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A TOTAL SYNTHESIS OF MYCOPHENOLIC ACID, SOME ANALOGUES AND SOME BIOGENETIC INTERMEDIATES

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Abstract—Mycophenolic acid (**28**) has been obtained by a convergent synthesis starting from methyl 6-bromo-4-methylhex-4-enoate (**13**) and 5,7-dihydroxy-4-methylphthalide (**24**). For the total synthesis of 5,7-dihydroxy-4-methylphthalide, 1-carbethoxy-2,3-dimethylcyclohexa-4,6-dione (**14**) was aromatized and transformed into 4,6-dimethoxy-2,3-dimethylbenzamide. The photolysis of the corresponding N-chloroamide and subsequent hydrolysis gave 5,7-dimethoxy-4-methylphthalide which was hydrolysed to 5,7-dihydroxy-4-methylphthalide (**24**).

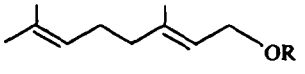
The bromoester (**13**) was obtained starting from geraniol. Condensation between **13** and **24** with silver oxide in dioxane afforded the methyl ester of nor-O-methyl mycophenolic acid. Selective methylation and hydrolysis furnished mycophenolic acid.

THE antibiotic and anti-tumor properties of mycophenolic acid have stimulated efforts toward the synthesis of some analogues and derivatives.[1]

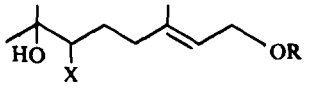
The use of mycophenolic acid itself as starting material for preparative purposes permits only a limited number of modifications and the total linear synthesis due to Birch,[2] cannot be easily modified to yield analogues and isotopically labelled biosynthetic intermediates.

We report here a convergent synthesis which has been explored in two preliminary communications.[9] It permits many significant structural modifications of its molecule. Starting materials for this scheme are an allylic bromide and a substituted phthalidic nucleus.

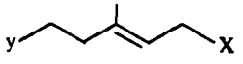
Synthesis of the key intermediate (**13**) was accomplished by reaction of geranyl acetate (**1**) with N-bromosuccinimide in aqueous t-butanol[3] to yield the bromohydrin (**2**). Treatment of this with potassium carbonate in aqueous methanol afforded the epoxide (**3**). Acetylation of **3** followed by treatment with perchloric acid in diglyme furnished the diol (**4**) which was transformed, with sodium periodate in aqueous THF, into the oily aldehyde (**5**). Alternatively, O-tritylgeraniol (**6**) was transformed into the epoxide (**7**) and subsequently into the corresponding triol monotrityl derivative (**8**) and the trityl aldehyde (**9**). Compound **5** was also obtained by the direct treatment of geranyl acetate (**1**) with an equimolecular amount of periodic acid in aqueous t-butanol in the presence of a catalytic quantity of potassium permanganate. The mixture was purified by silica-gel chromatography, and the acetyl aldehyde thus obtained was hydrolysed with sodium methoxide in methanol and transformed into **9** with trityl chloride in pyridine. The selective ozonolysis of geranyl acetate[4] yielded on the contrary poor results.



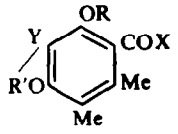
1: R = Ac
6: R = Ph_3C



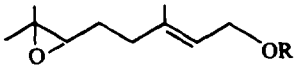
2: R = Ac; X = Br
4: R = Ac; X = OH
8: R = Ph_3C; X = OH



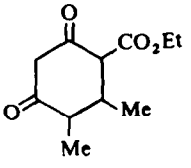
5: X = OAc; Y = CHO
9: X = $OCPh_3$; Y = CHO
10: X = $OCPh_3$; Y = CO_2H
11: X = OH; Y = CO_2H
12: X = OH; Y = CO_2CH_3
13: X = Br; Y = CO_2CH_3



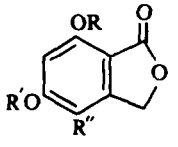
15: R = R′ = H; X = OEt; Y = Br
16: R = R′ = H; X = OEt; Y = H
17: R = R′ = Me; X = OEt; Y = H
18: R = R′ = Me; X = OH; Y = H
19: R = R′ = Me; X = Cl; Y = H
20: R = R′ = Me; X = NH_2; Y = H
21: R = R′ = Me; X = NHCl; Y = H



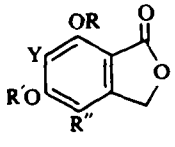
3: R = H
7: R = Ph_3C



14



22: R = R′ = R″ = Me
23: R = H; R′ = R″ = Me
24: R = R′ = H; R″ = Me
25: R = R′ = R″ = H



26: R = R′ = H; R″ = Me; Y = $H_3CO_2C(CH_2)_2C(CH_3)=CHCH_2$
27: R = H; R′ = R″ = Me; Y = $H_3CO_2C(CH_2)_2C(CH_3)=CHCH_2$
28: R = H; R′ = R″ = Me; Y = $HO_2C(CH_2)_2C(CH_3)=CHCH_2$
29: R = R′ = R″ = H; Y = $HO_2C(CH_2)_2C(CH_3)=CHCH_2$
30: R = R′ = H; R″ = Me; Y = t,t-farnesyl
31: R = R′ = R″ = H; Y = t,t-farnesyl
32: R = R′ = H; R″ = Me; Y = geranyl
33: R = R′ = R″ = H; Y = geranyl
34: R = R′ = Y = H; R″ = geranyl

Compound **9** was oxidised to the acid (**10**) by means of silver oxide in aqueous basic medium. Less satisfactory results were obtained by different oxidation procedures[5] either of aldehyde **5** or **9**. Hydrolysis of **10** in aqueous 80% acetic acid led to the oily compound (**11**) which was methylated with diazomethane in ethyl ether to give **12**. The bromide (**13**) was then obtained by allylic bromination with triphenyl phosphine and carbon tetrabromide in acetonitrile.[6]

The reaction sequence above exposed was also used for the transformation of geraniol and *trans,trans*-farnesol into the corresponding bromides.

The second key intermediate, 5,7-dihydroxy-4-methylphthalide (**24**), was obtained by the following route. Condensation between sodium diethyl malonate and 3-methylpent-3-en-2-one in ethanol afforded **14**, alternatively obtained from the reaction between sodium ethyl acetoacetate and ethyl tiglate. 1-Carbethoxy-2,3-dimethylcyclohexa-4,6-dione (**14**) was aromatised to **16** either by conversion into the bromo derivatives (**15**) and subsequent hydrogenolysis on Pd–C or by direct treatment with ferric chloride in refluxing acetic acid. Compound **16** was transformed into **17** by treatment with CH_2N_2 in methanol or with MeI and K_2CO_3 in acetone; the hydrolysis of **17** furnished the acid (**18**) which yielded the amide (**20**) by reaction of its chloride (**19**) with ammonia. Treatment of **20** with t-butylhypochlorite in CH_2Cl_2 gave the corresponding N-chloroamide (**21**) which was photolysed.[7] The intermediate

TABLE 1

Allylic bromide	Phthalide	Product	Time	Yield %
$H_3CO_2CC_2H_4C(CH_3)=CHCH_2Br$	(24)	(26)	30′	36
$H_3CO_2CC_2H_4C(CH_3)=CHCH_2Br$	(25)	(29)*	120′	36
Farnesyl bromide	(24)	(30)	30′	28
Farnesyl bromide	(25)	(31)	120′	30
Geranyl bromide	(24)	(32)	30′	34
Geranyl bromide	(25)	(33)	120′	32

* Characterized as acid.

TABLE 2

Compound	m.p.	Physical data ν_{max}/cm^{-1} ($CHCl_3$)	Analytical data
26*	107–110°	3440, 2940, 1735, 1635	C, 62·76; H, 6·00 (C, 62·74; H, 5·92)
29†	193–194°	1710, 1670, 1620‡	C, 61·58; H, 5·60 (C, 61·64; H, 5·52)
30*	98–100°	3440, 2920, 1735, 1635	C, 74·90; H, 8·41 (C, 74·97; H, 8·39)
31*	101–103°	3390, 2940, 1730, 1637	C, 74·60; H, 8·26 (C, 74·56; H, 8·16)
32*	98–100°	3440, 2930, 1735, 1635	C, 72·16; H, 7·75 (C, 72·12; H, 7·65)
33*	140–142°	3400, 2930, 1727, 1640	C, 71·53; H, 7·38 (C, 71·50; H, 7·33)

* Crystallized from MeOH–water.

† Condensation performed with methyl ester. The reaction product was characterized as acid which was crystallized from heptane–EtOAc

‡ Registered in nujol.

TABLE 3

Comp.	Solv.	$(CH_2)_n$	$CH_3C{=}CH{-}$	$-HC{=}C$	δ (ppm) Ar—H	$-OCH_3$	$ArCH_3$	$ArCH_2O$	$ArCH_2{-}CH{=}$
28	$CDCl_3$	2·38m n = 2	1·81s	5·18 cov.	—	3·76s	2·15s	5·18s	3·32; 3·43 d; *J* = 7
28	C_5D_5N	2·62m n = 2	1·95s	5·7t *J* = 7	—	3·73s	2·00s	5·08s	3·64; 3·75 d; *J* = 7
30	$CDCl_3$	2–2·16 n = 4; m	1·60s 6H 1·68s 3H 1·87s 3H	4·95–5·3 3H m	—	—	2·06s	5·18s	3·41; 3·53 d; *J* = 7
32	$CDCl_3$	2·06m *n* = 2	1·60s 3H 1·69s 3H 1·83s 3H	5·12 cov. 2H	—	—	2·06s	5·14s	3·40; 3·52 d; *J* = 7
26	$CDCl_3$	2·41m n = 2	1·85s	5·2 cov.	—	3·66s	2·07s	5·16s	3·39; 3·49 d; *J* = 6
29	C_5D_5N	2·63m n = 2	2·03s	5·89t *J* = 8	6·69s	—	—	5·08s	3·80; 3·93 d; *J* = 8
33	$CDCl_3$	2·06m n = 2	1·60s 3H 1·67s 3H 1·83s 3H	5·1–5·4 2H m	6·45s	—	—	5·2s	3·39; 3·50 d; *J* = 7
33	C_5D_5N	2·05m n = 2	1·53s 3H 1·63s 3H 2·03s 3H	5·84–6·06 2H m	6·74s	—	—	5·11s	3·86; 3·97 d; *J* = 7
31	$CDCl_3$	2·04m *n* = 4	1·58s 6H 1·66s 3H 1·82s 3H	5·02–5·38 3H m	6·44s	—	—	5·2s	3·38; 3·51 d; *J* = 7·8

iminolactone was immediately hydrolysed to **22**.[8] Treatment of the dimethoxyphthalide (**22**) with hydriodic acid in acetic acid in the presence of red phosphorus or with BBr_3 in CH_2Cl_2 yielded the corresponding dihydroxy derivative (**24**).[9] The selective hydrolysis of **22** to the 5-methoxy-7-hydroxy-4-methylphthalide (**23**) was performed by treatment with BCl_3 in CH_2Cl_2.

The critical problem of the introduction of the side-chain on the aromatic nucleus of phthalides **24** and **25** demanded a complex investigation.

Birch pointed out that attempts to introduce directly a geranyl side-chain on the compound **23** had failed.[2]

In our hands, biosynthetic evidence indicated that *in vivo*[10] C-alkylation occurred preferentially in compounds having the *m*-dihydroxybenzene structure. However attempts to alkylate the aromatic nucleus of **24** and **25** at C-6 either with the hydroxyester (**12**) or with geraniol, or with *trans,trans*-farnesol in methylene chloride in the presence of *p*-toluenesulphonic acid or in an aqueous solution of acetic acid, or using zinc chloride and boron trifloride etherate as catalyst, failed. Treatment of **25** in dioxane solution with a strong excess of geraniol in the presence of *p*-toluenesulphonic acid gave mainly C-4 alkylation. The same reaction with **24** failed. Alkylation with prenyl bromides in benzene or dimethoxyethane in the presence of sodium hydride or in methanol containing sodium methoxide gave a mixture of O-alkylated products and only traces of C-alkylated compounds. C-alkylation favouring solvents such as CF_3CH_2OH could not be used for their poor solvent power toward the phthalides.

Reaction of the allylic bromide (**13**) with the phthalide (**24**) in dioxane in the presence of silver oxide yielded a nearly equimolecular amount of the C-6 alkylated product and the 5-O-alkylated compound. On the contrary the condensation between **13** and **23** in these conditions yielded only an O-alkylation product.

For the synthesis of mycophenolic acid using condensation between **13** and **24** the dioxane/silver oxide system yielded a mixture from which the 6-alkylation product (**26**) was separated by column chromatography. Selective methylation of the C-5 OH with diazomethane in benzene or with MeI and K_2CO_3 in acetone gave mycophenolic acid methyl ester[11] (**27**), which was hydrolysed to the acid (**28**) with NaOH in methanol-water.

With the same procedure the phthalides **24** and **25**[12] were alkylated with geranyl bromide and *trans,trans*-farnesyl bromide. Coupling experiments between **13** and **25** showed an analogous course and indicated the possibility of introducing different groups at C-4 after the preparation of the whole skeleton.

This possibility together with that deriving from the C-6 alkylation of various 4-substituted phthalides, opens the way to the synthesis of unnatural C-4 substituted mycophenolic acids.

EXPERIMENTAL

Microanalyses were performed on a Perkin–Elmer 240 Elemental Analyser. IR spectra were measured with a Perkin–Elmer 257 spectrophotometer. NMR spectra were recorded with a Perkin–Elmer R10 spectrometer with TMS as internal reference. Values are reported in δ/ppm M.ps were determined on a Büchi apparatus and are uncorrected. Mass spectrometric investigations were conducted on a LKB 9000 (70 eV) GLC-mass spectrometer system.

1-Acetoxy-6-bromo-7-hydroxy-3,7-dimethyloct-2-ene (2)

A soln of **1** 10·35 g in 120 ml of t-BuOH and 20 ml water was added to 9·4 g of N-bromosuccinimide

and kept at 15–20° in the dark for 90 min. Then the mixture was poured into 500 ml water and extracted with EtOAc (3 × 200 ml). After evaporation of the collected organic extracts the residue (16·1 g) was dissolved into 60 ml EtOAc and the soln was added to 100 g silica-gel G Merck containing 15% water. The mixture thus obtained, after evaporation to dryness under vacuum, gave a residue which was added to the top of a column of 1·1 kg of the same adsorbent. Elution was then performed with the mixture heptane–EtOAc 6/4 (100 ml fractions). Fractions 4–8 eluted 11·4 g of pure **2**, yellow viscous oil, b.p. 180° at 5 mm; ν_{max}(CHCl$_3$) 1740, 1670, 1240 cm^{-1}. (Found: C, 49·21; H, 7·48. $C_{12}H_{21}O_3Br$ requires: C, 49·30; H, 7·21%).

1-*Hydroxy*-6,7-*epoxy*-3,7-*dimethyloct*-2-*ene* (3)

A soln of **2** (13 g) in 350 ml MeOH was added to a soln of 19·5 g of K_2CO_3 in 55 ml water. After 2 hr stirring at room temp the resulting mixture was filtered and the filtrate evaporated to a small volume under vacuum. Diluting with 100 ml water, extraction with chloroform (3 × 100 ml), evaporation of the collected and dried (Na_2SO_4) organic extracts gave **3**, b.p. 125° at 5 mm; ν_{max} (CHCl$_3$) 3400, 1670 cm^{-1}. (Found: C, 70·68; H, 10·64. $C_{10}H_{18}O_2$ requires C, 70·54; H, 10·66%).

1-*Acetoxy*-6,7-*epoxy*-3,7-*dimethyloct*-2-*ene* (3; R = Ac)

A soln of **3** (8·68 g) in 9 ml pyridine was added to 18 ml Ac_2O and left at room temp overnight. Evaporation to dryness under vacuum gave **3** (R = Ac), b.p. 135° at 4 mm Hg; ν_{max} (CHCl$_3$) 1740, 1670, 1245 cm^{-1}. (Found: C, 67·70; H, 9·36. $C_{12}H_{20}O_3$ requires: C, 67·89; H, 9·50%).

1-*Trityloxy*-6,7-*epoxy*-3,7-*dimethyloct*-2-*ene* (7)

(a) A soln of **3** (10 g) in 100 ml pyridine was added to 15·5 g trityl chloride and left at room temp for 24 hr. The mixture was then poured into 1 kg ice and extracted with chloroform (3 × 200 ml). The organic extracts were washed with 100 ml sat $NaHCO_3$ aq, then with water (100 ml), dried over Na_2SO_4 and evaporated to dryness under vacuum. The obtained residue had b.p. 171–175° at 0·2 mm (18·5 g); δ 1·25 (s, 6H, CH_3C), 1·48 (s, 3H, CH_3C), 3·61 (cov., 1H, —CHO—), 3·5 and 3·61 (d, 2H, J = 6·6, —CH_2O—), 5·48 (t, 1H, J = 6·6, =CH—). (Found: C, 84·52; H, 7·93. $C_{29}H_{32}O_2$ requires: C, 84·42; H, 7·82%).

(b) A soln of 22·5 g trityl geraniol in 250 ml t-BuOH was added to a soln of 12·5 g N-bromosuccinimide in 32 ml water. The resulting soln was kept under stirring at 15° in the dark for 90 min, then poured into 500 ml water and extracted with chloroform (3 × 250 ml). The organic extracts were then evaporated to dryness and the residue extracted 3 times with hexane (100 ml portions). Evaporation of the filtrate yielded crude **8** (R = Ph_3C, X = Br) which was dissolved into 400 ml MeOH and added to 19 g K_2CO_3 in 60 ml water. After a further 2 hr at room temp under stirring, the suspension was concentrated to a small volume and added to water until complete solubility, then extracted with CH_2Cl_2. The collected organic extracts were washed with water until neutral, dried over Na_2SO_4 and evaporated to dryness under vacuum. The residue thus obtained (23·4 g) was chromatographed over silica-gel 0·05–0·2 mm (234 g) eluting with benzene (1500 ml), benzene–EtOAc 1/1 (1500 ml) and EtOAc (2000 ml). The fractions eluted with the last solvent contained pure **7** (12·5 g).

1-*Acetoxy*-6,7-*dihydroxy*-3,7-*dimethyloct*-2-*ene* (4)

A soln of **3** (R = Ac) (19 g) in 190 ml diglyme was added to 4 ml 70% perchloric acid in 50 ml water and kept at room temp under stirring over a period of 2 hr. Neutralisation with Amberlite IR 4B resin and evaporation under vacuum of the solvent gave a residue which was chromatographed over silica-gel G Merck containing 15% of water (420 g) eluting with heptane EtOAc 1/1 (300 ml) and EtOAc. The latter eluent gave 12·5 g of pure **4**, b.p. 155° at 5 mm. (Found: C, 62·42; H, 9·75. $C_{12}H_{22}O_4$ requires: C, 62·58; H, 9·63%).

1-*Trityloxy*-6,7-*dihydroxy*-3,7-*dimethyloct*-2-*ene* (8)

A soln of **7** (14 g) in 150 ml diglyme was added to 2·8 ml 70% perchloric acid in 35 ml water. Working-up and chromatographic purification as descrived above gave 9·2 g of **8**, b.p. 235–238° at 0·2 mm Hg. (Found: C, 80·91; H, 8·01. $C_{29}H_{34}O_3$ requires: C, 80·89; H, 7·96%).

6-*Trityloxy*-4-*methylhex*-4-*enal* (9)

(a) A soln of **8** (9·2 g) in 60 ml THF was added to 5·5 g sodium periodate in 20 ml water. After 60 min reaction in the dark at room temp, the resulting suspension was filtered and the filtrate extracted 3 times

with chloroform (100 ml portions). Evaporation to dryness under vacuum of the collected organic extracts yielded 7 g of **9**, which, crystallized from hexane-ethyl ether, had m.p. 90–92°, ν_{max} ($CHCl_3$) 1720 cm^{-1}. (Found: C, 84·36; H, 7·10. $C_{23}H_{26}O_3$ requires: C, 84·29; H, 7·07%).

(b) A soln of **4** (2 g) in 5 ml MeOH was added to 0·4 g NaOH in 5 ml water. After 12 hr reaction at room temp the resulting soln was diluted with 25 ml MeOH and treated with Amberlite IR 4B resin until neutral. Filtration and evaporation to dryness under vacuum yielded a residue which was dissolved in 13 ml THF, and 2·18 g sodium periodate in 8 ml water was added and left at room temp for 1 hr. Work-up as in (a) gave 1·04 g of the oily hydroxyaldehyde **5** (X = OH). Compound **5** (X = OH) dissolved in 10 ml pyridine, was added to 2·17 g trityl chloride and left overnight at room temp. Pouring the soln in 20 g ice, extraction with chloroform (3 × 20 ml), washing of the organic extracts with water until neutral and evaporation to dryness under vacuum yielded a residue which was chromatographed over silica-gel 0·05–0·2 mm Merck (R = 40) eluting with benzene. The fractions which were shown to contain pure compound **9** by TLC were evaporated to give 0·8 g of this product.

6-Acetoxy-4-methylhex-4-enal (**5**)

(a) A soln of **4** (800 mg) in 10 ml dioxane was added to 1·34 g sodium periodate in 15 ml water. After 1 hr stirring at room temp in the dark the resulting suspension was filtered and the filtrate concentrated under vacuum to 5 ml. Dilution with 20 ml chloroform, washing with 10 ml water, drying over Na_2SO_4 and evaporation to dryness under vacuum yielded **5**, 0·55 g, b.p. 85° at 1 mm Hg; ν_{max} ($CHCl_3$) 1725 cm^{-1}. (Found: C, 63·40; H, 8·39. $C_9H_{14}O_3$ requires: C, 63·51; H, 8·29%).

(b) A soln of **1** (5 g) in 40 ml t-BuOH was added to 0·1 g $KMnO_4$ in 10 ml water and subsequently to 6 g periodic acid in 10 ml water. After 27 hr reaction at room temp, extraction with diethyl ether (5 × 100 ml), washing the organic extracts with water until neutral and evaporation to dryness under vacuum yielded a residue (3·34 g) which was chromatographed over silica-gel 0·05–0·2 mm Merck (150 g) eluting with benzene (50 ml fractions). Fractions 3–8 contained 1·4 g of **5**.

6-Trityloxy-4-methylhex-4-enoic acid (**10**)

A soln of **9** (0·5 g) in 10 ml of the mixture dioxane-water 1/1 was added under stirring at room temp to 0·46 g $AgNO_3$ in 2 ml water followed by 0·76 g KOH in 2 ml water. After 90 min reaction under the same conditions the resulting suspension was filtered over celite and this was washed with water and then with EtOAc. The filtrate and the washings were collected, acidified to pH 5 with N H_2SO_4 and extracted with EtOAc (3 × 50 ml). Evaporation of the organic extracts to dryness under vacuum gave a residue which was chromatographed over silica-gel 0·05–0·2 mm Merck eluting with the mixture $CHCl_3$/EtOAc/AcOH 55/45/1 (2 ml fractions). Fractions 1–9 contained 200 mg of **5**; fractions 10–24, 250 mg of **10** which, crystallized from MeOH–water had m.p. 135–136°; δ (ppm) ($CDCl_3$) 1·5 (s, 3H, CH_3—C=), 2·43 (m, 4H, —C_2H_4—), 3·60 and 3·70 (d, 2H, J = 6, —CH_2—CH=), 5·47 (t, 1H, J = 6, =CH—). (Found: C, 80·65; H, 6·64. $C_{23}H_{26}O_3$ requires: C, 80·80; H, 6·78%).

6-Hydroxy-4-methylhex-4-enoic acid (**11**)

A soln of **10** (1·037 g) in 120 ml aqueous 80% AcOH was left overnight at room temp. Evaporation under vacuum gave a residue which was dissolved in 30 ml AcOH and 120 ml water and kept at room temp for further 30 min, then filtered from the precipitated triphenyl carbinol which was washed with 5 ml water. Evaporation under vacuum to dryness of the filtrate and the washing afforded 377 mg of **11**, b.p. 85–90° at 0·1 mm; ν_{max} ($CHCl_3$) 3330, 1710 cm^{-1}; δ (ppm) 1·7 (s, 3H, CH_3—C), 2·4 (m, 4H, —C_2H_4—), 4·2 (m, 2H, —CH_2OH), 5·4 (m, 1H, CH_3—C=CH—). (Found: C, 58·45; H, 8·26. $C_7H_{12}O_3$ requires: C, 58·31; H, 8·39%).

Methyl 6-hydroxy-4-methylhex-4-enoate (**12**)

A soln of **11** (233 mg) in 5 ml chloroform was added, at 0°, to an excess of an ethereal soln of diazomethane. Evaporation to dryness under vacuum and distillation under 0·1 mm Hg vacuum gave **12**, b.p. 80–82°; ν_{max} (nujol) 3400, 1725 cm^{-1}, δ (ppm) ($CDCl_3$) 1·7 (s, 3H, CH_3—C=), 2·4 (m, 4H, —C_2H_4—), 3·7 (s, 3H, —OCH_3), 4·1 and 4·22 (d, 2H, J = 7, =CHC$\underline{H}_2$—), 5·4 (t, 1H, J = 7, =C$\underline{H}$$CH_2$—). (Found: C, 60·84; H, 8·97. $C_8H_{14}O_3$ requires: C, 60·74; H, 8·92).

Methyl 6-bromo-4-methylhex-4-enoate (**13**)

A soln of **12** (0·245 g) in 4 ml acetonitrile was added to 0·565 g CBr_4 followed by 0·45 g triphenylphosphine. After 150 min under stirring at room temp in the dark the mixture was added to 50 ml hexane. The hexane phase thus separated was evaporated to dryness under vacuum and the residue, dissolved in 5 ml hexane and filtered from some insoluble material, was chromatographed over silica-gel G Merck containing 15% water eluting with heptane–EtOAc 7/3 (20 ml fractions). Fractions 3–9 contained 0·29 g of **13**, b.p. 90–92° at 0·1 mm Hg; ν_{max} ($CHCl_3$) 1725 cm^{-1}; δ (ppm) ($CDCl_3$) 1·75 (s, 3H, CH_3—C=), 2·41 (m, 4H, —C_2H_4—), 3·67 (s, 3H, —OCH_3), 3·90 and 4·04 (d, 2H, J = 8·4, —CH_2Br), 5·54 (t, 1H, J = 8·4, =C<u>H</u>CH_2Br). (Found: C, 43·42; H, 5·98. $C_8H_{13}O_2Br$ requires: C, 43·45; H, 5·92%).

1-*Carbethoxy*-2,3-*dimethylcyclohexan*-4,6-*dione* (**14**)

(a) A soln prepared by dissolving 0·549 g Na in 8·1 ml abs EtOH was added to 4·125 g diethyl malonate and, after 20 min, to 2·295 g 3-methylpent-3-en-2-one during a period of 45 min. The resulting mixture was refluxed under stirring for 2 hr, then cooled with ice, acidified with dilute H_2SO_4 (indicator Congo red) and extracted with diethyl ether (3 × 30 ml). The collected organic extracts were washed with water until neutral, dried over Na_2SO_4 and evaporated under vacuum to dryness to give a residue which was dissolved in 15 ml sat $NaHCO_3$ aq. The neutral products were extracted with diethyl ether (80 ml) and the aqueous phase, acidified with dil H_2SO_4 and extracted with diethyl ether (3 × 30 ml) gave **14**, 3·4 g, as a viscous yellow oil, which had ν_{max} ($CHCl_3$) 1735, 1720, 1650 and 1560 cm^{-1}.

(b) The reaction between diethyl malonate and ethyl tiglate was conducted with the same molar ratio and the same work-up procedure. The reaction time was 5 hr and **14** was obtained in 50% yield.

Ethyl 5-*bromo*-4,6-*dihydroxy*-2,3-*dimethylbenzoate* (**16**)

A soln of Br_2 (3·39 g) in AcOH (3 ml) was added under stirring at −5° to 2 g of **14** in 5 ml AcOH. After further 12 hr at room temp, the ppt obtained was filtered and crystallized from AcOH–water, to yield 2·9 g of **15**, m.p. 67–69°. (Found: C, 45·82; H, 4·58. $C_{11}H_{13}O_4Br$ requires: C, 45·69; H, 4·53%).

Ethyl 4,6-*dihydroxy*-2,3-*dimethylbenzoate* (**16**)

(a) A soln of **15** (2 g) in 9 ml 2 N NaOH was hydrogenated at ambient temp and pressure in the presence of 300 mg 10% Pd-C. After 4 hr, the catalyst was filtered off and the filtrate acidified to pH 4 with conc HCl. The resulting mixture was extracted with EtOAc (3 × 100 ml) and the collected organic extracts evaporated to dryness under vacuum to yield **16**, 1·9 g which, crystallized from EtOH–water, had m.p. 115–117°. (Found: C, 62·79; H, 6·75. $C_{11}H_{14}O_4$ requires: C, 62·84; H, 6·71%).

(b) A soln of **14** (27 g) in 270 ml aqueous 20% AcOH was added to 54 g sublimed $FeCl_3$. After 120 min at reflux temp, the soln was cooled and added, at room temp, to 300 ml water. Extraction with diethyl ether (5 × 250 ml), washing with water of the collected organic extracts, drying over Na_2SO_4 and evaporation under vacuum to dryness gave a residue which was crystallized from EtOH–water to yield 11 g of **16**.

Ethyl 4,6-*dimethoxy*-2,3-*dimethylbenzoate* (**17**)

(a) A soln of **16** (4 g) in 80 ml anhyd acetone was added to 12 g K_2CO_3 and 8 ml MeI in 3 portions and refluxed under vigorous stirring for 18 hr. The resulting suspension was then cooled and filtered, the ppt washed with 25 ml acetone and the collected filtrate and washing evaporated under vacuum to dryness. The residue was then dissolved in 50 ml water and extracted 3 times with diethyl ether (100 ml portions). The collected organic extracts were dried over Na_2SO_4, evaporated to dryness and the residue crystallized from EtOH–water yielding 3·6 g of **17**, m.p. 62–63°; ν_{max} ($CHCl_3$) 1720, 1595 cm^{-1}. (Found: C, 65·73; H, 7·43. $C_{13}H_{18}O_4$ requires: C, 65·53; H, 7·61%).

(b) A soln of **16** (4 g) in 100 ml MeOH was added at 0° to an excess of an ethereal soln of diazomethane, then left under the same conditions for 8 days. Evaporation to dryness under vacuum and crystallisation of the residue from EtOH–water yielded 3·8 g of **17**.

4,6-*Dimethoxy*-2,3-*dimethylbenzoic acid* (**18**)

A soln of **17** (4 g) in 100 ml aqueous EtOH 75% was added to 10 ml 10% NaOH aq and left under N_2 at room temp for 18 hr. The resulting soln was then acidified with conc HCl and the ppt obtained (3·5 g) was filtered and washed with water until neutral. Crystallization from EtOH–water gave **18**, m.p. 208–210°; ν_{max} ($CHCl_3$) 3480, 3140, 1725, 1700, 1595 cm^{-1}. (Found: C, 62·64; H, 6·87. $C_{11}H_{14}O_4$ requires: C, 62·84; H, 6·71%).

4,6-Dimethoxy-2,3-dimethylbenzamide (**20**)

A soln of **18** g (1 g) in 10 ml $SOCl_2$ was refluxed for 3 hr and then left at room temp for 12 hr. Evaporation of the solvent under vacuum gave an oily residue which was dissolved in 5 ml anhyd benzene and evaporated to dryness under vacuum. This operation was repeated until no trace of $SOCl_2$ remained. At the end the residue constituted of **19** was dissolved in 30 ml anhyd benzene and poured into a soln of gaseous ammonia in 100 ml benzene. Gaseous ammonia was then bubbled into the soln for further 8 hr; the resulting ppt was filtered, washed with 10 ml water and crystallized from EtOH yielding 0·84 g of **20**, m.p. 225–229°; ν_{max} (nujol) 3405, 3175, 1650, 1595 cm^{-1}; δ (ppm) (DMSO) 2·0 and 2·1 (s, 3H, CH_3Ar), 3·74 and 3·79 (s, 3H, CH_3O—), 6·47 (s, 1H, H—Ar), 7·16 and 7·35 (b.s., 1H, —NH_2). (Found: C, 63·09; H, 7·63; N, 6·71. $C_{11}H_{15}NO_3$ requires: C, 63·14; H, 7·23; N, 6·69%).

5,7-Dimethoxy-4-methylphthalide (**22**)

A soln of **20** (1 g) in 150 ml CH_2Cl_2 was added to 0·503 g t-butyl hypochlorite and left for 20 min at room temp. Photolysis of the resulting soln in a quartz vessel with a Philips HPK 125 W high pressure mercury discharge lamp for 20 hr, and evaporation of the solvent under vacuum gave a residue which was dissolved in 160 ml EtOH–water 1/1 containing 1 g KOH. This soln was refluxed on a water bath for 1 hr, then cooled, acidified with dil HCl, extracted with EtOAc (3 × 100 ml) and the collected organic extracts evaporated to dryness under vacuum. The resulting residue was chromatographed over silica-gel 0·05–0·2 mm Merck (100 g) eluting with benzene–EtOAc 9/1. The fractions containing **22** were collected and crystallized from heptane–EtOAc yielding 0·34 g of the pure compound, m.p. 202–203°; ν_{max} ($CHCl_3$) 1735, 1610 cm^{-1}. (Found: C, 63·51; H, 5·61. $C_{11}H_{12}O_4$ requires: C, 63·45; H, 5·80%).

7-Hydroxy-5-methoxy-4-methylphthalide (**23**)

A soln of **22** (1 g) in 300 ml CH_2Cl_2 was cooled at −10° and added with stirring to 25 ml BCl_3 precooled at −60°. The resulting mixture was left at room temp for 10 days. Decomposition with water, separation of the organic layer, washing with water, drying over Na_2SO_4 and evaporation to dryness afforded a residue which was crystallized from MeOH to yield 0·81 g of **23**, m.p. 216–218°; ν_{max} ($CHCl_3$) 3450, 1730, 1635 cm^{-1}. (Found: C, 61·96; H, 5·20. $C_{10}H_{10}O_4$ requires: C, 61·90; H, 5·20%).

5,7-Dihydroxy-4-methylphthalide (**24**)

(a) A soln of **22** (1 g) in 150 ml CH_2Cl_2 was cooled at −10° and added with stirring to 6 ml BBr_3. After 8 days at room temp, work-up as for **23** yielded, after crystallization from MeOH–water, 0·61 g of **24**, m.p. 252–254°; ν_{max} ($CHCl_3$) 3420, 3330, 1725, 1630 cm^{-1}; δ (ppm) 2·17 (s, 3H, CH_3Ar), 5·17 (s, 3H, $ArCH_2O$—), 6·96 (s, 1H, ArH). (Found: C, 60·15; H, 4·71. $C_9H_8O_4$ requires: C, 60·00; H, 4·48%).

(b) A soln of **22** (0·5 g) in 2·9 ml Ac_2O and 2·9 ml 57% HI was refluxed in presence of 1·2 g of red P at a bath temp of 150° under stirring for 12 hr. The mixture was then cooled and filtered, washing the ppt with 5 ml 50% aqueous AcOH. The filtrate and washing were evaporated under vacuum to dryness to give a residue which was dissolved in 8 ml water and adjusted to pH 4 with 5% aqueous ammonia. The ppt thus obtained was filtered and the mother liquor extracted with EtOAc (3 × 25 ml). The solid and the extraction residue were then crystallized from MeOH–water to obtain 0·21 g of **24**, m.p. 242–254°.

6-Prenylation of **24** *and* **25**

A soln of 1·3 mmoles allylic bromide in 20 ml dioxane was added to 1 mmole phthalide and 1·5 mmoles Ag_2O and allowed to react under stirring at room temp. Filtering off the solid and evaporation of the filtrate under vacuum yielded a residue which was chromatographed over silica-gel 0·05–0·2 mm Merck (60 g) eluting with hexane–EtOAc 4/1. Fractions obtained (15 ml each) were investigated by TLC (eluent heptane-EtOAc 7/3) and showed that C-alkylation products were eluted as first. Further fractions separated O-alkyl derivatives and subsequently traces of starting material. In the Table 1 are reported time and yield of each reaction. In Tables 2 and 3 are quoted the physical and NMR data.

Hydrolysis of the methyl ester (**26**)

A soln of 1·2 mmoles of **26** in 20 ml EtOH–water 2/1 was added to 2 ml 1 N NaOH and after 12 hr at room temp it was acidified with AcOH, evaporated to dryness under vacuum and extracted with EtOAc. Nor-O-methyl mycophenolic acid thus obtained had m.p. 147–149° (heptane–EtOAc). (Found: C, 62·65;

H, 5·98. $C_{16}H_{18}O_6$ requires: C, 62·74; H, 5·92%). Compound **29** similarly prepared in 90% yield had m.p. 193–194°.

Mycophenolic acid methyl ester (**27**)

(a) A soln of **26** (0·5 g) in 15 ml acetone was added to 2 ml MeI and 2 g anhyd K_2CO_3 and refluxed with stirring until TLC ($CHCl_3$/AcOEt/AcOH 55/45/1) showed that a significant amount of the desired product had been formed (24–36 hr). The mixture was filtered and the solid residue acidified with 2 N HCl and extracted 3 times with EtOAc. The washed (H_2O) and dried (Na_2SO_4) EtOAc soln was evaporated to dryness under vacuum and the residual solid subjected to column chromatography on 0·5 kg silica-gel 0·05–0·2 mm Merck eluting with $CHCl_3$/AcOEt/AcOH 55/45/1). **27** thus obtained (205 mg), crystallized from EtOH–water had m.p. 104–105°. (Found: C, 64·80; H, 6·76. $C_{18}H_{22}O_6$ requires: C, 64·7; H, 6·6%).

(b) Ethereal diazomethane (1 eq) was added to a soln of **26** in C_6H_6 and the mixture was left at room temp (2 hr) until TLC showed that a significant amount of the desired product had been formed. After evaporation, the residue was worked-up as under (a). **27** was obtained in 25% yield.

6-Geranyl- and 4-geranyl-5,7-dihydroxyphthalide (**33**)

A soln of **25** (100 mg) in 2 ml dioxane was added to 100 mg *p*-toluenesulphonic acid and 2 ml geraniol and refluxed for 48 hr. Evaporation of the solvent under vacuum gave a residue which was dissolved in warm 2 N NaOH. After cooling the soln and extraction with diethyl ether, the resulting soln was acidified with dil H_2SO_4 and extracted with EtOAc. The crude product thus obtained (0·215 mg) was dispersed in 1·2 g silica-gel G Merck and added to the top of a column of 22·2 g silica-gel 0·05–0·2 mm Merck which was eluted with $CHCl_3$–EtOAc (9/1; 8/2; 1/1). The first and the second solvent mixture gave 12 mg of **33**. Subsequently, 37 mg of **34** was eluted with the second and the third elution system. Amorphous compound **34** had δ in $CDCl_3$ at 1·6 (s, 3H, CH_3C), 1·67 (s, 3H, CH_3C), 1·74 (s, 3H, CH_3C), 3·19 and 3·32 (d, 2H, J = 7·8, $=CHCH_2Ar$), 5·1–5·3 (m, 2H, $=CH-$), 5·18 (s, 2H, $ArCH_2O-$), 6·44 (s, 1H, ArH) and in C_5D_5N δ 1·56 (s, 3H, CH_3C), 1·65 (s, 3H, CH_3C), 1·81 (s, 3H, CH_3C), 3·46 and 3·59 (d, 2H, J = 7·8, $=CH-$), 5·1–5·4 (m, 2H, $=CH-$), 5·25 (s, 2H, $ArCH_2O-$), 6·82 (s, 1H, ArH).

Mycophenolic acid (**28**)

Hydrolysis of **27** to mycophenolic acid was performed as described for **26** yielding **28** in 90% yield.

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