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SOME NEW METABOLITES RELATED TO MYCOPHENOLIC ACID

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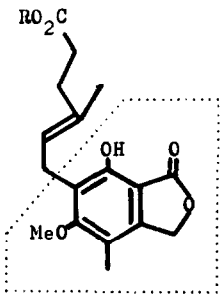
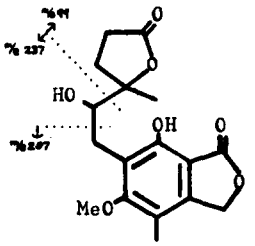
Mycophenolic acid (I, R=H), produced by various strains of fungi of the Penicillium brevicompactum group, is known to show considerable antifungal activity.[1] This property is almost entirely absent in three derived compounds which we have now isolated from the culture filtrates of a strain which produces mycophenolic acid.

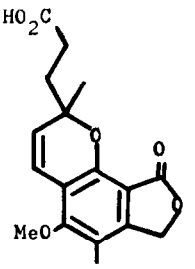
The least polar of these compounds, $C_{19}H_{24}O_6$, m.p. 88-90°, showed the characteristics of a carbethoxyl group [$\nu_{max}^{CHCl_3}$ 1737 cm^{-1}; triplet-quartet system (J = 7·2 c/s) at τ 8·80 (3H) and 5·95 (2H) respectively; ions at m/e 303 and 302 representing losses of 28 and 29 mass units from the (P-18) ion] and of a hydrogen bonded phenolic grouping. [$\nu_{max}^{CHCl_3}$ 3460 cm^{-1}; long wavelength band at 303 mμ (ε 4,700) shifting to 341 mμ (ε 7,900) in base, blue ferric chloride reaction]. This product was deduced from N.M.R., I.R. and M.S. data to be the ethyl ester of mycophenolic acid. A sample prepared by esterification of mycophenolic acid with ethanolic hydrochloric acid was identical in all respects.



I



II



III

Ethyl mycophenolate has previously been encountered in extracts derived from a strain of P. brevicompactum by Raistrick et. al.,[2] but was shown to have been formed during the extraction process by interaction of mycophenolic acid and ethanol. In the case of the strain used in the present work, this ester was shown by thin layer chromatography to be present in the culture fluid of the growing organism and appears to be a true metabolite. The rarity of naturally occurring ethyl esters was discussed recently in connection with the isolation of ethyl curvulinate.[3]

The second new metabolite $C_{17}H_{20}O_7$, m.p. 218-220° was slightly less polar than mycophenolic acid. I.R., U.V., N.M.R. and M.S. similarities with the latter compound indicated that the substituted phthalide nucleus (delineated in formula I) was also present in this metabolite. In addition it was possible to deduce the presence of a γ-lactone function ($\nu_{max}^{CHCl_3}$ 1768 cm^{-1}), a secondary hydroxyl group [$\nu_{max}^{CHCl_3}$ 3620 cm^{-1}, double doublet at 5·63 τ (hydrogen geminal to OH)], and a tertiary methyl group geminal to an oxygen function (singlet at 8·27 τ).

The data and the observed facile fragmentation into ions m/e 237 (base peak) and m/e 99 (abundance 51%) and to the ion m/e 207 (abundance 51%) are accommodated in the structure II.

The configuration of the glycol system was determined by synthesis of both geometric isomers. The erythro isomer (obtained by treatment of mycophenolic acid with osmium tetroxide, followed by acid catalysed lactonisation) had its m.p. 216-217° depressed 10° by admixture with the metabolite, and the I.R. and N.M.R. spectra of the two compounds were distinct. The threo isomer (obtained by epoxidation of mycophenolic acid with metachloroperbenzoic acid, followed by acid catalysed rearrangement) was identical (m.p., mixed m.p., I.R., N.M.R., T.L.C.) with the metabolite.

The third and most polar metabolite ("mycochromenic acid") is a carboxylic acid $C_{17}H_{18}O_6$ (parent molecular ion m/e 318) m.p. 163-165° which was assigned the structure III on the following grounds. This compound like I and II shows the resonances corresponding to a methyl methoxy phthalide nucleus (τ values 7·84, 6·17 and 4·76). In this case however the phthalide carbonyl group ($\nu_{max}^{CHCl_3}$ 1763 cm^{-1}) does not have a free phenolic grouping in the peri position (U.V. spectrum unchanged on basification).* The position of the U.V. maxima [λ_{max} 246 mμ (ε 20,500); 280 mμ (ε 3,200); 321·5 mμ (ε 3,500); 332·5 mμ (ε 3,000)] and the occurrence in the N.M.R. of doublets at 4·37 and 3·32 τ (J = 10·2 c/s) suggests the presence of the chromophore of mycophenolic acid extended by conjugation with a

* The compound III nevertheless shows a transient blue ferric chloride coloration.

cis disubstituted double bond. The remaining features of the N.M.R. spectrum are also consistant with the formulation as III, a singlet at 8·50 τ being assigned to the $CH_3-\overset{|}{\underset{|}{C}}-O-$ grouping and multiplets at 7·45 and 7·80 τ to CH_2 groups respectively α and β to the carboxyl group, the latter CH_2 group suffering additional

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2. P.W. Clutterbuck, A.E. Oxford, H. Raistrick, G. Smith, Biochem. J., 26, 1451 (1932).

3. A. Kamal, A. Ali Qureshi, A. Ahmad, R.W. Rickards, Tetrahedron, 21, 1411 (1965).

4. cf. The biogenesis of 2,2-dimethylchromenes discussed by A.B. Turner, Quart. Rev., 18 No. 4, p.356 (1964).

5. We are grateful for the biological assays carried out in this department by Dr. S. Clarke (activity against fungi) and Dr. A. Berry (germination of seedlings).