S-Alkenyl Cysteine Sulfoxide and Its Antioxidant Properties from *Allium cepa* var. *tropeana* (Red Onion) Seeds

Irene Dini,* Gian Carlo Tenore, and Antonio Dini

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", Via D. Montesano, 49, 80131 Naples, Italy

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A new cysteine sulfoxide, (S_SR_C)-*S*-(3-pentenyl)-L-cysteine sulfoxide (**1**), was identified from the seeds of *Allium cepa* var. *tropeana*, together with the known methiin, etiin, alliin, isoalliin, propiin, and butiin. The structure of compound **1** was established by analysis of its physical and spectroscopic data. The antioxidant activity of an extract containing cysteine sulfoxides and compound **1** was evaluated using the FRAP and DPPH tests.

Since ancient times, onion (*Allium cepa* L.) and related species of the leek family (Alliaceae) have been used as foods, spices, and herbal remedies in many parts of the world.[1] The bulbs are the main edible part, with a distinctive strong flavor and pungent odor. The seeds are also eaten, expecially in some Indian dishes, but their commercial availability is currently limited. The medicinal properties associated with members of *Allium* are attributed to sulfur-containing flavor compounds.[2−5] Their precursors are odorless, nonprotein sulfur amino acids, namely, *S*-alk(en)yl cysteine sulfoxides. Only the L-(+)-isomers of these substances have been found in nature.[6] To date, four major and two minor cysteine sulfoxide derivatives have been identified in the genus *Allium*: (+)-*S*-methyl-L-cysteine sulfoxide (methiin), (+)-*S*-ethyl-L-cysteine sulfoxide (ethiin), (+)-*S*-propyl-L-cysteine sulfoxide (propiin), (+)-*S*-allyl-L-cysteine sulfoxide (alliin), (+)-*S*-(*trans*-propenyl)-L-cysteine sulfoxide (isoalliin), and (+)-*S*-butyl-L-cysteine sulfoxide (butiin).[7] Usually, these compounds are not found together in a single species.

This paper describes an investigation on the amino acid odor precursors present in *Allium cepa* var. *tropeana* seeds and their antioxidant properties. The antioxidant activities in *Allium* tissue extracts have been of particular interest because of the relationship between oxidative stress and disease conditions, such as atherosclerosis and cancer, in which free radicals and reactive oxygen species are implicated as having a role. Antioxidant activity was evaluated in the present investigation using DPPH and FRAP tests, since the use of more than one method is recommended to give a more comprehensive determination of antioxidant capacity, as there is no simple universal method by which this can be measured accurately and quantitatively.

A. cepa var. *tropeana* seed flour was extracted with boiling hydromethanolic solutions in order to inhibit enzymatic reactions. Successively, the extract was chromatographed by passage over a cationic ion-exchange resin, gel filtration, and HPLC. Cysteine sulfoxide derivatives were identified on the basis of their molecular ion peaks and characteristic mass spectrometric fragmentation patterns. Methiin, ethiin, alliin, propiin, and buthiin were revealed in HPLC fractions from Sephadex pool 15−20, while isoalliin was obtained from HPLC fractions of Sephadex pool 21−34 together with an unknown cysteine sulfoxide derivative, **1** (t_R 13.0 min). This amino acid was isolated using a modified procedure first described by Kubec et al.[8,9] and characterized by polarimetry, IR, NMR, and MS techniques. From the ^{1}H data of **1**, two discrete spin systems could be discerned. The proton sequence within each spin system was elucidated by the series of cross-peaks in the COSY spectrum. All the proton resonances of this compound were associated unambiguously with the relevant carbon atoms from the HSQC spectrum, while data arising from the HMBC experiment (Figure 1) were used to interconnect the partial structures. Thus, the proton at δ 3.78 (m), which coupled to the proton at δ 2.94 (m, J = 6.1 Hz), coupled to the proton at δ 5.82 (m, J = 13.7, 8 Hz), which in turn coupled to the protons δ 1.55 (t, J = 8 Hz) of the C-1 methyl group (δ 18.0), giving rise to the C-5 to C-9 fragment, the pentenyl group. The double-bond isomerism was *E* on the basis of the coupling constant (J = 13.7 Hz). The proton resonances at δ 3.06 (dd, J = 14.0, 8.0 Hz) and 3.40 (dd, J = 14.0, 6.0 Hz) were located as part of an ABX system; they formed the second proton spin system and were assigned to the methylene group of cysteine. Finally, ^{1}H spectroscopy and the optical rotation were employed to determine the absolute configuration at the two chiral centers of the compound (at the sulfur and the α-carbon). In the ^{1}H spectrum, the pattern of the S(O)CH_2CH(NH_2) methylene protons appeared as two distinctive doublets of doublets (δ 3.06 and 3.40), with coupling constants of J_{AX} = 14.0 Hz and J_{BX} = 6.0 Hz. These values are a typical feature of all *S*-substituted cysteine sulfoxide derivatives that have the sulfoxide oxygen and the amino group on the same face of the molecule. The correct structural assignment of compound **1** was further verified by comparing its specific rotation value $[\alpha]^{20}_D$ +29 with those reported positive for (S_SR_C)-*S*-*n*-cysteine and negative for (R_SR_C)-*S*-*n*-cysteine sulfoxide derivatives, which confirmed the presence of a (S_SR_C)-*S*-*n*-cysteine sulfoxide derivative.[10] Moreover, the L-(+)-cysteine sulfoxide isomer is the most common form in nature.[7] Therefore, compound **1** was characterized as (S_SR_C)-*S*-(3-pentenyl)-L-cysteine sulfoxide. No attempt was made to purify the remaining cysteine derivatives detected by MS and MS-MS, since these cysteine derivatives are most probably sulfoxide forms with absolute configurations analogous to compound **1**, as supported by previous reports.[7]

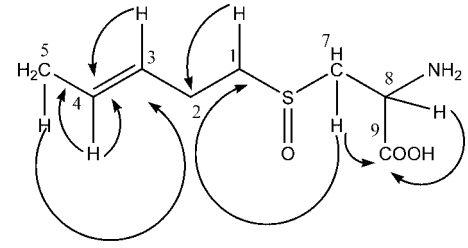

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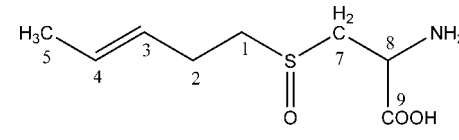
Figure 1. Significant HMBC correlations for compound **1**.



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Therefore, the antioxidant activity of a cysteine sulfoxide extract of *A. cepa* var. *tropeana* was studied by DPPH and FRAP methods. The results showed that antioxidant activity was particularly due to reducing activity (FRAP: 927.65 ± 0.6 TEs μM, $p < 0.0001$) rather than radical-scavenging effects (DPPH: 411.41 ± 1.0 TEs μM, $p < 0.005$). The antioxidant activity of the new compound **1**, which was also evaluated, showed a low antioxidant activity, when

* To whom correspondence should be addressed. Tel: 039-81-678537. Fax: 039-81-678552. E-mail: irdini@unina.it.

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compared with the standards used (FRAP 178 ± 0.8 μM; DPPH 107 ± 0.9 μM).

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P-100 polarimeter (Tokyo, Japan), equipped with a sodium lamp (589 nm) and a 10 mm microcell. UV spectra were recorded on a JASCO V-530 UV−vis spectrophotometer (Tokyo, Japan). The FTIR spectra were obtained on a Bruker IFS-48 spectrophotometer (Billerica, MA) using a KBr matrix. NMR spectra were determined at 25 °C on a Varian Unity INOVA 500 NMR spectrometer (Palo Alto, CA) and processed using the Varian VNMR software package; chemical shifts are referenced to the residual solvent signal (CH_3OD: δ_H 3.34, δ_C 49.0). Homonuclear ^1H connectivities were determined by COSY experiments. The reverse-detected, gradient-enhanced HSQC spectra were optimized for an average $^1J_{CH}$ of 140 Hz. The gradient-enhanced HMBC experiments were optimized for a $^3J_{CH}$ of 8 Hz. HRFABMS data, recorded in a glycerol matrix, were measured on a Prospec Fisons mass spectrometer (Danvers, NJ). Positive-mode ESIMS were recorded in formate buffer (pH 2.5) on an Applied Biosystems API 2000 mass spectrometer. Operational parameters were as follows: vaporizer, 350 °C; heated capillary, 150−200 °C; carrier gas, nitrogen, at a sheath pressure of 70 psi; auxiliary gas, nitrogen, to assist in nebulization, at a pressure of 30 psi; declustering potential, 44.0 eV; focusing potential, 340.0 eV; entrance potential, 10.0 eV; collision enegy, 33.0 eV for ion decomposition in the collision cell at 0.8 mTorr. HPLC separations were performed on a Hewlett-Packard 1100 series apparatus including a photodiode array detector set at 210 nm (Palo Alto, CA).

Plant Material and Chemicals. Red onion seeds (*Allium cepa* var. *tropeana*), available in the market, growing in the Capo Vaticano (Calabria-Italy), were purchased in September 2007 and identified by Prof. Daniela Rigano, Plant and Animal Biology, Faculty of Pharmacy, University "Federico II", Naples (Italy). A voucher was deposited at the Department of Chimica delle Sostanze Naturali, University "Federico II", Naples (Italy).

DPPH (1,1-diphenyl-2-picrilhydrazyl), ferric chloride (dry), 2,4,6-tris-2,4,6-tripyridyl-2-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), L-ascorbic acid, and *tert*-butyl-4-hydroxy toluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO). *S*-Methyl-L-cysteine and *S*-ethyl-L-cysteine were purchased from Sigma Chemical Co. (St. Louis, MO), while the other *S*-alk(en)yl-L-cysteines were provided by Wakunaga of America Co. Ltd. (Mission Viejo, CA). (±)-*S*-Alk(en)yl-L-cysteine sulfoxides were prepared by oxidation of the corresponding (±)-*S*-Alk(en)yl-L-cysteine derivatives with hydrogen peroxide following the procedure of Yu et al.[11]

Extraction and Isolation. The whole flour from the seeds (580 g) was extracted with boiling MeOH−H_2O (50:50) (2 L, four times). The hydromethanolic extract was treated with formate buffer (pH 2.5), added to a sulfuric acid resin column (30 × 2.0 cm), and eluted with 5% NH_4OH. The eluate was chromatographed on a Sephadex LH-20 column (100 × 5 cm), with MeOH as eluent. Two hundred fractions (9 mL) were collected and checked by TLC, using Macherey-Nagel silica gel plates (Bethlehem, PA) in *n*-BuOH−HOAc−H_2O (60:15:25) and pooled to give five major fractions, of which only 15−20 (441.0 mg) and 21−34 (68.6 mg) showed typical UV λ_{max} values for cysteine sulfoxide derivatives. Fraction 15−20 was subjected to RP-HPLC using a Thermoquest Hypersyl C_{18} column and the following eluting solution: CH_3CN−phosphate buffer (pH 7.0) gradient 0−20 min, 0−70% CH_3CN; 20−23 min, 70−100% CH_3CN; 23−26 min, 100−0% CH_3CN; 26−35 min, 0% CH_3CN. Fraction 21−34 was chromatographed by an Eclipse XDB C_8 column, with a CH_3CN−phosphate buffer (pH 7.0) gradient eluent: 0−5 min, 0% CH_3CN; 5−10 min, 0−30% CH_3CN; 10−15 min, 30−100% CH_3CN; 15−16 min, 100% CH_3CN; 16−22 min, 100−0% CH_3CN.

(S_SR_C)-*S*-(3-Pentenyl)-L-cysteine Sulfoxide (1): white, amorphous powder, $[\alpha]^{20}_D$ +23 (*c* 0.24, H_2O); IR (KBr) ν_{max} 3420, 2940, 1580, 1020 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 5.82 (1H, m, J = 13.7, 8.0 Hz, H-4), 5.82 (1H, m, J = 13.7, 8.0 Hz, H-3), 3.78 (2H, m, H-1), 3.76 (1H, dd, J = 6.0, 8.0 H-8), 3.06, 3.40 (2H, dd, J = 4.0, 8.0 Hz, H-7), 2.94, 3.06 (2H, m, J = 6.1 Hz, H-2), 1.55 (3H, t, J = 8.0 Hz, H-5); ^{13}C NMR (CD_3OD, 500 MHz) δ 173.2 (C, C-9), 132.0 (CH, C-3), 120.4 (CH, C-4), 55.0 (CH, C-8), 54.3 (CH_2, C-7), 53.8 (CH_2, C-1), 33.7 (CH_2, C-2), 18.0 (CH_3, C-5); positive ESIMS-MS *m/z* 206 $[M + H]^+$ (100), 189 (44), 160 (44), 128 (28), 147 (20), 134 (19), 88 (19), 145 (16), 132 (16), 102 (16), 174 (12), 119 (8), 74 (8), 173 (4); positive HRFABMS *m/z* 206.08512 $[M + H]^+$ (calcd for 206.08509).

Determination of Antioxidant Capacity. Antioxidant activity was determined for the cysteine sulfoxide derivative extract and for compound **1**, obtained from red onion seeds. After preliminary studies of the different concentrations, the extract was adjusted to 1 mg/mL (on a dried basis), and pure compound diluted to 1 mM, which was an appropriate concentration for assessing their antioxidant activity. For each antioxidant assay, a trolox aliquot was used to develop a 50−500 μmol/L standard curve. All data for pure compounds were then expressed as trolox equivalents (TEs, μM). Assay results were obtained using a JASCO V-530 UV/vis spectrophotometer (Easton, MD) set at wavelengths appropriate to each assay. All assays were performed in triplicate.

Determination of Reducing Power. The total antioxidant potential of samples was determined using the ferric reducing antioxidant power (FRAP) assay of Benzie and Strain.[12] A solution of 10 mM TPTZ in 40 mM HCl and 12 mM ferric chloride was diluted in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. Solutions of the onion seed extract and pure compound **1** (60 μL) were added to 3 mL of the FRAP solution, and the absorbance at 593 nm was determined every 10 min, for 90 min. The data for the standards L-ascorbic acid and *tert*-butyl-4-hydroxytoluene (BHT) in this assay were 824 ± 2.0 and 677 ± 1.5 μM, respectively.

Determination of Free-Radical-Scavenging Ability. The ability of samples to scavenge the DPPH (1,1-diphenyl-2-picrilhydrazyl) radical was measured using the method of Brand-Williams.[13] Onion seed extract and pure compound solutions (60 μL) were added to 3 mL of DPPH solution (6×10^{-5} mol/L), and the absorbance was determined at 515 nm every 10 min for 90 min. The data for the standards L-ascorbic acid and *tert*-butyl-4-hydroxy toluene (BHT) in this assay were 903 ± 2.6 and 637 ± 1.4 μM, respectively

Statistics. Triplicate analyses for each measurement were conducted for each sample. To establish the reproducibility of the analytical method, sample preparation was repeated three times. Differences between the means were evaluated with ANOVA, using the Graph Pad Instat 3 (Microsoft Software) statistics program. The significance of the model was evaluated by ANOVA. The significance of the regression coefficients was evaluated by Student's *t* test. The significance level was fixed at 0.05 for all the statistical analyses.

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References and Notes

(1) Carson, J. F. *Food Rev. Int.* **1987**, *3*, 71–103.
(2) Singh, S. V.; Pan, S. S.; Srivastava, S. K.; Xia, H.; Hu, X.; Zaren, H. A.; Orchard, J. L. *Biochem. Biophys. Res. Commun.* **1998**, *244*, 917–920.
(3) Kyo, E.; Uda, N.; Suzuki, A.; Kakimoto, M.; Ushujima, M.; Kasuga, S.; Itakura, Y. *Phytomedicine* **1998**, *5*, 259–267.
(4) Siegers, C. P.; Röbke, A.; Pentz, R. *Phytomedicine* **1999**, *6*, 13–16.
(5) Yin, M. C.; Cheng, W. S. *J. Agric. Food Chem.* **1998**, *46*, 4097–4101.
(6) Koch, H. P.; Lawson L. D. *Garlic: The Science and Therapeutic Applications of Allium sativum L. and Related Species*; Williams & Wilkins: Baltimore, MD, 1996; p 20.
(7) Rose, P.; Whiteman, M.; Moore, P. K.; Zhu, Y. Z. *Nat. Prod. Rep.* **2005**, *22*, 351–368.
(8) Kubec, R.; Kim, S.; Musah, R. A.; Rabi, A. *Phytochemistry* **2002**, *61*, 675–680.
(9) Kubec, R.; Velíšek, J. J.; Musah, R. A.; Rabi, A. *Phytochemistry* **2001**, *60*, 21–25.
(10) Kubec, R.; Kim, S.; McKeon, D. M.; Musah, R. A. *J. Nat. Prod.* **2002**, *65*, 960–964.
(11) Yu, T. H.; Wu, C. M.; Rosen, R. T.; Hartman, T. G.; Ho, C.-T. *J. Agric. Food Chem.* **1994**, *42*, 146–153.
(12) Benzie, I. F.; Strain, J. J. *Anal. Biochem.* **1996**, *239*, 70–76.
(13) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. *Food Sci. Technol.* **1995**, *28*, 25–30.

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