ENDOR Study of the Cation Radicals of Vitamin E Derivatives. Relation between Antioxidant Activity and Molecular Structure

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An ENDOR study of vitamin E derivatives was carried out. The molecular structures of the cation radicals of models for vitamin E derivatives were determined by means of ENDOR. The relation between the antioxidant activity and the molecular structure of vitamin E was discussed.

It is well known that vitamin E (α-, β-, γ-, and δ-tocopherols, Fig. 1) inhibits the autoxidation of organic molecules, and the mechanism has been studied extensively by numerous investigators.[1-4] Furthermore, vitamin E is present in cellular membranes and edible oils and acts as an antioxidant by protecting polyunsaturated lipids or fatty acids from peroxidation.

The antioxidant properties of tocopherols (TocH's) have been ascribed to proton transfer (hydrogen transfer) from the OH group in TocH's by a peroxyl radical (LOO·). The proton transfer (hydrogen transfer) produces a tocopheroxyl radical (Toc·), which combines with another peroxyl radical (reactions 1 and 2).[5,6]

$$\mathrm{LOO\cdot + TocH \xrightarrow{k_1} LOOH + Toc\cdot} \quad (1)$$

$$\mathrm{LOO\cdot + Toc\cdot \rightarrow Non\text{-}radical\ products.} \quad (2)$$

Recently, we measured the second-order rate constant of TocH with stable phenoxyl radical (2,6-di-*t*-butyl-4-(4-methoxyphenyl)phenoxyl), k_s, with a stopped-flow spectrophotometer.[7-15] The decreasing order of k_s is as follows: α-TocH>β-TocH $\approx\gamma$-TocH>δ-TocH>tocol. Burton et al. suggested that the difference in antioxidant activity between α-TocH and 4-methoxytetramethylphenol is due to the extent of orbital overlap between the 2p-type lone pair on the ring oxygen or the methoxyl oxygen and the aromatic π electron system.[16-19]

In this report, we have made an ENDOR (electron nuclear double resonance) study of cation radicals of TocH's in order to clarify whether or not the orbital overlap is a dominant factor governing the antioxidant activity of TocH's as explained by Burton et al. ENDOR is now a well-established method to study molecular and electronic structures of radicals. Thus, we have tried to determine the molecular structures of the cation radicals of TocH's by means of ENDOR. Since the radicals of TocH's are not stable and dimerize at low temperature, we have used model molecules shown in Fig. 2 instead of TocH's. The results for a part of molecules were given previously by Sutcliffe et al.[20,21] and us.[22-24]

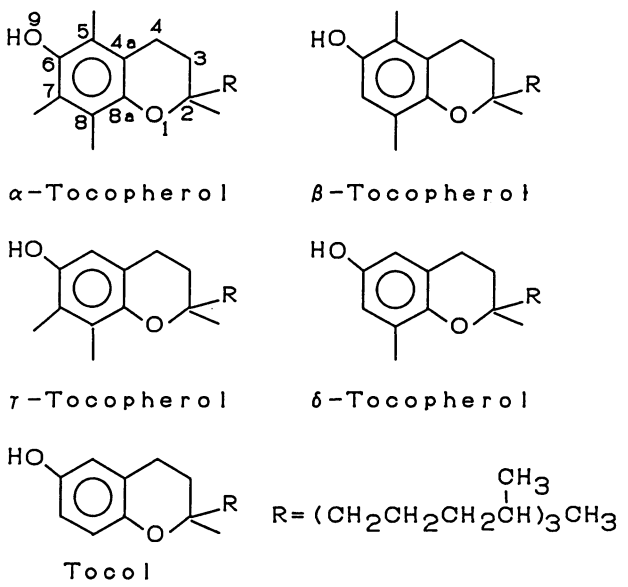

Fig. 1. Structures of TocH's.

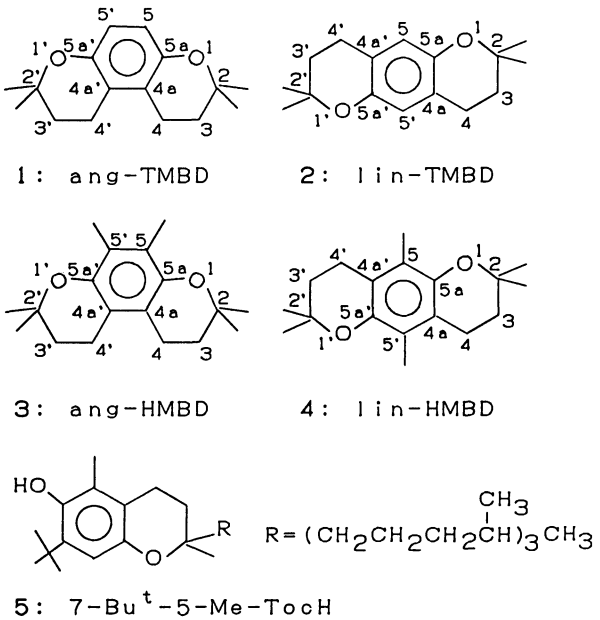

Fig. 2. Structures of models for TocH's studied in this work.

Experimental

Benzo[1,2-*b*:4,3-*b*'] and [1,2-*b*:4,5-*b*']dipyran compounds, 2,2,2',2'-tetramethyl-*angular*-benzodipyran (*ang*-TMBD, **1**, Fig. 2) and 2,2,2',2'-tetramethyl-*linear*-benzodipyran (*lin*-TMBD, **2**, Fig. 2), were synthesized by condensation of 2-methyl-3-buten-2-ol to hydroquinone in formic acid, according to the method of Nilsson et al.,[25] and the products were isolated by column chromatography on silica gel. The cation radicals of **1** and **2** were prepared by the oxidation of **1** and **2** in a degassed $AlCl_3$–CH_2Cl_2 solution, respectively.

The ESR (electron spin resonance) measurements were carried out using a JEOL JES-FE-2XG spectrometer equipped with a Takeda-Riken microwave frequency counter. The ENDOR spectra were recorded by a JEOL JES-EDX-1 spectrometer, operated with 80 kHz magnetic field modulation. All the ESR and ENDOR spectra were measured at −100 °C in a sealed and degassed system.

Results and Discussion

Figure 3 shows ENDOR spectra of the cation radicals of **1** and **2** in CH_2Cl_2 at −100 °C, where the free proton frequency ν_0 (13.8 MHz) is shown by an arrowhead in the diagram. If all the proton hyperfine couplings due to 5-H, 4-CH_2, 3-CH_2, and 2,2-$(CH_3)_2$ are different from each other, we can expect seven different hyperfine couplings for each of the cation radicals of **1** and **2**, as a maximum, assuming the free rotation of CH_3 groups. In our ENDOR measurements, we could resolve six different hyperfine splittings for each of the cation radicals of **1** and **2**.

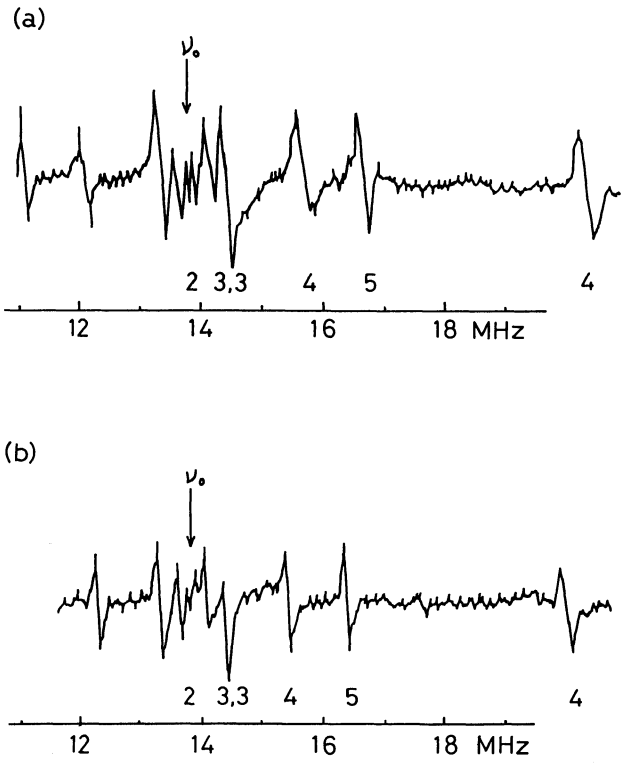

Fig. 3. ENDOR spectra of cation radicals of **1** (a) and **2** (b) in CH_2Cl_2 at −100 °C.

Assignments of the hyperfine couplings were performed, taking account of the result of the McLachlan molecular orbital calculations described later. The spin densities on C_5 and C_5' were calculated from McConnell's equation (Eq. 3).[26]

$$a_5^H = Q\rho_5^\pi, \quad (3)$$

where Q denotes an empirical parameter and was taken to be 24 G. The hyperfine couplings (a) and the spin densities (ρ) of the cation radicals of **1** and **2** thus obtained are given in Table 1, together with the results for those of 2,2,2',2'5,5'-hexamethyl-*angular*-benzodipyran (*ang*-HMBD, **3**, Fig. 2) and 2,2,2',2',5,5'-hexamethyl-*linear*-benzodipyran (*lin*-HMBD, **4**, Fig. 2) and for the

Table 1. Hyperfine Couplings (a) in G and Spin Densities of Radicals Obtained by ENDOR (ρ(Exptl)) and by McLachlan Molecular Orbital Calculations (ρ(Calcd)) of **1**—**5**

Position	a	ρ(Exptl)	ρ(Calcd)
Cation radical of **1**			
2, 2'	0.047[a,b]		
3, 3'	0.197		
	0.395		
4, 4'	1.318	0.0874[c]	0.0742
	4.719		
5, 5'	2.028	0.0845	0.0827
Cation radical of **2**			
2, 2'	0.057[b]		
3, 3'	0.172		
	0.399		
4, 4'	1.144	0.0844[c]	0.1087
	4.554		
5, 5'	1.849	0.0770	0.0491
Cation radical of **3**[22]			
2, 2'	0.040[b]		
3, 3'	0.199		
	0.389		
4, 4'	1.444	0.0932[c]	0.0787
	5.024		
5, 5'	1.547	0.0573	0.0731
Cation radical of **4**[22]			
2, 2'	0.038[b]		
3, 3'	0.141		
	0.355		
4, 4'	2.349	0.0593[c]	0.0548
	2.452		
5, 5'	2.986	0.1106	0.0976
Neutral radical of **5**[23]			
3	0.076		
	0.234		
4	1.448	−0.0376[c]	−0.0389
	1.592		
5	6.074	0.2250	0.2123
7	0.076		
8	0.608	−0.0225	−0.0286

a) Experimental errors$<\pm$0.010 G. b) It is not clear at present whether the smallest hyperfine coupling is due to two or four methyl groups at C_2 and $C_{2'}$. c) Spin density on C_{4a}($C_{4a'}$).

neutral radical of 7-*t*-butyl-5-methyltocopherol (7-Bu^t-5-Me-TocH, **5**, Fig. 2) obtained previously.[22-24]

McLachlan spin densities of the cation radicals of **1** and **2** were calculated using the same parameters for the oxygen atom and methyl group ($\alpha_{O1}=\alpha_{O1'}=\alpha+2.0\beta$, $\beta_{C5a\text{-}O1}=\beta_{C5a'\text{-}O1}=1.0\beta$, $\alpha_{C4a}=\alpha_{C4a'}=\alpha-0.06\beta$, $\alpha_{C5}=\alpha_{C5'}=\alpha-0.1\beta$, $\lambda=1.2$) as those used for α-Toc$\cdot$ radical.[27] The calculated results of the spin densities of the cation radicals, ρ(Calcd), of **1** and **2** are given in Table 1, together with the results for **3**—**5** obtained previously.[22-24] The results of McLachlan molecular orbital calculations were found to be in satisfactory agreement with the experimental spin densities, ρ(Exptl), evaluated from the hyperfine coupling constants.

The magnitude of the β-methylene proton hyperfine coupling (a_β^H) can often be calculated using Heller-McConnell's equation.[28]

$$a_\beta^H=(B_0+B_2\cos^2\theta_0)\rho_i^\pi, \quad (4)$$

where ρ_i^π denotes the spin density on the carbon atom to which an alkyl group is attached and θ_0 stands for the dihedral angle between the axis of the $2p_z$ orbital on the carbon atom to which the alkyl group is attached and the aliphatic C–H bond of the alkyl group. B_0 and B_2 are empirical parameters and were taken to be 0 and 54 G, respectively, in Eq. 4.

In the ENDOR spectra for the cation radical of **1**, two kinds of hyperfine couplings ($a_4^{CH_2}=1.318$ and 4.719 G) at $-100\,^\circ$C were attributable to the magnetically inequivalent β-methylene protons at the 4-position. Rotation of the β-methylene residue would be tightly rocked at low temperature. Consequently, the dihedral angles, θ_A and θ_B shown in Fig. 4, of the inequivalent β-methylene protons were calculated to be 1.9 and 58.1° for **1**, respectively, assuming that the sum of the two dihedral angles is equal to 60°. The result shows that the dihedral angle between the C_3–C_4 bond and the aromatic ring, θ_γ (Fig. 4), is 28.1°, if the C_3 local symmetry is assumed around the C_4–C_{4a} bond in the heterocyclic ring. The experimental value of the spin density on C_{4a} ($C_{4a'}$) was estimated using Eq. 4 and is given in Table 1. Similarly, the dihedral angles (θ_A, θ_B, and θ_γ) and the spin density on C_{4a} ($C_{4a'}$) of the cation radical of **2** were calculated from the observed β-methylene proton hyperfine splittings. These values are listed in Tables 1 and 2, together with those for the radicals of **3**—**5** obtained previously.[22-24]

As listed in Table 2, the values of dihedral angles (θ_A, θ_B, and θ_γ) observed for the cation radical of **2** are very different from those for the radicals of **4** and **5**. The above change in dihedral angle will be due to the difference of the steric repulsion forces between the β-methylene protons at C_4 and the neighboring proton at $C_{5'}$ in **2** and between the β-methylene protons at C_4 and neighboring methyl protons at $C_{5'}$(C_5) in **4**(**5**). In **2**, γ-, δ-TocH's, and tocol, the steric repulsion forces between the β-methylene protons at C_4 and neighboring protons (at $C_{5'}$ in **2** and C_5 in γ-, δ-TocH's, and tocol) will be similar to each other, and thus the conformation of the heterocyclic ring in **2** is considered to be similar to those of γ-, δ-TocH's, and tocol. In contrast, in **4**, **5**, α-, and β-TocH's, the steric repulsion forces between the β-methylene protons at C_4 and neighboring methyl protons (at $C_{5'}$ in **4** and at C_5 in α-, β-TocH's, and **5**) will be similar to each other, and thus the conformation of the heterocyclic ring in **4** is considered to be similar to those of α-, β-TocH's, and **5**. It is noted that the dihedral angles of the *cation* radical of **4** are similar to those of the *neutral* radical of **5** (Table 2). This result suggests that to use the cation radicals of the model compounds as models for the neutral radicals of TocH's is appropriate.

As shown in Table 2, θ_γ's of **4** and **5** are near to 0° and that of **2** is near to 30°. Thus, the dihedral angles between the 2p-type lone pair on O_1 ($O_{1'}$) and the π

Table 2. Dihedral Angles (θ_A, θ_B, and θ_γ) of Cation Radicals of **1**—**4** and Neutral Radical of **5** Obtained by ENDOR

	θ_A/°	θ_B/°	θ_γ[a]/°
1	1.9	58.1	28.1
2	0.1	59.9	29.9
3[22]	2.4	57.6	27.6
4[22]	28.9	31.1	1.1
5[23]	27.6	32.4	2.4

a) Calculated assuming $\theta_\gamma=|\theta_A-\theta_B|/2$.

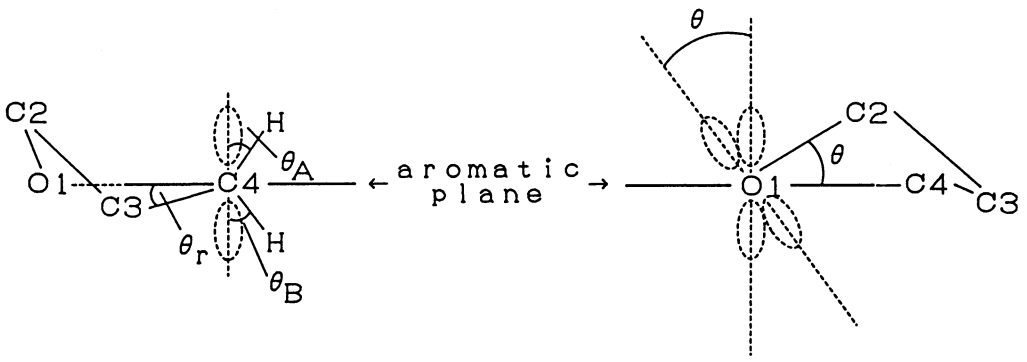

Fig. 4. Conformation of the β-methylene group in radicals of **1**—**5**.

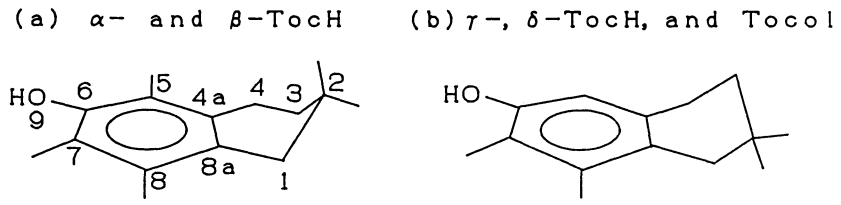

Fig. 5. Schematic sketch of molecular structures of (a) α- and β-TocH's and (b) γ-, δ-TocH's, and tocol.

orbital of the adjacent aromatic carbon (θ's, Fig. 4) of **4** and **5** are larger than that of **2**. Since the conformations of the heterocyclic rings in **4** and **5** are similar to those of α- and β-TocH's, it seems that θ_γ's of α- and β-TocH's are near to 0°. Similarly, θ_γ's of γ-, δ-TocH's, and tocol seem to be near to 30°. Accordingly, it is considered that the heterocyclic rings of TocH's adopt half-chair forms such as those shown in Fig. 5; Fig. 5a shows the schematic sketch of the molecular structures of α- and β-TocH's, and Fig. 5b shows that of γ-, δ-TocH's, and tocol. Then θ's of α- and β-TocH's are larger than those of γ-, δ-TocH's, and tocol.

As shown in Fig. 5, the extent of orbital overlap between the 2p-type lone pair on the ring oxygen and the aromatic π electron system in α- and β-TocH's is smaller than that in γ-, δ-TocH's, and tocol. As described in a previous section, Burton et al. suggested that the larger the orbital overlap, the higher the antioxidant activity.[16–19] If the orbital overlap were a dominant factor governing the antioxidant activity of TocH, k_s's of α- and β-TocH's would be smaller than those of γ-, δ-TocH's, and tocol. Actually, the decreasing order of k_s is as follows: α-TocH>β-TocH≈γ-TocH>δ-TocH>tocol.[7–15] Therefore, at least within the scope of these TocH's, the orbital overlap may not be a dominant factor governing the antioxidant activity.

Since θ's of TocH's with a 5-membered heterocyclic ring are smaller than those with a 6-membered ring,[14] the extent of orbital overlap in the former is larger than that in the latter. k_s increases on going from TocH with a 6-membered heterocyclic ring to the corresponding TocH with a 5-membered heterocyclic ring.[7–15] Accordingly, it is considered that, in those cases, the increase in the extent of orbital overlap between the 2p type lone pair on the ring oxygen and the aromatic π electron system induces the increase in k_s.

In previous papers,[7–15] we showed that the antioxidant activity of TocH in reaction 1 depends on the facility of the charge transfer from TocH to LOO·. This view is consistent with our previous experimental results that the decreasing order of k_s is as follows: α-TocH>β-TocH≈γ-TocH>δ-TocH>tocol. The ratio of k_s of α-TocH to that of tocol is about 9.1.[7–15] The ratio of k_1 of α-TocH to that of 4-methoxytetramethylphenol is about 8.2.[16–19] Thus, it seems that both of the facility of the charge transfer and the extent of orbital overlap contributes to the high antioxidant activity of α-TocH.

In the present work, we have discussed the relation between the antioxidant activity and the molecular structure of vitamin E. This discussion is based on the observed molecular structure of the cation and neutral radicals of model molecules. However, the molecular structure of the neutral molecule is not necessarily the same as that of the cation or neutral radical of the model molecule. Further investigation on the molecular structure of the neutral molecule of vitamin E derivatives (for example, X-ray analysis) is clearly needed.

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