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Occurrence and taxonomic significance of cysteine sulphoxides in the genus *Allium* L. (Alliaceae)

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Abstract

Methiin was present in all investigated samples. In the genus *Allium*, methiin-dominated species (rarely used by man) were common, but the occurrence of the other cysteine sulphoxides was variable and was largely correlated with use as spices or vegetables. Two major chemical types (named according to the species where they occur) could be distinguished, and at least two more may be recognized. Isoalliin dominates in the widely used "onion-type", which includes chive (*A. schoenoprasum*) and top onion (*A.* × *proliferum*). Pearl onion and leek (*A. ampeloprasum*) have higher relative amounts of methiin and propiin, respectively. Alliin dominates in the widely used "garlic-type", which includes wild leek (*A. obliquum*) and sand leek (*A. scorodoprasum*). Alliin and isoalliin rarely co-dominate, being only found in the cultivated Chinese leek (*A. tuberosum*). A triple mix of almost equal amounts of methiin, alliin and isoalliin is present in ramson (*A. ursinum*). General trends in the three evolutionary lines of the genus *Allium* were apparent. In the first lineage, high amounts of methiin were more frequent, and propiin, although being a minor component in all three evolutionary lines, was present in the highest amounts. Most of the species in the second line showed only traces of cysteine sulphoxides. In the third line, the "onion-type" dominates, the "garlic type" is characteristic for subgenus *Allium*, and co-dominating alliin and isoalliin also occur. Generally, the total cysteine sulphoxide amount increased, and the complexity of cysteine sulphoxide patterns decreased in the transition from the first to the third evolutionary line.

Keywords: *Allium*; Alliaceae; Onion; Garlic; Spice plants; Chemical analysis; Sulphur-containing compounds; Alliin; Isoalliin; Methiin; Propiin

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1. Introduction

Common onion (*Allium cepa* L.), garlic (*A. sativum* L.) and shallot (*A. cepa* L. aggregatum group) are major crop species. They are well known (but not always appreciated) throughout the world for their specific and commonly intense smell and taste. Prominent smell and taste is characteristic for a further 20 *Allium* species which are only regionally or locally cultivated (Hanelt, 2001), and for still more species collected in the wild. Chive (*A. schoenoprasum* L.) is the only cultivated *Allium* which occurs naturally in Europe. The other crops are historic introductions: the common onion reached the countries north of the Alps during the Roman Empire, and garlic and leek possibly arrived somewhat later. Welsh onion (*A. fistulosum* L.) reached Europe during the Middle Ages, and Chinese leek (*A. ramosum* L./*A. tuberosum* Rottl.) more recently (Hanelt, 2001). In addition, certain hybrids such as top onion (*A.* × *proliferum* [Moench] Schrad.) and St. John's onion (*A.* × *cornutum* Clem.), both of which were introduced a few centuries ago, were occasionally grown in European kitchen gardens.

Since the early stages of scientific botany, the smell and taste characterizing onion, garlic, and leek have been used as primary characters in order to relate taxa to the genus *Allium*. During taxonomic history, the accepted numbers of species and infrageneric groups has risen

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continuously from 30 species in 3 informal groups accepted by Linnaeus in 1753, to 256 species (plus about 260 synonyms) affiliated to six more or less heterogeneous sections by the last monographer (Regel, 1875). Beginning in the second half of the 19th century, species lacking a conspicuous smell have been described, thus leading to a decrease in importance of olfactory features in the characterizations of certain groups. In the 20th century, many more species have been described. Additionally recognized morphological, anatomical, karyological, and phytochemical characters have favoured more classifications with smaller but more homogenous groups. The "Gatersleben classification approach" of Hanelt et al. (1992) marked the final stage of this premolecular taxonomy by classifying the genus *Allium* (about 750 species) into 6 subgenera and 57 sections. Recent molecular studies have confirmed most of the basic tenets of this approach. The genus *Allium* has proven to be monophyletic, having differentiated through a number of evolutionary steps into three evolutionary lines. All three evolutionary lines contain species with easily visible or even visually dominant rhizomes ("rhizomatous species") as well as those with very short rhizomes hidden inside the bulb ("bulbous species"). The most recent classification proposal is based primarily on sequences of the ITS1 region of nuclear DNA relating about 780 species to 15 subgenera and 56 sections (Fritsch and Friesen, 2002; Friesen et al., 2005). This classification (Fig. 1) is used in the present paper.

Onions and garlic are universally used spice plants, and their medicinal properties are well known. Chive and the more recently introduced species are also much appreciated as spices, but leek and Welsh onion (in East Asia) are more important as vegetables with additional flavouring properties. Such a general acceptance of introduced *Allium* species in the daily diet certainly reflects an old tradition, and it is likely that they replaced indigenous species used for the same reasons in more ancient time. Some of the old botanical names for European wild *Allium* taxa such as *A. oleraceum* L. ("vegetable onion") indeed point to their former use. Ancient European written sources may be informative as to their traditional use.

In Central Asia, the diversity of *Allium* species is much larger than in Europe. Many taxa are exploited as food, spices and medicine in this region, although published data are rather scarce (Kochkareva and Chukavina, 1985; Khassanov and Umarov, 1989; Hanelt, 1994; Navruzshoev, 1994). There are clear indications that some species, which are collected in small amounts for the preparation of traditional foods, are spice plants like common onion and garlic. Others are esteemed as tasty vegetables and are collected in large amounts or bought at the local markets. Occasionally these plants are also stored in dried form. Several of the species belong taxonomically to subgenera and sections other than common onion and garlic. These taxa display either a different taste and/or smell, or lack any particular taste and smell.

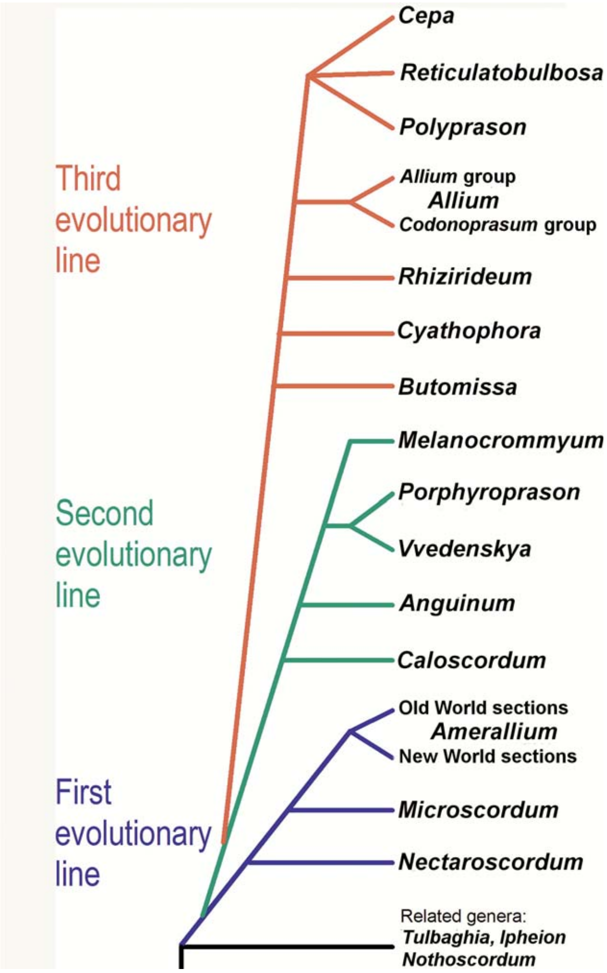

Fig. 1. Classification scheme of the genus *Allium* (Friesen et al., 2005). The three evolutionary lines (left) and the subgenera and informal subgroup names (right) are shown.

Cysteine sulphoxides possibly play a critical role in determining the characteristic smell and taste of these plants. Starting with odourless, non volatile cysteine sulphoxide derivatives, such as (+)-*S*-(2-propenyl)-L-cysteine sulphoxide (alliin) or (+)-*S*-(1-propenyl)-L-cysteine sulphoxide (isoalliin), which undergo reactions to yield alk(en)yl thiosulphinates in the presence of the enzyme alliinase. Finally, the corresponding alk(en)yl (poly)sulphides are formed from the thiosulphinates especially if the *Allium* species are heated/cooked. The thiosulphinates play a very important role in flavor and aroma of fresh garlic. Additionally, the cepeanes which are also formed from thiosulphinates in onion provide a 'sweet flavour note' (Block, 1992; Koch and Lawson, 1996). These substances are physiologically active and are used as antibiotic and antitumor agents, especially in the context of stomach cancer treatment (Koch and Lawson, 1996; Gao et al., 1999). Investigations of various wild species of the genus *Allium* have shown that some contain higher amounts of the cysteine sulphoxides than the cultivated species, and thus they may have considerable potential as spice, vegetable, and medicinal plants (Krest et al., 2000; Keusgen et al., 2002).

Several authors have suggested (see Keusgen, 1999) that the content of the four major cysteine sulphoxides (alliin, isoalliin, methiin and propiin) underlies the different tastes of common onion, garlic, and leek. After alliinase digestion, alliin is converted into allicin, which is characteristic of garlic-type alliums. In combination with small amounts of other cysteine sulphoxides, isoalliin is responsible for the typical onion-smell. If isoalliin is combined with measurable amounts of propiin (the *S*-propyl derivative), the typical leek-taste occurs. Methiin (the *S*-methyl-derivative) is associated with an unpleasant and "hard" taste and smell. The absolute content of these cysteine sulphoxides is less important for their olfactorial properties than their relative composition (Keusgen, 1999). Some other cysteine sulphoxides, including ethiin and butiin, have also been reported as being present in *Allium* (Kubec et al., 1999, 2002). Ethiin was only reported in traces to date. Butiin was not included in this study, despite its occurrence in substantial amounts in *A. siculum*, which is the only natural source for this compound until now.

Finally, the concentration of cysteine sulphoxides may show intraspecific variation (Keusgen et al., 2002). In contrast, the species-specific pattern of content and relative concentration of these substances is much more stable but does show some ontogenetic changes (Schmitt et al., 2005). The present report is thus focused on comparing the relative amounts of the four dominant cysteine sulphoxides ('pattern' of cysteine sulphoxides), related to the fresh weight of plant material. Because intraspecific variations of the absolute amounts of cysteine sulphoxides can be expected as stated above, four broad concentration categories were defined: above 0.5%: 'very high'; 0.25–0.5%: 'high'; 0.1–0.25%: 'low to medium'; below 0.1%: 'trace'.

2. Results and discussion

2.1. Species of the first evolutionary line

This is the oldest phylogenetic group of the genus *Allium* consisting of the subgenera *Nectaroscordum* (about 3 species), *Microscordum* (one species) and *Amerallium* (about 135 species belonging to 12 sections) (see Table 1).

Apart from dominating methiin, alliin and isoalliin were detected in a number of species, but with strongly varying concentrations. Propiin occurred usually in traces, but a few species (no geographic correlation) showed remarkable amounts of propiin combined with traces of isoalliin (relative amounts: *A. mobilense* 51%, *A. canadense* 37%, *A. scorzonerifolium* 27%). A number of species (*A. mobilense*,

Table 1
Content of cysteine sulphoxides of taxa of the first evolutionary line

Taxonomic groups or source or remarks	Total contents (average) (%)	Relative methiin (%)	Relative alliin (%)	Relative isoalliin (%)	Relative propiin (%)	Origin of material	Species name
Subg. *Amerallium*							
Bulbous New World species							
	0.136 ± 0.006	36	7	6	51	TAX 3251	*Allium mobilense* Regel
	0.031 ± 0.004	41	18	4	37	TAX 2031	*Allium canadense* L.
Rhizomatous New World species							
	0.436 ± 0.135	79	7	9	5	TAX 1353	*Allium unifolium* Kellogg
	0.172 ± 0.015	50	27	21	1	TAX 0380	*Allium cernuum* Roth
Old World species (all bulbous)							
	0.245 ± 0.069	39	24	37	0	Urft in der Eifel	*Allium ursinum* L.
Keusgen (1999)	0.164	35	28	37	0		*Allium ursinum* L.
	0.059	54	0	46	0	BG Frankfurt	*Allium paradoxum* (M. Bieb.) G. Don
Keusgen (1999)	0.040	80	0	20	0		*Allium paradoxum* (M. Bieb.) G. Don
	0.114 ± 0.019	56	21	19	4	BG Köln	*Allium triquetrum* L.
Keusgen (1999)	0.097	43	31	26	0		*Allium triquetrum* L.
	0.021 ± 0.001	57	12	4	27	TAX 3428	*Allium scorzonerifolium* Desf. ex DC.
Keusgen (1999)	0.033	79	21	0	0		*Allium subhirsutum* L.
	0.135 ± 0.121	98	2	0	1	TAX 1161	*Allium neapolitanum* Cyr.
	0.172 ± 0.092	90	9	1	0	TAX 3368	*Allium trifoliatum* Cyr.
	0.099	22	8	70	0	TAX 1117	*Allium moly* L.
	0.231 ± 0.018	80	17	2	0	BG Neapel	*Allium roseum* L.
Subg. *Nectaroscordum*							
Keusgen (1999)	0.010	100	0	0	0	BG Leipzig	*Allium tripedale* Trautv.

If standard deviations are given, analysis was repeated at least three times.

A. unifolium, *A. cernuum*, and A. *triquetrum*) contained varying but low levels of alliin and isoalliin (relative amounts of these cysteine sulphoxides typically below 30%). This seems to be a unique feature, although no correlation with either taxonomic classification or geography were apparent. *A. ursinum* contained a mixture of methiin, alliin and isoalliin in almost equal amounts. Only methiin and alliin have been detected in *A. ursinum* and *A. moly* to date (Bernhard, 1970; Freeman and Whenham, 1975). Except for *A. unifolium*, *A. ursinum*, and *A. roseum*, the total content of cysteine sulphoxides is low or very low (traces only), and *A. tripedale* showed only traces of methiin. We suspect that *A. tripedale* contains other cysteine sulphoxides not assayed in our investigations, since butiin has been reported to be present in considerable amounts in the closely related species *A. siculum* (Kubec et al., 2002).

2.2. Species of the second evolutionary line

This lineage contains the small rhizomatous subg. *Anguinum* (about 12 species) and four bulbous subgenera: *Caloscordum* (three species), *Porphyroprason* (one species), *Vvedenskya* (one species) and the large and taxonomically complex subgenus *Melanocrommyum* (140 species, 15 sections) which is phylogenetically the most advanced in this lineage (see Table 2).

Methiin was detected as a major and propiin as a minor component (relative amounts) in the second evolutionary line. With respect to *Melanocrommyum*, these findings are in accordance with previous investigations (Bernhard, 1970; Freeman and Whenham, 1975). However, the relative concentrations of methiin were below the 20% previously demonstrated for *A. suworowii* and *A. orientale*. This composition was characteristic of the third evolutionary line described below. In contrast to the first evolutionary line, species of the second line contained significant amounts of either alliin or isoalliin (alliin: *A. victorialis* averagely 42%, *A. sarawschanicum* 37%; isoalliin: *A. suworowii* 86%, *A. macleanii* 47%, *A. hollandicum* 31%, *A. cyrilli* 51%, *A. orientale* 77%).

Only traces of cysteine sulphoxides (below 0.1%) were present in the species of the bulbous subgenera *Porphyroprason* and *Melanocrommyum*. Most of these species do not have any special smell. However, *A. stipitatum* and *A. suworowii* emit a strong (and unpleasant) smell despite containing less cysteine sulphoxides than odourless taxa such as *A. jesdianum* and *A. hollandicum*. We interpret these results as follows: (a) there are cysteine sulphoxides present which were not assayed for, and which are acted on by a C-S lyase to produce derivatives other than those expected when methiin, alliin, isoalliin or propiin are present, or (b) the odour producing compounds are not derived from C-S lyase mediated breakdown of cysteine sulphoxides.

The relative amounts of all four cysteine sulphoxides were highly variable and were not clearly correlated with taxonomic classification or ecology. However, it will be

Table 2
Content of cysteine sulphoxides of taxa of the second evolutionary line

Taxonomic groups or source or remarks	Total contents (average) (%)	Relative methiin (%)	Relative alliin (%)	Relative isoalliin (%)	Relative propiin (%)	Origin of material	Species name
Subg. *Anguinum*							
	0.129 ± 0.017	38	57	0	5	Hohe Tauern	*Allium victorialis* L.
Keusgen (1999) bulbs	0.104	75	25	0	0		*Allium victorialis* L.
Keusgen (1999) rhizoms	0.117	56	44	0	0		*Allium victorialis* L.
Subg. *Porphyroprason*							
	0.027 ± 0.004	49	2	49	0	TAX 0115	*Allium oreophilum* C. A. Mey.
Subg. *Melanocrommyum*							
	0.003	100	0	0	0	TAX 5027	*Allium dasyphyllum* Vved.
	0.049 ± 0.007	12	2	86	0	TAX 3652	*Allium suworowii* Regel
	0.020 ± 0.001	53	0	47	0	TAX 2413	*Allium macleanii* Bak.
	0.007	100	0	0	0	TAX 5715	*Allium caspium* (Pall.) M. Bieb.
	0.046 ± 0.007	65	0	31	4	TAX 1631	*Allium hollandicum* R.M. Fritsch
	0.070	98	0	2	0	TAX 1033	*Allium jesdianum* ssp. *angustitepalum* (Wdb.) Khass. & R.M. Fritsch
Keusgen (1999)	0.062	97	0	3	0		*Allium jesdianum* ssp. *angustitepalum* (Wdb.) Khass. & R.M. Fritsch
	0.025	100	0	0	0	TAX 1900	*Allium rosenorum* R.M. Fritsch
	0.050	98	0	2	0	TAX 5475	*Allium stipitatum* Regel
Keusgen (1999)	0.044	98	0	2	0		*Allium stipitatum* Regel
	0.030 ± 0.003	62	37	2	0	TAX 1326	*Allium sarawschanicum* Regel
	0.171 ± 0.058	46	1	51	2	BG Neapel	*Allium cyrilli* Ten.
	0.028 ± 0.001	6	0	77	17	TAX 5371	*Allium orientale* Boiss.

If standard deviations are given, analysis was repeated at least three times.

necessary to broaden the number of taxa and/or accessions per species analysed to confirm this conclusion. Methiin is typically the dominant cysteine sulphoxide and may be combined with alliin or isoalliin. Additionally, more analyses of *A. cyrilli* need to be performed since the amount and pattern of cysteine sulphoxides were surprisingly similar to rhizomatous species in the third evolutionary line.

The only investigated species of subg. *Anguinum*, *A. victorialis*, showed equally high contents of methiin and alliin, which is typical for many other rhizomatous subgenera.

2.3. Species of the third evolutionary line

This is the largest and most advanced lineage. The relatively primitive subgenera *Butomissa* (4 species), *Cyathophora* (4 species) and *Rhizirideum* s. str. (37 species) are rhizomatous, the most species-rich subgenus *Allium* (300 species, 15 sections) is bulbous, and the most advanced subgenera *Polyprason* (c. 50 species, 4 sections), *Reticulatobulbosa* (c. 80 species, 5 sections), and *Cepa* (30 species, 5 sections) are also rhizomatous (see Table 3).

The relative concentration of methiin, though present in all samples investigated, is lower than in the other evolutionary lines. It is less than 20% for many of the species including *A. ampeloprasum*, *A. sativum*, *A. cepa*, *A. galanthum*, *A. proliferum* and *A. tuberosum*. A number of these are used as vegetables or medicinal plants. On the other hand, several contain either alliin and/or isoalliin in considerable amounts. This is a clear difference to the second evolution line. Garlic is alliin-dominated, as are *A. obliquum*, *A. scorodoprasum*, *A. cupanii* and some accessions of *A. ampeloprasum*. The "onion-type" species are characterized by dominance of isoalliin (*A. schoenoprasum*, *A. galanthum*, *A. altyncolicum*, *A. chevsuricum*, *A. senescens*, *A. caesium*, *A. griffithianum*, *A. umbilicatum* and most *A. ampeloprasum* accessions). Note that Freeman and Whenham (1975) characterized *A. scorodoprasum* as an "onion-type" *Allium*.

The high levels of methiin characteristic of the first evolutionary line were found in the subgenera *Allium*, *Butomissa* and *Rhizirideum*. The "onion-type" was also present in these subgenera, but only *A. vineale*, *A. kochii* and *A. tuberosum* contained significant relative levels of both alliin and isoalliin.

It can be assumed that differences in the biosynthesis of individual species also cause differences in the pattern of cysteine sulphoxides. However, the biosynthetic pathway of the cysteine sulphoxides is not well understood. It is assumed that in *Allium* both alliin and isoalliin are synthesized by different routes (Lancaster and Shaw, 1989), which appear to be most strictly regulated in the third evolutionary line. Significant propiin concentrations were found in some members of the *A. ampeloprasum* group, including leek and horse garlic, *A. senescens* and some *A. cepa* specimens (potato onions). Bernhard (1970) reported that *A. senescens* contained considerable amounts of not only methiin, but alliin and isoalliin also. All species of the subgenus *Cepa* belong to the isoalliin-type. We have been unable to confirm the presence of relatively large amounts of propyl sulphides, as identified by GC analysis in the earlier literature (Bernhard, 1970; Freeman and Whenham, 1975).

Shallots and potato onions are morphologically distinct, but genetically very close to the common onion (Fritsch and Friesen, 2002). They have distinct tastes, and only shallots are usable for specific culinary purposes. Although showing a similar general pattern of cysteine sulphoxide content as other cultivar groups (Fig. 2), our sample of shallots had a somewhat higher relative propiin (>10%) and lower isoalliin level than other onion types. We suggest that this may explain the remarkable difference in taste which has also been reported for leek in comparison to other species of the *A. ampeloprasum* group.

Very high (with one exception) to high levels of cysteine sulphoxides were confined to the third evolutionary line (subgenera *Allium*, *Butomissa*, *Rhizirideum* and *Cepa*), the members of which are characterized by a very hot taste (thiosulphinates and related compounds after alliinase reaction). These species are assumed to be well protected against herbivory, and thus are likely good candidates as medicinal plants. However, some widely used spice plants such as pearl onions, chives, leek, potato onions and shallots, have only low to moderate contents of cysteine sulphoxides. These species are mainly appreciated for their aromatic rather than for their hot taste, although the possibility cannot be excluded that high alliinase activity may also have an important influence on their taste.

2.4. Other genera

The occurrence of cysteine sulphoxides is not restricted to the genus *Allium*, as some onion-like smelling members of other genera of Alliaceae contain minor amounts of cysteine sulphoxides. Methiin is universally present, as well as other cysteine sulphoxides in varying amounts. It seems therefore that the ability to produce such compounds evolved early in the speciation of these genera (see Table 4).

Alliaria petiolata (family Brassicaceae) also showed traces of cysteine sulphoxides. These plants are not related to Alliaceae, and thus their ability to synthesize identical compounds probably evolved independently.

2.5. General discussion and conclusions

Cysteine sulphoxides are accessible to analysis by HPLC, but less so by earlier gas chromatography technology, as used by Bernhard (1970) and Freeman and Whenham (1975). Alk(en)yl-monosulphides, -disulphides, and -trisulphides can be analysed by GC, and are formed by alliinase digestion followed by further degradation processes (particularly thermal degradation inside the GC analyser). Thus although GC profiles allow for the characterization of general patterns of cysteine sulphoxides, the analysis depends strongly on alliinase activity, and especially on the amount and activity of the enzyme

Table 3
Content of cysteine sulphoxides of taxa of the third evolutionary line

Taxonomic groups or source or remarks	Total contents (average) (%)	Relative methiin (%)	Relative alliin (%)	Relative isoalliin (%)	Relative propiin (%)	Origin of material	Species name
Subg. *Allium* (bulbous)							
	0.143 ± 0.005	40	4	49	7	BG Neapel	*Allium ampeloprasum* L.
Pearl onion landrace	0.142 ± 0.028	42	1	49	8	garden origin	*Allium ampeloprasum* L.
Leek ‘pollux’	0.073 ± 0.002	13	1	79	7		*Allium ampeloprasum* L.
Cultivar ‘elephant garlic’	0.148 ± 0.010	40	43	3	15		*Allium ampeloprasum* L.
Italy, wild origin	0.280 ± 0.039	9	60	28	3	TAX 0004	*Allium ampeloprasum* L.
Georgian “horse garlic”	0.199 ± 0.065	21	4	62	13	TAX 1027	*Allium ampeloprasum* L.
Keusgen (1999)	0.255 ± 0.005	10	4	69	17	TAX 1027	*Allium ampeloprasum* L.
Pearl onion landrace	0.278 ± 0.026	23	2	62	12	All 554	*Allium ampeloprasum* L.
Pearl onion landrace	0.136 ± 0.076	33	0	56	10	All 559	*Allium ampeloprasum* L.
	0.485	36	41	23	0	TAX 5299	*Allium guttatum* ssp. *sardoum* (Moris) Stearn
Keusgen (1999)	0.229	14	76	10	0		*Allium sativum* L.
	0.751	8	90	1	1	TAX 1337	*Allium sativum* L. longicuspis group
	0.152 ± 0.076	25	69	5	0	Bonn, Siegauen	*Allium scorodoprasum* L.
	0.050	42	18	39	0	TAX 0863	*Allium sphaerocephalon* L.
	0.107 ± 0.015	69	19	10	2	TAX 3974	*Allium subvineale* Wendelbo
	0.117 ± 0.008	29	31	41	0	TAX 0454	*Allium vineale* L.
	0.266 ± 0.105	8	39	52	2		*Allium kochii* Lange
	0.302	16	0	84	0	TAX 2646	*Allium umbilicatum* Boiss.
	0.358	2	0	98	0	TAX 5072	*Allium griffithianum* Boiss.
	2.141 ± 0.109	44	56	0	0	TAX 3829	*Allium cupanii* Raf.
	0.204 ± 0.0006	24	12	60	4	TAX 3675	*Allium caesium* Schrenk
	0.213 ± 0.081	89	3	7	0	Bonn-Oberkassel	*Allium oleraceum* L.
	0.438	65	20	15	0	TAX 0652	*Allium rupestre* Stev.
	0.401 ± 0.277	82	0	18	0	TAX 3514	*Allium carinatum* L.
Rhizomatous subgenera							
Subg. *Butomissa*							
	0.306	9	48	42	0	TAX 0583	*Allium tuberosum* Rottl. ex Spr.
Subg. *Cyathophora*							
	0.146 ± 0.017	24	0	74	2	TAX 0165	*Allium cyathophorum* var. *farreri* (Stearn) Stearn
Subg. *Rhizirideum* s.str.							
Incorrectly named “*A. turkestanicum*”	0.055 ± 0.007	31	1	48	21	BG Leipzig	*Allium senescens* L.
	0.077 ± 0.006	41	1	43	15	All 1122	*Allium senescens* L.
	0.559	96	1	2	2	TAX 2773	*Allium angulosum* L.
Subg. *Polyprason*							
	0.154 ± 0.023	48	3	49	1	TAX 1736	*Allium chevsuricum* Tscholok.
	0.062	82	2	17	0	TAX 0320	*Allium hymenorrhizum* Ledebour
Keusgen (1999)	0.057	79	2	19	0		*Allium hymenorrhizum* Ledebour
	0.153 ± 0.255	82	7	10	1	TAX 0632	*Allium saxatile* M. Bieb.
Keusgen (1999)	0.661	94	2	4	0		*Allium saxatile* M. Bieb.
	0.173 ± 0.04	69	26	6	0	All 1123	*Allium globosum* M. Bieb. ex DC.
	0.883 ± 0.705	30	57	4	9	TAX 0094	*Allium obliquum* L.
Keusgen (1999)	0.064	44	56	0	0		*Allium obliquum* L.
Subg. *Cepa*							
	0.251 ± 0.046	11	10	79	0	bought in a shop	*Allium cepa* L.
‘Summit’	0.291 ± 0.028	9	4	86	0		*Allium cepa* L.
‘Ruden’ Iowa	0.03 ± 0.018	1	0	95	3	All 731	*Allium cepa* L. aggregatum group
German landrace	0.034 ± 0.008	23	1	70	6	All 638	*Allium cepa* L. aggregatum group
German landrace	0.052 ± 0.014	22	0	75	3	All 585	*Allium cepa* L. aggregatum group
‘Featherston’ USA	0.054 ± 0.009	1	2	90	8	All 732	*Allium cepa* L. aggregatum group
Georgian landrace	0.057 ± 0.009	13	1	75	10	All 1333	*Allium cepa* L. aggregatum group
Italian landrace	0.081 ± 0.021	26	2	55	18	All 548	*Allium cepa* L. aggregatum group
Georgian landrace	0.094 ± 0.018	4	0	84	12	All 710	*Allium cepa* L. aggregatum group
Korean landrace	0.107 ± 0.025	5	1	90	4	All 734	*Allium cepa* L. aggregatum group
Georgian landrace	0.121 ± 0.011	11	1	79	9	All 708	*Allium cepa* L. aggregatum group
German landrace	0.125 ± 0.027	17	0	78	5	All 1049	*Allium cepa* L. aggregatum group
	0.248 ± 0.002	4	3	88	5	TAX 5009	*Allium galanthum* Kar. et Kir.

Table 3 (*continued*)

Taxonomic groups or source or remarks	Total contents (average) (%)	Relative methiin (%)	Relative alliin (%)	Relative isoalliin (%)	Relative propiin (%)	Origin of material	Species name
	0.206 ± 0.009	12	16	72	0	BG Köln	*Allium* × *proliferum* (Moench) Schrad.
	0.059 ± 0.001	31	5	63	0	TAX 0702	*Allium schoenoprasum* L.
	0.343 ± 0.033	21	0	74	4	TAX 0042	*Allium altyncolicum* Friesen

If standard deviations are given, analysis was repeated at least three times.

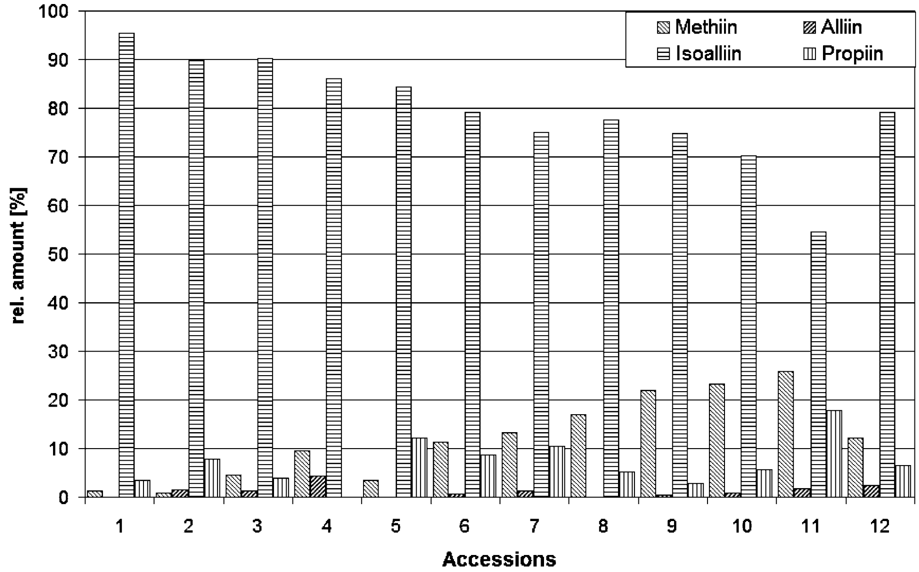

Fig. 2. Cysteine sulphoxides in various *A. cepa* accessions. 1, All 731 *Aggregatum* group, Illinois, USA; 2, All 732 *Aggregatum* group, Illinois, USA; 3, All 734 *Aggregatum* group, Korea; 4, *Cepa* group cultivar 'Summit'; 5, All 710 *Aggregatum* group, Georgian landrace; 6, All 708 *Aggregatum* group, Georgian landrace; 7, All 1333 *Aggregatum* group, Georgian landrace; 8, All 1049 *Aggregatum* group, German landrace; 9, All 585 *Aggregatum* group, German landrace; 10, All 638 *Aggregatum* group, German landrace; 11, All548 *Aggregatum* group, Italy; 12, *A. cepa* average.

Table 4
Content of cysteine sulphoxides in related genera and other plant families

Taxonomic groups or source or remarks	Total contents (average) (%)	Relative methiin (%)	Relative alliin (%)	Relative isoalliin (%)	Relative propiin (%)	Origin of material	Species name
Alliaceae							
	0.031 ± 0.001	97	0	0	3	TAX 2469	*Triteleia laxa* Bentham
	0.006	70	0	7	23	S. Africa, Pretoria region	*Tulbaghia acutiloba* Harv.
	0.058 ± 0.038	1	1	98	0	S. Africa, Durban region	*Tulbaghia violacea* Harv.
	0.089 ± 0.040	2	13	77	8	S. Africa, Durban region	*Tulbaghia simmleri* Beauverd
Brassicaceae							
	0.015 ± 0.022	75	25	0	0	Bonn, Rheinbach	*Alliaria petiolata* (M. Bieb.) Cavara et Grande
Keusgen (1999)	0.006	67	0	17	17		*Alliaria petiolata* (M. Bieb.) Cavara et Grande

If standard deviations are given, analysis was repeated at least three times.

in plant tissue and its substrate specificity. It has been demonstrated in previous investigations that there are significant differences between alliinases from wild *Allium* species (Krest et al., 2000). Furthermore, sulphides derived from isoalliin can only be detected in low amounts (Freeman and Whenham, 1975). Interestingly, isoalliin-rich species such as *A. cepa* and closely related species gave high levels of propyl sulphides by GC analysis (Bernhard, 1970; Freeman and Whenham, 1975). However, neither the relative concentrations of methiin, alliin, isoalliin, and propiin, nor the absolute concentration of these cysteine sulphoxides can be deduced from the results obtained by GC. Nevertheless, the "onion-type" alliums can be identified by their high levels of propyl sulphides, in addition to other species with high levels of alliin. The data presented here are, for the most part, concordant with other investigations of methiin and alliin, although we surveyed a larger number of species than has been covered in previous work.

Methiin is the dominant cysteine sulphoxide in all evolutionary lines of *Allium*, and in addition occurs in the Brassicaceae (Keusgen, 1999). Methiin can be also found in some fungi (Kreuzberg and Keusgen, 2004). Therefore, the content of methiin can only complement taxonomic interpretations based on the presence of additional sulphur containing compounds (e.g., glucosinolates for Brassicaceae, and other cysteine sulphoxides for *Allium*).

The elevated content of cysteine sulphoxides in the third evolutionary line is remarkable. Cysteine sulphoxides, in combination with the enzyme alliinase, are thought to be responsible for chemical protection from herbivory (Keusgen, 2002). High levels of cysteine sulphoxides have also been shown to have antibacterial and antifungal properties which are probably beneficial during extreme environmental conditions. From a phylogenetic point of view, it is likely that the ancestors of *Allium* were able to produce methiin. Later, the ability to synthesize large amounts of the higher homologues evolved independently in several groups (or failed to do so, as in subg. *Melanocrommyum* in the second evolutionary line). The consequent chemical diversity has resulted in differences in the uses of these species by man: The isoalliin-dominated "onion-type" and the alliin-dominated "garlic-type" are widely used, and the Chinese leek with equivalent content of both alliin and isoalliin is used in East Asia. Species with dominating methiin are rarely used, and the two propiin-dominated species are not used by man.

Increasing amounts of total cysteine sulphoxides, and a decreasing complexity in the total cysteine sulphoxide patterns are clear trends in the transition from the first to the third evolutionary lines. However, these trends are not without exceptions. For example, *Allium oleraceum*, *A. rupestre*, *A. carinatum*, *A. angulosum*, *A. hymenorrhizum*, *A. saxatile* and *A. globosum* (third evolutionary line) have the high methiin content typical of plants in the first evolutionary line, but retain a significant variety of cysteine sulphoxides. But in contrast to the first evolutionary line, the total amount of cysteine sulphoxides is high (over 0.5% for *A. saxatile* and *A. angulosum*). Although relative levels of propiin higher than 20% were only found in the first evolutionary line, several members of the subgenera *Allium* and *Rhizirideum* also exhibited relatively high relative levels, with *A. senescens* (up to 21%) being an exception.

Several factors may influence the highly variable and flexible cysteine sulphoxide content in the genus *Allium*. First the growth conditions affect these data. Koch and Lawson (1996) reported significant variation in the content of alliin in *A. sativum*, and for this reason, all material was grown on two similar non-fertilized fields (University of Bonn and IPK Gatersleben) before analysis. Bulbs were harvested at the dormant phase at both sites. Sulphur deficiency, which may reverse the proportion of methiin and isoalliin in common onion (Randle et al., 1995), was not observed. Secondly, the content of cysteine sulphoxides is naturally rather variable, which results in high coefficients of variation (Koch and Lawson, 1996, also our data). This variation can only be compensated for by the analysis of large numbers of samples. Although we have only been able to study less than one tenth of the currently recognized *Allium* species, the present data set includes twice as many accessions as in all former reported investigations combined. Intraspecific variation was noted but the identification of characteristic patterns and typical concentration ranges were still possible. We regard these results as preliminary, and intend further investigations to examine larger numbers of species.

Overall, about 40% of accessions had a low to medium content of cysteine sulphoxides, which would be expected to be associated with a characteristic and intense aroma, 30% contained low amounts, and should exhibit a mild flavour, while 25% contained high amounts and would be expected to have a rather hot taste. Four accessions had particularly high cysteine sulphoxide content, and these would probably taste unpleasantly hot.

3. Experimental

Samples for these investigations were obtained from the Botanical Garden of the Institute for Pharmaceutical Biology, Bonn. No fertilization was applied. The few species cultivated there were obtained by seed exchange from other botanical gardens (marked by "BG" in the tables). Because several of the taxa were not correctly named and had to be re-determined, investigations after 1997 used well defined material from the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. These *Allium* accessions were taken from the living collection of the Gene Bank (marked by "All" in the tables) and from the Taxonomic *Allium* Reference Collection (marked by "TAX" in the tables). All accessions were grown in the same field area without any fertilization. Herbarium vouchers as well as documentation is available at the IPK (http://www.ipk-gatersleben.de/en/). Plant material was analysed at the end of the dormant phase.

All chemicals, unless otherwise mentioned, were purchased from Merck (Darmstadt, Germany) or Fluka (Neu-Ulm, Germany). When necessary, they were purified by standard procedures (distillation of solvents). Synthetic L-(+)-alliin and homologous sulphoxides were prepared following procedures already described (Krest et al., 2000; Koch and Keusgen, 1998). Briefly, L-cysteine was *S*-alkylated followed by oxidation of the sulphur-atom. L-(±)-Alliin was separated into the two diastereomers by fractional recrystallization. Native isoalliin was isolated from *A. cepa* (Krest et al., 2000).

Bulbs, and in some cases rhizomes, were used for analyses of cysteine sulphoxide content. About 0.2–0.8 g (depending on the cysteine sulphoxide content of the material) of fresh plant material were weighed and extracted with 20 ml methanol by heating under reflux for 10 min. The plant material was crushed in a mortar and returned for further extraction with the addition of 20 ml H_2O (Krest et al., 2000; Storsberg et al., 2004). The resulting extract was filtered and the residue washed three times with 3 ml methanol. The combined filtrates were evaporated to dryness under reduced pressure and stored at −20 °C until further use. For HPLC analyses, the residue was redissolved in a phthaldialdehyde-(OPA)-derivatization reagent to give a final volume of 5 ml (Krest et al., 2000). Volumes of 15 μl each were analyzed after 30 min of incubation in the dark. HPLC-separation was carried out according to Krest et al. (2000). Briefly, a Sperimarge 80-ODS2 RP column (5 μm particle size; 250 × 4 mm with integrated guard column) was operated at a flow rate of 1.0 ml min^{-1}. Detection was carried out at 335 nm. A gradient starting at 20% acetonitrile in phosphate buffer (pH 6.5, 0.05 M) up to 30% after 60 min was used as solvent system. The total amount of cysteine sulphoxides as a proportion of the fresh weight of samples, as well as the relative amounts of each of the four cysteine sulphoxides, were determined. If only a single sample was available, standard deviations for the total amount of cysteine sulphoxides were not given in the tables.

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