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Allium Species from Central and Southwest Asia Are Rich Sources of Marasmin

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ABSTRACT: Marasmin, which is especially known from the two South African species *Tulbaghia alliacea* and *Tulbaghia violacea*, but was also described for the garlic mushroom *Marasmius alliaceus*, is the precursor of the thiosulfinate marasmicin. Marasmicin has attracted considerable attention because of its antifungal and tuberculostatic activities. However, many *Allium* species of the subgenus *Melanocrommyum*, especially *Allium suworowii*, are also very rich in marasmin. *A. suworowii* revealed concentrations of marasmin up to 1.6%, related to the fresh weight of bulbs, and up to 3.0%, related to air-dried fruiting bodies, of the corresponding γ-glutamylmarsmin was found in *M. alliaceus*. Both species show much higher amounts of marasmin as *Tulbaghia* and could be considered as natural sources for the isolation of this compound. Further promising *Allium* species with considerable amounts of marasmin besides other cysteine sulfoxides are *Allium stipitatum* and *Allium altissimum*. (R_S,R_C)-Marasmin is typical for the investigated species of the subgenus *Melanocrommyum*, whereas γ-glutamyl-(S_S,R_C)-marasmin is the only cysteine sulfoxide for the genus *Marasmius* known until now. Both cysteine sulfoxides were isolated and described as *o*-phthaldialdehyde (OPA) derivatives. Furthermore, the cysteine sulfoxides methiin, propiin, *S*-(2-pyrrolyl)-cysteine sulfoxide, eventually *S*-(2-pyridyl)-cysteine sulfoxide and *S*-(2-pyridyl)-L-cysteine *N*-oxide were found.

KEYWORDS: cysteine sulfoxides, marasmin, *Allium suworowii*, *Allium stipitatum*, *Marasmius alliaceus*, *Allium* subg. *Melanocrommyum*

INTRODUCTION

The plant family Alliaceae is well-known for many bulbous plants carrying a typical garlic smell. Most prominent is the genus *Allium*, which is distributed over the whole northern hemisphere. The taxonomic classification of the genus *Allium* is rather complex and still in progress. Besides classical morphological characterization, DNA internal transcribed spacer (ITS) sequences[1] are used to allow differentiation among more than 850 different species. The genus *Allium* is divided into several subgenera. The rather large and diverse subgenus *Melanocrommyum* is currently reexamined under taxonomic and chemical aspects.[2]

Because many plant species belonging to this subgenus are used as traditional medicinal plants or spicy vegetables,[3] focus was put on the chemical composition of secondary metabolites, mainly cysteine sulfoxides (CSOs). CSOs are precursors of thiosulfinates and further volatile sulfur compounds, which are formed by the action of the enzyme alliinase. The alliinase cleaves the CSOs between the β carbon and the sulfur. In the case of garlic (*Allium sativum*), the direct enzymatic product of the CSO alliin is the unstable alkanesulfenic acid.[4,5] Two molecules will condense to one molecule of thiosulfinate allicin. Thiosulfinates and related compounds show an antibiotic activity.[6] It is most likely that thiosulfinates interact with disulfide bonds and free thiol groups of proteins and enzymes of bacteria and fungi, significantly disturbing the function of many proteins.[7]

The important CSO (R_S,R_C)-marasmin (Figure 1, substance **1**) carrying two sulfur atoms and a methylthiomethyl moiety as aliphatic residue was first described for *Tulbaghia* species.[8] *Tulbaghia* is naturally distributed in South Africa and is also used as a traditional medicinal plant by the aborigines. Alliinase induces formation of the thiosulfinate marasmicin. Extracts of *Tulbaghia alliacea* were found to be highly active against tuberculosis and fungi.[9,10] Furthermore, marasmin (Figure 1, substance **2**) was identified as its corresponding γ-glutamyl derivative (γ-glutamyl-(S_S,R_C)-marasmin) in different mushroom species belonging to the genus *Marasmius*.[11] The absolute configuration of **2** was determined, and a total synthesis was performed by van den Broeck et al.[12]

CSO **1** can be also expected in the genus *Allium*, especially in the subgenus *Melanocrommyum*.[13] This subgenus is widely used in Central and Southwest Asia. Special dishes, which are much esteemed for strong tonic properties, are prepared from the leaves of three species of the subgenus *Melanocrommyum*:[3] *Allium motor* Kamelin et Levichev (= *Allium tschimganicum* O. Fedtsch.), *Allium rosenbachianum* Regel, and *Allium rosenorum* R.M. Fritsch. These dishes are explicitly consumed because of their tonic properties. These plants form a red dye after wounding, which is regarded as a "sign of authenticity" when leaves are collected in April and May. The term "motor" means "health", but the local names of the other two species are not related to application. Those persons questioned in the local population always denied that these plants are used as spice.

Furthermore, bulbs of *Allium chitralicum* Wang et Tang s. str. are used against a sense of fear, and the whole plant is used for the Tajik national dish "atolla".[3] Fresh or dried leaves of *Allium hissaricum* Vved. are applied against headache and fever. Moreover, *Allium karataviense* Regel should support wound healing and is used against pneumonia and lung problems. As a curious application, *Allium komarowii* Lipsky is used as an anabolic drug for horses in Tajikistan.

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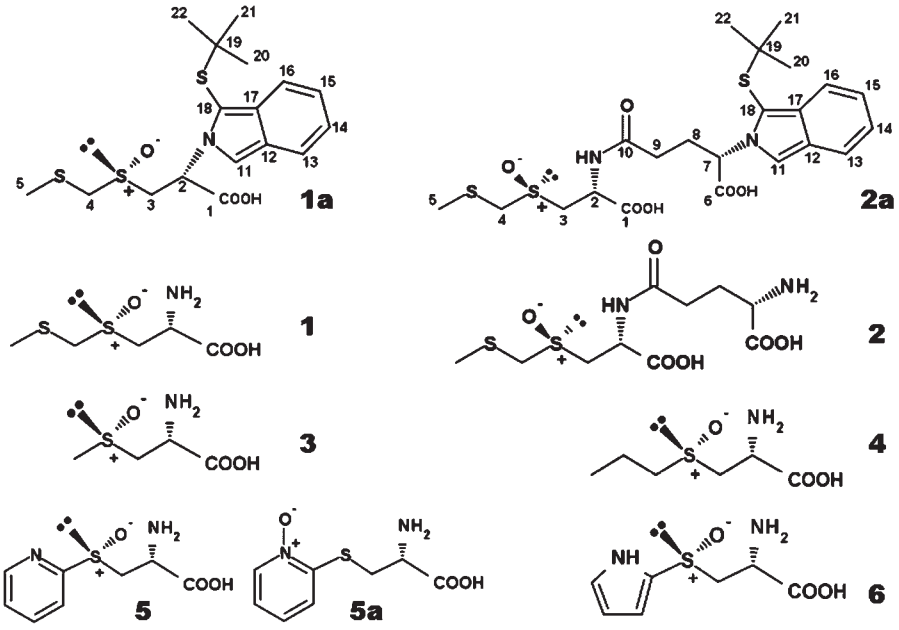

Figure 1. Chemical structures of (R_S,R_C)-marasmin **1**, γ-glutamyl-(S_S,R_C)-marasmin **2**, and their corresponding OPA derivatives **1a** and **2a**, respectively. Additional structures: methiin **3**, propiin **4**, *S*-(2-pyridyl)-L-cysteine sulfoxide **5**, *S*-(2-pyridyl)-L-cysteine *N*-oxide **5a**, and *S*-(2-pyrrolyl)-L-cysteine sulfoxide **6**.

Pickled young bulbs of *Allium stipitatum* Regel are used mainly as a spicy vegetable.[3] However, young bulbs of *A. suworowii* Regel are identically prepared but also medicinally used. Recently, extracts from *A. stipitatum* were tested on their antibiotic activity. *N*-Oxypyridyl sulfur compounds were tested by O'Donnell et al.[14] against species of *Mycobacterium* and methicillin-resistant *Staphylococcus aureus* (MRSA). *N*-Oxides had a minimum inhibitory concentration (MIC) of 1 μg/mL, whereas the common antibiotic norfloxacin had an MIC of 32 μg/mL.

The goal of this investigation is the analysis of CSOs of *Allium* species belonging to the subgenus *Melanocrommyum*. The investigated plants occur in Central and Southwest Asia. Preliminary investigations showed that CSOs of these plants can be related to volatile sulfur compounds, which are responsible for reported bioactivities.[13] Furthermore, the mushroom *Marasmius alliaceus* (Jacquin: Fr.) Fr. should be investigated for comparison reasons.

MATERIALS AND METHODS

Chemicals. Chemicals of analytical grade were purchased either from Merck (Darmstadt, Germany), Fluka (Deisenhofen, Germany), or Sigma (Munich, Germany). Millipore-grade water was used for all experiments.

Derivatization Reagent *o*-Phthaldialdehyde (OPA Reagent). For derivatization of amino acids and CSOs, 140 mg of OPA was solubilized in 5 mL of methanol under magnetic stirring. Then, 200 μL of 2-methylpropane-2-thiol was added. Afterward, 50 mL of borate buffer (pH 9.5) was slowly mixed with the obtained solution. The reagent was stored for at least 12 h under light protection before usage.

Plant Material. The *Allium* plant material was obtained from Iran (accession numbers starting with 1), Uzbekistan (accession numbers starting with 4), and Tajikistan (accession numbers starting with 6) during several expeditions between 2005 and 2009. Sample 0653h was taken from the living plant collection in IPK Gatersleben. The identification was done by Dr. R. M. Fritsch of IPK Gatersleben, Germany. Also, reference plant material for quantitative analysis was obtained from IPK Gatersleben. Voucher specimens are cultivated in the living *Allium* collections in Tehran (Iran), Tashkent (Uzbekistan), Dushanbe (Tajikistan), and IPK Gatersleben (Germany). For all *Allium* samples, a full record about the place of origin including GPS coordinates exists. The origin of plants is also available via an IPK database (http://www.ipk-gatersleben.de/databases/genetic_resources/allium). *M. alliaceus* material was collected in the state forest of Marburg, Germany, in October 2010 (Figure 2b). Voucher specimens are deposited at the Institute or Pharmaceutical Chemistry, University of Marburg.

Extraction and Derivatization Procedure for Quantitative Analysis. Fresh bulbs or entire air-dried mushrooms were cleaned and sliced into pieces of about 500 mg and exactly weighed. These pieces were instantly placed in 20 mL of methanol and heated at 65 °C under reflux to inhibit enzymatic activity. After 10 min, the pieces were homogenized and extracted again with a mixture of methanol/water (1:1 v/v) by heating under reflux for 10 min with 20 mL of water and the used methanol. The extract was cooled and filtered, and the filter was washed carefully with methanol/water. The filtrate was evaporated until dryness under reduced pressure and stored at −20 °C before further use.

Quantitative Analysis. The extract was dissolved in 4.93 mL of OPA reagent, and 50 μL of 2-methylpropane-2-thiol was added. After reaction for 30 min in darkness at room temperature, 20 μL of iodoacetamide (1 M) was added. For quantitative measurements, 200 μL of the sample was diluted with 800 μL of water to give a final volume of 1.00 mL. For qualitative measurements, 500 μL of the sample was diluted in 500 μL of water. Of this, 20 μL was injected into the HPLC device. A Merck Hitachi 7000 series HPLC system equipped with a VP 250/4 Nucleodur 100-5 C18 EC column (250 mm × 4 mm, Macherey Nagel, Düren, Germany) was used for quantitative analysis. The column oven was set to 30 °C, and the UV detector was set to 334 nm. Calibration of the system was done with L-(+)-alliin standard synthesized according to the method of Koch and Keusgen.[15] The HPLC conditions were as follows: a 50 mmol of phosphate buffer (pH 6.5) (A)/acetonitrile (B) gradient with a constant flow rate of 1 mL/min was used as mobile phase; gradient, 78% A for 20 min; 78−75% A over 29 min; 75% A for 1 min; 75−71% A over 4 min; 71% A for 1 min; 71−68%

Figure 2. Flowers of *Allium suworowii* (a; from Zomin valley, Uzbekistan) and fruiting bodies of *Marasmius alliaceus* (b; Marburg, Germany).

A over 8 min; 68−63% A over 2 min; 63% A for 10 min; 78% A for 10 min. Additionally, HPLC ESI-MS/MS measurements were performed on a Shimadzu LC 20 HPLC system consisting of an autosampler, a high-pressure mixing pump, a column oven, a UV detector (334 nm), and a QTrap 2000 equipped with a TurboIonspray ion source (Applied Biosystems/MDS Sciex, Toronto, Canada). ESI-MS operating conditions for the qualitative analysis of the extracts were as follows: positive ionization mode, scan range 30−1000 amu; source temperature, 200 °C; ion spray voltage, 5.500 V; curtain gas setting, 10; declustering potential, 110 V; entrance potential, 11 V; flow rate, 0.25 mL/min (HPLC separation). The following HPLC conditions were applied: 50 mmol of ammonium acetate buffer (pH 6.5) (A) and acetonitrile (B) with a constant flow rate of 0.25 mL was applied; gradient, isocratic 78% A for 20 min; 78−75% A over 29 min; 75% A for 1 min; 75−71% A over 4 min; 71% A for 1 min; 71−68% A over 8 min; 68−63% A over 2 min; 63% A for 10 min; 78% A for 10 min. A 250/2 Nucleodur 100-5 C18 EC column (250 mm × 2 mm, Macherey Nagel) was used for all experiments. Identification of peaks was based on MS data and spectroscopic data[16−18] and synthesized reference compounds according to ref 15.

Preparative Extraction of OPA-Derivatized (R_S,R_C)-Marasmin (1a). Eight bulbs (4.23 g fresh weight in total) of *A. suworowii* (Figure 2a) were treated by the following method: each bulb was cleaned, sliced, and then transferred into 20 mL of methanol. After extraction under reflux for 10 min, the extract was cooled and homogenized. Another 10 mL of methanol was added, and extraction under reflux was continued for an additional 10 min. After filtration, the solvent was removed under reduced pressure at 30 °C. The obtained residue was redissolved in 4.93 mL of OPA reagent, and 50 μL of 2-methylpropane-2-thiol was added. After 30 min of incubation in the dark, 20 μL of a methanolic iodoacetamide solution (1 M) was added. After 3 min, the solution was filtered through a 0.45 μm cellulose acetate filter. All filtered extracts from individual bulbs were combined. The solvent was carefully evaporated under reduced pressure, and the residue was stored at −20 °C for further processing.

Preparative Extraction of OPA-Derivatized γ-Glutamyl-(S_S,R_C)-Marasmin (2a). Air-dried *M. alliaceus* material (3.96 g fresh weight in total) was divided in four parts and was treated in a similar method as described above. All four parts of the sample were treated in this manner, and extracts were finally combined. The solvent was carefully evaporated under reduced pressure, and the residue was stored at −20 °C for further processing.

Preparative Separation of Compound 1a. The same Waters HPLC system (600 E System controller and a Waters 991 PDA) was used for separation. Preparative HPLC was performed on a VP 250/21 Nucleodur 100-5 C18 EC column (250 mm × 21 mm, Macherey Nagel). A methanol/ammonium acetate buffer (50 μM, pH 6.5) gradient with a constant flow rate of 10 mL/min was applied (A, ammonium acetate buffer; B, methanol); gradient, isocratic 60% A for 30 min; 60−55% A over 10 min; isocratic 55% A for 20 min. UV detection was performed at 334 nm. Compound **1a** eluted at 49 min. The purity of fractions was checked by direct injections into the MS. The collected fractions of the OPA-derivatized CSO were instantly evaporated under reduced pressure and stored at −20 °C.

Preparative Separation of Compound 2a. A Waters HPLC system (600 E system controller and a Waters 991 PDA) was used for separation. Preparative HPLC was performed on a VP 250/21 Nucleodur 100-5 C18 EC column (250 mm × 21 mm, Macherey Nagel). A methanol/ammonium acetate buffer (50 μM, pH 6.5) gradient with a constant flow rate of 10 mL/min was applied (A, ammonium acetate buffer; B, methanol); gradient, isocratic 60% A for 10 min; 60−55% A over 30 min. UV detection was performed at 334 nm. Compound **2a** eluted at 25 min. The purity of fractions was checked by direct injections into the MS. The collected fractions of the OPA-derivatized CSO were instantly evaporated until dryness and stored at −20 °C. Obtained yields were 6.8 mg for **1a** and 7.4 mg for **2a**.

Structure Elucidation. ESI-MS/MS measurements were conducted using a Shimadzu LC20 HPLC system containing an autosampler, a high-pressure mixing pump, a column oven, and a UV detector in combination with a QTrap 2000 equipped with a TurboIonspray ion source (Applied Biosystems/MDS Sciex). The ESI-MS operating conditions for qualitative determination of OPA-derivatized CSOs were positive ion mode or negative ion mode; scan range, 30−1000 amu; source temperature, 200 °C; ion spray voltage, 5.5 kV; curtain gas, 10; declustering potential, 80 or 110 V; entrance potential, 10 V; and flow rate, 10 μL/min (direct infusion) or 0.25 mL/min (HPLC separation). ESI-MS operating conditions for fragmentation experiments were positive ion or negative ion mode; scan range, 30−1000 amu; source temperature, off; ion spray voltage, 5.5 kV; curtain gas, 10; declustering potential, 110 V; entrance potential, 10 V; collision energy setting, 22−52; collision cell entrance potential, 25.42 V; collision cell exit potential, 3 V; and flow rate, 10 μL/min (direct injection). HR-ESI-MS experiment of compound **1a** was performed with a Micromass Autospec (Manchester, U.K.). The HR-ESI experiment of **2a** was carried out on a Finnigan LTQ-FT hybrid mass spectrometer (Bremen, Germany). NMR experiments were conducted on a JEOL-ECA 500 spectrometer (Tokyo, Japan). With the aid of standard correlation experiments (COSY, HMQC, HMBC), structure elucidation of isolated compounds was performed. The samples were dissolved in methanol. IR spectra were recorded on a Bruker Alpha-P FT-IR (Ettlingen, Germany). UV spectra were performed on a Shimadzu UV-2401 PC (Kyoto, Japan).

Analytical Data of Identified Compounds 1a and 2a. OPA derivative of (R_S,R_C)-marasmin (**1a**, Figure 1): NMR data are given in Table 1; FT-IR [ν_{max}, cm^{-1}, intensity given as strong (s), medium (m), or weak (w)] 721 (m), 747 (m), 1015 (s, S═O), 1161 (m), 1364 (s),

Table 1. NMR Data of Compounds 1a and 2a

	compound **1a** (methanol D4)			compound **2a** (methanol D4)	
no.	^{1}H	^{13}C	no.	^{1}H	^{13}C
1		172.9	1		174.8
2	6.08 (t, 1, J = 6.9 Hz)	56.2	2	4.58 (dd, 1, J = 9.2 Hz)	49.7
3	3.35 (dd, 1, J = 6.6, 13.2 Hz)	57.0	3	3.20 (dd, 1, J = 9.2, 13.2 Hz)	55.5
	3.95 (dd, 1, J = 7.5, 12.9 Hz)			3.25 (dd, 1, J = 9.2, 13.2 Hz)	
4	3.64 (d, 1, J = 13.8 Hz)	55.3	4	3.76 (d, 1, J = 13.8 Hz)	55.3
	3.86 (d, 1, J = 14.3 Hz)			3.96 (d, 1, J = 13.8 Hz)	
5	2.22 (s, 3)	15.8	5	1.94 (s, 3)	15.7
6			6		175.5
7			7	5.71 (dd, 1, J = 5.2, 10.0 Hz)	60.7
8			8	2.34 (m, 1) 2.65 (m, 1)	31.1
9			9	2.17 (m, 1) 2.28 (m, 1)	32.7
10			10		174.0
11	7.67 (s, 1)	114.9	11	7.70 (s, 1)	115.0
12		124.6	12		124.4
13	7.50 (dd, 1, J = 0.9, 8.3 Hz)	120.2	13	7.50 (dd, 1, J = 8.3 Hz)	119.9
14	6.88 (ddd, 1, J = 2.0, 8.6 Hz)	121.0	14	6.85 (ddd, 1, J = 6.6, 8.3 Hz)	120.4
15	6.94 (ddd, 1, J = 0.9, 8.6 Hz)	122.1	15	6.91 (ddd, 1, J = 6.6, 8.3 Hz)	121.5
16	7.58 (dd, 1, J = 0.9, 8.3 Hz)	120.1	16	7.58 (dd, 1, J = 8.6 Hz)	120.0
17		131.4	17		130.9
18		109.8	18		109.7
19		50.0	19		49.9
20, 21, 22	1.25 (s, 9)	30.3	20, 21, 22	1.23 (s, 9)	30.2

1598 (s, C═O), 1672 (w, C—N amide), 2923 (m, CH, CH_2), 2960 (m, CH, CH_2), 3233 (w); UV (λ_{max} nm, methanol) 202, 231, 298, 337; ESI-MS/MS, positive ionization mode, m/z (relative intensity) 55 (20), 79 (8), 103 (16), 121 (16), 143 (8), 156 (16), 170 (100), 174 (28), 182 (36), 203 (84), 220 (16), 228 (20); HR-ESI-MS [M + H] 386.0912; molecular formula $C_{17}H_{24}N_1O_3S_3$; MS calculated $[M + H]^+$ 386.0918.

OPA derivative of γ-glutamyl-(S_S,R_C)-marasmin (**2a**, Figure 1): NMR data are given in Table 1; FT-IR [ν_{max}, cm^{-1}, intensity given as strong (s), medium (m) or weak (w)] 747 (m), 857 (w) 1003 (s, S═O), 1162 (m), 1317 (w), 1362 (s), 1585 (s, C═O), 2921 (m, CH, CH_2), 2958 (m, CH, CH_2), 3040 (m, CH, CH_2), 3188 (w).; ESI-MS/MS, positive ionization mode, m/z (relative intensity) 61 (27), 132 (4), 149 (5), 234 (15), 244 (11), 262 (100), 331 (17), 349 (22), 381 (53), 441 (17), 459 (91), 515 (8); HR-ESI-MS $[M + H]^+$ 515.1338; molecular formula $C_{22}H_{31}N_2O_6S_3$; MS calculated $[M + H]^+$ 515.1344.

RESULTS AND DISCUSSION

Origin of Plant Material and Usage. With the exception of reference material from IPK Gatersleben, bulbs were collected during expeditions in the years 2005−2009 in Iran, Tajikistan, and Uzbekistan. All bulbs with the exception of accession no. 1086, *A. stipitatum*, which was bought from a local Iranian trader but probably also collected in the wild, stem from natural places. These were typically located in mountainous areas in a zone between 1080 and 2750 m above sea level. Referring to Keusgen et al.,[3] several species, for example, *A. suworowii*, are used in folk medicine. This was testified to for *A. suworowii* by local tribes of Tadjikistan and Uzbekistan. The bulbs, flowers (Figure 2a), and seeds of *A. suworowii* are prepared in a way comparable to that used for *A. stipitatum*. As "piozi anzur" the flowers and seeds of *A. stipitatum* are used against headache and cold, whereas the bulbs are eaten pickled ("niyazi ansul"). This niyazi ansul is also used against tuberculosis and bronchitis. Further applications, especially of bulbs with high concentrations of CSO **6**, were already mentioned in the Introduction.[3] In Iran it was reported that *A. stipitatum* is used against tuberculosis and as an analgesic herbal drug.[19]

Isolation and Identification of the OPA-Derivatized (R_S,R_C)-Marasmin 1a. HPLC analysis of the CSOs of *A. suworowii* gave evidence that a sulfur compound in high concentration must be present, which was not described for the genus *Allium* until now. The separation of this compound was performed by the corresponding OPA derivative to allow separation from further amino acids with similar retention times. A representative HPLC chromatogram is given in Figure 3. Iodoacetamide has to be added after the OPA derivatization to capture the excess of OPA reagent and to minimize side reactions disturbing structure elucidation. The above-described procedure has been already applied to further CSOs.[16,17] The main advantages of this method are (i) only about 4 g of fresh bulb material is necessary for full structure elucidation and (ii) OPA derivatives of amino acids allow a sufficient HPLC separation even of rather similar compounds. Furthermore, no extraction and separation steps using strong acidic or alkaline conditions are necessary. The latter point is of huge importance, guaranteeing that truly authentic compounds were analyzed.

Structure elucidation was based on NMR analysis using ^{1}H, ^{13}C, COSY, HMQC, and HMBC experiments. The results were validated with OPA-derivatized synthetic L-(+)-alliin and other aliphatic CSOs. The residue of **1a** was assigned by comparison with the above-mentioned model compounds. NMR results are summarized in Table 1. The numbering of carbon atoms is identical to the numbering of compound **1a** in Figure 1.

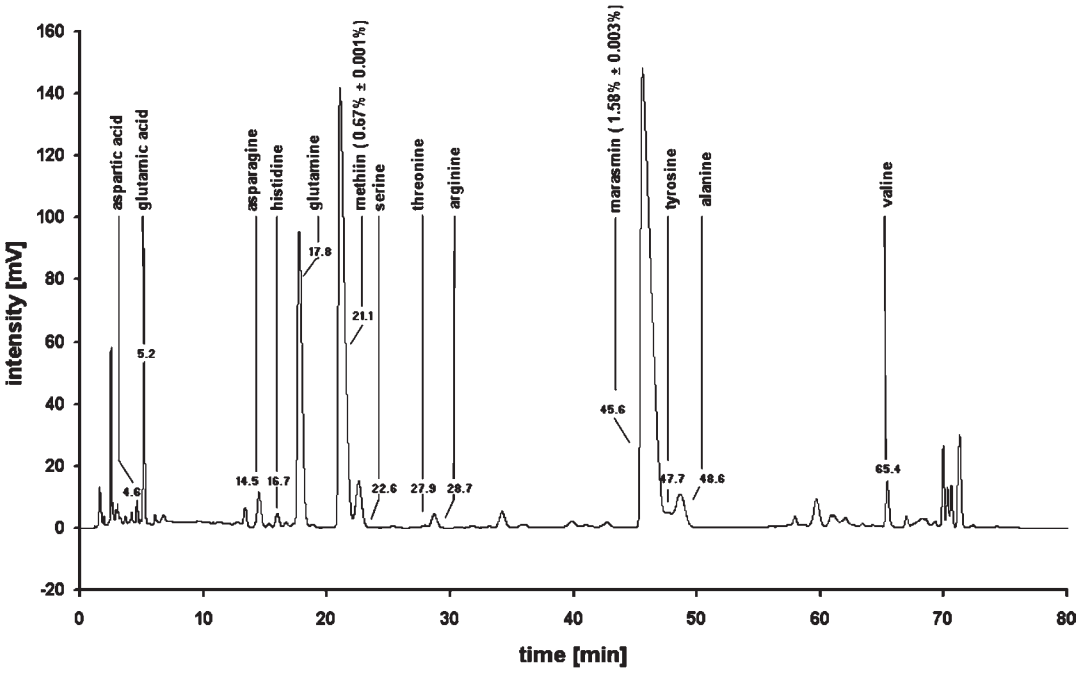

Figure 3. HPLC chromatogram of an OPA-derivatized *Allium suworowii* extract (detection at 334 nm). Peaks are labeled with the corresponding amino acid derivatives. Structures of CSOs are given in Figure 1.

The isolated compound turned out to be the OPA derivative of the previously described marasmin, which was first found in *Tulbaghia* species.[8] In the ^{1}H NMR, the methylthiomethyl residue was characterized by a singlet of the terminal methyl group (C5) at δ 2.22. Protons (δ 3.64/δ 3.86) at C4 (δ 55.3) gave a J_3 coupling with C5 (δ 15.8) in the HMBC. Chemical shifts of the cysteine residue also correlate with data already published for aliphatic CSOs.[12,15−18]

One important question is the configuration of the sulfinyl (sulfoxy) group. Because of derivatization, the optical rotation of the entire molecule does not give valid information. As was demonstrated for other CSOs, the proton shifts at C3 are strongly influenced by the stereochemistry of the neighboring sulfoxy group. In the case of *S*-(−)-configuration, both protons have nearly the same chemical shift of about δ 3.4. For *S*-(+)-configured sulfoxides, these protons show clearly separated signals.[15] Substance **1a** has a chemical shift difference at position 3 of about 0.6 ppm, which emphasizes the (+)-configuration of this sulfoxide. Therefore, the sulfoxide in marasmin is *R*-configured, due to the additional sulfur in the moiety. This finding is in accordance with already published data for marasmin **1** of *Tulbaghia* species.[8] Furthermore, the IR spectrum also showed the strong sulfoxide band at 1015 cm^{-1}. (−)-Configured sulfoxides have an intensive band at 1000 cm^{-1}, whereas (+)-configured sulfoxides have a intensive band at 1015−1020 cm^{-1}.[15] Consequently, compound **1** described for the genus *Tulbaghia* and the compound now described for the genus *Allium* have to be identical. These findings are also in accordance with those recently described for *A. stipitatum*.[20]

Isolation and Identification of the OPA-Derivatized γ-Glutamylmarasmin (2a). As already explained in the Introduction, marasmin has been also reported for fruiting bodies of the mushroom *M. alliaceus*. In contrast to marasmin from *Tulbaghia*, the sulfur atom is *S*-configured. This (S_S,R_C)-marasmin was observed in nature as the γ-glutamyl derivative.

Again, structure elucidation was based on NMR analysis using ^{1}H, ^{13}C, COSY, HMQC, and HMBC experiments (Table 1). The methylthiomethyl residue was characterized by a singlet for protons of the terminal methyl group (C5 δ 15.7; H5 δ 1.94). In the HMBC experiment, a J_3 coupling between the protons at C4 (H4 δ 3.76, 3.96) and C5 could be observed. In contrast to **1a**, additional signals for the γ-glutamyl moiety are visible in the spectra, which is characterized by two methylene groups (C8, δ 31.1; H8 δ 2.34, 2.65; C9, δ 32.7; H9, δ 2.17, 2.28) as well as the chiral carbon at position 7 (C7 δ 60.7; H7 δ 5.71). The two carboxy groups of **2a** were assigned by HMBC (C6, δ 175.5; C10, δ 174.0).

Again, the stereochemistry of the sulfur atom is of huge importance. In contrast to **1a**, protons at C3 have nearly the same chemical shift of about δ 3.2, indicating the (−)-configuration. The IR spectrum showed the strong sulfoxide band at 1003 cm^{-1}, also supporting the *S*-configuration of the sulfoxide. Reported spectroscopic data are in good correlation with literature data.[12] In conclusion, the opposite configurations at the SO group in **1** and **2** could be confirmed.

Quantitative Determination of Cysteine Sulfoxides. Results are summarized in Tables 2 and 3 in alphabetic order. Concentrations are related to the fresh weight of the bulbs (*Allium*) and the dry weight of mushrooms (*Marasmius*). Preliminary results are given in ref 13. In total, 74 *Allium* samples belonging to 30 species and subspecies of the subgenus *Melanocrommyum* and two *Marasmius* samples were analyzed. Besides CSOs, the amino acid composition was also analyzed (Figures 3 and 4). Glutamine is the most prominent amino acid for *A. suworowii*, whereas aspartic acid and glutamic acid as well as glutamine were found in high concentrations in *M. alliaceus*.

Structures of identified CSOs are given in Figure 1. Besides the well-described methiin **3** and propiin **4**, recently discovered marasmin **1**, *S*-(2-pyridyl)-L-cysteine *N*-oxide **5a**, eventually

Table 2. Amounts of Cysteine Sulfoxides (CSOs) in Various *Allium* Samples Belonging to the Subgenus *Melanocrommyum*

species	sample accession no.	CSO total amount (%)	RSD (%)	**1** (rel %)	**1** (%)	**3** (%)	**4** (%)	**5**/**5a** (%)	**6** (%)
A. akaka S.G. Gmelin ex Schult. et Schult. f.	1054a	0.630	12.54	4.310	0.027	0.522			0.081
A. akaka S.G. Gmelin ex Schult. et Schult. f.	1054b	0.564	1.42	5.818	0.033	0.474			0.057
A. alexeianum Regel	4242a	0.226	4.39	14.999	0.033	0.082			0.111
A. alexianum Regel	4242b	0.195	0.51	28.653	0.056	0.083			0.056
A. altissimum Regel	4206a	1.544	2.20	18.852	0.291	0.859		0.394	
A. altissimum Regel	4206b	1.189	3.87	17.374	0.207	0.486		0.496	
A. austroiranicum R.M. Fritsch	1235a	0.048	4.17			0.028			0.020
A. austroiranicum R.M. Fritsch	1235b	0.138	7.25			0.115			0.023
A. bakhtiaricum Regel	1156a	0.318	1.26			0.290			0.028
A. bakhtiaricum Regel	1156b	0.332	2.11			0.304			0.028
A. bakhtiaricum Regel	1158a	0.172	0.58			0.139			0.033
A. bakhtiaricum Regel	1158b	0.309	2.59			0.219			0.090
A. bisotunense R.M. Fritsch	1093a	0.146	0.68	9.540	0.014	0.094			0.038
A. bisotunense R.M. Fritsch	1093b	0.141	2.84	10.067	0.014	0.081			0.046
A. costatovaginatum Kamelin et Levichev	4213a	0.271	1.48	22.941	0.062	0.209			
A. cupuliferum Regel subsp. *cupuliferum*	4199a	0.269	0.37			0.210			0.059
A. derderianum Regel	1207a	0.059	5.08	14.247	0.008	0.045			0.006
A. elburzense Wendelbo	1206b	0.090	8.89	8.124	0.007	0.052			0.031
A. graveolens (R.M. Fritsch) R.M. Fritsch	1142a	0.181	2.76	22.663	0.041	0.034			0.106
A. jesdianum Boiss. et Buhse	1178a	0.200	86.43			0.028			0.172
A. jesdianum Boiss. et Buhse	1178b	0.487	0.82			0.077			0.410
A. jesdianum Boiss. et Buhse subsp. *angustitepoalum* F.O. Khass. et R.M. Fritsch	4241b	0.434	0.92			0.116			0.318
A. jesdianum Boiss. et Buhse subsp. *remediorum* R.M. Fritsch	1172a	0.139	3.60			0.103			0.036
A. jesdianum Boiss. et Buhse subsp. *remediorum* R.M. Fritsch	1172b	0.126	10.32			0.041			0.085
A. jesdianum Boiss. et Buhse subsp. *remediorum* R.M. Fritsch	1180b	0.707	0.71			0.186			0.521
A. karataviense Regel	4248a	0.325	0.92	8.411	0.027	0.172			0.126
A. keusgenii R.M. Fritsch	1198a	0.147	4.79	13.375	0.020	0.093			0.034
A. keusgenii R.M. Fritsch	1198b	0.122	2.46	25.977	0.032	0.054			0.036
A. koelzii (Wendelbo) K. Persson et Wendelbo	1110b	0.306	2.94	18.066	0.055	0.086		0.165	
A. koelzii (Wendelbo) K. Persson et Wendelbo	1107b	0.036	42.86	10.036	0.004	0.023			0.009
A. koelzii K. Persson et Wendelbo	1095b	0.105	0.94			0.089			0.016
A. macleanii Baker	6240a	0.209	1.91			0.025			0.184
A. macleanii Baker	6240b	0.150	20.00			0.029			0.121
A. macleanii Baker	6256a	0.228	1.75			0.056			0.172
A. macleanii Baker	6256b	0.328	1.52			0.035			0.293
A. materculae Bordz.	1060b	0.296	0.68	13.034	0.039	0.123			0.134
A. materculae Bordz.	1064a	0.525	0.38	12.395	0.065	0.300			0.160
A. materculae Bordz.	1064b	0.403	0.50	13.670	0.055	0.232			0.116
A. nevskianum Vved	4286a	0.064	1.56	4.698	0.003	0.055			0.006
A. nevskianum Vved	4286b	0.066	2.99	5.903	0.004	0.059			0.003
A. rosenorum R.M. Fritsch	0653 h	0.208	1.92			0.009			0.199
A. rosenorum R.M. Fritsch	4293a	0.089	1.12			0.020			0.069
A. rosenorum R.M. Fritsch	4293b	0.122	1.64			0.021			0.101
A. shelkovnikovii Grossh.	1062a	0.240	1.25			0.167			0.073
A. shelkovnikovii Grossh.	1062b	0.169	4.73	4.198	0.007	0.123			0.039
A. shelkovnikovii Grossh.	1063a	0.288	1.05			0.194			0.094
A. stipitatum Regel	1086b	0.214	2.80	12.670	0.027	0.044		0.143	
A. stipitatum Regel	1090b	0.298	1.67	10.444	0.031	0.045		0.222	
A. stipitatum Regel	1240b	0.315	0.95	24.092	0.076	0.095		0.144	
A. stipitatum Regel	4238b	0.424	0.24	10.877	0.046	0.160		0.218	
A. suworowii Regel	4247a	2.251	0.13	70.070	1.577	0.674			
A. suworowii Regel	4247b	1.125	7.82	96.357	1.084	0.041			

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Table 2. Continued

species	sample accession no.	CSO total amount (%)	RSD (%)	**1** (rel %)	**1** (%)	**3** (%)	**4** (%)	**5**/**5a** (%)	**6** (%)
A. suworowii Regel	4247c	1.121	1.25	95.966	1.076	0.045			
A. suworowii Regel	4247d	0.319	0.31	95.782	0.306	0.013			
A. suworowii Regel	4276a	0.572	0.52	55.432	0.317	0.145			0.110
A. taeniopetalum Popov et Vved	4246b	0.090	3.30	12.237	0.011	0.032			0.047
A. tashkenticum F.O. Khass. et R.M. Fritsch	4250a	0.105	12.26	27.709	0.029	0.035			0.041
A. tashkenticum F.O. Khass. et R.M. Fritsch	4250b	0.116	0.86	28.653	0.033	0.049			0.034
A. tashkenticum F.O. Khass. et R.M. Fritsch	4211b	0.994	0.30	15.464	0.154	0.804			0.036
A. tschimganicum O. Fedtsch. s. str.	4218a	0.405	6.17			0.178			0.227
A. tschimganicum O. Fedtsch. s. str.	4218b	0.237	0.85			0.120			0.117
A. ubipetrense R.M. Fritsch	1112a	0.261	0.77	4.370	0.011	0.164			0.086
A. ubipetrense R.M. Fritsch	1149b	0.042	13.95	6.181	0.003	0.033			0.007
A. ubipetrense R.M. Fritsch	1153a	0.859	2.68	6.252	0.054	0.759			0.046
A. ubipetrense R.M. Fritsch	1153b	0.190	0.53	4.523	0.009	0.119			0.062
A. ubipetrense R.M. Fritsch	1153c	0.183	1.65	6.062	0.011	0.104	0.006		0.062
A. ubipetrense R.M. Fritsch	1153d	0.488	2.46	5.063	0.025	0.425	0.009		0.029
A. ubipetrense R.M. Fritsch	1154a	0.418	2.15	7.155	0.030	0.349			0.039
A. ubipetrense R.M. Fritsch	1154b	0.209	1.44	8.075	0.017	0.160			0.032
A. ubipetrense R.M. Fritsch	1155a	0.303	0.33	2.544	0.008	0.295			
A. verticillatum Regel	4284a	0.258	6.98	10.076	0.026	0.218			0.014
A. verticillatum Regel	4284b	0.450	5.54	7.835	0.035	0.370			0.045
A. zagricum R.M. Fritsch	1184a	0.730	4.93	7.309	0.053	0.567			0.110
A. zagricum R.M. Fritsch	1184b	0.437	2.52	6.679	0.029	0.301			0.107

Table 3. Amounts of γ-Glutamylmarasmin 2 in *Marasmius* Samples

species	sample	CSO total amount (%)	SD (%)	**2** (rel %)
M. alliaceus	24.10.01	2.99	0.012	100
M. alliaceus	24.10.02	2.45	0.010	100

S-(2-pyridyl)-L-cysteine sulfoxide **5**, and also *S*-(2-pyrrolyl)-L-cysteine sulfoxide **6** were found in the investigated bulbs. All other known CSOs, especially alliin and isoalliin as well as γ-glutamyl derivatives, could not be detected.

Obviously, there are two possibilities for the structure of pyridyl-cysteine derivatives: As described in ref16, the oxygen can be located on sulfur leading to the structure of **5**. According to refs 14 and 20, the oxygen can be also located at the nitrogen atom of the pyridine ring, leading to compound **5a**. It is assumed by the authors that compound **5** is synthesized by the plant and later converted into **5a**. It must be considered that these *Allium* species are growing at very hot places with air temperatures of 40 °C and more in the shadow. This might lead to an internal oxygen migration, which is energetically favored (Figure 5): The pyridinyl-*N*-oxide has a bond dissociation energy of 302 kJ/mol, whereas the bond dissociation energy of the sulfoxide is 420 kJ/mol at 298.15 K.[16] This leads to the assumption that the *N*-oxide is energetically favorable for this molecule. An initially occurring sulfoxide might be converted into the more stable *N*-oxide over the storage time inside cells.

As also observed for further wild *Allium* species,[21] methiin **3** is present in all investigated samples. The highest amount was found in *A. suworowii* 4247a (0.67%) and the lowest amount in *A. rosenorum* 0653h (0.01%). The second most widespread CSO is compound **6**, which is also present in nearly all investigated species.[18,22] If plant material containing **6** is wounded, a coloring into orange and red occurs within minutes. The resulting red thiopyrrole is a unique compound in nature. Many of the species containing high amounts of **6** are used as vegetables and medicinal plants.[3] Most prominent are *A. jesdianum* Boiss. et Buhse subsp. *remediorum* R.M. Fritsch (1180b, 0.52%), *A. macleanii* Baker (6256b, 0.29%), *A. tschimganicum* (4218a, 0.23%), and *A. rosenorum* (0653h, 0.20%). Interestingly, all of these samples are free of marasmin **1**. As a further interesting fact, which is probably related to the biogenesis of **6**, no compound **5** or **5a** is present. Only **6**-free samples contain **5** or **5a**. Because **5** and **5a** differ only in the position of the oxygen, no unambiguous differentiation was possible.

Highest amounts of up to 0.50% were found in *A. altissimum* 4206. Furthermore, pyridyl derivatives were found in *A. stipitatum* (up to 0.22%) and in one sample of *A. koelzii* (Wendelbo) K. Persson et Wendelbo (0.17%). These plants are of great medicinal interest, because pyridyl derivatives are related to tuberculostatic activity.[14] In contrast, the health benefits of **6** and derived sulfur compounds are still unknown.

As a rather surprising finding, CSO **1** was present in 49 of the 74 investigated samples, which represent 21 different *Allium* species and subspecies. Compound **1** seems to be a major constituent in many *Allium* species, but had not been found in this genus before these investigations. Most interesting in terms of total amount of CSOs as well as relative concentration of **1** are *A. suworowii* (up to 2.25% total CSOs), *A. stipitatum* (up to 0.42% total CSOs), *A. altissimum* (up to 1.54% total CSOs), and *A. tashkenticum* F.O. Khass et R.M. Fritsch (up to 0.99% total CSOs), all of them showing >0.15% of **1**, related to the fresh weight of the bulbs (Table 2). Three samples of *A. suworowii* contained even more than 1% of **1**, related to fresh weight. The highest amount was found in *A. suworowii* 4247a (1.58%, corresponding to ca. 70% of the CSOs). This sample had a very

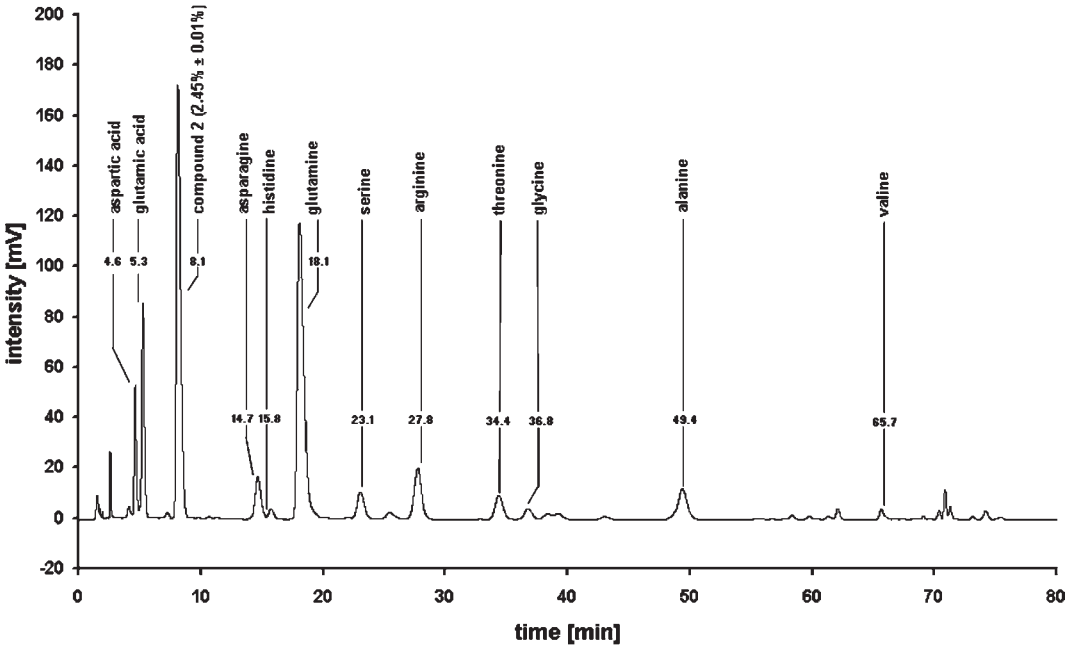

Figure 4. HPLC chromatogram of a derivatized *Marasmius alliaceus* extract (detection at 334 nm). Peaks are labeled with the corresponding amino acid derivatives. Compound **2**: γ-glutamyl-marasmin. Structures of CSOs are given in Figure 1.

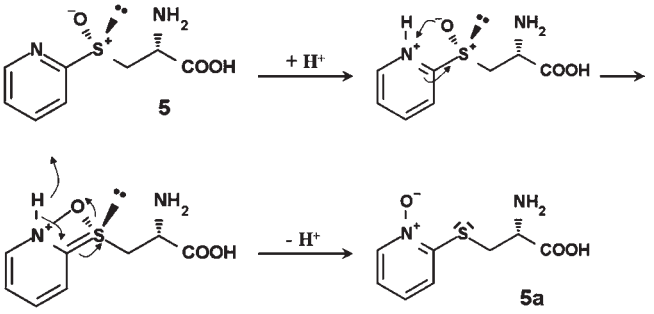

Figure 5. Proposed intramolecular migration of oxygen, which might explain the occurrence of the pyridyl-*N*-oxide **5a** in *A. stipitatum* and *A. altissimum*.

hot taste. Samples of *A. stipitatum* showed amounts between 0.02 and 0.07%. However, huge variations were found, for example, 0.004% for *A. koelzii* 1107b and 0.055% for *A. koelzii* 1110b.

The two samples of *M. alliaceus* showed very high amounts of γ-glutamylmarasmin **2** (2.45 and 2.99%, corresponding to 1.79 and 1.47% of **1**). However, neither **1** nor further CSOs could be detected with MS methods. Nevertheless, the mushrooms had a very intense taste and smell. Besides the enzyme alliinase, also a γ-glutamyltransferase must be present to give volatile sulfur compounds such as marasmicin.[11]

A rather interesting species is *A. ubipretense* R.M. Fritsch, which grows in Iran. The total amount of CSOs was up to 0.86% (1153). Besides **1**, **3**, and **6**, also propiin **4** in rather low concentrations (<0.01%) was found in two samples. Propiin is typical for leek (*Allium porrum* L.).[5] Substance **4** was not present in any other investigated *Allium* species.

In conclusion, marasmin **1** and its alliinase product marasmicin, which has attracted considerable attention because of its tuberculostatic and antifungal properties, were previously found in concentrations not higher than 0.02% (related to fresh weight) in *T. violacea*.[8] Besides the mushroom *M. alliaceus*, which is very rich in **2**, 15 different *Allium* species belonging to the subgenus *Melanocrommyum* showed concentrations of **1** of >0.02%. The presence of **1** in such high concentrations seems to be a unique feature of this subgenus and can be probably used as a chemical marker. Other investigated *Allium* subgenera, such as *Allium*, *Reticulatobulbosa*, and *Polyprason*, showed only trace amounts of **1**.[13]

Most prominent is *A. suworowii*, which grows in Central Asia in semiruderal, grassy habitats (Figure 2a). However, many areas, for example, in Uzbekistan, are overgrazed by millions of sheep and goats so that this species is vanishing. As a consequence, *A. suworowii* should be considered for cultivation. Because of the special climate (wet and cold winter, very hot and dry summer), cultivation should be done in Central Asia. As also shown in this investigation, differences between samples are rather large. Especially the total amount of CSOs is probably strongly influenced by the environment. Tuberculosis is a huge problem of Asian countries currently, and extracts of cultivated *A. suworowii* might support local therapy against tuberculosis in these countries.

It must be pointed out that most of the *Allium* species containing **1** in high concentrations also contain CSO **5** or **6** besides **3**. An interesting variety of "mixed" thiosulfinates and further alliinase-derived compounds can be expected in these species. It also must be pointed out that pyridyl *N*-oxides such as **5a** do occur in *A. stipitatum*, giving an even larger variety of potentially bioactive compounds.[14] Therefore, cultivation of *A. altissimum* and *A. stipitatum* seems to be a valuable task; however, as already mentioned above, intraspecific variations in the concentration of CSOs require careful selection of material prior to multiplication.

In earlier investigations, isoalliin was detected in subgenus *Melanocrommyum*.[21] These findings were based on cochromatography with isoalliin standards. However, isoalliin and marasmin show the same retention times as the corresponding OPA derivatives. Because the occurrence of marasmin in *Allium* was not known at the time of this investigation, peaks with a retention time of about 45 min were assigned as isoalliin (compare Figure 3).

With the now presented LC-MS investigations, the presence of isoalliin, $[M + H]^+$ at 366 amu, could not be confirmed. All CSO peaks at a retention time at about 45 min turned out to be marasmin indicated by $[M + H]^+$ at 386 amu.

It is a still unanswered question as to why the mushroom *M. alliaceus* (Figure 2b) contains huge amounts of γ-glutamylmarasmin **2** but no **1** and no other CSO. In contrast, **2** was not detected in any sample of *Melanocrommyum* species. According to Lancaster et al.,[23] γ-glutamyl-CSOs are the storage forms of the "free" CSOs, which are usually liberated over time.

Cultivation of *M. alliaceus* should be considered. First experiments showed that this mushroom can be grown on substrates containing beechwood. However, yields could not be estimated at the current stage of experiments. As is clearly visible in Figure 4, the amino acid composition of *Marasmius* is less complex than that found in *A. suworowii* (Figure 3).

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Notes

Safety. The toxicity and smell of *tert*-butylthiol require the preparation of the OPA reagent to be performed under a fume hood.

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ABBREVIATIONS USED

OPA, *o*-phthaldialdehyde; ITS, internal transcribed spacer; MRSA, methicillin-resistant *Staphylococcus aureus*; MIC, minimum inhibitory concentration; MS, mass spectrometry; ESI, electrospray ionization; HR-MS, high-resolution mass spectrometry; FT-IR, Fourier transformation infrared; COSY, correlated spectroscopy; HMQC, heteronuclear multiple-quantum correlation; HMBC, heteronuclear multiple-bond correlation; HIV, human immunodeficiency virus; RSD, relative standard deviation; SD, standard deviation; CSO, cysteine sulfoxide; GPS, global positioning system.

REFERENCES

(1) Friesen, N.; Fritsch, R. M.; Blattner, F. R. Phylogeny and new intrageneric classification of *Allium* (Alliaceae) based on nuclear ribosomal DNA ITS sequences. *Aliso* **2006**, *22*, 372–395.

(2) Fritsch, R. M.; Blattner, F. R.; Gurushidze, M. New classification of *Allium* L. subg. *Melanocrommyum* (Webb & Berthel.) Rouy (*Alliaceae*) based on molecular and morphological characters. *Phyton* **2010**, *49*, 145–220.

(3) Keusgen, M.; Fritsch, R. M.; Hisoriev, H.; Kurbonova, P. A.; Khassanov, F. O. Wild *Allium* species (Alliaciae) used in folk medicine of Tajikistan and Uzbekistan. *J. Ethnobiol. Ethnomed.* **2006**, *2*, 18.

(4) Block, E.; Dane, A. J.; Thomas, S.; Cody, R. B. Application 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.

(5) Block, E. *Garlic and Other Allium. The Lore and the Science*; ; RCS Publishing: Cambridge, U.K., 2010; 454pp, ISBN 978-0-85404-190-9.

(6) Keusgen, M. In *Allium Crop Science: Recent Advantages*; Rabinowitch, H. D., Currah, L., Eds.; CABI Publishing: New York, 2002; pp 357−378, ISBN 0851995101.

(7) Cavallito, C.; Bailey, J. H. Allicin, the antibacterial principle of *Allium sativum*. Isolation, physical properties and antibacterial action. *J. Am. Chem. Soc.* **1944**, *66*, 1944–1952.

(8) Kubec, R.; Velisek, J.; Musah, R. A. The amino acid precursors and odor formation in society garlic (*Tulbaghia violacea* Harv.). *Phytochemistry* **2002**, *60*, 21–25.

(9) Thamburan, S.; Klaasen, J.; Mabusela, W. T.; Cannon, J. F.; Folk, W.; Johnson, Q. *Tulbaghia alliacea* phytotherapy: a potential anti-infective remedy for candidiasis. *Phytother. Res.* **2006**, *20*, 844–50.

(10) Thamburan, S.; Cannon, J. F.; Mabusela, T.; Folk, W.; Johnson, Q. *Tulbaghia alliaceae*: a potential anti-tuberculosis phytotherapy. *Planta Med.* **2009**, *75*, 922.

(11) Gmelin, R.; Luxa, H.-H.; Roth, K.; Höfle, G. Dipeptide precursor of garlic odour in *Marasmius* species. *Phytochemistry* **1976**, *15*, 1717–1721.

(12) Van den 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.

(13) Kusterer, J. *Neue Erkenntnisse der Schwefelchemie und Chemotaxonomie in Arten des Genus Allium*; Institute of Pharmaceutical Chemistry, University of Marburg: Marburg, Germany, 2010; abstract http://archiv.ub.uni-marburg.de/diss/z2010/0127/; full text (German) http://archiv.ub.uni-marburg.de/diss/z2010/0127/pdf/djk.pdf.

(14) O'Donnell, G.; Poeschel, R.; Zimhony, O.; Gunaratnam, M.; Moreira, J. B. C.; Neidle, S.; Evangelopoulos, D.; Bhakta, S.; Malkinson, J. P.; Boshoff, H. I.; Lenaerts, A.; Gibbons, S. Bioactive pyridine-*N*-oxide disulfides from *Allium stipitatum*. *J. Nat. Prod.* **2009**, *72*, 360–365.

(15) Koch, I.; Keusgen, M. Diastereoselective synthesis of alliin by an asymmetric sulfur oxidation. *Pharmazie* **1998**, *53*, 668–671.

(16) Kusterer, J.; Vogt, A.; Keusgen, M. Isolation and identification of a new cysteine sulfoxide and volatile sulfur compounds from *Allium* subgenus *Melanocrommyum*. *J. Agric. Food Chem.* **2010**, *58*, 520–556.

(17) Kusterer, J.; Keusgen, M. Cysteine sulfoxides and volatile sulfur compounds from *Allium tripedale*. *J. Agric. Food Chem.* **2010**, *58*, 1129–1137.

(18) Jedelská, J.; Vogt, A.; Reinscheid, U. M.; Keusgen, M. Isolation and identification of a red pigment from *Allium* subgenus *Melanocrommyum*. *J. Agric. Food Chem.* **2008**, *56*, 1465–1470.

(19) Abbasi, M.; Fritsch, R. M.; Keusgen, M. Wild *Allium* species used as food and folk medicine in Iran. In *Marburg & Gatersleben*; Proceedings, First Kazbegi workshop on Botany, Taxonomy and Phytochemistry of Wild *Allium* L. Species of the Caucasus and Central Asia, June 4−8, 2007, Kazbegi, Caucasus, Georgia; Keusgen, M., Fritsch, R. M., Eds.; distributed by Koeltz Scientific Books: Koenigstein, Germany, 2008; pp 25−30, ISBN 978-3-9813096-0-7.

(20) Kubec, R.; Krejcova, P.; Simek, P.; Vaclavik, V.; Hajslova, 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.

(21) Fritsch, R. M.; Keusgen, M. Occurrence and taxonomic significance of cysteine sulphoxides in the genus *Allium* L. (Alliaceae). *Phytochemistry* **2006**, *67*, 1127–1135.

(22) Kucerova, P.; Kubec, R.; Simek, P.; Vaclavik, 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.

(23) Lancaster, J. E.; Shaw, M. L. γ-Glutamyl peptides in the biosynthesis of *S*-alk(en)yl-L-cysteine sulphoxides (flavour precursors) in *Allium*. *Phytochemistry* **1989**, *28*, 455–460.