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Electron-transfer mechanism in radical-scavenging reactions by a vitamin E model in a protic medium

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The scavenging reaction of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) or galvinoxyl radical (GO$^{\bullet}$) by a vitamin E model, 2,2,5,7,8-pentamethylchroman-6-ol (**1H**), was significantly accelerated by the presence of $Mg(ClO_4)_2$ in de-aerated methanol (MeOH). Such an acceleration indicates that the radical-scavenging reaction of **1H** in MeOH proceeds *via* an electron transfer from **1H** to the radical, followed by a proton transfer, rather than the one-step hydrogen atom transfer which has been observed in acetonitrile (MeCN). A significant negative shift of the one-electron oxidation potential of **1H** in MeOH (0.63 V *vs.* SCE), due to strong solvation as compared to that in MeCN (0.97 V *vs.* SCE), may result in change of the radical-scavenging mechanisms between protic and aprotic media.

Introduction

Recently, much attention has been paid to the mechanisms of radical-scavenging reactions of phenolic antioxidants, such as vitamin E (α-tocopherol) and flavonoids, with regard to the development of chemopreventive agents against oxidative stress and associated diseases. There are two mechanisms for the radical-scavenging reactions of phenolic antioxidants: a one-step hydrogen atom transfer from the phenolic OH group; and an electron transfer followed by a proton transfer.[1–3] Metal ions are a powerful tool that can be used to distinguish between these two mechanisms, since electron-transfer reactions are known to be significantly accelerated by their presence.[4] In fact, we have recently reported that scavenging reactions of the galvinoxyl radical (GO$^{\bullet}$) and the cumylperoxyl radical by (+)-catechin in aprotic media, such as acetonitrile (MeCN) and propionitrile, proceed *via* an electron transfer from (+)-catechin to the radicals (which is significantly accelerated by the presence of metal ions, such as Mg^{2+} and Sc^{3+}) followed by a proton transfer.[5,6] On the other hand, no effect of Mg^{2+} on the hydrogen-transfer rate from a vitamin E model, 2,2,5,7,8-pentamethylchroman-6-ol (**1H**), to 2,2-bis(4-*tert*-octylphenyl)-1-picrylhydrazyl radical (DOPPH$^{\bullet}$) or GO$^{\bullet}$ in de-aerated MeCN has been observed, indicating that the radical-scavenging reactions of **1H** in MeCN proceed *via* a one-step hydrogen atom transfer rather than *via* electron transfer.[7,8] However, the effects of solvents on the mechanism of radical-scavenging reactions of phenolic antioxidants have yet to be clarified. Leopoldini *et al.* have reported that the bond dissociation enthalpies for O–H bonds and the adiabatic ionization potentials for phenolic antioxidants, calculated with use of density functional theory, do not follow the same trends in gas, water and benzene.[2] Thus, it is of considerable importance to investigate the effects of metal ions on radical-scavenging reactions in various solvents with different polarity.[9]

We report herein that the scavenging reactions of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) or GO$^{\bullet}$ by the vitamin E model **1H** in de-aerated methanol (MeOH) proceed *via* an electron transfer mechanism rather than *via* a one-step hydrogen atom transfer, which has been observed in de-aerated MeCN. Effects of bases on the radical-scavenging rates were also examined, to clarify whether the actual electron donor is **1H** or the corresponding phenolate anion $\mathbf{1}^-$ in MeOH. Different mechanisms in protic and aprotic solvents are discussed based on kinetic, electrochemical, and EPR data obtained in this study, providing valuable and fundamental information about the radical-scavenging mechanism of phenolic antioxidants.

Experimental

Materials

2,2,5,7,8-Pentamethylchroman-6-ol (**1H**) was purchased from Wako Pure Chemical Ind. Ltd., Japan. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) and galvinoxyl radical (GO$^{\bullet}$) were commercially obtained from Aldrich. Tetra-*n*-butylammonium perchlorate (Bu_4NClO_4), used as a supporting electrolyte for the electrochemical measurements, was purchased from Tokyo Chemical Industry Co., Ltd., Japan, recrystallized from ethanol, and dried under vacuum at 313 K. $Mg(ClO_4)_2$ and methanol (MeOH; spectral grade) were purchased from Nacalai Tesque, Inc., Japan and used as received. Pyridine and 2,6-lutidine were commercially obtained from Wako Pure Chemical Ind. Ltd., Japan and purified by the standard procedure.[10]

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Spectral and kinetic measurements

Since the phenoxyl radical of **1H** (**1**$^{\bullet}$) generated in the reaction of **1H** with radicals readily reacts with molecular oxygen (O_2), reactions were carried out under strictly de-aerated conditions. A continuous flow of Ar gas was bubbled through a MeOH solution (3.0 mL) containing DPPH$^{\bullet}$ (4.8×10^{-5} M) and $Mg(ClO_4)_2$ (0–0.3 M) in a square quartz cuvette (10 mm id) with a glass tube neck for 10 min. Air was prevented from leaking into neck of the cuvette with a rubber septum. Typically, an aliquot of **1H** (2.0×10^{-2} M), which was also in de-aerated MeOH, was added to the cuvette with a microsyringe. This led to a reaction of **1H** with DPPH$^{\bullet}$. UV-vis spectral changes associated with the reaction were monitored using an Agilent 8453 photodiode array spectrophotometer. The rates of the DPPH$^{\bullet}$-scavenging reactions of **1H** were determined by monitoring the absorbance change at 516 nm due to DPPH$^{\bullet}$ ($\varepsilon = 1.13 \times 10^4$ M^{-1} cm^{-1}) using a stopped-flow technique on a UNISOKU RSP-1000-02NM spectrophotometer. The pseudo-first-order rate constants (k_{obs}) were determined by a least-squares curve fit using an Apple Macintosh personal computer. The first-order plots of $\ln(A - A_\infty)$ *vs.* time (A and A_∞ are denoted as the absorbance at the reaction time and the final absorbance, respectively) were linear until three or more half-lives with the correlation coefficient $\rho > 0.999$. The reaction of **1H** with GO$^{\bullet}$ was carried out in the same manner and the rates were determined from the absorbance change at 428 nm due to GO$^{\bullet}$ ($\varepsilon = 1.32 \times 10^5$ M^{-1} cm^{-1}). The rate constants of the reactions in the presence of base (pyridine or 2,6-lutidine) were determined in the same manner.

Electrochemical measurements

The cyclic voltammetry (CV) and second-harmonic alternating current voltammetry (SHACV)[11–16] measurements were performed on an ALS-630A electrochemical analyzer in de-aerated MeOH containing 0.10 M Bu_4NClO_4 as a supporting electrolyte. The Pt working electrode (BAS) was polished with BAS polishing alumina suspension and rinsed with acetone before use. The counter electrode was a platinum wire. The measured potentials were recorded with respect to an $Ag/AgNO_3$ (0.01 M) reference electrode. The $E_{1/2}$ values (*vs.* $Ag/AgNO_3$) were converted to those *vs.* SCE by adding 0.29 V.[17] All electrochemical measurements were carried out at 298 K under 1 atm Ar.

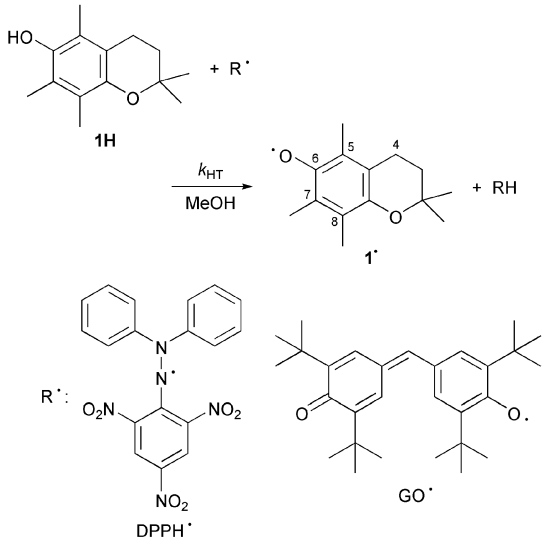
EPR measurements

Typically, an aliquot of a stock solution of **1H** (2.0×10^{-2} M) in de-aerated MeOH was added to the EPR sample tube (0.8 mm id) containing a de-aerated MeOH solution of DPPH$^{\bullet}$ (2.0×10^{-4} M) with a microsyringe under 1 atm Ar. EPR spectra of the phenoxyl radical **1**$^{\bullet}$ produced in the reaction between **1H** and DPPH$^{\bullet}$ were taken on a JEOL X-band spectrometer (JES-RE1XE). The EPR spectra were recorded under non-saturating microwave power conditions. The magnitude of modulation was chosen to optimize the resolution and the signal-to-noise ratio of the observed spectra. The g values and the hyperfine splitting constants were calibrated with a Mn^{2+} marker. Computer simulation of the EPR spectra was carried out using Calleo ESR Version 1.2 program (Calleo Scientific Publisher) on an Apple Macintosh personal computer.

Results and discussion

Radical-scavenging reactions of the vitamin E model in de-aerated MeOH

Upon addition of **1H** to a de-aerated MeOH solution of DPPH$^{\bullet}$, the absorption band at 516 nm due to DPPH$^{\bullet}$ disappeared immediately, accompanied by an appearance of the absorption band at 427 nm. Since the absorption band at 427 nm is diagnostic of the phenoxyl radical derived from **1H** (**1**$^{\bullet}$) in MeOH,[18] this spectral change indicates that hydrogen transfer from the phenolic OH group of **1H** to DPPH$^{\bullet}$ takes place to produce **1**$^{\bullet}$ (eqn. (1)). The absorption band of **1**$^{\bullet}$ was shifted from 423 nm in MeCN to 427 nm in MeOH.[7,8] Such a shift in the absorption band of **1**$^{\bullet}$ may be due to a stronger solvation of **1**$^{\bullet}$ in MeOH than in MeCN.



(1)

The rate of the DPPH$^{\bullet}$-scavenging reaction of **1H** was measured by monitoring the decrease in absorbance at 516 nm due to DPPH$^{\bullet}$ using a stopped-flow technique. The decay of the absorbance at 516 nm due to DPPH$^{\bullet}$ obeyed pseudo-first-order kinetics when the concentration of **1H** ([**1H**]) was maintained at more than a 10-fold excess of the DPPH$^{\bullet}$ concentration. The pseudo-first-order rate constants (k_{obs}) increase with increasing [**1H**], exhibiting first-order dependence on [**1H**]. From the slope of the linear plot of k_{obs} *vs.* [**1H**], the second-order rate constant (k_{HT}) was determined for the radical-scavenging reaction as 1.07×10^3 M^{-1} s^{-1}, in de-aerated MeOH at 298 K. The k_{HT} value thus obtained in de-aerated MeOH is significantly larger than that determined in de-aerated MeCN (4.35×10^2 M^{-1} s^{-1}).[7] A similar result has been reported by Litwinienko and Ingold.[19] Intermolecularly hydrogen-bonded phenolic OH groups of hydrogen-bond accepting solvents, such as alcohols, are known to be essentially unreactive against radicals.[19] Thus, the enhanced k_{HT} value in MeOH suggested that the reaction mechanism in MeOH may be different from that in MeCN. The GO$^{\bullet}$-scavenging rate constant by **1H** in de-aerated MeOH has also been determined in a same manner by monitoring the decrease in absorbance at 428 nm due to GO$^{\bullet}$ as 2.54×10^3 M^{-1} s^{-1}, which is slightly smaller than that in de-aerated MeCN (3.32×10^3 M^{-1} s^{-1}).

Effect of magnesium ion on the rates of radical scavenging reactions

If the radical-scavenging reactions of **1H** involve an electron-transfer process as the rate-determining step, the rates of radical scavenging would be accelerated by the presence of metal ions.[5,6] This was investigated by examining the effect of $Mg(ClO_4)_2$ on the radical-scavenging rates by **1H** in de-aerated MeOH. When $Mg(ClO_4)_2$ is added to the **1H**–DPPH$^{\bullet}$ system in de-aerated MeOH, the rate of DPPH$^{\bullet}$-scavenging reaction by **1H** was significantly accelerated. Such an acceleration was not observed for the DPPH$^{\bullet}$-scavenging reaction by **1H** in MeCN.[7] The k_{HT} value increases linearly with increasing Mg^{2+} concentration ([Mg^{2+}]) as shown in Fig. 1a. A similar acceleration effect of Mg^{2+} has been observed for the GO$^{\bullet}$-scavenging reaction by **1H** in de-aerated MeOH (Fig. 1b). Thus, the radical-scavenging reactions in de-aerated MeOH may proceed *via* an electron transfer from **1H** to DPPH$^{\bullet}$ or GO$^{\bullet}$, which is accelerated by the presence of Mg^{2+}, followed by proton transfer from **1H**$^{\bullet+}$ to $DPPH^-$ or GO^- as shown in Scheme 1. In such a case,

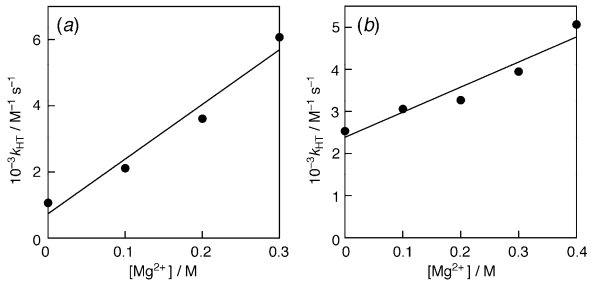
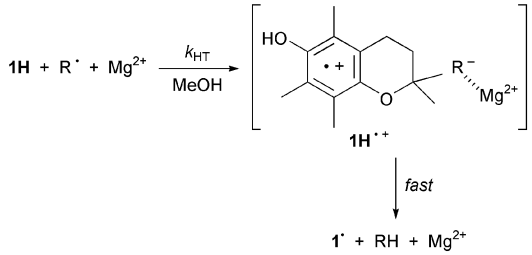


Fig. 1 Plots of k_{HT} *vs.* [Mg^{2+}] in the reaction of **1H** with (a) DPPH$^{\bullet}$ and (b) GO$^{\bullet}$ in de-aerated MeOH at 298 K.



Scheme 1 Radical-scavenging reaction by **1H** *via* an electron transfer in MeOH.

the coordination of Mg^{2+} to DPPH^{-} or GO^{-} may stabilize the product, resulting in the acceleration of the electron transfer.

Effect of base on the rates of radical scavenging reactions

In protic media, such as alcohols and water, **1H** may be in equilibrium with the corresponding phenolate anion **1**$^{-}$, which is a much stronger electron donor as compared to the parent **1H**.[20] In such a case, **1**$^{-}$ may act as an electron donor rather than the parent **1H** in MeOH.

In order to clarify an actual electron donor in MeOH, the effect of base on the radical-scavenging rates of **1H** was examined. The addition of pyridine to the **1H**–DPPH$^{\bullet}$ system results in a significant increase in the rate of the DPPH$^{\bullet}$-scavenging reaction by **1H**. The k_{HT} value increases with increasing pyridine concentration to reach a constant value as shown in Fig. 2. When pyridine is replaced by 2,6-lutidine, a stronger base than pyridine, the limiting k_{HT} value is larger than that in the case of pyridine, as shown in Fig. 2. If the rate of acceleration is due to the deprotonation of the phenolic OH group of **1H** in the presence of base, the limiting k_{HT} value should be the same regardless of the basicity of pyridines. The different limiting k_{HT} values between pyridine and 2,6-lutidine in Fig. 2 suggest that little deprotonation occurs to produce **1**$^{-}$ and that the actual electron donor is the parent **1H** rather than **1**$^{-}$ in MeOH, as shown in Scheme 1. In such a case, the coordination of pyridines

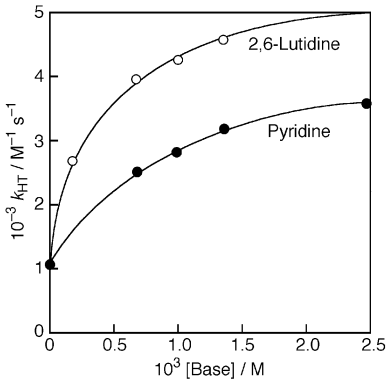


Fig. 2 Plot of k_{HT} *vs.* [base] for the reaction of **1H** with DPPH$^{\bullet}$ in the presence of pyridine (black circles) or 2,6-lutidine (white circles) in de-aerated MeOH at 298 K.

to **1H**$^{\bullet+}$ may stabilize the product, resulting in the acceleration of the initial electron-transfer process. In the presence of a large amount of a strong Lewis acid, such as $Mg(ClO_4)_2$, no deprotonation of **1H** occurs in MeOH.

Solvent effect on the one-electron oxidation potential of the vitamin E model

The solvent effect on the one-electron oxidation potential (E^0_{ox}) of **1H** was examined by cyclic voltammetry (CV) and second-harmonic alternating current voltammetry (SHACV) measurements.[11–16] Very recently, Williams and Webster have reported that the one-electron oxidation of α-tocopherol itself occurs at about 0.97 V *vs.* SCE in MeCN (0.25 M Bu_4NPF_6) based on the detailed electrochemical analyses.[21] A similar cyclic voltammogram was observed for the electrochemical oxidation of **1H** in MeCN (0.1 M Bu_4NClO_4) (data not shown), from which was determined the E^0_{ox} value (*vs.* SCE) of **1H** in MeCN as 0.97 V. On the other hand, the CV wave of **1H** in MeOH (0.1 M Bu_4NClO_4) was irreversible. Thus, SHACV measurement was carried out to determine the E^0_{ox} value of **1H** in MeOH. The E^0_{ox} value (*vs.* SCE) of **1H** in MeOH (0.1 M Bu_4NClO_4), determined from the intersection of an SHACV wave (Fig. 3), is located at 0.63 V, which is significantly more negative than the value in MeCN (0.97 V). Such a negative shift of the E^0_{ox} value in MeOH as compared to that in MeCN may be ascribed to a stronger solvation of **1H**$^{\bullet+}$ in MeOH than in MeCN. Thus, the ease of one-electron oxidation of **1H** in MeOH as compared to in MeCN may result in the difference in the radical-scavenging mechanism.

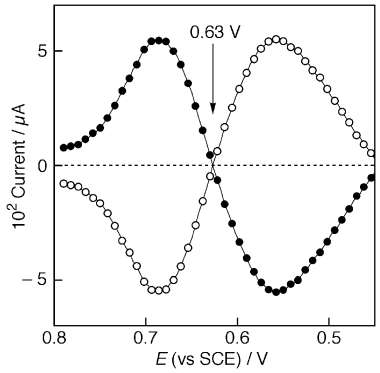


Fig. 3 SHACV of **1H** recorded at the scan rate of 4 mV s^{-1} on Pt working electrode in de-aerated MeOH (0.1 M Bu_4NClO_4) at 298 K.

EPR spectrum of the phenoxyl radical derived from the vitamin E model in de-aerated MeOH

The EPR detection of radical species derived from **1H** would provide valuable information about the solvation of the radical species.[22,23] The EPR spectrum of **1**$^{\bullet}$ in de-aerated MeOH at 298 K is shown in Fig. 4a. It should be noted that the *g* value of the EPR spectrum of **1**$^{\bullet}$ in MeOH (2.0040) is apparently smaller than that in MeCN (2.0047).[7] The observed hyperfine structure in Fig. 4a is well reproduced by the computer simulation (Fig. 4b) with four hyperfine splitting constants (*hfc*) listed in Table 1. Table 1 also shows the *hfc* values of **1**$^{\bullet}$ in MeCN.[7] All the *hfc* values in MeOH are also smaller than those in MeCN. The smaller *g* value of the EPR spectrum of **1**$^{\bullet}$ as well as the smaller *hfc* values in MeOH than those in MeCN indicates that the stronger solvation of **1**$^{\bullet}$ may occur in MeOH than in MeCN. Although the EPR spectrum of **1H**$^{\bullet+}$ could not be observed because of the fast deprotonation to produce **1**$^{\bullet}$ (Scheme 1), stronger solvation of **1H**$^{\bullet+}$ may also occur in MeOH than in MeCN, resulting in the ease of one-electron oxidation of **1H** in MeOH than in MeCN.

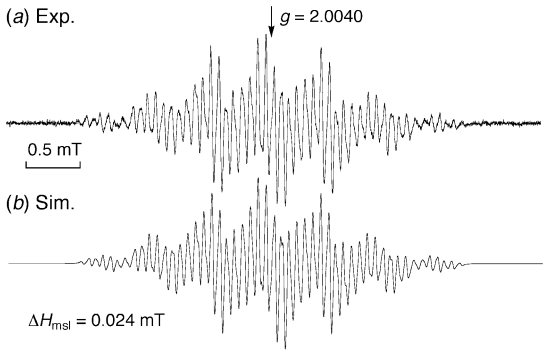


Fig. 4 (a) EPR spectrum of **1**• generated in the reaction of **1H** (1.0×10^{-3} M) with DPPH• (2.0×10^{-4} M) in de-aerated MeOH at 298 K. (b) The computer simulation spectrum. The *hfc* values used for the simulation are listed in Table 1.

Table 1 Hyperfine splitting constants (*hfc*; in mT) and *g* values of **1**• in de-aerated solvents

Solvent	*g*	$a(3H^5)$	$a(3H^7)$	$a(3H^8)$	$a(2H^4)$
MeOH	2.0040	0.577	0.423	0.073	0.126
MeCN	2.0047[a]	0.587[a]	0.440[a]	0.086[a]	0.139[a]

[a] Taken from ref. 7.

In conclusion, the scavenging reaction of DPPH• or GO• by **1H** in MeOH proceeds *via* the electron transfer from **1H** to DPPH• or GO• followed by proton transfer rather than *via* the one-step hydrogen atom transfer, which has been observed in MeCN. Such a difference in the mechanism of radical-scavenging reactions by the vitamin E model depending on the solvents provides valuable information for the biological antioxidative reactions.

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