Electron Spin Resonance Spectra of the Chromanoxyl Radicals Derived from Tocopherols (Vitamin E) and Their Related Compounds[1]

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ABSTRACT

The well resolved electron spin resonance (ESR) of the tocopheroxyl and chromanoxyl radicals derived from α-, β-, γ- and δ-tocopherols (vitamin E), 5,7-dimethyltocol, tocol and their model compounds in degassed toluene by treatment with 2,2-diphenyl-1-picrylhydrazyl were recorded. Their hyperfine coupling constants were determined and assigned using spectrum simulation. Their g-factors were also measured. On the basis of these parameters, the α-tocopheroxyl radical is similar to the 2,2,5,7,8-pentamethylchroman-6-oxyl, 5,7-dimethyltocoxyl and 2,2,5,7-tetramethylchroman-6-oxyl radicals. This suggests that the presence of methyl groups at C-5 and C-7 in tocopherols and chroman-6-ols is of great importance to their antioxidant action. The ESR parameters obtained here are very useful for the identification and quantification of a variety of tocopheroxyl radicals.
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INTRODUCTION

Vitamin E (mainly α-tocopherol) has been used as one of the safest antioxidants for the prevention of lipid deterioration in foodstuffs (1) and further, suggested to play an important role in the inhibition of lipid peroxidation in vivo (2,3). It is known that the effectiveness of various tocopherols in vitro depends on the reactivity of their phenolic groups (4), and that the first step in the tocopherol-induced chain-breaking process of autoxidation is the formation of tocopheroxyl radicals by abstraction of the phenolic hydrogens (5). So it requires the knowledge of the properties of tocopheroxyl radicals to understand the action of vitamin E.

Although the ESR spectra of tocopheroxyl radicals have been studied (6,7), their resolution was rather poor. Previously we recorded the considerably resolved ESR spectra of the radicals derived from α-tocopherol and its model compound with superoxide ion (8). However, we had difficulty in analyzing them completely because of their insufficient resolution. Recently, Mukai and the others observed the well resolved ESR spectrum of the α-tocopheroxyl radical generated by lead dioxide oxidation of α-tocopherol and determined its hyperfine coupling constants (9).

Now we wish to report the ESR spectra of the tocopheroxyl and chromanoxyl radicals formed by hydrogren abstraction of α-, β-, γ- and δ-tocopherols, 5,7-dimethyltocol, tocol and their model compounds with DPPH, and their hyperfine coupling constants and g-factors.

MATERIALS AND METHODS

Materials

d-α-, d-γ- and d-δ-Tocopherols were obtained from Eisai Research Laboratories, Tsukuba, Japan. dl-β-Tocopherol, dl-5,7-dimethyltocol, dl-tocol, and 2,2,5,7,8-pentamethyl-, 2,2,5,7-tetramethyl-, 2,2,5,8-tetramethyl-, 2,2,7,8-tetramethyl- and 2,2-dimethylchroman-6-ols were synthesized in our laboratory as described previously (10). Potassium nitrosodisulfonate, so-called Fremy's salt, was prepared by a known method (11). Toluene (Dotite Spectrosol) was purchased from Dojin Chemical Laboratory, Kumamoto, Japan, and DPPH from Wako Pure Chemical Industries, Osaka, Japan.

Procedure

Under nitrogen, 1 ml of a 2 mM tocopherol or chromanol solution in toluene was placed in a 5-mm quartz sample tube equipped with both an adjustable Teflon plunge and a side arm containing 1 μmol DPPH; for δ-tocopherol, tocol and 2,2-dimethylchroman-6-ol, however, their 12 mM solutions were used. The toluene solution was degassed under 10^{-2} torr by a freezing-thawing procedure. Immediately after mixing the DPPH in the degassed solution under the reduced pressure, we set the sample tube in an ESR cavity.

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Abbreviations: ESR = electron spin resonance; DPPH = 2,2-diphenyl-1-picrylhydrazyl.

Spectroscopic Measurements

ESR spectra were recorded on a Varian E-109 spectrometer (X-band) with an E-233 large access cylindrical cavity. All spectra were taken at room temperature under the following settings: modulation frequency 100 kHz, microwave power 10 mW, modulation amplitude 0.0125 or 0.025 mT, time constant 0.128 sec, scan time 8 min. Magnet fields were calibrated by the use of a Fremy's salt standard (a_N = 1.3091 ± 0.0004 mT, g = 2.0054) (12, 13).

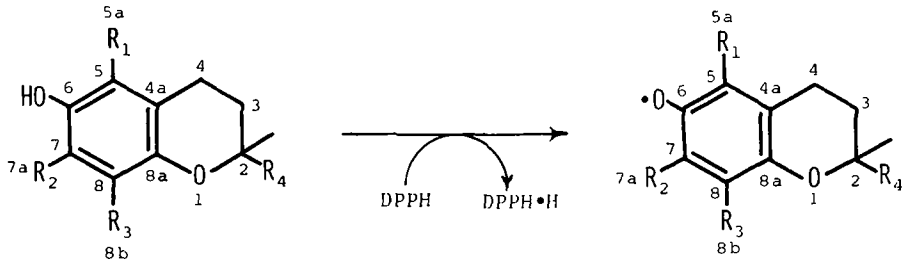
Calculations

Spectrum simulations were carried out employing a Varian E-900 data aquisition system. For computer-simulated spectra, line shape is Lorentzian and line widths are 0.025 (the γ-tocopheroxyl and model radicals), 0.028 (the α-tocopheroxyl and model radicals), 0.030 (the δ-tocopheroxyl radical) and 0.035 mT (the other radicals).

RESULTS AND DISCUSSION

We observed the well resolved ESR spectra of the radicals derived from tocopherols and their related compounds in degassed toluene by treatment with DPPH. The structures of tocopherols, their related compounds and the tocopheroxyl and chromanoxyl radicals together with the numbering of their atoms are given in Figure 1.

Figure 2 shows the ESR spectra of the α-tocopheroxyl (a) and model (b) radicals and the computer-simulated spectrum (c). The α-tocopheroxyl and model radicals gave the same spectrum, having four different hyperfine coupling constants: a_H's = 0.607 (5a-CH_3),



Parent compounds	Radicals	R_1	R_2	R_3	R_4
α-Tocopherol	α-Tocopheroxyl	CH_3	CH_3	CH_3	$C_{16}H_{33}$
α-Toc. model, 2,2,5,7,8-pentamethylchroman-6-ol	2,2,5,7,8-Pentamethylchroman-6-oxyl	CH_3	CH_3	CH_3	CH_3
5,7-Dimethyltocol	5,7-Dimethyltocoxyl	CH_3	CH_3	H	$C_{16}H_{33}$
2,2,5,7-Tetramethylchroman-6-ol	2,2,5,7-Tetramethylchroman-6-oxyl	CH_3	CH_3	H	CH_3
β-Tocopherol	β-Tocopheroxyl	CH_3	H	CH_3	$C_{16}H_{33}$
β-Toc. model, 2,2,5,8-tetramethylchroman-6-ol	2,2,5,8-Tetramethylchroman-6-oxyl	CH_3	H	CH_3	CH_3
γ-Tocopherol	γ-Tocopheroxyl	H	CH_3	CH_3	$C_{16}H_{33}$
γ-Toc. model, 2,2,7,8-tetramethylchroman-6-ol	2,2,7,8-Tetramethylchroman-6-oxyl	H	CH_3	CH_3	CH_3
δ-Tocopherol	δ-Tocopheroxyl	H	H	CH_3	$C_{16}H_{33}$
Tocol	Tocoxyl	H	H	H	$C_{16}H_{33}$
2,2-Dimethylchroman-6-ol	2,2-Dimethylchroman-6-oxyl	H	H	H	CH_3

FIG. 1. The structures of tocopherols, the related compounds and the tocopheroxyl and related radicals together with the numbering of their atoms.

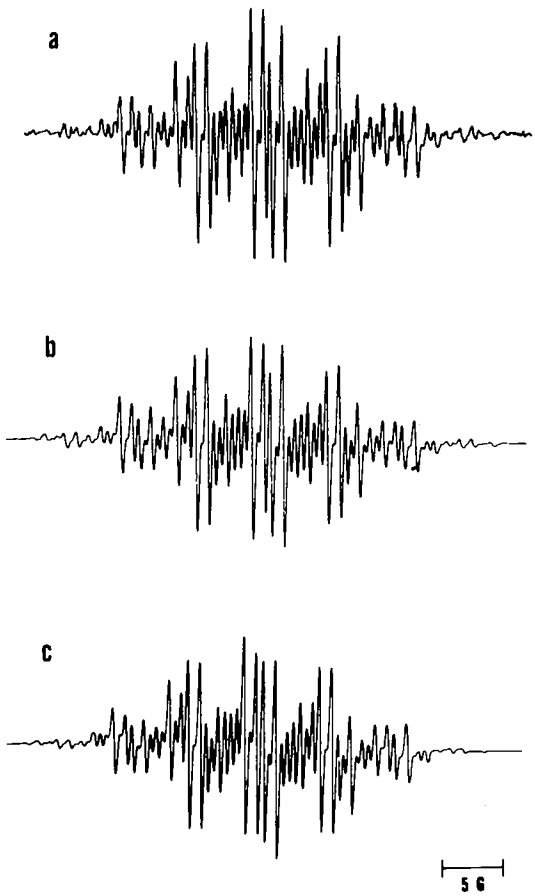


FIG. 2. The ESR spectra of the α-tocopheroxyl (a) and 2,2,5,7,8-pentamethylchroman-6-oxyl (b) radicals and the computer-simulated spectrum (c).

0.455 (7a-CH_3), 0.098 (8b-CH_3) and 0.152 mT (4-CH_2). The assignment of the hyperfine coupling constants has been confirmed by the analysis of spectra of the 5a-, 7a- and 8b-CD_3-isotopomers of the model radical (14). The values of the hyperfine coupling constants were very close to those reported by Mukai and the others (9).

Figures 3, 4, 5 and 6 show the ESR spectra (a) of the 5,7-dimethyltocoxyl, β- and γ-tocopheroxyl and tocoxyl radicals, respectively, with the spectra (b) of their model radicals and the computer-simulated spectra (c). There is no difference between the spectra of each of the 5,7-dimethyltocoxyl, β- and γ-tocopheroxyl and tocoxyl radicals and the corresponding model radical. The spectra of the 5,7-dimethyltocoxyl, β- and γ-tocopheroxyl radicals were reconstructed provided that an unpaired electron interacted with the four groups of inequivalent protons, each of which had one, two, three or three equivalent protons. The hyperfine coupling constants of the 5,7-dimethyltocoxyl and model radicals are a_H's = 0.591 (5a-CH_3), 0.464 (7a-CH_3), 0.090 (8b-H) and 0.140 mT (4-CH_2), of the β-tocopheroxyl and model radicals a_H's = 0.640 (5a-CH_3), 0.460 (7a-H), 0.091 (8b-CH_3) and 0.174 mT (4-CH_2), and of the γ-tocopheroxyl and model radicals a_H's = 0.603 (5a-H), 0.482 (7a-CH_3), 0.112 (8b-CH_3) and 0.130 mT (4-CH_2); they were assigned on the basis of the hyperfine coupling constants of the α-tocopheroxyl radical. The spectra of the tocoxyl and model radicals were also simulated by the use of the following hyperfine coupling constants: a_H's = 0.592 (5a-H), 0.513 (7a-H), 0.079 (8b-H) and 0.138 mT (4-CH_2).

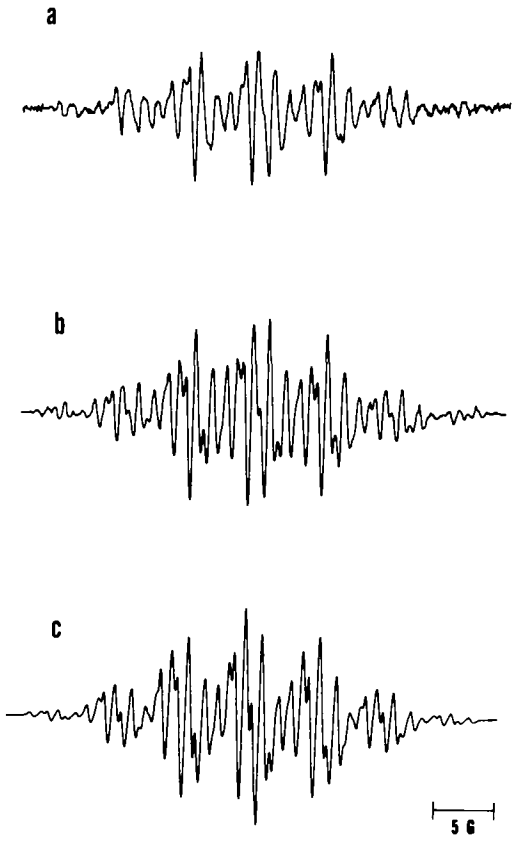


FIG. 3. The ESR spectra of the 5,7-dimethyltocoxyl (a) and 2,2,5,7-tetramethylchroman-6-oxyl (b) radicals and the computer-simulated spectrum (c).

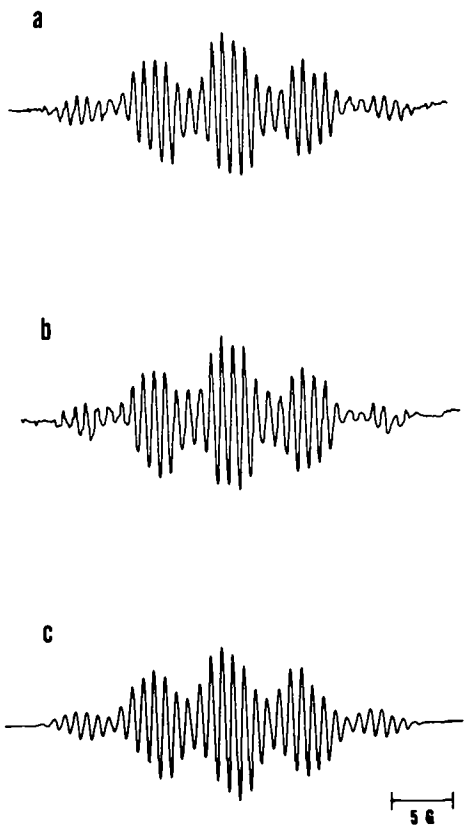


FIG. 4. The ESR spectra of the β-tocopheroxyl (a) and 2,2,5,8-tetramethylchroman-6-oxyl (b) radicals and the computer-simulated spectrum (c).

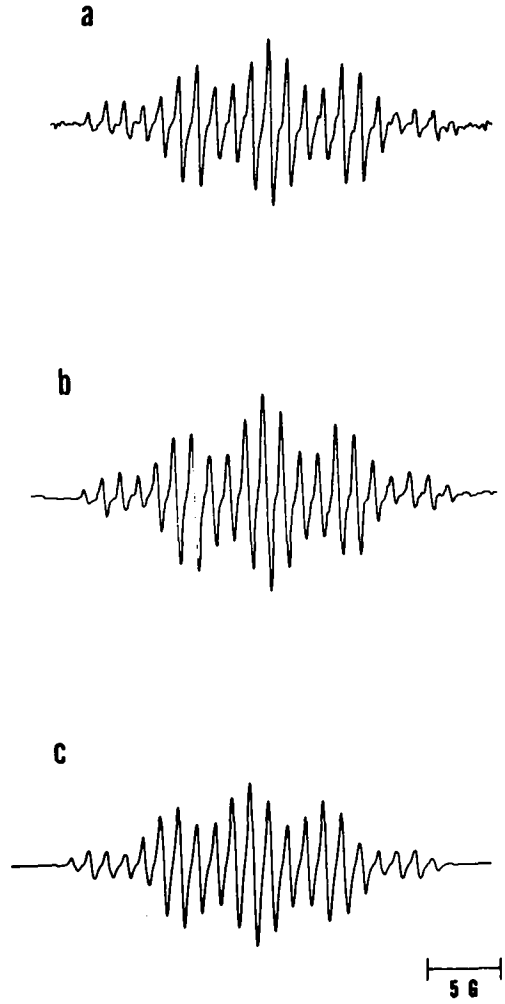


FIG. 5. The ESR spectra of the γ-tocopheroxyl (a) and 2,2,7,8-tetramethylchroman-6-oxyl (b) radicals and the computer-simulated spectrum (c).

The ESR and computer-simulated spectra of the δ-tocopheroxyl radical are shown in Figure 7. The hyperfine coupling constants of the δ-tocopheroxyl radical were estimated to be a_H's = 0.619 (5a-H), 0.482 (7a-H), 0.103 (8b-CH_3) and 0.145 mT (4-CH_2).

All the hyperfine coupling constants of the tocopheroxyl and chromanoxyl radicals are listed in Table 1. The magnitude of hyperfine coupling constants due to aromatic protons in the radicals is similar to that due to the protons of a methyl group substituted for the aromatic proton. The magnitude of the hyperfine coupling constants due to the aromatic and methyl protons at the α- and β-positions of C-5, C-7 and C-8 in the radicals decreases in that order of the aromatic carbon atoms; theoretically, it is proportional to the spin density at the aromatic carbon atoms (15). The values of the hyperfine coupling constants due to the methylene protons at C-4 in the radicals drop characteristically into the narrow range from 0.130 to 0.174 mT. For the γ-tocopheroxyl and model radicals, the hyperfine coupling constants (both, 0.112 mT) due to the protons of a methyl group at C-8 may be somewhat overestimated and those (both, 0.130 mT) due to the methylene protons at C-4 somewhat underestimated, because their values appear to be deviated from the values of the corresponding hyperfine coupling constants of the other radicals.

As given in Table 1, the g-factors of the radicals were measured on the basis of the g-factor of Fremy's salt. The g-factors of the tocopheroxyl radicals agree closely with those

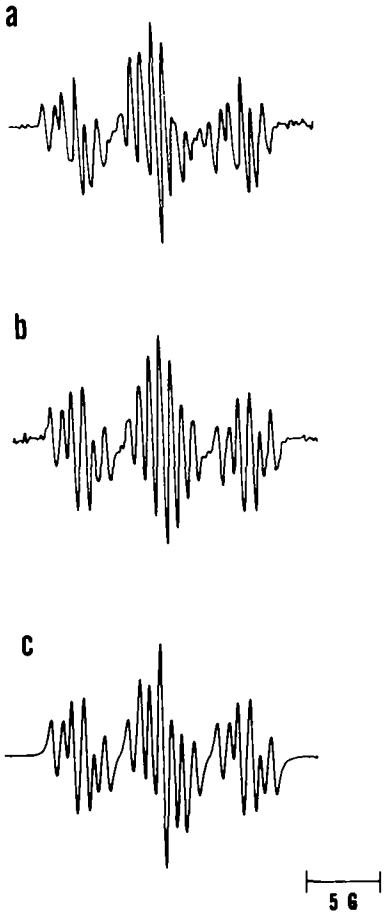


FIG. 6. The ESR spectra of the tocoxyl (a) and 2,2-dimethylchroman-6-oxyl (b) radicals and the computer-simulated spectrum (c).

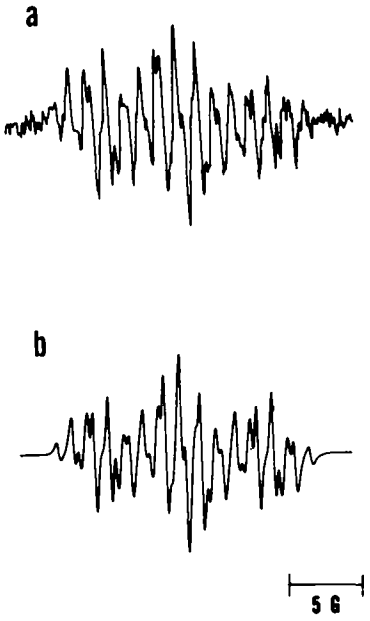


FIG. 7. The ESR spectrum of the δ-tocopheroxyl radical (a) and the computer-simulated spectrum (b).

TABLE 1

ESR Parameters of the Radicals Derived from Tocopherols and their Related Compounds

Radicals	Hyperfine coupling constants (mT)				g-Factors
	R_1[a]	R_2[a]	R_3[a]	4-CH_2	
α-Tocopheroxyl and 2,2,5,7,8-pentamethylchroman-6-oxyl	0.607	0.455	0.098	0.152	2.0046
5,7-Dimethyltocoxyl and 2,2,5,7-tetramethylchroman-6-oxyl	0.591	0.464	0.090	0.140	2.0046
β-Tocopheroxyl and 2,2,5,8-tetramethylchroman-6-oxyl	0.640	0.460	0.091	0.174	2.0047
γ-Tocopheroxyl and 2,2,7,8-tetramethylchroman-6-oxyl	0.603	0.482	0.112	0.130	2.0047
δ-Tocopheroxyl	0.619	0.482	0.103	0.145	2.0049
Tocoxyl and 2,2-dimethylchroman-6-oxyl	0.592	0.513	0.079	0.138	2.0049

[a]R_1, R_2 and R_3 = H or CH_3. See Figure 1.

of the corresponding model radicals. The magnitude of the g-factors is in the following order: the α-tocopheroxyl and 2,2,5,7,8-pentamethylchroman-6-oxyl radicals = the 5,7-dimethyltocoxyl and 2,2,5,7-tetramethylchroman-6-oxyl radicals > the β-tocopheroxyl and 2,2,5,8-tetramethylchroman-6-oxyl radicals = the γ-tocopheroxyl and 2,2,7,8-tetramethylchroman-6-oxyl radicals > the δ-tocopheroxyl radical = the tocoxyl and 2,2-dimethylchroman-6-oxyl radicals.

In regard to the magnitude of hyperfine coupling constants and g-factors, the α-tocopheroxyl radical is very similar to the 2,2,5,7,8-pentamethylchroman-6-oxyl, 5,7-dimethyltocoxyl and 2,2,5,7-tetramethylchroman-6-oxyl radicals. We have already found that the radical scavenging ability of α-tocopherol and 5,7-dimethyltocol is much higher than that of the other tocopherols (16). These findings make us attach importance to the presence of methyl groups at C-5 and C-7 in tocopherols and chroman-6-ols functioning as antioxidants.

On the basis of the ESR parameters obtained here, a variety of tocopheroxyl radicals can be identified and quantified easily. This is very useful for the analysis of the antioxidant action of tocopherols in various systems.

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