In some of the runs with ketene acetal and hydrogen fluoride the trimer fraction that was obtained failed to solidify. In such cases the above pyrolysis to the ether of phloroglucinol was used to estimate the amount of the cyclic trimer that was present in the trimer fraction. An 8.5 g. sample of this fraction was placed in a small distilling flask, a drop of concentrated sulfuric acid added, and the mixture heated (free flame) until no more ethyl alcohol distilled out. The residue then was transferred to a small 3-necked flask fitted with an efficient mechanical stirrer, treated with approximately 50 ml. of 20% sodium hydroxide solution and the mixture refluxed with stirring for two to three hours. The resulting mixture then was steam-distilled until no more oil came over. The oily distillate was taken up in ether, separated from the water layer, and dried. The phloroglucinol triethyl ether, after removal of the ether solvent, distilled at 98–106° (0.4 mm.), and weighed 2.12 g. On cooling in an ice-bath, it solidified to a product that melted at 41–43°. This weight of the ether corresponds to 3.68 g. of cyclic trimer. Assuming an 85% recovery of the triether (see above pyrolysis) this trimer fraction contained 4.34 g. of the cyclic trimer. This procedure also may be used to estimate the amount of cyclic trimer left in the filtrate after the initial crop of crystals of the solid trimer has been filtered off.

Hydrolysis of Cyclic Trimer to Phloroglucinol.—Two grams of the cyclic trimer was placed in a small flask with 10–15 cc. of water. Enough ethyl alcohol was added to give a clear solution when hot. Dry-ice (3–4 g.) was dropped into this solution over a period of fifteen to twenty minutes. During this time sufficient heat was applied to the flask to keep its contents just above room temperature. About one-third of the solvent was evaporated from the solution on a steam-bath and the remainder filtered into a small evaporating dish and the filtrate evaporated to complete dryness under a current of air. The remaining residue of phloroglucinol was dried in an oven at about 150° for an hour, after which it weighed 0.60 g. (83%) and melted at 195–205°. The product was recrystallized from water, after boiling with charcoal, filtering and adding a few drops of dilute hydrochloric acid just before cooling. The white crystals of phloroglucinol so obtained lost water of recrystallization without melting at 110–115° but did not melt until a temperature of 214–217° was reached.

Summary

Ketene acetal in dilute (1%) ethereal solution is converted by anhydrous hydrogen fluoride into the cyclic trimer, 1,1,3,3,5,5-hexaethoxycyclohexane, in approximately 22% yield.

This trimer is pyrolyzed in the presence of a trace of acid into phloroglucinol triethyl ether and hydrolyzed by aqueous carbonic acid into phloroglucinol. These products are obtained in 80–85% yields.

The homologous methylketene acetal is not polymerized by hydrogen fluoride or other catalysts such as cadmium chloride that are effective for the polymerization of ketene acetal. Hydrogen fluoride adds to methylketene acetal to form ethyl propionate and ethyl fluoride.

MADISON, WISCONSIN RECEIVED AUGUST 23, 1943

[CONTRIBUTION FROM THE SCHOOL OF CHEMICAL ENGINEERING OF PURDUE UNIVERSITY]

Studies in Azo Dyes. I. Preparation and Bacteriostatic Properties of Azo Derivatives of 2,6-Diaminopyridine[1]

BY R. NORRIS SHREVE, M. W. SWANEY[2a] AND E. H. RIECHERS[2b]

Many dyes exhibit the common characteristic of possessing a certain specificity in their properties. Some exert specific dyeing action on animal fibers, while others act specifically toward vegetable fibers or give characteristic precipitates with metals (analytical reagents). Likewise, a great many dyes have been observed to exhibit a similar specificity with respect to various organisms and have found widespread use in medicine. Typical of this class is 2,6-diamino-3-phenylazo-pyridine hydrochloride which, when ingested orally, goes via the blood stream to the kidneys whence it is eliminated by the urine to which it imparts a marked red color.

With a fundamental specificity existing in a dye, or in class of dyes, it then becomes of interest to consider the alteration of some portion of the molecule in order to modify its individual effect in medicine or in dyeing textiles. Thus, certain derivatives of diaminopyridine and hydroxyquinoline[3] have been prepared and their properties studied, with the object of ascertaining whether a wider field of application is possible for compounds of this type. This particular paper concerns itself with the synthesis and properties of azo dyes prepared by coupling various diazotized aromatic amines with 2,6-diaminopyridine, and naturally follows in line with the study carried on in these laboratories pertaining to the production of heterocyclic amines,[4] and the production of germicidal mercury compounds containing the pyridine nucleus.[5]

Chichibabin and Zeide[6] first published information concerning the first member of this series of dyes from diaminopyridine. This compound, 2,6-diamino-3-phenylazo-pyridine, possesses the formula

(1) Abstracted from the Ph.D. theses of M. W. Swaney and E. H. Riechers. Details of the yields of each dye and of the color of the hydrochloride and of the base are given in a fuller tabulation deposited with the American Documentation Institute, 1719 N St., N.W., Washington, D. C.; order Document 1801, remitting 50¢ for microfilm or 50¢ for photocopies. Original manuscript received May 1, 1942.

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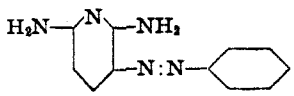
(3) R. Norris Shreve and R. B. Bennett, THIS JOURNAL, **65**, 2248 (1943).

(4) R. Norris Shreve, E. H. Riechers, H. Rubenkoenig and A. Goodman, *Ind. Eng. Chem.*, **32**, 172 (1940).

(5) M. W. Swaney, M. J. Skeeters and R. Norris Shreve, *ibid.*, **32**, 360 (1940).

(6) A. F. Chichibabin and O. A. Zeide, *J. Russ. Phys.-Chem. Soc.* **46**, 1216 (1914); *cf.* A. E. Chichibabin and E. O. Issitrowa, THIS JOURNAL, **56**, 1711 (1934).



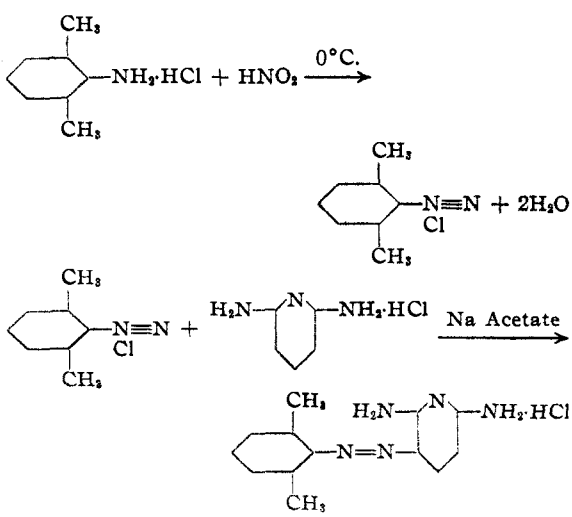
The hydrochloride of this original material[7] is being used extensively in medicine at the present time as a urinary antiseptic. Thus it was decided to prepare a number of derivatives of this present azo dye and attempt to obtain a correlation between chemical structure, bacteriostatic activity and other properties.

Experimental

2,6-Diaminopyridine, though it contains two primary and one tertiary amino groups, forms only a monohydrochloride with dilute hydrochloric acid. With strong acid it can be made to form a dihydrochloride. All these products are very soluble in water. The 2,6-diaminopyridine used in this work was prepared in our Laboratories by methods described earlier,[4] and was recrystallized from benzene until a constant melting point (120°) was attained. Of the various aminopyridines, only those with the —NH_2 group in the 3- or beta-positions are capable of being diazotized with nitrous acid. It is possible, under certain special conditions, to diazotize alpha-aminopyridines, *e.g.*, by the use of amyl nitrite. 2,6-Diaminopyridine, therefore, does not react with nitrous acid to form diazo compounds, but rather to give substitution in the 3- or beta-position, producing the compound 3-nitroso-2,6-diaminopyridine.[8] However, it couples quite easily with diazotized aromatic amines to give true azo dyes, the azo grouping likewise attaching itself to the pyridine ring in the beta-position. In this manner a considerable number of azo dyes of diaminopyridine were prepared in which substitution in the benzene ring was varied.

The aromatic amines employed in this work were either prepared in our laboratories or obtained from the Eastman Kodak Company. These amines were diazotized in aqueous solution, generally in the cold, using sodium nitrite and hydrochloric acid, and in every case diazotized amine was added to the solution containing diaminopyridine monohydrochloride (3% molar excess).

The reactions involved in the diazotization of 2,6-dimethylaniline and its coupling with diaminopyridine to form 2,6-diamino-3-(2,6-dimethylphenylazo)-pyridine, are illustrated below



(7) Trade-marked names: "Mallophene" and "Pyridium."

(8) A. E. Chichibabin and O. A. Zeide, *J. Russ. Phys.-Chem. Soc.*, **50** 522 (1920).

All of the dyes included in this work were prepared in the form of their hydrochlorides (most were obtained in yields of 90% or higher), although most were later converted to the free bases and purified by recrystallization from alcohol and other solvents. In the accompanying table are listed some of their more important properties. All dyes were analyzed for total nitrogen by the Dumas method. Closely checking analyses were obtained, agreeing with the stated formulas.

Bacteriostatic Testing of Dyes

In most cases the dyes were tested bacteriologically as aqueous solutions of their hydrochlorides. In a few cases it was necessary to resort to ethylene glycol in order to put the dyes into solution, although blank tests were made on the glycol used, in order to demonstrate its lack of bacteriostatic effect at the subsequent dilutions obtained during actual bacteriostatic tests. The tests with organisms were carried out as follows.

To a sterile tube containing 0.5 ml. of a dye solution of known strength was added 9.5 ml. of sterile agar solution (*p*H adjusted to 6.5) containing 2% agar, 1% peptone and 0.3% beef extract. To the tube was then added a 2-ml. loopful of a twenty-four-hour culture or organism. Each

TABLE I

PHYSICAL AND BACTERIOSTATIC PROPERTIES OF SUBSTITUTED PHENYLAZO DYES OF 2,6-DIAMINOPYRIDINE[a]

Dye name,[a] -pyridine	M. p. dye base, °C., cor.	Sol. of dye hydrochloride in water at 25°, grams/liter	Bacteriostatic index E. coli	Staph. aureus
R-(phenylazo)-	137	10.0	5000	6000
R-(*o*-toluylazo)-	184	2.0	2000	2000
R-(*m*-toluylazo)-	123.2	0.76	6000	12000
R-(*p*-toluylazo)-	151.3	1.57	6000	14000
R-(*o*-methoxyphenylazo)-	193	2.2	550	550
R-(*m*-methoxyphenylazo)-	99.5	1.06	1100	1500
R-(*p*-methoxyphenylazo)-	192	1.24	1100	1100
R-(*o*-ethoxyphenylazo)-	127	2.6	2000	8000
R-(*m*-ethoxyphenylazo)-	114.3	1.6	6000	8000
R-(*o*-hydroxyphenylazo)-	189	2.25	2000	8000
R-(*m*-hydroxyphenylazo)-	209.4	1.56	2000	2000
R-(*p*-hydroxyphenylazo)-	232	0.57	4000	5000
R-(*m*-chlorophenylazo)-	259	0.5	6000	20000
R-(*o*-iodophenylazo)-	209.5	0.1 Pptd. with agar solns		
R-(*m*-iodophenylazo)-	141	0.1	3000	28000
R-(*p*-iodophenylazo)-	198	0.1	1000	60000
R-(4-hydroxy-2-methylphenylazo)-	222	1.6	3000	6000
R-(4-hydroxy-3-methylphenylazo)-	203–4	2.5	2000	5000
R-(4-methyl-2-nitrophenylazo)-	233	0.1	15000	10000
R-(2-methyl-5-nitrophenylazo)-	265	.1	10000	10000
R-(2-methyl-4-nitrophenylazo)-	251	.1	15000	10000
R-(5-chloro-2-methoxyphenylazo)-	204	.1	2000	2000
R-(2-methoxy-5-nitrophenylazo)-	226	.1	5000	5000
R-(2-methoxy-5-methylphenylazo)-	174.5	5.0	2000	2000
R-(2,6-dimethylphenylazo)-	122	0.1	12000	24000
R-(biphenyl-2-azo)-	135.6	.1	4500	6000
R-(biphenyl-4-azo)-	230.5	.1	7500	5000
R-(*p*-phenylazo-phenylazo)-	203–4	.1	5000	5000
Methyl 2-(2,6-diaminopyridyl-3-azo)-benzoate	177	1.8	2000	2000
Ethyl 2-(2,6-diaminopyridyl-3-azo)-benzoate	170	3.3	2500	2500

[a] R = 2,6-Diamino-3-. Due to the deep colors of most dyes, the true colors in the solid state are often concealed, being best observed when the solid dyes are streaked across a clean porous plate. The present dyes are generally of yellow, red or brown shades.

loopful contained from 300,000 to 500,000 *E. coli* organisms or from 150,000 to 175,000 *Staphylococcus aureus* organisms. The contents of the tube were then poured into a Petri dish and incubated at 37° for forty-eight hours. At the end of this time the specimen was examined microscopically for growth of organism. These tests were repeated with different dilutions of the dye until the dilution was found beyond which growth occurred. The results of the bacteriostatic tests are summarized in the accompanying table, wherein the "bacteriostatic index" means the maximum effective dilution which would completely prevent growth.

Discussion of Results

Phenylazo-diaminopyridine hydrochloride, the first member of the series, possesses a bacteriostatic index of 5000 toward *E. coli* and 6000 toward *Staph. aureus*. Of the numerous derivatives prepared, tested in the present investigation, and detailed in the accompanying tabulations, some possessed bacteriostatic indices lower than those of this reference dye, while a considerable number exhibited much higher potencies. For example, the dye prepared from 2,6-dimethylaniline and 2,6-diaminopyridine possessed bacteriostatic indices of 12,000 and 24,000, respectively. In most cases the dyes showed higher growth-prevention powers toward *Staph. aureus* than toward *E. coli*, and in some instances markedly so (the iodoaniline dyes), although in a few instances the reverse was true (*e. g.*, derivatives of 4-methyl-2-nitroaniline and 2-methyl-4-nitroaniline). In a number of cases it was observed that great differences existed between the potencies of dyes prepared from *o*-, *m*- and *p*-isomers. This is particularly outstanding in the case of the three toluidine dyes. In the case, however, of the 2,4- and 3,4-methylhydroxyaniline and the 4,2- 2,5- and 2,4-methylnitroanilines, special arrangements of the various substituent groups in the benzene ring appear not to have exerted any marked effect on the relative potencies of the corresponding azo dyes. It is believed that the present data do not permit the formation of very steadfast conclusions regarding the dependence of bacteriostatic activity on chemical structure, although the data obtained do indicate enhanced bacteriostatic activities attributable to certain of the substituting groups. For example, methylation appears to enhance bacteriostatic activity, when alone or co-substituted with nitro groups. In general, methoxy groups appear somewhat less effective. When the dye molecule becomes too bulky, the bacteriostatic activity becomes suppressed. Increasing the complexity of the molecule greatly reduces its solubility in water.

Acknowledgment.—The authors wish to acknowledge their appreciation to Professor P. A. Tetrault for aid with the bacteriostatic tests, and to the Mallinckrodt Chemical Works for their active interest in the work.

Summary

Thirty dyes, derivatives of 2,6-diaminopyridine were prepared, purified and properties determined. Some were found to be very potent bactericidal agents.

LAFAYETTE, INDIANA RECEIVED AUGUST 4, 1943

[CONTRIBUTION FROM THE SCHOOL OF CHEMICAL ENGINEERING OF PURDUE UNIVERSITY]

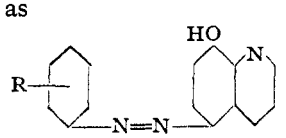
Studies in Azo Dyes. II. Preparation and Bacteriostatic Properties of Azo Derivatives of 8-Quinolinol[1a,b,c]

BY R. NORRIS SHREVE AND ROBERT B. BENNETT[2]

8-Quinolinol was coupled with twenty-eight diazotized amines to give products with marked bacteriostatic action but with no indication of commercial value as textile dyes. It was hoped that at least some fair correspondence might be established between chemical structure and bacteriostatic activity but no definite indications were found. Some of the dyes show rather strong activity but their low solubility in water limits their use in certain directions. Such insolubility may be desirable under some circumstances to prevent too rapid physiological elimination.

These dyes, tested as textile coloring agents, gave on animal fibers various tints of yellow, orange and brown-red similar to the colors of the powdered bases and hydrochlorides. Dyeings on cotton were very unsatisfactory. Preliminary tests on wool indicated poor fastness to light and washing. In view of this and the present high cost of starting materials, this phase of the application of the dyes was pursued no farther.

The generalized formula of these dyes may be represented as



and follows from the previous work of Fox[3] who

(1a) The first paper in this series appeared in THIS JOURNAL, **65**, 2241 (1943). In the first paper are detailed the methods employed for the bacteriostatic testing.

(1b) Abstracted from the Ph.D. thesis of Robert B. Bennett. Details of the yields of each dye and of the color of the hydrochloride and of the bases, are given in a fuller tabulation deposited with the American Documentation Institute, 1719 N St., N.W., Washington, D. C.; order Document 1802, remitting 50¢ for microfilm or 50¢ for photocopies.

(1c) Original manuscript received May 1, 1942.

(2) Present address, American Brakeblok Division of the American Brake Shoe & Foundry Company, Detroit, Michigan.

(3) J. J. Fox, *Proc. Chem. Soc.*, **26**, 177 (1910).