

Preparation and Antitumor Properties of Analogs and Derivatives of Mycophenolic Acid

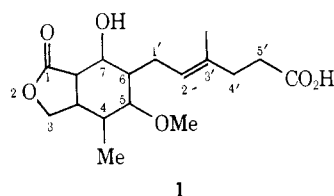
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Sixty-five derivatives and analogs of the antitumor agent, mycophenolic acid (**1**), have been obtained by modifying all positions of the molecule except C-1'. None of these compounds was as effective as **1** in suppressing cell division in mouse 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 groups² to have antitumor properties and the compound is presently undergoing clinical trial. Structure **1** was proposed in 1952³ and confirmed in 1957.⁴ A recent total synthesis⁵ has confirmed the trans configuration at the double bond.

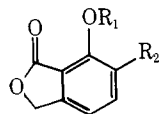


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The present paper reports derivatives and analogs of **1** which were prepared to determine the structural features necessary for antitumor activity.

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 $\text{Ph}_3\text{P}=\text{C}(\text{Me})\text{CO}_2\text{Et}$; 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 unex-



2, $\text{R}_1 = \text{H}$; $\text{R}_2 = \text{CH}_2\text{CH}=\text{CH}_2$

3, $\text{R}_1 = \text{COCH}_3$; $\text{R}_2 = \text{CH}_2\text{CH}=\text{CH}_2$

4, $\text{R}_1 = \text{COCH}_3$; $\text{R}_2 = \text{CH}_2\text{CHO}$

5, $\text{R}_1 = \text{H}$; $\text{R}_2 = \text{CH}_2\text{CH}=\text{C}(\text{CH}_3)\text{CO}_2\text{H}$

6, $\text{R}_1 = \text{COCH}_3$; $\text{R}_2 = \text{CH}=\text{CHC}(\text{CH}_3)=\text{C}(\text{CH}_3)\text{OCOCH}_3$

(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).

pectedly 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.

Modification of the C-6 Substituent of 1.—Aldehyde **7** was prepared by ozonolysis⁷ of **1**. Treatment of **7** with the appropriate phosphorane [$\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$, $\text{Ph}_3\text{P}=\text{C}(\text{CH}_3)\text{CO}_2\text{Et}$, or $\text{Ph}_3\text{P}=\text{C}(\text{CH}_3)\text{CHO}$] 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 $\text{NaCH}(\text{CO}_2\text{Et})_2$ 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 \rightarrow 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 $\text{Ph}_3\text{P}=\text{C}(\text{CH}_3)\text{CO}_2\text{Et}$ 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 $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$ followed by alkaline hydrolysis.

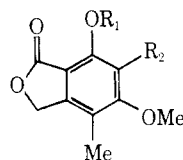
Alcohol **20**⁸ was obtained in low yield by microbial transformation of **1** with *Trichoderma viride* or with an unidentified bacterium. A good yield of **20** was obtained by treating **1** with ClCO_2Et and reducing the resulting anhydride with NaBH_4 .⁹ A similar reduction of *O*-acetylmycophenolic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

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

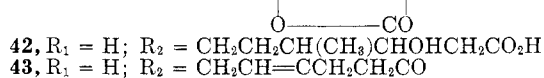
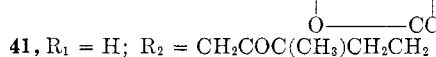
(8) D. F. Jones and R. H. Moore, British Patent Application, 44, 435/69 (1969).

(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).



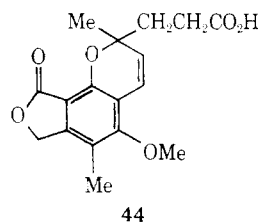
- 7, R₁ = H; R₂ = CH₂CHO
 8, R₁ = H; R₂ = CH₂CH=CHCO₂Et
 9, R₁ = H; R₂ = CH₂CH=CHCO₂H
 10, R₁ = H; R₂ = CH₂CH=C(CH₃)CO₂Et
 11, R₁ = H; R₂ = CH₂CH=C(CH₃)CO₂H
 12, R₁ = H; R₂ = CH₂CH=C(CH₃)CHO
 13, R₁ = H; R₂ = CH₂CH=C(CH₃)CH₂OH
 14, R₁ = COCH₃; R₂ = CH₂CH=C(CH₃)CH₂Br
 15, R₁ = H; R₂ = CH₂CH=C(CH₃)CH₂OH
 16, R₁ = H; R₂ = CH₂CH=C(CH₃)CH₂CONH₂
 17, R₁ = H; R₂ = CH₂CH=C(CH₃)(CH₂)₃CO₂H
 18, R₁ = H; R₂ = CH₂CH=C(CH₃)CH₂CH₂C(CH₃)CO₂H
 19, R₁ = H; R₂ = CH₂CH=C(CH₃)CH=CHCO₂H
 20, R₁ = H; R₂ = CH₂CH=C(CH₃)(CH₂)₃OH
 21, R₁ = COCH₃; R₂ = CH₂CH=C(CH₃)(CH₂)₃OH
 22, R₁ = COCH₃; R₂ = CH₂CH=C(CH₃)(CH₂)₃OCOCH₃
 23, R₁ = H; R₂ = CH₂CH=C(CH₃)(CH₂)₃OCOCH₃
 24, R₁ = COCH₃; R₂ = CH₂CH=C(CH₃)(CH₂)₃Cl
 25, R₁ = H; R₂ = CH₂CH=C(CH₃)(CH₂)₃Cl
 26, R₁ = COCH₃; R₂ = CH₂CH=C(CH₃)(CH₂)₃OSO₂CH₃
 27, R₁ = H₃; R₂ = CH₂CH=C(CH₃)(CH₂)₃OSO₂CH₃
 28, R₁ = COCH₃; R₂ = CH₂CH=C(CH₃)(CH₂)₃NO₂
 29, R₁ = H; R₂ = CH₂CH=C(CH₃)(CH₂)₃NO₂
 30, R₁ = H; R₂ = CH₂CH=C(CH₃)CH₂CH₂CHO
 31, R₁ = H; R₂ = CH₂CH=C(CH₃)CH₂CH₂CH₃
 32, R₁ = H; R₂ = CH₂CH=C(CH₃)(CH₂)₃NH-*n*-Pr
 33, R₁ = H; R₂ = CH₂CH=C(CH₃)CH₂CH₂CONH₂
 34, R₁ = H; R₂ = CH₂CH=C(CH₃)CH₂CH₂CON(Et)₂
 35, R₁ = H; R₂ = CH₂CH=C(CH₃)CH₂CH₂CONHCH₂CO₂H
 36, R₁ = H; R₂ = CH₂CH=C(CH₃)CH₂CH₂CO₂CH₃
 37, R₁ = H; R₂ = CH₂CH₂CH(CH₃)CH₂CH₂CO₂H
 38, R₁ = H; R₂ = CH₂CH₂C(CH₃)CH₂CH₂



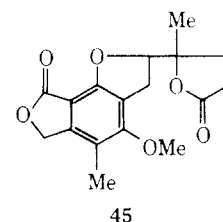
with EtOH-Et₂NH to give **23**. Treatment of **21** with MeSCl in pyridine gave **24** and **26** which were deacetylated by EtOH-Et₂NH to give **25** and **27**, respectively. Nitro compound **28** was prepared by heating **24** with NaI in Me₂CO and treating the resulting iodo derivative with AgNO₂ in Et₂O. Deacetylation of **28** with EtOH-Et₂NH gave **29**. Conversion of alcohol **20** into aldehyde **30** was accomplished with dipyridine chromium(VI) oxide in CH₂Cl₂.¹¹ Reduction of **30** by the Huang-Minlon procedure gave **31** and treatment of **30** with *n*-PrNH₂ in the presence of NaBH₄ gave **32**. Amides **33** and **34** were prepared from the acid chloride of *O*-acetylmecophenolic acid; amide **35** was obtained by microbial modification¹² of **1** and by treatment of **1** with NH₂CH₂CO₂Et in the presence of DCC followed by alkali hydrolysis.¹¹ Methyl mecophenolate¹⁰ **36** was prepared conveniently by heating **1** with MeOH containing a trace of concd H₂SO₄.

Apart from catalytic reduction, which gave dihydro-mecophenolic acid (**37**),¹⁰ attempts to effect additions to the double bond of **1** resulted in cyclic products. At-

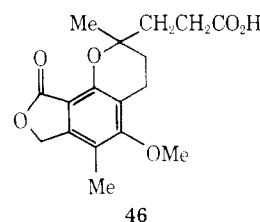
tempted hydration of **1** with hot dil mineral acid gave lactone **38**.¹² Bromination of **1** gave **39** and treatment of **1** with *m*-chloroperbenzoic acid gave either **40**^{12,13} or **45**¹² depending upon the conditions of work-up. Oxidation of **40** gave **41**.¹² Other compounds obtained during the study of microbial modification of mecophenolic acid¹² were hydroxy acid **42** and the cyclic derivatives **43**, **44** (mecochromenic acid),¹³ **45**, and **46**.



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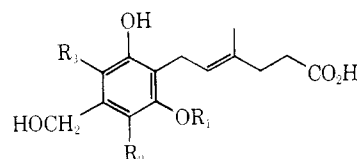


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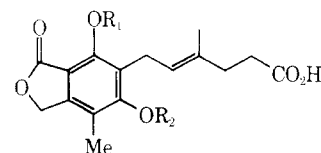
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Modification of the C-4 Substituent of 1.—Functionalization of the 4-Me group of **1** by conversion into HOCH₂ in **47** was achieved by microbial transformation of **1** with a variety of microorganisms¹² and by one-electron oxidation of **1** with alkaline K₃Fe(CN)₆.¹² Methanolysis of **47** followed by alkaline hydrolysis gave the ether **48**¹² and oxidation of **1** with CrO₃-H₂SO₄ gave aldehyde **49**.¹² Decarbonylation of the latter with RhCl(Ph₃P)₃¹⁴ in MeCN under reflux gave **50**.



- 47, R = CH₂OH
 48, R = CH₂OCH₃
 49, R = CHO
 50, R = H

Modifications of the C-5 and C-7 Substituents of 1.—*O*-Acetylmecophenolic acid (**51**) and *O*-methylmecophenolic acid (**52**) have been previously described.¹⁰ Although attempts to effect *O*-demethylation of **1** with the usual acidic reagents gave cyclic products¹⁵ an effi-



- 51, R₁ = COCH₃; R₂ = CH₃
 52, R₁ = CH₃; R₂ = CH₃
 53, R₁ = H; R₂ = H
 54, R₁ = COCH₃; R₂ = COCH₃
 55, R₁ = H; R₂ = COCH₃
 56, R₁ = H; R₂ = CH₂CH₃
 57, R₁ = H; R₂ = CH₂CH₂CH₃

(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).

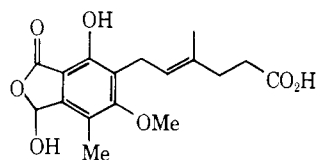
(13) I. M. Campbell, C. H. Calzadilla, N. J. McCorkindale, *Tetrahedron Lett.*, 5107 (1966).

(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).

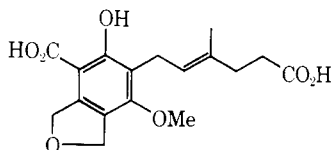
cient conversion of **1** into **53** was achieved using LiI in boiling collidine.¹⁶ This method was also used to convert **49** into **61**. Acetylation of **53** with Ac₂O in pyridine gave **54** which was selectively deacetylated to **55** with EtOH-Et₂NH. Compounds **56** and **57** were prepared from **53** by partial alkylation (Rl, Me₂CO, K₂CO₃) followed by chromatography.

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

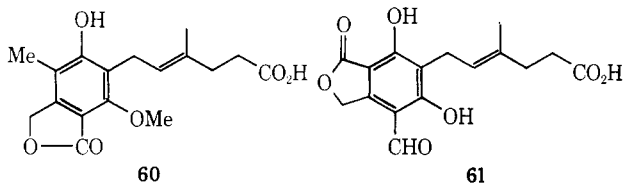


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and phthalan **59** by treatment of **47** with hot alkali.¹² Compound **60** was prepared from **55** by methylation with CH₂N₂ followed by ester hydrolysis with cold 3 N NaOH. Heating **60** in 3 N NaOH effected decarboxylation giving **62**. Similar 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.

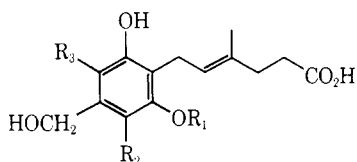


59



60

61



62, R₁ = Me; R₂ = H; R₃ = Me
63, R₁ = Me; R₂ = Me; R₃ = H
64, R₁ = H; R₂ = H; R₃ = Me
65, R₁ = H; R₂ = H; R₃ = H
66, R₁ = Me; R₂ = H; R₃ = 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 HOCH₂ (**47**), HCO (**49**), or MeOCH₂ (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 with 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 CO₂H of **1** is important for high antimitotic activity. The CO₂H 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	Compound								
		Tumor wt (% control)								
		1	20	30	35	11	37	51	53	56
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 dihydro-mycophenolic 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 Cook¹⁸ 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 Section¹⁹

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

(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-HCO₂H (66:33:1) for development and uv (254 mμ), FeCl₃-EtOH, or CrO₃-H₂SO₄ 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.

6-Allyl-7-hydroxyphthalan-1-one (2).—A soln of 7-hydroxyphthalan-1-one²⁰ (780 mg) and allyl bromide (1.6 g) in Me₂CO was heated under reflux in the presence of anhyd K₂CO₃ (700 mg) for 24 hr. H₂O and Et₂O were added and the Et₂O layer was sepd, washed with 2 N NaOH and H₂O, and dried (MgSO₄). Evapn of the Et₂O gave a solid (600 mg) which crystd from Et₂O-petr ether (bp 40–60°) as needles, mp 78–79°. This solid was heated in PhN(Et)₂ (10 ml) under reflux under N₂ for 2 hr. The reaction mixt was acidified with 3 N HCl and extd with Et₂O. Evapn of the washed (H₂O) and dried (MgSO₄) Et₂O soln and crystn of the residue from PhH-petr ether (bp 40–60°) gave **2**, mp 102–103°. Anal. (C₁₁H₁₀O₃) C, H.

7-Acetoxy-6-formylmethylphthalan-1-one (4).—A soln of **2** (750 mg) in Ac₂O (5 ml) and pyridine (5 ml) was kept at room temp for 18 hr. H₂O was added and the ppt collected by filtration. The solid was dissolved in EtOAc and the soln washed with 3 N HCl and H₂O. Evapn of the dried (MgSO₄) 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 H₂O (1 ml) was treated with OsO₄ (10 mg) at room temp with stirring. After 5 min and during 0.5 hr NaIO₄ (430 mg) was added in small portions. After stirring for 1.5 hr the reaction mixt was extd with EtOAc and the ext washed (H₂O), dried (MgSO₄), and evapd. The residue was chromatographed on a column of silica gel in PhH-CHCl₃ (3:1). Elution with PhH-CHCl₃ (1:1) and crystn of the solid residue from PhH-petr ether (bp 60–80°) gave **4**, mp 120–122° (190 mg). Anal. (C₁₂H₁₀O₅) C, H.

7-Hydroxy-6-(3-carboxybut-2-enyl)phthalan-1-one (5).—A soln of **4** (215 mg) and Ph₃P=C(Me)CO₂Et²¹ (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-CHCl₃ (3:1). Elution with PhH-CHCl₃ (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 MsSO₃H (95 mg) in 90% HCO₂H were heated at 100° for 5.5 hr. After cooling H₂O was added and the precipitated solid collected. Crystn from Me₂CO gave **5**: mp 190–191° (100 mg); τ(CDCl₃-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. (C₁₃H₁₂O₅) C, H.

7-Acetoxy-6-(2-acetoxy-3-methylpenta-2,4-dienyl)phthalan-1-one (6).—A soln of **5** (580 mg) in Ac₂O (2 ml) and pyridine (2 ml) was kept at room temp for 40 hr. H₂O and EtOAc were added, the sepd EtOAc layer was washed with 3 N HCl and H₂O, and the dried (MgSO₄) soln was evapd. Crystn of the residue from PhH gave **6**: mp 192–193° (160 mg); τ(CDCl₃) 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. (C₁₈H₁₈O₆) 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 Ph₃P=CHCO₂Et²¹ (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

(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.* (C₁₆H₁₅O₆) C, H.

A similar experiment using Ph₃P=C(Me)CO₂Et²¹ in place of Ph₃P=CHCO₂Et gave **10**, mp 131–132° (from PhH–petr ether, bp 60–80°) (60% yield). *Anal.* (C₁₇H₂₀O₆) 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 NaHCO₃ and the alkaline ext acidified and the product isolated in EtOAc. Evapn of the dried (MgSO₄) EtOAc and crstn from Me₂CO gave **9**: mp 177–179° (49 mg); τ (CDCl₃–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.* (C₁₄H₁₃O₆) C, H.

6-(3-Carboxybut-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (11).—A soln of **10** (240 mg) and MeSO₃H (70 mg) in 90% HCO₂H (0.75 ml) was heated at 100° for 6 hr. H₂O was added to the cooled soln and the pptd solid was collected and crstn from AcOH to give **11**: mp 219–222° (180 mg); τ (CDCl₃–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.* (C₁₅H₁₅O₆) C, H.

6-(3-Formylbut-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (12).—A soln of **7** (400 mg) and Ph₃P=C(Me)CHO²² (600 mg) in PhH (30 ml) was heated under reflux for 24 hr. Evapn of the soln and crstn of the residue from EtOH gave **12**: mp 110–112° (215 mg); τ (CDCl₃) 0.64 (s, 1 H, CHO). *Anal.* (C₁₅H₁₅O₅) 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 NaBH₄ (500 mg) in portions over 0.5 hr and kept at room temp for 24 hr. The soln was coned to 20 ml and treated with 3 *N* HCl and EtOAc. The EtOAc layer was sepd, washed (H₂O), dried (MgSO₄), and evapd to give a solid. Crstn from PhH–petr ether (bp 60–80°) gave **13**, mp 98–108° (1.8 g). *Anal.* (C₁₅H₁₅O₅) 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 crstn from PhH to give the *O*-acetate of **13**: mp 148–155° (135 mg); τ (CDCl₃) 6.05 (s, 2 H, CH₂O) and 7.6 (s, 3 H, CH₃CO). *Anal.* (C₁₇H₂₀O₆) C, H. This material (100 mg) in PhH (2.5 ml) and CHCl₃ (2.5 ml) was added dropwise to PBr₃ (32 mg) in PhH (3 ml) at 5° and the mixt was kept at room temp for 2 hr. H₂O and CHCl₃ were added and the CHCl₃ layer was sepd, washed (H₂O), and dried (MgSO₄). Evapn of the soln and crstn 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, CH₂Br). *Anal.* (C₁₇H₁₉BrO₅) 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 (H₂O), dried (MgSO₄), and evapd. Crstn of the residue from PhH–petr ether (bp 60–80°) gave **15**: mp 164–168° (33 mg); $\gamma_{\text{max}}^{\text{Nujol}}$ 2280 (C≡N); τ (CDCl₃) 6.95 (s, 2 H, CH₂CN). *Anal.* (C₁₆H₁₇NO₄) 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 coned HCl (1.5 ml) was kept at room temp for 24 hr. H₂O and CHCl₃ were added and the sepd CHCl₃ layer was washed (H₂O), dried (MgSO₄), and evapd. Prep tlc of the residue and crstn of the product from Me₂CO–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.* (C₁₆H₁₉NO₅) C, H, N.

Mycophenolic Acid from 14.—A soln of **14** (380 mg) in PhMe (5 ml) was added to a suspension of NaCH(CO₂Et)₂ in PhMe, prepd from Na (23 mg) and CH₂(CO₂Et)₂ in PhMe (5 ml) under reflux for 1 hr, and the mixt was heated under reflux for 7 hr. H₂O was added and the PhMe layer was sepd, washed (H₂O), dried (MgSO₄), and evapd. The residue and KOH (500 mg) were heated in MeOH (5 ml) under reflux for 7 hr. HCl (3 *N*) and CHCl₃ were added and the CHCl₃ layer was sepd and extd with aq NaHCO₃. The alk ext was acidified with 3 *N* HCl and extd with CHCl₃. Evapn of the washed (H₂O) and dried (MgSO₄) CHCl₃ soln gave a solid residue (81 mg) which was heated at 140–150° for 0.5 hr. Prep

tlc of the product and crstn 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 CH₂N₂ in Et₂O–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 SOCl₂ (15 ml) was heated under reflux for 1.5 hr. The residue obtained on complete removal of SOCl₂ was dissolved in Et₂O and added over 0.5 hr to excess CH₂N₂ in Et₂O. After being kept at room temp for 2.5 days the Et₂O was evapd to give an oil, $\gamma_{\text{max}}^{\text{dioxane}}$ 2100 cm^{−1}, which was dissolved in MeOH (7 ml). To this soln was added over 15 min a soln of PhCO₂Ag (100 mg) in Et₃N (900 mg). The mixt was heated under reflux for 0.5 hr and filtered and the solvent was evapd. The residue was treated with NH₃ in Me₂CO, 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 CHCl₃ were added and the sepd CHCl₃ layer was washed (H₂O), dried (MgSO₄), and evapd. Crstn 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 CH₂N₂ in Et₂O 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 Ph₃P=CHCO₂Et²¹ (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 NaHCO₃ and this ext was acidified with 3 *N* HCl and extd with EtOAc. Evapn of the washed (H₂O) and dried (MgSO₄) EtOAc ext gave a solid (2 g). Crstn from Me₂CO 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.* (C₁₇H₁₈O₆) 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 acid⁹ (**51**) (1 g) was converted into its acid chloride with SOCl₂ and this was triturated with NH₄OH (sp g, 0.88) to give a yellow solid. Crstn from MeOH gave prisms of **33**, mp 196–200° (800 mg). *Anal.* (C₁₇H₂₁NO₅) C, H, N.

The acid chloride (700 mg) in PhH (10 ml) was treated with Et₃NH (2 ml) in PhH (10 ml) at room temp. H₂O was added and the PhH layer was sepd, washed with H₂O, and dried (MgSO₄). Evapn of the PhH and crstn of residue from PhH–petr ether (bp 60–80°) gave prisms of **34**, mp 93–95° (580 mg). *Anal.* (C₂₁H₂₅NO₅) 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 ClCO₂Et (1.92 ml) in THF (5 ml) was added to soln of **1** (3.2 g) and Et₃N (2.8 ml) in THF (50 ml) at −5° and the mixt was stirred for 0.5 hr. Et₃NH⁺Cl[−] was removed by filtration and the filtrate was added over 0.5 hr to NaBH₄ (1.9 g) in H₂O (20 ml) at 10–15°. The mixt was stirred at room temp for 18 hr, acidified with 3 *N* HCl and extd with EtOAc. The EtOAc ext was washed (H₂O), dried (MgSO₄), 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 NaHCO₃ and H₂O and dried (MgSO₄). Evapn of the solvent and crstn of the residue from EtOAc–petr

(23) Using procedures described elsewhere¹² **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 (CDCl₃) 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.* (C₁₇H₂₂O₅) C, H.

Repetition of this procedure with *O*-acetylmecophenolic acid (**51**) in place of **1**, and using 1 molar equiv of ClCO₂Et and omitting the NaOH hydrolysis, gave **21**: mp 75° [from EtOAc–petr ether (bp 60–80°)]; τ (CDCl₃) 6.5 (t, 2 H, CH₂OH) and 7.7 (s, 3 H, ArOAc). *Anal.* (C₁₉H₂₄O₆) 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 Ac₂O (5 ml) and pyridine (5 ml) was kept at room temp for 24 hr. H₂O and EtOAc were added and the sepd EtOAc layer was washed with 3 *N* HCl and H₂O. Evapn of the dried (MgSO₄) EtOAc soln gave a gum which on crystn from EtOAc–petr ether (bp 60–80°) gave **22**: mp 75–76° (1 g); τ (CDCl₃) 6.1 (t, 2 H, CH₂OAc). *Anal.* (C₂₁H₂₆O₇) C, H. A soln of **22** (500 mg) in EtOH (20 ml) and Et₃NH (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 (H₂O) and dried (MgSO₄) EtOAc soln gave **23** as a sticky solid which could not be obtained cryst: $\gamma_{\text{max}}^{\text{CHCl}_3}$ 3300 and 1720 cm⁻¹; τ (CDCl₃) 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 MeSO₂Cl (0.3 ml) in pyridine (10 ml) was kept at room temp overnight. H₂O and EtOAc were added and the sepd EtOAc layer was washed with 3 *N* HCl and H₂O. Evapn of the dried (MgSO₄) 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 (CDCl₃) 5.86 (t, 2 H) and 7.05 (s, 3 H) (CH₂OSO₂CH₃). *Anal.* (C₂₀H₂₆O₆S) 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 Et₃NH (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 (H₂O) and dried EtOAc soln and crystn of the residue from EtOAc–petr ether (bp 60–80°) gave **25**, mp 110–112° (60 mg). *Anal.* (C₁₇H₂₁ClO₄) C, H.

A similar procedure was used to convert **26** into **27** which was obtained as a gum: τ (CDCl₃) 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 Me₂CO 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 (CDCl₃) 6.88 (t, 2 H, CH₂I). This was dissolved in Et₂O and the solution was added to a stirred suspension of freshly prepared AgNO₂ (900 mg) in Et₂O 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 Et₂O–petr ether (60–80°) gave **28**, mp 98–100° (400 mg); 5.7 (t, 2 H, CH₂NO₂), $\gamma_{\text{max}}^{\text{NaNO}_2}$ 1560 cm⁻¹ (NO₂). *Anal.* (C₁₈H₂₃NO₇) C, H, N.

Treatment of **28** (50 mg) with Et₃NH (3 ml) in EtOH (3 ml) at room temperature gave **29** which crystallized from Et₂O: mp 95–98°; $\gamma_{\text{max}}^{\text{NaNO}_2}$ 1560 cm⁻¹ (NO₂). *Anal.* (C₁₇H₂₁O₆N) C, H, N.

6-(5-Formyl-3-methylpent-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (30).—Dipyridinechromium(VI) oxide¹¹ (8 g) was added to CH₂Cl₂ (100 ml) whereupon a brown precipitate formed. The supernatant solution was decanted into a solution of **20** (1 g) in CH₂Cl₂ (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 (CDCl₃) 0.3 (t, 1 H, CH₂CHO). *Anal.* (C₁₇H₂₀O₅) 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 NH₂NH₂·H₂O (20 mg) in diethylene glycol was heated at 100° for 1.5 hr, then maintained at 150° for 0.5 hr to remove H₂O, and finally at 190° for 4 hr. HCl (3 *N*) and EtOAc were added and the sepd EtOAc soln washed (H₂O), dried (MgSO₄), 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 (CDCl₃) 9.2 (t, 3 H, CH₂CH₃). *Anal.* (C₁₇H₂₂O₄) C, H.

7-Hydroxy-5-methoxy-4-methyl-6-(3-methyl-6-*n*-propylamino-hex-2-enyl)phthalan-1-one (32).—A soln of **30** (570 mg) and *n*-PrNH₂ (114 mg) in MeOH (60 ml) was maintained at 0° for 0.5 hr and then treated with NaBH₄ (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 (H₂O), dried (MgSO₄), and evapd. Stirring of the residue with Me₂CO gave **32**, mp 153–154° (80 mg). *Anal.* (C₂₆H₃₉NO₄) 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 Ph₃P=CHCO₂Et¹² (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 (FeCl₃ 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 (H₂O), dried (MgSO₄), and evapd. Crystn of the residue from EtOAc–petr ether (bp 60–80°) gave **18**, mp 130–133° (450 mg); τ (CDCl₃) 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.* (C₂₀H₂₄O₆) C, H.

6β-Bromo-β-(2-methyl-5-oxotetrahydrofuran-2-yl)ethyl-7-hydroxy-5-methoxy-4-methylphthalan-1-one (39).—Br₂ was added dropwise to a soln of **1** (2 g) in CHCl₃ (80 ml) at 0° until a slight excess was present. After 0.5 hr the CHCl₃ soln was washed with H₂O, dried (MgSO₄), and evapd. Crystn of the residue from EtOAc–petr ether (bp 60–80°) gave **39**, mp 178–180° (3.0 g). *Anal.* (C₁₇H₁₉BrO₆) C, H, Br.

6-(5-Carboxy-3-methylpent-2-enyl)-7-hydroxy-5-methoxy-phthalan-1-one (50).—A soln of **49**¹² (2.4 g) in MeCN (100 ml) was heated with RhCl (PPh₃)₃¹⁴ (4.8 g) under reflux under O₂-free N₂ 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 NaHCO₃ and the alkaline ext acidified with 3 *N* HCl and extd with EtOAc. The EtOAc soln was washed (H₂O), dried (MgSO₄), and evapd and the residue, in EtOH (20 ml), was treated with NaBH₄ (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 (H₂O), dried (MgSO₄), and evapd. The residue was subjected to prep tlc and the phenolic (FeCl₃ spray) component isolated and crystd from EtOAc–petr ether (bp 60–80°) to give **50**: mp 158–162° (350 mg); τ (Me₂CO-*d*₆) 3.29 (s, 1 H, ArH). *Anal.* (C₁₆H₁₈O₆) 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 N₂, 1 hr) (25 g) in collidine (60 ml) maintained at reflux under N₂. 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 (H₂O) and dried (MgSO₄) EtOAc soln gave **53**, mp 147–149° (12 g). *Anal.* (C₁₆H₁₈O₆) C, H.

A similar procedure using **49**¹² in place of **1** gave 60% yield of **61**, mp 218–220°. *Anal.* (C₁₆H₁₆O₇) 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 Ac₂O (20 ml) and pyridine (20 ml) was kept at room temp for 48 hr. H₂O 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.* (C₂₀H₂₂O₈) C, H. This solid in EtOH (80 ml) was treated with Et₃NH (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 (H₂O) and dried (MgSO₄)

soln and crystn of the residue from EtOAc-petr ether (bp 60–80°) gave **55**, mp 142–143° (3.3 g). *Anal.* (C₁₈H₂₀O₇) 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 Me₂CO (125 ml) was heated under reflux in the presence of anhyd K₂CO₃ (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 (H₂O) and dried (MgSO₄) 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 (CDCl₃) 6.18 (q, 2 H) and 8.62 (t, 3 H) (CH₂CH₂O). *Anal.* (C₁₈H₂₂O₈) 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 (CDCl₃) 6.25 (t, 2 H), 8.1 (m, 2 H), and 8.91 (t, 3 H) (CH₂CH₂-CH₂O). *Anal.* (C₁₈H₂₄O₈) 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 CH₂N₂ in Et₂O for 1 hr at 5°. The Et₂O 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 (H₂O) and dried (MgSO₄) EtOAc soln and crystn of the residue from EtOAc-petr ether (bp 60–80°) gave **60**, mp 156–158° (310 mg). *Anal.* (C₁₇H₂₀O₈) C, H.

3-(5-Carboxy-3-methylpent-2-enyl)-2-hydroxy-6-hydroxy-methyl-4-methoxytoluene (62).—A soln of **60** (100 mg) in 3 *N* NaOH (30 ml) was heated under reflux under N₂ 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 (Me₂CO-*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.* (C₁₈H₂₂O₈) C, H.

3-(5-Carboxy-3-methylpent-2-enyl)-4-hydroxy-6-hydroxy-methyl-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 N₂ 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 (H₂O), dried (MgSO₄), and evapd and the residue was subjected to prep tlc. Isolation of the component giving an immediate response to CrO₃-H₂SO₄ 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, ArCH₂OH). *Anal.* (C₁₈H₂₂O₈) C, H.

3-(5-Carboxy-3-methylpent-2-enyl)-2,4-dihydroxy-6-hydroxy-methyltoluene (64).—A soln of **53** (1 g) in 3 *N* NaOH (80 ml) was heated under reflux under N₂ for 18 hr. It was acidified with 3 *N* HCl and extd with EtOAc. Concn of the washed (H₂O) and dried (MgSO₄) EtOAc soln gave **64**; mp 152–154° (800 mg); τ (Me₂CO-*d*₆) 3.5 (s, 1 H, ArH) and 5.52 (s, 2 H, ArCH₂-OH). *Anal.* (C₁₈H₂₀O₈) 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 N₂ 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 (Me₂CO-*d*₆) 3.67 (s, 2 H, ArH) and 5.64 (s, 2 H, ArCH₂OH). *Anal.* (C₁₄H₁₈O₈) 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 N₂ for 18 hr. After acidification with 3 *N* HCl the reaction mixt was extd with EtOAc and the ext washed (H₂O), dried (MgSO₄), 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 (Me₂CO-*d*₆) 3.57 (s, 1 H), 3.58 (s, 1 H) (ArH), and 5.6 (s, 2 H, ArCH₂OH). *Anal.* (C₁₈H₂₀O₈) C, H.

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Potential Antitumour Agents. 11. 9-Anilinoacridines

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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 proposed.

In the last paper of this series¹ 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 = CH₃) 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 earlier¹ (*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

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