Tetrahedron. Vol. 25, pp. 4915 to 4918. Pergamon Press 1969. Printed in Great Britain

A REINVESTIGATION OF THE DEMETHYLATION OF MYCOPHENOLIC ACID

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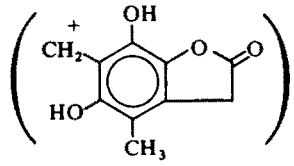
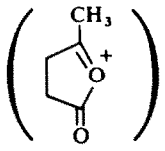
(*Received in the UK* 26 *June* 1969; *Accepted for publication* 1 *July* 1969)

Abstract—The reaction of mycophenolic acid (I, R = Me, R^1 = H) with hydriodic acid has been shown to give the isomeric chromans (II and III) and the lactone IV. Chroman II was previously thought to be desmethylmycophenolic acid (I, R, R^1 = H). With hydriodic acid both chromans, (II and III) give a similar equilibrium mixture.

INTEREST in the antitumour activity of mycophenolic acid[1] (I), (R = Me, R^1 = H) prompted an examination of compounds derived from this fungal metabolite. Raistrick[2] has reported that demethylation of mycophenolic acid with hydriodic acid gives the nor-compound (I, R, R^1 = H). This reaction seemed unlikely to us since under strongly acidic conditions chroman formation might be expected.[3] A re-investigation of this reaction has shown that none of the demethylated compound is in fact formed.

Treatment of mycophenolic acid with hydriodic acid was found to produce three compounds, which were readily separated by a combination of crystallization and thick plate chromatography. The least polar compound formed in 47% yield, was identical with Raistrick's nor-compound and has been assigned the chroman structure (II). The compound was isomeric with the required demethylated compound, but displayed no olefin proton in its NMR spectrum, five methylene groups however were discernible. The hydroxyphthalide structure was supported by the facts that the compound, like mycophenolic acid, gave an intense blue ferric chloride colouration and that the IR spectrum of a dioxan solution showed a lactone CO at 1740 cm^{-1}, mycophenolic acid exhibiting a lactone CO at 1745 cm^{-1}. The least polar product, formed in 14% yield, was found to be isomeric with II, and was given the chroman structure III. This acid possessed a very similar NMR spectrum to the chroman (II), but did not give a ferric chloride colouration. The IR spectrum of a dioxan solution showed a lactone CO at 1765 cm^{-1} indicating the absence of H-bonding to a 7-OH substituent in the aromatic ring. The third compound, again having the empirical formula, $C_{16}H_{18}O_6$, was isolated in 1% yield, and has been assigned the 5,7-dihydroxy-4-methyl-6-(β-2-methyl-5-oxotetrahydrofur-2-ylethyl)phthalon-1-one structure(IV). The NMR spectrum was very similar to that of the isomeric chromans five methylene groups again being present. The IR spectrum showed two CO bonds at 1755 cm^{-1} and 1730 cm^{-1} and the compound gave an intense blue ferric chloride colouration, characteristic of the 7-hydroxyphthalide grouping. The mass spectrum gave further evidence in favour of this structure, since fragmentation ions at 193

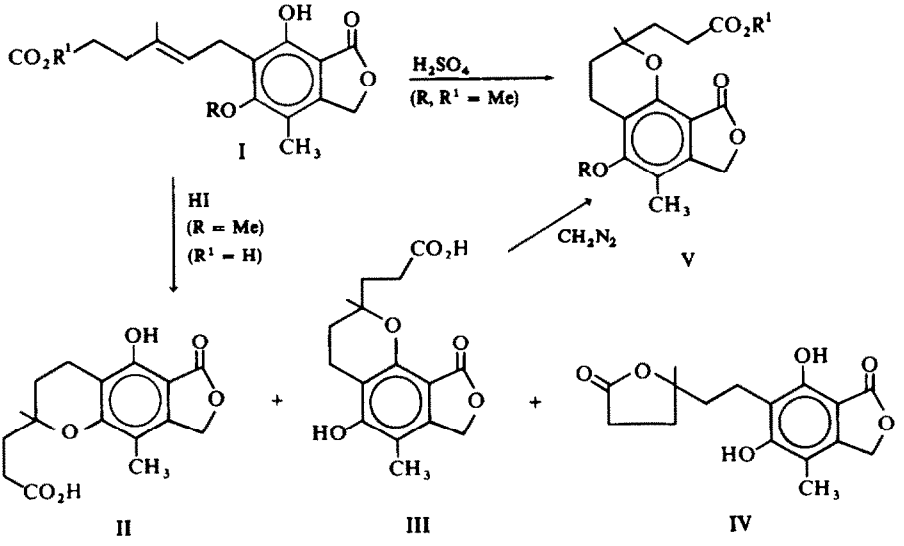


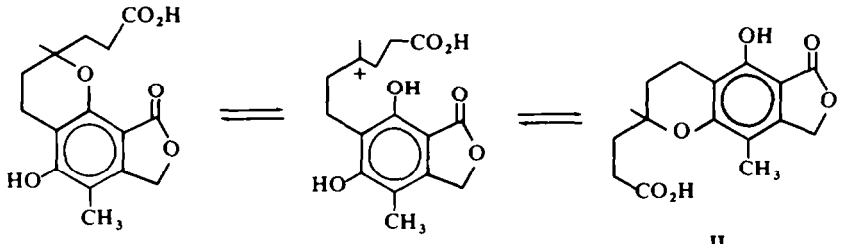
and 99  were observed.

The assignment of structure II to Raistrick's compound and the isomeric chroman structure III to the least polar product was conveniently verified by the concentrated sulphuric acid cyclization of mycophenolic acid methyl ester (I, R, R^1 = Me) to the chroman (V, R, R^1 = Me), which was identical with the product obtained by reacting III with excess diazomethane.

Treatment of Raistrick's compound (II) with hydriodic acid caused partial isomerization to III, an equilibrium mixture in ratio II/III of 2·9 to 1 was obtained. Reaction of the isomeric chroman (III) with hydriodic acid gave an equilibrium mixture of II/III in a similar 2·6 to 1 ratio. 2-(β-Carboxyethyl)-2,6-dimethyl-5-methoxy-9-oxo-3,4,79-tetrahydro-2H-furo[3.4-h][1]benzopyran (V, R = Me, R^1 = H) formed by treatment of mycophenolic acid with concentrated sulphuric acid, gave an equilibrium mixture of II/III in a 3 to 1 ratio, demethylation followed by formation of the equilibrium mixture occurring. The two chromans, (II and III) and the intermediate carbonium ion (VI), must be in equilibrium under these strongly acidic conditions.

Attempts to demethylate mycophenolic acid with pyridine hydrochloride gave similar results to the hydriodic acid experiment, the chroman (II), its isomer (III), and the lactone (IV) being obtained in 33, 10 and 7% yield respectively.





III VI II

EXPERIMENTAL

IR spectra were obtained on a Perkin Elmer 21; NMR spectra on a Varian H-A100. Mass spectral data was recorded on an A.E.I. M.S.9 instrument.

Reaction of mycophenolic acid with hydriodic acid

Mycophenolic acid (2·0 g) and 55% HI (30 ml) were heated at 80° in N_2 atm for 3 hr and then poured into ice-water, and left overnight. The black gum was extracted with EtOAc and the extract washed with 5% $Na_2S_2O_3$ aq. The EtOAc was evaporated to give a white solid (1·6 g) which was extracted in a soxlet overnight with benzene (20 ml) to give 2-(β-*carboxyethyl*)-2,9-*dimethyl*-5-*hydroxy*-6-*oxo*-3,4,6,8-*tetrahydro*-2H-*furo*[3,4-g][1] *benzopyran* (II; 1·0g), colourless prisms, m.p. 163–165, ν_{max}^{dioxan} 1740 cm^{-1} (C=O); NMR ($CDCl_3$)2·40 τ (2H, D_2O exchangeable), 4·80 τ (S, 2H, CH_2-O), 7·35 τ (M, 4H, $2CH_2$) 8·05 τ (M. 7H, CH_3 and $2CH_2$) 8·67 τ (S, 3H, CH_3), Mass spectrum M^+ 306 ($C_{16}H_{18}O_6$ requires 306). (Found: C, 62·4; H, 6·0, $C_{16}H_{18}O_6$ requires: C, 62·8; H, 5·9%). The benzene insoluble residue was recrystallized from EtOH to give 2-(β-*carboxyethyl*)-2,6-*dimethyl*-5-*hydroxy*-9-*oxo*-3,4,7,9-*tetrahydro*-2H-*furo*[3.4-h][1]*benzopyran* (III, 0·29 g), colourless prisms, m.p. 215°, ν_{max}^{dioxan} 1765 cm^{-1} (C=O); NMR ($(CD_3)_2SO$) −3·1 τ (1H, D_2O exchangeable), 0·53 τ (S, 1H, D_2O exchangeable), 4·97 τ (S, 2H, CH_2-O), 7·55 τ (M, 4H, $2CH_2$), 8·10 τ (M, 7H, $2CH_2$ and CH_3) 8·77 τ (S, CH_3); mass spectrum M^+ 306; $C_{16}H_{18}O_6$ requires: 306. (Found: C, 62·4; H, 6·0, $C_{16}H_{18}O_6$ requires: C, 62·8; H, 5·9%). The benzene filtrate was chromatographed on 1 mm silica plates in benzene/EtOAc/formic acid (66:33:1) to furnish a further 45 mg of II and 5,7-*dihydroxy*-4-*methyl*-6-(β-2-*methyl*-5-*oxotetrahydrofur*-2-*ylethyl*)*phthalan*-1-*one* (IV; 20 mg), colourless needles (from acetone/pet. ether b.p. 60–80), m.p. 171–173°; ν $CHCl_3$ 1755 cm^{-1} (C=O), 1730 cm^{-1} (C=O); NMR ($CDCl_3$) 4·9 τ (S, 2H, CH_2-O), 7·35 τ (M, 4H, —CH_2—Ar, —CH_2C=O), 8·0 τ (M, 7H, CH_3—Ar, $2CH_2$—) 8·55 τ (S, 3H, CH_3). (Found C, 62·2; H, 5·8; $C_{16}H_{18}O_6$ requires: C, 62·2; H, 5·9%); mass spectrum M^+ 306; $C_{16}H_{18}O_6$ requires 306.

2-(β-*Carboxyethyl*)-2,6-*dimethyl*-5-*methoxy*-9-*oxo*-3,4,7,9-*tetrahydro*-2H-*furo*[3.4-h][1]*benzopyran* (V, R = CH_3, R^1 = H)

Mycophenolic acid (10·0 g) was added in small portions with constant stirring to conc H_2SO_4 (35 ml), the temp being kept at −10°. After the addition the mixture was stirred for a further 4 hr at 18°. The reaction mixture was added to ice/water and extracted with $CHCl_3$. Evaporation of the extract furnished a solid which was recrystallized from $CHCl_3$/pet. ether b.p. 60–80° to give the *benzopyran* (6·8 g), colourless prisms, m.p. 186–188°, ν_{max} (dioxan) 1750 cm^{-1} (C=O lactone); NMR ($CDCl_3$) 1·5 τ (1H, CO_2H), 4·9 τ (S, 2H, —CH_2—O) 6·2 τ (S, 3H, OCH_3), 6·3 τ (M, 4H, 2-CH_2—), 7·85 τ (S, 3H, CH_3—Ar) 8·05 τ (M, 4H, $2CH_2$), 8·63 τ (S, 3H, CH_3), (Found: C, 63·5; H, 6·5; $C_{17}H_{20}O_6$ requires: C, 63·7; H, 6·3%).

2-(β-*Methoxycarboxyethyl*)-2,6-*dimethyl*-5-*methoxy*-9-*oxo*-3,4,7,9-*tetrahydro*-2H-*furo*[3.4-h][1]*benzopyran* (V, R, R^1 = CH_3)

(a) Mycophenolic acid methyl ester (0·5 g) was added to well stirred conc H_2SO_4 (2 ml) at 0°. The mixture was allowed to warm to room temp and was stirred for another hr. The reaction mixture was poured into ice water and extracted with EtOAc. The resultant gum was chromatographed on 1 mm silica plate in benzene/EtOAc/formic acid (66:33:1). The bottom band furnished the *acid* V (R = CH_3, R^1 = H) (0·02 g), colourless plates (acetone/pet. ether), m.p. 186–187°, and the top band gave the *methyl ester* (0·34 g), colourless prisms (acetone/pet. ether, b.p. 60–80°), m.p. 89°, ν_{dioxan} 1765 cm^{-1} (C=O), 1735 cm^{-1} (C=O); NMR ($CDCl_3$), 5·02 τ (S, 2H —CH_2—O), 6·28 τ (S, 3H, —OCH_3), 6·42 τ (S, 3H, OCH_3), 7·35 τ (m, 4H, Ar—CH_2,

—CH_2CO—) 7·94 τ (S, 3H, Ar—CH_3) 8·15 τ (m, 4H, 2-CH_2—), 8·70 τ (S, 3H, CH_3), (Found: C, 64·8; H, 6·6, $C_{18}H_{22}O_6$ requires: C, 64·7; H, 6·6%).

(b) Compound V (R = CH_3, R^1 = H) (0·10 g) was treated with excess of an ethereal soln of diazomethane at room temp, and stirred overnight. Addition of a little AcOH and evaporation gave a gum, which furnished the methyl ester (0·071 g), prisms (acetone/pet. ether b.p. 60–80°), m.p. 89°.

(c) Compound III (0·10 g) was treated with diazomethane in an analogous way to give the methyl ester (0·06 g), prisms (acetone/pet. ether b.p. 60–80°), m.p. 89°.

The reaction of 2-(β-*carboxyethyl*)-2,9-*dimethyl*-5-*hydroxy*-6-*oxo*-3,4,6,8-*tetrahydro*-2H-*furo*[3.4-g][1]-*benzopyran* (II) *with hydriodic acid*

The benzopyran (0·10 g) in 55% HI (5 ml) was heated on a steam bath for 7 hr in N_2 atm, then poured into ice/water, and extracted with EtOAc. The extract was washed with 5% $Na_2S_2O_3$ aq and evaporated. Chromatography of the residue on 1 mm silica plates in benzene/EtOAc/formic acid (66:33:1) gave starting material (0·065 g) and its isomer (III) (0·022 g) colourless prisms (EtOH) m.p. 215°.

Reaction of 2-(β-*carboxyethyl*)-2,6-*dimethyl*-5-*hydroxy*-9-*oxo*-3,4,7,9-*tetrahydro*-2H-*furo*[3.4-h][1]*benzo-pyran*, (III) *with hydroiodic acid*

The benzopyran (0·10 g) was treated with HI in an analogous manner described in the preceding reaction. Thick layer chromatography gave starting material (0·023 g) and its isomer (II) (0·063 g), colourless prisms (benzene) m.p. 165°.

The reaction of 2-(β-*carboxyethyl*)-2,6-*dimethyl*-5-*methoxy*-9-*oxo*-3,4,7,9-*tetrahydro*-2H-*furo*[3.4-h][1]-*benzopyran* (V, R = CH_3, R^1 = H) *with hydriodic acid*

The benzopyran (0·10 g) was treated with HI as in the preceeding examples. Thick layer chromatography gave II (0·055 g), colourless prisms (benzene) m.p. 165° and III (0·018 g), prisms (EtOH) m.p. 215°.

Attempted demethylation of mycophenolic acid utilising pyridine hydrochloride

Mycophenolic acid (0·70 g) and pyridine hydrochloride (7·0 g) was heated under reflux for 30 min in a N_2 atm. The mixture was added to ice/water and extracted with $CHCl_3$. Chromatography of the product on 1 mm silica plates in benzene/EtOAc/formic acid (66:33:1) gave II (0·24 g) m.p. 165[3] (benzene) its isomer III (0·07 g), m.p. 215° (EtOH), and the IV (0·05 g), m.p. 171–172°, (acetone/pet. ether b.p. 60–80°).

Mycophenolic acid methyl ester (I, R, R^1 = CH_3)

Ethereal diazomethane was added to a soln of mycophenolic acid (0·57 g) in ether until a faint yellow colour persisted and the solvent was then immediately removed. The residue was taken up in $CHCl_3$ and washed with $NaHCO_3$ aq. Evaporation of the $CHCl_3$ and recrystallization of the residue from aqueous EtOH furnished *mycophenolic acid methyl ester* (0·22 g), colourless plates, m.p. 104–105°, (Found: C, 64·6; H, 6·8; $C_{18}H_{22}O_6$ requires: C, 64·7; H, 6·6%).

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