Structural Characterization of the Oligosaccharide Antibiotics Everninomicins by Negative Ion FAB and ESI/MS

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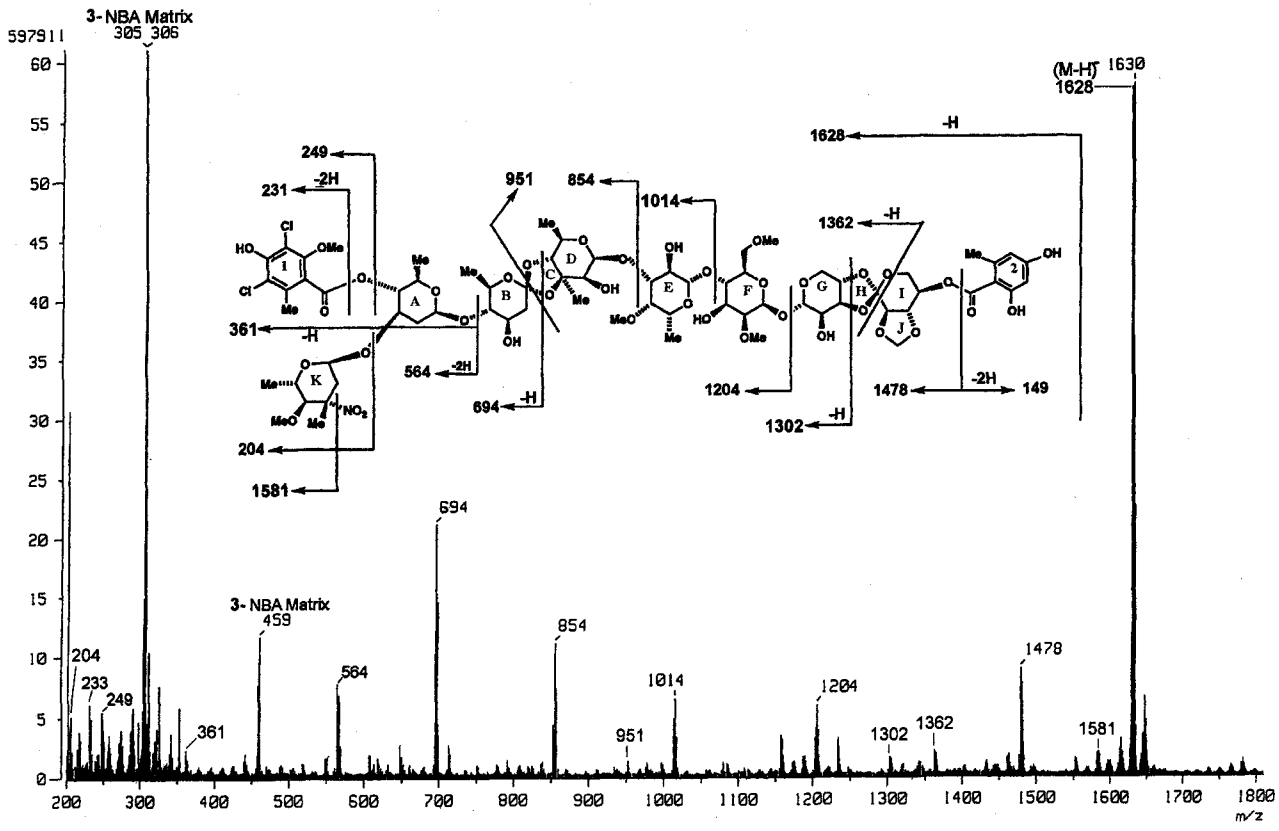
Everninomicins are an important group of oligosaccharide antibiotics, isolated from the fermentation broth, *Micromonospora carbonaceae*, and are found to be highly active against Gram-positive bacteria including methicillin resistant *Staphylococci* and vancomycin resistant *Enterococci*.[1] With the emergence of drug resistant strains of bacteria, this class of antibiotics is gaining an increasing importance. Currently, the everninomicin component ziracin (13-384-1, SCH 27899, Schering-Plough Corporation) is the most promising drug candidate from the everninomicin family and is undergoing extensive trials to determine its clinical efficacy.

Everninomicins are non-volatile compounds that yield no molecular ion information by EI, CI and DCI mass spectrometry. In earlier structural elucidation studies[2~4] with various members of the everninomicin family (B, C, D, 6, ziracin and 13-384-5), we have shown that positive ion FAB/MS yields strong pseudo-molecular ions plus useful sugar sequencing information. In this paper we will show the usefulness of negative ion FAB and ESI for analysis of everninomicins or any other orthosomycin antibiotics, and compare the advantages and disadvantages of each method. In addition, negative ion FAB exact mass measurements for the parent ion and all major fragment ions of ziracin are reported to support structure

Fig. 1. Negative ion FAB/MS fragmentation pattern of ziracin.



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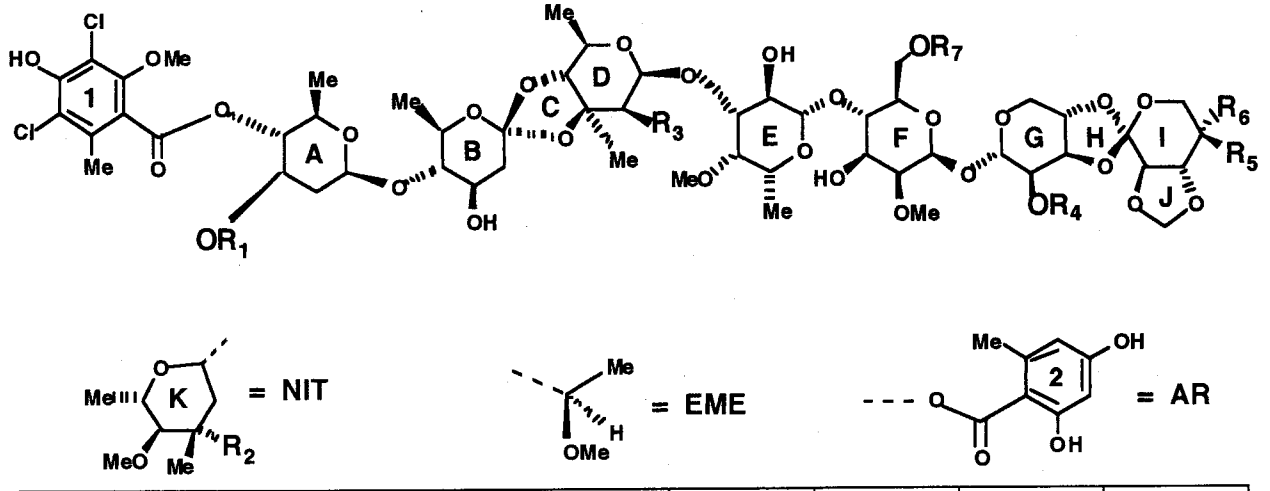
assignments.

Negative ion FAB mass spectra were obtained on a JEOL JMS-HX110A mass spectrometer equipped with a FAB gun using xenon gas. The resolution was set at 3,000 for full range scan (100~2000 amu) with a source accelerating voltage of −10 kV, the FAB gun accelerating voltage was set at 3 kV with an emission current of 10 mA. DMSO was used as a solvent and 3-NBA as a matrix for all FAB experiments. Exact mass measurements were performed at a resolution of 10,000 on a JEOL MStation JMS-700; source accelerating voltage was set at −10 kV, the FAB energy at 6 kV with an emission current of 10 mA. Ultramark 1621, PEG 1000 and PEG 600 were used as internal standards. ESI spectra were acquired on a Micromass Quattro LC where 0.5 mg/ml ziracin solution (75 : 25 acetonitrile : 0.1% acetic acid for positive ion and 75 : 25 methanol : 2 mM ammonium acetate for negative ion analysis) was infused at a flow rate of 10 μl/minute from a Harvard Apparatus syringe pump. The cone voltage was 100 V.

We have previously reported in detail the structural identification of ziracin by positive ion FAB/MS.[3] Negative ion FAB/MS was investigated as an alternative method of analysis because it provides certain advantages such as less matrix and background interference, and no alkali metal adducts. The negative ion FAB mass spectrum of ziracin was found to display a simple, abundant and fully informative fragmentation scheme (Figure 1), very different from its positive ion analog. Although the positive ion spectrum is rich in information, interpretation of the data is time consuming. In contrast, the negative ion spectrum shows a predominant step-wise fragmentation starting at the ester bond of ring I, followed by successive ether linkage cleavages along the sugar back bone to nitro-sugar K. In general, each sugar is lost as a neutral fragment while the charge is retained on the rest of the molecule. This results in a simple spectrum from which the complete sequence of sugar residues can be easily determined.

Table 1. Structures of the seven different everninomicin compounds analyzed by negative ion FAB/MS.

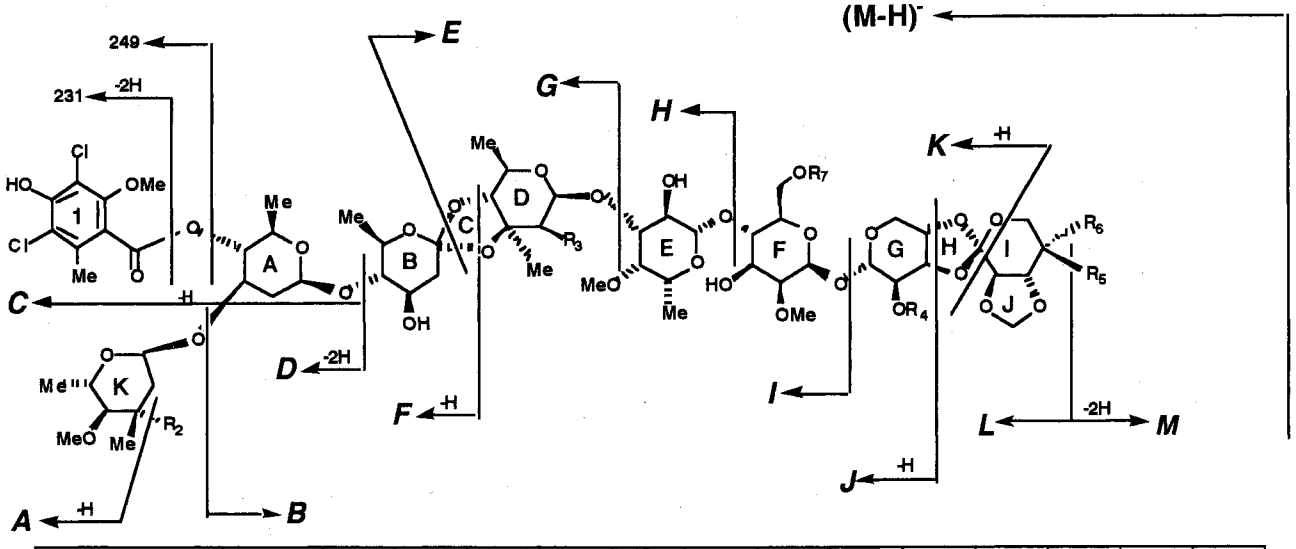


Components	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Ziracin	NIT	NO_2	OH	H	AR	H	Me
13384-5	NIT	NH_2	OH	H	AR	H	Me
Des evernitrose-ziracin	H	-	OH	H	AR	H	Me
EV-D	NIT	NO_2	H	Me	EME	OH	Me
EV-B	NIT	NO_2	OH	Me	EME	OH	Me
NHAc EV-D	NIT	NHAc	H	Me	EME	OH	Me
EV-6	H	-	H	Me	EME	OH	H

In the negative ion FAB spectrum of ziracin (Figure 1), the pseudo-molecular ion $(M-H)^-$ is observed at *m/z* 1628. The complete cleavage sequence for the sugar backbone is provided by the series of ions, *m/z* 1478, 1362, 1302, 1204, 1014, 854, 694, 564 and 249. Sugar rings A, B and K and terminal aromatic group **1** are defined by the fragment ions at *m/z* 361, 231 and 204, while terminal aromatic group **2** is defined by the ion at *m/z* 149. High resolution mass measurements were obtained for the $(M-H)^-$ ion and for all major ziracin fragment ions (*m/z* 1478, 1204, 1014, 854, 694, 564) to confirm the proposed fragment structures.

The structures of the seven different everninomicin compounds, chosen to illustrate the application of negative ion mass analysis, are shown in Table 1. Compounds EV-B,[5] EV-D,[6] 13384-5,[3] ziracin,[3] NHAc EV-D[2] and EV-6[4] have all been previously identified and reported in the literature. Des evernitrose-ziracin has not been previously reported and its structure was identified based on high resolution mass measurements, comparison of the observed fragmentation patterns with those of previously identified everninomicins and NMR data (unpublished results). Table 2 describes the detailed negative ion fragmentation schemes for the seven everninomicins. All compounds follow the same basic fragmentation pathways, however it should be noted that all everninomicins generate fragments A, D, F, G, H, I and J, while fragments B, C, E, K, L and M are specific to each compound. The data in Table 2 suggests that there are two major fragmentation pathways involved. The dominant pathway involves the sequential cleavage of ester and ether linkages along the sugar backbone, accounting for fragments L, I, H, G, and the ion at *m/z* 249, common for all everninomicins. The second pathway involves cleavage with lass of hydrogen to produce fragments K, J, F, D, and the ion at *m/z* 231 (Tables 1, 2). Interestingly, the three everninomicin structures containing terminal aromatic group **2** (SCH 27899, 13384-5 and Des evernitrose-ziracin) all show a peak at *m/z* 951 representing the complete ring sequence D→I in addition to the terminal

Table 2. Negative ion FAB/MS fragmentation patterns for the seven everninomicin compounds.



Components	(M-H)⁻	A	B	C	D	E	F	G	H	I	J	K	L	M
Ziracin	1628	1581	-	361	564	951	694	854	1014	1204	1302	1362	1478	149
13384-5	1598	1581	-	361	534	951	664	824	984	1174	1272	1332	1448	149
Des evernitrose-ziracin	1441	-	-	-	377	951	507	667	827	1017	1115	1175	1291	149
EV-D	1534	1487	1347	361	564	-	694	838	998	1188	1300	-	-	-
EV-B	1550	1503	1363	361	564	-	694	854	1014	1204	1316	-	-	-
NHAc EV-D	1546	1487	1347	-	576	-	706	850	1010	1200	1312	-	-	-
EV-6	1333	-	-	-	377	-	507	651	811	987	1099	-	-	-

group itself. The compounds without terminal aromatic group **2** do not show a corresponding ion, suggesting that the aromatic moiety is most likely the charge carrier.

The application of positive and negative ion electrospray ionization (ESI) were also investigated for fast and convenient analysis of everninomicins. Similar to FAB, positive ion ESI produced significant fragmentation, however, most of the fragment ions appeared as alkali metal adducts which further complicated the interpretation. In addition, many are generated *via* neutral loss of 150 amu (terminal aromatic group **2**) from other fragment ions, making the spectrum more complex while providing no additional structural information. Therefore, positive ion electrospray analysis provides limited information and it may prove to be insufficient for the rapid analysis of unknown species.

Of the ionization modes investigated, negative ion electrospray was found to be the most sensitive technique for the analysis of ziracin. Compared to positive ion electrospray, negative ion ESI exhibits a simpler, more informative fragmentation spectrum. The negative ion spectrum does not have the complication of alkali metal adduct formation which is observed in the positive ion mode and shows a fragmentation pattern similar to negative ion FAB. However, the negative ion ESI spectrum contains additional fragment ions, requiring further interpretation relative to that in FAB/MS.

Comparing ESI and FAB, the negative ion FAB spectrum shows a step-wise fragmentation occurring exclusively at sugar ether and *ortho* ester linkages that presents a less complicated spectrum for determining the sequence of sugar groups. Therefore, negative ion FAB proved to be the most effective technique for rapid identification of new everninomicins. Although negative ion ESI fragmentation is not as straight forward and easy to interpret as the FAB fragmentation, it does provide advantages of greater sensitivity and, more importantly, compatibility with on-line separation techniques such as HPLC. Negative ion ESI is, therefore, the method of choice for everninomicin characterization when separation is required.

Acknowledgement

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