[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NEW YORK UNIVERSITY COLLEGE OF MEDICINE]

THE REACTION OF ALKALINE PICRATE SOLUTION WITH AN EXCESS OF METHYLCREATININE, DIMETHYLCREATININE, AND 5-BENZYLCREATININE

ISIDOR GREENWALD

Received February 5, 1952

The writer (1) has previously described a compound of two moles of creatinine, one mole of picric acid, three moles of sodium hydroxide, and three moles of water. This was obtained by precipitation with alcohol from a strongly alkaline solution containing two moles of creatinine per mole of picrate. The creatinine in this compound was present in a form in which it did not react with additional alkaline picrate solution to give the well-known Jaffé reaction. In fact, this reaction could be obtained only after the new compound had been dissolved in dilute acetic acid and allowed to stand.

Later (2), it was found that treatment of the strongly alkaline reaction mixture with an excess of acetic acid and subsequent precipitation with ethanol yielded a compound consisting of two moles of creatinine, one mole of picric acid, 2.5 moles of sodium hydroxide, and two moles of water. Treatment of the reaction mixture with a moderate excess of hydrochloric acid precipitated the free acid, analyzing for 2 moles of creatinine, one mole of picric acid, and one or two moles of water. However, when the reaction mixture was run into a large excess of hydrobromic acid, there was no precipitate but subsequent addition of *n*-propanol produced a precipitate containing two moles of creatinine, one mole of picric acid, one mole of hydrobromic acid, and five or six moles of water. Evidently, the compound of creatinine and picric acid was an ampholyte dissociating three protons but also associating one proton in strongly acid solutions.

It seemed of interest to ascertain what would be the effect of substituting one or more of the hydrogens in creatinine by methyl or by benzyl groups.

It was found that substitution of one or both of the imide hydrogens did not prevent the formation of analogous compounds but that substitution of one of the methylene hydrogens did. Since such substitution does not interfere with the appearance of the Jaffé reaction, it is apparent that creatinine and its N-substituted derivatives may undergo two types of tautomerization; one, in the Jaffé reaction in the presence of an excess of picrate and another, quite different, in the presence of an excess of creatinine or its homolog.

It was also found that the full color of Jaffé's reaction was demonstrable with only three equivalents of picrate to one of methylcreatinine or of dimethylcreatinine. With 5-benzylcreatinine the color was intensified more than three-fold by further increasing the picrate concentration thirty-fold.

The depth of color of the reaction mixtures was diminished by the presence of an excess of methylcreatinine or of dimethylcreatinine but, with 5-benzylcreatinine, it was increased. With this last, subsequent addition of excess alkaline picrate led to a prompt intensification of the color. This was not the case with methylcreatinine nor with dimethylcreatinine. With these, the original Jaffé color was obtained only after acidification with acetic acid, standing overnight, and subsequent treatment with the alkaline picrate. The fact that the mixtures containing excess of 5-benzylcreatinine had the red color characteristic of the Jaffé reaction and also reacted promptly and completely with additional alkaline picrate to give an intensified color is evidence that the second type of tautomerization, observed with N-substituted creatinines, is not obtained with 5-benzylcreatinine.

We have not been able to devise satisfactory structural formulae for these compounds.

TABLE I

COLOR DEVELOPMENT IN MIXTURES OF ALKALINE PICRATE AND SUBSTITUTED CREATININES IN DIFFERENT PROPORTIONS

a. Composition of mixtures

FLASK →	1	2	3	4	5	6
0.01 *M* "creatinine", ml.	1	1	1	2	3	1
0.01 *M* picric acid, ml.	3	2	1	1	1	(10)[a]

b. Comparison of readings in visual colorimeter

SUBSTANCE						
Methylcreatinine	100	89	69	66	57	100
Dimethylcreatinine	100	89	77	66	40	100
5-Benzylcreatinine	100	42	25	42	47	350

[a] Added as 2 ml. of 0.05 *M* picric acid.

EXPERIMENTAL

Color development in mixtures of alkaline picrate and homologs of creatinine, in varying proportions. Into a series of six 10-ml. volumetric flasks, there were measured the amounts of 0.01 *M* methylcreatine hydriodide, dimethylcreatinine hydriodide, or 5-benzylcreatinine hydrochloride and of 0.01 *M* picric acid indicated in Table I.

To each flask there was added 1 ml. of 2 *N* NaOH and the contents of the flask were diluted to the mark, allowed to stand ten minutes, and the depth of color was then read in a visual colorimeter against the contents of flask 1 as a standard. Giving the color developed in this flask a value of 100, the values for the colors developed in flasks were as indicated in Table I.

Formation of tautomer that does not give the Jaffé reaction. Mixtures similar to those in flask 5 of Table I were prepared but were not diluted to 10 ml. After 10 minutes, 5 ml. of 0.05 *M* picric acid and 1.5 ml. of 2 *M* NaOH were added. The mixtures containing the excess of methylcreatinine or of dimethylcreatinine turned light brown but that containing 5-benzylcreatinine remained red. After another 10 minutes, the mixtures were diluted to *25 ml.* and read in the colorimeter against mixtures of similar composition, in which all the ingredients had been added in rapid succession. The apparent "creatinine" contents were: for methylcreatinine 6×10^{-6} moles, and for dimethylcreatinine 3×10^{-6} moles, instead of the 30×10^{-6} moles expected. 5-Benzylcreatinine gave the full color value of 30×10^{-6} moles.

Other mixtures similar to those in flask 5 were prepared and, after 20 minutes, 2 ml. of

2 *M* acetic acid was added and the mixtures were allowed to stand overnight. Then, 5 ml. of 50 *mM* picric acid and 2.5 ml. of 2 *M* NaOH were added and, after 10 minutes, the mixtures were diluted to 25 ml. The apparent "creatinine" contents were: for methylcreatinine, 28 × 10^{-6} moles and for dimethylcreatinine 30 × 10^{-6} moles.

Preparation and analysis of a compound containing 2 moles of methylcreatinine, 1 mole of picric acid, 3 moles of NaOH, and 2 moles of water. Methylcreatinine picrate (2 g., 5.52 × 10^{-3} moles) was dissolved in 10 ml. of hot water. The mixture was cooled rapidly and 1.78 g. (5 × 10^{-3} moles) of methylcreatinine hydriodide and 10 ml. of 2 *M* NaOH were then added. The picrate dissolved giving a dark red solution which became much lighter within ten minutes. It was then run into 400 ml. of 99% ethanol. The orange-yellow precipitate was filtered out after 30 minutes, washed with alcohol, and dried over sulfuric acid at about 30 mm.

Weighed amounts of the dried material were dissolved in dilute acetic acid and allowed to stand overnight. Aliquots were then taken for the determination of methylcreatinine and of picric acid. Methylcreatinine was determined colorimetrically by the Jaffé reaction, using methylcreatinine hydriodide as a standard. Picric acid was determined gravimetrically by precipitation with nitron.

The filtrate from the nitron picrate was treated with nitric acid, the nitron nitrate was filtered out, and the filtrate, after the addition of a few drops of sulfuric acid, was evaporated, at first in porcelain and then in platinum, ignited, and weighed.

Methylcreatinine, Found: 41.0%; Calc'd: 39.8%.
Picric acid, Found: 35.6%; Calc'd: 35.8%.
Sodium, Found: 10.8%; Calc'd: 10.8%.
Yield, 2.1 gm., 3 millimols, or 60%.

Preparation and analysis of a compound of 2 moles of methylcreatinine, 1 mole of picric, 1 mole of hydrobromic acid, and 5 moles of water. To a mixture of 1.78 g. (5 × 10^{-3} moles) of methylcreatinine picrate and 1.27 g. (10 × 10^{-3} moles) of methylcreatinine, there were added 10 ml. of water and 10 ml. of 2 *M* NaOH. The mixture was placed in ice. After 10 minutes, the temperature had fallen to 5° and the mixture was run into 15 ml. of 6 *M* hydrobromic acid at 4°. This mixture was kept in ice for an hour. The yellow precipitate was then filtered off, washed with cold *n*-propanol, and dried at 30 mm. over H_2SO_4 and KOH.

Weighed samples were dissolved in 0.1 *M* NaOH, then acidified with acetic acid and, after standing overnight, diluted to a definite volume. Aliquots were then taken for the determination of methylcreatinine, picric acid, and bromine. The latter substance was determined by precipitation with silver nitrate in the filtrate from the nitron nitrate. The precipitated silver bromide was weighed. Other weighed samples were used for the determination of nitrogen by the Kjeldahl method, after reduction with tin and hydrochloric acid. The values for bromine were found to be higher, and those for the other constituents lower, than expected. Other samples were analyzed for sodium by direct treatment with sulfuric acid and ignition. Sodium sulfate equal in amount to 9.1% of the material taken was obtained. Upon calculation to the equivalent of sodium bromide and correcting the results of the analyses accordingly, the following values were obtained:

Nitrogen, Found: 19.65%; Calc'd: 19.23%.
Methylcreatinine, Found: 38.9%; Calc'd: 38.85%.
Picric acid, Found: 34.9%; Calc'd: 35.01%.
Bromine, Found: 12.5%; Calc'd: 12.37%.

Preparation and analysis of a compound of 2 moles of dimethylcreatinine, one mole of picric acid, three moles of sodium hydroxide, and three moles of water. To 0.57 g. (2.5 × 10^{-3} moles) of picric acid there were added in order, 20 ml. of water, 5 ml. of 40% NaOH, and 1.34 g. (5 × 10^{-3} moles) of dimethylcreatinine hydriodide. The initial red color faded to an orange. After 10 minutes, the mixture was run into 250 ml. of 99% ethanol. There was an initial turbidity but this disappeared and further addition of ethanol produced no precipitate. However, the addition of anhydrous ether caused a flocculent precipitate which was filtered out, washed, and dried over sulfuric acid. Weighed portions were dissolved in acetic acid, allowed to stand, and then analyzed.

Dimethylcreatinine, Found: 41.6%; Calc'd: 41.17%.
Picric acid, Found: 34.7%; Calc'd: 33.42%.
Sodium, Found: 10.5%; Calc'd: 10.06%.

Preparation of a similar compound containing only two moles of sodium hydroxide but four moles of water. A mixture of 2 g. (7.4×10^{-3} moles) of dimethylcreatinine hydriodide, 0.57 g. (2.5×10^{-3} moles) of picric acid, 25 ml. of water, and 6 ml. of 2 *M* NaOH was prepared. The red color faded slowly. After 5 minutes, the mixture was placed in ice. After another 15 minutes, the mixture was run into 350 ml. of *n*-propanol at 0°. A red oil separated. After an hour, the supernatant was decanted, the oil was washed with propanol, and the flask was then placed in a vacuum desiccator over sulfuric acid. The red material dried and could be scraped from the glass. It was analyzed in the usual manner.

Dimethylcreatinine, Found: 44.0%; Calc'd. 43.72%.
Picric Acid, Found: 35.7%; Calc'd: 35.49%.
Sodium, Found: 7.0%; Calc'd: 7.13%.
Nitrogen, Found: 19.2%; Calc'd: 19.54%.

SUMMARY

Creatinine and certain of its derivatives undergo two types of tautomerization when mixed with alkaline picrate solutions. With an excess of picrate, the red color or Jaffé's reaction is obtained. With an excess of creatinine, or a derivative, there is a brown color. Subsequent addition of an excess of alkaline picrate does not produce a red color. The first type of change requires the presence of but one methylene hydrogen; the second requires both. Complexes of methylcreatinine and dimethylcreatinine with picric acid and sodium hydroxide, or hydrobromic acid, have been obtained in the solid state.

NEW YORK 16, N. Y.

BIBLIOGRAPHY

(1) GREENWALD, *J. Biol. Chem.*, **77**, 539 (1928).
(2) GREENWALD, *J. Biol. Chem.*, **86**, 333 (1930).