526. *The Effect of Co-ordination on Ionization. Part I. Iron*(II) *and Iron*(III) *Complexes of Pyridine-2-aldoxime.*

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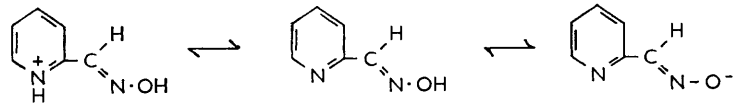
An attempt has been made to measure the extent by which the co-ordination of a ligand to a metal affects the ionization of acidic side groups on the ligand. It is shown that when ferrous iron forms a complex with pyridine-2-aldoxime the acid strength of the oxime (N·OH) group increases from $pK^0 = 10.22 \pm 0.02$ in the free ligand to $pK_3{}^0 = 7.13 \pm 0.02$ in the tris-complex ($T = 25°$). Thermodynamic data have been obtained for the acid ionizations in the free ligand and in the iron(II)–tris-complex. The overall formation constant has also been measured, and its thermodynamic data have been evaluated.

In certain co-ordination compounds, such as hæmoproteins and enzymes, it is known that there is a " linked effect " involving the metal–ligand bond and proton dissociation of an ionizable side group,[1a] but little attention has been given to the phenomenon. Harkins and Freiser [2] have examined a series of bivalent metal complexes of 2-2′-pyridylbenzimidazole in 50% dioxan–water mixture and have noted that co-ordination of a metal to a ligand " decreases the electron availability on the imino-nitrogen (through resonance interaction), thereby facilitating the dissociation." Our preliminary work on the iron(II) and iron(III) complexes of pyridine-2-aldoxime [3] showed that the iron(II) system was a suitable one for detailed study of the effect of co-ordination to iron on the ionization of the adjacent oxime group.

Ionizations in Pyridine-2-aldoxime.—(a) *The pyridinium* =NH⁺ *group.* We have shown [3] that pyridine-2-aldoxime exhibits two series of reversible changes in its ultraviolet absorption spectrum, corresponding to two acid ionizations with p*K* values of about 3·2 and 10·2, respectively. The first ionization was associated with the pyridinium =NH⁺ group (normal acid p*K* values for this group in pyridine 2-derivatives lie between 5·8 and 6·0) and the second with the oxime group (for which normal p*K* values lie between 12·5 and 13·0). These ionizations can be represented as follows:



$(^{+}HPAOH)$ (PAOH) (PAO^{-})

We now report more accurate determinations of these ionizations. The ionization constant, *K*, of the pyridinium =NH⁺ group, refers to the equilibrium

$$^{+}HPAOH + H_2O = PAOH + H_3O^{+}. \qquad (1)$$

The value of *K* at finite ionic strength, *I*, is defined by the relation

$$K = a_{H^+}(\text{base})/(\text{acid}), \qquad (2)$$

where a_{H^+} is the activity of hydroxonium ions obtained from pH measurement using the relation $pH = -\log_{10}a_{H^+}$, and (base)/(acid) is the ratio of concentrations of conjugate base to acid obtained spectrophotometrically. At 25·0°, the values of p*K* are 3·52 ($I = 0.050$M), 3·48 ($I = 0.010$M), 3·46 ($I = 0.0051$M), and 3·43 ($I = 0.0011$M), the mean deviation being

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[1] (*a*) Edsall and Wyman, " Biophysical Chemistry," Vol. 1, Academic Press, New York, 1958, p. 653; (*b*) *ibid.*, p. 452; (*c*) *ibid.*, p. 464.

[2] Harkins and Freiser, *J. Amer. Chem. Soc.*, 1956, **78**, 1143.

[3] Hanania and Irvine, *Nature*, 1959, **183**, 40–42.

0·02 pK unit in each case. As expected, the variation of observed pK values with ionic strength is consistent with the limiting relation

$$pK = pK^0 + 0{\cdot}5I^{\frac{1}{2}}/(1 + I^{\frac{1}{2}}), \quad . \ . \ . \ . \ . \ . \ . \quad (3)$$

where K^0 is the thermodynamic ionization constant obtained by extrapolation to $I = 0$.

Similar measurements of K were made at three other temperatures and the following extrapolated pK^0 values were obtained: 3·51 (15·0°), 3·42 (25·0°), 3·34 (34·0°), 3·29 (41·5°), the mean deviation in each case being 0·02 pK unit. A plot of pK^0 against $1/T$ (°K) was linear and yielded $\Delta H^0 = 3{\cdot}32 \pm 0{\cdot}18$ kcal. mole^{-1}, from which ΔS^0 (25°) $= -4{\cdot}5 \pm 0{\cdot}9$ cal. mole^{-1} deg.$^{-1}$. The low value of ΔS^0 is characteristic of the acid ionization of the $=NH^+$ group in a variety of compounds.[1b] The similarity in ΔS^0 and the marked difference in pK^0 values between pyridine and pyridine-2-aldoxime suggest that it is increased conjugation rather than any particular structural feature which accounts for the greater acid strength of the $=NH^+$ group in pyridine-2-aldoxime.

(b) *The oxime group.* For the acid ionization of this group, the following pK values were obtained at 25·0°: 10·11 ($I = 0{\cdot}025$), 10·16 ($I = 0{\cdot}010$), and 10·18 ($I = 0{\cdot}005$), the mean deviation being 0·02 unit of pK in each case. These values are consistent with the relation

$$pK = pK^0 - 0{\cdot}5I^{\frac{1}{2}}/(1 + I^{\frac{1}{2}}), \quad . \ . \ . \ . \ . \ . \ . \quad (4)$$

corresponding to the ionization

$$PAOH + H_2O = PAO^- + H_3O^+. \quad . \ . \ . \ . \ . \ . \ . \quad (5)$$

By adopting the same procedure as that for the $=NH^+$ ionization the following extrapolated values of pK^0 were obtained: 10·29 (18·5°), 10·22 (25·0°), and 10·06 (34·0°), the mean deviation being 0·02 unit of pK in each case. These values give $\Delta H^\circ = 6{\cdot}8 \pm 0{\cdot}8$ kcal. mole^{-1}, and hence ΔS^0 (25·0°) $= -24{\cdot}0 \pm 2{\cdot}8$ cal. mole^{-1} deg.$^{-1}$. It is interesting to note that these thermodynamic constants are similar to the corresponding values for the hydroxyl group in a variety of compounds.[1c]

Mason[4] has reported pK values for both ionizations in pyridine-2-aldoxime. Although ionic strength was not specified, his results appear to be in agreement with ours.

Ionizations in the Tri(pyridine-2-aldoxime)–Iron(II) *complex.*—Preliminary work,[3] using the method of continuous variation, had shown that the composition of the complex at pH 9·1 and also at pH 5·9 was 3 moles of ligand to 1 mole of ferrous ion. There was also fairly conclusive evidence that co-ordination occurs through the two nitrogen atoms of the ligand. With change of pH the complex underwent a series of reversible colour changes; in alkaline solution the absorption maximum was at 525 mμ, in slightly acidic solution at 515 mμ, and in more acidic solution at 475 mμ. The absorption spectrum in the visible region for the fully formed complex was taken over the pH range 10 to 3 and showed two different sets of isosbestic points. One set at 570 mμ and 490 mμ, appears in the pH region 9 to 5 and corresponds to the first colour change; the other, around 475 mμ and 425 mμ, in the pH range 5 to 3, corresponding to the second colour change.

On the above basis these colour changes were attributed to the successive ionization of the three oxime groups in the complex, as represented by the following equations:

$$Fe(PAOH)_3^{2+} = Fe(PAO)_3H_2^{+} + H^+ \quad (K_1) \quad . \ . \ . \ . \quad (6)$$

$$Fe(PAO)_3H_2^{+} = Fe(PAO)_3H^0 + H^+ \quad (K_2) \quad . \ . \ . \ . \quad (7)$$

$$Fe(PAO)_3H^0 = Fe(PAO)_3^{-1} + H^+ \quad (K_3) \quad . \ . \ . \ . \quad (8)$$

This was substantiated by measurements of changes of hydrogen-ion concentration accompanying the formation reaction in unbuffered solutions, and the charges on the ions

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[4] Mason, *J.*, 1960, 22.

were confirmed by their electrophoretic mobility in buffered solutions at various pH's.[3] pK_3 could easily be measured, pK_2 with some difficulty, but pK_1 could not be measured because of the rapid dissociation of the complex in solutions of pH < 3. At 25·0°, the following values of pK_3 were obtained: 7·01 ($I = 0{\cdot}055$M), 7·02 ($I = 0{\cdot}048$M), and 7·07 ($I = 0{\cdot}0125$M), the mean deviation in each case being 0·02 unit of pK. These results are consistent with a limiting relation of the same form as in eqn. 4, which is to be expected for the ionization in eqn. 8. Extrapolation to zero ionic strength gives p$K_3^0 = 7{\cdot}13 \pm 0{\cdot}02$ at 25·0°. The corresponding values at 17·5° and 33·5° are $7{\cdot}14 \pm 0{\cdot}02$ and $7{\cdot}11 \pm 0{\cdot}01$, respectively. ΔH^0 is thus $0{\cdot}8 \pm 0{\cdot}4$ kcal. mole^{-1}, and ΔS^0 -30 ± 2 cal. mole^{-1} deg.$^{-1}$.

In the case of pK_2 the optical density of a solution containing 100% conjugate acid could not be measured because of the rapid dissociation of the complex at pH < 3. The ionization constant was obtained by using eqn. (2), the ratio (base)/(acid) being calculated from the measured optical densities and the optical density of 100% conjugate acid which gave the best fit of the data. The extrapolated values, pK_2^0, are 3·36 at 24·5° and 3·33 at 33·0°, the mean deviation being 0·02 unit of pK in both cases. ΔH^0 is thus -1 ± 1 kcal. mole^{-1} and ΔS^0 -18 ± 6 cal. mole^{-1} deg.$^{-1}$.

The difference between the pK for the oxime group in pyridine-2-aldoxime (10·22) and pK_3 in the iron(II) complex (7·13) is unexpectedly large considering that the charge types are the same for both ionizations. The difference, which is clearly the result of co-ordination of the ligand to a metallic cation, shows the extent to which co-ordination can increase the acid strength of adjacent groups. The above thermodynamic data show that this difference arises almost entirely from a change in enthalpy, the entropy values being substantially the same. Another point worth noting is that ΔS^0 for the second oxime-group ionization of the complex (eqn. 7) has a large negative value. This is unusual for an ionization in which a positively charged species goes to a neutral molecule. Other experimental evidence relating to complex-ion equilibria also indicates that entropy changes are not always characteristic of the charge types involved.[5]

Ionization in the Tri(pyridine-2-aldoxime)-Iron(III) *Complex*.—This complex is brown in neutral solution. It undergoes rapid dissociation in acidic solution below pH 4. Only one reversible colour change, from brown to orange, could be observed. This probably refers to the third ionization of the complex

$$\mathrm{Fe(PAO)_3H^+} = \mathrm{Fe(PAO)_3^0} + \mathrm{H^+} \quad (K_3') \quad . \quad . \quad . \quad . \quad . \quad (9)$$

At 25° and $I \sim 0$, an average value of p$K_3' = 3{\cdot}5$ was obtained. Because of the rapid dissociation of the complex in this pH region optical-density readings had to be extrapolated back to zero time, and the ionization constant obtained by the same method as that used for obtaining pK_2 of the iron(II) complex.

It is interesting that the difference between pK_3' of the iron(III) complex and pK_3 of the iron(II) complex is of the same order of magnitude as that between pK_2 and pK_3 of the iron(II) complex. This could well be a reflection of the charge types' being similar in the two cases.

Formation Constant of the Tri(pyridine-2-aldoxime)-Iron(II) *Complex*.—This quantity is important for two reasons. First, its variation with pH provides a useful check on the ionizations represented by eqns. (6)—(8). Secondly, it can be used along with similar data, for the same ligand and a series of metallic cations, to find out whether there is, in fact, a correlation between formation constant of complex and acid ionization constant of a ligand. Harkins and Freiser[2] have reported that a linear relationship exists between the quantity log (formation constant) of the complexes of 2-2′-pyridylbenzimidazole with Cu(II), Zn(II), Mn(II), and Co(II), and the pH at half-neutralization of the =NH$^+$ group, but not so in the case of nickel(II). The case of nickel could be exceptional,

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[5] George, Hanania, and Irvine, *Rec. trav. chim.*, 1956, **75**, 759.

for steric or other reasons, but clearly a study of more systems of this type is necessary if any conclusion is to be reached.

The formation constant, K_{obs}, of the iron(II) complex of pyridine-2-aldoxime was measured at three temperatures, over a range of pH, and at constant ionic strength ($I = 0{\cdot}045$M). The theoretical overall equilibrium is

$$Fe^{2+} + 3PAOH = Fe(PAOH)_3{}^{2+} \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

for which the measured formation constant, K_{obs}, is defined in terms of concentration. However, since the concentration of the species PAOH varies with pH in accordance with the equilibria in eqns. (1) and (5), and that of $Fe(PAOH)_3{}^{2+}$ in accordance with the equilibria in eqns. (6)—(8) it follows that K_{obs} will be markedly dependent on pH, as indeed the data in the Table show.

Variation of K_{obs} with pH.

pH	5·87	5·04	4·15	4·04
$10^{-9}K_{obs}$	29,000	310	4·01	2·71
$10^{-24}K_T{}'$	9·5	5·2	6·0	7·5

$T = 25{\cdot}0°$, $I = 0{\cdot}045$M; $K_T{}'$ calculated from K_{obs} by using eqn. (13) and the following data: $pK_3 = 7{\cdot}02$, $pK_2 = 3{\cdot}45$, $pK_N = 3{\cdot}52$, $pK_0 = 10{\cdot}10$.

For $K_1 \gg K_2 \gg K_3$, the equation relating K_{obs} to the pH-independent formation constant K_T (which refers to eqn. 10) can be written in the form

$$K_T = K_{obs} \cdot \frac{h}{K_3 + h} \cdot \frac{h}{K_2 + h} \cdot \frac{h}{K_1 + h} \cdot \left(\frac{K_N + h}{K_N}\right)^3 \cdot \left(\frac{K_0 + h}{h}\right)^3 \quad . \quad (11)$$

where h represents a_{H^+}, the activity of the hydronium ion obtained from pH measurement, K_N the acid ionization constant of the pyridinum $=NH^+$ group (identical with K of eqn. 2), K_0 the ionization constant of the oxime group (identical with K of eqn. 5), and K_1, K_2, K_3 are as already defined in equations (6)—(8).

K_T can thus in principle be obtained from the values of K_{obs} and the ionization constants of pyridine-2-aldoxime and of complex. Unfortunately, K_1 cannot be measured because of rapid dissociation of the complex at low pH. However since K_3 is $\sim 10^{-7}$ and $K_2 \sim 10^{-3}$, it seems reasonable to assume that K_1 is of the order of 10^{-1}. On this basis K_T should be of the order of 10^6. Likewise, since $\Delta H_3 \sim 1$ kcal. mole^{-1} and $\Delta H_2 \sim -1$ kcal. mole^{-1}, it seems reasonable to assume that ΔH_1 is of the order of -2 kcal. mole^{-1}. These considerations suggest that the reaction represented by eqn. 10 is approximately thermoneutral, and that its free energy arises mainly from a favourable entropy change of about 30 e.u. It is interesting to compare these results with those for the corresponding bipyridyl–iron(II) system.[6] In both cases the ligand is bonded to the metal through the $-N{=}C{-}C{=}N-$ group. However, whereas in the case of bipyridyl the large free energy of formation ($K_T \sim 10^{17}$ at 25°) arises almost entirely from a large negative enthalpy of reaction ($\Delta H \sim -24$ kcal. mole^{-1}) with very little contribution from the entropy ($\Delta S \sim -4$ e.u.), the reverse is true for pyridine-2-aldoxime.

An alternative pH-independent formation constant, $K_T{}'$, which can be calculated from the data with precision refers to the equation

$$Fe^{2+} + 3PAO^- = Fe(PAO)_3{}^- \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

It can be shown that, within the range of pH covered in these experiments (4·0—6·0), the relation between $K_T{}'$ and K_{obs} can be written in the form

$$K_T{}' = K_{obs} \cdot \frac{K_3}{K_3 + h} \cdot \frac{K_2}{K_2 + h} \cdot \left(\frac{K_N + h}{K_N}\right)^3 \cdot \left(\frac{K_0 + h}{K_0}\right)^3 \quad . \quad . \quad . \quad (13)$$

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[6] Baxendale and George, *Trans. Faraday Soc.*, 1950, **46**, 55.

The Table, which shows the variation of K_{obs} with pH, also includes the calculated (constant) values of K_T'. In view of the number of variables involved the reasonably constant value of K_T is further support for the ionizations suggested in eqns. 6—8. The mean value of K_T at 25·0° and $I = 0{\cdot}045$ is $(7{\cdot}1 \pm 1{\cdot}4) \times 10^{24}$. Similar experiments carried out at three other temperatures and the same ionic strength gave the following results: $10^{-24}K_T' = 13{\cdot}4 \pm 1{\cdot}8$ (18·0°); $5{\cdot}0 \pm 1{\cdot}0$ (28·5°); and $2{\cdot}7 \pm 0{\cdot}5$ (32·5°). These results yield a value of $\Delta H = -20 \pm 4$ kcal. mole^{-1} and $\Delta S = 47 \pm 14$ e.u. This large exothermicity and favourable entropy change are perhaps to be expected in view of the charge types involved in the above reaction.

EXPERIMENTAL

Reagents and Materials.—Pyridine-2-aldoxime was obtained from L. Light & Co. Ltd. It was recrystallized thrice from conductivity water. The recrystallized product had a melting point of 113° (lit., 113°). All other chemicals were of analytical reagent grade. Conductivity water was used for making solutions, and ionic strengths were adjusted with sodium chloride or sodium perchlorate.

Determination of Ionization Constants.—The ionization constants of pyridine-2-aldoxime as well as of its iron complexes were obtained spectrophotometrically. In the case of the free ligand, optical-density measurements at various pH values were made at 260 mμ for the pyridinium $=NH^+$ ionization and at 294 mμ for the ionization of the oxime group. Values of pK at finite ionic strength were calculated by using eqn. (2), where the measured pH is assumed to be approximately equal to −log(activity of the hydronium ion), and the concentration ratio (base)/(acid) is calculated from optical densities. A similar procedure was followed in the determination of the ionization constants of the iron complexes. For pK_3 of the iron(II) complex optical-density measurements were made at 525 mμ, for pK_2 at 510 mμ, and for pK_3 of the iron(III) complex at 475 mμ.

Formation Constant of the Iron(II) *Complex.*—In order to ensure rapid attainment of equilibrium aliquot portions of concentrated stock solutions of ferrous ammonium sulphate and pyridine-2-aldoxime were mixed in water, set aside for a few minutes, and then diluted with buffer. Optical-density and pH measurements were then made on the dilute solution, which was approximately 5×10^{-5}M in complex. The wavelength chosen (between 520 and 510 mμ) depended on the pH. In all measurements the concentration of pyridine-2-aldoxime was sufficiently in excess of that of ferrous ion to ensure that only the tris-complex was involved in the equilibrium.

All optical-density measurements were made either on a Unicam S.P. 500 quartz spectrophotometer or on a Beckman D.U. spectrophotometer. Absorption spectra in very acidic solutions were taken on a Beckman DK 1 recording spectrophotometer. pH measurements were made either on a Doran pH-meter or on a Beckman pH-meter model G, readings being precise to ±0·01. Primary standards of pH were used, as recommended by the National Bureau of Standards. In all experiments temperature was controlled to ±0·05° by circulating water from a thermostat.

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