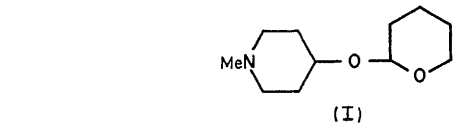
Fragmentation Process Occurring in Proton-transfer Chemical-ionization Mass Spectrometry

By RODGER L. FOLTZ* and ALLISON F. FENTIMAN, JUN.
(*Battelle, Columbus Laboratories, Columbus, Ohio* 43201)
and LESTER A. MITSCHER and H. D. HOLLIS SHOWALTER
(*College of Pharmacy, The Ohio State University, Columbus, Ohio* 43210)

Summary The isobutane chemical-ionization mass spectra of deuterium-labelled model compounds show that fragmentation can occur at locations remote from the site of protonation.

CHEMICAL-IONIZATION (CI) mass spectrometry is a useful complement to electron-impact (EI) mass spectrometry, primarily because of its ability to give prominent peaks in the molecular ion region. Further, the fragmentation observed in CI mass spectra can provide useful information for identification and structure determination. The fragmentation occurring when methane or isobutane is used as the reactant gas is generally simpler and easier to interpret than that displayed in the corresponding EI mass spectra.

The mechanisms by which complex molecules fragment after ionization by proton transfer under chemical-ionization conditions have received little attention. Field, Munson, and Becker[1] have proposed a process of random attack followed by localized reaction to rationalize the methane CI mass spectra of aliphatic hydrocarbons; in contrast, the CI fragmentation of compounds containing nucleophilic centres is generally considered to involve protonation at a nucleophilic centre, with fragmentation occurring at that location.[2,3] While the latter mechanistic view permits rationalization of the CI mass spectra of many compounds, it does not adequately explain some of the prominent fragment ions observed in the CI mass spectra of many polyfunctional molecules. The isobutane CI mass spectra of certain macrolide antibiotics show prominent fragment ions resulting from sequential loss of several neutral fragments.[4,5] We have suggested that formation of the ions corresponding to loss of neutral sugar fragments from the protonated molecule ion of a macrolide antibiotic involves protonation remote from the glycosidic linkage followed by a four-centred fragmentation of the glycosidic bond.[5] We have now obtained data on deuterium-labelled model systems which support this mechanism.



(I)

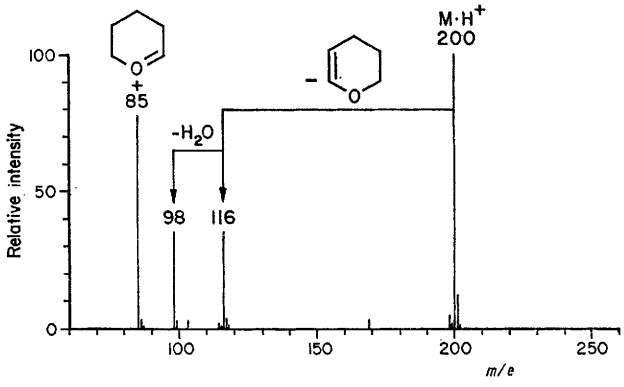


FIGURE. Isobutane chemical-ionization mass spectrum of the model compound.

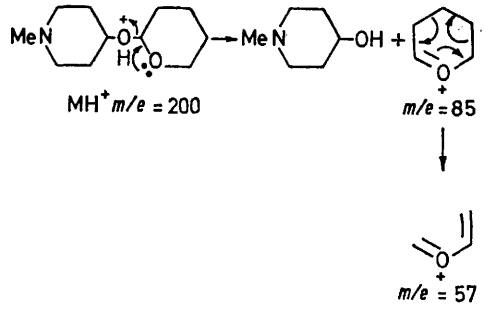
The tetrahydropyranyl ether of *N*-methylpiperidin-4-ol (I) was chosen as a model because it contains both a basic amine and an ether linkage of a glycosidic type. The isobutane CI mass spectrum (see Figure) of (I) shows the

TABLE. Comparison of ion-abundance ratios for deuteriated and undeuteriated (I)[a]

Ion-abundance ratios	Measured ratios[b] undeuteriated (I)	Calculated ratios based on natural abundance isotopes	Measured ratios[b] deuteriated (I)	Percentage of ions containing one deuterium
201/200	0·114 ± 0·006	0·123	0·340 ± 0·012	22·6
117/116	0·073 ± 0·002	0·067	0·178 ± 0·005	10·5

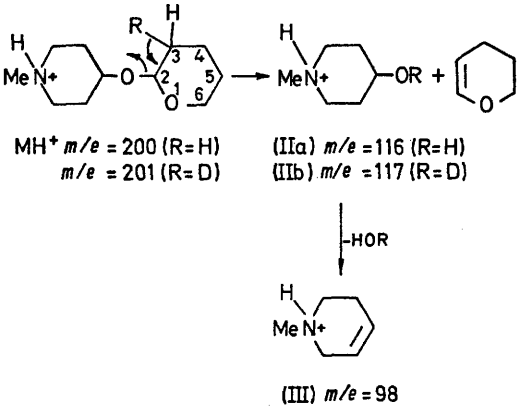
[a] The samples were introduced into a Finnigan 1015 quadrupole mass spectrometer *via* the gas chromatograph inlet using methane as the carrier–reactant gas: ion source temperature, 200 °C; pressure, 0·5 Torr; ionizing voltage, 100 eV; and ion repeller, 0 V.
[b] The ± values represent the standard deviation for three consecutively recorded mass spectra.

expected prominent fragment ions corresponding to fragmentation of the ether linkage. The abundant *m/e* 85 ions undoubtedly result from fragmentation *via* Scheme 1, while Scheme 2 provides a plausible rationalization for the formation of the ions at *m/e* 116 and 98. Protonation on the ring oxygen cannot easily lead to fragmentation of (I), so this process would contribute intensity only to MH^+.



SCHEME 1

The ion at *m/e* 57, formed by a *retro*-Diels–Alder cleavage of the *m/e* 85 ion, is isobaric with the t-butyl carbonium ion, so it is not detected in the isobutane CI mass spectra. However, it is prominent in the methane CI mass spectra of the tetrahydropyranyl ethers examined.

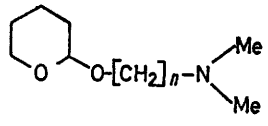


SCHEME 2

Metastable ion peaks were observed at the calculated masses for both fragmentations in Scheme 2 (*m/e* 200 $\xrightarrow{[67\cdot3]}$ 116 $\xrightarrow{[82\cdot8]}$ 98), thereby indicating that fragment ion (IIa) is formed directly from the protonated molecule ion and not by reprotonation of the neutral amino-alcohol formed in Scheme 1. Consequently, one of the hydrogen atoms in (IIa) must have migrated from the tetrahydropyranyl nucleus. In order to establish the origin of the migrating hydrogen, compound (I) was prepared containing deuterium in the 3-position of the tetrahydropyranyl ring, and its isobutane CI mass spectrum was examined.

If Scheme 2 is the dominant mechanism for the formation of fragment ion (II), the abundance of the deuterium-containing ion, (IIb) (*m/e* 117), relative to the undeuteriated ion, (IIa) (*m/e* 116), should be one-half the abundance of the deuterium-containing protonated molecule ion (*m/e* 201) relative to the undeuteriated protonated molecule ion (*m/e* 200), since either of the two hydrogens at the 3-position could migrate. Within the accuracy of the experiment, this is exactly what is observed. Furthermore (III) formed from (IIb) is substantially deuterium-free.

In order to investigate the potential generality of these processes, model compounds (IV) were prepared and examined. In these cases, the distance between the nitrogen and the ether linkage is varied by varying the length of the alkyl chain. The samples were introduced into the mass spectrometer *via* the gas chromatographic inlet. Methane



(IV) n = 2, 3, 5

was used as the reactant gas because it is a more convenient carrier gas than isobutane. These changes did not fundamentally alter the fragmentation patterns. Methane gives greater fragmentation and correspondingly smaller MH^+ peaks, although increased chain length increases the MH^+ intensity once again. Thus, moving the basic site further away from the anomeric site exerts only a quantitative effect on the peaks observed. With a single exception, the same cleavages occur with macrolide antibiotics, cyclic, and linear models. That exception is the occurrence of an α-cleavage fragment ($CH_2 = \overset{+}{N}Me_2$) that is prominent in the linear models but not observed in the cyclic molecules.

In none of the cases examined by us have we evidence of double protonation.

These results are consistent with the CI fragmentation of nucleosides using CH_4 and C_2H_4 as reactant gases.[6] It is evident that the protonated molecule ions resulting from chemical ionization using isobutane or methane as reactant gases have sufficient internal excitation energy to cause loss of labile substituents as neutral molecules, even when the substituents are located at positions remote from the site of protonation.

(*Received*, 23*rd July* 1973; *Com.* 1063.)

[1] F. H. Field, M. S. B. Munson, and D. A. Becker, Advances in Chemistry Series, No. 58, American Chemical Society, Washington, 1966, pp. 167–192.
[2] M. S. B. Munson, *Analyt. Chem.*, 1971, **43**, 28A.
[3] G. W. A. Milne, T. Axenrod, and H. M. Fales, *J. Amer. Chem. Soc.*, 1970, **92**, 5170.
[4] L. A. Mitscher, H. D. H. Showalter, and R. L. Foltz, *J.C.S. Chem. Comm.*, 1972, 796.
[5] L. A. Mitscher, H. D. H. Showalter, and R. L. Foltz, *J. Antibiotics*, 1973, **26**, 55.
[6] M. S. Wilson, I. Dzidic, and J. A. McCloskey, *Biochim. Biophys. Acta*, 1971, **240**, 623.