THE STRUCTURE OF VARIOSE – A NEW SUGAR FROM THE ANTIBIOTIC VARIAMYCIN

G. B. Lokshin, Yu. V. Zhdanovich,
A. D. Kuzovkov, and V. I. Sheichenko

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The acid degradation of the antibiotic variamycin [1] forms chromomycinone and three sugars: oliose, olivose, and variose (I). The present paper gives information on the determination of the structure of variose. This sugar gives positive reactions with triphenyltetrazolium chloride and with aniline hydrogen phthalate and, consequently, is an aldose.

As the NMR spectrum of methyl varioside (II) shows, the molecule of this substance contains the following groups: $\begin{matrix}O\\ O\end{matrix}\!\!>\!CH\!=\!CH_2$ (two-proton multiplet at δ 2.15 ppm and one-proton quartet at δ 5.16 ppm), 3 OCH (three-proton multiplet at δ 4 ppm), $CH_3CH\!<\!\begin{matrix}O\\ C\end{matrix}$ (three-proton doublet at δ 1.3 ppm), 2 OCH_3 (three-proton singlets at δ 3.44 and 3.5 ppm) and 1 OH (singlet at δ 2.01 ppm). Thus, the molecule of the methyl glycoside (II) contains 16 protons and four oxygen atoms (2 OCH_3, 1 OH, and one atom in the ring), mol. wt. 176 (mass spectrometry). With the given ratio of H and O, only one empirical formula can correspond to this substance – $C_8H_{16}O_4$. The methyl glycoside (II) may have pyranose (IIa) or furanose (IIb) structures (see scheme on following page).

According to the double-resonance spectrum (Fig. 1) of the benzoyl derivative of the methyl glycoside (III), the six protons in the molecule of (III) are bound to the carbon atoms of the ring, which shows the pyranose structure of methylvarioside.

The position of the hydroxy group at C_3 follows from the fact that variose is not oxidized by periodic acid, which means that it does not contain a vicinal diol grouping at C_4 and C_5. It follows from this that the only possible position remaining for the OCH_3 groups is at C_4.

Thus, variose is a 4-O-methyl-2,6-dideoxyhexose. A similar sugar, called olivomose (chromose A) has been isolated by the acid hydrolysis of chromomycin and olivomycin [2]. A direct comparison of variose and olivomose obtained by a published method [2] showed that they differed in chromatographic mobility and in specific rotation. Consequently, variose is an epimer of olivomose.

The configuration of the asymmetric centers in variose was determined from the NMR spectra of methyl varioside (II) and its benzoate (III) (see Fig. 1). (See scheme on following page.)

In the NMR spectrum of (II), the H_1 signal is located at 5.16 ppm and is a quartet in which the distance between the first and second lines is 2 Hz and that between the first and third is 4.5 Hz. The smaller of these two figures is close to the coupling constant of the C_1 proton with the C_2 protons in methyl olivomoside [2]. This means that in the NMR spectrum of the glycoside (II) the overlapping of the signals of the methylene group (2.1-2.4 ppm) does not affect the position of the lines of the quartet of the signal of the C_1 proton, and the distances between the components of the quartet correspond to the coupling constants. The value of J of 4.5 Hz is intermediate between the values of $J_{1,2a}$ and $J_{1,2e}$ in methyl olivomoside and apparently shows the existence of the glycoside (II) in the form of two conformers (with the axial and the equatorial orientation of the anomeric proton). The assignment of the signals of the other protons was made from the results of the double resonance of the benzoate (III) (see Fig. 1). Attention is attracted by the large downfield shift of the H_5 signal (by ~ 1.5 ppm) on passing from the glycoside (II) to the benzoate (III).

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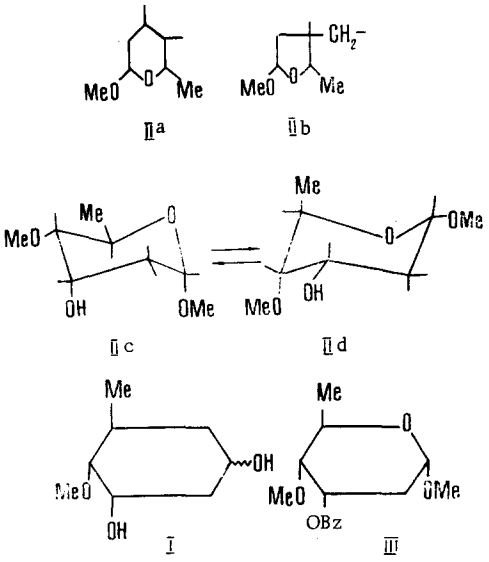

It can be seen from a consideration of molecular models that the substituent at C_3 and the proton at C_5 can be close in space only when they have the diaxial orientation. Consequently, the methyl and benzoyl groups are located on different sides of the plane of the pyranose ring. The value of the coupling constant between H_3 and H_4 is comparatively small (3.5 Hz) and shows that in neither of these conformations do these protons occupy mutually diaxial positions. Consequently, the substituents at C_3 and C_4 are in the cis configuration to one another. Since the substituents at C_3 and C_5 occupy the trans position, the substituent at C_4 is in the trans position to the methyl group at C_5. This is confirmed by the value of the J constant, which is 4.5 Hz. According to the literature [2], of the possible conformations that one is fixed in which the methyl group at C_5 is equatorial. The existence of the glycoside (II) in the form of two conformers – (IIc) and (IId) – is apparently connected with the destabilizing effect of the 1,3-diaxial interaction of the substituents at C_1 and C_3 in that one of the conformations in which the substituent at C_5 is in the equatorial position. In both possible conformations, the C_1-OMe and C_5-Me groups occupy the trans position and, consequently, methyl varioside with $[\alpha]_D^{20}+121°$ is the α-glycoside. Since the methyl α-glycoside of variose has a positive rotation, it belongs to the D series. These conclusions are also confirmed by the position of the H_1 signal in the NMR spectrum of the glycoside (II) (at δ 5.16 ppm), while for the β anomer in the D series it should be in a weaker field [3].

The facts given enable variose to be assigned the structure of 4-O-methyl-2,6-dideoxy-D-riboaldohexose (see scheme above).

The literature available to us does not report the isolation of such a sugar from natural sources or its synthesis. Thus, variose is a new monosaccharide.

EXPERIMENTAL

Chromatography was performed on silica gel of the "aqueous silicic acid" type, on alumina (activity grade III) and on "Leningrad S [medium]" paper. The chromogenic agents used were a 50% solution of H_2SO_4 in methanol and 1% $SbCl_3$ in chloroform. The IR spectra were recorded on a UR-10 spectrophotometer (Zeiss) using mulls of the substances in paraffin oil, the NMR spectra on a Varian HA-100 spectrometer in $CDCl_3$.

Methyl α-Varioside (II). A mixture of 1 g of variamycin and 100 ml of 0.05 N methanolic HCl was boiled for 2.5 h, and, after cooling, the mixture was neutralized with Ag_2CO_3 and filtered, and the filtrate was evaporated in vacuum to dryness. The residue was dissolved in 30 ml of water and extracted with ethyl acetate (3×10 ml). The aqueous solution was evaporated in vacuum to dryness and chromatographed in a thin layer of silica gel in benzene–acetone (4:1). A substance with R_f 0.5 was eluted by acetone, and the eluate was evaporated to dryness and the residue was chromatographed in a thin layer of Al_2O_3 in methylene chloride. The product was eluted from the zone with R_f 0.2 with acetone, and the eluate was evaporated. This gave a colorless syrup with $[\alpha]_D^{20}+121\pm2°$ (c 0.5; chloroform).

Variose (I). A solution of 20 mg of the glycoside (II) in 5 ml of 0.2 N H_2SO_4 was heated at 70°C for 3 h and, after cooling, it was neutralized with $BaCO_3$. The precipitate was separated off and the filtrate

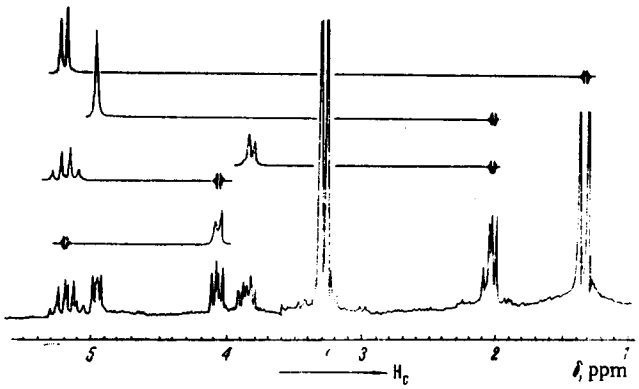

Fig. 1. Double-resonance spectrum of methyl 3-O-benzoylvarioside.

was evaporated to dryness. A colorless syrup with $[\alpha]_D^{20}$ +53° was formed, this rotation not changing in the course of an hour and longer (c 0.5; water). The product obtained was chromatographically identical with the variose isolated by the hydrolysis of variamycin [1]. On descending paper chromatography in the butanol–ethanol–water (4:1:5) system it had R_f 0.74. The R_f value of olivomose, isolated from olivomycin by a published method [2], was 0.66 under the same conditions.

Methyl 3-O-Benzoylvarioside (III). A solution of 100 mg of methyl varioside in 2 ml of pyridine was treated with 0.25 ml of benzoyl chloride. After 72 h, the reaction mixture was poured into cold water and extracted with chloroform, and the chloroform extract was washed with 5% solutions of $NaHCO_3$ and of H_2SO_4, and also with water, and it was then evaporated and the residue was chromatographed in a thin layer of Al_2O_3 in benzene–acetone (20:1). The zone with R_f 0.6-0.65 yielded the benzoate of the glycoside (II) in the form of a colorless syrup with $[\alpha]_D^{20}$ +60± 2° (c 0.2; chloroform), IR spectrum: 1722 cm^{-1} (C=O in esters).

SUMMARY

Variose – a new sugar isolated from the antiobiotic variamycin – is 4-O-methyl-2,6-dideoxy-D-ribo-hexose.

LITERATURE CITED

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