Chemical Ionization Mass Spectra of Macrolide Antibiotics

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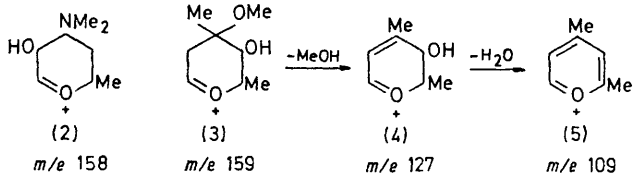
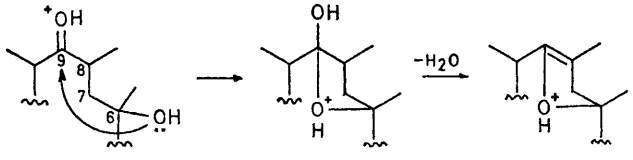
Summary Chemical ionization mass spectrometry is shown to be a valuable tool for structural analysis of certain macrolide antibiotics.

RECENT reports have shown that chemical ionization mass spectrometry is an extremely useful technique for the structural analysis of complex organic molecules such as polypeptides,[1] nucleosides,[2] alkaloids,[3] and drugs.[4] The

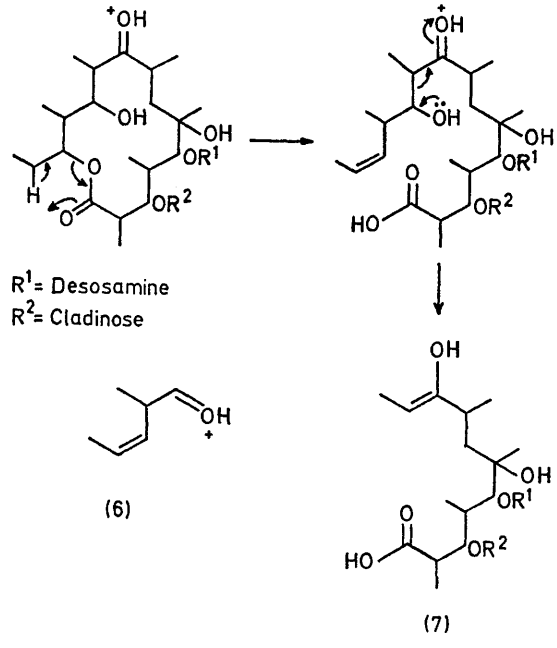


technique has now been applied to a number of clinically significant macrolide antibiotics. We present our most striking findings in advance of a detailed exposition for we feel the method has such promise that its use will soon become standard.



SCHEME 1

The electron impact mass spectra of the macrolide antibiotics are very complex with nearly all of the abundant ions in the low mass region. Consequently, the spectra are difficult to interpret. The chemical ionization mass spectra of the macrolide antibiotics, using isobutane as the reactant gas, normally show prominent protonated molecule ion peaks (MH^+). The fragmentation patterns are relatively simple but provide important structural information. The glycosidic bonds are particularly subject to cleavage under chemical ionization conditions giving abundant MH^+ -sugar ions as well as sugar fragment ions. Most of the remaining prominent fragment ions are due to loss of oxygenated substituents as neutral molecules, such as H_2O, MeOH, and $MeCO_2H$. The few important fragmentations involving C–C bonds that we have observed have well established solution chemistry counterparts.



SCHEME 2

The Figure compares the chemical ionization mass spectrum of erythromycin B (**1**) with its electron impact mass spectrum.[5] The base peak at *m/e* 718 is the protonated molecule ion, while the peaks at *m/e* 756 and 774 are due to adduct ions typically observed in isobutane c.i. mass spectra ($M^+ + C_3H_3$ and $M^+ + C_4H_9$ respectively). Consequently, assignment of the molecular weight is no

problem. The direct loss of cladinose from the protonated molecule ion, either with or without its glycosidic oxygen gives peaks at *m/e* 542 and 560 respectively. These fragmentations are substantiated by metastable ion peaks at the appropriate mass values (409·1 and 436·8). Loss of a neutral desosamine fragment from the molecule ion is not observed. However, the presence of the desosamine

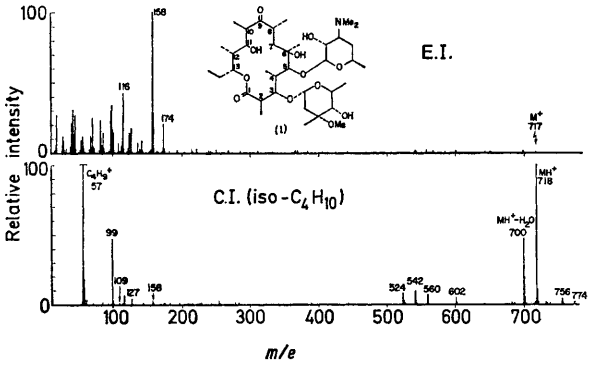


FIGURE. *Comparison of the electron impact (e.i.) and chemical ionization (c.i.) mass spectra of erythromycin B.*

fraction is indicated by the peak at *m/e* 158 corresponding to (**2**). Ions due to the cladinose are also observed in the low mass region of the spectrum [(**3**), (**4**), and (**5**)] and provide evidence for the sugar's OH and OMe substituents.

The abundant $MH^+ - H_2O$ ion (*m/e* 700) appears to be primarily due to a reaction sequence illustrated by Scheme 1, which is analogous to a known acid-catalyzed reaction which occurs rapidly in solution in those macrolides having a C(6)–OH conformationally suited for attack of the C(9)-carbonyl.[6] The c.i. mass spectra of macrolide molecules which lack the C(6)–OH such as oleandomycin[7] do not show a $MH^+ - H_2O$ peak of comparable intensity.

The intense peak at *m/e* 99 can be rationalized as resulting from protonation of the C(9)-ketone, ring-opening at the lactone linkage, and cleavage of the C(10)–C(11) bond *via* a retro-aldol type fragmentation (Scheme 2) to give ion (**6**). The prominent peak at *m/e* 602 corresponds to the protonated counterpart of fragment (**7**) minus one molecule of H_2O. The expected mass shifts for these fragmentations are observed in the c.i. mass spectra of other macrolide antibiotics. For example, in the c.i. mass spectrum of oleandomycin[7] the abundant retro-aldol generated ions occur at *m/e* 85 and 604, while erythromycin A[8] gives prominent peaks at *m/e* 115 and 602. Lankamycin[9] does not contain an OH group at C(11) (OAc instead) and ions resulting from the retro-aldol cleavage are not detected in its c.i. mass spectrum.

We thank the Abbott Laboratories for many of the macrolide samples, the National Institutes of Health and the American Foundation for Pharmaceutical Education for support of this work and Dr. W. D. Celmer (Pfizer) for the oleandomycin.

(*Received, 24th March* 1972; *Com.* 492.)

[1] W. R. Gray, L. H. Wojcik, and J. H. Futrell, *Biochem. Biophys. Res. Comm.*, 1970, **41**, 1111; A. A. Kiryushkin, H. M. Fales, T. Axenrod, E. J. Gilbert, and G. W. A. Milne, *Org. Mass Spectrom.*, 1971, **5**, 19.
[2] M. S. Wilson, I. Dzidic, and J. A. McCloskey, *Biochem. Biophys. Acta*, 1971, **240**, 623.
[3] H. M. Fales, H. A. Lloyd, and G. W. A. Milne, *J. Amer. Chem. Soc.*, 1970, **92**, 1590.
[4] H. M. Fales, G. W. A. Milne, and T. Axenrod, *Anal. Chem.*, 1970, **42**, 1432.
[5] L. A. Mitscher, R. L. Foltz, and M. I. Levenberg, *Org. Mass Spectrom.*, 1971, **5**, 1229.
[6] T. J. Perun, *J. Org. Chem.*, 1967, **32**, 2324.
[7] W. D. Celmer, *J. Amer. Chem. Soc.*, 1965, **87**, 1797; F. A. Hochstein, H. Els, W. D. Celmer, B. L. Shapiro, and R. B. Woodward, *ibid.*, 1960, **82**, 3225.
[8] D. R. Harris, S. G. McGeachin, and H. H. Mills, *Tetrahedron Letters*, 1965, 679; P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, jun., O. Weaver, U. C. Quark, R. R. Chauvette, and R. Monahan, *J. Amer. Chem. Soc.*, 1957, **79**, 6062.
[9] W. Keller-Schierein and G. Roncari, *Helv. Chim. Acta*, 1964, **47**, 78; R. S. Egan and J. R. Martin, *J. Amer. Chem. Soc.*, 1970, **92**, 4129.