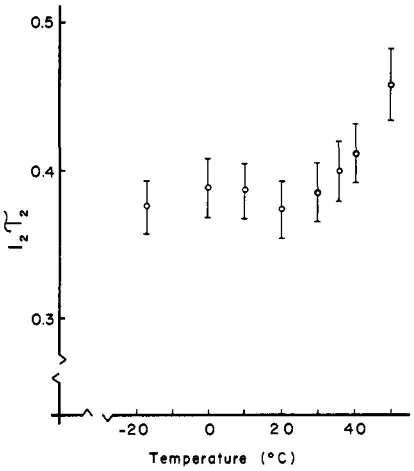

Figure 3. The intensity-lifetime product as function of temperature for the long-lived component of positron annihilation in carbonic anhydrase.

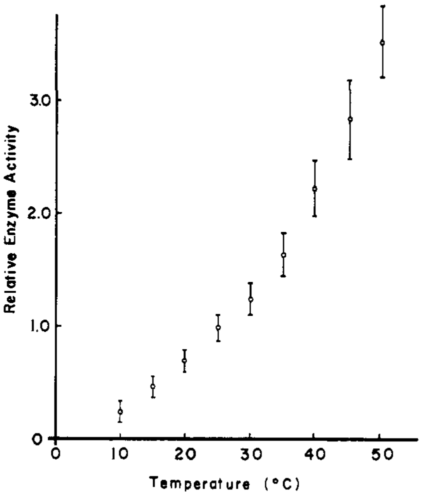

Figure 4. The relative esterase activity of carbonic anhydrase at various temperatures using *p*-nitrophenyl acetate as substrate. The measurements were made using the stopped-flow technique. The reaction mixture contained bovine carbonic anhydrase, 1.0 × 10^{-4} M; *p*-nitrophenyl acetate, 2.0 × 10^{-4} M; Tris-HCl, pH 8.0, 0.009 M; at ionic strength, 0.09 M (NaCl).

in which free volume regions rearrange or combine.[13] Thermal denaturation may be associated with the overall expansion of the molecule.[9] It appears to us that over a certain temperature range the positron is a selective probe of protein conformation. Further work is in progress to expand this correlation into a quantitative model by studying the effect of temperature, inhibitor binding, concentration, and pH on the positron annihilation parameters in a variety of polypeptides and proteins of well-defined conformation.

Acknowledgment. Support for this work by the North Dakota Agricultural Experiment Station, Fargo, N.D. 58102, is gratefully acknowledged.

References and Notes

(1) (a) J. Green and J. Lee, "Positronium Chemistry", Academic Press, New York, N.Y., 1964; (b) A. T. Stewart and L. O. Roellig, Ed., "Positron Annihilation", Academic Press, New York, N.Y., 1967; (c) J. A. Merrigan, J. H. Green, and S. J. Tao in "Physical Methods of Chemistry", Part IIID, Vol. I, A. Weissberger and B. W. Rossiter, Ed., Wiley-Interscience, New York, N.Y., 1972, pp 501–586; (d) H. J. Ache, *Angew. Chem., Int. Ed. Engl.*, **11**, 179 (1972).

(2) A. S. Garay, L. Keszthelyi, T. Demeter, and P. Hrasko, *Chem. Phys. Lett.*, **23**, 549 (1973).

(3) I. Dezsi, D. Horvath, and Zs. Kajcsos, *Chem. Phys. Lett.*, **24**, 514 (1974).

(4) (a) P. C. Jain, S. C. Pancholi, and M. M. Gupta, *Indian J. Biochem. Biophys.*, **10**, 85 (1973); (b) S. Y. Chuang and S. J. Tao, *J. Phys. Chem.*, **78**, 1261 (1974); (c) P. C. Jain, R. R. Jain, P. Joshi, and M. M. Gupta, *Indian J. Biochem. Biophys.*, **11**, 340 (1974).

(5) S. Lindskog, L. E. Henderson, K. K. Kannan, A. Liljas, P. O. Nyman, and B. Strandberg in "The Enzymes", Vol. V, 3d ed, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1971, pp 587–665.

(6) The positron annihilation in the source holder was negligible. Our data in Figures 1 and 2 show a small effect near 0° which might be attributed to residual bulk water. The overall temperature effect, however, is quite different from that observed in bulk water. Studies of bulk water have been discussed in ref 1b.

(7) G. Graf, J. C. Glass, and L. L. Richer in "Protein-Metal Interactions", M. Friedman, Ed., Plenum Publishing, New York, N.Y., 1974, pp 639–679.

(8) J. B. Cumming, BNL Report 6470. The authors are grateful for H. J. Ache and R. Wild for supplying their version of this program.

(9) E. D. Handel, unpublished results for esterase activity. (For CO_2 hydration see J. C. Kernohan, *Adv. Exp. Med. Biol.*, **28**, 189 (1972)). Prolonged incubation at 40° and at higher temperatures produces inactivation. In the irreversibly denatured protein τ_2 and $I_2\tau_2$ remained high even after returning to lower temperatures.

(10) Interactions with water are essential for conformational stability. Under conditions of our experiments protein concentrations are probably too high to allow direct comparison with thermal unfolding in dilute carbonic anhydrase solutions. However, experimental studies of the protein-water system are possible if the results are compared to a reproducible standard such as biological activity. The effect of protein concentration on τ_2 and I_2 is under investigation in our laboratory.

(11) (a) W. Brandt, S. Berko, and W. W. Walker, *Phys. Rev.*, **120**, 1289 (1960); (b) B. V. Thosar, R. G. Lagu, V. G. Kulkarni, and G. Chandra, *Phys. Status Solidi B.*, **55**, 415 (1973).

(12) The average lifetime of the o-Ps is reduced by various interactions with the substrate. Our lifetime measurements indicate pick-off annihilation to be the major process. In pick-off annihilation the positron is removed from the o-Ps under the influence of the electron cloud of the surrounding and undergoes free annihilation. In the free volume model τ_2 is related to the size of the cavities in which the o-Ps atoms are confined, and I_2 represents the number of o-Ps atoms which are formed and subsequently quenched at these sites. In our discussion, I_2 depends primarily on the number of free volume sites associated with the temperature dependent structural characteristics of the protein. The concept is being further tested in our laboratory.

(13) See R. Lumry and S. Rosenberg, "Proceedings of the Conference on Water at Roscoff, France", A. Alfsen and A. Berteaud, Ed., French National Research Center, Paris, France (in press); accordingly in folded proteins there are poorly H-bonded regions called defects providing a reservoir for potential energy. Some defects will be mobile due to the easy translocation of defect situations linked to conformation.

Edward D. Handel, G. Graf*
Department of Biochemistry, North Dakota State University
Fargo, North Dakota 58102

James C. Glass
Department of Physics, North Dakota State University
Fargo, North Dakota 58102

Received December 1, 1975

A Comparison of Translational Energies Released during Metastable Decomposition Following Electron Impact and Field Ionization. A Test of the QET Model and Mechanistic Probe

Sir:

We report here the first comparison between translational (kinetic) energies released during the decomposition of metastable ions formed by electron impact at 70 eV (EI) and field ionization (FI). We suggest that this is important for the following two reasons. The central tenet of the quasi-equilibrium theory (QET) is that of energy equilibration.[1] Failure to randomize vibrational energy[2] and/or nonrecognition of isolated electronic states[3] would complicate or undermine the QET approach. Rosenstock et al.[4] have outlined the underlying argument which predicts that if a particular molecule is ionized by two different methods, provided the internal energy of the molecular ion is equilibrated, the decomposition characteristics at times $>10^{-7}$ s in both cases should be the same. The two methods, EI at 70 eV and FI, provide a particularly stringent test of this point. Only the very lowest electronic states are expected to be reached by FI whereas EI at 70 eV should also yield more highly excited states. Further, even where the distributions of the total excitation energy overlap, the initial

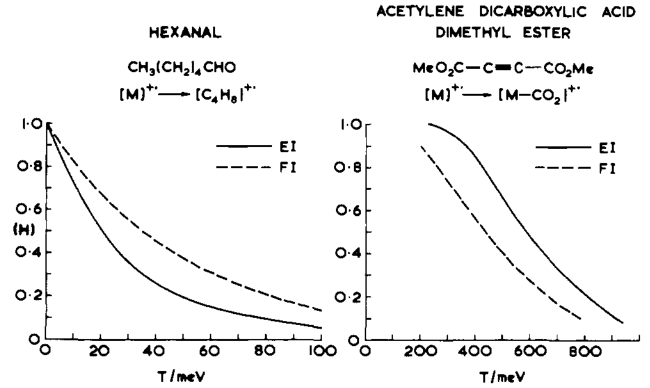

Figure 1. Translational energy releases ($T(H)$) following EI (70 eV) (—) and FI (- - -) against fractional metastable peak height (H).

distribution of this energy among the vibrational modes is unlikely to be the same. At its simplest, EI produces no change in geometry during ionization whereas FI occurs at the distorted geometry appropriate to the very high field conditions (10^9 to 10^{10} V m^{-1}) obtaining. Further, the time window for observable decompositions in the first field free region of our MS9 extends from 1×10^{-7} to 1.4×10^{-6} s for FI, but under EI it lies wholly in the microsecond time-frame (m/e 100 and 8 kV (FI), 4 kV (EI) accelerating voltages). This time difference gives important, mechanistically significant, information about the nature of the $k(E)$ vs. E curves.

The different methods used for the measurement of the translational energies released after EI and FI are being reported elsewhere.[5,6] Both sets of measurements were made at the same relatively low energy resolution (1 in 800), this (10 eV) being less than the full width at half height for molecular ions under FI.[7] The uncorrected[7] peak widths[5,6] at various fractional peak heights (H) were converted into energy releases[8] and plotted against H in order to compare the FI and EI results.[9]

The loss of methane from 2-methylpropane, which is seemingly adequately described by the QET model,[10] shows the same energy release after EI ($T_{EI}(H)$) and FI ($T_{FI}(H)$). Some other simple rearrangements show the same behavior. The loss of hydrogen cyanide from the molecular ion of benzonitrile is a further example where $T_{FI}(H) = T_{EI}(H)$. Measurements[11] suggest that an extra 0.6 eV excitation energy is required to raise $k(E)$ from 10^6 to 10^7 s^{-1}. This result in particular, those mentioned above, and others[12] indicate that shifting the observation time by an order of magnitude does not increase the translational energy release for a process with a reasonably steep $k(E)$ *vs.* E *curve,*[11] being consistent with a weak dependence of energy release on E.[13]

By contrast the formation (McLafferty rearrangement) of $[C_4H_8]\cdot^+$ from hexanal (Figure 1) and the formation of the $[M-CH_3]\cdot^+$ ion from hex-1-yne have $T_{FI}(H) > T_{EI}(H)$. The formation of the $[C_4H_8]\cdot^+$ ion from hexanal is a complex process involving an intermediate ion and three distinct rearrangement steps.[14,15] The $[M-CH_3]^+$ ion from hex-1-yne probably has the cyclopentenyl structure,[15] in which case its formation is associated with a very large kinetic shift.[15] Both of these facts would suggest a complex mechanism. We attribute the larger energy release following FI in these and other cases to the shift to shorter observation times and suggest that this variation of kinetic energy release with lifetime is indicative of a very slow rise of $k(E)$ with E.

Unexpectedly, in other cases, for example, the loss of water from the molecular ions of cyclohexanol and pentan-1-ol, $T_{EI}(H)$ is considerably greater than $T_{FI}(H)$ and this would seem to indicate that, despite the formal similarity, the decomposition processes following EI and FI do not proceed over the same energy surfaces. In the case of cyclohexanol this agrees with earlier work[16] in which it has been suggested that loss of water after EI occurs through a high energy process involving acyclic isomers of the molecular ion, whereas the loss of water after FI is due to a low energy process. Examples in which there is a large energy release ($T_{EI}(0.5) > 200$ meV) after EI but a smaller one under FI are the loss of nitric oxide from p-nitrotoluene (although for the loss of NO from nitrobenzene $T_{EI}(H) = T_{FI}(H)$) and the loss of carbon dioxide from dimethyl acetylenedicarboxylate (Figure 1). Large energy releases are observed, however, after FI as exemplified by the loss of carbon monoxide from diphenyl ether where $T_{FI}(0.5) = 450$ meV $= T_{EI}(0.5)$. In general terms the most likely explanation of the cases where $T_{EI}(H)$ is considerably greater than $T_{FI}(H)$ would seem to lie in the existence of high energy processes occurring under electron impact which are not possible under FI. Among other reasons may be the existence of isolated vibronic states or possibly noninterconverting isomeric structure (e.g., cyclohexanol).

Acknowledgment. We gratefully acknowledge the Ramsay Memorial Trust (P.J.D.), The Central Research Fund, University of London (P.J.D. and A.G.L.), and technical assistance from Dr. M. A. Baldwin and Mr. R. P. Morgan. We thank Professor J. L. Holmes for suggesting a study of hex-1-yne.

References and Notes

(1) H. M. Rosenstock, M. B. Wallenstein, A. L. Wahrhaftig, and H. Eyring, *Proc. Natl. Acad. Sci. U.S.A.*, **38**, 667 (1952).
(2) (a) J. F. Meagher, K. J. Chao, J. R. Barker, and B. S. Rabinovitch, *J. Phys. Chem.*, **78**, 2535 (1974); (b) S. Nordholm and S. A. Rice, *J. Chem. Phys.*, **62**, 157 (1975).
(3) H. M. Rosenstock, J. T. Larkins, and J. A. Walker, *Int. J. Mass Spectrom. Ion Phys.*, **11**, 309 (1973).
(4) H. M. Rosenstock, V. H. Dibeler, and F. N. Harlee, *J. Chem. Phys.*, **40**, 591 (1964).
(5) P. J. Derrick, R. P. Morgan, J. T. Hill, and M. A. Baldwin, *Int. J. Mass Spectrom. Ion Phys.*, **18**, 393 (1975).
(6) M. A. Baldwin, P. J. Derrick, and R. P. Morgan, *Int. J. Mass Spectrom. Ion Phys.*, accepted for publication.
(7) M. A. Baldwin, P. J. Derrick, and R. P. Morgan, *Org. Mass Spectrom.*, accepted for publication.
(8) R. G. Cooks, J. H. Beynon, R. M. Caprioli, and G. R. Lester "Metastable Ions", Elsevier, Amsterdam, 1973.
(9) The distributions in Figure 1 are therefore not the actual distributions of translational energy released during decomposition. See D. T. Terwilliger, J. H. Beynon, and R. G. Cooks, *Proc. R. Soc. London, Ser. A*, **341**, 135 (1974).
(10) P. J. Derrick, A. M. Falick, and A. L. Burlingame, *J. Chem. Soc., Faraday Trans. 1*, **71**, 1503 (1975).
(11) B. Andlauer and Ch. Ottinger, *Z. Naturforsch.*, **27**, 293 (1972).
(12) R. G. Cooks, K. C. Kim, T. Keough, and J. H. Beynon, *Int. J. Mass Spectrom. Ion Phys.*, **15**, 271 (1974).
(13) C. E. Klots, *J. Chem. Phys.*, **58**, 5364 (1973).
(14) R. P. Morgan and P. J. Derrick, *J. Chem. Soc., Chem. Commun.*, 836 (1974).
(15) J. L. Holmes and F. P. Lossing, personal communication, 1975.
(16) P. J. Derrick, J. L. Holmes, and R. P. Morgan, *J. Am. Chem. Soc.*, **97**, 4936 (1975).

Peter J. Derrick,* Alexander G. Loudon*
Christopher Ingold Laboratories, University College
London WC1H OAJ, Great Britain
Received January 20, 1976

Stereoselective One-Pot Dialkylation of *gem*-Dihalocyclopropanes. A Simple Route to *dl*-Sesquicarene and *dl*-Sirenin

Sir:

Double alkylation on the same carbon has been one of important synthetic problems.[1] The reaction of *gem*-dihalides with dialkylcuprates has proven to be a method of choice for this purpose, especially for dimethylation,[2] in which the intermediacy of a copper carbenoid of type **1** has been postulated.[3] We wish to disclose that the reaction of *gem*-dihalocyclopropanes (**3**) with lithium dibutylcuprate proceeds as