acid (I) we have identified the key aromatic intermediate (II) onto which the terpenoid side chain is introduced.

Under our normal experimental conditions (transformation time of 7 d at 26° on a reciprocal shaker in a semi-synthetic medium) this compound had never been isolated from the culture medium, probably owing to its further rapid transformation. However, interruption after 48 h of the fermentation conducted in the presence in the culture medium of a large amount of unlabelled (II) and [*Me*-^{14}C]-methionine, leads to the isolation of compound (II) containing 3% of the introduced radioactivity. This result can be explained on the basis of an exchange, through the cell wall, of added compound (II) with biosynthesised (II), which bears at C-4 a ^{14}*C*-methyl group originating from [*Me*-^{14}C]methionine.[2]

The methylation of the β-tetraketide enzyme-bound intermediate (III), prior to its aromatisation and lactonisation to (II), is indicated by the observation of a high incorporation of the acid (IV) into mycophenolic acid, as compared with the very low incorporation of orsellinic acid (V) (Table). This fact leads to the exclusion of the various

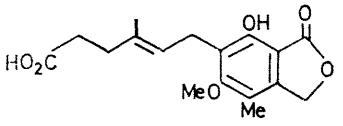
Incorporations of intermediates into mycophenolic acid

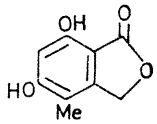
Intermediate	Total Inc. %	Molar Inc. %
[1-^{14}C]-4,6-dihydroxy-2,3-dimethylbenzoic acid (IV)	83·5	54·8
[6-^{14}C]-4,6-dihydroxy-2-methylbenzoic acid (V)	0·3	1·2
6-farnesyl-5,7-dihydroxy-4-[*Me*-^{14}C]-phthalide (VI)	33·6	38·6

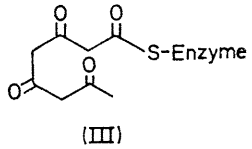
mechanisms suggested[3] for the formation of the phthalide moiety, but is consistent with the biosynthesis of other methylacetogenins.[4]

It has been suggested[5] that the isoprenoid side-chain of mycophenolic acid derives from a C_{10} unit; however, products bearing a geranyl-type side-chain have never been isolated from the cultures.

Feeding of (II) to the culture of *Penicillium brevi-compactum* permits, after 48 h, the isolation of the new intermediate (VI), $C_{24}H_{32}O_4$; m.p. 98—100°; *m/e* 384, 369, 351, 341, 328, 315, 301, 273, 247, 233, and 193; n.m.r. ($CDCl_3$) δ 1·85br (3H, s, Me–C(=)CH–), 1·68br (3H, s, Me–C(=)CH–), 1·6br (6H, s, Me–C(=)C*H*–), 2·06 (3H, s, MeAr), 3·47 (2H, d, *J* 8 Hz., ArCH_2-CH=), 4·24 (1H, m, olefinic H), 4·85—5·5 (2H, m, olefinic H), and 5·2 p.p.m. (2H, s, lactone CH_2),


(I)


(II)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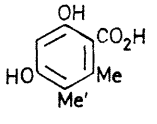
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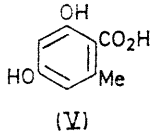
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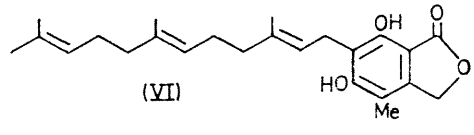
(VI)

which strongly suggests that the most important process for the biosynthesis of the side-chain of mycophenolic acid is the introduction of a C_{15} terpene chain and oxidative fission at the appropriate double bond with the subsequent loss of eight carbon atoms.

In fact, reintroduction of labelled (VI) into the culture of *Penicillium brevi-compactum* gives mycophenolic acid with a very high incorporation (Table).

From these experiments with [*Me*-^{14}C]methionine, we have also isolated (VI) and (I) with incorporations of the introduced radioactivity, of 1·8 and 10% respectively.

Finally, feeding of labelled compound (II) leads, after two days, to (I)† (total incorporation 62%, molar incorporation 97%), of (VI) (total incorporation 9%, molar incorporation 73%), and of diluted starting material.

These data are in accordance with a very slow biosynthesis of the aromatic ring followed by a fast introduction and subsequent partial removal of the terpenoid chain.

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† In a previous paper[1] we reported an incorporation of about 39%, after 7 d, of (II) into mycophenolic acid.

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