CHEMICAL IONIZATION MASS SPECTROMETRY OF SOME REPRESENTATIVE 16-MEMBERED RING MACROLIDE ANTIBIOTICS

LESTER A. MITSCHER and H. D. HOLLIS SHOWALTER

Division of Natural Products Chemistry,
The Ohio State University
Columbus, Ohio 43210, U.S.A.

RODGER L. FOLTZ

Battelle's Columbus Laboratories
Columbus, Ohio 43210, U.S.A.

(Received for publication November 6, 1972)

Chemical ionization mass spectrometry, using isobutane as a reactant gas, is shown to be useful in the structural characterization of representative 16-membered ring macrolide antibiotics. The spectra of spiramycin I, spiramycin III, and niddamycin contain relatively intense protonated molecule ion peaks (MH^+) making establishment of molecular formulae straightforward. There are relatively few fragment ion peaks in comparison with the corresponding electron impact mass spectra, but these peaks are nonetheless highly significant. The major fragmentations observed involve sequential loss of the sugar moieties and loss of other small molecules such as water, acetic acid, and methanol. Scission of carbon-carbon bonds is uncommon. The nature of these reactions and the mechanisms responsible are discussed briefly.

Interest in the therapeutic properties of the macrolide antibiotics remains high and new chemical entities are being described at a substantial rate.[1~5] Recent discoveries frequently have been mixtures of closely related antibiotics differing from one another primarily in the number and nature of the acyl groups attached to the aglycone or to the various sugar moieties. Differentiation of these products from each other and from previously known substances, especially when only very limited quantities of some of the minor components are available, presents a substantial analytical challenge. Electron impact mass spectrometry (EI-MS) has proven extremely useful in this context.[1~5] We have recently reported that the chemical ionization mass spectrometry (CI-MS)[6] of 14-membered ring macrolide antibiotics provides an especially useful adjunct to the more traditional spectrometric methods in that abundant protonated molecule ions (MH^+) are observed, rendering establishment of molecular weights extremely facile.[7] Although relatively few prominent fragment ions are observed, they are easily interpreted and can provide very useful structural information. Because of the higher molecular weights and greater molecular complexity of many of the 16-membered ring macrolide antibiotics, it was of interest to see if CI-MS would be as useful in characterizing selected members of this group of antibiotics.

Materials and Methods

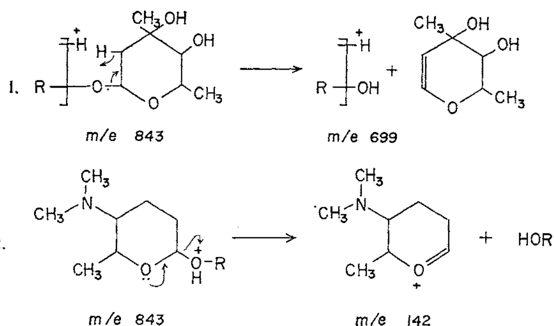
Spiramycins I and III[8] were obtained from Dr. T. NARA of the Kyowa Hakko Kogyo Company, and niddamycin[9] was obtained from Mr. L. THERIAULT of Abbott Laboratories (U.S.A.).

The mass spectra were obtained using an AEI MS-9 mass spectrometer equipped with a SRIC CIS-2 chemical ionization source. The isobutane pressure within the ion block was maintained at approximately 1 torr. The mass spectrometer was operated at 450 eV ionization voltage, 200 mA filament emission, OV ion repeller, and 8 KV accelerating voltage. The samples were introduced into the ion source by means of a heated probe and volatillized at probe temperatures of approximately 250°C. The ion source block was maintained at 200°C.

The mass spectra were recorded both on magnetic tape with subsequent computer-processing, and on an oscillographic recorder.

Results and Discussion

A variety of reactant gases have been used in CI-MS; however, we find that isobutane offers signficant advantages. Electron bombardment of the isobutane results in formation of a relatively stable, proton-rich and sterically hindered carbonium ion ($C_4H_9^+$) which readily transfers protons to atoms bearing nonbonded electron pairs.[10] The resulting protonated molecule ions (MH^+) are relatively stable, resulting in intense peaks in the CI-MS. The fragmentations which do occur most frequntly involve heterolysis of carbon-oxygen bonds by mechanisms which closely parallel those encountered in proton reactions in solution.[11] These considerations are best illustrated by examining the spectra of several examples.



The CI-MS of spiramycin I[8] (Fig. 1) shows an intense protonated molecule ion peak at *m/e* 843. The small peak at *m/e* 899 results from attachment of a *t*-butyl carbonium ion to the spiramycin molecule. Adduct ions of this type are normal in CI-MS, consequently, they do not

Fig. 1. Isobutane chemical ionization mass spectrum of spiramycin I.

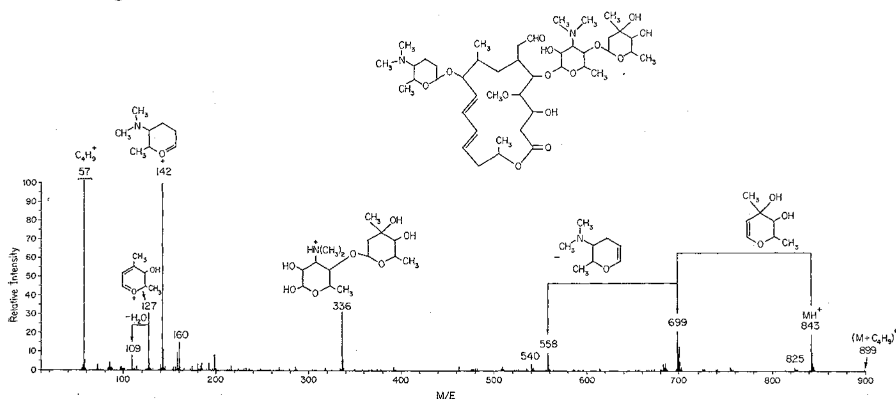


Fig. 2. Isobutane chemical ionization mass spectrum of spiramycin III.

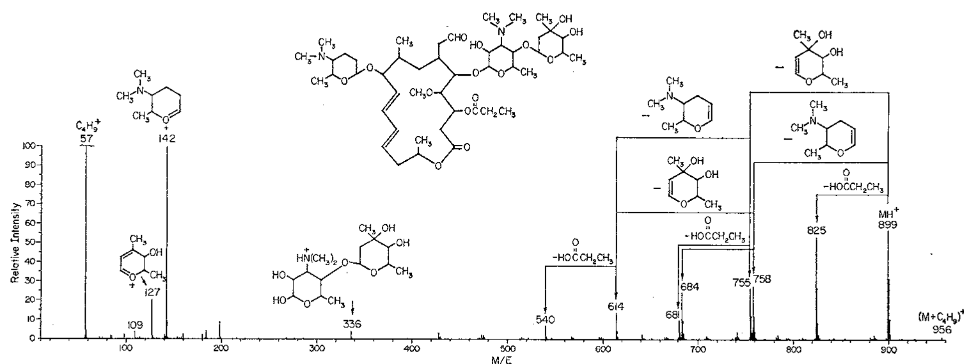
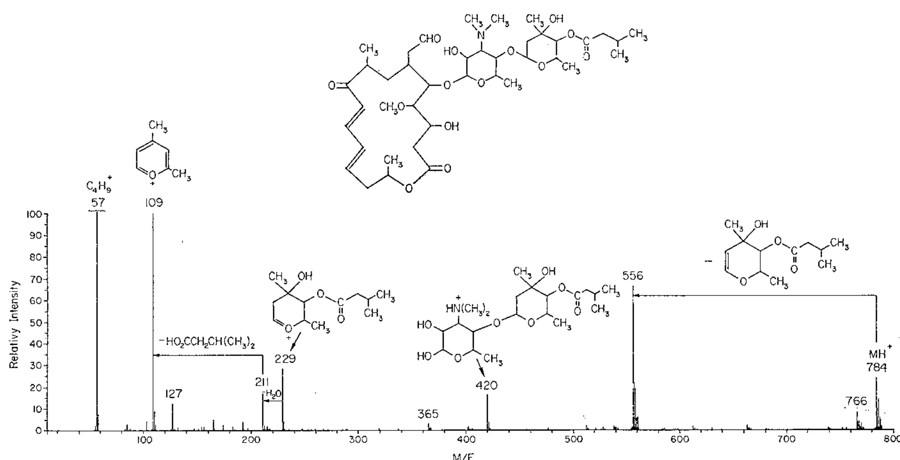


Fig. 3. Isobutane chemical ionization mass spectrum of niddamycin.



confuse interpretation of the spectrum. The most abundant fragment ions result from cleavage at glycosidic bonds, thereby facilitating identification of the sugar residues. The actual mechanism involved in the formation of these ions is being investigated; however, it appears to involve protonation at a heteroatom followed by a four-centered fragmentation of a glycosidic bond such as illustrated in Reaction (1).

Additional evidence regarding the structures of the sugars is provided by prominent sugar fragment ions in the low-mass region of the specrum. The *m/e* 142 ion is the base peak and probably results from protonation of the anomeric oxygen of the sugar followed by bond cleavage (Reaction 2).

The same process involving the other terminal sugar is the probable source of the *m/e* 145 ion, which readily loses a molecule of water to give the *m/e* 127 ion. The ion at *m/e* 336 corresponds to the protonated disaccharide and conveniently provides sugar-sequencing information.

Unlike EI-MS, doubly charged ions do not appear in isobutane CI-MS. If double protonation could occur, one would expect to see evidence for it in the CI-MS of the spiramycins since they have two basic centers (Me_2N-) relatively remote from each other. Even at high amplification no peak was detected at *m/e* 422.5 corresponding to the ^{13}C-isotope peak of MH_2^{++}.

Spiramycin III differs from spiramycin I in having a propionyl ester β to the lactone carbonyl rather than a hydroxyl group. This kind of substituent difference is common among the 16-membered ring macrolides; for example, the leucomycins and maridomycins. Under the isobutane CI-MS conditions fragmentation involving loss of propionic acid competes effectively with loss of the terminal sugars. Consequently, the CI-MS (Fig. 2) contains a series of prominent peaks (*m/e* 825, 758, 755, 684, 681, 614 and 540) corresponding to all possible combinations of losses of the propionic acid and the two terminal sugars from the protonated molecule ion (*m/e* 899). Therefore, the fragment ions in the isobutane CI-MS of spiramycin III not only identify the acyl group, but also clearly indicate that the acyl group is not attached to one of the sugars. Attachment of various acyl functions to the terminal sugar of a variety of macrolide antibiotics in this class is quite common, and the ease with which this distinction can be made is satisfying.

The isobutane CI-MS of niddamycin[9] (Fig. 3) is comparatively simple, partly because only two sugars are present. Loss of the terminal sugar from the abundant protonated molecule ion (*m/e* 784) gives the intense *m/e* 556 ion. Additional structural information regarding the terminal sugar is provided by the prominent series of peaks consisting of *m/e* 229, 211, and 109. These ions result from cleavage of the glycosidic bond by the process shown in Reaction 2, followed by loss of water and 2-methylbutyric acid from the sugar oxonium ion. The protonated disaccharide again gives a prominent peak (*m/e* 420) which establishes the sequential arrangement of the sugars and the 2-methylbutyrate ester. The intensity of the M+2 ion (*m/e* 786) indicates that there is some dihydro-niddamycin present in this sample.

From these representative examples it should be evident that isobutane chemical ionization mass spectrometry can provide useful structural information for the analysis of 16-membered ring macrolide antibiotics. The combination of this newer technique with the well-established methods of ir, uv, nmr and electron impact mass spectroscopy is a very powerful tool for structural elucidation and, combined with judicious degradation reactions, can lead to the very rapid determination of structure of new compounds in this and related series.

Acknowledgments

The authors wish to acknowledge the support of Abbott Laboratories, the National Institutes of Health, Division of Research Resources (Contract NIH-71-2483), and the American Foundation for Pharmaceutical Education for financial support.

References

1) SUZUKI, M.; I. TAKAMORI, A. KINUMAKI, Y. SUGAWARA & T. OKUDA: The structure of antibiotics YL-704 A and B. Tetrahedron Letters 1971: 435~438, 1971
2) SUZUKI, M.; I. TAKAMORI, A. KINUMAKI, Y. SUGAWARA & T. OKUDA: The structure of antibiotics YL-704 C_1,C_2, and W_1. J. Antibiotics 24: 904~906, 1971
3) MUROI, M.; M. ISAWA, H. ONO, E. HIGASHIDE & T. KISHI: Isolation of maridomycins and structure of maridomycin II. Experientia 28: 501~502, 1972
4) MUROI, M.; M. ISAWA & T. KISHI: Structures of maridomycin I, III, IV, V, and VI, macrolide antibiotics. Experientia 28: 129~131, 1972
5) KINUMAKI, A. & M. SUZUKI: Proposed structure of angolamycin (shincomycin A) by mass spectrometry. J. Antibiotics 25: 480~482, 1972

6) FIED, F. M.: Chemical ionization mass spectrometry. Accounts Chem. Research 1 : 42~49, 1968
7) MITSCHER, L. A.; H. D. H. SHOWALTER & R. L. FOLTZ: Chemical ionization mass spectra of macrolide antibiotics. J. Chem. Soc. Chem. Commun. 1972 : 796~797, 1972
8) OMURA, S.; A. NAKAGAWA, M. OTANI, T. HATA, H. OGURE & K. FURUHATA: Structure of the spiramycins (foromacidines) and their relationship with the leucomycins and carbomycins (magnamycins). J. Amer. Chem. Soc. 91 : 3401~3404, 1969
9) HUBER, G.; K. H. WALLHAEUSSER, L. FRIES, A. STEIGLER & H. L. WEIDENMUELLER: Niddamycin, ein neues Makrolid-Antibiotikum. Ärzneimittelforsch. 12 : 1191~1195, 1962
10) FIELD, F. M.: Chemical ionization mass spectrometry. IX. Temperature and pressure studies with benzyl acetate and *t*-amyl acetate. J. Amer. Chem. Soc. 91 : 2827~2839, 1969
FIELD, F. H.: Chemical ionization mass spectrometry. X. Temperature studies with substituted benzyl acetates. J. Amer. Chem. Soc. 91 : 6334~6341, 1969
11) MILNE, G. W. A.; T. AXENROD & H. M. FALES: Chemical ionization mass spectrometry of complex molecules. IV. Amino acids. J. Amer. Chem. Soc. 92 : 5170~5175, 1970