CXVII. THE DISSOCIATION CONSTANTS OF ARGININE.

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Considering the interest, chemical as well as physiological, attaching to the complex amino acid arginine (α-amino-δ-guanido-valeric acid) it is surprising how incomplete is our information concerning its dissociation constants. The single attempt hitherto made to ascertain their magnitude is that of Kanitz [1906]; but of one only—the second basic—does Kanitz claim to have made an accurate measurement. For the two others—the acidic and the principal basic—he offers, as he himself is careful to state, merely the roughest estimates. The gap thus left in our knowledge we have endeavoured to fill by carrying out an electrometric titration of arginine, the data of which, subjected to appropriate methods of calculation, have yielded satisfactory approximations to the desired constants.

Since it would be extremely difficult to prepare a solution of arginine free from carbonate, we chose as the starting point of our titrations not the base itself, but a salt—the monohydrochloride—which it is easy to obtain in a state of practically absolute purity. The preparation used contained 6·67 % of amino and 26·45 % of total nitrogen (theory, 6·65 and 26·61 respectively); the ratio of amino to total nitrogen was therefore 1:3·97 (theory, 1:4); and the specific rotatory power, $[\alpha]_D^{20°}$, in the presence of seven moles of HCl was + 21·95° [Hunter and Morrell, 1922].

Of this salt we prepared two solutions of nearly identical concentrations. The first (solution *A*) contained 0·8810, the second (solution *B*) 0·8860 g. in 50 cc.; their molar concentrations were therefore 0·0836 and 0·0841 respectively. Of each of these solutions 30 cc. were transferred to a suitable titrating bottle. Each bottle bore a closely-fitting stopper pierced by four holes. The first admitted the tip of a burette, the second the stem of a Moloney [1921] hydrogen electrode, the third a glass tube passing to the bottom of the bottle and serving for the introduction of hydrogen, and the fourth a short tube permitting the exit of the gas. The bottles were immersed in a water-bath of which the temperature, during the titration, was held at 20 ± 0·25°.

For the determination of the first basic and the acid dissociation constants the 30 cc. of solution *A* were titrated with a standard solution of barium hydroxide, protected in the usual way from any access of carbon dioxide.

The strength of this solution was 0·2086N, so that 12·0 cc. were the equivalent of the arginine hydrochloride taken. Barium hydroxide was preferred to sodium hydroxide because of the ease with which it may be prepared and maintained free from carbonate. Before titration was started, the titration vessel and its contents were freed from CO_2 by a rapid current of hydrogen, and the flow of gas was maintained uninterruptedly, although at a slower rate, until the entire operation was finished. Titration was effected in steps of 0·5 cc., ample time being given, before each potentiometer reading, for thorough mixing and the establishment of temperature equilibrium. No reading was accepted until it had been thrice repeated without significant alteration. The total amount of baryta solution added was 17 cc., about 1·5 equivalents; this, it will be noted, involved an increase of the volume in the titration bottle by not much more than 50 %.

The procedure described ensures the absence of carbonate, but it involves of course the production of barium chloride. It is open therefore to the objection that the p_H of a buffer mixture, and consequently the apparent dissociation constant of a buffer acid or base, are not unaffected by the presence of a neutral salt. Michaelis and Krüger [1921] found, for example, that a mixture of sodium acetate and acetic acid, each in 0·02N concentration, had its p_H reduced from 4·665 to 4·382 by the addition of 0·5N barium chloride. But in our titration the concentration of barium chloride at its maximum was no greater than that of the total arginine. It seems, therefore, rather unlikely that it would exert any considerable influence upon the result. Certainly the series of calculated values for $p_{K'_{b_1}}$, exhibited below in Table I, gives no indication of any progressive trend in one direction; yet that is the only kind of effect that the increasing concentration of barium chloride could produce.

The results of the baryta titration, as far as the addition of one equivalent of base, will be found in Table I; while Table II exhibits the effect of carrying the titration further.

The second basic dissociation constant was determined by titrating solution B with 0·1815N hydrochloric acid. Of this acid 13·9 cc. were equivalent to the 30 cc. of arginine hydrochloride. The titration was carried out in the same way as before, except that the precautions taken to avoid access of CO_2, being here unnecessary, were omitted. The results are shown in Table III.

In all three tables the acid or base in combination with arginine at each step of the titration has been represented in terms of equivalents. This is done chiefly in order to make easy the graphical combination of the separate experiments in the single titration curve of Fig. 1. Since the two solutions, A and B, were of practically the same concentration, there can be no objection to representing the two experiments thus as one continuous titration. In the figure the circular dots represent experimental points; while the curve as drawn is a theoretical one deduced, from the constants adopted, by the use between p_H 4 and 10 of the well-known Henderson-Hasselbalch equation, and outside that range of the formulae of Van Slyke (see below).

Table I. *Titration of First Basic Group.*

30 cc. of 0·0836M arginine hydrochloride titrated with 0·2086N barium hydroxide

cc. of barium hydroxide	Equivalents of arginine liberated	Equivalents of HCl left in combination with arginine	$\log \frac{[\text{arginine HCl}]}{[\text{arginine}]}$*	p_{H} observed	p_{OH}†	$p_{K'_{b1}}$
0·0	0·000	1·000	—	5·04	9·03	—
0·5	0·0417	0·958	1·36	7·73	6·34	4·98
1·0	0·0833	0·916	1·04	8·08	5·99	4·95
1·5	0·125	0·875	0·85	8·25	5·82	4·97
2·0	0·167	0·833	0·70	8·42	5·65	4·95
2·5	0·208	0·792	0·58	8·52	5·55	4·97
3·0	0·250	0·750	0·48	8·61	5·46	4·98
3·5	0·292	0·708	0·39	8·70	5·37	4·98
4·0	0·333	0·667	0·30	8·81	5·26	4·96
4·5	0·375	0·625	0·22	8·88	5·19	4·97
5·0	0·416	0·583	0·15	8·94	5·13	4·98
5·5	0·458	0·542	0·07	9·03	5·04	4·97
6·0	0·500	0·500	0·00	9·11	4·96	4·96
6·5	0·542	0·458	−0·07	9·20	4·87	4·94
7·0	0·583	0·416	−0·15	9·26	4·81	4·96
7·5	0·625	0·375	−0·22	9·35	4·72	4·94
8·0	0·667	0·333	−0·30	9·42	4·65	4·95
8·5	0·708	0·292	−0·39	9·47	4·60	4·99
9·0	0·750	0·250	−0·48	9·54	4·53	5·01
9·55	0·796	0·204	−0·59	9·67	4·40	4·99
10·0	0·833	0·167	−0·70	9·79	4·28	4·98
10·5	0·875	0·125	−0·85	9·87	4·20	(5·05)
11·0	0·916	0·0833	−1·04	10·04	4·03	(5·07)
11·5	0·958	0·0417	−1·36	10·31	3·76	(5·12)
12·0	1·000	0·000	—	10·67	3·30	—

* The ratio of salt to base is obtained by dividing the figure in column 3 by that in column 2. It may also be calculated as $\frac{12-y}{y}$ where y is the figure in column 1.

† Since the temperature was 20° p_{K_w} is 14·07 [Michaelis, 1922, p. 23] and p_{OH} is 14·07 − p_{H}.

First basic dissociation constant. The p_{H} observations recorded in Table I come within the range of applicability of the well-known formula of Henderson [1909] which, as modified by Hasselbalch [1917] and adapted to the case of a base, takes the form

$$p_{K'_b}=p_{\mathrm{OH}}-\log\frac{[\text{salt}]}{[\text{base}]}.$$

In this formula K'_b is a constant related to the true dissociation constant, K_b, by the equation $K_b = \gamma K'_b$, in which γ stands for the degree of ionisation of the salt. As γ is seldom far removed from unity, K'_b may for most purposes be taken as a close enough approximation to K_b. Applied to the experimental data, the formula yields, as set forth in the table, consistent results at all points of the titration except those near the end. The divergence of the last three values must apparently be attributed to experimental error, for, as an inspection of Fig. 1 will demonstrate, it cannot be accounted for by the slight overlapping of the basic by the acid titration curve. If these values, bracketed in the table, are rejected, there remain twenty very satisfactory determinations of $p_{K'_{b1}}$ showing an extreme variation of 0·07 and yielding an average of 4·97. This would give to K'_{b1} the magnitude $1{\cdot}07 \times 10^{-5}$.

The true dissociation constant, K_{b1}, may be deduced from the hydrogen ion concentration of the untreated arginine hydrochloride solution (first line of Table I)by the approximative equation [Michaelis, 1922, p. 75].

$$K_b = \frac{K_w \times C}{[H^{\cdot}]^2},$$

where C is the molecular concentration of the salt, and $\frac{[H^{\cdot}]}{C}$ represents accordingly the degree to which it is hydrolytically dissociated. K_w at 20° is $0{\cdot}86 \times 10^{-14}$, $[H^{\cdot}]$ as determined is $0{\cdot}91 \times 10^{-5}$, and C is $0{\cdot}0836$. K_{b1} therefore

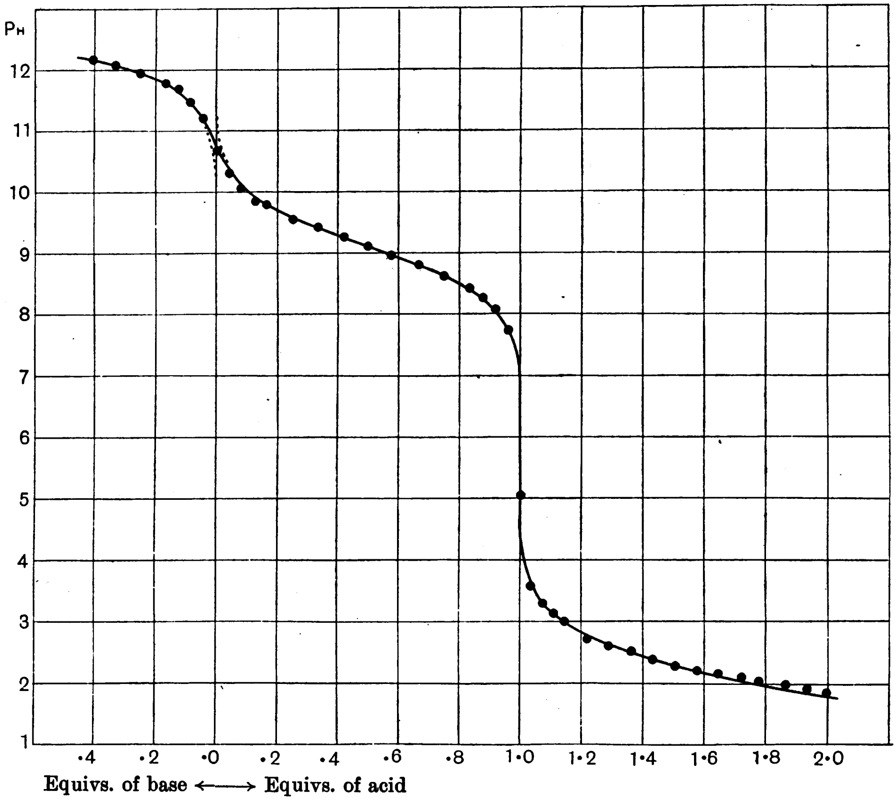

Fig. 1 Titration curve of arginine.

is $0{\cdot}87 \times 10^{-5}$, and arginine hydrochloride in the concentration employed is hydrolysed to the extent of 0·011 %. On this basis γ, the degree of ionisation of the salt, is evidently $\frac{0{\cdot}87}{1{\cdot}07}$, i.e. 0·81 (or 81 %). While these values have all, in relation to K'_{b1}, an entirely reasonable magnitude, it must be remembered that the p_H determination upon which they depend is peculiarly liable to experimental error; for, as the titration curve makes evident, the buffer value of the pure hydrochloride is practically nil.

Table II. *Titration of Acid Group.*

Continuation of the titration recorded in Table I

Barium hydroxide 0·2086N												
	In excess of 12 cc., i.e. of one equivalent											
Total cc.	cc.*	equivalents*	V cc.	C	B	p_H observed	p_{OH}	$[OH']$	B'	$C-B'$	$\log \frac{B'}{C-B'}$	$p_{K'_a}$
12·0	0·0	0·000	42·0	·0597	·0000	10·67	3·40	·0004	—	—	—	—
12·5	0·5	0·0417	42·5	·0590	·00245	11·18	2·89	·00129	·00116	·0578	−1·70	12·88
13·0	1·0	0·0833	43·0	·0583	·00485	11·46	2·61	·00245	·0024	·0559	−1·37	12·83
13·5	1·5	0·125	43·5	·0577	·0072	11·67	2·40	·0040	·0032	·0545	−1·23	12·90
14·0	2·0	0·167	44·0	·0570	·0095	11·78	2·29	·0051	·0044	·0526	−1·08	12·86
14·6	2·6	0·217	44·6	·0562	·0122	11·87	2·20	·0063	·0059	·0503	−0·93	12·80
15·0	3·0	0·250	45·0	·0557	·0139	11·95	2·12	·0076	·0063	·0494	−0·89	12·84
15·5	3·5	0·292	45·5	·0551	·0160	12·02	2·05	·0089	·0071	·0480	−0·83	12·85
16·0	4·0	0·333	46·0	·0545	·0181	12·07	2·00	·0100	·0081	·0464	−0·76	12·83
17·0	5·0	0·416	47·0	·0534	·0222	12·16	1·91	·0123	·0099	·0435	−0·64	12·80

* This gives, of course, the quantity of barium hydroxide free to combine with arginine.

Acid dissociation constant. When, as in Table II, the titration passes the stage of free arginine and barium argininate begins to appear, we reach a region of $[OH']$ where the simple formula employed in the last section no longer furnishes a satisfactory approximation. We have used here therefore an equation developed by Van Slyke [1922] in which the buffer effect of a high concentration of $H^{\cdot}$ or OH' is expressly allowed for. This equation, as applied to the case now in question—that of a very weak acid—takes the form

$$K'_a = \frac{[H^{\cdot}](B-[OH'])}{C-(B-[OH'])}$$

where C is the total molecular concentration of the arginine in all forms present, and B is the concentration, in terms of normality, of the added base. For purposes of calculation we have found it convenient to represent $B - [OH']$ by the symbol B' and to put the whole equation in the logarithmic form

$$p_{K'_a} = p_H - \log \frac{B'}{C-B'}.$$

The steps of the calculation are set forth in the table, in which V signifies the total volume of liquid in the titration bottle at each step, C is accordingly $\frac{0·0836 \times 30}{V}$ and B is $\frac{0·2086}{V}$ multiplied by the figure in column 2. The nine values found for $p_{K'_a}$ show, it will be seen, a satisfactory agreement. Their average is 12·84, indicating an acid dissociation constant of approximately $1·4 \times 10^{-13}$.

Second basic dissociation constant. This may be obtained from the titration data of Table III by means of the Van Slyke equation for a very weak base

$$K'_{b2} = \frac{[OH'](A-[H^{\cdot}])}{C-(A-[H^{\cdot}])}$$

in which C has the same meaning as before and A stands for the concentration of added acid. We have preferred to use this formula also in the logarithmic shape

$$p_{K'_{b_2}} = p_{OH} - \log \frac{A'}{C-A'}$$

where A' signifies $A - [\mathrm{H}^{\bullet}]$. C is calculated in the table as $\frac{0\cdot0841\times30}{V}$ and A as $\frac{0\cdot1815}{V}$ multiplied by the figure in the first column. The formula yields, it will be seen, values of $p_{K'_{b_2}}$ which show a rather greater variability than those obtained for the other constants. The later values in particular show a decided downward tendency. For this reason we have rejected the last three, and taken as a probable approximation the average of the remaining thirteen. This average is 11·90, corresponding to a K'_{b2} of $1\cdot3 \times 10^{-12}$.

Table III. *Titration of Second Basic Group.*

30 cc. of 0·0841*M* arginine hydrochloride titrated with 0·1815*N* HCl.

HCl, 0·1815*N*											
cc. added	Total equivalents present*	V cc.	C	A	p_{H} observed	$[\mathrm{H}^{\bullet}]$	p_{OH}	A'	$C-A'$	$\log\frac{A'}{C-A'}$	$p_{K'_{b_2}}$
0·5	1·036	30·5	·0827	·00297	3·57	·00027	10·50	·0027	·0800	−1·47	11·97
1·0	1·072	31	·0814	·00584	3·28	·00053	10·79	·0053	·0761	−1·15	11·94
1·5	1·108	31·5	·0801	·00864	3·14	·00073	10·93	·0079	·0722	−0·96	11·89
2·0	1·144	32	·0789	·0113	2·98	·0011	11·09	·0102	·0687	−0·83	11·92
3·0	1·216	33	·0765	·0165	2·72	·0019	11·35	·0146	·0619	−0·63	11·98
4·0	1·288	34	·0742	·0213	2·60	·0025	11·47	·0188	·0554	−0·47	11·94
5·0	1·360	35	·0721	·0259	2·52	·0030	11·55	·0229	·0492	−0·33	11·88
6·0	1·432	36	·0701	·0302	2·39	·0041	11·68	·0261	·0440	−0·23	11·91
7·0	1·504	37	·0682	·0343	2·30	·0050	11·77	·0293	·0389	−0·12	11·89
8·0	1·575	38	·0664	·0382	2·20	·0063	11·87	·0319	·0345	−0·05	11·92
9·0	1·647	39	·0647	·0419	2·16	·0069	11·91	·0350	·0297	+0·07	11·84
10·0	1·719	40	·0631	·0454	2·11	·0078	11·96	·0376	·0255	+0·17	11·79
11·0	1·791	41	·0616	·0487	2·03	·0093	12·04	·0394	·0222	+0·25	11·79
12·0	1·864	42	·0601	·0519	2·00	·0100	12·07	·0419	·0182	+0·36	(11·71)
13·0	1·935	43	·0587	·0548	1·91	·0123	12·16	·0425	·0162	+0·42	(11·74)
14·0	2·006	44	·0574	·0577	1·84	·0145	12·23	·0432	·0142	+0·48	(11·75)

* Including the one already combined in the hydrochloride.

Iso-electric point. The iso-electric point of a mono-acid, mono-basic ampholyte may be calculated by the formula

$$p_I = \frac{p_{K_a} + p_{k_b}}{2},$$

in which p_{k_b} means $p_{K_w} - p_{K_b}$. The second basic dissociation constant of arginine is so far removed from the first that this simple formula may be applied here without sensible error [Levene and Simms, 1923]. The iso-electric point of arginine is therefore approximately $\frac{12\cdot84+9\cdot10}{2} = 10\cdot97$ in terms of p_{H}, or, in terms of $[\mathrm{H}^{\bullet}]$, $1\cdot1 \times 10^{-11}$.

Discussion

From measurements (not reported in detail) of the conductivity of arginine mononitrate solutions (prepared by mixing the dinitrate with one equivalent of sodium hydroxide) Kanitz concluded that this salt is not measurably hydrolysed. The conclusion was confirmed by the failure of a *M*/10 solution to cause appreciable hydrolysis of methyl acetate. Kanitz[1] made the only deduction which such observations could justify—that the principal basic dissociation constant of arginine is "at least 1×10^{-7}." The hydrolysis of the hydrochloride, and presumably therefore of the nitrate, is indeed, as our results have shown, exceedingly slight, and Kanitz's statement with regard to the constant is accordingly literally correct; but the words "at least" must, in the light of our measurements, be now interpreted to mean "one hundred times greater than."

Our value for K'_{b1} ($1{\cdot}07 \times 10^{-5}$) shows arginine to be a base nearly as strong as ammonia, of which the dissociation constant (at 18°) is $1{\cdot}75 \times 10^{-5}$. Among acids acetic acid has a constant ($1{\cdot}82 \times 10^{-5}$) of a similar order of magnitude.

The second basic dissociation constant was derived by Kanitz from measurements of the catalytic action of arginine dinitrate upon the saponification of methyl acetate. The value found was $2{\cdot}2 \times 10^{-12}$ ($p_{K_{b_2}} = 11{\cdot}66$). This was the only one of his determinations in which Kanitz himself expressed particular confidence. It stands in very fair agreement with our own estimate of $1{\cdot}3 \times 10^{-12}$ ($p_{K'_{b_2}} = 11{\cdot}90$). If both estimations had been carried out at the same temperature, the agreement might have been better still; for K_b generally increases rather rapidly with rising temperature [Lundén, 1906], and Kanitz's result was obtained at a temperature of 25°.

The second basic constant of arginine, though far smaller than the first, is still of as high an order of magnitude as the basic constants of many mono-amino acids; thus for aspartic acid $K_b \times 10^{12}$ has the value 1·2, for phenylalanine 1·3, for leucine 2·3, for tyrosine 2·6, for glycocoll 2·7.

In an attempt to measure the strength of arginine as an acid Kanitz mixed two moles of arginine dinitrate with three of sodium hydroxide and from the conductivity of the mixture deducted that of two moles of sodium nitrate. The conductivity of sodium-arginine, thus obtained, appeared to be approximately the same as that of sodium hydroxide. Kanitz concluded that K_a is less than $1{\cdot}11 \times 10^{-14}$ and that arginine has in fact no acid properties at all. It is not surprising that the method described led to uncertain results, or that the conclusion reached has not been entirely confirmed by our own observations. The latter lead to a constant, $1{\cdot}4 \times 10^{-13}$, which is not quite so small as Kanitz believed it. It is small enough, nevertheless, to characterise arginine as a very weak acid indeed, much weaker even than phenol, hydrocyanic acid, or asparagine, which have acid dissociation constants of 1·3, 4·7 and 8·8 times 10^{-10} respectively, and comparable only with such excessively feeble acids as sucrose ($K_a = 1{\cdot}14 \times 10^{-13}$), glucose ($3{\cdot}6 \times 10^{-13}$) and fructose ($6{\cdot}6 \times 10^{-13}$). It may be of further interest to note that the strength of the di-acid base arginine as an acid is of an even lower order than the strength of the di-basic acid aspartic acid as a base; for the basic dissociation constant of the latter is $1{\cdot}2 \times 10^{-12}$.

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[1] As pointed out by Harris [1924], Kanitz's tabulation of his results contains misprints which completely distort some of his actual conclusions.

The constants for substances other than arginine, mentioned in the preceding paragraphs, have been taken from the compilations of Lundén [1906] and Michaelis [1922].

SUMMARY.

Arginine mono-hydrochloride was titrated with (*a*) barium hydroxide, and (*b*) hydrochloric acid. From the hydrogen-ion concentrations of the resulting mixtures the following approximations to the dissociation constants and isoelectric point of arginine were derived:

$$\begin{aligned} K'_{b1} &= 1{\cdot}07 \times 10^{-5};\ p_{K'_{b_1}} &&= 4{\cdot}97 \\ K'_{b2} &= 1{\cdot}3 \times 10^{-12};\ p_{K'_{b_2}} &&= 11{\cdot}90 \\ K'_{a} &= 1{\cdot}4 \times 10^{-13};\ p_{K'_{a}} &&= 12{\cdot}84 \\ I &= 1{\cdot}1 \times 10^{-11};\ p_{I} &&= 10{\cdot}97 \end{aligned}$$

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