Biosynthesis of Mycophenolic Acid. The Synthesis of 6-Farnesyl-5,7-dihydroxy-4-methylphthalide in a Cell-free Preparation from *Penicillium brevicompactum*

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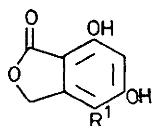
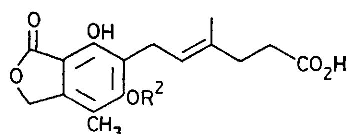
Summary A dialysed ammonium sulphate fraction of a cell-free preparation from *Penicillium brevicompactum* catalyses the synthesis of 6-farnesyl-5,7-dihydroxy-4-methylphthalide (**10**) from 5,7-dihydroxy-4-methylphthalide (**1**) and either (3*RS*)mevalonic acid (**5**) or farnesyl pyrophosphate (**8**).

THE proposed biosynthetic pathway[1,2] for mycophenolic acid (**4**) is based on the incorporation of labelled compounds *in vivo* in which 5,7-dihydroxy-4-methylphthalide (**1**),[2] 6-farnesyl-5,7-dihydroxy-4-methylphthalide (**10**),[2] and demethylmycophenolic acid (**3**)[3] are sequential intermediates. We report the isolation of an enzyme system from *P. brevicompactum* that catalyses the synthesis of (**10**). This is the first report of an aromatic prenyl transferase that utilises farnesyl pyrophosphate as the substrate.

P. brevicompactum was grown on Czapek-Dox medium in shake culture for 72 h at 25 °C and the mycelium was mixed with ballotine beads, suspended in 0·1 M Tris-HCl pH 7·8 containing 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, and 10% glycerol, and macerated for 5 min. The filtered homogenate was centrifuged and the soluble fraction was brought to 70% saturation with ammonium sulphate (F_0^{70}). Enzymic activity was determined by following the production of (**11**) in an incubation mixture that contained (**2**)[2,4,5] and (**5**) in the presence of 0·18 M Tris–HCl pH 7·6, 18 mM $MgCl_2$, 18 mM β-mercaptoethanol, 14 mM ATP, 2·3% glycerol, and *ca.* 12·5 mg of dialysed F_0^{70} protein in 2·2 ml. Product and substrate were isolated by t.l.c. and assayed for radioactivity. The optimal conversion of (**2**) and (**5**) into (**11**) was 90 and 14%, respectively and was obtained with substrate concentrations of 0·09 mM (**2**), with 3·18 mM (**5**) and 0·045 mM (**2**) with 0·32 mM (**5**), respectively.

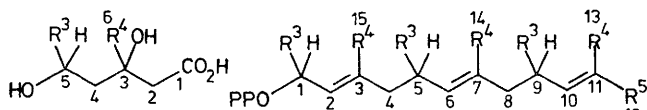
The enzyme preparation was used in large scale incubations of (**2**) with (3*RS*)mevalonate (**5**), with (3*RS*)[6-$^{2}H_3$]-mevalonate (**6**), and again with (**5**) but in a deuteriated incubation mixture. [4,8,12-^{14}C]Farnesyl pyrophosphate (**8**) and [4,8,12-^{14}C,1,5,9-$^{2}H_1$]farnesyl pyrophosphate (**9**) were prepared from (3*RS*)[2-^{14}C]mevalonate and (3*RS*,5*RS*)-[2-^{14}C,5-$^{2}H_1$]mevalonate (**7**), respectively using published procedures[6] and similarly incubated with (**1**) but without ATP. The products (**11**), (**12**), (**13**), (**14**), and (**15**), respectively, were purified by silica gel and Sephadex LH20 chromatography and compounds (**11**) and (**14**), after methylation, showed coincidence of mass and radioactivity with a methylated sample of (**10**) when subjected to thin-layer and gas-liquid radiochromatography.





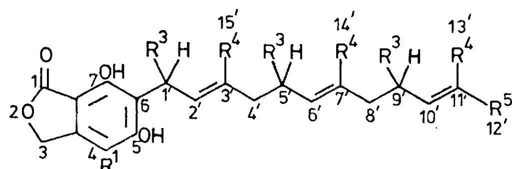
(1) $R^1 = CH_3$
(2) $R^1 = {}^{14}CH_3$

(3) $R^2 = H$
(4) $R^2 = CH_3$



(5) $R^3 = H, R^4 = CH_3$
(6) $R^3 = H, R^4 = C^2H_3$
(7) $R^3 = {}^2H, R^4 = CH_3$; 2-^{14}C

(8) $R^3 = H, R^4 = R^5 = CH_3$; 4,8,12-$^{14}C$
(9) $R^3 = {}^2H, R^4 = R^5 = CH_3$; 4,8,12-$^{14}C$



(10) $R^1 = R^4 = R^5 = CH_3, R^3 = H$
(11) $R^1 = {}^{14}CH_3, R^4 = R^5 = CH_3, R^3 = H$
(12) $R^1 = {}^{14}CH_3, R^4 = C^2H_3, R^5 = CH_3, R^3 = H$
(13) $R^1 = {}^{14}CH_3, R^4 = CH_3, R^5 = CH_2{}^2H, R^3 = H$
(14) $R^1 = R^4 = R^5 = CH_3, R^3 = H$; 4',8',12'-$^{14}C$
(15) $R^1 = R^4 = R^5 = CH_3, R^3 = {}^2H$; 4',8',12'-$^{14}C$

The ^1H n.m.r. spectrum of (**11**) was identical to published data[2] but in (**12**) the signals at δ 1·59 (13'- and 14'-Me) and δ 1·84 (15'-Me) were absent and represent the contribution of the deuteriated methyl arising from C-6 in (**6**). In compound (**13**) the ^1H n.m.r. signal at δ 1·67 (12'-Me) was reduced in intensity by 40% and in (**15**) the integral of the methylene doublet at δ 3·47 (1'-CH_2) showed the expected presence of one hydrogen which was consistent with the ^1H n.m.r. data (δ 4·4, J 6Hz, 1H) and mass spectra (m/e 225, $^2H_3$) on the farnesol recovered from the incubation following chromatography on silica gel. The principal ions in the mass spectrum of products (**11**), (**12**), (**13**), and (**15**) complement the ^1H n.m.r. data and gave peaks at m/e 384 (M^+), 393 ($^2H_9$), 385 ($^2H_1$), and 387 ($^2H_3$), respectively. Consecutive allylic cleavage of the farnesyl side chain (8', 9'; 4', 5') in (**11**), (**12**), (**13**) and (**15**) gave three sets of peaks at m/e 315, 69, 247; 321 ($^2H_6$), 72 ($^2H_3$), 250 ($^2H_3$); 315, 70 ($^2H_1$), 247 and 317 ($^2H_2$), 70 ($^2H_1$), and 248 ($^2H_1$), respectively. The base peak (m/e 193) due to the benzylic 1', 2' cleavage was present in (**11**), (**12**), and (**13**) but was replaced by a monodeuteriated species in the fragmentation of (**15**) which gave m/e 194 ($^2H_1$). These results show that (**10**) is synthesised in the cell-free system in such a way that the methyl groups at 13', 14', and 15' arise from C-6 of mevalonate and that the methyl group at 12' is formed from C-2 of mevalonate in accordance with the action of isopentenyl pyrophosphate isomerase on isopentenyl pyrophosphate.[7]

The data presented are consistent with the hypothesis that the synthesis of the farnesyl side chain of (**10**) in *P. brevicompactum* follows the documented pathway[8] for the synthesis of farnesyl pyrophosphate and that farnesyl pyrophosphate is a substrate for the synthesis of (**10**); the results do not rule out the possibility that farnesyl pyrophosphate is first converted into another intermediate, *e.g.* nerolidyl pyrophosphate.

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