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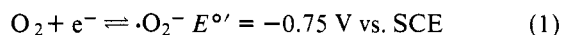
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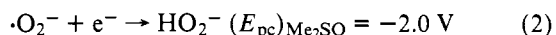
On the Chemical Reactivity of Superoxide Ion

Sir:

During the past decade strong interest has developed in the chemistry of superoxide ion. Electrochemical investigations in aprotic solvents¹⁻⁶ have established that molecular oxygen is reduced by a reversible one-electron process to superoxide ion



which in turn is reduced by a second irreversible one-electron process (with the solvent involved).⁵



Of the range of reactions for $\cdot\text{O}_2^-$ that have been described recently, two properties, its nucleophilicity⁷ and its reducing power⁸⁻¹¹ (roughly equivalent to dithionite ion),¹² seem well-documented. On the other hand, its propensity to act as an oxidizing agent^{9,13-18} is much more equivocal. Although solutions of superoxide can cause a net oxidation of substrates, we present evidence that $\cdot\text{O}_2^-$ cannot act directly as an oxidant for solution conditions where it is stable, namely aprotic media. Furthermore, although the $\text{p}K_{\text{a}}$ of HO_2^- in water has been shown to be 4.88 (i.e., $\cdot\text{O}_2^-$ is a weak base),¹⁹ we present arguments and preliminary data to show that solutions of $\cdot\text{O}_2^-$ behave as if they are strongly basic.

Nonoxidizing Properties. Equations 1 and 2 indicate that from a thermodynamic standpoint *superoxide ion is a moderate reducing agent, but a pitifully weak oxidizing agent (roughly the equivalent of sodium ion)*.^{2,5} However, in the presence of proton sources, superoxide ion disproportionates rapidly¹⁹ to form peroxide ion and oxygen. Because all the reports that discuss the oxidizing properties of superoxide ion have involved systems with proton sources, a reasonable explanation of the observed oxidation reactions is that they actually involve products of the disproportionation reaction.²⁰

The oxidative inertness of $\cdot\text{O}_2^-$ is confirmed by its non-reactivity with a wide variety of functional groups, including benzaldehyde, in rigorously aprotic solutions (dry pyridine).²²

In support of these considerations, we have undertaken a series of studies of 3,5-di-*tert*-butyl-*o*-quinone, 3,5-di-*tert*-butylcatechol, and catechol mono- and dimethyl ethers in acetonitrile. (The catechol, in the presence of KO_2 in acetonitrile and THF, recently has been shown to be oxidized to a complex mixture of products, including the *o*-quinone.¹³) Figure 1a illustrates the electrochemical reduction of oxygen to superoxide ion (upper peak), and the reoxidation of the latter (lower peak). Reduction of $\cdot\text{O}_2^-$ does not occur at potentials less negative than those which bring about solvent reduction (ca. -2.0 V). The lower peaks of curves b and c represent the oxidation of 3,5-di-*tert*-butylcatechol and its anion (formed as described in the legend), respectively. Curve d represents the reversible reduction of 3,5-di-*tert*-butyl-*o*-quinone to its semiquinone. These results indicate that it is thermodynamically unfavorable for $\cdot\text{O}_2^-$ to oxidize the catechol or its anion in acetonitrile by direct electron transfer.

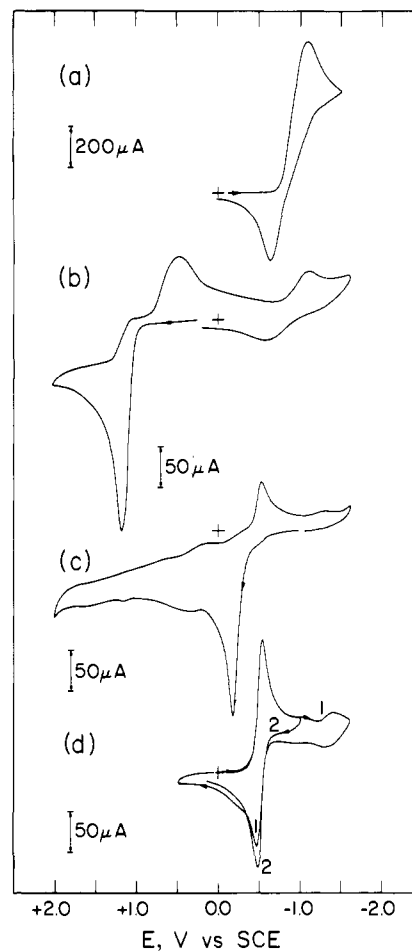
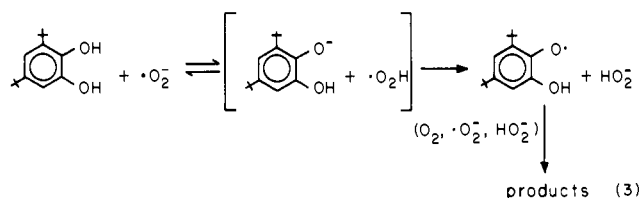
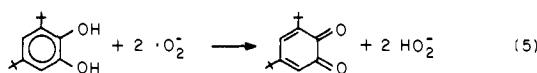
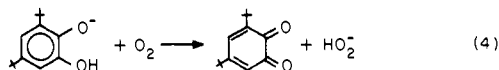


Figure 1. Cyclic voltammograms in acetonitrile that contains 0.1 M tetraethylammonium perchlorate (scan rate, 0.1 V/s at a platinum electrode (0.23 cm²)): (a) molecular oxygen at 1 atm, (b) 2 mM 3,5-di-*tert*-butylcatechol, (c) 2 mM 3,5-di-*tert*-butylcatechol plus 4 mM tetraethylammonium hydroxide, and (d) 2 mM 3,5-di-*tert*-butyl-*o*-quinone.

Indeed, we find that combining a solution of superoxide ion with 3,5-di-*tert*-butylcatechol anion in pyridine results in no reaction within 15 min. As previously reported,^{2,14,15} superoxide ion reacts rapidly with the un-ionized catechol. We also find that neither the monomethyl nor the dimethyl ether of catechol reacts with $\cdot\text{O}_2^-$ to yield the quinone or semiquinone, but the former promotes the disproportionation of $\cdot\text{O}_2^-$.²⁰ Taken together with the electrochemical data these chemical results show that the initial reaction of superoxide with 3,5-di-*tert*-butylcatechol must be a proton transfer from catechol to the superoxide ion followed by further chemistry of the HO_2^- so produced. Several subsequent paths to products can be envisioned. One is a cage reaction between catechol anion and HO_2^- , as depicted in eq 3, with subsequent reaction



between the semiquinone radical and any of a variety of potential further reactants. Another path, if the catechol anion becomes free, is attack by the molecular oxygen that results from disproportionation of HO_2^- and $\cdot\text{O}_2^-$.^{20,23} In this case the overall reaction would be represented by eq 4. The net reaction, starting with $\cdot\text{O}_2^-$, is then eq 5 which accounts not just for the



reported overall reaction stoichiometry, but also for several peculiar aspects of O₂ uptake and generation.^{1,3}

Other reported cases of apparent oxidation by $\cdot\text{O}_2^-$ include hydrazines,^{9,16,17} thiols,^{7b,18,22} and certain alcohols.^{7b,22} For each of these, proton transfer to $\cdot\text{O}_2^-$ appears to be a necessary first step (dialkyl sulfides and ethers are inert, and alkoxides are inert). In fact, Misra and Fridovich²⁴ have established that protons or metal cations are necessary to catalyze the oxidation of hydrazine by superoxide.

Effective Basicity. A second property of superoxide solutions is their capacity to effect proton removal from substrates.²⁴ For example, eq 6, which represents the acid-catalyzed disproportionation of superoxide, has a *K* value of 2.5×10^8 when the substrate is H₂O.²⁰ Obviously, even much weaker acids than water will produce an exothermic reaction here.



The overall reactions represented by eq 6 may not be rapid with weak acids (HB), but neither are many of the reported substrate reactions with $\cdot\text{O}_2^-$. Indeed, recent data in our laboratory establish that a number of weakly acidic organic compounds are deprotonated efficiently in the presence of superoxide, especially when the organics are solvents or in high concentrations.²²

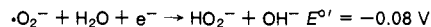
The cleavage of esters by superoxide ion recently has been described as a nucleophilic attack.^{7b} However, in the presence of water or other proton sources superoxide ion could lead to the formation of strong Brønsted bases via reaction 6 to give ester hydrolysis without direct attack by $\cdot\text{O}_2^-$.

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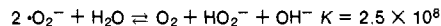
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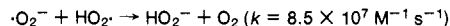
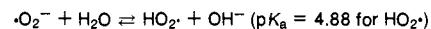
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- (20) The combination of the reduction half-reaction for $\cdot\text{O}_2^-$ in water²¹



with eq 1 (the redox potential of O₂/O₂⁻ in water, E^{0'}, is -0.58 V)²¹ yields the net expression



This indicates that superoxide ion solutions can promote proton transfer from substrates to an extent equivalent to that for the conjugate base of an acid with an approximate p*K*_a value of 23 (assuming a p*K*_a of 15 for water). This equilibrium expression is the result of the reactions



which are well established.¹⁹ Note that the last reaction is rapid and highly exothermic. Because of this, the lifetime of HO₂⁻ should be sufficiently short to preclude it as a major reactant with most substrates in solution.

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Structure of Shikodonin, a Unique Anti-Tumor Spirosecokarene Diterpenoid

Sir:

Recently we have reported the structure of a new *ent*-kaurane diterpenoid, shikokianidin,¹ which was isolated in addition to shikokianin and oridonin from the dry leaves of the ether extracts of *Isodon shikokianus* (Labiatae).² Further examination of the same plant has now resulted in the isolation of another new diterpenoid, shikodonin, in 0.00005% yield. This minor bitter principle could only be separated via chromatography on silica gel followed by HPLC using μ -Bondapak-C₁₈, 4-mm i.d. \times 30 cm, methanol-water (42.5:57.5 v/v).

Shikodonin possesses significant in vitro cytotoxicity (KB) and in vivo antitumor activity against Ehrlich ascites carcinoma inoculated into mice.^{3,4} It also exhibits insect growth inhibitory activity specifically against Lepidopteran larvae. For example, shikodonin much more strongly inhibits the respiratory reactions of mitochondria from *Bombyx mori* than from mammalian tissue (rat liver).⁵

We have now established the unique spirosecokarene structure **1** for shikodonin, with the following physical properties: C₂₀H₂₆O₆ (chemical ionization-mass spectroscopy in isobutane and elemental analysis); mp 206–209 °C; UV (EtOH) 233.5 nm (ϵ 8880); IR (Nujol) 3550 (hydroxyl), 1730 (six-membered lactone), 1700 and 1640 cm⁻¹ (five-membered ring ketone conjugated with an exocyclic methylene). The ¹³C NMR data of shikodonin showed the presence of one methyl, seven methylenes, five methines, and three tetrasubstituted carbons, together with two olefinic and two carbonyl carbon atoms.⁶ The pertinent ¹H NMR and ¹³C NMR data are shown in Chart I.

Addition of the ¹H NMR shift reagent Eu(dpm)₃ caused a clear downfield shift of the 9 β -H (d, 6 H). The magnitude of the coupling constants, *J*_{9 β ,11 β} = 6, *J*_{11 β ,12 α} = 15, and