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Catalytic Antioxidants: Regenerable Tellurium Analogues of Vitamin E

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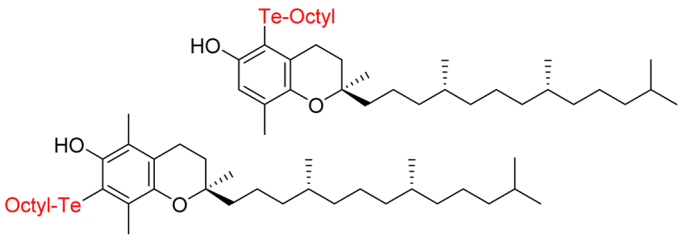
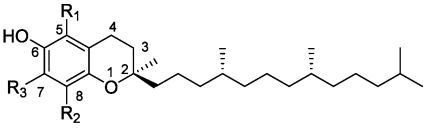
ABSTRACT



In an effort to improve the chain-breaking capacity of the natural antioxidants, an octyltelluro group was introduced next to the phenolic moiety in β- and δ-tocopherol. The new vitamin E analogues quenched peroxyl radicals more efficiently than α-tocopherol and were readily regenerable by aqueous *N*-acetylcysteine in a simple membrane model composed of a stirring chlorobenzene/water two-phase system. The novel tocopherol analogues could also mimic the action of the glutathione peroxidase enzymes.

For a long time, vitamin E has been considered the most important lipophilic chain-breaking antioxidant in vivo.[1] There are numerous studies in vitro that witness its ability to rapidly quench peroxyl radicals, and it is likely that vitamin E has a role as a chain-breaking antioxidant in biological membranes[2] to minimize polyunsaturated fatty acid oxidation and keep membrane fluids in the highest possible quality. However, more recent findings concerning uptake, transport, and metabolism, along with the scarcity of clinical evidence for a protective effect in vivo, has led to speculations that vitamin E could also have a nonantioxidant function[3] and serve as a regulator of transduction and gene expression.[4] Vitamin E is a collective name for a group of eight structurally related 6-chromanols carrying a variable number of methyl substituents in the chromanol entity along with either a branched $C_{16}H_{33}$ alkyl group (tocopherols **1**, **2**, **3**, and **4**) or a similar but triply unsaturated $C_{16}H_{27}$ substituent (tocotrienols) in position 2.



1 α-Tocopherol $R_1 = R_2 = R_3 = Me$
2 β-Tocopherol $R_1 = R_2 = Me$; $R_3 = H$
3 γ-Tocopherol $R_1 = H$; $R_2 = R_3 = Me$
4 δ-Tocopherol $R_1 = R_3 = H$; $R_2 = Me$

The stoichiometric number *n* for α-tocopherol is 2. This means that each molecule of the antioxidant can quench two peroxyl radicals. Regeneration and recycling of this valuable molecule would therefore seem to be a good idea. Rate constants as high as 3×10^6 $M^{-1}s^{-1}$ were recorded for the bimolecular reaction of ascorbate with α-, β-, and γ-tocopheroxyl radicals.[5] In membranes it is generally accepted that this reaction is occurring at the aqueous lipid interphase and that ascorbate is serving as the stoichiometric reducing agent for quenching of lipidperoxyl radicals. During azo-initiated oxidation of phosphatidyl choline

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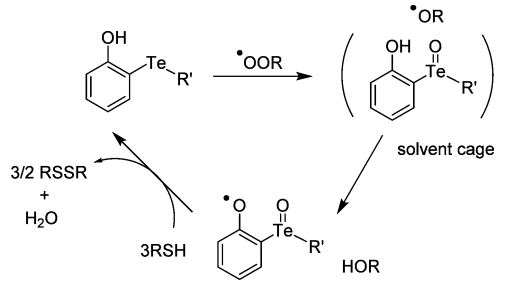
liposomes in an aqueous dispersion, α-tocopherol was not consumed until aqueous-phase ascorbate was used up.[6] In vivo data in support of regeneration are scarce, though. Biological hydroquinones, such as ubiquinole, have also been shown to act as regenerating agents toward the tocopheroxyl radical in vitro.[7] This is also true for phenothiazines,[8] and certain phenolic compounds with sufficiently low O−H bond dissociation enthalpies.[8,9] During the past decade we have studied the antioxidative properties of organochalcogen compounds. The most notable advance was the recent finding that introduction of an alkyltelluro group into the ortho-position of a phenolic compound caused a dramatic increase in the rate constant for quenching of peroxyl radicals. Thus, 2-(octyltelluro)phenol quenched lipid peroxyl radicals some 4 orders of magnitude more rapidly than phenol itself.[10] This cannot be accounted for by weakening of the O−H bond as a result of a substitutent effect[11] but rather suggests an unconventional mechanism involving the chalcogen. Both experiment and calculations were in support of the suggestion that quenching of peroxyl radicals occurs via initial oxygen transfer to tellurium, followed by hydrogen atom transfer in a solvent cage from the nearby phenol to the resulting alkoxyl radical (Scheme 1).[12]

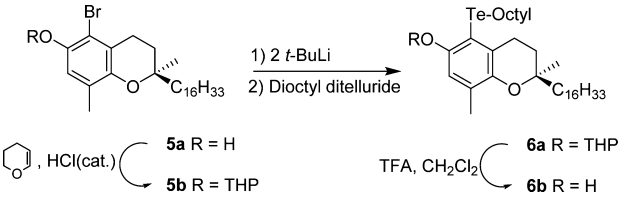
Scheme 1. Proposed Catalytic Mechanism for Quenching of Peroxyl Radicals by 2-(Alkyltelluro)phenols in the Presence of Thiols



In the presence of a suitable thiol reducing agent RSH, the alkyltelluro moiety could also facilitate regeneration of the phenolic antioxidant[10,12] to allow for a catalytic mode of action. Here, we report on our attempts to introduce alkyltelluro groups into the tocopherol scaffold and the catalytic chain-breaking and hydroperoxide decomposing activity of the products.

Alkyltelluro functionalization of phenols is commonly effected by a sequence of reactions involving *ortho*-bromination, lithium−halogen exchange/deprotonation, and reaction of the resulting dianion with a dialkyl ditelluride.[13] Since (2*R*,4′*R*,8′*R*)-δ-tocopherol (**4**) is commercially available in quantity at a reasonable price we thought it would be a suitable starting material for such an approach. Work by Rosenau and co-workers has shown that Br_2 selectively causes a monobromination of δ-tocopherol in position 5.[13] We found that tetrabutylammonium tribromide was a more convenient reagent for this transformation affording a 98% isolated yield of compound **5a** when the reaction was carried out in chloroform at room temperature. However, after lithium−halogen exchange/deprotonation effected by treatment with 3 equiv of *t*-BuLi in dry THF at −78 °C, none of the expected (alkyltelluro)-phenol **6b** was formed upon addition of dioctyl ditelluride as a source of electrophilic tellurium. Tetrahydropyran (THP) protection of the phenol changed the situation favorably (Scheme 2). Compound **5b** (93% yield) on treatment with 2 equiv of *t*-BuLi and then dioctyl ditelluride returned the protected target molecule **6a** (50% yield). Stirring in dichloromethane containing 0.5 equiv of trifluoroacetic acid released the desired tocopherol derivative **6b** (64% yield).

Scheme 2. Synthesis of Compound **6b**



β-Tocopherol (**2**) was also considered as a starting material for alkyltelluro functionalization in the remaining aromatic position. It is available from commercial suppliers but prohibitively expensive for large-scale synthesis. We found that the chemistry developed for obtaining **6b**, using methyl iodide instead of a ditelluride as an electrophile, produced THP-protected β-tocopherol (90% yield) and after deprotection, β-tocopherol (86% yield), identical in all respects with a sample obtained by aminoalkylation of δ-tocopherol followed by borohydride reduction.[14] By using the strategy shown in Scheme 2 as a blueprint, β-tocopherol was brominated (83% yield of **7a**) in position 7 using tetrabutylammonium tribromide. However,

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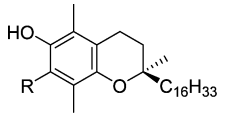
attempted THP or MOM protection of the phenol failed in this case.



7a R = Br
7b R = Te-Octyl

Much to our surprise, unprotected compound **7a** underwent smooth lithium−halogen exchange/deprotonation by treatment with 3 equiv of *t*-BuLi in dry THF at −78 °C to produce the desired 7-(octyltelluro)-*β*-tocopherol (**7b**; 30% yield) on trapping with dioctyl ditelluride.

The antioxidant capacity and regenerability of the novel tocopherol derivatives **6b** and **7b** were studied in a stirring two-phase system where azo-initiated peroxidation of linoleic acid was occurring in the lower chlorobenzene layer and the water-soluble reducing agent *N*-acetylcysteine (NAC) was contained in the upper, aqueous phase (Figure 1).

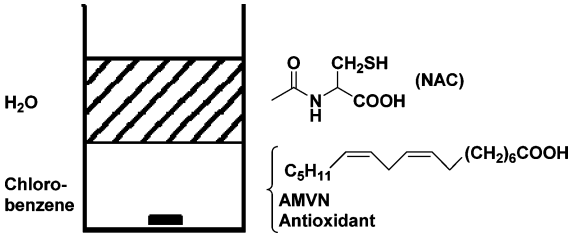


Figure 1. Two-phase model used for studying regeneration of antioxidants.

The progress of linoleic acid peroxidation was monitored for 680 min by HPLC (conjugated diene formation). For comparison of catalyst efficiency, the inhibited rate of peroxidation, R_{inh}, was determined as the slope during the inhibited phase and the inhibition time, T_{inh}, as the cross-point for the inhibited and the uninhibited lines (with α-tocopherol this happens after ca. 100 min; Figure 2). α-Tocopherol has previously been used as a benchmark in this system representing a potent (R_{inh} was as low as 25 ± 1 μM/h) but **not** regenerable antioxidant (the recorded T_{inh} was 97 ± 5 min in the presence and 109 ± 2 min in the absence of NAC). *β*- and *δ*-Tocopherol showed almost similar antioxidant characteristics as α-tocopherol (Table 1).

The relative quenching capacities (α > *β* > *δ*-tocopherol) is in accord with absolute rate constants for H-atom abstraction from the tocopherols as recorded by Ingold.[15] In the absence of NAC in the aqueous phase, **6b** and **7b** inhibited peroxidation less efficiently (R_{inh} = 34 ± 3 and 39 ± 1 μM/h, respectively) than the tocopherols **1**, **2**, and **4** but for longer times (135 ± 5 and 175 ± 7 min, respectively). This is because tellurium is oxidized to the tetravalent state (telluroxide) by residual hydroperoxide always contained in the commercial sample of linoleic acid and the electron withdrawing substituent increases the O−H bond dissociation enthalpy of the compound. In the presence of NAC, tellurium is always kept in the divalent oxidation state where it can quench peroxyl radicals as outlined in Scheme 1. The inhibited rates of peroxidation for **6b** and **7b** (1.3 and 5.0 μM/h, respectively) are notably lower than recorded for the parent tocopherols. Facile regeneration of the antioxidant is another effect of the chalcogen substitution. The inhibition times for **6b** and **7b** (458 and 591 min, respectively) are the longest we have ever recorded in this model system. It should be clear from the peroxidation traces recorded for **1**, **6b**, and **7b** (Figure 2) that introduction of tellurium into the 5- and 7-positions of the tocopherols dramatically improves both chain-breaking capacity and regenerability.

Table 1. Inhibited Rates of Conjugated Diene Formation (R_{inh}) and Inhibition Times (T_{inh}) with and without NAC (1 mM)

	with NAC		without NAC	
antioxidants (40 μM)	R_{inh}[a] (μM/h)	T_{inh}[b](min)	R_{inh}[a] (μM/h)	T_{inh}[b](min)
α-tocopherol	25 ± 1	97 ± 5	28 ± 2	109 ± 2
β-tocopherol	28 ± 1	105 ± 3	28 ± 1	119 ± 2
δ-tocopherol	30 ± 1	85 ± 3	33 ± 4	93 ± 3
6b	1.3 ± 0.1	458 ± 6	34 ± 3	135 ± 5
7b	5.0 ± 1	591 ± 10	39 ± 1	175 ± 7

[a] Rate of peroxidation during the inhibited phase (uninhibited rate ca. 425 μM/h). Errors correspond to ± SD for triplicates. [b] Inhibited phase of peroxidation. Reactions were monitored for 680 min. Errors correspond to ± SD for triplicates.

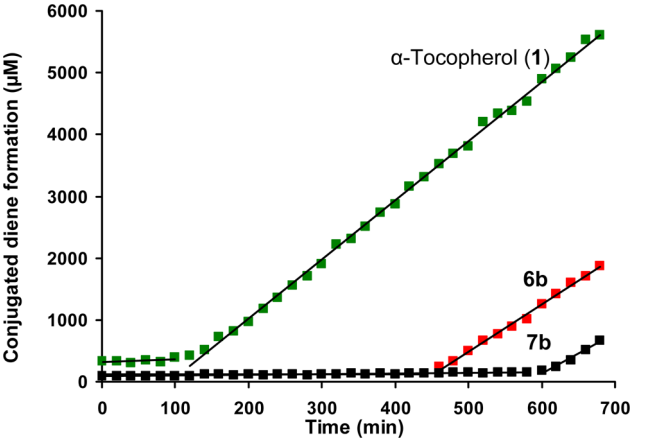


Figure 2. Peroxidation traces (conjugated diene formation vs time) recorded using compounds **1**, **6b**, and **7b** (40 μM) as antioxidants in the chlorobenzene layer in the presence of NAC (1 mM) in the aqueous phase.

The restriction is likely to be the amount of thiol contained in the aqueous phase. We speculate that a more

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thiol-efficient, catalytic mechanism than the one shown in Scheme 1 could come into play with the novel vitamin E analogues. Due to the substituent effect of the *o*-alkyltelluro group,[11] formal hydrogen atom transfer from phenol to peroxyl radical will contribute to quenching. The resulting phenoxyl radical is then reduced by electron/proton transfer from aqueous-phase thiol to regenerate the antioxidant at the expense of only 1 equiv (instead of 3; Scheme 1) of the reducing agent. The chalcogen would also impose on the vitamin a capacity to serve as a preventive, hydroperoxide decomposing antioxidant. This is because tellurium is easily redox cycled between the divalent and tetravalent states. Hydrogen peroxide and alkyl hydroperoxides cause oxidation and mild reducing agents such as thiols regenerate the divalent organotellurium compound. This catalytic, glutathione peroxidase-like activity of compounds **6b** and **7b** was evaluated by using the assay of Tomoda.[16] The initial rates (v_0) for the reduction of H_2O_2 (3.75 mM) by PhSH (1 mM) in the presence of catalyst (0.01 mM) were determined in methanol by monitoring the formation of diphenyl disulfide (PhSSPh) by UV spectroscopy at 305 nm for the first 10 s of reaction (eq 1).

$$2\mathrm{PhSH} + \mathrm{H_2O_2} \xrightarrow[\mathrm{MeOH}]{\text{catalyst}} \mathrm{PhSSPh} + 2\mathrm{H_2O} \qquad (1)$$

Initial rates of PhSSPh formation in this system were corrected for the spontaneous oxidation of PhSH induced by H_2O_2. The initial rates for **6b** ($v_0 = 23.8 \pm 0.9\,\mu\mathrm{M\ min^{-1}}$) and **7b** ($v_0 = 8.5 \pm 1.2\,\mu\mathrm{M\ min^{-1}}$) were ca. 20 and 7 times, respectively, higher than recorded for diphenyl diselenide ($v_0 = 1.2 \pm 0.5\,\mu\mathrm{M\ min^{-1}}$), a commonly used benchmark in this system.

In conclusion, we have found that introduction of alkyltelluro groups into the tocopherol scaffold causes a significant improvement of the antioxidant capacity. The novel chalcogen analogues are better quenchers of peroxyl radicals than the parent compounds. In contrast to the natural vitamins, they were highly regenerable by aqueous phase *N*-acetylcysteine in a simple membrane model system. As a further consequence of the chalcogen substitution, the novel vitamin E derivatives could catalyze hydroperoxide reduction in the presence of thiol reducing agents (glutathione peroxidase-like activity). Although the toxicity of organotelluriums has only been little studied,[17] it would seem interesting to explore the effects of the novel vitamin E analogues in biological systems under conditions where there is an overproduction of reactive oxygen and nitrogen species (oxidative stress).

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Supporting Information Available. Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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