at 22–27°, weight 6.5 g. This was shaken with mercury until colorless, and then redistilled, taking the fraction b. p. 45.0–45.6° (9 mm.), 3.8 g., $\alpha^{23}D$ −19.42°, $n^{25}D$ 1.4878. The *s*-hexyl chloroacetate, crude yield 9.9 g., boiled at 80.0–80.3° (9 mm.), $\alpha^{23}D$ +8.06°, $n^{25}D$ 1.4235, d^{25}_4 1.0212. The lower boiling products were collected in appropriately cooled traps and identified as methyl iodide, b. p. 42–43°, and methyl chloroacetate, b. p. 129–130°, contaminated with a little olefin. The yields of *s*-hexyl iodide and chloroacetate, 28.8 and 52.4%, indicated that the cleavage proceeded approximately one-third to the hexyl iodide, and two-thirds to the hexyl chloroacetate. The activity of the hexyl iodide, had optically pure carbinol been used to prepare the ether, would have been $\alpha^{23}D$ −26.6°, $(M)^{23}D$ −39.6°. This value is about 50% of that reported by Pickard and Kenyon,[9] using the optically pure carbinol and hydrogen iodide. The lower activity found here may be due to racemization by the iodide ion, and/or to decomposition of complex II by S_Ni.

Summary

1. Cleavage of *d*-methyl *s*-hexyl ether with chloroacetyl iodide yields *l*-*s*-hexyl iodide.

2. The mechanism of ether cleavage has thus been shown to proceed mainly through an ionized oxonium salt.

(9) Pickard and Kenyon, *J. Chem. Soc.*, **99**, 45 (1911).

MONTREAL, CANADA RECEIVED MARCH 27, 1940

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

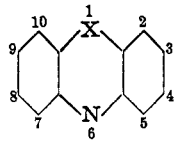
Semiquinones of Oxazines, Thiazines and Selenazines

BY S. GRANICK, L. MICHAELIS AND MAXWELL P. SCHUBERT

1. After it had been shown[1] that thionine and methylene blue yield free semiquinone radicals on partial reduction, a more systematic study of the dyestuffs of this class was suggestive. It will be shown in this paper that its various representatives vary greatly with respect to the magnitude of the formation constant and the optical properties of the semiquinone. The investigations were extended over suitable representatives not only of thiazines, but also oxazines and selenazines. The better known dyestuffs with two auxochromic amino groups such as methylene blue were compared with those containing only one, either an amino or a hydroxy group. The experiments will show that all of these compounds easily form semiquinones under similar conditions as does thionine, namely, in sufficiently acid solution. In part the separation of the two steps of oxidation is very distinct even in weakly acid solutions, especially for 3-hydroxythiazine and 3-hydroxyoxazine. In these two compounds, on increasing the acidity, it is easy to reach the value 10^{+6} for the semiquinone formation constant, by far the largest constant of this type encountered as yet. All these semiquinones show a characteristic absorption spectrum in the visible range of wave lengths. There are two types of absorption spectra: either there is one rather intense and sharp band; or there is such a complicated series of bands as recently described for thionine and methylene blue. It is not yet possible to correlate the chemical structure with the type of absorption spectrum. In every case the spectrum of the semiquinone is quite different from that of the quinonoid form of the dye. The high stability of those radicals, often largely exceeding that of the thionine radical under comparable conditions, was rather unexpected from the theoretical point of view as tentatively adopted in the previous paper, and will be commented on in the discussion.

(1) L. Michaelis, M. P. Schubert and S. Granick, THIS JOURNAL, **62**, 204 (1940).

2. **Material.**—The nomenclature of these dyestuffs is rather cumbersome. Bernthsen's nomenclature as applied in his classical papers is not systematic enough to cover derivatives in addition to those investigated by him. The nomenclature of the leuco compounds is always quite easy, so we recommend using always the name of the leuco dye. The prefix "pheno" in phenothiazine, phenoxazine or phenoselenazine may be omitted. Whenever it is necessary to distinguish the leuco compounds from their oxidation products, we add the prefix r-, s- or t-, to distinguish the reduced form, the semi-oxidized or semiquinone form, and the totally oxidized or quinonoid form. For instance, thionine then is t-3,9-diaminothiazine, and when no misunderstanding arises the t- is omitted. The numbering of the skeleton as shown is the customary one. "X" stands for O in oxazine, for S in thiazine, and for Se in selenazine



The substances were all prepared according to methods already described in the literature and only a few notes need be added where modifications were made.

Phenoxazine.[2]—Best results were obtained when the *o*-aminophenol and its hydrochloride were both recrystallized until almost white before proceeding with the condensation. Eighteen grams of *o*-aminophenol and 22 g. of its hydrochloride are heated under carbon dioxide at 240° for thirty minutes. The reaction mixture is extracted with 1200 cc. of hot benzene and the residue after evaporating the benzene is dissolved in 250 cc. of hot alcohol. To the hot alcoholic solution are added 100 cc. of water and 100 cc. of concentrated hydrochloric acid and the solution is filtered hot from a tarry deposit.[3] Addition of 2 liters of water to the filtrate deposits the crude phenoxazine. This is dried, distilled at 215° and 4 mm. and the product recrystallized from 100 cc. of hot alcohol by addition of 200 cc. of water. Thirteen grams is obtained with a m. p. of 151–152°.

Phenothiazine was prepared by the method of Sakom.[4] After distillation at 190° and 3 mm. it is recrystallized from benzene with petroleum ether.

Phenoselenazine[5] was distilled at 200° and 1–2 mm. and recrystallized from alcohol and then from benzene. The selenium monochloride required was prepared by the method of Lenher and Kao.[6]

t-3-Hydroxythiazine was prepared by the method of Pummerer and Gassner[7] though the crude product reported by these authors to have a melting point of 162–163° was further purified. About 4 g. of the crude product was first extracted with 100 cc. of hot alcohol and the crystalline product obtained by chilling this extract was further recrystallized from 1500 cc. of boiling water. The crystalline product, 0.7 g., had a melting point of 161°.

t-3-Hydroxyoxazine was prepared by a similar method from phenoxazine. After two recrystallizations from alcohol, using about 50 cc. per g., a crystalline product is obtained which decomposes at 214°.

t-3-Aminophenothiazine[8] and **t-3-aminophenoselenazine**[4] were used as their simple hydrochlorides.

t-3-Amino-9-hydroxythiazine can be obtained easily although in small yield by saturating with hydrogen sulfide a solution of 20 g. of *p*-aminophenol in 1 liter of water containing 20 cc. of concentrated hydrochloric acid. A solution of 100 g. of $FeCl_3 \cdot 6H_2O$ in 150 cc. of water is added, the mixture is aerated and another equal amount of ferric chloride is added. The crude precipitate is extracted with 1 liter of boiling water containing 50 cc. of concentrated hydrochloric acid and the crystalline product resulting on cooling is recrystallized from 300 cc. of water containing 15 cc. of concentrated hydrochloric acid. The crystalline product is the hydrochloride of Bernthsen's thionolin[9] and contains one mole of water.

t-3,9-Diaminophenoxazine hydrochloride is obtained in brilliant crystals by the method described by Kehrmann and Saages[10] and t-3,9-diaminophenoselenazine hydrochloride as described by Cornelius.[5]

The selenium analog of methylene blue can be made simply as described by Karrer[11] although the product obtained by crystallization from weak hydrobromic acid and dried in vacuum at room temperature contains an extra half mole of hydrogen bromide. Calcd. for $C_{16}H_{18}N_3SeBr \cdot H_2O \cdot {}^1/_2HBr$: N, 8.94; Br, 25.52. Found: N, 9.04; Br, 25.85.

t-3,9-Dihydroxyphenothiazine could not be prepared in any pure form. The method of De Eds and Eddy[12] gave a product of little promise. The original method of Bernthsen gives poorly crystalline products and uncertain reproducibility. In one trial only a well-crystallized product was obtained which only roughly approximated in composition the hydrochloride. Calcd. for $C_{12}H_8O_2NSCl$: N, 5.28; S, 12.05; Cl, 13.35. Found: N, 5.57; S, 10.88; Cl, 11.68. Since none of our preparations showed steady potentials in potentiometric titrations, this substance was not included in the following studies. It is mentioned only with reference to the paper by De Eds and Eddy.

t-3,9-bis-Phenylaminothiazine Bromide.—Five grams of the perbromide of thiazine prepared as described by Kehrmann[13] is suspended in 15 cc. of methanol, 10 cc. of aniline is added and the mixture thoroughly ground up. The product crystallizes out directly. Twenty-five cc. of ether is added, the product is filtered off and washed with ether. It is recrystallized once or twice from 800 cc. of hot alcohol from which it separates as large shining crystals on chilling; yield 1.0 g.; no m. p. up to 298°. Calcd. for $C_{24}H_{18}N_3SBr$: N, 9.13; S, 6.95; Br, 17.36. Found: N, 9.05; S, 6.26; Br, 17.88. Of this compound only the absorption spectrum of the semiquinone is shown in Fig. 8.

3. Methods of Potentiometric Titration.—Titrations in strongly acid solutions were performed with liquid junctions such as described before, in the form of agar bridges previously adapted to the acid solution so as to establish non-drifting liquid junction potentials. Titrations were made in part reductively, in part oxidatively. For reductive titration, titanous chloride dissolved in the corresponding acid was used. Or, chromous sulfate was used as follows. A suitable amount of chromium metal powder was put in deaerated sulfuric acid of the desired concentration. From one to eleven *N* sulfuric acid may be used; in higher concentrations the metal dissolves as a chromic instead of a chromous salt. The dissolving process is speeded up by heating in a stream of nitrogen. The solution of chromous sulfate in sulfuric acid is directly transferred by nitrogen-pressure to the buret. Whenever the potential range was suitable this reductant was very satisfactory. However, contrary to expectation, the potential range in sulfuric acid of higher concentration is less negative than that of titanous sulfate. Oxidative titrations also were performed.

(2) F. Kehrmann and A. A. Neil, *Ber.*, **47**, 3107 (1914).
(3) F. Kehrmann, *Ann.*, **322**, 9 (1902).
(4) D. Sakom, *J. prakt. Chem.*, **89**, 11 (1914).
(5) W. Cornelius, *ibid.*, **88**, 395 (1913).
(6) V. Lenher and C. H. Kao, THIS JOURNAL, **47**, 772 (1925).
(7) R. Pummerer and S. Gassner, *Ber.*, **46**, 2324 (1913).
(8) A. Bernthsen, *Ann.*, **230**, 100 (1885).
(9) A. Bernthsen, *ibid.*, **230**, 201 (1885).
(10) F. Kehrmann and A. Saages, *Ber.*, **36**, 475 (1903).
(11) P. Karrer, *ibid.*, **51**, 190 (1918).
(12) F. De Eds and C. W. Eddy, THIS JOURNAL, **60**, 1446 (1938).
(13) F. Kehrmann, *Ber.*, **49**, 53 (1916).

TABLE I

3-AMINOTHIAZINE

Titrations in 1 × 10^{-4} M solution; 50 cc. Increase in volume during titration, 2 cc. approx.

Solvent	Manner of titration	pH measured at the H_2 electrode after titration	E_m Potential at 50% oxidation, referred to the standard H_2 electrode	E_i Index potential Left	E_i Index potential Right	Comments
Phosphate + NaOH	Reduced with Pd-H_2 Titrated with $K_3Fe(CN)_6$	11.25	+.013	15.5	15.7	
Veronal buffer	Same	8.66	+.152	?	16.0	In the first half of the titration potentials have little poise. E_m is probably reliable
Phosphate	Same	7.16	+.205	?	?	Poise not sufficient for a reliable E_i. E_m is approximately correct
Phosphate	Reductively titrated with Leuco-Rosinduline GG	6.73	+.219	15.3	15.4	Unobjectionable in every respect
Phosphate	Reduced with Pd-H_2, titrated with $K_3Fe(CN)_6$ dissolved in same buffer	6.15	+.236	16.0	15.5	
Acetate	Reductively titrated with Leuco-Rosinduline GG	4.62	+.293	15.4	15.6	
Citrate	Reduced with Pd-H_2, titrated with $K_2Cr_2O_7$, dissolved in pure H_2O	1.91	+.452	15.4	15.3	
Citrate	Same	1.02	+.506	15.9	15.8	
0.990 N HCl	Same	0.07	+.558	18.5	16.4	Slight drifts, rendering E_i unreliable
1.11 N H_2SO_4	Reduced with Pd-H_2, titrated with ferric ammonium sulfate dissolved in same acid	0.29	+.548	15.6	15.3	Establishment of potential takes about one minute, but final values appear to be reliable
3.58 N H_2SO_4	Reductively titrated with chromous sulfate in same acid	(−0.22?)	(+.573)	16.3	16.6	
6.64 N H_2SO_4	Reductively titrated with titanous sulfate		(+.583)	20.0	19.2	
8.88 N H_2SO_4	Same		(+.581)	30.5	30.2	
11.1 N H_2SO_4	Same		(+.569)	54.5	54.0	Similar experiment with chromous sulfate gave less sharp end-point due to overlapping, but otherwise the same
15.3 N H_2SO_4	Same			94 approx.		End-point overlapping with Ti, yet recognizable

They often permitted covering even a larger potential range. They were carried out by reducing the dye solution with a small drop of 1% colloidal palladium and hydrogen gas, thoroughly displacing the hydrogen by nitrogen, and titrating with a deaerated solution of potassium bichromate dissolved in the same acid.

Titrations in less acid solutions, within the pH range of the customary buffers, were performed either reductively with leuco-rosinduline GG, or, oxidatively, after reducing with palladium and hydrogen, with potassium ferricyanide, and sometimes with ferric ammonium sulfate.

4. Results of Potentiometric Titrations

(A) Thiazine Derivatives. (1) 3-Amino-thiazine.—The results are shown in Table I and Fig. 1.

(2) 3-Hydroxythiazine.—This dyestuff is of particular interest because a distinct step formation becomes manifest already in rather weakly acid solutions, well definable in terms of pH. This fact itself would not be so exceptional since it has been observed before for other cationic dyestuffs, as for pyocyanine and related compounds. However, on increasing the acidity, for the latter dyestuffs very soon there arises the condition that the potential range of the lower step overlaps with, or even far exceeds, the potential of hydrogen for the same acidity. This overvoltage renders a full titration over both steps impossible. For 3-hydroxythiazine there prevails the rare oppor-

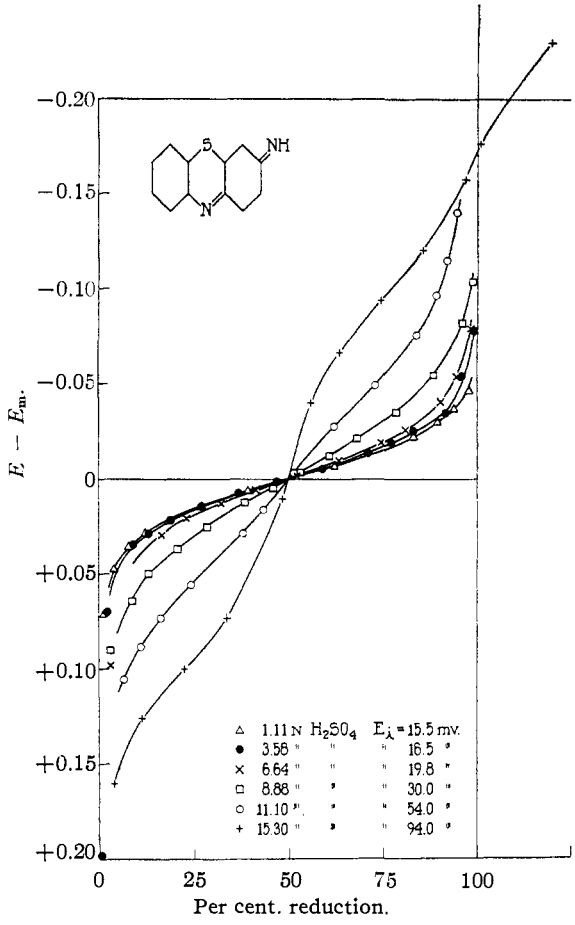


Fig. 1.

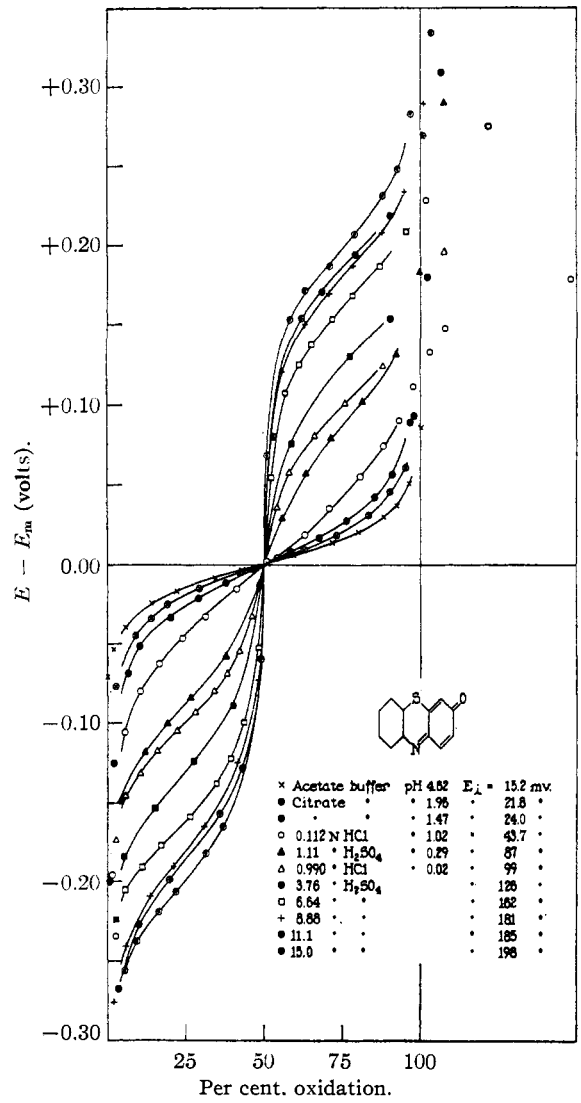


Fig. 2.

tunity that both steps can be titrated over an enormous range of acidity without any overlapping with the hydrogen potential occurring. The results are shown in the same fashion as with 3-aminothiazine, in Table II, and Figs. 2 and 3. Figure 2 corresponds to Fig. 1. Since the separation of the steps spreads into the well measurable *p*H range, not only E_m, but also E_1 and E_2 can be plotted against *p*H (Fig. 3) in the same way as has been done previously for pyocyanine and many other dyestuffs. It can be seen that at *p*H around 1 the uncertainty due to the liquid junction potentials begins. The observed points no longer fit the theoretical curves. For instance, the experimental points for E_1 at *p*H values < 1 apparently indicate that E_1 decreases a little with decreasing *p*H. This is impossible. There is obviously something wrong with the absolute potential values. This uncertainty sets a limit to extending the diagram, Fig. 3, further to the left, so the abundant experimental material can only be demonstrated in the way of Fig. 2. One might have attempted to extend the graph, Fig. 3, into the region of negative *p*H values by expressing the *p*H of the stronger sulfuric acid concentrations according to Hammett's[14] extended acidity scale. This problem is too important to discuss in a perfunctory way. It is rather to be considered as a special topic for a later investigation and will not be entered upon now.

(3) **3-Hydroxy-9-aminothiazine.**—This dyestuff is too little soluble within the ordinary *p*H range to allow really accurate titration experiments. Two titration experiments are shown, one at *p*H 7.11, the other at *p*H 2.92 (Table III), to show approximately the normal potentials in comparison with the other compounds. In more acid

(14) Hammett, *Chem. Rev.*, **16**, 67 (1935).

TABLE II

3-HYDROXYTHIAZINE

Concentration always 1×10^{-4} M, except for the experiment at pH 12.59

Solvent	Manner of titration	pH	E_m	E_i Left	E_i Right	E_2-E_1	Comments
Sec. phosphate + NaOH	Reductive titration with leuco-rosinduline GG	12.59	−0.103 approx.				(See footnote[a])
Phosphate	Same	6.77	+ .159	15.2	15.0	−0.12	
Acetate	Same	4.62	+ .288	14.9	15.0	− .12	
Acetate	Same (another sample of the dye)	4.62	+ .287	15.3	15.4	− .12	
Citrate	Reduced with Pd-H_2, titrated with $K_2Cr_2O_7$	1.96	+ .449	19.4	19.5	− .022	
Citrate	Same	1.47	+ .477	26.0	25.8	+ .024	
0.112 N HCl	Dye dissolved in 0.5 cc. of alcohol; added 50 cc. of 0.112 N HCl. Reduced with Pd-H_2, titrated with ferric ammonium sulfate	+1.02	+ .506	43.6	43.8	+ .081	End-point overlaps somewhat with Fe-potential
1.11 N H_2SO_4	Same procedure, with 1.11 N H_2SO_4, and titrated with $K_2Cr_2O_7$	+0.29	+ .543	87		+ .174	
0.990 N HCl	Dissolved similarly in 0.990 N HCl, titrated with titanous chloride dissolved in HCl of same concn.	+0.02	+ .555	99	99	+ .198	
3.76 N H_2SO_4	Reduced with Pd-H_2, titrated with $K_2Cr_2O_7$		+ .553			.252	
3.76 N H_2SO_4	Reductive titration with Ti^{+++}		+ .553			.242	
6.64 N H_2SO_4	Same					.324	Overlapping with Ti-potential at the end of titration
8.88 N H_2SO_4	Reduced with Pd + H_2, titrated with $K_2Cr_2O_7$					.363	
11.1 N H_2SO_4	Same					.371	
15.0 N H_2SO_4	Same					.397	

[a] At this pH the dye was very little soluble. The solution was filtered, the poise of the potential not good enough to yield reliable values for E_1.

solutions the solubility is somewhat better so that the desired concentration of approximately 1×10^{-4} M can be reached. Figure 4 shows the family of titration curves for varied concentration of sulfuric acid.

(B) **Oxazine Derivatives.** (5) **3,9-Diamino-oxazine.**—3,9-Diamino-oxazine is quite analogous to thionine (Lauth's violet). Its aqueous solution shows a gorgeous red fluorescence. Its normal potential E_m can be read from Fig. 6, and the separation into two steps is distinct in acid solution as shown in Table IV. The separation is smaller than that for thionine, compared in solutions of the same acidity. The index potential is 22 millivolts in 20.8 N sulfuric acid; it was 79 millivolts for thionine in sulfuric acid of nearly the same concentration (20.4 N).

(6) **3-Hydroxy-oxazine.**—The results are described in Table V and Fig. 5. The separation of the two steps is quite similar to that in 3-hydroxythiazine.

(C) **Selenazine Derivatives.** (7) **3,9-Diaminoselenazine.**—The experiments tabulated

TABLE III

3-HYDROXY-9-AMINOTHIAZINE

Solvent	Manner of titration	pH	E_m	E_i Left	E_i Right	Comments
Phosphate	Reductively with leuco-rosinduline GG	7.11	−0.220 approx.	..	..	Satd. solution, $\ll 10^{-4}$ M
Citrate	Same	2.92	+ .072	16.0	16.5	Concentration 6×10^{-5} M
4.52 N H_2SO_4	Reduced with Pd + H_2, titrated with $K_2Cr_2O_7$	..	(+ .263)	24	24	Concentration 5×10^{-5} M
7.60 N H_2SO_4	Reduced with Ti^{+++}	..	(+ .261)	48	52	Concentration approximately 1×10^{-4} M
15.0 N H_2SO_4	Reduced with Pd + H_2, titrated with $K_2Cr_2O_7$	..	(+ .245)	110		Concentration approximately 1×10^{-4} M

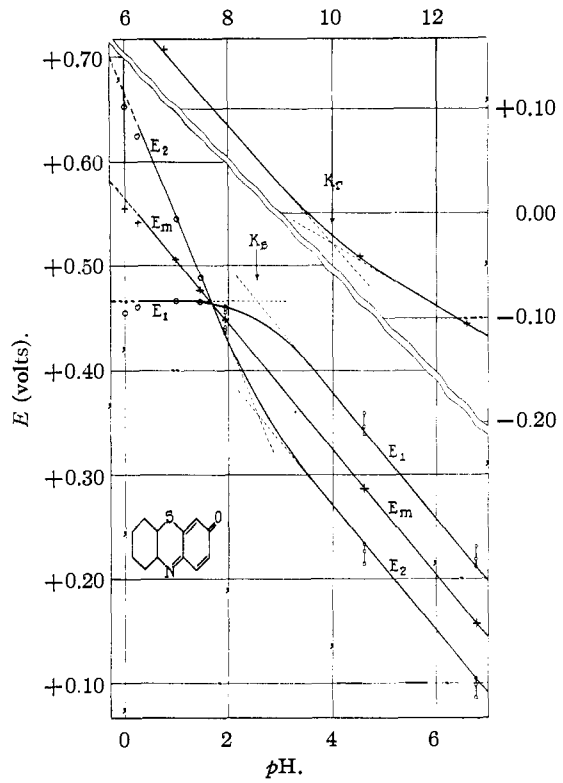


Fig. 3.

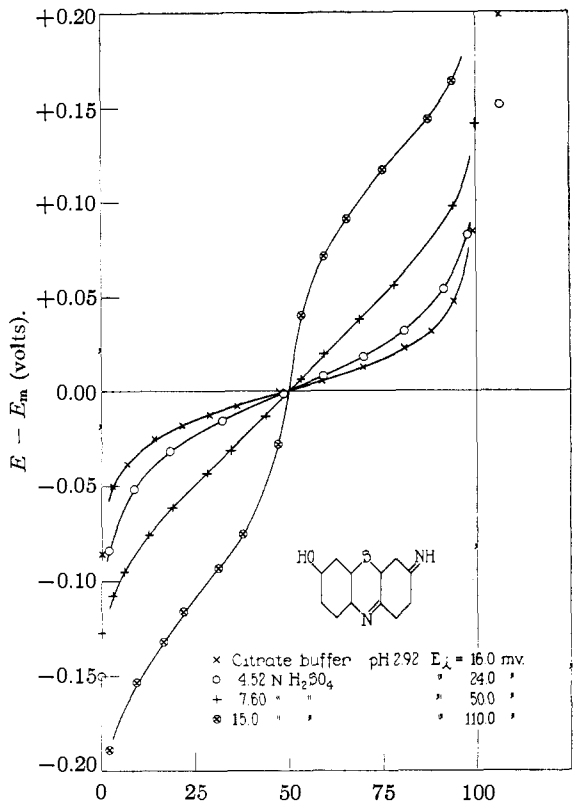


Fig. 4.

in Table VI show a great similarity in every respect to thionine.

(8) **3,9-Di-(dimethylamino)-selenazine** (Selenium-methylene blue).—The semiquinone formation for a given acidity is much smaller than in the preceding dyestuff. This difference is just the same as that between methylene blue and thionine.

Figure 6 is a plot of the mean normal potential, E_m, against pH for several of the dyestuffs investigated in this paper, and some others for comparison.

5. The Optical Properties of the Dyes.—Comparing the spectrum of the various radicals

TABLE IV

3,9-DIAMINO-OXAZINE

Solvent	Manner of titration	pH	E_m	E_i Left	E_i Right	Concn.
Phosphate	Reductive titration with leuco-rosinduline GG	6.79	−0.003	15.3	15.5	1×10^{-4}
Phosphate	Same	6.16	+ .018			1×10^{-4}
Citrate	Same	3.73	+ .169	14.9	14.6	1×10^{-4}
Citrate	Reduced with Pd + H_2; titrated with $K_2Cr_2O_7$	2.32	+ .294	14.3	14.3	1×10^{-4}
15.6 N H_2SO_4	Reduced with Cr^{++}			15.5	15.6	2×10^{-4}
20.8 N H_2SO_4	Same			23	22	2×10^{-4}

TABLE V

3-HYDROXYOXAZINE

Solvent	Procedure	pH	E_m	E_i Left	E_i Right	$E_2 - E_1$
Acetate buffer	Titrated with leuco-rosinduline GG	4.62	−0.0284	15.3	15.1	
Citrate	Reduced with Pd + H_2; titrated with $K_2Cr_2O_7$	2.02	+ .1304	15.1	15.3	
4.52 N H_2SO_4	Same					0.206
Same	Same					.205
7.60 N H_2SO_4	Same					.262
16.6 N H_2SO_4	Same					.301

TABLE VI

3,9-DIAMINOSELENAZINE

Solvent	Manner of titration	pH	E_m	E Left	E Right
Phosphate	Reduced with Pd + H_2; titrated with $K_3Fe(CN)_6$	6.76	−0.107	14.8	15.0
Acetate	Titrated with leuco-rosinduline GG	4.62	− .097	15.3	15.5
Citrate	Same	3.09	+ .319	15.4	15.5
16.6 N H_2SO_4	Titrated with Ti^{+++}		+ .615	22.3	22.1
18.9 N H_2SO_4	Titrated with Ti^{+++}		+ .626	46.5	46.5

TABLE VII

3,9-DI-(DIMETHYLAMINO)-SELENAZINE

Solvent	Manner of titration	pH	E_m	E_i Left	E_i Right
Phosphate	Titrated with leuco-rosinduline GG	6.73	+0.049	17	17
Acetate	Reduced with Pd + H_2; titrated with $K_3Fe(CN)_6$	4.62	+ .159	14 (Approx.)	
Citrate	Titrated with leuco-rosinduline GG	1.94	+ .382	15.8	16
23.0 N H_2SO_4	Titrated with Ti^{+++}		+ .538	27	28

of the thiazines, oxazines and selenazines, two general types of absorption spectra may be distinguished, and no, or at least no distinct, transitional types have been observed. One type is that described for the semiquinones of thionine and methylene bue before, with a series of bands in the green region of the spectrum exhibiting a fine structure not often encountered in organic dyestuffs and, in addition, a broader band within the far blue without finer structure. The other type shows only one intense and rather sharp band.

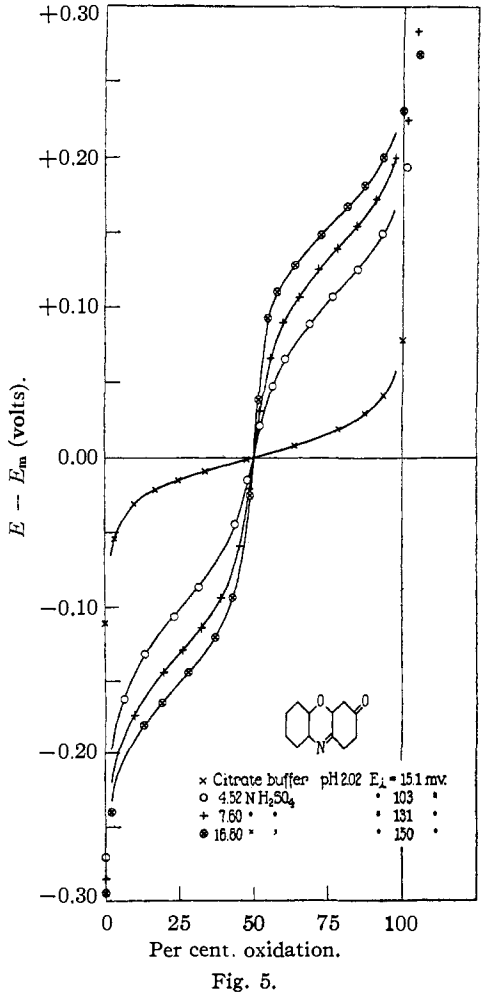


Fig. 5.

Figure 9 shows the absorption of the quinonoid forms of some of the dyestuffs. This figure is to show especially that thionine (in the form of its simply charged cation as existing in mildly acid solution), does not obey Beer's law.[15] The same to a lesser degree holds for t-diamino-oxazine. In addition it is shown in two examples how strongly the absorption is diminished by removing one of the two symmetrically located amino groups, or replacing one of them by OH, making the molecule unsymmetric with respect to the two "auxochromic" groups. The absorption spectra plotted in Fig. 9 hold for those states of ionization of the quinonoid form as indicated in the formulas of the legend. It is noteworthy that the great difference in the molar extinction coefficient according to the nature of the auxochromic groups holds only for the quinonoid forms, and not for the semiquinones (Fig. 7). In the quinonoid forms, the absorption is strongest if there are two amino groups in position 3 and 9 so as to establish an *equivalent* benzene–quinone resonance. Absorption is much weaker when there is only one auxochromic group, or two such of opposite character (one NH_2 and one OH), whereby the resonance is decreased. No such distinction prevails among the semiquinones.

Discussion

On attempting to explain a relatively high stability of a free radical, or any other molecular

(15) *Cf.* quotation (1), footnote on page 210.

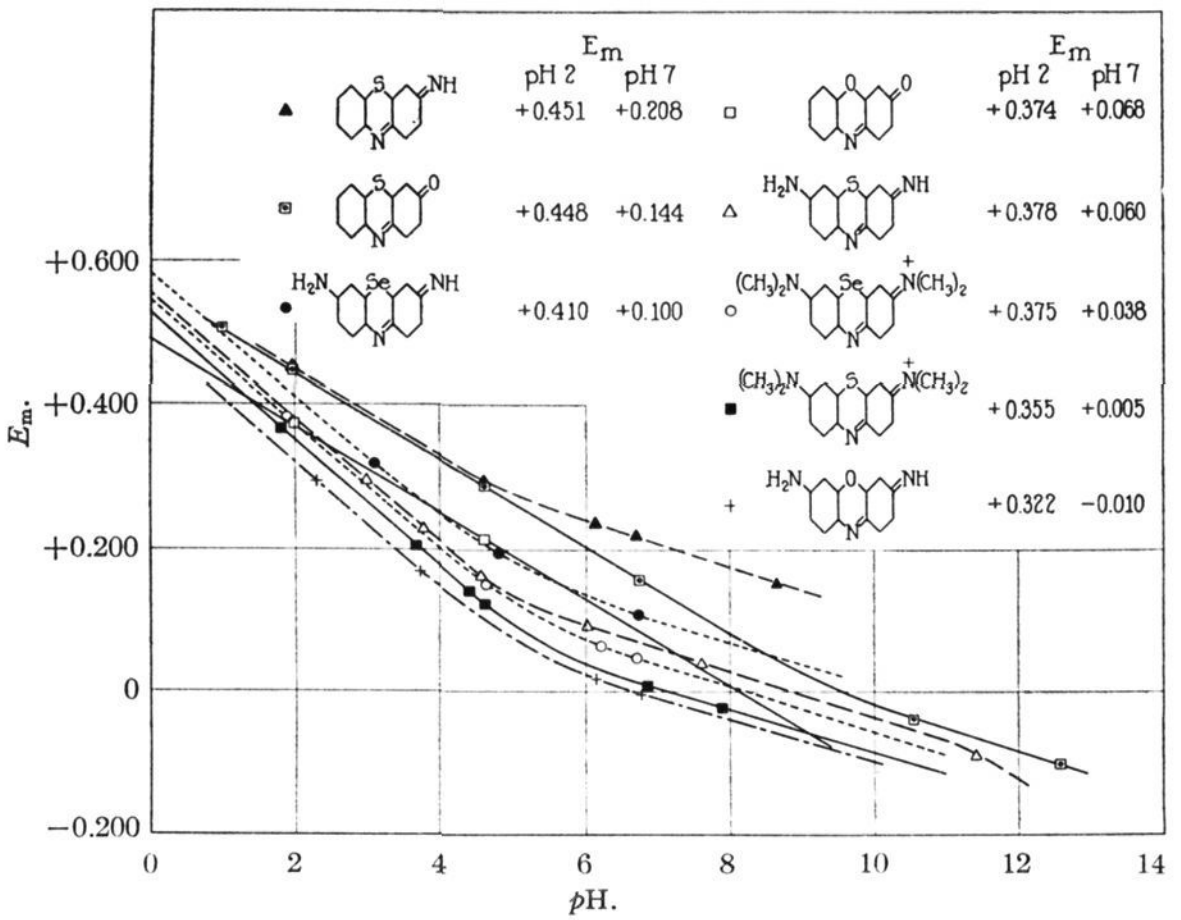


Fig. 6.—Normal potentials, E_m, plotted against pH.

species, which according to classical structural formulas should be expected to be unstable, the principle of resonance may account for an increase of stability beyond the expected extent. This is, at the present stage of the theory, a rather vague qualitative statement since it is practically impossible at the present time to apply this principle quantitatively for compounds of such complicated structure as we have to deal with. The case of triphenylmethyl is easier for quantum mechanical treatment and was successfully dealt with by Pauling and Wheland,[16] and by Hückel,[17] using two different methods of approximation. It will be much more difficult to use these methods for the compounds described in the present paper. Some qualitative considerations have to suffice for the time being. In the preceding paper it was suggested that the resonance structure of thionine is analogous to that of Wurster's dyes which because of their structural simplicity were used as

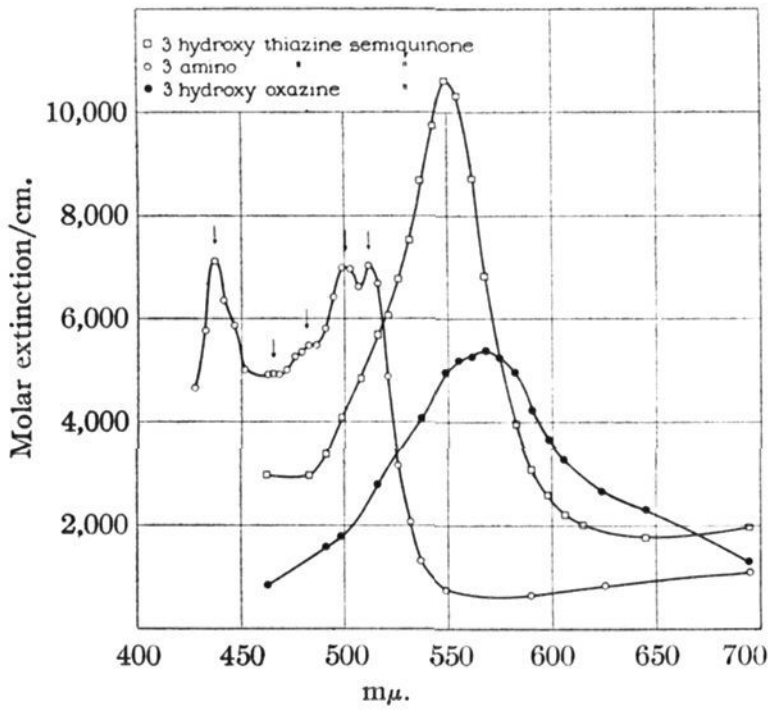


Fig. 7.—The dyes were dissolved in 15 N sulfuric acid. The concentration of the S-form was 1.5×10^{-4} M, and a 1-cm. cell was used in the König–Martins spectrophotometer. The S-forms were prepared by mixing equal parts of the fully oxidized deaerated form, with the fully reduced form (reduced with Pd + H_2, then hydrogen displaced by nitrogen). The S-forms are scarcely autoxidizable under these conditions.

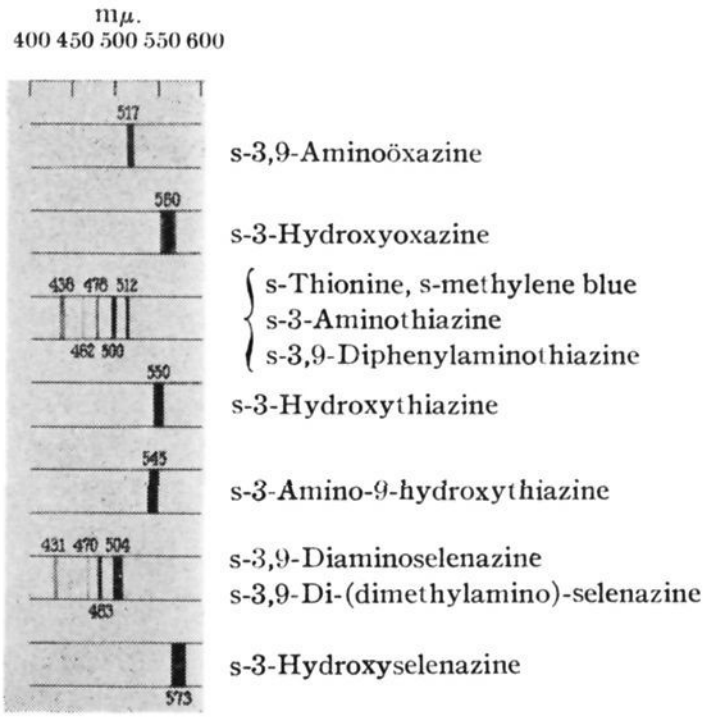


Fig. 8.—The S-forms were prepared by dissolving the oxidized form of the dye in suitable concentrations of sulfuric acid and partially reducing with titanous sulfate. The positions of the bands were located with a hand spectroscope. The 512 mμ band of s-thionine and s-methylene blue is more diffuse than represented in the figure.

(16) L. Pauling and Wheland, *J. Chem. Phys.*, **1**, 362 (1933).
(17) E. Hückel, *Z. Physik*, **83**, 632 (1933).

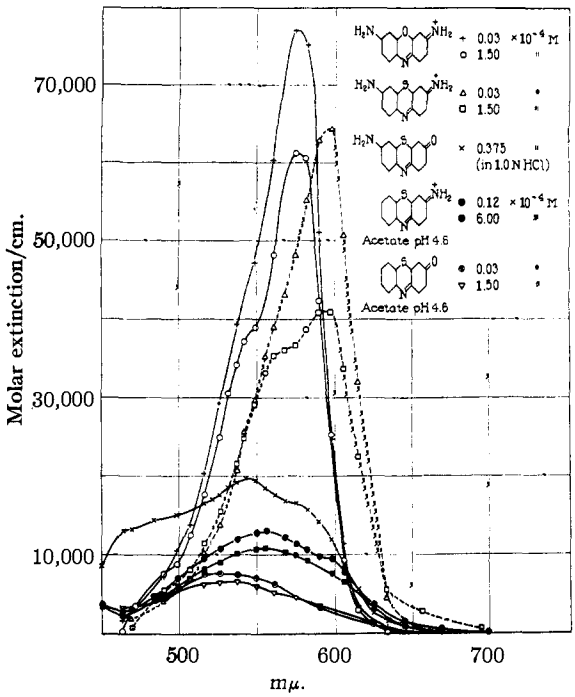
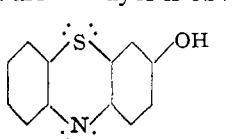
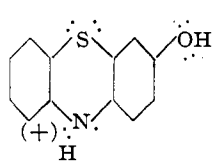


Fig. 9.

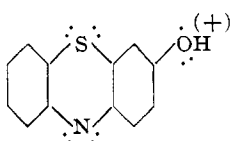
models. However, in going over the compounds investigated in this paper, we encounter very stable radicals for which no immediate analogy with Wurster's dyes is obvious.



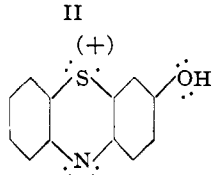
I



II



III



IV

Experience as obtained from the examples of this paper shows that a radical such as I is stabilized by acidity, by attaching a proton, as in II. The latter structure is in resonance with III. This, however, is no longer an *equivalent* resonance. Although it may be generally true that equivalent resonance is a highly stabilizing factor there is no reason why a non-equivalent resonance sometimes should not exert a highly stabilizing effect, too. If so, there is no longer any reason to restrict the limiting structures to the types previously shown. Another considerable contribution to stability may be attributed to a resonance between a pair of limiting structures, one of which is II, the other IV, with the odd electron in II at the N, in IV at the S. With this type of resonance, no side chain in phenothiazine would be necessary at all to establish a fairly well stable radical.

The logical continuation of these ideas would lead to an investigation of radicals derived by partial oxidation from unsubstituted phenothiazine, phenoxazine or phenoselenazine. Experiments along these lines will be the subject of a later publication.

Summary

The formation of free semiquinone radicals in a number of oxazine, thiazine and selenazine dyestuffs is investigated. All of these dyes easily form semiquinones in sufficiently acid solutions. The semiquinone formation constant for some of these dyestuffs are the largest encountered as yet, *e. g.*, amounting to 10^{+6} for 3-hydroxythiazine in 15 *N* sulfuric acid. Two types of absorption spectra for the semiquinones of these classes of dyestuffs are described, one consisting of a single rather sharp band, the other consisting of a complicated band system. High stability of these radicals is encountered not only when the resonance is an equivalent one, but also under certain conditions, when the resonance is not quite equivalent and a complete analogy to the structure of Wurster's dye is missing.

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