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OLIVOMYCIN. I. METHANOLYSIS

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IN 1961 an antibiotic mixture was isolated from Streptomyces olivoreticuli and given the name "olivomycin" (1). We separated it by chromatography on silica gel and counter current distribution (2) as a result of which the principal antibiotic of this complex designated further as olivomycin was isolated in 60-65% yield. It is a yellow cristalline substance of m.p. (3) 160-165° (from EtOAc-n.C_6H_{14}), $[\alpha]_D^{25}$ -35.5° (c 0.5, EtOH), R_f 0.85 (4), ν_{max}^{THF} 1588, 1639, 1715 (shoulder), 1745, 3380 cm^{-1}, λ_{max}^{EtOH} 227, 277, 308 (shoulder), 320, 408 mμ ($E_{1cm}^{1\%}$ 230, 470, 60, 70, 110). The UV spectrum undergoes almost no change on adding acid or alkali. The molecular weight of olivomycin determined thermoelectrically (in ethyl acetate) lies in the region of 1250-1350, which together with its elementary analysis allows one to ascribe it the formula $C_{61-65}H_{90-98}O_{27-29}$. Based on a molecular weight of 1300 olivomycin has been found to contain two CH_3O groups (by the

Zeisel procedure), 8 CH_3C groups (by the Kuhn-Roth procedure), 13 active hydrogens (by means of $LiAlH_4$ in pyridine) and three O-acyl groups, the latter being formyl (δ 9.3 ppm) (5), acetyl and isobutyryl (see below).

Olivomycin readily undergoes acid hydrolysis and alcoholysis. We found that by methanolysis even under mild conditions (refluxing for 3 hrs. in 0.1 N methanolic H_2SO_4) it could be smoothly cleaved into an aglycone and several carbohydrate derivatives, of which some are described in this paper.

The aglycone, which we have called olivin, has the formula $C_{19-21}H_{22-24}O_{9-10}$, m.p.189-191° (from EtOAc-C_6H_6 or EtOH-$CHCl_3$-n.C_6H_{14}), $[\alpha]_D^{25}$ +60.5° (c 0.5, EtOH). It displays the characteristic IR and UV features of the initial antibiotic: ν_{max}^{THF} 1592, 1637, 1725, 3360 cm^{-1}, λ_{max}^{EtOH} 230, 277, 324, 408 mμ ($E_{1cm}^{1\%}$ 580, 1000, 160, 320), $\lambda_{max}^{0.01\ N\ KOH\ in\ EtOH}$ 286, 310 (shoulder), 413 mμ ($E_{1cm}^{1\%}$ 1180, 320, 490). As with olivomycin, the UV spectra of olivin in acid and neutral solution are almost identical. Functional analysis and the NMR spectrum indicate the presence of 5-6 active hydrogens in the olivin molecule (by $LiAlH_4$ in pyridine), CH_3O (δ 3.35 ppm), CH_3CH group (doublet at 1.3 ppm) and a proton which judging from its position in the NMR spectrum should be in a cyclopropane ring (multiplet at 1.0 ppm). Olivin contains no low molecular O-acyl groups.

On acetylation (Ac_2O + Py, 24 hrs. at 20°) olivin is converted into a colorless acetate $C_{31-33}H_{34-36}O_{15-16}$, m.p. 200-202° (from $CHCl_3$-EtOAc), $[\alpha]_D^{22}$ -7.3° (c 1.3, $CHCl_3$),

ν_{max}^{THF} 1631, 1703, 1741, 1752, 1778 cm^{-1}, λ_{max}^{EtOH} 252 (shoulder), 258, 303, 315 (shoulder), 360 mμ ($E_{1cm}^{1\%}$ 750, 960, 115, 95, 30). This substance contains six acetyl groups (several peaks of 18 protons over-all intensity in the range 2.0-2.5 ppm), some of which are bound to an aromatic ring through oxygen. Possibly conversion of olivin to the acetate may involve C-acylation or addition of AcOH.

From the mixture of carbohydrate derivatives obtained in the methanolysis of olivomycin several components were isolated by adsorption chromatography on Al_2O_3, the most important of them being derivatives of three sugars we have called olivomycose, olivomose and olivose.

Of these compounds the highest chromatographic mobility is displayed by two substances of the composition $C_{12}H_{22}O_5$, which proved to be anomeric methyl glycosides of O-isobutyrylolivomycose: methyl isobutyrylolivomycoside A, $[\alpha]_D^{25}$ -123° (c 0.6, EtOH), and methyl isobutyrylolivomycoside B, $[\alpha]_D^{25}$ +29° (c 1.5, EtOH). Their saponification by 0.4 N aqueous alcoholic NaOH (20°, 4 hrs.) gives isobutyric acid (identified both as such and as the anilide, m.p.104-105°) and two methyl olivomycosides $C_8H_{16}O_4$ [methyl olivomycoside A, $[\alpha]_D^{22}$ -147° (c 1.0, EtOH), R_f 0.77, and methyl olivomycoside B, m.p.93-94° (from n.C_6H_{14}), $[\alpha]_D^{23}$ +50° (c 1.0, EtOH), R_f 0.73], which yielded olivomycose $C_7H_{14}O_4$ on hydrolysis with 0.2 N H_2SO_4. The sugar, m.p.103-106° (from Me_2CO-Et_2O), $[\alpha]_D^{26}$ -13° (immediately after dissolution) and -22° (after 20 min. and 1.5 hrs.) (c 1.1, H_2O), R_f 0.58, contained three hydroxyls (including the glycoside hydroxyl) and two C-methyl groups.

Evidently this sugar is a branched chain trideoxyheptose $C_5H_5O(CH_3)_2(OH)_3$.

Two other carbohydrate products of the methanolysis of olivomycin are anomeric methyl olivomosides $C_8H_{16}O_4$, which besides a glycoside methoxyl each contain an OH, CH_3O and CH_3CH group. One of these anomers, olivomoside A, has a m.p. 98° (from n.C_6H_{14}), $[\alpha]_D^{26}$ +150° (c 0.4, EtOH); the other, olivomoside B, a m.p.152-153° (from n.C_6H_{14}), $[\alpha]_D^{26}$ -37.5° (c 0.4, EtOH). Acid hydrolysis of both glycosides gives olivomose $C_7H_{14}O_4$, m.p.158-162° (from Me_2CO), $[\alpha]_D^{23}$ +98.5° (immediately after dissolution) and +89° (after 1 and 1.5 hrs.) (c 0.5, H_2O), R_f 0.65. Olivomose does not undergo HJO_4 oxydation which, in view of its other properties, permits one to assign it the structure 2,6-dideoxy-4-O-methyl-D-hexose and the olivomosides A and B that of the α- and β-methyl glycosides respectively (6).

Still another pair of carbohydrate methanolysis products of this antibiotic are the methyl olivosides $C_7H_{14}O_4$. They contain a CH_3CH group (δ 1.2 ppm) and two vicinal hydroxyls (consumption of 1 mole of periodate). Of these two glycosides only one, methyl olivoside A, was isolated in the pure state. It has $[\alpha]_D^{25}$ +131° (c 0.75, EtOH), R_f 0.75. Olivoside B (R_f 0.70) could not as yet be isolated without the accompanying anomer. The interrelation between these two glycosides was shown by transformation of olivoside A by means of methanolic HCl into a mixture of the olivosides A and B (without appreciable spectral changes) on the one hand and by hydrolysis of both compounds to the same sugar, olivose $C_6H_{12}O_4$, $[\alpha]_D^{25}$

+45° (c 0.5, H_2O), R_f 0.54, on the other hand. According to the chemical properties and the spectra of the latter, it is 2,6-dideoxy-D-hexose.

The acid hydrolysis of olivomycin (0.1 N H_2SO_4 in aqueous tetrahydrofuran at 75°), besides olivin, affords a mixture of carbohydrates which contains, among other substances, olivomycose (together with O-isobutyrylolivomycose), olivomose and olivose. However owing to the lability of the free deoxy sugars under the hydrolytic conditions, this degradation is less convenient than methanolysis.

It thus follows that the olivomycin molecule consists of the aglycone olivin and deoxy sugar residues, at least partly bound by phenolic or enolic glycoside bonds.

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2. Counter current distribution was carried out by 400 transfers in the system EtOH-EtOAc-n.C_6H_{14}-H_2O 10:14:10:13. The authors are greately indebted to Prof. A.S.Khokhlov and P.D.Reshetov for their valuable aid.
3. All m.p. determinations were carried out on a Kofler block.
4. The R_f values were determined by paper chromatography on Whatman No.2 in the system BuOH-EtOH-H_2O 4:1:5.
5. The NMR spectra for which the authors are greately indebted to Dr. G.Peck were obtained on a 60 Mc instrument with Me_4Si as internal reference.

6. The properties of olivomose are similar to those of chromose A (M.Miyamoto, Y.Kawamatsu, M.Shinohara, Y.Asahi, Y.Nakadaira, H.Kakisawa, K.Nakanishi, N.S.Bhacca, Tetrahedron Letters, 1963, 693), but they display some substantial differences. It is quite possible that under the names of chromose A and chromosides A were described insufficiently purified preparations of olivomose and olivomosides.