vealed that the nitrobenzenesulfonyl group shows a spectral shift in the direction of a more hydrophobic environment. There is nothing inconsistent with these results and the more polar environment of the OH group reported here. The explanation lies in the heterogeneity of the active site and the particular effectiveness of reporter groups in probing micro regions. The specificity pattern of chymotrypsin certainly indicates that hydrophobic bonding must play a key role in the attraction of substrates, e.g., the increased activity of phenylalanine and tyrosine derivatives in preference to derivatives of alanine and glycine. Thus the specificity site undoubtedly contains a hydrophobic region and this hydrophobic region probably accounts for the spectral shifts observed by Kallos and Avatis.[35] The same hydrophobic bonding probably occurs in our compound which contains a nitrobenzene portion. This hydrophobic bonding of the aromatic portion of the phenol, however, must place the OH of the phenolic group in a microscopic environment more polar than water, e.g., next to the positive charge of histidine. The combination of hydrophobic and hydrophilic regions is a key to active site behavior, the subtleties of their juxtaposition undoubtedly playing a key role in enzyme action. Reporter groups tailored to explore different facets of the active site will therefore be particularly valuable in exploring these subtleties. The recent demonstration that a change in the relative orientation of bromacetamido and phenolic groups causes changes in the difference spectra is consistent with this interpretation.[36] Different techniques may also provide special new insights. The nuclear magnetic techniques of Cohn,[37] McConnell,[38] Baldeschwieler,[39] and their co-

workers look particularly attractive in this regard. The use of fluorescent probes exploited so well by Weber,[40] Stryer,[41] and Edelman[42] also provide new information about the binding of small molecules in specific locations.

The elegant X-ray crystallographic studies of Phillips, et al.,[43] and Kendrew, et al.,[44] describe specific substrate–enzyme and small molecule–protein interaction in ways that would never be possible by more indirect physical probes, but the existence of conformational changes in solution and the need to probe the relationship of a wide variety of ligand–protein interactions mean that X-ray crystallography alone may not be able to probe all the interactions at the enzyme surface. Thus, a demand is created for complementary tools of which the reporter group may represent a powerful example.[45]

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(40) G. Weber, Biochem. J., 51, 155 (1952); E. Daniel and G. Weber, Biochemistry, 5, 1893 (1966).
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(43) C. C. F. Blake, D. F. Koenig, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, Nature, 206, 757 (1965).
(44) J. Kendrew, Brookhaven Symp. Biol., 15, 216 (1962).
(45) NOTE ADDED IN PROOF. The structure of chymotrypsin has recently been reported by B. W. Mathews, P. B. Sigler, R. Henderson, and D. M. Blow (Nature, 214, 652 (1967)). There is no conflict between the conclusions of this article and that structure where the facts are relevant. On the other hand, the deductions about binding and the pK perturbations cannot be established or rejected by mere observation of the structure, and, therefore, must await further work.

(36) A. Conway and D. E. Koshland, Jr., Biochim. Biophys. Acta, 133, 593 (967).
(37) W. J. O'Sullivan and M. Cohn, J. Biol. Chem., 241, 3116 (1966).

Communications to the Editor

Metastable Ion Characteristics. II.[1]
Variation of Metastable Ion Abundances in Mass Spectra with Vibrational Degrees of Freedom[2]

Sir:

Recent communications have described the use of the mass, relative abundance, and kinetic energy of formation of "metastable ions" resulting from the decomposition of a particular ion in order to characterize the structure and energy state of that ion.[1,3] The abundances of the different ions resulting from the metastable decompositions of ions of a particular structure were

constant relative to each other, but there was indication that the abundances of these ions relative to that of the precursor ion decreased with molecular weight.[1] Careful abundance measurements for 17 different metastable ion transitions now indicate that the ratio of the log abundance of a particular "metastable ion" relative to the abundance of ions of the same elemental composition as the precursor is *inversely proportional to the number of vibrational degrees of freedom in the original molecule*, as shown in Figures 1 and 2.[4] This relationship is a distinctive characteristic of the structure of the precursor ion and can be measured for many systems for which the other "metastable ion characteristics[1,3]

(1) Part I: T. W. Shannon and F. W. McLafferty, J. Am. Chem. Soc., 88, 5021 (1966).
(2) The generous financial support of the National Institutes of Health (GM 12755) is gratefully acknowledged. The Hitachi RMU-6D mass spectrometer used in this work was purchased through National Science Foundation Grant GP 4335.
(3) (a) F. W. McLafferty, M. M. Bursey, and S. M. Kimball, J. Am. Chem. Soc., 88, 5022 (1966); (b) F. W. McLafferty and M. M. Bursey, ibid., 88, 5023 (1966); P. Brown and C. Djerassi, ibid., 89, 2711 (1967).

(4) Relative peak heights measured from spectra recorded on a Hitachi RMU-6D mass spectrometer with an electron energy of 75 v. Data for 2-nonadecanone were measured on a CEC 21-110 with defocusing of the normal ions by the electrostatic analyzer;[5] relative abundances obtained by this technique for the other 2-alkanones were essentially identical with data from the Hitachi instrument.
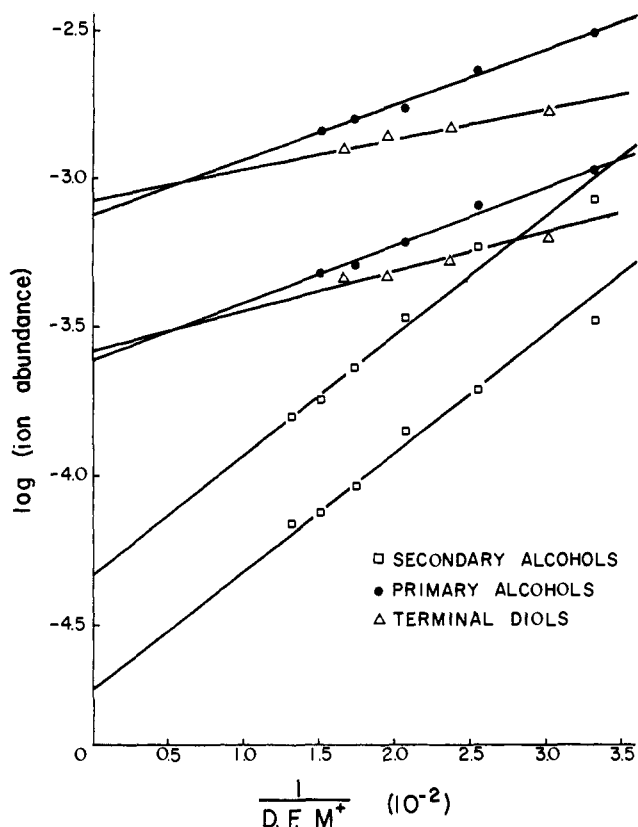(5) T. W. Shannon, T. E. Mead, C. G. Warner, and F. W. McLafferty, Anal. Chem., 39, 1748 (1967).

Figure 1. Correlation of "metastable ion" abundances with the number of vibrational degrees of freedom in homologous alkanols. The upper line of each pair corresponds to the metastable transition $C_2H_5O^+ \rightarrow H_3O^+ + C_2H_2$ ($m^*_{8.0}$), and the lower to $C_2H_5O^+ \rightarrow CHO^+ + CH_4$ ($m^*_{18.7}$).

Figure 2. Correlation of "metastable ion" abundances with the number of vibrational degrees of freedom. n-2-Alkanones plus 2,4-pentanedione, 4-hydroxy-4-methyl-2-pentanone, and 5-methyl-2-hexanone: $m^*_{3.9}$, $C_3H_6O^{·+} \rightarrow CH_3^+ + C_2H_3O^·$ (lower line); $m^*_{31.9}$, $C_3H_6O^{·+} \rightarrow C_2H_3O^+ + CH_3^·$ (upper line). n-3-Alkanones, $m^*_{25.7}$, $C_4H_8O^{·+} \rightarrow C_2H_3O^+ + C_2H_5^·$ (upper line); $m^*_{45.1}$ $C_4H_8O^{·+} \rightarrow C_3H_5O^{·+} + CH_3^·$ (lower line). n-4-Alkanones, $m^*_{21.5}$, $C_5H_{10}O^{·+} \rightarrow C_2H_3O^{·+} \rightarrow C_3H_7^·$ (lower line; 4-decanone is omitted and other data are of poorer accuracy because of interference by $m^*_{21.75}$); $m^*_{39.1}$, $C_5H_{10}O^{·+} \rightarrow C_3H_6O^{·+} + C_2H_4$ (upper line).

are not distinctive; application of this relationship to a particular problem of ion structure is described in an accompanying communication.[6] Of additional importance, we feel that an understanding of the basis of this rather surprising relationship should provide additional insight into the origin of mass spectra.

According to the quasi-equilibrium theory,[7] the rate of a decomposition reaction is a function of the internal energy of the precursor ion. The precursor ions decomposing in the "metastable" drift region will have a characteristic range of energies, so that the "metastable ion" abundances will be indicative of the abundance of precursor ions formed with these internal energies. Thus the $m^*_{8.0}$ and $m^*_{18.7}$ ions of Figure 1, formed from the common precursor $C_2H_5O^+$, exhibit a constant abundance ratio; this also indicates nearly identical activation energies for these transitions. Lowering the energy of the bombarding electrons does not change this ratio[8] nor the kinetic energy released in the "flat-topped" metastable transition $m^*_{18.7}$, supplying further evidence that the precursor ions have a characteristic energy distribution.

Thus for the formation of a particular primary ion product, such as the $C_2H_5O^+$ ions of Figure 1, in a homologous series of compounds, these data indicate

(6) F. W. McLafferty and W. T. Pike, *J. Am. Chem. Soc.*, **89**, 5953 (1967).

(7) A. L. Wahrhaftig, "Mass Spectrometry," R. I. Reed, Ed., Academic Press Inc., New York, N. Y., 1965, p 137, and references cited therein.

(8) But a lower value is observed for the "metastable" abundance relative to the total $C_2H_5O^+$ ions.
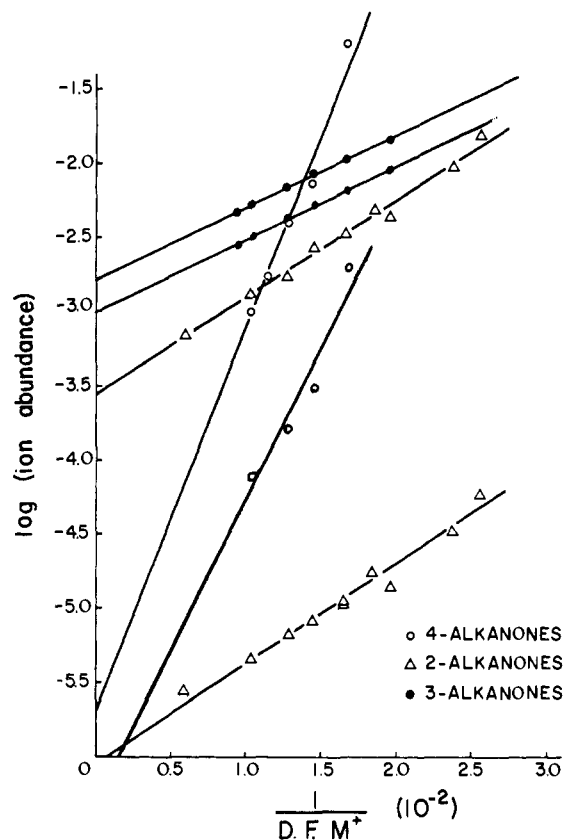
that the log abundance of ions formed with a particular energy (at least, those energies giving rise to metastable transitions) is inversely proportional to the number of vibrational degrees of freedom in the parent molecular ion. A possible explanation requires that a substantial characteristic part of the internal energy of the molecular ion arises from the presence of the functional group.[9] An increase in the molecular size which adds higher energy molecular orbitals then mainly affects the internal energy by providing more degrees of freedom over which the energy can be randomized.[10] Thus if the energy in a fragment ion is a function of the energy originally in the corresponding portion of the molecular ion, the energy of the fragment ion will also be a function of the number of degrees of freedom in the molecular ion.

(9) H. J. Svec and G. A. Junk (*J. Am. Chem. Soc.*, **89**, 790 (1967), and references cited therein) show that the ionization potentials of a homologous series of substituted alkanes are nearly identical; in the same way for these compounds the lowest lying molecular orbital may be of almost the same energy for each homolog, as this orbital should involve the same functional group in each.

(10) Note that the correlation of Figure 2 appears to be independent of chain branching. The variable effect (often small) of adding another functional group may be due to the addition of a new low-energy molecular orbital, to the interaction of the substituents, or to the difference in the density of states; this effect is being investigated further.

The abundance of the primary product ions, such as $C_2H_5O^+$ of Figure 1, vs. that of the molecular ions generally increases with an increase in the number of degrees of freedom; this could be due to multiple paths of formation and decomposition and to the effect of structure on bond strengths. This emphasizes the advantage of using the product ion yield of the same metastable decomposition reaction as a measure of internal energy distribution in the precursor.

Note in Figure 1 that the primary alcohols show different slopes than those of the secondary alcohols, despite the fact that the other "metastable ion characteristics" of their $C_2H_5O^+$ ions are identical.[1] A possible explanation is that the $C_2H_5O^+$ ions with sufficient energy to undergo metastable decomposition have rearranged to a common structure, such as the oxirane ion postulated previously,[1] but that the low-energy $C_2H_5O^+$ ions from the primary and secondary alcohols have the unrearranged structures $HOC_2H_4^+$ and $CH_3CH=OH^+$, respectively.[11]

Thus the degree of freedom effect appears to offer a useful way to correct for differences in energies so that the abundances of "metastable ions" can be used to characterize the structure of the precursor. Also the ratio of abundances of different metastable ions from the same precursor ion can be used to characterize the structure of that ion independent of its energy, with the qualification that isomerization during the metastable decompositions can yield the same abundance ratios for precursor ions of different structure.

Acknowledgment. We are indebted to A. L. Wahrhaftig, T. W. Shannon, and H. W. Brown for very helpful discussions on many important aspects of this work.

(11) Dr. T. W. Shannon points out for the metastable transitions

$$CH_3CH=OH^+ \xrightarrow{m^*_{18.7}} CHO^+ + CH_4$$

$$CH_3CH=OH^+ \xrightarrow{m^*_{8.0}} H_3O^+ + C_2H_2$$
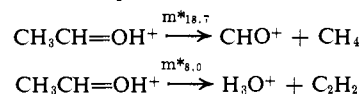
that the heats of reaction involving ground-state species are $\Delta H_1 = 58$ and $\Delta H_2 = 66$ kcal/mole.[12] This agrees quite well with the observed kinetic energy released[1] of 10 and 0 kcal, respectively, for these reactions assuming that these kinetic energies represent the activation energies for the reverse process and that activation energies for the forward process are the same.

(12) Using heats of formation for CH_3CHOH^-, CHO^+, and H_3O^+ of 145,[13] 122, and 157[14] kcal/mole, respectively.

(13) A. G. Harrison, A. Ivko, and D. Van Raalte, Can. J. Chem., 44, 1625 (1966).

(14) D. Van Raalte and A. G. Harrison, ibid., 41, 3118 (1963).

(15) Postdoctoral Fellow, 1966–1967.

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**Metastable Ion Characteristics. III.[1]
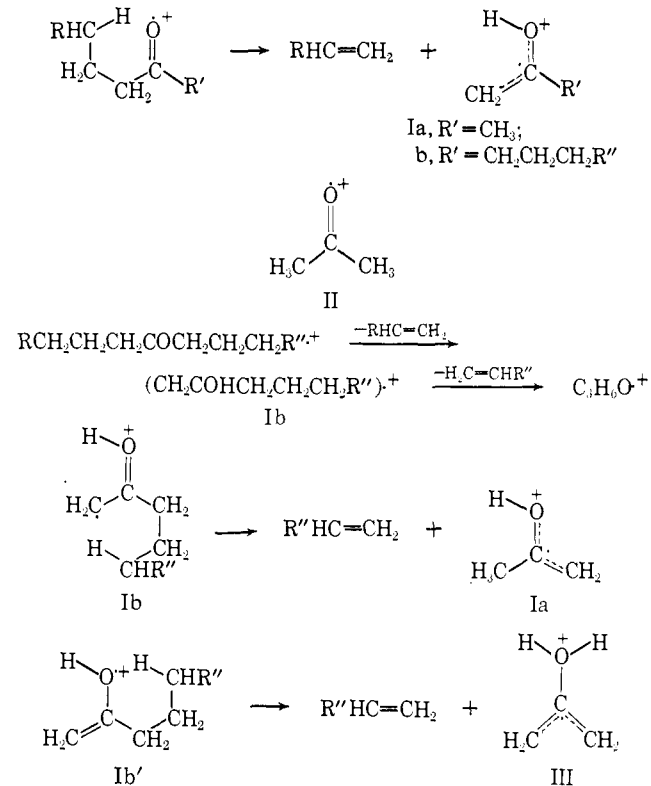Structures of $C_3H_6O \cdot^+$ Ions in the Mass Spectra of Aliphatic Ketones[2]**

Sir:

For the electron-impact-induced rearrangement of carbonyl compounds involving migration of a γ-hy-

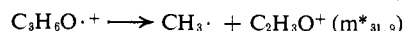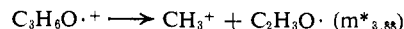(1) Part II: F. W. McLafferty and W. T. Pike, J. Am. Chem. Soc., 89, 5951 (1967).

(2) The generous financial support of the National Institutes of Health (GM 12755 and FR 00354) is gratefully acknowledged. The Hitachi RMU-6D mass spectrometer used in this work was purchased through National Science Foundation Grant GP 4335.

drogen atom[3] there is ample evidence[4] in favor of the formation of the enol radical ion I rather than the keto form (such as II, the molecular ion of acetone). However, when two such rearrangements[5,6] of γ-hydrogen

Ia, R′ = CH₃;
b, R′ = CH₂CH₂CH₂R″

II

$RCH_2CH_2CH_2COCH_2CH_2CH_2R'' \cdot^+ \xrightarrow{-RHC=CH_2}$

$(CH_2COHCH_2CH_2CH_2R'') \cdot^+ \xrightarrow{-H_2C=CHR''} C_3H_6O \cdot^+$

Ib

atoms occur from both alkyl chains of a higher alkanone (reaction 1), structures Ia or III are possible,[5–7] depending on whether the second hydrogen atom migrates to the carbon or oxygen atom, respectively. The driving forces for such rearrangements have been of considerable interest recently because of their importance in applying mass spectrometry to molecular structure determination. We report here evidence from metastable ion characteristics[1,8] in support of the formation of the allylic-radical oxonium-ion structure III.

Ions of the formula $C_3H_6O \cdot^+$ in the spectra of acetone, 2-alkanones, and 4- and 5-alkanones decompose to yield two metastable peaks, $m^*_{3.88}$ and $m^*_{31.9}$, whose abundances relative to that of $C_3H_6O \cdot^+$ are shown in Figure 1. Abundance ratios, $[m^*_{31.9}]/[m^*_{3.88}]$, of ap-

$$C_3H_6O \cdot^+ \longrightarrow CH_3^+ + C_2H_3O \cdot \quad (m^*_{3.88})$$

$$C_3H_6O \cdot^+ \longrightarrow CH_3 \cdot + C_2H_3O^+ \quad (m^*_{31.9})$$

proximately 23,[9] 280, and 110 are found for $C_3H_6O \cdot^+$

(3) A McLafferty rearrangement: F. W. McLafferty, Anal. Chem., 31, 82 (1959).

(4) S. Meyerson and J. D. McCollum, "Advances in Analytical Chemistry and Instrumentation," Vol. 2, C. N. Reilley, Ed., Interscience Publishers, Inc., New York, N. Y., 1963, p 179, and references cited therein.

(5) Termed "consecutive rearrangements": (a) F. W. McLafferty, "Interpretation of Mass Spectra," W. A. Benjamin, Inc., New York, N. Y., 1966; or "double rearrangements": ref 3 or H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964. The former name will be used here to avoid confusion with reactions in which two hydrogen atoms are rearranged in a single step.

(6) H. Budzikiewicz, C. Fenselau, and C. Djerassi, Tetrahedron, 22, 1391 (1966).

(7) J. H. Beynon, Endeavour, 25, 79 (1966).

(8) T. W. Shannon and F. W. McLafferty, J. Am. Chem. Soc., 88, 5021 (1966).

(9) Because of the interference of the m/e 32 peak, the $m^*_{31.9}$ value for acetone is a maximum; therefore this ratio is also a maximum value.