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Oxidation of aliphatic hydroxylamines in aqueous solutions

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The effects of pH, buffer constituents, duration of storage, presence of air, heavy metal ions, extracting solvents and various additives and cofactors on the aerial oxidation of some aliphatic primary and secondary hydroxylamines were investigated. Copper ions were particularly effective catalysts of the oxidation reaction. Conditions to minimize this transformation are described.

Hydroxylamines are important metabolic products of *N*-oxidation of aliphatic primary and secondary amines (Beckett, 1971; Beckett & Al-Sarraj, 1972; Cho, Lindeke & Hodshon, 1972; Beckett & Bélanger, 1974); many are unstable in aqueous solutions in the presence of oxygen, especially at alkaline pH values (Beckett & Al-Sarraj, 1973; Cho, Lindeke & Sum, 1974) and in the presence of metal ions (Johnson, Rogers & Trappe, 1956; Lindeke, Anderson & others, 1975). Hence, their oxidation may occur before extraction into organic solvents, in which they are generally more stable.

Compounds I–III (see Fig. 1) which possess varying degrees of stability towards oxidation were chosen for this investigation. In aqueous solutions, primary hydroxylamines possessing at least one α-hydrogen atom, e.g. I and II, are converted into oximes (Beckett & Al-Sarraj, 1973; Beckett & Chidomere, unpublished results); recently Lindeke & others (1975) isolated both oxime and nitroso compound from autoxidation of II and stated that the nitroso compound was the primary oxidation product which subsequently either wholly (at pH = 13) or partially (at pH = 7·5) isomerized to the oxime.

Secondary hydroxylamines are known to be converted into nitrones in alkaline solutions (Hamer & Macaluso, 1964). Also, Beckett & Gibson (1976), using similar conditions, reported trace amounts of benzaldoxime in addition to the major product, the nitrone, from *NN*-dibenzylhydroxylamine.

Certain analytical techniques used for measuring hydroxylamines produce decomposition (Beckett & Al-Sarraj, 1973; Beckett, 1974), but suitable derivatization can give stable compounds amenable to quantitative analysis by g.l.c. (Lindeke, Cho & others, 1973; Gal, Gruenke & Castagnoli, 1975; Beckett, Haya & others, 1975; Beckett & Achari, 1977). The possibility of the breakdown of hydroxylamines, before and during analytical procedures leads to uncertainties in the interpretation of the metabolism of many amine drugs. The various factors involved in the oxidation were investigated to define conditions producing greater stability of aqueous solutions of these compounds, before extraction into organic solvents, thus gaining clearer

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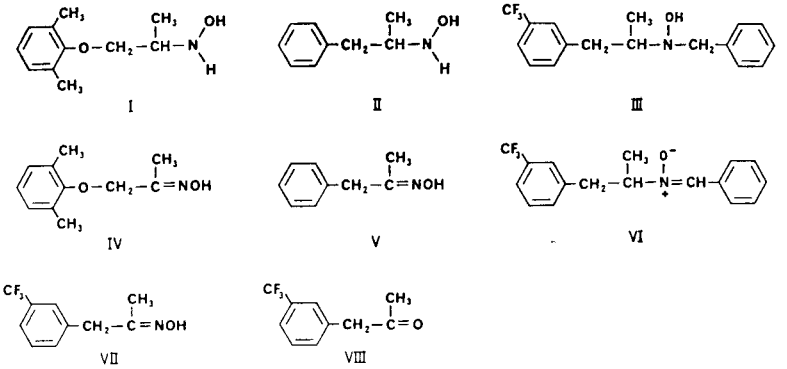

FIG. 1. Structures of the compounds investigated.

distinctions between metabolic and chemical changes taking place in such molecules.

MATERIALS AND METHODS

Reagents and chemicals. Compounds I–III were synthesized (Beckett & others, 1975); the oximes V and VII were synthesized by Dr K. Haya and the nitrone, VI, by Dr M. Mezei. The ketone, VIII, was a gift from Servier Laboratories, France, and compound IV was kindly supplied by Boehringer (Ingleheim); *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Pierce Chemical Co.; *N*-(trimethylsilyl)imidazole (TMSI) and *p*-chlorophenyl cyclopropyl ketone from Aldrich Chemical Co., diphenylmethylcyanide from BDH and bovine serum albumin (BSA) from Sigma Chemical Co. The co-factors, flavin-adenin-dinucleotide monosodium salt (FAD), flavin-mononucleotide monosodium salt (FMN), β-nicotinamid-adenin-dinucleotide (NAD), NADH disodium salt, β-nicotinamid-adenin-dinucleotide-phosphate disodium salt (NADP) and NADPH tetrasodium salt were purchased from Boehringer Mannheim GmbH. 8-Hydroxyquinoline (oxine) and all other chemicals were of Analar grade. Solvents were distilled before use. Acetonitrile was dried over $CaCl_2$.

Washing of the glass apparatus. Metal ions were removed from the inner surfaces of all glass apparatus by soaking overnight in 50% HNO_3 followed by thorough washing under tap water and rinsing with distilled water and trace heavy metal-free distilled water in succession before drying.

Preparation of trace heavy metal-free buffer solutions (Waring & Werkman, 1943; Beckett, Vahora & Robinson, 1958). Phosphate and borate buffer solutions (0·1 M) of varying pH values were prepared according to directions given in the Handbook of Biochemistry (1968). To 250 ml of the buffer solution in a 500 ml separating funnel was added 1 ml of oxine solution (2·5 mg ml^{-1} in chloroform), the mixture shaken vigorously, allowed to stand for about 1 h and the oxine-heavy metal complexes extracted with 3 × 25 ml of chloroform and discarded. The procedure was repeated thrice, and after the fourth treatment the aqueous layer was extracted with 6 × 25 ml of chloroform to remove final traces of oxine. Last traces of chloroform were removed by warming the trace heavy metal-free buffer solution with occasional shaking. Trace heavy metal-free distilled water was prepared similarly.

Buffer solutions containing added metal ions (Buffer soluion A). Trace heavy metal-free buffer solutions containing added metal ions were prepared by adding such metal ions in proportions reported to be present as impurities in the constituents from which the buffer solutions were prepared.

Gas-liquid chromatography (g.l.c.)

A. Instrumentation. A Perkin Elmer model F11 gas chromatograph equipped with a flame ionization detector was used. Chromatographic conditions: System A; 2 m × 4 mm i.d. glass column, packed with 2% XE60 on Chromsorb G, AW, DMCS, 80–100 mesh. System B; 2 m × 4 mm i.d. glass column, packed with 3% SE30 on Chromosorb G, AW, DMCS, 80–100 mesh. In both systems, nitrogen was 140 kPa and the injection port temperature was 50–60° above the respective column temperatures which are given in Table 1.

B. G.l.c. analysis of oxidation products. (*a*) Internal standard solutions: The following solutions were prepared in 20% methanol for use as internal standards; (A) *p*-chlorophenyl cyclopropyl ketone (50 μg ml^{-1}) for analysing I, IV and VII, (B) benzyl methyl ketone (50 μg ml^{-1}) for analysing II and V, and (C) diphenylmethylcyanide (75 μg ml^{-1}) for analysing III.

(*b*) *Calibration curves.* To 1 ml of the solutions of I, II, III, IV or V (0·1–1·25 μmol) and 1 ml of appropriate internal standard solution in a glass centrifuge tube (of *ca* 12 ml) was added 4 ml of trace heavy metal-free phosphate buffer solution (pH 7·4) and the free base was extracted immediately with 3 × 5 ml of ether and the extracts transferred to a glass evaporating tube. The extract was concentrated

Table 1. *G.l.c. and t.l.c. characteristics of the hydroxylamines, their oxidation products and markers.*

Compound	G.l.c. Rt values (min) Column A	Column B	T.l.c. *RF* values Solvent system A	B	C
I	5·7*a* (140°)	—	0·56	—	—
II	7·8*a* (100°)	—	0·45	—	—
III	—	10·2*a* (186°)	—	—	0·83
IV	4·2*a* (140°)	—	0·72	0·12	—
V	6·1*a* (100°)	—	0·66	0·13	—
VI	—	—	—	—	0·88
VII	—	9·8*a* (120°)	0·70	—	—
VIII	—	—	0·85	—	—
IX*b*	—	—	0·87	0·54	—
X*c*	—	—	8·82	0·41	—
Marker A	6·9 (140°)	13·0 (120°)			
Marker B	10·0 (100°)	—			
Marker C	—	4·0 (185°)			

a Trimethylsilyl derivative.
b 1 (2,6-Dimethylphenoxy)-2-nitrosopropane.
c 1-Phenyl-2-nitrosopropane.

to about 0·2 ml in a water bath (44°), the contents cooled by dipping the tube into an ice bath, the inside of the tube was washed down with a small volume (0·5 ml) of solvent and finally the solvent was removed under a stream of nitrogen at room temperature. The residue was dissolved in 50 μl of acetonitrile and 25 μl of BSTFA or TMSI was added; after 2–3 min, 2–3 μl of the resulting solution was injected onto the column and the remaining solution kept cold if further required.

(*c*) *Product analysis.* To 1 ml of the solution of the hydroxylamine (0·5 μmol) and 1 ml of the appropriate internal standard solution was added 4 ml of standard buffer of desired pH and the constituents and any other reagent under investigation (metal ions, solvents); after the appropriate treatment (storage or shaking for a certain length of time in the presence of 6 ml of enclosed air, oxygen, or nitrogen), the solution was extracted with ether and subsequently analysed as described above. (When the solution was to be stored under nitrogen or oxygen the hydroxylamine solution was added after all the other solutions and after the gas had been passed through the solution for 5–10 min). The amount of oxime formed and/or the amount of the hydroxylamine remaining was determined by g.l.c. by employing the peak-height ratio technique (Beckett & Rowland, 1965) and the percentage oxidation of the hydroxylamine was calculated. The column conditions and retention times of the various compounds are listed in Table 1.

Ultraviolet spectrometry

Measurement. Measurement of absorbance of III and VI was at 208 and 290 nm, respectively. ϵ for III was 18 000 and for VI was 18 750.

Calibration curve. To 1 ml of the solution of the hydroxylamine (III) or its nitrone (VI) (0·0125 to 0·125 μmol) in a glass centrifuge tube (volume = 12 ml) was added 5 ml of trace heavy metal-free phosphate or borate buffer solution and the absorbance recorded immediately.

Analysis of oxidation products. To 1 ml of a solution containing III (0·05 μmol) in a 12 ml centrifuge tube was added 5 ml of the buffer solution of desired pH and constituents and any other reagent (metal ions). After the appropriate treatment (storage for a certain length of time in presence of 6 ml of enclosed air, oxygen, or nitrogen), the appearance of nitrone (VI) was determined by measuring absorbance at 290 nm and the percentage of VI formed was calculated from its calibration curve. The amount of III remaining after any of the respective treatments above (and hence a measure of its oxidation) could not be determined by the ultraviolet method in the presence of VI and thus was analysed by g.l.c.

Thin layer chromatography (t.l.c.)

Glass plates (20 × 20 cm) were spread to a thickness of 0·25 mm with a mixture of silica gel G or F_{254} (Merck) and water (1:2). The plates were allowed to dry at room temperature and then activated by heating for 1 h at 110° before use. The solvent systems used were: A, chloroform–methanol (16:1); B, chloroform; and C, chloroform–ethanol (7:2). The various spots were visualized with ultraviolet light (254 nm), iodine vapour, or by spraying with Dragendorff's reagent, ammoniacal $AgNO_3$, $CuCl_2$, or 2,4-dinitrophenylhydrazine solutions. The identification of the compounds was confirmed by comparison of their R_F values with those of reference compounds.

RESULTS AND DISCUSSION

Identification and analysis of products

Good calibration curves were obtained for all the compounds I–VI and peak height ratios (g.l.c.) and absorbances (ultraviolet) were linearly proportional to the concentrations in the ranges, 0·1 to 1·25 μmol and 0·0125 to 0·125 μmol, respectively. Regression analyses of the replicate lines yielded correlation coefficients of not less than 0·99. The oximes IV, V, and VII were identified by both t.l.c. and g.l.c. analysis by comparison with authentic compounds (see Table 1). Although nitroso compounds were detected as oxidation products of I and II by t.l.c. analysis (IX and X, respectively), in agreement with Lindeke & others (1975), they were analysed as the corresponding oximes by g.l.c. Nitroso compounds isomerize to oximes in the g.l.c. column and subsequently form derivatives with the excess silylating reagent present in the injection solution (Beckett, Jones & Coutts, 1976). The oximes thus analysed accounted for more than 98% of the total oxidation products of I and II.

Of the oxidation products of the secondary hydroxylamine, III, 80–85% was present as the nitrone, VI, identified and measured by ultraviolet, 10–15% was the oxime, VII, identified by t.l.c. and g.l.c. and quantitated by g.l.c. Trace amounts of the ketone, VIII, were also detected by t.l.c. The nitrone (VI) and oxime (VII) always accounted for more than 95% of the oxidation products of III.

Extractability of the hydroxylamine and stability of their silyl derivatives

The hydroxylamines, I–III, were satisfactorily extracted from aqueous solutions (pH 7·4) by shaking

with three portions of either ether, benzene, or chloroform (g.l.c. evidence); with n-pentane it was necessary to saturate the aqueous phase with NaCl to obtain similar results. Ethyl acetate was not a satisfactory solvent as some breakdown (10–16%) occurred during extraction and subsequent removal of the solvent. The hydroxylamines, I and II, as well as the corresponding oximes, IV and V, each produced single peaks on g.l.c. accompanied by complete disappearance of the peaks of the parent compounds immediately after being mixed with BSTFA. However, for measurement of mixtures of III and the oxime, VII, derivatization was effected by TMSI, since BSTFA did not rapidly and completely react with III.

Effect of pH and duration of storage

The pH of the aqueous solution exhibited a marked effect on the stability of the hydroxylamines; whereas the hydroxylamines generally are stable in acidic conditions, the amount of decomposition of I–III increased rapidly as the pH was increased from neutrality to alkaline values (Fig. 2) as well as increasing steadily with duration of storage (Fig. 3). At a pH of 9–10 the decomposition approached the maximum within 30 min (Fig. 2). The degree of oxidation at any of the pH values investigated and for any duration of storage was in the order of I> II > III (Fig. 2), e.g., at pH 8·5 in phosphate buffer in presence of air, 32% of I was oxidized compared with 20% of II over 30 min.

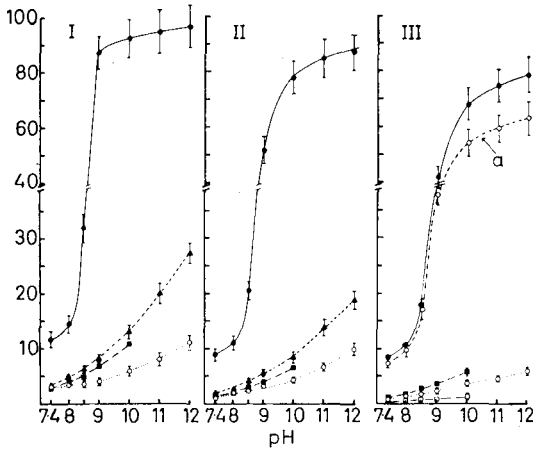

FIG. 2. The effect of pH on the oxidation of hydroxylamines, I, II and III at room temperature (22·7 ± 1·2°). Abscissa—% oxidation per 30 min. a—% nitrone formed. The conditions used and the corresponding symbols in the graphs are as follows: Storage of the respective hydroxylamines (0·5 μmol for g.l.c. analysis and or 0·05 μmol for ultraviolet analysis) in untreated buffer solution (4 or 5 ml) in the presence of enclosed air (6 ml) ●——●, or nitrogen (6 ml) ○ .... ○, and in trace heavy metal-free buffer solution (4 or 5 ml) in the presence of enclosed air (6 ml) ■ – – ■, or nitrogen (6 ml) □ – – □. Shaking of the hydroxylamines, I and II in untreated buffer solution (4 ml) with ether (4 ml) in the presence of enclosed air (2 ml) ▲- - - -▲. The percentage of oxidation of I and II was calculated on the basis of the percentage of oxime formed whereas that of III was calculated from the amount of III left after the prescribed treatment. The percentage of nitrone formed from III was measured by u.v. analysis (○– – –○). Each point in the graph is the mean of 3–4 determinations and the vertical bars represent scatter.

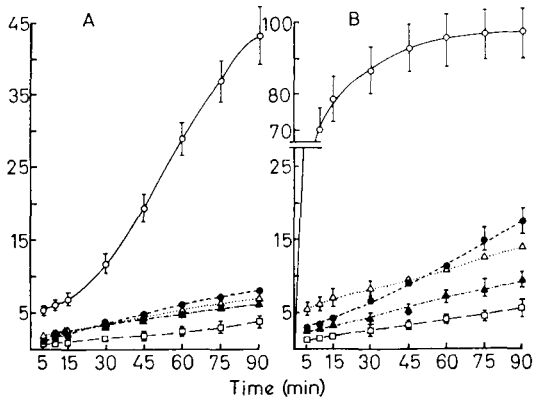

FIG. 3. The effect of the duration of storage on the oxidation of the hydroxylamine, I, at pH 7·4 (A) and at pH 9·0 (B) at room temperature (22·7 ± 1·2°). Abscissa—per cent oxidation. The conditions used and the corresponding symbols in the graph are as follows: Storage of I (0·5 μmol) in untreated buffer solution (4 ml) in the presence of enclosed air (6 ml), ○——○ or nitrogen (6 ml), ▲-----▲ and in trace heavy metal-free buffer solution (4 ml) in the presence of enclosed air (6 ml), ●-----●. Shaking of I in untreated buffer solution (4 ml) with ether (4 ml) in the presence of enclosed air (2 ml) △ .... △ or nitrogen (2 ml) □– –□. The percentage of oxidation was calculated on the basis of the percentage of oxime formed. Each point in the graph is the mean of 3–4 determinations and the vertical bars represent scatter.

The secondary hydroxylamine, III, but not the primary ones, I and II, was oxidized to a greater extent in phosphate than in borate buffer solutions at identical pH values over identical periods of time. There was a significant amount of oxidation of III in phosphate buffer even at pH 7·4 (7% in 30 min), whereas oxidation became detectable only above pH 8·0 in borate buffer solution; however, this result must be viewed with some caution since the ionic strength of the two buffers was not equivalent.

Effect of metal ions

The removal of trace heavy metal ions from the aqueous solutions dramatically reduced the amount

of oxidation of the dissolved hydroxylamines; e.g., at pH 7·4 oxidized products were either not detected or detected only at the levels of 1–3% after 30 min and at more alkaline pH values only minor breakdown <5%) occurred (Fig. 2). Dissolution of the hydroxylamines in Buffer Solution A produced similar amounts of oxidation as occurred under identical conditions with untreated buffer solutions of the same pH and constituents, e.g., after 30 min in phosphate buffer (pH 7·4), 12% of I was oxidized in untreated buffer solution as compared to 15% in Buffer Solution A.

Since it appeared that the trace heavy metal ions were serving as catalysts in the oxidation, as suggested for Cu^{2+} and Mn^{2+} by Johnson & others (1956) and for Cu^{2+} by Lindeke & others (1975), the efficiency of various metal ions in increasing the amount of oxidation of I and III was evaluated. Varying proportions of metal ions to a fixed quantity of I or III were plotted against the percentage oxidation over a 30 min time period. A large variation in the effectiveness of different metal ions as catalysts was observed. Rapid increases in the amount of oxidation were observed at the smaller ratios of Cu^{++} or Mn^{++} to I or III and when the ratio reached the range of 1:50 to 1:100 the increases in amounts of oxidation became progressively less marked (Fig. 4).

Identical levels of oxidation were obtained irrespective of whether cupric or cuprous ions were used which was not unexpected since cuprous ions are rapidly oxidized to cupric ions in aqueous solutions. Manganese, lead, nickel and ferric ions were respectively less effective than copper ions in the order as listed, while magnesium, zinc, silver and ferrous ions produced only minor increases in the levels of oxidation (Fig. 4).

Trace heavy metal-free buffer solutions, stored for one month either in glass or plastic containers were not significantly different in behaviour on the dissolved hydroxylamines than freshly prepared ones. The level of oxidation of I and III in contact for 30 min in the presence of air with freshly prepared or one month old trace heavy metal-free buffer solutions stored as above varied between 2–3%. Under identical conditions, slight increases (4–7% instead of the normal 2–3%) in the amount of oxidation of I and III occurred with buffer solutions stored for longer than one month. Buffer solutions stored in plastic containers consistantly gave slightly higher levels of oxidation of I and III than buffer solutions stored in glass containers over a range of ten analyses, although these differences fell within the margin of error.

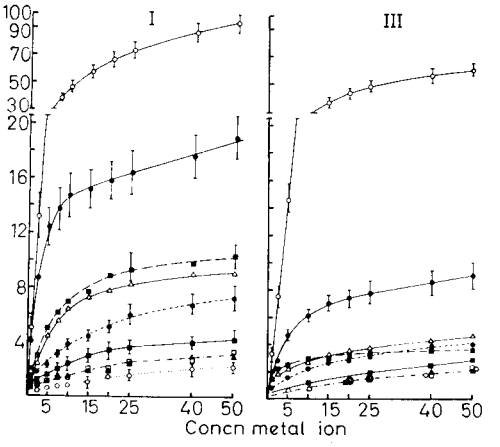

FIG. 4. The effect of various added metal ions (μg atom $\times 10^3$ per μmol hydroxylamine) on the oxidation of I and III in trace heavy metal-free phosphate buffer solution (pH 7·4) at room temperature (22·7 ± 1·2°). Abscissa—% oxidation per 30 min. The conditions used and the corresponding symbols in the graph are as follows: Storage of I and III (0·5 μmol) in the presence of enclosed air (6 ml) in trace heavy metal-free buffer solution (4 ml) containing Cu^{2+}, ○——○; Mn^{2+}, ●——●; Pb^{2+}, ■——■; Ni^{2+}, △——△; Fe^{3+}, ● - - ●; Mg^{2+}, ■——■; Zn^{2+}, □ · - · □; Fe^{2+}, ▲ · — · ▲ and Ag^{+}, ○ · · · · · · ○. The percentage of oxidation of I was calculated on the basis of the percentage of oxime formed and that of III was calculated from the amount of III left after the prescribed treatment. Each point in the graph is the mean of 3–4 determinations and the vertical bars represent scatter.

Effect of oxygen, light, and solvent

The oxidation of the hydroxylamines dissolved in untreated buffer solutions was much diminished when dissolved oxygen was removed by purging the solutions with nitrogen, the hydroxylamine added, and the resulting solution stored under an atmosphere of nitrogen (Fig. 2). This is in agreement with results reported by other workers for different hydroxylamines under similar conditions (Manson, 1974). As expected, shaking a solution of III in phosphate buffer (pH 7·4) in a stoppered centrifuge tube with the enclosed air for 30 min, as opposed to standing, increased the amount of oxidation from 8 to 11%. The role of oxygen was further demonstrated by the fact that the level of oxidation of I increased from 12 to 17% in untreated phosphate buffer (pH 7·4) and from 3 to 7% in trace heavy metal-free buffer, respectively, when the solutions were stored for 30 min under an atmosphere of oxygen. The corresponding figures for III under similar conditions were increases from 7 to 13% and from about 1 to 5%, respectively. Although the oxidation was shown to require aerial oxygen, it

appeared however, not to proceed via formation of singlet oxygen since addition of β-carotene, a powerful quencher of singlet oxygen (Foote & Denny, 1968), caused only small decreases in the level of oxidation of I; e.g., the level of oxidation during 30 min decreased from 11·7 to 10% in phosphate buffer (pH 7·4) and from 73 to 67% if 0·025 μg-atom of Cu^{2+} was present per μmol of I when a three-fold excess of β-carotene to I was added to the solutions.

The oxidation was independent of light exposure since the level of oxidation of I was 11·7% in the presence of light and 12% in the dark when the respective phosphate buffered solutions (pH 7·4) were stored for 30 min in the presence of air.

As expected, shaking the aqueous and buffer solutions containing the hydroxylamines with organic solvents such as ether, benzene or chloroform greatly diminished the levels of oxidation since the hydroxylamines were partitioned into the organic phase and protected, to some extent, from the aqueous environment containing the heavy metal ion catalysts and dissolved oxygen.

Effect of macromolecules, chelating agents and cofactors

Bovine serum albumin (BSA), used in three fold excess of the hydroxylamines, appreciably decreased the amounts of oxidation even in the presence of added cupric ions (Fig. 5). Stabilization of certain hydroxylamines by biological constituents was also reported by Ziegler, McKee & Paulsen (1973) and Gal & others (1975). Chelating agents such as EDTA, also in three-fold excess, completely negated the catalytic effect of cupric ions on the oxidation (Fig. 5); benzoin-α-oxime also reduced the level of oxidation, but not to the same extent as EDTA at a similar concentration (Fig. 5). The reduction in oxidation caused by ascorbic acid (Fig. 5), was possibly related to chelation of the cupric ions in the solution since cupric ions have long been known to catalyse the aerial oxidation of aqueous solutions of ascorbic acid (Weissburger & LuValle, 1944; Hayakawa, Minami & Nakamura, 1973); the concentration of ascorbic acid necessary to eliminate the catalytic effect of cupric ions on the oxidation of I was nearly ten times the molar concentration of Cu^{2+}, implicating a competitive uptake of oxygen and binding of the Cu^{2+} catalyst by ascorbic acid.

Of the different cofactors such as FMN, FAD, NAD, NADP and the reduced forms of NAD and NADP, only FMN caused an increase (about 20%) in the amount of oxidation of I when stored for 30 min in untreated phosphate buffer solution (pH 7·4) in contact with air (Fig. 5). The mechanism by which this increase was manifested is not known.

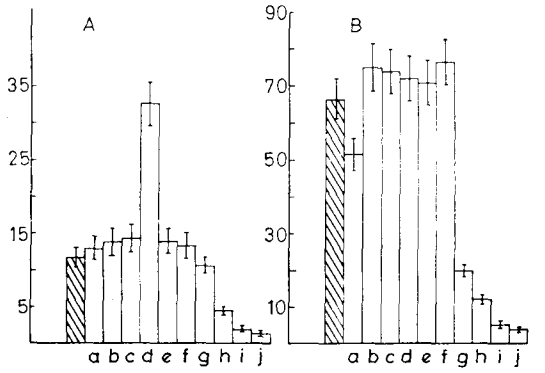

FIG. 5. The effect on oxidation of various added cofactors and other agents during storage of I in phosphate buffer (pH 7·4) at room temperature (22·7 ± 1·2°). Abscissa—% oxidation per 30 min. A—Storage of I (0·5 μmol) in untreated buffer solution (4 ml) in the presence of enclosed air (6 ml). B—Storage of I (0·5 μmol) in trace heavy metal-free buffer solution (4 ml) containing 0·0125 μg-atom of Cu^{2+} in the presence of enclosed air (6 ml). Concentration of each cofactor and other agents was 1–2 μmol. The percentage of oxidation was calculated on the basis of the percentage of oxime formed. Each column is the mean of 3–4 determinations and the vertical bars represent scatter. Control-hatched columns. a—NAD, b—FAD, c—NADP, d—FMN, e—NADH, f—NADPH, g—BSA, h—benzoin-α-oxime, i—ascorbic acid, j—EDTA.

CONCLUSIONS

This investigation confirms that primary and secondary hydroxylamines such as I, II and III are readily oxidized in nonacidic buffered or non-buffered aqueous solutions by aerial oxygen. In accord with other studies (Johnson & others, 1956, Beckett & Al-Sarraj, 1973; Cho & others, 1974; Lindeke & others, 1975), the oxidation was pH dependent, time dependent and to a lesser extent dependent on the buffer species; the hydroxylamines, I, II and III, were stable at acidic pH, were oxidized to the extent of 7–12% at neutral pH, and the levels of oxidation increased rapidly with increasing alkalinity up to pH 9–10 when it approached the maximum. Additionally, the levels of oxidation of I, II and III were greatly enhanced by the presence of trace heavy metal ions, particularly Cu^{2+}, which serve as catalysts in the reaction. In the absence of metal ions the levels of oxidation were markedly decreased, even in alkaline solutions up to pH 10.

Previous investigations (Johnson & others, 1956; Lindeke & others, 1975) of the effect of metal ions

on the oxidation of hydroxylamines in aqueous solutions have concluded that Cu^{2+} and Mn^{2+} act as catalysts to the reaction and have reported descriptions of the kinetics. While it was not our purpose to evaluate the kinetics of the oxidation reaction, previous workers appear not to have taken into account or attempted to remove the metal impurities in the reaction media. The effect of trace metal impurities will vary in magnitude depending on the pH, the concentration of hydroxylamine, the reaction time, the buffer used and the amounts and species of metal impurities present.

The major oxidation products of the primary hydroxylamines, I and II, were probably the nitroso compounds initially, which subsequently isomerized in solution and under conditions of analysis to the oximes, IV and V. Oxidation of the secondary hydroxylamine, III, yielded the nitrone, VI, as the major product.

On the basis of our findings, we recommend that, whenever possible, investigations of unstable alkylhydroxylamines be conducted in trace heavy metal-free aqueous or buffer solutions, preferably in the absence of air.

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