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ENDOR Studies of Vitamin E Model Radical. The Solution Structure and Dynamic Behavior of Chromanyloxyl Ring

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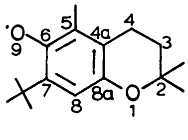
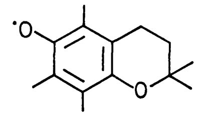
ENDOR technique has been used to elucidate the solution structure and dynamic behavior of chromanyloxyl ring in vitamin E radical. We have measured the ENDOR spectrum of a stable vitamin E model radical (7-*t*-butyl-2,2,5-trimethyl-6-chromanyloxyl (**1**)) in toluene in the temperature range −30°—−100 °C. From detailed analysis of the temperature dependence of hyperfine coupling of β-methylene protons in the heterocyclic ring, the equilibrium conformation of the β-methylene group and activation energy, enthalpy and entropy for the conformational interconversion of the heterocyclic ring have been determined. The observed positive temperature dependence of the hyperfine coupling constants ($a_5^{CH_3}$ and a_8^H) may be explained as a consequence of the restricted motion about the C_{8a}–O_1 bond in the chromanyloxyl ring, based on the McLachlan MO calculation.

Tocopherols (vitamin E) are well known as scavengers of lipid peroxide radicals generated in biological membranes.[1,2] The ESR studies of the tocopheroxyl radicals obtained by the oxidation of tocopherols are of biological interest since they are involved in radical-scavenging reactions of tocopherols.[3–6] In a previous paper, we reported the ESR spectra of α-, β-, γ-, and δ-tocopheroxyl and their model compounds in toluene at 20 °C, and found that all the two β-methylene protons at C-4 position are magnetically equivalent.[4] Further, from the results of the ENDOR measurements of α-tocopheroxyl and its model, it was observed that each of the two β- and γ-methylene protons in the heterocyclic ring shows an equivalent hyperfine coupling.[7] This suggests that the two carbon atoms C-4 and C-3 in the heterocyclic ring are coplanar with the aromatic ring. The result is inconsistent with the X-ray structure of α-tocopherol model,[8,9] which shows that the dihedral angle, θ_γ, between the C_3–C_4 bond and the aromatic ring is 11.1° and 11.9° (see Fig. 3).[10] However, in a previous paper, the clear ENDOR spectra of the α-tocopheroxyl and its model radicals were observed in the comparatively high and narrow temperature range (from −25° to −35 °C for α-tocopheroxyl and from −40° to −55 °C for α-tocopheroxyl model), because these radicals are not so stable and dimerize at low temperature region.[7]



Tocopheroxyl **1**



α-Tocopheroxyl model

In the present work, we have measured the ENDOR spectrum of stable vitamin E model radical (7-*t*-butyl-2,2,5-trimethyl-6-chromanyloxyl, hereafter called tocopheroxyl **1**) in toluene in the temperature range −30°—−100 °C. From detailed analysis of the temperature dependence of the hyperfine coupling constants, the conformation and dynamic behavior of the heterocyclic ring in tocopherol in solution have been discussed.

Experimental

The preparation of tocopherol model compound (7-*t*-butyl-2,2,5-trimethyl-6-chromanol) was reported in a previous paper.[11] The tocopheroxyl **1** was obtained by the PbO_2 oxidation of the above tocopherol model compound in toluene under vacuum.

The ENDOR spectra were recorded by a JES-EDX-1 spectrometer, operated with 80 Hz magnetic field modulation.

Results

The ENDOR spectrum of tocopheroxyl **1** was observed in the temperature range −30° to −100 °C. As shown in Fig. 1 and 2, notable temperature effects

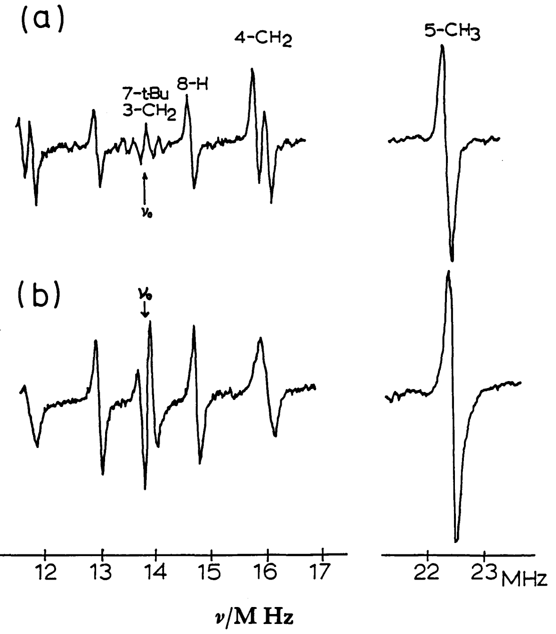


Fig. 1. ENDOR spectra of tocopheroxyl **1** in toluene at (a) −94 °C and (b) −72 °C.

Table 1. Hyperfine couplings (a_i^H) (in gauss) and spin densities (ρ_i) of tocopheroxyl **1** in toluene

	$a_5^{CH_3}$	$a_4^{CH_2}$	a_8^H	$a_3^{CH_2}$	a_7^{tBu}	T/°C
ESR	6.05[a)]	1.55	0.72	——	—	20
ENDOR	6.086[b)]	1.525	0.635	— , 0.073	0.073	−72
	6.074	1.592, 1.448	0.608	0.234, 0.076	0.076	−94
ρ(Exptl.)	0.2250	−0.0376[c)]	−0.0225	——	—	−94
ρ(Calcd.)[d)]	0.2123	−0.0389[c)]	−0.0286	——	—	

a) Experimental errors ±0.04 G. 1G=10^{-4} T. b) Experimental errors ±0.010 G. c) Spin density at C-4a. d) $\beta_{C_{8a}\text{-}O_1} = 1.0\beta$.

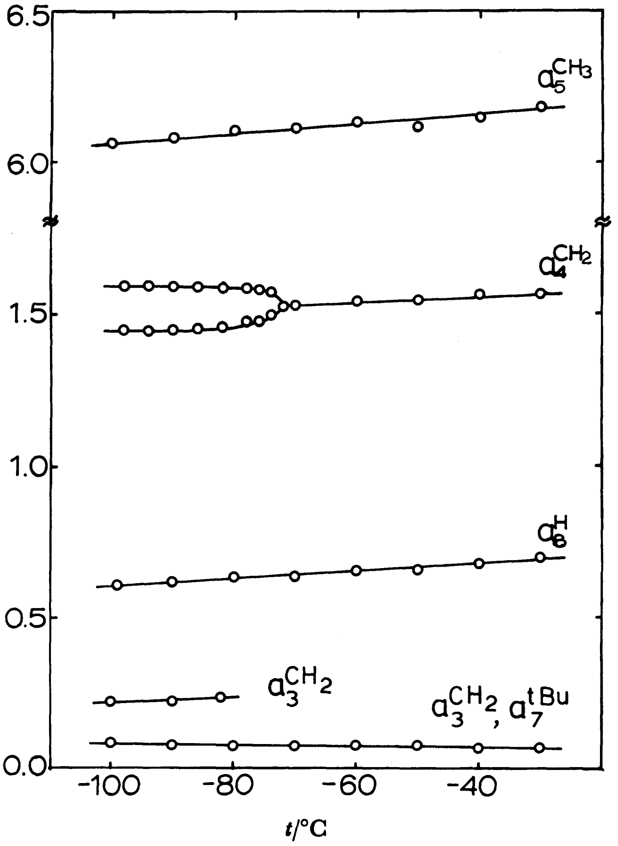


Fig. 2. Temperature dependence of the hyperfine couplings of tocopheroxyl **1** in toluene.

were observed. The ENDOR spectrum at −94 °C (see Fig. 1(a)) clearly shows six different proton hyperfine couplings (6.074, 1.592, 1.448, 0.608, 0.234, and 0.076 G). The larger four hyperfine couplings (6.074, 1.592, 1.448, and 0.608 G) are easily assigned to the protons at C-5, C-4, C-4, and C-8 positions, respectively, taking its ESR hyperfine couplings (see Table 1) into account.[11)] By comparing the ENDOR spectrum of **1** with that of α-tocopheroxyl model,[7)] the smallest hyperfine coupling of 0.076 G, which has never been observed in the ESR spectrum of **1**, will be assigned to the γ-methylene proton hyperfine coupling at C-3 position and/or *t*-butyl proton at C-7 position. The hyperfine coupling of 0.234 G was tentatively assigned to the γ-methylene proton at C-3. The ENDOR spectrum of **1** remains essentially unchanged upon warming from −100° to −80 °C except for small changes of the hyperfine couplings. When the temperature is increased further, the weak absorption due to γ-methylene proton ($a_3^{CH_2}$=0.234 G) has disappeared at −81 °C, and the two lines of alkyl β-protons ($a_4^{CH_2}$) were gradually merged into a single line at −72 °C, as is shown in Fig. 1(b) and Fig. 2. The values of the hyperfine couplings observed at −72 °C are $a_5^{CH_3}$=6.086, $a_4^{CH_2}$=1.525, a_8^H=0.635, and a_7^{tBu} and/or $a_3^{CH_2}$=0.073 G. These values are listed in Table 1, together with the values of the experimental spin densities (ρ_i) calculated from the hyperfine coupling.[4,11)]

Discussion

Conformation of β-Methylene Group in Tocopheroxyl 1. As described in a previous section, in the ENDOR spectrum for the tocopheroxyl **1** in toluene, two signals attributable to the inequivalent splittings ($a_{4A}^{CH_2}$=1.592 G and $a_{4B}^{CH_2}$=1.448 G) of the β-methylene protons were recorded at −94 °C. The inequivalent β-methylene proton splittings have been understood by assuming a tightly rocking model of the methylene group, based on the Heller-McConnell relationship (Eq. 1).[12)] In these cases, the sum of the two dihedral angles (θ_A, θ_B) of the inequivalent β-protons is about $\pi/3$. A similar situation probably exists in the present case.

$$a_4^{CH_2} = \langle Q(\theta)\rangle \rho_{4a}, \qquad Q(\theta) = B_0 + B_2 \cos^2\theta \qquad (1)$$

where B_0 and B_2 are empirical parameters, and θ is the dihedral angle between the axis of the $2p_z$ orbital and the C–H bond of the β-methylene group. B_0 and B_2 were taken to be 0 and 54 G, respectively, in Eq. 1. Consequently, based on the observed β-methylene proton couplings, and assuming the sum of the two dihedral angles of the inequivalent β-protons is $\pi/3$, the values of the dihedral angles, θ_A and θ_B, were calculated to be 27.6° and 32.4° for **1**, respectively. These values show that the dihedral angle, θ_γ, between the C_3–C_4 bond and the aromatic ring is 2.4° (see Fig. 3). From the results of the estimated dihedral angles, a preferable equilibrium conforma-

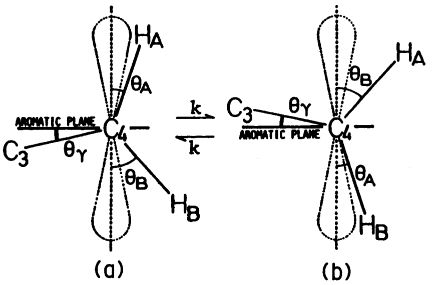


Fig. 3. Possible equilibrium conformations of the β-methylene group in tocopheroxyl **1** in toluene.

tion of C–H and C_3–C_4 bonds was assumed as illustrated in Fig. 3.

On the other hand, as the result of the analysis of the ENDOR spectrum indicates, the two β-methylene protons of **1** show an equivalent hyperfine splitting at higher temperatures. Therefore, the spin density (ρ_{4a}) at the C-4a position was tentatively calculated from $a_4^{CH_2}=1.525$ G at −72 °C, assuming $\theta=30°$ for two β-methylene protons in Eq. 1.[7] Using this spin density (ρ_{4a}), the splittings of $a_{4A}^{CH_2}=1.592$ and $a_{4B}^{CH_2}=1.448$ G are found to correspond respectively to angles of $\theta_A=27.8°$ and $\theta_B=32.5°$, which add up very nearly to $\pi/3$. These values (θ_A and θ_B) are very similar to those ($\theta_A=27.6°$ and $\theta_B=32.4°$) calculated above assuming $\theta_A+\theta_B=\pi/3$.

Very recently, we have succeeded in measuring the ENDOR spectrum of cation radical obtained by the oxidation of benzo[1,2-*b*:4,5-*b*′]dipyran, which has heterocyclic ring common to that of tocopherols, in $AlCl_3$–CH_2Cl_2 mixtures.[13] In the cation radical, it was found that the two β-methylene protons in the heterocyclic ring become magnetically inequivalent at low temperature, showing two kinds of hyperfine couplings. The observed dihedral angles are $\theta_A=28.9°$ and $\theta_B=31.1°$, and θ_γ is calculated to be 1.1°. These values are very similar to those of the tocopheroxyl **1**, because the steric repulsion force between the β-methylene protons at C-4 and the neighboring methyl protons is equivalent in **1** and the above cation radical. On the other hand, the values of the dihedral angle, θ_γ, (2.4° in **1** and 1.1° in cation radical) in solution are different from those ($\theta_\gamma=11.1°$ and 11.9° in α-tocopherol model[8,9]) in the crystal. The result suggests that the crystal and solution conformations of the heterocyclic ring attached to the aromatic ring in α-tocopherol are different.

Dynamic Behavior of Heterocyclic Ring. In the ENDOR spectra for the **1** in toluene, two signals due to the inequivalent splittings of the β-methylene protons were recorded at −100 °C. The β-methylene proton hyperfine coupling constants remain almost unchanged upon warming from −100° to −90 °C. They collapsed into a single line as the temperature was raised to −72 °C (see Fig. 1 and 2). In such a case, we can calculate the activation energy (E_{act}) for the conformational interconversion of the heterocyclic ring, applying the Gutowsky-Holm equation.[14–16]

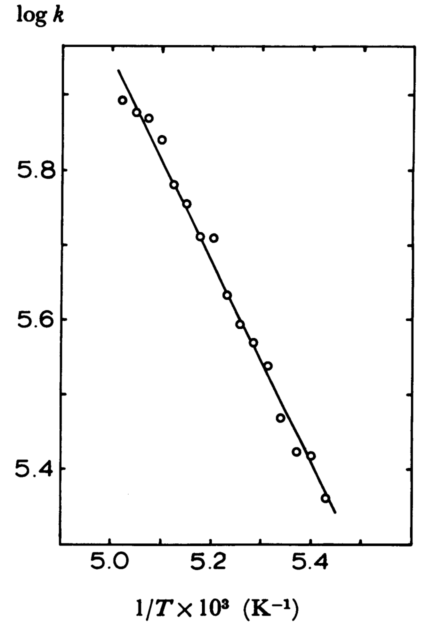


Fig. 4. The Arrhenius plot of the rate constant for the conformational interconversion process of the heterocyclic ring of tocopheroxyl **1** in toluene.

In a two-jump dynamic process, in which two nuclear spins of $I=1/2$ exchange their different hyperfine couplings, the observed separation $\Delta\omega$ of the two ENDOR lines is given by

$$\Delta\omega = (\Delta\omega_{max}^2 - 2k^2)^{1/2} \tag{2}$$

where $\Delta\omega_{max}$ is $\gamma_e|(a_{4A}^{CH_2}-a_{4B}^{CH_2})/2|$, and $a_{4A}^{CH_2}$ and $a_{4B}^{CH_2}$ (measured in Gauss) are the hyperfine coupling constants for the two β-methylene protons observed at −94 °C, and $k=1/\tau$. Here, τ is the mean lifetime of each conformation. We obtained k values as a function of the temperature between −89° and −74 °C.

Dynamic processes of this type can be described by the well-established theories of activated chemical reactions. Empirically the temperature dependence of the rate constant k for such an interconversion is given by the Arrhenius equation.

$$k = A_0 \exp(-E_{act}/RT) \tag{3}$$

where k is the jump rate of Eq. 2. Figure 4 shows the Arrhenius plots of log k *vs.* 1/T for tocopheroxyl **1** from which E_{act} and A_0 were determined. The obtained values are $E_{act}=25.9\pm1.7$ kJ mol^{-1} and $A_0=5.6\times10^{12\pm0.4}$ s^{-1}. It may be seen that the activation energy, E_{act}, obtained in the present work for **1** is similar to that (29.9 kJ mol^{-1}) reported for such an interconversion in 1,2,3,6,7,8-hexahydropyrene anion radical.[16] From the Eyring theory of the activated

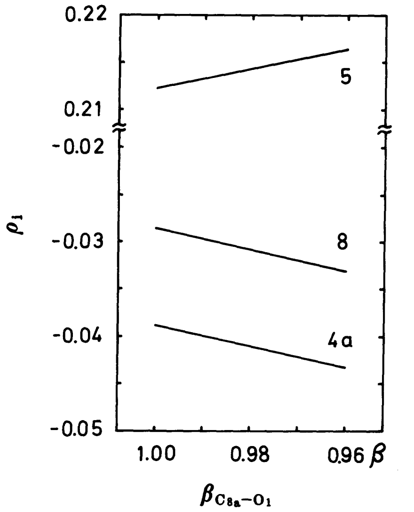


Fig. 5. The variation of the McLachlan spin densities (ρ_i) in tocopheroxyl **1** as a function of the resonance integral $\beta_{C_{8a}\text{-}O_1}(=\beta\cos\theta_{CO})$ between O_1 and C_{8a} atoms.

transition state, on the other hand, one obtains

$$k = (kT/h)\cdot\exp(\Delta S^{*}/R)\cdot\exp(-\Delta H^{*}/RT) \qquad (4)$$

where ΔH^{*} is the activation enthalpy and ΔS^{*} is the activation entropy.[15] The thermodynamic quantities ΔH^{*} and ΔS^{*} were deduced from plots of ln k/T *vs.* 1/T which yielded straight lines according to Eq. 4. The obtained values are $\Delta H^{*}=24.2\pm1.7$ kJ mol^{-1} and $\Delta S^{*}=-5.4\pm3.4$ J mol^{-1} K^{-1}.

Temperature Dependence of the Proton Hyperfine Couplings of the Tocopheroxyl 1. As is shown in Fig. 2, the proton hyperfine couplings, $a_5^{CH_3}$, $a_4^{CH_2}$, and a_8^{H}, of the tocopheroxyl radical **1** are temperature dependent. These effects are considered to be the restricted motion about the C_{8a}–O_1 bond which causes changes in the unpaired spin density (ρ_i) throughout the radical.[17,18] McLachlan MO calculations successfully predict the changes in the above proton hyperfine couplings from −100° to −30 °C. Following Stone and Carrington,[17] we have used the resonance integral, $\beta_{C_{8a}\text{-}O_1}=\beta\cos\theta_{CO}$, as an adjustable parameter to account quantitatively for the changes in the average dihedral angle (θ_{CO}) of the C_{8a} carbon $2p_z$ orbital with respect to the C_{8a}-O_1-C_2 plane. The other Coulomb and resonance integrals employed in the calculations are as follows: $\alpha_{O_1}=\alpha+2.0\beta$, $\alpha_{O_9}=\alpha+1.3\beta$, $\alpha_{C_5}=\alpha-0.1\beta$, $\alpha_{C_{4a}}=\alpha-0.06\beta$, $\alpha_{C_7}=\alpha-0.04\beta$, $\beta_{C_6\text{-}O_9}=1.5\beta$, and $\lambda=1.2$, where α and β are, respectively, the standard carbon Coulomb and resonance integrals. These parameters are the same as those used previously for the α-tocopheroxyl radical.[7]

The McLachlan spin densities (ρ_5, ρ_{4a}, and ρ_8) calculated for the tocopheroxyl **1** are plotted against $\beta_{C_{8a}\text{-}O_1}$ in Fig. 5. The result indicates that the absolute values of ρ_i increase linearly with increasing the value of $\beta_{C_{8a}\text{-}O_1}$. The observed temperature dependence of the hyperfine couplings may be reconciled with the variation of calculated hyperfine couplings since $\beta_{C_{8a}\text{-}O_1}$ is expected to decrease with increasing temperature due to increased torsional amplitudes.[17,18] For instance, the change of $\beta_{C_{8a}\text{-}O_1}$ required to explain the change (0.118 G) of the methyl hyperfine couplings ($a_5^{CH_3}$) observed between −100° and −30 °C is 0.04 β. Recently, the result of the X-ray analysis of α-tocopherol model was reported by Burton *et al.*; an X-ray structure for α-tocopherol model gives the dihedral angle $\theta_{CO}=$ 18.5° and 15.2°.[8–10] Therefore, if we assume that the tocopheroxyl **1** has $\theta_{CO}=16.9°$ at −100 °C, the average dihedral angle θ_{CO} at −30 °C is calculated to be 23.5°.

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