Carbon-13 Nuclear Magnetic Resonance Spectral Analysis of Tobramycin and Related Antibiotics[1]

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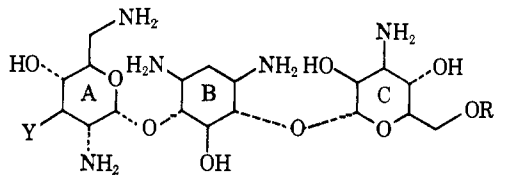
Abstract: The natural abundance ^{13}C nmr spectra of the antibiotics tobramycin, kanamycin B, and their carbamates were recorded at several pHs. The chemical shifts of the four antibiotics, of nebramine, neamine, deoxystreptamine, and of the methyl 2-amino-, 3-amino-, and 6-amino-α-D-glucopyranosides, were assigned and the conformation of all compounds in water solution was determined.

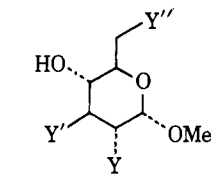
A mixture of aminoglycoside antibiotics[3] whose main components are nebramycin factors 2, 4, and 5′ is produced by *Streptomyces tenebrarius*. These substances have been shown to be apramycin[4] and carbamates[5] of kanamycin B (**1c**)[6] and of tobramycin (**1a**).[7]



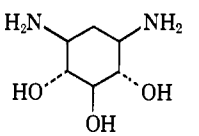
1a, R = Y = H
b, R = $CONH_2$; Y = H
c, R = H; Y = OH
d, R = $CONH_2$ Y = OH

A ^{13}C nmr spectral analysis of the carbamates and their hydrolysis products now was undertaken in order to discern the position of the carbamyl moiety and to lay the groundwork for structure analysis of aminoglycoside antibiotics by this new, diagnostically powerful research tool.[8]

Natural abundance ^{13}C nmr spectra of 0.3–0.5 *M* aqueous solutions of componds **1** and others (*vide infra*),[9] containing 5% dioxane as internal reference, were recorded on a Fourier transform spectrometer operating at 15.08 MHz.[10] Proton resonance decoupled spectra were sufficient for the ^{13}C chemical shift assignments indicated in Table I. The spectral analysis of the tricyclic compounds was aided by the spectra of the amino monosaccharides methyl 2-amino-2-deoxy-α-D-glucopyranoside (**2a**), methyl 3-amino-3-deoxy-α-D-glucopyranoside (**2b**) and methyl 6-amino-6-deoxy-α-D-glucopyranoside (**2c**), of the aminocyclitol 2-deoxystreptamine (**3**) and of the bicyclic substances nebramine (**4a**) and neamine (**4b**) as well as by known cmr data.[11]



2a, Y = NH_2, Y′ = Y″ = OH
b, Y = Y″ = OH; Y′ = NH_2
c, Y = Y′ = OH; Y″ = NH_2



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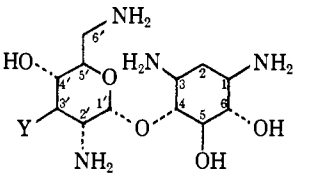


4b, Y = H
a, Y = OH

Chemical shift assignment of the aminoglucoside models **2** for rings A and C of the antibiotics are based on the shifts of methyl α-D-glucopyranoside[11] and the known effect of replacement of an equatorial hydroxyl group by an amino function on the substituent carbon (a *ca.* 18-ppm upfield shift) and on its immediate neighbors (an up to ± 1 ppm shift).[12] Thus comparison of the spectra of the 2-aminoglucoside **2a** and methyl α-glucoside[13] reveals the introduction of the amino group to deshield C-1′ and C-3′ by 0.7 and 1.0 ppm, respectively, and to shield C-2′ by 16.5 ppm. Similarly, the spectrum of the 3-aminoglucoside **2b** exhibits minor shifts of C-2″ and C-4″ but a 19.0 ppm upfield shift

(1) Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances. XXV. For the preceding paper, see N. J. Bach, H. E. Boaz, E. C. Kornfeld, C.-J. Chang, H. G. Floss, E. W. Hagaman, and E. Wenkert, *J. Org. Chem.*, **39**, 1272 (1974).

(2) (a) Eli Lilly and Company; (b) Indiana University.

(3) R. Q. Thompson and E. A. Presti, *Antimicrob. Ag. Chemother.*, 332 (1967); W. E. Wick and J. S. Welles, *ibid.*, 341 (1967); W. M. Stark, N. G. Knox, and R. M. Wilgus, *Folia Microbiol.*, **16**, 205 (1971).

(4) S. O'Connor and L. K. T. Lam, Abstracts, 165th National Meeting of the American Chemical Society, Dallas, Texas, Apr 1973, MEDI-6.

(5) K. F. Koch, F. A. Davis, and J. A. Rhoades, *J. Antibiot.*, in press.

(6) H. Umezawa, "Index of Antibiotics from Actinomycetes," University of Tokyo Press, Tokyo, Japan, 1967.

(7) K. F. Koch and J. A. Rhoades, *Antimicrob. Ag. Chemother.*, 309 (1970).

(8) For the first, albeit incomplete, cmr study of an aminoglycoside antibiotic, hygromycin B, see N. Neuss, K. F. Koch, B. B. Molloy, W. Day, L. L. Huckstep, D. E. Dorman, and J. D. Roberts, *Helv. Chim. Acta*, **53**, 2314 (1970).

(9) While the solutions were not degassed, no chemical shift changes by amine complexation with absorbed carbon dioxide were observed.

(10) The nmr instrument consisted of a Varian Associates DP-60 magnet working at 14 kG with an external ^{19}F lock, a white-noise generator and adjustable, home-built crystal oscillator for proton decoupling, a Fabri-Tek 1074 time-averaging computer and Digital Electronics Corp. PDP-8/1 computer for signal averaging and Fourier transformation of the free induction decay. The samples were spun in 13-mm o.d. tubes.

(11) (a) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, N. Y., 1972; (b) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972; (c) A. S. Perlin, B. Casu, and H. J. Koch, *Can. J. Chem.*, **48**, 2596 (1970); (d) D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, **92**, 1355 (1970).

(12) E. Conway, R. D. Guthrie, S. D. Gero, G. Lukacs, A.-M. Sepulchre, E. W. Hagaman, and E. Wenkert, *Tetrahedron Lett.*, 4879 (1972).

(13) The values in ref 11d, as corrected by H. J. Koch and A. S. Perlin [*Carbohyd. Res.*, **15**, 403 (1970)] and translated into the TMS scale, were used for this sugar derivative.

Table I. ^{13}C Chemical Shifts[a]

	2a[b]	2b[b]	2c[b]	3	4a	1a	1b	1c	1d	4b
C-1′	99.7		99.0		99.6	99.2	99.8	100.1	100.1	100.7
C-2′	54.9		71.6[c]		49.3[c]	49.5[c]	49.5[c]	55.3	55.3	55.2
C-3′	74.1		73.1		34.9	34.7	35.0	73.4	73.5	73.5
C-4′	69.7		71.2[d]		66.0	65.9	66.2	71.2	71.3	71.2
C-5′	71.7		71.2[c]		73.5	73.1	73.3	72.8	72.8	72.9
C-6′	60.6		41.4		41.6	41.5	41.6	41.6	41.7	41.7
C-1				50.7	50.2	50.2	50.6	50.2	50.4	50.3
C-2				36.3	35.7	35.5	35.5	35.4	35.6	35.6
C-3				50.7	49.0[c]	49.0[c]	49.3[c]	49.2	49.3	49.3
C-4				77.7	86.7	86.0	86.3	86.4	86.7	87.1
C-5				75.8	75.8	74.4	74.1	74.2	74.0	75.8
C-6				77.7	77.5	87.8	87.7	87.7	87.4	77.4
C-1″		98.7				99.1	99.2	99.5	99.5	
C-2″		71.7				71.6	71.6	71.7	71.6	
C-3″		54.1				54.2	54.3	54.2	54.2	
C-4″		69.6				69.2	69.2	69.1	69.2	
C-5″		71.3				71.9	70.1	71.9	70.0	
C-6″		60.6				60.2	63.5	60.2	63.5	
C=O							158.9		158.6	
Me	54.9	54.5	54.8							

[a] Spectra of water solutions at pH 11 or higher recorded in ppm downfield from TMS; $\delta^{TMS} = \delta^{dioxane} + 66.3$ ppm. [b] The prime symbols of the carbons point to their relationship to like carbon sites in compounds **1**. [c] Signals within a vertical column may be reversed. [d] This shift is unambiguous (in contrast to the C-2′ and C-5′ shifts) in view of the signal appearing as a triply intense line in a spectrum of a 1:1 mixture of anomers and in view of the C-4′ shift being known to be impervious to anomeric change.

for C-3″, while the spectrum of the 6-aminoglucoside **2c** shows the deshielding of C-4′ by 1.6 ppm and the shielding of C-6′ by 19.2 ppm. Replacement of a hydroxy group by an amino unit at C-6′ leaves C-5′ unaffected but appears to deshield C-4′ anomalously. This characteristic shift pattern for 6-aminoglucosides reflects a dissimilarity of rotamer populations of the hydroxymethyl and aminomethyl side chains.[14]

The symmetry of 2-deoxystreptamine (**3**), a model for ring B of the antibiotics, leads to a four-line cmr spectrum. The intensity and field position of the signals permit their assignments to specific carbons.

Compounds **2a**, **2c**, and **3** serve as models for the shift analysis of nebramine (**4a**) and neamine (**4b**). The shifts of C-6′, C-1, C-2, and C-6 expectedly are unfazed despite the attachment of two rings to each other.[8] In analogy with the 9–10-ppm shift imposed on the carbon site of attachment of a glycosyl unit in disaccharides[15] C-4 in **4a** and **4b** is deshielded by 9.0 and 9.4 ppm, respectively. Only one aminomethine and oxymethine signal each remaining identical in the spectra of **4a** and **4b** establishes their being associated with C-3 and C-5, respectively. Carbons 1′, 2′, and 3′ of nebramine (**4a**) are unique and their signals are easily recognized, while differentiation of the C-4′ and C-5′ signals is based on an evaluation of the C-4′ shift by subtraction of a β effect from the δ value of C-4′ of model **2c**. The $\Delta\delta$ value of 5.2 ppm for the **2c**–**4a** pair is similar to the 4.7 ppm difference for C-4 of methyl α-D-glucopyranoside and methyl 3-deoxy-α-D-glucopyranoside 4,6-*O*-benzylidene derivatives.[12] Calculation of the expected α and β effects for the introduction of a 3′-hydroxy group into nebramine (**4a**) fixes all chemical shifts of the remaining five carbons of the diaminoglucosyl unit of neamine (**4b**).

The assignment of the carbon shifts of the antibiotic tobramycin (**1a**) relies on the shift data for nebramine (**4a**) and the 3-aminoglucoside **2b**. Thus the δ values of all the ring A carbons and the ring B carbons 2 and 3 of the antibiotic are the same as those of nebramine, while the shifts of all ring C carbons are identical with those of **2b**. The remaining unassigned signals, those of C-1, C-4, C-5, and C-6, are composed of the unique, easily identifiable resonances of an aminomethine (*i.e.*, C-1) and hydroxymethine (*i.e.*, C-5) and two alkoxymethine lines whose unambiguous differentiation depends on the analysis of the effect of pH variation on chemical shift behavior (*vide infra*). All shifts of the antibiotic kanamycin B (**1c**) can be ascertained with the help of the δ values of tobramycin (**1a**) and neamine (**4b**). Since the spectra of the antibiotics **1b** and **1d** differ from those of **1a** and **1c**, respectively, only by the C-5″ and C-6″ shifts and the presence of an extra carbonyl signal, the two sets of antibiotics are distinguished from each other by a dissimilar C-6″ substitution pattern. Furthermore, **1b** and **1d** being C-6″ *O*-carbamyl derivatives of respectively **1a** and **1c** is shown by the 3.3-ppm deshielding of C-6″ on derivatization, reminiscent of the $\Delta\delta$ (acetate − alcohol) value of primary alcohols,[11] and the similarity of the carbonyl shift of the carbamate units and that of ethyl urethane (158.8 ppm). Thus structures **1b** and **1d** are assured.

pH Study

With the chemical shift data for the antibiotics (except for the differentiation of C-4 and C-6) and their individual ring components in hand attention was turned to the effect of pH on the carbon shifts. It could be anticipated that as in the peptide field[16] a pH study of the aminoglycosides would strengthen confidence in the cmr signal assignments. Furthermore, in view of the dissimilarity of orientation of polar groups toward each other in different pH environments a pH study could be envisaged to reveal information on the conformation of the antibiotics in solution.

(14) *Cf.* H. Eggert and C. Djerassi, *J. Amer. Chem. Soc.*, **95**, 3710 (1973).

(15) D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, **93**, 4463 (1971).

(16) F. R. N. Gurd, P. J. Lawson, D. W. Cochran, and E. Wenkert, *J. Biol. Chem.*, **246**, 3725 (1971).

Table II. ^{13}C Chemical Shifts and Shift Differences[a]

	5a		5b		5c		6		7a		7b		8[b]	
	δ	Δδ	δ	Δδ	δ	Δδ	δ	Δδ	δ	Δδ	δ	Δδ	δ	Δδ
C-1	41.0	1.4	40.7	1.1	49.5	−0.9	42.6	0.3	40.7	1.3	40.5	1.5	38.8	2.0
C-2	32.7	6.1	34.7	6.1	35.3	5.0	13.8	3.2	31.8	5.9	29.3	5.5	31.6	9.0[c]
C-3	26.3	0.9	19.4	0.5	24.7	1.1			23.3	0.9			46.2	1.7
C-4	31.4	0.9	13.2	0.4	25.2	1.1								
C-5	22.0	0.5											48.5	1.6
C-6	13.0	0.0											26.4	2.8[c]

[a] δ values are from spectra of water solutions at pH 11 or higher recorded in ppm downfield from TMS; $\delta^{TMS} = \delta^{dioxane} + 66.3$ ppm; $\Delta\delta = \delta(pH > 11) - \delta(pH < 1)$. [b] The numbering system is based on spermine being considered 1,12-diamino-4,9-diazadodecane. [c] Since the δ values of C-2 and C-6 for the salt may be interchanged, the Δδ values may need alteration to 8.0 and 3.8, respectively.

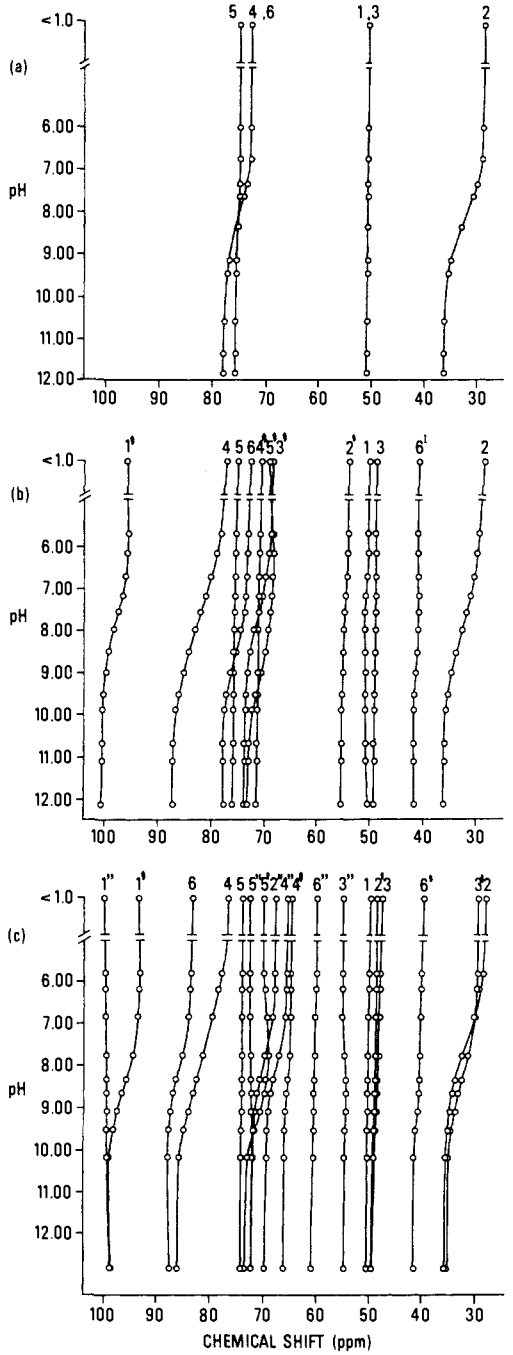


Figure 1. Representative pH profiles of ^{13}C chemical shifts of (a) 2-deoxystreptamine, (b) neamine, and (c) tobramycin.

Several water-soluble amines and diamines were chosen as models for an investigation of the magnitude and sign of the effect of their N-protonation and for a determination of the possible additivity of the protonation parameters.[17] The carbon shifts derived from spectra of water solutions of the amino compounds and their hydrochlorides or of the former at pH <1 are recorded in Table II.[18,19] The data indicate the difference of α-, γ-, and δ-carbon shifts between a primary amine and its protic salt to be small (0.5–1.5 ppm), while, contrastingly, the Δδ value for the β carbon ($\Delta\delta^{\beta}$) is large and hence diagnostically valuable in structure analysis. The $\Delta\delta^{\beta}$ value is dependent on the number of substituents of the amino carbon (*e.g.*, 6 ppm for *n*-hexylamine and 5 ppm for cyclohexylamine) and the β carbon[17] (*e.g.*, 5 ppm for cyclohexylamine and 4 ppm for the aminoglycosides, *vide infra*) and on the number of amine substituents (*e.g.*, 6 ppm for *n*-butylamine *vs.* one-half that amount for each of the β carbons of diethylamine). The $\Delta\delta^{\beta}$ values of the naturally occurring tetraamine, spermine, and of deoxystreptamine (**3**) (*vide infra*) suggest the protonation-induced shift are additive.

RNH_2	Et_2NH	$H_2N(CH_2)_nNH_2$
5a, R = *n*-C_6H_{13}	**6**	**7a**, *n* = 5
b, R = *n*-C_4H_9		**b**, *n* = 4
c, R = *c*-C_6H_{11}		

$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$

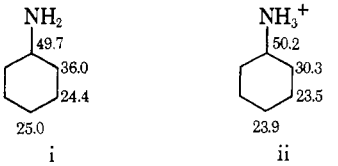
8

The protonation parameters were used to anticipate the shifts of the antibiotics and their models in acid medium, while the limiting shifts were determined by titration experiments (Figure 1).[20] The resultant list of

(17) Only a limited number of examples of the cmr shifts of amines and their protonated salts have been reported: T. Pehk and E. Lippmaa, *Org. Magn. Resonance*, **3**, 679 (1971); I. Morishima, K. Yoshikawa, K. Okada, T. Yonezawa, and K. Goto, *J. Amer. Chem. Soc.*, **95**, 165 (1973); E. Wenkert, D. W. Cochran, and F. M. Schell, unpublished observations.

(18) The shifts of the hydrochlorides are the same as those of the amines at pH <1.

(19) The chemical shifts of cyclohexylamine and its hydrochloride in chloroform are shown on i and ii, respectively. The difference of β-



i ii

carbon shifts is 0.7 ppm greater than the Δδ value for water solution. In view of the identity of the shifts of the salt in the two media the solvent effect must reflect protonation of the amine in water solution. A similar solvent effect (Δδ 1.0 ppm) is noted by comparison of the β-carbon shifts of *n*-butyl- and *n*-hexylamines in benzene[14] and in water (Table II).

(20) Cmr data on tobramycin (**1a**) at low pH were obtained with a variety of mineral acids. Since the spectra are superimposable, the negative ions have no effect on the chemical shifts.

Table III. ^{13}C Chemical Shift Differences[a]

	2a[b]	2b[b]	2c[b]	3	4a	1a	1b[c]	1c	1d[c]	4b
C-1′	4.2		0		5.9	5.9	6.5	4.8	5.4	5.5
C-2′	0.8		0.4[d]		0.7	0.7	0.8	1.8	1.8	1.6
C-3′	4.4		0.3		5.6	5.5	5.8	4.4	4.6	4.6
C-4′	0.6		0		1.5	1.4	1.6	0.7	0.8	0.6
C-5′	0.1		3.9[e]		3.8	3.1	3.1	4.9	4.9	4.8
C-6′	0.5		0.7		1.5	1.6	1.4	1.4	1.3	1.3
Subtotal	10.6		5.3		19.0	18.2	19.2	18.0	18.8	18.4
C-1				0.5	0.4	0.6	0.7	0.6	0.6	0.5
C-2				8.1	7.5	7.8	7.8	7.7	8.0	7.5
C-3				0.5	1.2	1.3	1.4	0.8	0.8	0.8
C-4				5.4	9.7	9.2	9.9	9.6	10.6	10.3
C-5				1.2	0.8	0.5	0.1	0.2	0.1	0.9
C-6				5.4	5.1	4.5	4.4	4.4	4.3	5.0
Subtotal				21.1	24.7	23.9	24.3	23.3	24.4	25.0
C-1″		0.8				−1.0	−1.0	−0.6	−0.7	
C-2″		4.1				3.7	3.7	3.8	3.7	
C-3″		−0.8				−0.7	−0.6	−0.7	−0.6	
C-4″		4.0				3.9	3.8	3.8	3.9	
C-5″		0.2				−0.6	−0.5	−0.6	−0.5	
C-6″		0.8				0.4	0.6	0.4	0.7	
Subtotal		9.1				5.7	6.0	6.1	6.5	
Total						47.8	49.5	47.4	49.7	

[a] $\Delta\delta$ values are from spectra of water solutions recorded in ppm downfield from TMS; $\delta^{TMS} = \delta^{dioxane} + 66.3$ ppm; $\Delta\delta = \delta(pH > 11) - \delta(pH < 1)$. [b] $\Delta\delta$ value of the methyl group omitted to permit comparison of the subtotal with that of the other compounds. [c] $\Delta\delta$ value of the carbonyl group omitted for a similar reason. [d] Alternatively 0. [e] Alternatively 4.3 ppm.

$\Delta\delta$ values (Table III) reveals some interesting trends among the amino sugars. The protonation effect on the α carbon of model **2b** and of ring C of the antibiotics (*i.e.*, C-3″) is negative. The $\Delta\delta^{\beta}$ values of the aminoglucosides (**2**) and of ring C of the antibiotics are constant (4.2 ± 0.2 and 3.8 ± 0.1 ppm, respectively). As expected, C-2 of the antibiotics is shielded nearly twice as strongly as C-6 in view of the former carbon being placed β to two amino groups. Placement of ring C upon the C-6 oxygen alters the magnitude of the $\Delta\delta^{\beta}$ value characteristic for deoxystreptamine (**3**) and the bicyclic compounds **4** (5.2 ± 0.2 ppm) to 4.4 ± 0.1 ppm. Similarly, the protonation parameter is sensitive to the presence of a substituent at C-3′ in the antibiotics (**1**), nebramine (**4a**) and neamine (**4b**). Thus $\Delta\delta^{\beta}$ of 5.6 ± 0.2 ppm reflects an unsubstituted C-3′ and 4.5 ± 0.1 ppm an oxycarbon-3′.

Summation of the $\Delta\delta$ values for the individual carbons of the monoamino compounds **2a** and **2c** yields calculated values that fit the experimental data for the ring A diamino substances **1c**, **1d**, and **4b** within 0.5 ppm at all ring A carbons except C-1′. The exception is not surprising, if we assume the replacement of a methoxy group by a *sec*-alkoxy unit forces upon C-1′ some γ effects from ring B. Inversely, γ effects from ring A on C-4 make the latter's environment different from that of C-6 despite their otherwise being disposed symmetrically toward each other within ring B. As a consequence the difference of the $\Delta\delta^{\beta}$ values of C-4 and C-6 in compounds **3**, **4**, and **1** remove the ambiguity regarding the chemical shift assignment of these carbons in the antibiotics (*vide supra*). Finally, it is noteworthy that the total of the $\Delta\delta$ values is the same for each of the antibiotics, that the subtotal of rings A and B is identical for each of the antibiotics and for nebramine (**4a**) and neamine (**4b**), and that the subtotal for ring B of compounds **1** and **4** differ from that for deoxystreptamine (**3**) by 4 ppm due mostly to the aforementioned γ effects induced by ring A. The calculation of subtotals of $\Delta\delta$ values for individual rings verifies the differentiation of chemical shifts for carbons of different rings.

Conformational Analysis

Comparison of the cmr data for the antibiotics (**1**) with those recorded for a variety of saccharides leads to revealing insight into the conformation of the antibiotics in water solution. The average chemical shift of the anomeric carbon of the α-glucopyranoside or structurally, closely related unit is 99.5 ppm[21] and that of a β-glucopyranoside moiety or its equivalent, 102.8 ppm.[24] Not only does the stereochemistry of the C-1 substituent and the C-2 and C-3 environments affect the anomeric carbon shifts, but also the nature of the alkyl group on the C-1 oxygen plays a role in affecting the basic δ values. Thus while a 1α-methoxy group is the foundation of the 99.5 ppm value and many 1α-oriented cyclitol and glycoside units retain this value, a 1α-isopropoxy function, the simplest model of a *sec*-alkoxy substituent, exhibits a 95.6-ppm anomeric carbon shift.[25] This difference of behavior is explained most readily on the basis of a difference of rotamer populations around the *O*-alkyl bond. If it be assumed that the favored orientation of an alkoxy group toward the ring bonds places the alkyl unit gauche to the ring oxygen and C-1 hydrogen (the exo-anomeric effect),[26]

(21) Methyl α-D-glucopyranoside,[11d] 99.5; methyl β-maltoside,[15] 99.5; amylose,[15] 99.4; nigerose,[22a] 99.0 glucan (1 → 4α, 1 → 6α),[22b] 100.8–101.6, 99.5 ppm.[23]

(22) (a) N. Yamaoka, T. Usui, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *Tetrahedron Lett.*, 2047 (1971); (b) P. A. J. Gorin, *Can. J. Chem.*, **51**, 2375 (1973).

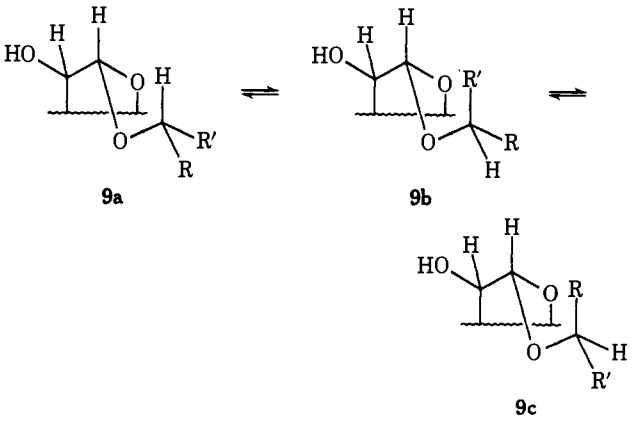
(23) The shifts recorded in the references have been translated into the TMS scale, when necessary.

(24) Methyl β-D-glucopyranoside,[11d] 103.1; methyl β-D-cellobioside,[15] 102.9, 102.4; methyl β-D-lactoside,[15] 102.8; and laminaribiose,[22a] 102.7 ppm.[23]

(25) This is the δ value of C-1 in isopropyl α-D-glucopyranoside.

(26) R. U. Lemieux, A. A. Pavia, J. C. Martin, and K. A. Watanabe, *Can. J. Chem.*, **47**, 4427 (1969); R. U. Lemieux and J. C. Martin, *Carbohyd. Res.*, **13**, 139 (1970); R. U. Lemieux, T. L. Nagabhushan, K. J. Clemetson, and L. C. N. Tucker, *Can. J. Chem.*, **51**, 53 (1973).

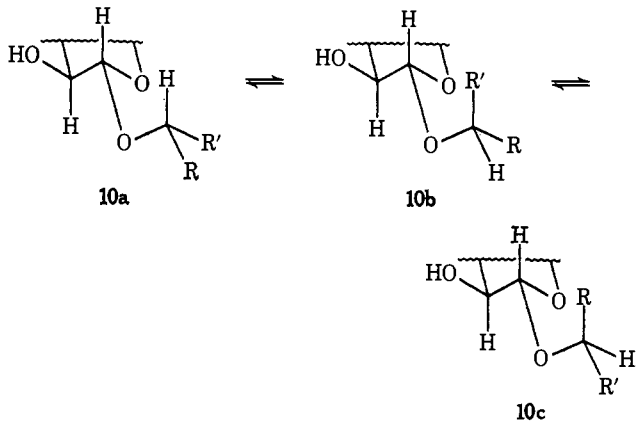
conformation **9** (R = R′ = H) represents a 1α-methoxy group. Since the isopropoxy group, an equilibrium mixture of rotamers **9a–c** (R = R′ = Me), introduces



9a **9b** **9c**

acyclic γ effects acting on C-1 which among hydrocarbons amount to 2.5 ppm per alkyl group[27] and among ethers to 2.0 ppm,[28] the 4 ppm difference of the anomeric carbon shift of methyl and isopropyl α-D-glucopyranosides follows expectations. The identity of the C-1 shifts for 1α-methoxyglucopyranoside, 1α-methoxygalactopyranoside, and related 1α-linked di- and polysaccharides reflects therefore a preference of rotamer population **9a** for the dimer and polymer systems, since only in this form does H-1 feel no effect from a γ-alkyl group. The parallel or closely parallel relationship of the C(1)–H bond and the first carbon–hydrogen bond of the attached *sec*-alkoxy moiety depicted in **9a** is present also in one of the three low-energy rotamers derived from a conformation placing the alkoxy group gauche to C(1)–H and C-2. Hence in the absence of consideration for the exo-anomeric effect[26] two rotamers are represented by the C-1 shifts. The same rotamer favoritism is shown by the antibiotics **1** in the disposition of their deoxystreptamine moiety toward C-1′ and C-1″.

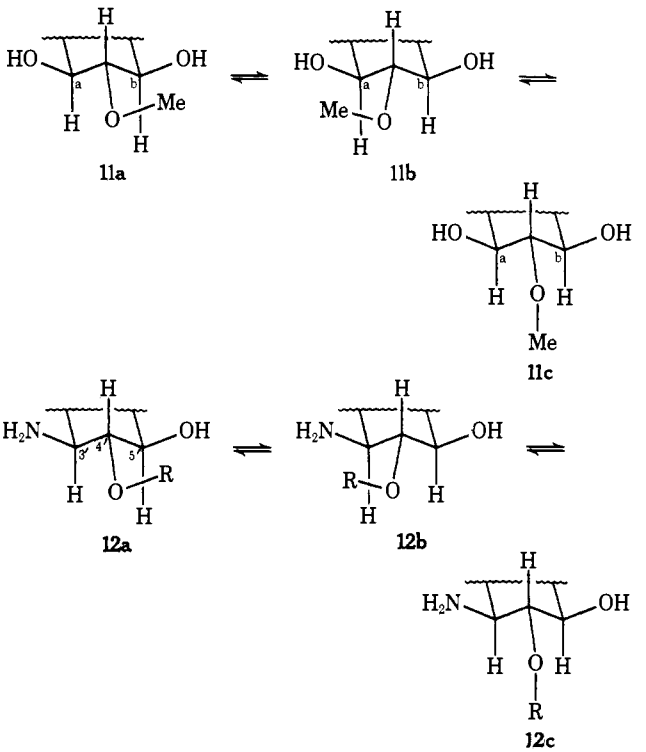
While β-D-glucopyranosides and their equivalents in principle can assume any of the conformations **10**,[26] the nearly identical anomeric carbon shifts of the 1β-methoxy and several 1β-glucosyloxy systems[24] indicate the latter's predisposition toward rotamer **10a**. Thus



10a **10b** **10c**

both 1α- and 1β-glycosyloxy glucopyranosides seem to favor a conformation in which the carbons attached to the oxygen bridge possess a parallel carbon–hydrogen bond arrangement. However, such rotamer preference may not be general and be dependent on the configuration of the substituents on the carbons flanking the oxygen-bridged carbon of the 1-alkoxy group. While these substituents and the oxygen bridge itself are equatorially oriented in the alkoxy groups of the compounds whose anomeric carbon shifts formed the basis for the 1α and 1β values of 99.5 and 102.8 ppm, respectively (*vide supra*),[21,24] an example of an α-D-glucopyranosyl system possessing one axial and one equatorial substituent on the flanking carbons and an equatorial oxygen bridge in its glucopyranoside residue is α-kojibiose. The anomeric carbon shift of this disaccharide is 96.3 ppm[22a] and indicative of a preference of conformers **9b** or **9c**. Saccharides whose 1α-alkoxy substituents resemble *tert*-butoxy groups exhibit their anomeric carbon signal 7.7 ppm upfield of that of 1α-methoxy substances in view of the imposition of the equivalent of three acyclic γ effects on C-1.[29]

The conformational disposition of the two glycosyl units toward the central deoxystreptamine moiety in the antibiotics **1** can be ascertained from a comparison of the cmr data of these substances with those of the inositols. It has been shown that O-methylation of equatorial hydroxyl groups induces a mild upfield shift (0.7 ± 0.2 ppm) of equatorial hydroxyl-bearing β carbons among inositols.[15,33] This effect has been interpreted to be the consequence of the energetic equality of rotamers **11a** and **11b** and their preference over



11a **11b** **11c**

12a **12b** **12c**

(27) D. M. Grant and E. G. Paul, *J. Amer. Chem. Soc.*, 86, 2984 (1964).

(28) This value is calculated from recorded chemical shifts of ethers.[11b]

(29) Sucrose,[30] 91.7; stachyose,[30] 91.8; raffinose,[30] 91.7; and nystose,[31] 92.0 ppm.[23] The anomeric carbon shift of a 1β-*tert*-butoxy compound is 97.7 ppm, 5.4 ppm above the C-1 resonance of the corresponding methyl glycoside.[32]

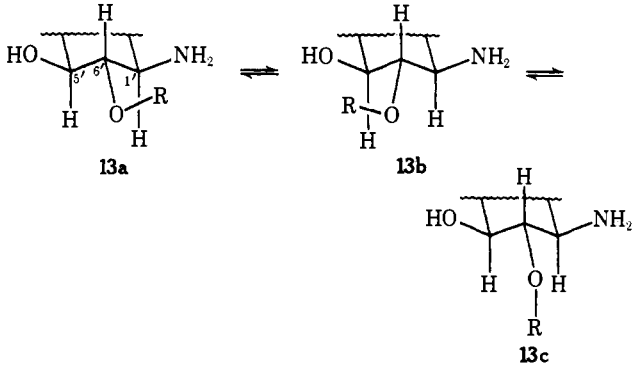
(30) A. Allerhand and D. Doddrell, *J. Amer. Chem. Soc.*, 93, 2777 (1971).

(31) W. W. Binkley, D. Horton, N. S. Bhacca, and J. D. Wander, *Carbohyd. Res.*, 23, 301 (1972).

(32) P. O. Larson, H. Sorensen, D. W. Cochran, E. W. Hagaman, and E. Wenkert, *Phytochemistry*, 12, 1713 (1973).

(33) D. E. Dorman, S. J. Angyal, and J. D. Roberts, *J. Amer. Chem. Soc.*, 92, 1351 (1970).

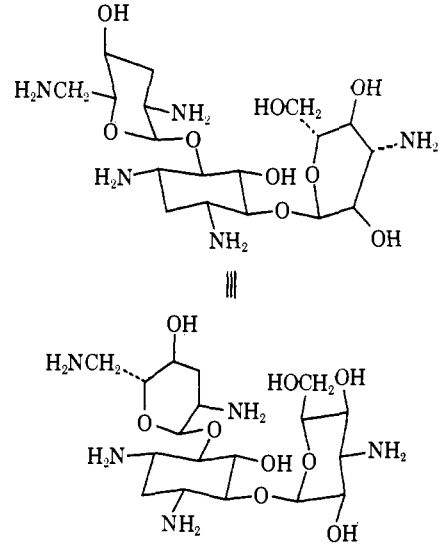
11c.[15,33] Since replacement of the *O*-methyl group by an *O*-glycosyl function can be expected to impose negligible, acyclic δ effects upon the β carbons, the shift change for inositol glycosides or their equivalents should remain *ca.* 0.7 ppm/β carbon, should no other factors affect the conformational equilibrium. The attachment of a glycosyl unit to the oxygen of C-4 of deoxystreptamine causes a shift change of 1.6 ± 0.2 ppm for C-3 of nebramine (**4a**) and neamine (**4b**) and of ±0.1 ppm for C-5 of the two substances. The dissimilarity of shift modification on the two β carbons implies the preponderant presence of rotamer **12b** in water solutions of the two bicyclic compounds. Furthermore, the joining of ring C to the deoxystreptamine portion of neamine (**4b**) leads to a shift change of ±0.1 ppm for C-1 in the antibiotics **1c** and **1d** and of 1.7 ± 0.1 ppm for C-5 of the two natural products. Analogous shift patterns emerge from the spectra of the antibiotics **1a** and **1b** upon comparison with the spectrum of nebramine (**4a**). These shift variations favor rotamer **13b** for the antibiotics. Since the attachment



13a **13b** **13c**

of rings A and B imposes a shift change of 1.6 ± 0.2 ppm at C-3 of all the antibiotics, their rotamer preference, as that of **4a** and **4b**, is **12b**.

The above arguments of the conformational constraints imposed on ring B and the aforementioned parallelism of the C(1′)–H and C(4)–H bond and C(6)–H and C(1″)–H bond pairs resolve the spatial disposition of the three rings toward each other in the antibiotics (**1**) as well as of the two rings in nebramine (**4a**) and neamine (**4b**). The exo-anomeric effect[26] is maintained among these compounds. The preferred, overall equilibrium conformation of the kanamycin antibiotics is illustrated by formula **14** for tobramycin.[34]



14

It appears to differ in water solution and in the crystalline state. As the X-ray analysis of kanamycin A (**1c** with a hydroxyl group replacing the C-2′ amino function) indicates,[35] the exo-anomeric effect is maintained in both physical states, while the orientation of rings A and C toward ring B differs.[36]

(34) The high $\Delta\delta^{\beta}$ values of C-1′ and C-4 indicate that the antibiotics assume a different conformational stance around rings A and B at low pH. The origin of this effect is under investigation.

(35) G. Koyama, Y. Iitaka, K. Maeda, and H. Umezawa, *Tetrahedron Lett.*, 1875 (1968).

(36) Note Added in Proof. Three studies of relevance to the present work have just appeared in the literature: J. B. Morton, R. C. Long, P. J. L. Daniels, R. W. Tkach, and J. H. Goldstein, *J. Amer. Chem. Soc.*, **95**, 7464 (1973); S. Omoto, S. Inouye, M. Kojima, and T. Niida, *J. Antibiot.*, **26**, 717 (1973); P. W. K. Woo and R. D. Westland, *Carbohyd. Res.*, **31**, 27 (1973).