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S-Substituted cysteine derivatives and thiosulfinate formation in *Petiveria alliacea*—part II

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Abstract

Three cysteine derivatives, (*R*)-*S*-(2-hydroxyethyl)cysteine, together with (R_SR_C)- and (S_SR_C)-*S*-(2-hydroxyethyl)cysteine sulfoxides, have been isolated from the roots of *Petiveria alliacea*. Furthermore, three additional amino acids, *S*-methyl-, *S*-ethyl-, and *S*-propylcysteine derivatives, were detected. They were present only in trace amounts (<3 μg g^{-1} fr. wt), precluding determination of their absolute configurations and oxidation states. In addition, four thiosulfinates, *S*-(2-hydroxyethyl) (2-hydroxyethane)-, *S*-(2-hydroxyethyl) phenylmethane-, *S*-benzyl (2-hydroxyethane)- and *S*-benzyl phenylmethanethiosulfinates, have been found in a homogenate of the roots. The formation pathways of various benzyl/phenyl-containing compounds previously found in the plant were also discussed.

Keywords: *Petiveria alliacea*; Phytolaccaceae; *S*-(2-Hydroxyethyl)cysteine; *S*-(2-Hydroxyethyl)cysteine sulfoxide; 6-Hydroxyethiin; Petiveriin; Petivericin; Thiosulfinate

1. Introduction

Petiveria alliacea L. (Phytolaccaceae) is an herbaceous perennial indigenous to the Amazon Rainforest and widely distributed in other areas including tropical and Central America, Africa, Sri Lanka, and the southeastern United States. Preparations from this plant have been used extensively in the traditional medicine of South and Central America for the treatment of many disorders. They reportedly exhibit antiinflammatory, antimicrobial, anticancer and stimulant effects, among others.

In a previous contribution (Kubec and Musah, 2001), identification of two diastereoisomers of *S*-benzylcysteine sulfoxide in *P. alliacea*, petiveriins A and B was reported. We also proposed that these amino acids serve as precursors of the benzyl/phenyl-containing compounds previously detected in this plant (e.g. dibenzyl sulfides, benzaldehyde, stilbenes, benzyl alcohol, benzoic acid, benzyl benzoate) (Adegosan, 1974; Sousa et al., 1990; Ayedoun et al., 1998; Benevides et al., 2001). The recently reported studies of Benevides et al. (2001), who isolated di-*n*-propyl disulfide, benzyl hydroxymethyl sulfide and several other antifungal polysulfides from the roots of *P. alliacea*, as well as the work of Szczepanski et al. (1972) and Mata-Greenwood et al. (2001) who isolated benzyl 2-hydroxyethyl disulfide and benzyl 2-hydroxyethyl trisulfide, respectively, encouraged us to search for additional amino acid precursors that might be present in this plant.

The present paper describes our investigations on identification of additional *S*-substituted cysteine derivatives from the root of *P. alliacea*. Identification and isolation of their enzymatic breakdown products, thiosulfinates, are also reported.

2. Results and discussion

An amino acid fraction from the roots was isolated by cation-exchange chromatography. As revealed by GC analysis of the extract, *P. alliacea* appears to be quite a rich source of several *S*-substituted cysteine derivatives. Besides *S*-benzylcysteine sulfoxides (petiveriins A and B) which were by far the most abundant components,

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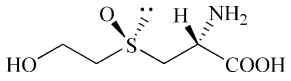
four additional cysteine derivatives were detected in the extract. These were *S*-methyl-, *S*-ethyl-, *S*-*n*-propyl- and *S*-(2-hydroxyethyl)cysteine derivatives. Whereas the first three amino acids were present only in minute amounts (<3 μg g^{-1} fr. wt), the content of the fourth one was considerably higher (ca. 0.2 mg g^{-1} fr. wt). Therefore, we focused our attention primarily on isolation and structural elucidation of the *S*-(2-hydroxyethyl)cysteine derivative.

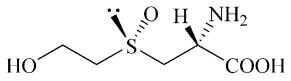
Another extract was prepared and analyzed using C_{18} reversed phase HPLC. As revealed by comparison with authentic samples, both *S*-(2-hydroxyethyl)cysteine sulfoxide (**1**) and *S*-(2-hydroxyethyl)cysteine (**2**) seemed to be present in the extract. The corresponding fractions were subsequently isolated by prep. C_{18} HPLC and purified by means of prep. C_8 HPLC, affording **1** and **2** as white solids.

The MALDI-HRMS data of **1** confirmed the anticipated molecular formula for *S*-(2-hydroxyethyl)cysteine sulfoxide ([MH^+] of 182.0483, $C_5H_{11}NO_4S$ req. 182.0482). Although the ^1H NMR signals are complex and overlapping, the spectrum does fit the structure of **1a**/**1b**. The peaks representing the methine protons on C-2, and the methylene protons on C-3 show ^1H–^1H COSY crosspeaks, consistent with the structure, as do the peaks representing the methylene protons on carbons 5 and 6 (see Experimental). Additionally, the ^{13}C NMR spectrum contained nine signals, indicating that two diastereomeric forms of the sulfoxide were present. The presence of two diastereomers of **1** was subsequently confirmed by HPLC analysis after derivatization with *ortho*-phthaldehyde (OPA), showing two nicely separated peaks that had the retention characteristics identical to the two components of **1** obtained by synthesis. The amount of **1** isolated from the roots was too small to allow preparative separation into the corresponding diastereomers to determine their absolute configurations. However, since the absolute configuration of **2** is (*R*) (see later), it is reasonable to assume that the same configuration about the α-carbon occurs also in both diastereomers of **1**. Based on the data obtained, we can therefore conclude that both (R_SR_C)- and (S_SR_C)-*S*-(2-hydroxyethyl)cysteine sulfoxides (**1a** and **1b**, respectively, Fig. 1) are present in the roots of *P. alliacea*. Since these compounds can be viewed as 6-hydroxy analogues of *S*-ethylcysteine sulfoxide (ethiin), we suggest the trivial names 6-hydroxyethiin A and 6-hydroxyethiin B for **1a** and **1b**, respectively. According to the HPLC/OPA analysis and the ^{13}C NMR signal intensities observed, the relative ratio of **1a**/**1b** in the root was approximately 1/1. It is however possible that different relative proportions of **1a** and **1b** may be observed in various parts of the plant as is the case for petiveriins A and B (Kubec and Musah, 2001).

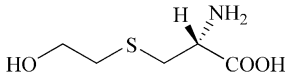
The IR, ^1H and ^{13}C NMR spectroscopic data of **2** were virtually identical with those of an authentic sample of *S*-(2-hydroxyethyl)cysteine. Furthermore, the



1a, (R_SR_C)-*S*-(2-hydroxyethyl)cysteine sulfoxide (6-hydroxyethiin A)



1b, (S_SR_C)-*S*-(2-hydroxyethyl)cysteine sulfoxide (6-hydroxyethiin B)



2, (*R*)-*S*-(2-hydroxyethyl)cysteine

Fig. 1. Structure of *S*-(2-hydroxyethyl)cysteine derivatives.

MALDI-HRMS of **2** gave [MH^+] of 166.0537 ($C_5H_{11}NO_3S$ req. 166.0532), unambiguously confirming the identity of the amino acid as *S*-(2-hydroxyethyl)cysteine. The absolute configuration of **2** at the α-carbon was determined by means of CD spectrometry and polarimetry, using authentic samples of (*R*)- and (*S*)-*S*-(2-hydroxyethyl)cysteines for comparison. Both the CD spectrum ($\Delta\varepsilon$ +1.03, 219 nm) and optical rotation value ($[\alpha]_D^{20}$ +29°) of **2** were in perfect agreement with those of the (*R*) isomer. The structure of **2** was therefore determined to be (*R*)-*S*-(2-hydroxyethyl)cysteine (Fig. 1).

To the best of our knowledge, the occurrence of **1a**, **1b** or **2** in nature has not been reported thus far. However, **2** is a well known metabolite occurring in the urine and blood of individuals who have been exposed to ethylene oxide (oxirane), ethylene bromide, vinyl chloride, and 2-bromo- or 2-chloroethanol (Nachtomi et al., 1966; Jones and Wells, 1981; Hefner et al., 1975).

Due to their very low content, no effort was made to isolate the minor *S*-substituted cysteine derivatives detected by GC, i.e. *S*-methyl-, *S*-ethyl- and *S*-*n*-propylcysteine derivatives. Since the GC method used for the screening does not permit distinction between *S*-substituted cysteines and their sulfoxides, it is not known whether the derivatives detected in this study were thioether or sulfoxide forms. Indeed, both may have been present. Nevertheless, the presence of the sulfoxides seems to be more likely. Since the total content and relative proportions of *S*-substituted cysteine sulfoxides are known to depend on many factors (e.g. growth conditions and sulfur and nitrogen supply), higher amounts of *S*-methyl-, *S*-ethyl-, *S*-propylcysteine derivatives might be present in plants grown at different locations.

All attempts to synthesize *S*-(hydroxymethyl)cysteine, a possible precursor of the hydroxymethyl-containing

sulfide found by Benevides et al. (2001), were unsuccessful. In accordance with the report of Ratner and Clarke (1937), the reaction of formaldehyde with cysteine gave thiazolidine-4-carboxylic acid under all conditions studied (pH 2–10, temp. 2–25 °C). Also reactions of formaldehyde with *N*-acetylcysteine or *N*-(*tert*-butoxycarbonyl)cysteine led to the formation of *N*-substituted analogues of thiazolidine-4-carboxylic acid. Interestingly, even the reaction of bromomethyl methyl ether (CH_3OCH_2Br) with cysteine yielded thiazolidine-4-carboxylic acid as the only ninhydrin-positive product. Apparently, *S*-(hydroxymethyl)cysteine is an extremely unstable compound with a high tendency towards self-cyclization. Its oxidized form, *S*-(hydroxymethyl)cysteine sulfoxide, is likely to be more stable. However, if present in the extract, at least a part of the sulfoxide would have also been converted into the cyclized form during the reduction step of the GC derivatization procedure. No sign of thiazolidine-4-carboxylic acid was found, implying that there was neither *S*-(hydroxymethyl)cysteine nor its sulfoxide present at levels >1 μg g^{-1} fr. wt in the sample we analyzed. In addition, none of the following compounds were detected by GC at the level of 1 μg g^{-1} fr. wt: *S*-isopropyl-, *S*-allyl-, *S*-(1-propenyl)-, *S*-*n*-butyl-, *S*-isobutyl, *S*-*sec*-butyl-, *S*-*n*-pentylcysteine derivatives. The next higher homologue of **2**, *S*-(3-hydroxypropyl)cysteine, was also not found. However, the presence of some additional analogues cannot be ruled out.

There are two major *S*-substituted cysteine sulfoxides present in the intact tissue of *P. alliacea*, namely *S*-benzyl- and *S*-(2-hydroxyethyl)cysteine sulfoxides. In theory, these amino acids should give rise to four thiosulfinates (two symmetrical and two unsymmetrical ones) upon the action of a C–S lyase (Fig. 2). All four of these thiosulfinates were synthesized to facilitate their HPLC detection and subsequent isolation. An HPLC method employing a C_8 column was developed. This methodology allowed an excellent separation, even of the regioisomers **4** and **5**. Indeed, all four of these thiosulfinates were detected in a diethyl ether extract of a root homogenate. The thiosulfinates **4–6** were subsequently isolated by preparative C_8 HPLC and fully characterized by spectroscopic methods (NMR, IR, MALDI/MS, and UV). The content of **3** in the extract was too low to allow its isolation. It was, however, detected and unambiguously identified by comparing its retention time and UV spectrum with that of an authentic sample prepared by synthesis. As expected, *S*-benzyl phenylmethanethiosulfinate (**6**) was the most abundant thiosulfinate found in the extract, followed by **4** and **5**. Analogous to the alliin/allicin and marasmin/marasmicin systems in garlic (*Allium sativum* L.) and society garlic (*Tulbaghia violacea* Harv.), respectively, we suggest using the trivial name petivericin for **6**. To the best of our knowledge, none of the thiosulfinates **3–6** has been observed in nature thus far. Furthermore, compounds **4–6** represent the first naturally occurring benzyl-containing thiosulfinates to be reported.

As revealed by HPLC/OPA analysis of a root homogenate after 30 min of standing at room temperature, both petiveriin A and petiveriin B are enzymatically cleaved. Since we did not have a fresh sample containing a fully native enzymatic system, no precise kinetic study was carried out to determine whether both petiveriins were depleted at the same rate. Because of partial overlap of the peaks of OPA-derivatives of **1a** and **1b** in HPLC chromatograms by other amino acid components present in the homogenate, we were also not able to determine whether both diastereomers of **1** were enzymatically cleaved. A more detailed study on the enzymatic system of *P. alliacea* is required to establish whether there is only one C–S lyase present, capable of cleaving both **1a**/**1b** and petiveriins A/B, or whether there are several specific lyases for each cysteine derivative.

Generally, thiosulfinates are quite thermolabile and usually decompose when analyzed by common GC procedures (employing high injection/oven temperatures and long, narrow capillary columns) (Block, 1992). As we found out, thiosulfinates **3–6** do not survive GC analysis. Even when very mild injection/oven temperatures were used (injection temp. as low as 100 °C), these labile compounds decomposed during the run. They gave rise to the parent disulfides and, in the case of **4** and **6**, also to a compound that was tentatively identified as thiobenzaldehyde [EIMS, 70 eV, *m*/*z* 124 (2, $M^+ + 2$), 122 (52, M^+), 121 (100), 93 (3), 84 (6), 78 (15), 77 (26)]. Based on the above observations, we conclude that GC analyses cannot provide a true representation of the compounds present in a fresh homogenate of *P. alliacea*.

As we proposed in the previous study (Kubec and Musah, 2001), the benzyl/phenyl-containing compounds identified in this plant (e.g. dibenzyl sulfides, benzaldehyde, trithiolaniacin, stilbenes, benzyl mercaptan, benzoic acid) are not present in the intact tissue of *P. alliacea*. In fact, these compounds are only degradation products of the thiosulfinates **4–6** that arise by the action of a C–S lyase on *S*-substituted cysteine sulfoxides upon cellular disruption. Indeed, we did not find significant amounts of dibenzyl sulfides, benzaldehyde or stilbenes in a Et_2O extract of a fresh root homogenate by means of C8 HPLC. The predominant thiosulfinate of *P. alliacea*, *S*-benzyl phenylmethanethiosulfinate (**6**, petivericin), seems to be the precursor of the majority of the benzyl/phenyl-containing compounds. It was shown that **6** can decompose into dibenzyl disulfide and trisulfide, benzaldehyde, stilbenes and benzyl mercaptan, among others (Furukawa et al., 1973). Analogous to other thiosulfinates, petivericin can also disproportionate into the corresponding disulfide and thiosulfo-

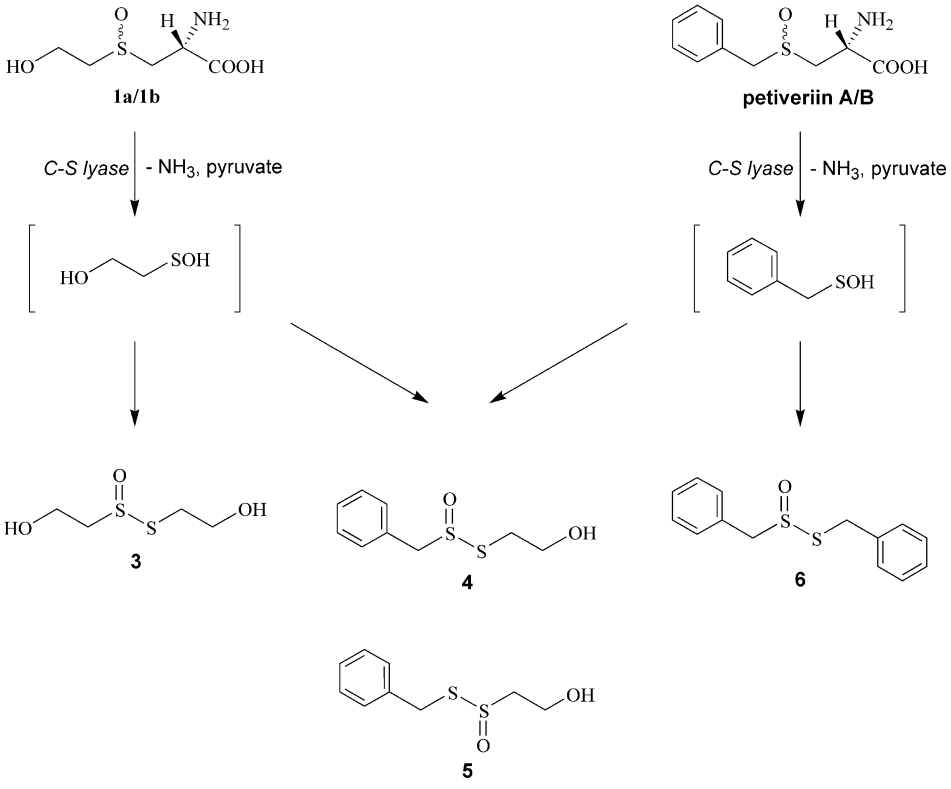


Fig. 2. Enzymatic formation of thiosulfinates in *P. alliacea* [**3**—*S*-(2-hydroxyethyl) 2-(hydroxyethane)thiosulfinate; **4**—*S*-(2-hydroxyethyl) phenylmethanethiosulfinate; **5**—*S*-benzyl 2-(hydroxyethane)thiosulfinate; **6**—*S*-benzyl phenylmethanethiosulfinate (petivericin)].

nate. The latter compound can undergo further decomposition, yielding mainly sulfur dioxide, stilbenes, benzyl mercaptan, and dibenzyl sulfide and disulfide (Smythe, 1922; Kice et al., 1960; Chatgilialoglu et al., 1980; White and Dellinger, 1985). It remains to be seen whether other likely decomposition products, such as *S*-benzyl phenylmethanethiosulfonate, phenylmethanesulfinic and phenylmethanesulfonic acids are present in *P. alliacea* extracts. Benzoic acid and benzyl alcohol might be formed by the Canizzaro reaction from abundantly occurring benzaldehyde and subsequently give rise to benzyl benzoate. The thiosulfinates **4** and **5** are possible precursors of benzyl 2-hydroxyethyl disulfide and trisulfide previously identified by Mata-Greenwood et al. (2001) and Szczepanski et al. (1972), respectively. However, the precursor and formation pathway of benzyl hydroxymethyl sulfide recently detected by Benevides et al. (2001) still remain to be determined.

Most of the above mentioned compounds (thiosulfinates **3**–**6**, the corresponding thiosulfonates and mono-, di- and trisulfides) are currently being tested in our laboratory for their antibacterial and antifungal activities, as well as for their antiinflammatory and anticancer properties. We believe that these compounds are responsible for many of the reported physiological effects of *P. alliacea*.

3. Experimental

3.1. General experimental procedures and plant material

All apparatus used in the present study were identical with those described previously (Kubec and Musah, 2001). The roots of *P. alliacea* originated from the same batch that was analyzed in the previous study. They had been stored in a freezer at −30 °C for several months before the experiments.

3.2. Extraction and purification

An amino acid fraction was obtained from the roots (315 g) as described previously (Kubec and Musah, 2001). It was subjected to prep. C_{18} HPLC and the fractions eluting at 3.5 and 4.2 min were collected, evaporated and purified by reinjection onto a prep. C_8 HPLC column to yield **1a**/**1b** (6 mg) and **2** (11 mg), respectively. The HPLC conditions employed were as follows: 10 mM KH_2PO_4 buffer (A, pH 7.0) and MeCN (B) as the mobile phase (14 ml min^{-1}), A/B 99/1 from $t = 0$–7 min, then to 20/80 from $t = 7$–15 min, with this being held for 25 min. HPLC/OPA analyses were performed according to the method of Ziegler and Sticher (1989).

Thiosulfinates **3**–**6** were isolated from a root homogenate that was prepared as follows. The roots (190 g)

were mixed with H_2O (300 ml) and homogenized using a blender. The resulting slurry was allowed to stand at room temp. for 30 min, filtered and the filtrate extracted with Et_2O (2×300 ml). Additional Et_2O (300 ml) was used for washing the filter cake. The Et_2O fractions were combined, evaporated to dryness and the yellow, strongly smelling residue was redissolved in MeCN (20 ml). The extract was subjected to prep. C_8 HPLC using the following conditions: H_2O (A), MeCN (B) as the mobile phase (18 ml min^{-1}), from A/B 98/2 at $t=0$ its 0/100 in 25 min, this being held for 15 min. The fractions eluting at 13.9, 14.4 and 22.0 min were collected, **4** (12 mg), **5** (4 mg) and **6** (98 mg), respectively.

3.3. GC analyses

An amino acid extract was prepared from the roots as described above and it was analyzed by GC after derivatization with ethyl chloroformate (ECF) and reduction with sodium iodide (Kubec et al., 1999). *S*-(4-Chlorobenzyl)cysteine, added to the samples before extraction, was used as an internal standard.

Thiosulfinates were analyzed using a Rtx-1 fused silica capillary column (30 m×0.25 mm; 25 μm; Restek Corp.), employing the following temperature program: 100 °C (held for 2 min), raised linearly to 260 °C at 6 °C min^{-1}. Helium (0.7 ml min^{-1}) was used as the carrier gas. Different injection temperatures were studied (100–260 °C).

3.4. Reference compounds

S-(2-Hydroxyethyl)cysteine and *S*-(3-hydroxypropyl) cysteine were synthesized by the reaction of L-cysteine with 2-bromoethanol or 1-chloro-3-propanol, respectively (Shiraiwa et al., 1998). All of the other *S*-substituted cysteine derivatives were obtained analogously. A mixture of *S*-(2-hydroxyethyl)-L-cysteine sulfoxide diastereomers (**1a**, **1b**) was synthesized by oxidation with H_2O_2 as described by Stoll and Seebeck (1949). Thiazolidine-4-carboxylic acid was prepared by condensation of formaldehyde with L-cysteine (Ratner and Clarke, 1937). 2-Hydroxyethyl benzyl disulfide and bis(2-hydroxyethyl) disulfide were prepared according to the method of Ayodele et al. (2000). Dibenzyl disulfide was purchased from Aldrich. *S*-(2-Hydroxyethyl) phenylmethane- (**4**), *S*-benzyl (2-hydroxyethane)- (**5**) and *S*-benzyl phenylmethanethiosulfinates (**6**) were synthesized by oxidation of the parent disulfides with an equimolar amount of 3-chloroperoxybenzoic acid (in $CHCl_3$, at −20 °C for 30 min). *S*-(2-Hydroxyethyl) (2-hydroxyethane)thiosulfinate (**3**) was obtained by oxidation of bis(2-hydroxyethyl) disulfide with 30% H_2O_2. All of the thiosulfinates were purified using prep. C_8 HPLC.

3.5. (R_SR_C)/(S_SR_C)-R-(2-Hydroxyethyl)cysteine sulfoxide (**1a**/**1b**)

White solid; mp 137–138 °C; UV λ_{max} (H_2O) nm (log ε): 192 (3.56), 218 (*sh*) (3.01); IR (KBr) ν_{max} cm^{-1}: 3670–2900 (*s*, *br*), 1633 (*vs*), 1503 (*m*), 1384 (*m*), 1352 (*m*), 1062 (*m*), 1021 (*m*), 990 (*m*); ^1H NMR (400 MHz; D_2O): δ 3.08–3.45 (4H, m, H-3, H-5), 3.87–3.98 (2H, *m*, H-6), 4.124.18 (1H, *m*, H-2); ^{13}C NMR (75 MHz; D_2O): δ 50.3 (C-2), 51.0 (C-3), 51.1 (C′-2), 51.5 (C′-3), 54.4 (C-5), 54.6 (C-6; C′-6), 55.1 (C′-5), 171.4 (C-1), 171.5 (C′-1); MALDI-HRMS [MH^+] 182.0483 ($C_5H_{11}NO_4S$ req. 182.0482); TLC R_f 0.20 (*n*-BuOHH_2OHOAc, 4:1:1), R_f 0.47 (*n*-PrOHH_2O, 7:3).

3.6. (R)-S-(2-Hydroxyethyl)cysteine (**2**)

White solid; mp 183–184 °C; $[\alpha]_D^{22}$: +29° (H_2O; *c* 0.1); CD $\Delta\varepsilon_{max}$ (H_2O; *c* 0.1; 22 °C): +1.0 (219 nm); UV λ_{max} (H_2O) nm (log ε): 190 (3.37), 204 (*sh*) (3.22); IR (KBr) ν_{max} cm^{-1}: 3331 (*m*, *br*), 3060–2900 (*s*, *br*), 1602 (*vs*), 1431 (*s*), 1410 (*s*), 1353 (*s*), 1317 (*s*), 1062 (*m*), 1000 (*m*), 850 (*m*); ^1H NMR (300 MHz; D_2O): δ 2.79 (2H, *t*, $J=6.2$ Hz, H-5), 3.07 (1H, *dd*, $J=7.4$, 14.7 Hz, H-3a), 3.17 (1H, *dd*, $J=4.3$, 14.7 Hz, H-3b), 3.78 (2H, *t*, $J=6.1$ Hz, H-6), 3.95 (1H, *dd*, $J=4.3$, 7.4 Hz, H-2); ^{13}C NMR (75 MHz; D_2O): δ 32.3 (C-3), 33.9 (C-5), 53.9 (C-2), 60.2 (C-6), 173.0 (C-1); MALDI-HRMS [$MH]^+$ 166.0537 ($C_5H_{11}NO_3S$ req. 166.0532); TLC R_f 0.38 (*n*-BuOH-H_2OHOAc, 4:1:1), R_f 0.61 (*n*-PrOHH_2O, 7:3).

3.7. (S)-S-(2-Hydroxyethyl)cysteine (**2b**)

White solid; $[\alpha]_D^{22}$: −27° (H_2O; *c* 0.1); CD $\Delta\varepsilon_{max}$ (H_2O; *c* 0.1; 22 °C): −0.94 (219 nm); all other analytical data identical with those of **2**.

3.8. S-(2-Hydroxyethyl) 2-(hydroxyethane)thiosulfinate (**3**)

Yellowish oil; UV λ_{max} (EtOH) nm (log ε): 204 (3.70), 252 (3.37); IR (neat) ν_{max} cm^{-1}: 3380 (*s*, *br*), 2933 (*m*), 2879 (*m*), 1399 (*m*), 1285 (*m*), 1052 (*vs*), 1002 (*s*); ^1H NMR (300 MHz; CD_3OD): δ 3.25–3.38 (4H, *m*, H-2, H-5), 3.84 (2H, *m*, H-1), 3.98 (2H, *t*, $J=5.7$ Hz, H-6); ^{13}C NMR (75 MHz; CD_3OD): δ 36.8 (C-5), 56.5 (C-2), 60.0 (C-1), 62.7 (C-6); MALDI-HRMS [$MH]^+$ 171.0142 ($C_4H_{10}O_3S_2$ req. 171.0144).

3.9. S-(2-Hydroxyethyl) phenylmethanethiosulfinate (**4**)

White solid; mp 48–50 °C; UV λ_{max} (EtOH) nm (log ε): 206 (4.26), 224 (4.09), 266 (3.23); IR (KBr) ν_{max} cm^{-1}: 3413 (*m*, *br*), 1452 (*m*), 1406 (*m*), 1400 (*m*), 1072 (*vs*), 1053 (*vs*), 1011 (*m*), 762 (*m*), 696 (*s*); ^1H NMR (300

MHz; CD_3OD): δ 3.23 (2H, *m*, SCH_2), 3.76 (2H, *m*, CH_2OH), 4.39 (1H, *d*, $J=12.9$ Hz, CH_2S(O)-a), 4.49 (1H, *d*, $J=12.9$ Hz, CH_2S(O)-b), 7.327.45 (5H, *m*, H_{arom}); ^{13}C NMR (75 MHz; CD_3OD): δ 36.1 (SCH_2), 62.7 (CH_2OH), 63.1 (CH_2S(O)), 129.7, 129.8, 131.6, 131.7 (C_{arom}); MALDI–HRMS $[MH]^+$ 217.0352 ($C_9H_{12}O_2S_2$ req. 217.0351).

3.10. S-Benzyl (2-hydroxyethane)thiosulfinate (**5**)

White solid; mp 50–51 °C; UV λ_{max} (EtOH) nm (log ε): 206 (4.25), 224 (3.99), 260 (3.37); IR (KBr) ν_{max} cm^{-1}: 3387 (*m*, *br*), 1493 (*w*), 1453 (*m*), 1069 (*vs*), 1055 (*vs*), 995 (*m*), 701 (*s*); ^{1}H NMR (300 MHz; CD_3OD): δ 3.22–3.37 (2H, *m*, S(O)CH_2), 3.93–3.97 (2H, *m*, CH_2OH), 4.39 (2H, *s*, CH_2S), 7.24–7.43 (5H, *m*, H_{arom}); ^{13}C NMR (75 MHz; CD_3OD): δ 37.7 (CH_2S), 56.5 (S(O)CH_2), 59.6 (CH_2OH), 128.8, 129.8, 130.2, 138.3 (C_{arom}); MALDI-HRMS $[MH]^+$ 217.0352 ($C_9H_{12}O_2S_2$ req. 217.0351).

3.11. S-Benzyl phenylmethanethiosulfinate (**6**)

White solid; mp 77–78 °C; UV λ_{max} (EtOH) nm (log ε): 208 (4.43), 226 (4.30), 266 (3.36); IR (KBr) ν_{max} cm^{-1}: 3445 (*w*, *br*), 2968–2900 (*w*), 1493 (*w*), 1451 (*m*), 1077 (*s*), 1055 (*s*), 761 (*m*), 695 (*s*); ^{1}H NMR (300 MHz; acetone): δ 4.29 (2H, *s*, SCH_2), 4.35 (1H, *d*, $J=12.9$ Hz, CH_2S(O)-a), 4.43 (1H, *d*, $J=12.9$ Hz, CH_2S(O)-b), 7.24–7.42 (5H, *m*, H_{arom}); ^{13}C NMR (75 MHz; acetone): δ 35.7 (SCH_2), 62.6 (CH_2S(O)), 128.2, 129.0, 129.2, 129.4, 129.9, 131.2, 131.8 (C_{arom}); MALDI-HRMS $[MH]^+$ 263.0564 ($C_{14}H_{14}OS_2$ req. 263.0559).

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