CXXXV. NOTE ON THE ELECTROMETRIC TITRATION OF *dl*-2-THIOLHISTIDINE.

By GEORGE MAXWELL RICHARDSON.

*From the Biochemistry Department, Imperial College of
Science and Technology, S.W. 7.*

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dl-2-THIOLHISTIDINE [Harington and Overhoff, 1933] has proved somewhat intractable to electrometric investigation. Its behaviour however has been ascertained as far as was possible without very comprehensive investigation. In spite of an unsatisfactory individual definition of points, a general concordance of three separate titrations in each direction has nevertheless been obtained. Data are depicted in Table I and Fig. 1 A.

Table I. *Calculation of titration constants.*

dl-2-Thiolhistidine approx. 0.03 *M*. 0.0995 *M* HCl. 0.1012 *M* NaOH. 25°. Italicised figures represent a fresh electrode.

Estimated p_H	Sat. KCl cc.	Titre HCl cc.	Corr. titre cc.	p_{G_1}	Estimated p_H	Titre NaOH cc.	Corr. titre cc.	p_G
(a) Indigo electrode. 0.0504 g. in 10 cc. + 1 cc. 0.01 <i>M</i> indigo carmine.					(c) H ₂ electrode. 0.0590 g. in 10 cc.			
4.711	0	0			4.734	0		
3.460	0.01	0.11	0.06	1.82	7.236	0.31	0.23	8.34
3.107	0.01	0.25	0.145	1.86	7.549	0.50	0.42	8.36
2.868	0.01	0.40	0.23	1.84	7.934	0.80	0.72	8.46
2.695	0.02	0.59	0.33	1.84	8.258	1.31	1.23	8.45
2.556	0.02	0.80	0.435	1.84	8.650	1.99	1.905	8.47
2.449	0.02	1.00	0.53	1.84	8.983	2.49	2.395	8.48
2.324	0.03	1.31	0.665	1.84	9.239	2.80	2.695	8.47
2.229	0.03	1.61	0.785	1.84	9.480	3.00	2.88	8.45
2.125	0.03	2.00	0.92	1.84				
1.928	0.04	3.00	1.15	1.80				
						(Equiv. = 3.15)		
					10.163	3.40	0.21	11.31
					10.570	3.69	0.43	11.37
					10.822	3.99	0.67	11.39
					11.095	4.49	1.06	11.39
					11.298	5.00	1.435	11.38
					11.456	5.51	1.78	11.35
(b) H ₂ electrode. 0.0602 g. in 10 cc.					(d) H ₂ electrode. 0.0372 g. in 10 cc.			
3.508	0.02	0.10	0.07	1.86	7.209	0.16	0.10	8.48
3.239	0.02	0.20	0.14	1.89	7.491	0.25	0.19	8.47
2.967	0.03	0.35	0.23	1.85	7.771	0.40	0.34	8.45
2.728	0.045	0.55	0.335	1.79	8.092	0.65	0.59	8.46
2.585	0.06	0.75	0.44	1.78	8.424	0.98	0.92	8.48
2.419	0.06	1.01	0.545	1.73	8.716	1.31	1.245	8.49
2.150	0.075	2.01	1.055	1.84	9.479	1.89	1.805	8.47
						(Equiv. = 1.98)		

It is seen that the relation of these constants to the molecular structure of thiolhistidine is obscure, since the molecule has four dissociable groups whereas only three constants have been detected. This means that, since the material has p_H about 5 in pure aqueous solution, the molecule must be either a di-zwitterion with basic groups at p_K 8.5 and 11.4 and with $-\text{COOH}$ and $-\text{SH}$ groups both strong acids (one being undetectable), or else it is a simple zwitterion with the cyclic nitrogen undetectable.

Fig. 1. Titration of 0.03 *M* dl-2-thiolhistidine. 25°. A. p_H titration. B. Effect of acetone.

Calculated titre, corrected —. Hydrogen electrode data ○.
 Calculated titre, uncorrected Indigo carmine electrode data ×.
 Titration curve of histidine HCl - - - .

Differentiation was made by addition of acetone to thiolhistidine solutions of appropriate degrees of neutralisation (Fig. 1 B). Groups 1 and 3, having their apparent p_H elevated by acetone addition are acid groups; group 2 is a basic group. Thus it is the cyclic basic group which is missing (it is interesting to note that calculation in very acid solution shows defective requirements of HCl rather than the reverse). Consequently, the allocation of constants to the separate groups of thiolhistidine (Table II) is analogous to that of cysteine and tyrosine and exhibits no direct relation to histidine or other basic amino-acids.

Table II. *Dissociation constants at 25°.*

	Mol. conc.	$-\text{COOH}$	Cyclic N	$-\text{NH}_2$	$-\text{SH}$	Reference
dl-2-Thiolhistidine	0.03	1.84	<1.5	8.47	11.4	This paper
Cysteine	0.02	1.96	—	8.18	10.28	Cannan and Knight [1927]
Histidine	0.1	1.77	6.10	9.18	—	Greenstein [1931]

EXPERIMENTAL.

Experimental and mathematical procedure was the same as for β -hydroxy-glutamic acid [Harington and Randall, 1931, Note]. Standardisation was effected however against 0.05 *M* potassium hydrogen phthalate of ascertained purity.

In both cases, junction was through a capillary goose-neck, widened to a cup of about 1 mm. diameter at the tip. In measurements at p_{H} extremes, occasional flushing with minute measured amounts of saturated KCl ensured a relatively sharp contact surface.

Recrystallised *dl*-2-thiolhistidine was dissolved in 10 cc. hot water under nitrogen. Solutions were about 0.03 *M* and may have been supersaturated [Ashley and Harington, 1930]; precipitation did not occur however during any recorded titration. After hydrogen saturation, junction was made and freshly plated electrodes immersed. It was found invariably that initial potentials after establishment underwent a slow hyperbolic drift, producing a decrease of as much as 5 mv. in 30 to 40 mins. Fresh electrodes registered the original potential. Duplicate electrodes immersed together might remain in disagreement by some tenths of a millivolt, and observed potentials might be unsteady by the same amount. The situation was unaltered by varying the depth of active electrode coating, or by pre-saturation with hydrogen before immersion. Deteriorated electrodes registered a correct potential in potassium hydrogen phthalate. As this behaviour was suggestive of the presence of the oxidant of an O/R system, all precautions against fortuitous oxidation of the material were observed. It is, however, doubtful whether thiolhistidine on oxidation would form a thermodynamically reversible O/R system capable of interference.

In a trial O/R experiment in 0.0025 *M* solution at p_{H} 7, gold-plated and bright platinum electrodes registered vague potentials about 0.5 v. positive to the hydrogen electrode, but fortuitous variations between individual electrodes amounting to 0.03 v. occurred. Addition of 0.02 *N* ferricyanide or quinone as oxidising agent produced initially the more positive potentials characteristic of these agents, followed by moderately rapid negative drifts as interaction proceeded. Distinctively different behaviour with iodine was suggestive of molecular substitution, since each addition merely produced a gradual positive drift without stepwise potential change, and no starch colour or iodine potential was given after even three equivalents. There would seem to be little apparent reason why direct p_{H} determinations should be less straightforward than those for cysteine [Cannan and Knight, 1927].

Titration at the quinhydrone electrode as an alternative to the hydrogen electrode is rendered invalid by the reactivity of quinone. The more negative 1:5-anthraquinonedisulphonate was tried but found to be insufficiently facile in reversibility. Finally, an indigo carmine electrode was used. Potentials of thiolhistidine solutions at this electrode were entirely satisfactory and stable. Standardisation was rendered difficult however by large concentration and salt errors. The actual electrode adopted was an approximately half-reduced 0.001 *M* indigo carmine solution standardised electrometrically against 0.01 *M* potassium hydrogen phthalate. Duplicate gold-plated electrodes were used and gave perfect agreement. During titration, the indigo concentration was maintained fairly constant, intermediate values being corrected by interpolation. A 50 % dilution of indigo, in either acid or alkaline solution, was found to involve a potential change of the order + 2.5 mv. Unfortunately, the electrode became subject to extensive negative drifts from p_{H} 7.5 onwards, but within this range general confirmation of hydrogen electrode measurements was obtained. That the drift was due to loss of oxidant was confirmed by a positive change of potential occurring upon further addition of half-reduced solution. In general therefore, it was necessary to use a hydrogen electrode, occasionally replated, and aged in solution for about 20 mins. In calculation, allowance has been made for some 0.03 equivalent of material titratable between p_{H} 3.5 and 7, though

the possibility is not ruled out that this is an artefact due to electrode peculiarity. For thiolhistidine the titration constant p_G is identical with the dissociation constant p_K .

SUMMARY.

Dissociation constants of *dl*-2-thiolhistidine have been reasonably well substantiated at p_{K_1} , 1·84, p_{K_2} , 8·47, p_{K_3} , 11·4, corresponding respectively to the —COOH, —NH₂, and —SH groups of the amino-acid.

No evidence of the basic dissociation of a cyclic nitrogen atom has been found.

As might be anticipated, the pure amino-acid does not form a readily reversible oxidation-reduction system.

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