SPECIAL TECHNIQUES OF FAST ATOM BOMBARDMENT MASS SPECTROMETRY FOR THE STUDY OF OLIGOSACCHARIDE CONTAINING MACROTETRONOLIDE ANTIBIOTIC, KIJANIMICIN

Sir:

Oligosaccharide containing antibiotics usually have complex structures having high molecular weights. At the early stages of structural studies, very small quantities of material are usually available. We describe here certain techniques which provide information not only about molecular weight, but special structural features as well.

Four classes of nitrosugar containing antibiotics are already known and in probability other such antibiotics will be discovered in the future. In particular the nitrosugars are difficult to recognize using microgram quantities of materials with conventional techniques. We have discovered that under fast atom bombardment mass spectrometry (FABMS) condition nitrosugars are characterized by unique fragmentation pathways that produce strong fragment ions, not only as $(M+Na-NO_2)^+$ but also as $(M+Na-16)^+$. We illustrate the usefulness of our techniques by discussing the structure of kijanimicin.

The structure of kijanimicin (**1**), a novel tetronic acid containing antibiotic having five sugars, one of which is a rare nitrosugar, has been established[1,2,3]. Attempts to determine the molecular weight of kijanimicin (**1**) by EI, CI and FDMS were unsuccessful. The ^{252}Cf-PDMS of 26-*O*-methylkijanimicin (**2**)[1,3] afforded a molecular ion at *m/z* 1,353 $(M+Na)^+$ and a negative ion at *m/z* 1,329 $(M-H)^-$, however only limited structural information was obtained[1], including the presence of a negative ion corresponding to (M−16). We report here success in obtaining both molecular ions and useful structural information by the application of FAB techniques[4,5].*

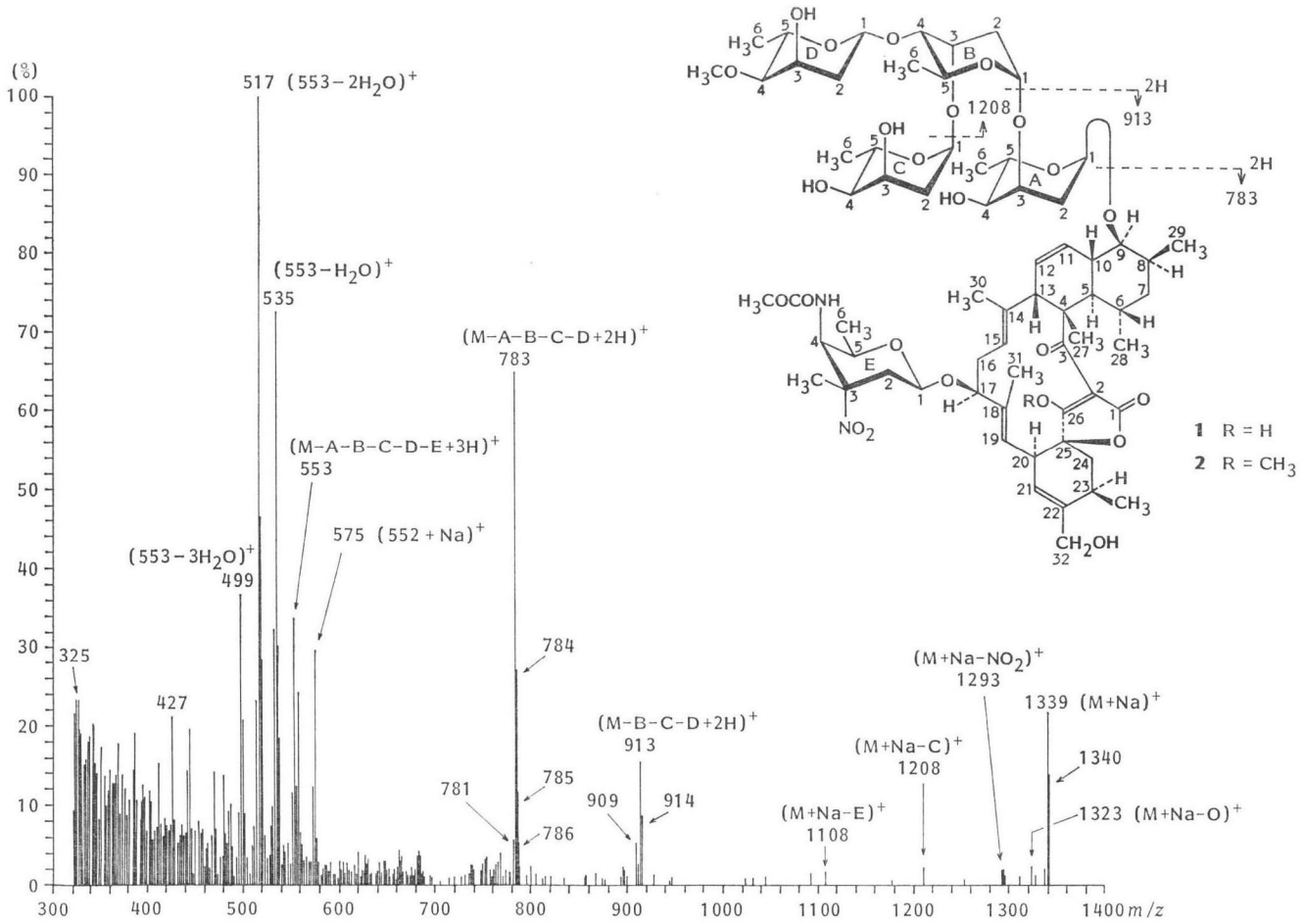
The FAB spectrum of the methyl ether (**2**) showed an intense ion at *m/z* 1,353 $(M+Na)^+$, while kijanimicin itself displayed a strong ion at *m/z* 1,339 $(M+Na)^+$. In both cases, ions corresponding to $(M+Na-16)^+$, $(M+Na-NO_2)^+$ and $(M+Na-HNO_2)^+$ were observed. The spectrum of both compounds exhibited a number of high molecular weight fragment ions which afforded useful structural information and these are indicated for **1** in Fig. 1. Noteworthy are the fragments corresponding to $(M+Na-C)^+$, $(M+Na-E)^+$, $(M-B-C-D+2H)^+$, $(M-A-B-C-D+2H)^+$, $(M-A-B-C-D-E+3H)^+$, $(552+Na)^+$, $(553-H_2O)^+$, $(553-2H_2O)^+$ and $(553-3H_2O)^+$ which were in full accord with the structure. In each case the glycosidic unit was lost through cleavage of the glycosidic bond and useful sugar sequencing information was available from such spectral data[6,7].

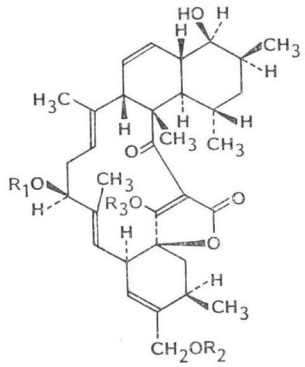
The FAB spectrum of 3$^\beta$-*O*-dedigitoxosyl-kijanimicin gave an ion at *m/z* 1,209 $(M+Na)^+$ and contained fragment ions at *m/z* 913 $(M-B-D+2H)^+$ and *m/z* 783 $(M-A-B-D+2H)^+$ arising from cleavage of the O−C_1B and O−C_1A glycosidic bonds respectively. Fragment ions were also visible at *m/z* 553 $(M-A-B-C-D-E+3H)^+$, 535 $(553-H_2O)^+$ and 517 $(553-2H_2O)^+$. The FAB spectrum of 3$^\beta$-*O*-dedigitoxosyl-26-*O*-methylkijanimicin gave an ion at *m/z* 1,223 $(M+Na)^+$ and showed similar fragment ions to those described above.

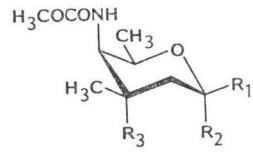
The FABMS of 17-*O*-β-D-kijanosylkijanolide (**3**), which lacks the tetrasaccharide unit, showed an intense ion at *m/z* 783 $(M+H)^+$, with fragment ions at *m/z* 767 $(M+H-16)^+$, 737 $(M+H-NO_2)^+$, 553 $(M-E+2H)^+$, 535 $(553-H_2O)^+$, 517 $(553-2H_2O)^+$ and 499 $(553-3H_2O)^+$. The composition of the ion at *m/z* 767 was established under high resolution FABMS conditions and it was found to correspond to the loss of an oxygen atom from the ion at *m/z* 783. The corresponding methyl ether (**4**) exhibited similar fragment ions, all at 14 mass units higher than those of **3**. The FABMS of 26,32-di-*O*-methylkijanolide (**5**) showed prominent ions at *m/z* $(M+H)^+$, 563 $(M+H-H_2O)^+$ and 545 $(M+H-2H_2O)^+$, but no ion corresponding to a loss of 16 amu. This suggested that the ion was arising by the loss of an

* Samples were dissolved in MeOH (2~5 μg/μl) and deposited on a copper probe tip. A thin layer of glycerol/thioglycerol was applied to the probe tip containing the samples and mixed thoroughly with a pasteur pipet before insertion into the source. The primary atom being comprised of xenon and was produced using a saddle field ion source (Ion Tech LTD, BIINF) operating with a tube current of 2 mA at an energy of 6 KeV. MS were obtained on a Finnigan MAT 312 instrument operating at an accelerating voltage of 3 kV.

Fig. 1. The FABMS of kijanimicin (**1**) (MW 1,316) in thioglycerol.







3 $R_1=\beta$-D-Kijanosyl, $R_2=R_3=H$
4 $R_1=\beta$-D-Kijanosyl, $R_2=H$, $R_3=CH_3$
5 $R_1=H$, $R_2=R_3=CH_3$
6 $R_1=H$, $R_2=OCH_3$, $R_3=NO_2$
7 $R_1=OCH_3$, $R_2=H$, $R_3=NH_2$

oxygen atom from the nitro group of the kijanose unit.

This was confirmed by comparing the FABMS of methyl-D-kijanoside (**6**) with that of the amino sugar (**7**). The nitro sugar (**6**) showed an intense ion at *m/z* 263 $(M+H)^+$ with a fragment ion at *m/z* 247 $(M+H-16)^+$, while the amino sugar (**7**) gave rise to a mere trace of a fragment ion corresponding to a loss of 16 amu from the molecular ion. We therefore conclude that under FAB conditions nitro sugars exhibit a characteristic loss of one oxygen atom from the nitro group. It appears that the loss of 16 amu may be used as a diagnostic test for the presence of a nitro group in a compound available in microgram quantities, as several nitro compounds other than nitro sugars showed $(M-16)^+$ ions in their FABMS (PRAMANIK, B. N.; unpublished observations). In each of these compounds the nitro group itself was also lost.

Recently we have reported[8,9] the utility of ammonium chloride for enhancing EI and CIMS of multifunctional, non-volatile compounds. In-beam MS of some of these compounds using polyethyleneglycol have also been reported[10]. We therefore also studied the FABMS of kijanimicin (**1**) mixed with ammonium chloride[11]*. This spectrum showed an $(M+Na)^+$ ion and a much more intense $(M+NH_4)^+$ ion. Interestingly the fragment ion at *m/z* 913 now became more intense than the molecular ion.

We have also developed a simple technique that can provide additional information from FABMS of compounds expected to contain exchangeable hydrogens. By using deuterated glycerol as a matrix for recording FABMS, we observed that the spectrum of **3** exhibited a series of new fragment ions. Thus the ion at *m/z* 783 became 787, 767 became 771, 737 became 741, 553 became 557, 535 became 538, 517 became 519 and 499 became 500. This clearly indicated the maximum number of exchangeable hydrogens in both the molecular and fragment ions.**

We have also found that substitution of thioglycerol for glycerol as the matrix, resulted in a substantial increase in the intensity of the molecular ions for kijanimicin (**1**) as well as its degradation products. We have also tested diethanolamine matrix[12] and observed that our samples gave very intense adduct ions $[M+DEAH]^+$. Thus FABMS, using special techniques such as those described above, can afford molecular weight data as well as provide much useful structural information in highly complex, non-volatile molecules. The application of these techniques to the solution of other structural problems will be reported elsewhere.

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* A saturated solution of ammonium chloride (2~3 μl) was added to 10 μl of sample solution (conc 2~5 μg/μl of MeOH); a few microliters of this mixture were deposited on a copper probe and the procedure described in the footnote * on the previous page, was followed for recording the FABMS.

** Application of this useful technique to the structure determination of other antibiotics such as oligopeptides and orthosomycins (everninomicins) will be reported elsewhere.

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