of such kinds of simple molecules as would permit the reliable prediction of reasonable configuration of the polypeptide chain. In this connection the result of the present experiment would be used to derive a conclusion that glycine, alanine and leucine residues take both E and B forms in a polypeptide chain, while proline residue takes only B-form.

HONGO, TOKYO, JAPAN

[CONTRIBUTION FROM THE NATIONAL BUREAU OF STANDARDS]

The Relation between the Absorption Spectra and the Chemical Constitution of Dyes. XXV. Phototropism and *cis-trans* Isomerism in Aromatic Azo Compounds[1]

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The photochemical *cis–trans* isomerization of a number of amino and hydroxy azo dyes in benzene solution was studied spectrophotometrically. The isomerization reaction was found to take place at such a rapid rate with most of these dyes that, in order to obtain reproducible spectral absorption curves, it was necessary to measure them with the aid of a rotating shutter which permitted the essentially simultaneous irradiation of the solutions during their measurement. The most probable spectral absorption curves of the *cis* forms of these dyes were calculated. The absorption curves of the *cis* forms of two typical dyes are shown and the spectral absorption data for the remaining dyes are tabulated. The spectra of *o*-hydroxyazobenzene and its derivatives were only slightly, or not at all, affected by irradiation, probably because of hydrogen bonding. Correlations between the absorption spectra, chemical structure, and spatial configuration of these dyes are discussed.

Introduction

The photochemical *cis–trans* isomerization of thioindigo dyes dissolved in organic solvents has recently been reported from this Laboratory,[2,3] and the results of that investigation indicated the desirability of re-examining the absorption spectra of organic compounds containing one or more double bonds for evidence of such *cis–trans* isomerizations. Azo dyes were chosen for this investigation because of their wide use in industry and because the phototropic behavior of some azo dyes had been observed previously.[4]

The unstable *cis* isomer of azobenzene was first isolated by Hartley by repeated fractional crystallizations of irradiated solutions of azobenzene.[5,6] The isolation of the *cis* isomers of azobenzene and of a number of its alkyl, nitro and halogen derivatives by the chromatographic separation of irradiated solutions of the appropriate azo compounds was carried out by Cook shortly afterwards.[7,8] Both investigators reported that solutions of 4-amino-, 4-acetylamino- and 4-hydroxyazobenzene (derivatives of which constitute the majority of azo dyes) exhibited considerable darkening in color upon irradiation, thus indicating *trans* to *cis* isomerization. These *cis* isomers appeared to be so short-lived, however, that they could not be estimated photometrically,[6] and, during the attempted chromatographic analyses, only an indefinite "tailing" and the appearance of subsidiary zones indicated their presence, but none could actually be isolated.[7,8]

Preliminary observations in this Laboratory indicated that irradiation of benzene solutions of 4-aminoazobenzene and of 4-dimethylaminoazobenzene by the same technique as had been used for thioindigo dyes[2] results in a change in their absorption spectra, but that there was a rapid reversal of the spectrum in the darkness[9] of the cell compartment during the measurement. In order to overcome this rapid reversal and thus to enable one to obtain reproducible results, a rotating shutter was constructed. The function of this shutter is to permit the irradiation of the solution for a fraction of a second (at the same moment when the measuring beam is cut off by the light chopper of the Cary Spectrophotometer) and, on the other hand, to shut off the irradiating beam when the measuring beam impinges upon the sample photocell.[10]

Experimental

(a) **Purification of Dyes.**—The dyes used for this investigation, which had been prepared by coupling diazotized aromatic amines with the appropriate amino or hydroxy compounds, were purified by repeated recrystallizations from aqueous alcohol. The dyes and their melting points are listed in Table I.

(b) **Preparation of Solutions.**—Approximately 0.010 g. of each dye was weighed and dissolved in 300–400 ml. of freshly distilled benzene and diluted to 500 ml. in a volumetric flask. Appropriate dilutions of these stock solutions were used whenever necessary.

(c) **Measurement of Absorption Spectra.**—The absorption spectra were determined by means of a Cary Recording Quartz Spectrophotometer (Model 12) with matched fused quartz absorption cells and the solvent as the reference standard.

(d) **Irradiation of Solutions.**—The dye solution contained in the absorption cell placed in the sample compartment was first exposed for about 10 minutes to uninterrupted filtered radiation from a 100-watt incandescent lamp, in order to accelerate the attainment of photochemical equilibrium. During this time the light was prevented from impinging upon the photocell. The rotating shutter was then placed in position[10] and intermittent irradiation continued until equilibrium was reached, that is, until no further changes in the absorption spectra occurred. Irradiation of the solution was maintained during the actual measurement of the absorption spectrum.

(1) Presented at the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., September, 1951.

(2) G. M. Wyman and W. R. Brode, THIS JOURNAL, **73**, 1487 (1951).

(3) W. R. Brode and G. M. Wyman, *J. Research Natl. Bur. Standards*, **47**, 170 (1951).

(4) E. I. Stearns, *J. Optical Soc. Am.*, **32**, 282 (1942).

(5) G. S. Hartley, *Nature*, **140**, 281 (1937).

(6) G. S. Hartley, *J. Chem. Soc.*, 633 (1938).

(7) A. H. Cook, *ibid.*, 876 (1938).

(8) A. H. Cook and D. G. Jones, *ibid.*, 1309 (1939).

(9) The intensity of the measuring beam is too low to cause any photochemical effects.

(10) For a detailed description of the rotating shutter, *cf.* J. H. Gould and W. R. Brode, *J. Optical Soc. Am.*, **42**, 380 (1952).

TABLE I

MELTING POINTS OF AZO DYES

Substituents	M.p., °C., cor. Obsd.	Lit.	Substituents	M.p., °C., cor. Obsd.	Lit.
(a) Substituted azobenzenes					
None	67.5–68.0	68[a]	4-Chloro-4'-hydroxy-	157.5–158.0	157[a]
4-Amino-	124 –125	126[a]	2,4-Dichloro-4'-hydroxy-	142 –143	....
4-Dimethylamino-	116 –117	116 –117[a]	2,2',4,6-Tetrachloro-4'-hydroxy-	142.0–142.5	....
4-Dimethylamino-4'-phenyl-	219.0–219.5	220.0–220.5[b]	2,6-Dimethyl-4-hydroxy-2',4',6'-trichloro-	143.0–143.5	....
4-Dimethylamino-4'-(*p*-aminophenyl)-	223.5–224.5	226 –227[c]	4-Dimethylamino-4'-hydroxy-	203 –204	203 –204[a]
4-Dimethylamino-4'-(*p*-acetylaminophenyl)-	280 –281	280 –281[c]	2-Hydroxy-	82.5– 83.0	82.5– 83.0[a]
4-Dimethylamino-4'-[β-(*p*-acetylaminophenyl)-ethyl]-	247 –248	248 –249[c]	2-Hydroxy-5-methyl-	108– 109	108[a]
4-Dimethylamino-4'-(*p*-aminophenyl)-methyl-	167.0–167.5	132 –139[c]	2-Hydroxy-2',5-dimethyl-	99.0– 99.5	98[a]
4-Hydroxy-4'-methyl-	152.5–153.0	151[a]	2-Hydroxy-2',5-dichloro-	133.5–134.0	....
4-Hydroxy-2,2',4',6'-tetramethyl-	96.5– 97.0	....			
4-Hydroxy-2,2',4',6,6'-pentamethyl-	136.0–136.5	....			
(b) Substituted benzeneazonaphthalenes					
4-Benzeneazo-1-naphthol	Tautomeric mixture		1-Benzeneazo-2-naphthol	133.0–133.5	134[a]
4-Benzeneazo-1-naphthylamine	125.0–125.5	123[a]	1-(*o*-Hydroxybenzeneazo)-2-naphthol	193.5–194.5	193[a]
1-Benzeneazo-2-naphthylamine	103 –104	103[a]	1-(*p*-Hydroxybenzeneazo)-2-naphthol	194.5–195.5	194[a]
1-*p*-Toluneazo-2-naphthylamine	114.0–114.5	113[a]	1-(*p*-Nitrobenzeneazo)-2-naphthol	256 –257	250 –252[a]

[a] "Beilstein," Vol. XVI. [b] J. D. Piper and W. R. Brode, THIS JOURNAL, **57**, 135 (1935). [c] W. R. Brode and J. D. Piper, *ibid.*, **63**, 1504 (1941).

Results

The changes induced in the absorption spectra of azo dyes in benzene solution by irradiation from a tungsten lamp were found to be reversible and dependent on the structure of the dyes concerned. In general, derivatives of 4-aminoazobenzene were most strongly phototropic. The spectral absorption curves of a typical example of such a dye (4-dimethylaminoazobenzene) in benzene solution are shown in Fig. 1. A number of other derivatives of 4-aminoazobenzene (Type I) have similar absorption curves, except that in the *p*-phenyl substituted compounds a new secondary absorption band appears in the ultraviolet region. The dyes of Type I are listed in Table II. The rapid rate at which these changes occur is demonstrated graphically in Fig. 2.[11]

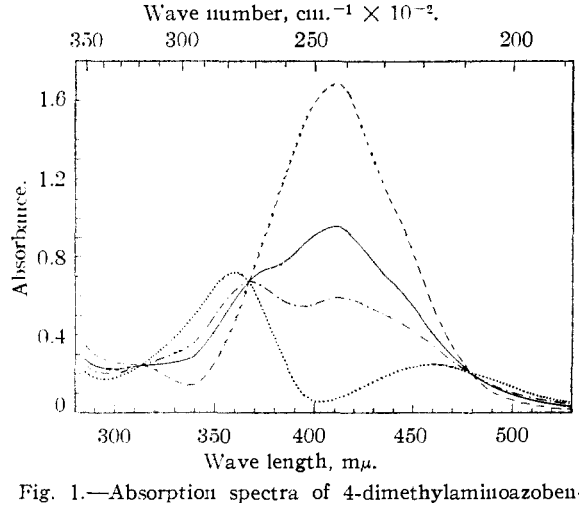

Fig. 1.—Absorption spectra of 4-dimethylaminoazobenzene in benzene exposed to: darkness, (- - -); green light ($\lambda > 350$ mμ), (——); blue light ($\lambda < 495$ mμ), (-·-·-·); calculated curve for *cis* isomer (. . .). Concentration: 0.0067 g./l.; cell length: 2.00 cm.

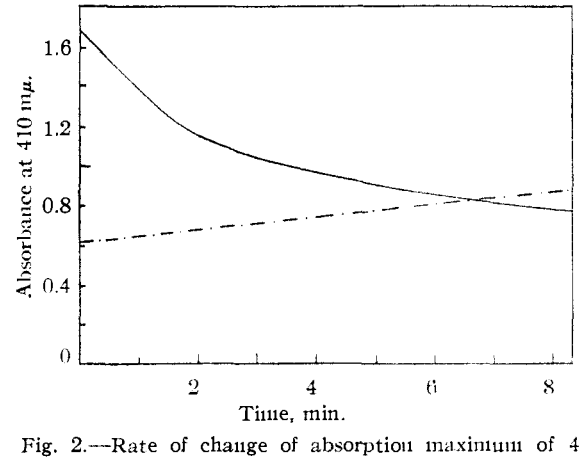

Fig. 2.—Rate of change of absorption maximum of 4-dimethylaminoazobenzene: ——, exposed to blue light; - - - -, on standing in darkness, after irradiation.

The results are consistent with the idea that solutions of these phototropic azo dyes contain the *cis* and *trans* forms in equilibrium with each other. Since it is probable that the *cis* isomer is present only in negligible amounts in a solution that has stood overnight in the dark, it is possible to

(11) When irradiating through the rotating shutter, light strikes the sample only about 12% of the total time of exposure (*cf.* reference 10).

TABLE II

SPECTRAL ABSORPTION DATA OF AMINOAZO DYES IN BENZENE (TYPE I)

Substituted azobenzene	*trans*-form λ_{max}[a]	ϵ_{max}[c]	λ_2[b]	ϵ_2[d]	*cis*-form λ_{max}	ϵ_{max}	λ_2	ϵ_2	Max., %[e] *cis*-form
4-Amino-	377	25,800			332	9,200	450	2,700	68
4-Dimethylamino-	410	28,300			362	12,000	460	4,300	68
4-Dimethylamino-4'-[β-(*p*-acetylaminophenyl)-ethyl]-	410	28,400			360	10,700	463	4,200	68
4-Dimethylamino-4'-(*p*-aminophenyl)-methyl-	410	30,600			360	10,900	462	4,200	78
4-Dimethylamino-4'-phenyl-	413	35,600	313	9,400	362	12,200	461	5,000	75
4-Dimethylamino-4'-*p*-aminophenyl-	426	35,900	323	6,800	345	14,200	472	4,900	73
4-Dimethylamino-4'-*p*-acetylaminophenyl-	425	35,600	320	10,600	360	11,900	480	4,800	71

[a] Wave length of most intense absorption band (in mμ). [b] Wave length of secondary absorption band (in mμ). [c] Molar absorption coefficient at λ_{max}. [d] Molar absorption coefficient at λ_2. [e] Per cent. *cis* isomer at conditions of optimum conversion.

TABLE III

SPECTRAL ABSORPTION DATA OF 4-HYDROXYAZO DYES IN BENZENE (TYPE II)[a]

Substituted 4-hydroxyazobenzene	*trans*-form λ_{max}	ϵ_{max}	λ_2	ϵ_2	*cis*-form λ_{max}	ϵ_{max}	λ_2	ϵ_2	Max., % *cis*-form
4'-Methyl-	347	25,600	440	900	304	7,700	446	1,700	58
2,2',4',6'-Tetramethyl-	348	20,800	470	1,200	305	8,900	450	1,600	50
2,2',4',6,6'-Pentamethyl-	340	18,100	470	1,200	301	7,600	467	1,900	42
4'-Chloro-	350	26,700	450	1,000	305	7,500	445	1,800	58
2',4'-Dichloro-	334	16,800	440	900	311	8,200	425	1,300	45
2,2',4',6'-Tetrachloro-	342	17,000	450	900	305	7,400	430	2,000	42
2,6-Dimethyl-2',4',6'-trichloro-	338	16,500	460	1,000	334	8,200	455	1,900	43
4'-Dimethylamino-	408	29,500			335	9,900	461	4,600	41
Azobenzene	321	19,000	440	300	325[b]	14,000[b]	440[b]	1,000[b]	

[a] For symbols used, see Table II. [b] Reported for solution in chloroform (*cf.* reference 15).

calculate the probable spectral absorption curves for the *cis* isomer. This may be done by assuming that, even in the absorption curve that corresponds to the solution richest in the *cis* isomer ("blue," Fig. 1), all but about 5% of the absorption in the wave length region of the main absorption band of the *trans* form (405–420 mμ, in Fig. 1) is due to the *trans* isomer. On the basis of this assumption it is possible to assign a probable absorbance value (A') at a specific wave length (λ) in this region to the *trans* form present in such a solution. It follows that

$$\frac{A'}{A} = \frac{C'}{C} = R$$

where

A = absorbance of the "dark" solution at wave length λ
C = total concentration
C' = concn. of *trans* isomer present in "blue" soln.

Since the total concentration is the same under all conditions of illumination, R is then the fraction of *trans* isomer present in solution after it had been irradiated with blue light.

The absorption due to the *trans* isomer in the "blue" absorption curve can be calculated by multiplying the absorption curve of the pure *trans* compound ("dark") by this fraction, R. The absorption curve of the *cis* isomer may be obtained by subtracting the contribution of the *trans* form from the "blue" absorption curve. In the solution richest in the *cis* form ("blue" curve in Fig. 1) the relative amount of the *trans* isomer will be 100 R per cent. and the *cis* form will account for 100(1 − R) per cent. of the total concentration. The calculated absorption curve for the *cis* form of 4-dimethylaminoazobenzene is shown in Fig. 1. The spectral absorption data of the two isomers of a number of aminoazo dyes in benzene (the absorption curves of which are similar in shape to the curves for 4-dimethylaminoazobenzene) are given in Table II.

Derivatives of *p*-hydroxyazobenzene possess absorption curves that are somewhat different from curves of the dyes of Type I, and they are also affected to a lesser degree by such irradiation. Absorption curves of a typical dye of this class (4-chloro-4'-hydroxyazobenzene) are shown in Fig. 3. In general, the principal absorption band is at a shorter wave length, and there is a weak, but distinct secondary band at the long wave length end of the spectrum of each dye of this type (Type II). It is again possible to calculate the probable spectral absorption curve for the *cis* isomer of each dye by the method described above, but because the absorption curve obtained with irradiation is not as drastically different from the normal "dark" curve, the error inherent in the assignment of R is greater. The calculated curve for the *cis* form of 4-chloro-4'-hydroxyazobenzene is shown in Fig. 3. The optical properties of a number of other azo dyes which have similar absorption curves in benzene solution are given in Table III.

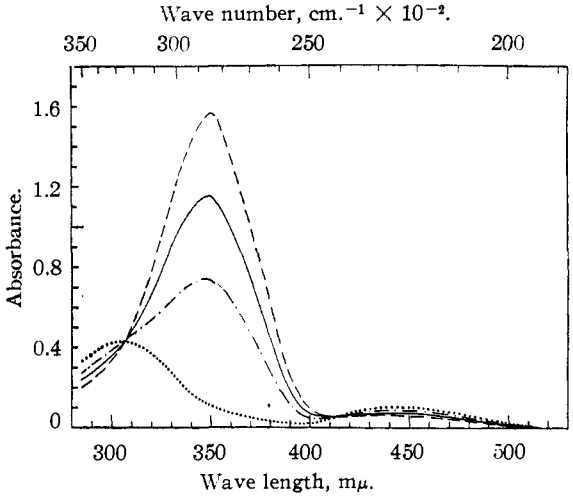

Fig. 3.—Absorption spectra of 4-chloro-4'-hydroxyazobenzene in benzene exposed to: darkness, (- - -); green light ($\lambda > 350$ mμ), (——); blue light ($\lambda < 495$ mμ), (-·-·-); calculated curve for *cis* isomer (. . . .); concentration: 0.0068 g./l.; cell length, 2.00 cm.

o-Hydroxyazobenzene and its derivatives (Type III) have a significantly different absorption in benzene, as shown in Fig. 4, and the absorption curves are unaffected by the irradiation used. It was observed, however, that these dyes also show phototropism when exposed to uninterrupted ultraviolet radiation (emitted by a G.E. H-4 lamp with the glass envelope removed), but reversal of the spectrum to that of the stable form is so rapid that it is im-

possible to obtain anything but a qualitative indication of a change.

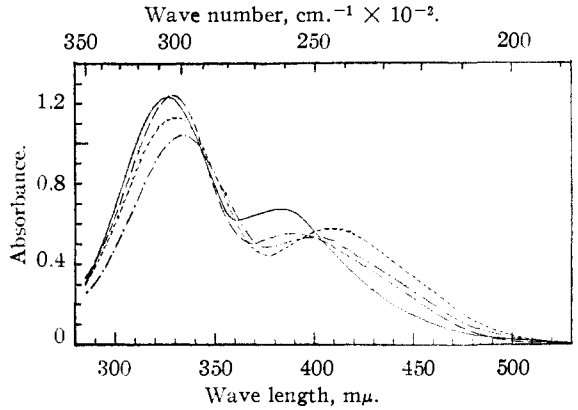

Fig. 4.—Absorption spectra of 2-hydroxyazobenzenes: ——, 2-hydroxyazobenzene (*c*, 0.0068 g./l.); — — —, 2-hydroxy-5-methylazobenzene; (*c*, 0.0067 g./l.); - - - -, 2-hydroxy-2′,5-dichloroazobenzene (*c*, 0.0068 g./l.); ·····, 2-hydroxy-2′,5-dichloroazobenzene (*c*, 0.0095 g./l.); cell length, 2.00 cm.

The absorption spectra of a number of weakly phototropic α-naphthyl azo dyes are shown in Fig. 5. The principal change in the absorption spectra of these four dyes when irradiated by visible radiation consists of a decrease in the intensities of the absorption bands in the visible region. Because of the relatively small changes induced by irradiation, it was impossible to calculate probable absorption curves for the *cis* isomers of these dyes. Although the spectra of these dyes are dissimilar, their similar structures and phototropic behavior permits them to be classified together in Type IV.

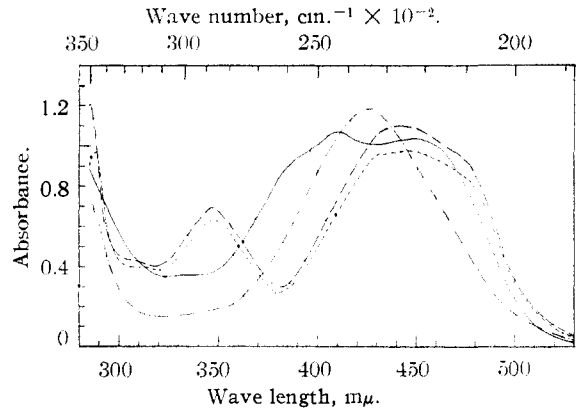

Fig. 5.—Absorption spectra of α-benzeneazonaphthalenes: ——, 4-benzeneazo-1-naphthol (*c*, 0.0100 g./l.); ·····, 4-benzeneazo-1-naphthylamine (*c*, 0.0067 g./l.); — — —, 1-benzeneazo-2-naphthylamine (*c*, 0.0101 g./l.); - - - - - -, 1-*p*-tolueneazo-2-naphthylamine (*c*, 0.0090 g./l.); cell length, 2.00 cm.

The absorption spectra of four azo dyes derived from β-naphthol (Type V) and thus containing at least one hydroxy group in *o*-position to the azo-group, are shown in Fig. 6. The spectra of these dyes appear to be unaffected by exposure to either ultraviolet or visible radiation.

Discussion of Results

The behavior of the 4-amino and 4-hydroxy azo compounds toward irradiation agrees with earlier observations on the effect of light on azobenzene derivatives; irradiation causes partial isomerization to the unstable *cis* isomer, subsequent standing in the dark results in reversal to the stable form. The high rate at which this reversal takes place is in marked contrast with the behavior of azo compounds which had been reported in the literature and also of thioindigo dyes; irradiated solutions of these latter two classes of dyes which contain mixtures of isomers have been observed to be stable enough to permit chromatographic separation of the two isomers.[2,8] The rate of isomerization is so fast for dyes of Type I that it is impossible to obtain reproducible measurements of the absorption spectrum, unless the dye solution has been permitted to reach equilibrium in the dark before measurement or unless it had been exposed to irradiation long enough to reach an equilibrium beforehand and the irradiation is also continued during the measurement.[12] For this reason the isosbestic points should be used in the spectrophotometric analysis of these dyestuffs.

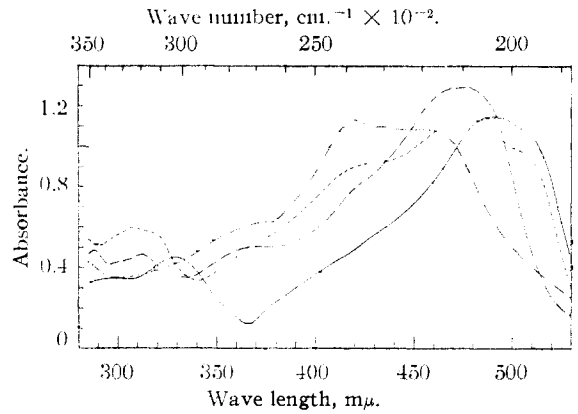

Fig. 6.—Absorption spectra of α-benzeneazo-β-naphthols: - - - - - -, 1-benzeneazo-2-naphthol (*c*, 0.0104 g./l.); — — —, 1-(*o*-hydroxybenzeneazo)-2-naphthol (*c*, 0.0098 g./l.); ·····; 1-(*p*-hydroxybenzeneazo)-2-naphthol; (*c*, 0.0101 g./l.); ——, 1-(*p*-nitrobenzeneazo)-2-naphthol (*c*, 0.0067 g./l.); cell length, 2.00 cm.

Although the dependence of the change of the absorption spectra on the wave length of the irradiation is not as clearcut in the case of these azo compounds as was observed for thioindigo dyes,[2] it still appears to be true that light absorbed in the wave length range of the main absorption band of the *trans* isomer is most effective in bringing about *trans* to *cis* isomerization. In accord with this generalization, it was observed that those dyes which have their main absorption band below 350 mμ show the greatest change when exposed to ultraviolet radiation.

The absorption spectra of the stable forms of azo dyes of this type had been measured previously,[13,14] and, as is also apparent from our data (*cf.* Tables II and III), in general, the introduction of an auxochrome group into the 4-position in the *trans*-azobenzene structure has the expected bathochromic effect; the position of the absorption band shifts toward longer wave lengths and the intensity of the absorption is considerably increased. The

(12) Azo dyes of Type II are less sensitive to irradiation, probably because their main absorption bands are at shorter wave lengths and incandescent lamps have relatively low intensity in that region.

(13) A. Pongratz, *et al.*, *Ber.*, **71A**, 1287 (1938).

(14) W. R. Brode and L. E. Herdle, *J. Org. Chem.*, **6**, 713 (1941).

order of increasing bathochromic effect appears to be: H < OH < NH_2 < $N(CH_3)_2$. From the data in Table II, it is apparent that a phenyl group has an additional bathochromic effect when in conjugation with the azo group,[15] but has practically no effect when separated from the azobenzene part of the molecule by one or more "insulating" methylene groups.[16]

The series of substituted 4-hydroxyazobenzenes contained in Table III had been originally synthesized for the purpose of evaluating the inhibition of resonance caused by the accumulation of substituents in the 2-positions. Introduction of two methyl groups or Cl-atoms in these positions has, indeed, a hypsochromic effect in the case of the *trans* forms, indicating that the accumulation of these relatively large substituents in the 2-positions has forced one of the benzene rings slightly out of the plane of the molecule and has thus inhibited the resonance through the molecule as a whole. It is of interest to examine our data on the *cis* isomers, in the light of these observed regularities in the absorption spectra of the *trans* forms of azo dyes of Type I and II. The calculated spectra of all of the *cis* forms of these dyes possess the same double-band structure, with the main absorption band in the near ultraviolet and a less intense band to the long wave length side of the principal band (*cf.* Figs. 1 and 3), similar to that of *cis*-azobenzene.[17,18] The bathochromic effect due to the amino groups in the *cis* forms of dyes of Type I is much less pronounced than that observed for the *trans* forms (*cf.* Table II). There is a slight shift of the band toward longer wave lengths, but the intensity of the absorption remains about the same as that for *cis*-azobenzene.[19] The reason for this probably lies in the inability of the amino group to enter into resonance with the entire molecule, because of the non-coplanarity of *cis*-azobenzene.[20] It is difficult to explain, however, the increased intensity of the secondary (long-wave) absorption bands of dyes of Type I.

The weak bathochromic effect of the 4-hydroxy group apparent in the *trans* isomers of dyes of Type II has changed to a weak hypsochromic effect in the *cis* forms. This is understandable in the case of the 2-substituted compounds where the substituents ortho to the azo group would force the molecule to deviate even more strongly from coplanarity than is the case for *cis*-azobenzene and thus result in a hypsochromic and hypochromic effect; however, this fails to account for shifts of this type observed in the absorption spectra of hydroxyazo compounds that do not contain substituents in the 2-positions.

(15) An auxiliary absorption band also appears in the ultraviolet region.

(16) W. R. Brode, "Chemical Spectroscopy," Second Edition, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 218.

(17) A. H. Cook, D. G. Jones and J. B. Polya, *J. Chem. Soc.*, 1315 (1939).

(18) It is this double band shape, reported for *cis*-azobenzene and shown by the calculated absorption curves of the *cis* forms of all dyes of Types I and II, that lends credence to the calculated curves of Type II.

(19) Since the intensity of absorption of these dyes has been found to be roughly the same in benzene as in chloroform, it is believed to be proper to use the value reported by Cook, *et al.*, for *cis*-azobenzene in chloroform for comparison (*cf.* reference 15).

(20) G. C. Hampson and J. M. Robertson, *J. Chem. Soc.*, 409 (1941).

The absorption spectra of 2-hydroxyazobenzene and its derivatives (Type III) differ from the spectra of the other azobenzene derivatives by a considerable increase in the relative intensity of the auxiliary band at the long wave length end of the spectrum. Since the spectra are the same in several solvents of different polarities, this could not be due to tautomerism between azoid and quinoid forms of these compounds.[21] It is probable that this feature in their absorption spectra stems from the two alternate paths of resonance that are possible in a molecule where the auxochromic hydroxy group is in the *o*-position with respect to the chromophoric azo group as suggested by Morris and Brode.[22] The introduction of methyl groups or chlorine atoms appears to have the weak bathochromic effect usually associated with substituents of this type.

The spectra of azo dyes of Type III are not affected by irradiation from an incandescent light source. It was found possible, however, to effect about a 10% decrease in the intensity of the strongest absorption band by 10 minutes of steady irradiation from an H-4 mercury vapor lamp. Reversal of this change was practically instantaneous indicating that, although there was some isomerization of these compounds, the difference in the stabilities of the two isomers was considerably greater than in the case of the 4-hydroxy azo compounds (Type II), probably because of the additional stabilization of the *trans* isomer by hydrogen bonding,[23]

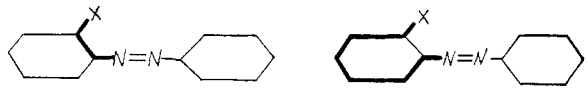

Fig. 7.—The alternate paths of resonance possible between the azo group and a substituent in the *o*-position

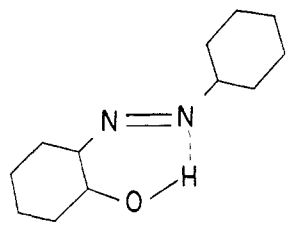

Fig. 8.—Hydrogen bonding in 2-hydroxyazobenzene.

as shown in Fig. 8. The spectra of the 1-naphthylazo dyes of Type IV have the main absorption bands in the blue end of the spectrum, at considerably longer wave lengths than those of the azobenzene derivatives, probably because of the added resonance contributed by the additional benzene ring. The compounds are weakly phototropic indicating that there is some *trans* to *cis* isomerization under the conditions used. One would expect less of a tendency for the formation of *cis* isomers in the naphthylazo dyes than in the phenylazo compounds, because the *trans* isomers of the former are stabilized by more resonance energy than are the *trans* isomers of the latter, and the loss of coplanarity inherent in conversion to a *cis*

(21) In this investigation only 4-benzeneazo-1-naphthol gave evidence for such tautomerism, in accordance with the observations of Kuhn and Baer, *Ann.*, **516**, 143 (1935).

(22) R. J. Morris and W. R. Brode, THIS JOURNAL, **70**, 2487 (1948)

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configuration would lead to a correspondingly larger decrease in the stability of the naphthylazo compounds. The two dyes which contain an amino group in ortho position to the azo group again show a double band spectrum, probably as a result of the two possible paths of resonance, as described above. These compounds also show some phototropism (although their rate of reversal is also high), indicating that the hydrogen bond which is possible between the amino group and the azo nitrogen in these compounds is not as strong as that in dyes of Type III and V. The normal bathochromic effect of a methyl group is not discernible from the spectra of these two compounds.

The spectra of the four azo dyes derived from β-naphthol (Type V) are not affected by irradiation of this type, probably because of the existence of a strong hydrogen bond in these molecules, similar to that shown in Fig. 8. The strong displacement of the main absorption band toward longer wave lengths and the absence of a second band in the spectra of these compounds is consistent with this structure, since the formation of an additional chelate ring would be expected to cause a large bathochromic shift[24] and to provide a single preferred path for the resonance in the molecule.

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Polarography of Glutathione

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The polarography of glutathione in the reduced (GSH) and oxidized form (GSSG) has been studied. Reduced glutathione gives two anodic waves at the dropping mercury electrode. The normal wave is well defined and corresponds to the formation of a mercurous compound (GSHg). The current–voltage curve in the *p*H region from 1 to 10.5 has been found to obey the equation for the reaction: $Hg + GSH \rightleftarrows GSHg + H^+ + e$. Normal diffusion currents have been observed with GSH over the entire *p*H range investigated. The diffusion coefficient of reduced glutathione is calculated to be 5.6×10^{-6} and 4.7×10^{-6} cm.^{2} sec.$^{-1}$ at *p*H 1 and 10.82, respectively, at an ionic strength 1 and at 25°. The characteristics of the second wave greatly depend upon the ionic strength of the medium. At an ionic strength of 1 it is fairly well defined and its height is of the same order of magnitude as that of the normal wave. It is suggested that the second wave corresponds to the formation of GSHg(II). Oxidized glutathione gives well defined reduction waves. Surface active substances like gelatin or thymol at low concentrations hardly affect the GSSG wave. Larger concentrations of thymol shift the waves to more negative potentials. This is accounted for by the electro-capillary behavior of thymol and GSSG. GSH is more capillary active at the dropping electrode than GSSG. The characteristics of the GSSG waves in the presence and absence of an excess of GSH are accounted for quantitatively by the sequence of reactions: (14) + (15) = (12) in which reaction (14) is the rate and potential determining step and equation (12) is the over-all reaction. The diffusion coefficient of oxidized glutathione at ionic strength 1 and *p*H 10.3 is calculated to be 4.5×10^{-6} cm.^{2} sec.$^{-1}$ at 25°.

A polarographic study of glutathione in the reduced (denoted as GSH) and oxidized form (denoted as GSSG) is described in this paper. The results are compared with those obtained with the amino acids cysteine and cystine.[1,2]

Catalytic polarographic waves obtained with glutathione have been described by Brdicka.[3] More recently Reiser,[4] Coulson, *et al.*,[5] and Tachi, *et al.*,[6] reported on diffusion-controlled current–voltage curves of glutathione.

Materials.—Glutathione in the reduced state was a Pfanstiehl product. The purity of this product was 99% as determined by titration with cupric copper.[7] Stock solutions 0.01 and 0.1 *M* in GSH were prepared in air-free water. Only freshly prepared stock solutions were used. A 10^{-2} *M* stock solution of oxidized glutathione (GSSG) was prepared by passing purified air through a 2×10^{-2} *M* GSH solution in an ammonia buffer (0.1 *M* in NH_4Cl, 0.1 *M* in NH_3) which contained a trace of copper (2×10^{-7} *M*) as a catalyst. The progress of the oxidation was followed polarographically. The air bubbling was continued until the GSH wave had disappeared. Toward the end of the reaction ammonia was driven out from the solution with air. The solution, which was stored in a refrigerator, was found to be stable for several months. Cysteine which was used in the form of its hydrochloride was a Pfanstiehl product. Cystine, C.P., was from Merck and Co., Inc. Stock solutions of cysteine and cystine were prepared in the same way as described in a previous paper.[8] All the other chemicals used were commercial C.P. reagent grade products.

Experimental Methods

Current–voltage curves were measured at 25.0 ± 0.1° with the manual apparatus and circuit described by Lingane and Kolthoff[9] and automatically with a Heyrovsky self-recording polarograph. All potentials were measured against the saturated calomel electrode (S.C.E.). Oxygen was removed from the solution in the cell with a stream of oxygen-free nitrogen which was purified by bubbling through vanadous sulfate.[10] During an experiment an atmosphere of nitrogen was maintained over the solution. Corrections were made for the residual current.

The characteristics of the capillary used were: $m = 1.56$ mg. sec.$^{-1}$, $t = 4.82$ sec. (open circuit); $m^{2/3}t^{1/6} = 1.748$ mg.$^{2/3}$sec.$^{-1/2}$; $h = 80$ cm.

The *p*H was measured with a Beckman *p*H meter, Laboratory Model G. A glass electrode made of the usual 015 type electrode glass was used for solutions with *p*H below

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