Biosynthesis of Mycophenolic Acid. Substrate Stereochemistry of Farnesyl Pyrophosphate: 5,7-Dihydroxy-4-methylphthalide Farnesyl Transferase from *Penicillium brevicompactum*

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Summary The enzymic synthesis of 6-farnesyl-5,7-dihydroxy-4-methylphthalide (**4a**) from 5,7-dihydroxy-4-methylphthalide (**2**) and (3*RS*)-mevalonic acid (**1a**) proceeds with inversion of configuration at C-5 in (**1a**).

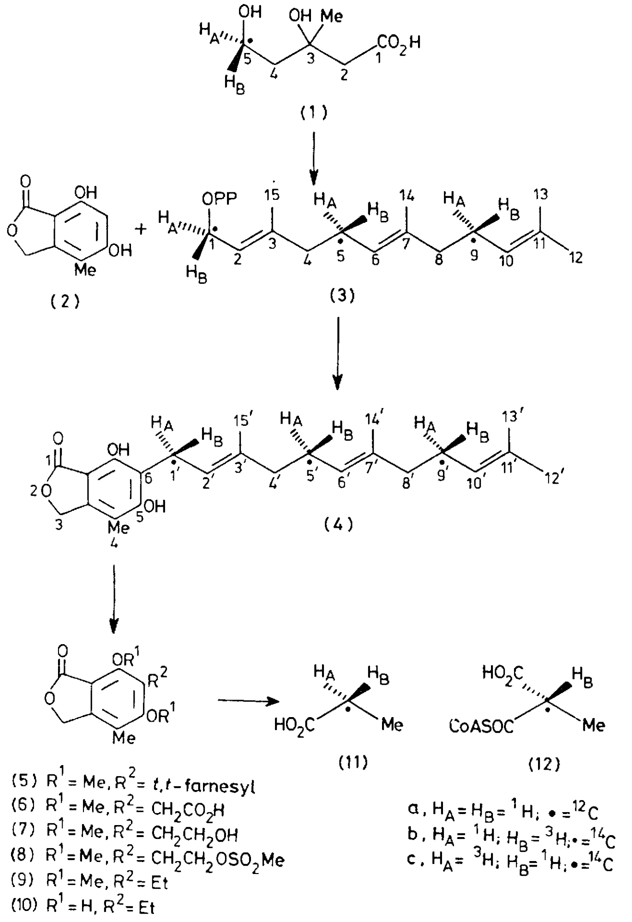
6-FARNESYL-5,7-DIHYDROXY-4-METHYLPHTHALIDE (**4a**) is biosynthesised by the alkylation of 5,7-dihydroxy-4-methylphthalide (**2**) with a C_{15}-prenylated intermediate which is derived from (3*RS*)-mevalonic acid (**1a**).[1] Farnesyl pyrophosphate (**3a**) is a substrate for this reaction in a cell-free system from *Penicillium brevicompactum*.[1] During the biosynthesis of (**4a**) the C-5 methylene of (**1a**) is transformed into the C-1′ methylene in (**4a**); we report on the substrate stereochemistry at C-5 in (**1a**) that accompanies this process. This is the first report on the substrate stereochemistry of an enzymic *C*-alkylation that involves the replacement of the pyrophosphate group of a prenyl pyrophosphate by a phenol.

(3*RS*)-[5-^{14}C,(5*R*)-5-^{3}H$_1$]mevalonate (**1b**) and (3*RS*)-[5-^{14}C,(5*S*)-5-^{3}H$_1$]mevalonate (**1c**) were separately incubated with (**2**) in a cell-free preparation[1] from *P. brevicompactum*. The products (**4b**) and (**4c**), respectively, were isolated after purification on silica gel and Sephadex LH20 chromatography. After methylation, the chiral compounds were chemically degraded by the pathway that had been developed for unlabelled materials. The methyl ether (**5**) was ozonised in methylene chloride at —70 °C and the ozonide was oxidatively decomposed with performic acid to give (**6**).[2] The possibility that proton exchange occurred during this oxidation was excluded following analysis of the mass and ^{1}H n.m.r. spectra of (**6**) which had been prepared in deuteriated performic acid. The acid (**6**) was reduced with borane[3] in tetrahydrofuran to the alcohol (**7**) which was converted into the mesylate (**8**) and reduced with NaBH$_4$ in dimethyl sulphoxide[4] to give (**9**). Demethylation of (**9**) with boron tribromide[5] in methylene chloride at —10 °C gave (**10**) which was oxidised with chromium trioxide[6] to a mixture of propionic (*ca.* 60%) and acetic acids (*ca.* 20%) that were isolated by steam distillation and completely separated by partition chromatography. The radioactive compounds from the degradation of (**4b**) and (**4c**) were purified by t.l.c. and gave identical R_F values with those of unlabelled compounds for which satisfactory ^{1}H n.m.r. spectra, mass spectra and elemental analyses had been obtained. The degradation products from (**4b**) and (**4c**) gave satisfactory ^{3}H/^{14}C ratios and constant specific radioactivities.

The configuration at C-2 in chiral propionate was determined by using a modification of published procedures[7,8] in which propionic acid (**11a**) was chemically converted into the coenzyme A ester and transformed into *S*-methylmalonyl-CoA (**12a**)[7,9] using propionyl-CoA carboxylase (EC 6.4.1.3). This carboxylation removes the *pro*-(2*R*)hydrogen (H$_\mathrm{A}$) from propionyl-CoA (**11a**)[7,8] and the process is not accompanied by a significant tritium isotope effect.[7,10] In our modification of this procedure the conversion of propionyl-CoA into *S*-methylmalonyl-CoA was allowed to proceed to *ca.* 50% and the substrate and product were rapidly separated by chromatography on ECTEOLA-cellulose at 4 °C over 3 h, in order to eliminate the exchange[11] of the proton at C-2 in *S*-methylmalonyl-CoA. Using this procedure, (*RS*)-[1-^{14}C,2-^{3}H$_1$]propionyl-CoA was converted into (*S*)-[1-^{14}C, 2-^{3}H$_1$]methylmalonyl-CoA that retained 49·2% of the tritium originally present in propionyl-CoA.

(3*RS*)-[5-^{14}C, (5*R*)-5-^{3}H$_1$]mevalonate (**1b**) was converted into (**4b**) which was degraded to propionic acid (**11b**). The corresponding propionyl-CoA was converted into [2-^{14}C, (2*S*)-2-^{3}H$_1$]methylmalonyl-CoA (**12b**) that contained 120% of the tritium present in the propionyl-CoA; thus the configuration of this propionate was (2*S*). Similarly (3*RS*)-[5-^{14}C, (5*S*)-5-^{3}H$_1$]mevalonate (**1c**) was converted *via* propionic acid (**11c**) into (*S*)-[2-^{14}C]methylmalonyl-CoA (**12c**) that retained 20% of the tritium present in propionyl-CoA; thus the configuration of this propionate was predominantly (2*R*).

The stereochemical interpretation of this result is that the configuration of the C-5 methylene in mevalonate (**1a**) is inverted during its conversion into the C-1′ methylene of (**4a**). The stereochemical course is shown in the Scheme [by reference to the (b) or (c) series]. Since the stereochemistry at C-5 of mevalonate (**1a**) remains unchanged during conversion into C-1 of farnesyl pyrophosphate (**3a**)[12,13] it follows that the methylene bearing the pyrophosphate group undergoes inversion of configuration during the alkylation of (**2**) by farnesyl pyrophosphate (**3a**). The overall stereochemistry of this aromatic prenylation is the same as that reported[14] for the enzymic olefin alkylation reactions in the mammalian system in which the C-1 methylene of isopentenyl pyrophosphate is inverted.



(5) R¹ = Me, R² = *t,t*-farnesyl
(6) R¹ = Me, R² = CH$_2$CO$_2$H
(7) R¹ = Me, R² = CH$_2$CH$_2$OH
(8) R¹ = Me, R² = CH$_2$CH$_2$OSO$_2$Me
(9) R¹ = Me, R² = Et
(10) R¹ = H, R² = Et

a, H$_\mathrm{A}$ = H$_\mathrm{B}$ = ^{1}H; • = ^{12}C
b, H$_\mathrm{A}$ = ^{1}H; H$_\mathrm{B}$ = ^{3}H; • = ^{14}C
c, H$_\mathrm{A}$ = ^{3}H; H$_\mathrm{B}$ = ^{1}H; • = ^{14}C

SCHEME.

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