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Oxidation-Reduction Potentials of β-Hydroxyphenazine and N-Methyl-β-oxyphenazine[1]

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The rapidly increasing number of type oxidation-reduction systems[2] shown to follow the mathematical formulations of the potentials of the two-step oxidation-reduction process[3] suggest that all electromotively active reversible oxidation-reduction systems are probably of this same general character. To gain further evidence on the important questions of the nature of the two-step process and of the effect on the potentials of the structure of the molecule and of substitution, a survey of the various types of systems is being conducted.

The potentials of β-hydroxyphenazine and of N-methyl-β-oxyphenazine (methyl aposafranone), because of their structural relation to the natural pigment pyocyanine, are of particular interest and are reported here. The oxidation-reduction reactions of β-hydroxyphenazine appear to involve both nitrogens of the phenazine nucleus, while those of N-methyl-β-oxyphenazine involve the oxygen and its para-position nitrogen. Both systems show clearly the two-step process in solutions more acid than *p*H 3 and a transformation into an apparent one-step process in solutions less acid. They illustrate the fundamental characteristics of their respective type systems and the effects of the ionizations of the components upon the oxidation-reduction potentials.

Experimental

β-Hydroxyphenazine was prepared[4] by condensing *o*-phenylenediamine and 2-hydroxy-1,4-quinone.[5] The resulting dark brown material was extracted repeatedly with boiling water and the extract was filtered off and allowed to cool slowly. The dark red hydrated crystals which were obtained were changed to the golden yellow anhydrous compound by heating to 110°.

N-Methyl-β-oxyphenazine was prepared by treating the acetyl derivative[4] of this yellow compound with methyl sulfate, decomposing the addition compounds so formed, and separating the desired material from isomeric substances. Recrystallization from water gave the characteristic dark red needle-like crystals.

The potentials of two platinum electrodes, immersed in the buffer containing the oxidant and its reduction products, were measured against a saturated calomel half-cell connected to the solution by a 5% agar-saturated potassium chloride bridge. Electrodes usually agreed to within 0.0002 volt and equilibrium conditions were attained rapidly. The calomel cell was standardized against a hydrogen electrode in 0.05 *M* potassium hydrogen phthalate. The *p*H values of the buffers were determined by a hydrogen electrode.

The substances used for buffers were: citrates from *p*H 1.48 to 4.73, phosphates from *p*H 5.79 to 6.71 and 10.69 to 11.69, carbonates from *p*H 9.13 to 9.54, and veronal from *p*H 7.48 to 8.52. Hydrochloric acid or sodium hydroxide was used to make up the more acid or more alkaline solutions.

The buffers were 0.05 to 0.10 *M* in buffering ion and the reactants about 0.0005 to 0.0001 *M*, as indicated. No corrections were made for the *p*H changes accompanying the reduction. An equivalent amount of sodium hydroxide was added to the titanous chloride titrating solutions to compensate for the excess of hydrochloric acid (7.493 *N*) in the commercial titanous chloride (1.29 *M*) preparations. Titanous chloride was used as reducing agent in buffers from *p*H 0.04 to 4.73 and sodium hydrosulfite in buffers less acid.

Commercial tank nitrogen, deoxygenated by passing over heated copper, was used to deoxygenate and stir the solutions. Temperature was maintained to within 0.1° of 30.0° by immersing the electrode vessels in a suitable bath.

The mixture of oxidant and its reduction products was made by titrating the deoxygenated, buffered oxidant solution with reducing agent similarly prepared. The solutions of N-methyl-β-oxyphenazine consisted of 50 cc. of buffer and 5 cc. of water containing 5 mg. of compound. The solutions of β-hydroxyphenazine consisted of 50 cc. of buffer, and 4 cc. of water and 1 cc. of an alcoholic solution containing 5 mg. for all buffers *p*H 0.04 to 1.48 and 8.40 to 9.54; 4.9 cc. water and 0.1 cc. for *p*H 2.05 to 5.79; and 4.8 cc. water and 0.2 cc. for *p*H 6.71 to 7.48. From 10 to 15 cc. of titrating solution was usually added.

Titrations of β-hydroxyphenazine by reducing agents, in buffers of higher *p*H than 9.54, and the alternative of reductions at lower *p*H and subsequent titrations at higher *p*H by oxidizing agents, did not give satisfactory results, probably because of the relatively high reducing intensity or the low solubility of the compounds.

The titration curves obtained for solutions at or below *p*H 2.05 show partial merging of the second step of the titrated system with the titanic-titanous system, however,

(1) Presented before the American Society of Biological Chemists, Washington, D. C., March, 1936; Abstract, *J. Biol. Chem.*, **114**, lxxxi (1936).

(2) L. Michaelis, *Chem. Rev.*, **16**, 243 (1935); E. S. Hill and P. A. Shaffer, *J. Biol. Chem.*, **114**, li (1936); L. Michaelis, THIS JOURNAL, **58**, 873 (1936); P. W. Preisler and L. H. Hempelmann, *ibid.*, **58**, 2305 (1936).

(3) L. Michaelis, *J. Biol. Chem.*, **96**, 703 (1932); B. Elema, *Rec. trav. chim.*, **50**, 807, 1004 (1931); *J. Biol. Chem.*, **100**, 149 (1933).

(4) F. Kehrmann and F. Cherpillod, *Helv. Chim. Acta*, **7**, 973 (1924).

(5) R. Willstätter and F. Müller, *Ber.*, **44**, 2180 (1911).

by employing the proper geometrical method, a close estimation of the mid-point of the second step was attained.

The colors of the oxidants and the reductants in the hydrochloric acid solutions are various shades of yellow and the semiquinones are yellow-green. N-Methyl-β-oxyphenazine is red in solutions more alkaline than *p*H 5; a colorimetric determination of the ionization constant gave $pK = 3.05$, in close agreement with the electrometrically determined value of 3.0. Although the ionized forms of the oxidant and the reductant of β-hydroxyphenazine are deeper yellow than the un-ionized, the low solubility of the un-ionized forms prevent the accurate estimation of the ionization constants by visual methods.

Discussion

The characteristic points of the titration curves are given in the table and figures, $E'_{0\text{oxid-red}}$ being the mid-point or the 50% reduction point and $E'_{0\text{oxid-semi}}$ and $E'_{0\text{semi-red}}$ being the 25 and 75% reduction points of the curves of E'_0 against per cent. of reduction at the respective *p*H values. The quantities in parentheses were calculated[3] from the "index potentials," $(E_{1/4} - E_{3/4})/2$, and represent the mid-points of the separate one-equivalent curves, which in this region overlap or merge into each other.

species. Each essentially straight-line portion represents a region in which particular ionic or molecular species predominate and each intermediary curved portion represents a region of transition mixtures. The chemical reactions which probably predominate in these straight line portions reveal that the slope of any curve of E'_0 against *p*H is apparently determined by the number of hydrogen ions and electrons involved in the equilibria, being at 30° where $RT/F = 0.06$ volt

$$\frac{E'_0}{p\text{H}} = 0.06 \text{ volt} \times \frac{(\text{number of } H^+)}{(\text{number of electrons})}$$

The chemical equations for β-hydroxyphenazine for the sections as numbered at the top of Fig. 1, using "(pz)" to indicate the two phenylene nuclei connected by two nitrogen atoms without designating the positions of the double bonds or the unusual valencies or configurations of the semiquinone, are as follows:

The first equation of each pair represents the oxidant–semiquinone system and the second the semiquinone–reductant system: the sum of both would represent the oxidant–reductant system whose slope would be the mean value of the other two.

1. $HO(pz)H^+ + e + H^+ = HO(pz)H^+\cdot H$
   (slope = 0.06 × 1/1 = 0.06)
   $HO(pz)H^+\cdot H + e + H^+ = HO(pz)H^+\cdot HH$
   (slope = 0.06 × 1/1 = 0.06)

POTENTIALS OF MIXTURES OF EQUIVALENT AMOUNTS OF OXIDANTS AND OF REDUCTANTS IN BUFFERS AT VARIOUS *p*H

	β-Hydroxyphenazine, volts				N-Methyl-β-oxyphenazine, volts			
*p*H	$E'_{0\text{oxid-red}}$	$\frac{E_{1/4} - E_{3/4}}{2}$	$E'_{0\text{oxid-semi}}$	$E'_{0\text{semi-red}}$	$E'_{0\text{oxid-red}}$	$\frac{E_{1/4} - E_{3/4}}{2}$	$E'_{0\text{oxid-semi}}$	$E'_{0\text{semi-red}}$
0.00			+0.297[a]	+0.079[a]			+0.316[a]	+0.078[a]
.04			.295	.077			.314	.076
.38			.274	.057			.295	.058
.78			.246	.033			.270	.042
1.09			.228	.020			.250	.036
1.48			.199	.007			.225	.029
2.05			.161	− .009			.187	.020
2.97	+0.027	+0.050	(+ .075	(− .021)	+0.070	+0.049	(+ .118)	(+ .022)
3.56	− .001	.030	(+ .020)	(− .022)	.039	.030	(+ .060)	(+ .018)
4.00	− .026	.022	(− .026)	(− .026)	.015	.022	(+ .015)	(+ .015)
4.46	− .053	.017	(− .081)	(− .025)				
4.73	− .069	.015			− .028	.017	(− .056)	(.000)
5.79	− .137	.014			− .092	.016	(− .127)	(− .057)
6.71	− .194	.014			− .151	.016	(− .186)	(− .116)
7.48	− .248	.014			− .193	.015		
8.40	− .316	.015						
8.52					− .225	.015		
9.13	−. 393	.014						
9.54	−. 436				− .314	.015		
10.69					− .361	.014		
11.69					− .397			
12.60					− .426	.015		

[a] Calculated.

By plotting the E'_0 values of the three systems against *p*H and projecting the straight line portions of the curves, intersections are formed which represent the ionization constants of the various

2. $HO(pz)H^+ + e + H^+ = HO(pz)H^+\cdot H$
(slope = 0.06 × 1/1 = 0.06)
$HO(pz)H^+\cdot H + e = HO(pz)\cdot HH$
(slope = 0.06 × 0/1 = 0.00)
3. $HO(pz) + e + 2H^+ = HO(pz)H^+\cdot H$
(slope = 0.06 × 2/1 = 0.12)
$HO(pz)H^+\cdot H + e = HO(pz)\cdot HH$
(slope = 0.06 × 0/1 = 0.00)
4. $HO(pz) + e + H^+ = HO(pz)\cdot H$
(slope = 0.06 × 1/1 = 0.06)
$HO(pz)\cdot H + e + H^+ = HO(pz)\cdot HH$
(slope = 0.06 × 1/1 = 0.06)
5. $^-O(pz) + e + 2H^+ = HO(pz)\cdot H$
slope = 0.06 × 2/1 = 0.12)
$HO(pz)\cdot H + e + H^+ = HO(pz)\cdot HH$
(slope = 0.06 × 1/1 = 0.06)

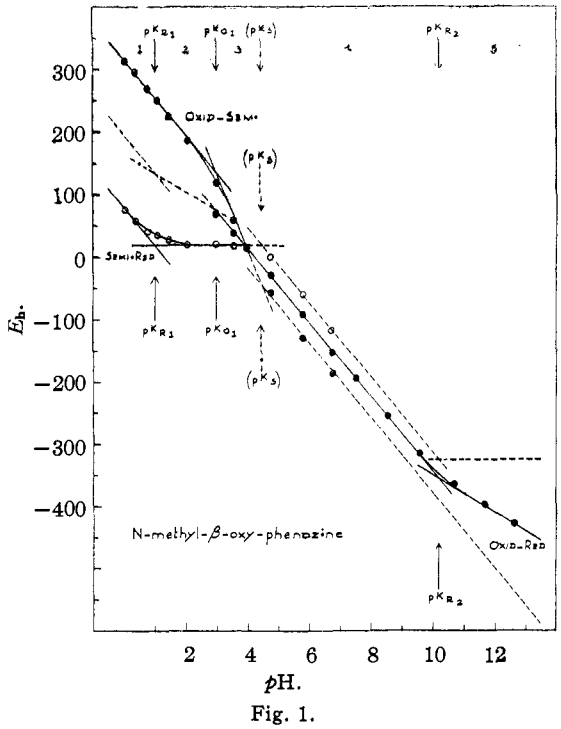


Fig. 1.

The ionization constants are: pK_{O_1} = 2.6 cation to free base; pK_{O_2} = 7.5, free base to anion; pK_S = (4.9) (approximate) cation to free base; pK_{R_1} = 1.8, cation to free base. The ionizations are accompanied by a noticeable change in color and an increase in solubility. The one additional anionic ionization in the semiquinone and the two in the reductant, which might occur at the hydrogens added in the reduction process, and the anionic ionization of the H from the OH group of the semiquinone and of the reductant are not evident in the pH regions studied.

The equations for N-methyl-β-oxyphenazine are of the same general character for the first four sections shown in Fig. 2, with the exception that the HO-group is replaced by an O⁻ with consequent rearrangement of the double bonds and loss of one point of ionization, and the addition of a CH_3– group to the nitrogen atom meta to the oxygen. The equations for section 5 are

5. $(pzCH_3) + e + H^+ = (pzCH_3)\cdot H$
(slope = 0.06 × 1/1 = 0.06)
$(pzCH_3)\cdot H + e = (pzCH_3)\cdot{}^-H$
(slope = 0.06 × 0/1 = 0.00)

The ionization constants are: pK_O = 3.0, cation to free base; pK_S = (4.4) (approximate) cation to free base; pK_{R_1} = 1.0, cation to free base; pK_{R_2} = 10.1, free base to anion. The one anionic ionization of the semiquinone and the second in the reductant, which might occur at the hydrogens added by the reduction, were not evident in the pH regions investigated.

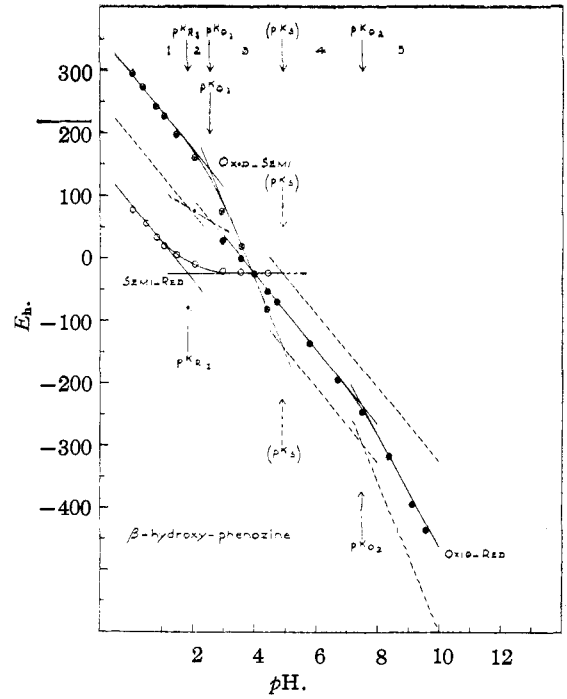
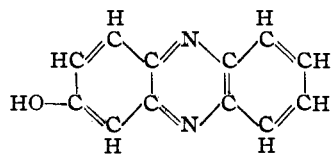


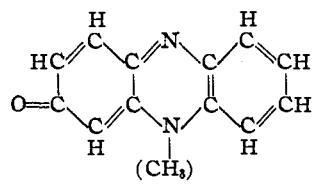
Fig. 2.

Only approximate values for the ionization constants for the semiquinones could be estimated, because the titration curves did not show a sufficiently high "index potential" to locate precisely the E_0' againts pH curves for the systems involving semiquinones. The effect of an ionization of a semiquinone is to change the solpe of the oxidant-semiquinone curve exactly to the same amount and in the opposite direction as the slope of the semiquinone-reductant curve, thereby causing no change in the slope of the resultant oxidant-reductant curve and preventing the detection there of any semiquinone ionizations.

The structure of β-hydroxyphenazine indicates that the oxidation–reduction system could involve either the two nitrogens or the oxygen and the nitrogen para to it. In the case of the N-methyl derivative, only one structure is possible, assuming that the methyl group does not have the power to shift its position.



β-Hydroxyphenazine



N-Methyl-β-oxyphenazine
(methylaposafranone)

Pyocyanine, N-methoxyl-α-oxyphenazine, and α-hydroxyphenazine have a similar relationship involving the ortho positions. The semiquinones in these cases have one additional hydrogen atom or electron, and the reductants two. The oxidants and reductants have the same structural relationship as quinone-imine to aminophenol; the structure of the semiquinones is discussed in the literature cited.[2,3]

In buffers at *p*H 6, the region of lowest semiquinone formation and where the un-ionized forms predominate, the E_0' of N-methyl-α-oxyphenazine is about 0.125 volt more positive than the E_0' of N-methyl-β-oxyphenazine. This amount is greater than the separation, 0.088 volt, of the ortho-quinone and the para-quinone systems, indicating that they are probably related as ortho–para isomers. The difference between the α- and the β-hydroxyphenazine is only 0.030 volt and they lie in the same general region where the E_0' of phenazine[6] would be expected, so that they are probably behaving as hydroxy substituted phenazines.

The low solubility and lack of contrasting color changes makes β-hydroxyphenazine unsuitable as a colorimetric indicator of oxidation–reduction potential. N-Methyl-β-oxyphenazine is useful as an indicator of oxidation–reduction potential from *p*H 5.5 to 12 in a zone about 0.05 volt more negative than that of indigo disulfonate, changing from red to colorless or light yellow, and as an indicator of hydrogen ion concentration in the acid region from about *p*H 2 to 4, changing from yellow to red. Because of the similarity of the color changes, care must be exercised, when it is used as an indicator, to distinguish one change from the other by suitably adjusting conditions in the material to be tested.

Summary

The oxidation–reduction potentials of β-hydroxyphenazine and N-methyl-β-oxyphenazine have been determined by an electrometric method and the ionization constants of the oxidants and of the reductants have been calculated from the data. The N-methyl-β-oxyphenazine has properties suggesting its use as an indicator for oxidation–reduction potential and for hydrogen ion concentration. The chemical structure of the compounds and certain theoretical aspects of semiquinone formation and oxidation–reduction potentials are discussed.

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(6) L. Michaelis and E. S. Hill, THIS JOURNAL, 55, 1493 (1933).