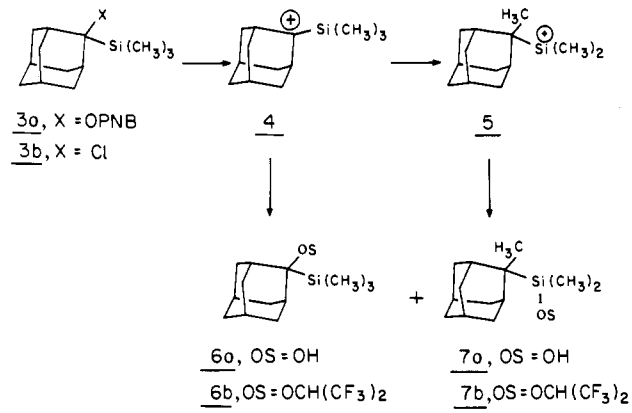
Scheme I

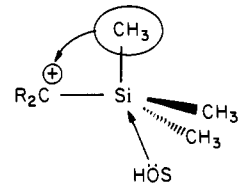


the *same* $C_5H_{13}Si^+$ ion, most likely $(CH_3)_2CHSi^+(CH_3)_2$.[8] Therefore, we decided to investigate similar rearrangements in the condensed phase.

We have recently shown[9] that solvolysis of **3** generates as the first intermediate an α-silyl carbenium ion **4** that is trapped by the solvent to give **6**. Rearrangement of **4** to the silicenium ion **5** and its capture by the solvent would give **7** as the reaction product (Scheme I). Solvolysis of **3a** in 80% acetone indeed yields a mixture of **6** and **7** (OS = OH) but the **7**:**6** product ratio is small, i.e., 9:91.[10] However, the yield of the rearranged products and thus the reaction fraction that proceeds via **5** (Scheme I) increases dramatically as the solvent nucleophilicity (N) is lowered.[11] The rearranged, **7**, to unrearranged, **6**, product ratios are 36:64, 68:32, and >99:1 in 93% trifluoroethanol (TFE), 98% TFE, and hexafluoroisopropyl alcohol (HFIP), respectively.[12] Similar results are observed with the chloride **3b**. Addition of water to the fluorinated solvents which increases its nucleophilicity,[11] as expected, reduces the **7**:**6** ratio. For example, in 95%, 87.5%, and 80% HFIP, **3b** yields **7**:**6** ratios of 85:15, 59:41, and 35:65, respectively (**7**:**6** is 64:36 in the solvolysis of **3a** in 95% HFIP). Similarly, with **3b** the addition of 40 equiv of 2,6-lutidine, which increases the nucleophilicity of the solvolysis medium, strongly reduces the **7**:**6** product ratio to 4:96 in 95% HFIP, compared to the unbuffered ratio of 85:15.

All the above data are *fully consistent* with the mechanism presented in Scheme I and *strongly support the formation of a silicenium ion* (either as a "free" ion or as an "ion pair"[2,13]) *in the solvolysis process*. The product–solvent dependency argues strongly against an alternative mechanism which involves a solvent-assisted methyl migration, depicted schematically in **8**,[5c] and which bypasses the silicenium ion **5** as a precursor to **7**. If rearrangement occurs via **8** the expectation is that increase in the solvent nucleophilicity would enhance the Si to C methyl migration and would increase the **7**:**6** product ratio,[14] but the opposite is observed (vide supra).

Some information on the selectivity of cationoid intermediates can be acquired by studying the product ratios (e.g., alcohol:ether)



8

in binary solvent mixtures[2,15] (e.g., aqueous HFIP). Using the usual assumption[2,15] that the solvent selectivity $S = k_{H_2O}/k_{HFIP}$ = [ALCOHOL][HFIP]/[ETHER][H_2O], we obtain (in the range of 80–95% aqueous HFIP mixtures) a selectivity of S(**4**) = *7.8* ± 2.6 for carbenium ion **4**, in contrast to the "inverse selectivity" for silicenium ion **5** of S(**5**) = *0.42* ± 0.01.[16]

The value of S(**4**) is consistent with the selectivities in aqueous TFE[15a,b] of other carbenium ions (e.g., for the 1-adamantyl cation k_{H_2O}/k_{TFE} = 0.83–2.60[15a]), taking into consideration the lower nucleophilicity of HFIP.[11] In contrast the silicenium ion **5** exhibits an "inverse selectivity", S(**5**) < 1; i.e., **5** is captured by HFIP ca. 2.4 times faster than by water. Further experiments are needed to clarify the reasons for this somewhat unusual behavior of **5**, and at this point we refrain from further speculation.

In conclusion, we have presented evidence which is fully consistent with the generation in solution of a silicenium ion via a 1,2-methyl shift in an α-silyl carbenium ion. Our study, coupled with the recent contributions by Lambert[4a] and Barton,[5d] calls for a reconsideration of silicenium ions as viable reaction intermediates in solution. We continue to explore the properties of **5** and extend our attempts to generate silicenium ions to other potential precursors and methods.

Acknowledgment. This research was supported by the Fund for Basic Research, administered by the Israel Academy of Sciences and Humanities. A.S. thanks Dr. Dorit Arad and Dr. Miriam Karni for their continuous support and collaboration.

(8) Apeloig, Y.; Drewello, T.; Karni, M.; Stanger, A.; Schwarz, H. *J. Chem. Soc., Chem. Commun.*, to be submitted.
(9) Apeloig, Y.; Stanger, A. *J. Am. Chem. Soc.* **1985**, *107*, 2806.
(10) Determined by gas chromatography. Reactions were conducted in a sealed tube at 90 °C and were quenched after 12–14 h (solvolysis rates are given in ref 9). The solvent was buffered with 2 equiv of 2,6-lutidine. There was no significant change in the product composition with reaction time. The amount of unidentified products did not exceed 3%.
(11) Bentley, T. W.; Schleyer, P. v. R. *Adv. Phys. Org. Chem.* **1977**, *14*, 1. N = 0.51, −2.78, and −3.93 for 80% acetone, TFE, and 97% HFIP, respectively.
(12) The products consisted of mixtures of alcohols (OS = OH) and ethers (OS = OCH_2CF_3 or $OCH(CF_3)_2$); see below.
(13) A methyl-bridged **5** as the product-forming intermediate cannot be ruled out. Additional experiments are needed to clarify this subtle mechanistic point.
(14) The possibility (suggested by a referee) that in analogy to the 3-coordinated Si atom in **5** (vide infra), the 4-coordinated Si atom in **8** is also more selective for the fluorinated alcohols as compared to H_2O is unlikely. Thus, t-Bu_3SiCl reacts much faster with water or methanol than with TFE; see: Eaborn, C.; Saxena, A. K. *J. Organomet. Chem.* **1984**, *271*, 33.
(15) (a) Kaspi, J.; Rappoport, Z. *J. Am. Chem. Soc.* **1980**, *102*, 3829. (b) Richard, J. P.; Rothenberg, M. E.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1361. Richard, J. P.; Jencks, W. P. *Ibid.* **1984**, *106*, 1373.
(16) As calculated from the observed product distributions; e.g., in 80% HFIP **3b** yields **6a** (59.1%), **6b** (5.8%), **7a** (13.4%), and **7b** (21.7%).[10]

^{1}H NMR Spectra of Rubredoxins: New Resonances Assignable to α-CH and β-CH_2 Hydrogens of Cysteinate Ligands to Iron(II)

Mark T. Werth and Donald M. Kurtz, Jr.*

Department of Chemistry, Iowa State University Ames, Iowa 50011

Isabel Moura† and Jean LeGall

Department of Biochemistry, University of Georgia Athens, Georgia 30602

Received July 21, 1986

As the simplest member of the iron–sulfur class of metalloproteins, rubredoxin (Rd), serves as a prototype for the study of protein-bound high-spin iron in tetrahedral coordination by sulfur atoms.[1-3] Rds have been examined by a variety of physical

* To whom inquires about the paper should be addressed. Present address: Department of Chemistry, University of Georgia, Athens, GA 30602.
† On leave of absence from Centro de Quimica Estrutural, UNL, 1096 Lisboa-Codex, Portugal.

(1) Eaton, E. A.; Lovenberg, W. In *Iron Sulfur Proteins*; Lovenberg, W., Ed.; Academic: New York, 1973; Vol. II, pp 131–162.
(2) Watenpaugh, K. D.; Sieker, L. C.; Jensen, L. H. *J. Mol. Biol.* **1979**, *131*, 509–522.
(3) Adman, E. T.; Sieker, L. C.; Jensen, L. H.; Bruschi, M.; LeGall, J. *J. Mol. Biol.* **1977**, *112*, 113–120.

0002-7863/87/1509-0273$01.50/0

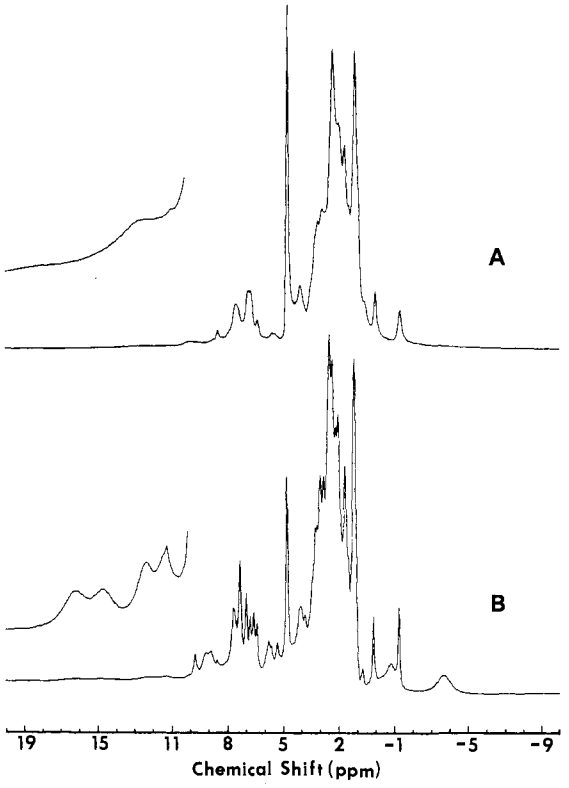


Figure 1. 300-MHz ^{1}H NMR spectra of *D. gigas* Rd in the 20 to −10 ppm region. Spectra were obtained at 55 °C as described:[6,8] (A) air-oxidized protein; (B) reduced protein prepared by addition of a small amount of solid $Na_2S_2O_4$. Insets show 16-fold vertical expansions of the 10–20 ppm region.

techniques, including ^{1}H NMR.[4,5] Their relatively small sizes (MW ~ 6000) make Rds particularly attractive candidates for the latter studies. We report the discovery of two groups of isotropically shifted resonances in ^{1}H NMR spectra of reduced *Desulfovibrio gigas* Rd[6] assignable to β-CH_2 (150–260 ppm) and α-CH (11–17 ppm) hydrogens of cysteinate ligands to Fe(II). We support these assignments by showing that a series of synthetic Fe(II)–alkylthiolate complexes give ^{1}H NMR resonances with chemical shifts and temperature dependences similar to those cited above for Rd.

Figures 1 and 2 show the 300-MHz ^{1}H NMR spectra of *D. gigas* Rd in the regions of 20 to −10 ppm and 260 to 100 ppm, respectively.[8] The spectra of Figure 1 in the region of 10 to −10 ppm resemble those previously published for *C. pasteurianum* Rd.[4,5] However, vertical expansions of the 20–10 ppm region in the spectrum of reduced *D. gigas* Rd reveal four new resonances at 16.2, 14.8, 12.4, and 11.4 ppm (at 55 °C), whose positions shift upfield with increasing temperature. These resonances are replaced in the spectrum of oxidized Rd by a single broad feature at ~13 ppm. The region between 260 and 100 ppm in the

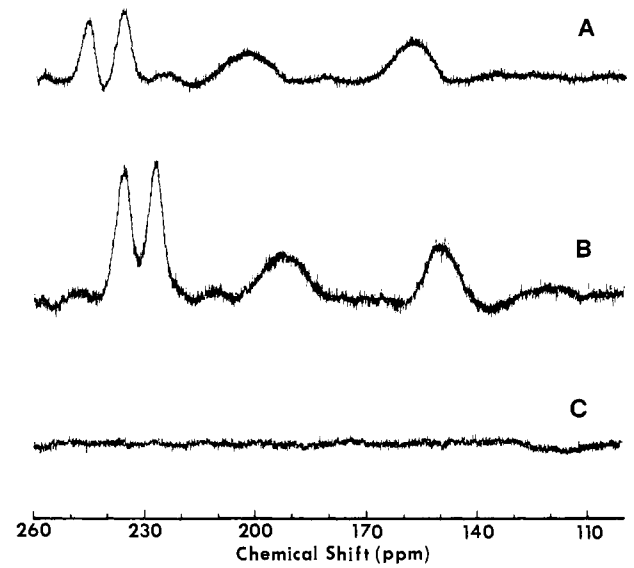
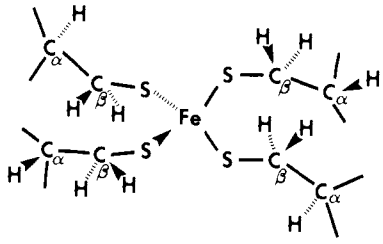


Figure 2. 300-MHz ^{1}H NMR spectra of *D. gigas* Rd in the 260–100 ppm region. (A) reduced, 40 °C; (B) reduced, 55 °C; (C) oxidized, 55 °C. These spectra are of the same solutions used to obtain the spectra of Figure 1, and spectra have been vertically expanded 256-fold compared to those of Figure 1. Spectra B and C are portions of the spectra in Figure 1 parts B and A, respectively, omitted from Figure 1.

spectrum of reduced Rd (Figure 2) reveals four additional new resonances of roughly equal areas at 236, 227, 192, and 150 ppm (at 55 °C). The positions of these resonances shift upfield with increasing temperature, and each exhibits a linear dependence of the chemical shift on T^{-1} between 25 and 65 °C.[9] These resonances disappear upon oxidation of the iron in *D. gigas* Rd, presumably due to the increased paramagnetism of high-spin Fe(III) over that of high-spin Fe(II). No resonances were detected between the regions shown in Figures 1 and 2 in spectra of either oxidized or reduced *D. gigas* Rd. For the spectra of reduced Rd, we assign the resonances between 236 and 150 ppm and between 17 and 11 ppm to the β-CH_2 and α-CH hydrogens, respectively, of the four cysteinate ligands to Fe(II). Perhaps because of the approximate D_{2d} site symmetry indicated by other spectroscopies[10,11] and by the crystal structures,[2,3] all eight β-CH_2 hydrogens are not resolved (cf. the structure).



Support for our assignments is as follows. First, the relative areas of the four resonances between 260 and 150 ppm to the four between 11 and 17 ppm are ~2:1. Second, it has been proposed that the isotropic shifts of the aforementioned β-CH_2 and α-CH hydrogens should be predominantly contact in origin.[5] Both the Curie temperature dependences of these new resonances and attenuations of the shifts with distance from the metal atom without sign reversal are exactly the behaviors expected for dominant contact interactions, assuming σ-spin transfer.[12,13]

(4) Phillips, W. D.; Poe, M.; Weiher, J. F.; McDonald, C. C.; Lovenberg, W. *Nature (London)* **1970**, *227*, 574–577.
(5) Krishnamoorthi, R.; Markley, J. L.; Cusanovich, M. A.; Przysieki, C. T. *Biochemistry* **1986**, *25*, 50–54.
(6) Lypohilized Rd from *D. gigas*, which had been purified by a standard procedure to A_{280}/A_{490} = 2.46 (lit. 2.47),[7] was dissolved in D_2O buffered with 50 mM phosphate, pH 7.5 (uncorrected). Solutions approximately 2 mM in Rd were used for NMR.
(7) Moura, I.; Bruschi, M.; LeGall, J.; Moura, J. J. G.; Xavier, A. V. *Biochem. Biophys. Res. Commun.* **1977**, *75*, 1037–1044.
(8) ^{1}H NMR spectra were collected on either Nicolet NT-300 or Bruker WM-300 spectrometers, the latter equipped with variable-temperature capability. Chemical shifts were referenced to the residual HDO solvent signal, which was assigned a value of 4.76 ppm downfield of DSS at 25 °C. The solvent signal was suppressed by a 50-ms presaturation pulse. For the protein spectra 64 000 transients were collected over a bandwidth of 125 kHz by using 16 384 data points and a center field of 150 ppm. The repetition rate was ~120 ms. Downfield and upfield shifts are reported as positive and negative, respectively.
(9) Details are included in the supplementary material.
(10) Yachandra, V. K.; Hare, J.; Moura, I.; Spiro, T. G. *J. Am. Chem. Soc.* **1983**, *105*, 6455–6461.
(11) Czernuszewicz, R. S.; LeGall, J.; Moura, I.; Spiro, T. G. *Inorg. Chem.* **1986**, *25*, 696–700.
(12) La Mar, G. N. In *NMR of Paramagnetic Molecules*; La Mar, G. N., Horrocks, W. D., Jr., Holm, R. H., Eds; Academic: New York, 1973; pp 86–123.

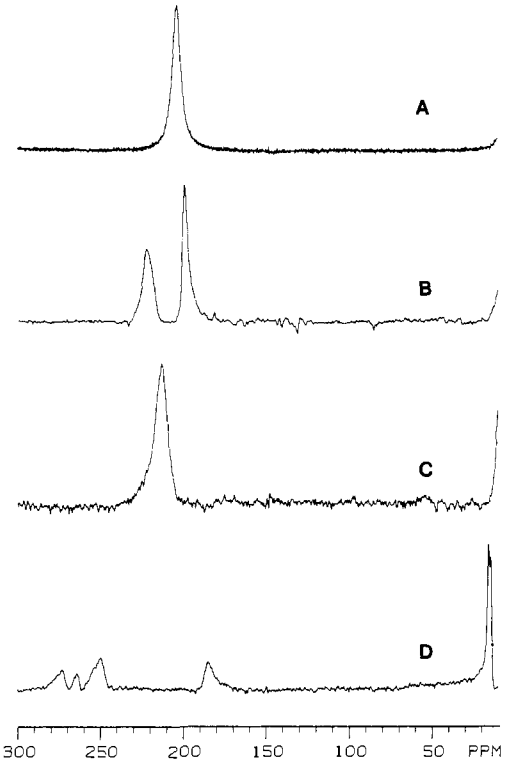


Figure 3. 300-MHz ^{1}H NMR spectra of tetrakis(alkylthiolate)-Fe(II) complexes in the region of 300-10 ppm. Complexes were prepared in D_2O as described.[14] Fe(II) complex with (A) 2-mercaptoethanol, (B) dithiothreitol, (C) glutathione, and (D) D,L-dihydrolipoate. Spectrum D is of a 2-fold diluted solution. All spectra were obtained at 25 °C.

Third, the spectra of Figure 3 show that similar isotropically shifted resonances can be obtained for a series of alkylthiolate-Fe(II) complexes.[14] We formulate these complexes as $[Fe(SR)_4]^{2-}$ (neglecting charges on the R groups) on the basis of the strong resemblance of their UV-vis[9] and ^{1}H NMR spectra to those of $[Fe(SCH_2CH_3)_4]^{2-}$.[15] The magnetic and spectral properties of this latter complex are indicative of tetrahedral coordination geometry.[16] For the complexes of Figure 3 we obtain resonances at 203 (β-mercaptoethanol); 221 and 199 (dithiothreitol); 213 (glutathione); and 271, 264, 250, and 185 ppm (D,L-dihydrolipoate), all at 25 °C. Each of the aforementioned resonances shifts upfield with increasing temperature and exhibits a linear dependence of the chemical shift on T^{-1} in the region of 5-55 °C. These resonances are all assigned to methylene hydrogens α to coordinated sulfur and, hence, correspond to the β-CH_2 groups of coordinated cysteinate in reduced Rd.[17] The multiple peaks observed in the cases of dithiotheitol and D,L-dihydrolipoate are presumably due to the S,S'-bidentate chelates formed with these dithiols. The chemical (though not necessarily magnetic) environment of α-CH of coordinated cysteinate in Rd would be expected to be most accurately approximated by the corresponding α-CH of S-coordinated glutathione (γ-glutamylcysteinylglycine). For this complex we observe a broad resonance centered at ~8 ppm (not shown), which we tentatively assign to the α-CH's of coordinated cysteinyl residues in glutathione. S,S'-Chelated dihydrolipoate has two methylenes β to coordinated sulfur, and the group of resonances between 13 and 16 ppm in Figure 3D we assign to these methylene hydrogens. These resonances shift upfield with increasing temperature. Thus, the resonances between 11 and 17 ppm in ^{1}H NMR spectra of reduced Rd have counterparts in at least one synthetic complex.

The results reported here represent a new spectroscopic probe of the iron site in at least one Rd and set the stage for examinations of iron sites in other Rds and in related proteins such as desulforedoxin[18] by ^{1}H NMR. Finally, this work clearly illustrates the usefulness of "synthetic analogues" in the clarification of properties of a metal site in a protein.[15]

Note Added in Proof. We have obtained ^{1}H NMR spectra of oxidized and reduced Rds from *Desulfovibrio vulgaris*. These spectra are similar to those in Figures 1 and 2.

Acknowledgment. This work was supported by the National Science Foundation (D.M.K., DMB-8216447, and J.L.G., DMB-8602789) and by a grant from Junta Nacional de Investigacão Cientifica e Tecnologica (Portugal). We thank the staff of the University of Georgia Fermentation Plant for growing the bacterial cells that were used in this study.

Registry No. Fe, 7439-89-6; L-cysteine, 52-90-4.

Supplementary Material Available: Plots of chemical shifts vs. T^{-1} for isotropically shifted resonances of reduced *D. gigas* Rd and UV-vis absorption spectra of alkylthiolate-Fe(II) complexes (2 pages). Ordering information is given on any current masthead page.

(13) Horrocks, W. D., Jr. In *ESR and NMR of Paramagnetic Species in Biological and Related Systems*; Bertini, I., Drago, R., Eds.; D. Reidel: Boston, MA, 1979; pp 55-87.

(14) Solutions of these complexes were prepared under Ar by first dissolving thiols in D_2O with sufficient LiOH to deprotonate the thiol groups. The resulting solutions, 80 mM in thiol (40 mM in dithiol), were added to solid $FeCl_2$ to give solutions 20 mM in Fe(II).

(15) A stimulus to the present work was the report by Hagen et al.[16] of ^{1}H NMR resonances of $[Fe(SCH_2CH_3)_4]^{2-}$ at 196 (CH_2) and 10 (CH_3) ppm downfield of Me_4Si.

(16) Hagen, K. S.; Watson, A. D.; Holm, R. H. *J. Am. Chem. Soc.* **1983**, *105*, 3905-3913.

(17) Attempts to prepare these complexes with Fe(III) resulted in species with only transient stability, making it impossible to obtain ^{1}H NMR spectra.

(18) Moura, I.; Huynh, B. H.; Hausinger, R. P.; LeGall, J.; Xavier, A. V.; Münck, E. *J. Biol. Chem.* **1980**, *255*, 2493-2498.

The Hydrated Methoxide Ion, $CH_3O^-\cdot 6H_2O$

Avi Bino

Department of Inorganic and Analytical Chemistry
The Hebrew University of Jerusalem
91904 Jerusalem, Israel
Received August 19, 1986

The interactions of very strong bases such as hydroxide and methoxide anions with water have been the subject of numerous experimental and theoretical studies. Experiments and calculations in the gas phase and in solutions have demonstrated the important role of solvation in determining molecular properties such as relative acidities, basicities, and nucleophilicities.[1-5]

While the hydrated hydroxide ion, $OH^-\cdot nH_2O$, has been characterized in several crystalline compounds,[6] the structure of the hydrated methoxide ion, $CH_3O^-\cdot nH_2O$, has not been reported in single-crystal X-ray studies.

We report here the results of an X-ray structural analysis of such a species, $CH_3O^-\cdot 6H_2O$, found in the crystal of $Na_5[Cr-$

(1) Salem, L. *Electrons in Chemical Reactions*; Wiley: New York, 1982; p 214.

(2) (a) Hehre, W. J.; Pople, J. A. *Tetrahedron Lett.* **1970**, 2959. (b) Kraemer, W. P.; Dierksen, G. H. F. *Theor. Chim. Acta* **1972**, *23*, 398. (c) Roos, B. O.; Kraemer, W. P.; Dierksen, G. H. F. *Theor. Chim. Acta* **1976**, *42*, 77. (d) Tel, L. M.; Wolfe, S.; Csizmadia, I. G. *J. Chem. Phys.* **1973**, *59*, 4047. (e) Heidrich, D.; Volkmann, D.; Zurawski, B. *Chem. Phys. Lett.* **1981**, *80*, 60.

(3) Jorgensen, W. L.; Ibrahim, M. *J. Comput. Chem.* **1981**, *2*, 7.

(4) (a) Bartmess, J. E.; Scott, J. A.; McIver, R. T., Jr. *J. am. Chem. Soc.* **1979**, *101*, 6056. (b) Kebarle, P. *Ann. Phys. Chem.* **1977**, *28*, 445. (c) Arnett, E. M.; Small, L. E.; McIver, R. T., Jr.; Miller, J. S. *J. Am. Chem. Soc.* **1974**, *96*, 5638.

(5) (a) Ikuta, S. *J. Comput. Chem.*, **1984**, *5*, 374. (b) Madura, J. D.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1986**, *108*, 2517. (c) MacKay, G. I.; Bohme, D. K. *J. Am. Chem. Soc.* **1978**, *100*, 327.

(6) (a) McMullan, R. K.; Mak, T. C. W.; Jeffrey, G. A. *J. Chem. Phys.* **1966**, *44*, 2338. (b) Boer, F. P.; Neuman, M. A.; van Remoortere, F. P.; Steiner, E. C. *Inorg. Chem.* **1974**, *13*, 2826. (c) Abu-Dari, K.; Freyberg, D. P.; Raymond, K. N. *Inorg. Chem.* **1979**, *18*, 3037.

0002-7863/87/1509-0275$01.50/0