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

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Abstract

Proton-coupled electron transfer (PCET) reactions and thermochemistry of 5,6-isopropylidene ascorbate (***i*AscH**$^-$) have been examined in acetonitrile solvent.***i*AscH**$^-$ is oxidized by 2,4,6-^{t}Bu$_3$C$_6$H$_2$O$^\bullet$ and by excess TEMPO$^\bullet$ to give the corresponding 5,6-isopropylidene ascorbyl radical anion (***i*Asc**$^{\bullet-}$), which persists for hours at 298 K in dry MeCN solution. The stability of ***i*Asc**$^{\bullet-}$ is surprising in light of the transience of the ascorbyl radical in aqueous solutions, and is due to the lack of the protons needed for radical disproportionation. A concerted proton-electron transfer (CPET) mechanism is indicated for the reactions of ***i*AscH**$^-$. Redox potential, pK_a and equilibrium measurements define the thermochemical landscape for 5,6-isopropylidene ascorbic acid and its derivatives in MeCN. These measurements give an O–H bond dissociation free energy (BDFE) for ***i*AscH**$^-$of 65.4 ± 1.5 kcal mol^{-1} in MeCN. Similar studies on underivatized ascorbate indicate a BDFE of 67.8 ± 1.2 kcal mol^{-1}. These values are much lower than the aqueous BDFE for ascorbate of 74.0 ± 1.5 kcal mol^{-1} derived from reported data.

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 thermo-chemical 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 the 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.

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*-butyl phenoxyl radical (^{t}Bu$_3$PhO$^\bullet$)[7] in MeCN (eq 1). Rapid and quantitative reduction of ^{t}Bu$_3$PhO$^\bullet$ to

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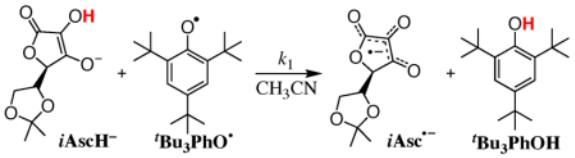


(1)

$^{t}Bu_3PhOH$ is observed by UV-Vis and ^1H NMR spectroscopies. 5,6-Isopropylidene ascorbic acid (***i*AscH$_2$**) is an organic soluble ascorbate analog,[8] available from Sigma-Aldrich. ***i*AscH**$^-$ can be isolated as $^{n}Bu_4N^+$***i*AscH**$^-$, a low melting solid, or generated *in situ* by addition of one equivalent of the strong base DBU to ***i*AscH$_2$** in MeCN (DBU = l,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-visible, EPR, and ESI-mass spectra. By stopped flow spectrophotometry, the disappearance of $^{t}Bu_3PhO^{\bullet}$ (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}).[1b,10] ESI-mass spectra of **iAscH**$^-$ + $^{t}Bu_3PhO^{\bullet}$ 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.

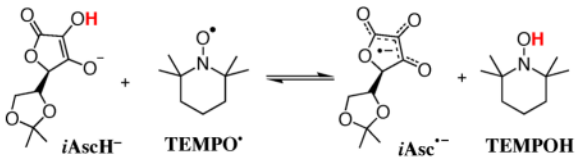
The room temperature X-band EPR spectrum of ***i*Asc**$^{\bullet-}$ in MeCN (Fig. 1b), prepared by mixing a slight excess of ***i*AscH**$^-$ with $^{t}Bu_3PhO^{\bullet}$, is centered at g_{MeCN} = 2.0059 with hyperfine couplings of 1.91 [H4], 0.05 [H5], and 0.12, 0.16 G [H6].[9] These are close to the values reported for ***i*Asc**$^{\bullet-}$ in DMSO and in water (e.g., g_{dmso} =2.00563, g_{H2O}=2.00520).[13]

***i*Asc**$^{\bullet-}$ generated via reaction 1, at *ca*. 0.1 mM concentrations, decays over about two hours 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}; $t_{1/2}$ ~3 ms at 0.1 mM).[14] The decay of ***i*Asc**$^{\bullet-}$ in MeCN forms ***i*AscH**$^-$ and ***i*Asc** by ^1H NMR and ESI-MS (eq 2), analogous to the

$$2\,i\mathbf{Asc}^{\cdot-} + H^+ \rightleftharpoons i\mathbf{AscH}^- + i\mathbf{Asc} \qquad (2)$$

decay of the ascorbyl 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. The 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 289 K.[15] The analogous reaction of 2,6-di-*tert*-butyl-4-methoxy-phenoxyl forms ***i*Asc**$^{\bullet-}$ and the corresponding phenol, about six times slower: k = (5.3 ± 0.5) × 10^5 M^{-1} s^{-1}; k_H/k_D = 3.5 ± 0.6 The reaction of ***i*AscH**$^-$ and the nitroxyl radical 2,2,6,6-tetramethyl-piperidin-1-oxyl (TEMPO) in MeCN results in production of ***i*AscH**$^{\bullet-}$ and TEMPOH (eq 3) as observed by UV-Vis and ^1H NMR spectroscopies. Aqueous ascorbic acid has long been known to reduce nitroxyl radicals.[16] Kinetic measurements in MeCN using excess TEMPO yielded k_3 = 1720 ± 150 M^{-1} s^{-1} at 298 K, and $\Delta H_3^{\ddagger}$ = 3.4±0.5 kcal mol^{-1},



(3)

$\Delta S_3^{\ddagger} = -32 \pm 2$ cal K[1] mol^{-1}. Spectrophotometric titration of ***i*AscH**$^-$ with limited TEMPO 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*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*AscH$_2$** with *N*-methyl morpholine (pk_a = 15.59 in MeCN),[17] and ***i*AscH**$^-$ with 1,5,7-triazabicyclo[4.4.0]dec-5-ene (pk_a = 26.03),[18] give pk_a(***i*AscH$_2$**) = 18.3 ± 0.3 and pk_a(***i*AscH**$^-$) = 28.8 ± 0.5. Cyclic voltammetry of ***i*AscH**$^-$ and ***i*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 *vs.* $Cp_2Fe^{+/0}$. The pk_a(**AscH**$^\bullet$) is calculated from these $E_{1/2}$ and pk_a values, using the square in Scheme 1 as a thermochemical cycle: $1.37[pk_a(\textbf{\textit{i}AscH}^-) - pk_a(\textbf{\textit{i}AscH}^\bullet)] - 23.1[E(\textbf{\textit{i}AscH}^-) - E(\textbf{\textit{i}Asc}^{2-})] = 0$.

The pk_a and $E_{1/2}$ values indicate, using eq 4, that the O-H bond dissociation free energies (BDFEs) for **iAscH$_2$** and ***i*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 versus $Fc^{0/+}$).[20] The equilibrium in eq 3

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

(K_3 = 1.2) and the known[21] BDFE(TEMPO-H) of 66.5 ± 1 kcal mol^{-1} give an independent measure of BDFE(**iAscH**$^-$), 66.4 ±1.0 kcal mol^{-1}. This value is in good agreement with that obtained from $E_{1/2}$ and Pk_a data, and indicates a consensus BDFE for **iAsc**$^-$ 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]

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 (λ_{max} = 377 nm), which then decays slowly over ca. 1 hour. This reaction has $k_{eq} = 0.11 \pm 0.07$ which implies BDFE(ascorbate) = 67.8 ± 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 versus 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*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.[4b] 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: $\Delta G°_{PT} \cong 40$ kcal mol^{-1} and $\Delta G°_{ET} \cong 35$ 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 ($\Delta G_3^{\ddagger} \geq \Delta G°$) 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 **iAscH**$^-$ + ^{t}Bu$_3$PhO$^\bullet$ (eq 1) also likely proceeds via CPET.[9]

In conclusion, the 5,6-isopropylidene ascorbyl radical (***i*Asc**$^{\bullet-}$) is readily generated from the corresponding ascorbate by phenoxyl and nitroxyl radicals. Remarkably, this radical is stable 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 bond dissociation free energy (BDFE) of ***i*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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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24. $pk_a(TEMPOH^+) \sim -3$, and $E_{1/2}(TEMPO^0/-) = -1.91$ V vs. $Fc^{+/0.9}$

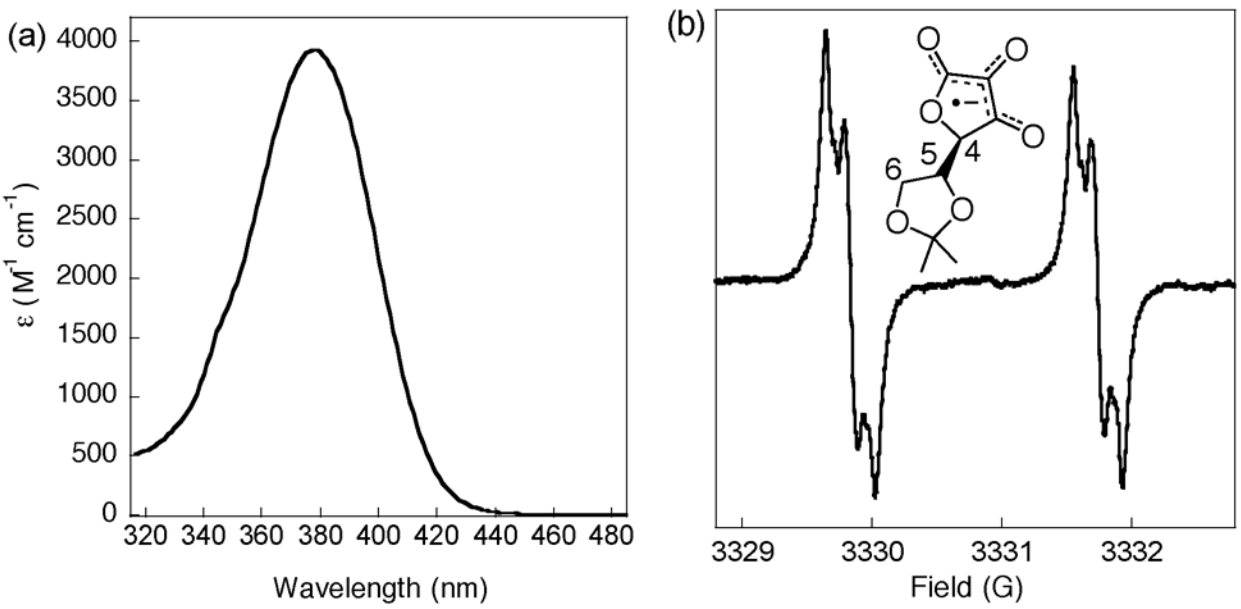
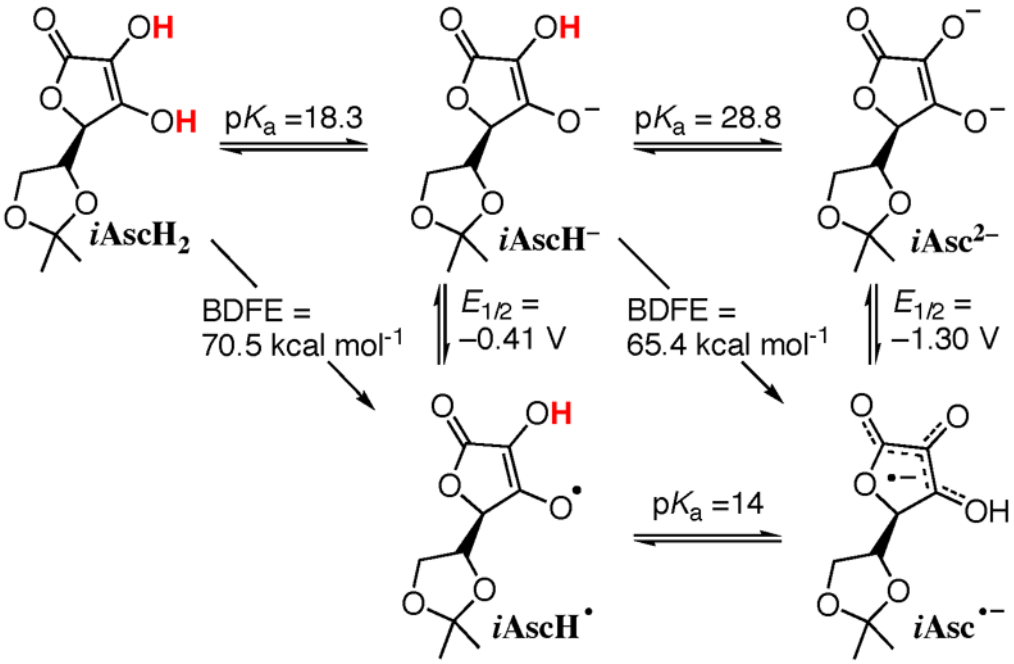


Figure 1.
(a) UV-Visible spectrum and (b) X-band EPR spectrum of iAsc'" in CH_3CN at 298K, generated via reaction (1)



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