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OLIVOMYCIN AND RELATED ANTIBIOTICS
XI. Structure of Olivomycose and its Acyl Derivatives*

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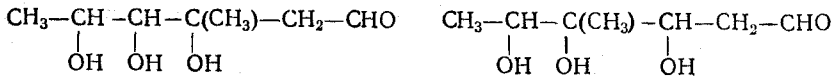
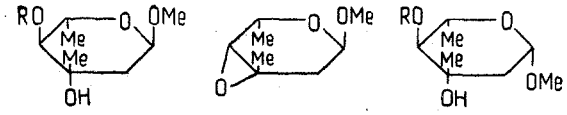
In a preceding paper, we have described the acid degradation of olivomycin antibiotics with the formation of the aglycone olivin and derivatives of a number of sugars—olivomycose, olivomose, olivose, and oliose [2]. In the present work it has been shown that the first of these sugars, olivomycose, has the structure XI and its acyl derivatives, in the form of which it is present in olivomycin, have the structures X and XI.

Among the products of the methanolysis of olivomycins A and C, two anomeric glycosides with the empirical formula $C_{12}H_{22}O_5$ have the highest chromatographic mobility. They contain an ester group (ν_{CO} 1735–1740 cm^{-1}) and on successive hydrolysis with 0.4 N KOH and 0.2 N H_2SO_4 are decomposed with the formation of isobutyric acid and olivomycose and are therefore isobutyrates of its methyl glycosides.

Olivomycose, $C_7H_{14}O_4$, gives characteristic reactions with aniline hydrogen phthalate and triphenyltetrazolium chloride, i.e., it is an aldose. As can be seen from the empirical formula, it contains three deoxy groups. One of them is a methylene group in position 2, as follows from the presence in the NMR spectrum of methyl β-olivomycoside of a two-proton multiplet in the 1.80 ppm region (CH_2) and a one-proton quadruplet at 4.35 ppm (O–CH–O), the presence of which confirms that the substance is an aldose. The two other deoxy links represent C-methyl groups, as is shown by the results of Kuhn-Roth oxidation and by the NMR spectrum, which also permits the conclusion that both carbon atoms bearing methyl groups are attached to oxygen, while one of the methyl groups is tertiary (singlet at 1.25 ppm) and the other is secondary (doublet at 1.30 ppm with J = 6 Hz). Thus, olivomycose has CH_3–CH(OH), –$C(CH_3)$(OH), and –CH_2CHO groupings. The remaining four atoms (C, 2H, and O) can form only a hydroxymethylene grouping in a six-membered chain, thanks to which the number of possible structures is limited to two:



The selection between these possibilities in favor of the first of them is determined by the fact that when olivomycose is oxidized with periodate, formic acid is formed and not malondialdehyde. Consequently, olivomycose is a 3-C-methyl-2, 6-dideoxyhexose.



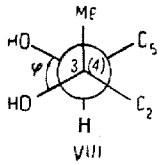
I R = Me_2CHCO
II R = Ac
III R = H
IV R = Ts

V

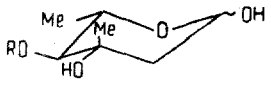
VI R = Me_2CHCO
VII R = H

The size of the oxide ring in methyl olivomycosides was established by periodate titration (1 mole of IO_4^- was consumed, which corresponds only to a pyranose structure), and the configuration of the asymmetric centers at C-3, C-4, and C-5 was determined as follows. Tosylation of methyl α-olivomycoside (III) led to the monotosylate (IV), which was readily converted by the action of methanolic alkali into the 3, 4-epoxide (V), thus showing the trans arrangement of the hydroxyls at C-3 and C-4. On the other hand, a polarometric study of methyl β-olivomycoside showed that the passage from an aqueous solution of the substance to its solution in Schweitzer's reagent was accompanied by a considerable negative shift in the molecular rotation $[M]_{436}$ ($\Delta Cu - 1670°$), which shows a value of $-60°$ for the projected valence angle φ between the C-3–O and C-4–O bonds (VIII) (cf. [3]). These data are compatible only with the trans-diequatorial arrangement of the hydroxyls at C-3 and C-4 and, consequently, an axial arrangement of the methyl at C-3 in the 1C conformation of the pyranose ring. This conformer can be stable only if the C-5 methyl is in the equatorial position; in the opposite case, the repulsion of the spatially adjacent methyl groups at C-3 and C-5 would cause inversion of the chair 1C → C1 and an increase in the projected angle O–C-3(C-4)–O to $-180°$. But an equatorial position of the C-5 methyl group in conformation 1C means the L-configuration of olivomycose. Thus, olivomycose is 3-C-methyl-2, 6-dideoxy-L-arabino-hexose (IX).

*For preliminary communication, see [1].



VIII



IX R = H
X R = Me_2CHCO
XI R = Ac

As mentioned above, in olivomycins A and C, the olivomycose is present in the form of the isobutyrate. It has been found that the latter is stable to periodate oxidation and its methyl glycosides do not undergo acetylation under mild conditions; consequently, the acyl residues in them and in isobutyrylolivomycose is located at the C-4 OH (X). So far as concerns the C-1 asymmetric center in these glycosides, its configuration follows from Hudson's rule: the methyl isobutyrylolivomycoside with $[\alpha]_D$ −115° and the methyl olivomycoside with $[\alpha]_D$−137° are the α-glycosides I and II and the methyl isobutyrylolivomycoside with $[\alpha]_D$+27° and the methyl olivomycoside with $[\alpha]_D$ +46° belong to the β-series, VI and VII.

Earlier, in a study of the carbohydrate composition of olivomycin B, we found that the antibiotic contains not only isobutyrylolivomycose (X) but also another sugar [2] with the empirical formula $C_9H_{18}O_4$. This contains an acetoxy residue (ν_{CO} 1730 cm^{-1}, δ = 2.15 ppm), is converted into olivomycose (IX) on hydrolysis, and, consequently, is its O-acetate. Like the isobutyryl derivative, (X) acetylolivomycose is stable to periodate oxidation, which shows that it contains the acetyl group at O-4. Finally, partial synthesis of this sugar by the acetylation of methyl α-olivomycoside (III) with subsequent acid hydrolysis has confirmed structure (XI) for it. It is important that the conclusions given above on the structure and stereochemistry of olivomycose and its derivatives agree satisfactorily with the results of the nuclear magnetic resonance of these substances.

Thus, the passage from unsubstituted methyl olivomycosides to their mono-O-isobutyrates or acetates is accompanied by the appearance in the NMR spectrum of a one-proton doublet in the 5 ppm region. (In the NMR spectra taken in $CDCl_3$ or CCl_4, this doublet is masked by the multiplet of the methyl at C-1, but the two signals can be clearly differentiated if benzene is used as the solvent.) This shows that in the acyl derivatives of olivomycose it is secondary (and not the tertiary) hydroxyl that is substituted, while this hydroxyl is located on the fourth (and not on the third) carbon atom, since otherwise the nature of the signal of the methine group H−C−OCOR would be more complex because of interaction with the two vicinal protons. The magnitude of the spin-spin splitting content of this doublet ($J_{4,5}$ = 10 Hz) shows the trans-diaxial arrangement of the H atoms at C-4 and C-5, i.e., the equatorial orientation of the C-4 OH and C-5 Me groups. Furthermore, while in the spectra of the unsubstituted olivomycosides the peaks of the C-3 Me and C-5 Me almost coincide, the acetylation of the 4-OH causes a considerable paramagnetic shift of the signal of the C-3 methyl but has almost no effect on the position of the peak of the C-5 methyl. Thus, the descreening effect of the acetyl residue is exhibited differently in relation to the two C-methyl groups, which shows that they are arranged unsymmetrically with respect to C_4−OAc, i.e., that the C-3 methyl group has the axial orientation.

In conclusion, it must be mentioned that olivomycose, its 4-acetate, and its 4-isobutyrate have also been isolated by Japanese workers from the chromomycins, antibiotics related to the olivomycins, and have been called, respectively, deacetylchromose B (from deacetylchromomycin A_3) [4, 5], chromose B (from chromomycin A_3 [6, 7]), and isobutyryldeacetylchromose B (from chromomycin A_2) [4, 5], while racemic olivomycose has been obtained synthetically and has been described under the name of DL-epimycarose [8].

Experimental

The characteristics of the absorbents and the conditions of chromatography are given in [2].

1. Methyl α-4-O-isobutyrylolivomycoside (I) was obtained by the methanolysis of olivomycin A [2]. Yield 47%; $[\alpha]_D^{26}$ −115° (c 0.7; in alcohol); ν_{CO} 1740 cm^{-1}.

Found, %: CH_3O 12.5, $(CH_3)_2CHCO$ 26.6. Calculated for $C_{12}H_{22}O_5$, %: 1 CH_3O 12.6, 1 $(CH_3)_2CHCO$ 28.8.

2. Methyl β-4-O-isobutyrylolivomycoside (VI) was obtained by the methanolysis of olivomycin A [2]. Yield 16%; $[\alpha]_D^{23}$ +27° (c 1.7; in ethanol); ν_{CO} 1735 cm^{-1}.

3. Isobutyrylolivomycose (X). A solution of 246 mg (1 mmole) of methyl α-isobutyrylolivomycoside (I) or the corresponding β-glycoside, VI, in 20 ml of 0.2 N H_2SO_4 was heated at 70° C for 2 hr, and after cooling it was neutralized with freshly precipitated $BaCO_3$, centrifuged, and evaporated to dryness in vacuum. The yield of isobutyrylolivomycose was 202 mg (88%), R_f 0.84 (on paper), $[\alpha]_D^{23}$ −43° (5 min after dissolution), $[\alpha]_D$ −34° (after 30 min), $[\alpha]_D$ −33° and $[\alpha]_{578}$ −37° (after 1 hr, without further change) (c 0.5; in water); ν_{max} 1728, 1745, 3415 cm^{-1}; see also [2].

The action of a 0.02 N aqueous solution of $NaIO_4$ on isobutyrylolivomycose led to no decomposition of the oxidizing agent.

4. Methyl α-olivomycoside (III). A solution of 246 mg (1 mmole) of methyl α-isobutyrylolivomycoside (I) in 4 ml of ethanol and 2 ml of 1.2 N KOH was left at 20° C for 5 hr, neutralized with gaseous CO_2, evaporated in vacuum, and diluted with water to its original volume. The resulting soltuion was continuously extracted with ether for 15 hr, the extract was dried with Na_2SO_4 and evaporated, and the residue (144 mg) was chromatographed on Al_2O_3 in ethyl acetate. This gave 107 mg (58%) of methyl α-olivomycoside (III), $[\alpha]_D^{22}$ −137° (c 1; in ethanol), R_f 0.77 (under the conditions of isolation).

Found, %: C 54.3, H 9.3. Calculated for $C_8H_{16}O_4$, %: C 54.5, H 9.2.

After the extraction of the glycoside, the aqueous solution was acidified to pH 1 and extracted with ether. This gave 70 mg of isobutyric acid, part of which was esterified with diazomethane (the methyl isobutyrate was identified by gas-liquid chromatography), while part was converted by the action of $SOCl_2$ and then $PhNH_2$ into the anilide, mp 105° C (from a mixture of hexane and ethyl acetate), which was shown to be identical with an authentic sample by chromatography, by a mixed melting point, and by its NMR spectrum.

5. Methyl β-olivomycoside (VII). The alkaline hydrolysis of methyl β-isobutyrylolivomycoside (VI) under the conditions of the preceding experiment gave methyl β-olivomycoside (VII). Yield 80%, mp 93–94° C (from hexane), $[\alpha]_D^{23}$ +46° (c 1; in ethanol), R_f 0.73 (under the conditions of isolation).

Found, %: C 54.6, H 9.3. Calculated for $C_8H_{16}O_4$, %: C 54.5, H 9.2.

When this glycoside was converted into the copper-ammonia complex (molar ratio of substance to copper 4:7) $[\alpha]_{436}^{25}$ changed from +81° (c 1; in water) to −867° (c 1; in Schweitzer's reagent, containing 12.7 g/*l* of Cu and 227 g/*l* of NH_3). $\Delta Cu = 176 \times (-867° - 81°)/100 = -1670°$.

6. Olivomycose (IX). 264 mg of methyl α-olivomycoside (III) or the corresponding β-glycoside, (VII), or a mixture of them, was hydrolyzed under the conditions of Exp. 3. The yield of olivomycose (IX) was 220 mg (90%); mp 103–106° C (from a mixture of acetone and ether); $[\alpha]_D^{26}$ −13° (immediately after dissolution) and −22° (after 20 min and 1.5 hr) (c 1; in water); R_f 0.58 (on paper).

Found, %: C51.9, H 8.9, CH_3(C) 16.0. Calculated for $C_7H_{14}O_4$, %: C 51.8, H 8.7, $2CH_3$(C) 18.5.

7. Periodate oxidation of methyl β-olivomycoside (VII) and olivomycose (IX). A solution of 13.4 mg (0.075 mmole) of the glycoside (VII) in 50 ml of a 0.02 N solution of KIO_4 was left in the dark at 20° C; an aliquot of the solution was made alkaline with $NaHCO_3$ and then an excess of KI was added and the iodine liberated was back-titrated with 0.02 N Na_3AsO_3. The consumption of oxidizing agent (in moles/mole) was: after 1.5 hr 0.82; after 3 hr 0.88; after 4.5 hr 0.99; after 24 hr 0.99. In the similar oxidation of olivomycose (IX), the consumption of periodate was: after 2.5 hr 1.63; after 5 hr 1.95; after 35 hr 2.08 mole/mole. Titration of the formic acid liberated with 0.01 N NaOH (to phenolphthalein) led to a figure of 1.18 mole/mole, and the gravimetric determination (calomel method [9]) to 1.13 mole/mole; no malondialdehyde was found in the solution (negative reaction with p-nitro-aniline).

8. Methyl α-4-O-p-toluenesulfonylolivomycoside (IV). At 0° C, 700 mg (3.5 mmole) of p-toluenesulfonyl chloride was added to a solution of 350 mg (2 mmole) of methyl α-olivomycoside (III) in 25 ml of anhydrous pyridine, and the mixture was left at 0° C for a day and at room temperature for another 3 days. The excess of sulfonyl chloride was decomposed with ice water, and the reaction solution was diluted with chloroform, washed with 5% H_2SO_4 and evaporated. The yield of tosylate (IV) was 426 mg (64%); R_f 0.70 [on Al_2O_3 in the benzene–acetone (9:1) system].

Found, %: C 54.5, H 6.7 S 9.7. Calculated for $C_{15}H_{22}O_6S$, C 54.3, H 6.9, S 9.4.

9. Methyl α-3, 4-anhydroolivomycoside (V) and its β-anomer. A solution of 166 mg (0.5 mmole) of the tosylate (IV) in 2 ml of 0.5 N methanolic NaOH was left at 20° C for 12 hr and was then neutralized with CO_2 and chromatographed on Al_2O_3 in the benzene–acetone (9:1) system. This gave 62 mg (80%) of the epoxide (V) with R_f 0.73.

Found, %: C 60.6, H 8.7. Calculated for $C_8H_{14}O_3$, %: C 60.7, H 8.9.

The same epoxide was obtained from a mixture of the α- and β-glycosides (III) and (VII) by tosylation, and subsequent treatment with alkali, and the chromatographic separation of the anomers on Al_2O_3 in the system given above. This also gave methyl β-3, 4-anhydroolivomycoside with mp 104–105° C and R_f 0.61 (after sublimation at 60° C/20 mm).

Found, %: C 60.7; H 8.8.

10. Methyl α-4-O-acetylolivomycoside (II). A solution of 150 mg (0.85 mmole) of methyl α-olivomycoside (III) in 2 ml of acetic anhydride and 2 ml of absolute pyridine was kept at 20° C for 5 hr, diluted with ice water, and extracted

with chloroform. The extract was washed with 1 N H_2SO_4, a saturated solution of $NaHCO_3$, and water, and was dried with Na_2SO_4 and evaporated. The yield of methyl α-4-O-acetylolivomycoside (II) was 130 mg (73%); mp 93–94° C (from hexane); $[\alpha]_D^{23}$ −141° (c 0.5; in ethanol, ν_{CO} 1723 cm^{-1}.

Found, %: C 55.2, H 8.5; H_{act} 0.57. Calculated for $C_{10}H_{18}O_5$, %: C 55.0, H 8.3, $1H_{act}$ 0.46.

11. Acetylolivomycose (XI). A solution of 100 mg of the acetylolivomycoside (II) in 20 ml of 50% acetic acid was heated at 75° C for 5 hr. after which it was evaporated in vacuum and the residue was chromatographed on silica gel in the benzene–acetone (1:1) system. For the zone with R_f 0.41–0.52* was isolated 71 mg (76%) of acetylolivomycose (XI); $[\alpha]_D^{23}$ −34° (c 1; in water), ν_{CO} 1730 cm^{-1}; R_f 0.81 (on paper).

Found, %: C 53.3, H 8.3. Calculated for $C_9H_{16}O_5$, %: C 52.9, H 7.9.

Summary

It has been shown that olivomycose has the structure of 3-C-methyl-2, 6-dideoxy-L-arabino-hexose. In olivomycins A and C, this sugar is present in the form of the 4-O-isobutyrate, and in olivomycin B in the form of the 4-O-acetate.

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*On adsorption chromatography, this substance moves in the form of a mixture of two components, apparently anomers.