equipped with and without a uranyl glass filter.
(13) R. Huisgen, *Angew. Chem., Int. Ed. Engl.*, **2**, 633 (1963); R. Huisgen, *J. Org. Chem.*, **33**, 2291 (1968); **41**, 403 (1976).
(14) N. R. Bertoniere and G. W. Griffin in "Carbenes", Vol. I, M. Jones, Jr., and R. A. Moss, Ed., Wiley, New York, N.Y., 1972, p 305.
(15) E. F. Ullman, *J. Am. Chem. Soc.*, **85**, 3529 (1963).
(16) A. Robert and B. Moisan, *J. Chem. Soc., Chem. Commun.*, 337 (1972).
(17) T. Jakobiec, *Arch. Immunol. Ther. Exp.*, 261 (1961); *Chem. Abstr.*, **71**, 49669e (1969). No melting point is cited for **14**.
(18) Y. Izawa and Y. Ogata, *J. Org. Chem.*, **35**, 3192 (1970).
(19) Farbenbriken Bayer A. G., British Patent 877 407; *Chem. Abstr.*, **58**, 1403*d* (1963).

The Unusually Mild and Facile Basic Hydrolysis of *N*-Nitroso-2-(methylamino)acetonitrile[1]

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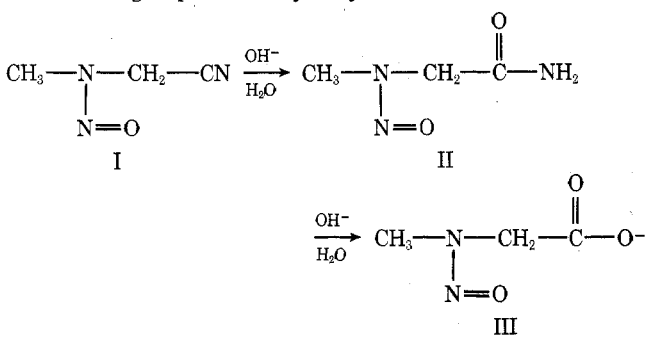
At pH 13 and room temperature, *N*-nitroso-2-(methylamino)acetonitrile (I) undergoes two unusually fast and successive hydrolytic changes that can be detected quantitatively by differential pulse polarography. The final hydrolysis product is *N*-nitrososarcosine (III), via the intermediate amide (II). The kinetics and activation parameters of the transformations have been determined. A mechanism has been proposed to account for these rapid reactions involving anchimeric assistance to the hydrolyses by the appropriately placed nitroso group. Isotopic labeling studies using ^{18}O enriched water and mass spectrometry confirm the proposed mechanism involving exclusive attack on carbon by hydroxide ion.

During the course of electroanalytical studies of a series of *N*-nitrosamines using differential pulse polarography,[2] one *N*-nitrosamine displayed unusual behavior. In aqueous solution at pH 13, *N*-nitroso-2-(methylamino)acetonitrile (I) displayed the anticipated current–potential peak at a negative potential vs. the saturated calomel electrode (SCE) but the expected peak was followed by a second peak, an unusual result for a *N*-nitrosamine.[2] In addition to the second peak, the situation was even more unusual by the observation that the peaks varied in height in some regular way as a function of time. A careful study yielded the results shown in Figure 1 where curves 1–5 are the results of repetitive scans on the same solution recorded over a period of approximately 200 min. Since the peak potential (E_p) of a *N*-nitrosamine is a function of pH and molecular structure, the results suggested that a chemical change was occurring resulting possibly in the formation of nitrosamines different from the original.

In this paper we report the results of an investigation to interpret the observed changes.

As Figure 1 shows, the initial scan yields two peaks at −1.26 and −1.42 V vs. SCE. The second scan taken about 5 min later shows a decrease in the first peak and an increase in the second with the suggestion of a third ill-defined peak at a more negative potential. Curve 5, recorded about 200 min after curve 1, shows that the species giving rise to the peaks at −1.26 and −1.42 V have completely disappeared; the only species left is that giving rise to the ill-defined peak at about −1.8 V.

Although it is well known that nitriles do not undergo basic hydrolysis rapidly at room temperature,[3] the most logical hypothesis to explain the polarographic results seemed to be the following sequence of hydrolytic reactions:



The final product (III) in the suggested sequence is the anion of *N*-nitrososarcosine. To establish the validity of the hydrolysis sequence, *N*-nitrososarcosine was prepared[4] and its properties were compared with those of the final hydrolysis product (III).

Figure 2, curve 1, shows the differential pulse polarogram obtained after acidifying (pH 1) the solution that yielded curve 5, Figure 1. The anodic shift of E_p with lower pH is characteristic of *N*-nitrosamines.[2,5] Curve 2, Figure 2, was obtained after addition of authentic *N*-nitrososarcosine to the solution that yielded curve 1. The increase in peak height without shift in potential strongly suggested that *N*-nitrososarcosine is the electroactive species in Figure 2, curve 1.

Since the polarograms were run on dilute solutions (ca. 10^{-4} M) and product isolation and identification would be difficult, reactions modeled after the polarographic runs were repeated on a preparative scale. The organic product was isolated by evaporation of the water and extraction of the residue with acetone. Evaporation of the acetone yielded a yellow oil which crystallized only after being held at 0 °C overnight. (In some cases the oil did not crystallize.) The crystals had a melting point of 66–67 °C. The melting point and crystallization behavior are those previously reported for nitrososarcosine.[4]

This result confirms the findings of Lijinsky et al. concerning the melting point of this compound as contrasted to the values of 73–74 °C reported by Hammick et al.[6] and 75–77 °C reported by Bergel et al.[7]

To confirm the identity of the hydrolysis product, the NMR, uv, and ir spectra of the final product were obtained; they were identical with those of authentic *N*-nitrososarcosine (Tables I and II). These results show unequivocally that the final product was, in fact, *N*-nitrososarcosine.

The unnitrosated parent amine, 2-(methylamino)acetonitrile, was subjected to the same alkaline reaction conditions as I. No change occurs over a period of 48 h, as would be expected for a simple nitrile. Thus, the *N*-nitroso group in I is clearly having an unusual activating effect on the nitrile group. To understand this effect, the kinetics of the reactions were determined using the rate of decay of the peak currents in the differential pulse polarograms. Both reactions (I → II and II → III) are second order overall, first order in nitrosamine and first order in OH^-. Rate constant data and calculated activation parameters are given in Table III. The most significant

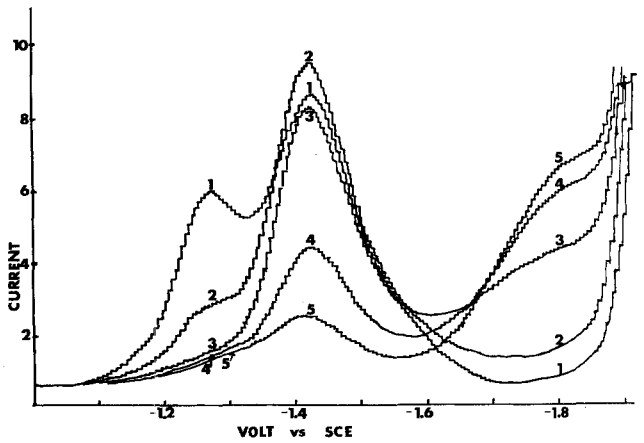


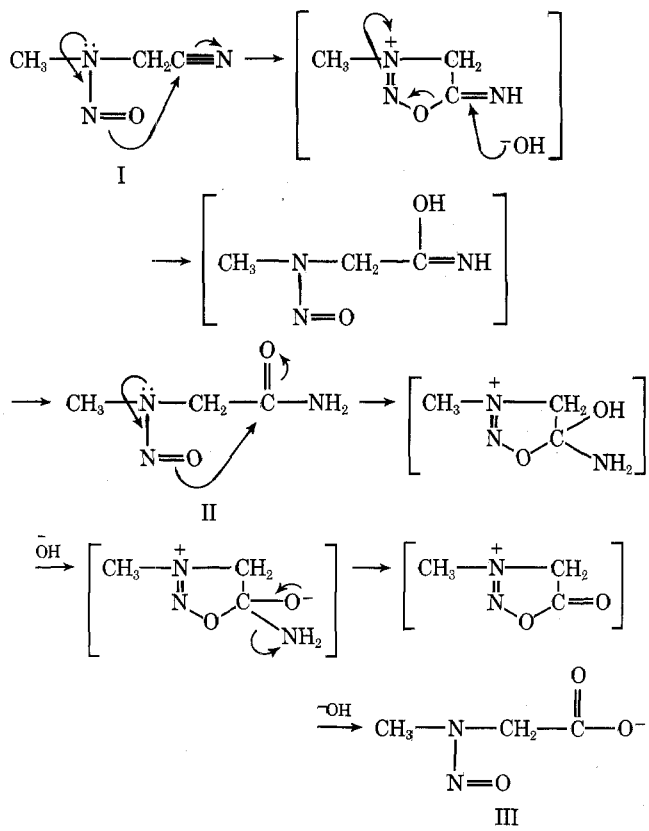
Figure 1. Differential pulse polarograms of *N*-nitroso-2-(methylamino)acetonitrile (I), pH 13.0, T = 25.3 °C: curve 1, 0 min; curve 2, 5 min; curve 3, 23 min; curve 4, 112 min; curve 5, 214 min. Scan 5 mV/s, pulse height 50 mV, drop time 1 s.

data in Table III are the large negative entropies of activation.

A mechanism that agrees with the experimental observations is shown in Scheme I. The activated complex, consisting of a five-membered ring, is consistent with the observed entropies of activation and the known polarities of the nitroso and nitrile groups. Two features of the hydrolytic mechanism are (a) the anchimeric assistance provided by the nitroso group and (b) the suggestion that the base attacks the cyclic complex at carbon.

Support for this mechanism involving a five-membered cyclic transition state is provided by comparing the kinetic results (Table IV) obtained for the analogous alkaline hydrolysis of $CH_3N(N{=}O)CH_2CH_2CN$ (IV) in which two methylene groups, rather than one, are between the *N*-nitroso

Scheme I



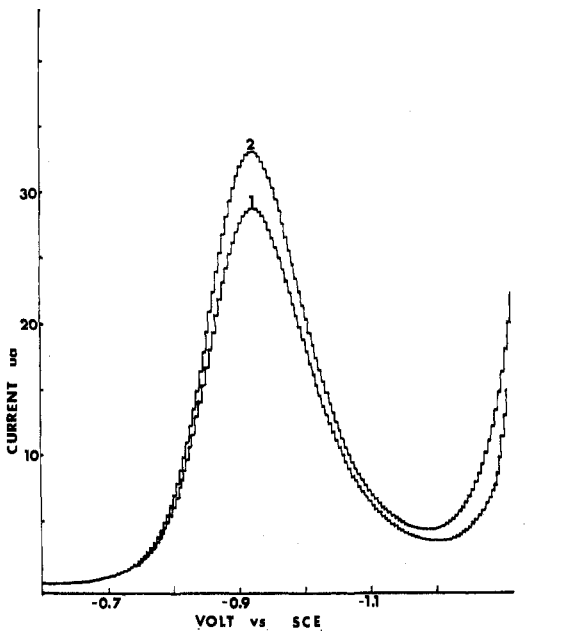


Figure 2. Curve 1: differential pulse polarogram of solution yielding curve 5, Figure 1, at pH 2.0 T = 25.3 °C. Curve 2: differential pulse polarogram resulting from addition of *N*-nitrososarcosine to solution yielding curve 1.

Table I. NMR Identification of Hydrolysis Product of *N*-Nitroso-2-(methylamino)acetonitrile (I)

	Hydrolysis product		*N*-Nitroso-sarcosine		
Solvent	ppm	Proton ratio	ppm	Proton ratio	Ref 4
Acetone-d_6	3.06	3:2	3.07	3:2	
	5.02		5.02		
	3.87	3:2	3.89	3:2	
	4.33		4.34		
Pyridine-d_5	3.22	3:2	3.21	3:2	*anti*-CH_3
	5.20		5.20		*anti*-CH_2
	3.88	3:2	3.89	3:2	*syn*-CH_3
	4.59		4.58		*syn*-CH_2

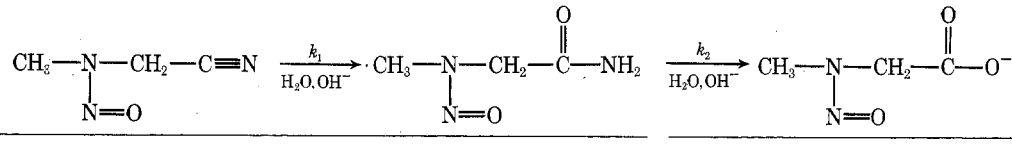
Table II. Uv Identification of Hydrolysis Product of *N*-Nitroso-2-(methylamino)acetonitrile (I)

	λ_{max}, nm			
Nitrososarcosine	340		229	
Hydrolysis product in H_2O (pH 7)	339	310	237	
Hydrolysis product in H_2O (pH 2)	335		230	
Lijinsky et al.[4] in H_2O (pH ?)	340		233	
Hydrolysis product in ether	372	361	351	235
Lijinsky et al.[4] in ether	373	361	352	234

and nitrile groups. The presence of the second methylene group results in a decrease in the overall rate by a factor of about 500. This decrease would be expected if a cyclic activated complex is the intermediate since a six-membered ring is required. The entropy of activation for the hydrolysis of IV is also in accord with such an activated complex.

Scheme I requires attack by OH^- to occur initially on the ring carbon associated with either the nitrile or the amide group. An alternative is attack on the ring nitrogen derived from the nitroso group, resulting in cleavage of the N–O bond rather than a C–O bond. To establish which pathway is, in fact, followed, the alkaline hydrolysis of I was conducted in

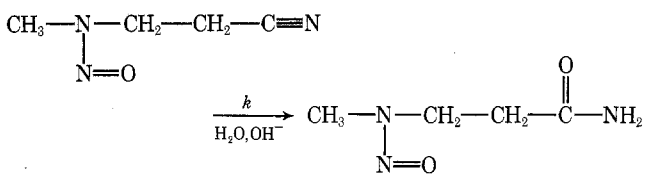
Table III. Hydrolysis of *N*-Nitroso-2-(methylamino)acetonitrile (I)



Temp, °C	k_1	k_2
0 ± 0.2	(3.73 ± 0.38) × 10^{-3}	
25.3 ± 0.2	(4.72 ± 0.59) × 10^{-2}	(1.15 ± 0.30) × 10^{-3}
37.3 ± 0.2	(1.10 ± 0.10) × 10^{-1}	(2.97 ± 0.24) × 10^{-3}
48.6 ± 0.2		(5.96 ± 0.27) × 10^{-3}
	$\Delta H^{\ddagger}$ = 13.3 ± 0.2 kcal/mol	$\Delta H^{\ddagger}$ = 11.3 ± 0.3 kcal/mol
	$\Delta S^{\ddagger}$ = −22.4 eu	$\Delta S^{\ddagger}$ = −35.8 eu

k_1, k_2 = average second-order rate constants (six concns), M^{-1} s^{-1}

Table IV. Hydrolysis of *N*-Nitroso-3-(methylamino)propionitrile. Kinetic Data



Temp, °C	k, M^{-1} s^{-1}
25.0	(5.28 ± 4.9) × 10^{-5}
34.8	(13.3 ± 1.2) × 10^{-5}
43.2	(27.8 ± 2.3) × 10^{-5}
$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$
16.6 ± 0.7 kcal/mol	−24.8 eu

Table V. Mass Spectrometric Results for ^{18}O Incorporation

	Species	amu	Intensities	%
Expt A	M^+	120	133 120	16.2
(see text)		118	823 808	100.0
	M − NO	90	118 784	16.5
		88	720 896	100.0
	M − COOH	75	71 680	2.3
		73	3 021 824	100.0
Expt B	M^+	120	35 840	2.9
(see text)		118	121 246	100.0
	M − NO	90	17 920	2.1
		88	868 352	100.0
	M − COOH	75	34 304	1.0
		73	3 351 552	100.0

water containing about 8% ^{18}O. The hydrolysis product was then examined by mass spectrometry to determine the location of incorporated ^{18}O, if any (Table V). The results in Table V are from low-resolution semiquantitative studies.

The data in Table V contain only the results used to determine the location of the ^{18}O. *N*-Nitrososarcosine yields a very complex mass spectrum on electron impact due, apparently, to considerable self-protonation. This is noted by the presence of unusually large MH^+ peaks for all samples and is probably associated with the fact that the compound was run as an oil, hence it has a high vapor pressure.

Neglecting the chemical ionization effects noted above, *N*-nitrososarcosine yields a molecular ion (mass 118) and two fragments resulting from loss of NO and COOH. These fragments correspond to mass 88 and 73, respectively. The mass spectral data show that incorporation of ^{18}O has occurred in the hydrolysis of I but, of great significance, loss of NO does *not* result in loss of ^{18}O (experiment A). As seen in the table, the peak at mass 120 is 16.2% of the peak at mass 118. After losing NO the peak at mass 90 *is still* 16.5% of the peak at mass 88. This constancy in relative percentages would not have occurred if the NO group contained any ^{18}O. The fragment resulting from loss of COOH, however, *does* result in loss of ^{18}O as shown by the drop in relative percentage of the mass 75 peak compared to the mass 73 peak. The remaining 2% at mass 75 is probably due to chemical ionization effects coupled with normal isotope effects. Further corroboration is found in the relative intensity of the peak at mass 73 to that at mass 118. Loss of COOH (^{16}O) yields the peak at mass 73 and loss of COOH (^{18}O) should also yield a peak at mass 73 (i.e, 120 − 47 = 73).

Data are also given in the table (experiment B) that exclude the incorporation of ^{18}O by simple exchange between ^{16}O *N*-nitrososarcosine and a basic solution containing $H_2{}^{18}O$. If any exchange occurs it cannot exceed 1%. The peaks at mass 120 and 90 are again consistent with the conclusion that ^{18}O is absent in the NO group and, subtracting the 1% at mass 75 as due to chemical ionization and normal isotope effects, the incorporation due to simple exchange is about 1%.

The presence in the *N*-nitrososarcosine of about 16% ^{18}O from ^{18}O water agrees nicely with the percentage predicted by Scheme I. Incorporation can occur twice, I → II and II → III. Simple calculations predict approximately 14% incorporation when exchange effects are considered.

The mass spectral results show that the *N*-nitroso group is unmodified during the course of the reaction and that its role is to speed up hydrolysis of the nitrile by anchimeric assistance. Furthermore, the exclusive site of attack by OH^- in I is at carbon as opposed to nitrogen.

Experimental Section

Spectra. Uv spectra were obtained using a Cary Model 14 spectrophotometer. Infrared spectra were obtained on a Perkin-Elmer 225 or a Pye Unicam AP 1000 spectrophotometer. NMR were recorded on a Varian XL-100 spectrometer using Me_4Si as internal standard.

Differential Pulse Polarography. Differential pulse polarograms were obtained as previously described[2] except that a Princeton Applied Research Corp. (PAR) Model 174 electroanalyzer was used in place of the PAR Model 171. Temperature studies were performed in a PAR Model 9350 jacketed cell. Temperature was controlled by a Cole-Parmer proportional controller, No. 2163.

Kinetic Studies. Buffer solutions of constant ionic strength containing 0.2 N KCl and 0.2 N KOH were made at pH 13.2, 12.9, 12.6, 12.3, 12.0, and 11.7. The *N*-nitrosamine was dissolved in the solutions and placed in the electrochemical cell at specified temperatures (Table III). The potential was set at −1.26 V vs. SCE and the current recorded as a function of time. Since the II → III reaction was about 1/40 as fast as the I → II reaction, the kinetics of II → III could be studied independently after the I → II reaction was completed. After the I_p of I disappeared the potential was changed to −1.42 V to monitor the decay of II. In all cases log i_p vs. time (s) plots were linear indicating a first-order reaction with respect to *N*-nitrosamine. The slopes of these plots were obtained as pseudo-first-order rate constants (k'). Log k' vs. pH plots were made at each temperature and the slopes of these plots were determined yielding the order with respect to OH^-. The values were 1.00 ± 0.1 in all cases. The second-order rate con-

stants reported in the tables were then calculated by substituting OH^- concentration into the overall rate equation. Activation parameters were calculated in the usual fashion.[8]

Mass Spectrometry. Mass spectra were run by Battelle Memorial Laboratories, Columbus, Ohio.

***N*-Nitroso(2-cyanoethyl)methylamine (IV).** To a chilled, stirred solution of 3-(methylamino)propionitrile (20.0 g, 0.238 mol) (Aldrich) and concentrated HCl (25 ml) in H_2O (120 ml), $NaNO_2$ (19.7 g, 0.276 mol) in H_2O (30 ml) was added dropwise. After addition was complete, the solution was stirred near 0 °C for 0.5 h and then at room temperature for 1.5 h. The solution was concentrated to ~50 ml by rotatory evaporation, filtered (to remove the inorganic salts which precipitated during concentration), and extracted three times with methylene chloride (75 ml). The methylene chloride extracts were combined, dried ($MgSO_4$), and concentrated in vacuo leaving 23.0 g of a yellow liquid. Distillation gave 21.3 g (79%) of a light yellow liquid: bp 102–103 °C (0.04 Torr); ir (CH_2Cl_2) 2255 (C≡N), 1470 (N=O), and 1040 cm^{-1} (N–N). Anal Calcd for $C_4H_7N_3O$: C, 42.47; H, 6.24; N, 37.15; O, 14.14; Found: C, 42.37; H, 6.50; N, 37.28; O, 13.85. In the absence of information on the potential carcinogenicity of IV and I in humans, every precaution should be taken to protect laboratory investigators.

Synthesis of *N*-Nitroso-2-(methylamino)acetonitrile (I). To a stirred solution of 2-(methylamino)acetonitrile hydrochloride (10.0 g, 0.093 mol) (Aldrich) in H_2O (100 ml), $NaNO_2$ (6.9 g, 0.10 mol) in H_2O (15 ml) was added dropwise at room temperature. A few drops of concentrated HCl were added to ensure an excess, and the solution was then stirred for 3 h. The workup procedure was the same as that used in the previous preparation. Distillation gave 4.2 g (46%) of a light yellow oil: bp 52.5–53 °C (0.004 Torr) [lit.[9] 119 °C (13 Torr)]; ir ($CHCl_3$) 2260 (C≡N, 1470 (N=O), and 1025 cm^{-1} (N–N).

Basic Hydrolysis of *N*-Nitroso-2-(methylamino)acetonitrile (I) in ^{18}O-Enriched Water. Recovery of *N*-Nitrosarcosine for Mass Spectral Analysis. A preliminary experiment using the procedure described below except with nonenriched water gave a yellow oil which exhibited an ir spectrum (CH_2Cl_2) identical with that of an authentic sample of *N*-nitrososarcosine.

To ^{18}O-enriched water (5.0 ml) (ca. 8% enrichment) made alkaline to pH > 13 by 0.25 N NaOH solution was added *N*-nitroso-2-(methylamino)acetonitrile (56.1 mg, 0.567 mmol). The clear solution was stirred for 24 h at room temperature and the pH was then adjusted to ~1.5 with concentrated HCl. The water was evaporated under reduced pressure, and the residue was extracted with acetone (10 ml). The solution was filtered to remove NaCl and was evaporated under a stream of N_2. The resulting yellow oil was dried over P_2O_5; it weighed 66.1 mg (98.8%).

Recovery of Nitrososarcosine in Basic ^{18}O-Enriched Water for Mass Spectral Analysis. Attempted Exchange Experiment. To ^{18}O-enriched water (2.5 ml) made alkaline to pH >13 by 0.25 N NaOH solution was added *N*-nitrososarcosine (34.0 mg, 0.284 mmol). The clear solution was stirred for 24 h at room temperature and the pH was then adjusted to ~1.5 with concentrated HCl. The workup procedure was the same as that just described; the yield was 28.3 mg of a yellow oil.

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Registry No.—I, 3684-97-7; III, 60153-48-2; IV, 60153-49-3; 3-(methylamino)propionitrile, 693-05-0; 2-(methylamino)acetonitrile hydrochloride, 25808-30-4.

References and Notes

(1) Presented at the 10th Middle Atlantic Regional Meeting of the American Chemical Society, Philadelphia, Pa., Feb 1976.
(2) S. K. Chang and G. W. Harrington, *Anal. Chem.*, **47**, 1857 (1975).
(3) D. J. Cram and G. S. Hammond, "Organic Chemistry", McGraw-Hill, New York, N.Y., 1964, p 358.
(4) W. Lijinsky, L. Keefer, and J. Loo, *Tetrahedron*, **26**, 5137 (1970).
(5) L. Holleck and R. Schindler, *Z. Elektrochem.*, **62**, 942 (1958).
(6) D. L. Hammick and D. J. Voaden, *J. Chem. Soc.*, 3303 (1961).
(7) F. Bergel, S. S. Brown, C. L. Leese, C. M. Timmis, and R. Wade, *J. Chem. Soc.*, 846 (1963).
(8) W. J. Moore, "Physical Chemistry", 4th ed, Prentice-Hall, Englewood Cliffs, N.J., 1972, p 297.
(9) H. Druckery, R. Preussman, S. Ivankovic, D. Schmähl, J. Afkham, G. Blum, H. D. Mennel, M. Müller, P. Petropoulos, and H. Schneider, *Z. Krebsforsch.*, **69**, 103 (1967).