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Autoxidation of *N*-hydroxyphenylalkylamines: the inhibitory effect of some anions on copper catalysed autoxidation of *N*-hydroxyphentermine*

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The inhibitory effect of certain electrolytes and buffers on the copper catalysed autoxidation of *N*-hydroxyphentermine (2-hydroxylamino-2-methyl-1-phenylpropane) has been investigated. The presence of ions such as SO_4^{2-}, Cl^- or Br^- markedly reduced the rate of oxidation. Phosphate and carbonate buffers had a similar effect with halides and phosphate buffers being the most inhibitory. The occurrence of 2-methyl-2-nitro-1-phenylpropane and 2-methyl-1-phenylpropene-(1) as secondary oxidation products was also established.

The occurrence of *N*-hydroxy compounds as metabolites of primary and secondary aliphatic amines is well known (Gorrod, 1973; Lindeke, Cho & others, 1973; Beckett & Bélanger, 1974; Caldwell, Köster & others, 1975). Aliphatic hydroxylamines are often unstable and are further metabolized or undergo chemical conversion. Evaluation of the kinetics of hydroxylamine formation in biological systems might therefore be complicated by the instability of the products.

The reaction of aliphatic hydroxylamines with oxidizing agents has been thoroughly investigated but comparatively little attention has been paid to the mechanism and factors that influence autoxidation. It has been shown (Johnson, Rogers & Trappe, 1956; Moews & Audrieth 1959; Anderson, 1964; Yagil & Anbar, 1964; Hughes & Nicklin, 1971; Hughes, Nicklin & Shrimanker, 1971; Lunak & Veprek-Siska, 1974) that (a) aliphatic hydroxylamines are readily autoxidized in alkaline and neutral solution, (b) trace quantities of transition state metal ions, especially Cu(II), catalyse the reaction and (c) the reaction is arrested by exclusion of oxygen, and almost stopped by metal sequestering agents.

We previously presented some preliminary kinetic results from studies on the copper catalysed autoxidation of *N*-hydroxyphentermine (Lindeke, Anderson & others, 1975) and showed the drug to be easily oxidized by air, especially in the presence of small amounts of Cu(II), forming primarily the nitroso mono- and dimer. The concentration and type of buffer greatly influenced the rate of oxidation. To avoid such influences a pH-stat method was developed in which constant ionic strength was maintained primarily with sodium chloride. *N*-Hydroxyphentermine was, however, very stable in this medium, i.e. chloride ions also influence the rate of oxidation.

We then investigated the effect of chloride and some commonly used buffers on the reaction rate, and this report describes the inhibitory effect of certain anions on the copper catalysed autoxidation.

MATERIALS AND METHODS

Instrumentation

The pH was kept constant with a pH stat (Radiometer SBR 3 Titrigraph, TTT 2b Titrator and a Methrom combined glass-calomel microelectrode). The reference electrolyte in the electrode was changed from KCl (3 M) to $NaClO_4$ (0·4 M) + NaCl (0·1 M), thereby preventing the formation of solid $KClO_4$ at the diaphragm of the electrode and also diminishing leakage of chloride ions into the solution. A thermostated glass reaction vessel, volume 30 ml, equipped with the microelectrode, a port for adding sodium hydroxide from the pH-stat, and a port for adding solution, was used. The solution in the vessel was stirred magnetically and was pumped through Teflon tubes into a flow cell in the photometer. The total volume of the reaction mixture was 20·0 ml of which only 1·2 ml was outside the reaction vessel at any time. The ultraviolet absorbance was measured.

Mass spectra were recorded on a LKB 9000 mass spectrometer using the direct probe technique. When extracts containing volatile products in low concentrations were analysed the sample was adsorbed

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on silica gel in the sample holder and then introduced into the ion source. The ionizing potentials were 70 and 12 eV, the trap current 60μA and the accelerating voltage 3·5 kV. The temperature of the ion source was at 270°.

T.l.c. plates were prepared from Merck's silica gel 60 PF spread on glass plates to a thickness of 0·25 mm. The plates were developed in chloroform (system I) or chloroform–light petroleum (1:1) (system II).

Materials

2-Hydroxylamino-2-methyl-1-phenylpropane, 2-methyl-2-nitroso-1-phenylpropane and 2-methyl-2-nitro-1-phenylpropane were synthesized as previously described (Lindeke & others, 1973, 1975). All other chemicals were obtained from commercial sources and were of analytical grade. The water used was distilled twice in quartz equipment.

Identification of oxidation products

To ensure that the formation of secondary oxidation products (Lindeke & others, 1975) did not interfere with the primary oxidation process, aliquots of the solutions from the reaction mixture were analysed by t.l.c. and ms analysis. The aliquots were extracted twice with an equal volume of CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4), concentrated under nitrogen, spotted on t.l.c. plates, and the spots were located by ultraviolet radiation and subsequently extracted with CH_2Cl_2 and the extracts analysed by m.s.

Determination of acidity constants

The pKa and pKw values reported are stoichiometric values, determined in a medium with an ionic strength of 0·5 ($NaClO_4$) containing 29% (v/v) ethanol (Lindeke & others, 1975). The pKw was 14·17 and the $[H^+]$ and $[OH^-]$ values were calculated using: $\log [H^+] = 0{\cdot}02 - pH$ and $\log [OH^-] = pH - 14{\cdot}19$. The pKa value for *N*-hydroxyphentermine was 5·87 (EtOH 29% (v/v), $\mu = 0{\cdot}5$ ($NaClO_4$)).

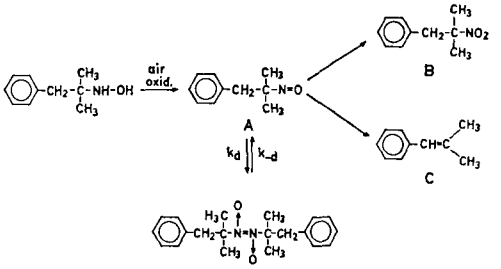
Kinetic studies

The medium contained 29% (v/v) ethanol and unless otherwise stated, $CuCl_2$ to a total concentration of 8×10^{-6} M. The substrate concentration was 3×10^{-4} M in all experiments.

The *N*-hydroxyphentermine HCl solution was added to the ethanol (29% v/v) containing the appropriate anion and sufficient sodium perchlorate to keep the ionic strength at 0·1 and 0·5 respectively. After thermal equilibrium (25·0 ± 0·1°) had been attained, the pH-stat was started and sodium hydroxide added to the solution until the desired pH was obtained. The reaction was then started by the addition of 1·6 ml of a stock solution of $CuCl_2$ (1×10^{-4} M) and 30 s after mixing the pump was started and the absorbance at 236 nm recorded. When the infinite absorption value was reached a spectrum in the range 330–220 nm was recorded as a control. Blanks were obtained by measuring the absorbance at 236 nm in solutions containing no $CuCl_2$. By plotting $\log (A_\infty - A)$ *vs* time (where A denotes the absorption at 236 nm) the pseudo first order rate constant was calculated.

RESULTS

Autoxidation of *N*-hydroxyphentermine gives the nitroso compound (A) (Lindeke & others, 1975) as the primary oxidation product. Two secondary oxidation products (B) and (C), identified as 2-methyl-2-nitro-1-phenylpropane and 2-methyl-1-phenylpropene-(1) respectively, are also formed (Scheme 1). For t.l.c. and m.s. characteristics see Table 1 and Lindeke & others (1975). The reactions leading to the formation of (B) and (C) are slow as



Scheme 1

Table 1. *T.l. c. and m.s.-characteristics of oxidation products A-C.*

	R_F value		*m/e* % (70 eV)						
	System I	System II	91	115	116	117	131	132	133
Product A	0·87	0·57	100	8	2	9	2	6	37
2-Methyl-2-nitroso-1-phenyl propane	0·86	0·59	100	10	3	12	4	11	41
Product B	0·85	0·73	100	6	3	13	2	34	29
2-Methyl-2-nitro-1-phenyl propane	0·84	0·70	100	7	3	14	2	35	30
Product C	0·87	0·89	39	33	11	100	18	65	11
2-Methyl-1-phenyl-propene-(1)	0·88	0·89	36	35	13	100	21	72	10

compared to the formation of (A) and these secondary oxidation products were not detected during the early phase of the autoxidation.

In a medium of 29% ethanol in 0·1 M $NaClO_4$ the formation of the nitroso monomer showed good first order kinetics in substrate for at least two half lives. The rate is sensitive to pH and the presence of catalytically active Cu(II) species (Table 2). The blank data show that metal ions, possibly present as impurities in the buffer solutions, cause no significant increase in the reaction rate at Cu(II) concentrations of 8×10^{-6} M and above. The experimental error of the rate constants is within ± 10%.

Table 2. *The effect of pH and Cu(II)-concentration on the oxidation rate constant (min^{-1}).*

	Cu(II) × 10^5 M			
pH	Blank	0·8	1·6	3·2
6·0	~ 1×10^{-3}	$4{\cdot}2 \times 10^{-2}$	$8{\cdot}4 \times 10^{-2}$	$1{\cdot}4 \times 10^{-1}$
6·6	~ 1×10^{-3}	$1{\cdot}9 \times 10^{-1}$	—	—
7·3	~ 1×10^{-3}	$3{\cdot}3 \times 10^{-1}$	—	—

The effect of electrolytes

The effect of ClO_4^-, SO_4^{2-}, Cl^- and Br^- ions on the oxidation rate was studied at pH 6·0 and 6·6. The results are summarized in Table 3. Compared with the ClO_4^- ion, SO_4^{2-}, Cl^- or Br^- ions markedly reduced the oxidation rate, the halides being the most inhibitory. The effect of different chloride concentrations on the *initial* reaction rate is shown in Fig. 1. This shows that the rate was reduced ~50% at chloride concentrations of 5×10^{-3} M. Also, with increasing chloride concentrations, the rate of oxidation changed gradually from pseudo first order kinetics towards zero order kinetics.

Table 3. *The effect of different electrolytes on the oxidation rate constant at pH 6·0 and 6·6 (μ = 0·1).*

		$K_{obs} \times 10^3$ min^{-1}	
Electrolyte	Concn M	pH = 6·0	pH = 6·6
$NaClO_4$	0·1	42	190
Na_2SO_4	0·033	18	72
NaCl	0·1	~2	~2
NaBr	0·1	~1	~1

The effect of buffers

The effect of different concentrations of phosphate and carbonate buffers, at an ionic strength of 0·5, on reaction rate was studied at pH 7·3 and 9·0 (Table 4). These data give curves similar to that for chloride (Fig. 1). Such curves show that the reaction rate is more sensitive to small amounts of phosphate than to chloride or carbonate species. Only 4×10^{-5} M of phosphate is needed to reduce the rate by 50% as compared with 5×10^{-3} M for chloride and 2×10^{-2} M for hydrogen carbonate. In contrast to chloride, no deviation from pseudo first order kinetics was found when the buffer concentrations were increased.

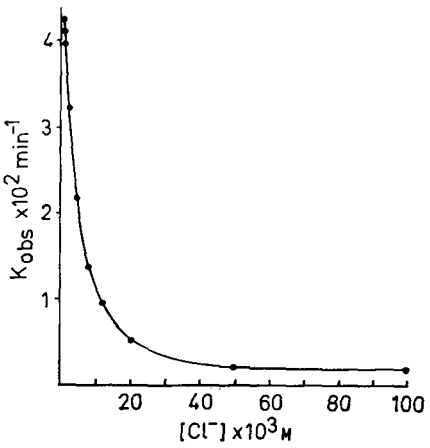


FIG. 1. The effect of chloride ions on the oxidation rate constant at pH 6·0. Every experimental point represents the initial rate at a given chloride concentration.

DISCUSSION

The results show that the autoxidation of *N*-hydroxyphentermine is very sensitive to the composition of the aqueous medium. The presence of heavy metal ions, *e.g.* Cu(II) ions, is needed for the promotion of autoxidation. Not only the presence of metal sequestering agents (Johnson & others, 1956) but any factor affecting the availability of active metal ions will have a great influence on the reaction rate.

Halides can affect copper-catalysed autoxidations, e.g. ascorbic acid (De Caro & Giani, 1934; Kellie & Zilva, 1935; Mapson, 1941, 1945; Ogata & Kosugi, 1969; Jameson & Blackburn, 1976). Mapson showed that the catalytic activity of copper was augmented by small concentrations of halide ions but diminished by higher concentrations. He stated that the inhibitory effect of chloride ions was probably due to complex formation between Cu^+ and Cl^-, which inhibited the reoxidation of Cu^+ by O_2.

Eberson & Persson (1962) reported an effect of chloride ions on the autoxidation of β-phenylisopropylhydrazine (the hydrazine analogue of *N*-

Table 4. *The effect of chloride, phosphate and hydrogen carbonate on the oxidation rate constant at different pH-values.*

pH = 6·6 (μ = 0·1)		pH = 7·3 (μ = 0·5)		pH = 9·0 (μ = 0·5)	
$(Cl^-) \times 10^3$M	K_{obs} $\times 10^3$ min^{-1}	$(HPO_4^{2-}, H_2PO_4^-) 10^5$M	K_{obs} $\times 10^2$ min^{-1}	$(HCO_3^-) \times 10^3$M	K_{obs} $\times 10^1$ min^{-1}
0·3*	190	2·9	21·0	5	18·0
1·3	160	5·8	14·2	25	7·9
2·3	127	11·5	10·1	50	5·2
4·3	50	57·5	4·8	100	2·6
8·3	23	115	3·3	150	1·9
12·3	19	23000	3	—	—
20·3	10	—	—	—	—
50·3	3·6	—	—	—	—
100·3	~2	—	—	—	—

* The Cl^-concentration 0·3 $\times 10^{-3}$ M originates from *N*-hydroxyphentermine-HCl.

hydroxyamphetamine). When halides were substituted for perchlorate the rate of oxidation increased drastically. They believed the effect was due to an alteration in the oxidation-reduction potential of the Cu^{2+}/Cu^+ system. Buffers can also influence copper-catalysed autoxidations (Butt & Hallaway, 1961; Schulert, 1961). The inhibitory effect of the halides on the autoxidation of *N*-hydroxyphentermine can probably be explained in terms of complex formation between Cu^+ and the halide ion, while the effect of the buffers could be due to interference with Cu^{2+} (Fig. 2).

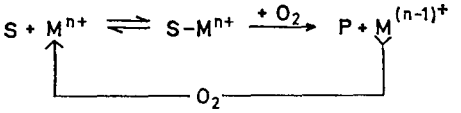


FIG. 2. General scheme for a metal-catalysed autoxidation. S = substrate, M = metal and P = product(s). The rate determinating step is the oxidation of $S-M^{n+}$.

Factors effecting the retardation of a metal-catalysed autoxidation can be summarized as follows:

1. M^{n+} can form a complex with buffer species. 2. If the formation of a $S-M^{n+}$ complex is necessary for the exchange of electrons a change of ligands to this complex (e.g. ClO_4^- to Cl^-) can affect this exchange. 3. $M^{(n-1)+}$ can form a complex with halides. 4. The product(s) can form a complex with M^{n+} or $M^{(n-1)+}$.

That the rate of oxidation deviates from first order kinetics with increasing chloride concentrations, but not with increasing buffer concentrations, is not inconsistent with such mechanisms.

That autoxidation of *N*-hydroxyphenylalkylamines can modify the interpretation of the results of metabolic studies on the corresponding amines has been known for some time (Lindeke & others, 1973; Beckett & Al-Sarraj, 1973; Beckett & Mida, 1974; Gal, Gruenke & Castagnoli, 1975). The influence of various electrolytes and buffers on the stability of the hydroxylamines accentuates further the uncertainties associated with the quantitative determination of these compounds.

The present study also shows that when the stability of hydroxylamines are evaluated in different biological preparations, not only the protective properties of the biological constituents *per se* (Kellie & Zilva, 1935; Gal, Gruenke & Castagnoli, 1975; Sternson, 1975) should be taken into consideration, but attention must also be paid to the effects caused by such agents as buffer species and other anions present in the solutions.

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REFERENCES

ANDERSON, J. H. (1964). *Analyst*, **89**, 357–362.
BECKETT, A. H. & AL-SARRAJ (1973). *J. Pharm. Pharmac.*, **25**, 328–334.
BECKETT, A. H. & BÉLANGER, P. M. (1974). *Ibid.*, **26**, 205–206.
BECKETT, A. H. & MIDA, K. K. (1974). *Xenobiotica*, **4**, 279–311.

BUTT, V. S. HALLOWAY, M. (1961). *Arch. Biochem. Biophys.*, **92**, 24–32.
CALDWELL, J., KÖSTER, U., SMITH, R. L. & WILLIAMS, R. T. (1975). *Biochem., Pharmac.*, **24**, 2225–2232.
DE CARO, L. & GIANI, M. (1934). *Z. Physiol. Chem.*, **228**, 13–24.
EBERSON, L. E. & PERSSON, K. (1962). *J. medl pharm. Chem.*, **5**, 738–752.
GAL, J., GRUENKE, L. D. & CASTAGNOLI Jr., N. (1975). *J. medl Chem.*, **18**, 683–688.
GORROD, J. W. (1973). *Chem.-Biol. Interactions*, **7**, 289–303.
HUGHES, M. N., NICKLIN, H. G. (1971). *J. chem. Soc., A.* 164–168.
HUGHES, M. N., NICKLIN, H. G. & SHRIMANKER, K. (1971). *Ibid.*, 3485–3487.
JAMESON, R. F. & BLACKBURN, N. J. (1976). *J. chem. Soc. Dalton*, 534–541.
JOHNSON, D. H., ROGERS, M. A. T. & TRAPPE, G. (1956). *J. chem. Soc.*, 1093–1103.
KELLIE, A. E. & ZILVA, S. S. (1935). *Biochem. J.*, **29**, 1028–1035.
LINDEKE, B., CHO, A. K., THOMAS, T. L. & MICHELSON, L. (1973). *Acta pharm. suecica*, **10**, 493–506.
LINDEKE, B., ANDERSON, E., LUNDKVIST, G., JONSSON, U. & ERIKSSON, S. O. (1975). *Ibid.*, **12**, 183–198.
LUNAK, S. & VEPREK-SISKA, J. (1974). *Colln. Czech. chem. Commun.*, **39**, 391–395.
MAPSON, L. W. (1941). *Biochem. J.*, **35**, 1332–1353.
MAPSON, L. W. (1945). *Ibid.*, **39**, 228–236.
MOEWS, Jr., P. C. & AUDRIETH, L. F. (1959). *J. inorg. nucl. Chem.*, **11**, 242–246.
OGATA, Y. & KOSUGI, Y. (1969). *Tetrahedron*, **25**, 4633–4641.
SCHULERT, A. R. (1961). *Archs int. Pharmacodyn. Thér.*, **132**, 1–15.
STERNSON, L. A. (1975). *Experientia*, **31**, 268–270.
YAGIL, G. & ANBAR, M. (1964). *J. inorg. nucl. Chem.*, **26**, 453–460.