Semisynthetic Aminoglycoside Antibacterials. Part 9.[1,2] Synthesis of Novel 1- and 3-Substituted and 1- and 3-*epi*-Substituted Derivatives of Sisomicin and Gentamicin from the 1- and 3-Oxo-derivatives

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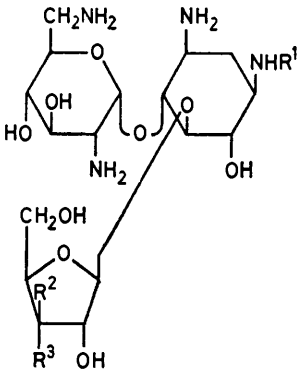
The conversion of selectively protected gentamicin and sisomicin derivatives into the 1- and 3-oxo-compounds by reaction with 3,5-di-t-butyl-1,2-benzoquinone is described. By application of suitable reductive techniques these oxo-aminoglycosides have been converted into novel 1- and 3-*epi*-, 1- and 3-deamino-1- and -3-hydroxy-, 1- and 3-deamino-1- and -3-*epi*-hydroxy-, and 1-deamino-derivatives. A study of the ^{13}C n.m.r. parameters of the 1-*epi*- and 1-deamino-derivatives has led to the assignment of novel solution conformations for these new aminoglycosides.

THE search for novel semisynthetic aminoglycoside antibacterials that would hopefully exhibit an improved spectrum of activity with decreased toxicity relative to the parent antibiotics, has been vigorously pursued in many laboratories during the last decade. Modification of the 1-amino-group of the 2-deoxystreptamine ring has proved to be particularly fruitful. The occurrence in nature of butirosin A (3) and B (4) [3-5] which may formally be regarded as the (1*S*)-1-*N*-(4-amino-2-hydroxybutyryl) derivatives of xylostasin (1) [6] and ribostamycin (2) [7] respectively, coupled with the recognition that such modification of the 1-amino-group resulted in an improved spectrum of activity, particularly against *Pseudomonas* strains, led to the preparation of a variety of 1-*N*-substituted ribostamycin derivatives,[8,9] none of which unfortunately proved to be better than (3) and (4). The preparation of the (1*S*)-1-*N*-(4-amino-2-hydroxybutyryl) derivative of kanamycin A (5) led to the discovery of a clinically useful drug, amikacin (6),[10] and a number of analogues have been prepared.[11] A variety of 1-*N*-acyl derivatives of gentamicin and sisomicin (7) have also been prepared.[12,13] Reductive alkylation of the 1-amino-group in sisomicin (7) led to the discovery of the important semisynthetic aminoglycoside, netilmicin (8),[14] which exhibits an improved spectrum of activity against resistant strains of bacteria and which has reduced toxicity relative to sisomicin (7).[15] We describe here an alternative route for the preparation of these 1-*N*-alkyl derivatives from novel 1-oxoaminoglycosides. The latter intermediates have also been converted into unique 1-*epi*-, 1-deamino-1-hydroxy-, 1-deamino-1-*epi*-hydroxy-, and 1-deamino-derivatives [2] which do not occur in nature. The preparation of a 3-deamino-3-oxo-derivative of gentamicin C_{1a} and its conversion into 3-*epi*-3-deamino-3-hydroxy-, and 3-deamino-3-*epi*-hydroxy-derivatives will also be described.

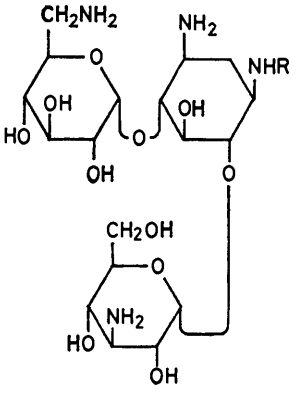
Several literature procedures are available for the transformation of a primary amino-group into a carbonyl group and from these methods the deamination procedure developed by Corey [16] was chosen in view of the mild reaction conditions used, the high yields, and absence of side reactions observed with this method. Thus gentamicin C_1 (20) was converted into the 3,2′-bis-*N*-trifluoroacetate (21),[12] which on treatment with 3,5-di-t-butyl-1,2-benzoquinone [16] followed by acidic hydrolysis, afforded a high yield of 1-deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (22) which was isolated as the sulphate salt. Reduction of the ketone (22) with sodium cyanoborohydride at pH 3 followed by hydrolysis of the protecting groups with concentrated ammonium hydroxide, gave 1-deamino-1-hydroxygentamicin C_1 (23) and the 1-*epi*-analogue (24). The mass-spectral data (Table 1) were consistent with replacement, of the 1-amino-group with a 1-hydroxy-group [17] and did not enable (23) and (24) to be distinguished. The epimers could readily be distinguished by the presence of a multiplet in the ^1H n.m.r. spectrum at δ_H 4.25 due to the equatorial proton at C-1 in the *epi*-derivative (24). The ^{13}C n.m.r. spectrum of the *epi*-derivative (24) was also highly characteristic of the structure (Table 2) and will be discussed later.

Reductive amination of the ketone (22) with ammonia and sodium cyanoborohydride at pH 6 followed by hydrolysis with concentrated ammonium hydroxide gave a 1 : 1 mixture of gentamicin C_1 (20) and its 1-*epi*-derivative (25), which could not be separated in a number of chromatographic systems. The ^{13}C n.m.r. spectrum of the mixture was recorded (Table 2). The use of morpholinoborane as the reducing agent gave similar results. In the above reaction as well as in subsequent reductive amination reactions, both 1-deamino-1-hydroxygentamicin C_1 (23) and 1-deamino-1-*epi*-hydroxygentamicin C_1 (24) were isolated as well. When the reductive amination was carried out using a variety of alkylamines in the presence of sodium cyanoborohydride at pH 5.5—6 there were obtained after base hydrolysis, from methylamine, 1-*N*-methylgentamicin C_1 (26) and 1-*epi*-N-methylgentamicin C_1 (27); from isopropylamine, 1-*N*-isopropylgentamicin C_1 (28) and 1-*epi*-*N*-isopropylgentamicin C_1 (29); from 2-hydroxyethylamine, 1-*N*-(2-hydroxyethyl)gentamicin C_1 (30) and 1-*epi*-*N*-(2-hydroxyethyl)gentamicin C_1 (31); and from 2-phenylethylamine, 1-*N*-(2-phenylethyl)gentamicin C_1 (32) and 1-*epi*-*N*-(2-phenylethyl)gentamicin C_1

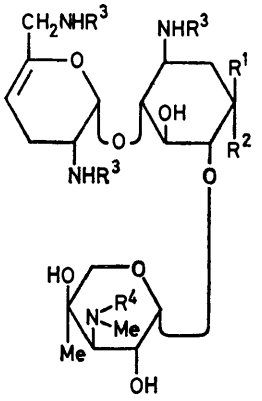
(33). The above *N*-alkyl derivatives were all readily separated by column chromatographic techniques. In contrast to the 1-*epi*-alcohol (24), the above 1-*epi*-amino-derivatives could not be distinguished from their ^{1}H n.m.r. spectra as the multiplets due to the equatorial



(1) $R^1 = R^3 = H, R^2 = OH$
(2) $R^1 = R^2 = H, R^3 = OH$
(3) $R^1 = CO-\underset{H}{\overset{OH}{C}}-CH_2CH_2NH_2, R^2 = OH, R^3 = H$
(4) $R^1 = CO-\underset{H}{\overset{OH}{C}}-CH_2CH_2NH_2, R^2 = H, R^3 = OH$



(5) R = H
(6) $R = CO-\underset{H}{\overset{OH}{C}}-CH_2CH_2NH_2$

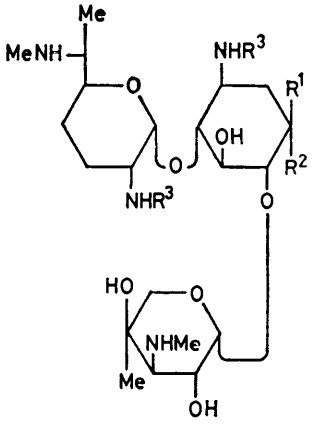


(7) $R^1 = NH_2, R^2 = R^3 = R^4 = H$
(8) $R^1 = NHEt, R^2 = R^3 = R^4 = H$
(9) $R^1 = NH_2, R^2 = R^4 = H, R^3 = Ac$
(10) R^1 $R^2 = O, R^3 = Ac, R^4 = H$
(11) $R^1 = NH_2, R^2 = R^4 = H, R^3 = Bz$
(12) $R^1 = NH_2, R^2 = H, R^3 = Bz, R^4 = Ac$
(13) R^1 $R^2 = O, R^3 = Bz, R^4 = Ac$
(14) $R^1 = OH, R^2 = R^3 = R^4 = H$
(15) $R^1 = R^3 = R^4 = H, R^2 = OH$
(16) $R^1 = R^3 = R^4 = H, R^2 = NH_2$
(17) $R^1 = R^3 = R^4 = H, R^2 = NHEt$
(18) $R^1 = NHCH_2CH_2CH_2NMe_2, R^2 = R^3 = R^4 = H$
(19) $R^1 = R^3 = R^4 = H, R^2 = NHCH_2CH_2CH_2NMe_2$

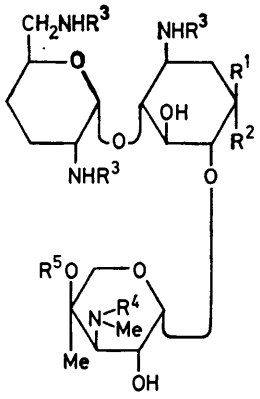
1-protons overlapped with other signals. However, the ^{13}C n.m.r. spectra were very characteristic (Table 2) and could be used to distinguish the 1-*epi*-amino-derivatives. The mass-spectral fragmentation patterns are given in Table 1 and all of the compounds showed the characteristic F_1 and F_2 ions [17] associated with 1-*N*-alkylation.

The application of transition-metal complexing for the selective protection of aminoglycosides has led to the development of elegant, high yielding procedures for the preparation of selectively protected aminoglycosides having the 1-amino-group free.[18] Thus gentamicin C_{1a} (35) has been converted into 3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (36) [18] in high yield, and the latter on treatment with 3,5-di-t-butyl-1,2-benzoquinone followed by acidic hydrolysis gave 1-deamino-1-oxo-3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (37). A fully *N*-protected keto-intermediate was also desired and this was prepared as follows. It has been shown [19] that 3,2′,6′-tri-*N*-acetyl-

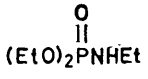
sisomicin (9) [18] reacts preferentially with 1-acetylimidazole to give 3,2′,6′,3″-tetra-*N*-acetylsisomicin. It was hoped therefore that 3,2′,6′-tris-*N*-(2,2,2-trichoroethoxycarbonyl)gentamicin C_{1a} (36) would react with 1-(2,2,2-trichloroethoxycarbonyl)imidazole to give the 3″-*N*-substituted derivative. However, the only product isolated from the reaction was the 3″-*N*,4″-*O*-carbonyl derivative (38) which presumably formed by cyclization of the vicinal *cis*-4″-hydroxy-group with the 3″-*N*-



(20) $R^1 = NH_2, R^2 = R^3 = H$
(21) $R^1 = NH_2, R^2 = H, R^3 = COCF_3$
(22) $R^1 R^2 = =O, R^3 = COCF_3$
(23) $R^1 = OH, R^2 = R^3 = H$
(24) $R^1 = R^3 = H, R^2 = OH$
(25) $R^1 = R^3 = H, R^2 = NH_2$
(26) $R^1 = NHMe, R^2 = R^3 = H$
(27) $R^1 = R^3 = H, R^2 = NHMe$
(28) $R^1 = NHPr^i, R^2 = R^3 = H$
(29) $R^1 = R^3 = H, R^2 = NHPr^i$
(30) $R^1 = NHCH_2CH_2OH, R^2 = R^3 = H$
(31) $R^1 = R^3 = H, R^2 = NHCH_2CH_2OH$
(32) $R^1 = NHCH_2CH_2Ph, R^2 = R^3 = H$
(33) $R^1 = R^3 = H, R^2 = NHCH_2CH_2Ph$
(34) $R^1 = R^2 = R^3 = H$



(35) $R^1 = NH_2, R^2 = R^3 = R^4 = R^5 = H$
(36) $R^1 = NH_2, R^2 = R^4 = R^5 = H, R^3 = CO_2CH_2CCl_3$
(37) $R^1 R^2 = =O, R^3 = CO_2CH_2CCl_3, R^4 = R^5 = H$
(38) $R^1 = NH_2, R^2 = H, R^3 = CO_2CH_2CCl_3, R^4R^5 = >C=O$
(39) $R^1 = NH_2, R^2 = R^5 = H, R^3 = CO_2CH_2CCl_3, R^4 = Ac$
(40) $R^1 = R^2 = =O, R^3 = CO_2CH_2CCl_3, R^4 = Ac, R^5 = H$
(41) $R^1 = OH, R^2 = R^3 = R^4 = R^5 = H$
(42) $R^1 = R^3 = R^4 = R^5 = H, R^2 = OH$



(43)

(2,2,2-trichloroethoxycarbonyl) group in the presence of imidazole. Reaction of (36) with 1,1′-carbonyldi-imidazole afforded the identical product (38). When (36) was treated with 1-acetylimidazole a smooth conversion into 3″-*N*-acetyl-3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (39) was obtained. The deshielding of the 3′-*N*-methyl group to δ_H 3.15 in the latter was characteristic of a 3″-*N*-acetyl substitution in the molecule. Application of the Corey deamination procedure to (39) afforded an excellent yield of 3″-*N*-acetyl-1-deamino-1-oxo-3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (40).

Reduction of the ketone (37) with either sodium cyanoborohydride, or sodium borohydride followed by deprotection with zinc in acetic acid afforded 1-deamino-1-hydroxygentamicin C_{1a} (41) and 1-deamino-1-*epi*-hydroxygentamicin C_{1a} (42). Both gave similar mass spectra (Table 1) and the latter could again be distinguished by the presence of a multiplet in the ^1H n.m.r. spectrum at δ_H 4.24 due to the equatorial 1-proton. The ^{13}C n.m.r. data are given in Table 2 and will be discussed later.

Application of the Corey deamination reaction to 3,2′,6′-tri-*N*-acetylsisomicin (9) [18] afforded 3,2′,6′-tri-*N*-acetyl-1-deamino-1-oxosisomicin (10) in quantitative yield. In order to prepare a fully *N*-protected keto-sisomicin derivative, 3,2′6′-tri-*N*-benzoylsisomicin (11) prepared by the transition-metal complexing method [18] was treated with 1-acetylimidazole in aqueous tetrahydrofuran to give 3″-*N*-acetyl-3,2′,6′-tri-*N*-benzoylsisomicin (12). The latter was then deaminated by the Corey procedure to give 3″-*N*-acetyl-3,2′,6′-tri-*N*-benzoyl-1-deamino-1-oxosisomicin (13). Reduction of the keto-derivative (10) with sodium cyanoborohydride at pH 3 followed by basic hydrolysis afforded both 1-deamino-1-hydroxysisomicin (14) and the 1-*epi*-analogue (15). The mass-spectral fragmentation patterns were consistent with the presence of the 1-hydroxy-group (Table 1).[17] The 1-*epi*-alcohol (15) exhibited a characteristic signal at δ_H 4.34 due to the equatorial 1-proton. The ^{13}C n.m.r. parameters are given in Table 2. In order

to achieve greater selectivity for the 1-*epi*-derivative (15) by using L-Selectride as the reducing agent, it was necessary to use the fully *N*-protected ketone (13), which was soluble in dry tetrahydrofuran. Thus (13) on reduction with L-Selectride followed by basic hydrolysis gave predominantly the 1-*epi*-derivative (15).

Reductive amination of the ketone (10) at pH 5—5.7 using sodium cyanoborohydride and a variety of amines afforded after suitable deprotection, from ammonium deamino-1-oxosisomicin (13) on treatment with the ylid derived from diethyl *N*-ethylphosphoramidate (43) [20,21] followed by reduction with L-Selectride and basic hydrolysis gave only the desired *epi*-derivative (17), but the yield was low.

It was of interest to prepare a 1-deamino-derivative of gentamicin C_1 and this was achieved in the following manner. Conversion of 1-deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (22) into the tosylhydrazone

TABLE 1

Mass-spectral fragment ions (*m/e* (%))

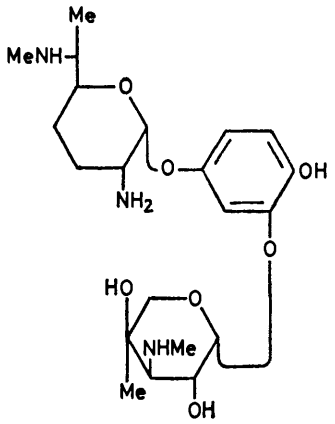
Compound	$(M+1)^+$	$M^{+\cdot}$	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}	A_{11}	A_{12}	B_1	C_1	D_1
(23)	479(2)	478(1)	348(23)		320(7)	302(5)	351(5)	333(3)	323(23)	305(13)	192(19)	174(27)	164(12)	146(30)	157(100)	160(70)	461(0.5)
(24)	479(0.5)	478(0.5)	348(7)		320(0.5)	302(2)	351(3)	333(2)	323(8)	305(9)	192(9)	174(7)	164(5)	146(7)	157(100)	160(58)	461(0.5)
(26)	492(1)	491(1)	361(8)	343(1)	333(2)	315(2)	364(2)	346(2)	336(5)	318(7)	205(27)	187(11)	174(24)	159(37)	157(100)	160(80)	474(0.5)
(27)	492(2)	491(0.5)	361(0.5)	343(2)	333(2)	315(0.5)	364(2)	346(0.5)	336(3)	318(9)	205(11)	187(4)	177(11)	159(6)	157(100)	160(64)	474(0.5)
(28)	520(0.5)	519(0.3)	389(5)	371(0.5)	361(2)	343(0.5)	392(1)	374(0.5)	364(2)	346(4)	233(14)	215(3)	205(8)	187(8)	157(100)	160(25)	502(0.2)
(29)	520(1)	519(0.5)	389(2)	371(3)	361(3)	343(2)	392(2)	374(1)	364(6)	346(20)	233(36)	215(8)	205(33)	187(15)	157(100)	160(70)	502(0.2)
(30)	522(0.5)	521(0.3)	391(7)	373(3)	363(3)	345(3)	394(3)	376(0.5)	366(3)	348(7)	235(17)	217(5)	207(10)	189(10)	157(100)	160(30)	
(31)	522(3)	521(1)	391(2)	373(6)	363(4)	345(1)	394(3)	376(2)	366(8)	348(22)	235(30)	217(9)	207(21)	189(10)	157(100)	160(80)	
(32)	582(0.5)	581(0.3)	451(7)	433(1)	423(1)	405(1)	454(2)	436(2)	426(2)	408(6)	295(16)	277(2)	267(10)	249(9)	157(100)	160(55)	564(0.1)
(33)	582(0.3)	581(0.2)	451(0.2)	433(0.5)	423(0.5)	405(0.3)	454(2)	436(0.5)	426(2)	408(7)	295(9)	277(2)	267(10)	294(4)	157(100)	160(63)	564(0.1)
(41)	451(0.2)	450(0.1)	320(3)		292(0.3)	274(3)	351(6)	333(4)	323(25)	305(8)	192(14)	174(10)	164(7)	146(22)	129(100)	160(90)	433(0.5)
(42)	451(0.2)	450(0.1)	320(2)		292(0.5)	274(0.5)	351(7)	333(6)	323(28)	305(4)	192(9)	174(5)	164(9)	146(10)	129(89)	160(100)	433(3)
(14)	449(2)	448(3)	318(10)	300(20)	290(9)	272(25)	351(10)	333(34)	323(7)	305(22)	192(38)	174(16)	164(19)	146(40)	127(65)	160(100)	
(15)	449(2)	448(2)	318(17)	300(10)	290(4)	272(32)	351(4)	333(23)	323(4)	305(7)	192(45)	174(22)	164(21)	146(60)	127(40)	160(100)	
(16)	448(0.5)	447(0.3)	317(2)	299(7)	289(4)	271(10)	350(2)	332(7)	322(2)	304(13)	191(48)	173(30)	163(52)	145(59)	127(45)	160(100)	
(17)	476(3)	475(1)	345(4)	327(15)	317(10)	299(15)	378(3)	360(11)	350(2)	332(33)	219(35)	201(56)	191(53)	173(53)	127(70)	160(100)	
(18)	533(4)	532(2)	402(15)	384(7)	374(6)	356(7)	435(3)	417(7)	407(4)	389(12)	276(15)	258(20)	248(12)	230(27)	127(38)	160(100)	
(19)	533(4)	532(2)	402(2)	384(14)	374(8)	356(4)	435(4)	417(8)	407(4)	389(23)	276(7)	258(39)	248(18)	230(20)	127(60)	160(100)	
(34)	463(3)	462(2)	332(57)		304(6)	286(11)	335(12)	317(7)	307(38)	289(25)	176(16)	158(26)	148(7)	130(23)	157(100)	160(81)	445(2)
(44)	442(15)	441(10)													157(100)	160(80)	424(1)
(47)	451(1)	450(0.5)	320(10)		292(4)	274(2)	351(8)	333(5)	323(35)	305(21)	192(20)	174(6)	164(9)	146(16)	129(80)	160(100)	433(0.3)
(48)	451(1)	450(0.5)	320(6)		292(3)	274(2)	351(9)	333(4)	323(18)	305(4)	192(12)	174(4)	164(7)	146(8)	129(100)	160(68)	433(0.5)
(50)	450(0.5)	449(0.1)	319(2)		291(2)	273(2)	350(2)	332(5)	322(18)	304(3)	191(15)	173(5)	163(16)	145(7)	129(100)	160(69)	432(0.5)

Compound	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9	D_{10}	E_1	E_2	E_3	E_4	F_1	F_2	Other fragments
(23)						421(10)						361(8)		286(27)	290(27)	
(24)						421(2)						361(4)		286(8)	290(12)	
(26)						434(1)				416(2)	260(5)	374(3)		286(27)	303(9)	
(27)						434(0.3)				416(0.5)	260(2)	374(0.5)		286(9)	303(6)	
(28)						462(0.4)				444(1)	288(2)	402(0.8)		286(21)	331(5)	
(29)						462(0.2)				444(0.5)	288(6)	402(2)		286(23)	331(16)	
(30)						464(1)				446(2)	290(2)	404(3)		286(21)	333(7)	490(0.5)
(31)						464(1)				446(1)	290(4)	404(5)		286(21)	333(15)	490(1)
(32)										506(2)		464(1)		286(20)	393(7)	490(15)
(33)										506(0.3)		464(0.5)		286(4)	393(4)	490(2)
(41)										375(0.2)	247(0.1)	333(3)	205(2)	258(3)	290(5)	
(42)										375(0.2)	247(0.1)	333(6)	205(3)	258(2)	290(4)	
(14)	272(25)	431(16)	272(25)	431(16)	272(25)			363(26)	204(44)	373(3)		331(6)		256(16)	290(9)	
(15)	272(32)	431(6)	272(32)	431(6)	272(32)			363(15)	204(25)	373(2)		331(4)		256(8)	290(4)	
(16)		430(0.6)	271(10)					362(5)	203(21)	372(0.2)	246(7)	330(2)	204(18)	256(7)	289(4)	
(17)		458(2)	299(15)	458(2)	299(15)			390(3)		400(2)		358(3)		256(36)	317(10)	
(18)		515(2)	356(7)	515(2)	356(7)			448(2)	288(6)	457(4)		415(4)		256(52)	374(6)	
(19)		515(2)	356(4)	515(2)	356(4)			448(2)	288(6)	457(3)		415(1)		256(29)	374(8)	
(34)	286(11)					405(22)	246(3)					345(26)		271(21)	274(36)	
(44)						384(2)						324(8)				285(80) 282(38) 126(80)
(47)										375(2)	247(9)	333(5)	205(7)	259(2)	289(8)	
(48)										375(1)	247(6)	333(4)	205(3)	259(2)	289(9)	
(50)										374(0.5)	246(4)	332(5)	204(3)	258(2)	289(2)	

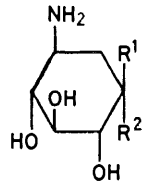
acetate, sisomicin (7) and 1-*epi*-sisomicin (16); from ethylamine, 1-*N*-ethylsisomicin (netilmicin) (8) and 1-*epi*-*N*-ethylsisomicin (1-*epi*-netilmicin) (17); and from 3-dimethylaminopropylamine, 1-*N*-(3-dimethylaminopropyl)sisomicin (18) and 1-*epi*-*N*-(3-dimethylaminopropyl)sisomicin (19). The 1-*epi*-amino-derivatives of sisomicin again could not be distinguished from their ^{1}H n.m.r. spectra; however, the ^{13}C n.m.r. spectra were quite characteristic (Table 2). The amino-derivatives exhibited the expected mass-spectral fragmentation patterns (Table 1) and the 1-*N*-alkyl derivatives showed the characteristic F_1 and F_2 ions [17] associated with 1-*N*-alkylation. In order to increase the stereoselectivity in the preparation of 1-*epi*-*N*-ethylsisomicin (17) an alternative synthesis of the intermediate imine was investigated. Thus 3″-*N*-acetyl-3,2′,6′-tri-*N*-benzoyl-1-followed by reduction with sodium cyanoborohydride [22] and basic hydrolysis afforded 1-deaminogentamicin C_1 (34). The mass-spectral data (Table 1) were consistent with the absence of the 1-amino-group in the molecule. A novel analogue (44) of gentamicin C_1 (20) in which the 2-deoxystreptamine ring had been replaced by a phenolic moiety was prepared from the ketone (22) by β-elimination and basic hydrolysis with concentrated ammonium hydroxide. The ^{1}H n.m.r. spectrum of (44) contained signals at δ_H 6.72 and 6.87 due to the aromatic protons. The mass spectrum of the phenol (44) showed the expected molecular ion and very few of the normal aminoglycoside fragment ions (Table 1). The spectrum did however contain a diagnostic series of fragment ions shown in Figure 1.

We next turned our attention to the preparation of

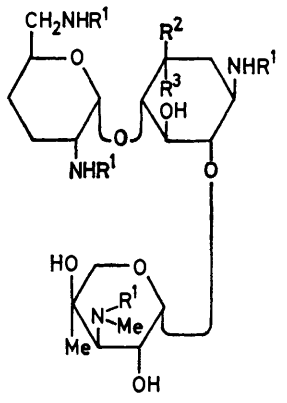
a 3-deamino-3-oxo-derivative. Thus 1,2′,6′,3″-tetrakis-*N*-benzyloxycarbonylgentamicin C_{1a} (45), prepared by the application of the transition-metal process,[18] on treatment with 3,5-di-t-butyl-1,2-benzoquinone afforded the intermediate 3-deamino-3-oxo-derivative (46). Reduction of (46) with sodium borohydride followed by deprotection with sodium in liquid ammonia gave 3-de-



(44)

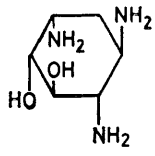


(51) R^1 = OH, R^2 = H
(52) R^1 = H, R^2 = OH
(53) R^1 = NH_2, R^2 = H
(54) R^1 = H, R^2 = NH_2
(55) R^1 = NHEt, R^2 = H
(56) R^1 = H, R^2 = NHEt

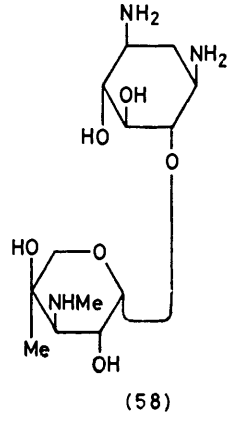


(45) R^1 = Z, R^2 = NH_2, R^3 = H
(46) R^1 = Z, R^2R^3 = =O
(47) R^1 = R^3 = H, R^2 = OH
(48) R^1 = R^2 = H, R^3 = OH
(49) R^1 = Z = R^2 = H, R^3 = NH_2
(50) R^1 = R^2 = H, R^3 = NH_2

Z = $PhCH_2O{\cdot}CO$



(57)



(58)

amino-3-hydroxygentamicin C_{1a} (47) and 3-deamino-3-*epi*-hydroxygentamicin C_{1a} (48). The mass spectra of (47) and (48) (Table 1) supported the assigned structures, but did not enable them to be distinguished. The equatorial alcohol (47) showed a broad multiplet at *ca.* δ_H 4.0 with $W_{\frac{1}{2}}$ *ca.* 20 Hz due to the axial 3-proton. The axial alcohol on the other hand exhibited a narrower multiplet at δ_H 4.23 with $W_{\frac{1}{2}}$ 8 Hz which was assigned to 3*eq*-H. The ^{13}C n.m.r. data (Table 2) further supported the above assignments and will be discussed later.

Reductive amination of the 3-oxo-derivative (46) using ammonium acetate and sodium cyanoborohydride afforded a mixture of the equatorial amine (45) and the axial amine (49) which were separated by chromatography. The axial amine (49) was reduced with sodium in liquid ammonia to give 3-*epi*-gentamicin C_{1a} (50). The mass-spectral data were in accord with the assigned structure (Table 1). The signal due to 3*eq*-H was not clearly visible in the ^1H n.m.r. spectrum. The ^{13}C n.m.r. data given in Table 2 clearly indicated that the assigned structure was correct and these data will be discussed later.

The 1-*epi*- and 1-deamino-derivatives exhibited very characteristic ^{13}C n.m.r. spectra (Table 2) which enabled them to be readily identified. Pronounced shielding was observed for these derivatives at C-1″, C-6, and C-1 relative to their 1-equatorially-substituted counterparts. The $\Delta\delta_C$ values for these derivatives relative to their 1-equatorial analogues are given in Table 3 and are consistent with epimerization, or removal of the 1-equatorial substituent, with concomitant clockwise

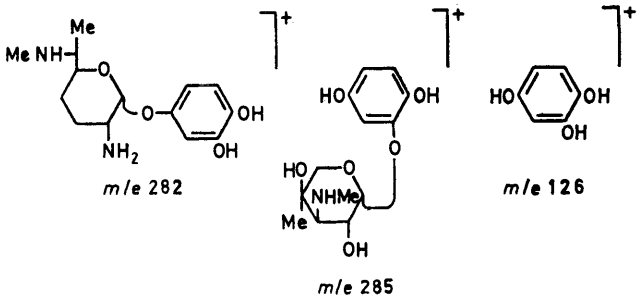


FIGURE 1 Mass-spectral fragments

rotation of the 6-*O*-glycoside about the O–C-6 glycosidic bond due to the absence of the 1-equatorial substituent in these derivatives. It is evident from Table 3 that epimerization of the 1-hydroxy-group in 1-deamino-2-deoxy-1-hydroxystreptamine (51) to give (52) resulted in moderate shielding at C-1, C-2, C-3, and C-5, with more pronounced shielding at C-6. Deshielding was also observed at C-4 in going from (51) to (52). These results are in good agreement with what is observed in going from *scyllo*-inositol to *myo*-inositol.[23] In the aminoglycosides we see a somewhat different picture when the 1-hydroxy-group is epimerized as in going from (23) to (24), (41) to (42), and (14) to (15) (Table 3). In these examples we again observe shielding at C-3 and C-5, and deshielding at C-4, similar to that observed in going from (51) to (52). Somewhat greater shielding is observed at C-2 and markedly greater shielding is also observed at C-1 and C-6, than can be accounted for by simply epimerizing the 1-hydroxy-group. Pronounced shielding is also observed at C-1″ when the 1-hydroxy-group is epimerized. If we now consider the $\Delta\delta_C$ values for the free bases for the 1-equatorial hydroxyaminoglycosides (23), (41). and (14) relative to 1-deamino-2-deoxy-1-hydroxystreptamine (51), and for the 1-axial hydroxyaminoglycosides (24), (42), and (15) relative to

TABLE 2

^{13}C N.m.r. chemical shifts (δ_C, p.p.m., downfield from Me_4Si in D_2O)

Carbon	(23)	(23)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(24)	(20)[a]	(25)[b]	(26)	(27)	(28)	(29)	(30)	(30)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(31)	(31)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(32)	(32)H$^+$	$\Delta\delta_C$(Base→H$^+$)
C-1	70.8	69.7	−1.1	65.3	51.8	45.4	58.9	54.6	54.5	50.5	57.3	56.4	−0.9	52.4	54.9	+2.5	57.1	56.9	−0.2
C-2	36.9	33.3	−3.3	34.6	36.8	33.4	32.5	30.3	33.5	32.2	33.2	26.3	−6.9	31.1	24.8	−6.3	33.1	26.3	−6.8
C-3	49.3	49.6	+0.3	48.0	50.9	47.8	50.3	47.8	50.3	48.0	50.3	49.6	−0.7	49.0	49.3	+0.3	50.4	49.5	−0.9
C-4	87.6	78.3	−9.3	88.7	88.6	88.9	87.4	88.8	87.4	88.3	88.0	77.1	−10.9	88.2	77.9	−10.3	87.7	77.1	−10.6
C-5	74.8	74.5	−0.3	72.8	75.4	73.1	75.6	73.2	75.9	73.3	75.5	75.3	−0.2	73.2	72.2	−1.0	75.5	75.2	−0.3
C-6	85.3	83.5	−1.8	79.6	87.9	78.7	86.4	78.7	87.2	77.9	86.3	83.9	−2.4	78.7	77.0	−1.7	86.3	83.9	−2.4
C-1′	102.5	95.9	−6.6	102.9	102.6	102.6	102.2	102.7	102.2	102.5	102.6	96.0	−6.6	102.2	95.9	−6.3	102.4	95.8	−6.6
C-2′	50.7	49.1	−1.6	50.8	51.1	50.8	50.6	50.8	50.7	50.7	50.8	49.4	−1.4	50.7	49.6	−1.1	50.7	49.5	−1.2
C-3′	26.8	21.3	−5.5	26.8	27.2	26.9	26.6	26.8	26.6	26.6	26.8	21.3	−5.5	26.5	21.3	−5.2	26.9	21.3	−5.6
C-4′	25.7	23.2	−2.5	25.7	26.1	25.9	25.5	25.8	25.5	25.6	25.7	23.2	−2.5	25.5	23.3	−2.2	25.7	23.3	−2.4
C-5′	72.6	70.0	−2.6	72.7	72.8	72.6	71.8	72.6	72.1	72.2	72.7	70.1	−2.6	71.8	70.1	−1.7	72.3	70.1	−2.2
C-6′	58.1	58.5	+0.4	58.0	58.2	58.1	58.3	58.1	58.2	58.2	58.0	58.5	+0.5	58.3	58.5	+0.2	58.2	58.5	+0.3
C-7′	14.3	10.8	−3.5	14.6	15.0	14.6	13.5	14.5	13.8	13.9	14.5	10.8	−3.7	13.6	10.8	−2.8	14.2	10.8	−3.4
6′-NCH_3	33.2	32.0	−1.2	33.3	33.7	33.4	32.2	33.3	32.9	33.0	33.2	32.1	−1.1	32.9	32.0	−0.9	33.1	32.0	−1.1
C-1″	100.5	99.2	−1.3	96.7	101.4	95.8	101.8	96.1	102.2	95.7	101.5	102.5	+0.8	96.0	98.7	+2.7	101.0	102.1	+1.1
C-2″	70.3	67.2	−3.1	70.0	70.3	69.7	70.1	69.6	70.4	69.7	70.3	67.2	−3.1	69.7	66.2	−3.5	70.2	66.7	−3.5
C-3″	64.1	65.0	+0.9	64.0	64.5	63.9	64.2	63.8	64.4	65.9	64.3	64.3		64.0	64.3	+0.3	64.4	64.3	−0.1
C-4″	73.2	70.8	−2.4	73.4	73.3	73.1	73.1	73.1	73.2	73.3	73.2	70.7	−2.5	73.2	70.7	−2.5	73.0	70.7	−2.3
C-5″	68.6	67.8	−0.8	68.4	68.7	68.2	68.5	68.3	69.0	68.3	68.9	68.8	−0.1	68.3	68.4	+0.1	68.7	68.7	
3″-NCH_3	37.7	35.9	−1.8	37.8	38.1	37.8	37.5	37.7	37.6	37.6	37.7	35.5	−2.2	37.7	35.6	−2.1	37.8	35.7	−2.1
4″-CH_3	22.3	21.7	−0.6	22.2	22.9	22.6	22.4	22.4	22.4	22.3	22.4	21.7	−0.7	22.3	21.8	−0.5	22.6	21.8	−0.8
C-1‴							32.8	33.8	45.6	48.0	48.0	47.8	−0.2	47.7	48.1	+0.4	48.2	47.2	−1.0
C-2‴									23.7	23.6	61.2	57.2	−4.0	61.0	57.2	−3.8	36.1	32.7	−3.4
C-3‴									21.1	22.1									
Ar(C-1)																	141.0	137.0	−4.0
Ar(C-3, C-5)																	129.8	130.1	+0.3
Ar(C-2, C-6)																	129.8	129.9	+0.1
Ar(C-4)																	127.5	128.5	+1.0
$N(CH_3)_2$																			

Carbon	(33)	(33)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(41)	(42)	(14)	(15)	(15)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(7)[a]	(16)	(16)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(8)[c]	(8)H$^+$[c]	$\Delta\delta_C$(Base→H$^+$)	(17)	(17)H$^+$	$\Delta\delta_C$(Base→H$^+$)
C-1	52.4	55.2	+2.8	69.5	65.3	71.0	65.3	65.0	−0.3	51.8	47.4	47.9	+0.5	58.0	56.9	−1.1	52.7	54.8	+2.1
C-2	31.0	24.8	−6.2	36.9	34.6	36.6	34.5	31.1	−3.4	36.4	34.1	27.1	−7.0	32.7	26.2	−6.5	30.6	24.6	−6.0
C-3	49.1	48.8	−0.3	49.1	47.9	49.0	47.8	48.5	+0.7	50.4	47.7	47.9	+0.2	50.2	49.2	−1.0	47.7	47.9	+0.2
C-4	88.7	77.8	−10.9	86.2	87.8	84.7	85.7	80.7	−5.0	85.5	85.3	79.8	−5.5	85.3	79.6	−5.7	85.7	79.6	−6.1
C-5	73.2	72.3	−0.9	74.9	73.2	74.8	72.9	72.1	−0.8	75.4	72.7	71.6	−1.1	75.7	74.3	−1.4	73.4	71.7	−1.7
C-6	78.6	76.6	−2.0	85.2	79.6	85.2	79.8	79.0	−0.8	87.8	78.9	77.0	−1.9	86.7	83.5	−3.2	78.4	77.1	−1.3
C-1′	102.6	95.7	−6.9	100.9	101.9	100.8	101.0	97.8	−3.2	100.6	100.7	97.9	−2.8	100.8	97.8	−3.0	100.9	97.9	−3.0
C-2′	50.8	49.6	−1.2	50.5	50.6	47.3	47.4	47.1	−0.3	47.6	47.7	47.0	−0.7	47.4	47.1	−0.3	47.4	47.0	−0.4
C-3′	26.8	21.8	−5.0	25.9	26.4	25.3	25.3	23.8	−1.5	25.6	25.3	24.0	−1.3	25.5	26.2	+0.7	25.2	24.0	−1.2
C-4′	25.7	23.5	−2.2	27.9	28.1	97.5	96.9	101.5	+4.6	96.5	97.8	101.5	+3.7	96.7	101.6	+4.9	97.3	101.5	+4.2
C-5′	72.4	70.1	−2.3	70.8	70.7	150.4	150.6	144.3	−6.3	150.4	150.2	144.1	−6.1	150.4	144.1	−6.3	150.4	144.1	−6.3
C-6′	58.2	58.5	+0.3	45.1	45.5	43.1	43.3	41.5	−1.8	43.5	43.1	41.5	−1.6	43.4	41.6	−1.8	43.2	41.6	−1.6
C-7′	14.4	10.7	−3.7																
6′-NCH_3	33.2	32.0	−1.2																
C-1″	96.0	98.6	+2.6	100.3	96.6	100.5	96.8	96.1	−0.7	101.5	95.8	98.1	+2.3	102.1	102.2	+0.1	95.8	98.2	+2.4
C-2″	69.7	66.4	−3.3	69.9	69.9	70.2	69.9	66.9	−3.0	70.0	69.6	66.3	−3.3	70.2	67.4	−2.7	69.7	66.3	−3.4
C-3″	64.0	64.4	+0.4	64.2	64.1	64.1	64.3	64.4	+0.1	64.3	64.0	64.3	+0.3	64.3	64.3		63.4	64.4	+1.0
C-4″	73.2	70.8	−2.4	72.9	72.8	73.2	73.3	70.8	−2.5	73.0	73.1	70.8	−2.3	73.1	70.8	−2.3	73.3	70.8	−2.5
C-5″	68.4	68.2	−0.2	68.5	68.4	68.5	68.5	67.6	−0.9	68.5	68.2	68.1	−0.1	68.7	68.6	−0.1	68.3	68.2	−0.1
3″-NCH_3	37.8	35.9	−1.9	37.4	37.7	37.6	37.9	36.0	−1.9	37.9	37.7	35.7	−2.0	37.7	35.7	−2.0	37.6	35.8	−1.8
4″-CH_3	22.5	21.8	−0.7	22.3	22.2	22.3	22.5	21.8	−0.7	22.9	22.4	21.9	−0.5	22.6	21.9	−0.7	22.3	21.9	−0.4
C-1‴	47.8	48.3	+0.5											41.1	41.9	+0.8	42.3	43.5	+1.2
C-2‴	35.9	32.5	−3.4											14.7	11.9	−2.8	14.4	11.6	−2.8
C-3‴																			
Ar(C-1)	141.2	137.2	−4.0																
Ar(C-3, C-5)	129.8	130.0	+0.2																
Ar(C-2, C-6)	129.8	130.0	+0.2																
Ar(C-4)	127.4	128.4	+1.0																

TABLE 2 (*continued*)

Carbon	(18)	(19)	(34)	(47)	(47)H⁺	Δδ_C(Base→H⁺)	(48)	(48)H⁺	Δδ_C(Base→H⁺)	(50)	(50)H⁺	Δδ_C(Base→H⁺)	(51)	(51)H⁺	Δδ_C(Base→H⁺)	(52)	(52)H⁺	Δδ_C(Base→H⁺)	(54) [d]	(54)H⁺ [d]
C-1	57.9	52.7	26.1	50.5	50.4	−0.1	49.2	50.1	+0.9	49.7	50.3	+0.6	70.3	69.5	−0.8	69.3	68.3	−1.0	50.4	48.2
C-2	32.7	30.7	28.0	37.0	33.2	−3.8	35.6	31.6	−4.0	34.8	27.6	−7.2	36.8	33.2	−3.6	35.5	32.0	−3.5	33.9	26.9
C-3	50.1	47.6	52.9	69.2	65.8	−3.4	68.5	66.1	−2.4	49.1	49.6	+0.5	50.3	50.8	+0.5	49.0	50.5	+1.5	49.8	50.5
C-4	85.1	85.8	88.9	85.8	82.5	−3.3	82.9	80.3	−2.6	82.8	74.5	−8.3	78.0	73.7	−4.3	78.9	73.9	−5.0	76.1	69.2
C-5	75.7	73.2	77.0	74.6	74.7	+0.1	72.1	72.6	+0.5	72.0	72.6	+0.6	75.8	75.2	−0.6	73.9	73.7	−0.2	72.1	72.2
C-6	86.8	78.5	77.6	87.4	84.8	−2.6	88.2	85.2	−3.0	88.3	84.3	−4.0	77.8	77.0	−0.8	74.7	74.1	−0.6	74.8	68.9
C-1′	100.8	100.9	103.1	101.3	96.0	−5.3	102.6	97.1	−5.5	102.3	96.8	−5.5								
C-2′	47.3	47.4	50.8	50.7	49.8	−0.9	50.8	49.8	−1.0	50.8	49.4	−1.4								
C-3′	25.4	25.3	26.8	26.8	22.0	−4.8	26.9	22.0	−4.9	27.0	23.0	−4.0								
C-4′	97.4	97.1	25.7	28.1	26.6	−1.5	28.4	26.7	−1.7	28.5	26.7	−1.8								
C-5′	150.1	150.1	72.8	70.8	67.8	−3.0	71.1	67.9	−3.2	71.5	67.1	−4.4								
C-6′	43.1	43.3	58.0	45.6	43.6	−2.0	45.7	43.6	−2.1	45.9	43.7	−2.2								
C-7′			14.5																	
6′-NCH_3			33.2																	
C-1″	102.2	95.9	95.9	101.3	101.6	+0.3	101.4	101.6	+0.2	101.3	101.7	+0.4								
C-2″	70.3	69.7	69.8	70.2	67.1	−3.1	70.2	67.2	−3.0	70.3	66.4	−3.9								
C-3″	64.3	64.0	64.0	64.2	64.2		64.2	64.3	+0.1	64.2	64.2									
C-4″	73.1	73.2	73.3	73.2	70.8	−2.4	73.3	70.8	−2.5	73.3	70.8	−2.5								
C-5″	68.8	68.3	68.2	68.5	68.6	+0.1	68.8	68.5	−0.3	68.5	68.7	+0.2								
3″-NCH_3	37.7	37.7	37.8	37.8	35.6	−2.2	37.8	35.6	−2.2	37.8	35.6	−2.2								
4″-CH_3	22.4	22.5	22.2	22.6	22.0	−0.6	22.6	22.0	−0.6	22.6	21.5	−1.1								
C-1‴	44.7	45.9																		
C-2‴	26.8	26.7																		
C-3‴	57.1	57.3																		
$N(CH_3)_2$	44.4	44.5																		

Carbon	Δδ_C(Base→H⁺)	(55) [e]	(55)H⁺ [e]	Δδ_C(Base→H⁺)	(56)	(56)H⁺	Δδ_C(Base→H⁺)	(57)	(57)H⁺	Δδ_C(Base→H⁺)
C-1	−2.2	57.5	56.4	−1.1	57.0	56.1	−0.9	49.8	50.5	+0.7
C-2	−7.0	33.8	26.9	−6.9	30.1	24.9	−5.2	33.9	26.9	−7.0
C-3	+0.7	51.3	51.1	−0.2	49.3	49.8	+0.5	50.4	48.2	−2.2
C-4	−6.9	78.4	72.9	−5.5	77.5	72.6	−4.9	74.8	68.9	−5.9
C-5	+0.1	76.9	75.6	−1.3	73.2	73.0	−0.2	72.1	72.2	+0.1
C-6	−5.9	76.3	72.4	−3.9	73.9	70.1	−3.8	76.1	69.2	−6.9
C-1‴		41.1	41.4	+0.3	42.6	43.1	+0.5			
C-2‴		14.8	11.6	−3.2	13.5	11.0	−2.5			

[a] Ref. 24. [b] Chemical shifts for (25) were obtained by subtraction of the signals due to (20) from the spectrum of the mixture (1 : 1). [c] Ref. 19. [d] Tentative assignments as no titration from base to pH 1 was run. [e] D. Rane, unpublished observations.

TABLE 3

$\Delta\delta_C$ Values for the free bases ($eq \longrightarrow ax$, and $eq \longrightarrow$ deamino)

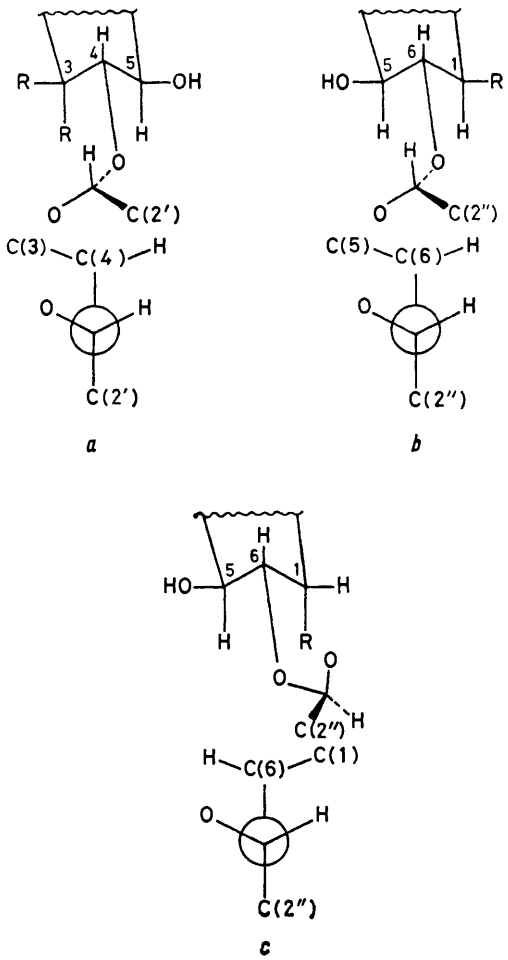
Carbon	1-OH ($eq \rightarrow ax$) (23)→(24)	(41)→(42)	(14)→(15)	(51)→(52)	3-OH ($eq \rightarrow ax$) (47)→(48)	1-NH_2 ($eq \rightarrow ax$) (20)→(25)	(7)→(16)	(53)→(54)	1-NH Alkyl ($eq \rightarrow ax$) (26)→(27)	(28)→(29)	(30)→(31)	(32)→(33)	(8)→(17)	(18)→(19)	(55)→(56)	3-NH_2 ($eq \rightarrow ax$) (35)→(40)	1-NH_2 →1-deNH_2 (20)→(34)
C-1	−5.5	−4.2	−5.7	−1.0	−1.3	−6.4	−4.4	−1.2	−4.3	−4.0	−4.9	−4.7	−5.3	−5.2	−0.5	−2.0	−25.4
C-2	−2.3	−2.3	−2.1	−1.3	−1.4	−3.4	−2.3	−3.1	−2.2	−1.3	−2.1	−2.1	−2.1	−2.0	−3.7	−1.9	−8.5
C-3	−1.3	−1.2	−1.2	−1.3	−0.7	−3.1	−2.7	−1.8	−2.5	−2.3	−1.3	−1.3	−2.4	−2.5	−2.0	−1.5	+2.5
C-4	+1.1	+1.6	+1.0	+0.9	−2.9	+0.3	0	−2.4	+1.4	+0.9	+0.2	+1.0	+0.4	+0.7	−0.9	−5.5	+0.5
C-5	−2.0	−1.7	−1.9	−1.9	−2.5	−2.3	−2.7	−4.5	−2.4	−2.6	−2.3	−2.3	−2.3	−2.5	−3.7	−3.4	+1.8
C-6	−5.7	−5.6	−5.4	−3.1	+0.8	−9.2	−8.9	−3.7	−7.7	−9.3	−7.6	−7.7	−8.3	−8.3	−2.4	+0.5	−10.1
C-1′	+0.4	+1.0	+0.2		+1.3	0	+0.1		+0.5	+0.3	−0.4	+0.2	+0.1	+0.1		+0.1	+0.4
C-1′′	−3.8	−3.7	−3.7		+0.1	−5.6	−5.7		−5.7	−6.5	−5.5	−5.0	−6.3	−6.3		0	−5.4

TABLE 4

$\Delta\delta_C$(DOS ⟶ Trisaccharide) for the free bases

Carbon	1-OH(eq) (51)→(23)	(51)→(41)	(51)→(14)	1-OH(ax) (52)→(24)	(52)→(42)	(52)→(15)	3-OH(eq) (51)→(47)	3-OH(ax) (52)→(48)	1-NH_2(eq) (53)→(20)	(53)→(7)	1-NH_2(ax) (54)→(25)	(54)→(16)	1-NHC_2H_5(eq) (55)→(8)	1-NHC_2H_5(ax) (56)→(17)	3-NH_2(eq) (53)→(35)	3-NH_2(ax) (54)→(50)
C-1	+0.5	−0.8	+0.7	−4.0	−4.0	−4.0	+0.2	+0.2	+0.2	+0.1	−5.0	−3.0	+0.5	−4.3	+0.1	−0.1
C-2	+0.1	+0.1	−0.2	−0.9	−0.9	−1.0	+0.2	+0.1	−0.2	−0.6	−0.5	+0.2	−1.1	+0.5	−0.3	+0.9
C-3	−1.0	−1.2	−1.3	−1.0	−1.1	−1.2	−1.1	−0.8	−0.7	−1.3	−2.0	−2.1	−1.1	−1.6	−1.0	−1.3
C-4	+9.6	+8.2	+6.7	+9.8	+8.9	+6.8	+8.0	+8.2	+10.1	+6.7	+12.8	+9.2	+6.9	+8.2	+9.8	+8.0
C-5	−1.0	−0.9	−1.0	−1.1	−0.7	−1.0	−1.2	−1.8	−1.2	−1.3	+1.0	+0.6	−1.2	+0.2	−1.2	−0.1
C-6	+7.5	+7.4	+7.4	+4.9	+4.9	+5.1	+9.4	+9.3	+9.4	+9.2	+3.9	+4.1	+10.4	+4.5	+9.3	+12.2

1-deamino-2-deoxy-1-*epi*-hydroxystreptamine (52) (Table 4) the reason for this pronounced shielding at C-1, C-6, and C-1″ becomes apparent. In (23), (41), (24), and (42) we observed deshielding at C-4 and shielding at C-3 indicating that in these compounds the 4-*O*-glycoside adopts the usual rotamer *a* about the O–C-4 glycosidic bond.[1,24-33] In the sisomicin derivatives (14) and (15) we again observe shielding at C-3 and reduced net deshielding at C-4 indicating that the 4-*O*-glycoside in these compounds has undergone a modest clockwise rotation about the O–C-4 glycosidic bond relative to rotamer *a*.[33] In the 1-equatorial alcohols (23), (41), and (14) we also observe shielding at C-5 and a reduced net deshielding at C-6 relative to that observed for gentamicin C_{1a} (35), C_1 (20), or sisomicin (7).[33] This indicates that the 6-*O*-glycoside in these derivatives adopts a rotamer about the O–C-6 glycosidic bond in which a modest clockwise rotation has occurred about the O–C-6 glycosidic bond relative to a rotamer *b*.[33] A similar clockwise rotation of the 6-*O*-glycoside has also been observed in the case of a series of 1-*N*-acyl aminoglycosides.[32,33] Presumably reduced steric and/or dipolar interactions between these substituents at C-1 and the 6-*O*-glycoside, relative to a 1-equatorial amino-group, are responsible for the observed rotation of the glycoside unit.[32,33] In the case of the 1-axial hydroxy-derivatives (24), (42), and (15) we again observed shielding at C-5 due to the 6-*O*-glycoside. However, we also observe pronounced shielding at C-1 (−4.0) and a marked reduction in the net deshielding at C-6 to +4.9 to +5.1 indicating that a strong shielding component is present at C-6. The anomeric carbon C-1″ is also shielded. The observed shieldings at C-1, C-6, and C-1″ are best explained by assuming that the 6-*O*-glycoside in these 1-*epi*-hydroxy-derivatives adopts a rotamer approaching that represented by *c* [34] about the O–C-6 glycosidic bond. In such a rotamer we would expect C-6 to be shielded by the 1,3-diaxial-type interaction between C-1″–O-5″ and C-6–H-6.[34-37] A non-bonded interaction between 1*eq*-H and 1*eq*″-H of the type shown in Figure 2 would be expected to shield both C-1 and C-1″ as was observed.[38] Shielding of C-1′ in a series of 5-deoxy- and 5-*epi*-aminoglycosides has been used earlier as diagnostic proof of a change in rotamer population about the O–C-4 glycosidic bond.[39] In all the rotamers *a*, *b*, and *c* we expect the normal *exo*-anomeric effect [40-43] to be operating.



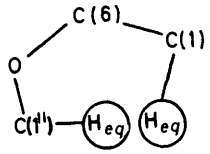


FIGURE 2 Non-bonded hydrogen interaction

Epimerization of the 1-amino-group of 2-deoxystreptamine (53) as in 1-*epi*-2-deoxystreptamine (54) resulted in shielding of all of the carbon atoms in the molecule (Table 3). Similarly in going from 1-*N*-ethyl-2-deoxystreptamine (55) to 1-*epi*-*N*-ethyl-2-deoxystreptamine (56) we also observed shielding of all of the carbon atoms (Table 3). When we consider the aminoglycosides we see a somewhat different picture when the 1-amino- or 1-alkylamino-group is epimerized as in going from (20) to (25), (7) to (16), (26) to (27), (28) to (29), (30) to (31), (32) to (33), (8) to (17), and (18) to (19) (Table 3). In these examples we observe shielding at C-2, C-3, and C-5 which is similar to, or slightly less than that observed in going from (53) to (54), and from (53) to (56). Modest deshielding also occurs at C-4 in these examples. However, greatly enhanced shielding is observed at C-1 and C-6 which is much more than can be accounted for by simply epimerizing the 1-substituent. Pronounced shielding is also observed at C-1″ when the 1-amino-group is epimerized. The shielding of C-6 and C-1″ was also greater than that observed for the 1-*epi*-alcohols discussed earlier. Once again when we consider the $\Delta\delta_C$ values in going from the appropriate 1-amino-, 1-*epi*-amino-, 1-alkylamino-, or 1-*epi*-alkylamino-2-deoxystreptamine to the corresponding aminoglycoside (20), (7), (25), (16), (8), or (17), the reason for this pronounced shielding at C-1, C-6, and C-1″ becomes apparent. Gentamicin C_1 (20) adopts rotamer *a* about the *O*–C-4 glycosidic bond, while sisomicin (7) and

netilmicin (8) both adopt a rotamer in which modest clockwise rotation of the sisosamine moiety has occurred about the O–C-4 glycosidic bond relative to rotamer *a*.[24,33] This follows from the reduced net deshielding observed at C-4 in (7) and (8).[33] In all three compounds shielding is observed at C-3. It is also evident from the shielding observed at C-5 and from the net deshielding at C-6 that the 1-equatorially substituted amines (20), (7), and (8) all adopt a rotamer approximating *b* for the 6-*O*-glycoside about the O–C-6 glycosidic bond.[24,33] In the case of the 1-axial amino-derivatives (25), (16), and (17) we observe enhanced net deshielding at C-4 and also some deshielding at C-5 permits the sugar to rotate in a clockwise direction about the O–C-6 glycosidic bond to give rotamer *c*.[33]

We shall now consider what happens in the case of 3-deamino-3-hydroxygentamicin C_{1a} (47), 3-deamino-3-*epi*-hydroxygentamicin C_{1a} (48), and 3-*epi*-gentamicin C_{1a} (50). If we first consider the $\Delta\delta_C$ values (Table 3) in going from the equatorial 3-hydroxy-derivative (47) to the axial 3-hydroxy-derivative (48) we see shielding at C-1, C-2, C-3, C-4, and C-5, with slight deshielding at C-6 as anticipated.[23] Deshielding of C-1′ was also evident in going from (47) to (48) (Table 3). When we consider the $\Delta\delta_C$ values for the free bases in going from 3-deamino-2-deoxy-3-hydroxystreptamine (51) to (47), and from

TABLE 5

γ-Effects of the 1-*N*-alkyl groups

Gentamicin C_1	1-NH_2			1-$NHCH_3$			1-$NHCH(CH_3)_2$			1-$NHCH_2CH_2OH$			1-$NHCH_2CH_2C_6H_5$		
	Compd.	C-2	C-6	Compd.	C-2	C-6	Compd.	C-2	C-6	Compd.	C-2	C-6	Compd.	C-2	C-6
δ_C *eq*	(20)	36.8	87.9	(26)	32.5	86.4	(28)	33.5	87.2	(30)	33.2	86.3	(32)	33.1	86.3
ax	(25)	33.4	78.7	(27)	30.3	78.7	(29)	32.2	77.9	(31)	31.1	78.7	(33)	31.0	78.6
$\Delta\delta_C$ *eq*				(20)→(26)	−4.3	−1.5	(20)→(28)	−3.3	−0.7	(20)→(30)	−3.6	−1.6	(20)→(32)	−3.7	−1.6
ax				(25)→(27)	−3.1	0	(25)→(29)	−1.2	−0.8	(25)→(31)	−2.3	0	(25)→(33)	−2.4	−0.1

Sisomicin	1-NH_2			1-$NHCH_2CH_3$			1-$NHCH_2CH_2CH_2N(CH_3)_2$		
	Compd.	C-2	C-6	Compd.	C-2	C-6	Compd.	C-2	C-6
δ_C *eq*	(7)	36.4	87.8	(8)	32.7	86.7	(18)	32.7	86.8
ax	16)	34.1	78.9	(17)	30.6	78.4	(19)	30.7	78.5
$\Delta\delta_C$ *eq*				(7)→(8)	−3.7	−1.1	(7)→(18)	−3.7	−1.0
ax				(16)→(17)	−3.5	−0.5	(16)→(19)	−3.4	−0.4

2-Deoxystreptamine	1-NH_2			1-$NHCH_2CH_3$		
	Compd.	C-2	C-6	Compd.	C-2	C-6
δ_C *eq*	(53)	37.0	78.5	(55)	33.8	76.3
ax	(54)	33.9	74.8	(56)	30.1	73.9
$\Delta\delta_C$ *eq*				(53)→(55)	−3.2	−2.2
ax				(54)→(56)	−3.8	−0.9

which appears to be characteristic of these derivatives. The origin of these shifts is not known as changes in the rotamer population about the O–C-4 glycosidic bond would not be expected in these derivatives relative to the gentamicins and sisomicin (7). In the 1-axial amino-derivatives we also observe pronounced shielding at C-1, C-6, and C-1″. The latter arises for the same reasons as discussed for the 1-*epi*-hydroxy-derivatives earlier, leading to the introduction of a strong shielding component at C-6 which results in an observed net deshielding of +3.9 to +4.5 in these compounds. The 6-*O*-glycoside therefore adopts a rotamer about the O–C-6 glycosidic bond in these 1-*epi*-amino-compounds, which closely approximates *c*.[33] The magnitude of the γ-effects at C-2 and C-6 upon introduction of the 1-*N*-alkyl substituents are given in Table 5, greater shielding being evident at C-2 than at C-6 in all instances.

1-Deaminogentamicin C_1 (34) exhibited δ_C values (Table 2) and $\Delta\delta_C$ values (Table 3) that were consistent with removal of the equatorial 1-amino-group. The 4-*O*-glycosyl resonances remained unchanged indicating that the 4-*O*-glycoside was present as the usual rotamer *a* about the O–C-4 glycosidic bond.[1,24-33] Pronounced shielding of C-1″ was observed in (34) indicating that the 6-*O*-glycoside adopts rotamer *c* about the *O*-glycosidic bond as was observed previously for the 1-*epi*-hydroxy- and 1-*epi*-amino-compounds. It is evident from these results that removal of the critical interaction between the equatorial 1-substituent and the 6-*O*-glycoside, 3-deamino-2-deoxy-3-*epi*-hydroxystreptamine (52) to (48) (Table 4) we observed shielding at C-3 as expected and also a reduced net deshielding at C-4 relative to that observed in gentamicin C_{1a} (35). This indicates that in these molecules the 4-*O*-glycoside has undergone a modest clockwise rotation about the O–C-4 glycosidic bond relative to rotamer *a*.[33] Some shielding of C-1′ is also evident in (47). We also observe shielding at C-5 and a net deshielding at C-6 of +9.4 and +9.3 in (47) and (48), similar to that observed in gentamicin C_{1a} (35) which indicates that the 6-*O*-glycoside adopts rotamer *b* about the O–C-6 glycosidic bond as expected.[1,24-33] Epimerization of the 3-amino-group in going from gentamicin C_{1a} (35) to 3-*epi*-gentamicin C_{1a} (50) resulted in shielding at C-1, C-2, C-3, C-4, and C-5, with slight deshielding evident at C-6 (Table 3). When we consider the $\Delta\delta_C$ values for the free bases in going from 2-deoxy-3-*epi*-streptamine (57) to (50) (Table 4) we observed similar shielding at C-3 and net deshielding at C-4 to that observed in the case of the 3-*epi*-hydroxy-derivative (48) indicating that both adopt the same rotamer about the O–C-4 glycosidic bond. Negligible shielding occurs at C-5 in (50) and the net deshielding observed at C-6 is greater than usually observed. The 6-*O*-glycoside adopts rotamer *b* about the O–C-6 glycosidic bond in (50). Further data on the solution conformations of these novel 1- and 3-substituted aminoglycosides at acidic pHs will be discussed in one of the following papers.[33] Similar steric effects to those discussed above

have been observed for glycosides having β-methyl groups on the aglycone [44-47] and our results with aminoglycosides are in good agreement with what has been observed previously.

The 1-hydroxy-, 1-*epi*-hydroxy-, 1-alkylamino-, and 1-*epi*-alkylamino-derivatives described above were all highly potent antibacterials and their biological activity has been described.[2] Epimerization of the 1-substituent resulted in no loss of biological potency. On the other hand the 3-hydroxy-, 3-*epi*-hydroxy-, and 3-*epi*-amino-derivatives were all essentially devoid of antibacterial activity.

EXPERIMENTAL

All physical data were recorded as described in Part 7.[31]

1-Deamino-1-oxo-3,2′-bis-N-trifluoroacetylgentamicin C_1 (22).—3,2′-Bis-*N*-trifluoroacetylgentamicin C_1 [12] (21) (3.34 g) was dissolved in anhydrous methanol (60 ml). 3,5-Di-t-butyl-1,2-benzoquinone (1.12 g) was added and the solution was stirred under dry nitrogen at 25 °C for 24 h. The solution was acidified to pH 2.5—3.0 using 1M-sulphuric acid and the mixture was stirred at 25 °C. The hydrolysis was judged to be complete by t.l.c. after 4 h and the mixture was diluted with distilled water and the solids were filtered off. The aqueous filtrate was extracted with chloroform (2 × 200 ml) and then neutralized to pH 6 with Amberlite IRA 40IS (OH^-) resin. The resin was removed by filtration and the filtrate was evaporated *in vacuo* to give 1-deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (22) as the sulphate salt (3.3 g, 86%) the product being an amorphous solid, $[\alpha]_D$ +129.5° (H_2O), $\nu_{max.}$ (KBr) 3 200, 1 680, 1 540, and 1 100 cm^{-1}, δ (D_2O) 1.21 (3 H, d, *J* 7 Hz, 6′-CH_3), 1.28 (3 H, s, 4″-CH_3), 2.69 (3 H, s, 6′-NCH_3), and 2.89 (3 H, s, 3″-NCH_3).

1-Deamino-1-hydroxygentamicin C_1 (23) *and* 1-*Deamino*-1-epi-*hydroxygentamicin* C_1 (24).—1-Deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (sulphate salt) (22) (1 g) was dissolved in distilled water (30 ml) and the solution was acidified to pH 3 using 0.5M-sulphuric acid. Sodium cyanoborohydride (0.6 g) was added and the solution was stirred at 25 °C for 18 h. Concentrated ammonium hydroxide (10 ml) was added and the solution was stirred at 25 °C for 30 h. The reaction mixture was evaporated to dryness and the residue was chromatographed on a silica gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-*deamino*-1-*hydroxygentamicin* C_1 (23) (285 mg, 46%) as a solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 52.6; H, 8.85; N, 11.5. $C_{21}H_{42}N_4O_8$ requires C, 52.70; H, 8.85; N, 11.71%), $[\alpha]_D$ +154.0° (H_2O), $\nu_{max.}$ (KBr) 3 300 and 1 050 cm^{-1}, δ (D_2O) 1.00 (3 H, d, *J* 7 Hz, 6′-CH_3), 1.14 (3 H, s, 4″-CH_3), 2.29 (3 H, s, 6′-NCH_3), 2.45 (3 H, s, 3″-NCH_3), 5.09 (1 H, d, $J_{1''eq.2''ax}$ 4 Hz, 1″*eq*-H), and 5.22 (1 H, d, $J_{1'eq.2'ax}$ 4 Hz, 1′*eq*-H), and the 1-epi-*analogue* (24) (105 mg, 17%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: $M^{+\cdot}$ 478.3010. $C_{21}H_{42}N_4O_8$ requires *M*, 478.3002), $[\alpha]_D$ +164.2° (H_2O), δ (D_2O) 1.00 (3 H, d, *J* 6.5 Hz, 6′-CH_3), 1.15 (3 H, s, 4″-CH_3), 2.29 (3 H, s, 6′-NCH_3), 2.46 (3 H, s, 3″-NCH_3), 4.25 (1 H, m, 1-H), 5.00 (1 H, d, $J_{1''eq.2''ax}$ 4 Hz, 1″*eq*-N), and 5.11 (1 H, d, $J_{1'eq.2'ax}$ 3.5 Hz, 1′*eq*-H).

Gentamicin C_1 (20) *and* 1-epi-*Gentamicin* C_1 (25).—(*a*) Ammonium chloride (486 mg) was dissolved in dry methanol (23 ml) and the pH was adjusted to 6 using ammonia in methanol. 1-Deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (sulphate salt) (22) (1 g) was added and the pH was readjusted to 6 by addition of ammonia in methanol. Sodium cyanoborohydride (460 mg) was added and the reaction mixture was stirred at 25 °C for 20 h, the pH being maintained at 5.5—6. Concentrated ammonium hydroxide (20 ml) was added and the mixture was allowed to remain at 25 °C for 72 h. The solution was concentrated to dryness and the residue was chromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give a mixture of 1-deamino-1-hydroxygentamicin C_1 (23) and 1-deamino-1-*epi*-hydroxygentamicin C_1 (24) (194 mg, 27%) which was obtained as a colourless solid after lyophilization. The more polar fraction from the column consisted of a *ca.* 1 : 1 mixture of gentamicin C_1 (20) and 1-*epi*-gentamicin C_1 (25) (219 mg, 31%) which was obtained as a solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization. The latter could not be separated in any of the usual chromatographic systems and the ^{13}C n.m.r. properties are given in Table 2.

(*b*) Ammonium chloride (121 mg) was dissolved in dry methanol (6 ml) and the pH was adjusted to 6 using ammonia in methanol. 1-Deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (sulphate salt) (22) (250 mg) was added and the pH was readjusted to 6 by addition of ammonia in methanol. Morpholinoborane (183 mg) was added and the reaction mixture was stirred at 25 °C for 20 h, the pH being maintained at 5.5—6. Concentrated ammonium hydroxide (5 ml) was added and the mixture was allowed to remain at 25 °C for 72 h. The solution was concentrated to dryness and the residue was chromatographed on a silica-gel column (140 × 1.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give a mixture of 1-deamino-1-hydroxygentamicin C_1 (23) and 1-deamino-1-*epi*-hydroxygentamicin C_1 (24) (83 mg, 46%) which was obtained as a solid after lyophilization. The more polar fraction from the column consisted of a *ca.* 1 : 1 mixture of gentamicin C_1 (20) and 1-*epi*-gentamicin C_1 (25) (20 mg, 11%) which was obtained as a solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization.

1-N-*Methylgentamicin* C_1 (26) *and* 1-epi-N-*Methylgentamicin* C_1 (27).—The pH of a solution of methylamine (480 mg) in dry methanol (23 ml) was adjusted to pH 6 by addition of a solution of methanol saturated with dry hydrogen chloride gas. 1-Deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (sulphate salt) (22) (1 g) was added and the pH was readjusted to 6 by addition of methylamine. Sodium cyanoborohydride (460 mg) was added and the reaction was stirred at 25 °C for 20 h, the pH being maintained at 5.5—6. Concentrated ammonium hydroxide (20 ml) was added and the mixture was allowed to remain at 25 °C for 100 h. The solution was concentrated to dryness and the residue was chromatographed on a silica-gel column (165 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant. The less polar fractions were rechromatographed on a silica-gel column (160 × 2.5 cm) using initially a chloroform–methanol–concentrated

ammonium hydroxide solution (30 : 10 : 1 v/v) as the eluant, followed by the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-epi-N-*methylgentamicin* C_1 (27) (132 mg, 18%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 53.55; H, 9.1; N, 14.05. $C_{22}H_{45}N_5O_7$ requires C, 53.75; H, 9.23; N, 14.25%), $[\alpha]_D$ +193.6° (H_2O), $\nu_{max.}$ (KCl) 3 300, 1 060, and 1 030 cm^{-1}, δ (D_2O) 0.99 (3 H, d, *J* 6.5 Hz, 6′-CH_3), 1.13 (3 H, s, 4″-CH_3), 2.25 (6 H, s, 1-NCH_3 and 6′-NCH_3), 2.44 (3 H, s, 3″-NCH_3), 2.44 (3 H, s, 3″-NCH_3), 4.93 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.07 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz, 1′*eq*-H).

The intermediate polarity fractions from the initial column were rechromatographed on a silica-gel column (110 × 1 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-N-*methylgentamicin* C_1 (26) (25 mg, 3%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: M^+, 491.3340. $C_{22}H_{45}N_5O_7$ requires *M*, 491.3319), $[\alpha]_D$ +122.4° (H_2O), δ (D_2O) 1.04 (3 H, d, *J* 6.5 Hz, 6′-CH_3), 1.18 (3 H, s, 4″-CH_3), 2.29 (3 H, s, 6′-NCH_3), 2.32 (3 H, s, 1-NCH_3), 2.49 (3 H, s, 3″-NCH_3), 4.95 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.13 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz, 1′-*eq*-H).

The most polar fraction from the initial column afforded a mixture of 1-deamino-1-hydroxygentamicin C_1 (23) and 1-deamino-1-*epi*-hydroxygentamicin C_1 (24) (90 mg, 13%) as an amorphous solid after lyophilization.

1-N-*Isopropylgentamicin* C_1 (28) *and* 1-epi-N-*Isopropylgentamicin* C_1 (29).—The pH of a solution of isopropylamine (600 mg) in dry methanol (60 ml) was adjusted to pH 6 by addition of a solution of methanol saturated with dry hydrogen chloride gas. 1-Deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (sulphate salt) (22) (1 g) was added and the pH was readjusted to 6 by addition of a drop of isopropylamine. Sodium cyanoborohydride (500 mg) was added and the reaction mixture was stirred at 25 °C for 16 h the pH being maintained at 5.5—6. Concentrated ammonium hydroxide (30 ml) was added and the mixture was allowed to remain at 25 °C for 72 h. The solution was concentrated to dryness and the residue was chromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-epi-N-*isopropylgentamicin* C_1 (29) (25 mg, 3%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: M^+, 519.3625. $C_{24}H_{49}N_5O_7$ requires *M*, 519.3632), δ (D_2O) 1.00 (3 H, d, *J* 6.5 Hz, 6′-CH_3), 1.03 [3 H, d, *J* 6.5 Hz, $CH(CH_3)_2$], 1.06 [3 H, d, *J* 6.5 Hz, $CH(CH_3)_2$], 1.15 (3 H, s, 4″-CH_3), 2.39 (3 H, s, 6′-NCH_3), 2.47 (3 H, s, 3″-NCH_3), 4.87 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.14 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz, 1′*eq*-H), and 1-N-*isopropylgentamicin* C_1 (28) (20 mg, 3%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: M^+, 519.3610. $C_{24}H_{49}N_5O_7$ requires *M*, 519.3632), δ (D_2O) 1.00 (3 H, d, *J* 6.5 Hz, 6′-CH_3), 1.02 [6 H, d, *J* 6.5 Hz, $CH(CH_3)_2$], 1.15 (3 H, s, 4″-CH_3), 2.44 (3 H, s, 6′-NCH_3), 2.49 (3 H, s, 3″-NCH_3), 4.95 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.20 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz, 1′*ax*-H).

The more polar fractions from the column afforded a mixture of 1-deamino-1-hydroxygentamicin C_1 (23) and 1-deamino-1-*epi*-hydroxygentamicin C_1 (24) (120 mg, 17%) as an amorphous solid after lyophilization.

1-N-(2-*Hydroxyethyl*)*gentamicin* C_1 (30) *and* 1-epi-N-(2-*Hydroxyethyl*)*gentamicin* C_1 (31).—The pH of a solution of 2-hydroxyethylamine (720 mg) in dry methanol (30 ml) was adjusted to pH 6 by addition of a solution of methanol saturated with dry hydrogen chloride gas. 1-Deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (sulphate salt) (22) (1.3 g) was added and the pH was readjusted to 6 by addition of a few drops of 2-hydroxyethylamine. Sodium cyanoborohydride (600 mg) was added and the reaction mixture was stirred at 25 °C for 17 h, the pH being maintained at 5.5—6. Concentrated ammonium hydroxide (10 ml) was added and the mixture was allowed to remain at 25 °C for 48 h. The solution was concentrated to dryness and the residue was chromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant. The major products were each rechromatographed on a silica-gel column (160 × 2 cm) using chloroform–methanol–7% ammonium hydroxide solution (1 : 2 : 1 v/v) as the eluant in each case to give as the more polar product 1-N-(2-*hydroxyethyl*)*gentamicin* C_1 (30) (180 mg, 17%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 52.8; H, 8.95; N, 13.6. $C_{23}H_{47}N_5O_8$ requires C, 52.95; H, 9.08; N, 13.43%), $[\alpha]_D$ +98.0° (H_2O), $\nu_{max.}$ (KBr) 3 300 and 1 060 cm^{-1}, δ (D_2O) 0.99 (3 H, d, *J* 6.5 Hz, 6′-CH_3), 1.13 (3 H, s, 4″-CH_3), 2.28 (3 H, s, 6′NCH$_3$), 2.45 (3 H, s, 3″-NCH_3), 4.97 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.11 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz, 1′*eq*-H), and as the less polar product the 1-epi-*analogue* (31) (150 mg, 15%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: M^+, 521.3469. $C_{23}H_{47}N_5O_8$ requires *M*, 521.3469), $[\alpha]_D$ +154.3° (H_2O), $\nu_{max.}$ (KBr) 3 330 and 1 060 cm^{-1}, δ (D_2O) 0.99 (3 H, d, *J* 6.5 Hz, 6′-CH_3), 1.14 (3 H, s, 4″-CH_3), 2.27 (3 H, s, 6′-NCH_3), 2.45 (3 H, s, 3″-NCH_3), 4.96 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.08 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz, 1′*eq*-H).

The more polar fractions from the initial column afforded traces of a mixture of 1-deamino-1-hydroxygentamicin C_1 (23) and 1-deamino-1-*epi*-hydroxygentamicin C_1 (24).

1-N-(2-*Phenylethyl*)*gentamicin* C_1 (32) *and* 1-epi-N-(2-*Phenylethyl*)*gentamicin* C_1 (33).—The pH of a solution of 2-phenylethylamine (1.44 g) in dry methanol (30 ml) was adjusted to pH 6 by addition of a solution of methanol saturated with dry hydrogen chloride gas. 1-Deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (sulphate salt) (22) (1.3 g) was added and the pH was readjusted to 6 by addition of a few drops of 2-phenylethylamine. Sodium cyanoborohydride (600 mg) was added and the reaction mixture was stirred at 25 °C for 19 h, the pH being maintained at 5.5—6. Concentrated ammonium hydroxide (20 ml) was added and the mixture was allowed to remain at 25 °C for 116 h. The solution was concentrated to dryness and the residue was chromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–propan-2-ol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-epi-N-(2-*phenylethyl*)*gentamicin* C_1 (33) (182 mg, 16%) as an amorphous solid after passage over Amerblite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 59.85; H, 8.6; N, 11.65. $C_{29}H_{51}N_5O_7$ requires C, 59.87; H, 8.84; N, 12.04%), $[\alpha]_D$ +152.1° (H_2O), $\nu_{max.}$ (KBr) 3 330, 1 065, and 1 030 cm^{-1}, δ (D_2O) 0.99 (3 H, d, *J* 6.5 Hz, 6′-CH_3), 1.12 (3 H, s, 4″-CH_3), 2.27 (3 H, s, 6′-NCH_3), 2.44 (3 H, s, 3″-NCH_3), 4.81 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), 5.05 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz,

1′*eq*-H), and 7.24 (5 H, s, C_6H_5), and 1-N-(2-*phenylethyl*)-*gentamicin* C_1 (32) (107 mg, 9%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 59.05; H, 8.5; N, 12.7. $C_{29}H_{51}N_5O_7$ requires C, 59.87; H, 8.48; N, 12.04%), $[\alpha]_D$ +99.4° (H_2O), $\nu_{max.}$ (KBr) 3 300, 1 050, and 1 030 cm^{-1}, δ (D_2O) 0.99 (3 H, d, *J* 6.5 Hz, 6′-CH_3), 1.10 (3 H, s, 4″-CH_3), 2.28 (3 H, s, 6′-NCH_3), 2.43 (3 H, s, 3″-NCH_3), 4.88 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), 5.08 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz, 1′*eq*-H), and 7.33 (5 H, s, C_6H_5).

The more polar fractions from the column afforded a mixture of 1-deamino-1-hydroxygentamicin C_1 (23) and 1-deamino-1-*epi*-hydroxygentamicin C_1 (24) (290 mg, 31%) as an amorphous solid after lyophilization.

1-*Deamino*-1-*oxo*-3,2′,6′-*tris*-N-(2,2,2-*trichloroethoxycarbonyl*)*gentamicin* C_{1a} (37).—3,2′,6′-Tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} [18] (36) (3.5 g) was dissolved in anhydrous methanol (70 ml). 3,5-Di-t-butyl-1,2-benzoquinone (770 mg) was added and the solution was stirred under dry nitrogen at 25 °C for 7 h. The solution was acidified to pH 3 using 1M-sulphuric acid and the mixture was stirred at 25 °C for 17 h The solution was neutralized to pH 7 using Amberlite IR 45 resin and filtered, and the filtrate was evaporated to dryness. The residue was chromatographed on a silica-gel column (20 × 3.5 cm) by gradient elution using chloroform (1.5 l), 1% methanol in chloroform (1 l), and 5% methanol in chloroform (1 l) as the eluant. Evaporation of the latter fractions afforded the *ketone* (37) (2.35 g, 68%) as an amorphous solid (Found: C, 32.9; H, 4.3; Cl, 32.0; N, 5.3. $C_{28}H_{40}Cl_9N_4O_{14}\cdot 2H_2O$ requires C, 33.2; H, 4.4; Cl, 31.5; N, 5.5%), $[\alpha]_D$ +86.4° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 425, 3 350, 1 720, 1 520, 1 100, and 1 040 cm^{-1}.

1-(2,2,2-*Trichloroethoxycarbonyl*)*imidazole*.—Imidazole (1 g) was dissolved in tetrahydrofuran (10 ml) and the solution was cooled to 0 °C. 2,2,2-Trichloroethyl chloroformate (1.55 g) in tetrahydrofuran (10 ml) was added dropwise over 1 h. The mixture was then stirred at 25 °C for 1 h, filtered, and concentrated. The resulting solid was washed with water and dried to afford 1-(2,2,2-trichloroethoxycarbonyl)-imidazole (1.96 g, 96%), m.p. 80 °C, $\nu_{max.}$ ($CHCl_3$) 3 000, 1 780, 1 400, 1 310, 1 280, 1 235, 1 165, and 1 015 cm^{-1}, δ ($CDCl_3$) 5.03 (2 H, s, CH_2CCl_3), 7.13 (1 H, m, 4-H), 7.48 (1 H, m, 5-H), and 8.20 (1 H, s, 2-H).

3″-N,4″-O-*Carbonyl*-3,2′,6′-*tris*-N-(2,2,2-*trichloroethoxycarbonyl*)*gentamicin* C_{1a} (38).—(*a*) 1-(2,2,2-Trichloroethoxycarbonyl)imidazole (624 mg) was added to a solution of 3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (36) [18] (500 mg) in dry tetrahydrofuran (10 ml) and the solution was allowed to remain at 25 °C for 24 h. The solution was evaporated and the residue was dissolved in ethyl acetate and washed with water. The ethyl acetate layer was dried ($MgSO_4$), filtered, and evaporated to dryness, and the residue was chromatographed on a silica-gel column (110 × 2.5 cm) using 4% v/v methanol–chloroform as the eluant to give the 3″-N,4″-O-*carbonyl derivative* (38) (290 mg, 56%) as an amorphous solid (Found: C, 33.9; H, 4.0; Cl, 32.6; N, 6.65. $C_{29}H_{40}Cl_9N_5O_{14}\cdot H_2O$ requires C, 34.15; H, 4.15; Cl, 31.29; N, 6.87%), $[\alpha]_D$ +78.6° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 375, 2 940, 1 730, and 1 520 cm^{-1}, δ ($CDCl_3$), 1.38 (3 H, s, 4″-CH_3), 2.98 (3 H, s, 3″-NCH_3), 4.77br (6 H, s, CH_2CCl_3), 5.08 (1 H, m, 1″-H), and 5.43 (1 H, m, 1′-H).

(*b*) 1,1′-Carbonyldi-imidazole (5 mg) was added to a solution of 3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)-gentamicin C_{1a} [18] (36) (30 mg) in tetrahydrofuran (5 ml) and the solution was allowed to remain at 25 °C for 0.25 h. The mixture was worked up as in (*a*) above to give the carbonyl derivative (38).

3″-N-*Acetyl*-3,2′,6′-*tris*-N-(2,2,2-*trichloroethoxycarbonyl*)-*gentamicin* C_{1a} (39).—3,2′,6′-Tris-*N*-(2,2,2-trichloroethoxycarbonylgentamicin C_{1a} [18] (36) (1.8 g) was dissolved in a solution of tetrahydrofuran (75 ml) and water (38 ml). 1-Acetylimidazole (248 mg) was added and the reaction mixture was kept at 25 °C for 65 h. The solution was evaporated to dryness and the residue was azeotroped with benzene. Chromatography on a silica-gel column (30 × 3 cm) using 10% v/v methanol–chloroform as the eluant afforded 3″-N-*acetyl*-3,2′,6′-*tris*-N-(2,2,2-*trichloroethoxycarbonyl*)*gentamicin* C_{1a} (39) (1.32 g, 70%) as an amorphous solid (Found: C, 35.5; H, 4.3; Cl, 31.2; N, 6.5. $C_{30}H_{44}Cl_9N_5O_{14}$ requires C, 35.4; H, 4.4; Cl, 31.4; N, 6.9%), $[\alpha]_D$ +72.3° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 350, 2 930, 1 725, and 1 620 cm^{-1}, δ_H ($CDCl_3$), 1.10br (3 H, s, 4″-CH_3), 2.17 (3 H, s, 3″-NAc), 3.15 (3 H, s, 3″-NCH_3), and 4.72 (6 H, m, OCH_2CCl_3).

3″-N-*Acetyl*-1-*deamino*-1-*oxo*-3,2′,6′-*tris*-N-(2,2,2-*trichloroethoxycarbonyl*)*gentamicin* C_{1a} (40).—3″-*N*-Acetyl-3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (39) (8.57 g) was dissolved in methanol (225 ml) and 3,5-di-t-butyl-1,2-benzoquinone (2.04 g) was added. After 16 h at 25 °C, tetrahydrofuran (20 ml), and water (10 ml) were added. Oxalic acid was then added until pH 3 and the mixture was stirred at 25 °C for 24 h. The solution was evaporated and the residue was taken up in chloroform and filtered and the filtrate was evaporated. Chromatography on a silica-gel column (45 × 3 cm) using 3% v/v methanol–chloroform as the eluant afforded the *ketone* (40) (8.1 g, 94%) as an amorphous solid (Found: C, 35.0; H, 4.4; Cl, 30.8; N, 5.4. $C_{30}H_{41}Cl_9N_4O_{15}\cdot H_2O$ requires C, 34.8; H, 4.2; Cl, 30.8; N, 5.4%), $[\alpha]_D$ +95.3° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 425, 3 325, 2 950, 1 730, and 1 620 cm^{-1}, δ_H ($CDCl_3$) 1.05br (3 H, s, 4″-CH_3), 2.12 (3 H, s, 3″-NAc), 3.15 (3 H, s, 3″-NCH_3), and 4.74br (6 H, s, OCH_2CCl_3).

1-*Deamino*-1-*hydroxygentamicin* C_{1a} (41) *and* 1-*Deamino*-1-epi-*hydroxygentamicin* C_{1a} (42).—(*a*) 1-Deamino-1-oxo-3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (37) (1.7 g) was dissolved in methanol (50 ml) and the solution was adjusted to pH 3 using 1M-sulphuric acid. Sodium cyanoborohydride (408 mg) was added and the solution was stirred at 25 °C for 18 h. The reaction mixture was evaporated to dryness and the residue was taken up in 10% acetic acid in methanol (50 ml). Powdered zinc (428 mg) was added and the mixture was heated under reflux for 3 h. The solution was filtered and the filtrate was evaporated to dryness and the residue was then azeotroped with toluene. The solid was dissolved in water and the solution was adjusted to pH 3 by addition of 2M-hydrochloric acid. The solution was reconcentrated, azeotroped with toluene, and then dissolved in water and passed over Amberlite IRA 40IS (OH^-) resin. The eluate was evaporated to dryness and the residue was chromatographed first on a silica-gel column (160 × 2.5 cm) using chroform–methanol–10% ammonium hydroxide solution (1 : 2 : 1 v/v) as the eluant and then again on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-*deamino*-1-*hydroxygentamicin* C_{1a} (41) (54 mg, 7%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: $M^{+\cdot}$, 450.2641. $C_{19}H_{38}N_4O_8$ requires *M*, 450.2689), $[\alpha]_D$ +150.7° (H_2O), $\nu_{max.}$ (KBr) 3 340 and 1 050 cm^{-1}, δ (D_2O) 1.17 (3 H,

s, 4''-CH_3), 2.50 (3 H, s, 3''-NCH_3), 5.18 (1 H, d, $J_{1''ax,2''ax}$ 4 Hz, 1''*eq*-H), and 5.24 (1 H, d, $J_{1'eq,2'ax}$ 3.8 Hz, 1'*eq*-H), and the 1-epi-*analogue* (42) (33 mg, 4%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: $M^{+\cdot}$, 450.2667. $C_{19}H_{38}N_4O_8$ requires *M*, 450.2689), $[\alpha]_D$ +158.3° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 055, and 1 020 cm^{-1}, δ (D_2O) 1.17 (3 H, s, 4''-CH_3), 2.50 (3 H, s, 3''-NCH_3), 4.24 (1 H, m, 1-H), 5.03 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1''*eq*-H), and 5.20 (1 H, d, $J_{1'eq,2'ax}$ 3.8 Hz, 1'*eq*-H).

(*b*) 1-Deamino-1-oxo-3,2'6'-tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (37) (1.3 g) was dissolved in ethanol (50 ml) and the solution was adjusted to pH 7 using 1M-sulphuric acid in ethanol. Sodium borohydride (494 mg) was added and the mixture was stirred under argon for 3 h at 25 °C. The excess of hydride was destroyed by dropwise addition of acetic acid and the solution was evaporated to dryness. The resulting solid was dissolved in 10% acetic acid in methanol (50 ml) and powdered zinc (338 mg) was added. The mixture was heated under reflux for 4.5 h. The mixture was filtered and the combined filtrate and methanol washings were evaporated to dryness and azeotroped with toluene. The solid was dissolved in water (10 ml) and the solution was adjusted to pH 4 with 2M-hydrochloric acid. The solution was evaporated to dryness and the residue was dissolved in water and passed over Amberlite IRA 40IS (OH^-) resin. The eluate was evaporated to dryness and the residue was chromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–10% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization, 1-deamino-1-hydroxygentamicin C_{1a} (41) (43 mg, 7%) and 1-*epi*-analogue (42) (65 mg, 11%) as amorphous solids, which were identical with samples prepared in (*a*) above.

3,2'6'-Tri-N-acetyl-1-deamino-1-oxosisomicin (10).—3,2'6'-Tri-*N*-acetylsisomicin [18] (9) (5 g) was dissolved in anhydrous methanol (200 ml). 3,5-Di-t-butyl-1,2-benzoquinone (1.92 g) was added and the solution was stirred under dry nitrogen at 25 °C for 25 h. The solution was acidified to pH 3 using 1M-sulphuric acid and the mixture was stirred at 25 °C. The hydrolysis was judged to be complete (t.l.c.) after 15 h, and the mixture was diluted with distilled water and extracted with chloroform (3 × 50 ml). The aqueous layer was neutralized to pH 7 with 2M-ammonium hydroxide and then passed over Amberlite IR 45 resin. The aqueous eluate was concentrated *in vacuo* and lyophilized to give 3,2',6'-tri-*N*-acetyl-1-deamino-1-oxosisomicin (10) (5 g, 100%) as an amorphous solid, $[\alpha]_D$ +186.1° (H_2O), $\nu_{max.}$ (KCl) 3 200 and 1 020 cm^{-1}, δ (D_2O) 1.30 (3 H, s, 4''-CH_3), 1.87, 1.91, and 1.96 (9 H, 3 s, NHAc), and 2.90 (3 H, s, 3''-NCH_3).

3''-N-Acetyl-3,2',6'-tri-N-benzoylsisomicin (12).—3,2'6'-Tri-*N*-benzoylsisomicin (11) [18] (2.22 g) was dissolved in a mixture of tetrahydrofuran (90 ml) and water (45 ml). 1-Acetylimidazole (483 mg) in tetrahydrofuran (20 ml) was added and the solution was stirred at 25 °C for 16 h. The solution was evaporated to dryness. The residue was azeotroped with benzene and then chromatographed on a silica-gel column (30 × 3 cm) using 10% v/v methanol–chloroform as the eluant to give the 3''-*N-acetyl derivative* (12) as an amorphous solid (2.01 g, 85%) (Found: C, 61.6; H, 6.4; N, 8.6. $C_{42}H_{51}N_5O_{11}\cdot H_2O$ requires C, 61.5; H, 6.5; N, 8.5%), $[\alpha]_D$ +120.5° (DMSO), $\nu_{max.}$ (KBr) 3 325, 2 920, and 1 640 cm^{-1}, δ_H ([$^2H_6$]DMSO) 1.08 (3 H, m, 4''-CH_3), 2.05 (3 H, s, 3''-NAc), 3.10 (3 H, s, 3''-NCH_3), 7.50 (9 H, m, Ar), and 7.95 (6 H, m, Ar).

3''-N-Acetyl-3,2',6'-tri-N-benzoyl-1-deamino-1-oxosisomicin (13).—Freshly decarbonated 3''-*N*-acetyl-3,2',6'-tri-*N*-benzoylsisomicin (12) (767 mg) was dissolved in methanol (20 ml) and tetrahydrofuran (5 ml). 3,5-Di-t-butyl-1,2-benzoquinone (232 mg) was added and the reaction mixture was stirred at 25 °C for 18 h. An aqueous solution of malonic acid was added to pH 4 and the mixture was allowed to remain at 25 °C for 24 h. The solution was evaporated to dryness and chromatographed on a silica-gel column (30 × 2.5 cm) using 3% v/v methanol–chloroform as the eluant to give the *ketone* (13) (651 mg, 85%) as an amorphous solid (Found: C, 62.8; H, 6.2; N, 6.9. $C_{42}H_{48}N_4O_{12}$ requires C, 63.0; H, 6.0; N, 7.0%), $[\alpha]_D$ +157.1° (DMSO), $\nu_{max.}$ (KBr) 3 300, 2 920, 1 720, and 1 650 cm^{-1}, δ_H ($CDCl_3$) 1.00br (3 H, s, 4''-CH_3), 2.10br (3 H, s, 3''-NAc), 3.10br (3 H, s, 3''-NCH_3), 7.50 (9 H, m, Ar), and 7.90br (6 H, s, Ar).

1-Deamino-1-hydroxysisomicin (14) *and 1-Deamino-1-epi-hydroxysisomicin* (15).—(*a*) 3,2',6'-Tri-*N*-acetyl-1-deamino-1-oxosisomicin (10) (2 g) was dissolved in methanol–water (8 : 2 v/v; 100 ml) and the solution was acidified to pH 3 using 1M-sulphuric acid. Sodium cyanoborohydride (880 mg) was added and the solution was stirred under dry argon at 25 °C for 18 h. The solution was filtered and the filtrate was evaporated to dryness *in vacuo*. The gum was dissolved in 5% w/v aqueous sodium hydroxide (70 ml) and the solution was heated under reflux under argon for 60 h. The solution was cooled and neutralized with Amberlite IRC 50 (H^+) resin, and the latter was washed with water (2 l). The resin was then eluted with 7% ammonium hydroxide solution (2.5 l) and the basic eluate was evaporated to dryness. The resulting gum was chromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-*deamino-1-hydroxysisomicin* (14) (170 mg, 11%) as a solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 49.65; H, 8.0; N, 12.8. $C_{19}H_{36}N_4O_8\cdot 0.5H_2O$ requires: C, 49.90; H, 8.15; N, 12.25%), $[\alpha]_D$ +168.3° (H_2O), $\nu_{max.}$ (KCl) 3 350, 1 680, and 1 000 cm^{-1}, δ (D_2O) 1.22 (3 H, s, 4''-CH_3), 2.52 (3 H, s, 3''-NCH_3), 4.90 (1 H, m, 4'-H), 5.29 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1''*eq*-H), and 5.38 (1 H, d, $J_{1'eq,2'ax}$ 2 Hz, 1'*eq*-H). The more polar fractions were rechromatographed on a silica-gel column (160 × 2.5 cm) using chloroform–methanol–7% ammonium hydroxide solution (1 : 2 : 1 v/v) as the eluant to give the 1-epi-*analogue* (15) (117 mg, 7%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: $M^{+\cdot}$, 448.2517. $C_{19}H_{36}N_4O_8$ requires *M*, 448.2533), $[\alpha]_D$ +145.4° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 680, and 1 075 cm^{-1}, δ (D_2O) 1.23 (3 H, s, 4''-CH_3) 2.52 (3 H, s, 3''-NCH_3), 4.34 (1 H, m, 1-H), 4.91 (1 H, m, 4'-H), 5.08 (1 H, d, $J_{1''eq,2''ax}$ 3.5 Hz, 1''*eq*-H), and 5.39 (1 H, d, $J_{1'eq,2'ax}$ 2.5 Hz, 1'*eq*-H).

(*b*) L-Selectride (1M) (2.5 ml) was added dropwise to a solution of 3''-*N*-acetyl-3,2',6'-tri-*N*-benzoyl-1-deamino-1-oxosisomicin (13) (1 g) in dry tetrahydrofuran (12 ml) at −78 °C under nitrogen. After 2 h at − 78 °C the mixture was oxidized with 5% sodium hydroxide and 50% hydrogen peroxide solutions and then diluted with water and extracted with chloroform. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was heated under reflux with 5% aqueous sodium hydroxide under nitrogen for 40 h. The mixture was cooled, and neutralized

with hydrochloric acid and Amberlite IRC 50 (H^+) resin. The resin was washed with water and the latter was discarded. Washing with 7% aqueous ammonium hydroxide afforded the crude product which was chromatographed on a silica-gel column (160 × 3 cm) using the lower phase of a chloroform–methanol–15% ammonium hydroxide solution as the eluant to give 1-deamino-1-hydroxysisomicin (14) (14 mg, 3%) and its 1-*epi*-analogue (15) (108 mg, 19%) which were identical with the samples prepared in (*a*) above.

Sisomicin (7) *and* 1-epi-*Sisomicin* (16).—Ammonium acetate (2.7 g) was dissolved in dry methanol (100 ml) and the pH was adjusted to 5 using dry hydrogen chloride in methanol. 3,2′,6′-Tri-*N*-acetyl-1-deamino-1-oxosisomicin (10) (2 g) was added and the mixture was stirred at 25 °C for 7 h. Sodium cyanoborohydride (1.8 g) was added and the reaction mixture was stirred at 25 °C for 18 h. The mixture was filtered and the filtrate was evaporated to dryness. The solid was dissolved in 5% aqueous sodium hydroxide (*100* ml) and the mixture was heated under reflux for 50 h under argon. The mixture was cooled, neutralized with Amberlite IRC 50 (H^+) resin, and the resin was washed with water (2 l). The resin was then eluted with 7% ammonium hydroxide solution (2.5 l) and the basic eluate was evaporated to dryness and the residue was chromatographed on a silica-gel column (160 × 5 cm) using chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1 v/v) as the eluant to give a mixture of 1-deamino-1-hydroxysisomicin (14) and 1-deamino-1-*epi*-hydroxysisomicin (15) as well as a mixture of sisomicin (7) and 1-*epi*-sisomicin (16).

The amines (7) and (16) were rechromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give sisomicin (7) (76 mg, 5%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization, which was identical (t.l.c. and mass and ^1H n.m.r. spectra) with an authentic sample. The more polar fractions from the column afforded 1-epi-*sisomicin* (16) (156 mg, 10%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization [Found: $(M + 1)^+$, 448.2728. $C_{19}H_{38}N_5O_7$ requires $M + 1$, 448.2771], $[\alpha]_D$ +117.8° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 670, and 1 050 cm^{-1}, δ (D_2O) 1.29 (3 H, s, 4″-CH_3), 2.61 (3 H, s, 3″-NCH_3), 5.02 (1 H, m, 4′-H), 5.10 (1 H, d, $J_{1''eq,2''ax}$ 3.5 Hz, 1″*eq*-H), and 5.46 (1 H, d, $J_{1'eq,2'ax}$ 2 Hz, 1′*eq*-H).

The alcohols (14) and (15) were rechromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-deamino-1-hydroxysisomicin (14) (70 mg, 4%) and 1-deamino-1-*epi*-hydroxysisomicin (15) (15 mg, 3%) which were identical with authentic samples (t.l.c.).

1-N-*Ethylsisomicin* (8) *and* 1-epi-N-*Ethylsisomicin* (17).—(*a*) The pH of a solution of ethylamine (1.62 g) in dry methanol (100 ml) was adjusted to pH 5.5 by addition of a solution of methanol saturated with dry hydrogen chloride gas. 3,2′,6′-Tri-*N*-acetyl-1-deamino-1-oxosisomicin (10) (4 g) was added and the reaction mixture was stirred at 25 °C for 7 h. Sodium cyanoborohydride (1.76 g) was added and the reaction mixture was stirred at 25 °C for 18 h during which time the pH of the solution gradually rose from pH 5 to pH 6.9. The solution was concentrated and the residue was taken up in 5% aqueous sodium hydroxide (200 ml) and the mixture was heated under reflux for 50 h under argon. The mixture was cooled and neutralized with Amberlite IRC 50 (H^+) resin, and the resin was washed with water (2 l). The resin was then eluted with 7% ammonium hydroxide solution (2.5 l) and the basic eluate was evaporated to dryness and the residue was chromatographed on a silica-gel column (160 × 7 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-epi-N-*ethylsisomicin* (17) (633 mg, 19%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 48.95; H, 8.35; N, 13.15. $C_{21}H_{41}N_5O_7 \cdot H_2O \cdot CO_2$ requires C, 49.14; H, 8.06; N, 13.02%), $[\alpha]_D$ +195.9° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 680, 1 050, and 1 020 cm^{-1}, δ (D_2O) 1.12 (3 H, t, *J* 7.5 Hz, $NHCH_2CH_3$), 1.27 (3 H, s, 4″-CH_3), 2.57 (3 H, s, 3″-NCH_3), 4.96 (1 H, m, 4′-H), 5.03 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.41 (1 H, d, $J_{1'eq,2'ax}$ 2 Hz, 1′*eq*-H), and 1-*N*-ethylsisomicin (8) (339 mg, 10%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization, $[\alpha]_D$ +129.5° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 680, 1 050, and 1 020 cm^{-1}, δ (D_2O) 1.07 (3 H, t, $NHCH_2CH_3$), 1.21 (3 H, s, 4″-CH_3), 2.53 (3 H, s, 3″-NCH_3), 4.89 (1 H, m, 4′-H), 5.00 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.36 (1 H, d, $J_{1'eq,2'ax}$ 2 Hz, 1′*eq*-H).

The more polar fractions from the column afforded 1-deamino-1-hydroxysisomicin (14) (320 mg, 10%), 1-deamino-1-*epi*-hydroxysisomicin (15) (176 mg, 6%), and sisomicin (7) (159 mg, 4%) as amorphous solids.

(*b*) To a suspension of sodium hydride (379 mg, 57%; washed with hexane) in dry dimethoxyethane (4 ml) under nitrogen, was added a solution of diethyl *N*-ethylphosphoramidate (43) (814 mg) in dimethoxyethane (6 ml). The mixture was heated to 50 °C for 1.5 h and allowed to cool. The clear solution was transferred *via* a syringe to a clean flask under nitrogen. A solution of 1-*N*-acetyl-3,2′,6′-tri-*N*-benzoyl-1-deamino-1-oxosisomicin (13) (340 mg) in dimethoxyethane (5 ml) was added dropwise and the mixture was heated at 50 °C for 1 h. The solution was cooled to −78 °C and L-Selectride (0.85 ml) was added dropwise. After 2 h at −78 °C the excess of L-Selectride was quenched with methanol. 5% Sodium hydroxide (1.5 ml) and 50% hydrogen peroxide (0.75 ml) solutions were added and the reaction was warmed slowly to 25 °C. Water was added and the mixture was extracted with chloroform. The organic extracts were washed with brine, dried (Na_2SO_4), and evaporated. The residue was heated under reflux with 5% aqueous sodium hydroxide under nitrogen for 40 h. The mixture was cooled and neutralized with hydrochloric acid and Amberlite IRC 50 (H^+) resin. The resin was washed with water and the latter was discarded. Elution with 7% aqueous ammonium hydroxide followed by evaporation afforded 1-*epi*-*N*-ethylsisomicin (17) (30 mg, 15%) which was identical with that prepared in (*a*) (t.l.c.).

1-N-(3-*Dimethylaminopropyl*)*sisomicin* (18) *and* 1-epi-N-(3-*Dimethylaminopropyl*)*sisomicin* (19).—The pH of a solution of 3-dimethylaminopropylamine (2.08 g) in dry methanol (100 ml) was adjusted to pH 5.7 by addition of a solution of methanol saturated with dry hydrogen chloride gas. 3,2′,6′-Tri-*N*-acetyl-1-deamino-1-oxosisomicin (10) (2.13 g) was added and the reaction mixture was stirred under dry argon at 25 °C for 7 h. Sodium cyanoborohydride (857 mg) was added and the reaction mixture was stirred at 25 °C for 18 h. The solution was concentrated and the residue was taken up in 5% aqueous sodium hydroxide (100 ml) and the mixture was heated under reflux for 50 h under argon. The solution was cooled, neutralized with Amberlite IRC 50 (H^+) resin, and the resin was washed with

water (1.5 l). The resin was eluted with 7% ammonim hydroxide solution (2 l) and the basic eluate was evaporated to dryness. The residue was chromatographed on a silica-gel column (160 × 5 cm) and then again on a silica-gel column (160 × 3.5 cm) using in each case the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant, to give 1-epi-N-(3-*dimethylaminopropyl*)*sisomicin* (19) (139 mg, 7%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: $M^{+\cdot}$, 532.3541. $C_{24}H_{48}N_6O_7$ requires M, 532.3584), $[\alpha]_D^{26}$ +162.5° (H_2O), $\nu_{max.}$ (KBr) 3 325, 1 675, 1 050, and 1 020 cm^{-1}, δ (D_2O) 1.21 (3 H, s, 4″-CH_3), 2.19 [6 H, s, $-N(CH_3)_2$], 2.52 (3 H, s, 3″-NCH_3), 4.90 (1 H, m, 4′-H), 5.00 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1′*eq*-H), and 5.38 (1 H, d, $J_{1'eq,2'ax}$ 2 Hz, 1′*eq*-H), and 1-N-(3-*dimethylaminopropyl*)*sisomicin* (18) (106 mg, 5%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: $M^{+\cdot}$, 532.3543. $C_{24}H_{48}N_6O_7$ requires M, 532.3584), $[\alpha]_D$ +112.3° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 680, 1 055, and 1 020 cm^{-1}, δ (D_2O), 1.20 (3 H, s, 4″-CH_3), 2.19 [6 H, s, $-N(CH_3)_2$], 2.50 (3 H, s, 3″-NCH_3), 4.88 (1 H, m, 4′-H), 4.97 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.34 (1 H, d, $J_{1'eq,2'ax}$ 2 Hz, 1′*eq*-H).

The more polar fractions from the column afforded 1-deamino-1-hydroxysisomicin (14) (68 mg, 4%) and 1-deamino-1-*epi*-hydroxysisomicin (15) (68 mg, 4%) as amorphous solids.

1-*Deaminogentamicin* C_1 (34).—1-Deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (22) (990 mg) was dissolved in dimethylformamide (10 ml) and sulpholan (10 ml). Toluene-*p*-sulphonyl hydrazide (300 mg) and toluene-*p*-sulphonic acid (30 mg) were added and the mixture was heated at 70 °C for 30 min. Sodium cyanoborohydride (300 mg) was added and the mixture was heated at 85 °C for 15 h. After cooling to 25 °C, concentrated ammonium hydroxide (12 ml) was added and the solution was allowed to remain at 25 °C for 16 h. The mixture was neutralized by addition of Amberlite IRC 50 (H^+) resin and the resin was washed with water and then eluted with 20% aqueous ammonium hydroxide which on evaporation gave a gum. Chromatography on a silica-gel column (100 × 3.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant, followed by rechromatography of the appropriate fractions on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant afforded 1-*deaminogentamicin* C_1 (34) (23 mg, 3%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: $M^{+\cdot}$, 462.3053. $C_{21}H_{42}N_4O_7$ requires M, 462.3078), $[\alpha]_D$ +155.9° (H_2O), δ (D_2O) 0.97 (3 H, d, $J_{6',7'}$ 6.5 Hz, 7′-CH_3), 1.14 (3 H, s, 4″-CH_3), 2.23 (3 H, s, 6′-NCH_3), 2.44 (3 H, s, 3″-NCH_3), 2.54 (1 H, d, $J_{2''ax,3''ax}$ 10.5 Hz, 3″*ax*-H), 3.25 (1 H, d, $J_{5''ax,5''eq}$ 12.5 Hz, 5″*ax*-H), 3.67 (1 H, dd, $J_{1''eq,2''ax}$ 4, $J_{2''ax,3''ax}$ 10.5 Hz, 2″*ax*-H), 3.93 (1 H, d, $J_{5''ax,5''eq}$ 12.5 Hz, 5″*eq*-H), 4.98 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.05 (1 H, d, $J_{1'eq,2'ax}$ 4 Hz, 1′*eq*-H).

O-2-*Amino*-2,3,4,6,7-*pentadeoxy*-6-*methylamino*-α-D-glycero-D-erythro-*heptopyranosyl*-(1 ⟶ 4)-O-[3-*deoxy*-4-C-*methyl*-3-*methylamino*-β-L-*arabinopyranosyl*-(1 ⟶ 2)]-1,2,4-*trihydroxybenzene* (44).—1-Deamino-1-oxo-3,2′-bis-*N*-trifluoroacetylgentamicin C_1 (22) (2.4 g) was dissolved in concentrated ammonium hydroxide (30 ml) and the mixture was allowed to remain at 25 °C for 24 h. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (110 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the *phenol* (44) (800 mg, 51%) as an amorphous solid (Found: C, 57.0; H, 7.9; N, 9.4. $C_{21}H_{35}N_3O_7$ requires C, 57.1; H, 8.0; N, 9.5%), $[\alpha]_D$ +227.8° (H_2O), $\lambda_{max.}$ (MeOH) 220 (ε 6 200) and 285 nm (3 500), $\nu_{max.}$ (Nujol) 3 350, 1 630, 1 515, 1 470, and 1 060 cm^{-1}, δ (D_2O) 1.05 (3 H, d, $J_{6',7'}$ 7 Hz, 7′-CH_3), 1.17 (3 H, s, 4″-CH_3), 2.28 (3 H, s, 6′-NCH_3), 2.52 (3 H, s, 3″-NCH_3), 5.27 (1 H, d, $J_{1''eq,2''ax}$ 3 Hz, 1″*eq*-H), 5.46 (1 H, d, $J_{1'eq,2'ax}$ 4 Hz, 1′*eq*-H), 6.72 (2 H, m, Ar), and 6.87 (1 H, m, Ar).

1,2′,6′,3″-*Tetrakis*-N-*benzyloxycarbonyl*-3-*deamino*-3-*oxogentamicin* C_{1a} (46).—1,2′,6′3″-Tetrakis-*N*-benzyloxycarbonylgentamicin C_{1a} (45) [18] (4 g) and 3,5-di-t-butyl-1,2-benzoquinone (1 g) were dissolved in methanol (35 ml) and the mixture was allowed to remain at 25 °C for 17 h. Oxalic acid (1.8 g) in 50% (v/v) aqueous methanol (16 ml) was added and the mixture was stored at 25 °C for 6 h and at 0—5 °C for 18 h. The solution was diluted with water, extracted with chloroform, and the chloroform layer was evaporated to dryness. The residue was chromatographed rapidly on silica gel (100 g) using 0.25% v/v methanol–chloroform as the eluant to elute the impurities followed by 1.5% v/v methanol–chloroform to give the 3-deamino-3-oxo-derivative (46) (3.3 g, 90%) as a pale yellow amorphous solid which was homogeneous on t.l.c.

3-*Deamino*-3-*hydroxygentamicin* C_{1a} (47) *and* 3-*Deamino*-3-epi-*hydroxygentamicin* C_{1a} (48).—1,2′,6′,3″-Tetrakis-*N*-benzyloxycarbonyl-3-deamino-3-oxo-gentamicin C_{1a} (46) (1.1 g) in methanol (10 ml) was treated with sodium borohydride (0.2 g) in water (1 ml). After 1 h at 25 °C, the mixture was diluted with chloroform, washed with water, dried ($MgSO_4$), and evaporated. The residue in dry tetrahydrofuran (15 ml) was added to a solution of sodium (0.5 g) in redistilled liquid ammonia (50 ml) at −80 °C. After 10 min, methanol (5 ml) was added and the ammonia was allowed to evaporate. The residue was dissolved in water (20 ml) and the solution was adsorbed onto Amberlite IRC 50 (H^+) resin. The resin was washed with water and then eluted with 2M-ammonium hydroxide solution. The eluate was evaporated to dryness and the residue was chromatographed on silica gel (60 g) using chloroform–methanol–concentrated ammonium hydroxide (4 : 2 : 1 v/v) as the eluant to give first 1-*deamino*-1-*hydroxygentamicin* C_{1a} (47) (190 mg, 34%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 47.9; H, 8.75; N, 11.6. $C_{19}H_{38}N_4O_6 \cdot 1.5H_2O$ requires C, 47.8; H, 8.65; N, 11.7%), $[\alpha]_D$ +163.9° (H_2O), δ_H (D_2O) 1.17 (3 H, s, 4″-CH_3), 2.48 (3 H, s, 3″-NCH_3), *ca.* 4.0 (1 H, m, $W_{\frac{1}{2}}$ *ca.* 20 Hz, 3*ax*-H), 5.05 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.12 (1 H, d, $J_{1'eq,2'ax}$ 4 Hz, 1′*eq*-H), and then the 1-epi-*analogue* (48) (90 mg, 16%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 48.0; H, 8.7; N, 11.85. $C_{19}H_{38}N_4O_6 \cdot 1.5H_2O$ requires C, 47.8; H, 8.65; N, 11.7%), $[\alpha]_D$ +140.6° (H_2O), δ_H (D_2O) 1.17 (3 H, s, 4″-CH_3), 2.49 (3 H, s, 3″-NCH_3), 4.23 (1 H, m, $W_{\frac{1}{2}}$ *ca.* 8 Hz, 3*eq*-H), 4.99 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.08 (1 H, d, $J_{1'eq,2'ax}$ 4 Hz, 1′*eq*-H).

3-epi-*Gentamicin* C_{1a} (50).—1,2′,6′,3″-Tetrakis-*N*-benzyloxycarbonyl-3-deamino-3-oxogentamicin C_{1a} (46) (2.1 g), ammonium acetate (5 g), and sodium cyanoborohydride (0.5 g) were dissolved in methanol (20 ml) and acetic acid (0.5 ml) and the mixture was stirred at 25 °C for 2.5 h. The

reaction mixture was diluted with 1M-ammonium hydroxide and extracted with chloroform. The chloroform layer was evaporated to dryness and the residue was chromatographed on silica gel (80 g) using 3% v/v methanol–chloroform as the eluant to give 1,2′,6′,3″-tetrakis-*N*-benzyloxycarbonyl-3-*epi*-gentamicin C_{1a} (49) (0.44 g, 21%) and 1,2′,6′,3″-tetrakis-*N*-benzyloxycarbonylgentamicin C_{1a} (45) (0.59 g, 28%). The latter was identical to the material used to prepare the 3-oxo-derivative (46). The axial amine (49) was dissolved in dry tetrahydrofuran (5 ml) and the solution was added to sodium (0.3 g) in distilled liquid ammonia (20 ml) at −80 °C. After 5 min, methanol was added, and the mixture was evaporated. The residue in water (20 ml) was adsorbed into Amberlite IRC 50 (H^+) resin. The resin was washed with water and then eluted with 2M-ammonium hydroxide solution to give 3-*epi*-gentamicin C_{1a} (50) (0.12 g, 57%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization, $[\alpha]_D$ +130.4° (H_2O), δ_H (D_2O) 1.17 (3 H, s, 4″-CH_3), 2.48 (3 H, s, 4″-NCH_3), 4.97 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.05 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1′*eq*-H).

1-*Deamino*-2-*deoxy*-1-*hydroxystreptamine* (51).—A crude sample of (51) (143 mg) obtained by reduction of 1-deamino-2-deoxy-1-oxo-3-*N*-(4-methoxybenzyloxycarbonyl)-streptamine followed by deprotection with trifluoroacetic acid, was chromatographed on a silica-gel column (40 × 1 cm) using chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1 v/v) as the eluant to give 1-*deamino*-2-*deoxy*-1-*hydroxystreptamine* (51) (23 mg) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 40.25; H, 6.15; N, 6.52. $C_6H_{13}NO{\cdot}CO_2$ requires C, 40.6; H, 6.33; N, 6.76%), $[\alpha]_D$ +101.9° (H_2O), *m/e* 164 (M + 1), δ_H (D_2O) 1.37 (1 H, ddd, $J_{1ax,2ax}$ 12, $J_{2eq,2ax}$ 12, $J_{2ax,3ax}$ 12 Hz, 2*ax*-H) and 2.11 (1 H, ddd, $J_{1ax,2eq}$ 4, $J_{2eq,2ax}$ 12, $J_{2eq,3ax}$ 4 Hz, 2*eq*-H).

1-*Deamino*-2-*deoxy*-1-epi-*hydroxystreptamine* (52).—1-Deamino-1-*epi*-hydroxysisomicin (15) (200 mg) was dissolved in 6M-hydrochloric acid (50 ml) and the solution was heated under reflux for 18 h. The solution was cooled and Amberlite IRA 40IS (OH^-) resin was added until the pH reached 10.0. The resin was then filtered off and washed with water (1 l) and the combined filtrates were evaporated to dryness. The residue was chromatographed on a silica-gel column (40 × 1 cm) using chloroform–methanol–3% ammonium hydroxide (1 : 2 : 1 v/v) as the eluant to give 1-*deamino*-2-*deoxy*-1-epi-*hydroxystreptamine* (52) (73 mg, 77%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 39.85; H, 8.2; N, 7.5. $C_6H_{13}NO_4{\cdot}H_2O$ requires C, 39.77; H, 8.34; N, 7.73%), $[\alpha]_D$ +97.7° (H_2O), *m/e* 164 (M + 1), $\nu_{max.}$ (KBr) 3 400 cm^{-1}, δ_H (D_2O) 1.35 (1 H, ddd, $J_{1eq,2eq}$ 4, $J_{2eq,2ax}$ 14, $J_{2ax,3ax}$ 11 Hz, 2ax-H) and 1.93 (1 H, ddd, $J_{1eq,2eq}$ 4, $J_{2eq,2ax}$ 14, $J_{2eq,3ax}$ 4 Hz, 2*eq*-H).

1-epi-2-*Deoxystreptamine* (54).—A mixture of garamine (58) and 1-*epi*-sisomicin (16) (150 mg) was dissolved in 6M-hydrochloric acid (50 ml) and the solution was heated under reflux for 18 h. The solution was cooled and Amberlite IRA 40IS (OH^-) resin was added until the pH reached 10.0. The resin was filtered off and washed with distilled water (1 l) and the combined filtrates were evaporated to dryness. The residue was chromatographed on a silica-gel column (60 × 2.5 cm) using chloroform–methanol–3% ammonium hydroxide (1 : 2 : 1 v/v) as the eluant to give a mixture of 1-*epi*-2-deoxystreptamine (54) and 2-deoxystreptamine (53) (23 mg) which could not be separated. The sample was obtained as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization which was used to obtain the ^{13}C n.m.r. data for (54), *m/e* 163 (M + 1).*

1-epi-N-*Ethyl*-2-*deoxystreptamine* (56).—1-*epi*-*N*-Ethylsisomicin (17) (200 mg) was dissolved in 6M-hydrochloric acid (60 ml) and the solution was heated under reflux for 2 h. The solution was cooled and Amberlite IRA 40IS (OH^-) resin was added until the pH reached 10.8. The resin was filtered off and washed with water (1 l) and the eluate was concentrated. The resulting solid was chromatographed on a silica-gel column (50 × 1.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution as the eluant to give 1-epi-N-*ethyl*-1-*deoxystreptamine* (56) (52 mg). The latter was rechromatographed on a silica-gel column (110 × 1.5 cm) using the same solvent system to give pure (56) (35 mg, 44%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization [Found: (M + 1)$^+$, 191.1396. $C_8H_{19}N_2O_3$ requires M + 1, 192.1393], $[\alpha]_D$ +52.2° (H_2O) δ_H (D_2O) 1.11 (3 H, t, J 7 Hz, $NHCH_2CH_3$), and 2.63 (2 H, q, J 7 Hz, $NHCH_2CH_3$).

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* As a pure sample of (54) free of (53) could not be obtained the ^{13}C n.m.r. assignments for (54) should be regarded as tentative.

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