Synthesis and Stopped-Flow Investigation of Antioxidant Activity of Tocopherols. Finding of New Tocopherol Derivatives Having the Highest Antioxidant Activity among Phenolic Antioxidants

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Several new tocopherol derivatives have been synthesized, and second-order rate constants, k_s, for H atom abstraction by substituted phenoxyl radicals (PhO•) have been measured spectrophotometrically by the stopped-flow method as a model reaction of tocopherols with unstable free radicals (LOO•, LO•, and HO•) in biological systems. Vitamin K_1-chromanol (1) and K_1-chromenol (2) were found to be 6.9 and 4.5 times more active than α-tocopherol, which has the highest antioxidant activity among natural tocopherols. Thus, these compounds have the highest antioxidant activity among phenolic antioxidants including natural tocopherols, tocopherol derivatives, and related phenols in solution. Two new tocopherol derivatives with five-membered heterocyclic rings were also found to be 1.8 and 1.1 times more active than the α-tocopherol. However, both the ubichromanol (3) and ubichromenol (4) having two methoxy substituents at the aromatic ring were only ca. 10% as reactive as α-tocopherol. From detailed analysis of the temperature dependence of k_s values in the tocopherols, the activation energy, E_{act}, for the reaction has been determined. Half-peak oxidation potentials, $E_{p/2}$, for tocopherol compounds have also been measured with cyclic voltammetry.

Introduction

It is well known that tocopherols (vitamin E) are localized in biomembranes and function as efficient inhibitors of lipid peroxidation. The antioxidant properties of the tocopherols have been ascribed to the initial oxidation by a oxyradical of the phenolic hydroxyl group, producing a tocopheroxyl radical, which in turn combines with another oxyradical. Burton et al. have measured the second-order rate constants, k_1, for the reactions of the α-, β-, γ-, and δ-tocopherols and other related phenols with poly(styrylperoxyl)peroxyl radicals by the inhibited autoxidation of styrene method (reaction 1).[1-3] The results

$$\mathrm{LOO^{\bullet}} + \mathrm{TocH} \xrightarrow{k_1} \mathrm{LOOH} + \mathrm{Toc^{\bullet}} \qquad (1)$$

showed that α-tocopherol and α-tocopherol model compound (2,2,5,7,8-pentamethyl-6-hydroxychroman) were the best phenolic antioxidants known at that time and, in particular, that they were very much better antioxidants than the major commercial antioxidants, such as 2,6-di-*tert*-butyl-4-alkylphenols. Further, they found that a better tocopherol compound is 2,3-dihydro-5-hydroxy-2,2,4,6,7-pentamethylbenzofuran (tocopherol **9**, see Figure 1), which has a five-membered heterocyclic ring instead of a six-membered one in α-tocopherol. They reported that the reaction rate of tocopherol **9** is 1.8 times higher than that of α-tocopherol. The high reactivity of tocopherol **9** has been attributed to stereoelectric factors relating to the orientation of the p-type lone pair on the oxygen in position 1 with respect to the aromatic ring.

Recently, we have determined spectrophotometrically the rates of reaction of α-, β-, γ-, and δ-tocopherols with stable substituted phenoxyl radical (2,6-di-*tert*-butyl-4-(4-methoxyphenyl)phenoxyl (PhO•) (see Figure 1)) in ethanol solution by using the stopped-flow technique, as a model reaction of tocopherols with unstable free radicals, such as lipid peroxyl (LOO•), alkoxyl (LO•), and hydroxyl (HO•) radicals, in biological systems.[4] The second-order

$$\mathrm{PhO^{\bullet}} + \mathrm{TocH} \xrightarrow{k_s} \mathrm{PhOH} + \mathrm{Toc^{\bullet}} \qquad (2)$$

rate constants, k_s, obtained are 5.12×10^3 (α-Toc), 2.24×10^3 (β-Toc), 2.42×10^3 (γ-Toc), and 1.00×10^3 (δ-Toc) M^{-1} s^{-1} in ethanol at 25.0 °C. The relative rates (α:β:γ:δ = 100:44:47:20) agree well with those obtained from studies of the reactivities of tocopherols toward poly(styrylperoxyl)peroxyl radicals (100:41:44:14) by the inhibited autoxidation of the styrene method. The result suggests that, while the absolute rates are considerably different from each other, the relative reactivities, that is, the relative antioxidant activities, of tocopherols in homogeneous solution do not depend on the kinds of oxyradicals (substituted phenoxyl and peroxyl radicals) used. Further, we prepared many tocopherol compounds and studied the structure–activity relationship in antioxidant action of tocopherols.[5-7] However, tocopherol derivatives with higher antioxidant activity than tocopherol **9** or its derivatives with five-membered heterocyclic rings have not been obtained.

The vitamin K_1-chromanol (1) and K_1-chromenol (2) and ubichromanol (3) and ubichromenol (4) have a structure similar to that of vitamin E chromanol (tocopherol), and, as we reported in our previous papers, produce corresponding chromanoxyl and chromenoxyl radicals.[8,9] Therefore, we can expect that these chromanols and chromenols function as efficient inhibitors of lipid peroxidation, but there is no report regarding their absolute antioxidant effectiveness in solution.

In the present work, we have synthesized several new tocopherol derivatives **1–8**, which include vitamin K_1-chromanol (1) and K_1-chromenol (2), and ubichromanol (3) and ubichromenol (4). We have measured the reaction rates k_s of tocopherol derivatives with substituted phenoxyl in ethanol solution, using stopped-flow spectrophotometer. From detailed analysis of the temperature dependence of k_s values, activation energy, E_{act}, for the reaction between tocopherols and substituted phenoxyl radical has been

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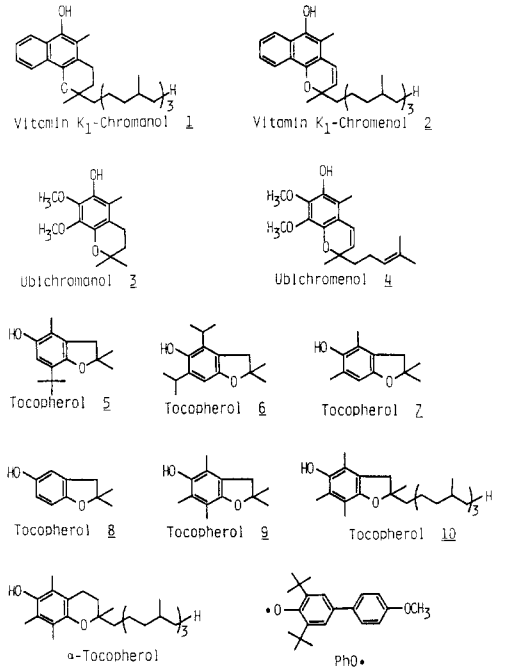

Figure 1. Molecular structures of tocopherols and substituted phenoxyl radical (PhO•).

Table I. Second-Order Rate Constants (k_s) and Relative Rate Constants for Oxidation of Tocopherols by Substituted Phenoxyl in Ethanol at 25.0 °C and Half-Peak Oxidation Potentials ($E_{p/2}$) for Tocopherols

tocopherols	$10^{-3}k_s$,[a] M^{-1} s^{-1}	k_s(Toc)/k_s(α-Toc)	$E_{p/2}$ vs SCE,[b] mV
K_1-chromanol (1)	35.4	6.91	780
K_1-chromenol (2)	24.8	4.45	750
ubichromanol (3)	0.42	0.08	870
ubichromenol (4)	0.56	0.11	920
tocopherol 5	9.10	1.77	900
tocopherol 6	5.40	1.05	870
tocopherol 7	3.49	0.68	850
tocopherol 8	0.88	0.17	1030
tocopherol 9	6.99	1.36	810
α-tocopherol	5.12	1.00	860

[a] Experimental errors <±5%. [b] Experimental errors <±20 mV.

determined. Half-peak oxidation potentials, $E_{p/2}$, for tocopherol compounds have also been measured, by using cyclic voltammetry technique.

Results

The oxidation rates of tocopherols by substituted phenoxyl were studied spectrophotometrically by the stopped-flow technique in the presence of excess tocopherol in ethanol. The details of these experiments are reported in a previous paper.[4] The rate was measured by following the decrease in absorbance at 376 and 580 nm of substituted phenoxyl radical. The values of the second-order rate constants, k_s, at 25.0 °C are listed in Table I, together with those of α-tocopherol and tocopherol **9**.

We obtained k_s values as a function of the temperature between 10.0 and 35.0 °C in ethanol. The k_s values increased depending on the temperature. Empirically the temperature dependence of the rate constants k_s for such a reaction is given by the Arrhenius equation:

$$k_s = A_0 \exp(-E_{act}/kT) \quad (3)$$

where k_s is the reaction rate of eq 2. From the Arrhenius plots of ln k_s vs $1/T$ for tocopherols **1–9**, E_{act} and A_0 values were determined. The values obtained are listed in Table II. From the Eyring theory of the activated transition state, one obtains

$$k_s = (kT/h) \exp(\Delta S^{\ddagger}/R) \exp(-\Delta H^{\ddagger}/RT) \quad (4)$$

where $\Delta H^{\ddagger}$ is the activation enthalpy and $\Delta S^{\ddagger}$ is the activation entropy. The thermodynamic quantities $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were deduced from plots of ln k_s/T vs $1/T$, which yielded straight lines according eq 4.

Table II. Summary of Activation Parameters for Reactions of Tocopherols with Substituted Phenoxyl in Ethanol

tocopherols	E_{act},[a] kJ mol^{-1}	$10^{-7}A_0$, M^{-1} s^{-1}
K_1-chromanol (1)	14.3[b]	1.04
K_1-chromenol (2)	17.0	2.35
ubichromanol (3)	27.7	3.09
ubichromenol (4)	23.9	0.77
tocopherol 5	17.5	1.05
tocopherol 6	17.2	0.55
tocopherol 7	17.3	0.37
tocopherol 8	28.8	10.1
tocopherol 9	15.8	0.40
α-tocopherol	18.7	1.01

[a] For each tocopherol, experimental error in E_{act} was <±5%. For a plot of ln k_s vs $1/T$, correlation coefficient was <−0.997. [b] 3.42 kcal mol^{-1}.

The half-peak oxidation potentials, $E_{p/2}$, for tocopherols **1–9** have been measured in acetonitrile by using cyclic voltammetry. The details of the experiment are reported in a previous paper.[7] The observed results are listed in Table I.

Discussion

The results listed in Table I demonstrate that the vitamin K_1-chromanol (**1**) and K_1-chromenol (**2**) are 6.9 and 4.5 times as reactive as α-tocopherol, which has the highest antioxidant activity yet reported among natural tocopherols. Two new tocopherols **5** and **6** with a five-membered heterocyclic ring were also found to be 1.8 and 1.1 times more active than the α-tocopherol, respectively. However, tocopherols **7** and **8** showed less reactivity than α-tocopherol. Further, both the ubichromanol (**3**) and ubichromenol (**4**) having two methoxy substituents at the aromatic ring are only ca. 10% as reactive as α-tocopherol.

As reported in previous papers,[5,7] absolute reactivities of tocopherols to PhO• increase as the total electron-donating capacity of the alkyl substituents at the aromatic ring increases. For the tocopherol derivatives log k_s was found to roughly correlate with the sum of the Hammett's σ constants (Σσ) or the Brown's σ^+ constants (Σσ^+), although the two cases could not be distinguished. Further, the log of the second-order rate constants, k_s, obtained for the tocopherols was found to correlate with their half-peak oxidation potentials, $E_{p/2}$; the tocopherols that have smaller $E_{p/2}$ values show higher reactivities.[7]

As described above, both the ubichromanol (**3**) and ubichromenol (**4**) having two methoxy substituents at the aromatic ring are only ca. 10% as reactive as α-tocopherol. In fact, as listed in Table II, the E_{act} values of ubichromanol (**3**) (27.7 kJ mol^{-1}) and ubichromenol (**4**) (23.9 kJ mol^{-1}) are larger than that of α-tocopherol (18.7 kJ mol^{-1}). The methoxy group can be a powerful electron-donating group (σ = −0.268), if it is ortho or para to the hydroxy group and has the correct orientation for conjugative interaction of its oxygen lone pair with the aromatic π-orbital. Otherwise, it is electron-withdrawing (σ = +0.115). The low reactivities observed for **3** and **4** suggest that the methoxy group acts as electron-withdrawing group in these compounds.

It was observed that tocopherol **5** (7-*tert*-butyl-2,3-dihydro-5-hydroxy-2,2,4-trimethylbenzofuran) having a

five-membered heterocyclic ring is 1.8 times as reactive as α-tocopherol in ethanol solution (see Table I). Further, the reactivity of tocopherol **5** is 1.3 times higher than that of tocopherol **9**, which has three electron-donating methyl substituents at the aromatic ring. For instance, the $\Sigma\sigma$ value (−0.270) of tocopherol **5** is larger than that (−0.409) of tocopherol **9**. However, the k_s value of the former is larger than that of the latter. The electronic and steric interactions between the bulky *tert*-butyl group at the C-7 position and the oxygen in position 1 in tocopherol **5** may increase the extent of orbital overlap between the 2p type lone pair on the para oxygen atom and the aromatic π electron system, and, thus, induce the increase in the second-order rate constants.[1-3,10]

The vitamin K_1-chromanol (**1**) and K_1-chromenol (**2**) were found to be 6.9 and 4.5 times more active than the α-tocopherol; that is, the vitamin K_1-chromanol (**1**) and K_1-chromenol (**2**) have the highest antioxidant activity among phenolic antioxidants including natural tocopherols, tocopherol derivatives, and related phenols. In fact, the E_{act} values of K_1-chromanol (**1**) and K_1-chromenol (**2**) are smaller than that of α-tocopherol (see Table II). Further, as expected from the larger π-electron system in K_1-chromanol (**1**) and K_1-chromenol (**2**) than that in α-tocopherol, the half-peak oxidation potentials of **1** and **2** are smaller than that of α-tocopherol.

The importance of vitamin K in a variety of biological processes such as blood coagulation, oxidative phosphorylation, and electron transport has been well recognized in recent years.[11,12] The photolysis of vitamin K_1 in ethanol solution under anaerobic conditions has been investigated, and the primary product was observed to be its chromenol.[13,14] The chromanol derivative of vitamin K_1 was identified as product of the enzymatic reduction of vitamin K_1.[12] Wilson et al. have studied the tetraphenylporphin-sensitized photooxidation of vitamin K chromanol and chromenol derivatives.[15] It was observed that tocopherols **5**, **6**, and **9** with a five-membered heterocyclic ring are 1.1–1.8 times as reactive as α-tocopherol in homogeneous solution. However, these tocopherols have no phytyl side chain, $C_{16}H_{33}$, which is necessary for membrane penetration. On the other hand, both vitamin K_1-chromanol and K_1-chromenol have higher antioxidant activity than α-tocopherol and have a phytyl side chain (see Figure 1). Therefore, these compounds may function as better antioxidants in vivo than α-tocopherol. Recently, Burton et al. have found a tocopherol derivative **10** (see Figure 1) having 1.5–1.9 times higher biological activity than α-tocopherol.[16] Here, the tocopherol **10** is a derivative of tocopherol **9** in which one of the methyl groups at the 2-position has been replaced by a phytyl group, $C_{16}H_{33}$. It will be interesting to study the biological activity of vitamin K_1-chromanol (**1**) and K_1-chromenol (**2**), in detail.

Experimental Section

Materials. Ubiquinones Q_1 and Q_2 were kindly supplied from Takeda Chemical Industries Ltd. The vitamin K_1-chromanol (**1**) and K_1-chromenol (**2**) were prepared according to the method of Fujisawa et al.[13] and Wilson et al.,[15] respectively. The synthesis of the ubichromanol (**3**) has been reported in a previous paper.[9] The ubichromenol (**4**) was prepared according to the method of Imada et al.[17] 2,3-Dihydro-5-hydroxy-2,2,4,6-tetramethylbenzofuran (tocopherol **7**) and 2,3-dihydro-5-hydroxy-2,2-dimethylbenzofuran (tocopherol **8**) were prepared according to the method of Nilsson et al.[18] 2,3-Dihydro-5-hydroxy-2,2,4,6,7-pentamethylbenzofuran (tocopherol **9**) was prepared according to the method of Burton et al.[3] 7-*tert*-Butyl-2,3-dihydro-5-hydroxy-2,2,4-trimethylbenzofuran (tocopherol **5**) and 2,3-dihydro-5-hydroxy-2,2-dimethyl-4,6-diisopropylbenzofuran (tocopherol **6**) were synthesized by condensation of 2-methyl-2-propen-1-ol to the corresponding alkylhydroquinone, according to a procedure similar to that used by Nilsson et al. to prepare tocopherols **7** and **8**.[18]

The 2,6-di-*tert*-butyl-4-(4-methoxyphenyl)phenoxyl (abbreviated to substituted phenoxyl (PhO•)) was prepared according to the method of Müller et al.[19] Radical concentration of substituted phenoxyl was obtained from the results of the paramagnetic susceptibility measurements at 20 °C. The value was 100% for substituted phenoxyl, assuming the Curie law.

Vitamin K_1-Chromanol (1). Compound **1** was prepared by refluxing the vitamin K_1 in dioxane with stannous chloride and concentrated HCl, according to the method of Fujisawa et al.:[13] viscous oil; ^{1}H NMR ($CDCl_3$, 270 MHz)[20,21] δ 0.82–1.78 (m, 21 H, 1′–12′-H), 0.85 (s, 6 H, 4′a- and 8′a-CH_3), 0.88 (s, 6 H, 12′a- and 13′-CH_3), 1.32 (s, 3 H, 2a-CH_3), 1.90 (octet, 2 H, J = 6.8 Hz, 3-CH_2), 2.30 (s, 3 H, 5-CH_3), 2.74 (t, 2 H, J = 6.8 Hz, 4-CH_2), 4.62 (s, 1 H, 6-OH), 7.43 (quintet, 2 H, J = 8.1 Hz, aromatic H), 7.98 (d, 1 H, J = 8.1 Hz, aromatic H), 8.17 (d, 1 H, J = 8.1 Hz, aromatic H); UV spectrum λ_{max} = 248 nm (log ε = 4.51), 324 nm (log ε = 3.68), 338 nm (log ε = 3.68 in ethanol). Anal. Calcd for $C_{31}H_{48}O_2$: C, 82.24; H, 10.69. Found: C, 81.81; H, 10.66.

Vitamin K_1-Chromenol (2). Compound **2** was synthesized by refluxing the vitamin K_1 in pyridine under nitrogen, according to a procedure similar to that by Wilson et al. to prepare vitamin K_3-chromenol:[15] viscous oil; ^{1}H NMR ($CDCl_3$, 270 MHz)[20,21] δ 0.82–1.76 (m, 21 H, 1′–12′-H), 0.86 (s, 6 H, 4′a- and 8′a-CH_3), 0.89 (s, 6 H, 12′a- and 13′-CH_3), 1.42 (s, 3 H, 2a-CH_3), 2.36 (s, 3 H, 5-CH_3), 4.72 (s, 1 H, 6-OH), 5.67 (d, 1 H, J = 7.6 Hz, 3- or 4-H), 6.64 (d, 1 H, J = 7.6 Hz, 3- or 4-H), 7.43 (s, 2 H, aromatic H), 7.98 (s, 1 H, aromatic H), 8.16 (s, 1 H, aromatic H); UV spectrum λ_{max} = 228 nm (log ε = 4.74), 272 nm (log ε = 4.64), 281 nm (log ε = 4.67 in ethanol). Anal. Calcd for $C_{31}H_{46}O_2$: C, 82.61; H, 10.29. Found: C, 82.33; H, 10.15.

Ubichromenol (4). Compound **4** was synthesized by refluxing the coenzyme Q_2 in trimethylamine under nitrogen, according to a procedure similar to that used by Imada et al. to prepare ubichromenol (Q_7):[17] viscous oil; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.58 (s, 3 H, 4′a- or 4′b-CH_3), 1.60–1.85 (m, 4 H, 1′- and 2′-CH_2), 1.67 (s, 3 H, 4′a- or 4′b-CH_3), 2.16 (s, 3 H, 5-CH_3), 3.88 (s, 3 H, 7- or 8-OCH_3), 3.93 (s, 3 H, 7- or 8-OCH_3), 5.11 (t, 1 H, J = 5.9 Hz, 3′-H), 5.45 (s, 1 H, 6-OH), 5.59 (d, 1 H, J = 10 Hz, 3- or 4-H), 6.52 (d, 1 H, J = 10 Hz, 3- or 4-H); UV spectrum λ_{max} = 232 nm (log ε = 4.29), 275 nm (log ε = 3.94), 283 nm (log ε = 3.93), 332 nm (log ε = 3.47 in ethanol). Anal. Calcd for $C_{19}H_{26}O_4$: C, 71.62; H, 8.23. Found: C, 72.02; H, 8.48.

7-*tert*-Butyl-2,3-dihydro-5-hydroxy-2,2,4-trimethylbenzofuran (Tocopherol 5). Tocopherol **5** was prepared by the reaction of 2-*tert*-butyl-5-methylhydroquinone with 2-methyl-2-propen-1-ol in anhydrous formic acid in the presence of H_2SO_4, according to the method of Nilsson et al.:[18,22] mp 174.0–175.0 °C;

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(22) Condensation of 2-*tert*-butyl-5-methylhydroquinone with 2-methyl-2-propen-1-ol gave only 7-*tert*-butyl-2,3-dihydro-5-hydroxy-2,2,4-trimethylbenzofuran (tocopherol **5**), and the possible isomer, that is, 4-*tert*-butyl-2,3-dihydro-5-hydroxy-2,2,7-trimethylbenzofuran (tocopherol **5′**) has not been obtained in the reaction. The structure of tocopherol **5** was supported by the ESR hyperfine splittings ($a_3^{CH_2}$ = 0.30 G (1 H), $a_4^{CH_3}$ = 5.27 G (3 H), a_6^{H} = 5.60 G (1 H)) of tocopheroxyl radical obtained by the PbO_2 oxidation of **5** in toluene.[3]

^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.30 (s, 9 H, 7-t-Bu), 1.44 (s, 6 H, 2-CH_3), 2.07 (s, 3 H, 4-CH_3), 2.85 (s, 2 H, 3-CH_2), 4.13 (s, 1 H, 5-OH), 6.46 (s, 1 H, 6-H); UV spectrum λ_{max} = 299 nm (log ε = 3.66 in ethanol). Anal. Calcd for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found: C, 76.95; H, 9.63.

2,3-Dihydro-5-hydroxy-2,2-dimethyl-4,6-diisopropylbenzofuran (Tocopherol 6). Tocopherol 6 was prepared by the reaction of 2,6-diisopropylhydroquinone with 2-methyl-2-propen-1-ol in anhydrous formic acid in the presence of H_2SO_4, according to the method of Nilsson et al.:[18] mp 96.5–97.0 °C; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.20 (d, 6 H, J = 6.5 Hz, 4- or 6-CH-(CH_3)$_2$), 1.25 (d, 6 H, J = 6.5 Hz, 4- or 6-CH(CH_3)$_2$), 1.43 (s, 6 H, 2-CH_3), 2.98 (s, 2 H, 3-CH_2), 3.05 (sep, 2 H, J = 6.5 Hz, 4- and 6-CH(CH_3)$_2$), 4.17 (s, 1 H, 5-OH), 6.34 (s, 1 H, 7-H); UV spectrum λ_{max} = 298 nm (log ε = 3.69 in ethanol). Anal. Calcd for $C_{16}H_{24}O_2$: C, 77.38; H, 9.74. Found: C, 77.49; H, 9.73.

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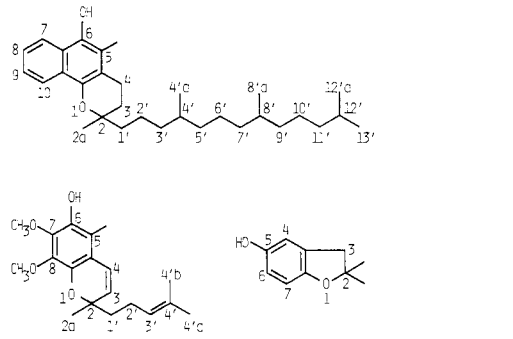

Registry No. 1, 4208-84-8; 2, 19274-66-9; 3, 3626-85-5; 4, 3625-63-6; 5, 118111-99-2; 6, 118112-00-8; 7, 118112-01-9; 8, 6956-76-9; 9, 84574-05-0; PhO•, 6257-34-7; α-tocopherol, 59-02-9; vitamin K_1, 84-80-0; coenzyme Q_2, 606-06-4; 2-*tert*-butyl-5-methylhydroquinone, 2349-76-0; 2-methyl-2-propen-2-ol, 513-42-8; 2,6-diisopropylhydroquinone, 1988-10-9.

Chiral Effects on the ^{13}C Resonances of α-Tocopherol and Related Compounds. A Novel Illustration of Newman's "Rule of Six"[1]

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The 100-MHz ^{13}C NMR spectrum of (2*R*,4′*R*,8′*R*)-α-tocopherol (natural vitamin E) has been completely assigned with the aid of a number of selectively deuteriated (2*R*,4′*R*,8′*R*)-α-tocopherols. The ^{13}C NMR spectrum of (2*RS*,4′*RS*,8′*RS*)-α-tocopherol (all-racemic, synthetic vitamin E) has also been measured. Many of the individual carbons in this all-racemic mixture of eight α-tocopherol stereoisomers give more than one resonance with eight of the carbons (2-CH_3, 2′, 3′, 4′, 4′-CH_3, 5′, 8′, and 9′) giving the maximum number of four resonances from each of the four enantiomeric pairs; these resonances have also been assigned. The structurally related 5′-hydroxy-2-(4′,8′,12′-trimethyltridecyl)-2,4,6,7-tetramethyl-2,3-dihydrobenzofuran (HTDBF) has been synthesized for the first time in the 2*R*,4′*R*,8′*R* and 2*S*,4′*R*,8′*R* configurations and their ^{13}C resonances have been assigned. In its all-racemic form this compound also shows up to four resonances from a single carbon. Related observations have been made with phytol and isophytol. A careful examination of these chirally induced chemical shift differences for the individual carbon atoms, Δ, reveals a bond-alternation effect with maxima at a separation of one, three, and five bonds from the closest chiral center and with the maximum at a five-bond separation being greater than that at a three-bond separation. For example, the total Δ, $\sum\Delta$, averaged over the number of carbon aroms, n, which are separated from the nearest chiral center by the same number of bonds has been conservatively calculated for α-tocopherol to be 54, 106, 43, 66, 40, and 75 ppb at separations from the closest chiral center of zero, one, two, three, four, and five bonds, respectively. For HTDBF the corresponding $\sum\Delta/n$ values are 45, 67, 12, 0, 0, and 20 ppb. We attribute these remarkable long-range (five-bond) effects to differences in 1,6 nonbonded repulsions for different enantiomeric pairs and consider that it provides direct evidence for the operation of Newman's classic "rule of six".

Nuclear magnetic resonance spectrscopy provides a unique and sensitive tool for studying the propagation down a hydrocarbon chain of the effect of a change in the chirality of an asymmetric center. This is a subject of considerable scientific interest and of practical utility. Thus, we required a simple and unequivocal method for identifying the chirality of α-tocopherol (vitamin E) for our in vivo studies on the uptake, transport, and elimination of the various stereoisomers of (specifically deuteriated) α-tocopherol in animals[2,3] and in man.[4,5] Principally, we needed a means to distinguish between the natural, 2*R*,4′*R*,8′*R* (*RRR*) stereoisomer and the synthetic, all-racemic compound (see Scheme I). We also needed to be able to distinguish between natural, *trans*-(7*R*,11*R*)-phytol and synthetic, *all-rac*-phytol (see Scheme I) since this compound is frequently employed in the synthesis of α-tocopherol. Literature data suggested that ^{13}C NMR would prove suitable for both tasks. Thus, Bremser and

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