dine is likewise a stimulator of fermentation. Increased "yield" of gas is caused by fermentation in the presence of these substances. The vitamin assay method described (gas method) is a rapid and reliable one for research or control purposes. Details are given for the performance of the above tests.

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The Equilibrium of the Semiquinone of Phenanthrene-3-sulfonate with its Dimeric Compound

BY L. MICHAELIS AND E. S. FETCHER, JR.

Previous studies of the oxidation–reduction intermediates of various dyestuffs[1] have resulted in the conclusion that these intermediates are monomolecular radicals. By analogy to radicals of the triphenylmethyl and diphenylnitride type, which have long been known to exist in equilibrium with their dimeric, valence-saturated compounds, it is of interest to examine the possibilities of a similar dimerization of the radicals in question.

Dimerization, a bimolecular reaction, will depend upon concentration. Hitherto all studies have been made in the rather dilute solutions imposed by the limited solubility of most of the substances used. The concentration usually has varied from 10^{-4} to 3×10^{-3} mole/liter. Few dyestuffs of otherwise suitable properties are sufficiently water soluble to permit of any great extension of this concentration range. One such, however, has been found in potassium phenanthrenequinone-3-sulfonate, recently studied in dilute solution.[2] It is the purpose of this paper to extend the investigation of the course of the potentiometric titration of this dye to comparatively high concentrations.

Critical Review of the Experimental Technique.—In order to obtain comparable titration curves for widely varied concentrations of the dye and thus to examine the influence of the concentration upon these curves, it is necessary that two conditions be held constant, the ionic strength and the *p*H. The difficulty in maintaining these is increased by a high concentration of the dye, and it is virtually impossible to do this exactly.

In acid solution, the influence of the ionic strength is negligible within the range of its variation occurring in the experiments. This is not quite so in alkaline solutions, in which the dye is soluble enough to allow of a wider range in ionic strength. In two otherwise comparable experiments at *p*H 12.2, the index potential increased 5 to 7 mv. on increasing the ionic strength from $\mu = 0.1$ to 1.0. Hence, all titrations to be compared with each other should represent experiments at an approximately equal ionic strength. In order to overcome as much as possible the effect of the polyvalent ferricyanide ion added during the titration, the initial ionic strength of the dye solution was chosen as high as feasible. Two ranges of *p*H had to be selected for the experiments, one around *p*H 12.2, the other around 4.6. For *p*H 12.2, a 0.05 *M* solution of sodium hydroxide was made 1.0 molar with potassium chloride. At *p*H 4.6, we had to be satisfied with ionic strength $\mu = 0.2$. At a higher salt concentration the solubility of the dye is not great enough to reach the dye concentration desired. Acetate buffer of $\mu = 0.1$ was used, and μ was doubled by addition of potassium chloride. These salt concentrations were high enough to maintain the ionic strength sufficiently constant during any one titration, as well as over the range of dye concentrations used.

The second condition, that the *p*H should be the same in all experiments to be compared, need be fulfilled only approximately for the following reason. We wish to compare not the absolute values of the potentials, which of course depend on *p*H, but the shape of the curve, as expressed by the slope at comparable points. This shape does not depend at all on minor variations of *p*H in certain ranges. There is one such range from about *p*H 3 to 5, in which the shape of the titration curve is practically identical with that of a dye with no intermediate step. There is a second range at *p*H > 11.5, where the three normal potentials E_1, E_m, and E_2, plotted against *p*H,

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[1] L. Michaelis, *Chem. Rev.*, **16**, 244 (1935).

[2] L. Michaelis and M. P. Schubert, *J. Biol. Chem.*, **119**, 133 (1937).

run parallel. Hence, a set of experiments, each one performed at a *p*H constant during the titration, the *p*H being within one of the above ranges mentioned, is suitable for comparison. In plotting a titration curve it is simply necessary to refer all potentials to the potential at 50% oxidation as the zero point.

All titrations were carried out by first reducing the dye with hydrogen and colloidal palladium, replacing the hydrogen with nitrogen and titrating with potassium ferricyanide. For the technique of this procedure, we refer to previous descriptions.[3] Potassium ferricyanide was chosen because its high solubility made it possible to minimize the volume change during the titration. It was made up in the same buffer solution as the dye to a concentration such that the total volume change during each titration never exceeded 8%, which may be disregarded.

For the acid solutions no difficulties arising from decomposition were encountered. For the alkaline solutions some comments with regard to the stability of the compounds are necessary. This dye is relatively stable in alkaline solution as compared with most quinones, but is not absolutely stable. The titer of the dye at *p*H 12.2 averaged about 95% of the theoretical, as obtained in acid solutions. A small part of the loss may be due to the decomposition comparable to the benzilic acid rearrangement of benzil, which converts phenanthrenequinone to diphenylidene-glycolic acid. This rearrangement takes place at a fairly high rate only on boiling with alkali, but may proceed to a slight extent even at 30°. According to our own studies this rearrangement during the time necessary for a titration at 30°, would reduce the concentration of the dye by at most 0.5%. The rest of the loss seems to be due to a decomposition of the reduced form of the dye, for it was observed that if this were left in contact with the alkali for more than the usual sixty to ninety minutes necessary for the whole experiment, the titer was decreased correspondingly. To avoid as much as possible the deleterious effect of the alkali the *p*H = 12.2 was chosen, using 0.05 *M* sodium hydroxide as buffer. This *p*H is as low as is safely compatible with the conditions specified above. Furthermore, it should be mentioned that at this high *p*H, at the end-point of titration, the potential, though rising sharply and giving a distinct end-point, drifts back due to an irreversible over-oxidation of the dye. No serious error arises from this.

Altogether, the titration experiments in the alkaline solutions may be taken as fair approximations and can be used for the purpose required with the restriction that the claim for accuracy of the constants computed from them should not be too high. It will be observed that in general the two halves of these curves, from 0 to 50%, and from 50 to 100% oxidation, are not exactly symmetrical as they ought to be. The potential difference from 25 to 50%, and that from 50 to 75%, differ by 1 or 2 mv., the latter usually being the smaller one. This irregularity is obviously the effect of the alkali. This first half of the curve was used in the calculations.

Experiments

All experiments were performed at least in duplicate with the technique previously described, at 30.0 ± 0.1°. The results are recorded in Figs. 1 and 2 and in the legends of these diagrams, and Tables I and II. The 0.0033 *M* and 0.001 *M* curves are omitted from Fig. 2 to avoid confusion.

TABLE I

a, Concn., mole/liter	*p*H	Normal potential, E_m, referred to hydrogen electrode	Index potential, E_i	
			In 1st half of titration	In 2d half of titration
0.020	4.61	+0.197	0.0274	0.0277
.020	4.62	.198	.0274	.0277
.010	4.61	.195	.0228	.0228
.010	4.62	.195	.0228	.0228
.00167	4.62	.195	.0170	.0171
.00167	4.63	.194	.0170	.0171
.00033	4.63	.195	.0143	.0143
.00033	4.60	.195	.0143	.0143

TABLE II

a, Concn., mole/liter	*p*H	Normal potential, E_m, referred to hydrogen electrode	E_i, Index potential	
			In 1st half of titration E_i 0.5	In 2d half of titration E_i 1.5
0.020	12.11	−0.154	0.0594	0.0598
.020	12.09	.153	.0594	.0598
.010	12.24	.166	.0542	.0550
.010	12.19	.162	.0542	.0550
.0033	12.32	.170	.0484	.0470
.0033	12.30	.169	.0484	.0470
.00167	12.27	.166	.0468	.0463
.00167	12.32	.169	.0468	.0463
.0010	12.29	.166	.0468	.0453
.0010	12.29	.166	.0468	.0453
.00033	12.32	.167	.0461	.0443
.00033	12.32	.164	.0461	.0443

Whenever the curve is steeper than the limiting curve for extremely dilute acid solutions, the

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[3] L. Michaelis, M. P. Schubert and C. V. Smythe, *J. Biol. Chem.*, **116**, 596 (1936).

color goes through an intermediate brown. Since there is always a mixture of the oxidized, the reduced, and the intermediate forms, it is difficult to account for the characteristic spectrum of the two co-existing modifications of the intermediate form. No distinct bands can be seen in the mixture in the visible region, and one cannot distinguish the two intermediate species by their color. A detailed spectroscopic analysis would be difficult in this case, and will not be entered upon in this paper.

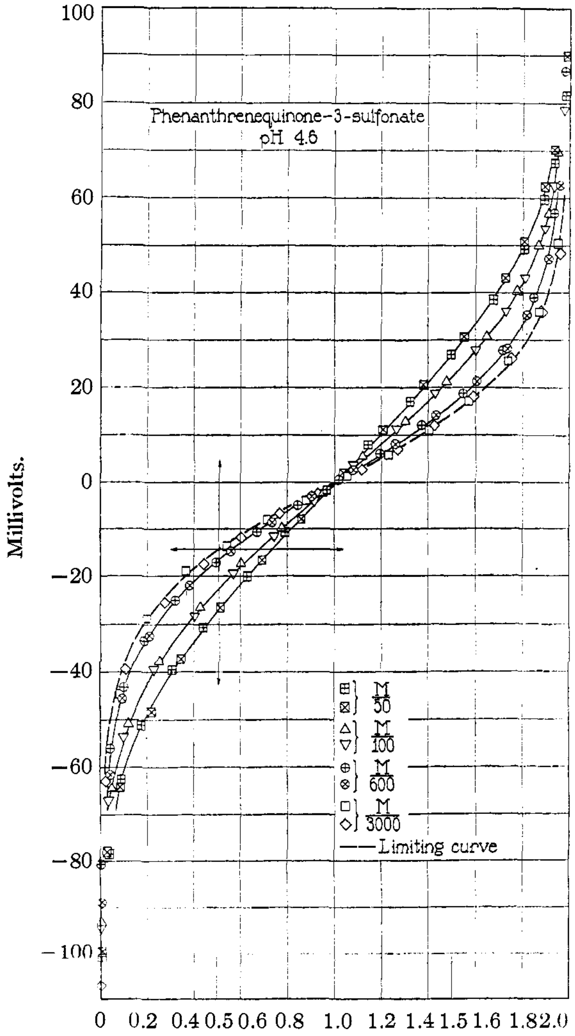

Fig. 1.—Titration curves at *p*H 4.6, for various concentrations of the dye. Abcissa: *x*, equivalents of potassium ferricyanide added. The percentage of oxidation equals $(x/2) \times 100$. Ordinates: potential, in millivolts, referred to the normal potential as zero point. The limiting curve —— is the curve as calculated for a bivalent system with no intermediate step.

Discussion

The observed variation in the shape of the curves can only be attributed to the change in concentration of the dye. It indicates that some reversible polymolecular reaction is taking place. This reaction might consist of an association to higher molecular units of the oxidized or the reduced forms of the dye, which increases with concentration. However, this would produce an effect just opposite to that observed. The curves would become flatter with increasing concentration, instead of steeper. Another reaction might be the polymerization of the semiquinone radical. The simplest possibility, which alone will be considered here, is to assume an equilibrium between the radical and its dimeric, valence-saturated compound. This reaction would shift

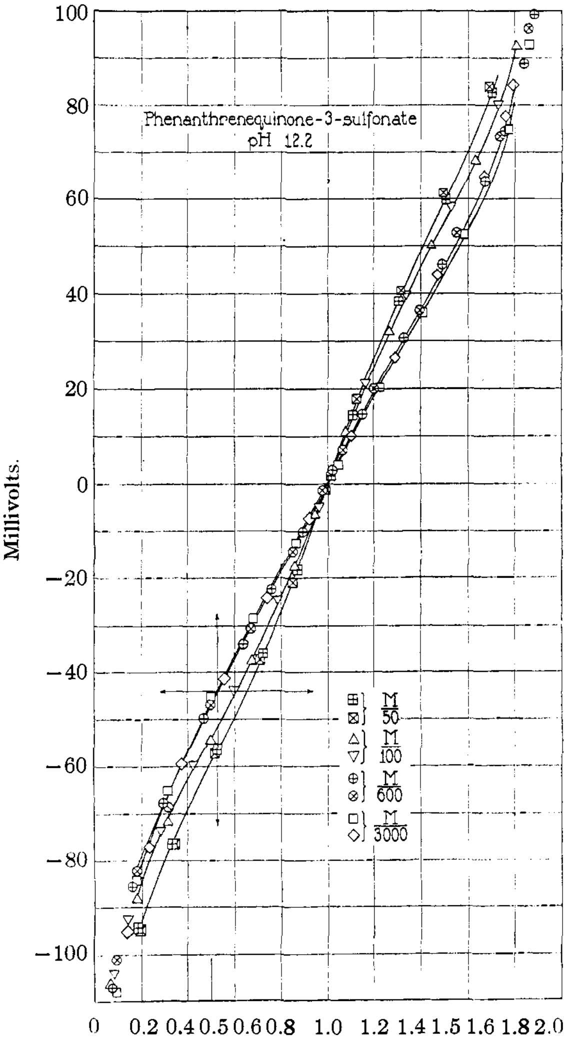

Fig. 2.—Titration curves at *p*H 12.2 for various concentrations of the dye. The experiments at 0.001 *M* and 0.0033 *M* concentrations as tabulated in Table IV, are omitted for the sake of clarity.

the curves in the observed direction. We shall now develop the theory on the basis of this assumption. It is to be noted that this theory is by no means a return to the previously accepted assumption that the intermediate forms of oxidation–reduction are essentially bimolecular compounds, but is an extension and generalization of the semiquinone theory. The latter, in its simple form as developed in the preceding papers, is still valid for very diluted solutions. Its extended form is to account for a property of the semiquinone hitherto unknown which occurs at higher concentrations, and is comparable to the dimerization of triphenylmethyl.

The reduced, the semi-oxidized radical, and the totally oxidized forms will be designated as **R**, S, and T, respectively; the dimeric molecule, formed by two molecules of the radical, as D. Since the radical S is in equilibrium with the R and T forms, the dimer, D, may also be considered as a molecular compound of R and T. The concentrations of these species will be designated by small letters, r, s, t, and d; the total concentration of the dye as a. We have the relation

$$a = r + s + t + 2d \qquad (1)$$

The following constants of equilibrium may be distinguished:

$$s^2/rt = k \quad \text{the semiquinone formation constant} \qquad (2)$$
$$d/rt = q \quad \text{the dimeric formation constant} \qquad (3)$$
$$d/s^2 = \gamma \quad \text{the dimerization constant} \qquad (4)$$

Only two of these are independent of each other, for

$$q = k\gamma \qquad (5)$$

These are not true constants, but are dependent upon pH, since the molecules involved may be in various states of ionization.

The development of an exact and general mathematical analysis of these curves would be extremely complicated and of little practical value. We shall therefore set forth an approximate solution of the problem, not based on any general method, but rather on special approximation methods adapted to the experimental conditions prevailing. That is, for the family of curves at $p\text{H} = 4.6$, the simplifying assumption will be the neglect of the S-form; at $p\text{H} = 12.2$, the T-form will be neglected. There are two methods of reasoning applicable to each of the two sets of curves, both based on the assumption that the displacement of the curves for higher concentration from the limiting curve, as obtained for infinitely small concentration, is due to the formation of the dimer. One method makes use of the horizontal displacement for the calculation of the concentration of the dimer, and hence the constants. The error is smallest when this displacement is measured from the point at 25% oxidation of the limiting curve. The other method makes use of the vertical displacement. Here also the displacement from the 25% oxidation point of the limiting curve will be used for the calculation. The constants derived from these two independent methods should agree with each other, whereas the constants for different pH need not.

1. The Family of Curves for pH 4.6

(a) Method of Horizontal Displacement.—The shape of the limiting curve for extremely low concentration is, within the limits of error, identical with that for a dye without intermediate form. Therefore we may assume with sufficient accuracy that at 25% oxidation (when $x = 0.5$), $r{:}t = 3$. This ratio unambiguously determines the potential. Whenever, in any one of the other curves of the family, the potential equals that of the limiting curve at 25% oxidation, $r{:}t$ will always be equal to 3 at this point. These points lie on the horizontal line marked with an arrow in Fig. 1, and will be designated as the characteristic points. At each characteristic point we have the following conditions

$$r{:}t = 3 \qquad (6)$$
$$r + t + 2d = a \qquad (7)$$
$$d + t = \frac{x}{2}a \qquad (8)$$

Here x is the equivalent amount of oxidizing agent added, per mole of dye. This set of approximately correct equations presupposes that s is negligible compared with r or t. Solving for d, one obtains

$$d = a(x - {}^1/_2) \qquad (9)$$

That fraction of the dye existing in the D-form equals $2\,d/a$; it is

$$2d/a = 2x - 1 \qquad (10)$$

Having the values for x correlated to each characteristic point, we calculate d from equation (9). Furthermore, from equations (3), (6), and (7) one can express q in terms of a and d and obtains

$$q = \frac{16}{3} \times \frac{d}{(a - 2d)^2} = \frac{2(2x - 1)}{3a(1 - x)^2} \qquad (11)$$

The result of the calculation carried out for the various curves of this family is shown in Table III.

TABLE III

FOR pH = 4.6

a concentration of dye. x number of equivalents of oxidizing agent added to attain the potential of -0.0143 v. E_i potential at $x = 0.5$ (= 25% oxidation), or index potential. q dimeric formation constant. The subscripts h and v designate the method of calculation; *i. e.*, from the horizontal, and the vertical displacements, respectively.

a	x	E_i	q_h	q_v
0.000333	0.500	−0.0143	...	...
.00167	.572	− .0170	(315)	(300)
.0100	.678	− .0228	228	227
.0200	.732	− .0274	216	228

At this place it may be mentioned that one item of the previous paper[2] requires modification owing to the fact that, at the time, it was not recognized that the intermediate brown color present in high concentration of the dye in acid solution is mainly due to the dimer and not to the radical. It is easy to see that the parallelism of the three normal potentials plotted against pH, in low pH ranges (see Fig. 1 in (2)) should be maintained since no further step of ionization is imaginable. But we can no longer be sure whether the bend leading to this parallelism, corresponding to the pK of the S-form, lies at pH 7.5, or at an appreciably smaller pH. The possibility that pK might be less than 7.5 was conceded also in the previous paper, but it may be considerably less. If so, the distance of the three parallels from one another in the range of small pH's will be greater than drawn in Fig. 1 of the previous paper, and the semiquinone-formation constant, k, may be even smaller than 0.001 in acid solution.

(b) Method of Vertical Displacement.—We again start from the assumption that at the point $x = 0.5$, for the limiting curve $t:r = 1/3$. The potential depends on this ratio, namely,

$$E = \text{Const.} + 0.03 \log (t/r)$$

In the other curves, where the potential at $x = 0.5$ is displaced by, say, p volts, the ratio $t:r$ can no longer be $1/3$, but $1/3 \times 10^{p/0.03} = 10^{(p - 0.0143)/0.03} = P$. Here, p is always a negative number. It is easy to see that P is simply

$$P = 10^{E_i/0.030}$$

where E_i is the index potential of the particular curve. Using the latter equation in combination with equations 7 and 8, which are valid for any of these curves, we obtain

$$r = \frac{a}{2(1 - P)} \qquad (12)$$

$$t = \frac{aP}{2(1 - P)} \qquad (13)$$

$$d = \frac{a(1 - 3P)}{4(1 - P)} \qquad (14)$$

$$q = \frac{d}{rt} = \frac{(1 - P)(1 - 3P)}{aP} \qquad (15)$$

We obtain for q the values of Table III. The value for the concentration 0.00167 M is based on a very small displacement and subject to a large error. It may be considered only as confirmation of the order of magnitude. We shall adopt 230 as the value for q. It is to be noted that 1 mv. displacement of the curves changes q by about 18%.

In the previous paper the semiquinone formation constant, K, was found to be about 10^{-3}. As discussed above, it may be even less. If we adopt 10^{-3}, we have, for the pH range around 4.6

$$q = 230 \text{ with fair approximation, say, } \pm 30$$

$$\left.\begin{aligned} k &= 10^{-3} \\ \gamma &= 2 \times 10^{6} \end{aligned}\right\} \text{accurate only to the order of magnitude}$$

The numerical values of these three constants cannot be compared as they differ in their dimensions. Whereas k is a dimensionless number, q and γ have the dimension of reciprocal concentration.

2. The Family of Curves for pH = 12.2

(a) Method of Vertical Displacement.—The slope at the midpoint of the curves in this pH range is much steeper than in acid solution and shows that some intermediate form of oxidation arises to an appreciable extent even in extremely dilute solution. The shape of the curves depends on the concentration. A practical measure of this is the index potential. A change of the concentration from 0.00033 to 0.00167, a fivefold increase, hardly shifts the index potential beyond the limits of error, whereas a change from 0.01 to 0.02, a twofold change, increases the index potential by 4.2 mv. This justifies taking the curve for 0.00033 as characteristic of an infinitely dilute solution within the limits of error. That the curves tend, with the decrease of concentration, toward a rather steep limiting slope, indicates, first, that even at very high dilution the intermediate form does exist, second, that at very low concentrations, it exists entirely in the S-form, and that there is none in the D-form; or that the dissociation of the D-form into radicals is complete. Under these circumstances we can treat this limiting curve according to the theory developed for semiquinone formation.[4] The index potential

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(4) In the previous paper,[2] at concentrations about 0.0005 M, E_i was found = 40 mv., corresponding to k = 16 and $(s/a)_{\text{max.}}$ = 0.67. This difference will be due mainly to the difference in ionic strength; μ = 0.1 formerly, and 1.0 in these experiments.

E_i is 46.1 mv., hence the semiquinone-formation constant $k = 28.6$ and $(s/a)_{\text{max.}} = 0.727$.[5]

Considering now the curves for higher concentrations, a distinct steepening of the middle portion is obvious. The limiting curve has only one point of inflection. If there be more, they are obscured by the experimental errors. In the other curves three points of inflection appear very distinctly. This indicates that the maximum fraction of the intermediate form must be greater than 0.727. We claim that this excess of the intermediate form is due to a partial dimerization of the radical at higher concentrations. In order to calculate the equilibrium constants q and γ, besides k, which is already known we proceed as follows.

First of all, let us find that point in the limiting curve (0.00033 M) where $r = s$, in other words, that point at which the potential equals E_1, the normal potential of the lower step of oxidation. There are two equations,[6] one expressing r, the other t, in terms of x, a, and k. Equating these two equations we obtain as the value of x at the point desired

$$x = (k + 2)/(2k + 1) = 0.525 \qquad (16)$$

As k approaches infinity, x approaches 0.500, which differs but little from 0.525. This fact may be expressed thus: although there is a distinct overlapping of the two steps of oxidation in the middle portion of the limiting curve (around $x = 1$), there is only a very slight overlapping in the region around $x = 0.5$. That is to say, in this region the T-form exists only to a negligible extent in comparison to R and S. This state of affairs entitles us to simplify equation 1 by neglecting not only d, but also t, and we have approximately

$$r + s = a \qquad (17)$$

for the point of this curve at $x = 0.525$, which will be designated as x^*.

We now consider the other curves at the same point x^*. The potential of any of these curves at this point differs from that of the limiting curve by, say, n volts. This potential can be expressed in terms of the ratio $s:r$, namely: $E = \text{Const.} + 0.06 \log s/r$. In the limiting curve $s:r$ was 1. In any of the other curves we have

$$s/r = 10^{n/0.06} = N \qquad (18)$$

N is always less than 1. For these curves t may be neglected as well as in the limiting curve, to even a closer approximation, since these curves are still steeper; on the other hand, we no longer can neglect d, and we have to write

$$r + s + 2d = a \qquad (19)$$

Furthermore, we have

$$s + 2d + 2t = ax^*$$

or, again neglecting t

$$s + 2d = ax^* \qquad (20)$$

Equations 18, 19, and 20 can be solved for r, s, and d in terms of a and x^*, hence also for γ. The solutions are

$$r = a(1 - x^*) \qquad (21)$$

$$s = Na(1 - x^*) \qquad (22)$$

$$d = {}^1/_2 a[x^* - N\,(1 - x^*)] \qquad (23)$$

$$\gamma = \frac{d}{s^2} = \frac{x^* - N(1 - x^*)}{2aN^2(1 - x)^2} \qquad (24)$$

where x^* is always $= 0.525$. From this we calculate the values for r, s, d, γ, and q. So we obtain the values of Table IV.

(b) Method of Horizontal Displacement.—Draw a horizontal line through the point $x = 0.525$ of the limiting curve. For each point of intersection with the other curves we have

$$r = s$$
$$r + s + 2d = a$$
$$s + 2d = xa$$

Hence

$$s = r = a(1 - x)$$
$$d = {}^1/_2 a(2x - 1)$$
$$\gamma = \frac{d}{s^2} = \frac{2x - 1}{2a(1 - x)^2}$$

Here x is the value of the abscissa at the point of intersection defined above. The results are given in Table IV.

TABLE IV

FOR pH 12.2, $k = 28.6$

a	x	Potential $x = 0.525$	N	γ_h	γ_v	q_h	q_v
0.000333	0.525	0.0439	1	...	...	..	..
.00100	.532	.0447	0.970	(146)	(150)	..	..
.00167	.532	.0447	.970	(85)	(84)	..	..
.00333	.548	.0462	.916	68	71	1950	2030
.0100	.612	.0520	.733	74	73	2120	2090
.0200	.654	.0571	.603	64	73	1830	2090

The values calculated from the smallest displacements are not expected to be reliable. They have been put in parentheses, and serve merely to confirm the order of magnitude of γ. The other values show a satisfactory agreement. They depend neither upon the concentration, a, nor upon the method of calculation, to any extent which exceeds the limits of error of this approximate method. We may take the value of

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(5) L. Michaelis, THIS JOURNAL, **58**, 873 (1936).

(6) L. Michaelis, *J. Biol. Chem.*, **96**, 703 (1932); equations 4 and 6.

γ as 70; or q as 2000. Among the figures utilized for the calculations of these constants the index potential of the limiting curve is especially important. As this curve is obtained by experiments at extremely low concentration in alkaline solution, the limits of error may be greater than in other experiments. We have retraced all calculations assuming this index potential to be different by a millivolt from the value adopted above. The difference of the constants resulting herefrom, from those adopted above, is small and not worthy of detailed comment.

Using the constants obtained we may calculate the distribution of the various forms of the dye for various total concentrations, at 50% oxidation, where both s and d are at their maximum, and $r = t$. For this calculation we start from any arbitrarily chosen value of r; this equals t; and using the above constants we obtain the corresponding values for s, d, and a. From these calculations Figs. 3 and 4 have been derived. For the sake of demonstration, the range of concentration as plotted greatly exceeds that really obtainable. These curves are only rough sketches.

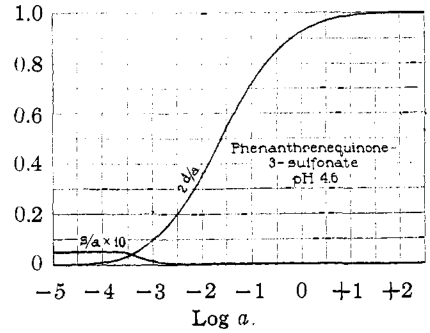

Fig. 3.—s/a and $2d/a$, plotted against the logarithm of a, at 50% oxidation, at pH 4.6.

Conclusion and Additional Comments

The data of this, and the previous paper[2] indicate (1) that in acid solution the concentration of the radical is always exceedingly small, but the concentration of the dimer may be quite large for higher total concentrations; (2) that in *alkaline* solution the intermediate form is almost entirely in the radical form, if the total concentration is small, say, <0.001 M, while in higher total concentrations the two forms of the intermediate form are more nearly equal; (3) that the intermediate form may exist in different states of ionization according to the pH. The radical exists as an anion in alkaline solution; as an undissociated acid in acid solution; pK was estimated $\gtrless 7.5$. The dimeric compound, when completely ionized, in extremely alkaline solution, then, is a bivalent anion, and will shift, with decreasing pH, first to a univalent anion and then to an undissociated acid. The ionization constants for the dimer are not known. It is quite probable that at pH 12.2 it exists as a univalent anion. Certainly, in acid solution it exists as an undissociated acid. The results lead to the conclusion that the ionized form of the radical has a much smaller tendency to dimerization than the non-ionized. This again is compatible with the hypothesis that the stability of the radical is, to a large extent, due to its resonance. Without attempting a more refined account, it appears plausible that the free exchange of the odd electron between the two oxygen atoms —which probably proceeds across the bridge of C-atoms between them—is interfered with when a proton is attached to this electron. The ionic form of the radical has a strong symmetrical resonance and is more stable than the non-ionized form. The tendency of the radical to exist in its ionic, more resonating form is so great that it is a stronger acid ($pK \gtrless 7.5$) than the hydroquinone ($pK = 8.0$) although the (non-ionized) radical has only one OH group, and the hydroquinone two.

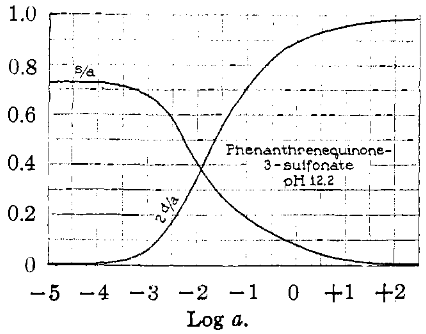

Fig. 4.—s/a and $2d/a$, plotted against log a, at 50% oxidation, at pH 12.2.

It must be emphasized that this theory has little in common with the old quinhydrone theory which neglected the radical form, inasmuch as we postulate a mobile equilibrium between the two intermediate forms—the radical and its polymer—and between the fully oxidized and reduced forms.

By analogy to certain other types of radicals it would seem justifiable, *a priori*, to extend the concept developed for this particular substance into a general theory for oxidation–reduction intermediates. How far it is really applicable can only be determined by further experiment. Nevertheless, it is interesting to consider a few of the possible consequences if we do assume that the phenomenon is general. First, the scope of the semiquinone theory is considerably broadened. Thus it may now be possible to treat, in a more satisfactory manner than has hitherto been possible, systems where no intermediate form has been shown to exist. In the light of the present theory it is entirely possible that an intermediate form does exist, but that the maximum concentration of the radical is not great enough to raise the index potential beyond the limits of error. In the case presented here, the high value of the dimerization constant enables us to recognize the intermediate form in acid solution, which scarcely would be possible if there were no dimerization. Second, we have dealt only with homogeneous systems. It is suggestive that under physiological conditions and with biologically occurring oxidation–reduction systems, adsorption at interfaces, or combination with specific proteins or enzymes, may have a similar influence as variation in concentration or *p*H has in a simple and homogeneous system. Finally, this theory removes the question which of the intermediate forms exists in any given case; for the radical and its polymer can exist at all times in equilibrium, the constants of which are determined by the substances in question and by the conditions of the surrounding medium. The resonance of the radical is one of the essential factors determining the equilibrium constants.

Summary

The intermediate form of oxidation–reduction of phenanthrenequinone-3-sulfonate may be present in two forms, as a semiquinone radical, or as a valence-saturated dimeric compound of the radical. There is an equilibrium between the four forms of the dye: the oxidized, the reduced, and the two intermediate forms. According to this equilibrium the existence of the dimer is favored by increasing the total concentration of the dye. In alkaline solution the intermediate form is represented mainly by the radical even in moderately concentrated solution, and practically entirely so at the concentration of 0.001 *M* and below. In acid solution the radical exists in any case only in extremely small amounts. The dimeric form is just as scarce when the total concentration of the dye is low; but it may increase very considerably if the total concentration of the dye is high. The values of the equilibrium constants are

		at *p*H 4.6	at *p*H 12
Semiquinone formation constant	$k =$	$\leqq 10^{-3}$	28.6
Dimer formation constant	$q =$	230	2000
Dimerization constant	$\gamma =$	$\geqq 2 \times 10^6$	70

Increase of alkalinity not only increases the total intermediate form, but also the fraction of this which is in the form of a radical.

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NOTES

The Identity of Solancarpine with Solanine-*s*

BY LINDSAY H. BRIGGS

A comparison, by the method of mixed melting points, of "solancarpine" from *Solanum xanthocarpum*[1] and solanine-*s* both from *S. sodomaeum*[2] and *S. auriculatum*[3] as well as of the related aglyco alkaloids and their derivatives has shown that the alkaloids from all three botanical sources are identical.[4] The names solancarpine and solancarpidine should therefore be removed from the literature.

This observation tends to confirm the formulas $C_{44}H_{75}O_{18}N$ or $C_{44}H_{77}O_{19}N$ for solanine-*s* and $C_{26}H_{43}O_3N$ for solanidine-*s*[5] with which the following additional analyses[6] are also in agreement.

Solanine-*s* (*ex S. sodomaeum*): Calcd. for $C_{44}H_{75}O_{18}N \cdot 2H_2O$: C, 56.11; H, 8.39; N, 1.47. Found: C, 56.10, 55.98; H, 8.64, 8.68; N, 1.83, 1.75.

Solanidine-*s* (*ex S. sodomaeum*): Calcd. for $C_{26}H_{43}O_3N$: C, 74.82; H, 10.31; N, 3.35. Found: C, 75.18, 75.10; H, 10.51, 10.57; N, 3.32, 3.38.

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(1) Saiyed and Kanga, *Proc. Indian Acad. Sci.*, **4A**, 255 (1936).

(2) Oddo and Caronna, *Ber.*, **69B**, 283 (1936), and earlier papers.

(3) Anderson and Briggs, *J. Chem. Soc.*, 1036 (1937).

(4) *Cf.* Briggs, THIS JOURNAL, **59**, 1404 (1937).

(5) *Cf.*, however, Rochelmeyer, *Arch. Pharm.*, **275**, 336 (1937).

(6) Analyses by Dr.-Ing. A. Schoeller.