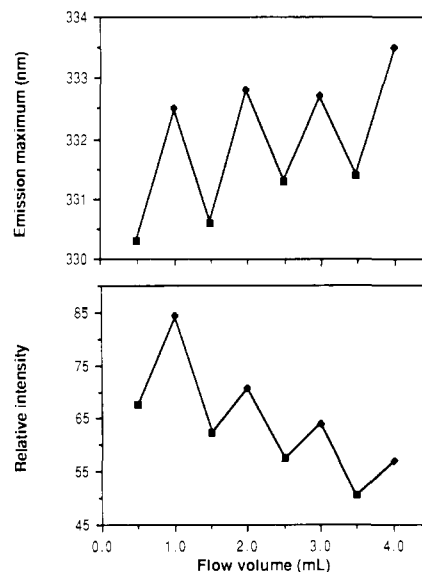**Figure 1.** Fluorescence spectra of apomyoglobin adsorbed to Zorbax silica from pH 4.0 buffer: (A) after clearing the flowcell void volume with 1.0 mL of pH 4.0 buffer; (B) after pumping 2.0 mL of pH 7.5 buffer through the cell. The emission maxima of (A) and (B) occur at 330.3 and 332.3 nm, respectively; the intensity units are relative.

Zorbax prior to each experiment. Fluorescence spectra were acquired at stopped flow on a Perkin-Elmer MPF-66 spectrofluorimeter (Ex = 295; slits = 3 nm) at 22 °C. Scattering and silica background luminescence were treated as previously described.[9] After preequilibration with buffer and acquisition of the background spectrum for correction of spectra, a solution of 4.8 μM protein (pH 4.0) was pumped through the cell. When the luminescence from the adsorbed protein became constant, the column was flushed free of solution-phase protein with fresh pH 4.0 buffer. The background-corrected spectrum was obtained, the cell flushed with 2.0 mL of pH 7.5 buffer, and the spectrum obtained again. In another experiment, after apomyoglobin adsorption, the cell was flushed 8 times alternately with fresh pH 4.0 and 7.5 buffer and spectra were recorded.

The emission maxima of apomyoglobin in solution in pH 7.5 and pH 4.0 buffer are 330 and 335 nm.[10] The 5-nm red shift observed upon acidification of the solution is associated with destruction of the heme-binding pocket and loss of approximately half of the α-helical content of the protein.[11] Fluorescence spectra of apomyoglobin adsorbed to Zorbax at pH 4.0 and 7.5 are shown in Figure 1. The emission maximum of apomyoglobin at pH 4.0 is 330 nm, a 5-nm blue shift from the solution value. This suggests that the two tryptophans (positions 7 and 14 in the A α-helix) are in a more hydrophobic environment compared to the solution conformation. This could occur if the protein is either folded into a more compact configuration upon adsorption or is unfolded to allow interaction of the exposed tryptophans with the octadecyl chains bound to the silica gel. We believe the latter case is more likely in light of the red shift in the emission maximum to 332 nm and the increase in intensity observed after pumping pH 7.5 buffer through the cell. It is highly unlikely that apomyoglobin in the adsorbed state is more unfolded at pH 7.5 than at pH 4.0. The 2-nm red shift must represent partial refolding of the adsorbed protein, resulting in less interaction between the tryptophan residues and the hydrophobic surface. The 332-nm emission maximum is 2 nm to the red from that in solution at pH 7.5, indicating partial unfolding of the adsorbed protein at neutral pH. The emission maximum of apomyoglobin adsorbed from pH 7.5 buffer is 334 nm (a 4-nm red shift from the solution maximum of 330 nm). This suggests that the pH 4.0 surface form can be only partially refolded to a pH 7.5 surface form when adsorption has taken place at the more acidic pH.

The correlated fluctuation of the emission maximum and relative intensity (at the maximum) of adsorbed apomyoglobin with the pH of the contact buffer suggests that interconversion between the folded and unfolded states is at least partially reversible (Figure 2). Repeated interconversion is accompanied by a gradual red shift and an overall decrease in fluorescence intensity. The latter may be due to UV photodegradation (ob-

Figure 2. Fluorescence emission maxima and relative intensity (at the maximum) of apomyoglobin adsorbed to Zorbax silica from pH 4.0 buffer: (■) after pumping 0.5 mL of pH 4.0 buffer through the flowcell; (♦) after pumping 0.5 mL of pH 7.5 buffer through the cell.

served for albumin adsorbed on silica[1]). A gradual decline in luminescence which cannot be attributed to UV degradation but is associated with adsorption for long periods of time was also observed.

A Novel Hypervalent Antimony Ate Complex (12-Sb-6)[1]

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The chemistry of hypervalent ate complexes has recently attracted increasing interest.[2] Concerning that of antimony, there is no example of stable organic 12-Sb-6 compounds other than $Ph_6Sb^-Li^+$,[3a] although several 12-Sb-6 species have been proposed as intermediates in the reactions of Sb(V) with nucleophiles.[4]

(1) The *N*-X-*L* designation was proposed previously: X, central atom; *N*, formal valence shell electrons about an X; *L*, the number of ligands. (See: Perkins, C. W.; Martin, J. C.; Arduengo, A. J.; Lau, W.; Algeria, A.; Kochi, J. K. *J. Am. Chem. Soc.* **1980,** *102,* 7753).

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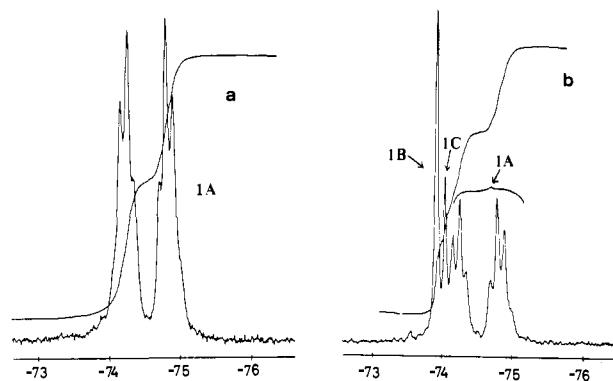
Figure 1. ^{19}F NMR spectra of 12-Sb-6 ate complexes **1**: (a) **1A**, after treatment of **2** with 1 equiv of MeLi at −78 °C; (b) a mixture of **1A**/**1B**/**1C** at 24 °C before the equilibrium. Both spectra were measured at −50 °C.[7]

Scheme I. Kinetic Ratio of Formation of Ate Complexes **1A**–**1C** at −78 °C and Their Equilibration
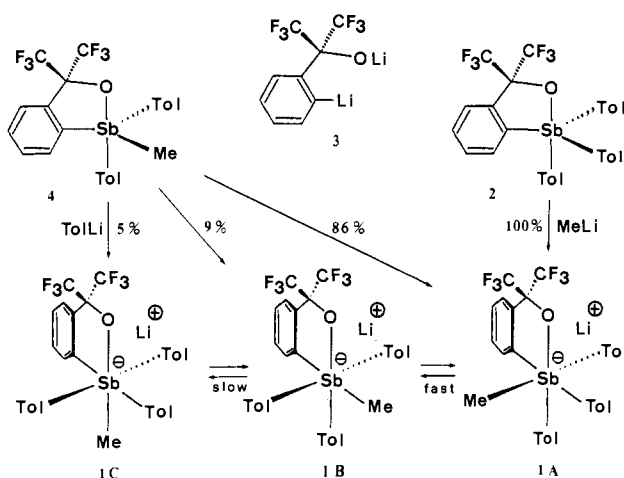
Now we wish to report convincing spectroscopic evidence for a stable unsymmetrical ate complex of antimony (**1**) and its geometrical isomerization.

1,1,1-Tris(*p*-tolyl)-3,3-bis(trifluoromethyl)-3*H*-2,1-benzoxastibole(V) (**2**)[5] was prepared by the reaction of (*p*-tolyl)$_3$SbBr$_2$ and the dilithiated reagent **3** of bis(trifluoromethyl)benzyl alcohol.[6] Compound **2** is thermally stable (<250 °C, 3 h) and does not react with electrophiles such as EtCOCl, CH$_2$=N$^+$Me$_2$I$^-$, and (N-H$_4$)$_2$Ce(NO$_3$)$_6$ at 60–70 °C for over 3 h.

Upon treatment with 1 equiv of methyllithium at −78 °C in THF, **2** yielded a single adduct. The clear quartets of the adduct in ^{19}F NMR showed the presence of nonequivalent CF$_3$ groups (δ −74.2 and −74.9, $^4J_{F-F}$ = 9 Hz at −50 °C; δ −74.0 and −74.3 at 35 °C), thus the structure of the adduct was assigned to be **1A** as shown in Scheme I. Other isomers such as **1B** and **1C** could not be observed at −50 °C during 5 h. When the solution was warmed to −20 °C, a new singlet **1B** (δ −73.8 at −50 °C; δ −74.0 at 35 °C) appeared to give an equilibrium mixture of **1A**/**1B** (3.8:1.0) within 1.5 h and then another singlet **1C** (δ −74.0 at −50 °C; δ −73.8 at 35 °C) appeared at around 0 °C.[7] Equilibrium ratio of the three isomers, **1A**/**1B**/**1C**, was 61:23:16 at 20 °C.[8]

When another benzoxastibole(V) **4** was treated with *p*-tolyllithium, three isomers were formed during the addition at −78 °C and the ratio was observed at −40 °C (**1A:1B:1C** = 86:9:5)[9] and the equilibration gave a mixture with the same ratio as that of **2** and MeLi at 20 °C. The greater kinetic selectivity seen for the formation of **1A** from **2** can be ascribed to the complexation of methyllithium with the oxygen atom of **2** and also to the least steric hindrance during the approach of the nucleophile to **2**.

One possible mechanism for the equilibration is an intermolecular equilibrium, in which **4** and *p*-tolyllithium are produced in situ via **1** as was reported quite recently for the tin 10-Sn-5 complexes Me$_{5-n}$Ph$_n$Sn$^-$Li$^+$.[2f] We checked for the presence of free MeLi and TolLi by ^{13}C NMR but could not detect the presence of either until more than 1 equiv of MeLi was added (only overfed MeLi was detected). In order to examine the mechanism more closely, we compared the rate of the equilibration of **1A** ⇌ **1B**[10] and that of quenching of the equilibrium mixture with ethanol at −20 °C in THF. The initially formed **1A** reached equilibrium with **1B** completely in 1.5 h (**1A:1B** = 3.8:1). The first-order rate constant for the equilibration could be calculated as (1.9 ± 0.1) × 10^{-4} s^{-1}. The quenching of the mixture of **1A** and **1B** at the same temperature with 10 and 100 equiv of ethanol (the resulting concentration of ethanol, 0.6–1.4 M) was relatively slow to give **4** and **2** (total yield 95%; **4:2** = 95:5). The second-order rate constant[11] was (1.8 ± 0.1) × 10^{-5} L·mol^{-1}·s^{-1}, indicating that quenching with 1.0 M ethanol is ca. 10 times slower than the isomerization. This result clearly excludes the possibility that an intermolecular dissociative mechanism plays a major role, because any free MeLi or *p*-TolLi should be quenched with ethanol instantaneously and also because *p*-TolLi, if any, should give a mixture of **1A**, **1B**, and **1C** by the reverse addition to **4**. As yet we cannot conclude which intramolecular path (dissociative or nondissociative) takes place in the equilibration process.[12]

The reactivity of **1** with electrophiles was examined preliminarily. Treatment of **1** with 3,*N*-dimethoxycarbonylpyridinium chloride yielded 31% of an isolated mixture of dihydronicotinates **5** (the yields of 1,2-, 1,4-, and 1,6-dihydronicotinates were 6%, 8%, and 17%, respectively) and 34% of isolated **6**. Compounds

Tol = *p*-CH$_3$C$_6$H$_4$

2 and **4** were recovered in 46% and 49% yields. The reaction products are quite different from those of the reaction of *p*-tolyl(or methyl)lithium,[13] thus confirming that the "ate" complex **1** is the

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(5) **2**: colorless crystals (recrystallized from benzene), mp 192–193 °C; ^{19}F NMR (THF) δ −75.0 (s), upfield from CFCl$_3$, at 35 °C. Anal. Calcd for C$_{30}$H$_{25}$F$_6$OSb: C, 56.54; H, 3.95. Found: C, 56.79; H, 3.94.
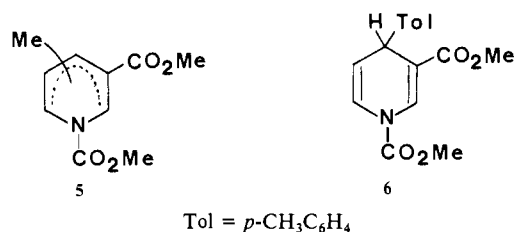
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(7) Chemical shifts of three peaks shift slightly according to the temperature, so spectra were measured at −50, −20, 20, and 35 °C. For example, the equilibrium mixture at 24 °C was cooled rapidly to −50 °C and the spectrum was measured to obtain a well-resolved one.

(8) Although we do not have any evidence to discriminate between the structures of **1B** and **1C**, we assume that the ate complex **1** with the *p*-tolyl group trans to the oxygen atom is preferable to that with methyl group trans, thus **1B** and **1C** are depicted as shown in Scheme I, based on the equilibrium ratio. When the equilibrium mixture at 20 °C was cooled to −20 °C, the ratio **1A:1B:1C** becomes 3.8:1.0:0.4 (73:19:8) after 1.5 h (**1C** was still decreasing). Thus the ratio **1A:1B** (3.8:1.0) at −20 °C described in the text was confirmed to be the equilibrium ratio. We thank the comment of referee III.

(9) **4**: colorless crystals (recrystallized from benzene), mp 176–178 °C; ^{19}F NMR (THF) δ −75.5 (s), at 35 °C. Anal. Calcd for C$_{24}$H$_{21}$F$_6$OSb: C, 51.37; H, 3.77. Found: C, 51.55; H, 3.78. Kinetic preference for **1A** cannot be understood if based only on tight complexation of TolLi to oxygen; there may be some (steric) reason for TolLi to attack Sb of **4** directly from the side between Tol and Me.

(10) At −20 °C no detectable amount of **1C** was observed after the equilibration between **1A** and **1B** was completed. We can calculate the rate of the equilibration, therefore, by the reversible first-order kinetics.

(11) The value can be calculated from the pseudo-first-order kinetics.

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(13) The reaction of *p*-tolyllithium with 3,*N*-dimethoxycarbonylpyridinium salt did not give the dihydropyridines at all but instead afforded methyl nicotinate and 3-(*p*-toluoyl)pyridine in 45% and 26% yield, respectively.

actual reactive species. The conclusion was also supported by the reaction of **1** with *p*-methoxybenzoyl chloride, which resulted in the formation of 4-methyl-4'-methoxybenzophenone (52% yield).[14] These features of **1** will offer some interesting possibilities for further manipulation of 12-Sb-6 "ate" complexes.

(14) The reaction of 0.7 equiv of *p*-tolyllithium with *p*-methoxybenzoyl chloride did not give the ketone but instead yielded (4-methoxyphenyl) bis-(4-methylphenyl)carbinol (68%).

(15) Partial support of this research is acknowledged for a Grant-in-Aid for Special Project Research (No. 61225019) by the Ministry of Education, Science, and Culture of the Japanese Government.

Bimolecular Reactions of Doubly Charged Metal Ions in the Gas Phase. Formation of NbCH₂²⁺

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There has been much interest in the reactivity of gas-phase metal ions with small organic molecules as seen in the extensive literature relating to this topic.[1] This interest has, until now, exclusively involved singly charged metal ions. This trend is similar to that seen for gas-phase organic ions where there is a far greater amount of literature related to singly charged ions.[2,3]

Multiply charged metal ions have not received much attention due in part to the belief that rapid charge exchange would occur exclusively. This has, in fact, been observed for multiply charged, later transition metals.[4] In a very interesting recent paper, however, Tonkyn and Weisshaar[5] have observed clustering reactions of Ti²⁺ with methane and hydride abstraction from ethane in a flowing afterglow. This prompted us to look at some bimolecular reactions of multiply charged early transition metals with various organic molecules under low-pressure conditions in a Fourier transform mass spectrometer (FTMS). The early transition metals have relatively low second ionization potentials.

All experiments were performed on a prototype Nicolet FTMS-1000 equipped with a 5.2-cm cubic trapping cell situated between the poles of a Varian 15-in. electromagnet maintained at 0.9 T. Laser desorption[6] was used to generate Nb²⁺ from a Nb target. Nb⁺ was also generated in varying amounts relative to Nb²⁺ depending on experimental conditions, but it could be cleanly ejected from the cell eliminating any interference.[7] A typical reaction sequence is shown in Figure 1. A Bayard–Alpert ionization gauge was used to monitor the pressure and was calibrated for the kinetics experiments using reaction 1 with a rate constant of 1.14×10^{-9} cm³/molecule s.[8]

$$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3^\bullet \qquad (1)$$
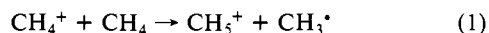
Multiply charged metal ions produced by laser desorption can have considerable excess electronic and kinetic energy.[4,9,10] In order to minimize these effects, the Nb²⁺ was trapped for 500 ms in the presence of $\sim 6 \times 10^{-7}$ torr of reagent and enough Ar to

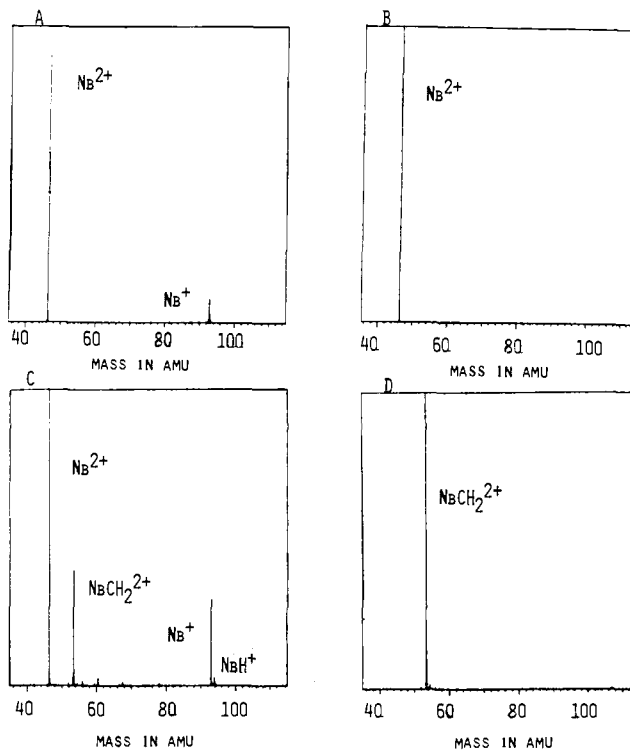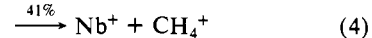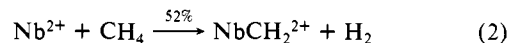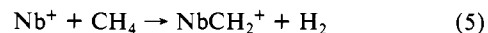(1) For a review of gas-phase metal ion chemistry, see: Allison, J. In *Progress in Inorganic Chemistry*; Lippard, S. J., Ed.; Wiley-Interscience: New York, 1986; Vol. 34, p 628.

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Figure 1. (A) Laser ionization of Nb foil; (B) isolation of Nb²⁺; (C) reaction of Nb²⁺ with CH₄; (D) isolation of NbCH₂²⁺.

bring the total pressure to $\sim 1 \times 10^{-5}$ torr, after which the doubly charged ions were reisolated and permitted to react.[11] Observation of a simple first-order reaction decay of Nb²⁺ for at least two half-lives suggested a predominantly ground-state population. The presence of nonthermal ions, however, cannot be completely ruled out.

Nb²⁺ (2nd IP(Nb) = 14.3 eV)[12] is observed to react via many other routes besides charge exchange, as in reactions 2 and 3 with methane (IP = 12.6 eV) (see Figure 1C). Observation of NbCH₂²⁺ from methane implies D^0(Nb²⁺–CH₂) > 112 kcal/mol.[13]

$$Nb^{2+} + CH_4 \xrightarrow{52\%} NbCH_2^{2+} + H_2 \qquad (2)$$

$$\xrightarrow{7\%} NbH^+ + CH_3^+ \qquad (3)$$

$$\xrightarrow{41\%} Nb^+ + CH_4^+ \qquad (4)$$

The overall reaction rate constant for reactions 2–4 is (0.95 ± 0.19) $\times 10^{-9}$ cm³/molecule s, giving a calculated reaction efficiency of ~ 0.47.[14] This reaction is more than 2 orders of magnitude faster than the reaction of Nb⁺ with methane, reaction 5, which occurs with a rate constant of (0.9 ± 0.2) $\times 10^{-11}$

$$Nb^+ + CH_4 \rightarrow NbCH_2^+ + H_2 \qquad (5)$$

cm³/molecule s, which gives a calculated reaction efficiency of ~ 0.008. Reaction 5 is believed to be slightly endothermic on the basis of the slow reaction rate and from photodissociation experiments.[15] The fast rate for reaction 2, however, indicates that the thermodynamic limit of D^0(Nb²⁺–CH₂) > 112 kcal/mol is

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