937. *Prototropic Equilibrium and Fluorescence of Some 8-Hydroxyquinoline Derivatives.*

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That the fluorescence of 8-hydroxyquinoline (oxine) in concentrated acids is quenched by water is shown to be due to the displacement of a prototropic equilibrium, resulting in the ionisation of the hydroxyl group of the excited molecule. Prototropic equilibrium constants for lowest, singlet, excited states are reported. The hydroxyl group of 8-hydroxyquinoline-5-sulphonic acid is more acidic than the sulphonic acid group.

8-Hydroxyquinoline(oxine) fluoresces only in strongly acid solution,[1] whereas its complexes with non-transition metals fluoresce in many solvents,[2,3] a property useful for

[1] Popovych and Rogers, *Spectrochim. Acta*, 1959, 584.
[2] Hercules and Rogers, *Spectrochim. Acta*, 1959, 393.
[3] Haar and Umland, *Z. analyt. Chem.*, 1962, **191**, 81.

quantitative analysis.[4–6] That the oxinium cation (C in Fig. 1, $R^1 = R^2 = R^3 = H$) does not fluoresce in dilute acid has been attributed to hydrogen bonding between water and the 8-hydroxyl group, such bonding being supposed absent in concentrated acid.[1] An alternative explanation is that a *prototropic equilibrium* exists between the excited states of two ionic species in concentrated acid, *e.g.*, between the zwitterion, Z, and cation, C, of Fig. 1, the protonated species being fluorescent and the other non-fluorescent.

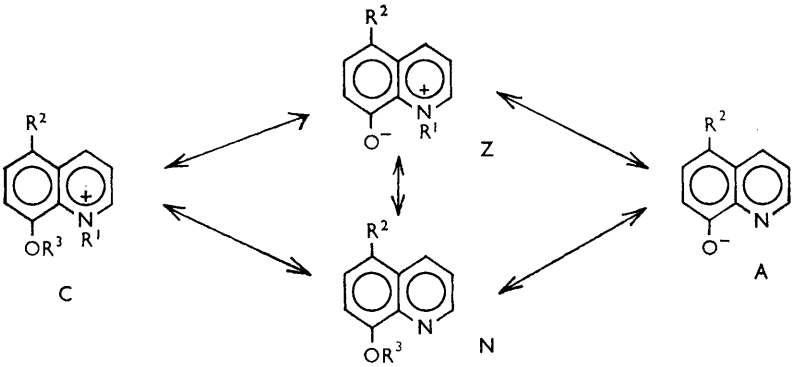

FIG. 1. The prototropic equilibria of oxine; C being the cation, Z the zwitterion, A the anion and N the neutral molecule.

For such an equilibrium to exist the time for protonation of the excited state must be small compared to its life-time (about 10^{-8} sec.), and conditions are favourable for this in concentrated mineral acid on account of the high activity and chain-transfer mechanism of the proton.[7]

Equation 1 governs the prototropic equilibrium between Z and C in concentrated acid, where H_0 is Hammett's acidity function,[8] pK^* is the logarithmic dissociation constant of the protonated species in the excited state, and [Z*] and [C*] are the concentrations of the excited species.

$$H_0 = pK^* + \log_{10} [Z^*]/[C^*] \quad (1)$$

A test of the prototropic equilibrium hypothesis is possible if the variation of fluorescence intensity with H_0 can be measured and translated into values of [Z*] and [C*] for substitution into equation 1 (see equations 2 to 5 below).

The total concentration of excited species present in concentrated acid is given by equation 2 if the concentration of the cation, [C], is large, and approximately by equation 3 if [C] is small, where ε is the extinction coefficient of C, l the path-length through the solution, I the intensity of illumination and Q a constant.

$$[Z^*] + [C^*] = QI\{1 - \exp(-\varepsilon l[C])\} \quad (2)$$

$$\simeq QI\varepsilon l[C] \quad (3)$$

Supposing that the relative fluorescence intensity, f, is some function, ϕ, of [C], as in equation 4,

$$f = \phi([C]) \quad (4)$$

then if ϕ is determined experimentally at some value of H_0 such that $[C^*] \gg [Z^*]$ we may write,

$$f \simeq ([C^*])/QI\varepsilon l \quad (5)$$

The denominator of equation 5 need not be evaluated so long as it is constant, [C*] then being known in arbitrary units which cancel out in equation 1.

[4] Bishop, *Analyt. Chim. Acta*, 1963, 172.
[5] Watanabe, Frantz, and Trottier, *Anal. Biochem.*, 1963, **5**, 345.
[6] Haitanger, *Mikrochem.*, 1935, **16**, 331.
[7] Robinson and Stokes, "Electrolyte Solutions," Butterworths, London, 1955, p. 365.
[8] Paul and Long, *Chem. Rev.*, 1957, **57**, 1.

Since oxine itself was found to be rapidly sulphonated in concentrated sulphuric acid, we have made measurements on 8-hydroxyquinoline-5-sulphonic acid in that solvent and on oxine itself—over a limited range of acidity—in perchloric acid.

Results and Discussion

In Fig. 2 experimental values of the relative fluorescence intensity, f, are plotted against the concentration of 8-hydroxyquinoline-5-sulphonic acid in sulphuric acid of $H_0 = -9{\cdot}3$,

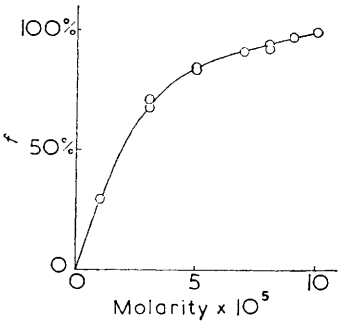

Fig. 2. Relative fluorescence intensity, f, plotted against the concentration of 8-hydroxyquinoline-5-sulphonic acid in concentrated sulphuric acid ($H_0 = -9{\cdot}3$).

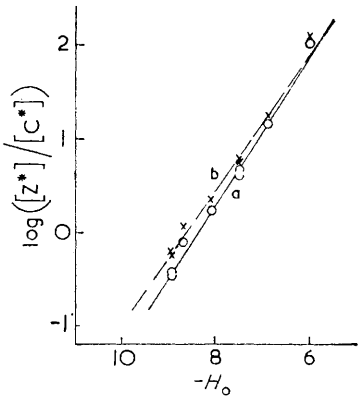

Fig. 3. The logarithm of the concentration ratio of the excited species (equation 1) plotted against H_0 for 8-hydroxyquinoline-5-sulphonic acid in sulphuric acid. Curve a, first approximation; curve b, second approximation.

and in Table 1 measurements of f at various values of H_0 are given for 10^{-4}M solutions of 8-hydroxyquinoline-5-sulphonic acid.

The results in Fig. 2 and Table 1 enable a plot to be made of H_0 against the last term

Table 1.

Relative fluorescence efficiency, f, expressed as percentage of the intensity at $H_0 = -9{\cdot}3$ for solutions (10^{-4}M) of 8-hydroxyquinoline-5-sulphonic acid in different concentrations of sulphuric acid. The filter was Ilford 623.

$-H_0$	f (%)		$-H_0$	f (%)	
9·3	100		7·5	48·8	47·2
8·95	92·8	92·6	6·9	16·6	15·8
8·70	86·6	87·4	6·0	2·6	2·6
8·1	74·4	74·8	5·2	0·4	0·4

of equation 1 (see Fig. 3a), a linear relationship being obtained, thus supporting the hypothesis of prototropic equilibrium. The intercept on the abscissa of Fig. 3a gives $pK^* = -8{\cdot}4$, a value suggesting that the equilibrium is not entirely on the side of the excited cation at $H_0 = -9{\cdot}3$, the acidity at which Fig. 2 was determined (*i.e.*, the condition $[C^*] \gg [Z^*]$ was not met). However, the approximate value of $-8{\cdot}4$ can be taken for the starting point of an iteration procedure in which Fig. 2 is corrected by equation 1 and used to obtain a new curve (Fig. 3), only one iteration being required to give Fig. 3b and $pK^* = -8{\cdot}6$, which is unchanged by further repetition. The experimental values of [C*], in arbitrary units, are plotted against H_0 in Fig. 4a, together with the theoretical line for $pK^* = -8{\cdot}6$. The experimental errors are those of measurement ($\pm 5\%$), and an unknown error due to the charged nature of the species in equilibrium, the H_0 scale having been defined for uncharged bases.

Several equilibria could be established under these conditions; in either the excited or

the ground states, one could envisage protonation of an aromatic ring, carbonium-ion formation by extraction of the hydroxyl ion, and radical-cation formation by removal of an electron. In the excited state, either the equilibria of Fig. 1 or ionisation of the sulphonic acid group could occur.

The possibility that a ground-state species, protonated in the ring, is involved can be discounted, since the absorption spectrum of 8-hydroxyquinoline-5-sulphonic acid in concentrated sulphuric acid is very like that in dilute acid. Ring protonation of the excited state can also be rejected, since there is a mirror-image relationship between the emission and absorption spectra of 8-hydroxyquinoline-5-sulphonic acid, with the maxima 126 mμ apart, the corresponding separation for the aluminium chelate being 136 mμ,[1] whereas ring protonation of the excited state would alter the separation of the two maxima and destroy the mirror-image relationship. Carbonium-ion formation by extraction of the hydroxyl group is also excluded by the above considerations. Since no e.s.r. signal could be detected in either dilute or concentrated sulphuric acid, radical-cation formation in the ground state is eliminated, although if it were formed from the excited state its concentration would be too low for detection. There is evidence that electron removal from excited states is important in some systems [9] but the resulting doubly-charged radical is likely to be less fluorescent than the singly-charged cation, since unpaired electrons appear to quench oxine derivatives.[3] That ionisation of the hydroxyl group is involved in the quenching equilibrium is probable, for in strong acid solution no quenching by water is observed, when ionisation is prevented by replacement of the proton by methyl. Further experiments were made on the basis of the analogy between the protonated and methylated compounds [10] so as to eliminate other equilibria.

8-Methoxyquinoline (N in Fig. 1, $R_3 = CH_3$, $R_2 = H$) was found to give weak blue fluorescence in alkaline solution and intense green in dilute or concentrated acid. By appropriate choice of filter, the blue colour was almost completely removed, and the intensity of the green emission alone was measured for a range of concentrations in 0·01M acetic acid and for a range of pH values at constant concentration in the presence of acetate ion (0·01M). In Fig. 5a experimental values of the concentration of the excited, protonated species are plotted against pH with the theoretical line for $pK = 5{\cdot}14$, the ground state value [11] for equilibrium between C ($R_1 = R_2 = R_3 = H$) and N ($R_2 = R_3 = H$) of Fig. 1. Hence the protonation of the nitrogen atom is too slow to attain equilibrium within the life-time of the excited state, probably because it is diffusion-controlled.[12] The large change in intensity of fluorescence upon protonation makes 8-methoxyquinoline suitable for use as a fluorescent indicator for acid–base titrimetry.

In order to study the ionisation of the 5-sulphonic acid group, it was necessary to block the nitrogen atom and hydroxyl groups by methylation. To this end, 1-methyl-8-methoxyquinolinium iodide was prepared (C in Fig. 1, $R_1 = R_3 = CH_3$, $R_2 = H$); it was found to be non-fluorescent in the solid state and was destroyed by concentrated sulphuric acid with the liberation of iodine. However, the perchlorate fluoresced intensely green, both as the solid and in aqueous solution, and dissolved in concentrated sulphuric acid with rapid sulphonation, presumably in the 5-position, the absorption spectrum being very similar to that of 8-hydroxyquinoline-5-sulphonic acid. In concentrated sulphuric acid the 5-sulphonic acid of 1-methyl-8-methoxyquinolinium perchlorate fluoresced an intense blue that became green upon the addition of water, *with little change in intensity*. Since the presumed ionisation of the sulphonic acid group caused no quenching and the protonation of nitrogen occurs with $pK = 5{\cdot}14$, the quenching equilibrium with $pK^* = -8{\cdot}6$ must have been due to ionisation of the hydroxyl group. The emission bands were rather broad, and the best separation that could be effected with filters still allowed some 40%

[9] Allan and Scholes, *Nature*, 1960, **187**, 218.
[10] Albert and Barlin, *J.*, 1959, 2384.
[11] Albert and Phillips, *J.*, 1956, 1294.
[12] Weller, *Z. Elektrochem.*, 1957, **61**, 956.

of the intensity of the green band into the blue and vice versa, and this involved a fall in sensitivity of the fluorimeter due to the high absorbance of the filters. The titration curves for both emission bands are plotted in Figs. 4b and c as concentrations of the excited species, in arbitrary units, against H_0 together with the theoretical lines for $pK^* = -7{\cdot}4$.

In order to follow the ionisation of the hydroxyl group without the complication of sulphonation, experiments were made with oxine in perchloric acid. Unfortunately the practical upper limit of acidity in perchloric acid ($H_0 = -7{\cdot}4$) allowed only half of the titration to be followed; moreover the H_0 scale was known beyond the value $-5{\cdot}8$ only by extrapolation, but the titration curve (Fig. 5b) agrees fairly well with $pK^* = -7{\cdot}0$.

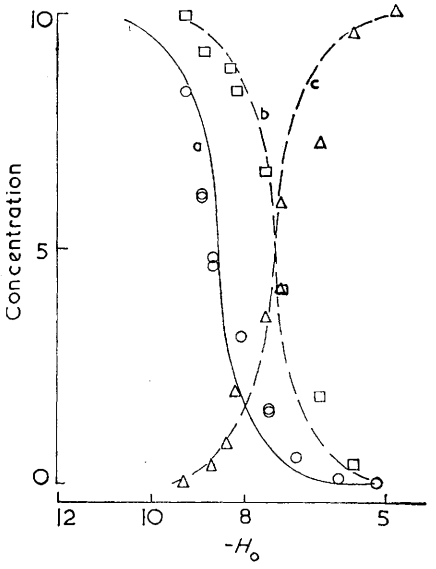

FIG. 4.

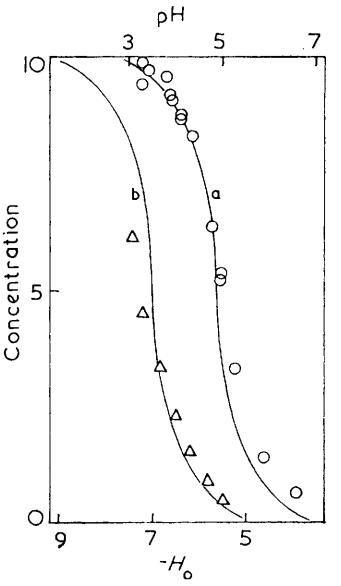

FIG. 5.

FIG. 4. The concentrations of excited species, in arbitrary units, plotted against H_0. Curve a, theoretical line for $pK^* = -8{\cdot}6$, experimental points (circles) are for the blue emission of 8-hydroxyquinoline-5-sulphonic acid. Curves b and c are the theoretical lines (dashed) for $pK^* = -7{\cdot}4$, the experimental points being squares for the blue emission (acid form) of the 5-sulphonic acid of 1-methyl-8-methoxyquinoline and triangles for the green emission (ionised form). The concentrations were 10^{-4}M, the solvent was sulphuric acid.

FIG. 5. The concentration of excited species, in arbitrary units, as functions of H_0 and pH. Curve a, theoretical line for $pK = 5{\cdot}14$ (upper scale), experimental points (circles) are for the green emission of 8-methoxyquinoline. Curve b, theoretical line for $pK = -7{\cdot}0$ (lower scale), experimental points (triangles) are for the green emission of oxine in perchloric acid.

By means of Weller's term diagram,[13] the excited-state enthalpy changes of the equilibria of Fig. 1 may be estimated from the absorption spectra (equation 6).

$$E_1 + \Delta H^* = E_2 + \Delta H \qquad (6)$$

where E_1 is the energy of the lowest energy absorption band of the thermodynamically more stable species and E_2 that of the other species, ΔH^* is the enthalpy change upon protonation in the excited state, ΔH that in the ground state. If the entropy changes are the same in the ground and excited states the reaction isotherm may be used to find pK^* (equation 7).

$$pK - pK^* = (E_1 - E_2)/\boldsymbol{R}T \log_e 10 \qquad (7)$$

[13] *Progr. Reaction Kinetics*, ed., Porter, Pergamon, London, 1961, Vol. I, p. 187.

In Table 2 are given the pK values for the equilibria of Fig. 1 together with values of pK* calculated by equation 7 from the experimental wavelengths of the appropriate bands, which are listed in Table 3.

TABLE 2.

Values of pK from ref. (14) and pK* calculated from the absorption data of Table 3 for the equilibria of Fig. 1.

Equilibrium	pK	pK *	Equilibrium	pK	pK *
N ⇌ Z	1·46	−19	Z ⇌ A + H$^+$	8·42	20·8
C ⇌ Z + H$^+$	6·6	−3·9	C ⇌ N + H$^+$	5·14	15·3
N ⇌ A + H$^+$	9·88	1·8			

TABLE 3.

Absorption data for the species of Fig. 1. Concentrations were 10^{-4}M except for oxine in water which was saturated, the concentration being unknown, ν being the frequency in cm.$^{-1}$ and ε the extinction coefficient.

Compound	Species	Solvent	ν	Log ε	ν	Log ε	ν	Log ε
Oxine	N	Water	31,800	—	37,000	—	41,700	—
Oxine	C	0·01M-$HClO_4$	27,000	3·21	31,500	3·20	39,700	4·61
Oxine	A	0·01M-NaOH	28,000	3·45	29,600	3·45	39,300	4·48
1-Methyl-8-hydroxyquinolinium iodide	Z	0·01M-NaOH	22,300	3·18	29,000 30,000	3·08 3·04	36,500	4·52

The value, pK* = −3·9 for the cation–zwitterion equilibrium derived from absorption spectra differs markedly from the value, pK* = −7·0 obtained by titration. Since the ground-state equilibrium takes place in aqueous solution and the excited equilibrium in concentrated acid good agreement is not to be expected. The absorption spectrum of oxine in concentrated acid appears to be somewhat different from that in dilute acid (Fig. 6), implying significantly different environments in the two solvents. Another reason for the divergence in the two values lies in the zwitterionic nature of the base, since the H_0 and H_z (zwitterionic base) acidity scales might be expected to diverge as the acidity increases. The slope of Fig. 3 would, by equation 1, be unity, and the observed slope of 0·695 indicates that H_0 increases more rapidly than H_z. Correction of the titration value of pK* by the factor 0·695 leads to the value −4·9, in better agreement with the value derived from absorption spectra.

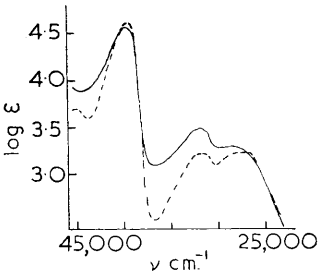

FIG. 6. Absorption spectra of oxine in concentrated sulphuric acid (full line) taken as soon as possible after preparation and in 0·01M acid (dashed line).

The relative pK* values of Table 2 are such that the zwitterion is the dominant excited species in any solvent other than concentrated acid. The rate of change from neutral molecule, N, to zwitterion, Z, in Fig. 1 would be very rapid since the proton transfer is intramolecular and the non-fluorescence of oxine in ordinary solvents must therefore be ascribed to quenching of the zwitterion. Any explanation of the non-fluorescence of the zwitterion must take into account the intense fluorescence of the zwitterion of 3-hydroxyquinoline.[15] The difference between the two compounds might be related to the intramolecular hydrogen bonding present in the former and absent in the latter.[16]

[14] Mason, *J.*, 1958, 674.
[15] S. F. Mason, private communication.
[16] Mason, *J.*, 1957, 4874.

The enormous increase in the acidity of the hydroxyl group upon excitation, and the increase in basicity of the nitrogen atom, is in accordance with the aromatic-anion model proposed [17] to explain qualitative features of hydroxy-*N*-heteroaromatic absorption spectra. According to this model the optical electron in the ground state occupies an orbital that correlates with the non-bonding molecular orbital of the α-methylnaphthyl anion, with one-electron charge densities of 0·45 and zero upon the oxygen and nitrogen atoms, respectively.[18] In the lowest excited state the optical electron occupies the lowest anti-bonding orbital of the α-methylnaphthyl ion, with charge densities of 0·13 and 0·24. The large changes in electron density upon the two functional groups are responsible for the observed changes in proton affinity.

Experimental

Materials.—8-Hydroxyquinoline-5-sulphonic acid was recrystallised three times with water and dried at 105°. 8-Methoxyquinoline was prepared according to the directions of Kaufmann and Rothlin.[19] 8-Hydroxyquinoline was purified by steam distillation followed by sublimation *in vacuo*. 1-Methyl-8-methoxyquinolinium iodide was prepared by dissolving 8-methoxyquinoline in methyl iodide and collecting the yellow crystals that separated. The perchlorate was prepared by double decomposition in ethanol with silver perchlorate, followed by precipitation with ether and recrystallisation from ethanol.

AnalaR grades of sulphuric and perchloric acids were used.

Measurement of Fluorescence Intensity.—The " Eel " fluorimeter made by Evans Electroselenium & Co. was used; the exciting source being a 125 w mercury lamp. The 365 mμ line was isolated with a Chance OX filter. Ilford spectrum filters were used to isolate the fluorescent light.

Measurement of Absorption Spectra.—An " Optika " automatically recording instrument was used, with concentration 10^{-4}M, and with 1 cm. and 1 mm. silica cells.

U.K.A.E.A., Harwell, Berks. [*Received, October 24th*, 1963.]

[17] Mason, *J.*, 1959, 1253.
[18] Coulson and Daudel, " Dictionary of Values of Molecular Constants," Vol. III, p. 32.
[19] Kaufmann and Rothlin, *Ber.*, 1956, **49**, 578.