575. Cinnolines. Part XXIX.* 8-Hydroxycinnoline and Some of its Derivatives.

By E. J. Alford, H. Irving, (Miss) H. S. Marsh, and K. Schofield.

8-Hydroxy- and 8-methoxy-cinnoline have been synthesised, together with a number of 4-substituted derivatives. The absorption spectra of 8-hydroxy- and 8-hydroxy-4-methyl-cinnoline in acid, neutral, and alkaline solutions have been measured and compared with those of 8-methoxycinnoline and corresponding compounds of the quinoline series. The acidic and basic dissociation constants of the 8-hydroxycinnolines are compared with those of the 8-hydroxyquinolines, and discussed in relation to their respective bacteriostatic actions and abilities to chelate with metals. The quantitative dibromination of 8-hydroxycinnoline has been used to determine the solubility of the compound.

8-Hydroxyquinoline (" oxine ") (XVII) forms chelated complexes with a large number of metals, and many attempts have been made to improve its selectivity as an analytical reagent by the introduction of substituents or by annelation (Irving, Butler, and Ring, *J.*, 1949, 1489, and refs. therein). However, apart from the effect of 2-substituents which prevent the formation of insoluble tris-complexes with aluminium, possibly for steric reasons, no instances of exceptional behaviour have been reported. Derivatives of oxine have also been used extensively in medicine, and the relation between the bacteriostatic action of compounds of this type and the strength of the complexes they form with metals of biological interest has been thoroughly studied (Albert *et al.*, *Biochem. J.*, 1947, **41**, 529, 534; *Brit. J. Exp. Path.*, 1945, **26**, 160; 1947, **28**, 69).

The significance of the acidic and basic dissociation constants of ampholytes such as oxine for interpretations of their antibacterial activity and their role in analytical chemistry is now beginning to be appreciated, and a more extensive knowledge of the capacities to form metal complexes and related properties of other heterocyclic systems which incorporate the characteristic chelating group of oxine would clearly be of interest. To this end we have begun an investigation of some analogues in which a methine group is replaced by a nitrogen atom. Of the three possible isomers this paper deals with the preparation and properties of 8-hydroxycinnoline (VI) and some of its derivatives. Preliminary measurements on 8-hydroxyquinoxaline (X) have been carried out by Freeman and Spoerri (*J. Org. Chem.*, 1951, **16**, 438), and our results for this reagent and for 8-hydroxyquinazoline (XI) will be communicated later.

Preparation of 8-*Hydroxycinnoline and its Derivatives.*—Synthesis of 8-hydroxycinnoline presented an interesting problem. 2-Amino-3-methoxyacetophenone, a compound of fully authenticated structure, was readily available (Simpson, Atkinson, Schofield, and Stephenson, *J.*, 1945, 646), but previous attempts (Schofield and Simpson, *ibid.*, p. 520) to convert it into 4-hydroxy-8-methoxycinnoline (I ⟶ II) by diazotisation in dilute acid failed. Later work on this type of cyclisation (*idem, J.*, 1948, 1170) clearly demonstrated that its success depended largely upon enolisation of the ketone group, an acid-catalysed process. Accordingly (I) was now diazotised in concentrated hydrochloric acid, whereupon it gave 92% of (II). Comments on the large-scale preparation of (I) will be found in the Experimental section.

Other experiments in the cinnoline series (Alford and Schofield, to be published) made it probable that removal of the hydroxyl group from (II) would be possible by converting the latter into 4-chloro-8-methoxycinnoline (III), which would then react with toluene-*p*-sulphonylhydrazide giving (IV), whence 8-methoxycinnoline (V) would arise by alkaline decomposition. In fact each of these steps proceeded without difficulty, and 8-methoxycinnoline was readily demethylated by hydrobromic acid to 8-hydroxycinnoline (VI). The overall yield of (VI) from *m*-nitroacetophenone (10 steps) was 7·4%, or from *m*-hydroxyacetophenone (8 steps) 16·6%.

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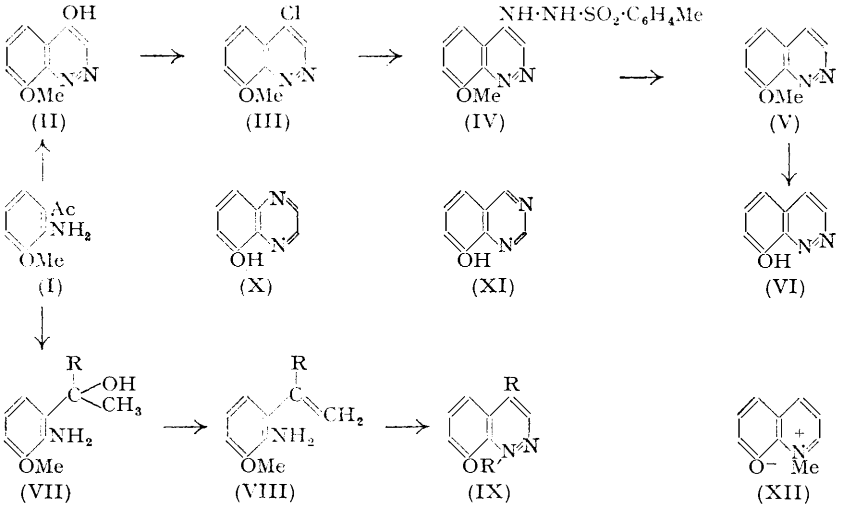
2-Amino-3-methoxyacetophenone is a useful intermediate for the synthesis of 4-substituted 8-hydroxycinnolines. With methylmagnesium iodide and phenylmagnesium bromide it gave the alcohols (VII; R = Me or Ph), which were readily dehydrated. Diazotisation of the ethylenes (VIII; R = Me or Ph) produced 8-methoxy-4-methyl- (IX; R = R′ = Me) and 8-methoxy-4-phenyl-cinnoline (IX; R = Ph, R′ = Me), each of which was converted by hydrobromic acid into the 8-hydroxy-compound (IX; R = Me or Ph, R′ = H).



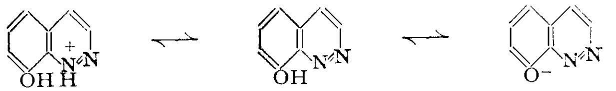
8-Methoxycinnoline, which formed an orange picrate, was a colourless crystalline solid which quickly liquefied and became green in the air. Cinnoline itself behaves in the same way (Morley, *J.*, 1951, 1971). In contrast, 8-methoxy-4-methyl- and 8-methoxy-4-phenyl-cinnoline were orange and yellow respectively. The former was stable, but the latter slowly became sticky in the air.

8-Hydroxycinnoline melted at a higher temperature than the two 4-substituted derivatives, and also much higher than 8-hydroxyquinoline. Whilst the latter is colourless, 8-hydroxycinnoline was bright yellow. 8-Hydroxy-4-methyl- and 8-hydroxy-4-phenyl-cinnoline were light yellow and fawn-coloured, respectively.

8-Hydroxycinnoline formed an orange-red hydrobromide, and the reddish-orange solution of the compound in sodium hydroxide solution deposited a sodium salt on cooling. A crimson dye was formed by coupling with benzenediazonium chloride. Woroshtzow and Kogan (*Ber.*, 1932, **65**, 142) converted oxine " almost quantitatively " into 8-aminoquinoline by means of the Bucherer reaction and we have found conditions which gave 87% of 8-aminocinnoline (isolated as its acetyl derivative) from the hydroxy-compound by this method.

8-Aminocinnoline, a yellow crystalline solid obtained by hydrolysis of its acetyl derivative, gave deep purple solutions in dilute acid, and formed a deeply coloured picrate : Woroshtzow and Kogan (*loc. cit.*) noted that acid solutions of 8-aminoquinoline were deep red. 8-Hydroxycinnoline dissolved slowly in ethereal diazomethane to the accompaniment of brilliant colour changes, and formed a very dark blue product which could not be purified. It is likely that this substance possesses a structure similar to that of the brick-red product obtained by this method from oxine, for which the expression (XII) has been proposed (Caronna and Sansone, *Gazzetta*, 1939, **69**, 24; H. and M. Schenkel-Rudin, *Helv. Chim. Acta*, 1944, **28**, 1456).

Absorption Spectra of 8-*Hydroxycinnolines*.—The absorption spectrum of 8-methoxycinnoline in 95% methyl alcohol is extremely similar to that of 8-hydroxycinnoline, but is displaced slightly towards shorter wave-lengths while the subsidiary maximum at 315 mμ is lacking. The spectrum of the methoxy-compound in 0·01N-sodium hydroxide is virtually unchanged in position, and the apparent decrease in molecular extinction coefficient shown in Table 1 is certainly not a real effect but reflects only the difficulty of handling this very unstable substance. Ionisation of the phenolic group of 8-hydroxycinnoline in 0·01N-sodium hydroxide produces the anion (XIV) and there is a pronounced bathochromic shift of 11, 27, and 65 mμ for the prominent ultra-violet, medium, and long-wave peaks respectively. On solution in 0·01N-hydrochloric acid, the formation of the cation (XIII) again causes a bathochromic shift in the spectrum of 8-hydroxycinnoline amounting to 7, 1, and 70 mμ for the corresponding peaks. The behaviour of the methoxy-compound (V) is almost identical. Table 1 summarises the salient features of these spectra which show remarkable analogies to those of 8-hydroxy- and 8-methoxy-quinoline (Ewing and Steck, *J. Amer. Chem. Soc.*, 1946, **68**, 2181) although they are displaced considerably towards longer wave-lengths, as might have been expected from the introduction of the grouping –N:N– in place of –N:CH–. Phillips, Huber, Chung, and Merritt (*ibid.*, 1951, **73**, 630) have discussed the absorption spectra of the 8-hydroxyquinolines in terms of the structures (XV) to (XVIII) and point out correctly that the inability of 8-methoxyquinolines to undergo comparable structural changes on passing from a neutral to a basic medium explains the virtual identity of their absorption spectra in *cyclo*hexane and aqueous sodium hydroxide. They do not, however, comment on the fact that 8-methoxyquinoline cannot undergo structural changes comparable to (XVII ⟶ XVI ⟶ XV), and we suggest that the close similarity of the absorption spectra of 8-methoxy- and -hydroxy-quinoline in an acidic solvent is strong evidence against the presence in appreciable quantity of the keto-form (XV). The occurrence of this keto-form, first postulated on chemical grounds by Stone and Friedman (*ibid.*, 1947, **69**, 209) and supported by magnetic-susceptibility measurements (Seguin, *Bull. Soc. chim.*, 1946, **13**, 566), is not confirmed by infra-red studies of oxine and its derivatives (Phillips, *J. Amer. Chem. Soc.*, 1949, **71**, 3984), or by our present measurements in the related cinnoline series. The success of the Bucherer reaction in both series might have been counted as support for the existence of the keto-forms according to one theory of the mechanism of this reaction (*Org. Reactions*, **1**, 105; but see Cowdrey, *J.*, 1946, 1046).



(XIII; H_2Cnx^+) (VI; HCnx) (XIV; Cnx^-)

TABLE 1. *Absorption spectra of* 8-*hydroxycinnoline and its derivatives.*

In 0·01N-HCl		*In* 95% *methanol*		*In* 0·01N-NaOH	
λ (mμ)	log ε (mol.)	λ (mμ)	log ε (mol.)	λ (mμ)	log ε (mol.)
8-*Hydroxycinnoline.*					
—	—	—	—	240 (min.)	3·95
252 (max.)	4·62	245 (max.)	4·53	256 (max.)	4·40
289	3·00	270	2·67	290	2·74
304 (max.)	3·07	303 (max.)	3·03	330 (max.)	3·43
—	—	306	3·00	—	—
—	—	315 (max.)	3·04	—	—
335	2·63	320	3·00	359	2·94
430 (max.)	3·32	360 (max.)	3·43	425 (max.)	3·49
8-*Methoxycinnoline.*					
247 (max.)	4·34	242 (max.)	4·64	242 (max.)	4·38
290	3·06	261	3·05	270	2·99
300 (max.)	3·08	295 (max.)	3·32	295 (max.)	3·12
328	2·74	310	3·25	310	3·00
422 (max.)	3·28	350 (max.)	3·64	358 (max.)	3·44
8-*Hydroxy*-4-*methylcinnoline.*					
250 (max.)	4·38	246 (max.)	4·42	255 (max.)	4·40
290	3·04	268	2·68	287	2·76
308 (max.)	3·14	310 (max.)	3·09	330 (max.)	3·46
334	2·46	320	3·05	355	3·00
420 (max.)	3·43	360 (max.)	3·50	415 (max.)	3·60

The formation of metal chelates of 8-hydroxycinnoline (see below) may be taken to indicate the probability of intramolecular hydrogen bonding in the neutral molecule (Hunter, *Ann. Reports*, 1946, **43**, 141). No such bonding is, however, possible in the 8-methoxy-derivative, and the close similarity of the absorption spectra of 8-hydroxy- and 8-methoxy-cinnoline in neutral solvents affords no support for this hypothesis.

The absorption spectra of 8-hydroxy-4-methylcinnoline in neutral, acid, and alkaline media show the same features as those of 8-hydroxycinnoline itself, but the introduction of the 4-methyl group causes a slight hypsochromic shift (Table 1) of the peak of longest wave-length and an increase in the intensity of absorption of the ionised forms. The same effect has been noted by Phillips (*loc. cit.*) and Phillips and Merritt (*J. Amer. Chem. Soc.*, 1952, **74**, 552) among 4-methyl-oxines for which comparative data are given in Table 2.

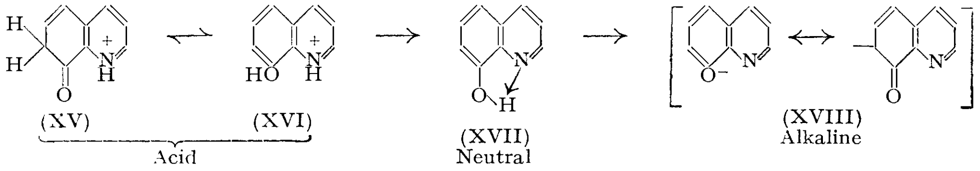

(XV) (XVI) Acid (XVII) Neutral (XVIII) Alkaline

TABLE 2.

	In 0·01N-HCl		*In* 95% MeOH		*In* 0·01N-NaOH	
	$\lambda_{max.}$	log $\varepsilon_{max.}$	$\lambda_{max.}$	log $\varepsilon_{max.}$	$\lambda_{max.}$	log $\varepsilon_{max.}$
8-Hydroxycinnoline	430	3·32	360	3·43	425	3·49
8-Hydroxy-4-methylcinnoline	420	3·43	360	3·50	415	3·60
8-Hydroxyquinoline	358	3·23	320	3·38	345	3·43
8-Hydroxy-4-methylquinoline	350—353	3·38	319	3·52	343	3·59

Dissociation Constants of 8-*Hydroxy- and* 8-*Hydroxy*-4-*methyl-cinnoline.*—Owing to the general similarity in the absorption spectra of 8-hydroxycinnoline in acid and alkali solutions, change of pH produces less marked alterations in the spectra of its aqueous solutions than is often the case in acid–base systems. Nevertheless, by careful selection of wave-lengths it is possible to determine its dissociation constants by the method described by Irving, Ewart, and Wilson (*J.*, 1949, 2672) for oxine. Values for the Brønsted dissociation constants defined by $K_1' = [H^+][HCnx]/[H_2Cnx^+]$ and $K_2' = [H^+][Cnx^-]/[HCnx]$ obtained from spectrophotometric measurements on a series of buffer solutions of constant ionic strength (0·3M), covering the pH range 2—10, are given in Table 3. Assuming that the activity coefficient f_{HCnx} does not differ appreciably from unity, while $f_{H_2Cnx^+}$ (or f_{Cnx^-}) is equal to the mean activity coefficient of a typical univalent ion of radius 2·5 Å, at the same ionic strength, we obtain $\log f_{\pm} = -0{\cdot}19$ whence the thermodynamic dissociation constants of the species H_2Cnx^+ (XIII) and HCnx (VI) at 14°±3° are given by $pK_1 = 2{\cdot}86 \pm 0{\cdot}06$ and $pK_2 = 8{\cdot}13 \pm 0{\cdot}07$. Potentiometric titration at 25° of $7{\cdot}6 \times 10^{-3}$M-8-hydroxycinnoline solutions in 0·036M-perchloric acid with carbonate-free 1·25N-alkali gave the values 2·90 and 8·30. Spectrophotometric measurements with 8-hydroxy-4-methylcinnoline gave Brønsted dissociation exponents of 3·47 and 8·48 (Table 3) for $\mu = 0{\cdot}3$, whence $pK_1 = 3{\cdot}28 \pm 0{\cdot}02$ and $pK_2 = 8{\cdot}67 \pm 0{\cdot}04$ at 14°±3°.

TABLE 3.

Wave-length (mμ)	300	330	320	370	410	Mean value
8-Hydroxycinnoline						
pK_1'	3·15	—	2·95	—	3·05	3·05
pK_2'	8·01	—	8·00	—	7·80	7·94
8-Hydroxy-4-methylcinnoline						
pK_1'	—	3·50	—	3·45	3·45	3·47
pK_2'	—	8·55	—	8·45	8·45	8·48

EXPERIMENTAL

(A temperature given in parentheses before a melting point refers to the characteristic temperature of sintering.)

Unless otherwise stated, extracts were dried with anhydrous sodium sulphate.

m-*Hydroxyacetophenone*.—By the method of Simpson *et al.* (*loc. cit.*), except that the acid copper sulphate solution was boiled during the addition of the diazonium solution, *m*-aminoacetophenone (54 g.) gave the phenol (30 g., 56%), m. p. 94—96°.

m-*Methoxyacetophenone*.—*m*-Hydroxyacetophenone (175 g.), water (1750 c.c.), and half of a solution of sodium hydroxide (87 g.) in water (500 c.c.) were stirred on the steam-bath whilst the remaining alkali and methyl sulphate (175 c.c.) were added dropwise during 5 minutes. After a further ½ hour's heating the product was extracted with ether. Distillation gave a colourless oil (161 g., 83%), b. p. 130—132°/15 mm.

3-*Methoxy*-2-*nitroacetophenone*.—Small-scale experiments indicated that the heating at the end of the nitration recommended by Simpson *et al.* (*loc. cit.*) caused only insignificant improvement in yield. More satisfactory was the use of longer nitration periods. *m*-Methoxyacetophenone (60 g.) was added rapidly (there was no appreciable evolution of heat) to nitric acid (360 c.c.; *d* 1·42) stirred at 0—5°, and the solution was set aside for 54 hours at room temperature. The product (68·8 g.) was precipitated by water, and crystallised from alcohol, giving 3-methoxy-2-nitroacetophenone (34·2 g., 44%), m. p. 129—130°. The acid liquor slowly deposited colourless needles (m. p. 42—45°), which could not be purified by crystallisation. When reduced in the way described for the 2-nitro-compound this material gave an oily solid which benzoylation converted into a product which could be separated by crystallisation from methanol and mechanical sorting into yellow prisms, m. p. (105°) 111—116°, and soft white needles, m. p. (129°) 133—136°. Sufficient of these compounds was not obtained for complete purification, but the former was very probably impure 6-benzamido-3-methoxyacetophenone, which forms yellow prisms, m. p. 117—118° (Simpson *et al.*, *loc. cit.*).

2-*Amino*-3-*methoxyacetophenone*.—The powdered nitro-ketone (36 g.) was added in portions during 50 minutes to a stirred suspension of iron filings (52 g.), water (193 c.c.), and acetic acid (193 c.c.), at 75—80°. The mixture was then refluxed and stirred for 1 hour, and, after cooling, extracted with ether. Evaporation of the solvent after drying (Na_2CO_3), and recrystallisation of the residue from alcohol gave the amine (28 g., 92%) as large yellow crystals, m. p. 64—66°.

4-*Hydroxy*-8-*methoxycinnoline*.—Powdered 2-amino-3-methoxyacetophenone (20 g.) in hydrochloric acid (*d* 1·16; 1 l.) was diazotised at 0—5° by sodium nitrite (10 g.) in water (20 c.c.). After the addition of further concentrated hydrochloric acid (2 l.) the solution was left overnight at room temperature and then heated at 60° until it no longer gave a coupling reaction (*ca.* 6 hours). Evaporation under reduced pressure on the steam-bath, dissolution of the residue in water (250 c.c.), and neutralisation with anhydrous sodium acetate, followed by boiling (charcoal) and filtration, gave a crystalline product (19·7 g., 92%), m. p. 162—164·5°. 4-*Hydroxy*-8-*methoxycinnoline* gave white needles of its *monohydrate*, m. p. 164—165° (Found: C, 55·8; H, 5·4. $C_9H_8O_2N_2,H_2O$ requires C, 55·7; H, 5·2%), after crystallisation from water and drying at 110°/2 mm.

4-*Chloro*-8-*methoxycinnoline*.—The 4-hydroxy-compound (15 g.) and phosphorus oxychloride (60 c.c.) were heated for ¼ hour at 95°, and the solution was then decomposed with ice and basified with sodium hydroxide solution. Extraction with ether and removal of the solvent gave clusters of faint yellow needles (14·4 g., 87%), m. p. 140—142°. 4-*Chloro*-8-*methoxycinnoline* crystallised from acetone in long, thick, light yellow needles, m. p. 142—143° (Found: C, 55·6; H, 3·5. $C_9H_7ON_2Cl$ requires C, 55·6; H, 3·6%), which slowly darkened.

By standard methods the chloro-compound gave 8-*methoxy*-4-*phenoxycinnoline*, which formed stout colourless needles or plates, m. p. 123—124° (Found: C, 71·7; H, 4·6. $C_{15}H_{12}O_2N_2$ requires C, 71·4; H, 4·8%), from aqueous acetone.

8-*Methoxy*-4-N′-(*toluene*-p-*sulphonyl*)*hydrazinocinnoline Hydrochloride*.—Toluene-*p*-sulphonylhydrazide (4·5 g.) in warm chloroform (125 c.c.) was treated with 4-chloro-8-methoxycinnoline (2·4 g.) in the same solvent (10 c.c.). After the solution had been kept overnight, scratching initiated the separation of an orange solid, which was collected after 1 week [4·4 g.; m. p. 176—179° (decomp.)]. Crystallisation from acetic acid–chloroform gave yellowish-orange crystals of 8-*methoxy*-4-N′-(*toluene*-p-*sulphonyl*)*hydrazinocinnoline hydrochloride*, m. p. 169—172° (decomp.) (Found: C, 47·7; H, 4·75. $C_{16}H_{17}O_3N_4ClS,H_2O$ requires C, 48·2; H, 4·8%). In experiments using 40 g. of the chloro-compound the yield reached 96%.

8-*Methoxycinnoline*.—The powdered toluenesulphonyl compound (19·5 g.) was added to sodium carbonate (195 g.; anhydrous) and water (1950 c.c.) at 95°, and the mixture was then refluxed for ¼ hour, cooled, filtered, and extracted with chloroform. The extract provided a dark brown oil (8·1 g.) which readily solidified and was suitable for demethylation. Distillation gave a yellow oil (6·8 g.; b. p. 144—145°/0·3—0·4 mm.) which crystallised. 8-*Methoxycinnoline* formed, from benzene–ligroin (b. p. 60—80°), colourless needles, m. p. (in a sealed tube) 67—70·5° (Found: C, 67·2; H, 5·2. $C_9H_8ON_2$ requires C, 67·5; H, 5·0%), which quickly liquefied, darkened, and became green when kept. The *picrate* formed orange needles, m. p. (183°) 189—191·5° (with darkening) (Found: C, 46·5; H, 2·45. $C_9H_8ON_2,C_6H_3O_7N_3$ requires C, 46·3; H, 2·85%).

8-*Hydroxycinnoline*.—The methoxy-compound (4·4 g. of crude material) was refluxed with hydrobromic acid (*d* 1·5; 40 c.c.) for 2 hours. The solution was cooled rapidly to produce a finely divided precipitate (slow cooling gave clusters of large orange needles of the hydrobromide), and neutralised with ammonia whilst being cooled in ice. The yellow precipitate (2·8 g.; m. p. 175—185°) of 8-*hydroxycinnoline* crystallised from benzene in long yellow needles, m. p. (177°) 185—186° (Found: C, 65·5; H, 4·2; N, 19·9. $C_8H_6ON_2$ requires C, 65·75; H, 4·1; N, 19·2%). A little more of the substance was obtained by extracting the neutral filtrate with chloroform.

2-(2-*Amino*-3-*methoxyphenyl*)*propan*-2-*ol*.—2-Amino-3-methoxyacetophenone (10 g.) in dry ether (100 c.c.) was added slowly to a Grignard reagent [from magnesium (5·2 g.), ether (125 c.c.), and methyl iodide (15 c.c.)], and the mixture was refluxed for ½ hour and then decomposed with ice and ammonium chloride. Extraction with ether gave a solid (10·9 g.; m. p. 76—77·5°). The *alcohol* formed glistening leaflets, m. p. 78—79·5° (Found: C, 66·2; H, 8·1. $C_{10}H_{15}O_2N$ requires C, 66·3; H, 8·3%), from ether–light petroleum (b. p. 40—60°).

2-(2-*Amino*-3-*methoxyphenyl*)*propylene*.—The alcohol (2·5 g.), benzene (25 c.c.), and phosphoric oxide (5 g.) were refluxed for 3 hours. The mixture was decomposed with ice and ammonia and extracted with ether. Removal of the solvent gave an oil (2·3 g.) which was used in the next stage. The *benzamido*-derivative, prepared from the oil in a little acetone by treatment with benzoyl chloride and aqueous sodium hydroxide, crystallised from alcohol as white prisms, m. p. 206—206·5° (Found: C, 76·6; H, 6·35. $C_{17}H_{17}O_2N$ requires C, 76·4; H, 6·4%).

8-*Methoxy*-4-*methylcinnoline*.—The propylene (6 g.) in sulphuric acid (2·7 c.c. of concentrated acid and 24 c.c. of water) was diazotised with sodium nitrite (2·6 g.) in water (5·5 c.c.) at 0—5°. The solution was diluted with iced water (400 c.c.) and set aside in the dark for 4 days at room temperature, and then neutralised with sodium hydroxide solution (it became green), and extracted with chloroform. The dark red residue remaining on removal of the solvent gave dark red needles (4·3 g.), m. p. 123—129°, when crystallised from benzene–light petroleum (b. p. 60—80°). Pure 8-*methoxy*-4-*methylcinnoline* formed orange needles, m. p. 130—132° (Found: C, 68·5; H, 6·0. $C_{10}H_{10}ON_2$ requires C, 68·95; H, 5·8%).

8-*Hydroxy*-4-*methylcinnnoline*.—The above compound (crude, 4·2 g.) was heated under reflux with hydrobromic acid (*d* 1·5; 40 c.c.) for 2 hours, and the solution was then neutralised with aqueous ammonia whilst being cooled, and was then extracted with chloroform. The extract on evaporation gave a dark residue which was crystallised from benzene (charcoal) and gave a product (2·1 g.), m. p. 172—174°. Pure 8-*hydroxy*-4-*methylcinnoline* separated from benzene as light yellow prisms, m. p. 177—178·5° (Found: C, 67·9; H, 5·3. $C_9H_8ON_2$ requires C, 67·5; H, 5·0%).

1-(2-*Amino*-3-*methoxyphenyl*)-1-*phenylethyl Alcohol*.—Prepared from 2-amino-3-methoxyacetophenone (5 g.) by the use of phenylmagnesium bromide (4 equivalents), as with the methyl compound, this *alcohol* (6·3 g.; m. p. 93—95°, after one crystallisation) formed clusters of slightly impure chunky prisms, m. p. 94—95° (Found: C, 75·3; H, 6·6. $C_{15}H_{17}O_2N$ requires C, 74·0; H, 7·05%), from light petroleum (b. p. 60—80°).

α-(2-*Amino*-3-*methoxyphenyl*)*styrene*.—The alcohol (2 g.), when dehydrated as above, gave the crude oily ethylene (1·7 g.), suitable for use in the next stage. The *benzamido*-derivative crystallised from aqueous alcohol in clusters of colourless needles, m. p. 99—100·5° (Found: C, 80·0; H, 5·75. $C_{22}H_{19}O_2N$ requires C, 80·2; H, 5·8%).

8-*Methoxy*-4-*phenylcinnoline*.—The ethylene (1 g.) in sulphuric acid (0·33 c.c. of concentrated acid in 3 c.c. of water) was diazotised with sodium nitrite (0·32 g.) in water (0·7 c.c.) at 0—5°. Iced water (45 c.c.) was added and the solution was left 3 days at room temperature before being basified with sodium hydroxide solution and extracted with chloroform. Evaporation of the extract gave an oil (1·4 g.) which slowly solidified and crystallised from chloroform–light petroleum (b. p. 60—80°) as yellow plates (0·9 g.), m. p. 65—70°. Further crystallisation gave

8-methoxy-4-phenylcinnoline as opaque, light yellow plates, m. p. (78°) 85—89°. The compound became sticky in air, and a satisfactory specimen for analysis could not be obtained.

8-*Hydroxy*-4-*phenylcinnoline*.—The methoxy-compound (0·8 g.) was heated under reflux with hydrobromic acid (*d* 1·5; 8 c.c.) for 2 hours, and worked up as before. The tarry red solid which separated solidified overnight and was then extracted with boiling light petroleum (b. p. 60—80°). Evaporation of the extract yielded dull yellow prisms (0·37 g.), m. p. 136—140°. 8-*Hydroxy*-4-*phenylcinnoline* formed clusters of fawn-coloured needles, m. p. 142—143·5° (Found: C, 75·9; H, 4·7. $C_{14}H_{10}ON_2$ requires C, 75·7; H, 4·5%), from benzene–light petroleum.

Reactions of 8-*Hydroxycinnoline*.—(a) *With diazomethane*. When 8-hydroxycinnoline (0·5 g.) was added to an ethereal solution of diazomethane (from 1 c.c. of nitrosomethylurethane) it slowly dissolved with effervescence, producing a brilliant emerald-green colour which changed to deep blue. The dark blue precipitate (0·35 g.), m. p. *ca.* 119—121°, which separated overnight could not be crystallised. It was insoluble in cold water, but dissolved slightly on boiling to give a red-brown solution. The solid dissolved readily in dilute mineral acids giving yellow solutions, and addition of alkali caused darkening and precipitation of a solid.

(b) *The Bucherer reaction*. The cinnoline (0·5 g.), aqueous ammonia (*d* 0·88; 7 c.c.), and ammonium sulphite (7 c.c. of a saturated solution) were heated in a sealed tube at 100—120° for 17 hours. The product was diluted with water, treated with aqueous sodium hydroxide, and extracted with ether. Removal of the solvent left a black oil (0·42 g.) which was dissolved in boiling acetic anhydride (6 c.c.), and the solution was then heated at 95° for 1 hour. The solution was decomposed with water (charcoal), filtered, and extracted with ether. The residue from the dried (K_2CO_3) extract formed yellow crystals (0·42 g.), m. p. 171—174°, from benzene–light petroleum (b. p. 60—80°). Further crystallisation gave 8-acetamidocinnoline as yellow prisms, m. p. 173—175° (Found: C, 63·8; H, 4·8. Calc. for $C_{10}H_9ON_3$: C, 64·2; H, 4·85%). Morley (*loc. cit.*) gave m. p. 177—178°. Higher reaction temperatures lowered the yield and produced much tar.

8-Acetamidocinnoline (0·3 g.) and hydrochloric acid (5N; 7 c.c.) were refluxed for 40 minutes, and the deep purple solution was then cooled in ice and basified with sodium hydroxide solution. The colour was discharged, and golden-yellow crystals separated. 8-*Aminocinnoline* crystallised from benzene–light petroleum as golden-yellow needles, m. p. 89—92° (Found: C, 62·2; H, 5·4. $C_8H_7N_3,\frac{1}{2}H_2O$ requires C, 62·3; H, 5·2%). The *picrate* crystallised from acetic acid, first as fluffy dark brown needles, and later as hard jet-black needles, m. p. (230°) 236—238° (Found: C, 45·0; H, 2·9. $C_8H_7N_3,C_6H_3O_7N_3$ requires C, 44·9; H, 2·7%).

(c) *Reactions with metals*. Sensitivity tests in buffers of pH 5, 8, and 12 were carried out as described by Irving, Butler, and Ring (*loc. cit.*), and stability constants were measured in 73 : 27 dioxan–water, the Bjerrum–Calvin technique of potentiometric titration being used. Full details will be reported elsewhere.

Absorption spectra were measured with a Beckman Model DU Spectrophotometer, with minimum slit widths. " 95% Methyl alcohol " was prepared by adding redistilled water (5 ml.) to methyl alcohol (95 ml.). " AnalaR " reagents were used with water redistilled from an all-Pyrex apparatus in making up solutions, and every precaution was taken to prevent the intrusion of metallic impurities. Using 1-cm. matched silica cells it was shown that Beer's law was obeyed by 8-hydroxycinnoline and its ions at 370 and 410 mμ for concentrations up to at least 36 mg./l., and by 4-methyl-8-hydroxycinnoline and its ions at 294, 370, and 470 mμ up to at least 120 mg./l. The actual concentrations used varied from 10^{-3} to 10^{-5}M, according to the part of the spectrum under examination. Buffer solutions prepared from sodium acetate and hydrochloric acid, potassium dihydrogen and disodium hydrogen phosphate, borax and hydrochloric acid, or sodium carbonate and hydrochloric acid, were made up with sufficient sodium chloride to give a constant ionic strength of 0·3M. The appropriate buffer containing no organic solute was used in the comparison cell, and measurements of pH were made to 0·01 unit with a Cambridge pH meter.

Solubility measurements were carried out by shaking mechanically in large stoppered Pyrex test-tubes at room temperature (i) distilled water and excess of 8-hydroxycinnoline, and (ii) a solution saturated with solute at the b. p. and chilled to produce fine crystals. At intervals portions of the clear solution were filtered through sintered glass, diluted 10-fold, and treated (10 ml.) in turn with hydrochloric acid (2N; 15 ml.), potassium bromide (1 g.), and potassium bromate (0·00253M; 2·0 ml.). After 5 minutes, potassium iodide solution (10%; 10 ml.) was added and liberated iodine was titrated with sodium thiosulphate (0·00987N). Preliminary measurements with known weights of 8-hydroxycinnoline showed that quantitative dibromination was achieved under these conditions. After 6 and 12 hours' shaking the concentration of hydroxycinnoline in (i) was 0·500 and 0·612 g./l., showing that solution was proceeding slowly, but consistent figures of 0·778 and 0·778 g./l. in (ii) after the same lapse of time showed the absence of supersaturation when equilibrium was approached from this side.

DISCUSSION

The introduction of a second nitrogen atom into a six-membered ring already containing one nitrogen atom greatly reduces the basic strength (Albert, Goldacre, and Phillips, *J.*, 1948, 2240), the pK values of the acids conjugate to quinoline, cinnoline, quinazoline, and quinoxaline being 4·94, 2·7, 3·5, and *ca.* 0·8 respectively. The basic strengths of the corresponding 8-hydroxy-derivatives follow the same pattern, values for pK_1 being 5·00, 2·86, 3·41, and 2·15, while values for pK_2 of 9·85, 8·13, 8·65, and 8·73 respectively reflect the increasing tendency of the phenolic hydrogen atom to dissociate. It is noteworthy that the acid strengths of the corresponding 4-hydroxy-quinoline, -cinnoline, and -quinazoline, follow the same sequence since in 50% aqueous alcohol pK_1 is 2·41, 1·77, and 2·07, and pK_2 12·43, 9·53, and 9·98 respectively (Keneford, Morley, Simpson, and Wright, *J.*, 1949, 1356). With the exception of quinoxaline these relative orders are those predictable from the electron densities on $N_{(1)}$ calculated by Longuet-Higgins and Coulson (*J.*, 1949, 971; cf. Keneford, Morley, Simpson, and Wright, *J.*, 1950, 1104) who discuss similar discrepancies in the case of pyridine, quinoline, and acridine (*loc. cit.*, p. 979).

In so far as it is possible to correlate the stability of a metal complex with the dissociation constants of the ligand (cf. Bjerrum, *Chem. Reviews*, 1950, **46**, 381; Irving, Discussion on Co-ordination Chemistry, 1951, I.C.I. Report No. BRL/149) we might expect 8-hydroxycinnoline to act like 8-hydroxyquinoxaline, especially at high pH, and for both of these reagents to chelate much less strongly with metals than does oxine itself. The stability constants of magnesium, nickel, zinc, and copper 8-hydroxycinnoline complexes in 73% aqueous dioxan (Irving and Marsh, to be published shortly) are found to be less than those of the corresponding oxine complexes, and the results of sensitivity tests, some of which are given in Table 4, confirm the general prediction. A full account of the interaction of metals with 8-hydroxycinnoline will be published elsewhere, but of the metals studied the only ones with which it shows a greater sensitivity than oxine are Hg(II) and Ga (but not In) in buffer A, Hg(II) and In (but not Ga) in buffer B, and Pd in buffer C. Apart from reactions of moderate sensitivity with Cu(I), Ag, Ru, and Rh in buffer A, and with Cu(I) and Pt in buffer B, the substance gave no evidence of potential value in analytical chemistry. As expected the low stability of its metal complexes is reflected in the relative activity of 8-hydroxy-quinoline and -cinnoline towards micro-organisms (Table 5).

TABLE 4.

Metal used:		Cu^{++}	Zn^{++}	Fe^{+++}	Al^{+++}	Mn^{++}	Co^{++}
8-Hydroxyquinoline [a]	A	1·6	1·0	1·0	3·0	3·0	3·0
	B	2·0	4·0	4·0	6·0	N.P.	18
	C	3·7	7·0	N.P.	N.P.	,,	18
8-Hydroxycinnoline [b]	A	N.P.*	N.P.*	6·6	N.P.*	N.P.	71
	B	,,	,,	N.P.	,,	,,	19
	C	,,	,,	,,	,,	,,	N.P.
8-Hydroxyquinoxaline [c]	A	4·0	6·5	N.P.§	N.P.	N.P.	3·0
	B	,,	,,	,,	,,	5·5	4·0
	C	N.P.	N.P.	,,	,,	700	N.P.

[a] Berg, "Die Analytische Verwendung von *o*-Oxychinolin," Stuttgart, 1938; the slight difference in buffer compositions does not affect the general conclusions. [b] Present authors. [c] Freeman and Spoerri (*loc. cit.*).

Sensitivity limits are given in μg./ml. and represent the lowest concentrations of metals to give visible precipitates under the conditions of test. The letters A, B, and C refer to buffers of pH 5·2, 8·4, and 12·4 (Irving, Butler, and Ring, *loc. cit.*). N.P. signifies that there was no precipitation of complex. The symbol § signifies no precipitation up to 1000 μg./ml.

* Under conditions of potentiometric titration with more concentrated solutions, insoluble aluminium, zinc, and copper complexes are precipitated.

The favourable effect of a 4-substituent, revealed by tests *in vitro*, has not yet been correlated with factors such as the lipoid solubility and stability of the metal complexes; but it is a striking fact that all three hydroxycinnolines proved to be inactive when mice infected with hæmolytic streptococci, *P. berglei*, or *T. equiperdum* were treated intraperitoneally with the maximum tolerated dose (2·0, 2·0, and 5·0 mg./20-g. mouse respectively) once daily for 4 days.

After this work had been completed we learned that Albert and Hampton (to whom we are indebted for the dissociation constants of 8-hydroxyquinazoline) had undertaken and completed the synthesis of all six possible 8-hydroxyazaquinolines, and were engaged in studies of their bacterial action and ability to form complexes with metals. Their route to 8-hydroxycinnoline differs from ours, and they have generously suggested the independent publication of results.

TABLE 5. *Bacteriostatic indices.*

	Hæmolytic streptococcus	*Staph. aureus*	*B. coli*
8-Hydroxyquinoline [a]	—	100,000	1,600
8-Hydroxycinnoline [b]	7,300	730	290
8-Hydroxy-4-methylcinnoline [b]	29,200	2,900	730
8-Hydroxyquinoxaline [c]	—	1,200	—
8-Hydroxy-4-phenylcinnoline [b]	111,000 *	44,400 *	§

[a] Albert, Rubbo, Goldacre, and Balfour, *Brit. J. Exp. Path.*, 1947, **28**, 69. [b] Data provided by the National Institute for Medical Research, for Hedley Wright broth after 12 hours' incubation at 37°. [c] Freeman and Spoerri (*loc. cit.*). * The figures are only approximate as the compound is so slightly soluble. § The compound was not inhibitory in saturated solution at pH 7·5, the solubility being approx. 10 mg./l.

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WASHINGTON SINGER LABORATORIES,
PRINCE OF WALES RD., EXETER.
THE INORGANIC CHEMISTRY LABORATORY, OXFORD UNIVERSITY.
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