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THE ACTION OF NITROUS ACID UPON CREATININE AND SOME OF ITS DERIVATIVES

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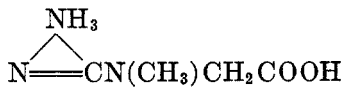
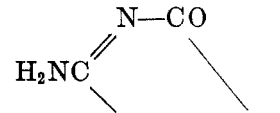
The work discussed in the present paper is an attempt to determine the reactivity of nitrous acid with creatinine and several of its derivatives and to consider the significance of the results for the formulation of the constitution of these substances.

Wilson (1) and Plimmer (2) found that creatine, for which the usual formula indicates an amino group, did not react with nitrous acid or did so only slowly and incompletely; whereas creatinine, for which the usual formula does not indicate an amino group, reacted quite rapidly.

Plimmer (2) added the observation that, in the presence of hydrochloric acid, creatine did liberate gas, whereas the yield from creatinine was diminished. He, therefore, proposed the alternative formulas I and II for creatine and creatinine, respectively, as expressing the state of the substance in aqueous solution and suggested that the usual formulas express the structures existing in solutions containing hydrochloric acid.



I



II

We confirmed Plimmer's report as to the effect of hydrochloric acid upon the reactivity of creatinine and nitrous acid. We mixed the creatinine with hydrochloric acid before adding it to the reaction mixture and used such small quantities of hydrochloric acid that they could not have affected the *p*H of the reaction mixture appreciably. We are, therefore, of the opinion that there are two tautomeric forms of creatinine, one of which is stable at low *p*H and does not react with nitrous acid.

However, if Plimmer's formulation were correct, methylcreatinine and N-benzylcreatinine should not react with nitrous acid or do so but slowly. Actually, they do (Table I, entries 6 to 12, 14 and 15), and rapidly and without apparent interference by small amounts of hydrochloric acid.

Since the *p*H of a solution of creatinine picrate is about 3.9 (glass electrode) and since that of the reaction mixture of acetic acid and nitrous acid cannot be far removed from this, it would appear that about ten per cent of the creatinine exists in the non-reactive form at this *p*H (entry 4). Moreover, since some reconversion to the reactive form may be expected to have occurred in the reaction

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TABLE I

LIBERATION OF NITROGEN BY ACETIC ACID AND SODIUM NITRITE FROM CREATININE AND ITS DERIVATIVES

(The figures represent percentages of calculated yield of one atom per molecule. Only one value is reported if duplicate analyses yielded concordant results.)

NO.	SUBSTANCE	SOLVENT	NITROGEN LIBERATED IN, MINUTES		
			3	15	30
			Molar percntage		
1	Creatine	H_2O	0.4	7.2	7.5
2	Creatinine	H_2O	22–45	88	101
3	Creatinine HCl	0.04 *M* HCl [a]	16–23	46	51
4	Creatinine picrate	H_2O	21	80	90
5	Creatinine	0.1 *M* NaOH [b]	19–28	83–91	97
6	Methylcreatinine HI	H_2O	33	88	99
7	Methylcreatinine HCl [c]	H_2O	50	96	100
8	Methylcreatinine picrate	H_2O	39	87	107
9	Methylcreatinine picrate	0.04 *M* HCl [a]	45		
10	Methylcreatinine HCl [c]	0.9 *M* HCl [a]		90	
11	Methylcreatinine HI	0.04 *M* HCl [a]	41		
12	Methylcreatinine H_2SO_4	H_2O	51	94	94
13	Methylcreatinine HI	0.04 *M* NaOH [a, b]			14
14	N-benzylcreatinine	H_2O		90	103
15	N-benzylcreatinine HCl	H_2O	62	92	111
16	Creatinine oxime	0.04 *M* NaOH [a]	81–97	104	
17	Creatinine oxime	0.44 *M* NaOH [a]	56		102
18	Methylhydantoin oxime	0.065 *M* NaOH [a]			10–30
19	Dimethylhydantoin oxime	0.068 *M* NaOH [a]		85	95
20	Methylhydantoin	H_2O			2
21	Acetylcreatinine	H_2O	13	24	32
22	Benzoylcreatinine [e]	0.96 *M* NaOH [a]	4	10	10[f]
23	Benzalcreatinine, 2 HCl	H_2O	36	108	136
24	Benzalcreatinine	10% Acetic acid	24	83	99
25	5-Benzylcreatinine	H_2O		90	103
26	Dimethylcreatinine HCl [c]	H_2O		52	58
27	Dimethylcreatinine HI	H_2O	22–36	34–45	46–53
28	Dimethylcreatinine HI	0.07 *M* HCl [a]		34	
29	Dimethylcreatinine HI	0.07 *M* HCl [a, d]		41	
30	Glycocyamine	H_2O	5	13	26
31	Glycocyamidine HCl	H_2O	37	77	90
32	3-Methylglycocyamidine picrate	H_2O	38	66	70
33	Dicreatinine picrate HBr	H_2O	47–68	110	125

[a] The concentration of hydrochloric acid and of sodium hydroxide are those calculated as remaining after displacement of picric acid or of hydroiodic acid from the salts or of one proton from benzoylcreatinine or any of the oximes; or the addition of one proton to creatinine.

[b] The mixtures were prepared one hour before use.

[c] Prepared from the hydriodide by treatment with freshly precipitated silver chloride.

[d] After standing eight days.

[e] In a previous paper (10) it had been assumed, because of the different crystal forms, that the benzoylcreatinine made from creatinine and benzoyl chloride (plates) was an isomer of that obtained by heating creatinine with benzoic anhydride (needles). Ing (7) claimed that the supposed isomer was a mixture of benzoylcreatinine and tribenzoyl creatinine. Our preparations did not contain tribenzoylcreatinine. However, upon recrystallization from alcohol, particularly in the presence of a few needles, the plates changed to needles. Determinations of the solubility of the two materials, separately or together indicated that they were different crystal forms of the same substance.

[f] The low yield of gas when treated with nitrous acid was probably due to precipitation of benzoylcreatinine in the reaction chamber.

vessel in the experiments summarized in entry 3, it would appear that more than half of the creatinine at *p*H 1.4 was in this non-reactive form. At the lower *p*H prevailing in Plimmer's mixtures containing 5, 6, 7, and 8 ml. of concentrated hydrochloric acid, still more of the creatinine would have been in this inactive form and the approximately 0.5 atom of nitrogen liberated might well have been formed during the first few minutes (as Plimmer recognized), before all the acid had been added. Whatever the change may be, it does not seem to occur if the two hydrogens in position 5 are replaced by a benzal group (entries 23 and 24).

The removal of one atom of nitrogen from creatinine should result in the formation of methylhydantoin. Indeed, Schmidt (3), thirteen years before Plimmer, had shown that creatinine in approximately 23% nitric acid, to which an excess of sodium nitrite was added, deposited a mixture of a small quantity of creatinine oxime and a large quantity of methylhydantoin oxime.

Experiment showed that gas was liberated from creatinine oxime more rapidly than from creatinine, indicating that the reaction might be creatinine oxime → methylhydantoin oxime (entries 16 and 17). However, methylhydantoin oxime, dissolved in NaOH, in order to get it into the apparatus, also liberated gas (entry 18). Dimethylhydantoin oxime yielded even more (entry 19). Apparently, the addition of alkali to either of these oximes produces a change in configuration which is not readily reversed by the acetic acid in the reaction mixture. The postulated isomers are acted upon by nitrous acid, with the liberation of gas. Methylhydantoin, itself, dissolved in water, gave only traces of gas (entry 20).

It seems, therefore, quite likely that the reaction is creatinine → methylhydantoin in the dilute solution employed in the determination, and to creatinine oxime and then to methylhydantoin oxime in the more concentrated mixtures. The methylhydantoin oxime isolated could scarcely have been formed from methylhydantoin because the latter, when added to a mixture of sodium nitrite and acetic acid, yielded no oxime but was recovered unchanged upon extraction with chloroform.

When the same preparative technic was applied to methylcreatinine hydrochloride, no methylcreatinine oxime was obtained. Instead, 60% of the material employed was accounted for as dimethylhydantoin oxime, identified by analysis and by conversion to dimethylparabanic acid. However, Zeile and Meyer (4) have obtained and described the hydrochloride of methylcreatinine oxime. This was formed in the presence of an excess of hydrochloric acid; when an excess of sodium nitrite was present, the product was dimethylhydantoin oxime.

The fact that methylcreatinine reacted so readily indicated that the second methyl group is in position 3 and not in 2. Zeile and Meyer (4), came to a similar conclusion in spite of the opposite view held by Nicolet and Campbell (5) and by Cornthwaite (6).

Ing (7) has presented evidence for regarding acetylcreatinine and benzoylcreatinine as being substituted in position 2. Since neither of these (entries 21, 22) reacted rapidly and since, in large-scale experiments, benzoylcreatinine yielded only benzoylcreatinine oxime, Ing's views would seem to have been

confirmed. Such liberation of nitrogen as did occur might be considered as having taken place after hydrolysis.

However, this interpretation is contradicted by the fact that dimethylcreatinine (entries 26 to 29) reacted to a greater extent than did acetylcreatinine. In dimethylcreatinine, all the nitrogen atoms are methylated and there can be no question of hydrolysis.

Other compounds related to creatinine were also tested with nitrous acid (entries 30–33). Glycocyamine reacted to a greater extent than did creatine; glycocyamidine hydrochloride almost as much as did creatinine; methylglycocyamidine picrate to a greater extent than did creatinine picrate. The compound of two molecules of creatinine and one of picric acid, in which the creatinine is present in a form that does not give Jaffe's reaction (8) yielded more gas than did the equivalent amount of creatinine, added as the free base, the picrate or the hydrochloride.

In contrast to the apparent stability of creatinine to 0.1 *M* sodium hydroxide for one hour, is the great lability of methylcreatinine to an even lower concentration (entry 13). Colorimetric analysis, using Jaffe's reaction and creatinine as a standard, of some of the same mixture as was used for the gasometric determinations, showed it to contain 23% of the expected amount of methylcreatinine. However, at the end of an hour, when the diluted alkaline picrate solutions were again compared with one another, the readings indicated the presence of 37% of the calculated amount of methylcreatinine. In another experiment, in which the undiluted mixtures of creatinine, or methylcreatinine, picric acid and sodium hydroxide were allowed to stand an hour before dilution and comparison, the readings indicated the presence of 79% of the calculated amount.

Apparently, the ring in methylcreatinine is more readily opened by hydroxyl ions than is that in creatinine but, as enolization takes place in more concentrated alkali and as complex formation with picrate occurs, it tends to close again.

The same ready opening of the ring in methylcreatinine was observed by Zeile and Meyer (4) when they attempted to prepare methylcreatinine by Cornthwaite's method. We succeeded once in the preparation by Cornthwaite's method but, thereafter, always encountered opening of the ring, whether we used that method or Korndorfer's (9), which had previously given good results. Finally a satisfactory product was obtained by treating methylcreatinine sulfate with ammonia, as described in the experimental part.

Attempts were made to isolate the products of the reaction between nitrous acid and creatinine and some of its derivatives. Under the conditions chosen, which except for time and the concentration of the "creatinine", were similar to those obtaining in the Van Slyke analysis, the following substances were isolated and identified: from creatinine, the oximes of creatinine and methylhydantoin; from methylcreatinine, dimethylhydantoin oxime; from N-benzylcreatinine, benzylmethylhydantoin oxime; from benzoylcreatinine, benzoylcreatinine oxime.

Dimethylcreatinine yielded a mixture of substances, which were not separated satisfactorily. In one experiment, what appeared to be two different substances, of widely different melting points, were obtained. The empirical constitution

of both was that of dimethylhydantoin oxime but neither yielded dimethylparabanic acid upon hydrolysis. In another experiment, the bulk of the product had a melting point which was different from that of either of the other two materials or from that of pure dimethylhydantoin oxime but it yielded dimethylparabanic acid upon hydrolysis.

We wish to thank Mr. D. Rigakos for the determinations of carbon, hydrogen, and nitrogen.

EXPERIMENTAL

Except as mentioned below, all the derivatives of creatinine were prepared by the methods used in previous work (10).

Benzalcreatinine and its dihydrochloride were prepared by the method of Nicolet and Campbell (5).

Methylcreatinine. To 18 g. of methylcreatinine hydrogen sulfate (6), dissolved in 200 ml. of hot absolute alcohol, 12 ml. of concentrated NH_4OH was added with constant shaking. The mixture was cooled in ice, filtered, and the precipitate was washed with absolute alcohol. The filtrate and washings were evaporated, under diminished pressure, with a bath temperature of 35°, to about 15 ml. Some crystals had separated. Fourteen milliliters of anhydrous ether was added and, after a few hours, the mixture was filtered and the precipitate was dried over H_2SO_4 and then over P_2O_5. The yield was practically the theoretical. The crystals melted at 70°, and recrystallization either from alcohol (9) or from acetone (6) raised the m.p. only to about 73°, not the previously reported 80°. However, even the crude product could be used for the preparation of dimethylcreatinine hydriodide with perfectly satisfactory results.

Creatinine oxime and methylhydantoin oxime. A suspension of 5 g. of creatinine in 10 ml. of water was added in small quantities with constant stirring to a mixture of 8.4 g. of sodium nitrite, 25 ml. of H_2O and 7 ml. of glacial acetic acid. After standing at 10° for two days, the precipitate was filtered off, washed, and dried over KOH. It weighed 4.4 g. Extraction with hot alcohol yielded 2.3 g. of methylhydantoin oxime (m.p. 196°; diacetyl deriv., m.p. 187°). The material insoluble in alcohol was dissolved in 0.25 *M* NaOH, reprecipitated with acetic acid, washed and dried. It weighed 1.5 g., decomposed above 252° and yielded a diacetyl derivative of m.p. 208°.

Dimethylhydantoin oxime. Similar experiments in which methylcreatinine hydrochloride or hydrogen sulfate were used yielded no material insoluble in alcohol but only an alcohol-soluble material, of m.p. 236°.

Anal. Calc'd for $C_5H_7N_3O_3$: C, 38.19; H, 4.49; N, 26.76.
Found: C, 38.66; H, 4.2; N, 25.90.

Upon hydrolysis with HCl (4) it yielded dimethylparabanic acid; crystals, from alcohol, of m.p. 150–151°.

Oxime of 1-methyl-3-benzylhydantoin. N-benzylcreatinine hydrochloride yielded only a small amount of material that was insoluble in alcohol. This dissolved in NaOH and was reprecipitated by acetic acid. It darkened above 262° but did not melt even at 355°. The alcohol-soluble material was crystallized twice from 50% alcohol, and then melted at 183°.

Anal. Calc'd for $C_{11}H_{11}N_3O_3$: C, 56.64; H, 4.75; N, 18.01.
Found: C, 56.79; H, 4.34; N, 17.97.

Oximes from dimethylcreatinine. A solution of dimethylcreatinine hydriodide was treated with freshly precipitated silver chloride. The mixture was filtered and the filtrate and washings were evaporated, under diminished pressure, to small volume. Upon treatment with sodium nitrite and acetic acid, a crystalline material was obtained. This was fractionated into two portions, one less soluble in alcohol (A) of m.p. 206° and one more soluble (B) of m.p. 140–142°.

(A) Found: C, 37.98; H, 4.43; N, 26.25.
(B) Found: C, 38.28; H, 4.54; N, 26.48.

These figures agree very well with those calculated for dimethylhydantoin oxime. Some of each fraction was mixed with pure dimethylhydantoin oxime. The m.p. of the mixture with A was 208–210°; of that of the mixture with B was 185–195°. Neither A nor B yielded dimethylparabanic acid upon treatment with HCl.

In another experiment, the bulk of the material melted above 175°. This was not analyzed but, upon hydrolysis, yielded typical crystals of dimethylparabanic acid, m.p. 150°, unchanged by mixture with material obtained from pure dimethylhydantoin oxime.

Benzoylcreatinine oxime. Benzoylcreatinine (1.76 g.) was ground with 30 ml. of 0.04 *M* sodium hydroxide. There were then added 10 ml. of 30% sodium nitrite and 50 ml. of 20% acetic acid. After standing 24 hours, the mixture was filtered and the precipitate was washed, air-dried, and extracted with benzene to remove a small quantity of unchanged benzoylcreatinine. The residue was dissolved in 0.1 *M* sodium hydroxide and reprecipitated with acetic acid. After being filtered, washed, and dried, it melted at 129–130°; yield 1.79 g., or 90% of that calculated. Four-tenths gram was dissolved in 5 ml. of warm concentrated hydrochloric acid and the solution was then placed in a vacuum desiccator containing potassium hydroxide. The dry residue was extracted with ether. The ether was evaporated and the residue was recrystallized from water. It melted at 120° and 0.0786 g. required 0.61 ml. of 0.1014 *M* sodium hydroxide for a titration to *p*H 8.5; calculated for benzoic acid, 0.636 ml. The aqueous mother liquids from the benzoic acid were evaporated to dryness. The residue, after crystallization from alcohol, melted at 152–153°, unchanged upon mixing with pure methylparabanic acid. The material insoluble in ether weighed 0.11 g., melted at 147° and contained 48.4% chlorine and 19.0% nitrogen. It was, apparently, hydroxylamine hydrochloride, containing 51% chlorine and 20% nitrogen, contaminated with some material of lower chlorine and nitrogen content.

SUMMARY

The reaction between nitrous acid and creatinine and several of its derivatives was studied. The results indicate the existence of a tautomer of creatinine that is stable at low *p*H and that does not react with nitrous acid. This type of tautomerism does not occur with methylcreatinine, which reacts readily to form dimethylhydantoin oxime. Dimethylcreatinine is less reactive than methylcreatinine but also forms a compound of the composition of dimethylhydantoin oxime.

A method for the preparation of free methylcreatinine is described.

Attention is directed to the ready opening of the ring in methylcreatinine.

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BIBLIOGRAPHY

(1) WILSON, *J. Biol. Chem.*, **56**, 183 (1923).
(2) PLIMMER, *J. Chem. Soc.*, **127**, 2651 (1925).
(3) SCHMIDT, *Arch. Pharm.*, **25**, 330 (1912).
(4) ZEILE, AND MEYER, *Z. physiol. Chem.*, **252**, 101 (1938).
(5) NICOLET, AND CAMPBELL, *J. Am. Chem. Soc.*, **50**, 1155 (1928).
(6) CORNTHWAITE, *J. Am. Chem. Soc.*, **59**, 1616 (1937).
(7) ING, *J. Chem. Soc.*, 2047 (1932).
(8) GREENWALD, *J. Biol. Chem.*, **86**, 333 (1930).
(9) KORNDORFER, *Arch. Pharm.*, **242**, 641 (1904).
(10) GREENWALD, *J. Am. Chem. Soc.*, **47**, 1443 (1925).