Autoxidation of Biological Molecules. 1. The Antioxidant Activity of Vitamin E and Related Chain-Breaking Phenolic Antioxidants in Vitro[1]

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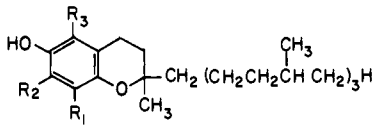
Abstract: The rate constants, k_5, for abstraction by peroxyl radicals of the phenolic hydrogens from a number of phenols have been measured at 30 °C. Values of k_5 for α-, β-, γ-, and δ-tocopherols are 23.5, 16.6, 15.9, and 6.5×10^5 M^{-1} s^{-1}, respectively. Like most other phenols these tocopherols react with two peroxyls per molecule and the reaction with peroxyls exhibits a substantial deuterium kinetic isotope effect. α-Tocopherol is the most reactive chain-breaking, phenolic antioxidant known. The simple model compound 4-methoxy-2,3,5,6-tetramethylphenol (TMMP) is much less reactive, with a k_5 value of only 2.1×10^5 M^{-1} s^{-1}. A better model compound is 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC) which has a k_5 value of 21.4×10^5 M^{-1} s^{-1}. The low reactivity of TMMP compared with α-tocopherol and PMHC is attributed to stereoelectronic factors. In TMMP the methoxy group is twisted out of the plane of the aromatic ring by steric forces and, in consequence, the p-type lone pair on the methoxyl oxygen cannot help stabilize the phenoxyl formed upon abstraction of the phenolic hydrogen. However, in the tocopherols and in PMHC the chroman ring system holds the ethereal oxygen's p-type lone pair nearly perpendicular to the aromatic ring, thereby providing additional stabilization for the resultant phenoxyls. This hypothesis has been confirmed by X-ray analysis of TMMP and PMHC and of 4-methoxyphenol and 2,6-di-*tert*-butyl-4-methoxyphenol.

There is now rather general agreement that Vitamin E functions



α-Tocopherol (α-T) : $R_1 = R_2 = R_3 = CH_3$

β-Tocopherol (β-T) : $R_1 = R_3 = CH_3$; $R_2 = H$

γ-Tocopherol (γ-T) : $R_1 = R_2 = CH_3$; $R_3 = H$

δ-Tocopherol (δ-T) : $R_1 = CH_3$; $R_2 = R_3 = H$

Vitamin E

as an efficient inhibitor of lipid peroxidation in vivo.[3-6] However, there appears to be widespread confusion concerning both the *relative* and the *absolute* antioxidant effectiveness in vitro of the individual tocopherols that go to make up Vitamin E. Thus, some workers[7-13] have reported that γ-tocopherol (γ-T) is more effective than α-tocopherol (α-T) at preventing in vitro lipid peroxidation. Others[14,15] have reported that α-T is more effective than γ-T. Still others[16-19] have reported that relative effectiveness depends on the experimental conditions. In vitro comparisons of α-T with other natural and synthetic phenols have usually led to the conclusion that α-T has only a rather modest antioxidant activity.[20]

The apparent "discrepancy" between the high in vivo Vitamin E activity of α-T and its *apparently* low in vitro antioxidant activity has generally been accepted uncritically. This acceptance has produced some remarkable rationalizations of the "discrepancy"[21] and has even been used to argue against the antioxidant theory for the role of Vitamin E in biological systems.[22-24] However, most of the experiments from which conclusions have been drawn regarding the relative effectiveness of the various tocopherols as antioxidants and their effectiveness relative to other phenols were, in fact, incapable of yielding the requisite information since they merely involved a comparison of induction periods for the self-initiated autoxidation of some natural product. In such experiments reproducibility is poor because the autoxidation process is initiated by minute and highly variable quantities of impurities (e.g., peroxides, transition-metal ions, etc.). Furthermore, in a self-initiated autoxidation the length of an induction period will depend also on the reactivity toward peroxy radicals of the phenol, on the number of radicals which are trapped by each molecule of phenol (the stoichiometric factor), and on the occurrence or otherwise of chain transfer. A meaningful comparison between antioxidants requires that these factors be individually evaluated.

The autoxidations of organic materials at temperature below ca. 100 °C and their inhibition by added phenols are very well-understood chemical processes.[25-28] Briefly, autoxidation is a chain

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reaction which can be described by the reaction scheme given in eq 1–4. In this scheme RH represents the organic substrate and

initiation: production of R· or ROO· (1)

propagation: $R\cdot + O_2 \rightarrow ROO\cdot$ (2)

$ROO\cdot + HR \rightarrow ROOH + R\cdot$ (3a)

$ROO\cdot + RH \rightarrow ROO\dot{R}H\ (\equiv R\cdot)$ (3b)

termination: $ROO\cdot + ROO\cdot \rightarrow$ nonradical products (4)

ROO· the peroxyl radical which it yields. The majority of organic compounds autoxidize to form hydroperoxides (reaction 3a), but certain polymerizable olefins yield polyperoxides (reaction 3b). In the presence of a chain-breaking phenolic antioxidant, ArOH, the oxidation chains are shortened, chain termination by reaction 4 is suppressed, and termination occurs instead by reactions 5 and 6, where n represents the stoichiometric factor for the phenol. The

$$ROO\cdot + ArOH \rightarrow ROOH + ArO\cdot \quad (5)$$

$$(n-1)ROO\cdot + ArO\cdot \rightarrow \text{nonradical products} \quad (6)$$

rate of an uninhibited autoxidation can be represented by eq I

$$\frac{-d[O_2]}{dt} = \frac{k_3[RH]R_i^{1/2}}{(2k_4)^{1/2}} \quad (I)$$

where R_i is the rate of chain initiation. For an inhibited autoxidation in which all ArO· are destroyed by reaction 6 (i.e., there is no chain transfer) the rate of autoxidation can be represented by eq II.

$$\frac{-d[O_2]}{dt} = \frac{k_3[RH]R_i}{nk_5[ArOH]} \quad (II)$$

On structural grounds the tocopherols are all expected to be rather good chain-breaking antioxidants. That is, the most comprehensive survey of the effect of ring substituents on the rate of reaction 5[29] has shown that this reaction is accelerated by a 4-methoxy group and by the presence of methyl groups in the 2- and 6-position or, better yet, in the 2-, 3-, 5-, and 6-positions. Furthermore, chain transfer is retarded when the phenoxyl oxygen is sterically protected by two ortho alkyl groups[26,27] and by the electron-donating 4-methoxy group.[26] It is therefore difficult to reconcile the structure of α-T with its purported low in vitro antioxidant activity. The few measurements of the rate constant for reaction 5 for α-T indicate that it is fairly reactive toward peroxyl radicals.[30-32,73] However, the k_5 values reported range from a low of 2×10^5 M^{-1} s^{-1} for $C_6H_5C(CH_3)_2OO\cdot$ at 60 °C[30] to a high of 5×10^8 M^{-1} s^{-1} for $Cl_3COO\cdot$ at room temperature[31] (see Table I). Such a large range of values is unexpected since most previous work[26-28,33] suggests that k_5 for a particular phenol is essentially independent of the nature of the peroxyl radical. However, it must be added that $Cl_3COO\cdot$ does appear to be more reactive in hydrogen atom abstractions than most alkylperoxyls.[34] Nevertheless, it is clear that the magnitude of k_5 for α-T is still uncertain. Since the techniques so far employed to measure k_5 for α-T (see Table I) are indirect and have been known to yield misleading results, we decided to measure this quantity by the most reliable and direct method known to us. This method involves a simple measurement of the rate of autoxidation of a pure hydrocarbon which has been inhibited with the phenol, under conditons where the rate of chain initiation is known, the oxidation chain is not completely suppressed, and the hydrocarbon is one for which k_3 has been determined. This method, though more time consuming than pulse radiolysis or chemiluminescence, has proved to be highly successful and reliable in a great deal of earlier work.[26-29,35-39]

Table I. Inhibition Rate Constants, k_5, and Stoichiometric Factors, n, for α-Tocopherol from the Literature

peroxyl	technique	T/°C	k_5/M^{-1} s^{-1}	n	ref
$C_6H_5C(CH_3)_2OO\cdot$	O_2 consumption	60	2.0×10^5	2.0	30
$C_6H_5CH(CH_3)OO\cdot$	chemiluminescence	37	1.8×10^6	3.2	32a
$C_6H_5CH(CH_3)OO\cdot$	chemiluminescence	60	3.3×10^6	3	32b
$c\text{-}C_6H_{11}OO\cdot$	pulse radiolysis	*a*	2.3×10^7	*b*	73
$Cl_3COO\cdot$	pulse radiolysis	*a*	5.0×10^8	*b*	31

[a] Not given, presumed to be room temperature. [b] Not measured.

Experimental Section

Materials. Commercial *dl*-α-T (ICN Pharmaceuticals, Inc.) and *d*-γ-T (Eastman) were generally used without further purification. A small sample of α-tocopherol that had been purified by high-pressure liquid chromotography gave results (induction period and k_5 values) identical with those for the commercial sample. Samples of β-T and δ-T were kindly provided by Dr. J. N. Thompson (Health Protection Branch, Department of National Health and Welfare, Ottawa) and samples of 2,6-di-*tert*-butyl-4-methylphenol and 2,6-di-*tert*-butyl-4-methoxyphenol by Dr. J. A. Howard (N.R.C.C.). Commercial 2,3,5,6-tetramethylphenol (Aldrich) and pentamethylphenol (Aldrich) were recrystallized twice from hexane, mp 116.4–116.7 °C (lit.[40] 117 °C) and 124–125 °C (lit.[41] 125 °C), respectively. 1-Naphthol (BDH, Analar) and *N,N'*-diphenyl-*p*-phenylenediamine (zone refined some time earlier) were used without further purification. 1-Hydroxypyrene was synthesized by the method of Vollmann et al.[42] and recrystallized from aqueous methanol, mp 178 °C (lit.[42] 179 °C). Tetramethyl-*p*-methoxyphenol was synthesized from duroquinone and trimethylphosphite by the method of Ramirez et al.[43] and recrystallized from hexane, mp 112–113 °C (lit.[44] 111–112 °C). 2,2,5,7,8-Pentamethyl-6-hydroxychroman was prepared from trimethyl-*p*-hydroquinone and isoprene by the method of Smith et al.[45] and was recrystallized three times from hexane, mp 91.8–92.2 °C [lit.[45] 94–94.5 °C).

Chlorobenzene (Fisher) was washed with concentrated sulfuric acid, 2% aqueous sodium bicarbonate, and water and was dried over anhydrous sodium sulfate and distilled from barium oxide. Styrene (99%, Aldrich) was percolated through basic alumina to remove peroxides and most of the added inhibitor (4-*tert*-butylcatechol) and was then trap-to-trap distilled on a vacuum line immediately prior to use. The oxidations were initiated with 2,2'-bis(isobutyronitrile) (AIBN, Eastman), which was recrystallized from warm methanol (ca. 35 °C), dried over P_2O_5 under vacuum, and stored in chlorobenzene as a 0.3623 M solution at −20 °C. Heavy water, D_2O (99.7%, Merck, Sharp and Dohme), was used as supplied.

The antioxidants were stored as standard solutions (2×10^{-4}–7×10^{-2} M) in chlorobenzene at −20 °C in the dark. The tocopherol solutions were further protected from oxidation by freeze–thaw degassing and storage under vacuum immediately after each use.

Autoxidation Procedure. Oxidations were carried out at 30 °C under 760 torr of O_2 in an automatic recording gas absorption apparatus of the

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type descried previously,[46] using a Validyne P 10 pressure transducer (±5 psi) and C 12 transducer indicator connected to a strip-chart recorder. The transducer as well as the reaction vessel were thermostated at 30 °C.

In a typical experiment, 250 μL of the chlorobenzene solution of AIBN was transferred to the reaction vessel and the chlorobenzene was removed on a vacuum line. The AIBN was then dissolved in 2 mL of styrene. After the reaction vessel was connected to the pressure transducer and sufficient time was allowed for the solution to come to thermal equilbrium, the reference and test halves of the transducer were isolated by closing the appropriate stopcocks. The pressure change created by the uninhibited oxidation of the styrene was recorded for ca. 20 min and then a small volume (1–15 μL) of the chlorobenzene solution of the antioxidant was introduced directly into the styrene by using a microsyringe controlled by a repeating dispenser. In some experiments, the styrene was diluted with chlorobenzene but the total liquid volume in all runs was maintained at 2 mL. The apparatus was calibrated by studying some oxidations for which the rate is known reliably.[28] The accuracy of the calibration was confirmed by Dr. J. A. Howard (N.R.C.C.), who kindly oxidized some identical samples on his apparatus. In this way, the continuously recorded changes in pressure could be converted, after correction for the nitrogen evolved and oxygen consumed by the AIBN initiator, to rates of oxidation of the styrene in mol L^{-1} s^{-1} units.

Both α-T and 2,2,5,7,8-pentamethyl-6-hydroxychroman reacted too rapidly with peroxyl radicals for their k_5 values to be measured at low temperature by the technique of kinetic electron spin resonance spectroscopy.[33]

Measurement of R_i. The rate of chain initiation due to the thermal decomposition of the AIBN was determined by the induction period method[47] by using 2,6-di-*tert*-butyl-4-methoxyphenol as the antioxidant. The stoichiometric factor, n, for this phenol is close to 2.0.[48] The induction period, τ, was determined in the usual way[47,48] from the length of time between antioxidant injection and the point of intersection of tangents to the oxidation curve corresponding to the initial inhibited and final uninhibited rates of oxidation. The efficiency, e, of radical formation (i.e., of chain initiation) by AIBN in styrene at 30 °C was found to be 0.64 from the known rate constant for its decomposition[35] and the measured R_i.

Measurement of n. The stoichiometric factors for the antioxidants used in this work were determined by using the measured value of R_i and the induction period, τ, obtained from a known antioxidant concentration $[ArOH]_0$.

$$n = \frac{R_i\tau}{[ArOH]_0} \qquad \text{(III)}$$

Deuterium Kinetic Isotope Effect. The magnitude of this effect for α-T and some other phenols were determined by comparison of inhibited autoxidations carried out in the presence of 50 μL of H_2O or D_2O as described previously.[35] In the presence of D_2O the phenolic hydrogen is rapidly replaced by deuterium. The deuterated antioxidants react more slowly with peroxyl radicals than the undeuterated materials.

Results

Some typical traces showing the uninhibited oxidation and some tocopherol inhibited oxidations of 3.95 M styrene in chlorobenzene initiated with 3.29×10^{-2} M AIBN are shown in Figure 1. The inhibited oxidation curves show a break when the antioxidant has been completely consumed. These breaks are very sharp for a highly efficient antioxidant (see curve for α-T) and provide a very reliable measure of the stoichiometry of the antioxidant. The stoichiometric factors for all the antioxidants examined in this work are listed in Table II. They are all equal to 2.0 within experimental error. This means, in particular, that each of the four tocopherols reacts with exactly two peroxyl radicals.

Our choice of styrene as the oxidizable substrate was based on the fact that it has proved highly successful in earlier quantitative kinetic studies of phenol-inhibited autoxidations.[29,35-37] It has a number of advantages over most other potential substrates. First, the rate constant for chain propagation, k_3, is comparatively large [41 M^{-1} s^{-1} at 30 °C].[49] This means that even with a very good

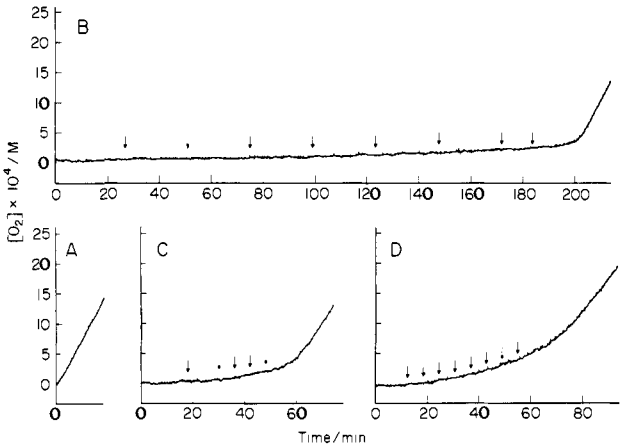

Figure 1. The uninhibited (curve A) and tocopherol inhibited (curves B, C, and D) autoxidation of 3.95 M styrene in chlorobenzene initiated with 0.0329 M AIBN at 30 °C. The arrows on curves B, C, and D are points from which the data given in Table III were derived. Curve B is α-T at 2.34×10^{-5} M. Curve C is γ-T at 6.67×10^{-6} M. Curve D is δ-T at 7.34×10^{-6} M.

Table II. Stoichiometric Factors for Some Antioxidants

antioxidant	n^a
2,6-di-*tert*-butyl-4-methoxyphenol	(2.00)[b]
α-T	2.04 ± 0.16 (17)
β-T	2.07 ± 0.07 (5)
γ-T	1.81 ± 0.07 (5)
δ-T	1.96 ± 0.18 (5)
2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC)	1.89 ± 0.09 (7)
2,3,5,6-tetramethyl-4-methoxyphenol (TMMP)	1.89 ± 0.08 (4)
pentamethylphenol	1.86 ± 0.03 (3)
2,3,5,6-tetramethylphenol	1.86 ± 0.11 (5)
2,6-di-*tert*-butyl-4-methylphenol[c]	2.04 ± 0.13 (3)
1-naphthol	1.96 ± 0.19 (4)
1-hydroxypyrene	2.09 ± 0.07 (5)
N,N'-diphenyl-*p*-phenylenediamine[c]	2.10 ± 0.30 (5)

[a] Errors are standard deviations. The number of determinations of n is given in parentheses. [b] Assumed. [c] This compound has been suggested as a standard to measure n values.[47]

antioxidant the oxidation occurs at a measurable rate and the chain length is greater than one; i.e., the inhibited autoxidation is still a *chain* reaction. Secondly, because styrene contains no readily abstracted hydrogen atom, it forms a polyperoxide rather than a hydroperoxide.[35,49-51] The reverse of reaction 5, i.e.

$$ArO\cdot + ROOH \rightarrow ArOH + ROO\cdot \qquad (-5)$$

a reaction which complicates kinetic studies of inhibited autoxidations in most organic substrates[26,27,38,52,53] is therefore unimportant in styrene. Third, the chain-transfer reaction

$$ArO\cdot + RH \rightarrow ArOH + R\cdot \qquad (7)$$

which also complicates kinetic studies in most other substrates[26,27,38,39,54] is unimportant. Chain transfer by phenoxyl addition to styrene does occur with phenol itself at relatively high concentrations ($> \sim 5 \times 10^{-4}$ M) and with substituted phenols that are as poor (or worse) antioxidants.[36] For thermochemical reasons this process becomes much less important with substituted phenols that are significantly better antioxidants than phenol. Thus, chain transfer is relatively unimportant for 4-methoxyphenol[36] and for most ortho-alkylated phenols.[26] The tocopherols all proved to be such reactive antioxidants that no sign of chain transfer was apparent in the kinetics of tocopherol-inhibited styrene aut-

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Table III. Determination of k_5 for α-T, γ-T, and δ-T at 30 °C from Oxidation Curves Shown in Figure 1 ([AIBN] = 0.0329 M; $R_i = 3.91 \times 10^{-9}$ M s^{-1}; [styrene] = 3.95 M)

α-T (2.34×10^{-5} M)				γ-T (6.67×10^{-6} M)				δ-T (7.34×10^{-6} M)			
t/τ[a]	$10^5\Delta[O_2]$[b]	ν[c]	$10^{-6}k_5$[d]	t/τ[a]	$10^5\Delta[O_2]$[b]	ν[c]	$10^{-6}k_5$[d]	t/τ[a]	$10^5\Delta[O_2]$[b]	ν[c]	$10^{-5}k_5$[d]
0.134	1.15	1.97	2.03	0.317	5.03	14.5	1.22	0.199	4.42	16.9	8.13
0.255	2.30	2.24	2.07	0.529	7.53	15.9	1.61	0.294	6.31	17.5	8.93
0.376	3.46	2.51	2.21	0.634	11.3	23.0	1.43	0.390	11.4	25.8	7.01
0.497	5.90	3.65	1.89	0.741	15.2	32.6	1.44	0.487	14.6	29.0	7.40
0.618	8.33	4.84	1.87	0.846	20.9	54.4	1.45	0.583	20.3	37.9	6.96
0.740	12.1	7.38	1.81					0.680	28.0	52.3	6.58
0.861	17.7	13.8	1.80					0.776	36.9	75.0	6.56
0.921	20.2	21.5	2.03					0.873	43.9	114	7.60
mean			(1.96 ± 0.14)				(1.43 ± 0.14)				(7.32 ± 0.76)

[a] Ratio of elapsed time to induction period; indicates fraction of inhibitor consumed. [b] Total O_2 (M) absorbed by the solution, corrected for evolution of N_2 and consumption of O_2 by the initiator. Values obtained from Figure 1. [c] Chain length. [d] In M^{-1} s^{-1} units.

Table IV. Measurement of k_5 for α-T at 30 °C

10^5-[α-T]/M	[styrene]/M	10^2-[AIBN]/M	$10^{-6}k_5$[a]/M^{-1} s^{-1}
2.17	3.95	3.29	2.34 ± 0.31
2.17	3.95	3.29	1.96 ± 0.14
0.72	3.95	3.29	2.61 ± 0.13
0.79	8.51	4.53	2.42 ± 0.16
0.80	8.70	4.53	2.86 ± 0.12
0.80	8.70	4.53	2.85 ± 0.10
0.80	8.70	4.53	2.81 ± 0.37
3.11	8.51	18.1	1.57 ± 0.18
3.17	8.68	18.1	1.52 ± 0.14

[a] Errors are standard deviation from ca. 8 determinations of k_5 per run.

Table V. Values of k_5 for Tocopherols and for Selected Synthetic Antioxidants at 30 °C

antioxidant	$10^{-4}k_5$[a]
α-T	235 ± 50
β-T	166 ± 33
γ-T	159 ± 42
δ-T	65 ± 13
2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC)	214 ± 81
2,3,5,6-tetramethyl-4-methoxyphenol (TMMP)	21 ± 2[b]
pentamethylphenol	14 ± 1[b]
2,3,5,6-tetramethylphenol	5.6 ± 0.0[1][b]
2,6-di-*tert*-butyl-4-methoxyphenol	7.8 ± 0.2[b]
2,6-di-*tert*-butyl-4-methylphenol	1.2 ± 0.0[2][b]
1-naphthol	24 ± 3
1-hydroxypyrene	186 ± 70
N,N'-diphenyl-*p*-phenylenediamine	117 ± 42

[a] Errors are standard deviations; units are M^{-1} s^{-1}. [b] Mean of two separate measurements.

oxidation. Finally, under the conditions employed, the autoxidation of styrene is not autocatalytic.

The rate constant for inhibition, k_5, was calculated from the quantity of oxygen absorbed after time, t, during the induction period (length = τ) using the integrated form of eq II, viz.[30,55]

$$[O_2]_{t=0} - [O_2]_t = -(k_3[RH]/k_5)[\ln(1 - t/\tau)] \quad \text{(IV)}$$

It should be noted that the stoichiometric factor does not appear in this equation. The values of $[O_2]_t$ were calculated after allowing for nitrogen release by the initiator and oxygen absorption by the radicals derived from the initiator.[35] The value of k_5 was calculated at several points throughout a run. If kinetic expressions II and III are valid, then k_5 should remain constant. This can be seen to be the case for the tocopherol inhibited oxidation runs shown in Figure 1 from the data listed in Table III. This table also includes values of the chain length, ν, throughout each run which have been calculated from eq V.

$$\nu_t = \left(\frac{-d[O_2]}{dt} \Big/ R_i\right)_t = \frac{[O_2]_t - [O_2]_{t=0}}{n[AH]_0(1 - t/\tau)\ln(1 - t/\tau)} \quad \text{(V)}$$

Table VI. Deuterium Kinetic Isotope Effects for Some Phenolic Antioxidants at 30 °C

antioxidant	$(k_5)_{OH}/(k_5)_{OD}$	ref
α-T	4.0 ± 0.5[a]	this work
γ-T	9.1	this work
2,2,5,7,8-pentamethyl-6-hydroxychroman	5.1 ± 0.5[a]	this work
2,3,5,6-tetramethyl-4-methoxyphenol	10.6 ± 3.7[a]	this work
2,6-di-*tert*-butyl-4-methoxyphenol	9.4[b]	33
2,6-di-*tert*-butyl-4-methylphenol	6.8[b]	33
	10.6[c]	35
1-naphthol	4.3[b]	33

[a] Mean of two or more separate measurements. [b] Extrapolated to 30 °C. [c] At 65 °C.

The "fit" to the simple kinetic equations (II) and (IV) was also verified for each antioxidant by carrying out measurements at different concentrations of styrene, antioxidant, and AIBN. For the more efficient antioxidants the concentration ranges that could be covered while maintaining a reasonable chain length were rather limited. However, even with α-T, which was the most efficient antioxidant, the range was sufficient to confirm its "fit" to the equations by the constancy (within our experimental accuracy) of the computed k_5 values (see Table IV).

Values of k_5 for the four tocopherols and for nine synthetic antioxidants are summarized in Table V. Where comparison is possible, the present results with the synthetics are in satisfactory agreement with literature values: viz., pentamethylphenol, 8.6×10^4 M^{-1} s^{-1}, 2,3,5,6-tetramethylphenol, 3.0×10^4 M^{-1} s^{-1}, 2,6-di-*tert*-butyl-4-methoxyphenol, 7.0×10^4 M^{-1} s^{-1}, 2,6-di-*tert*-butyl-4-methylphenol, $1.7_8 \times 10^4$ M^{-1} s^{-1}, and *N,N'*-diphenyl-*p*-phenylenediamine, ca. 1.9×10^6 M^{-1} s^{-1}, all in styrene at 65 °C;[29,56-58] 1-naphthol, 1.4×10^5 M^{-1} s^{-1}, and 1-hydroxypyrene, 3.7×10^6 M^{-1} s^{-1}, in 9,10-dihydroanthracene at 60 °C.[57,59]

It is now well established that phenols owe their antioxidant ability to reaction 5 and that this reaction exhibits a substantial deuterium kinetic isotope effect.[29,33,35,36] In order to be certain that the tocopherols also owe their antioxidant properties to a rate-controlling transfer of their phenolic hydrogen to a peroxyl radical, we measured the rate constant ratio $(k_5)_{OH}/(k_5)_{OD}$ for α-T, γ-T, and two model compounds. These compounds all exhibited a substantial deuterium isotope effect. The results are compared in Table VI with some data from the literature.

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Table VII. Relative Reactivities toward Peroxyl Radicals of Some 4-Methoxy and 4-Methyl-Substituted Phenols

ortho and meta substituents	$(k_5)_{MeO}/(k_5)_{Me}$	ref
none	5.2	36
2-*tert*-butyl	4.6	29
2,6-di-*tert*-butyl	3.9	29
2,6-di-*tert*-butyl	6.5	this work
2,3,5,6-tetramethyl	1.5	this work

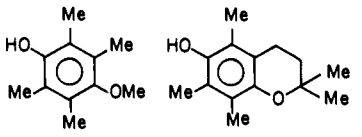
Discussion

The results summarized in Table V show that all the tocopherols are exceptionaly good chain-breaking antioxidants in vitro. In particular, α-T has a larger rate constant for H-atom transfer to a peroxyl than any synthetic antioxidant (including 1-hydroxypyrene),[59] while β-T and γ-T are only slightly less reactive. The k_5 value for α-T is in good agreement with the values found by chemiluminescence[32] (see Table I). However, it should be noted that the reported[32a,60] stoichiometric factor for α-T was equal to 3.2. This does not agree with our finding that $n = 2$ for this compound, as for the other tocopherols and all the synthetic antioxidants (see Table II). Furthermore, Aristarkhova et al.[32a] reported that their autoxidation still showed signs of inhibition after all the α-T had been consumed. This phenomenon was attributed to inhibition by some oxidation product of α-T with a k_5 value ca. 5% of that of α-T. We found no such extended inhibition. The rate of oxidation always returned to its uninhibited value after the α-T was consumed. This was also the case with all the other antioxidants we examined.

Values for k_5 of 2.0×10^6, 1.5×10^6, and 9.0×10^5 M^{-1} s^{-1} at 60 °C have been reported for β-T, γ-T, and δ-T, respectively.[32b] A value for k_5 of 9×10^5 M^{-1} s^{-1} at 37 °C has been reported for δ-T.[61]

The in vitro relative reactivities of the tocopherols reported here are in agreement with in vivo tests of their relative biological activities which yield: α-T > β-T > γ-T > δ-T,[62] and with in vitro tests of their relative reactivities toward singlet oxygen which yield the same order.[63] We could not, however, detect any truly significant difference in the reactivities of β-T and γ-T.

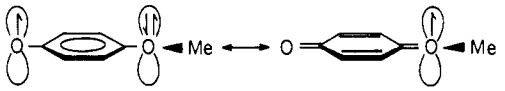
The question remains: what makes α-T so reactive toward peroxyl radicals? The observation of a significant, primary deuterium kinetic isotope effect (Table VI) shows that the in vitro reactive moiety of α-T must reside in the phenolic portion of the molecule. At first sight, this would seem to be no different from 2,3,5,6-tetramethyl-4-methoxyphenol (TMMP). However, this

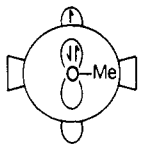


TMMP PMHC

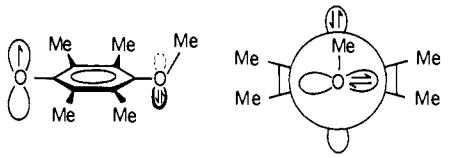
phenol is only ca. 9% as reactive as α-T (see Table V). By way of contrast, 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC) is, within our limits of error, as reactive as α-T. The 6-hydroxychroman system contained in α-T and PMHC is therefore not "equivalent" to the structurally related TMMP.

The intitial clue as to the origin of this structural difference came from a comparison of the ratio of the k_5 values for TMMP and pentamethylphenol with the ratios of k_5 values for other pairs of 4-methoxyphenols and 4-methylphenols (see Table VII). It is clear that for all classes of phenols *except* the 2,3,5,6-tetramethylphenols the 4-methoxy derivative is about 5 times as reactive as the 4-methyl derivative. This normal enhancement of k_5 by a 4-methoxy group is, of course, due to stabilization of the phenoxyl radical formed in reaction 5 by delocalization of the unpaired electron to the p-type lone pair of the methoxyl oxygen. For 4-methoxyphenol this stabilization amounts to 4.3 kcal/mol relative to phenol or 2.5 kcal/mol relative to 4-methylphenol.[64]



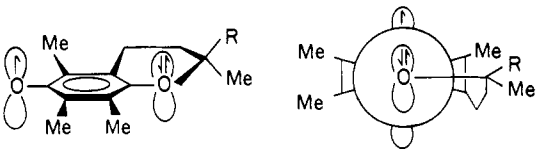


In TMMP the methoxyl group would not provide this enhanced phenoxyl stabilization if, for steric reasons, the two meta methyl groups twisted the methoxyl methyl out of, and the methoxyl oxygen's p-type lone pair into, the plane of the aromatic ring.



Supporting evidence for steric hindrance to conjugation is provided by the electron spin resonance spectrum of the phenoxyl from TMMP. This spectrum shows no hyperfine splitting by the methoxyl's hydrogens,[65] which implies that there is very little spin density on the methoxyl oxygen. In contrast, the methoxyl hydrogens in 4-methoxyphenoxyl and in 4-methoxy-2,6-dialkylphenoxyls produce hyperfine splittings in the range 1.5–2.1 G[65] which shows that there must be appreciable spin on the methoxyl oxygens of these radicals. Additional evidence supporting an out-of-plane methoxyl in TMMP comes from both carbon-13 chemical shifts[66] and from the UV spectra[67-69] of 2,6-dimethylanisole and related compounds.

In PMHC and the tocopherols, the oxygen in the ring would be prevented from twisting its p-type lone pair into the aromatic plane. These phenoxyls should therefore gain at least some, and perhaps most, of the stabilization which is available to those 4-methoxyphenoxyls which do not contain alkyl groups in the 3- and 5-positions. The magnitude of the stabilization of the phenoxyl radicals derived from α-T and PMHC relative to that from TMMP can be estimated to be ca. 3 kcal/mol. This energy difference is based on the fact that the k_5 values for α-T and PMHC are about 10 times the value for TMMP, plus the known relationship[64] between k_5 and phenolic O–H bond strengths.



This stereoelectronic explanation for the high in vitro reactivity of the tocopherols and PMHC relative to TMMP was tested by

(60) Note that Burlakova et al.[32b] give $n = 3$ for α-, γ-, and δ-T, but $n = 1$ for β-T!
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Table VIII. Some X-ray Diffraction Data on Some 4-Alkoxyphenols

structural parameter	PMHC		TMMP	phenol	
	A[a]	B[a]		4-MeO	2,6-*t*-Bu$_2$-4-MeO
		Angles, deg			
dihedral Ar-O-C	14.5	18.0	88.6	8.3	25.3
dihedral Ar-O-H	53.9, 45.3[b]	59.2, 57.1[b]	30.9	27.3	4.8
interbond Ar-O-C	117.0	116.7	113.5	117.6	117.2
interbond Ar-O-H	123.7, 120.0[b]	123.4, 119.2[b]	114.5	108.5	118.5
		Bond Lengths, Å			
ArO-H	1.37, 1.29[b]	1.42, 1.33[b]	0.89	1.05	0.80
Ar-OH	1.402	1.405	1.400	1.391	1.381
ArO-C	1.463	1.448	1.443	1.428	1.420
Ar-OC	1.389	1.392	1.408	1.377	1.397

[a] There are two symmetry-unrelated molecules in the unit cell for PMHC. [b] Duplicate values arise from the presence of two symmetrical hydrogen bonds to each phenolic oxygen.

X-ray analysis of PMHC and TMMP. Some of the more significant structural parameters for these two phenols are listed in Table VIII together with data for 4-methoxyphenol and 2,6-di-*tert*-butyl-4-methoxyphenol which provide model compounds which do not have hindering alkyl substituents in positions 3 and 5. It can be seen that the Ar–O–C dihedral angle is indeed ca. 90° for TMMP but is only ca. 16° for PMHC, which is of similar magnitude to the angles found for 4-methoxyphenol (8.3°) and 2,6-di-*tert*-butyl-4-methoxyphenol (25.3°).

Although α-T and PMHC have similar in vitro antioxidant activity, the latter compound generally shows little or no Vitamin E activity in vivo.[70,71] The major, and possibly the only, role of the phytyl moiety of the tocopherols would therefore seem to be to increase the solubility of the hydroxychroman moiety in those regions of biological systems which require protection against autoxidation, e.g., biomembranes. In fact, the phytyl side chain has been shown to have the optimum length in assisting 6-hydroxytetramethylchromans to penetrate monolayers of phospholipid molecules.[72]

In conclusion, it is now clear that those factors which enhance the peroxyl radical trapping ability of phenols, i.e., their chain-breaking antioxidant activity, are maximized in α-T. These factors are, first, alkyl substitution at both ortho and both meta positions and, second, a para-alkoxy group held in an orientation which permits stabilization by interaction of the unpaired electron with the p-type lone pair on the para oxygen. Such optimization of structure implies that the *primary* (and possibly only) function of α-T, in vivo, is to act as an antioxidant. The phytyl side chain is presumably attached to the hydroxychroman moiety to ensure that the reactive center is present where it is most needed in a living system.

Acknowledgment. We thank Dr. J. A. Howard for helpful discussions and Dr. J. N. Thompson for his generous gifts of β-T and δ-T and for his continuing interest in this work. We also thank Dr. Helmut Woynar for writing the computer program used for treating the kinetic data and Drs. Y. Le Page and E. J. Gabe for the X-ray analysis.

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