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Preparation and Antitumor Properties of Analogs and Derivatives of Mycophenolic Acid

D. F. JONES* AND S. D. MILLS

Imperial Chemical Industries Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, England

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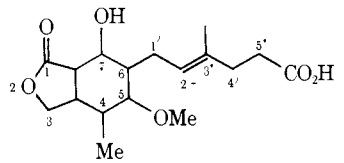
Sixty-five derivatives and analogs of the antitumor agent, mycophenolic acid (1), have been obtained by modifying all positions of the molecule except C-1′. None of these compounds was as effective as **1** in suppressing cell division in mouse fibroblasts cultured *in vitro*. The most active analog (**56**), in which the MeO of 1 was replaced by EtO, had about 12% of the activity of 1. Although *O*-acetate **51**, alcohol **20**, and aldehyde **30** were equiactive with 1 against transplanted tumors in rodents, presumably because of *in vivo* conversion into 1, no compound having a greater antitumor effect than that of **1** was obtained.

Mycophenolic acid (**1**), a mold metabolite first isolated in 1896,[1] has been shown by several groups[2] to have antitumor properties and the compound is presently undergoing clinical trial. Structure **1** was proposed in 1952[3] and confirmed in 1957.[4] A recent total synthesis[5] has confirmed the trans configuration at the double bond.[5]



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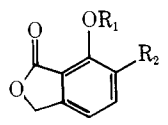
The present paper reports derivatives and analogs of **1** which were prepared to determine the structural features necessary for antitumor activity.

Chemistry.—It was established early that a variety of simple substituted phenols including 7-hydroxyphthalan-1-one and 6-allyl-7-hydroxyphthalan-1-one (**2**) were devoid of antitumor properties. Compound **5**, prepared from **3** by the sequence (a) oxidation with OsO_4–$NaIO_4$ to give **4**, (b) condensation of **4** with $Ph_3P{=}C(Me)CO_2Et$; and (c) hydrolysis of the resulting ester with $MeSO_3H$–90% HCO_2H,[6] was also devoid of antitumor properties indicating that most of the structural features of **1** were probably required for biological activity. Accordingly each of the substituents of **1** was modified in turn as described below.

Attempted O-acetylation of **5**, as a preliminary to further modification of the C-6 substituent unexpectedly gave the enol acetate **6**, presumably by initial removal of a benzylic proton followed by (i) migration of the double bond and attack of acetylium on the new carbanion, (ii) decarboxylation of the β-keto acid, and (iii) acetylation of the enol.



2, R_1 = H; R_2 = $CH_2CH{=}CH_2$
3, R_1 = $COCH_3$; R_2 = $CH_2CH{=}CH_2$
4, R_1 = $COCH_3$; R_2 = CH_2CHO
5, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CO_2H$
6, R_1 = $COCH_3$; R_2 = $CH{=}CHC(CH_3){=}C(CH_3)OCOCH_3$

Modification of the C-6 Substituent of 1.—Aldehyde **7** was prepared by ozonolysis[7] of **1**. Treatment of **7** with the appropriate phosphorane [$Ph_3P{=}CHCO_2Et$, $Ph_3P{=}C(CH_3)CO_2Et$, or $Ph_3P{=}C(CH_3)CHO$] gave the unsaturated compounds **8**, **10**, and **12**. Hydrolysis of **8** with cold 3 *N* NaOH gave **9**; hydrolysis of **10** with $MeSO_3H$–90% HCO_2H gave **11**. Reduction of **12** with $NaBH_4$ gave **13**. Acetylation of the phenolic OH of **13** followed by treatment with PBr_3 gave the bromoacetate **14**. Treatment of **14** with $NaCH(CO_2Et)_2$ followed by hydrolysis and pyrolysis gave material which was indistinguishable from **1** by tlc, ir, nmr, and mass spectroscopy, but which was resolved into 2 components by glc of the product of methylation (CH_2N_2). The major component (87%) corresponded in retention time to the di-*O*-Me derivative of **1**: the minor component was presumably the di-*O*-Me derivative of the cis isomer of **1**. Some trans → cis isomerization presumably occurred during the production or subsequent manipulation of **13**. Treatment of **14** with NaCN gave nitrile **15** and this on treatment with AcOH–HCl gave amide **16**. Homologation of **1** by the Arndt–Eistert method gave **17** contaminated by 5–10% of **1** (indicated by glc of the methylation product). This mixture could not be separated by conventional chromatography. Extension of the terpenoid chain of **1** to that of **18** was achieved by condensation of aldehyde **30** with $Ph_3P{=}C(CH_3)CO_2Et$ followed by alkali hydrolysis. The diunsaturated compound **19** has been briefly reported by Birch and Wright.[5] It was prepared by us by condensation of **12** with $Ph_3P{=}CHCO_2Et$ followed by alkaline hydrolysis.

Alcohol **20**[8] was obtained in low yield by microbial transformation of **1** with *Trichoderma viride* or with an unidentified bacterium. A good yield of **20** was obtained by treating **1** with $ClCO_2Et$ and reducing the resulting anhydride with $NaBH_4$.[9] A similar reduction of *O*-acetylmycophenolic acid (**51**)[10] gave **21**. Acetylation of **20** gave **22** which was selectively deacetylated

(1) B. Gosio, *Riv. Igiene Sanita Publica Ann.*, **7**, 825 (1896).

(2) (a) S. B. Carter, British Patent 1,157,100 (1969); S. B. Carter, T. J. Franklin, D. F. Jones, B. J. Leonard, S. D. Mills, R. W. Turner, and W. B. Turner, *Nature (London)*, **233**, 848 (1969). (b) R. H. Williams, D. A. Lively, D. C. DeLong, J. A. Cline, M. J. Sweeney, G. A. Doore, and S. H. Larsen, *J. Antibiot.*, **21**, 463 (1968). (c) K. Ando, S. Suzuti, G. Tarima, and K. Arima, *ibid.*, **22**, 649 (1968). (d) D. N. Planterose, *J. Gen. Virol.*, **4**, 629 (1969).

(3) J. H. Birkinshaw, H. Raistrick, and D. J. Ross, *Biochem. J.*, **50**, 630 (1952).

(4) W. R. Logan and G. T. Newbold, *J. Chem. Soc.*, 1946 (1957).

(5) A. Birch and J. J. Wright, *Chem. Commun.*, 788 (1969).

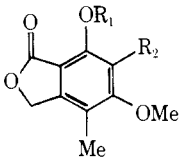
(6) B. Loev, *Chem. Ind. (London)*, 193 (1964).

(7) J. H. Birkinshaw, A. Bracken, E. N. Morgan, and H. Raistrick, *Biochem. J.*, **43**, 216 (1948).

(8) D. F. Jones and R. H. Moore, British Patent Application, 44, 435/69 (1969).

(9) K. Ishizumi, K. Koga, and S. Yamada, *Chem. Pharm. Bull. (Tokyo)*, **16**, 492 (1968).

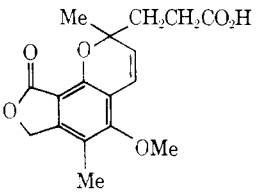
(10) P. W. Clutterbuck and H. Raistrick, *Biochem. J.*, **27**, 654 (1933).



7, R_1 = H; R_2 = CH_2CHO
8, R_1 = H; R_2 = $CH_2CH{=}CHCO_2Et$
9, R_1 = H; R_2 = $CH_2CH{=}CHCO_2H$
10, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CO_2Et$
11, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CO_2H$
12, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CHO$
13, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH_2OH$
14, R_1 = $COCH_3$; R_2 = $CH_2CH{=}C(CH_3)CH_2Br$
15, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH_2OH$
16, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH_2CONH_2$
17, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3CO_2H$
18, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)CO_2H$
19, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH{=}CHCO_2H$
20, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3OH$
21, R_1 = $COCH_3$; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3OH$
22, R_1 = $COCH_3$; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3OCOCH_3$
23, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3OCOCH_3$
24, R_1 = $COCH_3$; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3Cl$
25, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3Cl$
26, R_1 = $COCH_3$; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3OSO_3CH_3$
27, R_1 = H_3; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3OSO_2CH_3$
28, R_1 = $COCH_3$; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3NO_2$
29, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3NO_2$
30, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH_2CH_2CHO$
31, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH_2CH_2CH_3$
32, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)(CH_2)_3NH$-*n*-Pr
33, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH_2CH_2CONH_2$
34, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH_2CH_2CON(Et)_2$
35, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH_2CH_2CONHCH_2CO_2H$
36, R_1 = H; R_2 = $CH_2CH{=}C(CH_3)CH_2CH_2CO_2CH_3$
37, R_1 = H; R_2 = $CH_2CH_2CH(CH_3)CH_2CH_2CO_2H$
38, R_1 = H; R_2 = $CH_2CH_2C(CH_3)CH_2CH_2$ (O———CO)
39, R_1 = H; R_2 = $CH_2CHBrC(CH_3)CH_2CH_2$ (O———CO)
40, R_1 = H; R_2 = $CH_2CHOHC(CH_3)CH_2CH_2$ (O———CO)
41, R_1 = H; R_2 = $CH_2COC(CH_3)CH_2CH_2$ (O———CO)
42, R_1 = H; R_2 = $CH_2CH_2CH(CH_3)CHOHCH_2CO_2H$
43, R_1 = H; R_2 = $CH_2CH{=}CCH_2CH_2CO$ (CH_2———O)

with EtOH–Et_2NH to give **23**. Treatment of **21** with MesCl in pyridine gave **24** and **26** which were deacetylated by EtOH–Et_2NH to give **25** and **27**, respectively. Nitro compound **28** was prepared by heating **24** with NaI in Me_2CO and treating the resulting iodo derivative with $AgNO_2$ in Et_2O. Deacetylation of **28** with EtOH–Et_2NH gave **29**. Conversion of alcohol **20** into aldehyde **30** was accomplished with dipyridine chromium(VI) oxide in CH_2Cl_2.[11] Reduction of **30** by the Huang-Minlon procedure gave **31** and treatment of **30** with *n*-$PrNH_2$ in the presence of $NaBH_4$ gave **32**. Amides **33** and **34** were prepared from the acid chloride of *O*-acetylmycophenolic acid; amide **35** was obtained by microbial modification[12] of **1** and by treatment of **1** with $NH_2CH_2CO_2Et$ in the presence of DCC followed by alkali hydrolysis.[11] Methyl mycophenolate[10] **36** was prepared conveniently by heating **1** with MeOH containing a trace of concd H_2SO_4.

Apart from catalytic reduction, which gave dihydromycophenolic acid (**37**),[10] attempts to effect additions to the double bond of **1** resulted in cyclic products. Attempted hydration of **1** with hot dil mineral acid gave lactone **38**.[12] Bromination of **1** gave **39** and treatment of **1** with *m*-chloroperbenzoic acid gave either **40**[12,13] or **45**[12] depending upon the conditions of work-up. Oxidation of **40** gave **41**.[12] Other compounds obtained during the study of microbial modification of mycophenolic acid[12] were hydroxy acid **42** and the cyclic derivatives **43**, **44** (mycochromenic acid),[13] **45**, and **46**.



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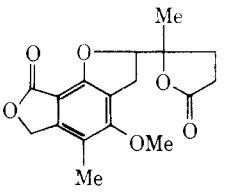

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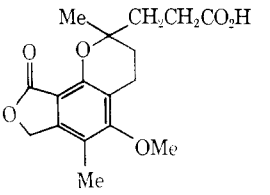

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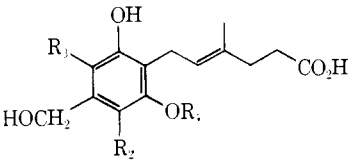
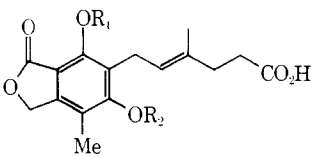
Modification of the C-4 Substituent of 1.—Functionalization of the 4-Me group of **1** by conversion into $HOCH_2$ in **47** was achieved by microbial transformation of **1** with a variety of microorganisms[12] and by one-electron oxidation of **1** with alkaline $K_3Fe(CN)_6$.[12] Methanolysis of **47** followed by alkaline hydrolysis gave the ether **48**[12] and oxidation of **1** with CrO_3–H_2SO_4 gave aldehyde **49**.[12] Decarbonylation of the latter with $RhCl(Ph_3P)_3$[14] in MeCN under reflux gave **50**.



47, R = CH_2OH
48, R = CH_2OCH_3
49, R = CHO
50, R = H

Modifications of the C-5 and C-7 Substituents of 1.—*O*-Acetylmycophenolic acid (**51**) and *O*-methylmycophenolic acid (**52**) have been previously described.[10] Although attempts to effect O-demethylation of **1** with the usual acidic reagents gave cyclic products[15] an effi-



51, R_1 = $COCH_3$; R_2 = CH_3
52, R_1 = CH_3; R_2 = CH_3
53, R_1 = H; R_2 = H
54, R_1 = $COCH_3$; R_2 = $COCH_3$
55, R_1 = H; R_2 = $COCH_3$
56, R_1 = H; R_2 = CH_2CH_3
57, R_1 = H; R_2 = $CH_2CH_2CH_3$

(11) J. C. Collins, W. W. Hess, and F. J. Frank, *Tetrahedron Lett.*, 3363 (1968).

(12) D. F. Jones, R. H. Moore, and G. C. Crawley, *J. Chem. Soc. C*, 1725 (1970).

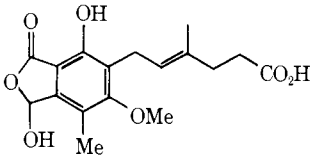
(13) I. M. Campbell, C. H. Calzadilla, N. J. McCorkindale, *Tetrahedron Lett.*, 5107 (1966).

(14) J. A. Asbom, F. H. Jardine, J. F. Young, and G. Wilkinson, *J. Chem. Soc. A*, 1711 (1966).

(15) T. Seden, R. W. Turner, and W. B. Turner, *Tetrahedron*, **25**, 4915 (1969).

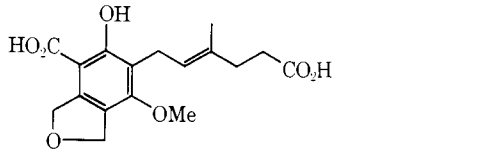
cient conversion of **1** into **53** was achieved using LiI in boiling collidine.[16] This method was also used to convert **49** into **61**. Acetylation of **53** with Ac_2O in pyridine gave **54** which was selectively deacetylated to **55** with EtOH–Et_2NH. Compounds **56** and **57** were prepared from **53** by partial alkylation (RI, Me_2CO, K_2CO_3) followed by chromatography.

Modifications Involving the Lactone Ring of 1.—Lactol **58** was obtained by microbial modification of **1**,[12]



58

and phthalan **59** by treatment of **47** with hot alkali.[12] Compound **60** was prepared from **55** by methylation with CH_2N_2 followed by ester hydrolysis with cold 3 *N* NaOH. Heating **60** in 3 *N* NaOH effected decarboxylation giving **62**. Similar alkali-induced decarboxylations gave **63** from **1**, and **64** from **53**. Hot 3 *N* NaOH effected decarboxylation and decarbonylation of **61** and **49** giving **65** and **66**, respectively.



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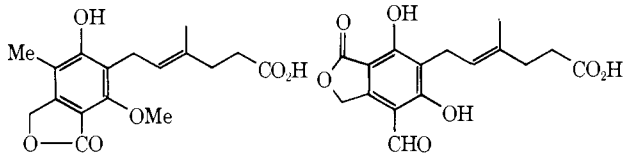
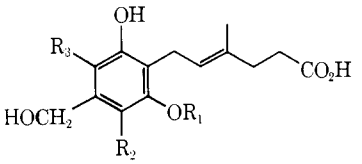


60 **61**



62, R_1 = Me; R_2 = H; R_3 = Me
63, R_1 = Me; R_2 = Me; R_3 = H
64, R_1 = H; R_2 = H; R_3 = Me
65, R_1 = H; R_2 = H; R_3 = H
66, R_1 = Me; R_2 = H; R_3 = H

Structure–Activity Relationships.—Compounds were examined for their effects on mitosis of mouse fibroblasts using the *in vitro* test system previously described.[2a] Those which showed significant antimitotic activity are given in Table I; the remaining compounds mentioned in this report were inactive.

A striking feature of the results given in Table I is the marked decrease in antimitotic properties when any of the substituents of mycophenolic acid (**1**) are modified. Thus simply replacing the MeO of **1** by EtO (**56**) resulted in an eightfold decrease in biological activity and replacement of MeO by PrO (**57**), AcO (**55**), or OH (**53**) reduced activity still further. Substitution of H for the 4-Me of **1** to give **50** resulted in a marked decrease in antimitotic activity, possibly because of an

TABLE I

ACTIVITY OF MYCOPHENOLIC ACID (**1**) AND RELATED COMPOUNDS IN SUPPRESSING MITOSIS IN MOUSE FIBROBLASTS *in Vitro*

Compd[a]	Concentration needed to suppress mitosis, ppm[b]
1	1
30	4
33	4
51	4
56	4
17[c]	8
36	8
57	8
11	15
20	15
16	15
37	15
53	15
15	30
19	30
34	30
50	30
55	30

[a] Compds not tabulated but reported in this paper were inactive at 100 ppm. [b] Compds were tested at the following dilutions (ppm): 0.5, 1, 2, 4, 8, 15, and 30. [c] Contaminated by approx 5–10% of 1 (as shown by glc).

increase in the susceptibility of the phthalide system to ring opening. Mycophenolic acid was found to be unchanged by conditions which effected ring opening of 5,7-dihydroxyphthalan-1-one and related compounds.[17] However the stability of the phthalan-1-one ring cannot be the only factor influenced by 4-Me because substitution of this group by $HOCH_2$ (**47**), HCO (**49**), or $MeOCH_2$ (sterically analogous to *n*-Pr) (**48**) resulted in loss of antimitotic activity.

Although an analog differing from **1** only in the absence of the phthalan-1-one carbonyl group was not prepared the absence of antimitotic activity in **59** and **60** and in **62–66** strongly suggests that this CO is important for biological activity. Furthermore the biological inactivity of lactol **58** suggests that an unsubstituted lactone ring is necessary for antimitotic properties. The phenolic OH of **1** seems necessary for antimitotic properties because *O*-methylmycophenolic acid (**52**) and the cyclic derivatives **44**, **45**, and **46** were inactive and *O*-acetylmycophenolic acid (**51**) was less active than **1**. The antimitotic effect of **51** may be due in part to its conversion into **1** under the conditions of the test system. The sensitivity to hydrolysis of the 7-OAc is shown by the ease which the diacetates **22** and **54** were selectively deacetylated.

The reduced biological activity of **21**, **30**, **33**, **34**, and **36** and the lack of activity of **23**, **25**, **27**, **29**, **31**, **32**, **35**, and **43** shows that the CO_2H of **1** is important for high antimitotic activity. The CO_2H of the weakly antimitotic **11** is also important because the corresponding aldehyde **12** and hydroxymethyl derivative **13** were inactive. Table I shows that modification of the chain length of the terpenoid substituent of **1** is detrimental to antimitotic activity. Thus **11** was significantly less active than **1** and amide **16** was less active than the corresponding mycophenolic acid derivative **33**. Although homologation of **1** gave **17** contaminated by 5–10% of **1** the relatively low antimitotic activity of the mixture

(16) I. T. Harrison, *Chem. Commun.*, 616 (1969).

(17) J. Blair, J. J. Brown, and G. T. Newbold, *J. Chem. Soc.*, 208 (1955).

TABLE II

EFFECT OF MYCOPHENOLIC ACID AND RELATED COMPOUNDS ON SOLID TRANSPLANTABLE TUMORS IN RATS AND MICE

Tumor	Compound[a] dose (oral), mg/kg per day	Compd 1	20	30	35	11	37	51	53	56
		Tumor wt (% control)								
Sarcoma 183 (mouse[b])	500	1.6	2.8		32.0					
	250	11.2		15.6		83.8	50.9	5.1	51.0	28.6
	150	21.4		25.5						
Yoshida tumor (rat[c])	25	10.3	16.9							
	15	22.6	37.7							
Ehrlich ascites (mouse[b])	250	47	49							
	150	70	49							
Landschütz ascites (mouse[b])	250	50	48							
	150	65	58							
	250	35		56	61					
	150	68		70	90					

[a] Compounds 12, 24, 26, 31, 32, 33, 44, and 47 showed no inhibitory effect on Sarcoma 180 (mouse[b]) at 250 mg/kg per day. [b] Chester Beatty strain. [c] Alderley Park specific pathogen free.

indicated that **17** was at most only weakly active. The importance for antimitotic effect of the olefinic double bond of **1** was shown by the reduced activity of dihydromycophenolic acid (**37**) and the lack of activity of **42** and the lactones **38**, **39**, **40**, and **41**. Increasing the degree of unsaturation to that of **19** reduced antimitotic activity and modification of the terpenoid substituent to that of the diunsaturated substituent of **18** abolished activity.

To confirm that the results obtained in the antimitosis test reflected antitumor properties a selection of the compounds described in this paper were examined for their effect against transplanted tumors in rodents (Table II) using the procedures previously reported.[2a] The similarity in the antitumor properties of **1** and **20**, **30**, and **51** was expected because it seemed likely that these compounds would be readily metabolized to **1** *in vivo*. The weak antitumor properties of **35** were also probably due to partial hydrolysis to **1** *in vivo*.

The antimitotic activity and antitumor properties of mycophenolic acid are probably due to an interference with purine metabolism. Franklin and Cook[18] have established that mycophenolic acid blocks a biosynthetic route to guanylic acid by inhibiting inosinic acid dehydrogenase, the enzyme catalyzing the oxidation of inosinic acid to xanthylic acid. It is possible that the spatial distribution of at least some of the polar groups in **1** closely simulates that in a purine derivative concerned with the action of inosinic acid dehydrogenase either at the active site or an allosteric site. Wherever the locus of action of **1** is, the results reported in this paper suggest that it is sensitive to the slightest alteration in the structure of **1**.

Experimental Section[19]

Compounds **36**, **37**, **51**, and **52**,[10] and **35**, **38**, **40–49**, **58**, and **59**[12] have been described elsewhere.

6-Allyl-7-hydroxyphthalan-1-one (**2**).—A soln of 7-hydroxyphthalan-1-one[20] (780 mg) and allyl bromide (1.6 g) in Me_2CO was heated under reflux in the presence of anhyd K_2CO_3 (700 mg) for 24 hr. H_2O and Et_2O were added and the Et_2O layer was sepd, washed with 2 *N* NaOH and H_2O, and dried ($MgSO_4$). Evapn of the Et_2O gave a solid (600 mg) which crystd from Et_2O–petr ether (bp 40–60°) as needles, mp 78–79°. This solid was heated in $PhN(Et)_2$ (10 ml) under reflux under N_2 for 2 hr. The reaction mixt was acidified with 3 *N* HCl and extd with Et_2O. Evapn of the washed (H_2O) and dried ($MgSO_4$) Et_2O soln and crystn of the residue from PhH–petr ether (bp 40–60°) gave **2**, mp 102–103°. *Anal.* ($C_{11}H_{10}O_3$) C, H.

7-Acetoxy-6-formylmethylphthalan-1-one (**4**).—A soln of **2** (750 mg) in Ac_2O (5 ml) and pyridine (5 ml) was kept at room temp for 18 hr. H_2O was added and the ppt collected by filtration. The solid was dissolved in EtOAc and the soln washed with 3 *N* HCl and H_2O. Evapn of the dried ($MgSO_4$) soln and crystn of the residue from PhH–petr ether (40–60°) gave **3**, mp 111–112° (615 mg). A soln of **3** (208 mg) in dioxane (3 ml) and H_2O (1 ml) was treated with OsO_4 (10 mg) at room temp with stirring. After 5 min and during 0.5 hr $NaIO_4$ (430 mg) was added in small portions. After stirring for 1.5 hr the reaction mixt was extd with EtOAc and the ext washed (H_2O), dried ($MgSO_4$), and evapd. The residue was chromatographed on a column of silica gel in PhH–$CHCl_3$ (3:1). Elution with PhH–$CHCl_3$ (1:1) and crystn of the solid residue from PhH–petr ether (bp 60–80) gave **4**, mp 120–122° (190 mg). *Anal.* ($C_{12}H_{10}O_5$) C, H.

7-Hydroxy-6-(3-carboxybut-2-enyl)phthalan-1-one (**5**).—A soln of **4** (215 mg) and $Ph_3P{=}C(Me)CO_2Et$[21] (400 mg) in PhH (10 ml) was heated under reflux for 6 hr. The soln was evapd and the residue chromatographed on silica gel in PhH–$CHCl_3$ (3:1). Elution with PhH–$CHCl_3$ (3:1) and crystn of the solid residue from PhH–petr ether (bp 60–80°) gave prisms, mp 120–122° (170 mg). This solid and $MsSO_3H$ (95 mg) in 90% HCO_2H were heated at 100° for 5.5 hr. After cooling H_2O was added and the precipitated solid collected. Crystn from Me_2CO gave **5**: mp 190–191° (100 mg); τ($CDCl_3$–TFA) 2.5 (d, 1 H), 2.8 (d, 1 H), 3.0 (m, 1 H), 4.6 (s, 2 H), 6.35 (d, 2 H), and 8.0 (sb, 3 H). *Anal.* ($C_{13}H_{12}O_5$) C, H.

7-Acetoxy-6-(2-acetoxy-3-methylpenta-2,4-dienyl)phthalan-1-one (**6**).—A soln of **5** (580 mg) in Ac_2O (2 ml) and pyridine (2 ml) was kept at room temp for 40 hr. H_2O and EtOAc were added, the sepd EtOAc layer was washed with 3 *N* HCl and H_2O, and the dried ($MgSO_4$) soln was evapd. Crystn of the residue from PhH gave **6**: mp 192–193° (160 mg); τ ($CDCl_3$) 2.2 (d, 1 H), 2.7 (d, 1 H), 3.2 (q, 2 H), 4.8 (s, 2 H), 7.59 (s, 3 H), 7.8 (s, 3 H), 7.94 (s, 3 H), and 8.1 (s, 3 H). *Anal.* ($C_{18}H_{18}O_6$) C, H.

6-(3-Ethoxycarbonylprop-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (**8**) **and 6-(3-Ethoxycarbonylbut-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one** (**10**).—A soln of **7**[7] (3 g) and $Ph_3P{=}CHCO_2Et$[21] (5.4 g) was heated in PhH (150 ml) under reflux for 4 hr. Evapn of the soln and column chromatography of the residue gave a solid. Crystn from EtOAc–petr

(18) T. J. Franklin and J. M. Cook, *Biochem. J.*, **113**, 515 (1969).

(19) Melting points were taken on a Kofler hot stage microscope. Anal. and prep tlc was carried out on silica gel GF using PhH–EtOAc–HCO_2H (66:33:1) for development and uv (254 mμ). $FeCl_3$–EtOH, or CrO_3–H_2SO_4 for detection. Hopkin-Williams (MFC Grade) silica gel was used for column chromatography. Satisfactory ir spectra (Perkin-Elmer Model 157), nmr spectra (Varian instruments A60 and HA 100, using TMS as internal standard) and mass spectra (Hitachi, Perkin-Elmer RMU 6D) were obtained for all compds reported. In nmr description s = singlet, sb = singlet broadened by spin–spin coupling through the double bond, d = doublet, t = triplet, q = quartet, m = multiplet. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

(20) E. L. Eliel, D. E. Rivard, and A. W. Burgstahler, *J. Org. Chem.*, **18**, 1679 (1953).

(21) O. Isler, H. Gutmann, M. Montavon, R. Ruegg, G. Ryser, and P. Zeller, *Helv. Chim. Acta*, **40**, 1242 (1957).

ether (bp 60–80°) gave **8**, mp 112–114° (1.3 g). *Anal.* ($C_{16}H_{18}O_6$) C, H.

A similar experiment using $Ph_3P{=}C(Me)CO_2Et$[21] in place of $Ph_3P{=}CHCO_2Et$ gave **10**, mp 131–132° (from PhH–petr ether, bp 60–80°) (60% yield). *Anal.* ($C_{17}H_{20}O_6$) C, H.

6-(3-Carboxyprop-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (9).—A soln of **8** (100 mg) in 3 *N* NaOH was kept at room temp for 0.5 hr. The reaction mixt was acidified with 3 *N* HCl and extd with EtOAc. The EtOAc ext was extd with satd aq $NaHCO_3$ and the alkaline ext acidified and the product isolated in EtOAc. Evapn of the dried ($MgSO_4$) EtOAc and crystn of the residue from Me_2CO gave **9**: mp 177–179° (49 mg); τ ($CDCl_3$–DMSO-d_6) 3.0 (m, 1 H), 4.3 (d, 1 H), 4.81 (s, 2 H), 6.28 (s, 3 H), 6.44 (d, 2 H), and 7.87 (s, 3 H). *Anal.* ($C_{14}H_{14}O_6$) C, H.

6-(3-Carboxybut-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (11).—A soln of **10** (240 mg) and $MeSO_3H$ (70 mg) in 90% HCO_2H (0.75 ml) was heated at 100° for 6 hr. H_2O was added to the cooled soln and the pptd solid was collected and crystd from AcOH to give **11**: mp 219–222° (180 mg); τ ($CDCl_3$–TFA) 3.0 (t, 1 H), 4.68 (s, 2 H) 6.16 (s, 3 H), 6.32 (d, 2 H), 7.8 (s, 3 H), and 7.95 (sb, 3 H). *Anal.* ($C_{15}H_{16}O_6$) C, H.

6-(3-Formylbut-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (12).—A soln of **7**[7] (400 mg) and $Ph_3P{=}C(Me)CHO$[22] (600 mg) in PhH (30 ml) was heated under reflux for 24 hr. Evapn of the soln and crystn of the residue from EtOH gave **12**: mp 110–112° (215 mg); τ ($CDCl_3$) 0.64 (s, 1 H, CHO). *Anal.* ($C_{15}H_{16}O_5$) C, H.

6-(4-Hydroxy-3-methylbut-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (13).—A soln of **12** (2 g) in EtOH (120 ml) was treated with $NaBH_4$ (500 mg) in portions over 0.5 hr and kept at room temp for 24 hr. The soln was concd to 20 ml and treated with 3 *N* HCl and EtOAc. The EtOAc layer was sepd, washed (H_2O), dried ($MgSO_4$), and evapd to give a solid. Crystn from PhH–petr ether (bp 60–80°) gave **13**, mp 98–108° (1.8 g). *Anal.* ($C_{15}H_{18}O_5$) C, H.

6-(4-Cyano-3-methylbut-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (15).—A soln of **13** (278 mg) in MeOH (1 ml) was treated with 1 *N* NaOH (10 ml) and the soln was evapd. After evapn from PhH several times the residue was suspended in PhH (5 ml) and treated with AcCl (78 mg) in PhH. After 1 hr the reaction mixt was filtered and residue from the filtrate crystd from PhH to give the *O*-acetate of **13**: mp 148–155° (135 mg); τ ($CDCl_3$), 6.05 (s, 2 H, CH_2O) and 7.6 (s, 3 H, CH_3CO). *Anal.* ($C_{17}H_{20}O_6$) C, H. This material (100 mg) in PhH (2.5 ml) and $CHCl_3$ (2.5 ml) was added dropwise to PBr_3 (32 mg) in PhH (3 ml) at 5° and the mixt was kept at room temp for 2 hr. H_2O and $CHCl_3$ were added and the $CHCl_3$ layer was sepd, washed (H_2O), and dried ($MgSO_4$). Evapn of the soln and crystn of the residue from PhH–petr ether (bp 60–80°) gave needles of **14**: mp 115–120° (52 mg); τ 6.06 (s, 2 H, CH_2Br). *Anal.* ($C_{17}H_{19}BrO_5$) C, H, Br. A soln of **14** (82 mg) in DMF (2 ml) was treated with NaCN (25 mg) in DMF (3 ml) and kept at room temp for 24 hr. HCl (1 *N*) and EtOAc were added and the EtOAc layer was sepd, washed (H_2O), dried ($MgSO_4$), and evapd. Crystn of the residue from PhH–petr ether (bp 60–80°) gave **15**: mp 164–168° (33 mg); γ_{max}^{Nujol} 2280 (C≡N); τ ($CDCl_3$) 6.95 (s, 2 H, CH_2CN). *Anal.* ($C_{16}H_{17}NO_4$) C, H, N.

6-(4-Carbamoyl-3-methylbut-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (16).—A soln of **15** (74 mg) in AcOH (1 ml) and concd HCl (1.5 ml) was kept at room temp for 24 hr. H_2O and $CHCl_3$ were added and the sepd $CHCl_3$ layer was washed (H_2O), dried ($MgSO_4$), and evapd. Prep tlc of the residue and crystn of the product from Me_2CO–petr ether (bp 60–80°) gave **15**: mp 186–189° (32 mg); τ (DMSO-d_6) 4.73 (s, 2 H), 4,75 (m, 1 H), 6.2 (s, 3 H), 6.65 (d, 2 H), 7.28 (s, 2 H), 7.9 (s, 3 H), and 8.2 (sb, 3 H). *Anal.* ($C_{16}H_{19}NO_5$) C, H, N.

Mycophenolic Acid from 14.—A soln of **14** (380 mg) in PhMe (5 ml) was added to a suspension of $NaCH(CO_2Et)_2$ in PhMe, prepd from Na (23 mg) and $CH_2(CO_2Et)_2$ in PhMe (5 ml) under reflux for 1 hr, and the mixt was heated under reflux for 7 hr. H_2O was added and the PhMe layer was sepd, washed (H_2O), dried ($MgSO_4$), and evapd. The residue and KOH (500 mg) were heated in MeOH (5 ml) under reflux for 7 hr. HCl (3 *N*) and $CHCl_3$ were added and the $CHCl_3$ layer was sepd and extd with aq $NaHCO_3$. The alk ext was acidified with 3 *N* HCl and extd with $CHCl_3$. Evapn of the washed (H_2O) and dried ($MgSO_4$) $CHCl_3$ soln gave a solid residue (81 mg) which was heated at 140–150° for 0.5 hr. Prep tlc of the product and crystn from EtOH gave prisms, mp 126–132° (21 mg). This solid could not be distinguished from **1** by tlc, ir, nmr, and mass spectroscopy. However permethylation of the solid with CH_2N_2 in Et_2O–MeOH gave a gum which was sepd by glc (5% silicone rubber gum SE 30 on Chromosorb G) into 2 components; the major component (87% of product) corresponded in retention time to methyl *O*-methylmycophenolate, the minor product was presumed to be the permethyl derivative of the cis isomer of **1**.

Attempted Preparation of 17.—A soln of *O*-acetylmycophenolic acid **51**[10] (1.3 g) in $SOCl_2$ (15 ml) was heated under reflux for 1.5 hr. The residue obtained on complete removal of $SOCl_2$ was dissolved in Et_2O and added over 0.5 hr to excess CH_2N_2 in Et_2O. After being kept at room temp for 2.5 days the Et_2O was evapd to give an oil, $\gamma_{max}^{dioxane}$ 2100 cm^{-1}, which was dissolved in MeOH (7 ml). To this soln was added over 15 min a soln of $PhCO_2Ag$ (100 mg) in Et_3N (900 mg). The mixt was heated under reflux for 0.5 hr and filtered and the solvent was evapd. The residue was treated with NH_3 in Me_2CO, the solvent was evapd, and the residue was chromatographed on a column of silica gel in EtOAc–petr ether (bp 60–80°) (1:6). Elution with EtOAc–petr ether (bp 60–80°) (1:3) gave a gum which was treated with 5 *N* NaOH (1 ml) and MeOH (8 ml) under reflux for 2 hr. HCl (3 *N*) and $CHCl_3$ were added and the sepd $CHCl_3$ layer was washed (H_2O), dried ($MgSO_4$), and evapd. Crystn from EtOAc–petr ether (bp 60–80°) gave prisms: mp 128–130° (145 mg); τ 4.8 (s, 2 H,), 4.8 (m, 1 H), 6.25 (s, 3 H), 6.62 (d, 2 H), 7.7 (m, 4 H) 7.83 (s, 3 H), 8.06 (m, ~2 H), and 8.2 (sb, 3 H). The solid gave a single spot on tlc and gave the correct elementary analysis for **17** but mass spectrometry showed ions due to **1** in addition to the ions expected for **17**. Methylation of the solid with excess CH_2N_2 in Et_2O gave a gum which was resolved by analytical glc into 2 components, one corresponding in retention time to methyl *O*-methylmycophenolate and the other (90–95% of the product) having a longer retention, was presumed to be the di-*O*-Me derivative of **17**.

6-(5-Carboxy-3-methylpenta-2,4-dienyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (19).—A soln of **12** (2.4 g) and $Ph_3P{=}CHCO_2Et$[21] (3 g) in PhH was heated under reflux for 24 hr. The PhH was evapd and the residue was treated with MeOH (40 ml) and 3 *N* NaOH (100 ml) under reflux for 3 hr. The cooled reaction mixt was acidified with 3 *N* HCl and extd with EtOAc. The EtOAc soln was extd with aq $NaHCO_3$ and this ext was acidified with 3 *N* HCl and extd with EtOAc. Evapn of the washed (H_2O) and dried ($MgSO_4$) EtOAc ext gave a solid (2 g). Crystn from Me_2CO gave **19**: mp 176–177°; τ (pyridine-d_5) 2.1 (d, 1 H), 3.0 (d, 1 H), 3.74 (t, 1 H), 4.9 (s, 2 H), 6.32 (s, 3 H), 6.52 (d, 2 H), and 8.02 (s, 6 H). *Anal.* ($C_{17}H_{18}O_6$) C, H.

6-(5-Carbamoyl-3-methylpent-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (33) and 6-(5-*N*,*N*-Diethylcarbamoyl-3-methylpent-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (34).—*O*-Acetylmycophenolic acid[9] (**51**) (1 g) was converted into its acid chloride with $SOCl_2$ and this was triturated with NH_4OH (sp g, 0.88) to give a yellow solid. Crystn from MeOH gave prisms of **33**, mp 196–200° (800 mg). *Anal.* ($C_{17}H_{21}NO_5$) C, H, N.

The acid chloride (700 mg) in PhH (10 ml) was treated with Et_2NH (2 ml) in PhH (10 ml) at room temp. H_2O was added and the PhH layer was sepd, washed with H_2O, and dried ($MgSO_4$). Evapn of the PhH and crystn of residue from PhH–petr ether (bp 60–80°) gave prisms of **34**, mp 93–95° (580 mg). *Anal.* ($C_{21}H_{29}NO_5$) C, H, N.

7-Hydroxy-6-(6-hydroxy-3-methylhex-2-enyl)-5-methoxy-4-methylphthalan-1-one (20)[8,23] **and 7-Acetoxy-6-(6-hydroxy-3-methylhex-2-enyl)-5-methoxy-4-methylphthalan-1-one (21).**—A soln of $ClCO_2Et$ (1.92 ml) in THF (5 ml) was added to soln of **1** (3.2 g) and Et_3N (2.8 ml) in THF (50 ml) at −5° and the mixt was stirred for 0.5 hr. $Et_3NH^+Cl^-$ was removed by filtration and the filtrate was added over 0.5 hr to $NaBH_4$ (1.9 g) in H_2O (20 ml) at 10–15°.[9] The mixt was stirred at room temp for 18 hr, acidified with 3 *N* HCl and extd with EtOAc. The EtOAc ext was washed (H_2O), dried ($MgSO_4$), and evapd and the residue was heated in 3 *N* NaOH (30 ml) at 90° for 0.5 hr. After acidification with 3 *N* HCl the reaction mixt was extd with EtOAc. The EtOAc soln was washed with aq $NaHCO_3$ and H_2O and dried ($MgSO_4$). Evapn of the solvent and crystn of the residue from EtOAc–petr

(22) S. Trippet and D. M. Walker, *J. Chem. Soc.*, 1266 (1961).

(23) Using procedures described elsewhere[12] **20** was obtained in about 3% yield from fermentations of **1** with *Trichoderma viride* (A.C.C. 718) and with an unidentified bacterium (A.C.C. 2237).

ether (bp 60–80°) gave **20**: mp 105–107° (2.7 g); τ ($CDCl_3$) 4.82 (t, 1 H), 4.88 (s, 2 H), 6.3 (s, 3 H), 6.47 (t, 2 H), 7.9 (s, 3 H), 8.01 (m, 2 H), 8.24 (sb 3 H), and 8.38 (m, 2 H). *Anal.* ($C_{17}H_{22}O_5$) C, H.

Repetition of this procedure with *O*-acetylmycophenolic acid (**51**) in place of **1**, and using 1 molar equiv of $ClCO_2Et$ and omitting the NaOH hydrolysis, gave **21**: mp 75° [from EtOAc–petr ether (bp 60–80°)]; ($CDCl_3$) 6.5 (t, 2 H, C**H**$_2$OH) and 7.7 (s, 3 H, ArOAc). *Anal.* ($C_{19}H_{24}O_6$) C, H.

7-Acetoxy-6-(6-acetoxy-3-methylhex-2-enyl)-5-methoxy-4-methylphthalan-1-one (22) and Its Conversion into 23.—A soln of **20** (1 g) in Ac_2O (5 ml) and pyridine (5 ml) was kept at room temp for 24 hr. H_2O and EtOAc were added and the sepd EtOAc layer was washed with 3 *N* HCl and H_2O. Evapn of the dried ($MgSO_4$) EtOAc soln gave a gum which on crystn from EtOAc–petr ether (bp 60–80°) gave **22**: mp 75–76° (1 g); τ ($CDCl_3$) 6.1 (t, 2 H, C**H**$_2$OAc). *Anal.* ($C_{21}H_{26}O_7$) C, H. A soln of **22** (500 mg) in EtOH (20 ml) and Et_2NH (20 ml) was kept at 0° for 10 min. The reaction mixt was acidified with 3 *N* HCl with cooling and extd with EtOAc. Evapn of the washed (H_2O) and dried ($MgSO_4$) EtOAc soln gave **23** as a sticky solid which could not be obtained cryst: $\gamma_{max}^{CHCl_3}$ 3300 and 1720 cm^{-1}; τ ($CDCl_3$) 4.78 (m, 1 H), 4.83 (s, 2 H), 6.0 (t, 2 H), 6.24 (s, 3 H), 6.62 (d, 2 H), 7.84 (s, 3 H), 7.9 (m, 2 H) 7.98 (s, 3 H), 8.2 (s, 3 H), and 8.22 (m, 2 H); the mass spectrum was as expected for **23**.

7-Acetoxy-6-(6-chloro-3-methylhex-2-enyl)-5-methoxy-4-methylphthalan-1-one (24) and 7-Acetoxy-6-(6-methanesulfonyloxy-3-methylhex-2-enyl)-5-methoxy-4-methylphthalan-1-one (26).—A soln of **21** (1.16 g) and $MeSO_2Cl$ (0.3 ml) in pyridine (10 ml) was kept at room temp overnight. H_2O and EtOAc were added and the sepd EtOAc layer was washed with 3 *N* HCl and H_2O. Evapn of the dried ($MgSO_4$) EtOAc soln gave a gum which was subjected to preparative tlc to give **24**: mp 85–86° [from EtOAc–petr ether (bp 60–80°)] (318 mg); τ ($CDCl_3$) 5.86 (t, 2 H) and 7.05 (s, 3 H) (C**H**$_2$·OSO$_2$C**H**$_3$). *Anal.* ($C_{20}H_{26}O_8S$) C, H.

6-(6-Chloro-3-methylhex-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (25) and Conversion of 26 into 27.—A soln of **24** (100 mg) in EtOH (10 ml) and Et_2NH (10 ml) was kept at 0° for 10 min. The reaction mixt was acidified with 3 *N* HCl and extd with EtOAc. Evapn of the washed (H_2O) and dried EtOAc soln and crystn of the residue from EtOAc–petr ether (bp 60–80°) gave **25**, mp 110–112° (60 mg). *Anal.* ($C_{17}H_{21}ClO_4$) C, H.

A similar procedure was used to convert **26** into **27** which was obtained as a gum: τ ($CDCl_3$) 4.76 (t, 1 H), 4.83 (s, 2 H) 5.86 (t, 2 H), 6.24 (s, 3 H), 6.62 (d, 2 H) 7.08 (s, 3 H), 7.9 (m, 2 H) 8.17 (m, 2 H), and 8.22 (sb, 3 H). The compound gave a single spot on tlc and gave the expected mass spectrum.

7-Acetoxy-5-methoxy-4-methyl-6-(3-methyl-6-nitrohex-2-enyl)phthalan-1-one (28) and Its Conversion into 29.—A mixture of **24** (1.8 g) and NaI (1.2 g) was heated in Me_2CO under reflux for 20 hr. Solid was removed by filtration and the filtrate was evaporated to give a pale yellow solid: mp 66–70°; τ ($CDCl_3$) 6.88 (t, 2 H, CH_2**I**). This was dissolved in Et_2O and the solution was added to a stirred suspension of freshly prepared $AgNO_2$ (900 mg) in Et_2O at 0°. The suspension was stirred at 0° for 5 hr and at room temperature for 40 hr. The reaction mixture was filtered and the filtrate was evaporated. Crystallization of the residue from Et_2O–petrol ether (60–80°) gave **28**, mp 98–100° (400 mg); 5.7 (t, 2 H, C**H**$_2$NO$_2$), γ_{max}^{Nujol} 1560 cm^{-1} (NO_2). *Anal.* ($C_{19}H_{23}NO_7$) C, H, N.

Treatment of **28** (50 mg) with Et_2NH (3 ml) in EtOH (3 ml) at room temperature gave **29** which crystallized from Et_2O: mp 95–98°, γ_{max}^{Nujol} 1560 cm^{-1} (NO_2). *Anal.* ($C_{17}H_{21}O_6N$) C, H, N.

6-(5-Formyl-3-methylpent-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (30).—Dipyridinechromium(VI) oxide[11] (8 g) was added to CH_2Cl_2 (100 ml) whereupon a brown precipitate formed. The supernatant solution was decanted into a solution of **20** (1 g) in CH_2Cl_2 (80 ml). After 10 min at room temperature the brown precipitate was removed by filtration and the filtrate evaporated. The residue was stirred with EtOAc and filtered and the filtrate evaporated. The residue was adsorbed on silica gel and placed on top of a column of silica gel in petr ether (bp 60–80°). Elution with EtOAc–petr ether (bp 60–80°) (1:3) and crystallization of the product from EtOAc–petr ether (bp 60–80°) gave **30**: mp 89–91° (370 mg); τ ($CDCl_3$) 0.3 (t, 1 H, CH_2C**H**O). *Anal.* ($C_{17}H_{20}O_5$) C, H.

7-Hydroxy-5-methoxy-4-methyl-6-(3-methylhex-2-enyl)-phthalan-1-one (31).—A soln of **30** (50 mg), KOH (30 mg), and $NH_2NH_2·H_2O$ (20 mg) in diethylene glycol was heated at 100° for 1.5 hr, then maintained at 150° for 0.5 hr to remove H_2O, and finally at 190° for 4 hr. HCl (3 *N*) and EtOAc were added and the sepd EtOAc soln washed (H_2O), dried ($MgSO_4$), and evapd. The residue was chromatographed on a column of silica gel, elution with EtOAc–petr ether (bp 60–80°) (1:9) giving a colorless solid. Crystn from EtOAc–petr ether (bp 60–80°) gave **31**: mp 98–100° (35 mg); τ ($CDCl_3$) 9.2 (t, 3 H, CH_2C**H**$_3$). *Anal.* ($C_{17}H_{22}O_4$) C, H.

7-Hydroxy-5-methoxy-4-methyl-6-(3-methyl-6-*n*-propylaminohex-2-enyl)phthalan-1-one (32).—A soln of **30** (570 mg) and *n*-$PrNH_2$ (114 mg) in MeOH (60 ml) was maintained at 0° for 0.5 hr and then treated with $NaBH_4$ (360 mg), added portionwise over 1 hr. The reaction mixt was maintained at 0° for a further 1.5 hr and then acidified with AcOH. Most of the MeOH was evapd and 3 *N* HCl and EtOAc were added. The sepd aq layer was made alkaline with 3 *N* NaOH and extd with EtOAc. The EtOAc ext was washed (H_2O), dried ($MgSO_4$), and evapd. Stirring of the residue with Me_2CO gave **32**, mp 153–154° (80 mg). *Anal.* ($C_{20}H_{29}NO_4$) N. The mass spectrum was that expected for **32**.

6-(7-Carboxy-3,7-dimethylhepta-2,6-dienyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (18).—A soln of **30** (800 mg) and $Ph_3P{=}C(Me)CO_2Et$[21] (1 g) in PhH (40 ml) was heated under reflux for 4 hr, and the soln was evapd. The residue was subjected to prep tlc and the phenolic ($FeCl_3$ spray) material was isolated and heated in 3 *N* NaOH (15 ml) and MeOH (15 ml) under reflux for 3 hr. After acidification with 3 *N* HCl the reaction mixt was extd with EtOAc and the EtOAc soln was washed (H_2O), dried ($MgSO_4$), and evapd. Crystn of the residue from EtOAc–petr ether (bp 60–80°) gave **18**, mp 130–133° (450 mg); τ ($CDCl_3$) 3.25 (t, 1 H), 4.84 (t, 1 H) 4.89 (s, 2 H), 6.3 (s, 3 H), 6.68 (d, 2 H), 7.8 (m, 4 H), 7.9 (s, 3 H), and 8.24 (sb, 6 H). *Anal.* ($C_{20}H_{24}O_6$) C, H.

6β-Bromo-β-(2-methyl-5-oxotetrahydrofur-2-yl)ethyl-7-hydroxy-5-methoxy-4-methylphthalan-1-one (39).—Br_2 was added dropwise to a soln of **1** (2 g) in $CHCl_3$ (80 ml) at 0° until a slight excess was present. After 0.5 hr the $CHCl_3$ soln was washed with H_2O, dried ($MgSO_4$), and evapd. Crystn of the residue from EtOAc–petr ether (bp 60–80°) gave **39**, mp 178–180° (3.0 g). *Anal.* ($C_{17}H_{19}BrO_6$) C, H, Br.

6-(5-Carboxy-3-methylpent-2-enyl)-7-hydroxy-5-methoxyphthalan-1-one (50).—A soln of **49**[12] (2.4 g) in MeCN (100 ml) was heated with $RhCl(PPh_3)_3$[14] (4.8 g) under reflux under O_2-free N_2 for 18 hr. Solvent was evapd and the residue triturated with EtOH (20 ml) and filtered. The filtrate was evapd and the residue was dissolved in EtOAc (100 ml). The EtOAc soln was extd with aq $NaHCO_3$ and the alkaline ext acidified with 3 *N* HCl and extd with EtOAc. The EtOAc soln was washed (H_2O), dried ($MgSO_4$), and evapd and the residue, in EtOH (20 ml), was treated with $NaBH_4$ (150 mg) to convert any **49** into **47**: on tlc R_f of **50** = R_f of **49**). The reaction mixt was kept at room temp for 0.5 hr and then treated with 3 *N* HCl and EtOAc. The EtOAc layer was sepd, washed (H_2O), dried ($MgSO_4$), and evapd. The residue was subjected to prep tlc and the phenolic ($FeCl_3$ spray) component isolated and crystd from EtOAc–petr ether (bp 60–80°) to give **50**: mp 158–162° (350 mg); τ (Me_2CO-d_6) 3.29 (s, 1 H, ArH). *Anal.* ($C_{16}H_{18}O_6$) C, H.

6-(5-Carboxy-3-methylpent-2-enyl)-5,7-dihydroxy-4-methylphthalan-1-one (53) and 6-(5-Carboxy-3-methylpent-2-enyl)-5,7-dihydroxy-4-formylphthalan-1-one (61).—A suspension of **1** (15 g) in collidine (60 ml) was added to a stirred soln of LiI (dried at 300° under N_2, 1 hr) (25 g) in collidine (60 ml) maintained at reflux under N_2. After heating under reflux for 2.5 hr the cooled reaction mixt was acidified with 3 *N* HCl and extd with EtOAc. Concn of the washed (H_2O) and dried ($MgSO_4$) EtOAc soln gave **53**, mp 147–149° (12 g). *Anal.* ($C_{16}H_{18}O_6$) C, H.

A similar procedure using **49**[12] in place of **1** gave 60% yield of **61**, mp 218–220°. *Anal.* ($C_{16}H_{16}O_7$) C, H.

5-Acetoxy-6-(5-carboxy-3-methylpent-2-enyl)-7-hydroxy-4-methylphthalan-1-one (55).—A soln of **53** (4 g) in Ac_2O (20 ml) and pyridine (20 ml) was kept at room temp for 48 hr. H_2O was added and the ppt was collected and crystd from EtOAc–petr ether (bp 60–80°) to give **54**, mp 146–148° (4.1 g). *Anal.* ($C_{20}H_{22}O_8$) C, H. This solid in EtOH (80 ml) was treated with Et_2NH (80 ml) and the soln was kept at room temp for 10 min. The reaction mixt was acidified with 3 *N* HCl with cooling and extd with EtOAc. Evapn of the washed (H_2O) and dried ($MgSO_4$)

soln and crystn of the residue from EtOAc–petr ether (bp 60–80°) gave **55**, mp 142–143° (3.3 g). *Anal.* ($C_{18}H_{20}O_7$) C, H.

6-(5-Carboxy-3-methylpent-2-enyl)-5-ethoxy-7-hydroxy-4-methylphthalan-1-one (56) and 6-(5-Carboxy-3-methylpent-2-enyl)-7-hydroxy-4-methyl-5-*n*-propyloxyphthalan-1-one (7).—A soln of **53** (5 g) and EtI (20 ml) in Me_2CO (125 ml) was heated under reflux in the presence of anhyd K_2CO_3 (20 g) until tlc showed that a significant amount of the desired product had been formed (36–48 hr). The reaction mixt was filtered and the solid residue acidified with 2 *N* HCl and extd with EtOAc. The washed (H_2O) and dried ($MgSO_4$) EtOAc soln was evapd and the residual solid subjected to prep tlc. Product **56** (500 mg) had mp 143–145° [from EtOAc–petr ether (bp 60–80°)]; τ ($CDCl_3$) 6.18 (q, 2 H) and 8.62 (t, 3 H) (CH_2CH_2O). *Anal.* ($C_{18}H_{22}O_6$) C, H.

Using a similar procedure **53** (3 g) was converted into **57**: mp 125–127° (from EtOAc–petr ether (bp 60–80°) (240 mg); τ ($CDCl_3$) 6.25 (t, 2 H), 8.1 (m, 2 H), and 8.91 (t, 3 H) ($CH_3CH_2CH_2O$). *Anal.* ($C_{19}H_{24}O_6$) C, H.

6-(5-Carboxy-3-methylpent-2-enyl)-5-hydroxy-7-methoxy-4-methylphthalan-1-one (60).—A soln of **55** (500 mg) in MeOH (70 ml) was allowed to stand with excess of CH_2N_2 in Et_2O for 1 hr at 5°. The Et_2O was evapd, 3 *N* NaOH (10 ml) was added, and the soln was kept at room temp for 20 min. The reaction mixt was acidified with 3 *N* HCl and extd with EtOAc. Evapn of the washed (H_2O) and dried ($MgSO_4$) EtOAc soln and crystn of the residue from EtOAc–petr ether (bp 60–80°) gave **60**, mp 156–158° (310 mg). *Anal.* ($C_{17}H_{29}O_6$) C, H.

3-(5-Carboxy-3-methylpent-2-enyl)-2-hydroxy-6-hydroxymethyl-4-methoxytoluene (62).—A soln of **60** (100 mg) in 3 *N* NaOH (30 ml) was heated under reflux under N_2 for 24 hr. After acidification of the reaction mixt with 3 *N* HCl the product was isolated in EtOAc and crystd from EtOAc–petr ether (bp 60–80°) to give **62**: mp 128–132° (55 mg); τ (Me_2CO-d_6) 3.42 (s, 1 H), 4.7 (t, 1 H), 5.5 (s, 2 H), 6.3 (s, 3 H), 6.7 (d, 2 H), 7.76 (m, 4 H), 7.92 (s, 3 H), and 8.28 (sb, 3 H). *Anal.* ($C_{18}H_{22}O_5$) C, H.

3-(5-Carboxy-3-methylpent-2-enyl)-4-hydroxy-6-hydroxymethyl-2-methoxytoluene (63).—A soln of 1 (2 g) in 3 *N* NaOH (100 ml) was heated under reflux for 18 hr during which time a fast stream of N_2 caused a reduction in vol to 20 ml and the production of a ppt. The reaction mixt was acidified with 3 *N* HCl and extd with EtOAc. The EtOAc soln was washed (H_2O), dried ($MgSO_4$), and evapd and the residue was subjected to prep tlc. Isolation of the component giving an immediate response to CrO_3–H_2SO_4 spray and crystn from EtOAc–petr ether (bp 60–80°) gave **63**: mp 126–128° (150 mg); τ 3.28 (s, 1 H, ArH) and 5.54 (s, 2 H, $ArCH_2OH$). *Anal.* ($C_{16}H_{22}O_5$) C, H.

3-(5-Carboxy-3-methylpent-2-enyl)-2,4-dihydroxy-6-hydroxymethyltoluene (64).—A soln of **53** (1 g) in 3 *N* NaOH (80 ml) was heated under reflux under N_2 for 18 hr. It was acidified with 3 *N* HCl and extd with EtOAc. Concn of the washed (H_2O) and dried ($MgSO_4$) EtOAc soln gave **64**; mp 152–154° (800 mg); τ (Me_2CO-d_6) 3.5 (s, 1 H, ArH) and 5.52 (s, 2 H, $ArCH_2OH$). *Anal.* ($C_{15}H_{20}O_5$) C, H.

2-(5-Carboxy-3-methylpent-2-enyl)-5-hydroxymethylresorcinol (65).—A soln of **61** (100 mg) in 3 *N* NaOH was heated under reflux under N_2 for 18 hr. Acidification of the reaction mixt with 3 *N* HCl, isolation of the product in EtOAc, and crystn from EtOAc–petr ether (bp 60–80°) gave **65**: mp 135–138° (46 mg); τ (Me_2CO-d_6) 3.67 (s, 2 H, ArH) and 5.64 (s, 2 H, $ArCH_2OH$). *Anal.* ($C_{14}H_{18}O_5$) C, H.

2-(5-Carboxy-3-methylpent-2-enyl)-5-hydroxymethyl-3-methoxyphenol (66).—A soln of **49**[12] (400 mg) in 3 *N* NaOH was heated under reflux under N_2 for 18 hr. After acidification with 3 *N* HCl the reaction mixt was extd with EtOAc and the ext washed (H_2O), dried ($MgSO_4$), and evapd. Prep tlc of the residue gave **59** (24 mg) and a solid which was crystd from EtOAc–petr ether (bp 60–80°) to give **66**: mp 115–118° (52 mg); τ (Me_2CO-d_6) 3.57 (s, 1 H), 3.58 (s, 1 H) (ArH), and 5.6 (s, 2 H, $ArCH_2OH$). *Anal.* ($C_{15}H_{20}O_5$) C, H.

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Potential Antitumour Agents. 11. 9-Anilinoacridines

B. F. CAIN,* G. J. ATWELL, AND R. N. SEELYE

Cancer Chemotherapy Laboratory, Cornwall Geriatric Hospital, Auckland, New Zealand

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It is suggested that the group of coplanar, fully ionized, cationic agents which demonstrate high activity against the L1210 leukemia may lodge temporarily in the minor groove of a polynucleotide helix as a site of residence. A later intercalated mode of drug binding leads to cell death. From a consideration of binding to these sequential sites and to modes of cellular entry certain nonquaternary bis bases active against the L1210 have been prepared.

In the last paper of this series[1] we developed a view of the structure–activity relationships of a large series of cationic agents which are essentially fully ionized at physiological pH values. It was then suggested that the structural requirements for activity with these agents [for example, **1** (R = CH_3) and isometamidium **2**] could indicate a site equivalent to the minor groove in a polynucleotide duohelix. Further, such molecules might reside in this site until unwinding of the helix caused separation of the purine–pyrimidine pairs. The agents could then intercalate between adjacent base pairs with the planar aromatic nuclei contacting the purine–pyrimidine nuclei and the cationic charges matching to negative charges on the sugar–phosphate chains.

Intercalation has been well documented as regards homidium (**3**).[1] It also appears reasonable to expect derived agents such as isometamidium (**2**) to intercalate. Extension of such views to the coplanar biscationic agents grouped earlier[1] (*e.g.*, **1**) leads to the speculation that intercalation may be an important event with this group also. Combining this concept with structure–activity relationships concerned with matching of drug structure to the minor groove of a polynucleotide helix, a novel viewpoint emerges that two sets of structure–activity relationships may operate for these agents: (a) structural characteristics allowing binding in the minor groove of a polynucleotide helix as a site of residence; (b) features consonant with binding in a later available intercalation site–the actual site of action. Such views could conveniently explain the requirement for an approximate charge separation of 20 Å in molecules such as **1**[1] for high antileukemic activity. Such dimensions would allow the agent to extend through a

(1) B. F. Cain, G. J. Atwell, and R. N. Seelye, *J. Med. Chem.*, **12**, 199 (1969).