## The Stability of Iron Complexes. Part III.<sup>1</sup> A Comparison 50. of 1:1 Ferric and Ferrous Amino-acid Complexes.

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The stability constants of twenty 1:1 ferrous complexes with  $\alpha$ -aminoacids were obtained in water at  $20^{\circ}$  and I = 1, in a medium consisting essentially of potassium chloride, by potentiometric titration. The constants vary with the proton, acid, dissociation constants of the amino-acids, but to a much smaller extent than for the corresponding ferric complexes. Molar electrode potentials for the  $Fe^{3+}$ :  $Fe^{2+}$  amino-acid complexes, calculated from these results, are linearly related to the logarithms of the dissociation constants of the amino-acids. The slope of the line is probably somewhat greater than similar slopes for the iron 1:1 polyaza-1-naphthol and 5substituted 1:10-phenanthroline complexes.

LITTLE quantitative information exists on the relative stabilities in aqueous solutions of ferric and ferrous complexes with a series of similar ligands. Albert and Hampton<sup>2</sup> obtained stability constants of 1:1 ferric and ferrous complexes with seven polyaza-1naphthols (" aza-oxines ") and Smith and Richter 3 measured the oxidation-reduction

<sup>&</sup>lt;sup>1</sup> Parts I and II, J., 1958, 3120, 3125.

 <sup>&</sup>lt;sup>2</sup> Albert and Hampton, J., 1954, 505.
 <sup>3</sup> Smith and Richter, Ind. Eng. Chem. Anal., 1944, 16, 580.

potentials of iron chelates of four substituted 1:10-phenanthrolines. Although stability constants for both ferric and ferrous iron with a further eighteen organic ligands have been tabulated.<sup>4</sup> the ligands were of so many different types, and experimental conditions varied so widely, that few quantitative comparisons are possible. Qualitatively, as is to be expected from entropy effects <sup>5</sup> and electrostatic considerations, ferric complexes are more stable than ferrous complexes when the ligand is an anion; the relative stability appears to increase with the charge on the ligand. In the few cases so far studied where the ligand is a neutral molecule the ferrous complexes are the more stable.

The dissociation constant, K', of the ligand is also important. In many cases, including the 1:1 ferric-amino-acid complexes,<sup>1</sup> the stability constants of a cation with a series of similar ligands follow approximately the relation <sup>6</sup> log  $K = \alpha p K' + c$ , where  $\alpha$  and c are constants. It has been suggested that this relation is somewhat fortuitous and is obeyed so widely only because most ligands are at the same time both  $\pi$ - and  $\sigma$ -electron donors (or acceptors).<sup>7</sup> Although the value of  $\alpha$  appears to increase with the electronegativity of the cation, from about 0.3 for Ag<sup>+</sup> in complexes with secondary amines <sup>8</sup> to 1.7 for  $Fe^{3+}$  in amino-acid complexes,<sup>1</sup> factors affecting  $\alpha$  have not been studied in detail. It has also been suggested that from thermodynamic reasoning  $\alpha$  should be unity; <sup>9</sup> on the other hand it has been stated that there is no reason to expect this value.<sup>5</sup> From a simple electrostatic model  $\alpha$  should increase with increasing cationic charge and decreasing distance of separation of the cation and ligand.<sup>7</sup> The effect of the dissociation constants of similar ligands on the relative stabilities of ferric and ferrous complexes, and hence on their standard oxidation-reduction potentials, has been very little studied, except that it has been observed that the potentials of iron complexes of 5-substituted 1:10-phenanthrolines varied with pK' of the corresponding *para*-substituted benzoic acids <sup>10</sup> and of the ligands themselves.<sup>11</sup> Such a study has recently been commenced in mixed aqueous organic solvents.12

The results now reported for 1:1 ferrous complexes with  $\alpha$ -amino-acids are part of an investigation of the effects of the charge and the dissociation constant of a ligand on the stability in aqueous solution of its ferric and ferrous complexes. Amino-acids are convenient complex-forming species for this purpose because the stability constants of the corresponding ferric complexes are known. However, although stability constants of many 1:2 ferrous-amino-acid complexes have been recorded,<sup>4</sup> values for the 1:1 ferrous complexes have been published only for glycine,<sup>13</sup> glutamic acid,<sup>13</sup> lysine,<sup>14</sup> cysteine,<sup>14</sup> arginine,<sup>14</sup> and ornithine.<sup>14</sup>

## EXPERIMENTAL

The amino-acids were of the same quality as in Part II. All other reagents were of analytical grade. Ferrous perchlorate solutions were prepared by double decomposition of carefully neutralised barium perchlorate and ferrous sulphate solutions. Ferric ion was removed by passage through a column of the ferrous salt of a cation-exchange resin (Amberlite IR-120). The eluates, which gave only weak tests for ferric ion on addition of sodium thiocyanate, were stored under nitrogen. Ferrous concentrations were determined by titration

Bjerrum, Schwarzenbach, and Sillén, "Stability Constants. Part 1: Organic Ligands," The Chemical Society, London, Spec. Publ. No. 6, 1957.
 Williams, in "Special Lectures in Biochemistry, 1954—1955," University College, London, p. 43.

<sup>6</sup> Keller and Parry, in "The Chemistry of the Co-ordination Compounds," ed. Bailar, Chapman and Hall, London, 1956, p. 180. <sup>7</sup> Jones, Poole, Tomkinson, and Williams, J., 1958, 2001.

- <sup>1</sup> Jones, Foole, Fonkinson, and Winnans, J., 1950, 2001.
  <sup>8</sup> Bjerrum, Chem. Rev., 1950, 46, 381.
  <sup>9</sup> Irving and Rosotti, J., 1954, 2910.
  <sup>10</sup> Ewens, Nature, 1945, 155, 398.
  <sup>11</sup> Brandt and Gullstrom, J. Amer. Chem. Soc., 1952, 74, 3532.
  <sup>12</sup> Turificant of Williams J. 1959, 2010.
- <sup>12</sup> Tomkinson and Williams, J., 1958, 2010.
   <sup>13</sup> Albert, Biochem. J., 1953, 54, 646.
   <sup>14</sup> Idem, ibid., 1952, 50, 690.

with potassium dichromate in phosphoric acid, sodium diphenylamine-4-sulphonate being used as indicator.

Stability constants of the 1:1 ferrous-amino-acid complexes were obtained potentiometrically by Bjerrum's method <sup>15</sup> as developed by Calvin and Wilson,<sup>16</sup> by use of the relationship,

$$K_1 = \frac{\overline{n}}{(1-\overline{n})[L^-]} - K_s \left(\frac{2-\overline{n}}{1-\overline{n}}\right)[L^-] \quad . \quad . \quad . \quad . \quad (i)$$

where  $K_{\rm g}$  is the overall stability constant of the 1:2 metal-amino-acid complex. The rigorous graphical treatment of eqn. (i) described by Irving and Rossotti (J., 1953, 3397) could not be used to evaluate  $K_1$  accurately because over the selected range of  $\overline{n}$  values (0.06-0.40), the quantity,  $\bar{n}/\{(1-\bar{n})[L^-]\} - K_1$ , was too sensitive to experimental error for an estimate of  $K_s$ to be obtained. Thus for all values of  $\bar{n} \leq 0.20$ , the range  $K_1/K_2 = 10$  to  $K_1/K_2 = \infty$  is covered by a difference of less than 0.03 log unit, which is about half the experimental error of a single estimate of log  $K_1$ ; even at  $\bar{n} = 0.40$  the difference is less than 0.08 log unit. Accordingly, the approximation 17

$$\log K_{\rm s} = 2 \log K_1 - 1 \qquad \dots \qquad \dots \qquad \dots \qquad (\rm ii)$$

was used in evaluating the final, small, term in eqn. (i). Table 1 gives experimental results for the complexes studied. Values of  $\log K_1$  have been calculated on the assumption that complex formation takes place through the uncharged  $\alpha$ -amino-nitrogen atom of the aminoacid anion. Probably in the compound so formed the ferrous ion is chelated between the  $\alpha$ -amino-nitrogen and one or more of the carboxylic oxygen atoms.<sup>14, 17, 18</sup> However, because the carboxyl groups are already fully ionised under the experimental conditions, any bonding to them is not detected potentiometrically. Solutions 0.0100M in amino-acid and 0.0050M in ferrous ion were titrated, under nitrogen, with 0.1M-potassium hydroxide (carbonate free). A stream of nitrogen, free from oxygen and saturated with water vapour, was used for mixing the solutions. All measurements were made at  $20^\circ \pm 0.1^\circ$ , with a Cambridge bench model pH meter and a glass electrode-saturated calomel electrode assembly. A constant ionic strength of 1.0 was maintained by adding potassium chloride. Sodium perchlorate, which was used for this purpose in studying ferric complex formation (Part II), was not satisfactory for the present investigation because, in the near-neutral solutions, it oxidised ferrous ion. From the similarity of the activity coefficients of hydrochloric acid in sodium perchlorate and potassium chloride solutions <sup>19</sup> it was assumed that these salts are comparable in their effects on the stability constants of the iron-amino-acid complexes.

For the amino-acids studied, ferrous and ferric complexes are believed to be structurally similar.<sup>1</sup> Hence, from the equation,

$$E_{\rm L}^{\circ} = E^{\circ}_{\rm Fe^{i+}, \rm Fe^{i+}} - 2.3026 \, (\mathbf{R}T/\mathbf{F}) \, (\log K_{\rm Fe^{i+}} - \log K_{\rm Fe^{i+}}) \, . \qquad . \qquad (iii)$$

the standard oxidation–reduction potential,  $E_{L}^{\circ}$ , of any corresponding pair of 1 : 1-iron–aminoacid complexes can be calculated once the stability constants,  $K_{Fe^{0}+}$  and  $K_{Fe^{0}+}$ , of the ferric and the ferrous complexes are known. At 20° the standard potential of the ferric-ferrous electrode, in a sodium perchlorate solution of unit ionic strength, was 0.4956 v against a saturated calomel electrode, an ammonium nitrate-sodium nitrate-agar bridge being used. Taking  $E_{\text{sat. calc.}}$  as 0.244 v at  $20^{\circ}$ , 20 we get  $E^{\circ}_{Fe^{1+}}$ ,  $Fe^{1+} = 0.740$  v at  $20^{\circ}$  and I = 1, in good agreement with the figure <sup>21</sup> of 0.741 v at 25°. Values of  $E_{\rm L}^{\circ}$  for 1 : 1 iron-amino-acid complexes, calculated from eqn. (iii) by using data  $^{1}$  for the ferric complexes and the present results for the ferrous complexes, are given in Table 2.

- Hawkins, J. Amer. Chem. Soc., 1932, 54, 4480; Bates and Urmston, ibid., 1953, 55, 4068.
   Ewing, J. Amer. Chem. Soc., 1925, 47, 301; Chateau, J. Chim. phys., 1954, 51, 590.
- <sup>21</sup> Schumb, Sherrill, and Sweetser, J. Amer. Chem. Soc., 1937, 59, 2360.

<sup>&</sup>lt;sup>15</sup> Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

<sup>&</sup>lt;sup>16</sup> Calvin and Wilson, J. Amer. Chem. Soc., 1945, 67, 2003.
<sup>17</sup> Albert, Biochem. J., 1950, 47, 531.
<sup>18</sup> Flood and Lorås, Tidsskr. Kjemi Bergvesen Met., 1945, 5, 83; Maley and Mellor, Nature, 1950, 165, 453.

 TABLE 1. Derivation of stability constants for 1:1 ferrous-amino-acid complex.

 $[Fe^{2+}] = 0.005 \text{m}, [HL] = 0.01 \text{m}, I = 1 \text{m} (KCl)$ 

$[Fe^{2+}] = 0.005M, [HL] = 0.01M, I = 1M$ (KCI)										
$\bar{n} =$ (a) Glycine.	= 0.06	0.08	0.10	0.12	0.16	0.20	0.24	0.30	0.40	0.50
$\begin{array}{c} \text{pH} \dots \\ -\log [L^{-}] \dots \\ \log K_1 \dots \end{array}$	. —	6·80 4·980 3·91	6∙95 4∙835 3∙87	7·08 4·710 3·83	7·24 4·560 3·82	7·38 4·430 3·80	7·48 4·341 3·80	7·62 4·217 3·80	7·80 4·065 3·81	
(b) Alanine. pH -log [L <sup>-</sup> ]		7∙08 4∙730	7·26 4·555	7·40 4·420	7∙58 4∙250	7·72 4·120	7·80 4·051	7·89 3·977	8∙00 3∙896	_
$\log K_1  \dots  \dots  \dots  \dots  \dots  \dots  \dots  \dots  \dots  $		(3.66)	<b>3</b> ∙59	<b>3∙54</b>	<b>3</b> ·51	3.48	3.52	<b>3</b> ∙57	(3.68)	
(c) α-Amino- <i>n</i> -butyric pH -log [L <sup>-</sup> ]		7·20 4·480 3·41	7·34 4·345 3·38	7·44 4·250 3·37	7·60 4·100 3·36	7·74 3·970 3·34	7∙83 3∙891 3∙36	7·95 3·787 3·38	8·10 3·656 3·43	
$\log K_1$										
$\begin{array}{c} \mathbf{p}\mathbf{H} \dots \\ -\log \left[\mathbf{L}\right]^{-} \dots \\ \log K_1 \dots \end{array}$	4.725	7·61 4·500 3·43	7·72 4·395 3·42	7·81 4·305 3·42	7·88 4·250 3·51	7·96 4·180 3·56	 	8·06 4·107 (3·72)	8·15 4·046 (3·85)	 
(e) Valine. pH $-\log [L^{-}]$ $\log K_1$	4.655	7·14 4·470 3·40	7·30 4·315 3·37	7∙39 4∙230 3∙35	7·55 4·080 3·34	7∙68 3∙960 3∙34		7·87 3·797 3·39	8·02 3·676 3·46	 
(f) Leucine.								0.00	0 10	
pH —log [L <sup>-</sup> ] log K <sub>1</sub>	<b>4·67</b> 5	7·16 4·480 3·41	7·28 4·365 3·40	7·37 4·280 3·40	7·52 4·140 3·40	7.63 4.040 3.42		7·86 3·837 3·43	7·96 3·766 (3·56)	
(g) β-Phenylalanine pH		6.78	6.96	7.08	7.24	7.36	7.48	7.60	7.77	
$-\log [L^-]$ $\log K_1$		4·420 3·35	$4 \cdot 215 \\ 3 \cdot 25$	4·130 3·25	3∙980 3∙24	3·870 3·24	3·761 3·23	3.657 3.25	$3.516 \\ 3.28$	
(h) Serine.	6.44	6.69	6.74	6.96	7 09	7.15		7 90	<b>7</b> 50	
$\begin{array}{c} \text{pH} \dots \\ -\log \left[ L^{-} \right] \dots \\ \log K_{1} \dots \end{array}$	0.44 4.695 3.50	6∙62 4∙520 3∙45	6·74 4·405 3·44	6·86 4·290 3·41	7·02 4·140 3·40	4·020 3·39		7·39 3·807 3·40	7·56 3·666 3·43	
(i) Threonine.										
$\begin{array}{c} \mathrm{pH} \dots \\ -\log \left[ \mathrm{L}^{-} \right] \dots \\ \log K_{1} \dots \end{array}$	6∙31 4∙565 3∙37	6·50 4·375 3·31	6∙63 4∙255 3∙29	6·73 4·160 3·28	6·88 4·020 3·28	7.00 3.910 3.28		7·25 3·687 3·28	7∙42 3∙546 3∙31	
(j) Aspartic acid.										
pH $-\log [L^-]$ $\log K_1$	6∙08 5∙524 4∙32	6·20 5·409 4·34		6∙38 5∙239 4∙36	6∙54 5∙089 4∙35	6∙66 4∙979 4∙35		6∙92 4∙746 4∙33	7•12 4∙575 4∙33	7·30 4·413 4·31
(k) Glutamic acid.										
$\begin{array}{c} \text{pH} \dots \\ -\log [L^{-}] \dots \\ \log K_1 \dots \end{array}$	6·78 4·775 3·58	6∙97 4∙590 3∙52		7·20 4·370 3·49	7·34 4·240 3·50	7·47 4·120 3·49		7·70 3·917 3·51	7·86 3·786 3·56	
(l) Asparagine.										
$\begin{array}{c} \mathbf{p}\mathbf{H} \dots \\ -\log \left[\mathbf{L}^{-}\right] \dots \\ \log K_{1} \dots \end{array}$		6·26 4·550 3·48	6·40 4·415 3·45	6·52 4·300 3·42	6·69 4·140 3·40	6·84 4·000 3·37	6·95 3·901 3·37	7·10 3·767 3·35	7·30 3·596 3·34	
(m) Arginine.								0.00	001	
$\begin{array}{c} \mathrm{pH} \dots \\ -\mathrm{log} \ [\mathrm{L}^{-}]  \dots \\ \mathrm{log} \ K_1  \dots \end{array}$	6·70 4·525 (3·33)	6·90 4·330 3·26		7·15 4·090 3·21	7·32 3·930 3·19	7·44 3·820 3·19		7·68 3·607 3·20	7·82 3·496 3·27	
(n) Ornithine.	(0.00)	0 10		~ - 1	0 10	0.0		0 40	5 - 1	
$\begin{array}{c} pH \dots \\ -\log [L^{-}] \dots \\ \log K_1 \dots \end{array}$	6·48 4·465 (3·27)	6·75 4·200 3·13	6·88 4·075 3·11	6·98 3·980 3·10	7·17 3·800 3·10	7·29 3·690 3·06		7·53 3·477 3·06	7·70 3·336 3·10	
(o) Proline.	/				•					
pH $-\log [L^-]$ $\log K_1$	7·20 5·335 4·14	7·42 5·220 4·05	7·57 4·975 4·01	7·66 4·890 4·01	7·76 4·800 4·06	7·82 4·750 4·13		7·96 4·637 (4·25) (	8·05 4·576 (4·38)	
			_						/	

TABLE 1.    (Continued.)										
$\bar{n} =$	0.06	0.08	0.10	0.12	0.16	0.20	0.24	0.30	0.40	0.50
(\$\nterf{p}) Hydroxyproline.										
рН	6.41	6.60	6.70	6.80	6.96	7.08	<u> </u>	7.32	7.50	
$-\log [L^-]$	5.185	5.000	4.905	<b>4</b> ·810	4.660	4.550		4.337	<b>4</b> ·186	
$\log K_1$	3.99	3.93	3.94	3.93	3.92	3.92		3.93	3.95	
(q) Tryptophan.										
рН	6.77	6.95	7.10	7.20	7.35	7.47		7.68	<b>7·84</b>	
$-\log [L^{-}]$	4.686	4.511	4.366	4.271	<b>4</b> ·131	4.021		3.838	3.707	
$\log K_1$	3.49	3.44	3.40	3.39	3.39	3.40		3.44	3.50	
(r) Methionine.										
рН	6.64	6·84	6.98	7.07	7.24	7.36		7.58	7.75	_
-log [L <sup>-</sup> ]	4.516	4.321	4.186	4.101	3.941	3.831		3.638	3.497	<del></del>
$\log K_1$	3.32	3.25	3.22	3.22	3.20	3.20		3.23	3.27	
(s) Sarcosine.										
рН	7.18	7.42	7.56	7.68	7.82	7.90		8.02	8.08	
-log [L <sup>-</sup> ]	4.855	4.620	4.485	4.370	4.240	4.170		4.077	4.046	<del></del>
$\log K_1$	(3.66)	3.55	3.52	<b>3·4</b> 9	3.50	3.55		(3.69)	(3.86)	
(t) Glycylglycine.										
рН	6.29	6.52	6.64	6.76	<b>6</b> ∙94	7.07		7.30	7.46	
-log [L <sup>-</sup> ]	3.955	3.730	3.612	3.500	<b>3·33</b> 0	$3 \cdot 210$		3.007	2.876	
$\log K_1$	(2.76)	2.66	2.65	2.62	2.59	2.58	$\rightarrow$	2.60	2.65	<u> </u>

Figures in parentheses have been omitted in obtaining average values.

 TABLE 2. Stability constants of 1:1 ferrous-amino-acid complexes, and molar electrodepotentials of ferric-ferrous couples.

(In water at 20° and unit ionic strength of potassium chloride.)

	pK' (Ref. 1	)	pK' (Ref. 1)					
Amino-acid	$(\alpha - NH_2)$	$\log K_1$	$E_{L}^{o}(v)$	Amino-acid	$(\alpha - \dot{N}H_2)$	$\log K_1$	$E_{\mathbf{L}}^{\mathbf{o}}(\mathbf{v})$	
Glycine	9.76	3.83	0.380	Glutamic acid	9.54	3.52	0.240	
Alanine	9.79	3.54	0·340	Asparagine	8.79	3.40	0.440	
α-Amino- <i>n</i> -butyric acid	9.66	3.37	0.370	Arginine	9.21	$3 \cdot 22$	0.420	
α-Amino <i>iso</i> butyric acid	10.09	3.48	0.345	Ornithine	8.93	3.09	0.412	
Valine	9.59	3.39	0.380	Proline	10.52	4.07	0.395	
Leucine	9.62	3.42	0.362	Hydroxyproline	9.58	3·9 <b>4</b>	0.445	
$\beta$ -Phenylalanine	<b>9·18</b>	3.26	0.412	Tryptophan	9.43	3.43	0.412	
Serine	9.12	3.43	0.402	Methionine	9.13	$3 \cdot 24$	0.400	
Threonine	8.86	3.30	0.430	Sarcosine	10.02	3.52	0.380	
Aspartic acid	9.56	4.34	0.330	Glycylglycine	8.23	$2 \cdot 62$	0.360	

## DISCUSSION

The approximation (ii) provides a rough check on the results if it is assumed that the effect of ionic strength on the stability constants is the same for all amino-acids. From results for glycine (present work and ref. 12) this effect is found to be a decrease of about 0.5 in log  $K_1$  as *I* increases from 0.01 to 1.0 (KCl). For eight of the ten amino-acids where such a comparison is possible,<sup>14, 17</sup> estimated and observed values of log  $K_1$  do not differ by more than 0.1; only for tryptophan (0.37) is the difference greater than 0.2. On the other hand, previous values <sup>14</sup> for the 1: 1 ferrous complexes with ornithine and glutamic acid are much higher than were found in the present work (which now places the ferrousornithine complex in its correct position in the Irving-Williams <sup>22</sup> series).

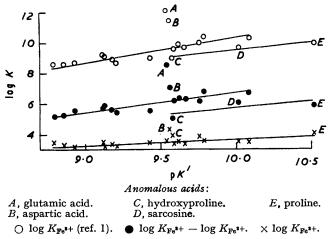
From the Figure the complex-forming ability of an amino-acid with ferrous ion varies much less with the proton, acid, dissociation constant of the amino-acid than was found for ferric ion. In both cases, however, approximately linear relationships are obtained. For the ferrous complexes the slope,  $\alpha$ , of the line drawn by the method of least squares is about 0.4. Although subject to considerable uncertainty because of the scatter of the experimental points, values of  $\alpha$  of this order of magnitude for the 1:1 copper and zinc

<sup>22</sup> Irving and Williams, Nature, 1948, 162, 746.

amino-acid complexes are obtained from published  $^{14, 17, 23}$  data if the approximation (ii) is assumed. On the other hand, for the corresponding ferric complexes,  $\alpha$  is about 1.7.

The ferrous-aspartic acid complex is more stable than would be predicted from its dissociation constant, probably indicating additional chelation through the  $\beta$ -carboxyl group. The increase in stability is less than for the ferric complex, possibly because of the difference in the charges on the metal ions. The lower charge, and the requirement that the ring formed must be seven-membered, may explain why enhanced stability is not observed for the ferrous-glutamic acid complex. Similarly, and unlike the ferric complex, 1:1 ferrous-glycylglycine is less stable than predicted from the dissociation constant of glycylglycine. In contrast to the ferric complexes, the 1:1 ferrous-proline and -hydroxy-proline complexes are somewhat more stable than expected.

Dependence of stability constants and oxidation-reduction potentials of iron complexes on dissociation constants of amino-acids.



The difference of 1.3 between  $\alpha_{Fe^{s+}}$  and  $\alpha_{Fe^{s+}}$  for the 1:1 amino-acid complexes gives directly from eqn. (iii) their change of standard oxidation-reduction potential with dissociation constant as -0.075 volt/pK' unit. The basic skeleton,  $-N\cdot C\cdot C\cdot O$ -, of the aminoacid chelating system is also found in oxine (8-hydroxyquinoline) and its aza-derivatives. However, probably because of large individual variations in resonance energies of the ligands as different ring carbon atoms are replaced by nitrogen, the stability constants of neither the 1:1 ferric nor the 1:1 ferrous series comprising 8-hydroxyquinoline.<sup>2,13</sup> 8-hydroxyquinoline-5-sulphonic acid,<sup>2,13</sup> and polyaza-1-naphthol<sup>2</sup> complexes show much correlation with dissociation constants. On the other hand, the difference, log  $K_{\rm Fe^{a+}}$  -- $\log K_{\rm Fe^{++}}$ , is roughly linear with pK' and of slope about 0.8, giving  $dE_{\rm L}^{\circ}/d(pK') \sim -0.045$  v. From recent measurements,<sup>12</sup> the plot of oxidation-reduction potentials of 1:3 iron complexes of substituted 8-hydroxyquinolines in 50% dioxan-water against pK' ( $pK_{OH}$ ) gives a straight line with a slope,  $dE_{\rm L}^{\circ}/d(pK')$ , ~ -0.080 v; except for 5-cyano-8-hydroxyquinoline  $(\delta \sim 0.12 \text{ v})$  the deviation,  $\delta$ , of points from this line averages 30 mv. However, if potentials are plotted against the sum,  $pK_{NH} + pK_{OH}$ , which has been suggested as a measure of ligand basicity,<sup>7</sup> no simple relation is found.<sup>12</sup> If the factors governing the binding of protons and cations are similar, it seems reasonable to expect that the stability constants of metal chelates might depend on the dissociation constant of the more strongly proton-binding of the two chelating groups. Hence, for the substituted 8-hydroxyquinolines where  $pK_{OH}$  is 5–8 log units greater than  $pK_{NH}$ ,  $pK_{OH}$  rather than  $pK_{NH}$  +  $pK_{OH}$  should be used in comparing the observed oxidation-reduction potentials. If this

<sup>23</sup> Perkins, Biochem. J., 1954, 57, 702; Monk, Trans. Faraday Soc., 1951, 47, 292, 297.

is done, 5-formyl- and 5-cyano-8-hydroxyquinoline complexes are found to have potentials much nearer the predicted values than if the comparison uses the sums of the pK values. Similarly, if an error of about 10 mv is assumed, the oxidation-reduction potentials of five 1:3 iron-substituted phenanthroline complexes (probably in dioxan-water) <sup>12</sup> are linear with  $pK_{\rm NH}$ . Results for some 1:3 iron complexes of 5-substituted 1:10-phenanthrolines in M-sulphuric acid <sup>3,11</sup> show  $E_{\rm L}^{\circ}$  to vary directly with pK', within experimental uncertainty, giving  $dE_{\rm L}^{\circ}/d(pK') = -0.120$  v. If it is assumed that, because the ligands are neutral molecules, the pK' effect is roughly the same for successive complex formation,  $dE_{\rm L}^{\circ}/d(pK')$  for the corresponding 1:1 complexes thus becomes of the order of -0.040 v. For all three groups (amino-acid, 8-hydroxyquinoline, and phenanthroline) of 1:1 iron complexes in aqueous solution it appears, therefore, that as the basic strength of the ligand is increased the firmness with which the valency electron is bound to ferrous ion varies in approximately the same manner. Whether this is also true of ligands containing other skeletons, such as  $-0.{\rm C}\cdot{\rm C}\cdot{\rm C}\cdot{\rm O}-$ , is at present being examined.

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