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]

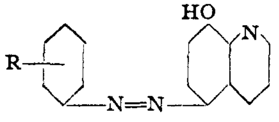
Studies in Azo Dyes. II. Preparation and Bacteriostatic Properties of Azo Derivatives of 8-Quinolinol[1a,b,c]

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).

TABLE I

PROPERTIES OF 8-QUINOLINOL DYES

Dye name (R_1 is quinolinol) (R_2 is 8-Hydroxyquinolyl-5-azo)	Ref.	Melting point, °C. Base	Melting point, °C. HClide	Solubility, grams/100 liters Base in 95% EtOH at 25.0°	Solubility, grams/100 liters Hydrochloride in Glycol at 24°	Solubility, grams/100 liters Hydrochloride in 0.1 *N* HCl at 24°	Bacterial growth *E. coli* Diln.	Bacterial growth *E. coli* %	Bacterial growth *S. aureus* Diln.	Bacterial growth *S. aureus* %
5-Phenylazo-8-R_1	*h*	172.4[a]	202.0	*e*			12,000	0	150,000	0
5-(*o*-Chlorophenylazo)-8-R_1		211.6	241.2	15.1	140	0.01	17,000	0	17,000	0
5-(*m*-Chlorophenylazo)-8-R_1		192.2	236.6	33.6	80	.25	42,000	0	52,000	0
5-(*p*-Chlorophenylazo)-8-R_1		232.8	238.7	9.5	66	.01	42,000	0	48,000	0
5-(2,5-Dichlorophenylazo)-8-R_1		246.8	239.3	1.2	100	.01	24,000	100	24,000	100
5-(*o*-Tolylazo)-8-R_1		178.1	213.2				5,400	0	11,700	0
5-(*m*-Tolylazo)-8-R_1		167.8	226.1	86.3	200	.10	11,000	0	22,000	0
5-(*p*-Tolylazo)-8-R_1	*j*	189.1[b]	sinter 207							
5-(2,6-Xylylazo)-8-R_1		196.5	223.3	58.4[f]	220	.01	9,100	0	16,800	0
5-(*o*-Nitrophenylazo)-8-R_1		221.1	206.6	57.0	66	.66	22,000	0	13,500	0
5-(*m*-Nitrophenylazo)-8-R_1		249.4	221.3	12.8[f]	17	.33	28,000	0	28,000	0
5-(*p*-Nitrophenylazo)-8-R_1	*h*	283.5[c]	225.8				24,000	90	24,000	20
5-(*o*-Hydroxyphenylazo)-8-R_1		219.2	228.4	19.8[f]	200	.31	10,000	60	28,000	0
5-(*m*-Hydroxyphenylazo)-8-R_1		237.0	258.3	247.2	330	5.01	7,000	0	6,000	0
5-(*p*-Hydroxyphenylazo)-8-R_1	*i*	228.5	248.2				10,000	10	14,000	0
o-R_2-benzoic acid	*k*	250	sinter 198							
m-R_2-benzoic acid	*i*	256.0	*d*				24,000	100	24,000	0
p-R_2-benzoic acid	*i*	287.7	*d*							
m-R_2-benzenesulfonic acid	*k*	*d*	*d*				24,100	100	24,000	100
p-R_2-benzenesulfonic acid	*j*	*d*	*d*				24,000	100	24,000	100
p-R_2-benzenearsonic acid		235.4	219.8	20.9	140	7.06	14,000	90	14,000	100
5-(4′-Aminobiphenyl-4-azo)-8-R_1		sinter 265	*d*	400[g]	466	1.01	5,600	0	2,250	0
4,4′-BisR_2-biphenyl	*l*	*d*	*d*							
5-(4′-Amino-3,3′-dimethoxybiphenyl-4-azo)-8-R_1		285.6	*d*	0.4	66	0.01	42,000	0	12,000	0
4-R_2-1-naphthalenesulfonic acid	*i*	*d*	*d*							
2-R_2-1-naphthalenesulfonic acid	*m*	*d*	*d*							
2-R_2-6-naphthalenesulfonic acid	*m*	*d*	*d*							
8-R_2-1-naphthol-3,6-disulfonic acid		*d*	*d*	582.4[f]	140	25.0	2,000	0	2,000	0

[a] Fox (*h*) gives 174°. [b] Matsumura (*j*) gives 185–186°. [c] Fox (*h*) gives 281°. [d] Indicates not melting below 300°. [e] Accurate solubilities and nitrogen percentages were run principally on compounds not mentioned in the literature. [f] Suspensions would not settle. [g] Decomposition took place during drying at 60°. [h] J. J. Fox, *Proc. Chem. Soc.*, **26**, 177 (1910). [i] G. Gutzeit and R. Monnier, *Helv. Chim. Acta*, **16**, 478–486 (1933). [j] K. Matsumura, THIS JOURNAL, **52**, 4164 (1930). [k] C. Courtot and H. Hartman, *Compt. rend.*, **194**, 1949–1952 (1932). [l] J. Ebert, U. S. Patent 1,979,678 (Nov. 6, 1934). [m] I. G. Farbenindustrie A. G., British Patent 430,287 (June 17, 1935).

reduced the dye made by diazotizing and coupling sulfanilic acid with 8-quinolinol, to produce 5-amino-8-quinolinol and proved the latter compound to be identical with that studied by Fischer and Renouf.[4]

Experimental

The coupling of the diazotized amines with 8-quinolinol was carried out as is customary for phenolic compounds, in alkaline solution. Ordinary tap water could not be substituted for distilled water in making up the alkaline 8-quinolinol solution due to precipitates formed, presumably calcium and magnesium salts.

The filtered and dried halogen containing dyes could be vacuum sublimated to obtain a small yield of quite pure dye but for reasonable purity and yields for most of the dyes, it was found best to extract the finely divided product from the first filtration with boiling water or 0.5% potassium hydroxide followed by hot filtration. The potassium salt came down in high yields of beautiful needles on cooling and was converted to the dye base (invariably some form of needles) by stirring in an excess of acetic acid added to a suspension in water of the washed salt.

After drying, the dye was crystallized from an organic solvent. The solvent best suited for recrystallization in most cases was benzene although in individual cases the best medium was found to be ethanol, pyridine, nitrobenzene, water, ethylene glycol, or dioxane. The hydrochloride was easily prepared by bubbling dry hydrogen chloride gas through a solution of the pure base in benzene.

Properties

The accompanying table summarizes the properties of these azo compounds. The ability of these high molecular weight dyes to form in most cases crystals larger than micro size is unusual for azo dyes. The percentage nitrogen was determined for those dyes not mentioned in the literature, by the Dumas method and indicated a close agreement with the calculated formula and hence that there was present a negligible amount of the diazo compounds.

The bacteriostatic power was measured in terms of the extent of water dilution of the hydrochloride that would just prevent growth of a culture of the bacterium listed. In several cases, due to the low solubility of the dye in the media, the saturated solution was so dilute that the lowest dilution supported some growth. In these cases the percentage growth, as compared with the blanks run at the same time, is stated in the table.

Some of these dyes have been recommended[5] as delicate analytical tests, sometimes quantitative or for metallic ions. On all new dyes (see tabulated dyes with no literature reference) nitrogen determinations were made by the Dumas method with good agreement with the formula listed. In most cases the yields were around 80% and up though the dyes derived from the following amines gave lower yields: *m*-chloroaniline, *p*-chloroaniline, arsanilic acid, 2-amino-1-naphtha-

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(4) O. Fischer and E. Renouf, *Ber.*, **17**, 1642 (1884).

(5) T. Boyd, E. Degering and R. N. Shreve, *Ind. Eng. Chem., Anal. Ed.*, **10**, 606 (1938).

lenesulfonic acid and 8-amino-1-naphthol-3,6-disulfonic acid.

Acknowledgment.—The authors wish to acknowledge their appreciation for bacteriostatic determinations by Dr. P. A. Tetrault of the Purdue Bacteriology Department and to the Mallinckrodt Chemical Works for its generous support.

Summary

8-Quinolinol was coupled with twenty-eight diazotized amines to give azo dyes. A detailed table of properties and yields of these 8-quinolinol dyes is deposited with the American Documentation Institute.

LAFAYETTE, INDIANA RECEIVED AUGUST 4, 1943

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Magnetic Measurements on Some Catalytically Active Substances

BY HUMBERT MORRIS AND P. W. SELWOOD

It is well known that copper hydrogenation catalysts are activated by small amounts of nickel, and that these catalysts undergo thermal deactivation, poisoning, and activation by minute amounts of substances, such as lead, which normally act as poisons.[1,2] The system copper–nickel is peculiarly convenient for magnetochemical investigation, because nickel is ferromagnetic and copper is diamagnetic. The principal purpose of this work was to examine the environment and behavior of the nickel in such catalysts, by the aid of magnetic susceptibility measurements. The use of magnetic methods to study catalytically active substances has been described by several workers.[3-9]

Experimental Methods

Catalytic Measurements.—The reaction studied with all catalysts was the hydrogenation of benzene. Chemically pure benzene, thiophene free, was dried with sodium and fractionated through an efficient column, the middle third being used in the hydrogenation reaction.

The hydrogenation train consisted of copper gauze at 550°, followed by anhydrous calcium chloride, ascarite and anhydrone. The mixture of hydrogen and benzene was obtained by bubbling hydrogen through benzene contained in a spiral wash-bottle in a constant temperature bath at 25°. The catalyst was packed into a glass tube in a furnace held at constant temperature. The liquid catalysate was collected in two receivers which were cooled in a bath of toluene and solid carbon dioxide. In all the hydrogenation tests the catalyst was at 175°, except where otherwise stated.

The composition of the catalysate, a mixture of benzene and cyclohexane, was determined from its refractive index, according to the data of Ipatieff, Corson and Kurbatov.[1]

Typical treatment of the catalytic data is shown in Table I. The contact time in seconds was calculated as the free space divided by the gas rate per second. The gas volume was taken as the sum of the benzene and cyclohexane volumes plus the arithmetical mean of the inlet and outlet volumes of hydrogen. The free space was taken as the difference between the volume of the reaction tube and the volume of the catalyst as calculated from its weight, and the density of the metal. In those cases where it was desirable to compare the activity of a catalyst at different times it was convenient to calculate the proportion of cyclohexane that would have been produced in a given contact time by assuming a direct proportion between the time and the amount of cyclohexane. This was valid over short ranges only, so care was taken to have results which are so compared be of approximately the same contact time.

TABLE I

TYPICAL CALCULATION

Hours on test		0.5
Excess pressure at inlet, mm.		12.0
Temperature of benzene, °C.		25.0
Vapor pressure of benzene at 25.0°, mm.		94
Barometer (corrected), mm.		749
Average pressure in system, mm.		755
Hydrogen–benzene volume ratio		7.0
Liquid catalysate	Grams	0.5857
	$n^{20}\mathrm{D}$	1.4300
Weight composition, 7% benzene and 93% cyclohexane		
Gas volumes at 175° and 755 mm.		
Benzene + cyclohexane, cc.		260
Inlet hydrogen, cc.		1830
Outlet hydrogen, cc.		1110
Average hydrogen, cc.		1469
Benzene + cyclohexane + average hydrogen, cc.		1729
Contact time, sec. (free space, 22 cc.)		23

Because the catalysts were very sensitive to impurities, all apparatus was scrupulously cleaned with hot concentrated nitric acid or a hot mixture of concentrated nitric and sulfuric acids, and then rinsed with distilled water.

Preparation of the several catalysts is more conveniently described *seriatim* below.

Magnetic Measurements.—The magnetic susceptibilities were measured by the Gouy method.[10] The sample tube was surrounded by a silica tube which could be heated electrically as desired. The details of this apparatus will be clear from Fig. 1. A few magnetic measurements were made on a more sensitive magnetic balance which has been described elsewhere.[11]

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(1) Ipatieff, Corson and Kurbatov, *J. Phys. Chem.*, **43**, 589 (1939).

(2) Corson and Ipatieff, *ibid.*, **45**, 431, 440 (1941).

(3) Welo and Baudisch, *Chem. Ztg.*, **49**, 661 (1925).

(4) Veiel, *Compt. rend.*, **180**, 932 (1925); **182**, 1028 (1926).

(5) Merck and Wedekind, *Z. anorg. allgem. Chem.*, **186**, 49 (1930); **192**, 113 (1930).

(6) Baudisch, *Ber.*, **68B**, 769 (1935).

(7) Hüttig, *et al.*, *Acta Physicochem. U. R. S. S.*, **2**, 1929 (1935), *et seq.*

(8) Bhatnagar, *et al.*, *J. Indian Chem. Soc.*, **17**, 125 (1940); **18**, 350, 371, 390 (1941).

(9) Woodman, Taylor and Turkevich, THIS JOURNAL, **62**, 1397 (1940).

(10) Selwood, *ibid.*, **61**, 3168 (1939).

(11) Preckel and Selwood, *ibid.*, **63**, 3397 (1941).