We have also made Mössbauer studies using ammonia, isobutane, benzene, and hydrogen-argon as the matrix material for condensed iron vapor. In all these cases the normal spectrum of both Fe and Fe_2 disappears. New absorptions are found with positive isomer shifts characteristic of chemically bound iron. The nature of these products has not yet been completely probed by infrared spectroscopy. However, H_2 gives absorption bands in the metal-hydrogen stretching region, and NH_3 does not. If methane has indeed been oxidatively cleaved, then it is likely that i-C_4H_{10} and H_2 also have been cleaved. In chemisorption studies, both of these molecules are more reactive than is CH_4.[9] Benzene and NH_3 are also more reactive, but can form simple complexes on metal films. The enhanced reactivity of Fe_2 compared with Fe occurs with N_2 and with CH_4, the two most difficult molecules to chemisorb of those we have examined.

Very recently it has been shown that small nickel clusters, but not atoms, react with pentane between 77 and 143 K.[11] Organometallic species of unknown nature are formed.

Acknowledgment. This work was supported in part by the National Science Foundation under Grant DMR 76-23329.

References and Notes

(1) (a) See a series of papers in *Angew. Chem., Int. Ed. Engl.* **14**, 193 ff, 273 ff (1975); (b) G. A. Ozin and A. Vander Voet, *Acc. Chem. Res.*, **6**, 313 (1973).
(2) (a) K. J. Klabunde, *Acc. Chem. Res.*, **8**, 393 (1975). (b) P. L. Timms, *Adv. Inorg. Radiochem.*, **14**, 121 (1972); *Angew. Chem.*, **87**, 295 (1975).
(3) P. H. Barrett and P. A. Montano, *J. Chem. Soc., Faraday Trans. 2*, **73**, 378 (1977).
(4) S. S. Lin and A. Kant, *J. Phys. Chem.*, **73**, 2450 (1969).
(5) W. E. Klotzbucher, S. A. Mitchell, and G. A. Ozin, *Inorg. Chem.*, **16**, 3063 (1977).
(6) C. Klee, T. K. McNab, F. J. Litterst, and H. Micklitz, *J. Phys.*, **270**, 31 (1974).
(7) A detailed description of the apparatus and methods used is given in T. K. McNab and P. H. Barrett, "Mössbauer Effect Methodology", Vol. 7, Plenum Press, New York, 1971.
(8) For example, see S. D. Ittel, C. A. Tolman, A. D. English, and J. P. Jesson, *J. Am. Chem. Soc.*, **98**, 6073 (1976). These authors report on the oxidative cleavage of C-H bonds by $Fe(dmp)_2$, the most reactive homogeneous metal complex known for such reactions.
(9) "Chemisorption and Reaction on Metallic Films", Vol. 2, J. R. Anderson, Ed., Academic Press, New York, 1971, Chapter VIII.
(10) For details, see ref 3.
(11) S. C. Davis and K. J. Klabunde, *J. Am. Chem. Soc.*, **100**, 5973 (1978).
(12) (a) Physics Department. (b) Chemistry Department.

Paul H. Barrett,[12a] Moshe Pasternak,[12a] Ralph G. Pearson*[12b]
Physics and Chemistry Departments, University of California
Santa Barbara, California 93106

Received August 17, 1978

Structure and Electrochemistry of a Stable Enantiomeric Metalloflavin Complex

Sir:

Flavin coenzymes transfer electrons to and from heme, iron-sulfur, and molybdenum centers in proteins and serve as a required electron-pair splitting or -joining interface between metal one-electron donors or acceptors and organic two-electron oxidants or reductants.[1-3] Direct inner-sphere electron transfer has long been suspected between flavins and iron-sulfur or molybdenum sites,[1-11] but definitive evidence is lacking. Numerous model systems have been used to explain the nature of metal-flavin interactions,[5-19] but none have employed well-characterized metalloflavin complexes which maintain their integrity in both aqueous and nonaqueous solvents and incorporate a redox active metal ion with the same d-electron configuration as low-spin iron(II). We now report structural, spectroscopic, and electrochemical studies of stable ruthenium(II) isoalloxazine complexes which possess these properties and provide direct evidence for chirality and sequential one-electron reductions of the flavin due to metal coordination.

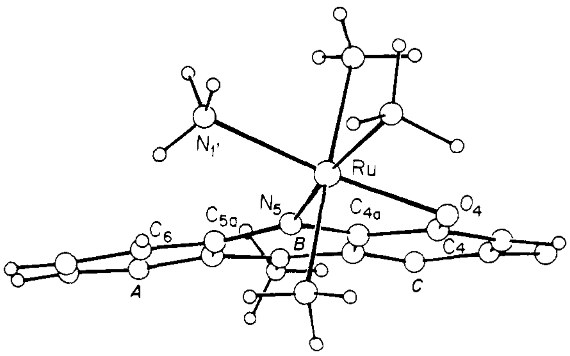

Figure 1. View of the $(10\text{-MeIAlo})(NH_3)_4Ru^{2+}$ complex ion.

The complexes were prepared by direct combination of $(H_2O)(NH_3)_5Ru^{2+}$, *cis*-$(H_2O)_2(NH_3)_4Ru^{2+}$, or *cis*-$(H_2O)_2(NH_3)_4Ru^{3+}$ with the isoalloxazine ligand and purified by ion-exchange chromatography. Crystallographic quality crystals of $[(10\text{-MeIAlo})(NH_3)_4Ru](PF_6)_2 \cdot 2H_2O$ (10-MeIAlo = 10-methylisoalloxazine) were grown by ethanol diffusion into saturated aqueous solutions of the complex seeded with small single crystals. These crystals belonged to the triclinic space group $P\bar{1}$ with unit cell dimensions of a = 9.631 (3), b = 10.618 (3), c = 13.216 (4) Å; α = 113.86 (2), β = 100.19 (2), γ = 94.12 (2)°.[20]

The structure of the $(10\text{-MeIAlo})(NH_3)_4Ru^{2+}$ ion is shown in Figure 1 and represents the first molecular structure determination of a 1:1 octahedral metalloflavin complex of the type postulated as an intermediate for inner-sphere electron transfer[16] and the first in which back-bonding and steric distortions of the heterocyclic ring are evident. The flavin ring chelates the metal ion via the N_5 and O_4 atoms with a N_5-Ru-O_4 angle of 80.9 (5)°. The four Ru-NH_3 bond lengths are a typical 2.12 Å,[21] while the Ru-N_5 distance is only 1.980 (6) Å. The latter bond length is significantly shorter than expected for a single bond to nitrogen for either Ru(II) or Ru(III)[21-23] and indicates some degree of multiple bonding. Back-bonding interactions were predicted for this series of metalloflavin complexes on the basis of their occurrence in a number of Ru(II) complexes with unsaturated heterocyclic molecules.[22-27] Remarkably, this bond length is shorter than the 1.991- and 2.058-Å distances reported for ammineruthenium(II) complexes with pyrazine[22] and isonicotinamide,[23] respectively, in which strong steric repulsion involving the heterocyclic ligand does not occur. The only shorter Ru(II)-N bond distance reported for a neutral nitrogen ligand is the 1.928-Å bond length for complexation by dinitrogen,[28] which is known to be a powerful π-acceptor ligand for this metal ion.

The Ru-O_4 bond distance of 2.088 (7) Å is similar to the 2.10-Å length expected for a Ru-O single bond.[27] The C_4-O_4 distance of 1.269 (10) Å is similar to those of other metalloflavin complexes which do not involve retrodative bonding,[15-19] so that back-bonding probably does not occur through the carbonyl group. Instead the metal back-donation of electron density appears to be localized in the N_5-C_{4a} bond via d-π^*-orbital overlap. This is evidenced by the fact that the N_5-C_{4a} bond distance of 1.351 (11) Å is ~0.05 Å longer than the corresponding bond lengths in either the free ligand[29] or other metalloflavin complexes,[15-19] whereas the N_1-C_{10a} bond distance of 1.309 (11) Å remains essentially unchanged.

Strong cooperative back-bonding and steric interactions induce significant departures from the coplanarity normally present between the benzene (A), bridging (B), and pyrimidine (C) rings of isoalloxazines.[29] The dihedral angles between the A and B rings and between the B and C rings are 6.2 and 3.7°,

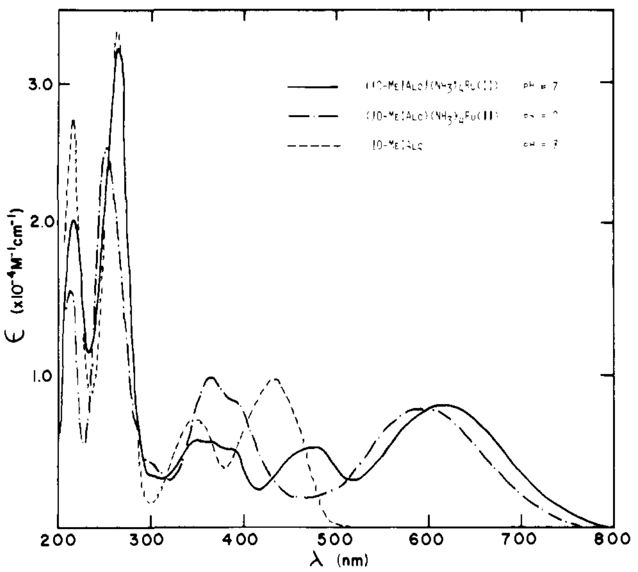

Figure 2. Spectra of (a) the (10-MeIAlo)(NH$_3$)$_4$Ru^{2+} ion in 0.1 M LiCl at pH 7, (b) protonated (10-MeIAlo)(NH$_3$)$_4$Ru^{II} in 1 M HCl, and (c) 10-methylisoalloxazine at pH 7 in 0.1 M LiCl.

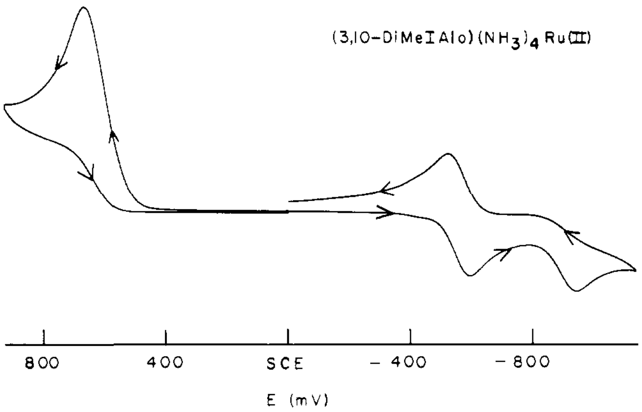

Figure 3. Cyclic voltammetry scan of (3,10-Me$_2$IAlo)(NH$_3$)$_4$Ru^{2+} taken at 125 mV/s with a carbon paste electrode in 0.1 M LiCl at pH 7.

respectively. The angle between the A and B rings is attributed largely to steric repulsion between the C$_6$ proton and the N$_{1'}$-coordinated ammonia group. The C$_6$–N$_{1'}$ repulsion also displaces the metal ion 0.05 Å from the plane of the bridging ring. However, this displacement is probably assisted by the large degree of retrodative bonding which would tend to alter the geometry around N$_5$. This is consistent with the C$_{5a}$–N$_5$–C$_{4a}$ bond angle of 114.8°, which can be compared with the corresponding angle of 117.5° in the free ligand.[29] These back-bonding and steric interactions also join to induce the canting of the B and C rings. Depending on whether the N$_{1'}$ and Ru atoms are above or below the organic ligand, the net 10° bend in the isoalloxazine ring is either up or down, yielding two enantiomeric complex ions with one of each enantiomer included per unit cell.

A prominent feature of the spectra of the Ru–Fl$_{ox}$ complexes (Figure 2) is the intense absorption centered around 617 nm, which can be attributed to a metal-to-ligand charge transfer (MLCT) band. The energies of these transitions for Ru(II) complexes correlate with the reduction potential of the heterocyclic ligand and, therefore, with the ease of back-donation of electron density onto the ligand,[24,26,30] so that a low energy MLCT band is to be expected with Fl$_{ox}$ ligands. Protonation of the heterocyclic ligand at a site which is in conjugation with Ru(II) usually induces a bathochromic shift in the MLCT band;[24,26,30] however, in cases where the retrodative bonding is extensive, this band has been observed to shift toward higher energy.[31,32] Spectra of the Ru(II)–Fl$_{ox}$ complexes taken in 1 M HCl, where the ligand is partially protonated, reveal a shift in the visible absorption band from 617 to 590 nm, and so are consistent with the other indicators of extensive back-bonding.[33]

It has been suggested that shifts in the isoalloxazine band, normally centered around 444 nm in the uncomplexed molecule, toward lower energy correlates with the amount of metal back-donation.[2] By this standard the occurrence of this band at 475 nm in the neutral ligand complex (Figure 2) is consistent with the postulate of considerable retrodative bonding. Unlike previously reported Fl$_{ox}$ complexes, in which a small rapidly exchangeable equilibrium quantity of Fl· prevents characterization by NMR,[1,5] the Ru(II)–Fl$_{ox}$ complexes are truly diamagnetic and have proven amenable to NMR studies.

Cyclic voltammetry studies of these complexes indicate a single reversible and two irreversible redox processes. For the (3,10-Me$_2$IAlo)(NH$_3$)$_4$Ru(II) complex (Figure 3) the potentials of the three processes are pH dependent and peaks occurred at nearly identical potentials on carbon paste and platinum disk electrodes (scanning positive of the SCE potential) and a hanging drop mercury electrode (scanning negative of the SCE potential). The reversible wave centered at −584 mV is attributed to the formation and oxidation of the metallosemiquinone species. This occurs at a potential more negative than might be expected for a metalloflavin complex,[38,39] since the presence of metal ions usually stabilizes Fl· in aqueous solution[1,2,5,8] and sterically induced bending of the isoalloxazine ring should favor flavin reduction as well.[6] The low potential for the reduction of the isoalloxazine ring must, therefore, be due to considerable delocalization of electron density from the metal onto this ligand.

Addition of a second electron to the heterocyclic ring occurs at −844 mV, which is more negative than the corresponding reduction potential for any neutral flavin or metalloflavin reported to date. The two-electron reduction of the complex, presumably yielding the fully reduced isoalloxazine product, results in decomposition of the complex. This is not surprising in view of the fact that Fl$_{red}$ species are not good ligands and protonation becomes strongly favored over metal coordination at the N$_5$ site.[1]

The anodic peak at 620 mV (862 mV vs. NHE) represents the minimum potential necessary to oxidize the metal center. However, the large peak current and the irreversibility of the anodic wave indicates that other redox precesses may be incumbent upon metal oxidation. The oxidation potential for Ru(II) complexed by isoalloxazine is higher than that reported for any ruthenium ammine or amine ion coordinated by a single, neutral heterocyclic molecule.[24,25,40] Since Ru(III–II) electrochemical potentials correlate with the π-acceptor ability of the nitrogen ligand, these results are also consistent with a large amount of metal-to-ligand back-donation of electron density.

The steric and electronic properties evident in these complexes indicate how a distorted octahedral metal ion, of the type postulated for iron–sulfur cluster interactions with flavins,[16] can alter the reduction potentials of the flavin and so cause it to favor one-electron rather than two-electron transfers. Although no structural parameters are available for the semiquinone form of flavins, it can be assumed that the A–B–C angle of Fl· is between the 180° observed for Fl$_{ox}$ and the 144–160° measured for Fl$_{red}$.[6] The coordinated ammonias, chelation, and tight metal binding evident in the present structure may limit the possible range of this dihedral angle, so as to favor the one-electron reduction of the ring. This is consistent with the cyclic voltammetry studies of these complexes which indicate two sequential one-electron reductions. Strong back-bonding interactions lower the electrochemical potentials for addition of electrons to the heterocyclic ligand, even though the presence of a metal ion at N_5 and the sterically imposed bending of the isoalloxazine ring would be expected to increase the potential for addition of at least the first electron.

Finally, this and related work[25] now make it feasible to selectively label either the isoalloxazine or adenine rings of the FAD coenzyme by addition of Ru(II) under strictly anaerobic or redox catalytic conditions, respectively. It is hoped that coordination of Ru^{103} or Ru^{97} to FAD and related coenzymes at either of these sites will yield radiopharmaceuticals which may be of use as organ-imaging agents for diagnostic purposes.[41]

Acknowledgments. We are grateful to Dr. Gilbert Brown (Brookhaven National Laboratories) for several helpful discussions. Funding for this work at Boston College was initially provided by a Cottrell Research Grant from the Research Corporation and, in later phases, by a grant from the American Cancer Society, Massachusetts Division, Inc. Funding at Northeastern University was provided by DHEW Grant No. RR07143.

References and Notes

(1) P. Hemmerich in "Bioinorganic Chemistry-II", K. N. Raymond, Ed., American Chemical Society, Washington, D.C., 1977, p 312.
(2) P. Hemmerich and J. Lauterwein in "Inorganic Biochemistry", G. L. Eichhorn, Ed., Elsevier, New York, 1973, p 1168.
(3) O. Groudinsky in "In Flavins and Flavoproteins", H. Kamin, Ed., University Park Press, Baltimore, Md., 1970, p 581.
(4) J. S. Olson, D. P. Ballow, G. Palmer, and V. Massey, *J. Biol. Chem.*, **249**, 4363 (1974).
(5) A. Ehrenberg, *Vitamins Hormones*, **28**, 489 (1970).
(6) P. Hemmerich, G. Nagelschneider, and C. Veeger, *FEBS Lett.*, **869**, (1970).
(7) J. Lauterwein, P. Hemmerich, and J. M. Lhoste, *Z. Naturforsch. B*, **27**, 1047 (1972).
(8) P. Hemmerich, D. V. Devartanian, C. Veeger, and J. D. Van Voorst, *Biochim. Biophys. Acta*, **77**, 504 (1963).
(9) L. E. Erikson, *Biochim. Biophys. Acta*, **208**, 528 (1970).
(10) J. Selbin, J. Sherrill, and C. H. Bigger, *Inorg. Chem.*, **13**, 2544 (1974).
(11) F. Muller, L. E. G. Erikson, A. Ehrenberg, *Eur. J. Biochem.*, **12**, 93 (1970).
(12) D. T. Sawyer and R. L. McCreery, *Inorg. Chem.*, 11, 779 (1972).
(13) L. S. DeHayes, H. C. Faulkner, W. H. Daub, and D. T. Sawyer, *Inorg. Chem.*, **14**, 2110 (1975).
(14) (a) M. M. Morrison, E. T. Seo, J. K. Howie, and D. T. Sawyer, *J. Am. Chem. Soc.*, **100**, 207 (1978); (b) M. M. Morrison and D. T. Sawyer, *ibid.*, **100**, 211, (1978).
(15) T. D. Wade and C. J. Fritchie, *J. Biol. Chem.*, **248**, 7516 (1973).
(16) C. J. Fritchie, *J. Biol. Chem.*, **247**, 7459 (1972).
(17) C. J. Fritchie, *J. Biol. Chem.*, **248**, 7516 (1973).
(18) G. W. T. Garland and C. J. Fritchie, *J. Biol. Chem.*, **249**, 2228 (1974).
(19) P. Kierkegaard, M. Leijon, and P. Werner, *Acta Chem. Scand.*, **26**, 2080 (1972).
(20) Intensity data were collected on an automated Syntex $P2_1$ diffractometer using (Mo $K\alpha$) radiation out to a 2θ limit of 48°. The structure was solved by heavy-atom and direct methods and refined to an R value of 0.054 for 3523 reflections with $I_0 > 1.5\sigma(I_0)$.
(21) H. Stynes and J. A. Ibers, *Inorg. Chem.*, **10**, 2304 (1971).
(22) M. E. Gress, C. Creutz, and C. O. Quicksall, unpublished work.
(23) D. Richardson, J. Walker, J. Sutton, K. Hodgson, and H. Taube, unpublished work.
(24) H. Taube, *Survey Prog. Chem.*, 6, 1 (1973).
(25) M. J. Clarke, *J. Am. Chem. Soc.*, **100**, 5068 (1978).
(26) P. C. Ford, De F. P. Rudd, R. Gaunder, and H. Taube, *J. Am. Chem. Soc.*, **90**, 1187 (1968).
(27) P. T. Cheng, B. R. Loesther, and S. C. Nyburg, *Inorg. Chem.*, **10**, 1275 (1971).
(28) I. M. Treitel, M. T. Flood, R. E. Marsh, and H. B. Gray, *J. Am. Chem. Soc.*, **91**, 6512 (1969).
(29) M. Wang and C. J. Fritchie, *Acta Crystallogr., Sect. B*, **29**, 2040 (1973).
(30) A. M. Zwickel and C. Creutz, *Inorg. Chem.*, **10**, 2395 (1971).
(31) K. Rieder, U. Hauser, H., Siegenthaler, E. Schmidt, and A. Ludi, *Inorg. Chem.*, **14**, 1902 (1975).
(32) R. H. Magnuson and H. Taube, *J. Am. Chem. Soc.*, **97**, 5129 (1975).
(33) It should be noted that the semiquinone form of flavins also absorbs strongly in the visible region of the spectrum and that the semiquinone radical is stabilized by the presence of a positive charge at N_5.[1,2,5,34-37] Extensive retrodative bonding would have the effect of partially reducing the flavin ring, so that the band characteristic of the flavosemiquinone[34] might appear. Addition of a proton to the free flavosemiquinone anion causes a shift in the visible absorption band from 480 to 580 nm. Further addition of a proton to the neutral semiquinone radical results in a hyperchromic shift to 490 nm.[34] Since the latter spectral change is consistent with that seen for protonation of the Ru-Fl_{ox} complex, an intraligand transition with the flavin behaving like a semiquinone radical cannot be entirely ruled out.
(34) E. J. Land and A. J. Swallow, *Biochemistry*, **8**, 2117 (1969).
(35) H. Beinert and P. Hemmerich, *Biochem. Biophys. Res. Commun.*, **18**, 212 (1965).
(36) G. R. Penzer, G. K. Radda, J. A. Taylor, and M. B. Taylor, *Vitamins Hormones*, **28**, 441 (1970).
(37) G. Kemal and T. C. Bruice, *J. Am. Chem. Soc.*, **98**, 3955 (1976).
(38) B. Janik and P. J. Elving, *Chem. Rev.*, **68**, 295 (1968).
(39) G. Dryhurst, "Electrochemistry of Biological Molecules", Academic Press, New York, 1977, p 365.
(40) H. S. Lim, D. J. Barclay, and F. C. Anson, *Inorg. Chem.*, 11, 1460 (1972).
(41) L. J. Anghileri, *Strahlentherapie*, **149**, 173 (1975); M. Tanabe, *Radioisotopes*, **25**, 44 (1976); G. Subramanian and J. G. McAfee, *J. Nucl. Med.*, 11, 365 (1970).

Michael J. Clarke,* Michael G. Dowling
Department of Chemistry, Boston College
Chestnut Hill, Massachusetts 02167

Alfred R. Garafalo
Department of Chemistry
Massachusetts College of Pharmacy
Boston, Massachusetts 02115

Thomas F. Brennan*
Department of Chemistry, Northeastern University
Boston, Massachusetts 02115

Received August 11, 1978

Thermochemistry and Generation of Vinylketene

Sir:

Neutral vinylketene has so far eluded observation, although its possible formation as a red compound, stable up to −160 °C, was reported by Conia et al.[1] in some flash thermolysis studies of spiro[2.3]hexan-4-ones. Vinylketene has been postulated as an intermediate in [2 + 2] cycloaddition reactions of cyclopentadiene with the dehydrochlorination product of *trans*-2-butenoyl and 3-butenoyl chloride[2] and in other related systems.[3]

We report here that vinylketene is obtained in high yield from the gas-phase thermal decomposition of the en-yne ether[4,5] $CH_2{=}CHC{\equiv}COCH_2CH_3$ (I).

$$\mathrm{I} \rightarrow \underset{\mathrm{II}}{C_4H_4O} + C_2H_4$$

Ionized vinylketene has been proposed[6] as resulting from the electron impact induced losses of (i) H_2O from crotonic acid (and some of its isomers), (ii) CH_3OH from methyl crotonate, and (iii) C_2H_4 from cyclohexen-2-one. The latter reaction had an appearance energy (AE) which yielded a heat of formation, $\Delta H_f(C_4H_4O)^{+\cdot} = 194 \pm 1$ kcal mol^{-1}, a value close to but significantly lower than that of the most stable isomer[7] $(\text{furan})^{+\cdot}$, $\Delta H_f = 197$ kcal mol^{-1}. However, the metastable peak characteristics of the reacting and nonreacting $(C_4H_4O)^{+\cdot}$ ions produced by i, ii, and iii (see Table I) show that they cannot have the structure of $(\text{furan})^{+\cdot}$.

The ionic heat of formation of II and the metastable characteristics of ionized II are consistent with the structural assignment given to $[C_4H_4O]^{+\cdot}$ ions derived from i, ii, and iii. Ethoxyethyne readily thermally decomposes to yield ketene and C_2H_4.[11] A similar 1,5-hydrogen shift in I would produce vinylketene and C_2H_4. When I is introduced into the ion source of an AEI-GEC MS902S mass spectrometer via a heated glass inlet system ($T = 100$ °C), the highest mass in the resulting 70-eV mass spectrum has m/z 68, $[C_4H_4O]^{+\cdot}$ (67%). Prominent ions are observed at m/z 42, $[CH_2CO]^{+\cdot}$ (14%); m/z 40, $[C_3H_4]^{+\cdot}$ (37%); m/z 39, $[C_3H_3]^{+}$ (78%); m/z 38, $[C_3H_2]^{+\cdot}$ (17%); m/z 37, $[C_3H]^{+}$ (11%); m/z 28, $[C_2H_4]^{+\cdot}$ (100%); m/z

0002-7863/79/1501-0225$01.00/0