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Biosynthetic Intermediates *en route* to Mycophenolic Acid in *Penicillium brevicompactum*

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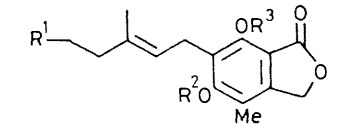
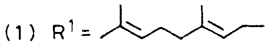
Summary The farnesyl phthalide (**1**; R^2 = Me, R^3 = H) and the mycophenolic acid prenylogue (**4**; R^2 = Me, R^3 = R^4 = H), but not the acyloin (**3**; R^2 = H or Me, R^3 = H), have been found by radiogas chromatography–mass spectrometric analysis of *P. brevicompactum* cultures that were active in mycophenolic acid biosynthesis.

It appears that at least two pathways lead from the farnesyl phthalide (**1**; R^2 = R^3 = H) to mycophenolic acid (MA, **2**, R^2 = Me, R^3 = H). Cell-free extracts of *Penicillium brevicompactum* convert the phthalide (**1**; R^2 = R^3 = H) into the acyloin (**3**; R^2 = R^3 = H);[1] whole cells of the fungus convert this acyloin effectively into MA.[1] The prenylogue of MA, compound (**4**; R^2 = R^3 = R^4 = H),[2] is also converted into MA by the fungus.[3] As would be required by a role for this compound, acetone and levulinic acid are produced in parallel with MA as the fermentation develops.[4,5]

The existence of two pathways to MA raises the question of the relative importance of each. The basic metabolic opportunism[6,7] of fungal cells coupled with uncertainties regarding relative rates of transport into cells make it doubtful that this question can be answered unambiguously by feeding isotopically labelled (**3**) and (**4**) (in both, R^2 = R^3 = H) to *P. brevicompactum*. This communication reports preliminary results with an alternative method, based on radiogas chromatography–mass spectrometry (r.g.c.–m.s.),[8] in which cells are exposed to primary precursors such as ^{14}C-acetate and total cell extracts are then scanned to determine (a) if the biosynthetic pathway is active and (b) what pathway-related compounds are present in the tissue.

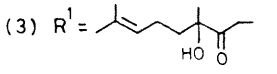


(1) R^1 =

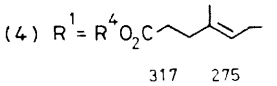


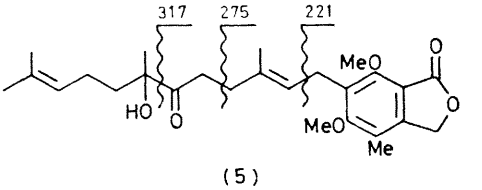
(2) R^1 = CO_2H

(3) R^1 =



(4) R^1 = R^4O_2C





(5)

P. brevicompactum was grown from spores in medium B of reference 9. At the 60th hour the culture was exposed to [1-^{14}C-]acetate for 3 h. Medium and biomass were separately extracted with ethyl acetate. A portion of the extracts was treated with diazoethane; the bulk was treated with diazomethane. The two alkylation procedures should allow us to determine if R^2 was H or Me, in compounds (**1**), (**3**), and (**4**), *in vivo*.

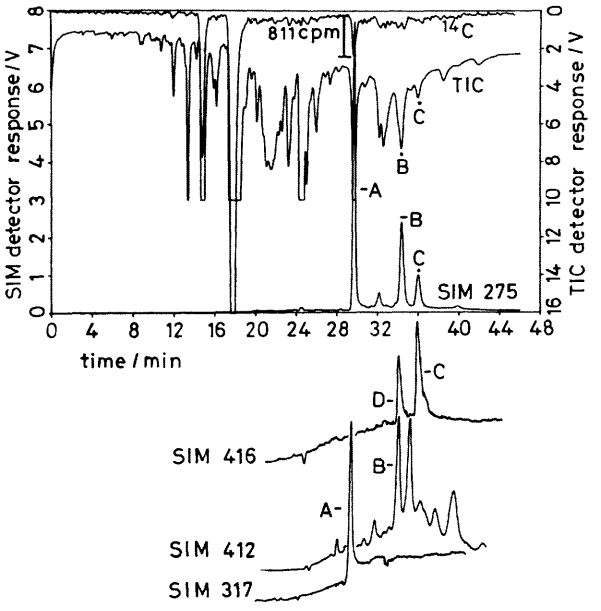


FIGURE. Radioactivity (^{14}C), total ion current (TIC), and selected ion monitoring (*m/z* 275, 416, 412, and 317) outputs from an r.g.c.–m.s. analysis of a total tissue extract of *P. brevicompactum*. The maximum disintegration rate in the mycophenolic acid peak (peak A) was 811 counts per minute. (Peak D in the *m/z* 416 profile is produced by a steroid.)

The Figure gives results obtained from the methylated tissue extract and shows the radioactivity, total ion current, and *m/z* 275, 317, 412, and 416 selected ion monitor (SIM) profiles. The *m/z* 412 and 416 ions are the parent molecular ions of (**1**; $R^2 = R^3 = Me$) and (**4**; $R^2 = R^3 = R^4 = Me$) respectively. We expect the ion *m/z* 317 to be formed from the methylated acyloin as shown in (**5**). The origin of the *m/z* 275 ion is also shown in structure (**5**); it, together with an *m/z* 221 ion, is highly characteristic of the methylated MA nuclear skeleton.

In the Figure, peak A is due to MA. It is radiolabelled indicating that MA biosynthesis is active. The *m/z* 221 (not shown), 275, 412, and 416 SIM profiles establish that peaks B and C are due to (**1**) ($R^2 = R^3 = Me$) and (**4**) ($R^2 = R^3 = R^4 = Me$) respectively. No trace of the acyloin (**3**; $R^2 = R^3 = Me$) could be found in this or other experiments of a similar type.

Failure to find the acyloin does not preclude its involvement. However, since compound (**4**), together with acetone and levulinate are found routinely while the acyloin and 2-oxo-6-methylhept-5-ene[5] (the presumed residue from cleaving the MA skeleton out of the acyloin in a single step) are not, it is unlikely that the biosynthetic route through the acyloin is a major one.

R.g.c./m.s. analysis of the ethylated tissue extracts reveals that the parent molecular ions associated with former peaks B and C move to *m/z* 426 and 444 respectively. This establishes that in the cells only the methylated forms (**1**;$R^2 = Me$, $R^3 = H$) and (**4**;$R^2 = Me$, $R^3 = R^4 = H$) exist. It confirms the fears of Muth and Nash[10] that their methyltransferase is not specific for demethylmycophenolic acid.

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