The Antioxidant Profile of 2,3-Dihydrobenzo[*b*]furan-5-ol and Its 1-Thio, 1-Seleno, and 1-Telluro Analogues

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Abstract: A novel synthesis of 2,3-dihydrobenzo[*b*]thiophene-5-ol based on intramolecular homolytic substitution on sulfur was reported. The "antioxidant profile" of the series of 2,3-dihydrobenzo[*b*]furan-5-ol (**2a**) its 1-thio (**2b**), 1-seleno (**2c**) and 1-telluro (**2d**) analogues was determined by studies of redox properties, the capacity to inhibit stimulated lipid peroxidation, the reactivity toward *tert*-butoxyl radicals, the ability to catalyze decomposition of hydrogen peroxide in the presence of glutathione, and the inhibiting effect on stimulated peroxidation in liver microsomes. The one-electron reduction potentials of the aroxyl radicals corresponding to compounds **2a**−**2d**, $E°$ ($ArO^{\bullet}/ArO^{-}$) were 0.49, 0.49, 0.49, and 0.52 V vs NHE, respectively, as determined by pulse radiolysis. With increasing chalcogen substitution the compounds become slightly more acidic (pK_a = 10.6, 10.0, 9.9, and 9.5, respectively, for compounds **2a**−**2d)**. By using Hess' law, the homolytic O−H bond dissociation enthalpies of compounds **2a**−**2d** (340, 337, 336, and 337 kJ mol^{-1}, respectively) were calculated. The reduction potentials for the proton coupled oxidation of compounds **2a**−**2d** ($ArOH \rightarrow ArO^{\bullet} + H^{+}$) as determined by cyclic voltammetry in acetonitrile were 1.35 (irreversible), 1.35 (quasireversible) 1.13 (reversible), and 0.74 (reversible) V vs NHE, respectively. As judged by the inhibited rates of peroxidation, R_{inh}, in a water/chlorobenzene two-phase lipid peroxidation system containing *N*-acetylcysteine as a thiol-reducing agent in the aqueous phase, the antioxidant capacity increases (**2d** > **2c** = **2b** > **2a**) as one traverses the group of chalcogens. Whereas the times of inhibition, T_{inh}, were slightly reduced for the oxygen (**2a**) and sulfur (**2b**) derivatives in the absence of the thiol-reducing agent, they were drastically reduced for the selenium (**2c**) and tellurium (**2d**) derivatives. This seems to indicate that the organochalcogen compounds are continuously regenerated at the lipid aqueous interphase and that regeneration is much more efficient for the selenium and tellurium compounds. The absolute rate constants for the oxidation of compounds **2a**−**2b** by the *tert*-butoxyl radical in acetonitrile/di-*tert*-butyl peroxide (10/1) were the same−2 × 10^8 M^{-1} s^{-1}. Whereas the oxygen, sulfur, and selenium derivatives **2a**−**2c** were essentially void of any glutathione peroxidase-like activity, the organotellurium compound **2d** accelerated the initial reduction of hydrogen peroxide, *tert*-butyl hydroperoxide, and cumene hydroperoxide in the presence of glutathione 100, 333, and 213 times, respectively, as compared to the spontaneous reaction. Compounds **2a**−**2d** were assessed for their capacity to inhibit lipid peroxidation in liver microsomes stimulated by Fe(II)/ADP/ascorbate. Whereas the oxygen, sulfur, and selenium compounds showed weak inhibiting activity (IC_{50} values of ∼250, 25, and 13 μM, respectively), the organotellurium compound **2d** was a potent inhibitor with an IC_{50} value of 0.13 μM.

Introduction

α-Tocopherol (**1a**), the main component of vitamin E, other 6-hydroxychromanes as well as the corresponding ring-contracted analogues—2,3-dihydrobenzofurans—are some of the most active peroxyl radical trapping agents known.[1] Ingold and co-workers attributed the improved antioxidant activity of these compounds (as compared to other phenols lacking an oxygen substituent para to the phenolic oxygen) to a stereoelectronic effect,[2] involving the p-type lone pair orbital of the nonphenolic oxygen. Sulfur is considered to be more effective than oxygen at stabilizing a neighboring radical center.[3] It was therefore surprising that 1-thio-α-tocopherol (**1b**) and related 6-hydroxythiochromanes were found slightly less reactive toward peroxyl radicals than their corresponding chromane derivatives.[4] 1-Seleno-α-tocopherol (**1c**) and 1-telluro-α-tocopherol (**1d**) have not yet been prepared. Organoselenium and organotellurium compounds show many interesting antioxidative properties. As one traverses group 16 of the periodic table, the elements become

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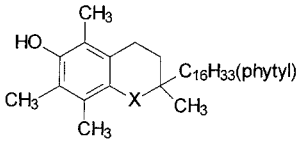

1 a X=O
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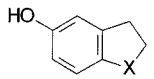
c X=Se
d X=Te

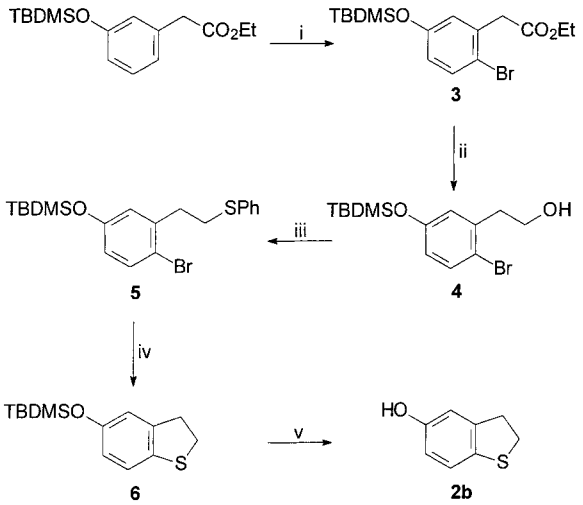


2 a X=O
b X=S
c X=Se
d X=Te

less electronegative. Thus, electron transfer to reactive alkoxyl and peroxyl radicals becomes more likely to occur. In addition to this chain-breaking antioxidative capacity, organochalcogens are preventive antioxidants in the sense that they reduce organic hydroperoxides to the corresponding alcohols. The selenium-containing glutathione peroxidases[5] catalyze the reduction of hydrogen peroxide, fatty-acid hydroperoxides, and phospholipid and cholesterol hydroperoxides using the tripeptide glutathione and other thiols as stoichiometric reducing agents. We and others have reported organoselenium and organotellurium compounds with chain-breaking donating[6,7] or thiol peroxidase activity.[8]

Recently, we described a tandem $S_{RN}1/S_Hi$ sequence for radical-based synthesis of 5-hydroxy-2,3-dihydrobenzo[*b*]selenophene (**2c**) and 5-hydroxy-2,3-dihydrobenzo[*b*]tellurophene.[9] It occurred to us that these materials, together with the known oxygen (**2a**) and sulfur (**2b**) analogues, constitute a nice series of compounds for studying antioxidant capacity as a function of the chalcogen substituent para to the phenolic group. In the following we report the "antioxidant profile" of compounds **2** as determined by studies of their redox properties, their capacity to inhibit stimulated lipid peroxidation, their reactivity toward *tert*-butoxyl radicals, their ability to catalyze decomposition of hydrogen peroxide in the presence of glutathione, and their inhibiting effect on peroxidation in liver microsomes.

Scheme 1[a]



[a] Conditions: (i.) Br_2, KOAc, HOAc, 15−17 °C, 2.5 h, 97%. (ii.) $LiAlH_4$, Et_2O, 0 °C, 70 min, 93%. (iii.) $(PhS)_2$, Bu_3P, C_6H_6, ambient temperature, 8 h, 76%. (iv.) *n*-Bu_3SnH, AIBN, C_6H_6, reflux, 44 h. (v.) TBAF, THF, ambient temperature, 62% from **5**.

Results and Discussion

Synthesis. 5-Hydroxy-2,3-dihydrobenzo[*b*]furan (**2a**) is readily prepared according to the literature.[10] However, for the known[11] sulfur derivative **2b** we developed an efficent alternative protocol based on homolytic substitution at sulfur (Scheme 1). TBDMS-protected ethyl 3-hydroxyphenylacetic acid was regioselectively brominated in the 6-position and the ester **3** reduced to the corresponding alcohol **4**. The required radical precursor **5** formed when the alcohol was treated with diphenyl disulfide in the presence of tri-*n*-butyl phosphine. Homolytic substitution at sulfur with expulsion of a phenyl radical occurred during heating in benzene in the presence of tri-*n* butyltin hydride and AIBN. Final deprotection using tetra-*n*-butylammonium fluoride afforded compound **2b**.

Acidity and Redox Properties. Data obtained from pK_a and redox studies of compounds **2** in aqueous solution are summarized in Table 1. Primary oxidation of the phenolates ArO^- corresponding to compounds **2** (pH 12, NaOH) was achieved by $N_3^\bullet$ produced in pulse radiolysis experiments by the reaction of $OH^\bullet$ with N_3^- (10^{-2} M NaN_3). To determine one-electron reduction potentials of the phenoxyl radicals, the redox equilibrium between the radical of interest and a redox couple with a known one-electron reduction potential was studied (eq 1).

$$ArO^\bullet + Ref \rightleftarrows ArO^- + Ref^{+\bullet} \qquad (1)$$

The equilibrium constant can be derived from the rate constants of the electron-transfer reaction and the back reaction and/or

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Table 1. Acidity and Redox Properties of Compounds **2** in Aqueous Solution

compd	pK_a (H_2O	λ_{max} ArO$^\bullet$ (nm)	reference substance	K	$E°$ (ArO$^\bullet$/ArO$^-$) (V vs NHE)	BDE O−H (kJ mol^{-1})
2a	10.6	450	**2b**	1	0.49	340
2b	10.0	560	4-MeOC_6H_4OH	7.86	0.49	337
2c	9.9	630	**2a**	1	0.49	336
2d	9.5	800	4-MeOC_6H_4OH	2.53	0.52	337

the equilibrium concentrations of the two redox couples.[12] The one-electron reduction potential of interest is then calculated from the equilibrium constant and the one-electron reduction potential of the redox reference couple using Nernst's equation ($\Delta E° = 0.0591 \log K$).

The phenolic O−H bond dissociation enthalpies given in Table 1 were calculated from eq 2, which is derived from Hess' law ($C = 232$ kJ mol^{-1} for phenols).[13,14]

$$\text{BDE (O−H)} = 96.48E° + 5.70\text{p}K_a + C \qquad (2)$$

As can be seen in Table 1, the pK_a values and the one-electron reduction potentials do not differ dramatically in the series of compounds **2**. Consequently, the O−H bond dissociation enthalpies are also essentially independent of the chalcogen (336−340 kJ mol^{-1}) and significantly higher than that reported for α-tocopherol (323 kJ mol^{-1}).[15] Interestingly, the experimentally determined O−H bond dissociation enthalpy for compound **2a** (relative to phenol) is identical to the corresponding relative value determined from DFT calculations by Wright et al.[16]

The large differences in the absorption maxima of the aryloxyl radicals corresponding to compounds **2a**−**d** (Table 1) can probably be attributed to the involvement of heavier atoms in the delocalization of the radical, resulting in a red-shift. This does not imply that the radical is more delocalized for heavier substituents since this would result in considerably lower one-electron reduction potentials. For the same reason, λ_{max} for 4-I-$C_6H_4O^\bullet$ is considerably red-shifted as compared to other phenoxyl radicals, for example, 4-Cl-$C_6H_4O^\bullet$ and 4-Br-$C_6H_4O^\bullet$; however, the one-electron reduction potentials are identical for these three radicals.[13]

It has previously been found that the one-electron reduction potentials of substituted phenoxyl radicals are linearly dependent on the Brown substituent constant, σ^+(eq 3).[13]

$$E° = 0.82 + 0.38\,\sigma_p^+ \qquad (3)$$

On the other hand, the phenolic pK_a's are linearly dependent on σ^-(eq 4).[17]

$$\text{p}K_a = 10.0 - \sigma_p^- \qquad (4)$$

Unfortunately, substituent constants for chalcogens contained in a five-membered ring are not available, and it is therefore not possible to make direct quantitative comparisons with the linear relationships derived for substituted phenols and phenoxyl radicals. However, if we compare the substituent constants for −OCH_3 ($\sigma^+ = -0.78$, $\sigma^- = -0.26$)[17] and −SCH_3 ($\sigma^+ = -0.60$, $\sigma^- = 0.06$)[17] we can conclude that the S-containing aryloxyl radical should have a somewhat higher one-electron reduction potential than the O-containing one and that the S-containing phenol should be somewhat more acidic than the O-containing one. Whereas the reduction potentials are identical for the O- and S-containing aryloxyl radicals, there is a significant difference in the phenolic pK_a values, pointing in the direction expected from the substituent constants of −OCH_3 and −SCH_3. The substituent constants for −$SeCH_3$ and −$TeCH_3$ are not known, but it is reasonable to assume that σ^+ and σ^- will increase somewhat with atomic number of the chalcogen. This is also reflected in the experimental data.

Table 2. Oxidation Potentials as Determined by Cyclic Voltammetry (E_p and $E_{1/2}$) and Pulse Radiolysis ($E°$)

compd	E (V vs Fc)	E (V vs NHE)	solvent
2a	0.66[a]	1.35[a]	MeCN
2b	0.66[b]	1.35[b]	MeCN
2c	0.44[b]	1.13[b]	MeCN
2d	0.05[b]	0.74[b]	MeCN
Ph-O-Me	−	1.62[c] (from ref 18)	H_2O
Ph-S-Me	−	1.45[c] (from ref 19)	H_2O
Ph-Se-Me	−	1.09[c] (from ref 19)	H_2O
Ph-Te-Me	−	0.74[c] (from ref 19)	H_2O
PhOH	−	1.5[c] (from ref 13)	H_2O
4-MeOC_6H_4OH	−	∼1.3[c] (from ref 13)	H_2O

[a] E_p. [b] $E_{1/2}$. [c] $E°$.

The oxidation potentials determined by cyclic voltammetry in acetonitrile are given in Table 2 along with the data for some related compounds. It should be noted that, whereas the oxidation potential for compound **2a** is irreversible (peak potential, E_p) and the one for compound **2b** is quasireversible, the potentials for compounds **2c** and **2d** are fully reversible. The potentials given for the other compounds in Table 2 are thermodynamically derived potentials.

If we compare the oxidation potentials of compounds **2a**−**2d** (recorded in acetonitrile) with those for the corresponding aryl methyl chalcogenides (recorded in water), assuming the solvent effect can be neglected,[20] some interesting conclusions can be drawn (Figure 1). For the selenium- and tellurium-containing compounds **2c** and **2d**, the oxidation potentials are essentially identical to those recorded for Ph-Se-Me and Ph-Te-Me, respectively. For the O- and S-containing analogues **2a** and **2b**, the potentials are lower than those recorded for Ph-O-Me and Ph-S-Me, respectively. The oxidation potentials for compounds **2a** and **2b** are approaching the value recorded for 4-MeOC_6H_4OH, though. Consequently, from a redox point of view, these compounds behave like phenols while the Se- and Te-containing compounds behave like methyl phenyl chalcogenides. In the latter case, the heteroatom is likely to be the redox center.

With the notable exception of strongly electron-donating substituents, the one-electron reduction potentials of 4-substi-

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(14) If we adopt 365.3 kJ mol^{-1} as the correct O−H bond dissociation energy for phenol, the constant C is 232 kJ mol^{-1}.
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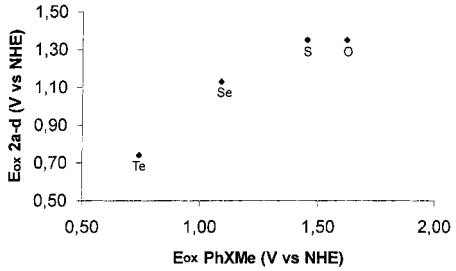
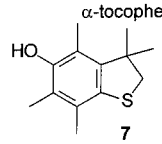
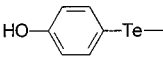


Figure 1. Oxidation potentials of compounds **2a**−**2d** (*y*-axis) plotted against the oxidation potentials of the corresponding methyl phenyl chalcogenides (PhOMe, PhSMe, PhSeMe, PhTeMe; *x*-axis).

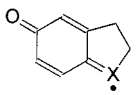
Table 3. Inhibited Rate of Peroxidation, R_{inh}, and Time of Inhibition, T_{inh}, for Antioxidants Tested

			without *N*-acetylcysteine in the aqueous phase	
antioxidant	R_{inh} (μM/h)[a]	T_{inh} (min)[b]	R_{inh} (μM/h)[a]	T_{inh} (min)[b]
2a	79	140	81	90
2b	64	180	53	85
2c	64	>300	113	50
2d	30	60	655	0
α-tocopherol 	19	90	18	80
7 	37	70	35	65
8	60	160	655	0

[a] Rate of peroxidation during the inhibited phase (uninhibited rate ca. 655 μM/h). [b] Duration of the inhibited phase of peroxidation.

tuted aryl alkyl chalcogenides were found to be insensitive to the nature of the substituent.[19] The rationale for this is that a strongly electron-donating substituent is required for the charge of the radical cation to become delocalized on the phenyl ring (to become an aromatic radical cation) rather than on the chalcogen. Since $-O^-$ is an extremely strong electron-donating substituent, this also explains why compounds **2a**−**2d** seem to behave as ordinary phenolates in alkaline aqueous solution.

Inhibition of Lipid Peroxidation. Azo-initiated peroxidation of linoleic acid or derivatives thereof has been often used for studying the antioxidative properties of synthetic and natural compounds.[21] Some time ago we reported a two-phase variant of this method which allows regeneration of the antioxidant by a water soluble thiol.[6] In the procedure used, linoleic acid and the antioxidant to be evaluated were vigorously stirred in chlorobenzene at 42 °C with an aqueous solution of *N*-acetylcysteine. 2,2′-Azobis(2,4-dimethylvaleronitrile) was added as an initiator in the organic phase and the progress of peroxidation monitored by HPLC with UV detection of conjugated diene at 234 nm. For comparison of catalyst efficiency, the inhibited rate of peroxidation, R_{inh}, was determined by least-squares methods from absorbance/time plots. The progress of peroxidation was followed for 300 min and the duration of the inhibited phase, T_{inh}, determined graphically as the cross-point for the inhibited and the uninhibited lines. R_{inh} and T_{inh} values for compounds **2a**−**2d** are shown in Table 3 together with those recorded for α-tocopherol, 3,3,4,6,7-pentamethyl-2,3-dihydrobenzo[*b*]thiophene-5-ol (**7**) and bis(4-hydroxyphenyl) telluride (**8**). For comparison, R_{inh} and T_{inh} values for all compounds were also recorded in the absence of thiol-reducing agent in the aqueous phase. It is clear from the inhibited rates of peroxidation in the absence of *N*-acetylcysteine that none of the synthetic compounds are as good as α-tocopherol as an antioxidant (R_{inh} = 18 μM/h). In fact, organotellurium compounds **2d** and **8** do not inhibit peroxidation at all (R_{inh} = 655 μM/h = uninhibited rate), and the organoselenium compound **2c** is a poor inhibitor (R_{inh} = 113 μM/h). Probably, the organotellurium compounds are rapidly deactivated (oxidized) by residual hydroperoxide contained in the linoleic acid. It is also noteworthy that introduction of methyl groups into the 3- and aromatic positions (compound **7**) significantly increases antioxidant efficiency (R_{inh} = 35 μM/h) as compared with the parent (compound **2b).** Under conditions where selenoxides and telluroxides are reduced by a thiol-reducing agent in the aqueous phase, the antioxidant efficiency *increases* in the series of compounds **2** as one traverses the periodic table from oxygen to tellurium (R_{inh} = 79, 64, 64, and 30 μM/h, respectively, for compounds **2a**−**2d)**. Except for α-tocopherol and compound **7**, all antioxidants investigated inhibit peroxidation for significantly longer times when *N*-acetylcysteine is present in the aqueous phase. This seems to indicate that the thiol is capable of regenerating the phenol at the aqueous lipid interphase. Obviously, this process is much less efficient with the two sterically hindered ortho-disubstituted phenolic compounds. Whereas the relative increase in inhibition time is only moderate for the oxygen and sulfur analogues **2a** and **2b**, it is substantial for organoselenium derivative **2c** and for tellurides **2d** and **8** (see Table 3). This could be indicative of an additional mechanism of regeneration for the Se- and Te-containing antioxidants. Whereas compounds **2a**−**b** are likely to be regenerated by H-atom donation to their respective phenoxyl radicals, compounds **2c**−**2d** may also be regenerated by reduction of their corresponding 1-oxides. These species are conceivably formed by hydroperoxide oxidation of the divalent chalcogenides or by further oxidation/hydrolysis of hypervalent chalcogen-centered radicals **9**. In contrast to the less efficient



9 X=Se, Te

organotellurium antioxidant bis(4-hydroxyphenyl) telluride (**8**),[6] T_{inh} for compound **2d** could not be extended by increasing the amount of thiol-reducing agent in the aqueous phase. This could indicate that compound **2d** (or its corresponding telluroxide) is somehow destroyed under the conditions of the peroxidation assay, for example via telluroxide syn elimination.

Reactivity toward *tert*-Butoxyl Radicals. Photodecomposition of di-*tert*-butyl peroxide by 355-nm laser pulses yields *tert*-butoxyl radicals within the duration of the pulse. This radical is known to abstract phenolic hydrogen atoms rapidly in comparison with decay by β-scisson.[22] The resulting phenoxyl radicals can be directly monitored by their spectral absorption. Buildup of the signal follows pseudo-first-order kinetics (eq 5)

$$k_{obs} = k_0 + k[\mathrm{ArOH}] \qquad (5)$$

where k_{obs} is the observed rate constant and k the absolute second-order rate constant for reaction of the *tert*-butoxyl radical

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with the phenolic compound (eq 6).

$$Me_3CO^{\bullet} + ArOH \xrightarrow{k} Me_3COH + ArO^{\bullet} \qquad (6)$$

Absolute rate constants for reaction of compounds **2** with the *tert*-butoxyl radical were measured in acetonitrile/di-*tert*-butyl peroxide (10/1, v/v). Plots according to eq 5 with monitoring at the λ_{max} of the corresponding phenoxyl radical (Table 1) gave the absolute rate constants *k*. Within experimental error, the rate constant for the oxygen (**2a**) and sulfur (**2b**) derivatives were the same: 2×10^8 M^{-1} s^{-1} (due to laser photoexitation of compounds **2c** and **2d**, assessment of the corresponding rate constants was impossible). For comparison, α-tocopherol was found to react under the same conditions with a rate of 6×10^8 M^{-1} s^{-1} and compound **7** with a rate of 4×10^8 M^{-1} s^{-1}. With the limited rate data at hand, it seems that the reactivity of the *tert*-butoxyl radical toward the series of compounds **2** and α-tocopherol is reflected by the phenolic O−H bond dissociation enthalpies given in Table 1 and consistent with a H-atom transfer mechanism.

Hydroperoxide-Decomposing Capacity. Hydroperoxide decomposition is maybe the most important duty for preventive antioxidants. In biological systems, this task is fulfilled by the Se-containing glutathione peroxidases and catalase. Third-row or higher divalent organochalcogen compounds react with hydrogen peroxide or organic hydroperoxides to form the corresponding oxides. Reactivity toward hydroperoxides usually increases as one traverses the group of chalcogens (sulfides < selenides < tellurides). Regeneration of the divalent organochalcogen compounds from their oxides by reducing agents also occur in a similar order. Thus, telluroxides and selenoxides are far more reactive than sulfoxides toward mild reducing agents such as thiols or ascorbate. For obtaining compounds that could decompose hydroperoxides in a catalytic fashion in the presence of thiols (thiol peroxidase activity), it seems that incorporation of selenium and tellurium rather than sulfur into the catalyst is likely to be successful. Thiol peroxidase activity can be conveniently assessed by using the coupled reductase method.[23] In this assay, hydroperoxide, glutathione, and the catalyst to be evaluated are allowed to react at pH 7.4 in the presence of glutathione reductase and NADPH. As soon as glutathione is oxidized to the corresponding disulfide, it will be enzymatically reduced. The reaction can be conveniently followed spectrophotometrically by observing the consumption of NADPH at 340 nm. The results with compounds **2** are shown in Table 4. Not unexpectedly, the oxygen and sulfur derivatives **2a** and **2b** were essentially void of any glutathione peroxidase activity. Catalyst activity (% catalysis) as determined by the initial rate increase (as compared with the uncatalyzed process) in the reaction between glutathione and hydroperoxide was also insignificant for the organoselenium derivative. In contrast, the organotellurium compound **2d** was a highly active catalyst. When hydrogen peroxide was used as the oxidant, NADPH was consumed almost 100 times faster than in the control experiment. To be sure that the organotellurium compounds act in a truely catalytic fashion under the conditions used, more NADPH, hydrogen peroxide, and glutathione were added to the incubation, and the consumption of NADPH was recorded again. After five repeated additions/recordings, no significant change in catalyst efficiency was seen. When *tert*-butyl hydroperoxide and cumene hydroperoxide were used as oxidants, the reaction was accelerated even more (333 and 213 times, respectively, as compared to the uncatalyzed reaction; Table 4).

Table 4. Glutathione Peroxidase-Like Activity of Compounds **2** with Hydroperoxide Substrates

catalyst	hydroperoxide	NADPH consumption[a] (μM/min)	% catalysis[b]
−	H_2O_2	6.3 ± 0.1	−
2a	H_2O_2	6.1 ± 0.3	97
2b	H_2O_2	6.0 ± 0.2	95
2c	H_2O_2	6.5 ± 0.3	103
2d	H_2O_2	624 ± 56[c]	9900
−	*t*-BuOOH	1.1 ± 0.2	−
2c	*t*-BuOOH	1.4 ± 0.2	127
2d	*t*-BuOOH	365 ± 17	33300
−	CuOOH[d]	2.4 ± 0.4	−
2c	CuOOH[d]	3.7 ± 0.4	156
2d	CuOOH[d]	511 ± 26	21300

[a] Calculated over a 20 s period of stable decline of absorption at 340 nm. [b] The catalyst percentage increase of the basal reaction rate between GSH and H_2O_2 was calculated as rate of NADPH consumption + 5 mol % catalyst (μM/min)/rate of NADPH consumption + vehicle (μM/min) × 100. [c] The following values [μM NADPH/min (% catalysis)] were recorded after repeated additions of 250 μM NADPH, 250 μM H_2O_2 and 500 μM GSH: 628.1 (9968), 640.2 (10161), 635.3 (10079), 622.8 (9885), 612.9 (9728). [d] The incubation contained 20% DMSO.

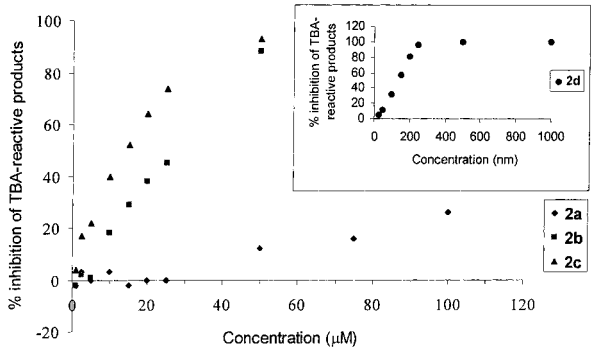

Figure 2. Inhibition of TBARS formation in Fe(II)/ADP/ascorbate-treated microsomes as a function of the concentration of compounds **2**. For compound **2d**, see inset.

The exceptional (as compared to the other chalcogen derivatives) nucleophilicity of compound **2d** toward hydroperoxide substrates is also reflected in its substantially lower oxidation potential (see Table 2).

Lipid Peroxidation in Microsomes. Microsomal fractions prepared from animal tissue undergo lipid peroxidation when incubated with Fe(II) salts or Fe(III) salts plus ascorbate or plus NADPH.[24] During peroxidation, cytochromes b_5 and P450 are attacked, and the heme groups are degraded. The progress of peroxidation can be conveniently followed by assessment of thiobarbituric acid-reactive substances (TBARS).[25] Compounds **2a**−**2d** were assessed for their capacity to inhibit lipid peroxidation in liver microsomes stimulated by Fe(II)/ADP/ascorbate. Whereas the oxygen, sulfur, and selenium compounds showed weak inhibiting activity (IC_{50} values of ∼250, 25, and 13 μM, respectively), the organotellurium compound **2d** was a potent inhibitor with an IC_{50} value of 0.13 μM. The concentration dependency of the antioxidant activity of compounds **2a**−**2d** are shown in Figure 2. The outstanding performance of the organotellurium compound **2d** seems to indicate that peroxide-decomposing capacity may be more important than chain-breaking ability to inhibit stimulated lipid peroxidation in

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microsomes under the conditions used. Also, the stimulant—ascorbate—may serve to amplify the protective effect by continuously regenerating the organotellurium and organoselenium compounds from their corresponding oxides.

Conclusions

From experimental pK_a and redox data, the homolytic O−H bond dissociation enthalpies of compounds **2a**−**2d** were estimated to be very similar (336−340 kJ mol^{-1}). These values were also reflected in the similar absolute rate constants (k = 2 × 10^8 M^{-1} s^{-1}) for reaction of two of the compounds with the *tert*-butyloxyl radical generated in laser flash photolysis experiments in acetonitrile/di-*tert*-butyl peroxide. As judged by the inhibited rates of peroxidation, R_{inh}, in a two-phase lipid peroxidation system containing a thiol-reducing agent in the aqueous phase, the antioxidant capacity increases as one traverses the group of chalcogens (**2d** > **2c** = **2b** > **2a**). This is probably because of facile regeneration of the organoselenium and organotellurium compounds at the lipid aqueous interphase. Thus, because of increasingly facile redox cycling, substitution with heavier chalcogens in the series of compounds **2** set the scene for a catalytic mode of action. Also, the introduction of tellurium (compound **2d**) imposes another antioxidative capacity on the molecule—the ability to catalytically decompose hydroperoxides in the presence of a stoichiometric reducing agent. As compared with the other chalcogen analogues, compound **2d** showed a far superior glutathione peroxidase-like behavior and an outstanding ability to protect liver microsomes subjected to stimulated lipid peroxidation. With the perspective to obtain antioxidants with similarly good H-atom donating capacity as α-tocopherol, but with a catalytic mode of action in the presence of mild reducing agents and glutathione peroxidase-like activity, we have recently embarked on the synthesis of the "real" selenium and tellurium analogues of α-tocopherol.

Experimental Section

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at 400 and 100 MHz, respectively. For proton spectra the residual peak of $CHCl_3$ was used as the internal reference (7.26 ppm), while the central peak of $CDCl_3$ (77.0 ppm) was used as the reference for carbon spectra. Silica gel was used for flash column chromatography. 5-hydroxy-2,3-dihydrobenzofuran,[10] 3,3,4,6,7-pentamethyl-2,3-dihydrobenzo[*b*]thiophene-5-ol,[26] bis(4-hydroxyphenyl)-telluride,[27] ethyl 3-(*tert*-butyldimethylsilyloxy)phenyl acetate, 5-hydroxy-2,3-dihydrobenzo[*b*]selenophene, and 5-hydroxy-2,3-dihydrobenzo-[*b*]tellurophene were prepared according to literature procedures.[9] Tetrahydrofuran was distilled under nitrogen from sodium/benzophenone. Benzene and dichloromethane were distilled under nitrogen from calcium hydride. Elemental analyses were performed by Analytical Laboratories, Lindlar, Germany.

Ethyl 6-Bromo-3-(*tert*-butyldimethylsilyloxy)phenyl Acetate (3). A solution of bromine (525 μL, 10.2 mmol) in acetic acid (30 mL) was added dropwise to a magnetically stirred solution of ethyl 3-(*tert*-butyldimethylsilyloxy)phenyl acetate (3.00 g, 10.2 mmol) and potassium acetate (1.00 g, 10.2 mmol) in acetic acid (60 mL) maintained at 15 °C. The reaction was then stirred at 15−17 °C for 2.5 h. After filtration of salt, water and diethyl ether were added, and the organic layer was separated. The aqueous phase was then extracted with diethyl ether (3×). The combined organic phases were washed with saturated $NaHCO_3$ solution (6×), water, and brine. The organic phase was then dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give the title compound (3.69 g, 97%) as a colorless oil, which was used in the next step without further purification. ^{1}H NMR δ 0.19 (6H, s), 0.97 (9H, s), 1.26 (3H, t, J = 7.1 Hz), 3.70 (2H, s), 4.18 (2H, q, J = 7.1 Hz), 6.63 (1H, dd, J = 8.6, 2.9 Hz), 6.79 (1H, d, J = 2.9 Hz), 7.38 (1H, d, J = 8.6 Hz). ^{13}C NMR δ −4.5, 14.2, 18.2, 25.6, 41.8, 61.0, 116.1, 120.6, 123.2, 133.2, 135.2, 155.0, 170.4. Anal. Calcd for $C_{16}H_{25}BrO_3Si$: C, 51.47; H, 6.75. Found: C, 51.39; H, 6.68.

4-Bromo-3-(2-hydroxyethyl)phenyl *tert*-Butyldimethylsilyl Ether (4). To a solution of compound **3** (3.15 g, 8.41 mmol) in dry diethyl ether (95 mL) was added $LiAlH_4$ (0.390 g, 10.3 mmol) in one portion at −20 °C under an atmosphere of dry nitrogen. After 70 min of stirring at 0 °C, the reaction was quenched by addition of aqueous HCl (1 M), and the organic layer was separated. The aqueous layer was extracted with diethyl ether (3×), and the combined organic phases were washed with water and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give the title compound (2.59 g, 93%) as a pale yellow oil, which was used in the next step without further purification. ^{1}H NMR δ 0.19 (6H, s), 0.97 (9H, s), 1.41 (1H, br s), 2.95 (2H, t, J = 6.7 Hz), 3.86 (2H, t, J = 6.8 Hz), 6.60 (1H, dd, J = 8.6, 2.9 Hz), 6.77 (1H, d, J = 2.9 Hz), 7.37 (1H, d, J = 8.6 Hz). ^{13}C NMR δ −4.4, 18.2, 25.6, 39.4, 62.1, 115.8, 120.0, 123.0, 133.4, 138.7, 155.0.

4-Bromo-3-[2-(phenylthio)ethyl]phenyl *tert*-Butyldimethylsilyl Ether (5). To a solution of compound **4** (1.76 g, 5.33 mmol) in dry benzene (45 mL) was added diphenyl disulfide (1.28 g, 5.86 mmol) and tributylphosphine (1.45 mL, 5.86 mmol) under an atmosphere of dry nitrogen. The reaction mixture was then stirred at ambient temperature for 8 h. $NaHCO_3$ (5% aq) was added, and the layers were separated. The aqueous phase was extracted with diethyl ether (3×). The combined organic phases were washed with $NaHCO_3$ (5% aq), water, and brine. After drying ($MgSO_4$), filtration, and concentration in vacuo, the residue was purified by flash chromatography (pentane:EtOAc, 99:1) to furnish 1.71 g (76%) of the title compound as a colorless oil. ^{1}H NMR δ 0.19, (6H, s), 0.97 (9H, s), 2.97 (2H, m), 3.15 (2H, m), 6.59 (1H, dd, J = 8.6, 2.9 Hz), 6.71 (1H, d, J = 2.9 Hz), 7.19 (1H, m), 7.28−7.40 (5H, m). ^{13}C NMR δ −4.4, 18.2, 25.6, 33.2, 36.3, 115.4, 120.1, 122.6, 126.0, 128.9, 129.3, 133.4, 136.1, 140.3, 155.1. Anal. Calcd for $C_{20}H_{27}$-BrOSSi: C, 56.72; H, 6.43. Found: C, 56.77; H, 6.41.

5-(*tert*-Butyldimethylsilyloxy)-2,3-dihydrobenzo[*b*]thiophene (6). To a solution of compound **5** (0.300 g, 0.708 mmol) in dry degassed benzene (75 mL) under nitrogen was added AIBN (8.7 mg, 0.053 mmol). The reaction mixture was then heated to reflux, and tributyltin hydride (230 μL, 0.850 mmol) was added. Heating was then continued at reflux for 17 h, and another 1.2 equiv of tin hydride was added together with another 0.075 equiv of AIBN. The reaction was then refluxed for 10 h and another 0.075 equiv of AIBN was added, and the reaction was refluxed for 17 h. According to ^{1}H NMR, the starting material was now almost consumed. After cooling of the flask, the sovent was removed in vacuo. Purification of the crude material by flash chromatography (pentane) afforded the title compound as a colorless oil together with an inseparable impurity. The crude material was subjected to deprotection. ^{1}H NMR δ 0.17 (6H, s), 0.97 (9H, s), 3.21 (2H, m), 3.35 (2H, m), 6.61 (1H, m), 6.70 (1H, m), 7.02 (1H, m). ^{13}C NMR δ −4.5, 18.2, 25.7, 33.9, 36.5, 116.8, 119.0, 122.3, 132.8, 141.6, 153.0.

5-Hydroxy-2,3-dihydrobenzo[*b*]thiophene (2b). To a solution of compound **6** (165 mg, 0.617 mmol) in dry THF (20 mL) was added tetra-*n*-butylammonium fluoride (650 μL, 1.0 M in THF, 0.648 mmol) under an atmosphere of dry nitrogen. The reaction mixture was then stirred at ambient temperature for 1 h. Water was added, and the mixture was extracted with diethyl ether (3×). The combined organic phases were washed with water and brine and dried over $MgSO_4$, and the solvent was removed in vacuo. Flash chromatography (EtOAc/pentane 10:90) afforded the title compound (67 mg, 62% from compound **3**) as white crystals; mp 86−88 °C (lit. 87−89 °C).[11b] ^{13}C NMR δ 33.8, 36.4, 112.3, 114.4, 122.6, 132.3, 141.9, 153.0.

pK_a Values. The pK_a's of the phenols were determined by recording the UV−vis spectra at a number of different pH values. The spectral differences between the phenols and the corresponding phenolates were used to generate pK_a plots from which the pK_a could be determined.

Pulse Radiolysis. Radiolysis of water results in the formation of OH$^\bullet$, e_{aq}^-, H_2O_2, H_2, and H_3O^+, with OH$^\bullet$ and e_{aq}^- being the major

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radical species with primary radiation chemical yields of 0.28 μmol/J each above pH 3. N_2O-saturated solutions were used throughout in order to convert the reducing solvated electron into the oxidizing hydroxyl radical ($G_{OH} = 5.6 \times 10^{-7}$ mol/J).[28] Millipore Milli-Q filtered water was used throughout. The pulse radiolysis equipment consists of a linear accelerator delivering 3-MeV electrons and a computerized optical detection system.[29] The pulses were of 5−10 ns duration, giving doses of 3−6 Gy. For dosimetry a N_2O-saturated 10^{-2} KSCN solution was used.[30] The $G\epsilon$ value of $(SCN)_2^{\bullet-}$ was taken to be 4.78×10^{-4} m^2/J at 500 nm.

Cyclic Voltammetry. Cyclic voltammetry was performed with a PAR 263A Potentiostat/Galvanostat interfaced to a base PC using the EG&G model 270 software package. The cell was a standard three-electrode setup using a 3 mm diameter glassy carbon working electrode, a platinum coil counter electrode, and a calomel reference electrode. Acetonitrile was of the purest grade available, and the supporting electrolyte, 0.1 M tetrabutylammonium perchlorate (TBAP), was recrystallized from 10% hexane in ethyl acetate. The scan rate was 500 mV/s, and full IR compensation was employed in all measurements.

Peroxidation Assay. An HPLC (Waters 600 E) equipped with an autoinjector (5 μL samples were injected every 10 min) with a sample holder at 42.0 °C (Gilson 233 XL with thermostated sample rack), Photodiode Array Detector (Waters 996), and a Millenium 32 chromatography data system was used for the peroxidation studies. In a typical experiment linoleic acid in chlorobenzene (7.5 mL, 36.2 mM) was stirred (1100 rpm) in a 20 mL thermostated reaction vessel. To this solution the inhibitor in ethanol (107 μL, 3.0 mM; 40 μM final concentration) was added by syringe followed by an aqueous thermostated solution of NAC (8.0 mL, 1.0 mM). After 25 min of stirring/equilibration, a thermostated solution of AMVN in chlorobenzene (0.5 mL, 22.4 mM) was added. Samples were withdrawn (after interruption of the stirring and phase separation during 30 s) from the lower chlorobenzene layer and injected onto a Waters Resolve Silica 90 Å column (5 μM, 3.9 × 150 mm) eluted with hexane/ethanol (90/10) at a flow rate of 1.0 mL/min. After sampling, stirring was immediately resumed. The formation of conjugated dienes (retention time 2.8−3.2 min) was monitored at 234 nm and the concentration determined by integration using an experimentally determined response factor (a weighted amount of linoleic acid containing a trace of linoleic acid hydroperoxide was allowed to react in $CDCl_3$ with bis[4-(dimethylamino)phenyl]telluride, and the conversion to the corresponding telluroxide was determined by integration in the ^{1}H NMR spectrum).

The inhibited rate of peroxidation, R_{inh} (Table 3), was calculated by least-squares methods from plots of linoleic acid hydroperoxide concentration versus time during the inhibited phase of peroxidation. In a blank experiment, in the absence of inhibitor, the uninhibited rate of peroxidation was ~655 μM/h. T_{inh} values were determined graphically as the cross-point for the inhibited and the uninhibited lines. Linoleic acid hydroperoxide concentration increased in a linear fashion for $0 < t < \sim 0.75\ T_{inh}$. Chain lengths, ν_t, in the beginning of the inhibited reaction ($t = 0.1 \times T_{inh}$) were calculated as described in the literature[1b] assuming $n = 2$ for all antioxidants:

Antioxidant nr; ν_t in the presence of NAC (ν_t in the absence of NAC): **2a**; 2.6 (1.7); **2b**; 2.7 (1.0), **2c**; 4.4 (1.3), **2d**; 0.42 (−), α-tocopherol; 0.40 (0.33), **7**; 0.60 (0.53), **8**; 2.2 (−).

In some of these experiments $\nu_t < 1$. This means the inhibited autoxidation is not a chain reaction. However, since the rate constant for the scavenging of peroxyl radical with α-tocopherol calculated using a similar procedure[21c] to the one described in this paper is in satisfactory agreement with earlier rate determinations,[21a] we believe that the observed R_{inh} values reflect antioxidant activities of the compounds studied.

Laser Flash Photolysis. The laser flash photolysis (LFP) experiments were carried out using a modification of the procedure described in ref 31. Briefly, experiments were carried out using a LKS60 flash photolysis spectrometer from Applied Photophysics. For excitation, the frequency tripled fundamental at 355 nm from a YAG-laser (Quantel Brilliant) was used. The pulses had a duration of ~6 ns and an energy of ~200 mJ/pulse. The pulses were defocused to somewhat reduce the excitation intensity. A cross-beam overlap with the analyzing light (150 W pulsed Xe-lamp) was obtained using a cylendrical lens. The time resolution of the instrument was <10 ns. *tert*-Butoxyl radicals were generated by 355 nm LFP of di-*tert*-butyl peroxide in acetonitrile (1/10, v/v) in the presence of the H-atom donating substrate. The reaction was followed by monitoring the growth in the absorption of the respective aryloxyl radical at the appropriate wavelength (440 nm for α-tocopherol; for the other compounds see Table 1). Pseudo-first-order rate constants (k_{obs}) were determined at 298 °C using digitally averaged growth curves from five to 10 laser flashes. Absolute second-order rate constants (k) were calculated by least-squares fitting of k_{obs} vs [ArOH] for five different ArOH concentrations (0.3−5 mM).

Coupled Reductase Assay. The glutathione peroxidase-like activity of the compounds under study was assessed by their ability to catalyze the reaction between hydroperoxides and glutathione in an aqueous buffer at physiological pH. The oxidation of GSH to GSSG was measured indirectly by spectrophotometrically assessing the stimulated oxidation of NADPH in the presence of glutathione reductase. Incubations were conducted at room temperature in a Shimadzu model 160 spectrophotometer recording at 340 nm with air as a reference. They were constructed in the following manner: Incubations in quartz cuvettes were with 50 mM potassium phosphate buffer pH 7.4 containing 20% DMSO where necessary (1 mL). Additions were made in the order (all final concentrations): NADPH (250 μM), GSH (1 mM), test substance (50 μM), record baseline, GSSG reductase (1 unit), record, hydroperoxide (1 mM), record the decline in absorbance. Rate assessments were performed when the decline in absorbance was constant for at least 20 s. Control experiments revealed that the observed catalytic action of the compounds was not influenced by increasing amounts of GSSG reductase (0.5, 1.0 and 2.0 units) in the incubation. Controls also showed that none of the compounds directly interacted with the reduction of GSSG (250 and 500μM) by the reductase.

Lipid Peroxidation in Microsomes. The livers of male Sprague−Dawley rats were exsanguinated, excised, and homogenized in ice-cold sucrose (250 mM)/phosphate (50 mM) buffer, pH 7.4, using a polytron. The homogenate was centrifuged once at 12000*g*, at 4 °C for 60 min. The pellets were resuspended and washed twice with 150 mM KCl before being used in the experiments. Microsome preparations were always freshly prepared.

Microsomal lipid peroxidation was performed in incubations constructed as follows: incubations (1 mL) in phosphate buffer (50 mM), pH 7.4, containing microsomal protein (1 mg), ADP (200 μM), $FeSO_4$ (1 mM), and DMSO vehicle/test substance were preincubated for 5 min at 37 °C before addition of the initiation stimulus ascorbate (50 μM). For screening experiments, the accumulation of thiobarbituric acid reactive substances over 30 min of incubation in antioxidant-treated samples was compared to control levels in microsomes treated with DMSO vehicle only.[32] The DMSO concentration of the incubations never exceeded 0.5% (v/v). Aliquots, (0.5 mL) of incubation were mixed with equal amounts of trichloroacetic acid (10% aq) containing 10 mM butylated hydroxytoluene and then reacted with thiobarbituric acid at 95 °C for 15 min. The samples were then centrifuged (1000*g*, 5 min), and the absorbance was determined at 535 nm. The concentration of malondialdehyde was determined using $\epsilon = 1.56 \times 10^5$ M^{-1} cm^{-1}. Individual 50% inhibition concentrations (IC_{50} values) were calculated from best-fit curves of the inhibitory effect as a function of concentration. Controls demonstrated that the compounds did not react with thiobarbituric acid reactive compounds in the system.

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