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THE CHEMISTRY OF JAFFE'S REACTION FOR CREATININE II. THE EFFECT OF SUBSTITUTION IN THE CREATININE MOLECULE AND A POSSIBLE FORMULA FOR THE RED TAUTOMER[1]

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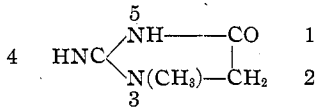
In a previous communication,[2] it has been shown that the red color obtained when creatinine solutions are treated with picric acid and sodium hydroxide is due to the formation of a red tautomer of creatinine picrate. A method for the preparation of this tautomer was presented and some of its properties were described.

There seem to be at least two places in the creatinine molecule at which the shifting of a hydrogen atom would give rise to a tautomer. There



might be a lactam-lactim rearrangement between Positions 1 and 5 or there might be a keto-enol change between Positions 1 and 2.

It seemed that it would be of interest to prepare derivatives of creatinine and to test their behavior with picric acid and sodium hydroxide.

Table I gives, in succeeding columns, the names of the compounds prepared, their graphic formulas, references to the published methods of preparation and the results of the test with picric acid and sodium hydroxide. The names of new compounds are printed in italics and the methods of preparation are described in the experimental part of this paper.

The red tautomers were actually isolated in the case of the glycocyamidine, methylglycocyamidine and methylcreatinine picrates. This was not attempted with the other substances because of lack of material.

Examination of these results shows that a lactam-lactim rearrangement cannot be responsible for the formation of the red tautomer because dimethylcreatinine, in which such a change has been made impossible by the substitution of all the hydrogens attached to nitrogen, also gives a red color with picric acid and sodium hydroxide.

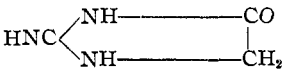
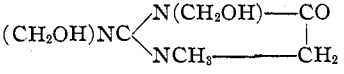
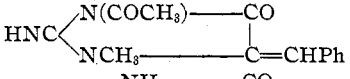
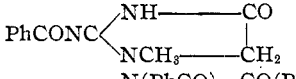
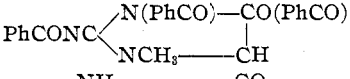
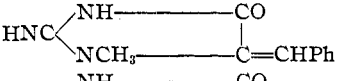
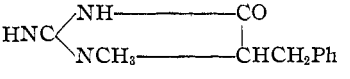
That a keto-enol change is responsible for the reaction is indicated by the fact that creatinine oxime, benzylidene-acetylcreatinine, benzylidene-creatinine and tribenzoyl-creatinine, in all of which such a change is impossible, fail to give the reaction. After treatment of solutions of benzyl-

[1] A preliminary report was read before the American Chemical Society, at Ithaca, September, 1924.

[2] Greenwald and Gross, *J. Biol. Chem.*, **59**, 601 (1924).

TABLE I

JAFFE'S REACTION WITH HOMOLOGS AND DERIVATIVES OF CREATININE

Substance	Formula	Reference	Color with picric acid and sodium hydroxide
Glycocyamidine		3	Red
5-Methylglycocyamidine		3	Red
5-Methylcreatinine		4	Red
4,5-Dimethylcreatinine		5	Red
5- (or 4-)Benzoyl-creatinine[a]		6	Red
5- (or 4-)Benzyl-creatinine		7	Red
Dimethylol-creatinine		8	Yellow (picrate color)
2-Creatinine oxime		9	Yellow (picrate color)
Benzylidene-acetylcreatinine		10	Yellow (picrate color)
4- (or 5-)Benzoyl-creatinine[a]		New	Red
Tribenzoyl-creatinine		New	Yellow (picrate color)
Benzylidene-creatinine		New	Yellow (picrate color)
2-Benzyl-creatinine		New	Orange

[a] The compositions indicated for the two forms of benzoylcreatinine have been arbitrarily chosen. In both, the benzoyl group is attached to nitrogen but in which it is in Position 4 and which in Position 5 is not known.

idene-creatinine or of benzylidene-acetylcreatinine with zinc dust and acetic acid,[11] they yielded a picrate which had the composition indicated for 2-benzylcreatinine picrate and which gave an orange color when treated with picric acid and sodium hydroxide. The reduction had made a keto-enol change possible. The fact that the color obtained was not red, but orange, may have been due to the introduction of a benzyl group so close

[3] Korndörfer, *Arch. Pharm.*, **242**, 620 (1904).
[4] Korndörfer, *ibid.*, **242**, 641 (1904).
[5] Kunze, *ibid.*, **248**, 578 (1910).
[6] Urano, *Beitr. chem. Physiol. Pathol.*, **9**, 183 (1907).
[7] Hennig, *Arch. Pharm.*, **251**, 396 (1913).
[8] Jaffe, *Ber.*, **35**, 2896 (1902).
[9] Hennig, *Arch. Pharm.*, **250**, 370 (1911–1912).
[10] Erlenmeyer, *Ann. Chem. Phys.*, **284**, 49 (1895).
[11] This experiment was suggested by Professor Treat B. Johnson, to whom I wish to express my thanks.

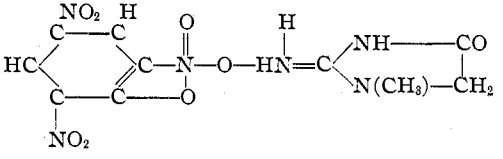
to the chromophore. In all the other positively-reacting derivatives, the substitution occurred at a greater distance.

It may be objected that dimethylol-creatinine, which retains the possibility of a keto-enol tautomerism, fails to give the reaction. But this compound cannot form a salt with picric acid. The basic nitrogen has been combined with a CH_2OH group.

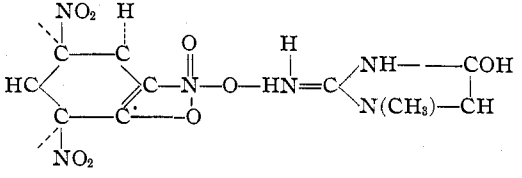
Apparently, then, we may conclude that the production of the red color when creatinine is treated with picric acid and sodium hydroxide depends upon the formation of the enolic form of creatinine and upon the retention of the basic properties of the molecule.

In the previous communication to which reference has already been made[2] it was shown that not one of a considerable number of substances that are more or less similar to picric acid reacts with creatinine and sodium hydroxide to give a red color. Not even 2,4-dinitrophenol, nor 2,4,6-trinitro-*m*-cresol, does so. Apparently, the two *o*-nitro groups are essential as is, also, the hydrogen in the *meta* position. Accordingly, the formation of the red creatinine picrate would seem to involve changes in all of these groups.

If the picric acid, in picrates, be assumed to have the *o*-quinone form, the formula for the ordinary yellow, creatinine picrate might be written thus,



The similar compound with trinitro-*m*-cresol, which is readily prepared, would have the same constitution, except for the presence of a methyl group instead of hydrogen in one of the *meta* positions; but this does not form the red tautomer. It is, therefore, highly probable that at least one of these hydrogen atoms migrates to a new position in the formation of the red tautomer. We cannot as yet determine, however, whether this migration is to the enolic creatinine molecule or to the oxygen of the nitro groups. For the present, we may write the formula of the red tautomer as



The dotted lines indicate that the exact positions of these hydrogens and the disposition of the remaining valences of the carbon atoms are not known.

For the purpose of more fully identifying benzylidene-acetylcreatinine

and benzoylcreatinine, they were oxidized with alkaline permanganate. From the products of the reactions, picric acid precipitated *acetylmethylguanidine picrate* and *benzoylmethylguanidine picrate*, respectively. The latter was compared with that obtained from *benzoylmethylguanidine hydrochloride*, prepared by the action of benzoyl chloride upon methylguanidine hydrochloride.

Experimental Part

Glycocyamidine, methylglycocyamidine (*iso*creatinine), methylcreatinine, dimethylcreatinine, dimethylol-creatinine and creatinine oxime were prepared as such, or as salts, by the methods described in the literature.[3,4,5,8,9]

For the purposes of identification, the bases were generally converted into picrates. The picric acid content of these was then determined by precipitation with nitron.[12] This offers a simple and convenient method for determining the molecular weight of the base. Taken into consideration with the method of preparation, it offers, in the author's opinion, a better indication of identity than does the determination of nitrogen in a picrate and at least as satisfactory a method as the determination of the halide in a halide salt. As compared with the latter, it has the advantage that the picrates, while readily soluble in hot water, are much less soluble in cold water than the corresponding halides and, consequently, may be recrystallized more readily and with less loss. In some later experiments, only one of which is here reported (acetyl-methylguanidine), the identity of the picrate was further established by using a known amount of nitron for the determination of the picric acid content and then quantitatively precipitating the excess of nitron in the filtrate with picric acid and using the final filtrate for a determination of nitrogen.

Methylcreatinine picrate was obtained as yellow needles; m. p., 183° (uncorr.).

Anal. Calcd. for $C_5H_9ON_3.C_6H_2(NO_2)_3OH$: $C_6H_2(NO_2)_3OH$, 64.3. Found: 64.4.

The **dimethylcreatinine hydriodide** obtained melted at 187° (uncorr.). Kunze[5] gives 179–180°.

Anal. Calcd. for $C_6H_{11}ON_3.HI$: I, 47.6. Found: 47.2.

Dimethylcreatinine did not form a difficultly soluble picrate.

Benzoyl-creatinine was prepared according to the directions of Urano[6] by heating creatine with benzoic anhydride and was obtained as yellow needles; m. p., 190°.

Anal. Calcd. for $C_{11}H_{11}N_3O_2$: N, 19.35. Found: 19.43.

After oxidation with sodium permanganate in alkaline solution, picric acid precipitated *benzoyl-methylguanidine picrate* as yellow needles; m. p., 180°.

Anal. Calcd. for $C_9H_{11}N_3O.C_6H_2(NO_2)_3OH$: $C_6H_2(NO_2)_3OH$, 56.4. Found: 56.4.

For purposes of comparison, methylguanidine hydrochloride was benzoylated according to Korndörfer's directions[13] for guanidine hydrochloride and *benzoyl-methylguanidine hydrochloride* obtained; m. p., 222°.

[12] Busch and Blume, *Z. angew. Chem.*, **21**, 354 (1908).

[13] Korndörfer, *Arch. Pharm.*, **241**, 449 (1903).

Anal. Calcd. for $C_9H_{11}N_3O.HCl$: Cl, 16.6. Found: 16.6.

Benzoyl-methylguanidine picrate, prepared from the hydrochloride, was identical with that obtained from the oxidation of benzoyl-creatinine.

Attempts were made to prepare dibenzoyl-creatinine by adding benzoyl chloride to mixtures of creatinine and pyridine, with and without the addition of benzene. With small amounts of benzoyl chloride, only a monobenzoyl-creatinine was obtained. This crystallized from alcohol in the form of light brown plates, quite different from the yellow needles obtained by Urano's method; m. p., 190°.

Anal. Calcd. for $C_{11}H_{11}N_3O_2$: N, 19.33. Found: 19.43.

When mixed with the benzoyl-creatinine prepared by Urano's method, the melting point remained unchanged. On oxidation and treatment with picric acid the same benzoyl-methylguanidine picrate that has already been described was obtained. According to Schenk,[14] methods of preparation that might be expected to yield derivatives of $CH_3NC(NH_2)_2$ always yielded derivatives of $HNCNH_2.NHCH_3$. Similarly, benzoyl-creatinines containing the benzoyl group attached to different nitrogen atoms would gave the same benzoyl-methylguanidine. The fact that the melting points of the two forms of a mixture are identical may indicate that a similar shifting occurs, at high temperatures, even within the creatinine molecule.

The following procedure gave a yield of 77%. To a mixture of 1.13 g. of creatinine and 15 cc. of pyridine in a test-tube at 75° was added 0.5 cc. of benzoyl chloride, drop by drop, at 30-minute intervals, until 2.5 cc. had been added. The temperature of the bath was then raised to 120°, until all of the creatinine had dissolved. The tube was then allowed to cool and water was added. The insoluble material was filtered out, washed and dried in the air. After extraction with ether, the residue was crystallized from absolute alcohol.

Tribenzoyl-creatinine.—When an excess of benzoyl chloride was used, even if the temperature of the bath was only about 90°, tribenzoyl-creatinine was obtained. Recrystallized from alcohol, this formed colorless crystals, melting at 238–240°, which did not give Jaffe's reaction.

Anal. Calcd. for $C_6H_5CONC\begin{cases} N(C_6H_5CO)—CO(C_6H_5CO) \\ NCH_3\text{———}CH \end{cases}$: N, 9.88. Found: 9.89.

Benzylidene-acetylcreatinine was prepared by Erlenmeyer's method.[10] It was crystallized from glacial acetic acid. The mother liquor was diluted with water and some of it was mixed with an aqueous solution of picric acid. Benzylidene-acetyl-creatinine picrate separated in the form of yellow needles, softening at 230°, melting at 250° (uncorr.).

Anal. Calcd. for $C_{13}H_{13}N_3O_2.C_6H_2(NO_2)_3OH$: $C_6H_2(NO_2)_3OH$, 48.5. Found: 48.0.

When recrystallized from water, alcohol or glacial acetic acid, the melting point and picric acid content gradually rose to 260° and 53.1% respectively. The same result was obtained by prolonged boiling in aqueous solution. Apparently, the acetyl group was split off, forming benzylidene-creatinine picrate.

Anal. Calcd. for $C_{11}H_{11}N_3O.C_6H_2(NO_2)_3OH$: $C_6H_2(NO_2)_3OH$, 53.3. Found: 53.1.

The dilute acetic acid solution of benzylidene-acetylcreatinine did not give Jaffe's reaction. Some of it was allowed to stand in contact with zinc dust at room temperature for one week. The solution then gave an orange color when mixed with picric acid

[14] Schenk, *Arch. Pharm.*, **249**, 463 (1911).

and sodium hydroxide. Addition of picric acid, alone, produced a yellow precipitate, apparently 2-benzylcreatinine picrate which, recrystallized from hot water, formed yellow needles and melted at 206–208° (uncorr.). These crystals gave an orange color when mixed with picric acid and sodium hydroxide.

Anal. Calcd. for $C_{11}H_{13}N_3O.C_6H_2(NO_2)_3OH$: $C_6H_2(NO_2)_3OH$, 53.0. Found: 53.6.

This picrate was quite different from that obtained from the benzyl-creatinine hydrochloride prepared by the action of benzyl chloride upon creatinine.[7] The latter picrate separated as a gum when the solutions of the hydrochloride and sodium picrate were mixed. It was recrystallized from methyl alcohol and then melted at 174–175° (uncorr.).

Anal. Subs., dried at 100°. Calcd. for $C_6H_2(NO_2)_3OH$: 53.0. Found: 52.8.

5 (or 4) benzyl-creatinine picrate.—According to Hennig,[7] this benzyl-creatinine yields benzyl-methyl-guanidine on oxidation with alkaline permanganate and is, therefore, 4-(or 5-)benzyl-creatinine. Our 2-benzyl-creatinine was not isolated except as the picrate and was, therefore, not treated with alkaline permanganate. However, some of the benzylidene-acetyl-creatinine from which it had been prepared was oxidized with alkaline sodium permanganate. After removal of the excess of permanganate with alcohol, acidification with acetic acid and filtration, picric acid precipitated *acetyl-methyl-guanidine picrate*, which after recrystallization from hot water melted at 160–162° (uncorr.).

Anal. Calcd. for $C_4H_9ON_3.C_6H_2(NO_2)_3OH$: $C_6H_2(NO_2)_3OH$, 66.5. Found: 66.8.

The nitron in the filtrate was quantitatively precipitated with picric acid and the filtrate was used for a nitrogen determination.

Anal. Calcd. for $C_4H_9ON_3.C_6H_2(NO_2)_3OH$: base N, 12.2. Found: 12.4.

A Compound of Creatinine and Trinitro-*m*-cresol, from the constituents, was obtained as yellow needles; m. p., 218° (uncorr.). The content of trinitro-*m*-cresol was determined by precipitation with nitron, with which it forms an insoluble compound very similar to nitron picrate.

Anal. Calcd. for $C_4H_7ON_3.C_6HCH_3(NO_2)_3OH$: $C_6HCH_3(NO_2)_3OH$, 68.3. Found: 68.2.

I am indebted to Mr. Joseph Gross for most of the analyses here reported and to the Valentine's Meat Juice Company for a generous supply of creatine, without which this work would not have been completed.

Summary

A study of the behavior of derivatives of creatinine with picric acid and sodium hydroxide and of creatinine with various substances related to picric acid indicates that the formation of the red tautomer responsible for Jaffe's reaction is dependent upon the formation of a salt, a keto-enol change within the creatinine molecule, and a change in the picric acid molecule involving the hydrogens in the *meta* positions and, probably, all three nitro groups.

NEW YORK, N. Y.