374. *Lactones. Part V.* Experiments relating to Mycophenolic Acid.*

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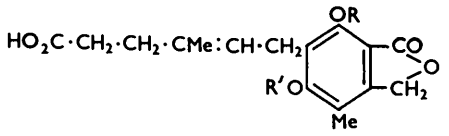
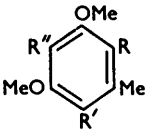
The structure of mycophenolic acid has been confirmed as (I) by infrared spectroscopy and by the synthesis of the degradation product, 6-carboxymethyl-5 : 7-dimethoxy-4-methylphthalide (X; R = $CH_2 \cdot CO_2H$).

From degradative studies by Raistrick and his co-workers [1-4] the structure of mycophenolic acid, a metabolic product of strains of *Penicillium brevi-compactum*, is almost certainly structure (I). Birkinshaw, Raistrick, and Ross,[4] however, admitted slight doubts as to which of the two nuclear hydroxyl groups in normycophenolic acid (II) was methylated in mycophenolic acid and as to whether the potential hydroxymethyl group in the phthalide ring was *ortho* or *meta* to the nuclear methyl group. Though the fact that mycophenolic acid gives a ferric chloride colour † suggests the hydroxy-phthalide system given in structure (I), the phthalide carbonyl stretching frequency region of the infrared spectra shows maxima at 1742 ($CHCl_3$), 1748 (CCl_4), and 1751 cm.$^{-1}$ (dioxan) while the dimethoxy-compound (III) shows maxima at 1763, 1776, and 1770 cm.$^{-1}$ in the same solvents, respectively, clearly indicating intramolecular hydrogen bonding as expected from structure (I).[7]



(I) R = H, R′ = Me
(II) R = R′ = H
(III) R = R′ = Me



(IV) R = CO_2H, R′ = R″ = H
(V) R = CO_2H, R′ = Br, R″ = H
(VI) R = R″ = H, R′ = Br
(VII) R = H, R′ = Br, R″ = CO_2H

In order further to confirm the orientation of groups in the dimethoxyphthalide (III) it was aimed to synthesise 6-carboxymethyl-5 : 7-dimethoxy-4-methylphthalide (X; R = $CH_2 \cdot CO_2H$). This compound has been obtained [3] by ozonolysis of the dimethoxyphthalide (III) or by oxidation of the corresponding aldehyde with alkaline iodine. Our starting material was orsellinic acid dimethyl ether (IV); [8] this compound on treatment with 1½ mol. of bromine according to the photobromination technique [9] gave 3-bromo-4 : 6-dimethoxy-2-methylbenzoic acid (V) as major product. The orientation of the bromine atom was readily established by decarboxylation which gave 2-bromo-3 : 5-dimethoxytoluene (VI) which has been prepared by decarboxylation [10] of the acid (VII) and by bromination of orcinol followed by methylation.[11] On photobromination with 2 mol. of bromine followed by alkaline hydrolysis orsellinic acid dimethyl ether gave

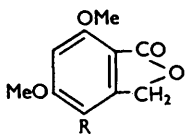
* Part IV, *J.*, 1956, 4980.

† Mycophenolic acid [2] gives a blue colour; cf. 7-hydroxyphthalide [5] which gives a purple colour while 4-hydroxyphthalide [6] gives no colour.

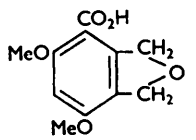
[1] Clutterbuck, Oxford, Raistrick, and Smith, *Biochem. J.*, 1932, **26**, 1441.
[2] Clutterbuck and Raistrick, *ibid.*, 1933, **27**, 654.
[3] Birkinshaw, Bracken, Morgan, and Raistrick, *ibid.*, 1948, **43**, 216.
[4] Birkinshaw, Raistrick, and Ross, *ibid.*, 1952, **50**, 630.
[5] Blair, Brown, and Newbold, *J.*, 1955, 708.
[6] Buehler, Powers, and Michels, *J. Amer. Chem. Soc.*, 1944, **66**, 417.
[7] Duncanson, Grove, and Zealley, *J.*, 1953, 1331.
[8] Herzig, Wenzel, and Kurtzweil, *Monatsh.*, 1903, **24**, 894; Robertson and Robinson, *J.*, 1927, 2196.
[9] Eliel, Rivard, and Burgstahler, *J. Org. Chem.*, 1953, **18**, 1679; Eliel and Rivard, *ibid.*, 1952, **17**, 1252.
[10] Asahina and Fuzikawa, *Ber.*, 1934, **67**, 163; Fuzikawa, *Ber.*, 1935, **68**, 72.
[11] Chakravarti and Mukerjee, *J. Indian Chem. Soc.*, 1937, **14**, 725; Asahina and Fuzikawa, ref. 10.

4-bromo-5 : 7-dimethoxyphthalide (VIII; R = Br) as principal product with some 3-bromo-4 : 6-dimethoxy-2-methylbenzoic acid. Photobromination of methyl orsellinate dimethyl ether with 2 mol. of bromine or of the methyl ester of the acid (V) with 1 mol. of bromine gave in good yield methyl 3-bromo-2-bromomethyl-4 : 6-dimethoxybenzoate, alkaline hydrolysis of which gave the phthalide (VIII; R = Br). The latter has also been prepared from orsellinic acid dimethyl ether by reaction with 1 mol. of *N*-bromosuccinimide to give the acid (V) whose methyl ester was treated with a further mol. of the reagent followed by hydrolysis. These results show that nuclear bromination must occur first and the presence of the *ortho*-bromo-group is necessary to activate the methyl group for side-chain bromination.

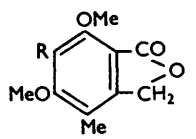
The phthalide (VIII; R = Br) on catalytic hydrogenation afforded 5 : 7-dimethoxyphthalide (VIII; R = H), alkaline permanganate oxidation of which gave the known 3 : 5-dimethoxyphthalic acid.[12] On chloromethylation at 0° by a modification of the method of Wilson *et al.*,[13] the phthalide (VIII; R = H) gave 4-chloromethyl-5 : 7-dimethoxyphthalide (VIII; R = CH_2Cl). The position of the entering chloromethyl group was proved by the ability of corresponding hydroxymethyl compound (VIII; R = CH_2·OH), formed from the chloromethyl compound (VIII; R = CH_2Cl) by hydrolysis with aqueous carbonate, to be rearranged [14] by methanolic sodium methoxide to 5 : 7-dimethoxyphthalan-4-carboxylic acid (IX) which was also formed during the hydrolysis with carbonate. Reductive dehalogenation of the chloromethyl compound (VIII; R = CH_2Cl) gave 5 : 7-dimethoxy-4-methylphthalide (VIII; R = Me) into which a 6-chloromethyl group was introduced by vigorous chloromethylation forming 6-chloro-



(VIII)



(IX)



(X)

methyl-5 : 7-dimethoxy-4-methylphthalide (X; R = CH_2Cl) which reacted readily with potassium cyanide to yield 6-cyanomethyl-5 : 7-dimethoxy-4-methylphthalide (X; R = CH_2·CN), alkaline hydrolysis of which afforded 6-carboxymethyl-5 : 7-dimethoxy-4-methylphthalide (X; R = CH_2·CO_2H). This acid was identical with that obtained by degradation of mycophenolic acid methyl ether.

Hydrolysis of the chloromethylphthalide (X; R = CH_2Cl) with aqueous sodium carbonate gave 6-hydroxymethyl-5 : 7-dimethoxy-4-methylphthalide (X; R = CH_2·OH) which on oxidation with chromic acid gave the corresponding aldehyde (X; R = CHO), m. p. 131·5—133°; while it has not been possible to make a direct comparison between the synthetic aldehyde and a crystalline sample of that, m. p. 125—126°, obtained [3] by Birkinshaw *et al.*, as end product of the side-chain degradation of the dimethoxyphthalide (III), we feel that the latter authors' aldehyde was incompletely purified.

The same conclusion regarding the structure of mycophenolic acid has been reached independently by Professor A. J. Birch,[15] whose helpful co-operation we acknowledge.

Experimental

Ultraviolet absorption spectra were determined in ethanol.

Orsellinic Acid Dimethyl Ether (*4 : 6-Dimethoxy-2-methylbenzoic Acid*).—Ethyl everninate

[12] Fritsch, *Annalen*, 1897, **296**, 344.
[13] Cf. Wilson, Zirkle, Anderson, Stehle, and Ullyot, *J. Org. Chem.*, 1951, **16**, 792.
[14] Cf. Brown and Newbold, *J.*, 1952, 4878; Blair and Newbold, *J.*, 1954, 3935; Blair, Logan, and Newbold, *J.*, 1956, 3608.
[15] Birch and Massy-Westropp, personal communication.

(5·84 g.), prepared [17] from ethyl orsellinate [16] in 71% yield, was heated on the steam-bath for 1½ hr. with aqueous sodium hydroxide (58 c.c.; 2N). The cooled solution was stirred with dimethyl sulphate (2·5 c.c.). After 1½ hr. dimethyl sulphate (2·5 c.c.) was added and thereafter five additions of dimethyl sulphate (each 2·5 c.c.) together with aqueous sodium hydroxide (each 11 c.c.; 2N) were made at hourly intervals. 2 hr. after the last addition, aqueous sodium hydroxide (11 c.c.; 2N) was added and the solution refluxed for 1½ hr. The cooled solution was acidified (Congo red) with hydrochloric acid (*d*, 1·15). The orsellinic acid dimethyl ether (5·2 g.) crystallised from aqueous ethanol as plates, m. p. 142—143° (lit.,[8] m. p. 143—144°).

3-Bromo-4 : 6-dimethoxy-2-methylbenzoic Acid.—(*a*) A partial solution of orsellinic acid dimethyl ether (1·0 g.) in dry carbon tetrachloride (25 c.c.), heated under reflux and irradiated by a 150 w lamp, was treated dropwise with bromine (0·527 c.c.; 2 mol.) during 10 min. and refluxing continued for 1½ hr. The solvent was removed under reduced pressure and the solid residue heated under reflux for 3 hr. with aqueous sodium hydroxide (50 c.c.; 2N). The cooled solution was extracted with chloroform (50 c.c.) and acidified (Congo red) with hydrochloric acid (*d*, 1·15). The precipitate was extracted with chloroform (3 × 50 c.c.) and the extract (*A*) washed with aqueous sodium hydrogen carbonate (3 × 50 c.c.; 10%). Acidification of the aqueous washings gave *3-bromo-4 : 6-dimethoxy-2-methylbenzoic acid* (0·48 g.) which separated from aqueous ethanol as needles, m. p. 210° (decomp.) (Found: C, 43·7; H, 4·0%; equiv., 276. $C_{10}H_{11}O_4Br$ requires C, 43·65; H, 4·0%; equiv., 275). Light absorption: max. at 2070 (ε = 34,000) and 2860 Å (ε = 3550). The *methyl ester*, prepared with diazomethane, crystallised from aqueous methanol as needles, m. p. 120—121° (Found: C, 45·5; H, 4·7. $C_{11}H_{13}O_4Br$ requires C, 45·7; H, 4·5%), light absorption max. at 2065 (ε = 32,000) and 2895 Å (ε = 3350). Photobromination of orsellinic acid dimethyl ether (1·0 g.) with bromine (0·395 c.c.; 1·5 mol.) gave the bromo-acid (0·74 g.).

(*b*) (With Dr. JOHN BLAIR). A solution of orsellinic acid dimethyl ether (300 mg.) in carbon tetrachloride (20 c.c.) and benzene (10 c.c.) was refluxed with *N*-bromosuccinimide (1·05 mol.) for 5 hr. with irradiation from a 60 w lamp. The cooled reaction mixture was evaporated and the residual solid shaken with ether and aqueous sodium hydrogen carbonate (10%). Acidification of the aqueous phase and crystallisation of the precipitate from aqueous ethanol gave the bromo-acid (100 mg.), m. p. 210—211° (decomp.) alone or mixed with preparation from (*a*) (Found: C, 43·65; H, 4·0%).

Methyl 3-Bromo-2-bromomethyl-4 : 6-dimethoxybenzoate.—A solution of methyl 3-bromo-4 : 6-dimethoxy-2-methylbenzoate (250 mg.) in carbon tetrachloride (10 c.c.) was photobrominated as in (*a*) above with bromine (0·138 g.; 1 mol.). Refluxing was continued for 1 hr. after addition had been completed. Removal of the solvent gave an oil which rapidly solidified. Crystallisation from light petroleum (b. p. 60—80°) gave *methyl 3-bromo-2-bromomethyl-4 : 6-dimethoxybenzoate* (300 mg.) as needles, m. p. 118·5—119° (Found: C, 36·3; H, 3·5. $C_{11}H_{12}O_4Br_2$ requires C, 35·9; H, 3·3%), light absorption: max. at 2200 (ε = 25,000) and 3070 Å (ε = 3900). The same compound, m. p. and mixed m. p. 118—119°, was obtained in almost quantitative yield by photobromination of methyl orsellinate dimethyl ether with bromine (2 mol.).

4-Bromo-5 : 7-dimethoxyphthalide.—(i) The foregoing bromomethyl compound (0·6 g.) was refluxed with aqueous sodium hydroxide (25 c.c.; 2N) for 2 hr. The almost complete solution was cooled, extracted once with chloroform (25 c.c.) and the aqueous phase acidified (Congo red) with hydrochloric acid (*d*, 1·15); the resulting precipitate was extracted with chloroform (50 c.c.), and the extract washed with aqueous sodium hydrogen carbonate (2 × 25 c.c.) and water (25 c.c.) and dried (Na_2SO_4). Evaporation of the chloroform and crystallisation of the solid from chloroform–methanol gave *4-bromo-5 : 7-dimethoxyphthalide* (330 mg.) as fine needles, m. p. 246—248° (Found: C, 44·5; H, 3·5. $C_{10}H_9O_4Br$ requires C, 44·0; H, 3·3%). Light absorption max. at 2205 (ε = 35,400), 2590 (ε = 12,400), and 2985 Å (ε = 5900). It showed a strong infrared band at 1767 cm.$^{-1}$ in chloroform (phthalide carbonyl stretching frequency). The photobromination of orsellinic acid dimethyl ether (1 g.) with 1½ mol. of bromine gave the bromophthalide (0·29 g.). (ii) The chloroform extract *A*, above, was washed with water, dried (Na_2SO_4), and evaporated, and the residue hydrolysed as in (i). The bromo-phthalide thus obtained (0·6 g.) separated from chloroform–methanol as needles, m. p. and mixed m. p. 246—248° (Found: C, 44·2; H, 3·4%). (iii) Methyl 3-bromo-4 : 6-dimethoxy-2-methylbenzoate

[16] St. Pfau, *Helv. Chim. Acta*, 1933, **16**, 282.
[17] Fischer and Hoesch, *Annalen*, 1912, **391**, 347.

(250 mg.) in carbon tetrachloride (10 c.c.) and benzene (10 c.c.) was heated under reflux and irradiated by a 150 w lamp for 1½ hr. with *N*-bromosuccinimide (162 mg.; 1·05 mol.). The mixture was evaporated to dryness under reduced pressure and the residue refluxed for 2 hr. with aqueous sodium hydroxide (20 c.c.; 2N). The filtered solution was treated as in (i) to give the bromo-phthalide (66 mg.) as needles, m. p. and mixed m. p. 243·5—244·5°, from chloroform–methanol. From the bicarbonate washings the bromo-acid (129 g.), m. p. 206·5—207°, was recovered.

2-Bromo-3 : 5-dimethoxytoluene.—3-Bromo-4 : 6-dimethoxy-2-methylbenzoic acid (200 mg.) was heated with quinoline (5 c.c.) and copper powder (100 mg.) at 210—230° (bath temp.) for 1 hr. The cooled mixture was diluted with ether (20 c.c.) and the ethereal solution successively washed with 3N-hydrochloric acid, 10% aqueous sodium hydroxide, and water and dried (Na_2SO_4). Removal of the ether gave a light-brown oil which solidified. Crystallisation from aqueous ethanol followed by sublimation at 70°/10^{-3} mm. gave 2-bromo-3 : 5-dimethoxytoluene (60 mg.), m. p. 53·5—54·5° undepressed on mixing with a synthetic specimen,[10] m. p. 51° (lit., 57°).

5 : 7-Dimethoxyphthalide.—A suspension of 4-bromo-5 : 7-dimethoxyphthalide (1·56 g.) in dry ethyl acetate (250 c.c.) was shaken with hydrogen at room temperature and pressure in the presence of palladised charcoal (1·2 g.; 2½% of palladium chloride on charcoal) and magnesium oxide (3·0 g.). When absorption was complete (*ca.* 12 hr.) the mixture was filtered (filtrate *B*) and the insoluble material extracted with boiling chloroform (3 × 100 c.c.). The combined extracts and filtrate *B* were evaporated to give a solid which crystallised from chloroform–methanol to give *5 : 7-dimethoxyphthalide* (1·02 g.) as stout needles, m. p. 151—153° (Found : C, 61·5; H, 5·25. $C_{10}H_{10}O_4$ requires C, 61·85; H, 5·2%), light absorption : max. at 2165 (ε = 37,600), 2560 (ε = 15,900), and 2900 Å (ε = 5300).

3 : 5-Dimethoxyphthalic Acid.—A solution of 5 : 7-dimethoxyphthalide (50 mg.) in hot aqueous sodium hydroxide (10 c.c.; 2N) was treated with aqueous potassium permanganate (4·6 c.c.; 5%). After being heated on the steam-bath for 10 min., the mixture was cooled and filtered. The filtrate and washings were evaporated to small bulk under reduced pressure and acidified (Congo red) with hydrochloric acid (*d*, 1·15) to give 3 : 5-dimethoxyphthalic acid (50 mg.) as needles, m. p. 159° (decomp.) alone or mixed with an authentic sample [lit.,[12] m. p. 158° (decomp.)].

4-Chloromethyl-5 : 7-dimethoxyphthalide.—5 : 7-Dimethoxyphthalide (100 mg.) was suspended in hydrochloric acid (0·2 c.c.; *d*, 1·15) and aqueous formaldehyde (0·1 c.c.; 40%), and the mixture treated with dry hydrogen chloride at 0° for 35 min. After being kept overnight at room temperature the solid was triturated with water and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), and evaporated and the solid crystallised from ethyl acetate–light petroleum (b. p. 60—80°) to give *4-chloromethyl-5 : 7-dimethoxyphthalide* (110 mg.) as prisms, m. p. 187·5—189° (Found : C, 54·4; H, 4·7. $C_{11}H_{11}O_4Cl$ requires C, 54·45; H, 4·6%), light absorption : max. at 2240 (ε = 38,000), 2590 (ε = 14,400), and 2950 Å (ε = 5000).

4-Hydroxymethyl-5 : 7-dimethoxyphthalide.—4-Chloromethyl- 5 : 7-dimethoxyphthalide (147 mg.) was refluxed for 1½ hr. with a solution of sodium carbonate (480 mg.) in water (10 c.c.). The solution was worked up as described for analogous cases by Blair and Newbold [14] and the lactone fraction crystallised from ethanol to give *4-hydroxymethyl-5 : 7-dimethoxyphthalide* (48 mg.) as needles, m. p. 233·5—240° (Found : C, 59·15; H, 5·4. $C_{11}H_{12}O_5$ requires C, 58·9; H, 5·4%), light absorption : max. at 2210 (ε = 35,000), 2580 (ε = 13,800), and 2930 Å (ε = 6150).

5 : 7-Dimethoxyphthalan-4-carboxylic Acid.—(*a*) The acid fraction from the foregoing experiment, crystallised from ethyl acetate, gave the acid (51 mg.) as short needles, m. p. 221—222·5° (Found : C, 59·2; H, 5·6. $C_{11}H_{12}O_5$ requires C, 58·9; H, 5·4%), light absorption : max. at 2160 (ε = 26,000), 2550 (ε = 10,400) and 2945 Å (ε = 4400).

(*b*) 4-Hydroxymethyl-5 : 7-dimethoxyphthalide (110 mg.) was refluxed with methanolic sodium methoxide [from sodium (50 mg.) and dry methanol (10 c.c.)] for 7½ hr. The acid was isolated as described for an analogous case by Blair, Logan, and Newbold.[14] It separated from ethyl acetate as needles, m. p. 221—222° alone or mixed with the preparation from (*a*).

5 : 7-Dimethoxy-4-methylphthalide.—4-Chloromethyl-5 : 7-dimethoxyphthalide (200 mg.) was shaken in dry ethyl acetate (50 c.c.) with hydrogen at room temperature and pressure in the presence of palladised charcoal (174 mg.; 2·5% of palladium chloride on charcoal) and magnesium oxide

(400 mg.). Hydrogen uptake was complete in 4½ hr. The reaction mixture was filtered and the residue well washed with boiling ethyl acetate; the filtrate and washings were evaporated and the residue, crystallised from ethyl acetate–light petroleum (b. p. 60—80°), gave the *phthalide* (160 mg.) as prismatic needles, m. p. 202—203° (Found: C, 63·1; H, 5·6. $C_{11}H_{12}O_4$ requires C, 63·45; H, 5·8%), light absorption: max. at 2220 (ε = 31,000), 2600 (ε = 14,000), and 2970 Å (ε = 7200).

6-*Chloromethyl*-5 : 7-*dimethoxy*-4-*methylphthalide*.—5 : 7-Dimethoxy-4-methylphthalide (200 mg.) was refluxed for 1 hr. with aqueous formaldehyde (2·06 c.c.; 40%) and hydrochloric acid (3·44 c.c.; *d*, 1·15), dissolution being accompanied by separation of a brown oil. The mixture was cooled, diluted with water (30 c.c.), and extracted with chloroform (3 × 50 c.c.); the combined extracts were washed with aqueous sodium hydrogen carbonate (2 × 30 c.c.; 10%) and water, and dried (Na_2SO_4). Removal of the chloroform gave a yellow gum which crystallised from benzene–light petroleum (b. p. 60—80°) to give 6-*chloromethyl*-5 : 7-*dimethoxy*-4-*methylphthalide* (150 mg.) as needles, m. p. 107—107·5°; the compound sublimed at 100°/10^{-3} mm. (Found: C, 56·8; H, 5·35. $C_{12}H_{13}O_4Cl$ requires C, 56·15; H, 5·1%), light absorption: max. at 2210 (ε = 40,000), 2450 (ε = 8600), and 2960 Å (ε = 2400).

6-*Cyanomethyl*-5 : 7-*dimethoxy*-4-*methylphthalide*.—A solution of 6-chloromethyl-5 : 7-dimethoxy-4-methylphthalide (200 mg.) in ethanol (10 c.c.) was added during ½ hr. to a cold solution of potassium cyanide (65 mg.) in water (1 c.c.) and the mixture heated on the steam-bath for 4 hr. The cooled mixture was filtered and the filtrate and ethanol washings were acidified (Congo red) with hydrochloric acid (*d*, 1·15). The filtered solution was concentrated to *ca.* 5 c.c. and diluted with water. The precipitate was washed with aqueous ethanol and crystallised from benzene–light petroleum (b. p. 60—80°) to give the *cyanomethyl compound* (96 mg.) as needles, m. p. 129—131·5° (Found: C, 63·2; H, 5·3. $C_{13}H_{13}O_4N$ requires C, 63·15; H, 5·3%), light absorption: max. at 2140 (ε = 41,000), 2460 (ε = 10,000), and 2915 Å (ε = 3150). The compound shows a weak band at 2222 cm.$^{-1}$ in carbon tetrachloride (C≡N stretching frequency).

6-*Carboxymethyl*-5 : 7-*dimethoxy*-4-*methylphthalide*.—The foregoing cyanomethyl compound (60 mg.) was refluxed for 2 hr. with aqueous potassium hydroxide (10 c.c.; 10%). The cooled solution was acidified (Congo red) with hydrochloric acid (*d*, 1·15) and the precipitate extracted with chloroform (2 × 20 c.c.). Isolation through aqueous sodium hydrogen carbonate gave the *acid* (47 mg.) which separated from benzene–light petroleum (b. p. 60—80°) as needles, m. p. 152—153° (Found: C, 58·6; H, 5·5. $C_{13}H_{14}O_6$ requires C, 58·6; H, 5·3%), light absorption: max. at 2160 (ε = 43,000), 2500 (ε = 11,000), and 2930 Å (ε = 3300). The acid on being mixed with 6-carboxymethyl-5 : 7-dimethoxy-4-methylphthalide, m. p. 152—153° (lit.,[3] m. p. 153°), obtained by degradation of mycophenolic acid methyl ether, had m. p. 152—153° and the infrared spectra of both acids determined in Nujol were identical. The methyl esters, prepared with diazomethane and crystallised from methanol had m. p. 92—93·5° (synthetic) and 93—94° (from degradation; lit.,[3] 94—95°) and were undepressed on mixing.

6-*Hydroxymethyl*-5 : 7-*dimethoxy*-4-*methylphthalide*.—6-Chloromethyl-5 : 7-dimethoxy-4-methylphthalide (69 mg.) was refluxed with aqueous sodium carbonate (10 c.c.; 10%) for 1 hr. The cooled solution was filtered and acidified (Congo red) and the product isolated with chloroform. The *hydroxymethyl compound* (50 mg.) separated from benzene–light petroleum (b. p. 60—80°) as needles, m. p. 103—104° (Found: C, 60·5; H, 6·3. $C_{12}H_{14}O_5$ requires C, 60·5; H, 5·9%), light absorption: max. at 2150 (ε = 31,000), 2490 (ε = 7500), and 2935 Å (ε = 2500).

6-*Formyl*-5 : 7-*dimethoxy*-4-*methylphthalide*.—A solution of crude 6-hydroxymethyl-5 : 7-dimethoxy-4-methylphthalide (420 mg.) in acetic acid (20 c.c.) was treated, at room temperature with stirring, during 3 min. with a solution of chromium trioxide (500 mg.) in acetic acid (10 c.c.). 5 min. after the addition had been completed the solution was diluted with water (20 c.c.) and extracted with chloroform (3 × 30 c.c.). The combined extract was washed with aqueous sodium hydrogen carbonate (2 × 80 c.c.; 10%) and water, and dried (Na_2SO_4). Removal of the chloroform gave a yellow gum which rapidly solidified. The solid was extracted with boiling light petroleum (b. p. 60—80°) and thrice crystallised from this solvent, separating as needles. Sublimation at 100°/10^{-3} gave 6-*formyl*-5 : 7-*dimethoxy*-4-*methylphthalide* (60 mg.), m. p. 131·5—133° (Found: C, 60·9; H, 5·2. $C_{12}H_{12}O_5$ requires C, 61·0; H, 5·1%), light absorption: max. at 2320 (ε = 28,000) and 3100 (ε = 2700) and inflexion at 2040—2100 Å (ε = 13,000).

We gratefully acknowledge the assistance of Mr. J. G. C. Campbell and his staff of the Department of Microbiology in obtaining mycophenolic acid. Analyses were performed by Dr. A. C. Syme and Mr. W. McCorkindale and spectra determined by Miss P. Adams (ultraviolet) and Miss N. Caramando (infrared). Professor H. Raistrick, F.R.S., kindly supplied cultures of *Penicillium brevi-compactum*.

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