CHEMICAL IONIZATION MASS SPECTROMETRY OF MACROLIDE ANTIBIOTICS II[1].

——— Platenomycin and Related Compounds ———

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Abstract —— The chemical ionization (CI) mass spectra of four intact 16-membered ring macrolide antibiotics have been examined using i-C_4H_{10} and NH_3 as the reagent gases. All CI mass spectra show the abundant protonated molecular ions (MH^+) and the aglycone and sugar derived ions arised from the cleavage of the glycosidic linkages. The fruitful information available from above ion species increases the capabilities of chemical ionization mass spectrometry (CIMS) in the structural characterization of this class of compounds.

It is hard to obtain satisfactory results in elemental analysis and molecular weight determination of the macrolide antibiotics, because they combined with some solvent molecules very tightly. In addition, the macrolide family is usually constituted by large number of components, hence it has been required to establish an effective analytical technique for the discrimination of their components.

Electron ionization mass spectrometry (EIMS) has been successfully used in the elucidation of the structures of a number of macrolides, particularly when the shifting method between peracetyl and perdeuteroacetyl derivatives is employed[2]. However, the recent publication[3] of the CI mass spectra of the some basic macrolide antibiotics prompted us to present our own results on the 16-membered ring macrolide antibiotics, M-4365 A_2 and G_2, containing only amino sugar[1]. We now wish to present the results of CIMS of several intact macrolides which contain two or more sugars, using both i-C_4H_{10} and NH_3 as the reagent gases.

Chemical ionization mass spectra of Platenomycin A_1(1) and B_1(2)

The structure of the 16-membered aglycone is the same in Platenomycin A_1(1) and B_1(2), while terminal acyl groups of the sugar moiety are isovaleryl and propionyl, respectively[4]. CI(i-C_4H_{10}/NH_3) mass spectra of 1 are shown in Fig. 1.

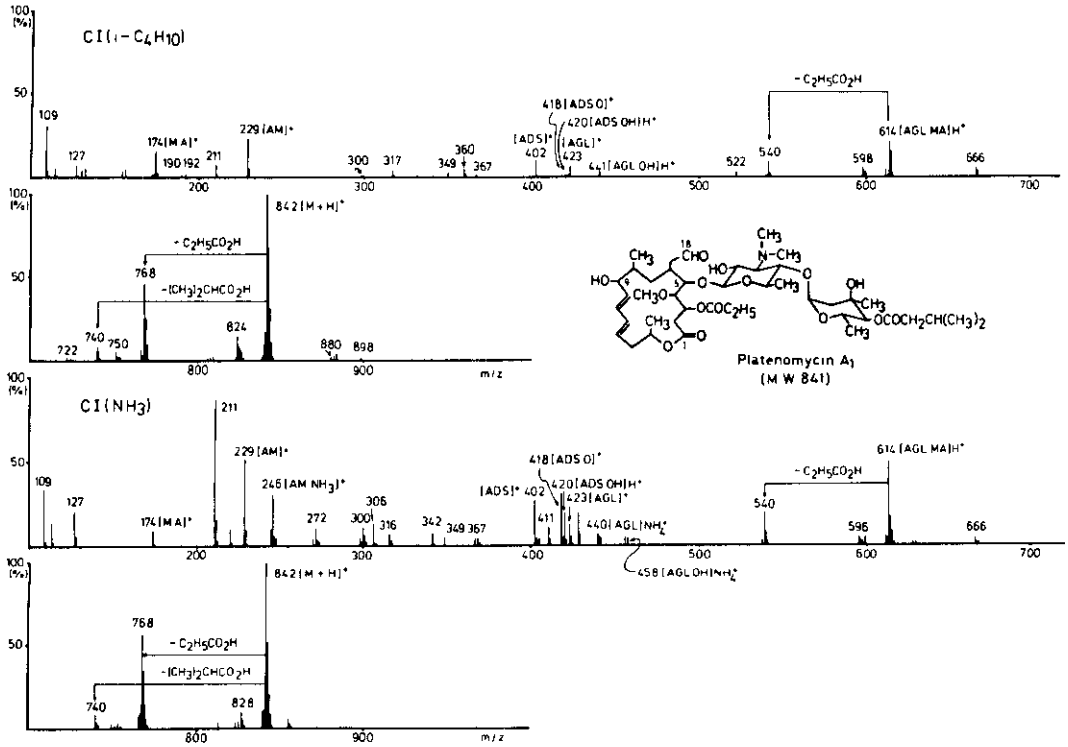


Fig. 1. Chemical ionization mass spectra of platenomycin A_1(1).

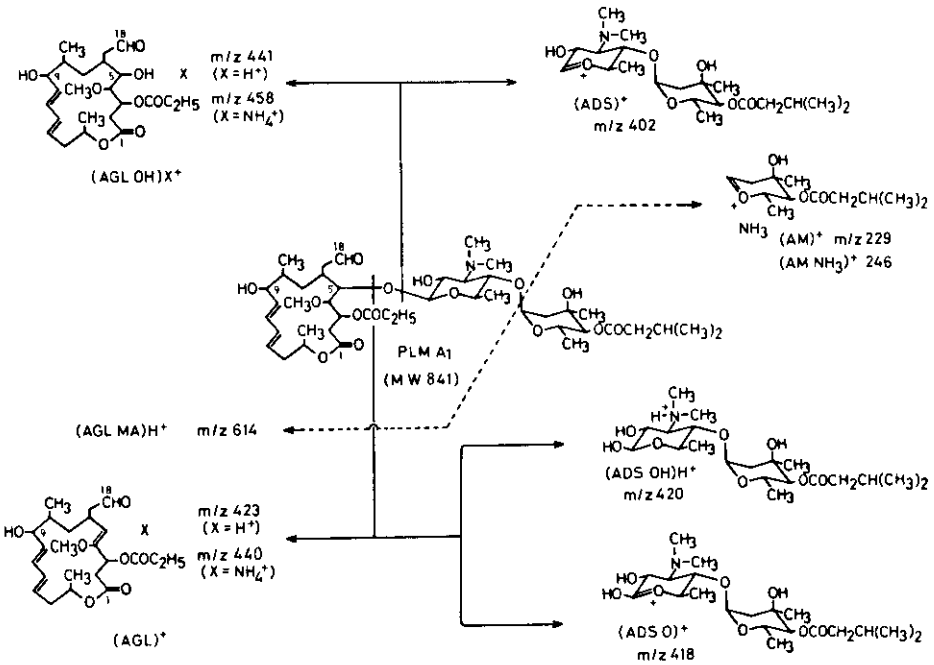


Fig. 2. Major fragmentation pathways of platenomycin A_1(1).

In both CI mass spectra, the protonated molecular ion (MH^+, m/z 842) forms the base peak, and in the CI(i-C_4H_{10}) mass spectrum, the molecular-ion cluster, such as $M\cdot C_3H_3^+$; m/z 880, $M\cdot C_3H_5^+$; m/z 882, $M\cdot C_3H_7^+$; m/z 884 and $M\cdot C_4H_9^+$; m/z 898 is weakly demonstrated. By contrast, no $M\cdot NH_4^+$ ion is observed in the CI(NH_3) mass spectrum. This behavior can be attributed to that the proton affinity (PA) of the macrolide molecule, which contains a dimethylamino group in sugar moiety, is higher than that of NH_3[5]. Formations of the ions at m/z 768 and 740 in both CI mass spectra correspond to loss of $C_2H_5CO_2H$ and $(CH_3)_2CHCH_2CO_2H$ from MH^+, respectively. The principal fragmentation pattern is illustrated in Fig. 2. The cleavage of glycosidic carbon-oxygen bond of the terminal sugar leads to the ions of aglycone-mycaminose $(AGL\cdot MA)H^+$; m/z 614 and acylmycarose $(AM)^+$; m/z 229. The former ion eliminates $C_2H_5CO_2H$ giving rise to the ion at m/z 540. The latter loses $(CH_3)_2CHCH_2CO_2H$ to give the ion at m/z 127. Fission of a glycosidic linkage between aglycone and acyldisaccharide, in principle, occurs in two ways. Cleavage at the aglycone side of the glycosidic linkage leads to the aglycone-derived ion, $(AGL)^+$; m/z 423 and the acyl-disaccharide ions $(ADS\cdot OH)H^+$; m/z 420 and $(ADS\cdot O)^+$; m/z 418. Similarly, the cleavage at the sugar side of the glycosidic linkage leads to $(AGL\cdot OH)H^+$; m/z 441 and $(ADS)^+$; m/z 402. The loss of acylmycarosyl moiety from $(ADS)^+$, $(ADS\cdot O)^+$ and $(ADS\cdot OH)H^+$ ions yields amino sugar

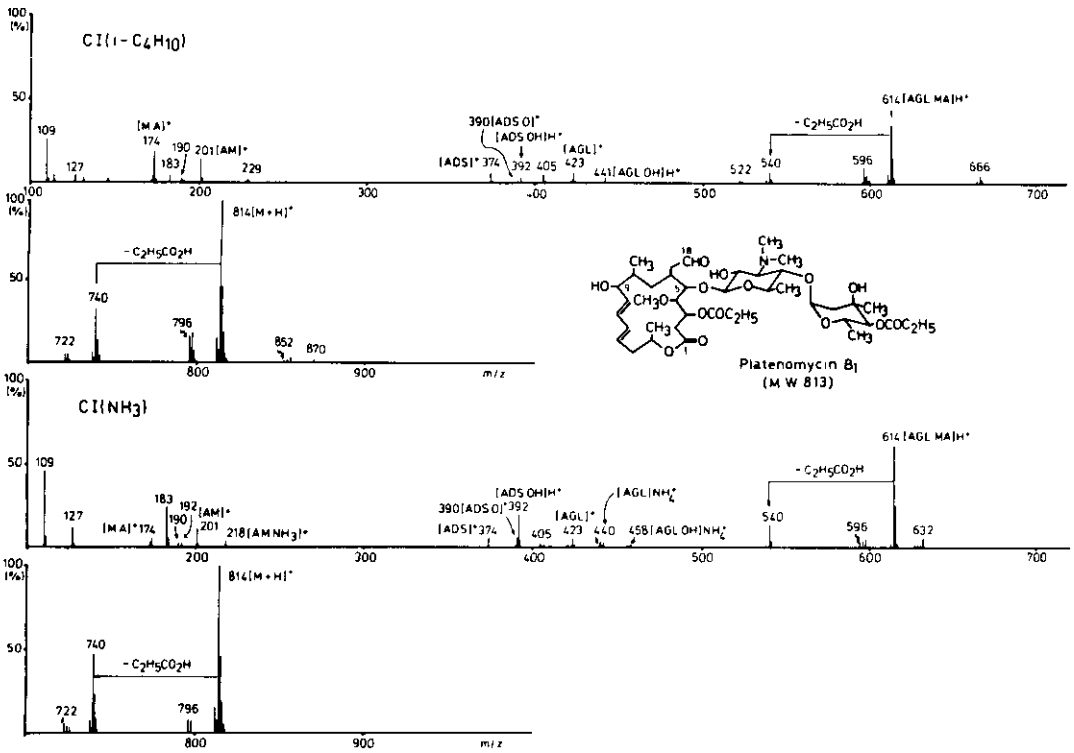
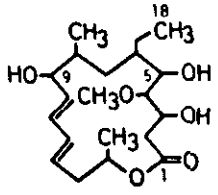


Fig. 3. Chemical ionization mass spectra of platenomycin B_1(2).

ions at m/z 174, 190 and 192, respectively. A set of these three ions derived from the amino sugar (mycaminose) has been analogously observed in the CI mass sprctra of desosamine containing macrolides[1]. In the CI(NH_3) mass spectrum, the major fragmentation pattern is similar to the case of the CI(i-C_4H_{10}) mass spectrum, except for the occurrence of the ions at m/z 246, 440 and 458 (Fig. 2). The even mass number of these ions suggests that they should contain a nitrogen atom.

CI mass spectra of 2 using i-C_4H_{10} and NH_3 as the reagent gases are shown in Fig. 3. The major fragmentation pattern is consistent with that of 1. The CI(i-C_4H_{10}/NH_3) mass spectra show the MH^+ ion at m/z 814 as the base peak, and the ion at m/z 740 corresponds to the loss of $C_2H_5CO_2H$ from MH^+. Aglycone-derived ions in the CI(i-C_4H_{10}) are observed at m/z 423 $(AGL)^+$ and 441 $(AGL{\cdot}OH)H^+$, whereas they appear at m/z 423 $(AGL)^+$, 440 $(423+17)^+$ and 458 $(441+17)^+$ in the CI (NH_3) mass spectrum. In both CI(i-C_4H_{10}/NH_3) mass spectra, acyldisaccharide-derived ions are found at m/z 374, 390 and 392, which correspond to $(ADS)^+$, $(ADS{\cdot}O)^+$ and $(ADS{\cdot}OH)H^+$, respectively, as shown in the CI mass spectra of 1. $(AM)^+$ ion at m/z 201 is acylmycarose ion and the ion at m/z 218 is also observed together with $(AM)^+$ ion in the case of CI(NH_3).

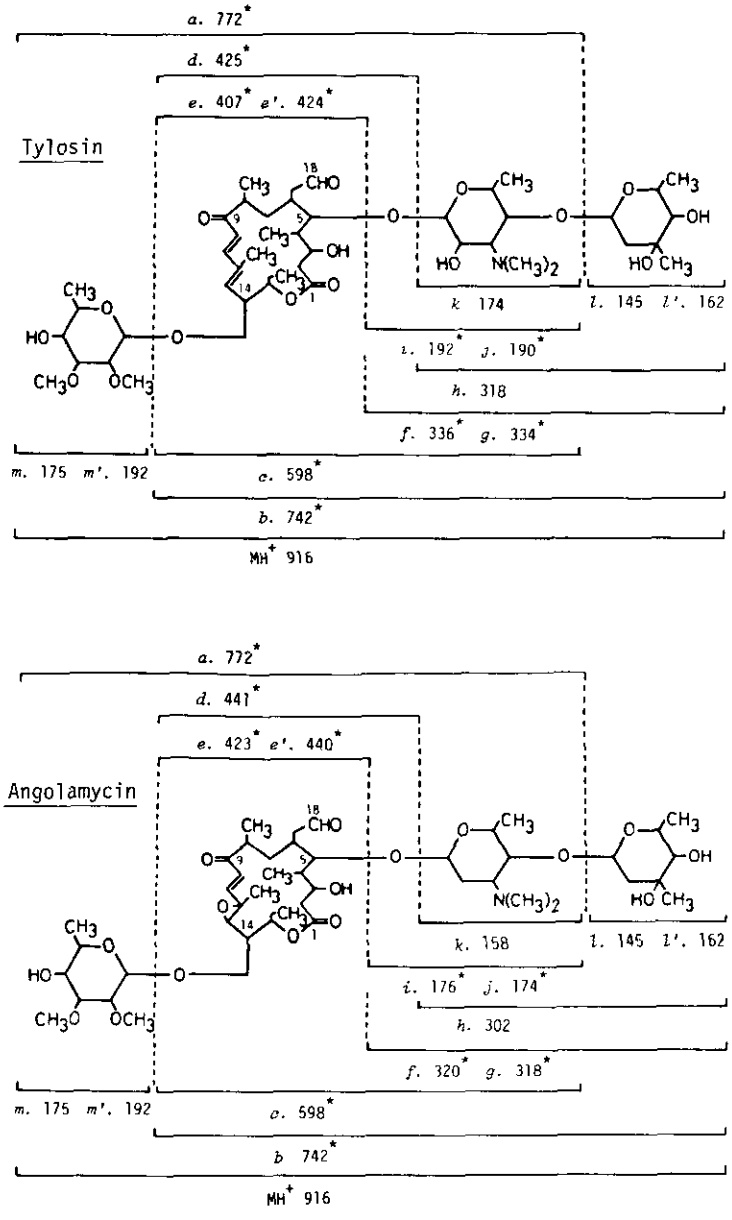
As already outlined, diagnostic fragmentations of the CI mass spectra of 1 and 2 with NH_3 reagent gas are similar to those observed with i-C_4H_{10}. However, fragment ions derived from acylmycarose and aglycone parts are observed to be shifted to a higher m/z value by 17 u in the CI (NH_3) mass spectra. The former (m/z 218) may be explained as $(AM{\cdot}NH_3)^+$ produced by participation of the reactant ion plasma. Proof of this phenomenon will be discussed later in connection with the CI mass spectra of Tylosin (3) and Angolamycin (4). The latter ions (m/z 440 and 458) can be expressed as $(AGL\text{-}H)NH_4^+$ and $(AGL{\cdot}OH)NH_4^+$. This observation can be attributed to that the PA of the aglycone part is rather low compared with that of NH_3. This result is also supported by the fact that the CI(NH_3) mass spectrum of Platenolide II (5)[6], which is structurally analogous to the aglycone part of 1 and 2, shows only $M{\cdot}NH_4^+$.



Platenolide II (5)

Chemical ionization mass spectra of Tylosin (3) and Angolamycin (4)

Tylosin (3) and Angolamycin (4) are the most complex 16-membered macrolides and are structurally isomeric with each other[7,8]. Diagnostic fragmentations of 3 and 4 can be assigned as illustrated in Fig. 4. The resulting CI mass spectra using i-C_4H_{10} and NH_3 differ in showing a much-reduced MH^+ intensity compared with those of 1 and 2. However, they have enough intensity



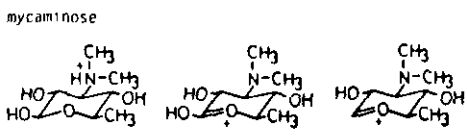
Asterisk indicate the ions accompained by hydrogen transfer

Prime indicate the characteristic ions in the CI(NH_3) mass spectrum

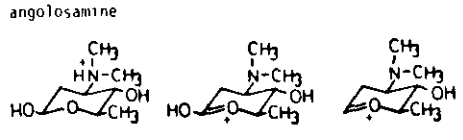
Fig. 4. Diagnostic fragmentations of Tylosin (3) and Angolamycin (4).

to determine their molecular weights. The expected cleavages of the glycosidic linkage lead to the formation of the ions a - m in the CI mass spectra of 3 and 4 (Fig. 4). The ions a, b and c corresponding to the loss of mycarose, mycinose and both neutral sugar units from MH^+ are common to the CI(i-C_4H_{10}/NH_3) mass spectra. However, the ions d and e derived from aglycone part are observed at the different m/z values for 3 and 4. Namely, above ions are detected at m/z 425 and 407 for 3, and at m/z 441 and 423 for 4 in the CI(i-C_4H_{10}). In the CI(NH_3), ammonium adduct ion e' is demonstrated in addition to the ion e. The difference of the m/z values of the ions d and e observed between in 3 and 4 is attributed to their individual chromophores in the aglycone parts. Disaccharide-derived ions f, g and h are also observed in the CI(i-C_4H_{10}/NH_3) mass spectra of 3 and 4. These three ions observed in 3 are found upwards 16 u in comparison with those observed in 4. This result is apparently consistent with their disaccharide structures. A similar rationalization can be advanced for the amino sugar-derived ions i, j and k. Cleavage of the glycosidic bonds at neutral sugars leads to the ions l and m corresponding to the mycarosyl- and the mycinosyl-derived ions, respectively. In the CI(NH_3) mass spectra, these fragments are observed as the NH_3-adduct ion anticipated from the results of 1 and 2.

Consequently, cleavage of the glycosidic linkage between the aglycone and the amino sugar occurs at both sides of glycosidic oxygen giving rise to the protonated type ion i and the oxonium type ions j and k, whereas cleavage of the glycosidic linkage with the neutral sugar moiety

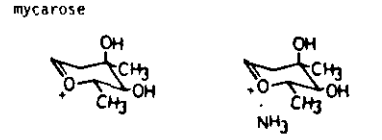


	i	j	k
CI(i-C_4H_{10})	m/z 192	m/z 190	m/z 174
CI(NH_3)	192	190	174
CI(ND_3)	196	193	176

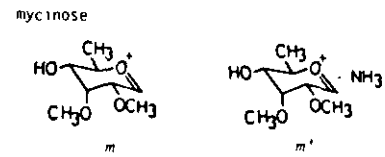


	i	j	k
CI(i-C_4H_{10})	m/z 176	m/z 174	m/z158
CI(NH_3)	176	174	158
CI(ND_3)	179	176	159

Fig. 5.



	l	l'
CI(i-C_4H_{10})	m/z 145	CI(NH_3) m/z 162
		CI(ND_3) 167



	m	m'
CI(i-C_4H_{10})	m/z 175	CI(NH_3) m/z 192
		CI(ND_3) 196

Fig. 6.

occurs at one side of the glycosidic oxygen giving rise to the only oxonium type ions *l* and *m*, or *l'* and *m'*.

In order to prove the ion structures of the sugar units in 3 and 4, shifting technique using ND_3 as the reagent gas has been performed[1]. Plausible structures of amino sugar-derived ions thus obtained, *i*, *j* and *k* are depicted in Fig. 5. The shifting values observed in the CI(NH_3/ND_3) mass spectra are consistent with the values expected for the ion structures. Similarly, plausible ion structures of the neutral sugar-derived ion *l'* and *m'* are shown in Fig. 6. The shifting values in this case can be explained in terms of the H-D exchange of active hydrogen and the addition of ammonia-d_3 molecule.

In conclusion, all CI mass spectra of the macrolides 1 - 4 examined using both i-C_4H_{10} and NH_3 as the reagent gas definitely give the MH^+ ion indicating the molecular weight. Major fragmentations are arisen from the cleavages of the glycosidic linkage. Therefore, the resulting fragment ions provide the useful information concerning the structures of the aglycone and sugar moieties. The glycosidic cleavages associated with the amino sugar moiety occur at both sides of glycosidic oxygen giving rise to the three types of the ions, whereas, with the neutral sugar moiety occur at the sugar side of glycosidic oxygen yielding the oxonium type ion. Besides, in the CI(NH_3) mass spectrum, the latter ion is also observed as the ammonia adduct ion. Above findings suggest that the CI(i-C_4H_{10}/NH_3) mass spectra provide easy discrimination between amino sugars and neutral sugars in natural glycosides. These results show that CIMS is practically potential for the characterization of basic macrolide intact molecules, comparing with EIMS.

EXPERIMENTAL

The chemical ionization mass spectra were obtained using a Shimadzu LKB 9000A mass spectrometer equipped with a high-pressure chemical ionization source. The source temperature was 250-260°C, while the samples were introduced through a direct inlet system. Reagent gas pressures were in the range of 0.2-0.3 Torr.

ACKNOWLEDGEMENT

We would like to thank Dr. M. Kawanishi, manager of Microbial Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd., for his generosity in providing macrolide samples.

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Received, 13th October, 1980