solution showed methyl aniline to be absent. This indicates that the —MgBr group did not add to nitrogen.

In one experiment when diethyl sulfate was used instead of dimethyl sulfate, the ether was replaced by dry xylene and the reaction mixture refluxed for three hours. After hydrolyzing, and then washing the ether solution with water, a reddish oil was obtained which could not be induced to crystallize. Accordingly, it was distilled in a vacuum and the major part came over at 194° (16 mm.)

This oil resisted hydrolysis by hot concentrated potassium hydroxide. However, it was readily hydrolyzed when refluxed with about 20% hydrochloric acid, giving aniline and ethyl thiolbenzoate. The ethyl thiolbenzoate in turn was identified by the products resulting from alkaline hydrolysis, namely, benzoic acid and ethyl mercaptan.

Accordingly, the oil boiling at 194° (16 mm.) must be S-ethyl-thiobenzanilide. The yield was 60%; n^{28}, 1.6110; d_4^{28}, 1.084.

Analysis.[12] Calc. for $C_{15}H_{15}NS$: S, 13.27. Found: 13.11.

An examination of the several solutions failed to reveal any ethyl aniline.

Summary

It has been proved that the Grignard reagent adds to the thiocarbonyl group in isothiocyanates. By experiment and by analogy it is virtually certain that the Grignard reagent also adds to the carbonyl group in isocyanates.

AMES, IOWA

[CONTRIBUTION FROM THE DEPARTMENTS OF PHARMACOLOGY AND OF TROPICAL MEDICINE, HARVARD MEDICAL SCHOOL]

N,N'-DIMETHYLENESULFUROUS ACID-3,3'-DIAMINO-4,4'-DIHYDROXY-AZOBENZENE: A NITROGEN COMPOUND ANALOGOUS TO SULFARSPHENAMINE[1]

BY WALTER G. CHRISTIANSEN

RECEIVED NOVEMBER 21, 1923

Since nitrogen is in the same group of the periodic table as arsenic and antimony, and many classes of physiologically active substances are organic nitrogen compounds, it seemed desirable to examine an azo dye closely related to arsphenamine and to compare its trypanocidal power with that of an arseno compound. If the nitrogen atom in certain physiologically active substances be replaced successively by phosphorus, arsenic and antimony, the products still retain the same type of activity when injected into animals but the activity per unit weight of material decreases as the atomic weight of the element replacing the nitrogen atom increases.[2] It seemed possible, therefore, that since arsphenamine and its antimony

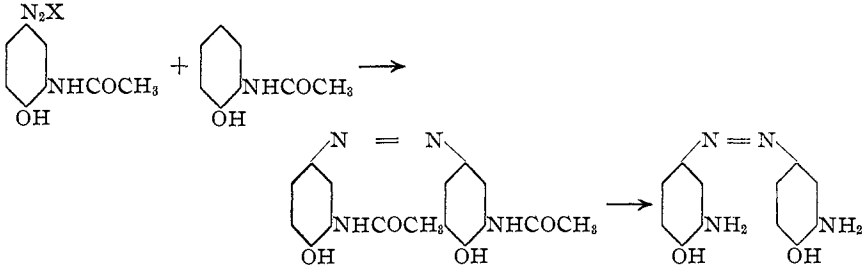
[12] Analysis made by Mr. N. J. Beaber.

[1] This is a continuation of a study of arsphenamine which was made under a grant from the United States Interdepartmental Social Hygiene Board to the Harvard Medical School and which was under the general direction of Doctor Reid Hunt.

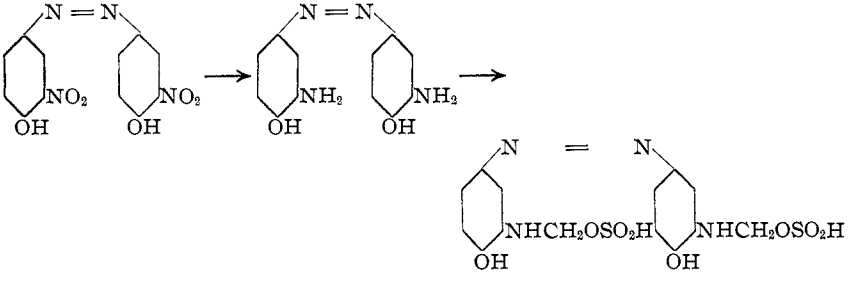
[2] Unpublished results of Doctors Reid Hunt and R. R. Renshaw.

analog are powerful trypanocidal agents, the corresponding azo compound might behave similarly toward trypanosomes. Also, an examination of the nitrogen analog of arsphenamine in relation to trypanosomiasis would indicate to some extent whether the arsenic in arsphenamine is essential to the therapeutic value of this substance.

Unsuccessful attempts were made to prepare the nitrogen analog of arsphenamine by coupling diazotized 3-amino-6-hydroxy-acetanilide with N-acetyl-*o*-aminophenol to produce N,N′-diacetyl-3,3′-diamino-4,4′-dihydroxy-azobenzene and subsequently removing the acetyl groups by hydrolysis.



Although solutions of 3,3′-diamino-4,4′-dihydroxy-azobenzene can be obtained by reduction of the corresponding dinitro-dihydroxy-azobenzene, the diamine cannot be isolated easily, due to its extreme susceptibility to oxidation. Even if it were possible to isolate the hydrochloride of this substance, it would be necessary to convert it into the sodium salt by the addition of sodium hydroxide prior to its use in animals, and under these conditions, it would oxidize with great rapidity. When the solution of the azo compound is treated with formaldehyde and sodium bisulfite a condensation occurs, and the reaction product, N,N′-dimethylenesulfurous acid-3,3′-diamino-4,4′-dihydroxy-azobenzene can easily be isolated in a fairly pure condition.



This substance is much more resistant toward oxidation than the unsubstituted diamino compound, and its sodium salt is the nitrogen analog of sulfarsphenamine.

This azo compound is tolerated in doses of at least 500 mg. per kilogram

body weight when injected intravenously into rats and has no therapeutic value when injected into rats infected with trypanosomes. The introduction of a large dose into the blood stream causes the rat's ears and nose to become distinctly brown immediately; the urine which is excreted while the last portions of the solution are being injected is very dark brown, indicating that the body disposes of this compound very readily. However, the total amount of material introduced is not eliminated at once because the urine which is excreted four hours after treatment is still colored dark brown. In addition to showing that the simple azo compound analogous to sulfarsphenamine is valueless therapeutically, these experiments indicate that the arsenic in the arsphenamines is essential for the development of trypanocidal activity, and that in producing trypanocidally active nitrogen compounds a class of substances different from the simple azo dyes should be investigated. As the azo dye is exactly like sulfarsphenamine except that two nitrogen atoms have been substituted for the arsenic atoms and as the dye has no trypanocidal activity, the groups attached to the arsenic in sulfarsphenamine have no action on the trypanosomes, but merely modify the action of the arsenic.

Experimental Part

N,N'-Dimethylenesulfurous Acid-3,3'-diamino-4,4'-dihydroxy-azobenzene.—Since 3,3'-diamino-4,4'-dihydroxy-azobenzene is formed as an intermediate in the production of the above mentioned azo compound from 3,3'-nitro-4,4'-dihydroxy-azobenzene and as *o*-aminophenols oxidize with extreme ease, these reactions must be carried out in an inert atmosphere; the apparatus employed is shown in Fig. 1.

Two g. of 3,3'-dinitro-4,4'-dihydroxy-azobenzene,[3] 30 cc. of alcohol and 13 cc. of concd. ammonia water are placed in a 100cc. cylinder which is closed with a 3-hole stopper through which are inserted a glass tube extending to the bottom of the cylinder, a thistle tube extending about 5 cm. below the stopper, and a right-angle tube extending just below the stopper. The cylinder is immersed in a water-bath maintained at 60–65°, and a rapid stream of hydrogen sulfide is introduced. As the reduction proceeds the solid, which is not dissolved at the outset, gradually dissolves, and after three hours a clear, deep reddish-brown solution of 3,3'-diamino-4,4'-dihydroxy-azobenzene is obtained. Without discontinuing the stream of hydrogen sulfide, the cylinder is now cooled in ice and water and 21 cc. of hydrochloric acid (d., 1.19) is introduced through the thistle tube; this produces a heavy precipitate of finely divided sulfur. In order to drive out the major part of the hydrogen sulfide which is still present in the cylinder, a rapid stream of carbon dioxide is passed through the mixture for 1.5 hours.

While the hydrogen sulfide is being expelled, a filter is prepared for removal of the precipitated sulfur. A percolator of about 100cc. capacity is stoppered at the bottom with a rubber stopper through which a glass tube passes so that the end of the tube is flush with the inside surface of the stopper. A wad of moist absorbent cotton is placed in the bottom of the percolator, and a suspension of filtering carbon in water is allowed to percolate through the cotton, thereby forming a mat of carbon over the latter. The

[3] Robertson, *J. Chem. Soc.*, **113**, 1476 (1913).

top of the percolator is closed with a stopper through which two tubes pass; one is a right-angle tube which is bent so that the end is close to the wall of the percolator; this will prevent the material, when forced into the vessel, from dropping directly onto the cotton. The other tube is simply a straight tube to the outer end of which a rubber tube and a clamp are attached and serves as a vent. After the air in the percolator has been displaced with carbon dioxide, the outlet at the bottom is closed with rubber tube and clamp and the bent tube at the top is connected by means of a rubber tube to the tube through which carbon dioxide has been passed into the solution in the cylinder. After the addition of 20 cc. of water through the thistle tube, the latter is closed with a stopper and carbon dioxide is passed into the cylinder through the short tube,

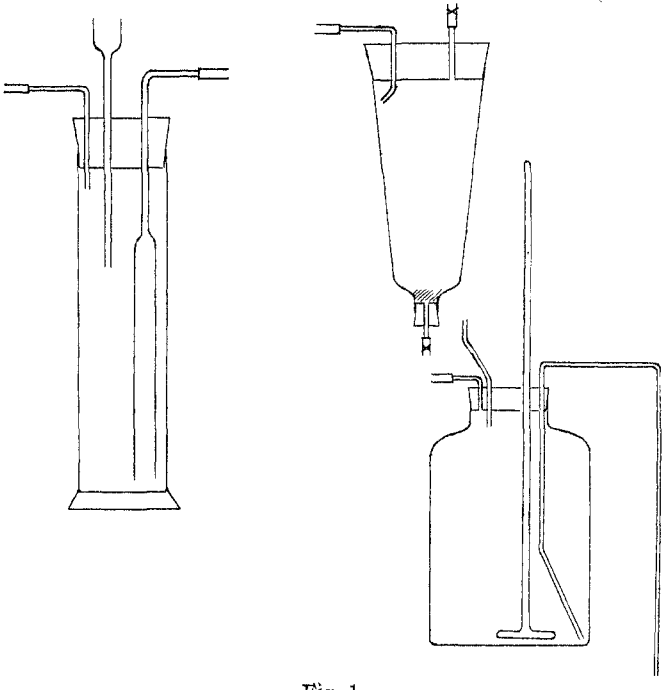


Fig. 1.

thereby forcing the contents of the cylinder into the percolator. The cylinder is disconnected and a slow stream of carbon dioxide is passed into the percolator through the bent tube, while the vent is still open.

The rubber tube on the outlet of the percolator is attached to a short glass tube which passes through a 4-hole stopper into a 150cc. wide-mouth bottle. A stirrer of such diameter that it will rotate freely passes through one of the other holes; a short right-angle tube is inserted through the third hole. Through the fourth hole a siphon passes to the bottom of the bottle; the outer end of the siphon is 10–15 cm. below the bottom of the bottle. The air in the bottle is displaced by introducing carbon dioxide through the siphon. With slow streams of carbon dioxide passing into the top of the percolator and through the bottle, the vent at the top of the percolator is closed, the solution now being under pressure. The clamp on the rubber tube which joins the percolator to the bottle is gradually opened so that the liquid drops slowly into the bottle. When this filtration is carried out properly, the finely divided sulfur is completely re-

moved and a clear, red solution is obtained in the bottle; this step requires several hours. The percolator is now disconnected, leaving the rubber tube and clamp on the inlet to the bottle.

The clear filtrate contains the dihydrochloride of 3,3′-diamino-4,4′-dihydroxy-azobenzene, some ammonium chloride and a small amount of free hydrochloric acid. With the carbon dioxide still passing through the solution and the stirrer running, 1 cc. of an aqueous solution of formaldehyde (about 37% formaldehyde) is added through the tube which previously joined the bottle to the percolator, and is rinsed in with a little water. Five minutes later, 2.6 cc. of an aqueous solution of sodium bisulfite containing 0.98 g. of bisulfite is added, and after another 5-minute interval, 1.3 cc. more of the bisulfite solution is added. During these steps, changes in the appearance of the solution are noticeable. Ten minutes after the last addition of bisulfite the agitator is stopped and the small space around the stirrer is plugged with cotton and coated with paraffin. The rubber tube through which the formaldehyde and bisulfite solutions were added is closed by a clamp, and the carbon dioxide which has been passing through the siphon is stopped. An Erlenmeyer flask containing 600 cc. of alcohol is placed under the end of the siphon and carbon dioxide is passed into the bottle through the short right-angle tube, thereby forcing the red solution from the bottle into the alcohol. After 1.5 hours the precipitate is filtered out, washed first with 95% alcohol and then with absolute alcohol. After drying in a vacuum over sodium hydroxide, 0.5 g. of N,N′-dimethylene-sulfurous acid-3,3′-diamino-4,4′-dihydroxy-azobenzene is obtained as an orange powder. The product is the free acid rather than the sodium salt because there was a slight excess of hydrochloric acid during the condensation with formaldehyde and bisulfite.

The product is insoluble in hot or cold water, alcohol, acetic acid and aqueous hydrochloric acid, but is very soluble in dil. sodium hydroxide solution, sodium carbonate and ammonium hydroxide, forming very deep red solutions. The solid when mixed with zinc dust and treated with hydrochloric acid gives off hydrogen sulfide. When suspended in dil. hydrochloric acid, the solid is reduced by stannous chloride forming a clear, very slightly pink solution. A solution of the material in sodium carbonate is reduced by sodium hydrosulfite to a clear, very slightly pink solution. When a sodium carbonate solution of the product is treated with alcohol, the sodium salt of the azo compound is precipitated. An ammoniacal solution when heated with magnesia mixture or barium hydroxide gives deeply colored precipitates of the magnesium and barium salts, respectively. To prove that the precipitate obtained with barium hydroxide was the barium salt, it was separated by centrifuging, washed twice with water, and decomposed with dil. hydrochloric acid. The precipitate dissolved and then another deeply colored precipitate formed. After being centrifuged the liquor gave a very heavy precipitate of barium sulfate when treated with sodium sulfate; the colored precipitate after being washed with water dissolves readily in sodium carbonate. Therefore, the original precipitate was decomposed by dil. hydrochloric acid to form the original acid azo dye and barium chloride. The properties, such as solubility, behavior toward reducing agents, and formation of salts, are exactly those which one would expect from the formula assigned to the material prepared above.

Analyses. Calc. for $C_{14}H_{16}O_8N_4S_2$: N, 12.9; S, 14.8; N:S, 2:1. Found: N, 12.0; S, 12.7; N:S, 2:0.93.

Although the analyses are slightly low, indicating that the product was not absolutely pure, they show, when considered in connection with the nitrogen-sulfur ratio and properties described above, that the material obtained has the structure ascribed to it. Even in the preparation of sulfarsphenamine where the technique is far less involved than in the present case, the precipitated material seldom contains the calculated amounts of arsenic and sulfur.

In examining this substance toxicologically, 90 mg. was dissolved in 1 cc. of sterile water and 0.42 cc. of *N* sodium hydroxide solution, and the mixture diluted to 1.8 cc. This 5% solution was injected intravenously into albino rats in doses of 300 and 500 mg. per kilogram of body weight. Although the ears, nose and urine became brown very rapidly, the animals showed no undesirable symptoms and appeared to tolerate this substance with great ease.

To determine the trypanocidal power of this substance, three rats were infected with *Trypanosoma Nagana*. The first was not treated and died of trypanosomiasis in 90–100 hours after inoculation. The second was treated intravenously with a 5% solution of sulfarsphenamine at a dose of 100 mg. per kilogram of body weight 67 hours after infection; at this time the blood was swarming with trypanosomes. Five and a half hours later the blood was still highly infected, but 22 hours after the time of treatment no trypanosomes could be found in the blood. The blood remained free from the parasites for the next 12 days and the animal gained weight in the normal way. On the following day, the trypanosomes reappeared in the peripheral blood and the rat died 4 days later of trypanosomiasis. The third rat was treated with a 5% solution of the azo dye which has been described above; the solution was prepared in the same way as that used in the toxicological test. This solution was injected intravenously at a dose of 300 mg. per kilogram body weight 67 hours after infection. This rat's blood did not show any decrease in the number of trypanosomes at any time and the animal died in 31–44 hours after it was treated, that is, 98–111 hours after infection. These results prove conclusively that the nitrogen analog of sulfarsphenamine has little or no trypanocidal power, and it seems that the arsenic in sulfarsphenamine is the essential factor in developing the trypanocidal activity of this substance.

Antimonyl compounds when injected into rats which are very highly infected with trypanosomes and which are very nearly dead will rapidly free the peripheral blood of the parasites, and the rat will live for some days but, if not treated again, will suffer a relapse and die. When the azo compound prepared above was injected into a rat which was nearly dead from trypanosomiasis the disease was not arrested in the least, thus showing that this compound also lacked the therapeutic value of the antimonials.

In the preparation of the azo compound outlined above the yield was poor and the product was isolated as the free acid. It seemed that a larger yield and the sodium salt might be obtained by treating the diaminodihydroxy-azobenzene with larger quantities of formaldehyde and bisulfite. When the experiment was repeated using 2.25 cc. of the formaldehyde solution and two 4cc. portions of the bisulfite solution, 1.5 g. of the disodium salt was obtained but it was contaminated with sodium bisulfite

and was only about 70% pure. Qualitatively, toxicologically and trypanocidally, this material was similar to that obtained in the first case.

It would undoubtedly be possible by further experimentation to secure good yields of the disodium salt but the trouble involved in the preparation of 3,3′-dinitro-4,4′-dihydroxy-azobenzene and the apparent lack of therapeutic value of the product indicate that the results would hardly be worth the time necessary to carry out the experiments.

Summary

By reduction of 3,3′-dinitro-4,4′-dihydroxy-azobenzene to the corresponding diamino-dihydroxy-azobenzene, and by subsequent condensation with formaldehyde and sodium bisulfite, N,N′-dimethylenesulfurous acid-3,3′-diamino-4,4′-dihydroxy-azobenzene has been prepared. Owing to extreme susceptibility of the intermediate diamino compound to oxidation, it is impracticable to attempt to isolate it, and the entire process is carried out in an inert atmosphere.

The product, which is the nitrogen analog of sulfarsphenamine, has a very low toxicity, is excreted very rapidly when injected intravenously and is apparently devoid of trypanocidal power.

BOSTON 17, MASSACHUSETTS

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

A NEW TYPE OF CYCLIC COMPOUNDS

BY E. P. KOHLER

RECEIVED NOVEMBER 23, 1923

The following investigation was undertaken for the purpose of ascertaining the reason for the extremely variable yields that are obtained in the preparation of cyclopropane derivatives which have a nitro group attached to one of the carbon atoms of the ring. The reaction by which substances of this type[1] are prepared is represented by the equation

$$\begin{matrix} RCHCHBrCOR \\ | \\ CH_2NO_2 \end{matrix} + CH_3CO_2K = \begin{matrix} RCH{-}CHCOR \\ \diagdown\ \diagup \\ CHNO_2 \end{matrix} + CH_3CO_2H + KBr$$

When primary nitro compounds are used, the yields range from 25 to 75%. Secondary nitro compounds give even lower yields and in many cases no cyclopropane at all. The by-products usually appear as uncrystallizable and undistillable oils.

It has not been possible, thus far, to identify all the products of this reaction, but it has now been found that a number of other solid products can be obtained by operating under very specific conditions. These are all due to a second reaction in which hydrogen bromide is eliminated from

[1] THIS JOURNAL, **41**, 1379, 1644, 1698 (1919); **44**, 624 (1922).