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OLIVOMYCIN. III. THE STRUCTURE OF OLIVIN

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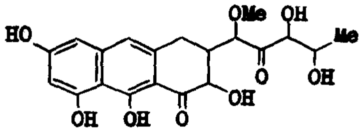
IN 1962 a new antitumor antibiotic, olivomycin, was isolated from Streptomyces olivoreticuli (1). Later the acid degradation of this compound was found to yield several sugars and the aglycone, olivin, retaining the chromophore system of the original glycoside (2,3,4). In this paper it will be shown that olivin possesses structure I.

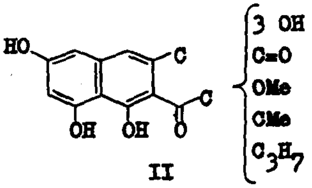


I

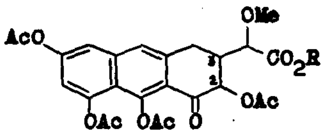
Molecular weight measurements (5) and NMR determination of the total number of protons made possible refinement of the earlier proposed tentative formula of olivin $C_{19-21}H_{22-24}O_{9-10}$ (3) to $C_{20}H_{22}O_9$. Olivin was found to contain six hydroxyls, a conjugated (chelated) and a non-conjugated carbonyl group and a methoxy and C-methyl group.

It augments the acidity of boric acid solutions, the magnitude of the effect (ΔpH 2.5 in 0.1 M H_3BO_3) bearing evidence of the presence of a 1,8-dihydroxynaphthalene system in the molecule (cf. (6)). At the same time it is less susceptible to oxidation than ordinary peri-dihydroxynaphthalenes, which may be accounted for by assuming that this system is stabilized by chelation of one of the peri-hydroxyls with the o-keto group. This is also borne out by the ability of olivin to form metal complexes and by disappearance of the strong chelate carbonyl band in the 1640-1650 cm^{-1} region on exhaustive acetylation of olivin to the hexa-acetate (3) or on hydrogenolysis (with PtO_2 in EtOH) to the desoxo-olivin (hexa-acetate: m.p. 195°, from AcOEt; $[\alpha]^{22}_{D}$ -25° in $CHCl_3$ (here and elsewere c = 1). It is this ketodiphenolic grouping which is responsible for the first ionization constant of olivin (pK_a' 6.3), the second constant (pK_a'' 9.3) being associated with the presence of another phenolic hydroxyl in the naphthalene nucleus. The position of this hydroxyl can be defined from the NMR spectra which show that of the three aromatic protons, two are in meta position to each other, while the third is in peri position to one of them (see below). On this basis olivin may be ascribed the partial formula II.

Olivin smoothly forms an acetonide with Me_2CO in the presence of $CuSO_4$ ($[\alpha]^{22}_{D}$ -25° in EtOH), whose peracetate ($[\alpha]^{22}_{D}$ -42° in $CHCl_3$) yields olivin tetra-acetate ($[\alpha]^{22}_{D}$ -7° in $CHCl_3$) on selective cleavage of the isopropylidene group by 50% AcOH. The tetra-acetate readily undergoes periodate (or $(AcO)_4Pb$) oxidation, with consumption of 2 moles of the oxidant and formation of MeCHO, HCO_2H and the monocarboxylic tetra-acetylolivinic acid $C_{25}H_{24}O_{12}$ (dihydrate: m.p. 127°, from EtOH; $[\alpha]_D^{22}$ -45° in $CHCl_3$). This bears evidence of the existence in olivin of a COCH(OH)CH(OH)Me grouping, which is confirmed by the presence of a three-proton doublet at δ 1.3 ppm (J 6 cps) in the NMR spectrum of hexa-acetylolivin and by the non-conjugated carbonyl absorption (ν 1723 cm^{-1}) in the IR spectrum of olivin, itself. As tetra-acetylolivinic acid has no ethylenic bonds, it must, in accord with its molecular formula, possess a saturated ring, which,



II



III

a: R = H, b: R = Me

judging from the total number of carbon atoms, must contain the keto group. Hence the acid has three linearly fused rings, the frequency (1695 cm^{-1}) of the ketonic carbonyl indicating the third ring to be six-membered.

Decisive information on the structure of tetra-acetylolivinic acid was obtained from the NMR spectrum (Fig.1) of its methyl ester (m.p. 171°, from EtOH; $[\alpha]_D^{22}$ -50° in $CHCl_3$). Indeed, three one-proton peaks in the region of 7 ppm show the presence of three aromatic protons, the pair of doublets centered at 6.95 and 7.41 being due to meta hydrogens (J 3 cps), and the singlet at 7.50 to a peri hydrogen. Of the four three-proton singlets in the region of 2.2-2.5 ppm, three (2.30, 2.35 and 2.44) may be ascribed to aromatic acetoxy groups, and the fourth (2.25) to an acetylated alcoholic hydroxyl. The peak at 3.76, absent in the spectrum of the acid itself, is due to the methyl ester protons, whereas that at 3.39 may be assigned to a methoxy group attached to a saturated carbon atom. The remaining five protons in the molecule give rise to two isolated one-proton doublets at 5.51 and 4.12 and to a three-proton multiplet in the region of 3 ppm, two of the protons, as follows from the carbon to hydrogen ratio, being in the form of a methylene group. Spin decoupling showed that the protons absorbing at 5.51 and 4.12 both interact with the same H-atom (designated on Fig.1 by an arrow). Since both these protons do not participate in any other interaction, the complex three-proton multiplet must be the result, at

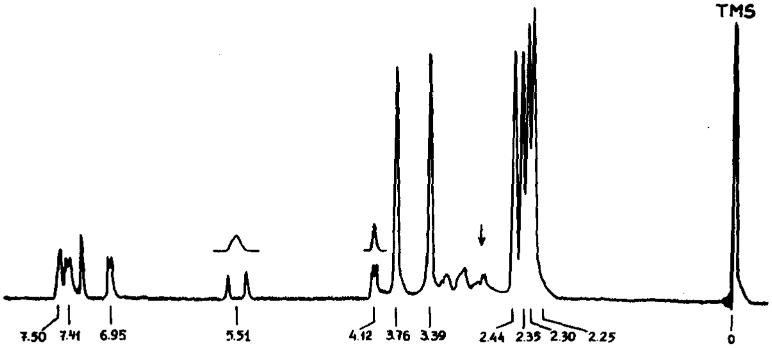


Fig.1. NMR spectrum of methyl tetra-acetylolivinate (IIIb) in $CDCl_3$ at 60 Mc.

least partly, of coupling of the methylene group also with this same H-atom; in other words the latter must be vicinal to the two methines and to a methylene. The molecule, therefore, possesses the branched grouping $CH_2CH(OH)CH$, i.e. there is a side chain on C_3. Furthermore, the position and character of the multiplet indicate that the methylene is presumably in the saturated ring and adjacent to the aromatic nucleus. Consequently the side chain of the molecule must contain a methine connected to two of the three functions, CO_2Me, OMe and OAc. Actually they are CO_2Me and OMe, for the arrangement CH(OMe)OAc would have corresponded to the unstable hemi-ketal group non-existent in olivin, and the $CH(OAc)CO_2Me$ grouping was excluded by a comparison of the NMR spectra of the olivin and desoxo-olivin hexa-acetates, which showed that an acetoxy rather than methoxy group is adjacent to the hydrogenolyzable (i.e. aromatic) keto group. These considerations leave as the only possible structure for tetra-acetylolivinic acid that expressed by formula IIIa with trans-diaxial arrangement of the hydrogens at C_2 and C_3 ($J_{H_2H_3}$ 12 cps). Consequently the structure of olivin (with COCH(OH)CH(OH)Me instead of CO_2H) is given by formula I.

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