[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY AND THE GRADUATE DEPARTMENT OF BIOCHEMISTRY, BRANDEIS UNIVERSITY[1]]

The Reaction of Hydroxylamine with Activated Acyl Groups. II. Mechanism of the Reaction

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Kinetic data are presented for the rapid acylation of hydroxylamine to give O- and N-acylhydroxylamines and for the further reaction of O-acylhydroxylamine with hydroxylamine to give hydroxamic acid. The initial acylation appears to be a bimolecular reaction involving a species of hydroxylamine which carries no net charge. The second reaction shows a *p*H optimum, proceeds at a rate proportional to more than the first power of the hydroxylamine concentration, and is accelerated by certain buffers, particularly phosphate It is suggested that the unusual reactivity of the hydroxylamine hydroxyl group in the initial reaction may be due to concerted attack by the amino group.

In the previous paper[2] it was shown that the reaction of hydroxylamine with a number of acylating agents at neutral *p*H proceeds largely to form an unstable O-acylhydroxylamine as well as hydroxamic acid

$$RCB + NH_2OH \longrightarrow RCONH_2 + B^- + H^+ \quad (1a)$$

$$RCB + NH_2OH \longrightarrow RCNHOH + B^- + H^+ \quad (1b)$$

The O-acylhydroxylamine in turn will react more slowly with hydroxylamine to form hydroxamic acid

$$RCONH_2 + NH_2OH \longrightarrow RCNHOH + NH_2OH \quad (2)$$

Acylation of hydroxylamine on oxygen rather than the generally much more nucleophilic nitrogen atom and the rapid rate of this reaction are unexpected and prompted an investigation into the mechanism and kinetics of these reactions.

Results

The kinetics of the reaction of *p*-nitrophenyl acetate with hydroxylamine fall into two distinct phases corresponding to equations 1 and 2. At low hydroxylamine concentration it is possible to follow the initial rapid reaction by measuring the liberation of *p*-nitrophenolate ion (equations 1a and 1b), which follows (pseudo) first-order kinetics and proceeds to completion (Fig. 1a). At a higher concentration of hydroxylamine the formation of hydroxamic acid from O-acetylhydroxylamine proceeds at a measurable rate and, after an initial burst due to the formation of some hydroxamic acid from reaction 1b, also follows (pseudo) first-order kinetics (Fig. 1b). These phases will be considered separately.

The Reaction of Nitrophenyl Esters with Hydroxylamine.—The linear increase of the first-order rate constant for *p*-nitrophenol liberation from *p*-nitrophenyl acetate with increasing hydroxylamine concentration indicates that the over-all reaction is second order (Fig. 2). With varying *p*H the rate increases with *p*H over the range of hydroxylamine dissociation (pK_a 6.0)[3] and then levels off; the rates agree within experimental error with those calculated for a reaction in which the species of hydroxylamine which carries no net charge is the reactant (Fig. 3).[4] In contrast, the logarithm of the rate of reaction of hydroxamic acids with *p*-

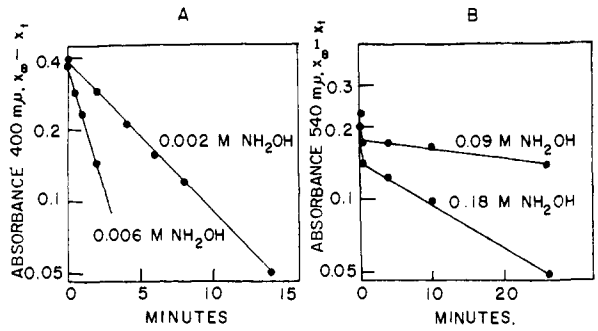


Fig. 1.—Semi-logarithmic plot of the rate of *p*-nitrophenol liberation (A) and the rate of hydroxamic acid formation (B) in the reaction of *p*-nitrophenyl acetate with various concentrations of hydroxylamine at 25°: A, 5×10^{-5} *M* *p*-nitrophenyl acetate, 0.1 *M* NaCl; B, 5.5×10^{-4} *M* *p*-nitrophenyl acetate, 0.5 *M* NaCl.

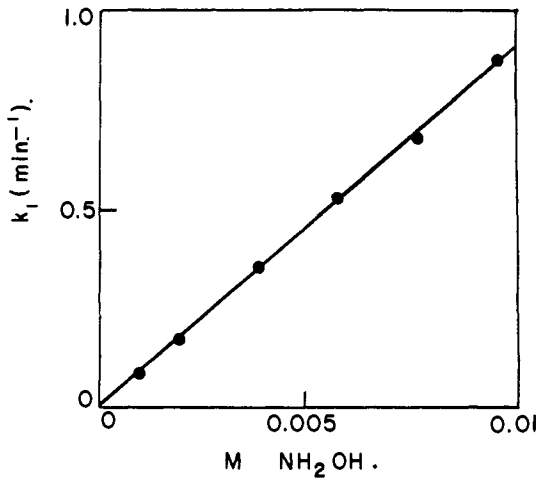


Fig. 2.—Rate of *p*-nitrophenol liberation from *p*-nitrophenyl acetate as a function of hydroxylamine concentration in 0.1 *M* potassium phosphate buffer, *p*H 6.7, at 25°.

(1) Publication #5 of the Graduate Department of Biochemistry, Brandeis University, Waltham 54, Mass.

(2) W. P. Jencks, THIS JOURNAL, **80** 4581 (1958).

(3) T. C. Bissot, R. W. Parry and D. H. Campbell, *ibid.*, **79**, 796 (1957).

(4) Recent experiments by Miss Kay McGarrahan in this Laboratory have shown that the rate of this reaction remains constant up to *p*H 9.0. This is in marked contrast to the strongly base-catalyzed reaction of hydroxylamine with such weakly activated acyl groups as acetylcholine (S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949)) and amino acid esters (I. D. Raacke, *Biochim. et Biophys. Acta*, **27**, 416 (1958)).

TABLE I

RATE OF *p*-NITROPHENOL LIBERATION IN THE REACTION OF *p*-NITROPHENYL ACETATE WITH VARIOUS COMPOUNDS AT 25°

Substance, *M*		*p*-Nitrophenyl acetate, *M*	Additions	*p*H	k_1, min.$^{-1}$	$k_2 = (k_1 - k_0)/(X)$,[a] l. mole^{-1} min.$^{-1}$
NH_2OH	0.0134	5×10^{-5}	0.1 *M* K phosphate	7.6	1.47	110
CH_3ONH_2	.067	5×10^{-5}	.1 *M* K phosphate	6.8	0.019	0.19
CH_3ONH_2	.133	5×10^{-5}	.1 *M* K phosphate	6.9	.034	0.20
Histamine	.01	5×10^{-5}	.1 *M* NaCl, 0.5% acetone	7.0	.030	2.8
Imidazole	.002	1×10^{-4}	.1 *M* NaCl, 0.5% acetone	7.3	.048	29.4[b]
			5% dioxane			28.5[c]
$NaHCO_3$	.09	1×10^{-4}	.1 *M* NaCl, 1% acetone	8.2	.0032	0.018
$NaHCO_3$	.01	1×10^{-4}	.1 *M* NaCl, 1% acetone	8.2	.0017	
K phosphate	.1	1×10^{-4}	.1 *M* NaCl, 1% acetone	6.6	~ .0004	~0.003
H_2O	55	1×10^{-4}	.1 *M* NaCl, 1% acetone	6.6	~ .0001[d]	$\sim 2 \times 10^{-6}$
H_2O_2	0.128	1.7×10^{-4}	.1 *M* phosphate	6.72	.373	2.8×10^{5}[e]
H_2O_2	.128	1.7×10^{-4}	.1 *M* phosphate	6.25	.122	2.7×10^{5}[e]

[a] (X) = total concentration of base added; k_0 was negligible in measurements with NH_2OH and imidazole. [b] Calculated for free base. [c] M. L. Bender and B. W. Turnquest, THIS JOURNAL, **79**, 1652 (1957). [d] k_0 for phosphate. [e] Calculated for HOO^- using $K_a = 2 \times 10^{-12}$.

nitrophenyl acetate (to form diacylhydroxylamines), which is also rapid, increases linearly with *p*H suggesting that the hydroxamate anion is the reactive species. The reaction with hydroxylamine displays little sensitivity to variation of the ionic strength or phosphate concentration, but is depressed by addition of alcohol; omission of phosphate buffer did not alter the linear dependence of the rate on hydroxylamine concentration.

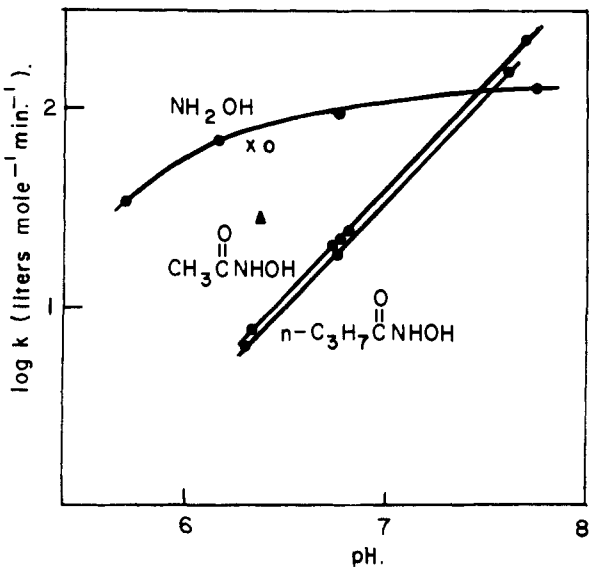


Fig. 3.—Rates of reactions of hydroxylamine and hydroxamic acids with *p*-nitrophenyl acetate at varying *p*H; 25°, 0.1 *M* potassium phosphate buffer, 3×10^{-5} *M* to 1.3×10^{-4} *M* *p*-nitrophenyl acetate: X, without phosphate buffer; O, 0.2 *M* KCl (without phosphate); ▲, 50% alcohol (without phosphate).

For comparison with the hydroxylamine reaction, the rate of the reaction of *p*-nitrophenylacetate with a number of other oxygen and nitrogen-containing bases is shown in Table I. Similar studies of the reaction of *p*-nitrophenyl benzoate and 2,4-dinitrophenyl benzoate with hydroxylamine showed a linear dependence of the rate upon hydroxylamine concentration and revealed second-order rate constants of 11.0 and 316 l. mole.$^{-1}$ min.$^{-1}$, respectively (12.6 and 360 l. mole^{-1} min.$^{-1}$ if calculated for hydroxylamine present as the free base). These rates show the expected effects of conjugation of the carbonyl group with an aromatic nucleus and electron withdrawal by an additional nitro group.

The Reaction of O-Acetylhydroxylamines with Hydroxylamine.—The formation of hydroxamic acid from O-acetylhydroxylamine could be studied by following the slow phase of the reaction of *p*-nitrophenyl acetate with hydroxylamine (Fig. 1b); however, similar results were obtained without interference from the initial rapid hydroxamic acid formation due to reaction 1b by using O-acetylhydroxylamine solutions which had been purified by distillation with alcohol.[2] Although the appearance of hydroxamic acid in a given run follows first-order kinetics, the rate at a given *p*H is not proportional to the hydroxylamine concentration but is more nearly proportional to the product of the hydroxylamine and hydroxylammonium ion concentrations (Table II). With varying *p*H the rate passes through a maximum at *p*H 6.2 (Fig. 4), but does not follow exactly the calculated curve for a reaction rate which is proportional to the product of hydroxylamine and hydroxylammonium ion concentrations alone (dashed line, Fig. 4).

TABLE II

THE REACTION OF HYDROXYLAMINE WITH *o*-ACETYLHYDROXYLAMINE AS A FUNCTION OF HYDROXYLAMINE CONCENTRATION AT 25°, *p*H 6.5[a]

Hydroxylamine concn., *M*	k_1, min.$^{-1}$	$k_1/[NH_2OH]_{tot.}$, l. mole^{-1} min.$^{-1}$	$k_1/[NH_2OH]^+$, l.^{2} mole^{-2} min.$^{-1}$
0.1	0.013	0.13	6.9
.2	.043	.22	5.7
.3	.090	.30	5.3
.4	.154	.39	5.1
.5	.230	.46	4.9

[a] NaCl added to maintain constant ionic strength in all tubes. The reaction mixtures contained 4% alcohol and 10^{-4} *M* EDTA (ethylenediaminetetraacetic acid).

In contrast to the reaction with *p*-nitrophenyl acetate, the reaction of hydroxylamine with O-acetylhydroxylamine is accelerated markedly by phosphate buffer (Table III). This stimulation is not due to an ionic strength effect since addition of 0.5 *M* KCl has only a small effect on the rate, nor can

it be ascribed to a single ionic species of phosphate since it occurs over a wide range of *p*H. Less striking increases were found, at *p*H 6.5, with other buffers including 0.1 *M* citrate and succinate which cause 19% increases in rate and 0.1 *M* acetate which gives an 11% increase; 0.1 *M* phenol or tris-(hydroxymethyl)-aminomethane have no effect on the rate.

TABLE III

EFFECT OF BUFFERS ON THE RATE OF HYDROXAMIC ACID FORMATION FROM 2 × 10^{-3} *M* O-ACETYLHYDROXYLAMINE IN 0.2 *M* HYDROXYLAMINE, 10^{-4} *M* EDTA, 4% ALCOHOL AT 30°

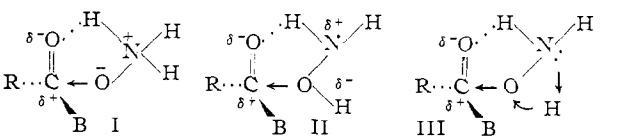
Buffer	Rates given as $k_1 \times 10^2$, min.$^{-1}$ *p*H 5.8	6.5	8.0
None	5.3	4.7	2.9
None, 0.5 *M* KCl		5.5	
0.01 *M* phosphate		5.9	
.03 *M* phosphate	7.2	8.0	4.9
.1 *M* phosphate	9.3	12.8	10.3
.3 *M* phosphate		23.9	
.03 *M* pyrophosphate			4.8[a]
.085 *M* pyrophosphate			6.4[a]
.03 *M* bicarbonate			3.9[b]
.10 *M* bicarbonate			6.1[b]

[a] *p*H 8.1. [b] *p*H 8.6.

The reaction of O-benzoylhydroxylamine with hydroxylamine to form benzohydroxamic acid is similar to that of the acetyl compound, but much slower; the rate with 1.8 *M* hydroxylamine (85% as the free base) in 40% alcohol was found to be 0.036 min.$^{-1}$.

Discussion

The rapid O-acylation of hydroxylamine is in striking contrast to the much slower reactions of *p*-nitrophenyl acetate with water, oxygen anions and even nitrogen bases of comparable basicity (Table I). The reaction with hydroxylamine is over ten million times faster than that with water, which has a basicity which would be expected to be equal to or greater than that of the hydroxyl group of hydroxylamine since nitrogen is more electronegative than hydrogen. These differences indicate that the neighboring amino group in the hydroxylamine molecule must increase the reactivity of the hydroxyl group and suggest that the reaction may proceed with concerted acid–base attack, as proposed by Swain for the α-pyridine-catalyzed mutarotation of glucose in organic solvents[5] or with intramolecular neighboring group participation as in the solvolysis reactions studied by Winstein, *et al.*[6] It recently has been suggested that the reactions in aqueous solution of the catecholate monoanion,[7] the hydroxamic acid anion[8] and the perhydroxyl anion[9] (which also reacts rapidly with *p*-nitrophenyl acetate; *cf.* Table I) with certain phosphate anhydrides and of hydroxylamine with oximes[10] involve concerted acid–base attack. If the addition step is the rate-limiting step of the reaction, the acylation of hydroxylamine may accordingly be formulated as (I) attack of hydroxylamine in the zwitterionic form, with hydrogen bonding of the carbonyl group to the ammonium group of hydroxylamine increasing the polarization and reactivity of the carbonyl group; (II) attack of the uncharged hydroxyl group, with oxygen attack favored by the high electronegativity of oxygen and carbonyl polarization aided by hydrogen bonding to the amino group; or (III) the amino group may act as a more efficient proton acceptor than water in the transition state.



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If the rate-limiting step is the decomposition of a tetrahedral intermediate, the amino group might be expected to exert its rate-accelerating effect in a similar manner. The existence of an appreciable concentration of hydroxylamine in the zwitterionic form in aqueous solution is not unexpected in view of the known dissociation constants of hydroxylamine derivatives and trimethylamine oxide,[3,11] although the zwitterion does not appear to exist in the hydroxylamine crystal.[12] The high reactivity of the hydroxylamine oxygen atom in this reaction requires some specific interaction of hydroxylamine

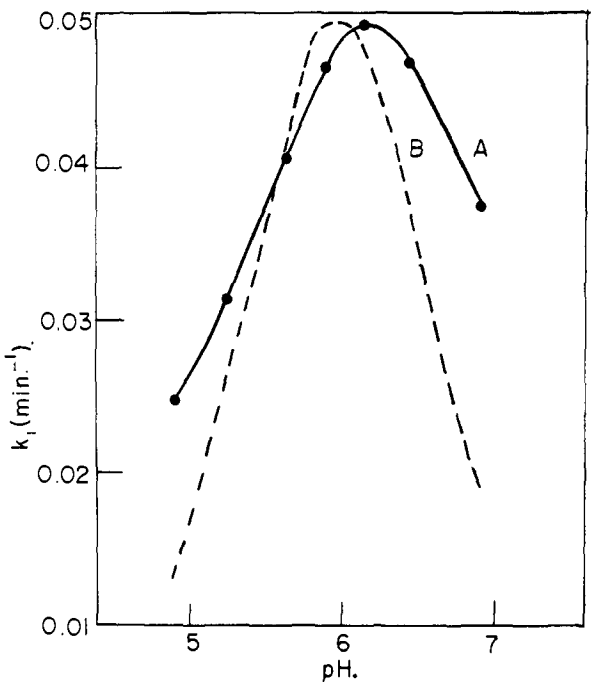


Fig. 4.—Effect of *p*H on the rate of hydroxamic acid formation from 2 × 10^{-3} *M* O-acetylhydroxylamine in 4% alcohol, 10^{-4} *M* EDTA at 25°; total hydroxylamine concentration, 0.207 *M*; NaOH added to hydroxylamine hydrochloride solution to obtain desired *p*H: A, observed rates; B, calculated for $k_1 = 4.6[NH_2OH][NH_3OH]^+$.

(5) C. G. Swain and J. F. Brown, THIS JOURNAL, **74**, 2534, 2538 (1952).

(6) S. Winstein, E. Grunwald, *et al.*, *ibid.*, **70**, 812, 816, 821, 828 (1948).

(7) J. Epstein, D. H. Rosenblatt and M. M. Demek, *ibid.*, **78**, 341 (1956).

(8) R. Swidler and G. M. Steinberg, *ibid.*, **78**, 3594 (1956).

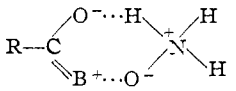
(9) J. Epstein, M. M. Demek and D. H. Rosenblatt, *J. Org. Chem.*, **21**, 796 (1956).

(10) Personal communication from R. B. Woodward.

(11) T. D. Stewart and S. Maeser, THIS JOURNAL, **46**, 2583 (1924).

(12) R. E. Nightingale and E. L. Wagner, *J. Chem. Phys.*, **22**, 203 (1954).

with the carbonyl group and is not an intrinsic property of the oxygen atom since alkylation of hydroxylamine occurs on the nitrogen atom. Furthermore, a mechanism of this kind provides an explanation for the increase in the ratio of O- to N-acylation observed with acylating agents containing bases with increasing electron-donating ability[2] since an increase in the positive character of the base through either inductive or resonance effects would be expected to aid the preliminary alignment of the hydroxylamine molecule favoring oxygen attack



The rapid rate of this reaction, which occurs in aqueous solution, is somewhat analogous to the rapid rates of enzymatic reactions and provides some experimental analogy for the proposed acylation of a serine hydroxyl group in the active site of a number of enzymes (*cf.* ref. 13 and references therein). Although the serine hydroxyl group is ordinarily only weakly nucleophilic, its reactivity may be increased markedly if it is located on the enzyme surface in proper spatial relationship to a nitrogen-containing group which can perform the same function as does the amino group of hydroxylamine.

The reaction of hydroxylamine with O-acylhydroxylamine, in contrast to that with *p*-nitrophenyl acetate, shows a rate maximum at *p*H 6.2, a rate proportional to more than the first power of the hydroxylamine concentration, and a marked rate acceleration with phosphate buffer. These effects, which are similar to those observed in oxime and semicarbazone formation,[14] suggest that this reaction is subject to general acid catalysis by the hydroxylammonium ion and by phosphate, although the lack of precise agreement with the rates calculated for such a mechanism alone as well as the remarkable effectiveness of phosphate in accelerating the reaction over a wide range of *p*H suggest that other factors which are as yet unexplained may be of importance in this reaction. The observation that acid catalysis does not appear to be of importance in the reaction with *p*-nitrophenyl acetate lends additional support to the hypothesis that the hydroxylamine molecule is itself acting as an acid as well as a base in this reaction. The possibility that the reaction of hydroxylamine with ketones and aldehydes is initiated by a rapid step not requiring a separate acid catalyst is currently under investigation.

(13) H. Gutfreund and J. M. Sturtevant, *Proc. Natl. Acad. Sci.*, **42**, 719 (1956).

(14) E. Barrett and A. Lapworth, *J. Chem. Soc.*, **93**, 85 (1908); J. B. Conant and P. D. Bartlett, THIS JOURNAL, **54**, 2881 (1932).

Experimental

Preparations and determinations were carried out as described in the preceding paper.[2] Methoxyamine hydrochloride (Eastman Kodak Co.) was neutralized with NaOH immediately before use. Acetohydroxamic acid, m.p. 90–91°, from acetic anhydride and neutralized aqueous hydroxylamine hydrochloride, was recrystallized from ethanol and from ethyl acetate. Incubations were carried out in a water-bath at 25.0 ± 0.1° except where otherwise specified. Ethylenediaminetetraacetic acid was added to reaction mixtures containing O-acylhydroxylamine to avoid occasional erratic results apparently due to a trace metal-catalyzed decomposition of O-acylhydroxylamine; this chelating agent did not itself otherwise affect the reaction. First-order rate constants, k_1, were calculated, using the formula $k_1 = (0.693/t_{1/2})$, from the half-times of reaction which were determined graphically from plots of the extent of the reaction, $x_\infty - x_t$, against time on semilogarithmic graph paper. Second-order rate constants were calculated from the slope of the plot of k_1 against concentration of the second reactant. The rate constants are reproducible to within approximately ±5%.

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WALTHAM MASS.

[CONTRIBUTION FROM AVERY LABORATORY, UNIVERSITY OF NEBRASKA]

Reactions of Amines. III. Pyrolysis of N-Alkylacetamides[1,2]

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The pyrolyses of a number of N-alkyl- and N-alkyl-N-methylacetamides have given the corresponding olefins. The reactions required somewhat higher temperatures than the pyrolyses of the corresponding alkyl acetates and first indications were that the former were less selective in the direction of elimination.

The pyrolysis of simple amides to form nitriles is a well known reaction, but the pyrolysis of N-alkylamides appears to have received little study. As far as we have been able to determine Ritter and Minieri[5] were the first to observe that the liquid phase pyrolysis of N-*t*-alkylacetamides gave the corresponding olefins and acetonitrile. They mentioned also the facile acid-catalyzed decomposition of these amides into apparently the same olefins. Wiley, Ketterer and Reed[6] have reported a similar ready elimination in the acid-catalyzed decomposition of N-(*t*-octyl)-*p*-ethylbenzenesulfonamide, which gave isoöctene (2,3,4-trimethyl-2-pentene) and *p*-ethylbenzenesulfonamide. Cook, Dickson, Ellis and Loudon[7] have described, among other

(1) Paper II, THIS JOURNAL, **79**, 3145 (1957).

(2) This work was supported in part by grant G-3689 of the National Science Foundation.

(3) Standard Oil C . (of Indiana) Fellow, 1951–1953.

(4) Minnesota Min ng and Manufacturing Co. Fellow, 1955–1956.

(5) J. J. Ritter and P. P. Minieri, THIS JOURNAL, **70**, 4045 (1948).

(6) R. H. Wiley, C. C. Ketterer and S. F. Reed, *ibid.*, **76**, 4996 (1954).

(7) J. W. Cook, C. T. Dickson, D. Ellis and J. D. Loudon, *J. Chem. Soc.*, 1078 (1949).