ORGANIC LETTERS

2008
Vol. 10, No. 21
4895−4898

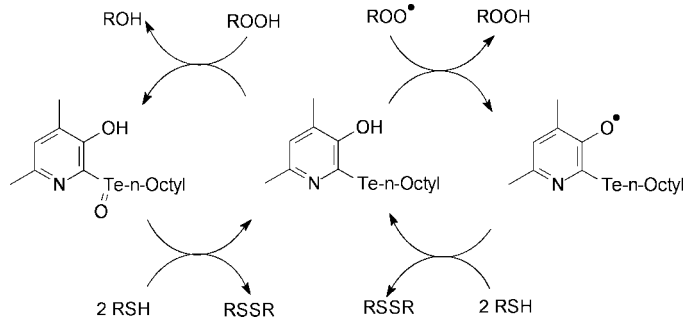
Catalytic Chain-Breaking Pyridinol Antioxidants

Sangit Kumar, Henrik Johansson, Takahiro Kanda, Lars Engman,* Thomas Müller,† Mats Jonsson,† Gian Franco Pedulli,‡ Silvia Petrucci,‡ and Luca Valgimigli‡

Department of Biochemistry and Organic Chemistry, Uppsala University, Box 576, SE-75123 Uppsala, Sweden
lars.engman@biorg.uu.se

Received August 25, 2008

ABSTRACT



When assayed for their capacity to inhibit azo-initiated peroxidation of linoleic acid in a water/chlorobenzene two-phase system, tellurium-containing 3-pyridinols were readily regenerable by *N*-acetylcysteine contained in the aqueous phase. The best inhibitors quenched peroxyl radicals more efficiently than α-tocopherol, and the duration of inhibition was limited only by the availability of the thiol reducing agent. The compounds were also found to catalyze reduction of hydrogen peroxide in the presence of thiol reducing agent.

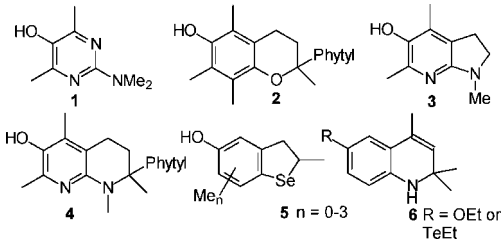
The majority of chain-breaking antioxidants, both in Nature[1] and in man-made materials,[2] is phenolic. The antioxidant activity of these compounds stems from their ability to transfer the phenolic hydrogen to lipidperoxyl radicals at a rate much faster than the chain-propagating H-atom transfer step of lipid peroxidation. It is well-known that electron-donating ortho- and para-substituents in the phenolic moiety weaken the O−H bond[3] and thus increase the rate of hydrogen atom transfer. However, improving the antioxidant activity of phenolic compounds along these lines will only be successful until the ionization potential of the compounds (which is also decreased by introduction of electron-donating groups) becomes so low[4] that the material will be consumed in a spontaneous electron-transfer reaction with molecular oxygen. Recently, by incorporating one or two nitrogens into the hydroxyaromatic ring, the groups of Pratt and Valgimigli found a way to circumvent the problem with the low ionization potential. Thus, whereas the O−H bond dissociation enthalpies (BDEs) of substituted 5-pyrimidinols and 3-pyridinols differed only marginally from those of the corresponding phenols, the ionization potentials were substantially higher. The O−H bond of 5-pyrimidinol **1** (78.2 kcal/mol) has almost the same strength as that of α-tocopherol (**2**; 78.3 kcal/mol), but the calculated ionization potential is 7.7 kcal/mol higher (167.0 kcal/mol).[5] Furthermore, as judged by the capacity to inhibit autoxidation of styrene, pyrimidinol **1** ($k_{inh} = 8.6 \times 10^6$ $M^{-1}s^{-1}$) transfers its phenolic hydrogen atom to peroxyl radicals almost three

† School of Chemical Science and Engineering, Nuclear Chemistry, Royal Institute of Technology, 10044 Stockholm, Sweden.
‡ Dipartimento di Chimica Organica "A. Mangini", Università di Bologna, 40126 Bologna, Italy.

(1) Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194–201.
(2) Zweifel, H. *Stabilization of Polymeric Materials*; Springer: Berlin, Germany, 1997.
(3) (a) Lucarini, M.; Pedrielli, P.; Pedulli, G. F. *J. Org. Chem.* **1996**, *61*, 9259–9263. (b) Wright, J. S.; Johnson, E. R.; DiLabio, G. A. *J. Am. Chem. Soc.* **2001**, *123*, 1173–1183.
(4) Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053–7065.
(5) Pratt, D. A.; DiLabio, G. A.; Brigati, G.; Pedulli, G. F.; Valgimigli, L. *J. Am. Chem. Soc.* **2001**, *123*, 4624–4626.

10.1021/ol801983n CCC: $40.75
Published on Web 10/02/2008

times as fast as α-tocopherol ($k_{inh} = 3.2 \times 10^6$ $M^{-1}s^{-1}$). It has been hypothesized that this rate enhancement is due to polar effects in the transition state of the atom-transfer reaction.[6] Incorporation of an electron-donating amino substituent para to the hydroxyl group in the 3-pyridinol scaffold in the form of a fused five-membered ring (compound **3**) resulted in the most effective phenolic chain-breaking antioxidant reported to date. Its ionization potential is 7.0 kcal/mol lower than calculated for α-tocopherol, and therfore, it is slowly decomposing when exposed to atmospheric oxygen. Due to the 2.8 kcal/mol weaker O−H BDE of compound **3** than α-tocopherol, the reactivity toward peroxyl radicals is an impressive 88-fold higher ($k_{inh} = 280 \times 10^6$ $M^{-1}s^{-1}$).[7,8] The synthesis and antioxidative properties of more Vitamin E-like derivatives of this kind,[9] such as the naphthyridinol **4**, have recently been described.[10]



α-Tocopherol, the most reactive component of Vitamin E, is known to trap two peroxyl radicals before it is converted into nonradical products. Nature has therefore arranged for its regeneration to allow for a catalytic mode of action of the antioxidant. In biological membranes, this process is thought to occur by donation of a hydrogen atom from ascorbate (AscOH) to the α-tocopheroxyl radical at the lipid−aqueous interphase (eq 1).[11−14]

$$\alpha\text{-TO}^{\bullet} + \text{AscOH} \rightarrow \alpha\text{-TOH} + \text{AscO}^{\bullet} \qquad (1)$$

The tripeptide glutathione (GSH) is present in much higher concentrations than ascorbate in human plasma. It is known to act as a biological antioxidant and reducing agent both by one-electron (hydrogen atom) and two-electron (e.g., as a cofactor for the glutathione peroxidase enzymes) chemistry. However, early studies by Barclay[15] showed that GSH is incapable of regenerating α-tocopherol from the α-tocopheroxyl radical in simple model systems. In our search for other chain-breaking antioxidants which could perform in a catalytic fashion in the presence of thiols, we recently found that 2,3-dihydrobenzo[*b*]selenophene-5-ols **5**[16] as well as ethoxyquins **6**[17] were regenerable by *N*-acetylcysteine when assayed for their capacity to inhibit azo-initiated peroxidation of linoleic acid in a two-phase system. Considering that none of these antioxidants quenched peroxyl radicals as efficiently as α-tocopherol, we thought it would be interesting to modify the efficient pyridinol antioxidants in such a way that they could also act in a catalytic fashion in the presence of stoichiometric amounts of a thiol reducing agent. Initially, 3-pyridinols **7a**−**c** (Table 1) substituted in the 6-position with octyltelluro-, octylseleno-, and octylthio groups, respectively, and the corresponding 2-substituted derivatives **8a**−**c** were prepared starting from readily available 6-bromo- and 2-bromo-3-pyridinol. The capacity of these antioxidants to inhibit 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN)-initiated peroxidation of linoleic acid (L-H) to the corresponding hydroperoxide (L-OOH) in a biphasic chlorobenzene/water system was very dependent on the experimental conditions (eq 2 and Table 1). In the absence of *N*-acetylcysteine in the aqueous phase, the two organotelluriums **7a** and **8a** did not inhibit peroxidation at all. However, in the presence of the thiol reducing agent, the rate of linoleic acid hydroperoxide formation in the chlorobenzene layer, R_{inh}, was only slightly higher (32 and 27 μM/h, respectively) than recorded using α-tocopherol

$$\text{L-H} \xrightarrow[\text{oxidant in chlorobenzene/}\ N\text{-acetylcysteine in } H_2O]{\text{AMVN, } O_2\text{, anti-}} \text{L-OOH} \qquad (2)$$

($R_{inh} = 24$ μM/h) as an antioxidant under identical conditions. α-Tocopherol is a nonregenerable antioxidant which inhibits peroxidation for 90 min under the standard conditions of our assay. Whereas the inhibition period for compound **7a** was 70 min only, antioxidant **8a** was clearly regenerable with an inhibition time, T_{inh}, of 200 min. The selenium (**7b**, **8b**) and sulfur (**7c**, **8c**) analogues were markedly poorer quenchers of peroxyl radicals ($115 < R_{inh} < 203$ μM/h) than the organotelluriums, and the addition of thiol to the aqueous phase always caused an increase (17−100%) in T_{inh} (Table 1).

Introduction of additional electron-releasing groups was thought to further improve the chain-breaking capacity of our antioxidants. Compounds **9a**−**c** were therefore prepared from commercially available 2-iodo-6-methyl-3-pyridinol and evaluated together with the corresponding phenylchalcogeno compounds **10a**−**c** that we recently prepared.[18] As noted above,

(6) Valgimigli, L.; Brigati, G.; Pedulli, G. F.; DiLabio, G. A.; Mastragostino, M.; Arbizzani, C.; Pratt, D. A. *Chem.−Eur. J.* **2003**, *9*, 4997–5010.

(7) Wijtmans, M.; Pratt, D. A.; Valgimigli, L.; DiLabio, G. A.; Pedulli, G. F.; Porter, N. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 4370–4373.

(8) Wijtmans, M.; Pratt, D. A.; Brinkhorst, J.; Serwa, R.; Valgimigli, L.; Pedulli, G. F.; Porter, N. A. *J. Org. Chem.* **2004**, *69*, 9215–9223.

(9) (a) Kim, H.-Y.; Pratt, D. A.; Seal, J. R.; Wijtmans, M.; Porter, N. A. *J. Med. Chem.* **2005**, *48*, 6787–6789. (b) Nam, T.; Wijtmans, M.; Pratt, D. A.; Porter, N. A. *Synthesis* **2005**, 1397–1404.

(10) Nam, T.; Rector, C. L.; Kim, H.; Sonnen, A.F.-P.; Meyer, R.; Nau, W. M.; Atkinson, J.; Rintoul, J.; Pratt, D. A.; Porter, N. A. *J. Am. Chem. Soc.* **2007**, *129*, 10211–10219.

(11) Halliwell, B.; Gutteridge, J. M. C. *Free radicals in biology and medicine*, 4th ed.; Oxford University Press: Oxford, England, 2007; p 168.

(12) Kamal-Eldin, A.; Appelquist, L.-Å. *Lipids* **1996**, *31*, 671–701. See refs 266−286. See also: Sato, K.; Niki, E.; Shimasaki, H. *Arch. Biochem. Biophys.* **1990**, *279*, 402–405. Bisby, R.; Parker, A. W. *Arch. Biochem. Biophys.* **1995**, *317*, 170–178. Noguchi, N.; Niki, E. *Free Rad. Res.* **1998**, *28*, 561–572. Niki, E.; Noguchi, N. *Acc. Chem. Res.* **2004**, *37*, 45–51.

(13) Niki, E.; Saito, T.; Kawakami, A.; Kamiya, Y. *J. Biol. Chem.* **1984**, *259*, 4177–4182.

(14) Niki, E.; Kawakami, A.; Yamamoto, Y.; Kamiya, Y. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1971–1975. Doba, T.; Burton, G. W.; Ingold, K. U. *Biochem. Biophys. Acta* **1985**, *835*, 298–303.

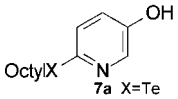
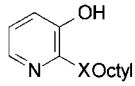
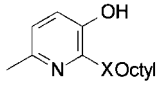
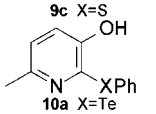
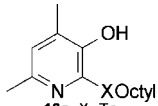
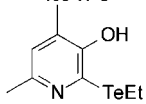
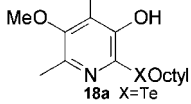
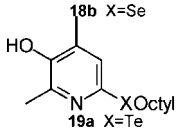
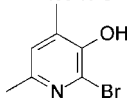
(15) Barclay, L. R. C. *J. Biol. Chem.* **1988**, *263*, 16138–16142.

(16) Kumar, S.; Johansson, H.; Engman, L.; Valgimigli, L.; Amorati, R.; Fumo, M. G.; Pedulli, G. F. *J. Org. Chem.* **2007**, *72*, 2583–2595.

(17) Kumar, S.; Engman, L.; Valgimigli, L.; Amorati, R.; Fumo, M. G.; Pedulli, G. F. *J. Org. Chem.* **2007**, *72*, 6046–6055.

(18) Kumar, S.; Engman, L. *J. Org. Chem.* **2006**, *71*, 5400–5403.

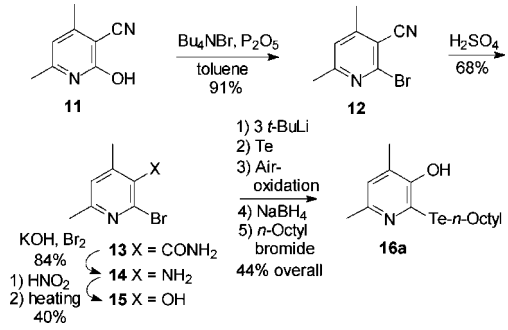
Table 1. Inhibited Rate of Linoleic Acid Peroxidation (R_{inh}) and Inhibition Times (T_{inh}) for Antioxidants and Reference Compounds Tested in the Two-Phase Model

antioxidant	R_{inh}[a] (μM/h)	T_{inh}[b] (min)	without *N*-acetylcysteine in the aqueous phase: R_{inh}[a] (μM/h)	without *N*-acetylcysteine in the aqueous phase: T_{inh}[b] (min)
 **7a** X=Te	32	70	542	0
7b X=Se	163	200	119	100
7c X=S	115	130	121	110
 **8a** X=Te	27	200	726	0
8b X=Se	120	150	152	90
8c X=S	203	140	115	120
 **9a** X=Te	23	>360	652	0
9b X=Se	65	170	57	80
9c X=S	45	130	68	90
 **10a** X=Te	27	280	627	0
10b X=Se	231	170	85	80
10c X=S	328	140	166	110
 **16a** X=Te	8	>400	600	0
16b X=Se	73	130	58	70
16c X=S	88	70	99	70
 **17** X=Te	22	240	619	0
 **18a** X=Te	8	>400	479	0
18b X=Se	45	110	54	60
 **19a** X=Te	27	50	490	0
19b X=Se	75	90	78	70
19c X=S	82	70	78	70
 **15**	584	0	613	0
α-Tocopherol (**2**)	24	90	22	80

[a] Rate of peroxidation during the inhibited phase (uninhibited rate ca. 650 μM h^{-1}). [b] Inhibited phase of peroxidation. Reactions were monitored for <400 min.

the organotellurium derivatives clearly outperformed the other chalcogen analogues when it comes to rate of inhibition and regenerability. Pyridinol **9a** inhibited peroxidation as efficiently as α-tocopherol with an inhibition time exceeding 360 min in the presence of the thiol regenerating agent. Thus, regenerability was also improved by introduction of the methyl group. It is noteworthy that the phenyltelluro derivative **10a** performed poorer (T_{inh} = 280 min) than its octyltelluro counterpart in this respect. Introduction of additional methyl groups into the 3-pyridinol scaffold was more demanding from a synthetic point of view. Shown in Scheme 1 is the preparation of a 4,6-dimethylated derivative **16a** from the commercially available 2-pyridinol **11**. In this approach, the 3-cyano group serves as a source of a phenol via hydrolysis to an amide, Hofmann rearrangement, and diazotization/aromatic nucleophilic substitution. The chalcogen is introduced into the 2-position in the final step by lithiation, insertion of tellurium, and alkylation of an arenetellurolate. Compound **16a** quenched peroxyl radicals three times as efficiently as α-tocopherol (R_{inh} = 8 μM/h) in the presence of *N*-acetylcysteine in the aqueous phase. Regenerability was also excellent (T_{inh} > 400 min). Again, chain-breaking capacity and regenerability of the corresponding organoselenium and −sulfur compounds **16b** and **16c** could not match those of the organotellurium derivative. Bromopyridinol **15** which was tested as a chalcogen-free reference did not inhibit peroxidation at all under the conditions of the assay. It is also noteworthy that compound **17**, carrying a less lipophilic group (ethyl) bonded to tellurium, showed a poorer regenerability than **16a**. Organotellurium **18a**, prepared in analogy with **16a**, showed similar antioxidant characteristics and clearly outperformed its selenium analogue **18b**. On the other hand, compound **19a**, carrying the phenolic moiety para to tellurium, showed surprisingly poor regenerability.

Scheme 1. Preparation of Compound **16a**



The peroxidation traces in Figure 1 show that compound **16a** can clearly outperform α-tocopherol when it comes to the duration of the inhibited phase. In fact, the limitation is the availability of the thiol reducing agent. Sampling of the aqueous (instead of the chlorobenzene) phase at intervals during a normal peroxidation experiment showed that the thiol/disulfide ratio decreased linearly with time until complete consumption of *N*-acetylcysteine after ca. 400 min. While keeping the concentration of *N*-acetylcysteine constant (1 mM), the concentration of antioxidant **16a** was lowered from the 40 μM used in the standard assay. The inhibition of peroxidation did not change at the 20 μM level, decreased at 10 μM (T_{inh} = 310 min), and could not be observed at the 5 μM level. Inhibition by compound **16a** of homogeneous-phase autoxidation of 4.3 M styrene in chlorobenzene at 303 K was also investigated by monitoring the oxygen consump-

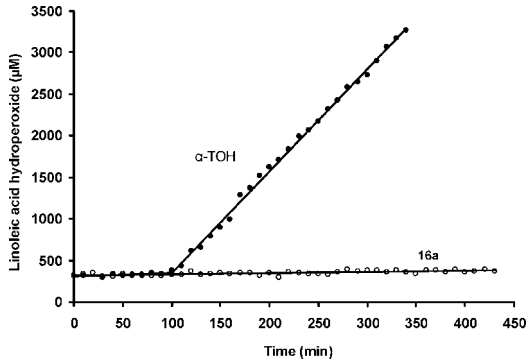


Figure 1. Peroxidation traces (linoleic acid hydroperoxide concentration vs time) recorded using compound **16a** (40 μM) or α-TOH (40 μM) as antioxidants in the chlorobenzene layer in the presence of NAC (1 mM) in the aqueous phase.

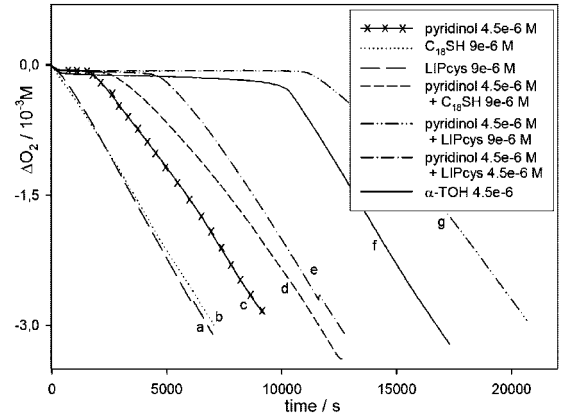


Figure 2. Typical oxygen uptake kinetic traces during homogeneous phase autoxidation of 4.3 M styrene in chlorobenzene, initiated by 5 mM AIBN at 303 K (initiation rate, $R_i = 9.0 \times 10^{-10}$ Ms^{-1}), in the presence of pyridinol **16a**, or α-TOH (reference), and/or octadecanethiol ($C_{18}SH$) or *tert*-butoxycarbonyl cysteine methyl ester (LIPcys). Inhibition times T_{inh} at the indicated concentrations were: ~ 0 s (a, b), 1825 s (c), 2870 s (d), 4850 s (e), 10 350 s (f), and 11 600 s (g).

tion kinetics in a two-channel oxygen uptake apparatus. A neat inhibited period was observed from which slope the absolute k_{inh} for reaction with peroxyl radicals was obtained as $9.2 \pm 2.0 \times 10^6$ $M^{-1}s^{-1}$, i.e., about 3 times larger than with α-TOH. However, the inhibition time, T_{inh}, corresponded to a stoichiometric factor n of only ~ 0.4 peroxyl radical *per* antioxidant molecule (for α-TOH, $n = 2$) possibly suggesting that during autoxidation the octyltelluro moiety is oxidized to the corresponding telluroxide, which would then be electron withdrawing, impairing the reactivity of the OH group. Indeed, after the inhibited period, the autoxidation was still "retarded" affording a second-rate constant for reaction with peroxyl radicals as $k_{inh} = 2.1 \pm 0.4 \times 10^4$ $M^{-1}s^{-1}$. Addition of 1−2 equiv of alkylmercaptans was found to partially prevent such oxidation at tellurium and slightly extend the length of the inhibition period (Figure 2). On the other hand, addition of 2 equiv of *N*-*tert*-butoxycarbonyl cysteine methyl ester (LIPcys), a lipid soluble analogue of *N*-acetylcysteine, was found to completely suppress oxidation at tellurium and partly regenerate the pyridinol from the corresponding aryloxyl radical as judged from the extension of the inhibition time corresponding to a stoichiometric factor n larger than 2.[19] No inhibition of autoxidation was observed with the alkylmercaptans or LIPcys alone at the same concentrations (see Figure 2).

The detailed mechanism for the catalytic chain-breaking antioxidant activity of 3-pyridinols carrying suitably positioned organylyltelluro groups is not known in detail. We feel it has to involve initial hydrogen atom transfer for quenching of peroxyl radicals, and we hypothesize that regeneration of the antioxidant occurs via electron transfer from thiol to phenoxyl radical at or near the aqueous−lipid interphase, accompanied by transfer of a proton to produce disulfide.

Organotellurium(II) compounds are quite reactive toward hydrogen peroxide, organic hydroperoxides, and a variety of other two-electron oxidizing agents. The resulting tetravalent organotelluriums obtained in the process can be reduced to the divalent state under mild conditions by reductants such as thiols and ascorbate. This facile redox cycling allows for their use as peroxide decomposers. In fact, they mimic the action of the selenium-containing glutathione peroxidase enzymes. We have measured the initial rate of reduction of hydrogen peroxide (ν_0) by monitoring the formation of diphenyl disulfide from thiophenol by UV spectroscopy at 305 nm as described by Tomoda.[20] Thus, what is measured is thiol peroxidase activity rather than glutathione peroxidase activity. Initial rates recorded for the reduction of hydrogen peroxide (3.75 mM) in methanol by thiophenol (1 mM) in the presence of compounds **16a** and **18a** (0.01 mM) as recorded by UV spectroscopy at 305 nm were 15.4 and 17.8 μM/min, respectively. For comparison, the thiol peroxidase activity of diphenyl diselenide under these conditions was only 0.67 μM/min.

The above results demonstrate that 3-pyridinols suitably substituted with organyltelluro groups can act as catalysts not only for the reduction of peroxyl radicals but also for decomposition of hydroperoxides in the presence of stoichiometric amounts of thiol reducing agents. We feel that our catalytic multifunctional antioxidant compositions would be useful for the prevention or treatment of disorders caused by or involving free-radical-mediated or oxidative tissue damage or for the stabilization of man-made and natural materials during processing and use.

Acknowledgment. The Swedish Research Council and MIUR (Rome) are acknowledged for financial support.

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

OL801983N

(19) Amorati, R.; Ferroni, F.; Lucarini, M.; Pedulli, G. F.; Valgimigli, L. *J. Org. Chem.* **2002**, *67*, 9295–9303.
(20) Iwaoka, M.; Tomoda, S. *J. Am. Chem. Soc.* **1994**, *116*, 2557–2561.