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MASS SPECTRA OF PARTIALLY *N*-ACETYLATED DERIVATIVES OF KANAMYCIN A†

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Abstract—As part of a program concerned with the chemistry and biochemistry of aminocyclitol antibiotics, a number of selectively *N*-acetylated kanamycins have been prepared from kanamycin monosulfate and characterized by a study of the electron impact induced fragmentation of two types of derivatives. In one of these, the remaining free amino groups were *N*-trideuteroacetylated and the *N*-acylated kanamycins thus obtained, were *N,O*-methylated. The spectra of these derivatives were useful for the location of the *N*-acetyl and *N*-trideuteroacetyl groups, except in the 2-deoxystreptamine unit. In a second type of derivative, the partially *N*-acetylated kanamycins were *N,O*-permethylated converting the free amino groups into dimethylamino groups. In this form, it was possible to locate the site of *N*-acetylation on the 2-deoxystreptamine ring. The partially *N*-acetylated kanamycins have been identified as 1,3,6′-tri-*N*-acetyl, 3,6′,3″-tri-*N*-acetyl, 3,6′-di-*N*-acetyl, 1,6′-di-*N*-acetyl and 6′-*N*-acetyl kanamycin, from a study of the mass spectra of these two types of derivatives.

INTRODUCTION

Partially *N*-acylated derivatives of kanamycin A[1] and other aminocyclitol antibiotics[2] have gained unprecedented importance in recent years with the discovery that the antibacterial activity of some analogs is greatly enhanced and broadened compared to the corresponding unacylated derivative.[3] For example, the attachment of a 4-amino-2(*S*)-hydroxybutyric acid residue to the 1-amino group of the 2-deoxystreptamine unit of many aminocyclitol antibiotics seems to confer unusually enhanced antibacterial properties to the modified compounds. The earliest such observation was made with the naturally-occurring butirosins,[4] which provided much impetus to chemically introduce the above-mentioned *N*-acyl unit in a large number of aminocyclitols, with good results in most, if not all, of the cases studied.

As part of a program concerned with the chemistry and biochemistry of aminocyclitol antibiotics,[5] we have had occasion to prepare a number of selectively *N*-acylated kanamycins.

Thus acetylation of commercially available "kanamycin monosulfate" with acetic anhydride in aqueous medium leads to a mixture in which the 6′-*N*-acetyl, 3,6′-di-*N*-acetyl and 1,3,6′-tri-*N*-acetyl derivatives are relatively major components. These can be conveniently separated by chromatography. Although, the 6′-amino group appears to be the most extensively protonated in kanamycin monosulfate, as indicated by ^{13}C NMR spectral data,[6] the high proportion of 6′-*N*-acetylation in the partially *N*-acetylated derivatives under the conditions of the reaction is presumably due to the much higher reactivity of the 6′-amino group in its unprotonated form relative to the other amino groups.

Our long-standing interest in the structural analysis of aminocyclitol antibiotics by mass spectrometry prompted us to study the fragmentation of some of these derivatives, particularly since the data were expected not only to locate the sites of *N*-acetylation, thereby identifying the products, but also to complement our previous studies in this area.[7] The location of the *N*-acetylated amino groups has also been studied by ^{13}C NMR.[6]

Mass spectrometry has been widely used in the study and structural determination of a large number of aminocyclitol antibiotics.[7–20] They have been studied in their free, underivatized form by electron-impact[8] and by chemical-ionization and field-desorption[18–19] mass spectrometry. In addition, their *N*-acetyl derivatives, which have been *O*-trimethylsilylated[7,13] and *N,O*-methylated,[7,15] and their Schiffs' base-oxazolidine,[16] salicylidene,[17] and *N,O*-trimethylsilyl[12] derivatives have also been studied by mass spectrometry. In this article, we present the mass spectra of some partially *N*-acetylated derivatives of kanamycin A which were suitably protected. Derivatization was necessary, as some of the parent compounds decomposed under the conditions used in the electron-impact ionization chamber.

*Mass spectra of per-*N*-acetyl, per-*N,O*-methyl kanamycin A* **1** *and its* N-*COCD*$_3$ *derivatives* **1A-E**. The mass spectrum of the per-*N*-acetyl, per *N,O*-methyl derivative **1** of kanamycin A has been recorded and discussed previously.[7] With the partially *N*-acetylated kanamycins on hand, it was of interest to complete the *N*-acetylation with acetic anhydride-d_6 and then to prepare the per-*N,O*-methyl derivatives. In this manner, it has been possible to study the sites of *N*-acetylation and to derive valuable information pertaining to the use of mass spectrometry as a diagnostic analytical tool in this area. The compounds resulting from this derivatization are **1A-E** and the shifts of major peaks, compared to the mass spectrum of **1**, are given in Table 1.

The most important peaks for recognizing structural features of kanamycins are those associated with the cleavage of bonds between the aminosugar units and the 2-deoxystreptamine unit.[7] In Scheme 1, these fragments are illustrated, as found in the mass spectrum of the least polar major component in the mixture of *N*-acetyl kanamycins, to which structure **1A** has been assigned. It can be seen that the partial shift of the fragments of mass 530 to 533, corresponding to cleavage of bonds to the 2-deoxystreptamine unit, and of the fragments of

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Table 1. Shifts of some of the major peaks in the mass spectra of *N*-acetyl, *N*-trideuteroacetyl kanamycin derivatives taken at 70 eV

	1	1-A	1-B	1-C	1-D	1-E
m/e	806	809	812	812	815	809
m/e	576	579 / 576 1:1	582 / 579 1:1	582 / 579 1:1	585 / 582 1:1	579
m/e	530	533 / 530 1:1	533 / 536 1:1	533 / 536 1:1	536 / 539 1:1	533
m/e	317	317	320	320	323	320
m/e	299	299	302	302	305	302
m/e	289	289	292	292	295	292
m/e	271	271	274	174	277	274
m/e	260	263 / 260 2:1	263 / 260 2:1	263 / 260 2:1	263 / 260 2:1	260
m/e	228	228	228	228	228	228
m/e	187	187	187	187	187	187
m/e	155	155	155	155	155	155
m/e	142	142	142	142	142	142
m/e	129	132	132	132	132	129

mass 260 to 263, corresponding to the aminosugar units, confirms the original proposal[7] that these fragments originate from both sides of the 2-deoxystreptamine unit. Additionally, metastable peaks present for the formation of the peak at *m/e* 228, 196 and 155, which originate from the 6-aminohexose unit, and the fact that these peaks do not shift in the mass spectrum of **1A**, are in agreement with interpretations presented earlier[7] and shown in Scheme 1.

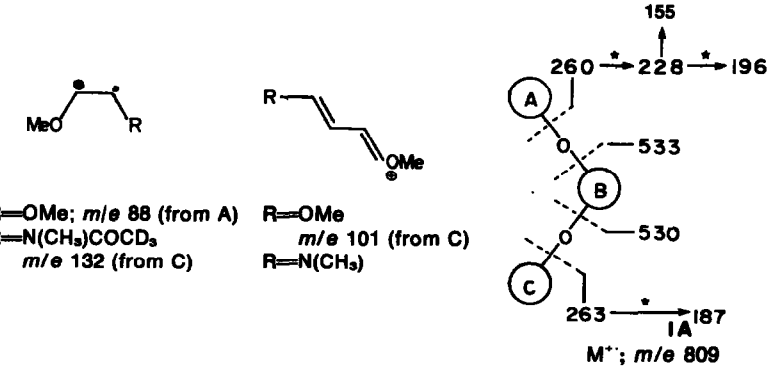
The fact that the peak found at *m/e* 129 in the spectrum of **1** shifts to *m/e* 132 in the spectrum of **1A** demonstrated that it is formed exclusively from the 3-aminohexose unit. On the other hand, it is interesting to note that the peak at *m/e* 142 does not shift in the spectrum of **1A**, upon deuteroacetylation; thus, it originates from the 6-aminohexose unit. The shifts of these peaks can be diagnostic for the presence of 3-acetamidohexose and 6-acetamidohexose units, respectively, in partially *N*-acetylated kanamycins.

With these considerations in mind, **1D** can readily be identified as being derived from 6′-*N*-acetyl kanamycin A. The shift of the molecular-ion peak from *m/e* 806 in the case of **1** to *m/e* 815 in the case of **1D** shows that the latter is mono-*N*-acetylated and tri-*N*-deuteroacetylated. The presence of the peak at *m/e* 132 in its mass spectrum denotes that the 3-aminosugar unit is *N*-deuteroacetylated (compare *m/e* 129, **1**), whereas the presence of the peak at *m/e* 142 (and not *m/e* 145) shows that the 6-aminosugar unit is already *N*-acetylated in the parent, partially *N*-acetylated kanamycin. The locations of other peaks in the mass spectrum of **1D**, compared with the mass spectrum of **1**, confirm this structural assignment.

As can be seen in Table 1, the mass spectra of **1B** and **C** are essentially identical. Their molecular ions differ from that of **1** by 6 atomic mass units (u), showing that two-*N*-deuteroacetyl groups are present. From the shift of the fragment of *m/e* 129 by 3u to 132, it can be seen that the parent compounds were not *N*-acetylated in the 3-aminohexose unit. However, the fragment of mass 142 is present in the mass spectra of these compounds, demonstrating that, in both cases, the parent compounds contain *N*-acetyl groups in the 6-aminohexose unit. Thus, one *N*-acetyl group is at the 6′-position and the other *N*-acetyl group has to be located in the 2-deoxystreptamine unit. Although it is possible to determine that the compounds from which **1B** and **C** were derived are 1,6′-*N*-diacetyl and 3,6′-*N*-diacetyl kanamycin, it is not possible to distinguish between these two isomers on the basis of the mass spectra of their derivatives **1B** and **C**.

During the selective acetylation of kanamycin A, a product that had an R_f very close to that of **1A** was isolated in very low yield. The corresponding derivative, after *N*-acetylation with acetic anhydride-d_6 and *N,O*-methylation, was identified as being 1,6,3″-tri-*N*-acetyl kanamycin or 3,6′,3″-tri-*N*-acetyl kanamycin. Its molecular-ion peak is found at *m/e* 809, shifted by 3u from that of **1** (see Table 1), showing that one amino group is trideuteroacetylated and that three amino groups were already *N*-acetylated in the product from which the derivative was prepared. The presence of a peak at *m/e* 260 (and none at *m/e* 263) indicates that the amino groups at positions 3″ and 6′ are acetylated in the original compound. This conclusion is confirmed by the fact that the fragments of *m/e* 129 and of *m/e* 142 are once again present. The shift of the fragment of mass 530 in the spectrum of **1** by 3u to mass 533 in the spectrum of **1E** leads to the deduction that the *N*-trideuteroacetyl group in **1E** is located in the 2-deoxystreptemine part of the molecule. Once again, it is possible to say which of the two positions of the 2-deoxystreptamine unit remains unacetylated in the original tri-*N*-acetyl kanamycin.

In summary, the use of the fully *N*-acetylated, *N,O*-methylated derivatives in conjunction with deuterium labeling is very useful for determining structural features



Scheme 1.[a]

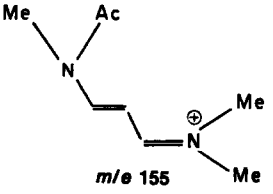
[a]The amino sugar units are abbreviated Ⓐ and Ⓒ and the 2-deoxystreptamine unit, Ⓑ.

of the partially *N*-acetylated kanamycins by mass spectrometry. However, it suffers from a major limitation as it is not possible to distinguish between the two amino groups in the 2-deoxystreptamine unit with the *N*-acetylated derivatives. In the case of the partially acetylated kanamycins bearing one *N*-acetyl group on the 2-deoxystreptamine ring, the site of *N*-acetylation was located by a study of the corresponding permethylated derivatives where free amino groups are converted to dimethylamino groups.

Mass spectra of N,O-*methylated* N-*acetyl kanamycins* **2-6**. The mass spectrum of the per-*N*,*O*-methyl derivative **2** of the product identified as 1,3,6'-tri-*N*-acetyl kanamycin by means of the mass spectrum of its derivative **1A**, is found in Table 1, and its fragmentations are summarized in Scheme 2. Fragment ions arising from glycosidic cleavage give rise to peaks at *m/e* 260 and 232, and decompose further by loss of methanol. Cleavages around the 2-deoxystreptamine unit lead to the peaks found at *m/e* 530 and 502, as shown in Scheme 2. The fragment-ion peak located at *m/e* 142 in the mass spectra of **1** and **1A** is once again found at *m/e* 142, in the mass spectrum of **2**. However, the fragment arising from the 3-aminohexose unit now has a mass of 101 (compared to *m/e* 129, Scheme 1). At high resolution, the peak at *m/e* 101 in the mass spectrum of **2** is resolved into a doublet, consisting of 5 parts of $C_5H_{11}ON$ and 1 part of $C_5H_9O_2$ (see Scheme 2). Thus, the conclusion reached from the study of derivative **1A**, i.e. that the 3"-amino group is free in this partially *N*-acetylated kanamycin, has been confirmed by the mass spectrum of derivative **2**.

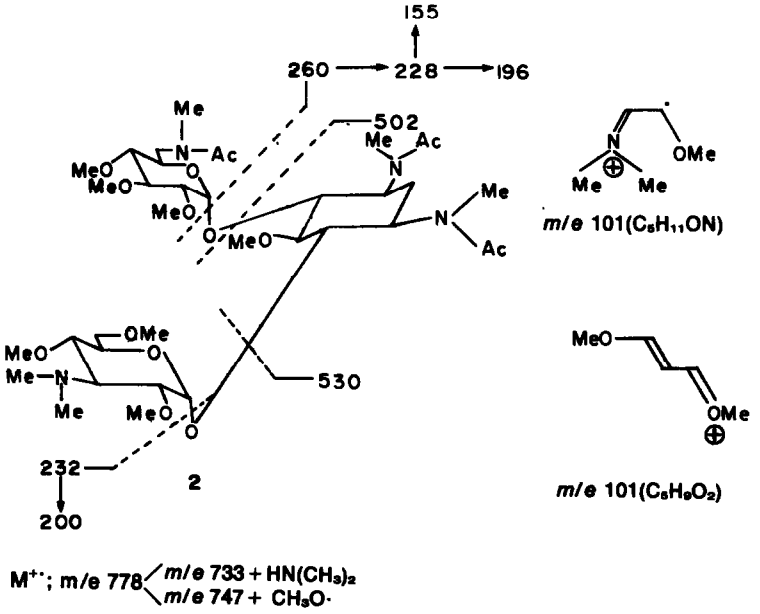
The mass spectra of the permethylated derivatives of the two di-*N*-acetyl kanamycins are substantially different (**3** and **4**, Table 2), whereas the mass spectra of their derivatives **1B** and **C** (Table 1) were essentially identical. The fragmentations involving the aminosugar units (giving peaks at *m/e* 260 and 232) are the same as those found in the mass spectrum of **2** (Scheme 2), whereas peaks from the ions formed from the disaccharide units are found at *m/e* 474 and 502 in each case (Scheme 3). Displacements of peaks in the mass spectra of the compounds after permethylation with CD_3I, compared to the spectra of the unlabeled analogs, and formulas obtained from exact-mass measurements made at high resolution are in agreement with this interpretation.

The results of exact-mass measurements obtained at high resolution show that the peak at *m/e* 155 is in fact a doublet (ratio, 1:6) in the mass spectrum of **3**. The less abundant component, $C_8H_{11}O_3$, corresponds to an ion formed from an aminosugar unit (see Scheme 2), and is shifted to *m/e* 161 in the mass spectra of the labeled compounds, prepared with CD_3I. The major component of the peak at *m/e* 155, $C_8H_{15}N_2O$, which shifts to *m/e* 164, can be attributed to a fragment originating from the 2-deoxystreptamine unit and retaining the two substituted amino groups, along with, presumably, carbons C_1, C_2 and C_3.

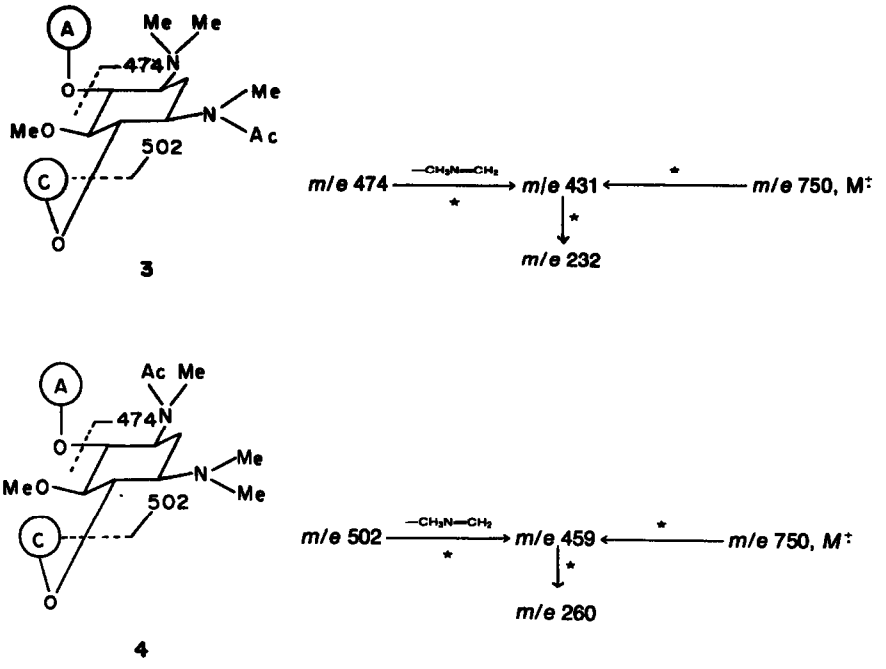


m/e 155

The formation of this fragment from the 2-deoxystreptamine component has considerable diagnostic value. The corresponding fragment from derivatives **1** and **2**, with two *N*-methyl, *N*-acetyl groups and having a mass of 183, is of very low abundance in their spectra. On the other hand, when a dimethylamino group is present in the 2-deoxystreptamine unit, as is the case with derivatives **3** and **4**, this fragment has a much higher probability of formation, presumably due to the better stabilization of charge by the dimethylamino group than by the *N*-methylacetamido group. The importance of this fragment for structural determination is that its presence is dependent on the substitution of the amino groups of the 2-deoxystreptamine unit; however, its formation is not dependent on whether or not the dimethylamino group is at C-1 or C-3.



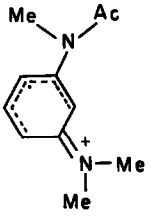
Scheme 2.



Scheme 3.[a]

[a]Metastable peaks were obtained by scanning the accelerating voltage with fixed electric sector and fixed magnetic field.

Another fragment-ion peak apparently arising from the 2-deoxystreptamine component is found at *m/e* 192 in the mass spectrum of **4**. Deuteromethylation leads to a shift to *m/e* 201 and exact-mass determination gives a formula of $C_{11}H_{16}N_2O$, prompting us to propose the following structure:



m/e 192

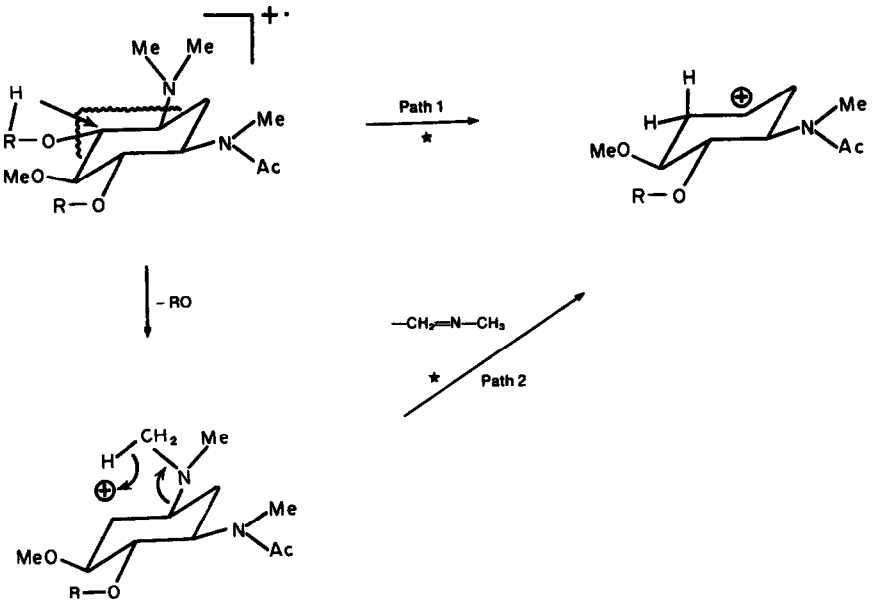
It is not evident why this ion should be more abundant in the mass spectrum of **4** than in that of **3**; however, it may be useful for distinguishing the presence of the *N*-acetyl group on C-1 from its presence on C-3 of parent compounds containing only one *N*-acetyl group in the 2-deoxystreptamine component.

The fragments giving rise to the peak at *m/e* 101 are abundant in the mass spectra of **3** and **4**, as they were in the spectrum of **2** (see Scheme 2). The major fragment of mass 101 once again arises from the 3-aminosugar unit, as can be deduced from its location at *m/e* 110 in the mass spectra of the deuteromethylated analogs.

By far the most interesting feature of the mass spectra of **3** and **4** is a rearrangement involving elimination of $CH_3H{=}CH_2$ from fragments containing a positive charge on the carbon adjacent to the carbon bearing the dimethylamino group of the 2-deoxystreptamine component. Normal and metastable peaks associated with this rearrangement are summarized in Scheme 3. The important factor, from the point of view of structural determination, is that the rearrangement-ion peak is found at *m/e* 431 in the mass spectrum of **3** and at *m/e* 459 in that of **4**, due to the fact that the 3-aminohexose unit is retained in the ion of mass 431 whereas the 6-aminohexose unit is retained in the ion of mass 459. Exact-mass determinations are in agreement with the fragmentations given in Scheme 3. These fragment-ion peaks, which are relatively abundant at 70 eV (Table 2), become even more relatively abundant at low electron voltages: *m/e* 431 has a relative intensity of 93% in the 15 eV spectrum of **3**, and *m/e* 459 is the base peak in the 15 eV spectrum of **4**.

A duality of mechanisms for the formation of the peaks of *m/e* 431 and 459 in the mass spectra of **3** and **4**, is indicated by the data obtained from metastable peaks and deuterium labeling. Metastable peaks are present for formation of these rearrangement ions directly from the molecular ions as well as from the fragment ions of mass 474 and 502; in fact, in the mass spectrum of **4**, the metastable peak for the formation of *m/e* 459 from *m/e* 750 is more intense than the metastable peak for its formation from *m/e* 502.

Additionally, data obtained from the deuterated analogs prepared with CD_3I are best interpreted by the operation of two competing mechanisms (Scheme 4). For example, the peak at *m/e* 431 in the spectrum of **3** shifts to *m/e* 452 and 453 in the spectrum of **3**-d_{39}. The fragment of mass 452 must contain 7 *N,O*-CD_3 groups, since it is 21u higher than in the spectrum of unlabeled **3**; this can be explained by path 1 in Scheme 4. On the other hand, the fragment of mass 453 in the mass spectrum of labeled **3** must contain 7 *N,O*-CD_3 groups along with a rearranged deuterium atom; this can be rationalized by path 2 in Scheme 4. Likewise, the peak at *m/e* 459 in the spectrum of **4** shifts to *m/e* 477 and 478 in the spectrum of **4**-d_{39}. The formation of the peaks at *m/e* 477 and 478



Scheme 4.

"These data were obtained from deuteromethylation.

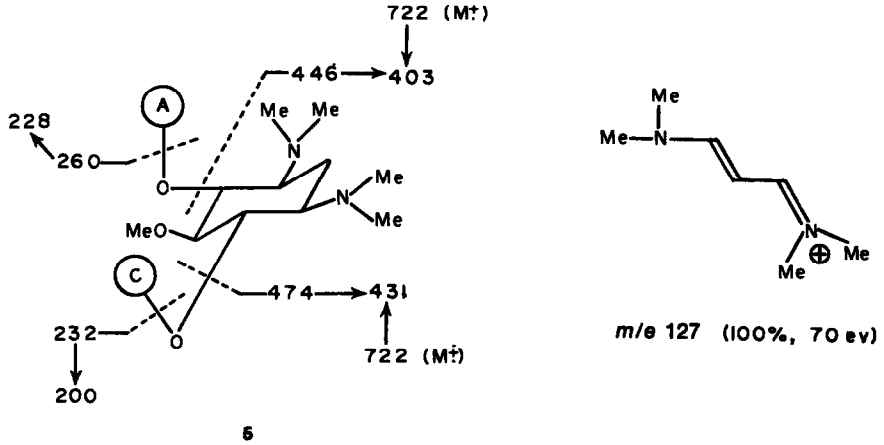
can be accounted for by paths 1 and 2, respectively. The peaks at *m/e* 452 and 453 are of approximately equal intensity; *m/e* 478 is about 3/4 as intense as *m/e* 477. Additional support for the formation of *m/e* 477 *via* path 1 and *m/e* 478 *via* path 2 comes from study of metastable peaks in the mass spectrum of labeled **4**: a metastable peak is present for the formation of *m/e* 477 from the molecular ion (*m/e* 789) and another is present for the formation of *m/e* 478 from the fragment ion (*m/e* 526).

A related mass-spectral fragmentation of gentamycins with a *N*-ethyl group and a free amino group on the 2-deoxystreptamine unit has been reported.[20] An amino group on the 2-deoxystreptamine ring is lost together with the glycosyloxy group vicinal to it, which would correspond to path 1 in Scheme 4.

It is this rearrangement process, a major aspect of the fragmentation of **3** and **4**, that has permitted us to assign structures to these compounds. When the aminohexose unit is located next to a carbon bearing a *N*-methyl, *N*-acetyl substituent, this rearrangement does not occur. Therefore, we conclude that the presence of a dimethylamino group on the carbon next to the aminohexose unit is a requirement for the rearrangement. If that is the case, **4** can be seen to have a dimethylamino group at C-1 and methylacetamido group at C-3 of the 2-deoxystreptamine component, whereas **3** has a methylacetamido group at C-1 and a dimethylamino group at C-3.

This requirement that a dimethylamino group be located on a carbon adjacent to the carbon bearing an aminohexose unit for the rearrangement to occur can be seen to be operating also in the mass spectrum of the permethylated derivative **5** of 6'-*N*-acetyl kanamycin (Table 2). In this spectrum, two rearrangement-ion peaks are observed, due to the presence of two dimethylamino groups in the 2-deoxystreptamine component. One rearrangement ion (*m/e* 403) contains the 3-di-methylamino-hexose unit, whereas the other (*m/e* 431) contains the 6-methylacetamidohexose unit (Scheme 5).

Other fragments and fragmentations found in the mass spectrum of **5** can be elucidated by reference to those found in the spectra of **2**-**4**. The absence of *N*-acetyl groups in the 2-deoxystreptamine component of the



5

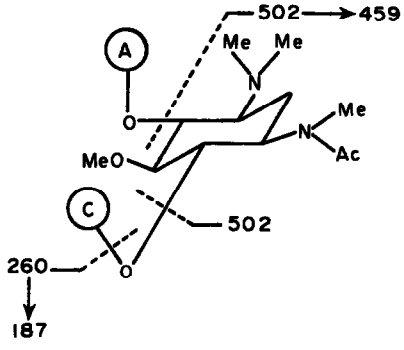
Scheme 5.

parent compound is evident from the base peak at *m/e* 127 in the 70 eV spectrum of derivative **5**. Fragment 127 corresponds to a three carbon segment of the 2-deoxystreptamine part, retaining the two dimethylamino groups which provide excellent stabilization of the charge.

It is evident in the 70 eV mass spectra of compounds **2** and **5** that the 3-aminohexose component attached to C-6 of the 2-deoxystreptamine unit is more readily cleaved than the 6-aminohexose attached to C-4. For example, the peaks at *m/e* 530 and 232 are more intense than those at *m/e* 502 and 260, in the mass spectrum of **2** (see Table 2 and Scheme 2). Likewise, in the spectrum of **5**, the peaks at *m/e* 474 and 232 are more intense than those at *m/e* 446 and 260 (see Table 2 and Scheme 5). Also, in the mass spectrum of **5**, where rearrangement ions involving the loss of the 3-aminohexose portion as well as the 6-aminohexose portion are formed, the peak at *m/e* 431 is far more intense than *m/e* 403 (see Table 2 and Scheme 5). The formation of the former ion (of mass 431) requires that the 3-aminohexose unit be eliminated.

Thus, two general trends are observed. Firstly, there is the preferential loss of the 3-aminohexose unit over the 6-aminohexose unit, everything else being equal. Secondly, the presence of a dimethylamino group favors the rearrangement involving the loss of the aminohexose unit on the adjacent carbon, as shown in Scheme 4. These two effects appear to work together when a dimethylamino group in the 2-deoxystreptamine component is adjacent to the 3-aminohexose component, as is the case in **4** which form the rearrangement ion of mass 459 (56%). On the other hand, in the case of **3**, the dimethylamino group is not adjacent to the 3-aminohexose unit and the relative intensity of the rearrangement-ion peak at *m/e* 431 at 70 eV is only 8%, the 6-aminohexose unit being lost. (At 15 eV, the peaks corresponding to the rearrangement ions of mass 431 from **3** and of mass 459 from **4** are both very intense.)

The *N,O*-methyl derivative **6** of the tri-*N*-acetyl kanamycin studied above as derivative **1E** was also prepared and examined by mass spectrometry. The data given in Table 2 and Scheme 6 demonstrate that the free



M^+, *m/e* 778

Scheme 6.

amino group in the parent compound is indeed located in the 2-deoxystreptamine component, confirming the conclusion reached earlier. The peak at *m/e* 155 is a doublet, consisting of 2 parts of $C_8H_{15}N_2O$ to 1 part of $C_8H_{11}O_3$, as was also the case for **3**, discussed above. On the other hand, the much less intense peak at *m/e* 155 in the mass spectrum of **2**, an isomer of **6** with 3″-amino group unacetylated, is almost entirely composed of ions of the formula $C_8H_{11}O_3$ (see Scheme 2).

Thus, we once again come upon the need to be able to distinguish between the two amino groups of the 2-deoxystreptamine unit. To do this, we compared the spectrum of **6** with those of **3** and **4**, which also have a *N*-methyl, *N*-acetyl and a *N,N*-dimethylamino group in the 2-deoxystreptamine unit. It is not possible to make the distinction by the presence or absence of peaks. For example, the ions due to glycosidic cleavage in **6** give peaks at 502 and 260 regardless of which of the aminosugar units is lost; likewise, the rearrangement ions give a peak at *m/e* 459 ($C_{22}H_{39}O_8N_2$ at high resolution) whether it is the 3- or the 6-aminohexose which is lost in its formation. At 15 eV, the relative intensity of *m/e* 459 is 56%.

In order to distinguish between the two amino groups and assign the structure 1,6′,3″-tri-*N*-acetyl kanamycin to the compound from which derivative **6** was prepared, it became necessary to compare relative intensities of peaks in the mass spectrum of **6** with those in the spectra of **3** and **4** (see Table 2). If the intensities in the spectrum of **6** resembled those of **3** more than those of **4**, it would indicate that the 3-aminohexose unit is not lost in the formation of the rearrangement ion and that the dimethylamino group is at C-3. On the other hand, if the peak due to the rearrangement ion were intense in the spectrum of **6**, as in the spectrum of **4**, it would indicate that the dimethylamino group is at C-1, adjacent to the 3-aminohexose unit. In fact, the relative intensities in the spectrum of **6** are similar to those found in the spectrum of **3**. In both spectra, peaks are present at *m/e* 501 as well as at *m/e* 502 and the rearrangement-ion peaks (*m/e* 431 from **3** and 459 from **6**) are not very abundant at 70 eV, whereas the rearrangement-ion peak at *m/e* 459 is very abundant in the spectrum of **4** and a peak is not present at *m/e* 501.

Another important point of comparison of the intensities in the spectrum of **6** with those in the spectra of **3** and **4** revolves around the formation of the ions giving a peak at *m/e* 228. This is the base peak in the spectrum of **4**, but is less intense in the spectra of **6** and **3**. The ion of mass 228 arises from the loss of methanol from the ion of mass 260, which originates from the 6-aminohexose unit. The similarity in the relative intensities of *m/e* 228 in the spectra of **3** and **6** can be interpreted to say that the dimethylamino group of the 2-deoxystreptamine unit is at the same position in **3** and **6** and influences in the same manner the formation of the 6-aminohexose ion of mass 260 from which the ion of mass 228 is formed.

The peak at *m/e* 260 is more intense in the spectrum of **6** than in the spectrum of **3** because the ion of mass 260 forms from the 3-aminohexose as well as from the 6-aminohexose, whereas in the case of **3**, the formation of the 3-aminohexose ion gives rise to a peak at *m/e* 232. However, the ions of mass 260 arising from the 3-aminohexose unit do not decompose further to ions of mass 228.

Thus on the basis of mass spectrometry of these derivatives we have been able to identify the partially *N*-acetylated kanamycins as 1,3,6′-tri-*N*-acetyl, 1,6′,3″-tri-*N*-acetyl, 3,6′-di-*N*-acetyl, 1,6′-di-*N*-acetyl and 6′-*N*-acetyl kanamycin. The identification of all except the 1,6′,3″-tri-*N*-acetyl kanamycin was readily accomplished from fragmentations and rearrangements in the mass

spectra of derivatives **1–5**. In the case of the exception, a 1,6,3″-tri-*N*-acetyl rather than 3,6′,3″-tri-*N*-acetyl structure was assigned on the basis of similarities in relative intensities between the mass spectra of **6** and **3**.

EXPERIMENTAL

Preparation of partially N-*acetylated kanamycins.* Kanamycin monosulfate (582 mg, 1 mmole) was dissolved in 30 ml of distilled water and the solution was treated with acetic anhydride (1.9 ml, 20 mmoles). After stirring for 6 h at room temperature, the solution was slowly poured into a 1:1 mixture of ether and acetone (300 ml), and the resulting sticky solid was repeatedly washed with the same solvent mixture by decantation (total 200 ml). The residue was dissolved in 2 ml of water, enough silica gel was added to give a slurry, and the slurry was evaporated to dryness. The resulting mass was mechanically applied to a column (3×9 cm) containing silica gel GF 254, equilibrated with the solvent system, chloroform–methanol (1:3). The column was developed (under mild suction from an aspirator), using the above solvent mixture (100 ml), then with chloroform–methanol–0.05N ammonium hydroxide (1:3:0.1, 600 ml), and (1:3:0.2, 300 ml). Fractions (~10 ml) were analysed by TLC and pooled according to their composition. Very good separations were thus obtained, although slight contamination of some tail-end fractions could not be avoided. Such fractions were pooled and rechromatographed. The following weight distribution was obtained from solutions that were first evaporated on a rotary evaporator to eliminate organic solvents, and subsequently lyophilized: fractions 55–66, 174 mg (28.5%) of 1,3,6′-tri-*N*-acetylkanamycin; fractions 85–95, 117 mg (21%) of 1,6′-di-*N*-acetylkanamycin; fractions 105–110, 63 mg (11.0%) of 3,6′-di-*N*-acetylkanamycin; fractions 118–122, 66 mg (12.5%) of 6′-*N*-acetylkanamycin; fractions 128–132, 50 mg (10.3%) of kanamycin free base. A very small amount of 3,6′,3″-tri-*N*-acetylkanamycin could also be isolated by reprocessing fractions 45 and 46. All the *N*-acetylated kanamycin derivatives were obtained as the free bases, in the form of colorless amorphous solids. These were kept in tightly stoppered vials under nitrogen, to avoid absorption of carbon dioxide. Samples were derivatized for mass spectral studies within a few hours of their isolation; otherwise they were treated with dilute hydrochloric acid, then with an anion exchange resin to convert them into the free bases, immediately before derivatization.

Preparation of the derivatives. N-Trideuteroacetylation was carried out by treating the partially *N*-acetylated kanamycins with a large excess of methanolacetic anhydride-d_6 (3:1), at room temperature, for a period of 4 h and then evaporating the solution to dryness. A modification of our previous procedure[7] was employed for the *N,O*-permethylation of partially and fully *N*-acetylated kanamycins. To a solution of the sample (1 mg) in DMSO (0.2 ml) was added à solution of methylsulfinyl carbanion in DMSO (*ca.* 1 molar) (0.2 ml) and after 1 min, methyl iodide (0.02 ml) was added with cooling. The reaction was quenched after 1 min by adding ice-cold water (2 ml) and the resulting solution was immediately extracted with chloroform. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was exposed to a high vacuum before recording the mass spectrum to remove traces of solvents and other volatile impurities.

N,O-Pertrideuteromethyl derivatives were prepared by an essentially similar procedure, methyl iodide being replaced with methyl iodide-d_3.

Mass spectra. Mass spectra were recorded on an AEI MS 902 mass spectrometer. The spectra were obtained at 70 eV, unless stated otherwise. All the spectra were recorded at source temperatures below 180°C. Exact mass measurements were carried out at a resolution of 12,500 (10% valley).

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