CHEMICAL IONIZATION MASS SPECTRA OF NEW MACROLIDE ANTIBIOTICS, M-4365 A_2 AND G_2

Makoto Suzuki*, Ken-ichi Harada, Naohito Takeda and Akira Tatematsu

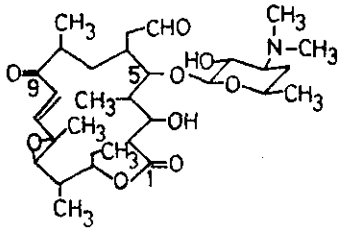
Faculty of Pharmacy, Meijo University, Tempaku-ku, Nagoya 468, Japan

Ammonia and isobutane chemical ionization (CI) mass spectra of basic 16-membered ring macrolide antibiotics, M-4365 A_2 and G_2, are described.

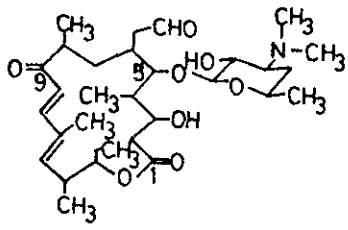
The spectra show protonated molecular ions (MH^+, m/e 582 and 566, respectively) as the base peak in all cases. Cleavage of desosamine moiety from M-4365 A_2 and G_2 affords the protonated (m/e 176)- and oxonium (m/e 174)-type ions. These ions are shown to be useful in the structural characterization of new macrolide antibiotics.

Chemical ionization mass spectrometry (CIMS) is useful for molecular-weight determination and structural characterization of relatively non-volatile natural products. Macrolide antibiotics, which possess a large ring lactone with sugar moieties, are also polar compounds and very difficult to get reliable EI mass spectra using their intact molecules.[1] There are only few papers of CIMS for this group, however, Mitscher has reported CI mass spectra of previously known 14- and 16-membered ring macrolide antibiotics using isobutane as a reactant gas.[2,3]

Basic macrolide antibiotics, M-4365 A_2 and G_2 are produced by *Micromonospora capillata* MCRL 0940 and their structures have recently been proposed as (I) and (II), respectively.[4] Therefore, we were interested to confirm the



M-4365 A_2 (I)



M-4365 G_2 (II)

structures of M-4365 A_2 (I) and G_2 (II) by CIMS. CIMS is the gas-phase reaction of reactant ions and the substrate, and is varied with the varieties of reactant gases. For the reactant gases, methane and isobutane have been widely used, however, ammonia and dimethylamine are employed effectively to polar natural products. In macrolide antibiotic chemistry, only isobutane has been used as a reactant gas of CIMS, therefore, we have determined to use ammonia besides isobutane to our further studies of M-4365 A_2 (I) and G_2 (II).

CI mass spectra of M-4365 A_2 (I, Fig.I) show an abundant protonated molecular ion (MH^+) peak at m/e 582 as the base peak using both reactant gases. With isobutane, small peaks at m/e 620, 624 and 638 are observed in higher mass region. They are a molecular-ion cluster resulting from the attachment of $C_3H_3^+$, $\underline{i}$-$C_3H_7^+$ and $\underline{t}$-$C_4H_9^+$-reactant ions, respectively to M-4365 A_2 molecule. In contrast to the behavior in isobutane, no molecular-ion cluster ($M{\cdot}NH_4^+$ and $M{\cdot}(NH_3)NH_4^+$) are observed in the ammonia CI mass spectrum, as is to be expected for high proton affinity of ammonia.[5,6] In both spectra, a peak appeared at m/e 566 corresponds to the loss of oxygen from MH^+ ion and such de-epoxydation process has been observed also in EI mass spectrum of peracetyl-angolamycin.[7]

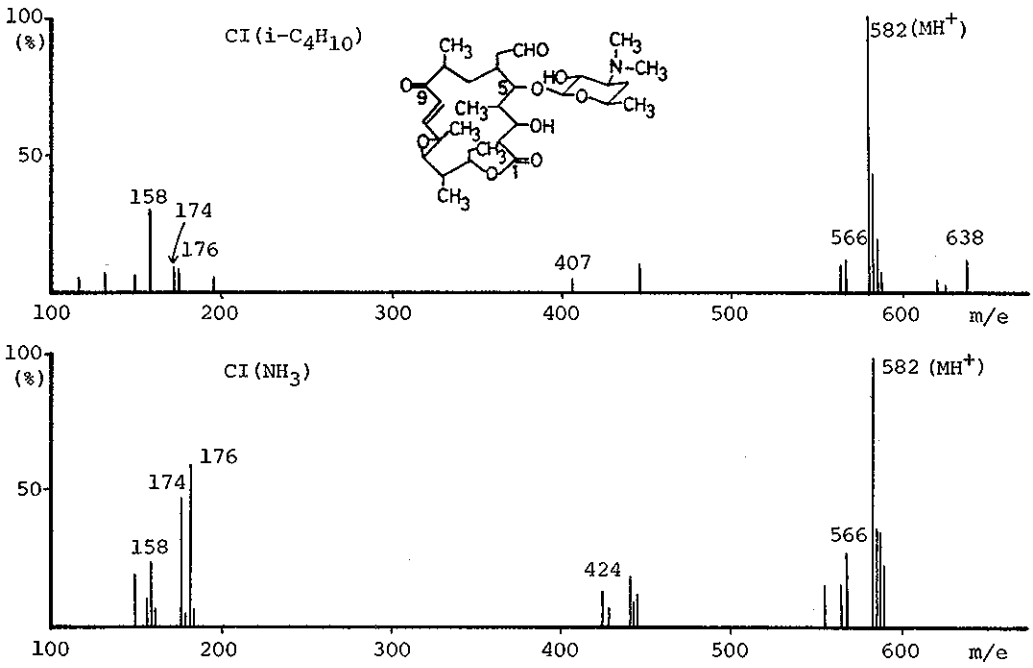


Fig.1. Chemical ionization mass spectra of M-4365 A_2.

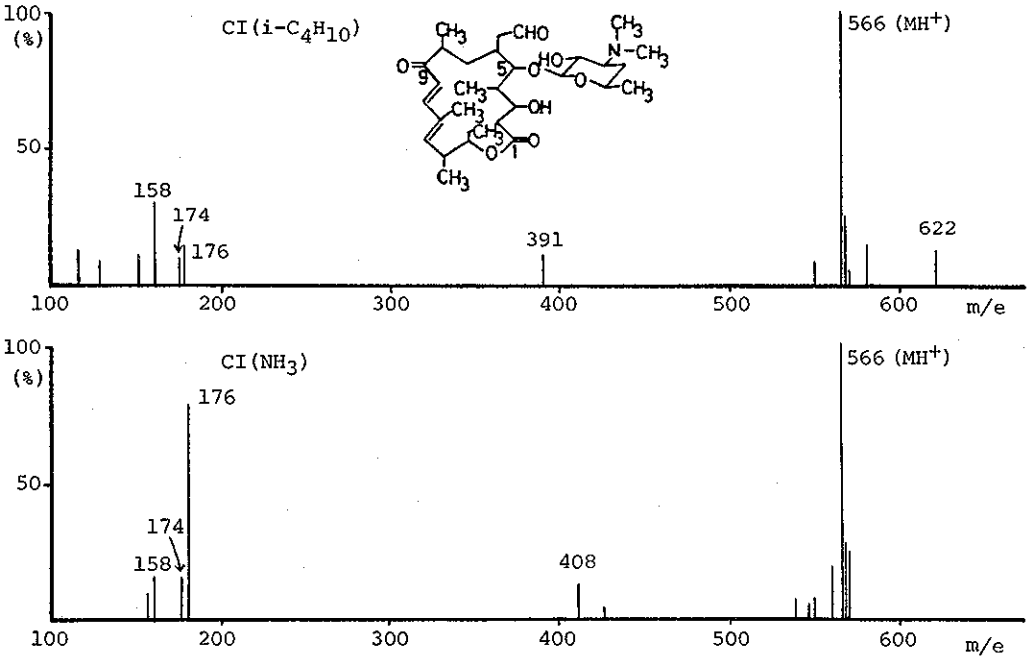
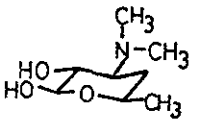


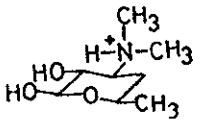
Fig.2. Chemical ionization mass spectra of M-4365 G_2.

As the major fragment ions, we consider that the aglycone ion at m/e 407 arises from the cleavage of the glycosidic bond and the protonation in isobutane CI mass spectrum, whereas the corresponding ion at m/e 424 in ammonia CI mass spectrum is formed by the addition of an ammonium ion (NH_4^+). The detailed mechanisms of these fragmentations are under investigation using model compounds.

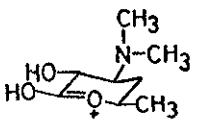
On the other hand, sugar (desosamine) ions are prominently observed at m/e 176, 174 and 158. The structures of these ions may be assigned as the protonated ion (IV, m/e 176) and the oxonium-type ions (V, m/e 174 and VI, m/e 158).



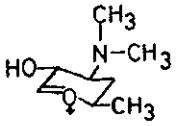
desosamine (III)



m/e 176 (IV)



m/e 174 (V)



m/e 158 (VI)

Isobutane and ammonia CI mass spectra of M-4365 G_2 (II) are quite similar to that of M-4365 A_2 (Fig.2). Namely, a protonated molecular ion peak at m/e 566 is observed as a base peak in both spectra. The cluster ion formed by attachment of t-butylcarbonium ion to M-4365 G_2 molecule appeared at m/e 622 in isobutane CI mass spectrum, however, no cluster ion is observed in ammonia CI mass spectrum. Aglycone ion of M-4365 G_2 at m/e 391 results from the cleavage of the glycosidic bond and the protonation in isobutane CI mass spectrum. However, the corresponding ion observed at m/e 408 in ammonia CI mass spectrum follows ammonium ion (NH_4^+) attachment. The sugar-derived ions in the low mass region are also similar to the case of M-4365 A_2.

As mentioned above, it is remarkably suggested that the cleavage of the glycosidic bond of M-4365 A_2 and G_2 gives two different sugar ions, one is a protonated ion at m/e 176, the other is a oxonium-type ion at m/e 174. In order to investigate these peaks, we measured CI mass spectrum of M-4365 G_2 (II) using

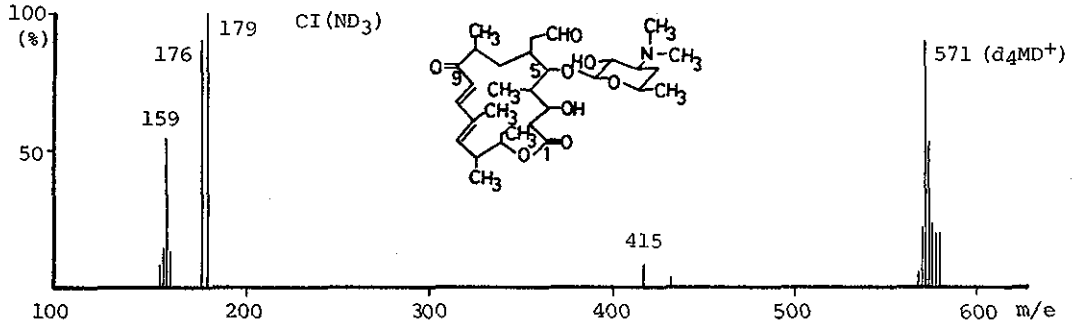


Fig.3. Chemical ionization mass spectrum of M-4365 G_2.

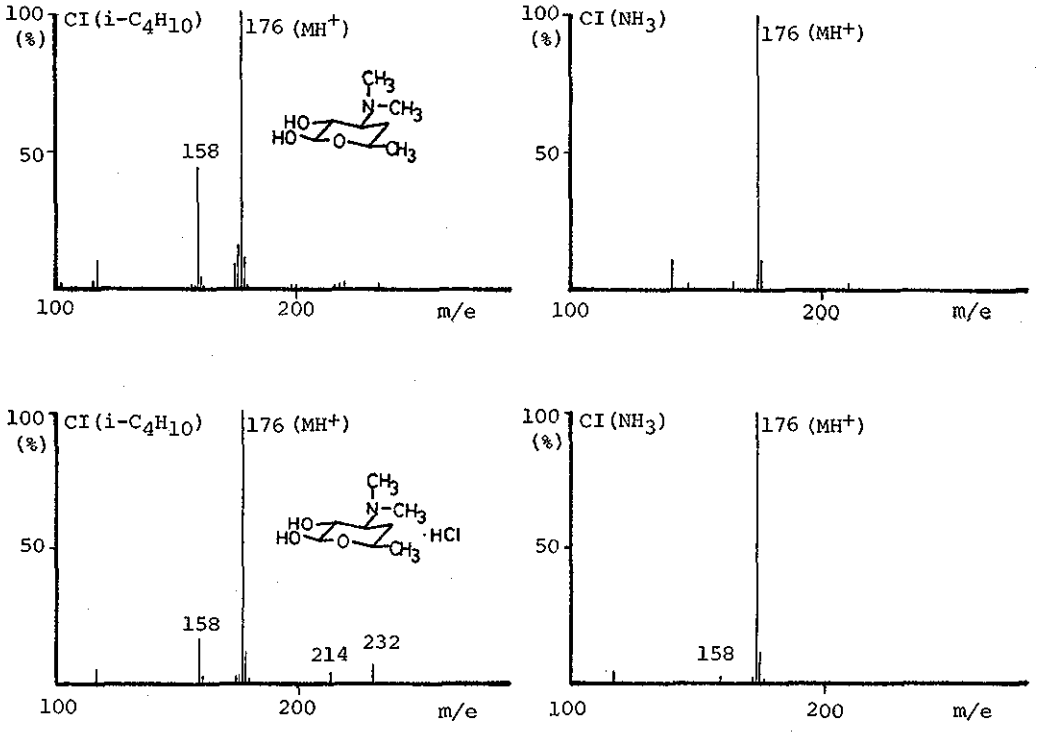


Fig.4. Chemical ionization mass spectra of desosamine and desosamine hydrochloride.

ammonia-d_3 (ND_3) as shifting reactant gas. Ammonia-d_3 CI mass spectrum of M-4365 G_2 (II) is shown in Fig.3. As is to be expected, each peak at m/e 566 (MH^+), 176, 174 and 158 in the ammonia CI mass spectrum shifts to m/e 571 (d_4MD^+), 179, 176 and 159, respectively in this spectrum. Consequently, the structures of the sugar-derived ions (IV, V and VI) are finally estimated by shifting technique. The detailed study of the hydrogen-deuterium exchange reaction on active hydrogens in this case will be published elsewhere. On similar view-point, we are interested in investigating CI mass-spectrometric behavior of desosamine molecule (III) itself. Isobutane and ammonia CI mass spectra of (III)-free base and hydrochloride are shown in Fig.4. It is quite unexpected that only protonated ion (m/e 176) is observed. This result suggests that an aglycone participates significantly in the cleavage of the glycosidic bond.

In conclusion, under isobutane and ammonia CI mass-spectrometric condition, M-4365 A_2 (I) and G_2 (II) give the protonated molecular ion (MH^+) as the most abundant peak and the fragmentation involving carbon-carbon bond fission does not occur. As the major fragmentation pathway, glycosidic bond cleavage is only observed. There is no difference between isobutane and ammonia CIMS for M-4365 A_2 (I) and G_2 (II) except cluster ion formation.

Experimental

The mass spectra were obtained using a Shimadzu LKB-9000B mass spectrometer equipped with chemical ionization source. Operating conditions were as follows: ion source temperature, 190°C ; electron energy, 500 eV ; accelerating voltage, 3.5 KV ; reactant gas pressure, 0.3 torr (isobutane, ammonia and ammonia-d_3) ; emission current, 500 μA. The samples were introduced into the ion source by means of a direct probe and volatilized at probe temperatures of approximately 230-240°C.

REFERENCES

1) (a) S. Inouye, T. Tsuruoka, T. Shomura, S. Omoto and T. Niida, J. Antibiotics, 1971, 24, 460 and 526.

(b) M. Suzuki, I. Takamori, A. Kinumaki, Y. Sugawara and T. Okuda, Tetrahedron Letters, 1971, 435.

(c) R. S. Jaret, A. K. Mallams and H. F. Vernay, J. Chem. Soc. Perkin I, 1973, 1389.

(d) H. Achenbach and W. Karl, Chem. Ber., 1975, 108, 759.

2) L. A. Mitscher and H. D. H. Showalter, J. Chem. Soc. Chem. Comm., 1972, 796.

3) L. A. Mitscher, H. D. H. Showalter and R. L. Foltz, J. Antibiotics, 1973, 26, 55.

4) A. Kinumaki, K. I. Harada, T. Suzuki, M. Suzuki and T. Okuda, J. Antibiotics, 1977, 30, 450.

5) D. F. Hunt, C. N. McEwen and R. A. Upham, Tetrahedron Letters, 1971, 4539.

6) I. Dzidic and J. A. McCloskey, Org. Mass Spectrom., 1972, 6, 939.

7) A.Kinumaki and M. Suzuki, J. Antibiotics, 1972, 25, 480.

Received, 18th July, 1977