SOME NEW COLOR REACTIONS OF CREATININE[1]

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Benedict and Behre (1936), Langley and Evans (1936), and Bolliger (1936), working independently, have reported a color reaction between an alkaline solution of 3, 5-dinitrobenzoic acid and creatinine. Riesser (1923, p. 892), Anslow and King (1929), Bolliger (1936), and Schindel (1940) have reported positive reactions between creatinine and nine nitroaromatic compounds. On the other hand, a number of investigators (von Bitto 1892, Greenwald and Gross 1924, Greenwald 1925a, 1925b) have reported failure to obtain a color reaction between creatinine and nitroaromatic compounds other than picric acid. The compounds reported as reacting with creatinine are; 3, 5-dinitrobenzoic acid, 1, 3, 5-trinitrobenzene, 2, 4, 6-trinitrotoluene, 2, 4, 6-trinitrobenzoic acid, *m*-dinitrobenzene, 2, 4-dinitrophenol, trinitro-*m*-cresol, 2, 4-dinitro-1-naphthol-7-sulfonic acid, and dinitrochlorobenzene. The color obtained was in all cases less intense than with picric acid. The compounds reported as not reacting with creatinine are: *o*-nitrophenol, *p*-nitrophenol, 2, 4-dinitrobenzoic acid, 3, 5-dinitrobenzoic acid, 3, 5-dinitrosalicylic acid, trinitro-*m*-cresol, picramic acid, 2, 4-dinitrophenol, 2, 6-dinitrophenol, 3, 5-dinitro-*p*-cresol, *m*-dinitrobenzene, *o*-nitrobenzoic acid, *m*-nitrobenzoic acid, *p*-nitrobenzoic acid, *p*-nitrotoluene, 1-nitronaphthalene, 1, 5-dinitronaphthalene, 1, 8-dinitronaphthalene, and trinitroresorcinol.

The purpose of this paper is to present semi-quantitative data as to the sensitivity of 91 nitrobenzene derivatives and 16 nitropolycyclic compounds in producing color with creatinine.

EXPERIMENTAL

Reagents. The nitro compounds studied were of "C. P." grade when available. No attempt was made to purify them further, except in the case of picric acid, which was recrystallized according to the directions of Folin (1934, p. 285). Unless otherwise indicated, a saturated aqueous solution of the reagent was prepared and mixed with 0.5 volume of 10 percent sodium hydroxide solution immediately before use.

Creatinine Solutions. A series of solutions containing from 0.0001 mg to 10.0 mg of creatinine per ml in multiples of ten were prepared in 0.1 N hydrochloric acid.

Method. The procedure used for obtaining the data here reported was as follows: 5 ml of a fresh alkaline solution of the compound to be tested was added to each of a series of tubes containing 1 ml of several standard creatinine solutions. In the experiments with di- and tri-nitro compounds the mixtures were allowed to stand 10 minutes at room temperatures before comparing their colors with those of control blanks (5 ml of the alkaline reagent plus 1 ml of 0.1 N hydrochloric acid). Repeated observations showed that in most cases a color developed in ten minutes or not at all.

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The reaction was much slower with mono- than with di- and tri- nitrobenzene derivatives. The color reaction mixtures with the mononitrobenzene derivatives were compared with the control blanks after 18 hours to 24 hours.

Some of the alkaline solutions of the nitrobenzene derivatives were so highly colored that it was difficult to detect any change in color upon the addition of creatinine. In these instances it was often possible to remove much of the color due to the alkali by the addition of 10 percent acetic acid solution. The amount of acid added was the minimum necessary to produce maximal bleaching in the control tube. This quantity of acetic acid (2 to 4 ml, depending upon the reagent) was added to each of the test solutions after the period of color development. Excess acid must be avoided since it causes rapid bleaching of the colors due to creatinine.

RESULTS

The results of these experiments are summarized in table I. In the first column of this table are given the smallest amounts of creatinine, in milligrams, which will produce an unmistakable change in the intensity of the color of the alkaline reagent. In the last group in this table are listed the nitro compounds which produce no change in color when treated with 10 mg. of creatinine.

The trinitrobenzene derivatives are, in general, the most sensitive in the detection of creatinine and the mononitrobenzene derivatives are the least sensitive. All trinitrobenzene derivatives investigated gave color reactions with creatinine with the exception of 2,4,6-trinitromesitylene and 2,4,6-trinitro-1,3-dimethyl-5-tert.-butylbenzene. In both of these compounds the hydrogen at all six benzenoid positions is substituted. Under the experimental conditions 2,4,6-trinitrobenzene, 2,4,6-trinitrobenzoic acid, 2,4,6-trinitroanisole, 2,4,6-trinitrotoluene and picric acid were found to be about equally sensitive in the detection of creatinine.

Of all the dinitrobenzene derivatives examined only dinitroresorcinol produced no recognizable change in color when treated in alkaline solution with creatinine. The reagent was highly colored and may have masked a feeble reaction. However, inasmuch as the analogous trinitro derivative 2,4,6-trinitroresorcinol gave poor results, it could be expected that the dinitro compound would react feebly if at all. *para*-Dinitrobenzene was sensitive to 0.01 mg of creatinine, while the corresponding *meta* and *ortho* compounds required 0.1 and 1.0 mg of creatinine respectively to produce a change in color intensity. *para*-Nitrobenzoic acid was the only one of the isomeric mononitrobenzoic acids which reacted at room temperature.

Creatinine reduced the color intensity of the following alkaline reagents: 2,4,6-trinitrobenzene, 2,4,6-trinitrobenzaldehyde, 2,4,6-trinitro-*m*-cresol, 2,4,6-trinitrophenylhydrazine, 2,4-dinitroaniline, dinitrohydroquinone diacetate, dinitrophydroquinone, and *p*-nitrophenylacetic acid. After bleaching with acetic acid the creatinine-reagent mixtures were generally more highly colored than the blanks.

DISCUSSION

These experiments show that creatinine can react with a considerable number of nitro compounds in alkaline solution to produce distinct colorations. The deep colors frequently encountered in the alkaline reagents probably account for numerous reported negative results. After the deep colors produced by sodium hydroxide are bleached by acetic acid the slight colorations produced by small amounts of creatinine can be observed.

The color reactions here reported develop at room temperature. When the reaction mixtures are heated in a boiling water bath many of the reagents show a marked increase in sensitivity, some as much as a thousandfold. This relationship does not hold for all compounds since some are not affected at all by heat.

Introduction of an additional radical into a di- or tri- nitro- benzene compound either has no effect on the sensitivity or decreases it. There is one apparent exception: 2,6-dinitrotoluene is sensitive to 0.01 mg of creatinine while *m*-dinitrobenzene is sensitive to 0.1 mg under the conditions employed.

The facts that completely substituted trinitrobenzene compounds (2,4,6-trinitromesitylene and 2,4,6-trinitro-1,3-dimethyl-5-tertiary-butylbenzene) do not react, and that the trinitro compounds with two additional hydrogens substituted (2,4,6-trinitroresorcinol and 2,4,6-trinitro-m-cresol) react only weakly indicate that hydrogens attached to the benzene ring are concerned in the color reactions.

SUMMARY

The color reactions between creatinine and alkaline solutions of 11 trinitrobenzene derivatives, 24 dinitrobenzene derivatives, 55 mononitrobenzene derivatives, and 16 nitropolycyclic compounds have been studied semiquantitatively. Of these, 9 trinitro-, 23 dinitro-, 10 mononitrobenzene derivatives, and 7 nitropolycyclic compounds give positive color reactions at room temperature.

In general trinitrobenzene derivatives are more sensitive in the detection of creatinine than are dinitrobenzene derivatives, and the latter are more sensitive than mononitrobenzene derivatives. The nitropolycyclic compounds tested are poor reagents for the detection of creatinine.

TABLE I

Minimum concentration of creatinine detected by various nitro compounds in alkaline solutions

Creatinine concentration *mg/ml*	Nitro compounds
0.001	2, 4, 6-trinitroanisole, 2, 4, 6-trinitrobenzene, 2, 4, 6-trinitrobenzoic acid, 2, 4, 6-trinitrotoluene, 2, 4, 6-trinitrophenol.
0.01	*p*-dinitrobenzene, 2, 6-dinitrotoluene.
0.1	2, 4, 6-trinitrobenzaldehyde, 2, 4, 6-trinitroresorcinol, 2, 4-dinitroaniline, *m*-dinitrobenzene, 2, 4-dinitrobenzoic acid, 3, 5-dinitrobenzoic acid, 2, 4-dinitrobromobenzene, 2, 4-dinitrochlorobenzene, 2, 6-dinitro-4-chlorophenol, dinitrohydroquinone diacetate, 2, 4-dinitrophenylhydrazine, picramic acid, 3, 5-dinitrosalicylic acid, 3, 5-dinitrosalicylaldehyde, nitroanthraquinone 8-sulfonic acid.

1.0	2, 4, 6-trinitrophenylhydrazine, 2, 4-dinitrotoluene, *o*-dinitrobenzene, 3, 5-dinitro-*o*-cresol, dinitrohydroquinone, *l*-3,5-dinitro-2-hydroxymandelic acid, 2, 4-dinitrophenol, 2, 6-dinitrophenol, 2, 4-dinitrophenylacetic acid, *d*-3, 5-dinitro-2-hydroxymandelic acid, nitrobenzyl chloride, 4-nitrochlorobenzene-2-sulfonic acid, *o*-nitrophenol, *p*-nitrophenylacetic acid, tripotassium nitrophenoldisulfonate, *m*-nitrobenzenesulfonic acid, 2, 4-dinitro-1-naphthol, 1, 3, 8-trinitronaphthalene.
10.0	2, 4, 6-trinitro-*m*-cresol, *p*-nitrobenzoic acid, *o*-nitrophenetole, nitrophenylpropiolic acid, sodium 4-nitrotoluene-2-sulfonate, dinitroanthraquinone, nitroanthraquinone, nitroanthraquinone-5-sulfonic acid, 6-nitroquinoline.
No reaction with 10 mg creatinine.	2, 4, 6-trinitromesitylene, 2, 4, 6-trinitro-1, 3-dimethyl-5-tert.-butylbenzene, 2, 4-dinitroresorcinol, *p*-nitrobenzeneazoresorcinol, 2, 4-dinitro-1-naphthol-7-sulfonic acid, *p*-nitrodiphenyl, *p*-nitrophenyl salicylate, 1-nitronaphthalene, 1, 8-dinitronaphthalene, 1, 5-dinitronaphthalene, sodium 4-nitrodiphenylamine-2-sulfonate, *p*-nitroacetanilide, *m*-nitroacetophenone, 3-nitro-4-acetylaminophenyl acetate, 3-nitro-4-acetylaminotoluene, 3-nitro-4-aminoanisole, 5-nitro-2-aminoanisole, 5-nitro-2-aminotoluene, 3-nitro-4-aminotoluene, 4-nitro-2-aminotoluene, *m*-nitroaniline, *o*-nitroaniline, *p*-nitroaniline, *o*-nitroaniline-*p*-sulfonic acid, nitrobenzene, *m*-nitrobenzene sulfochloride, *m*-nitrobenzhydrazide, *m*-nitrobenzoic acid, *o*-nitrobenzoic acid, *p*-nitrobenzyl bromide, 2-nitrobromobenzene-4-sulfonic acid, *m*-bromonitrobenzene, *o*-bromonitrobenzene, *p*-bromonitrobenzene, 2, 5-dichloronitrobenzene, 2, 6-dichloro-4-nitrophenol, *m*-nitrocinnamic acid, *o*-nitrocinnamic acid, nitrocymene, *m*-nitrodimethylaniline, *p*-nitrodimethylaniline, *m*-nitrophenol, *p*-nitrophenol, *p*-nitrophenylhydrazine hydrochloride, *p*-nitrophenylglycine, *m*-nitrophenylhydrazine, 3-nitrophthalic acid, 2-nitroresorcinol, 5-nitrosalicylaldehyde, 5-nitrosalicylic acid, *o*-nitrotoluene, *m*-nitrotoluene, *p*-nitrotoluene, sodium *o*-nitrophenoxide.

LITERATURE CITED

Anslow, W. K. and H. King. 1929. J. Chem. Soc. 1210.

Benedict, S. R. and J. Behre. 1936. J. Biol. Chem. 114: 515.

Bolliger, A. 1936. J. Proc. Roy. Soc. N. S. Wales 69: 224.

Folin, O. 1934. Laboratory manual of biological chemistry. 5th ed. New York.: Appleton-Century.

Greenwald, I. 1925a. J. Am. Chem. Soc. 47: 1443.

Greenwald, I. 1925b. *Ibid.*: 2620.

Greenwald, I. and A. Gross. 1924. J. Biol. Chem. 59: 601.

Langley, W. D. and M. Evans. 1936. *Ibid.* 115: 333.

Riesser, O. 1923. Abderhalden's Handbuch der biologischen Arbeitsmethoden, Abt. I, Teil 7. Berlin: Julius Springer.

Schindel, L. 1940. J. Lab. Clin. Med. 25: 515.

Von Bitto, B. 1892. Ann. Chem. 269: 377.