Quinone–quinol equilibria in solutions of 2-(β-hydroxyethyl)aminobenzo-1,4-quinones. A spectrophotometric and polarographic study

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2-(β-Hydroxyethyl)aminobenzo-1,4-quinones are electrochemically reduced in two steps. The more positive wave corresponds to the reduction of the quinone form, the second at a potential more negative by 0.7 V is attributed to the reduction of the less conjugated quinol. Electronic spectra enabled determination of equilibrium constants (K = [quinone]/[quinol]) as well as the equilibrium constants for the addition of hydroxide ions to the 1-carbonyl group. Changes of polarographic limiting current with pH indicated that the conversion of the quinone into quinol is base catalyzed. The addition of OH$^-$ ions is followed by a ring formation, resulting from an attack of the -O$^-$ group on the β-carbon in the side chain. Structural effects on the measured overall constant K indicate that substituents on the quinone ring and in the side chain exert different effects on addition of OH$^-$ and on the rate-determining step. Oxidation–reduction potentials of these quinones follow linear free energy relationships as expected. As 2-(β-hydroxyethyl)aminobenzoquinones are formed by hydrolysis of ethyleneiminoquinones, used as cancerostatics, observed equilibria may occur under physiological conditions.

Hydrolysis of ethyleneiminoquinones, which are important cancerostatic agents, yields 2-(β-hydroxyethylamino)benzo-1,4-quinones.[1,2] These hydrolytic products can be electrochemically reduced and, when a dropping mercury electrode is used, yield two well separated polarographic waves.[3] The more positive wave was attributed[4] to the reduction of the quinonoid form, the more negative one to the quinol form. This latter form results from ring formation involving the β-hydroxyethylamino side chain and a carbonyl group. The evaluation of the role of reaction conditions and some structural effects on the quinone–quinol equilibria and comparison of oxidation–reduction potentials have enabled us to propose a plausible mechanism of reactions involved. Some aspects of the chemistry and electrochemistry of such compounds have been reported earlier,[4–14] but this contribution offers a re-evaluation of all available, reliable data and their interpretation together with new experimental evidence. The present discussion is limited to the behavior of β-hydroxyalkylaminobenzo-1,4-quinones at pH larger than 3 but lower than 10. At pH below 3 these compounds undergo elimination.[15] At $\mathrm{pH} > 10$, on the other hand, a cleavage of the quinone ring takes place, initiated probably by a Michael addition, as with other quinones.

Experimental

Instrumentation

Current–voltage curves were recorded using a Polariter PO4 (Radiometer, Copenhagen), the Universal Polarograph OH-105 (Radelkis, Budapest) and the polarographs LP55 and LP60 (Laboratorní, Přístroje, Prague). The capillaries used for the dropping mercury electrode had an outflow velocity m between 1.5 and 2.5 mg s^{-1} and a drop-time $t_1 \approx 3$ s at pressure $h = 700$ mmHg. The electrolytic cell was thermostatted at (25 ± 0.05) °C unless the effect of temperature variation was studied. It contained a mercurous sulfate reference electrode, which prevented traces of chloride, which, especially at lower pH-values, interfered with the exact measurement of half-wave potentials.

pH Measurements were carried out with the PHM4 pH-meter (Radiometer, Copenhagen) and the 202B glass electrode.

Instruments for cyclic voltammetry designed by J. Vogel and J. Říha, as well as that for rectangular voltage polarization for the Kalousek commutator,[16] were self-constructed.

The instrumentation used for temperature-jump kinetics,[14] fluorescence measurement,[11] and ^{13}C-NMR spectrometry[10] has been described elsewhere.

The UV-visible absorption spectra were recorded using spectrophotometers SP-700 (Unicam, Cambridge) and Specord UV/VIS (Carl Zeiss, Jena) using 10 and 20 mm cells.

Materials

All quinones used were prepared in the Central Institute of Microbiology and Experimental Therapy in Jena[17] and were recrystallized before use. Chemicals for preparation of buffers and supporting electrolytes were of analytical grade. The solvents used were of spectroscopic quality. Stock solutions of quinones (0.01 M) were prepared either in DMF (for polarography) or in ethanol (for both polarography and spectrophotometry).

Procedures

For electroanalytical measurements (polarography, voltammetry and rectangular voltage polarization in the Kalousek commutator), 10 cm^3 of an aqueous acetate, phosphate or borate buffer was deoxygenated by a stream of nitrogen, then 0.1 or 0.2 cm^3 of the 0.01 M stock solution of the quinone was added and after a sharp, short burst of nitrogen (for 30 s) the current–voltage curve was recorded. The final concentration of the quinone was thus practically 0.1 or 0.2 mM in a solution containing about 1 or 2% of DMF. At $\mathrm{pH} < 3.5$ and $\mathrm{pH} > 9$ the curve was again recorded after 10 min to prove the absence of a homogeneous reaction.

Composition of solutions for UV-visible spectrophotometry was similar, with ethanol, in some instances, replacing DMF.

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Results and discussion

Most benzo- and naphtho-1,4-quinones substituted by alkyl, amino, halogen, hydroxy, or alkoxy groups are reduced in buffered protic solvents in a single, two-electron step.[18–22] The half-wave potentials for the reduction of these quinones are identical with those of the oxidation of the corresponding hydroquinones indicating that within the frame of the polarographic measurement the process can be described as reversible. This means that the rate of both the electron transfer and that of the establishment of accompanying acid–base equilibria are fast when compared with the time-window of the measurement. Whereas on platinum electrodes another sequence may apply,[23,24] the role of adsorption is not predominant when the dropping mercury electrode is used.

For a reversible system, where the reduced form undergoes a two-step dissociation with constants $K_{1,\mathrm{red}}$ and $K_{2,\mathrm{red}}$, the half-wave potentials depend on pH, following the equation: $E_{1/2} = E^\circ + RT/nF \ln([\mathrm{H}^+]^2 + K_{1,\mathrm{red}}[\mathrm{H}^+] + K_{1,\mathrm{red}}K_{2,\mathrm{red}})$. The $E_{1/2}$ of such a system is thus independent of pH only at pH > p$K_{2,\mathrm{red}}$, where the ionic form predominating in the bulk of the solution is also the electroactive species. In all other pH ranges the half-wave potentials of such reversible systems are shifted per pH-unit by $-0.059\ p/n$ V, where p is the number of protons needed in a given pH range to convert the species predominant in the bulk of the solution into the electroactive form and n is the number of the transferred elections.

The observed shifts of half-wave potentials with pH having slopes of 0.059 V per pH unit at pH < p$K_{1,\mathrm{red}}$, 0.029 V per pH unit at p$K_{1,\mathrm{red}}$ < pH < p$K_{2,\mathrm{red}}$ and 0.0 V per pH unit at pH > p$K_{2,\mathrm{red}}$ indicate that the two-electron transfer yields a dianion. Depending on the pH range, this transfer is followed by a fast transfer of two (at pH < p$K_{1,\mathrm{red}}$), one (at p$K_{1,\mathrm{red}}$ < pH < p$K_{2,\mathrm{red}}$) or zero (at pH > p$K_{2,\mathrm{red}}$) protons.

Most quinones that are substituted in position 2 by a β-hydroxyethylamino group show a behavior[3–14] different from those quinones mentioned above. Their polarographic and voltammetric reduction occurs in two waves (i_1 and i_2), the half-wave potentials of which differ by more than 700 mV. The more positive wave, i_1, has all the characteristics typical of reductions of quinonoid species, including the identity of the half-wave potentials of the reduction of quinone and of the oxidation of the hydroquinone. As samples of β-hydroxyethylamino-1,4-hydroquinones were not available, they were synthesized *in situ* by hydrogenation of the corresponding quinone in the presence of palladium sol as catalyst. The reversibility of the process occurring in wave i_1 has been further confirmed by the independence of the half-wave potential of the reduction wave i_1 of the concentration of the quinone over at least two orders of magnitude, from 0.01 to 1.0 mM. The system is reversible even under conditions of cyclic voltammetry, between $v = 10$ mV s^{-1} and 200 mV s^{-1}. Cyclic voltammograms of the studied quinones showed a cathodic peak (with current i_{pc} at potential E_{pc}) and an anodic peak (with current i_{pa} at potential E_{pa}) on the reverse sweep. The quantities $E_{\mathrm{pc}} - E_{\mathrm{pa}} = (0.030 \pm 0.003$ V) and $i_{\mathrm{pc}}/i_{\mathrm{pa}}$ close to 1.0 were appropriate for a reversible two-electron process. Finally, the half-wave potentials of the oxidized and reduced form, obtained by rectangular voltage polarization using the commutator by Kalousek and Rálek[16] agreed to within ± 0.003 V.

The wave i_2 at more negative potentials corresponds, on the other hand, to a two-electron irreversible process. The number of electrons transferred in this more negative process is indicated by the sum of $i_1 + i_2$, which remains constant between pH 5 and 10. As the process occurring in wave i_1 corresponds to a two-electron transfer, the process in wave i_2 must also involve two electrons. The irreversibility of this process was demonstrated by an absence of an anodic wave in the potential range where wave i_2 was observed in a solution saturated with hydrogen in the presence of palladium sol. The irreversibility was further confirmed by the absence of an anodic peak on cyclic voltammograms at potentials close to that of the second cathodic peak (E_{pc2}) in solutions of these β-hydroxyethylaminoquinones as well as by the absence of an anodic wave when the commutator method[16] was used.

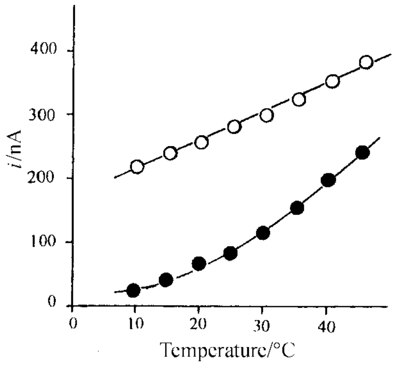


Fig. 1 Temperature dependence of the diffusion controlled total current ($i_1 + i_2$, ○) and of the first, kinetic wave i_1 (●) in 2 × 10^{-4} M solution of 2-(*N*-methyl-*N*-β-hydroxyethyl)aminobenzo-1,4-quinone in a phosphate buffer at pH 7.0 containing 20% methanol.

The ratio $i_1 : i_2$ is independent of concentration of the parent quinone, indicating an equilibrium involving two reactions of the same order. As the independence of $E_{1/2}$ and i_1 of concentration of the quinone indicates absence of dimerization of the quinone, the equilibrium involved must be first order in both directions (Q$\rightleftharpoons$C). Over the entire pH range from 3 to 10, where homogeneous reactions do not interfere, the sum $i_1 + i_2$ remains constant and corresponds to a two-electron diffusion-controlled process. The ratio $i_1 : i_2$ increases at a given pH with increasing temperature, as the relative height of wave i_1 gradually increases and that of i_2 decreases (Fig. 1). The relative height of wave i_1 also increases with increasing pH. For some of the studied compounds the limiting current i_1 is well developed (Fig. 2a), in others the limiting current decreases with increasingly negative potentials (Fig. 2b). In the latter case the current i_1 was measured at most positive potentials. In all cases the plot of the current i_1 as a function of pH has the shape of a dissociation curve, increasing with increasing pH (Fig. 3). At the pH where the current i_1 is less than 15% of the total wave-height ($i_1 + i_2$), the current is independent of mercury pressure and shows a temperature coefficient, a, larger than 5% deg^{-1}. This dependence causes a change in the ratio $i_1 : i_2$ with temperature, as mentioned above. When the instantaneous current during the life of a single mercury drop was measured and plotted as a function of time, a parabola with a 2/3 exponent was observed. All these results indicate that the current i_1 at pH *ca.* 3 to 6 is limited by the rate of a chemical reaction occurring in the vicinity of the electrode surface (kinetic current).

In the interpretation of the dependence of the current i_1 on pH it is possible to exclude the most frequently encountered type of chemical reactions preceding the electron transfer, namely the generation of a reducible acid from its conjugate base at the electrode surface. For such reactions, where the conjugate acid is more readily reduced than the base, the limiting current of the more positive wave decreases with increasing pH. The observed increase in i_1 with increasing pH is thus attributed to an increase in the rate of a base catalyzed reaction producing the more easily reducible species. All reactions involved must be equilibria which are perturbed by a removal of the product at the electrode surface by reduction. The rate of reestablishment of such equilibria governs i_1. This corresponds to equilibria (1)–(4).

$$\mathrm{Q} + \mathrm{OH}^- \underset{\text{fast}}{\rightleftharpoons} \mathrm{C{-}OH}^- \quad K_\mathrm{a} \tag{1}$$

$$\mathrm{C{-}OH}^- \underset{\text{rds}}{\rightleftharpoons} \mathrm{C} \tag{2}$$

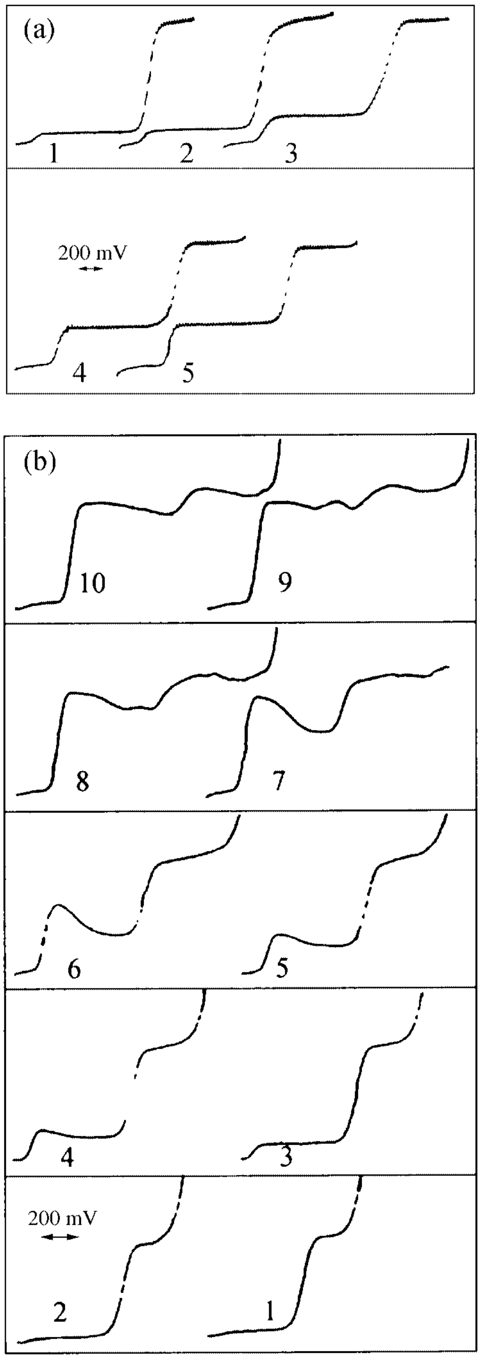


Fig. 2 Dependence of current–voltage curves of 4 × 10^{-4} M quinones on pH in Britton–Robinson buffers. (a) 2-Bis(β-hydroxyethyl)-aminobenzo-1,4-quinone (**10**, Table 1) at pH: (1) 6; (2) 7; (3) 8; (4) 9; (5) 10. Full current scale: 0.8 μA, curves starting at 0.0 V. (b) 2-*N*-methyl-(β-hydroxyethyl)aminobenzo-1,4-quinone (**11**, Table 1) at pH: (1) 3.2; (2) 4.1; (3) 5.2; (4) 5.9; (5) 6.5; (6) 7.2; (7) 8.0; (8) 8.7; (9) 9.2; (10) 10.0. Full current scale: 0.8 μA, curves starting at 0.0 V, $T = 25$ °C.

$$Q + ne \rightarrow P_1 \quad i_1 \tag{3}$$

$$C + me \rightarrow P_2 \quad i_2 \tag{4}$$

In agreement with the theory for electrolysis of systems involving addition of OH^- ions,[25] the pH at the point where $i_1 = (i_1 + i_2)/2$ (denoted by pK') is smaller than the equilibrium pK_a determined spectrophotometrically. This can be demonstrated for 2-(*N*-2′-hydroxyethyl-*N*-methylamino)benzo-1,4-quinone, where p$K' = 7.2$ and p$K_a = 9.6$.

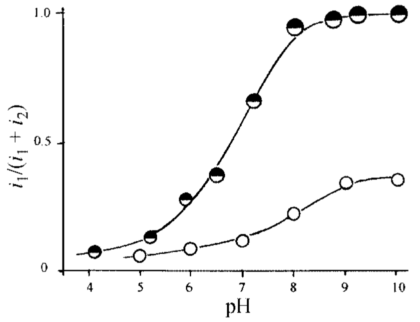


Fig. 3 Dependence of the relative current $i_1/(i_1 + i_2)$ on pH in 4 × 10^{-4} M solutions of quinones in Britton–Robinson buffers. ○ 2-Bis(β-hydroxyethyl)aminobenzo-1,4-quinone (**10**, Table 1) and ◓ 2-*N*-methyl-(β-hydroxyethyl)aminobenzo-1,4-quinone (**11**, Table 1).

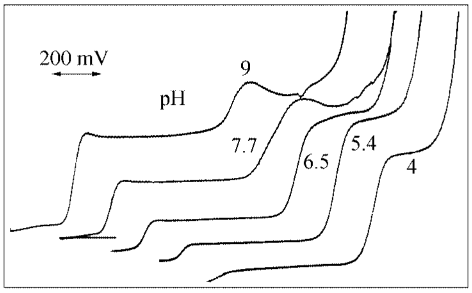
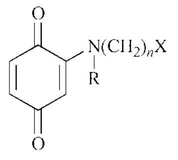


Fig. 4 Dependence of current–voltage curves of 4 × 10^{-4} M 2-*N*-phenyl-(β-hydroxyethyl)aminobenzo-1,4-quinone (**15**, Table 1) in Britton–Robinson buffers containing 20% DMF on pH. Full scale: 0.8 μA, curves starting at 0.0 V, $T = 25$ °C.

If chemical reactions preceding the electron transfer occur in the vicinity of the electrode as homogeneous processes, it is possible from known values of pK' and pK_a to calculate the rate constants of the reaction generating the electroactive species.[26] The current–voltage curves of most of the studied β-hydroxyethylaminobenzoquinones show a decrease in the limiting current of wave i_1 with increasingly negative potentials (Fig. 2b). This indicates that the rate of formation of the quinonoid form decreases with increasingly negative potentials. Such decreases are attributed to a desorption of reactants from the electrode surface. The shape of the current–voltage curves also serves as proof that the chemical reaction occurs as a heterogeneous process at the electrode surface. The role of desorption is further supported by the absence of the current drop in buffers containing 20% DMF (Fig. 4), where the organic solvent minimizes adsorption effects.

For such heterogeneous processes involving a preceding chemical reaction, rigorous analysis enabling determination of the rate constant is currently not available. An exception is the behavior of 2-bis(β-hydroxyethyl)aminobenzo-1,4-quinone[14] (**I**, $n = 2$, X = OH, R = CH_2CH_2OH) in buffered solutions containing 22% EtOH. Under these conditions the shape of the *i*–*E* curves is ideal (Fig. 2a), which indicates that the addition of the hydroxide ion to the quinone form occurs as a homogeneous system. Weber *et al.*[14] attempted to calculate rate constants, using the overall equilibrium constant, *K*, which makes it uncertain which two of the four rate constants involved were actually calculated. It seems plausible that the rate constants reported, obtained by polarography and a temperature jump method, correspond to equilibrium (2).



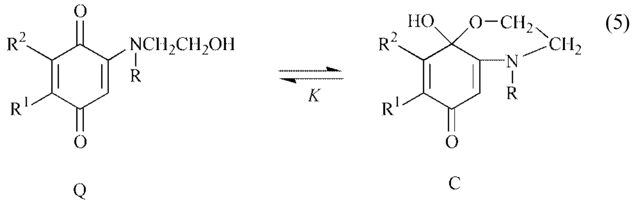
I

For reaction (1) involving addition of OH^- ions to a carbonyl group (see the Reaction mechanism section) it is possible to calculate the rate constant using the dependence of i_1 on pH (Fig. 3). Denoting the pH where $i_1 = i_1/(i_1 + i_2)$ is equal to 0.5 by pK', the expression[26] $\log k_{OH} = 2\ pK' - pK_a - 2 \log 0.886 - \log t_1$ for $pK' = 8.1$ (Fig. 2a), $pK_a = 10$ and $t_1 = 3.0$ s then yields the value $k_{OH} = 6.3 \times 10^5$ s. This value is comparable with rate constants obtained for addition of hydroxide ions to benzaldehydes[25] and tropylium ions.

Nevertheless, even for heterogeneous processes, the dependences of polarographic currents i_1 as a function of pH indicate the presence of equilibria (1) and (2) and prove that the rate determining step, equilibrium (2), is base catalyzed.

To distinguish structural conditions for observations of equilibria (1) and (2), manifested by two separate polarographic waves (i_1 and i_2), the reduction of numerous benzo-1,4-quinones substituted in the 2 position (**I**) was tested. The presence of a β-hydroxyethylamino group (**I**, $n = 2$, X = OH) in position 2 has been proved to be the necessary condition for the appearance of two waves. In the absence of the amino group, as well as in the presence of $-NH_2$ (**I**, R = H, $n = 0$, X =H), -NH alkyl (**I**, R = alkyl, $n = 0$, X = H) or $-N(alkyl)_2$ (**I**, R = alkyl, X = H) groups, only a single wave of the reduction of the quinone ring was observed. Similarly, a single quinone wave was observed when the terminal hydroxy group in the side-chain was replaced by an alkoxy group (**I**, $n = 2$, X = O alkyl) or a halide (**I**, $n = 2$, X = Cl or Br). Moreover, the separation of the two waves did not occur in **I** where $n = 1$ and X = OH or when $n = 3$ and X = OH. All the above mentioned compounds are reduced in a single two-electron reversible diffusion-controlled wave corresponding to the reduction of the quinonoid system in a hydroquinone.

Product C in equilibrium (2) is hence not a quinone, and it has been proved that a 2′-OH group is necessary for the reaction which results in formation of the intermediate $C\text{-}OH^-$, and that the forward reactions (1) and (2) result in the formation of a species where the carbocyclic ring is annulated by the six-membered ring. Formation of a five- or seven-membered ring is not favored. Hence equilibrium (5) is proposed.



(5)

To determine spectrophotometrically the value of the tautomeric equilibrium constant of equilibrium (5), defined as $K = [Q]/[C]$, it is first necessary to find the optimum pH range, where equilibrium (1), involving addition of OH^- ions, is shifted to the left hand side. The molar absorptivity (ε) of the quinone form Q can be estimated from the spectra of 2-aminoquinones which do not participate in equilibria of the type given in equilibrium (5). Such quinones are characterized by an absorption band at about 500 nm ($\log \varepsilon \approx 3.5$) accompanied by a even stronger band at about 285 nm ($\log \varepsilon \approx 4$).

The product of the addition of the OH^- ions ($C\text{–}OH^-$ in equilibrium (1)) shows a weak band at about 500 nm (log ε between 1.5 and 2.5) and a strong band ($\log \varepsilon > 4$) at about 380 nm. The addition of OH^- with increasing pH is best followed by measuring absorbance at the minimum at 270 nm, where the contribution of absorbance due to the form Q is negligible (Fig. 5). The absorbance at 270 nm remains unchanged between pH 5 and 7. A plot of the increase of absorbance at 270 nm as a function of pH has the shape of a dissociation curve, for which 2-(*N*-2′-hydroxyethyl-*N*-methylamino)benzo-1,4-quinone (**I**, $R = CH_3$, $n = 2$, X = OH) has an inflexion point corresponding to $pK_a = 9.6$ for the equilibrium (6). This pK_a is lower than that observed for the addition of hydroxide ions to benzaldehydes[25b] due to higher reactivity of the quinonoid system.

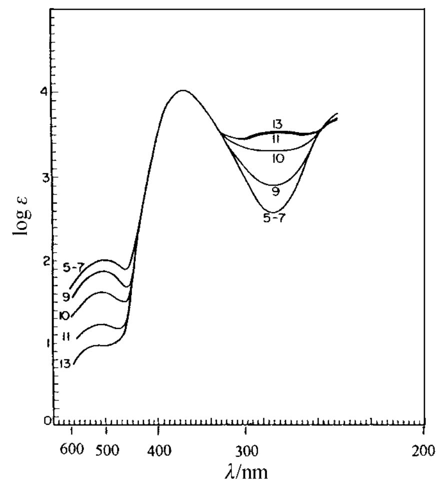
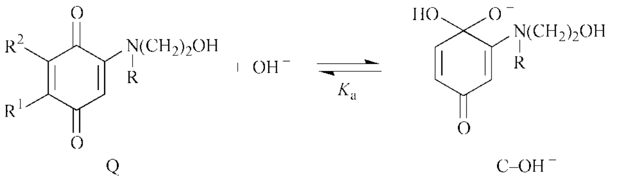


Fig. 5 Dependence of electronic spectra of 1×10^{-4} M solution of 2-*N*-methyl-(β-hydroxyethyl)aminobenzo-1,4-quinone (**11**, Table 1) in Britton–Robinson buffers containing 20% methanol on pH.



(6)

An alternative, namely protonation of the amino nitrogen in the side chain can be excluded, because such protonations of 2-aminoquinones have[22] $pK_a < 1.0$. Between pH 11 and 13 the absorbance of the adduct $C\text{–}OH^-$ is again pH-independent.

The measurement of the absorbance between pH 5 and 7 is thus best suited for evaluation of the tautomeric equilibrium constant K (equilibrium (5)). As at 500 nm the molar absorptivity of the quinol (C) is at least one (but usually up to two) order of magnitude smaller than that of the parent quinone, it is possible to estimate the equilibrium concentration of the quinone form Q by measuring absorbance at 500 nm and assuming $\log \varepsilon = 3.5$. Using this approach it was possible to calculate approximate values of the tautomeric constants $K = [Q]/[C] = [Q]/([Q]_{init} - [Q])$ (where [Q] is the equilibrium concentration of the quinonoid form and $[Q]_{init}$ is the initial, analytical concentration of the quinone) summarized in Table 1. Good agreement with data obtained from the temperature dependence of absorbance[6] (for **11**) indicates the validity of the above approximation. The role of the equilibrium (5) between the quinonoid and quinol forms has been further supported by fluorimetry[7,9,11] of quinone **3**, as the quinol form that absorbs at 280 nm yields a π→π* fluorescence band at about 510 nm. The quinonoid form manifested itself at about 500 nm only by an n→π* absorption and yields no fluorescence emission. With decreasing temperature the equilibrium is shifted in favor of the quinol form and at temperatures between −30 and −60 °C the fluorescence corresponds only to the quinol form.[11] The structure of the quinol form has also been confirmed by ^{13}C NMR.[10]

Reaction mechanism

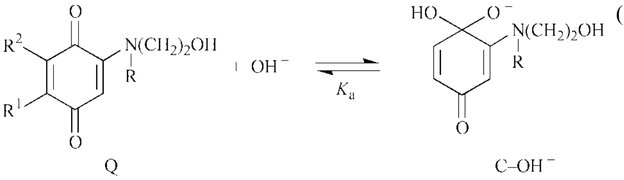
Based on the available evidence, particularly the spectrophotometrically investigated acid–base and tautomeric equilibria, as well as the dependence of the rate of the establishment of the latter equilibrium on pH, as manifested by the pH-dependence of polarographic waves, we propose the mechanisms given in equilibria (7) and (8) for the conversion of the quinonoid form.

Both of these reactions involve a nucleophilic attack, in (7) on an sp^2-carbon, in (8) on an sp^3-carbon. Distinguishing whether equilibrium (7) or (8) is the rate determining step is

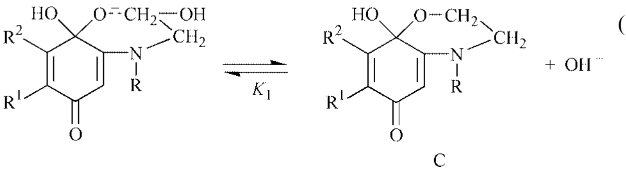
Table 1 Values of tautomeric equilibrium constants $K = [Q]/[C]$ for 2-(β-hydroxyethyl)aminobenzo-1,4-quinones from spectrophotometric measurements and of half-wave potentials of wave i_1 $(E_{1/2})_1$ and wave i_2 $(E_{1/2})_2$ at pH 7.0

No.	Substituents on benzo-1,4-quinone	K	log K	$(E_{1/2})_1$/V *vs.* SCE	$(E_{1/2})_2$/V *vs.* SCE
1	2-NHCH$_2$CH$_2$OH	0.63	−0.2	0.31	—
	5,6-(CH$_3$)$_2$				
2	2-N(CH$_2$CH$_2$OH)$_2$	—	—	−0.26	−1.25
	5,6-(CH$_3$)$_2$			−0.26[a]	−1.18
3	2-N(CH$_3$)CH$_2$CH$_2$OH	0.57	−0.24	−0.25	−1.29
	5,6-(CH$_3$)$_2$	—	—	−0.26[a]	−1.31[a]
		0.60[b,c]	−0.22[b,c]	−0.28[d]	−1.05[d]
		0.70[e]	−0.15[e]	−0.30[f]	−1.12[f]
4	2-N(CH$_2$CH$_2$CH$_3$)CH$_2$CH$_2$OH	—	—	−0.25[a]	−1.09[a]
	5,6-(CH$_3$)$_2$				
5	2-N(CH$_2$CH$_2$OH)$_2$	0.08	−1.10	−0.23	−1.25
	5-CH$_3$			−0.21	−1.01
6	2-N(CH$_3$)CH$_2$CH$_2$OH	0.28	−0.55	−0.21	−1.23
	5-CH$_3$	0.17[d]	−0.77[d]	−0.24[d]	−1.02[d]
		—	—	−0.22[a]	−1.16[a]
7	2-N(CH$_2$C$_6$H$_5$)CH$_2$CH$_2$OH	—	—	−0.21	−1.07
				−0.20[c]	−1.04[a]
8	2-N[CH$_2$CH(CH$_3$)$_2$]CH$_2$CH$_2$OH	0.01	−2.00	−0.18	−1.14
				−0.18[a]	−1.09[a]
9	2-N(CH$_2$CH$_2$C$_6$H$_5$)CH$_2$CH$_2$OH	—	—	−0.18	−1.05
		—	—	−0.17[a]	−1.00[a]
10	2-N(CH$_2$CH$_2$OH)$_2$	0.016	−1.80	−0.16	−1.16
		0.01[d]	−2.00	−0.17[a]	−1.01[a]
		0.005[g]	−2.3	—	—
11	2-N(CH$_3$)CH$_2$CH$_2$OH	0.063	−1.20	−0.16	−1.16
		0.06[h]	−1.22[h]	−0.18[a]	−1.06[a]
		0.028[d]	−1.55[d]	−0.21[d]	−1.10[d]
12	2-N(CH$_2$CH=CH$_2$)CH$_2$CH$_2$OH	0.032	−1.49	−0.16	−1.15
		—	—	−0.17[a]	−1.09
13	2-N(CH$_2$CH$_2$CH$_3$)CH$_2$CH$_2$OH	0.016	−1.80	−0.14	−1.15
		—	—	−0.18[a]	−1.04[a]
		—	—	−0.20[d]	−1.05[d]
14	2-N(CH$_2$CH$_3$)CH$_2$CH$_2$OH	0.016	−1.80	−0.13	−1.14
		—	—	−0.18[a]	−1.12[a]
		—	—	−0.20[d]	−1.05[d]
15	2-N(C$_6$H$_5$)CH$_2$CH$_2$OH	0.05	−1.30	−0.11	−1.05
				−0.10	−0.97
16	2,5-(NHCH$_2$CH$_2$OH)$_2$	—	—	−0.50	—
		—	—	−0.52[d]	—
17	2,5-(NHCH$_2$CH$_2$OH)$_2$	—	—	−0.42	—
	3,6-(Cl)$_2$				
18	2,5-[N(CH$_3$)CH$_2$CH$_2$OH]$_2$	0.13	−0.89	−0.34	

[a] Ref. 15. [b] Ref. 9. [c] Ref. 11. [d] Ref. 5, 20% MeOH. [e] Ref. 10. [f] Ref. 8. [g] Ref. 14, 22% EtOH. [h] Ref. 6.



(7)



(8)

possible based on the dependence of the rate of formation of the Q-form on pH, as manifested by the pH-dependence of wave i_1. Were the addition of OH$^-$ to the carbonyl group the slower process, a linear dependence of the measured rate constant k' on a_{OH^-} would be expected. The pH-dependence of i_1, which is in the shape of a dissociation curve, indicates that the acid–base equilibrium (7) is rapidly established preceding the slower step (8).

For equilibria (7) and (8) $[Q]_e = [C]_e[OH^-]/K_aK_1[OH^-]$, so that $[Q_e]/[C]_e = 1/K_aK_1$. This indicates that the equilibrium concentrations of both the quinonoid form and the quinol are pH-independent, as has been proven experimentally for pH $\ll$ pK_a.

Structural effects

Equilibria

Both the values of the overall equilibrium constant $K = [Q]/[C]$ for equilibrium (5) and of the half-wave potentials $(E_{1/2})_1$ of the quinonoid form depend on substituents both in the quinone ring and in the side-chain (Fig. 6). There is no linear free energy relationship between the available values of log K and the values of $(E_{1/2})_1$. Substituent effects on both processes must be, therefore, discussed separately.

Effects of substituents on the quinonoid ring on the value of log K are not additive: the introduction of the first methyl group in position 5 for compounds **5** and **10** (Table 1) results in an increase in log K of +0.70 units, for compounds **6** and **11** (in aqueous solutions) an average +0.66 units and +0.78 units in 20% methanolic solutions. The introduction of the second methyl group at the 6-position results in an increase of only +0.32 units, when the values for compounds **3** and **6** are compared. This can be attributed to the steric effect of the 6-methyl group on the coplanarity of the adduct. Substitution of hydrogen at position 5 by a N(CH$_3$)CH$_2$CH$_2$OH group results in an increase in log K of +0.32 units (comparison of log K for compounds **11** and **18**), which is considerably smaller than the effect of a 5-methyl group.

Replacing the methyl group in 2-(*N*-methyl-*N*-(2′-hydroxyethyl)amino)benzo-1,4-quinone (compound **11**) by alkyl or aryl

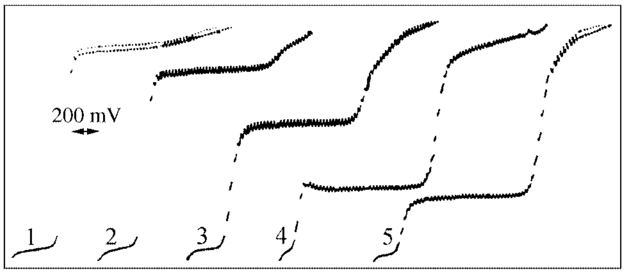


Fig. 6 Comparison of current–voltage curves of 4×10^{-4} M solutions of various 2-(β-hydroxyethyl)aminobenzo-1,4-quinones in Britton–Robinson buffer pH 7.0 containing 40% propan-2-ol. (1) 5,6-Dimethyl-2-(β-hydroxyethyl)aminobenzo-1,4-quinone (**1**, Table 1); (2) 5,6-dimethyl-2-(*N*-methyl-β-hydroxyethyl)aminobenzo-1,4-quinone (**3**, Table 1); (3) 5-methyl-2-(*N*-methyl-β-hydroxyethyl)aminobenzo-1,4-quinone (**6**, Table 1); (4) 2-(*N*-phenyl-β-hydroxyethyl)aminobenzo-1,4-quinone (**15**, Table 1); (5) 2-(*N*-methyl-β-hydroxyethyl)aminobenzo-1,4-quinone (**11**, Table 1). Full scale 0.8 μA, curves starting at 0.0 V, $T = 25$ °C.

groups results in a shift of log K to more negative values. Comparing the differences in log K for those substituents with the value of the methyl derivative **11**, the difference of $\Delta\log K = \log K_{CH_3} - \log K_R$ decreases in the following order: **8** ($\Delta\log K = -0.8$) > **10** (-0.6) = **13** (-0.6) = **14** (-0.6) > **12** (-0.3) > **15** (-0.1).

These changes cannot be rationalised by simple polar or inductive effects, as no correlation can be found with Taft polar substituent (σ^*) or inductive (σ^I) constants.[27] The contribution of steric effects on equilibrium (7) seems plausible. The effect of the nature of R is little affected by the substitution of a methyl group in position 5 of the quinone ring. Thus the replacement of N–CH_3 in compound **6** by a N–CH_2CH_2OH group in **5** results in $\Delta\log K = -0.55$, comparable with the value of $\Delta\log K = -0.6$ observed in the absence of 5-methyl group for compound **9**. Comparison of log K for compounds **1** and **3** shows $\Delta\log K + 0.03$ for the replacement of the methyl group by hydrogen.

Redox properties. Effects of a methyl group in positions 5 and 6 of the quinone ring seem to be additive. Comparable shifts were observed for the introduction of the first and the second methyl group. Substitution by the first methyl group resulted in shifts of -0.07 V (comparing compounds **5** and **10**, Table 1) and -0.05 V (for compounds **6** and **11**). Introduction of a second methyl group in position 6 caused shifts of -0.04 V (for compounds **3** and **6**) and -0.03 V (for compounds 2 and 5). Accuracy of ± 0.02 V can be expected for available data. Similar shifts due to the introduction first of a 5-methyl and then of a 6-methyl group were observed[15] also for 2-(β-chloroethyl)-aminobenzoquinones, which have no tendency to form the quinol. Thus for **I** ($n = 2$, R = CH_3, X = Cl), $\Delta(E_{1/2})_1$ for the 5-CH_3 group was -0.06 V and for the additional 6-CH_3 -0.04 V, whereas for **I** ($n = 2$, R = CH_2CH_2Cl, X = Cl) such shifts were -0.04 V for 5-CH_3 and -0.05 V for the additional 6-CH_3. Substitution by $N(CH_3)CH_2CH_2OH$ in position 5 causes a shift of $\Delta E_{1/2} = -0.12$ V, as found by comparison for compounds **18** and **11**.

The shifts $\Delta E_{1/2}$ due to a substituent R on the 2-amino nitrogen relative to the value for R = CH_3 (compound **11**), are as follows: **7** ($\Delta E_{1/2}$ -0.05) > **8**,**9** (-0.02) > **10**,**12** (0.0) > **13** (+0.02) > **14** (+0.03) > **15** (+0.05).

Additive effects of quinone ring substituents are in agreement with linear free energy relationships, which have been proved to be applicable to substituted quinones.[28] Substituents on the quinonoid ring affect directly the electron distribution both in the oxidized quinone and reduced quinone (hydroquinone). This is then reflected in changes of the values of $E_{1/2}$ which correspond to changes in differences in energies of the oxidized and reduced forms. Smaller effects of substituents on the 2-amino nitrogen correspond to relatively smaller changes in distribution of electron density in the quinone ring due to side-chain substitution.

Conclusions

Combination of polarographic and spectrophotometric studies enabled proof of the existence of an equilibrium between the 2-(β-hydroxyethyl)aminobenzoquinones and corresponding bicyclic quinols. These quinols are formed by the addition of a hydroxide ion to the carbonyl group adjacent to the 2-amino group [equilibrium (7)]. In the resulting adduct nucleophilic attack of the -O^- group on the β-carbon of the side chain [equilibrium (8)] takes place. Spectrophotometry enabled determination of the overall equilibrium constant of the reaction between quinone and quinol. Polarography proved that formation of the quinol from the quinone is base catalyzed. With decreasing temperature the equilibrium is shifted in favor of the quinol form, which also shows fluorescence emission. The studied equilibria may play a role in the physiological activity of cancerostatic ethyleneiminoquinones, which produce 2-(β-hydroxyethyl)aminoquinones on hydrolysis.

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