then the bridgehead and ring C–C distances, and the dihedral angle, α, between the ring planes, may be chosen to reproduce the experimentally observed rotational constants. For this purpose we have chosen all $R(CH) = 1.08$ A, and $\angle HCH = 116°$, with the HCH angles bisected by the ring planes. The bridgehead CCH angles are much more uncertain but were chosen such that each bridgehead C–H bond was oriented at identical angles to all three adjacent C–C bonds.[9] With these assumptions, the best fit gives $\angle CCH$ (bridgehead) = 130°, $R(CC)_{bridge} = 1.49$ A, $R(CC)_{ring} = 1.51$ A, and $\alpha = 121°$. If $\angle CCH$ (bridgehead) is opened up to 145° (Haller and Srinivasan[4] suggested 160°), the major change is to force $R(CC)_{bridge}$ to become even smaller, about 1.44 Å. It seems likely that $R(CC)_{bridge}$ is indeed smaller than $R(CC)_{ring}$, indicating that the bridgehead bond is somewhat stronger and has a somewhat higher electron density. This would be in agreement with some chemical reactivity results discussed by Wiberg[3] and would also be an important factor in contributing to the large dipole moment. We are currently studying other isotopic species of bicyclobutane in order to resolve the structural problem completely.

Acknowledgment. Grateful acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We are also grateful for the computing facilities made available to us by the University of Kansas Computation Center.

(9) The assumption is arbitrary but might have physical reality if nonbonded interactions of the bridgehead hydrogens with the nearest neighbor carbon atoms were important.

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Energy Transfer in Ion-Impact Mass Spectra. Application to Structural Mass Spectrometry[1]

Sir:

We have investigated the problem of energy transfer in ion-impact mass spectra to determine its potential as a technique for structural studies on complex molecules. Electron-impact ionization techniques have been used extensively to provide information on molecular structures.[2-4] Electron impact produces excited ionized species which decompose unimolecularly to yield mass spectra, but many isomeric and closely related molecules are virtually indistinguishable because the excitation deposited in electron-impact ionization is frequently sufficient to obscure subtle differences in molecular structure. Ion impact, on the other hand, provides a means of depositing energy by an over-all mechanism which is significantly different from that operative in electron impact and which permits a more precise control of the magnitude of the excitation in the target molecule. The bulk of the energy deposited in ion-impact processes, if relatively low velocity ions are used as projectiles, comes from the recombination energy of the projectile ion.[5] Additional energy can be deposited locally at the point of impact between the colliding molecules by conversion of projectile ion translational energy into target molecule internal energy.[6] If it is desired to demonstrate differences in decomposition patterns between isomeric molecules, the ideal projectile must have a recombination energy very close to the ionization potential of target isomers, and the additional energy to produce the ion fragments should then come from translational energy of the projectile. The success of such an approach depends on the validity of the assumption that kinetic energy deposited locally in a molecule ion sets up a forced vibration which favors dissociation prior to redistribution of this excess energy among other internal degrees of freedom in the target molecule ion. If a statistical distribution of energy takes place prior to decomposition then mass spectra similar to those obtained by electron impact would be expected.

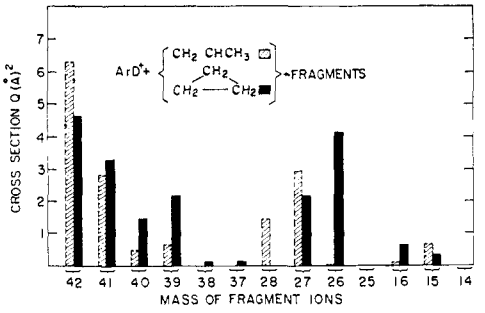

Figure 1. Ion-impact mass spectrum produced in collisions of 10-ev ArD^+ ions with propylene and cyclopropane. Reaction cross sections, in units of square angstroms, are plotted for the different fragment ions.

We have determined ion-impact mass spectra of isomeric butenes, propylene, and cyclopropane using low-velocity ArD^+, COD^+, and Ar^+ ions as projectiles. These data[7] will be discussed in more detail in a subsequent publication. Results obtained with 10-ev ArD^+ ions on propylene and cyclopropane are presented in Figure 1. Electron-impact mass spectra

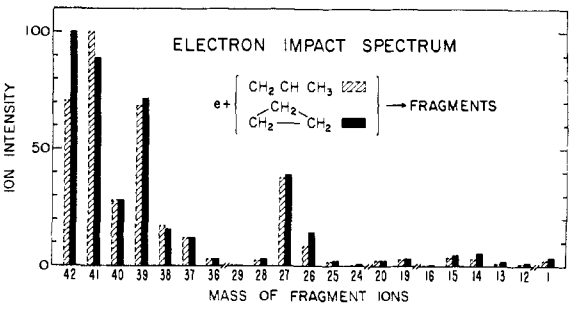

Figure 2. Electron-impact spectrum of propylene and cyclopropane.

(1) Research performed under the auspices of the U. S. Atomic Energy Commission.

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(obtained with 70-v ionizing electrons) are shown in Figure 2. Significant differences between the two sets of mass spectra support the conclusion that while extensive dissociation of parent molecule ions takes place in both experiments, fundamentally different energy-transfer and dissociation mechanisms must operate. The role of kinetic to internal energy transfer is established by the fact that the recombination energy in ArD^+ is insufficient to bring about some of the decomposition processes observed if one accepts the validity of electron-impact, appearance-potential thresholds as a measure of actual minimum energy requirements. The most striking differences in the spectra are found in the two carbon fragment ion region where $C_2H_4^+$ or CH_3CH^+ ions are detected only from propylene while $C_2H_2^+$ is seen only from cyclopropane.

Studies with isomeric butenes show significant differences in spectra between butene-1 and *cis*- and *trans*-butene-2. Spectra obtained with Ar^+, where the recombination energy is approximately 5 ev greater than target ionization energies, tend to obscure differences between isomeric species. The use of ArD^+ reduces the recombination energy from that of the ionization potential of Ar (15.7 ev) to that of D (13.6 ev) minus the binding energy of $Ar–D^+$ (approximately 3 ev). No evidence in any of these experiments is found for D exchange or the incorporation of D in the target molecule ions. There is the possibility that HD and neutral CH_3D products may be formed in the ion-impact collision. If this were a very probable process, we would expect a relatively smaller yield of parent molecule ion.

Details of mechanisms operative in producing these spectra are so far not clearly understood. The potential of this technique in studies which attempt to correlate molecular structure with mass spectra is evident. Low-velocity ion-impact techniques provide a very sensitive method of energy deposition in target molecules and hence are capable of reflecting in mass spectra relatively subtle differences in molecular structure.

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The Synthesis of Bovine Insulin by the Solid Phase Method[1]

Sir:

We wish to report the synthesis of bovine insulin by the solid phase method.[2] This polypeptide hormone has already been synthesized independently by three other laboratories,[3] using the classical methods of peptide chemistry. With these methods, many months are required for the synthesis of each of the protected component peptide chains, and the over-all yields are low. Using the solid phase method we have, in a matter of days and in high yields, synthesized both protected chains of insulin. The chains were then combined to form the active hormone.

The B chain was synthesized in a stepwise manner beginning with 1.9 mmoles of *t*-butyloxycarbonyl-L-alanine esterified to 8 g of the supporting cross-linked polystyrene resin.[2,4] Twenty-nine cycles of deprotection, neutralization, and coupling were carried out with appropriate Boc-amino acids[5] according to previously developed procedures,[4] producing the fully protected triacontapeptide esterified to the resin. Boc-amino acids with protected side chains were O-Bzl-Glu, S-Bzl-Cys, O-Bzl-Ser, O-Bzl-Tyr, N^{im}-Bzl-His, N^{ϵ}-Z-Lys, and N^{G}-Tos-Arg. All coupling reactions to form peptide bonds were mediated by dicyclohexylcarbodiimide[6] except those involving the carboxyl groups of Asn and Gln which were used as the nitrophenyl esters.[7] Cleavage of the peptide from the resin by hydrogen bromide was done as described[4] except that the HBr was first bubbled through a solution of resorcinol[8] in TFA in order to prevent bromination of tyrosine residues and the peptide resin was suspended in TFA containing methionine in order to prevent benzylation of S-Bzl-Cys. The peptide was precipitated from water to remove the methionine. The yield of partially protected peptide was 64%, based on the amount of alanine originally esterified to the resin. Amino acid analysis gave:[9] Asp, 0.78; Thr, 0.99; Ser, 0.83; Glu, 3.16; Pro, 0.97; Gly, 3.26; Ala, 2.08; Val, 2.81; Leu, 4.06; Tyr, 1.76; Phe, 3.22; Lys, 0.90; Arg, 1.15; Bzl-His, 1.67; Bzl-Cys, 2.37 (Cys, 0; His, 0). The total time required for synthesis and cleavage was 11 days. The partially protected peptide was thoroughly dried and then treated with sodium in liquid ammonia as described by Niu, *et al.*,[10] except that the stable light blue end point was limited to exactly 15 sec in order to prevent excessive cleavage of the Thr-Pro bond (B_{27-28}).[11] Under these conditions this cleavage was only 20–25%,[12] whereas 80% was lost during a 60-sec treatment. The shorter time was adequate for complete debenzylation of His and Cys and complete detosylation of Arg (as determined by direct amino acid analyses for the protected amino acids). The deprotected triacontapeptide was converted to the S-sulfonate.[13] On electrophoresis,[13] there was a major Pauly-positive spot with the same mobility as that of the B-chain S-sulfonate ($BSSO_3$) obtained by sulfitolysis of natural bovine insulin, and a

(1) Presented at the 8th European Peptide Symposium, Noordwijk-aan-Zee, The Netherlands, Sept 18–23, 1966. This work was supported in part by Grant A1260 from the U. S. Public Health Service.

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