1139. *The Ionization Constant of the Carboxyl Group of Creatine.*

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Accurate measurements of the first acid ionization constant of creatine have been made at 15°, 25°, 35°, 45°, and 55° by an e.m.f. method, by use of cells without liquid junction of the type

$$Pt,H_2|creatine,HCl,KCl|AgCl,Ag.$$

The following equation in temperature for the thermodynamic ionization constants has been obtained:

$$pK_{1a} = 1175{\cdot}0236/T - 4{\cdot}98827 + 0{\cdot}0123344T.$$

The significance of the results is discussed.

The ionization of creatine is interesting because of its biological importance and because in solution it undergoes ring-closure, with the elimination of water, to give an equilibrium mixture with creatinine. The technique developed for the measurement of the ionization constant of creatinine [1] has now been used with creatine.

The ionization constant, K_{1a}, for the process, $R^+ \rightleftharpoons {}^-R^+ + H^+$, is given by:

$$K_{1a} = a_{H^+}a_{-R^+}/a_{R^+}, \quad (1)$$

where R^+ and $^-R^+$ represent the cationic and the dipolar ion form of the creatine molecule. To determine the values of K_{1a}, measurements were made, at different temperatures, of the e.m.f.'s of cells without liquid junction of the type:

$$Pt,H_2(1\ atm.)|creatine(m_1),HCl(m_2),KCl(m_3)|AgCl,Ag, \quad (2)$$

where m_1, m_2, and m_3 are molal concentrations; both electrodes were in the same compartment. To overcome the drift in e.m.f. caused by the conversion of creatine into creatinine, the cells were initially filled with solutions containing hydrochloric acid and potassium chloride only. After the e.m.f. had become steady, solid creatine was added and rapidly dissolved by stirring with a stream of hydrogen. The e.m.f. was then read at timed intervals and extrapolated back to the time when the solid creatine was added; this was taken as the e.m.f. of the cell. Plots of the e.m.f. against time showed, after an initial equilibration period (40—60 minutes), a linear variation, similar to that seen with creatinine.[1] Linear equations were fitted by least squares to the straight portions of these plots, which covered 60—80 minutes (readings every 2 minutes). From these equations the e.m.f. at zero time was calculated.

From the usual equation for the e.m.f. of cell (2) and from equation (1) we can write:

$$pK_{1a} = \frac{(E - E^\circ)\boldsymbol{F}}{\boldsymbol{R}T \ln 10} + \log \frac{m_{R^+}m_{Cl^-}}{m_{-R^+}} + \log \frac{\gamma_{R^+}\gamma_{Cl^-}}{\gamma_{-R^+}}, \quad (3)$$

where the concentration terms have the values:

$$m_{R^+} = m_2 - m_{H^+};\ m_{-R^+} = m_1 - m_2 + m_{H^+};\ \text{and } m_{Cl^-} = m_2 + m_3.$$

If the activity coefficient of the dipolar ion, γ_{-R^+}, is assumed to be unity, the last term on the right of equation (3) may be represented by: [2]

$$\log \frac{\gamma_{R^+}\gamma_{Cl^-}}{\gamma_{-R^+}} = -2\boldsymbol{A}I^{\frac{1}{2}} - BI - CI^{\frac{3}{2}}, \quad (4)$$

where $\boldsymbol{A}$ is the Debye–Hückel slope and B and C are arbitrary parameters.

[1] Grzybowski and Datta, *J.*, in the press.
[2] Datta and Grzybowski, *Trans. Faraday Soc.*, 1958, **54**, 1179.

Combining equations (3) and (4) and the values of the concentration terms we have:

$$y = \frac{(E - E^\circ)\boldsymbol{F}}{\boldsymbol{R}T \ln 10} + \log \frac{(m_2 - m_{H+})(m_2 + m_3)}{(m_1 - m_2 + m_{H+})} - 2\boldsymbol{A}I^{\frac{1}{2}}$$
$$= pK_{1a} + BI + CI^{\frac{3}{2}}. \quad (5)$$

The values of m_{H+} used in equation (5) were obtained from the relation:

$$-\log m_{H+} = \frac{(E - E^\circ)\boldsymbol{F}}{\boldsymbol{R}T \ln 10} + \log (m_2 + m_3) + 2 \log \gamma_\pm,$$

where $\gamma_\pm$ is the mean activity coefficient of hydrogen chloride, calculated from the values given by Bates and Bower.[3] The ionic strength is given by $I = (m_2 + m_3)$.

The thermodynamic values of pK_{1a} were obtained by fitting experimental values of y to eqn. (5) by least squares and extrapolating to $I = 0$. The thermodynamic pK_{1a}'s were fitted to Harned and Robinson's equation:[4]

$$pK_{1a} = A/T - D + CT, \quad (6)$$

where T is the Kelvin temperature (t°C + 273·15°), from which the thermodynamic quantities associated with the ionization were calculated in the usual way.

The molal concentrations of the solutions used, the e.m.f.'s of cell (2), and the values for the extrapolation function y of eqn. (5) are given in Table 1. The values of the thermodynamic pK_{1a}'s and other thermodynamic quantities and their errors are shown in Table 2.

TABLE 1.

Molalities of solutions, e.m.f.'s of the cell (see text), and the extrapolation functions y [equation (5)]. $I = m_2 + m_3$.

At 15°.								
$10^2 m_1$	2·0041	2·6964	3·0732	3·8564	4·3708	5·0516	5·4310	6·1044
$10^2 m_2$	1·0246	1·3054	1·5906	1·8888	2·1849	2·4943	2·7298	3·0524
$10^2 m_3$	1·0058	1·2815	1·5614	1·8542	2·1449	2·4486	2·6798	2·9965⁻
$10^5(E-E^\circ)$	26,167	25,697	24,898	24,672	24,206	23,944	23,618	23,391
y	2·7561	2·7725	2·7760	2·7896	2·7944	2·8064	2·8054	2·8171
At 25°.								
$10^2 m_1$	2·0363	2·7811	3·1317	3·5534	4·2290	5·0411	5·4583	6·2731
$10^2 m_2$	0·9933	1·2792	1·5714	1·8535	2·1554	2·4529	2·7380	3·0105⁻
$10^2 m_3$	0·8581	1·1050	1·3575⁻	1·6012	1·8620	2·1190	2·3653	2·6007
$10^5(E-E^\circ)$	27,417	26,906	26,012	25,426	25,102	24,976	24,556	24,525⁻
y	2·7408	2·7553	2·7632	2·7753	2·7815	2·7938	2·7976	2·8078
At 35°.								
$10^2 m_1$	1·9081	2·1674	3·0192	3·7275	4·1216	5·0568	5·4566	6·1064
$10^2 m_2$	0·9933	1·2791	1·5714	1·8535	2·1554	2·4529	2·7380	3·0105⁻
$10^2 m_3$	0·8581	1·1050	1·3575⁻	1·6012	1·8620	2·1190	2·3653	2·6007
$10^5(E-E^\circ)$	28,156	26,941	26,788	26,529	25,883	25,877	25,417	25,255
y	2·7490	2·7646	2·7719	2·7863	2·7903	2·8029	2·8047	2·8121
At 45°.								
$10^2 m_1$	2·0681	2·5894	3·2187	3·4539	4·5115	4·2792	5·6623	5·8836
$10^2 m_2$	0·9852	1·2873	1·5657	1·8469	2·1313	2·5061	2·6938	3·0653
$10^2 m_3$	0·8511	1·1120	1·3526	1·5955	2·1213	2·4552	2·7152	3·1090
$10^5(E-E^\circ)$	29,436	28,506	27,962	27,137	27,094	25,684	26,425	25,640
y	2·7543	2·7757	2·7762	2·7935	2·8059	2·8110	2·8221	2·8264
At 55°.								
$10^2 m_1$	1·9749	2·3304	3·3046	3·7608		4·8329	5·3613	
$10^2 m_2$	0·9852	1·2873	1·5657	1·8469		2·4274	2·7152	
$10^2 m_3$	0·8511	1·1120	1·3526	1·5955		2·3678	2·7010	
$10^5(E-E^\circ)$	30,238	29,033	29,025	28,409		27,438	27,070	
y	2·7624	2·7845⁻	2·7899	2·8024		2·8314	2·8403	

[3] Bates and Bower, *J. Res. Nat. Bur. Stand.*, 1954, **53**, 283.
[4] Harned and Robinson, *Trans. Faraday Soc.*, 1940, **36**, 973.

The errors were calculated by Please's method,[5] from the average value of $V(y_0) = 2{\cdot}0 \times 10^{-4}$, the variance of the points about the fitted lines of equation (5). The variance of the values of pK_{1a} about the temperature equation (6), $V(pK) = 3{\cdot}0 \times 10^{-8}$, is, however, much lower than $V(y_0)$; therefore the errors given for $\Delta S°$, $\Delta H°$, and $\Delta C_p°$ are probably too large, since these quantities are calculated from the temperature coefficient of pK_{1a}.

TABLE 2.

Values of pK_{1a} of creatine and the thermodynamic functions for the first dissociation.

$pK_{1a\,(obs.)}$ was obtained from extrapolation of equation (5), $\Delta = pK_{1a\,(obs.)} - pK_{1a\,(calc.)}$ where $pK_{1a\,(calc.)} = 1175{\cdot}0236/T - 4{\cdot}98827 + 0{\cdot}0123344T$. For errors see text.

Temp.	$pK_{1a\,(obs.)}$	$10^4\Delta$	$\Delta G°$ (kJ mole^{-1})	$\Delta H°$ (kJ mole^{-1})	$\Delta S°$ (J mole^{-1} deg.$^{-1}$)	$-\Delta C_p°$ (J mole^{-1} deg.$^{-1}$)
15°	2·6435	−2	14·58 ± 0·08	+2·9 ± 2·8	40·6 ± 9·4	136 ± 129
25	2·6308	+6	15·01 ± 0·05	+1·5 ± 1·5	45·3 ± 5·2	141 ± 134
35	2·6252	−5	15·49 ± 0·06	+0·1 ± 0·8	50·0 ± 2·7	146 ± 138
45	2·6293	+1	16·01 ± 0·05	−1·4 ± 1·7	54·8 ± 5·2	150 ± 143
55	2·6400	0	16·59 ± 0·08	−2·9 ± 3·0	59·5$^-$ ± 9·4	155$^-$ ± 147

$\sqrt{V(pK)} = 0{\cdot}00055$. $V(y_0)_{av.} = 2{\cdot}0 \times 10^{-4}$.

TABLE 3.

Comparison of the pK_{1a}'s of creatine obtained in this work and by other investigators.

Method *	Conditions	Temp.	pK_{1a}	Ref.	Method *	Conditions	Temp.	pK_{1a}	Ref.
A	0·05M	17°	2·61	*a*	A	$I \longrightarrow 0$	30°	2·63	*a*
B	0·04M	17	2·85	*b*	C	$I \longrightarrow 0$	30	2·627	*c*
C	$I \longrightarrow 0$	17	2·640	*c*	D	0·1M	40·2	2·68	*e*
A	$I \longrightarrow 0$	20	3·05	*d*	C	$I \longrightarrow 0$	40·2	2·627	*c*
C	$I \longrightarrow 0$	20	2·636	*c*					
A	0·1M	25	2·66	*a*					
C	$I \longrightarrow 0$	25	2·630	*c*					

* A, E.m.f. measurements of cells with hydrogen electrodes and liquid junction during titration of creatine with HCl. B, Electrometric. C, E.m.f. measurements of cells without liquid junction. D, Acid catalysis.

References: (*a*) Cannan and Shore, *Biochem. J.*, 1928, **22**, 920 (recalc. for 30°). (*b*) Hahn and Barkan, *Z. Biol.*, 1920, **72**, 25. (*c*) This work. (*d*) Recalc. from data by Eadie and Hunter, *J. Biol. Chem.*, 1926, **67**, 237. (*e*) Wood, *J.*, 1903, **83**, 568.

In Table 3 are given values for pK_{1a} of creatine obtained by other workers. The best agreement is with the values of Cannan and Shore, approximately within the limits of error of their experimental method. Wood's very early value, determined from experiments on the acid-catalysed hydrolysis of methyl acetate and the inversion of sucrose, can also be regarded as satisfactory in view of the difference in the methods used. Hahn and Barkan's, and particularly Eadie and Hunter's, results lie far outside the limits of reasonable error, possibly owing to failure to allow for cyclization of creatine.

The carboxyl dissociation of creatine may be compared with that for the simpler related compound glycine.[6] pK_{1a} is higher for creatine than for glycine and it passes through a minimum at a lower temperature (35·5° for creatine and 51·4° for glycine). The variation of pK_{1a} with temperature is similar to that found for most carboxylic acids.[7,8] Simple α-amino-acids all have pK_{1a} values near that for glycine,[8] while the insertion of a methylene group between the carboxyl and the carbon atom bearing the primary amino-group in an amino-acid raises the pK of the carboxyl group by >1 unit. Thus, for glycine [6] pK_{1a} is 2·351 and for β-alanine [9] 3·550, both at 25°. Creatine, therefore, with $pK_{1a} = 2{\cdot}630$ at 25°, is intermediate between these two other acids though nearer to

[5] Please, *Biochem. J.*, 1954, **56**, 196.
[6] King, *J. Amer. Chem. Soc.*, 1951, **73**, 155.
[7] Harned and Owen, "The Physical Chemistry of Electrolytic Solutions," Reinhold Publ. Corp., New York, 3rd edn., 1958, p. 758.
[8] Smith, Taylor, and Smith, *J. Biol. Chem.*, 1937, **122**, 109.
[9] May and Felsing, *J. Amer. Chem. Soc.*, 1951, **73**, 406.

glycine, presumably owing to differences in the distribution and position of the positive charge in the nitrogen-containing portions of glycine and creatine.

The thermodynamic quantities for the carboxyl dissociations of creatine and glycine at 25° are shown in Table 4: $\Delta H°$ and $\Delta S°$ are less positive for the creatine dissociation. The crystal structure of creatine [10] reveals that the distance between the positive and the negative charge in the dipolar ion (~3 Å) is virtually the same as in glycine; [11] this indicates that the parts of the thermodynamic quantities due to electrostatic interactions should be the same for both compounds, since the contribution of the guanidinium group in creatine, which may be larger than the ammonium group in glycine, is likely to cancel out between the creatine cation and the dipolar ion; the other charged groups are the same.

To verify this, we must compare the so-called " environmental " parts of the thermodynamic quantities of the dissociations for these compounds, that is to say, the parts which

TABLE 4.

Thermodynamic quantities for the first (carboxyl) dissociations of creatine and glycine, together with the " non-environmental " parts of $\Delta S°$ and $\Delta H°$, at 25°.

	$\Delta G°_{obs.}$	$-\Delta S°_{obs.}$	$-\Delta S°_{non.}$	$\Delta H°_{obs.}$	$\Delta H°_{non.}$	$-\Delta Cp°_{obs.}$
	kJ mole^{-1}	J mole^{-1} deg.$^{-1}$		kJ mole^{-1}		J mole^{-1} deg.$^{-1}$
Creatine	15·01	45·3	22·8	1·5	−5·8	141
Glycine	13·42 *	31·6 *	8·6	4·0 *	−3·5	144 *
Creatine − Glycine	+1·59	+13·7	+14·2	−2·5	−2·3	−3

* See King (ref. 6).

are sensitive to factors in the environments of the relevant species, mainly the temperature.[12] It can be shown that:

$$\Delta S°_{env.} = p[\mathrm{d}/\mathrm{d}T(1/D)],$$

and

$$\Delta H°_{env.} = p[1/D - T\mathrm{d}/\mathrm{d}T(1/D)],$$

where p is a parameter determined by the shape and dimensions of the molecules, the number of charges, and their magnitude and distribution. The values of p can be derived theoretically, or found experimentally from the relation, $p = \boldsymbol{R} \ln 10C/c$, where C is the parameter occurring in equation (6) and c is the parameter in the equation relating $1/D$ to temperature: $1/D = a - bT + cT^2$. By using the value of C found in this work for creatine and that of King [6] for glycine, together with $1{\cdot}4741 \times 10^{-7}$ for c, calculated from the values of $1/D$ given by Malmberg and Maryott,[13] p is found to be $16{\cdot}02 \times 10^5$ for creatine and $16{\cdot}42 \times 10^5$ for glycine. Using these values gives $-\Delta S°_{env.} = 22{\cdot}5$ and 23·1 J mole^{-1} deg.$^{-1}$ for creatine and glycine, respectively, at 25°. Similar calculations may be made for $\Delta H°_{env.}$.

Non-environmental parts of $\Delta S°$ and $\Delta H°$ may be obtained by subtracting the environmental parts from the observed values. These are shown in Table 4, from which it can be seen that most of the differences between the observed values of $\Delta S°$ and $\Delta H°$ for creatine and glycine are due to non-environmental factors in these compounds themselves and not to differences in their interactions with the solvent. These non-environmental factors could, for example, include the suppression of the resonance in the guanidinium group of creatine dipolar ion. The Raman spectrum of creatine hydrochloride is consistent with spread of the positive charge equally over all three nitrogen atoms in the cation; [14] unfortunately no similar studies have been made of the dipolar ion.

Experimental.—Creatine. Commercial creatine monohydrate was recrystallized several times from water and the product dried *in vacuo* over P_2O_5 to give the unhydrated compound.

[10] Mendel and Hodgkin, *Acta Cryst.*, 1954, **7**, 443.
[11] Cohn, *Ann. Rev. Biochem.*, 1935, **4**, 93.
[12] Datta and Grzybowski, *J.*, 1962, 3068.
[13] Malmberg and Maryott, *J. Res. Nat. Bur. Stand.*, 1956, **56**, 1.
[14] Garfinkel, *J. Amer. Chem. Soc.*, 1958, **80**, 3827.

Several batches were prepared and their purity checked by heating weighed samples at 120—140° for 20—30 hr. Samples were examined spectrophotometrically in alkaline solution before and after heating to determine how much creatinine was present—the latter shows a well marked absorption at 234 mμ.[1] The weight loss, corrected for any creatinine formed during heating, was assumed to be due to water. In none of the batches was this more than 0·1% and no creatinine could be detected in the unheated material. Allowance for the water was made in calculating the concentrations of creatine in the solutions.

Miscellaneous. The preparation of hydrochloric acid and potassium chloride and the technique of measurements in cells without liquid junction were as in the experiments with creatinine.[1]

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