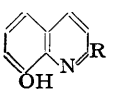
317. *Steric Hindrance in Analytical Chemistry. Part I.*

By HARRY IRVING, E. J. BUTLER, and M. F. RING.

8-Hydroxy-2-, -5-, and -6-methylquinoline, 8-hydroxy-1-phenylquinoline, 1-hydroxyacridine, and 9-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroacridine* have been prepared as potential precipitating reagents in metal analysis and found to have much the same sensitivity as 8-hydroxyquinoline (" oxine "), whilst that of 8-hydroxy-7-methylquinoline is somewhat greater. All gave insoluble complexes with Cr^{+++}, Fe^{+++}, Ga^{+++}, Cu^{++}, and Zn^{++}, but 8-hydroxy-2-methyl-, -2 : 4-dimethyl-, and -2-phenyl-quinoline, 1-hydroxyacridine, and 9-hydroxy-1 : 2 : 3 : 4-tetrahydroacridine differed from the others in failing to give an insoluble complex with Al^{+++}. A possible explanation in terms of the stereochemistry of these complexes is advanced, and its implications are considered in connexion with the behaviour of various charged and uncharged complexes of analytical importance. The efficiency as bacteriostatic agents of several of these derivatives and analogues of oxine has been measured and discussed in terms of a contemporary theory of drug action.

IT is well known that aluminium forms a stable complex of stoicheiometric composition with 8-hydroxyquinoline [" oxine " (I; R = H)] which permits of its determination by gravimetric, volumetric, and absorptiometric procedures (Berg, " Die Analytische Verwendung von *o*-Oxychinolin," Stuttgart, 1938; Sherrington and Gentry, *Analyst*, 1946, **71**, 433). Since very many other elements behave similarly, lack of selectivity is an adverse feature in the use of oxine necessitating careful adjustment of pH and choice of masking agents when the constituents of certain mixtures are to be determined. It is often difficult and sometimes impossible to avoid co-precipitation of metal–oxine derivatives [Chirnside, Pritchard, and Rooksby, *Analyst*, 1941, **66**, 399; Moyer and Remington, *Ind. Eng. Chem.* (*Anal.*), 1938, **10**, 212]. That the introduction of substituents might produce a reagent more sensitive or selective was first considered by Berg (*Z. anorg. Chem.*, 1932, **204**, 208), and 5 : 7-dibromo-8-hydroxyquinoline was developed as a special reagent for copper, iron, and titanium (Berg and Küstenmacher, *ibid.*, p. 215). A number of 5-arylazo-derivatives of oxine were examined by Gutzeit and Monnier (*Helv. Chim. Acta*, 1933, **16**, 233, 478), and many others by Boyd, Degering, and Shreve [*Ind. Eng. Chem.* (*Anal.*), 1938, **10**, 606]; like the 7-arylazo-derivatives of 8-hydroxy-5-methylquinoline (Irving and Taylor, unpublished), they are more sensitive than oxine itself, and several of them may be recommended as selective reagents for palladium, mercury, vanadium, and, possibly, copper. 7-Iodo-8-hydroxyquinoline-5-sulphonic acid has been carefully studied as a colorimetric reagent for iron (Yoe and Hill, *J. Amer. Chem. Soc.*, 1937, **59**, 872).

Now, whilst 8-hydroxy-5-methylquinoline appears to resemble oxine very closely so far as it has yet been studied (Gietz and Sa, *Anal. Asoc. Quím. Argentina*, 1935, **23**, 45), 8-hydroxy-2-methylquinoline (I; R = CH_3) displays a remarkable difference, for Merritt and Walker [*Ind. Eng. Chem.* (*Anal.*), 1944, **16**, 387] found that it would not give a precipitate with aluminium, a peculiarity which they exploited by introducing it as a reagent for zinc in the presence of aluminium and magnesium. Comparing 2-methyloxine (I; R = CH_3) with oxine they remark: " Probably because of its increased size, it is a more selective reagent. If the size of the molecule is the determining factor, the larger molecule might be expected not to react with the smaller ions because of the difficulty of grouping three large molecules around the small ion. If a complex is formed it might be less stable." No further communication has appeared in this field and, since the observation is of considerable analytical importance and no less interesting academically, we have considered its implications in some detail. To this end we prepared 8-hydroxy-2-, -5-, -6-, and -7-methyl-, -2 : 4-dimethyl-, and -2-phenyl-quinoline, 1-hydroxyacridine (II), and 9-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroacridine* (III), and compared the sensitivity of their reactions with those of oxine towards aluminium and a number of other metals.



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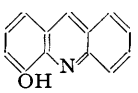

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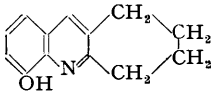


(III.)

Preparation of Reagents.—8-Hydroxy-2-, -6-, and -7-methylquinolines were prepared by modifications of published syntheses (cf. Merritt and Walker, *loc. cit.*; Herzfeld, *Ber.*, 1884, **17**, 905, 1552; Fischer, *ibid.*, p. 441; Noelting and Trautmann, *Ber.*, 1890, **23**, 3663). Although *o*-aminophenol and acetylacetone condensed smoothly to give 4-(o-*hydroxyanilo*)*pentan*-2-*one*,

o-OH·C_6H_4·N:CMe·CH_2·COMe, this anil resisted all attempts at ring-closure to form 8-hydroxy-2 : 4-dimethylquinoline. However, aniline and acetylacetone readily condensed to give 2 : 4-dimethylquinoline (Scheibe, Merkel, and Müller, *J. pr. Chem.*, 1919, **100**, 91), which was readily sulphonated, but gave only a very poor yield of the desired hydroxyquinoline on alkali fusion. 8-Hydroxy-2-phenylquinoline, prepared by decarboxylation of 8-hydroxy-2-phenylquinoline-4-carboxylic acid, formed almost colourless needles, m. p. 85·5° (Doebner and Fettbach, *Annalen*, 1894, **281**, 9, give m. p. 59°). A more direct synthesis by the action of phenyl-lithium upon oxine gave plates m. p. 58—59°. Both preparations give satisfactory analytical figures for $C_{15}H_{11}ON$ and are probably polymorphs; the picrate from each melted at 159° and did not depress the melting point of the other.

1-Hydroxyacridine has already been synthesised by several routes (cf. Jensen and Rethwisch, *J. Amer. Chem. Soc.*, 1928, **50**, 1144; Albert and Ritchie, *J.*, 1943, 458). On heating 2-ethoxydiphenylamine-2′-carboxylic acid with concentrated sulphuric acid, Matsumara (*J. Amer. Chem. Soc.*, 1927, **49**, 811) obtained a mixture of 1-hydroxy- and 1-ethoxy-acridone, from which the former was separated and then reduced by sodium in amyl alcohol to 1-hydroxyacridine. Starting from 2-methoxydiphenylamine-2′-carboxylic acid, Freeman and Lions (*Proc. Roy. Soc. N.S. Wales*, 1940, **74**, 520) found difficulty in repeating this preparation, but claimed a 60% yield of hydroxyacridine by a modified process which they did not describe in detail. Ullmann (*Annalen*, 1907, **355**, 342) believed the yellow substance, m. p. 293°, which he obtained by the action of concentrated sulphuric acid on 2-methoxydiphenylamine-2′-carboxylic acid to be 1-methoxyacridone. In our hands this yellow product of ring-closure always contained sulphur and gave analytical figures approaching those for a monosulphonated methoxyacridone. Variation in the concentration of acid, temperature, and duration of heating, and the use of acetic–sulphuric acid mixtures failed to give sulphur-free products (cf. Matsumara, *J. Amer. Chem. Soc.*, 1935, **57**, 1533). Authentic 1-*methoxyacridone* was, however, obtained as buff-coloured needles, m. p. 284—285° by heating 2-methoxydiphenylamine-2′-carboxylic acid with phosphorus oxychloride and hydrolysing the intermediate 5-chloro-1-methoxyacridine with boiling dilute hydrochloric acid (cf. Albert and Linnell, *J.*, 1936, 1614). Though reduction with sodium amalgam (Bradbury and Linnell, *J.*, 1942, 377) did not prove satisfactory, the product obtained by reducing 1-methoxyacridone with sodium and amyl alcohol (Kranzlein, *Ber.*, 1937, **70**, 1785) was oxidised smoothly by silver nitrate to 1-methoxyacridine, identical with an authentic specimen. Demethylation with fuming hydrobromic acid (cf. King and Sherred, *J.*, 1942, 415) gave 1-hydroxyacridine (II) in 50% overall yield. 9-Methoxy-1 : 2 : 3 : 4-tetrahydroacridine, prepared by a modification of Petrow's method (*J.*, 1942, 693), was similarly demethylated to 9-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroacridine* (III).

Testing of Reagents.—Following Lutz (*Z. anal. Chem.*, 1920, **59**, 145), it has become the practice to record the sensitivity of a reagent in terms of the smallest amount of metal which will give a perceptible precipitate in a fixed volume of a test solution under standardised conditions. Berg (*loc. cit.*) used three test solutions containing respectively (i) 1 ml. of saturated sodium acetate and 0·5 ml. of 2N-acetic acid, (ii) 1 ml. of 2N-ammonia, and (iii) 0·5 ml. of saturated sodium tartrate and 0·5 ml. of 2N-sodium hydroxide, in a total volume of 5 ml. When the reactions of kations precipitable by ammonia were examined, a few drops of saturated sodium tartrate were added to solution (ii). In each test a few drops of a 2% alcoholic solution of the organic reagent were added.

Now a complex MR_n will not be precipitated until $[M^{n+}][R']^n$ equals or exceeds S_{MR_n}, the solubility product. The observed sensitivity will thus be influenced by the "visibility" of the precipitate and will depend on the concentration of reagent, HR, employed and especially upon the pH of the solution, since the pH will determine what fraction of the reagent is present as the participating ion, R′, and what fraction of the metal added is present as the (hydrated) kation, M^{n+} — a factor of importance if it shows amphoteric character. Masking reagents which form competitive complexes will naturally reduce the measured sensitivity, and small variations in the amount of saturated sodium tartrate added to test solutions (see above) may thus produce a disproportionately large effect on the apparent sensitivity. Berg does not record the pH of his test solutions, which may well have been liable to variation unless the volume measurements were reproduced with some accuracy.

In the present work the three test solutions of pH 5·2, 8·4, and 12·4, respectively, were prepared in bulk from known weights of pure reagents. In each test the total volume was identical, *viz.*, 6·2 ml., and each organic reagent was then present in 0·00323M. concentration. Table I records for each combination the smallest concentration (in μg./ml.) of metal which gave a perceptible precipitate, together with the largest concentration which just failed to do so

TABLE I.

Metal used :	Al^{+++}.			Cr^{+++}.			Fe^{+++}.			Cu^{++}.			Zn^{++}.			Ga^{+++}.		
Test solution :	*A.*	*B.*	*C.*	*A.*	*B.*	*C.*	*A.*	*B.*	*C.*	*A.*	*B.*	*C.*	*A.*	*B.*	*C.*	*A.*	*B.*	*C.*
8-Hydroxyquinoline.	4·4— 1·7 (yellow)	4·4— 1·7 (yellow)	N.P.	8·4— 3·4 (yellow-brown)	16·8— 8·4 (yellow-brown)	N.P.	1·8— 0·9 (greenish-black)	1·8— 0·9 (greenish-black)	N.P.	1·0— 0·4 (yellow-green)	1·0— 0·4 (yellow-green)	1·0— 0·4 (yellow-green)	1·1— 0·4 (yellow)	1·0— 0·4 (yellow)	10·6— 4·2 (yellow)	2·3— 1·1 (yellow)	2·3— 1·1 (yellow)	N.P.
8-Hydroxy-2-methylquinoline.	N.P.	N.P. (—)	N.P.	3·4— 1·7 (yellow-brown)	1·7— 0·8 (yellow-brown)	N.P.	1·8— 0·9 (greenish-black)	0·9— 0·4 (greenish-black)	N.P.	1·0— 0·4 (salmon)	1·0— 0·4 (salmon)	20·5— 10·3 (salmon)	21·0— 10·6 (yellow)	1·0— 0·4 (yellow)	2·1— 10·6 (yellow)	22·5— 11·3 (yellow)	2·3— 1·1 (yellow)	N.P.
8-Hydroxy-5-methylquinoline.	0·9— 0·4 (lemon-yellow)	*	N.P.	1·7— 0·8 (orange)	*	N.P.	3·64 1·8 (greenish-black)	0·9— 0·4 * (greenish-black)	N.P.	1·0— 0·4 (yellow)	*	1·0— 0·4 (yellow)	10·6— 4·2 (yellow)	*	N.P.* (yellow)	2·3— 1·1 (yellow)	*	N.P.
8-Hydroxy-6-methylquinoline.	0·4— 0·2 (yellow)	(—)	N.P.	3·4— 1·7 (orange)	—	N.P.	0·9— 0·36 (dark green)	1·8— 0·9 § (dark green)	N.P.	1·0— 0·4 (yellow)	—	2·1— 1·0 (yellow)	2·1— 1·1 (yellow)	10·6— 2·1 § (yellow)	10·6— 4·2 § (yellow)	1·1— 0·45 (yellow)	2·3— 1·1 § (yellow)	N.P.
8-Hydroxy-7-methylquinoline.	0·4— 0·09 § (lemon-yellow)	0·44— 0·17 § (lemon-yellow)	N.P.§	0·34— 0·17 § (yellow)	0·8— 0·34 § (yellow)	N.P.	0·9— 0·36 § (greenish-black)	0·9— 0·36 § (greenish-black)	36— 18 § (greenish-black)	0·1— 0·04 § (yellow)	1·0— 0·4 § (yellow)	2·1— 1·0 § (yellow)	21·0— 10·6 § (yellow)	4·2— 2·1 § (yellow)	2·1— 1·1 § (yellow)	1·1— 0·23 § (yellow)	1·1— 0·45 § (yellow)	22·5— 11·3 § (yellow)
8-Hydroxy-2-phenylquinoline.	N.P.	N.P. (—)	N.P.	0·84— 0·34 * (yellow-brown)	34— 17 * (yellow-brown)	N.P.	0·9— 0·36 * (yellow-brown)	0·9— 0·36 * (yellow-brown)	N.P.	1·0— 0·4 * (yellow)	1·0— 0·4 * (yellow)	2·0— 1·0 * (yellow)	10·6— 4·2 * (yellow)	2·1— 1·1 * (yellow)	4·2— 2·1 (yellow)	1·1— 0·45 * (yellow)	11·0— 4·5 * (yellow)	N.P.
1-Hydroxyacridine.	N.P.§	N.P.§ (—)	N.P.§	0·84— 0·34 § (red)	8·4— 3·4 § (red)	N.P.§	1·8— 0·9 § (purplish-brown)	0·9— 0·36 § (purplish-brown)	N.P.§	2·1— 1·0 § (red)	1·0— 0·4 § (red)	4·1— 2·0 § (red)	21·1— 10·6 § (red)	1·1— 0·42 § (red)	N.P.§	1·1— 0·45 § (orange red)	0·45— 0·23 § (orange red)	N.P.§
9-Hydroxy-1 : 2 : 3 : 4-tetrahydroacridine.	N.P.	N.P.§ (—)	N.P.	0·84— 0·34 (yellow-brown)	0·84— 0·34 § (yellow-brown)	N.P.	0·9— 0·36 (dark green-brown)	0·9— 0·36 § (dark green-brown)	N.P.	1·0— 0·41 (brown)	1·0— 0·41 § (brown)	2·1— 1·0 (brown)	42·2— 21·1 (yellow)	1·0— 0·41 § (yellow)	211— 105 (yellow)	4·5— 2·3 (pale yellow)	1·1— 0·45 § (pale yellow)	N.P.

N.P. signifies that there was no precipitate of complex.
— signifies that no observation was made.
§ This symbol denotes observations made on a hot solution in those cases in which the organic reagent was insoluble in the cold test solution but soluble at 80°.
* Where the reagent was insoluble in both hot and cold solutions evidence of complex formation could sometimes be obtained if its colour or appearance was sufficiently distinctive.
Sensitivity limits (cf. p. 1490) are given as μg./ml.
The colour of the precipitated complex is recorded below the sensitivity limits.
8-Hydroxy-2 : 4-dimethylquinoline was tested only with aluminium and failed to form a complex in any of the three test solutions.

under the standardised conditions of testing detailed below (p. 1497). These seldom differed by a factor greater than two, and the larger value was adopted as a conservative estimate of sensitivity since more precise values were not required for the present work and, indeed, may well be without real significance (cf. Feigl, *Z. angew. Chemie*, 1930, **43**, 550). One expected result should be recorded. When oxine was tested with aluminium in the ammoniacal buffer (*B*), precipitation failed with concentrations of metal less than 4·4 μg./ml., but with 175 μg./ml. very little precipitate appeared, and with 220 μg./ml. or stronger aluminium solutions no precipitate at all was obtained. Calculation showed that in these concentrated solutions there was insufficient oxine to form the normal 3 : 1 complex, $AlOx_3$ (Ox = C_9H_6ON), and the metal is probably held in solution (as salt of the ions, $[Al(H_2O)_2Ox_2]^+$, $[Al(H_2O)_4Ox]^{++}$, or $[Al(OH)_2Ox_2]^-$, etc.

Discussion.—In agreement with Merritt and Walker, we find that 2-methyloxine (I) will not give a precipitate with aluminium under any conditions. However, since this peculiarity is not shared by any of the other methyloxines, the effect cannot be due simply to a general increase in size as they suggest. Since 2 : 4-dimethyloxine, 2-phenyloxine (I; R = C_6H_5), 1-hydroxyacridine (II), and 9-hydroxy-1 : 2 : 3 : 4-tetrahydroacridine (III) likewise fail to form insoluble complexes with aluminium, whilst forming insoluble complexes with a number of other bi- and ter-valent kations, it must be concluded that this specific effect is caused by substitution adjacent to the nitrogen atom.

Now it has been shown (Goto, *J. Chem. Soc. Japan*, 1933, **54**, 725; 1935, **56**, 314; Fleck and Ward, *Analyst*, 1933, **58**, 388; 1937, **62**, 378) that, above a pH value characteristic for each element, the percentage of a metal precipitated by oxine rises rapidly from zero to its maximum value as the alkalinity increases. Over a range of hydrogen-ion concentrations, precipitation is complete, but the fraction precipitated rapidly decreases again to zero at high alkalinities with metals of amphoteric character. Similar characteristics are displayed by other organic precipitants. Table II records a sensitivity exponent p*L*, defined as $pL = -\log_{10}$.(limiting

TABLE II.

	Al^{+++}.	Cr^{+++}.	Fe^{+++}.	Cu^{++}.	Zn^{++}.	Ga^{+++}.	Average p*L* values with that for aluminium excluded.	Average p*L* values with that for aluminium included.
Oxine	3·3	3·3	4·0	4·5	4·5	4·0	4·1	3·9
2-Methyloxine	N.P.	4·0	4·3	4·5	4·5	4·0	4·3	—
5-Methyloxine	4·0	4·0	4·3	4·5	3·5	4·0	4·1	4·1
6-Methyloxine	4·3	3·7	4·3	4·5	4·2	4·3	4·2	4·2
7-Methyloxine	4·3	4·7	4·3	5·3	4·2	4·3	4·6	4·5
2-Phenyloxine	N.P.	4·3	4·3	4·5	4·2	4·3	4·4	—
1-Hydroxyacridine	N.P.	4·5	4·5	4·5	4·5	4·9	4·6	—
9-Hydroxytetrahydroacridine	N.P.	4·5	4·5	4·5	4·5	4·8	4·6	—

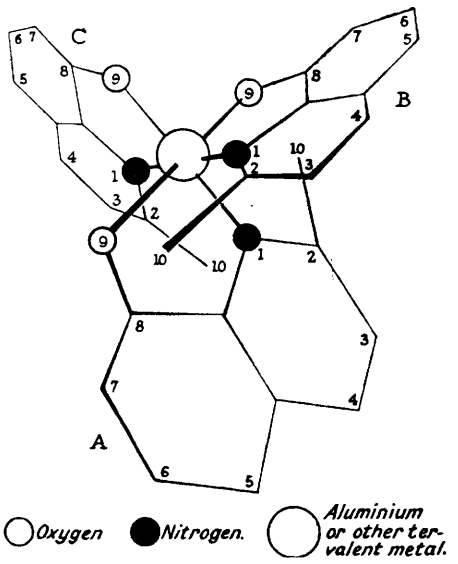
Values for oxine and derivatives cannot be compared directly with those for the acridines as these form 2 : 1 complexes. The composition of the complexes formed by 2-phenyloxine has not been determined.

concentration in gram-equivalents/l.). (In default of comprehensive data relating the percentage of element precipitated to the pH of the solution for each metal and reagent studied, and where the sensitivities in the three test-solutions *A*, *B*, and *C* were not identical, we have adopted values from the medium where the greatest sensitivity was shown.) When sensitivity is expressed in the customary manner as μg./ml., a false impression may be conveyed of the difference in sensitivities of the reactions of the same reagent with various metals of widely differing atomic weights or of different valency. This is to some extent corrected by the above device which, moreover, facilitates the comparison of one reagent with another.

It is perhaps surprising to note from Table II how small are the individual variations of sensitivity among the oxine derivatives from the average value, p*L* = 4·2. The most striking result is the superior average sensitivity of 7-methyloxine which we attribute to a steric effect discussed below (page 1495). The introduction of a methyl group elsewhere in the oxine skeleton produces only a slight general increase in sensitivity which, like that shown by 2-phenyloxine and the acridine derivatives (II) and (III) may well be related to the increased molecular weight and decreased solubility of these reagents, though the validity of such generalisations must await studies with a wider range of metals and an exact knowledge of the compositions of the complexes actually precipitated.

Whilst the oxine complexes of bivalent elements are often hydrated, those of tervalent

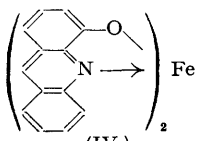
aluminium, iron, gallium, and indium are invariably anhydrous. *Gallium* and *indium* derivatives of 8-hydroxy-2-methylquinoline have now been prepared and, like the *thallic* derivative of 5 : 7-dibromo-8-hydroxyquinoline, these yellow complexes are also anhydrous and conform to the general formula, MR_3; they are all soluble in chloroform. Though Chirnside (*loc. cit.*) has used *X*-ray powder photographs in studying the co-precipitation of metal–oxine complexes, no complete determination of structure has yet been carried out, but it seems reasonable to suppose that with tervalent elements of co-ordination number six the three heterocyclic ligands form chelate rings in planes mutually at right angles. Apart from mirror-image isomerism two arrangements are possible. The figure depicts one of these possible arrangements for the 2-methyloxine complex of a tervalent metal. In the alternative configuration the three nitrogen atoms are located in the same quadrant diametrically opposite to the three oxygen atoms; but, since this arrangement would bring all three methyl groups into one and the same quadrant, the less crowded arrangement of the figure is more probable on energetic grounds. It will be noted that the 2-methyl group of each ligand is directed towards the oxygen (or nitrogen) atom of the neighbouring chelate ring at right-angles to it. The actual separation could be estimated by projections to scale in the planes of A, B, and C if reliable data for the appropriate atomic parameters were available. The octahedral covalent radius of aluminium is not recorded, but by use of a value of 1·30 A. (obtained by increasing the quadrivalent covalent radius of 1·26 A. by 3%, this being the order of increase for certain other elements) together with conventional atomic radii for the remaining elements the lengths C_{methyl}–Oxygen (B_{10} to A_9, and A_{10} to B_9) and C_{methyl}–Nitrogen (C_{10} to A_1) are nearly equal at about 2·7 A., *i.e.*, shorter than the distance of approach accepted for non-bonded atoms. The hydrogen atoms of the methyl group are naturally still closer, and it would appear that purely steric considerations would prohibit the formation of the aluminium complex of 2-methyloxine. However, judging from electronegativity differences (Pauling), the Al–O and Al–N bonds show some 65% and 45% ionic character. Magnetic data certainly show that the nickel complex of oxine contains ionic bonds ($\mu = 3{\cdot}4$ Bohr magnetons; Mellor and Craig, *Proc. Roy. Soc. N.S. Wales*, 1940, **74**, 479). Great uncertainty must therefore attach to the choice of bond angles and distances appropriate to the central chelate rings of complexes such as those depicted in the figure. Reference to a model shows how markedly any variation in these parameters may influence possible steric hindrance to complex formation, and, though simultaneous changes in the dimensions of the ring systems of the reagent may well be of subsidiary importance, the possibility of in-plane or out-of-plane displacement of the interfering 2-substituent cannot be ignored.



Whilst the ionic radius of aluminium is 0·50 A., that of gallium (which is reported as having the same tetrahedral covalent radius as aluminium, *viz.*, 1·26 A.), tervalent iron, chromium, indium, and thallium increase in the order 0·60, 0·62, 0·64, 0·81 and 0·95 A. The fact that we have found both gallium and indium to form well-defined 3 : 1 *complexes* with 2-methyloxine must not, however, be taken as evidence that mere increase of size has alone sufficed to reduce steric hindrance to negligible proportions. All these kations are less electropositive than aluminium and their bonds to oxygen and nitrogen should be more covalent in character; in certain cases (*i.e.*, with iron and chromium) additional orbitals are available which should lead to stronger bonds. The distinctive behaviour of aluminium in *not* forming complexes with certain derivatives of oxine substituted adjacent to the nitrogen atom is thus to be attributed to its (normally) small tendency towards such complex formation being still further reduced by the additional energy barrier imposed by unfavourable steric factors, a hypothesis capable of being tested by measurements of the instability constants of a series of tervalent metal complexes with oxine and methyloxines variously substituted. It seems reasonable to suppose that with 2-substituents bulkier than methyl the energy barrier imposed by steric factors might be

sufficient to prevent the formation of, *e.g.*, aluminium, chromium, and gallium complexes, whilst permitting those of indium and thallium. For the present the development of such reagents which would owe their greater selectivity to controlled steric hindrance must proceed empirically.

Steric hindrance to complex formation of the type discussed in the case of 2-methyloxine must occur also when the interfering group forms part of an attached ring system, as shown by the failure of the hydroxyacridines (II) and (III) to give insoluble complexes with aluminium. Our speculations are strengthened by interesting results obtained by Freeman and Lions (*loc. cit.*) in a preliminary study of the potentialities of 1-hydroxyacridine (II) as an analytical reagent, though they did not interpret them. It was found that ferrous and ferric ions gave the same 2 : 1 complex (IV); presumably steric hindrance does not permit the formation of the expected 3 : 1 complex, but reduction of ferric ions by the phenolic reagent gives ferrous ions from which (IV) is obtained. The non-existence of a 3 : 1 complex from chromic ions is likewise to be expected; but the formation of an analogue of (IV) is equally unlikely in view of the difficulty of reducing Cr^{+++} to Cr^{++}. It is thus of great interest that the complex actually obtained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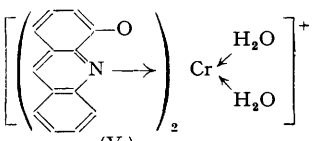
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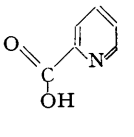
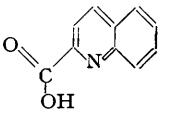

(VI.)


(VII.)

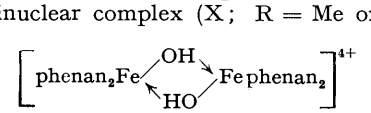
could be formulated as (V). In (IV) and (V) the cyclic ligands need not be coplanar and do not interfere sterically with one another. The soluble complex formed by aluminium and 2-methyloxine, and aluminium in the presence of a deficiency of oxine (page 1492) may well be similarly constituted. That certain metals do react with 2-methyloxine, thereby displacing protons, is demonstrable by electrometric titration, and we will discuss the exact constitution of these complexes in the sequel. We have confirmed Freeman and Lions's statement that aluminium and bismuth are not precipitated by 1-hydroxyacridine, but disagree in obtaining an orange-red precipitate with stannous tin in (hot) test solutions *A* and *B*. In addition to those cases mentioned in Table I, 9-hydroxy-1 : 2 : 3 : 4-tetrahydroacridine (III) gave yellow complexes with Bi^{+++}, Pb^{++}, Hg^{++}, Co^{++}, and Ni^{++}, and a black precipitate with Ag^{+}. The anhydrous *zinc* complex, $Zn(C_{13}H_{12}ON)_2$, was readily obtained pure. Gallium is incompletely precipitated by 1-hydroxyacridine, and the composition of the basic *precipitate* agrees most closely with that of the 1 : 1 complex, $[Ga(C_{13}H_8ON)(OH)_2(H_2O)_2]$.

The relation between 8-hydroxyquinoline (I; R = H) and 1-hydroxyacridine (II) is paralleled by that between picolinic acid (VI) and quinaldinic acid (VII). The steric factors which inhibit 3 : 1 complex formation with the hydroxyacridine (II) may likewise be expected to operate with quinaldinic acid, and it is significant that, whilst the latter forms normal 2 : 1 complexes of considerable analytical importance with cadmium, copper, zinc, and other bivalent metals, the complexes with aluminium and chromium are described as "basic" (Flagg, "Organic Reagents," New York, 1948, 246) and their compositions are probably based on, *e.g.*, (V) and (VIII) (see below). Picolinic acid, on the other hand, forms the expected 3 : 1 complexes with tervalent iron, chromium, and cobalt of type, $M(C_6H_4O_2N)_3$, as well as basic complexes such as $Fe(C_6H_4O_2N)_2OH$ (VIII; Ley and Ficken, *Ber.*, 1917, **50**, 1133; Ley, Schwarte, and Münnich, *Ber.*, 1924, **57**, 355; Gorvin, *J.*, 1944, 25). A compound with aluminium does not seem to have been described, and no study has as yet been published of the complexes of 6-substituted picolinic acids or 3-substituted *iso*quinoline-1-carboxylic acids which, as analogues of 2-substituted oxines, might be expected to reveal the influence of steric factors on the ease of complex formation.

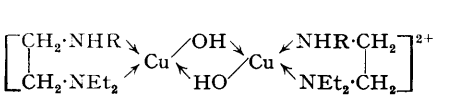
Failure to obtain a *precipitate* of the expected composition has hitherto provided the only indication of the operation of steric factors in the particular examples of the uncharged inner complexes discussed above, but steric factors may be equally important in the formation and stability of hydrophilic complexes carrying a net charge—where the simple diagnostic of solubility fails. That this is in fact the case is seen in the reactions of polypyridyls and *o*-phenanthrolines with ferrous ions, which have been extensively studied on account of their analytical importance both in the colorimetric estimation of iron and as oxidation–reduction indicators. Though 2 : 2′-dipyridyl and *o*-phenanthroline give red tris-bidentate complexes, steric hindrance operates to reduce the colour intensity with 3 : 3′-dimethyl-2 : 2′-dipyridyl

(Cagle and Smith, *J. Amer. Chem. Soc.*, 1947, **69**, 1860), 6-substituted 2 : 2′-dipyridyls (Burstall, *J.*, 1938, 1664), and 2-methyl-1 : 10-phenanthroline (Pfeiffer and Christeleit, *J. pr. Chem.*, 1938, **151**, 127), and completely inhibits complex formation in the cases of many 6 : 6′-disubstituted 2 : 2′-dipyridyls (Burstall, *loc. cit.*), 2-2′-quinolylpyridine, and 2 : 2′-diquinolyls (Smirnoff, *Helv. Chim. Acta*, 1921, **4**, 802). Similar behaviour in the case of bivalent kations which give colourless complexes with substituted dipyridyls and analogous ligands can be inferred from their ultraviolet absorption spectra or from their influence upon the spectra of other, coloured complexes, or more directly by measurements of their consecutive instability constants. The extent to which steric factors modify the stability of such complexes and cause departure from the " natural sequence " of stability already noted among different metals with varied ligands (Mellor and Maley, *Nature*, 1947, **159**, 370; 1948, **161**, 436; Irving and Williams, *ibid.*, 1948, **162**, 764) will be discussed in later papers.

Where 3 : 1 complexes are sterically inhibited, an alternative solution to that adopted in the case of, *e.g.*, chromium and 1-hydroxyacridine [page 1494 and formula (V)] is illustrated by the case of ferric ions and *o*-phenanthroline (phenan = $C_{12}H_8N_2$). Red tris-*o*-phenanthroline–ferrous ions, $[Fe\ phenan_3]^{++}$ are oxidised with difficulty to feebly-blue tris-*o*-phenthroline–ferric ions, $[Fe\ phenan_3]^{+++}$; the change is reversible with an oxidation potential of 1·14 volts. When, however, phenanthroline is allowed to react directly with ferric ions, the above tris-complex is not formed, but the binuclear complex (IX) results. An analogous case is provided by cupric ions, which react normally with ethylenediamine, *N*-methyl-, and *NN*′-diethyl ethylenediamine to give 2 : 1-complexes, whilst *N*-methyl (or -ethyl)-*N*′-diethylethylenediamine gives the binuclear complex (X; R = Me or Et).



(IX.)



(X; R = Me or Et.)

It is worth noting that simple 3 : 1 complexes of dimethylglyoxime (dmg = $C_4H_7O_2N_2$), or of sulphamide, $NH_2{\cdot}SO_2{\cdot}NH_2$, appear to be incapable of existence (cf. Mann, *J.*, 1933, 412). This explains the remarkable result of great analytical importance that, for the two so closely related elements cobalt and nickel, *o*-nitrosophenols are specific reagents for the former and dialkylglyoximes and analogously constituted substances for the latter. For, though the stability of complexes increases along the transition series Fe^{++}, Co^{++}, Ni^{++}, and Cu^{++}, with cobaltous complexes less stable than those of bivalent nickel (Irving and Williams, *loc. cit.*) the former is readily oxidised to the tervalent state by the phenolic reagent and in this state forms a far more stable complex (cf. the sequence $[Co\ en_3]^{+++} \gg [Ni\ en_3]^{++} > [Co\ en_3]^{++}$ for which the decadic logarithms of the instability constants are −48·7, −18·6, and −13·8, respectively; Bjerrum, " Metal Ammine Formation in Aqueous Solution," Copenhagen, 1941). On the other hand, whilst nickel forms with dimethylglyoxime an insoluble, planar inner-complex, $[Ni(dmg)_2]$, which carries no net charge, the corresponding cobaltous complex would have an unpaired 5*d* electron. After oxidation to the cobaltic state, the formation of a tris-complex with dimethylglyoxime is presumably hindered sterically, and instead there must result soluble complexes of the type, $[Co(dmg)_2(NH_3)_2]^+Cl^-$, studied by Tschugaieff (*Z. anorg. Chem.*, 1905, **46**, 144; *Ber.*, 1908, **41**, 2226).

Craig (*J.*, 1946, 534) has shown that the feebly basic character of the ring nitrogen atom in 1-amino-9-methyl- (or 1 : 9-diamino-)acridine can be explained in terms of steric hindrance by the 1 and 9 substituents to the approach of oxonium ions. The presence of a 7-substituent in a tris-8-hydroxyquinoline complex (cf. Fig.) may similarly (if less effectively) restrict the accession of oxonium ions and so permit the complex to persist in solutions of greater acidity. It is suggested that this explains, at least in part, the enhanced stability of 8-hydroxy-7-methylquinoline (page 1492) and the stability towards acid of the metal complexes of 5 : 7-dibromo-(or -dichloro)-8-hydroxyquinoline, 7-iodo-8-hydroxyquinoline-5-sulphonic acid, and the 7-arylazo-8-hydroxy-5-methylquinolines mentioned above (page 1489).

A final aspect of steric hindrance remains to be mentioned. Albert *et al.* (*Brit. J. Exp. Path.*, 1947, **28**, 69) have advanced the hypothesis that the bacteriostatic action of certain drugs arises from their ability to chelate with certain trace-metals present in parts of enzyme systems, thus interfering with the metabolic cycle of the bacterium. They showed that 2-methyloxine, 1-hydroxyacridine, and 1-hydroxyphenazine were less effective drugs than oxine [which did not differ greatly from 5-methyl- or 5-propyl-oxine or 8-hydroxypyridino(2′ : 3′-5 : 6)quinoline] and suggested that substitution adjacent to the nitrogen atom of the oxine ring

system caused steric hindrance to such chelation. Similar measurements have been carried out with some of the reagents tested above. The results, given in Table III, confirm the reduced activity of 1-hydroxyacridine (as compared with oxine) and establish similar behaviour for 2-phenyloxine. However, our figure for 2-methyloxine scarcely differs from that for oxine,

TABLE III.

Bacteriostatic Indexes.

Compound.	Albert *et al.*	This paper.
Oxine	100,000	102,400
2-Methyloxine	13,000	51,200
5-Methyloxine	51,000	—
5-Propyloxine	51,000	—
6-Methyloxine	—	51,200
7-Methyloxine	—	51,200
2-Phenyloxine	—	12,800
1-Hydroxyacridine	4,000	12,800
9-Hydroxy-1 : 2 : 3 : 4-tetrahydroacridine	—	102,400

The bacteriostatic index given represents the smallest concentration expressed as 1/molarity which completely inhibited the growth of *Staph. aureus* under the conditions of test used by Albert *et al.* (*loc. cit.*).

whilst that for the reduced hydroxyacridine is identical and shows no indication of the operation of steric factors in its bacteriostatic action. The problem is clearly too complex to warrant a further discussion of the meagre evidence at present available.

EXPERIMENTAL.

4-o-*Hydroxyanilopentan*-2-*one*.—Acetylacetone (5 g.; 0·05 mol.) and *o*-aminophenol (2·75 g.; 0·025 mol.) were heated for 4 hours on a water-bath under reflux. The resultant light-brown solid was rubbed with portions of ether (30 ml. in all) to remove unchanged starting material, and recrystallised from aqueous alcohol; 4-o-*hydroxyanilopentan*-2-*one* separated as light-buff-coloured needles, m. p. 184° (Found: C, 69·0; H, 7·1; N, 7·25. $C_{11}H_{13}O_2N$ requires C, 69·1; H, 6·8; N, 7·3%). Attempts at ring closure by warming with 90% sulphuric acid at 100°, heating under reflux with phosphoric oxide in dry toluene, or heating with a phosphoric oxide–phosphoric acid mixture at 140° or under reflux with *o*-aminophenol hydrochloride and zinc chloride in ethyl alcohol gave unchanged starting materials.

Action of Phenyl-lithium on 8-*Hydroxyquinoline*.—Lithium (1·5 g.; 2 equivalents) was cut up and stirred under dry nitrogen with ether (200 ml.) during the addition of bromobenzene (31·5 g.). After 40 minutes the flask was cooled to 0° and 8-hydroxyquinoline (14·5 g.; one equivalent) in dry ether (300 ml.) was slowly added, whereupon the orange colour changed to lime-yellow. After 10 minutes, water (150 ml.) was added, and the solution neutralised partly with hydrochloric acid and finally with acetic acid. On steam-distillation ether and unchanged oxine were removed. The residue was acidified, boiled with animal charcoal, filtered, and neutralised. The slate-grey product gave on distillation at atmospheric pressure a pale yellow oil, b. p. 320—325°, which solidified on cooling. Recrystallisation from methyl alcohol gave 8-hydroxy-2-phenylquinoline (I; R = C_6H_5) as faintly yellow plates, m. p. 58—59° (Found: C, 81·3; H, 5·0. Calc. for $C_{15}H_{11}ON$: C, 81·45; H, 5·0%). The picrate (yellow needles from alcohol) had m. p. 158—159°. Doebner and Fettbach (*Annalen*, 1894, **281**, 9) give 59° and 152°, respectively. A sample prepared from 8-hydroxy-2-phenylquinoline-4-carboxylic acid by decarboxylation, following Doebner and Fettbach (*loc. cit.*), formed almost colourless needles, m. p. 85·5° after repeated crystallisation from ethyl alcohol (Found: C, 81·8; H, 5·0. Calc.: C, 81·45; H, 5·0%). Admixed with the sample, m. p. 58—59°, the mixed m. p. was 58° and the picrate, m. p. 159—160°, did not depress the melting point of the former specimen.

Ring-closure of 2-*Methoxydiphenylamine*-2′-*carboxylic Acid*.—(*a*) The diphenylamine (16 g.; Ullmann, *loc. cit.*) was refluxed at 120° with freshly distilled phosphorus oxychloride (90 ml.) until evolution of hydrogen chloride had ceased (2 hours). After removal of excess of the oxychloride by distillation under reduced pressure the viscous residue was triturated with ice–water and dilute ammonia. Crude 5-chloro-1-methoxyacridine was collected and at once hydrolysed by boiling for ½ hour with 5% hydrochloric acid (700 ml.). 1-*Methoxyacridone* which separated on cooling was collected (yield, 12·8 g.) and recrystallised from 50% aqueous acetic acid from which it separated in light-buff needles, m. p. 284—285° (Found: C, 74·5; H, 5·1; N, 6·3. $C_{14}H_{11}O_2N$ requires C, 74·6; H, 4·9; N, 6·3%). The acridone was insoluble in water and dilute acids and alkali. Solutions in glacial acetic acid and concentrated sulphuric acid showed a blue-green fluorescence.

(*b*) When ring-closure was attempted by heating 2-methoxydiphenylamine-2′-carboxylic acid with 75% sulphuric acid or various sulphuric–acetic acid mixtures at 105° for 15 minutes, the starting material was recovered unchanged. With concentrated sulphuric acid no reaction took place at room temperature (1 hour) or at 55° (20 minutes), but at 70° and 98° heating for 5—55 minutes gave a yellow *product*, m. p. 293° from 50% aqueous acetic acid; the properties agreed with those of Ullmann's "9-methoxyacridone." However, it contained sulphur, a typical analysis being C, 56·2; H, 3·5; N, 5·2 S, 10·7 [Calc. for $C_{14}H_{11}O_2N$: C, 74·6; H, 4·9; N, 6·3%. $C_{14}H_{10}O_2N(SO_3H)$ requires C, 55·1; H, 3·6; N, 4·6; S, 10·5%].

Reduction of 1-*Methoxyacridone to* 1-*Methoxyacridine*.—A solution of 1-methoxyacridone (11·3 g.

in 600 ml. of amyl alcohol) was boiled under reflux, and sodium (30 g.) was added gradually in small pieces until the initial fluorescence had vanished. The amyl alcohol was removed by steam-distillation and the resulting crude 1-methoxyacridane extracted with ether. After removal of the solvent, the sticky yellow residue was dissolved in alcohol (150 ml.) and oxidised by the slow addition of 2N-silver nitrate (30 ml.); the reaction was completed by gentle warming and, after the mixture had been kept overnight, excess of dilute hydrochloric acid was added, and the mixture filtered (after warming to coagulate the silver chloride). On basification, 1-methoxyacridine separated from the filtrate and gave light yellow needles (9 g.), m. p. 130°, from aqueous alcohol. Jensen and Rethwisch (*loc. cit.*) give m. p. 130—131°. Demethylation by boiling under reflux with hydrobromic acid (*d* 1·48) for 3—4 hours gave 1-hydroxyacridine (II) in 97% yield; m. p. 116—117° (from 75% ethyl alcohol).

Preparation of 9-*Hydroxy*-1 : 2 : 3 : 4-*tetrahydroacridine* (III).—9-Methoxy-1 : 2 : 3 : 4-tetrahydroacridine (5 g.; Petrow, *J.*, 1942, 693) was heated under reflux for 3½ hours with hydrobromic acid (45 ml.; *d* 1·48). After removal of excess of acid by distillation under reduced pressure the residue was neutralised with sodium carbonate, and 9-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroacridine* (III) was collected (yield, 4·5 g.). It crystallised from alcohol–water in colourless needles, m. p. 89—90° (Found : C, 78·3; H, 6·5; N, 7·0. $C_{13}H_{13}ON$ requires C, 78·3; H, 6·6; N, 7·0%).

Testing of Reagents.—(a) *Composition of test solutions.* Solution *A* was prepared from 250 ml. of 2N-acetic acid, 225 g. of sodium acetate (trihydrate), and 500 ml. of distilled water. Solution *B* contained 40 ml. of 2N-ammonium hydroxide, 320 g. of ammonium acetate, 30 g. of sodium potassium tartrate, and 850 ml. of distilled water. Solution C contained 500 ml. of 2N-sodium hydroxide, 275 g. of sodium potassium tartrate, and 500 ml. of distilled water. Stock solutions of metals containing 0·01 g.-atom/l. were prepared from "Spekpure" metals or "AR" salts. Each organic reagent was used in the form of a 0·1M. solution in ethyl alcohol.

(b) *Procedure.* 4·0, 0·4, and 0·04 Ml. of 0·01N. metal solution were placed severally in three test-tubes (selected 6 × ½-inch tubes which had been previously boiled in nitric acid, repeatedly rinsed in distilled water, and finally cleaned and dried with live steam); 2 ml. of buffer *A*, 0·2 ml. of organic reagent, and sufficient water to make the total volume up to 6·2 ml. were then added. A fourth tube containing 2 ml. of buffer, 0·2 ml. of reagent, and 4 ml. of water, but no metal, served to determine the behaviour of the reagent, whilst an additional control tube containing 0·4 ml. of metal solution, 2 ml. of buffer, and 3·8 ml. of water, but no reagent, ensured that the possible precipitation of metal as hydroxide or basic salt under the conditions of testing employed could not be mistaken for complex formation. Each tube was then heated for 15 minutes at 80° and examined, when cold, for precipitation. If results were positive with as little as 0·04 ml. of 0·01N. metal solution, the whole series was repeated with 0·001N. metal solutions and, if necessary, with still more dilute solutions, until precipitation did not occur. The limits of sensitivity were then more exactly delimited by carrying through a series of tests with 0·4, 0·2, 0·1, and 0·04 ml. of metal solution of the appropriate strength. The sequence of testing was then repeated with buffer solutions *B* and *C*, in turn, and the whole procedure repeated for each reagent and every metal studied. In a few cases the reagent was insoluble in the cold buffer, though freely soluble at 80°, whilst the metal complex was sufficiently insoluble for its formation to be detected with certainty when observations were made on the hot solution. In some cases, where the reagent was insoluble in a particular buffer both hot and cold, the colour or appearance of the metal complex was often sufficiently distinctive to enable its formation to be detected despite the presence of excess of reagent. Sensitivities obtained under these exceptional conditions are liable to have been under-estimated.

The Complex of Gallium and 8-*Hydroxy*-2-*methylquinoline.*—A solution of gallium chloride (0·0516 g. of metal) in 25 ml. 0·2N-hydrochloric acid was diluted to 250 ml., and a 5% excess of 2-methyloxine added in the form of a 2% alcoholic solution. A yellow colour developed. After heating to 60—70°, 2N-ammonia solution was added dropwise until the separation of a flocculent precipitate was complete. After digestion for 1½ hours, this became granular and was collected on a sintered-glass crucible, washed thoroughly to remove excess of reagent, and dried at 130° for 1 hour. The following weights of precipitate were obtained : 0·3759, 0·3763, 0·3828, 0·3885, and 0·3906 g.; calc. for $Ga(C_{10}H_8ON)_3$, 0·4025 g. The defect in weight might be caused by incomplete precipitation, losses during washing due to the solubility of the *complex*, or to the formation of a basic complex or of a stoicheiometric 3 : 1 complex mixed with gallium hydroxide. The last two alternatives were excluded by determining the amount of 2-methyloxine present in the heaviest precipitate. This was dissolved in 500 ml. of 2N-hydrochloric acid, and 50 ml. containing 1 g. of potassium bromide aliquots were titrated with M/60-bromate, any excess over that required to form 2-methyl-5 : 7-dibromo-8-hydroxyquinoline being determined iodometrically [Found : $C_{10}H_8ON$, 87·2. $Ga(C_{10}H_8ON)_3$ requires 87·2%]. The procedure was first checked against pure 2-methyloxine. It is noteworthy that hot water was used to wash the first two precipitates and cold water for the others. Like the gallium–oxine complex (Geilmann and Wrigge, *Z. anorg. Chem.*, 1932, **209**, 129), the 2-methyloxine complex appears to be appreciably soluble in cold water.

The Complex of Indium and 8-*Hydroxy*-2-*methylquinoline* (with Miss M. Tutt.)—A yellow precipitate formed when 2N-sodium acetate solution was added to 150 ml. of a solution of indium (30—40 mg.) in dilute acid containing a 2% excess of 2-methyloxine. Addition of ammonia produced a further precipitate and after digestion at 70° for 1 hour it was collected, washed with the acid wash-liquor recommended by Berg (*Z. anorg. Chem.*, 1932, **204**, 208), and dried at 135°. The low results (Found : 0·1385, 0·1833, and 0·1688 g. Calc. : 0·1953 g.) were partly due to the solubility of the precipitate in the acid wash-liquor, and partly to the co-precipitation of indium hydroxide since the pH of the filtrate was found to be 5·5 or greater. Subsequent samples, made by adding ammonia dropwise to an indium solution containing 1 g. of sodium potassium tartrate, weighed 0·1951 and 0·1938 (calc., 0·1953 g.), 0·1935 and 0·1936 (calc., 0·1956 g.), and 0·2034 and 0·2023 g. (calc., 0·2031 g.). Though quantitative precipitation and recovery of the *complex* was not attained, its composition was established as before by bromination [Found : $C_{10}H_8ON$, 80·6. $In(C_{10}H_8ON)_3$ requires $C_{10}H_8ON$, 81·5%].

The Complex of Zinc and 9-*Hydroxy*-1 : 2 : 3 : 4-*tetrahydroacridine.*—A 3% excess of an alcoholic solution of the reagent was added to 50 ml. of zinc sulphate solution (containing 0·0525 g. of metal)

acidified with 5 ml. of 2N-hydrochloric acid. After addition of 2 g. of ammonium acetate and heating to 80—90°, 2N-ammonium hydroxide was added dropwise until precipitation was complete. After digestion at 90° for 1 hour, the precipitate was collected, washed with hot water, and dried at 135° [Found: 0·3593, 0·3524, and 0·3554 g.; C, 67·4; H, 5·1; N, 6·2; Zn, 14·2. $Zn(C_{13}H_{12}ON)_2$ requires C, 67·6; H, 5·2; N, 6·1; Zn, 14·2%].

The Complex of Gallium and 1-*Hydroxyacridine.*—0·0205 G. of gallium was precipitated with 1-hydroxyacridine as described above for the zinc complex. The weights of precipitate were 0·1060, 0·0981, and 0·0895 g., and a typical analysis gave C, 53·9; H, 3·6; Ga, 22·6%.

The Complex of (Tervalent) Thallium and 5 : 7-*Dibromo*-8-*hydroxyquinoline* (with E. J. RISDON).—Thallium (15 mg.), as nitrate, in 100 ml. of 0·01N-hydrochloric acid was oxidised by 15—30 ml. of bromine water. After heating to 50°, dibromo-oxine (80 mg. in 150 ml. of acetone) was added and 2N-ammonium hydroxide run in dropwise until precipitation was complete (pH ~8·6). The solution was digested for 1 hour and cooled, and the yellow complex collected and dried (at 120°). In successive experiments it weighed 78·4 and 78·6 mg. [Calc. for $Tl(C_9H_5ONBr_2)_3$: 81·5 mg.].

Bacteriological Tests (with Miss M. N. JENNINGS).—Oxine and other reagents were tested, by the serial-dilution method, for their power to inhibit the growth of one strain each of *Staph. aureus* and *Bact. coli*. Aqueous M/80 solutions of each substance, after being sterilised in the autoclave, were diluted in two-fold series, and 0·2 ml. of each dilution was added to 1·8 ml. of nutrient broth. The tubes were inoculated with 1 drop of a 1 in 1,000 dilution of an overnight culture of the organism. The results were observed after incubation overnight. As already noted by Albert *et al.* (*loc. cit.*), oxine and its analogues proved to be relatively ineffective towards *Bact. coli*.

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