Thiol Peroxidase Activity of Diaryl Ditellurides As Determined by a ^1H NMR Method

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Received February 25, 1992

Abstract: A ^1H NMR method was developed for the assessment of the glutathione peroxidase-like activity of synthetic compounds. In this assay, thiols (*N*-acetylcysteine, *tert*-butyl mercaptan and 1-octyl mercaptan) were oxidized to the corresponding disulfides in CD_3OD or CD_3OD/D_2O in the presence of hydrogen peroxide and the catalyst to be evaluated. The time required to reduce the thiol concentration with 50%, t_{50}, was determined as a measure of the thiol peroxidase activity of the catalyst. Several diaryl ditellurides were efficient catalysts when present in low concentrations (0.3 mol %), whereas compounds with well-documented glutathione peroxidase-like activity in other assays were inactive (Ebselen, diaryl diselenides). The glutathione peroxidase-like activity of diaryl ditellurides was also assessed by using the classical coupled reductase assay. A mechanistic study showed that diaryl ditellurides, in the presence of hydrogen peroxide and a thiol, were rapidly converted to tellurosulfides. These species were stable enough to be isolated in some cases. The tellurosulfides reacted very slowly with added thiol, but in the presence of thiol/hydrogen peroxide the thiol was rapidly converted to its corresponding disulfide. On the basis of these observations, a mechanism involving a tellurinic acid thiol ester was proposed for the thiol peroxidase reaction of ditellurides. In contrast to tellurosulfides, selenosulfides, obtained either from diphenyl diselenide/hydrogen peroxide/1-octyl mercaptan or from Ebselen and 1-octyl mercaptan, were found to react very slowly with thiols in the presence of hydrogen peroxide.

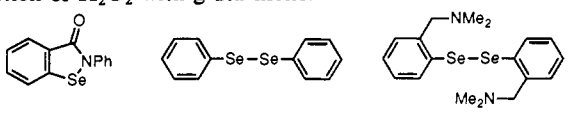
Introduction

Cells are constantly under pressure from the oxidative environment of aerobic life. This burden arises mainly from the production of reduced oxygen metabolites[1] ($O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$) during normal metabolism (Figure 1). The concentration of these reactive species is controlled within the organism by enzymes such as superoxide dismutase, catalase, and the glutathione peroxidases. Reduced oxygen metabolites also serve a function in the defense system against bacteria, viruses, and other exogenic compounds. Since the discovery by Folz and Schwarz[2] in 1957 that selenium is an essential microelement in humans, it has been recognized that this element exerts its most important biological function in the enzyme glutathione peroxidase (GSH-Px). This enzyme comprises four identical subunits, each of 21 000 Da and each containing a selenocysteine residue. In addition to reducing hydrogen peroxide to water, the enzyme also serves to convert organic hydroperoxides to the corresponding alcohols. Both processes require the presence of the tripeptide glutathione (GSH; γ-glutamylcysteinylglycine), which is oxidized to its corresponding disulfide, GSSG (eqs 1 and 2).

$$H_2O + 2GSH \xrightarrow{\text{GSH-Px}} GSSG + H_2O \quad (1)$$

$$ROOH + 2GSH \xrightarrow{\text{GSH-Px}} GSSG + ROH \quad (2)$$

Several attempts have been made recently to produce synthetic compounds which mimic the properties of GSH-Px. Ebselen, 2-phenylbenzisoselenazol-3(2*H*)-one (**1**), was the first compound of this kind.[3] Derivatives of Ebselen are presently undergoing clinical trials with rheumatic arthritis and liver injury as major indications.[4] The use of Ebselen for the treatment of other disease states has also been patented.[5] More recently, it has been reported that diphenyl diselenide (**2**) and diaryl diselenides carrying basic (dialkylamino)methyl groups in the ortho position (e.g. compound **3**) were 2-10 times more efficient than Ebselen in catalyzing the reaction of H_2O_2 with glutathione.[6]



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A synthetic selenoprotein, selenosubtilisin, has been reported to be 70 000 times more efficient than diphenyl diselenide in catalyzing the oxidation of 3-carboxy-4-nitrophenyl mercaptan to its corresponding disulfide in the presence of *tert*-butyl hydroperoxide.[7]

In this article we report a simple NMR method for measuring the ability of synthetic compounds to catalyze the oxidation of certain thiols to the corresponding disulfides in the presence of H_2O_2 (thiol peroxidase activity). We also report that diaryl ditellurides in these systems are much more efficient catalysts than Ebselen or any of the compounds based on diaryl diselenides.

Methods for the Assessment of Glutathione Peroxidase-like Activity

The glutathione peroxidase-like behavior of a compound is commonly studied in a rather indirect manner by recording the disappearance of the UV absorption of NADPH (340 nm) at pH 7 in a coupled system containing GSH, H_2O_2, glutathione disulfide (GSSG) reductase, and the catalyst to be evaluated (eq 3).[8] A prerequisite condition for obtaining reliable results with this assay is that all intermediates in the reaction are inert toward GSSG reductase/NADPH.[9] This is not easy to check, especially when the reaction mechanism is not known in detail. Therefore, the use of direct methods (e.g., determination of hydrogen peroxide

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(2) Schwarz, K.; Foltz, C. M. *J. Am. Chem. Soc.* **1957**, *79*, 3292.
(3) Müller, A.; Cadenas, E.; Graf, P.; Sies, H. *Biochem. Pharmacol.* **1984**, *33*, 3235. Wendel, A.; Fausel, M.; Safayhi, H.; Tiegs, G.; Otter, R. *Biochem. Pharmacol.* **1984**, *33*, 3241. Parnham, M. J.; Kindt, S. *Biochem. Pharmacol.* **1984**, *33*, 3247.
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(6) Wilson, S. R.; Zucker, P. A.; Huang, R.-R. C.; Spector, A. *J. Am. Chem. Soc.* **1989**, *111*, 5936.
(7) Wu, Z.-P.; Hilvert, D. *J. Am. Chem. Soc.* **1990**, *112*, 5647.
(8) Wendel, A. *Methods Enzymol.* **1981**, *77*, 325.
(9) We have found that the selenosulfide formed from Ebselen and GSH serves as a substrate for GSSG reductase. Thus, as compared with a direct method (H_2O_2 removal), the glutathione peroxidase-like activity of Ebselen will be overestimated by using the coupled assay. See also: Cotgreave, I. A.; Moldéus, P.; Brattsand, R.; Hallberg, A.; Andersson, C. M.; Engman, L. *Biochem. Pharmacol.* **1992**, *43*, 793.

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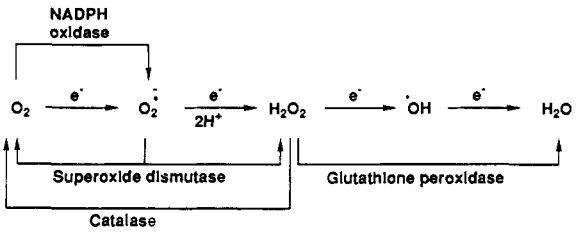


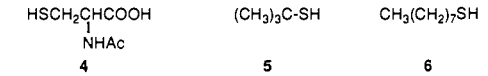
Figure 1. Formation and interconversion of reduced oxygen metabolites.

removal[9,10] or thiol removal[7,9]) seems more reliable when new catalysts are to be evaluated.

$$2GSH + H_2O_2 \xrightarrow{\text{catalyst}} GSSG + 2H_2O$$

$$GSSG \xrightarrow[NADPH]{\text{GSSG reductase}} 2GSH \qquad (3)$$

We thought it should be worthwhile to develop a direct method based on ^1H NMR spectroscopy for the assessment of thiol peroxidase activity. The most serious problem that we encountered in this work was the rapid spontaneous oxidation of many thiols to their corresponding disulfides by hydrogen peroxide (the half-life of glutathione at pH 7 in an aqueous solution containing H_2O_2 is approximately 15 min).[9,10] The autoxidizability of a thiol depends on several factors, the most important being thiol acidity, anion stability, radical stability, and steric effects.[11] Most of these factors also seem relevant to peroxide-induced oxidation of thiols, which has been suggested to involve a sulfenic acid intermediate.[12] We have found that *N*-acetylcysteine (**4**), *tert*-butyl mercaptan (**5**), and 1-octyl mercaptan (**6**), the two latter in CD_3OD and the former in $D_2O/CD_3OD = 4/1$, react very slowly in clean NMR tubes with a stoichiometric amount of hydrogen peroxide (the basal oxidation rate was studied by ^1H NMR for 40 h, and the time required to reduce the thiol concentration with 50%, t_{50}, of the respective thiols was extrapolated as follows: *N*-acetylcysteine, $t_{50} \approx 55$ h; *tert*-butyl mercaptan, $t_{50} \gg 100$ h; 1-octyl mercaptan, $t_{50} \approx 100$ h). In contrast to D_2O-based NMR solvents, the use of CD_3OD avoids solubility problems with added catalysts. On the other hand, the pH of the solution is not defined. Our D_2O/CD_3OD system offers the possibility of pH control via added buffers. The unbuffered *N*-acetylcysteine system had a pH of 2.0 as determined with a pH meter using water as solvent. This value was unchanged as the thiol was oxidized to its corresponding disulfide. Therefore, no buffer was added under the standard conditions of the assay. Attempts to increase the pH to physiological levels resulted in a rapid basal oxidation ($t_{50} \approx 5$ min). *tert*-Butyl mercaptan and 1-octyl mercaptan have similar pK_a values (11.05[13] and 10.8,[14] respectively). The slower basal oxidation of the former probably reflects the more severe steric hindrance around the sulfur atom.



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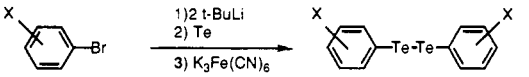
Thiol Peroxidase Activity of Diaryl Ditellurides

Spector and co-workers recently reported that diphenyl diselenide and several derivatives thereof showed glutathione peroxidase-like behavior in the coupled assay using GSSG reductase/NADPH.[6] We were curious to compare some of these compounds with the analogous organotellurium compounds and other ditellurides in our NMR system for thiol peroxidase activity.

Diaryl ditellurides **7–12** (Table I) were prepared from aryl bromides via lithiation, tellurium insertion, and ferrocyanide oxidation according to, or in analogy with, a literature procedure (eq 4).[15] In the case of 2-bromoaniline 3 equiv of *tert*-butyllithium was used. Bis(4-aminophenyl) ditelluride (**13**) was obtained by reduction of the 2/1 complex[16] of aniline and $TeCl_4$.



(4)

X= 4-H, 4-OMe, 2-OMe, 2,4,6-tri Me, 2-NH2 4-NMe2

An attempted synthesis of the tellurium analog of diselenide **3** was recently reported by Sing and co-workers.[17] In this synthesis, ortholithiated benzyldimethylamine was treated with elemental tellurium and the insertion product allowed to air-oxidize. The isolated product was apparently overoxidized and tentatively assigned a tellurinic anhydride structure. We prepared the tellurium analog of compound **3** from (2-bromobenzyl)dimethylamine following the procedure shown in eq 4. The crude ditelluride was then passed through a SiO_2 column and treated with hydrochloric acid to give the crystalline bis-hydrochloride **14** in 33% overall yield. Dibutyl ditelluride (**15**) was prepared as an example of a dialkyl ditelluride.

The thiol peroxidase activities of compounds **7–15** were determined by recording the times required to reduce the thiol concentration with 50%, t_{50}, for the thiols **4–6** as outlined above in the presence of a catalytic amount (0.3 mol %) of ditelluride. The results are presented in Table I. When the progress of the reaction could not be clearly distinguished from the basal oxidation rate, the catalyst was classified as inactive. It was noted that the t_{50} data could be well reproduced in parallel experiments, whereas the reproducibility between different experiments was poorer, especially for active catalysts (see the Experimental Section). Ebselen (**1**), diphenyl diselenide (**2**), and the bis-hydrochloride of diselenide **3**, compound **16**, were also tested for thiol peroxidase activity. In contrast to most of the ditellurides, all selenium compounds were inactive (Table I). The glutathione peroxidase-like activity of compounds **1**, **2**, and **7–16** was also assessed by using the coupled reductase assay[9] (Table I and eq 3). The activity of the catalyst (% GSH) was determined as the increase of the basal reaction rate between GSH and H_2O_2, as compared with a control, over the first 20 s of the reaction. Due to solubility problems, DMSO was often added in the experiments.

In order to gain some insight into the reaction mechanism of the thiol peroxidase reaction of ditellurides, diphenyl ditelluride was treated in an NMR tube ($CD_3OD/CDCl_3$) with 2 equiv of a thiol (*tert*-butyl mercaptan or 1-octyl mercaptan) and 1.0 equiv of hydrogen peroxide. In both cases the thiol peak almost disappeared in the spectrum and another major peak appeared in addition to a small peak corresponding to disulfide. Whereas the compound obtained from 1-octyl mercaptan was partly decomposed during attempted chromatographic purification, the one from *tert*-butyl mercaptan was isolable in pure form and assigned a tellurosulfide structure **17a** (eq 5). The reactivity of octylthio phenyl telluride (**17b**), prepared in situ as described above, was further studied in order to disclose pathways whereby it could be readily transformed into dioctyl disulfide. Since the compound was stable for many hours in $CD_3OD/CDCl_3$, its disproportionation to give disulfide and ditelluride is not likely to account for the thiol peroxidase activity of diphenyl ditelluride. Similarly, when 1 equiv of 1-octyl mercaptan or 1 equiv of hydrogen peroxide was added to the tellurosulfide solution, dioctyl disulfide was formed only very slowly. On the other hand, when thiol *and*

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(11) Munday, R. *Free Rad. Biol. Med.* **1989**, *7*, 659.
(12) Lim, P. K.; Giles, D. W.; Cha, J. A. *Chem. Eng. Sci.* **1986**, *41*, 3141.
(13) Danehy, J. P.; Parameswaran, K. N. J. *Chem. Eng. Data* **1968**, *13*, 386.
(14) Estimated value from a determination in 23% aqueous *tert*-butyl alcohol[13] and comparison with 1-butyl mercaptan. For a dissociation constant of 1-butyl mercaptan in water, see: Crampton, M. R. In *The Chemistry of the Thiol Group*; Patai, S., Ed.; Wiley: New York, 1974; p 398.
(15) Engman, L.; Persson, J. *J. Organomet. Chem.* **1990**, *388*, 71.
(16) Morgan, G. T.; Burgess, H. *J. Chem. Soc.* **1929**, 1103.
(17) Singh, H. B.; Sudha, N.; West, A. A.; Hamor, T. A. *J. Chem. Soc., Dalton Trans.* **1990**, 907.

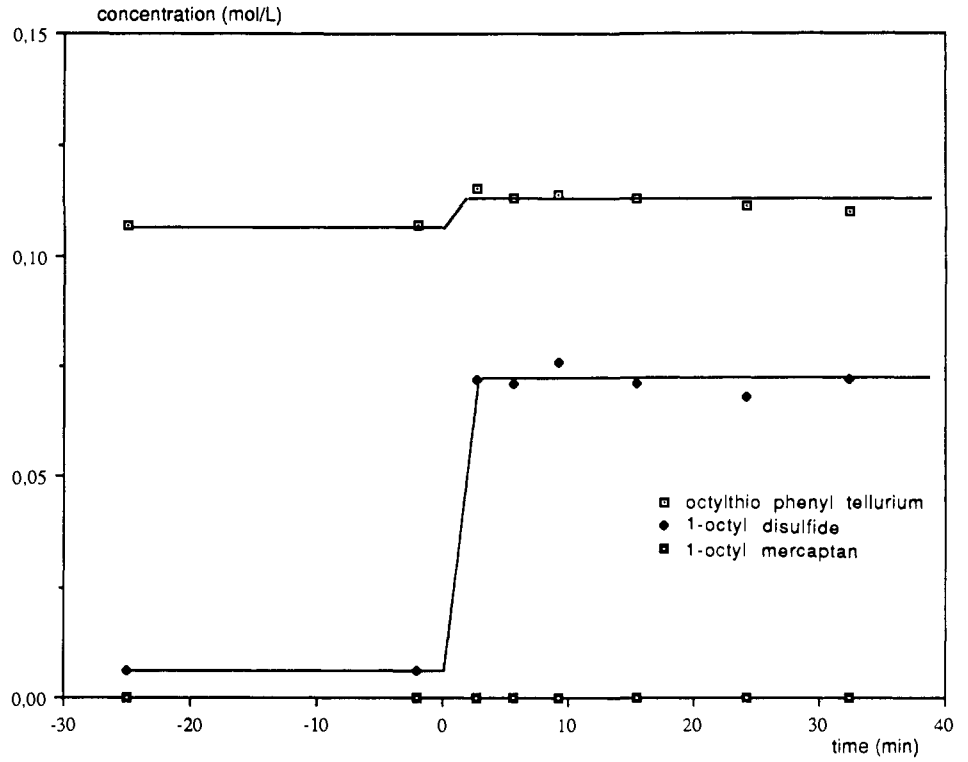


Figure 2. Treatment of 1-octylthio phenyl telluride prepared in situ with 1-octyl mercaptan/hydrogen peroxide. 1-Octylthio phenyl telluride was prepared from diphenyl ditelluride (0.0445 mmol), 1-octyl mercaptan (0.089 mmol), and hydrogen peroxide (0.0445 mmol). At the point $t = 0$, a mixture of 1-octyl mercaptan (0.089 mmol) and hydrogen peroxide (0.0445 mmol) was added. Anisole was used as an internal standard.

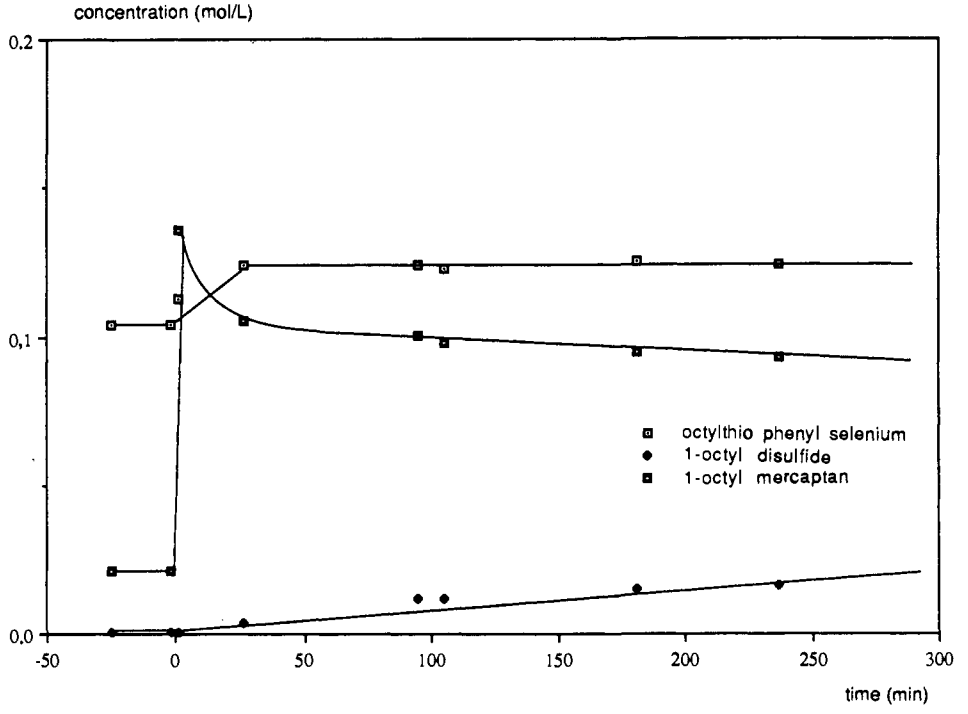


Figure 3. Treatment of 1-octylthio phenyl selenide prepared in situ with 1-octyl mercaptan/hydrogen peroxide. 1-Octylthio phenyl selenide was prepared from diphenyl diselenide (0.0445 mmol), 1-octyl mercaptan (0.089 mmol), and hydrogen peroxide (0.0445 mmol). At the point $t = 0$, a mixture of 1-octyl mercaptan (0.089 mmol) and hydrogen peroxide (0.0445 mmol) was added. Anisole was used as an internal standard.

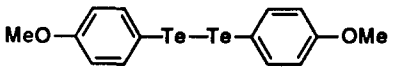
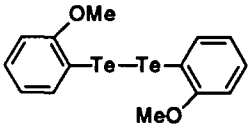
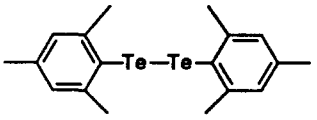
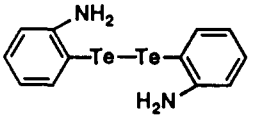
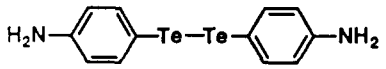
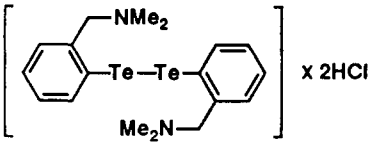
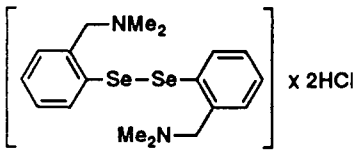
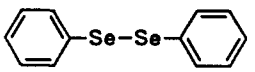
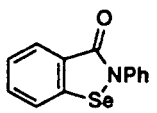
hydrogen peroxide were added, all of the thiol was rapidly (<2 min) converted to dioctyl disulfide while the tellurosulfide concentration remained essentially constant. This is shown graphically in Figure 2.

$$\mathrm{PhXXPh + 2RSH + H_2O_2 \longrightarrow 2\ PhXSR + 2H_2O} \quad (5)$$

17 a X=Te; R=t-Bu
b X=Te; R=C_8H_{17}
c X= Se; R=t-Bu
d X= Se; R=C_8H_{17}

In the presence of hydrogen peroxide, diphenyl diselenide reacted with thiols to give selenosulfides (eq 5). The product from 1-octyl mercaptan, **17d**, was readily formed but difficult to isolate due to partial decomposition during chromatographic separation. The compound **17c** from *tert*-butyl mercaptan was more slowly formed but was isolable in pure form. When the selenosulfide prepared in situ from 1-octyl mercaptan was treated as described above with 1-octyl mercaptan and H_2O_2, the rate of disulfide formation was considerably slower than that of the corresponding

Table I. Glutathione Peroxidase-like Activity of Diorganyl Ditellurides, Diaryl Diselenides, and Ebselen as Determined by ^{1}H NMR Spectroscopy and the Coupled Reductase Assay[a]

catalyst	compound number	^{1}H NMR t_{50}[b] (min)			coupled reductase assay	
		N-acetyl cysteine	t-butyl mercaptan	1-octyl mercaptan	%GSH[c]	DMSO (%)
	7	144[d]	327	132	570	20
	8	58	232	106	380	30
	9	143	880	-[e]	239	30[d]
	10	490	inactive	-[e]	175	30[d]
	11	50	228	-[e]	428	10
	12	34	137	149	120	30[d]
	13	13	125	47	2450	0
	14	86	14	88	2080	0
Bu_2Te_2	15	22	227	35	1185	10
	16	inactive	inactive	inactive	2190	0
	2	inactive	inactive	inactive	1457	10
	1	inactive	inactive	inactive	531	0

[a] For details see the Experimental Section. [b] t_{50} is the time required to reduce the thiol concentration with 50%. [c] The catalyst's percentage increase of the basal reaction rate between GSH and H_2O_2 was calculated as rate of NADPH consumption + catalyst (μM/min)/rate of NADPH consumption + vehicle (μM/min) × 100. Vehicle controls were performed on the day (n = 4 for all concentrations of DMSO). [d] Solubility problems, cloudy solutions. [e] Not determined.

tellurosulfide reaction (Figure 3).

Ebselen was an inactive catalyst in the NMR assay with all three thiols when present in a catalytic amount (0.3 mol %). When the amount of catalyst was increased (11%) in the 1-octyl mercaptan system, disulfide formation was still slow. However, it was concluded from the ^{1}H NMR spectra, and by comparison with an authentic sample, that the catalyst was present as a selenosulfide **18**. This is not surprising, since Ebselen is known to be readily ring-opened by thiols to give selenosulfides.[18] Furthermore, two recent investigations of the mechanism of the glutathione peroxidase-like reaction of Ebselen have found evidence for selenosulfide formation.[10,19] When selenosulfide **18** was treated in an NMR tube with 1-octyl mercaptan, only a trace amount of dioctyl disulfide was detected after 2h. However, in the presence of both

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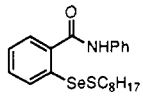
(19) Fischer, H.; Dereu, N. *Bull. Soc. Chim. Belg.* **1987**, *96*, 757.

1-octyl mercaptan and hydrogen peroxide, dioctyl disulfide was formed considerably faster (20% conversion after 2 h) whereas the amount of selenosulfide remained constant.



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Discussion

Although it is not easy to rationalize all of the data from the ^{1}H NMR method and the coupled reductase assay (Table I), it is clear that diorganyl ditellurides show a glutathione peroxidase-like activity in both systems. According to the coupled reductase assay, the best ditelluride catalysts (compounds **13** and **14**) are equally or more efficient than the best catalysts based on diselenides (compounds **2** and **16**). For the diorganyl ditelluride catalysts there is a consistency between the results obtained from the three thiol systems in the ^{1}H NMR assay and the coupled reductase method. Compounds **13–15**, which are the three most efficient catalysts according to the coupled reductase assay, also turn out to be the best catalysts in one of the thiol systems and highly ranked in the other two.

Although parameters like pH, solvent, and thiol structure vary in the two systems, the strikingly different results obtained in the ^{1}H NMR and the coupled reductase assay with diselenide catalysts are difficult to understand. Thiols are much less nucleophilic than their corresponding thiolate ions. Since the thiols used in the ^{1}H NMR assay are probably less dissociated than GSH in the coupled assay, the t_{50} data may reflect the reactivities of thiols while the coupled reductase data reflect the combined thiol and thiolate reactivities. In support of this idea, the catalytic activity of diselenide **16** was substantially increased (t_{50} = 116 min) in the 1-octyl mercaptan system when the catalyst was added in its deprotonated form, **3**, where the amino groups could serve to deprotonate thiol. In order to eliminate potential complicating factors in the coupled reductase assay,[9] both the enzyme and NADPH were removed, and the progress of the reaction of GSH with H_2O_2, in the presence of catalysts **13**, **14**, and **16**, was determined at pH 7.4 by glutathione removal (derivatization with monobromobimane, HPLC separation, and fluorescence quantitation).[20] The activities of catalysts **13**, **14**, and **16** (% GSH) were calculated as outlined in Table I (rate of GSH consumption instead of NADPH consumption): catalyst (% GSH), **13** (1693), **14** (997), **16** (2670). As compared with the results presented in Table I, compounds **13** and **14** are less active in the absence of reductase/NADPH, whereas the activity of compound **16** is potentiated. However, all three compounds are still very active.

Concerning the t_{50} data, the reactivities of the three thiols **4–6** in their catalyzed reactions seem to match their basal oxidation rates fairly well (*N*-acetylcysteine > 1-octyl mercaptan > *tert*-butyl mercaptan). In general, electron-donating substituents on the aromatic rings seem to improve the catalytic activity of diaryl ditellurides. This is also true with amine substituents at low pH (*N*-acetylcysteine system), where these groups are present essentially in their protonated form. If steric hindrance is introduced into the diaryl ditelluride catalyst, the activity is reduced as determined by both assays (compound **10**). This steric effect is also reflected in the lower activity of diaryl ditellurides containing an ortho substituent (compounds **9** and **11**) as compared with the analogous para-substituted derivatives (compounds **8** and **13**, respectively).

Concerning the reaction mechanism of the thiol peroxidase reaction of ditellurides, we have good reasons to believe that tellurosulfides are key intermediates in the catalytic cycle. Several tellurosulfides are described in the literature,[21] but their formation from ditellurides in the presence of thiols and hydrogen peroxide has (eq 5), to the best of our knowledge, not been previously

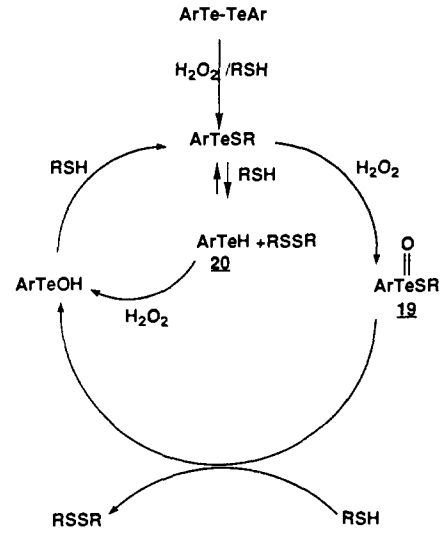


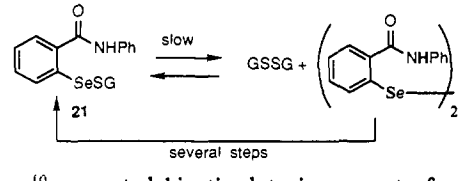
Figure 4. Proposed mechanism of the thiol peroxidase reaction of ditellurides.

described. As indicated above, tellurosulfides require oxidative activation to readily give disulfides in the presence of thiols. Although we were unable to isolate any oxidation products, we suggest a tellurinic acid thiol ester, **19** as an intermediate in our mechanistic scheme for the thiol peroxidase activity of ditellurides (Figure 4). Nucleophilic attack by thiol on sulfur would then give disulfide and a tellurenic acid, which would react with thiol to regenerate the tellurosulfide. When tellurosulfide **17a** (0.6 mol %) was used as a catalyst in the *tert*-butyl mercaptan system, a slightly shorter t_{50} value was recorded (292 min) as compared with catalyst **7** (327 min). An alternate mechanism, involving an equilibrium of tellurosulfide and thiol with disulfide and an arenetellurol **20** (Figure 4) and H_2O_2 oxidation of the latter to give a tellurenic acid, would also accommodate the observed reactivity of the tellurosulfide in the presence of H_2O_2/thiol. However, since attempts to trap a tellurol under the conditions of the NMR experiment by replacing the H_2O_2 with an alkylating agent (methyl iodide or 2,4-dinitrochlorobenzene) did not result in the formation of any diorganyl tellurides, we consider this pathway less likely.

Diaryl diselenides probably exert their very weak thiol peroxidase activity (^{1}H NMR system) by a mechanism similar to that shown for diaryl ditellurides (Figure 4; Te replaced by Se). As indicated above, the organoselenium compounds react slower in one or several of the reactions involved in the catalytic cycle. Spector[6] has previously suggested a tentative mechanism for the glutathione peroxidase-like behavior of diaryl diselenides. According to his proposal, disulfide is formed via nucleophilic attack of thiol on a selenosulfide intermediate (not isolated).

In support of the idea of oxidative activation of the selenosulfide, Kice and co-workers reported some time ago that benzeneseleninic acid thiol esters react with thiols to form disulfides and benzeneselenenic acid. The selenenic acid then readily condenses with excess thiol to form a selenosulfide.[22,23]

In the first mechanistic study of the glutathione peroxidase-like behavior of Ebselen, Fischer and Dereu[19] proposed a disproportionation reaction of the readily formed selenosulfide **21** as the slow step of the catalytic cycle (eq 6). Later Haenen and co-



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workers[10] presented kinetic data in support of an alternative mechanism involving a selenol intermediate **22** (eq 7). Our results with selenosulfide **18** seem to indicate that a third mechanism has

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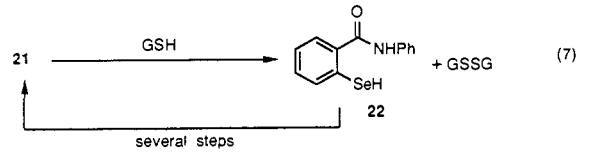
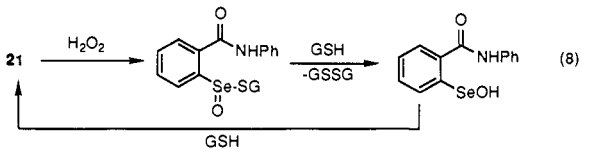


(7)

to be considered, involving slow oxidation of selenosulfide **21** followed by rapid attack by GSH to give disulfide and a selenenic acid which could reform the selenosulfide in the presence of thiol (eq 8).



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Experimental Section

Melting points (uncorrected) were determined by using a Büchi 510 melting point apparatus. ^{1}H NMR spectra were obtained with a Bruker AC-F250 instrument and, unless otherwise stated, recorded in $CDCl_3$ solutions containing tetramethylsilane as the internal standard. Elemental analyses were performed by Analytical Laboratories, Engelskirchen, Germany. Hydrogen peroxide, 30 wt % solution in water, was obtained from Aldrich. *tert*-Butyl mercaptan and 1-octyl mercaptan were distilled before use to remove trace amounts of disulfides. *tert*-Butyl disulfide and diphenyl diselenide were commercial samples. 1-Octyl disulfide was prepared in analogy with a literature procedure[24] by oxidation of 1-octyl mercaptan with iodine, bp 156–60 °C/0.8 mmHg (lit.[25] bp 178–83 °C/5 mmHg). *N*-Acetylcysteine and its corresponding disulfide were provided by Astra Draco AB, Lund, Sweden. Reduced glutathione, glutathione reductase (grade 1, from yeast), and NADPH were obtained from Sigma. Diphenyl ditelluride,[15] bis(4-methoxyphenyl) ditelluride,[15] bis(2,4,6-trimethylphenyl) ditelluride,[26] bis[4-(dimethylamino)phenyl] ditelluride,[15] dibutyl ditelluride,[27] 2,2′-diselenobis[[(*N*,*N*-dimethylamino)methyl]benzene] bis(hydrochloride)[6], and Ebselen[28] were prepared according to literature methods.

Bis(2-methoxyphenyl) Ditelluride (9). To a stirred solution of 2-bromoanisole (1.59 g, 8.5 mmol) in dry THF (40 mL) under N_2 at −78 °C was added *tert*-butyllithium (10 mL, 1.7 M; 17.0 mmol). After 1 h, the cooling bath was removed and freshly crushed finely ground elemental tellurium (1.09 g, 8.5 mmol) was added while a brisk stream of nitrogen was passed through the open system. After 1 h, when only trace amounts of tellurium remained, the dark solution was poured into a beaker containing $K_3Fe(CN)_6$ (2.80 g, 8.5 mmol) in water (150 mL). After extraction with CH_2Cl_2, filtration, separation, drying of the organic phase, evaporation, and flash chromatography (SiO_2, hexanes/CH_2Cl_2 = 2/1), 1.79 g (90%) of compound **9**, mp 87–9 °C (EtOH), was obtained: ^{1}H NMR δ 3.91 (s, 6 H), 6.73–6.82 (several peaks, 4 H), 7.24 (m, 2 H), 7.64 (dd, 2 H, J = 1.5 and 7.5 Hz). Anal. Calcd for $C_{14}H_{14}O_2Te_2$: C, 35.82; H, 3.01. Found: C, 35.80; H, 2.92.

Bis(2-aminophenyl) ditelluride (11) was prepared from 2-bromoaniline (1.95 g, 11.3 mmol), *tert*-butyllithium (20 mL, 1.7 M; 34.0 mmol), elemental tellurium (1.45 g, 11.3 mmol), and $K_3Fe(CN)_6$ (3.74 g, 11.3 mmol) following the procedure for the preparation of compound **9**. After evaporation of the CH_2Cl_2 solution, the crude product was recrystallized (without chromatographic purification) from EtOH to give 1.48 g (60%) of brownish-red crystals: mp 104–5 °C (lit.[29] mp 100 °C); ^{1}H NMR δ 4.18 (s, 4 H), 6.52 (m, 2 H), 6.73 (dd, 2 H), 7.13 (m, 2 H), 7.69 (dd, 2 H).

Bis(4-aminophenyl) Ditelluride (13). The 2/1 complex of aniline and $TeCl_4$ was prepared in analogy with a literature procedure[16] by mixing $TeCl_4$ with 2 equiv of aniline in dry ether. When added to a two-phase system comprising $K_2S_2O_5$ (6.0 g, 17.0 mmol) in water (50 mL) and CH_2Cl_2 (50 mL), the green complex (6.2 g, 13.6 mmol) turned red, but did not dissolve until $NaHCO_3$ was added in excess (gas evolution). The deep red organic phase was separated after filtration from some insoluble material. Drying and evaporation afforded a red oil which was dissolved in EtOH. Addition of hexane and cooling (−20 °C) caused precipitation of golden yellow crystals of compound **13**: 0.50 g (17%); mp 79–81 °C; ^{1}H NMR δ 3.74 (s, 4H), 6.51 (d, 4H), 7.56 (d, 4H). Anal. Calcd for $C_{12}H_{12}N_2Te_2$: C, 32.80; H, 2.75. Found: C, 32.67; H, 2.79.

2,2′-Ditellurobis[[(*N*,*N*-dimethylamino)methyl]benzene] Bis(hydrochloride) 14. To a stirred solution of (2-bromobenzyl)dimethylamine (1.83 g, 8.5 mmol) in dry THF (40 mL) under N_2 at −78 °C was added *tert*-butyllithium (10 mL, 1.7 M; 17.0 mmol). After 1 h, the cooling bath was removed and freshly crushed finely ground elemental tellurium (1.09 g, 8.5 mmol) was added while a brisk stream of nitrogen was passed through the open system. After 1 h, when only trace amounts of tellurium remained, the reaction mixture was poured into water (100 mL) containing $K_3Fe(CN)_6$ (2.80 g, 8.5 mmol). After extraction with CH_2Cl_2, filtration, separation, drying ($MgSO_4$), and evaporation together with a small amount of silica, flash chromatography (SiO_2; CH_2Cl_2/MeOH = 97/3) of the product afforded a crude ditelluride as a red viscous oil. This was dissolved in 50% aqueous EtOH (25 mL) containing concentrated HCl (0.7 g), and the solution was filtered and evaporated. Recrystallization of the crude product from EtOH afforded 0.84 g (33%) of the bis(hydrochloride) **14**: mp 134–5 °C; ^{1}H NMR δ 2.84 (s, 12 H), 4.01 (s, 4 H), 7.15–7.23 (several peaks, 4 H), 7.34 (m, 2 H), 8.05 (d, 2 H, J = 7.7 Hz). Anal. Calcd for $C_{18}H_{26}N_2Cl_2Te_2$: C, 36.25; H, 4.39. Found: C, 36.41; H, 4.16).

***tert*-Butylthio Phenyl Telluride (17a).** *tert*-Butyl mercaptan (0.035 g, 0.39 mmol), diphenyl ditelluride (0.080 g, 0.20 mmol), and hydrogen peroxide (22 μL, 30%, 0.22 mmol) were stirred in a mixture of MeOH (2 mL) and CH_2Cl_2 (1 mL) for 2 h. The reaction mixture was then evaporated together with a small amount of silica and put on top of a flash chromatography column. Elution with hexanes afforded 50 mg (44%) of pure compound **17a** as a yellowish red oil: ^{1}H NMR (CD_3OD) δ 1.36 (s, 9 H), 7.21–7.26 (several peaks, 3 H), 7.72–7.77 (several peaks, 2 H). Anal. Calcd for $C_{10}H_{14}STe$: C, 40.87; H, 4.80. Found: C, 40.76; H, 4.69.

1-Octylthio phenyl telluride (17b) was similarly prepared. However, after chromatography the product was contaminated by ca. 15% of dioctyl disulfide. ^{1}H NMR data for compound **17b** are reported: δ (CD_3OD) 0.88 (s, 3 H), 1.22–1.40 (several peaks, 10 H), 1.57 (m, 2 H), 2.98 (t, 2 H), 7.23–7.30 (several peaks, 3 H), 7.74–7.79 (m, 2 H).

***tert*-Butylthio Phenyl Selenide (17c).** *tert*-Butyl mercaptan (0.088 g, 0.98 mmol), diphenyl diselenide (0.150 g, 0.48 mmol), and hydrogen peroxide (55 μL, 30%, 0.54 mmol) were stirred for 13 days in a mixture of MeOH (5 mL) and CH_2Cl_2 (1 mL). The reaction mixture was then evaporated together with a small amount of silica and put on top of a flash chromatography column. Elution with hexanes afforded 100 mg (43%) of pure compound **17c**. The ^{1}H NMR spectrum of the compound was in good agreement with literature data.[23]

1-Octylthio phenyl selenide (17d) was prepared from 1-octyl mercaptan, diphenyl diselenide, and hydrogen peroxide following the procedure for compound **17a**. The product obtained after flash chromatography was contaminated by 1-octyl disulfide. ^{1}H NMR data for compound **17d** are reported: δ (CD_3OD/$CDCl_3$ = 1/1) 0.88 (t, 3 H), 1.18–1.50 (several peaks, 10 H), 1.64 (m, 2 H), 2.84 (t, 2 H), 7.27–7.35 (several peaks, 3 H), 7.60–7.64 (m, 2 H).

1-Octylthio 2-(*N*-Phenylcarboxamido)phenyl Selenide (18). 2-Phenyl-1,2-benzisoselenazol-3(2*H*)-one (0.099 g, 0.36 mmol) was dissolved in a mixture of CH_2Cl_2 (4 mL) and 1-octyl mercaptan (0.055 g, 0.38 mmol). After 2 h at ambient temperature, hexanes (20 mL) were added, and the sample was cooled in a freezer (−20 °C) to give 0.128 g (85%) of compound **19**: mp 99–100 °C; ^{1}H NMR δ 0.87 (t, 3 H), 1.24–1.50 (several peaks, 10 H), 1.65 (m, 2 H), 2.78 (m, 2 H), 7.18 (t, 1 H), 7.30–7.42 (several peaks, 3 H), 7.50–7.71 (several peaks, 4 H), 7.87 (s, 1 H), 8.35 (d, 1 H). Anal. Calcd for $C_{21}H_{27}NOSSe$: C, 59.99; H, 6.47. Found: C, 59.96; H, 6.35.

^{1}H NMR Assays. To obtain reproducible results in the NMR experiments, we found it essential to use rigorously cleaned NMR tubes. The tubes were left in a chromium cleaning mixture[30] for 1–2 h and then in nitric acid (concentrated) for 3–4 h before they were washed with distilled water (1.5 L/tube) and dried in an oven.

A. *N*-Acetylcysteine System. In a typical experiment *N*-acetylcysteine (0.015 g, 0.092 mmol) was dissolved in a mixture of D_2O (0.6 mL) and CD_3OD (0.15 mL) in an NMR tube, and hydrogen peroxide (30%, 4.7 μL, 0.046 mmol) was added by syringe. After 1 h, the basal oxidation was checked by ^{1}H NMR, and the catalyst to be evaluated (2.7×10^{-7} mol) was added dissolved in CD_3OD or a CD_3OD/$CDCl_3$ mixture (~10 μL). The ^{1}H NMR spectrum of the solution was then recorded at intervals over 2 h, and the conversion of thiol to disulfide (as determined by integration of the methine peaks at 4.62 and 4.73 ppm, respectively) was plotted against time to give a linear correlation (at least to 50%

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conversion; $0.95 < r < 1.0$ for $5 < n < 20$). In some experiments followed to 100% conversion, the conversion vs time plot started to deviate from the straight line only after 60% conversion. The time required to reduce the thiol concentration with 50%, t_{50}, in the presence of different catalysts was determined, usually by extrapolation, from the equation of the line. When the rate of thiol disappearance could not be clearly distinguished from the basal oxidation rate, the catalyst was classified as "inactive" (Table I).

B. *tert*-Butyl Mercaptan System. Except for the replacement of *N*-acetylcysteine in D_2O/CD_3OD by *tert*-butyl mercaptan (10 μL, 0.089 mmol) dissolved in CD_3OD (0.75 mL), the procedure was as described above. The peaks at 1.40 (*tert*-butyl mercaptan) and 1.29 ppm (*tert*-butyl disulfide) in the ^1H NMR spectrum were used for calculation of the t_{50} values.

C. 1-Octyl Mercaptan System. Except for the replacement of *tert*-butyl mercaptan by 1-octyl mercaptan (15.4 μL, 0.089 mmol), the procedure was as described above (*tert*-butyl mercaptan system). The methylene peaks at 2.49 (1-octyl mercaptan) and 2.68 ppm (1-octyl disulfide) were used for calculation of the t_{50} values.

The t_{50} values reported in Table I were in most cases the average of several determinations. In a series of experiments carried out to examine the reproducibility of the method, the t_{50} values varied ±15% between different experiments for moderately active ($t_{50} > 100$ min) catalysts. For more active catalysts, larger variations between experiments were observed (although the reproducibility in parallel experiments with the same catalyst solution was satisfying). For example, catalyst **14** was tested 10 times in the *N*-acetylcysteine system, and the t_{50} values (min) were recorded as follows: 17, 13, 18, 33, 41, 9, 11, 41, 14, 17.

The experiment described in Figure 2 was carried out as follows. Diphenyl ditelluride (0.0184 g, 0.044 mmol) and 1-octyl mercaptan (15.4 μL, 0.089 mmol) were dissolved in a mixture of $CDCl_3$ (0.375 mL) and CD_3OD (0.375 mL) in an NMR tube containing anisole as an internal standard. Hydrogen peroxide (30%, 4.5 μL, 0.044 mmol) was then added and the sample left 20 min to allow formation of 1-octylthio phenyl telluride. After the ^1H NMR spectrum was recorded, a mixture of 1-octyl mercaptan (15.4 μL, 0.089 mmol) and hydrogen peroxide (30%, 4.5 μL, 0.044 mmol) was added, and the ^1H NMR spectrum was recorded at intervals over the next 60 min. It was shown by comparison with authentic samples that the triplet at 2.99 ppm corresponded to 1-octylthio phenyl telluride, the triplet at 2.69 ppm to 1-octyl disulfide, and the triplet at 2.50 ppm to 1-octyl mercaptan. The concentrations of the three species were determined by integration and comparison with the internal standard. When only 1-octyl mercaptan (15.4 μL, 0.089 mmol) or only hydrogen peroxide (30%, 4.5 μL, 0.044 mmol) was similarly added to the tellurosulfide solution, the formation of 1-octyl disulfide was very slow.

The experiment shown in Figure 3 was carried out as described above (Figure 2), except that diphenyl diselenide (0.013 g, 0.044 mmol) replaced diphenyl ditelluride and the time allowed for selenosulfide formation was 1.5 h. The triplet in the ^1H NMR spectrum at 2.84 ppm was characteristic of 1-octylthio phenyl selenide.

D. Reaction of Selenosulfide 18 with 1-Octyl Mercaptan and 1-Octyl Mercaptan/H_2O_2. Two samples of 1-octylthio 2-(*N*-phenylcarboxamido)phenyl selenide (**18**) (0.010 g, 0.024 mmol) dissolved in a 1/1 mixture of CD_3OD and $CDCl_3$ (0.75 mL), containing acetone as an internal standard, were prepared in NMR tubes. To one of the samples was added 1-octyl mercaptan (8.2 μL, 0.048 mmol) and to the other one a mixture of 1-octyl mercaptan (8.2 μL, 0.048 mmol) and hydrogen peroxide (30%, 2.4 μL, 0.024 mmol). After 2 h, ^1H NMR analysis showed 20% conversion of thiol to disulfide in the H_2O_2/thiol-treated sample, whereas the thiol-treated one contained only trace amounts (<3%) of disulfide.

Coupled Reductase Assay/Direct Glutathione Removal. The glutathione peroxidase-like activity of the compounds under study was assessed as their ability to catalyze the reaction between hydrogen peroxide and glutathione in an aqueous buffer at pH 7.4 using an indirect enzymatic assay and a direct assay based on glutathione removal. The experiments were performed as described in a literature procedure.[9]

In the coupled reductase assay, the consumption of NADPH in the absence of catalyst (= control) varied with DMSO concentration as follows: % DMSO [NADPH consumption (μM/min) ± standard deviation; $n = 4$], 0 [10 ± 2], 10 [14 ± 1], 20 [20 ± 2], 30 [23 ± 3].

In the catalyzed reactions, the following NADPH consumptions were recorded: compound no. [NADPH consumption (μM/min) ± standard deviation; $n = 3$], **7** [114 ± 2], **8** [88 ± 3], **9** [55 ± 3], **10** [35 ± 2], **11** [60 ± 1], **12** [28 ± 1], **13** [245 ± 10], **14** [208 ± 9], **15** [166 ± 15], **16** [219 ± 8], **2** [204 ± 15], **1** [53 ± 8].

The following control experiments were carried out using diaryl ditelluride **13**. A normal peroxidation experiment was carried out using varying amounts of GSSG reductase (0.5, 1.0, and 2.0 units). The NADPH consumption (μM/min ± standard deviation; $n = 3$) did not vary significantly (174 ± 8, 172 ± 3, and 183 ± 6; control rate = 8 ± 1 μM/min) in the three experiments. This shows that the measured rate is independent of the amount of GSSG reductase.

A normal peroxidation experiment was carried out in the absence of H_2O_2. No consumption of NADPH was observed. This shows that the ditelluride is not a substrate for GSSG reductase.

The reduction of GSSG (250 μM) in the presence of NADPH and GSSG reductase (0.1 units) in a 50 mM potassium phosphate buffer (pH = 7.4) was studied in the absence and presence of diaryl ditelluride (50 μM). The initial consumption of NADPH (μM/min ± standard deviation; $n = 3$) was 273 ± 3 and 266 ± 5, respectively. This shows that the ditelluride does not interfere with the assay.

Acknowledgment. The National Swedish Board for Technical Development, the Swedish Natural Science and Medical Research Councils, and Carl Tryggers Stiftelse are gratefully acknowledged for financial support.