^{13}C NMR SPECTRA OF THE AMINOGLYCOSIDE ANTIBIOTICS STREPTOMYCIN AND DIHYDROSTREPTOMYCIN. COMPLETE ASSIGNMENT OF THE SIGNALS

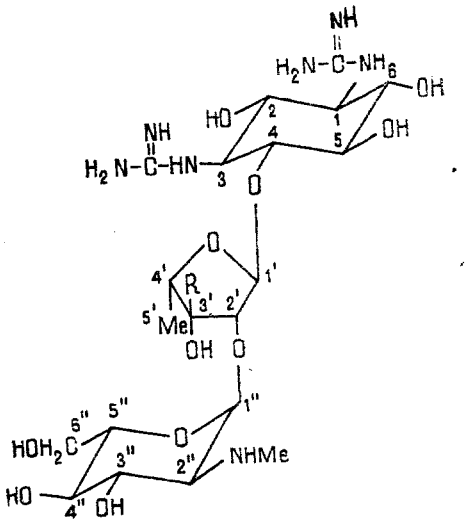
A. I. Chernyshev, V. S. Kartashov, A. P. Arzamastsev, S. E. Esipov, and P. L. Senov

UDC 543.422:615.332

On the basis of the results of a study of the dependence of the chemical shifts of the ^{13}C NMR spectra streptomycin and dihydrostreptomycin over a wide pH range (from 1.0 to 10.0) and a comparison with the spectrum of streptidine in an acid medium, a complete assignment of the spectra of the antibiotics investigated has been made. The optimum pH values of solutions at which the clearest separation of the resonance signals in the spectra of streptomycin and dihydrostreptomycin have been determined (pH 1.0-7.0 and pH $\leq$ 8.6, respectively).

As has been shown for a number of amines, including aminoglycoside antibiotics [1], the protonation of an amino group with a lowering of the pH values of solutions leads to considerable upfield displacements ($\Delta\delta$ 4-10 ppm) of the resonance signals of carbon atoms present in the β position to an amino group. The signals of α- and γ-carbon atoms are shifted upfield to a smaller extent: $\Delta\delta$ 0.5-1.6 ppm. The characteristic features of the displacement of the ^{13}C NMR signals of the aminoglycoside antibiotics neamine, kanamycin B, and tobramycin [1], and also of neomycin B, monomycin A, and kanamycin A [2] as functions of the pH of the solutions investigated previously have permitted the complete assignment of the absorption signals in the spectra of these compounds and the determination of the positions of the phosphate groups in neomycin 3'-phosphate and neamine 3'-phosphate [2, 3].

In the present paper we consider the results of a study of the ^{13}C NMR spectra of the aminoglycoside antibiotics streptomycin (I) and dihydrostreptomycin (II) over a wide pH range with the aim of a complete assignment of the signals to the corresponding carbon atom and the determination of the optimum pH values of solutions at which a clear separation of the resonance signals of all the carbon atoms is observed. Previously [4], for the ^{13}C NMR spectra of (I) and (II) an unambiguous assignment was made of the signals of the C-2" and C-6",



I. R = CHO
II. R = CH_2OH

All-Union Scientific-Research Institute of Antibiotics, Moscow. I. M. Sechinov 1st Moscow Medical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 695-700, September-October, 1980. Original article submitted April 28, 1980.

 0009-3130/80/1605-0508$07.50

C-3" and C-4", C-5 and C-5", C-2' and C-4, C-1 and C-3, and C-2 and C-6 carbon atoms, and also of the signals of the carbon atoms of the guanidine residues of the streptidine fragments.

To perform the complete assignment of the signals to the corresponding carbon atoms we have compared the chemical shifts (CSs) of the ^{13}C NMR signals of the antibiotics (I) and (II) recorded in the range of pH values of the solutions of from 1.0 to 10.0, and also the shifts of the signals of the spectrum of streptidine in an acid medium. The measured values of the chemical shifts in the pH range from 1.0 to 10.0 for the corresponding carbon atoms of (I) and (II) and the observed displacements of the resonance signals ($\Delta\delta = \delta_{pH\ 10.0} - \delta_{pH\ 1.0}$) are given in Tables 1 and 2.

Analysis of the ways in which the chemical shifts depend on the pH (Tables 1 and 2 and Fig. 1) permit the isolation of two resonance signals in each of the spectra of (I) and (II) which shift downfield by $\Delta\delta$ 2.3-3.7 ppm with an increase in the pH value and, consequently, are assigned to the C-1" and C-3" atoms, which are present in the β positions to methylamine groups. The different shifts of the signals in the $\delta \sim 70$ ppm region (at pH < 5.3) with a rise in the pH permits their assignment to C-3" and C-4" carbon atoms. Strongly shifted signals ($\Delta\delta$ 2.3 ppm) related to the C-3" carbon atoms, and signals displaced by $\Delta\delta$ 0.7 ppm to the C-4" carbon atoms, which are present in the γ positions to amino groups. The assignment of the closely located C-2" and C-6" signals was based on the large shifts of the signals of the C-2" α-carbon atoms ($\Delta\delta$ 1.6 ppm for (I) and 1.7 ppm for (II)) as compared with the signals of the C-6" carbons, which are separated from amino groups by five chemical bonds ($\Delta\delta$ 0.3 ppm for (I) and 0.4 ppm for (II)). The signals of the carbon atoms of the N-methyl groups shift on alkalinization by values of $\Delta\delta$ of 1.4-1.5 ppm, which are close to the shifts of the C-2" signals.

A comparison of the ^{13}C NMR spectra of antibiotics (I) and (II) with the spectrum of streptidine recorded for an acid solution (see Tables 1 and 2 and Fig. 2) enables the assignment of the signals of the carbon atoms of the streptidine fragments to be refined.

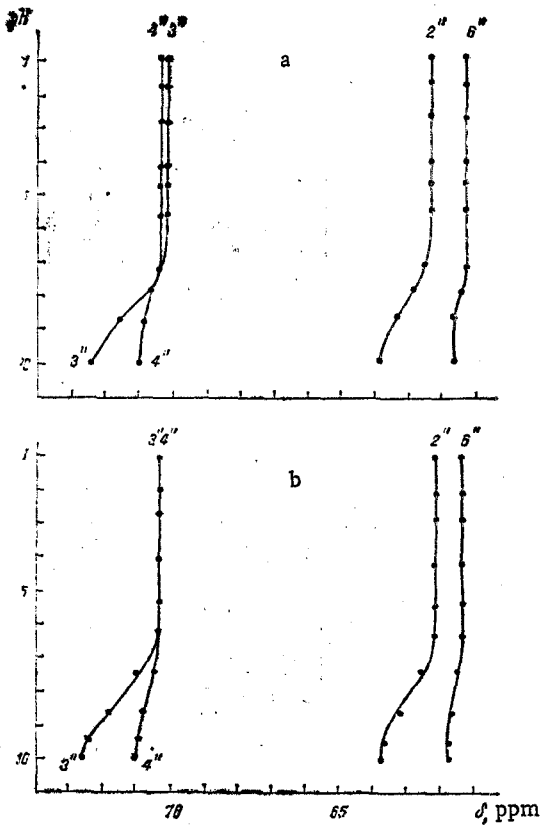


Fig. 1. Dependence of the chemical shifts of the ^{13}C NMR signals of streptomycin (a) and of dihydrostreptomycin (b) on the pH of the solutions.

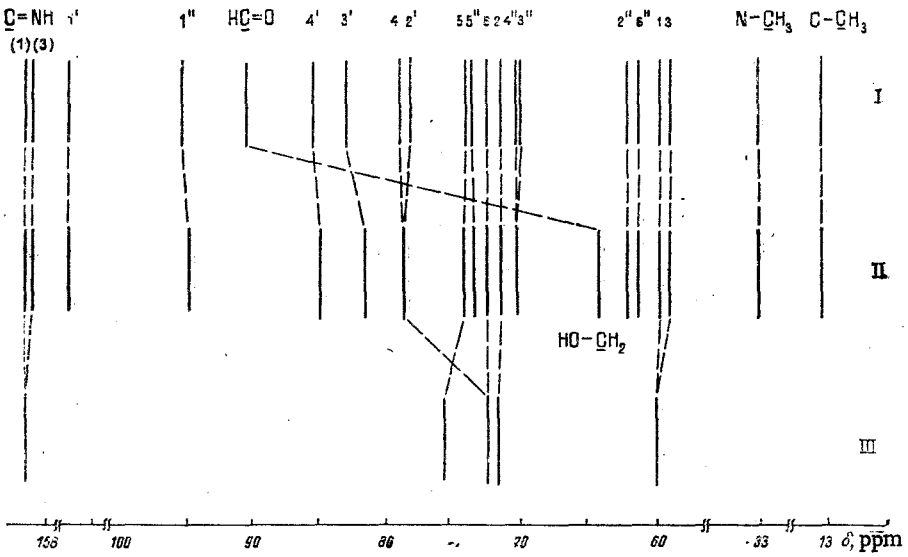


Fig. 2. Diagram of the chemical shifts of the ^{13}C NMR spectra of acid solutions of streptomycin (I), dihydrostreptomycin (II), and streptidine (III).

The spectrum of streptidine consists of five resonance signals, three of which have a double relative intensity. The signals of the six nuclei magnetically equivalent in pairs are assigned to the carbon atoms of the guanidine groups, δ 159.2 ppm, to the C-4 and C-6 carbon atoms bearing hydroxy groups, δ 72.5 ppm, and to the C-1 and C-3 carbon atoms bearing the guanidine substituents, δ 60.0 ppm. Of the two signals with unit intensity at δ 71.8 and 75.7 ppm, the strong-field one is assigned to the C-2 carbon having the voluminous guanidine substituent on the C-1 and C-3 carbon atoms and the weak-field one to C-5.

The substitution of the streptidine hydroxyl at C-4 by the streptobiosaminyl substituent should lead to more considerable changes in the CS values of this carbon atoms and of those closest to it, C-3 and C-5, and also of the carbon atom of the guanidine group at C-3 and, to a smaller extent of the more remote C-1, C-2, and C-6 carbon atoms of the streptidine ring and of the carbon atom of the guanidine group at C-1. On this basis, the positions of the signals with chemical shifts of δ 159.2 ppm in the spectra of (I) and (II) can be assigned to the carbon atoms of the guanidine groups at C-1, and the signals at δ 158.7 ppm in (I) and δ 158.6 ppm in (II) to the carbon atoms of the C-3 guanidine groups. Similarly, the signals at δ 59.7 ppm closest to the CS values of the signals of the C-1 and C-3 carbon atoms in the spectrum of streptidine have been assigned to the C-1 carbons, and the signals at δ 59.0 ppm with a greater upfield shift, to the C-3 atom. To the C-2 and C-6 carbon atoms remote from the position of substitution have been assigned the resonance signals at δ 71.5 and 72.4 ppm, which are close to the CSs of the signals of the corresponding atoms in the streptidine spectrum (see Fig. 3).

The presence of voluminous streptobiosaminyl substituents on the C-4 carbon atoms in (I) and (II) should be shown in a downfield shift of the signals of the C-5 carbon atoms in comparison with the spectrum of streptidine, in analogy with the signals of the C-3 carbon atoms. In view of this, the signals with CSs of δ 74.1 and 74.2 ppm in the spectra of (I) and (II), shifted upfield by 1.6 and 1.5 ppm in comparison with the spectrum of streptidine, were assigned to the C-5 carbon atom. In this case, the resonance signals with chemical shifts of δ 73.7 ppm in the spectrum of (I) and 73.5 ppm in the spectrum of (II) are assigned to the C-5" carbon atoms. The correctness of the assignment of the C-5 and C-5" signals is confirmed by the presence in the ^{13}C NMR spectrum of methyl streptobiosaminide of a signal with a chemical shift of δ 73.5 ppm from the C-5" carbon atom [4]*, and by the absence of a signal in the 74.1-74.2 ppm region which is characteristic for C-5 in the spectra of antibiotic (I) and (II).

Of the two remaining unassigned signals in the ^{13}C NMR spectrum of the antibiotic (I) with CSs of δ 78.2 and 78.9 ppm, the first is located considerably closer to the signal of the C-2' carbon atom of the spectrum of methyl streptobiosaminide (δ 77.6 ppm [4]). On this

*In this paper, a different numbering of the carbon atoms was used.

TABLE 1. ^{13}C NMR Chemical Shifts of Streptomycin at Various pH Values

pH	Carbon atom																			
	1	2	3	4	5	6	1′	2′	3′	4′	1″	2″	3″	4″	5″	6″	C–$\underline{C}H_3$	N–$\underline{C}H_3$	HC=O	(1)$\underline{C}$=NH(3)
1,0	59,7	71,5	59,0	78,9	74,1	72,4	106,7	78,2	83,0	85,4	95,3	62,3	70,1	70,3	73,7	61,3	13,4	33,1	90,6	159,2	158,7
1,8	59,7	71,5	59,0	78,9	74,1	72,4	106,7	78,2	83,0	85,4	95,4	62,3	70,1	70,3	73,7	61,3	13,4	33,1	90,6	159,2	158,7
2,8	59,7	71,5	59,0	78,9	74,1	72,4	106,7	78,2	83,0	85,4	95,4	62,3	70,1	70,3	73,7	61,3	13,4	33,1	90,6	159,1	158,7
4,1	59,7	71,5	59,0	78,9	74,1	72,4	106,7	78,2	83,0	85,4	95,4	62,3	70,1	70,3	73,7	61,3	13,4	33,1	90,6	159,1	158,7
4,7	59,7	71,5	59,0	78,9	74,1	72,4	106,7	78,2	83,0	85,4	95,4	62,3	70,1	70,3	73,7	61,3	13,4	33,1	90,6	159,1	158,7
5,5	59,7	71,5	59,0	78,9	74,1	72,4	106,7	78,2	83,0	85,4	95,4	62,3	70,3	70,4	73,7	61,3	13,4	33,1	90,6	159,1	158,7
7,2	59,7	71,5	59,0	78,9	74,1	72,4	106,7	78,2	83,0	85,4	95,5	62,6	70,4	70,4	73,7	61,3	13,4	33,2	90,6	159,1	158,7
7,9	59,7	71,5	59,0	78,9	74,2	72,4	106,7	78,2	83,0	85,4	96,0	62,7	70,6	70,6	73,7	61,4	13,4	33,4	90,6	159,1	158,7
8,7	59,7	71,5	59,0	79,0	74,2	72,4	106,9	78,2	83,0	85,9	97,2	63,3	70,7	70,7	73,7	61,6	13,6	33,9	90,9	159,1	158,7
10,0	59,7	71,5	59,0	79,1	74,2	72,4	107,0	78,2	83,0	86,0	98,5	63,9	71,0	71,0	73,7	61,6	13,8	34,6	91,9	159,1	158,7
Δδ	0	0	0	0,2	0,1	0	0,3	0	0	0,6	3,2	1,6	2,3	0,7	0	0,3	0,4	1,5	0,4	—0,1	0

TABLE 2. ^{13}C NMR Chemical Shifts of Dihydrostreptomycin for Various pH Values

pH	Carbon atom																			
	1	2	3	4	5	6	1′	2′	3′	4′	1″	2″	3″	4″	5″	6″	C–$\underline{C}H_3$	N–$\underline{C}H_3$	$\underline{H}_2$COH	(1)$\underline{C}$=NH(3)
1,0	59,7	71,5	59,0	78,7	74,2	72,4	106,7	78,7	81,6	85,0	94,7	62,1	70,3	70,3	73,5	61,3	13,4	32,9	64,2	159,2	158,6
2,0	59,7	71,5	59,0	78,7	74,2	72,4	106,7	78,7	81,6	85,0	94,7	62,1	70,3	70,3	73,5	61,3	13,4	32,9	64,2	159,2	158,6
2,7	59,7	71,5	59,0	78,7	74,2	72,4	106,7	78,7	81,6	85,0	94,7	62,1	70,3	70,3	73,5	61,3	13,4	32,9	64,2	159,2	158,6
4,1	59,7	71,5	59,0	78,7	74,2	72,4	106,7	78,7	81,6	85,0	94,7	62,1	70,3	70,3	73,5	61,3	13,5	32,9	64,2	159,2	158,6
5,5	59,7	71,5	59,0	78,7	74,2	72,4	106,7	78,7	81,6	85,0	94,7	62,1	70,3	70,3	73,5	61,3	13,5	32,9	64,2	159,2	158,6
6,2	59,7	71,5	59,0	78,7	74,2	72,4	106,7	78,7	81,6	85,0	94,7	62,1	70,3	70,3	73,5	61,3	13,5	32,9	64,2	159,2	158,6
7,3	59,7	71,5	59,0	78,7	74,2	72,4	106,7	78,7	81,6	85,3	95,7	62,6	71,0	70,5	73,5	61,4	13,5	33,3	64,2	159,2	158,7
8,6	59,7	71,5	59,0	78,9	74,2	72,4	106,8	78,7	81,6	85,5	96,9	63,1	71,7	70,7	73,5	61,6	13,6	33,8	64,2	159,2	158,7
9,4	59,7	71,5	59,0	79,1	74,2	72,4	106,9	78,7	81,5	85,7	98,0	63,6	72,4	70,9	73,5	61,7	13,6	34,2	64,0	159,2	158,7
10,0	59,8	71,5	59,0	79,1	74,2	72,4	106,9	78,7	81,5	85,7	98,2	63,8	72,6	71,0	73,5	61,7	13,7	34,3	63,8	159,2	158,7
Δδ	0,1	0	0	0,4	0	0	0,2	0	—0,1	0,7	3,5	1,7	2,3	0,7	0	0,4	0,3	1,4	—0,4	0	0,1

basis, the signal in the higher field can be assigned to the C-2' carbon atom and the weak-field signal at δ 78.9 ppm to the C-4 atom. A differentiation of the resonance signals of C-2' and C-4 in the spectrum of (II) was made by analogy with the spectrum of (I) (see Tables 1 and 2). Reduction of the aldehyde group at C-3' of streptomycin to the hydroxymethyl group of dihydrostreptomycin led to a larger change in the CS value of the adjacent C-2' atom (by 0.5 ppm) as compared with the remote C-4 atom (by 0.2 ppm), which confirms the correctness of the assignment made. The downfield shift of the C-4 resonance signals in the spectra of the antibiotics (I) (Δδ 0.2 ppm) and (II) (Δδ 0.4 ppm) with a rise in the pH of 7.9 can be explained by the effect of the deprotonation of the guanidine group on the neighboring C-3 atom (see Tables 1 and 2). The positions of the resonance signals of the C-2' carbon atoms, which are remote from the nitrogen-containing substituents, do not depend on the pH of the solution.

The analysis of the ^{13}C NMR spectra of aqueous solutions of the antibiotics (I) and (II) performed over a wide pH range shows that the most complete separation of the resonance signals is observed at pH values of from 1.0 to 7.0 for streptomycin (I) and at pH ≥ 8.6 for dihydrostreptomycin (II).

EXPERIMENTAL

The ^{13}C NMR spectra of 30% aqueous solutions of streptomycin (I) and dihydrostreptomycin (II) and of a 10% solution of streptidine in 1 N hydrochloric acid were recorded at 40°C on a WH-90 pulsed spectrometer (Bruker) with a working frequency of 22.26 MHz in the regime of complete suppression of spin-spin coupling of protons with carbon atoms. To stabilize the resonance conditions we used deuterated water sealed into a coaxial glass capillary with a diameter of 2.5 mm. The numerical resolution of the spectrum at a width of 3600 Hz and with the accumulation of the data in 16 K points of a computer amounted to 0.44 Hz per point, which corresponded to an accuracy of the measurement of the chemical shifts of 0.02 ppm. The shifts were measured in relation to an internal standard — dioxane (~3%) — in the δ scale, δ_{do} 67.4 ppm.

The solutions investigated were acidified and alkalinized with 0.5 N solutions of sulfuric acid and caustic soda. The pH values were varied in steps of 0.2-0.3; Tables 1 and 2 and Fig. 1 give the results only for selected pH values. The pH measurements were carried out on a pH 121 instrument.

The streptidine was obtained by the hydrolysis of streptomycin with 2 N hydrochloric acid at room temperature (2 days) followed by precipitation and washing of the precipitate with ethanol.

SUMMARY

On the basis of the results of a study of the dependence of the chemical shifts of the ^{13}C NMR signals of streptomycin and dihydrostreptomycin over a wide pH range (from 1.0 to 10.0) and a comparison with the spectrum of streptidine in an acid medium a complete assignment of the spectra of the antibiotics has been made.

LITERATURE CITED

1. R. F. Koch, J. A. Rhoades, E. W. Hagaman, and E. Wenkert, J. Am. Chem. Soc., 96, 3300 (1974).
2. A. I. Chernyshev, V. S. Kartashov, A. P. Arzamastsev, S. E. Esipov, P. L. Senov, and S. M. Navashin, Khim. Prir. Soedin, 686 (1980).
3. R. M. Petyushenko, V. L. Ganelin, A. I. Cherinyshev, A. S. Demina, S. E. Esipov, Yu. O. Sazykin, and S. M. Navashin, Antibiotiki, No. 6, 430 (1979).
4. K. Bock, C. Pederson, and H. Heding, J. Antibiot., 27, 139 (1974).