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OLIVOMYCIN. IV. THE STRUCTURE OF OLIVOMYCIN

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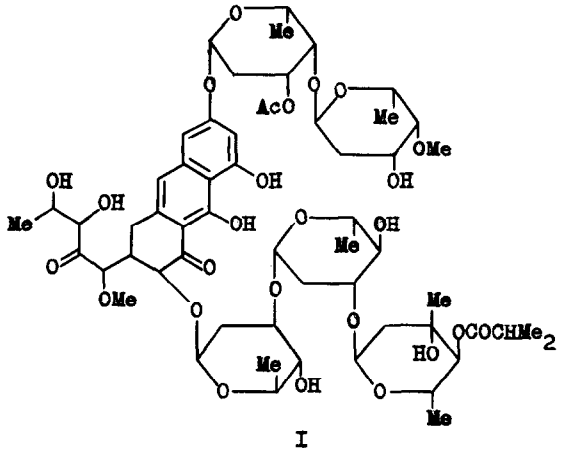
IN previous communications on the chemistry of olivomycin we reported on the structure of its aglycone, olivin (II) (1), and of three of its carbohydrate components, namely, isobutyrylolivomycose (IV), olivomose (VI) and olivose (IX) (2). The present paper is devoted to identification of the fourth, last carbohydrate component (XIII) and to elucidation of the number and sequence of the sugar residues in the antibiotic, which resulted in assigning to olivomycin the structure I.



I

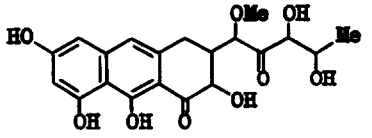
Olivomycin

On hydrolysis of olivomycin with 50% acetic acid (3 hrs., 75°) besides the sugars (IV), (VI) and (IX) still another sugar was isolated from the hydrolysate. This sugar proved to be an aldose containing an O-acetyl group (ν 1722 cm^{-1}, δ 2.1 ppm) and was called acetyloliose ($[\alpha]_D^{22}$ +76°, c 0.6 in water; R_f 0.67 (3)). Oliose, itself, $C_6H_{12}O_4$ ($[\alpha]_D^{20}$ +46°, c 0.7 in water; R_f 0.44), contains the groupings: CH_3-CH(O-)-C (doublet at 1.3 ppm, J 6 cps) and CH_2-CH(O-)-O (multiplet at 4.8 ppm), and consumes two moles of periodate with formation of malonic dialdehyde, which shows it to possess the structure 2,6-dideoxyhexose. The ability of the methyl oliosides to readily form isopropylidene derivatives indicates a cis arrangement of the hydroxyls at C_3 and C_4 and leaves the ribo and lyxo configurations as the only possible ones. The choice between the two, in favor of the lyxo configuration, was made by direct identification of the oliose (XII) with 2,6-dideoxy-D-lyxo-hexose, synthesized from D-fucose by the glycal method (cf. (4)). Acetyloliose reduces 1 mole of periodate, which shows it to be the 3-O-acetyl derivative (XIII).

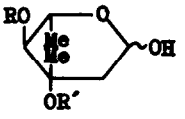
Quantitative determination of the sugars in the aforementioned olivomycin hydrolysate showed that for every olivin residue there was one residue each of isobutyrylolivomycose, olivomose and acetyloliose, and two residues of olivose. At the same time, on milder treatment of olivomycin with dilute acetic acid, partial cleavage products were obtained for which the following carbohydrate composition was established: (olivosyl-olivosyl)-(olivomosyl-acetyloliosyl)-olivin (XIV) ($[\alpha]_D^{20}$ -25°, c 0.8 in EtOH), (olivosyl-olivosyl)-(acetyloliosyl)-olivin (XV)

($[\alpha]_D^{20}$ -59°, c 0.7 in EtOH) and acetyloliosyl-olivin (XVI) ($[\alpha]_D^{20}$ -19°, c 0.5 in EtOH).

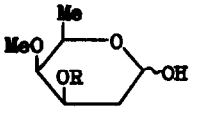
Isolation of the tetroside (XIV) showed that isobutyryl-olivomycose is the terminal sugar. This was confirmed by a study of the degradation of olivomycin benzoate (m.p. 170-175°, from $CHCl_3$-C_6H_{14}; $[\alpha]_D^{20}$ -16°, c 1 in $CHCl_3$) prepared by means of benzoyl chloride in pyridine. Hydrolysis of this



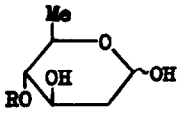
II
(olivin)



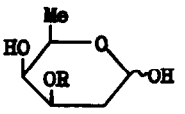
III: R = R' = H
(olivomycose)
IV: R = Me_2CHCO, R' = H
V: R = Me_2CHCO, R' = Bz



VI: R = H
(olivomose)
VII: R = Bz
VIII: R = Ac



IX: R = H
(olivose)
X: R = Bz
XI: R = Ac



XII: R = H
(oliose)
XIII: R = Ac

compound with 75% AcOH at 75° yielded isobutyrylolivomycose benzoate (V), olivomose benzoate (VII), olivose 4-benzoate (X) (stable towards HIO_4) and unchanged acetyloliose (XIII) (5). These results, moreover, show that, in contrast to chromomycin A_3, which possesses a single branched tetrasaccharide chain (6), olivomycin contains two unbranched carbohydrate chains,

ending with isobutyryloliyomycose (IV) and olivomose (VI).

The attachment of these chains to the aglycone was established in the following way. Like olivin, olivomycin contains a peri-dihydroxynaphthalene system, as follows from the characteristically high acidity of its borate complex (ΔpH 2.5 in 0.1 M H_3BO_3; cf. (1)). At the same time, in contrast to olivin (pK_a 6.3 and 9.3), olivomycin possesses only one dissociation constant (pK_a 7.2) and therefore its phenol hydroxyl at C_6 must be blocked by one of the carbohydrate chains. On the other hand, both olivomycin and olivin give an acetonide, whence it follows that the two hydroxyls of the aglycone side chain remain free in the antibiotic. This is in accord also with the fact that in the oxidation of olivomycin with excess periodate only two moles of the oxidant are consumed, with the formation of MeCHO and HCO_2H, while the UV spectrum remains unchanged, and hence the acyloin grouping at C_1-C_2 is unaffected by this treatment. Consequently the second carbohydrate chain must be attached <u>via</u> the hydroxyl at C_2.

The sugar sequence in both carbohydrate chains of olivomycin followed from its partial degradation products (XIV)-(XVI). Indeed, the UV spectra of acetyloliosyl-olivin (XVI) in neutral and alkaline media are almost identical, whereas olivin, itself, displays a considerable bathochromic shift on basification (7). This shows that acetyloliose (XIII) is attached to the aglycone through the phenolic hydroxyl at C_6. Periodate oxidation of the trioside (XV) causes degradation of one of the olivose residues, leaving the second intact,

whereas after mild alkaline hydrolysis of (XV), the same treatment not only destroys the olivose residue, but also the oliose residue. In addition, acetylation of (XV) followed by acid cleavage, yields inter alia diacetyloliose. From this it follows that 4-OH of acetyloliose in the trioside (XV) is free, whereas both olivose residues form a disaccharide chain glycosidizing the 2-OH of olivin. On periodate treatment of the tetroside (XIV), differing from olivomycin in the absence of the isobutyryllolivomycose residue, one of the two olivose fragments is oxidized. Completely analogous is the periodate oxidation of deacetyl-XIV. This indicates that in (XIV) olivomose is bound to acetyloliose and consequently in the antibiotic, itself, isobutyryllolivomycose is attached to an olivose residue. Since only the 4-benzoate (X) of olivose is formed in the hydrolysis of benzoyllolivomycin, it is apparent that the isobutyryllolivomycose-olivose and olivose-olivose glycoside bonds are effected via the 3-OH of olivose. Finally, calculation according to Klyne's rule showed that the glycoside bonds of the acetyloliose and both olivose residues are of β-configuration and those of the olivomose and isobutyryllolivomycose residues of α-configuration. Hence, olivomycin contains a α-olivomosyl-β-acetyloliosyl chain on 6-OH and a α-isobutyryllolivomycosyl-β-olivosyl-β-olivosyl chain on 2-OH of olivin, i.e. possesses the structure I.

REFERENCES

1. Yu.A.Berlin, O.A.Chuprunova, S.E.Esipov, B.A.Klyashchitskii, M.N.Kolosov, G.Yu.Peck, L.A.Piotrovich, M.M.Shemyakin, I.V. Vasina, Tetrahedron Letters, this issue.

2. Yu.A.Berlin, S.E.Esipov, M.N.Kolosov, M.M.Shemyakin, M.G. Brazhnikova, Tetrahedron Letters, 1964, 3513.

3. The R_f values were determined by descending paper chromatography on Whatman No.2 in the system n-BuOH-EtOH-H_2O 4:1:5.

4. B.Iselin, T.Reichstein, Helv.Chim.Acta, 27, 1200 (1944).

5. Analogous results were obtained in the acetic acid hydrolysis of olivomycin acetate (m.p. 218-220°; cf. (8)). In that case acetyloliose (XIII), acetylolivomose (VIII) (R_f 0.84) and acetylolivose (XI) (R_f 0.70) but not olivose, itself, were found among the degradation products.

6. M.Miyamoto, K.Morita, Y.Kawamatsu, M.Sasai, A.Nohara, K.Tanaka, S.Tatsuoka, K.Nakanishi, Y.Nakadaira, N.S.Bhacca, Tetrahedron Letters, 1964, 2367.

7. Yu.A.Berlin, S.E.Esipov, M.N.Kolosov, M.M.Shemyakin, M.G. Brazhnikova, Tetrahedron Letters, 1964, 1323.

8. A.S.Mezentsev, E.B.Kruglyak, V.N.Borisova, G.B.Fedorova, M.G.Brazhnikova, Antibiotiki, 10, 410 (1965).