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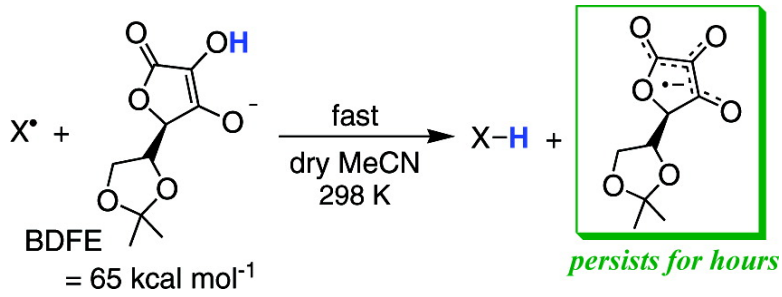

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Surprisingly Long-Lived Ascorbyl Radicals in Acetonitrile: Concerted Proton−Electron Transfer Reactions and Thermochemistry

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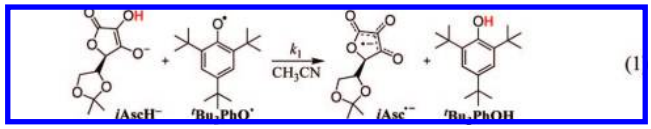
Ascorbic acid (vitamin C) is involved in a wide range of biochemical processes.[1] It is primarily a redox cofactor, being oxidized to the ascorbyl radical and then to dehydroascorbate. Studies of the ascorbyl radical in aqueous media are complicated by its transient nature.[1c] Here we report that in "dry" acetonitrile, the soluble 5,6-isopropylidene ascorbyl radical (*i*Asc$^{\bullet-}$, eq 1) is surprisingly long-lived, as is the parent ascorbyl radical.[2] These long lifetimes have facilitated detailed reactivity and thermochemical studies. While ascorbate reactivity is often described as electron transfer,[1] at pH 7, one-electron oxidation to the ascorbyl radical occurs with concomitant loss of a proton (a proton-coupled electron transfer (PCET) process).[3] Ascorbate often reacts by *concerted* transfer of e^- and H^+, including reactions with cytochrome b_{561}, nitrosobenzenes, quinones, and reduction of tocopheroxyl radicals to tocopherols (vitamin E).[4,5] These were historically called hydrogen atom transfer reactions, but they are probably better termed PCET or (our preference) concerted proton−electron transfer (CPET) because the ascorbate proton is in the molecular plane while the electron is removed from a π-orbital.[6] Described here are a number of CPET reactions of ascorbates in MeCN, which reveal an unusual solvent dependence of the O−H bond dissociation free energy.



(1)

5,6-Isopropylidene semidehydroascorbyl radical (*i*Asc$^{\bullet-}$) is conveniently generated by reaction of 5,6-isopropylidene ascorbate (*i*AscH$^-$) with the 2,4,6-tri-*tert*-butylphenoxyl radical (^{t}Bu$_3$PhO$^\bullet$)[7] in MeCN (eq 1). Rapid and quantitative reduction of ^{t}Bu$_3$PhO$^\bullet$ to ^{t}Bu$_3$PhOH is observed by UV−vis and ^{1}H NMR spectroscopies. 5,6-Isopropylidene ascorbic acid (*i*AscH$_2$) is an organic soluble ascorbate analogue,[8] available from Sigma-Aldrich. *i*AscH$^-$ can be isolated as ^{n}Bu$_4$N$^+$*i*AscH$^-$, a low melting solid, or generated in situ by addition of 1 equiv of the strong base DBU to *i*AscH$_2$ in MeCN (DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene).[9]

The ascorbyl radical product of reaction 1 (*i*Asc$^{\bullet-}$) has been characterized by its UV−vis, EPR, and ESI-mass spectra. By stopped flow spectrophotometry, the disappearance of ^{t}Bu$_3$PhO$^\bullet$ (λ_{max} = 382, 401, 634 nm) is accompanied by growth of a band at 377 nm (Figure 1a). The λ_{max} and ε_{377} (3900 ± 200 M^{-1} cm^{-1}) of this species are similar to those reported for the ascorbyl radical anion in water (360 nm, 3300 M^{-1} cm^{-1}).[1c,10] ESI-mass spectra of *i*AscH$^-$ + ^{t}Bu$_3$PhO$^\bullet$ reaction mixtures[9] show a negative ion at *m*/*z* = 214, as well as major peaks at *m*/*z* = 213 and 215 due to *i*AscH$^-$ and dehydroascorbate (*i*Asc)[11] (from loss of H^+ from the 4-position[12]). The *m*/*z* = 214 ion is the ascorbyl radical anion.

The room temperature X-band EPR spectrum of *i*Asc$^{\bullet-}$ in MeCN (Figure 1b), prepared by mixing a slight excess of *i*AscH$^-$ with ^{t}Bu$_3$PhO$^\bullet$, is centered at g_{MeCN} = 2.0059 with hyperfine couplings of 1.91 [H4], 0.05 [H5], and 0.12, 0.16 G [H6],[9] close to the values

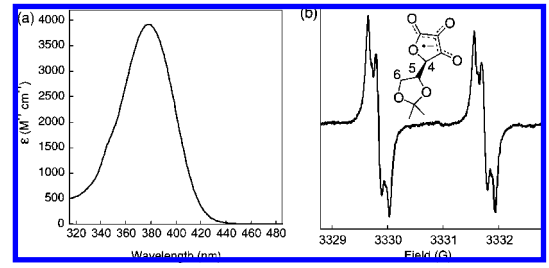


Figure 1. (a) UV−visible spectrum and (b) X-band EPR spectrum of *i*Asc$^{\bullet-}$ in CH_3CN at 298 K, generated via reaction 1.

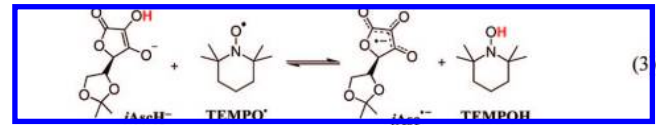
reported for *i*Asc$^{\bullet-}$ in DMSO and in water (e.g., g_{DMSO} = 2.00563, g_{H_2O} = 2.00520).[13]

*i*Asc$^{\bullet-}$ generated via reaction 1, at ca. 0.1 mM concentrations, decays over about 2 h at room temperature. This stability is surprising given that the underivatized radical anion disproportionates rapidly in aqueous solutions at pH 7 ($k \sim 3 \times 10^6$ M^{-1} s^{-1}; $t_{1/2} \sim 3$ ms at 0.1 mM).[14] The decay of *i*Asc$^{\bullet-}$ in MeCN forms *i*AscH$^-$ and *i*Asc by ^{1}H NMR and ESI-MS (eq 2), analogous to the decay of the ascorbyl

$$2\ i\text{Asc}^{\bullet-} + H^+ \rightleftharpoons i\text{AscH}^- + i\text{Asc} \qquad (2)$$

radical in H_2O.[14] The decay of *i*Asc$^{\bullet-}$ in MeCN appears to be highly dependent on the proton activity: drying the MeCN with activated alumina extends the lifetime, and addition of 56 mM H_2O causes complete decay within 30 min. Addition of 0.1 mM CF_3CO_2H results in immediate loss of the absorbance at 377 nm. This indicates that the stability of *i*Asc$^{\bullet-}$ arises from effectively "starving" the reaction of protons since, unlike the decay of most organic radicals, disproportionation stoichiometrically requires H^+ (eq 2). Analogous preparations of *i*Asc$^{\bullet-}$ in dry CH_2Cl_2 and DMSO give similarly stable solutions.

Stopped-flow kinetic studies of reaction 1 give k_1 = (3.4 ± 0.5) × 10^6 M^{-1} s^{-1} and k_H/k_D 3.2 ± 0.6 at 298 K.[15] The analogous reaction of 2,6-di-*tert*-butyl-4-methoxyphenoxyl forms *i*Asc$^{\bullet-}$ and the corresponding phenol about 6 times slower: k = (5.3 ± 0.5) × 10^5 M^{-1} s^{-1} and k_H/k_D = 3.5 ± 0.6. The reaction of *i*AscH$^-$ and the nitroxyl radical 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) in MeCN results in the production of *i*Asc$^{\bullet-}$ and TEMPOH (eq 3) as observed by UV−vis and ^{1}H NMR spectroscopies. Aqueous ascorbic acid has long



(3)

been known to reduce nitroxyl radicals.[16] Kinetic measurements in MeCN using excess TEMPO yield k_3 = 1720 ± 150 M^{-1} s^{-1} at 298 K, and $\Delta H_3^\ddagger$ = 3.4 ± 0.5 kcal mol^{-1}, $\Delta S_3^\ddagger$ = −32 ± 2 cal K^{-1} mol^{-1}. Spectrophotometric titration of *i*AscH$^-$ with limited TEMPO

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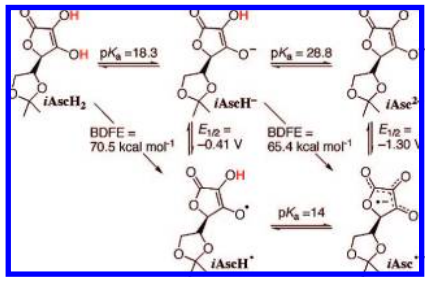
Scheme 1. Thermochemistry of the $AscH_2$ System in MeCN



gives the equilibrium constant for eq 3, $K_3 = 1.2 \pm 0.2$ ($\Delta G°_3 = -0.15 \pm 0.10$ kcal mol^{-1}).[9]

The stability of $i\text{Asc}^{\bullet -}$ allows the ready determination of the thermochemistry of electron, proton, and hydrogen atom transfers in this system (Scheme 1; all data in MeCN).[9] Titrations of $i\text{AscH}_2$ with *N*-methylmorpholine ($pK_a = 15.59$ in MeCN)[17] and $i\text{AscH}^-$ with 1,5,7-triazabicyclo[4.4.0]dec-5-ene ($pK_a = 26.03$)[18] give $pK_a(i\text{AscH}_2) = 18.3 \pm 0.3$ and $pK_a(i\text{AscH}^-) = 28.8 \pm 0.5$. Cyclic voltammetry of $i\text{AscH}^-$ and $i\text{Asc}^{2-}$ [19] show quasi-reversible waves ($\Delta E_P \sim 0.2$ V) at -0.410 ± 0.015 and -1.30 ± 0.02 V versus $Cp_2Fe^{+/0}$. $pK_a(i\text{AscH}^{\bullet})$ is calculated from these $E_{1/2}$ and pK_a values, using the square in Scheme 1 as a thermochemical cycle: $1.37[pK_a(i\text{AscH}^-) - pK_a(i\text{AscH}^{\bullet})] - 23.1[E(i\text{AscH}^-) - E(i\text{Asc}^{2-})] = 0$.

The pK_a and $E_{1/2}$ values indicate, using eq 4, that the O−H bond dissociation free energies (BDFEs) for $i\text{AscH}_2$ and $i\text{AscH}^-$ in MeCN are 70.5 ± 2.0 and 64.4 ± 2.0 kcal mol^{-1}, respectively ($C_G = 54.9$ kcal mol^{-1} in MeCN vs $Fc^{0/+}$).[20] The equilibrium in eq 3 ($K_3 = 1.2$) and the known[21] BDFE(TEMPOH) of 66.5 ± 1 kcal mol^{-1} give an independent measure of BDFE($i\text{AscH}^-$), 66.4 ± 1.0 kcal mol^{-1}, in good agreement with that obtained from $E_{1/2}$ and pK_a data and indicating a consensus BDFE for $i\text{Asc}^-$ of 65.4 ± 1.5 kcal mol^{-1} in MeCN (Scheme 1). This corresponds to a bond dissociation *enthalpy* of roughly 70 ± 1 kcal mol^{-1}.[20b]

$$\text{BDFE[X−H]} = 23.06E° + 1.37pK_a + C_G \text{ (in kcal mol}^{-1}\text{)} \qquad (4)$$

With underivatized ascorbate, the tetrabutylammonium salt is sufficiently soluble in MeCN to examine its similar reactivity. Reaction with TEMPO forms the parent ascorbyl radical, based on its characteristic optical spectrum ($\lambda_{max} = 377$ nm), which then decays over ca. 1 h. This reaction has $K_{eq} = 0.11 \pm 0.04$ which implies BDFE(ascorbate) $= 67.8 \pm 1.2$ kcal mol^{-1} in MeCN.[9] Aqueous thermochemical data for ascorbate[22] yield an aqueous O−H BDFE of 74.0 ± 1.5 kcal mol^{-1} (eq 4 with $C_G = 57.5$ kcal mol^{-1} in H_2O vs NHE[20]).[9] The BDFE is 6.2 ± 1.8 kcal mol^{-1} higher in H_2O than in MeCN. Preliminary studies of the aqueous ascorbate + TEMPO reaction (pH 7 phosphate buffer) seem consistent with these conclusions. The difference in BDFE of 6.2 ± 1.8 kcal mol^{-1} is a very large given that homolytic bond strengths are typically not very sensitive to the solvent.[23]

The mechanism of the reaction of $i\text{AscH}^-$ and TEMPO in MeCN is indicated to be concerted H^+/e^- transfer following the analysis used by Njus to implicate this mechanism for ascorbate + tocopheroxyl radicals.[5b] Alternative pathways of (i) initial proton transfer (PT) followed by electron transfer (ET) or (ii) initial ET followed by PT are not possible because of the high free energies of the initial steps: $\Delta G°_{PT} \geq 40$ kcal mol^{-1} and $\Delta G°_{ET} \geq 35$ kcal mol^{-1} from the data in Scheme 1 and the known thermochemical values for TEMPO(H).[24] These ground-state free energy changes are the *minimum* activation barriers for these pathways ($\Delta G^{\ddagger} \geq \Delta G°$) and both are much larger than the observed Eyring barrier, $\Delta G_3^{\ddagger} = 13.0 \pm 0.1$ kcal mol^{-1}. The same treatment indicates that $i\text{AscH}^-$ + $^t\text{Bu}_3\text{PhO}^{\bullet}$ (eq 1) also likely proceeds via CPET.[9]

In conclusion, $i\text{Asc}^{\bullet -}$ is readily generated from the corresponding ascorbate by phenoxyl and nitroxyl radicals. Remarkably, this radical persists for hours in dry acetonitrile, likely because protons are required for radical disproportionation (eq 2). Mechanistic and thermochemical data indicate that these reactions proceed by concerted H^+/e^- transfer rather than a stepwise path involving separate electron and proton transfers. The O−H BDFE of $i\text{AscH}^-$ in MeCN is determined to be 65.4 ± 1.5 kcal mol^{-1} from two different approaches, and the BDFE of underivatized ascorbate in MeCN is 67.8 ± 1.2 kcal mol^{-1}. These values are significantly lower than the BDFE of ascorbate, 74.0 ± 1.5 kcal mol^{-1}, derived from aqueous thermochemical measurements. Future studies will explore the origin of this large variation in ascorbate O−H BDFEs, which could reflect an unusual sensitivity to local solvation effects and could be important for enzymatic reactions of ascorbate.

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Supporting Information Available: Experimental details for kinetic and thermochemical studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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