Table I. Ab Initio Results for *syn*- and *anti*-Butane

calculation	basis set	E(syn), au	E(anti), au	ΔE, kcal/mol
SCF	6-31G	−157.218 797	−157.228 929	6.36
SCF	6-31G+P[a]	−157.298 189	−157.308 520	6.48
CISD[b,c]	6-31G	−157.312 984	−157.320 828	4.92
CISD[b,d]	6-31G	−157.428 038	−157.435 338	4.58

[a] Polarization functions have been added to each atom as follows: C, an sd set (0.75); H, a p set (1.00). [b] Configuration interaction over selected singly and doubly excited configurations (33 234 possible, see text). [c] 3782 configurations: the active space is MO's 5–30. [d] 11 665 configurations: the active space is MO's 5–40.

mechanics method but missing from the HFR calculations.

We report herein results of a molecular orbital (MO) study of the syn and anti conformations of *n*-butane using the split-valence 6-31G basis set.[7] The geometries used are taken from the molecular mechanics optimizations.[4] Possible basis set limitations have been further explored by carrying out HFR calculationx in which polarization functions[8] have been added to all atoms (6-31G+P). Correlations effects have been studied by the method of configuration interaction (CI). In the CI calculations (6-31G) selected singly and doubly excited configurations were included (CISD) at two different levels. In both cases all occupied valence MO's (5–17) were included. Configurations were selected by the graphical unitary group approach, the size of the calculation being controlled by truncation of the virtual space (see Table I for the virtual space truncations).[9] These results are summarized in Table I. The calculated energy difference at the HFR level is 6.36 kcal/mol for the 6-31G basis set. Addition of polarization functions induces little change in this quantity, +0.12 kcal/mol. The CISD calculations, conversely, produce a diminution of the energy difference to 4.92 kcal/mol for 3782 configurations, and 4.58 kcal/mol for 11 665 configurations. From these values we have extrapolated that the energy difference will be 4.54 kcal/mol at the CISD limit for the 6-31G basis set. We have compared these results to those obtained for ethane at the 6-31G level. The rotational barriers found for MM1[10] geometries are 2.83 kcal/mol at the HFR level and 3.03 kcal/mol for the full CISD calculation. Thus, the correlation effects that we find reflect the unique features of *syn-n*-butane. We were unable, unfortunately, to carry out CISD calculations for the butane conformations with the 6-31G+P basis set due to disk storage limitations.

It is clear that, when correlation effects are taken into account, the energy difference is predicted to match closely that obtained from the molecular mechanics studies. This is reassuring, as the large thermodynamic data base used to parameterize the molecular mechanics method made it seem unlikely that the error was in the MM2 value. Most previous estimates of this energy difference have not been helpful. Ito suggested a value of 6.1 kcal/mol,[11] but this was derived from a thermodynamic analysis assuming a planar cyclopentane. Ultrasonic relaxation studies[12] have led to a proposed value of 6.7 kcal/mol, but this value draws upon questionable data for 2-methylbutane. Durig[13] has analyzed the far-infrared spectrum of *n*-butane and derived a value of 3.02 kcal/mol for this energy difference. (This value is suspiciously low and probably reflects the limited torsional data available for the analysis.) Recent Raman studies of the relevant spectral lines at higher resolution have led to a more reasonable value, 4.52 kcal/mol.[14] This last value implies that the MM2 and CISD values probably provide the most accurate theoretical assessments of this energy difference thus far.

The significance of these results extends far beyond the case of *n*-butane itself. When molecular mechanics calculations indicate large dispersion interaction energy differences, it is quite likely that calculations at the HFR level will be in error, as for butane. This will generally be true for congested molecules. At the practical level, a simple dispersion energy calculation may suffice to overcome this difficulty. It follows that care must be exercised in the use of HFR calculations for generation of data required in the development of molecular mechanics force fields.

Acknowledgment. We thank Dr. J. J. Wendoloski, who brought the program HONDOG to Du Pont. Helpful comments were also obtained from Dr. L. L. Shipman.

Registry No. Butane, 106-97-8.

(7) (a) R. Ditchfield, W. J. Hehre, and J. A. Pople, *J. Chem. Phys.*, **54**, 724 (1971); (b) W. J. Hehre, R. Ditchfield, and J. A. Pople, *ibid.*, **56**, 2257 (1972); (c) P. C. Hariharan and J. A. Pople, *Chem. Phys. Lett.*, **16**, 217 (1972).

(8) F. A. Van-Catledge, unpublished results.

(9) All calculations were performed by using the program HONDOG, the Du Pont version of the General Atomic and Molecular Electronic Structure System (GAMESS) developed by the staff of the National Resource for Computing in Chemistry (NRCC). This work was carried out on the VAX 11/780 computer of the Central Research and Development Department at Du Pont.

(10) N. L. Allinger, *Adv. Phys. Org. Chem.*, **13**, 1 (1976).

(11) K. Ito, *J. Am. Chem. Soc.*, **75**, 2430 (1953).

(12) J. E. Piercy and M. G. S. Rao, *J. Chem. Phys.*, **46**, 3951 (1967).

(13) J. R. Durig and D. A. C. Compton, *J. Phys. Chem.*, **83**, 265 (1979).

(14) D. A. C. Compton, S. Montero, and W. F. Murphy, *J. Phys. Chem.*, **84**, 3587 (1980).

Oxidation of Ascorbic Acid and Dehydroascorbic Acid by Superoxide Ion in Aprotic Media

Donald T. Sawyer,* Glaico Chiericato, Jr., and Tohru Tsuchiya

Contribution from the Department of Chemistry, University of California, Riverside, California 92521. *Received April 12, 1982*

Abstract: In dimethylformamide, superoxide ion (O_2^-) oxidizes ascorbic acid (H_2A) to dehydroascorbic acid (A). The rate-limiting step is first order for each reactant and has a second-order rate constant (k) of 2.8×10^4 M^{-1} s^{-1}; the overall stoichiometry is $3H_2A$ molecules per $2O_2^-$ ions. Addition of O_2^- to dehydroascorbic acid (A) results in its rapid oxidation ($k = 3.3 \times 10^4$ M^{-1} s^{-1}), with an overall stoichiometry of $2O_2^-$ per A. The major products of the process are oxalate ion and the anion of threonic acid. On the basis of the reaction stoichiometries, kinetics, and products, self-consistent mechanisms are proposed for the oxidation of ascorbic acid and dehydroascorbic acid.

The oxidation of ascorbic acid (H_2A) and its anion (HA^-) by perhydroxyl radical ($HO_2\cdot$) and by superoxide ion (O_2^-) in aqueous solutions has been demonstrated to be a direct one-electron transfer process ($k_1 \sim 10^5$ M^{-1} s^{-1}),[1,2]

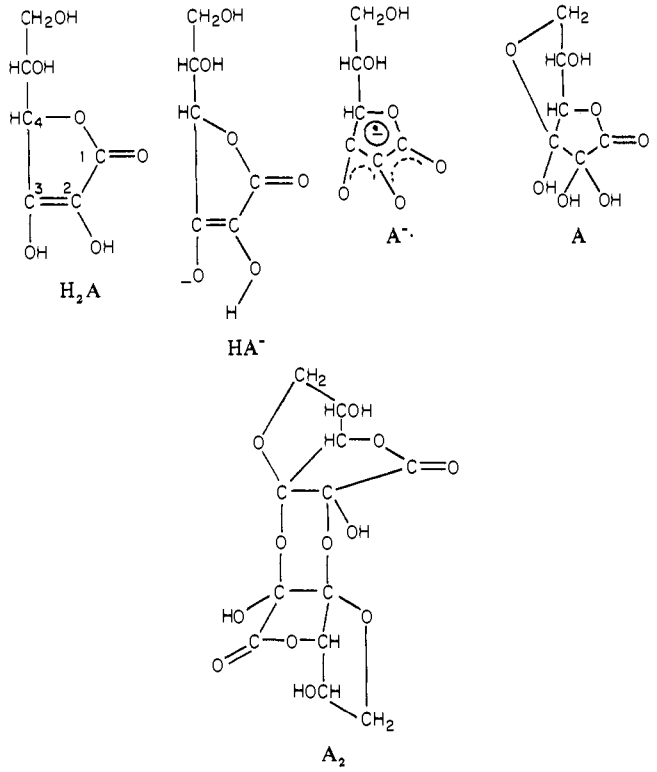
0002-7863/82/1504-6273$01.25/0

$$O_2^- + HA^- \xrightarrow[k_1]{H^+} H_2O + \text{oxidation products} \qquad (1)$$

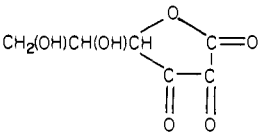
with the anion radical of ascorbic acid ($A^{-}\cdot$) assumed to be the initial product.[2] A subsequent study has suggested that the $A^{-}\cdot$ species disproportionates via an initial dimerization.[3] These results are consistent with those from a biochemical study of ascorbate oxidation at pH 7.4 by O_2^- ($k \sim 10^5$ M^{-1} s^{-1}) that was generated by the xanthine–xanthine oxidase system.[4] A recent analysis[5] discusses the thermodynamics and kinetics for ascorbate as a one-electron reducing agent in aqueous media.

Comparable studies in aprotic media have not been reported, nor are we aware of studies of the reactivity of O_2^- with dehydroascorbic acid in any medium. However, in a previous paper[6] we addressed (without resolution) the question as to whether O_2^- directly oxidizes ascorbic acid (in dimethylformamide) or undergoes a proton-induced disproportionation to O_2 and H_2O_2 with a subsequent redox reaction between O_2 and HA^-.

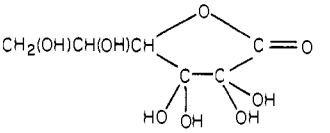
The stable structure of ascorbic acid (H_2A) has been known since 1933[7] and has been confirmed by recent NMR studies[8] to be the same in aqueous, dioxane, and dimethyl sulfoxide solutions as it is in the solid phase. The NMR results[8] demonstrate that deprotonation occurs at the C_3 hydroxyl to give the HA^- anion.



For aqueous conditions, oxidation of H_2A or HA^- by ·OH radicals yields the ascorbate anion radical ($A^{-}\cdot$) on the basis of ESR studies;[9] the approximate pK_a of $HA\cdot$ is 0.5.[10] Although dehydroascorbic acid (A) traditionally has been viewed as a tricarbonyl with a C_1–C_4 lactone ring,[11]



X-ray[12] and recent NMR studies[8,13] provide compelling evidence that its crystalline structure is a symmetric dimer (A_2), which persists in dry dimethyl sulfoxide and dimethylformamide solutions (at room temperature isomerization occurs between the C_2–O–link and the C_2–OH group to give asymmetrical anomers of A_2). Addition of water causes the dimer to dissociate to the bicyclic, hydrated monomer of dehydroascorbic acid (A); for aqueous solutions at room temperature the hemiketal ring slowly hydrolyzes to give the hydrated lactone[13]



Ascorbic acid was recognized as an essential nutritional component for primates prior to its structural characterization,[14] but its biochemical function in animal[15] and plant[16] metabolism is not understood. There is persuasive evidence that ascorbic acid (a) is associated with respiration processes in plants[16] and animals,[17] (b) acts as an antioxidant,[18] (c) is a protective agent in plants against O_2^- and peroxides,[18,19] and (d) is autoxidized under alkaline conditions.[6] The observation that C_1-labeled (^{14}C) ascorbic acid and dehydroascorbic acid are oxidatively metabolized by a number of plants to yield substantial fractions (3–58%) of labeled oxalate[20,21] is especially intriguing.

Clearly, ascorbic acid has unique interactions with the activated derivatives of dioxygen ($HO_2\cdot$, O_2^-, H_2O_2, HO_2^-, and ·OH). The preceding observations have prompted the present studies of the electron-transfer reactions of ascorbic acid and dehydroascorbic acid, and of the stoichiometries, kinetics, and mechanisms for oxidation of H_2A and A by O_2^- under aprotic conditions.

Experimental Section

Instrumentation. The electrochemical experiments were made with either a three-electrode potentiostat–amperostat constructed with operational amplifiers[22] or a Princeton Applied Research Model 173/179 potentiostat with digital integration. The voltammograms were recorded with a Houston Instruments Omnigraph 2000 X-Y recorder. The details of the electrochemical cell and its electrodes were the same as in a previous study.[6]

The UV–vis spectra were recorded with either a Cary Model 17D or Model 219 spectrophotometer. The concentration of supporting electrolyte in the matched reference and sample cells was the same as in the electrochemical experiments (0.1 M tetraethylammonium perchlorate).

Chemicals and Reagents. Burdick and Johnson "distilled in glass" dimethylformamide (DMF) (0.008% H_2O) was obtained in quart bottles to minimize contamination by water. Tetraethylammonium perchlorate (TEAP) (G. Frederick Smith Chemical Co.) was used as the supporting electrolyte in the electrochemical experiments. High-purity argon and oxygen were obtained from the Chemetron Corp.

(1) Bielski, B. H. J.; Richter, H. W. *J. Am. Chem. Soc.* **1977**, *99*, 3019.
(2) Nadezhdin, A. D.; Dunford, H. B. *Can. J. Chem.* **1979**, *57*, 3017.
(3) Bielski, B. H. J.; Allen, A. O.; Schwarz, H. A. *J. Am. Chem. Soc.* **1981**, *103*, 3516.
(4) Nishikimi, M. *Biochem. Biophys. Res. Commun.* **1975**, *63*, 463.
(5) Creutz, C. *Inorg. Chem.* **1981**, *20*, 4449.
(6) Nanni, E. J., Jr.; Stallings, M. D.; Sawyer, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 4481.
(7) Herbert, R. W.; Hirst, E. L.; Percival, E. G. V.; Reynolds, R. J. W.; Smith, F. *J. Chem. Soc.* **1933**, 1270.
(8) Matusch, R. *Z. Naturforsch, B: Anorg. Chem. Org. Chem.* **1977**, *32B*, 562.
(9) Schuler, M. A.; Bhatia, K.; Schuler, R. H. *J. Phys. Chem.* **1974**, *78*, 1063.
(10) Kirino, K. Y.; Schuler, R. H. *J. Am. Chem. Soc.* **1973**, *95*, 6926.
(11) Stecher, P. G., Ed. "The Merck Index"; Merck & Co.: Rahway, N.J.; 9th ed., p 325.
(12) Hvoslef, J. *Acta Crystallogr., Sect. B* **1972**, *B28*, 916.
(13) Hvoslef, J.; Pedersen, B. *Acta Chem. Scand., Ser. B* **1979**, *B33*, 503.
(14) Sharman, I. M. "Vitamin C"; Birch, G. G., Parker, K., Eds.; Wiley: New York, 1974; pp 1–15.
(15) Tolbert, B. M.; Harkrader, R. J.; Loux, S. J. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1978**, *37*, 589.
(16) Loewus, F. A. "The Biochemistry of Plants"; Preiss, J., Ed.; Academic Press: New York, 1980; pp 77–99.
(17) Boyer, P. D.; Chance, B.; Ernster, L.; Mitchell, P.; Racker, E.; Slater, E. C. *Annu. Rev. Biochem.* **1977**, *46*, 955.
(18) Halliwell, B.; Foyer, C. H. *Biochem. J.* **1976**, *155*, 697.
(19) Foyer, C. H.; Halliwell, B. *Planta* **1976**, *133*, 21.
(20) Yang, J. C.; Loewus, F. A. *Plant Physiol.* **1975**, *56*, 283.
(21) Nuss, R. F.; Loewus, F. A. *Plant Physiol.* **1978**, *61*, 590.
(22) Goolsby, A. D.; Sawyer, D. T. *Anal. Chem.* **1967**, *39*, 411.

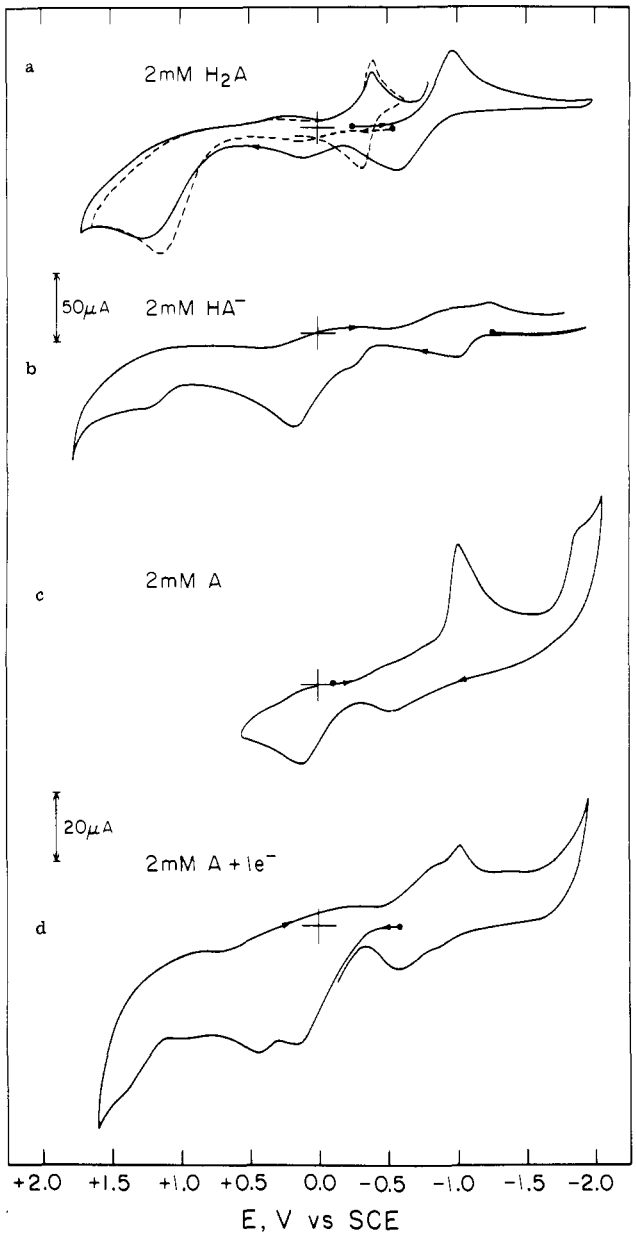


Figure 1. Cyclic voltammograms in dimethylformamide (0.1 M tetraethylammonium perchlorate) of 2 mM ascorbic acid (H_2A), 2 mM HA^- (from 2 mM H_2A and 2 mM TEAOH), 2 mM dehydroascorbic acid (A), and the product from the one-electron reduction of 2 mM A at −1.2 V vs. SCE. Measurements were made with a platinum electrode (area, 0.23 cm^2) at a scan rate of 0.1 V s^{-1}; temperature, 25 °C. $E_{NHE} = E_{SCE} + 0.25$ V.

Superoxide ion (O_2^-) was generated by electrochemical reduction of a solution through which oxygen was continuously bubbled. Upon completion of the electrolysis the solution of O_2^- was degassed with argon to remove residual oxygen. The concentration of O_2^- was monitored by cyclic voltammetry with the anodic peak current measured at −0.7 V vs. SCE. Standardization of the current relative to the concentration of O_2^- was accomplished by controlled-potential coulometric analysis. As an alternative source of O_2^-, tetramethylammonium superoxide ($TMAO_2$) was synthesized and isolated from liquid ammonia.[23] Tetraethylammonium hydroxide (TEAOH) was obtained from Eastman Kodak Co. as a 25% solution in ethanol. L(+)-Ascorbic acid was purchased from Matheson Coleman and Bell. Its purity was confirmed by acid–base titration, electrochemistry, and infrared spectra. The symmetrical dimer of L(+)-dehydroascorbic acid[13] was prepared by oxidation of L(+)-ascorbic acid with benzoquinone and isolated as a crystalline solid.[24] Its purity was confirmed by its infrared spectrum.

(23) McElroy, A. D.; Hashman, J. S. *Inorg. Chem.* **1964**, *3*, 1798.
(24) Muller-Mulot, W. *Hoppe-Seyler's Z. Physiol. Chem.* **1970**, *351*, 52.

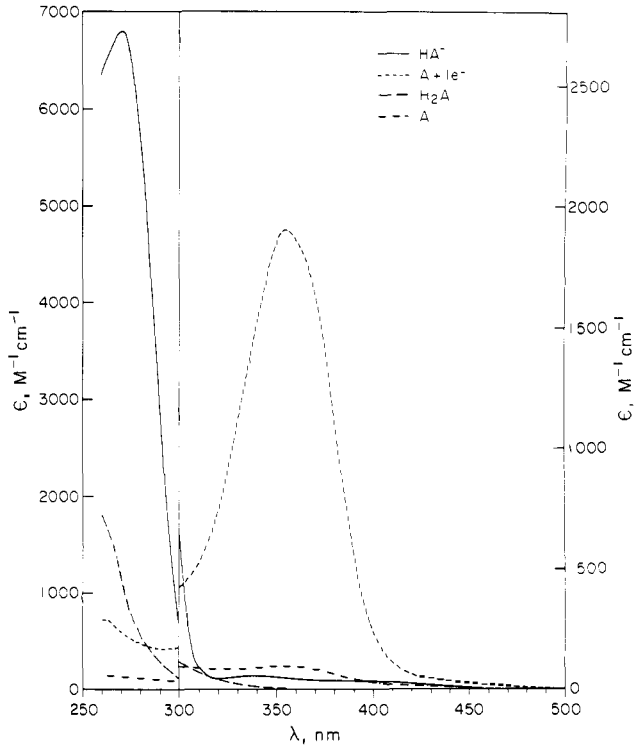


Figure 2. Absorption spectra in dimethylformamide (0.1 M TEAP) of ascorbic acid (H_2A), HA^-, dehydroascorbic acid (A), and the product from the one-electron reduction of A at −1.2 V vs. SCE. The molar absorptivities, ε, are in relation to the total concentration of the ascorbate species as mononuclear molecules.

Results

Redox Chemistry for Ascorbic Acid and Dehydroascorbic Acid. Figure 1 illustrates cyclic voltammograms for both an initial negative scan and an initial positive scan of 2 mM ascorbic acid (H_2A) in dimethylformamide (DMF). Other curves in this figure illustrate the electrochemical behavior of the anion of ascorbic acid (HA^-), dehydroascorbic acid (A), and the one-electron reduction product of A. Controlled-potential electrolysis of H_2A at the potential of its first major reduction peak consumes one electron per H_2A. The resulting solution has electrochemistry identical with that for HA^-.

The spectrophotometric characteristics of these species in DMF are illustrated by Figure 2. The most noteworthy feature is the intense absorption band at approximately 355 nm for the one-electron-reduction product of A. This band corresponds to that for the dehydroascorbate anion radical ($A^{-\cdot}$); in aqueous media its molar absorptivity is 3300 M^{-1} cm^{-1} at 360 nm.[3]

Oxidation of Ascorbic Acid by Superoxide Ion. When superoxide ion is added to ascorbic acid (H_2A) in DMF the O_2^- species is rapidly destroyed without production of molecular oxygen. Titration of H_2A by O_2^- confirms that molecular oxygen is not a product of the reaction up to a stoichiometry of two superoxide ions per three H_2A molecules. (Cyclic voltammetry has been used to monitor the O_2^- and O_2 concentrations during the course of the titrations.) Figure 3 illustrates the cyclic voltammogram for such a titration solution at the stoichiometric equivalence point. This and the spectrophotometric data of Figure 4 confirm that dehydroascorbic acid (A) is a major product from the 2:3 O_2^-:H_2A reaction.

When a titration of H_2A by O_2^- is continued until there is an unreacted excess of superoxide ion, the stoichiometry is 2.7 equiv of O_2^- per H_2A. The middle cyclic voltammogram of Figure 3 is for a solution with this reaction stoichiometry; the reduction peak at −0.9 V vs. SCE is due to molecular oxygen. Combination of O_2^- and H_2A in an 8:3 molar ratio in a sealed-cell assembly yields one oxygen molecule per H_2A.

Oxidation of Dehydroascorbic Acid by O_2^-. Addition of superoxide ion to dehydroascorbic acid in DMF results in a rapid

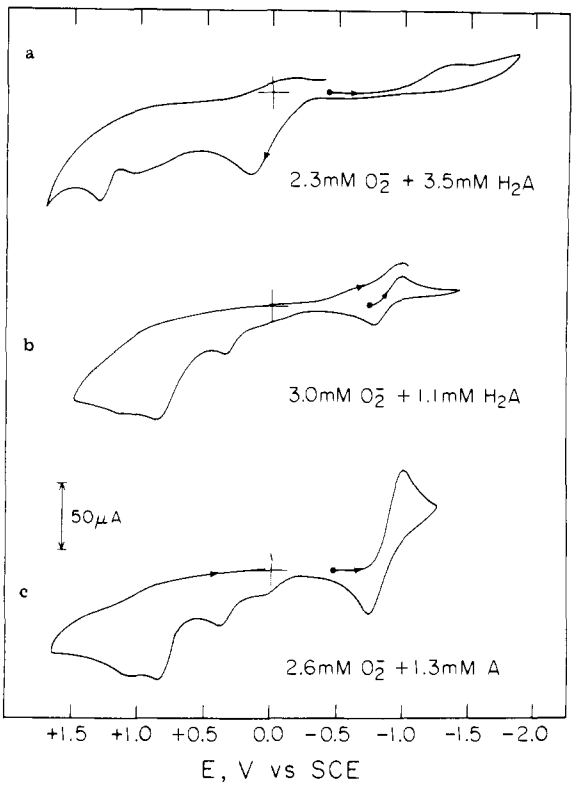


Figure 3. Cyclic voltammograms in dimethylformamide (0.1 M TEAP) of (a) 2.3 mM O_2^- plus 3.5 mM H_2A; (b) 3.0 mM O_2^- plus 1.1 mM H_2A; and (c) 2.6 mM O_2^- plus 1.3 mM A. Measurements were made in a sealed cell with a platinum electrode (area, 0.23 cm^2) at a scan rate of 0.1 V s^{-1}; temperature, 25 °C.

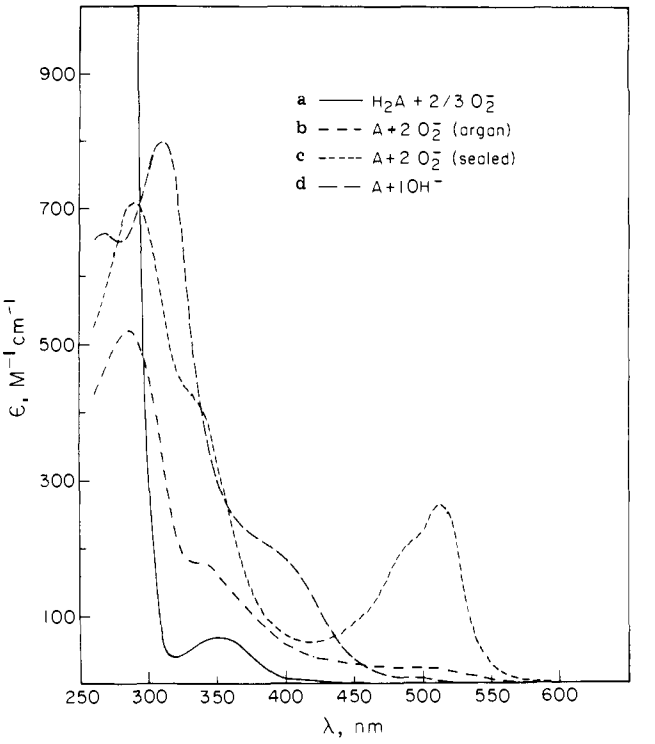


Figure 4. Absorption spectra in dimethylformamide (0.1 M TEAP) for the product solutions from the molar combination of (a) $3H_2A$ plus $2O_2^-$; (b) A plus $2O_2^-$ (with the system purged with argon during the reaction); (c) A plus $2O_2^-$ (in a sealed cell); and (d) A plus OH^- (as TEAOH). The molar absorptivities, ε, are in relation to the total concentration of the ascorbate species (as a mononuclear molecule) and the assumption that each yields a single absorbing one-for-one product molecule.

reaction to give a product solution that contains carboxylate anions and molecular O_2, but negligible amounts of A, HA^-, $A^{-\cdot}$, or 2,3-diketogulonate. On the basis of titrations of A by O_2^- (with cyclic voltammetry used to monitor the concentrations of reactants and products), the reaction stoichiometry is 2 equiv of O_2^- per A to give 1 equiv of O_2 per A. The bottom cyclic voltammogram of Figure 3 is for such a stoichiometric combination and illustrates a redox couple at −0.9 V vs. SCE for O_2; the couple is absent after the solution is bubbled with argon.

Figure 4 illustrates the UV–vis absorption spectra for the product solution from the combination of 2 equiv of O_2^- per A in DMF. When the reaction mixture is continuously purged with argon, a less intense product spectrum results than that obtained for a sealed reaction cell. The use of such a sealed cell results in product species with a substantial absorption band at 512 nm (with a shoulder at 480 nm). Those solutions that exhibit this band have a distinctive color that ranges from pinkish-orange to pink. (Several TLC analyses of the product solutions indicate that these colored species represent less than 3 mol % of the original A.) If this experiment is repeated in dimethyl sulfoxide solutions, the coloration develops much more slowly after the addition of two superoxide ions per A.

The product solution from the combination of two O_2^- per A in DMF only exhibits a series of diffuse anodic peaks in its electrochemistry (bottom curve, Figure 3), which are indicative of organic bases such as carboxylate ions. The addition of hydroxide ion to dehydroascorbic acid results in hydrolysis of the lactone to yield the anion of 2,3-diketogulonic acid; its spectrum is illustrated in Figure 4.

Addition of excess aqueous calcium acetate to a DMF solution in which 2 equiv of O_2^- have reacted with 1 equiv of A results in a pale yellow precipitate of calcium oxalate monohydrate (confirmed by IR spectra and redox titrations). On the basis of three separate experiments, at least 80% of the dehydroascorbic acid is oxidized to oxalate ion.

Reaction Kinetics. Because previous studies[6] have shown that superoxide ion can be rapidly decomposed by acidic substrates and also can act as a nucleophile toward carbonyl carbons with adequate leaving groups,[25] a series of kinetic measurements has been made with a stopped flow spectrophotometer for (O_2^-)–(H_2A) and (O_2^-)–(A) combinations in DMF. Both the disappearance of O_2^- (~1 mM $TMAO_2$ in DMF) and the appearance of product species have been monitored at the appropriate wavelengths. Analysis of the data indicates that both the reaction of O_2^- with H_2A and that of O_2^- with A follow pseudo-first-order rate laws. In each case the process is first order in O_2^- and first order in H_2A or A. Because both H_2A and A contain a lactone ring, a similar kinetic study has been made for the reaction of O_2^- with the δ lactone of gluconic acid. The second-order rate constants, k_2, for ascorbic acid, dehydroascorbic acid, and δ-gluconolactone in DMF at 25 °C are $(2.8 \pm 0.3) \times 10^4$ M^{-1} s^{-1}, $(3.3 \pm 0.3) \times 10^4$ M^{-1} s^{-1}, and $(2.6 \pm 0.3) \times 10^3$ M^{-1} s^{-1}, respectively. In a previous study[26] the observed value for k_2 for H_2A in DMF was 2×10^4 M^{-1} s^{-1}.

Discussion and Conclusions

Reference to the structures for H_2A, HA^-, $A^{-\cdot}$, A, and A_2 in the introduction indicates that each of these ascorbate derivatives has numerous acidic as well as redox active functional groups for potential reactivity with O_2^-. Furthermore, a recent review[27] confirms that O_2^- under aprotic conditions is a strong Brønsted base and nucleophile, a moderate one-electron reducing agent, and a hydrogen-atom oxidant. Hence, the reaction possibilities from the combination of O_2^- and ascorbic acid, and of O_2^- and dehydroascorbic acid, are diverse. To discover the dominate processes requires that the primary acid–base and electron-transfer process for the various ascorbate species in DMF solution be characterized.

(25) Gibian, M. J.; Sawyer, D. T.; Ungermann, T.; Tangpoonpholvivat, R.; Morrison, M. M. *J. Am. Chem. Soc.* **1979**, *101*, 640.
(26) Chin, D.-H.; Chiericato, G., Jr.; Nanni, E. J., Jr.; Sawyer, D. T. *J. Am. Chem. Soc.* **1982**, *104*, 1296.
(27) Sawyer, D. T.; Valentine, J. S. *Acc. Chem. Res.* **1981**, *14*, 393.

Acid–Base and Electron-Transfer Reactions. The data of Figures 1 and 2 confirm previous NMR results; namely, the stoichiometric addition of hydroxide ion (as TEAOH) to H_2A in DMF results in deprotonation of the C_3 hydroxyl to give HA^- (without opening the lactone ring)[28]

$$H_2A + OH^- \rightarrow HA^- + H_2O \quad (2)$$

Electrochemical reduction of H_2A yields the same anion on the basis of its electrochemical and spectrophotometric properties.

$$H_2A + e^- \rightarrow HA^- + {}^1/_2H_2 \quad (3)$$

$$E_{p,c} = -0.98 \text{ V vs. SCE}$$

When a solution of HA^- in DMF is oxidized electrochemically (Figure 1), the voltammetric peak height is characteristic of a process that involves more than one electron; controlled-potential coulometry at +0.25 V vs. SCE is consistent with a two-electron oxidation (when excess base is present). A reasonable sequence involves an ECE process (excess base will convert H_2A to more HA^-).

$$HA^- \rightarrow HA\cdot + e^- \quad (4)$$

$$E_{p,a} = +0.17 \text{ V vs. SCE}$$

$$2HA\cdot \rightarrow A + H_2A \quad (5)$$

The data of Figure 1 and from controlled-potential coulometry confirm that ascorbic acid is oxidized to dehydroascorbic acid in DMF via a two-electron process

$$H_2A \rightarrow A + 2H^+ + 2e^- \quad (6)$$

$$E_{p,a} = +1.13 \text{ V vs. SCE}$$

Addition of OH^- to dehydroascorbic acid in DMF rapidly hydrolyzes the lactone to give the anion of 2,3-diketogulonic acid (Figure 4).

$$A + OH^- \rightarrow CH_2(OH)CH(OH)CH(OH)C(O)C(O)C(O)O^- \quad (7)$$

In contrast to H_2A, electrochemical reduction of A only yields the anion radical of dehydroascorbic acid ($A^-\cdot$) in an irreversible and apparently complicated electron-transfer process (Figure 1).

$$((1/2)A_2 \xrightarrow{H_2O} A) + e^- \rightarrow A^-\cdot \quad (8)$$

$$E_{p,c} = -1.01 \text{ V vs. SCE}$$

Both the electrochemistry of Figure 1 and the spectra of Figure 2 confirm that $A^-\cdot$ is reasonably stable in dry DMF. However, water appears to promote formation of a dehydroascorbic acid–ascorbate [$A(HA)^-$] adduct via a disproportionation process

$$2A^-\cdot + H_2O \rightarrow A(HA)^- + OH^- \quad (9)$$

The electrochemistry of Figure 1 indicates that this adduct is oxidized to two A molecules by a two-electron process

$$A(HA)^- \rightarrow 2A + H^+ + 2e^- \quad (10)$$

$$E_{p,a} = +0.44 \text{ V vs. SCE}$$

Comparison of the electrochemistry and spectroscopy for the product solutions from the combination of O_2^- with H_2A and with A (Figures 3 and 4) to those for the acid–base and electron-transfer products for H_2A and A (Figures 1 and 4) provides compelling evidence that the Brønsted basicity and one-electron-reducing capacity of O_2^- are not significant in its reactions with H_2A and A.

Oxidation and Oxygenation by O_2^-. Although ascorbic acid is a moderately strong acid that should promote the disproportionation of superoxide ion to hydrogen peroxide and molecular oxygen, the present results as well as those of a previous study[6] have failed to exhibit any O_2 during the facile second-order reaction. This is true up to the stoichiometric equivalence point of two O_2^- ions per three H_2A molecules. On the basis of the electrochemical and spectrophotometric evidence, a reasonable set of reactions can be proposed for the process. The primary step appears to be a concerted transfer to the attacking O_2^- ion of a proton and hydrogen atom from the C_2 and C_3 hydroxyls of H_2A to yield dehydroascorbate anion radical plus hydrogen peroxide

$$O_2^- + H_2A \rightarrow A^-\cdot + H_2O_2 \quad (11)$$

$$k_{11} = 2.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$$

Subsequent reactions involve the proton-induced disproportionation of the dehydroascorbate anion radical and the base-catalyzed oxidation of HA^- by H_2O_2 to yield A (probably via the base-catalyzed disporportionation of H_2O_2).[29]

$$2A^-\cdot + H_2A \rightarrow A + 2HA^- \quad (12)$$

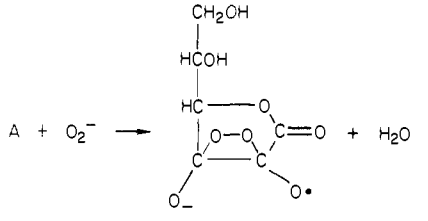
$$HA^- + H_2O_2 \rightarrow A + H_2O + OH^- \quad (13)$$

Summation of eq 11–13 yields an overall reaction stoichiometry that is in accord with the O_2^-–H_2A titration results and product yield.

$$2O_2^- + 3H_2A \rightarrow 3A + 2H_2O + 2OH^- \quad (14)$$

This reaction sequence also is consistent with the absence of any intermediate O_2.

The final challenge is to develop a rational mechanism for the observed $2O_2^-$-per-A oxidation (with oxalate as a major product). Reference to Figures 3 and 4 provides convincing evidence that hydrolysis of the lactone of A by O_2^- is not a significant process. (The observed rate of reaction for the O_2^-–A process is an order of magnitude faster than the rate of lactone hydrolysis for δ-gluconolactone.) Also, the weak acidity of A precludes proton-induced disproportionation of O_2^- as a viable path to account for the rapid second-order O_2^-–A reaction. Previous studies of the reactivity of O_2^- with substrates that contain adjacent electrophilic carbons (benzil[30,31] and methyl viologen cation radical[32]) are consistent with a nucleophilic addition to one of the carbons with subsequent attack of the second carbon to give a dioxetane intermediate, followed by cleavage to give two carbonyl groups. In the case of benzil, the reaction kinetics are second order and the stoichiometry is two O_2^- ions per substrate to give two benzoate ions plus one O_2 molecule. Consideration of the monomeric structure of A, the second-order kinetics for the O_2^-–A reaction, and the reaction stoichiometry of two O_2^- per A to give one oxalate and one O_2 provides a striking parallel to the benzil system. Hence, a reasonable conclusion is that the initial rate-controlling step involves nucleophilic addition of O_2^- at the C_3 carbon of A with subsequent closing to give a dioxetane radical anion



(15)

$A(O_2)^-\cdot$

$$k_{15} = (3.3 \pm 0.3) \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$$

The latter apparently is reduced by a second O_2^- to give oxalate

(28) Acerete, C.; Garrigos, L.; Guilleme, J.; Diez, E.; Aldaz, A. *Electrochim. Acta* **1981**, *26*, 1041.

(29) Roberts, J. L., Jr.; Morrison, M. M.; Sawyer, D. T. *J. Am. Chem. Soc.* **1978**, *100*, 329.

(30) San Fillipo, J., Jr.; Chern, C.-I.; Valentine, J. S. *J. Org. Chem.* **1976**, *41*, 1077.

(31) Stamp, J. J.; Menton, K. N.; Sawyer, D. T. *J. Am. Chem. Soc.*, to be submitted. In DMF O_2^- rapidly oxidizes benzil to two benzoate ions ($k \approx 10^4 \text{ M}^{-1} \text{ s}^{-1}$), $2O_2^- + PhC(O)C(O)Ph \rightarrow 2PhC(O)O^- + O_2$, apparently via a dioxetane intermediate.

(32) Nanni, E. J., Jr.; Angelis, C. T.; Dickson, J.; Sawyer, D. T. *J. Am. Chem. Soc.* **1981**, *103*, 4268.

($HC_2O_4^-$), the anion of threonoic acid (T^-), and O_2

$$A(O_2)^{-}\cdot + O_2^- \xrightarrow{H_2O} CH_2(OH)CH(OH)CH(OH)C(O)O^- + HC_2O_4^- + O_2 \quad (16)$$

The overall reaction is

$$A + 2O_2^- \rightarrow T^- + HC_2O_4^- + O_2 \quad (17)$$

Hence, in DMF O_2^- acts as a dioxygenase of dehydroascorbic acid via cleavage of its C_2, C_3 bond.

Combination of eq 14 and 17 yields an overall reaction whose stoichiometry is consistent with the experimental results when H_2A is limiting relative to O_2^- (the observed stoichiometry is $2.7O_2^-$ per H_2A).

$$3H_2A + 8O_2^- \rightarrow 3T^- + 3HC_2O_4^- + 3O_2 + 2OH^- \quad (18)$$

Under alkaline conditions HA^- reacts facilely with molecular oxygen in both aqueous and aprotic media. This represents another dimension of the unique ability of H_2A to serve as an antioxidant and protective agent from activated dioxygen species. On the basis of previous studies[6] and the observation of transient levels of O_2^- by ESR, a reasonable reaction sequence in alkaline DMF is

$$2O_2 + 2HA^- \rightarrow 2O_2^- + H_2A + A \quad (19)$$

$$2O_2^- + A \xrightarrow{H_2O} T^- + HC_2O_4^- + O_2 \quad (20)$$

The overall reaction has a stoichiometry of one dioxygen per ascorbate ion to give threonoate and oxalic acid.

$$O_2 + HA^- \rightarrow T^- + H_2C_2O_4 \quad (21)$$

Although oxidation and oxygenation of H_2A by O_2^- in DMF yields oxalate, which is consistent with the metabolic fate of H_2A in many plants,[20,21] a direct O_2^-–H_2A process in plant metabolism is unreasonable. However, a dioxygenase enzyme (possibly a copper protein) may activate O_2 in a manner to promote the chemistry of reactions 11–21. This proposition is under active investigation.

The present results confirm that ascorbic acid, its anion, and dehydroascorbic acid are unique in their ability to destroy superoxide ion without the production of reactive intermediate radicals. Especially noteworthy is that one H_2A molecule can deactivate more than 2.5 superoxide ions. However, the production of oxalate, which results from such a stoichiometry, probably does not occur under most biological conditions because ascorbic acid usually occurs at concentrations that are at least three orders of magnitude greater than superoxide ion. When O_2^- is limiting, eq 11–13 will prevail; thus, the most hazardous product from ascorbic acid deactivation of superoxide ion is dehydroascorbic acid and hydrogen peroxide; under alkaline conditions HA^- destroys H_2O_2.

Work in progress is directed to the effect of transition-metal complexes on the activation of O_2, O_2^-, and H_2O_2 for reaction with H_2A and A. Some of this chemistry may prove relevant to the in vivo mechanism of oxidation and oxygenation of H_2A to oxalate in plants.[20,21]

Acknowledgment. This work was supported by the National Science Foundation under Grant No. CHE79-22040 and by the University of California Agricultural Experiment Station under Project No. CA-R-BPS-4080. We are grateful to Dr. Der-Hang Chin of this department and to Professor Henry Po of the California State University at Long Beach for assistance with the stopped-flow kinetic measurements.

Registry No. H_2A, 50-81-7; HA^-, 299-36-5; A^-, 34481-26-0; A, 490-83-5; A_2, 72691-25-9; $A(O_2)^-$, 83313-12-6; T^-, 83313-10-4; $HC_2O_4^-$, 920-52-5; O_2^-, 12185-08-9; H_2O_2, 7722-84-1; benzoquinone, 106-51-4; 2,3-diketogulonic acid anion, 83313-11-5; δ-gluconolactone, 90-80-2.

Porphyrins. 43.† Triplet Sublevel Emission of Platinum Tetrabenzoporphyrin by Spectrothermal Principal Component Decomposition

Thijs J. Aartsma,‡ Martin Gouterman,* Clemens Jochum, Alvin L. Kwiram, Barry V. Pepich, and Loren D. Williams‡

Contribution from the Department of Chemistry, University of Washington, Seattle, Washington 98195. *Received February 9, 1982*

Abstract: The synthesis and absorption spectra are reported for Pt^{II}(TBP) (peaks at 394 and 595 nm) and for $Pt^{IV}(TBP)X_2$ (peaks at 417 and 619 nm) (TBP = tetrabenzoporphyrin). The former shows strong phosphorescence at 745 nm with a quantum yield in degassed pyridine at room temperature of 0.18 ± 0.04; the latter is dark. The phosphorescence spectrum and lifetime of Pt(TBP) vary in the temperature range 4–84 K, but all decays are exponential. The decay rates are fitted with the assumption of thermal equlibrium among three triplet levels: Two levels at energy E_1 have an average decay rate $\bar{k}_1 = (418\ \mu s)^{-1}$; a third level at energy E_3 has a decay rate $k_3 = (22\ \mu s)^{-1}$; $E_3 - E_1 = 22.6\ cm^{-1}$. The temperature-dependent emission spectra are studied by principal component analysis, which shows two distinct spectra are present. These two spectra are determined within narrow limits. From the spectral decomposition we also determine the ratio $2\bar{k}_{1r}/k_{3r} = 0.0369$, where $\bar{k}_{1r}$ is the average radiative decay rate for the lower two levels and k_{3r} is that of the third level, and obtain an independent estimate that $E_3 - E_1 = 22.7\ cm^{-1}$.

Recently Vogler et al.[1] reported the synthesis and optical spectra of palladium and platinum tetrabenzoporphyrin (TBP). The relation between the spectra of the reported Pd(TBP) and Pt(TBP) was contrary to the known relation between the spectra in the porphyrin series.[2,3] In the porphyrin series the change Pd → Pt is characterized by a blue shift to the optical absorption and emission and an enhancement of the phosphorescence intensity. For the tetrabenzoporphyrin reported by Vogler et al., the change Pd → Pt gave rise to a red shift of the optical absorption and emission and a strong decrease in phosphorescence emission. This

† Part 42: Rawlings, D. C.; Davidson, E. R.; Gouterman, M. *Theor. Chim. Acta* **1982**, *61*, 227.

‡ Present addresses: T.J.A., Department of Chemistry, Florida State University, Tallahassee, FL 32306; L.D.W. Department of Chemistry, Duke University, Durham, NC 27706.

(1) Vogler, A.; Kunkely, H.; Rethwisch, B. *Inorg. Chim. Acta* **1980**, *46*, 101.
(2) Eastwood, D.; Gouterman, M. *J. Mol. Spectrosc.* **1970**, *35*, 359.
(3) Buchler, J. W.; Lay, K.-L.; Stoppa, H. *Z. Naturforsch.* **1980**, *35b*, 433.

0002-7863/82/1504-6278$01.25/0