The Choice of a Chelating Agent for Inactivating Trace Metals

2. DERIVATIVES OF OXINE (8-HYDROXYQUINOLINE)

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Oxine has been shown to retain its avidity for a number of biologically interesting metallic ions at pH 7 and 37° (Albert & Gledhill, 1947) and to possess a much more powerful antibacterial activity than other commercial chelating agents (Albert, Rubbo, Goldacre & Balfour, 1947). Hence it seemed desirable to investigate the isomerides and derivatives of oxine in order to correlate chelating activity, under physiological conditions of pH and temperature, with molecular structures. It seemed that such an elementary study might pave the way for the discovery of more delicate or selective reagents for depriving cells of essential trace metals. In addition, it was hoped to shed some light on the antibacterial action of oxine, which has been used as an antiseptic since 1895, mainly in the form of 'Chinosol' (Potassii Hydroxyquinolini Sulphas, B.P.C.), an acidic powder containing 50% oxine.

Forty-five analogues and derivatives of oxine have accordingly been investigated, with the result that the ability to chelate with a number of metals at pH 7 and 37° has been found to be widely distributed throughout the series. Certain of the derivatives gave visible evidence of activity at greater dilution than does oxine, but no outstanding instance of specificity was found. Some fundamental correlations between chemical constitution and chelating power and antibacterial activity have been brought to light.

METHOD AND MATERIALS

The tests were carried out exactly as in Part I (Albert & Gledhill, 1947). The derivatives and analogues of oxine were synthesized by the methods cited by references in Tables 1–3. The following details concern improved syntheses and new compounds.

4-Hydroxyquinoline. Aniline (10 ml.), aniline hydrochloride (0·1 g.) and freshly distilled ethyl oxalacetate (20 ml.) were mixed in a wide dish and kept *in vacuo* over $CaCl_2$ for 2 days. The product was diluted with ether (100 ml.) and extracted with 0·2 N-HCl (two lots of 100 ml.), then with 0·2 N-NaOH (three lots of 100 ml.); then with water (two lots of 50 ml.). The upper layer was dried over Na_2SO_4, then over K_2CO_3, filtered and completely freed from ether by vacuum distillation. The orange residue of diethyl anilinofumarate (20 g.) was poured into mechanically stirred medicinal paraffin (300 ml.) held at 250–255° (internal temp.) during 3 min., the flask being rinsed with a little light petroleum (b.p. 90–120°). The mixture was stirred for 10 min. more at 250–260° giving a golden liquid with white crusts. Light petroleum (60 ml.; b.p. 90–120°) was stirred in after cooling and the white crystals of ethyl-4-hydroxyquinoline-2-carboxylate were filtered off giving 13 g. (m.p. 210°) after washing with light petroleum.

This ester was heated with 2·5 N-NaOH (200 ml.) at 97° for 2 hr. and the clear solution was acidified with HCl while hot, then cooled and filtered. This gave 11 g. of the acid as small, cream-coloured crystals, m.p. 280° (placed in bath at 260°). This acid and 0·01 g. of copper bronze were added to mechanically stirred paraffin at 260–265° (100 ml.) as quickly as effervescence permitted and stirred for 5 min. more. The mixture was cooled to 100° and light petroleum (25 ml.) added. After cooling to room temp., the mixture was filtered and the white 4-hydroxyquinoline (7·7 g.; m.p. 197°; 50% yield) washed with light petroleum and recrystallized from water (about 25 ml.) giving white crystals, m.p. 200°, not depressed by admixture with 4-hydroxyquinoline prepared by older procedures.

5-Propyloxine. *p*-Propylphenol, obtained in 80% yield by a Clemmensen reduction of *p*-hydroxypropiophenone, was nitrated according to the directions of Baranger (1931), the reaction being initiated by the addition of a crystal of $NaNO_2$. The 2-nitro-4-propylphenol (65% yield) was hydrogenated, using Raney nickel, in ethanol (10 parts) giving 2-amino-4-propylphenol, which was purified by recrystallizing the hydrochloride from HCl (yield of base: 60%, m.p. 139°). Nitrobenzenesulphonic acid mixture (3 g. of nitrobenzene and 13 g. of 17% oleum, heated at 100° until a drop gave only a faint turbidity on dilution) was added to 2 g. of 2-amino-4-propylphenol, followed by water (7·5 ml.) and glycerol (4 ml.). The mixture was refluxed for 8 hr. with continuous stirring, the internal temperature being adjusted to 135±5° by evaporation (or addition) of water. The mixture was diluted with water and fractionally precipitated with ammonia, rejecting the tars first formed. Eventually a pale solid (2 g.) was obtained; this was dissolved in N-HCl (15 ml.), boiled with charcoal, and the filtrate precipitated with an equal volume of 10 N-HCl. This gave 1·12 g. of 5-propyloxine hydrochloride, m.p. 245–246°; the liberated base formed white crystals, m.p. 60–61°. The only previous synthesis involved the action of propionyl chloride on oxine followed by selective reduction of the keto group: the yield was very poor (Rosenmund & Karst, 1941).

7-Chlorooxine. Previous preparations (Claus & Giwartowsky, 1896; Howitz & Witte, 1905) gave products of m.p. 145 and 146°, lower than that now recorded (156°). 2-Chloro-6-nitrophenol ,m.p. 70–71° (10 g.) (Barton & Linnell, 1945), was hydrogenated in ethanol (125 ml.) at

atmospheric temperature and pressure over Raney nickel. The solution was filtered and taken to dryness under CO_2. The crude product was continuously extracted (Soxhlet) with light petroleum which deposited buff crystals of a new compound, *2-chloro-6-aminophenol* in 80% yield, m.p. 80°. Recrystallization from light petroleum (b.p. 30–60°) gave white crystals, m.p. 81°, which were much more soluble in hot water than in cold and soluble in acetone, ethanol and benzene without temperature gradient. (Found: C, 50·1; H, 4·2; N, 9·7. C_6H_6ONCl requires C, 50·2; H, 4·2; N, 9·8%.)

2-Amino-6-chlorophenol (3·5 g.), nitrobenzenesulphonic acid mixture (27 g.), water (14 ml.) and glycerol (7·5 ml.) were heated for 7 hr. as described for 5-propyloxine. The mixture was cooled, diluted and fractionally precipitated with ammonia. The 7-chlorooxine (3·6 g.; 80% yield, m.p. 150°) was purified from benzene until the m.p. (156°, i.e. 158° corr.) remained constant. The other properties agreed with those given by the early workers. (Found: C, 60·0; H, 3·4; N, 7·75. Calc. for C_9H_6ONCl: C, 60·2; H, 3·4; N, 7·8%.)

4-Aminooxine. 4-Hydroxyoxine (3 g.) was refluxed with phosphorus oxychloride (70 ml.) for 2 hr. and the excess reagent was distilled off *in vacuo*. The residue, added to a mixture of ammonia and ice, gave a yellow solid which was filtered off and dried *in vacuo*, giving 3 g. of crude 4-chlorooxine, m.p. 235–240°. This was heated with phenol (30 g.) for 2 hr. at 160° while a stream of NH_3 was bubbled through. Conc. HCl was then added and the phenol removed by steam distillation. The residual solution was concentrated until crystals of 4-aminooxine hydrochloride were deposited (2·2 g.; m.p. 344°). These were dissolved in N-NaOH, treated with charcoal, filtered and the filtrate acidified with acetic acid. After removing a dark impurity the filtrate was concentrated to a small bulk and cautiously treated with $NaHCO_3$ until precipitation was complete. *4-Aminooxine* was obtained (35% yield) as creamy-white needles from water, m.p. 208°. It is moderately soluble in water to a pale yellow solution and gives no marked colour changes with acid or alkali. (Found: C, 67·5; H, 5·1; N, 17·4. $C_9H_8ON_2$ requires C, 67·4; H, 5·0; N, 17·5%.) This substance does not diazotize in aqueous solution and hence the $-NH_2$ group is in the 4- and not in the 8-position.

5-Nitrosooxine. A solution of $NaNO_2$ (12·5 g.) in water (45 ml.) was slowly added to a well cooled, and mechanically stirred, solution of oxine (24 g.) in 10N-HCl (45 ml.). The mixture was allowed to stand overnight, diluted with water (50 ml.) and filtered. The precipitate of 5-nitrosooxine hydrochloride was washed with ice water and dried *in vacuo* (yield, 75%). The free base was obtained by dissolving the salt in N-Na_2CO_3 and adding acetic acid at 60° until precipitation was complete. The crystals were filtered off and dried *in vacuo*. 5-Nitrosooxine decomposes about 235° and has no definite m.p. Hence this batch was recrystallized from ethanol until material consuming the theoretical amount of hydrogen was obtained.

5-Aminooxine. The published syntheses having proved unsatisfactory, 5-nitrosooxine (5 g.) in acetone (100 ml.) was hydrogenated over Raney nickel at room temp. and pressure. The catalyst was filtered off by gravity under CO_2 and the filtrate treated with 10N-HCl (6·5 ml.). The orange crystals (5·3 g.; 80% yield) were filtered off and dissolved in a little hot 0·01N-HCl. The solution was treated with charcoal, filtered and diluted with an equal volume of 10N-HCl while hot. Orange crystals of 5-aminooxine dihydrochloride, obtained on refrigeration, were dried *in vacuo* over KOH (m.p. 294–295°, 60% yield). The hydrochloride is fairly stable, but the base is easily oxidized. (Found: C, 46·5; H, 4·35; N, 11·95; Cl, 30·5. Calc. for $C_9H_8ON_2$, 2HCl: C, 46·3; H, 4·3; N, 12·0; Cl, 30·4%.)

5:7-Dinitrooxine. As this substance decomposes rather than melts at 320°, it was submitted to analysis. (Found: N, 17·8. Calc. for $C_9H_5O_5N_3$: N, 17·9%.)

5:7-Diaminooxine. There is no satisfactory preparation of this substance in the literature. 5:7-Dinitrooxine (1·0 g.) was hydrogenated as 5-nitrosooxine (above). The orange precipitate of 5:7-diaminooxine dihydrochloride was purified by dissolving in water at 50°, adding one drop of 10N-HCl and diluting to double volume with acetone. The red needles of *5:7-diaminooxine monohydrochloride hemihydrate* (yield 60%), m.p. 275° (dec.), were dried *in vacuo* over KOH. (Found: C, 49·1; H, 5·0; N, 19·2; Cl, 15·9. $C_9H_9ON_3$, HCl, $0{\cdot}5H_2O$ requires C, 49·0; H, 5·0; N, 19·05; Cl, 16·1%.)

5-Diguanidinooxine. 5-Aminooxine dihydrochloride (2 g.), dicyandiamide (0·8 g.) and water (10 ml.) were heated at 97° for 2 hr. in CO_2. The solution was cooled, saturated with HCl and precipitated with acetone. The yellow solid was filtered off, dissolved in a little boiling N-HCl and poured into excess acetone which deposited yellow crystals of *5-diguanidinooxine dihydrochloride* which were dried *in vacuo* over KOH (65% yield), m.p. 262–263°. (Found: C, 41·3; H, 4·5; Cl, 22·2. $C_{11}H_{12}ON_6$, 2HCl requires C, 41·6; H, 4·45; Cl, 22·35%.)

7-Acetamido-8-acetoxyquinoline. This was obtained whenever an attempt was made to prepare 7-acetamidooxine by acetylating 7-aminooxine, whether by the use of acetic anhydride and sodium acetate in ether (Matsumura, 1927) or by boiling with one equivalent of acetic anhydride in benzene. *7-Acetamido-8-acetoxyquinoline* formed white crystals from benzene (m.p. 172°). (Found: C, 63·5; H, 4·9; N, 11·4. $C_{13}H_{12}O_3N_2$ requires C, 63·9; H, 4·95; N, 11·5%.) An attempt to hydrolyze the *O*-acetyl group with 0·5N-NaOH gave a mixture of diacetylated and unacetylated material.

Ethyl ester of oxine-5-carboxylic acid. As the method of von Niementowski & Sucharda (1916) gave only a 30% yield, oxine-5-carboxylic acid (1 g.), H_2SO_4 (1 ml.) and absolute ethanol (20 ml.) were refluxed for 8 hr. The solution was concentrated to 7 ml., poured into water, made faintly alkaline with Na_2CO_3 and filtered. The precipitate was taken up in 40% aqueous ethanol, treated with charcoal, filtered and boiled until crystals began to form. The mixture was cooled in ice, filtered and the crystals extracted with hot benzene, giving white needles; m.p. 125°, in 70% yield.

5-Hydroxybenzo-f-quinoline (*5:6-benzooxine*, Fig. 2*c*). 2-Amino-3-methoxynaphthalene (3 g.), nitrobenzenesulphonic acid mixture (17·5 g.), water (8·5 ml.) and glycerol (5 ml.) were heated for 6 hr. as described under 5-propyloxine. Aqueous ammonia was then added and the plastic precipitate was dried and refluxed with HBr (30 ml.; sp.gr. 1·5) for 10 hr. The HBr was recovered and the residue diluted with water. Fractional precipitation with ammonia gave tar (discarded), followed by orange plastic material which became solid on drying and was extracted with N-NaOH. The filtrate was precipitated with acetic acid and the pale 5:6-benzooxine recrystallized from light petroleum (b.p. 60–90°). The yield was 6% of ivory-coloured crystals, m.p. 106–107°. The use of arsenic acid as oxidizing agent gave lower yields. (Found: C, 79·9; H, 4·6

Calc. for $C_{13}H_9ON$: C, 79·95, H, 4·65%.) Barnum & Hamilton (1942), who prepared this substance from 2-amino-3-naphthol in poor yield, give a lower m.p. (104–106°).

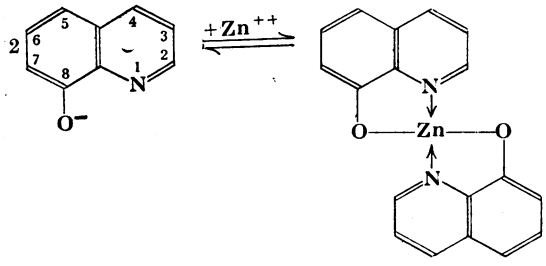

Fig. 1. Reaction between two oxine anions and one zinc ion to give oxine metal chelate complex.

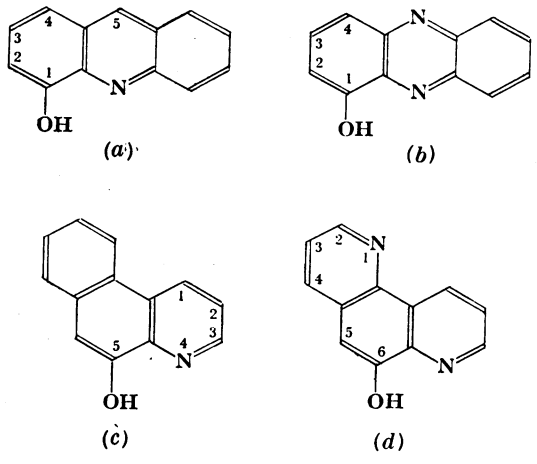

Fig. 2. (*a*) 1-Hydroxyacridine. (*b*) 1-Hydroxyphenazine. (*c*) 5-Hydroxy-benzo-*f*-quinoline (5:6-benzooxine). (*d*) 6-Hydroxy-*m*-phenanthroline.

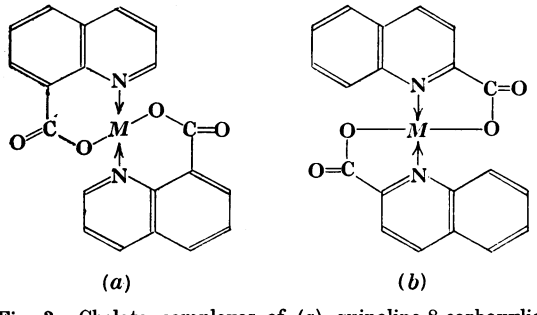

Fig. 3. Chelate complexes of (*a*) quinoline-8-carboxylic acid and (*b*) quinoline-2-carboxylic acid (quinaldinic acid) with a divalent metal (*M*).

RESULTS

The results of these tests are given in Tables 1–5 which record, semi-quantitatively, the precipitation of a number of metallic ions of biological interest by oxine and 45 substances related to it. This precipitation is caused by chelation involving the combination of one polyvalent metallic cation with as many anions of oxine as the cation has positive charges. For example, one zinc cation unites with two anions of oxine as in Fig. 1 (Fleck & Ward, 1933) and the combination involves new bonds, from nitrogen to metal and from metal to oxygen, (Morgan & Burstall, 1936).

When a metal combines with oxine the only water-attracting groups present (—OH and =N—) become masked, resulting in a strongly hydrophobic substance which is forced out of solution. Hence, among closely related substances, the amount of precipitation is likely to be roughly proportional to the extent of the chelation; conversely, the absence of precipitation usually implies that chelation has not occurred. Only when, in addition to the chelating groups, a strongly hydrophilic group (in particular —SO_3H or —COOH) is introduced, can the absence of precipitation be consistent with chelation having occurred in the concentrations dealt with here. When precipitation did not occur another test for chelation was applied, viz. an investigation as to whether hydrogen ions were liberated in the clear solution formed when solutions of the reagent and the salt of a divalent metal were mixed. This effect is exemplified in the following equation:

$$CuCl_2 + 2N{-}X{-}OH = 2N{-}X{-}O\overset{Cu}{\diagup\diagdown} + 2H^+ + 2Cl^-$$

where *N*—*X*—OH stands for the derivative of oxine. Actually this liberation of hydrogen ions increases when alkali is added to the system so that, in the presence of the metal, the normal titration curve of the compound is very considerably displaced.

In Part I (Albert & Gledhill, 1947) this type of chelate activity was designated 'hydrophilic chelation' to distinguish it from the more common 'hydrophobic chelation' and is exemplified by oxine-5-sulphonic acid (Table 2, no. 31) which does not give precipitates with metals. The chelating tendencies of this substance, although not necessarily equal to those of oxine, are clearly revealed by the abnormal acid-base titration curve obtained in the presence of copper, as in Fig. 4. Quinoline-5-sulphonic acid, which cannot chelate, did not behave in this way.

Table 1 summarizes the interaction between the inorganic ions dealt with in Part I (Albert & Gledhill, 1947) and the seven isomeric monohydroxyquinolines. Quinoline is included for purposes of comparison. It can be seen that oxine alone gives precipitates with the metals. Further tests showed that copper salts did not liberate hydrogen ions from the isomerides of oxine. Hence it is established that oxine alone of its isomerides is capable of chelation.

Table 2 deals similarly with simple derivatives of oxine. A variety of substituents, both lipophilic and hydrophilic, electron-attracting and electron-repelling, acid-strengthening and acid-weakening, have been inserted in various positions in the oxine molecule. It can be seen that a high degree of chelation persists in spite of considerable variation in the type of substituent. Even in the cases of nos. 26, 29, 31 and 32 which give little or no precipitation, evidence of hydrophilic chelating powers was obtained from the copper test (*v.s.*). However,

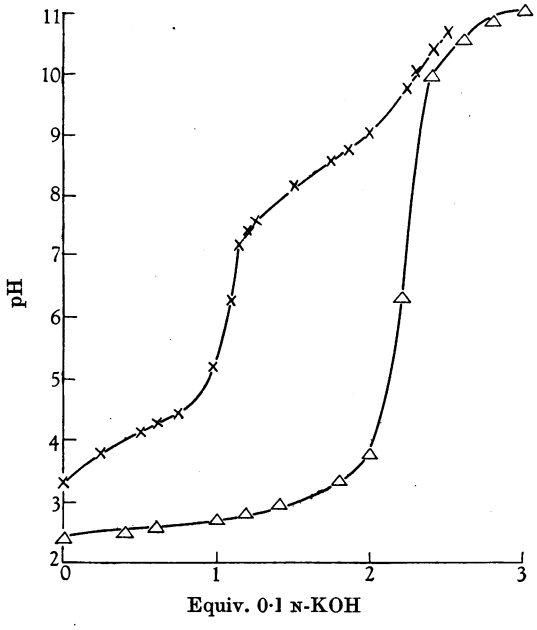

Fig. 4. Evidence of chelation from liberation of hydrogen ions (20° C.). × M/320 Oxine-5-sulphonic acid (pH 3·3). △ M/320 Oxine-5-sulphonic acid (pH 3·3) + M/640 cupric sulphate (pH 5·7).

it was found that the blocking of either the oxygen or the nitrogen atom (as in nos. 9, 10 and 12) completely prevented any union between the substance and the metal. A few of the compounds (nos. 22–28, inclusive) were found to undergo aerial oxidation catalyzed by the metal present. For example, 1:2:3:4-tetrahydrooxine (no. 28), in the presence of air, gave red solutions with Mn, Zn, Cd, Co, Pb and Cu instead of the stable yellow precipitates given by oxine and many of its derivatives.

Table 3 records the chelating properties of some substances having structural features in common with oxine. Four of these compounds belong to different heterocyclic series but possess the configuration of chelating atoms found in oxine (1-hydroxyacridine, 1-hydroxyphenazine, 5-hydroxybenzo-*f*-quinoline and 6-hydroxy-*m*-phenanthroline,

Table 1. *Oxine and isomerides: visible response to metals at* pH 7 *and* 37° *with reagent in excess*

No.	Substance	Source	Criterion of purity	Mol. wt.	Ca^{++}	Mg^{++}	Mn^{++}	Zn^{++}	Fe^{++}	Fe^{+++}	Cd^{++}	Co^{++}	Pb^{++}	Cu^{++}
1	8-Hydroxyquinoline (oxine)	B.D.H., recryst. from dilute ethanol and from light petroleum	m.p. 75°	145	–	–	+ +	+ +	+	+	+ +	+ +	+ +	+ +
2	7-Hydroxyquinoline	Claus & Massau (1892)	m.p. 235°	145	–	–	–	–	–	–	–	–	–	–
3	6-Hydroxyquinoline	Skraup (1882)	m.p. 193°	145	–	–	–	–	–	–	–	–	–	–
4	5-Hydroxyquinoline	Claus & Howitz (1893)	m.p. 223°	145	–	–	–	–	–	–	–	–	–	–
5	4-Hydroxyquinoline	For synthesis, see text	m.p. 200°	145	–	–	–	–	–	–	–	–	–	–
6	3-Hydroxyquinoline	Mills & Watson (1910)	m.p. 198°	145	–	–	–	–	–	–	–	–	–	–
7	2-Hydroxyquinoline*	Einhorn & Lauch (1886)	m.p. 199°	145	–	–	–	–	–	–	–	–	–	–
8	Quinoline	B.D.H., refractionated in hydrogen	b.p. 238°	129	–	–	–	–	–	–	–	–	–	–

Key: – No ppt.; + trace of ppt. with 0·0001 M-metal and 0·001 M-reagent; + + marked ppt. with 0·0001 M-metal and 0·001 M-reagent; + + + as + + but trace of ppt. also with 0·00001 M-metal and 0·0001 M-reagent.

* No precipitate even with 0·005 M-reagent and 0·0005 M-Ca, Mg, Mn, Zn, Cd, Co, Cu (Fe and Pb were not tested at this strength as they are precipitated by the buffer).

Table 2. *Substituted oxines: visible response to metals at* pH 7 *and* 37° *with reagent in excess*

No.	Substance	Source	Criterion of purity	Mol. wt.	Ca^{++}	Mg^{++}	Mn^{++}	Zn^{++}	Fe^{++}	Fe^{+++}	Cd^{++}	Co^{++}	Pb^{++}	Cu^{++}
9	*O*-Methyloxine (8-methoxyquinoline)*	Bedall & Fischer (1881)	m.p. 46°	159	–	–	–	–	–	–	–	–	–	–
10	Oxine methochloride (8-hydroxy-*N*-methylquinolinium chloride)*	Claus & Howitz (1890)	m.p. 232° (dec.)	196	–	–	–	–	–	–	–	–	–	–
11	2-Methyloxine	Doebner & Miller (1884)	m.p. 74°	159	–	–	+ + +	+ + +	+	+	+ + +	+ + +	+ + +	+ + +
12	2-Methyl-8-methoxyquinoline	Doebner & Miller (1884)	m.p. 125°	173	–	–	–	–	–	–	–	–	–	–
13	5-Methyloxine	Nölting & Trautmann (1890)	m.p. 122–123°	159	–	–	+ +	+ + +	+ +	+ +	+ +	+ +	+ +	+ + +
14	5-*n*-Propyloxine	For synthesis, see text	m.p. 60–61°	187	–	–	+ +	+ +	+ +	+ +	+ +	+ +	+ + +	+ + +
15	5-Chlorooxine	D.R.P. (1930)	m.p. 125°	179·5	–	–	+ +	+ +	+	+	+ +	+ + +	+ +	+ + +
16	7-Chlorooxine	For synthesis, see text	m.p. 156°	179·5	–	–	+ +‡	+ +‡	+ +	+ + +	+ +	+ + +	+ +	+ + +
17	5:7-Dichlorooxine	D.R.P. (1930)	m.p. 181°	214	–	–	+‡	+ +	+ +	+ +	+ +	+ +	+ +	+ +
18	5-Chloro-7-iodooxine (Vioform)	CIBA, recryst.	m.p. 178–179°	305·5	–	–	+‡	+‡	+ +	+ +	+ +	+ +	+ +	+ +
19	5-Nitrooxine	Kostanecki (1891)	m.p. 178°	190	–	–	–	+	+ +	+ +	–	+ + +	+ +	+ + +
20	5:7-Dinitrooxine	Bennett & Grove (1945)	m.p. 320° (dec.), see also text	235	–	–	+	–	+	+	+	+	+	+
21	5-Nitrosooxine	For synthesis, see text	See text	174	–	–	–	+	(+)†	–	+	+ +	+	+ + +
22	4-Aminooxine§	For synthesis, see text	m.p. 208°	160	–	–	–	–	+	+	–	–	–	+
23	5-Aminooxine§	For synthesis, see text	See text	160										
24	7-Aminooxine§	Matsumura (1927), but hydrolysis conducted at 190° instead of 170°	m.p. 131°	160					Difficult to assess because of oxidation					
25	5:7-Diaminooxine§	For synthesis, see text	See text	175	–	–	–	–	+	–	–	–	–	–
26	5-Diguanidinooxine§	For synthesis, see text	See text	244	–	–	–	–	(+)†	(+)†	–	(+)†	–	–
27	4-Hydroxyoxine (8-hydroxy-4-keto-1:4-dihydroquinoline)§	Musajo & Minchilli (1941)	m.p. 314–315°	161	–	–	–	–	(+ +)†	–	–	(+)†	–	–
28	1:2:3:4-Tetrahydrooxine§	Bedall & Fischer (1881)	m.p. 121–122°	149	–	–	–	–	(+)†	(+)†	–	(+)†	–	–
29	Oxine-5-carboxylic acid*	Lippmann & Fleissner (1886)	m.p. 273° (dec.)	189	–	–	(+)†	–	(+)†	(+)†	–	(+)†	–	–
30	Ethyl ester of no. 29	Niementowski & Sucharda (1916)	m.p. 125–126°	217	–	–	+	+	+	+	+	+	+	+
31	Oxine-5-sulphonic acid*	Claus & Posselt (1890)	m.p. 321–322°	225	–	–	–	–	(+)†	(+)†	–	–	–	–
32	7-Iodooxine-5-sulphonic acid (Chiniofon; Ferron; Yatren)*	B.D.H., recryst.	m.p. 285° (dec.)	351	–	–	–	–	(+ +)†	(+)†	–	–	–	–

Key: – No ppt.; + trace of ppt. with 0·0001 M-metal and 0·001 M-reagent; + + marked ppt. with 0·0001 M-metal and 0·001 M-reagent; + + + as + + but trace of ppt. also with 0·00001 M-metal and 0·0001 M-reagent.

* No precipitate even with 0·005 M-reagent and 0·0005 M-Ca, Mg, Mn, Zn, Cd, Co, Cu (Fe and Pb were not tested at this strength as they are precipitated by the buffer).

† Colour change, no precipitate.

‡ Exact reading difficult as reagent is poorly soluble and controls become cloudy.

§ Metals appear to catalyze oxidation of these compounds causing colour changes.

Table 3. *Substances chemically related to oxine: visible response to metals at* pH 7 *and* 37° *with reagent in excess*

No.	Substance	Source	Criterion of purity	Mol. wt.	Ca^{++}	Mg^{++}	Mn^{++}	Zn^{++}	Fe^{++}	Fe^{+++}	Cd^{++}	Co^{++}	Pb^{++}	Cu^{++}
			(*a*) Same chelation mechanism as in oxine, but different nucleus											
33	1-Hydroxyacridine (Fig. 2*a*)	Jensen & Rethwisch (1928)	m.p. 116°	195	–	–	+ +	+ +	+	+ +	+ +	+	+ +	+ + +
34	1-Methoxyacridine	Jensen & Rethwisch (1928)	m.p. 134°	209	–	–	–	–	–	–	–	–	–	–
35	2-Hydroxyacridine	Albert & Ritchie (1943)	m.p. 285°	195	–	–	–	–	–	–	–	–	–	–
36	1-Hydroxyphenazine (Fig. 2*b*)	Wrede & Strack (1929)	m.p. 157°	196	–	–	+ + +	+	–	–	+	+ + +	+ + +	+
37	1-Methoxyphenazine	Wrede & Strack (1929)	m.p. 169°	210	–	–	–	–	–	–	–	–	–	–
38	2-Hydroxyphenazine	Kehrmann (1924)	m.p. 253°	196	–	–	–	–	–	–	–	–	–	–
39	5-Hydroxybenzo-*f*-quinoline (5:6-benzoxine; Fig. 2*c*)	For synthesis, see text	m.p. 106–107°	195	–	–	+ + +	+ + +	+ +	+ +	+ + +	+ + +	+ + +	+ + +
40	6-Hydroxy-*m*-phenanthroline (5:6-pyridoxine; Fig. 2*d*)	Haworth & Sykes (1944)	m.p. 160°	196	–	–	+ + +	+*	+ +	+ +	+ + +	+ +	+ +	+ +
			(*b*) Same nucleus (quinoline) as in oxine, but different chelating mechanism											
41	8-Mercaptoquinoline	Edinger (1908)	m.p. 58–59°	161	–	–	+	+	+	+	+	+ + +	+	+ + +
42	8-Aminoquinoline	Hydrogenation of 8-nitroquinoline (Raney nickel)	m.p. 65°	144	–	–	–	–	–	–	–	–	–	–
43	Quinoline-2-carboxylic acid (quinaldinic acid; Fig. 3*b*)	B.D.H., recryst.	m.p. 157°	173	–	–	–	+	+	–	+	–	+	+
44	Quinoline-4-carboxylic acid (cinchoninic acid)	Pfitzinger (1902)	m.p. 253–254°	173	–	–	–	–	–	–	–	–	–	–
45	Quinoline-8-carboxylic acid (Fig. 3*a*)	Schlosser & Skraup (1881)	m.p. 187°	173	–	–	–	–	–	–	–	–	+	+ + +
46	4:8-Dihydroxyquinoline-2-carboxylic acid (xanthurenic acid)†	Musajo & Minchilli (1941)	m.p. 284–285°	205	Difficult to assess because of oxidation									

Key: – No ppt.; + trace of ppt. with 0·0001 M-metal and 0·001 M-reagent; + + marked ppt. with 0·0001 M-metal and 0·001 M-reagent; + + + as + + but trace of ppt. also with 0·00001 M-metal and 0·0001 M-reagent.

* Exact reading difficult as reagent is poorly soluble and controls become cloudy.

† Metals appear to catalyze oxidation of this compound causing colour changes.

nos. 33, 36, 39 and 40, see Fig. 2). It is evident that these substances have chelating powers very similar to those of oxine. Further, it is seen that, as with oxine, chelating ability is lost when the hydroxyl group is blocked (nos. 34 and 37) or else removed to a distant position (nos. 35 and 38). Table 3 also lists some quinoline compounds which differ from oxine in the chemical nature of the groups available for chelation. 8-Mercaptoquinoline (no. 41), in which the O of oxine has been replaced by S, retains chelating power, but 8-aminoquinoline (no. 42) shows no chelating properties under the conditions of these tests as its complexes are weakly held, water-soluble cations (Burrows & Ritchie, 1939). As chelation does not usually take place unless a 5- or 6-membered ring can be formed, it is understandable that 2-hydroxyquinoline (no. 7) does not chelate. Among the quinolinecarboxylic acids (nos. 43–45) both the 2- and 8-isomerides could reasonably be expected to chelate as in Fig. 3. Actually these substances chelate less avidly than oxine, quinoline-8-carboxylic acid (no. 45) being almost a specific reagent for Cu.

The results of Tables 1–3 were extended by the examination of some other cations of possible biological interest (Al, Ni, MoO_2 and UO_2). The results (Table 4) show selectivity, but confirm the impression that oxines are capable of chelation with many different metals, even under the restricted conditions of these tests.

equal to the maximal amount bound by the metal present in the earlier tests. There is substantial agreement between the results of the two tests.

All the tests described so far have been carried out at pH 7·3 and it was thought desirable to examine oxine under more alkaline conditions (pH 8·6). The results differed from those of Tables 1 and 4 in only one detail, viz. Mg was now precipitated.

At the request of our biological collaborators, we examined the effect of oxine on a series of complex cobaltic salts (conditions as in Table 1): sodium cobaltinitrite, chloropentamminocobaltic chloride, nitratopentamminocobaltic nitrate, dinitrotetramminocobaltic chloride, carbonatotetramminocobaltic nitrate and *trans*dichlorodiethylenediamine cobaltic chloride. In no case was there any reaction with oxine. However, freshly prepared cobaltic sulphate reacted similarly to any cobaltous salt.

To provide data for discussion of the effect of ionization upon chelation, the pK_a values (i.e. the negative logarithms of the acidic ionization constants) of oxine and 19 related substances were obtained and the percentages of the four species (anion, cation, zwitterion and neutral molecule) calculated for pH 7·3. It can be seen from Table 6 that the proportions of the four species vary greatly from compound to compound. Oxine (no. 1) is present principally as neutral molecule with only 0·5 % of cation and 0·7 % of anion, at 20°, the temperature of

Table 4. *Other metals: visible response to some other metals of possible biological interest at* pH 7 *and* 37°, *reagent in excess*

No.	Substance	Al^{+++}	Ni^{++}	MoO_2^{++}	UO_2^{++}
1	Oxine	+ +	+	+	+ +
15	5-Chlorooxine	+ + +	+ + +	+	+ + +
19	5-Nitrooxine	+ + +	–	–	–
21	5-Nitrosooxine	–	(+)*	–	–
40	6-Hydroxy-*m*-phenanthroline	+	+ + +	+†	+ + +

Key: Signs have same meaning as in Tables 1-3 (inclusive).

* Colour change, no precipitate.

† Exact reading difficult as reagent is poorly soluble and controls are cloudy.

Table 5. *Metals in excess: visible response of oxine to metals at* pH 7 *and* 37° *with metals in excess*

Ca^{++}	Mg^{++}	Al^{+++}	Mn^{++}	Zn^{++}	Fe^{++}	Fe^{+++}	Cd^{++}	Co^{++}	Ni^{++}	Pb^{++}	Cu^{++}	MoO_2
–	–	+	+	+ +	+ +	+ +*	+ + +	+ + +	+ +	+*	+ +	–

Key: – No ppt.
\+ Trace of ppt. with 0·0004 M-metal and 0·0002 M-oxine.
\+ + Marked ppt. with 0·0004 M-metal and 0·0002 M-oxine.
\+ + + As + + but trace of ppt. also with 0·00004 M-metal and 0·00002 M-oxine.

* Exact reading difficult as control is cloudy.

As preliminary work indicated that oxine-metal reactions obeyed the mass action law, an excess of organic reagents had been used in all the tests described so far. It is interesting now to record the results (Table 5) of using the metals in excess. The quantity of oxine used in such tests has been made the determinations. At 37° the proportions undergo only a small change (0·3 % cation and 1·5 % anion).

Surveying the table, it will be seen that the percentage of anion varies from $<0·01$ % (no. 26) to 83 % (no. 18) or even, if non-phenolic anions are considered, 98 % (no. 29). Likewise the percentage

Table 6. *Ionization of oxine and related compounds at 20°*

No.	Substance	pK_a		Reference	Percentage of species present at pH 7·3 in water: Anion	Kation	Zwitterion	Neutral molecule
1	Oxine	—OH =N—	9·45 5·03	(1)	0·7 (1·5 at 37°)	0·5 (0·3 at 37°)	Nil	98·8
8	Quinoline	=N—	4·94	Albert & Goldacre (1944)	Nil	0·4	Nil	99·6
14	5-*n*-Propyloxine	—OH =N—	(9·7) (5·0)	(1), (2)	0·4	0·5	Nil	99·1
15	5-Chlorooxine	—OH =N—	(8·7) (3·8)	(1), (2)	4·3	<0·1	Nil	95·7
16	7-Chlorooxine	—OH =N—	7·7 (4·0)	(1), (3) (1), (2)	28·5	<0·1	Nil	71·5
17	5:7-Dichlorooxine	—OH =N—	(6·7) (*c.* 3·6)	(1), (2)	80·0	<0·1	Nil	20·0
18	5-Chloro-7-iodooxine	—OH	(6·6)	(1), (2)	83·4	—	Nil	16·6
22	4-Aminooxine	—OH =N—	6·91 10·71	(1)	0·03	28·5	71·5	Nil
23	5-Aminooxine	—OH =N—	11·24 5·67	(1)	<0·1	2·5	Nil	97·5
25	5:7-Aminooxine	—OH =N—	6·96 10·83	(1)	0·02	28·9	71·1	Nil
26	5-Diguanidinooxine	—OH —C(=NH)NH$_2$ =N—	8·89 11·8 3·97	(1)	<0·01	97·9 <0·1 (=N$^+$—)	2·1	Nil
28	1:2:3:4-Tetrahydrooxine	—OH =N—	(9·4) (5·5)	(1), (2)	0·7	2·0	Nil	97·3
29	Oxine-5-carboxylic acid	—COOH —OH =N—	(4·8) 9·32 (4·0)	(1), (2) (1) (1), (2)	98·4 (—COO$^-$) 1·0 (—O$^-$ + —COO$^-$)	<0·1	Nil	0·6
31	Oxine-5-sulphonic acid	—SO$_3$H —OH =N—	2·04 8·48 4·19	(1)	94·1 (—SO$_3^-$) 5·9 (—O$^-$ + —SO$_3^-$)	<0·1	Nil	Nil
32	7-Iodooxine-5-sulphonic acid	—SO$_3$H —OH =N—	<2 7·15 2·45	Feldman & Powell (1940)	44·3 (—SO$_3^-$) 55·7 (—O$^-$ + —SO$_3^-$)	<0·1	Nil	Nil
33	1-Hydroxyacridine	—OH =N—	(9·0) (4·7)	Albert & Goldacre (1943)	1·8	0·3	Nil	97·9
34	1-Methoxyacridine	=N—	(4·7)	Albert & Goldacre (1946)	Nil	0·3	Nil	99·7
36	1-Hydroxyphenazine	—OH =N—	8·5 1·44	Michaelis, Hill & Schubert (1932)	5·9	<0·01	Nil	94·1
40	6-Hydroxy-*m*-phenanthroline	—OH =N—	(8·5) (3·9)	(1), (2)	5·9	<0·1	Nil	94·1
42	8-Aminoquinoline	=N–	3·93	Albert & Goldacre (1944)	Nil	<0·1	Nil	*c.* 100

(1) Specially determined for the present studies by Mr R. Goldacre and Mr J. Phillips.

(2) The pK values in brackets had to be calculated approximately from values obtained in 50% ethanol, because of the sparing solubility of the substances in water. The corrections of −1·7 and +0·5 were made to the —OH and =N– values respectively as was suggested by the pK values of oxine (10·92 and 4·57 respectively), and the acid pK of 7-chlorooxine (9·61), when determined in 50% ethanol.

(3) Determined in 50, 40, 30, 20 and 10% ethanol and extrapolated to pure water.

of cation varies from $< 0{\cdot}1\,\%$ (several examples) to 29 % (no. 22) or even, if the ionization of a group other than the ring nitrogen is considered, 98 % (no. 26). Only two examples of substances which yield a large proportion of zwitterion are found in the series (nos. 22 and 25). Among the various members, the percentage of neutral molecule varies from nil to practically 100 %.

DISCUSSION

Interest in the biological properties of oxine goes back to about 1895 in which year the firm of Fritzsche and Co., Hamburg, brought out a product, Chinosol, for use as an antiseptic and disinfectant. The composition of this product was found by Brahm (1899) to be a mixture of 8-hydroxyquinoline and potassium pyrosulphate, the acidity of the latter helping to dissolve the former. The great dilution at which oxine acts against bacteria and fungi (often M/80,000, i.e. 1 part in 500,000) would suggest that its mode of action depends on some specific interference with a vitally important metabolic reaction. Until recently there has been no attempt to investigate the mode of action of oxine which has usually been classed with the phenolic disinfectants (e.g. Hata, 1932). However, it was later suggested that oxine exerts its antibacterial action by withdrawing essential trace metals from bacteria (Albert, 1943). This hypothesis has been largely substantiated not only for oxine but for all derivatives shown in the present work to have the property of chelating hydrophobically with metals (Albert *et al.* 1947). Zentmyer (1944) similarly explained the fungistatic power of oxine.

Little was known about the nature of the metallic complexes of oxine until Berg (1927) introduced it and some of its simpler derivatives into analytical practice for the determination of various metals. The reaction is very delicate (e.g. 1 μg. of zinc can be detected in 1 ml. of water, i.e. at a dilution of 1 p.p.m.), and is much used when traces only of metals are present. The ease with which small amounts of metal-oxine complexes can be shaken out of aqueous solutions by chloroform has increased the general utility of this reaction. Berg found that oxine precipitated different metals at different hydrogen ion concentrations and this differentiation was put on a scientific basis by Fleck & Ward (1933; also Fleck, 1937) who determined the pH limits between which metals would precipitate from M/300 solution, the majority of heavy metals being completely precipitable only between pH 4·5 and 14·5. However, magnesium was not precipitated at all below pH 7, to the extent of 3 % only at pH 7·7 and to the extent of 75 % at pH 8·5. The molybdyl, uranyl and tungstyl cations were precipitated completely only between narrow pH limits (e.g. pH 5·0–5·7 for tungstyl).

Mechanism of chelation by oxines

Theoretical considerations suggest that no isomeride of 8-hydroxyquinoline should be capable of chelation because the formation of a 5- or 6-membered ring, coplanar with the other rings, becomes impossible when the hydroxyl group is transferred to other positions in the quinoline nucleus. This postulate received some confirmation when it was found that iron, nickel, copper, palladium and platinum chelated with no hydroxyquinoline other than the 8-isomeride (Bargellini & Bellucci, 1923). That this is equally true of the metals investigated in the present work follows from the fact (Table 1) that oxine alone of its isomerides gave precipitates. Independent testing for hydrophilic chelation, as described in the foregoing text and exemplified by Fig. 4, substantiated the conclusion that oxine alone of the seven isomeric hydroxyquinolines is capable of chelation.

To provide a basis for the control of chelating power among derivatives of oxine, it becomes necessary to look more deeply into the mechanism of chelation. What is already known on this topic is summarized by Fig. 1, viz. that oxine complexes are formed by the metal (i) becoming linked to the oxygen atom through a primary valence and (ii) completing the ring by forming a co-ordinate bond with the nitrogen atom, utilizing the latter's lone pair of electrons for this purpose. Further, it is known that, in at least one example, the bond between the oxygen and the metal is more polar than covalent (Mellor & Craig, 1941). Concordant with these views are our findings that oxine will no longer chelate with metals when either the O or N is firmly blocked, as by a methyl group in nos. 9, 10 and 12 (see Table 2). By far the most likely first step in the formation of oxine-metal complexes is that the ion of the metal combines with oxine anions and not that the metallic ion breaks the O—H bond in the course of random collisions with the unionized hydroxyl group. Accordingly, factors which modify the ionization of the hydroxyl group in oxine could conceivably interfere with chelation. For example, a graded repression of ionization ought to be procurable by the introduction of electron-repelling groups (such as $-CH_3$, $-NH_2$ and —OH), particularly if these were to be placed in the ring containing the —OH group, and it was thought of interest to note whether these occasioned a proportionate drop in ability to chelate.

As the literature offered little information on the ionization of oxine derivatives, the examination of a number of our compounds from this point of view was arranged (Table 6). It was found that the electron-repelling effect of an alkyl group (as exemplified by 5-propyloxine, no. 14, Table 6), affected ionization in the expected direction to too slight an

extent to interfere with chelation (nos. 11, 13 and 14, Table 2). When more strongly electron-repelling groups (e.g. —OH and —NH_2; nos. 23 and 27) were inserted in the oxine molecule, the connexion between ionization and chelation was somewhat obscured by the susceptibility of the new compounds to aerial oxidation in the presence of traces of metals (e.g. zinc ions). For example, 5-aminooxine (no. 23) shows the expected marked decrease in ionization of the hydroxyl group and it appears not to chelate with metals in the manner of oxine, but it gives solutions of various colours in proportion to the amount of air admitted and the nature of the metal. Fortunately some other oxines with a low percentage of anion (i.e. $<0{\cdot}1\,\%$) at pH 7·3 are not so prone to metal-catalyzed oxidation (nos. 22 and 25; Tables 2 and 6). Here too, there is a virtual disappearance of chelating properties, e.g. no. 22 gives a slight precipitate with copper but does not precipitate or give the hydrophilic chelation test with zinc.*

On the other hand, no outstanding improvement in chelating properties seems to have been effected by increasing the percentage of anion present in oxines. Attempts to favour chelation by increasing the electron flow towards a participating nitrogen, as recommended by Calvin & Bailes (1946), can give equivocal results when, as here, the nitrogen can ionize at the pH of the experiment. In fact, until it is possible to determine stability constants for metal-oxine complexes (as discussed in Part I; Albert & Gledhill, 1947), treatment of all such questions can be no more than semiquantitative.

Some other factors involved when comparing the effects of substituents on the chelation of oxines will now be briefly referred to. Precipitation phenomena are affected, favourably or otherwise, by substituents which render the metal-oxine complexes less, or more, soluble. The alkyl and halogen derivatives of oxine in Table 2 provide several examples where precipitation can be recognized at a tenfold higher dilution than is the case with oxine. On the other hand, the insertion of a sulphonic acid group does not prevent chelation from taking place although the metal complexes become water soluble, an effect which has been described at length above. It should be noted that compounds of this type (nos. 26, 29, 31 and 32, Tables 2 and 6) have a peculiarity in that the chelating ion carries two charges. Perhaps the principal value of Table 6 lies in its demonstration that a highly complex ionic situation can arise when a few common substituents are introduced into the molecule of a potential ampholyte such as oxine.

The remaining compounds of Table 2 have some points of interest; 5:7-dinitrooxine (no. 20) has the hydrogen-bonded *o*-nitrophenol structure; 5-nitrosooxine (no. 21) is tautomeric with the monoxime of 5:8-quinolinequinone; tetrahydrooxine (no. 28) is catalytically oxidized in the presence of metals; such features may explain the lowered chelating powers of these compounds.

All the structural features responsible for the chelation of oxine can be reproduced in heterocyclic ring systems other than quinoline (e.g. nos. 33, 36, 39 and 40, Table 3). The chelating powers of 1-hydroxyacridine have already been recorded (Freeman & Lions, 1940); the other examples are new. As with oxine, ability to chelate is lost by isomerization (nos. 35 and 38) or by blocking the hydroxyl group (nos. 34 and 37). It may be added that pyocyanin, an *N*-methylated derivative of 1-hydroxyphenazine, does not chelate. The remaining compounds in Table 3 show that systematic departure from the arrangement of atoms responsible for chelation in oxine leads to diminishing degrees of ability to chelate.

One of the most interesting findings of this survey is that with very few exceptions oxine, and substances similarly constituted, chelate with the same selection of metals. Disregarding those substances which are destroyed by air in the presence of metals (nos. 22–28 inclusive, 46 and perhaps 21), one sees a tendency to chelate with a limited range of metals only in 5-nitrooxine (no. 19, Tables 2 and 4) and 1-hydroxyphenazine (no. 36, Table 3). Even here there is not the high specificity that often appears in other classes of chelating compounds (e.g. nos. 41 and 45). Throughout the entire series, no case occurred where calcium or magnesium was precipitated at pH 7·3. The colours of the complexes formed by iron and various oxines were always blue or green in contrast to those of other metals which partook of the colour of the chelating agent (usually pale yellow).

Biological and biochemical implications

The data presented above have a bearing on the use of oxines as drugs in chemotherapy and as reagents in biological research. Although the antibacterial action of oxine has been known so long and so many modifications of the molecule have been made (Altpeter, 1929), it had been difficult to

* In nos. 22 and 25, the phenomenon of extra ionic resonance, associated with the presence of amino groups in certain positions (Albert & Goldacre, 1944) has caused the basic centres to become ionized at the pH studied. In these compounds the positive charge, by attracting electrons, increases the ionization of the hydroxyl groups, but the net effect has been to decrease the amount of available anion owing to its inevitable participation in zwitterion formation through internal neutralization. In no. 26, which chelates hydrophilically owing to the diguanide cation being a powerful water-attracting group, the cationic species (present to the extent of 98 %) is free to chelate as the ring nitrogen does not carry a charge.

visualize the possibilities and limitations of this series until the mode of action was discovered. In a collateral publication (Albert *et al.* 1947) it is shown that the antibacterial action of oxine derivatives runs remarkably parallel with their ability to chelate and hence bacteriostasis probably depends on the removal of trace metals which are essential to bacteria. Certain cases, where inert groups in particular positions interfere with antibacterial action without interfering with chelation *in vitro*, are discussed from the viewpoint of steric hindrance and it is concluded that the antibacterial action occurs, not in solution, but at a bacterial surface.

For use as a biological tool, to remove a catalytic or a protective metal, the value of any oxine can be roughly gauged from the present work. The comparative lack of metal specificity is unfortunate but it may be permissible in some cases to remove the oxine-metal complexes by shaking out with chloroform and when this can be done at various pH values, a degree of metal specificity is attainable (Moeller, 1943). We have observed that chloroform does not remove the metal complexes of amino-acids (e.g. glycine, arginine, tryptophane). As it is known that some metals which catalyze the oxidation of thiol groups can do so even when bound to oxine (Bernheim & Bernheim, 1939), one must not regard metal complexes as being necessarily inert and their removal (e.g. by chloroform) may be imperative in some types of work.

SUMMARY

1. Forty five analogues and derivatives of oxine (8-hydroxyquinoline) have been investigated with reference to their ability to combine, by chelation, with trace metals in neutral solution.

2. It was found that oxine alone of the seven isomeric hydroxyquinolines had chelating ability and that this property persisted throughout all derivatives having the essential chelating mechanism of oxine, viz. a phenolic group *peri* to a heterocyclic tertiary nitrogen atom. Chelate activity was found to be partly or totally suppressed in proportion as this arrangement of atoms was modified.

3. Several derivatives of oxine were found to give precipitates with metals at a greater dilution than did oxine. No outstanding instances of specificity for any metal were encountered.

4. The somewhat complex influence of electronic distribution, and hence ionization, on chelating in this series is briefly discussed.

5. Some applications of the experimental results to problems in biology and biochemistry are indicated.

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The Potentiation of the Toxicity of some Vesicants to the Pyruvate Oxidase System *in vitro* by certain Dithio-compounds

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One of the more puzzling features of the *in vivo* action of mustard gas has always been the small amount which can prove toxic to animals. In this laboratory, for instance, it was reported (Holiday, Ogston, Peters, Philpot & Stocken, 1940) that 2 mg. of mustard gas placed upon the skin of most rats weighing 200 g. would kill. Allowing for loss by evaporation, hydrolysis and fixation in the skin, this means that the toxicity is < 10 μg./g. of rat, and is of a different order from that associated with the *in vitro* action of mustard gas upon certain enzymes and enzyme systems which is *c.* 300 μg./ml. (Peters & Wakelin, 1940; Dixon & Needham, 1941*a*; van Heyningen, 1941); it is also quite different from the toxicity of arsenicals and of divinyl sulphone and 2:2′-dichlorodiethyl sulphone, which approaches *in vitro* more closely the concentration of *in vivo* toxicity. There is much evidence now of causal connexion between lewisite action and enzyme poisoning (see review by Peters, Stocken & Thompson, 1945). It would be satisfactory also to interpret the activity of mustard gas in terms of an enzyme poisoning; Dixon, van Heyningen & Needham (1942) have indeed developed the theory that mustard gas and other vesicants owe their action to toxic effects upon hexokinase. In the course of this work, in which the pyruvate oxidase system was used as a test for possible thiol-containing therapeutic agents (Holiday *et al.* 1940), it was found that *N*:*N*-diethyldithiocarbamate or *N*:*N*-di-(2-hydroxyethyl)-dithiocarbamate had a special action. Instead of protecting the pyruvate oxidase system against mustard gas it much increased its toxicity; this effect was called 'potentiation'. The important point was made that in the presence of some dithio compounds, mustard gas becomes almost 20 times more toxic, and some evidence was produced that the action was due to a compound of the dithio substance and mustard gas. While there is no reason to think that the potentiating action of these dithio compounds occurs *in vivo*, it was felt that a closer study was advisable. We were reinforced in our suspicion that there might be unsuspected ways in which the action of mustard gas was altered *in vivo* by our finding that mustard gas was often toxic to the succinic dehydrogenase enzyme in a brain *brei*, whereas it was not so in the same enzyme in ground-muscle preparations (Peters & Wakelin, 1941). The opposite is true of phosphocreatine phosphokinase, which is sensitive to mustard gas in the isolated state (Cori, Colowick, Berger & Slein, 1945), whereas it has not been found to be poisoned by treating skin with mustard gas *in vivo* (Needham, quoted by Dixon, 1943).

Following our initial observations, Dixon & Needham (1941*b*), using the pyruvate oxidase of *E. coli*, confirmed the effect of diethyldithiocarbamate, and the toxicity of the incubated product; they added the further observation that addition of diethyldithiocarbamate (not itself toxic), after hydrolysis of mustard gas was complete, could still cause an inhibition. Hence they thought that the potentiations could not be explained simply by the formation of a more toxic addition compound.

In this report, modified from the original (Peters & Wakelin, 1943*b*), we present a closer study of the potentiating effect upon the pyruvate oxidase system of diethyldithiocarbamate and diethanoldithiocarbamate; no essential difference in the action of the two compounds has been found. The toxic effects are due to the formation of a compound of mustard gas with the diethyldithiocarbamate ion, which is more toxic than the sum of the toxicities of the two compounds.