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Antioxidant Functions of Selected *Allium* Thiosulfinates and *S*-Alk(en)yl-L-Cysteine Sulfoxides

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Pure thiosulfinates, R−S(O)S−R (**2**), where R = Me (**2a**), Pr (**2b**), or All (**2c**), at levels up to 4 mM were not capable of scavenging hydrogen peroxide or superoxide anion. Relative to standard antioxidants (ascorbic acid, *n*-propyl gallate, butylated hydroxytoluene, Trolox, and reduced glutathione), these thiosulfinates were 1−3 orders of magnitude less efficient at reducing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 0.5−2 orders of magnitude less efficient at quenching singlet oxygen, and about equally effective at scavenging hydroxyl radical. Generally, AllS(O)SAll (**2c**) was the most effective and PrS(O)SPr (**2b**) was the least effective thiosulfinate in these assays, except that MeS(O)SMe (**2a**) exhibited no quenching effect toward singlet oxygen. These thiosulfinates were also incapable at levels up to 0.1 mM (where they were toxic) of in vitro induction of quinone reductase (QR) in murine hepatoma (hepa 1c1c7) cells. However, *S*-1-propenyl-L-cysteine sulfoxide (isoalliin, **1a**) and cycloalliin (**3**) induced QR in this system at 2 mM and 1 mM, respectively, although doubling of QR required levels of 10−15 mM.

KEYWORDS: Thiosulfinate; allicin; S-1-propenyl-L-cysteine sulfoxide; isoalliin; cycloalliin; antioxidant; quinone reductase; hepa 1c1c7 cells

INTRODUCTION

Garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) have been cultivated for many centuries because of their characteristic flavors and medicinal properties (*1*−*3*). A lengthy list of health-promoting and related biological effects has been ascribed to the organosulfur components of tissue preparations from garlic and onion. Many of these applications are purported in accounts of traditional medicine (*2*), and many of these biological activities have also been demonstrated in various *Allium* tissue preparations by research in the past decade, including anticarcinogenic (*4*, *5*), antitumorigenic (*6*, *7*), antimutagenic (*8*), cardiovascular-protective (*9*, *10*), antimicrobial (*11*), immunomodulatory (*6*), and antioxidant (*12*, *13*) effects.

The antioxidant activities in *Allium* tissue extracts have been of particular interest because of the relationship between oxidative stress and pathologies such as atherosclerosis, cancer, and aging, in which free radicals and reactive oxygen species are implicated as having a role (*14*−*17*). Many recent studies on antioxidant activities of *Allium* tissue components have used crude extracts or tissue derivatives. Despite the complexity of such preparations, many investigators have reached unequivocal conclusions (*12*, *18*, *19*) or have insinuated (*7*, *20*, *21*) that the thiosulfinates (**2**) or related organosulfur components (**Figure 1**) are primarily responsible for the observed antioxidant effects, even though many other endogenous components may have antioxidant properties (*13*). Indeed, pure thiosulfinates (*22*) and related organosulfur compounds (*23*) exhibit antioxidant properties under specific conditions. However, it remains equivocal and premature to attribute a host of antioxidant properties to the organosulfur components in crude *Allium* preparations, simply because they are present. A case in point was the finding that pure alliin (**1c**, *S*-allyl-L-cysteine sulfoxide) has no antioxidant activity in a linoleic acid emulsion and has only weak reducing power, despite earlier claims that (**1c**) is an effective antioxidant (*24*). Thus, the antioxidant functionality of thiosulfinates (which are not present in intact tissue *(1)*) and related organosulfur compounds in Alliums will remain ambiguous until pure compounds are assessed.

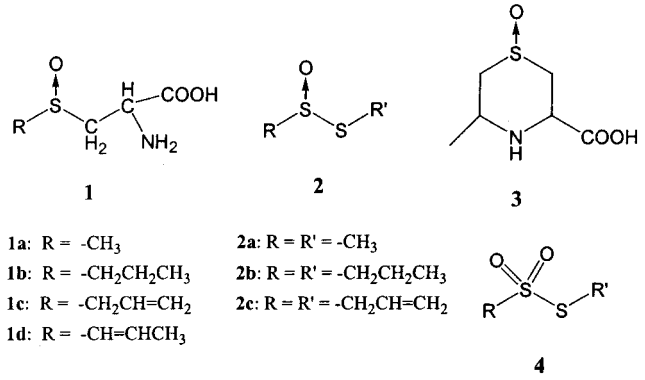

Figure 1. Chemical structures of organosulfur compounds used in this study.

In this study, we investigated the antioxidant function of pure thiosulfinates and selected *S*-alk(en)yl-L-cysteine sulfoxides (**1d** and **3**) (representative of Alliums) using a panel of assays to

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assess various types of antioxidant responses or functions. These activities include both the ability to scavenge various reactive oxygen species and the ability to induce a phase II enzyme (quinone reductase, QR; [NAD(P)H/(quinone-acceptor) oxidoreductase, EC 1.6.99.2]) in vitro through an antioxidant/electrophile response element (*25*, *26*). The objective was to develop a structure−function basis for the types of antioxidant activities that may exist for thiosulfinates.

EXPERIMENTAL PROCEDURES

Materials. All chemicals were obtained from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI) Chemical Companies, unless otherwise noted. White onion bulbs (cultivar and origin unknown) were obtained on multiple occasions from a local retailer.

Preparation of Thiosulfinates (2) and *S*-Alk(en)yl-L-cysteine Sulfoxides (1, 3). Three representative thiosulfinates were prepared using the model reaction system described previously (*27*, *28*). Crude alliinase action on *S*-methyl-L-cysteine sulfoxide (**1a**), *S*-propyl-L-cysteine sulfoxide (**1b**), and *S*-2-propenyl-L-cysteine sulfoxide (**1c**) individually allowed for the respective production of MeS(O)SMe (**2a**), PrS(O)SPr (**2b**), and AllS(O)SAll (allicin, **2c**). These thiosulfinates were obtained by extracting the reaction mixture with chloroform, followed by phase separation, evaporation, and reconstitution of the residue in slightly acidified water (pH ∼5) as rapidly as possible. Thiosulfinates were prepared daily, and have a half-life of >50 days at 20 °C when prepared under these conditions (*28*). The amount of thiosulfinate products was monitored by HPLC analysis (model 2300 pumps, V[4] detector set at 254 nm, and with peak integration by ChemResearch software; Isco, Lincoln, NE) of the chloroform extract (*27*, *28*).

S-1-Propenyl-L-cysteine sulfoxide (isoalliin, **1d**) was isolated from onion bulbs using ion-exchange chromatography, and its identity was confirmed by ^{1}H NMR, as described in an earlier report from this laboratory (*27*). Cycloalliin (**3**), the cyclic (imino acid) form of isoalliin (**1d**), was prepared as a distinct fraction during isolation of (**1d**). The identity of (**3**) was confirmed by ^{1}H NMR (Bruker Instruments, Inc., Billerica, MA) (model AM-300, 300 MHz, D_2O): δ 4.14 (1H, *dd*, J = 2.4, 13.2 Hz, C3−H), 3.19−3.91 (1H, *m*, C5−H), 2.60−3.50 (4H, *m*, C2−H_2, C6−H_2), 1.30 (3H, *d*, J = 6.6 Hz, C5−CH_3) and (Bruker model DMX-400, 400 MHz, CF_3COOD, sodium 3-(trimethylsilyl)-propane sulfonate as internal standard): δ 5.22 (1H, *dd*, C3−H), 2.8−5.0 (5H, *m*, C2−H_2, C5−H, C6−H_2), 1.74 (3H, *d*, C5−CH_3). These spectroscopic data were consistent with those of a previous analysis of pure cycloalliin (**3**) (*29*). Electrospray ionization-mass spectrometry analysis (triplequadrapole, model API 365, PE Sciex, Toronto, ON; applied voltages of 5000, 5, and 50 for needle, orifice, and ring, respectively; nebulizer and curtain settings were 10 and 8, respectively; mass range was *m*/*z* 100 to 500 by 0.1 amu steps) revealed signals of 178 MH^+ and 355 M_2H^+, also consistent with a cycloalliin (**3**) structure. Dimers (in this case, 355 M_2H^+) are often observed with this type of analysis (*30*).

Scavenging of Superoxide Anion ($O_2^{\bullet-}$). The ability of test compounds to scavenge superoxide anion ($O_2^{\bullet-}$) was determined on the basis of inhibiting the reduction of nitro blue tetrazolium (NBT) (*31*). The reaction mixture contained, at final concentrations, NADH (117 μm), NBT (37.5 μm), and phenazine methosulfate (PMS, 15 μm) in sodium phosphate (0.1 M, pH 7.4). The test compounds (or standard antioxidant) were added at various levels and the reaction was initiated by the addition of PMS. A five-minute reaction end-point was used, whereupon absorbance at 560 nm was measured against appropriate blank samples.

Scavenging of H_2O_2. Test compounds were incubated in the presence of H_2O_2 (0.5 mM) in sodium phosphate-buffered saline (PBS, pH 7.4) for 10 min. The level of H_2O_2 remaining after incubation was measured by the Fe^{3+}-xylenol orange (FOX) complex assay (*32*). After proper dilution, 0.1 mL of reaction mixture was mixed with 0.9 mL of FOX reagent that contained 90% methanol, 100 μM xylenol orange, 4 mM butylated hydroxytoluene (BHT), and 25 mM H_2SO_4, and incubated for 30 min at 20−22 °C prior to measurement of absorbance at 560 nm.

Scavenging of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical. Test compounds were incubated in 1.2 mL of solution containing DPPH radical (40 μM) in 40% ethanol for 30 min, and the absorbance of the solution was measured at 517 nm.

Scavenging of Singlet Oxygen ($^1O_2$). Singlet oxygen was generated by photosensitization of rose bengal using the approach and apparatus described previously (*33*). Reaction mixtures containing PBS (10 mM, pH 7.4), linoleic acid (0.03% v/v), Tween 20 (0.12% v/v), rose bengal (0.167 mg mL^{-1}) and the test compounds were placed in 16 × 10-mm glass tubes suspended in a temperature-controlled water bath (25 °C). An incandescent lamp (60 W) was placed in a 600-mL glass beaker and suspended in the center of the water bath such that the sample tubes were situated equidistant (4 cm) from the lamp. After 60 min of illumination, the level of linoleic acid hydroperoxide generated from photooxidation was measured by the FOX assay described earlier (*32*). Preliminary experiments revealed that AllS(O)SAll (**2c**) yielded FOX-reactive products upon exposure to $^1O_2$. Also, because some of the standard antioxidants tested are known to form (endo)peroxides in $^1O_2$-generating systems (*34*−*36*), nonlinoleic acid blanks were required to account for FOX-reactive products that could be formed from the test or standard antioxidant compounds alone.

Scavenging of Hydroxyl Radical (·OH). The iron-catalyzed oxidation of ascorbic acid at 20−22 °C was used to generate ·OH (*37*, *38*), and coumarin-3-carboxylic acid (3-CCA) was used as the detector/reporter for ·OH generated (*39*). The reaction mixture contained, at final concentrations, 100 mM sodium phosphate (pH 7.4) 167 μM iron-EDTA (1:2 molar ratio), 0.1 mM EDTA, 2 mM ascorbic acid, 0.1 mM 3-CCA, and test compound. Reactions were initiated by the addition of ascorbic acid and the progress of hydroxylation of 3-CCA was monitored up to an end point of 60 min reaction. Fluorescence of hydroxylated 3-CCA was measured in appropriately diluted samples using an LS-5B luminescence spectrometer (Perkin-Elmer, Norwalk, CT) with respective excitation and emission wavelengths of 395 and 450 nm, with corrections made from background contributions of reaction components.

Induction of Quinone Reductase (QR). A bioassay based on cultured murine hepatoma cells (Hepa 1c1c7) (ATCC, Rockville, MD) was used to assess QR induction as described earlier (*40*). Fetal bovine serum (FBS) was treated with activated charcoal to remove any traces of endogenous QR inducers prior to use. Cells were placed in 96-well plates at an inoculum of 10^5 cells per well and grown for 24 h, and then the test compound was added (in 200 μL of minimal essential medium), and the cells were induced for an additional 48 h at 37 °C in 5% CO_2 in air. Cells were lysed by adding 50 μL of 0.08% (w/v) aqueous digitonin prior to measuring QR activity in 200 μL of an assay cocktail containing FBS (0.066%, w/v), Tris−Cl (2.5%, v/v), Tween 20 (0.67%, v/v), FAD (0.67%, v/v), glucose-6-phosphate (0.1%, v/v), NADP (0.002%, w/v), glucose-6-phosphate dehydrogenase (0.0007%, w/v), 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (0.03%, w/v), menadione (0.0008%, w/v), and acetonitrile (0.1%, v/v; as the carrier for menadione) to each well. The absorbance of the reduced tetrazolium dye was measured over a 10-min period using an optical microtiter plate scanner (SPECTRA MAX plus, Molecular Devices, Sunnyvale, CA) set at 490 nm.

Samples from a duplicate 96-well plate were decanted and assayed for protein by immersion in a crystal violet (0.2% in 2% ethanol) bath for 10 min followed by addition of 200 μL of 0.5% (w/v) SDS solution (prepared in 50% aqueous ethanol). After incubation for 1 h in a shaker oven set at 37 °C, absorbance of each well was measured at 610 nm. The degree of staining with crystal violet was used as a measure of cell density and provided the basis for reporting specific activity of QR for each sample.

Statistical Analysis. Results are expressed as mean values ± SD for three separate experiments. Statistical analysis was afforded by analysis of variance (ANOVA) employing completely randomized block design with subsampling (*41*), and *t*-test, using the general linear model (SAS System for Windows Version 8, 1999−2000, SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Scavenging of $O_2^{\bullet-}$ and H_2O_2. No $O_2^{\bullet-}$ scavenging activity based on inhibition of NBT reduction was observed for any of the three thiosulfinates examined at levels up to 4.33 mM (data

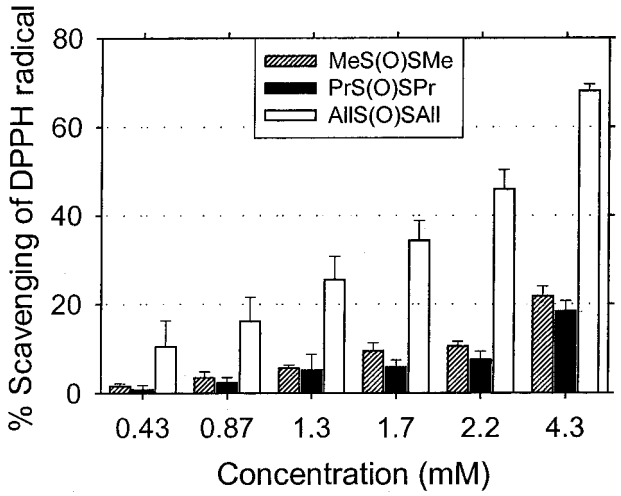

Figure 2. Scavenging activity of thiosulfinates toward DPPH radical. The standard reaction mixture contained 40 μM DPPH in 40% ethanol. Thiosulfinates were added to achieve the final concentrations indicated on the axis. The results represent the mean values ± SD from three independent experiments.

not shown). However, because oxidizing thiols and thiyl radicals can generate $O_2^{\bullet-}$ (*42*), it is possible that thiosulfinates do scavenge $O_2^{\bullet-}$ and yield $O_2^{\bullet-}$ or some corresponding species that can also reduce NBT. In fact, at the 4.3 mM level of PrS(O)SPr (**2b**), the amount of reduced NBT formed was 30−50% greater ($P < 0.05$) than that of the control samples. Although this may indicate a pro-oxidative activity of **2b** at this concentration, it is difficult to speculate on a mechanism of action that would be specific to **2b** and not MeS(O)SMe (**2a**) or AllS(O)SAll (**2c**).

None of the thiosulfinate species examined at levels up to about 2 mM was capable of scavenging H_2O_2. Because thiosulfinates were not capable of scavenging either $O_2^{\bullet-}$ or H_2O_2, no corresponding analyses for any common antioxidants were conducted.

Preliminary experiments (data not shown) also indicated that thiosulfinates were not capable of reversing/inhibiting oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) mediated by metmyoglobin/H_2O_2 (based on the assay described in ref *43*), or β-carotene bleaching mediated by *t*-BuOOH/metmyoglobin (based on the assay described in ref *44*). An inability of thiosulfinates to impede oxidation of fatty acids by direct attack of oxy- or peroxyl-radicals was reported previously (*45*).

Scavenging of DPPH Radical. All of the tested thiosulfinates could reduce DPPH radical in a concentration-dependent response ($P < 0.01$; **Figure 2**). AllS(O)SAll (**2c**) was the most effective thiosulfinate in this system, and MeS(O)SMe (**2a**) was more effective than PrS(O)SPr (**2b**) ($P < 0.01$). Although the −S(O)S− functional unit may have a role in DPPH radical reduction, it was evident that the allyl functional units of AllS(O)SAll provided for a more effective electron donating ability than saturated alkyl groups within the thiosulfinate structure. Dose-dependent responses (data not shown) obtained with known antioxidants indicated that the relative effectiveness of thiosulfinates as reducing agents on an equimolar basis was 2−3 orders of magnitude less than that of more commonly used antioxidants (**Table 1**). Approximate levels of test compounds to quench 50% DPPH radical were 2,400 μM for allicin (**2c**), compared to 11, 19, 11, 3.6, and 31 μM for L-ascorbic acid, BHT, Trolox, *n*-propyl-gallate, and reduced glutathione, respectively.

Table 1. Relative Antioxidant Potential of Some Known Antioxidants and Thiosulfinates[a]

	relative quenching/scavenging effects		
antioxidant	DPPH radical	singlet oxygen	hydroxyl radical
BHT	129.0	34.3	ND*
n-propyl-gallate	679.8	3.24	0.781
L-ascorbic acid	225.2	2.57	ND*
Trolox	220.5	5.07	0.791
glutathione (red.)	77.9	5.63	0.635
AllS(O)SAll (allicin)	1.00	1.00	1.00
MeS(O)SMe	0.243	NA**	0.843
PrS(O)SPr	0.195	0.271	0.609

[a] Results are shown as relative activities by adjusting the antioxidant potential of AllS(O)SAll (allicin) to 1.00, and represent the average of three independent experiments. ND*, not determined. NA**, no antioxidant activity detected.

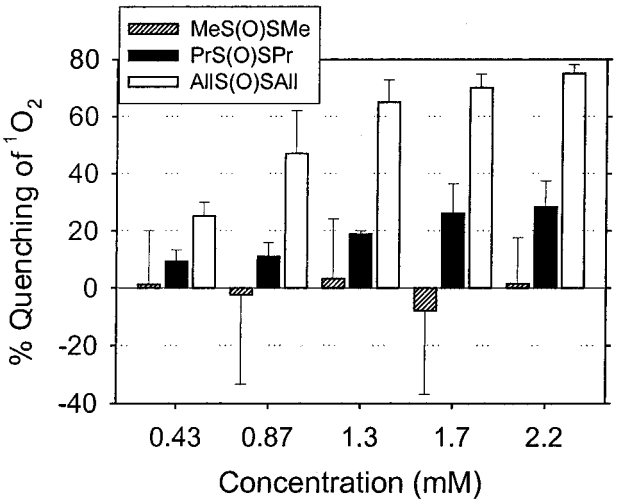

Figure 3. Quenching activities of thiosulfinates toward singlet oxygen. The standard reaction mixture contained, at final concentrations, 10 mM PBS, 0.03% (v/v) linoleic acid, 0.12% (v/v) Tween 20, and 0.167 mg Rose Bengal mL^{-1}. Thiosulfinates were added to achieve the final concentrations indicated on the axis. The results represent the mean values ± SD from three independent experiments.

The relative effectiveness in DPPH radical quenching that we observed for the common antioxidants were quantitatively similar to what has been reported from previous studies: gallic acid derivatives > Trolox/tocopherols ∼ ascorbic acid ≥ BHT/butylated hydroxyanisole > cysteine (mercaptans) with respective molar reducing activities of about 3:1:1:0.6:0.4 (*46*−*50*; **Table 1**). Differences between studies may exist because of the time frame used for the assay, and the known differences among antioxidants in their kinetics for quenching DPPH radical (*46, 47*). The ineffectiveness of the thiosulfinates (**2**) in the DPPH radical quenching assay was likely associated with the lack of reactivity of the −S(O)S− functional unit with DPPH radical and/or the relative oxidation−reduction potentials for the alk(en)yl groups of thiosulfinates (e.g., allyl•, H^+/allyl-H; E°′/mv = 960) compared to respective tocopherol, ascorbic acid, and phenolic (catechol) oxidation−reduction couples (E°′/mv = 500, 530, and 280, respectively) (*51*).

Quenching of Singlet Oxygen ($^1O_2$). Quenching of $^1O_2$, expressed as percent inhibition of linoleic hydroperoxide production, was observed in a concentration-dependent manner for AllS(O)SAll (**2c**) and PrS(O)SPr (**2b**) but not for MeS(O)SMe (**2a**) ($P < 0.01$) (**Figure 3**). Quenching activity of **2c** was almost 4-fold greater than that of **2b** ($P < 0.01$) (**Table 1**).

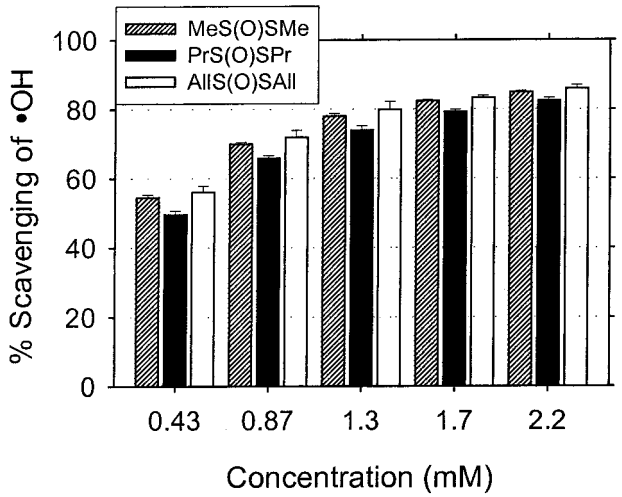

Figure 4. Scavenging activities of thiosulfinates toward hydroxyl radical. The standard reaction mixture contained, at final concentrations, 100 mM sodium phosphate (7.4), 0.167 μM iron-EDTA (1:2 molar ratio), 0.1 mM EDTA, 2 mM ascorbic acid, and 0.1 mM coumarin-3-carboxylic acid. Thiosulfinates were added to achieve the final concentrations indicated on the axis. The results represent the mean values ± SD from three independent experiments.

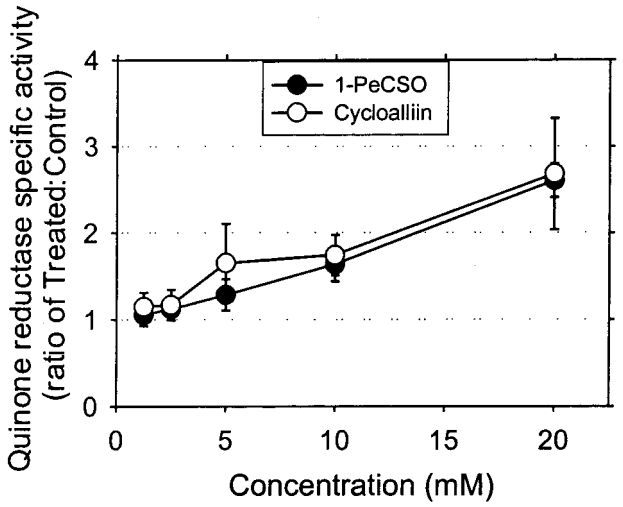

Figure 5. Quinone reductase induction in Hepa 1c1c7 cells by *S*-alk(en)yl-L-cysteine sulfoxides. Culture conditions are specified in Experimental Procedures. Test compounds were added to achieve the final concentrations indicated on the axis and cells were induced for 48 h at 37 °C, prior to lysing by 0.08% (w/v) aqueous digitonin prior to measuring QR activity or 0.5% SDS in 50% ethanol for protein measurement. The results represent the mean values ± SD from three independent experiments.

The inhibitory effect of added thiosulfinate was manifested as a reduced rate of peroxide formation; the additional appearance of a lag period of peroxide accumulation was observed at the greater levels of thiosulfinate examined. It seems likely that the dominant inhibitory effect from thiosulfinates in this system is derived from the unsaturated R group (allyl in **2c**), and quenching of $^1O_2$ could thereby be afforded by a typical ene-addition reaction (*52*). The −S(O)S− functional unit is likely to play a lesser role in quenching $^1O_2$ because thiosulfinates (**2**) and thiosulfonates (**4**) are isolable products of the reaction of $^1O_2$ with disulfides (*53, 54*).

All of the common antioxidants tested were better inhibitors of $^1O_2$-mediated linoleic acid oxidation than were the thiosulfinates (**Table 1**). With the exception of BHT, the common antioxidants tested were of similar effectiveness in this assay system. The compilation of $^1O_2$ quenching rate constants (k, in $M^{-1}s^{-1}$) from various studies reveals the following order of effectiveness: carotenoids, 10^9-10^{10}; amino acids, 10^6-10^8; tocopherol and other phenols, 10^7-10^9; and sulfides 10^6 (*55, 56*). A corresponding rate constant of 10^8 has been estimated for ascorbic acid (*57*). These published studies were commonly done in organic solvents, and the choice of solvent can influence the lifetime of $^1O_2$, as well as influencing the quenching ability of the test compound (*55*). Despite this qualification, based on these published rate constants, the relative effectiveness of AllS(O)SAll (**2c**) and PrS(O)SPr (**2b**) is surprising, as is the relative effectiveness of BHT. We attribute the unexpected effectiveness of these three compounds to their apolarity (*27, 57*) and their ability to preferentially partition into the lipid phase of this assay system, resulting in an increase in their effective molarities proximal to the target of $^1O_2$ oxidation, linoleic acid.

Scavenging of ·OH. All thiosulfinate species tested were capable of inhibiting ·OH-mediated hydroxylation of 3-CCA, based on the % inhibition of accumulation of fluorescent products (**Figure 4**). The relative degree of effectiveness observed was AllS(O)SAll (**2c**) > MeS(O)SMe (**2a**) > PrS(O)SPr (**2b**) ($P < 0.01$), and the inhibitory properties of these thiosulfinates toward ·OH rivaled those of the common antioxidants tested (**Table 1**). The similar degree of effectiveness of the thiosulfinates implies that the scavenging of ·OH is based on the −S(O)S− functional unit, with less influence of the R group.

A recent study demonstrated the ·OH-scavenging activity of pure allicin (**2c**) using a spin trapping and electron spin resonance approach (*22*). The commercial preparation "Garlicin" also has ·OH-scavenging activity, and this was attributed to (**2c**) (*12*), despite the presence of many other potentially inhibitory substances. In fact, ·OH reacts with virtually all organic compounds at or near the rate of diffusion (*59*), so it is not surprising that the components evaluated had similar inhibitory effects.

Induction of Quinone Reductase (QR). None of the three thiosulfinates (**2a**, **2b**, and **2c**) tested was capable of inducing QR in hepa 1c1c7 cells. At levels of 0.1 mM thiosulfinate addition, ≥50% toxicity of the cells was observed, based on reduced levels of cellular protein after the 48-h induction period. Some mixed thiosulfinates, and specifically those comprised of 1-propenyl residues, are capable of a 2-fold induction of QR in this bioassay at 10−14 μM level of addition (*60*). Effective inducers of QR contain structures characterized as Michael acceptors (*26, 61*).

Two *S*-alk(en)yl-L-cysteine sulfoxides (**1**) were examined for their QR-inducing effect, and both isoalliin (**1d**) and cycloallin (**3**) were capable of doubling the level of QR in hepa 1c1c7 cells at levels of 10−15 mM (**Figure 5**). No toxicity (indexed by a reduction of protein in induced cells) was observed for these two test compounds over the range of concentrations evaluated. The concentrations of **1d** and **3** required to double QR activity were much greater than those of compounds recognized as potent QR-inducers (*61*), and some 3 orders of magnitude greater than the levels of 1-propenyl mixed thiosulfinates required for this effect (*60*). However, a significant induction of QR over control samples was observed in this bioassay at 2 mM isoalliin (**1d**) ($P < 0.01$) and 1 mM cycloalliin (**3**) ($P = 0.01$). Furthermore, because **1d** and **3** are amino acid derivatives, they may be more bioavailable than thiosulfinates, the latter of which are rapidly metabolized by bodily fluids (*3*).

Considering that the per capita annual utilization of onions in the U.S. is about 19 pounds (*62*), and that the combined levels of **1d** and **3** in cooked onion is about 10−30 mM (*63*−*65*), a tangible health benefit may be derived from the regular and sustained consumption of onions if compounds **1d** and **3** are ultimately shown to be efficacious phase II enzyme inducers in humans.

CONCLUSIONS

Relative to some common antioxidants, thiosulfinates (**2**) were found to be effective antioxidants only in terms of scavenging ·OH. This activity was previously reported only for pure allicin (**2c**) (*22*). Although thiosulfinates had some ability to scavenge DPPH radical and $^1O_2$, their abilities in these systems were often 1−3 orders of magnitude less than those of known antioxidant compounds. Thus, we conclude that the observation of antioxidant properties in crude extracts of *Allium* tissues are more likely due to components other than thiosulfinates (**2**) and related organosulfur compounds (**1**). If thiosulfinates are biologically active in humans, other mechanisms are likely responsible for their physiological effects in biological systems, such as modulation of −SH functionality of biological components (*22*), or by serving as precursors to other biologically active agents.

Onion and other Alliums have long been associated with reduced risk of cancer and disease (*2*, *4*, *5*), and the agent(s) responsible for this benefit remain(s) to be identified. The results from the present study indicate that the in vitro phase II enzyme inducing properties of isoalliin (**1d**) and cycloalliin (**3**), compounds present in onion extracts (*40*), merit further assessment in this regard.

Supporting Information Available: ^{1}H NMR scan of cycloalliin. This material is available free of charge via the Internet at http://pubs.acs.org.

ABBREVIATIONS USED

RS(O)SR, thiosulfinate; BHT, butylated hydroxytoluene; Trolox, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid; QR, quinone reductase; FBS, fetal bovine serum; NBT, nitro blue tetrazolium; PMS, phenazine methosulfate; $O_2^{\bullet-}$, superoxide anion; $^1O_2$, singlet oxygen; ·OH, hydroxyl radical; DPPH, 1,1-diphenyl-2-picrylhydrazyl; *t*-BuOOH, *ter*-butyl hydroperoxide; PBS, sodium phosphate-buffered saline; FOX, Fe^{3+}-xylenol orange; 3-CCA, coumarin-3-carboxylic acid.

LITERATURE CITED

(1) Carson, J. F. Chemistry and biological properties of onions and garlic. *Food Rev. Int.* **1987**, *3*, 71−103.

(2) Augusti, K. T. Therapeutic and medicinal values of onions and garlic. In *Onions and Allied Crops. Vol. III, Biochemistry, Food Science and Minor Crops*; Brewster, J. L., Rabinowitch, H. D., Eds.; CRC Press: Boca Raton, FL, 1990; pp 93−108.

(3) Lawson, L. D. Garlic: a review of its medicinal effects and indicated active compounds. In *Phytomedicines of Europe. Chemistry and Biological Activity*; Lawson, L. D., Bauer, R., Eds.; American Chemical Society: Washington, DC, 1998; pp 176−209.

(4) Lau, B. H. S.; Tadi, P. P.; Tosk, J. M. *Allium sativum* (garlic) and cancer prevention. *Nutr. Res.* **1990**, *10*, 937−948.

(5) Singh, S. V.; Pan, S. S.; Srivastava, S. K.; Xia, H.; Hu, X.; Zaren, H. A.; Orchard, J. L. Differential induction of NAD(P)H: quinone oxidoreductase by anticarcinogenic organosulfides from garlic. *Biochem. Biophys. Res. Commun.* **1998**, *244*, 917−920.

(6) Kyo, E.; Uda, N.; Suzuki, A.; Kakimoto, M.; Ushujima, M.; Kasuga, S.; Itakura, Y. Immunomodulation and antitumor activities of aged garlic extract. *Phytomedicine* **1998**, *5*, 259−267.

(7) Siegers, C.-P.; Röbke A.; Pentz, R. Effects of garlic preparations on superoxide production by phorbol ester activated granulocytes. *Phytomedicine* **1999**, *6*, 13−16.

(8) Ishikawa, K.; Naganawa, R.; Yoshida, H.; Iwata, N.; Fukuda, H.; Fujino, T.; Suzuki, A. Antimutagenic effects of ajoene, an organosulfur compound derived from garlic. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 2086−2088.

(9) Mirhadi, S. A.; Singh, S. Effect of garlic supplementation to cholesterol-rich diet on development of atherosclerosis in rabbits. *Ind. J. Exp. Biol.* **1991**, *29*, 162−168.

(10) Morimitsu, Y.; Morioka, Y.; Kawakishi, S. Inhibitors of platelet aggregation generated from mixtures of *Allium* species and/or *S*-Alk(en)yl-L-cysteine sulfoxide. *J. Agric. Food Chem.* **1992**, *40*, 368−372.

(11) Weber, N. D.; Anderson, D. O.; North, J. A.; Murray, B. K.; Lawson, L. D.; Hughes, B. G. *In vitro* virucidal effects of *Allium sativum* (garlic) extract and compounds. *Planta Med.* **1992**, *58*, 417−423.

(12) Prasad, K.; Laxdal, V. A.; Yu, M.; Raney, B. L. Antioxidant activity of allicin, an active principle in garlic. *Mol. Cell. Biochem.* **1995**, *148*, 183−189.

(13) Yin, M.-C.; Cheng, W.-S. Antioxidant activity of several *Allium* members. *J. Agric. Food Chem.* **1998**, *46*, 4097−4101.

(14) Maeda, H.; Katsuki, T.; Akaike, T.; Yasutake, R. High correlation between lipid peroxide radical and tumor-promoter effect: suppression of tumor promotion in the Epstein−Barr virus/B-lymphocyte system and scavenging of alkyl peroxide radicals by various vegetables extracts. *Jpn. J. Cancer Res.* **1992**, *52*, 923−928.

(15) Halliwell, B.; Gutteridge, J. M. C.; Cross, E. E. J. Free radicals, antioxidants, and human disease: where are we now? *J. Lab. Clin. Med.* **1992**, *119*, 598−620.

(16) Cavalieri, E. L.; Rogan, E. G. The approach to understanding aromatic hydrocarbon carcinogenesis. The central role of radical cations in metabolic action. *Pharmacol. Ther.* **1992**, *55*, 183−199.

(17) Salvemini, D.; Botting, R. Modulation of platelet function by free radicals and free-radical scavengers. *Trends Pharmacol. Sci.* **1993**, *14* (2), 36−42.

(18) Kourounakis, P. N.; Rekka, E. A. Effect on active oxygen species of alliin and *Allium sativum* (garlic) powder. *Res. Commun. Chem. Pathol. Pharmacol.* **1991**, *74*, 249−252.

(19) Rekka, E. A.; Kourounakis, P. N. Investigation of the molecular mechanism of the antioxidant activity of some *Allium sativum* ingredients. *Pharmazie* **1994**, *49*, 539−540.

(20) Popov, I.; Blumstein, A.; Lewin, G. Antioxidant effects of aqueous garlic extract. 1st communication: direct detection using the photochemiluminescence. *Arzneim.-Forsch./Drug Res.* **1994**, *44*, 602−604.

(21) Kim, S. M.; Kubota, K.; Kobayashi, A. Antioxidative activity of sulfur-containing flavor compounds in garlic. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1482−1485.

(22) Rabinkov, A.; Miron, T.; Konstantinovski, L.; Wilchek, M.; Mirelman D.; Weiner, L. The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. *Biochim. Biophys. Acta* **1998**, *1379*, 233−244.

(23) Imai, J.; Ide, N.; Nagae, S.; Moriguchi, T.; Matsuura, H.; Itakura, Y. Antioxidant and radical scavenging effects of aged garlic extract and its constituents. *Planta Med.* **1994**, *60*, 417−420.

(24) Hirata, R.; Matsushita, S. Reducing activity level of alliin. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 484−485.

(25) Rushmore, T. H.; Morton, M. R.; Pickett, C. B. The antioxidant response element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *J. Biol. Chem.* **1991**, *266*, 11632−11639.

(26) Prestera, T.; Talalay, P. Electrophile and antioxidant regulation of enzymes that detoxify carcinogens. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8965−8969.

(27) Shen, C.; Parkin, K. L. In vitro biogeneration of pure thiosulfinates and propanethial-*S*-oxide. *J. Agric. Food Chem.* **2000**, *48*, 6254−6260.

(28) Shen, C.; Xiao, H.; Parkin, K. L. In vitro stability and chemical reactivity of thiosulfinates. *J. Agric. Food Chem.* **2002**, *50* (in press).

(29) Sakai, K.; Yoneda, N. Convenient synthesis of 1,4-thiazane-3-carboxylic acid derivatives. *Chem. Pharm. Bull. (Tokyo)* **1981**, *29*, 1554−1560.
(30) Draper, W. M. Electrospray liquid chromatography quadrapole ion trap mass spectrometry determination of phenyl urea herbicides in water. *J. Agric. Food Chem.* **2001**, *49*, 2746−2755.
(31) Yen, G.-C.; Chen, H.-Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27−32.
(32) Jiang, Z.-Y.; Hunt, J. V.; Wolff, S. P. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low-density lipoprotein. *Anal. Biochem.* **1992**, *202*, 384−389.
(33) Lowum, S. E.; Parkin, K. L. Characterizaton of dye-sensitized photooxidation of mushroom tyrosinase. *J. Food Biochem.* **1989**, *13*, 391−401.
(34) Kaiser, S.; Di Mascio, P.; Murphy, M. E.; Sies, H. Physical and chemical scavenging of singlet molecular oxygen by tocopherols. *Arch. Biochem. Biophys.* **1990**, *277*, 101−108.
(35) Bradley, D. G.; Min, D. B. Singlet oxygen oxidation of foods. *CRC Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 211−236.
(36) Yasaei, P. M.; Yang, G. C.; Warner, C. R.; Daniels, D. H.; Ku, Y. Singlet oxygen oxidation of lipids resulting from photochemical sensitizers in the presence of antioxidants. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1177−1181.
(37) Cohen, G. A novel route for the metabolism of ethanol: the oxidation of ethanol by hydroxyl free radicals. In *Alcohol and Aldehyde Metabolizing Systems*; Thurman, R. G., Williamson, J. R., Drott, H., Chance, B., Eds.; Academic Press: New York, 1977; pp 403−412.
(38) Klein, S. M.; Cohen, G.; Cederbaum, A. I. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical generating system. *Biochemistry* **1981**, *20*, 6006−6012.
(39) Manevich, Y.; Held, K. D.; Biaglow, J. E. Coumarin-3-carboxylic acid as a detector for hydroxyl radicals generated chemically and by gamma radiation. *Radiation Res.* **1997**, *148*, 580−591.
(40) Prochaska, H. J.; Santamaria, A. B.; Talalay, P. Rapid detection of inducers of enzymes that protect against carcinogens. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2394−2398.
(41) Snedecor, G. W.; Cochran, W. G. *Statistical Methods, 7th ed.*; The Iowa State University Press: Ames, IA, 1980.
(42) Winterbourne, C. C.; Metodiewa, D. Reaction of superoxide with glutathione and other thiols. In *Methods in Enzymology. Biothiols, Part A, Monothiols and Dithiols, Protein Thiols, and Thiyl Radicals*, Vol. 251; Packer, L., Ed.; Academic Press: New York, 1995; pp 81−86.
(43) Rice-Evans, C.; Miller, N. J. Total antioxidant status in plasma and body fluids. In *Methods in Enzymology. Oxygen Radicals in Biological Systems*, Vol. 234; Packer, L., Ed.; Academic Press: New York, 1994; pp 279−293.
(44) Akaike, T.; Ijiri, S.; Sato, K.; Katsuki, T.; Maeda, H. Determination of peroxyl radical-scavenging activity in food by using bacteriocidal action of alkyl peroxyl radical. *J. Agric. Food Chem.* **1996**, *43*, 1864−1870.
(45) Bateman, L.; Cain, M.; Colclough, T.; Cunneen, J. I. Oxidation of organic sulfides. Part XIII. The antioxidant action of sulphoxides and thiosulphinates in autoxidizing squalene. *J. Chem. Soc.* **1962**, 3570−3578.
(46) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25−30.
(47) von Gadow, A.; Joubert, E.; Hansmann, C. F. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT, and BHA. *J. Agric. Food Chem.* **1997**, *45*, 632−638.
(48) Moon, J.-H.; Terao, J. Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low-density lipoprotein. *J. Agric. Food Chem.* **1998**, *46*, 5062−5065.
(49) Sanchez-Moreno, C.; Larrauri, J. A.; Saura-Calixto, F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. *Food Res. Int.* **1999**, *32*, 407−412.
(50) Lissi, E. A.; Modak, B.; Torres, R.; Escobar, J.; Urzua, A. Total antioxidant potential of resinous exudates from *Heliotropium* species, and a comparison of the ABTS and DPPH methods. *Free Radical Res.* **1999**, *30*, 471−477.
(51) Buettner, G. R. The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. *Arch. Biochem. Biophys.* **1993**, *300*, 535−543.
(52) Frimer, A. A.; Stephenson, L. M. The singlet oxygen "ene" reaction. In *Singlet O_2. Vol. II: Reaction Modes and Products, Part 1;* Frimer, A. A., Ed.; CRC Press: Boca Raton, FL, 1985; pp 67−91.
(53) Murray, R. W.; Jindal, S. L. The photosensitized oxidation of disulfides related to csystine. *Photochem. Photobiol.* **1972**, *16*, 147−151.
(54) Clennan, E. L.; Wang, D.; Clifton, C.; Chen, M.-F. Geometry-dependent quenching of singlet oxygen by dialkyl disulfide. *J. Am. Chem. Soc.* **1997**, *119*, 9081−9082.
(55) Belluš, D. Quenchers of singlet oxygen − a critical review. In *Singlet Oxygen. Reactions with Organic Compounds and Polymers*; Rånby, B., Rabek, J. F., Eds.; John Wiley & Sons: New York, 1978; pp 61−110.
(56) Foote, C. S. Mechanisms of photooxidation. In *Singlet Oxygen. Reactions with Organic Compounds and Polymers*; Rånby, B., Rabek, J. F., Eds.; John Wiley & Sons: New York, 1978; pp 135−163.
(57) Bodannes, R. S.; Chan, P. C. Ascorbic acid as a scavenger of singlet oxygen. *FEBS Lett.* **1979**, *105*, 195−197.
(58) Schwarz, K.; Frenkel, E. A.; German, J. B. Partition behavior of antioxidative phenolic compounds in heterophasic systems. *Fett/Lipid* **1996**, *98*, 115−121.
(59) Youngman, R. J. Oxygen activation: is the hydroxyl radical always biologically relevant? *Trends Biochem. Sci.* **1984**, *9*, 280−283.
(60) Block, E.; Gillies, J. Z.; Gillies, C. W.; Bazzi, A. A.; Putnam, D.; Revelle, L. K.; Wang, D.; Zhang, X. *Allium* chemistry: microwave spectroscopic identification, mechanism of formation, synthesis, and reactions of (*E, Z*)-propanethial *S*-oxide, the lachrymatory factor of the onion (*Allium cepa*). *J. Am. Chem. Soc.* **1996**, *118*, 7492−7501.
(61) Talalay, P.; De Jong, M. J.; Prochaska, H. J. Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8261−8265.
(62) U.S. Department of Agriculture, National Agriculture Statistics Service. Chapter IV. Statistics of vegetables and melons. **2000**, www.nass.usda.gov.
(63) Matikkala, E. J.; Virtanen, A. I. On the quantitative determination of the amino acids and γ-glutamylpeptides of onion. *Acta Chem. Scand.* **1967**, *21*, 2891−2893.
(64) Thomas, D. J.; Parkin, K. L. Quantificatiin of alk(en)yl-L-cysteine sulfoxides and related amino acids in Alliums by high-performance liquid chromatography. *J. Agric. Food Chem.* **1994**, *42*, 1632−1638.
(65) Lancaster, J. E.; Shaw, M. L.; Randle, W. M. Differential hydrolysis of alk(en)yl cysteine sulphoxides by alliinase in onion macerates: flavour implications. *J. Sci. Food Agric.* **1998**, *78*, 367−372.

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