the Central Asian cobra. The efficiency of the hemolytic action of a mixture of the venom or of the phospholipases A_2 with cytotoxins depends on the concentration and activities of both components of the mixture.

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^{13}C NMR SPECTRA OF THE AMINOGLYCOSIDE ANTIBIOTIC NEOMYCIN B, MONOMYCIN A, AND KANAMYCIN A. COMPLETE ASSIGNMENT OF THE SIGNALS

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On the basis of the results of a study of the dependence of the chemical shifts of the signals in the ^{13}C NMR spectra of neomycin B, monomycin A, and kanamycin A, over a wide pH range (from 1.0 to 10.0) a complete assignment of the spectra signals of these antibiotics has been made. Optimum pH values of solutions are proposed for which the more complete resolution of the resonance signals in the spectra of neomycin B, monomycin A, and kanamycin A is observed.

At the present time, great attention is being devoted to the directed modification of aminoglycoside antibiotics and the study of the mechanism of their inactivation in the organism, which is leading to the creation of preparations possessing increased activity in relation to pathogenic microorganisms. The method of ^{13}C NMR spectroscopy is being used successfully for the structural identification of aminoglycoside antibiotics and of their modified derivatives and inactivation products. However, literature information on the assignment of the ^{13}C NMR resonance signals to the corresponding carbon atoms in the molecule of such aminoglycoside antibiotics widely used in practice as neomycin B, monomycin A (paromomycin I), and kanamycin A do not agree with one another; an unambiguous assignment of the signals is lacking for a number of atoms of structurally related fragments.

The conditions for recording the ^{13}C NMR spectra of neomycin [1] do not permit an assignment to be made of the mutually overlapping absorption signals of the C-4 and C-5, C-1' and C-1'", and C-6' and C-6"' carbon atoms. Because of the inadequacy of the spectral information, no unambiguous assignment has been made of the four signals of the C-3', C-4', C-3'", and C-4'" of the structurally isomeric fragments of the α-D-gluco- and β-L-idopyranosides, and also of the pairs of C-5' and C-5" signals. In later publications, the assignment of the

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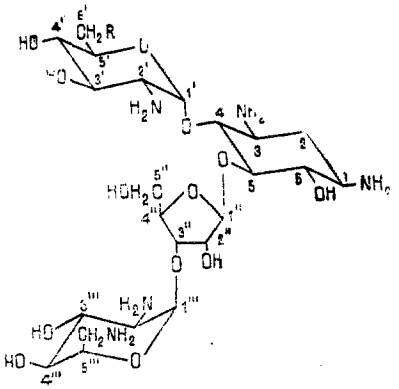
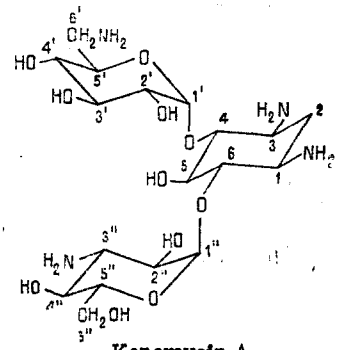


Monomycin A: R = OH
Neomycin B: R = NH_2



Kanamycin A

signals of the ^{13}C NMR spectra of neomycin [2] and of monomycin A [3] was made by comparing the spectra of these antibiotics with simpler fragmentary analogs of them for alkaline solutions at a single pH value ≥ 11.0 with no discussion of the experimental results and with no comparison with those published previously.

The lack of agreement in the assignment of the resonance signals of the C-5 and C-3" carbon atoms of neomycin [1, 2] and monomycin [3], and of the C-1' and C-1" atoms of kanamycin in [1] and [4], together with the above-mentioned ambiguities of the assignment of the signals of neomycin, considerably complicates the complete analysis of the spectra of the products of the modification and inactivation of these antibiotics.

A study of the dependence of the chemical shifts (CSs) of tobramycin, kanamycin B, and neamine and their analogs on the pH of the medium over a wide pH range has permitted a complete assignment to be made of the signals in the ^{13}C NMR spectra of these aminoglycoside antibiotics [5]. It has been shown that the protonation of an amino group when solutions of these compounds are acidified (from pH ≥ 11 to pH < 1.1) leads to considerable upfield shifts (Δδ 4-10 ppm) of the resonance signals of the carbon atoms present in the β position to this amino group. The signals of the α- and γ-carbon atoms also shift upfield, but to a smaller degree (Δδ 0.5-1.6 ppm). The values of the shifts of the resonance signals with a change in the pH of the solutions have been used in the structural identification of the products of the inactivation of neomycin by aminoglycoside phosphotransferase from the ^{13}C NMR spectra of neamine 3'-phosphate — the product of the methanolysis of phosphorylated neomycin B [6].

In the present paper we consider the results of a study of the dependence of the ^{13}C NMR CSs of neomycin B, monomycin A, and kanamycin A on the medium over a wide pH range (from 1.0 to 10.0) with the aim of a complete assignment of the spectral signals to the corresponding carbon atoms and of the selection of the optimum pH values at which the clearest separation of the ^{13}C NMR signals of al the carbon atoms is observed.

The values of the CSs for ^{13}C NMR signals of the corresponding carbon atoms of neomycin B, monomycin A, and kanamycin A at various pH values, and also the shifts in the resonance signals with a change in the pH values of the solutions from 10.0 to 1.0 ($\Delta\delta = \delta_{pH\ 10.0} - \delta_{pH\ 1.0}$) are given in Tables 1-3. The dependences of the CSs on the pH for the sections of the spectra of neomycin B and monomycin A most complicated by the overlapping of the signals are given in Fig. 1.

TABLE 1. ^{13}C NMR Chemical Shifts (δ, ppm) of Neomycin B at Various pH Values and of Neomycin 3-Phosphate at pH 5.2

pH	Carbon atom																					
	1	2	3	4	5	6	1′	2′	3′	4′	5′	6′	1″	2″	3″	4″	5″	1‴	2‴	3‴	4‴	5‴′	6‴′
Neomycin-3′-phosphate																							
5,2	50,8	28,9	49,4	75,8	75,9	73,5	95,9	53,7	73,5	70,4	70,2	41,4	110,7	74,3	82,1	85,2	61,1	95,5	51,8	70,9	68,1	68,5	40,9
Neomycin																							
1,0	51,0	28,9	49,5	75,6	75,9	73,4	96,0	54,7	70,4	71,9	69,0	41,5	110,9	74,3	82,1	85,5	61,3	95,8	51,9	71,3	68,1	68,6	41,5
5,2	51,0	28,9	49,6	75,6	75,9	73,4	96,0	54,7	70,4	71,9	69,0	41,5	110,9	74,3	82,1	85,5	61,3	95,8	51,9	71,3	68,1	68,6	41,5
7,2	51,3	30,6	49,7	77,7	76,0	73,9	96,3	54,9	70,2	71,9	69,6	41,5	110,8	74,3	82,1	85,8	61,4	96,1	52,1	71,3	68,2	68,8	41,5
8,2	51,5	32,3	50,1	79,5	76,1	74,2	97,1	55,4	70,8	71,9	70,0	41,5	110,6	74,2	82,1	85,8	61,5	96,8	52,3	71,5	68,5	69,4	41,5
8,7	51,5	33,1	50,2	80,1	76,1	75,2	97,9	55,5	71,6	72,0	70,2	41,6	110,4	74,2	82,1	85,6	61,7	97,5	52,6	72,0	68,8	70,0	41,6
9,0	51,5	33,3	50,3	80,3	76,2	75,4	98,2	55,5	72 0	72,0	70,2	41,6	110,3	74,2	82,1	85,5	61,8	97,7	52,7	72,0	68,8	70,2	41,6
9,3	51,5	34,3	50,6	80,9	76,4	76,4	99,0	55,8	72,7	72,1	70,8	41,7	109,9	74,2	82,1	85,4	61,9	98,4	53,0	72,7	69,2	70,8	41,7
9,5	51,4	34,3	50,6	81,1	76,6	76,6	99,0	55,8	72,7	72,1	70,8	41,7	109,9	74,2	82,1	85,3	61,9	98,4	53,0	72,7	69,2	70,8	41,7
9,7	51,4	35,4	50,8	81,7	76,6	77,4	99,6	56,1	73,4	72,1	71,8	42,1	109,6	74 2	82,1	85,2	62,1	99,0	53,0	73,9	69,3	71,2	41,9
10,0	51,3	35,7	50,9	82,1	76,7	77,7	99,8	56,2	73,7	72,2	72,2	42,2	109,4	74,2	82,1	85,2	62,2	99,2	53,4	74,5	69,4	71,3	41 9
Δδ*	0,3	6,8	1,4	6,5	0,8	4,3	3,8	1,5	3,3	0,3	3,2	0,7	—1,5	—0,1	0	—0.3	0,9	3,4	1,5	3,2	1,3	2,7	0,4

*Here and in Tables 2 and 3 $\Delta\delta = \delta_{pH\ 10.0} - \delta_{pH\ 1.0}$.

TABLE 2. ^{13}C NMR Chemical Shifts (δ, ppm) of Monomycin A at Various pH Values

pH	Carbon atom																					
	1	2	3	4	5	6	1′	2′	3′	4′	5′	6′	1″	2″	3″	4″	5″	1‴	2‴	3‴	4‴	5‴	6‴
1,0	50,9	29,0	50,0	78,2	76,0	73,1	96,7	55,0	69,9	70,3	74,6	61,3	110,7	74,2	82,2	84,9	61,3	96,2	51,9	71,2	68,1	68,6	41,5
2,1	50,9	29,0	50,0	78,2	76,0	73,1	96,7	55,0	69,9	70,3	74,6	61,3	110,7	74,2	82,2	84,9	61,3	96,2	51,9	71,2	68,1	68,6	41,5
3,1	50,9	29,0	50,0	78,2	76,0	73,1	96,7	55,0	69,9	70,3	74,6	61,3	110,7	74,2	82,2	84 9	61,3	96,2	51,9	71,2	68,1	68,6	41,5
4,3	50,9	29,0	50,0	78,2	76,0	73,1	96,7	55,0	69,9	70,3	74,6	61,3	110,7	74,2	82,2	84.9	61,3	96,2	51,9	71,2	68,1	68,6	41,5
5,0	50,9	29,1	50,0	78,2	76,0	73,1	96,7	55,0	69,9	70,3	74,6	61,3	110,7	74 2	82,2	84.9	61,3	96,2	51,9	71,2	68,1	68,6	41,5
5,8	50,9	29,1	50,0	78,2	76,0	73,1	96,7	55,0	69,9	70,3	74,6	61,3	110,7	74,2	82,2	84.9	61,3	96,2	51,9	71,2	68,1	68,6	41,5
7,0	51,0	29,8	50,0	79,1	76,0	73,4	96,9	55,1	70,1	70,3	74,6	61,3	110,7	74,2	82,2	84,9	61,3	96,2	52,0	71,2	68,2	68,6	41,5
7,9	51,3	31,3	50,2	80,9	76,1	74,1	97,3	55,3	70,8	70,4	74,1	61,4	110,5	74,1	82,1	84.9	61,4	96,6	52,1	71,3	68,3	68.9	41,5
9,1	51.4	33,9	50,7	82,1	76,3	75,9	99,2	56,0	72,3	70,6	74,1	61,5	109,8	74,1	82.1	84,8	61.7	98,7	52,9	72,9	69,0	70,6	41,7
10,0	51,4	36,1	51,1	83,8	76,8	77,9	100	56,3	73,8	70,8	74,1	61,6	109,1	74,0	82.1	84.8	62,2	100	53,5	75,0	69,4	71,4	41,9
Δδ	0,5	7,1	1,1	5.6	0,8	4,8	3,3	1,3	3,9	0,5	—0,5	0,3	—1,6	—0,2	—0,1	—0.1	0,9	3,8	1,6	3,8	1,3	2,8	0,4

TABLE 3. ^{13}C NMR Chemical Shifts (δ, ppm) of Kanamycin A at Various pH Values

pH	Carbon atom																	
	1	2	3	4	5	6	1′	2′	3′	4′	5′	6′	1″	2″	3″	4″	5″	6″
1,0	50,8	28,4	48 9	78,8	73,6	84,5	97,0	71,8	73,0	71,8	69,6	41,6	10 ,1	69,2	56,2	66,6	73,6	61,0
2,4	50,9	28,6	40,1	78,8	73,9	84,6	97,1	71,9	73,0	71 9	69,7	41,7	101,1	69,2	56,2	66,7	73,6	61,0
3,4	50,9	28,6	49,1	78,8	73,9	84,6	97,1	71,9	73,0	71,9	69,7	41,7	101,1	69,3	56,2	66,7	73,6	61,0
6,0	51,0	28,7	49,1	78,9	74,1	84,7	97,2	72,0	73,1	72,0	69,8	41,8	101,1	69,4	56,2	66,8	73,6	61,0
7,4	51,1	30,1	49,1	80,3	74,2	85,3	97,4	72,0	73,1	72,0	69 6	41,6	101,0	69,5	56,1	66,9	73,5	61,0
7,5	51,1	30,8	49,2	81,1	74,3	85,5	97,6	72,0	73,1	72,0	69,6	41,6	100,9	69,6	56,1	67,1	73,5	61,0
7,8	51,3	31,8	49,4	82,1	74,5	86,0	98,0	72,0	73,2	72,0	69,7	41,6	100,9	70,0	56,1	67,4	73,4	61,0
8,4	51,3	32,6	49,4	83,0	74,6	86,4	98,2	72,0	73,2	72,0	69,8	41,6	100,9	70,5	56,0	68,0	73,3	61,1
8,5	51,3	32,8	49,4	83,1	74,6	86,5	98,3	72,1	73,3	72,0	69,8	41,6	100,8	70,6	55,8	68,1	73,3	61,1
9,0	51,3	34,0	49,7	84,4	74,8	87,2	98,8	72,4	73,4	72,0	70,3	41,8	100,8	71,5	55,8	69,0	73,3	61,1
9,4	51,3	34,9	49,7	85,3	74,8	87,7	99,1	72,4	73,5	72,0	70,8	41,8	100,8	71,9	55,6	69,6	73,3	61,3
10,0	51,3	35,8	49,7	86,8	74,9	88,2	99,6	72,5	73,7	72,0	71,9	42,1	100,7	72,5	55,4	70,1	73,2	61,4
Δδ	0,5	7,4	0,8	8,0	1,3	3,7	2,6	0,7	0,7	0,2	2,3	0,5	−0,4	3,3	−0,8	3,5	−0,4	0,4

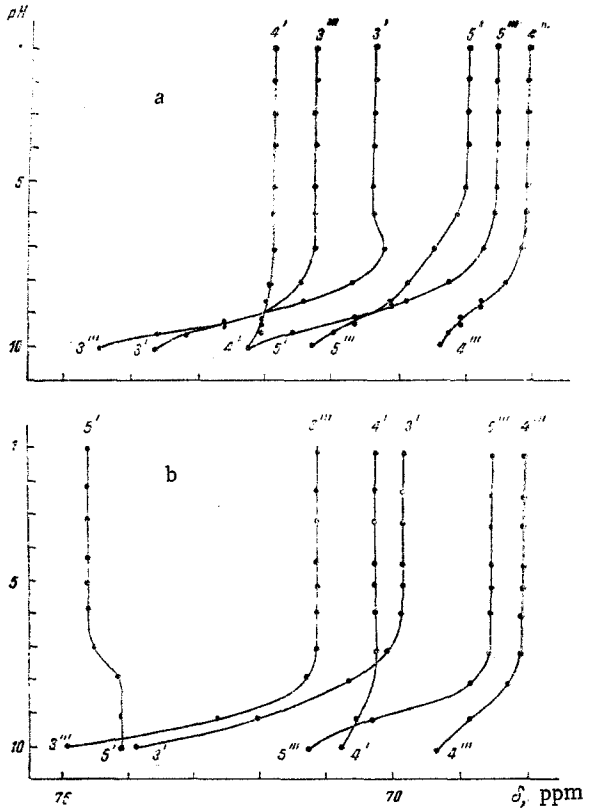


Fig. 1. Dependence of the chemical shifts of the ^{13}C NMR signal of neomycin B (a) and monomycin A (b) on the pH of the solutions.

With a rise in the pH of the solution, nine of the resonance signals of neomycin B and eight of those of monomycin A shifted downfield by 2.3-7.1 ppm (see Tables 1 and 2) and, consequently, relate to carbon atoms present in β positions to amino groups. The facts given permit the assignment of the signal of the C-4, C-5, and C-3" carbon atoms to be refined. The signal of the C-4 carbon atom, which is located in the β position to an amino group, should undergo a considerable downfield shift with the alkalinization of the solution (Δδ 6.5 ppm) in comparison with the signal of the C-5 carbon atom (Δδ 0.8 ppm), which is in the γ position to two amino groups, and the C-3" carbon atom of the riboside fragment, which has no amino group (Δδ 0.0 ppm).

The difference in the α-D-glucopyranoside fragments of neomycin B and monomycin A is shown in the ^{13}C NMR spectra of these antibiotics. A comparison of the spectra at the same pH values and an analysis of the Δδ values permits a clear differentiation of the signals of the α-D-glucopyranoside and β-L-idopyranoside carbon atoms C-4' and C-4"', C-5' and C-5"', and C-6' and C-C"' to be made. The replacement of the protonated amino group at C-6' of the 2,6-diamino-2,6-dideoxy-α-D-glycopyranoside moiety of neomycin B by a hydroxy radical in the monomycin molecule should, by analogy with a comparison of the CSs of the carbon atoms of amino sugars with nonamino sugars [1], lead to a downfield displacement of the signals of the α- and β-carbon atoms by approximately 20 and 3 ppm, respectively. The experimental results for solutions at pH 5.2 (Fig. 2 and Tables 1 and 2) show that the passage from neomycin B to monomycin A is accompanied by considerable changes (of 1.6 ppm and more) in the CSs of the signals of four carbon atoms. The resonance signal shifted upfield by 19.8 ppm is assigned to the C-6' carbon, while the C-6"' signal of the β-L-idopyranoside fragment does not change its position in the spectra of these antibiotics. The signals of the C-6' and C-6"' carbon atoms, which overlap one another in the spectra of solutions of neomycin B up to pH 9.7, separate with further alkalinization of the solutions. A comparison of the CS values of the C-6' and C-6"' carbon atoms of neomycin B and the C-6"' carbon atom of monomycin A at pH 10 (see Tables 1 and 2) permits the assignment of the signal of neomycin B at δ 42.2 ppm to the C-6' carbon atom and the signal in a stronger field (δ 41.9 ppm) to C-6"'. Similarly,

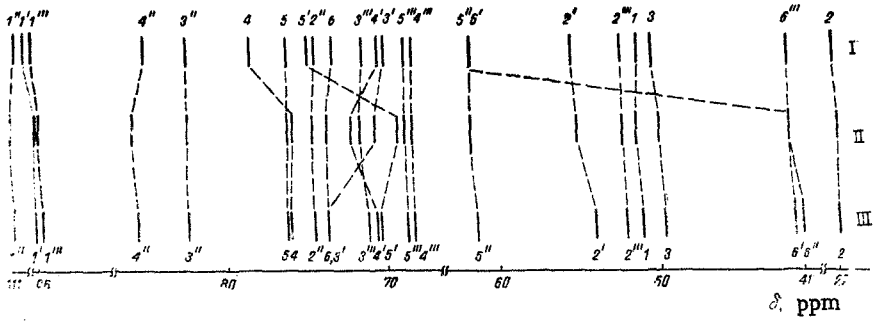


Fig. 2. Diagram of the chemical shifts of the ^{13}C NMR spectra of acid solutions of monomycin A (I), neomycin B (II), and neomycin 3'-phosphate (III).

by comparing the spectra of neomycin B and monomycin A at pH 10.0 it is possible to make an assignment of the individual signals of the spectrum of monomycin A to the C-5'' and C-6' carbon atoms (see Table 2); at pH > 9.1, the signals of these atoms are superimposed on one another.

Of the two signals that shift downfield by 5.6 and 2.6 ppm on passing from neomycin B to monomycin A (see Fig. 2), the first is assigned to the C-5' carbon atom and the second to C-4. The assignment made is confirmed by a downfield displacement by Δδ 3.2 ppm of the signal of the resonance of the C-5' carbon atom of neomycin B with a rise in the pH and the retention of the position of the signal of this carbon atom in the spectrum of monomycin A in the pH range studied (Δδ −0.5 ppm). The chemical shifts of the corresponding C-5''' carbon atoms in β-L-idopyranosides have practically the same values in the ^{13}C NMR spectra of neomycin B and monomycin A and similar tendencies to changes in the shifts with a change in the pH (see Fig. 1). The signals of the C-4 atoms of the 2-deoxystreptamine fragments of neomycin B and monomycin A, which are in each case in the β position to an amino group, demonstrate a clear dependence of the increase in the CS on a rise in the pH of the solution that is similar to what has been observed previously for neamine [5]. The fourth signal, changing its position in the spectrum by 1.6 ppm with the replacement of the amino group at the C-6' carbon atom by a hydroxy group (see Fig. 2), is assigned to the C-4'. The assignment of the C-4' resonance signal and its differentiation from C-4''', C-3', and C-3''' signals is confirmed by the way in which the CSs of the signals depend on the pH (see Tables 1 and 2 and Fig. 1) and by the ^{13}C NMR spectral characteristics of neomycin 3'-phosphate (see Table 1). The ways in which the CSs of the signals of the C-3', C-3''', C-4', and C'4''' carbons depend on the pH show considerable downfield shifts of the signals of the C-3' and C-3''' carbon atoms, which are present in the β positions to an amino group, in comparison with the C-4' and C-4''' signals, which are located in the γ positions. While the signals of the C-3''' and C-4''' carbon atoms in the β-L-idopyranoside moiety scarcely change their positions in the spectra of neomycin B and monomycin A at the present pH value, the signals of the corresponding carbon atoms of the α-D-glucopyranoside fragment in the spectra of monomycin A are recorded in a weaker field, reflecting the influence of the replacement of an amino group of neomycin B by a hydroxyl.

The introduction of a phosphate group at the C-3' carbon atom of α-D-glucopyranoside of neamine is reflected in a shift of the doublet resonance signal of the C-3' carbon atom ($J^2_{^{13}C\ldots{}^{31}P}$ = 5.0 Hz) and of the singlet C-5' signals downfield by 3.3 and 0.7 ppm, and of the signals of the C-2' and C-4' carbon atoms ($^3J_{^{13}C\ldots{}^{31}P}$ = 3.2 Hz) by 0.2 and 0.5 ppm [6]. This characteristic behavior of the changes of the CSs of the ^{13}C NMR signals permits a complete assignment to be made of the resonance signals of the carbon atoms of neomycin B 3'-phosphate (see Table 1 and Fig. 1), thereby confirming the correctness of the assignment of the resonance signals of the carbon atoms of the α-D-glucopyranoside and β-L-idopyranoside fragments of neomycin A. A comparison of the ^{13}C NMR spectra of neomycin B and of neomycin B 3'-phosphate at pH 5.2 shows considerable changes (by 1.0 ppm and more) in the positions of four signals. Three doublet signals, one of which is shifted downfield by 3.1 ppm and the other two upfield by 1.0 and 1.5 ppm, relate to carbon atoms C-3', C-2', and C-4', respectively. The resonance signals of the C-3''', C-2''', anc C-4''', carbon atoms in the β-L-idopyranoside fragment, having no phosphate substituent, scarcely change their positions in the spectrum. The fourth, singlet, signal, which is shifted upfield in the spectrum of neomycin B 3'-phosphate, is assigned to the C-5' carbon atom.

In contrast to the spectra of neomycin B, the resonance signals of the C-6' and C-6''' carbon atoms of neomycin B 3'-phosphate are readily separated even in an acid medium (pH 5.2, see Table 1 and Fig. 2). A comparison of the chemical shifts of the C-6' signals of the α-D-glucopyranoside fragments of neamine (δ 41.5 ppm*) and of neamine 3'-phosphate (δ 41.5 ppm*) in acid solutions (pH $\leqslant$ 6.0) [5, 6] shows that the introduction of a phosphate group at the C-3' carbon does not affect the position of this signal in the NMR spectrum. In view of this, the weaker-field signal of the spectra of neomycin B 3'-phosphate with a shift of δ 41.4 ppm can be assigned to the C-6' carbon atom of the α-D-glucopyranoside 3'-phosphate fragment, while the signal in the stronger field at δ 40.9 ppm can be assigned to the C-6''' atom of the β-L-idopyranoside fragment. It must be mentioned that the assignment made implies a greater influence of the phosphate substituent on the chemical shift of the C-6''' carbon atom in the remote fragment, while the C-6' shift of the fragment including the substituting group does not change. This phenomenon does not agree with the usual idea of the short-range effects of substituent, but can be explained by spatial intra- or intermolecular interactions of the phosphate substituent with the functional groups of the β-L-idopyranoside residue.

The assignment of two signals in the weak field of the spectrum of neomycin B to the C-1' and C-1''' carbon atoms can be made by comparing the CS values of the signals being analyzed with the shifts of the anomeric carbon atoms of model compounds — neamine and methyl neobiosaminide in alkaline solutions at pH $\geqslant$ 11.0 [3]. The resonance signal of the C-1' carbon atom of neamine (δ 101.9 ppm) is present in a weaker field than the signal of the anomeric carbon atom of the β-L-idopyranoside residue of methyl neobiosaminide (δ 99.5 ppm). On the basis of this fact, the signal present in the weaker field in the spectrum of neomycin B can be assigned to the C-1' carbon atom and that in the stronger field to the C-1'' carbon atom (see Table 1 and Fig. 2). The slight divergence in the values of the CSs of the resonance signals under discussion from those given in the literature is due to a difference in the pH values of the solutions analyzed. It has been shown experimentally (see Tables 1 and 2 and Fig. 1) that the positions of the absorption signals of β-carbon atoms in relation to an amino group change most strongly at high pH values.

The correctness of the assignment of the resonance signals to the corresponding carbon atoms of the α-D-glucopyranoside and β-L-idopyranoside fragments was confirmed by a measurement of the ^{13}C NMR spectrum of solutions of an artificial mixture of neomycin B with neamine in a molar ratio of 2:1 at various pH values (from 1.8 to 10.0). In the spectrum of an alkaline solution of the mixture, at pH 10.0, a clear arrangement in pairs of the absorption signals of the C-2' and C-6' carbon atoms of the α-D-glucopyranoside fragments of neamine and neomycin B is observed, which is shown in an increase in the relative intensities of these signals. The corresponding C-2''' and C-6''' signals of the β-L-idopyramoside fragment of neomycin B do not overlap the signals of the neamine carbon atoms. A partial arrangement of the resonance signals of the C-4' and C-5' carbon atoms in pairs is observed in the spectrum of a solution of a mixture of neomycin B and neamine at pH 1.8. In the spectra of solutions of neamycin B and of the artificial mixture at pH 10.0 the signals of the C-4' and C-5' carbon atoms have identical CSs, which complicates their relative identification. The resonance signals of the corresponding C-4''' and C-5''' carbon atoms of the β-L-idopyranoside moiety of neomycin B do not overlap with the signals of the neamine, which confirms the correctness of the assignments made. No complete coincidence of the absorption signals of the C-1' and C-3' carbon atoms was observed for neomycin B and neamine over the whole pH range, although the signals of the α-D-glucopyranoside fragment of neamine were located in the immediate vicinity of the corresponding signals of the carbon atoms of neomycin B and could be unambiguously distinguished from the signals of the C-1''' and C-3''' carbon atoms of the β-L-idopyranoside fragment of neomycin B.

The assignment of the other resonance signals of the ^{13}C NMR spectra of neomycin B and monomycin A (see Tables 1 and 2 and Figs. 1 and 2) coincides completely with that given previously [1-3], even when account is taken of the measured displacements of the individual signals with a change in the pH of the solutions from 1.0 to 10.0.

The dependence of the CSs of the absorption signals of individual carbon atoms in the ^{13}C NMR spectra of kanamycin A on the pH (see Table 3) coincides completely with the assign-

*To compare the values of the CSs with those published previously for neamine [5] and neamine 3'-phosphate [6] a correction of 1.1 ppm must be made because of the different CS values of the standard — dioxane. In [5, 6], a value of δ_{do} of 66.3 ppm was used.

ment given previously [4]. The considerable downfield shift of the signal of the anomeric C-1' carbon atom of the 6-amino-6-deoxy-α-D-glucopyranoside moiety of kanamycin A on alkalinization (Δδ 2.6 ppm), in contrast to the C-1" signal of the 3-amino-3-deoxyglucopyranoside fragment (Δδ -0.4 ppm), permits the signals of these carbon atoms to be distinguished in the spectra. A displacement of the signal of the C-1' carbon, which has no amino groups in β positions, was observed previously [7] and was explained by the 4-R-1'-R-axial absolute configuration of the anomeric center, in contrast to the 6-S-1"-R-axial configurations for which only a slight upfield displacement of the signal of the anomeric carbon atom of alkalinization has been reported.

The analysis that has been given of the ^{13}C NMR spectra of aqueous solutions of the antibiotics under investigation for a wide pH range (see Tables 1-3 and Figs. 1 and 2) shows that the most complete separation of the signals of the carbon atoms of neomycin B and monomycin A is observed at pH values between 1.0 and 7.0, and for kanamycin A over the narrower range of 9.0-9.5. A separation of the absorption signals of the C-6' and C-6"' carbon atoms of neomycin B and the C-6' and C-5" carbon atoms of monomycin A is observed in strongly alkaline solutions pH ≥ 10.0. Thus, the optimum conditions for recording the ^{13}C NMR spectra of neomycin B and monomycin A are pH 1.0-7.0 and 10.0, and for kanamycin A pH 9.0-9.5.

EXPERIMENTAL

The ^{13}C NMR spectra of 25-28% aqueous solutions of the substances under investigation were recorded on a WH-90 pulsed spectrometer (Bruker, 22.62 MHz) 40°C in the regime of complete suppression of the spin–spin coupling of protons with carbon atoms. To stabilize the NMR conditions we used deuterated water sealed into a coaxial glass capillary with a diameter of 2.5 mm. The numerical resolution at a spectral width of 3000 Hz and with the accumulation of the figures in 8 K points of the computer memory was 0.73 Hz per point, which corresponded to an accuracy of the measurement of the chemical shifts of 0.03 ppm. The chemical shifts were measured with respect to an internal standard — dioxane — and were recalculated to the δ scale using a figure for δ_{do} of 67.4 ppm. The acidification and alkalinization of the solutions under investigation were carried out 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 3 and Fig. 1 give the results only for selected pH values. The pH measurements were carried out on a pH-121 instrument. The artificial mixture of neomycin B and neamine contained 800 g of neomycin B sulfate and 130 mg of neamine base, the molar ratio being 2:1.

SUMMARY

1. On the basis of the results of a study of the dependence of the chemical shifts of the signals of the ^{13}C NMR spectra of neomycin B, monomycin A, and kanamycin A over a wide pH range (from 1.0 to 10.0) a complete assignment of the spectral signals of these antibiotics has been made.

2. The optimum pH values of solutions for which the most complete separation of the resonance signals in the spectra of neomycin B, monomycin A, and kanamycin A is observed have been proposed.

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