Mass Spectral Studies on Aminocyclitol–Aminoglycoside Antibiotics [1]

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The electron impact mass spectral fragmentation patterns of a series of underivatized aminocyclitol–aminoglycoside antibiotics are reported. and their utility in making structural assignments is discussed. The compositions of the fragment ions were confirmed by high resolution mass measurements, and in some cases the origins of the ions were determined by the " direct analysis of daughter ions " technique. The chemical ionization mass spectra of representative compounds of this class are also reported. In general the mass spectra of the underivatized compounds, at least up to the pseudotrisaccharide size. were simpler to interpret and afforded more useful diagnostic information than those of their more volatile permethyl, trimethylsilyl, and per-*N*-aralkylidene derivatives studied by others.

SINCE the advent of mass spectrometry, numerous studies describing the fragmentation pathways of compounds containing carbohydrates have been published.[2] Although the mass spectra of simple sugars have been obtained, their low volatility and thermal instability has often rendered direct mass spectral determination impractical, and the use of derivatives, notably methyl ethers, acetates, trifluoroacetates, and trimethylsilyl ethers, has often proved advantageous. This has been particularly true in the case of therapeutically important aminocyclitol–aminoglycoside antibiotics. Mass spectrometry as applied to these compounds has been confined to studies on their *N*-acetyl-*O*-trimethylsilyl,[3-6]

[1] Preliminary communication, P. J. L. Daniels, M. Kugelman, A. K. Mallams, R. W. Tkach, H. F. Vernay, J. Weinstein, and A. Yehaskel, *Chem. Comm.*, 1971, 1629

[2] T. Radford and D. C. DeJongh, ' Biochemical Applications of Mass Spectrometry,' ed. G. R. Waller, Wiley, New York, 1972, p. 313.

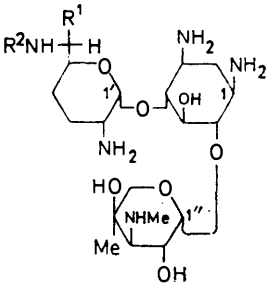
[3] D. C. DeJongh, J. D. Hribar, S. Hanessian, and P. W. K. Woo, *J. Amer. Chem. Soc.*, 1967, **89**, 3364.

[4] W. T. Schier, K. L. Rinehart, and D. Gottleib, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **63**, 198.

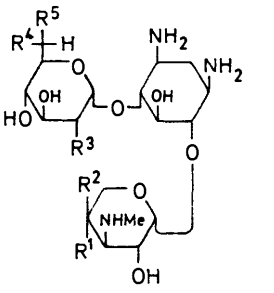
[5] P. W. K. Woo, *Tetrahedron Letters*, 1971, 2621.

[6] D. C. DeJongh, E. B. Hills, J. D. Hribar, S. Hanessian, and T. Chang, *Tetrahedron*, 1973, **29**, 2707.

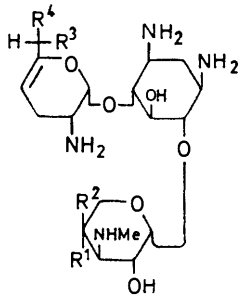
NO-trimethylsilyl,[7] *N*-acetyl-*NO*-methyl,[6,8] Schiff's base-oxazolidine,[9] and salicylidene [10] derivatives. Although much useful information has been obtained from mass

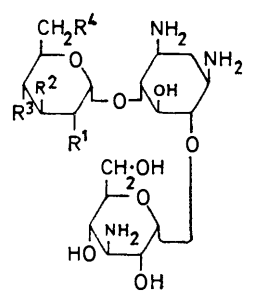


(1) $R^1=R^2=H$
(2) $R^1=R^2=Me$
(3) $R^1=Me$, $R^2=H$
(4) $R^1=H$, $R^2=Me$



(5) $R^1=R^4=OH$, $R^2=R^5=H$, $R^3=NH_2$
(6) $R^1=Me$, $R^2=R^3=OH$, $R^4=NH_2$, $R^5=H$
(7) $R^1=Me$, $R^2=R^4=OH$, $R^3=NH_2$, $R^5=H$
(8) $R^1=R^5=Me$, $R^2=R^4=OH$, $R^3=NH_2$



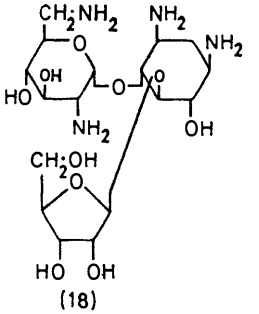


(9) $R^1=Me$, $R^2=OH$, $R^3=NH_2$, $R^4=H$
(10) $R^1=OH$, $R^2=R^4=H$, $R^3=NH_2$
(11) $R^1=R^4=H$, $R^2=OH$, $R^3=NH_2$
(12) $R^1=R^4=Me$, $R^2=OH$, $R^3=NH_2$
(13) $R^1=Me$, $R^2=OH$, $R^3=NHMe$, $R^4=H$
(14) $R^1=R^2=R^3=OH$, $R^4=NH_2$
(15) $R^1=R^4=NH_2$, $R^2=R^3=OH$
(16) $R^1=R^4=NH_2$, $R^2=H$, $R^3=OH$
(17) $R^1=R^4=NH_2$, $R^2=R^3=H$

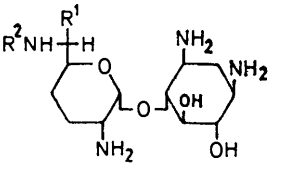
spectra of these derivatives their use has had its drawbacks. Apart from the obvious need to prepare the derivatives, often on a micro scale, they are of substantially higher molecular weight than the parent compounds and their spectra are complicated by fragmentations more closely associated with the nature of the derivatizing groups than with that of the parent compounds themselves. This is especially so for trimethylsilyl derivatives, whose molecular weights are over 1 000 and which have the added disadvantages of sensitivity to moisture and a high propensity for rearrangement on electron impact.

In the course of structural studies on aminocyclitol–aminoglycoside antibiotics carried out in our own laboratories [1,11-13] we have shown that satisfactory mass spectra can be obtained with the underivatized free bases up to the pseudotrisaccharide level by the direct inlet technique, provided that sufficient care is taken in sample preparation. By using samples that have been freed from carbon dioxide by passage over a strongly basic ion-exchange resin, good spectra can be obtained even for such polyhydroxylic compounds as gentamicin B (6) and kanamycin A (14). However, satisfactory spectra could not be obtained under these conditions for pseudotetrasaccharides such as neomycin, and for these compounds derivatization is a prerequisite for obtaining electron impact (e.i.) spectra. The information available from the spectra of the underivatized compounds is easier to analyse and is diagnostically more useful than that obtained from spectra of higher molecular weight derivatives.

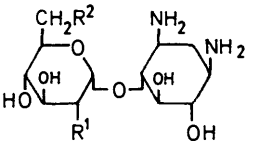
The structures of the aminocyclitol–aminoglycoside antibiotics used in this study, together with some of their hydrolysis and methanolysis products, are depicted in formulae (1)—(28). The names and principal e.i. mass spectral fragmentations of these compounds are given in Table 1. In general, the underivatized aminoglycosides gave MH^+ peaks as the highest mass ions, with less intense $M^{+\cdot}$ peaks, except in the case of the unsaturated antibiotics (9)—(13) where the $M^{+\cdot}$ peaks



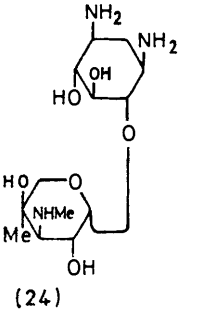
(18)



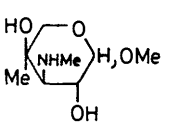
(19) $R^1=R^2=H$
(20) $R^1=R^2=Me$
(21) $R^1=Me$, $R^2=H$



(22) $R^1=R^2=NH_2$
(23) $R^1=NH_2$, $R^2=OH$



(24)



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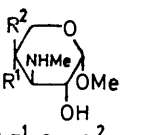
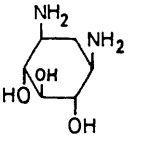


(26) $R^1=OH$, $R^2=H$
(27) $R^1=H$, $R^2=OH$



(28)

were stronger. Increasing the repeller potential increased the relative abundance of $M^{+\cdot}$ as expected.

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[8] D. J. Cooper, P. J. L. Daniels, M. D. Yudis, H. M. Marigliano, R. D. Guthrie, and S. T. K. Bukhari, *J. Chem. Soc.* (*C*), 1971, 3126.
[9] D. J. Cooper, J. Weinstein, and J. A. Waitz, *J. Medicin. Chem.*, 1971, **14**, 1118.
[10] S. Inouye, *Chem. and Pharm. Bull.* (*Japan*), 1972, **20**, 2331.
[11] D. J. Cooper, M. D. Yudis, H. M. Marigliano, and T. Traubel, *J. Chem. Soc.* (*C*), 1971, 2876.
[12] D. J. Cooper, R. S. Jaret, and H. Reimann, *Chem. Comm.*, 1971, 285.
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Even in the case of polyhydroxy-compounds such as gentamicins A (5), B (6), and X_2 (7) and antibiotic G-418 (8), MH^+ could be readily discerned and was sufficiently abundant for unequivocal assignment of molecular composition by accurate mass measurement. In contrast the usual microanalytical techniques often gave variable results due to hydration and carbonation of the samples.

All the aminocyclitol–aminoglycosides in this study gave rise to three prominent series of deoxystreptamine-

TABLE 1

E.i. mass spectral data for aminocyclitol–aminoglycoside antibiotics

Compound	m/e (% of base peak)										
	$(MH)^+$	$M^{+\cdot}$	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9
Gentamicin C_{1a}†(1) [6,11,14]	450 (1)	449 (0.5)	319 (5) *a*		291 (2) *b*	273 (2)	350 (4)	332 (4)	322 (22)	304 (22)	191 (24)
Gentamicin C_1†(2) [6,11,14]	478 (2)	477 (2)	347 (14) *c*		319 (3) *d*	301 (3)	350 (3)	332 (2)	322 (11)	304 (11)	191 (17)
Gentamicin C_2(3) [6,11,14]	464 (0.3)	463 (0.3)	333 (5) *e*		305 (7) *f*	287 (3)	350 (6)	332 (2)	322 (28)	304 (20)	191 (20)
Gentamicin C_{2b}(4) [15]	464 (0.8)	463 (0.8)	333 (7) *g*		305 (5) *h*	287 (3)	350 (2)	332 (1)	322 (5)	304 (8)	191 (7)
Gentamicin A (5) [16]	469 (0.2)	468 (0.1)	352 (1)	334(0.8)	324 (2)	306 (2)	336 (10)	318 (3)	308 (2)	290 (10)	191 (58)
Gentamicin B† (6) [14]	483 (1)	482 (0·2)	352 (16)	334 (0.5)	324 (8)	306 (6)	350 (0.1)	332 (0.2)	322 (6)	304 (9)	191 (85)
Gentamicin X_2(7) [14]	483 (1)	482 (0.2)	352 (10)	334 (2)	324 (7)	306 (4)	350 (14)	332 (4)	322 (5)	304 (10)	191 (74)
Antibiotic G-418† (8) [17]	497 (4)	496 (1)	366 (10)	348 (1)	338 (4)	320 (4)	350 (13)	332 (4)	322 (4)	304 (13)	191 (62)
Sisomicin†(9) [13]	448 (0.5)	447 (1)	317 (5) *i*	299 (6) *j*	289 (3) *k*	271 (13) *l*	350 (3)	332 (13)	322 (4)	304 (22)	191 (25)
Antibiotic 66-40B†(10) [18]	434 (0.1)	433 (0.3)	317 (9) *m*	299 (2) *o*	289 (0.4) *p*	271 (9) *q*	336 (2)	318 (9)	308 (4)	290 (16)	191 (23)
Antibiotic 66-40D†(11) [18]	434 (0.3)	433 (1)	317 (2) *s*	299 (1) *t*	289 (1) *u*	271 (12) *v*	336 (3)	318 (10)	308 (2)	290 (14)	191 (17)
Verdamicin (12) [19]	462 (0.5)	461 (0.8)	331 (6) *x*	313 (3) *y*	303 (25) *z*	285 (8) *aa*	350 (7)	332 (17)	322 (11)	304 (31)	191 (27)
Antibiotic G-52†(13) [20]	462 (2)	461 (5)	331 (17) *cc*	313 (10) *dd*	303 (32) *ee*	285 (57) *ff*	350 (2)	332 (9)	322 (10)	304 (32)	191 (27)
Kanamycin A(14) [21–23]	485 (0.2)		352 (0.4)	334 (0.5)	324 (3)	306 (4)	352 (0.4)	334 (0.5)	324 (3)	306 (4)	191 (20)
Kanamycin B(15) [23–24]	484 (0.1)	483 (0.03)	351 (0.3)	333 (0.3)	323 (1)	305 (1)	352 (1)	334 (2)	324 (5)	306 (12)	191 (33)
Tobramycin (16) [25]	468 (0.4)		335 (2)	317 (0.3)	307 (3)	289 (1)	352 (3)	334 (2)	324 (9)	306 (19)	191 (24)
3′,4′-Dideoxykanamycin B (17) [26]	452 (0.6)		319 (2)		291 (4) *hh*	273 (3)	352 (6)	334 (3)	324 (25)	306 (40)	191 (54)
Ribostamycin (18) [27]	455 (0.2)		351 (0.6)		323 (2)	305 (2)	323 (2)	305 (2)	295 (3)	277 (7)	191 (38)
Gentamine C_{1a} (19) [8]	291 (3)	290 (0.7)									191 (82)
Gentamine C_1† (20) [8]	319 (5)	318 (6)									191 (37)
Gentamine C_2 (21) [8]	305 (3)	304 (1)									191 (52)
Neamine (22) [28]	323 (1)										191 (13)
Paromamine, 3HCl (23) [29]	324 (2)	323 (0.2)									191 (100)
Garamine (24) [13,30]	322 (2)										191 (69)

Compound	m/e (% of base peak)										
	A_{10}	A_{11}	A_{12}	B_1	C_1	D_1	D_2	D_3	D_4	D_7	D_8
Gentamicin C_{1a}†(1) [6,11,14]	173 (9)	163 (19)	145 (31)	129 (100)	160 (92)	432 (2)					
Gentamicin C_1†(2) [6,11,14]	173 (16)	163 (11)	145 (20)	157 (100)	160 (67)	460 (2)				420 (10)	261 (2)
Gentamicin C_2(3) [6,11,14]	173 (8)	163 (15)	145 (21)	143 (100)	160 (81)	446 (1)				420 (0.5)	
Gentamicin C_{2b}(4) [15]	173 (2)	163 (7)	145 (14)	143 (100)	160 (43)	446 (0.3)				420 (0.3)	261 (1)
Gentamicin A (5) [16]	173 (18)	163 (25)	145 (76)	162 (35)	146 (69)						
Gentamicin B†(6) [14]	173 (12)	163 (60)	145 (100)	162 (67)	160 (77)						
Gentamicin X_2(7) [14]	173 (22)	163 (50)	145 (100)	162 (39)	160 (80)						
Antibiotic G-418† (8) [17]	173 (14)	163 (35)	145 (62)	176 (38)	160 (100)						
Sisomicin†(9) [13]	173 (18)	163 (19)	145 (84)	127 (41)	160 (100)			430 (4) *m*	271 (13)		
Antibiotic 66-40B†(10) [18]	173 (22)	163 (35)	145 (100)	127 (32)	146 (100)			416 (2) *r*	271 (9)		
Antibiotic 66-40D†(11) [18]	173 (24)	163 (24)	145 (100)	127 (25)	146 (58)			416 (5) *w*	271 (12)		
Verdamicin (12) [19]	173 (16)	163 (18)	145 (69)	141 (27)	160 (100)			444 (5) *bb*	285 (5)		
Antibiotic G-52†(13) [20]	173 (22)	163 (26)	145 (60)	141 (80)	160 (100)			444 (18) *gg*	285 (57)		
Kanamycin A(14) [21–23]	173 (11)	163 (37)	145 (82)	162 (60)	162 (60)						
Kanamycin B(15) [23,24]	173 (21)	163 (55)	145 (100)	161 (42)	162 (38)						
Tobramycin (16) [25]	173 (9)	163 (42)	145 (100)	146 (18)	162 (27)						
3′,4′-Dideoxykanamycin B (17) [26]	173 (14)	163 (95)	145 (100)	129 (95)	162 (42)	434 (0.6)					
Ribostamycin (18) [27]	173 (62)	163 (57)	145 (100)	161 (45)	133 (10)						
Gentamine C_{1a} (19) [8]	173 (22)	163 (88)	145 (100)	129 (90)			273 (14)				261 (1)
Gentamine C_1† (20) [8]	173 (40)	163 (75)	145 (79)	157 (73)			301 (8)				261 (75)
Gentamine C_2 (21) [8]	173 (25)	163 (90)	145 (96)	143 (92)			287 (13)				261 (10)
Neamine (22) [28]	173 (13)	163 (28)	145 (100)	161 (30)							
Paromamine, 3HCl (23) [29]	173 (39)	163 (37)	145 (80)	162 (53)							
Garamine (24) [13,30]	173 (6)	163 (38)	145 (54)		160 (19)						

Compound	m/e (% of base peak)									
	D_9	D_{10}	E_1	E_2	E_3	E_4	F_1	F_2	F_3	F_4
Gentamicin C_{1a}†(1) [6,11,14]			374 (1)	246 (5)	332 (4)	204 (7)	258 (4)	289 (2)	130 (17)	112 (17)
Gentamicin C_1†(2) [6,11,14]			402 (2)	246 (5)	360 (6)		286 (22)	289 (13)	130 (13)	112 (16)
Gentamicin C_2(3) [6,11,14]			388 (1)	246 (5)	346 (5)	204 (4)	272 (5)	289 (4)	130 (9)	112 (12)
Gentamicin C_{2b}(4) [15]			388 (0.8)	246 (3)	346 (3)	204 (1)	272 (9)	289 (4)	130 (6)	112 (18)
Gentamicin A (5) [16]	348 (1)	203 (6)	407 (1)	246 (6)	365 (1)	204 (10)	291 (10)	275 (2)	130 (26)	112 (14)
Gentamicin B† (6) [14]			407 (2)	246 (10)	365 (3)	204 (10)	291 (12)	289 (5)	130 (40)	112 (30)
Gentamicin X_2(7) [14]	362 (2)	203 (3)	407 (4)	246 (10)	365 (4)	204 (13)	291 (10)	289 (7)	130 (40)	112 (47)
Antibiotic G-418† (8) [17]	362 (1)	203 (4)	421 (1)	246 (6)	379 (2)	204 (8)	305 (14)	289 (4)	130 (26)	112 (26)
Sisomicin†(9) [13]	362 (8)	203 (28)	372 (1)	246 (4)	330 (3)	204 (6)	256 (8)	289 (3)	130 (15)	112 (31)
Antibiotic 66-40B†(10) [18]	348 (7)	203 (25)	372 (0.2)	246 (17)	330 (2)	204 (28)	256 (6)	275 (1)	130 (58)	112 (50)
Antibiotic 66-40D†(11) [18]	348 (8)	203 (38)	372 (0.2)	246 (2)	330 (3)	204 (8)	256 (8)	275 (1)	130 (14)	112 (12)
Verdamicin (12) [19]	362 (16)	203 (30)	386 (0.5)	246 (5)	344 (3)	204 (6)	270 (8)	289 (1)	130 (11)	112(26)
Antibiotic G-52† (13) [20]	362 (10)	203 (70)	386 (1)	246 (7)	344 (7)	204 (12)	270 (37)	289 (3)	130 (23)	112 (35)
Kanamycin A(14) [21–23]					365 (0.4)	204 (20)	291 (2)	291 (2)	130 (46)	112 (10)
Kanamycin B(15) [23,24]	364 (0.6)	203 (68)			364 (0.6)	204 (39)	290 (2)	291 (1)	130 (38)	112 (8)
Tobramycin (16) [25]	364 (0.8)	203 (10)			348 (0.8)	204 (7)	274 (4)	291 (2)	130 (19)	112 (7)
3′,4′-Dideoxykanamycin B (17) [26]					332 (3)	204 (9)	258 (7)	291 (4)	130 (34)	112 (65)
Ribostamycin (18) [27]		203 (8)								
Gentamine C_{1a} (19) [8]									130 (50)	112 (74)
Gentamine C_1† (20) [8]									130 (60)	112 (19)
Gentamine C_2 (21) [8]									130 (41)	112 (11)
Neamine (22) [28]		203 (7)							130 (9)	
Paromamine, 3HCl (23) [29]		203 (11)							130 (14)	
Garamine (24) [13,30]				246 (11)		204 (8)			130 (34)	112 (12)

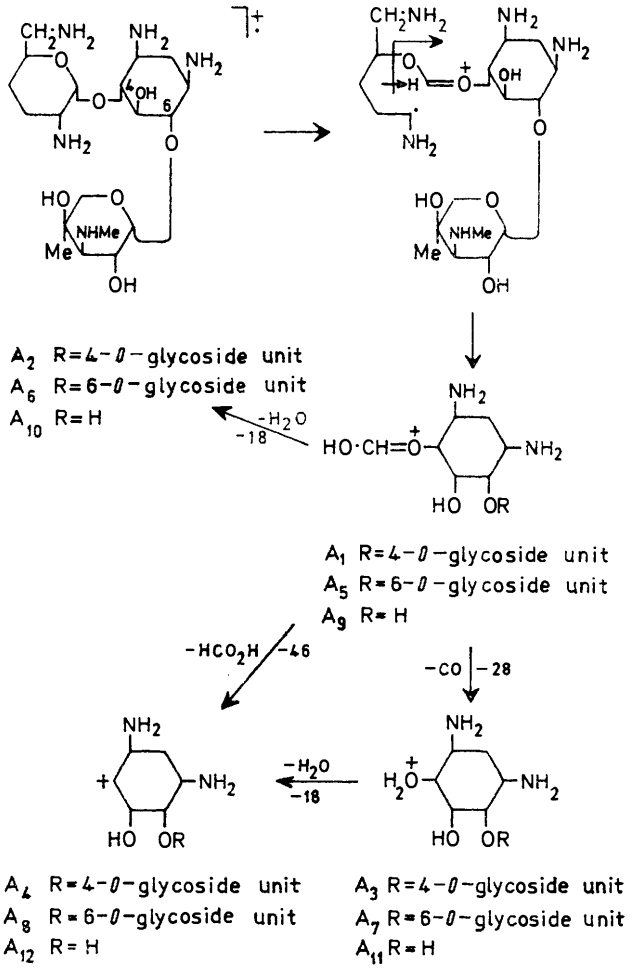
† The compositions of the fragment ions were confirmed by high resolution mass measurements.

a $A_1 - NH_3$: 302 (2). *b* $A_3 - NH_3$: 274 (2). *c* $A_1 - NH_3$: 330 (6). *d* $A_3 - NH_3$: 302 (4). *e* $A_1 - NH_3$: 316 (2). *f* $A_3 - NH_3$: 288 (4). *g* $A_1 - NH_3$: 316 (3). *h* $A_3 - NH_3$: 288(4). *i* $A_1 - NH_3$: 300 (2). *j* $A_2 - NH_3$: 282 (5). *k* $A_3 - NH_3$: 272 (4). *l* $A_4 - NH_3$: 254 (4). *m* *m**413.6 ± 0.2 (theory 413.65). *n* $A_1 - NH_3$: 300 (2). *o* $A_2 - NH_3$: 282 (2). *p* $A_3 - NH_3$: 272 (4). *q* $A_4 - NH_3$: 254 (3). *r* *m**399.7 ± 0.2 (theory 399.67). *s* $A_1 - NH_3$: 300 (0.3). *t* $A_2 - NH_3$: 282 (0.5). *u* $A_3 - NH_3$: 272 (1). *v* $A_4 - NH_3$: 254 (0.5). *w* *m**399.8 ± 0.2 (theory 399.67) *x* $A_1 - NH_3$: 314 (3). *y* $A_2 - NH_3$: 296(4). *z* $A_3 - NH_3$: 286 (5). *aa* $A_4 - NH_3$: 268 (4). *bb* *m**427·5 ± 0.2 (theory 427.63). *cc* $A_1 - NH_3$: 314 (4). *dd* $A_2 - NH_3$: 296 (7). *ee* $A_3 - NH_3$: 286 (18). *ff* $A_4 - NH_3$: 268 (7). *gg* *m**427.7 ± 0.2 (theory 427.63). *hh* $A_3 - NH_3$: 274 (2).

containing fragment ions, depicted in Scheme 1 for gentamicin C_{1a} (1).*

The protonated formyl ions A_1 and A_5 may be rationalized as arising by cleavage of the C(1)–C(2) bonds of the respective sugar units, followed by fission of the C(5)–O bond with hydrogen transfer. Loss of water from these ions then gives ions A_2 and A_6, respectively. Ions A_3 and A_7 can arise formally from ions A_1 and A_5 by loss of carbon monoxide. Loss of water from ions A_3 and A_7, or a molecule of formic acid from ions A_1 and A_5, gives rise to ions A_4 and A_8. The intensity of the A_2 peak was variable, and in cases in which the 4-*O*-glycosyl group is saturated and possesses no hydroxy-substituent, such as in compounds (1)—(4) and (17), the peak was very weak or absent. In these latter compounds prominent ions corresponding to losses of ammonia from A_1 and A_3 were observed. The ions formed by loss of ammonia from the A_7 ions were also prominent in many compounds. The unsaturated antibiotics (9)—(13) undergo ready loss of ammonia in the mass spectrometer, presumably from the enopyranoside moiety and give, in addition to the ions A_1—A_4, an analogous series of ions with loss of ammonia. It seems equally likely that these latter ions could arise from initial loss of ammonia from the molecular ion followed by the process of Scheme 1, or by direct loss of ammonia from the ions A_1—A_4. Metastable ion studies, described later, indicated that the ions A_7 and A_8 also arise directly from the molecular ion, and the ion A_3 directly from MH^+.

The A_9—A_{12} series of peaks (*m/e* 191, 173, 163, and 145) is always intense in 2-deoxystreptamine-containing compounds and is diagnostic for the presence of this subunit in an aminocyclitol–aminoglycoside antibiotic.



SCHEME 1

Of these peaks, that of A_{12} (*m/e* 145) is almost always the most intense and forms the base peak in the spectra of a number of the compounds studied. The formation of these ions is most easily envisaged by glycosidic cleavage of ions A_1 and A_5 with hydrogen transfer to give ion A_9.

* We are aware of the problems associated with assigning structures to mass spectrometric ions,[31] and in this text an ion structure represents only one of several isomeric possibilities. In the absence of other evidence we do not claim that the structures shown are necessarily the 'most plausible'; they are intended only to illustate the process involved. Similarly the arrows used in the text should not be construed as implying any knowledge of the mechanism of fragmentation. They do however provide a method of 'electron book-keeping' and, together with the ion structures shown, form a useful mnemonic for the fragmentations described.

[31] T. W. Bentley and R. A. W. Johnstone, *Adv. Phys. Org. Chem.*, 1970, **8**, 151.

References 14—30 refer to Table 1 on page 1080.

[14] P. J. L. Daniels, 'Drug Action and Drug Resistance in Bacteria,' ed. S. Mitsuhashi, University Park Press, Tokyo, 1975, p. 77.
[15] P. J. L. Daniels, C. Luce, T. L. Nagabhushan, R. S. Jaret, D. Schumacher, H. Reimann, and J. Ilavsky, *J. Antibiotics*, 1975, **28**, 35.
[16] H. Maehr and C. P. Schaffner, *J. Amer. Chem. Soc.*, 1970, **92**, 1697.
[17] P. J. L. Daniels, A. S. Yehaskel, and J. B. Morton, Abstracts, 13th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington D.C., U.S.A., 19—21 September, 1973, paper 137.
[18] D. H. Davies, D. Greeves, A. K. Mallams, J. B. Morton, and R. W. Tkach, *J.C.S. Perkin I*, 1975, 814.
[19] P. J. L. Daniels and A. S. Yehaskel, *Abstracts*, 13th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington D.C., U.S.A., 19—21 September, 1973, paper 135.
[20] J. A. Marquez, G. H. Wagman, R. T. Testa, J. A. Waitz, and M. J. Weinstein, Abstracts, 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, California, U.S.A., 11—13 September, 1974, paper 164.
[21] M. J. Cron, D. L. Evans, F. M. Palermiti, D. F. Whitehead, I. R. Hooper, P. Chu, and R. U. Lemieux, *J. Amer. Chem. Soc.*, 1958, **80**, 4741.
[22] H. Ogawa, T. Ito, S. Kondo, and S. Inoue, *J. Antibiotics*, 1958, **11A**, 169.
[23] M. Hichens and K. L. Rinehart, *J. Amer. Chem. Soc.*, 1963, **85**, 1547.
[24] T. Ito, M. Nishio, and H. Ogawa, *J. Antibiotics*, 1964, **17A**, 189.
[25] K. F. Koch, F. A. Davis, and J. A. Rhoades, *J. Antibiotics*, 1973, **26**, 745.
[26] S. Umezawa, H. Umezawa, Y. Okazaki, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 1972, **45**, 3624.
[27] E. Akita, T. Tsuruoka, N. Egaki, and T. Niida, *J. Antibiotics*, 1970, **23**, 173.
[28] H. E. Carter, J. R. Dyer, P. D. Shaw, and K. L. Rinehart, *J. Amer. Chem. Soc.*, 1961, **83**, 3723.
[29] T. H. Haskell, J. C. French, and Q. R. Bartz, *J. Amer. Chem. Soc.*, 1959, **81**, 3480.
[30] M. Kugelman, A. K. Mallams, and H. F. Vernay, *J. Antibiotics*, 1973, **26**, 394.

Subsequent losses of water, carbon monoxide, or formic acid (see Scheme 1) would then give rise to ions A_{10}—A_{12}. The pseudodisaccharides (19)—(24), as anticipated, also give rise to the A_9—A_{12} series of ions.

The tetra-*N*-acetyl derivatives of gentamines C_{1a}, C_1, and C_2 [(19)—(21)] gave the A_9—A_{12} series of ions displaced to appropriately higher mass by the acetyl substituents. In the case of tetra-*N*-acetylgentamine C_2, the loss of carbon monoxide from the protonated formyl ion (ion A_9 having acetylated amino-groups) (*m/e* 275 ⟶ 247) was accompanied by the appropriate metastable transition (222.0 ± 0.2; theory 221.85). In order to gain further insight into the origin of ions shown

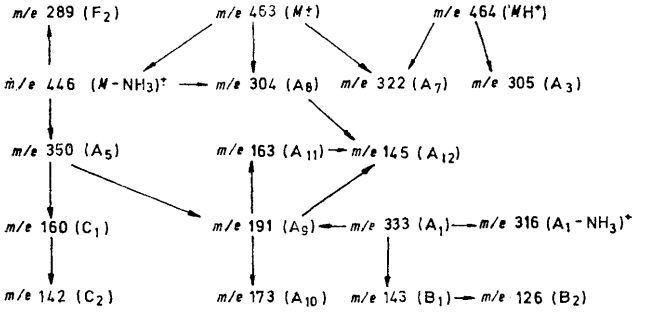


FIGURE 1 Some fragmentation pathways for gentamicin C_2 (3)

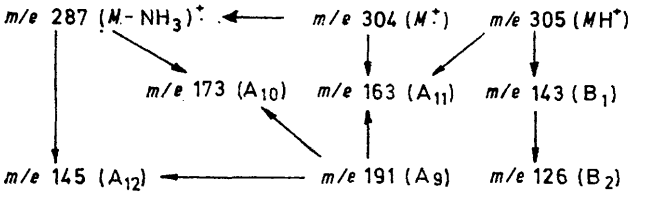
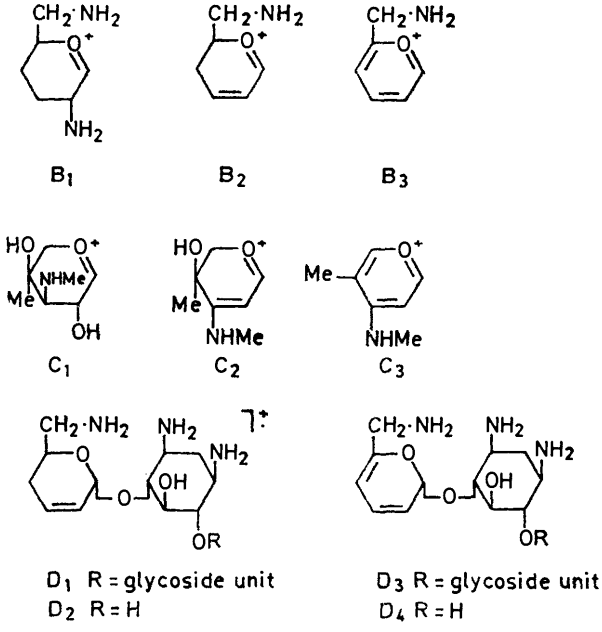


FIGURE 2 Some fragmentation pathways for gentamine C_2 (21)

in Scheme 1, several compounds in this series were examined by the ' direct analysis of daughter ions ' (DADI) technique described by Maurer and his co-workers.[32] This technique, which utilises a double-focussing mass spectrometer of reversed geometry (in which the magnetic sector precedes the electric sector), analyses the products of metastable decompositions occurring in the second field-free region of the spectrometer. All daughter ions arising from a chosen parent focussed in the magnetic sector of the instrument are analysed by scanning the electric sector. This technique has also been investigated by Beynon and his co-workers,[33] who termed the technique ' mass analysed ion kinetic energy spectroscopy ' (MIKES). Certain selected ions in the mass spectra of gentamicin C_2 (3) and gentamine C_2 (21) were investigated by this technique. The fragmentation pathways revealed are shown in Figures 1 and 2. The DADI data clearly support the sequence of fragmentations shown in Scheme 1 for ions A_9—A_{12}. Although no evidence for the analogous fragmentations of ions A_1—A_4 and A_5—A_8 was revealed by this work, this may simply mean that metastable ions for the proposed fragmentations were not observed under the experimental conditions used, and does not necessarily invalidate the proposed sequences shown for these ions in Scheme 1. It is also clear, and not unexpected, that some ions have more than one origin. For example the ion A_7 can arise by fragmentation of the molecular ion, or of MH^+. This latter fact seems noteworthy since fragmentations of electron-impact-produced ions of greater mass-to-charge ratio than the molecular ion have seldom been discussed in the literature.

The formation of protonated formyl ions such as A_1, A_5, and A_9 in the mass spectra of glycosides has been reported previously in nucleosides,[34,35] permethylated flavonoid trisaccharides,[36] terpene glycosides,[37] and, independently of our studies, in aminocyclitol–aminoglycosides [38] and derivatized aminocyclitol–aminoglycosides.[6] In our experience the protonated formyl ion peaks were either weak, or absent in several amino-



cyclitol–aminoglycosides in which no amino- or hydroxy-substituents were present at the 2-position of the sugar. A similar observation was made earlier in connection

[32] K. H. Maurer, C. Brunce, G. Kappus, K. Habfast, U. Schroder, and P. Schulze, Abstracts, 19th Annual Conference on Mass Spectrometry and Allied Topics, Atlanta, Georgia, U.S.A., 2—7 May, 1971, paper K9.

[33] For a review, see R. C. Cooks, J. H. Beynon, R. M. Caprioli, and G. R. Lester ' Metastable Ions,' Elsevier, London and New York, 1973, p. 42.

[34] K. Biemann and J. A. McCloskey, *J. Amer. Chem. Soc.*, 1962, **84**, 2005.

[35] S. Hanessian, D. C. DeJongh, and J. A. McCloskey, *Biochim. Biophys. Acta*, 1966, **117**, 482.

[36] R. D. Schmid, P. Varenne, and R. Paris, *Tetrahedron*, 1972, **28**, 5037.

[37] W. R. Chan, D. R. Taylor, C. R. Willis, R. D. Bodden, and H. W. Fehlhaber, *Tetrahedron*, 1971, **27**, 5081.

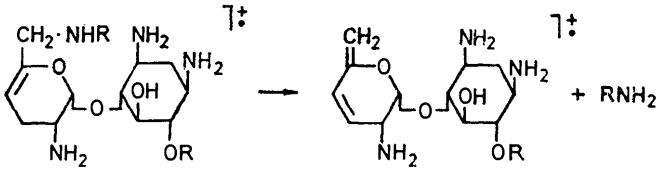
[38] K. L. Rinehart, P. Schaefer, J. C. Cook, C. P. Schaffner, and A. Kershner, Abstracts, 19th Annual Conference on Mass Spectrometry and Allied Topics, Atlanta, Georgia, U.S.A., 2—7 May, 1971, paper F5.

with the mass spectra of the megalomicin antibiotics,[39] where protonated formyl ions were observed to arise only from cleavage of the desosamine unit, which has a 2-hydroxy-substituent, but not from the mycarose or rhodosamine units, which are both 2-deoxy-sugars. Similarly it has been noted previously that protonated formyl ions are missing from the spectra of 2′-deoxy-nucleosides.[34] In this latter case it was suggested that the hydrogen atom of the 2′-hydroxy-group of a nucleoside was the one transferred to give the protonated formyl ions, thus explaining their absence in the 2′-deoxy-compounds. Other workers,[37] however, have written a mechanism in which a different hydrogen atom is transferred in this type of fragmentation. In the absence of other evidence, a more likely explanation seems to be that the initial C(1)–C(2) bond cleavage of the glycosyl unit (see Scheme 1) would be energetically less favoured in the case of 2-deoxy-sugars, as a less stable ion radical would be produced.

The formation of ions A_1—A_{12}, together with ions B_1 and C_1, produced as a result of normal glycosidic cleavage, has proved extremely useful in determining the compositions and linkage sequence of aminosugar and aminocyclitol units in natural and semisynthetic aminocyclitol–aminoglycoside antibiotics, particularly when only very small quantities of the samples were available. A simple but most useful example has been in the study of antibiotics inactivated by bacterial enzymes.[40-42] In these cases sites of inactivation by *N*-acetylation have been located by observing the fragment ions described here.

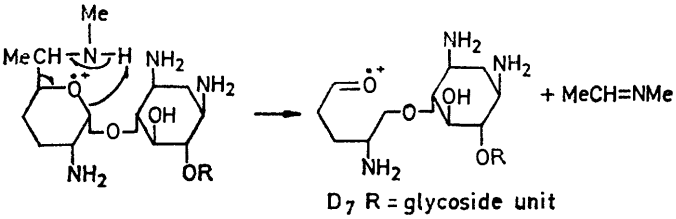
The fragment ions arising from glycosidic cleavage of the sugar units, exemplified by ions B_1 and C_1 from gentamicin C_{1a} (1), are normally very intense, one or other of these ions often forming the base peak of the spectrum. In the case of compounds (1)—(4) and (17), having a purpurosamine sugar unit, loss of ammonia from ion B_1 occurs to give ion B_2. In the unsaturated antibiotics (9)—(13) similar loss of ammonia gives ions of the type B_3. The peak due to the ion C_1 (*m/e* 160) is very intense in all garosamine-containing compounds. Loss of water from this ion gives a prominent peak at *m/e* 142 (ion C_2). All the foregoing fragmentations are supported by the metastable ions, or by the results of DADI experiments. The ion C_3 (*m/e* 124) formed by loss of water from C_2 is always observed, but is of relatively low abundance. Similar ions, fourteen mass units lower, are formed from the gentosamine units of gentamicin A (5) and antibiotic 66—40B (10) and the *arabino*-sugar of antibiotic 66—40D (11).

In general the aminoglycosides studied exhibited ions in the high mass region due to loss of ammonia and loss of water, and occasionally due to losses of ammonia and water, and of two molecules of water from the molecular ion. These peaks were usually quite weak in intensity except for those due to the loss of ammonia from compounds having no hydroxy-substituents in the 4-*O*-glycosyl unit. Of these compounds the gentamicins (1)—(4), 3′,4′-dideoxykanamycin B (17), and the gentamines (19)—(21) gave $(M - NH_3)^{+\cdot}$ peaks of intensity comparable to those of the molecular ions. The unsaturated compounds (9)—(13) on the other hand showed quite prominent ions corresponding to loss of ammonia from the molecular ion and were accompanied by appropriate metastable transitions. Since many compounds containing garosamine and deoxystreptamine do not show prominent losses of ammonia in their mass spectra, and since antibiotics having a 6′-methylamino-substituent, such as (2) and (13), show more intense



D_5 R = glycoside unit
D_6 R = H

SCHEME 2



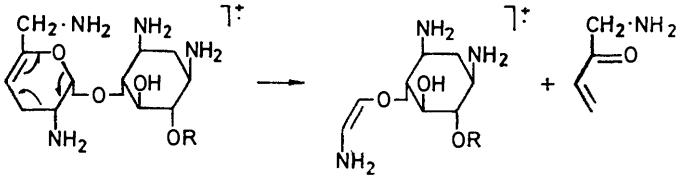
D_7 R = glycoside unit
D_8 R = H

SCHEME 3

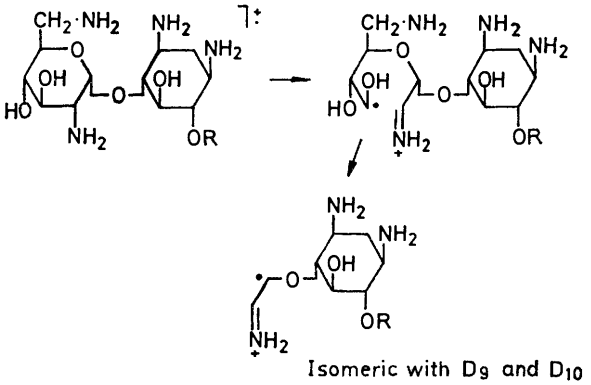
peaks for loss of ammonia than for loss of methylamine, it is believed that the loss of ammonia from the molecular ion occurs primarily from the 2′-position to give ions such as D_1 (from the saturated aminoglycosides) and D_3 (from the unsaturated compounds). Ions D_2 and D_4, corresponding to glycosidic cleavage of ions D_1 and D_3 with hydrogen transfer, are also observed. These ions occur at the same unit masses as the A_4 ion in low resolution scans, but can be differentiated at high resolution. In the case of the unsaturated antibiotic G-52 (13) loss of methylamine from the 6′-position occurs to give ion D_5 (Scheme 2). Other related 6′-*N*-alkyl unsaturated aminoglycosides show similar cleavages.[43] Saturated compounds having a 6′-methylamino-substituent, as in (2) and (4), show only weak peaks corresponding to loss of methylamine from the molecular ion, but have a more abundant ion one mass unit higher. DADI measurements with gentamicin C_1 (2) suggest that this peak arises by loss of methylamine from MH^+.

[39] R. S. Jaret, A. K. Mallams, and H. F. Vernay, *J.C.S. Perkin I*, 1973, 1389.
[40] M. Brzezinska, R. Benveniste, J. Davies, P. J. L. Daniels, and J. Weinstein, *Biochemistry*, 1972, **11**, 761.
[41] M. Chevereau, P. J. L. Daniels, J. Davies, and F. LeGoffic, *Biochemistry*, 1974, **13**, 598.
[42] H. Umezawa, M. Yagisawa, Y. Matsuhashi, H. Naganawa, H. Yamamoto, S. Kondo, T. Takeuchi, and Y. A. Chabbert, *J. Antibiotics*, 1973, **26**, 612.
[43] A. K. Mallams and P. J. L. Daniels, unpublished observations.

Another useful fragmentation of the purpurosamine side chain in compounds (1)—(4) is shown in Scheme 3 leading to ion D_7. This ion, *m/e* 420, is particularly



SCHEME 4



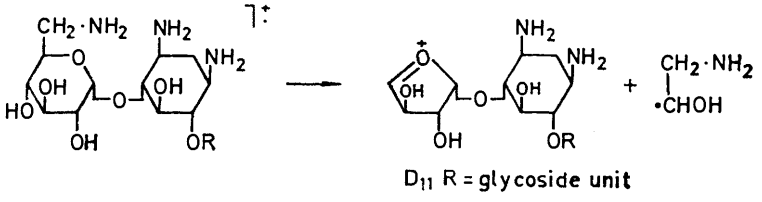
SCHEME 5

abundant in the spectrum of gentamicin C_1 (2). The analogous ion D_8, *m/e* 261, is also present in the spectra of the gentamines (19)—(21), being particularly prominent for gentamines C_1 (20) and C_2 (21). The presence of the ion D_7 has been used to distinguish between 6′-*N*- and 2′-*N*-substitution in gentamicin C_1 (2).[44] In the unsaturated antibiotics (9)—(13) a similar distinction between substituents at the 6′- and 2′-positions has been made [41] based on the position of the prominent fragment ion D_9 in the mass spectra of these compounds. This ion, which is considered to arise by the retro-diene cleavage of the enopyranoside unit as shown in Scheme 4, also establishes the location of the double bond in the unsaturated aminoglycosides, at the 4′,5′-position.[12] Similar fragmentations have been used to assign the positions of double bonds in other unsaturated carbohydrates.[45] The ion D_{10}, which could formally arise by glycosidic cleavage of ion D_9 with a hydrogen transfer, is also prominent in the mass spectra of compounds (9)—(13). Fragmentation of certain other antibiotics containing a 2′-amino-2′-deoxypyranosyl unit [(5)—(8), (15), and (16)] also gave the ions D_9 and D_{10}. These ions may be viewed as arising by dehydration followed by the process of Scheme 4, or possibly *via* a different route such as that illustrated in Scheme 5. These peaks were very weak, or absent in the spectra of the gentamicin C antibiotics. The ion D_{10} was also observed in the spectra of the pseudodisaccharides (22) and (23).

Another useful diagnostic fragment appearing in the high mass region of the spectrum of gentamicin B (6) corresponds to ion D_{11} as shown in Scheme 6. This fragment ion was prominent also in the spectra of the related antibiotics gentamicin A_3 [46] and gentamicin B_1 (6′-*C*-methylgentamicin B).[14] Interestingly, however, this peak was very weak in the spectra of corresponding 2,6-diamino-2,6-dideoxyglucopyranosyl-containing antibiotics such as 6′-amino-6′-deoxygentamicin A.[47]

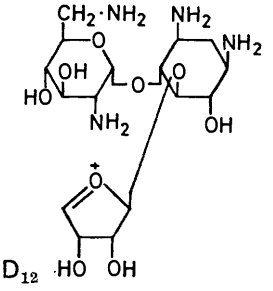
In ribostamycin (18), which contains a β-D-ribofuranosyl unit, cleavage of the 5″-hydroxymethylene group occurs to give the ion D_{12} at *m/e* 423. Although somewhat weak in the case of ribostamycin (18), this peak occurs in the high mass region of the spectra of several semisynthetic aminoglycosides containing pentofuranosyl units.[48]

Aminocyclitol–aminoglycosides containing a garosaminyl [(1)—(4), (6)—(9), (12), (13), and (24)], gentosaminyl [(5) and (10)], or 3-deoxy-3-methylaminoarabinopyranosyl (11) unit give rise to prominent ions E_1 in the



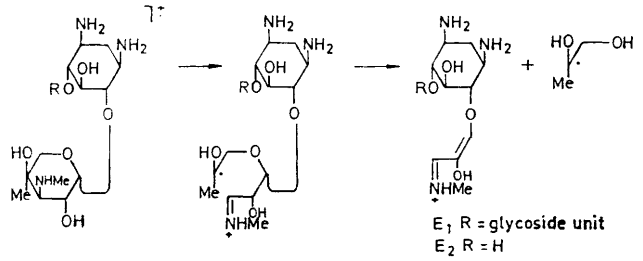
SCHEME 6

high mass region. This ion presumably arises *via* cleavage of the 3″,4″-bond as shown in Scheme 7. In



[44] P. J. L. Daniels, J. Weinstein, and T. L. Nagabhushan, *J. Antibiotics*, 1974, **27**, 889.
[45] R. J. Ferrier, N. Vethaviyasar, O. S. Chizov, V. I. Kadentsev, and B. M. Zolotarev, *Carbohydrate Res.*, 1970, **13**, 269.
[46] T. L. Nagabhushan, W. N. Turner, P. J. L. Daniels, and J. B. Morton, *J. Org. Chem.*, 1975, **40**, 2830.
[47] T. L. Nagabhushan and P. J. L. Daniels, *J. Medicin. Chem.*, 1974, **17**, 1030.
[48] A. K. Mallams, S. S. Saluja, D. F. Crowe, G. Detre, M. Tanabe, and D. M. Yasuda, *J.C.S. Perkin I*, 1976, 1135.

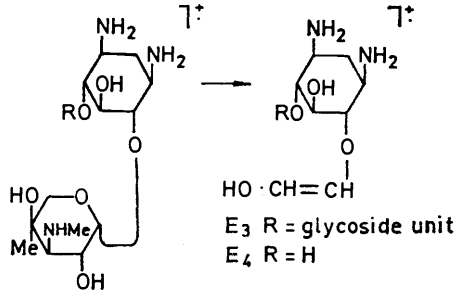
all these compounds the corresponding lower mass ion E_2 (*m/e* 246) was also present. It was evident that these



SCHEME 7

ions are associated with a 3-deoxy-3-methylamino-sugar, since in compounds lacking substitution on the amino-group, these ions are very weak, or absent altogether. Thus 3',4'-dideoxykanamycin B (17), which contains a 3-amino-3-deoxypyranose sugar, gives rise to very low intensity E_1 and E_2 peaks, and in the kanamycins (14) and (15) and tobramycin (16) the ions are absent.

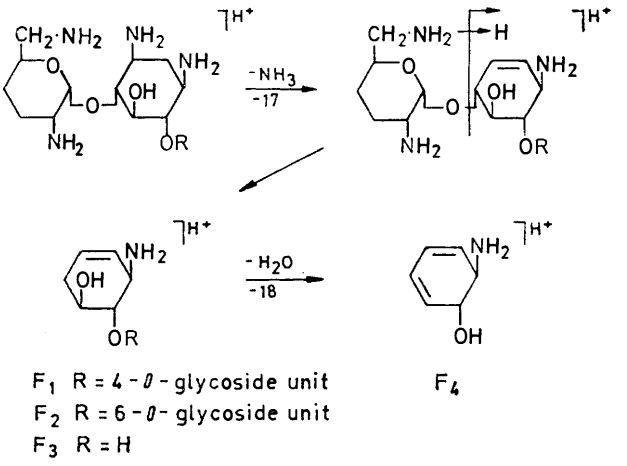
An alternative fragmentation of the 3-amino-3-deoxypyranose sugar affords ion E_3, arising possibly as shown in Scheme 8. Formal loss of the 4-*O*-glycosyl group from ion E_3 with a hydrogen transfer affords ion E_4 (*m/e* 204). This is often accompanied by an even-electron ion of similar intensity at *m/e* 205, and accurate



SCHEME 8

mass measurements indicate this ion to be the protonated E_4 ion. Ions analogous to E_3 and E_4 have been observed previously, albeit of low intensity, in the mass spectra of trimethylsilyl derivatives of simple glycosides.[49]

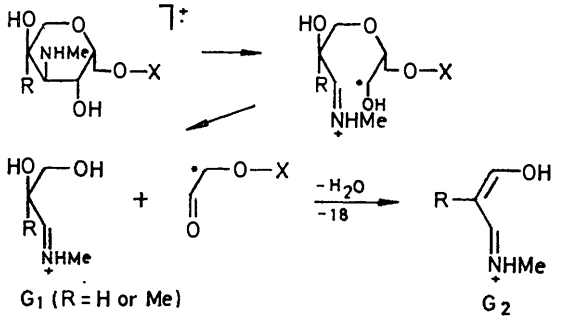
A further useful series of fragmentations is shown in Scheme 9. The net process involves loss of one of the deoxystreptamine amino-groups together with its vicinal glycosyloxy-group to give ions F_1 and F_2. The specificity of these cleavages has been demonstrated by comparison of the spectra of aminoglycoside derivatives selectively alkylated at the 1- and 3-amino-groups of the deoxystreptamine ring,[50] and Scheme 9 is written in such a way as to rationalize this specificity. Thus loss of ammonia from the MH^+ ion, followed by cleavage of the allylic glycosyloxy-group with hydrogen transfer, would give ions F_1 and F_2. These ions have proved very useful in differentiating between the products of alkylation at the 1- and 3-amino-groups of the deoxystreptamine ring.[50] The ion F_3 (*m/e* 130), which is formally the product of glycosidic cleavage of ions F_1 and F_2 with hydrogen transfer, and the ion F_4 (*m/e* 112), its dehydration product, are relatively abundant in the spectra of all deoxystreptamine 4,6-diglycosides in this study. Ribostamycin (18), a 4,5-diglycoside, however, showed no intense peaks corresponding to the F series of



SCHEME 9

ions. Peaks arising by loss of water from ions F_1 and F_2 were observed in many cases, but were often very weak and in some cases even absent.

All the aminoglycosides studied containing a garosamine unit gave rise to a very abundant low-mass ion at *m/e* 118. Examination of the high resolution spectra of several compounds indicated that this peak was composed of two ions having compositions $C_5H_{12}NO_2$, corresponding to ion G_1 (Scheme 10), and $C_4H_8NO_3$, corresponding to ion H_1 (Scheme 11), in a *ca.* 3 : 1 ratio. Both ions lose a molecule of water to give ions G_2 and H_2, respectively, at *m/e* 100. Aminoglycosides containing a gentosaminyl [(5) and (10)] or 3-deoxy-3-methylaminoarabinopyranosyl (11) unit exhibited the G_1 and G_2 ions displaced fourteen units to lower mass, at *m/e*



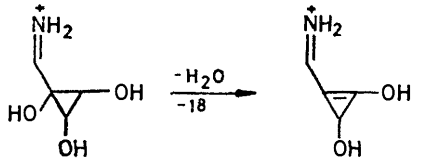
SCHEME 10

104 and 86, respectively. These compounds also displayed the H_1 and H_2 ions in their mass spectra. The

[49] D. C. DeJongh, T. Radford, J. D. Hribar, S. Hanessian, M. Bieber, G. Dawson, and C. C. Sweeley, *J. Amer. Chem. Soc.*, 1969, **91**, 1728.

[50] J. J. Wright, Abstracts, 15th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington D.C., U.S.A., 24—26 September, 1975, paper 91.

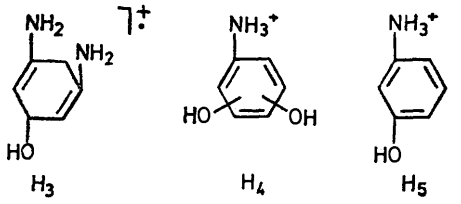
mass spectrum of methyl β-garosaminide (25) showed prominent G_1 and G_2 ions at *m/e* 118 and 100, whilst methyl α-gentosaminide (26) [51] and methyl 3-deoxy-3-methylamino-β-L-arabinopyranoside (27) [51] both showed these ions at *m/e* 104 and 86, respectively. The observation that compounds not containing garosamine gave rise to the H_1 and H_2 ions led to the conclusion that these ions were derived from the deoxystreptamine portion of the molecule. Possible structures are given in Scheme 11. Peaks were also observed at *m/e* 126



SCHEME 11

and 110 in the spectra of all compounds studied, and must arise from the deoxystreptamine unit. Accurate mass measurements indicated that the peak at *m/e* 126 consisted of two ions having the compositions $C_6H_{10}N_2O$ and $C_6H_8NO_2$, for which ions H_3 and H_4, respectively, are possibilities. The peak at *m/e* 110 was shown to have the composition C_6H_8NO, for which the ion H_5 is suggested. This ion occurs in the spectra of all 2-deoxystreptamine-containing compounds, and corresponds to loss of water and ammonia from ion A_{12}.

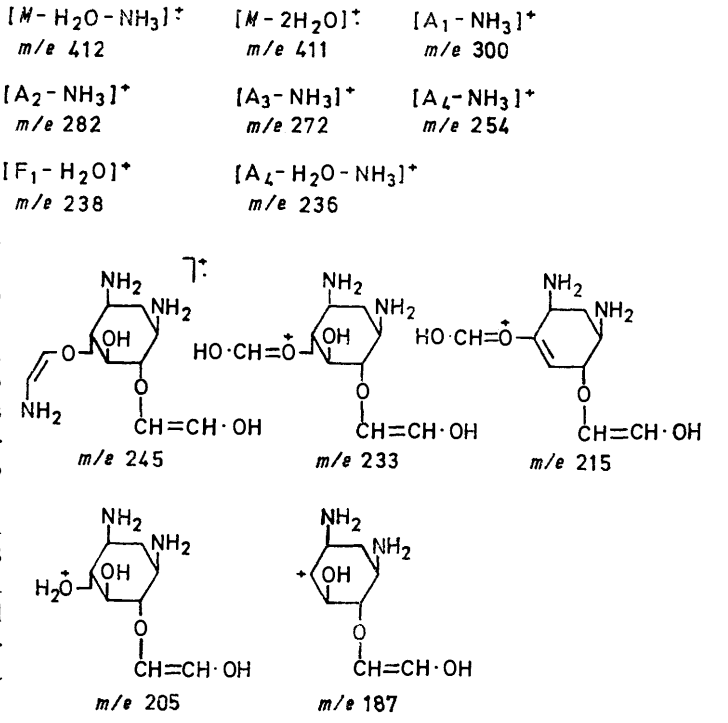
Two representative e.i. mass spectra are shown in Figures 3 and 4 for sisomicin (9) and antibiotic G-418 (8). The ions are labelled according to the Schemes in the text. Additional ions for sisomicin are labelled with their *m/e* values in Figure 3, and structures consistent with the accurate compositions of these extra



ions are shown in Scheme 12. The ions at *m/e* 233, 215, 205, and 187 are found also in the spectrum of antibiotic G-418 (Figure 4).

Selected aminocyclitol–aminoglycoside antibiotics have also been studied by using the milder ionising conditions afforded by chemical ionisation (c.i.) with isobutane or methane as the reagent gas. Representative data for compounds (1)—(3) and (5)—(7) are given in Table 2 and for sisomicin (9) in Table 3. The antibiotics studied each contain at least four nitrogen and seven oxygen atoms, and all these electron-rich atoms are potentially susceptible to protonation by reagent ions such as $C_4H_9^+$ and CH_5^+. Since aliphatic amino-groups are known to be relatively stable towards c.i. conditions,[52] protonation at many of these sites produces ions which do not fragment rapidly and the compounds studied uniformly provide relatively abundant protonated molecular ions MH^+, along with a few structurally informative fragment ions.

Apart from the high intensity of the MH^+ peaks, the most notable feature of the c.i. spectra was the effect of source temperature on the amount of fragmentation observed. This is in contrast to the e.i. spectra in



SCHEME 12

which the extent of fragmentation was not markedly temperature-sensitive. The temperature sensitivity of isobutane c.i. mass spectra has been noted previously [53] and is clearly shown for sisomicin in Table 3. If methane is used as the reagent gas, even at lower temperatures, considerable fragmentation is observed and the spectrum resembles one measured with isobutane at higher temperatures.

The data in Tables 2 and 3 show that, at temperatures in the 160—190 °C range, the gentamicin C components exhibit little or no fragmentation, loss of the 2,6-diaminosugar unit with hydrogen transfer to give the ion A_7 being the only fragmentation observed. The unsaturated aminoglycoside (9) and the polyhydroxylic compounds (5)—(8) fragment more extensively, giving both glycosidic cleavage ions B_1 and C_1, as well as the pseudodisaccharide ions A_3 and A_7 and the protonated

[51] D. J. Cooper, D. H. Davies, A. K. Mallams, and A. S. Yehaskel, *J.C.S. Perkin I*, 1975, 785.
[52] P. Longevialle, G. W. A. Milne, and H. M. Fales, *J. Amer. Chem. Soc.*, 1973, **95**, 6666.
[53] F. H. Field, 'MTP International Review of Science,' ed. A. Maccoll, Butterworths, London 1972, vol. 5, p. 133.

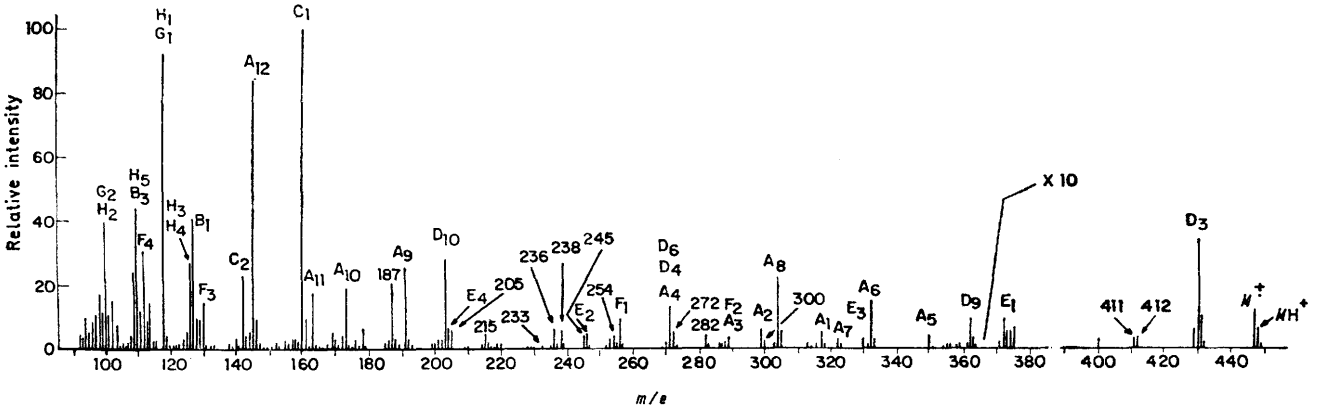


FIGURE 3 E.i. mass spectrum of sisomicin (9)

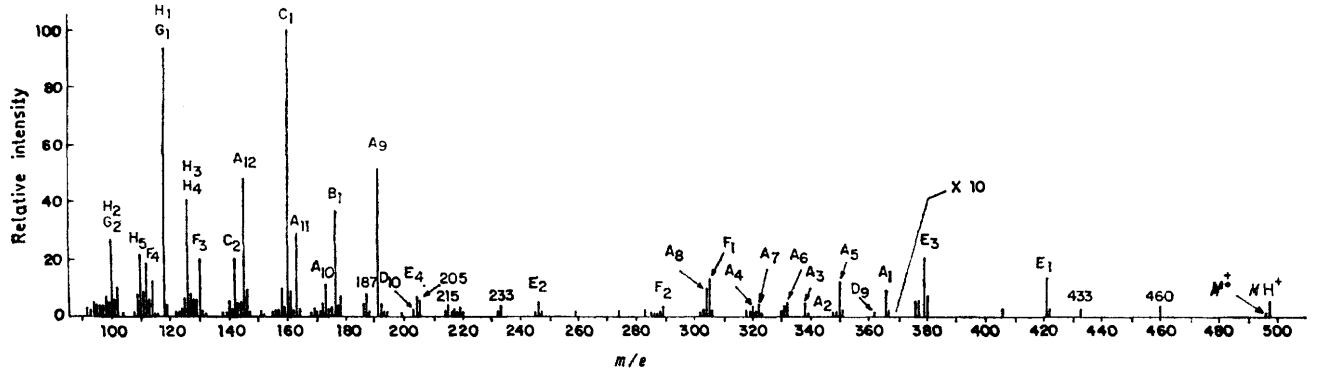


FIGURE 4 E.i. mass spectrum of antibiotic G-418 (8)

TABLE 2

The isobutane c.i. mass spectra of some aminocyclitol–aminoglycoside antibiotics

	Source temp (°C)	*m/e* (% of base peak)					
		$(MH)^+$	A_3	A_7	A_{11}	B_1	C_1
Gentamicin C_{1a} (1)	160	450 (100)					
Gentamicin C_1 (2)	165	478 (100)		322 (12)			
Gentamicin C_2 (3)	170	464 (100)		322 (32)			
Gentamicin A (5)	180	469 (20)	324 (20)	308 (30)	163 (100)	162 (21)	146 (63)
Gentamicin B (6)	185	483 (26)	324 (16)	322 (51)	163 (52)	162 (14)	160 (100)
Gentamicin X_2 (7)	190	483 (70)	324 (34)	322 (95)	163 (77)	162 (30)	160 (100)

TABLE 3

The isobutane c.i. mass spectra of sisomicin (9) at various temperatures

	150 °C		180 °C		220 °C	
Ion (*m/e*)	Rel.[a] intensity	% of ion[b] current	Rel.[a] intensity	% of ion[b] current	Rel.[a] intensity	% of ion[b] current
B_1 (127)	38	14.4	46	12.1	60	14.0
C_1 (160)	4	1.5	35	9.2	85	19.8
A_{11} (163)	2	0.8	21	5.5	42	9.8
A_7 (322)	38	14.4	97	25.5	100	23.3
D_3 (430)	9	3.4	12	3.1	12	2.8
MH^+ (448)	100	38.0	100	26.2	27	6.3

[a] As % of base peak. [b] Relative intensity expressed as % of the total ion current above *m/e* 120. Since minor ions and isotope peaks are not reported here the totals are less than 100%.

deoxystreptamine ion A_{11}. Sisomicin (9) also loses ammonia to give the ion D_3.

C.i. mass spectra of some aminoglycosides have recently been reported,[54] and the field desorption spectrum of neomycin B, which gives essentially only the MH^+ peak, has been published.[55]

[54] D. Horton, Abstracts, 169th National American Chemical Society Meeting, Philadelphia, 1975, CARB 17.

[55] K. L. Rinehart, jun., J. Carter Cook, jun., K. H. Maurer, and U. Rapp, *J. Antibiotics*, 1974, **27**, 1.

EXPERIMENTAL

The aminocyclitol–aminoglycoside antibiotics used were freed from carbon dioxide by passage over Amberlite IRA 401S (OH^-) resin; the samples were then lyophilized and the mass spectra determined. Low resolution e.i. spectra were run at 70 eV with a Varian MAT CH5 spectrometer, and the *m/e* values of relevant ions, together with their intensities expressed as a percentage of the base peak as 100%, are given in Table 1. High resolution mass measurements were carried out on either an A.E.I. MS902B or a CEC 21-110B spectrometer by the photoplate recording method. Compounds for which high resolution data were recorded are indicated by means of an obelus (†) in Table 1. In the latter spectra the *m/e* values for the ions of the compositions shown were in agreement with the calculated values.

All c.i. mass spectra were measured with an A.E.I. MS902 instrument modified for operation in the c.i. mode as previously described.[56] In all cases, the sample was admitted to the source on a direct insertion probe which was not heated independently of the source block. The temperatures given in the Tables are those of the source block when the spectrum was measured.

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[5/1894 *Received*, 30*th September*, 1975]

[56] F. H. Field, *J. Amer. Chem. Soc.*, 1969, **91**, 2827.