possible to calculate the increment for either solution if the value for the other is known.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA]

THE IONIZATION CONSTANT OF CREATININE[1]

BY C. P. MCNALLY

RECEIVED NOVEMBER 25, 1925 PUBLISHED APRIL 5, 1926

Introduction

In view of the fact that the values for the ionization constant of creatinine, $NH{=}CNHCOCH_2NCH_3$, appearing in the literature are few and in very bad agreement, it was decided to attempt to measure this constant more exactly.

The values appearing in the literature at present are 1.85×10^{-10} at 17°, and 3.57×10^{-11} at 40.2°. The first is that of Hahn and Barkan,[2] obtained from hydrogen-ion measurements in solutions of the hydrochloride. The second is that of J. K. Wood,[3] obtained by measuring the degree of hydrolysis of the hydrochloride by its catalytic effect on the hydrolysis of methyl acetate. It can be seen that a more exact determination of this constant is needed.

Many different methods, such as conductivity measurements, hydrogen-ion determinations, catalytic methods, distribution, solubility and colorimetric determinations, have been used for determining the ionization constants of weak organic bases of this type. In this set of investigations it was decided to use several of the above-mentioned methods, so as to obtain a check on our results, as well as to compare the various methods used as to their ease of application and probable accuracy. Conductivity measurements and hydrogen-ion determinations were decided on. It was also considered best to apply two of the less generally used methods, and distribution and solubility were selected. Results should thus be obtained representing with a good degree of accuracy the true ionization constant of creatinine.

The creatinine used throughout was prepared by the method of Edgar and Hinegardner,[4] from purified creatine. A white, finely crystalline odorless compound was obtained.

---

[1] This investigation completes the series on the physicochemical properties of creatine and creatinine undertaken in this Laboratory. It was outlined and begun under the direction of Dr. Graham Edgar and finished under the direction of Dr. Robert N. Pease.

[2] Hahn and Barkan, *Z. Biol.*, **72,** 25 (1920).

[3] Wood, *J. Chem. Soc.*, **83,** 568 (1903).

[4] Edgar and Hinegardner, *J. Biol. Chem.*, **56,** 886 (1923).

Procedure

Hydrogen-Ion Determinations in Creatinine Hydrochloride Solutions.—The apparatus was the regular hydrogen-ion determination set-up, using the Leeds and Northrup Type K potentiometer. The saturated calomel electrode was used, as it seems to possess several advantages over the normal and tenth-normal electrodes. The solutions were made up by dissolving a known amount of creatinine in water, adding an equivalent amount of standard hydrochloric acid, and making up to a known volume. The temperature was kept constant by means of a thermostat. From the e.m.f. readings the ionization constant was calculated through the equation, $(E-E_0)/[(RT/nF) \times 2.303] = \log 1/C_H$. Complete dissociation of the hydrochloric acid and the creatinine hydrochloride was assumed, and the liquid-junction potential was taken to be zero. The value for E_0 at 25° is 0.2458 volt,[5] and K_{H_2O} at this temperature[6] is 1.005×10^{-14}.

Preliminary determinations led to results in which the calculated ionization constant showed a steady drift upward as the solutions became more dilute. This was due to a slight impurity in the creatinine, the effect being the presence of an excess of hydrochloric acid in the solution, which was more apparent as the solutions were diluted. This was corrected in subsequent measurements in the weight of creatinine added.

Table I

Hydrogen-Ion Determination

Concn. Cr.HCl Equiv./liter	E.m.f.	$C_H \times 10^4$	X	$K \times 10^{10}$
0.02736	0.4336	6.31	0.0231	6.72
.01368	.4424	4.46	.0326	6.69
.00684	.4523	3.09	.0452	6.87
.00342	.4610	2.19	.0640	6.71
.00171	.4702	1.51	.0883	6.87

Temperature, 25 ± 0.1°. X is the degree of hydrolysis.

Another series on solutions in which the concentration of creatinine hydrochloride ranged from 0.03377 to 0.00211 equivalent per liter gave 6.67, 6.55, 6.79, 6.64 and 6.74 for $K \times 10^{10}$. The mean of the ten determinations is 6.73×10^{-10}. Two different standard acids were used in making up the two sets of solutions, and each solution was made up separately. The ten values vary closely around the mean value of 6.73×10^{-10}, which is given as the ionization constant of creatinine at 25° as obtained from hydrogen-ion determinations.

A series was carried out at 40° in order to determine the effect of temperature upon the ionization of creatinine. The potential of the saturated calomel cell at this temperature is 0.2340 volt from Clark,[5] and the

---

[5] Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., 1922, p. 200.

[6] Lewis and Randall, "Thermodynamics," McGraw-Hill Book Co., 1923, p. 486.

ionization constant of water at this temperature is 2.879×10^{-14} from Lewis and Randall.[6]

TABLE II

HYDROGEN-ION DETERMINATION

Concn. Cr.HCl Equiv./liter	E.m.f.	$C_H \times 10^4$	X	$K \times 10^{10}$
0.03377	0.4208	9.78	0.0290	9.85
.01689	.4302	6.92	.0410	9.73
.00844	.4401	4.69	.0568	9.97
.00422	.4496	3.39	.0803	9.73
.00211	.4596	2.35	.1114	9.77

Temperature, 40 ± 0.1°. Mean value for K is 9.81×10^{-10}. X is the degree of hydrolysis.

The Degree of Hydrolysis of Creatinine Hydrochloride by the Conductance Method.—This series consisted of measurements of the conductivity of creatinine hydrochloride solutions, both alone and in the presence of an excess of creatinine equal to the concentration of the salt. The ionization of creatinine is calculated from the degree of hydrolysis obtained.

The set-up consisted of a Leeds and Northrup drum type Wheatstone bridge, a Leeds and Northrup variable resistance box, a conductivity cell of the usual type consisting of two platinum circular plates, coated with platinum black, and a thermostat. The solutions were made up as in the hydrogen-ion determinations, being successively diluted as the readings were made. The cell constant was determined in the usual manner with 0.02 N potassium chloride solutions, the conductance of which is 2.768×10^{-3} at 25°. The values for the equivalent conductance of hydrogen chloride used in the calculations are from Bray and Hunt,[7] given in the Landolt-Börnstein Tables.[8] From these, by interpolation, values were obtained which were used in the calculation of the degree of hydrolysis.

TABLE III

CONDUCTANCE DATA

Concn. Cr.HCl Equiv./liter	Equiv. cond.	X	$K \times 10^{10}$
	CREATININE HYDROCHLORIDE		
0.03407	102.72	0.0206	6.81
.01704	109.95	.0279	7.36
.00852	117.65	.0404	6.94
.00426	125.96	.0558	7.16
	CREATININE HYDROCHLORIDE-CREATININE		
.03407	96.42	....	..
.01704	101.40	....	..
.00852	105.25	....	..
.00426	108.82	....	..

Temperature, 25 ± 0.1°. X is the degree of hydrolysis.

---

[7] Bray and Hunt, THIS JOURNAL, **33,** 781 (1911).

[8] Landolt-Börnstein, "Tabellen," Julius Springer, Berlin, **1923,** p. 1093.

Another series gave 6.92, 7.21, 7.12, 6.85 for $K \times 10^{10}$. The average value for the eight determinations is 7.05×10^{-10}.

A series was made at 40° with solutions of approximately the same concentrations of creatinine hydrochloride. The values ranged from 8.60×10^{-10} to 12.18×10^{-10}. The mean value for the entire series is 10.5×10^{-10}. The values for the equivalent conductance of hydrogen chloride at this temperature were obtained from the 25° values by applying the temperature coefficients as calculated from the data of Arrhenius[9] and given in the Landolt-Börnstein Tables.[10] The results do not agree as closely, but the average value is not far different from that previously obtained, and the value of 10.5×10^{-10} is given for K.

Distribution.—The distribution method, though not so generally applied, is comparatively simple. The chief difficulty lies in the selection of a suitable system. The method consists principally in the determination of the concentration of free acid or base in a solution of a salt by means of the distribution of the acid or base between the solution and a liquid immiscible with it. From the amount of free acid or base in the latter, and its distribution coefficient, the concentration in the solution can be calculated, and from this the degree of hydrolysis and the ionization constant are readily obtained.

The system creatinine picrate-water-benzene seemed to be suitable, free picric acid being measured by distribution. However, the results were entirely unsatisfactory. The distribution of the undissociated picric acid in the two layers is greatly affected by the presence of the other substances in the solution, as we determined by adding various amounts of sodium picrate to the solution. The ionization constant of picric acid, which plays a large part in the calculations, is not very accurately known, and varies with concentration. Consistent results could not be obtained, and the study was abandoned.

It was then decided to study the system creatinine acetate-water-ether. At first thought this may not seem to be a very good system, due to a slight mutual solubility of water and ether. However, the work of Löfman[11] shows that the distribution ratio of acetic acid between water and ether for concentrations less than 0.0667 N, well within our range, is independent of the concentration of acid in the water layer. Löfman also gives data on the effect of other salts present, and shows that sodium acetate, in concentration as high as 3 N, has a negligible effect when the acetic acid concentration is below 0.125 N. The data of Löfman show that although water and ether are mutually soluble to some extent, this mutual solubility is not changed by low concentrations of solute, and that the

[9] Arrhenius, *Z. physik. Chem.*, **9**, 339 (1892).

[10] Ref. 8, p. 1095.

[11] Löfman, *Z. anorg. Chem.*, **107**, 248 (1919).

distribution ratio is constant over the range of concentrations with which we work. The uncertainty in the results would lie in the possible effect of the dissolved ether on the progress of the hydrolysis of the salt.

Distilled water free from carbon dioxide, and ether twice distilled from sodium, were used; 100cc. portions of each were placed in ground-glass stoppered bottles of slightly over 200 cc. capacity. Known amounts of creatinine and acetic acid were added, and the bottles revolved for several days in a thermostat held at 25 ± 0.1°. Samples were then withdrawn from the ether layer, and titrated for acetic acid with barium hydroxide solution, using phenolphthalein as the indicator. It was found that when equal volumes of water and ether were mixed, the volumes of the resulting water and ether layers were in the ratio of 110 to 88. These values were used in converting the amounts of creatinine and acetic acid to concentrations. The distribution ratio was checked with each run, being calculated from the expression, water titer/ether titer = $K \times 110/88$. K was always 2.18.

Calculations were made from the following relations:

$$\frac{[H^+]\,[Ac^-]}{[HAc]} = 1.85 \times 10^{-5};\ K_{H_2O} = 1 \times 10^{-14};\ \frac{[Cr.^+]\,[OH^-]}{[Cr.OH]} = K = \frac{[Cr.^+] \times 10^{-14}}{[Cr.OH]\,[H^+]}$$

$$\frac{[Ac^-]\,[Cr.^+] \times 10^{-14}}{[HAc]\,[Cr.OH]} = K \times 1.85 \times 10^{-5};\ K = \frac{[Cr.^+]\,[Ac^-] \times 5.4 \times 10^{-10}}{[HAc]\,[Cr.OH]}$$

Preliminary results were irregular and high, due probably to the fact that the ether samples were titrated directly with the barium hydroxide, the two liquids being immiscible and resulting in incomplete neutralization. In the final determinations, the ether samples were first placed in a beaker of warm water, and the ether was vaporized before titrating. A 25cc. sample from one bottle containing 0.00846 equivalent of creatinine and 0.00432 equivalent of acetic acid required 16.89 cc. of 0.00679 N barium hydroxide solution; $K \times 10^{10} = 6.92$.

The calculation was made as follows.

$(0.00679 \times 16.89 \times 40)/1000 = 0.00459$ mole/liter of HAc in ether
$0.00459 \times 2.18 = 0.01001$ mole/liter of HAc in water
$0.00459 \times 0.088 = 0.00040$ mole of HAc in 88 cc. of ether layer
$0.01001 \times 0.110 = \underline{0.00110}$ mole of HAc in 110 cc. of water layer
0.00150 mole of total HAc

0.00432 mole HAc originally present
$\underline{-0.00150}$ $0.00282/0.11 = 0.02564 = (Ac^-) = (Cr^+)$.
0.00282 mole Ac^-

0.00846 mole of creatinine originally added
$\underline{-0.00282}$
0.00564 mole of free creatinine

$0.00564/0.11 = 0.05127 = (CrOH)$

$$K = \frac{(0.02564) \times (0.02564) \times 5.4 \times 10^{-10}}{(0.05127) \times (0.01001)} = 6.92 \times 10^{-10}$$

There were fifteen determinations in all, giving the following results: creatinine: 0.04800 to 0.08200 mole/liter originally present. $K \times 10^{10}$: 7.2, 7.2, 7.0, 9.1, 7.1, 7.1, 10.0, 6.8, 6.8, 8.0, 9.0, 6.9, 7.1, 6.8, 6.9. These results, exclusive of the four high values due probably to some experimental error, average a value not far different from those obtained in the other determinations, and 7.0×10^{-10} is given as the ionization constant of creatinine at 25° as determined by distribution measurements.

Attempts were made to determine this constant by the solubility method of Löwenherz,[12] using benzoic acid. However, satisfactory results have not as yet been obtained. The values for the constant were all considerably higher than those obtained by the three methods already outlined, and were not in very good agreement among themselves. The solubility method has proved unsatisfactory so far in determining this ionization constant.

Discussion of Results

Table IV gives a summary of our results, together with the values to be found in the literature.

TABLE IV

SUMMARY OF RESULTS

Method	H-Ion	Cond.	Distrib.	H-Ion	Cond.	H-Ion (Hahn and Barkan)	Catal. (Wood)
Temp., °C.	25	25	25	40	40	17	40.2
$K \times 10^{10}$	6.73	7.05	7.0	9.80	10.5	1.85	0.357

It will be noted that our results agree much better with one another than with those of previous workers. Of the two other determinations, that of Hahn and Barkan agrees better with ours. Their value is somewhat lower, and this discrepancy may be due to impurities in their creatinine. Some of our preliminary results, before making correction for the impurity in the sample, ran as low as theirs. Wood's value of 0.357×10^{-10} at 40.2° is of another order of magnitude than ours (10×10^{-10}). Doubtless this discrepancy may be traced to the excessive difficulty of carrying out measurements of the rate of hydrolysis of methyl acetate at so high a temperature and at such low acid concentration.

Altogether, we believe that the average of our closely agreeing determinations by the hydrogen-ion, conductance and distribution methods comes nearest to the truth, and we give as the dissociation constant of creatinine the following values: 7.0×10^{-10} at 25°; 10.1×10^{-10} at 40°.

Summary

1. The ionization constant of creatinine has been determined by three methods; namely, hydrogen ion, conductance and distribution.

---

[12] Löwenherz, *Z. physik. Chem.*, **25**, 385 (1898).

2. The values found for this constant are 7.0 × 10^{-10} at 25°, and 10.1 × 10^{-10} at 40°.

University, Virginia

[Contribution from the Department of Physiological Chemistry, Johns Hopkins Medical School]

REACTIONS OF MERCURY DIPHENYL WITH SOME ACYL HALIDES

By Herbert O. Calvery

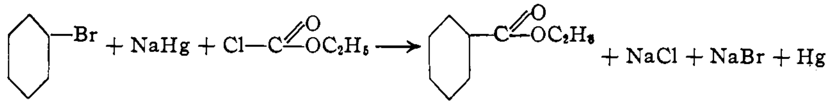
Received December 1, 1925 Published April 5, 1926

In the preparation of ethyl benzoate from bromobenzene, ethyl chloroformate and sodium amalgam,[1] Wurtz obtained some mercury diphenyl. It has been suggested that mercury diphenyl is an intermediate product in the formation of the ethyl benzoate. Since the action of ethyl chloroformate with mercury diphenyl has not been studied it was decided to investigate it in order to learn more about the mechanism of the reaction observed by Wurtz. In general, unsatisfactory results have been obtained by several investigators who have studied the reactions of acid halides and organic mercury compounds.[2]

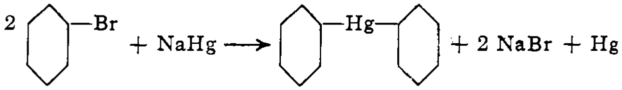
Whitmore and Thurman[3] have published the first of a series of papers in which they are studying in some detail the action of several organic halides on mercury *p*-ditolyl. They have chosen this particular mercury compound because of the ease of preparation and because of the fact that it differs in a number of its reactions from mercury diphenyl[4]. The first definite results they obtained were with *p*-toluene-sulfonyl iodide. It has been found to give a good yield of *p*-ditolyl-sulfone, tolylmercuric iodide and mercuric iodide. Whitmore and Thurman discuss the previous work in this general field and show that sulfonyl chlorides have been more thoroughly investigated than ordinary acyl halides.

Several mechanisms may be suggested for the action of bromobenzene, ethyl chloroformate and sodium amalgam. These may be illustrated as follows.

I.



II. (a).



---

[1] Wurtz, *Compt. rend.*, **68**, 1298 (1869).

[2] Steinkopf and Bauermeister, *Ann.*, **403**, 59 (1914). Otto, *Ber.*, **3**, 197 (1870); **18**, 246 (1885). Paterno, *Ber.*, **10**, 1749 (1877).

[3] Whitmore and Thurman, This Journal, **45**, 1068 (1923).

[4] Whitmore, private communication.