Preparation and ESR Studies of New Stable Tocopheroxyl Model Radicals

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New tocopherol (vitamin E) model compounds (tocopherol **1**; 7-*t*-butyl-2,2-dimethyl-6-chromanol, tocopherol **2**; 7-*t*-butyl-2,2,5-trimethyl-6-chromanol, and tocopherol **3**; 5,7-diisopropyl-2,2-dimethyl-6-chromanol) have been synthesized by condensation of 2-methyl-3-buten-2-ol to the corresponding alkylhydroquinone. Electron spin resonance measurements were performed for the tocopheroxyl radicals obtained from the above tocopherol model compounds by oxidizing the phenol precursors with PbO_2 in toluene. The proton hyperfine coupling constants and g_{iso}-values were determined. The tocopheroxyl radicals **2** and **3** having large alkyl substituents at both ortho positions (C-5 and C-7) are fairly stable, and the radical stability, as measured by the intensities of the ESR signals, increases in the order tocopheroxyl **1**<α-tocopheroxyl model<tocopheroxyl **2**<tocopheroxyl **3**. The result suggests that the *in vitro* antioxidant activities of tocopherols **2** and **3** are higher than that of α-tocopherol model.

It has been well recognized that tocopherols (vitamin E) function as natural antioxidants, particularly in biomembranes by protecting unsaturated lipids from peroxidation.[1-3] The ESR study of the tocopheroxyl radicals obtained by the oxidation of tocopherols is of biological interest since they are involved in vitamin E reactions. Therefore, recently, some workers have reported the ESR spectra of α-, β-, γ-, and δ-tocopheroxyl radicals and their model compounds, and determined the proton hyperfine splittings exactly.[4-10]

Generally, in these tocopheroxyl radicals, replacement of ortho methyl groups at C-5 and C-7 positions with protons lowers the concentration of the tocopheroxyl radicals in solution, as well as the longevity of the radicals. The approximate order of stability, as measured by the intensities of the ESR signals, was: α-, β-, γ-, and δ-tocopheroxyl.[8] The above relative stabilities of the tocopheroxyl radicals correlate well with their biological activities[11] which yield: α->β->γ->δ-tocopherol, and with *in vitro* tests of their relative reactivities toward peroxide radical[12] and singlet oxygen[13] which yield the same order. Further, α-tocopherol and α-tocopherol model, where the isoprenoid side chain is replaced by a methyl group, have similar *in vitro* antioxidant activity,[12] although the latter compound generally shows little or no vitamin E activity *in vivo*.[14,15] These findings suggest that the presence of methyl groups at C-5 and C-7 positions in tocopherols and their model compounds is of great importance to their antioxidant action.

In the present work, in order to obtain further information on such antioxidant action, we have prepared three kinds of new tocopherol model compounds (tocopherol **1**; 7-*t*-butyl-2,2-dimethyl-6-chromanol, tocopherol **2**; 7-*t*-butyl-2,2,5-trimethyl-6-chromanol, and tocopherol **3**; 5,7-diisopropyl-2,2-dimethyl-6-chromanol) (see Fig. 1), and have succeeded in measuring well-resolved ESR spectra of tocopheroxyl-type radicals obtained by the PbO_2 oxidation of the above tocopherol model compounds in toluene at room temperature. The proton hyperfine coupling constants and g_{iso}-values were correctly determined for each radical. From the results, the electronic structure of the tocopheroxyl radicals has been discussed. Especially, the tocopheroxyls **2** and **3** having large alkyl groups at both ortho positions (C-5 and C-7) showed higher radical stability than that of α-tocopheroxyl model, as measured by the ESR intensity change. The result suggests that tocopherols **2** and **3** have higher *in vitro* antioxidant activity than that of α-tocopherol model.

Experimental

Measurements. The ESR measurements were carried out using a JES-FE-2XG spectrometer equipped with a Takeda-Riken microwave frequency counter. The *g*-values were measured relative to the value of Li-TCNQ powder, calibrated with $(KSO_3)_2NO$ (g=2.0054).[16] All the ESR spectra have been measured in a sealed, degassed system. In all the cases, we made careful measurements of the decay of the ESR signal intensity for the tocopheroxyl radicals obtained by oxidizing the toluene solution of tocopherol precursors (1.0 ml, 5.0×10^{-3} M (1 M=1 mol dm^{-3})) with PbO_2 (0.7 g).

Preparation of Specimens. 2-*t*-Butyl-6-methylhydroquinone (mp 98.5—100.5 °C) (lit, 97—98 °C)[17] and 2,6-diisopropylhydroquinone (mp 108.5—110.0 °C) (lit, 105.5—106.0 °C)[17]

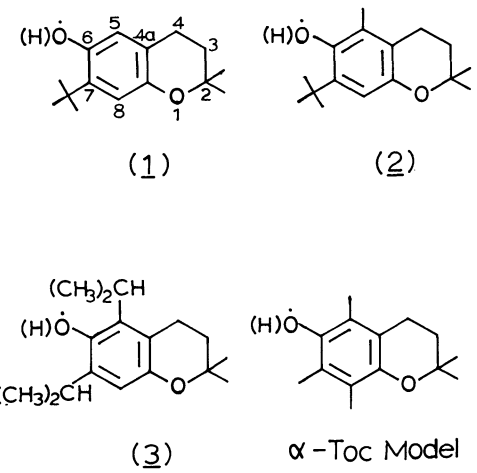

Fig. 1. Molecular structures of tocopherols and tocopheroxyls (**1**, **2**, **3**, and α-model), and the atomic numbering system.

were prepared from 2-*t*-butyl-6-methylphenol and 2,6-diisopropylphenol respectively by the standard procedure, as reported for the syntheses of the 2,3-, 2,5-, and 2,6-dimethylhydroquinone.[18]

2,2,5,7,8-Pentamethyl-6-chromanol (α-tocopherol model) and 2,2-dimethyl-6-chromanol (tocol model) were prepared according to the method of Nilsson *et al.*[19] Tocopherols **1**, **2**, and **3** were synthesized by condensation of 2-methyl-3-buten-2-ol to the corresponding alkylhydroquinone, according to a procedure similar to that used by Nilsson *et al.* to prepare α-, β-, γ-, and δ- tocopherol models.[19] Tocopherol **1** was also prepared by the reaction of 2,2-dimethyl-6-chromanol (tocol model) with isobutene in benzene in the presence of H_2SO_4.[20]

7-t-Butyl-2,2,5-trimethyl-6-chromanol (Tocopherol 2). 2-*t*-Butyl-6-methylhydroquinone (4.0 g, 22 mmol) was dissolved in formic acid (99%) (50 ml) and THF (tetrahydrofuran) (5 ml), and the solution was heated to reflux. A THF (2 ml) solution of 2-methyl-3-buten-2-ol (1.9 g, 22 mmol) was added dropwise slowly during 1 h, and the refluxing was continued for an additional 3 h with stirring. The reaction mixture was poured onto crushed ice (150 g), and extracted with diethyl ether (5×25 ml). Petroleum ether (bp 40—50 °C) (25 ml) was added to the combined diethyl ether extracts and the mixture was washed with water (5×25 ml). After removal of the solvent, the residue was dissolved in methanol (75 ml), 1 ml of concd HCl was added, and the solution was refluxed for 20 min to hydrolyze formate of **2** produced. The methanol was then evaporated, and the residue was taken up in diethyl ether (100 ml), washed with $NaHCO_3$ solution and with water, and the solution was dried over anhydrous sodium sulfate. The solvent was evaporated, and the residual oil was extracted three times with hot petroleum ether (bp 50—60 °C) (3×100 ml). After removal of the solvent, the oily solids obtained were recrystallized twice from benzene, giving white crystals (1.5 g). Mp 145.0—147.0 °C. Found: C, 77.39; H, 9.93%. Calcd for $C_{16}H_{24}O_2$: C, 77.38; H, 9.74%. UV spectrum (ethanol) λ_{max}=292 nm, log ε=3.55. NMR spectrum δ=1.29 ppm (6H, s, 2-CH_3), 1.37 (9H, s, 7-*t*-Bu), 1.77 (2H, t, 3-CH_2, J=6.7 Hz), 2.10 (3H, s, 5-CH_3), 2.60 (2H, t, 4-CH_2, J=6.7 Hz), 4.27 (1H, s, 6-OH), 6.54 (1H, s, 8-H); δ in $CDCl_3$ with TMS as an internal standard.

5,7-Diisopropyl-2,2-dimethyl-6-chromanol (Tocopherol 3). Tocopherol **3** was prepared from 2,6-diisopropylhydroquinone (4.0 g, 21 mmol) and 2-methyl-3-buten-2-ol (1.8 g, 21 mmol) in 50 ml of formic acid (99%) and 7 ml of THF, following a method similar to that used with tocopherol **2**. After hydrolysis of the formate of **3** and diethyl ether extraction, the diethyl ether was evaporated and the crude product was extracted several times with petroleum ether to remove the unreacted 2,6-diisopropylhydroquinone. After removal of the petroleum ether, a viscous oil remained. The residue was twice chromatographed on 50 g of silica gel with benzene as the eluting agent. The subsequent removal of the benzene from **3** solution left a slightly brownish oil; this was dissolved in diethyl ether (5 ml) and heated at 60 °C for 2 h under a vacuum (5×10^{-3} Torr (1 Torr≈133.322 Pa)) in order to completely remove the benzene solvent included in the crystal. Viscous oil (2.2 g). Found: C, 77.67; H, 10.26%. Calcd for $C_{17}H_{26}O_2$: C, 77.82; H, 9.99%. UV spectrum (ethanol) λ_{max}=295 nm, log ε=3.57. NMR spectrum δ=1.20 (6H, d, 5- or 7-$CH(CH_3)_2$, J=7.0 Hz), 1.28 (6H, s, 2-CH_3), 1.34 (6H, d, 5- or 7-$CH(CH_3)_2$, J=7.0 Hz), 1.73 (2H, t, 3-CH_2, J=6.6 Hz), 2.68 (2H, t, 4-CH_2 J=6.6 Hz), 2.64—3.46 (2H, m, 5- and 7-$CH(CH_3)_2$), 4.28 (1H, s, 6-OH), 6.48 (1H, s, 8-H); δ in $CDCl_3$ with TMS as an internal standard).

7-t-Butyl-2,2-dimethyl-6-chromanol (Tocopherol 1). Method A: To 1.0 g (5.6 mmol) of 2,2-dimethyl-6-chromanol (tocol model) in 13 ml of benzene, 5 drops of concd H_2SO_4 were added, and isobutene (6.0 g, 0.107 mol) was introduced for 3 h under stirring at 50 °C.[20] The reaction mixture was washed with water, and dried over anhydrous sodium sulfate. After removal of the benzene, the solids remained were recrystallized first from ligroin (bp 75—90 °C), and then from ethanol, giving white crystals (0.3 g). Mp 184.0—185.0 °C. Found: C, 76.70; H, 9.49%. Calcd for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46%. UV spectrum (ethanol) λ_{max}=226 nm, log ε=3.63, λ_{sh}=230 nm, log ε=3.59, λ_{max}=298 nm, log ε=3.65. NMR spectrum δ=1.25 (6H, s, 2-CH_3), 1.29 (9H, s, 7-*t*-Bu), 1.74 (2H, t, 3-CH_2, J=6.7 Hz), 2.65 (2H, t,4-CH_2, J=6.7 Hz), 4.48 (1H, s, 6-OH), 6.38 (1H, s, 5- or 8-H), 6.70 (1H, s, 5- or 8-H); δ in $CDCl_3$ with TMS as an internal standard.

7-t-Butyl-2,2-dimethyl-6-chromanol (Tocopherol 1). Method B: Tocopherol **1** was prepared from 2-*t*-butylhydroquinone (3.7 g, 22 mmol) and 2-methyl-3-buten-2-ol (1.9 g, 22 mmol) in 50 ml of formic acid (99%) and 7 ml of THF, following a method similar to that used with **2**. The product was extracted and the formates were hydrolyzed as before. The oily residue obtained was chromatographed on 50 g of silica-gel with ether–petroleum ether (1:5) as the eluting agent. After removal of the solvent, the oily crystals remained were washed with petroleum ether, and recrystallized from ligroin, giving white crystals (0.5 g). Mp 183.0—185.0 °C. The ultraviolet and NMR spectra of the compound were shown to be identical with the material prepared by method A.

Results and Discussion

As has been described in our previous paper, the oxidation product of the α-, β-, γ-, δ-tocopherols, tocol and their model compounds with PbO_2 in toluene gives well-resolved hyperfine patterns, and the spectra were assigned to the primary tocopheroxyl-type radicals formed from the parent tocopherols by abstraction of a phenolic hydrogen atom.[8]

The tocopherol **1** was similarly oxidized with PbO_2 in toluene under vacuum. The ESR spectrum of this solution at room temperature is shown in Fig. 2a. The spectrum can be reconstracted with three groups of 1, 2 and 1 equivalent protons, showing three different hyperfine couplings 5.91, 1.22, and 1.04 G (1 G=10^{-4} T), respectively. These couplings will be assigned to a ring proton at C-5 (a_5^H=5.91 G), two equivalently interacting methylene protons at C-4 ($a_4^{CH_2}$=1.22 G), and a ring proton at C-8 (a_8^H=1.04 G), taking the analysis of α-, β-, γ-, and δ-tocopheroxyls into account. No interaction was observed for the methylene protons at C-3 and the *t*-butyl protons at C-7.

Similarly, the toluene solution ESR spectrum of the tocopheroxyl **2** radical has been measured (see Fig. 2b). The spectrum at 20 °C can be easily analyzed, giving three different hyperfine couplings, $a_5^{CH_3}$= 6.05 G, $a_4^{CH_2}$=1.55 G, and a_8^H=0.72 G.

The ESR spectrum of the tocopheroxyl **3** measured at 20°C is given in Fig. 2c. The spectrum can be reconstructed with four groups of 1, 2, 1, and 1 equivalent protons, showing four different hyperfine splittings (2.72, 1.39, 0.75, and 0.65 G), respectively. The assignment of the hyperfine splittings has been performed by a comparison of experimentally obtained hyperfine couplings with reported ones for α-, β-, γ-, and δ-tocopheroxyls[8] and 2,6-diisopropyl-4-methoxyphenoxyl radical.[17] The tentative assignments are as follows: a_7^{CH}=2.72 G, $a_4^{CH_2}$=1.39 G, a_8^{H}=0.75 G, and a_5^{CH}=0.65 G.

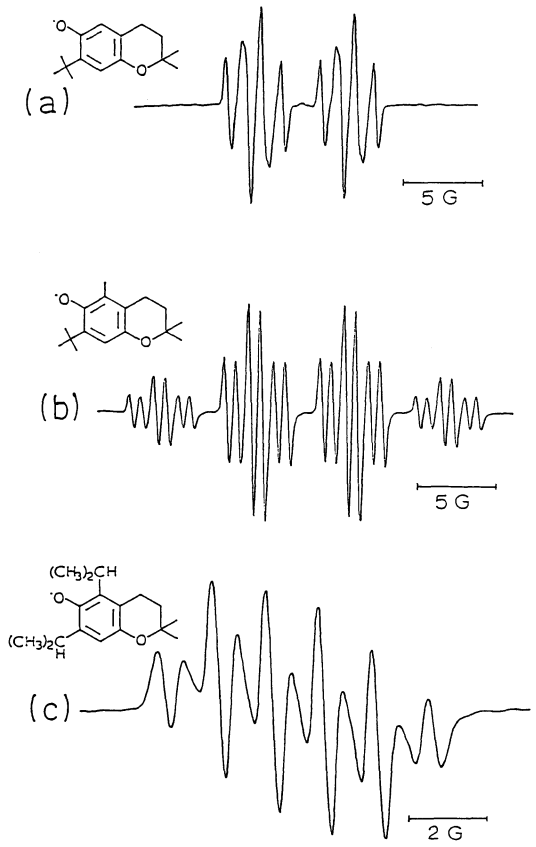

Fig. 2. ESR spectra of (a) tocopheroxyl **1**, (b) tocopheroxyl **2**, and (c) tocopheroxyl **3** in toluene at 20°C.

All the hyperfine couplings and g_{iso}-values obtained for these new tocopheroxyl model radicals are summarized in Table 1, together with those of α-tocopheroxyl model.[7]

The experimental values of spin densities (ρ_i^{π}) were estimated using the relations, $a_i^{H}=27\rho_i^{\pi}$, $a_i^{CH_3}=27\rho_i^{\pi}$, and $a_4^{CH_2}=54\cos^2 30°\times\rho_{4a}^{\pi}$, as performed for α-, β-, γ-, and δ-tocopheroxyl radicals.[7,8] All the experimental spin densities (ρ_i^{π}) calculated from the ESR hyperfine couplings are summarized in Table 1.

As is clear from the results listed in Table 1, unpaired spin density at C-5 position is similar to each other in these tocopheroxyl radicals. On the other hand, unpaired spin densities at C-8 position in **1**, **2**, and **3** are 8% higher, 25% lower, and 22% lower than that at C-8 position in α-tocopheroxyl model, respectively. Similarly, the unpaired spin densities at C-4a position are different each other in these tocopheroxyls. The result suggests that alkyl-substitution effect is considerable for the unpaired spin density at meta carbon atoms (C-4a and C-8 positions) with small negative spin densities. However, it is not clear at present how the above differences in the electronic structure of these tocopheroxyls relate to the antioxidant activity of tocopherols.

In order to obtain the information on the radical stability of these tocopheroxyls, radical decay measurements were performed on tocopheroxyls **1**, **2**, **3**, and α-tocopheroxyl model in toluene. Shown in Fig. 3 are typical plots of the ESR signal intensity as a function of time for the tocopheroxyls at 20°C. As it is clear from the results shown in Fig. 3, the order of stability, as measured by the intensities of the ESR signals, was tocopheroxyl **3**, **2**, and α-tocopheroxyl model. Especially, tocopheroxyl **3** is very stable and the ESR signal

Table 1. Hyperfine couplings (a_i^H) (in gauss), g_{iso}-values, and spin densities (ρ_i^{π}) of the tocopheroxyl radicals derived from tocopherol model compounds in toluene at 20°C

Tocopheroxyls		5	7	8	4	g_{iso}-values
		H	$C(CH_3)_3$	H	CH_2	
1	a_i^H	5.91[a]	—	1.04	1.22	2.00477[c]
	ρ_i^{π}	0.2189	—	−0.0385	−0.0301[b]	
		CH_3	$C(CH_3)_3$	H	CH_2	
2	a_i^H	6.05	—	0.72	1.55	2.00477
	ρ_i^{π}	0.2241	—	−0.0267	−0.0383	
		$CH(CH_3)_2$	$CH(CH_3)_2$	H	CH_2	
3	a_i^H	0.65	2.72	0.75	1.39	2.00472
	ρ_i^{π}	—	—	−0.0278	−0.0343	
		CH_3	CH_3	CH_3	CH_2	
α-Toc Model	a_i^H	5.94[d]	4.51	0.96	1.46	2.00471
	ρ_i^{π}	0.2200	0.1670	−0.0356	−0.0360	

a) Experimental errors ±0.04 G. 1 G=10^{-4} T. b) Spin densities at C-4a. c) Experimental errors ±0.00005. d) From Ref. 8.

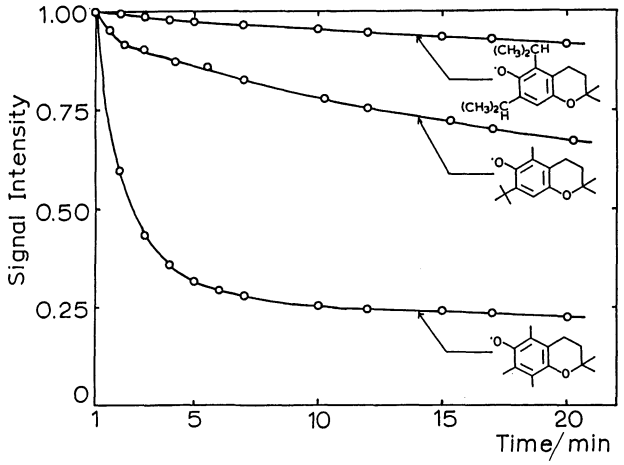

Fig. 3. Plots of the ESR signal intensity of tocopheroxyl **2**, tocopheroxyl **3**, and α-tocopheroxyl model at 20 °C as a function of time.

intensity decreased to about half of the initial intensity after 20 h, because **3** has large isopropyl groups at both C-5 and C-7 positions. On the other hand, tocopheroxyl **1** has no alkyl substituent at C-5 position which is the most reactive site of the tocopheroxyl, and thus showed fast radical decay.

As described in a previous section, the relative radical stability of α-, β-, γ-, and δ-tocopheroxyls[8] is in agreement with *in vivo* and *in vitro* tests of their relative antioxidant activities which yield: α->β->γ->δ-tocopherol.[3,11-13] The result of the present radical decay measurement shows that tocopheroxyls **2** and **3** are more stable than α-tocopheroxyl. Therefore, we can expect that tocopherols **2** and **3** have higher antioxidant activity than α-tocopherol. Measurements of *in vitro* antioxidant activity of these tocopherols are now in progress.

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