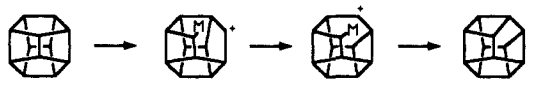
and the other with a cyclopropane ring, so both are plausible reaction paths. Initial cleavage of the other bond in the three-membered ring of **22** can lead to **21** directly.

(12)

21 20 22

Truncated tetrahedrane (**9**) can rearrange as well. The most likely path begins with cleavage of a cyclopropyl bond and leads to compound **22**. This creates a route from **9** to the most stable isomer, **8**.

Many other rearrangements of these hydrocarbons are possible, but they seem mechanistically less likely. Most, like the degenerate isomerization reaction of compound **21** (eq 13), involve initial cleavage of a relatively unstrained bond. Such reactions would require severe conditions, under which other processes would probably compete.



(13)

21 21

Conclusion

The chemistry of $(CH)_{12}$ hydrocarbons has been of interest for over 25 years, and recent results show no slowing of work in the area. Reports of preliminary investigations suggest that some very interesting members of this family will become available in the next several years. In advance of this, we have sought to discover likely chemical transformations of these molecules and to put our knowledge of their energetics on a more uniform footing. We believe that we have identified the most stable isomer of the family. Two other possibilities, **13** and **14**, have also been pointed out. Our relative energies for the benzene dimers should prove more accurate than those previously reported, and they can be compared to our results for other $(CH)_{12}$ hydrocarbons. We point out several relatively stable and symmetrical compounds, such as **20**, **21**, and **22**, which could prove of interest in the rearrangement chemistry of $(CH)_{12}$ hydrocarbons which will develop subsequently. It is particularly worth noting that compounds **5** and **9**, actively sought synthetic targets, may prove very susceptible to Lewis acid catalyzed rearrangement.

Acknowledgment. We thank the Tulane University Computing Service and IBM Corporation for their generous grants of computer time. We also thank Drs. B. Liu, W. Koch, and A. Scheiner, IBM Almaden Research Center, for their assistance in optimizing the performance of the GAUSSIAN programs on the E/SNSC 3090VF supercomputer system.

Supplementary Material Available: Electronic energies for molecules used in constructing Table II and tables of internal (z matrix) and Cartesian coordinates for the optimized structures of all molecules in Table I (17 pages). Ordering information is given on any current masthead page.

Interplay of Substituent Conformation and Electron Affinity in Quinone Models of Quinone Reductases

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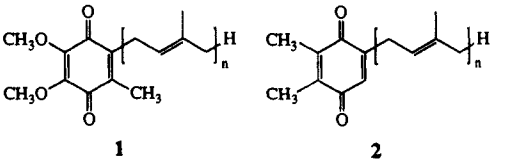
Contribution from the Departments of Biophysics and Chemistry, University of Illinois, Urbana, Illinois 61801. *Received August 9, 1989*

Abstract: Nonempirical molecular orbital calculations were used to explore the electron affinities of model systems for ubiquinones and plastoquinones. An interconnection between substituent conformation on the quinone and the potential of the quinone–semiquinone couple is indicated. The role of this effect is discussed in the context of allosteric regulation/control of electron transfer reactions and/or charge storage in quinone-bearing proteins.

Quinones are lipid soluble enzyme cofactors that function as oxidation/reduction intermediaries between assemblies of the membrane-bound proteins of the energy conversion systems of photosynthetic and respiratory systems.[1] Ubiquinone (**1**) and plastoquinone (**2**) are biologically significant quinones, which possess alkoxy and alkyl substituents bound to the ring of a 1,4-benzoquinone, respectively. Within an energy conversion pathway, one set of enzymes reduces the quinone with two electrons and two protons to the neutral hydroquinone and another set of enzymes oxidizes the hydroquinones back to the quinone and liberates two electrons and two protons.[1] These oxidative and reductive processes must involve considerable reorganization of the quinone's electronic structure where the various charged transition states and intermediates are stabilized by the protein environment. For example, the radical anionic form of the quinone is known to be stabilized at the photosynthetic reaction center reductase quinone-binding site for minutes under certain conditions.[2] To help understand the mechanisms of both the oxidative and reductive reactions and, in particular, to understand how the radical anion is stabilized, we have undertaken a quantum mechanical analysis of the ground-state quinone and radical anionic form, the semiquinone. Knowledge concerning the influence of reaction site topology on the stability of the radical anion was sought by probing the relationship between quinone-substituent conformation and the electronic properties of the quinone moieties. Such information is needed to develop insight into nature's ubiquitous usage of quinones in oxido reductases.[3-5]



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* To whom correspondence should be addressed.

(1) Crofts, A. R.; Wraight, C. A. *Biochim. Biophys. Acta* **1983**, *726*, 149-185.
(2) Robinson, H. H.; Crofts, A. R. *FEBS Lett.* **1983**, *153*, 221-226.
(3) Chipman, D. M.; Prebenda, M. F. *J. Phys. Chem.* **1986**, *90*, 5557-5560.
(4) Prince, R. C.; Halbert, T. R.; Upton, T. H. In *Advances in Membrane Biochemistry and Bioenergetics*; Kim, C. H., Tedeshi, H., Diwan, J. J., Salerno, J. C., Eds.; Plenum: New York, 1988; pp 469-478.

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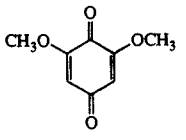
Computational Details

The interconnection between quinone topology and its electronic properties has been studied in a series of ab initio calculations on substituted quinones at the Hartree–Fock level with use of the 3-21G basis set.[6] All structures were fully optimized with the GAUSSIAN85/SPARTAN program system[7] as implemented on Silicon Graphics IRIS-4D workstations. Energy profiles for ring-substituent rotations involved complete reoptimization of the molecular system at 15° or 30° intervals of the dihedral angle being varied.

The large size of the naturally occurring benzoquinones required model systems to be chosen for the present study. 2,6-Dimethoxy-1,4-benzoquinone (**3**) and 2,3,5,6-tetramethyl-1,4-benzoquinone (**4**) were



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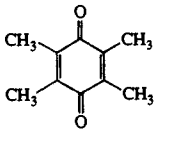

4

used to model ubiquinone and plastoquinone, respectively, in part for reasons of symmetry. Energies and structures for closed shell ground-state quinones were calculated within the restricted Hartree–Fock (RHF) formalism, whereas unrestricted Hartree–Fock (UHF) theory was used for calculations on the singly reduced open-shell quinone radical anions (i.e., semiquinone anions).[3,8] In all examples the expectation value of the spin operator suggested negligible spin contamination of the doublet radical anions with higher multiplicity states. Quoted electron affinities reflect the difference in calculated energies between the quinone and the semiquinone anion (i.e., $E_{RHF} - E_{UHF}$) in vacuo. Electron attachment energies were found not to change significantly upon inclusion of calculated zero-point vibrational energies. Furthermore, preliminary studies employing larger basis sets suggest little variation in the calculated electron affinities from those reported herein.

Results and Discussion

A conventional analysis of the average structures calculated for the quinone/semiquinone pairs reveals several subtle but distinct differences. For example, the characteristic carbonyl C–O bond length in each of the ground-state quinones lengthens by ~0.06 Å upon formation of the semiquinone. However, CO bond lengths in the semiquinone remain notably shorter than those found in phenols.[9] It is interesting that rather than localizing the perturbation caused by the one-electron reduction on the electronegative oxygen (i.e., destroying the CO π bond), the quinone distributes distortions evenly throughout the ring system. Moreover, the bond lengths involving the ring carbons are transformed from alternating single–double bonds in the quinone to a more "aromatic" arrangement, resembling phenol, in the semiquinone.

Calculated enthalpies of electron attachment should correlate closely to measurements of electron attachment performed in the gas phase at low pressures. In contrast to past work on 1,4-benzoquinone,[3,5] the two-substituted quinones in the present study have additional degrees of freedom that involve changes in the torsion angles of the substituents about the quinone rings. This paper addresses the influence of methyl rotor torsion angle on the electrical properties of **4** and the influence of the methoxy torsion angle in **3**. These studies are intended to serve as models for the conformational determinants of reduction potentials for ubiquinones and plastoquinones in quinone reductases.

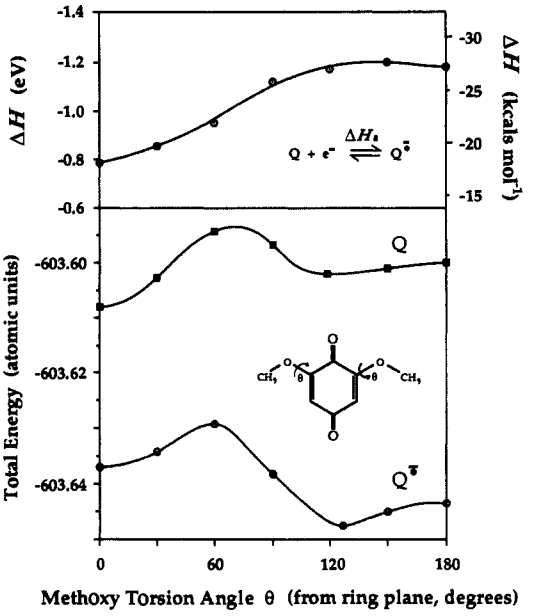

Figure 1. Rotational profiles and electron affinity of 2,6-dimethoxy-1,4-benzoquinone. Energies obtained via reoptimization of all geometric parameters exclusive of the fixed dihedral angle indicated (C_2 symmetry imposed).

It has been argued previously that adjacent methoxy groups on a 1,4-benzoquinone ring, as in ubiquinone, will experience a large, mutually repulsive steric interaction.[4] This has been supported by empirical calculations, which approximated steric interactions between the groups using nuclear-centered hard-wall potentials (bumping spheres). Additionally, generalized valence bond (GVB) calculations on 2-methoxy-1,4-benzoquinone were performed to test the importance of π-donation (from the methoxy oxygen to the quinone ring) on the aforementioned assertion. These calculations found a minimum energy structure for the ground-state quinone with the CO bond of the methoxy group eclipsing the CC double bond of the quinone ring. An evaluation of the energy profile for rigid rotation about the C_{ring}–O bond (i.e., without further reoptimization of the structure) suggested a large repulsive potential, presumably caused by interaction between the methoxy methyl and the carbonyl oxygen.

Reported in Figure 1 are the energy profiles obtained for rotation about the C_{ring}–O bond in **3**, wherein all geometric parameters have been reoptimized at fixed torsion angles for the methoxy groups (C_2 symmetry was enforced throughout). The curves labeled "Q" and "Q$^{\bullet-}$" display the energies for the ground-state quinone and semiquinone structures, respectively. In concurrence with Prince et al.,[4] a minimum-energy ground-state structure is indicated wherein the methoxy group eclipses the quinone ring (see insert molecule drawing of Figure 1). However, at larger dihedral angles, geometry relaxation (i.e., reoptimization) reveals significant deviations from the calculations of Prince et al.,[4] suggesting a more complex interplay of electronic structural determinants. The noted variations in stabilization with torsional angle alterations can be rationalized as a balance of three distinct effects: (1) the net delocalization of two electrons on oxygen onto the electron-poor quinone ring, (2) a Coulombic repulsion between the charge density of the methoxy oxygen lone pairs and the charge density of the carbonyl oxygen lone pairs that lie in the plane of the quinone ring, and (3) a steric interaction between the methoxy methyl group and the carbonyl oxygen. Orbital delocalization (effect 1) will be maximized at 0° and 180° and will be negligible at 90°. Coulombic repulsion (effect 2) will monotonically decrease over the range from 0° to 180°, whereas steric repulsion (effect 3) will monotonically increase over the same range.

The minimum-energy ground-state structure represents a geometry where the π-type oxygen lone pair can optimally overlap with the π-system of the quinone, at the expense of a severely repulsive Coulombic interaction. For the ground-state quinone, electron delocalization onto the ring dominates and overshadows

(5) Reynolds, C. A.; King, P. M.; Richards, W. G. *Nature* **1988**, *334*, 80–82.

(6) Binkley, J. S.; Pople, J. A.; Hehre, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 939–947.

(7) GAUSSIAN 85: Hout, R. F., Jr.; Francl, M. M.; Kahn, S. D.; Dobbs, K. D.; Blurock, E. S.; Pietro, W. J.; McGrath, M. P.; Steckler, R.; Hehre, W. J. University of Illinois (Urbana—Champaign) and University of California (Irvine), 1988. SPARTAN: Carpenter, J. E.; Hehre, W. J.; Kahn, S. D.; Weinhold, F. H. University of California (Irvine), University of Illinois (Urbana—Champaign), and University of Wisconsin (Madison), 1989.

(8) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986.

(9) (a) Gillier-Pandraud, H. *Bull. Soc. Chim. Fr.* **1967**, 1988–1995. (b) Scheringer, C. Z. *Kristallogr.* **1963**, *119*, 273–283. Phenol C–O linkages range from 1.346 to 1.410 Å, to be contrasted with bond lengths of 1.204 Å (1.217 Å) and 1.281 Å (1.275 Å) for the quinone and semiquinone of **3** (**4**), respectively.

the Coulombic repulsion. As the torsion angle is moved away from the minimum, the observed stabilization decreases sinusoidally in line with diminishing overlap between the oxygen lone pair and the π-system and reaches a maximum (i.e., least stabilization) at 60°. In the range from 60° to 120°, overlap is small and the energy profile reflects a decrease in the Coulombic repulsion between the lone pairs on the methoxy and carbonyl oxygens. Finally, in the range from 120° to 180°, steric interactions between the methoxy methyl and the carbonyl come to the fore and slightly outweigh the increasingly favorable overlap between the lone pair on the methoxy oxygen and the quinone π-system. Supporting the assignment of a steric interaction in the 180° structure, the C_{ring}–O–C angle widens by 6°, compared to the minimum at 0°. A consequence of this widening of bond angle is an increased p-character of the methoxy oxygen lone pair, thereby enhancing stabilization due to orbital overlap relative to the conformer at 0° (effect 1).[10]

One would anticipate that upon formation of the semiquinone (i.e., a one-electron reduction) the importance of Coulombic effects would be enhanced whereas contributions due to electron delocalization (effect 1) would be attenuated. Heightened Coulombic interactions result from increased electron density around the carbonyl oxygen due to the additional electron. In a similar vein, the additional electron on the semiquinone renders the aromatic ring less electron deficient and hence the lessened importance of overlap stabilization of the oxygen lone pair. The calculated energy profile for the semiquinone of **3** (curve labeled "$Q^{\cdot-}$" in Figure 1) is in accord.

Examination of the curve in Figure 1 for the semiquinone indicates that conformers that minimize Coulombic interactions between the methoxy and carbonyl oxygens are in general more stable. As in the case of the ground-state quinone, the decrease in stabilization in going from 0° to 60° has been ascribed to an attenuation of orbital overlap between the semiquinone π-system and the methoxy oxygen π-type lone pair. The range from 60° to 120° reflects a diminished Coulombic repulsion, and the energy increase for the range from 120° to 180° is consistent with an increasing steric interaction between the methoxy methyl and the semiquinone oxygen.

On the whole, all of the enthalpies for electron attachment for **3** are negative, although the magnitude is strongly a function of the methoxy torsional angle. The calculations indicate a variation of approximately 0.4 eV (~9 kcal·mol^{-1}) from torsion angle extrema to extrema. In the absence of large barriers to conformer interconversion, experimentally measured electron affinities (and in solution, measured oxidation–reduction potentials) will reflect the difference in free energy between the minimum energy forms of the various electronic states (i.e., a nonvertical process), rather than at fixed methoxy torsion angles where a vertical electron-transfer process would be observed. However, at a catalytic site in a protein, the quinone and the semiquinone radical anion are likely to be constrained to particular geometries by the shape of the binding site, and thus variations in the electron-stabilizing abilities of unique quinone conformers can be expressed. *That is, enforced conformations of an alkoxy group in alkoxy quinones (e.g., ubiquinone) could have a significant influence on the stability and electron-transer rates of the putative intermediates along reduction pathways through conformer interconversion.*

The energy differences calculated for **3** are for two essentially non-interacting methoxy groups; a monomethoxyquinone would likely have less of an effect. Relevant to the 2,3-dialkoxyquinone ubiquinone, it has been noted[4] that substituents at the 3-position sterically block access to the 0° methoxy conformer, based upon a survey of the crystallographic data for 3-methyl-2-methoxyquinones. The average torsion angle reported was 120°, in accord with the second minima found herein for the calculated ground-state quinone **3**.

In line with the conformations of other 2,3-dialkoxybenzoquinones,[11] two configurations for ubiquinone are suggested as likely minima (Figure 2). The first places one of the alkoxy groups syn to the double bond in the ring (i.e., the 0° form), with the adjacent alkoxy group adopting the 120° conformation. This configuration samples both of the minima found for **3** and in addition should experience additional stabilization due to the alignment of the two alkoxy oxygen dipoles. The second configuration finds both alkoxy groups in a 120° conformation, on opposite sides of the ring. While minimizing steric interactions involving the alkyl groups of the alkoxy substituents, this form is expected to be higher in energy due to a Coulombic repulsion between the oxygen lone pairs on the ring substituents. Given that there will be several opposing influences of the sort discussed above, the salient hypothesis is that both 0° and 120° forms are accessible. Thus, it is suggested that protein-bound ubiquinone has available two discrete reduction potentials that are determined by the shape of the binding pocket, which in turn determines the conformation of the methoxy substituents. Furthermore, given the ability of a protein to alter the shape of an incorporated binding site, e.g., through allosteric effects, the preferred conformation of a bound quinone may be purposefully regulated; it is proposed that this represents a simple means of energy exchange/transformation between electrical and conformational potential energies within quinone reductases. Alternatively proteins could utilize multiple pockets, with regulation of the "observed" quinone potential occurring via competitive inhibition of the competing sites.

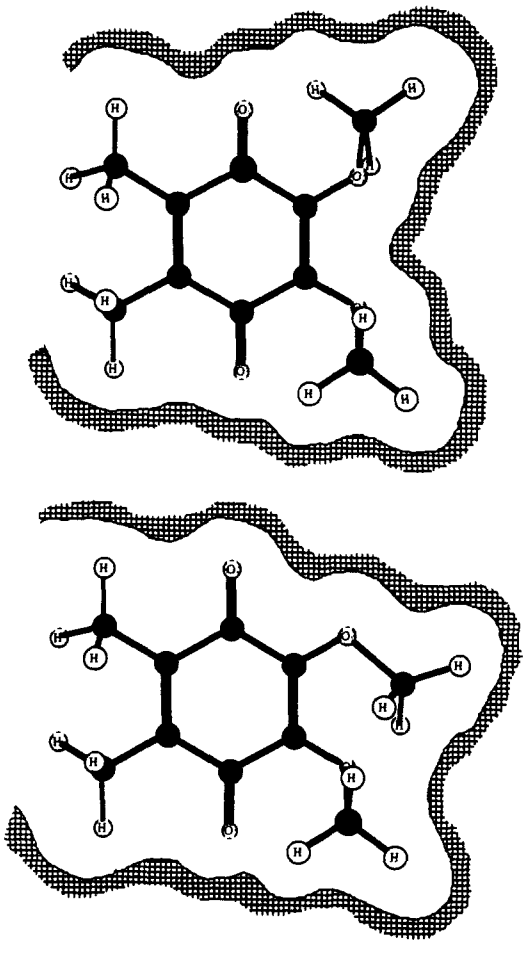

Figure 2. Proposed structures for 2,3-dimethoxy-5,6-dialkyl-1,4-benzoquinones. Model protein binding pockets for ubiquinone are included as hashed lines.

Upon examination of Figure 3, one may conclude that alterations in electron affinities in substituted quinones, with substituent conformation, are a common motif. For example, as a model for plastoquinone the peralkylated quinone **4** displays an interesting

(10) Kahn, S. D.; Keck, G. E.; Hehre, W. J. *Tetrahedron Lett.* **1987**, *28*, 279–280.

(11) (a) Silverman, J.; Stam-Thole, I.; Stam, C. H. *Acta Crystallogr.* **1971**, *B27*, 1846–1851. (b) Schmalle; Jarchow, O. H.; Hausen, B. M.; Schulz, K.-H. *Acta Crystallogr.* **1984**, *C40*, 1090–1092.

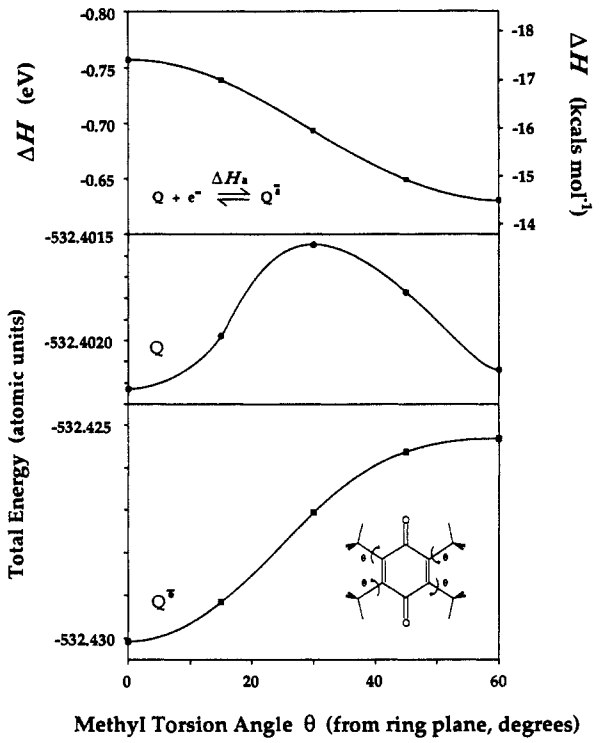

Figure 3. Rotational profiles and electron affinity of 2,3,5,6-tetramethyl-1,4-benzoquinone. Energies were obtained via reoptimization of all geometric parameters exclusive of the fixed dihedral angle indicated (C_2 symmetry imposed).

relationship between methyl group conformation and calculated electron affinity. The variation of calculated electron affinity of ~0.14 eV (~3 kcal·mol^{-1}) is consistant with the participation of two primary interactions involving the methyl substituents: (1) the delocalization of the π-type symmetry electrons of the methyl bonding orbitals[12] and (2) the steric interactions between adjacent methyl groups. Orbital delocalization will be at a maximum at 0° and 60° and will be zero by symmetry at 30°. However, in an absolute sense this effect is very small indeed (see Figure 3). Steric interactions, on the other hand, are most apparent for 60° forms, wherein hydrogens on adjacent methyl groups eclipse the double bond of the ring and in turn impinge upon one another. In direct analogy with the previous arguments concerning **3**, the importance of π-donation from a substituent to the quinone ring will be attenuated upon formation of the semiquinone, whereas the putative steric interactions will be accentuated by the one-electron reduction due to a stiffening of the underlying ring. Lastly, consideration of an intermediate case with one methyl group adopting the 0° form and the adjacent methyl the 60° form yields an electron affinity roughly midway between the 0° to 0° and 60° to 60° transitions shown in Figure 3. This is perhaps suggestive of simple group additivity, rather than group cooperativity, between the adjacent methyl groups.[13] While it seems unlikely that a methyl rotor can be constrained to adopt a single conformation,[14] a reasonable extension of these data suggests that the conformations of larger alkyl substituents could contribute to the overall redox potential of quinones in confined environments.

(12) Hehre, W. J.; Salem, L. *J. Chem. Soc., Chem. Commun.* **1973**, 754–755.

Conclusions

In conclusion, it would appear that quinones are highly functional oxidation–reduction intermediaries, which can alter electrical potentials through conformational changes in their substituents. The criteria for large alterations in potential include conformation-dependent π-donation by the substituent and Coulombic or dipolar interactions between the substituents and the carbonyl oxygens that correlate with substituent conformation. In the case of electron-transfer processes in quinone reductases, the calculations presented suggest the magnitude of the energetic misalignment (and alignment) between the energy levels of the donor and acceptor that can result from conformational adjustments in the quinone or semiquinone substituents. In the case of ubiquinone, methoxy conformation could act as an on–off switching mechanism, with the alkyl-substituent conformation allowing for the fine tuning of the usable electrical potential. This implies that careful consideration must be given to the gross structure of the quinone moiety when examining the mechanisms of electron transfer at a reductase or oxidase site. Perhaps more generally, the "switching" of quinone potential with substituent conformation may play a role in the allosteric control of quinone-based redox pathways and, for example, may be implicated in the mechanism of hormonal control of mamalian mitochondrial electron-transfer reactions.

Acknowledgment. We thank Professor Antony Crofts for the many stimulating discussions of the biophysics of quinone reductases that helped inspire this work. Partial support from the donors of the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged.

Registry No. 3, 530-55-2; 4, 527-17-3.

Supplementary Material Available: Listing of fully optimized structures for the quinone and quinone radical anion of **3** and **4** in Z-matrix format (4 pages). Ordering information is given on any current masthead page.

(13) It should be noted that the effects found for **3** are approximately three times greater than those found for **4**. Also, while it is difficult to image a binding pocket capable of expressing the two suggested reduction potentials of a methyl substituent (i.e., in **4**), the same effects are expected to operate in alkylated quinones by analogy.

(14) Lubitz, W.; Abresch, E. C.; Debus, R. A.; Isaacson, R. A.; Okamura, M. Y.; Feher, G. *Biochem. Biophys. Acta* **1985**, *808*, 464–469.