

The determining factor seems to be the boiling point of the reagent since the acetate was obtained in satisfactory yield by the use of acetic anhydride.

Seven and three-tenths grams of the alcohol was refluxed for one hour with excess acetic anhydride, the solution poured into water and, after the hydrolysis of the anhydride was complete, the product was separated by filtration and recrystallized from alcohol: yield 6.4 g., m. p. 114–115°.

Anal. Calcd. for $C_{23}H_{22}O_2$: C, 83.6; H, 6.67. Found: C, 83.3, 83.1, 83.7; H, 6.72, 6.70, 6.75.

Hydrolysis of the Acetate.—Two grams of the acetate was dissolved in 50 cc. of alcohol in which 3 g. of sodium had been dissolved and 10 cc. of water was added. After refluxing for two hours the solution was poured into water, neutralized with hydrochloric acid and filtered. After crystallization from commercial hexane the product was identified as the triphenylpropyl alcohol by a mixed melting point.

Preparation of Triphenylpropyl Acetate from the Iodide.—An attempt to prepare the acetate by refluxing a solution of the iodo compound in glacial acetic acid with silver acetate for ten and one-half hours proved unsuccessful, possibly because the initially formed silver iodide coated over the sparingly soluble silver acetate. The reac-

tion proceeded satisfactorily, however, when an excess of silver acetate was placed in the filter thimble and the acetic acid solution of 1.88 g. of triphenylpropyl iodide was placed in the flask of a Soxhlet apparatus to prevent precipitation of the silver iodide from occurring in the presence of solid silver acetate. After the Soxhlet apparatus had been operated for about twenty hours, the solution in the flask was cooled, filtered, evaporated to small volume, diluted with water, neutralized with sodium carbonate and filtered again. The black gummy solid was crystallized from alcohol by seeding with a sample of the previously prepared acetate. After recrystallization from alcohol it was found to be halogen free and identical (by mixed melting point) with the acetate obtained from γ,γ,γ -triphenylpropyl alcohol and acetic anhydride.

Summary

The product previously obtained by the action of hydriodic acid on γ,γ,γ -triphenylpropyl ethyl ether has been shown to be γ,γ,γ -triphenylpropyl iodide. Evidently no molecular rearrangement occurs during this reaction.

PROVIDENCE, R. I.

RECEIVED MAY 5, 1938

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

Semiquinone Radicals in the Indamine and Indophenol Groups

BY G. SCHWARZENBACH¹ AND L. MICHAELIS

The original task of this paper was to extend the search for semiquinone radicals as intermediate steps of reversible oxidation-reduction, to the indamine and indophenol groups. The result not only was successful but in addition presented an excellent opportunity for developing the general principles concerned with the degree of stability of such radicals.

In none of the many representatives of the indophenol dyes investigated by W. M. Clark² and associates, was there any evidence for the existence of an intermediate step of reduction. On the contrary, these authors took just these dyestuffs as models to prove their assumption that in reversible bivalent oxidations the two electrons always go on and off in pairs. The experimental results can be confirmed entirely for the dyestuffs selected by these authors and for the pH range covered by their experiments. Yet it would be unjustifiable to consider this state of affairs as general. We shall demonstrate for two dyestuffs rather closely related to the group investi-

gated by these authors, that semiquinone radicals are formed in fairly large concentrations as intermediate steps. These two dyestuffs are phenol blue and Bindschedler's green, the formulas of which are given by the T-forms in the table of formulas (Fig. 7). The experimental material will be presented first, and the cause for the different behavior will be treated in the discussion.

The formulas shown in the table are electronic formulas, each stroke representing an electron pair. The totally oxidized forms T, the semiquinone forms S, and the fully reduced forms R are given in the different possible states of ionization. T, R, and S, themselves, denote the forms existing only in very alkaline solutions, where all removable protons are removed. The order represented in this scheme corresponds to the order of the ionization steps as derived from the experiments.

An open structure has been chosen for the T-forms in which the central nitrogen appears surrounded by only six electrons. The fourth electron pair can be said to be delivered by the auxochromic groups $N(CH_3)_2$, OH, and O. These have

(1) Under a fellowship from the Rockefeller Foundation.

(2) Hygienic Laboratory Bulletin, No. 151, United States Public Health Service, Washington, D. C., 1928.

unshared electron pairs, which can form a double bond with the neighboring carbon atom, whereby the benzene ring, of which this carbon is a part, is converted into a quinoid ring. Both auxochromic groups take part in this electron delivery, the molecule is a resonance system. The valence chemistry and its connection with the absorption spectra and the acidimetric behavior of systems like these have been described by Schwarzenbach and co-workers.³ In the S-form the odd electron is represented as a dot attached to the central atom, but this should not be regarded as an expression of a definite position of the odd electron.

A. Experimental Part

1. Preparation of the Dyestuffs

Phenol blue was prepared by the method described by Heller,⁴ by oxidation of freshly prepared *p*-aminodimethylaniline and phenol with sodium hypochlorite in acetate buffer and at zero temperature. The dye was filtered off and crystallized three times from 95% ethanol. It then forms dark crystals, m. p. 167°.

Bindschedler's green was prepared by the method given by Wieland.⁵ *p*-Aminodimethylaniline and dimethylaniline are oxidized with bichromate in acid solution and in the presence of zinc chloride. The zinc double salt forms a beautifully crystallized green product which was not further purified. Recrystallization cannot be accomplished without some decomposition and the recrystallized product is rather less pure than the original material. In concentrated aqueous solutions this green salt is converted partially into red crystals, the longer known of the two modifications of Bindschedler's green, which behave in solution exactly like the green product. Both modifications have been described already by Wieland. Titrations have been carried out mainly with the green crystals, which show certain advantages owing to their greater solubility.

Phenolindophenol was prepared according to the method described by Clark² and recrystallized five times from a solution of sodium chloride. It was obtained in beautifully crystalline state of great purity and stability. All these specimens were kept constantly in the ice box to prevent decomposition.

2. The Titrations.—The titrations were mostly reductive. As reductant, reduced rosindulin GG, and in a few cases in the acid pH range, where rosindulin becomes insoluble, titanous chloride was used. These reductive titrations were controlled in some cases by a corresponding oxidative titration, the dyestuff being reduced previously by means of hydrogen on colloidal palladium, and bromine or ferricyanide was used as oxidant.

The technique of the titration has been described before. The only modification consisted in adding the dyestuff to the buffer solution just before the titration began. This makes the effect of a possible slow decomposition of these dyestuffs, which are not very stable, almost negligible.

Two microburets were introduced through the stopper of the titration vessel which contained 50 cc. of the buffer solution. One of the burets contained a strong solution of the dye (concentration about $4 \times 10^{-3} M$) in alcohol (in the case of phenol blue) or water (in the case of Bindschedler's green) and the other the reductant or oxidant in the same molar concentration. The air in the titration apparatus was then replaced by hydrogen. Colloidal palladium ensured the removal of the oxygen dissolved and produced a hydrogen potential, which made possible the measurement of the pH of the buffer. In the case of an oxidative titration 2 cc. of the dyestuff solution was now added and immediately reduced. After replacement of the hydrogen by nitrogen the titration was started. In the case of a reductive titration, the buffer was bubbled with nitrogen before adding the dyestuff and the titration begun immediately after its addition. After the titration was finished more colloidal palladium was added, hydrogen was passed through the vessel and the hydrogen potential measured. It was found that the change of pH value during the reduction was negligible in all titrations of Bindschedler's green, except for some few titrations with titanous chloride. In the experiments with phenol blue the final pH value was always somewhat lower than the value before the beginning of the titration. This difference of about 0.07 pH unit is due to the addition of alcohol to the buffer solution and not to a change caused by the oxidation-reduction process. In the following tables only the final pH value is registered.

In the case of phenol blue we could get excellent titration curves between pH 3.7 and 9.1. The potentials could be measured in this pH range with the same accuracy achieved in the case of lactoflavin,⁶ the limits of error being about ± 0.2 mv. In more acid solutions we had difficulties in the determination of the starting point of the reduction and end-point of the oxidation. The potentials at these points are very unsteady due to an irreversible decomposition of phenol blue. But even in solutions of a pH of 2 or 3 it could be shown definitely that the titration curve is much too steep for a two-electron process without intermediate. The semiquinone in these solutions could also be detected visually by its color, especially distinctly visible in a nearly completely reduced solution. At pH 10 the potentials were again unstable. This is due most probably to a hydrolytic elimination of the dimethylamino group, as can be inferred from the fact that the potentials dropped slowly to the potential range of phenolindophenol which is produced by this process.

Bindschedler's green is more stable in acid solutions and less stable in alkaline solutions in comparison with phenol blue. The potentials were perfectly stable between pH 3 and 7, the limits of error being not greater than in the case of phenol blue. At pH 7.5 and 8.0 fairly reliable titration curves were still obtained, but at pH 9 the dye stuff was hydrolyzed rather quickly to form phenol blue, a well-known reaction for Bindschedler's green. In the acid range fairly good curves were obtained down to a pH of 1.5. Here titanous chloride had to be applied as a reductant because rosindulin forms a precipitate. The strong acidity of the titanous chloride solutions impaired some-

(3) G. Schwarzenbach and co-workers, *Helv. Chim. Acta*, **20**, 490, 498, 627, 654, 1253, 1591 (1937).

(4) G. Heller, *Ann.*, **392**, 47 (1912).

(5) H. Wieland, *Ber.*, **48**, 1087 (1915).

(6) L. Michaelis and G. Schwarzenbach, *J. Biol. Chem.*, **123**, 527 (1938).

what the reliability of the measurements around pH 2 due to a change in pH during the reduction. Below pH 1 Bindschedler's green begins to become very unstable, the green solution being decolorized in a few hours. In phosphate buffer solutions which were used in the pH range around 7, zinc phosphate was precipitated, because the zinc double salt was used directly. In some cases this precipitate was filtered off, but it was found that it did not affect the potential measurements at all.

The final concentration of the dye in the titration experiments was usually between 2 and 4×10^{-4} mole per liter. The slight solubility of phenol blue did not allow of higher concentrations. Below a concentration of 1×10^{-4} the establishment of the potentials was no longer satisfactory.

In Table I the normal potentials E_m against the standard hydrogen electrode in volts and the index potentials E_i against the corresponding E_m value in millivolts are registered for the different buffer solutions. The values are mean values of several duplicate titration experiments at 30° .

TABLE I

Buffer	pH	Titration	E_m	E_i
Phenol blue				
Lactate	3.00	Oxidative (CrO_4^{2-} , Br_2)	+0.5007	(21)
Lactate	3.00	Reductive (rosindulin)	+ .5000	(22)
Lactate	3.72	Reductive (rosindulin)	.4538	19.5
Lactate	3.84	Reductive (rosindulin)	.4490	20.0
Acetate	4.12	Reductive (rosindulin)	.4262	19.7
Acetate	4.62	Reductive (rosindulin)	.3970	19.6
Acetate	5.04	Reductive (rosindulin)	.3623	18.2
Phosphate	5.96	Reductive (rosindulin)	.2923	15.6
Phosphate	6.86	Oxidative ($[Fe(CN)_6]^{3-}$)	.2300	15.0
Borate	9.13	Reductive (rosindulin)	.0945	14.6
Bindschedler's green				
HCl	1.10	Reductive ($TiCl_3$)	+0.6290	?
HCl	1.53	Reductive ($TiCl_3$)	.5927	15.3
Lactate	2.37	Reductive ($TiCl_3$)	.5325	(15)
Lactate	2.65	Reductive ($TiCl_3$)	.5185	(15)
Lactate	2.95	Reductive (rosindulin)	.4978	15.8
Acetate	3.97	Reductive (rosindulin)	.4152	15.4
Acetate	4.58	Reductive (rosindulin)	.3635	16.4
Acetate	5.50	Reductive (rosindulin)	.2945	17.1
Phosphate	6.78	Reductive (rosindulin)	.2294	17.2
Phosphate	7.18	Reductive (rosindulin)	.2157	16.4
Phosphate	7.47	Reductive (rosindulin)	.2071	16.1
Phosphate	8.03	Reductive (rosindulin)	.1853	16.2

The E_i values in brackets are not certain. Here the titration curves were not very reliable because the dye was not quite stable or the pH during the titration could not be kept quite constant.

The results indicate clearly that in both dyes the reduction leads through an intermediate product on an oxidation level of a quinhydrone, since the index potentials are distinctly greater than 14.3 mv. In the case of phenol blue below pH 5 the index potentials are so high as to indicate the formation of the quinhydrone in large quantities.

This quinhydrone may be a radical or its dimeric form, a hydrazine derivative. To decide

between these alternatives, we carried out some titration experiments with various concentrations of the dyestuff, keeping the pH value constant. Table II gives information about these.

TABLE II

Phenol blue pH 4.60		Bindschedler's green pH 4.58		Bindschedler's green pH 6.78	
C	E_i	C	E_i	C	E_i
0.9×10^{-4}	19.8	2.2×10^{-4}	16.4	2.8×10^{-4}	17.2
1.8×10^{-4}	19.3	4.0×10^{-4}	16.4	5.6×10^{-4}	17.2
3.5×10^{-4}	19.5	7.6×10^{-4}	16.4	9.0×10^{-4}	17.3

These figures show clearly that the quinhydrone is a radical and not a dimeric compound, since the index potential is independent of concentration. This result is not unexpected. The intermediate form of phenol blue has a definite blue color which would not be expected of a valence saturated hydrazine. Furthermore, Wieland states that the radical *bis*-(dimethylaminophenyl)-hydrazine at a concentration of $2 \times 10^{-2} M$ in benzene solution is 10% dissociated into radicals. We may expect that the dimerization constant in water is even smaller, but even if this be not the case, the dimeric form could not be formed in the low concentration range used in our titration experiments.

3. The Absorption Spectra.—Phenol blue is a base, which takes up a proton at pH 4.9 giving rise to a color change from pure blue to violet. There can be no doubt about the constitution of the molecules involved in this acidity equilibrium: namely, T and HT. The color of the ion and the neutral molecule indicate that both are resonance systems, the quinoid double bonds shifting from one nucleus to the other. This is only possible if we place the added proton in the indicated position. An addition to the electron pair of the dimethylamino nitrogen would destroy the resonance and give rise to the formation of a quinone of a very slight, probably yellow, color.⁷ Furthermore the order of magnitude of 10^{-6} must be expected for a dissociation constant of a phenolic hydroxide group which is acidified through a slightly asymmetrical resonance. The addition of the proton to the central nitrogen would give rise to a much smaller change in color as we will see in the case of Bindschedler's green. The absorption spectra of T and HT have been determined with König and Martens' spectrophotometer and are given in Fig. 1, together with the spectra of two forms of Bindschedler's green. It can be seen that the absorption maxima of phenol

(7) G. Schwarzenbach and co-workers, *Helv. Chim. Acta*, **20**, 490, 627 (1937).

blue in its T and HT state are separated far enough to make a spectrometric determination of the acidity constant possible. It was found by this method that in an acetate buffer solution of a pH of 4.55 the ratio of HT/T is 1.81 at 30° , a wave length of 6620 Å. being used for the measurements of the extinctions. This corresponds to a dissociation constant of $K_t = 10^{-4.81}$.

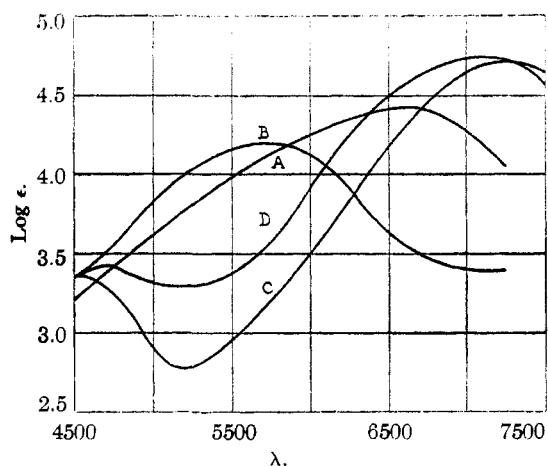


Fig. 1.—The logarithm of the molar extinction coefficient: Curve A, phenol blue pH 5.5 (T); Curve B, phenol blue pH 3.5 (HT); Curve C, Bindschedler's green pH 4.5 (T); Curve D, Bindschedler's green pH 2 (HT).

Bindschedler's green gives yellowish-green solutions which on acidifying turn to a more bluish-green. Both solutions have a strong absorption band at the infrared border of the visible part of the spectrum. The maximum of the absorption lies so far in the red that its determination with the visual method (König and Martens' apparatus) is scarcely possible. Photographs on infrared sensitized plates revealed, however, the exact position of the bands. Their maximum is in the infrared quite close to the visible red. The color change on acidifying takes place between pH 3 and 4. From Fig. 1 it can be seen that the change in the absorption spectrum is not very pronounced, making a colorimetric determination of the acidity constant difficult. But this acidity constant shows up clearly if we plot the E_m potentials against pH . From this curve it follows that this dissociation constant has a value of about $10^{-3.4}$. The place of addition of the proton can only be the central nitrogen atom of the molecule so that the molecules T and HT, with the formulas in the table of formulas, are involved in this acidity equilibrium. An addition to the free electron pair of the di-

methylamino nitrogen would give rise to the formation of a strongly asymmetrical structure, which would have a yellow color. Such a color change takes place only at much lower pH values, namely, in about 40% sulfuric acid.

Both forms of Bindschedler's green and of phenol blue have two more absorption bands in the region of shorter wave lengths. One lies in all cases in the neighborhood of 2900 Å. The other lies in the case of the acid form of phenol blue also in the ultraviolet at about 3550 Å., whereas in the case of Bindschedler's green and the neutral form of phenol blue this second band which is rather weak lies in the violet and blue region of the visible spectrum (Fig. 1). The whole absorption spectrum has therefore in all cases three bands showing a pronounced similarity to the spectra of the sulfonphthaleins.⁸ This is not surprising because the indophenols and indamines represent a resonance system very similar to that of the dyes of the Döbner's violet and benzaurin type (2). On reduction the bands in the visible part of the spectrum and in the nearer ultraviolet disappear, whereas the band at 2900 Å. is strongly increased in intensity.

It has been mentioned that the presence of the semiquinone of phenol blue can be demonstrated by its definite color. If small amounts of bromine are added to a solution of reduced phenol blue in an acid solution ($HCl = 0.005 N$), the color of the solution turns blue, not violet, which in acid solution is the color of the fully oxidized form. Also an absorption appears in the far red which disappears again on further oxidation and is replaced by the band of the fully oxidized substance HT. The absorption in the far red has to be attributed to the radical. Curve A in Fig. 2 represents the extinction of a solution in which 16% of the phenol blue has been reoxidized (concentration of the dyestuff = 10^{-5}). From the semiquinone formation constant, derived from the titration experiments, the concentration of the fully oxidized form HT has been calculated and the absorption due to its presence introduced as curve B in Fig. 2. Curve C is the difference between the curves A and B and represents the absorption of the semiquinone. In the other regions of the spectrum, including the ultraviolet, the presence of the semiquinone does not show up by any specific absorption.

(8) Mohler, Forster and Schwarzenbach, *Helv. Chim. Acta*, **20**, 654 (1937).

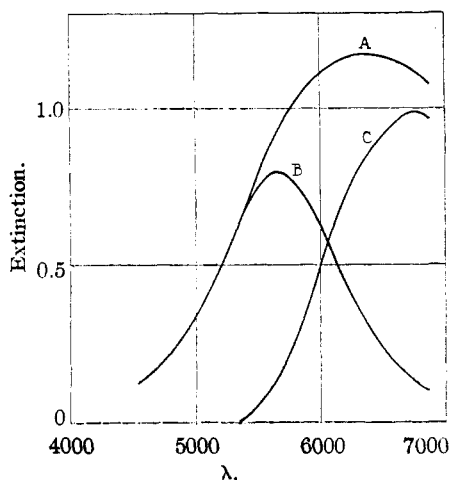
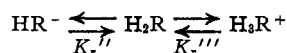


Fig. 2.—A, the extinction of a solution of 10^{-5} molar leuco-phenol blue in 5×10^{-3} molar hydrochloric acid of which 16% has been oxidized; B, the extinction due to the form HT; C, the extinction due to the form H_2S (the free radical).

It is somewhat surprising that the oxydimethylaminodiphenyl nitrogen, the radical derived from phenol blue, has a completely different color from *bis*-(dimethylaminophenyl)-nitrogen derived from Bindschedler's green, which has been prepared by Wieland⁵ and which is yellow. Since the absorption bands of Bindschedler's green are shifted toward the red in comparison with phenol blue, we think it likely that this will be the case also in the two corresponding radicals and we predict for the yellow radical of Wieland another absorption band in the infrared region.

4. **The Acid-Base Constants of Reduced Phenol Blue.**—The curve of E_m as a function of pH which is shown in Figs. 4 and 5, shows different points where it changes its direction owing to the dissociation of the reduced and oxidized forms. To confirm the exact positions of these bends, the direct determination of the acidity constants of the reduced forms also was desirable. In the case of Bindschedler's green this was not possible because the reduced form is very sparingly soluble, but it could be accomplished easily in the case of leuco-phenol blue, which is a phenol and an aniline at the same time



K_r''' was derived from an acid titration curve of the leuco-base which is shown in Fig. 3 and K_r'' was computed from the shift of the potential of a

hydrogen electrode in a sodium hydroxide solution by addition of phenol blue. The results are

$$K_r''' = 10^{-5.99} \quad K_r'' = 10^{-10.00}$$

Both figures hold for 30° and an ionic strength of about 0.1.

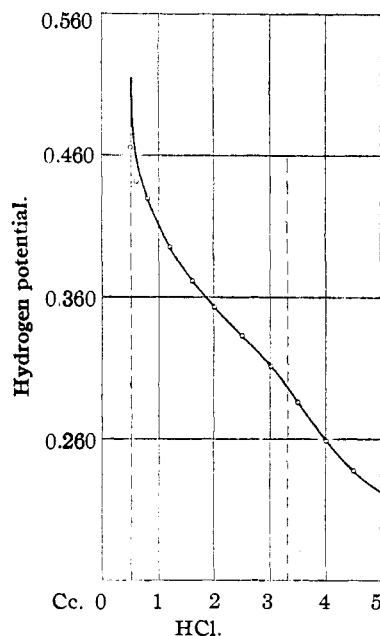


Fig. 3.—Titration of leuco-phenol blue with acid at the hydrogen electrode.

B. The Interpretation of the Potential Measurements

1. **The Mean Potentials E_m as a Function of pH and the Dissociation Constants of T and R.**—The curve drawn through the experimental points in Fig. 4 for phenol blue shows a bend at pH 4.8 and 6.0 owing to the acid dissociations of T and R described above. At pH 10 there must be another bend indicating K_r'' and then the curve will have, in the more alkaline range, a slope of 30 mv. per pH unit and can be extrapolated safely. In the acid range, where experimental points no longer can be obtained, the extrapolation is not so safe. We will probably soon reach another constant of the T form, a proton being attached to the central nitrogen.

The curve of Bindschedler's green represented in Fig. 5 has a slope of 30 mv. per pH unit in the alkaline solution. The reduced form contains, therefore, one proton more than the oxidized form in this pH range and must be represented by formula HR. The curve will extend with this slope far into the alkaline range, where the proton

attached to the central nitrogen atom finally will be lost, the potential becoming independent of pH. At pH 6.4 and 5.1 the reduced form takes

formed here with salt formation of the weakly basic diphenylamino nitrogen. This constant lies so far in the acid range that a confirmation by means of an acidimetric titration is impossible.

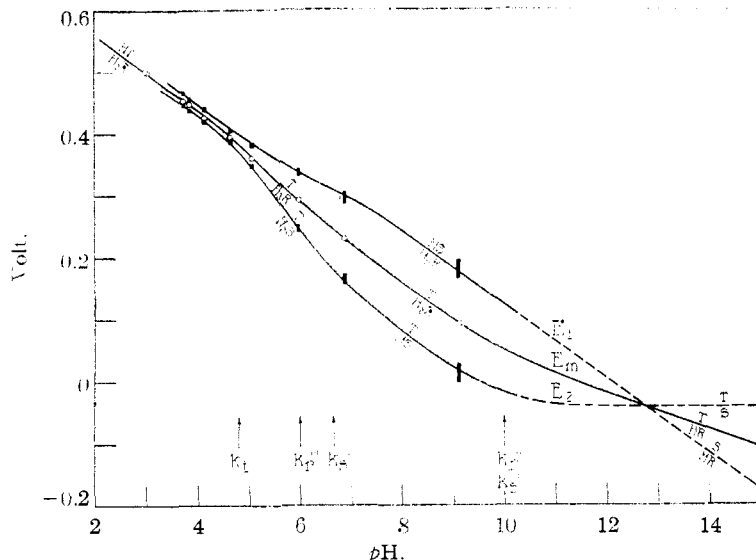


Fig. 4.—Phenol blue: the normal potentials E_m for the system T, R; E_2 for the system T, S; and E_1 for the system S, R. $K_1, K_2,$ and K_3 denote the acidity constants of the T, S, and R forms.

up two more protons, obviously forming the ions H_2R and H_3R . The constants $K_r'' = (H^+) \times (HR)/(H_2R)^+ = 10^{-6.4}$ and $K_r''' = (H^+) \times (H^+) \times (H_2R^+)/ (H_3R^{++}) = 10^{-5.1}$ are very reasonable for aromatic dimethylamino groups and also the ratio of K_r''/K_r''' , corresponding to 1.3 pH units is compatible with the expected value as influence of the ionic charge of the first proton on the acidity constant of the second in this distance. In the case of leuco-phenol-indophenol about the same ratio between K_r'' and K_r''' has been observed, the constants being $10^{-9.4}$ and $10^{-10.6}$. Unfortunately the solubility of the base HR is so slight that no good acidimetric titration curves could be obtained in a titration experiment with the hydrogen electrode to confirm the constants above.

At pH 3.4 the E_m curve bends from the 90 mv. slope back to the 60 mv. slope owing to the dissociation constant of the T-form described above.

At pH 1.5 there is once more a bend indicating a third constant of the reduced form. This is somewhat surprising. Only the ion H_4R can be

2. The Index Potentials, E_i , the Normal Potentials of the Lower and the Higher Step of Oxidation, E_1 and E_2 , and the Semiquinone Formation Constant, k .—From the index potentials we can calculate⁹ the semiquinone formation constant k and the maximum ratio of semiquinone to the total dye $(s/a)_{max}$. Table III contains these figures in solutions of different pH, as computed from the curves E_1 and E_2 which will be discussed later. Obviously both dyestuffs form free radicals to a large extent. One of these radicals, the one derived from Bind-

schedler's green, is identical with the radical is very sensitive toward water. We can

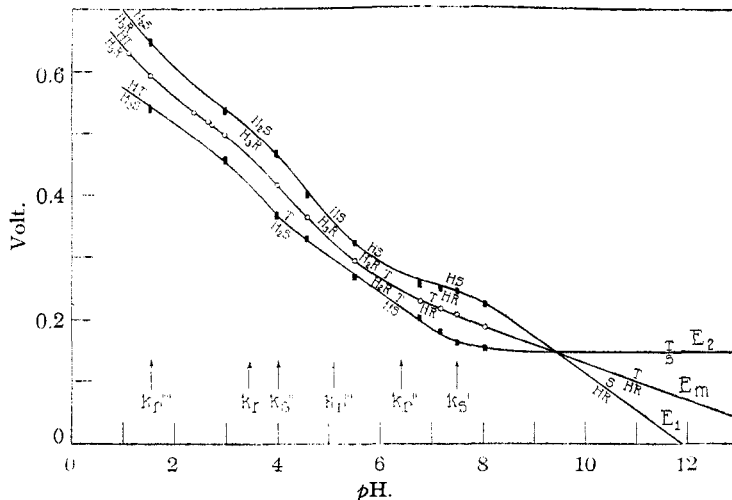


Fig. 5.—Bindschedler's green: The normal potentials E_m for the systems T, R; E_2 for the system T, S, and E_1 for the system S, R. $K_1, K_2,$ and K_3 denote the acidity constants of T, S, and R.

confirm this result but Wieland's viewpoint should be corrected somewhat. The radical does not disappear entirely in the presence of water. There is an equilibrium established according to $2S \rightleftharpoons$

(9) The methods of calculation as developed previously will be summarized in *Chem. Rev.*, in press (1938).

TABLE III

$k = s^2/rt$, where s , r and t denote the activities of the S, R and T-forms. $(s/a)_{\max.}$ = maximum ratio of $s : a$, that is to say the ratio $s : a$ in the half-reduced state.

pH		1	2	3	4	5	6	7	8	9	10	11	12
Bindschelder's green	k	0.0087	0.030	0.040	0.023	0.100	0.146	0.059	0.059	0.40	4.00	36	1550
	$100 \times (s/a)_{\max.}$	4.5	8.0	9.1	7.0	13.7	16.0	10.8	10.8	24	50	75	95
Phenol blue	k			0.48	0.48	0.25	0.027	0.0041	0.0022	0.0022	0.0047	0.022	0.18
	$100 \times (s/a)_{\max.}$			26	26	20	7.6	3.1	2.2	2.2	3.3	6.9	17.5

R + T, the constant of this equilibrium depending on pH. At pH 6 as much as 16% of the whole dyestuff is still present in the form of the radical.

It is worth while mentioning that this is the first case in which a radical of the semiquinone type is identical with a radical previously prepared by the classical method, by spontaneous dissociation of its dimeric form in an organic solvent.

If we compare the figures of Table III with the behavior of phenolindophenol we find at first sight a pronounced difference. As can be inferred from the data obtained by W. M. Clark,² the latter compound does not form a radical between pH 5 and 13. We also have found in these pH ranges an index potential not differing from the theoretical minimum value 14.3. But there is an indication that in very strongly alkaline solutions the formation of an intermediate does occur. Satisfactory titration curves could not be obtained owing to an instability of the system, but by reducing a very pure sample of phenolindophenol in strong alkali (> 2 *m* sodium hydroxide) with glucose, a pink color can be observed easily just before the solution decolorizes on forming the fully reduced compound. On re-oxidation with air, the pink color appears again before the solution becomes blue. We do not believe that this phenomenon is due to a decomposition product, but to the formation of a radical as intermediate product. It is analogous in appearance and behavior to the intermediate form of the indigosulfonate as described by Shaffer.¹⁰

We also have an indication that a semiquinone is formed in more acid solutions. Below pH 4 the dye is very unstable, the potentials always drift toward the positive side, probably because quinone-imide is formed with a normal potential above phenolindophenol. This decomposition has the effect of compressing the titration curve (potential plotted against degree of reduction), causing the index potentials to appear lower than they would be without secondary decomposition oc-

curing. So at pH 3.7 a curve was obtained with an index potential of only 11.0, but at pH 4.6 an index potential of 14.7 and at pH 4.02 even one of 15.2 was observed. The corrected values, therefore, would be distinctly greater than 14.3 although accurate figures cannot be given. This is a clear indication that a semiquinone is formed in these pH ranges.

We shall discuss the semiquinone formation in terms of the E_1 and E_2 values which, in addition to E_m , are plotted in Figs. 4 and 5. These potentials are the normal potentials of the systems T, S and S, R and defined by the equations

$$E = E_2 + \frac{RT}{F} \ln (t/s) = E_1 + \frac{RT}{F} \ln (s/r)$$

where E is the potential observed.

It is comparatively easy to give the correct E_1 and E_2 curves although the index potentials may not be absolutely certain, for these curves can only have a slope of 0.06 or 0.12 v. per pH unit. This restriction makes it possible to compute a mean value of the measured index potentials at different pH. The slope of the curve informs us about the number of protons by which the semiquinone differs from the t or r forms. We are therefore definitely sure about the state of ionization of the half reduced form. In the pH range investigated, the semiquinone formation constant, k , is in the case of both dyestuffs, and of course also in the case of phenolindophenol, so small, that $E_2 - E_1$ becomes negative, the E_1 curve being above the E_2 curve. Here, a wide separation of E_2 and E_1 is equivalent to a small k .

Figure 4 shows that between pH 7 and 10 the semiquinone of phenol blue has the form HS and that below pH 6.6 the form H₂S exists. These two forms will have the structures given in the table of formulas because it is reasonable to assume that the dissociation constant for an acidic group in the semiquinone lies between the corresponding constants for the T and R forms.

The last value experimentally obtained for phenol blue on the alkaline side is that for pH 9. Nevertheless we know, as has been pointed out, the course of the E_m curve beyond 10, because the

(10) P. A. Shaffer, *J. Biol. Chem., Proc.* **105**, 1xxviii (1934); cf. also L. Michaelis, *Chem. Rev.*, **16**, 243 (1935), on p. 282.

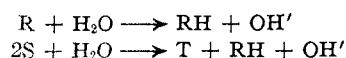
dissociation constant $H_2R \rightarrow HR$ has been measured. At pH 10, where the parallel course of these three curves is broken by the constant just mentioned, the semiquinone has still the central proton to lose. The semiquinone formation constant in very alkaline solutions depends on the magnitude of this unknown constant $HS \rightarrow S$. It is certain that this constant must be much greater than the corresponding one for the reduced form $RH \rightarrow R$, and reasonable to assume that it has to lie in the neighborhood of 10^{-10} . As we will see, we have in the case of Bindschedler's green a clear indication that this constant $HS \rightarrow S$ lies below pH 8. If we take account of all these arguments, we come to the extensions for the curves in the high pH ranges as indicated in Fig. 4, and reach the conclusion that in very strong alkaline solutions we must expect a very large semiquinone formation. The same holds true for the very acid range. At pH 3, where the last experimental points could be obtained, we have as semiquinone the form H_2S and a new constant will soon be due, addition of the proton taking place at the dimethylamino nitrogen. Such a constant makes the E_1 and E_2 curves again bend toward the E_m curve, producing an increase in the semiquinone formation.

The case of Bindschedler's green is very similar, but the conclusions reached for the behavior in very alkaline solutions are more certain because the constant of the semiquinone $RS \rightarrow S$ is indicated clearly at pH 7.5. It is clearly seen that the semiquinone formation in very alkaline solutions must be very large as indicated also in Table III.

The observations on all three dyestuffs, Bindschedler's green, phenol blue and phenolindophenol, indicate the following general behavior: strong semiquinone formation in alkaline and acid range and comparatively small semiquinone formation in the middle pH range. In the case of phenol blue the acid range of large semiquinone formation is reached exceptionally early and already begins at pH 4.

The above statement for the very alkaline range is not astonishing. We know that the formation of R and T forms in non-aqueous solutions, from a free radical, is generally negligible and that we find only the equilibrium between the dimeric compound and the radical. Such solutions are similar to a solution of very high pH , because the R form has not the possibility of taking up a proton. The sensitivity of these equilibria to-

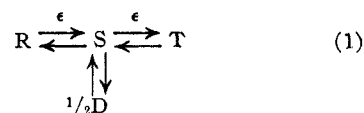
ward water now becomes quite clear. The addition of water, a proton donator, has an effect equivalent to that of a strong lowering in pH by converting the R form into HR



This is the main reason for the dismutation which has been found in many cases by addition of water to the radicals and has been especially described for the case of the radical derived from Bindschedler's green by Wieland. If we dissolve small quantities of the semiquinone or its dimeric product in water, Bindschedler's green is produced immediately and by the action of the OH ions, formed by the reaction just mentioned, hydrolyzed to phenol blue.

C. Discussion

The general treatment of oxidations and reductions of organic compounds has to take account of the following equilibria



The reaction $D \rightleftharpoons 2S$ has been the classical one to prepare radicals and has been investigated always in non-aqueous solutions. On the other hand, oxidations and reductions $R \rightleftharpoons T$ have been investigated extensively in aqueous solution by means of the potentiometric method. The general belief was that the bivalent reversible oxidation-reduction in organic compounds would occur in a single bivalent step. There was no reason to link the reversible oxidation with the problem of free organic radicals, until almost simultaneously Friedheim and Michaelis¹¹ and Elema¹² found that in some cases during such reductions and oxidations free radicals are formed even in aqueous solutions. Later on, Michaelis and Fetcher¹³ also found the dimeric form of the radical to exist in the equilibrium. Thus the complete picture of the scheme (1) was revealed.

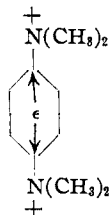
The semiquinones, however, still seemed to have little connection with the substances commonly known as free radicals. As pointed out by Michaelis, the semiquinones were in all cases compounds which contained two equal atoms (other

(11) E. A. H. Friedheim and L. Michaelis, *J. Biol. Chem.*, **61**, 355 (1931).

(12) B. Elema, *Rec. trav. chim.*, **50**, 907 (1931).

(13) L. Michaelis and E. S. Fetcher, *THIS JOURNAL*, **58**, 2460 (1937).

than carbon), symmetrically located in the molecule, as in the case of Wurster's blue



These two atoms could be regarded as carriers of the odd electron, ϵ , which was assumed to resonate symmetrically between the two positions. The older free radicals, on the other hand, contained one atom which might be considered as the carrier of the odd electron, so that it was reasonable to speak of trivalent carbon, or divalent nitrogen or univalent oxygen. These latter radicals were found to be very sensitive toward water.

The present paper has filled the gap between the two groups of radicals: radicals of the second type, with so called divalent nitrogen, have been found to exist in aqueous solution in equilibrium with the oxidized and reduced forms. These findings are quite in agreement with the theory of stability of the free radicals. As Hückel¹⁴ and Pauling¹⁵ have shown, all free radicals owe their existence to resonance energy. The odd electron and the π electrons of the aromatic nuclei cannot be placed in a definite position, rather their charge is on a time average distributed over a wide range of the molecule.¹⁶

The equilibria constants of the above scheme are of course dependent on pH . The equilibrium $R \rightleftharpoons T$ is expressed in the potential E_m and the equilibria $R \rightleftharpoons S$ and $S \rightleftharpoons T$ in the potentials E_1 and E_2 . To understand their magnitude, we must know something about the relative stability of the molecules involved in the equilibrium, this is to say, of all the molecules whose formulas are given in the table of formulas (Fig. 7) for the T-, R- and S-forms.

This stability cannot yet be calculated by quantum mechanical methods. Even in simpler cases, where the molecules contain no other atoms than carbon and hydrogen, different approximation methods (Hückel and Pauling) yield widely differing results. We will try here to express the stability of a molecule in terms of the more qualita-

tive valence chemistry, starting from the concept of an ideal state of a molecule exhibiting maximum stability. The instability of a molecule is then measured in terms of the tendency of its atoms to reach this ideal state.

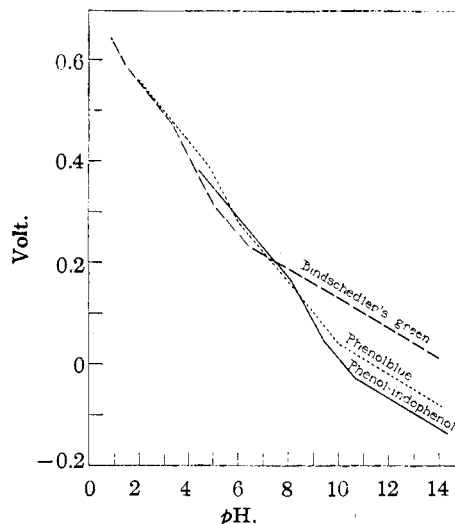
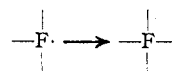


Fig. 6.—The mean normal potentials of Bindschedler's green, phenol blue and phenolindophenol.

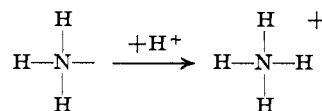
An atom in the ideal state would be surrounded by four electron pairs which are all equal and indistinguishable. For example, the C-atom in methane or the helium atom can be regarded as being in this ideal state. Any reactivity can be attributed to one of the two following reasons:

1. A deviation from the stable electron number 8, giving rise to a tendency for oxidation or reduction as for instance in the fluorine atom



This kind of instability will be called oxidation-reduction instability.

2. A deviation from the ideal state of equality of the four electron pairs, giving rise to acid-base reactions as in ammonia



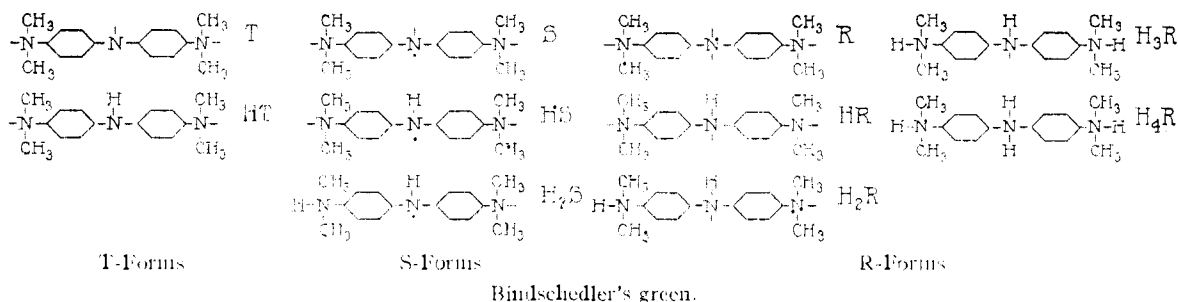
We will call this instability an acid-base instability.

Both tendencies must be regarded as equally important, because the free energies of acid-base reactions and oxidation-reduction reactions are of the same order of magnitude.

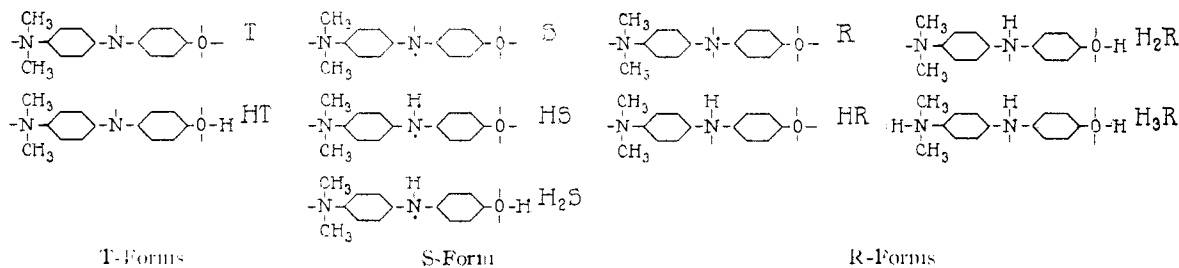
(14) E. Hückel, *Z. Physik*, **83**, 632 (1933); *Trans. Faraday Soc.*, **30**, 1, 41 (1934).

(15) L. Pauling and G. W. Wheland, *J. Chem. Phys.*, **1**, 362 (1933).

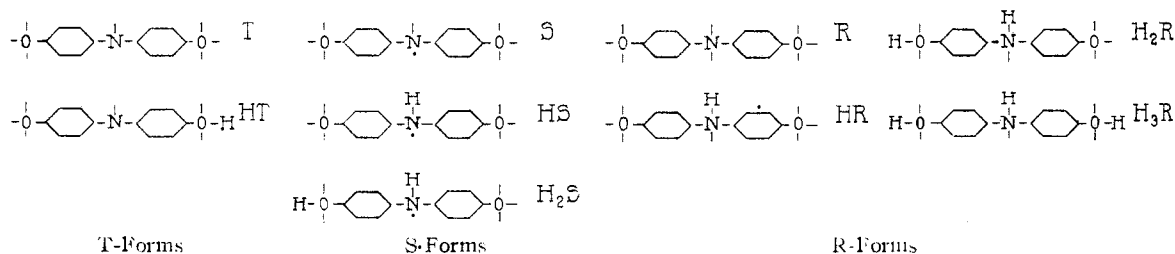
(16) C. K. Ingold, *Trans. Faraday Soc.*, **30**, 52 (1934).



Bimischdler's green.



Phenol blue.



Phenolindophenol.

Fig. 7.

If we regard the stability of all the ions and molecules involved in the equilibrium $\text{R} \rightleftharpoons \text{S} \rightleftharpoons \text{T}$ in all possible pH ranges, we arrive at the following conclusions.

1. **The Stability of the R-Forms.**—The R-forms of our dyestuffs show primarily an acid-base instability, all atoms being surrounded by an electron octet. (In the formulas in the table of formulas (Fig. 7) 4 strokes belong to every atom.) The instability is manifested in the basic character of these molecules, the central N-atom and the auxochromic groups having unshared electron pairs. It is easily understood that the instability will be great in strongly alkaline solution, where even the central N atom has lost a proton and is strongly basic. In extremely acid solutions a maximum stability is reached. This means that all equilibria will be displaced in favor of the R form on decreasing the pH .

2. **The Stability of the T-Forms.**—The fact that the R-forms can be oxidized to the S- and T-

forms even in acid solutions, is due to the fact that the oxidized forms exhibit a new type of resonance, which is missing in the R forms. The nature of this resonance is different for the S and for the T forms. In the T forms we have resonance between the two moieties of the molecule, one of quinoid structure, the other of benzenoid structure. Their acid-base instability is much decreased in comparison with the R forms, but instead an oxidation-reduction instability arises. In these substances the fourth electron pair of the central nitrogen is delivered by the auxochromic groups. The stability will therefore depend on the tendency of these groups to share their electron pairs with the neighboring carbon atom, in other words, to form a double bond with this carbon atom. This tendency is strongly related to the basicity of the auxochromic groups. The stability will be great if the auxochromic groups are strongly basic. We have, therefore, the maximum stability in solutions of a high pH . As we go

to more acid solutions we block the unshared electron pairs of the auxochromic groups ($T \rightarrow HT$) and the central nitrogen is more and more deprived of its electrons, giving rise to an increased tendency to take up an electron pair from outside. It has to be assumed that all symmetrical forms, in which the two auxochromic groups are equal, have a greater stability than the corresponding unsymmetrical forms. This is due to the larger resonance energy of the symmetrical forms.

The stabilities of the R and T-forms change, therefore, in just the opposite sense as we change the pH . In acid solutions R is especially stable and in alkaline solutions T is especially stable. This corresponds to the general fact that the normal potentials all become more negative as we raise the pH . A more positive potential means that T is more easily reduced to R and a more negative potential means that R is more easily oxidized to T.

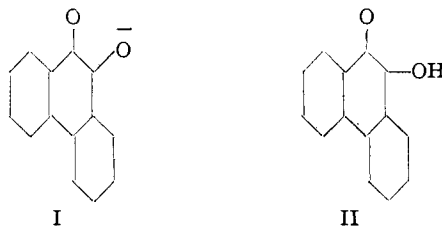
In this respect a comparison of the E_m values of phenolindophenol, phenol blue and Bindschedler's green, as demonstrated in Fig. 6, is of interest. Above pH 10, the three curves are parallel, the value for Bindschedler's green being the most positive and the one for phenolindophenol the most negative. The same must hold true for still more alkaline solutions, where the potentials become independent of pH . The relative values of the three normal potentials can be understood easily, because the instability (basicity) of the three R forms will increase in the following sequence

Bindschedler's green < Phenol blue < Phenolindophenol
 whereas the instability of the T forms will increase in just the opposite order, the more basic ionic oxygen group forming a more stable resonance system than the less basic amino group. This brings about the sequence in the normal potentials of these three dyestuffs.

3. **The Stability of the Radical S.**—It is much more difficult to predict the stability of a radical even qualitatively. Quite generally it must be assumed that a valence-unsaturated compound is less stable than R or T. The S form arises from the reaction $R + T \rightleftharpoons 2S$ only if R and T are relatively unstable; this means, in general, in very acid and in very alkaline solution. We have shown experimentally that this really is the case in our examples. However, although it is difficult to make any statement about the stability of a radical in a general way, there may ex-

ist one quite obvious electronic structure in a radical which greatly enhances its existence. We may say that the semiquinones, which are radicals stable to a well measurable extent even in an aqueous solution, show this type of electronic arrangement. This is a resonance phenomenon, which differs essentially from the resonance of the T form mentioned above. It is not concerned necessarily with a resonance between two rings, but may be confined entirely within one single ring which contains two atoms of the same kind, either two N atoms, or two O atoms in symmetrical positions—that is to say, either in ortho or in para positions; either attached to, or within this ring. They are within the ring in phenazine, attached to the ring in Wurster's dyes. Instead of one ring, it may be the double ring of the biphenyl type, as in benzidine and γ, γ' -dipyridyl. The experience with the Wurster's dyes shows that the atom groups attached to each of the two N atoms need not necessarily be alike. They are alike in Wurster's blue, which has a very great stability. They are not alike in Wurster's red, which still has a fair stability. Also the semiquinone of phenol blue may be considered as comparable to Wurster's red, symmetrical with respect to the two N atoms at one ring, but not symmetrical with respect to the groups attached to the two N atoms. Phenolindophenol does not show this type of symmetry at all, but Bindschedler's green does.

This type of resonance furthermore is greatly influenced by pH , as was formerly explained on several occasions, *e. g.*, for the semiquinone of phenanthrenequinone,¹⁸ and for the purple radical prepared by partial reduction of benzil.¹⁷



So, in alkaline solution (I), there is resonance with respect to the negative charge which may be at the one or the other O atom. But in acid solution (II), the attachment of the proton fixes the electron in one of the two possible positions thus decreasing the resonance and so the stability.

(17) L. Michaelis and E. S. Fetcher, *THIS JOURNAL*, **59**, 1246 (1937).

These considerations convey, at least qualitatively, an understanding about the stability of the radicals as depending on the constitution of the particular dye and on pH . We may emphasize once more, at this occasion, that practically none of the organic radicals that had been known prior to the discovery of the semiquinones, exhibit that particular type of strongly symmetrical resonance structure which has just been described as characteristic of the semiquinones. Correspondingly, those older radicals are usually not stable enough to be capable of any appreciable existence in the presence of water, in contrast to the semiquinone radicals.

Summary

Potentiometric oxidative and reductive titrations of Bindschedler's green and phenol blue at various pH 's have been carried out. The slope of the titration curves indicates that an intermediate semiquinone radical is formed. The ioni-

zation constants for the reduced, half-oxidized, and oxidized forms of these dyes as well as the semiquinone formation constant have been determined. The latter depends on pH .

The ionization constants have been checked by spectrophotometric measurements. The absorption bands of all the three forms of each of these two dyestuffs are shown.

The discussion correlates the stability of the three forms, and especially that of the free radical, with general ideas about molecular stability derived from the electronic theory of the chemical bond and the principle of resonance. The type of resonance stabilizing the free radical is very different from the type of resonance stabilizing the oxidized form of the dye itself. The relative stability of the three forms of a dyestuff (oxidized, half-reduced radical, and reduced) is accounted for, qualitatively at least. The results are summarized in Figs. 4, 5, 6.

NEW YORK, N. Y.

RECEIVED APRIL 22, 1938

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH AND THE PHYSICS DEPARTMENT OF COLUMBIA UNIVERSITY]

Potentiometric and Magnetometric Study of the Duroquinone System

BY L. MICHAELIS, M. P. SCHUBERT, R. K. REBER,¹ J. A. KUCK¹ AND S. GRANICK

Among the numerous reversible bivalent two-step oxidation-reduction systems studied so far only a few cases of simple quinones have been subjected to an experimental study. They are concerned with orthoquinones. This paper adds an example of a paraquinone. A suitable representative avoiding the difficulties due to the instability of most quinones in alkaline solution, is duroquinone (tetramethylbenzoquinone). Although there is a limit to the stability of duroquinone also, no observable irreversible alteration by alkali occurs at all, during the period of time necessary for the experiments. At the temperature, 30°, even at the highest pH that reasonably may be used for experiments of this type. Because of its slight solubility, the experiments were performed with buffer solution containing 20 volume per cent. of pyridine, which, though reacting chemically with other quinones, serves only as a solvent for duroquinone. The process of reduction was studied both by the potentiometric and the magnetometric methods. Both

methods agree in the final result. In sufficiently alkaline solution a free semiquinone radical of brown color is formed as intermediate step of the reversible reduction. This radical is strongly paramagnetic. In contrast to the semiquinone of phenanthrenequinonesulfonate, which is a representative of the orthoquinones, the semiquinone of duroquinone has no measurable tendency to form a valence-saturated, dimeric form in the dissolved state.

1. Potentiometric Experiments

Reductive titration with leuco-rosinduline G G was used. In sufficiently alkaline solutions, $pH > 9$, the potentials are instantaneously and reproducibly established at concentrations down to 6×10^{-5} molarity. At lower pH values, the establishment of the potentials in low concentrations, about $10^{-4} m$, is relatively sluggish, though eventually leading to definite and reproducible values. For this reason titration curves for duroquinone at $pH < 9$ are somewhat less accurate than those at $pH > 9$. This uncertainty has no

(1) Members of the staff of the College of the City of New York.