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A MASS SPECTRAL INVESTIGATION OF DERIVATIVES OF KANAMYCIN A

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Abstract—The high and low resolution mass spectra of the N-acetyl-N,O-methyl and N-acetyl-O-trimethylsilyl derivatives of kanamycin A have been determined and interpreted, along with those of deuterated analogs under conditions of electron impact. In addition, the chemical ionization mass spectrum of the N-acetyl-N,O-methyl derivative is presented. The use of these spectra for recognizing structural features, such as the sequence of the sugar units, is discussed. The characteristics of the two types of derivatives are compared. The value of the chemical ionization spectrum and the complementary nature of the data obtained from it are emphasized.

Several types of derivatives have been employed in mass spectrometric investigations of carbohydrates.[3] The methyl ethers and the trimethylsilyl ethers have been the most commonly reported. The latter derivatives have been widely employed despite the disadvantages of high molecular weights, complexity of fragmentation and propensity for rearrangement upon electron impact, and sensitivity to moisture. This preference for the trimethylsilyl derivatives has been due to their ease of preparation and excellent chromatographic behavior. The methyl ethers continue to be employed in mass spectrometric work on carbohydrates,[4,5] due in part to the use of Hakomori's methylation procedure which has to a large extent alleviated the problem of preparation of these derivatives,[5-9] and to the fact that methylation remains a conventional method of analysis of polysaccharides.[8,9] In addition, the methyl ethers chromatograph well,[5,10] are more stable than the trimethylsilyl ethers, have significantly smaller molecular weights, and exhibit simpler fragmentations upon electron impact.[3] For these reasons we have investigated the mass spectrometric behavior of the N-acetyl-N,O-methyl and N-acetyl-O-trimethylsilyl derivatives of aminocyclitol antibiotics.

The mass spectra of the N-acetyl-O-trimethylsilyl derivatives of some aminocyclitol antibiotics have been investigated.[11-13] Investigations of N-acetyl-N,O-methyl derivatives of pseudotrisaccharide gentamicins have included the use of mass spectrometry.[14] Also, the mass spectra of fifteen underivatized pseudotri- and pseudodisaccharides, including kanamycin A, are briefly summarized in a communication.[15]

The work reported here is a detailed investigation of the mass spectra of the N-acetyl-N,O-methyl (1) and N-acetyl-O-trimethylsilyl (2) derivatives of kanamycin A. The mass spectra of the N-acetyl-d_3 and N,O-methyl-d_3 derivatives, exact-mass measurements, and chemical ionization (CI) mass spectra have been used to aid in the elucidation of the fragmentation pathways.

The 70 eV mass spectrum of **1**, obtained under conditions of electron impact (EI), is given in Fig 1. The EI mass spectrum of **2** is given in Table 1. The proposals below are in each case in agreement with the results from the specifically deuterated compounds and with the measured exact masses.

N-Acetyl-N,O-methyl kanamycin (**1**). The first peak for which one generally searches in a mass spectrum is the molecular-ion peak. These peaks are very small in the EI spectra of **1** (*m/e* 806, 0·4%) and **2** (*m/e* 1156, 0·8%) and may be obscured by background or noise. Also, the use of TMS derivatives increases the molecular weight to such an extent that it is difficult to obtain exact-mass data, especially on peaks of such low abundance. Thus, the CI mass spectrum of **1** is very useful: the M + H peak at *m/e* 807 is intense, 13%. In the EI mass spectrum of underivatized kanamycin A, the (M + 1) peak is larger than the molecular ion.[15]

The gross aspects of the sequential arrangement of the saccharide units are readily apparent from the masses of the ions which form by cleavage of the glycosidic bonds and of the C—O bond connecting a hexose to the aminocyclitol unit. For example, in the mass spectrum of **1** these peaks are found

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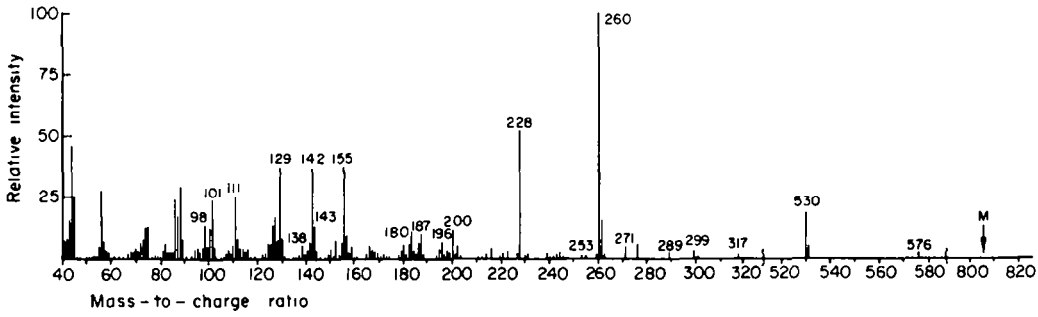
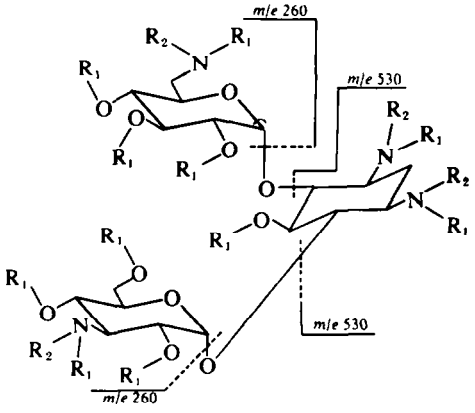


Fig 1. The 70 eV EI mass spectrum of N-acetyl-N,O-methyl Kanamycin A M.W. = 806.

at *m/e* 530 and 260 when $R_1 = CH_3$ and $R_2 = COCH_3$:

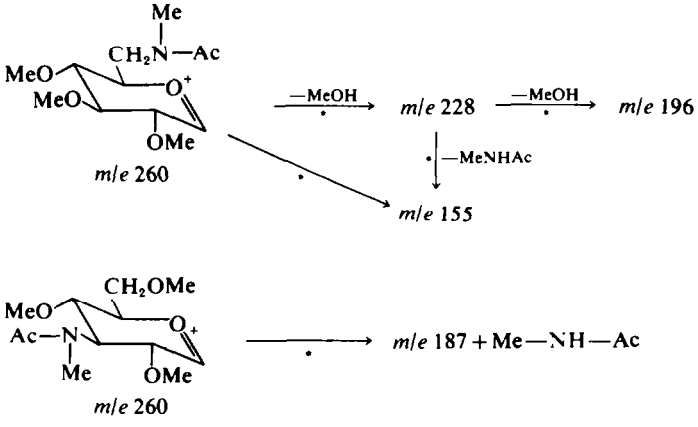


1 $R_1 = CH_3, R_2 = COCH_3$
$R_1 = CH_3, R_2 = COCD_3$
$R_1 = CD_3, R_2 = COCH_3$

the 6-aminohexose end; so it is not possible to determine the relative amount of fragmentation from each end with these data.

Labeling experiments involving the methoxyl groups in the monosaccharide methyl ethers have shown that these groups are lost in a characteristic sequential fashion upon electron impact.[3] In the hexose derivatives, loss of the C-1 methoxyl group occurs first followed by loss of the 3-methoxyl group as methanol,[16] followed by competitive loss of either the 4- or the 6-methoxyl group also as methanol.[17] The same sequence of losses is observed in the pentose derivatives, except that the 3- and 5-methoxyl groups are lost competitively, but less so than in the hexose case. This sequential loss of substituents is observed in the mass spectra of the N-acetyl-O-methyl derivatives of aminosugars,[18,19] and in the mass spectra of the compounds studied here.

The ions of this series commence with *m/e* 260 in the mass spectrum of **1**:



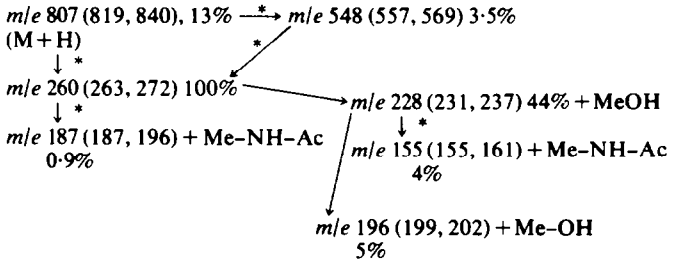
The shifts of the peaks in the mass spectra of the deuterated analogs ($R_1 = CH_3$, $R_2 = COCD_3$ and $R_1 = CD_3$, $R_2 = COCH_3$) and the elemental compositions of these ions are the same whether they are formed from the 3-aminohexose end or from

Exact-mass measurements, labeling data, and the presence of metastable peaks (*) are consistent with these assignments.

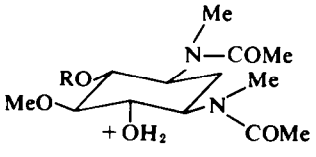
According to the substituent losses outlined above, the loss of 73 mass units corresponding to

N-methylacetamide from *m/e* 260 is consistent with the presence of a N-methylacetamido group in the 3-position of the 3-aminohexose end of kanamycin, while loss of 32 mass units as methanol from *m/e* 260 results from the presence of a 3-OMe substituent in the 6-aminohexose end. Competitive losses of 32 and 73 mass units from *m/e* 228 is characteristic of the presence of the 6-N-methylacetamido group. Thus, it is possible to see that *m/e* 260 is in fact two ions originating from different ends of the molecule.

Similar fragmentations are observed in the CI mass spectrum of **1**, using isobutane as the reactant gas:



The values in parentheses represent the peak locations obtained from the mass spectra of the derivatives containing N-$COCD_3$ and N-CD_3, O-CD_3, respectively. The fragment at *m/e* 548 corresponds to the sequence ion 530 plus —OH_2^+, one of the glycosidic oxygens. There is present also a small peak at *m/e* 289 for a similar fragment:

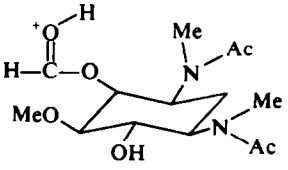


R = H *m/e* 289 (295,298) 1·4%
R = aminohexose *m/e* 548 (557,569) 3·5%

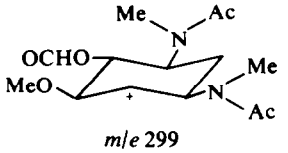
The CI technique has recently been shown to give (M+H) ions from pentoses, hexoses and simple glycosides of monosaccharides, and from O-acetyl derivatives of monosaccharides, maltose, trehalose and sucrose.[20]

The deoxystreptamine unit also undergoes fragmentations in the EI spectrum. The resulting ions are of reasonable intensity and many paths were studied by the metastable defocusing (dm*) technique.

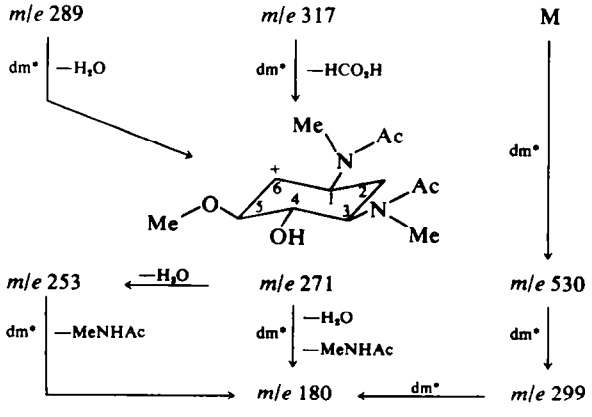
The scheme below is consistent with the exact-mass and the labeling data. It is likely that the roles of the positions 4 and 6 as shown below, are reversed in a large number of the *m/e* 271 ions and their precursors. Possible structures for *m/e* 317 and 299 are:



m/e 317

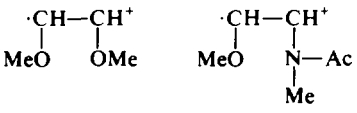


m/e 299

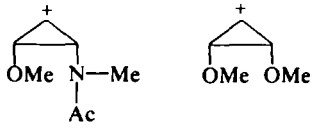


The peaks at *m/e* 317 and 299 retain C-1 of one of the sugars as a formyl group. The results of metastable defocusing studies show that at least some of the ions at *m/e* 299 are formed from *m/e* 530, which in turn can be formed directly from the molecular ion. There is a peak at *m/e* 576 which could be a precursor of *m/e* 317; this ion would be the same as shown for 317 except that the aminohexose unit rather than —OH is on C-4. A similar protonated formyl ion has been observed in the mass spectrum of kanamycin A.[15]

Other ions present in the Fig. are characteristic of the mass spectra of aminohexoses[3]:



m/e 88 *m/e* 129



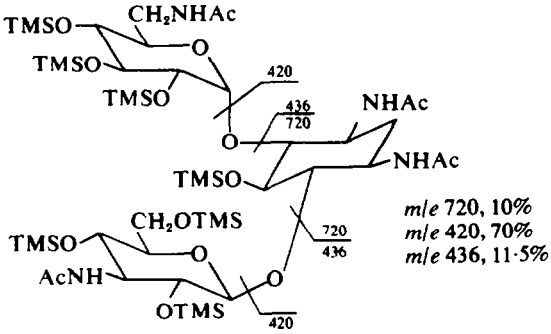
m/e 142 *m/e* 101

Ions identical or similar to these are not found in the CI spectrum.

N-Acetyl-O-trimethylsilyl kanamycin (**2**). The 70 eV EI mass spectrum of N-acetyl-O-trimethylsilyl kanamycin A (**2**) is given in the Table; also, exact-mass data were obtained with the aid of an on-line PDP 8 Computer and by the peak-matching technique. The mass spectrum of the acetyl-d_3 analog was also obtained.

The high mass region contains peaks of low relative intensity with a molecular ion just discernible. The molecular weight is easily calculated from the more intense peak resulting from loss of CH_3· at M-15 (*m/e* 1141, 8·5%), ubiquitous in the mass spectra of TMS derivatives of carbohydrates.[21] The base peak is found at *m/e* 73, due to $(CH_3)_3Si^+$. The large number of carbon atoms and especially the presence of silicon account for the abundant isotope peaks (compound **2** has the composition $C_{47}H_{100}O_{15}N_4\ Si_7$). Due to the high H/C ratio of 100/47, the profusion of peaks and the electron-gain capability of the instrument, it was possible to observe a peak at nearly every mass unit up to the molecular-ion region. To complete the counting in the high mass region, samples which were intentionally impure were used.

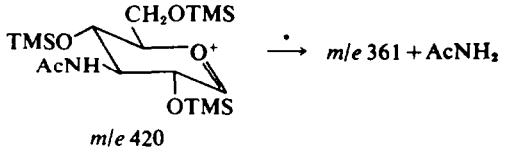
The mass spectrum of **2** also shows prominent peaks which are useful for sequencing the units:



2

The peak at *m/e* 420 is the precursor of two series of ions. In the first case, the fragmentations are the same as observed in our mass spectrum of methyl 6-acetamido-6-deoxy-α-D-glucopyranoside (**3**), as shown at the bottom of this page.

In addition, there is a peak at *m/e* 361 and a metastable peak for the loss of acetamide from *m/e* 420 to give 361. This presumably arises from the ion due to the presence of the 3-acetamido sugar:

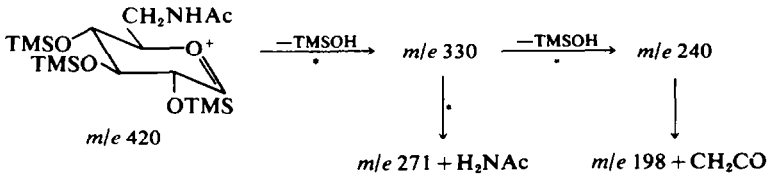


These assignments are consistent with the data obtained from studies at high resolution and from shifts in the mass spectrum of the acetyl-d_3 compound.

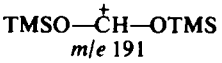
The ion at *m/e* 720 loses trimethylsilanol, and in further fragmentation, provides a source of *m/e* 420:

$$m/e\ 720 \xrightarrow{-TMSOH} m/e\ 630 \longrightarrow m/e\ 420$$

Rearrangement ions are well known in the mass spectra of TMS derivatives of sugars. One of the most prominent involves the migration of a substituent on C-3 to C-1, found, for example, in the

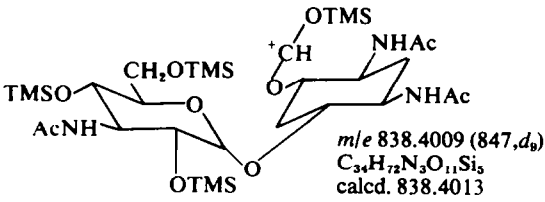


mass spectrum of the TMS derivative of glucose at *m/e* 191:[21]



m/e 191

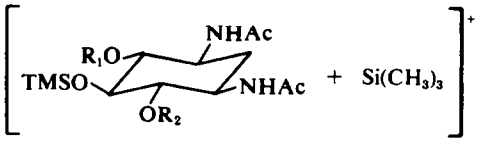
A similar peak is found in the mass spectrum of **2**, at *m/e* 838:



m/e 838.4009 (847,d_9)
$C_{34}H_{72}N_3O_{11}Si_5$
calcd. 838.4013

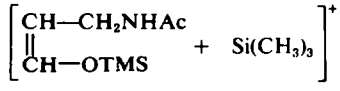
When a —NHAc group is on C-3, however, it does not rearrange as readily as —OTMS. Hence, the corresponding peak involving the transfer of the 3-NHAc group, expected at *m/e* 807, is negligible. Also a peak is found at *m/e* 766 (775)($C_{31}H_{64}O_{11}N_3Si_4$), corresponding to *m/e* 838 except with —CHOH in place of —CH—OTMS.

Another rearrangement ion is found at *m/e* 810 (819) ($C_{33}H_{72}O_{10}N_3Si_5$). This peak corresponds to:



m/e 810 R_1 = H, R_2 = aminohexose or R_1 = aminohexose, R_2 = H

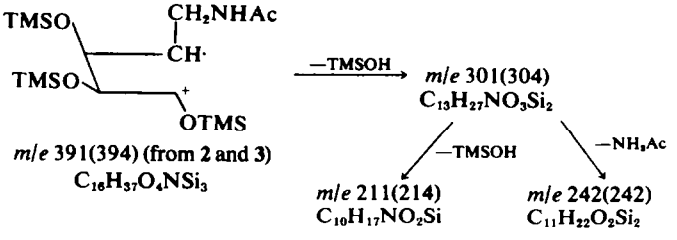
A strong peak is present at *m/e* 260 in the mass spectrum of the TMS derivative of methyl 6-acetamido-6-deoxy-α-D-glucopyranoside (**3**), and it is also found in the mass spectrum of **2**. It shifts to *m/e* 263 and has the formula $C_{11}H_{26}NO_2Si_2$. A possible representation is:



m/e 260(263)

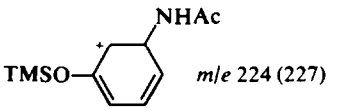
This ion is characteristic of the 6-acetamido group.

Another series of ions is found in the mass spectra of **2** and of the 6-acetamidohexose derivative (**3**):



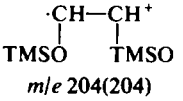
m/e 391(394) (from **2** and **3**)
$C_{16}H_{37}O_4NSi_3$

The peak at *m/e* 224(227) corresponds to the deoxystreptamine unit:

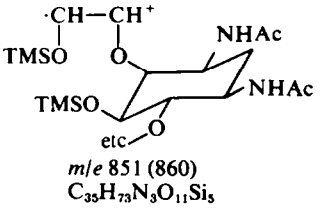


As discussed earlier, a similar ion is found in the mass spectrum of **1** at *m/e* 180.

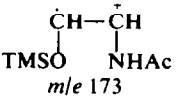
There are also several ions formed which contain 2 or 3 ring carbons of one of the three sugars present in kanamycin. For example the peak at *m/e* 204 is well known in the mass spectra of TMS derivatives of carbohydrates.[21] An analog of low intensity is found in the mass spectrum of **2** at *m/e* 851/(860):



m/e 204(204)

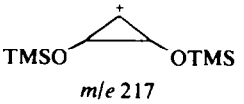


m/e 851 (860)
$C_{35}H_{73}N_3O_{11}Si_5$

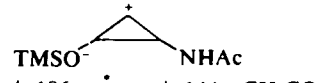


m/e 173

In the spectrum of the TMS derivative of methyl 3-acetamido-3-deoxy-α-D-glucopyronoside, this fragment is found at *m/e* 173.[21] Thus, it is likely that in the mass spectrum of **2**, *m/e* 173 originates from the 3-aminohexose end. The reported formation of *m/e* 131 and 116 from *m/e* 173 by losses of ketene and CH_3.[21] are observed in the mass spectrum of **2** also. Other well-known[21] fragments are *m/e* 217, 186, and 144, found here also.



m/e 217



m/e 186 ⟶ *m/e* 144 + CH_2CO

Similarly, the fragments at *m/e* 147, and 103 are present:[21]

$(CH_3)_3Si—O—\overset{+}{S}i(CH_3)_2$ *m/e* 147 $\quad CH_2=\overset{+}{O}—Si(CH_3)_3$ *m/e* 103

Thus, in spite of the complexity of the spectrum of 2 and the tendency for rearrangement in the mass spectra of TMS derivatives, it is possible to explain the fragmentations of 2, using the N-acetyl-d_3 compound, exact-mass measurements, and mass spectra of model compounds.

DISCUSSION

The technique reported in our initial communication[11] has been used for observing changes in sugar units[12] and for structure determination.[13,14] This potential can also be observed in the CI and EI mass spectra of kanamycin A. In the EI mass spectrum of the underivitized compound, the molecular ion is very small.[15] We have found the same situation to exist in the EI mass spectra of the N-acetyl-N,O-methyl and the N-acetyl-O-trimethylsilyl derivatives (1 and 2).

Table 1. Relative-intensity data from the mass spectrum of the N-acetyl-O-trimethylsilyl derivative (2) of kanamycin A.

m/e	rel. int.	*m/e*	rel. int.	*m/e*	rel. int.	*m/e*	rel. int.
1157	0·6[a]	843	0·5	630	4·4	223	5·5
1156	0·8	842	1·5			218	7·0
1155	0·9	841	3·5	467	5·0[c]	217	36·5
1145	3·4	840	9·0	436	11·5	211	7·0
1144	5·0	839	20·4	422	10·0	210	6·5
1142	7·0	838	34·0	421	29·0	205	6·5
1141	8·5	813	2·0	420	70·0	204	34·0
1085	0·6	812	3·5	419	5·0	200	5·5
1084	0·7	811	5·5	391	5·0	199	7·5
1071	0·8	810	8·5	362	9·5	198	8·0
1070	1·4	809	0·7	361	40·0	195	5·5
1069	1·7	808	1·5	348	5·5	191	5·0
978	0·8			347	5·0	186	10·5
977	1·4	792	2·0[b]	332	17·0	183	5·0
976	1·7	768	2·5	331	10·5	182	5·5
956	0·6	767	4·0	330	38·3	174	9·0
955	1·0	766	7·3	329	5·0	173	32·3
954	1·3	739	2·7	301	8·0	172	6·0
953	0·6	738	3·8	300	5·0		
952	0·7	723	2·0	272	7·5	169	10·0[d]
926	0·5	722	4·0	271	35·7	147	39·1
925	0·7	721	6·5	260	11·9	144	12·0
924	0·9	720	10·0	243	7·5	129	11·0
854	0·5	719	2·1	242	7·5	116	27·2
853	1·0	648	2·0	240	12·5	103	25·5
852	1·5	632	2·0	226	8·0	75	30·0
851	1·6	631	2·2	224	10·0	73	100·0[e]

[a]Only peaks 0·5% or greater are given.
[b]Only peaks 2·0% or greater are given.
[c]Only peaks 5·0% or greater are given.
[d]Only peaks 10·0% or greater are given; *m/e* 131 is 8%.
[e]The spectrum below *m/e* 70 is not given.

Nevertheless, these derivatives can be readily prepared and purified on a small scale, and obtaining their mass spectra presents no problems of volatility. The mass spectra of 1 and 2 can be explained with the aid of deuterated analogs and high resolution data, as well as with published data on model compounds.[3]

We have obtained the chemical ionization mass spectrum of 2 using isobutane as the reaction gas. The (M + H) ion is, in this case, relatively intense, making this technique a good complement to the EI spectrum. The fragmentations which occur are not numerous, but they are useful for recognizing structural features. It is especially the "sequence" ions that are abundant.

EXPERIMENTAL

Electron-impact mass spectra were determined using the direct inlet system of an AEI MS 902 mass spectrometer, at an ionizing potential of 70 eV, an ionizing current of 100 μA, and a resolution of approximately 1000 (10% valley). Some exact-mass data were obtained by peak matching against heptacosafluorotributylamine. For the calculations of elemental compositions all values were within ±0·0010 mass units of the theoretical values. Other exact-mass data were obtained directly from an MS 902-PDP8 combination, using perfluorokerosene as the standard. All experimental values were within ±0·0030 mass units of the theoretical values.

Chemical ionization mass spectra were obtained from an AEI MS 902 mass spectrometer equipped with a SRI Chemspect CIS-2 CI/EI Source. A pressure of 0·2 Torr of isobutane was used. Source temp, 250°; probe temp, 250°; 500 eV; 8 KV.

The free base kanamycin A[22] was selectively N-acetylated with Ac_2O in MeOH.[23] Evaporation of the soln to dryness under reduced pressure gave the N-acetate. Since Hakomori's methylation procedure converts an —OAc group to an —OMe group, the small amount of O-acetylation encountered here is of no consequence in the final derivatives, and the N-acetates were used without further purification.

A modified Hakomori procedure was employed.[24] The permethylated derivatives were prepared by dropwise addition of a soln of the N-acetates in dry, distilled DMSO to a solution of methylsulphinyl carbanion (1-2M).[25] The carbanion was maintained in excess throughout the addition using triphenylmethane as an indicator. After stirring for 1 hr, A 20–100 fold excess of MeI was added. Stirring was continued for 12 hr. All of the above procedures were carried out under dry N_2. The soln was then diluted approximately 20 times with water and extracted several times with chloroform. The chloroform was evaporated and the residue was washed twice with reagent hexanes. TLC of the remaining residue was carried out on glass plates coated with Silica Gel G and the compounds were detected by exposure to iodine vapors. The mass spectra of the materials before and after chromatography were essentially identical.

The deuterated derivatives were prepared by using trideuteroacetic anhydride in the N-acetylation procedure and trideuteromethyl iodide in the methylation procedure.

The procedure used in the preparation of the trimethylsilyl derivatives has been previously described.[11,21]

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