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4-METHYL- AND 7-METHYLPHTHALAN-1-ONE DERIVATIVES OF MYCOPHENOLIC ACID

USE IN ESTABLISHING THE DISTRIBUTION OF ACETATE DERIVED HYDROGENS

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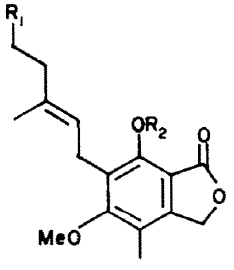
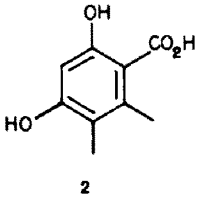
Abstract—Incorporation of acetate-[2-^{14}C, 2-^{3}H] into mycophenolic acid has been shown by degradative experiments to be consistent with a starter effect indicative of a tetraketide precursor.

Biosynthetic studies on mycophenolic acid, **1**, date from the early incorporations of acetate-[^{14}C][1] to recent work involving aromatic intermediates including **2** and **3**[2–4] and it now appears that the later stages can proceed via several different pathways.[4] A number of pentaketide phenolics co-occur with **1**,[5] a new addition being the dihydroxyphthalide **4**,[6] and an alternative pathway to **3** from a pentaketide could not be excluded.[7] However we now describe evidence from incorporation of sodium acetate-[2-^{14}C, 2-^{3}H] that both H-atoms of the phthalide methylene group in **1** are part of the starter acetate unit of a tetraketide derived moiety.

In order to be able to determine the tritium content in the phthalide methylene group, a derivative of **1** was sought in which these protons had been specifically removed. Apart from the chosen sequence described below, two other methods were found by which this could be achieved although in less satisfactory overall yields (Scheme 1). One involved oxidation of O-methyl mycophenolic acid (**5**) to give the *threo* anhydride **6**.‡ In the other, acid catalysed cyclization of **1** to mycochromanic acid **9**, followed by LAH reduction to the triol **11** and oxidation with active MnO_2 afforded a separable mixture of the isomeric lactones **13** and **14**. These afforded after oxidation and esterification, methyl mycochromanate **15** and the 7-methylphthalan-1-one **16** respectively. In similar fashion, MnO_2 oxidation of the triol **17** gave an inseparable 2:1 mixture of the lactones **18** and **19**. (A reference sample of the latter was prepared via the carbonate **20** which was obtained, rather than **21**,



1: $R_1 = CO_2H$, $R_2 = H$
5: $R_1 = CO_2H$, $R_2 = Me$
8; $R_1 = CO_2Me$, $R_2 = Me$
19; $R_1 = CH_2OH$, $R_2 = Me$
20: $R_1 = CH_2OCO_2Et$, $R_2 = H$
21: $R_1 = CH_2OH$, $R_2 = H$
22: $R_1 = CO_2CO_2Et$, $R_2 = H$
24: $R_1 = CONHC_6H_4Me\text{-}p$, $R_2 = H$
25: $R_1 = CONHC_6H_4Me\text{-}p$, $R_2 = Me$



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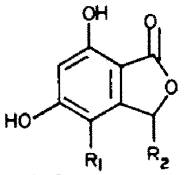
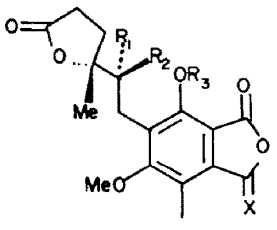


3: $R_1 = Me$, $R_2 = H$
4: $R_1 = H$, $R_2 = CHOHMe$



6: $R_1 = OH$, $R_2 = H$, $R_3 = Me$, $X = O$
7a: $R_1 = OH$, $R_2 = H$, $R_3 = Me$, $X = H_2$
7b: $R_1 = H$, $R_2 = OH$, $R_3 = Me$, $X = H_2$
10: $R_1 = R_2 = R_3 = H$, $X = H_2$

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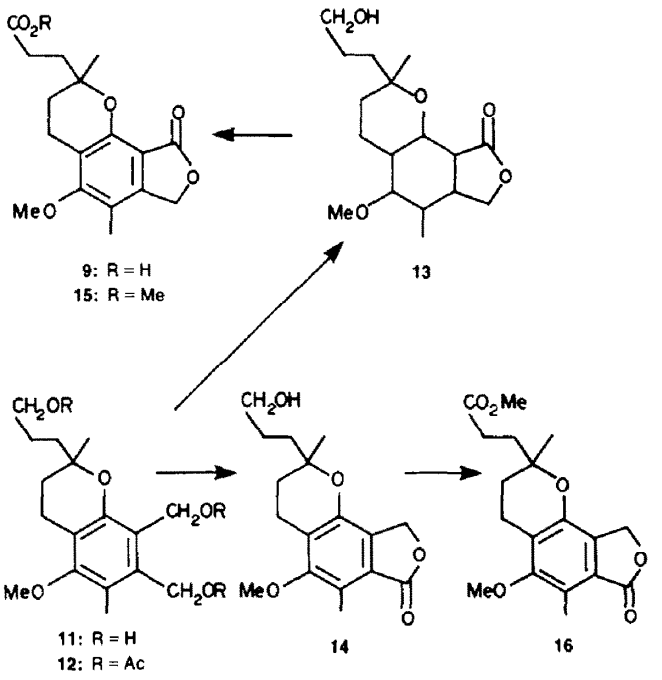
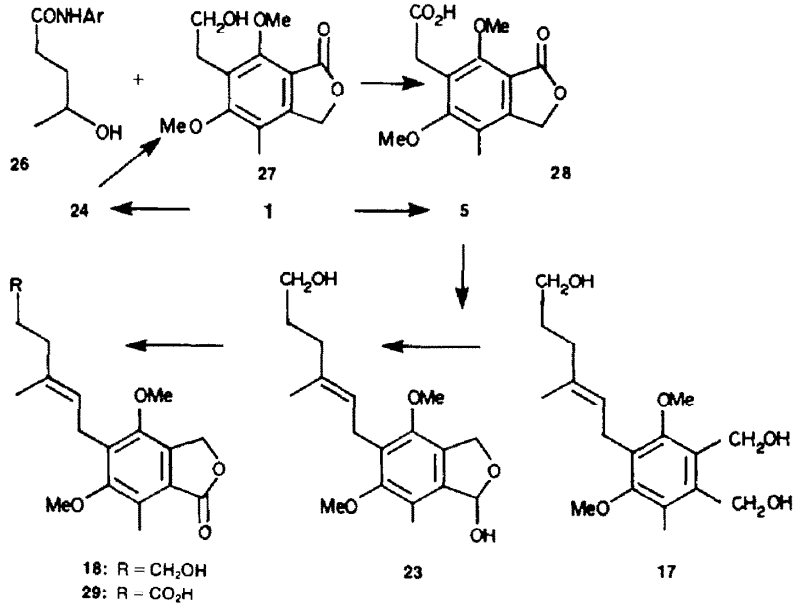
‡The *threo* lactone **7a** (a plausible intermediate) was obtained from this oxidation under milder conditions and was clearly distinct from the *erythro* isomer **7b** obtained via the epoxide of **8**.



Scheme 1.

by borohydride reduction of the mixed anhydride **22**). The desired regioselectivity in the oxidation was achieved by using less active MnO_2 under controlled conditions giving the cyclic hemiacetal **23** as the major product (82%) from which the 7-methylphthalan-1-one **18** was obtained in high yield by further oxidation. The NMR signal for the aromatic Me group in all the above 7-methylphthalan-1-one derivatives was shifted downfield by *ca.* 0.4 ppm from that for the Me group in the isomeric 4-methylphthalan-1-ones owing to deshielding by the *ortho* carbonyl function.

Doubly labelled **1** was readily obtained from 4-day old shake flask cultures of *P. stoloniferum* which had been fed on the second day after inoculation with sodium acetate-[2-^{14}C, 2-^{3}H] and this was degraded as indicated in Scheme 2. In order to assess the level of incorporation into the terpenoid side chain, **1** was converted into **2** and ozonized. Borohydride reduction of the product afforded the alcohols **26** and **27** which contained 60% and 39.7% respectively of the total ^{14}C incorporated. Since each of the three labelled carbon atoms in **26** presumably carries *ca.* 13% of the ^{14}C radioactivity, the hydroxymethyl group of **27** must be similarly labelled. In agreement with this removal of the ^{3}H in the hydroxymethyl group of **27** by oxidation gave **28** with loss of tritium corresponding to 12% of the total in **1**. The remaining 48% of ^{14}C activity is presumably distributed over the four appropriate C atoms of the polyketide



Scheme 2.

derived phthalide nucleus, i.e. this would have been 12% per C atom if the distribution had been uniform.[1] However the high level of tritium (34%) retained in the acid **28** indicates that the methylene group must carry a higher proportion of this than the three ring carbons. An independent measurement of the tritium content in the phthalide group was obtained by conversion of **1** via **8** into the triol **17** and thence as described above and in Scheme 2 into the lactone **18**. For convenience this was further oxidised with CrO_3 to the crystalline lactone acid **29** and the transformation **1**→**29** was accompanied by a loss of 28.5% of the tritium content, again showing the high level of tritium activity located in the phthalide methylene group.

The relative levels of radioactivity at the eight centres can be assigned as indicated in Fig. 1 and ^3H:C ratios and percentages radioactivity calculated from this provide a reasonable fit for those observed (Table 1). Both the hydrogen atoms in the phthalide CH_2 group are derived from acetate [eliminating the C-Me analogue of **4** (i.e. R' = Me) as a possible intermediate] and the starter effect observed is in accord with the precursor for the phthalide nucleus normally being a tetraketide.

EXPERIMENTAL

M.ps are uncorrected. R_fs refer to tlc on silica HF_{254} using the solvents indicated.

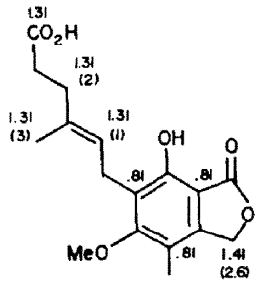


Fig. 1. Relative distribution of ^{14}C (^3H) in **1**.

The anhydride **6**. To a soln of **5**[6] (70 mg) in 2N KOH (2 ml), an excess of $KMnO_4$ in 0.45 N KOH was added at 100°. After heating for a further 2 hr, the soln was filtered, acidified with 6 N HCl and continuously extracted with ether to give the *anhydride* **6**. (10 mg, 13%), m.p. 149–151° from $CHCl_3$–pet. ether (lit[9] m.p. 153°), R_f 0.55 ($CHCl_3$); IR (KBr) 3560, 3460, 1840, 1770 cm^{-1}; NMR ($CDCl_3$) 1.48 (3 H, s, Me), 2.0–2.5 (2H, m), 2.55 (3H, s, Ar*Me*), 2.6–3.2 (5H, m, 1H exchangeable with D_2O, $2\times CH_2$ and OH), 3.65 (1H, m, CHOH), 4.17 and 3.84 (ea 3H, s, OMe); MS *m/e* 364 (6%, M^+), 346 (5), 266 (99, M-98), 99 (100, lactone ring, $C_5H_7O_2$). (Found: C, 59.4; H, 5.5. Calc. for $C_{18}H_{20}O_8$: C, 59.3; H, 5.5%).

The threo *lactone* **7a**. When the 2 hr heating was omitted in the above procedure, prep. tlc of the crude product gave the threo *hydroxylactone* **7a** (14% yield), m.p. 150–151° from $CHCl_3$–pet. ether, R_f 0.56 ($CHCl_3$); IR (KBr) 3480, 1755, 1604, 1203, 1130, 1075, 943, 870, 789 cm^{-1}; UV (EtOH) λ_{max} 226 nm (ϵ 20,000), 251 (ϵ 9930), 296 (ϵ 3180); NMR ($CDCl_3$) 1.51δ (3H, s, Me), 2.20 (3H, s, ArMe), 2.0–2.4 (2H, m), 2.85 (1H, exchangeable with D_2O, OH), 2.5–3.1 (4H, m), 3.64 (1H, m, CHOH), 3.84 and 4.08 (ea 3H, s, OMe), 5.15 (2H, s, $ArCH_2O$–); MS *m/e* 351 (13%, M^+), 251 (100, M-99), 221 (68), 99 (40, lactone ring $C_5H_7O_2$). (Found: C, 61.6; H, 6.3. $C_{18}H_{22}O_7$ requires C, 61.7; H, 6.3%).

The erythro *lactone* **7b**. Compound **8** (100 mg) was allowed to stand with *m*-chloroperbenzoic acid (100 mg) in $CHCl_3$ (15 ml) overnight at room temp. Prep. tlc of the neutral fraction of the product gave the corresponding epoxide (75 mg, 80%), R_f 0.72 ($CHCl_3$); IR (CCl_4) 1768, 1741, 1124 cm^{-1}; NMR ($CDCl_3$) 1.41 δ (3H, s, Me), 1.7–2.1 (2H, m), 2.19 (3H, s, ArMe), 2.2–2.6 (2H, m), 2.94 (3H, br, $2\times CH_2$), 3.62 (3H, s, MeO_2C–), 3.81 and 4.10 (ea 3H, s, OMe), 5.14 (2H, s, $ArCH_2O$–); MS *m/e* 364 (8%, M^+), 333 (25, M-MeO·), 235 (100), 221 (48, M-$MeO_2CCH_2CH_2CMe$–CH·), 143 (23), 115 (28), 99 (28).

A soln of the epoxide (45 mg) in CF_3CO_2H (5 ml) was allowed to stand at room temp for 2 hr. Evaporation gave the erythro *lactone* **7b** which crystallized from $CHCl_3$–pet. ether as colourless needles, m.p. 158–159°, R_f 0.53 ($CHCl_3$); IR ($CHCl_3$) 3500, 1764, 1604 cm^{-1}; IR (KBr) 3450 br, 1760, 1597, 1300, 1201, 1124 cm^{-1}; NMR ($CDCl_3$) 1.46 δ (3H, s, Me), 2.16 (3H, s, ArMe), 1.8–2.9 (6H, m), 2.8 (1H, s, exchangeable with D_2O, OH), 3.8 (1H, m, –CHOH), 3.78 and 4.02 (ea 3H, s, OMe), 5.05 (2H, s, $ArCH_2O$–). (Found: C, 61.7; H, 6.0. $C_{18}H_{22}O_7$ requires: C, 61.7; H, 6.3%).

Mycochromanic acid (**9**) *and mycophenolic acid lactone* (**10**). A soln of **1** (430 mg) in 3% conc H_2SO_4 in HOAc was refluxed for

Table 1.

Compound	Dpm/mmol x 10^{-7} ^3H	^{14}C	^3H : ^{14}C	Atomic ratio† ^3H : ^{14}C	% Radioactivity relative to 1† ^3H	^{14}C
NaOAc (fed)			178	3T : 1C		
1	69.5	1.25	55.6	8.52T : 9.08C (8.6T : 9.08C)	100 (100)	100 (100)
17	68.2	1.24	55.0	8.42T : 9.08C (8.6T : 9.08C)	98.1 (100)	99.2 (100)
29	51.0	1.25	40.8	6.25T : 9.08C) (6.0T : 9.08C)	73.4 (70)	100 (100)
27	32.1	0.75	42.6	3.70T : 5.15C (3.6T : 5.15C)	46.2 (41.9)	60.0 (56.7)
28	23.7	0.765	31.0	2.69T : 5.15C (2.6T : 5.15C)	34.1 (30.3)	61.2 (56.7)
26	35.6	0.496	71.7	4.75T : 3.93C (5T : 3.93C)	51.2 (58.1)	39.7 (43.3)

† Values in parenthesis are calculated on the basis of the relative distribution indicated in fig 1.

4 hr. Extraction with $CHCl_3$ gave **9** (380 mg, 90%), m.p. 172–175° from $CHCl_3$–pet. ether (lit[10] m.p. 186–188°), identical (mixed m.p., IR, NMR) with an authentic sample;[10] NMR ($CDCl_3$) 1.36 δ (3H, s, Me), 1.9 (4H, m, $-CH_2-C-O-$), 2.11 (3H, s, ArMe), 2.60 (4H, m, $ArCH_2$ and CH_2CO-O-), 3.81 (3H, s, OMe), 5.1 (2H, s, $ArCH_2O-$); MS *m/e* 320 (50%, M^+), 302 (40, $M-H_2O$), 247 (70, $M-HO_2CCH_2CH_2$), 207 (100), 159 (60) with m* corresponding to 320→302 and 302→207. (Found: C, 63.8; H, 6.2. Calc. for $C_{17}H_{20}O_6$: C, 63.7; H, 6.3%).

Addition of pet. ether to the mother liquor from crystallization of **9** gave the *lactone* **10** (20 mg, 5%), m.p. 161–162° from $CHCl_3$–pet. ether; IR (KBr) 1770, 1735, 1630, 1292, 1140 cm^{-1}; NMR ($CDCl_3$) 1.5 δ (3H, s, $-O-C-Me$), 2.0 (4H, m, CH_2-C-O-), 2.13 (3H, s, ArMe), 2.7 (4H, m, $ArCH_2$ and CH_2COO), 3.8 (3H, s, OMe), 5.2 (2H, s, $ArCH_2O$), 7.7 (1H, s, ArOH); MS *m/e* 320 (30% M^+), 302 (28, $M-H_2O$), 247 (50), 207 (100), 159 (40) with m* corresponding to 320→302 and 302→207. (Found: C, 63.8; H, 6.7. $C_{17}H_{20}O_6$ requires: C, 63.7; H, 6.3%). This lactone can be prepared more efficiently (68% yield) by the action of CF_3CO_2H on **1**.[11]

A soln of **10** (60 mg) in HOAc containing 3% conc H_2SO_4 was refluxed for 12 hr. $CHCl_3$ extraction and prep tlc using 5% MeOH in $CHCl_3$ as eluent gave unchanged **10** (25 mg) and **9** (30 mg, 50%).

The triol **11**. A suspension of LAH (100 mg) in dry THF was added slowly to a stirred soln of **9** (200 mg) in dry THF and the mixture was refluxed for 1 hr. After decomposition of excess reagent by addition of sat aq Na_2SO_4 (2 ml) and then water (10 ml), extraction with EtOAc gave the *triol* **10** as a colourless oil (200 mg, 98%) which decomposed on attempted distillation, R_f 0.45 (MeOH–$CHCl_3$, 1:19); IR (liquid film) 3500 (broad), 1630, 1580, 1320, 1110, 1000, 770 cm^{-1}; NMR ($CDCl_3$) 1.10 δ (3H, s, Me), 1.8 (6H, m), 2.25 (3H, s, ArMe), 2.40 (3H, s, exchangeable with D_2O), 2.5–2.8 (2H, m, $ArCH_2CH_2-$), 3.50 (2H, m, $-CH_2CH_2OH$), 3.64 (3H, s, OMe), 4.61 (4H, m, 2× $ArCH_2OH$). The triol was characterised as its oily *triacetate* **11**, b.p. 190°/0.03 mm, R_f 0.8 ($CHCl_3$); IR (CCl_4) 1747, 1604, 1590, 1331, 1241, 1118, 1028 cm^{-1}; IR (KBr) 1744, 1603, 1590, 1334, 1250, 1119, 1031 cm^{-1}; NMR ($CDCl_3$) 1.27 δ (3H, s, Me), 1.6–2.0 (4H, m), 2.02 (6H, s, 2× OAc), 2.03 (3H, s, OAc), 2.24 (3H, s, ArMe), 2.79 (2H, t, J = 6.5 Hz, $ArCH_2CH_2-$), 3.70 (3H, s, OMe), 4.08 (2H, t, J = 5.5 Hz, $-CH_2CH_2OAc$), 5.19 and 5.20 (ea 2H, s, $ArCH_2O-$); MS *m/e* 436 (67%, M^+), 408 (17), 376 (73, M–HOAc), 348 (M–28–HOAc), 334 (46, M–HOAc–CH_2=C=O), 316 (69, M–2x HOAc), 235 (67), 215 (100, M– 2x HOAc–$AcOCH_2CH_2$), 193(95) with m* corresponding to 436→376, 376→334, 376→316 and 316→215. (Found: C, 63.8; H, 7.5. $C_{23}H_{32}O_8$ requires: C, 63.3; H, 7.4%).

Oxidation of the triol **11** *to give the* 4-*methylphthalan*-1-*one* **13** *and the* 7-*methylphthalan*-1-*one* **14**. The triol **11** (160 mg) in dry acetone was stirred with freshly prepared active MnO_2[12] (1.6 g) for 48 hr under N_2. Filtration and evaporation gave an oil, prep tlc of which gave the isomeric lactones **13** and **14**.

The 4-*methylphthalan*-1-*one* **13** crystallized from $CHCl_3$–pet. ether in colourless prisms (33 mg, 20%), m.p. 148–150°, R_f 0.4 ($CHCl_3$); IR ($CHCl_3$) 3525, 1760, 1607 cm^{-1}; IR (KBr) 3425, 1760, 1604, 1325, 1133, 1104, 1032, 787 cm^{-1}; NMR ($CDCl_3$) 1.35 δ (3H, s, Me), 1.6–2.0 (2H, m, CH_2), 1.79 (4H, m, 2× CH_2), 2.05 (1H, m, exchangeable with D_2O), 2.17 (3H, s, ArMe), 2.79 (2H, t, J = 6 Hz, $ArCH_2CH_2-$), 3.75 (2H, m, $-CH_2OH$), 3.82 (3H, s, OMe), 5.07 (2H, s, $ArCH_2O-$); MS *m/e* 306 (37%, M^+), 288 (10, $M-H_2O$), 247 (98, $M-HOCH_2CH_2CH_2$), 207 (100), 159 (98) with m* corresponding to 306→288 and 207→159. (Found: C, 66.8; H, 7.4. $C_{17}H_{22}O_5$ requires: C, 66.7; H, 7.2%).

The 7 *methylphthalan*-1-*one* **14** was obtained as a colourless oil (48 mg, 30%), b.p. 160°/0.003 mm, R_f 0.48 ($CHCl_3$); IR ($CHCl_3$) 3620, 1755, 1618 cm^{-1}; IR (KBr) 3430, 1755, 1620, 1329, 1116, 1030, 782 cm^{-1}; NMR ($CDCl_3$) 1.32 δ (3H, s, Me), 1.68 (4H, m, 2× CH_2), 1.70 (1H, exchangeable with D_2O), 1.81 (2H, t, J = 6 Hz, CH_2), 2.50 (3H, s, ArMe), 2.80 (2H, t, J = 6 Hz, $ArCH_2CH_2-$), 3.62 (2H, m, CH_2OH), 3.75 (3H, s, OMe), 5.05 (2H, s, $ArCH_2O-$); MS *m/e* 306 (83%, M^+), 247 (67, M–$HOCH_2CH_2CH_2$), 207 (93), 177 (67), 85 (100). (Found: C, 66.4; H, 7.2. $C_{17}H_{22}O_5$ requires: C, 66.7; H, 7.2%).

Conversion of the triol **11** *into the lactones* **15** *and* **16**. A soln of the triol **11** (140 mg) in acetone (10 ml) was treated with Jones reagent (0.2 ml) for 5 min and then diluted with icewater (5 ml). Extraction with EtOAc gave a mixture of acids which was taken up in MeOH and esterified with ethereal CH_2N_2. Prep tlc gave two products, the first of which was **15** (28 mg, 19%), b.p. 165°/0.004 mm, R_f 0.45 ($CHCl_3$) identical (R_f, IR, NMR) with a sample prepared from **9**;[10] NMR ($CHCl_3$) including 2.12 δ (3H, s, ArMe); MS *m/e* 334 (58%, M^+), 316 (49, $M-H_2O$), 247 (100, $M-MeO_2CCH_2CH_2$), 207 (99), 159 (61) with m* corresponding to 334→316. (Found: C, 64.4; H, 6.6. Calc. for $C_{18}H_{22}O_6$: C, 64.7; H, 6.6%). The second product was the isomeric 7-*methylphthalan*-1-*one* **16** (30 mg, 20%), b.p. 160°/0.005 mm, R_f 0.50 ($CHCl_3$); IR (CCl_4) 1764, 1742, 1615, 1323, 1112, 1030, 1015 cm^{-1}; IR (KBr) 1760, 1740 sh, 1618, 1325, 1112, 1024, 993, 780 cm^{-1}; NMR ($CDCl_3$) 1.30 δ (3H, s, Me), 1.84 (2H, t, J = 7.5 Hz, CH_2), 1.9–2.2 and 2.3–2.6 (ea 2H, m, CH_2), 2.52 (3H, s, ArMe), 2.86 (2H, t, J = 7.5 Hz, $ArCH_2CH_2-$), 3.67 (3H, s, MeO_2C-), 3.79 (3H, s, OMe), 5.08 (2H, s, $ArCH_2O-$); MS *m/e* 334 (100%, M^+), 247 (59, $M-MeO_2CCH_2CH_2$), 208 (82), 207 (56), 177 (47), 149 (43). (Found: C, 64.4; H, 6.6. $C_{18}H_{22}O_6$ requires: C, 64.7; H, 6.4%).

The triol **17**. A suspension of LAH (153 mg) in dry THF (15 ml) was added at room temp during 15 min to a stirred soln of **8**[8] (570 mg) in dry THF (15 ml) and stirring continued for 2 hr. After decomposition of excess reagent by addition of sat Na_2SO_4aq (5 ml), extraction with EtOAc gave the *triol* **17** as colourless prisms (370 mg, 72%), m.p. 91° from $CHCl_3$–pet. ether, R_f 0.45 (MeOH–$CHCl_3$, 1:19); IR ($CHCl_3$) 3610, 3480, 1572 cm^{-1}; IR (KBr) 3300, 1579, 1101, 974, 722 cm^{-1}; UV (EtOH) λ_{max} 260 nm (ϵ 104), 275 (ϵ 4100), 283 (ϵ 4700); NMR ($CDCl_3$) 1.79 δ (3H, s, Me), 1.5–2.2 (4H, m, 2× CH_2), 2.31 (3H, s, ArMe), 3.35 (2H, t, J = 7 Hz, $ArCH_2CH$=), 3.47 (2H, t, J = 6 Hz, $-CH_2CH_2OH$), 3.2–3.7 (3H, br, exchangeable with D_2O, 3× OH), 3.66 and 3.73 (ea 3H, s, OMe), 4.67 and 4.70 (ea 2H, s, $ArCH_2OH$), 5.18 (1H, t, J = 7 Hz, olefinic H); MS *m/e* 324 (10%, M^+), 306 (80, $M-H_2O$), 288 (62, M– 2x H_2O), 275 (33), 247 (58, $M-H_2O-HOCH_2CH_2CH_2$), 207 (83, $M-H_2O-HOCH_2CH_2CH$=$CMeCH_2$), 193 (58), 85 (100) with m* corresponding to 306→288. (Found: C, 66.6; H, 8.6. $C_{18}H_{28}O_5$ requires: C, 66.6; H, 8.7%).

Attempted preparation of the 7-*methylphthalan*-1-*one* **18**. The triol **17** (60 mg) was stirred in $CHCl_3$ with active MnO_2[18] (600 mg) under N_2 for 24 hr, giving a product shown by IR and NMR to consist of a 2:1 mixture of the isomeric lactones **18** and **19** inseparable by tlc.

The carbonate **20**. Ethyl chloroformate (0.88 ml) in THF (2.5 ml) was added to a soln of **1** (1.46 g) and Et_3N (1.28 ml) in THF (30 ml) at −10°. After stirring for 1 hr, Et_3NHCl was removed by filtration and the soln of mixed anhydride added during 30 min to a stirred soln of $NaBH_4$ (1.05 g) in water (10 ml) at 10–15°. The mixture was then stirred at room temp for 18 hr, acidified with 3N HCl and extracted with EtOAc to give the *carbonate* **20** as a colourless oil (1.68 g, 97%), b.p. 180°/0.005 mm, R_f 0.47 ($CHCl_3$); IR (liquid film) 3550, 3000, 1760, 1725, 1620, 1245, 1218, 1048, 775 cm^{-1}; UV (EtOH) λ_{max} 222 nm (ϵ 13,600), 247 (ϵ 7830), 291 (ϵ 1960), 308 (ϵ 1730); NMR ($CDCl_3$) 1.40 δ (3H, t, J = 8 Hz, CH_3CH_2O-), 1.77 (3H, s, Me), 1.6–2.2 (4H, m, 2× CH_2), 2.24 (3H, s, ArMe), 3.40 (2H, t, J = 6.5 Hz, $ArCH_2CH$=), 3.55 (2H, t, J = 6 Hz, CH_2OCO_2Et), 3.84 (3H, s, OMe), 4.38 (2H, q, J = 8 Hz, CH_3CH_2O-), 5.17 (1H, t, J = 6.5 Hz, olefinic H), 5.18 (2H, s, $ArCH_2O-$); MS *m/e* 378 (<0.1%, M^+), 306 (35, $M-C_2H_4-CO_2$), 288 (31), 247 (99, $M-EtO_2COCH_2CH_2CH_2$), 229 (46), 207 (100, $M-EtO_2COCH_2CH_2CH$=$CMeCH_2$), 159 (57), 85 (93). (Found: C, 63.5; H, 7.1. $C_{20}H_{26}O_7$ requires: C, 63.5; H, 6.9%).

The alcohol **21**. The above **20** was heated with 3N NaOH (20 ml) under reflux for 3 hr. After acidification, extraction with EtOAc gave the alcohol **21** as colourless needles (970 mg, 70%), m.p. 105–106° from EtOAc (lit[13] m.p. 106–107°), R_f 0.45 ($CHCl_3$); IR ($CHCl_3$) 3630, 3100 br, 3016, 1742, 1631 cm^{-1}; IR (KBr) 3436, 3100 br, 1735, 1622, 1614, 1089, 1003 cm^{-1}; NMR ($CDCl_3$) 1.62 δ (1H, s, exchangeable with D_2O, –OH), 1.84 (3H, s, Me), 1.6–2.2 (4H, m, 2× CH_2), 2.21 (3H, s, ArMe), 3.46 (2H, d, J = 7.5 Hz, $ArCH_2CH$=), 3.65 (2H, t, J = 6 Hz, $-CH_2OH$), 3.85 (3H, s, OMe),

5.25 (2H, s, $ArCH_2O$–), 5.32 (1H, t, J = 7.5 Hz, olefinic H), 7.78 (1H, s, exchangeable with D_2O, ArOH); MS *m/e* 306 (27%, M^+), 288 (20, $M-H_2O$), 247 (87, $M-HOCH_2CH_2CH_2$), 229 (28), 207 (100, $M-HOCH_2CH_2CH{=}CMeCH_2$), 159 (45), 85 (100) with m* corresponding to 306→288, 288→270, 207→159. (Found: C, 66.5; H, 7.2. Calc. for $C_{17}H_{22}O_5$: C, 66.7; H, 7.2%).

The alcohol **19**. The above **21** (60 mg) in MeOH (10 ml) was treated with an excess of ethereal CH_2N_2 for 18 hr, giving the *dimethoxy alcohol* **19**, a colourless oil (62 mg), b.p. 165°/0.005 mm, R_f 0.4 (MeOH–$CHCl_3$, 1:49); IR (KBr) 3400, 1760, 1600, 1316, 1128, 1033, 994, 966, 914 cm^{-1}; NMR ($CDCl_3$) 1.80 δ (3H, s, Me), 1.5–2.2 (5H, m, 1H exchangeable with D_2O, 2 × CH_2 and –OH), 2.19 (3H, s, ArMe), 3.38 (2H, s, J = 8 Hz, $ArCH_2CH$=), 3.59 (2H, t, J = 6.5 Hz, $-CH_2OH$), 3.79 and 4.04 (ea 3H, s, OMe), 5.11 (2H, s, $ArCH_2O$–), 5.18 (1H, t, J = 8 Hz, olefinic H); MS *m/e* 320 (58%, M^+), 261 (40, $M-HOCH_2CH_2CH_2$), 221 (73, $M-HOCH_2CH_2CH{=}CMeCH_2$), 207 (60), 85 (100). (Found: C, 67.3; H, 7.5. $C_{18}H_{24}O_5$ requires: C, 67.5; H, 7.6%).

The 7-methylphthalan-1-ones **18** *and* **28**. The MnO_2 used in the following reaction was prepared as before[12] but had been stored for 12 months at room temp and humidity. A soln of **17** (97 mg) in dry $CHCl_3$ was stirred at room temp under N_2 with MnO_2 (1 g) until **17** could not be detected by tlc (*ca.* 5 hr). Prep tlc gave the *7-methylphthalan-1-ol* **23** as an unstable colourless oil (70 mg, 82%), R_f 0.5 (MeOH–$CHCl_3$, 1:19); IR ($CHCl_3$) 3500 br, 1655, 1600 cm^{-1}; NMR ($CDCl_3$) 1.78 δ (3H, br s, Me), 1.6–2.4 (6H, m, 2H exchangeable with D_2O, 2 × CH_2 and 2 × OH), 2.28 (3H, s, ArMe), 3.35 (2H, d, J = 7.5 Hz, $ArCH_2CH$=), 3.55 (2H, t, J = 6.5 Hz, $-CH_2OH$), 3.72 and 3.76 (ea 3H, s, OMe), 5.2 (2H, br s, irr at 6.4 δ→s, $ArCH_2O$–), 5.25 (1H, m, olefinic H), 6.4 [1H, br s, irr 5.2 δ→s, $ArCH(OH)O$–].

Compound **23** (70 mg) in dry $CHCl_3$ (15 ml) was stirred with active MnO_2 (700 mg) under N_2 for 12 hr. Filtration and evaporation gave the *7-methylphthalan-1-one* **18** as an oil (65 mg, 95%); IR ($CHCl_3$) 3500 br, 1760, 1600 cm^{-1}; NMR ($CDCl_3$) 1.80 δ (3H, br s, Me), 1.4–2.3 (5H, m, 1H exchangeable with D_2O), 2.53 (3H, s, ArMe), 3.42 (2H, d, J = 6.5 Hz, $ArCH_2CH$=), 3.59 (2H, t, J = 6.5 Hz, $-CH_2OH$), 3.75 and 3.86 (ea 3H, s, OMe), 5.19 (1H, m, olefinic H), 5.40 (2H, s, $ArCH_2O$–).

The above **18** (65 mg) in acetone (2 ml) was treated at 0° with Jones reagent (0.2 ml) for 15 min. Crystallization of the acidic fraction of the product from $CHCl_3$–pet. ether gave the *7-methylphthalan-1-one* **29** as colourless needles (55 mg, 83%), m.p. 107–110°, R_f 0.4 (MeOH–$CHCl_3$, 1:19); IR ($CHCl_3$) 3520 br, 1760, 1711, 1595 cm^{-1}; IR (KBr) 3200–2850, 1754, 1695, 1595, 1295, 1116, 1032 cm^{-1}; UV (EtOH) λ_{max} 210 nm (ε 16,000), 250 (ε 10,900), 295 (ε 5500); NMR ($CDCl_3$) 1.80 δ (3H, s, Me), 2.36 (4H, br s, 2 × CH_2), 2.56 (3H, s, ArMe), 3.42 (2H, d, J = 7 Hz, $ArCH_2CH$=), 3.72 and 3.85 (ea 3H, s, OMe), 5.12 (1H, m, olefinic H), 5.04 (2H, s, $ArCH_2O$–), 9.5 (1H, br, exchangeable with D_2O, $-CO_2H$). (Found: C, 64.4; H, 6.8. $C_{18}H_{22}O_6$ requires: C, 64.7; H, 6.6%).

The p-toluidide **24**. The acid **1** (520 mg), suspended in dry benzene (50 ml) was stirred with oxalyl chloride (1 ml) for 2 hr at room temp. After evaporation, the residue, in benzene (50 ml), was refluxed with *p*-toluidine (230 mg) for 2 hr. After washing successively with water, dil HClaq and sat $NaHCO_3$aq, evaporation gave the *phenolic p-toluidide* **24** which crystallized from EtOH as colourless prisms (590 mg, 90%), m.p. 103–104°, R_f 0.45 ($CHCl_3$); IR (Nujol 3450, 3360, 1752, 1660, 1618, 1600, 1535 cm^{-1}; UV (EtOH) λ_{max} 217 nm (ε 33,000), 250 (ε 17,400), 306 (ε 4500); NMR ($CDCl_3$) 1.82 δ (3H, s, Me), 2.05 (3H, s, $-C_6H_4Me$), 2.28 (3H, s, ArMe), 2.40 (4H, s, 2 × CH_2), 3.39 (2H, d, J = 7 Hz, $ArCH_2CH$=), 3.78 (3H, s, OMe), 5.11 (2H, s, $ArCH_2O$–), 5.30 (1H, t, J = 7 Hz, olefinic H), 7.14 (4H, AA'BB' system, J = *ca.* 3 Hz, $-C_6H_4NH$–), 7.40 (1H, s, exchangeable with D_2O–CF_3CO_2D, NH), 7.50 (1H, br, exchangeable with D_2O, –OH). (Found: C, 70.2; H, 6.6; N, 3.5. $C_{24}H_{27}NO_5$ requires C, 70.4; H, 6.7; N, 3.4%).

The p-toluidide **25**. The above **16** (180 mg) in toluene (10 ml) was treated with an excess of ethereal CH_2N_2 and allowed to stand 18 hr at room temp. Filtration and evaporation gave **25** as an oil, R_f 0.5 ($CHCl_3$); IR (liquid film) 3390, 1750, 1659, 1600, 1635, 1315, 1132, 770 cm^{-1}; NMR ($CDCl_3$) 1.83 δ (3H, s, Me), 2.11 (3H, s, $-C_6H_4Me$), 2.28 (3H, s, ArMe), 2.40 (4H, s, 2 × CH_2), 3.39 (2H, d, J = 7 Hz, $ArCH_2CH$=), 3.74 and 4.05 (ea 3H, s, OMe), 5.05 (2H, s, $ArCH_2O$–), 5.22 (1H, t, J = 7 Hz, olefinic H), 7.12 (4H, AA'BB' system, J = *ca* 3 Hz, $-C_6H_4NH$–), 7.37 (1H, br, exchangeable with D_2O–CF_3CO_2D, NH).

Ozonolyis of the p-toluidide **25**. Compound **25** (180 mg) was ozonized in EtOAc at −80° for 30 min and the ozonide reduced by stirring with $NaBH_4$ (50 mg) in aqueous MeOH (1:1) at room temp for 2 hr. Extraction with EtOAc and prep tlc gave, firstly, **26** (25 mg, 30%) as colourless prisms, m.p. 114–115° from $CHCl_3$–pet. ether, R_f 0.4 (MeOH–$CHCl_3$, 1:19); IR ($CHCl_3$) 3435, 1623, 1598, 1510 cm^{-1}; IR (KBr) 3330, 3250, 3180, 3120, 1670, 1608, 1550, 1511, 1310, 1128, 816 cm^{-1}; NMR ($CDCl_3$) 2.20 (3H, d, J = 6.5 Hz, CH_3CHOH–), 1.6–2.0 (2H, m, CH_2), 3.30 (3H, s, $-C_6H_4Me$), 2.43 (2H, t, J = 6.5 Hz, $-CH_2CO$–), 3.21 (1H, br, exchangeable with D_2O, –OH), 3.85 (1H, m, –CHOH–), 7.20 (4H, AA'BB' system, J = *ca.* 3 Hz, $-C_6H_4NH$–), 8.10 (1H, br, exchangeable with D_2O–CF_3CO_2D, NH). (Found: C, 69.4; H, 8.0; N, 6.9. $C_{12}H_{17}NO_2$ requires: C, 69.5; H, 8.3; N, 6.8%).

The second product was the *alcohol* **27** (30 mg, 30%) as colourless needles, m.p. 98–100° from $CHCl_3$–pet. ether, R_f 0.65 (MeOH–$CHCl_3$, 1:19); IR ($CHCl_3$) 1754, 1597 cm^{-1}; IR (KBr) 3240, 1761, 1738, 1590, 1301, 1124, 1100, 954 cm^{-1}; UV (EtOH) λ_{max} 217 nm, 249 (ε 5500), 295 (ε 1500); NMR ($CDCl_3$) 2.00δ (1H, br, exchangeable with D_2O, –OH), 2.19 (3H, s, ArMe), 2.99 (2H, t, J = 6.5 Hz, $ArCH_2CH_2$–), 3.75 (2H, m, $-CH_2OH$), 3.80 and 4.08 (ea 3H, s, OMe), 5.11 (2H, s, $ArCH_2O$–); MS *m/e* 252 (60%, M^+), 234 (26, $M-H_2O$), 221 (100, $M-HOCH_2$), 220 (57), 191 (80), 163 (59), 161 (57), 133 (74), 91 (51), 77 (44) with m* corresponding to 252→234, 252→221, 221→191, 191→163, 191→161 and 161→133. (Found: C, 62.0; H, 6.4. $C_{13}H_{16}O_5$ requires: C, 61.9; H, 6.4%).

The acid **28**. The alcohol **27** (17 mg) in acetone (1 ml) was treated at 5° with Jones reagent (0.05 ml) for 10 min. Crystallization of the acidic fraction of the product from $CHCl_3$–pet. ether gave **28** as colourless prisms (12 mg, 70%), m.p. 148–150° (lit[14] m.p. 150–151°), R_f 0.15 (MeOH–$CHCl_3$, 1:19), IR ($CHCl_3$) 1759, 1710, 1601 cm^{-1}; IR (KBr) 3000 br, 2940, 1752, 1699, 1599, 1297, 1228, 1200, 1129, 1100, 1070, 1035, 989, 965 cm^{-1}; UV (EtOH) λ_{max} 217 nm (ε 26,000), 250 (ε 5400), 295 (ε 1500); NMR ($CDCl_3$) 2.18 δ (3H, s, Me), 3.76 (2H, s, $ArCH_2O$–), 3.79 and 4.09 (ea 3H, s, OMe), 5.14 (2H, s, $ArCH_2O$–), 7.00 (1H, br, exchangeable with D_2O); MS *m/e* 266 (92%, M^+), 248 (77, $M-H_2O$), 221 (63, $M-HO_2C^·$), 207 (100), 163 (59), 161 (57), 133 (74), 91 (51), 77 (44) with m* corresponding to 266→248, 221→191, 191→163, 191→161 and 161→133. (Found: C, 58.3; H, 5.2. Calc. for $C_{13}H_{14}O_6$: C, 58.6; H, 5.3%).

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