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OLIVOMYCIN AND RELATED ANTIBIOTICS

XII. Structure of Olivomose

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Olivomose $C_7H_{14}O_4$ is contained in all the olivomycins known at the present time [1-4], i.e., it is one of the most characteristic carbohydrate components of the antibiotics of this group. It gives positive reactions with triphenyltetrazolium chloride and with aniline hydrogen phthalate and it contains one readily acetylatable alcoholic hydroxyl and one methoxyl and also a $CH_3-CH<^{O}_{C}$ grouping (three-proton doublet at $\delta_{1.3}$ ppm with J 6 Hz) and a $CH_2-CH<^{O}_{O}$ group (two-proton multiplet in the 1.5-2 ppm region and a one-proton multiplet in the 4-5 ppm region). Since olivomose is not oxidized by periodate, only two structures are possible for it: a 3,5-dihydroxy-4-methoxyhexanal and a 4-hydroxy-3-hydroxymethyl-3-methoxypentanal.

In the oxidation of the anomeric methyl olivomosides with chromic anhydride in pyridine, a monomethoxy ketone $C_7H_{10}O_3$ is smoothly formed.

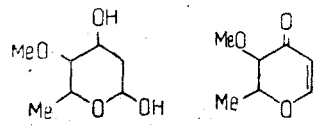
The spectral characteristics of this substance (ν_{max} 1670 cm^{-1}; λ_{max} 263 mμ) show that it consists of an α, β-unsaturated ketone the bathochromic shift of the absorption in which is explained by an oxygen-containing substituent on the double bond (see, for example [5]). Another characteristic of the substitution on this double bond follows from the NMR spectrum (figure, a), in which one-proton doublets at 5.15 and 7.15 ppm correspond to cis-olefinic protons present, respectively, in the α- and β-positions to a carbonyl group.

The spin-spin coupling constant of these protons (7 Hz) is lower than in six-membered carbocyclic systems (10 Hz), probably because of the electronic influence of the cyclic oxygen (in the case of 2,3-dihydropyran the corresponding figure is also 7 Hz [6]). The additional splitting of the doublet at 5.15 ppm with a constant of about 1 Hz is caused by the effect of long-range interaction through the carbonyl group (cf. [7]).

Since the two methyl olivomosides give the same compound on chromic oxidation, of the two methoxy groups present in them it is the glycosidic methoxyl that is split off in the process of oxidation.

An analogous splitting off of a glycosidic methoxyl in the chromic oxidation of methyl 4,6-benzylidene-2-deoxy-D-lyxo-hexopyranoside has recently been described by Overend et al. [8].

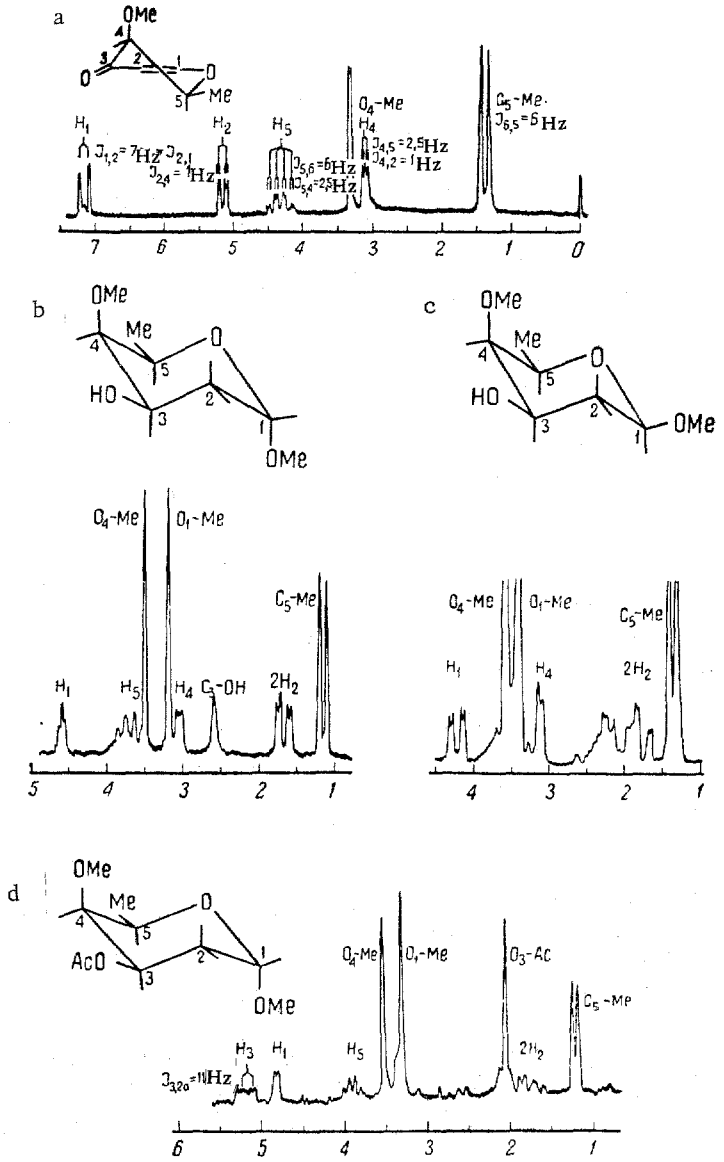
Since, then, the splitting out of methanol takes place in the oxidation of methyl olivomosides, and a similar conversion is not characteristic of the glycosides themselves, it is obvious that the tendency to elimination arises as a result of the appearance of the β-methoxy ketone grouping. In these circumstances, in the olivomosides the acetal methoxyl and the free hydroxyl are in the 1,3 positions. The data presented agree only with the first of the two possible structures of the sugar mentioned above, so that olivomose is the 4-O-methyl-2,6-dideoxyaldohexose I and the product of the chromic oxidation of its methyl glycosides has the structure of 3-dehydroolivomal (II).



I II

We elucidated the configuration of olivomose in the following way. In the NMR spectrum of the methyl olivomoside having $[\alpha]_D$ +160°, the signal of the H_1 proton forms a triplet at 4.65 ppm with a J value of about 3 Hz (figure, b). This means that the spin-spin coupling constants of H_1 with the two protons at C_2 are almost the same, i.e., the projected valence angles $H_1-C_1(C_2)-H_{2e}$ and $H_1-C_1(C_2)-H_{2a}$ differ little from one another in absolute magnitude. This shows the equatorial arrangement of H_1 and the axial orientation of the glycosidic methoxyl. Since the equatorial position of the C_5 methyl group is a factor determining the energetic suitability of the conformation, this shows the trans-ae arrangement of the C_1—OMe and C_5—Me groups, i.e., the α-configuration of the glycoside under consideration.

This conclusion is in good agreement with the NMR spectrum of the anomeric methyl olivomoside having $[\alpha]_D$ −36° (figure, c). In this, the H_1 proton is represented by a quadruplet at 4.8 ppm, and its spin-spin coupling constants

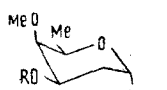


NMR spectra (at 60 MHz in $CDCl_3$ with Me_4Si as internal standard) of dihydroolivomal (II) (a); methyl α-olivomoside (III) (b); methyl β-olivomoside (V) (c); and methyl α-3-0-acetylolivomoside (V) (d).

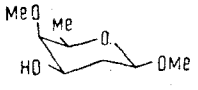
($J_{1,2a}$ 9 Hz, $J_{1,2e}$ 3 Hz) show the axial orientation of H_1 and, consequently, the β-configuration of the glycoside; at the same time, the position of the quadruplet under consideration in the higher-field region as compared with the triplet in the spectrum of methyl α-olivomoside gives additional confirmation of the axial and equatorial positions of the corresponding protons. Thus, in methyl α-olivomoside the contribution of the C_1 asymmetric atom to the molecular rotation is positive and in the β-olivomoside it is negative, which, according to the rules of isorotation, indicates the D-configuration for olivomose.

In the NMR spectrum of methyl α-olivomoside, the multiplet of the H_5 proton, which is partially overlapped by the peak of the 4-methoxy group, is in the region of weaker fields than in the spectrum of methyl β-olivomoside in which the signal of this proton is overlapped by the peaks of both methoxy groups. This paramagnetic shift on passing from methyl β-olivomoside to the α-anomer is due to the descreening effect of the axial C_1 methoxyl in the α-glycoside (see, for example [9]) and confirms the conclusion drawn above on the configuration of the C_1 and C_5 asymmetric centers.

The conversion of the olivomosides into dehydroolivomal profoundly changes the geometry of the $O-C_1-C_2-C_3$ moiety of the molecule, but has little effect on the arrangement of the substituents on the asymmetric C_4 and C_5 atoms, thanks to which the conclusions on the conformation of these substituents in dehydroolivomal may be extended to the initial olivomosides. In connection with this, we must consider the multiplet with a center at 4.30 ppm which is present in the NMR spectrum of dehydroolivomal (see figure, a), which corresponds to the H_5 proton interacting with the methyl and C_4 methine groups. Characteristic for this is quadruplet splitting with a constant of 6 Hz, equal to the distance between the components of the three-proton doublet of the C_5 Me (δ 1.35 ppm), each of the components of this quadruplet being split additionally as a result of interaction with H_4 (δ 3.15 ppm) with a constant of 2.5 Hz. Since the C_5 Me group, as already mentioned, occupies the equatorial position and, consequently, H_5 is axial, such a low value of $J_{4,5}$ shows the equatorial orientation of the H_4 proton, i.e., the axial arrangement of the 4-methoxy group. In the spectra of the methyl olivomosides the half-width of the H_4 peak (about 4 Hz), which forms a component of the quadruplet of a A system of protons close to a AB_2 system, likewise shows that this proton does not participate in any axial-axial interaction, i.e., it occupies the equatorial position. So far as concerns the asymmetric center at C_3, the stability of methyl α-olivomoside to the action of oxygen in the presence of a platinum catalyst, which dehydrogenates axial alcohols specifically [10], shows the equatorial position of the hydroxyl on this carbon atom. The facts presented lead to formulas III and V for methyl α- and β-olivomosides.



III R = H
IV R = Ac



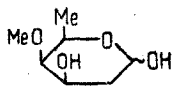
V

These formulas are in good agreement with some of the spectral properties of the olivomosides, in particular the similarity of the chemical shifts of H_3 and the authentically axial H_5 proton, (especially when account is taken of the diamagnetic shift of H_5 under the influence of a substituent at O_5) and the paramagnetic shift of the H_3 signal on passing from methyl β-olivomoside to the α-olivomoside, which is connected with the descreening effect of the C_1—OMe group, this being possible only in respect of an axial proton. Further, in the product of the acetylation of methyl α-olivomoside (IV) (figure, d) the H_3 signal, thanks to the strong paramagnetic shift (δ 5.2 ppm), is not overlapped by the H_5 peak, as is the case in the α-olivomoside itself and it may easily be analyzed. The values of the spin-spin splitting constants found for it (11.6 and 3 Hz) show the participation of this proton in a diaxial interaction (obviously, with H_{2a}).

An analysis of the resonance signals of the methylene group of olivomose leads to a similar conclusion. In the NMR spectrum of methyl β-olivomoside (see figure, c), the protons of this group are represented by a multiplet in the 1.3–2.6 ppm range which is partially overlapped by the peaks of the C_5—Me and C_3—OH groups. The nature of this multiplet is explained by the considerable difference in the chemical shifts of H_{2a} and H_{2e} and by the large value of J_{2a2e} (which is typical for nonequivalent geminal protons in six-membered rings), and also by the interaction of the methylene protons with H_1 and H_3 in the composition of a AKLX system. In the NMR spectrum of the α-olivomoside, in contrast to the β-anomer, the methylene protons form a two-proton quadruplet with a center at 1.75 ppm (J' 9 Hz, J" 3 Hz) which shows the conversion of a AKLX system into an AK_2X system.

This change in the resonance of the methylene protons on passing from the β- to the α-glycoside is important for stereochemical conclusions. In fact, the difference in the degree of screening by the bonds of the ring which is usual for axial and equatorial protons, because of which the first of them resonates in a stronger field than the second, changes little when an equatorial OR grouping is present on the neighboring carbon atom (the C_1 methoxyl in methyl β-olivomoside), the screening of these two protons being approximately the same. If, however, the alkoxy group occupies the axial position (like the C_1 methoxyl in methyl α-olivomoside), it screens H_{2e} and simultaneously descreens H_{2a} [11]; the combination of these effects, displacing the peaks of the two protons in opposite directions, leads, in the general case, to an approach, and in the case of methyl α-olivomoside to a coincidence, of the chemical shifts of H_{2a} and H_{2e}, as a result of which the spin-spin coupling between these protons ceases to appear in the spectrum. Consequently, the quadruplet with a center at 1.7 ppm mentioned above is due to the interaction of the equivalent H_2 protons with the H_1 and H_3 protons. A comparison of this quadruplet with the H_1 triplet at 4.65 ppm ($J_{1,2}$ 3 Hz) shows that the H_2, H_3 interaction corresponds to a J value of 9 Hz, from which the axial position of the H_3 proton follows.

Thus, olivomose is 4-O-methyl-2,6-dideoxy-D-lyxo-hexose (VI).



VI R = H
VII R = Ac

An independent proof of this formula is the synthesis of the 3-methyl ether of olivomose described in the following paper [12].

Experimental

Thin-layer chromatography was carried out in a nonfixed layer of alumina (activity grade V) or silica gel of "aqueous silicic acid" type (activity grade III–IV, dispersity > 150 mesh), and by partition chromatography on Whatman No. 2 paper in the n-BuOH–EtOH–H_2O (4 : 1 : 5) system.

Methyl α-olivomoside (III) was obtained by the methanolysis of olivomycin A [4]. Yield 42%; mp 97–98° C (from hexane); sublimes at 60° C/760 mm; $[\alpha]_D^{22}$ +160° (c 0.5; alcohol); R_f 0.61 [on Al_2O_3 in the benzene–acetone (1 : 1) system].

Found, %: C 54.7; H 9.1; CH_3(C) 8.2; CH_3O 35.9. Calculated for $C_8H_{16}O_4$, %: C 54.5; H 9.2; $1CH_3$(C) 8.5; $2CH_3O$ 35.2.

Methyl β-olivomoside (V) was obtained by the methanolysis of olivomycin A [4]. Yield 8%; mp 152–153° C (from hexane); sublimes at 110° C/760 mm; $[\alpha]_D^{26}$ −35° (c 0.4; ethanol); R_f 0.54 [on Al_2O_3 in the benzene–acetone (1 : 1) system].

Found, %: C 54.6; H 9.3.

Olivomose (VI). A solution of 88 mg of methyl α- or β-olivomoside (III or V), or a mixture of them, in 6 ml of 0.2 N H_2SO_4 was heated at 70° C for 3 hr, and after cooling it was neutralized with $BaCO_3$, filtered, and evaporated, and the residue was recrystallized from acetone. This gave 77 mg (95%) of olivomose (VI), mp 158–162° C; $[\alpha]_D^{23}$ +113° (5 min after dissolution), +98.5° (10 min after dissolution), and +89° (30 min after dissolution; no further change) (c 0.5; water); R_f 0.65 (on paper). The substance was chromatographically and spectroscopically identical with the olivomose isolated by the hydrolysis of the olivomycins [4].

When olivomose was treated with a 0.02 N solution of $NaIO_4$, no oxidizing agent was consumed.

Dehydroolivomal (II). A solution of 93 mg of CrO_3 in 18 ml of pyridine was added to a stirred solution of 280 mg of methyl α-olivomoside (III) in 10 ml of pyridine at 20° C, and the mixture was left for 48 hr, after which another 93 mg of CrO_3 in 18 ml of pyridine was added and it was heated at 70° C for 2 hr. After cooling, the reaction mixture was poured into 50 ml of ice water, acidified with 1 N H_2SO_4 to pH 4, and extracted with chloroform. The extract was washed with water, dried, and evaporated, and the residue was chromatographed on silica gel in the benzene–acetone (3 : 1) system. From the zone with R_f 0.35–0.45 was isolated 80 mg of the starting material, and a substance isolated from the zone with R_f 0.65–0.95 (100 mg) was rechromatographed under the same conditions. The yield of dehydroolivomal (II) was 50 mg (25%), R_f 0.85; $[\alpha]_D^{29}$ +100° (c 0.6; ethanol); λ_{max} 263 mμ (log ε 3.81); ν_{max} 1670 cm^{-1}; mol. wt. (m/e) 142; calculated for $C_7H_{10}O_3$: mol. wt. 142.

The same results were obtained in the oxidation of methyl β-olivomoside (V) and of a mixture of the anomers (III) and (V).

3-O-Acetylolivomose (VII). A solution of 88 mg of methyl α-olivomoside (III) in 5 ml of pyridine and 5 ml of acetic anhydride was kept at 20° C for 10 hr and evaporated in vacuum, and the residue was chromatographed on Al_2O_3 in the benzene–acetone (3 : 1) system. Methyl 3-O-acetyl-d-olivomoside (IV) was isolated. To obtain acetylolivomose (VII), the glycoside (IV), without chromatographic purification, was heated with 5 ml of 50% acetic acid at 75° C for 3 hr. The solution was evaporated and the residue was chromatographed on Al_2O_3 in the benzene–acetone (3 : 1) system. The yield of acetylolivomose (VII) was 80 mg (77%); R_f 0.42; $[\alpha]_D^{20}$ +69° (c 0.4; water) ν_{CO} 1732 cm^{-1}.

The action of 0.05 N HCl in methanol on 100 mg of acetylolivomose (VII) (3 hr at 20° C) gave 80 mg (92%) of a mixture of methyl α- and β-olivomosides (3 : 1) from which, by chromatography on Al_2O_3 in the benzene–acetone (1 : 1) system, 40 mg of methyl α-olivomoside (III) with mp 96–97° C was isolated.

Conclusions

It has been shown that olivomose, which forms part of the carbohydrate moiety of olivomycins A, B, C, and D, possesses the structure of 4-O-methyl-2,6-dideoxy-D-lyxo-hexose (VI).

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