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The Stereo Structures of Some Mycophenolic Acid Derivatives

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X-Ray crystallography affords the stereochemistry of three iodo compounds derived from mycophenolic acid. These included the crystallographic structure of a tertiary iodide.

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Introduction

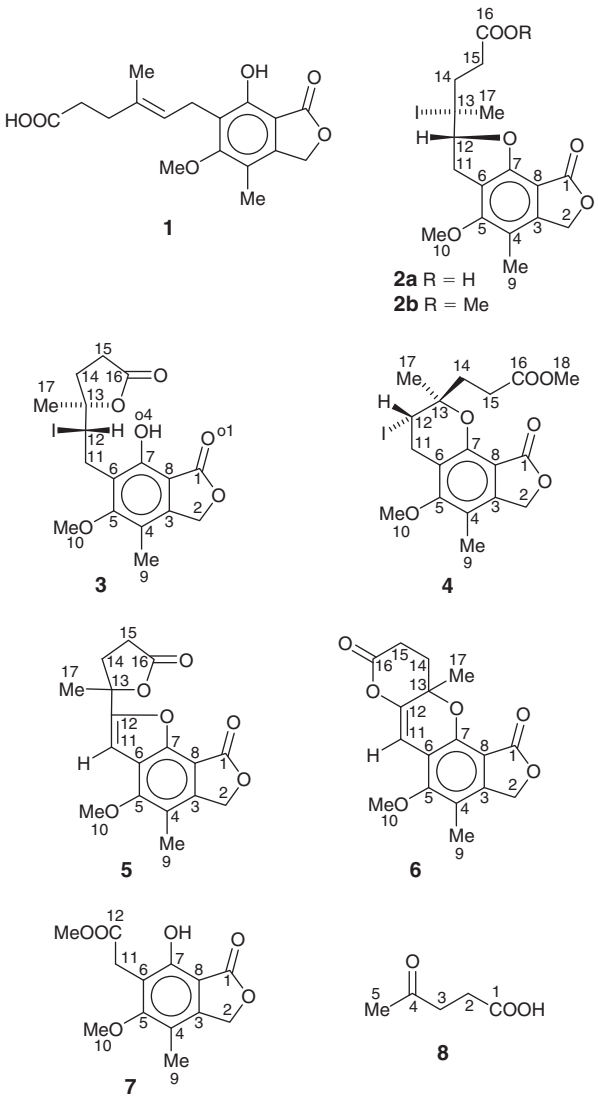
Mycophenolic acid **1** remains a compound of considerable medicinal interest.[1] It shows exceptional antimitotic properties, but is rapidly eliminated from the human body as the glucuronate derived from the phenolic hydroxyl group. Attempts to block this hydroxyl group lead to loss of biological activity, but various iodo compounds have been explored[2] with the aim to temporarily block the phenolic group to give derivatives which might then revert back to mycophenolic acid in vivo. These compounds, such as **2**–**4**, all have two new stereogenic centres (Scheme 1). The relative stereochemistry at these centres was previously assigned only on mechanistic grounds; and we now provide X-ray crystallographic evidence for the correct structures.

Tertiary iodides are normally unstable and unsuited to X-ray examination. The crystallographic structures of only 38 tertiary iodides are to be found in the Cambridge Structural Database,[3] and the large majority of these are bridgehead iodides which owe their relative stability to the protection that the bridgehead position provides against S_N2, S_N1, E2, or E1 loss of the iodine atom. It was hoped that the electronegative oxygen on the carbon atom vicinal to the iodine in compound **2** would provide a stabilizing influence, and that this tertiary iodide would be sufficiently stable for X-ray crystallographic examination.

Results and Discussion

As previously reported,[2] mycophenolic acid, when shaken in bicarbonate solution with iodine in ethyl acetate, provides almost equal amounts of the acid **2a** and the dilactone **3**. These two compounds, both crystalline, are easily isolated from the bicarbonate and ethyl acetate layers respectively. Both compounds are racemic as befits the achiral nature of the starting mycophenolic acid **1**. Common crystallographic numbering is used for the carbon skeletons in the following compounds as it enables a direct comparison of atoms to be made within the variety of chemical structures.

Compound **3** afforded crystals suitable for X-ray work. The structure comprises two independent molecules in the asymmetric unit. There are no significant differences between their respective bond lengths, angles, or dihedral angles. One of these molecules is shown in Fig. 1. Of most relevance to this study is that the stereochemistry 12*RS*,13*SR* is defined, which is consistent with the *trans* addition of iodine and the carboxyl group to



Scheme 1. Structures **1**–**8**.

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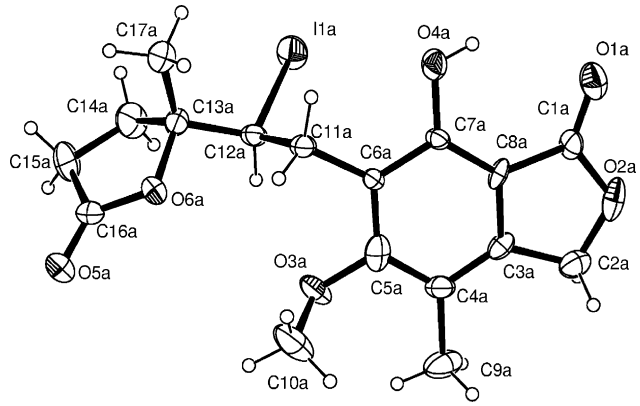

Fig. 1. Compound **3**.

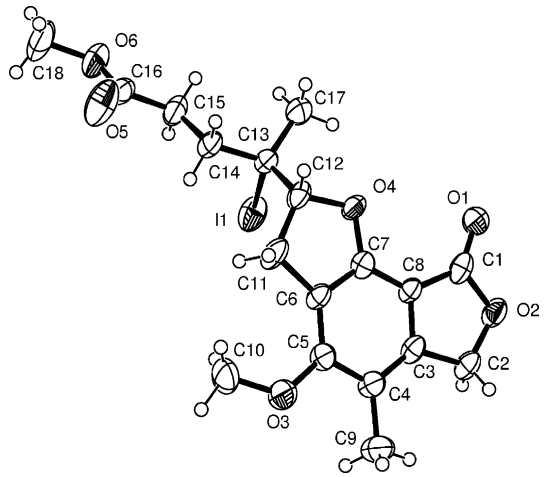

Fig. 2. Compound **2b**.

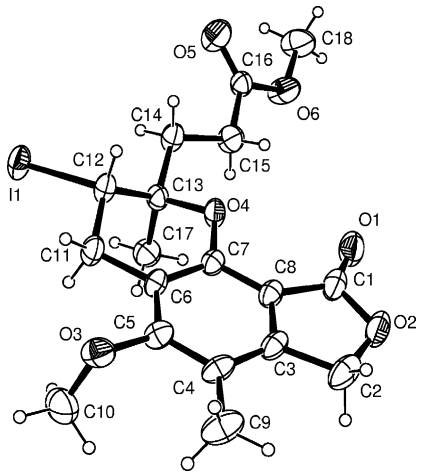

Fig. 3. Compound **4**.

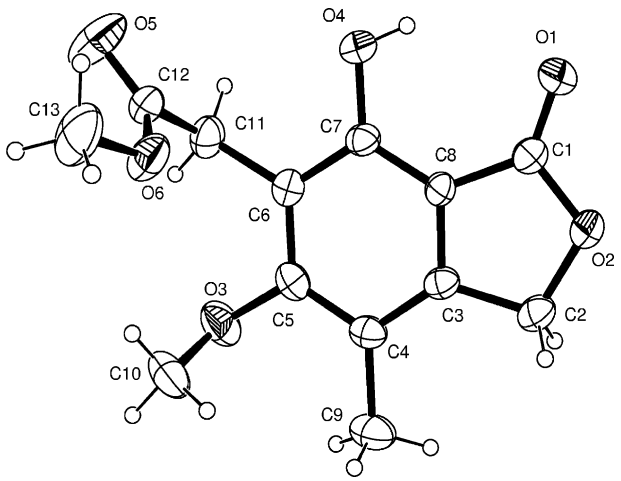

Fig. 4. Compound **7**.

the starting double bond. The hydroxyl groups of each molecule participate in both intramolecular (O4A–H4A···O1A 2.38 Å, 136.5°; O4B–H4B···O1B 2.52 Å, 124.2°) and intermolecular H-bonds (O4A–H4A···O5A $[-x+2, y, z+1/2]$ 2.27 Å, 138.7°; O4B–H4B···O5B $[-x+1, y, z+1/2]$ 2.20 Å, 135.1°), which generate two independent and racemic H-bonded chains that each follow a glide plane along the *c*-axis.

Acid **2a** crystallized as fine feathers from several solvents, and did not provide material suitable for X-ray examination. The compound was therefore converted into its methyl ester **2b**, either with diazomethane or through the mixed anhydride route. This product **2b** gave needles from acetone/water at room temperature, and provided the crystallographic structure shown in Fig. 2. Again the 12*RS*,13*SR* configuration is consistent with the *trans* addition of iodine and the phenolic oxygen to the starting mycophenolic double bond.

Perhaps surprisingly, given the presence of the tertiary iodide, compound **2a** cleanly dissolves in concentrated sulfuric acid. When this solution is poured into methanol the rearranged methyl ester **4** is formed[2] in near quantitative yield. X-Ray crystallography of this compound provided the stereochemistry depicted in Fig. 3. The configuration is again 12*RS*,13*SR*, consistent with inversion at both these carbon atoms during the rearrangement **2a** to **4**. In the most stable conformer, the methyl group in compound **4** is *quasi axial* to the pyran ring, while the iodine is *equatorial*.

During one attempt to convert acid **2a** into its methyl ester **2b** by the mixed anhydride route (ethyl chloroformate and triethylamine, followed by methanol), the reaction temperature of the first step was accidentally allowed to become too high. The crystalline product failed to provide suitable crystals for X-ray examination, but gave excellent NMR data that indicated that it had lost iodine to form a trisubstituted double bond. The compound is neutral and possesses no methyl ester group, and is formulated as either **5** or **6**. The carbon connectivites in these two compounds are identical, and NMR examination did not allow a final distinction between the two possibilities. A least-squares analysis of the chemical shift differences, for both the proton and carbon data, from those shifts predicted by *CHEMDRAW* also did not allow a clear distinction, although structure **5** was slightly favoured. However, the magnitude of the vicinal coupling constants $J_{14,15}$ (10, 9.8, 9.5, 2.1 Hz) might suggest that these protons are on a six-membered ring rather than a five, favouring structure **6**. We note that structure **5** has been tentatively proposed[2] for a compound isolated from a considerable mixture when acid **2a** was treated with hot pyridine. The product now obtained (mp 148°) is different from that previously described[2] (mp 192–194°).

Ironically, given the time we had spent in attempting to grow crystals of compound **5**/**6**, the NMR sample deposited very large crystals as colourless needles following the slow evaporation (about one week) of $CDCl_3$/methanol. X-Ray crystallographic examination of these crystals provided the decomposed structure **7** (Fig. 4). In contrast to the H-bonded polymer seen

Table 1. ^{1}H NMR chemical shifts and multiplicities

	Compound				
	2b	**3**	**4**	**5/6**	**7**
H2 (2)	5.10 (s)	5.19 (s)	5.05 (s)	5.23 (s)	5.21 (s)
H9 (3)	2.04 (s)	2.13 (s)	2.11 (s)	2.16 (s)	2.15 (s)
H10 (3)	3.96 (s)	3.78 (s)	3.79 (s)	4.12 (s)	3.76 (s)
H11a	3.59 (dd)	3.36 (dd)	3.50 (dd)	6.30 (s)	3.71 (s)
H11b	3.42 (dd)	3.25 (dd)	3.33 (dd)	–	3.71 (s)
H12	4.71 (dd)	4.66 (dd)	4.34 (dd)	–	–
H14a	2.29 (ddd)	2.36 (ddd)	2.36 (ddd)	2.70 (ddd)	–
H14b	2.07 (ddd)	2.19 (ddd)	2.17 (ddd)	2.35 (ddd)	–
H15a	2.71 (ddd)	2.63 (m)	2.67 (ddd)	2.82 (ddd)	–
H15b	2.59 (ddd)	2.63 (m)	2.56 (ddd)	2.51 (ddd)	–
H17 (3)	1.85 (s)	1.70 (s)	1.46 (s)	1.80 (s)	–
Other	3.69 (OMe)	7.75 (OH)	3.61 (OMe)	–	7.70 (OH) 3.70 (OMe)

in the (acentric) structure **3**, the compound **7** forms centrosymmetric H-bonded dimers involving both intramolecular (O4–H4···O1 2.38 Å, 139.5°) and intermolecular (O4–H4···O1 $[-x, -y+1, -z+2]$ 2.17 Å, 141.1°) interactions. The compound provided excellent NMR data consistent with structure **7**. The compound **7** as formed was contaminated with an equimolar mixture of 4-ketopentanoic acid (levulinic acid) **8**, identified through NMR spectroscopy and by comparison with an authentic sample.

The mechanism for the conversion of **5**/**6** in $CDCl_3$/methanol into a mixture of **7** and **8** is unclear. All the carbons are accounted for, and the products are consistent with an ozonolysis-type cleavage, but of material with a C12–C13 double bond. We note that levulinic acid **8** has been previously reported[4] as an ozonolysis product of mycophenolic acid **1**, but compound **7** now appears to be new.

Experimental

^{1}H and ^{13}C NMR spectra in $CDCl_3$ solution at ambient temperature were recorded using Bruker 500 MHz spectrometers. Assignments were made using DEPT, HSQC, HMBC, COSY, and double quantum filtered COSY pulse sequences.

*Reaction of Mycophenolic Acid **1** with Iodine*

Following the literature procedure,[2] iodine in ethyl acetate was added with shaking to mycophenolic acid dissolved in aqueous sodium bicarbonate. When the iodine colour persisted, the aqueous layer was acidified to give the (12*RS*,13*SR*) iodo acid **2a**, mp 168°C, identical with the literature,[2] in approx. 50% yield. The ^{1}H NMR spectrum ($(CD_3)_2SO$) showed peaks consistent with the literature,[2] but the compound decomposed too fast for elaborate pulse sequencing. Crystals suitable for X-ray crystallography could not be obtained.

The ethyl acetate layer from the above reaction was washed with sodium thiosulfate solution, with brine, and then taken to dryness to yield crystals of the (12*RS*,13*SR*) dilactone **3** (~50%). mp 168°C (dec.; lit.[2] 170°C). δ_H see Table 1, with $J_{11a,11b}$ −14.5, $J_{11a,12}$ 11.3, $J_{11b,12}$ 3.1, $J_{14a,14b}$ −13.2, $J_{14a,15a}$ 9.0, $J_{14a,15b}$ 9.0, $J_{14b,15a}$ 6.8, $J_{14b,15b}$ 8.4, $J_{15a,15b} \approx -13$ Hz, consistent with the literature,[2] but now with better resolution. δ_C see Table 2, with the compound >98% pure. Crystals for X-ray structure analysis were grown from acetone.

Table 2. ^{13}C NMR chemical shifts in $CDCl_3$

	Compound				
	2b	**3**	**4**	**5/6**	**7**
C1	168.6	172.7	168.3	168.4	171.7
C2	69.0	70.1	68.0	69.4	70.1
C3	147.3	145.4	147.9	143.9	145.6
C4	115.4[A]	116.9	116.2	116.0	116.7
C5	159.6	164.0	161.0	144.7	163.8
C6	115.3[A]	120.0	115.3	111.5	115.7
C7	157.4	153.5	151.0	156.6	153.6
C8	102.6	106.5	109.1	103.6	106.5
C9	11.0	11.6	11.1	11.2	11.6
C10	59.2	60.9	60.3	59.9	61.0
C11	34.3	29.07[A]	31.9	119.1	28.9
C12	91.2	40.4	28.1	155.9	172.6
C13	56.8	87.5	78.4	86.8	–
C14	38.7	35.1	35.1	34.2	–
C15	32.7	29.11[A]	28.0	28.0	–
C16	173.0	175.8	173.6	175.1	–
C17	27.6	21.9	20.3	23.3	–
Me ester	51.9	–	51.8	–	53.3

[A]Values bearing the same superscript within a column may be interchanged.

*Ester **2b***

Acid **2a** in acetone was treated overnight at 0°C with diazomethane in ether. The solution was taken to dryness to provide colourless crystals of the (12*RS*,13*SR*) ester **2b** from acetone at room temperature. mp 126°C (slow dec. ~108°C) (lit.[2] 133–135°C). δ_H see Table 1, with $J_{11a,11b}$ −16.0, $J_{11a,12}$ 9.6, $J_{11b,12}$ 7.0, $J_{14a,14b}$ −15.0, $J_{14a,15a}$ 4.9, $J_{14a,15b}$ 11.2, $J_{14b,15a}$ 11.0, $J_{14b,15b}$ 5.2, $J_{15a,15b}$ −16.2 Hz, consistent with the literature,[2] but now with better resolution. $^1\delta_C$ see Table 2, with the compound >98% pure. Crystals for X-ray structure analysis were grown from acetone by slow evaporation at room temperature.

The same ester **2b** was available from acid **2a** by the mixed anhydride route (tetrahydrofuran, triethylamine, ethyl chloroformate, and methanol; all at ice temperature).[2]

*Ester **4***

Acid **2a** was dissolved in conc. H_2SO_4 at room temperature. The solution was poured onto methanol at 0°C. Ice was added

to give a gummy semi-crystalline precipitate. The liquid was decanted off and the residue taken into ethyl acetate, washed with bicarbonate solution, and with brine to provide crystals of the (12*RS*,13*SR*) ester **4** from ethyl acetate. mp 168°C (lit.[2] 170°C). δ_H see Table 1, with $J_{11a,11b}$ −17.0, $J_{11a,12}$ 5.5, $J_{11b,12}$ 10.5, $J_{14a,14b}$ −14.0, $J_{14a,15a}$ 10.5, $J_{14a,15b}$ 5.5, $J_{14b,15a}$ 5.5, $J_{14b,15b}$ 10.5, $J_{15a,15b}$ −16.0 Hz, consistent with the literature,[2] but now with better resolution. δ_C see Table 2, with the compound >98% pure. Crystals for X-ray structure analysis were grown from ethyl acetate by slow evaporation at room temperature.

*Compound **5/6***

Acid **2a** in ether was treated with triethylamine. Ethyl chloroformate was added with inadequate cooling, when the ether boiled. Methanol was added followed by iced water to the clear solution. The product was extracted into ethyl acetate, washed with bicarbonate solution and brine, and was taken to dryness. The crystalline product went somewhat orange over the weekend (loss of iodine). Recrystallization (ethyl acetate) gave colourless feathery needles. mp 148°C. Found: C 64.4, H 5.2. $C_{17}H_{16}O_6$ requires C 64.6, H 5.1%. δ_H see Table 1, with H11 as a sharp singlet, $J_{14a,14b}$ −13.5, $J_{14a,15a}$ 9.5, $J_{14a,15b}$ 2.1, $J_{14b,15a}$ 10.0, $J_{14b,15b}$ 9.8, $J_{15a,15b}$ −17.7 Hz. δ_C see Table 2, with the compound >98% pure. Crystals suitable for X-ray structure analysis could not be obtained.

*Ester **7***

The above compound **5/6** by slow evaporation (∼1 week) of a $CDCl_3$/methanol solution produced large colourless needles in a residual oily base. ^1H and ^{13}C NMR analysis showed an approximately equimolar mixture of compounds **7** and **8**. Methyl 2-(4′-hydroxy-6′-methoxy-7′-methyl-3′-oxo-1′,3′-dihydroisobenzofuran-5′-yl)ethanoate **7** had mp 132°C (from chloroform). Found: C 58.4, H 5.5. $C_{13}H_{14}O_6$ requires C 58.7, H 5.3%. δ_H see Table 1, with no coupled protons. $^1\delta_C$ see Table 2, with the compound >98% pure apart from traces of levulinic acid **8**. Large needles for X-ray crystallography were grown from chloroform.

Levulinic acid (4-oxopentanoic acid) **8** had δ_H 2.72 (2H t, H3), 2.59 (2H t, H2), 2.16 (3H s, H5), with $J_{2,3}$ 6.5 Hz. δ_C 206.6 (C4), 178.4 (C1), 37.7 (C3), 29.7 (C5), 27.7 (C2). These assignments were confirmed by two-dimensional and long-range coupling experiments. The spectra were identical to those of an authentic sample of 4-oxopentanoic acid, and are consistent with the literature[5] provided that the assignments to H2 and H3, and to C2 and C3, are reversed in this database.[5]

Crystallography

*Compound **2b** (Fig. 2)*

$C_{18}H_{21}IO_6$: *M* 460.25. *T* 293(2) K, monoclinic, space group $P2_1/c$, *a* 6.437(1), *b* 23.369(2), *c* 12.520(3) Å, β 101.17(2)°, *V* 1847.7(4) Å^3, *Z* 4, *F*(000) 920, D_c 1.655 g cm^{-3}, μ 17.63 cm^{-1}, 3246 unique data ($2\theta_{max}$ 50°), *R* 0.0429 (for 1846 reflections with $I > 2\sigma(I)$), wR_2 0.1179 (all data).

*Compound **3** (Fig. 1)*

$C_{17}H_{19}IO_6$: *M* 446.22. *T* 293(2) K, orthorhombic, space group $Pc2_1b$, *a* 8.932(2), *b* 19.743(4), *c* 20.210(3) Å, *V* 3564(1) Å^3, *Z* 8, *F*(000) 1776, D_c 1.663 g cm^{-3}, μ 18.25 cm^{-1}, 3237 unique data ($2\theta_{max}$ 50°), *R* 0.0408 (for 1857 reflections with $I > 2\sigma(I)$), wR_2 0.0826 (all data).

*Compound **4** (Fig. 3)*

$C_{18}H_{21}IO_6$: *M* 460.25, *T* 293(2) K, monoclinic, space group $P2_1/a$, *a* 13.624(3), *b* 8.752(2), *c* 15.957(8) Å, β 104.66(3)°, *V* 1841(1) Å^3, *Z* 4, *F*(000) 920, D_c 1.661 g cm^{-3}, μ 17.70 cm^{-1}, 3226 unique data ($2\theta_{max}$ 50°), *R* 0.0410 (for 1814 reflections with $I > 2\sigma(I)$), wR_2 0.1191 (all data).

*Compound **7** (Fig. 4)*

$C_{13}H_{14}O_6$: *M* 266.24. *T* 293(2) K, triclinic, space group $P\bar{1}$, *a* 7.910(1), *b* 8.393(1), *c* 10.536(1) Å, α 66.07(1), β 83.32(1), γ 81.96(1)°, *V* 631.7(1) Å^3, *Z* 2, *F*(000) 280, D_c 1.400 g cm^{-3}, μ 1.12 cm^{-1}, 2224 unique data ($2\theta_{max}$ 50°), *R* 0.0448 (for 1144 reflections with $I > 2\sigma(I)$), wR_2 0.1452 (all data).

Intensity data were collected on an Enraf–Nonius CAD4 four-circle diffractometer using graphite monochromatized $Mo_{K\alpha}$ radiation (λ 0.71073 Å) in the ω–2θ scan mode. Data were measured at room temperature. Lattice dimensions were determined by a least-squares fit of the setting parameters of 25 independent reflections. Data reduction and empirical absorption corrections (ψ-scans) were performed with the *WINGX* package.[6] Structures were solved by direct methods with *SHELXS* and refined by full matrix least-squares analysis with *SHELXL97*.[7] All non-H atoms were refined with anisotropic thermal parameters, and H-atoms were constrained at estimated positions using a riding model. The atomic nomenclature is defined in Figs 1, 2, 3, and 4 drawn with *ORTEP3*.[8] Crystallographic data in CIF format are available from the Cambridge Crystallographic Data Base (CCDC deposition nos 629744, 629742, 629741, and 629743 for compounds **2b**, **3**, **4**, and **7**, respectively).

Acknowledgments

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