6-Farnesyl-5,7-dihydroxy-4-methylphthalide Oxidation Mechanism in Mycophenolic Acid Biosynthesis

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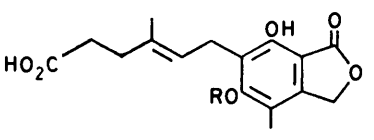
The farnesyl-chain oxidation-mechanism in mycophenolic acid biosynthesis has been studied by incorporation experiments and isotopic-trap experiments with advanced precursors. Two different approaches were used for the synthesis of the precursors: a semisynthetic route, starting from mycophenolic acid, and a total-synthesis route. Our results show that 6-farnesyl-5,7-dihydroxy-4-methylphthalide (3) is converted into mycophenolic acid (1) by at least two pathways: a direct oxidation of the central double bond, and a two-stage removal of the terminal and central groups.

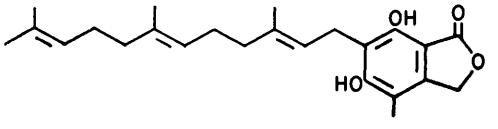
The incorporation percentages indicate that the two pathways are equally important. As regards the oxidation mechanism, our data suggest the sequence: epoxidation of the double bond, rearrangement of the epoxide to ketone, hydroxylation to α-hydroxyketone, and finally Woodward reaction with C–C bond cleavage and formation of the acid.

The last three biosynthetic steps leading to mycophenolic acid (1) in *P. brevicompactum* (prenylation of the aromatic nucleus, oxidation of the farnesyl chain, and *O*-methylation) are characterized by a very low enzyme specificity.

THE biosynthesis of mycophenolic acid (1), a metabolite of *Penicillium brevicompactum*, has been the subject of considerable investigation.[1,2] Current interest in the compound is centred primarily on its potential use in chemotherapy.[3] Previous research had established the origin of every carbon atom and the sequence of reactions and intermediates involved in the biosynthesis of this important mould metabolite. At the outset of our investigations little was known of the last three biosynthetic steps leading to mycophenolic acid (1) in *P. brevicompactum*. Recently, a considerable effort has been made by different research groups to elucidate the mechanism of nor-*O*-methylmycophenolic acid (2) methylation,[4] of the prenylation of the aromatic nucleus,[5,6] and of the oxidative demolition of the farnesyl chain.[6-14]



(1) R = Me
(2) R = H



(3)

6-Farnesyl-5,7-dihydroxy-4-methylphthalide (3) is converted into mycophenolic acid by at least two pathways: a direct oxidation of the central double bond,[6,13,14] and a two-stage removal of the terminal central groups.[8-12] Here we discuss the relative importance of each and present some details of the oxidation mechanism.

RESULTS

6-Farnesyl-5,7-dihydroxy-4-methylphthalide (3), nor-*O*-methylmycophenolic acid (2), and the prenylogue of the acid (1), compound (11a), were isolated from cultures of *P. brevicompactum*.[1,11]

Cell-free extracts of *P. brevicompactum* convert the phthalide (3) into the acyloin (6).[6] Whole cells of fungus convert the compounds (2), (3), (6), and (11a) into mycophenolic acid.[1,6,11] ^{14}C-Labelled-precursor feeding experiments were performed with the ketones (4) and (8), the hydroxy-ketones (6) and (9), and with the epoxide (10) (Table 1).

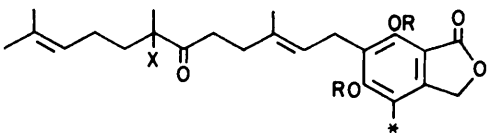
TABLE 1

Incorporation experiments into mycophenolic acid (1)

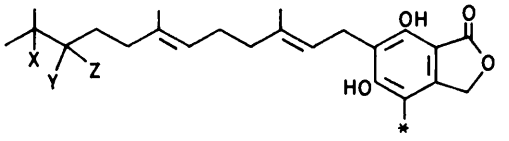
Expt.	Compd.	Total incorporation (%)	Molar incorporation (%)	Transformation (h)
1	(4)	8.30	12.82	72
2	(4)	2.16	3.31	18
3	(6)	17.39	21.59	72
4	(6)	2.05	2.72	18
5	(8)	4.69	1.25	72
6	(8)	0.62	2.37	18
7	(9)	5.11	1.03	72
8	(9)	0.68	0.59	18
9	(10)	4.40	1.30	72
10	(10)	0.60	0.96	18

Ozonolysis of mycophenolic acid (1), obtained by feeding all the labelled precursors, was performed in order to test the incorporation specificity.[1] This removal of the side chain afforded the aldehyde (12) [15] with a molar activity the same as that of the labelled acid (1)

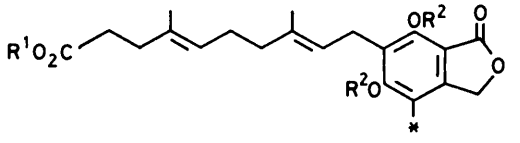
The terminal ketone (8), the hydroxy-ketone (9), and the epoxide (10) were also incorporated into the prenylogue of the acid (1) (Table 2). In these experiments, isolation and purification of prenylogous acid was facilitated by diazomethane treatment of the crude extracts, and by dilution with unlabelled (11c) obtained by total synthesis.



(4) R=H, X=H
(5) R=H, X=OOH
(6) R=H, X=OH
(7) R=Ac, X=H

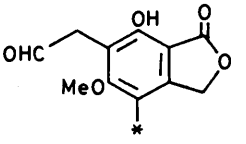


(8) X=H, Y,Z=O
(9) X=OH, Y,Z=O
(10) X,Y=O, Z=H



(11)
a; $R^1=R^2=H$
b; $R^1=Me, R^2=H$
c; $R^1=R^2=Me$

* = ^{14}C



(12)

'Isotopic-trap' experiments were performed with the central ketone (4) and the hydroxy-ketone (6), and with compound (3) as the labelled precursor. In a typical experiment, the unlabelled compounds (4) or (6) (300 mg)

TABLE 2

Incorporation experiments into the prenylogue of mycophenolic acid (1)

Expt.	Compd.	Total incorporation (%)	Molar incorporation (%)	Transformation (h)
11	(8)	0.31	6.97	18
12	(9)	0.16	5.95	18
13	(10)	0.92	43.8	18

together with the ^{14}C-labelled compound (3), were added to a 1-d culture broth of *P. brevicompactum*. After a further 12 or 24 h, the fermentation was interrupted and the compounds were isolated (Table 3). All the compounds were purified to constant activity by crystallization; in the case of the ketone (4) and the hydroxy-ketone (6) (Experiments 14, 15, and 16), reduction of compound (4) to the alcohol (13a) and of compound (6) to the diol (13b) permitted a final control.

TABLE 3

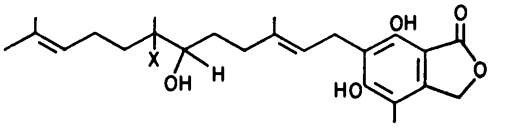
Isotropic-trap experiments

Expt.	Compd.	Isolated (mg)	Recovery of label (%)	Transformation (h)
14	(4)	205	2.5	24
	(1)	27	12.0	
15	(6)	192	0.3	24
	(1)	14	3.0	
16	(6)	208	0.2	12
	(1)	20	2.3	

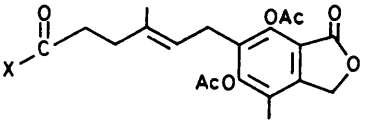
Synthesis of Precursors

Two different approaches were used for the synthesis of the precursors. The central ketone (4) and the hydroxy-ketone (6) were obtained by the semisynthetic route, starting from mycophenolic acid. The acid (1) was demethylated to compound (2) (LiI; collidine; 70% yield),[16] acetylated to compound (14) (87% yield),[17] and then treated with thionyl chloride at room temperature to give compound (15).

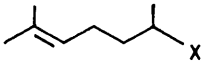
The acyl chloride (15) was treated with the organocopper compound (16) in diethyl ether–tetrahydrofuran (THF)



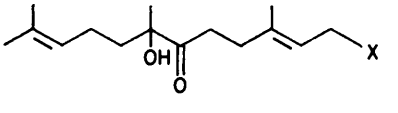
(13)
a; X=H
b; X=OH



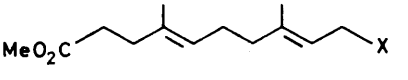
(14) X=OH
(15) X=Cl



(16) X=CuMgBrI
(17) X=Br
(18) X=OMs
(19) X=OH



(20) X=Br
(21) X=OH



(22) X=Br
(23) X=OH

(7 : 1) at −80 °C to −40 °C, to give the ketone (7) in 70% yield. Addition of the acyl chloride (15) in diethyl ether–THF (4 : 1) to a mixture of the Grignard reagent and cuprous iodide in diethyl ether at −80 °C proved to be the best method.[18] Attempts to improve the yields by using an *S*-phenyl thioester instead of acyl chloride were not satisfactory.[19] Mild, alkaline hydrolysis of the acetyl groups under nitrogen gave the ketone (4) (90% yield). Saponification under more drastic conditions in the presence of air gave the ketone (4) in lower yield, and the acid (2) as a by-product, probably *via* the intermediate α-keto-hydroperoxide (5).[20]

In the fermentation medium, without the micro-organism (pH 8.5), the ketone (4) was stable over a long period. The ketone (4), by treatment with Bu^tONa and O_2 [21] in Bu^tOH–dimethoxyethane (DME) (1 : 3) at −20° to −25 °C, led to the α-keto-hydroperoxide (5), which was reduced *in situ* with $(EtO)_3P$ to the α-hydroxy-ketone (6) in 80% overall yield. Reduction of compound (6) with $NaBH_4$ in ethanol gave a mixture of the two diastereoisomeric diols (13b) in quantitative yield. Treatment with tosyl chloride in dry pyridine gave two stereoisomeric tritosylates, but attempts to close the central epoxide with potassium hydroxide in ethanol were unsuccessful.

In the total synthesis route, reaction of the allylic bromides (20), (22), (24), (26), and (28) with the phthalide (30) in dioxan-water (24 : 1) in the presence of Ag_2O [22] gave a nearly equimolecular amount of the *C*-6-alkylated product and the 5-*O*-alkylated compound (Table 4). The allylic bromide (20) had been obtained [6] by reaction of the acyloin (21), obtained by total synthesis,[23] with CBr_4 and PPh_3 in acetonitrile,[24] and used without purification.

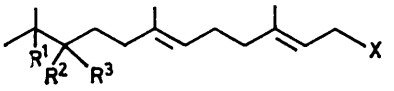
TABLE 4

Coupling reactions between the phthalide (30) and allylic bromides

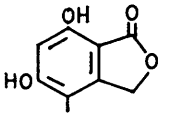
Allylic bromide	Product	Time (min)	Yield (%)
(20)	(6)	30	13
(22)	(11b)	30	20
(24)	(8)	30	15
(26)	(9)	30	13
(28)	(10)	30	16

The allylic bromides (22), (24), and (26) were obtained from the corresponding alcohols (23), (25), and (27) by bromination using the above mentioned method. In contrast, CBr_4–PPh_3 [24] and mesyl chloride–lithium chloride [25] did not convert the epoxy-alcohol (29) into the allylic halide. Bromination of compound (29) was effectively carried out using *N*-bromosuccinimide (NBS) and methyl sulphide.[26]

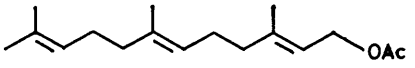
The allylic alcohols (23), (25), (27), and (29) were synthesized starting from (*E*,*E*)-farnesol by the following sequence:



(24) X = Br, R^1 = H, R^2, R^3 = O
(25) X = OH, R^1 = H, R^2, R^3 = O
(26) X = Br, R^1 = OH, R^2, R^3 = O
(27) X = OH, R^1 = OH, R^2, R^3 = O
(28) X = Br, R^1, R^2 = O, R^3 = H
(29) X = OH, R^1, R^2 = O, R^3 = H
(32) X = OAc, R^1 = OH, R^2 = Br, R^3 = H
(33) X = OAc, R^1, R^2 = O, R^3 = H
(34) X = OAc, R^1 = H, R^2, R^3 = O
(35) X = OAc, R^1 = OH, R^2 = OH, R^3 = H
(36) X = OAc, R^1 = OH, R^2, R^3 = O



(30)



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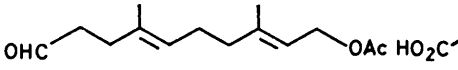

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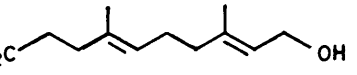

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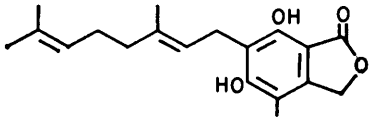

(39)

acetylation to compound (31) (Ac_2O; pyridine), oxidation to the bromohydrin (32) (NBS; Bu^tOH–H_2O),[27] and alkaline treatment to the epoxy-alcohol (29) (K_2CO_3; MeOH–H_2O). Acetylation of compound (29) (Ac_2O; pyridine) gave compound (33), which was treated with catalytic amounts of potassium hydrogensulphate and lithium perchlorate [28] in refluxing benzene to give a smooth rearrangement to the keto-acetate (34) in 60% yield.

Other Lewis acids gave lower yields (*e.g.* magnesium bromide–diethyl ether,[29] 20% yield). Alkaline hydrolysis of compound (34) (K_2CO_3; MeOH–H_2O) gave the keto-alcohol (25). Acid hydrolysis of the epoxide (33) ($HClO_4$; diglyme–H_2O) gave the diol (35) which was oxidised to form the hydroxy-ketone (36) (*N*-chlorosuccinimide; methyl sulphide; Et_3N; toluene).[30] Alkaline hydrolysis of compound (36) (K_2CO_3, MeOH–H_2O) gave the hydroxy-ketone (27). $NaIO_4$ oxidation of the diol (35) gave the aldehyde (37), which was, in turn, oxidised to form the acid (38) (Ag_2O, NaOH, dioxan–H_2O). Methylation of compound (38) gave the hydroxy-ester (23) (CH_2N_2, Et_2O).

DISCUSSION

^{14}C-Labelled-precursor feeding experiments and isotopic-trap experiments (Tables 1, 2, and 3) show, unambiguously, that 6-farnesyl-5,7-dihydroxy-4-methylphthalide (3) is converted into mycophenolic acid (1) by at least two pathways: a direct oxidation of the central double bond, and a two-stage removal of the terminal and central units.

Compounds (4) and (6), functionalized at the central double bond, were converted effectively into the acid (1). (Experiments 1—4), and were recovered, labelled, in the isotopic-trap experiments (Experiments 14—16) using the farnesyl-phthalide (3) as the labelled precursor. In these last experiments, the distribution of radioactivity can be ascribed to exchange through the cell wall of the labelled compounds (4) and (6), biosynthesized from compound (3), with the unlabelled ketones (4) and (6), present in excess in solution. However, the higher percentage recovery of label in the final product (1) is also due to the co-presence of an alternative biosynthetic pathway, *e.g.* the terminal–central one. As required by the terminal–central process, acetone and levulinic acid are produced in parallel with the acid (1) as the fermentation develops.[9] Failure to find 6-methylhept-5-en-2-one [9] and the hydroxy-ketone (6) [12] in the fermentation only means that the endogenous pool of these compounds is small and that they are immediately transformed as soon as they have been formed.

Compounds (8), (9), and (10), functionalized at the terminal double-bond, were incorporated into the acid (1) (Experiments 5—10) to a smaller extent. The labelled prenylogue of mycophenolic acid, (11c), was obtained, by diazomethane treatment and dilution with synthetic samples of (11c), when the labelled compounds (8), (9), and (10) were fed to the culture (Experiments 11—13). However, uncertainties regarding relative rates of transport into cells makes it doubtful as to whether the relative importance of the two pathways can be determined from the incorporation percentages. At the most our percentage label values indicate that the two pathways are equally important.

As regards the oxidation mechanism, it has been reported [7] that mycophenolic and nor-*O*-methylmycophenolic aldehydes are not intermediates. Therefore, the carboxy-group of mycophenolic acid derives directly from the oxidation process during the C–C bond cleavage. This result rules out the possibility of a C–C bond cleavage at the diol level, as for the C(20)–C(22) oxidative removal in steroids.[31]

For the 6-farnesyl-5,7-dihydroxy-4-methylphthalide oxidation mechanism, we propose the following sequence: epoxidation of the double bond, rearrangement of the epoxide to a ketone, hydroxylation to an α-hydroxy-ketone, and finally Woodward reaction [32] with C–C bond cleavage and formation of the acid.

We can say nothing about the possible role of the hydroperoxide (5) in the mechanism, in analogy with hydroperoxide role in the transformation of C(21)- into C(19)-steroids,[33] because compound (5) is not stable enough at room temperature to be used in biological experiments.

The micro-organism is able to oxidise either the double bond which is five carbons distant from the aromatic nucleus or the double bond which is nine carbons distant. The enzyme mechanisms for this process are not specific for one particular substrate: many different molecules,[1,34] *e.g.* geranylphthalide (39), although not obligatory intermediates in the normal biosynthetic pathway, are effectively converted into the acid (1). The attachment of the farnesyl unit can also occur onto many different aromatic nuclei,[34] and neither is *O*-methylation specific for nor-*O*-methylmycophenolic acid.[4,12]

It is noteworthy that the final three biosynthetic steps leading to mycophenolic acid in *P. brevicompactum* are characterized by a very low enzyme specificity.

EXPERIMENTAL

Culture Conditions and Incorporation Experiments.—Mycophenolic acid (1) was obtained from the strain of *Penicillium brevicompactum* Dierckx CBS 25729. Aliquots (150 ml) of Raulin–Thom medium [35] were inoculated with a suspension of *Penicillium brevicompactum* spores obtained from 1—2-week potato-agar slants, and incubated at 27 °C on a shaker. After 24 h incubation, the following labelled compounds were added to the cultures as solids or in acetone solution: ketones (4) and (8), the hydroxy-ketones (6) and (9), and the epoxide (10). After a suitable time interval the transformation was discontinued and each aliquot was filtered, crushed with Celite, and extracted with ethyl acetate (5 × 50 ml). The cultural broth was acidified to pH 1 with 6M hydrochloric acid and extracted with ethyl acetate (5 × 100 ml). The organic extracts were washed with water, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was chromatographed over silica gel (Merck; 70—230 mesh), with heptane–ethyl acetate (1 : 1), ethyl acetate, and ethyl acetate–chloroform–acetic acid (55 : 45 : 1) as eluants. Fractions eluted with the last eluant contained mycophenolic acid and were collected and evaporated to dryness under reduced pressure. The product was crystallised from heptane–ethyl acetate.

TABLE 5

No. of aliquots	Incubated compound (mg per aliquot)	Activity (disint. min^{-1} $mg^{-1} \times 10^4$)	Acid (1) isolated (mg)	Activity (disint. min^{-1} $mg^{-1} \times 10^4$)	Fermentation (h)	Activity of the aldehyde (12) (disint. min^{-1} $mg^{-1} \times 10^4$)
2	(4) 47.5	4.8	49.2	0.7692	72	1.0511
5	(4) 19.0	4.8	49.6	0.1986	18	0.2697
2	(6) 47.5	4.6	61.2	1.2410	72	1.6812
5	(6) 19.0	4.6	57.3	0.1564	18	0.2116
2	(8) 10.0	184.05	60.0	2.875	72	3.891
2	(8) 10.0	184.05	4.2	5.452	18	7.395
2	(9) 7.5	176.97	57.3	2.369	72	3.210
2	(9) 7.5	176.97	13.3	1.357	18	1.842
2	(10) 11.0	184.05	59.1	3.011	72	4.08
2	(10) 11.0	184.05	11.0	2.208	18	2.99

Table 5 gives the quantity and activities of ^{14}C-labelled compounds incubated, of mycophenolic acid isolated, and of 6-formylmethyl-7-hydroxy-5-methoxy-4-[^{14}C]methylphthalide (12) obtained by ozonolysis.[15]

In the experiments with ^{13}C-labelled compounds,[6,11] molar incorporation percentages were calculated by comparing integrals of the signals from ArMe (δ 10.8) and $ArCH_2O$ (δ 69.0) in unlabelled mycophenolic acid (1) (relative ratio *ca.* 1.4) and in labelled mycophenolic acid. The initial ratio in the labelled precursors (*ca.* 114, 90% enrichment) became *ca.* 52 (40.8% enrichment) in the acid (1) derived from the labelled compound (6) (45% molar incorporation; 5 d fermentation; ref. 6), and *ca.* 33 (26% enrichment) in the acid (1) derived from the labelled compound (11a) (28% molar incorporation; 3 d fermentation; ref. 11). Compounds (8), (9), and (10) were also incorporated into the prenylogue of the acid (1). In these experiments the labelled compounds were added to the cultures (10 aliquots) in acetone solution. After interruption of the transformation (18 h), the mycelium of each aliquot was filtered, crushed with Celite, and extracted with ethyl acetate (5 × 50 ml). The organic extracts were dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was treated with diazomethane and then chromatographed over silica gel with hexane and hexane–ethyl acetate (2 : 1) as eluants. Fractions which were eluted with the latter eluant contained a crude mixture which was, in turn, purified by preparative t.l.c. (hexane–chloroform, 3 : 10) to give compound (11c) (*ca.* 5 mg). This product was diluted with the unlabelled compound (11c) (10 mg), obtained by total synthesis and extensively purified by silica-gel chromatography. Table 6 gives the quantities and activities of the ^{14}C-labelled compounds incubated and of the prenylogous compound isolated.

TABLE 6

No. of aliquots	Incubated compound (mg per aliquot)	Activity (disint. min^{-1} $mg^{-1} \times 10^4$)	Isolated (11c) (mg)	Activity [a] (disint. min^{-1} $mg^{-1} \times 10^4$)	Fermentation (h)
10	(8) 10.16	184.05	4.7	3.943	18
10	(9) 16.0	176.97	4.3	3.166	18
10	(10) 69.5	184.05	5.2	77.513	18

[a] After dilution with 10 mg of unlabelled compound (11c), obtained by total synthesis.

'*Isotopic-trap*' *Experiments*.—The unlabelled compounds (4) or (6) (300 mg) together with the ^{14}C-labelled compound (3) (14×10^6 disint. min^{-1}) were added to a 1-d old culture broth of *P. brevicompactum*. After a further 12 or 24 h the fermentation was discontinued and the compounds were isolated. In the case of the ketone (4), the crude extracts were chromatographed over silica gel with hexane–ethyl acetate (4 : 1), ethyl acetate, and ethyl acetate–chloroform–acetic acid (55 : 45 : 1). Fractions which were eluted with the first eluant contained the ketone (4), which was crystallised many times from hexane–diethyl ether. After 9 crystallizations the product was reduced with $NaBH_4$ in methanol to give 5,7-dihydroxy-6-(6-hydroxy-3,7,11-trimethyldodeca-2,10-dienyl)-4-methylphthalide (13a) which was, in turn, crystallised from hexane–diethyl ether to constant activity. Fractions eluted with the last eluant contained mycophenolic acid (1) which was crystallised from heptane–ethyl acetate.

In the case of the hydroxy-ketone (6), the crude extracts were chromatographed over silica gel with hexane–ethyl acetate (7 : 3), ethyl acetate, and ethyl acetate–chloroform–acetic acid (55 : 45 : 1) as eluants. Fractions which were eluted with the first eluant contained the hydroxy-ketone (6) which was extensively purified by preparative t.l.c. The product was then reduced with $NaBH_4$ in methanol to give a mixture of the diastereoisomeric diols (11b) which were crystallised from hexane–diethyl ether to constant activity. Fractions which were eluted with the last eluant contained mycophenolic acid (1), which was crystallised from heptane–ethyl acetate. Table 7 gives the quantities and activities of the compounds incubated and of the products isolated. Labelled 6-(*E*,*E*)-farnesyl-5,7-dihydroxy-4-methylphthalide (3) (30 mg) was obtained as previously described,[1] by feeding 5,7-dihydroxy-4-[^{14}C]methylphthalide (30) (120 mg)

TABLE 7

No. of aliquots	Incubated compounds (mg)	Activity (disint. min^{-1} $mg^{-1} \times 10^5$)	Isolated compounds (mg)	Activity (disint. min^{-1} $mg^{-1} \times 10^4$)	Fermentation (h)
2	(4) 300	0	(4) 205	0.17	24
	(3) 10	14	(1) 27	6.2	
2	(6) 300	0	(6) 192	0.022	24
	(3) 10	14	(1) 14	3.0	
4	(6) 300	0	(6) 208	0.013	12
	(3) 10	14	(1) 20	1.6	

specific activity 4.09×10^6 disint. min^{-1} mg^{-1}, and isolation after fermentation (48 h).

Synthesis of Precursors

Nor-O-methylmycophenolic Acid Diacetate (14).—Mycophenolic acid was demethylated with anhydrous lithium iodide in collidine [16,17] to give nor-*O*-methylmycophenolic acid (2) (70% yield), which was crystallised from heptane–ethyl acetate, m.p. 149—150 °C (Found: C, 62.8; H, 5.9. $C_{16}H_{18}O_6$ requires C, 62.74; H, 5.88%). Acetylation of the acid (2) with acetic anhydride and pyridine [17] gave the *diacetate* (14) in 87% yield, which was crystallised from ethanol, m.p. 147—148 °C; δ ($CDCl_3$) 1.75br (3 H, s, MeC=C), 2.05 (3 H, s, ArMe), 2.2—2.5 (4 H, m, CH_2CH_2), 2.34 (3 H, s, AcO), 2.38 (3 H, s, AcO), 3.23 (2 H, d, *J* 7 Hz, $ArCH_2C$=C), 5.01 (1 H, t, CH=C), 5.16 (2 H, s, $ArCH_2O$), and 9.25br (1 H, s, CO_2H) (Found: C, 61.7; H, 5.7. $C_{20}H_{22}O_8$ requires C, 61.50; H, 5.65%).

6-*Bromo*-2-*methylhept*-2-*ene* (17).—6-Methylhept-5-en-2-one (18 ml) was reduced with $NaBH_4$ (6.22 g) in ethanol (288 ml) to give 6-*methylhept*-5-*en*-2-*ol* (19) (11.5 g) in 75% yield, b.p. 80 °C/16 mmHg (Found: C, 75.1; H, 12.7. $C_8H_{16}O$ requires C, 75.0; H, 12.5%). A solution of the alcohol (19) (11.5 g) in dry pyridine (22 ml) was treated at 0 °C with methanesulphonyl chloride (9 ml). After 2 h at room temperature, the reaction mixture was poured into ice, acidified with hydrochloric acid, and extracted with benzene. The organic extracts were washed with water until neutral, concentrated to a small volume under reduced pressure, and treated with a saturated solution of KBr (10.7 g) in water (15 ml) and hexadecyltributylphosphonium bromide (4.56 g). The mixture was stirred at 70 °C for 20 h, after which the organic phase was separated and the aqueous phase extracted with benzene. The organic extracts were dried (Na_2SO_4) and passed through a short silica-gel column with hexane (600 ml) as eluant. The solvent was removed at room pressure and the residue was distilled to give the *bromide* (17) (12.92 g, 75% yield), b.p. 80 °C/18 mmHg; δ($CDCl_3$) 1.72 (3 H, d, MeCBr), 1.70—2.40 (4 H, m, CH_2CH_2), 1.65 (6 H, s, MeC=C), 4.18 (1 H, m, CHBr), and 5.12 (1 H, t, CH=C) (Found: C, 50.1; H, 7.9. $C_8H_{15}Br$ requires C, 50.26; H, 7.85%).

5,7-*Diacetoxy*-4-*methyl*-6-[3,7,11-*trimethyl*-6-*oxododeca*-2-(E),10-*dienyl*]*phthalide* (7).—A solution of the acid (14) (1.0 g) in thionyl chloride (10 ml) was stirred at room temperature for 3 h. Thionyl chloride was distilled off under reduced pressure and the residue (15) was dissolved thrice in dry benzene which was, in turn, distilled off under reduced pressure. The residue was dissolved in dry tetrahydrofuran (THF) (4 ml) and dry diethyl ether (15 ml). The resulting solution was added as drops at −80 °C to the Grignard reagent prepared from magnesium (0.2 g) and the bromide (17) (1.5 g) in dry diethyl ether (11 ml) containing CuI (1.5 g). The temperature was slowly raised to −40 °C during 2 h and the reaction was quenched in the normal manner. The crude mixture was chromatographed (CH_2Cl_2) to give the *ketone* (7) (0.86 g, 70%); δ ($CDCl_3$) 1.15 (3 H, d, *J* 6 Hz, *Me*CH), 1.57 (3 H, s, MeC=C), 1.67 (3 H, s, MeC=C), 1.75 (3 H, s, MeC=C), 1.5—2.0 (4 H, m, CH_2CH_2), 2.05 (3 H, s, ArMe), 2.2—2.5 (4 H, m, CH_2CH_2), 2.32 (3 H, s, AcO), 2.37 (3 H, s, AcO), 3.24 (2 H, d, *J* 7 Hz, $ArCH_2C$=C), 4.7 (1 H, m, MeC*H*CO), 4.91 (1 H, t, CH=C), 5.03 (1 H, t, CH=C), and 5.16 (2 H, s, $ArCH_2O$); *m/e* 390 (0.6%), 356 (5.0), 349 (2.5), 348 (2.5), 347 (2.5), 341 (2.5), 331 (6.2), 330 (3.7), 329 (6.9), 313 (7.5), 306 (2.5), 305 (3.1), 289 (10), 288 (15), 287 (12.5), 259 (6.9), 257 (5), 247 (3.7), 235 (6.2), 193 (17.5), and 110 (100); $\nu_{max.}$ ($CHCl_3$) 1 765 and 1 725 cm^{-1} (Found: C, 69.3; H, 7.4. $C_{28}H_{36}O_7$ requires C, 69.42; H, 7.43%).

5,7-*Dihydroxy*-4-*methyl*-6-[3,7,11-*trimethyl*-6-*oxododeca*-2(E),10-*dienyl*]*phthalide* (4).—A solution of the ketone (7) (0.7 g) in ethanol (30 ml) was treated under nitrogen at room temperature with a solution of sodium hydroxide (0.358 g) in water (10 ml). After 3 h the reaction was quenched with acetic acid (0.5 ml) and concentrated under reduced pressure. The mixture was extracted with methylene dichloride and the organic extracts were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (hexane–ethyl acetate, 7 : 3) and then crystallised from hexane–diethyl ether to give the ketone (4) (0.52 g, 90%), m.p. 64—65 °C; δ($CDCl_3$) 1.13 (3 H, d, *J* 6 Hz, MeCH), 1.55 (3 H, s, MeC=C), 1.65 (3 H, s, MeC=C), 1.83 (3 H, s, MeC=C), 1.5—2.0 (4 H, m, CH_2CH_2), 2.04 (3 H, s, ArMe), 2.37br (4 H, s, CH_2CH_2), 3.43 (2 H, d, *J* 7 Hz, $ArCH_2C$=C), 4.87—5.3 (2 H, m, CH=C), 5.18 (2 H, s, $ArCH_2O$), 5.94br (1 H, s, ArOH), and 7.71 (1 H, s, ArOH); *m/e* 400 (M^+, 5%), 318 (8), 300 (10), 247 (16), 246 (31), 233 (23), 232 (18), 231 (100), 194 (47), and 193 (58); $\nu_{max.}$ ($CHCl_3$) 3 400, 1 735, and 1 720 cm^{-1} (Found: C, 72.15; H, 8.15. $C_{24}H_{32}O_5$ requires C, 72.0; H, 8.0%).

5,7-*Dihydroxy*-6-[7-*hydroxy*-3,7,11-*trimethyl*-6-*oxododeca*-2(E),10-*dienyl*]-4-*methylphthalide* (6).—Sodium hydride (1.068 g; 50% in oil) was treated under argon with Bu^tOH (13.5 ml) and then with 1,2-dimethoxyethane (DME) (20.7 ml). The mixture was stirred at room temperature for 1 h, after which it was treated with a solution of $(EtO)_3P$ (0.96 ml) in DME (9 ml). A solution of the ketone (4) (0.88 g) in anhydrous DME (10.5 ml) was added as drops to the mixture which was then cooled to −30 °C, and the argon replaced by oxygen. The mixture was stirred for 2 h at −20 °C to −30 °C and then quenched with acetic acid (1.35 ml) and water. The aqueous phase was extracted with CH_2Cl_2 and the organic extracts were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (pentane–diethyl ether, 7 : 3) to give the hydroxy-ketone (6) (0.733 g, 80%). The product was crystallised from methanol–water to give a deliquescent *solid*; δ($CDCl_3$) 1.40 (3 H, s, *Me*COH), 1.63 (3 H, s, MeC=C), 1.73 (3 H, s, MeC=C), 1.93 (3 H, s, MeC=C), 1.7—2.1 (4 H, m, CH_2CH_2), 2.16 (3 H, s, ArMe), 2.41 (2 H, t, CH_2CH_2), 2.72 (2 H, t, CH_2CH_2), 3.49 (2 H, d, *J* 7 Hz, $ArCH_2C$=C), 3.86 (1 H, s, OH), 5.01 (1 H, t, CH=C), 5.20 (2 H, s, $ArCH_2O$), 5.24 (1 H, t, CH=C), 6.1br (1 H, s, ArOH), and 7.7br (1 H, s, ArOH); $\nu_{max.}$ ($CHCl_3$) 3 400, 1 735, and 1 715 cm^{-1} (Found: C, 69.3; H, 7.75. $C_{24}H_{32}O_6$ requires C, 69.23; H, 7.69%); *m/e* 396 (0.87%), 368 (2.9), 336 (0.4), 313 (1.3), 305 (1.8), 257 (4.2), 245 (0.6), 237 (1.4), 233 (3.8), 231 (4.1), 219 (1.0), 207 (1.4), 193 (15), 111 (17.3), 99 (14.0), and 57 (100). The mass spectrum of compound (6) had previously been incorrectly reported.[6]

6-(6,7-*Dihydroxy*-3,7,11-*trimethyldodeca*-2,10-*dienyl*)-5,7-*dihydroxy*-4-*methylphthalides* (13b).—A solution of the hydroxy-ketone (6) (0.1 g) in ethanol (5 ml) was treated at 0 °C with a suspension of $NaBH_4$ (21 mg) in ethanol. After 15 min the reaction mixture was treated with 0.2M HCl, concentrated under reduced pressure, and extracted with CH_2Cl_2. The organic extracts were dried (Na_2SO_4) and evaporated to give a mixture of the two stereoisomeric *diols* (13b) (0.1 g, 100%); δ($CDCl_3$), 1.16 (3 H, s, *Me*COH), 1.4—1.7 (4 H, m, CH_2CH_2), 1.62 (3 H, s, MeC=C), 1.68 (3 H, s, MeC=C), 1.86 (3 H, s, MeC=C), 1.9—2.3 (4 H, m, CH_2CH_2), 2.06 (3 H, s, ArMe), 3.47 (d, 2 H, *J* 7 Hz, $ArCH_2C$=C), 5.0—

5.3 (2 H, m, CH=C), 5.18 (2 H, s, $ArCH_2O$), 6.1br (s, 1 H, ArOH), and 7.76br (1 H, s, ArOH); the other stereoisomer had an analogous ^1H n.m.r. spectrum, the only relevant difference being δ 1.12 (3 H, s, *Me*COH); $\nu_{max.}$ ($CHCl_3$) 3 400 and 1 735 cm^{-1} (Found: C, 68.1; H, 8.1. $C_{24}H_{34}O_6$ requires C, 68.89; H, 8.13%).

Attempts to Synthesize the 6,7-Epoxide.—A solution of the diols (13b) (0.08 g) in pyridine (1 ml) was treated with toluene-*p*-sulphonyl chloride (0.18 g). After 1 d at room temperature, the reaction mixture was treated with 2M HCl and extracted with diethyl ether. The organic extracts were dried (Na_2SO_4) and evaporated. The crude product was chromatographed (hexane–diethyl ether, 9 : 1) to give a tritosylated compound (0.110 g); δ($CDCl_3$) 7.2—8.1 (12 H, m, ArH). A solution of the tritosylated compound (0.1 g) in THF (1 ml) was treated at 0 °C with a solution of NaOH (0.03 g) in methanol (1 ml). A complex reaction mixture was obtained.

The 4-[^{14}C]*Methyl-ketone* (4) *and the Hydroxy-ketone* (6).—The labelled ketone (4) (0.19 g; 48 × 10^3 disint. min^{-1} mg^{-1}) and the hydroxy-ketone (6) (0.19 g; 46 × 10^3 disint. min^{-1} mg^{-1}) were prepared, as described above, from labelled mycophenolic acid (1) (1.0 g; 60 × 10^3 disint. min^{-1} mg^{-1}). Labelled mycophenolic acid (1.0 g; 60 × 10^3 disint. min^{-1} mg^{-1}) was obtained by dilution of the acid (1) (28 mg; 2.14 × 10^6 disint. min^{-1} mg^{-1}), prepared as previously described [1] by feeding the [^{14}C]methylphthalide (30) (25 mg; 4.09 × 10^6 disint. min^{-1} mg^{-1}), and isolation after fermentation (48 h).

3,7,11-*Trimethyl*-10,11-*epoxydodeca*-2(E),6(E)-*dienol* (29).—A solution of (*E*,*E*)-farnesol (8.75 g) in dry pyridine (8.75 ml) was treated with acetic anhydride (17.5 ml). The mixture was heated to 95 °C for 3 h and the usual work-up gave farnesol acetate (31) (10.34 g, 99%). A solution of farnesol acetate (31) (10 g) in Bu^tOH (130 ml) and water (20 ml) was treated in the dark, while being stirred, with *N*-bromosuccinimide (NBS) (7.7 g). After 1 h at room temperature, the mixture was diluted with water (1 l), and extracted with ethyl acetate. The organic extracts were dried (Na_2SO_4) and evaporated. The residue was taken up in hexane, filtered off from the succinimide, and evaporated under reduced pressure. The crude product was purified by flash-chromatography [36] (hexane–ethyl acetate, 9 : 1) to give 10-bromo-11-hydroxy-3,7,11-trimethyldodeca-2,6-dienyl acetate (32) (6.8 g, 50%). A solution of the bromohydrin (32) (6.7 g) in methanol (17 ml) and water (3.18 ml) was treated at room temperature, while being stirred, with K_2CO_3 (15.5 g). After 2 h the mixture was evaporated under reduced pressure, after which the residue was taken up in water and extracted with chloroform. The organic extracts were dried (Na_2SO_4) and evaporated to give the *epoxide* (29) (4.36 g, 100%); δ($CDCl_3$) 1.28 (6 H, s, 2 × $MeCOC$), 1.63 (6 H, s, 2 × MeC=C), 1.9—2.4 (8 H, m, 2 × CH_2CH_2), 2.67 (1 H, t, *J* 6 Hz, $COCH$), 4.13 (2 H, d, *J* 6 Hz, CH_2OH), 5.27 (1 H, t, *J* 6 Hz, CH=C), and 5.39 (1 H, t, *J* 6 Hz, CH=C) (Found: C, 75.7; H, 11.0. $C_{15}H_{26}O_2$ requires C, 75.63; H, 10.92%).

1-*Bromo*-3,7,11-*trimethyl*-10,11-*epoxydodeca*-2,6-*diene* (28).—To a solution containing NBS (0.68 g) in anhydrous methylene dichloride (13 ml) was added, as drops at 0 °C, methyl sulphide (0.4 ml). The mixture was cooled to −20 °C, and the epoxy-alcohol (29)(0.7 g) in methyl dichloride (2 ml) was added as drops. Then the reaction mixture was warmed to 0 °C and stirred for 2.5 h, diluted with pentane (4 ml), and poured into ice–water (10 ml). The organic extracts were dried (Na_2SO_4), filtered through silica gel, and evaporated under reduced pressure to give epoxy-bromide (28) (0.58 g, 66%); δ($CDCl_3$) 4.00 (2 H, d, *J* 8 H, CH_2Br).

5,7-*Dihydroxy*-4-*methyl*-6-[3,7,11-*trimethyl*-10,11-*epoxydodeca*-2(E),6(E)-*dienyl*]*phthalide* (10).—A solution of the epoxy-bromide (28) (0.5 g) in dioxan (20 ml) and water (0.8 ml) was treated with the phthalide (30) (0.24 g) and freshly prepared Ag_2O (0.57 g). The mixture was stirred in the dark for 30 min at room temperature and then filtered on a Celite cake. The filtrate was evaporated under reduced pressure and the residue was chromatographed on silica gel (hexane–ethyl acetate, 6 : 4) to give the *C*-alkylated product (10) (0.086 g, 16%); further fractions separated the *O*-alkylated compound and, subsequently the phthalide (30). Compound (10) was a deliquescent *solid* (Found: C, 71.9; H, 8.1. $C_{24}H_{32}O_5$ requires C, 72.0; H, 8.0%); δ($CDCl_3$) 1.26 (3 H, s, $MeCOC$), 1.30 (3 H, s, $MeCOC$), 1.60 (3 H, s, MeC=C), 1.80 (3 H, s, MeC=C), 2.06 (3 H, s, ArMe), 2.0—2.4 (8 H, m, CH_2CH_2), 2.7 (1 H, t, *J* 6 Hz, $COCH$), 3.44 (2 H, d, *J* 7 Hz, $ArCH_2$), 5.16 (2 H, m, 2 × CH=C), 5.16 (2 H, s, $ArCH_2O$), 6.4 (1 H, s, ArOH), and 7.7 (1 H, s, ArOH).

12-*Hydroxy*-2,6,10-*trimethyldodeca*-6(E),10(E)-*dien*-3-*one* (25).—A solution of the epoxy-alcohol (29) (4 g) in pyridine (4 ml) was treated with acetic anhydride (8 ml). The mixture was stirred overnight and then worked up as usual to give 3,7,11-trimethyl-10,11-epoxydodeca-2,6-dienyl acetate (33) (4.7 g, 100%); $\nu_{max.}$ ($CHCl_3$) 1 740 cm^{-1}. A solution of the epoxy-acetate (33) (3.0 g) in anhydrous benzene (70 ml) was treated with $KHSO_4$ (0.28 g) and $LiClO_4$ (1.32 g) and heated to 100 °C for 90 s. The reaction mixture was poured into ice–water and extracted with benzene. The organic extracts were dried and evaporated to give crude 3,7,11-trimethyl-10-oxododeca-2,6-dienyl acetate (34) (3.0 g); $\nu_{max.}$ ($CHCl_3$) 1 745 and 1 715 cm^{-1}. A solution of the crude acetate (34) (3.0 g) in methanol (29 ml) and water (5.6 ml) was treated with K_2CO_3 (6 g). The mixture was stirred for 2 h at room temperature and then evaporated under reduced pressure. The residue was taken up in water and extracted with chloroform. The organic extracts were dried (Na_2SO_4) and evaporated to give a crude product, which was chromatographed on silica gel (hexane–ethyl acetate, 7 : 3) to give the *keto-alcohol* (25) (1.53 g, 60%); $\nu_{max.}$ ($CHCl_3$) 3 400 and 1 715 cm^{-1}; δ ($CDCl_3$) 1.18 (6 H, d, *J* 8 Hz, *Me*CHCO), 1.72 (3 H, s, MeC=C), 1.77 (3 H, s, MeC=C), 1.8—2.8 (9 H, m,), 4.12 (2 H, d, *J* 7 Hz, CH_2O), 5.05br (1 H, t, CH=C), and 5.36br (1 H, t, CH=C) (Found: C, 75.4; H, 11.0. $C_{15}H_{26}O_2$ requires C, 75.63; H, 10.9 %).

5,7-*Dihydroxy*-4-*methyl*-6-[3,7,11-*trimethyl*-10-*oxododeca*-2(E),6(E)-*dienyl*]*phthalide* (8).—A solution of the keto-alcohol (25) (0.335 g) in anhydrous acetonitrile (4.1 ml) was treated with CBr_4 (0.56 g) and PPh_3 (0.44 g). The reaction mixture was stirred at room temperature in the dark for 3 h and then treated with hexane (90 ml). This mixture was stirred for 1 h, and then the hexane phase was separated off and evaporated under reduced pressure. The residue was taken up in hexane (5 × 5 ml), filtered from Ph_3PO, and evaporated under reduced pressure to give crude 12-bromo-2,6,10-trimethyldodeca-6,10-dien-3-one (24) (0.5 g), which was immediately used for the subsequent reaction without purification. A solution of the crude ketone (24) (0.5 g) in dioxan (18 ml) and water (0.73 ml) was treated with the phthalide (30) (0.2 g) and freshly prepared Ag_2O (0.51 g).

The mixture was stirred in the dark for 30 min at room temperature, then worked up as described above, and chromatographed on silica gel (hexane–ethyl acetate, 7 : 3) to give the *C-alkylated product* (8) (0.082 g, 15%) as a deliquescent solid; $\nu_{max.}$ ($CHCl_3$) 3 400, 1 735, and 1 715 cm^{-1}; δ($CDCl_3$) 1.10 (6 H, d, *J* 7.5 Hz, *Me*CHCO), 1.63 (3 H, s, MeC=C), 1.83 (3 H, s, MeC=C), 2.10 (3 H, s, ArMe), 2.0—2.7 (9 H, m), 3.45 (2 H, d, *J* 7 Hz, $ArCH_2C$=C), 5.13 (1 H, m, CH=C), 5.20 (2 H, s, $ArCH_2O$), 5.30 (1 H, m, CH=C), 6.36br (1 H, s, OH), and 7.76br (1 H, s, OH) (Found: C, 71.8; H, 8.2. $C_{24}H_{32}O_5$ requires C, 72.0; H, 8.0%).

2,12-*Dihydroxy*-2,6,10-*trimethyldodeca*-6(E),10(E)-*dien*-3-*one* (27).—A solution of the epoxy-acetate (33) (0.46 g) in diglyme (4.6 ml) and water (1.2 ml) was treated with 70% perchloric acid (0.093 ml). The mixture was stirred for 2 h at room temperature, after which it was treated with methanol (25 ml) and neutralised with Amberlite IR-45. The resulting mixture was filtered and evaporated under reduced pressure to give a crude product which was chromatographed on silica gel (hexane–ethyl acetate, 6 : 4) to give 10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienyl-acetate (35) (0.37 g, 75%). To a solution of *N*-chlorosuccinimide (1.45 g) in toluene (29.5 ml) was added, at 0 °C, methyl sulphide (0.85 ml) under argon. The mixture was cooled to −25 °C and a solution of the diol (35) (1.56 g) in toluene (10 ml) was added to the well-stirred mixture. The mixture was stirred for 3 h and then a solution of triethylamine (1.1 g) in toluene (5 ml) was added as drops. The cooling bath was removed and, after 5 min, diethyl ether (100 ml) was added. The organic phase was washed successively with 1% HCl (40 ml) and water, dried (Na_2SO_4), and evaporated under reduced pressure to give crude 11-hydroxy-3,7,11-trimethyl-10-oxododeca-2,6-dienyl acetate (36) (1.54 g). A solution of the crude acetate (36) (1.54 g) in methanol (16 ml) and water (3.2 ml) was treated with K_2CO_3 (3.4 g). The mixture was stirred for 2 h at room temperature and the usual work-up gave a crude product which was chromatographed on silica gel (hexane–ethyl acetate, 5 : 5) to give the *hydroxy-ketone* (27) (1.0 g, 75%); $\nu_{max.}$ ($CHCl_3$) 3 400 and 1 715 cm^{-1}; δ($CDCl_3$) 1.4 (6 H, s, *Me*COH), 1.65 (3 H, s, MeC=C), 1.70 (3 H, s, MeC=C), 2.1—2.8 (8 H, m), 3.9 (2 H, s, OH), 4.16 (2 H, d, *J* 7 Hz, CH_2O), 5.15br (1 H, t, CH=C), and 5.4br (1 H, t, CH=C) (Found: C, 70.4; H, 10.4. $C_{15}H_{26}O_3$ requires C, 70.86; H, 10.23%).

5,7-*Dihydroxy*-6-[11-*hydroxy*-3,7,11-*trimethyl*-10-*oxododeca*-2(E),6(E)-*dienyl*]-4-*methylphthalide* (9).—A solution of the hydroxy-ketone (27) (0.5 g) in anhydrous acetonitrile (6.1 ml) was treated with CBr_4 (0.83 g) and PPh_3 (0.66 g). The reaction mixture was stirred at room temperature in the dark for 3 h and the usual work-up gave 12-bromo-2-hydroxy-2,6,10-trimethyldodeca-6,10-dien-3-one (26) (0.59 g). A solution of the crude ketone (26) (0.59 g) in dioxan (25 ml) and water (1.0 ml) was treated with the phthalide (30) (0.2 g) and freshly prepared Ag_2O (0.72 g). The mixture was stirred in the dark for 30 min at room temperature, after which the usual work-up and chromatography on silica gel (hexane–ethyl acetate, 7 : 3) gave the *C-alkylated product* (9) (0.061 g, 13%) as a deliquescent solid; $\nu_{max.}$ ($CHCl_3$) 3 400, 1 735, and 1 715 cm^{-1}; δ($CDCl_3$) 1.37 (6 H, s, *Me*COH), 1.6 (3 H, s, MeC=C), 1.8 (3 H, s, MeC=C), 2.06 (3 H, s, ArMe), 2.0—2.7 (8 H, m), 3.43 (2 H, d, *J* 7 Hz, $ArCH_2C$=C), 3.76br (1 H, s, OH), 5.16 (2 H, m, CH=C), 5.16 (2 H, s, $ArCH_2O$), 6.32 (1 H, s, OH), and 7.72 (1 H, s, OH); *m/e* 399 (1.8%), 398 (1.9), 373 (3.8), 358 (2.5), 355 (1.9), 330 (5.1), 301 (2.5), 273 (7.7), 247 (11.5), 233 (25.6), 231 (19.2), 193 (100), and 59 (80.1) (Found: C, 69.1; H, 7.85. $C_{24}H_{32}O_6$ requires C, 69.23; H, 7.69%).

Methyl 10-*Hydroxy*-4,8-*dimethyldeca*-4(E),8(E)-*dienoate* (23).—A solution of the diol (35) (0.36 g) in THF (3.6 ml) was treated with a solution of $NaIO_4$ (0.31 g) in water (3.7 ml). The mixture was stirred at room temperature for 2 h and then filtered and extracted with chloroform. The organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give 9-formyl-3,7-dimethylnona-2,6-dienyl acetate (37) (0.28 g, 97%). To a solution of the aldehyde (37) (0.28 g) in dioxan–water (1 : 1; 8.9 ml) was added, at room temperature and in the dark, a solution of $AgNO_3$ (0.41 g) in water (1.8 ml), and of potassium hydroxide (0.68 g) in water (1.8 ml). The mixture was stirred for 1.5 h and then filtered on a Celite cake, acidified with 1M H_2SO_4 to pH 4 and extracted with ethyl acetate. The organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give a crude product which was chromatographed on silica gel (chloroform–ethyl acetate–acetic acid, 55 : 45 : 1) to give 10-hydroxy-4,8-dimethyl-4,8-dienoic acid (38) (0.22 g, 85%); δ($CDCl_3$) 1.60 (6 H, s, MeC=C), 2.0—2.5 (8 H, m, CH_2CH_2), 4.13 (2 H, d, *J* 7 Hz, CH_2O), 5.06 (1 H, m, CH=C), 5.33br (1 H, t, CH=C), and 6.26br (2 H, s, OH). A solution of the acid (38) (2.8 g) in diethyl ether (26 ml) was treated at 0 °C with diazomethane. The usual work-up gave a crude product which was chromatographed on silica gel (hexane–ethyl acetate, 65 : 35) to give the methyl ester (23) (2.2 g, 73%); δ($CDCl_3$) 1.60 (3 H, s, MeC=C), 1.65 (3 H, s, MeC=C), 1.9—2.5 (8 H, m, CH_2CH_2), 3.64 (3 H, s, OMe), 4.13 (2 H, d, *J* 7 Hz, CH_2O), 5.11 (1 H, m, CH=C), and 5.37br (1 H, t, CH=C).

6-[(E,E)-9-*Carboxy*-3,7-*dimethylnona*-2,6-*dienyl*]-5,7-*dihydroxy*-4-*methylphthalide* (11a).—A solution of the alcohol (23) (0.45 g) in anhydrous acetonitrile (5.1 ml) was treated with CBr_4 (0.73 g) and PPh_3 (0.57 g). The reaction mixture was stirred at room temperature in the dark for 3 h and the usual work-up gave crude methyl 10-bromo-4,8-dimethyldeca-4,8-dienoate (22) (0.57 g). A solution of the crude acetate (22) (0.57 g) in dioxan (25 ml) and water (1 ml) was treated with the phthalide (30) (0.28 g) and freshly prepared Ag_2O (0.69 g). The mixture was stirred in the dark for 30 min at room temperature, after which the usual work-up and chromatography on silica gel (hexane–ethyl acetate, 6 : 4) gave 5,7-dihydroxy-6-(9-methoxycarbonyl-3,7-dimethylnona-2,6-dienyl)-4-methylphthalide (11b) (0.121 g, 20%). Further fractions separated the *O*-alkylated compound; δ($CDCl_3$) 1.53 (3 H, s, MeC=C), 1.63 (3 H, s, MeC=C), 2.05 (3 H, s, ArMe), 2.0—2.5 (8 H, m, CH_2CH_2), 3.68 (3 H, s, MeO), 4.69 (2 H, d, *J* 5 Hz, $C=CCH_2O$), 5.1 (2 H, s, $ArCH_2O$), 5.1 (1 H, m, C=CH), 5.35 (1 H, m, C=CH), and 6.48 (1 H, s, ArH); $\nu_{max.}$ ($CHCl_3$) 3 320 and 1 730—1 750 cm^{-1}. A solution of compound (11b) (0.25 g) in 0.1M sodium hydroxide (32.2 ml) was stirred at room temperature for 1.5 h. The mixture was then acidified (0.1M HCl) to pH 3 and extracted with ethyl acetate; the organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give a crude product, which was chromatographed on silica gel (ethyl acetate–chloroform–acetic acid, 45 : 55 : 1) to give the *acid* (11a) (0.24 g, 90%), m.p. 132 °C (crystallised from methylene dichloride–hexane); δ($CDCl_3$) 1.64 (3 H, s, MeC=C), 1.85 (3 H, s, MeC=C), 2.09 (3 H, s, ArMe), 2.10—2.60 (8 H, m, CH_2CH_2), 3.47 (2 H, d, *J* 7 Hz, $C=CCH_2Ar$), 5.0—5.30 (2 H, m, CH=C), 5.20 (2 H, s, $ArCH_2O$), and 6.50br (3 H, s, OH) (Found: C,67.3; H, 6.95. $C_{21}H_{26}O_6$ requires C, 67.37; H, 6.95%). A solution

of the acid (11a) (0.2 g) in methanol (20 ml) was treated at 0 °C with diazomethane. The usual work-up gave a crude product which was chromatographed on silica gel (hexane–ethyl acetate, 7 : 3) to give 5,7-dimethoxy-6-(9-methoxycarbonyl-3,7-dimethylnona-2,6-dienyl)-4-methylphthalide (11c) (0.18 g, 81%); *m/e* 416 (10.4%), 385 (6.9), 384 (9.2), 275 (43.0), 221 (100), 207 (31.4), 195 (53), and 141 (44).

The [Me-^{14}C)- *and* [Me-^{13}C]-*Phthalides* (30).—The [*Me*-^{14}C]-phthalide (30) (3.177 g; 4.09×10^6 disint. min^{-1} mg^{-1}) was obtained by a modification of the literature procedure,[37] using labelled formaldehyde (50 mCi) in the chloromethylation step. The [*Me*-^{13}C]phthalide (30) was obtained using ^{13}C-labelled formaldehyde (90% isotopic enrichment).

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