JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Flavor Precursors and Sensory-Active Sulfur Compounds in Alliaceae Species Native to South Africa and South America

Roman Kubec,*[,†] Petra Krejčová,[†] Leví Mansur,[‡] and Nicolás García[§]

[†]Department of Applied Chemistry, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic

[‡]Faculty of Agronomy, Pontificia Universidad Católica de Valparaíso, Casilla 4-D, Quillota, Chile

[§]Department of Biology, University of Florida, Gainesville, Florida 32611, United States

Supporting Information

ABSTRACT: Profiles of S-substituted cysteine flavor precursors were determined in 42 Alliaceae species native to South Africa and South America. It was found that the pool of cysteine derivatives present in these plants is remarkably very simple, with *S*-((methylthio)methyl)cysteine 4-oxide (marasmin) being the principal flavor precursor, typically accounting for 93−100% of the pool. Out of the other cysteine derivatives, only minor quantities of methiin were present in some species. The marasmin-derived thiosulfinate marasmicin (2,4,5,7-tetrathiaoctane 4-oxide), a major sensory-active compound of the freshly disrupted plants, was isolated, and its organoleptic properties were evaluated. Furthermore, sulfur-containing volatiles formed upon boiling of these alliaceous species were studied by GC−MS. The profile of the volatiles formed was relatively simple, with 2,3,5-trithiahexane and 2,4,5,7-tetrathiaoctane being the major components. Despite the traditional belief, ingestion of the marasmin-rich plants was always accompanied by development of a strong "garlic breath". We believe that especially several *Tulbaghia* species deserve to attract much greater attention from the food industry thanks to their pungent garlicky taste and unusual yet pleasant alliaceous smell.

KEYWORDS: *marasmin, marasmicin, society garlic, flavor precursor, Tulbaghia, Leucocoryne, Ipheion, sulfur volatiles, Alliaceae taxonomy*

INTRODUCTION

The family Alliaceae comprises about 900 species growing worldwide. While some are only little more than botanical curiosities, others are attractive ornamental plants or economically very important vegetables (e.g., onion, garlic, and leek). Furthermore, numerous alliaceous species are medicinal plants widely used in both folk medicine and the pharmaceutical industry.

The family Alliaceae is taxonomically divided into three subfamilies, i.e., Allioideae Herb., Tulbaghioideae (Endl. ex Meisn.) Fay & Chase, and Gilliesioideae (Lindl.) Arn. The largest subfamily, Allioideae, is more or less restricted to the northern hemisphere and comprises the genus *Allium* L. (including several subgenera). The subfamily Tulbaghioideae includes only two genera, *Tulbaghia* L. (about 25 species) and *Prototulbaghia* Vosa (1 species), comprising plants native to southern parts of Africa. The third subfamily, Gilliesioideae, comprises all the South American taxa.[1−3]

Many Alliaceae species contain compounds that give rise to the characteristic onion- or garlic-like smell (sometimes referred to as "alliaceous chemistry") when their tissue is disrupted. The precursors of these volatile compounds are sulfur nonprotein amino acids *S*-alk(en)ylcysteine *S*-oxides or *N*-oxides. Eleven such derivatives (**1**−**11**) have thus far been found in Alliaceae plants (Figure 1).[4−11] Upon tissue disruption, these amino acids are enzymatically cleaved to give rise to a great number of various sensory-active compounds, including pungent thiosulfinates [RS(O)SR′] and lachrymatory sulfines (RCH═S═O).[7,12]

The vast majority of reports have been focused on sulfur compounds occurring in various members of the genus *Allium*, especially in economically important species such as onion (*Allium cepa* L.), garlic (*Allium sativum* L.), and leek (*Allium porrum* L.).[4,13−17] On the other hand, very little attention has thus far been paid to sulfur compounds present in Alliaceae species native to South Africa and South America.[11,18−20] Many of these plants are frequently used in folk medicine and consumed by local people as a substitute for garlic and chive.[21] The popularity of these species is increasing also in the western world due to the (erroneous) belief that their consumption is not accompanied by the development of bad breath as is the case with the consumption of garlic.[11] Hence, the South African plant *Tulbaghia violacea* Harv. is trivially called "society garlic" or "sweet garlic", and *Ipheion uniflorum* Raf., a leafy bulbous plant native to South America, is often sold as "bad-breath-free garlic".

This work was aimed at studying the flavor precursors, thiosulfinates and volatile sulfur compounds present/formed in Alliaceae species native to South Africa and South America (members of the subfamilies Tulbaghioideae and Gilliesioideae, respectively). Although many of these plants are frequently consumed, detailed information about their chemical composition is still lacking. A better knowledge about the structure

Received: June 8, 2012
Revised: January 18, 2013
Accepted: January 18, 2013
Published: January 18, 2013

dx.doi.org/10.1021/jf305179p | J. Agric. Food Chem. 2013, 61, 1335−1342

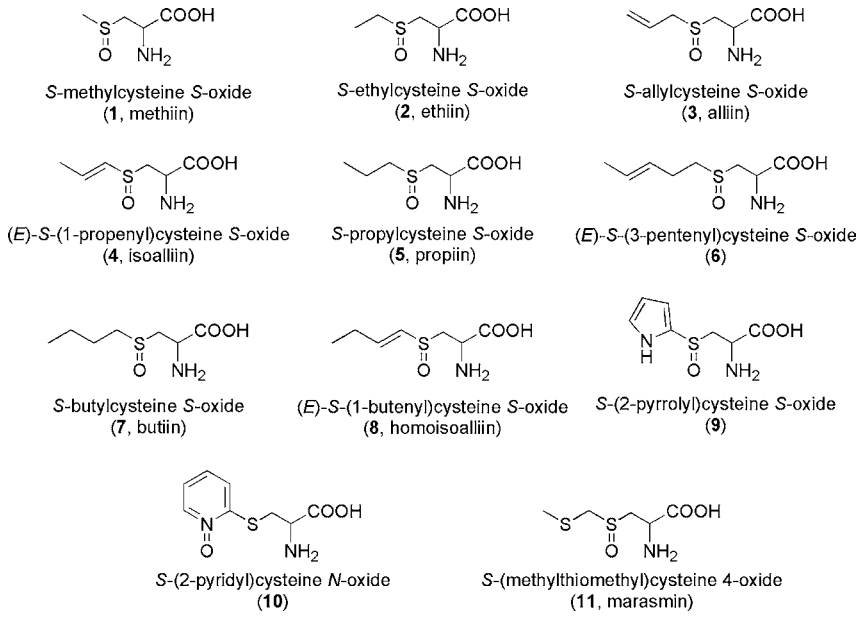

Figure 1. S-substituted cysteine derivatives found in Alliaceae species.

Table 1. S-Substituted Cysteine *S*-Oxides Found in Subfamily Tulbaghioideae Species

			relative proportion (%)				
genus	species	origin[a]	methiin (**1**)	marasmin (**11**)	total content (mg g^{-1} of fresh weight)	odor[b]	taste[c]
Prototulbaghia	*P. siebertii* Vosa	A	0	100	0.28 ± 0.03	++	++
Tulbaghia	*T. acutiloba* Harv.	B	tr[d]	100	0.01	−	−
	T. alliacea L.f.	B	0	100	1.68 ± 0.13	+++	+++
	T. capensis L.	C	5	95	1.62 ± 0.18	+++	+++
	T. cernua Fisch Mey Avé-Lall.	D	7	93	0.22 ± 0.03	−	−
	T. coddii Vosa Burb.	D	tr	100	1.18 ± 0.15	+++	++
	T. cominsii Vosa	D	tr	100	0.74 ± 0.08	++	++
	T. dregeana Kunth.	E	0	100	0.02	−	−
	T. galpinii Schltr.	D	tr	100	0.15 ± 0.02	++	+
	T. leucantha Baker	D	tr	100	1.05 ± 0.11	+++	+++
	T. ludwigiana Harv.	C	0	100	nq[e]	−	−
	T. montana Vosa	D	tr	100	0.12 ± 0.02	++	+
	T. natalensis Baker	D	0	100	0.98 ± 0.14	+++	+++
	T. simmleri Beauverd	D	0	100	0.12 ± 0.02	−	−
	T. simmleri var. *alba* Beauverd	D	0	100	0.07 ± 0.02	−	−
	T. violacea var. *maritima* Vosa	D	tr	100	1.26 ± 0.17	+++	+++
	T. violacea var. *obtusa* Baker	D	tr	100	1.17 ± 0.20	+++	+++
	T. violacea var. *violacea* Harv.	D	6	94	1.58 ± 0.22	+++	+++

[a]See the Materials and Methods for details. [b]Intensity of the garlic-like odor emitted upon comminuting the rhizomes (+++, strong; ++, moderate; +, faint but detectable; −, not detectable). [c]Intensity of the garlic-like taste (+++, strong; ++, moderate; +, faint but detectable; −, not detectable). [d]Traces (<0.01 mg g^{-1} of fresh weight). [e]Not quantified (<0.01 mg g^{-1} of fresh weight).

and formation of these sulfur compounds is important from several points of view. They are responsible for the attractive alliaceous aroma of these scientifically overlooked plants and may thus be of interest to the food industry still seeking novel sources of unusual aromas to satisfy increasing consumer demands. Furthermore, some of these sulfur compounds could also exhibit interesting biological properties, as indicated by the traditional usage of these plants in folk medicine.

MATERIALS AND METHODS

General Methods. HPLC separations were performed on a Dynamax SD-210 binary pump system (Varian, Palo Alto, CA), employing a Varian PDA 335 detector and a C-18 column (Rainin Microsorb-MV 100 Å, 250 × 4.6 mm, 5 μm). For the isolation of marasmicin, a preparative C-18 column (Rainin Microsorb-MV 100 Å, 250 × 21.4 mm, 5 μm) was used. GC analyses were conducted on a Varian 3800 chromatograph (Varian, Palo Alto, CA), equipped with a Varian 4000 MS detector and an HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Agilent Technologies, Santa Clara, CA). Mass spectra were obtained by EI ionization at 70 eV over the range of 15−350 mass units. For obtaining CI-MS data, acetonitrile was used as the reagent gas. NMR and HPLC−MS experiments were conducted using the instrumentation and settings described in our previous papers.[9,10]

Plant Material. All samples analyzed in this study were obtained from reliable sources, namely, from (A) Dr. Stefan Siebert (North-West University, Potchefstroom, South Africa), (B) the Hoyland Plant Centre (Hoyland, U.K.), (C) Silverhill Seeds (Cape Town, South Africa), (D) the NCCPG National Collection of *Tulbaghia* (Prime

Table 2. S-Substituted Cysteine *S*-Oxides Found in Subfamily Gilliesioideae Species

			relative proportion (%)				
genus	species	origin[a]	methiin (**1**)	marasmin (**11**)	total content (mg g^{-1} of fresh weight)	odor[b]	taste[c]
Ancrumia	*An. cuspidata* Harv. ex Baker	F			nd[d]	−	−
Gethyum	*G. atropurpureum* Phil.	E			nd	−	−
Gilliesia	*Gi. graminea* Lindl.	G			nd	−	−
Ipheion	*I. dialystemmon* Guagl.	E	0	100	0.02	−	−
	I. recurvifolium Traub.	E	tr[e]	tr	nq[f]	−	−
	I. uniflorum (Lindl.) Raf.	E	2	98	1.33 ± 0.18	+	+
Leucocoryne	*L. alliacea* Lindl.	H	tr	100	2.53 ± 0.36	+++	+++
	L. appendiculata Phil.	I	tr	100	0.08 ± 0.02	−	−
	L. coquimbensis Phil.	H	tr	100	2.70 ± 0.22	+++	+++
	L. ixioides (Sims) Lindl.	H	tr	100	2.28 ± 0.26	+++	++
	L. purpurea Gay	H	64	36	0.02	−	−
	L. talinensis Mansur	H	tr	100	3.51 ± 0.35	++	++
	L. violacescens Phil.	H	100	tr	0.03 ± 0.01	−	−
	L. vittata Ravenna	H	tr	tr	nq	−	−
Miersia	*M. tenuiseta* Ravenna	G	tr	tr	nq	−	−
Nothoscordum	*N. bivalve* (L.) Britton	J	100	0	0.03 ± 0.01	−	−
	N. felliponei Beauverd	E	100	0	nq	−	−
	N. inodorum (Aiton) Nicholson	J			nd	−	−
	N. montevidense var. *latipetalum*	E	100	tr	0.02	−	−
	N. montevidense var. *minarum*	E	100	tr	0.09 ± 0.02	−	−
	N. striatellum (Lindl.) Kunth	E	0	100	0.11 ± 0.02	−	−
Pabellonia	*Pa. incrassata* Phil.	E	tr	tr	nq	−	−
Solaria	*S. miersioides* Phil.	G	tr	tr	nq	−	−
Speea	*Sp. humilis* (Phil.) Loes.	G	49	51	0.02	−	−
Tristagma	*Tr. nivale* Poepp.	E	52	48	0.08 ± 0.01	−	−
	Tr. sessile (Phil.) Traub	G	tr	tr	nq	−	−
Zoellnerallium	*Z. andinum* (Poepp.) Crosa	E			nd	−	−

[a]See the Materials and Methods for details. [b]Intensity of the garlic-like odor emitted upon crushing the bulbs (+++, strong; ++, moderate; +, faint but detectable; −, not detectable). [c]Intensity of the garlic-like taste (+++, strong; ++, moderate; +, faint but detectable; −, not detectable). [d]Not detected (<1 μg g^{-1} of fresh weight). [e]Traces (<0.01 mg g^{-1} of fresh weight). [f]Not quantified (<0.01 mg g^{-1} of fresh weight).

Perennials Nursery, Llanilar, Aberystwyth, U.K.), (E) the NCCPG National Collection of *Ipheion* (Ian Hunt, Morton, Derbyshire, U.K.), (F) Inelia Escobar (Universidad de Concepción, Chile), (G) Nicolás García (University of Florida, Gainesville, FL), (H) the *Leucocoryne* collection of Dr. Leví Mansur (Pontificia Universidad Católica de Valparaíso, Chile), (I) Telos Rare Bulbs (Ferndale, CA), and (J) the Botanical Garden at Kew (U.K.). The origin of each sample is indicated in Tables 1 and 2. Voucher specimens are still cultivated in the Alliaceae species collection at the University of South Bohemia and can be accessed upon request. Rhizomes of *Prototulbaghia* and *Tulbaghia* species were analyzed, whereas bulbs were analyzed in the case of all other samples.

Reference Compounds. The possible presence of the following 21 derivatives was monitored: *S*-methyl-, *S*-ethyl-, *S*-propyl-, *S*-isopropyl-, *S*-allyl-, (*E*)-*S*-(1-propenyl)-, (*Z*)-*S*-(1-propenyl)-, *S*-propargyl-, *S*-butyl-, *S*-isobutyl-, *S*-(*sec*-butyl)-, (*E*)-*S*-(1-butenyl)-, (*E*)-*S*-(2-butenyl)-, (*Z*)-*S*-(2-butenyl)-, *S*-(3-butenyl)-, *S*-pentyl-, *S*-((methylthio)methyl)-, *S*-phenyl-, *S*-benzyl-, *S*-(2-pyrrolyl)-L-cysteine *S*-oxide, and *S*-(2-pyridyl)-L-cysteine *N*-oxide. These compounds were synthesized/isolated and fully characterized by spectral methods as described in our previous reports.[4,6,7,9−11]

Isolation and Derivatization Procedures. Amino acid containing fractions were obtained according to the method of Kubec and Dadáková.[13] Typically, about 5−10 g of carefully cleaned bulbs/rhizomes was homogenized in 150 mL of MeOH/H_2O/HCl (90/10/1, v/v/v) by using a tissue homogenizer. The homogenate was allowed to gently boil under reflux for 5 min, filtered, and repeatedly extracted with another 150 mL portion of MeOH/H_2O/HCl (90/10/1, v/v/v). The combined extracts were concentrated at reduced pressure to approximately 10−15 mL and stored at −28 °C until derivatization.

Dansyl derivatives were prepared and analyzed by HPLC as described by Kubec and Dadáková.[13] Phenyl isothiocyanate (PITC) derivatives of isoalliin and marasmin were obtained and analyzed by HPLC according to Lancaster et al.[18] For GC−MS determination, pH of the extract was adjusted to 2.5−3.0 and an aliquot of 3 mL was passed through a column (1 × 5 cm) of a cation-exchange resin (Dowex 50WX4, H^+ form, 50−100 mesh). After the column was washed with 10 mL of 1% HCl and 20 mL of H_2O, the amino acids were eluted with 50 mL of 1 M NH_4OH. The eluate was evaporated to dryness, and the residue was derivatized by ethyl chloroformate and analyzed by GC−MS as described by Kubec and Dadáková.[13]

Identification of the amino acids was achieved by matching of mass spectra and retention times of components in sample extracts with those of authentic standards and/or spiking sample extracts with standards. The quantification was done relative to the internal standard of *S*-isobutylcysteine *S*-oxide, which was added to the samples before homogenization. All analyses were conducted in triplicate.

Isolation of Marasmicin (13). Marasmicin was isolated according to the method of Kubec et al.[11] with some modifications. Freeze-dried rhizomes (37 g) of *T. violacea* were finely pulverized and mixed with water (800 mL), and the homogenate was allowed to stand at room temperature for 30 min. The slurry was then filtered and extracted with CH_2Cl_2 (2 × 500 mL). After centrifugation, the organic layers were combined and dried over $MgSO_4$, and the solvent was evaporated (25 °C). The oily residue was redissolved in acetonitrile, filtered through a syringe-tip PTFE filter (0.45 μm), and subjected to preparative HPLC with H_2O (solvent A) and acetonitrile (solvent B) as the mobile phase. The gradient was as follows, with a flow rate of 18 mL min^{-1}: A/B, 80/20 (0 min), 60/40 (in 16 min), 15/85 (in 20 min), 15/85 (in 30 min), and 80/20 (in 35 min). The fractions eluting at 15.2 min were collected, combined, and evaporated, affording 102

dx.doi.org/10.1021/jf305179p | J. Agric. Food Chem. 2013, 61, 1335−1342

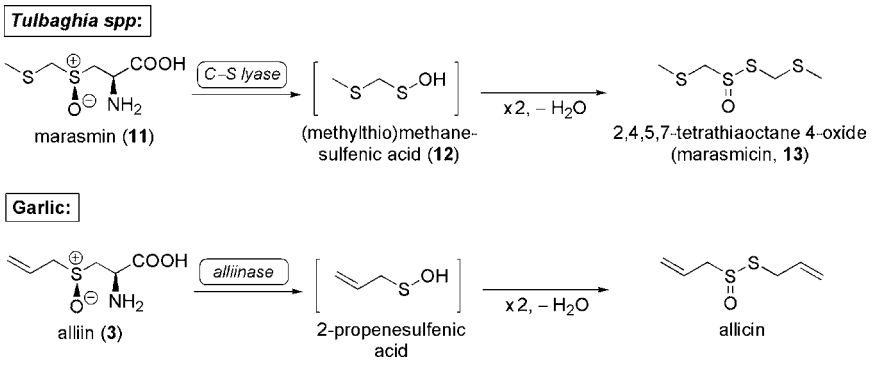

Figure 2. Enzyme-mediated formation of marasmicin from marasmin (above) and allicin from alliin (below).

mg of yellow viscous oil. On the basis of HPLC analysis, the purity of **13** was >98%. ^{1}H and ^{13}C NMR, ESI-MS, and UV spectra of marasmicin are given in the Supporting Information.

Sensory Evaluation of Plant Samples. A 2 g sample of fresh rhizomes (Tulbaghioideae) or peeled bulbs (Gilliesioideae) was finely bruised using a mortar. The odor emitted was immediately evaluated by five panelists who distinguished four levels of the odor intensity (not detectable, faint but detectable, moderate, and strong). For comparison, the intensity of odor emitted upon crushing a clove of garlic (*A. sativum*) was considered as strong. After the odor evaluation was finished, the bruised sample was divided into 100 mg portions that were administered to the panelists for taste analysis. As a reference, the intensity of garlicky taste of a crushed garlic clove (100 mg) was considered as strong. The garlic breath was evaluated 2 h after the ingestion of samples by two individuals who had not consumed them. The panelists did not consume any meal or drink during the 2 h evaluation period. In the case of sensory-active species, an at least 4 h delay was held between two evaluations.

Sensory Analysis of Marasmicin (13). Purified marasmicin was dissolved in 0.1 mL of ethanol and added to water (1 L). After being stirred for 10 min, this stock solution was diluted stepwise with water (1/1, v/v) and stirred for 5 min after each dilution step. The solutions were presented in glass beakers (100 mL) each containing 20 mL of liquid. The samples were evaluated in order of decreasing concentration. The taste threshold was determined by triangle tests by a panel of eight individuals experienced in sensory analysis.

Isolation and GC–MS Analysis of *Tulbaghia* Volatiles. Fresh rhizomes of *T. violacea* (25 g) were homogenized in 250 mL of H_2O using a blender. The slurry was placed in a 1 L round-bottomed flask equipped with a condenser and heated under reflux for 30 min. After the slurry was cooled to room temperature, the homogenate was filtered through a cheesecloth, and the filtrate was extracted with Et_2O (2 × 200 mL). The combined organic portions were dried over $MgSO_4$, and the extract was carefully concentrated using a Vigreaux column to 10 mL. The extract obtained was analyzed by GC–MS, emloying the following operating conditions: injector and detector temperatures of 180 and 250 °C, respectively; a helium carrier gas flow rate of 1.3 mL min^{-1}; a temperature linear gradient from 40 (3 min hold) to 250 °C at 4 °C min^{-1}.

RESULTS AND DISCUSSION

Flavor Precursors. In this study, 18 different Alliaceae subfamily Tulbaghioideae species native to South Africa were analyzed for the presence of *S*-substituted cysteine flavor precursors. Except for *Tulbaghia ludwigiana*, rhizomes of all samples analyzed contained (R_C,R_S)-*S*-((methylthio)methyl)-cysteine 4-oxide (**11**, marasmin) in amounts varying between 0.01 and 1.68 mg g^{-1} of fresh weight (Table 1). Marasmin was found to be the principal cysteine derivative present in these plants, accounting for 93–100% of the pool. Only a few *Tulbaghia* species (*T. capensis*, *T. cernua*, and *T. violacea* var. *violacea*) also contained minor quantities (5–7% of the pool) of (R_C,S_S)-*S*-methylcysteine *S*-oxide (**1**, methiin) (Table 1).

Furthermore, 24 Alliaceae subfamily Gilliesioideae species native to South America were evaluated. It was observed that most plants contained detectable quantities (i.e., >0.01 mg g^{-1} of fresh weight) of two S-substituted cysteine derivatives, namely, **1** and **11**. However, only some members of the *Leucocoryne* and *Ipheion* genera were found to accumulate significant amounts of these amino acids (between 1.33 and 3.51 mg g^{-1} of fresh weight), with marasmin being the principal derivative accounting for 98–100% of the pool (Table 2).

It should be mentioned that five *Leucocoryne* species were already analyzed for the presence of S-substituted cysteine *S*-oxides by Lancaster et al.[18] They reported that all five samples contained **1** and three of them also isoalliin (**4**) in total amounts varying between 0.41 and 3.2 mg g^{-1} of fresh weight. Isoalliin is the major flavor precursor present in onion, and it is responsible for its typical sensory properties. However, none of the *Leucocoryne* samples we analyzed contained detectable levels of this amino acid. Lancaster et al.[18] analyzed the samples by an HPLC method employing precolumn derivatization by PITC, and the identification of compounds was based on matching of retention times of standards with those of analytes present in the extracts (without verification by MS). However, we found out that both PITC-derivatized marasmin and isoalliin had nearly identical retention times when analyzed by the same HPLC method used by Lancaster et al.[18] We therefore suspect that they misidentified **11** with **4** and thus the reported identification of isoalliin in *Leucocoryne* species was most likely erroneous. This assumption is also supported by GC–MS analysis of *Leucocoryne ixioides* volatiles, revealing a total absence of 1-propenyl-containing sulfur compounds. Instead, various marasmin-derived (methylthio)methyl-containing volatiles were detected by GC–MS (not shown).

Another discrepancy between our results and literature data was observed in the case of *I. uniflorum*. We detected substantial amounts of **11** (1.33 mg g^{-1} of fresh weight) in *I. uniflorum*, accompanied by minor quantities of **1** (Table 2). On the other hand, Tsuno[20] reported the presence of another cysteine derivative, ethiin (**2**), in this species. He based his finding on TLC analysis of an *I. uniflorum* extract after the reaction with thiamine. Given the apparent limitations of the TLC method used by Tsuno, it is likely that he probably misidentified ethiin with some other compound present in the extract. However, the possibility that ethiin was indeed present in the sample he analyzed cannot be completely ruled out.

Thiosulfinate Profiles. As described in our previous papers,[10,11] **11** is enzymatically cleaved by a C–S lyase, yielding

(methylthio)methanesulfenic acid (**12**). The enzymatically catalyzed formation of this highly reactive, short-lived compound from **11** was recently confirmed by DART-MS.[10] Two molecules of this sulfenic acid subsequently condense to give the thiosulfinate marasmicin (2,4,5,7-tetrathiaoctane 4-oxide, **13**).[11] This formation pathway of marasmicin from marasmin is virtually analogous to that of allicin from alliin (**3**) in garlic (Figure 2).[12]

The presence of only a single S-substituted cysteine *S*-oxide precursor (i.e., marasmin) in most analyzed species results in exceptionally simple profiles of thiosulfinates formed upon tissue disruption. Indeed, marasmicin was found to be the predominant compound present in a CH_2Cl_2 extract of freshly homogenized *T. violacea* rhizomes (Figure 3). Such a simple

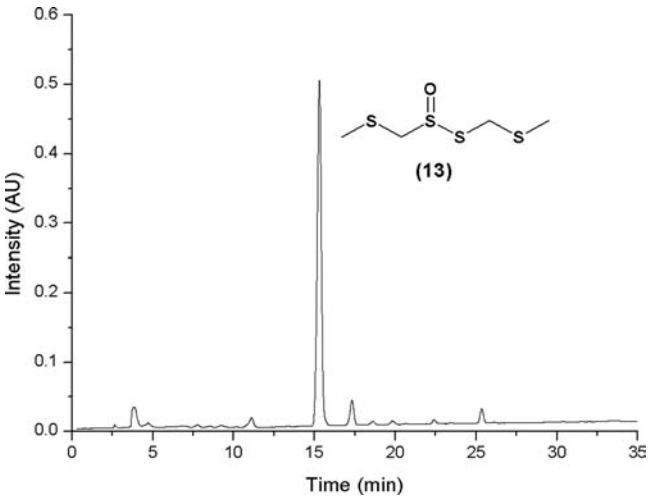

Figure 3. HPLC chromatogram of a dichloromethane extract of freshly homogenized *T. violacea* rhizomes.

thiosulfinate profile is remarkably different from those observed in garlic, onion, and other *Allium* species which typically consist of 8−12 various derivatives (including regiomers and stereomers).[22] Consistent with earlier reports,[7,12,22] HPLC−MS analysis did not reveal the presence of (methylthio)methyl oligosulfides in freshly homogenized plants. It can therefore be assumed that these compounds are formed by degradation of marasmicin as discussed below.

Organoleptic Properties. Marasmicin (**13**), an analogue of the alliin-derived thiosulfinate allicin from garlic, is expected to be the pungent principle of freshly comminuted *T. violacea* tissue.[11] In accordance with this assumption, the content of its precursor **11** in the samples analyzed correlated positively with the intensity of the alliaceous odor emitted upon bruising of their rhizomes (Table 1). In particular, *Tulbaghia* species *T. alliacea*, *T. capensis*, *T. leucantha*, *T. natalensis*, and *T. violacea* (including all of its varieties), when bruised, produced a very pungent garlicky odor. This odor was however markedly different from that of garlic. Interestingly, several *Tulbaghia* species (*T. acutiloba*, *T. cernua*, and *T. simmleri*) did not emit a detectable alliaceous odor, despite the fact they contained moderate amounts of marasmin (0.01−0.22 mg g^{-1} of fresh weight). We assume that the activity of the C−S lyase acting upon marasmin was very low in these three species, resulting in only a very limited formation of the pungent thiosulfinate **13**. Indeed, no significant levels of marasmicin were detected in CH_2Cl_2 extracts of these three species by HPLC.

Out of the plants native to South America, the formation of an alliaceous smell upon crushing the bulbs was detected only in four *Leucocoryne* species (*L. alliacea*, *L. coquimbensis*, *L. ixioides*, and *L. talinensis*) and *I. uniflorum* (Table 2). The smell was quite similar to that produced by *Tulbaghia* and *Prototulbaghia* species.

The content of **11** was also proportional to the intensity of the garlicky taste of the plants analyzed (Tables 1 and 2). It seems reasonable to assume that the compound primarily responsible for the sharp garlicky taste of these species is **13**. We thus isolated this sulfur-rich thiosulfinate from homogenized rhizomes of *T. violacea* by preparative HPLC and evaluated its organoleptic properties. The smell of this unusual thiosulfinate is quite pleasant, garlic-like, and sulfury with meat-like tones. The taste of marasmicin at high concentrations (200−500 ppb) could be described as very pungent and garlic-like, whereas at lower levels (10−50 ppb) was assessed as garlic-like with cooked kohlrabi-like tones. We determined the taste detection threshold in water for this compound to be 10 ppb. For example, the detection levels for analogous methyl/allyl/1-propenyl/propyl thiosulfinates from garlic and onion were reported to vary between 10 and 500 ppb, with that for allicin being 100 ppb.[23] Marasmicin is thus somewhat more sensory potent than allicin, the major taste and flavor principle of freshly crushed garlic. Chewing of rhizomes or bulbs of the marasmin-rich species also resulted in a strong "garlic breath" which usually persisted for several hours. Thus, none of the plants traditionally named as "sweet garlic", "society garlic", "wild garlic", or "bad-breath-free garlic" (some *Tulbaghia* species or *I. uniflorum*) deserve these trivial names.

It should also be mentioned that all species analyzed in this study were devoid of lachrymatory (tear-inducing) properties typically observed upon crushing of onion (*A. cepa*), *Allium* subg. *Nectaroscordum* species (*A. siculum* and *A. tripedale*), or the neotropical plant *Petiveria alliacea* L.[7,24,25] These sensory observations imply that no lachrymatory sulfines (RCH═S═

Table 3. Sulfur-Containing Volatiles Formed upon Boiling of *T. violacea* Rhizomes

compd no.	compd name	formula	KI[a]	rel amt[b] (%)
14	dimethyl disulfide[c]	CH_3SSCH_3	745	2.4 ± 0.6
15	(methylthio)methanethiol[c]	CH_3SCH_2SH	798	0.2 ± 0.1
16	dimethyl trisulfide[c]	CH_3SSSCH_3	972	0.9 ± 0.2
17	2,3,5-trithiahexane[c]	$CH_3SSCH_2SCH_3$	1146	20.1 ± 3.2
18	2,3,4,6-tetrathiaheptane[d]	$CH_3SSSCH_2SCH_3$	1351	3.2 ± 0.4
19	2,4,5,7-tetrathiaoctane[c]	$CH_3SCH_2SSCH_2SCH_3$	1490	38.5 ± 4.7
20	2,3,4,6,8-pentathianonane[d]	$CH_3SSSCH_2SCH_2SCH_3$	1684	3.3 ± 0.8

[a]Kovats indices on an HP-5 column. [b]Relative percentage based on the total peak area. [c]Identity confirmed by comparison with an authentic sample. [d]Tentatively identified by interpretation of MS data.

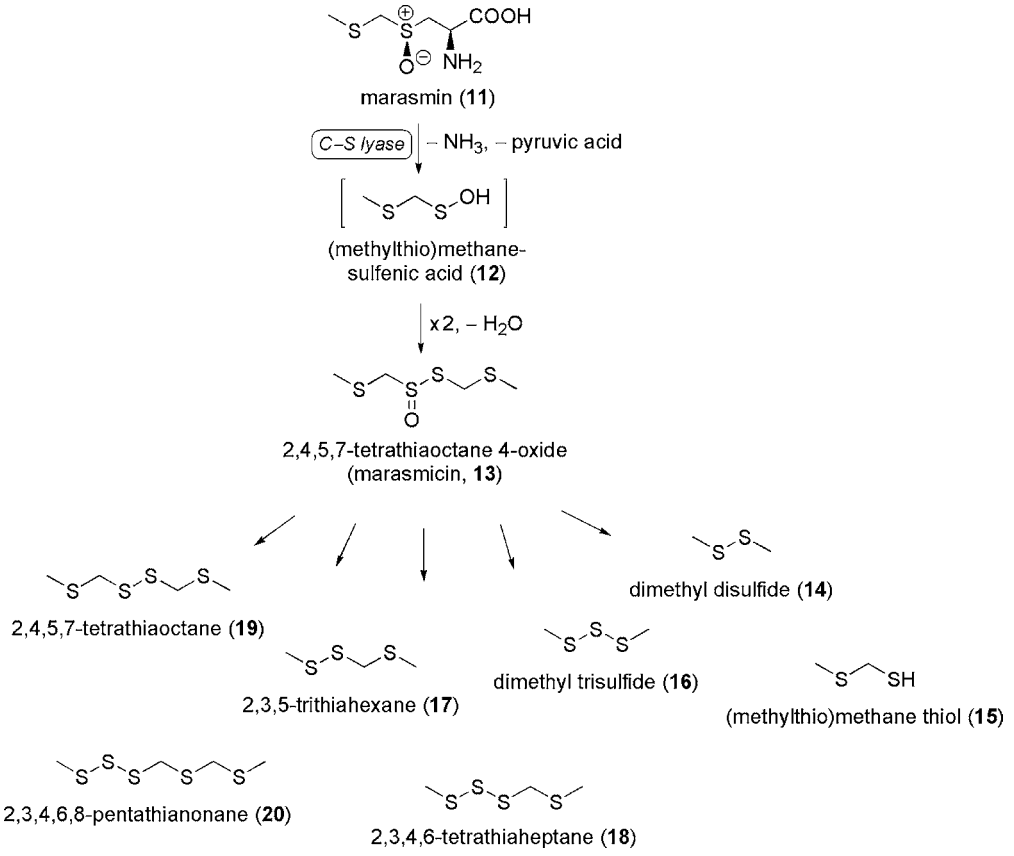

Figure 4. Formation of volatile sulfur compounds upon boiling of *T. violacea* rhizomes.

Table 4. Distribution of S-Substituted Cysteine Derivatives in Alliaceae Species

		S-substituted cysteine derivative[a]										
subfamily	(sub)genus	1	2	3	4	5	6	7	8	9	10	11
Allioideae	*Allium*	+	±[b]	+	+	+	+[c]					
	Caloscordum	+								+		
	Melanocrommyum	+								+[d]	+[d]	+
	Nectaroscordum	+						+	+			
Tulbaghioideae	*Prototulbaghia*											+
	Tulbaghia	±										+
Gilliesioideae	*Ipheion*	±										+
	Leucocoryne	±										+

[a]For structures see Figure 1. [b]Only a trace component. [c]Detected so far only in *A. cepa* var. *tropeana*.[5] [d]Compounds **9** and **10** are not present concurrently in a single species.[10,37]

O) are formed in these species by a concerted action of C−S lyase and lachrymatory factor synthase on a cysteine derivative following tissue disruption.[26−28]

Volatile Compounds Formed upon Boiling. Fresh rhizomes of *T. violacea* were homogenized and boiled in water (30 min) to simulate a typical cooking treatment. The volatiles formed were isolated by solvent extraction and analyzed by GC−MS. The number of sulfur compounds present in the extract at significant levels (>0.3% of the total peak area) was relatively low. 2,3,5-Trithiahexane (**17**) and 2,4,5,7-tetrathiaoctane (**19**) were found to be the major components accounting for 20.1% and 38.5% of the total peak area, respectively (Table 3). Minor sulfur volatiles identified in the extract were dimethyl disulfide (**14**), (methylthio)methanethiol (**15**), dimethyl trisulfide (**16**), 2,3,4,6-tetrathiaheptane (**18**), and 2,3,4,6,8-pentathianonane (**20**). It is noteworthy that most of the compounds detected in the extract were acyclic oligosulfides containing the CH_3SCH_2- moiety (mass spectra of these compounds always contained a very intense ion, m/z 61). As none of these sulfur volatiles were detected in freshly homogenized *T. violacea* tissue, it is very likely that they were formed by thermally induced decomposition of **13** (Figure 4). Given the profound thermal instability of many sulfur compounds, it cannot be ruled out that some of the volatiles detected in the extract were only artifacts formed during GC−MS analysis (e.g., in the injector). Sensory properties of the two major sulfur volatile components (**17** and **19**) were already evaluated in detail by Kubota and Kobayashi.[29] They determined the odor threshold values in water for these compounds to be 40 and 3.5 ppb, respectively. They reported that the odor of **17** resembled aged

garlic or onion, whereas that of **19** was onion-like and somewhat irritating.

Rapior et al.[30] found similar sulfur compounds among the volatiles isolated from the "garlic mushroom" *Marasmius alliaceus* Fr., which is known to contain γ-glutamyl-*S*-((methylthio)methyl)cysteine 4-oxide (i.e., γ-glutamylmarasmin).[31] Interestingly, the absolute configuration around the sulfur in this dipeptide is opposite that observed in marasmin from Alliaceae species analyzed in this study.[11,32] Similar profiles of volatile sulfur compounds were also observed in several "garlic trees", e.g., *Scorodophloeus zenkeri* Harms. and *Hua gabonii* Pierre ex De Wild. from Central Africa, *Scorodocarpus borneensis* Becc. from Malaysia, and *Gallesia gorazema* (Vell.) Moq. from South America. The bark and fruits of these trees (sometimes referred to as "wood garlic") are locally used for seasoning of food.[33−36] It is therefore very likely that marasmin is the precursor of the sulfur volatiles found in these trees. That would indicate that **11** is much more widespread in nature than had originally been expected.

Taxonomic Implications. Our findings also have implications for the taxonomy of Alliaceae species. As shown in Table 4, distribution of S-substituted cysteine derivatives **1**−**11** in Alliaceae plants significantly correlates with the current taxonomic classification of this family. **1** seems to be present abundantly in all Allioideae species, including members of the *Allium* subgenera *Melanocrommyum*, *Caloscordum*, and *Nectaroscordum*.[4,6−10,13−17,37,38] On the contrary, the present study revealed that Tulbaghioideae and Gilliesioideae plants accumulate **1** only at trace levels (if at all) and **11** is the principal derivative present in these species. Furthermore, the three-carbon side chain derivatives [i.e., **3**, **4**, and propiin (**5**)] are present only in *Allium* species, excluding members of the subgenera *Nectaroscordum*, *Caloscordum*, and *Melanocrommyum* which do not contain **3**−**5** at significant levels.[6−10,37,38] On the other hand, subgenus *Nectaroscordum* plants (i.e., *A. siculum* and *A. tripedale*) are significantly distinct from all other alliaceous species in their ability to synthesize two four-carbon side chain derivatives, namely, *S*-butyl- and (*E*)-*S*-(1-butenyl)cysteine *S*-oxides (**7** and **8**, butiin and homoisoalliin, respectively).[6−8] Finally, subgenus *Melanocrommyum* species are characterized by the presence of **11** and two unusual heterocyclic derivatives, i.e., *S*-(2-pyrrolyl)cysteine *S*-oxide (**9**) and *S*-(2-pyridyl)cysteine *N*-oxide (**10**).[9,10,37]

Conclusion. It can be concluded that several marasmin-rich *Tulbaghia* species (in particular, *T. alliacea*, *T. capensis*, *T. leucantha*, *T. natalensis*, and *T. violacea*) deserve much greater attention from the food industry. Thanks to their unusual, powerful yet pleasant garlic-like taste and odor, these plants (either fresh or dried) could find numerous applications in the food industry as an alternative to garlic and chive. Furthermore, *Tulbaghia* species are unassuming, perennial, easily cultivated plants that survive outdoors in most areas of the northern hemisphere or can be grown indoors year-round. On the other hand, the Alliaceae species native to South America (members of the subfamily Gilliesioideae) do not seem to have any appreciable value for the food industry as most of them do not exhibit interesting sensory properties. Only some members of the *Leucocoryne* and *Ipheion* genera emit alliaceous odor. However, these leafy bulbous plants are rather slowly growing and tender and thus more difficult to cultivate in the northern hemisphere. These plants, prized for their attractive and long-lasting inflorescences, seem to have a much better applicability in landscaping and production of cut flowers.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR, ^{13}C NMR, UV, and ESI-MS/MS spectra of marasmicin. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +420-38-7772664. Fax: +420-38-5310405. E-mail: kubecr@centrum.cz, kubecr@yahoo.com.

Funding

Financial support of the Grant Agency of the University of South Bohemia (Grants GAJU 067/2010/Z and GAJU 068/2011/P) is greatly appreciated.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Ian Hunt (Morton, Derbyshire, U.K.), Liz Powney (Prime Perennials Nursery, Llanilar, Aberystwyth, U.K.), Dr. Stefan Siebert (North-West University, Potchefstroom, South Africa), Inelia Escobar (Universidad de Concepción, Chile), and the Botanical Garden at Kew (U.K.) for providing some of the research material. Our thanks also belong to Dr. Jan Schraml (Institute of Chemical Process Fundamentals, Prague, Czech Republic) and Pavla Kružberská (Biology Centre, České Budějovice, Czech Republic) for their helpful technical assistance.

■ ABBREVIATIONS

CI, chemical ionization; DART-MS, direct analysis in real time mass spectrometry; EI, electron impact; ESI, electrospray ionization; GC−MS, gas chromatography−mass spectrometry; HPLC, high-performance liquid chromatography; HPLC−MS, high-performance liquid chromatography−mass spectrometry; MS, mass spectrometry; NMR, nuclear magnetic resonance; PDA, photo diode array; PITC, phenyl isothiocyanate; PTFE, poly(tetrafluoroethylene); subg., subgenus; TLC, thin-layer chromatography; UV, ultraviolet

■ REFERENCES

(1) Fay, M. F.; Rudall, P. J.; Chase, M. W. Molecular studies of subfamily Gilliesioideae (Alliaceae). *Aliso* **2006**, *22*, 367−371.

(2) Vosa, C. G. A revised cytotaxonomy of the genus *Tulbaghia* (Alliaceae). *Caryologia* **2000**, *53*, 83−112.

(3) Vosa, C. G. *Prototulbaghia*, a new genus of the Alliaceae family from the Leolo Mountains in Sekhukhuneland, South Africa. *Caryologia* **2007**, *60*, 273−278.

(4) Kubec, R.; Svobodová, M.; Velíšek, J. Distribution of *S*-alk(en)ylcysteine sulfoxides in some *Allium* species. Identification of a new flavor precursor: *S*-Ethylcysteine sulfoxide (ethiin). *J. Agric. Food Chem.* **2000**, *48*, 428−433.

(5) Dini, I.; Tenore, G. C.; Dini, A. *S*-Alkenyl cysteine sulfoxide and its antioxidant properties from *Allium cepa* var. *tropeana* (red onion) seeds. *J. Nat. Prod.* **2008**, *71*, 2036−2037.

(6) Kubec, R.; Kim, S.; McKeon, D. M.; Musah, R. A. Isolation of *S*-*n*-butylcysteine sulfoxide and six *n*-butyl-containing thiosulfinates from *Allium siculum*. *J. Nat. Prod.* **2002**, *65*, 960−964.

(7) Kubec, R.; Cody, R. B.; Dane, A. J.; Musah, R. A.; Schraml, J.; Vattekkatte, A.; Block, E. Applications of direct analysis in real time-mass spectrometry (DART-MS) in *Allium* chemistry. (*Z*)-Butanethial *S*-oxide and 1-butenyl thiosulfinates and their *S*-(*E*)-1-butenylcysteine *S*-oxide precursor from *Allium siculum*. *J. Agric. Food Chem.* **2010**, *58*, 1121−1128.

(8) Kusterer, J.; Keusgen, M. Cysteine sulfoxides and volatile sulfur compounds from *Allium tripedale*. *J. Agric. Food Chem.* **2010**, *58*, 1129−1137.
(9) Kučerová, P.; Kubec, R.; Šimek, P.; Václavík, L.; Schraml, J. *Allium* discoloration: The precursor and formation of the red pigment in giant onion (*Allium giganteum* Regel) and some other subgenus *Melanocrommyum* species. *J. Agric. Food Chem.* **2011**, *59*, 1821−1828.
(10) Kubec, R.; Krejčová, P.; Šimek, P.; Václavík, L.; Hajšlová, J.; Schraml, J. Precursors and formation of pyrithione and other pyridyl-containing sulfur compounds in drumstick onion *Allium stipitatum*. *J. Agric. Food Chem.* **2011**, *59*, 5763−5770.
(11) Kubec, R.; Velíšek, J.; Musah, R. A. The amino acid precursors and odor formation in society garlic (*Tulbaghia violacea* Harv.). *Phytochemistry* **2002**, *60*, 21−25.
(12) Block, E.; Dane, A. J.; Thomas, S.; Cody, R. B. Applications of direct analysis in real time mass spectrometry (DART-MS) in *Allium* chemistry. 2-Propenesulfenic and 2-propenesulfinic acids, diallyl trisulfane *S*-oxide, and other reactive sulfur compounds from crushed garlic and other Alliums. *J. Agric. Food Chem.* **2010**, *58*, 4617−4625.
(13) Kubec, R.; Dadáková, E. Chromatographic methods for determination of *S*-substituted cysteine derivatives—A comparative study. *J. Chromatogr., A* **2009**, *1216*, 6957−6963.
(14) Yoo, K. S.; Pike, L. M. Determination of flavor precursor compound *S*-alk(en)yl-L-cysteine sulfoxides by an HPLC method and their distribution in *Allium* species. *Sci. Hortic. (Amsterdam, Neth.)* **1998**, *75*, 1−10.
(15) Yamazaki, Y.; Iwasaki, K.; Mikami, M.; Yagihashi, A. Distribution of eleven flavor precursors, *S*-alk(en)yl-L-cysteine derivatives, in seven *Allium* vegetables. *Food Sci. Technol. Res.* **2011**, *17*, 55−62.
(16) Fritsch, R. M.; Keusgen, M. Occurrence and taxonomic significance of cysteine sulphoxides in the genus *Allium* L. (Alliaceae). *Phytochemistry* **2006**, *67*, 1127−1135.
(17) Krest, I.; Glodek, J.; Keusgen, M. Cysteine sulfoxides and alliinase activity of some *Allium* species. *J. Agric. Food Chem.* **2000**, *48*, 3753−3760.
(18) Lancaster, J. E.; Shaw, M. L.; Walton, E. F. *S*-Alk(en)yl-L-cysteine sulfoxides, alliinase and aroma in *Leucocoryne*. *Phytochemistry* **2000**, *55*, 127−130.
(19) Burton, S. G.; Kaye, P. T. Isolation and characterisation of sulphur compounds from *Tulbaghia violacea*. *Phytochemistry* **1992**, *58*, 295−296.
(20) Tsuno, S. Studies on the nutritional value of *Allium* plants. XVII. Production of allithiamine from thiamine by the use of *Ipheion uniflorum* Rof. *Bitamin* **1958**, *14*, 665−670.
(21) Lyantagaye, S. L. Ethnopharmacological and phytochemical review of *Allium* species (sweet garlic) and *Tulbaghia* species (wild garlic) from Southern Africa. *Tanzania J. Sci.* **2011**, *37*, 58−72.
(22) Block, E.; Naganathan, S.; Putman, D.; Zhao, S. H. *Allium* chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (ramsoms), leek, scallion, shallot, elephant (great-headed) garlic, chive, and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. *J. Agric. Food Chem.* **1992**, *40*, 2418−2430.
(23) Block, E.; Calvey, E. M.; Gillies, C. W.; Gillies, J. Z.; Uden, P. Organosulfur and -selenium phytochemicals in genus *Allium* plants. In *Functionality of Food Phytochemicals*; Johns, T., Romeo, J. T., Eds.; Plenum Press: New York, 1997; pp 1−30.
(24) Brodnitz, M. H.; Pascale, J. V. Thiopropanal *S*-oxide: A lachrymatory factor in onions. *J. Agric. Food Chem.* **1971**, *19*, 269−272.
(25) Kubec, R.; Kim, S.; Musah, R. A. The lachrymatory principle of *Petiveria alliacea*. *Phytochemistry* **2003**, *63*, 37−40.
(26) Imai, S.; Tsuge, N.; Tomotake, M.; Nagatome, Y.; Sawada, H.; Nagata, T.; Kumagai, H. An onion enzyme that makes the eyes water. *Nature* **2002**, *419*, 685.
(27) Musah, R. A.; He, Q.; Kubec, R. Discovery and characterization of a novel lachrymatory factor synthase (LFS) in *Petiveria alliacea* and its influence on alliinase-mediated formation of biologically active organosulfur compounds. *Plant Physiol.* **2009**, *151*, 1294−1303.
(28) He, Q.; Kubec, R.; Jadhav, A.; Musah, R. A. First insights into the mode of action of a "lachrymatory factor synthase"—Implications for the mechanism of lachrymator formation in *Petiveria alliacea*, *Allium cepa* and *Nectaroscordum* species. *Phytochemistry* **2011**, *72*, 1939−1946.
(29) Kubota, K.; Kobayashi, A. Sulfur compounds in wood garlic (*Scorodocarpus borneensis* Becc.) as versatile food components. In *Sulfur Compounds in Foods*; Mussinan, C. J., Keelan, M. E., Eds.; American Chemical Society: Washington, DC, 1994; pp 238−246.
(30) Rapior, S.; Breheret, S.; Talou, T.; Bessière, J. M. Volatile flavor constituents of fresh *Marasmius alliaceus* (garlic *Marasmius*). *J. Agric. Food Chem.* **1997**, *45*, 820−825.
(31) Gmelin, R.; Luxa, H. H.; Roth, K.; Höfle, G. Dipeptide precursor of garlic odour in *Marasmius* species. *Phytochemistry* **1976**, *15*, 1717−1721.
(32) Broek, L. A. G. M.; Breuer, M. L.; Liskamp, R. M. J.; Ottenheijm, H. C. J. Total synthesis and absolute configuration of the natural dipeptide γ-glutamylmarasmine. *J. Org. Chem.* **1987**, *52*, 1511−1517.
(33) Kubota, K.; Hirayama, H.; Sato, Y.; Kobayashi, A.; Sugawara, F. Amino acid precursors of the garlic-like odour in *Scorodocarpus borneensis*. *Phytochemistry* **1998**, *49*, 99−102.
(34) Barbosa, L. C. A.; Demuner, A. J.; Teixeira, R. R.; Madruga, M. S. Chemical constituents of the bark of *Gallesia gorazema*. *Fitoterapia* **1999**, *70*, 152−156.
(35) Jirovetz, L.; Buchbauer, G.; Ngassoum, M. B.; Geissler, M. Analysis of the headspace aroma compounds of the seeds of the Cameroonian "garlic plant" *Hua gabonii* using SPME/GC/FID, SPME/GC/MS and olfactometry. *Eur. Food Res. Technol.* **2002**, *214*, 212−215.
(36) Kouokam, J. C.; Zapp, J.; Becker, H. Oxygen-containing sulfur-rich compounds from the bark of the tropical garlic tree *Scorodophloeus zenkeri* Harms. *Phytochemistry* **2002**, *60*, 403−407.
(37) Kusterer, J.; Fritsch, R. M.; Keusgen, M. *Allium* species from Central and Southwest Asia are rich sources of marasmin. *J. Agric. Food Chem.* **2011**, *59*, 8289−8297.
(38) Kubec, R. *S*-Methylcysteine *S*-oxide (**1**) and *S*-(2-pyrrolyl)-cysteine *S*-oxide (**9**) were found as the only cysteine derivatives in the bulbs of *Caloscordum neriniflorum* in the ratio of 37/63. Unpublished results.