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Cysteine sulfoxide derivatives in *Petiveria alliacea*

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Abstract

Two diastereomers of *S*-benzyl-L-cysteine sulfoxide have been isolated from fresh roots of *Petiveria alliacea*. Their structures and absolute configurations have been determined by NMR, MALDI-HRMS, IR and CD spectroscopy and confirmed by comparison with authentic compounds. Both the R_S and S_S diastereomers of the sulfoxide are present in all parts of the plant (root, stem, and leaves) with the latter diastereomer being predominant. Their total content greatly varied in different parts of the plant between 0.07 and 2.97 mg g^{-1} fr. wt, being by far the highest in the root. *S*-Benzylcysteine has also been detected in trace amounts (<10 μg g^{-1} fr. wt) in all parts of the plant. This represents the first report of the presence of *S*-benzylcysteine derivatives in nature.

Keywords: *Petiveria alliacea*; Phytolaccaceae, *S*-Benzylcysteine sulfoxide; Petiveriin; *S*-Benzylcysteine; Flavor precursor

1. Introduction

Petiveria alliacea L. (family Phytolaccaceae) is a perennial shrub indigenous to the Amazon Rainforest and widely distributed in other areas including tropical America, the Caribbean, Africa, Sri Lanka, and the southeastern Unites States. It is known by many names among which "anamu", "apacin", "guiné", "pipi", "tipi", and "garlic guinea henweed" are noteworthy. It has commonly been used in folk medicine and various preparations made from this plant are considered to have antiinflammatory, antimicrobial, antispasmodic, diuretic, and stimulant effects, among others.

The presence in *P. alliacea* of several volatile compounds, most of them containing a benzyl or phenyl moiety has been reported. Whereas Szczepanski et al. (1972) identified benzyl-2-hydroxyethyl trisulfide, Adegosan (1974) found *cis*-3,5-diphenyl-1,2,4-trithiolan (trithiolaniacin), benzaldehyde, benzoic acid, elemental sulfur, and *trans*-stilbene. Sousa et al. (1990) isolated dibenzyl trisulfide and Ayedoun et al. (1998) observed benzaldehyde, benzyl alcohol, *cis*- and *trans*-stilbenes, benzyl benzoate, dibenzyl disulfide, and dibenzyl trisulfide in the root oil.

The present paper describes our investigations on isolation and identification of non-volatile precursors of the above mentioned compounds.

2. Results and discussion

Two abundant constituents of an amino acid fraction from the roots of *Petiveria alliacea* were isolated by ion-exchange chromatography and prep. HPLC as white crystalline solids. Their NMR, IR, and UV spectra as well as their chromatographic behavior were very similar. The ^1H and ^{13}C NMR spectra revealed that both compounds are α-amino acids containing an unsubstituted benzyl moiety and two pairs of heterosteric methylene protons. The IR spectra contained a very strong absorption band near 1020 cm^{-1}, indicating the presence of a sulfoxide group. The MALDI-HRMS data showed m/z $[M]^+$ of 227.0616 and 227.0626, respectively, corresponding to a molecular formula of $C_{10}H_{13}NO_3S$ for both compounds (req. 227.0616). In accordance with the above data, the two amino acids were identified as diastereomers of *S*-benzylcysteine sulfoxide.

There are two chiral centers in *S*-benzylcysteine sulfoxide (the sulfur and α-carbon atoms) and thus four stereoisomers exist. All of them were synthesized and their absolute configurations were determined by NMR and CD spectrometry. The CD spectra of corresponding

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diastereomers were almost mirror images, consisting of a single maximum around 224 nm (Fig. 1). Unfortunately, when strongly perturbing groups are present around the sulfur atom (e.g. alkylthio, allyl, benzyl), CD spectrometry cannot be used to directly determine the absolute configuration of sulfoxides (Axelrod et al., 1968; Ottenheijm et al., 1981). However, the diastereomers of all *S*-substituted cysteine sulfoxides display ^{1}H NMR spectra with a characteristic ABX pattern for the S(O)CH_2CH(NH_2) methylene protons. The isomers with both the amino group and the oxygen atom on the same side of the molecule show two distinct doublets of doublets at $\sim\delta$ 3.25 ($J_{AX} = \sim 7$–8.5 Hz) and δ 3.50 ($J_{BX} = \sim 5.5$–7 Hz), respectively. On the other hand, the methylene protons of derivatives with the opposite configuration typically resonate in the much narrower region of $\sim\delta$ 3.30–3.45 with the coupling constants of $J_{AX} = \sim 3.5$–4 Hz and $J_{BX} = \sim 8.5$–10.5 Hz. The coupling constants J_{AB} are similar for both diastereomers ($\sim$13.5–14 Hz) (Kuttan et al., 1974; Broek et al., 1987; Kubota et al., 1998). Employing this rule, the absolute configuration of all synthesized derivatives was unambiguously determined. Furthermore, the specific rotation values of the corresponding antipodes were nearly opposite, confirming the correct structural assignment. Along with NMR, CD spectrometry and polarimetry were also employed to determine the absolute configuration of the compounds isolated from the root of *P. alliacea*. The CD spectrum of the first eluting isomer showed a positive sign of the Cotton effect ($\Delta\varepsilon_{max}$ +12.7 at 222 nm) and a specific rotation of $[\alpha]_D^{22}$ +31°. The second isomer gave a CD spectrum with a negative sign of the Cotton effect ($\Delta\varepsilon_{max}$−14.6 at 224 nm) and a specific rotation of $[\alpha]_D^{22}$ −55°. On the basis of the above data and together with their other spectroscopic and chromatographic characteristics, the absolute configuration of the isolated amino acids were determined as (R_CR_S)-*S*-benzyl-L-cysteine sulfoxide (**1a**) and (R_CS_S)-*S*-benzyl-L-cysteine sulfoxide (**1b**), respectively. In close analogy to alliin (*S*-allylcysteine sulfoxide) and other related *S*-alk(en)ylcysteine sulfoxides, the new amino acids have trivially been named petiveriin A (the dextrorotary R_CR_S isomer, **1a**) and petiveriin B (the levorotary R_CS_S isomer, **1b**).

Aliphatic *S*-substituted cysteine sulfoxides have been isolated from various plants, fungi, and algae. They occur almost exclusively in the R_CS_S configuration (e.g. in *Allium* and *Brassica* species). So far, there are only two known *S*-substituted cysteine derivatives in which both diastereomeric forms have been found to occur in nature. (R_CR_S)-*S*-(*trans*-1-Propenyl)cysteine sulfoxide is widely distributed in onion and other alliaceous species, whereas its (R_CS_S) diastereomer was identified in *Santalum album* L. (in the form of a γ-glutamyl dipeptide; Kuttan et al., 1974). Similarly, (R_CS_S)-*S*-(methylthiomethyl) cysteine sulfoxide was found in *Marasmius* species (Gmelin et al., 1976; Broek et al., 1987) and the corresponding (R_CR_S) diastereomer has recently been isolated from *Scorodocarpus borneensis* Becc. (Kubota et al., 1998). However, the presence in a plant of both diastereomers has not been reported thus far. Using pure synthetic diastereomers, we have observed that the cysteine sulfoxides are not interconvertible under the conditions

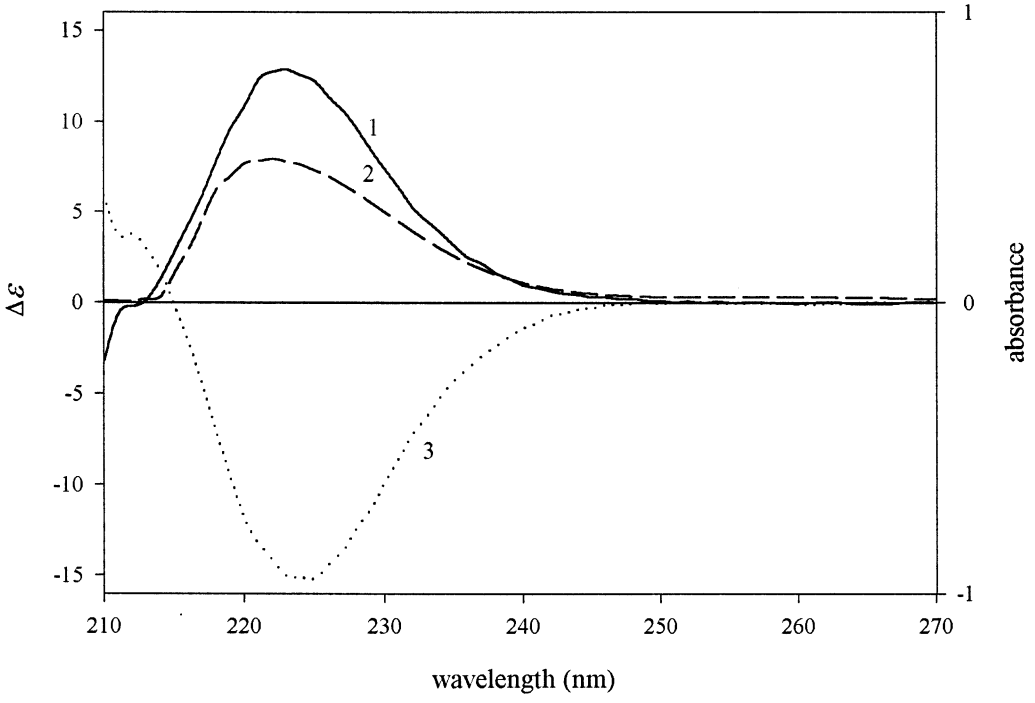


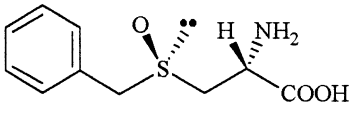
Fig. 1. The CD and UV spectra of petiveriin A (1 and 2, respectively) and the CD spectrum of petiveriin B (3).

used during extraction and isolation of the compounds (e.g. refluxing in MeOH for 20 min). Thus, they are not artifacts arising during isolation and they are naturally present in the tissue. To our knowledge, **1a** and **1b** are the first naturally occurring *S*-benzyl cysteine derivatives to be observed and the first naturally occurring aromatic *S*-substituted cysteines to be reported. In view of the presence of these aromatic cysteine derivatives, it appears likely that some aliphatic cysteine sulfoxide derivatives analogous to those observed in garlic, onion and other alliaceous plants may also be present in the *P. alliacea*.

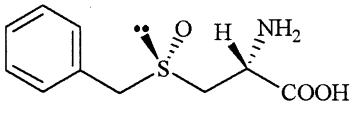
S-Benzyl-L-cysteine, a presumable biochemical precursor of petiveriin, has also been detected in all parts of the plant. However, due to its very low content (<10 μg g^{-1} fr. wt) it could not be isolated in sufficient quantity to confirm its structure by spectroscopic methods. Identification was made by comparing its chromatographic behavior (TLC and HPLC) with that observed for *S*-benzyl-L-cysteine obtained from laboratory synthesis (Fig. 2).

The total content of both *S*-benzylcysteine sulfoxide diastereomers has been determined in different parts of the plant (root, stem, and leaves) by HPLC. As shown in Table 1, the content ranges between 0.07 and 2.97 mg g^{-1} fr. wt, being the highest in the root. Interestingly, it was observed that not only the total content but also the relative amounts of petiveriin A and B vary significantly from 40:60 in the root to 22:78 in the leaves and 12:88 in the stem. However, the content of *S*-substituted cysteine derivatives depends on many factors including soil composition, climate, harvest date etc. (Fenwick and Hanley, 1985; Block, 1992). Thus, it can be assumed that the content of petiveriin is dependent on such factors and may vary considerably in plants growing in different areas.

Further research on *P. alliacea* is still being conducted in our laboratory. Our preliminary data indicate that both petiveriin A and B serve as non-volatile precursors of the phenyl/benzyl-containing volatile compounds



1a, petiveriin A



1b, petiveriin B

Fig. 2. The structure of petiveriin A and petiveriin B.

Table 1
The total content and relative ratios of *S*-benzylcysteine sulfoxides in different parts of *P. alliacea*

Tissue analyzed	mg g^{-1} fr. wt	**1a:1b**
Root	2.97±0.16	40:60
Stem	0.29±0.03	12:88
Leaves	0.07±0.01	22:78

previously found in this plant. We propose that these amino acids are enzymatically cleaved to give *S*-benzyl phenylmethanethiosulfinate in a fashion similar to that observed in the formation of allicin (diallyl thiosulfinate) and other thiosulfinates in *Allium* species (Stoll and Seebeck, 1949; Block, 1992). Our results also indicate that the vast majority of the volatiles that have so far been identified in extracts of *P. alliacea* are not present in a fresh tissue. They are, in fact, only degradation products generated during isolation procedures from labile *S*-benzyl phenylmethanethiosulfinate (Kice et al., 1960; Furukawa et al., 1973; White and Dellinger, 1985; Block, 1992).

3. Experimental

3.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 HC spectrometer, and IR spectra were recorded using a Perkin Elmer RX I FTIR spectrometer. UV spectra were measured on a Shimadzu UV-1601PC spectrophotometer and CD spectra on an Aviv 62DS circular dichroism spectrometer. Specific rotation values were determined by means of a Perkin-Elmer 243B polarimeter. HPLC separations were performed on a Dynamax SD-200 binary pump system, employing a Varian PDA 330 detector, a C-18 reverse phase column (Rainin Microsorb-MV 100 Å, 250×4.6 mm, 5 μm), using 10 mM KH_2PO_4 buffer (solv. A, pH 7.0) with MeCN (solv. B) as the mobile phase, with a flow rate of 1 ml min^{-1}. The gradient was as follows: A/B 100/0 (0 min), 95/5 (in 20 min), 60/40 (in 30 min) held for 15 min. Alternatively, a preparative C-18 reverse phase column (Rainin Dynamax-100 Å, 250×21.4 mm, 8 μm) was used. TLC was performed on precoated Aldrich plastic plates (silica gel polyester) with *n*-BuOH–H_2O–HOAc (4:1:1 v/v/v) or *n*-PrOH–H_2O (7:3 v/v/v) as the mobile phase. Ninhydrin (0.2% soln. in ethanol) was used for detection. Melting points (uncorr.) were determined using a Köfler hot stage.

3.2. Plant material

Whole fresh plants of *Petiveria alliacea* were obtained from Native Habitat Landscaping, FL, USA. They were

collected in Vero Beach, Indian River County, FL, USA in October 2000, immediately shipped, and stored in the freezer at −30 °C until analysis. A voucher specimen is deposited in the herbarium PIHG at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL, USA under the number 7801.

3.3. Extraction and purification

Fresh roots (69.0 g) were carefully cleaned and homogenized in MeOH using a blender, and extracted with boiling MeOH (2×600 ml). The extracts were combined, concentrated to ca. 400 ml by vacuum evaporation (40 °C), and adjusted to 500 ml by addition of 3% HCl. The precipitate that appeared on acidification was filtered off and the filtrate was passed through a cation-exchange column (19×2.1 cm; Amberlite 200, H^+ form, 20–50 mesh). After washing the column with 3% HCl (300 ml) and H_2O (300 ml), the amino acid fraction was eluted with 500 ml of 1 M NH_4OH. The yellow eluent obtained was concentrated to ca. 20 ml and subjected to prep. HPLC. The fractions eluting at 18.1 and 18.9 min were collected, evaporated, and the residues re-crystallized from boiling H_2O to yield 24 and 29 mg of white crystalline **1a** and **1b**, respectively.

3.4. Quantitative determination

Extracts were prepared from each part of the plant (leaves, stem, and root) as described above. These were analyzed by HPLC without derivatization. *S*-(4-Chlorobenzyl)cysteine, added to the samples before extraction, was used as an internal standard. At least three analyses of each extract were done. Calibration curves were computed using solutions prepared from the synthetic diastereomers of *S*-benzyl-L-cysteine sulfoxide (**1a** and **1b**).

3.5. Reference compounds

S-Benzylcysteine, *S*-(4-chlorobenzyl)cysteine (internal standard) and *S*-benzylcysteine sulfoxide were synthesized as described by Stoll and Seebeck (1949). Pure diastereomers of *S*-benzylcysteine sulfoxide were obtained by repeated fractional re-crystallization (H_2O–Me_2CO–HOAc 70:30:1 v/v/v) and by prep. HPLC.

(R$_C$R$_S$)-S-Benzyl-L-cysteine sulfoxide (**1a**, petiveriin A): small white plates; mp 172–173 °C; $[\alpha]_D^{22}$ +34° (1 M NaOH; *c* 1.0); CD $\Delta\varepsilon_{max}$ (0.1 M NaOH; *c* 0.2; 22 °C): +12.9 (223 nm); UV λ_{max} (0.1 M NaOH) nm (log ε): 220 (4.05), 259 (2.65); IR (KBr) ν_{max} cm^{-1}: 3463 (*w, br*), 3040–2900 (*s, br*), 1633 (*vs*), 1594 (*vs*), 1424 (*s*), 1359 (*s*), 1307 (*m*), 1071 (*s*), 1019 (*vs*), 696 (*s*); ^1H NMR (300 MHz; CD_3OD/DCl; DSS): δ 3.36 (1H, *dd*, *J* = 3.6, 14.4 Hz, H-3a), 3.46 (1H, *dd*, *J* = 8.4, 14.4 Hz, H-3b), 4.25 (1H, *d*, *J* = 13.2 Hz, H-5a), 4.37 (1H, *d*, *J* = 13.2 Hz, H-5b), 4.57 (1H, *dd*, *J* = 3.6, 8.4 Hz, H-2), 7.367.43 (5H, *m*, H_{arom}); ^{13}C NMR (75 MHz; CD_3OD/DCl; DSS): δ 49.6 (C-2), 49.7 (C-3), 58.9 (C-5), 129.8 (C-9), 130.0 (C-8), 130.8 (C-6), 131.6 (C-7), 169.7 (C-1); TLC R_f 0.50 (*n*-BuOH–H_2O–HOAc, 4:1:1), R_f 0.69 (*n*-PrOH–H_2O, 7:3). *ms* (M+)m/t: 227.0616 calc. for C_{10} H_{13} NO_3S: 227.0616.

(R$_C$S$_S$)-S-Benzyl-L-cysteine sulfoxide (**1b**, petiveriin B): long tiny white needles; mp 166–168 °C): $[\alpha]_D^{22}$ −59° (1 M NaOH; *c* 1.0); CD $\Delta\varepsilon_{max}$ (0.1 M NaOH; *c* 0.2; 22 °C): −15.1 (225 nm); UV λ_{max} (0.1 M NaOH) nm (log ε): 220 (3.98), 259 (2.60); IR (KBr) ν_{max} cm^{-1}: 3423 (*s, br*), 3040–2900 (*m, br*), 1664 (*vs*), 1590 (*s*), 1521 (*s*), 1425 (*m*), 1351 (*m*), 1299 (*m*), 1273 (*m*), 1071 (*m*), 1017 (vs), 696 (*m*); ^1H NMR (300 MHz; CD_3OD/DCl; DSS): δ 3.22 (1H, *dd*, *J* = 6.9, 13.5 Hz, H-3a), 3.51 (1H, *dd*, *J* = 6.7, 13.5 Hz, H-3b), 4.18 (1H, *d*, *J* = 13.2 Hz, H-5a), 4.38 (1H, *d*, *J* = 13.2 Hz, H-5b), 4.52 (1H, *t*, *J* = 6.8 Hz, H-2), 7.377.43 (5H, *m*, H_{arom}); ^{13}C NMR (75 MHz; CD_3OD/DCl; DSS): δ 50.5 (C-2), 50.7 (C-3), 59.6 (C-5), 129.7 (C-9), 130.0 (C-8), 130.8 (C-6), 131.6 (C-7), 169.5 (C-1); TLC: identical with **1a**. *ms*(M^+) *m*/*z*: 227.0626. Calc. for C_{16} H_{13} NO_3S: 227.0616

(S$_C$S$_S$)-S-Benzyl-D-cysteine sulfoxide (**1c**): small white plates; mp 173–174 °C; $[\alpha]_D^{22}$: −33° (1 M NaOH; *c* 1.0); CD $\Delta\varepsilon_{max}$ (0.1 M NaOH; *c* 0.2; 22 °C): −12.7 (223 nm); UV λ_{max} (0.1 M NaOH) nm (log ε): 220 (4.06), 259 (2.58); IR (KBr): identical with 1a; ^1H NMR (300 MHz; CD_3OD/DCl; DSS): δ 3.35 (1H, *dd*, *J* = 3.9, 14.7 Hz, H-3a), 3.42 (1H, *dd*, *J* = 8.1, 14.7 Hz, H-3b), 4.24 (1H, *d*, *J* = 12.9 Hz, H-5a), 4.35 (1H, *d*, *J* = 12.9 Hz, H-5b), 4.56 (1H, *dd*, *J* = 3.9, 8.1 Hz, H-2), 7.37–7.43 (5H, *m*, H_{arom}); ^{13}C NMR (75 MHz; CD_3OD/DCl; DSS): δ 49.6 (C-2), 49.7 (C-3), 58.9 (C-5), 129.8 (C-9), 130.1 (C-8), 130.8 (C-6), 131.6 (C-7), 169.7 (C-1); TLC: identical with **1a**.

(S$_C$R$_S$)-S-Benzyl-D-cysteine sulfoxide (**1d**): long tiny white needles; mp 165–167 °C; $[\alpha]_D^{22}$: +54° (1 M NaOH; *c* 1.0); CD $\Delta\varepsilon_{max}$ (0.1 M NaOH; *c* 0.2; 22 °C): +11.2 (224 nm); UV λ_{max} (0.1 M NaOH) nm (log ε): 220 (3.98), 259 (2.60); IR (KBr): identical with **1b**; ^1H NMR (300 MHz; CD_3OD/DCl; DSS): δ 3.20 (1H, *dd*, *J* = 6.9, 13.5 Hz, H-3a), 3.51 (1H, *dd*, *J* = 6.7, 13.5 Hz, H-3b), 4.17 (1H, *d*, *J* = 13.1 Hz, H-5a), 4.38 (1H, *d*, *J* = 13.1 Hz, H-5b), 4.52 (1H, *t*, *J* = 6.8 Hz, H-2), 7.36–7.42 (5H, *m*, H_{arom}); ^{13}C NMR (75 MHz; CD_3OD/DCl; DSS): δ 50.4 (C-2), 50.7 (C-3), 59.6 (C-5), 129.7 (C-9), 130.0 (C-8), 130.9 (C-6), 131.7 (C-7), 169.5 (C-1); TLC: identical with **1a**.

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