MYCINAMICINS, NEW MACROLIDE ANTIBIOTICS

IX.[1] CHEMICAL IONIZATION MASS SPECTRAL STUDIES ON MYCINAMICINS

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Chemical ionization (CI) mass spectra of new macrolide antibiotics, mycinamicins are reported. Protonated molecules (MH^+) are observed as base peaks in the CI mass spectra of all components. Fragmentations are mainly restricted to the glycosidic linkages and the resulting aglycone and sugar-derived ions appear regularly in their mass spectra. Moreover, characteristic fragment ions involving carbon-carbon bond fission are found in the CI mass spectra of the epoxyenone-containing components, mycinamicins I (**1**) and II (**2**). The mechanism for the formation of the ion species is also discussed.

In previous reports we have described the chemical ionization mass spectrometry (CIMS) of several known representative 16-membered basic macrolide antibiotics.[2,3] The results obtained throughout this series of investigations are summarized as follows:

1) A protonated molecule (MH^+) is intensely observed.

2) Fragmentation involving carbon-carbon bond fission is rarely recognized.

3) Fragmentations occur mainly at glycosidic linkages to produce aglycone ions and sugar-derived ions.

4) Shift techniques using deuterated reagent gases are very useful for characterizing the diagnostic ions.

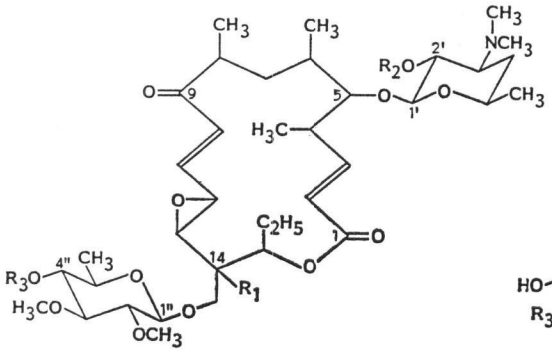
5) These diagnostic ions are valuable for structural characterization of 16-membered macrolide antibiotics.

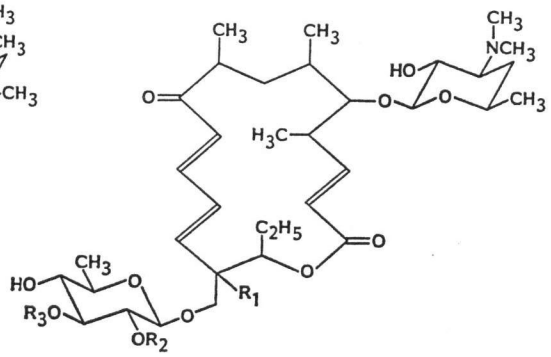
Mycinamicins, a new group of 16-membered macrolide antibiotics, have been obtained from fermentation broth of *Micromonospora griseorubida* sp. nov. and are composed of several components.[4] Although the structures have been already determined on the basis of chemical degradations and spectroscopic studies, CIMS played a very significant role in their structural characterization.

In this paper we would like to describe the mass spectrometric features of mycinamicins in connection with the structural determination and discuss the characteristic carbon-carbon bond fission of mycinamicins I (**1**) and II (**2**) under CI conditions.

Experimental

Low resolution CI mass spectra of all macrolides were obtained with a Shimadzu LKB 9000A





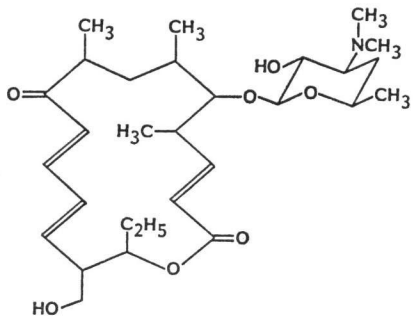
Mycinamicin I series

1 $R_1 = R_2 = R_3 = H$ (Mycinamicin I)
8 $R_1 = H$, $R_2 = R_3 = Ac$
10 $R_1 = H$, $R_2 = Ac$, $R_3 = H$
12 $R_1 = R_2 = H$, $R_3 = Ac$

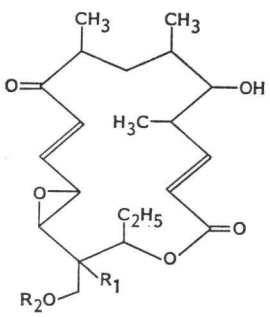
Mycinamicin II series

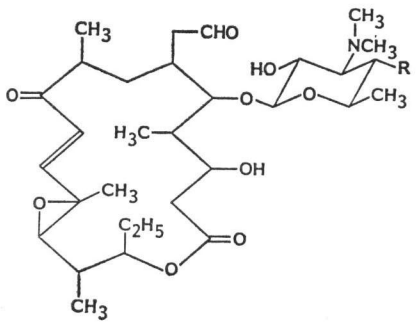
2 $R_1 = OH$, $R_2 = R_3 = H$ (Mycinamicin II)
9 $R_1 = OH$, $R_2 = R_3 = Ac$
11 $R_1 = OH$, $R_2 = Ac$, $R_3 = H$
13 $R_1 = OH$, $R_2 = H$, $R_3 = Ac$

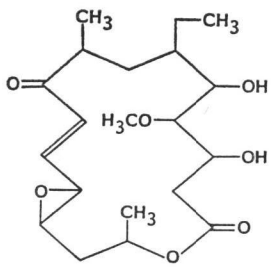
3 $R_1 = H$, $R_2 = CH_3$, $R_3 = H$ (Mycinamicin III)
4 $R_1 = H$, $R_2 = R_3 = CH_3$ (Mycinamicin IV)
5 $R_1 = OH$, $R_2 = R_3 = CH_3$ (Mycinamicin V)
6 $R_1 = R_2 = R_3 = H$ (Mycinamicin VI)



7 (Mycinamicin VII)







14 $R_1 = R_2 = H$
15 $R_1 = OH$, $R_2 = H$
16 $R_1 = OH$, $R_2 =$ Mycinosyl

17 R = H
18 R = OH

19

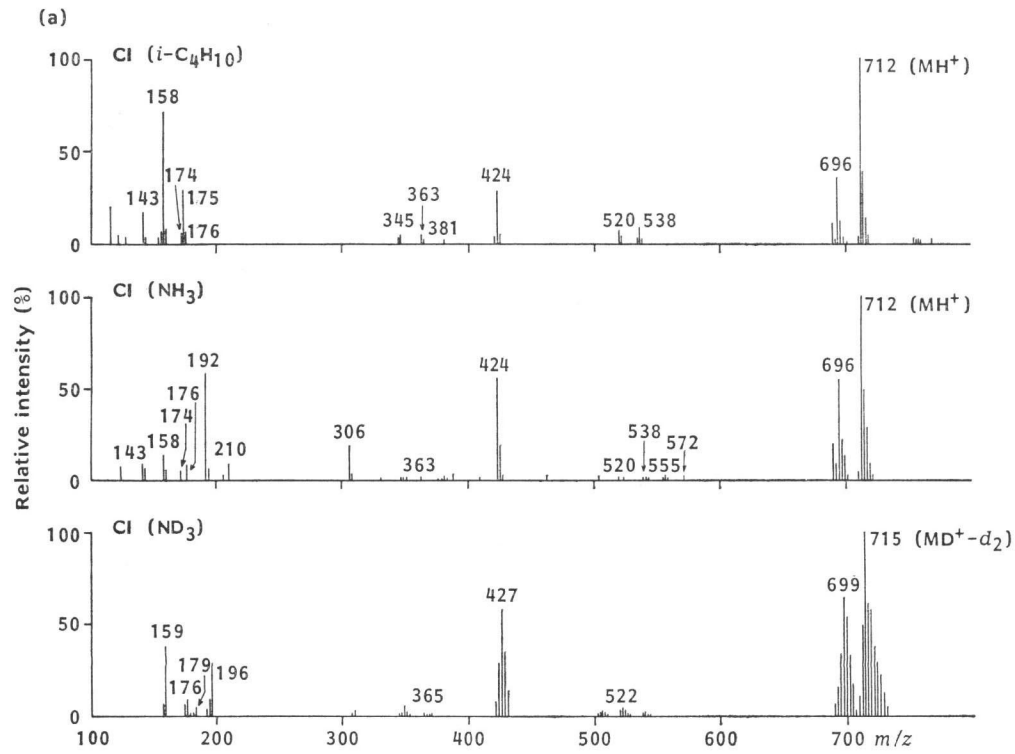
mass spectrometer. The ion source was maintained at 170~190°C with the gas pressure at approximately 0.3 mmHg. The spectra were recorded using an accelerating voltage of 3.5 kV, an emission current of 250 μA and an electron energy of 140 eV. High resolution CI data were recorded on a Jeol JMS-D 300 mass spectrometer on-line to a JMA-2000 computer at a resolution of approximately 5,000 under the following conditions: ion source temperature; 250°C, electron energy; 240 eV, accelerating voltage; 3 kV, emission current; 610 μA, reagent gas pressure; about 0.3 mmHg. The samples were introduced *via* direct probe and perfluoro-kerosine was admitted from a heated inlet system. The reagent gases used, *i*-C_4H_{10} and NH_3 (99.9% purity), were purchased from Takachiho Trading Co., Ltd. ND_3 (99 atom % D) was obtained from Merck Sharp and Dohme, Canada, Ltd. Field desorption (FD) mass spectra were measured with a Hitachi M-80 mass spectrometer.

The occurrence of the macrolides studied has been reported in Part II,[5] IV[6] and VII.[7] Each acetyl derivative was prepared according the following methods: Treatment of **1** and **2** with acetic anhydride in pyridine gave their 2′,4″-diacetates (**8** and **9**). Acetylation of **1** and **2** with acetic anhydride in acetone yielded the 2′-monoacetates (**10** and **11**). 4″-Monoacetates (**12** and **13**) were obtained by heating of the 2′,4″-diacetates in methanol.

Results and Discussion

Fig. 1 shows the CI mass spectra of mycinamicins I (**1**), II (**2**) and V (**5**) using isobutane, ammonia and ammonia-d_3 as reagent gases and the resulting diagnostic ions of all components (I~VII) under CI (*i*-C_4H_{10}, NH_3) conditions are listed in Table 1. All mass spectra mediated with isobutane and ammonia show clearly MH^+ as base peaks. Fragmentations are expectedly restricted at the glycosidic linkages to produce aglycone and sugar-derived ions except for the characteristic cleavage mentioned

Fig. 1. Chemical ionization mass spectra of (a) mycinamicins I (**1**), (b) II (**2**) and (c) V (**5**) mediated with isobutane, ammonia and ammonia-d_3 as reagent gases.



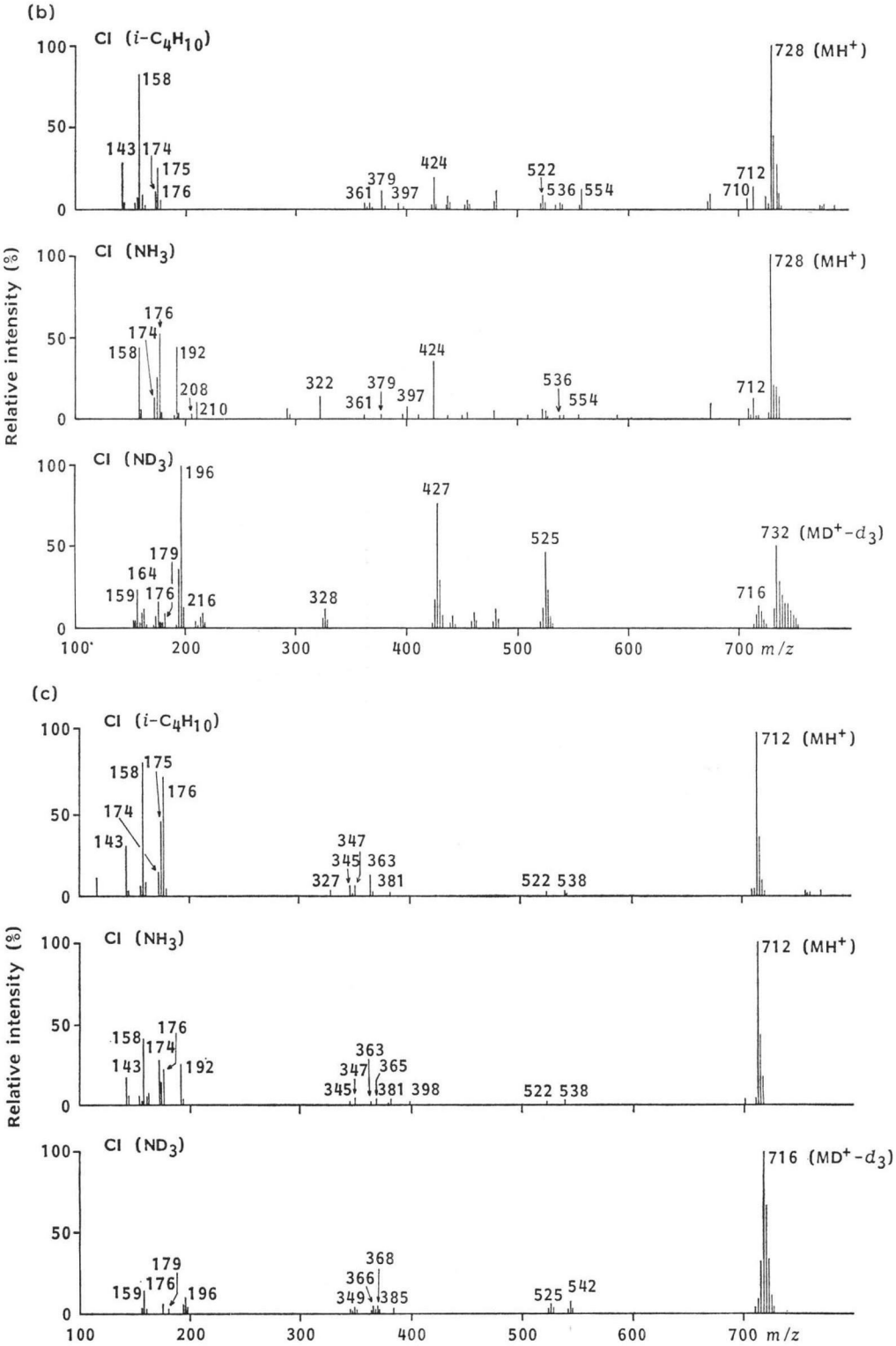
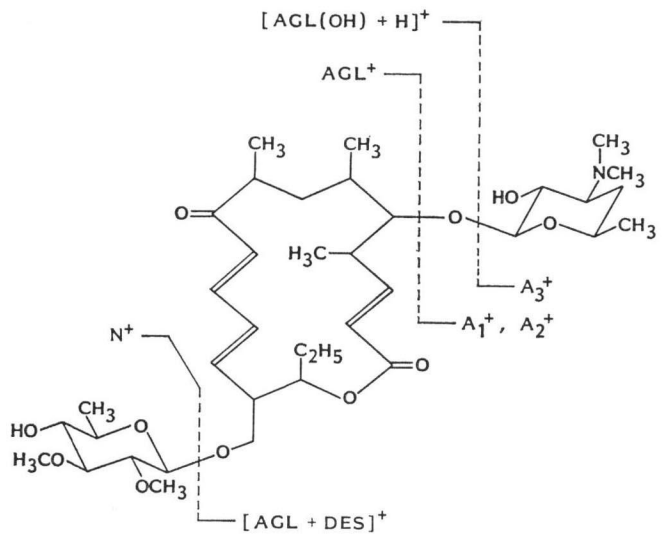

(b)
CI (i-C_4H_{10})
728 (MH^+)
158
143
174
175
176
361
379
397
424
522
536
554
710
712
CI (NH_3)
728 (MH^+)
176
174
158
192
208
210
322
361
379
397
424
536
554
712
CI (ND_3)
196
427
525
732 (MD^+-d_3)
179
164
159
176
216
328
716
Relative intensity (%)
100
200
300
400
500
600
700 m/z
(c)
CI (i-C_4H_{10})
712 (MH^+)
158
175
176
174
143
347
345
363
327
381
522
538
CI (NH_3)
712 (MH^+)
158
176
174
143
192
363
347
365
345
381
398
522
538
CI (ND_3)
716 (MD^+-d_3)
179
176
159
196
368
366
349
385
525
542
Relative intensity (%)
100
200
300
400
500
600
700 m/z

Table 1. Diagnostic ions (m/z) of mycinamicin series under chemical ionization.

Component	MH^+	$[AGL+DES]^+$	$[AGL(OH)+H]^+$	AGL^+	Amino sugar			Neutral sugar
					A_1^+	A_2^+	A_3^+	N^+
I	712	538	381	363	176	174	158	175 (192)
II	728	554	397	379	176	174	158	175 (192)
III	682	522	365	347	176	174	158	161 (178)
IV	696	522	365	347	176	174	158	175 (192)
V	712	538	381	363	176	174	158	175 (192)
VI	668	522	365	347	176	174	158	147 (164)
VII	522	522	365	347	176	174	158	—

( ): NH_3 as a reagent gas.

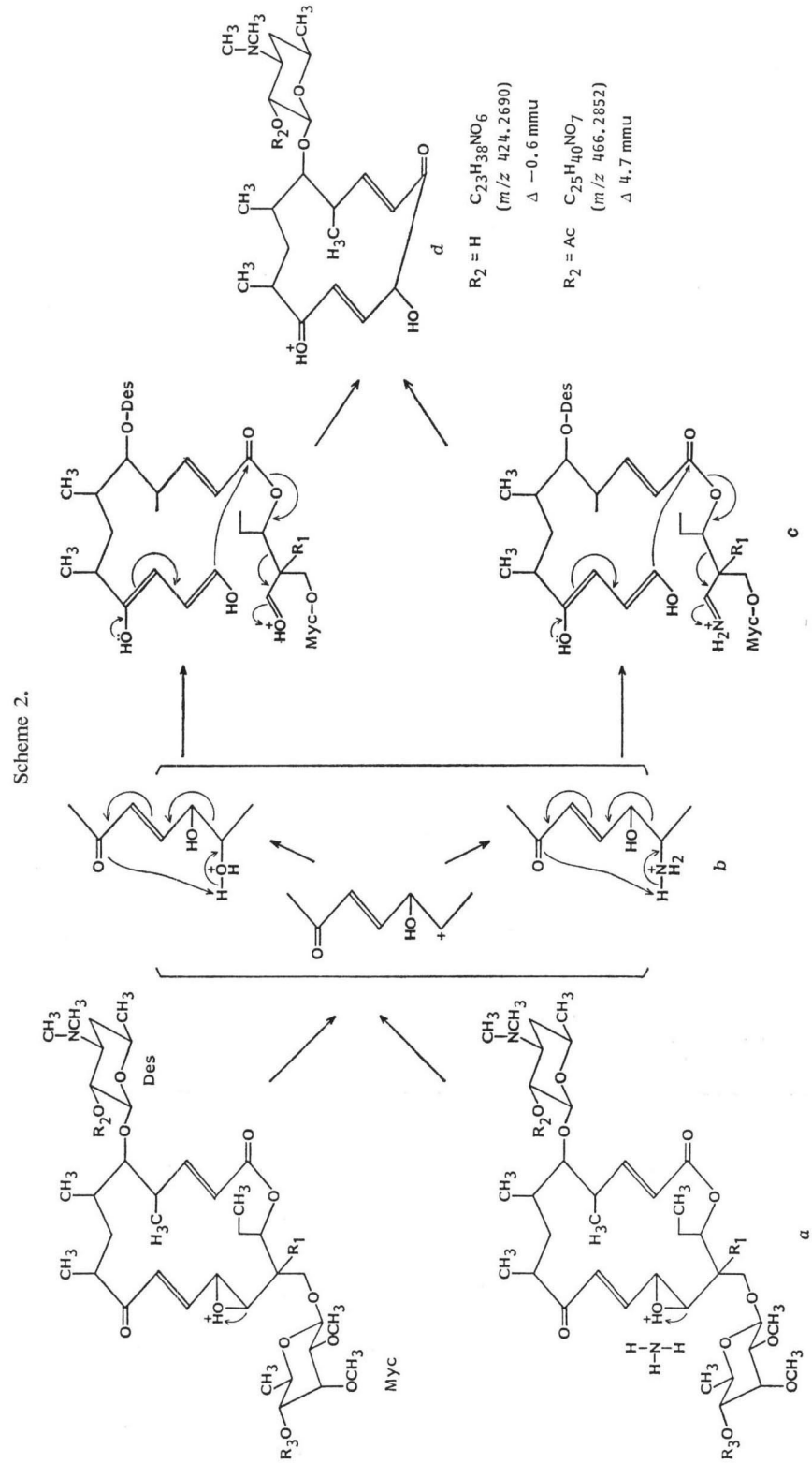
Scheme 1.



later. The appearance of the three ions at m/z 176 (A_1^+), 174 (A_2^+) and 158 (A_3^+) indicates the presence of a constituent amino sugar, desosamine in common to all components.[3] While the neutral sugar-derived ions (N^+) are observed at m/z 175 for **1**, **2**, **4** and **5** (mycinose), m/z 161 for **3** (javose) and m/z 147 for **6** (6-deoxyallose) in their CI (i-C_4H_{10}) spectra, these ions are shifted upwards (Δ17 u) in their CI (NH_3) mass spectra.[2]

Mycinamicins possess two glycosidic linkages, the aglycone-amino sugar and the aglycone-neutral sugar. Because the latter is apt to be cleaved rather than the former, the ions composed of the aglycone and desosamine, $(AGL+DES)^+$ appear in all spectra, but no glycosidic ions containing a neutral sugar are recognized. Concerning the aglycone-derived ions, two kinds of species, $[AGL(OH)+H]^+$ and AGL^+ are found in their mass spectra. The difference of these ions is derived from that of the cleavage of the glycosidic linkage containing desosamine. These results suggest that the glycosidic linkage containing desosamine is cleaved at both sides of the glycosidic oxygen atom, whereas the glycosidic linkage containing a neutral sugar is only cleaved between the glycosidic oxygen atom and the aglycone. The principal fragmentations illustrated in Scheme 1 are very useful for structural characterization of minor components and degradation products of mycinamicins.

Scheme 2.


Des
Myc
a
b
c
d
R2 = H C23H38NO6 (m/z 424.2690) Δ −0.6 mmu
R2 = Ac C25H40NO7 (m/z 466.2852) Δ 4.7 mmu

Further, a characteristic fragment ion (*d*-type ion), other than the regular ions mentioned above, is prominently found at *m/z* 424 in the CI mass spectra of **1** and **2**. This ion species is shifted to *m/z* 427 in their CI mass spectra mediated with ammonia-d_3, indicating that there are two active hydrogens in the ion. No such ions are detected in the CI mass spectra of mycinamicins III (**3**)~VII (**7**). Since the significant difference between **1**~**2** and **3**~**7** is their chromophores (**1**~**2**: epoxyenone, **3**~**7**: dienone), the formation of the ion is considered to be concerned with the epoxyenone system. Its composition is determined to be $C_{23}H_{33}NO_6$ by the use of the high resolution CI (*i*-C_4H_{10}) mass spectral data. The structural difference between **1** and **2** is due to the presence or absence of a hydroxyl group at C-14, which does not influence its appearance. Moreover, this type ion is not observed at all in the FD mass spectra of **1** and **2**, indicating that the ion is not derived from other compounds but it is an indoubted fragment ion of **1** and **2**. Consequently, it is proposed that the formation proceeds as shown in Scheme 2, in which includes a protonation of the epoxide and a fission of the bond at C-12~C-13 followed by a recombination between C-1 and C-12.

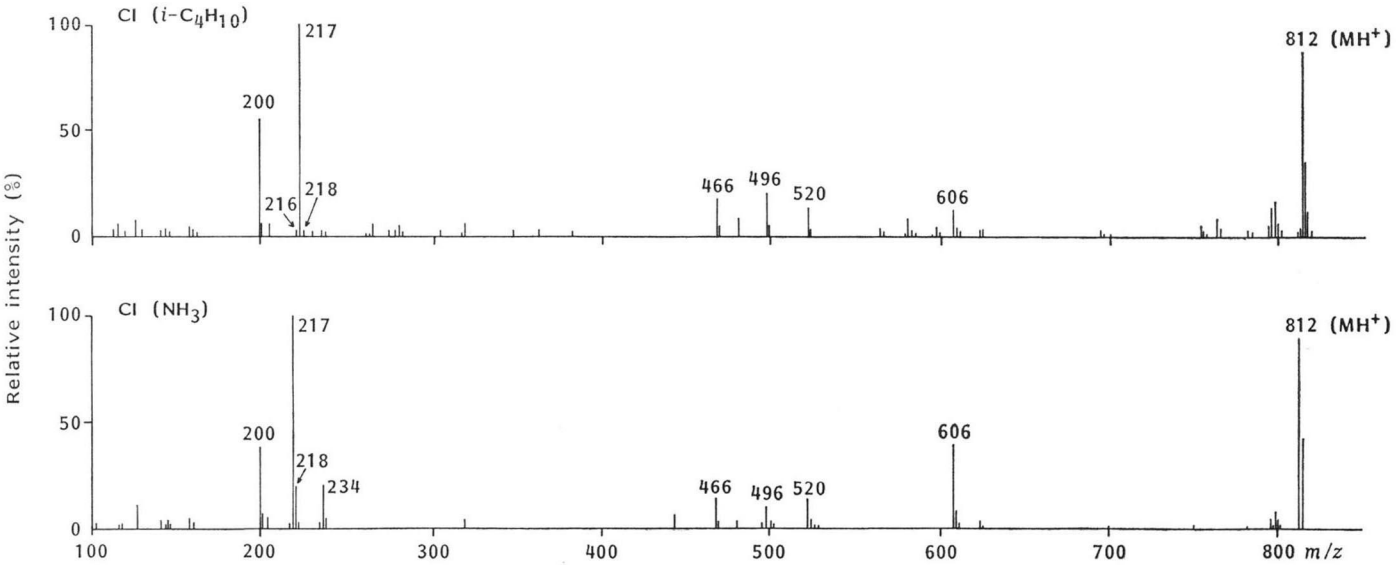
Because the first step for the formation of the ion species is considered to be a protonation and cleavage of the epoxide, an epoxyenone system is essential to detect the *d*-type ion. It would perhaps be expected that the *d*-type ions of the aglycones of **1** and **2**, mycinolides I (**14**) and II (**15**),[8] and de-desosaminylmycinamicin II (**16**)[8] appear at *m/z* 267 in their CI mass spectra. But their intensities are extremely low in contrast with the cases of the parent compounds. No appearance of the corresponding ions is also recognized in the CI mass spectra of other epoxyenone-containing macrolide antibiotics such as rosaramicin (M-4365 A_1, **17**), cirramycin A_1 (**18**) and platenolide III (**19**).[3] These results suggest that both of the constituent sugars of **1** and **2** are concerned with the formation of the *d*-type ion in which the amino sugar is required for the stabilization of the ion species and the presence of the neutral sugar allows the leaving group to eliminate readily.

In order to examine further this hypothesis, the CI mass spectra of the three acetates, 2′,4″-diacetates (**8**, **9**), 2′-monoacetates (**10**, **11**) and 4″-monoacetates (**12**, **13**), were determined. The CI mass spectra of **9** are shown in Fig. 2 as a typical example and the diagnostic ions of **8**~**13** together with the *d*-type ions are listed in Table 2. When the amino sugar moiety is acetylated, the *d*-type ion is shifted to *m/z* 466 whose composition is established to be $C_{25}H_{40}NO_7$ by the high resolution data.

Table 2. Diagnostic ions (*m/z*) of acetylated mycinamicins I and II under CI conditions.

Compound	Reagent gas	MH^+	Amino sugar	Neutral sugar	*d*-Type ion
2′,4″-Diacetate					
8	*i*-C_4H_{10}	796	218, 216, 200	217	466
	NH_3	796	218, 216, 200	234, 217	466
9	*i*-C_4H_{10}	812	218, 216, 200	217	466
	NH_3	812	218, 216, 200	234, 217	466
2′-Monoacetate					
10	*i*-C_4H_{10}	754	218, 216, 200	175	466
	NH_3	754	218, 216, 200	192, 175	466
11	*i*-C_4H_{10}	770	218, 216, 200	175	466
	NH_3	770	218, 216, 200	192, 175	466
4″-Monoacetate					
12	*i*-C_4H_{10}	754	176, 174, 158	217	424
	NH_3	754	176, 174, 158	234, 217	424
13	*i*-C_4H_{10}	770	176, 174, 158	217	424
	NH_3	770	176, 174, 158	234, 217	424

Fig. 2. Chemical ionization mass spectra of 2′,4″-diacetylmycinamicin II (**9**) mediated with isobutane and ammonia as reagent gases.



However, an acetyl group in the neutral sugar moiety does not participate in the formation of the ion. These results support further the mechanism shown in Scheme 2 for the production of the characteristic fragment ion.

MITSCHER *et al.* have reported the cleavage of a carbon-carbon bond *via* a retro-aldol type fragmentation for 14-membered macrolide antibiotics, erythromycin B and oleandomycin.[9] Therefore, the present fragmentation is the second example including the characteristic carbon-carbon bond cleavage in the CI mass spectra of macrolide antibiotics.

In summary, we have demonstrated that the regular fragmentation rule under CI conditions is applicable for the characterization of the constitution of unknown macrolide antibiotics. Further, we have found a characteristic fragmentation including carbon-carbon bond cleavage in the CI mass spectra of the epoxyenone-containing components of mycinamicins.

Acknowledgment

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