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Note

Mass spectrometric studies on the fragmentation and structural characterization of aminoacyl derivatives of kanamycin A

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Abstract

Described herein are the fragmentation pathways of kanamycin A and its 6′-*N*- and 1-*N*-acyl derivatives, as well as the determination of their positional isomers by FABMS and ESIMS in combination with tandem mass spectrometry. The presence or absence of key ions and the difference in abundance of common ions are correlated with the position of the substitution. © 1998 Elsevier Science Ltd. All rights reserved

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Kanamycins belong to a very important class of clinically used antibiotics referred to as aminoglycosides [1,2]. Their administration is strictly controlled due to a definite tendency to cause nephro- and ototoxic reactions, and chemical modifications have been made in order to produce compounds more active against resistant strains and less toxic than the parent antibiotics [3–8].

Kanamycin A (**1**) is a polyamino and polyhydroxy compound; therefore, positional isomers are formed during synthesis and differentiation among them is required. ^{13}C NMR spectroscopy and electron-impact mass spectrometry (EIMS) have been applied to assignment or structural elucidation of aminoglycosides [9,10]. However, NMR spectra must be determined at various pHs, while EI spectra are characterized by the absence of the molecular ion or its low abundance. Also, the identification of the substituent at C-6 position of hexoses by EIMS is not possible, because the fragment responsible for its determination appears with low abundance [11].

Californium-252 plasma desorption (^{252}CfPD), secondary-ion (SI), ammonia chemical ionization (CI, the moving belt interface) and atmospheric pressure chemical ionization (APCI, with corona discharge) mass spectrometry have been applied to aminoglycosides [12–16]. Moreover, ion-spray or electrospray-ionization mass spectrometry (ESIMS) are proved to be more sensitive than thermospray mass spectrometry (TSMS) [17,18].

In continuation of our studies on aminoglycosides, we have explored the use of fast-atom bombardment or electrospray and tandem mass spectrometric techniques for the mass spectral

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analysis of non-volatile compounds such as *N*-acyl derivatives of kanamycin A. The FAB mass spectra (Tables 1–4) were studied in detail both to illustrate the dissociation pathways that isomers followed and to propose a direct method for the determination of positional isomers at the 6′-*N*- and 1-*N*-positions of the antibiotics. The term 6′-*N*- and 1-*N*- is used to denote the amino group of the sugar moiety or aglycon of aminoglycoside that has been acylated by the carboxyl group of H-L-Ala-OH (**a**), H-Gly-OH (**b**), H-L-Leu-OH (**c**) and H-L-Ala-L-Ala-OH (**d**) and palmitic acid (**e**).

Under conventional FAB conditions in the positive-ion mode, 6′-*N*-derivatives of kanamycin A exhibited a $[M+H]^+$ ion of relative abundance between 8 and 30%, and 1-*N*-derivatives between 10 and 8%. Ions $[M+Na]^+$ in a low abundance (5–10%) have also been observed in some cases. However, the FAB spectra obtained for the compounds studied also contained ions derived from the glycerol matrix, and the use of *m*-nitrobenzyl

Table 1
FABMS data of the 6′-*N*-derivatives of kanamycin A [*m*/*z* values (relative abundance %)]

	$[M+H]^+$	A_1	A'_1	A_2	A_3	A_4	A_5	A_6	A_9	A_{10}	B_1	B_2	B_3	C_1
2a	556(25)	395(100)	324(10)	437(36)	419(20)	205(7)	163(35)	144(8)	377(8)	342(38)	233(57)	215(45)	197(10)	162(5)
2b	542(8)	381(100)	324(30)	423(30)	405(20)	205(8)	163(20)			328(20)	219(30)	201(40)		162(4)
2c	598(20)	437(100)	324(10)	479(15)	461(35)	205(8)	163(10)	144(4)	419(8)	384(35)	275(50)	257(60)	239(15)	162(5)
2d	627(30)	466(100)	324(15)	508(25)	490(20)	205(10)	163(10)	144(4)	448(5)	413(50)	304(10)	286(10)	268(10)	162(35)
2e	723(20)	562(100)	324(15)	604(20)	586(18)	205(5)				509(30)	400(30)	382(70)	364(18)	162(5)

Table 2
FABMS data of the 6′-*N*-Boc-protected derivatives of kanamycin A [*m*/*z* values (relative abundance %)]

	$[M+H]^+$	$[M+H-Boc]^+$	A_1	A_1-Boc	A'_1	A_2	A_2-Boc	A_3-Boc	A_4	A_5	$A_{10}-Boc$	B_1-Boc	B_2-Boc	B_3-Boc
3a	656(12)	556(20)	495(40)	395(100)	324(22)	537(12)	437(20)	419(35)	205(16)	163(22)	342(44)	233(85)	215(78)	197(15)
3b	642(10)	542(15)	481(40)	381(100)	324(15)	523(15)	423(25)	405(25)	205(15)	163(20)	328(40)	219(85)	201(70)	
3c	698(12)	598(15)	537(35)	437(100)	324(15)	579(12)	479(22)	461(23)	205(12)	163(15)	384(42)	275(80)	257(75)	239(10)
3d	727(10)	627(20)	566(38)	466(100)	324(18)	608(12)	508(24)	490(35)	205(15)	163(15)	413(44)	304(75)	286(70)	268(8)

Other fragments: A_6: 144(4), C_1: 162(4).

Table 3
FABMS data of the 1-*N*-derivatives of kanamycin A [*m*/*z* values (relative abundance %)]

	$[M+H]^+$	A_1	A'_1	A_2	A_4	A'_5	A'_6	A_5	A_6	A_7	A_8	A_9	A_{10}	C_1
4a	556(10)	395(100)	324(18)	437(10)	276(10)	233(60)	215(10)	163(40)	144(10)	289(18)	271(5)	377(7)	342(4)	162(10)
4b	542(8)	381(100)	324(15)	423(10)	262(15)	219(50)	201(8)	163(40)	144(10)	289(15)	271(5)			162(8)
4d	627(8)	466(100)	324(15)	508(12)	347(10)	304(55)	286(10)	163(35)	144(8)	289(15)	271(7)	448(5)	413(5)	162(10)

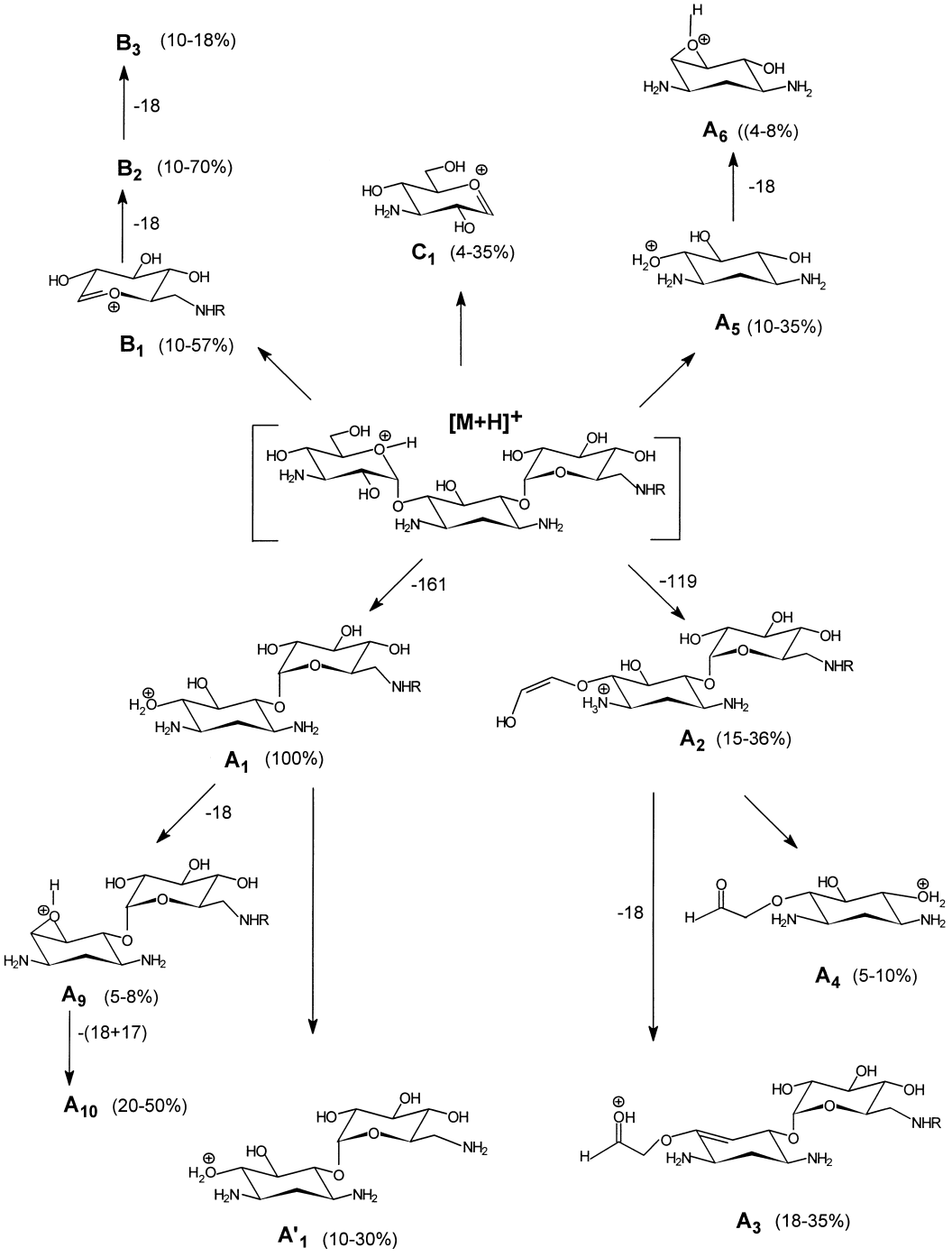
Table 4
FABMS data of the 6′,3-di-Boc-protected derivatives of kanamycin A [*m*/*z* values (relative abundance %)]

	$[M+H]^+$	A_1-Boc	A_1-2Boc	A_1-3Boc	A'_1	A_2-2Boc	A_4-Boc	A_4-2Boc	A_4-3Boc	A'_5-2Boc	A'_5-3Boc	A'_6-3Boc	C_1
5a	856(8)	595(16)	495(100)	395(75)	324(10)	537(40)	476(6)	376(10)	276(50)	333(15)	233(82)	215(20)	162(5)
5b	842(8)	581(15)	481(100)	381(70)	324(10)	523(35)	462(8)	362(10)	262(55)	319(12)	219(80)	201(18)	162(8)
5d	927(8)	666(15)	566(100)	466(75)	324(8)	708(40)	547(8)	447(12)	347(50)	404(15)	304(80)	287(20)	162(5)

Other fragments for **5a**: $[M+H-Boc]^+$: 756(10), $[M+H-2Boc]^+$: 656(35), $[M+H-3Boc]^+$: 556(18), $A_2-Boc-C(CH_3)_3$: 539(40).

alcohol (MNBA) instead of glycerol was unsuccessful. Tandem mass spectrometry (MS/MS) gave abundant fragments by collision of $[M+H]^+$ ions [19]. Applying the ESIMS in the positive-ion mode a much enhanced $[M+H]^+$ ion was obtained (30–45%). ESIMS/MS and FABMS/MS gave the same fragments but in ESIMS additional peaks corresponding to the partial cleavage of the *tert*-butoxycarbonyl-(Boc) group were observed. The typical fragmentation of the latter included loss of 56 daltons, followed by the loss of carbon dioxide.

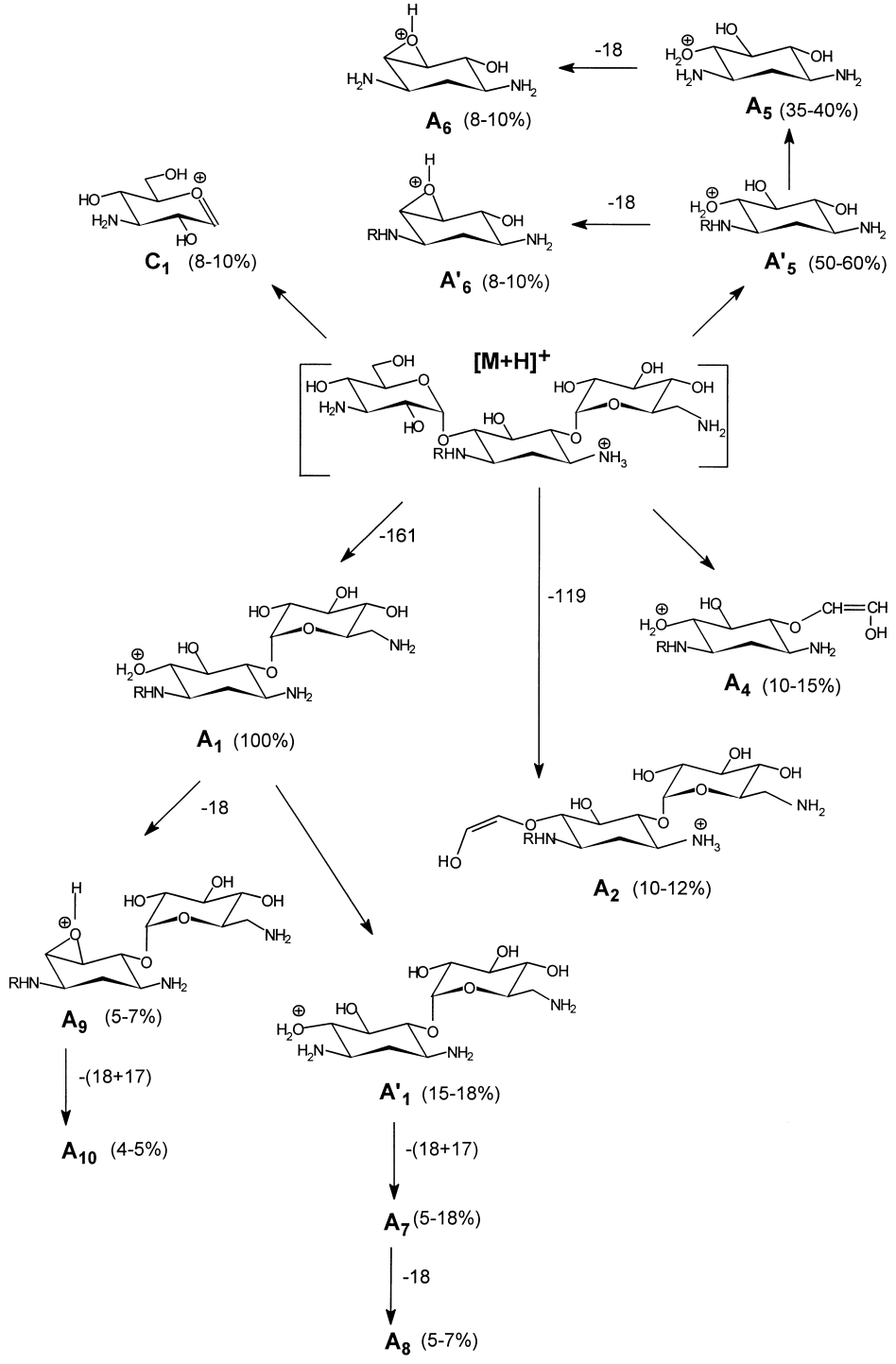
The main fragmentation pathway of the 6′-*N*-derivatives of kanamycin **2a–e** was the glycosidic cleavage at the C-6 position of 2-deoxystreptamine (2-DOS) with the loss of the kanosamine unit, forming ion A_1 (100%) (Scheme 1, Table 1). The structures of fragment ions are speculative. 6′-*N*-



Scheme 1. Fragmentation pathways of 6′-*N*-derivatives of kanamycin A.

derivatives **3a–d**, with a Boc protection at the amino acid moiety, gave A_1-Boc as the most intense peak (Table 2).

In the spectra of kanamycin (**1**) and its 1-*N*-derivatives (**4a,b** and **d**), glycosidic cleavage at the C-4 or C-6 position gave ions with exactly the same masses. Thus, it was not possible to determine the relative amount of fragmentation taking place from each end. All 1-*N*-derivatives gave an ion with m/z, depending on the substituent, which corresponded to elimination of 161 daltons from $[M+H]^+$, as the base peak of their spectra. This peak is also referred as A_1 throughout the paper (Scheme 2). 6′,3-Di-Boc-1-*N*-Boc-derivatives **5a,b**



Scheme 2. Fragmentation pathways of 1-*N*-derivatives of kanamycin A.

and **d** gave ions A_1-2Boc and A_1-3Boc in an abundance of 100% and 70–75%, respectively (Tables 3 and 4).

Ion B_1 (10–57%) produced as a result of a simple glycosidic cleavage at C-4 position of 2-DOS was also present in the spectra of 6′-*N*-derivatives. Loss of one or two molecules of water from B_1 gave ions B_2 and B_3. These ions contained the aminoacyl substituent were sufficiently abundant (10–70% and 10–18%, respectively) to serve as diagnostic peaks (Table 1). In the case of 6′-*N*-Boc-protected compounds **3a–d**, after loss of the Boc protecting group ions B_1-Boc (75–85%), B_2-Boc (70–78%) and B_3-Boc (8–15%) were obtained (Table 2).

An ion, C_1 with $m/z = 162$ and in low abundance (4–10%) for all compounds except **2d** and higher (35%) for **2d**, corresponding to the kanosamine unit was observed in the specta of all derivatives.

An important ion, also present in all derivatives, but with different relative abundance, was A_2 obtained from $[M+H]^+$ by elimination of 119 daltons. Ion A_2 was prominent in 6′-*N*-derivatives with an abundance of 15–36% compared with 10–12% in 1-*N*-derivatives. However, only in 6′-*N*-derivatives, after elimination of water from ion A_2, ion A_3 (18–35%) was obtained and could be used as a key ion for the discrimination between 6′-*N*- and 1-*N*-derivatives.

In 6′-*N*-derivatives, ion A_2 after a glycosidic cleavage at C-4 position of 2-DOS with hydrogen transfer gave the protonated ion A_4 (m/z 205, 5–10%). In 1-*N*-derivatives an ion at m/z 205 was also present, but it was not easy to distinguish which one of the sugar units at C-4 and C-2 position of 2-DOS is released.

However, the absence of B_1 and the presence of C_1 at low abundance may suggest the preference for release of the sugar at C-6 position.

A key ion for the isomers indicating the presence of 2-DOS was ion A_5 (m/z 163). In 1-*N*-derivatives, apart from the ion A_5 which was present at an abundance of 35–40%, an ion denoted A'_5 with m/z depending on the substituent at C-1, was observed in an abundance of 50–60%. Ion A_5 could be obtained from A'_5 by elimination of the aminoacyl substituent (Scheme 2). The ions A'_5-2Boc and A'_5-3Boc in an abundance of 12–15% and 80–82%, respectively, were present in the Boc-protected compounds **5a,b**, and **d**. Elimination of water from A'_5 or A'_5-2Boc could lead to ions A'_6 and A'_6-3Boc, respectively.

Elimination of water from ion A_5 was observed in FABMS for all isomers, leading to ion A_6 with a low abundance (8–10%) despite this peak being one of the base peaks in EI [10].

Another ion present in 1-*N*- and 6′-*N*-derivatives with an abundance of 15–18% or 10–30%, respectively, was ion A'_1 obtained from A_1 after elimination of the amino substituent. However, 1-*N*-derivatives, after the loss of a molecule of water and a molecule of ammonia, gave ion A_7 (15–18%), and with a further loss of water ion A_8 (5–7%).

In all derivatives elimination of a molecule of water from ion A_1 gave ions A_9 (5–8%), and with a further loss of a molecule of water and a molecule of ammonia gave A_{10}. The intensity of A_{10} in 6′-*N*-derivatives (20–50%) in comparison with 1-*N*-derivatives (4–5%) suggested that this is a key ion for the discrimination of the isomers.

Identification of kanamycin and its derivatives could be also readily achieved by examining ions containing the aminoacyl substituent, for example, A_1, A_2, A_4, and A'_5 for 1-*N*-derivatives and A_1, A_2, A_3, B_1, B_2, and A_{10} for 6′-*N*-derivatives.

For the derivatives of kanamycin, the position of the substitution determines their fragmentation pattern. Even although ion A_1 (or A_1-Boc and A_1-2Boc for the Boc-protected compounds) was the predominant peak for the isomers 6′-*N*- and 1-N- of kanamycin A, a subsequent fragmentation permitted their discrimination. Ion A_3 was present only in 6′-*N*-derivatives, while A_7 and A_8 in 1-*N*-derivatives. The higher abundance of ions A_2 and A_{10} were related to the 6′-*N*-substitution. Thus, there was an excellent correlation between the presence or absence of fragment ions and their relative intensity with the position of the substitution. FABMS/MS spectra of selected compounds are given in Figs. 1 and 2.

The difference in abundances of ions A_1 or A_1-Boc and A'_1 reflected the greater stability of the amide compared to the glycosidic bond. Thus, ions A_1 or A_1-Boc were the base peaks in all cases, while the abundance of A'_1 was between 8 and 30%.

The presence of B_1, B_2 and B_3 in 6′-*N*-derivatives suggested that the substituent at 6′-*N*-position prevented the elimination of a part of the substituted sugar.

Ions A_9 and A_{10} containing the aminoacyl substituent were present in all isomers formed directly from A_1, whereas A_7 and A_8 were present only in

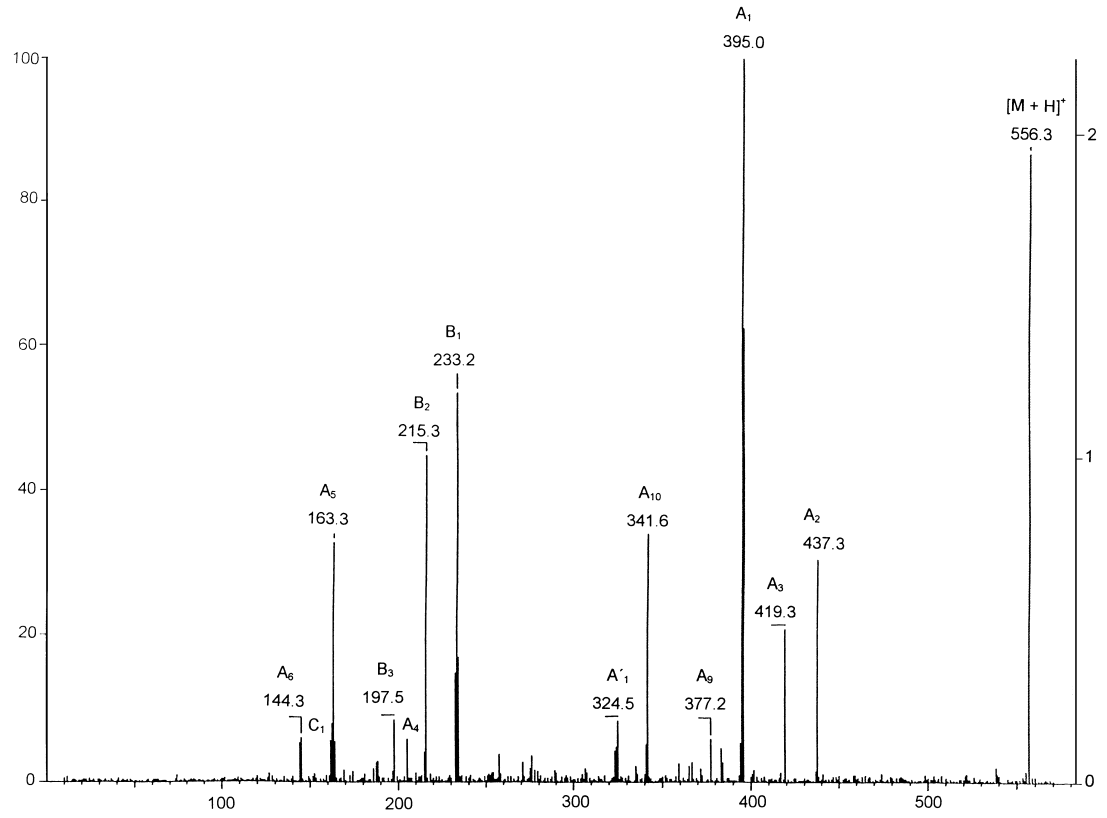


Fig. 1. FABMS/MS spectra obtained from $[M+H]^+$ of 6′-*N*-alanylkanamycin A (**2a**).

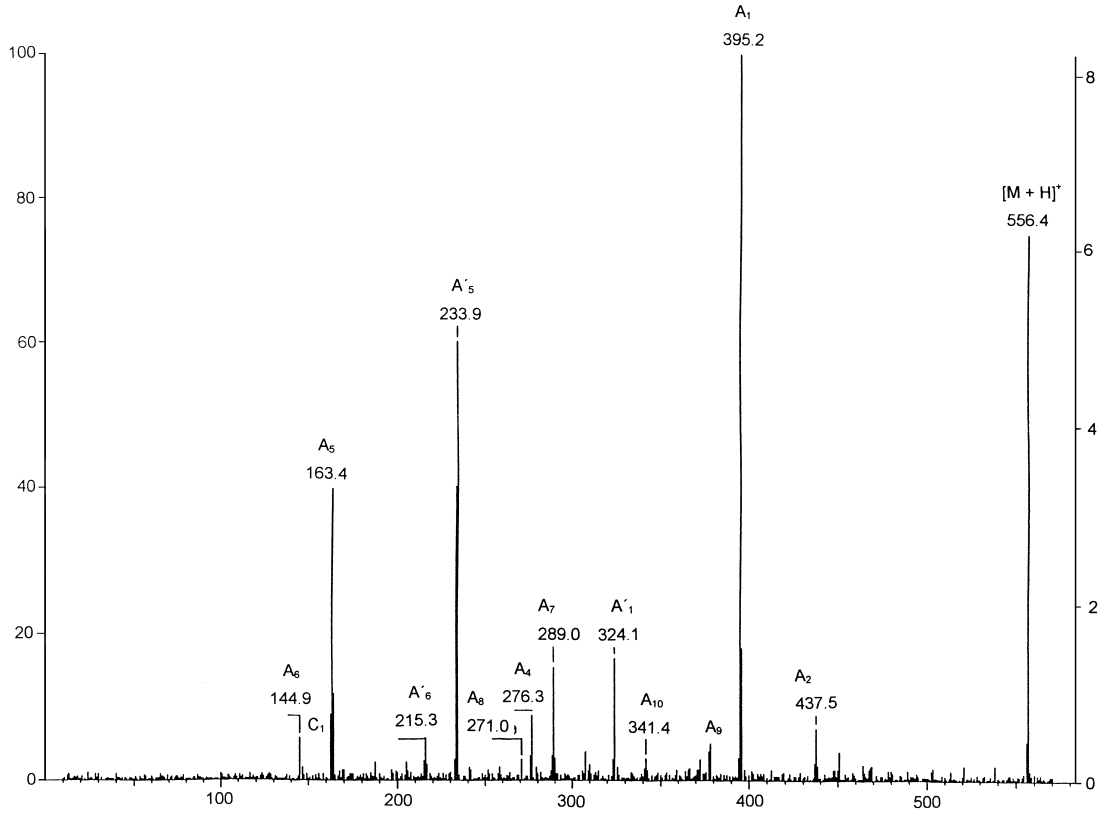


Fig. 2. FABMS/MS spectra obtained from $[M+H]^+$ of 1-*N*-alanylkanamycin A (**4a**).

the 1-*N*-derivatives formed from A'_1 with the same pattern after elimination of the substituent. The latter suggested the lower stability of the amide bond in the 1-*N*-position.

Thus, it was clearly demonstrated that FABMS and ESIMS in combination with tandem mass spectrometry were effective ionization methods for analysis of these non-volatile and thermally labile compounds.

1. Experimental

Kanamycin A was kindly provided by HELP Pharmaceutical Co, Athens, and its derivatives investigated in this study were synthesized as described in ref. [8]. FAB mass spectra were recorded on a TSQ 700 triple quadrupole spectrometer from a Finnigan MAT. An Ion-Tech FAB gun gave a beam of Xenon at 8 keV. The solution of samples in glycerol was injected at a flow rate of 3 μL/min and was bombarded with high-energy atoms, resulting in positive ions produced by desorption from the solution. In tandem mass spectrometry, a second analyzer was provided, separated from the first one by a collision cell. Thus, the ions corresponding to the molecular ions produced in the source were selected by the first analyzer, fragmented by collision with an inert gas (xenon) under a delivery pressure of 0.7 mtorr, and the fragments were analyzed by the second m/z analyzer. This allowed us to obtain additional structural information on these fragment ions.

ES spectra were taken with a TSQ 7000 from Finnigan MAT. The capillary temperature was 250 °C using a spray voltage of 5.5 kV. The pressure of the sheath and auxiliary gas was 50 and 10 psi, respectively. The samples were dissolved in a solution of 1:2 CH_3OH–DMSO and injected in the mass spectrometer through a Harvard pump syringe system at a flow rate of 3 μL/min.

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