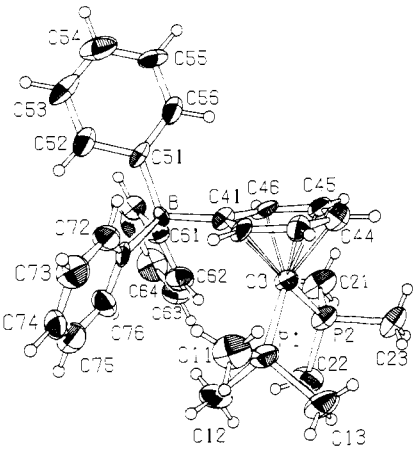

Figure 1. ORTEP drawing of the $Co(PMe_3)_2(BPh_4)$ molecule. Ellipsoids correspond to 50% probability. Hydrogens are represented as spheres of arbitrary size. Distances: Co–P1, 2.171 (3); Co–P2, 2.164 (3); Co–C41, 2.219 (9); Co–C42, 2.096 (9); Co–C43, 2.131 (11); Co–C44, 2.123 (12); Co–C45, 2.119 (10); Co–C46, 2.120 (9); Co–centroid, 1.602 Å. Angles: P1–Co–P2, 94.6 (1)°; P1–Co–centroid, 133.5°; P2–Co–centroid, 131.7°.

or dichloromethane solutions. X-ray diffraction[12] shows that they consist of monomeric molecules (Figure 1) in which the Co(I) center is σ-bonded with two PMe_3 ligands and π-bonded with one of the phenyl rings of the BPh_4^- ion. The Co–P distances (av 2.167 (3) Å) are normal. The C–C distances in the π-bonded ring (av 1.412 Å) are longer than those of the free rings (av 1.386 Å) as a result of metal-to-ligand back-bonding. The π-bonded ring is planar, except for the Ph_3B-bonded carbon, which is displaced by 0.044 Å from the plane of the remaining five carbon atoms, away from cobalt. This ring distortion, which probably minimizes steric hindrance, is different from those previously found. Except for this carbon, the Co–C distances (2.096 (9)–2.131 (11) Å, av 2.118 Å) are equal, indicating that the ring behaves as a normal 6-electron donor. Therefore, $Co(PMe_3)_2(BPh_4)$ can be regarded as an 18-electron system.

Its $^{31}P\{^1H\}$ spectrum at 295 K shows no signal as commonly found for Co–PMe_3 complexes.[16] At 183 K, a broad singlet is present at 8.3 ppm, which is consistent with two equivalent phosphorus atoms.[13] The ^{13}C and $^{13}C\{^1H\}$ spectra at 295 K exhibit only a small ill-resolved signal at 19 ppm, corresponding to the P-bonded methyl groups. At 223 K, however, well-resolved spectra are obtained, and decreasing the temperature has no supplementary effect on the spectra. Figure 2 shows the 183 K $^{13}C\{^1H\}$ spectrum in CD_2Cl_2. The ^{13}C data are consistent with the X-ray result. The five coplanar carbon atoms of the π-bonded ring are shifted upfield as expected, but only a small downfield shift is observed for the Ph_3B-bonded carbon, which is certainly related to the presence of a positive charge on the adjacent boron atom. The $^1H\{^{13}P\}$ spectrum is temperature dependent and indicates that a complicated exchange process is occurring between the hydrogens of the phenyl groups. At 183 K, exchange is still present, but two broad singulets are resolved at 5.0 and 5.3 ppm, in the range where the protons of the coordinated phenyl ring are expected. The last protons are probably masked by the broad signal at ~7 ppm for the free phenyl ring.

$Co(PMe_3)_2(BPh_4)$ is not inert toward nucleophilic substitution in contrast with the rhodium complexes. In solution, the

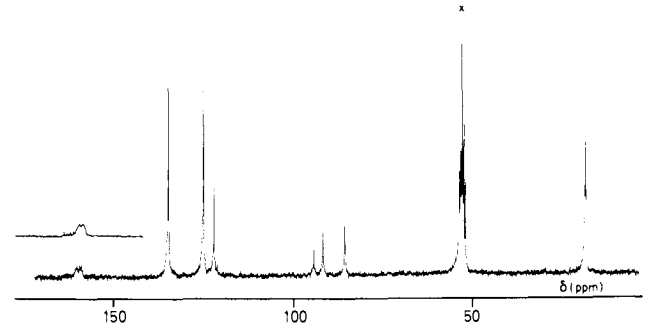

Figure 2. $^{13}C\{^1H\}$ spectrum, 183 K, of a CD_2Cl_2 solution of $Co(PMe_3)_2PBh_4$ ($\delta(SiMe_4)$ = 0) PMe_3, 20 (J_{CH} = 126, J_{CP} = 15 Hz); uncoordinated Ph, 123 (J_{CH} = 159 Hz), 125 (J_{CH} = 155 Hz), 135 (J_{CH} = 153 Hz), 159.5 (J_{CB} = unresolv.); coordinated Ph, 86 (J_{CH} = 163 Hz), 92 (J_{CH} = 163 Hz), 95 (J_{CH} = 160 Hz), 162.5 (J_{CB} = 50 Hz) (x = CD_2Cl_2).

Co–(C_6H_5) π-bond is easily broken in the presence of nucleophiles such as PMe_3, CO, ethylene, or acetylene. Investigations in this direction are in progress since we have at hand the reactive 12-electron $[Co(PMe_3)_2]^+$ moiety, which until now has been stabilized and studied only as cyclopentadienyl[14] or carbonyl species.[15]

Acknowledgment. We thank G. Commenges for running the low-temperature NMR spectra and M. J. Olivier for collecting the X-ray data. Support of this research through the Centre National de la Recherche Scientifique, le Conseil de Recherches en Sciences Naturelles et en Gēnie du Canada and la coopēration France-Quēbec, is gratefully acknowledged.

Registry No. $[Co(PMe_3)_4]BPh_4$, 53432-11-4; $Co(PMe_3)_2(BPh_4)$, 92220-66-1; $CoBr(PMe_3)_3$, 53432-07-8; $NaBPh_4$, 143-66-8.

Supplementary Material Available: Refined atomic coordinates and temperature factors for $[Co(PMe_3)_4]BPh_4$ and $Co(PMe_3)_2(BPh_4)$ (8 pages). Ordering information is given on any current masthead page.

(12) $Co(PMe_3)_2(BPh_4)$ is monoclinic, $P2_1/c$, a = 11.457 (3) Å, b = 13.804 (4) Å, c = 19.339 (8) Å, β = 115.21 (3)°, Z = 4. Structure refined on 2857 Cu Kα reflections ($I > 3\sigma(I)$)[10] to R = 0.051 and R_w = 0.052.

(13) The $^1H\{^{31}P\}$, ^{13}C, $^{13}C\{^1H\}$, and $^{31}P\{^1H\}$ NMR spectra were recorded at 250 MHz for ^1H, 101.27 MHz for ^{31}P, and 62.9 MHz for ^{13}C, with a Bruker WM-250 spectrometer.

(14) Werner, H. *Pure Appl. Chem.* **1982**, *54*, 177-188.

(15) Rigo, P.; Bressan, M.; Morvillo, A. *J. Organomet. Chem.* **1976**, *105*, 263-269.

(16) Kidd, R. G.; Goodfellow, R. J. In "NMR and the Periodic Table"; Harris, R. K., Mann, B. E., Eds.; Academic Press: New York, 1978; pp 225-244.

Novel Suicide Inhibitors of Serine Proteinases. Inactivation of Human Leukocyte Elastase by Ynenol Lactones†

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There has been considerable interest in developing specific proteinase inhibitors as pharmacological and therapeutic agents.[1-4] Scheme I depicts a strategy for serine proteinase inhibition, in which a new class of compounds, the ynenol lactones, are featured

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(1) *Ciba Found. Symp.* **1980**, *75*.

(2) Barrett, A. J., Ed. "Proteinases in Mammalian Cells and Tissues"; North-Holland Publishing Co.: Amsterdam, 1977.

(3) Reich, E., Rifkin, D. B., Shaw, E., Eds. "Proteases and Biological Control"; Cold Spring Harbor: New York, 1975.

(4) (a) White, E. H.; Roswell, D. F.; Politzer, I. R.; Branchini, B. R. *J. Am. Chem. Soc.* **1975**, *97*, 2290-2291. (b) Bechet, J.-J.; DuPaiz, A.; Blagoeva, I. *Biochimie* **1977**, *59*, 231-239. (c) Chakravarty, P. K.; Krafft, G. A.; Katzenellenbogen, J. A. *J. Biol. Chem.* **1982**, *257*, 610-612. Sofia, M. K.; Chakravarty, P. K.; Katzenellenbogen, J. A. *J. Org. Chem.* **1983**, *48*, 3318. Krafft, G. A.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 5459-5466. (d) Moorman, A. R.; Abeles, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 6785-6787. (e) Harper, J.; Hemmi, K.; Powers, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 6578-6520. (f) Westkaemper, R.; Abeles, R. H. *Biochemistry* **1983**, *22*, 3256-3264.

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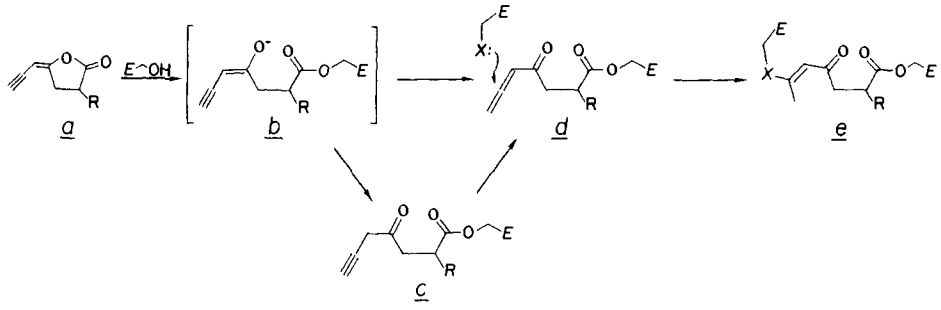
Scheme I



Scheme II

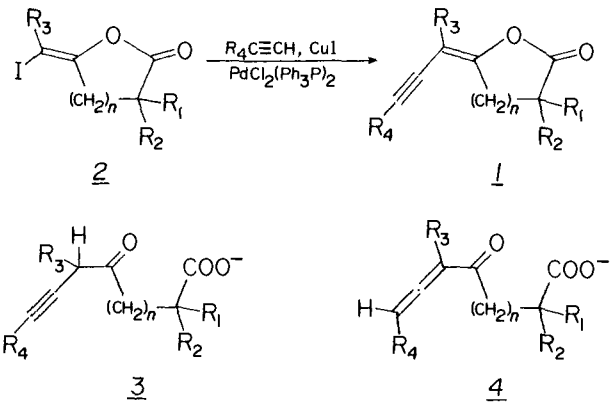


Table I

	R_1	R_2	R_3	R_4	n
a	H	H	H	H	1
b	*n*-Bu	H	H	H	1
c	$PhCH_2$	H	H	H	1
d	$PhCH_2$	H	H	Me	1
e	$PhCH_2$	H	Me	H	1
f	$PhCH_2$	Me	H	H	1
g	$PhCH_2$	H	H	H	2

as suicide inhibitors. Three conditions, (1) acylation of the enzyme (a → b), (2) unmasking of the allenone (b → d), and (3) capture of an enzyme nucleophilic residue, must be met in order to inactivate the enzyme.

(*E*)-Ynenol lactones **1** are conveniently synthesized[5-7] by coupling the corresponding (*E*)-iodo enol lactones[4c] **2** with the appropriate acetylide (Scheme II, Table I). Alkaline hydrolysis of ynenol lactones **1** results in the formation of allenone acids **4**.[8]

Ultraviolet spectra during the course of hydrolysis of the terminal alkynes (**1**, R_4 = H) are isosbestic (for **1a**, λ = 208 nm). In contrast, the terminally substituted alkynes (**1**, R_4 = alkyl) show biphasic, nonisosbestic spectra during alkaline hydrolysis, probably due to the intermediate formation of the propargyl ketone **3** (R_4 = alkyl), which is only slowly isomerized to the allenone **4** (R_4 = alkyl).

We have focussed on the inhibition of human leukocyte elastase[9] (HLE, E.C. 3.4.21.11.) because of the probable role of this enzyme in human disease.[10] Ynenol lactone **1a** is an inhibitor of HLE (K_i = 5.3 μM, based on initial rate assays) that does not show measurable time-dependent inactivation, suggesting that acylation by **1a** (Scheme I, a → b), but not trapping (d → e), occurs.

Substitution at C-3 has a profound effect on potency. Compound **1c** shows time-dependent, irreversible inactivation. The rate of inactivation is saturable (K_{inact} = 4.1 μM, k_{inact} = 0.09 s^{-1}, giving an apparent rate at low concentrations of **1c** of 22 000 M^{-1} s^{-1}). Inactivation is efficient; approximately two-thirds of all catalytic events lead to inactivation. Protection against inactivation by elastatinal, an active-site directed, reversible inhibitor,[11] shows that **1c** is active-site directed. That the *tethered* allenone (Scheme I, d) is required for inactivation is demonstrated by the inability of exogenous allenone **4c** (100 μM; prepared in situ by alkaline hydrolysis of **1c**) to inactivate the enzyme. Inactivation by **1c** is irreversible, since inactivated enzyme shows no recovery of activity after gel exclusion chromatography (Sephadex G-50), with or without 2-mercaptoethanol present. Ynenol lactones **1b** and **1g** inhibit HLE with potencies comparable to **1c** (apparent second-order inactivation rates of 23 000 and 28 000 M^{-1} s^{-1}, respectively).

On the other hand, the geminally substituted **1f** inhibits HLE 350-fold slower than **1c**. Terminal substitution of the alkyne **1d** significantly slows inhibition (1000-fold slower than **1c**). It is also noteworthy that the specificity constants (apparent k_{inact}/K_{inact}) for inhibition of the serine proteinases HLE, porcine pancreatic elastase, and bovine trypsin are, respectively, 22 000:730:17 for **1c** and 23 000:260:43, for **1b**.

Acyl enzyme formation by ynenol lactones has been demonstrated more directly with bovine α-chymotrypsin. Addition of a *catalytic* amount of chymotrypsin to **1a** at neutral pH results in ultraviolet spectra identical with those seen during alkaline hydrolysis of **1a**, showing that **1a** is a substrate and that **4a** is its accumulating product. With *stoichiometric* **1c** and chymotrypsin, proflavin (0.02 equiv) is rapidly displaced from the active site and rebinds in a time-dependent manner. The rate of this rebinding is a measure of product deacylation,[12] and these rates show a pH profile similar to other acylchymotrypsins.[13] These and other results will be elaborated in future publications.

Acknowledgment. We thank Valerie Robinson for her expert

(5) The iodo enol lactone **2** is treated with a terminal alkyne in the presence of a catalytic amount of cuprous iodide (0.05–0.1 equiv) and bis(triphenylphosphine)palladium(II) chloride (0.1–0.2 equiv) in triethylamine at 35 °C for 4–6 h. Yields vary from 44% to 66%. Deprotection of the trimethylsilylated ynenol lactones is achieved with $AgNO_3$/KCN in aqueous ethanol.

(6) In this study, only racemic ynenol lactones were tested. **1a**: ^1H NMR ($CDCl_3$) δ 2.8 (m, 2 H, H_3), 3.1 (d, 1 H, C≡CH), 3.1 (m, 2 H, H_4), 5.4 (ddd, 1H, C=CH); IR ($CHCl_3$) 3300, 1810, 1660, 2100 cm^{-1}; MS, 122 (M^+), 94 (M^+ - CO). **1b**: ^1H NMR ($CDCl_3$) δ 0.9 (br t, 3 H, CH_3), 1.4 (m, 6 H, $(CH_2)_3$), 2.8 (m, 3 H, H_3, H_4), 3.1 (d, 1 H, C≡CH), 5.25 (m, 1 H, C=CH); IR ($CHCl_3$) 3300, 1805, 1655 cm^{-1}; bp 108–110 °C (1 mmHg); MS, 178 (M^+), 111, 66, 28. **1c**: ^1H NMR ($CDCl_3$) δ 2.9 (d, 1 H, C≡CH), 2.5–3.2 (m, 5 H, $PhCH_2$, H_3, H_4), 5.2 (m, 1 H, C=CH), 7.2 (m, 5 H, Ar H); IR ($CHCl_3$) 3300, 1800, 1600, 2100 cm^{-1}; mp 64–65 °C; MS, 212 (M^+), 145, 66, 91 ($C_7H_7^+$).

(7) Coupling reactions carried out under these conditions proceed stereospecifically with retention of configuration. See: (a) Ratovelomanana, V.; Linstrumelle, G. *Tetrahedron Lett.* **1981**, 315–318. (b) Ando, T.; Vu, M.; Yoshida, S.; Takahashi, M. *Agric. Biol. Chem.* **1982**, *46*, 717–722.

(8) For example, **1a** (UV in H_2O: λ_{max} 229 nm (14 100 M^{-1} cm^{-1}) at pH 10, 25 °C, gives **4a** (λ_{max} 220 nm (9600 M^{-1} cm^{-1}) at a rate of 0.015 s^{-1}. In ^1H NMR, **1a** plus 1.8 equiv of KOH gives **4a** in situ (six-line ABX, δ_X 5.80, δ_A, δ_B centered at 5.37, $J_{AX} + J_{BX}$ = 13.4 Hz). (a) Covey, D.; Robinson, C. H. *J. Am. Chem. Soc.* **1976**, *98*, 5038–5040. (b) Carlson, R.; Henton, D. *J. Chem. Soc., Chem. Commun.* **1969**, 674–675. (c) Abraham, R. J. "The Analysis of High Resolution NMR Spectra"; Elsevier: Amsterdam, 1971; pp 75–77.

(9) HLE was prepared according to: Barrett, A. J. *Methods Enzymol.* **1981**, *80C*, 581–588. Engelbrecht, S.; Pieper, E.; Macartney, H. W.; Rautenberg, W.; Wenzel, H. R.; Tschesche, H. *Z. Physiol. Chem.* **1982**, *363*, 305–315.

(10) (a) Janoff, A. *Chest* **1983**, *83*, 54–58. (b) Janoff, A. *J. Lab. Clin. Med.* **1977**, *115*, 461–478.

(11) Umezawa, H. *Methods Enzymol.* **1976**, *45B*, 687–689.

(12) Bernhard, S.; Lee, B.; Tashjian, Z. *J. Mol. Biol.* **1966**, *18*, 405–420.

(13) Bender, M.; Schonbaum, G.; Zerner, B. *J. Am. Chem. Soc.* **1962**, *84*, 2562–2570.

NMR assistance and helpful discussions and Lynn Jacob for her many contributions.

Supplementary Material Available: Spectral characterization of **1f-1g** and a description of HLE assay (1 page). Ordering of information is given on any current masthead page.

Electrochemistry in Near-Critical and Supercritical Fluids. 1. Ammonia

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We report electrochemical studies in near-critical and supercritical ammonia and the behavior of solvated electrons and *m*-chloronitrobenzene in this medium. Thermodynamic and solubility studies of supercritical solutions have been an active area of research.[1] We are interested in applying electrochemical techniques to near-critical and supercritical fluids to obtain a better understanding of the thermodynamics and kinetics of reactions, particularly homogeneous and heterogeneous electron-transfer reactions, that occur in these media and perhaps to utilize these solutions for electrosynthetic purposes. The high temperatures and pressures necessarily associated with electrochemically useful supercritical solutions necessitate the use of highly specialized cells and electrodes. In addition, the increased reactivity of species and the highly corrosive environment of supercritical ammonia limits the types of useful electrolytes and redox couples that easily can be studied as test systems.

As the critical temperature of a liquid is approached, the gaseous and liquid phases merge into a single, space-filling phase called a supercritical fluid. For ammonia, the critical point occurs at 133 °C and 112.5 atm; addition of low concentrations of electrolyte do not change these values appreciably. The characteristics that typify these fluids include decreased viscosities, densities, and dielectric constants, unusual changes in inter- and intramolecular forces, and altered solvation characteristics. A primary question of interest is whether electrochemical studies can be carried out in a supercritical fluid containing an electrolyte. We show here that electrochemical techniques, such as cyclic voltammetry and chronocoulometry, with near-critical and supercritical ammonia can be used to probe changes in redox potentials, electrogenerated product stability, and diffusion coefficients. Of the limited number of electrochemical studies that have been carried out on supercritical solutions, most have dealt with the corrosion of metals in contact with water.[2] To our knowledge, the only previous attempt at obtaining quantitative electrochemical information from supercritical NH_3 involved a two-electrode, constant-current, electrodeposition of silver.[3]

There are two primary experimental difficulties associated with these types of experiments: containment of a high-pressure, space-filling, corrosive fluid and design of electrode feed throughs that can withstand the supercritical environment and remain insulated from the walls of the cell. The base of the electrochemical cell we have fabricated is of 316 stainless steel; the interior is cylindrical and has an internal volume of 75 mL. A detachable lid of the same material is bolted to the base by means of a flange arrangement incorporating a diamond-shaped copper gasket. Three electrodes, supported by standard Swagelok fittings, pass through the lid. Provisions are also made for evacuation, filling, sample addition, and a rupture disk. All electrode feed throughs consist of tungsten wire passed through a commercially available graded glass to Kovar seal. Construction of the feed through is completed by sealing the glass to the wire. The working electrode is a disk-shaped cross section of a tungsten wire, and the counter and quasi-reference (QRE) electrodes consist respectively of platinum and silver wires soldered onto the tungsten. These electrodes have withstood pressures of 340 atm at 160 °C.

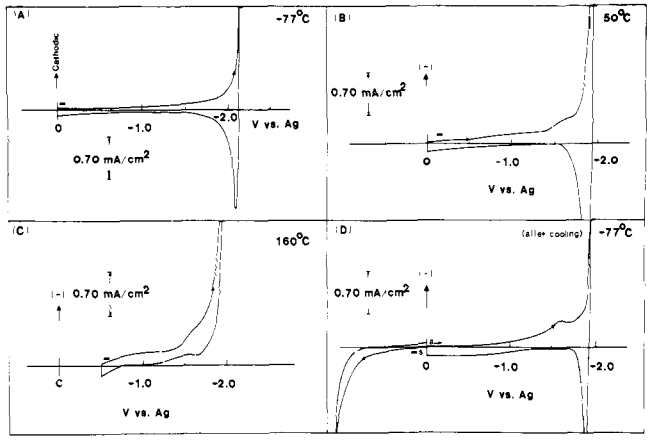

Figure 1. Cyclic voltammograms for generation and oxidation of solvated electrons in NH_3. (A) -77 °C, 0.2 M KI; (B) 50 °C, 20 atm, 0.2 M KI; (C) 160 °C, 252 atm, 0.1 M KI (supercritical); (D) -77 °C (after cooling from 160° C), 0.2 M KI. Scan rate, 200 mV s^{-1}.

A typical experiment involved evacuating the cell, to which electrolyte had previously been added, followed by the addition of sufficient dry ammonia under vacuum line conditions to generate the desired pressure and density at a specified temperature. The cell was isolated from the vacuum line by means of a high-pressure valve, and removed to an armored autoclave for heating above the critical temperature (~140 °C) of the electrolytic solution. To probe changes in the available potential range of NH_3 and the stability of solvated electrons (e_s^-) as a function of temperature, cyclic voltammetric scans were employed (Figure 1).[4]

As the temperature increased, the polarizable range of the ammonia solution decreases as evidenced by a positive shift of the solvated electron peak and a negative shift of the anodic background (vs. QRE). A small shoulder preceded the onset of solvated electron production at higher temperatures which persisted when the solution was cooled back to -77 °C (curve D). A similar wave was observed at -77 °C in single-compartment glass cells and thus is probably associated with the reduction of a species generated at the counter electrode. Above the critical temperature (curve C) generation of solvated electrons is still observed, although on the voltammetric time scale little, if any, oxidation is seen upon scan reversal. This can be attributed to a decrease in the stability of the solvated electrons, probably by reaction with NH_3 at the higher temperatures.[5] Upon cooling the solution to -77 °C, the system returns to essentially its initial condition, demonstrating no extensive contamination or irreversible changes in the solution. The apparent shift in potential of the solvated electron peak

(1) (a) Groenier, W. S.; Thodos, G. *J. Chem. Eng. Data* **1961**, *6*, 240-244. (b) Zander, M.; Thomas, W. *J. Chem. Eng. Data* **1979**, *24*, 1-2. (c) Franck, E. U.; Roth, K. *Discuss. Faraday Soc.* **1967**, *43*, 108-114. (d) Todheide, K. "Water: A Comprehensive Treatise"; Franks, F., Ed.; Plenum Press: New York, 1972; Vol. 1, pp 504-513. (e) Schneider, G. M. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 716-727. (f) Franck, E. U. "High Pressure Chemistry"; Kelm, H., Ed.; D. Reidel: Boston, 1978; pp 221-257.

(2) Cowan, R. L.; Kasnoff, A. I. *Proc. Int. Congr. Met. Corros., 5th, 1972* **1974**, 874-883.

(3) Silvestri, G.; Gambino, S.; Filardo, G.; Cuccia, C.; Guarino, E. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 101-102.

(4) For previous studies of e_s^- in liquid NH_3, see: (a) Teherani, T.; Itaya, K.; Bard, A. *J. Nouv. J. Chim.* **1978**, *2*, 481-487. (b) Schindewolf, U. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 190-202.

(5) (a) Jolly, W. L. "Metal-Ammonia Solutions"; Lagowski, J. J., Sienko, M. J., Eds.; Butterworths: London, 1970; p 168. (b) The lifetime of e_s^- in supercritical metal-ammonia solutions has been estimated to be ~1 h in glass cells. Transition metals catalyze the reaction $e_s^- + NH_3 \rightleftharpoons NH_2^- + {}^1/_2H_2$ (excess NH_2^- and H_2 will form e_s^- in supercritical NH_3). Naiditch, S. "Solutions Métal-Ammoniac, Propriétés Physicochimiques"; Lepoutre, G.; Sienko, M. J., Eds.; W. A. Benjamin: New York, 1964; pp 113-136. Schindewolf, U.; Vogelsgesang, R.; Böddeker, K. W. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 1076-1077.

0002-7863/84/1506-6851$01.50/0