Tetrahedron Vol. 49, No. 41, pp. 9221-9234, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Macrolide Antibiotic Structure Determination by Fast Atom Bombardment/ Tandem Mass Spectrometry#

Y. Shida,[1,2] L.J. Deterding,[1] K. O'Hara,[3] M. Kono,[3] and K.B. Tomer[1*]

[1]. Laboratory of Molecular Biophysics
National Institute of Environmental Health Sciences
Research Triangle Park, NC 27709, USA

[2]. Department of Chemical Analysis and [3]. Department of Microbiology
Tokyo College of Pharmacy
Tokyo 192-03, Japan

(*Received in USA* 17 *February* 1993; *accepted* 5 *May* 1993)

ABSTRACT - The combined techniques of fast atom bombardment and tandem mass spectrometry have been applied to the structural determination of macrolide antibiotics. Major fragmentation processes in the protonated molecule involve cleavage at the glycosidic linkages while more extensive ring fragmentation is also noted in the spectra of the sodiated molecules.

INTRODUCTION

Macrolides are a class of antibiotics produced by *Streptomyces* soil bacteria. Macrolides are widely-used broad-spectrum antimicrobial agents which are useful against gram-positive bacteria (1-4). While erythromycin is, perhaps, the most commonly-prescribed macrolide in the United States, other members of this class have been developed and are commonly used in other countries (5-9).

Research in our (Tokyo) laboratory has involved the mechanisms of bacterial macrolide resistance. This work, and that of other researchers, has indicated several mechanisms involving macrolide esterases and rRNA methylases (7,10-14) and 2'-phosphotransferase (15-19). During the course of these studies, however, the need has become apparent for a physico-chemical method capable of determining both macrolide and macrolide degradation product structures to form a basis for the determination of enzymatically-induced structural changes in the macrolide.

One of the most widely-applied methods of structural analysis has been mass spectrometry. A variety of ionization techniques have been applied to the analysis of members of this class including thermospray (20,21), fast atom bombardment (16,17,22-25), electrospray ionization (26), chemical ionization (27-29), and field desorption (13,25). As these compounds are very polar and thermally labile,

#Dedicated to Carl Djerassi on the occasion of his seventieth birthday

the 'soft' ionization techniques have gained the widest acceptance for their analysis. The lability of these compounds is reflected in their mass spectral behavior. The dominant fragmentation process observed is cleavage of the glycosidic bonds (15,27,29). This fragmentation process is characteristic of compounds in general containing glycosidic linkages (30,31).

Structural elucidation based on the fragment ions observed in mass spectra requires relatively pure samples. In addition, the spectra obtained by some of the desorption ionization techniques, such as FAB, can contain a high level of background ions and, in some cases, few fragment ions. This can hinder observation and/or identification of structurally-significant fragment ions. The most prevalent technique in current use to increase fragmentation and to identify parent ion-fragment ion relationships in complex mixtures is tandem mass spectrometry (30-36). In this technique, the parent ion of interest is isolated in the first mass spectrometer of the tandem system. This mass-selected ion is then induced to undergo fragmentation, typically by colliding the ion with an inert gas, such as helium. This converts some of the ion's translational energy into internal energy which is then available to induce bond cleavage. The fragment ions thus produced are analyzed by the second mass spectrometer of the tandem system. The tandem MS spectra of the macrolide antibiotics, rosaramicin, a monoaminoglycoside, and erythromycin, a doubly glycosylated macrolide, have been reported (37,38). As in the normal mass spectra, the predominant fragment ions observed arose via cleavage of the glycosidic bond.

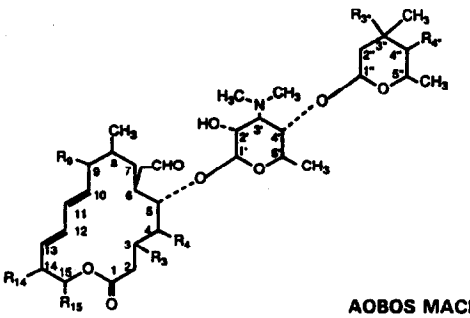
We have investigated the fragmentation reactions of macrolide antibiotics using skimmer-induced CID under electrospray ionization, and have observed structurally useful information (39). This information, however, was limited to information on the intact rings. To determine whether more detailed structural information could be obtained through a more rigorous and systematic MS/MS study, we have investigated the application of tandem mass spectrometry in conjunction with fast atom bombardment for the structure determination of macrolide antibiotics. In addition, because macrolide isolates may often be contaminated with sodium, we have also investigated the MS/MS behavior of a number of monosodiated macrolide ions. We report our results here.

EXPERIMENTAL

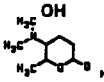
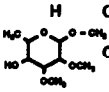
Instrumentation

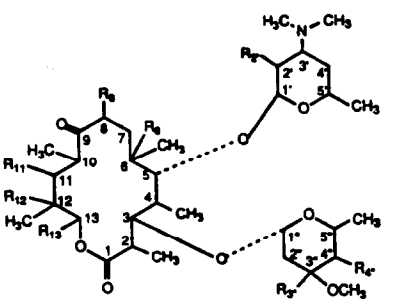
Mass Spectrometry. All spectra were obtained on a VG ZAB-4F of B_1E_1-B_2E_2 geometry (VG Analytical, Altrincham, UK). Full scan spectra were obtained using FAB ionization and a static FAB probe, a glycerol matrix and xenon gas. The instrument was typically operated at a resolution of 1500-2000. MS/MS spectra were acquired by selecting the appropriate parent ion with MS-I, B_1E_1, and colliding the selected ion at 8 keV translational energy in the 3rd field-free region with sufficient He to reduce the parent ion intensity by ca. 50%. The resulting product ions were determined (at a resolution of 1500-2000) by a B_2/E_2 linked scan of MS-II, B_2E_2. A static FAB probe with glycerol matrix was used for the determination of the MS/MS spectra of protonated molecules. For the determination of the MS/MS spectra of the sodiated molecules, the analyte and NaI were introduced in water through a coaxial continuous flow FAB probe that has been described previously (40). Data were acquired with a VG 11-250J data system. In some cases, additional data work-up was performed on a Sun 3/60 workstation using MACH 3 software (Kratos

Fig. 1. Structures of the macrolides studied.



AOBOS MACROLIDES

Compound	R_3	R_4	R_6	R_{14}	R_{15}	$R_{3'}$	$R_{4'}$
Midecamycin (MDM)	$OCOCH_2CH_3$	OCH_3	OH	H	CH_3	OH	$OCOCH_2CH_3$
Miokamycin (MOM)	$OCOCH_2CH_3$	OCH_3	$OCOCH_3$	H	CH_3	$OCOCH_3$	$OCOCH_2CH_3$
Leucomycin-A5 (LM-A5)	OH	OCH_3	OH	H	CH_3	OH	$OCOCH_2CH_2CH_3$
Rokitamycin (RKM)	OH	OCH_3	OH	H	CH_3	$OCOCH_2CH_3$	$OCOCH_2CH_2CH_3$
Josamycin (JM)	$OCOCH_3$	OCH_3	OH	H	CH_3	OH	$OCOCH_2CH(CH_3)_2$
Spiramycin (SPM)	OH	OCH_3		H	CH_3	OH	OH
Tylosin (TYL)	OH	CH_3	=O		CH_2CH_3	OH	OH



SOAOB MACROLIDES

Compound	R_6	R_8	R_{11}	R_{12}	R_{13}	$R_{3'}$	$R_{3''}$	$R_{4''}$
Erythromycin A (EMA)	OH	CH_3	OH	OH	CH_2CH_3	OH	CH_3	OH
Oleandomycin (OL)	H	CH_2O	OH	H	CH_3	OH	H	OH
Troleandomycin (TAO)	H	CH_2O	$OCOCH_3$	H	CH_3	$OCOCH_3$	H	$OCOCH_3$

Analytical, Urmston, Manchester UK).

Chemicals. Structures and names of the macrolide antibiotics studied are shown in Figure 1. Standard samples of EMA (erythromycin A), MDM (midecamycin), OL (oleandomycin), SPM (spiramycin), TAO (troleandomycin, formerly known as triacetyloleandomycin) and TYL (tylosin) were purchased from Sigma Chemical Co. (St. Louis, MO). JM (josamycin) was a gift from Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan. MOM (miokamycin) was a gift from Meiji Seika Kaisya, Ltd. (Tokyo, Japan). LM (leucomycin-A5) was purchased from Wako Pure Chemical Industries (Osaka, Japan). RKM (rokitamycin) was a gift from Asahi Chemical Industry Co., Ltd. (Tokyo, Japan). Compounds were used without further purification. The glycerol used as the FAB matrix was from Sigma Chemical Company.

RESULTS AND DISCUSSION

Macrolide antibiotics are comprised of an amino sugar (B), a nitrogen-free sugar (S), and a 14- or 16-membered ring aglycone portion (A) (see Figure 1). The nitrogen-free sugar (S) and the amino sugar (B) can either be both attached directly to the aglycone (A) to give a structure with the shorthand notation SOAOB, or the sugar (S) can be bound to the amino sugar (B) which is then bound to the aglycone (A) to give a structure with the shorthand notation AOBOS. All compounds in the SOAOB class that were investigated contain a 14-membered aglycone, while all compounds studied in the AOBOS class have a 16-membered aglycone. In addition, there are three macrolides, containing 16-membered aglycones, two containing two aminosugar groups, spiramycin I and III, with the shorthand notation BOAOBOS, and a third, tylosin, containing two sugar groups, with the shorthand notation SOAOBOS.

SOAOB macrolides

$(M + H)^+$ Ions. Three compounds in this class have been studied, OL, TAO, and EMA. The FAB/MS/CA/MS data are presented in Table 1. A representative MS/MS spectrum, that of the protonated molecule of troleandomycin (TAO), is shown in Figure 2. The major fragmentations are shown in Scheme

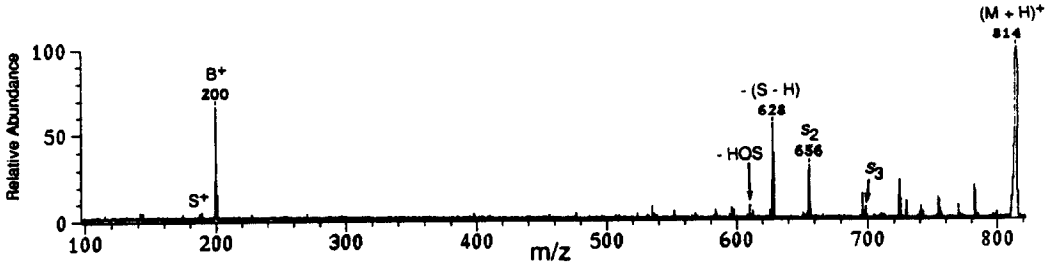
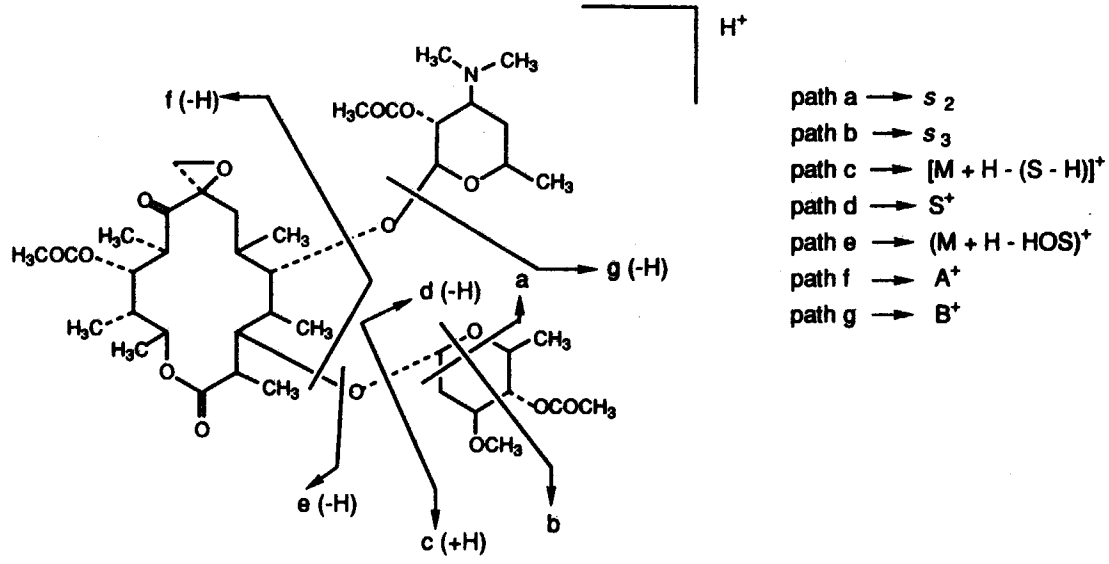


Fig. 2. MS/MS spectrum of the $(M + H)^+$ ion of TAO.

1. The proton in MH^+ is assumed to be located on the dimethylamino nitrogen. Loss of the sugar ring, S, with transfer of hydrogen is the major cleavage reaction observed for these compounds, $[M + H - (S - H)]^+$. In addition, formation of ions due to the aminosugar, B^+, the sugar, S^+, and fragmentation across the sugar ring, s_2 and s_3, are observed. The B^+ ion observed in this spectrum is unique to compounds in which no sugar ring is directly attached to the aminosugar ring. The relative abundance of the B^+ ion is quite variable,



Scheme 1. MS/MS fragmentations of $(M + H)^+$ ions of SOAOB macrolides using TAO as a model.

Table 1. Major Fragment Ions in the MS/MS Spectra of the (M + H)+ Ions of SOAOB Macrolides

	M+H [a]	s2 [b]	s3 [b]	- (S-H) [b]	- HOS [b]	A^+ [-HOB-HOS] [b]	B+ [b]	S+ [b]	[BOH - H]+ [b]	[SOH + H]+ [b]	-(HOB)-(S-H) [b]
OL	688	572 (4)	614 (3)	544 (100)	526 (25)	351 (0.5)	158 (6)	145 (1)	174 (2)	163 (0.5)	369 (1.5)
TAO	814	656 (72)	698 (24)	628 (93)	610 (26)	395 (0)	200 (100)	187 (14)	216 (0)	205 (0)	
EMA	734	604 (1)	660 (1)	576 (100)	558 (30)	417 (0)	158 (< 2)	159 (0)	174 (0)	177 (0)	

Additional Ions

OL

TAO 754(28) [-HO_2CCH_3]; 724(24) [-CH_2O -CH_3CO_2H]; 696(45) [-2(O_2CCH_3)]

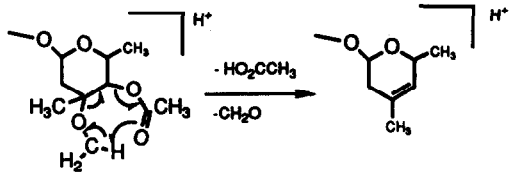
EMA

[a] m/z

[b] m/z(relative abundance)

from the base peak in the spectrum of TAO to less than 2% in EMA. Gross et al. have also observed this ion to be the base peak in the MS/MS spectrum of rosaramicin, a monoaminoglycoside macrolide (37). The s_2 fragmentation is similar to that observed in the MS/MS spectra of nucleosides (41) and of glycosides (42). The second sugar-ring fragmentation differs from the s_1 fragmentation found in nucleosides and glycosides and is thus designated as an s_3 fragmentation.

In addition to these common fragment ions, the MS/MS spectrum of TAO also contains a number of ions due to various combinations of losses of ketene and acetic acid due to the presence of the three acetate groups. An unusual ion observed in the spectrum of TAO is that due to loss of 90 dalton. The structural feature which distinguishes this compound from the other SOAOB macrolides is the proximity of the acetate group and tertiary methoxy group on the sugar ring. A mechanism such as that shown in Scheme 2 can be postulated for the formation of this ion. A similar loss is observed in RKM (see below) in which vicinal ester groups are present. In that case, however, the corresponding hydrogen transfer would be through a ten-membered ring, which would be sterically unlikely. Therefore, nearly simultaneous loss of the two groups are postulated for the origin of this ion.



Scheme 2. Possible mechanism for loss of 90 dalton from the $(M + H)^+$ ion of TAO.

Shifts in the mass of the sugar moiety lost in formation of the $[M + H - (S - H)]^+$ ion identify differences in substituents on the sugar ring. For example, this loss in the MS/MS spectrum of TAO is 42 dalton greater than in OL, indicative of the acetate difference, while the corresponding loss in EMA is 14 dalton higher due to the presence of the R-5" methyl group. These mass differences are confirmed in the mass differences in the S^+ ions. Mass differences in the losses giving rise to the s_2 and s_3 ions can help localize the position of the substituent. Thus, comparison of the losses giving rise to the s_2 and s_3 ions locates the methyl group in EMA on either C-4" or C-5", while the acetyl group on TAO can be localized to C-3" or C-4".

The shift in the mass of the B^+ ion by 42 dalton in TAO relative to the same ion in OL and EMA indicates the presence of an acetyl group on the amino sugar. No information about the location of substituents on the aglycone moiety is provided by the MS/MS data from the protonated molecule.

$(M + Na)^+$ Ions. The MS/MS spectrum of the $(M + Na)^+$ ion of OL is shown in Figure 3 and Scheme 3 as an example of the MS/MS spectra of sodiated SOAOB macrolides (Table 2). For the MS/MS spectrum of this molecule, the base peak is the same for the sodiated parent as for the protonated parent, i.e. loss of the sugar with transfer of a hydrogen. Ions arising from ring cleavages are, however, more prominent in the MS/MS spectrum of the sodiated species than in that of the protonated species. In addition, a new ring cleavage ion, arising from cleavage across the aminosugar, is observed. This ion is designated as a b_2 ion and corresponds to the same ring cleavage as the s_2 cleavage of the sugar ring. The relative abundance of ions due to the aglycone are enhanced in the MS/MS spectrum of the sodiated macrolide relative to those observed in the MS/MS spectrum of the protonated parent. In addition, fragments due to successive loss of small groups from the parent and from the $[M + Na - (S - H)]^+$ and $(A + 2OH + Na)^+$ are noted. The

Table 2. Major Fragment Ions in the MS/MS Spectra of the (M + H) + Ions of the AOBOS Macrolides

	M+H [a]	*s2*	*s3*	- (S-H)	- HOS	A [-HOBOS]	-(BOS-H)	S+	HOB+	BOS+	(HOBOS+H)
		[b]									
MDM	814	642 (16)	684 (2)	614 (100)	596 (18)	423 (3)	440 (0)	201 (10)	174 (8)	374 (6)	
MOM	898	684 (15)	768 (0)	656 (52)	638 (4)	465 (10)	484 (3)	243 (5)	174 (7)	416 (10)	
RKM	828	586 (12)	684 (0)	558 (60)	540 (52)	367 (9)	384 (0)	271 (0)	174 (15)	462 (1)	444 (18)
JM	828	628 (21)	670 (1)	600 (100)	582 (18)	409 (0)	426 (7)	229 (6)	174 (5)	402 (2)	
LM	772	586 (20)	628 (0)	558 (100)	540 (40)	367 (0)	384 (7)	215 (7)	174 (14)	388 (0)	
											-HOBOS
SPM-I	843	727 (20)	769 (30)	699 (100)	681 (8)	351 (0)	526 (2)	145 (0)	174 (4)	318 (5)	508 (0)
SPM-III	899	783 (0)	825 (17)	755 (86)	737 (93)	407 (0)	580 (73)	145 (0)	174 (0)	318 (28)	562 (20)
									S'+/HOB+	BOS+	
TYL	916	800 (3)	842 (2)	772 (100)	754 (20)	407 (1)	599 (4)	145 (1)	174 (3)	318 (2)	581(2)

ADDITIONAL IONS

MDM

MOM 838 (100) [-HOAc]; 824 (40) [-HO2CCH2CH3]; 766 (55) [-OAc-O2CCH2CH3]

RKM 668 (100) [-O2CCH3 - O2CCH2CH3]; 192 (70) [S+ - HO2CCH2CH3]; 289 (23) [SOH2+] 462 (15) [HOBOS+H]

754 (95) [-HO2CCH2CH3]; 740 (51) [-HO2CCH2CH2CH3]; 672 (45); 642 (55) [-O2CC3H7- (C2" + C3")]

JM

LM 743 (24) [-CHO]; 684 (22) [-HO2CC3H7]; 670 (21);

	-(B' - H)	- (BOH)	-[S-H-B'-H)	-[(S-H)-B'OH)]	-H20	-NH(CH3)2	*b2*	ADDITIONAL IONS
SPM-I	702 (7)	684 (90)	558 (1)	540 (26)	825 (13)	798 (9)	730 (16)	
SPM-III		740 (33)	612 (30)	594 (50)	881 (17)			698 (63) [-(S -H) -OCCHCH3]; 745 (72); 706 (33)
	s'2	-(S-H)	[BOS-H20]+	[A-H20]+				
TYL	770 (8)	742 (11)	300 (2)	389 (1)				

[a] m/z

[b] m/z(relative abundance)

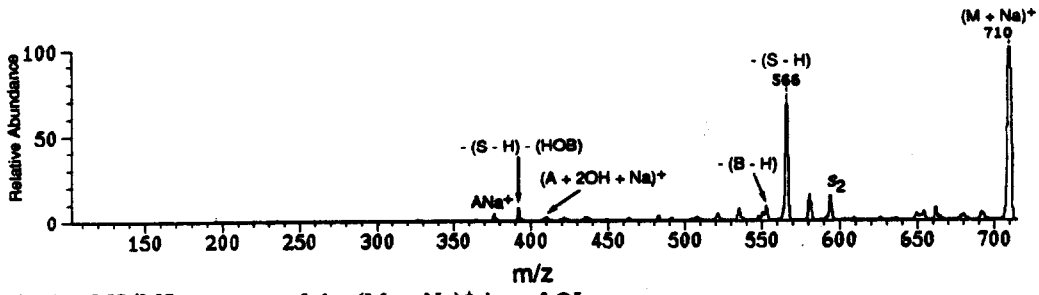
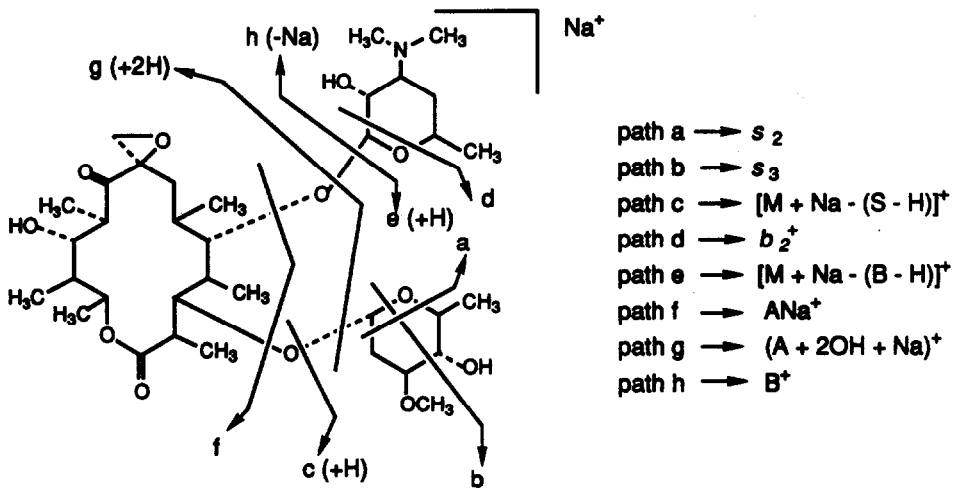


Fig. 3. MS/MS spectrum of the $(M + Na)^+$ ion of OL.

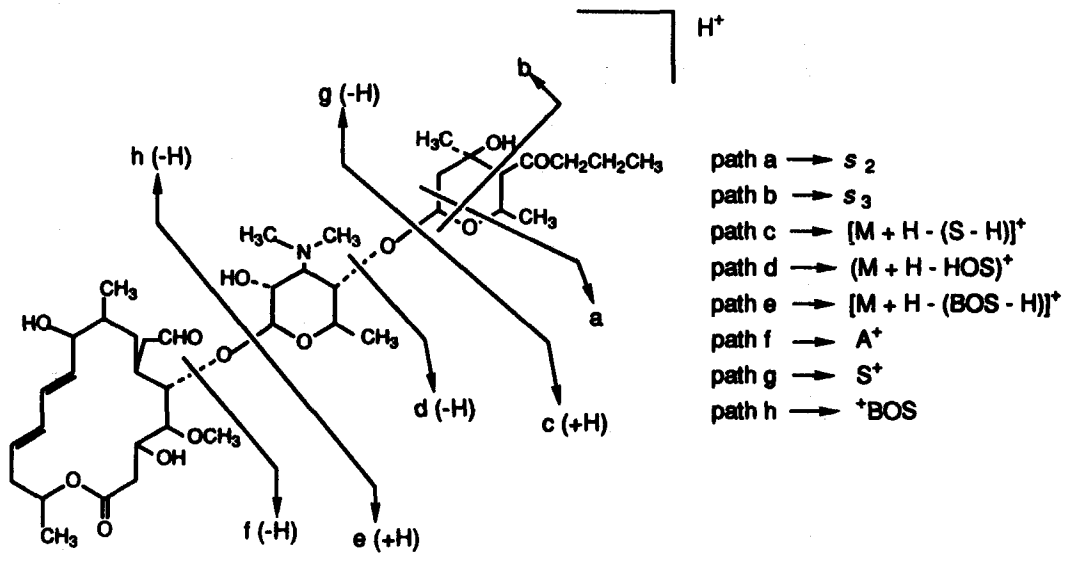


Scheme 3. MS/MS fragmentations of $(M + Na)^+$ ions of SOAOB macrolides using OL as a model.

observation of these ions are consistent with the hypothesis of Gross et al. (37) that the sodium ion is associated with the aglycone moiety rather than with the aminosugar group. The presence of a small B^+ ion of m/z 158 and an ion of m/z 553 due to an $[M + Na - (B - H)]^+$ ion also indicate that the sodium is associated with another part of the molecule.

AOBOS-Macrolides

$(M + H)^+$ Ions. The MS/MS spectra of the compounds in this class are given in Table 3 and the origin of the major ions is shown in Scheme 4. A representative spectrum (of LM) is shown in Figure 4. As for the SOAOB class of molecules, the major fragmentations observed in this class arise through glycosidic bond cleavages. Thus, the major ions are similar to that observed for the SOAOB class. Cleavage of the AOB glycosidic bond with charge retention on the aminoglycosidic part of the molecule now gives rise to BOS^+ and H_2OBOS^+ ions rather than B^+ ions and HOB^+ ions. The HOB^+ ions found in the MS/MS spectra



Scheme 4. MS/MS fragmentations of $(M + H)^+$ ions of AOBOS macrolides using LM as a model.

Table 3. Major Fragment Ions in the MS/MS spectra of the (M + Na)+ Ions of SOAOB Macrolides [a]

	M+Na	*s2*	*s3*	- (S-H)	- (B - H)	*b2*	ANa+	(A + 2OH + Na)+	B+	-(S-H)-(HOB)
OL	710 [b]	594(24) [c]	614 (3)	566 (100)	553(24)	351 (0.5)	375(9)	409 (8)	158 (2)	391(14)
EMA	756	626(53)	660 (1)	598 (100)	599	417 (0)	407 (11)	441 (6)	158(0)	

[a] Insufficient sample to obtain data for the (M + Na) of TAO

[b] m/z

[c] m/z(relative abundance)

of this class of molecules must now be formed via multiple glycosidic bond cleavages.

The extensive acylation of the hydroxyl groups that is characteristic of this class leads to a number of minor ions due to loss of carboxylic acids from the molecule. An unusual fragmentation is observed in

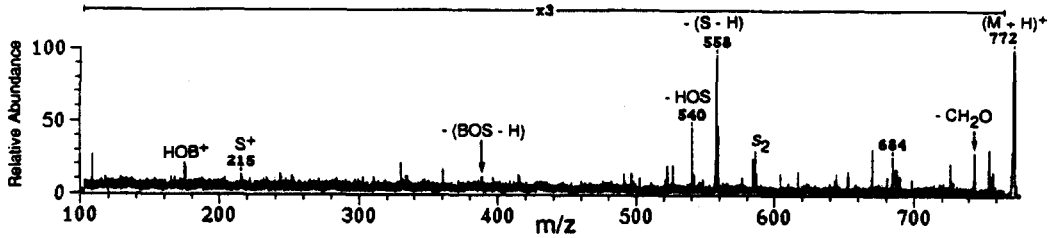


Fig. 4. MS/MS spectrum of the $(M + H)^+$ ion of LM.

MOM and RKM, presumably due to the presence of vicinal carboxylate functions on the sugar ring. This fragmentation yields an ion due to loss of the vicinal carboxylate groups with formation of a carbon-carbon double bond. This unusual process leads to the base peak in RKM (m/z 668) and a 55% relative abundance ion in MOM (m/z 766). Although there is insufficient information available to define the mechanistic details of the fragmentation, it presumably occurs through a rapid 2-step process as described above for loss of 90 daltons from TAO.

Differences in the masses lost in the formation of the s_2 and s_3 fragments provide information as to the location of substituents on the sugar ring as for SOAOB molecules. Substituents on C-2" and C-3" will be lost in formation of the formation of the s_2 fragment but not in the formation of the s_3 fragment while substituents on C-4" and C-5" will be lost in the formation of both fragments. Potential differences in the substitution pattern on the amino sugar would be observed as differences in the masses of the HOB^+ ions, but no information is generated as to the location of other substituents on the aglycone.

The two spiramycins and tylosin are special cases of the AOBOS macrolides in that they contain either an additional aminosugar ring (the spiramycins) or an additional glycoside ring (tylosin). The MS/MS spectra of these molecules are similar to those of the simpler members of this group except that fragmentations characteristic of the additional moiety are also observed. The fragmentations involving the additional moiety are designated by a prime (').

$(M + Na)^+$ Ions. In contrast to the AOBOS macrolides that contain a 14-membered ring, the MS/MS spectra of the $(M + Na)^+$ ions of the 16-membered ring macrolides contain prominent ions that involve cleavage of the aglycone ring in addition to the glycosidic bond cleavages noted above (Table 4). This is illustrated by the MS/MS spectrum of the $(M + Na)^+$ ion of LM (Fig. 5, Scheme 5). The spectrum is dominated by an ion due to loss of 150 dalton. We propose that this ion arises via cleavage of the aglycone ring at the C7-C8 bond and the C13-O bond with transfer of two hydrogen atoms and expulsion of a highly conjugated tetraene neutral (fragment a_1 Scheme 5). An additional ring cleavage between C5 and C6 and C1 and C2 is also noted for LM (a_2). The observation of cleavages involving loss of the dimethylamino group (m/z 493 and 517) indicates that the sodium is not complexed with the basic nitrogen. The cleavages through the aminoglycoside and the aglycone rings can be used to help locate substituents on these rings. Thus, the

Table 4. Major Fragment Ions in the MS/MS Spectra of the (M + Na)+ Ions of the AOBOS Macrolides[a]

	M+Na	*s*2	-(S-H)	*b*4	AOH + Na	*a*1	*a*2	(M+Na-*a*2+2H)+	*b*2
MDM	836[b]	664(45)[c]	636(100)		463(81)	686(32)		600(20)	491(58)
RKM	850	608(67)	580(220)		407(16)	700(100)			435(19)
JM	850	650(32)	622(100)		449(56)	700(26)			477(40)
LM	794	608(12)	580(27)	493(15)	407(17)	644(100)	259(23)	558(38)	435(10)
SPM-I	865	749(10)	721(100)		388(11)	574(32)			575(16)
SPM-III	921	805(27)	777(100)		444(14)	630(36)			671(15)
TYL	938	822(15)	794(100)						649(24)

ADDITIONAL IONS

MDM 762(93) [-HO2CC2H5]; 535(35); 458(99) [a1s2]
RKM 776(85) [-HO2CC2H5]; 688(26)[-HO2CC2H5-HO2CC3H7]; 522F(35) [-C2H5OS]; 551(47); 401(22)
JM
LM

	b'2	-B'OH	-(BOS - H	-HOBOS	HOB+	B+	OTHER
SPM-I	752(20)	705(57)	547(54)	529(15)	174(4)		
SPM-III	808(15)	761(35)	603(44)	585(12)	174(5)	156(5)	553(22)
	-(S'-H)	-(BOS-H)	-HOBOS	OTHER			
TYL	748(30)	621(72)	603(36)	(40); 472(17)			

[a] (M + Na)+ of MOM obscured by glycerol background

[b] m/z

[c] m/z(relative abundance)

MS/MS spectra of the $(M + Na)^+$ ions provides additional structural information not available from the MS/MS spectra of the $(M + H)^+$ ions.

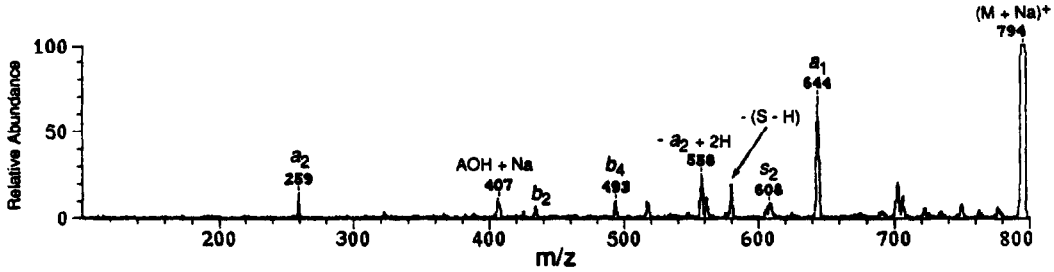
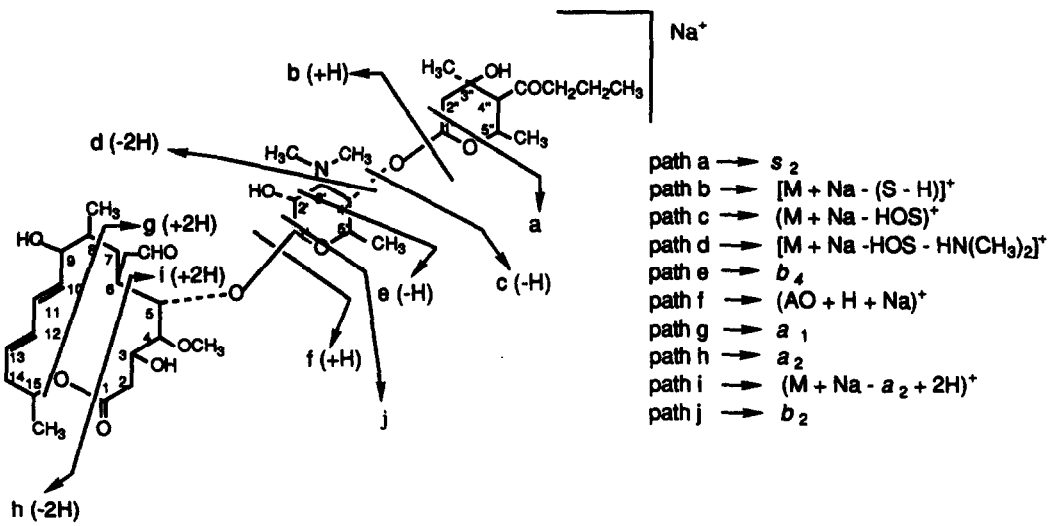


Fig. 5. MS/MS spectrum of the $(M + Na)^+$ ion of LM.



Scheme 5. MS/MS fragmentations of $(M + Na)^+$ ions of AOBOS macrolides using LM as a model.

Phosphorylated Oleandomycin

In the Tokyo laboratory, we have previously shown that oleandomycin undergoes enzymatic inactivation when treated with an extract of an erythromycin-resistant strain of *Escherichia coli* and have identified the product as a phosphorylated derivative. To investigate the utility of FAB/MS/MS for the structure determination of such enzymatic products, we investigated this lyophilized crude reaction mixture. The FAB/MS spectrum of the crude reaction mixture contains ions due to the sodium salt of monophosphorylated OL and an adduct of this salt with TMK buffer (at m/z 911). The MS/MS spectrum of the monosodiated salt is shown in Figure 6. Three major peaks are observed at high mass, m/z 670, 646 and 553. The ion of m/z 670 is due to loss of NaH_2PO_4. This confirms that the inactivation process is due to phosphorylation of the parent macrolide. The base peak, m/z 646, corresponds to the $[M + H - (S - H)]^+$ ion which was observed as the base peak in the MS/MS spectrum of the $(M + Na)^+$ ion of OL. This

indicates that the phosphate group is not attached to the sugar group, S. The second largest ion, m/z 553, corresponds to loss of phosphorylated aminosugar, $[M + Na - (BPO_3H_2 - H)]^+$. Although loss of the base was noted in the MS/MS spectrum of the protonated parent, $(OL + H)^+$, it was not as prominent. The presence of this ion is consistent with our previous work which showed that phosphorylation occurs on the 2'-hydroxyl group of the base (39). An ion of m/z 391 due to loss of (S - H) and (BOH) is also observed, confirming that phosphorylation did not occur on the aglycone portion of the molecule. Thus, the MS/MS spectrum clearly locates the site of phosphorylation as being on the base. Comparing the MS/MS spectra of

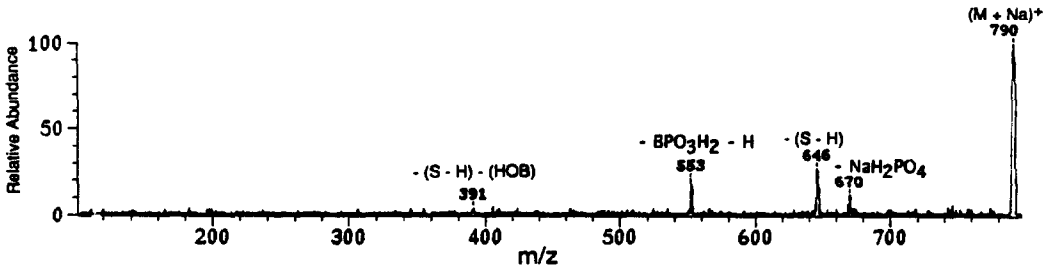


Fig. 6. MS/MS spectrum of the $(M + Na)^+$ ion of phosphorylated OL.

the phosphorylated and non-phosphorylated OLs, one can note that the presence of the phosphate group leads to a significant reduction in the relative abundances of the ions due to losses of small molecules from the major ions, presumably due to the lability of the phosphate group.

CONCLUSION

The combination of fast atom bombardment with MS/MS provides a wealth of structural information about macrolide antibiotics. The major fragmentation processes evident in the MS/MS spectra of the protonated molecules involve cleavages of the glycosidic linkages. Less abundant process involve ring cleavages as well. These cleavages can be used to locate substituents on the sugar, the aminosugar or the aglycone. More extensive ring cleavages are often noted in the MS/MS spectra of the sodiated molecules and can isolate the location of substituents to specific atoms or groups of atoms in the rings. The successful application of these techniques to locating the ring to which the phosphate group is attached during the enzymatic inactivation of oleandomycin illustrates that the combined techniques are applicable to biological samples.

REFERENCES

1. Abramowicz, M. (ed.), *Med. Lett.*, **1986**, *28*, 33.
2. Brittain, D.C., *Med. Clin. North Am.*, **1987**, *71*, 1147.
3. Nelson, J.D. (ed.), Proceedings of a symposium: the evolving role of erythromycin in medicine. *Pediatr. Infect. Dis.*, **1986**, *5*, 118.
4. Washington, J.A., Wildon, W.R., *Mayo Clin. Proc.*, **1985**, *60*, 189.
5. Nakayama, I., in S. Omura, (Ed.), Macrolide Antibiotics: chemistry, biology, and practice. Academic

Press, Orlando, FL, **1984**, p. 261.
6. Kirst, H.A., Sides, G.D., *Antimicrob. Agents Chemother.*, **1989**, 1413.
7. Andremont, A., Gerbaud, G., P. Courvalin, *Antimicrob. Agents Chemother.*, **1986**, *29*, 515.
8. Andremont, A., Railbaud, P., Tancrede, C., *J. Infect. Dis.*, **1983**, *148*, 579.
9. Andremont, A., Tancrede, C., *Ann. Microbiol. (Paris)*, **1981**, *132B*, 419.
10. Arthur, M., Andremont, A., Courvalin, P., *Antimicrob. Agents Chemother.*, **1987**, *31*, 409.
11. Arthur, M., Courvalin, P., *Antimicrob. Agents Chemother.*, **1986**, *30*, 396.
12. Barthelemy, P., Autisser, D., Gerbaud, G., P. Courvalin, *J. Antibiot.*, **1984**, *37*, 1692.
13. Brisson-Noel, A., Arthur, M., Courvalin, P., *J. Bacteriol.*, **1988**, *170*, 1739.
14. Ounissi, H., Courvalin, P., *Gene*, **1985**, *35*, 271.
15. O'Hara, K., Kanda,T., Kono, M., *J. Antibiot.*, **1988**, *41*, 823.
16. O'Hara, K., et al., *Antimicrob. Agents Chemother.*, **1989**, *33*, 1354.
17. Wiley, P.F., Baczynskyj, L., Dolak, L.A., Cialdella, J.I., Marshall, V.P., *J. Antibiotics*, **1987**, *40*, 195.
18. Kono, M., O'Hara, K., Ebisu, T., *FEMS Microbiol. Lett.*, **1992**, *97*, 89.
19. Marshall, V., Cialdella, J., Baczynskyj, L., Liggett, W., Johnson, R., *J. Antibiotics*, **1989**, *42*, 132.
20. Kratos Analytical, MS25 LC/MS Data Compilation, Ramsey, NJ, 1985.
21. Chapman, J.R., Pratt, J.A.E., *J. Chromatogr.*, **1987**, *394*, 231.
22. Kratos Analytical, Continuous Flow Fast Atom Bombardment, Ramsey, NJ, 50 pp.
23. Adachi, T., et al., *J. Antibiotics*, **1988**, *41*, 966.
24. Marshall, V., Cialdella, J., Baczynskyj, L., Liggett, W., Johnson, R., *J. Antibiotics*, **1989**, *42*, 132.
25. Yoshioka, T., Kiyoshima, K., Maeda, M., Sakamoto, M., Ishikura, T., Fukagawa, Y., Sawa, T., Hamada, M., Naganawa, H., Takeuchi, T., *J. Antibiotics*, **1988**, *41*, 1617.
26. Sakairi, M., Kambara, H., *Anal. Chem.*, **1988**, *60*, 774.
27. Mitscher, L.A., Showalter, H.D.H., Foltz, R.L., *J.C.S. Chem. Comm.*, **1972**, 796.
28. Mitscher, L.A., Showalter, H.D.H., Foltz, R.L., *J. Antibiotics*, **1973**, *26*, 55.
29. Egan, R.S., et al., *J. Antibiotics*, **1974**, *27*, 544.
30. Tomer, K.B., *Mass Spectrom. Rev.*, **1989**, *8*, 445.
31. Tomer, K.B., *Mass Spectrom. Rev.*, **1989**, *8*, 483.
32. Siu, K.W.M., Gardner, G.J., Berman, S.S. *Org. Mass Spectrom.*, **1989**, *24*, 931.
33. Quilliam, M., Thompson, Scott, G., Siu, K., *Rapid Commun. Mass Spectrom.*, **1989**, *3*, 145.
34. Hail, M., Lewis, S., Jardine, I., Liu, J., Novotny, M., *J. Microcol. Sep.*, **1990**, *4*, 285.
35. Loo, J.A., Edmonds, C.G., Smith, R.D., *Science*, **1990**, *248*, 201.
36. Huang, E.C., Henion, J.D., *Anal. Chem.*, **1991**, *63*, 735.
37. Gross, M.L., et al., *Anal. Chim. Acta*, **1991**, *250*, 105.
38. Pleasance, S., et al., *Biol. Mass Spectrom.*, **1992**, *21*, 675.
39. Parker, C.E., et al., *J. Amer. Soc. Mass Spectrom.*, **1992**, *3*, 563.
40. Deterding, L.J., Moseley, M.A., Tomer, K.B., Jorgenson, J.W., *Anal. Chem.*, **1989**, *61*, 2504.
41. Tomer, K.B., Gross, M.L., *Biomed. Environ. Mass Spectrom.*, **1988**, *15*, 89.
42. Crow, F.W., Tomer, K.B., Looker, J.H., Gross, M.L., *Anal. Biochem.*, **1986**, *155*, 286.