

EPR Study on Stable Magnesium Complexes of the Phenoxyl Radicals Derived from a Vitamin E Model and Its Deuterated Derivatives

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The phenoxyl radical **1**•, generated by the reaction of a vitamin E model, 2,2,5,7,8-pentamethylchroman-6-ol (**1H**), with 2,2-bis(4-*tert*-octylphenyl)-1-picrylhydrazyl (DOPPH•), was significantly stabilized by complex formation with Mg^{2+} in deaerated acetonitrile at 298 K. The assignments of the hyperfine coupling constants (hfc) obtained by computer simulations of the observed EPR spectrum of the Mg^{2+} complex of **1**• (Mg^{2+}–**1**•), were carried out using three deuterated isotopomers of **1**•, i.e., 5-CD_3-**1**•, 7-CD_3-**1**•, and 8-CD_3-**1**•, where a methyl group at the C5, C7, or C8 position is replaced by a CD_3 group, respectively. The decreased spin density of the benzene ring in the Mg^{2+}–**1**• complex indicates that delocalization of the unpaired electron in **1**• into Mg^{2+} by complexation between Mg^{2+} and **1**• results in the enhanced stability of **1**• in the presence of Mg^{2+}.

Most biological antioxidants, such as vitamin E (α-tocopherol) and flavonoids, have one or more phenolic hydroxy groups, and are converted into phenoxyl radical intermediates as the result of antioxidative radical-scavenging reactions with active oxygen radicals, such as hydroxyl radical (•OH), superoxide anion ($O_2^{\bullet-}$), and lipid peroxyl radical (LOO•).[1–4] Thus, it is of great importance to detect and characterize the phenoxyl radicals of such antioxidants in order to shed light on the mechanism of the antioxidative radical-scavenging reactions in biological systems as well as to develop novel antioxidants with more effective antioxidative activities than the natural occurring ones. However, the phenoxyl radical of α-tocopherol is known to be unstable, because of disproportionation, even in the absence of molecular oxygen (O_2).[5] Furthermore, in the presence of O_2, a radical coupling between the phenoxyl radical derived from vitamin E and O_2 is known to produced a wide variety of oxidation products of the phenoxyl radical.[6–23] On the other hand, we have recently reported that the phenoxyl radical **1**• of a vitamin E model, 2,2,5,7,8-pentamethylchroman-6-ol (**1H**), generated by hydrogen transfer from **1H** to 2,2-bis(4-*tert*-octylphenyl)-1-picrylhydrazyl radical (DOPPH•) or galvinoxyl radical (G•), is significantly stabilized by the presence of Mg^{2+} via the complexation of **1**• with Mg^{2+}.[24] The well-resolved 14 lines were observed in the EPR spectrum of the Mg^{2+} complex of **1**• (Mg^{2+}–**1**•).[24] However, the hyperfine structure of the observed EPR spectrum of the Mg^{2+}–**1**• complex has yet to be sufficiently characterized, since there are three methyl groups in the **1**• molecule, each of which gives a quartet hyperfine coupling structure. On the other hand, it is known that deuterium substitution at appropriate known sites in the molecule permits an experimental verification of the assignment of the hyperfine coupling constants (hfc) for the EPR spectrum of the observed radical species.[25–29]

Here, we report on an experimental assignment of the hfc values of the EPR spectrum of the Mg^{2+} complex of **1**• using three deuterated isotopomers of **1**•, i.e., 5-CD_3-**1**•, 7-CD_3-**1**•, and 8-CD_3-**1**•, where a methyl group at the C5, C7, or C8 position is replaced by a CD_3 group, respectively (Chart 1). A comparison of the hfc values of the Mg^{2+}–**1**• complexes obtained in this study with those of the parent **1**• provides fundamental information about the spin distribution and stabilization of the phenoxyl radical species of phenolic antioxidants in the presence of metal ions, as well as mechanistic insight into the antioxidative radical-scavenging reactions of phenolic antioxidants.

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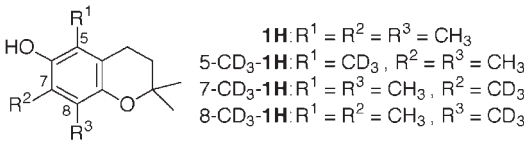


Chart 1. Vitamin E models.

Experimental

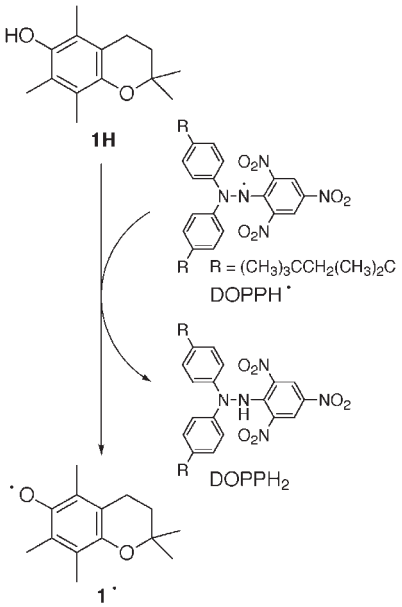
Materials. 2,2,5,7,8-Pentamethylchroman-6-ol (**1H**) was purchased from Wako Pure Chemical Ind. Ltd., Japan. 2,2-Bis(4-*tert*-octylphenyl)-1-picrylhydrazyl radical (DOPPH$^\bullet$) was obtained commercially from Aldrich. $Mg(ClO_4)_2$ and acetonitrile (MeCN; spectral grade) were purchased from Nacalai Tesque, Inc., Japan, and used as received. Three deuterated isotopomers (>98% isotopic purity) of **1H**, 5-CD_3-**1H**, 7-CD_3-**1H**, and 8-CD_3-**1H**, were synthesized according to the literature procedures.[30]

Spectral Measurements. A continuous flow of Ar gas was bubbled through a MeCN solution (3.0 mL) containing DOPPH$^\bullet$ (1.4×10^{-5} M) (1 M = 1 mol dm^{-3}) in a square quartz cuvette (10 mm i.d.) with a glass tube neck for 10 min. The neck of the cuvette was sealed to ensure that air would not leak into the cuvette by using a rubber septum. A microsyringe was used to inject **1H** (2.0×10^{-2} M), which was also deaerated, into the cuvette. This led to a hydrogen-transfer reaction from **1H** to DOPPH$^\bullet$. UV–vis spectral changes associated with this reaction were monitored using an Agilent 8453 photodiode array spectrophotometer.

EPR Measurements. Typically, an aliquot of a stock solution of **1H** (1.0×10^{-3} M) was added to LABOTEC LLC-04B EPR sample tube containing a deaerated MeCN solution of DOPPH$^\bullet$ (1.0×10^{-3} M) in the presence or absence of 0.1 M $Mg(ClO_4)_2$ under an atmospheric pressure of Ar. The EPR spectra of the phenoxyl radical **1**$^\bullet$ produced in the reaction between **1H** and DOPPH$^\bullet$ were taken on a JEOL X-band spectrometer (JES-RE1-XE). The EPR spectra were recorded under nonsaturating microwave power conditions. The magnitude of modulation was chosen so as to optimize the resolution and the signal-to-noise (S/N) ratio of the observed spectra. The *g* values and the hyperfine splitting constants were calibrated with a Mn^{2+} marker. A computer simulation of the EPR spectra was carried out using the Calleo ESR Version 1.2 program (Calleo Scientific Publisher) on an Apple Macintosh personal computer.

Results and Discussion

Upon the addition of vitamin E model **1H** to an acetonitrile (MeCN) solution of DOPPH$^\bullet$, the absorption band due to DOPPH$^\bullet$ ($\lambda_{max} = 543$ nm) decreased, accompanied by an increase in the absorption bands at 402 and 423 nm due to the phenoxyl radidal **1**$^\bullet$ with clear isosbestic points at 343, 374, and 437 nm. The absorption bands around 400 nm are typical for phenoxyl radical species of α-tocopherol.[5,31] Thus, this spectral change is ascribed to a hydrogen transfer from **1H** to DOPPH$^\bullet$ to produce **1**$^\bullet$ and hydrogenated DOPPH$^\bullet$ (DOPPH$_2$) (Scheme 1). In fact, the characteristic EPR spectrum due to **1**$^\bullet$ having a *g* value of 2.0047 was observed in the reaction of **1H** with DOPPH$^\bullet$ in deaerated MeCN at 298 K, as shown in Fig. 1(a), although the observed EPR signal gradually decreased because of the disproportionation of **1**$^\bullet$, even in the absence of O_2.[5] The hyperfine coupling constants (hfc) of the observed EPR spectrum of **1**$^\bullet$ were determined by a comparison of the observed spectrum with the computer-simulated spectrum, as shown in Fig. 1(a); the thus-obtained hfc values were assigned as listed in Table 1.[24,32,33]



Scheme 1. Hydrogen transfer from **1H** to DOPPH$^\bullet$ to produce **1**$^\bullet$.

On the other hand, in the presence of $Mg(ClO_4)_2$ (0.1 M), the absorption bands due to **1**$^\bullet$ were shifted from 402 and 423 nm to 412 and 437 nm, respectively.[24] Such a red shift of the absorption bands indicates a complex formation between Mg^{2+} and **1**$^\bullet$. The EPR spectrum of the Mg^{2+}–**1**$^\bullet$ complex was observed at $g = 2.0040$ [Fig. 1(b)], which is appreciably smaller than the *g* value of **1**$^\bullet$ (2.0047), indicating that the spin density on oxygen nuclei in **1**$^\bullet$ in the presence of Mg^{2+} is decreased by complexation with Mg^{2+}.[34] It should be noted that no decay of the EPR signal was observed, significantly demonstrating the enhanced stability of the phenoxyl radical species in the presence of $Mg(ClO_4)_2$.[24] This behavior is similar to that found for the α-tocopheroxyl radical in the presence of a fluorinated alcohol, which acts as a hydrogen-bond donor, by Lucarini et al.[35] The hyperfine structure can be reproduced by a computer simulation with the hyperfine coupling constants (hfc) of only two sets of methyl protons (0.486 and 0.335 mT), as shown in Fig. 1(b). However, the hfc values of the methylene protons and the remaining methyl protons become undetectably small. Since there are three methyl groups in the **1**$^\bullet$ molecule, the assignment of these two hfc values due to two sets of methyl protons is quite complex.

In order to assign these hfc values obtained for the Mg^{2+}–**1**$^\bullet$ complex, we synthesized three deuterated isotopomers of **1**$^\bullet$, i.e., 5-CD_3-**1**$^\bullet$, 7-CD_3-**1**$^\bullet$, and 8-CD_3-**1**$^\bullet$, where the methyl group at the C-5, C-7, or C-8 potision is replaced by the CD_3 group, respectively (Chart 1),[30] since deuterium substitution at appropriate known sites in the molecule permits an experimental verification of the assignment of the observed radical species (vide supra). A single deuteron gives a triplet (instead of a doublet) hyperfine pattern and the deuteron splitting should decrease due to the magnetogyric ratio of a proton to a deuteron (0.153).[25–29] In fact, deuterium substitution of the methyl group at the C-5 position of the Mg^{2+}–**1**$^\bullet$ complex resulted in a drastic change in the splitting pattern from the spec-

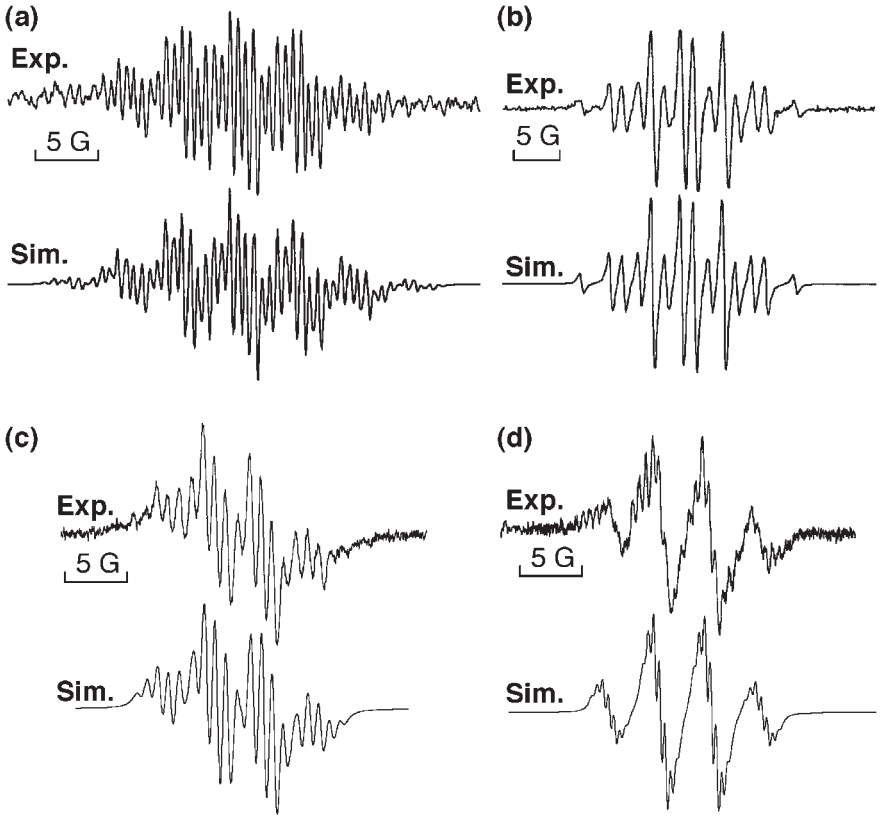


Fig. 1. EPR spectra of (a) **1**•, (b) Mg^{2+}–**1**•, (c) Mg^{2+}–5-CD_3-**1**•, and (d) Mg^{2+}–7-CD_3-**1**• in deaerated MeCN at 298 K with the corresponding computer simulation spectra. The hfc values used for the simulation are listed in Table 1.

Table 1. Hyperfine Splitting Constants (hfc) (in mT) of **1**• and the Mg^{2+} Complexes of **1**• and Its Deuterated Radicals in Deaerated MeCN

Radical	$a(3H^5)$	$a(3H^7)$	$a(3H^8)$	$a(2H^4)$
1•	0.587	0.440	0.086	0.139
Mg^{2+}–**1**•	0.486	0.335	—[a]	—[a]
Mg^{2+}–5-CD_3-**1**•	0.075[b]	0.335	—[a]	—[a]
Mg^{2+}–7-CD_3-**1**•	0.486	0.052[b]	—[a]	—[a]
Mg^{2+}–8-CD_3-**1**•	0.486	0.335	—[a]	—[a]

a) Too small to be determined. b) Deuterium splitting value.

trum in Fig. 1(b) to that in Fig. 1(c) for the Mg^{2+}–5-CD_3-**1**• complex generated in the same way. The hfc value of 0.486 mT of the Mg^{2+}–**1**• complex is decreased by the factor of the magnetogyric ratio of proton to deuterium (0.153) to 0.075 mT due to the CD_3 deuterons at the C-5 position of the Mg^{2+}–5-CD_3-**1**• complex, while the other hfc value (0.335 mT) remains identical, as shown in Fig. 1(c) and Table 1. From such a decrease in the hfc value at the C-5 position by the deuterium substitution, was assigned the hfc value at the C-5 position of the Mg^{2+}–**1**• complex as 0.486 mT.

A change in the splitting pattern was also observed upon deuterium substitution of the methyl group at the C-7 position of the Mg^{2+}–**1**• complex, as shown in Fig. 1(d). The computer simulation spectrum using the same hfc value, except for the deuterium at the C-7 position, which are reduced by a factor of 0.153, agrees well with the observed EPR spectrum of the Mg^{2+}–7-CD_3-**1**• complex [Fig. 1(d)]. On the other hand, a deuterium substitution of the methyl protons at the C-8 position of Mg^{2+}–**1**• resulted in no change in the splitting pattern in the EPR spectrum of the Mg^{2+}–8-CD_3-**1**• complex, as compared to the Mg^{2+}–**1**• complex (data not shown). From the above results, the hfc values of the Mg^{2+}–**1**• complex were assigned as listed in Table 1. The hfc values due to the methyl protons at the C-5 (0.587 mT) and C-7 (0.440 mT) positions are significantly decreased by complexation with Mg^{2+} (0.486 and 0.335 mT, respectively). No hyperfine structure was observed due to the methylene protons at the C-4 position, as well as the methyl protons at the C-8 protons in the Mg^{2+}–**1**• complex. It is clearly shown that the spin densities on the aromatic ring in the Mg^{2+}–**1**• complex are significantly decreased by the coordination of Mg^{2+}. Thus, an unpaired electron in **1**• is significantly delocalized into Mg^{2+} by complexation between **1**• and Mg^{2+} via the phenolic O atom.

In conclusion, the deuterium substitutions of the methyl group in **1H** enabled us to assign the hfc values of **1**• in the presence of Mg^{2+} experimentally. The decreased spin densities on the benzene ring in **1**•, the smaller g value of the EPR spectrum of **1**•, and the red shift of the absorption bands of **1**• in the presence of Mg^{2+} indicate that Mg^{2+} coordinates to the phenoxyl radical **1**• via the phenolic O atom of **1**•. Such complex formation between **1**• and Mg^{2+} precludes the disproportionation of **1**•, leading to the enhanced stability of **1**•. We are in the process of further exploring the effect of metal ions on the stability of phenoxyl radicals derived from phenolic antioxidants with a catechol moiety, such as (+)-catechin and quercetin.

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