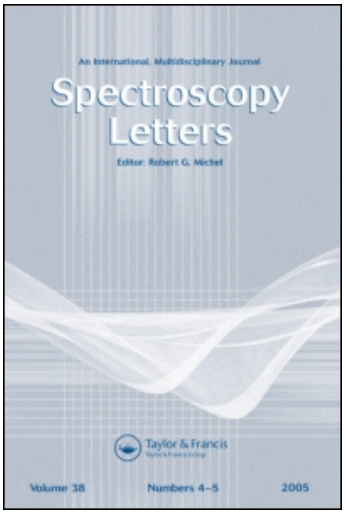


Spectroscopy Letters

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Complete ^{1}H and ^{13}C NMR Chemical Shift Assignments for Some N-Polyformylated Aminoglycoside Antibiotics

Galya I. Eneva[a]; Stefan L. Spassov[a]; Marietta A. Haimova[b]
[a] Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria [b] Faculty of Chemistry, University of Sofia, Sofia, Bulgaria

To cite this Article Eneva, Galya I. , Spassov, Stefan L. and Haimova, Marietta A.(1995) 'Complete ^{1}H and ^{13}C NMR Chemical Shift Assignments for Some N-Polyformylated Aminoglycoside Antibiotics', Spectroscopy Letters, 28: 1, 69 — 79

To link to this Article: DOI: 10.1080/00387019508011655

URL: http://dx.doi.org/10.1080/00387019508011655

SPECTROSCOPY LETTERS, 28(1), 69-79 (1995)

COMPLETE ^{1}H AND ^{13}C NMR CHEMICAL SHIFT ASSIGNMENTS FOR SOME N-POLYFORMYLATED AMINOGLYCOSIDE ANTIBIOTICS

KEY WORDS: Aminoglycoside Antibiotics, ^{1}H NMR, ^{13}C NMR, Formylation shifts.

Galya I. Eneva and Stefan L. Spassov

Institute of Organic Chemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Marietta A. Haimova

Faculty of Chemistry, University of Sofia, 1126 Sofia, Bulgaria

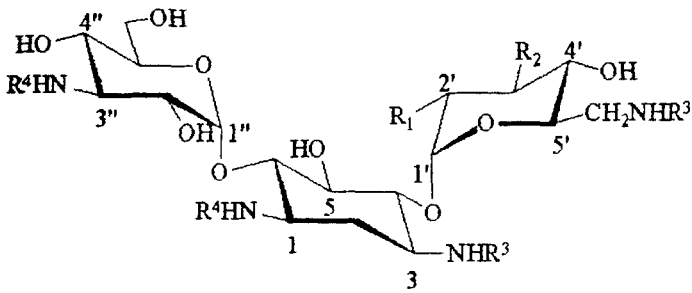
ABSTRACT

The complete ^{1}H and ^{13}C NMR assignments of a series of semisynthetic N-polyformyl derivatives of aminoglycoside antibiotics kanamycin A, kanamycin B, tobramycin, apramycin and sisomicin were achieved by 1D and 2D NMR methods (mainly ^{1}H-^{1}H and ^{1}H-^{13}C shift-correlation spectroscopy).

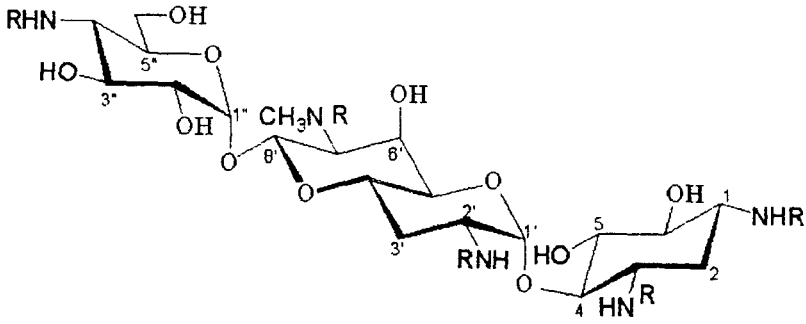
INTRODUCTION

Kanamycin A (**1**), kanamycin B (**2**), tobramycin (**3**), apramycin (**4**) and sisomicin (**5**) - *medicinally important members of the aminoglycoside* family of antibiotics have been studied extensively by ^{1}H and ^{13}C NMR methods [1 - 5].

We reported the complete assignments of the ^{1}H and ^{13}C NMR spectra of antibiotics **1-5** and of a series of their semisynthetic N-polyacetyl- and N,O-polyacetyl derivatives [6]. The aim of this work was to achieve a complete assignments of N-polyformylated derivatives **6-11** of aminoglycoside antobiotics **1-5** .

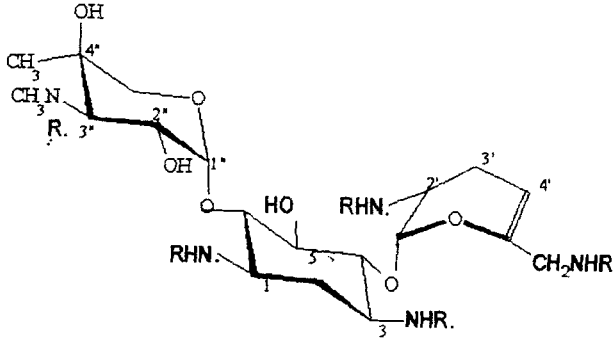


1	Kanamycin A	R^1=R^2=OH; R^3=R^4=H
2	Kanamycin B	R^1=NH_2; R^2=OH; R^3=R^4=H
3	Tobramycin	R^1=NH_2; R^2=R^3=R^4=H
6	Tetra-N-formylkanamycin A	R^1=R^2=OH; R^3=R^4=CHO
7	Penta-N-formylkanamycin B	R^1=NHCHO; R^2=OH; R^3=R^4=CHO
8	Tetra-N-formylkanamycin B	R^1=NHCHO; R^2=OH; R^3=CHO;R^4=H
9	Penta-N-formyltobramycin	R^1=NHCHO; R^2=H; R^3=R^4=CHO



4 Apramycin R=H

10 Penta-N-formylapramycin R=CHO



5 Sisomicin R=H

11 Penta-N-formylsisomicin R=CHO

EXPERIMENTAL

NMR Spectra

The NMR spectra were obtained on a Varian XL-300 spectrometer operating at 299.94 and 75.43 MHz for ^{1}H and ^{13}C, respectively, using 0.05-0.1 M solutions in D_2O. The proton chemical shifts are referenced to internal sodium 4,4-dimethyl-4-silapentane-1-sulphonate, and external tetramethylsilane (TMS) was used for the ^{13}C chemical shifts. The measurements were carried out at ambient temperatute (ca 300 K) and pH 5-7 for the formyl derivatives **6-11**. The 2D NMR spectra were obtained using the standard Varian software: COSY, phase-sensitive COSY (COSYPS) and phase-sensitive double-quantum filtered COSY (DQ COSY) for the ^{1}H-^{1}H correlations, HETCOR for the ^{1}H-^{13}C correlations.

Typical conditions for the 1D ^{1}H spectra were: pulse width 32^{o}, FT size 32K and digital resolution 0.2 Hz per point, and for the 1D ^{13}C spectra pulse width 60^{o}, FT size 64K and digital resolution 0.5 Hz per point. The COSY experiments were typically performed with a spectral width of ca 1500 Hz, relaxation delay 1.3 s, mixing pulse width 45 or 60^{o}, number of increments 256 or 512 and FT size 1K x 1K. The HETCOR experiments were carried out with a spectral width of ca 6000 Hz for ^{13}C and ca 1500 Hz for ^{1}H, relaxation delay 1.4 s, number of increments 128, FT size 4K x 256W, H-H decoupled multiplets.

Compounds

Samples of kanamycin A (**1**), kanamycin B (**2**), tobramycin (**3**) and sisomicin (**5**) were obtained commercially as sulphates and converted into the free bases via ion - exchange chromatography [8]. Apramycin (**4**) was isolated from the nebramycin complex (Antibiotics Factory, Razgrad, Bulgaria) and purified by ion-exchange chromatography [9].

The N-formylated compounds **6-11** were prepared by the method described for N-formylation of kanamycin A and B [7]. The compounds **6-11** were purified by ion-exchange chromatography (Amberlite CG-50, H^{+}-

or NH_4^+-form). The purities of the compounds were checked by thin-layer chromatography: Silica gel G-60, mobile phase - aceton : ethanol : acetic acid : water (1:1:0.5:0.5). Their structures were verified by ^1H and ^{13}C NMR studies. Compound **6**, m.p. 188-190°C (decomp.), [α]^{20}D +110° (c 0.5, H_2O), R_f 0.69 ; **7**, m.p. 197-200°C (decomp), [α]^{20}D +118° (c 0.5, H_2O), R_f 0.72 ; **8**, m.p. 195-198°C (decomp.), [α]^{20}D +102.7° (c 0.5, H_2O), R_f 0.57: **9**, m.p. 198-201°C (decomp.), [α]^{20}D +102.5° (c 0.5, H_2O), R_f 0.78; **10**, m.p. 258-261°C (decomp.), [α]^{20}D 0° (c 0.5, H_2O), R_f 0.50; **11**, m.p. 195-197°C (decomp.), [α]^{20}D +120.9° (c 0.5, H_2O), R_f 0.82.

RESULTS AND DISCUSSION

The ^1H and ^{13}C chemical shifts obtained for the N-polyformylated derivatives **6-11** are presented in Tables 1 and 2. For comparison purposes, data for the parent compounds **1-5** taken from Ref. 6 are also included. An additional complication in the cases of formyl derivatives **6-11** was the observation of signals for more than one rotamer due to hindered rotation about the C-N bonds at ambient temperature, as already reported for some aminoglycoside antibiotics and their acetyl derivatives [6]. Only the signals belonging to the major rotamer (amouting to ca. 70% in the various compounds) are reported.

^{13}C chemical shifts

The ^{13}C-NMR chemical shifts of compounds **1-11** are presented in Table 1. The ^{13}C-NMR chemical shifts for antibiotics **1-5** are in agreement with the data from our earlier paper [6] and with the literature data [2 - 5]. As can be seen from Table 1, the N-formylation of **1-5** in most cases causes upfield shifts of the ^{13}C signals. The shift is more pronounced for the carbons in a β-position to the amino groups, in agreement with earlier observations [6]. The strongest shifts (4.5-7.5 ppm) are observed for the deoxystreptamine carbons participating in the glycosidic bonds (C-4 and C-6). Anomeric carbons with β-amino groups

TABLE 1.

^{13}C NMR Chemical Shifts (ppm from TMS) for Compounds **1-11**.

C	**1**	**6**	**2**	**7**	**8**	**3**	**9**	**4**	**10**	**5**	**11**
1	51.3	48.6	50.6	48.4	50.7	50.4	48.4	50.2	48.7	50.9	48.8
2	36.3	33.5	35.4	33.5	34.8	35.5	33.5	35.4	31.9	35.3	33.2
3	49.8	47.5	49.4	47.4	47.3	49.2	47.5	49.3	47.7	49.3	46.8
4	88.2	82.8	86.1	80.7	80.9	86.5	80.7	86.7	82.3	84.3	79.8
5	74.9	75.9	74.6	76.0	76.0	74.5	76.1	75.9	77.0	74.5	75.8
6	88.6	81.1	88.0	81.1	88.2	88.1	81.1	77.3	74.9	86.9	80.5
1'	100.3	99.1	100.1	98.4	98.4	99.7	97.0	100.6	98.0	99.9	96.9
2'	72.7	73.3	55.4	52.8	52.9	49.4	47.1	48.9	46.9	46.5	44.9
3'	73.8	73.0	73.6	71.7	71.7	34.9	32.3	31.8	29.6	24.6	21.2
4'	71.9	72.5	71.5	71.2	71.2	66.2	65.8	67.1	67.2	94.4	97.7
5'	73.8	71.3	72.3	71.5	71.4	73.7	72.0	70.1	70.0	148.9	145.8
6'	42.4	38.9	41.6	38.8	39.0	41.6	38.9	65.3	70.0	42.3	40.2
7'								61.4	60.9		
8'								95.5	97.2		
1"	100.8	99.1	100.1	98.7	98.7	100.0	98.5	94.5	94.8	100.6	99.7
2"	72.7	70.2	71.9	70.1	70.1	71.8	70.1	70.8	71.3	69.3	64.2
3"	55.0	53.6	54.4	53.3	53.6	54.2	53.4	73.3	69.4	63.3	53.9
4"	70.2	68.0	69.3	68.0	67.9	69.3	68.0	52.2	52.5	72.3	73.8
5"	72.9	71.3	72.3	73.0	72.9	72.1	73.1	72.5	68.9	67.7	69.3
6"	61.2	60.9	60.4	60.9	60.7	60.3	60.8	60.9	63.3	21.6	22.1
7"								32.0	33.2	36.9	33.0

TABLE 2.
^{1}H NMR Chemical Shifts (ppm from TMS) of Compounds **1-11**.

H	**1**	**6**	**2**	**7**	**8**	**3**	**9**	**4**	**10**	**5**	**11**
1	2.90	4.05	2.86	4.05	2.90	2.90	4.10	2.74	4.00	2.80	4.10
2ax	1.22	1.65	1.22	1.65	1.45	1.23	1.70	1.23	1.70	1.18	1.65
2eq	1.96	2.05	1.94	2.05	2.00	1.96	2.05	2.00	2.05	1.95	2.10
3	2.89	4.05	2.87	4.05	4.00	2.90	4.10	2.87	3.90	2.76	3.95
4	3.33	3.53	3.32	3.55	3.50	3.33	3.50	3.31	3.60	3.44	3.60
5	3.66	3.81	3.65	3.75	3.75	3.63	3.80	3.49	3.60	3.55	3.70
6	3.25	3.63	3.24	3.61	3.25	3.25	3.65	3.16	3.40	3.25	3.60
1'	5.33	5.34	5.35	5.38	5.33	5.16	5.27	5.16	5.20	5.35	5.43
2'	3.59	3.50	2.77	4.00	4.00	2.90	4.10	3.02	4.10	3.07	4.12
3'ax	3.70	3.69	3.56	3.70	3.65	1.61	1.70	1.67	1.80	1.95	2.00
3'eq						2.03	2.05	2.12	2.10	2.20	2.10
4'	3.31	3.28	3.30	3.38	3.25	3.54	3.55	3.78	3.85	4.88	4.85
5'	3.75	3.70	3.75	3.70	3.70	3.58		3.67	3.50		
6'	2.78	3.50	2.87	3.50	3.50	2.73	3.50	4.28	4.30	3.17	3.82
	3.00		3.07			2.97					
7'								2.68	3.65		
8'								4.92	5.40		
1"	5.04	5.17	5.03	5.20	5.16	5.05	5.30	5.37	5.40	5.07	5.27
2"	3.50	3.55	3.49	3.53	3.54	3.51	3.58	3.59	3.60	3.80	4.20
3"	3.01	4.12	2.99	4.14	4.00	3.01	4.14	3.65	3.80	2.78	4.40
4"	3.33	3.56	3.32	3.51	3.52	3.33	3.50	2.74	3.70		
5"	3.92	4.07	3.90	4.05	4.00	3.92	4.05	3.65	3.80	3.31	3.35
										4.05	4.15
6"	3.75	3.75	3.75	3.79	3.75	3.77	3.78	3.72		1.20	1.10
								3.85			
7"								2.38	3.03	2.52	2.96
											3.11

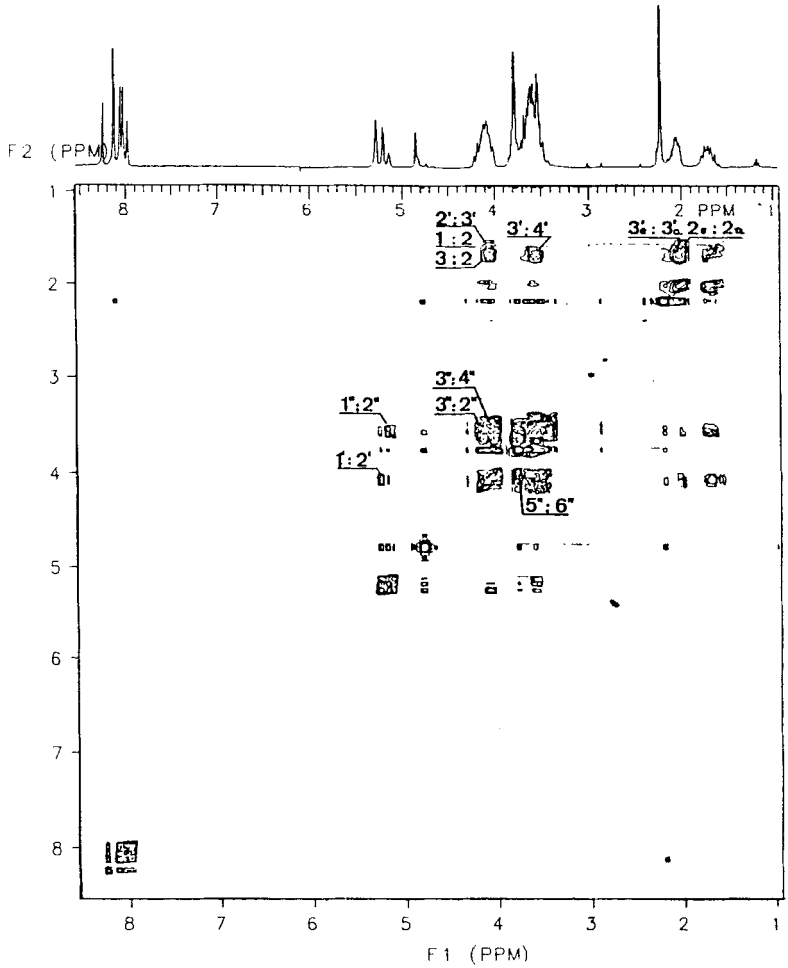


FIG. 1. 300 MHz 2D ^1H-^1H Correlation Spectrum (COSY) of Penta-N-formyltobramycin (**9**) in D_2O with Indication for Some of the Cross-peaks.

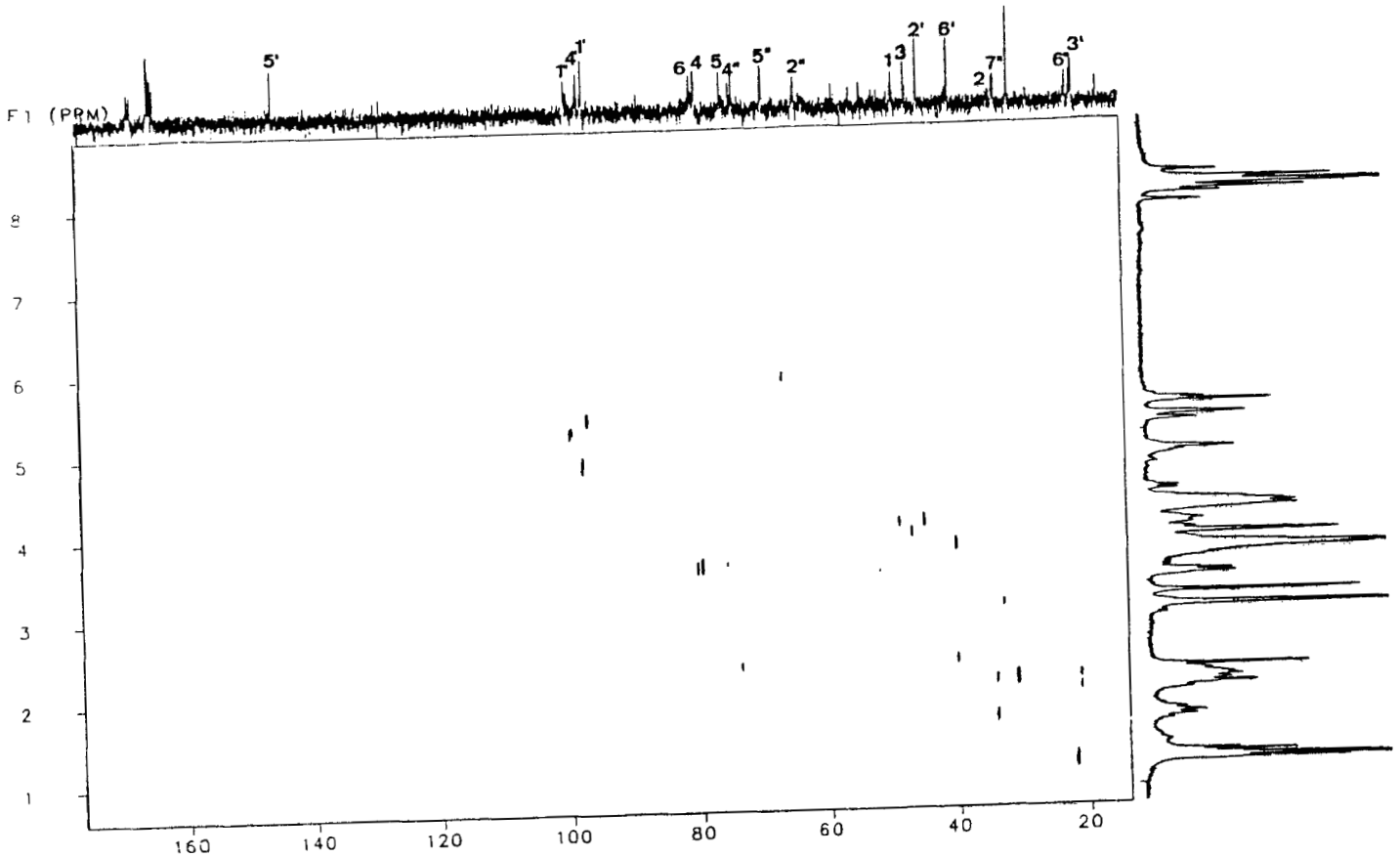


FIG. 2. 300/75 MHz 2D ^{1}H-^{13}C Correlation Spectrum of Penta-N-formylsisomicin (**11**) in D_2O.

are shifted upfield by 1.7 - 3 ppm, whereas C-2 is shifted by 2.0 - 2.8 ppm. The formylation effect on the α-carbons is less pronounced (0.8-3.5 ppm). It is strongest (2.7-3.5 ppm) for the methylene carbons, weaker (1.7 - 2.7 ppm) for carbons adjacent to a CH_2 and a C-OH group and weakest (0.8 - 1.4 ppm) for carbons between two C-OH groups. The data for tetra-N-formyl-kanamycin A (**6**) are in very good agreement with those published by Horii et al. [7] for some carbons of **6**.

^1H chemical shifts

The results of the analyses of the proton spectra of compounds **1-11** are presented in Table 2. The spectral assignment and multiplet analysis were based primarily on the ^1H-^1H 2D corelation spectra (COSY, FIG. 1) in the normal, phase-sensitive and double-quantum filtered versions. In the cases when the ^{13}C assignments were known (**1-6**) ^1H-^{13}C 2D heterocorrelated spectra (FIG. 2) were also helpful.

The N-formylation of **1-5** causes deshielding of the protons attached to α-carbons by 1.1 - 1.2 ppm for the CH, and by 0.4 - 0.6 ppm for the CH_2. Unusually large deshielding (1.6 ppm) was observed for H-3" of **10** (assignment also conformed via selective decoupling). A reliable determination of coupling constants was not possible due to the superposition of signals of the rotamers.

CONCLUSION

The results from the present investigation showed that the effect of N-formylation on the ^1H and ^{13}C chemical shifts of aminoglycoside antibiotics **1-5** exhibits qualitatively similar trends as in the case of the N-acetylated derivatives. However, the magnitude of the α-shifts is larger for the formyl derivatives as compared to the acetylated compounds, whereas in the case of the β-shifts the opposite is generaly true, although in the latter case the effects are less systematic.

REFERENCES

1. Koch K. F., Merkel K. E., O'Connor S.C., Occolowitz J. L., Paschal J. W. and Dorman D. E. Structures of Some of the Minor Aminoglycoside Factors of the Nebramycin Fermentation. J. Org. Chem. 1978; 43: 1430-1434.
2. Szilagyi L. Assignments of the ^{1}H- and ^{13}C- NMR Spectra of Tobramycin at Low and High pH. Carbohydr. Res. 1987; 170: 1-17.
3. Szilagyi L. and Pusztahelyi Z. Sz. Apramycin: Complete ^{1}H and ^{13}C NMR Assignments and Study of the Solution Conformation by ROESY Measurements. Magn. Res. Chem. 1992; 30: 107-117.
4. Chernyshev A. I., Kartashov, Arzamastsev A. P. and Esipov S. E. NMR Spectroscopy in Pharmaceutical Analysis. III. ^{13}C- NMR Spectroscopy of Aminoglycoside Antibiotics Kanamycin and Amicacin in Acidic and Basic Medium. Khim. Pharm. Zh. 1990; 24 (4): 84-86.
5. Chernyshev A. I., Kartashov V. S., Shorshnev S. V., Arzamastsev A. P. and Esipov S. E. NMR Spectroscopy in Pharmaceutical Analysis. IV. ^{13}C-NMR Spectroscopy of Aminoglycoside Antibiotics Sisomicin and Gentamycin in Acidic and Basic Medium. Khim. Pharm. Zh. 1990; 24 (5): 80-82.
6. Eneva G., Spassov S., Haimova M. and Sandstrom J. Complete ^{1}H and ^{13}C NMR Assignments for Apramycin, Sisomicin and Some N- and N,O-Polyacetylated Aminoglycosides. Magn. Res. Chem. 1992; 30: 841-846.
7. Horii S., Fukase H., Kameda Y. and Mizokami N. A New Method for Selective N-Acetylation of Aminoglycoside Antibiotics. Carbohydrate Research 1978; 60: 275-288.
8. Claes P., Vanderhaeghe H. Preparation of Octa-N-methyl- and Tetra-N-methylkanamycin. Bull. Soc. Chim. Belg. 1969; 78: 561-569.
9. Koch K. F., Davis F. A. and Rhoades J. A. Nebramycin: Separation of the Complex and Identification of Factors 4, 5, and 5'. J. Antibiot. 1973; 26:745- 751.

Date Received: July 29,1994
Date Accepted: September 13, 1994