phase. Isolation of the products was accomplished by extraction of the silica gel with ether, filtration, and evaporation of the ether. The material with the largest R_f value (0.91), ester **3c**, was recovered in 6% yield (3 mg). The material with the second largest R_f value (0.73), (±)-tabersonine, was isolated as a clear semisolid, 15 mg (30.2%), and was identical with a sample of natural origin by comparative tlc, solution infrared, ultraviolet, and mass spectrometry.

When the polar material isolated from the preparative tlc plate (25 mg) was stirred overnight in 5% aqueous acetic acid, an additional 7 mg (total yield of 44.5%) of tabersonine was obtained.

Acknowledgment. The authors wish to express their appreciation to the Natural Science Foundation (GP-11273) for their generous support of this work.

A Carbon-13 Magnetic Resonance Study of Aminoglycoside Pseudotrisaccharides. The Gentamicin Antibiotics

James B. Morton,*[1] Robert C. Long,[2] Peter J. L. Daniels,[1] Richard W. Tkach,[1] and J. H. Goldstein[2]

Contribution from the Research Division, Schering Corporation, Bloomfield, New Jersey 07003, and the Chemistry Department, Emory University, Atlanta, Georgia 30322. *Received March 15, 1973*

Abstract: The ^{13}C nmr spectra of some gentamicin aminoglycosides are tabulated, assigned, and discussed. Methods of assignment are detailed. The chemical shifts of many carbon nuclei are shown to be reasonably constant among the different compounds. The reproducibility of ^{13}C nmr data obtained under varying experimental conditions is demonstrated.

Within recent years, carbon-13 magnetic resonance (cmr) techniques have been applied to the study of carbohydrates by a number of workers. The chemical shifts of oxygenated carbons, substitution, and proximity effects have been noted by earlier workers, especially Hall, Dorman, and Roberts.[3-5] Perlin, Casu, and Koch have studied configurational and conformational effects on chemical shifts in some detail and have demonstrated the existence of several consistent patterns in the dependence of these shifts on carbohydrate stereochemistry.[6] These studies have been extended to disaccharides.[7] The cmr of an aminoglycoside, hygromycin B, has been described, although this spectrum was not fully assigned.[8] Extension of these results to more complex systems such as antibiotics is a matter of obvious interest and practical value. As is well known, cmr spectra provide many advantages over proton spectra in the characterization of such complex molecules, principally due to the relatively much greater range (up to 200 ppm) of ^{13}C chemical shifts.[9]

The goal of the present study was the complete assignment of all resonances in a series of gentamicin antibiotics. As has been reported by other workers,[7,8] the present study demonstrates that the chemical shifts for certain positions of carbon nuclei are sufficiently invariant to allow assignments to be made with a considerable degree of confidence. Accordingly, we have utilized the comparisons of shifts among similar aminoglycosides and with the mono- and pseudodisaccharide fragments of these antibiotics. This approach has been supplemented by the use of such spectroscopic techniques as specific frequency proton decoupling and single frequency off-resonance decoupling (SFOR).

The particular series of compounds studied here include gentamicins C_1, C_{1a}, and C_2 and sisomicin, as well as several one- and two-ring fragments from these aminoglycosides. These antibiotics have been described previously and are representative of a large family of aminoglycosides.[10-13] The gentamicin antibiotics are the subject of considerable chemical and biological research. The successful outcome of the present investigation suggests that cmr will be a routine and reliable instrumental method for the elucidation of similar structures.

The cmr data currently found in the literature have been generated on a variety of instruments operated in both the pulsed and continuous wave mode. It was, therefore, important to determine the dependence of the present data on the experimental conditions employed. The results presented in this paper have been obtained from three different laboratories, each using significantly different experimental techniques. The agreement among the data so obtained gives assurance that these data are truly independent of the technique by which they were obtained.

(1) Schering Corporation.
(2) Emory University.
(3) L. D. Hall and L. F. Johnson, *Chem. Commun.*, 509 (1969).
(4) D. E. Dorman, S. J. Angyal, and J. D. Roberts, *J. Amer. Chem. Soc.*, **92**, 1351 (1970).
(5) D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, **92**, 1355 (1970).
(6) A. S. Perlin, B. Casu, and J. J. Koch, *Can. J. Chem.*, **48**, 2596 (1970).
(7) E. Breitmaier, G. Jung, and W. Voelter, *Chimia*, **25**, 362 (1971).
(8) N. Neuss, K. R. Koch, B. B. Molloy, W. Day, L. L. Huckstep, D. E. Dorman, and J. D. Roberts, *Helv. Chim. Acta*, **53**, 2314 (1970).
(9) A brief comparison of carbon-13 and proton data is given by W. O. Crain, Jr., W. C. Wildman, and J. D. Roberts, *J. Amer. Chem. Soc.*, **93**, 990 (1971).
(10) D. J. Cooper, M. D. Yudis, R. D. Guthrie, and A. M. Prior, *J. Chem. Soc. C*, 960 (1971).
(11) D. J. Cooper, M. D. Yudis, H. M. Marigliano, and T. Traubel, *J. Chem. Soc. C*, 2876 (1971).
(12) D. J. Cooper, P. J. L. Daniels, M. D. Yudis, H. M. Marigliano, R. D. Guthrie, and S. T. K. Bukhari, *J. Chem. Soc. C*, 3126 (1971).
(13) D. J. Cooper, R. S. Jaret, and H. Reimann, *Chem. Commun.*, 285, 924 (1971).

Table I. The ^{13}C Chemical Shifts of the Pseudotrisaccharides and Their Fragments

Carbon	1[a]	1a[b]	1b[a]	1b[b]	1b[c]	2[b]	2[c]	3[b]	3[c]	3a[b]	3a[c]	3b[b]	3b[c]	4[c]	5[a]	5[c]	6[a]
A1	101.4	101.3	101.3	101.2	101.2	101.5	101.5								100.6	100.6	101.2
A2	70.3	70.2	70.2	70.1	70.1	70.0	70.0								70.1	70.1	70.2
A3	64.5	64.4	64.4	64.4	64.4	64.3	64.3								64.6	64.7	64.4
A4	73.3	73.3	73.2	73.1	73.1	73.0	73.0								73.4	73.5	73.3
A5	68.7	68.7	68.7	68.7	68.7	68.5	68.5								68.0	68.1	68.7
A6	22.9	23.0	22.8	22.9	23.0	22.9	22.9								22.5	22.7	22.4
A7	38.1	38.0	38.1	38.0	38.2	37.9	37.9								38.0	38.2	37.8
A8															56.0	56.0	
B1	51.8	51.7	51.8	51.7	51.8	51.8	51.7	51.3	51.3	51.3	51.3	51.3	51.3	51.6			51.6
B2	36.8	36.7	36.7	36.8	36.7	36.4	36.4	36.7	36.8	36.7	36.8	36.8	36.8	37.0			36.7
B3	50.9	50.6	50.8	50.7	50.8	50.4	50.3	50.7	50.8	50.5	50.5	50.6	50.7	51.6			50.5
B4	88.6	88.3	88.7	88.8	88.7	85.3	85.2	88.9	88.8	88.3	88.1	88.9	88.7	78.5			87.8
B5	75.4	75.4	75.3	75.4	75.3	75.4	75.3	76.7	76.8	76.9	76.8	76.9	76.8	76.6			75.4
B6	87.9	87.8	87.7	87.8	87.6	87.8	87.7	78.4	78.4	78.4	78.3	78.4	78.3	78.5			87.8
C1	102.6	102.2	102.6	102.7	102.6	100.6	100.6	102.9	102.8	102.3	102.1	102.9	102.9				101.8
C2	51.1	51.0	51.0	51.0	51.0	47.6	47.5	50.8	50.9	50.7	50.7	50.8	50.9				50.8
C3	27.2	27.1	27.0	27.1	27.1	25.6	25.6	27.3	27.4	27.0	27.1	27.0	27.0				26.6
C4	26.1	28.5	26.0	26.1	26.2	96.5	96.4	26.2	26.3	28.5	28.5	26.0	26.2				28.2
C5	72.8	71.5	74.4	74.4	74.5	150.4	150.2	72.6	72.6	71.3	71.3	74.3	74.3				71.0
C6	58.2	46.1	50.3	50.4	50.3	43.5	43.5	58.0	58.1	46.0	46.0	50.0	50.1				45.9
C7	15.0		19.0	19.0	19.2			15.0	15.1			19.0	19.1				
C8	33.7							33.6	33.7								

[a] Data were obtained at Emory University. [b] Data were obtained at Stevens Institute. [c] Data were obtained at Schering Corp.

Experimental Section

Samples under investigation at Emory University (Laboratory A) were prepared by weight in distilled H_2O previously degassed by N_2. Concentrations of gentamicin C_1, gentamicin C_2, and methyl β-garosaminide were 34, 38, and 20% by weight, respectively. The sample cell consisted of a 10-mm precision capillary spacing system. The ^{19}F lock material (C_6F_6) was contained in the 3-mm inner capillary. Cmr spectra were obtained in the continuous wave mode at 22.628 MHz employing a Bruker HFX-90 spectrometer. Signals were accumulated with a Nicolet 1074 signal averager. The protons were decoupled by means of noise modulation of the appropriate irradiating frequency. The chemical shifts were referred to the frequency of CS_2 measured in the same coaxial cell and converted to δ(TMS) by the following relation: $\delta_C^{TMS} = 194.0 - \delta_C^{CS_2}$.

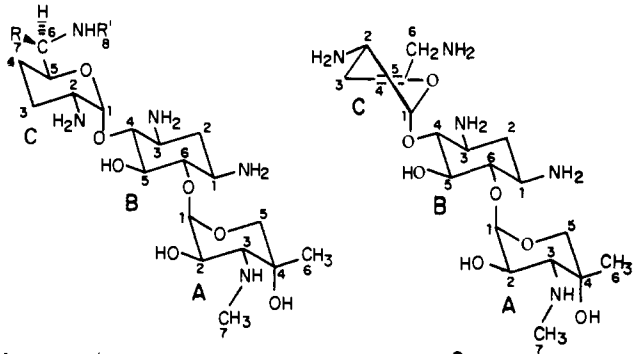
The cmr spectra taken at Stevens Institute (Laboratory B) were obtained at 22.628 MHz on a Bruker HX-90 spectrometer using a 10-mm sample tube. Spectra were obtained in the pulsed mode and accumulated with a Nicolet 1074 signal averaging computer. Fourier transform to the frequency domain was accomplished with a Digital Equipment Corp. PDP-8 computer, utilizing Nicolet software. Only the real part of the time domain data was transformed and a phase correction program was used. The free induction decay was not filtered. Proton decoupling was effected with noise modulation of the irradiating frequency centered at δ 3 in the center band of the proton resonance frequency (approximately 90 MHz). The bandwidth of the modulation envelope was approximately 1000 Hz. Shifts were referenced to internal dioxane and calculated from computer addresses. One address equaled 0.054 ppm. Shifts were reported relative to TMS using the following relation: $\delta_C^{TMS} = \delta_C^{diox} + 67.4$ ppm. The system was field frequency locked at 84.7 MHz to a sample of hexafluorobenzene contained in a coaxial 5-mm tube. The aminoglycosides were examined at concentrations of 0.5 to 1.0 *M*. The samples, which had been weighed prior to decarbonation (see below), were dissolved without reweighing into D_2O flushed with nitrogen.

The cmr spectra taken at the Schering Corp. (Laboratory C) were obtained at 25.2 MHz on a Varian XL-100-15 spectrometer using a 12-mm sample tube. Single-scan spectra were obtained at a scan rate of 1 Hz/sec. Shifts were referenced to internal dioxane and measured on the radiofrequency frequency counter. Shifts were reported relative to TMS using the following relations: $\delta_C^{TMS} = \delta_C^{diox} + 67.4$ ppm. The system was field frequency locked at 15.4 MHz to the deuterium nuclei of the solvent. Proton decoupling was effected with noise modulation of the irradiating frequency centered at δ 3 in the center band of the proton resonance frequency (approximately 100 MHz). The bandwidth of the modulation envelope was approximately 700 Hz. Off-resonance proton decoupled spectra (SFOR) were obtained by use of a monochromatic irradiating frequency removed from either the highest or lowest centerband proton resonance by no more than 100 Hz. The power of this irradiating frequency was set to the same level as that used for white noise irradiation. Selective proton decoupling on the XL-100-15 spectrometer was accomplished by use of monochromatic irradiation at the centerband resonance of the proton(s) which was to be decoupled. No attempt was made to alter this frequency by inspection of results. This frequency was determined from pmr spectra obtained with the magnet locked to the solvent deuterium resonance. The aminoglycosides were examined at concentrations of 0.5–1.0 *M*. The samples, which had been weighed prior to decarbonation (see below), were dissolved without reweighing into D_2O flushed with nitrogen. The sample temperature, which was not regulated, was 30 ± 5°.

Prior to cmr determination all samples were decarbonated by passage of their aqueous solutions through a column of Amberlite IR401S resin ($^-$OH form). The samples were collected under nitrogen and lyophilized. All subsequent handling of the samples was under a nitrogen atmosphere in a glove box. The gentamicins and sisomicin were carefully purified by chromatography. Gentamines C_1, C_2, and C_{1a} were prepared by methanolysis of the appropriate gentamicin followed by chromatography as described for the gentamicin C complex.[10]

Gentamine C_1 was obtained as an amorphous white solid: $[\alpha]^{26}D$ +68.7° (*c* 0.45 H_2O); mass spectrum, *m/e* (rel intensity) 319 (5, [M + 1]$^-$), 318 (6, M·$^-$), 191 (37), 173 (40), 163 (75), 157 (73, purpurosamine A ion), 145 (79).

Gentamine C_2 was obtained as an amorphous solid: $[\alpha]^{26}D$ +86.4° (*c* 0.38, H_2O); mass spectrum, *m/e* (rel intensity) 305 (3, [M + 1]$^+$), 304 (1, M·$^+$), 191 (52), 173 (25), 163 (90), 145 (96), 143 (92, purpurosamine B ion).



1 , R = R' = CH_3
1a, R = R' = H
1b, R = CH_3, R' = H

2

Figure 1. The pseudotrisaccharides: gentamicin C_1 (**1**), C_{1a} (**1a**), and C_2 (**1b**) and sisomicin (**2**).

Gentamine C_{1a} was obtained as an amorphous solid: $[\alpha]^{26}D$ +96.3 (*c* 0.39, H_2O): mass spectrum, *m/e* (rel intensity) 291 (3, $[M + 1]^+$), 290 (1, $M\cdot^+$), 191 (82), 173 (22), 163 (88), 145 (100), 129 (90, purpurosamine C ion).

Methyl β-garosaminide, prepared as described by Cooper *et al.*,[10] was obtained crystalline: mp 65–67°; $[\alpha]^{26}D$ +216.8° (*c* 0.40, H_2O) [literature[10] reports a syrup, $[\alpha]^{26}D$ +209° (*c* 0.3, H_2O)].

Anal. Calcd for $C_8H_{17}NO_4$: C, 50.24; H, 8.96; N, 7.33. Found: C, 50.23; H, 9.35; N, 7.10.

Mass spectra were determined on a Varian MAT CH5 spectrometer and rotations were obtained with a Bendix 1164 automatic polarimeter.

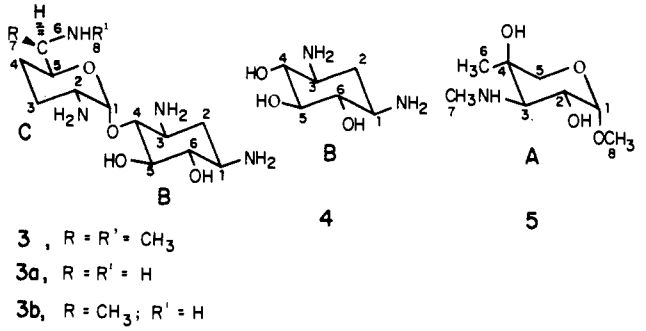
Results and Assignments

The spectral frequencies determined for all the compounds studied are presented in Table I. It will be observed that for some compounds measurements were determined in two or three laboratories. These multiple measurements permitted us to determine the reproducibility of the spectral data under different conditions. Experimental details varied significantly and are given in the Experimental Section. All shifts are reported in ppm downfield from TMS. The left-hand margin of the table indicates the assignments of each resonance; the manner in which these assignments were made is discussed below in detail. These assignments follow the labeling scheme indicated in Figures 1 and 2, which give the structures of the compounds studied. The three rings are labeled A, B, and C and the carbon nuclei within each ring are numbered from 1 to 8 in the normal manner.

Discussion

For the 86 resonances measured more than once during the investigation the overall average agreement fell within 0.08 ppm. In only 12 instances was the range of values as great as 0.2 ppm, the largest observed in this study. This is of general interest since it indicates the extent to which reproducible cmr data can be produced in different laboratories. In this study, insofar as reproducibility of data alone is concerned, experimentally determined shift differences of 0.3 ppm or greater were judged to be significant in the assignments reported here.

As can be seen from the data presented, the chemical shifts of similar carbon nuclei in the different aminoglycosides are remarkably constant, making unambiguous assignments possible by comparison of shifts from compound to compound. It should be noted,



3 , R = R' = CH_3
3a, R = R' = H
3b, R = CH_3; R' = H

4

5

Figure 2. Gentamine C_1 (**3**), C_{1a} (**3a**), and C_2 (**3b**), 2-deoxystreptamine (**4**), and methyl β-garosaminide (**5**).

however, that this observed constancy was not the sole basis upon which the assignments were made. Many assignments were verified by specific frequency proton decoupling and single frequency off-resonance decoupling (SFOR).[9] The gentamicin-type aminoglycosides are amenable to these techniques due to the presence of deoxygenated and nitrogen-bearing functions which spread the pmr spectrum out over a much larger range than that obtained for other oligosaccharides. Thus, in gentamine C_1 (**1**), for example, it was possible to selectively decouple the C3 and C4 protons centered at δ 1.6 ppm, yielding singlets for the respective carbon atoms (see Figure 3). The residual one-bond coupling is seen to increase for the other carbon resonances the further away the resonances of the adjacent protons fall from δ 1.6. Thus the B2 resonance is distinguished from those of C3 and C4, because this resonance occurs as an unresolved doublet of doublets. The 2-deoxystreptamine B2 axial and equatorial protons resonate at δ 1.10 and 1.90, respectively. The multiplicities of the various resonances are more easily seen in the SFOR spectrum. In this discussion, reference will be made to Table I and to the spectral correlation chart shown in Figure 4.

With these considerations in mind, it is now possible to proceed to a detailed discussion of the assignments. 2-Deoxystreptamine (**4**) is the simplest of cases to be considered because of the symmetry of the molecule (Figure 2). Carbon nuclei at B4 and B6 appear as a single resonance of double intensity at 78.5 ppm, in the region typical of oxygen-bearing carbon shifts.[4] A resonance of single intensity at 76.6 ppm also falls in the range of carbons adjacent to oxygen and is assigned to carbon B5. The remaining resonances are assigned by an analogous argument. The resonance of double intensity at 51.5 ppm is identified with carbons B1 and B3 and the single intensity resonance at 36.6 ppm with carbon B2.

These shifts compare well with those reported for *N*-methyl-2-deoxystreptamine by Roberts.[8] Carbons B3, -4, and -5 have resonances within 0.5 ppm of the corresponding carbons of the *N*-methyl homolog. In this homolog, the resonance of the carbon bearing the methylamino function lies 7.6-ppm downfield from the resonance of carbon B1. This downfield shift of a carbon resonance upon substitution at an atom adjacent to it, corresponding to the hydrocarbon β shift of Grant and Paul,[14] has been considered in detail for

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

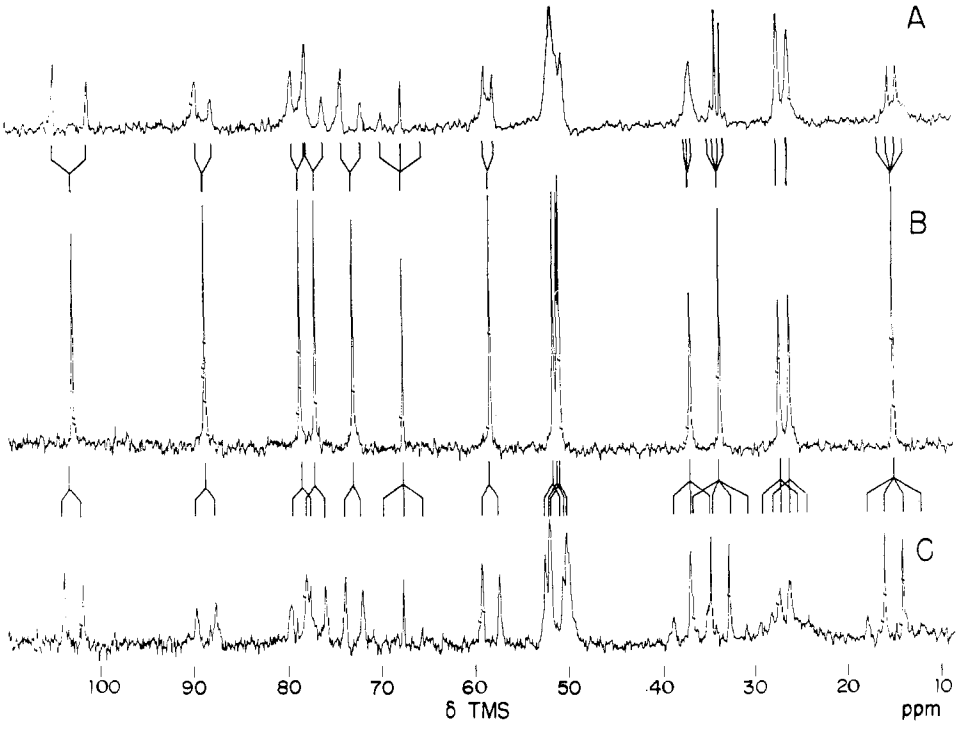


Figure 3. ^{13}C magnetic resonance spectra of gentamine C_1: (A) specific frequency decoupled at δ 1.6, (B) white noise decoupled, (C) SFOR decoupled. The sample was dissolved in D_2O with dioxane (67.4 ppm) as an internal reference. These spectra were obtained on the Varian XL-100 spectrometer at Springfield, N. J. Fourier transform was accomplished with a 620L computer after 100 pulses.

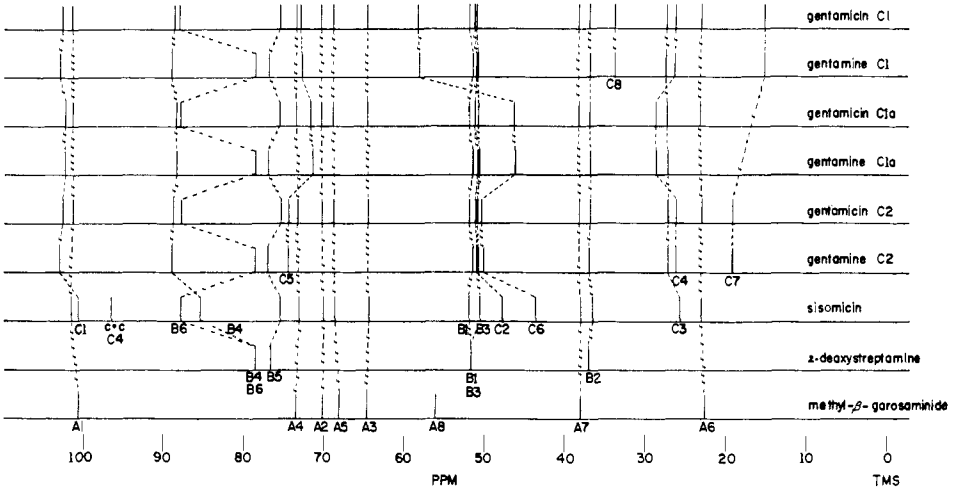


Figure 4. Correlation of the spectra of the pseudotrisaccharides and their fragments.

the case of substitution at a carbon atom.[14-17] Dorman and Roberts report downfield β shifts to be general in cyclitols and pyranosides upon O-methylation,[4,5] with a magnitude of 7–10 ppm compared with 9.4 ppm for C-methylation.[14] Note that these workers use the term "α carbon" hydroxy methylation. In the present paper, the atom to which a substituent has been added is termed the "α atom," be it a carbon, oxygen, or nitrogen.

The undecoupled and SFOR cmr spectra of methyl β-garosaminide show three doublets, one at 100.6 ppm, which is consistent with the anomeric carbon,[3,5-7] one at 70.1 ppm, which is typical of carbons bearing one oxygen[3,5-7] and is accordingly assigned to A2, and one at 64.6 ppm, which is assigned to A3. This latter resonance and a quartet found at 38.0 ppm are consistent with methine and methyl carbon atoms adjacent to nitrogen.[8,9] The quartet at 38.0 ppm, therefore, is assigned to A7. The quartet at 56.0 ppm was assigned to A8 by comparison with shifts reported for the methyl carbons of several methyl α-D-pyranosides.[6] The third quartet, found at 22.5 ppm, must belong to a carbon not adjacent to heteroatoms[18] and is assigned to A6. The remaining resonances, a singlet at 73.4

(15) H. Spiesecke and W. G. Schneider, *J. Chem. Phys.*, **35**, 722 (1961).
(16) B. V. Cheney and D. M. Grant, *J. Amer. Chem. Soc.*, **89**, 5319 (1967).
(17) D. M. Grant and B. V. Cheney, *J. Amer. Chem. Soc.*, **89**, 5315 (1967).
(18) M. Christl, H. J. Reich, and J. D. Roberts, *J. Amer. Chem. Soc.*, **93**, 3463 (1971).

ppm and a triplet at 68.0 ppm, are assigned to A4 and A5, respectively, on the basis of their multiplicities.

Proceeding next to consideration of the pseudodisaccharides and pseudotrisaccharides, the resonance falling near 23 ppm is assigned to A6 (see Figure 4). The nearly constant value of this resonance is consistent with the distance of the A6 position from the region of structural changes. The other resonance in the *C*-methyl region must be assigned to position C7. The 4.0-ppm upfield shift of this resonance in gentamine C_1 and gentamicin C_1 compared with the C_2 structures can be reasonably ascribed to the introduction of the *N*-methyl group γ to C7. The correlations given above are indicated by the dashed lines in Figure 4.

The resonances falling close to 38 ppm have all been assigned to carbons at A7 based on the assignment of the 38.0-ppm resonance to A7 in methyl β-garosaminide and the expected insensitivity of the A7 resonances to structural changes in the C ring. This assignment was also confirmed for the case of gentamicin C_2 by the selective decoupling of the A7 methyl protons at δ 2.47.

In gentamine C_1 the quartet found in the SFOR spectrum at 33.6 ppm can unambiguously be assigned to C8. Since the addition of ring A to form gentamicin C_1 is expected to have very little effect on the C8 resonance, this gentamicin C_1 resonance has been assigned to the peak found at 33.7 ppm.

The set of resonances at 36.4 to 36.8 ppm is close in chemical shift value only to B2 of 2-deoxystreptamine (37.0 ppm). These resonances in the pseudotrisaccharides and gentamines are assigned, therefore, to carbon B2. Gentamine C_1 yields a triplet for this resonance in the SFOR decoupled spectrum.

Selective proton irradiation of the ring-C methylene envelope, centered at δ 1.6, in gentamine C_1 demonstrated the two resonances between 26.2 and 27.3 ppm to belong to carbons C3 and C4 (Figure 3). The two remaining unassigned resonances between 10 and 40 ppm for each of the other gentamines and gentamicins must also arise from carbons at these positions. As examination of Figure 4 shows, there is a striking comparison between each gentamicin and the corresponding gentamine. It is not surprising that the presence or absence of ring A has little effect on these resonances. Furthermore, C3 is expected to be the least influenced by structural variation in the six compounds, since in a comparison of pyranose sugars with their 6-deoxy derivatives, Dorman and Roberts found only small differences between chemical shifts of the C-1, -2, and -3 carbons, while large changes occurred among the C-4 and C-5 resonances.[5] On this basis the C3 resonance is assigned to the peaks falling between 27.0 and 27.4 ppm. If this assignment were reversed in gentamicin C_{1a} and gentamine C_{1a}, which lack the C6-methyl group, it would follow that the C6-methyl substituent effect was the same at C3 and C4, about 1 ppm. By assigning C3 consistently to the resonances found at about 27 ppm, the upfield γ shift at C4 upon addition of a methyl group at C6 becomes about 2 ppm, which compares favorably with the results of Grant and Paul.[14] Although the methyl substituent effects were obtained by these workers for open chain hydrocarbons, the effects observed here are of roughly comparable magnitude and of the same sign.

The remaining A ring resonances, A1 through A5, are expected to be constant throughout the entire series of compounds. These resonances were unambiguously assigned in methyl β-garosaminide and have been correlated in the manner shown in Figure 4. The other resonances in the anomeric carbon region of each pseudotrisaccharide must belong to carbon C1. These ^{13}C chemical shifts fall in the range of 100.6 to 102.7 ppm, depending on the structure of ring C. The chemical shift of the resonance assigned to C1 for each gentamicin is very close to the value of the single resonance found in the anomeric region of the cmr spectrum of the respective gentamine, confirming the assignment of A1 and C1.

In the pseudotrisaccharides the two resonances falling between 85 and 89 ppm have been assigned to positions B4 and B6, since these values are typical of carbon atoms bearing an ether linkage.[4] Each gentamine has only one ether linkage and only one resonance within 10 ppm of this range; therefore, this resonance may be unambiguously assigned to carbon B4. Since the B4 resonance is not expected to vary significantly with introduction of the A ring, the corresponding resonances of the gentamicins (88.3 to 88.7 ppm) are also assigned to B4 as shown in Figure 4.

The other set of resonances, falling between 87.7 to 87.9 ppm, is assigned to B6. In sisomicin the resonance at 87.8 ppm is assigned to B6 and the resonance at 85.3 ppm to B4. Obviously the significant change in C-ring structure in sisomicin is more likely to exert its effect on the B4 resonance than on the more distant B6 resonance. In the gentamines the resonances at 78.3 and 78.4 ppm are assigned to B6, which resonance occurred at 78.5 ppm in 2-deoxystreptamine. The downfield β shifts upon substitution at B4 and B6 vary between 9.3 and 10.4 ppm. These values are only slightly larger than the values determined for methyl substitution.[4,5]

The resonance for B5 has been assigned as indicated in Figure 4, which also reveals the striking constancy for this resonance in the four trisaccharides and among the three gentamines. There is very little difference in chemical shift between the gentamine B5 resonances and the 2-deoxystreptamine resonance. Addition of the A ring to the gentamines produces an upfield γ shift of $\sim$1.3 ppm in the gentamicin B5 resonances.

Dorman and Roberts report shielding at carbons bearing equatorial substituents upon γ methylation of vicinal equatorial hydroxyl groups of 0.7 $\pm$ 0.2 ppm.[4,5,19] These workers also report marked differences in the shielding of carbons which interact sterically to different extents with a substituent introduced γ to them. Thus, in 1-*O*-methyl-*myo*-inositol, the C2 carbon, which bears an axial hydroxyl group, is shielded 4.2 ppm by the *O*-methyl substituent, while the C6 carbon is deshielded 0.5 ppm.[4] In general, the γ effect on the carbon which does not interact sterically with the substituent is small and variable.[19] The difference in substituent γ effects on two carbons in cyclitol or pyranose rings is thought to be indicative of rotamer population of ether linkages.[19] The regularity of these effects helped in the resonance assignments of methylated inositols and in the study of solution conformation of disaccharides.[4,19]

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

Since addition of ring C at B4 of 2-deoxystreptamine to form the gentamines did not cause shielding of the B5 carbon, a preferred rotamer population of the B4-oxygen bond as shown in Figure 5a is indicated. The comparatively large shielding of the B5 resonance upon addition of ring A to form the gentamicins, however, indicates a preferred rotamer at B6 as shown in Figure 5b. The rotamer shown in 5c is not considered likely due to the presence of two gauche interactions. It is shown below that addition of ring A to the gentamines does not produce shielding of the B1 resonances. Note that Figure 5a shows that ring C in the gentamines and pseudotrisaccharides should shield the B3 resonance. Use of this is made below in differentiating between the B1 and B3 resonances.

The only remaining unassigned resonance in the oxygen-bearing region must belong to C5. Comparison of the C_{1a} and C_2 compounds shows a downfield β C-methylation shift of 3.0 ppm for this resonance. Comparison of the C_1 and C_2 compounds yields an upfield γ N-methylation shift of 1.7 ppm.

The resonances lying from 43 to 59 ppm are the most difficult to assign and require detailed consideration of the probable effects of structural changes on the shifts. The remaining positions which are to be assigned are B1, B3, C2, and C6. Of these, the C6 resonance is expected to show large and relatively predictable changes upon substitution at C_6 and at the C6 nitrogen atom. Furthermore, the C6 resonances are expected to be similar for each gentamicin and its corresponding gentamine. The C2 resonances are expected to show very little variation among the gentamicins but should change appreciably with insertion of the double bond in sisomicin. Moreover, the C2 gentamicin resonances should be quite similar to the corresponding gentamine values.

As indicated in Figure 5a, the B3 resonance might be expected to show some variation among the pseudotrisaccharides, as do the B4 resonances, due to structural changes in ring C. Addition of ring C to 2-deoxystreptamine to form the gentamines is expected to shift the B3 resonance about 1-ppm upfield. Ring-C substitution at B4 should have an effect of less than 0.5 ppm on the B-1, -2, and -6 resonances.

The B1 resonance should show no significant variation with ring-C structure. The adjacent B2 and B6 resonances varied only 0.4 ppm or less among the four pseudotrisaccharides. Unfortunately, for purposes of positive identification of this resonance, addition of ring A to the gentamines is not expected to induce an upfield γ shift at B1, unless ring A lies as in Figure 5c, in the preferred rotamer.

On the basis of these considerations, the B1, B3, C2, and C6 resonances have been assigned and correlated as shown in Figure 4. These assignments for B1, B3, and C2 are based on relatively small experimental shift differences and are presented here as plausible though not necessarily conclusive. With the assignments as indicated, the B1 resonance falls at 51.3 ppm for all the gentamines, while in the four pseudotrisaccharides it lies at 51.7 to 51.8 ppm.

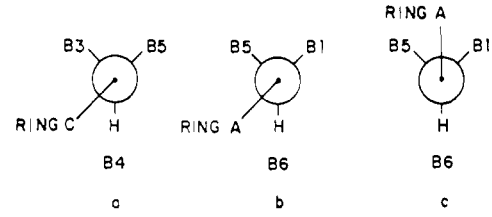


Figure 5. Rotamers about the B4–oxygen (a) bond and the B6–oxygen bond (b, c) in the pseudotrisaccharides.

In contrast, the B3 resonances of the pseudotrisaccharides lie in a wider range, 50.3 to 50.9 ppm. These resonances are not significantly different from those in the corresponding gentamines. As expected from consideration of Figure 5a, there is a 1-ppm upfield γ shift of the B3 resonance upon addition of ring C to 2-deoxystreptamine.

The set of resonances assigned to C2 shows a change of 0.3 ppm or less between the gentamines and corresponding gentamicins and no significant changes among the three pseudodi- or pseudotrisaccharides. In sisomicin, the only resonances within 3.4 ppm of this range have already been assigned to B1 and B3. The resonance at 47.6 ppm is tentatively assigned to the sisomicin C2 carbon. The C2 resonance, therefore, experiences a shift of at least 3.4 ppm upon introduction of the double bond into ring C.

Similarly, for the C6 resonance, the change upon going from the gentamines to the corresponding gentamicins is in the range of 0.1 to 0.3 ppm, while this resonance experiences an α-substituent effect of 4.0 to 4.2 ppm, comparing the C_{1a} and C_2 gentamines and gentamicins, and a β-substituent effect of about 8 ppm, comparing the C_2 and C_1 gentamines and gentamicins. The resonance at 43.5 ppm in sisomicin is tentatively assigned to this carbon.

Aminoglycosides react readily with carbon dioxide and one of the specific concerns of this study has been exclusion of carbon dioxide from the samples. Spectra obtained on samples exposed to atmospheric carbon dioxide show shifts outside the range of deviation reported in this study. Aminoglycosides form simple carbonate salts which are easily neutralized. Additionally, aminoglycoside–carbon dioxide adducts are formed which are not so readily destroyed. An example is given in the table for gentamicin C_{1a}–carbon dioxide adduct (**6**). All other samples for which data are reported in the table were treated to remove CO_2. Upon addition of excess CO_2 to a solution of gentamicin C_{1a}, multiple resonances were observed for most of the carbon nuclei.

The nature of these aminoglycoside–carbon dioxide adducts is not known; however, based on their properties and chemical reactivities it seems unlikely that they are simple carbonate salts.

Acknowledgment. The authors are grateful to Dr. George Gray of Varian Associates and Mr. Ray Brambrilla of Stevens Institute for the determination of many of the cmr spectra.