Selenium Compounds

The Isolation and Crystal Structure of a Cyclic Selenenate Ester Derived from Bis(2,6-diformyl-4-*tert*-butylphenyl)diselenide and its Glutathione Peroxidase-Like Activity**

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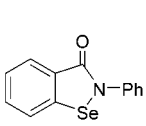
Glutathione peroxidase (GPx) is a well-known antioxidant selenoenzyme which protects biomembranes and other cellular components from oxidative stress by catalyzing the reduction of harmful peroxides at the expense of glutathione.[1] Recently, much attention has been directed toward the synthesis of simple organoselenium compounds that mimic the activity of GPx. These include: ebselen (**1**, PZ 51, 2-phenyl-1,2-benzisoselenazol-3(2*H*)-one),[2] ebselen analogues,[3] benzselenazolinones,[4] selenenamide **2** and related derivatives,[5] diaryl diselenides,[6] tellurides and ditellurides,[7] and the synthetic enzyme selenosubtilisin.[8] Diaryl diselenides with intramolecularly coordinating basic amino groups have been studied extensively, since Se···N nonbonded interactions are known to enhance the catalytic capacity of these antioxidants.[6a,d,9]



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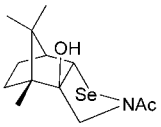

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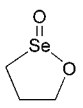

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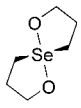

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Wirth studied a series of diselenides containing an oxygen atom in proximity to the selenium atoms, and observed that the nonbonded interactions play a role in enhancement of the catalytic activity.[10] Recently, Back et al. reported a seleninate ester **3** and related spirodioxaselenanonane **4** that exhibit remarkable GPx activity.[11] Seleninate **3**, which contains a Se–O bond instead of a Se–N bond, catalyzes the reaction by a different mechanistic pathway than that reported for ebselen and the diselenides. Herein, we report the synthesis and GPx mimetic activity of the diselenide **5** and its cyclic selenenate ester **6**.

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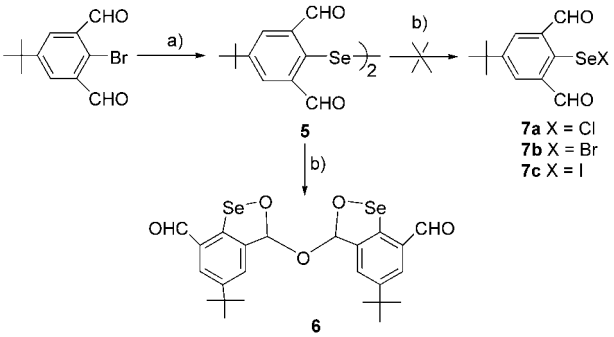
Synthesis of the diselenide **5** was accomplished by treating 2,6-diformyl-4-*tert*-butyl-1-bromobenzene with disodium diselenide (Scheme 1). In an attempt to synthesize selenenyl



Scheme 1. a) Na_2Se_2, THF, RT, 4 h; b) $SOCl_2$, Br_2, or $I_2/CHCl_3$.

halides **7** (RSeCl, RSeBr, RSeI), diselenide **5** was treated with SO_2Cl_2, Br_2, and I_2, respectively, in $CHCl_3$ under ambient conditions. Interestingly, the isolated product in all cases was an identical yellow crystalline compound along with recovered diselenide. Detailed spectroscopic analysis of the yellow solid and single-crystal structure determination revealed it to be the selenenate ester **6**.

To the best of our knowledge, only one example of the isolated selenenate ester **8** has been reported.[12] However, selenenate **8** was found to be susceptible to hydrolysis and decomposition. Selenenate **6**, in contrast, is stable in air and does not show any decomposition over a year. This extra stability presumably results from the strong intramolecular Se···O interactions. Selenenate **6** contains two chiral centers and can exist as a *meso* compound and a D,L pair. Thus, two sets of signals are expected in the ^{1}H NMR spectrum corresponding to these two diastereomers. The single-crystal X-ray structure clearly shows the D,L pair, which is the major diastereomer. Therefore, the signals at 10.20, 8.00, 7.75, and 6.90 ppm for the D,L pair can be assigned to aldehyde, aromatic, aromatic, and acetal protons, respectively. Next to these signals, at a slightly lower field (10.25, 8.10, and 8.80 ppm), are the corresponding aldehyde and aromatic proton signals for the *meso* diastereomer. There is fortuitous magnetic and chemical equivalency for the more remote signals from the "ether" bridge. However, the "acetal" CH moieties are not equivalent: one projects toward the lone pair of electrons on the ether oxygen atom and the other away from it, and two one-proton singlets are observed. The ratio of the two major *tert*-butyl signals (4:1) is also in accordance with the ratio of other proton signals. All attempts to purify and separate the diastereomers, including chromatographic separation, were unsuccessful. To confirm the purity two equivalents of benzenethiol (PhSH) were added to the solution of selenenate **6** in $CDCl_3$ and the mixture analyzed by ^{1}H NMR spectroscopy. The resulting spectrum indicated clean formation of the selenenyl sulfide **9** with three singlets corresponding to the formyl and *tert*-butyl groups and aromatic protons, respectively. The signal observed at $\delta =$ 1401 ppm in the ^{77}Se NMR spectrum of **6** is shifted upfield

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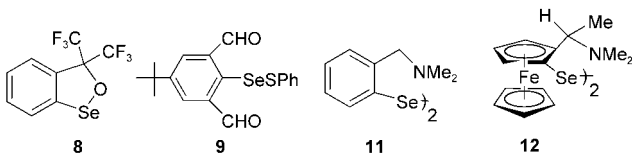

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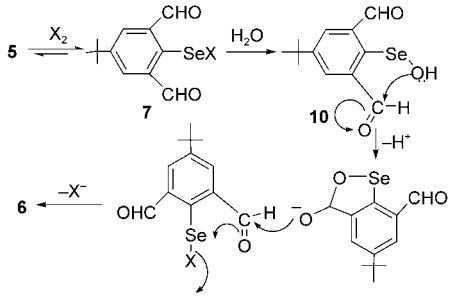
relative to that reported for selenenate **8** ($\delta = 1718$ ppm). This shift can be ascribed to the presence of strongly electron-withdrawing CF_3 groups in the five-membered ring in **8**.[12a] Selenenate **6** can be considered as a protected form of the highly unstable selenenic acid **10**. A plausible mechanism for the formation of **6** can be proposed via selenenic acid **10** as an intermediate (Scheme 2).[13]



Scheme 2. Plausible mechanism for the formation of **6**.

The molecular structure of selenenate **6**[14] shows the presence of intramolecular nonbonding Se···O interactions. The distances between the selenium atoms and the formyl oxygen atoms (Se(1A)···O(1A), 2.6042 Å; Se(1B)···O(1B), 2.4647 Å) are considerably smaller than the sum of the van der Waals radii (3.4 Å). The packing diagram of compound **6** reveals the intermolecular hydrogen-bonding interaction between the oxygen atom of the formyl group and the hydrogen atom of the *tert*-butyl group of the other molecule. The crystal packing contains the D,L pair of **6** (Figure 1).

The glutathione peroxidase-like catalytic activity was studied using the method reported by Iwaoka and Tomoda[6d] with PhSH as a glutathione alternative. The initial rates (v_0) for the reduction of H_2O_2 (5 mM) by thiol (0.75 mM) in the presence of catalysts **5** and **6** (0.01 mM) were determined in methanol by monitoring the UV absorption at 305 nm resulting from the formation of diphenyl disulfide. The initial rates for the diselenide **5** and selenenate ester **6** were 202 ± 9 and 167 ± 11 μmol dm^{-3} min^{-1}, respectively. Almost 7- and 6-fold enhancement in the initial reduction rate (v_0) of **5** and **6**, respectively, compared with the catalyst (**11**) reported by Wilson et al.,[6c] and 350- and 300-fold enhancement, respectively, compared with diphenyl diselenide (PhSeSePh) was observed under identical experimental conditions. Also, the v_0 value is roughly $^1/_3$ that of the most active diselenide **12**.[6a,b]

To investigate the mechanistic pathway, ^{77}Se NMR spectroscopic experiments were carried out in a mixture of $CDCl_3$ and CH_3OH (3:1). Four closely related signals were obtained when two equivalents of H_2O_2 were added to a solution of selenenate **6**. These signals can be assigned to the cyclic seleninate **13**.[11] Addition of two equivalents of PhSH resulted in one additional signal corresponding to selenenate **6**. The addition of two more equivalents of PhSH resulted in only the signal corresponding to selenenate **6** being observed. The formation of selenenate **6** can be explained by the opening of the seleninate ring by the thiol,[11] followed by breakdown of the hemiacetal to form thioseleninate **14**. Selenenic acid and PhSSPh were formed on treatment of thioseleninate **14** with a thiol.[15] Selenenic acid affords selenenate **6** (Scheme 2), and the ^{77}Se NMR spectrum of the reaction of **6** with PhSH showed a signal at $\delta = 435$ ppm. This signal can be assigned to the corresponding selenenyl sulfide **9**, formed by ring opening with PhSH. This was further confirmed by ^{1}H NMR studies. No other signals were observed even after addition of an excess of the thiol or H_2O_2 to this solution. However, diselenide **5** and selenenate **6** were isolated after allowing the solution of the selenenyl sulfide containing two equivalents of H_2O_2 to stand for a day. The ^{77}Se NMR studies and the mechanism of Back et al. for catalyst **3** enable the mechanistic pathway for selenenate **6** to be discussed in terms of the oxidation of **6** to seleninate **13**, followed by opening of the ring in **13** by a thiol molecule, and then subsequent steps (Scheme 3).

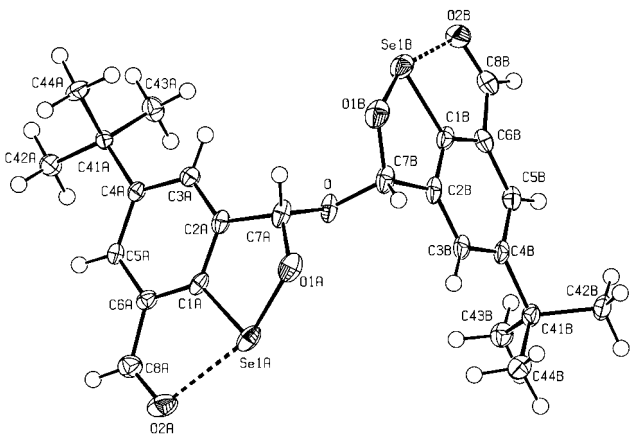
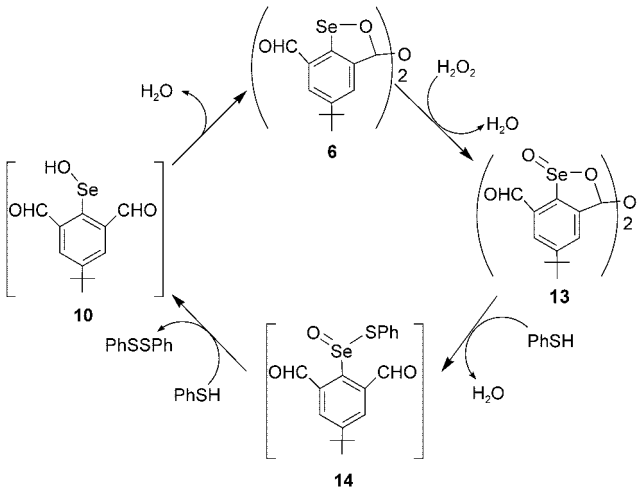


Figure 1. The molecular structure of **6**.



Scheme 3. Catalytic cycle for the GPx-like activity of **6**.

In summary, diselenide **5**, a GPx mimic with excellent activity, affords the unusual selenenate ester **6** simply by halogenation in the presence of water. The selenenate ester **6** also exhibits excellent catalytic activity. The facile isolation of **6** proved again the role of intramolecular nonbonding interactions between selenium atoms and heteroatoms in the stabilization and isolation of unstable species. This is the first structural report of a selenenate ester. These results corroborate the observations made by Back et al. that compounds containing Se–O bonds can be equally effective catalysts as the commonly studied Se–N derivatives. Further investigation toward the isolation and characterization of intermediates is in progress.

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[14] X-ray crystallographic data for **6**: $C_{12}H_{13}O_{2.50}Se$, $M_r = 276.18$, monoclinic, space group $P\text{-}1$, $a = 8.783(3)$, $b = 8.714(3)$, $c = 14.740(4)$ Å, $V = 1101.3(6)$ Å^3, $T = 93(2)$ K, 9209 reflections collected. CCDC-236477 (**6**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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