A NEW COLOR REACTION FOR SOLUBLE ORGANIC SULFUR COMPOUNDS*

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In view of the increasing importance of reduced sulfur compounds in biological chemistry, a study of the nitroprusside reaction was recently made in this laboratory in an attempt to render the reaction more specific for compounds of the type C-S-H, since sodium nitroprusside in alkaline solution not only gives coloration with compounds of this type but also with ketones, aldehydes, creatinine, thio ethers, and many alkaloids. Such interference was found to be practically eliminated by working at pH 8 to 9 with saturated sodium bicarbonate as the alkaline agent in place of the more generally used carbonate or hydroxide. In bicarbonate solution, the immediate coloration from ketones, aldehydes, etc. is so slight that the test for C-S-H may be carried out in aqueous acetone solution without interference. With potassium cyanide being used as a reducing agent, the test can also be applied to compounds of the disulfide type C-S-S-C.

During the course of this work the observation was made that sodium nitroprusside solution previously exposed to light for some time gives an intense blue color with compounds of the thiourea type, in addition to the normal purple-red color with C-S-H. A search of the literature revealed that while Hofmann¹ noted a blue color as an intermediate in the formation of the red compound, Na₄Fe(CN)₆NOSCNHNH₂, from thiourea and ordinary nitroprusside, we were unable to find further mention of a blue coloration.

Sodium nitroprusside solution after several days exposure to indirect light, or a few hours to direct sunlight, changes from a

cherry-red color to yellow, then green, and finally Prussian blue coagulates out and the solution may be filtered to yield a yellow-brown filtrate. This solution gives an intense blue color in bicarbonate solution with compounds of the thiourea type as well as the normal purple-red with C—S—H. During the reaction in the light a strong odor of cyanide is present and bubbles of nitrogen are given off. No further reaction apparently takes place upon further exposure to light for short periods. If placed in the dark without filtration for a few weeks, the light-treated solution will go back to the original cherry-red color and no longer give the new reaction with C=S but will again do so after reexposure to light.

The rate of this photochemical change may be observed by adding a small amount of thiourea to fresh sodium nitroprusside solution and noting the development of the blue coloration upon exposure to light. If a slight amount of sodium bicarbonate is added, the reaction is photochemically reversible, the solution turning blue in the light and crimson when then placed in the dark. This alternate change of color with light and darkness may be repeated a dozen or more times if free access to air is allowed. A further study of this interesting reversible reaction will be reported upon in a later publication.

A number of derivatives of sodium nitroprusside were prepared in an attempt to duplicate the action of sunlight. The procedures of Hofmann were followed. A reduction of sodium nitroprusside in alkaline solution by means of hydroxylamine hydrochloride yields a yellow compound, sodium aquoferrocyanide, Na₃Fe(CN)₅.H₂O. This compound fails to give either the usual nitroprusside reaction or the new reaction with thiourea.

Treatment of this yellow compound with bromine water in neutral solution gives an intense purple compound to which Hofmann assigns the formula Na₂Fe(CN)₃·H₂O (sodium aquoferrocyanide). This compound was found to give an intense blue with thiourea after several minutes, more quickly on warming, but not as promptly as the material prepared by sunlight. The intense color of the reagent was objectionable also.

On standing in solution for some time in the presence of sodium bicarbonate, the purple color of sodium aquoferrocyanide is objectionable also.

changed to a dark yellow-brown. This yellow compound reacts instantly with thiourea to give a blue color but does not give the usual nitroprusside reactions with mercaptans, etc.

**Preparation of Test Solution for C=S, C-SH, and C-S-S-C Compounds**

A satisfactory test solution giving both the new reaction with C=S compounds and the usual nitroprusside reactions may be prepared as follows: 0.5 gm. of sodium nitroprusside (sodium nitroferricyanide) is dissolved in 10 cc. of water at room temperature, 0.5 gm. of hydroxylamine hydrochloride is added, followed by 1 gm. of sodium bicarbonate. After evolution of gas has ceased, 2 drops of bromine are added. Excess bromine is removed by aeration and the dark greenish or black-brown solution filtered and made up to 25 cc. This solution behaves like that prepared by exposure of sodium nitroprusside to sunlight and contains a mixture of several compounds, one of which reacts with the thiourea type and another like ordinary nitroprusside. The solution is stable for about 2 weeks, gradually losing reactivity toward C=S. No further purification is necessary for general test use.

A black, hygroscopic compound, yellow in dilute solution and reacting instantly with thiourea with formation of an intense blue, can be separated from the solution prepared as above after several precipitations from aqueous solution by addition of an alcohol-ether mixture. The ordinary nitroprusside reactions are not given by this purified material, mercaptans in alkaline solution giving only a transient light green color, quickly turning yellow. The purified reagent offers no especial advantages over the crude solution and is much less stable. The active compound has not yet been identified.

The alcohol-ether filtrates discarded in this purification process, in addition to a small amount of the black compound and another compound reacting like ordinary nitroprusside, contain still another compound giving a blue coloration with C=S on slight warming or more slowly at room temperature. This reacts differently from that of the active alcoholic precipitant in that the blue coloration starts at the solution surface and works down while the former gives color throughout almost instantly. In
addition, the blue color obtained with the filtrate and thiourea in bicarbonate solution when allowed to stand for a few hours, or more rapidly at 50–60°, changes through a purple-red to an intense crimson which turns blue in slightly acid solution and again red when made weakly alkaline. This behavior of forming the blue to red coloration with thiourea is also shown by the general test reagent prepared above and that prepared by action of light upon nitroprusside but is not a property of the purified alcoholic precipitant. The preliminary blue color formed in alkaline solution does not show a color change when made slightly acid.

Method of Test—5 to 20 mg. of the compound to be tested are dissolved in 2 to 3 cc. of water and solid sodium bicarbonate added to excess. About 0.5 cc. of the general test reagent is then added. Purple-red given instantly or within 10 minutes indicates $\text{C} = \text{S} - \text{H}$ while an intense green or blue indicates $\text{C} = \text{E} - \text{S}$ (where E is any single non-metallic element). Both colors may fade more or less rapidly but often reappear upon addition of fresh reagent. If no color appears within 10 minutes an equal volume of 5 per cent potassium cyanide solution is added. $\text{C} - \text{S} = \text{S} - \text{C}$ compounds will give a pink to purple-red within $\frac{1}{2}$ hour. Ring-linked sulfur compounds of both the $\text{C} - \text{S} - \text{H}$ and $\text{C} - \text{S} - \text{S} - \text{C}$ type may fail to react, such as diparatolyl disulfide. Organo metallic compounds such as the type $\text{C} - \text{S} - \text{Bi}=\text{R}_2$ may require a preliminary warming with dilute ammonia before reacting.

The color with the type $\text{C} = \text{S}$ is usually first green, then turquoise, and finally deep blue. In some cases the color stops at the deep green stage, in others at the turquoise. In a few cases, as with thiourea previously mentioned, it may go through the blue stage to a purple-red and finally crimson after several hours. The test for $\text{C} = \text{S}$ cannot be carried out in acid solution as the reagent is decomposed with liberation of Prussian blue.

The blue color is given in bicarbonate solution with the new reagent not only by compounds of the type $\text{C} = \text{S}$ but also by $\text{N} = \text{S}$, $\text{S} = \text{S}$, and probably in general by the type $\text{E} = \text{S}$, where E is any single non-metallic element doubly linked to divalent sulfur. Potassium thiocyanate, of the type $\text{N} = \text{S}$, and sodium thiosulfate, of the type $\text{S} = \text{S}$, give the reaction beautifully.

The blue coloration in many cases is sufficiently stable for colorimetric quantitative assay. Care must be taken to provide...
dilutions sufficient to prevent maximum color formation as the reaction is extremely sensitive. Distinct green coloration is given by thiourea 1 to 1 million and excellent quantitative comparisons can be made 1:100,000 to 1:200,000. Sodium thiosulfate is best matched 1:1000 to 1:5000, the blue color in this case remaining stable for days.

Some compounds destroy the reagent but many of these give transient colors of the correct type. Thioacetic acid, which attacks the reagent, gives a flash of blue, indicating the formula CH$_3$CSOH rather than CH$_3$COSH. Compounds of the thiophenol type give only a fugitive purple-red.

If both C=S and C=S—H are present, the blue color alone is produced but fades rapidly. In this case more stable color is given in neutral solution. Potassium xanthate O—C—SK gives a blue with the new reagent as well as with ordinary sodium nitroprusside.

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\begin{align*}
C & \quad \text{C} \\
C-S-C & \quad \text{C-S-C}, \quad \text{sulfites, creatinine, alkaloids, acetoacetic ester, etc. develop at the most only a slight orange color in 10 minutes. This color is not easily confused with the pink or purple of C-S—H.}
\]

No color is given with sulfones, sulfonic acids, urea, barbituric acid, taurine, thiophene, or isothioureas.

Among the compounds tested and found in the proper class are the following: (1) C=S—H: cysteine, glutathione, thioglycolic acid, thiosalicylic acid, butyl mercaptan, bismuth sodium thioglycolate. Thiophenol and thiocresol give fugitive purplered colors only, the reagent being rapidly destroyed. (2) C=S—S—C: cystine, diglycylcystine, dialanyleystine, dithioglycolic acid, dibutyl disulfide, dibenzyl disulfide, diformidine disulfide. Negative tests were obtained with diparatrolyl disulfide, diphenyl disulfide, and several other aromatic compounds in which S is directly linked to the ring. (3) C=S and E=S: thiourea, allyl thiourea, tetramethyl thiourea, thiocarbanilide, thiobar bituric acid, thioacetic acid, potassium xanthate, sodium azido carbon disulfide, thioacetamide, potassium thioyanate, sodium thiosulfate. Phenylisocyanate destroys the reagent in alcohol solution with a
transitory blue. Sodium diethyldithiocarbamate (C\textsubscript{6}H\textsubscript{5})\textsubscript{2}NC-SNa destroys the reagent without coloration. Carbon disulfide gives only a faint green, probably because of lack of solubility but gives a blue after preliminary warming with sodium hydroxide solution.

Further work on the composition of the active reagent is in progress.

**SUMMARY**

1. A new color reaction has been found, apparently limited to compounds of divalent sulfur doubly linked to a single non-metallic element. The active reagent is produced by treatment of sodium nitroferricyanide in sodium bicarbonate solution with hydroxylamine followed by bromine.

2. With use of the new reagent, a method is described to distinguish soluble organic compounds of the types C-S-H, C-S-S-C, and C=S from other types and from one another.

3. The new reagent may be used for quantitative colorimetric estimation of thiosulfate, thiocyanate, thiourea, and other compounds of the C=S type.
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