THE STRUCTURE OF SIBIROSAMINE – A NEW AMINO SUGAR FROM THE ANTIBIOTIC SIBIROMYCIN

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In studying the structure of the antitumoral antibiotic sibiromycin produced by the actinomycete Streptosporangium sibiricum [1], we have established that sibiromycin is a glycoside of a new amino sugar, which we have called sibirosamine, and an aglycone which is transformed in the process of isolation into a biologically inactive bright-yellow substance with the composition $C_{16}H_{14}N_2O_3$ (mol. wt. 282). The present paper gives information on the determination of the structure of the carbohydrate fragment of the molecule of sibiromycin–sibirosamine.

On acid hydrolysis, the molecule of sibiromycin undergoes far-reaching decomposition, forming a large amount of dark-colored unidentified products. After methanolysis of sibiromycin, by ion-exchange chromatography we isolated the methyl glycoside of sibirosamine. After purification, the methyl sibirosaminide was obtained in the form of the hydrochloride, which is readily soluble in water and the lower alcohols, sparingly soluble in acetone, and insoluble in ether and benzene. Methyl sibirosaminide hydrochloride $C_9H_{19}NO_4 \cdot HCl$ is a white amorphous powder without a sharp melting point which decomposes on heating above 120°C, $[\alpha]_D^{20}$ – 45° (in water). Methyl sibirosaminide does not possess reducing properties, and gives positive ninhydrin and benzidine reactions. Like other amino sugars, methyl sibirosaminide is electrophoretically mobile. The glycosidic bond in the molecule of sibirosaminide was not hydrolyzed by 1 N HCl (3 h at 100°C), but on being heated with 6 N HCl the amino sugar decomposed completely. When the sibirosaminide was boiled in alcoholic solution, neither ammonia nor volatile amines were formed.

When methyl sibirosaminide was acylated with acetic anhydride in pyridine, the diacetate (II), $C_{13}H_{23}NO_6$, was formed with mp 135-136°C, $[\alpha]_D^{20}$ – 70° (in CH_3OH). The presence of two acetyl groups in it was shown by the PMR method (signal at 2.15 ppm corresponding in intensity to two CH_3–CO groups) and by IR spectroscopy. (The spectrum has the bands of the absorption of CO in an amide group – 1635 cm^{-1} – and in an ester group – 1748 cm^{-1} – which are not present in the spectrum of methyl sibirosaminide.) However, the IR spectrum of the diacetate has absorption at 3450 cm^{-1} (Fig. 1a), which shows the presence in the molecule of methyl sibirosaminide of a tertiary hydroxyl which is not acylated under ordinary conditions. When the diacetate was additionally acetylated by Steglich's method [2] in 4-dimethylaminopyridine, the triacetate of methyl sibirosaminide (III), $C_{15}H_{25}NO_7$, was obtained with mp 127-128°C, $[\alpha]_D^{20}$ – 25° (in CH_3OH). The fact that substance (III) was actually the triacetate was confirmed by the absence of absorption in the region of the stretching vibrations of hydroxy groups in the IR spectrum (Fig. 1b), by the presence of three signals of acetyl groups in the PMR spectrum (at 1.95, 2.07, and

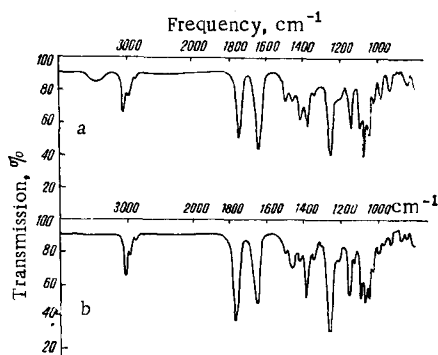

Fig. 1. IR spectra of methyl sibirosaminide diacetate (a) and triacetate (b) (in $CHCl_3$).

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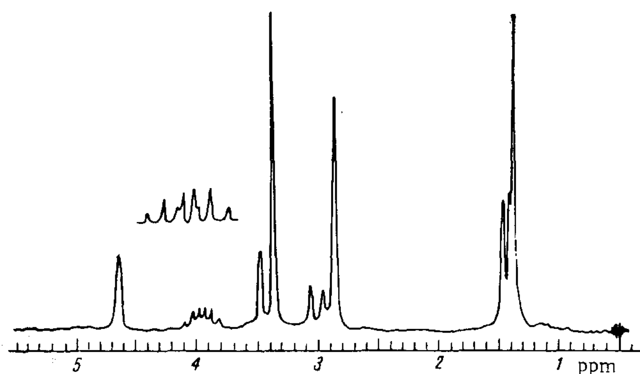


Fig. 2. PMR spectrum of methyl sibirosaminide (hydrochloride) (100 MHz).

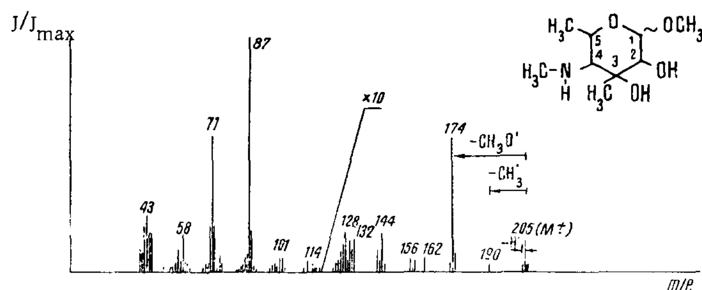


Fig. 3. Mass spectrum of methyl sibirosaminide.

2.26 ppm), and by the increase in the molecular weight of (III) (M^+ 331 m/e) by 126 units as compared with that of methyl sibirosaminide (M^+ 205 m/e).

In the IR spectra of neither acetates was there an "amide II" band in the 1500-1600 cm^{-1} region. Consequently, the amino group in methyl sibirosaminide is secondary. Since the oxidation of methyl sibirosaminide with periodate yielded methylamine in the reaction products (identified in the form of the 3,5-dinitrobenzoate), it may be assumed that one of the hydrogen atoms in the amino group has been replaced by a methyl group. On oxidation, the methyl sibirosaminide consumed two moles of periodate. No volatile carbonyl compounds were detected in the oxidation products. The results of periodate oxidation show the vicinal positions of a CH_3-NH and two OH groups in the molecule of the methyl glycoside. The kinetics of the oxidation show the presence of an α-glycol grouping. (The first mole of periodate is consumed in 1 h, and the second in 20 h.)

The glycoside bond in the molecule of methyl sibirosaminide is stable to the action of 1 N HCl. Such stability of this bond under conditions of acid hydrolysis is characteristic for glycosides of amino sugars having a OH group at C_2, and is due to the influence of the positively charged nitrogen atom. In contrast to methyl sibirosaminide itself, its diacetyl derivative is readily hydrolyzed by 1 N HCl, being converted into sibirosamine N-acetate ($\nu_{max}^{CHCl_3}$ 1630 cm^{-1}; no absorption in the 1740-1760 cm^{-1} region), which possesses reducing properties (positive reaction with aniline hydrogen phthalate) but does not give the ninhydrin or the benzidine reaction and is electrophoretically immobile.

Further information on the structure of methyl sibirosaminide was obtained by an analysis of its PMR and mass spectra (Figs. 2 and 3).

The signals of the methyl groups in the PMR spectrum show that the molecule of the methyl glycoside contains two $C-CH_3$ groups (singlet at 1.37 ppm and doublet at 1.41 ppm with a spin-spin coupling constant SSCC of 6.2 Hz), one $O-CH_3$ group (singlet at 3.38 ppm), and one $N-CH_3$ group (singlet at 2.86 ppm). The position of the $O-CH_3$ and one of the $C-CH_3$ groups was found from an analysis of the mass spectrum. It is known that a characteristic feature of the mass-spectrometric decomposition of compounds with a pyranose ring is the formation of fragments due to the cleavage of the α-bond with respect to the heterocyclic oxygen atom [3]. The elimination of the substituents attached to C_1 and C_5 of the methyl sibirosaminide molecule (I)

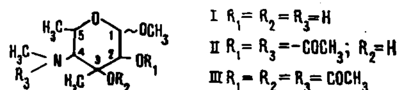
TABLE 1

Grouping	CS (δ, ppm)	SSCC, Hz
$CH_3-C_{(3)}$	1,37	
$CH_3-C_{(5)}$	1,41	$J_{CH_3,1} = 6,2$
$CH_3-\overset{+}{N}$	2,86	
CH_3-O	3,38	
H−1	4,65	$J_{1,2} = 1,7$
H−2	3,45	$J_{2,1} = 1,7$
H−4	2,98	$J_{4,5} = 10$
H−5	3,94	$J_{5\,CH_3} = 6,2$; $J_{5,4} = 10$

belongs to this type of decomposition. The mass spectrum of (I) (see Fig. 3) shows the peaks of the fragments M−H, $M-CH_3$, and $M-OCH_3$, indicating that these groupings are present in the C_1 and C_5 positions. It proved to be possible to determine the positions of the other functional groups and the spatial orientation of some of them from the values of the chemical shifts (CSs) and from the nature of the splitting of the signals of the cyclic protons in the PMR spectrum of methyl sibirosaminide (see Fig. 2 and Table 1).

In the PMR spectrum, the signal of the H-1 proton, which has two neighboring oxygen atoms, is in the weakest field (4.65 ppm). The splitting of this signal into a doublet with a SSCC of 1.7 Hz is due to equatorial−axial or equatorial−equatorial coupling with the proton on the neighboring C_2 atom. The signal of the H-2 proton (doublet with J = 1.7 Hz) is located at 3.45 ppm. In the 3.94 ppm region there is a multiplet which we assign from the nature of its splitting and the value of the CS to the H-5 proton. The SSCC of 6.2 Hz corresponds to coupling with protons of a neighboring methyl group, and the constant of 10 Hz is due to axial−axial interaction with the H-4 proton. The H-4 signal (doublet with J = 10 Hz) lies in a weaker field than the signals of the other cyclic protons (at 2.98 ppm). From its CS value, this signal can be ascribed to a proton adjacent to a $\overset{+}{N}-CH_3$ group or to a OH group. To solve this problem, we recorded the PMR spectrum of the base of methyl sibirosaminide obtained by the addition of a solution in CD_3ONa to a solution of methyl sibirosaminide hydrochloride in CD_3OD to a pH of 8.0-8.5. The neutralization of the charge on the nitrogen atom caused an upfield shift of the doublet with J = 10 Hz by 0.6 ppm. Simultaneously, the signal of the N-methyl group shifted upfield by 0.4 ppm. The other signals scarcely changed their positions. Thus, it was shown that a methylamino group is present on the C_4 atom of the sibirosaminide molecule and a hydroxy group on C_2. The absence of splitting of the signal of the second C-methyl group (at 1.37 ppm), and also the nature of the multiplicity of the H-2 and H-4 signals shows that the tertiary hydroxyl and the second $C-CH_3$ group are located on the C_3 atom.

From the results obtained it follows that methyl sibirosaminide has the structure of methyl 3-C-methyl-4-methylamino-4,6-dideoxyhexopyranoside (I).



Consequently, sibirosamine, a component of the antitumoral antibiotic sibiromycin, is a new amino sugar differing from all the amino sugars known hitherto by a branching of the carbon skeleton.

EXPERIMENTAL

The IR spectra were taken on a UR-10 instrument (solutions in chloroform), the PMR spectra on a JNM-4H-100 instrument [the spectrum of (I) in CD_3OD and those of (II) and (III) in $CDCl_3$; internal standard tetramethylsilane], and the mass spectra on an MKh-1303 instrument at an ionizing voltage of 50 V. The compounds obtained were purified chromatographically on type hydrated silicic acid silica gel.

Methyl Sibirosaminide (I). A solution of 3 g of sibiromycin in 20 ml of absolute ethanol saturated with HCl (9% of HCl by weight) was heated in a sealed tube in the boiling water bath for 1 h. After cooling, the reaction mixture was evaporated to dryness in vacuum at 30°C. The residue in the flask was extracted with 300 ml of water, the insoluble precipitate was filtered off, and the filtrate was extracted with butanol (3 × 5 ml). The aqueous fraction was diluted to 1 liter with water, neutralized with Dowex 2 × 10 anion-exchange resin (HCO_3^-) to pH 7.0-7.1, and passed through a column (2 × 15 cm) filled with Dowex 50 × 8 cation-exchange resin (NH_4^+). The amino sugar was desorbed from the resin with a 1 N solution of ammonia in water. The eluates (in 20-ml fractions) were analyzed by chromatography on FN-16 paper (German Democratic Republic) in the butan-1-ol−acetic acid−water (4 : 1 : 1) system. The methyl glycoside was detected on the chromatogram with ninhydrin or benzidine. The fractions containing the methyl glycosides were combined and evaporated in vacuum at 30-35°C. The residual, faintly colored, syrupy methyl glycoside base was freed from water in a desiccator over P_2O_5, dissolved in the minimum amount of benzene−acetone (1 : 1), and transferred to a column containing 50 g of silica gel. The methyl glycoside was eluted with the same system (20-ml fractions). After chromatographic checking on paper, the fractions containing the pure (I) were combined and evaporated (see above). The residue was dissolved in 10 ml of absolute methanol, methanol saturated

with HCl was added to bring the pH to 6.0-6.5, and the solution was evaporated. After drying in vacuum over KOH, 0.4 g of chromatographically homogeneous methyl sibirosaminide hydrochloride was obtained in the form of a white amorphous powder with $[\alpha]_D^{20} - 45°$ (c 2.0; water).

Found, %: C 44.96; H 8.52; N 5.55; Cl 14.4; OCH_3 12.0; C−CH_3 12.1. Mol. wt. 205 (M^+−HCl). $C_9H_{19}NO_4 \cdot HCl$. Calculated, %: C 44.71; H 8.34; N 5.79; Cl 14.68; 1 OCH_3 12.82; 2 C−CH_3 12.40. Mol. wt. 205.25 + HCl.

Diacetate of Methyl Sibirosaminide (II). A solution of 0.3 g of the hydrochloride of (I) in 15 ml of a mixture of pyridine and acetic anhydride (1 : 1) was left at room temperature for 24 h. Then it was evaporated in vacuum at 35°C, and the residual syrupy product was purified by chromatography on silica gel in the benzene−acetone (10 : 1) system. This gave 0.3 g of a chromatographically homogeneous colorless syrup of (II), which precipitated from a mixture of ether and n-hexane (1 : 3) in the form of colorless crystals with mp 135-136°C, $[\alpha]_D^{20} - 70°$ (c 0.4; methanol).

Found, %: C 54.06; H 8.12; N 5.02; OCH_3 10.19; $COCH_3$ 29.36; $C_{13}H_{23}NO_4$. Calculated, %: C 53.96; H 8.01; N 4.84; 1 OCH_3 10.72; 2 $COCH_3$ 29.76.

Triacetate of Methyl Sibirosaminide (III). A solution of 0.25 g of (II) in 5 ml of a mixture of triethylamine and acetic anhydride (1 : 1) was treated with 20 mg of 4-dimethylaminopyridine, and the mixture was left at room temperature for 24 h. The solvent was driven off, and the oily product was chromatographed on silica gel in the benzene−acetone (25 : 1) system. This gave 0.22 g of the chromatographically homogeneous substance (III) in the form of white crystals with mp 127-128°C [ether−n-hexane (1 : 25)], $[\alpha]_D^{20} - 25°$ (c 0.6; methanol).

Found, %: C 54.23; H 7.52; N 4.11; OCH_3 9.03; $COCH_3$ 38.01. Mol. wt. 331. $C_{15}H_{25}NO_7$. Calculated, % C 54.37; H 7.61; N 4.23; 1 OCH_3 9.36; 3 $COCH_3$ 38.93. Mol. wt. 331.35.

SUMMARY

1. It has been shown that the antitumoral antibiotic sibiromycin is a glycoside of a new amino sugar which has been called sibirosamine.

2. It has been established that sibirosamine is a 3-C-methyl-4-methylamino-4,6-dideoxyhexopyranose, i.e., a new amino sugar differing from all the amino sugars known hitherto by the branching of the carbon skeleton.

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