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Biochemistry

Some electrochemical and chemical properties of methoxatin and analogous quinoquinones

(phenanthroline quinones/semiquinones/quinols)

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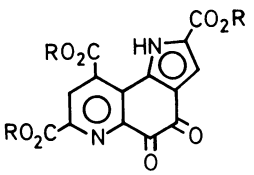
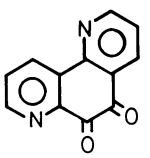
Contributed by Thomas C. Bruice, January 28, 1982

ABSTRACT **The present study establishes relationships between structure and reactivity for the pyrroloquinoline and phenanthroline quinones. The electrochemical reductions of 1,7- and 1,10-phenanthroline-5,6-quinones, like other quinones, are reversible and occur by $2e^-$ transfer in a single step in aqueous solution and by two $1e^-$-transfer steps in aprotic media. The electron-withdrawing pyridine moieties both increase their potentials and stabilize their aprotic semiquinones. The electrochemistry of the cofactor methoxatin and its trimethylester derivative is similar to the phenanthroline quinones in aqueous solution. However, the electrochemical reductions of methoxatin and its triester in aprotic solutions are characterized by at least three potentials, each accounting for less than $1e^-$. This has been explained by the proposal of semiquinone complexing with itself and with quinone. Despite an electron-donating pyrrole moiety, methoxatin and its trimethylester have relatively high potentials in aprotic solution. This is presumably due to stabilization of radical anions by the aforementioned complexing or by delocalization with carboxylic acid and ester groups. The reduction potential of methoxatin, in both aqueous and aprotic solvent, suggests that oxidation of methanol should be a thermodynamically favorable process. No evidence for an electrochemically reduced state lower than the quinol was found for any of the compounds. Chemical reactivity is influenced by the orientation of the pyridine nitrogen. The two quinones with a pyridine nitrogen peri to a quinone carbonyl add and oxidize nucleophiles most readily.**

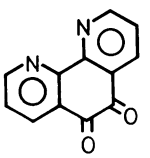
Methoxatin (compound **Ia**) plays the role of coenzyme in various bacterial NAD(P)-independent alcohol, glucose, aldehyde, and perhaps methylamine dehydrogenases



Ia: R = H
Ib: R = Me

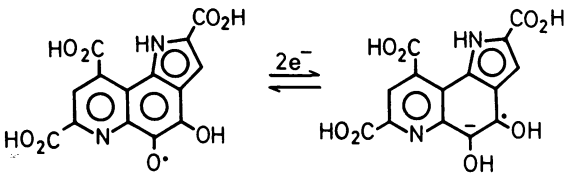


II



III

(1–5). Its structure, totally novel for a coenzyme, has been identified (1, 2) and its synthesis has been accomplished (6, 7), but little about its chemistry has been established. It has been proposed (8) that only the semiquinone form of the coenzyme in methanol dehydrogenase is catalytically active and that this form might undergo a $2e^-$ reduction to a second radical:



[1]

Because methanol dehydrogenase is found coupled to cytochrome *c* in a lipophilic cell membrane (9), the electrochemistry of the coenzyme, with and without metal, in water and aprotic solvents, should prove instructive. Compounds **II** and **III** are also quinoline *o*-quinones. Their high cytotoxicity against five mammalian cell lines,§ simple structure, and relative abundance make them analogues of biological and chemical interest. Thus, electrochemical and chemical studies of **Ia**, its triester (**Ib**), and compounds **II** and **III** have begun.

MATERIALS AND METHODS

Synthesis and Properties of I, II, and III. Quinones **Ia** and **Ib** were totally synthesized by an existing method (6). *Dione* **III** was obtained from Alfa-Ventron. 1,7-Phenanthroline dione (**II**) was synthesized as follows. A solution of 1,7-phenanthroline (Alfa-Ventron, 2.7 g) in fuming H_2SO_4 (18 ml) and fuming HNO_3 (9 ml) was heated at 180°C for 3.5 hr, poured onto crushed ice, and brought to pH 6 with water saturated with Na_2CO_3. The collected precipitate was washed with water and recrystallized from methanol (MeOH) to yield pure, orange-brown **II** (0.78 g): mp 258–62°C (dec.) [lit. (10) 255°C]. Analysis. Calculated for $C_{12}H_6O_2N_2$: 68.57% C, 2.88% H, 13.33% N. Found: 68.43% C, 2.93% H, 13.32% N. NMR, infrared, and mass spectra are consistent with the desired product.

pK_a values of the conjugate acids of **II** and **III** were determined spectrophotometrically at 30°C. The ionic strength, μ, for both **II** and **III** was 0.1. Equilibrium constants for the addition of hydroxide to **II** and **III** were determined spectrophotometrically at $\mu = 1.0$. Stability constants for the metal complexes of **III** were determined by the method of partitioning (11) between water (pH 5.6, 30°C, $\mu = 0.1$) and *n*-octanol.

Cyclic Voltammetry. A modified Princeton Applied Research model 174 Polarographic Analyzer was used for cyclic voltametry. Aqueous solutions were analyzed at 22–24°C at a scan rate of 2 mV/sec with a thin-layer platinum electrode (designed and built by A. T. Hubbard) and an aqueous Ag/AgCl/

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Abbreviations: MeCN, acetonitrile; MeOH, methanol; DMF, dimethylformamide; DBN, 1,5-diazabicyclo[4,3,0]non-5-ene.

§ ED_{50} values for the quinones were determined in the laboratories of S. J. Benkovic (38 nM, >190 nM, 34 nM for **III** and 4.0 mM, 7.6 mM, 1.7 mM for **II** against mammalian cell lines HK SV28, BALB 3T3, Swiss 3T3, respectively) and of D. V. Santi (10 nM, 10 nM for **III** and 1.0 mM, 1.0 mM for **II** against S-49 mouse lymphoma, 180 mouse sarcoma, respectively).

1 M NaCl reference electrode. Solutions at pH 0.30, 3.0, and 5.6 were made with 0.5 M $HClO_4$, 0.5 M KH_2PO_4, and 0.5 M NaOAc, respectively. For anaerobic aprotic solutions at 22–25°C, Ag/0.1 M $AgNO_3$ in acetonitrile (MeCN) provided the reference electrode, and 0.1 M tetraethylammonium perchlorate provided the electrolyte. The numbers of electrons exchanged were determined by thin-layer cyclic voltammetry (12) with a platinum electrode at a scan rate of 2 mV/sec. Redox potentials were determined with a stationary, 1-mm platinum-sphere electrode at a scan rate of 20 or 100 mV/sec.

Acetone Adducts of II and III. The dione (20 mg) and alumina (20 mg, neutral, activity III) were stirred in acetone for 2 hr. The supernatant and several acetone extracts of the alumina were concentrated to a solid, which was recrystallized from acetone/hexane to yield pure adduct. The adduct of **II** was obtained as 11 mg of greenish-white crystals: mp 163–8°C (dec.). Analysis. Calculated for $C_{15}H_{12}N_2O_3$: 67.16% C, 4.51% H, 10.44% N. Found: 67.10% C, 4.62% H, 10.36% N. The adduct of **III** was obtained as 7 mg of white crystals: mp 192–6°C (dec.). Analysis. Calculated as before. Found: 67.03% C, 4.45% H, 10.36% N. The infrared and mass spectra are consistent with the desired products.

Transamination of Cyclohexylamine with III. A pH 9.3 solution of **III** (34 mg), amine (1.0 ml), 1 M aqueous HCl (6.0 ml), and water (53 ml) was maintained anaerobically at 30°C for 7 days. The mixture was filtered and the filtrate was acidified to pH 1.3. Gas chromatography of hexane extracts showed the presence of cyclohexanone, as did the 2,4-dinitrophenylhydrazone derivative from ether extracts of the aqueous acid solution. Analysis. Calculated for $C_{12}H_{14}N_4O_4$: 51.80% C, 5.07% H, 20.13% N. Found: 51.80% C, 5.14% H, 20.16% N. The mass spectrum of the derivative also was consistent with the desired product.

Transamination of Cyclohexylamine with II. A solution of **II** (10.5 mg), amine (1.0 ml), 1 M aqueous HCl (8.0 ml), and water (91 ml) was maintained anaerobically at 30°C for 3 days. The mixture was filtered, and the filtrate was acidified to pH 1.3 and extracted with ether. The 2,4-dinitrophenylhydrazone derivative from the ether extract was found to have the same absorbance spectrum and chromatographic characteristics as the authentic 2,4-dinitrophenylhydrazone of cyclohexanone.

Hydrazine Reductions of Ia, II and III. Pseudo-first-order rate constants were determined for at least four half-lives by spectrophotometric monitoring of the formation of product in (anaerobic, 30°C) solutions of the quinone with 3.33 mM hydrazine in either aqueous 0.1 M HCl or 18% (wt/wt) MeCN in water buffered at pH* 7.2–7.3 with 0.1 M phosphate (13). Product from a scaled-up reaction with **II** was identified by a high resolution mass spectrum of the hydrochloride salt. Product from a scaled-up reaction with **III** was identified by comparison of its absorbance spectrum with that of the known 5,6-quinol (14) and by an elemental analysis of the hydrochloride salt. Calculated for $C_{12}H_9ClN_2O_2$: 57.96% C, 3.65% H, 11.27% N, 14.26% Cl. Found: 57.98% C, 3.79% H, 11.26% N, 14.35% Cl. The product from the reaction of **Ia** at pH 7 was identified by a spectrum identical to that of known 5,6-quinol (15). The identity of the product from the reaction of **Ia** in 0.1 M HCl was indicated by the similarity of its spectrum and that of the acidified product from the reaction at pH 7.

RESULTS AND DISCUSSION

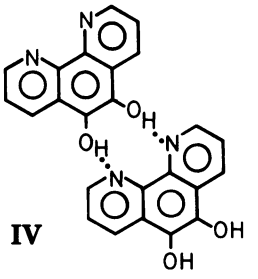
The influence of the quinone moiety, as well as carboxylic ester and carboxylic acid substituents, on the electron density of the pyridine rings of **Ia**, **Ib**, **II**, and **III** may be ascertained from pK_a data and metal ion binding constants. The pK_a value (2.16) of protonated **II**, designated IIH^+, was found to be less than the pK_a of $IIIH^+$ (2.40). The greater acidity of IIH^+ might be attributed to the inability of the 1,7-nitrogens of IIH^+ to act in concert. The pyridine ring in **Ib** should be the most electron deficient because of its carbomethoxy substituents. Because of their poor solubility and lack of spectrophotometric change upon protonation, **Ia** and **Ib** could not be titrated to determine pK_a values. Yet the pK_a for IbH^+ can be estimated to be about 0, in as much as the value for protonated 2,3-pyridine-dicarboxylic acid dimethyl ester is −0.15 (16), and the pyrrole and quinone carbonyl moieties of **Ib** would have opposite electronic effects. For the protonated pyridyl nitrogen of **Ia**, a much higher pK_a of about 5 would be expected because of the three carboxylate anion substituents. The protonated nitrogen of 2,3-pyridinedicarboxylic acid has a pK_a of 5.06 (17). Finally, a comparison of the pK_a of $IIIH^+$ to that of the conjugate acid of *o*-phenanthroline [pK_a, 4.98 (11)] points to the role of the quinone carbonyl groups in lessening the electron density of the pyridine rings. This also is shown by the finding that binding constants with divalent metal ions are 2–6 orders of magnitude larger for phenanthroline than for **III** (Table 1).

Table 1. Stability constants of bivalent cation complexes at μ = 0.1 M

Complex*	Zn(II)	Co(II)	Ni(II)
With 1,10-phenanthroline at 25°C†			
log β_1	6.3	7.0	8.0
log β_2	12.0	13.7	16.0
log β_3	17.1	20.1	23.9
With **III** at 30°C			
log β_1	4.5	5.0	5.3
log β_2	8.0	9.5	10.8
log β_3	11.2	13.2	16.3

* $\beta_n = [ML_n]/[M][L]^n$.
† Ref. 10.

It was determined by thin-layer cyclic voltammetry that the reductions of **Ia**, **Ib**, **II**, and **III** in aqueous solution, with but one exception, are thermodynamically and kinetically reversible (Table 2). At a pH (5.64) above the pK_a of the conjugate acid of the pyridine moieties, **III** was irreversibly reduced in a $2e^-$ step. Presumably this irreversibility in reduction of **III** results from intermolecular complexing of reduced **III** species through hydrogen bonding involving the 5,6-hydroxyl groups and unprotonated 1,10-nitrogens (**IV**).



IV

The likelihood of this is shown by the fact that addition of Fe(III) to a solution of **III** allows its reduction to be reversible. This finding would be in accord with the metal ion complexing with the 1,10-nitrogen atoms of $2e^-$-reduced **III**. By coulometry, the Fe(III) complex of **III** was reduced by $3e^-$. The triester **Ib** is reduced in a single $2e^-$ step. Duine and coworkers have determined a $2e^-$-reduction potential of **Ia** by potentiometric titration of the quinol using I_2 and ferricyanide as oxidants (15, ¶). According to the Nernst equation, the dependence of the

¶ In ref. 15, redox potentials for **Ia** at pH 2.0 and 7.0 are reported as 0.419 V and 0.090 V, respectively. By extrapolation these values are about 0.02 V higher than those reported for this study.

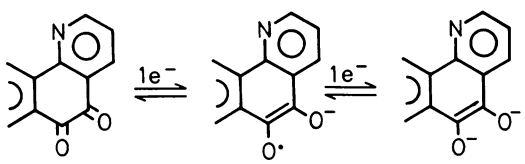
Table 2. Aqueous redox potentials of **Ia**, **Ib**, **II**, and **III**

Compound	E_m*, V	No. of e^-
Ia at pH 0.30	0.51	—
Ib at pH 0.30	0.53	2.0†
II at pH 0.30	0.59	1.9
III at pH 0.30	0.53	1.9
Ia at pH 2.98	0.34	—
Ia at pH 2.98 + excess Zn(II)	0.34	—
Ib at pH 3.01	0.38	—
Ib at pH 3.01 + excess Zn(II)	0.38	—
II at pH 3.01	0.41	1.9
III at pH 3.01	0.38	2.0
III at pH 3.01 + excess Zn(II)	0.36	2.1
Ia at pH 5.60	0.15	—
Ia at pH 5.60 + excess Zn(II)	0.23, 0.17	—
II at pH 5.62	0.24	2.1
II at pH 5.62 + excess Zn(II)	0.31	2.0
III at pH 5.64	ND	2.0
III at pH 5.64 + excess Fe(III)	0.32	2.9

ND, no anodic peak; peak potential cathodic E_p = 0.23 V.
* Versus the normal hydrogen electrode.
† Determined in 10% MeCN/90% 1 M $HClO_4$ (aq) (vol/vol).

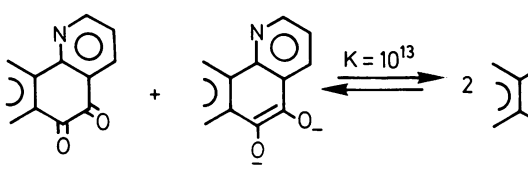
potentials upon the pH (Table 2) indicates that one proton is taken up per electron in the reduction of the four quinones in aqueous solution in the pH range studied. Of the four compounds, **II** showed slightly the highest oxidation potential, but all proved to be better oxidants than 9,10-phenanthraquinone, with $E_{1/2}$ = 0.00 V at pH 7.6 (18), and all could thermodynamically oxidize MeOH with its calculated $E^{0\prime}$ = −0.182 V (19).

Because the methoxatin-containing alcohol dehydrogenase resides in a lipophilic cell membrane (9), the electrochemistry of the quinones in aprotic media is particularly pertinent. Like other quinones in aprotic media, **Ia**, **Ib**, **II**, and **III** are reduced in two or more reversible steps, indicative of the formation of stable intermediates (Table 3). The reduction of **II** and **III** appeared to occur through two $1e^-$ steps with semiquinone anions as intermediates:



[2]

The second reduction steps for **II** and **III** occurred at E_m values indicating equilibrium constants on the order of 10^{13} for semiquinone formation from the quinone and quinol (Eq. 3).



[3]

The influence of the electron-withdrawing pyridine moieties of **II** and **III** on the first reduction potentials may be appreciated by comparison to 9,10-phenanthraquinone in MeCN with an $E_{1/2}$ of −0.42 V (20). Its second $E_{1/2}$ value of −0.98 V allows a calculation of its semiquinone formation constant to be on the order of 10^9. Thus, the substitution of the two phenyl rings of phenanthraquinone with pyridine rings, as in **II** and **III**, stabilizes the semiquinone anion relative to the quinone and quinol dianion by 5 kcal M^{-1}.

The aprotic electrochemistry of **Ia** and **Ib** is not as simple as that of **II** and **III**. In MeCN, **Ib** showed three reversible reduction steps. In dimethylformamide (DMF), **Ia** exhibited an additional fourth step at E_m = −1.53 V. The first reduction peaks accounted for $<1e^-$ in each case (Table 3). Coulometry on the peaks of lower potential was not possible by thin-layer cyclic voltammetry, perhaps because of the slow scan rate required by this technique. Nonetheless, it can be stated that the reductions of **Ia** and **Ib** do not occur through two $1e^-$ steps with the sole intermediacy of the semiquinone. The reactions of Scheme I provide a plausible explanation for the four reduction peaks observed with **Ia**.

Table 3. Redox potentials of **Ia**, **Ib**, **II**, and **III** in aprotic solvents

Compound	Solvent	E_m*, V (no. of e^-)
Ia	DMF	−0.05(0.8), −0.71, −0.94, −1.53
Ia + excess DBN	DMF	−1.09(2)
Ib	MeCN	+0.05(0.62), −0.69, −0.97
II	MeCN	−0.25(1.0), −0.97
II	DMF	−0.30(1.3), −1.23
II + excess DBN	DMF	−0.30(1.2), −1.34(0.95)
III	MeCN	−0.25(1.1), −1.00(0.82)
III	DMF	−0.30(1.2), −1.11

* Versus the normal hydrogen electrode.

Couple I $\quad I + e^- \rightleftarrows \text{semiquinone}^{\cdot-}$
$\quad I + \text{semiquinone}^{\cdot-} \rightleftarrows [(I)(\text{semiquinone}^{\cdot-})]$

Couple II $\quad [(I)(\text{semiquinone}^{\cdot-})] + e^- \rightleftarrows [(\text{semiquinone}^{\cdot-})_2]$
$\rightleftarrows 2\ \text{semiquinone}^{\cdot-}$

Couple III $\quad \text{semiquinone}^{\cdot-} + e^- \rightleftarrows \text{quinol}^{=}$

Couple IV $\quad [(\text{semiquinone}^{\cdot-})_2] + e^- \rightleftarrows \text{semiquinone}^{\cdot-}$
$+\ \text{quinol}^{=}$

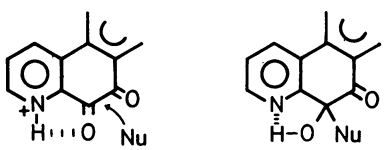
Scheme I

For **Ib** reduction, the observation of three reduction peaks might be due to Couple IV being obscured by the reduction of the MeCN solvent system. If Scheme I is valid, then, in the case of **Ia**, the formation of complexes of the semiquinone with quinone and with semiquinone should be disfavored by electrostatic repulsion upon ionization of its three carboxyl groups. To test this concept, the effects of excess 1,5-diazabicyclo-[4,3,0]non-5-ene (DBN), a strong nonnucleophilic base, on the electrochemistry of **Ia** were observed. The nonionizable **II** was used as a control. There was found to be little change (Table 3) in the reduction of **II** upon addition of DBN in DMF. In contrast, addition of excess DBN to ionizable **Ia** in DMF results in a change from multiple reduction potentials to a single $2e^-$ transfer. Apparently the trianion nature of ionized **Ia** both prevents complex formation and inhibits its first $1e^-$ reduction sufficiently to overlap with the reduction of the semiquinone. It should be noted that both **Ib** and **II** complex reversibly with DBN in DMF. Complexation of radical species with self and reduced species has been noted in the electrochemical investigation of N^5-ethyl-3-methyllumiflavinium cation (21). Abeles and coworkers (8) have considered the possibility that the mechanism of action of **Ia** as a cofactor for the oxidation of MeOH involves $2e^-$ transfer to the semiquinone radical of **Ia** to provide a second radical. We found no evidence, however, for a reduced state lower than the hydroquinone for **Ia** or **Ib** in water or aprotic solvents.

If enzyme-bound **Ia** must be in its semiquinone state for activity (8), then our results suggest that: (*i*) $1e^-$ reduction of substrate by **Ia** is followed by $1e^-$ transfer to cytochrome *c* prior to the second electron transfer, or (*ii*) enzyme-bound semiquinone arises from the presence of both quinol and quinone at the active site. Duine and coworkers have reported that meth-

anol dehydrogenase contains one molecule each of **Ia** and its quinol (15). Notable is the superior $1e^-$ oxidizing strength of **Ia** and **Ib** relative to that of **II** and **III** in aprotic media. This might result from increased stabilization of the anion radicals by complexation (Scheme I) or through resonance and inductive effects by the carboxyl or ester substituent groups. The first aprotic E_m values of −0.05 and +0.05 V for **Ia** and **Ib** exceed the oxidation potential of MeOH with its $E^{0'} = -0.182$ V (19). Perhaps, **Ia**, linked to cytochrome *c* in its lyophobic cell membrane (9), could be reduced by MeOH in a basic aqueous cell milieu in a thermodynamically favored process. The oxidation of MeOH by semiquinone should be more difficult.

As oxidants and electrophiles, **Ia**, **Ib**, **II**, and **III** proved chemically active. Acetone is known to add as a nucleophile to C-5 of **Ia** (1, 2), and so it was not surprising to find that stable adducts of acetone with **II** and **III** are formed also. Cyclohexylamine was transaminated by both **II** and **III** in water at pH 9–10 at 30°C under anaerobic conditions (for similar reactions, see ref. 22). The orientation of the pyridine nitrogen relative to the quinone function affects the addition of nucleophiles. The addition of MeOH to the quinones was observed by comparing the UV spectra taken in MeOH with those taken in CH_2Cl_2 and *t*-butanol. Whereas changing CH_2Cl_2 solvent to *t*-butanol alters the spectra of **Ib**, **II**, and **III** very little, spectra of **Ib** and **II** in MeOH resembled those of hydroxide and acetone adducts of **Ia** (23) and **II**, respectively. The spectrum of **III** was less affected by MeOH. There is little difference in the equilibrium constants for addition of the strong base, HO^-, to **II** (3.55×10^3 M^{-1}) and to **III** (2.57×10^3 M^{-1}) in water at 30°C. The reductions of the quinones in 0.1 M HCl (30°C) by hydrazine were associated with pseudo-first-order rate constants of 3.8×10^{-3}, 1.3×10^{-2}, and 6.8×10^{-4} min^{-1} for IaH^+, IIH^+, and $IIIH^+$, respectively. Similar reduction at pH 7 provided rate constants of 0.32, 25, and 0.26 min^{-1}. Thus, the ratio of rate constants for reduction of quinones to quinols with pyridine nitrogens protonated is IaH^+:IIH^+:$IIIH^+$ = 5.6:19:1, whereas the rate ratios for unprotonated quinones (and with **Ia** ionized) are 1.2:96:1. It would appear that the pyridine nitrogen, if peri to a quinone carbonyl group, provides an advantage for both the stability of carbonyl adducts and the rate of carbonyl reduction. This may be due to an inductive withdrawal of electrons by the pyridine nitrogen and to internal hydrogen bonding:



At pH 7, with its ionized carboxyl groups and electron-donating pyrrole group, **Ia** almost loses its kinetic advantage over **III**.

SUMMARY

Comparison of pK_a values establishes that the pyridine nitrogen of the triester of methoxatin (**Ib**) is more electron deficient than are the pyridine rings of **II** and **III**. The same conclusion is expected for methoxatin (**Ia**), providing the carboxyl substituents remain undissociated. The reductions of the protonated quinones are reversible in aqueous solution. At pH values where their pyridine rings are not protonated, the reductions of **Ia**, **Ib**, and **II**, but not **III**, are reversible. The irreversibility of the reduction of neutral **III** is attributed to the self-complexing of reduced **III** by hydrogen bonding of its 1,10-pyridine nitrogens and 5,6-hydroxyl substituents. The reductions of the four quinones in water in the pH range examined involves a single $IIH^+ + 2e^-$ step. Of the quinones, **II** has the greatest potential in aqueous solution.

In aprotic media, **II** and **III** are reduced in two $1e^-$ steps, which establishes the intermediacy of semiquinone intermediates. Quinone and quinol dianion species react quantitatively ($K = 10^{13}$) to yield two semiquinone radical species. The stabilizing influence of the heterocyclic nitrogens of **II** and **III** on semiquinones is shown by the fact that the semiquinone anions generated from **II** and **III** are far more stable than the semiquinone of 9,10-phenanthraquinone. The electrochemical reduction of **Ia** and **Ib** in aprotic medium involves the formation of complexes of semiquine + quinone and semiquinone + semiquinone. Ionization of the carboxyl groups of **Ia** prevents this complex formation by electrostatic repulsive forces. The reduction of ionized **Ia** in aprotic solvent occurs in a single $2e^-$ step.

Acetone forms stable covalent adducts with **II** and **III** as previously established with **Ia**. The transamination of cyclohexylamine by **II** and **III** has been established. MeOH addition to the carbonyl functions of **Ib**, **II**, and **III** and hydroxide addition to **II** and **III** also have been examined. The rate constants for the hydrazine reductions of nitrogen protonated and unprotonated species of **Ib**, **II**, and **III** were determined. Results of these studies establish that the reactivity of the quinone carbonyl groups is greatest when carbonyl function and pyridine nitrogen are peri. Thus, **Ia**, **Ib**, and **II** add nucleophiles and oxidize hydrazine more readily than does **III**.

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