THE STRUCTURE OF THE AMINO SUGARS FROM THE ANTIBIOTIC ACTINOIDIN

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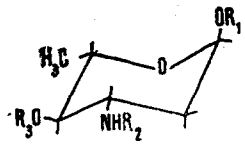
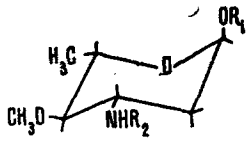
The antibiotic actinoidin is a glycoside. Its carbohydrate moiety contains glucose and mannose [1]. In addition to neutral sugars, in acid hydrolyzates of actinoidin we have found two previously unknown amino sugars, which have been called acosamine and actinosamine. The present paper gives proofs of the structure of acosamine as 3-amino-2,3,6-trideoxy-L-arabinohexose (I) and of that of actinosamine as 3-amino-4-O-methyl-2,3,6-trideoxy-L-arabinohexose (Ia).





I. $R_1=R_2=R_3=H$ (Acosamine)
II. $R_1=R_3=H$; $R_2=CH_3CO$
III. $R_1=CH_3$; $R_2=R_3=H$
IV. $R_1=CH_3$; $R_2=CH_3CO$; $R_3=H$
V. $R_1=CH_3$; $R_2=R_3=CH_3CO$
VI. $R_1=R_3=CH_3$; $R_2=CH_3CO$

Ia. $R_1=R_2=H$ (Actinosamine)
IIa. $R_1=H$; $R_2=CH_3CO$
IIIa. $R_1=CH_3$; $R_2=H$
IVa. $R_1=CH_3$; $R_2=CH_3CO$.

Homogeneous preparations of the amino sugars were obtained by the two-stage methanolysis of actinoidin. In 0.2 N methanolic solution, hydrogen chloride liberated only acosamine. To isolate the actinosamine, the glycosidic fraction not containing acosamine was subjected to methanolysis in a 3 N solution of hydrogen chloride. In this way we obtained crystalline preparations of methyl acosaminide (III), $C_7H_{15}NO_3$, mol. wt. 161 (titration), $p\overset{K}{\downarrow}a$ 8.6, $[\alpha]_D^{20}-118°$ (c 0.5; methanol), and methyl actinosaminide (IIIa), $C_8H_{17}NO_3\cdot HCl$, mol. wt. 211 (titration), $p\overset{K}{\downarrow}a$ 8.3, $[\alpha]_D^{20}-95°$ (c 0.6; methanol).

The empirical formula of (IIIa) has an additional CH_2 group as compared with the formula of (III). This is explained by the presence in (IIIa) of a second methoxy group, in addition to the glycosidic methoxy group, which was determined by the Zeisel method. Acosamine and actinosamine each contain a $C-CH_3$ group and give a positive iodoform test, which is characteristic for 6-deoxy sugars [2]. The nitrogen of both compounds is present in the form of primary amino groups: it is determined quantitatively by Tsuverkalov's method and is liberated in the form of ammonia when the sugars are heated in alkaline solutions. Under these conditions, (I) and (Ia) give (II) and (IIa), and (III) and (IIIa) give (IV) and (IVa), respectively. The acetylation of (III) with acetic anhydride in pyridine led to the formation of methyl N,O-diacetylacosaminide (V); under the same conditions, methyl actinosaminide gave only a N-monoacetyl derivative. It follows from these facts that, besides the amino group, acosamine has two hydroxyls while actinosamine has only one. The N-acetates of acosamine and of actinosamine (II and IIa), on being oxidized with bromine and subsequent deacetylation, are converted into acosaminic and actinosaminic acids, which shows that the sugars are aldoses. When their methanolic solutions containing hydrogen chloride are evaporated, the acids are readily converted into lactones.

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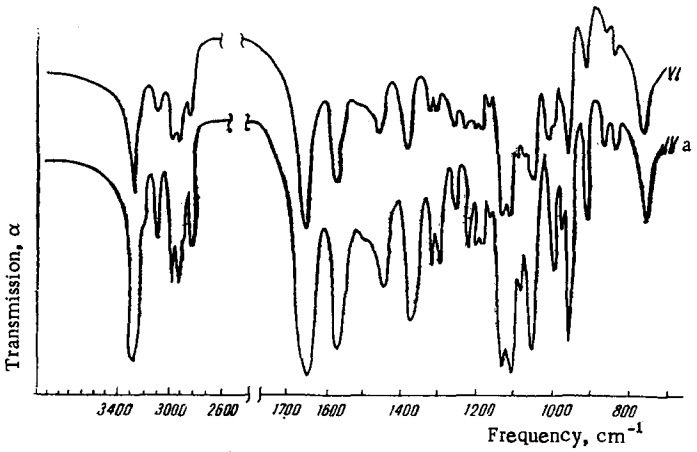
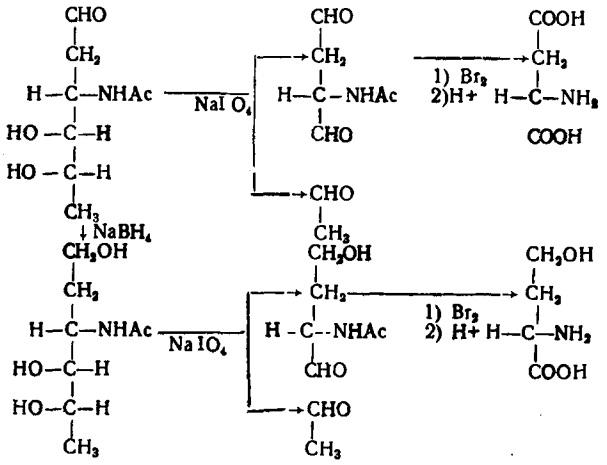


Fig. 1. IR spectra of methyl N-acetylactinosaminide (IVa) and methyl N-acetyl-4-O-methylacosaminide (VI).

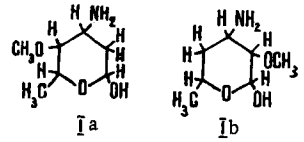
The most valuable information on the structure of the amino sugars was obtained by using periodate oxidation. Acosamine, N-acetylacosamine, and methyl acosaminide rapidly consume sodium periodate. The oxidation of N-acetylacosamine (II) formed acetaldehyde, which was identified as the 2,4-dinitrophenylhydrazone from its chromatographic mobility, melting point, and molar absorption coefficient. Since the amine group of the derivative (II) is blocked, the formation of acetaldehyde from it shows that there are hydroxyls at C_4 and C_5. The amine group cannot be located at C_2, since methyl acosaminide retains the capacity for being oxidized by periodate. Consequently, acosamine is a 3-amino sugar.

The oxidation of N-acetylacosamine first with sodium periodate and then with bromine led to the formation of N-acetylaspartic acid. Under the same conditions, N-acetylacosamine, previously reduced with sodium tetrahydroborate (N-acetylacosaminol) gave α-acetylamino-γ-hydroxybutyric acid (N-acetylhomoserine).



The formation of aspartic acid and homoserine agrees with the presence of a 2-deoxy group in acosamine and shows its structure as a 3-amino-2,3,6-trideoxyaldohexose.

Actinosamine contains, in addition to an aldehyde group, a methoxy, an amine, and a hydroxy group. The latter two are vicinal: actinosamine, unlike acosamine, is resistant to periodate oxidation. The positive iodoform test shows the presence of an oxygen-containing grouping in the C_5 position of actinosamine. This group cannot be a methoxy group, since in this case any position of the amine and hydroxy groups would lead to a structure oxidizable by periodate. This means that there is a hydroxyl at C_5. Then the C_3 position is the only possible one for the amino group. The methoxy group may be either at C_4 (Ia) or C_2 (Ic).



Ia Ib

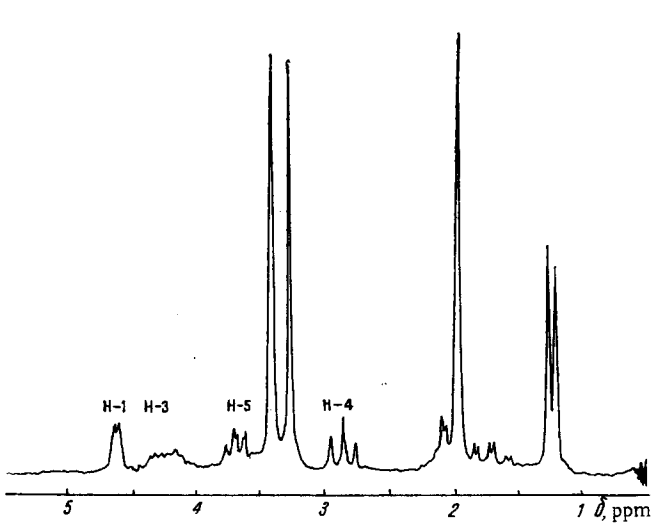


Fig. 2. PMR spectrum of methyl N-acetylactinosaminide (IVa).

The hypothetical formula (Ia) of actinosamine differs from that of acosamine (I) by a methyl group at C_4. In view of this, it appeared of interest to compare actinosamine with 4-O-methylacosamine. In order to perform the selective replacement of the hydroxy group at C_4, as the starting material we took methyl N-acetylacosaminide (IV), which was methylated in tetrahydrofuran with dimethyl sulfate in the presence of solid caustic soda. Substance (VI) was obtained. A crystalline sample of (VI) did not differ from methyl N-acetylactinosaminide (IVa) in its mobility in a thin layer of silica gel, its IR spectrum (Fig. 1), and its PMR spectrum. Consequently, it may be considered that actinosamine is identical with 4-O-methylacosamine both in its structure and in its configuration.

The PMR spectra of the methyl glycosides of N-acetylacosamine (IV) and N-acetylactinosamine (IVa) (Fig. 2) are very similar and differ by the presence in (IVa) of two signals of methoxy groups at δ 3.28 ppm (C_1-OCH_3) and δ 3.43 ppm (C_4-OCH_3), in place of the one (δ 3.30 ppm) in (IV). A doublet signal located in the 1.28-ppm region is due to the $C-CH_3$ protons, and a singlet at 1.97 ppm to the protons of the N-acetyl group. A multiplet of six lines at 1.59-1.87 ppm relates to the axial proton in position 2; its constants are $J_1 = J_2 = 12$ Hz, $J_3 = 3$ Hz. The first of them is geminal, and the second and third show the presence of axial and equatorial protons, respectively, in the neighboring positions of the ring. The signal of H-2 eq is partially masked by the singlet of the $N-COCH_3$ group. The signal of the H-1 proton, which is located between two oxygen atoms, is found in the weakest field (δ 4.65 ppm), has the constants $J_{1e\ 2a} \approx J_{1e\ 2e} \approx 2$-3 Hz and thus shows the equatorial position of this proton. It follows from this that the H_3 proton is axial. A triplet at δ 2.86 ppm with $J_{4a\ 3a} = J_{4a\ 5a} = 10$ Hz is due to H-4, and it confirms the axial orientation of H-3 and shows the axial position of H_5. In the 3.6-3.8-ppm region there is a multiplet which we have assigned from the value of the chemical shift (CS) and the nature of the splitting to H-5 ax ($J_{5a\,4a} = 10$ Hz, $J_{CH_3} = 6.5$ Hz). The upfield shift of the H-3 signal as compared with the H-4 signal is apparently caused by the influence of the carbonyl of the N-acetyl group. The proton at C_3 gives a broad multiplet in the 4.10-4.35-ppm region as a result of coupling with the H-2, H-4, and NH protons.

Thus, the H-1 atoms in acosamine and actinosamine are equatorial and H-3, H-4, and H-5 are axial and, consequently, these sugars possess the arabino configuration. The equatorial-axial arrangement of the protons at C_1 and C_5 shows that the glycosides of acosamine and actinosamine that we obtained were the α anomers. They can be assigned to the α-D (C1) or the α-L (1C) series; however, the negative optical rotations of (III) and (IIIa) permit a conclusion in favor of the L configuration and the 1C conformation. The same conformation is characteristic for the amino sugar daunosamine (3-amino-2,3,6-trideoxy-L-lyxohexose) isolated previously from the antibiotic daunomycin [3].

EXPERIMENTAL

The IR spectra were taken on a UR-10 instrument (KBr) and the PMR spectra on a JNM-4H-100 instrument (solvent: $CDCl_3$; internal standard: tetramethylsilane). Chromatographic analysis was performed on paper in the butan-1-ol–acetic acid–water (4 : 1 : 1) system, R_f I 0.35; Ia 0.6; III 0.59; IIIa 0.7 (spots revealed by the chlorine-benzidine method); in a thin layer of silica gel (Silufol) in the n-propanol–

ethyl acetate–water–1 N ammonia (5 : 1 : 3 : 2) system, R_f III 0.41; IIIa 0.49; and in the benzene–acetone (5 : 7) system – (IV), (IVa), (V), and (VI) (spots revealed with iodine vapor). The analyses of all the compounds corresponded to the calculated figures.

Methanolysis of Actinoidin. Preparation of methyl acosaminide (III). The actinoidin raw material (40 g) was dissolved in 200 ml of absolute methanol containing 0.2 N HCl, and the solution was boiled under reflux for 2 h. The cooled solution was evaporated in vacuum to a syrup, and this was dissolved in 20 ml of water and the solution was poured into a mixture of water, ice, and Dowex 2 × 4 (OH^-) anion-exchange resin. The precipitate that deposited was separated off, washed with water, dried, and used to obtain (IIIa) (see below).

A solution (750 ml) containing the (III) was passed at pH 7.5 through a column with 150 ml of Dowex 50 × 10 (NH_4^+) cation-exchange resin. The (III) was eluted with 0.25 N ammonia solution (600 ml). The eluate was evaporated in vacuum to a syrup, and fractionation was performed with methanol and ethanol, the insoluble precipitates being discarded. The alcoholic solution was evaporated, and the residue was dissolved in hot benzene. On cooling, (III) (free base) with the composition $C_7H_{15}O_3N$ deposited in the form of long broad plates. Yield 1.2 g.

Preparation of methyl actinosaminide (IIIa). A solution of 18 g of the dry residue obtained after the separation of (III) from actinoidin in 400 ml of a 2 N solution of HCl in methanol was boiled under reflux for 7 h. Compound (IIIa) was isolated from the methanolizate in the same way as (III). This gave 350 mg of the crystalline hydrochloride of (IIIa) (from a mixture of methanol and ether) with the composition $C_8H_{17}NO_3 \cdot HCl$.

Acetylation of the Sugars. Preparation of methyl N-acetylacosaminide (IV). A solution of 700 mg of (III) in 3 ml of water was treated with 1 ml of acetic anhydride, and the mixture was stirred vigorously for 30 min, after which it was evaporated in vacuum. The residue was dissolved in water to 50 ml (pH 6.0) and the solution was passed through a layer of SDV-3 (H^+) cation-exchange resin. The eluate was neutralized with Dowex 2 × 4 (OH^-) anion-exchange resin and evaporated in vacuum to dryness. Crystalline substance (IV) with the composition $C_9H_{17}NO_4$ was obtained from a mixture of chloroform and ether with cooling. Yield 420 mg, mp 160–162°C (Kofler), $[\alpha]_D^{20}$ –90° (c 0.1; methanol).

Methyl N-acetylactinosaminide, $C_{10}H_{19}NO_4$ (IVa). This was crystallized from a mixture of chloroform and ether, mp 165–168°C (Kofler), $[\alpha]_D^{20}$ –101° (c 0.6; methanol).

N-Acetylactinosamine (IIa). This was crystallized from a mixture of methanol and ether; $[\alpha]_D^{20}$ –49° (c 0.3; methanol); $[\alpha]_D^{20}$ –54° (c 1.0; water); composition $C_9H_{17}NO_4$, mol. wt. 203 (mass spectrometry).

Preparation of methyl N,O-diacetylacosaminide (V). A solution of 250 mg of (IV) in a mixture of 1 ml of pyridine and 1 ml of acetic anhydride was left to stand for 24 h and was then poured onto ice and the sugar was extracted with chloroform. From a mixture of chloroform and petroleum ether, 92 mg of crystalline (V) was obtained with the composition $C_{11}H_{19}NO_5$, mp 158–163°C, $[\alpha]_D^{20}$ –84° (c 0.5; methanol).

Oxidation of N-acetylacosamine (II) with sodium periodate. The actinoidin raw material (5 g) was hydrolyzed in 0.1 N aqueous HCl for 30 min (100°C). The acosamine was isolated and purified on Dowex 50 × 10 (NH_4^+) resin in the same way as methyl acosaminide (III). To the ammoniacal eluate, concentrated to 3 ml, was added 1 ml of acetic anhydride. This gave N-acetylacosamine (II) in the form of a syrup. The latter was dissolved in 3 ml of water. For the oxidation of (II) with sodium periodate, 1 ml of this solution was taken and was diluted to 10 ml with water, and then 0.5 g of $NaIO_4$ in 10 ml of water was added and the mixture was left at 18°C for 10 h. The volatile aldehydes were displaced from the reaction mixture into a solution of 2,4-dinitrophenylhydrazine. A precipitate of the crystalline 2,4-dinitrophenylhydrazone of acetaldehyde with mp 170–172°C, λ_{max} 360 nm (ε 20,600) was obtained (25 mg).

Then the reaction mixture was freed from mineral ions by means of ion-exchange resins. The resulting solution was treated with bromine water (0.1 ml of bromine in 100 ml of water) and was left in the dark for two days. The excess of bromine was eliminated, the solution was evaporated, and the acetate was hydrolyzed in 3 ml of 0.5 N HCl for 2.5 h (100°C). The hydrolyzate was found to contain a ninhydrin-positive substance not differing from aspartic acid in its electrophoretic and chromatographic mobilities.

N-Acetylacosaminol was obtained from N-acetylacosamine (II). To 1 ml of a solution of (II) was added 20 mg of $NaBH_4$, and the mixture was left at 18°C for 24 h. The reduced product was oxidized with sodium periodate and bromine in the same way as substance (II). After deacetylation, 6 mg of α-amino-

γ-hydroxybutyric acid was obtained; it did not differ from a standard sample in its electrophoretic and chromatographic mobilities.

Oxidation of the Sugars with Bromine. N-Acetylacosamine. A mixture of 100 mg of this substance and 100 ml of bromine water (1.7 mole of bromine to 1 mole of sugar) was left in the dark for two days. After the deacetylation of the oxidized product, 38 mg of acosaminic acid was obtained (crystallization from aqueous acetone); composition $C_6H_{13}NO_4$, R_f 0.25 (BAW, 4 : 1 : 1). On being heated in methanol–0.5 N HCl, it was converted into the δ-lactone with R_f 0.32 (BAW, 4 : 1 : 1), IR spectrum: ν_{max} 1735 cm^{-1}.

N-Acetylactinosamine (70 mg), on oxidation with bromine and deacetylation, gave 25 mg of actinosaminic acid (crystallization from acetone) with R_f 0.43 (BAW 4 : 1 : 1); on heating in methanol–0.5 N HCl it was converted into the δ-lactone with R_f 0.55 (BAW 4 : 1 : 1); IR spectrum: ν_{max} 1735 cm^{-1}.

Preparation of the 4-O-Methyl Derivative of Acosamine (VI). To 100 mg of methyl N-acetylacosaminide (IV) in 5 ml of tetrahydrofuran were added 0.5 g of ground NaOH and then, in drops over 3 h at 40-50°C, 0.6 ml of dimethyl sulfate. After the completion of the reaction, the precipitate was filtered off and washed with chloroform. The solution was evaporated to dryness, giving 40 mg of methyl N-acetyl-4-O-methylacosaminide (VI), mp 156-158°C (from a mixture of ethane and n-hexane); $[\alpha]_D^{20}$ −70° (c 0.4; methanol).

SUMMARY

1. Two new aminodeoxy sugars – acosamine and actinosamine – have been obtained from the antibiotic actinoidin.

2. Acosamine has the structure of 3-amino-2,3,6-trideoxy-L-arabinohexose.

3. Actinosamine is identical with 4-O-methylacosamine.

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