(*E*,*E*)-10-(1,3-Dihydro-4,6-dihydroxy-7-methyl-3-oxoisobenzofuran-5-yl)-4,8-dimethyldeca-4,8-dienoic Acid: Total Synthesis and Role in Mycophenolic Acid Biosynthesis

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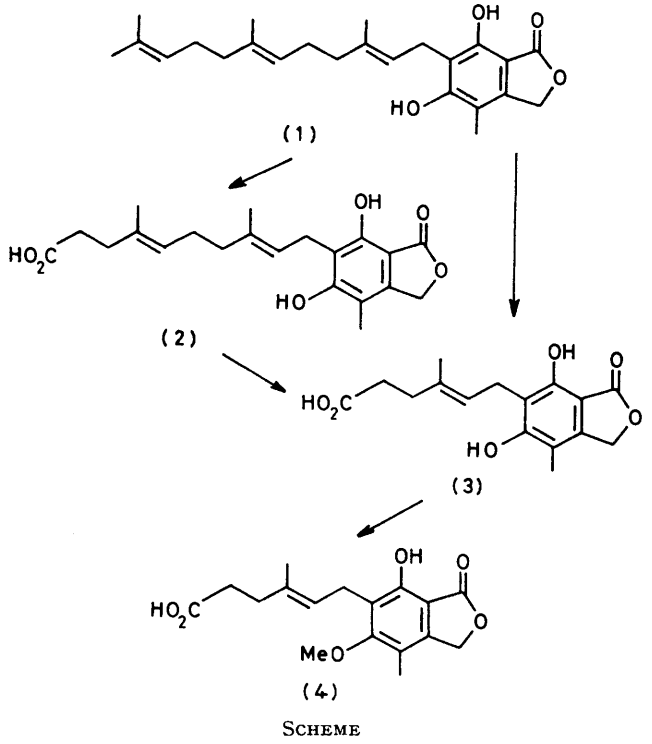
Summary The title compound (**2**), a prenylogue of mycophenolic acid (**4**), has been synthesized by two different routes; experiments show unambiguously that (**2**) is a biosynthetic intermediate of mycophenolic acid.

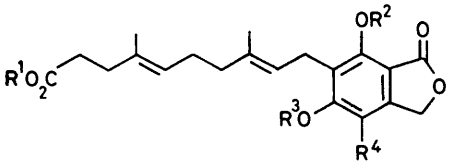
6-FARNESYL-5,7-DIHYDROXY-4-METHYLPHTHALIDE (**1**) is converted into mycophenolic acid (**4**) not only by direct oxidation of the central double bond,[1] but also by a two-stage, 'terminal–central, demolition process' (Scheme).



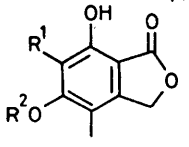
SCHEME

Previously reported radio g.l.c.–mass spectral data[2] are consistent with the latter process but alone do not prove the intermediacy of the title compound (**2**) in mycophenolic acid biosynthesis. Proof is given in this paper by isolating (**2**) and studying the incorporation of labelled (**2**) into mycophenolic acid. A synthetic sample of (**2**) was methylated (CH_2N_2; MeOH) to give (**5**). Comparison (g.l.c.–mass spectroscopy) between the synthetic compound (**5**) and the fermentation compound, obtained by methylation (CH_2N_2; MeOH) of the mycelium extracts and silica gel chromatographic purifications,† shows that (**2**) is a natural metabolite of *Penicillium brevicompactum* [g.l.c. conditions: 1% SE 30, 220 °C; *m/e* 416 (10·4), 385 (6·9), 384 (9·2), 275 (43·0), 221 (100), 207 (31·4), 195 (53·0), and 141 (44·0%)].

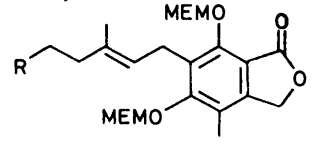
Administration of the ^{14}C-labelled acid (**6a**) to the *in vivo* culture led to the formation of labelled mycophenolic acid (total incorporation 22%).‡ Introduction of the ^{13}C-labelled acid (**6b**) yielded 28% molar incorporation into mycophenolic acid after the same fermentation time (3 days). These data show that (**2**) is a biosynthetic intermediate of mycophenolic acid. The whole oxidation sequence, in the conversion of (**1**) into (**4**), is currently under investigation, in particular the relative importance of the direct-central and terminal–central pathways.



(**5**) $R^1 = R^2 = R^3 = R^4 = Me$
(**6a**) $R^1 = R^2 = R^3 = H$; $R^4 = {}^{14}CH_3$
(**6b**) $R^1 = R^2 = R^3 = H$; $R^4 = {}^{13}CH_3$
(**7**) $R^1 = Et$; $R^2 = R^3 = MEM$; $R^4 = Me$
(**8**) $R^1 = Me$; $R^2 = R^3 = H$; $R^4 = Me$



(**9**) $R^1 = CH_2CHO$; $R^2 = Me$
(**10**) $R^1 = R^2 = H$



(**11**) R = $-CO_2MEM$
(**12**) R = $-CO_2H$
(**13**) R = $-CH_2OH$
(**14**) R = $-CHO$
(**15**) R = $-CH(OH)$–

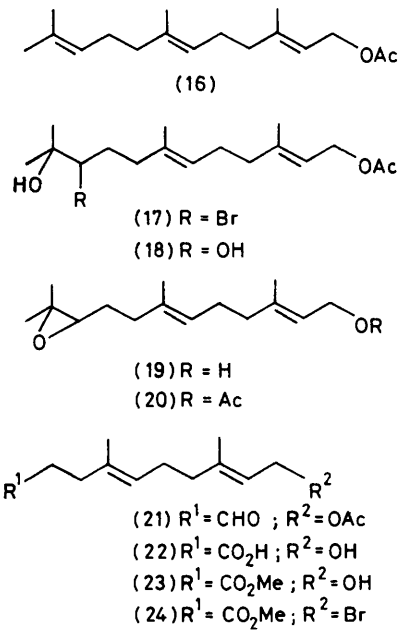
MEM = $MeOCH_2CH_2OCH_2$

† *Ca.* 10 mg of (**5**) were obtained from the mycelium extracts derived from ten flasks of culture (L. Canonica, W. Kroszczynski, B. M. Ranzi, B. Rindone, E. Santaniello, and C. Scolastico, *J.C.S. Perkin I*, 1972, 2639).

‡ The incorporation specificity was tested by ozonolysis of (**4**) to the aldehyde (**9**) (L. Canonica, W. Kroszczynski, B. M. Ranzi, B. Rindone, E. Santaniello, and C. Scolastico, *J.C.S. Perkin I*, 1972, 2639): the molar activity value did not change.

Two different approaches were used for the synthesis of the prenylogous acid (**2**). In the semisynthetic route, mycophenolic acid (**4**) was converted into the protected aldehyde (**14**) by the following sequence: demethylation to (**3**) (LiI; collidine; 70% yield),[3] β-methoxyethoxymethylation to (**11**) (MEMCl; Et_3N; CH_2Cl_2–THF 5:1; 100% yield),[4] hydrolysis to (**12**) (NaOH; H_2O–THF; 100% yield), reduction to (**13**) ($ClCO_2Me$; Et_3N; THF–$NaBH_4$; H_2O; 90% yield),[5] and oxidation of the alcohol (pyridinium chlorochromate; CH_2Cl_2; AcONa; 80% yield) (THF = tetrahydrofuran).[6] Treatment of (**14**) with prop-2-enyllithium (Et_2O–THF 5:1; −60 °C) gave the alcohol (**15**) (50% yield after Florisil chromatography) which underwent a Claisen-type transposition[7] [$MeC(OEt)_3$; $EtCO_2H$; 130 °C] to form the compound (**7**) in 40% yield. Hydrolysis of the MEM ethers (HCl; H_2O–THF; 50% yield) and of the ethyl ester (NaOH, H_2O; 100% yield) of (**7**) gave (*E*,*E*)-10-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxoisobenzofuran-5-yl)-4,8-dimethyldeca-4,8-dienoic acid (**2**).

In the total synthesis, (*E*,*E*)-farnesol was converted into the terminal diol (**18**) by the following sequence: acetylation to (**16**) (Ac_2O; pyridine), oxidation to the bromohydrin (**17**) (*N*-bromosuccinimide; Bu^tOH–H_2O),[8] alkaline treatment to (**19**) (K_2CO_3; MeOH–H_2O), acetylation to (**20**) (Ac_2O; pyridine), and acid hydrolysis of the epoxide ($HClO_4$; diglyme–H_2O). Oxidation of the diol (**18**) gave the aldehyde (**21**) ($NaIO_4$; H_2O–THF), which was in turn oxidised to form the acid (**22**) (Ag_2O; NaOH; dioxan–H_2O). Methylation of (**22**) gave the ester (**23**) (CH_2N_2; Et_2O) which was brominated (CBr_4; PPh_3; MeCN)[9] to give (**24**). The reaction of the allylic bromide (**24**) with the diphenol (**10**) and Ag_2O in dioxan–water (96:4)[10] gave the *C*-alkylated product (**8**) in 20% yield. Final hydrolysis (NaOH, H_2O) gave the prenylogous acid (**2**).



The second synthetic route is more convenient, especially for the introduction of the label. The same reactions, using labelled (**10**),§ gave the labelled acids (**6**).

Interest in the prenylogous acid (**2**) is not only limited to biosynthetic studies. Its pharmacological activity could be interesting, because of the structural analogy between (**2**), mycophenolic acid (**4**), and other anti-psoriasis agents.[11]

We thank Eli-Lilly Internat. Corp. for kindly giving us the mycophenolic acid.

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§ [*Me*-^{14}C] and [*Me*-^{13}C]-phthalides (**10**) were obtained by a modification of the literature procedure (W. R. Logan and G. T. Newbold, *J. Chem. Soc.*, 1957, 1946) using labelled formaldehyde in the chloromethylation step.

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[11] Drugs of the Future, 1978, Vol. III, No. 8, p. 594, and references therein; Unlisted Drugs, Nov. 1977, **29**: 176 o, and references therein.