Sequence Studies in Biosynthesis; Mycophenolic Acid

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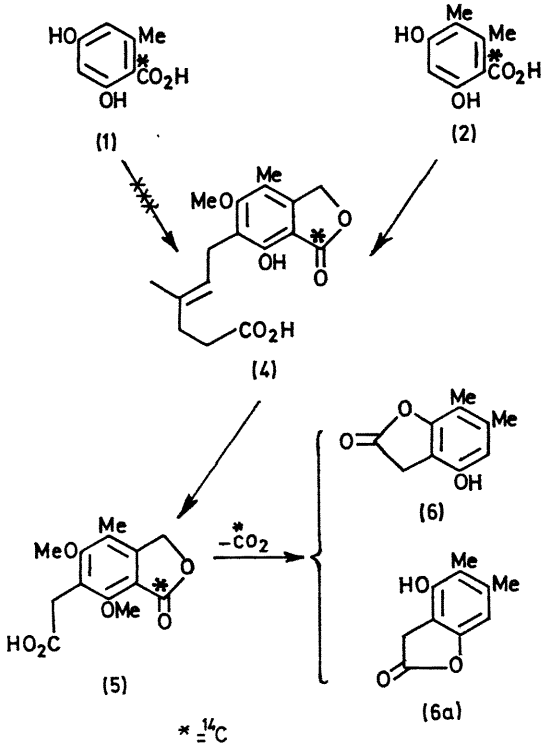
Summary Comparative incorporation experiments with [1′-^{14}C]orsellinic acid (1) and [1′-^{14}C]-2,4-dihydroxy-5,6-dimethylbenzoic acid (2) suggest that only the latter is a specific precursor of mycophenolic acid; the implication of this result in terms of the biosynthetic sequence is discussed, and preliminary studies on the mode of construction of the terpenoid side-chain are also reported.

A RECENT paper[1] on the biosynthesis of mycophenolic acid prompts us to record the results of our investigations in this area.

Previous studies[2,3] on the biosynthesis of mycophenolic acid (4)[4] have shown that the basic skeleton of the molecule is acetate-derived and that methionine and mevalonic acid serve as precursors of the methyl group and acidic side-chain attached to the aromatic nucleus. In spite of these results, however, little is known of the sequence of events leading to the construction of this important mould metabolite.[5] Our initial studies were designed to determine the nature of the least complex aromatic intermediate in the biosynthetic route. For this purpose we decided to test the precursor activity of [1′-^{14}C]orsellinic acid (1) and [1′-^{14}C]-2,4-dihydroxy-5,6-dimethylbenzoic acid (2).[6] The acid (1) ($2{\cdot}5 \times 10^{-3}$ mCi/mmole) and (2) ($5{\cdot}4 \times 10^{-4}$ mCi/mmole) were separately administered to shake cultures of *P. brevi-compactum* grown on Raulin–Thom medium when the pH of the broth was 5·2—5·6. When pH 7 was reached (14—20 days) mycophenolic acid was isolated from the parallel experiments and purified to constant radioactivity. Mycophenolic acid (4) from the orsellinic acid experiment was radioinactive† while that from the experiment using acid (2) as precursor had a specific activity of $6{\cdot}3 \times 10^{-5}$

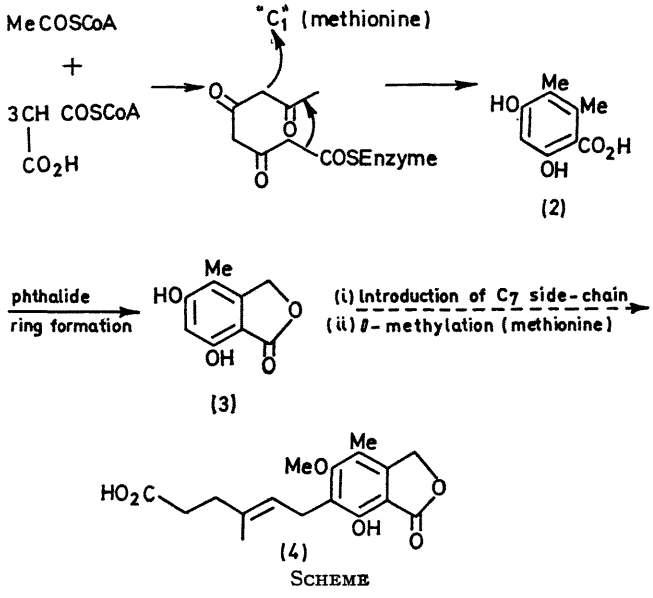
† Previous results (ref. 2) have indicated a low and partially random incorporation of orsellinic acid into mycophenolic acid.

mCi/mmole (*i.e.* specific incorporation 11·5%). Methylation of mycophenolic acid (after dilution $1·782 \times 10^{-5}$ mCi/mmole) with diazomethane followed by ozonolysis yielded the dimethoxy-acid (5)[4] ($1·77 \times 10^{-5}$ mCi/mmole)



with total retention of radioactivity. Further treatment of acid (5) with red phosphorus and hydriodic acid[4] yielded radio-inactive coumaran-2-one (6) and/or (6a) and one mole equivalent of carbon dioxide (as $BaCO_3$)[12] which possessed 96% of the original activity. These results show that specific incorporation of radioactivity from [1′-^{14}C]-2,4-dihydroxy-5,6-dimethylbenzoic acid (2) into mycophenolic acid had occurred and support the role of this acid as a precursor in the biosynthetic sequence. It is also interesting to note that we have detected this substance as a normal metabolite of *P. brevi-compactum*.[7,8] The results outlined above also indicate that *C*-methylation is occurring before aromatisation of the tetra-acetate chain and that other secondary transformations (*O*-methylation, phthalide ring formation, and construction of C_7 side-chain) are occurring after the formation of the aromatic acid (2).‡

The next step in the biosynthetic sequence seems to be the formation of the phthalide ring, since Canonica and his colleagues have reported[1] the high, specific incorporation of 5,7-dihydroxy-4-methylphthalide (3) into mycophenolic acid. A revised tentative scheme for the biosynthesis of mycophenolic acid is shown (Scheme) and work in progress is designed to provide further information regarding the nature of later intermediates in the sequence.



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The mode of construction of the C_7 acidic side-chain in mycophenolic acid has attracted some attention. Birch and his co-workers have shown[2] that mevalonic acid is involved and their results support the suggestion that C_{10}-alkylation occurs (presumably involving geranyl-pyrophosphate) followed by selective oxidative cleavage of the terminal alkene linkage. To test this possibility 1,1-ditritiogeraniol, 1,1-ditritiogeranyl pyrophosphate, and [1-^{14}C]geraniol were separately administered to *P. brevi-compactum*. Radioactive mycophenolic acid was isolated in each case but degradation, as outlined above, showed that non-specific incorporation of radioactivity had occurred. Further experiments are in progress to provide an adequate explanation for this surprising result.

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‡ By contrast, the biological conversion of (1) into (2) has been shown in the biosynthesis of flavipin.[11]

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[4] J. H. Birkinshaw, H. Raistrick, and D. J. Ross, *Biochem. J.*, 1952, **50**, 630 and references cited.
[5] For an account of the anti-cancer properties of this compound see S. E. Carter, T. J. Franklin, D. F. Jones, B. J. Leonard, S. D. Mills, R. W. Turner, and W. B. Turner, *Nature*, 1969, **223**, 848 and references cited.
[6] The synthesis of radioactive (1) and (2) from orcinol followed well recognised procedures (*cf.* R. Thomas, *Biochem. J.*, 1961, **78**, 748) and will be described in our full paper.
[7] C. T. Bedford, unpublished observations.
[8] 2,4-Dihydroxy-5,6-dimethylbenzoic acid (2) has previously been isolated from *A. terreus*[9] and *Gliocladium roseum*.[10]
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