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OLIVOMYCIN AND RELATED ANTIBIOTICS

XIII. The Structure of Olivose and Oliose*

Yu. A. Berlin, G. V. Borisova, S. E. Esipov, M. N. Kolosov, and V. A. Krivoruchko

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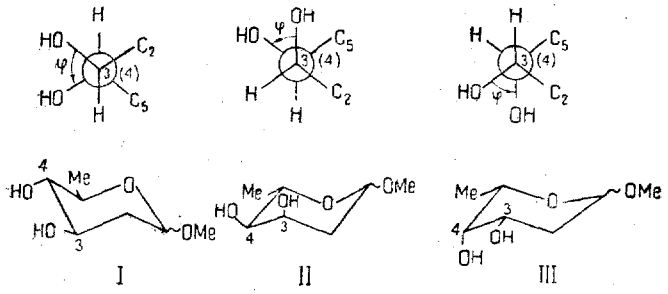
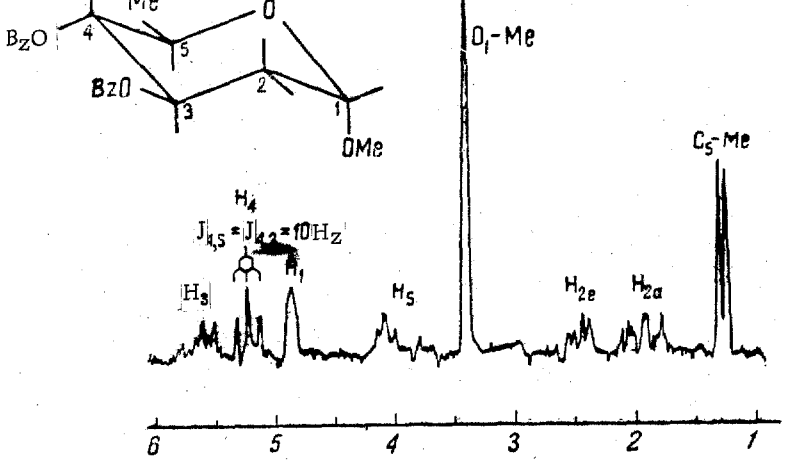
Olivose and oliose, unsaturated carbohydrate components of all the olivomycin antibiotics, have the same empirical composition $C_6H_{12}O_4$ [3]. They give positive reactions for reducing sugars (with aniline hydrogen phthalate and triphenyltetrazolium chloride) and, as can be seen from their NMR spectra, contain $CH_3-CH\langle{}^{C}_{O}$ and $CH_2-CH\langle{}^{O}_{O}$ groupings (see [2, 4]). In the periodate oxidation of these carbohydrates, two moles of oxidizing agent are consumed in the formation of acetaldehyde, formic acid, and malondialdehyde, and in the oxidation of their methyl glycosides one mole of periodide is consumed but no volatile substances are formed. This shows that olivose and oliose are stereoisomeric 2,6-dideoxyaldohexoses, and their methyl glycosides possess the pyranose structure.

In a study of methyl olivoside, with $[\alpha]_D$ −85°, we found that its conversion into the cuprammonium complex causes a strong positive shift of the molecular rotation ($\Delta[M]_{436}$ +2120°) which shows a value of φ of +60° for the projected O—C_3(C_4)—O valence angle (see [5]), i.e., an ea or an ee arrangement of the C_3—O and C_4—O bonds in the preferred conformation. In view of the equatorial position of the C_5 methyl group as a necessary condition for the stability of the conformer, in the case of the 2,6-dideoxyaldohexopyranosides this requirement is satisfied by only three formulas: (I), (III), and



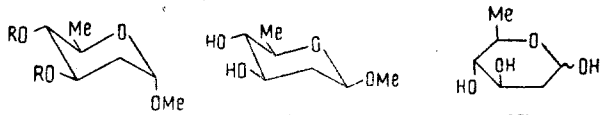
In the NMR spectrum of the methyl olivoside mentioned, the H_1 signal forms a quadruplet at 4.38 ppm with $J_{1,2a}$ 6 Hz and $J_{1,2e}$ 3 Hz, and in the NMR spectrum of its anomer ($[\alpha]_D$ +131°) the H_1 proton gives a quadruplet at 4.70 ppm with $J_{1,2e}$ 1.5 Hz and $J_{1,2a}$ 3 Hz. As already shown for the olivomosides, this demonstrates the β and α



NMR spectrum of methyl α-3,4-di-O-benzoylolivoside (V) (at 100 MHz in $CDCl_3$ with Me_4Si as internal standard).

*For preliminary communication, see [1]; for part XII, see [2].

configurations, respectively, for the glycosidic centers of the olivosides under consideration and, according to Hudson's rule, the fact that they belong to the D-series. Consequently, of the three formulas given above, (I) with the trans-ee arrangement of the hydroxyls at C_3 and C_4, is correct, so that methyl α- and β-olivosides possess the structures IV and VI. This conclusion is in good agreement with the NMR spectrum of methyl α-3,4-di-O-benzoylolivoside (V) (figure) since in this, the H_4 proton is the K part of a AKX system (with $K_{AX} \sim 0$) and forms a triplet at 5.22 ppm with $J_{KA} = J_{KX} = 10$ Hz, which shows the presence of $H_{4a,5a}$ and $H_{4a,3a}$ interactions. Thus, olivoside is 2,6-dideoxy-D-arabinohexose (VII).

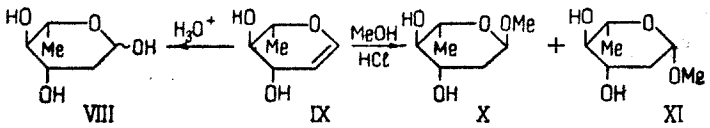


IV, R = H
V, R = Bz

VI

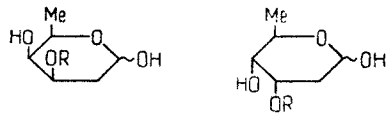
VII

To confirm this formula, we carried out the hydrolysis and methanolysis of the readily available L-rhamnal [6] with the formation of 2,6-dideoxy-L-arabinohexose (VIII) [6] and its methyl α- and β-pyranosides (X and XI). The latter proved to be identical with the methyl olivosides IV and VI in all respects apart from the sign of their optical activities.



VIII IX X XI

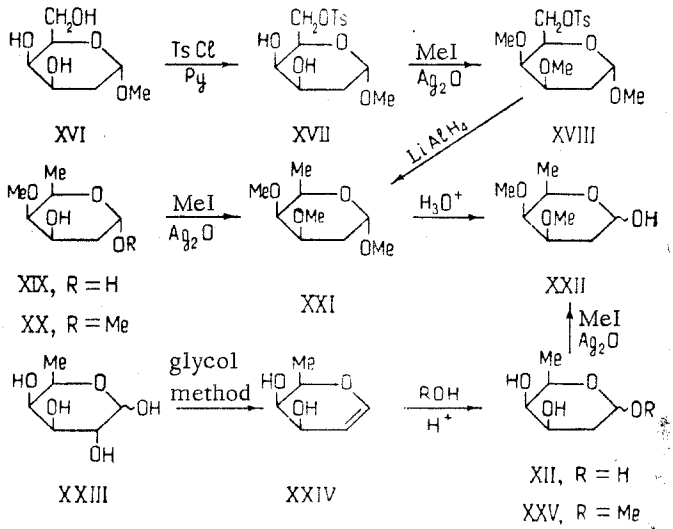
Oliose, like olivose, is also a 2,6-dideoxy-D-hexose but contains a cis-diol grouping, since its methyl glycosides readily give isopropylidene derivatives under the action of acetone in the presence of $CuSO_4$ [4]. Consequently, oliose can possess only the lyxo (XII) and ribo (XIV) configurations. Oliose is present in the molecule of olivomycin C as such, and in the other antibiotics of this group—olivomycins A, B, and D—it is present in the form of the monoacetyl derivative, from which it can readily be obtained by saponification. This monoacetate smoothly reduces one mole of periodate, from which it follows that it is 3-O-acetyloliose and has the structure XIII or XV.



XII, R = H
XIII, R = Ac

XIV, R = H
XV, R = Ac

In the course of a study of the relationship of the olivomycins to the antibiotic chromomycin A_3 by Japanese workers, chromoses A, B, C, and D were isolated [7,8]. Since it has been shown that olivomycose [1,4] and the olivose (VII) described in the present paper have the same structures, respectively, as deacetylchromose B, chromose A, and chromose C and acetyloliose has similar constants to chromose D, for which the lyxo configuration has been established [8], we have suggested that oliose and acetyloliose also possess the lyxo configurations (XII) and (XIII). We have shown the correctness of this hypothesis by the correlation of oliose (XII) with olivomose (XIX) by the reduction of both sugars to the dimethyl ether XXII that we have synthesized and also by the synthesis of oliose from D-fucose (XXIII) by the method previously used by Iselin and Reichstein to obtain 2-deoxy-L-fucose [9].



XVI XVII XVIII

XIX, R = H
XX, R = Me

XXI

XXII

XXIII XXIV

XII, R = H
XXV, R = Me

In conclusion, it must be mentioned that the structure of three other carbohydrate components of olivomycin-chromomycin has also been confirmed by synthesis: olivose (chromose C, canarose) has been synthesized by Zorbach and Ciandelli [10], and olivomose (chromose A) and acetyloliose (chromose D) by Brimacombe et al. [11].

Experimental

For general information on the conditions of chromatography, see [2]. The gas-liquid chromatography was carried out on a "Pye" (England) argon chromatograph with 5% of poly(ethylene glycol adipate) on Chromosorb W as stationary phase.

Methyl α-olivoside (IV) was obtained by the methanolysis of olivomycin A [3]. Yield 23%; $[\alpha]_D^{25}$ +131° (c 0.7; alcohol); R_f 0.38 [on Al_2O_3 in the benzene–acetone (1 : 1) system] and 0.75 (on paper).

Found, %: C 51.6; H 8.6; CH_3(C) 9.8; CH_3O 17.8. Calculated for $C_7H_{14}O_4$, %: C 51.8; H 8.7; 1 CH_3(C) 9.3; 1 CH_3O 19.1.

In the oxidation of this glycoside with 0.02 N $NaIO_4$ solution, 1 mole of periodate was consumed.

The dibenzoate (V) was obtained by the action of BzCl + Py with a yield of 88%, mp 94–96° C (from hexane).

Methyl β-olivoside (VI) was obtained by the methanolysis of olivomycin A [3]. Yield 6%; mp 84° C (from ethyl acetate–hexane); $[\alpha]_D^{22}$ −85° (c 1; ethanol); $[\alpha]_D^{26}$ −45° (c 0.6; water); R_f 0.27 [on Al_2O_3 in the benzene–acetone (1 : 1) system], and 0.70 (on paper).

Found, %: C 51.7; H 8.7.

In the action of periodate on methyl β-olivoside, the consumption of the oxidizing agent was as follows: after 1.5 hr, 0.80; after 3 hr, 0.88; after 4.5 hr, 0.99; after 24 hr, 0.99 mole/mole.

The conversion of methyl β-olivoside (VI) into the cuprammonium complex (molar ratio of the substance to copper 1 : 2) was accompanied by a change in $[\alpha]_{436}^{26}$ from −149° (c 0.6; water) to +1157° (c 0.7); the Schweitzer reagent contained 14.6 g/l of Cu and 227 g/l of NH_3. $\Delta Cu = 162 \times (+1157 + 149°)/100 = +2120°$.

Olivose (VII). A solution of 16.2 mg of methyl α- or β-methylolivoside (IV or VI) or a mixture of them in 3 ml of 0.2 N H_2SO_4 was heated at 70° C for 2 hr; after cooling the mixture was neutralized with $BaCO_3$ and filtered, and the evaporation residue was chromatographed on silica gel in the chloroform–acetone (1 : 2) system. The yield of olivose (VII) was 12.2 mg (82%); $[\alpha]_D^{23}$ +31°, $[\alpha]_{578}$ +32° (c 1; water); R_f 0.45 (under the conditions of isolation) and 0.54 (on paper); it was identical with the olivose obtained by the hydrolysis of olivomycin A [3].

When olivose was oxidized with $NaIO_4$, the consumption of oxidizing agent was as follows: after 2.5 hr, 1.63; after 5 hr, 1.90; after 35 hr, 2.05 mole/mole. Among the oxidation products were detected formic acid (1.18 mole found by titration with 0.01N NaOH, 1.12 mole/mole by the calomel method) and also acetaldehyde and malondialdehyde (identified by reactions with dinitrophenylhydrazine and p-nitroaniline).

2-Deoxy-L-rhamnose (VIII) and its methyl glycosides (X) and (XI). A solution of 3.08 g of L-rhamnal (IX) [6] in 30 ml of 1 N H_2SO_4 was left at 0° C for 8 hr and was then neutralized with $BaCO_3$. Barium salts were separated off by centrifuging, the solution was evaporated, and the residue was chromatographed on silica gel in ethyl acetate. From the zone with R_f 0.20–0.30 was isolated 1.42 g (40%) of 2-deoxy-L-rhamnose (VIII), identical chromatographically with olivose (VII).

A solution of 5 g of L-rhamnal (IX) in 5 ml of 3% methanolic HCl was kept at 20° C for 1.5 hr, neutralized with Ag_2CO_3, filtered, and evaporated in vacuum, and the residue was chromatographed on 200 g of Al_2CO_3 (column 500 × × 25 mm). This yielded 800 mg of the methyl α-glycoside X and 450 mg of the methyl β-glycoside XI.

Methyl 2-deoxy-α-L-rhamnopyranoside (X): $[\alpha]_D^{17}$ −132° (c 1.3; ethanol).

Found, %: C 51.5; H 8.5. Calculated for $C_7H_{14}O_4$, %: C 51.8; H 8.7.

Methyl 2-deoxy-β-L-rhamnopyranoside (XI): $[\alpha]_D^{20}$ + 70° (c 1; ethanol); mp 83–84° C (from ethyl acetate–hexane).

Found, %: C 51.6; H 8.9.

3-O-Acetyloliose (XIII). This was obtained by the hydrolysis of olivomycin A [3]. Yield 42%; R_f 0.38 [on silica gel in the benzene–acetone (1 : 1) system], 0.70 (on paper); $[\alpha]_D^{23}$ +89°, $[\alpha]_{578}$ +91° (c 1; water); ν_{max} 1722, 3380 cm^{-1}; Δ 2.1 ppm (3H, singlet).

Found, %: C 50.6; H 7.6; CH_3CO (O) 23.0. Calculated for $C_8H_{14}O_5$, %: C 50.5; H 7.4; 1 CH_3CO (O) 22.6.

In the periodate oxidation of acetyloliose, the consumption of oxidizing agent was: after 1 hr, 0.78; after 1.5 hr, 0.84; after 3 hr, 0.89; and after 5 hr, 0.92 mole/mole.

Oliose (XII). A) Under the conditions for the preparation of olivose (VII), 48 mg of a mixture of the anomeric methyl oliosides [3] was hydrolyzed, and the hydrolysate was chromatographed on silica gel in the chloroform—acetone (1 : 2) system. The yield of oliose (XII) was 40 mg (90%); $[\alpha]_D^{20}$ +51° (c 0.7; water); R_f 0.35 (under the conditions of isolation); 0.44 (on paper). Oliose was obtained with the same yield by the hydrolysis of a mixture of isopropylidene-oliosides, see [3].

B) A solution of 3 mg of acetyloliose (XIII) in 3 ml of 0.4N $Ba(OH)_2$ was kept at 20° C for 6 hr and was then neutralized with 1 N H_2SO_4, and the resulting sugar was identified as oliose by paper chromatography.

The periodate oxidation of oliose took place in a similar manner to the oxidation of olivose (see above) and led to the same degradation products.

2-Deoxy-D-fucose. By the method described previously for the L-enantiomer [9], D-fucose (XXIII) was converted via diacetylfucal (mp 49–50° C) and fucal (XXIV) [mp 70–71° C (from benzene)] into 2-deoxy-D-fucose. The over-all yield was 40%; $[\alpha]_D^{20}$ +50° (c 0.5; water; R_f 0.44 (on paper). In optical activity, chromatographic mobility, and IR spectrum, the substance obtained was identical with oliose (XII).

Methyl 3-O-methyl-α-olivomoside (XXI). A solution of 88 mg of methyl α-olivomoside (XX) in 2 ml of methyl iodide was stirred and boiled with 460 mg of freshly-prepared Ag_2O for 8 hr. The solids were filtered off and washed with chloroform, the filtrate was evaporated, and the residue was chromatographed on Al_2O_3 in the ethyl acetate—heptane (1 : 1) system and eluted with ether. From the zone with R_f 0.62–0.70 was obtained 84 mg (90%) of methyl 3-O-methyl-α-olivomoside (XXI) $[\alpha]_D^{23}$ +133° (c 0.6; ethanol); V_R^{abs} 5.5 min (125° C, 100 v × 10, 50 ml/min).

Methyl 6-O-tosyl-2-deoxy-α-D-galactopyranoside (XVII). A solution of 1.9 g of p-toluenesulfonyl chloride in 5 ml of absolute pyridine was added to a solution of 1.8 g of methyl 2-deoxy-α-D-galactopyranoside (XVI) [12] in 4 ml of pyridine at −5° C. The reaction mixture was kept at 0° C for 24 hr and was then poured into 100 ml of ice water and extracted with chloroform. The extract was washed with water, dried, and evaporated, and the residue was chromatographed on silica gel in the ethyl acetate—heptane (3 : 1) system. From the zone with R_f 0.30–0.40 was isolated 1.7 g (50%) of the monotosylate (XVII), $[\alpha]_D^{21}$ +70° (c 1.3; ethanol).

Found, %: C 50.7; H 6.1; S 9.7. Calculated for $C_{14}O_{20}O_7S$,%: C 50.6; H 6.1; S 9.6.

Methyl 3,4-di-O-methyl-6-O-tosyl-2-deoxy-α-D-galactopyranoside (XVIII). A solution of 3.0 g of the tosyldeoxygalactoside (XVII) in 18 ml of methyl iodide was stirred and boiled with 4.24 g of Ag_2O for 15 hr and filtered, the precipitate was washed with chloroform, and the filtrate was evaporated. A similar treatment was repeated twice, after which the substance was chromatographed on silica gel in the ethyl acetate—heptane (3 : 1) system. From the zone with R_f 0.71–0.75 was obtained 1.61 g (50%) of the dimethyl derivative XVIII; mp 78–80° C [from ethyl acetate—heptane (1 : 1)]; $[\alpha]_D^{21}$ +67° (c 1 : 1; ethanol).

Found, %: C 53.0; H 6.6; S 8.7. Calculated for $C_{16}H_{24}O_7S$, %: C 53.3; H 6.7; S 8.9.

From the zone with R_f 0.47–0.52 was isolated 0.6 g of a monomethylation product which was converted by retreatment with MeI + Ag_2O into compound XVIII.

Methyl 3,4-O-dimethyl-2,6-dideoxy-α-D-galactopyranoside (XXI). A solution of 258 mg of the methyl dimethyltosylgalactoside XVIII in 10 ml of tetrahydrofuran was treated with 7 ml of a 0.5 M tetrahydrofuran solution of $LiAlH_4$. The mixture was boiled for 3.5 hr and, after cooling, it was treated with 30 ml of ethyl acetate and 2 ml of water and the precipitate was filtered off and carefully washed with ethyl acetate. The filtrate was washed with water, dried, and evaporated, and the residue was chromatographed on silica gel in the ethyl acetate—heptane (3 : 1) system. From the zone with R_f 0.65–0.70 was isolated 40 mg (30%) of the methyl dimethyldideoxygalactopyranoside $[\alpha]_D^{20}$ + +115° C (c 1; ethanol), which, from the results of gas-liquid and thin-layer chromatography and also from its IR spectrum, was identical with the methyl 3-O-methyl-α-olivomoside (XXI) described above.

3,4-Di-O-methyl-2,6-dideoxy-D-lyxo-hexose (3-O-methylolivomose, 3,4-di-O-methyloliose) (XXII). A) A solution of 76 mg of methyl 3-O-methyl-α-olivomoside (XXI) in 2 ml of 0.1 N H_2SO_4 was heated at 50° C for 3 hr, neutralized with $BaCO_3$, filtered, and extracted with ethyl acetate; the extracted substance was chromatographed on Al_2O_3 in the benzene—acetone (2 : 1) system. The yield of 3-O-methylolivomose (XXII) was 60 mg (85%); R_f 0.37; mp 61–64° C (from hexane); $[\alpha]_D^{30}$ +117° (c 0.5; chloroform). Found, %: C 54.6; H 9.2. Calculated for $C_8H_{16}O_4$, %: C 54.5; H 9.2.

B) A mixture of the anomeric methyloliosides (XXV) [3] (160 mg) was methylated with methyl iodide and was then hydrolyzed with 0.1 N H_2SO_4 under the conditions described above. This gave 40 mg (23%) of 3,4-dimethyloliose (XXII); mp 65° C (from hexane); $[\alpha]_D^{30}$ +114° (c 0.5; chloroform).

C) A mixture of the anomeric methyl 2-deoxy-D-fucopyranosides (XXV) (720 mg) obtained by the methanolysis of D-fucal (XXIV) under the conditions for the methanolysis of IX was methylated with methyl iodide in the presence of Ag_2O. Chromatography on Al_2O_3 in the ethyl acetate—heptane (1 : 1) system yielded 180 mg of a mixture of methyl 3,4-dimethyl-2-deoxy-D-fucosides. Hydrolysis of this mixture with 0.1 N H_2SO_4 gave 120 mg (71%) of 3,4-di-O-methyl-2-deoxy-D-fucose. In its melting point $[\alpha]_D$, chromatographic mobility, and IR spectra, the substance was identical with the 3-O-methylolivomose and the 3,4-di-O-methyloliose described above in experiments A and B.

Conclusions

It has been shown that olivose possesses the structure of 2,6-dideoxy-D-arabino-hexose (VII) and oliose that of 2,6-dideoxy-D-lyxo-hexose (XII).

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Institute of the Chemistry of Natural Compounds, AS USSR