the sign of ΔV_{II}. Without such information further speculation concerning the significance of the trend would be premature.

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The Kinetics of Oxidation of Copper(I) by Molecular Oxygen in Perchloric Acid–Acetonitrile Solutions[1a]

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Abstract: Spectrophotometric and polarographic methods were used to study the oxidation of Cu(I) by dissolved O_2 in $HClO_4$–CH_3CN solutions of ionic strength 0.1 *M*. The reaction follows the rate law $-d[Cu(CH_3CN)_2^+]/dt = d[Cu^{2+}]/dt = k_c[Cu(CH_3CN)_2^+][O_2][H^+]/[CH_3CN]^2$, where $k_c = 6.9 \pm 0.3 \times 10^7\ M^{-2}\ sec^{-1}$ at 30°. The activation energy for the process is 8.4 ± 0.4 kcal/mole in the temperature range 9.8–48°. The data are interpreted in terms of a bimolecular reaction between a hypothetical CuO_2^+ complex and H^+ in the rate-limiting step of a sequence which involves the prior rapid dissociation of the $Cu(CH_3CN)_2^+$ complex followed by the formation of the oxygenated intermediate. The stoichiometry of the reaction indicates that under the reported conditions Cu(I) reacts more slowly with H_2O_2 than with O_2. A small amount of the Cu(I) ion can be detected at the conclusion of O_2 uptake, at which time the addition of a complexing agent preferential for Cu(I) results in the relatively slow formation of the Cu(I) complex in a pH-dependent reaction. Although the reaction sequence implies the participation of free-radical oxygen intermediates, attempts to obtain evidence for the appearance of these reactive species by the ability of the reaction mixture to hydroxylate benzoic acid were unsuccessful. In addition the ultraviolet spectra for the acetonitrile and aquo complex ions of Cu(I) were obtained; attempts to detect the CuO_2^+ intermediate spectroscopically failed.

The addition of copper ion to apoproteins to form copper enzymes and proteins is a reaction of importance in the study of the biosynthesis and the reconstitution of copper proteins. While virtually nothing is known about the biosynthesis of copper proteins, exploratory work is available on the reconstitution of several copper proteins,[2] particularly hemocyanin, ceruloplasmin, and cytochrome *c* oxidase. For these and several other copper proteins, the prosthetic copper can be removed to form a copper-free apoprotein. Restoration of the prosthetic copper to form biologically functional molecules in general requires the participation of Cu(I). A recent paper by Hemmerich[3] describing the properties of the water-soluble compound $Cu(CH_3CN)_4ClO_4$ prompted us to use this substance as a source of univalent copper in studying the reconstitution of apoceruloplasmin. It became apparent during the course of our study,[4] and it was also recently reported by Nair and Mason[2c] in the reconstitution of apocytochrome *c* oxidase, that the added Cu(I) is oxidized by dissolved O_2 in a reaction which is rapid compared to the apparent velocity of the reconstitution reactions.

In addition, the importance of Cu(II) as a catalyst in oxidation–reduction reactions involving molecular oxygen is well known in both enzymic[5] and nonenzymic[6] systems. The mechanism of catalytic action is generally attributed to the rapid regeneration of Cu(II) by the facile oxidation of Cu(I) by O_2. However, the rapidity of the oxygenation of Cu(I), the paucity of soluble Cu(I) salts, and the competing disproportionation reaction of Cu(I) have made difficult a quantitative study of the kinetics of the oxidation of Cu(I) by O_2.

Wieland and Franke[7] in 1929 observed that the ratio of the velocity of oxidation of CuCl by O_2 to the velocity of its oxidation by H_2O_2 is a function of pH, where higher acidity favors an increase in the magnitude of the ratio. Nord[8] found that in 0.04–0.4 *M* HCl, the velocity of the oxidation of $CuCl_2^-$ by O_2 depends on $[H^+]$ and is limited by the rate of diffusion of O_2 from

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[2] (a) Hemocyanin: R. Lontie, V. Blaton, M. Albert, and B. Peeters, *Arch. Intern. Physiol. Biochim.*, 73, 150 (1965); (b) ceruloplasmin: A. G. Morell, P. Aisen, W. E. Blumberg, and I. H. Scheinberg, *J. Biol. Chem.*, 239, 1042 (1964); (c) cytochrome *c* oxidase: P. M. Nair and H. S. Mason, *ibid.*, 242, 1406 (1967).

[3] P. Hemmerich in "The Biochemistry of Copper," J. Peisach, P. Aisen, and W. E. Blumberg, Ed., Academic Press, New York, N. Y., 1965, pp 15–32.

[4] R. D. Gray, Ph.D. Dissertation, Florida State University, 1968.

[5] E. Frieden, S. Osaki, and H. Kobayashi, *J. Gen. Physiol.*, 49 (2), 213 (1965).

[6] F. Basolo and R. G. Pearson, "Mechanisms of Inorganic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1967, p 500.

[7] H. Wieland and W. Franke, *Ann.*, 473, 291 (1929).

[8] H. Nord, *Acta Chem. Scand.*, 9, 430 (1955).

the gas to the liquid phase. In 1966, Henry[9] investigated the reaction in glacial acetic acid and reported a rate expression exhibiting first-order dependency on O_2 and second-order dependency on CuCl. More recently, Zuberbühler[10] reported evidence that the complex ions $Cu(imidazole)_2^+$ and $Cu(NH_3)_2^+$ react with O_2 *via* bimolecular pathways which were independent of pH.

A striking feature of the reaction of monovalent copper with oxygen is the nature of the product. H_2O_2 is not detected in reaction solutions containing either the chloride ion,[4,8] imidazole,[10] or 2-(N-morpholino)ethanesulfonic acid[4]; however, in reaction media buffered with phosphate, acetate, citrate, cacodylate,[4] or ammonia,[10] less than stoichiometric amounts of H_2O_2 can be detected at the conclusion of oxygen consumption. In enzymic systems containing redox active copper, a correlation has been suggested between the number of copper atoms per catalytic unit and the reduction of O_2 to H_2O_2 in a two-electron transfer or to H_2O in a four-electron step.[5]

The purpose of this investigation was then threefold: (1) to determine the stoichiometry of the reaction of Cu(I) with O_2; (2) to describe the kinetics of this reaction; (3) to explain the phenomenon in which the oxidation reaction is apparently reversed when a Cu(I)-preferring ligand is added to the system at the completion of oxygen consumption.

Experimental Section

Materials. $Cu(CH_3CN)_4ClO_4$ was synthesized from Cu_2O (Baker, Analyzed reagent), CH_3CN (Baker, Analyzed reagent), and $HClO_4$ (Mallinckrodt, 60% Analytical reagent) by the method of Hemmerich and Sigwart.[11] Stock solutions of about 0.1 *M* Cu(I) in CH_3CN were prepared from freshly recrystallized material and stored in the dark at 4°. The exact concentration of Cu(I) in the stock solution was measured colorimetrically at 454 mμ as the $Cu(neocuproine)_2^+$ complex in ethanol.[12] Since the addition of $NH_2OH \cdot HCl$ had no effect on the intensity of light absorption in the neocuproine assay, we infer that the stock solutions were free of Cu(II), even after several months of storage. In one series of experiments, the $Cu(CH_3CN)_4ClO_4$ was recrystallized five times from CH_3CN which had been redistilled[13] from $KMnO_4$.

$HClO_4$ stock solutions were prepared by diluting 60% $HClO_4$ with twice deionized water; $[H^+]$ was determined by titration with standard NaOH. Where necessary, $NaClO_4$ (Fisher, purified), which had been recrystallized from ethanol–water and stored over P_2O_5, was added to maintain an ionic strength of 0.10.

Colored impurities were removed from neocuproine (2,9-dimethyl-1,10-phenanthroline, Aldrich Chemical Co.) by passing a solution of the chelator in $CHCl_3$ over a column of silicic acid followed by recrystallization from low-boiling petroleum ether.

Benzoic acid-7-^{14}C and salicylic acid-7-^{14}C (New England Nuclear Corp.) were of specific activity 8.41 and 4.02 mCi/mmole, respectively. Both were reported to be >99% radiochemically pure by the manufacturer.

Methods. pH was determined with a Radiometer PHM 26 pH meter equipped with G2222C glass and K4112 reference electrodes; 0.05 *m* $KHC_2O_4 \cdot H_2C_2O_4 \cdot 2H_2O$ (National Bureau of Standards) served for calibration below pH 3. Absorption spectra were obtained either on a Cary-15 spectrophotometer equipped with 0–0.1 and 0–1.0 absorbance slide wires or on a Beckman DU instrument, using 10- or 1-mm silica cuvettes (Pyrocell, S18-260). Descending paper chromatography was conducted in a glass apparatus at room temperature. Aliquots (25 μl) of reaction mixtures were spotted on Whatman No. 3MM paper which was subsequently developed for 15 hr in 4:1 (v:v) 1-butanol–NH_4OH solvent.[14] The chromatograms were dried and the position of carrier salicylic acid was noted as a fluorescent spot under ultraviolet irradiation. Radioactivity was counted by a Packard Model 7700 radiochromatogram scanner with a ^{14}C counting efficiency of 22.8%.

Apparatus. Oxygen consumption was estimated using a Clark-type, Teflon membrane-covered oxygen electrode[15] (Beckman Instruments No. 39065). The electrode was mounted in a thermostated glass cylinder of 5.75-ml volume which could be isolated from the atmosphere by a rubber stopper. Reagents were added to the stirred reaction mixture by injection from Hamilton microliter syringes through a thin septum in the stopper. The output from the electrode was used to drive the pen of a millivolt recorder (Sargent Model SRL); the system was calibrated by determining dissolved $[O_2]$ in the reaction medium (0.10 *M* $NaClO_4$, pH 2.40) by the Winkler[16] method. A value of 235 μ*M* O_2 (std dev = ±17) was obtained as the mean of seven determinations for the air-saturated solution at 760 mm, 30°. Since this value agrees closely with the published values for the concentration of dissolved oxygen in air-saturated water at 30° (236 μ*M*), dissolved oxygen concentrations in the perchlorate media at temperatures other than 30° were taken to be the same as for water at the temperatures of interest.[17] H_2O_2 was converted to O_2 by acidified $KMnO_4$ and estimated as such in the oxygen electrode.

Kinetic Measurements. At pH values >2, the kinetic measurements at 240 mμ (slit = 0.32 mm) were accomplished in the Cary-15 using a micro-stopped flow apparatus modified from that described by Strittmatter[18] in which one of the two 1-ml reagent syringes of the original device was replaced by a 50-μl Hamilton syringe. The material in the microsyringe was diluted by a factor of 0.0471 (std dev = ±0.0026, 38 determinations) as determined by mixing a concentrated solution of methylene blue with water and recording the final absorbance of the solution at 635 mμ. The response time of this apparatus for a change in absorbance from 1.0 to 0.1 was 1 sec. The temperature within the reaction cuvette and reagent syringe was matched to within ±0.07° using a calibrated thermistor.

The velocity of autoxidation at pH 1 exceeded the response capabilities of the Cary-15 instrument; therefore, the stopped-flow unit was used with a Beckman DK-1 spectrophotometer optical system and light source which was operated in single-beam mode. The output from the 1P28 photomultiplier was fed directly to a Tektronix Type D high-gain differential preamplifier and Tektronix Type 549 storage oscilloscope. The oscilloscope time base was conveniently triggered by the abrupt change in light transmittance caused by activation of the stopped-flow apparatus. For the rate studies at 240 or 245 mμ, a hydrogen discharge lamp operated at 400 mA dc was used with a slit width of 2.0 mm. A filter circuit was necessary to reduce the noise level; the response time of this system was 40 msec as determined by a dye-mixing experiment. Oscilloscope traces were photographed and transmittance was determined as a function of time on enlarged prints.

Measurements of the rate of Cu^{2+} production were made at 800 mμ in a cell designed for simultaneous spectrophotometric and oxygen electrode measurements. A brief description of the apparatus follows. Light emerging from the monochromator of the Beckman DK-1 spectrophotometer was focused at one end of a flexible fiber optics light guide (American Optical Co., LGM-2X48) which transmitted the radiation to an edge of a cylindrical plexiglass container (volume = 2.50 ml) containing an oxygen electrode mounted as described above. A second receiving light guide, rigidly mounted 180° from the transmitting guide, returned the unabsorbed light to the cell compartment of the spectrophotometer. The light beam was then reflected to a Hamamatsu R-136 photomultiplier which was used because of its high sensitivity at 800 mμ. The amplified output from the photomultiplier was expanded 100-fold and the upper 0.8% (corresponding to a transmittance range of approximately 99.2-100%) was displayed on the oscilloscope as described above, or used with a recorder (Sargent Model SR) when slower rates of reaction were of interest. The light

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(9) P. M. Henry, *Inorg. Chem.*, **5**, 688 (1966).

(10) A. Zuberbühler, *Helv. Chim. Acta*, **50**, 466 (1967).

(11) P. Hemmerich and C. Sigwart, *Experientia*, **19**, 488 (1963).

(12) G. F. Smith and W. H. McCurdy, *Anal. Chem.*, **24**, 371 (1952).

(13) J. F. O'Donnell, J. T. Ayres, and C. K. Mann, *Anal. Chem.*, **37**, 1161 (1965).

(14) E. Lederer and M. Lederer, "Chromatography," Elsevier Publishing Co., Amsterdam, 1957, p 197.

(15) L. C. Clark, *Trans. Am. Soc. Artificial Internal Organs*, **2**, 41 (1956).

(16) L. W. Winkler, *Ber.*, **21**, 2843 (1888).

(17) "Standard Methods for the Examination of Water and Wastewater," 12th ed, American Public Health Association, New York, N. Y., 1965, pp 405–409.

(18) P. Strittmatter in "Rapid Mixing and Sampling Techniques in Biochemistry," B. Chance, R. H. Eisenhardt, Q. H. Gibson, and K. K. Lonberg-Holm, Ed., Academic Press, New York, N. Y., 1965, pp 71–84.

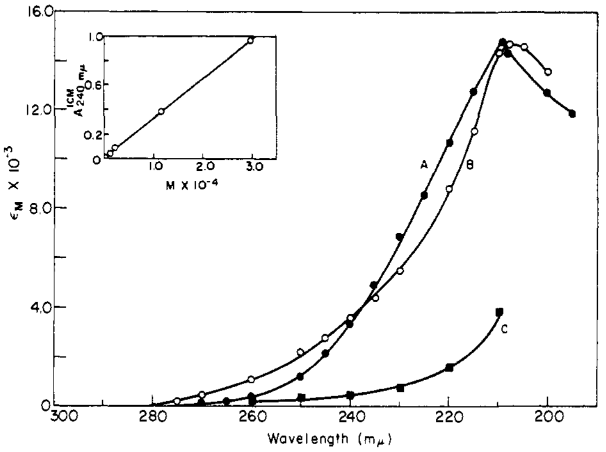

Figure 1. (A) Aerobic spectrum of $Cu(CH_3CN)_4ClO_4$ in 25 vol % CH_3CN–0.1 *M* $NaClO_4$, pH 2.40. (B) The anaerobic spectrum of Cu(I) in the absence of appreciable CH_3CN in 0.10 *M* $NaClO_4$, pH 2.40 (see text). (C) Spectrum obtained after admitting air to the system described for curve B. The inset shows Beer's law relationship of the absorption at 240 mμ.

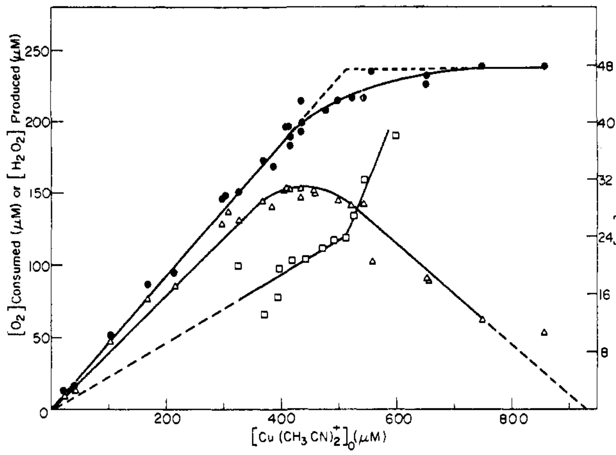

Figure 2. Plot illustrating $[O_2]$ consumed (●), $[H_2O_2]$ produced (△), and $[Cu^+]$ (□) remaining at the conclusion of oxygen consumption as a function of initial $[Cu(CH_3CN)_2^+]$. Cu(I) was measured at the conclusion of O_2 consumption by withdrawing aliquots of the reaction mixture into a syringe partially filled with a solution of neocuproine in ethanol, followed by measurement of the absorbance of the solution at 454 mμ. Conditions: 0.10 *M* $NaClO_4$, pH 2.40, 235 μ*M* O_2, 0.098 *M* CH_3CN, 30°.

source was a tungsten lamp operated at 6 V and 4.5 A from a storage battery. The apparatus was calibrated at 800 mμ (slit = 0.78 or 1.52 mm) with aqueous $Cu(ClO_4)_2$ solutions; Beer's law applied over the concentration range to at least 450 μ*M* Cu^{2+}.

Calculation of Rate Constants. Second-order rate constants (k_{obsd}) were estimated for the reactions followed in the ultraviolet from the integrated second-order rate expression

$$k_{obsd} = \frac{2.303}{t(B - {}^1/_2A)} \log\left\{\frac{A[B - {}^1/_2(A - X)]}{BX}\right\} \qquad (1)$$

where *B* is the initial $[O_2]$ (*M*), *A* is the initial $[Cu(CH_3CN)_2^+]$ (*M*), and *X* is $[Cu(CH_3CN)_2^+]$ (*M*) at time *t* (sec). The estimates of k_{obsd} obtained from eq 1 and *A* were taken as adjustable parameters, *B* was treated as a fixed parameter, and an iterative linear least-squares procedure was used to adjust k_{obsd} and *B* to best values. The calculations were performed by a Control Data 6400 computer on 15–20 concentration–time values extending from 5 to 95% of the reaction, using a FORTRAN IV program, LSKIN2.[19] The standard deviation of the fit of an individual data set was generally 5–10% for k_{obsd} and 2–3% for *A*.

Initial rates were estimated from tangents drawn to the initial portion of concentration–time curves. The reported stability constant[3] (2.24 × 10^4 M^{-1}) was utilized for the calculation of concentrations of uncomplexed Cu(I) in equilibrium with known $[Cu(CH_3CN)_2^+]$ and $[CH_3CN]$. The latter was estimated from the known added volumes and the density of CH_3CN at 30° (0.771 g/ml[20]).

Results

The Absorption Spectrum of $Cu(CH_3CN)_2^+$. Curve A of Figure 1 illustrates the absorption spectrum of $Cu(CH_3CN)_2^+$ (λ_{max} 210 mμ) obtained in a solution consisting of 75% 0.10 *M* $NaClO_4$ (pH 2.40) and 25% CH_3CN (v:v). An identical spectrum, within experimental error, was obtained in aqueous CH_3CN (25% by volume). The absorption at all wavelengths adhered to Beer's law as illustrated by the inset of Figure 1 for 240 mμ, the wavelength at which most of the kinetic data was obtained.

The spectrum labeled B in Figure 1, presumably that of Cu_{aq}^+ (λ_{max} 208 mμ) was obtained in 0.10 *M* $NaClO_4$ (pH 2.40) in an evacuated Thunberg cuvette in which the CH_3CN solvent had been removed from the side arm during the evacuation procedure. The anaerobic spectrum thus obtained after mixing did not change appreciably with time until air was admitted to the system, whereupon the spectrum reverted to that of Cu(II) (Figure 1C).

Stoichiometry. The stoichiometry of the oxidation of Cu(I) by dissolved O_2 exhibited some interesting features (Figure 2). The most striking of these indicates that the reaction proceeds to a point that is characterized by the presence of some Cu(I) which remains at the conclusion of O_2 uptake. Evident from Figure 2 is the fact that the amount of H_2O_2 present at this time is less than the amount of O_2 consumed during the reaction. This effect becomes more noticeable when O_2 is the limiting reagent; under these conditions the H_2O_2 production declined, apparently reflecting the much slower reaction of Cu(I) and H_2O_2. The observed stoichiometries taken from Figure 2 under conditions where Cu(I) is the limiting reagent are Cu(I)(consumed)/O_2(consumed) = 2.15 and H_2O_2 (produced)/O_2(consumed) = 0.86.

Hydroxylation of Benzoic Acid. The reported ability of Cu(I) salts in the presence of H_2O_2 to initiate the free-radical polymerization of acrylonitrile,[21] of $CuSO_4$ to initate the hydroxylation of benzoic acid in the presence of H_2O_2,[22] and of Cu(I), under oxidizing conditions, to elicit the hydroxylation of acetanilide[23] prompted us to test for the presence of the free-radical ·OH in this system. The air oxidation of Cu(I) was carried out in 0.10 *M* $NaClO_4$, pH 2.40 in the presence of 100 μmoles of benzoic acid-7-^{14}C (specific activity 1 mμCi/μmole) and the reaction mixture subjected to paper chromatography. Radioactive salicylic acid could not be identified positively on the chromatogram. In a control reaction consisting of $CuSO_4$ and ascorbic acid,

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(19) Provided by Professor D. F. DeTar, Department of Chemistry, Florida State University, Tallahassee, Fla. 32306.

(20) J. Timmermans, "Physico-Chemical Constants of Pure Organic Compounds," Vol. I, Elsevier Publishing Co., Amsterdam, 1950, p 527.

(21) J. H. Baxendale, M. Evans, and G. S. Park, *Trans. Faraday Soc.*, 42, 155 (1946).

(22) J. O. Konecny, *J. Am. Chem. Soc.*, 76, 4993 (1954).

(23) H. Staudinger, B. Kerekjarto, V. Ullrich, and Z. Zubrzycki in "Oxidases and Related Redox Systems," Vol. II, T. E. King, H. S. Mason, and M. Morrison, Ed., John Wiley and Sons, Inc., New York, N. Y., 1965, p 815.

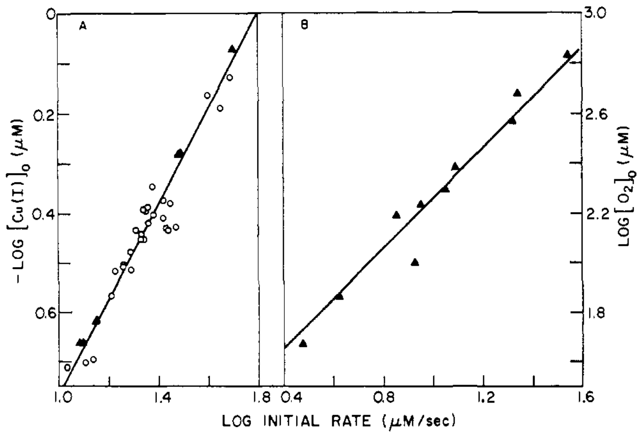

Figure 3. Initial rate of oxidation of Cu(I) by O_2 as a function of the initial concentration of Cu(I) (A) and O_2 (B) in 0.10 *M* $NaClO_4$ (pH 2.40), 30°: (○) measured at 240 mμ; (▲) measured at 800 mμ. Lines are drawn with slope = 1. Conditions: (A) 235 μ*M* O_2; (B) 0.22 μ*M* Cu^+ (calculated).

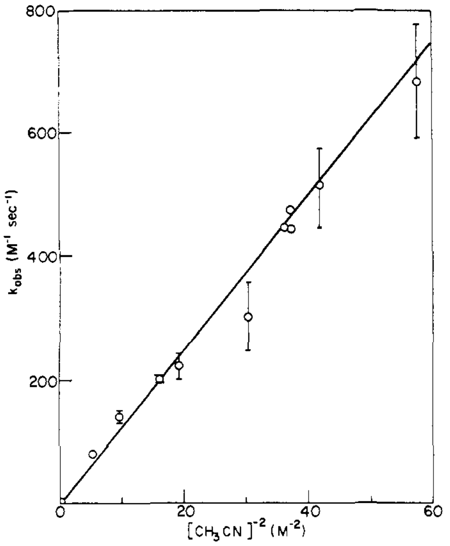

Figure 4. Plot of k_{obsd} *vs.* [CH_3CN]$^{-2}$. Error bars indicate standard deviations of the mean. Points without error bars represent single experiments. Conditions: 0.10 *M* $NaClO_4$, pH 2.40, 235 μ*M* O_2, 90–430 μ*M* $Cu(CH_3CN)_2^+$, 30°.

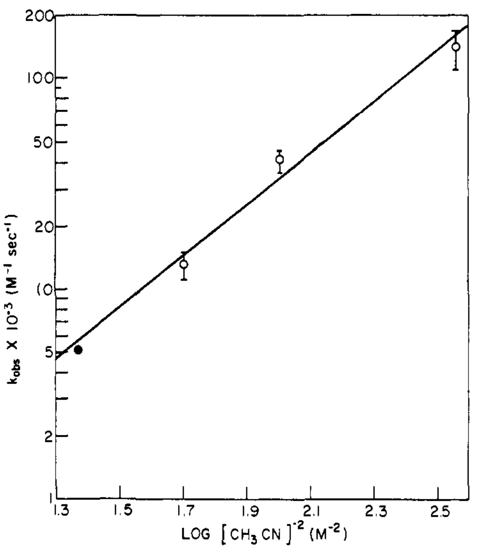

Figure 5. Double logarithmic plot of k_{obsd} *vs.* [CH_3CN]$^{-2}$. The point represented by the filled circle was obtained by extrapolation of data from Figure 6. Conditions: 0.106 *M* $HClO_4$, 235 μ*M* O_2, 360–430 μ*M* $Cu(CH_3CN)_2^+$, 30°.

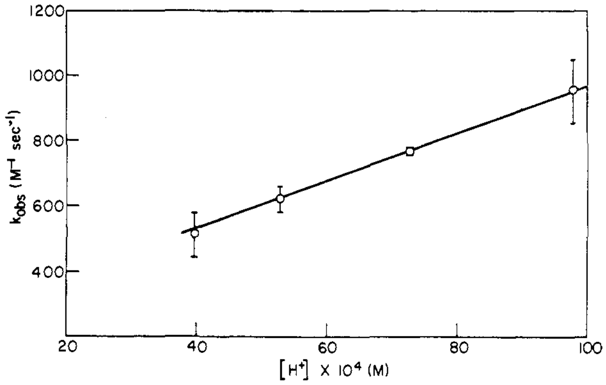

Figure 6. Plot of k_{obsd} *vs.* [H^+]. Conditions: 235 μ*M* O_2, 0.156 *M* CH_3CN, 250–300 μ*M* $Cu(CH_3CN)_2^+$, *I* = 0.10 *M* with $NaClO_4$, 30°.

181 mμmoles of salicylic acid was produced from 111.3 μmoles of benzoic acid in 60 min, an amount about ten times the lower limit of detection of labeled salicylic acid in this assay system. We conclude from these experiments that negligible amounts of ·OH are present when the oxidation of Cu(I) by O_2 proceeds in acid solution.

Kinetic Experiments. The order of the oxidation reaction with respect to Cu(I) and O_2 was determined by the method of initial rates. Log–log plots of the initial rate of oxidation as a function of the initial concentrations of the appropriate reactants are shown in Figure 3. The data of Figure 3A indicate a reaction first order with respect to Cu(I), while the companion Figure 3B shows that the oxidation is first order in O_2. The initial rates are independent of the method of measurement: both the appearance of Cu(II) or the disappearance of the Cu(I) species give comparable results.

The effect of [CH_3CN] on the rate of the autoxidation of Cu(I) was tested using the spectrophotometric assay method at 240 and 245 mμ. The results obtained at pH 2.40 in 0.10 *M* $NaClO_4$ are illustrated in Figure 4 and indicate that k_{obsd} is a linear function of [CH_3CN]$^{-2}$. Figure 5 presents the data obtained from experiments conducted in 0.106 *M* $HClO_4$; essentially the same trend is indicated in the latter as in Figure 4. Experiments which were carried out using redistilled[14] CH_3CN gave results comparable to those obtained when the reagent grade material was used.

The solid point in Figure 5 was obtained by extrapolation of the data in Figure 6 in which the variation in k_{obsd} as a function of [H^+] is depicted. A linear relationship between k_{obsd} and [H^+] is evident for $1 < \text{pH} < 3$. We were unable to conduct kinetic experiments at pH >3 because pH changes resulting from the uptake of H^+ during the reaction become significant in these less acidic solutions.

The effect of temperature on k_{obsd} was observed between 9.8 and 48°; the Arrhenius plot of the data is shown in Figure 7. The line was obtained by a least-squares procedure which resulted in the calculation of an activation energy of 8.4 ± 0.4 kcal/mole.

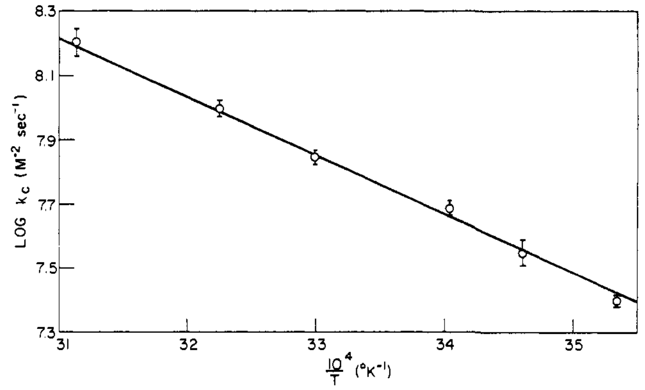

Figure 7. Arrhenius plot of log k_c (see text for definition) *vs.* $1/T$. Conditions: 0.10 *M* $NaClO_4$, pH 2.40, 145–260 μM $Cu(CH_3CN)_2^+$, 0.156 *M* CH_3CN.

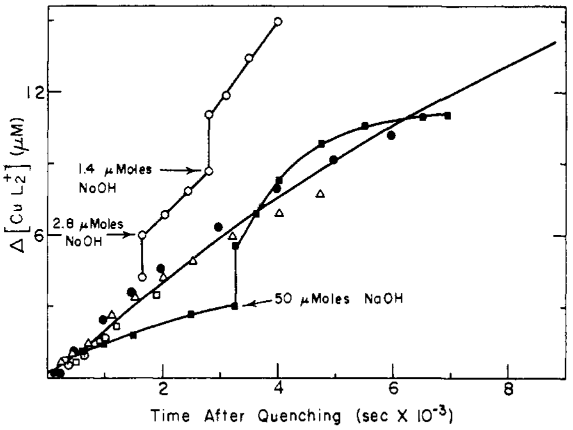

Figure 8. Plot of the change in $[CuL_2^+]$ (L = acetonitrile or neocuproine) *vs.* the time after quenching the reaction with complexing agent. Conditions: 0.23 μmole of $Cu(CH_3CN)_4ClO_4$ was oxidized by O_2 in 1 ml of 0.10 *M* $NaClO_4$, pH 2.40 at 30°. After 50 sec, 2 ml of CH_3CN was added and the increase in absorbance at 240 mμ recorded (○, △, □, and ● indicate replicate experiments). A second experiment (■) was carried out in an identical manner except that 1.69 m*M* neocuproine in ethanol was used to quench the oxidation; the increasing absorbance at 454 mμ was recorded. The experiment represented by the filled circles leveled off at approximately 24 μM $Cu(CH_3CN)_2^+$ after 5.7 × 10^4 sec.

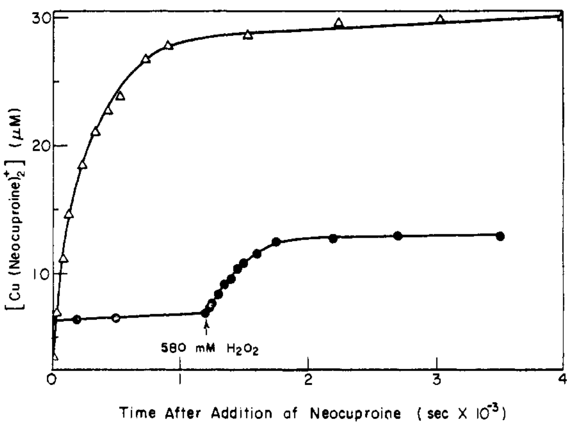

Figure 9. Plot of formation of $Cu(neocuproine)_2^+$ as a function of the time after addition of the neocuproine. Lower curve: 0.366 μmole of $Cu(CH_3CN)_4ClO_4$ was allowed to react with O_2 in 1 ml of 0.10 *M* $NaClO_4$, 30°; 38.6 sec after the addition of Cu(I), 2 ml of 1.69 m*M* neocuproine in ethanol was added; H_2O_2 was added at the arrow to give a final concentration of 590 m*M*. In the upper curve, 0.122 μmole of Cu(I) reacted with the O_2 dissolved in 1 ml of 0.08 *M* sodium acetate buffer, pH 5.9, for 120 sec. The reaction was quenched as described above with 2 ml of 1.69 m*M* ethanolic neocuproine.

The discussion of Williams[24] and of Morpurgo and Williams[25] concerning the oxidation of Cu(I) complexes by O_2 and the intense blue color of the crustacean respiratory pigment oxyhemocyanin (an oxygenated copper complex) suggested that it might be possible to detect spectroscopically the transient appearance of an oxygen–copper intermediate in the air oxidation of Cu(I). However, even with the considerable sensitivity available with the light-guide apparatus described here, no spectral change was observed from 400 to 700 mμ when $Cu(CH_3CN)_4ClO_4$ was mixed with air-saturated 0.10 *M* $NaClO_4$, pH 2.40 at 30°.

The Effect of Added Cu(I) Complexing Agents. In obtaining the data for the [Cu(I)] remaining at the end of oxygen consumption, it was found that the concentration of Cu(I) complex was not constant, but increased slowly as a function of the time after the addition of the complexing agent. The graphs in Figure 8 illustrate the phenomenon under various conditions. The appearance of the Cu(I) complex is accelerated by the addition of NaOH (Figure 8) and by H_2O_2 (Figure 9). The control reaction of mixing $CuSO_4$ with the complexing agent alone demonstrated that the reduction reaction does not result from the presence of a reducing impurity in the complexing agent. It was also shown that the addition of NaOH did not increase the amount of Cu(I) complex by means of a pH effect on the concentration of complex ion.

Discussion

Absorption Spectra. The spectrum obtained for $Cu(CH_3CN)_2^+$ (Figure 1, curve A) is qualitatively similar to that reported by Simpson and Waind[26] for $Cu(CN)_2^-$ (λ_{max} 234 mμ (ϵ_M 11,000)) except that the spectrum of the acetonitrile complex appears to lack the shoulder reported for the $Cu(CN)_2^-$ spectrum at 260 mμ. The spectrum for what presumably is the aquated Cu(I) (Figure 1, curve B) is evidently very similar to that for the complex with acetonitrile.[27] The invariance of the spectrum B with time is expected in view of the recent report[28] on the stability of the Cu(I) species toward the disproportionation reaction, which must be slow under these conditions. This reaction, therefore, has been eliminated from consideration in the following discussion.

Possible Mechanisms. It is possible to account for the experimental observations by reactions 2–6. The steps represented by (2) and (3) are assumed to be rapidly adjusted equilibria (expected for a d^{10} system[29]) described by the equilibrium constants K_2 and K_3,

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[24] R. J. P. Williams in "The Enzymes," Vol. I, P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press, New York, N. Y., 1959, p 433.

[25] G. Morpurgo and R. J. P. Williams in "Physiology and Biochemistry of Hemocyanins," Academic Press, London, 1968, pp 113–130.

[26] E. A. Simpson and G. W. Waind, *J. Chem. Soc.*, 1746 (1958).

[27] Discussion of the spectral properties of colorless Cu(I) complexes may be found in C. K. Jørgensen, "Absorption Spectra and Chemical Bonding in Complexes," Pergamon Press, Oxford, England, 1962, p 199.

[28] J. A. Altermatt and S. E. Manahan, *Anal. Chem.*, **40**, 655 (1968).

[29] F. Basolo and R. G. Pearson, ref 6, p 155.

$$\mathrm{Cu(CH_3CN)_2^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Cu^+ + 2CH_3CN} \qquad (2)$$

$$\mathrm{Cu^+ + O_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} CuO_2^+} \qquad (3)$$

$$\mathrm{CuO_2^+ + H^+ \xrightarrow{k_4} Cu^{2+} + HO_2} \qquad (4)$$

$$\mathrm{Cu^+ + HO_2 \longrightarrow Cu^{2+} + HO_2^-} \qquad (5)$$

$$\mathrm{H^+ + HO_2^- \rightleftharpoons H_2O_2} \qquad (6)$$

respectively. The participation of Cu^+ is suggested by the reciprocal square dependency of k_{obsd} on (CH_3CN) (Figures 4 and 5). Step 4, assumed rate limiting, is suggested by the linear dependence of k_{obsd} on $[H^+]$; it is also included by Nord[8] in his proposed mechanism for the reaction of $CuCl_2^-$ with O_2.

An alternative explanation of the $[H^+]$ effect is possible if the stability constant for $Cu(CH_3CN)_2^+$ is pH dependent. Increasing H^+ would then act to decrease the $Cu(CH_3CN)_2^+$ by the mass law and hence increase the $[Cu^+]$ available to react *via* reaction 3. It is pertinent to note that even in concentrated H_2SO_4, CH_3CN is not protonated[30] so that it at least appears likely that the pH effect on k_{obsd} does not result from a direct displacement of Cu^+ by H^+.

The rate expression corresponding to the reaction scheme 2–6 is given by

$$\text{rate} = k_{obsd}\,K_2K_3[\mathrm{Cu(CH_3CN)_2^+}] \times [\mathrm{O_2}][\mathrm{H^+}]/[\mathrm{CH_3CN}]^2 \qquad (7)$$

k_{obsd} can be obtained from

$$k_{obsd} = k_4K_2K_3[\mathrm{H^+}]/[\mathrm{CH_3CN}]^2 = k_c[\mathrm{H^+}]/[\mathrm{CH_3CN}]^2 \qquad (8)$$

k_c was calculated from experimental data to be $6.9 \pm 0.3 \times 10^7\ M^{-2}\ \mathrm{sec}^{-1}$ at 30°.

An alternative expression may be derived in which a reaction second order in Cu(I), under appropriate conditions, might exhibit apparent first-order kinetics.[31] Consider the sequence represented by reaction 2 above and reactions 9 and 10 below.

$$\mathrm{2Cu^+ + O_2 \xrightarrow{k_5} [Cu^+\text{–}O_2\text{–}Cu^+]} \qquad (9)$$

$$\mathrm{[Cu^+\text{–}O_2\text{–}Cu^+] + 2H^+ \xrightarrow{fast} 2Cu^{2+} + H_2O_2} \qquad (10)$$

Suppose that Cu^+ reaches a steady-state concentration given by

$$k_{-2}[\mathrm{Cu^+}][\mathrm{CH_3CN}]^2 + k_5[\mathrm{Cu^+}]^2[\mathrm{O_2}] = k_2[\mathrm{Cu(CH_3CN)_2^+}] \qquad (11)$$

At very high $[O_2]$

$$[\mathrm{Cu^+}]^2 = (k_2/k_5)[\mathrm{Cu(CH_3CN)_2^+}]/[\mathrm{O_2}] \qquad (12)$$

whereas in the limit as $[O_2]$ approaches zero

$$[\mathrm{Cu^+}] = K_2[\mathrm{Cu(CH_3CN)_2^+}]/[\mathrm{CH_3CN}]^2 \qquad (13)$$

The rate of the reaction 9 is given by

$$\text{rate} = k_5[\mathrm{Cu^+}]^2[\mathrm{O_2}] \qquad (14)$$

so that at high $[O_2]$

$$\text{rate} = (\text{constant})[\mathrm{Cu(CH_3CN)_2^+}] \qquad (15)$$

whereas at low $[O_2]$

$$\text{rate} = (\text{constant})[\mathrm{Cu(CH_3CN)_2^+}][\mathrm{O_2}]/[\mathrm{CH_3CN}]^4 \qquad (16)$$

The $[O_2]$ and $[CH_3CN]$ dependencies of the rate appear to rule out the possibility of the second-order mechanism described by steps 2, 9, and 10. At high $[O_2]$ the rate of the reaction is dependent upon $[O_2]$ (Figure 3B), contrary to the behavior predicted by eq 15. At low $[O_2]$ eq 11 predicts a $[CH_3CN]^{-4}$ rate dependence. This is not observed under the conditions of the data presented in Figures 4 and 5.

A third possibility for a direct two-electron transfer involves the transfer of two electrons from a single Cu(I) according to

$$\mathrm{Cu^+ + O_2 + 2H^+ \longrightarrow Cu^{3+} + H_2O_2} \qquad (17)$$

Cu(III) has been postulated as an intermediate in the oxidation of Cu(II)–peptide[32] complexes by $IrCl_6^-$ and in the radiolysis of ethylenediamine in the presence of Cu(II).[33] However, its presence appears to be ruled out in the case under discussion here since Cu(III) has been reported to react with water to give ·OH in acid solution.[34] It will be recalled that hydroxyl radicals could not be detected in these experiments.

The mechanism of the reduction of Cu(II) in this system is unclear, but it undoubtedly involves the interaction of Cu(I), Cu(II), and H_2O_2 in an equilibrium situation in which the addition of reagents which stabilize Cu(I) remove this species from participation in the equilibrium. This action would then require adjustment of the equilibrium, thus producing more Cu(I) at the expense of the other participants.

The following reactions explain the effect of pH and H_2O_2 without introducing the need to postulate ·OH.

$$\mathrm{H_2O_2 \rightleftharpoons H^+ + HO_2^-} \qquad (18)$$

$$\mathrm{HO_2^- + Cu^{2+} \longrightarrow HO_2 + Cu^+} \qquad (19)$$

$$\mathrm{HO_2 \rightleftharpoons H^+ + O_2^-} \qquad (20)$$

$$\mathrm{O_2^- + Cu^{2+} \longrightarrow Cu^+ + O_2} \qquad (21)$$

The acidity constant for the first ionization[35] of H_2O_2 is 2.4×10^{-12}; consequently, $[HO_2^-]$ under these circumstances of low pH must be minute. Small pH increases would result in only minor increases in $[HO_2^-]$ and hence only small rate increases. In fact, there is only a barely detectable reverse reaction at pH 1. The initial rapid increase in $[CuL_2^+]$ (L = acetonitrile or neocuproine) upon the addition of NaOH (Figure 8) is reasonably explained by an instantaneous shift in the equilibrium step (20) ($pK_a = 4.6$)[36] followed by very rapid reduction of Cu(II) by the increased $[O_2^-]$.[37] The slight apparent increase in the rate of formation of the Cu(I) complex subsequent to the addition of base might result from the expected small increase in $[HO_2^-]$. H_2O_2 would, of course, also be expected to accelerate the reduction of Cu(II); this was demonstrated by the experiment illustrated in Figure 9.

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[30] N. C. Deno, cited in E. M. Arnett, *Progr. Phys. Org. Chem.*, **1**, 279 (1963).

[31] I. W. Cohen and W. S. Caughey, *Biochemistry*, **7**, 636 (1968).

[32] A. Lovitzki, M. Anbar, and A. Berger, *ibid.*, 6, 3757 (1967).

[33] M. Anbar, R. A. Munoz, and P. Rona, *J. Phys. Chem.*, 67, 2708 (1963).

[34] M. Anbar, Advances in Chemistry Series, No. 49, American Chemical Society, Washington, D. C., 1965, p 129.

[35] P. George and J. S. Griffith in ref 24, p 375.

[36] G. Czapski and L. M. Dorfman, *J. Phys. Chem.*, 68, 1169 (1964).

[37] J. H. Baxendale, *Advan. Catalysis*, **4**, 31 (1952).

The observations noted here concerning the finding that simple Cu(I) complexing agents can reverse the oxidation of Cu(I) by O_2 afford a possible explanation for the ability of Cu(I), under oxidizing conditions, to serve as an effective source of univalent copper for the reconstitution of the copper-free proteins (apohemocyanin or apocytochrome *c* oxidase) as noted earlier in this communication. It is possible that the apoproteins react to reverse the autoxidation in the same fashion as the simple Cu(I) complexing agents reported here.

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Preparation and Characterization of Crystalline Long-Chain Ammonium Polyphosphates

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Abstract: Crystalline ammonium polyphosphates with a chain length greater than 50 were characterized for the first time. Control of the ammonia vapor pressure is the key to producing these compounds at elevated temperatures. Five forms were identified. Single crystals of forms II, IV, and V were isolated and their cell parameters determined. The long-chain crystalline ammonium polyphosphates are quite insoluble in cold water and show a slower degradation rate than Graham's salt.

It is well known that thermal dehydration of ammonium orthophosphates, instead of producing crystalline condensed phosphates, produces an amorphous mixture of polyphosphates,[1] from which most of the ammonia has been lost.[2,3] Early attempts to produce $(NH_4PO_3)_x$ varied from treating a copper or lead polyphosphate with ammonium sulfide[4,5] to tempering ammonium trimeta- or tetrametaphosphate at 200–250°. The products from these approaches, however, were impure as revealed by paper chromatograms and chemical analysis.[6]

Relatively pure and highly polymerized ammonium polyphosphates were reported to result from heating amidophosphates[7] and from reaction of ammonia with a condensed phosphoric acid containing about 83–84% P_2O_5.[8] The X-ray diffraction pattern given in the latter work is similar to that of an ammonium polyphosphate produced by heating an ammonium orthophosphate with urea.[9–11]

In the present work much more detailed information is developed on the formation and properties of a number of crystalline long-chain ammonium polyphosphates including forms not previously reported in the literature.

Experimental Methods

All chemicals used in the preparations were reagent grade without further purification. The products were first characterized by X-ray diffraction patterns of powdered samples. In those cases where sufficiently large single crystals could be isolated, oscillation and Weissenberg photographs were obtained to determine the unit cell parameters which were then refined using the powder data.

Total nitrogen in ammonium polyphosphate samples was determined by a Kjeldahl method for nitrate-free material.[12] Ammoniacal nitrogen was determined by a formaldehyde titration technique.[12] Total P_2O_5 content and phosphate species were determined by a combination molybdenum blue colorimetric paper chromatographic procedure.[13] Nuclear magnetic resonance spectra[14] were used to test for the presence of amido or imido phosphates and to estimate the relative amounts of ortho-, end-group, and middle-group phosphates.

Preparation and Characterization of Various Crystalline Forms

General Considerations. The long-chain ammonium polyphosphates (APP) described here were prepared either by tempering other forms and quenching or by reaction of urea with a less condensed phosphate. In either case it was necessary to provide some control

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(1) In this work, "polyphosphate" designates –P–O–P– chains and "metaphosphate" designates ring compounds.

(2) (a) G. V. Knorre, *Z. Anorg. Chem.*, 24, 369 (1900); (b) H. N. Terem and S. Akalan, *Compt. Rend.*, **228**, 1437 (1949).

(3) E. V. Margulis, L. J. Beisekeeva, N. I. Kopylov, and M. A. Fishman, *J. Appl. Chem.* (USSR), **39**, 2216 (1966).

(4) G. Tamman, *Z. Physik Chem.*, 6, 122 (1890); *J. Prakt. Chem.*, **45**, 417 (1892).

(5) S. J. Kiehl and T. H. Hill, *J. Am. Chem. Soc.*, **49**, 123 (1927).

(6) V. H. Grunze and E. Thilo, *Z. Anorg. Allgem. Chem.*, **281**, 262 (1955).

(7) M. Goehring and J. Sambeth, *Ber.*, **90**, 232 (1957).

(8) A. W. Frazier, J. P. Smith, and J. R. Lehr, *J. Agr. Food Chem.*, **13**, 317 (1965).

(9) S. Ueda, K. Oyama, and K. Koma, *Kogyo Kagaku Zasshi*, 66, 586 (1963).

(10) P. G. Sears and H. L. Vandersall, Belgian Patent 651,782 (Aug 13, 1964), and unpublished information.

(11) E. Kobayashi, *Kogyo Kagaku Zasshi*, 69, 2065 (1966).

(12) W. Horwitz, Ed., "Official Methods of Analysis of the Association of Official Agricultural Chemists," 8th ed, Association of Official Agricultural Chemists, Washington, D. C., 1955, Procedures 2:23 and 2:26.

(13) E. K. Kroupa, *Anal. Chem.*, **28**, 1091 (1956).

(14) M. Crutchfield, C. Dungan, J. Letcher, V. Mark, and J. R. Van Wazer, "Topics in Phosphorus Chemistry," Vol. 5, E. Griffith and M. Grayson, Ed., Interscience Publishers, New York, N. Y., 1967.