PERGAMON

Phytochemistry 60 (2002) 21–25

PHYTOCHEMISTRY

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The amino acid precursors and odor formation in society garlic (*Tulbaghia violacea* Harv.)

Roman Kubec[a,b], Jan Velíšek[b], Rabi A. Musah[a,*]

[a]*Department of Chemistry, State University of New York at Albany, Albany, NY, 12222, USA*
[b]*Department of Food Chemistry and Analysis, Institute of Chemical Technology, Technická 1905, Prague 6, 166 29, Czech Republic*

Received 20 November 2001; received in revised form 5 February 2002

Abstract

Identification and isolation of (R_SR_C)-*S*-(methylthiomethyl)cysteine-4-oxide from rhizomes of *Tulbaghia violacea* Harv. is reported. The structure and absolute configuration of the amino acid have been determined by NMR, MALDI–HRMS, IR, and CD spectroscopy. Its content varied in different parts of the plant (rhizomes, leaves, and stems) between 0.12 and 0.24 mg g^{-1} fr. wt, being almost equal in the stems and rhizomes. In addition, *S*-methyl- and *S*-ethylcysteine derivatives have been detected in minute amounts (<3 μg g^{-1} fr. wt) in all parts of the plant. The enzymatic cleavage of the amino acid and subsequent odor formation are discussed. 2,4,5,7-Tetrathiaoctane-4-oxide, the primary breakdown product, has been detected and isolated for the first time.
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Keywords: *Tulbaghia violacea*; Alliaceae; *S*-(Methylthiomethyl)cysteine-4-oxide; Society garlic; Marasmin; Marasmicin; Flavor precursor; Thiosulfinate

1. Introduction

Tulbaghia violacea Harv. (Alliaceae) is a small bulbous herb indigenous to Natal, Transvaal and the eastern Cape region in South Africa where it grows in rocky grasslands. The evergreen leaves of *T. violacea* exhibit a garlic-like smell when bruised and have been used in some cultures as a substitute for garlic and chive. The plant is known by several common names including "society garlic", "sweet garlic" and "wild garlic". These names originated from the belief that, in spite of its garlic-like flavor, the consumption of *T. violacea* is not accompanied by the development of bad breath as is the case with the consumption of the real garlic (*Allium sativum* L.). *T. violacea* has traditionally been used for the treatment of fever and colds, asthma, tuberculosis, and gastrointestinal ailments. However, extensive consumption of this plant has been associated with a variety of undesirable symptoms, such as abdominal pain, inflammation, and gastroenteritis. It has also been reported that society garlic deters moles and that the Zulus of South Africa grow this plant around their homes to repel snakes (Hutchings et al., 1996; Wyk et al., 2000).

To date, no systematic research on *T. violacea* has been conducted. To our knowledge, only two scientific articles dealing with the chemical constituents of society garlic have been published. Jacobsen et al. (1968) reported the presence of a C–S lyase and three unidentified *S*-substituted cysteine sulfoxide derivatives, whereas Burton and Kaye (1992) isolated 2,4,5,7-tetrathiaoctane-2,2-dioxide and 2,4,5,7-tetrathiaoctane from the leaves of *T. violacea*. This study was undertaken to identify the three unknown cysteine derivatives detected by Jacobsen et al. (1968) and to clarify their role in the organoleptic properties of *T. violacea*.

2. Results and discussion

An amino acid fraction was isolated from rhizomes of *T. violacea* by ion-exchange chromatography. The extract was subjected to GC–MS analysis after derivatization by ethyl chloroformate (ECF) and reduction with NaI (Kubec et al., 1999). This methodology revealed the presence of *S*-(methylthiomethyl)-, *S*-methyl-, and

* Corresponding author. Tel.: +1-518-437-3740; fax: +1-518-437-3741.
E-mail address: musah@csc.albany.edu (R.A. Musah).

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PII: S0031-9422(02)00065-1

S-ethylcysteine derivatives, with the first amino acid being by far the major component of the extract. None of the following *S*-alk(en)ylcysteine derivatives was detected: *S*-propyl-, *S*-isopropyl-, *S*-allyl-, *S*-(1-propenyl)-, *S*-butyl-, *S*-isobutyl-, *S*-(*sec*-butyl)-, *S*-pentyl-, *S*-isopentyl-, *S*-benzyl-, implying that their content in the samples analyzed was less than 1 μg g^{-1} fr. wt.

The GC method used for the preliminary screening, however, does not distinguish between *S*-substituted cysteines and their sulfoxides. To determine the oxidation state and the absolute configuration of the *S*-(methylthiomethyl)cysteine derivative, another extract was prepared and subjected to prep. HPLC. The predominant component of the extract (when monitored at the wavelength of 215 nm) was collected, affording 34 mg of a white crystalline compound. The IR spectrum contained a very strong absorption at 1026 cm^{-1} and MALDI–HRMS analysis showed [MH^+] of 198.0256 ($C_5H_{11}NO_3S_2$ req. 198.0253), revealing that the amino acid isolated was a sulfoxide form of *S*-(methylthiomethyl)cysteine. The position of the sulfoxide group was determined based on NMR spectroscopic data. Since the ^1H and ^{13}C chemical shifts of the terminal methyl group (δ 2.32 and 16.3 ppm, respectively) corresponded to a CH_3S– group rather than a $CH_3S(O)$– group, the structure of the amino acid was determined as *S*-(methylthiomethyl)cysteine-4-oxide. This compound, trivially named marasmin, was first isolated as a γ-glutamyl dipeptide from several basidiomycetous mushrooms of the genus *Marasmius* Fr. (Gmelin et al., 1976). Several years later, the absolute configuration of marasmin was determined to be S_SR_C (Broek et al., 1987). Recently, *S*-(methylthiomethyl)cysteine-4-oxide was also isolated from the fruits of the tropical tree of *Scorodocarpus borneensis* Becc. (Olacaceae) (Kubota et al., 1998). Interestingly, this latter amino acid was found to have an absolute configuration about the sulfoxide group opposite to that observed in marasmin from basidiomycetous mushrooms (i.e. R_SR_C).

To determine the absolute configuration of the derivative isolated from *T. violacea*, CD and ^1H NMR spectrometry were employed. *S*-Substituted cysteine sulfoxides are known to display ^1H NMR spectra with a characteristic ABX splitting pattern for the S(O) $CH_2CH(NH_2)$ methylene protons. Those having the amino and the sulfoxide groups on the same face show two distinct doublets of doublets with coupling constants of $J_{AX} = \sim 7$–8.5 Hz and $J_{BX} = \sim 5.5$–7 Hz. The methylene protons of derivatives with the opposite configuration typically resonate within a much narrower range, having coupling constants of $J_{AX} = \sim 8.5$–10.5 Hz and $J_{BX} = \sim 3.5$–4 Hz (Broek et al., 1987; Kubota et al., 1998; Kubec and Musah, 2001).

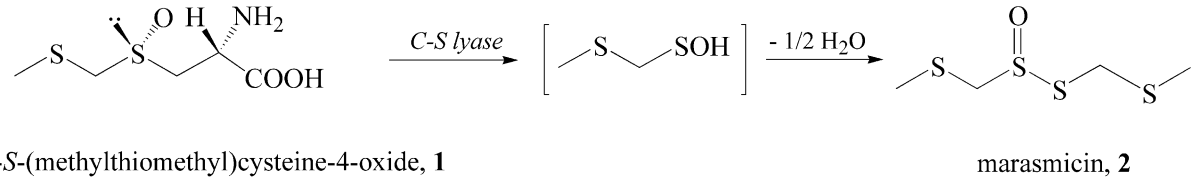
The CD spectrum of the isolated amino acid showed a negative sign of the Cotton effect with a single maximum at 228 nm. As reported by Broek et al. (1987), a negative sign of the Cotton effect correlates with R_S configuration. The ^1H NMR spectrum contained two distinct doublets of doublets centered at δ 3.32 and δ 3.55 (with both J_{AX} and J_{BX} being 6.9 Hz), indicating that the amino and the sulfoxide groups are on the same face of the molecule. The above data permitted the unambiguous determination of the absolute configuration of the amino acid as (R_SR_C)-*S*-(methylthiomethyl)cysteine-4-oxide (**1**, Fig. 1). This compound is therefore identical with that isolated from *Scorodocarpus borneensis* and is of opposite sulfoxide configuration to marasmin isolated from *Marasmius* species.

Due to their presence in very low concentrations, no attempt was made to determine the oxidation state and the absolute configuration of the two minor cysteine derivatives. However, it seems reasonable to assume that these are (S_SR_C)-*S*-methyl- and (S_SR_C)-*S*-ethylcysteine sulfoxides (MCSO and ECSO, respectively). That there is a difference in stereochemistry about the sulfur atom in these compounds relative to that observed in the isolated *S*-(methylthiomethyl)cysteine-4-oxide (i.e. S_SR_C vs R_SR_C respectively) is simply a consequence of reversed priority assignments of the substituents at the sulfur atom. Although these compounds have opposite *R*,*S* designations, the geometric arrangement of their substituents about the sulfur remains analogous to that observed in **1**. *S*-Methylcysteine sulfoxide (methiin, MCSO) is a very common secondary metabolite occurring in plants of many families including Alliaceae, Brassicaceae, and Leguminosae. The latter amino acid, *S*-ethylcysteine sulfoxide (ethiin, ECSO), has only recently been found as a minor component in some *Allium* species and in members of several genera of the Brassicaceae family (Kubec et al., 2000, 2001).

The content of the *S*-alkylcysteine sulfoxides was determined in different parts of the plant. As shown in Table 1, the total amounts ranged from 11.6 to 23.7 mg 100 g^{-1} fr. wt, being nearly equal in the stems and rhizomes. In all examined parts, *S*-(methylthiomethyl)cysteine-4-oxide was the predominant derivative, accounting for nearly 99% of the *S*-alkylcysteine sulfoxide pool.

Based on preliminary GC/MS data, Jacobsen (1965) proposed that one of the three unidentified *S*-substituted cysteine sulfoxides he detected by TLC might have been an ethyl derivative. We have confirmed his assumption. However, the quantities of ECSO we found were well below the limits he would have been able to detect by TLC. Thus, the sample he analyzed may have contained a substantially higher amount of ECSO (and MCSO as well) than that observed in this study.

Interestingly, Krest et al. (2000) found *S*-methyl-, (*E*)-*S*-(1-propenyl)-, and *S*-propylcysteine sulfoxides in a different member of the genus, *T. acutiloba* Harv., in a relative ratio of 4/1/1 (w/w/w). It is not certain whether the authors looked for *S*-(methylthiomethyl)cysteine-4-oxide, and indeed, it may have been present. However,



(R_SR_C)-*S*-(methylthiomethyl)cysteine-4-oxide, **1** marasmicin, **2**

Fig. 1. Formation of marasmicin in *Tulbaghia violacea* Harv.

Table 1
Quantitative determination of *S*-alkylcysteine sulfoxides in *Tulbaghia violacea* Harv.

	Content (mg 100 g^{-1} fr. wt)			
	MCSO[a]	ECSO[b]	MTMCSO[c]	Total
Rhizomes	tr[d]	tr	22.8±1.8	22.8±1.8
Leaves	0.1±0.02	tr	11.5±1.7	11.6±1.7
Stem	0.3±0.05	tr	23.4±1.9	23.7±2.0

[a] MCSO, *S*-methylcysteine sulfoxide.
[b] ECSO, *S*-ethylcysteine sulfoxide.
[c] MTMCSO, *S*-(methylthiomethyl)cysteine-4-oxide.
[d] tr, Traces (<0.1 mg 100 g^{-1} fr. wt).

even though some cysteine sulfoxide derivatives might have been overlooked in *T. acutiloba*, the study of Krest et al. (2000) implies that different members of the genus *Tulbaghia* contain a different cysteine sulfoxide pool. Similar species-to-species variation in relative proportions of *S*-alk(en)ylcysteine sulfoxides has also been observed in other genera of the Alliaceae family, most notably *Allium* and *Leucocoryne* (Fenwick and Hanley, 1985; Block, 1992; Kubec et al., 2000; Lancaster et al., 2000).

Thus, a more detailed study of the *S*-substituted cysteine sulfoxide derivatives in *Tulbaghia* species may reveal very useful chemotaxonomic information that would help to organize this genus.

Gmelin et al. (1976) were the first to propose that the enzymatic cleavage of marasmin is analogous to that of alliin (*S*-allylcysteine sulfoxide) in garlic and other alliaceous species. They suggested the formation of *S*-(methylthiomethyl) (methylthio)methanethiosulfinate (2,4,5,7-tetrathiaoctane-4-oxide, **2**) from marasmin as the primary breakdown product. They described this putative compound to be very unstable, decomposing rapidly into unidentified polysulfides. Although the formation of **2** from marasmin has been anticipated for a long time, this interesting thiosulfinate has not been isolated or synthesized thus far. The presence of a C–S lyase in *Tulbaghia* (Jacobsen et al., 1968) and the close genetic relationship with *Allium* species, make it reasonable to assume that a similar mechanism is operative in society garlic. In order to identify the product(s) of enzymatic decomposition of **1**, a dichloromethane extract of freshly homogenized *Tulbaghia* rhizomes was prepared and analyzed by HPLC. The predominant component of the extract (when monitored at 215–300 nm) was isolated by prep. HPLC, yielding a yellow viscous liquid with a very intense garlic-like smell. ^{1}H NMR revealed the presence of two isolated methyl groups and two pairs of heterosteric methylene protons. The ^{13}C APT NMR spectrum showed two methylene and two methyl carbon atoms. The signals of the latter two carbons had identical chemical shifts in $CDCl_3$ (δ 17.5 ppm) but were nicely separated in C_6D_6 (δ 15.0 and 17.0 ppm). MALDI-HRMS gave [MNa^+] of 224.9511 ($C_4H_{10}OS_4$ req. 224.9507). The IR spectrum contained a very strong absorbtion band at 1073 cm^{-1}, indicating the presence of a sulfoxide group. Based on the above data, this compound has been assigned as 2,4,5,7-tetrathiaoctane-4-oxide (**2**). Due to the relatively complicated IUPAC name of **2** and in close analogy to the alliin/allicin system, we have trivially named this compound marasmicin.

Very likely, marasmicin further decomposes giving various sulfur-containing degradation products, e.g. 2,4,5,7-tetrathiaoctane, 2,4,5,7-tetrathiaoctane-2,2-dioxide, 2,4,5,7-tetrathiaoctane-4,4-dioxide, or 2,4,5,7-tetrathiaoctane-2,2,7,7-tetraoxide. Many of these compounds have been shown to possess strong antimicrobial and antifungal activity as well as antithrombotic properties (Takazawa et al., 1982; Burton and Kaye, 1992; Kubota et al., 1994a,b; Block et al., 1994; Rapior et al., 1997; Lim et al., 1998, 1999).

Finally, we would like to comment on the legitimacy of the use of the name "society garlic" in reference to *T. violacea*. The absence of alliin, the precursor of the main volatile components detected in the breath after ingestion of real garlic (*A. sativum* L.) (Ruiz et al., 1994; Cai et al., 1995), may justify this trivial name of *T. violacea* Harv. However, we have observed *T. violacea* Harv. leaves to possess an exceptionally powerful garlicky taste. Chewing and subsequent ingestion of the leaves was accompanied by development of a strong breath that persisted for several hours. Apparently, the compounds generated are different from those detected in garlic breath. Furthermore, when the rhizomes or leaves are handled with bare hands, a strong organosulfur scent persists on the fingers for several hours. It seems reasonable to assume that the odor producing compounds are decomposition products of marasmicin (**2**), such as (methylthiomethyl) mercaptan as well as bis (methylthiomethyl) sulfides, sulfones, and thiosulfonates. Even though *T. violacea* Harv. apparently does

not deserve the trivial name "society garlic", it seems to be an ideal candidate for a new condiment. We believe that this plant might become a very popular seasoning for its pleasant, unique, and powerful flavor as well as for its original sharp garlic-like taste.

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. Matrix-assisted laser desorption/ionization (MALDI) FTMS experiments were performed on an IonSpec FTMS mass 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 and a C-18 reverse phase column (Rainin Microsorb-MV 100Å, 250×4.6 mm, 5 μm). Alternatively, a preparative C-18 reverse phase column (Rainin Dynamax-100Å, 250×21.4 mm, 8 μm) was used. The gradient used for amino acid analysis was as follows (A—10 mM KH_2PO_4 buffer, pH 7.0; B—MeCN): A/B 97/3 (0 min), 97/3 (in 10 min), 40/60 (in 20 min) held for 15 min. The gradient used for thiosulfinate analysis was as follows (A—H_2O; B—MeCN): A/B 85/15 (0 min), 85/15 (in 6 min), 20/80 (in 40 min). A flow rate of 1 ml min^{-1} (analytical) or 16 ml min^{-1} (prep. column) was employed. 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 *T. violacea* Harv. were obtained from Brays Bayou Nursery, Houston, TX, USA. They were collected in August 2001, immediately shipped and analyzed. A voucher specimen has been deposited in the New York State Museum Herbarium in Albany, NY, USA under the number A33087.

3.3. Amino acid isolation

Fresh rhizomes (266 g) were homogenized in MeOH using a blender and extracted with boiling MeOH (2 × 600 ml). The extracts were combined, concentrated to ca. 50 ml by vacuum evaporation (40 °C), and adjusted to 200 ml by addition of 3% HCl. The precipitate formed on acidification was removed by filtration 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 (200 ml) and H_2O (200 ml), the amino acid fraction was eluted with 1 M NH_4OH (400 ml). The yellowish eluent obtained was concentrated to ca. 10 ml, filtered and subjected to prep. HPLC. The fractions eluting at 5.1 min were collected, combined, evaporated and the residue recrystallized from boiling H_2O to yield 36 mg of white crystalline **1**.

3.4. Quantitative analysis

Quantitative determination was done by means of GC after derivatization with ethyl chloroformate (Kubec et al., 1999). (±)-*S*-Benzylcysteine sulfoxide was used as an internal standard.

3.5. Thiosulfinate isolation

A homogenate of fresh *Tulbaghia* rhizomes (345 g in 750 ml H_2O) was prepared and allowed to stand at room temperature for 30 min. The slurry was then filtered and immediately extracted with cold dichloromethane (2 × 500 ml). After centrifugation, the organic layer was evaporated and the oily residue re-dissolved in MeCN and subjected to prep. HPLC. The fractions eluting at 15.2 min were collected, combined and evaporated, affording 26 mg of a yellow viscous oil.

3.6. Analytical data

(R_SR_C)-*S*-(Methylthiomethyl)cysteine-4-oxide (**1**) was obtained as white small plates: mp 167–168 °C; $[\alpha]_D^{25}$−43.3° (*c* 0.0107, H_2O); UV (H_2O) λ_{max} (log ε) 198 (3.59), 233 (*sh*) (3.00); CD $\Delta\varepsilon_{max}$ (H_2O; *c* 0.02; 22 °C) −4.2 (228 nm); IR (KBr) ν_{max} 3445 (*br*, *m*), 3093–2902 (*br*, *m*), 1618 (*vs*), 1426 (*m*), 1353 (*m*), 1322 (*w*), 1275 (*w*), 1026 (*vs*), 855 (*w*) cm^{-1}; ^{1}H NMR (D_2O, DSS, 300 MHz) δ 2.32 (3H, *s*, H-7), 3.32 (1H, *dd*, *J* = 6.9, 13.8 Hz, H-3b), 3.55 (1H, *dd*, *J* = 6.9, 13.8 Hz, H-3a), 4.01 (1H, *d*, *J* = 14.1 Hz, H-5b), 4.21 (1H, *d*, *J* = 14.1 Hz, H-5a), 4.24 (1H, t, *J* = 6.9 Hz, H-2); ^{13}C NMR (D_2O, DSS, 75 MHz) δ 16.3 (C-7), 50.5 (C-3), 50.9 (C-2), 55.6 (C-5), 171.6 (C-1); MALDI–HRMS [MH^+] 198.0256 ($C_5H_{11}NO_3S_2$ req. 198.0253), TLC R_f 0.36 (*n*-BuOH–H_2O–HOAc, 4:1:1), R_f 0.50 (*n*-PrOH–H_2O, 7:3).

2,4,5,7-Tetrathiaoctane-4-oxide (**2**, marasmicin) was obtained as a yellow oil: UV (MeCN) λ_{max} (log ε) 206 (4.03), 232 (*sh*) (3.69); IR (neat) ν_{max} 2968 (*m*), 2916 (*m*), 1426 (*m*), 1302 (*s*), 1202 (*m*), 1135 (*m*), 1073 (*vs*), 969 (*m*) cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS, 300 MHz) δ 2.28 (3H, s, H-8), 2.39 (3H, *s*, H-1), 4.09 (1H, *d*, *J* = 13.8 Hz, H-3b), 4.18 (1H, *d*, *J* = 13.8 Hz, H-3a), 4.26 (2H, *d*, *J* = 2.7 Hz,

H-6); ^{13}C NMR ($CDCl_3$, TMS, 75 MHz) δ 17.5 (C-1, C-8), 37.5 (C-6), 59.6 (C-3); ^{13}C NMR (C_6D_6, 75 MHz) δ 15.0 (C-8), 17.0 (C-1), 36.7 (C-6), 59.3 (C-3); MALDI–HRMS [MNa^+] 224.9511 ($C_4H_{10}OS_4$ req. 224.9507).

Acknowledgements

The authors would like to thank Dr. Eric Block for helpful suggestions and critical reading of the manuscript. We also thank Dr. Gary Siuzdak at the Scripps Research Institute Center for Mass Spectrometry (La Jolla, CA) for technical support. Support for this work was provided by the State University of New York at Albany.

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