(6) (a) M. J. Robins, J. R. McCarthy Jr., R. A. Jones, and R. Mengel, *Can. J. Chem.*, **51**, 1313 (1973); (b) T. C. Jain, A. F. Russell, and J. G. Moffatt, *J. Org. Chem.*, **38**, 3179 (1973).
(7) E. M. Acton, K. J. Ryan, and D. W. Henri, Abstracts, VIIth International Symposium on Carbohydrate Chemistry, Bratislava, 1974, p 70.
(8) (a) F. L. Rose, *J. Chem. Soc.*, 4116 (1954); (b) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **23**, 191 (1958).
(9) (a) E. C. Taylor and A. L. Borror, *J. Org. Chem.*, **26**, 4967 (1961); (b) W. Ried and R. Giesse, *Justus Liebigs Ann. Chem.*, **713**, 149 (1968).
(10) T. Huynh-Dinh, A. Kolb, G. Barnathan and J. Igolen, *J. Chem. Soc., Chem. Commun.*, 680 (1973).
(11) R. K. Robins, F. W. Furcht, A. D. Grauer, and J. W. Jones, *J. Am. Chem. Soc.*, **78**, 2418 (1956).
(12) (a) J. A. McCloskey in "Basic Principles in Nucleic Acid Chemistry", P. O. P. Tso', Ed., Academic Press, New York, N.Y., 1974, p 209; (b) S. J. Shaw, D. M. Desiderio, K. Tsuboyama, and J. A. McCloskey, *J. Am. Chem. Soc.*, **92**, 2510 (1970).
(13) A. Kolb, Thèse de Doctorat d'Etat ès Sciences Physiques, Université Paris VI, 1974.
(14) L. B. Townsend and R. K. Robins, *J. Heterocycl. Chem.*, **6**, 459 (1969).
(15) P. F. Crain, J. A. McCloskey, A. F. Lewis, K. H. Schram, and L. B. Townsend, *J. Heterocycl. Chem.*, **10**, 843 (1973).
(16) D. C. Dejongh in "Synthetic Procedures in Nucleic Acid Chemistry", Vol. 2, W. W. Zorbach and R. S. Tipson, Ed., Wiley-Interscience, New York, N.Y., 1973, p 145.
(17) L. B. Townsend in ref 16, p 267.
(18) R. U. Lemieux and M. Hoffer, *Can. J. Chem.*, **39**, 110 (1961).
(19) M. J. Robins and R. K. Robins, *J. Am. Chem. Soc.*, **87**, 4934 (1965).
(20) T. Huynh-Dinh, A. Kolb, C. Gouyette, and J. Igolen, *J. Heterocycl. Chem.*, **12**, 111 (1975).
(21) U. Sequin and C. Tamm, *Helv. Chim. Acta*, **55**, 1196 (1972).
(22) S. David and J. C. Fisher, *Carbonhydr. Res.*, **18**, 39 (1971).
(23) This rule is not observed on 2'-*O*-methylformycin: M. J. Robins, S. R. Naik, and A. S. K. Lee, *J. Org. Chem.*, **39**, 1891 (1974).
(24) T. L. V. Ulbricht in ref 16, p 177.
(25) Unpublished results.
(26) G. Barnathan, Thèse de Doctorat 3° cycle, Université Paris VI, 1974.

The Gentamicin Antibiotics. 7.[1a] Structures of the Gentamicin Antibiotics A_1, A_3, and A_4

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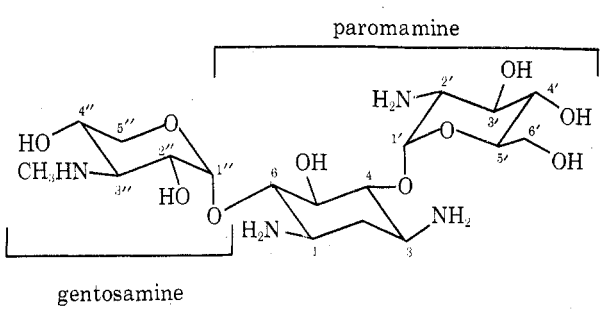
The structures of the gentamicin antibiotics A_1, A_3, and A_4 coproduced with other gentamicins in submerged fermentations of *Micromonospora purpurea* and *Micromonospora echinospora* have been elucidated by proton and carbon-13 magnetic resonance spectroscopy in conjunction with mass spectrometry. Gentamicin A_1 and A_3 are 4-*O*-(2'-amino-2'-deoxy-α-D-glucopyranosyl)-6-*O*-(3''-methylamino-3''-deoxy-β-L-arabinopyranosyl)deoxystreptamine and 4-*O*-(6'-amino-6'-deoxy-α-D-glucopyranosyl)-6-*O*-(3''-methylamino-3''-deoxy-β-L-arabinopyranosyl)deoxystreptamine, respectively. Gentamicin A_4 is 3''-*N*-formylgentamicin A.

Gentamicin A is coproduced with other gentamicins in submerged fermentations of *Micromonospora purpurea* and *Micromonospora echinospora*.[1b,2] Its structure was elucidated by Maehr and Schaffner[3,4] and is shown below.



gentamicin A

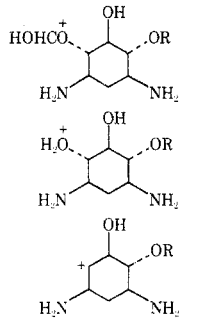
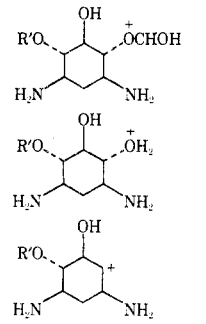
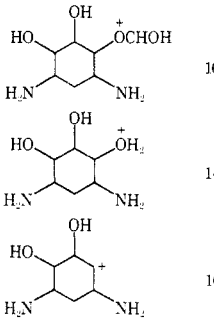
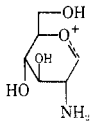
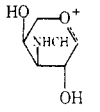
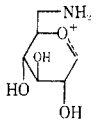
Recent investigations in this laboratory have revealed the presence of four new deoxystreptamine-containing antibiotics in crude preparations of gentamicin A which we have designated gentamicins A_1, A_2, A_3, and A_4. The elucidation of the structures of A_1, A_3, and A_4 is the subject of this communication. The structure of gentamicin A_2 is published in the accompanying note.[5a]

Gentamicins A_1, A_3, and A_4 could be separated from A and from each other by thin layer chromatography on silica gel using chloroform–methanol–ammonium hydroxide (3:4:2) as the developer. On a typical chromatogram A_1, A_3, and A_4 had R_A[5b] values of 0.78, 0.40, and 1.62, respectively. Isolation of these compounds in high states of purity was effected by chromatography of the crude mixture on a column of silica gel using the above-mentioned eluent, and in the case of A_1 and A_3 by rechromatography on Dowex 1-X2 ion exchange resin in the hydroxide cycle using water as the eluent.[6,7]

Structures of Gentamicins A_1 and A_3. The proton noise decoupled ^{13}C NMR spectra of A_1 and A_3 were very similar to that of A and indicated the presence of 18 carbon atoms in each compound (Table III). The mass spectra of A_1 and A_3 were also very similar to that of A. Each exhibited a peak at *m/e* 469 attributable to the $(MH)^+$ ion as previously indicated for A.[8] It was apparent, therefore, that A, A_1, and A_3 were isomers. The elemental analyses were consistent with the compositions $C_{18}H_{36}N_4O_{10}\cdot H_2O$ for A_1 and $C_{18}H_{36}N_4O_{10}\cdot 4HCl$ for the hydrochloride salt of A_3, further supporting the above contention.

Hydrolysis of gentamicins A, A_1, and A_3 with 6 *N* hydrochloric acid at 100° for 1 hr followed by paper chromatographic analysis of the hydrolyzate clearly indicated the presence of deoxystreptamine in all of them. Glucosamine and paromamine were present only in the hydrolyzates of A and A_1. The hydrolyzate of A_3 contained 6-amino-6-deoxyglucose. Furthermore, this comparative study indicated the absence of gentosamine (3-methylamino-3-deoxy-D-xylose), one of the hydrolysis products of gentamicin A, in the hydrolyzates of A_1 and A_3, but the presence of another sugar whose R_f was very close to, but not identical with, that of gentosamine. These data strongly suggested, therefore, that A_1 was an isomer of A in the gentosamine moiety, and A_3 was an isomer of A_1 in the glucosamine moiety. Recently, Mallams and coworkers[9a] in our laboratories isolated two new antibiotics named 66-40B and 66-40D from *Micromonospora inyoensis* and showed these to possess the following structures.

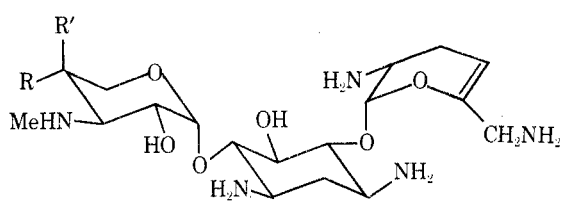
Table I
Prominent Mass Spectral Fragment Ions of Gentamicins A_1 and A_3[a]

m/e	Ion	m/e	Ion	m/e	Ion	m/e	Ion
352		336		191		162	
−CO		−CO		−CO			
324		308		163		146	
$-H_2O$		$-H_2O$		$-H_2O$			
306		290		145		162	
$-H_2O$		$-H_2O$		$-H_2O$			
334		318		173			

[a] In gentamicin A_1, R = 2′-amino-2′-deoxy-α-D-glucopyranosyl and R′ = 3″-methylamino-3″-deoxy-β-L-arabinopyranosyl; in gentamicin A_3, R = 6′-amino-6′-deoxy-α-D-glucopyranosyl and R′ = 3″-methylamino-3″-deoxy-β-L-arabinopyranosyl.

Table II
Proton Magnetic Resonance Parameters of Comparative Aminoglycosides

		Gentamicin A		Gentamicin A_1		Gentamicin A_3		6640-D	
H	J (H,H)	δ	Multiplicity, J, Hz	δ	Multiplicity, J, Hz	δ	Multiplicity, J, Hz	δ	Multiplicity, J, Hz
2ax		1.19	q	1.20	q	1.22	q		
	2ax, 2eq		12.5		12.5		12.5		
	1, 2ax		12.5		12.5		12.5		
	2ax, 3		12.5		12.5		12.5		
2eq		1.94	d,t	1.95	d,t	1.98	d,t		
	1, 2eq		3.5		3.5		3.5		
	2eq, 3		3.5		3.5		3.5		
1′		5.23	d	5.26	d	5.35	d		
	1′, 2′		3.5		3.5		3.8		
2′		2.77	d,d	2.78	d,d	Not observed			
	2′, 3′		10.0		10.0				
1″		4.99	d	5.08	d	5.09	d	5.05	d
	1″, 2″		3.5		4.0		4.0		4.0
2″		3.61	d,d	3.81	d,d	3.86	d,d	3.79	d,d
	2″, 3″		9.5		10.5		10.5		10.0
3″		2.70	t	Not observed		2.92	d,d	2.78	d,d
	3″, 4″		9.5				3.0		3.0
4″		3.26	d,t	4.12	br s	4.13	br s	4.10	br s
	4″, 5″ax		9.5						
	4″, 5″eq		5.0						
5eq″				4.18	br d	4.18	br d	4.15	br d
	5eq″, 5ax″				12.5		12.5		12.5
3″NMe		2.49	s	2.37	s	2.43	s	2.35	s



R = OH; R′ = H = 66-40B
R = H; R′ = OH = 66-40D

The presence of gentosamine in 66-40B and its 4″ epimer in 66-40D suggested that gentamicin A and A_1 might similarly be related. The unknown component in the acid hydrolyzates of both A_1 and A_3 had the same R_f on a thin layer chromatogram as that of 3-methylamino-3-deoxy-L-arabinose.[9b] On the basis of these findings and in view of the likely common biogenesis of these compounds, gentamicin A_1 was thought to be 6-*O*-(3″-methylamino-3″-deoxy-β-L-arabinopyranosyl)paromamine (**1**) and gentamicin A_3, 4-*O*-(6′-amino-6′-deoxy-α-D-glucopyranosyl)-6-*O*-(3″-methylamino-3″-deoxy-β-L-arabinopyranosyl)deoxystreptamine (**2**). Substantiating evidence was obtained from the physical methods delineated below.

The mass spectra of A_1 and A_3 showed the expected prominent fragment ions for the above structures and are explained in Table I.

The proton magnetic resonance parameters for gentamicins A, A_1, A_3 and antibiotic 66-40D are listed in Table II. It may be seen from Table II that the spectra of A, A_1, and A_3 have in common only the H-2_{ax} and H-2_{eq} resonances of deoxystreptamine and an *N*-methyl singlet. Gentamicin A_1 also gives rise to an anomeric doublet (H-1′) and a multi-

plet (H-2′) which are mutually coupled and compare well with the corresponding resonances in gentamicin A. This portion of the spectrum is therefore consistent with that expected from the presence of the paromamine moiety in gentamicin A_1. Similar resonances are not found in the spectrum of gentamicin A_3.

Resonances assigned to H-1″, H-2″, H-4″, and H-5″$_{eq}$ of gentamicins A_1, A_3 and antibiotic 66-40D are also listed in Table II. A resonance for H-3″ could not be distinguished in the spectrum of gentamicin A_1. In all other cases listed in the table, INDOR techniques were used to observe any protons not clearly resolved from the envelope of a spectrum. All assignments were confirmed by spin decoupling. Although the proton chemical shifts of aminoglycoside antibiotics are sensitive to changes in pH and therefore to sample preparation and handling, it may be seen that the characteristic resonances of the 4″-*epi*-gentosamine residue of the antibiotic 66-40D are also found in the spectra of gentamicin A_1 and A_3, supporting the presence of a 3-methylamino-3-deoxy-β-L-arabinopyranosyl moiety in their structures.

The unambiguous assignments of structure **1** to A_1 and **2** to A_3 were finally possible from proton noise decoupled carbon-13 magnetic resonance spectroscopy. The ^{13}C chemical shifts of gentamicins A, A_1, and A_3 and of the reference compounds methyl 3-methylamino-3-deoxy-α-D-xylopyranoside, methyl 3-methylamino-3-deoxy-β-L-arabinopyranoside, paromamine, and kanamycin A are given in Table III. Recent work of Morton and coworkers,[10] Lemieux and Koto,[11] Woo and Westland,[12] Omoto and his associates,[13] and Koch and her collaborators[14] clearly demonstrate that the carbon resonances in aminoglycoside antibiotics can readily be assigned by a combination of techniques including simple comparison of ^{13}C chemical shifts with those of adequate model substances and, as first shown by Lemieux and Koto[11] in the aminoglycoside antibiotics, by utilizing the β-carbon shifts that occur on protonation of the amino groups. The latter two techniques were employed for making assignments in the present work. The presence of the paromamine moiety in both A and A_1 is readily apparent when the chemical shifts of carbon atoms 1–5 and 1′–6′ in these compounds are compared with the corresponding resonances for paromamine. The chemical shifts of paromamine obtained in our laboratory agreed, within experimental errors, with those reported by Woo and Westland[12] and, except for the C-2′ resonance (56.1 ppm), also with those reported by Omoto and coworkers (52.2 ppm).[13] A comparison of the chemical shifts of carbon atoms 1″ to 5″ and the *N*-methyl of A_1 with the corresponding carbon atoms of methyl 3-methylamino-3-deoxy-β-L-arabinopyranoside clearly confirmed the presence of the latter sugar moiety in A_1. The validity of such a comparison in structural assignments is apparent from the close agreement of the chemical shifts of carbon atoms 1″–5″ and the *N*-methyl group of gentamicin A and the corresponding carbon atoms of methyl 3-*N*-methylamino-3-deoxy-α-D-xylopyranoside. The position of linkage of the 3-methylamino-3-deoxy-β-L-arabinopyranosyl unit to paromamine in gentamicin A_1 was readily established by comparison with gentamicin A, in which the linkage of the corresponding α-D-xylo unit to paromamine is at 6. As expected,[10] the C-6 of paromamine experienced a deshielding of 9.6 ppm and C-5 a shielding of 1.7 ppm on substituting the hydroxyl hydrogen atom at 6 by the α-gentosaminyl residue. At pD 2, these shifts were 9.7 and 1.1 ppm, respectively. Furthermore, C-6 in A experienced a shielding of 3.7 ppm on acidification. Similar analysis in the case of gentamicin A_1 revealed that $\delta_{C\text{-}6}(A_1) - \delta_{C\text{-}6}(Par) = 9.3$ ppm, $\delta_{C\text{-}5}(A_1) - \delta_{C\text{-}5}(Par) = -1.6$ ppm, $[\delta_{C\text{-}6}(A_1) - \delta_{C\text{-}6}(Par)]_{pD2} = 9.9$ ppm, $[\delta_{C\text{-}5}(A_1) - \delta_{C\text{-}5}(Par)]_{pD2} = -0.9$ ppm, and $\delta_{C\text{-}6}(A_1) - \delta_{C\text{-}6,pD2}(A_1) = 3.2$ ppm. These values are in excellent agreement with those of gentamicin A, and hence it can be concluded that linkage of 3″-methylamino-3″-deoxy-β-L-arabinopyranosyl residue to paromamine in gentamicin A_1 is at 6.

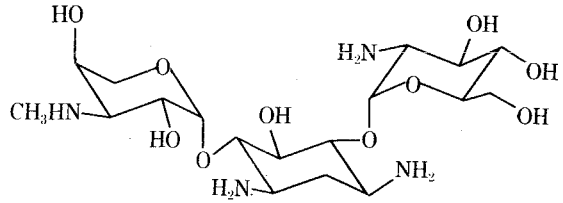
The chemical shifts of carbon atoms 1–6 and 1′–6′ in gentamicin A_3 agree extremely well with those of the corresponding carbon atoms of kanamycin A, confirming the presence of 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl) unit in the latter. The chemical shifts of carbon atoms 1″–5″ and *N*-methyl, on the other hand, are, within experimental error, identical with those of the corresponding atoms in gentamicin A_1, confirming the presence of 6-*O*-(3″-methylamino-3″-deoxy-β-L-arabinopyranosyl) moiety in A_3. The structures of A_1 and A_3 are shown above Table III.

It is noteworthy that in gentamicins A_1 and A_3, which have an axial hydroxyl group on C-4″, the β shifts of C-4″ on acidification are 1.4 and 1.3 ppm, respectively, compared to 4.4 ppm in the case of A in which the hydroxyl group at C-4″ is in the equatorial orientation. In kanamycins A and B the corresponding β shifts are 3.8 and 3.5 ppm, respectively.[11] Also, on acidification, the *N*-methyl carbon atoms in A_1 and A_3 are shielded by 1.7 and 1.6 ppm, respectively, as compared to 3.7 ppm in gentamicin A.

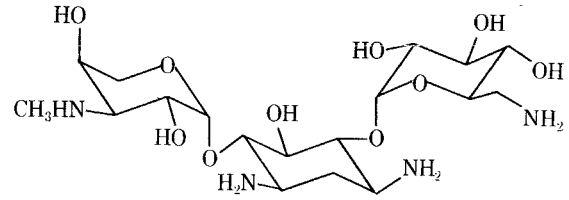
Structure of Gentamicin A_4. The ^{13}C NMR spectrum of gentamicin A_4 (Table III) showed the presence of 19 carbon atoms in the molecule, including a carbonyl carbon atom (167.5 ppm). The molecular weight of A_4 was found to be 28 units higher than that of A from mass spectrometry and the ^1H NMR spectrum showed a peak at 8.00 ppm attributable to a *N*-formyl proton. Therefore, it was apparent that A_4 was a formyl derivative of one of the gentamicin A's. The spectrum also showed the presence of two anomeric protons, a doublet at δ 5.21 ppm (H-1′, $J_{1',2'} = 3.8$ Hz) and a doublet at δ 5.11 ppm (H-1″, $J_{1'',2''} = 3.8$ Hz), and an *N*-methyl group as a singlet at δ 2.79 ppm. The characteristic signals due to the deoxystreptamine methylene protons were at δ 1.88 and 1.12 ppm. The presence of a quartet at δ 2.75 ppm with spacings of 9.0 ($J_{2',3'}$) and 3.75 Hz ($J_{1',2'}$) indicated a glucosamine moiety and therefore suggested that A_4 was a derivative of either A or A_1 but not of A_3.

Analysis of the ^{13}C NMR spectra of A_4 at basic and acidic pD's and comparison of the chemical shifts with the corresponding values of A indicated the location of the formyl group in A_4 at 3″. As seen in Table III, the C-1 to C-6 and C-1′ to C-6′ resonances of A_4 were almost identical with the corresponding resonances of gentamicin A, confirming the presence of the paromamine moiety in the latter. The *N*-methyl carbon in A_4 was shielded by 8.5 ppm relative to this resonance in gentamicin A. In contrast to an upfield shift of 3.7 ppm of the *N*-methyl carbon atom on acidification of gentamicin A, the chemical shift of the *N*-methyl carbon atom of A_4 did not change on acidification. These observations and the fact that the C-5″ and C-1″ resonances of A and A_4 were identical, within experimental error, and the C-2″ and C-4″ resonances in A_4 appeared shielded by 4.1 and 4.5 ppm, respectively (β shifts) in A_4, established the structure of A_4 to be 3″-*N*-formylgentamicin A (**3**). As predicted from the structure, on protonation of the amino groups only the paromamine moiety of A_4 showed the expected changes in the chemical shifts (Table III).

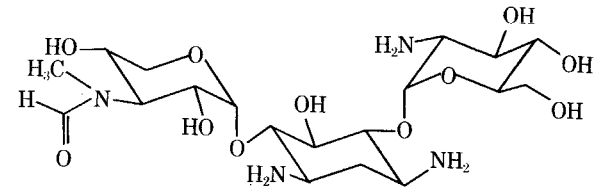
Finally, the mass spectrum of gentamicin A_4 gave a series of ions at *m/e* 352, 334, 324, 306, 191, 173, 163, 145, and 179 for the paromamine moiety[8] and ions at *m/e* 174 and 364, 346, 336, and 318 for the 3″-*N*-formylgentosaminyl deoxystreptamine unit consistent with the assigned structure.



gentamicin A_1 (1)



gentamicin A_3 (2)



gentamicin A_4 (3)

Table III
Carbon-13 Chemical Shifts of Comparative Aminoglycosides[a]

Carbon	G-A	Par	R[c]	G-A_1	R'[c]	G-A_3	K-A[b]	G-A_4	G-A pD 2	G-A_1 pD 2	G-A_3 pD 2	K-A[b] pD 3.6	Par pD 2	G-A_4 pD 2	$\delta_{base} - \delta_{salt}$ G-A	G-A_1	G-A_3	G-A_4	Par	K-A[b]
1	51.5	51.1		51.5		51.4	51.0	51.5	50.3	50.6	50.7	50.4	50.7	50.4						
2	36.5	36.7		36.4		36.2	36.1	36.5	28.5	29.4	28.4	28.1	29.3	28.6	8.0	7.0	7.8	7.9	7.4	8.0
3	50.3	50.3		50.3		49.8	49.6	50.2	49.6	49.7	48.6	48.3	50.3	49.6						
4	88.6	88.8		88.5		87.7	87.6	88.5	80.9	81.9	79.1	78.8	81.4	81.1	7.7	6.6	8.6	7.4	7.4	8.8
5	75.1	76.8		75.2		75.0	74.8	75.1	74.5	74.7	73.1	73.4	75.6	74.5						
6	87.9	78.3		87.6		88.1	88.4	88.0	84.2	84.4	84.7	84.3	74.5	84.2	3.7	3.2	3.4	3.8	3.8	4.1
1'	101.7	102.0		101.7		100.4	99.9	101.6	97.7	97.9	96.6	96.1	97.9	97.8	4.0	3.8	3.8	3.3	4.1	3.8
2'	56.2	56.1		56.2		72.6	72.4	56.2	54.8	55.0	71.7	71.4	55.1	54.8						
3'	74.7	74.6		74.6		73.7	73.5	74.6	69.8	70.2	73.1	72.8	70.1	69.8	4.9	4.4		4.8	4.5	
4'	70.9	70.8		70.8		71.9	71.7	70.8	70.3	70.4	71.7	71.4	70.4	70.3						
5'	73.8	73.8		73.8		73.2	72.9	73.8	74.5	74.5	69.5	69.3	73.3	74.5			3.7			3.6
6'	61.6	61.6		61.6		42.2	42.0	61.6	61.5	61.3	41.3	41.0	61.3	61.3						
1''	100.8		100.1	101.2	100.3	101.1	100.4	100.7	101.4	101.8	102.0	100.7		101.8						
2''	70.9		70.5	68.6	68.2	68.3	72.4	66.8	67.1	64.8	64.7	68.7		66.7	3.8	3.8	3.6			3.7
3''	62.8		62.8	59.3	59.2	59.3	54.9	64.2	61.3	59.1	59.1	55.7		63.8						
4''	68.7		68.6	64.6	63.9	64.6	70.0	64.2	64.3	63.2	63.3	66.2		63.8	4.4	1.4	1.3			3.8
5''	63.0		62.3	65.3	65.3	65.1	72.9	62.8	63.3	66.3	66.4	72.8		63.4						
6''							61.1					60.7								
N-CH_3	34.2		34.3	32.6	32.5	32.4		25.7	30.5	30.9	30.8			25.8	3.7	1.7	1.6			
N-CHO								167.5						167.8						

[a] G-A = gentamicin A; Par = paromamine; R = methyl 3-methylamino-3-deoxy-α-D-xylo-pyranoside; G-A_1 = gentamicin A_1; R' = methyl 3-methylamino-3-deoxy-β-L-arabinopyranoside; G-A_3 = gentamicin A_3; K-A = kanamycin A; G-A_4 = gentamicin A_4. [b] See ref 11. [c] See ref 9.

Experimental Section

Thin layer chromatography was performed on silica gel GP (Analtech, Inc., Newark, Del.) using, unless otherwise specified, chloroform–methanol–ammonium hydroxide (3:4:2) as the developing phase. Column chromatography was carried out on silica gel (60-200 mesh, J. T. Baker Chemical Co., Phillipsburg, N.J.) using, unless otherwise specified, the same solvent system as above and on Dowex 1-X2 (200–400 mesh, hydroxide form, Sigma Chemical Co., St. Louis, Mo.) with water as the eluent.

The ^1H NMR spectra were recorded on a Varian Associates XL-100-15 spectrometer. Chemical shifts are given in δ values for solutions in deuterium oxide, flushed with nitrogen, using DSS as the internal standard. ^{13}C NMR spectra were obtained at 25.2 MHz on a Varian XL-100-15 spectrometer in the pulsed mode and Fourier transform to the frequency domain was accomplished with a Varian 620L-16K computer. ^{13}C chemical shifts are given in parts per million downfield from Me_4Si for solutions in deuterium oxide. The spectra were recorded with an internal dioxane reference and the expression δ_C (Me_4Si) = δ_C (dioxane) + 67.4 was employed to express the chemical shifts downfield from Me_4Si.[10] Mass spectra were obtained on a Varian MAT CH5 spectrometer at 70 eV with a probe temperature of 200–250°.

Isolation of Gentamicin A_1. A sample of crude gentamicin A (5.5 g) isolated as previously described[1b,2] and enriched in A_1 (R_A 0.78) was chromatographed on silica gel (600 g) using a column 5 cm in diameter. Ten-milliliter fractions were collected. Tubes 395–441, which contained gentamicin A_1, were pooled, concentrated, and lyophilized to give 0.28 g. Fractions 292–394 contained both gentamicins A and A_1, and after removal of the solvents in vacuo the residue was rechromatographed on the same column used above. After 500 ml of eluate 10-ml fractions were collected. Tubes 222–282 contained pure gentamicin A_1 and after work-up as above gave 0.386 g. Tubes 194–217 (0.5 g) contained mainly gentamicin A_1 and after work-up as above this was combined with the 0.28 g of material obtained above and chromatographed on a 3 × 72 cm column of Dowex ion exchange resin collecting 10-ml fractions. The homogeneous fractions (tubes 36–42) were pooled and concentrated to dryness. The residue was dried and dissolved in a minimum amount of methanol. Addition of excess ether precipitated pure gentamicin A_1, which was isolated by filtration, washed with ether, and dried to give 0.35 g, $[\alpha]^{26}D$ +141° (*c* 0.44, water), $[\theta]_{TaCu}^{280}$ − 12,190.

Anal. Calcd for $C_{18}H_{36}N_4O_{10}\cdot H_2O$: C, 44.44; H, 7.87; N, 11.52. Found: C, 44.75; H, 7.75; N, 11.57.

Isolation of Gentamicin A_2. In a manner similar to that described above, crude gentamicin A (36 g)[1b,2] was chromatographed on silica gel (3 kg) collecting 25-ml fractions. Tubes 811–900 were pooled, concentrated, and lyophilized to give partially pure (3 g) gentamicin A_2 (R_A 1.18). This product was again chromatographed on a Dowex ion exchange resin column (5.5 × 49 cm). Five-milliliter fractions were collected. Tubes 75–105 were pooled, concentrated, and lyophilized to give 1.84 g of pure A_2, $[\alpha]^{26}D$ +141° (*c* 0.4, water).

Anal. Calcd for $C_{17}H_{33}N_3O_{11}\cdot 2H_2O$: C, 41.54; H, 7.59; N, 8.55. Found: C, 41.36; H, 6.94; N, 8.29.

Isolation of the Gentamicins A_3 and A_4. Crude gentamicin A (112 g)[1b,2] was chromatographed on two 5-kg silica gel columns (12 × 152 cm) attached in series. Two-liter fractions were collected. Fractions 53–62 contained mainly gentamicin A_3 (R_A 0.40) and after work-up as above gave 6.5 g of crude A_3. In a similar manner work-up of tubes 27–31 yielded 7.5 g of crude gentamicin A_4 (R_A 1.62).

The above product of gentamicin A_3 (2 g) was rechromatographed on Dowex ion exchange resin (300 ml) collecting 10-ml fractions. Tubes 41–48, which contained pure A_3, were pooled, concentrated, and freeze dried to afford 0.1 g of material. A sample of this material was converted to the tetrahydrochloride salt by dissolving in water, neutralizing with 0.1 *N* hydrochloric acid, and lyophilization, $[\alpha]^{26}D$ +130° (*c* 0.1, water).

Anal. Calcd for $C_{18}H_{36}N_4O_{10}\cdot 4HCl$: C, 35.19; H, 6.56; N, 9.12. Found: C, 34.87; H, 6.77; N, 9.12.

Crude gentamicin A_4 isolated above was rechromatographed on silica gel (630 g) using a chloroform–methanol–ammonium hydroxide (2:1:0.35) system as the eluent and taking 3-ml fractions. Tubes 1361–1951, which contained A_4, were pooled, concentrated, and lyophilized to give 3.8 g of partially pure A_4. A sample of the above material (0.357 g) was further purified by chromatography on silica gel (70 g) using a 2.7 × 30 cm column and a solvent system containing chloroform–methanol–ammonium hydroxide–formic acid (300:400:200:0.3). Two-milliliter fractions were collected. The homogeneous fractions (tubes 170–189) were pooled, concentrated, deionized with Amberlite IRA-401S ion exchange resin in the hydroxide form, and lyophilized to give 0.222 g of pure gentamicin A_4, $[\alpha]^{26}D$ +130° (*c* 0.1, water). Anal. Calcd for $C_{19}H_{36}N_4O_{11}\cdot CO_2\cdot 2H_2O$: C, 41.66; H, 6.99; N, 9.72. Found: C, 41.68; H, 6.99; N, 9.88.

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Registry No.—1, 55925-13-8; 2, 55925-14-9; 3, 55904-33-1; gentamicin A, 13291-74-2; gentamicin A_2, 55715-66-7.

References and Notes

(1) (a) See P. J. L. Daniels, C. Luce, T. L. Nagabhushan, R. S. Jaret, D. Schumacher, H. Reimann, and J. Ilvasky, *J. Antibiot.*, **28**, 35 (1975), for Part 6. (b) H. Maehr and C. P. Schaffner, *J. Chromatogr.*, **30**, 572 (1967).
(2) G. H. Wagman, J. A. Marquez, and M. J. Weinstein, *J. Chromatogr.*, **34**, 210 (1968).
(3) H. Maehr and C. P. Schaffner, *J. Am. Chem. Soc.*, **89**, 6787 (1967).
(4) H. Maehr and C. P. Schaffner, *J. Am. Chem. Soc.*, **92**, 1697 (1970).
(5) (a) T. L. Nagabhushan, P. J. L. Daniels, R. S. Jaret, and J. B. Morton, *J. Org. Chem.*, following paper in this issue. (b) R_A, $R_{gentamicin\ A}$.
(6) T. L. Nagabhushan and P. J. L. Daniels, *J. Med. Chem.*, **17**, 1030 (1974).
(7) P. W. Austin, F. E. Hardy, J. G. Buchanan, and J. Baddiley, *J. Chem. Soc.*, 5350 (1963).
(8) P. J. L. Daniels, M. Kugelman, A. K. Mallams, R. W. Tkach, H. F. Vernay, J. Weinstein, and A. Yehaskel, *Chem. Commun.*, 1629 (1971).
(9) (a) D. H. Davies, D. Greeves, A. K. Mallams, J. B. Morton, and R. W. Tkach, *J. Chem. Soc., Perkin Trans. 1*, in press. (b) The authors are grateful to Dr. A. K. Mallams for a sample of the methyl glycoside of this substance.
(10) J. B. Morton, R. C. Long, P. J. L. Daniels, R. W. Tkach, and J. H. Goldstein, *J. Am. Chem. Soc.*, **95**, 7464 (1973).
(11) R. U. Lemieux and S. Koto, Abstracts, 165th National Meeting of the American Chemical Society, Dallas, Texas, April 8–13, 1973, No. MEDI-022.
(12) P. K. Woo and R. D. Westland, *Carbohydr. Res.*, **31**, 27 (1973).
(13) S. Omoto, R. Inouye, M. Kojima, and T. Nilda, *J. Antibiot.*, **26**, 717 (1973).
(14) K. F. Koch, J. A. Rhoades, E. W. Hagman, and E. Wenkert, *J. Am. Chem. Soc.*, **96**, 3300 (1974).