PHARMACEUTICAL VALUE OF ONIONS (*ALLIUM* L.) AND RELATED SPECIES OF CENTRAL ASIA

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List of Abbreviations

Acc.No.	Accession number
amu	Atomic mass unit
A_S	Enzymatic activity
BHT	Butyrated hydroxytoluene
Con A	Concanavalin A
C_{prot}	Concentration of proteins
cps	Counts per second
CSO	Cysteine sulphoxides
Da	Dalton
DPPH	1,1-Diphenyl-2-picrylhydrazine
e.g.	For example
et al.	And others
Extr.	Extract
FIA	Flow injection analysis
Fig.	Figure
g	Gram
HPLC	High performance liquid chromatography
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung
IR	Infrared
LC	Liquid chromatography
LDH	Lactate dehydrogenase
m	Meter
m	Milli -
min	Minute
n	Nano -
NAD	Nicotinamide-adenine-dinucleotide
NCCLS	National Comitee for Clinical Laboratory Standards
NMR	Nuclear magnetic resonance
No.	Number
OPA	o-Phtaldialdehyde
ppm	Parts per million
RSD	Relative standard deviation
SD	Standard deviation
sect.	Section
So.	Solvent

ssp.	Subspecies
Tab.	Table
TAX.No.	Taxonomic number
TLC	Thin layer chromatography
UV	Ultraviolet
V_F	Dilution factor
VP	Volume parts
%	Percent
ε	Molar extinction coefficient
ΔE	Difference absorption unit
Δt	Difference time
°C	Degree Celsius
µ	Micro -

1. Introduction

1.1. *The Genus Allium*

The genus *Allium* is a very large genus (c. 750 species) and is widely spread over the holarctic region from the dry subtropics to the boreal zone. A region of exceptionally high species diversity stretches from the Mediterranean Basin to Central Asia and Pakistan. A second, less pronounced centre of species diversity occurs in western North America. The majority of species grows in open, sunny, rather dry sites in arid and moderately humid climates. However, *Allium* species have adapted to many other ecological niches as well, causing the development of an astonishing amount of different morphotypes. This is the main reason for the widely recognised difficulties in taxonomy and classification of *Allium* [Gregory et al. 1998].

Common onion and shallot (*A. cepa*) as well as garlic (*A. sativum*) are worldwide known as important species. Other cultivated species possess only regional importance. Well known in Europe are leek (*A. porrum)* and chives (*A. schoenoprasum*), in the eastern Mediterranean area kurrat and tarée (*A. ampeloprasum*) and in East Asia Welsh onion (*A. fistulosum*), rakkyo (*A. chinense*) and Chinese chives (*A. ramosum/A. tuberosum*). About two dozens other *Allium*-species are locally cultivated or collected as highly esteemed vegetables, seasonings, and/or medicinal plants [Hanelt 2001; Fritsch and Friesen 2002; Fritsch et al. 2007]. However, the knowledge about these species is rather incomplete.

1.1.1. *History of the Genus Allium*

Members of the genus *Allium* (onions) have been used by humans since the Neolithic age and are still used in recent times. In this long period there were always people who appreciated and consumed them in considerable amounts, but also those who rejected and detested them [Koch and Lawson 1996].

Ancient Times

Species belonging to the genus *Allium* L. have been already intensively used in ancient times. The place of origin is supposed to be in Central Asia. From there onions and garlic were brought to other countries, *e.g*., India, China, Korea, Japan and also to Mesopotamia and Old Egypt. The most prominent species were *A. sativum*, *A*. *fistulosum*, *A. porrum* and *A. cepa.* These plants were valued not only as seasoning and spices, but also as drugs. Usually the entire plant was utilized.

First written reports about onions are gained from Sumerians dating back to 2600 - 2100 BC. In the Papyrus Ebers, which is based on older Egyptian writings and knowledge, we can find out that leek played an important role in The Kingdom of Old Egypt. In the eyes of people, mostly garlic and onion were highly regarded as a daily diet and medicine.

The Israelites acquired various onion species from Egyptians. They were used medicinally for indigestion, as a diuretic, spasmolytic and also as an aphrodisiac.

Arabs became the knowledge about medicinal use of *Allium* plants from Jewish physicians. In Arabic countries these plants were usually used as a treatment of eye diseases, worms, snake bites, skin rashes, menstrual abnormalities and in veterinary medicine.

The Greeks became acquainted with *Allium* species through Egypt and Orient. Garlic, common onion and other *Allium* species found place in numerous Greek kitchen recipes. They were used as a medicine with other plants in ointments. The great Greek physician Hippocrates recommended *A. sativum*, *A. cepa* and *A. porrum* as diuretic, laxative and emmenagogue. He also used them for pneumonia and externally for putrid wounds [Koch and Lawson 1996].

The ancient Romans obtained the knowledge about the genus *Allium* from Greeks. To the consumers of onions in ancient Rome belonged mainly soldiers, seamen and slaves; the aristocracy detested them. The Romans believed that garlic and other onion species (*e.g*., *A. leucoprasum*, *A. subhirsutum* and *A. ampeloprasum*) have a magic power against evil spirits. Physicians recommended them mostly as diuretic and stomachic treatments and in other cases for expelling of intestinal worms. The famous physician Galen summarized the knowledge about various onions in his work Planta Medica.

Medieval Era

The Romans introduced cultivated garlic and other *Allium* species to Celts and to Germans. Germans and Anglo-Saxons knew about other *Allium* species, for instance *A. ursinum*.

In Middle Ages, various onions *e.g.*, *A. cepa*, *A. sativum*, *A. schoenoprasum*, A. *ascalonicum* and *A. porrum* were cultivated in gardens of monasteries. The monks followed the tradition of Hippocrates, Dioscurides and Galen and used them in a similar manner. Paracelsus prescribed onions as an antidote against the plague, as an expectorant and a diuretic plant and externally for treatment of abscesses and also for the expulsion of the afterbirth.

Various wild *Allium* species were also used intensely in folk medicine, *e.g.*, *A. ursinum* and *A. victorialis* [Koch and Lawson 1996].

1.1.2. *Botany and Taxonomy of the Genus Allium*

There are numbers of classifications of the genus *Allium*. Following hierarchy has been adopted from Friesen et al. [2006]. This large genus contains over 780 species with a huge morphological diversity making classifications into subgenera and sections necessary (see below).

1. Class *Liliopsida*
2. Subclass *Liliidae*
3. Superorder *Liliianaea*
4. Order *Amarylidales*
5. Family *Alliaceae*
6. Subfamily *Allioideae*
7. Tribe *Allieae*
8. Genus *Allium*

Plant morphology

To the underground organs belong roots, rhizomes and bulbs. From a juvenile stadium possess *Allium* plants a root-system, which grows until the adult stadium. Swollen roots serve as a storage organ. Rhizome fails by the species with swollen roots, but it has a similar function. Rhizome can have a diverse shape, orientation and embranchment. The shape of bulbs in genus *Allium* is also very variable,from thin bulbs with a low storage capacity to very thick bulbs with a huge storage capacity [Fritsch 2005].

Leaves are usually basally arranged. Their shape, length, width, surface character, composition of the nerve embranchment and vascular bundle is very diverse by the *Allium* plants. Blossoms create inflorescence, which fasciculate to umbel or head-like shapes. Flowers are bisexual and are composed of three external and three internal tepals, three external and three internal stamens and three carpels, which adhere to ovary and stigma. Shape, size, location, number and colour of the flowers are very diverse. Seeds occur in capsules. They have a black colour and are mostly globular or drop shape. Many species create bulbils, which are important in vegetative propagation.

The majority of species grow in open, sunny and rather dry sites in arid and moderately humid climates. However, *Allium* species have adapted to many other ecological niches [Fritsch and Friesen 2002].

Taxonomy of Genus *Allium*

The genus *Allium* is divided into various subgenera. The following Figure 1.1 shows the phylogenetic division of genus *Allium* according to Friesen et al. [2006]. The listed subgenera are also divided in numerous sections. This system is necessary, because the genus *Allium* contains more than 780 members. The division into subgenera and in the case of the large subgenus *Melanocrommyum* classification into sections is also respected in this investigation.

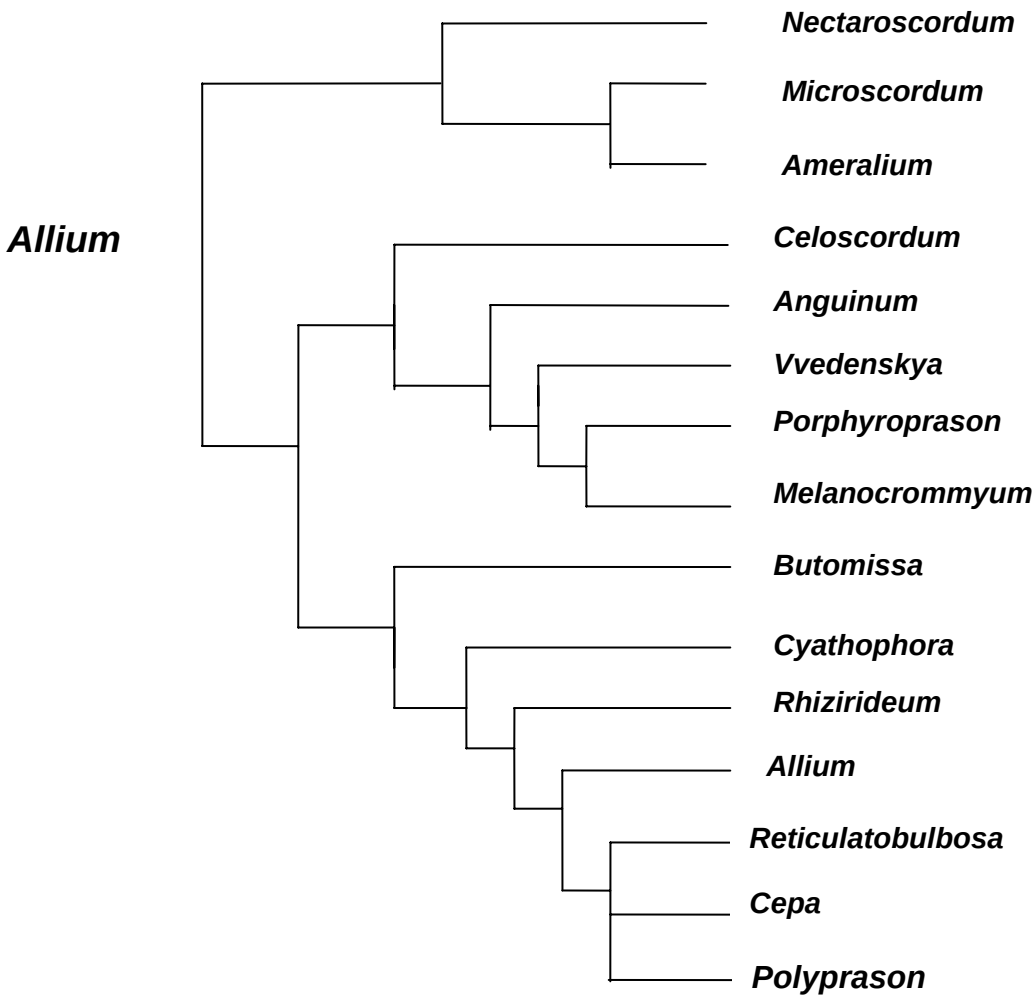


Figure 1.1: Classification scheme into subgenera of the genus *Allium* [Friesen et al. 2006].

1.1.3. *Sulphur Compounds of Allium*

The high amount of organosulphur compounds is one of the biggest characteristics of the genus *Allium*. Many health benefits of leek species are attributed to these compounds, which make up between 1 to 5 % of the dry weight of the bulbs [Block 1992]. The most important sulphur containing substances are the amino acid cysteine and its derivatives, especially the S-substituted cysteine sulphoxides and the γ-glutamyl peptides. There are four basic representatives of cysteine sulphoxides: (+)-S-methyl-L-cysteine sulphoxide, (+)-S-propyl-L-cysteine sulphoxide, (+)-S-(2-propenyl)-L-cysteine sulphoxide and (+)-S-(1-propenyl)-L-cysteine sulphoxide (Fig. 1.2).

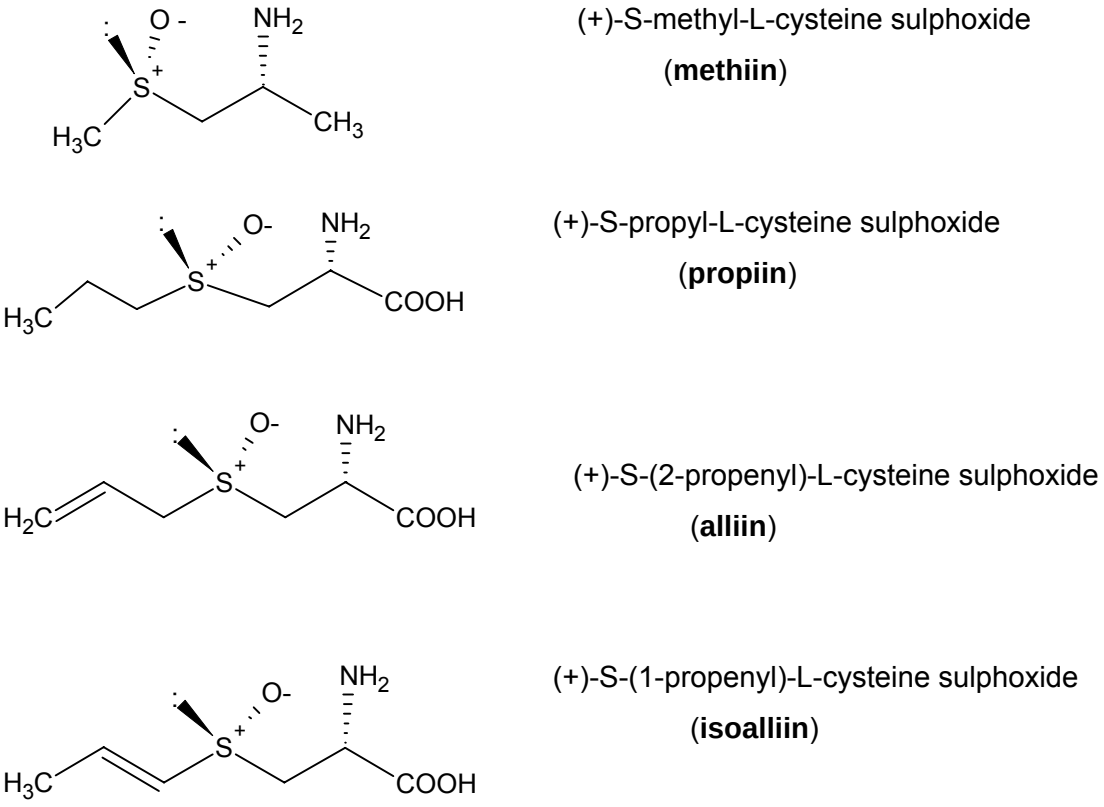


Figure 1.2: The four representatives of cysteine sulphoxides examined in this investigation.

Cysteine sulphoxides can form four diastereomers, but until now there have been only found the (+)-S-alk(en)yl-L-cysteine sulphoxides in the nature [Koch a Lawson 1996]. In garlic occur mainly alliin and methiin, in onion isoalliin and methiin.

The odourless cysteine sulphoxides are stored in an intact cell in the cytoplasm. After disruption of the cell, they are rapidly converted into alk(en)ylsulphenic acid, pyruvic acid and

ammonia. This reaction is catalysed by an enzyme called alliinase (E.C.4.4.1.4), which normally occurs in the vacuole. Products of alliinase cleavage are very unstable compounds, which are rapidly converted into either corresponding thiosulphinates or the lachrymatory factor, so-called primary aroma compounds (Fig. 1.3).

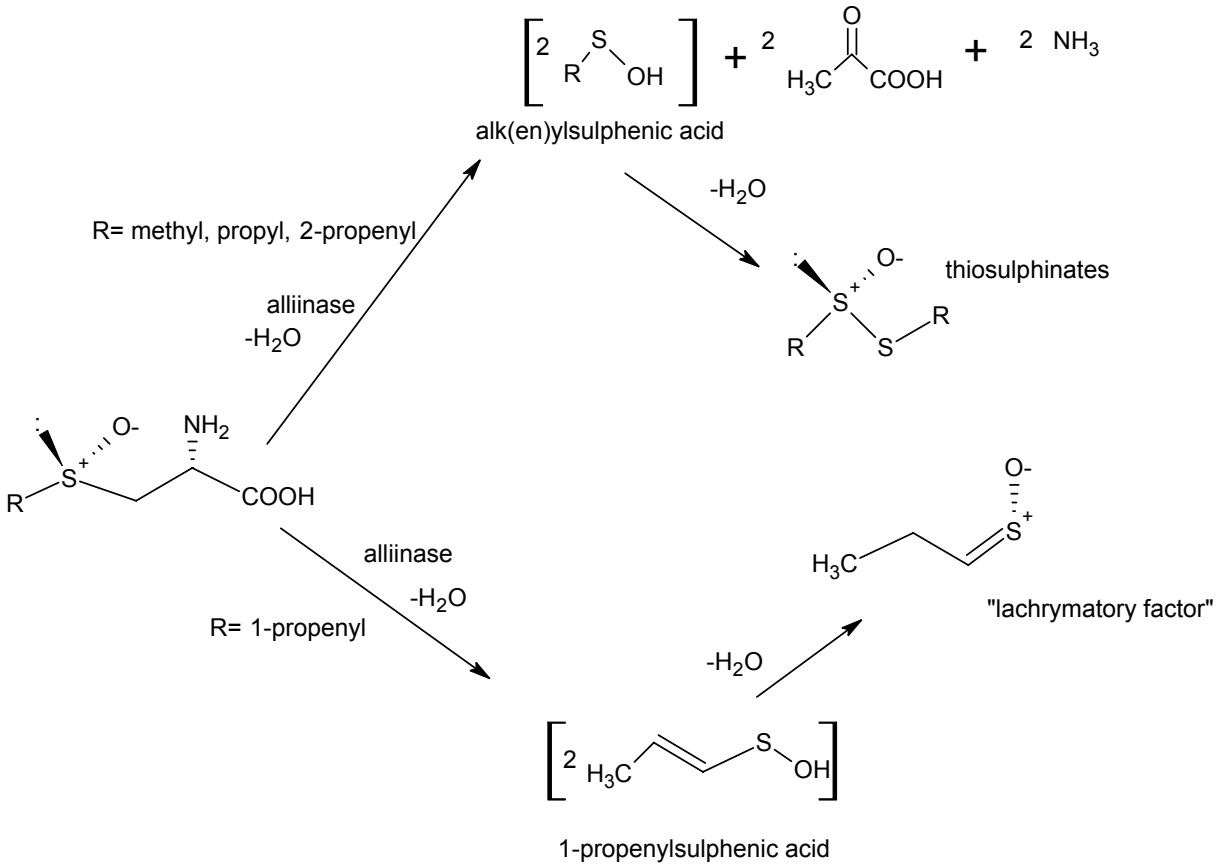
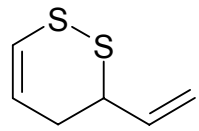
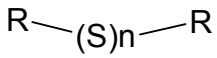


Figure 1.3: Formation of thiosulphinates and the lachrymatory factor.

Primary aroma compounds are also very unstable and highly reactive. They decompose almost completely at room temperature within hours in a variety of different sulphur containing compounds called secondary aroma compounds (Fig. 1.4 and Fig. 1.5). Formation of these compounds strongly depends on the reaction conditions as well as on the thiosulphinates formed by the alliinase reaction.

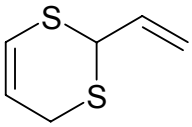


3-Vinyl-4H-1,2-dithiin

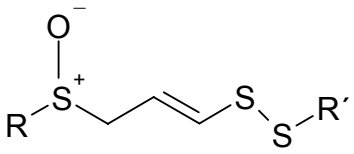


Sulphides:

R=Allyl,methyl

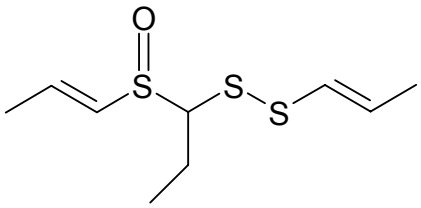


2-Vinyl-4H-1,3-dithiin

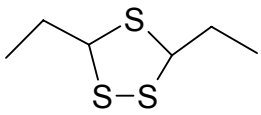


Ajoenes:
R,R´= Allyl: Ajoene
R,R´= Allyl, methyl: Methylajoene
R,R´= Methyl: Dimethylajoene

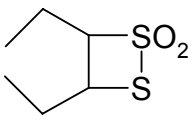
Figure 1.4: Secondary aroma compounds, which are characteristic for garlic.



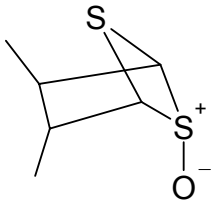
Caepaenes



3,5-Diethyl-1,2,4-trithiolanes



Dithietane-S-oxides



Zwiebelanes

Figure 1.5: Secondary aroma compounds, which are characteristic for onion.

1.1.4. *Non-Sulphur Compounds of Allium*

Apart from organosulphur compounds, *Allium* species also contain other important compounds. Except water (ranging between 65-95%), they contain mainly carbohydrates, flavonoids and saponins. The formation of carbohydrates by *Alliums* is very curious. They do not build starch as a storage substance, but so-called fructanes, which are fructose-based polysaccharides. The fructanes are similar to inulin; the polymerization grade lies between 10 and 15 units [Suzuki and Cutcliffe 1989; Ernst et al. 1998]. As a monosaccharide, glucose

can also occur and contribute to the sweet taste of some kind of onions. Carbohydrates do not influence the nutrition value of *Allium* species, but they play an important role in flavour properties of *Alliums.*

Flavonoids contain phenolic groups, which can interact with many proteins in human organism. Of great interest are the antioxidant effects of this substance class. The main representative of the flavonoids is quercetin.

Steroid saponins are common in *Liliaceae* family and closely related families. In *Alliums*, spirostanol and furostanol types of steroid saponins were found [Inoue 1995]. Additionally, *Allium* species contain malic, citric, succinic, fumaric and quinic acids as well as vitamins such as B_1, B_2, B_6, biotinic, nicotinic, folic, panthotenic and ascorbic acids [Breu 1996].

1.1.5. *Enzymes of Allium*

Enzymes mentioned in this section are only related to the formation and degradation of cysteine sulphoxides. One of the major protein fraction found in *Allium* is alliinase. It was first isolated from garlic by Stoll and Seebeck in 1949 and is probably presented in all members of *Allium* genus. The official name for this enzyme is alliin alkyl-sulphenate-lyase (EC 4.4.1.4) [Randle and Lancaster 2000]. Alliinase belongs to a family of C-S lyases, which cleave bonds between sulphur and carbon atoms. The active enzyme is a pyridoxal-5´-phosphate-dependent homodimeric glycoprotein of 2x 448 amino acids residues and a total molecular weight of 103,000 [Kuettner et al. 2002]. As mentioned above, alliinase is compartmentalized in the vacuole. After disruption of the intact cell, this enzyme catalyses the conversion of cysteine sulphoxides into alk(en)ylsulphenic acid, pyruvic acid and ammonia (Figure 1.6).

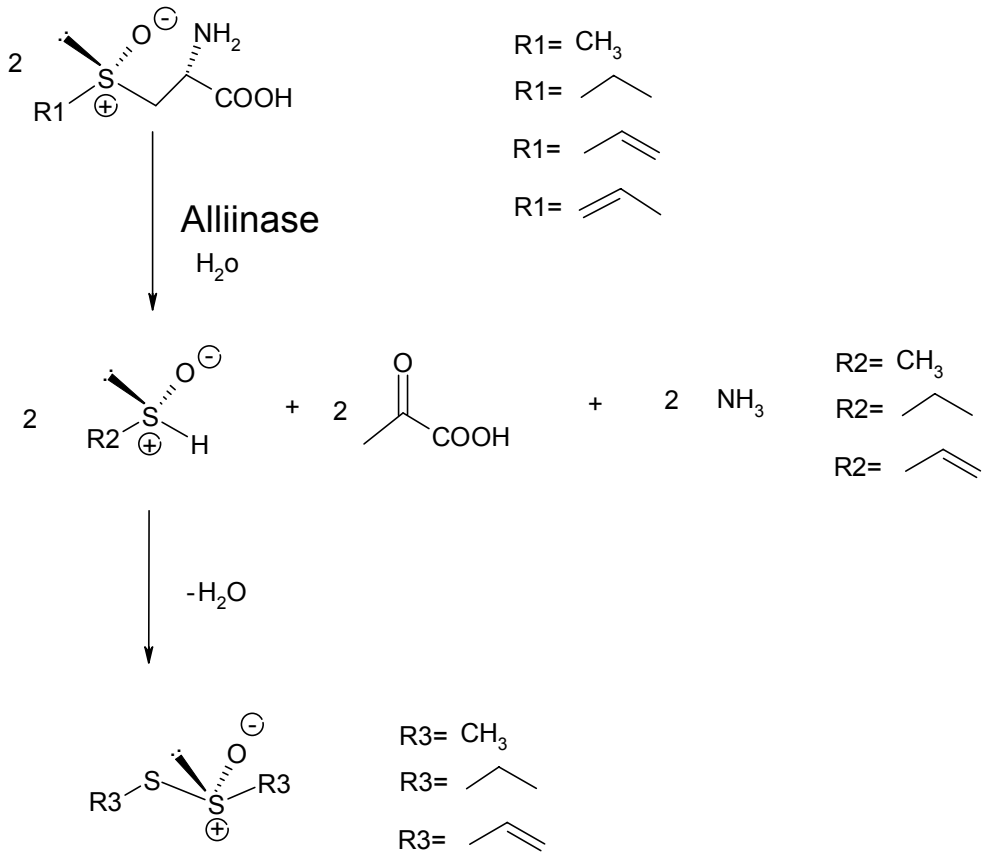


Figure 1.6: Alliinase-catalyzed cleavage of cysteine sulphoxides.

The irritating lachrymatory factor (responsible for stimulating tears), which is released by onions when they are chopped up, has been presumed to be produced spontaneously following the action of alliinase. Imai et al. [2002] however demonstrated that this factor is synthesized by an enzyme called lachrymatory factor synthase. The enzyme showed a high substrate specificity, producing lachrymatory factor from only *trans*-1-propenyl-L-cysteine sulphoxide.

The relationship between γ-glutamyl peptides and alk(en)yl cysteine sulphoxides requires an activity of enzymes to remove the glycyl and γ-gutamyl residues from the nascent alk(en)yl sulphoxide. γ -glutamyl transpeptidase catalyses the transfer of the γ-glutamyl group from γ-glutamyl peptides to either amino acids or other peptides [Jones et al. 2004].

The proposed biosynthesis of the γ-glutamyl cysteine sulphoxides is given in Figure 1.7. The sulphur atom has its origin in sulphate. Directly at the beginning of the biogenetic pathway, cysteine is transferred on glutamic acid. Then, the S-substituted side chain is attached and stepwise modified. Before the glutamic acid is enzymatically cleaved of, the sulphur atom is strictly stereo-specific oxidized [Lawson 1991; Block 1992].

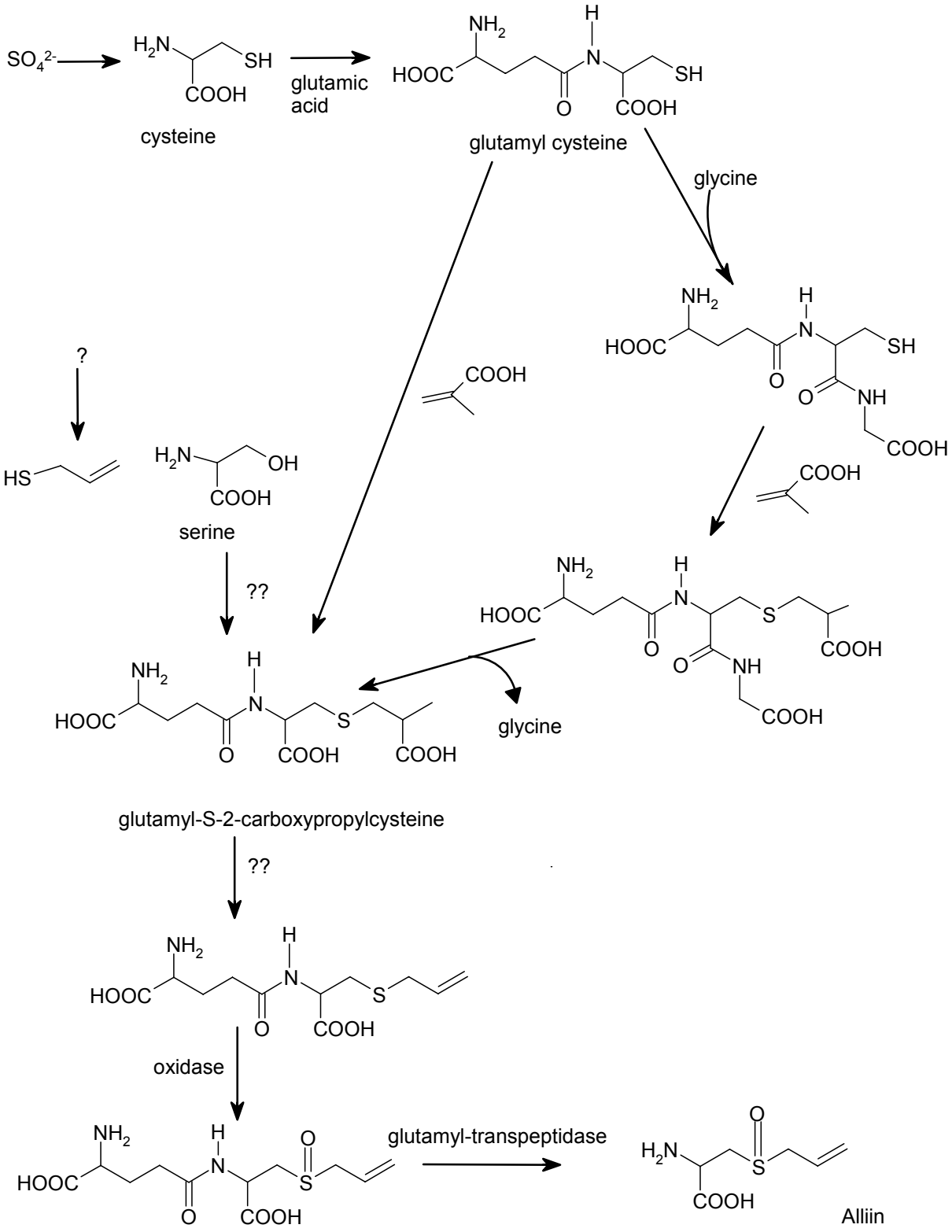


Figure 1.7: Proposed biosynthesis pathway of (+)-S-(2-propenyl)-L-cysteine sulphoxide (alliin). Sulphate is reduced and assimilated into cysteine and then into the glutathione cycle. Glutathione and γ-glutamylpeptides are the first intermediates in the biosynthetic pathway to flavour precursors in *Allium* species.

1.1.6. *Bioactivity and Medicinal Use of Garlic (Allium sativum L.)*

Garlic is one of the best-studied medicinal plants. As mentioned, antibacterial and antiseptic properties were already described by Egyptians, Greeks and Romans. The various effects of garlic on bacteria, fungi, protozoa and viruses have been shown in vitro as well as in vivo. The antibiotic activity is mainly due to allicin [Koch and Lawson 1996; Ankri and Mirelman 1999]. Allicin is formed from two molecules of allylsulphenic acid, which arise from the alliinase catalyzed cleavage of alliin. Antimicrobial activity has been demonstrated also by ajoene and diallyl disulphides [Naganawa et al.1996].

Studies on animal and humans, beginning the 1960s, show that garlic has an effect on heart and circulatory system. By appropriate application, garlic may protect the blood vessels from the deleterious effect of free radicals, exert a positive influence on blood lipids, increase capillary flow and lower elevated blood pressure levels.

According to recent studies, some of the antiatherosclerotic effects are based on the reduction of trombocyte adhesiveness and aggregation. The tendency of the platelets to aggregate and to form thrombi is significantly decreased by the effective constituents of garlic.

The most important risk factors for developing arteriosclerosis with its secondary effects, such as myocardial infarction, stroke and occlusive arterial disease, are hyperlipidemia and hypercholesterolemia, in addition to obesity, high blood pressure and diabetes [Koch and Lawson 1996]. An important role also plays an unhealthy life style (e.g., alcohol and nicotine abuse, bad feed habits), stress and genetic predisposition. The mode of action is probably the suppression of cholesterol biosynthesis. Allicin, ajoene and diallyl disulphide seem to be responsible for this action.

Garlic extracts are very reputed to reduce the incidence of tumours. Several sulphur-containing compounds of garlic were tested for their chemopreventive potential. Diallyl disulphide, allyl sulphide, ajoene but also alliin are responsible for the antiproliferative effect of garlic [Keusgen 2002a, Qi and Wang 2003].

1.1.7. *Bioactivity and Medicinal Use of Onion (Allium cepa L.)*

Antibiotic properties of onion are known since ancient times. In the second half of the 20th century, onion extracts, essential oils and onion juice have been intensively investigated for antimicrobial activities. Positive effects were observed almost against Gram-positive bacteria. Onion extracts showed only weak virostatic and antifungal activity.

Fresh onion juice is often recommended in the folk medicine of various countries for pain and swelling after bee or wasp stings. The observed high efficacy of this treatment for this so-called late cutaneous allergic reaction led to the discovery of various compounds in

onion with anti-inflammatory and antiasthmatic activity [Dorsch 1996]. Antiasthmatic properties of onion extracts were studied *in vitro* as well *as in vivo*. Thiosulphinates and cepaenes were identified as the active compounds.

Onion bulbs exhibit antiplatelet activity. Part of the antiaggregation activity of onion preparations seems to be mediated by the inhibition of thromboxane biosynthesis in platelets. Reducing platelet aggregation has a preventive effect on some cardiovascular disorders such as atherosclerosis. In addition, onion extracts posses some lipid-lowering and in higher concentrations also hypoglycaemic effects [Keusgen 2002a].

1.1.8. *Bioactivity and Medicinal Use of Further Onions (Allium spec.)*

Several studies have been carried out with other *Allium* species. It was demonstrated that a number of them contain cysteine sulphoxides in considerable amounts, as well as active alliinase [Keusgen 1999]. Certain antimicrobial effectiveness could be determined for instance by shallot (*A. ascalonicum* L.), chinese leek (*A. odorum* L.) and scallion (*A. fistulosum* L.) [Yin and Tsao 1998]. Leaves, bulbs and roots of *A. nutans* L. and *A. pskemense* B. Fedtsch. were tested for antioxidant activity. All investigated plant parts exhibited antioxidant properties [Stajner et al.1999; Stajner et al. 2002]. Steroid saponins isolated from *A. jesdianum* Boiss. et Buhse and *A. karataviense* Regel as well as steroidal glycosides obtained from *A. macleanii* Bak. and *A. senescens* L. showed cytostatic and cytotoxic effects against different tumour cells [Keusgen 2002a; Mimaki et al.1999].

1.2. *Analytical Methods for the Determination of Allium Compounds*

There are several established analytical methods, which are used for the determination of *Allium* secondary metabolites. Among these, chromatographic methods are most important. Volatile compounds are usually determined by GC and HPLC, non-volatile compounds by

- thin layer chromatography (TLC)
- low pressure liquid chromatography (LC)
- high performance liquid chromatography (HPLC).

New analytical methods for determination of *Allium* compounds are

- flow-through and flow-injection analysis (FIA)
- biosensor for FIA analysis.

Analytical methods used in this investigation were focused on non-volatile *Allium* compounds, such as cysteine sulphoxides.

1.2.1. *Thin layer Chromatography (TLC)*

The apparatus required for TLC separation consists of a plate and a developing chamber. In TLC, the adsorbent is relatively thin, uniform layer of dry, finely powdered material applied to a glass, plastic, metal, or plate (as adsorbent activated alumina or silica gel can be used). Separation proceeds in the developing chamber. The bottom of the developing chamber is covered with a developing solvent system. Walls of the chamber are usually lined with paper to saturate the chamber with the solvent system. The achieved separation is based on adsorption, partition, ion-exchange or on combination of these mechanisms [USP 2000; PhE-Supplement 2001]. After the separation, the developed plates can be sprayed with various reagents to allow the detection by daylight. Separated substances, which exhibit or extinct fluorescence, may be detected by UV light.

Sulfur-containing constituents of garlic have been already separated and detected via TLC analysis [Keusgen 1997]. TLC is also applied for identification of alliin in garlic powder in European Pharmacopoeia.

1.2.2. *Low Pressure Liquid Chromatography (LC)*

The apparatus required for LC separation consists only of the chromatographic tube itself. The tube is cylindrical and is made of glass or other materials such as nylon, quartz or metal. A smaller-diameter delivery tube is fused by a leak-proof joint to the lower end of the main tube. The delivery tube may include a stopcock for accurate control of the flow rate of solvents through the column. The adsorbent (such as activated alumina, silica gel, calcined diatomaceous silica, etc.) as a dry solid or as a slurry is packed into the chromatographic tube. An examined material (for example plant extract) either in solid or in a liquid form is added to the top of the column and allowed to flow into the adsorbent. Single components adsorb in a narrow transverse band at the top of the column. An additional solvent is allowed to flow through the column, either by gravity or by application of air pressure, each substance progresses down the column at a characteristic rate resulting in spatial separation [USP 2000]. The separation is mainly based on mechanisms of adsorption, mass distribution, ion exchange, size exclusion or stereochemical interaction [PhE-Supplement 2001]. The separated substances are usually examined by daylight or ultraviolet light. LC is usually applied for a preparative separation of active substances from a plant extract.

1.2.3. *High Performance Liquid Chromatography (HPLC)*

This separation method (sometimes named high pressure liquid chromatography) was developed from the LC to improve and accelerate the separation. The apparatus consists of a pumping system, an injector, a chromatographic column, a detector and a data acquisition system. The mobile phase is supplied from one or several reservoirs and is pumped through the column, usually at a constant rate, and then through the detector [PhE-Supplement 2001]. The size of particles of the stationary phase ranges between 3 and 10 µm in diameter [USP 2000]. Stationary phase is packed in a column, usually made from stainless steel, to hold out the huge pressure. The separation is achieved by partition, adsorption, or ion-exchange processes, depending on the type of stationary phase. Detectors commonly employed in HPLC are UV/VIS spectrophotometers, including diode array detectors. Fluorescence spectrophotometers, differential refractometers, electrochemical detectors, mass spectrometers, light scattering detectors or radioactivity detectors may also be used.

1.2.4. *Flow-Through and Flow-Injection Analysis (FIA)*

FIA is based on injection of sample plug into a carrier stream of reagent, which flows through a microreactor into a detector of choice. The apparatus consists of a high multichannel peristaltic pump, an injection valve, a coiled reactor, a detector such as photometric flow cell, and an autosampler.(As the reacted sample plug passes through the detector, a transient signal is observed which yields the readout, in the form of peak, usually within seconds).

A typical FIA is schematically depicted in Figure 1.8. In this example, two reaction coils are combined with each other, which would allow a two step chemical modification of the analyte. By this procedure, one compound can be selectively detected even out of a mixture of different substances.

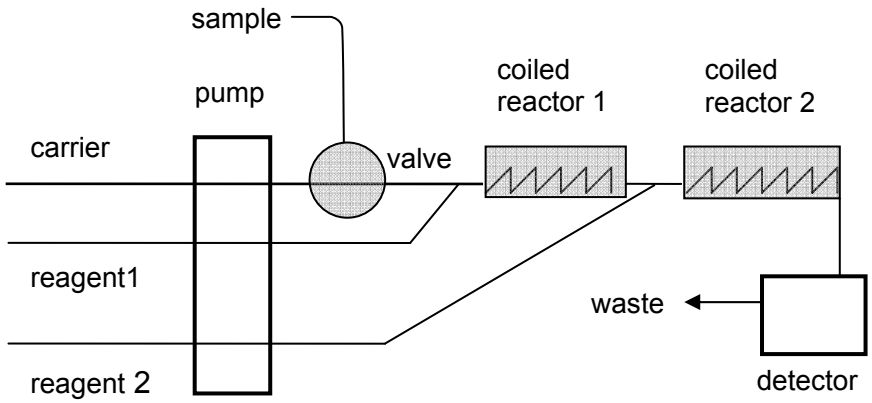


Figure 1.8: Schematic draw of a flow injection analyzer (FIA) containing two reaction coils. These coils can be also replaced by enzymatic reactors.

As a variation of this principle, reaction coils as well as the detector can be replaced by a biosensor. A biosensor is defined as a device consisting of a biological part and a physical transducer. Both parts are in direct contact and should last for a number of measurements, not only for single use. Biosensors were initially developed for clinical diagnosis, *e.g.*, in the determination of blood glucose. In recent years, biosensors have been developed for many additional applications, such as quality control of food, bioprocess control and environmental analysis. A principle of a biosensor is described in the Figure 1.9 below.

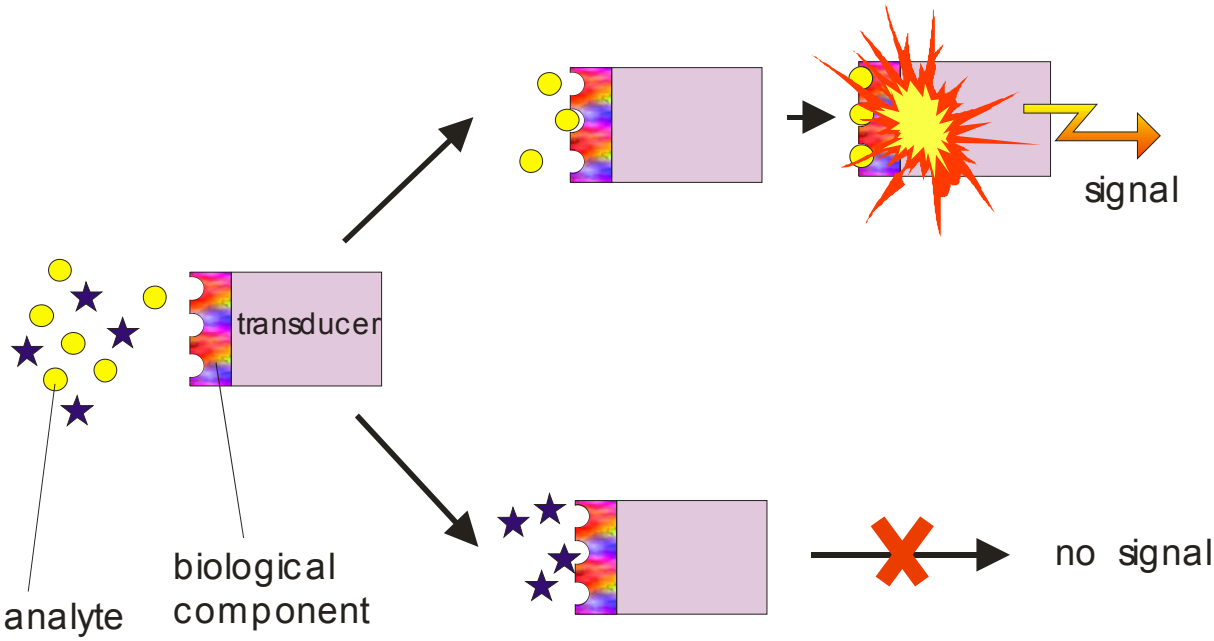


Figure 1.9: Principle of function of a biosensor. One compound (circles) of a mixture of substances specifically interacts with the biological part of the sensor. The resulting

biological signal is converted into a physical signal (*e.g*, electric or optical) by a transducer. Substances, which are not able to interact with the biological component, will not produce any signal [Keusgen 2002b]. As transducer elements, electrical devices such as electrodes, semiconductors, and optical components are often used, as biological components, biomolecules such as enzymes, receptors, antibodies, and whole cells. A biosensoric FIA for the determination of cysteine sulphoxides could be already realized by Krest [2002]. Instead of a reaction coil, an enzyme cartridge filled with alliinase was used. Then, the enzymatically formed ammonia was determined by an ionsensitive electrode.

2. Scope of Investigation

2.1. *Screening on Cysteine Sulphoxides*

The scope of this research project is the investigation of members of the leek family (*Allium*), which are characteristic for the Caucasus and Middle Asia. In these areas, occurrence of a great number of extremely diverse *Allium* taxa is a characteristic element of the vegetation. Use of these plants in the folk's medicine has been incompletely studied yet, and information about the pharmacologically active substances is extremely scarce. It is supposed that these plants are highly valuable in terms of their health benefits in the daily nutrition. However, it will be impossible to investigate all species during the course of this research project. But research results will be the basis for further joined projects.

Precursors of sulphur containing bioactive compounds are cysteine sulphoxides. Therefore, extensive analysis of these compounds is the main focus of interest. Samples from the area of investigations, mainly Iran, Uzbekistan, Turkmenistan and Tajikistan, as well as from the collection of IPK, Germany will be analysed for their contents of cysteine sulphoxides like alliin, isoalliin, methiin and propiin. Additionally, the formation of the red pigment will be investigated. It can be assumed, that this compound is also related to sulphur chemistry.

Analysis of cysteine sulphoxides should be based on two methods: i) biosensoric FIA and ii) HPLC. The biosensoric FIA should base on immobilized alliinase and will be used for the determination of the total amounts of cysteine sulphoxides. For this task, a new method grounded on fluorescence detection has to be developed. The method should be fast, robust and sensitive. For more detailed analysis, HPLC will be used. By this method, valuable wild *Allium* species containing high amounts of alliin and isoalliin should be identified.

2.2. *Testing for Bioactivity*

Further on, some selected *Allium* species will be tested for their bioactivity. From literature data, it can be assumed that many species will have an antibiotic and a radical scavenger effect. For a rapid testing on antibiotic effects, the agar diffusion test will be used. Effects against fungi, algae and different bacteria strains will be evaluated by the inhibition zone surrounding a sample on an agar plate.

For testing on radical scavenger activity, an appropriate test has to be established. The problem with *Allium* extracts is the high content in reactive sulphur compounds, which might disturb many standard assays containing iron or further sulphur compounds. If a suitable test system could be established, a variety of samples should be tested.

2.3. *Aspects of Chemotaxonomy*

The genus *Allium* is a very large genus (more than. 780 species) and is widely spread out over the holarctic region from the dry subtropics to the boreal zone. However, the knowledge about these species is rather incomplete. Especially division of this genus into subgenera as well as sections causes huge problems. Also the exact determination of certain species is often very difficult.

Results obtained from HPLC investigations should be compared with each other. If possible, results obtained for the same species collected from different places as well as results for bulbs and leaves should be compared with each other in order to gain information about intraspecific variations of cysteine sulphoxides. Further on, content of cysteine sulphoxides inside different subgenera and sections should be evaluated. The goal should be to find characteristic chemotaxonomic features for different subgenera.

3. Material and Methods

3.1. *List of Investigated Allium Species*

Samples respected in this investigation were collected in Georgia (only two single samples), Iran, Turkmenistan, Uzbekistan and Tajikistan. An overview of collecting sites between the years 2002 and 2004 is given in Figure 3.1. A complete list of investigated samples is given in Tables 3.1-3.5.

Figure 3.1: Collecting sites (dots) during the PharmAll-project. Samples were collected between 2002 and 2004. All samples were described in the PharmAll database. Some samples were collected by project partners in areas outside the marked districts. This map was kindly provided by Prof. Michael Keusgen.

Table 3.1: *Allium* species collected in Uzbekistan

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. aflatunenese B. Fedt.	4101•	06.06.2005	25.03.2004 [a]	Chatkal reserve
A. alaicum Vved.	4125	21.05.2003	06.02.2004 [a] 18.09.2003	Alaj range, Aravan mountains, between Osh and Aravan
A. aroides M. Pop. et Vved.	4168	29.05.2003	12.07.2003 [a]	West Hissar, village Kaltakul, 3 km by the road to Tashkurgan
A. caspium ssp. baissunense (Lipsky)Kh.& Fr.	4164	28.05.2003	03.09.2004 [a]	25 km eastern Bajssun, side valley to the north of the main road

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. aff. brevidentiforme Vved.	4169	29.05.2003	15.10.2004 [a] 12.08.2003 [c]	West Hissar, village Kaltakul, 6 km by the road to valley Kirgalma
A. caesium Schrenk	4187	03.06.2003	17.02.2004 [a]	Ugam mountains, north end of village Khumsan, opposite to sanatorium
	4130	22.05.2003	14.07.2003 [c]	Angren valley, Kurama mountain range, 4 km down of Irtashsaj
A. caspium (Pallas) M. Bieb.	4021	1.10.2002	30.04.2004 [a]	Buchara field station 30 km north from the City of Buchara
A. crystallinum Vved.	4160	28.05.2003	13.10.2004 [a]	25 km eastern Bajssun, side valley to the north of the main road
A. cupuliferum Regel	4176	31.05.2003	09.12.2003 [a]	Aktau mountains, slate slope near the way to Langar (2 km southern Langar)
A. drepanophyllum Vved.	4153	27.05.2003	13.08.2003 [a]	Pass between Derbend and Sajrob
A. filidens Regel	4144	26.05.2003	02.02.2004 [a]	Tamerlan gate 5 km south of Jizzakh
	4190	03.06.2003	13.08.2003 [b]	Karzhantau mountains, side valley above the sanatorium, north of Khumsan
	4185	02.06.2003	18.08.2003 [c]	Malguzar mountains, right bank of Sanzar river, hill near village Sanzar
	4147	26.05.2003	05.08.2003 [c]	5 km south-eastern Dehkkanabad
A. aff. gusaricum Regel	4120	20.05.2003	13.10.2004 [a] 28.07.2003 [c]	Village Chorkesar, granitic rocky slopes
A. gypsaceum M. Pop. et Vved.	4151	27.05.2003	02.02.2004 [a]	Pass between Derbend and Sajrob
A. haneltii F.O. Khassanov & R.M. Fritsch	4117	19.05.2003	09.08.2003 [a]	Soft slope c. 5 km SW of Chorkesar
A. barsczewskii Lipsky	4166	29.05.2003	30.03.2004 [a]	West Hissar, village Kaltakul, 3 km by the road to Tashkurgan
A. karataviense Regel	4139	24.05.2003	07.08.2003 [a]	Ugam range, Nauvalisaj, 3 km above the road
	4193	04.06.2003	11.09.2003 [b]	Chatkal mountains, north of village Chetsuv
A. komarowii Lipsky	4170	29.05.2003	20.07.2003 [a] 10.01.2004 [a] 10.09.2003 [b]	West Hissar, village Kaltakul, 7 km by the road to valley Kirgalma

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. sativum L.	4900	05.06.2003	15.04.2003 [a] 18.08.2003 [b] 19.08.2003 [c]	Botanical Garden Tashkent
A. giganteum Regel	4165	28.05.2003	12.07.2004 [a] 17.09.2003 [b]	25 km eastern Bajssun, side valley to the north of the main road
A. margaritiferum Vved.	4156•	27.05.2003	19.02.2004 [a] 18.08.2003 [b]	5 km northern Sherabad and 3 km from turn of old way to Zarabag
A. motor Kamelin et Levichev	4133	23.05.2005	12.07.2003 [a] 10.07.2003 [a] 10.09.2003 [b] 06.12.2004 [b]	Chimgan massif, Aksaj and Chimgansaj
A. ophiophyllum Vved.	4155	27.05.2003	09.02.2004 [a]	5 km northern Sherabad and 3 km from turn of old way to Zarabag
A. orunbaii F.O. Khass. et R.M. Fritsch	4118•	19.05.2003	09.08.2003 [a]	soft slopes c. 5 km SW of Chorkesar
A. oschaninii O. Fedt.	4123	21.05.2003	25.04.2004 [a]	Alaj range, Aravan mountains, between Osh and Aravan
A. pskemense B. Fedt.	4142	24.05.2003	09.08.2003 [a] 11.08.2003 [b] 11.08.2003 c	Ugam range, village Sijjak, house garden, collected in Nauvalisaj
A. severtzovioides R.M. Fritsch	4140	24.05.2003	30.08.2004 [a]	Ugam range, Nauvalisaj, 5 km above the road
A. taeniopetalum Popov & Vved.	4180	02.06.2003	06.02.2004 [a]	Pistalitau, middle section of western part, 25 m eastern of Yangikishlok
A. tashkenticum Khassanov et R. M. Fritsch	4131	23.05.2003	27.09.2004 [a] 10.08.2003 [a]	Chimgan massif, Galabasaj
A. stephanophorum Vved.	4183	02.06.2003	17.02.2004 [a]	Malguzar mountains, right bank of Sanzar river, hill near village Sanzar
A. verticillatum Regel	4175	31.05.2003	27.07.2004 [a]	Aktau mountains, slate slope near the way to Langar (5 km southern Langar)
A. talassicum R.M. Fr.ined.	4135 4134	23.05.2003	06.12.2004 [a] 06.12.2004 [b] 12.08.2003 [b]	Chimgan massif, Aksaj and Chimgansaj
	4116	19.05.2003	14.07.2003 [c]	E part of Kamchik pass, slopes near the way c. 5 km below the pass
A. turkestanicum Regel	4143	26.05.2003	10.08.2003 [a] 11.08.2003 11.08.2003 [c]	Tamerlan gate 5 km south of Jizzakh

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. pallasii Murr.	4124	21.05.2003	13.10.2003 [a]	Alaj range, Aravan mountains, between Osh and Aravan
A. jodanthum Vved.	4188	03.06.2003	12.08.2003 [b]	Karzhantau mountains, side valley above the sanatorium, north of Khumsan
A. barsczewskii Lipsky	4136	23.05.2003	05.08.2003 [c]	Chimgan massif, Chimgansaj
A. severtzovioides R.M. Fritsch	4137	23.05.2003	22.07.2003 [c]	Chimgan massif, Aksaj
A. protensum Wendelbo	4145	26.05.2003	22.07.2003 [c]	5 km south-eastern Dehkkanabad
A. sp. (*sect. oreiprason*)	4154	27.05.2003	18.08.2003 [c]	Pass between Derbend and Sajrob
A. sarawschanicum Regel	4172	29.05.2003	12.08.2003 [c]	West Hissar, village Kaltakul, 8 km by the road in the valley Kirgalma
A. talassicum Regel	4122	20.05.2003	28.07.2003 [c]	Village Chorkesar, granitic rocky slopes
A. litvinovii Drob. ex Vved.	4119	20.05.2003	09.09.2003 [c]	Village Chorkesar, granitic rocky slopes

[a] Extraction for determination of amount of cysteine sulphoxides using HPLC
[b] Extraction for determination of scavenger activity.
[c] Extraction for determination of amount of cysteine sulphoxides using biosensoric method.

- Botanical names of these Acc. numbers have not been finally determined yet

Table 3.2: *Allium* species collected in Tajikistan

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. jodanthum Vved.	6040	21.04.2003	17.05.2003 [a]	South part of Darai Odamkhur, stony steep slope, between Pistacia, Ferula
A. barsczewskii Lipsky	6085	29.04.2003	14.08.2003 [b]	By the way to Pamir prior to Jakhchipun, slopes along the way, different
	6168	29.06.2004	19.07.2004 [a] 18.03.2005 [a]	Valley of river Obi-borik, village Zindowud
	6105•	06.05.2003	10.07.2003	Slopes above Teguzak village, Sebiston W region of Wakhsh range

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. chitralicum Wang et Tang	6097	03.05.2003	21.05.2003 [a]	NW exposed stony slopes above Garm Chashma village
A. darwasicum Regel	6073	27.04.2003	04.07.2003 [a]	Loess terraces at the top of the hill between two soy 1.5 km W of Khojidara
	6074	27.04.2003	10.09.2003 [b] 28.10.2003 [c]	E exposed grassy slopes under Crataegus pontica
A. fedschenkoanum Regel	6911•	not given by collector	06.03.2003 [a] 07.03.2003 [a]	Gissar Mountain around Anzob
A. flavellum Vved**.**	6111	07.05.2003	07.05.2003 [a]	Hissar range, drainage of Varzob river, Kondara valley, left side-valley
A. hissaricum Vved**.**	6106	06.05.2003	19.07.2004 [a] 19.05.2003 [a] 18.09.2003 [b]	Slopes above Teguzak village, Sebiston W region of Wakhsh range, in Rosa
A. rosenbachianum ssp. Kwakense R.M. Fritsch	6107	06.05.2003	09.07.2003 [a] 10.07.2003 [a]	Slopes above Teguzak village, Sebiston W region of Wakhsh range
A. rosenbachianum R.M. Fritsch	6050	23.04.2003	17.09.2003 [b] 20.10.2003 [c]	Top region of Khodzha Mumin, near path from Mazor, place Archazor
A. rosenorum R.M. Fritsch	6110	07.05.2003	25.06.2003 [a] 19.06.2003 [a] 10.09.2003 [b] 06.12.2004 [b] 10.07.2003 [c]	Hissar range, drainage of Varzob river, Kondara valley, left side-valley
A. suworowii Regel	6076	27.04.2003	20.06.2003 [a]	Slopes between sandstone rocks, different exposition, rich soil
	6083	29.04.2003	29.06.2003 [a] 06.10.2003 [c]	By the way to Pamir prior to Jakhchipun, slopes along the way
	6090	30.04.2003	17.03.2004 [a] 07.10.2003 [c]	Shergovad village 23 km below Kalai Khumbgarden area
	6112	07.05.2003	16.02.2005 [a] 03.06.2003 [a] 07.03.2005 [b]	Hissar range, drainage of Varzob river, Kondara valley
A. alexeianum Regel	6136	24.06.2004	17.07.2004 [a] 10.02.2005 [a] 10.04.2005 [a] 20.03.2005 [b]	W steep slopes E Iskanderkul lake left side of Saritag river Mt. Qozkhona
A. caeruleum Pall.	6166	29.06.2004	17.07.2004 [a] 08.03.2005 [a] 07.03.2005 [b]	Valley of river Obi-borik, place Daroshka

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. carolinianum DC.	6150	26.06.2004	21.07.2004 [a] 07.02.2005 [a] 06.01.2005 [b]	Right side of Karakul river, N exposed slope of Mt. Saritag
A. giganteum Regel	6912		28.02.2005 [a] 06.01.2005 [b]	
A. hymenorrhizum Ledebour	6163	28.06.2004	08.04.2005 [a] 09.02.2005 [a] 06.01.2005 [b]	W side of the end of 7th lake
A. komarowii Lipsky	6142	24.06.2004	06.04.2005 [a] 23.02.2005 [a] 07.12.2004 [b]	Mountain Qozkhona next valley E of mouth of Saritag river
A. oschaninii O. Fedt.	6135	24.06.2004	21.07.2004 [a] 08.12.2004 [a] 08.12.2004 [b]	W steep slopes E Iskanderkul lake left side of Saritag river Mt. Qozkhona
	6084	29.04.2003	13.08.2003 [b]	By the way to Pamir prior to Jakhchipun, slopes along the way, 2 km higher than 6083
	6077	27.04.2003	14.10.2003 [c]	Slopes between sandstone rocks, different exposition, rich soil
A. schugnanicum Vved.	6094	02.05.2003	11.09.2003 [b] 20.10.2003 [c]	Ishkashim massif, gorge Gojak, N exposed stony slope
A. bucharicum Regel	6054	23.04.2003	17.09.2003 [b] 27.10.2003 [c]	W-facing steep loess slope 2 km north of village Kairagoj
A. verticillatum Regel	6113	07.05.2003	11.09.2003 27.10.2003 [c]	Hissar range, drainage of Varzob river, Kondara valley
A. stipitatum Regel	6101	04.05.2003	18.09.2003 [b]	Margzor, on the way Kalaikhumb to Shuroobod, 307 km to Khorog from Dushanbe
A. griffithianum Boiss.	6031	19.04.2003	14.08.2003 b 09.09.2003 [c]	Khodzhagii Khajriya 30.5 km W of Panj town loess slopes near way from Dusti
A. sativum L.	6103	05.05.2003	20.07.2004 [b]	Panj valley 2 km W of Devdara, SE exposed, wet area of a karst spring
	6102	05.05.2003	06.10.2003 [c]	Panj valley between Kalai Khumb and Shuroobod 344 km from Khorog Bot. Garde
A. winklerianum Regel s. lat.	6145	25.06.2004	17.07.2004 [a] 07.12.2004 [a] 07.12.2004 [b]	2 km W Saritag, left side of Karakul river, S slope, Tagoipista

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. winklerianum Regel s. lat.	6171•	30.06.2004	21.04.2005 [a] 21.07.2004 [a]	Anzob pass area above Ziddi village
A. winklerianum Regel	6081	28.04.2003	07.10.2003 c	W slopes of Kugi Frush, N exposition, open place in Prangos association
A. subg. Allium	6045	22.04.2003	14.10.2003 [c]	E slopes of Khodzha Mumin about 1 km above Kairagoj, near former oil
A. insufficiens Vved.	6042	21.04.2003	19.08.2003 [c]	Loess terraces S of Darai Odamkhur, grassy area without trees
A. filidens	6164	28.06.2004	22.03.2005 [a] 21.07.2004 [a]	near upper end of 6th lake, village Qijoguly

[a] Extraction for determination of amount of cysteine sulphoxides using HPLC
[b] Extraction for determination of scavenger activity.
[c] Extraction for determination of amount of cysteine sulphoxides using biosensoric method.
• Botanical names of these Acc. numbers have not been finally determined

Table 3.3: *Allium* species collected in Iran

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. pseudobodeanum R.M.Fritsch et Matin	1024	23.04.2004	16.03.2005 [a] 05.01.2005 [b]	Central Kopetdag chain NE of vill. Cheve Ly
A. cristophii Trautv.	1016	20.04.2004	15.02.2005 [a] 05.01.2005 [b]	Region near passJakhtikalon, SE-border of Golestan Reservation
A. chelotum Wendelbo	1006	19.04.2004	08.12.2004 [a] 08.12.2004 [b]	Golestan National Park, Golza valley
A. iranicum (Wendelbo) Wendelbo	1022•	22.04.2004	05.07.2004 [a] 14.07.2004 [a] 14.07.2004	Binalud Massif, slopes of the valley above Akhlamat, close to waterfal
A. jesdianum Boiss. et Buhse	1033	25.04.2004	19.04.2005 [a] 07.01.2005 [b]	Binalud massif near vill. Kharv e Olya, valley with river NE of village
A. paradoxum var. normale Stearn	1017	20.04.2004	06.07.2004 [a] 24.07.2004 [b]	Region near passJakhtikalon, SE-border of Golestan Reservation

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. giganteum Regel	1028	23.04.2004	07.07.2004 [a] 14.07.2004 [a] 07.01.2005 [b]	Central Kopetdag, slope near main road to Dargaz NE of main pass
	1035	25.04.2004	07.01.2005 [b]	Binalud massif near vill. Kharv e Olya, valley with river NE of village

[a] Extraction for determination of amount of cysteine sulphoxides using HPLC
[b] Extraction for determination of scavenger activity
• Botanical names of these Acc. numbers have not been finally determined

Table 3.4: *Allium* species collected in Turkmenistan. The date of harvest was not given by collector.

Botanical Name	Acc. No.	Date of Collection	Date of Extraction	Place of Collection
A. borszczowii Regel	0111•		06.01.2005 [a]	Badhyz, in the neighbourhood hollow Eroylanduz
A. caspium (Pallas) M. Bieb.	0030		05.01.2005 [a]	Special place Duslychai
A. isakulii ssp. subkopetdagense R.M.Fr.&Kh.	0046		26.10.2004 [a]	Kopetdag, upper valley of Chuli above the spring area
A. kopetdagense Vved.	0022		22.11.2004 [a]	Kopetdag, small and shallow valley ca. 5 km E Parowbibi
A. ophiophyllum Vved.	0082•		04.01.2005 [a]	Kugitang, in the neighbourhood settlement Bazartepe
A. regelii Trautv.	0006		01.11.2004 [a]	Kopetdag, border control station Gaudan-Bajigiran c. 10 km S Ashgabat
A. turcomanicum Regel	0114•		25.10.2004 [a]	Badhyz, in the neighbourhood hollow Eroylanduz
A. xiphopetalum Aitch. et Bak.	0116•		06.12.2004 [a]	Badhyz, canyon Torangali
A. cristophii Trautv.	0011		04.03.2005 [a]	Kopetdag, special place Kumyshdash
A. paradoxum var. normale Stearn	0135		03.03.2005 [a]	Western Kopetdag, Karakala (Yoldere)
A. yatei Aich. et Baker	0115•		03.03.2005 [a]	Badhyz, canyon Akrabat
A. brachyscapum Vved.	0014		04.03.2005 [a]	Kopetdag, special place Kumyshdash
A. vavilovii M. Pop. et Vved.	0047•		09.12.2003 [a]	Kopetdag, upper valley of Chuli above the spring area

[a] Extraction for determination of amount of cysteine sulphoxides using HPLC
[b] Extraction for determination of scavenger activity.

- Botanical names of these Acc. numbers have not been finally determined

Table 3.5: Wild *Allium* species with a different place of origin cultivated and harvested in IPK Gatersleben (Germany).

Botanical Name	Tax. No.	Date of Collection	Date of Extraction	Place of origin
A. aflatunense B. Fedt.	2657	01.10.2003	14.10.2003 [a]	Botanical garden of Dresden
	1211	01.10.2003	11.05.2004 [b]	Botanical garden of Leningrad Academy
A. altissimum Regel	2976	01.10.2003	15.12.2003 [a]	Expedition Middle Asia 1990 Fritsch & Pistrick No. K-640, agricultural research station Dr. Kamenetzkaja, collected from Zailijskij Alatau, Kurdai-Pass (cultivated plants)
A. angulosum L.	2806	01.10.2003	11.12.2003 [a]	Mansfeld,10 km west from Halle
	2773		03.04.2003 [a]	Nowosibirsk
A. hybrid (spontaneus hybrid of *A. nevskianum* Vved. ex Wendelbo)	5394	01.10.2003	18.12.2003 [a]	Garden of Dr.Fritsch, Gatersleben
A. jesdianum ssp.angustitepalum (Wdb.)Kh.&F	1222	01.10.2003	17.12.2003 [a]	Botanical Garden of Princip. Academy of Moscow
	3666	17.09.2004	27.01.2005 [a] 10.01.2005 [b]	Kugitang-mountains, 20 km west from Gulistan
	3951	1.10.2003	07.05.2004 [b]	Deh Balla, Province Yazd
A. kunthii G.Don	2158	24.09.2004	08.03.2004 [a]	Chihuahua near Panalachi, 2400 Mexico
A. longicuspis Regel	1125	24.09.2004	17.02.2005 [a] 11.11.2004 [b]	Botanical Garden of Minsk
	1337		04.04.2003 [a]	Chorongon, north-east from Dushanbe
A. macleanii Bak.	2218	24.09.2004	22.03.2005 [a] 09.12.2004 [b]	Botanical garden of Minsk
A. proliferum (Moench)Schrad.	2254	01.10.2003	29.10.2003 [a]	Bulgary
A. pskemense B. Fedt.	0514	01.10.2003	15.03.2005 [a] 11.11.2004 [b]	Botanical garden of Munich-Nymphenburg

Botanical Name	Tax. No.	Date of Collection	Date of Extraction	Place of origin
A. pskemense B. Fedt.	1297	1.10.2003	09.12.2003 [a]	Botanical garden of Strasbourg
	2724	1.10.2003	17.06.2004 [b]	Botanical garden of Academy of Alma-Ata
A. spirale Willd.	1968	01.10.2003	11.12.2003 [a]	Botanical garden in province S-Hwanghe Wonsan, Korea
A. tuberosum Rottl. ex Spr.	0583	01.10.2003	17.11.2003 [a]	Medicinal Plant Research Station Tsukuba
A. altaicum Pall.	1678	01.10.2003	11.04.2003 [a] 23.06.2004 [b]	Charchira-ul, Mongolia
	2746	24.09.2004	11.11.2004 [b]	Nowosibirsk
A. ampeloprasum L.	3605		21.10.2002 [a]	Tunisia
A. cyatophorum var. farreri Bur. et Franch.	2824	01.10.2004	04.10.2004 [a] 05.10.2004 [a]	Botanical Garden of Oslo
A. fistulosum L.	1120	01.10.2003	09.12.2003 [a]	Hadmersleben
A. nutans L.	0568	01.10.2003	06.01.2004 [a]	Botanical garden of Dnepropetrowsk
A. oleraceum L.	0382	24.09.2004	11.11.2004 [b]	Botanical garden of Genf

[a] Extraction for determination of amount of cysteine sulphoxides using HPLC
[b] Extraction for determination of scavenger activity.

3.2. *Reagents and Buffers*

The following Tables 3.6 – 3.7 shows reagents used for this work. Unless otherwise stated, reagents were purchased in p.a. quality.

Table 3.6: Chemicals and reagent used for the investigation of *Allium* species.

Reagent	**Source**
1,1-Diphenyl-2-picrylhydrazine	Sigma (Sigma-Aldrich Chemie), Steinheim
2-Methyl-2-propanthiol	Merck, Darmstadt
Acetic acid	Merck, Darmstadt
Acetonitrile HPLC gradient grade	SDS Peypin/France
Boric acid	Merck, Darmstadt
Bovine serum albumin	Merck, Darmstadt

Reagent	**Source**
Bradford reagent	Sigma (Sigma-Aldrich Chemie), Steinheim
Butanol	Merck, Darmstadt
Butylated hydroxytoluene	Sigma (Sigma-Aldrich Chemie), Steinheim
Calcium chloride dihydrate	Merck, Darmstadt
D-(+)-Saccharose	Sigma (Sigma-Aldrich Chemie), Steinheim
D-(+)-Glucose monohydrate	Merck, Darmstadt
Dichloromethane	Merck, Darmstadt
di-Sodium hydrogen phosphate dihydrate	Merck, Darmstadt
di-Sodium hydrogen phosphate dihydrate Suprapur® Sodium hydroxide Suprapur®	Merck, Darmstadt Merck, Darmstadt
di-Sodium hydrogen phosphate monohydrate	Merck, Darmstadt
Ethanol	Merck, Darmstadt
Ethyl acetate	Merck, Darmstadt
Folin-Ciocalteu´s phenol reagent	Fluka (Sigma-Aldrich Chemie), Steinheim
Chloroform	Merck, Darmstadt
Magnesium chloride hexhydrate	Merck, Darmstadt
Manganese chloride dihydrate	Merck, Darmstadt
Methanol	SDS Peypin/France
n-Hexane	Merck, Darmstadt
Ninhydrin	Merck, Darmstadt
o-Phtaldialdehyde for fluorimetry	Fluka (Sigma-Aldrich Chemie), Steinheim
o-Phtaldialdehyde for synthesis	Merck, Darmstadt
Potassium di-hydrogen phosphate	Merck, Darmstadt

Reagent	**Source**
Potassium-sodium-tartrate	Merck, Darmstadt
Sea sand	Fluka (Sigma-Aldrich Chemie), Steinheim
Sodium azide	Merck, Darmstadt
Sodium carbonate	Merck, Darmstadt
Sodium dihydrogen phosphate dihydrate	Merck, Darmstadt
Sodium dihydrogen phosphate dihydrate Suprapur ®	Merck, Darmstadt
Sodium hydroxide	Merck, Darmstadt
Sodium chloride	Merck, Darmstadt
Sodium chloride Suprapur®	Merck, Darmstadt
Sodium tetraborate decahydrate	Merck, Darmstadt
Thioglycolic acid	Fluka (Sigma-Aldrich Chemie), Steinheim
Thiomersal	Fluka (Sigma-Aldrich Chemie), Steinheim
Concanavalin A	Sigma (Sigma-Aldrich Chemie), Steinheim
NADH	Merck, Darmstad
Pethrolether (40-60 °C)	Riedel de Haen
Pyridoxal-5´-phosphate	Merck, Darmstadt
LDH suspension (18.5U/5 µl)	Sigma (Sigma-Aldrich Chemie), Steinheim
Silica gel 60	Merck, Darmstadt
Ammonium sulphate, 99,99%	Merck, Darmstadt

Table 3.7: Cysteine sulphoxides used as reference compound in this investigation (HPLC and FIA).

Substance	(+) Enantiomer [%]	(-) Enantiomer [%]	Lot
Methiin	38.9	61.1	
Ethiin	47.5	52.5	
Alliin	>97 >97 50	<3 <3 50	**MK-02-108-01** **MK-02-156-01**
Propiin	100 61.5	0 38.5	
Buthiin	49.1	50.9	
Hexiin	40.5	50.5	

For biosensoric FIA experiments, water for analysis (water p.a.) purchased from Merck, Darmstadt was used. For other experiments Millipore® water was used.

In the following Tables 3.8-3.9, buffers used for chromatographic separations, biosensoric FIA experiments and radical scavenger acitivity testing are listed.

Table 3.8: Buffers and solutions used for chromatographic purposes.

Name	Reagents	Quantity	Method of preparation
Borate-buffer pH 9.5	Sodium tetraborate decahydrate	4.77 g	The reagent was dissolved in 200 ml of Millipore water and pH was regulated with a solution of sodium hydroxide. The volume was adjusted to give 250.0 ml.
Phosphate-buffer pH 6.5	Sodium di-hydrogen phosphate dihydrate di-Sodium hydrogen phosphate monohydrate	1.93 g 2.53g	Both reagents were dissolved in 1000.0 ml of Millipore water. If needed, pH was regulated.
Solution for derivatization	o-Phtaldialdehyde for synthesis Methanol 2-Methyl-2-propanthiole	140 mg 5 ml 200 µl	o-Phtaldialdehyde was dissolved in methanol. 2-Methyl-2-propanthiole was added and then Borate buffer (in small portions). The work had to be performed in a fume hood.

Name	Reagents	Quantity	Method of preparation
	Borate buffer pH 9.5	50 ml	
Solution of sodium hydroxide (1M)	Sodium hydroxide	4.00 g	Reagent was dissolved in 100.0 ml of Millipore water
Solution of ninhydrin	Ninhydrin Butanol Acetic acid 98%	30 mg 10 ml 0.3 ml	After the complete dissolution of ninhydrin in butanol acetic acid was added.

Table 3.9: Buffers and solutions used for biosensoric FIA experiments and determination of radical scavenger activity.

Name	Reagents	Quantity	Method of preparation
Stock solution of allin 0.001 M	Alliin (99%)	18.8 mg	Alliin was dissolved in 100.0 ml of biosensoric eluent.
Stock solution of ammonium sulphate 0.001 M	Ammonium sulphate 99.99%	13.2 mg	Reagent was dissolved in 100.0 ml of biosensoric eluent
Biosensor buffer	Sodium dihydrogen phosphate dihydrate Suprapur ® di-Sodium hydrogen phosphate dihydrate Suprapur® Sodium chloride Suprapur® Thiomersal	888 mg 1.776 g 10 g 100 mg	Reagents were dissolved in 800 ml of water p.a. The volume was adjusted to give 1000.0 ml.
Con A-buffer	Phosphate-buffer pH 7 (600 mM) Sodium chloride Calcium chloride dihydrate Magnesium chloride hexyhydrate Manganese chloride dihydrate Pyridoxal-5´-phosphate Thiomersal	100 ml 58.4 g 147.0 mg 203.3 mg 161.8 mg 26.5 mg 200 mg	Phosphate buffer and the salts were dissolved in 800 ml of water p.a. After 10 minutes Pyridoxal-5´-phosphate and thiomersal were added. Solution was filtered. The volume was adjusted to give 1000 ml.
OPA buffer pH 9.0	o-Phtaldialdehyde for fluorimetry (OPA)	1.006 g	OPA was suspended in 200 ml of water p.a. Thioglycolic acid

Name	Reagents	Quantity	Method of preparation
(15 mM)	Thioglycolic acid	2.73 ml	was added. After dissolution of the OPA, the pH was regulated with solution of sodium hydroxide for FIA purposes. The volume was adjusted to give 500 ml. The work had to be performed in a fume hood.
Boric acid buffer (0.8 M) pH 12	Boric acid	24.732 g	Boric acid was suspended in 400 ml of water p.a. The pH was regulated with a solution of sodium hydroxide for FIA purposes. The volume was adjusted to give 500 ml.
Solution of sodium hydroxide for FIA purposes (1M)	Sodium hydroxide Suprapur®	4.0 g	Sodium hydroxide was dissolved in 100 ml of water p.a.
Phosphate buffer pH 7 (600 mM)	Potassium di-hydrogen phosphate di-Sodium hydrogen phosphate dihydrate	35.4 g 72.52 g	Reagents were dissolved in 800 ml of water p.a. The volume was adjusted to give 1000.0 ml. Whenever necessary, pH was regulated.
Sørensen buffer (60 mM)	Phosphate buffer pH 7 (600 mM) Thiomersal	100 ml 0.2 g	Thiomersal was dissolved in 200 ml of water p.a. and the phosphate buffer pH 7 was added. The volume was adjusted to give 1000.0 ml.
Alliinase Buffer	Phosphate buffer pH 7 (600 mM) Saccarose Sodium chloride Pyridoxal-5´-phosphate Thiomersal	100 ml 100 g 10 g 26.5 mg 0.2 g	Phosphate buffer pH 7 and the reagents were dissolved in 800 ml of water p.a. After 10 minutes, pyridoxal-5´-phosphate and thiomersal were added. The volume was adjusted to give 1000.0 ml.
NADH solution (8.5mM)	NADH Sørensen buffer	12.0 mg 2.0 ml	NADH was dissolved in Sørensen buffer.
LDH solution	LDH suspension Sørensen buffer	150 µl 2.0 ml	LDH was dissolved in Sørensen buffer.
DPPH solution	1,1-Diphenyl-2-picrylhydrazine	0.01 g	1,1-Diphenyl-2-picrylhydrazine was dissolved in 25 ml of methanol p.a. The dissolution took 2 hours. A dark graduated flask had to be used.

Name	Reagents	Quantity	Method of preparation
BHT solution	Butylated hydroxytoluene	0.08 g	Butylated hydroxytoluene was dissolved in a small amount of methanol p.a. The volume was adjusted to give 25.0 ml.

3.3. *Equipment Used for Investigation*

The following Table 3.10 lists especially equipment used for HPLC, FIA and bioactivity testing.

Table 3.10: Equipment used for the investigation of wild *Allium* species.

Device	Type
HPLC	Shimadzu LC – 4A, Detector SPD – 2 AM, with column oven CTO – 2AS and integrator CR -3A, Shimadzu, Düsseldorf
FIA Device	Bioservice, Halle
Plate Reader	Multiskan EX, Thermo Electron Corporation, Dreieich SLT Rainbow, SLT Labinstrument, Crailsheim
UV	Uvikon 810 with recorder 21, Kontron, Munich
pH Meter	pMX 3000, WTW, Weilheim
Peristaltic Pumps	Minipuls 3, Gilson, Ohio, USA
NMR	Bruker AMX 300, Ettlingen
MS	Kratos MS 50, Berlin
IR	Perkin -Elmer Spectrum BX with ART unit, Friedrichshafen
UV	Perkin – Elmer Lambda 40, Friedrichshafen

Device	Type
Heating -block	DRI – Block DB.2A, Biostep GmbH, Jahndorf
Vortexer	Janke + Kunkel, Staufen
Analytical Weight	H11W, Mettler, Giessen
Laboratory Weigt	K7, Mettler, Giessen
Ultrasound Water Bath	Bransonic 12, Branson, Dietzenbach

3.4. *LC-Separation*

LC separations were performed to obtain fractions of some of the selected *Allium* extracts. The apparatus consisted of a cylindrical glass tube. At the lower end of the tube a small delivery tube with a stopcock was fused. As a stationary phase (adsorbent), silica gel 60 was used (Figure 3.2).

In a case of elucidation of a red dye following procedure was performed: To reach a liquid form of the adsorbent, 70 g of silica gel 60 was mixed with 200 ml of petrol ether (40 - 60 °C). This slurry was poured into the cylindrical tube.

Before the sample (ethyl acetate and dichloromethane extract of *Allium giganteum)* was added into the column, it had been dissolved in a very small volume of ethyl acetate and a small amount of the silica gel 60 had been added. The sample was carefully placed on top of the column. Three tea spoons of sea sand were filled onto the column and 100 ml of petrol ether (40-60 °C) were added. The stopcock was open and the solvent was caught in a flask. When the level of the mobile phase fell to the sea sand layer, the next mobile phase as listed below was poured onto the column. Also, the next solvent fraction was collected. The following mobile phases were used:

1. 100 ml petrol ether (40-60 °C)
2. 100 ml petrol ether (40-60 °C)/ethyl acetate (80/20)
3. 100 ml petrol ether (40-60 °C)/ethyl acetate (60/40)
4. 100 ml petrol ether (40-60 °C)/ethyl acetate (30/70)
5. 100 ml petrol ether (40-60 °C)/ethyl acetate (10/90)
6. 100 ml ethyl acetate
7. 100 ml ethyl acetate/methanol (80/20)
8. 100 ml ethyl acetate/methanol (50/50)
9. 100 ml ethyl acetate/methanol (20/80)

10. 100 ml methanol

To keep a constant flow rate, low pressure (compressed air) was evolved at the top of the column. The flow rate ranged between 9-10 ml per minute.

In the case of elucidation of a red dye precursor the following procedure was performed: To reach a liquid form of the adsorbent, 75 g of silica gel 60 were mixed with 250 ml of n-hexane. This mixture was poured into a cylindrical tube. Before the sample (methanolic extract of *Allium giganteum)* was placed into the column, it had been dissolved in a very small volume of methanol and a small amount of the silica gel 60 had been added. The solvent was slightly evaporated. The sample was carefully placed on the top of column filling. The sample was covered by three tea spoons of sea sand and 100 ml of n-hexane were added. The stopcock was open and the solvent was caught into a flask. When the level of the mobile phase fell to the sea sand layer a new mobile phase as listed below was poured onto the column. Also, a new solvent fraction was collected. Following mobile phases were used:

1. 100 ml n-hexane
2. 100 ml ethyl acetate
3. 150 ml ethyl acetate/methanol (50/50)
4. 100 ml ethyl acetate/methanol (20/80)
5. 100 ml methanol
6. 100 ml methanol/water (50/50)
7. 100 ml water

To keep a constant flow rate, a low pressure (compressed air) was evolved at the top of the column. The flow rate was approximately 6 ml per minute. Single fractions were evaporated and stored by -20° before further investigations.

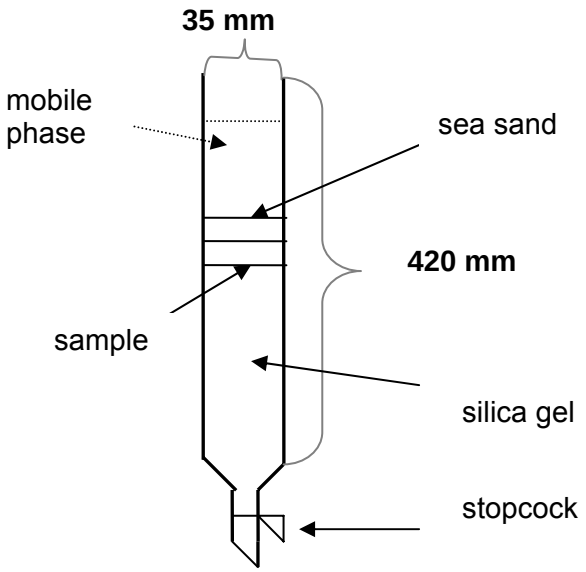


Figure 3.2: Chromatographic tube for LC separations

3.5. *TLC-Separation*

TLC analysis was used for the i) investigation of the content of single fractions obtained by LC separations and ii) in experiments with low molecular extracts (precursor of the red dye). For evaluation of the LC-separation, the evaporated fractions were further investigated. There was a possibility to find unknown substances. As a first step TLC separations were carried out. This method is quite fast and can give us an overview about substances which occur in the extract. Three different suitable developing solvent systems were developed for this purpose:

- lipophilic system: petrol ether/ethyl acetate/ethanol (60/30/10)
- medium lipophilic system with medium polarity: ethyl acetate/isopropanol/water (60/30/10)
- hydrophilic system: butanol/acetic acid/water (60/20/20)

As a stationary phase, silica gel F_{254} ready plates purchased from Merck Darmstadt were used. Plates were developed over a path of 10 cm.

100 ML of the mobile phase were poured into a developing chamber. The saturation of the chamber took 30 minutes. Duration of the separation depended on the solvent system. The detection was performed by UV light (254 nm and 366 nm) and by daylight. Developed plates were also sprayed with ninhydrin reagent to detect nitrogen molecules [Keusgen 1997].

3.6. *HPLC Separation*

3.6.1. *Sample Preparation*

Plant material (bulbs, leaves or inflorescences) was cleaned and dry parts were removed. 0.20-1.00 G of exactly weighted sample were directly put into 20 ml methanol to avoid the enzymatic reaction of cysteine sulphoxides with alliinase. The sample was heated by a reflux for 10 minutes; after cooling it was crushed in a mortar and returned quantitatively with the methanol for further extraction. By bringing the methanol to boil, 20 ml of distilled water were added. Then the sample was heated for 10 minutes. After cooling the extract was filtered and the residue was washed with 5 ml mixture of methanol/ water (1:1). The filtrate was then carefully evaporated under vacuum to dryness and stored at -20 °C before further use.

3.6.2. *HPLC Method*

HPLC analysis is based on a method of Ziegler and Sticher [1989]. This procedure was modified by Keusgen and Krest [2000].

Derivatisation of Samples

Due to their structure, cysteine sulphoxides show UV absorption at short wave lengths. Their absorption maximum lies at 220 nm [Keusgen 1999]. To obtain a better division of particular cysteine sulphoxides, the sample was derivatized with O-phtaldialdehyde and 2-methyl-2-propanthiol before analysis. The reaction runs at alkaline ambience (pH 9.5). Before analysis the sample was brought to room temperature; the residue in the flask was completely resolved in 5.00 ml of derivatization reagent using ultrasound water bath and 50 µl of 2-methyl-2-propanthiol were added. The sample was left in the dark for 30 minutes at room temperature. Before injection, the sample was filtrated using membrane filters (Ø= 0.45 µm).

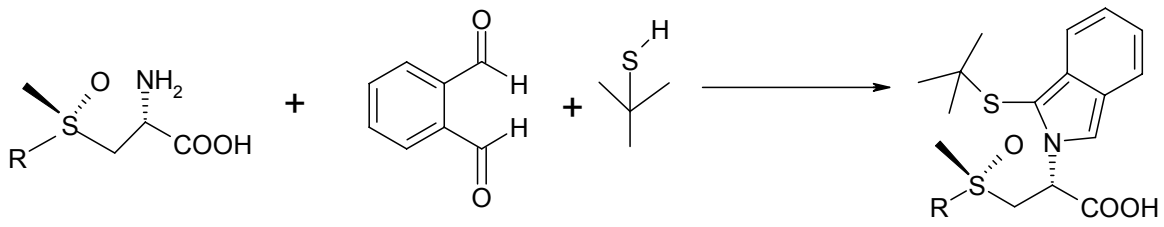


Figure 3.3: Derivatization of alk(en)yl cysteine sulphoxides with o-phtaldialdehyde and 2-methyl-2-propanthiol.

HPLC Equipment and Chromatographic Conditions

The quantitative analysis was performed on a Shimadzu LC-4A chromatograph equipped with a Chromatopac C-R3A integrator and SPD-2AM detector. As a stationary phase, a spherimage 80 ODS2 RP column, particle size 5 µm (250x4 mm) with integrated guard column purchased from Knauer, Berlin, was used. The operating flow rate ranged between 1.0-1.3 ml/min. Detection was carried out at 335 nm. Peaks were identified by cochromatography with standards.

Composition of the mobile phase:

Eluent A: 70 volume parts of phosphate buffer (pH 6.5)
30 volume parts of acetonitrile

Eluent B: 85 volume parts of phosphate buffer (pH 6.5)
15 volume parts of acetonitrile

A modified gradient system established by Keusgen [1999] was used in this trial (Fig. 3.4).

Table 3.11: Gradient system established by Keusgen [1999]

Time [min]	Eluent A [vp %]	Eluent B [vp %]
0	30	**70**
10	60	**40**
20	60	**40**
40	80	**20**
45	100	**0**
50	100	**0**
55	30	**70**
65	30	**70**

Table 3.12: A modified gradient system used for investigation of cysteine sulphoxides in wild *Allium* species.

Time [min]	Eluent A [vp %]	Eluent B [vp %]
0	20	**80**
5	20	**80**
15	60	**40**
25	60	**40**
45	80	**20**
50	100	**0**
55	100	**0**
60	20	**80**
65	**20**	**80**

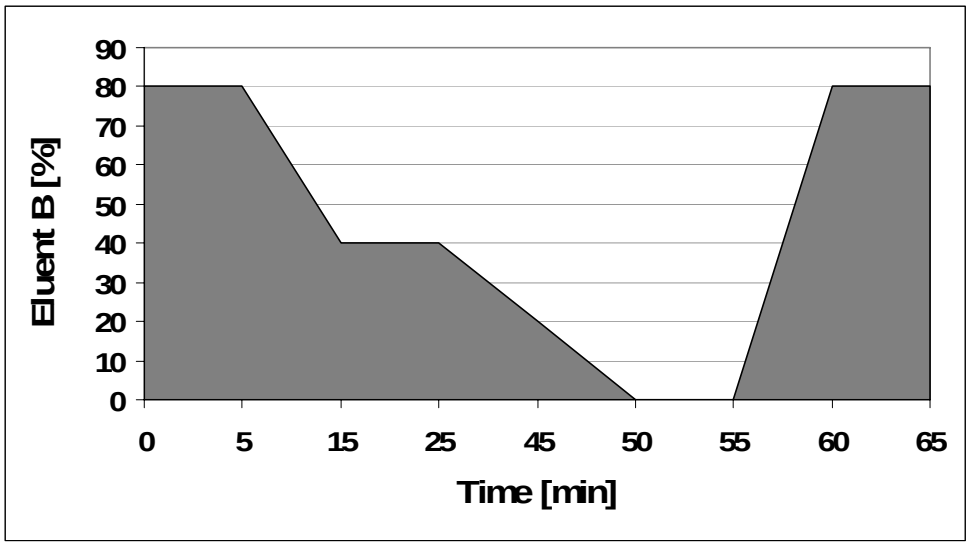


Figure 3.4: Modified gradient system used for investigation of cysteine sulphoxides in wild *Allium* species.

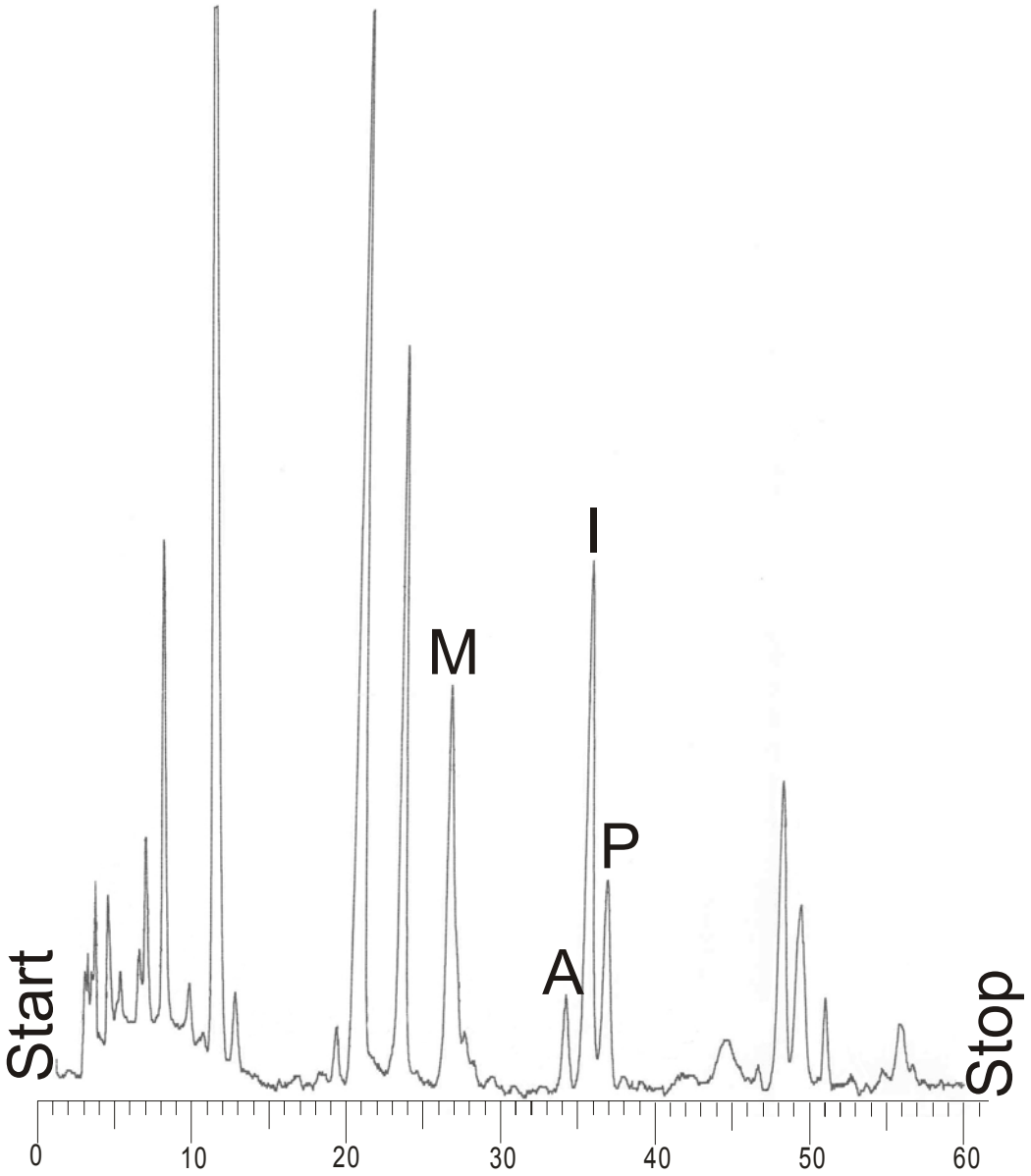


Figure 3.5: Example of a chromatogram acquired from the measurement of *A. pskemense*, **Acc.-No. 4142**, from Uzbekistan. M=methiin, A=alliin, I=isoalliin, P=propiin. Peaks were identified by cochromatography with reference substances (standards). With exception of isoalliin, which was isolated from authentic *A. cepa*, standards were synthesized.

Calibration of the Method

Synthetic cysteine sulphoxides were used for calibration. Standards were dissolved in water and derivatized with OPA reagent as also performed for real samples. It was found that molar absorption coefficients at 335 nm were the same for all tested cysteine sulphoxides because only the OPA moiety of the OPA derivative is responsible for the absorption. In the case of methiin, racemic (+/-) methiin [averagely 54% (+), 46% (-) methiin] was used, but only the peak obtained from the (+)-derivative was considered for the calculation. Because of the molar absorption coefficient of all OPA derivatives is the same, the amount of methiin,

alliin, isoallin and propiin was calculated in dependence on the calibration curve. The calibration was carried out every six weeks and was determined for (+) methiin. Standard compounds were diluted in Millipore water. Five different concentrations of (+) methiin ranging between 4 and 33 µg/ml were tested and each concentration was examined in triplicate. The calibration curve was calculated according to Fig. 3.6.

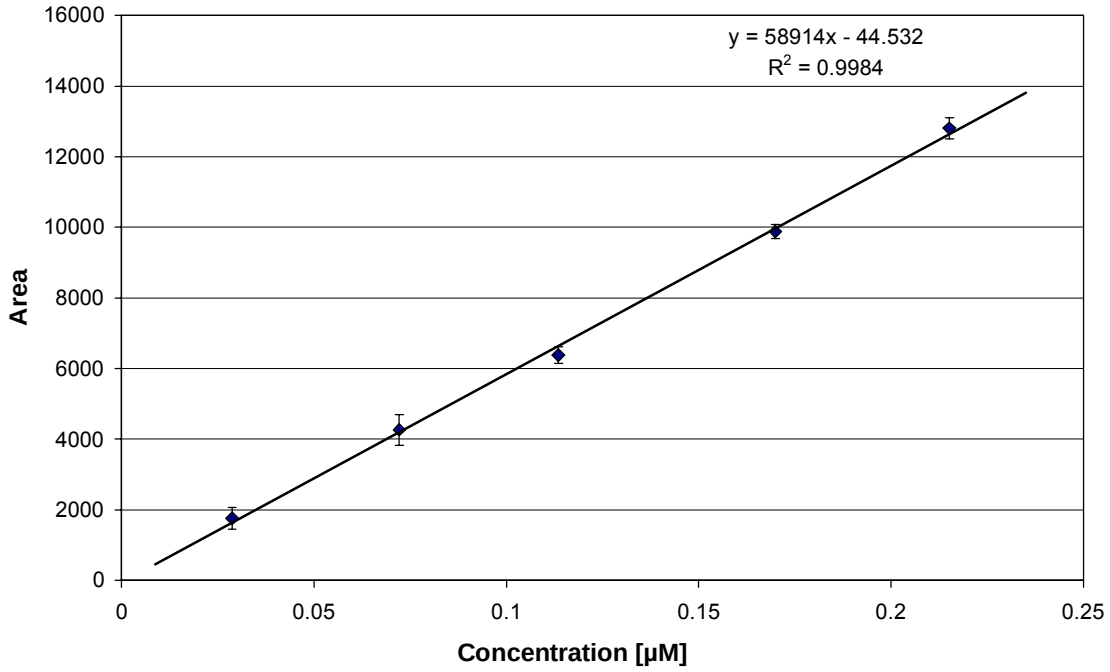


Figure 3.6: Calibration curve (for methiin) obtained for the quantitative determination of cysteine sulphoxides by means of HPLC.

3.7. *Flow Injection Analysis (FIA)*

3.7.1. *Sample Preparation*

Bulbs of various *Allium* species were cleaned and dry parts were removed. 0.20 - 0.40 G of an exactly weighted sample were directly put in a small test tube filled with 2.5 ml methanol to avoid the contact of cysteine sulphoxides with the enzyme alliinase. The test tubes were closed with a stopper. The sample was placed in a heating-block, where it was heated at 80 °C for 10 minutes. After cooling, the sample was crushed using a glass stick and 2.5 ml water were added. The sample was heated again in the heating-block at 80 °C for further 10 minutes and after cooling filtered through a filter paper. The residue was washed with 5 ml of biosensor buffer. The filtrate was adjusted with biosensor buffer to give a final volume of 25.0 ml. An exactly measured volume, which corresponded to a sample weight of

125 mg, was placed into a volumetric flask and the volume was adjusted with biosensor buffer to give the final volume of 25.0 ml. Samples were stored at 4 °C before further use.

Before measurements, 500 -1000 µl of the sample were filtered through a HPLC-filter (∅=0.45 µm). The sample could be diluted in dependence on the concentration of particular sample (1:5 or 1:10, depending on the total amount of cysteine sulphoxides).

3.7.2. *FIA Method*

Immobilization of Alliinase

The enzyme alliinase was obtained from *A. sativum* (*longicuspis* type) as described in chapter 3.8. 1.0 Ml of alliinase was mixed with a Con A buffer to give the final volume of 10.0 ml. At a bottom of the cartridge (Fig. 3.7), a filter paper was placed and the bottom screw was closed. The cartridge was filled with concanavalin A, which was immobilized on small spheres of agarose. The spheres of agarose did not pass through the filter paper at the bottom of the cartridge. Filter paper was also placed on the top of the cartridge and the top screw was closed.

The diluted alliinase was slowly delivered by a peristaltic pump with a flow rate of 0.1 ml/min through the cartridge for 80 minutes. To support the immobilization (alliinase formed stable complexes with the lektin concanavalin A), the cartridge was left at room temperature for further 40 minutes. Afterwards, the cartridge was washed with a constant flow rate of 0.1 ml/min with a Con A buffer for 15 minutes and stored at 4 °C before further use.

Figure 3.7: Cartridge (made by Bioservice, Halle) with immobilized alliinase. The inner volume of the cartridge was 150 µl.

Preparation of the FIA-Device

At the beginning of the measurements, the samples, buffers and the carrier were allowed to stay at room temperature for 30 minutes. Furthermore, the buffers and carrier were degassed. The tubes on peristaltic pumps were tightened and the volume pulsing through single tubes was determined. Finally, the device was washed with p.a. water followed by the carrier (biosensor buffer).

Standards and Calibration of the Method

Before measurements of samples could be started, calibrations with ammonium sulphate and alliin had to be performed. First, stock solutions of both standards were prepared (1.0 mM in biosensor buffer). The stock solutions were diluted with biosensor buffer into required concentrations shortly before use. Initially, a detailed calibration of ammonium and alliin was performed. The calibration of ammonium was carried out with concentrations of 5, 10, 20, 50, 75, 100, 150, and 200 µM, the calibration of alliin with concentrations of 5, 10, 30, 50, 60, 75, 80, and 95 µM. Each measurement was repeated three times. Every day a recalibration of ammonium and alliin was carried out. This time, only 3 concentrations were determined (for both standards 20, 50 and 95 µM). Again, each measurement was carried out three times.

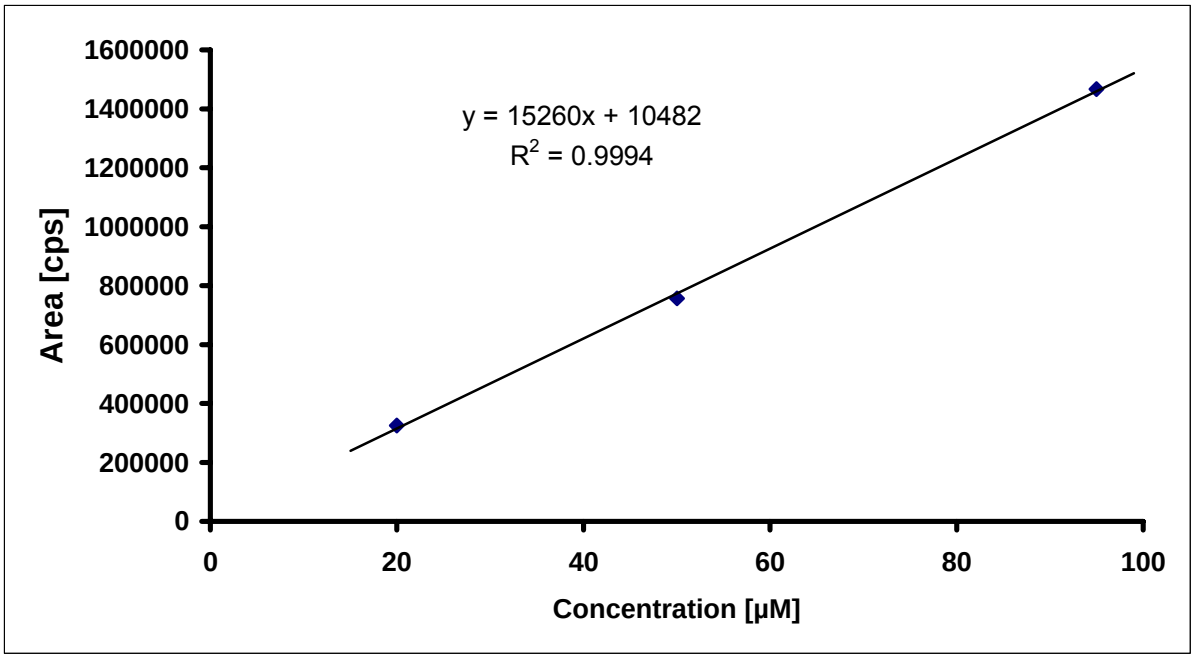


Figure 3.8: A typical recalibration curve obtained with ammonium standards. This measurement was performed every day before measurements of *Allium* samples had been started.

It is important to mention that the values of ammonium solutions as well as of samples were first measured without alliinase cartridge. Then, the cartridge was mounted into the system and the values of alliin solutions as well as of samples were measured. Usually, the measurement took eight minutes and was repeated three times. The Figure 3.8 below shows a common recalibration curve for ammonium.

Substrate Specificity

The substrate specificity of the immobilized alliinase for different cysteine sulphoxides was investigated. In the following Table 3.13, cysteine sulphoxides respected in this investigation are listed.

Table 3.13: Cysteine sulphoxides used in investigation of substrate specificity. Purity levels of single cysteine sulphoxides are listed in chapter 3.2. (Materials and Methods). Isoalliin was obtained from a purified amino acid extract, where the extract contained 15.0 % isoalliin. Other cysteine sulphoxides were synthesized in the working group of Prof. Keusgen.

Cysteine Sulphoxide
(+) alliin
(+/-) alliin
(+) isoalliin
butiin (butyl-cysteine sulphoxide)
propiin
ethiin (ethyl-cysteine sulphoxide)
Methiin
hexiin (hexyl-cysteine sulphoxide)

Initially, stock solutions of single cysteine sulphoxides were prepared; all cysteine sulphoxides were dissolved in biosensor buffer to give the concentration of 1.0 mM. Later on, stock solutions were diluted to give the final concentrations of 10, 20, 50, 75 and 90 µM. The values for each concentration were determined and single measurements were repeated three times. After each measurement of a particular cysteine sulphoxide, the FIA device was washed three times with the carrier (biosensor buffer) before further measurements.

3.8. *Enzyme Isolation and Partial Purification*

For investigation performed in this work, extracts of enzyme alliinase had to be obtained.

Extraction of Alliinase

2-4 G (fresh weight) of cleaned and peeled garlic cloves were chopped in an iced cold mortar filled with 10 ml of phosphate buffer. One tea spoon of sea sand was added and the chopped cloves were crushed with a pistil to a homogenous matter. This matter was centrifuged at 11 500 g and 4 °C for 30 minutes. The supernatant was dialyzed against 5000 ml of the phosphate buffer (Servapor dialysis tubing, 21 mm diameter, protein exclusion at 10 kDa) for12-14 hours at 4 °C. Dialyzed extract was divided into small portions and stored at -20 °C before further use.

Enzyme Characterization

To characterize the enzymatic activity, protein concentrations have to be determined. Protein concentrations of alliinase extracts were determined following the methods described by Lowry et al [1951].

At first, the enzyme-extract was diluted with Millipore-water consecutively:

1. 5-fold (100 µl extract + 400 µl water)
2. 10-fold (60 µl extract + 540 µl water)
3. 20-fold (300 µl of solution No.2. + 300 µl water)
4. 50-fold (300 µl of solution No.3. + 450 µl water)
5. 100-fold (300µl of solution No.4 + 300µl water)
6. 200-fold (300µl of solution No.5. + 300µl water)
7. 400-fold (300µl of solution No.6 + 300µl water)

For calibration, bovine serine albumin (BSA) was used. 10, 20, 30 …100 µl of BSA stock solution (1 mg/ml) were filled into a single-use cuvette. The volume was adjusted to give 100 µl. Seven additional cuvettes were filled with 100 µl of diluted extract. One cuvette was filled with the pure extract. Furthermore two blind samples filled only with 100 µl of Millipore water were prepared. Finally in each cuvette 335 µl of Lowry solution were added. After exactly 15 minutes, 1 ml of Folin-solution was added into each cuvette. After exactly 45 minutes, the solutions were measured at 540 nm.

It was very important to work precisely on time. After addition of a new solution into a cuvette, the content of the cuvette had to be stirred very well immediately. From the calibration curve, the protein concentration could be obtained.

Calculation of Enzymatic Activity

Principals of the calculation consist on the performance of a further enzyme reaction. Pyruvic acid, which arises by the cleavage of cysteine sulphoxides catalyzed by the enzyme alliinase, reacts further to lactic acid. This reaction is catalyzed by enzyme lactate dehydrogenase under consumption of $NADH_2$. The reaction can be measured using specrophotometry at 340 nm.

Two quartz cuevettes were filled with 800 µl of Sørensen buffer. 50 µL NADH solution, 50 µl LDH solution and 50 µl sample were added. The cuevettes were tempered at 25 °C within the spectrophotometer. 50 µL of Sørensen buffer were added into the reference cuevette and 50 µl of (+)-Alliin solution (c= 80 mM) into measuring cuevette. Contents of both cuevettes were immediately well stirred. The decrease of $NADH_2$ could be graphically recorded.

For the calculation of the enzyme activity following formula was applied:

$$A_s = (V_F * V_K / \varepsilon * d * V_P * C_{prot.}) * \Delta E / \Delta t$$

A_s	Enzymatic activity
V_F	Dilution factor of the sample (in our case 30)
ε	molar extinction coefficient for NADH at 340 nm (in our case 6,22 $cm^2/\mu mol$)
d	width of the cuevette [cm]
V_P	volume of the sample [ml]
$C_{prot.}$	concentration of proteins [mg/ml]
ΔE	difference absorption unit [none unit]
Δt	difference time [min]

The calculation was performed as described by Krest [2000].

3.9. *Isolation and Structure Elucidation of the Red Dye*

Bulbs of *Allium giganteum* (Acc.-No. 6089) were cleaned and peeled. 258.4 G (fresh weight) were chopped in a mixer (Braun KSM1) into a homogenous matter. This matter was transferred into 3 mortars. To prevent drying out, 20 ml of water were added into each mortar. The matter was well stirred and incubated at room temperature for 16 hours. The matter turned orange-red. After incubation, the water residue was filtered through linen. At first, the matter was extracted twice with 500 ml of dichloromethane and then with 800 ml of ethyl acetate. Both extracts (orange coloured) were filtered, evaporated and finally combined.

The integrated extracts were separated using LC. Gained fractions were further investigated by TLC separation.

As already mentioned, the colour of the matter obtained from the bulbs of *Allium giganteum* turned orange-red. One fraction gained from the LC separation was coloured light red. In this fraction, an unknown red substance was assumed. To elucidate the structure of this substance, this fraction was further examined using NMR, MS, UV and IR spectrometry.

3.10. *Isolation and Structure Elucidation of a Possible Precursor*

A bulb of *Allium giganteum* (Acc.-No. 6089) was cleaned and peeled. A mass of 19.8 g (fresh weight) were chopped into small pieces and immediately put into a beaker with 100 ml of methanol to inhibit the enzymatic activity. After 20 minutes, the pieces of the bulb were taken out of the beaker and transferred into a mortar. One tea spoon of sea sand and a small volume of methanol were added. The pieces were crushed to a homogenous matter. The matter was transferred back into the beaker with methanol and extracted at continuous stirring for 45 minutes (the matter and extract stayed colourless). The extract was filtered, evaporated and finally stored at -20 °C before further use.

3.11. *Antibiotic activity*

Antibiotic Activity of *Allium*-Extracts Using Agar Diffusion Test

Garlic, onion, leek and other species from the *Aliaceae* family have been used worldwide as a food, spice and medicinal plant since ancient times. Louis Pasteur was the first researcher to describe the antibacterial effect of onion and garlic juices [cited in Koch and Lawson 1996].

Antibiotic activity has been well described for garlic and onion [Keusgen 2002a]. In this work antimicrobial activity was tested on extracts acquired from 18 different wild *Allium* species growing in the Central Asia. As a control of effectiveness, garlic extract was used.

Agar Diffusion Test

Agar diffusion test is a microbiological method used to test resistance or sensitivity of bacteria strains towards antibiotics. For this test, a carrier (paper disk), impregnated with a known amount of antibiotic, is placed on the surface of a solid medium, which has previously been inoculated with a bacterial suspension of the pathogenic strain to be tested. The antibiotic diffuses from the carrier into the medium, producing a concentration gradient. [Urbašová 1998]. Bacterial growth in the vicinity of the carrier only occurs when the concentration of the antibiotic substance diffusing from the carrier is no longer sufficient to

inhibit bacterial replication, or when the pathogen strain is resistant to the tested antibiotic. With the described method, extracts obtained from various wild *Allium* species were tested on their antibiotic activity. As a control of effectiveness (positive control), defined antibiotics were used (see Table 3.14). As a control of ineffectiveness (negative control), paper disks soaked only with ethyl acetate were used.

In cooperation with the Institute of Pharmaceutical Biology in Bonn, Germany, *Allium* extracts were tested on their inhibition activity against bacteria, fungi and algae. The agar diffusion test as described above was used. The work was carried out by Mrs. Edith Neu from the working group of Prof. G. Koenig. Obtained results served as pre-screening, which was very important for our further investigation.

3.11.1. *Sample Preparation*

5.0-30.0 G (fresh weight) of cleaned and peeled bulbs of various *Allium* species were crushed in a mortar with sea sand to a homogenous matter. After 60 minutes incubation, the matter was transferred into a flask and extracted thrice with 200 ml of ethyl acetate. The ethyl acetate extracts were combined, filtrated through a glass wool and finally carefully evaporated nearly to complete dryness. 8-20 Mg of the oily extract were transferred into small HPLC-vials.

The following Table 3.13 shows wild *Allium* species used in the agar diffusion test. *Allium sativum* does not belong to wild *Allium* species collected in Central Asia. As already mentioned, its antibacterial activity is well known, described in literature and in this case serves as a confirmation of effectiveness of the chosen method.

Table 3.13: *Allium* species used in agar diffusion test. Numbers in brackets indicate the same *Allium* species collected in various regions and under various conditions.

Acc. No.	***Botanical Name***
1006	*A. chelotum*
1016	*A. cristophii*
1024	*A. pseudobodeanum*
1033	*A. jesdianum (1)*
1035	*A. giganteum (1)*
4133	*A. motor*

Acc. No.	***Botanical Name***
4135	*A. talassicum*
4165	*A. giganteum (2)*
4170	*A. komarowii (1)*
6110	*A. rosenorum*
6135	*A. oschaninii*
6136	*A. alexeianum*
6142	*A. komarowii (2)*
6145	*A. winklerianum*
6150	*A. carolinianum*
6163	*A. hymenorrhizum*
Tax 0514	*A. pskemense*
Tax 1125	*A. longicuspis*
Tax 2218	*A. macleanii*
Tax 3666	*A. jesdianum (2)*
Cz 001	*A. sativum longicuspis type*
6912	*A. giganteum (3)*

3.11.2. *Agar Diffusion Test*

Disk Preparation

Disks were labelled with numbers and afterwards sterilized in an autoclave at 121 °C for 21 minutes. Extracts in HPLC-vials were diluted with ethyl acetate to give a final concentration of 10 mg/ml. Each disk was soaked with the extract to provide a defined concentration in a range between 0.5-1.0 mg. After the evaporation of ethyl acetate, the discs were transferred into a sterile Petri-dish.

Antibiotics

The following table shows antibiotics used in the test. These antibiotics are commonly used as a positive control by not resistant Gram-positive and Gram-negative bacterial strains. The susceptibility depends on the size of inhibition zone and is different for each bacterial strain. Disks with antibiotics were stored at –20 °C and brought to room temperature before use.

Table 3.14: Antibiotics used in the agar diffusion test as a positive control. **Penicillin** was used for *Streptococcus pyogenes*, *Streptococcus agalactiae* and *Streptococcus pneumoniae* **Oxacyllin** for *Staphylococcus aureus* and *Staphylococcus epidermidis*, **Ampicillin** for *Salmonella enteritidis* and *Enterococcus faecalis*, and **Cefalothin** and **Cefalexin** for *Klebsiella pneumoniae* and *Escherichia coli*.

Antibiotic	Concentration/disk
Penicillin	10.0 iU
Oxacyllin	1.0 µg
Ampicillin	10.0 µg
Cefalothin	30.0 µg
Cefalexin	30.0 µg

Bacterial strains

Bacterial strains used in this test were acquired from a Laboratory of Clinical Microbiology in Sternberk, Czech Republic, as listed in Table 3.14. These bacterial strains were isolated from patients, selected from primary agar plates and tested for susceptibility. They did not show a considerable resistance against common antibiotics.

Table 3.14: Bacterial strains used in this investigation and their categorization according to Gram- coloration.

Bacterial Strain
Enterococcus faecalis (G+)
Streptococcus pyogenes (G+)
Streptococcus pneumoniae (G+)
Streptococcus agalactiae (G+)
Staphylococcus aureus (G+)
Staphylococcus epidermidis (G+)
Salmonella enteritidis (G-)
Escherichia coli (G-)
Klebsiella pneumoniae (G-)

Solid Mediums (Agars)

For their optimal growth and reproduction, different bacteria require different nutrition and cultivation conditions (Table 3.15). Agar plates used for the test were ready standard plates purchased from Biomerieux, Lyon, France. These are ready-to-use plates following

the NCCLS standard. For isolation of bacteria, Columbia plates with sheep blood (5%) were used. Agars used for testing of susceptibility are presented the in following Table 3.15.

Table 3.15: Bacterial strains and corresponding agars used for agar diffusion test.

Bacterial strain	Agar
Streptococcus pyogenes	Mueller-Hinton Agar with sheep blood (5%)
Streptococcus pneumoniae	
Streptococcus agalactiae	
Staphylococcus aureus	Mueller-Hinton Agar without blood
Staphylococcus epidermidis	
Salmonella enteritidis	
Escherichia coli	
Klebsiella pneumoniae	
Enterococcus faecalis	

Liquid Mediums (Broth Mediums) and Inoculation

For inoculation, Mueller-Hinton broth medium was used. For dilution of the Mueller-Hinton broth medium, physiological saline including glucose (0.1%) was used. Isolated bacterial strains were suspended in 2.0 ml of Mueller-Hinton broth medium and the suspension was left at room temperature until it achieved a turbidity of 0.5 McFarland standard. Afterwards, 1.0 ml of the suspension was diluted in 10.0 ml of physiological saline including glucose (0.1%). The diluted suspension (inoculum) was poured onto the sterile agar plate completely and the agar plate was slightly shaken. Then, the inoculum was poured onto another plate. Inoculum residues on the edges of the plate were dried with a paper towel. This procedure was repeated four times. Plates were left at room temperature for 5 minutes. Afterwards, the disks containing *Allium* extracts as well as the antibiotics were placed evenly on the surface of the plate (3-4 disks/plate) using a sterile needle. The plates were incubated at 36 °C for 16 hours. If required, plates were incubated in an environment containing CO_2.

Screening

In a first experiment, each *Allium* extract listed in the Table 3.13 was tested against each bacterium listed in Table 3.15. Disks contained 0.5 mg of *Allium* extract. The susceptibility was measured three times for each *Allium* extract.

In the second experiment the bacterial strains, which demonstrated certain sensitivity, were tested against effective *Allium* extracts identified in the first experiment. The tests were done in triplicate. In some cases (*A. pskemense*, *A. komarowii*, *A. giganteum* 1,3, *A. winklerianum*, *A*. *macleanii*, *A. longicuspis* and *A. carolinianum*), paper disks were impregnated with 1.0 mg of these *Allium*-extracts.

Evaluation of Results

After incubation, a complete zone of inhibition was evaluated. The manner of the measurement is shown in Figure 3.9.

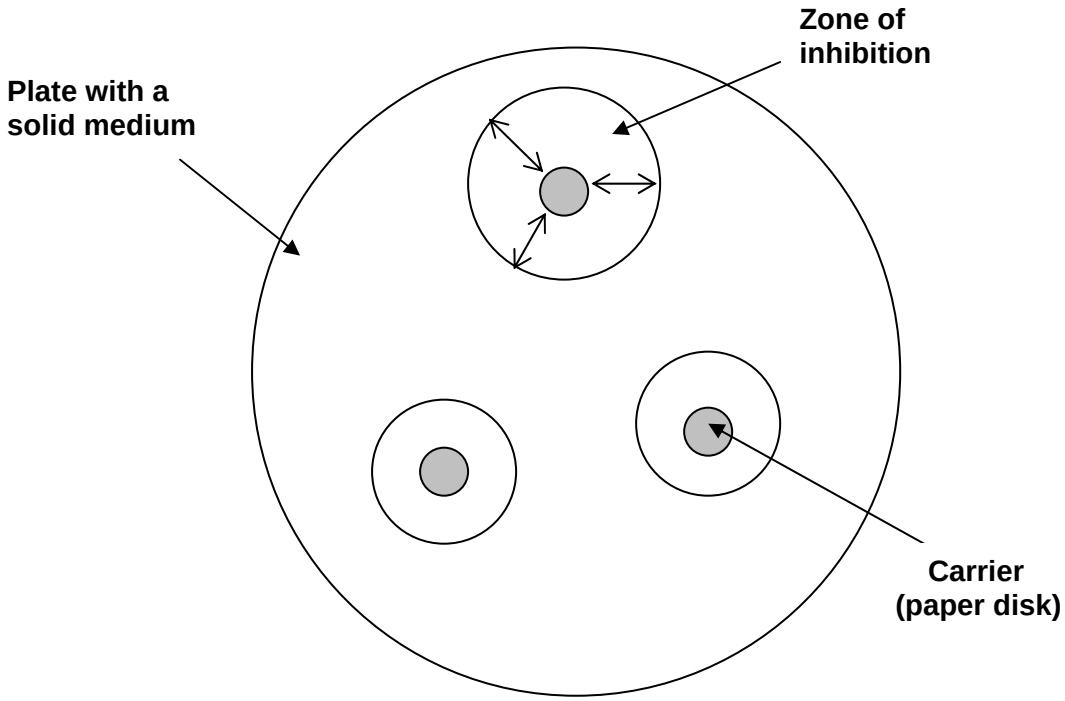


Figure 3.9: Schematic sketch of the performed agar diffusion test. As shown by arrows, the inhibition zone was measured at three different positions. An average of these three measurements was calculated and noted in a table.

3.12. *Radical scavenger activity*

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body and to prevent the deterioration of fats and other constituents of daily diet [Molineux 2004]. Extracts obtained from various *Allium* species were tested on their antioxidative activity. The used method is based on diphenylpicrylhydrazil (DPPH) [Blois 1958].

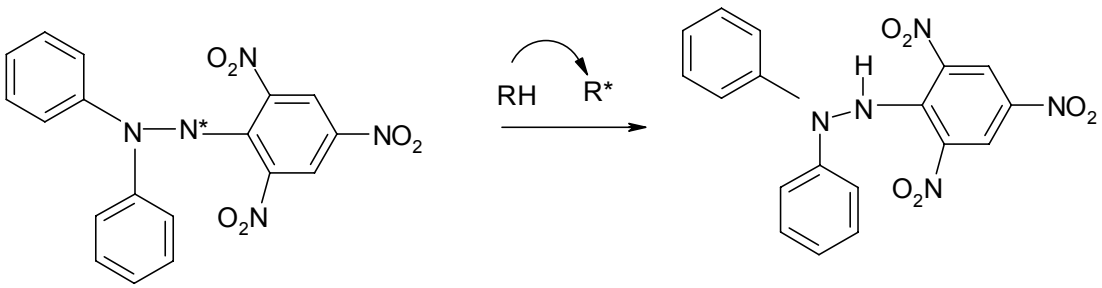


Figure 3.10: DPPH free radical and reduced form.

DPPH is a stable free radical because of a delocalisation of a spare electron over the whole molecule. The delocalisation gives rise to a deep violet colour, which is characterised by an absorption band in methanol solution at about 250 nm. When the solution of DPPH is mixed with a substance that can donate a hydrogen atom, then this gives rise to a reduced form. The colour of the solution turns to light yellow. This change in colour can be quantified.

3.12.1. *Sample Preparation*

0.3-1.0 G (fresh weight) of cleaned and peeled bulbs of various *Allium* species were crushed in a mortar filled with 5 ml of Sørensen buffer into a homogenous mixture. After 30 minutes of incubation, the mixture was filtrated through a glass wool. The filtrate was extracted thrice with 5 ml ethyl acetate each. The ethyl acetate extracts were combined and one spoon of sodium sulphate was added to dispose of water residue. After 10 minutes, the water-free ethyl acetate extracts were filtered and the filtrate was carefully evaporated nearly to dryness. The evaporated oily extract was resolved in 1 ml of methanol and transferred into a small vial. The solvent was evaporated again. The vial was closed and stored at -20 °C before further use.

Wild *Allium* species

In the following Table 3.16, wild *Allium* species are listed used for the determination of radical scavenger activity. *Allium sativum* used in this experiment as reference did not belong to wild *Allium* species.

Table 3.16: *Allium* species, for which radical scavenger activity was determined by the DPPH-assay

Acc. No.	Botanical Name
4125	*A. alaicum*
4133	*A. motor*
4134	*A. talassicum*
4142	*A. pskemense*
4143	*A. turkestanicum*
4156	*A. margaritiferum*
4165	*A. giganteum* (1)
4170	*A. komarowii* (1)
4188	*A. jodanthum*
4190	*A. filidens*
4193	*A. karataviense*
6031	*A. griffithianum*
6050	*A. rosenbachianum*
6054	*A. bucharicum*
6074	*A. darwasicum*
6084	*A. oschaninii* (1)
6085	*A. barsczewskii*
6094	*A. schugnanicum*
6101	*A. stipitatum* (1)
6106	*A. hissaricum*
6110	*A.rosenorum*
6113	*A. verticillatum*
4900	*A. sativum*
1006	*A. chelotum*
1016	*A. cristophii*
1017	*A. paradoxum*
1022	*A. iranicum*
1023	*A. oschaninii* (2)
1024	*A. pseudobodeanum*
1033	*A. jesdianum* (1)
1035	*A. giganteum* (2)

Acc. No.	Botanical Name
4133	*A. motor*
4135	*A. talassicum*
6103	*A. stipitatum* (2)
6112	*A. suworowii*
6135	*A. oschaninii* (3)
6136	*A. alexeianum*
6142	*A. komarowii* (2)
6145	*A. winklerianum*
6150	*A. carolinianum*
6163	*A. hymenorrhizum*
6166	*A. caeruleum*
Tax 1125	*A. longicuspis*
Tax 1211	*A. aflatunense*
Tax 1678	*A. altaicum* (1)
Tax 2218	*A. macleanii*
Tax 2724	*A. pskemense*
Tax 2746	*A. altaicum* (2)
Tax 3666	*A. jesdianum* (2)
Tax 3951	*A. jesdianum* (3)
Tax 5394	*A. hybrid*
Tax 0382	*A. oleraceum*
Tax 0514	*A. pskemense*
6912	*A. giganteum* (3)

3.12.2. *Determination of Radical Scavenger Activity using DPPH*

The extracts were solved in methanol to give a final concentration of 1.0 mg/ ml. As a positive control, BHT (butylated hydroxytoluene) solution (1.0 mg/ ml) was used and as a negative control methanol p.a. BHT is a known antioxidant, which is often used in the food industry. As a free radical, DPPH solution (0.4 mg/ml) was used. Solutions were disposed in the wells in a microtiter plate. Each microtiter plate was covered with a dark folio and kept at room temperature for 30 minutes. The absorbance was measured by a plate reader either at 517 nm or at 540 nm, depending on the used reader model. The dispensing scheme is given in Table 3.17.

Table 3.17: Dispensing scheme for two extracts on a microtiter plate in order to determine radical scavenger activity. Each test was done twice and controls were completed four times. Each *Allium* extract was tested three times.

	1	2	3	4	5	6
A	DPPH	NC	BHT D	BHT+So.	Extr.1 + DPPH	Extr.1 + So.
B	DPPH	NC	BHT D	BHT+So.	Extr.1 + DPPH	Extr.1 + So.
C	DPPH	NC	BHT D	BHT+So.	Extr.2 + DPPH	Extr.2 + So.
D	DPPH	NC	BHT D	BHT+So.	Extr.2 + DPPH	Extr.2 + So.

DPPH	100 µl So + 25 µl DPPH + 75 µl So
NC (negative control)	100 µl So + 25µl So + 75µl So
BHT D	100 µl BHT + 25µl DPPH + 75µl So
BHT+So.	100 µl BHT + 25µl Ro + 75 µl So
Extr. + DPPH	100 µl Extr. + 25µl DPPH + 75µl So
Extr. + So.	100 µl Extr. + 25µl Ro + 75µl So
So.	solvent (methanol)
Extr.	extract

Figure 3.14: Photo of the microtiter plate after performing the DPPH assay.

Evaluation of Results

As mentioned above, the absorbance at 517 nm or 540 nm was measured. Relative absorbance was calculated as follows:

$A_{DPPH} - A_{so.} = \mathbf{A_D}$

$(A_{Extr.} + A_{DPPH)} - (A_{Extr.} + A_{So)} = \mathbf{A}$

In the case of BHT $(A_{BHT} + A_{DPPH)} - (A_{BHT} + A_{SO.}) = \mathbf{A}$

A_{DPPH}	absobance of DPPH
$A_{so.}$	absobance of solvent
$A_{Extr.}$	absobance of extract
A_{BHT}	absobance of BHT

Relative absorbance = $100- (100*A/A_D)$

4. Results

4.1. *Flow Injection Analysis (FIA)*

Alternatively to HPLC, the total amount of cysteine sulphoxides was determined by a biosensoric method using a flow injection analyser (FIA). In contrast to the conventional HPLC-method, FIA is faster, allows a high throughput of samples and neither pre-column derivatization nor chromatographic separations are required. This method is based on the reaction of alliinase with cysteine sulphoxides as described in detail below (see Fig. 1.6., introductory section).

4.1.1. *FIA-System Development*

Previously, the content of cysteine sulphoxides (mostly alliin) was determined by the amount of enzymatically formed allicin. The Fig. 1.6 shows that the enzyme alliinase catalyzes the conversion of the cysteine sulphoxides not only into alk(en)ylsulphenic acid and further to thiosuphinates. Pyruvic acid and ammonia are also products of this reaction. The amount of enzymatically formed ammonia is proportional to the content of cysteine sulphoxides. Therefore, it can be used for indirect quantification of cysteine sulphoxides [Keusgen 2003].

In our experiments, enzymatically formed ammonia reacted further with OPA-reagent (pH 9.0), which contained o-phtaldialdehyde (OPA) and thioglycolic acid. This reaction is similar to that used for HPLC analysis; it had to be performed at alkaline milieu. Complete reaction is shown in the Figure 4.1 below. Theoretically, amino acids can also react, but this reaction needs much more time (several minutes) as the ammonia reaction depicted below.

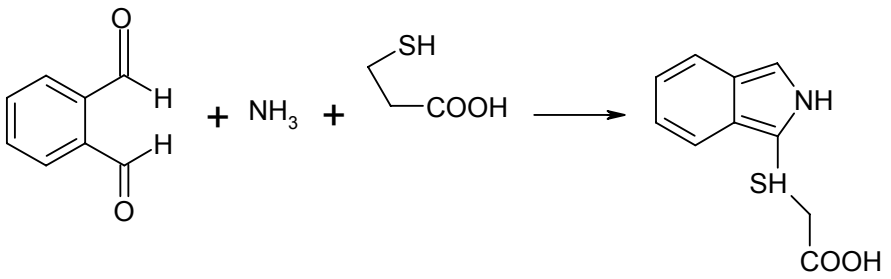


Figure 4.1: Reaction of OPA, ammonia and thioglycolic acid. The reaction leads to OPA derivatives. Alkaline milieu was adjusted by using boric acid buffer (pH 12.0).

Resulting OPA derivatives were determined by a FIA device (Bioservice, Halle), which consisted of two four-channel peristaltic pumps, autosampler (both Gilson Company, Ohio),

6 port switching valve and a fluorescence detector. The device was fully controlled by a personal computer equipped with a software prototype developed by Mr. Bodo Fuhrmann from Halle. The following scheme describes the FIA device (Fig. 4.2).

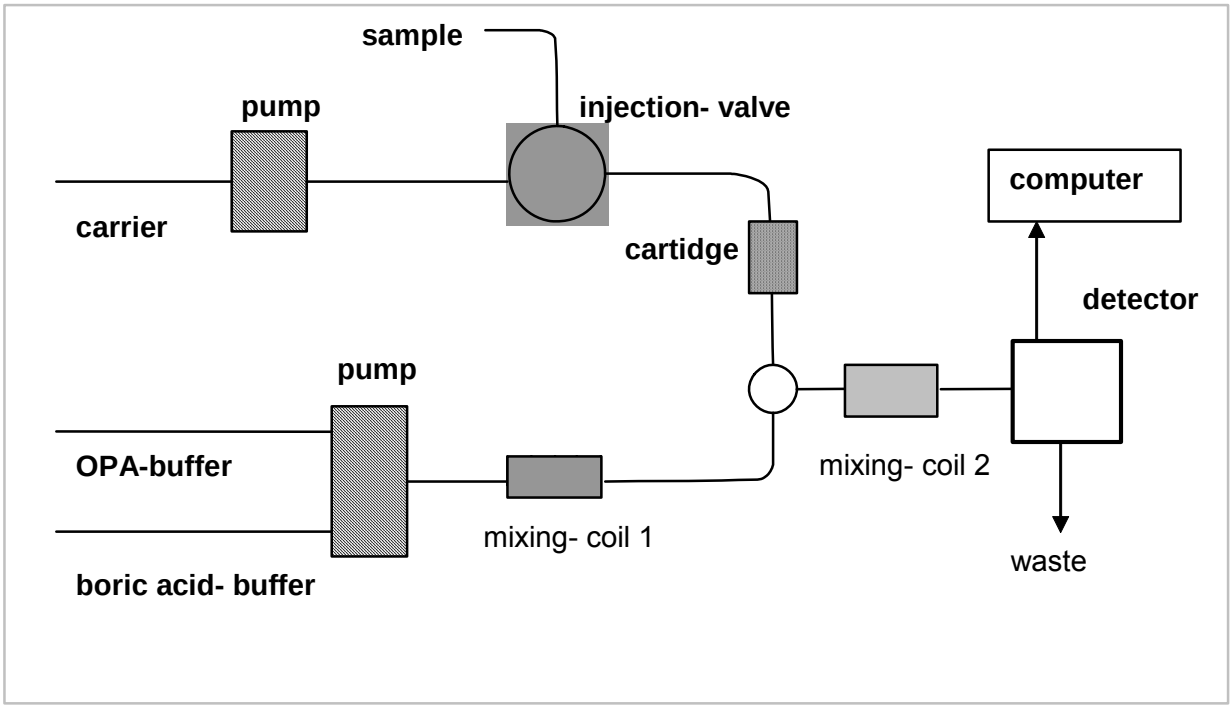


Figure 4.2: Scheme of the FIA used for determination of cysteine sulphoxides in wild *Allium* species.

Carrier and buffers were delivered with a peristaltic pump at a constant flow rates. Samples were taken in periodically by means of an autosampler and in certain intervals they were injected into the injection valve. Furthermore, the sample was firstly mixed with the carrier and later on with the derivatization reagents (OPA-buffer) in the mixing coils. Products of the reaction were determined by a fluorescence detector (emission λ= 485 nm; single photon counter). Directly behind the injection valve, a cartridge was mounted where the enzyme alliinase was immobilized.

As already mentioned, in contrast to a chromatographic method there was no separation step involved in the FIA method. Peak geometry is therefore somewhat different from that of chromatograms [Keusgen 1998]. Typical peaks acquired from such measurements are displayed in the following Figure 4.3.

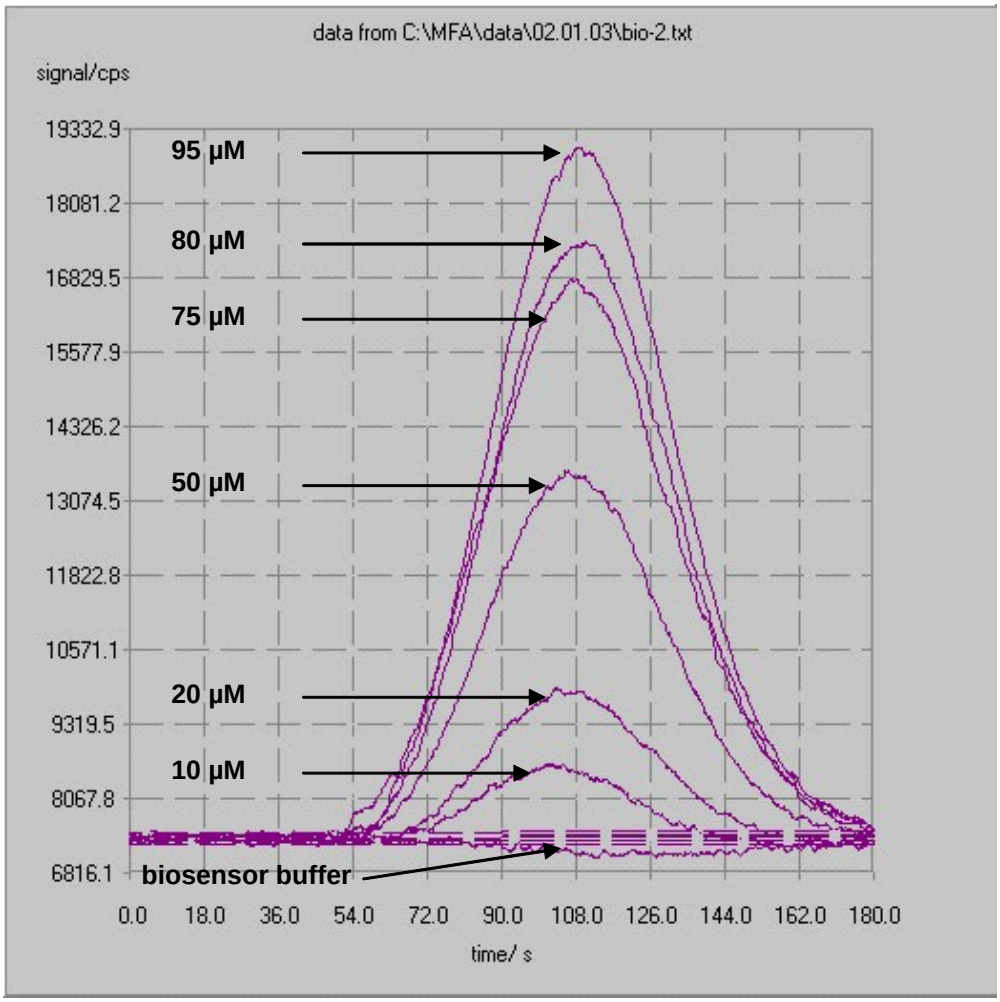


Figure 4.3: Typical peaks acquired from measurements (calibration) obtained by the FIA device given in Fig. 4.2. Depicted measurements were performed to obtain a calibration plot of alliin. Alliin concentrations are shown by arrows.

4.1.2. *Calibration of FIA with Ammonium and Alliin*

As already mentioned, a detailed calibration of eight alliin and ammonium standards was carried out. The following Figures 4.4 and 4.5 show the calibration curves obtained from these measurements.

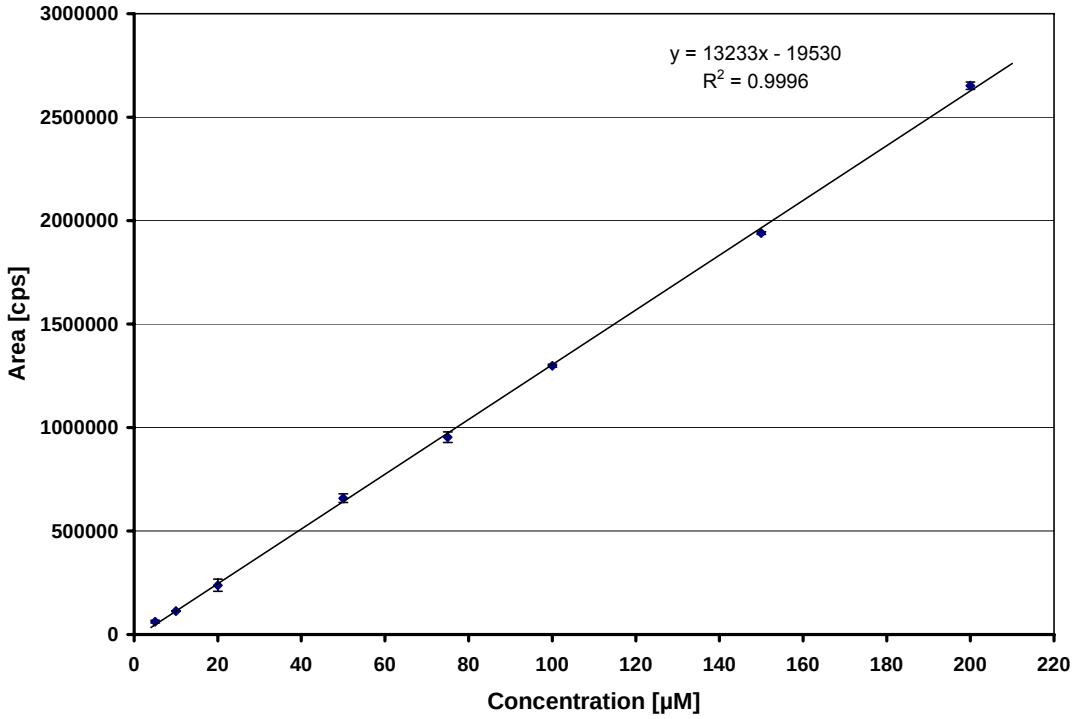


Figure 4.4: A calibration curve obtained from eight different concentrations of ammonium. Measurements were performed without the allinase cartridge (n=3).

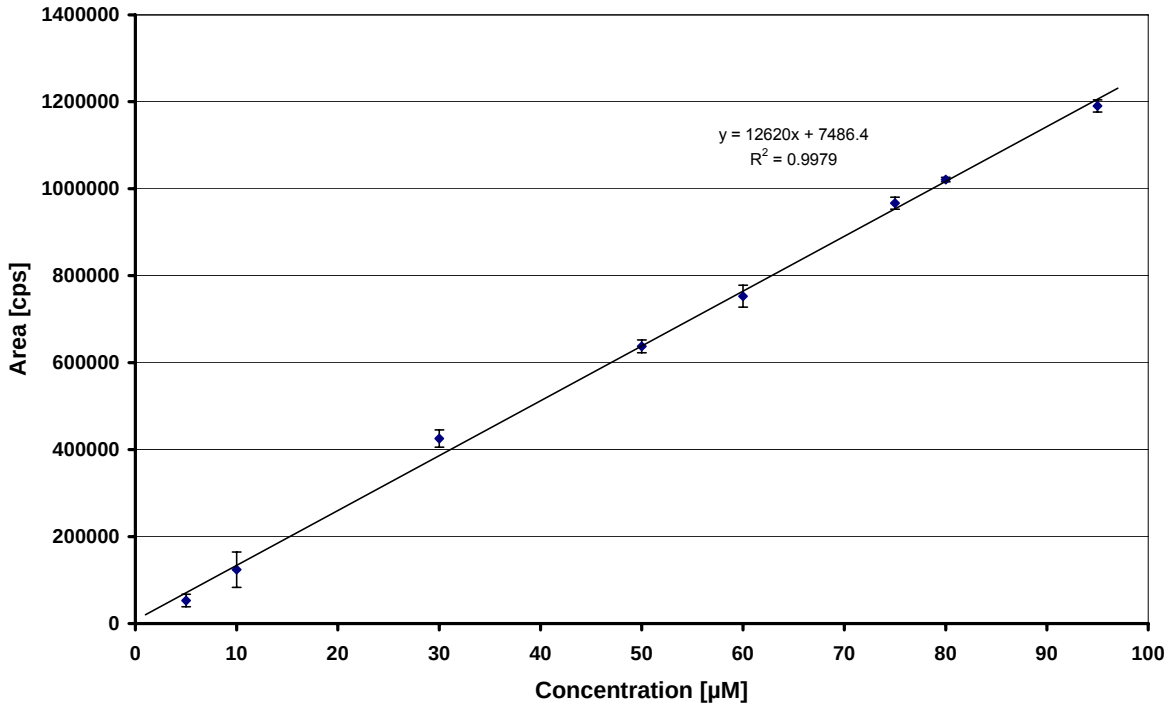


Figure 4.5: A Calibration curve obtained from eight different concentrations of alliin. Measurements were performed using the cartridge filled with immobilized alliinase (n=3).

A three-point recalibration was carried out every day before measurements of samples were started. The recalibration was necessary for several reasons:

- for determination of the activity of immobilized enzyme alliinase (the activity of alliinase decreases slowly over time [Keusgen 1998])
- as control of the FIA device (defects in the device could be fastly detected without wasting the samples)
- as control of the accuracy of single measurements.

Typically, further measurements with real samples were performed when the coefficient r^2 was found to be better than 0.99.

4.1.3. *FIA Substrate Specificity*

Previous investigations have demonstrated that the enzyme alliinase possess a high specificity towards alliin and isoalliin [Krest 2000]. Nevertheless, alliin is not the only cysteine sulphoxide obtained in bulbs of wild *Allium* species. Therefore, the substrate specificity of immobilized alliinase towards different cysteine sulphoxides was investigated. Substrate specificity was determined using a FIA device. The alliinase was immobilized in a cartridge (inner volume 150µl) as described in chapter 3.7.2. (Immobilization of Alliinase). All measurements, except those for (+)-alliin, were performed in one day. It has to be taken into account that alliinase activity might decrease during measurements [Keusgen 1998]. Measurements of cysteine sulphoxides were performed as follows: (+/-)-alliin, (+)-isoalliin, (+/-)-ethiin, (+/-)-methiin, (+/-)-propiin, (+/-)-hexiin, (+/-)-buthiin, and again (+/-)-alliin. With the exception of alliin and isoalliin, only mixtures of + and - cysteine sulphoxides were available. Isoalliin was isolated from *A. cepa*. The following Figures 4.6 - 4.9 show the obtained results.

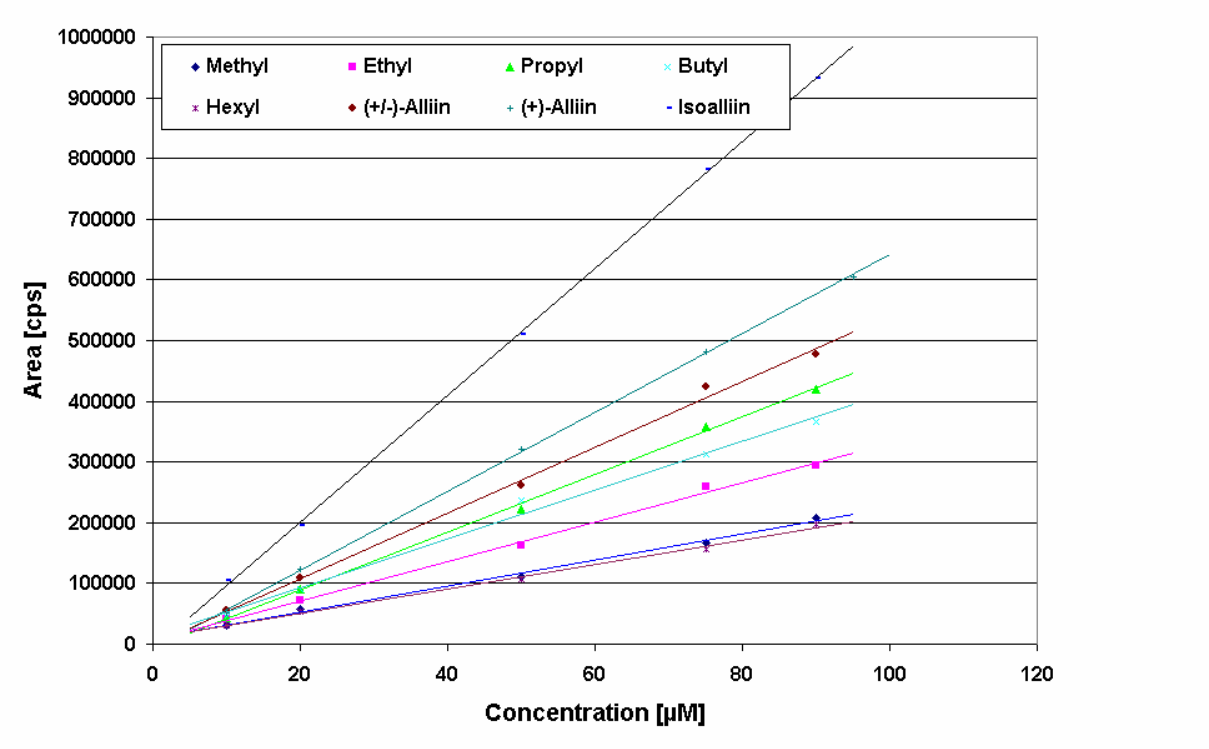


Figure 4.6: Substrate specificity of enzyme alliinase towards all investigated cysteine sulphoxides in an overview.

The next three graphics display in detail the substrate specificity of alliinase towards each cysteine sulphoxide with depicted standard deviations and coefficient r^2 in detail. Relative standard deviations ranged on average from 2.6 to 9.0%. The relative standard deviations (RSD) were found to be smaller at higher concentrations than at lower concentrations, *e.g.*, for alliin at a concentration of 95 µM the RSD was found to be 0.70% whereas at 10 µM 8.16% were determined.

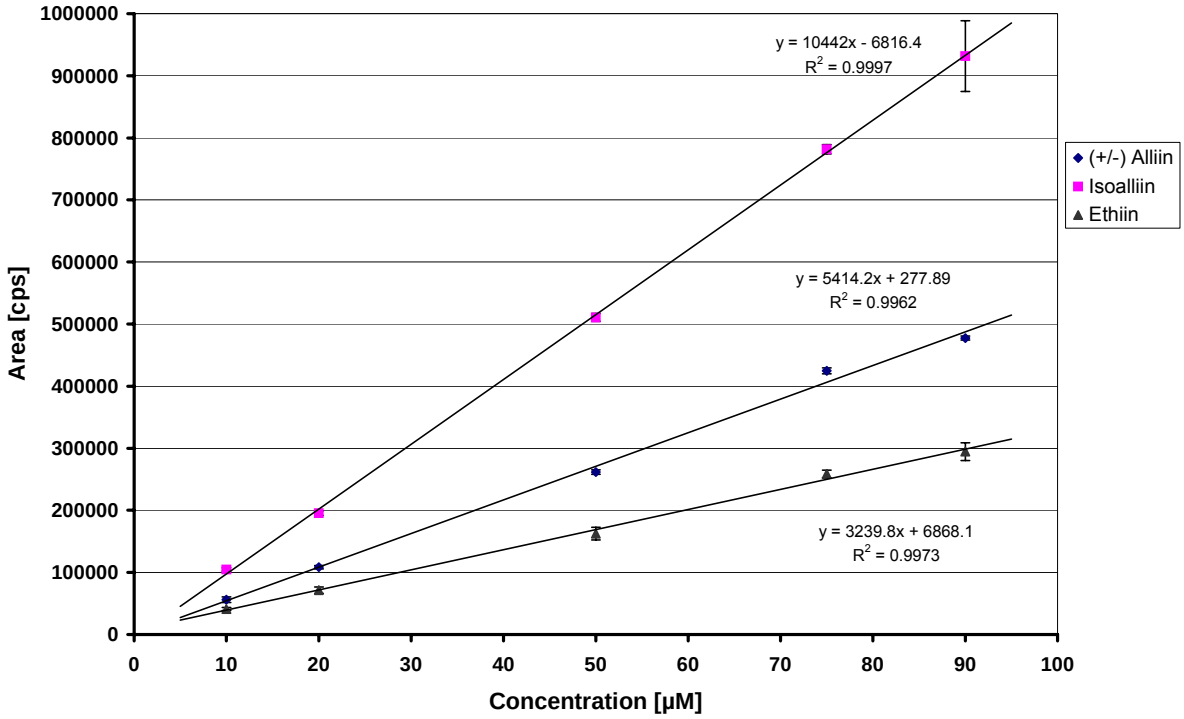


Figure 4.7: Substrate specificity of alliinase towards isoalliin, (+/-)-alliin and ethiin. For isoalliin, the relative standard deviation was found to be in the range between 0.95% and 6.12%, for (+/-) alliin in the range between 0.70% and 8.16% and for ethiin between 2.45% and 6.38%. The coefficient r^2 was found to be better than 0.99.

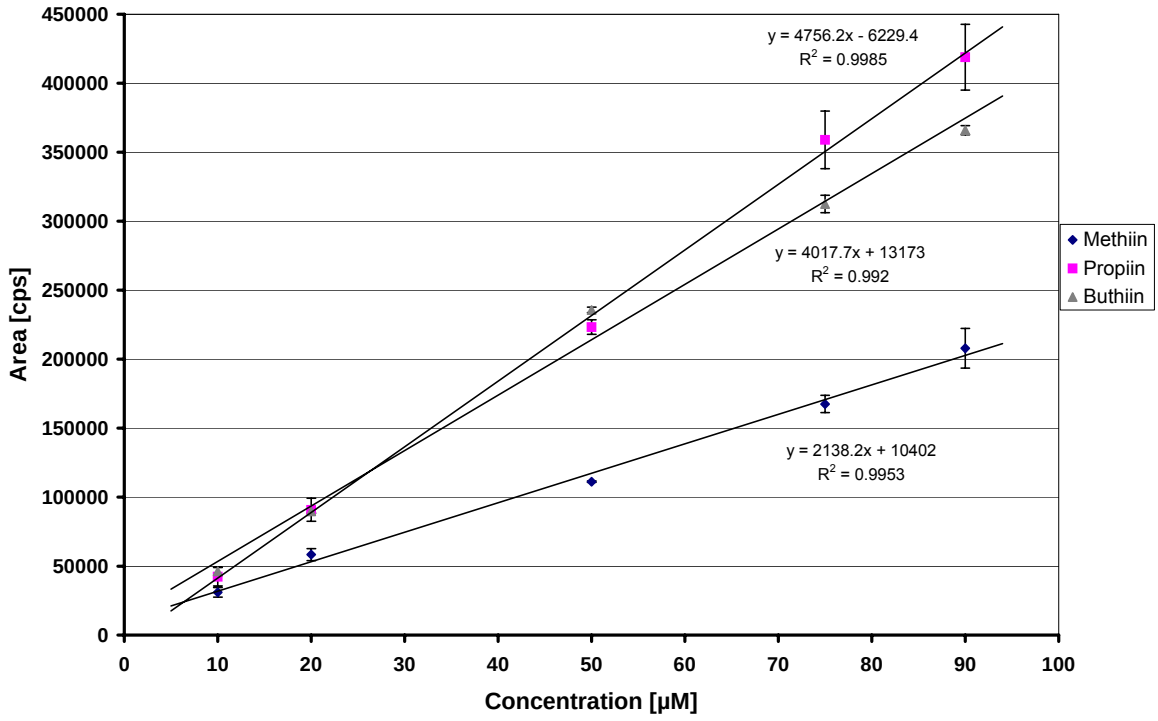


Figure 4.8: Substrate specificity of alliinase towards methiin, propiin and buthiin. For methiin, the values of relative standard deviation ranged from 0.45% to 11.31%, for propiin from 2.38% to 15.76% and for buthiin from 0.82% to 6.90%. The coefficient r^2 was found to be better than 0.99.

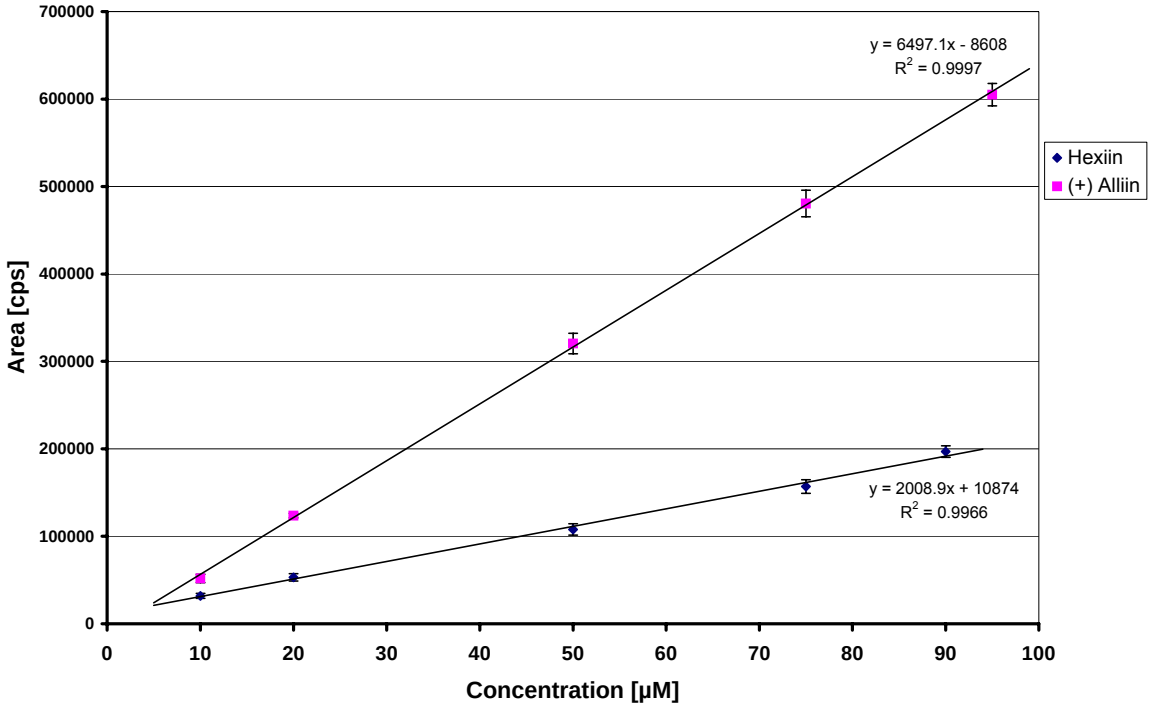


Figure 4.9: Substrate specificity of alliinase towards (+) alliin and hexiin. In the case of hexiin, the relative standard deviation was found in the range between 3.41% and 9.05% and in the case of (+) alliin in the range between 0.82% and 24.88%. Again, the coefficient r^2 was found to be better than 0.99.

These graphics show that alliinase possess the highest specificity towards isoalliin and the lowest specificity towards hexiin. To get a better overview of the acquired results, relative activity was calculated (relative activity was deduced from the slope of regression function). The following Figure 4.10 shows the relative activity of investigated cysteine sulphoxides in comparison to the (+/-)-alliin.

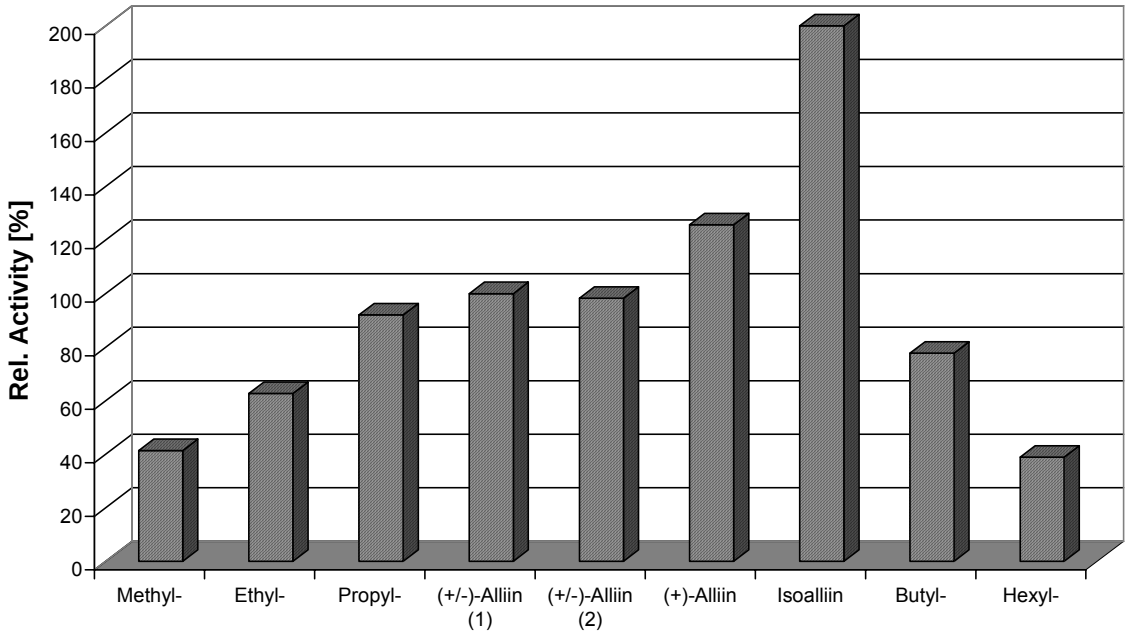


Figure 4.10: The relative activity of investigated cysteine sulphoxides (relative activity of (+/-) alliin = 100%).

The relative activity of (+/-) alliin was set to lie at 100%. As already mentioned, the highest relative activity was found for isoalliin (202.06%), further followed by (+)-alliin (125.72%), propiin (92.04%), buthiin (77.74%), ethiin (62.69%) and methiin (41.38%). The lowest relative activity was found for hexiin (only 38.87%). Previous investigations showed that the alliinase activity might decrease during measurements. Therefore, the activity for (+/-)-alliin as substrate was determined at the beginning and at the end of the measurements. The values of relative activity of (+/-)-alliin at the end of the measurements were found to be lower than at the beginning, but the difference was only 1.78% and can therefore be neglected.

4.1.4. *Limits of Detection and Quantitation*

In order to use the FIA method for standard measurements, the signal to noise ratio as well as the limit of detection and limit of quantitation had to be determined. These two limits are in dependence of the signal to noise ratio.

A typical base line is depicted in the Figure 4.11.The noise of the base line was determined to be approximately 200 cps. In contrast, typical signal intensity of a peak obtained from a 5 µM alliin standard was approximately 1250 cps. The signal to noise ratio

for this peak is therefore about 6. Limit of detection should have a signal to noise ratio of 3 [Rücker et al. 2001]. From this value it can be deduced that the limit of detection lies approximately at 3 µM. Limit of quantitation should have at least a signal to noise ratio of 10. Consequently, an alliin concentration of 10 µM was used as a limit of quantitation. Both limits allow a sufficient measurement of wild *Allium* samples even with a low level of cysteine sulphoxides.

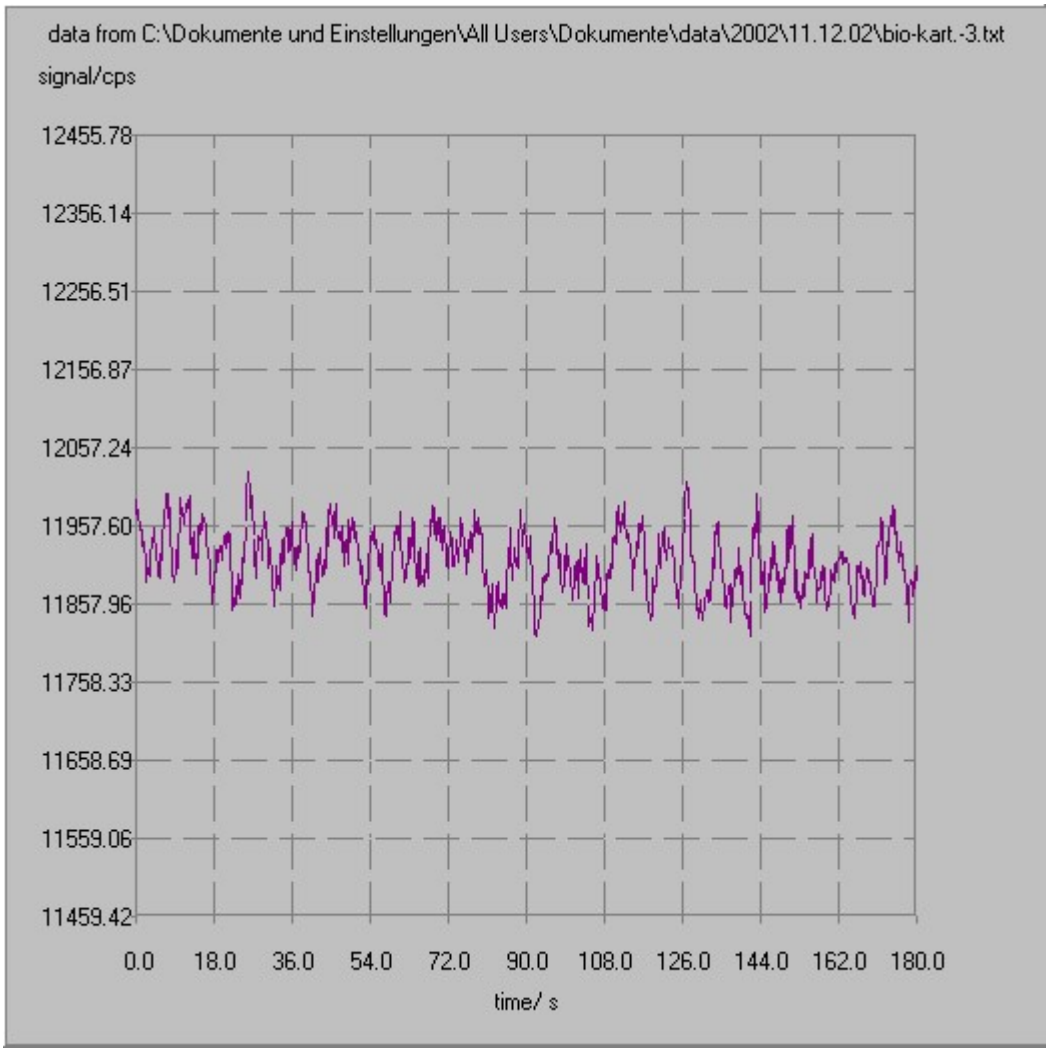


Figure 4.11: A typical plot of a base line during the calibration with alliin.

4.1.5. *Analysis of Allium Species by FIA*

A method using the FIA device was developed to allow a rapid prescreening in order to identify wild *Allium* species with a high concentration of cysteine sulphoxides (higher than 0.25%). Moreover, these species should be investigated by HPLC in detail. However, development of the FIA method was rather time consuming. Therefore, FIA and HPLC experiments were performed in parallel in the here presented investigations.

After calibration of the FIA device with standards, the total amount of cysteine sulphoxides was measured. Various bulbs of wild *Allium* species collected in the Republics of Uzbekistan and Tajikistan were investigated and acquired results were compared with concentrations obtained from the HPLC measurements. The samples were prepared as described in chapter 3.7.1. (Material and Methods) and they were usually measured directly after recalibration of the FIA device with standards. Single measurements were repeated three times. The following Table 4.1.and Figure 4.12 show results that were obtained.

Table 4.1: Total amounts of cysteine sulphoxides, calculated as (+)-alliin, in bulbs of different wild *Allium* species collected in Uzbekistan and Tajikistan. Concentrations are related to fresh weight.

Acc.-Number	Name	Total CSO [%]	SD. [%]
4122	*A. talassicum*	0.11	± 0.10
4119	*A. litvinovii*	0.05	± 0.01
6102	*A. sativum* (1)	0.61	± 0.09
6105	*A. barsczewskii*	0.12	± 0.02
4169	*A. brevidentiforme*	0.43	± 0.15
6054	*A. bucharicum*	0.02	± 0.01
4130	*A. caesium*	0.11	± 0.03
6074	*A. darwasicum*	0.03	± 0.02
4147	*A. filidens*	0.23	± 0.07
4185	*A. filidens*	0.10	± 0.02
6031	*A. griffithianum*	0.05	± 0.01
4120	*A. gusaricum*	0.55	± 0.15
6042	*A. insufficiens*	0.01	± 0.00
4136	*A. barsczewskii*	0.36	± 0.02
4900	*A. sativum* (2)	0.43	± 0.05
6077	*A. oschaninii*	0.09	± 0.02
4145	*A. protensum*	0.03	± 0.01
4142	*A. pskemense*	0.22	± 0.07
6050	*A. rosenbachianum*	0.04	± 0.01
6110	*A. rosenorum*	0.01	± 0.00
4172	*A. sarawschanicum*	0.04	± 0.00
4145	*A. sect. oreiprason*	0.02	± 0.00
6094	*A. schugnanicum*	0.08	± 0.00

Acc.-Number	Name	Total CSO [%]	SD. [%]
6045	*A. subg. Allium*	0.14	± 0.06
6083	*A. suworowii* (1)	0.06	± 0.01
6090	*A. suworowii* (2)	0.37	± 0.14
4116	*A. talassicum*	0.32	± 0.05
4137	*A. severtzovioides*	0.02	± 0.01
4143	*A. turkestanicum*	0.54	± 0.03
6113	*A. verticillatum*	0.06	± 0.04
6081	*A. winklerianum*	0.02	± 0.01

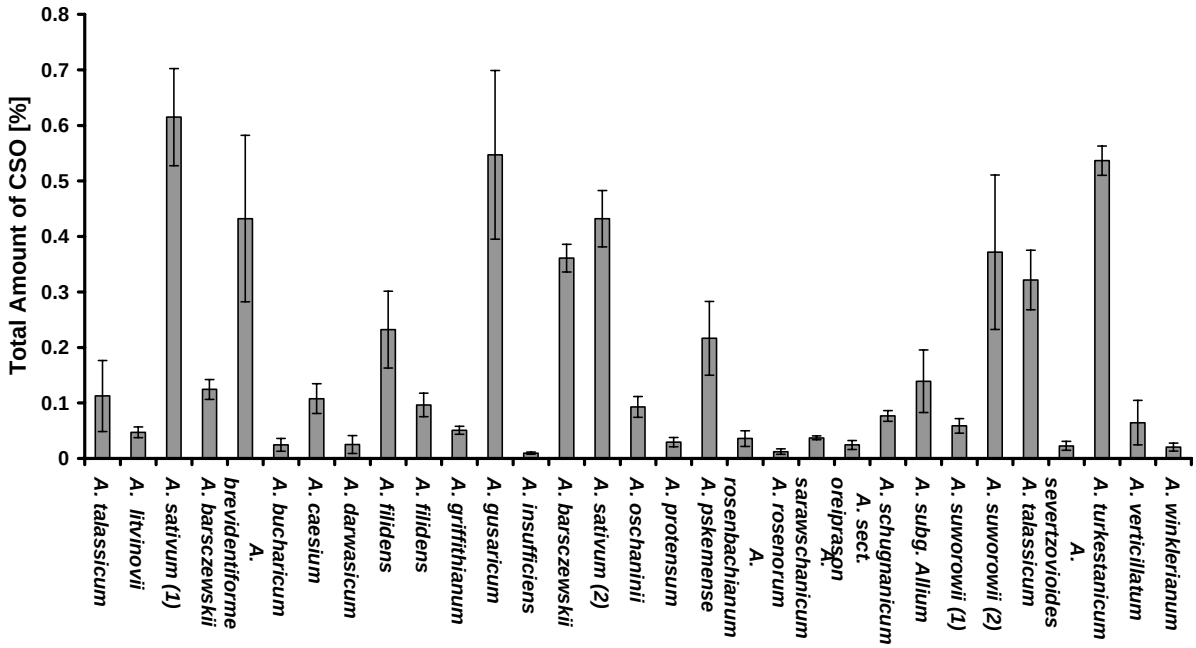


Figure 4.12: Total amounts of cysteine sulphoxides, calculated as (+)-alliin, in bulbs of various wild *Allium* species collected in Uzbekistan and Tajikistan. Standard deviations are given by error bars.

According to their amount of total cysteine sulphoxides, the investigated *Allium* species could be divided into three groups:

- In the first group, *Allium* species containing a low level of cysteine sulphoxides are included (amount of cysteine sulphoxides is **lower than 0.1%**). To this group belong, *e.g.*, *A. darwasicum* (0.03 ± 0.02%), *A. protensum* (0.03 ± 0.01%), and *A. rosenorum* (0.01 ± 0.01%).

- In the second group, *Allium* species containing a medium level of cysteine sulphoxides are included (amount of cysteine sulphoxides is **between 0.1 and 0.25%**). To this group belong, *e.g.*, species like *A. pskemense* (0.22 ± 0.07%), *A. barsczewskii* (0.12 ± 0.02%), and *A. caesium* (0.11± 0.03%).
- In the third group *Allium* species containing high levels of cysteine sulphoxides are included (amount of cysteine sulphoxides is **higher then 0.25%**). To this group belong, *e.g.*, *A. brevidentiforme* (0.43 ± 0.15%), *A. jodanthum* (0.36 ± 0.02%), and *A. sativum* (0.43 ± 0.05%).

4.2. *HPLC Analysis*

One of the disadvantages of the FIA-method is that no information about the composition of cysteine sulphoxides can be gained. Therefore, a complex and time consuming HPLC-method is necessary for the determination of the cysteine sulphoxide pattern. A pre-column derivatization is necessary for the separation of amino acids and cysteine sulphoxides. However, separation between alliin and isoalliin is rather crucial. Therefore, the method was adapted to this task as described in the introductory section (chapter 3.6). In this investigation, only main cysteine sulphoxides as methiin, alliin, isoalliin and propiin were taken into consideration. The investigation showed that the smaller cysteine sulphoxides like ethiin and buthiin do not play an important role and were mostly found below detection limit. The date as well as the exact place of collection of investigated *Allium* species are listed in Tables 3.1 - 3.5. A foto documentation of some selected *Allium* species as well as of places of collection are given in chapter 8.1. (Appendix).

4.2.1. *Analysis from Allium Species Collected in Iran*

Samples obtained from Iran were collected in 2004. Since there was only a limited quantity of plant material available, not all collected bulbs and leaves were analysed using HPLC. The flavour precursors (cysteine sulphoxides) of wild *Allium* species were analyzed by HPLC, allowing a differentiation between methiin, alliin, isoalliin and propiin. The following Table 4.2 shows total amounts as well as amounts of particular cysteine sulphoxides.

Table 4.2: Amounts of cysteine sulphoxides in bulbs of different wild *Allium* species collected in Iran. Concentrations are related to fresh weight.

Acc.-Number	Name	Total CSO [%]	SD [%]	Methiin [%]	SD [%]	Alliin [%]	SD [%]	Isoalliin [%]	SD [%]	Propiin [%]	SD [%]
1024	*A. pseudobodeanum*	0.63	± 0.32	0.45	± 0.24	0.12	± 0.05	0.06	± 0.02	0.00	± 0.00
1016	*A. cristophii*	0.34	± 0.10	0.24	± 0.08	0.01	± 0.01	0.09	± 0.03	0.00	± 0.00
1006	*A. chelotum*	0.50	± 0.01	0.41	± 0.01	0.03	± 0.00	0.04	0.00	0.02	± 0.00
1022	*A. iranicum*	0.13	± 0.05	0.13	± 0.05	0.00	± 0.00	0.00	0.00	0.00	± 0.00
1033	*A. jesdianum*	0.50	± 0.27	0.46	± 0.29	0.00	± 0.00	0.04	± 0.03	0.00	± 0.00
1017	*A. paradoxum var. normale*	0.82	± 0.42	0.38	± 0.20	0.00	± 0.00	0.40	± 0.21	0.04	± 0.01
1028	*A. giganteum*	0.45	± 0.06	0.31	± 0.04	0.00	± 0.00	0.15	± 0.01	0.00	± 0.00

Determination of the Total Amount of Cysteine Sulphoxides

The following Figure 4.13 displays the total amounts of flavour precursors in wild *Allium* species, which were collected in Iran.

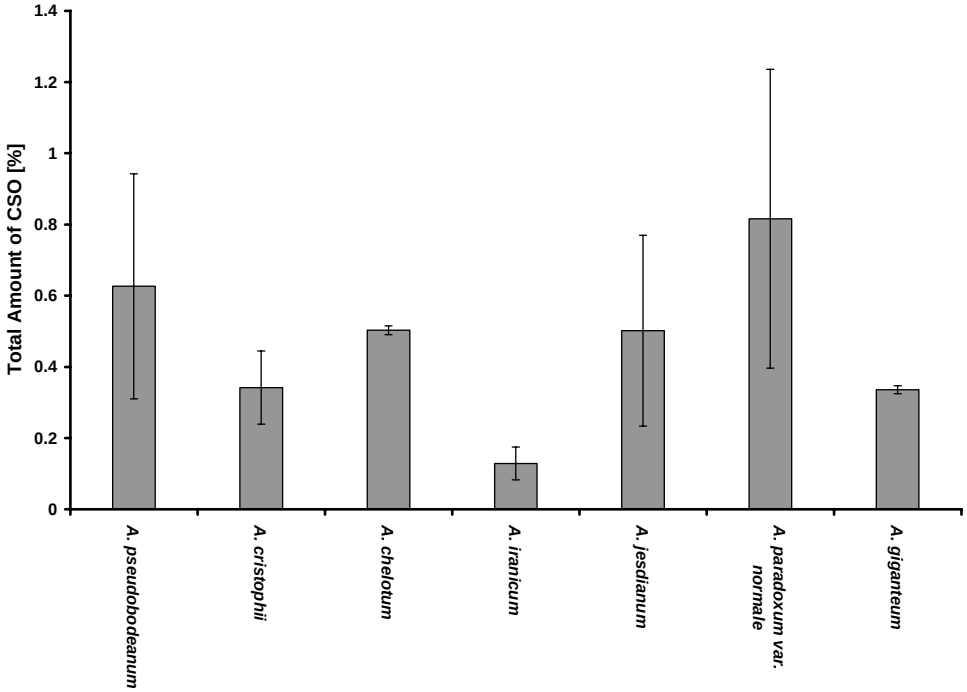


Figure 4.13: Total amounts of cysteine sulphoxides in wild *Allium* species collected in Iran. Concentrations are related to fresh weight. Standard deviations are given by error bars.

The total amounts of the cysteine sulphoxides were in the range between 0.13 ± 0.05% (*A. iranicum*) and 0.82 ± 0.42% (*A. paradoxum var. normale*). Remarkable values of total amounts were found in bulbs of *A. pseudobodeanum* (0.63 ± 0.32%), *A. jesdianum* (0.50 ± 0.27%) and *A. chelotum* (0.50 ± 0.01%). These species belong to the subgenus *Melanocrommyum*.

Pattern of Cysteine Sulphoxides

Patterns of cysteine sulphoxides were determined and are depicted in the following Figure 4.14. For a better overview the results are given as bars indicating relative amounts (total amount = 100%).

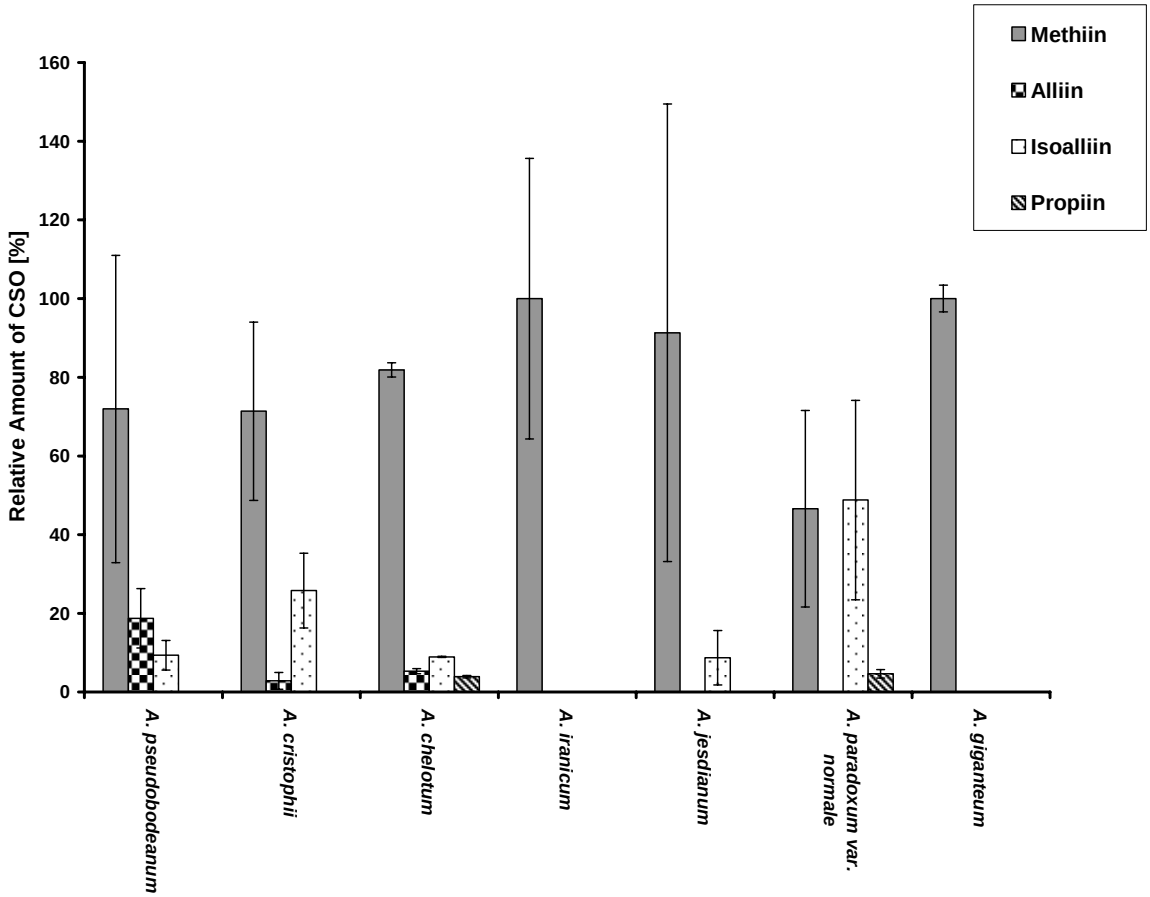


Figure **4.14**: Patterns of cysteine sulphoxides in wild *Allium* species, which were collected in Iran. Standard deviations are given by error bars.

Figure 4.14 shows that **methiin** was the most abundant cysteine sulphoxide in all investigated samples. In two cases, it was even the only cysteine sulphoxide, which was found in these wild *Allium* species (*A. iranicum* and *A. giganteum*). Therefore, the relative

amount of **methiin** ranged from 46.58 ± 25.0% (*A. paradoxum var*. *normale*) to 100% (*A. giganteum* and *A. iranicum*).

Alliin is the typical cysteine sulphoxide for garlic and it is also the main cysteine sulphoxide of this species. Alliin was detected only in three investigated wild *Allium* species: in *A. pseudobodeanum* (18.71 ± 7.55%), *A. cristophii* (2.84 ± 2.11%) and in *A. chelotum* (5.29 ± 0.67%).

Isoalliin is characteristic for the common onion. Apart from *A. giganteum* and *A. iranicum*, isoalliin was found in all investigated samples. Remarkable amounts were detected in bulbs of *A. paradoxum var. normale* (48.79 ± 25.34%) and *A. cristophii* (25.79 ± 9.50%). Small amounts were found in samples obtained from *A. chelotum*, *A. jesdianum* and *A. pseudobodeanum.*

Propiin is the typical cysteine sulphoxide of leek. **Propiin** was determined only in small amounts in bulbs of *A. chelotum* (3.9 ± 0.29%) and *A. paradoxum var. normale* (4.63 ±1.08%).

4.2.2. *Analysis from Allium Species Collected in Turkmenistan*

The following *Allium* samples were collected in year 2002 in Turkmenistan. Samples were transferred to the living *Allium* collection of Agabath and later on transferred to Germany on demand. The determination of cysteine sulphoxides was also carried out by means of HPLC. In the following Table 4.3, total amounts as well as amounts of particular cysteine sulphoxides are depicted.

Table 4.3: Amount of cysteine sulphoxides in bulbs,bulbils and leaves of different wild *Allium* species collected in Turkmenistan. Concentrations of the bulbs are related to fresh weight, concentration of leaves to dry weight.

Acc.-Number	Name	Total CSO [%]	SD [%]	Methiin [%]	SD [%]	Alliin [%]	SD [%]	Isoalliin [%]	SD [%]	Propiin [%]	SD [%]
0111	*A. borszczowii*	0.32	± 0.11	0.27	± 0.09	0.02	± 0.00	0.02	± 0.02	0.00	± 0.00
030	*A. caspium*	0.15	± 0.01	0.08	± 0.01	0.00	± 0.00	0.07	± 0.00	0.00	± 0.00
046	*A. isakulii ssp. subkopetdagense*	0.14	± 0.02	0.07	± 0.00	0.00	± 0.00	0.07	± 0.02	0.00	± 0.00
022	*A. kopetdagense*	0.12	± 0.01	0.02	± 0.00	0.00	± 0.00	0.10	± 0.00	0.00	± 0.00
082	*A. ophiophyllum*	0.08	± 0.02	0.05	± 0.02	0.00	± 0.00	0.03	± 0.01	0.00	± 0.00
006	*A. regelii*	0.34	± 0.08	0.27	± 0.07	0.00	± 0.00	0.08	± 0.01	0.00	± 0.00
0114	*A. turcomanicum*	0.85	± 0.01	0.61	± 0.01	0.21	± 0.00	0.02	± 0.00	0.00	± 0.00

Acc.-Number	Name	Total CSO [%]	SD [%]	Methiin [%]	SD [%]	Alliin [%]	SD [%]	Isoalliin [%]	SD [%]	Propiin [%]	SD [%]
	bulbils										
0114	*A. turcomanicum*	1.07	± 0.01	0.81	± 0.00	0.18	± 0.00	0.07	± 0.00	0.00	± 0.00
0116	*A. xiphopetalum*	0.97	± 0.04	0.91	± 0.04	0.04	± 0.00	0.02	± 0.00	0.00	± 0.00
011	*A. cristophii*	0.20	± 0.06	0.17	± 0.05	0.00	± 0.00	0.00	± 0.00	0.03	± 0.01
0135	*A. paradoxum var. normale*	0.16	± 0.03	0.11	± 0.02	0.00	± 0.00	0.04	± 0.02	0.01	± 0.01
0115	*A. yatei*	0.26	± 0.01	0.19	± 0.09	0.00	± 0.00	0.04	± 0.02	0.03	± 0.02
014	*A. brachyscapum*	0.44	± 0.22	0.43	± 0.21	0.00	± 0.00	0.00	± 0.00	0.01	± 0.01
047	*A. vavilovii* **leaves**	0.41	± 0.06	0.05	± 0.01	0.00	± 0.00	0.36	± 0.05	0.00	± 0.00

Determination of the Total Amount of Cysteine Sulphoxides

The following Figure 4.15 shows the total amount of flavour precursors obtained from wild *Allium* species, which were collected in Turkmenistan.

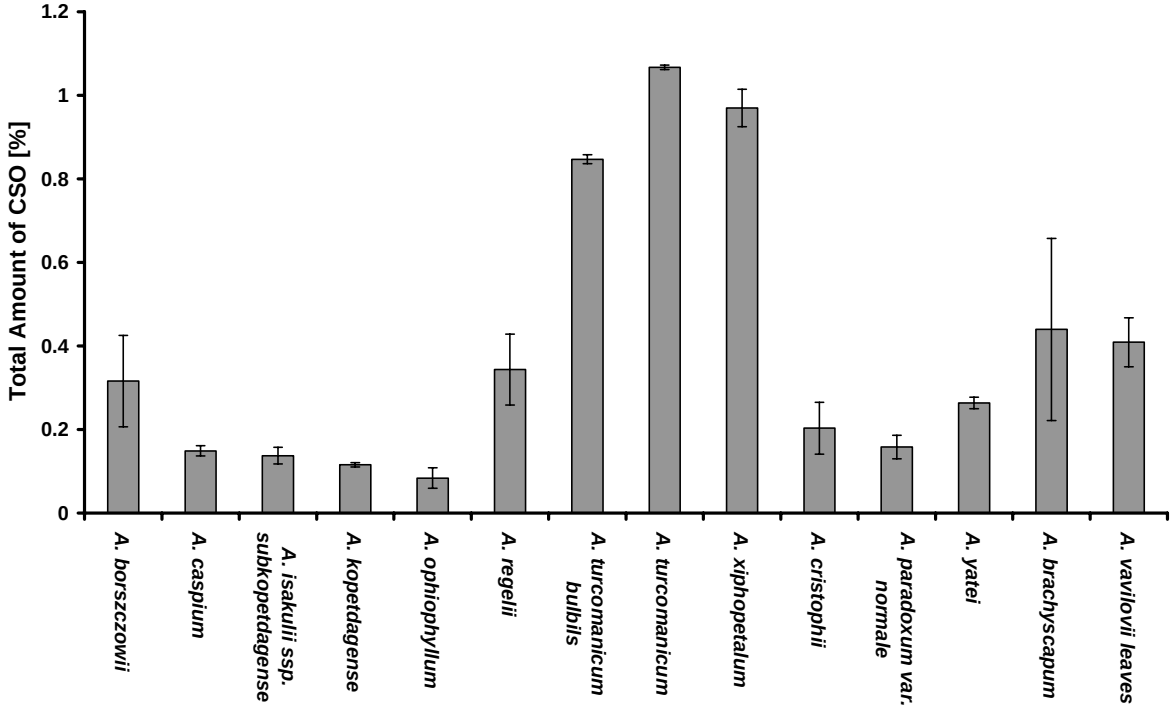


Figure 4.15: Total amounts of cysteine sulphoxides obtained from bulbs, bulbils and leaves of different wild *Allium* species collected in Turkmenistan. Standard deviations are given by error bars.

Total amounts of cysteine sulphoxides ranged from 0.08 ± 0.02% (*A. ophiophyllum*) to 1.07 ± 0.01% (bulbs of *A. turcomanicum*). Remarkably high amounts were found in bulbils of

A. turcomanicum (0.85 ± 0.01%), and in samples obtained from *A. xiphopetalum* (0.97 ± 0.04%) and *A. brachyscapum* (0.44 ± 0.22%). Interestingly, in bulbs and bulbils of *A. turcomanicum*, different values of the total amount of cysteine sulphoxides were detected.

Pattern of Cysteine Sulphoxides

The following Figure 4.16 depicts the relative composition of cysteine sulphoxides obtained from wild *Allium* species collected in Turkmenistan.

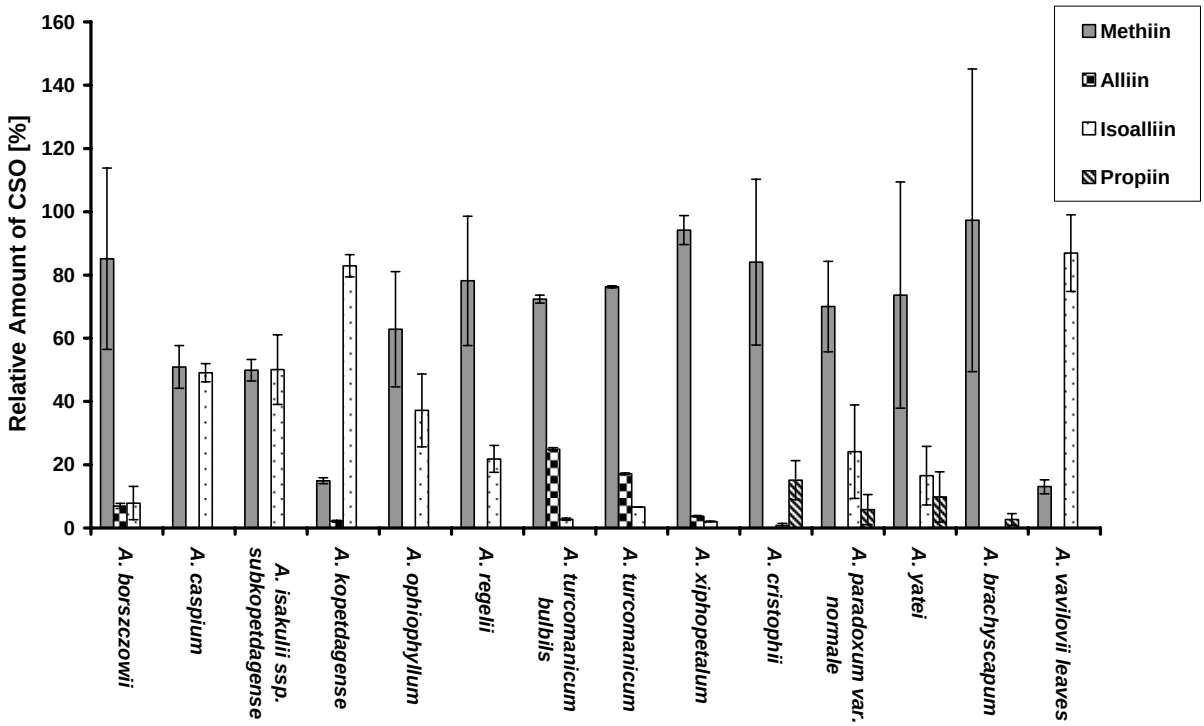


Figure 4.16: Relative amounts of cysteine sulphoxides obtained from bulbs, bulbils and leaves of different wild *Allium* species collected in Turkmenistan (total cysteine sulphoxides = 100%). Standard deviations are given by error bars.

Methiin was found to be the most abundant cysteine sulphoxide, which was detected in all investigated samples. The relative amount ranged between 13.08 ± 2.20% (leaves of *A. vavilovii*) and 85.15 ± 28.65% (bulbs of *A. borszczowii*). For this set of samples, methiin was found only in combination with other flavour precursors. The relative amount of **methiin** in bulbs and bulbils of *A. turcomanicum* was slightly different (bulbs 72.37 ± 1.29%, bulbils 76.23 ± 0.36%).

Alliin was detected only in five *Allium* species. The relative amount ranged between 2.15 ± 0.29% (*A. kopetdagense*) and 24.91 ± 0.47% (bulbils of *A. turcomanicum*). The

relative amounts of **alliin** in bulbs and bulbils of *A*. *turcomanicum* were, again, slightly different (bulbs 17.15 ± 0.25%, bulbils 24.91 ± 0.47%).

The relative amounts of **isoalliin** ranged from 0.83 ± 0.62% (*A. cristophii*) to 86.92 ± 12.15% (leaves of *A. vavilovii*). Remarkably relative amounts of **isoalliin** were found in samples obtained from *A. kopetdagense* (82.9 ± 3.49%), *A. isakulii* (50.09 ± 11.01%) and *A. caspium* (49.07 ± 2.87%). The relative amount of i**soalliin** of *A. turkmestanicum* was higher in bulbs (6.62 ± 0.03%) than in bulbils (2.76 ±0.35%). **Isoalliin** was not detected in bulbs of *A. brachyscapum* and *A. cristophii*.

Propiin was only found in four wild *Allium* species. The relative amounts ranged between 2.69 ± 1.84% (*A. brachyscapum*) and 15.1 ± 6.21% (*A. cristophii*).

4.2.3. *Analysis from Allium Species Collected in Uzbekistan*

The investigated *Allium* species were collected in year 2003 in Uzbekistan. Amounts of cysteine sulphoxides were analyzed by means of HPLC. Total amounts as well as amounts of particular cysteine sulphoxides are shown in the following Table 4.3.

Table 4.3: Amounts of cysteine sulphoxides in bulbs and leaves of different wild *Allium* species collected in Uzbekistan. Concentrations of the bulbs are related to fresh weight, concentrations of leaves to dry weight.

Acc.-Number	Name	Total CSO [%]	SD [%]	Methiin [%]	SD [%]	Alliin [%]	SD [%]	Isoalliin [%]	SD [%]	Propiin [%]	SD [%]
4101	*A. aflatunense*	0.61	± 0.06	0.55	± 0.06	0.00	± 0.00	0.06	± 0.02	0.00	± 0.00
4125	*A..alaicum*	0.12	± 0.01	0.10	± 0.02	0.00	± 0.00	0.02	± 0.01	0.00	± 0.00
4168	*A. aroides*	0.13	± 0.04	0.08	± 0.04	0.00	± 0.00	0.04	± 0.01	0.00	± 0.00
4164	*A. caspium ssp. baissunense*	0.08	± 0.02	0.02	± 0.01	0.06	± 0.02	0.05	± 0.03	0.00	± 0.00
4169	*A. brevidentiforme*	0.32	*	0.09	*	0.17	*	0.07	*	0.00	*
4187	*A. caesium*	0.19	± 0.01	0.08	± 0.03	0.03	± 0.02	0.08	± 0.02	0.00	± 0.00
4021	*A. caspium*	0.10	± 0.01	0.06	± 0.01	0.00	± 0.00	0.04	± 0.00	0.00	± 0.00
4160	*A. crystallinum*	0.63	± 0.29	0.08	± 0.03	0.48	± 0.33	0.06	± 0.01	0.00	± 0.00
4176	*A. cupuliferum*	0.21	± 0.01	0.17	± 0.10	0.02	± 0.01	0.03	± 0.00	0.00	± 0.00
4153	*A. drepanophyllum*	1.05	± 0.07	1.05	± 0.07	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
4144	*A. filidens*	0.60	± 0.06	0.49	± 0.05	0.11	± 0.02	0.00	± 0.00	0.00	± 0.00

Acc.-Number	Name	Total CSO [%]	SD [%]	Methiin [%]	SD [%]	Alliin [%]	SD [%]	Isoalliin [%]	SD [%]	Propiin [%]	SD [%]
4120	*A. aff. gusaricum*	0.03	± 0.00	0.01	± 0.00	0.00	± 0.00	0.02	± 0.00	0.00	± 0.00
4151	*A. gypsaceum*	0.07	± 0.04	0.05	± 0.03	0.00	± 0.00	0.01	± 0.01	0.00	± 0.00
4117	*A. haneltii*	0.02	± 0.00	0.01	± 0.00	0.00	± 0.00	0.00	± 0.00	0.01	± 0.00
4166	*A. barsczewskii*	0.94	± 0.14	0.45	± 0.02	0.44	± 0.16	0.05	± 0.04	0.00	± 0.00
4139	*A. karataviense* leaves	0.31	± 0.03	0.14	± 0.02	0.00	± 0.00	0.00	± 0.00	0.17	± 0.03
4170	*A. komarowii* **leaves**	0.52	± 0.12	0.02	± 0.01	0.00	± 0.00	0.29	± 0.08	0.21	± 0.04
4170	*A. komarowii*	0.10	± 0.01	0.08	± 0.01	0.00	± 0.00	0.01	± 0.00	0.01	± 0.00
4900	*A. sativum*	1.69	± 0.91	0.08	± 0.01	1.59	± 0.89	0.02	± 0.01	0.00	± 0.00
4165	*A. giganteum*	0.02	± 0.00	0.01	± 0.00	0.00	± 0.00	0.01	± 0.00	0.00	± 0.00
4156	*A. margaritiferum*	0.48	± 0.18	0.36	± 0.16	0.11	± 0.03	0.00	± 0.00	0.00	± 0.00
4133	*A. motor*	0.21	± 0.08	0.21	± 0.08	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
4133	*A. motor* **leaves**	0.07	± 0.02	0.07	± 0.02	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
4155	*A. ophiophyllum*	0.04	± 0.01	0.04	± 0.01	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
4118	*A. orunbaii*	0.05	± 0.01	0.05	± 0.01	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
4123	*A. oschaninii*	0.38	± 0.03	0.02	± 0.00	0.00	± 0.00	0.31	± 0.03	0.05	± 0.00
4142	*A. pskemense*	0.19	± 0.12	0.05	± 0.04	0.02	± 0.01	0.08	± 0.07	0.04	± 0.02
4140	*A. severtzovioides*	0.10	± 0.06	0.08	± 0.05	0.00	± 0.00	0.02	± 0.01	0.00	± 0.00
4183	*A. stephanophorum*	0.12	± 0.00	0.11	± 0.00	0.01	± 0.00	0.00	± 0.00	0.00	± 0.00
4180	*A. taeniopetalum*	0.02	± 0.00	0.01	± 0.00	0.00	± 0.00	0.01	± 0.00	0.00	± 0.00
4131	*A. tashkenticum*	0.17	± 0.01	0.09	± 0.01	0.00	± 0.00	0.08	± 0.01	0.00	± 0.00
4175	*A. verticilatum*	0.42	± 0.35	0.37	± 0.35	0.00	± 0.00	0.04	± 0.01	0.00	± 0.00
4135	*A. talassicum*	0.73	± 0.04	0.65	± 0.05	0.00	± 0.00	0.08	± 0.02	0.00	± 0.00
4143	*A. turkestanicum*	0.59	± 0.03	0.22	± 0.01	0.34	± 0.02	0.02	± 0.02	0.00	± 0.00
4124	*A. pallassii*	0.10	± 0.01	0.03	± 0.01	0.00	± 0.00	0.08	± 0.00	0.00	± 0.00

***** not determined (limited amount of plant material).

Determination of the Total Amount of Cysteine Sulphoxides

The following two Figures 4.17 and 4.18 depict the total amounts of flavour precursors in bulbs and leaves of wild *Allium* species, which were collected in Uzbekistan.

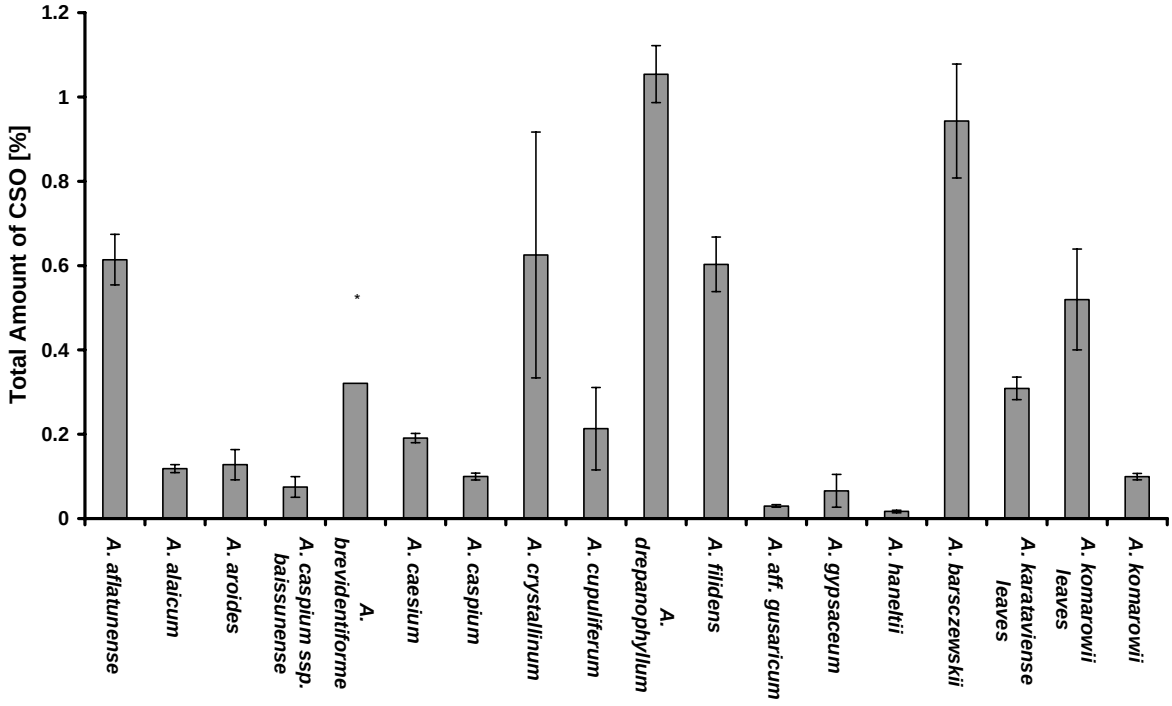


Figure 4.17: Total amounts of cysteine sulphoxides obtained from bulbs and leaves of different wild *Allium* species collected in Uzbekistan (part 1). Standard deviations are given by error bars.

* SD not determined (limited amount of plant material).

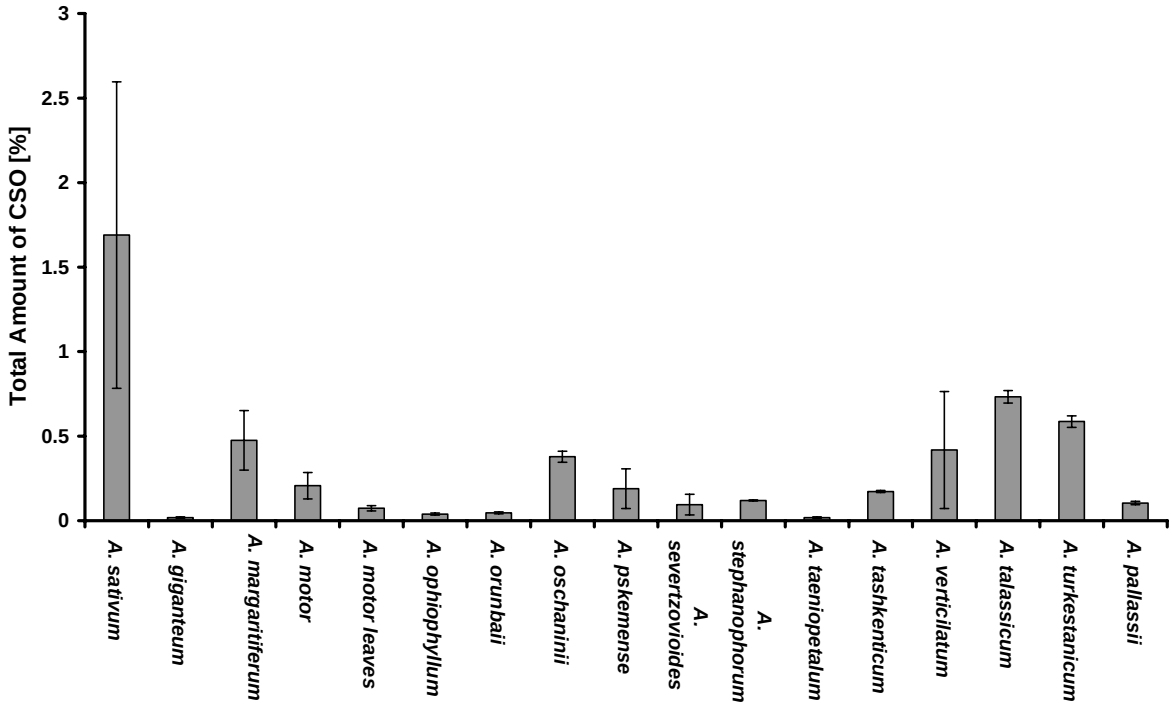


Figure 4.18: Total amounts of cysteine sulphoxides obtained from bulbs and leaves of different wild *Allium* species collected in Uzbekistan (part 2). Standard deviations are given by error bars.

Investigated wild *Allium* species demonstrated a high variation in the total amounts of cysteine sulphoxides. The total amounts ranged from 0.02 ± 0.003% (*A. haneltii*) to 1.69 ± 0.91% (of *A. sativum*). Remarkably high amounts of cysteine sulphoxides were detected in samples obtained from A*. drepanophyllum* (1.05 ± 0.07%), *A. barsczewskii* (0.94 ± 0.14%), *A. crystallinum* (0.63 ± 0.29%) and *A. aflatunense* (0.61 ± 0.06%). Significantly low amounts of flavour precursors were found in the bulbs of *A. giganteum* and *A. taeniopetalum* (both species 0.018 ± 0.004%), *A. ophiophyllum* (0.04 ± 0.01%) and *A. orunbaii* (0.046 ± 0.01%).

Pattern of Cysteine Sulphoxides

Following Figures 4.19 - 4.21 depict the patterns of cysteine sulphoxides of wild *Allium* species collected in Uzbekistan. The results are, again, given in relative amounts.

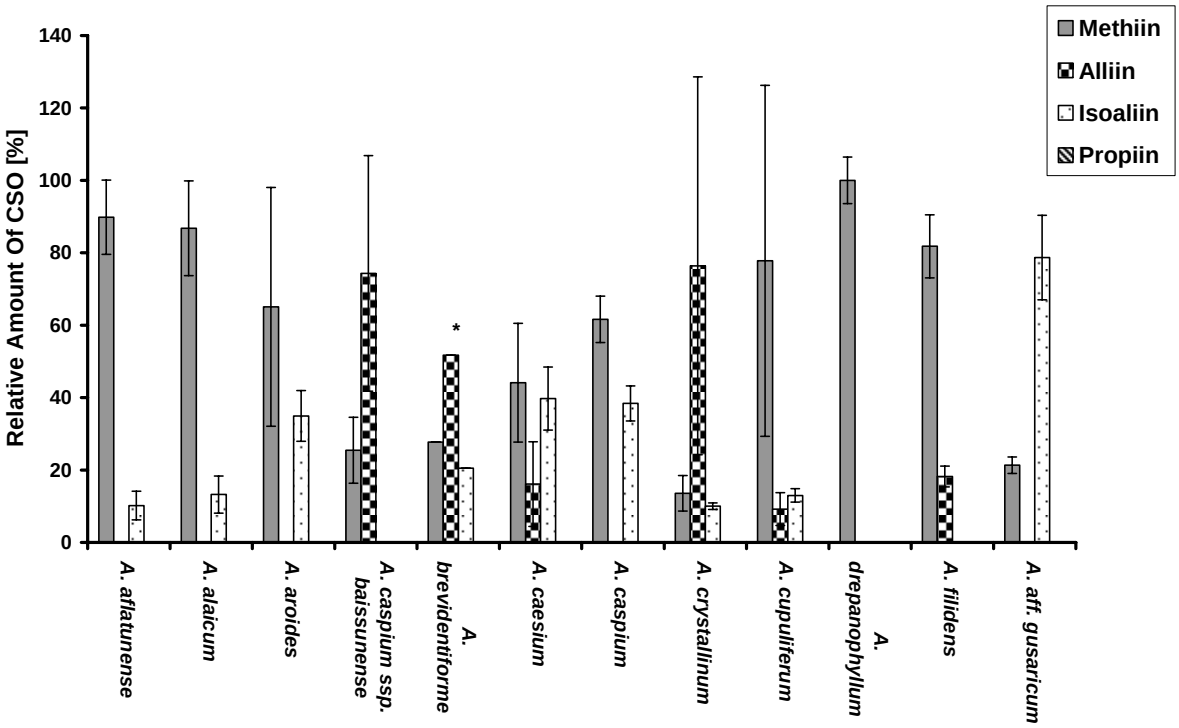


Figure 4.19: Relative amounts of cysteine sulphoxides obtained from bulbs of different wild *Allium* species collected in Uzbekistan (part 1). Total cysteine sulphoxides = 100%. Standard deviations are given by error bars.

* not determined (limited amount of plant material).

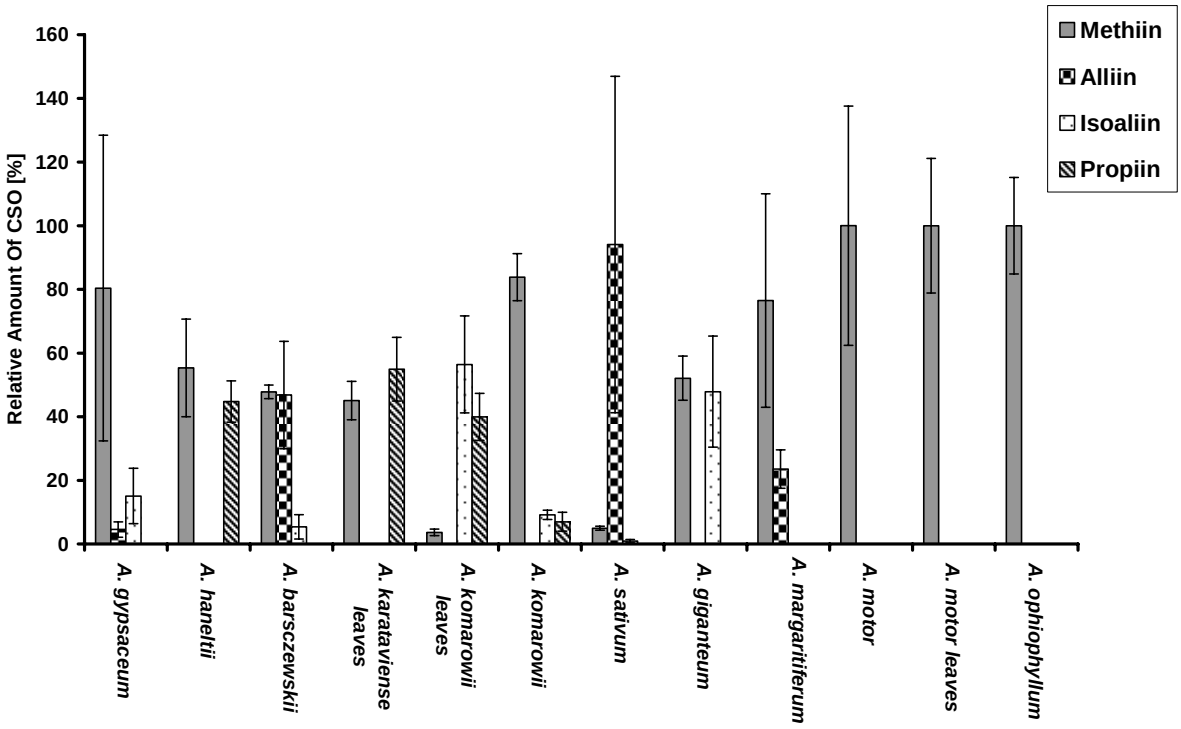


Figure 4.20: Relative amounts of cysteine sulphoxides obtained from bulbs and leaves of different wild *Allium* species collected in Uzbekistan (part 2). Total cysteine sulphoxides = 100%. Standard deviations are given by error bars.

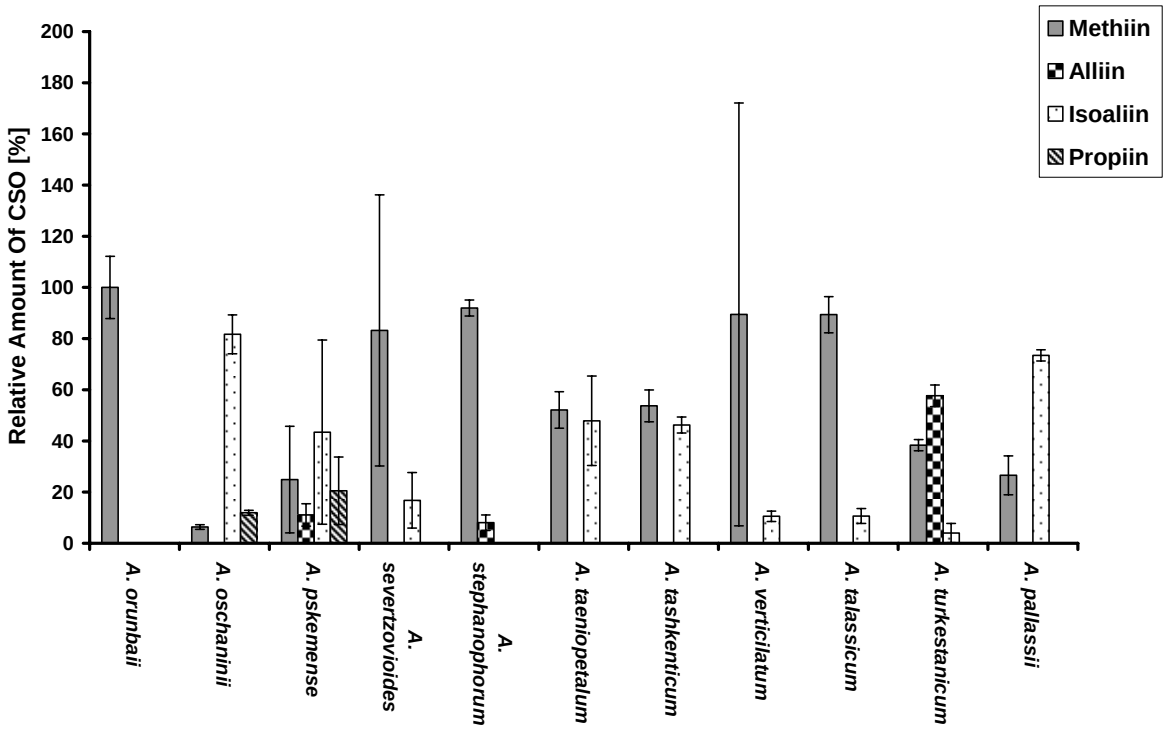


Figure 4.21: Relative amounts of cysteine sulphoxides obtained from bulbs of different wild *Allium* species collected in Uzbekistan (part 3). Total cysteine sulphoxides = 100%. Standard deviations are given by error bars.

As expected, **methiin** was detected in all investigated samples. **Methiin** was the only cysteine sulphoxide found in bulbs of *A. drepanophyllum*, *A. motor* and *A. orunbaii*. **Isoalliin** was determined as the second frequent flavour precursor. The highest relative amounts (over 50 %) of **isoalliin** were found in bulbs of *A. oschaninii* (81.69 ± 7.61%), *A. aff. gusaricum* (78.69 ± 11.67%), and *A. pallassii* (73.41 ± 2.20%) and in the leaves of *A. komarowii* (56.41 ± 15.24%). The highest relative amounts of **alliin** were detected in the bulbs of *A. sativum* (94.10 ± 52.83%), *A. crystallinum* (76.39 ± 52.17%), *A. caspium* ssp. *baissunense* (74.31 ± 32.53%) and *A. turkestanicum* (57.67 ± 4.20%). **Propiin** was detected only in six *Allium* species. The highest amounts were found in the leaves of *A. karataviense* (54.92 ± 10.00%) and in the bulbs of *A. haneltii* (44.76 ± 6.48%). Interestingly, the patterns of cysteine sulphoxides in leaves and bulbs of *A. komarowii* were found to be relatively identical (both plant parts contained **methiin**, **isoalliin** and **propiin**). The patterns of investigated leaves and bulbs of *A. motor* were also found to be identical (in both plant parts only methiin was detected).

4.2.4. *Analysis from Allium Species Collected in Tajikistan*

Wild *Allium* samples were collected in Tajikistan in 2003 and 2004. The amounts of cysteine sulphoxides were analyzed, identically as previous samples, by means of HPLC. The following Table 4.4 shows the total amounts as well as amounts of particular cysteine sulphoxides.

Table 4.4: Amounts of cysteine sulphoxides in bulbs, leaves and inflorescences of different wild *Allium* species collected in Tajikistan. Concentrations of the bulbs and inflorescences are related to fresh weight, concentration of leaves to dry weight.

Acc.-Number	Name	Total CSO [%]	SD [%]	Methiin [%]	SD [%]	Alliin [%]	SD [%]	Isoalliin [%]	SD [%]	Propiin [%]	SD [%]
6040	*A. jodanthum*	1.54	± 0.66	1.27	± 0.59	0.20	± 0.08	0.07	± 0.02	0.00	± 0.00
6097	*A. chitralicum*	0.09	± 0.08	0.09	± 0.08	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
6097	*A. chitralicum* **leaves**	0.94	± 0.22	0.35	± 0.07	0.00	± 0.00	0.60	± 0.23	0.00	± 0.00
6073	*A. darwasicum*	0.16	± 0.03	0.12	± 0.02	0.00	± 0.00	0.04	± 0.01	0.00	± 0.00
6911	*A. fedschenkoanum*	1.03	± 0.05	0.73	± 0.03	0.30	± 0.02	0.00	± 0.00	0.00	± 0.00
6911	*A. fedschenkoanum* **leaves**	0.70	± 0.02	0.24	± 0.01	0.00	± 0.00	0.46	± 0.02	0.00	± 0.00

Acc.-Number	Name	Total CSO [%]	SD [%]	Methiin [%]	SD [%]	Alliin [%]	SD [%]	Isoalliin [%]	SD [%]	Propiin [%]	SD [%]
6111	*A. flavellum*	0.57	± 0.13	0.28	± 0.06	0.29	± 0.07	0.00	± 0.00	0.00	± 0.00
6106	*A. hissaricum*	0.09	± 0.02	0.09	± 0.02	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
6106	*A. hissaricum* **leaves**	0.05	± 0.01	0.05	± 0.01	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
6107	*A. rosenbachianum ssp. kwakense*	0.05	± 0.03	0.05	± 0.03	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
6107	*A. rosenbachianum ssp. kwakense* **leaves**	0.08	± 0.00	0.08	0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
6110	*A. rosenorum*	0.03	± 0.01	0.03	± 0.01	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
6110	*A. rosenorum* **leaves**	0.04	± 0.00	0.04	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
6076	*A. suworowii* (1)	0.46	± 0.02	0.34	± 0.01	0.00	± 0.00	0.12	± 0.00	0.00	± 0.00
6083	*A. suworowii* (2)	0.05	± 0.01	0.00	± 0.00	0.00	± 0.00	0.05	± 0.01	0.00	± 0.00
6090	*A. suworowii* (3)	0.41	± 0.12	0.14	± 0.04	0.00	± 0.00	0.27	± 0.08	0.00	± 0.00
6136	*A. alexeianum* **inflorescence**	0.05	± 0.01	0.02	± 0.00	0.00	± 0.00	0.03	± 0.00	0.00	± 0.00
6136	*A. alexeianum*	0.09	± 0.00	0.05	± 0.00	0.00	± 0.00	0.04	± 0.00	0.00	± 0.00
6158	*A. alexeianum* **leaves**	2.48	± 0.25	0.92	± 0.12	0.00	± 0.00	1.56	± 0.37	0.00	± 0.00
6168	*A. barsczewskii* **inflorescence**	1.23	± 0.06	0.95	± 0.05	0.16	± 0.02	0.11	± 0.01	0.00	± 0.00
6168	*A. barsczewskii*	5.71	± 0.26	4.78	± 0.29	0.68	± 0.09	0.25	± 0.03	0.00	± 0.00
6166	*A. caeruleum* **inflorescence**	0.42	± 0.01	0.02	± 0.00	0.02	± 0.00	0.37	± 0.01	0.01	± 0.01
6166	*A. caeruleum*	0.51	± 0.22	0.22	± 0.14	0.03	± 0.02	0.26	± 0.07	0.00	± 0.00
6150	*A. carolinianum* **inflorescence**	0.61	± 0.02	0.50	± 0.16	0.04	± 0.00	0.08	± 0.01	0.00	± 0.00
6150	*A. carolinianum*	2.16	± 0.15	1.61	± 0.09	0.19	± 0.01	0.36	± 0.06	0.00	± 0.00
6912	*A. giganteum*	0.38	± 0.18	0.34	± 0.16	0.00	± 0.00	0.04	± 0.02	0.00	± 0.00
6163	*A. hymenorrhizum* **leaves**	4.83	± 0.85	2.28	± 0.42	0.85	± 0.15	1.65	± 0.26	0.04	± 0.02
6163	*A. hymenorrhizum*	1.39	± 0.06	0.73	± 0.03	0.35	± 0.04	0.29	± 0.02	0.02	± 0.01
6142	*A. komarowii* **leaves**	1.01	± 0.16	0.01	± 0.01	0.00	± 0.00	1.00	± 0.15	0.00	± 0.00
6142	*A. komarowii*	0.06	± 0.00	0.03	± 0.01	0.00	± 0.00	0.03	± 0.01	0.00	± 0.00
6135	*A. oschaninii* **inflorescence**	0.27	± 0.02	0.02	± 0.01	0.00	± 0.00	0.21	± 0.01	0.03	± 0.00
6135	*A. oschaninii*	0.23	± 0.04	0.01	± 0.00	0.00	± 0.00	0.20	± 0.04	0.02	± 0.00
6135	*A. oschaninii* **leaves**	1.47	± 0.35	0.51	± 0.11	0.00	± 0.00	0.97	± 0.24	0.00	± 0.00

Acc.-Number	Name	Total CSO [%]	SD [%]	Methiin [%]	SD [%]	Alliin [%]	SD [%]	Isoalliin [%]	SD [%]	Propiin [%]	SD [%]
6164	*A. filidens* **inflorescence**	1.58	± 0.63	1.13	± 0.66	0.45	± 0.07	0.00	0.00	0.00	± 0.00
6164	*A. filidens*	1.55	± 0.58	0.34	± 0.09	0.06	± 0.00	0.08	± 0.01	1.07	± 0.67
6112	*A. suworowii* (4)	1.81	± 0.37	1.14	± 0.24	0.00	± 0.00	0.68	± 0.14	0.00	± 0.00
6112	*A. suworowii* (4) **leaves**	0.06	± 0.03	0.06	± 0.03	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
6145	*A. winklerianum* **inflorescence**	0.28	± 0.01	0.28	± 0.01	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
6145	*A. winklerianum*	0.06	± 0.01	0.06	± 0.01	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
6171	*A. winklerianum* **leaves**	1.40	± 0.32	0.17	± 0.03	0.00	± 0.00	1.24	± 0.29	0.00	± 0.00
6171	*A. winklerianum* **inflorescence II**	0.19	± 0.05	0.19	± 0.05	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00

Determination of the Total Amount of Cysteine Sulphoxides

Because of high variations of values of total amounts of cysteine sulphoxides in investigated wild *Allium* species, the following two Figures 4.22 and 4.23 are composed according to the amount of cysteine sulphoxides. Samples containing values of the total amounts of cysteine sulphoxides higher than 0.6% are depicted in Figure 4.22. Figure 4.23 shows those samples, where the values of total amounts of cysteine sulphoxides were found to be lower than 0.6%.

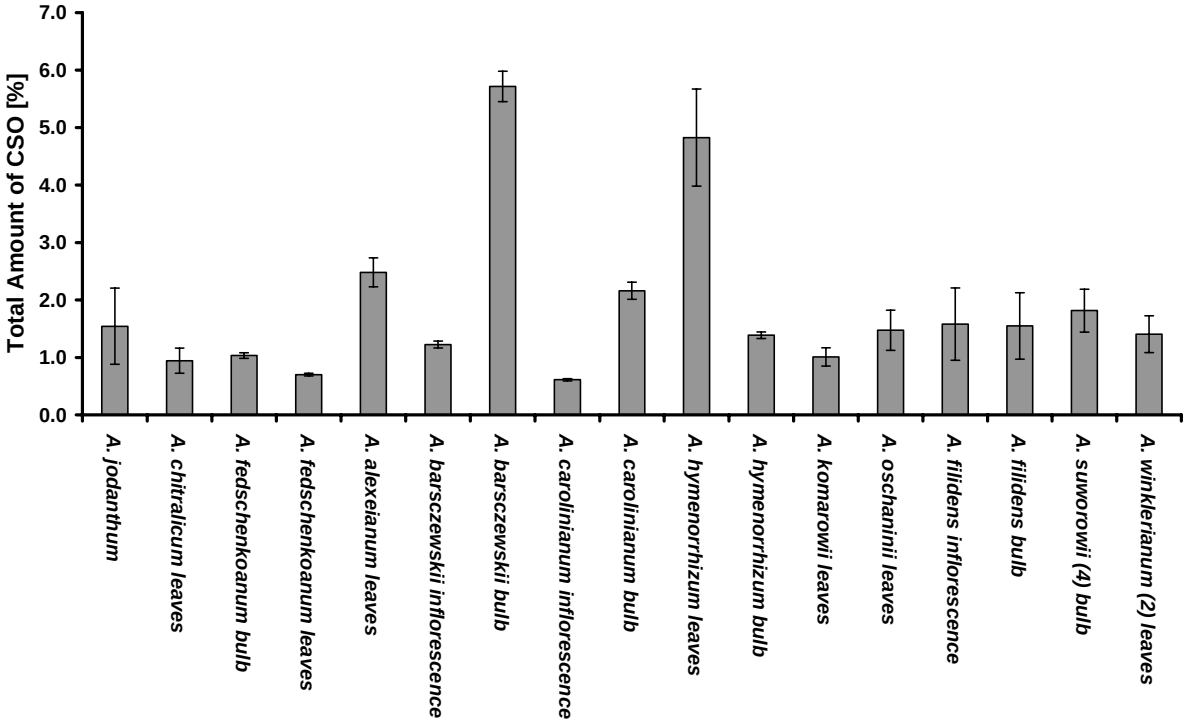


Figure 4.22: Total amounts of cysteine sulphoxides obtained from bulbs, leaves and inflorescences of different wild *Allium* species collected in Tajikistan, part 1.

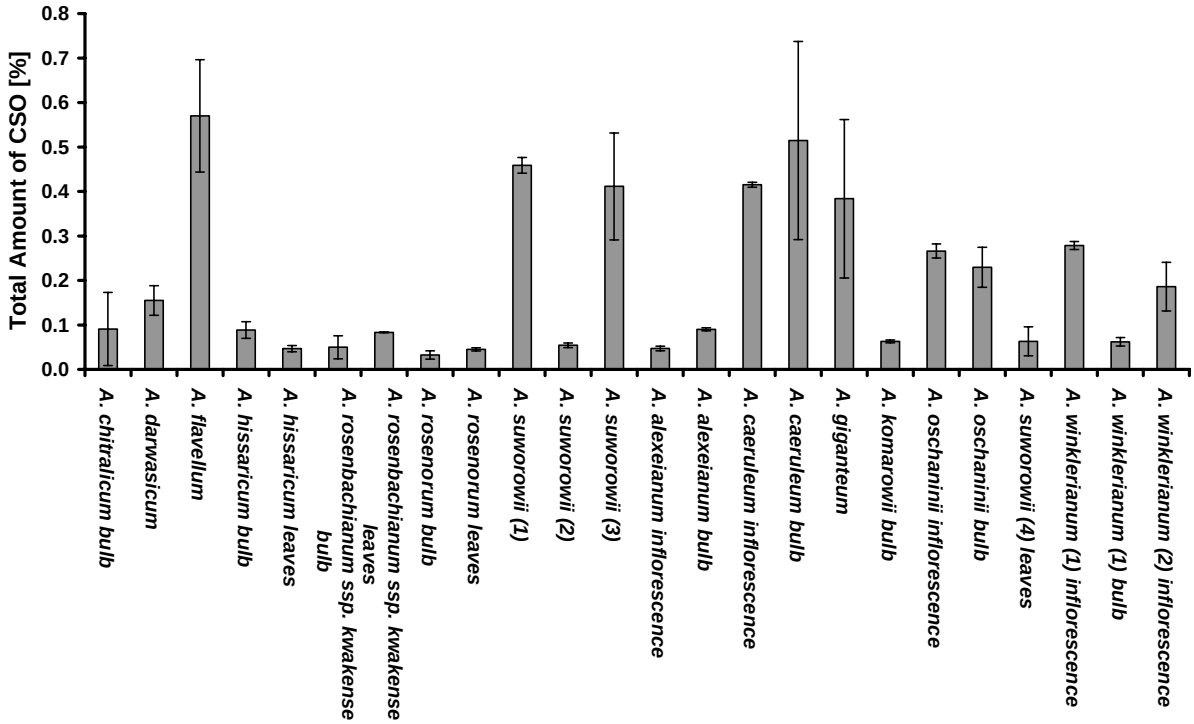


Figure 4.23: Total amounts of cysteine sulphoxides obtained from bulbs, leaves and inflorescences of different wild *Allium* species collected in Tajikistan, part 2. Standard deviations are given by error bars.

Wild *Allium* species collected in Tajikistan showed a very high variation of values of the total amount of flavour precursors. The total amounts of cysteine sulphoxides ranged from 0.03 ± 0.01 % (bulbs of *A. rosenorum*) to 5.72 ± 0.27% (bulbs of *A. barsczewskii*). High variations of total amounts were observed also for same *Allium* species possessing different Acc.-numbers, *e.g.*, bulbs of *A. suworowii* **Acc.-No. 6083** (2) contained only 0.054 ± 0.01%, **Acc.-No. 6076** (1) 0.46 ± 0.02% and **Acc.-No. 6112** (4) even 1.82 ± 0.37% of total cysteine sulphoxides.

The total amount of cysteine sulphoxides differentiated in various plant parts. In some cases, the difference was not very remarkable (*A. filidens* bulb 1.55 ± 0.58%, inflorescence 1.58 ± 0.63% and *A. oschaninii* bulb 0.23 ± 0.05%, inflorescence 0.27 ± 0.02%), in other cases, the difference was significant (*A. carolinianum* bulb 2.16 ± 0.15%, inflorescence 0.61 ± 0.02% and *A. alexeianum* bulb 0.09 ± 0.003%, inflorescence 0.047 ± 0.01%). It has to be pointed out that the concentrations of bulbs and inflorescences are related to fresh weight, whereas the amounts of cysteine sulphoxides of leaves are related to dry weight.

Pattern of Cysteine Sulphoxides

The following Figures 4.24 - 4.39 show the pattern of cysteine sulphoxides obtained from wild *Allium* species, which were collected in Tajikistan. Again, the results are given in relative amounts. The first Figure 4.24 exhibits relative composition of some bulbs of wild *Allium* species. For these species, no leaves or flowers were investigated.

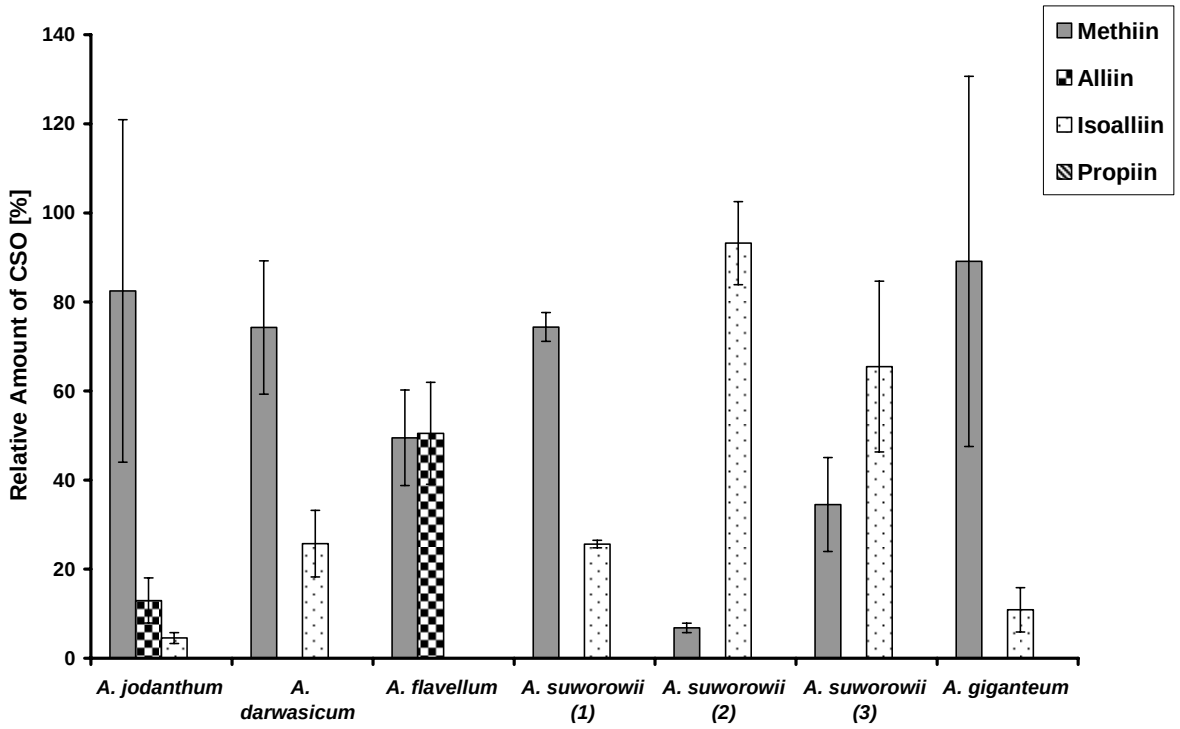


Figure 4.24: Relative amounts of cysteine sulphoxides obtained from bulbs of different wild *Allium* species collected in Tajikistan (total cysteine sulphoxides = 100%). Standard deviations are given by error bars.

Methiin was detected in all investigated bulbs (Fig. 4.24). Bulbs of *A. barsczewskii*, *A. darwasicum*, *A. jodanthum*, and *A. giganteum* contained **methiin** as major component. Variations in the amount of **methiin** were observed for *A. suworowii*. In bulbs of **Acc.-No. 6076** (1), 74.37 ± 3.23% were detected whereas in **Acc.-No. 6090** (3) only 34.51 ± 10.54% and in **Acc.-No.6083** (2) even 6.82 ± 1.06% were found.

Alliin was found in samples obtained from *A. jodanthum* (12.97 ± 5.06%) and *A. flavellum* (50.51 ± 11.43%). **Alliin** was even detected as a major cysteine sulphoxide of *A*. *flavellum*. **Isoalliin** was found in bulbs of *A. jodanthum* (4.54 ± 1.23%), A. *darwasicum* (25.73 ± 7.49%), *A. giganteum* (10.89 ± 4.98%) and *A. suworowii*. In the case of *A. suworowii*, significant variations of **isoalliin** amounts were observed, *e.g*., in bulbs of **Acc.-No. 6076**, 25.63 ± 0.86% were detected whereas in bulbs of **Acc.-No.6083** 93.19 ± 9.33% were found. **Propiin** was not detected in any of the investigated bulbs.

In the following Figures 4.24 - 4.38, the relative amounts of cysteine sulphoxides for different plant parts from the same *Allium* species are depicted.

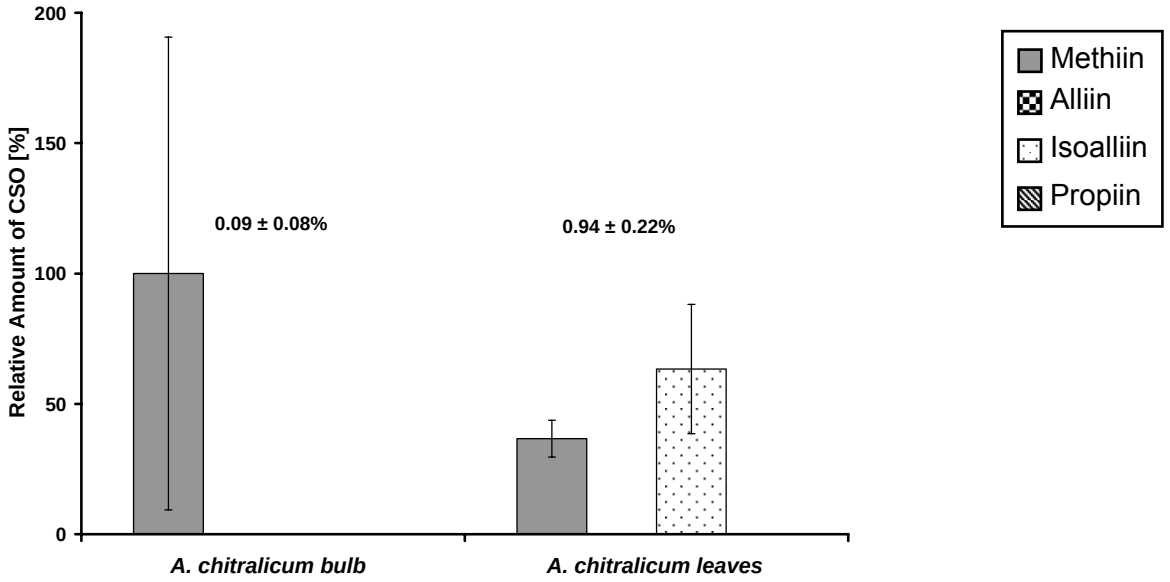


Figure 4.25: Relative amounts of cysteine sulphoxides obtained from bulbs and leaves of *A. chitralicum*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

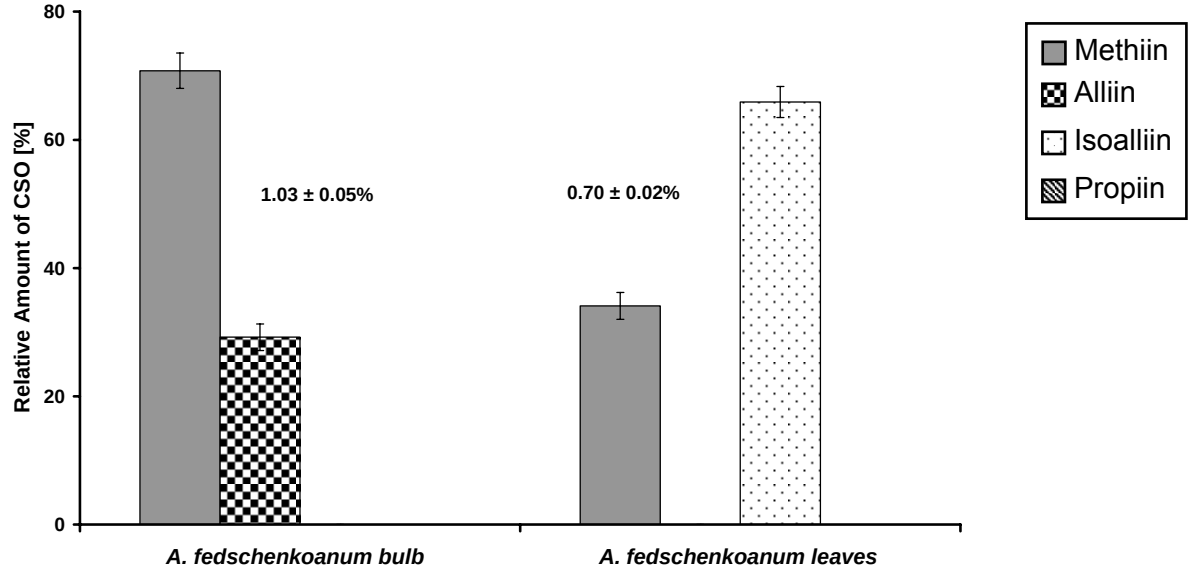


Figure 4.26: Relative amounts of cysteine sulphoxides obtained from bulbs and leaves of *A.fedschenkoanum*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

As evident from the Figure 4.25, there are differences in the pattern of flavour precursors in bulbs and leaves of *A. chitralicum*. Bulbs of this species contained **methiin** as the only cysteine sulphoxide. In the leaves of *A. chitralicum*, **methiin** and **isoalliin** were

detected. **Isoalliin** was found to be major flavour precursor (63.36 ± 24.83%). **Alliin** and **propiin** were found neither in the bulbs nor in the leaves of *A. chitralicum*.

In the case of *A. fedschenkoanum*, a high divergence in the pattern of cysteine sulphoxides in bulbs and leaves was observed (Fig. 4.26). In both plant parts, **methiin** was detected. In the bulbs of *A. fedschenkoanum*, **methiin** was determined as a major component (70.78 ± 2.75%).The leaves contained 34.10 ± 2.11% of **methiin**. **Alliin** was found only in the bulbs of *A. fedschenkoanum* (29.22 ± 2.08%). **Isoalliin** was detected in the leaves as a major cysteine sulphoxide (65.90 ± 2.43%).

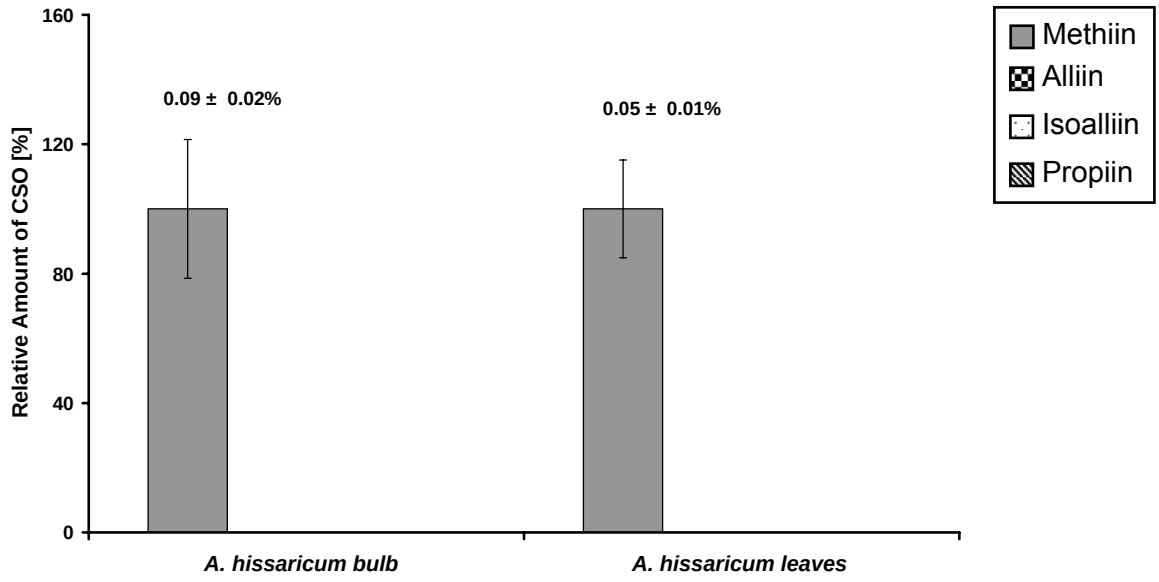


Figure 4.27: Relative amounts of cysteine sulphoxides obtained from bulbs and leaves of *A.hissaricum*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

The composition of flavour precursors in the bulbs and leaves of *A. hisaricum* was found to be identical (Fig. 4.27). In both plant parts, **methiin** was the only cysteine sulphoxide detected.

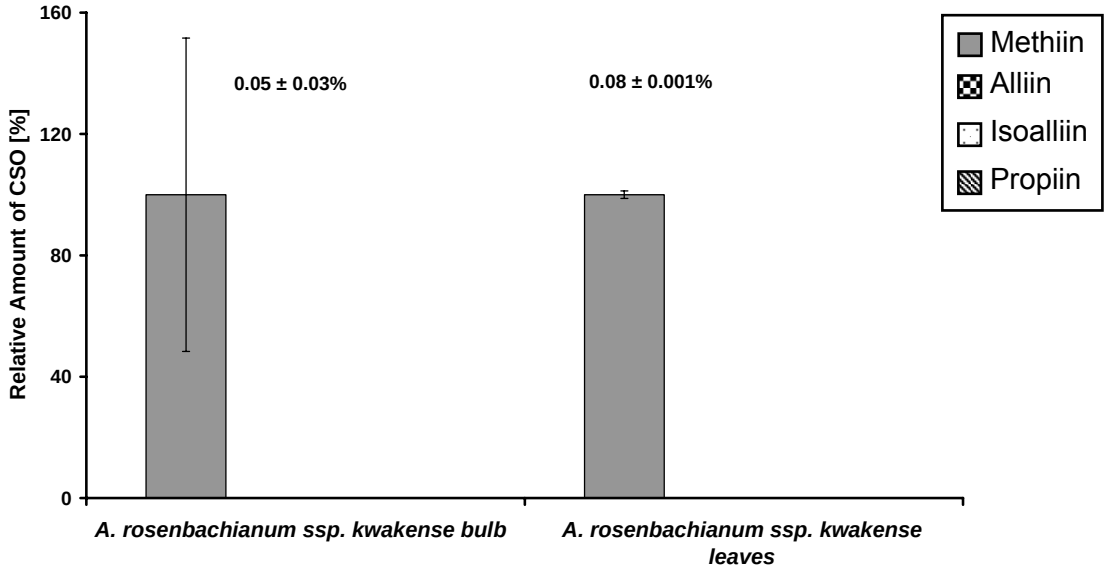


Figure 4.28: Relative amounts of cysteine sulphoxides obtained from bulbs and leaves of *A.rosenbachianum ssp. kwakense*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

Methiin was also found as the only cysteine sulphoxide in the bulbs and leaves of *A*. *rosenbachianum ssp. kwakense* (Fig. 4.28). Any other flavour precursor was detected.

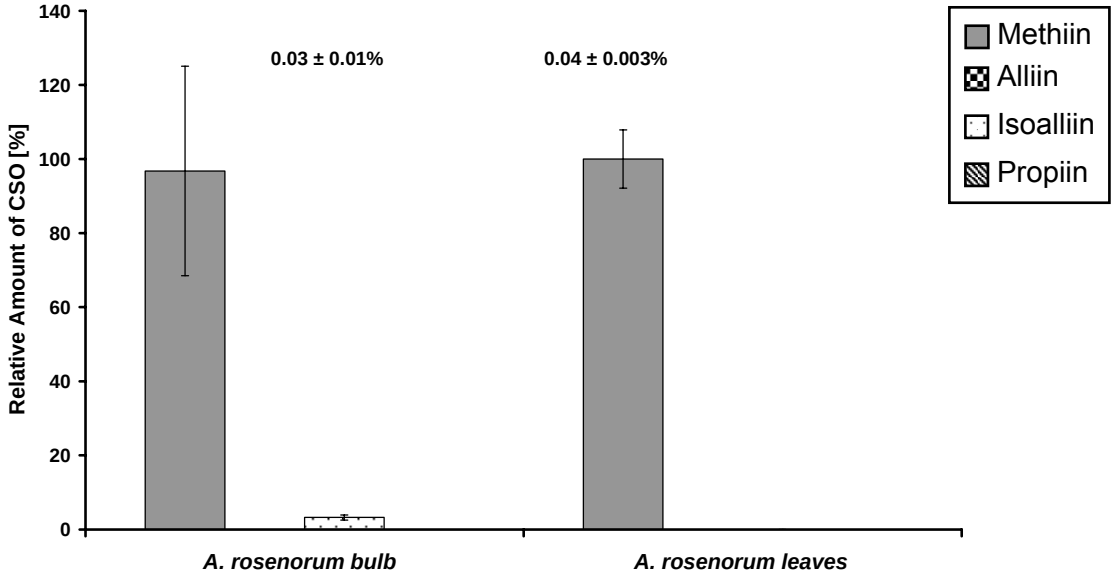


Figure 4.29: Relative amounts of cysteine sulphoxides obtained from bulbs and leaves of *A.rosenorum*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

In the bulbs and leaves of *A. rosenorum*, moderate differences in the cysteine sulphoxides pattern were determined (Fig. 4.29). In the bulbs of *A. rosenorum*, **methiin** and **isoalliin** were detected, whereas in the leaves only **methiin** was found. Nevertheless, the relative amount of **isoalliin** in bulbs was found to be rather low (3.26 ± 0.68%).

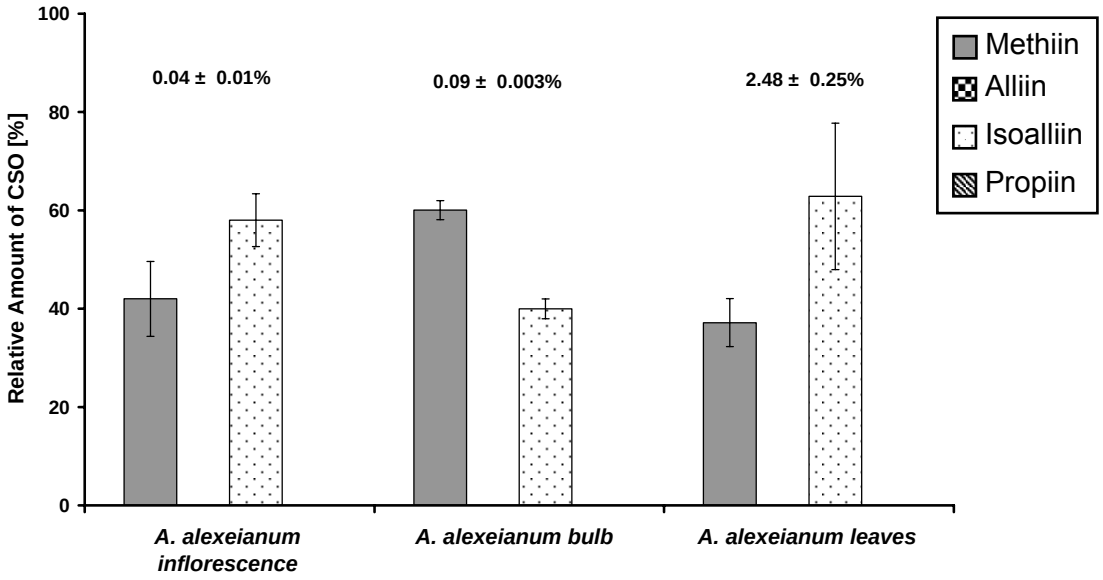


Figure 4.30: Relative amounts of cysteine sulphoxides obtained from inflorescence, bulbs and leaves of *A. alexeianum*. Total amounts of cysteine sulphoxides are displayed over bar charts.

The composition of cysteine sulphoxides in the inflorescences, bulbs and leaves of *A. alexeianum* corresponded rather well; **methiin** and **isoalliin** were detected in all examined plant parts (Fig. 4.30). The relative amount of **methiin** varied between 37.15 ± 4.88 (leaves) and 60.05 ± 1.96% (bulbs). **Isoalliin** was found to be the main flavour precursor in the inflorescences (58.01 ± 5.37%) and in the leaves (62.85 ± 14.91%) of *A. alexeianum*.

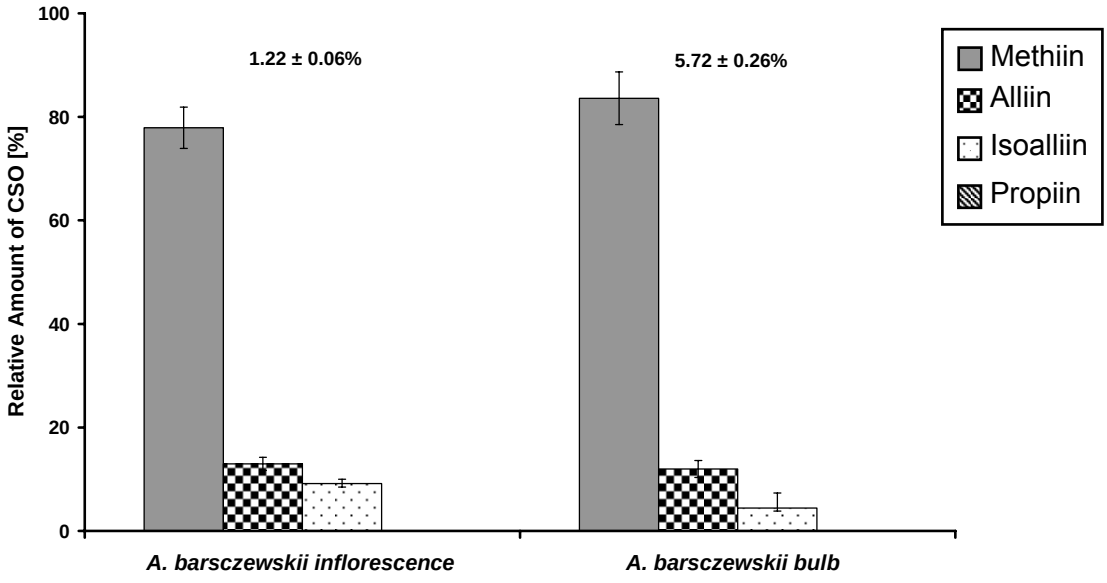


Figure 4.31: Relative amounts of cysteine sulphoxides obtained from inflorescence and bulbs of *A.barcsczewskii*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

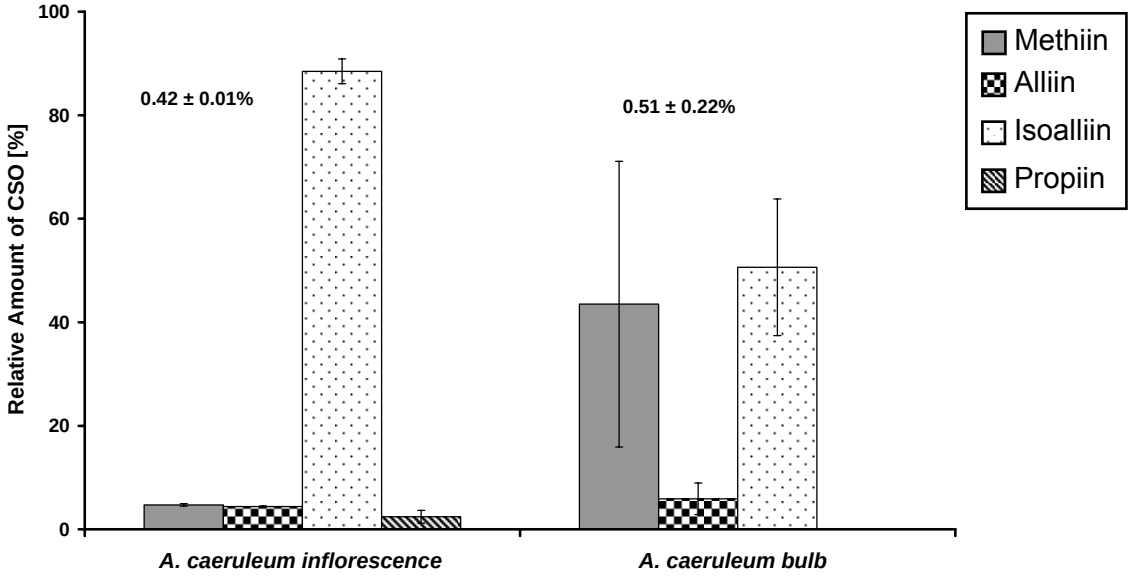


Figure 4.32: Relative amounts of cysteine sulphoxides obtained from inflorescence and bulbs of *A. caeruleum*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

In the case of *A. barsczewskii*, inflorescences and bulbs were examined. The pattern of flavour precursors corresponded in both plant parts very well (Fig. 4.31). **Methiin** was

detected as the main cysteine sulphoxide (77.89% ± 3.98% in the inflorescences and 83.59 ± 5.08% in the bulbs). The relative amount of **alliin** was found to be much lower (12.96 ± 1.26% in the inflorescences and 11.97 ± 1.63% in the bulbs). The lowest relative amounts were determined for **isoalliin** (9.14 ± 0.67 % in the inflorescences and 4.44 ± 0.61% in the bulbs).

In investigated plant parts of *A. caeruleum* slightly different patterns of flavour precursors were observed (Fig. 4.32). In the inflorescences, all four cysteine sulphoxides were detected, whereas in the bulbs only **methiin**, **alliin** and **isoalliin** were found. The values of **methiin** varied remarkably (inflorescences 4.72 ± 0.24% and bulbs 43.49 ± 27.59%).

In both plant parts, **isoalliin** was determined to be the main cysteine sulphoxide (inflorescences 88.48 ± 2.40% and bulb 50.62 ± 13.20%). **Alliin** was detected in small amounts (inflorescences 4.39 ± 0.12% and bulbs 5.89 ± 3.05%). Only traces of **propiin** were detected in the bulbs of *A*. *cearuleum* (2.40 ± 1.25%).

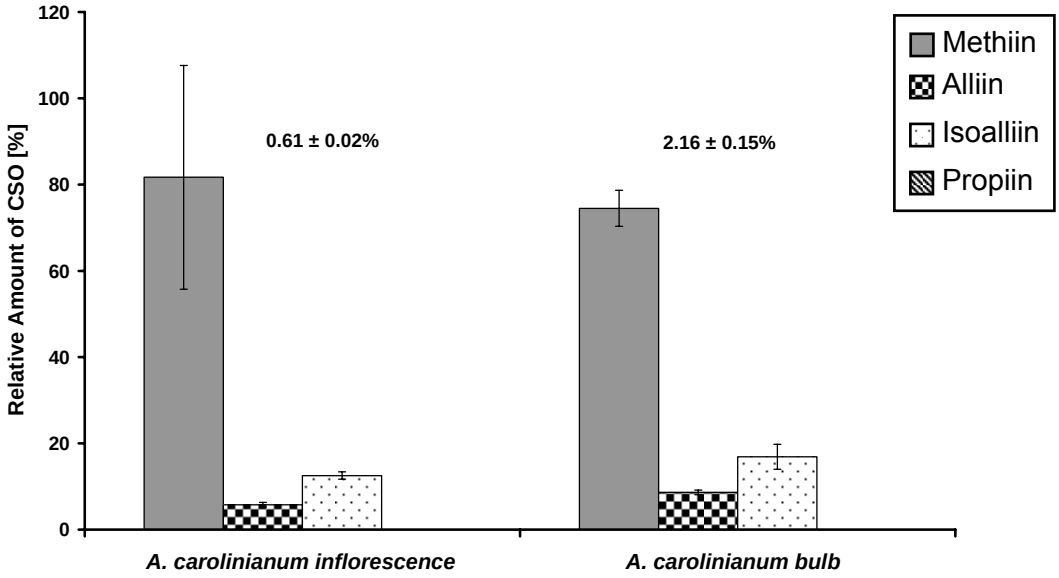


Figure 4.33: Relative amounts of cysteine sulphoxides obtained from inflorescence and bulbs of *A.carolinianum*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

The composition of flavour precursors in inflorescences and bulbs of *A. carolinianum* was found to be identical; in both plant parts, **methiin, alliin** and **isoalliin** were detected (Fig. 4.33). In both cases, **methiin** was detected to be the main cysteine sulphoxide

(inflorescence 81.69 ± 25.95% and bulbs 74.47 ± 4.18%). **Isoalliin** was found to be the second most abundant component even if in much lower relative amounts (inflorescences 12.54 ± 0.85% and bulbs 16.89 ± 2.88%). Also **alliin** was found only in small amounts (inflorescences 5.77 ± 0.52% and bulbs 8.64 ± 0.54%).

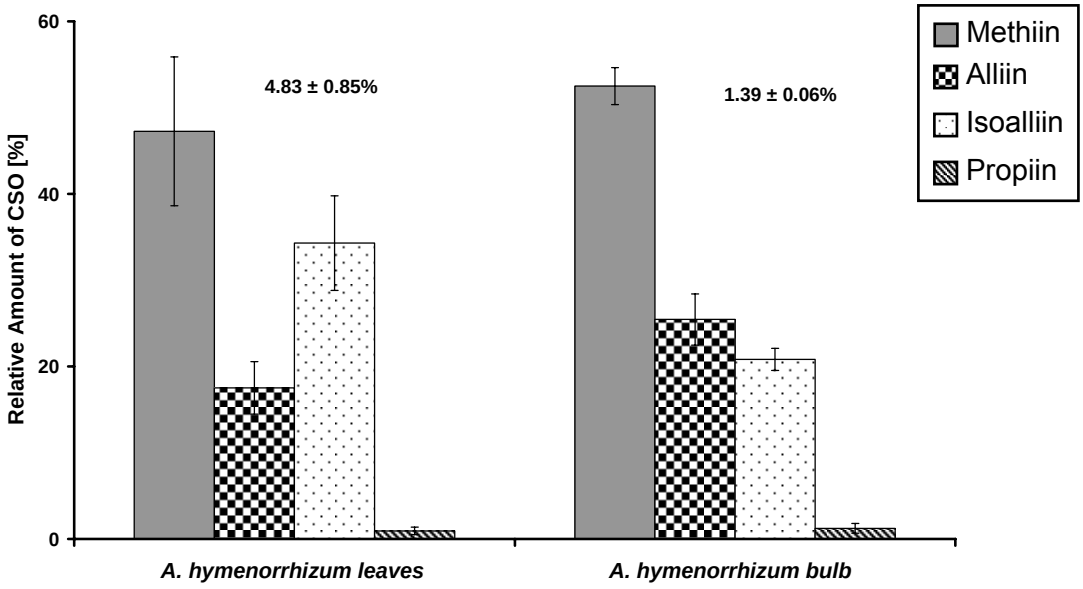


Figure 4.34: Relative amounts of cysteine sulphoxides obtained from leaves and bulbs of *A.hymenorrhizum*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

In the leaves and bulbs of *A. hymenorrhizum*, all four flavour precursors were detected (Fig. 4.34). The patterns of cysteine sulphoxides corresponded relatively well in both investigated plant parts. Again, **methiin** was found to be the main cysteine sulphoxide (leaves 47.26 ± 8.63% and bulbs 52.50 ± 2.15%). In the leaves, a different ratio between **alliin** and **isoalliin** was observed than in the bulbs. In the leaves of *A. hymenorrhizum*, **isoalliin** was determined to be the second most abundant flavour precursor (34.29 ± 5.48%) whereas in the bulbs, higher amounts of **alliin** than **isoalliin** were found (25.45 ± 2.98%). **Propiin** was detected only in trace amounts in both plant parts.

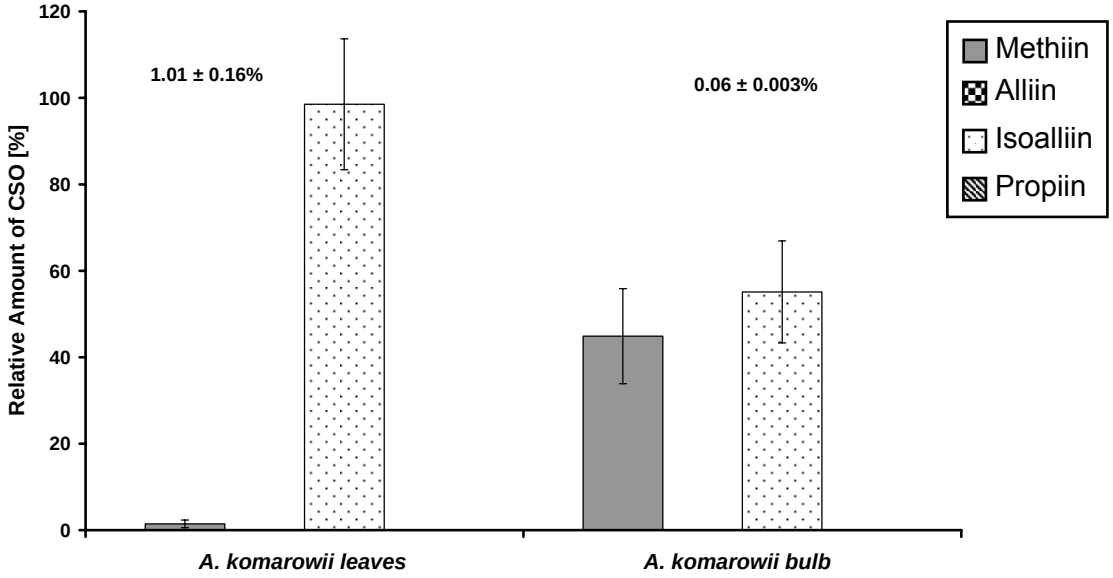


Figure 4.35: Relative amounts of cysteine sulphoxides obtained from leaves and bulbs of *A.komarowii*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

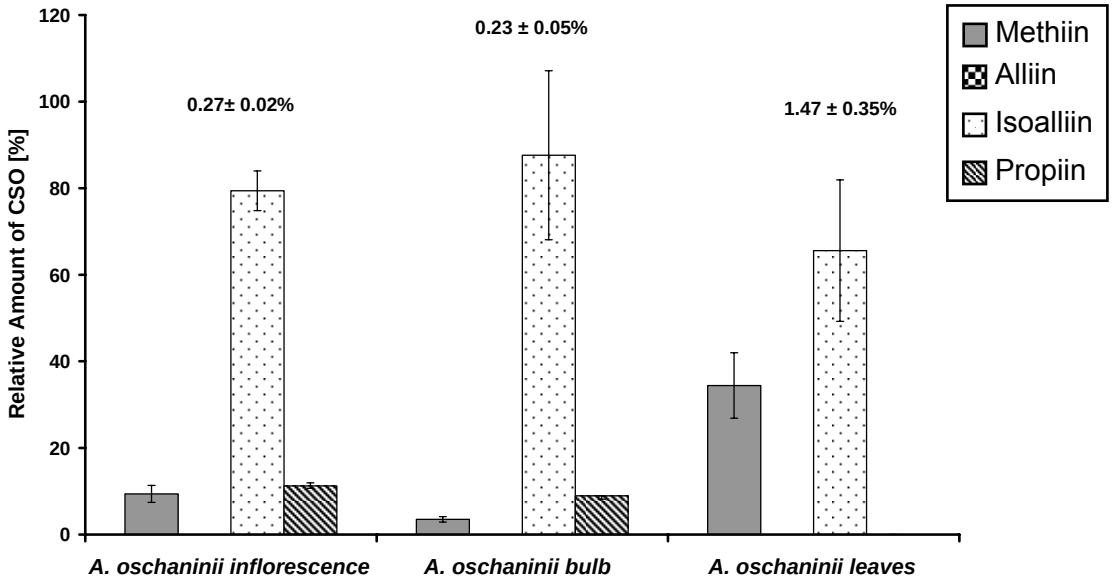


Figure 4.36: Relative amounts of cysteine sulphoxides obtained from inflorescences, bulbs and leaves of *A.oschaninii.* Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

In the leaves and bulbs of *A. komarowii*, the pattern of cysteine sulphoxides showed relative differences (Fig. 4.35). Although **isoalliin** was detected as the main cysteine

sulphoxide, its ratio in both plant parts varied remarkably (leaves 98.54 ± 15.13% and bulbs 55.12 ± 11.79%). The relative amount of **methiin** was 1.46 ± 0.89% in the leaves and 44.88 ± 10.98% in the bulbs. **Alliin** and **propiin** were not found in investigated plant parts.

In samples obtained from the inflorescences and bulbs of *A. oschaninii*, nearly identical patterns of flavour precursors were observed; they contained **methiin**, **isoalliin** and **propiin** (Fig. 4.36). In the leaves of *A. oschaninii*, only **methiin** and **isoalliin** could be detected. Nevertheless **isoalliin** was found to be the main cysteine sulphoxide in all investigated plant parts (inflorescences 79.39 ± 4.60%, bulbs 87.59 ± 19.50% and leaves 65.58 ± 16.34%). In the inflorescences and bulbs, **propiin** was detected as the second important cysteine sulphoxide (inflorescences 11.23 ± 0.58% and bulbs 8.91 ± 0.73%). Relative amounts of **methiin** were found to be 9.37 ± 1.98% (inflorescences), 3.50 ± 0.62% (bulbs) and 34.42 ± 7.35% (leaves). **Alliin** was not found in examined samples.

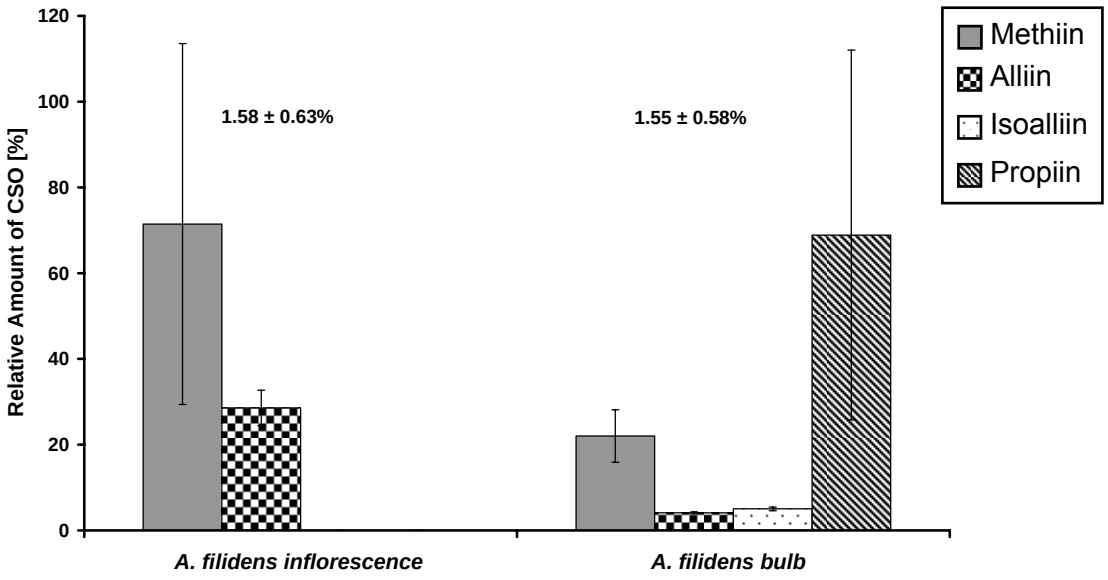


Figure 4.37: Relative amounts of cysteine sulphoxides obtained from inflorescences and bulbs of *A. filidens*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

Investigated plant parts of *A. filidens* showed different patterns of cysteine sulphoxides (Fig. 4.37). In the inflorescences **methiin** and **alliin** were detected whereas in the bulbs all four cysteine sulphoxides were found. The ratio of particular cysteine sulphoxides in the inflorescences and bulbs varied remarkably. In inflorescences, **methiin** was found to be the most abundant flavour precursor (71.44 ± 42.11%) whereas in bulbs, only 22.03 ± 6.12%

were detected. Interestingly, **propiin** was determined as the main cysteine sulphoxide (68.87 ± 43.10%) in the bulbs of *A. filidens*. **Alliin** and **isoalliin** were detected in trace amounts in the leaves of *A. filidens*.

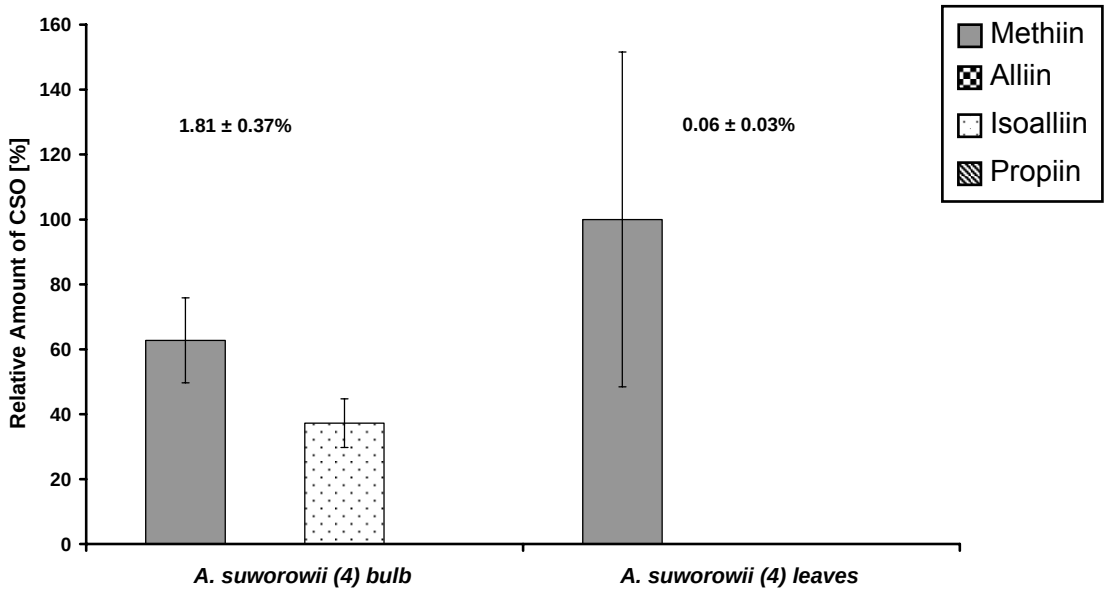


Figure 4.38: Relative amounts of cysteine sulphoxides obtained from bulbs and leaves of *A.suworowii*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

In the bulbs and leaves of *A. suworowii*, significantly different patterns of flavour precursors were observed (Fig. 4.38). In the bulbs, **methiin** and **isoalliin** were detected whereas in the leaves only methiin was found. In both plant parts, **methiin** was determined to be the main flavour precursor (bulbs 62.76 ± 13.09% and leaves 100 ± 51.57%). The relative amount of **isoalliin** was 37.24 ± 7.48% in the bulbs of *A. suworowii.*

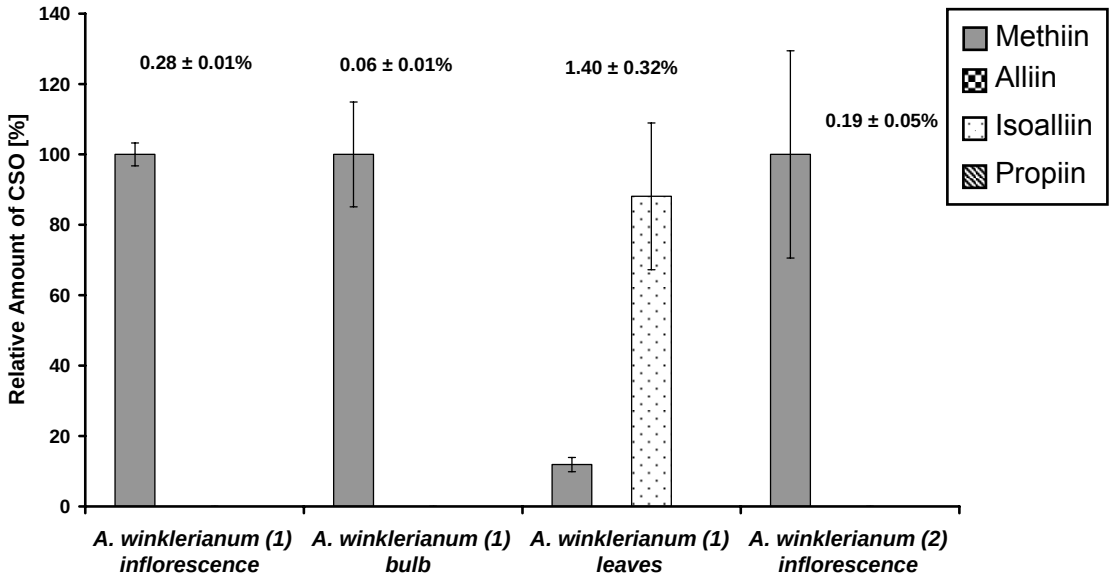


Figure 4.39: Relative amounts of cysteine sulphoxide obtained from inflorescences, bulbs and leaves of *A.winklerianum*. Total amounts of cysteine sulphoxides are displayed over bar charts. Standard deviations are given by error bars.

In the inflorescences and bulbs of *A. winklerianum*, an identical pattern of cysteine sulphoxides was observed (Fig. 4.39). In both mentioned plant parts, only **methiin** was detected. In the leaves of *A. winklerianum*, **methiin** and **isoalliin** were detected; the relative amount of **isoalliin** was found to be 88.11 ± 20.84%.

4.2.5. *Analysis from Allium Species from the IPK-Collection, Gatersleben*

Amounts of cysteine sulphoxides were also examined on *Allium* species, which were cultivated in IPK Gatersleben, Germany. These species were collected as wild *Allium* plants mostly in Central Asia. An overview of their places of origin as well as the date of collection is listed in chapter Material and Methods (Table 3.5).The determination of flavour precursors was carried out by means of HPLC analysis. The following Table 4.5 shows the total amounts as well as amounts of particular cysteine sulphoxides.

Table 4.5: Amounts of cysteine sulphoxides in bulbs or roots of *Allium* species cultivated in IPK Gatersleben, Germany. Concentrations of all plant parts are related to fresh weight.

Tax.-No.	Name	Total CSO [%]	SD [%]	Methiin [%]	SD [%]	Alliin [%]	SD [%]	Isoalliin [%]	SD [%]	Propiin [%]	SD [%]
2657	*A. aflatunense*	0.65	± 0.09	0.11	± 0.02	0.00	± 0.00	0.54	± 0.08	0.00	± 0.00
2976	*A. altissimum*	0.15	± 0.07	0.09	± 0.04	0.00	± 0.00	0.05	± 0.03	0.00	± 0.00
2806	*A. angulosum*	1.06	± 0.11	0.08	± 0.10	0.00	± 0.00	0.23	± 0.02	0.00	± 0.00
5394	*A. hybrid*	0.13	± 0.03	0.11	± 0.03	0.00	± 0.00	0.00	± 0.00	0.01	± 0.00
1222	*A. jesdianum*	0.14	± 0.03	0.11	± 0.02	0.00	± 0.00	0.00	± 0.00	0.03	± 0.01
3666	*A. jesdianum*	0.45	± 0.06	0.37	± 0.05	0.00	± 0.00	0.08	± 0.01	0.00	± 0.00
2158	*A. kunthii*	0.06	± 0.01	0.04	± 0.01	0.00	± 0.00	0.02	± 0.00	0.01	± 0.00
1125	*A. longicuspis*	2.98	± 0.21	0.44	± 0.01	2.54	± 0.20	0.00	± 0.00	0.00	± 0.00
2218	*A. macleanii*	1.34	± 0.38	0.71	± 0.22	0.33	± 0.07	0.28	± 0.08	0.02	± 0.01
2254	*A. proliferum*	0.24	± 0.01	0.06	± 0.02	0.00	± 0.00	0.12	± 0.05	0.06	± 0.03
0514	*A. pskemense*	0.38	± 0.02	0.05	± 0.01	0.01	± 0.00	0.32	± 0.03	0.01	± 0.00
1968	*A. spirale*	0.31	± 0.07	0.07	±0.06	0.00	± 0.00	0.22	± 0.02	0.01	± 0.00
583	*A. tuberosum*	1.14	± 0.30	0.56	± 0.10	0.49	± 0.18	0.06	± 0.02	0.03	± 0.01
1678	*A. altaicum*	0.61	± 0.13	0.12	± 0.02	0.00	± 0.00	0.46	± 0.09	0.03	± 0.03
3605	*A. ampeloprasum*	0.43	± 0.04	0.20	± 0.03	0.22	± 0.02	0.01	± 0.00	0.00	± 0.00
2824	*A. cyatophorum var. farreri* **root**	0.25	± 0.08	0.09	± 0.04	0.00	± 0.00	0.15	± 0.03	0.00	± 0.00
2824	*A. cyatophorum var. farreri* **veget. part**	0.64	± 0.07	0.31	± 0.04	0.01	± 0.00	0.34	± 0.04	0.02	± 0.00
1120	*A. fistulosum*	0.41	± 0.02	0.21	±0.01	0.00	± 0.00	0.18	± 0.01	0.03	± 0.01
0568	*A. nutans*	0.35	± 0.03	0.10	± 0.01	0.00	± 0.00	0.25	± 0.04	0.00	± 0.00
1297	*A. pskemense*	0.65	± 0.06	0.07	± 0.01	0.00	± 0.00	0.54	± 0.07	0.04	± 0.01
2773	*A. angulosum*	0.56	± 0.04	0.54	± 0.03	0.00	± 0.00	0.01	± 0.00	0.01	± 0.01
1337	*A. longicuspis*	0.75	± 0.13	0.06	± 0.00	0.68	± 0.13	0.00	± 0.00	0.00	± 0.00
3447	*A. stipitatum*	0.96	± 0.01	0.77	*	0.00	*	0.19	*	0.00	*

* not determined (limited amount of plant material)

A. hybrid (spontaneous hybrid of *A. nevskianum* Vved. ex Wendelbo)

Determination of the Total Amount of Cysteine Sulphoxides

Following Figure 4.40 depicts the total amounts of cysteine sulphoxides in bulbs and roots of *Allium* species which were cultivated in IPK Gatersleben.

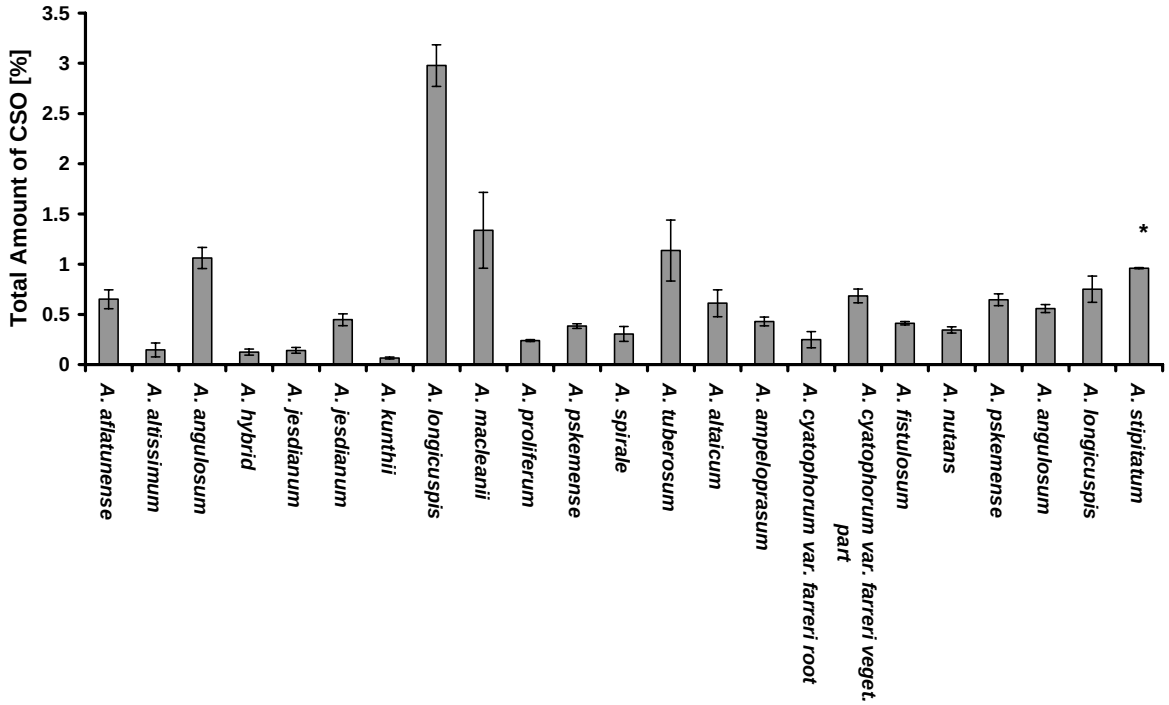


Figure 4.40: Total amounts of cysteine sulphoxides obtained from bulbs and roots of different *Allium* species cultivated in IPK Gatersleben, Germany. Standard deviations are given by error bars.

* SD not determined (limited amount of plant material)

In investigated *Allium* species, the total amounts of flavour precursors in the range between 0.07 ± 0.01% (bulbs of *A. kunthii*) and 2.98 ± 0.21% (bulbs of *A. longicuspis*) were observed. Significantly high total amounts (over 1.0%) were detected in the bulbs of *A. macleanii* (1.34 ± 0.38%), *A. tuberosum* (1.14 ± 0.30%) and *A. angulosum* (1.06 ± 0.11%). In comparison to wild *Allium* species, which were collected in Central Asia, remarkably high amounts of cysteine sulphoxides were detected in some *Allium* species cultivated in the IPK Gatersleben. In the bulbs of *A. jesdianum* possessing different Tax.-numbers**,** dissimilar values of total amount of cysteine sulphoxides were observed; in bulbs of **Tax.-No. 1222** 0.14 ± 0.03% were detected whereas in **Tax.-No. 3666** 0.45 ± 0.06% were found.

Pattern of Cysteine Sulphoxides

The following two Figures 4.41 and 4.42 depict the patterns of flavour precursors obtained from *Allium* species, which were cultivated in IPK Gatersleben. The results are, again, shown in relative amounts.

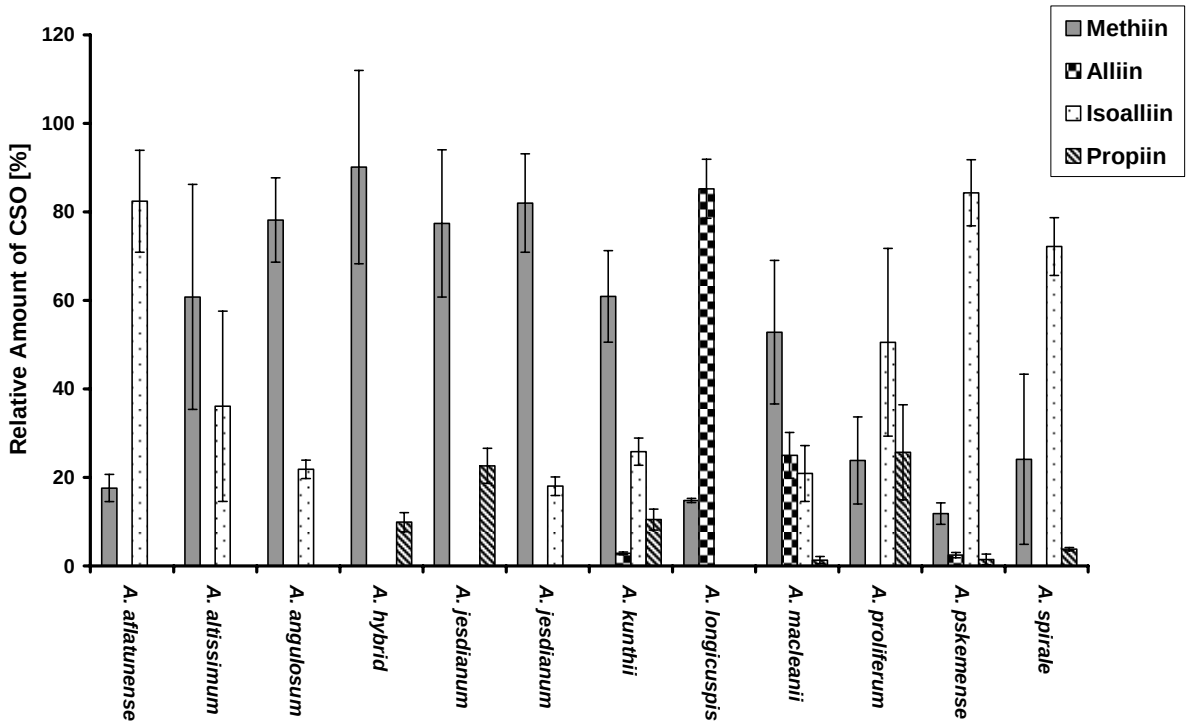


Figure 4.41: Relative amounts of cysteine sulphoxides obtained from bulbs of *Allium* species cultivated in IPK Gatersleben, Germany (part 1). Total cysteine sulphoxides = 100%. Standard deviations are given by error bars.

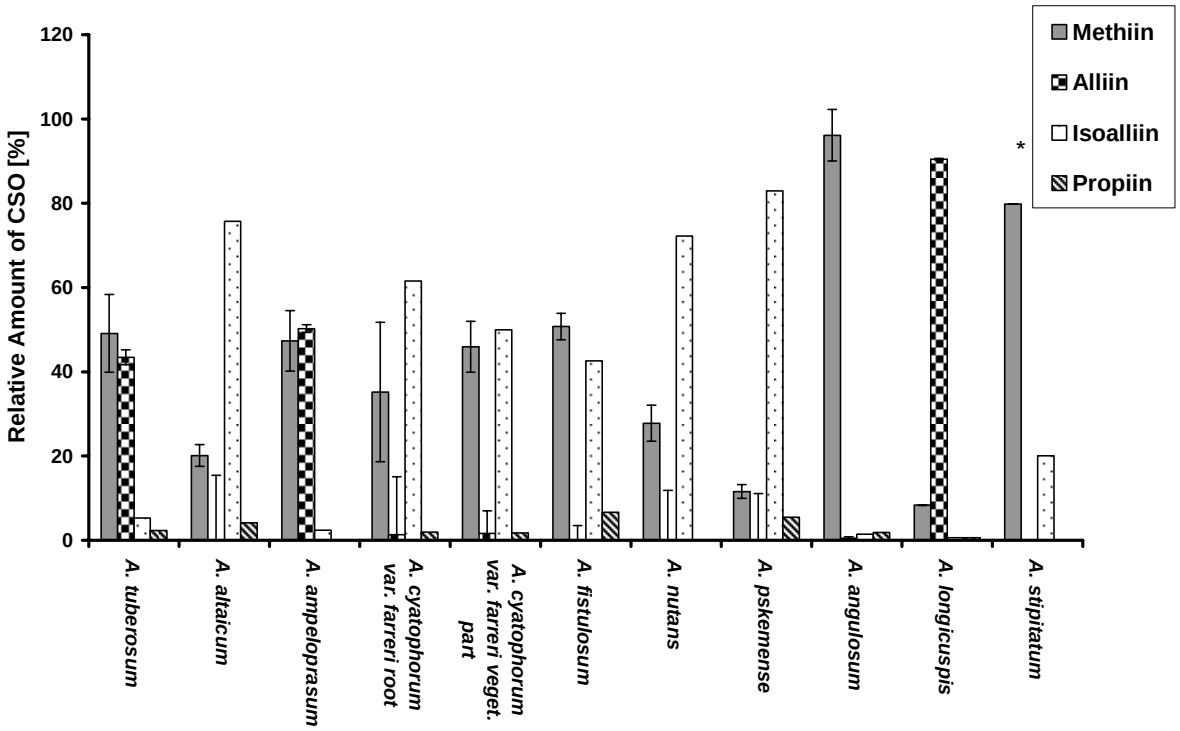


Figure 4.42: Relative amounts of cysteine sulphoxides obtained from bulbs of *Allium* species cultivated in IPK Gatersleben, Germany (part 2). Total cysteine sulphoxides = 100%. * SD not determined (limited amount of plant material)

Methiin was detected in all investigated samples. **Methiin** was found to be the main cysteine sulphoxide in the bulbs of *A. altissimum* (60.78 ± 25.40%), *A. angulosum* **Tax.-No. 2806** (78.17 ± 9.54%), *A. hybrid* (90.12 ± 21.84%), *A. jesdianum* **Tax.-No. 1222** (77.40 ± 16.62%), *A. jesdianum* **Tax.-No**. 3666 (82.00 ± 11.12%), *A. macleanii* (52.81 ± 16.23%), *A. tuberosum* (49.10 ± 9.23%), *A. fistulosum* (50.73 ± 3.15%), *A. angulosum* **Tax.-No. 2773** (96.14 ± 6.13%) and *A. stipitatum* 79.81%.

Isoalliin was found almost in all investigated samples. The highest amounts of isoalliin (over 50%) were detected in bulbs of *A. pskemense* **Tax.-No. 0514** (84.30 ± 7.45%), *A. pskemense* **Tax.-No. 1297** (82.94 ± 11.11%), *A. aflatunense* (82.40 ± 11.54%), *A. altaicum* (75.72 ± 15.44%), *A. nutans* (72.22 ± 11.85%), *A. spirale* (72.16 ± 6.56%), and *A. proliferum* (50.52 ± 21.20%) and in the roots of *A. cyatophorum var. farreri* (61.53 ± 13.78%).

Alliin was found to be the most abundant cysteine sulphoxide in the bulbs of *A. longicuspis* **Tax.-No. 1125** (85.23 ± 6.67%), *A. longicuspis* **Tax.-No. 1337** (90.45 ± 17.39%) and *A. ampeloprasum* (50.23 ± 3.88%).

Propiin was detected only in small amounts. The highest relative amounts of **propiin** were found in the bulbs of *A. proliferum* (25.66 ± 10.75), *A. jesdianum* **Tax.-No. 1222** (22.60 ± 3.96%), and *A. kunthii* (10.46 ± 2.35%). The composition of cysteine sulphoxides in the roots and vegetative part of *A. cyatophorum var. farreri* was found to be relatively identical.

In both plant parts, **methiin** and **isoalliin** were determined to be the most abundant flavour precursors whereas **alliin** and **propiin** were found in trace amounts.

4.2.6. *Comparison Between HPLC and FIA*

Total amounts of cysteine sulphoxides acquired from measurements with the FIA device were compared with results obtained from HPLC measurements. The table 4.6 below displays the comparison.

Table 4.6: The comparison of results obtained from measurements of cysteine sulphoxides in wild *Allium* species using FIA device and HPLC.

Preliminary Name	**Total CSO (FIA) [%]**	**Total CSO (HPLC) [%]**	**Ratio (FIA/HPLC)**
A. brevidentiforme	0.43	0.32	1.35
A. caesium	0.11	0.19	0.57
A. darwasicum	0.03	0.12	0.21
A. filidens	0.23	0.60	0.39
A. pskemense	0.22	0.19	1.14
A. rosenbachianum	0.04	0.05	0.72
A. rosenorum	0.01	0.03	0.41
A. suworowii(1)	0.06	0.05	1.18
A. suworowii (2)	0.37	0.41	0.91
A. talassicum	0.32	0.73	0.44
A. turkestanicum	0.54	0.57	0.94
		Average	0.75
		SD	0.38

The comparison of values of concentrations obtained from FIA device and HPLC showed a sufficient agreement. Values obtained from the measurements with FIA device were approximately 25% lower then values obtained from the measurements with HPLC. This is an expectable result, which follows from investigations of substrate specificity of enzyme alliinase. Investigations described in chapter 4.1.3. showed that the enzyme alliinase, which was immobilized in the cartridge utilized for these measurements, exhibited a high activity towards alliin and isoalliin. Activity towards other cysteine sulphoxides, which can also occur in wild *Allium* species, was found to be much lower. Methiin is a

representative of cysteine sulphoxide, which occurs in most of the wild *Allium* species in rather high amounts. However, alliinase activity towards methiin was found to be significantly lower,only 41.38% of (+/-)-alliin.

4.3. *Structure Elucidation of the Red Dye*

A number of *Allium* species belonging to the subgenus *Melanocrommyum* leads an intensively red dye, which is formed when the plant material is damaged. This red dye is also formed, when the plant material is heated or treated with basic reagents like diluted NaOH or KOH. Typical plants leading this red compound are *A. giganteum*, *A. jedianum*, *A. macleanii* or *A. rosenorum*.

A series of pre-experiments showed, that the formation of this dye after wounding of the plant material is catalysed by one or more enzymes. Inactivation of the enzymes by methanol or carefully drying of leaves does not yield to the red dye after damaging of the plant material.

The purified red dye could be obtained from an extract of bulbs from *A. giganteum*. The crunched bulb material was extracted several times with ethyl acetate and dichloromethane and the combined extracts were fractionated on a silica gel column.

The main fraction showed a maximum at 519 nm in the UV/VIS-spectrum. The high resolution mass spectrometry gave a molecular weight of 193.9984 amu, which fits to the molecular formula of $C_8H_6N_2S_2$ (MS calculated: 193.9996 amu).

The IR spectra showed some characteristic absorptions, which lead to the assumption, that a thio-pyrrole structure is involved in this molecule (wavenumbers 3370, 3108, 2360, 2341, 1697, 1524 and 1455 cm^{-1}, see chapter 8.2.).

Further on, a number of NMR experiments were performed. In the ^{1}H-NMR, only two protons with a chemical shift of δ=6.43 and 6.28 ppm (doublet, *J*=3.66 Hz) were visible. In the ^{13}C-NMR, a total of 4 carbons were observed (δ=107.98, 120.29, 125.53 and 131.97 ppm). All NMR data are given in chapter 8.2. An HMBC experiment allowed the conclusion, that all carbons had long distance couplings to the two protons and the proton with δ=6.43 ppm is attached to the carbon with δ=107.98 ppm as well as the proton with 6.28 ppm is attached to the carbon with δ=120.29 ppm. These results lead to the assumption, that the found protons were at neighbouring positions of a pyrrole ring, which is substituted at two positions with quaternary carbons. Taking the molecular formula into account, the red dye must contain two pyrrole moieties, which are directly linked with each other.

However, the position of the two sulphur atoms could not be clarified out of the existing data material. Therefore, all possibilities containing two pyrrole rings and two sulphur atoms

were simulated with the ACD calculator (Advanced Chemistry Development, Inc., Toronto, Canada). It turned out, that the only possibility would be a 3,3'-dithio-2,2'-dipyrrole as depicted in Fig. 4.43. The correlation of measured and calculated data set was found to be rather good.

It must be pointed out, that the stability of the dithiodipyrrole is rather poor. Even during long-period NMR measurements, the proton signal at δ=6.43 ppm got lost, meaning, that a polymerisation at the carbon directly linked to the pyrrol-nitrogen took place. After a while, the colour turned to deep red and than to blackish colours. Another observation showed that the colour switched to dark green, if the substance was solved in $CDCl_3$. It is not clear, which reaction might be responsible for this effect. But it must be noticed, that the red dye, which was found in herbarium vouchers of *Allium* species, always got green after several months.

In further time consuming experiments, strong attempts were undertaken to isolate the precursor of the red dye. At least four fractions, which are related to the thiopyrrole structure, could be identified by TLC. However, these compounds were found to rather instable and purification by preparative TLC as well as HPLC was nearly impossible. NMR experiments of partially purified fractions gave evidence, that at least one compound contained a sulphur-substituted pyrrole ring system, which was probably a condensation product between pyruvic acid and pyrrole-3-sulphenic acid.

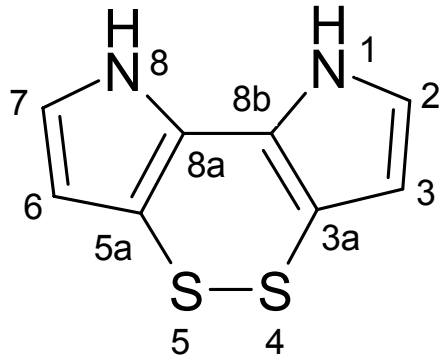


Fig. 4.43: Structure of the red dye, a dithiodipyrrole

4.4. *Antibiotic Activity of Allium Extracts*

4.4.1. *Pre-Screening on Antibacterial, Antifungal and Antialgal Activity*

In Institute of Pharmaceutical Biology in Bonn, Germany, some *Allium* extracts were tested on their inhibition effects towards bacteria, fungi and algae. The work was carried out by Mrs. Edith Neu from the working group of Prof. Koenig. Obtained results served as pre-screening that was very important for our further investigations. The following table shows wild *Allium* species used for this trial.

Table 4.7: Wild *Allium* species examined on inhibition activity towards certain microorganisms. The concentrations were 0.5 mg of *Allium* extract /disk.

Tax No.	Botanical Name
3666	*A. jesdianum*
2746	*A. altaicum*
3080	*A. pskemense*
0382	*A. oleraceum*
1125	*A. longicuspis*
Acc.-No. 6912	*A. giganteum*

Microorganisms used for the trial are listed in Table 4.8. They belong to bacteria, fungi and microalgae.

Table 4.8: Microorganisms used for pre-screening experiments.

Microorganism
Bacillus megaterium
Eurotium rubrum
Mycotypha microspora
Microbotryum violaceum
Chlorella fusca

As a positive control benzyl penicillin, miconazole and streptomycine in concentrations of 0.05 mg/ disk were used. The values of inhibition zones were determined as described in the chapter 3.11.1. Following Figures 4.44 - 4.46 show results acquired from the pre-screening experiment. Photos were kindly provided by Edith Neu.

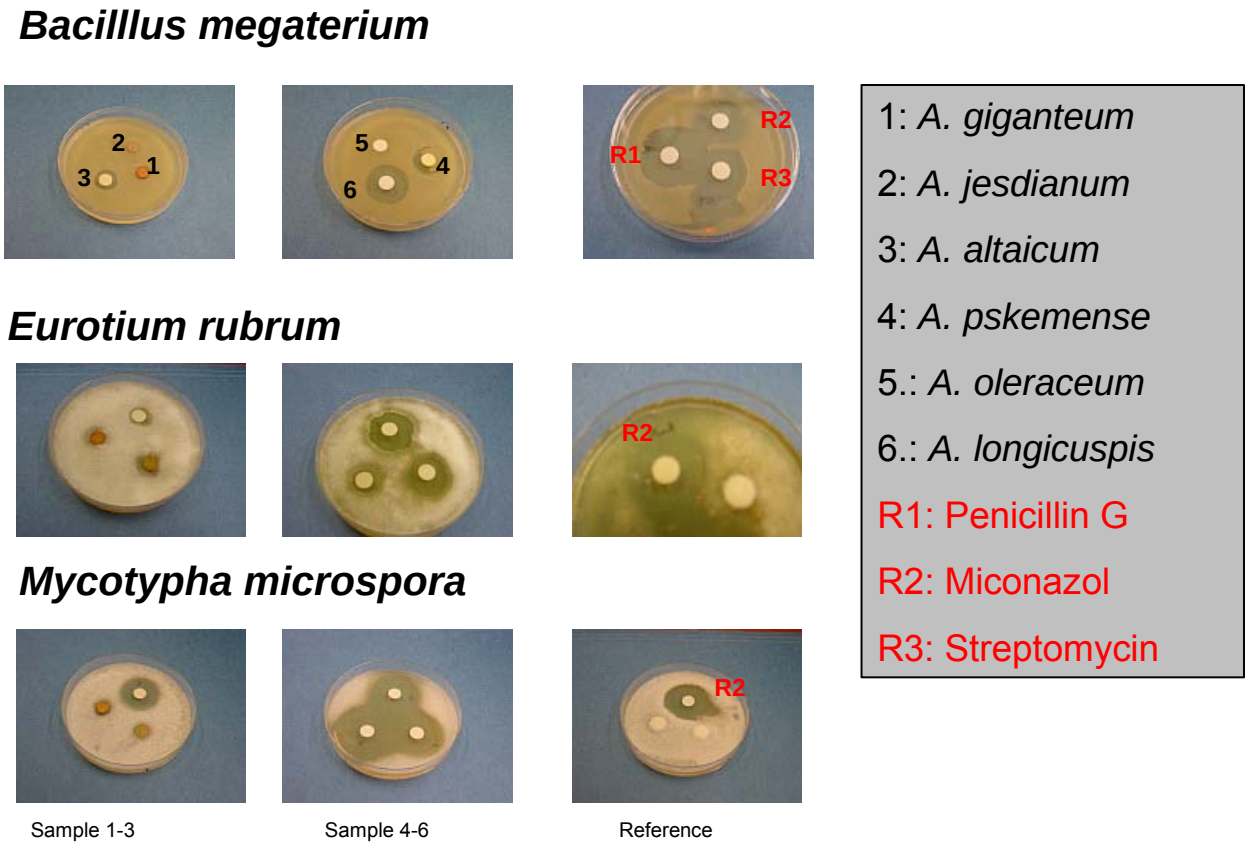


Fig 4.44: Performed agar diffusion tests. Plates were inoculated with *Bacillus megaterium*, *Eurotium rubrum* and *Mycotypha microspora*.

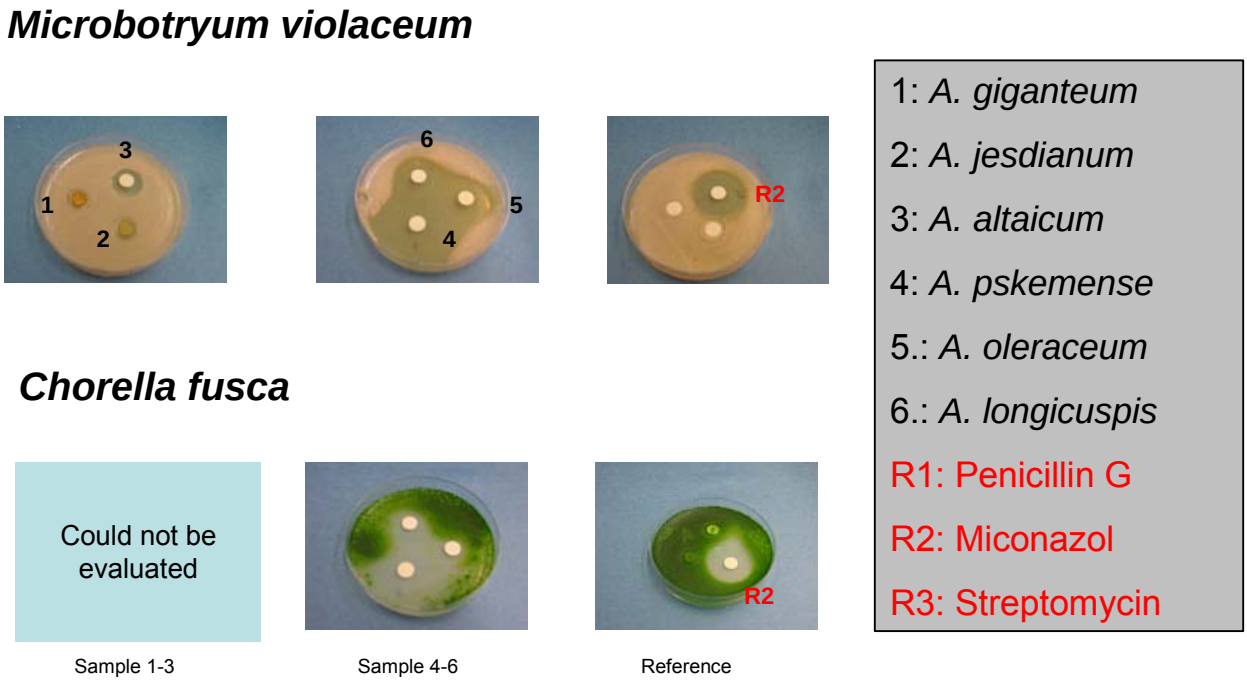


Fig. 4.45: Performed agar diffusion tests. Plates were inoculated with *Microbotryum violaceum* and *Chlorella fusca*.

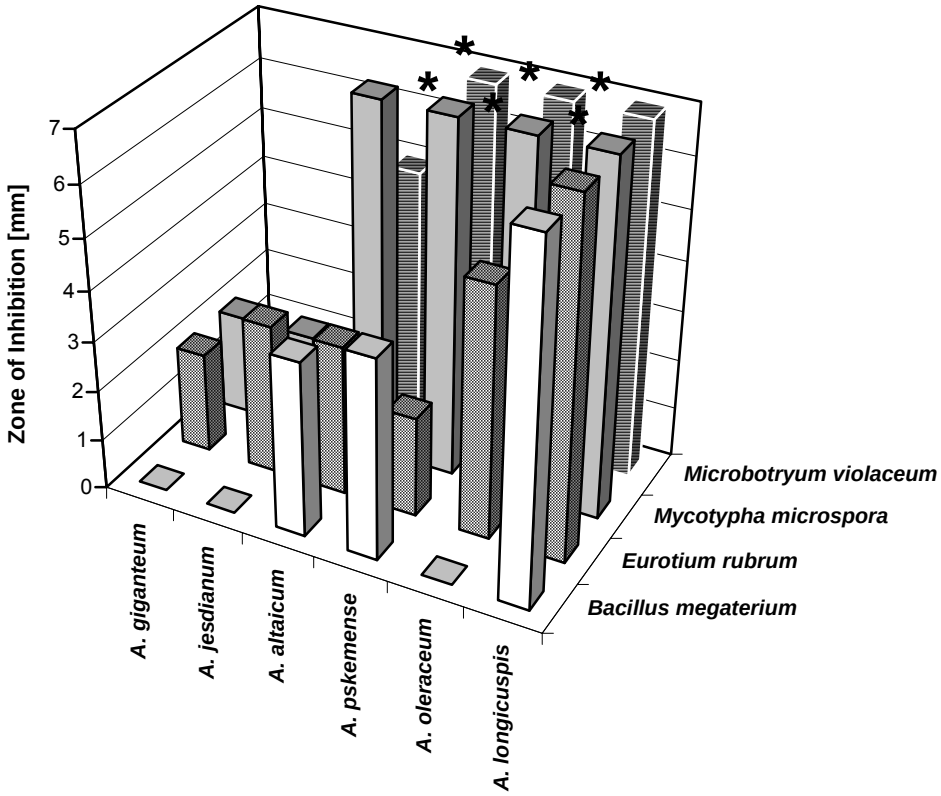


Figure 4.46: Results obtained from the pre-screening experiment.

Values of the inhibition zones in the case of *Chlorella fusca* are not presented, because of contamination of the first inoculated agar plate. On this agar plate, extracts obtained from *A. jesdianum*, *A. altaicum* and *A. pskemense* were placed. Values of inhibition zones in the second plate could not be measured properly, because they were overlapped (Fig. 4.45). The same problem was observed in experiments with *Mycotypha microspora* and *Microbotryum violaceum*. The inhibition zones were at least 7.0 mm and larger (in Fig. 4.46 marked by *).The inhibition zones of miconazole ranged between 10.0 and 14.0 mm. Inhibition zones of benzyl penicillin ranged between 8.0 and 12.0 mm. Experiments could not be repeated because of limited sample material.

Results of the pre-screening experiment show that extracts obtained from *A. longicuspis* possessed the highest inhibitory activity towards all tested microorganisms. The values of the inhibition zones were comparable with those of the positive control. Extracts obtained from *A. pskemense* and *A. oleraceum* exhibited the highest inhibitory effect towards *Mycotypha microspora*, *Microbotryum violaceum* and *Chlorella fusca*. Inhibition activity towards *Eurotium rubrum* was found to be also significant. In experiments with

Bacillus megaterium the extract obtained from *A. pskemense* showed lower inhibitory activity (4.0 mm) and for extract obtained from *A. oleraceum* any significant inhibitory effect was observed.

Extract acquired from *A. altaicum* exhibited inhibitory activity towards all tested microorganisms. The largest inhibition zones were observed in experiments with *Mycotypha microspora* and *Microbotryum violaceum* (7.0 mm and 5.0 mm). Grow of *Bacillus megaterium* as well as *Eurotium rubrum* was also inhibited with this extract (values of inhibition zones attained 3.5 and 3.0mm, respectively).

For extracts obtained from *A. giganteum* and *A. jesdianum*, significant inhibitory effects only towards *Eurotium rubrum* and *Mycotypha microspore* were found. Values of inhibition zones ranged between 2.0 and 3.0 mm. These extracts seemed to be ineffective towards other tested microorganisms.

4.4.2. *Broad Screening on Antibacterial Activity (Experiment No. 1)*

In the first experiment, each bacterial strain was tested toward each *Allium* extract. Results of the experiment were divided into two groups depending on the Gram-coloration of the particular bacteria. The following Figure 4.47 presents results of the experiment with Gram-negative bacterial strains.

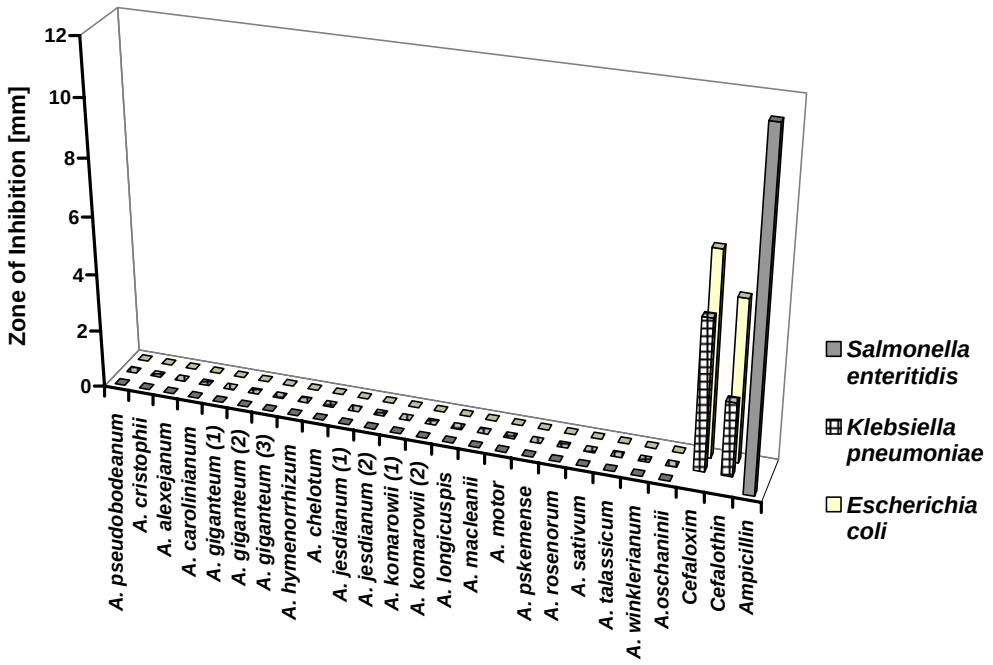


Figure 4.47: Results of the first experiment. The figure shows the susceptibility of Gram-negative bacterial strains towards *Allium* extracts.

As shown in Figure 4.47, tested *Allium* extracts did not demonstrate any effectiveness towards Gram-negative bacteria used in this test. Antibiotics used as a positive control exhibited effectiveness towards these bacteria. Inhibition zone of ampicillin, which was used as a positive control towards *Salmonella enteritidis*, was 12 mm. Furthermore, the zones of inhibition of cefaloxim and cefalothin (used in the case of *Klebsiella pneumoniae* and *Escherichia coli*) were in the range between 2.55 and 7.08 mm. In accordance with standard sheets used in the Laboratory of Clinical Microbiology in Sternberk, Czech Republic, all tested bacterial strains were susceptible towards the antibiotics but did not exhibit any susceptibility towards *Allium* extracts. In contrast to results in the literature, where the susceptibility of Gram-negative bacteria towards *A. sativum* is decribed, we could not confirm these findings.

The following Figure 4.48 depicts results of the experiment with Gram-positive bacterial strains.

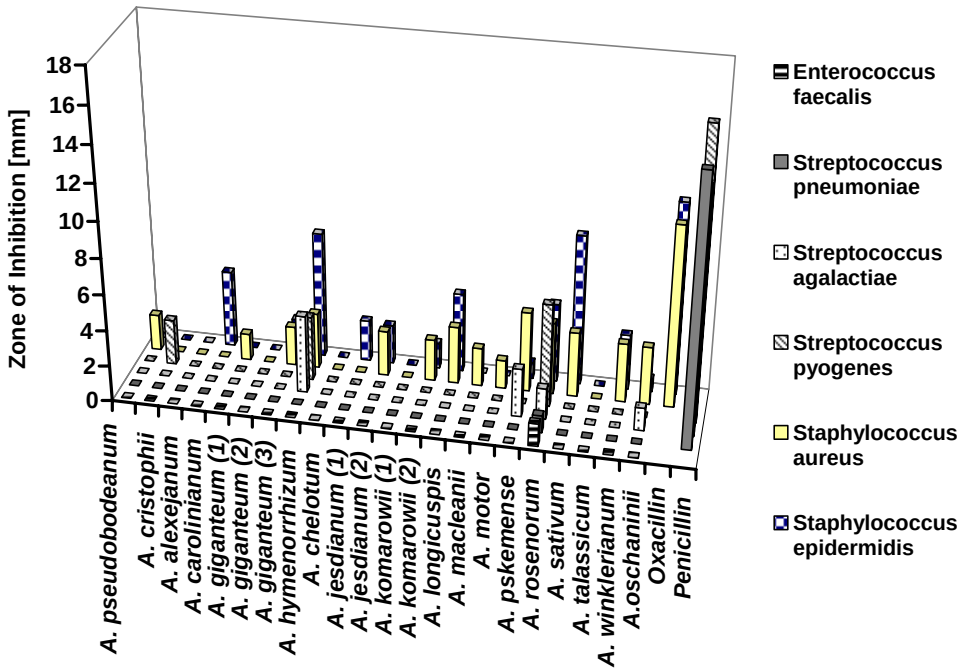


Figure 4.48: Results of the first experiment. The figure shows the susceptibility of Gram-positive bacterial strains towards *Allium* extracts.

Figure 4.48 shows that some Gram-positive bacterial strains exhibited a possible susceptibility towards numbers of *Allium* extracts. Considerable inhibition was observed for *A. rosenorum*. All Gram-positive bacterial strains demonstrated a possible sensitivity towards extract obtained from *A. rosenorum* (inhibition zones were in the range between 0.96 - 5.75 mm). Inhibitory effects were also found for extracts obtained from *A. hymenorrhizum*, *A.*

oschaninii, *A. longicuspis*, *A. sativum* and *A. pskemense*, mostly towards *Staphylococcus aureus Staphylococcus epidermidis* and *Streptococcus agalactiae*. Interesting results showed *A. cristophii*; extract obtained from this species inhibited only the growth of *Streptococcus pyogenes*. All tested bacterial strains were sensitive towards antibiotics used as a positive control (oxacilin, penicillin and in the case of *Enterococcus faecalis* ampicillin).

4.4.3. *Selective Screening on Antibacterial Activity (Experiment No. 2)*

In the second experiment, only those *Allium* extracts, which exhibited an inhibitory effect, were retested towards susceptible bacterial strains. While Gram-negative bacterial strains did not show any susceptibility, only Gram-positive bacterial strain were retested. *Enterococcus faecalis* was not retested, because only one extract (obtained from *A. rosenorum*) possessed a considerable inhibitory activity. Other *Allium* extracts did not show any activity. Following Tables 4.9 - 4.12 and Figures 4.48 - 4.54 demonstrate the results of each tested bacterial strain.

Effectiveness towards *Streptococcus pyogenes*

In the first experiment, it has been demonstrated that extracts acquired from *A. rosenorum*, *A. hymenorrhizum* and *A. cristophii* possessed certain inhibitory effects against *Streptococcus pyogenes*. These results could be confirmed in the second experiment (Fig. 4.50). The largest inhibition zone was found for extract obtained from *A. rosenorum* (5.91± 0.55 mm). The values of inhibition zones did not differ considerably from the values obtained in the first experiment. Other tested *Allium* extracts did not show any inhibitory effects. An increased concentration (1.0 mg/ml) did not increase the inhibitory effect of the extracts.

Table 4.9: Inhibition zones of particular *Allium*-extracts tested towards *Streptococcus pyogenes.* Standard deviations of penicillin and *A. hymenorrhizum* were not determined because of limited amounts of extracts and antibiotics.

Acc.-No.	Botanical Name	Zone of Inhibition (mm)	SD [mm]
Tax 0514	*A. pskemense*	0.00	± 0.00
6163	*A. hymenorrhizum*	2.39	0.00 *
Tax 1125	*A. longicuspis*	0.00	± 0.00
Cz001	*A. sativum longicuspis* type	0.00	± 0.00
6912	*A. giganteum* (3)	0.00	± 0.00
6110	*A. rosenorum*	5.91	± 0.55
6135	*A. oschaninii*	0.00	± 0.00
6145	*A. winklerianum*	0.00	± 0.00
6150	*A. carolinianum*	0.00	± 0.00
Tax 3666	*A. jesdianum*	0.00	± 0.00
6142	*A. komarowii*	0.00	± 0.00
1016	*A. cristophii*	1.62	± 0.15
	Penicillin	10.66	*

* SD not determined

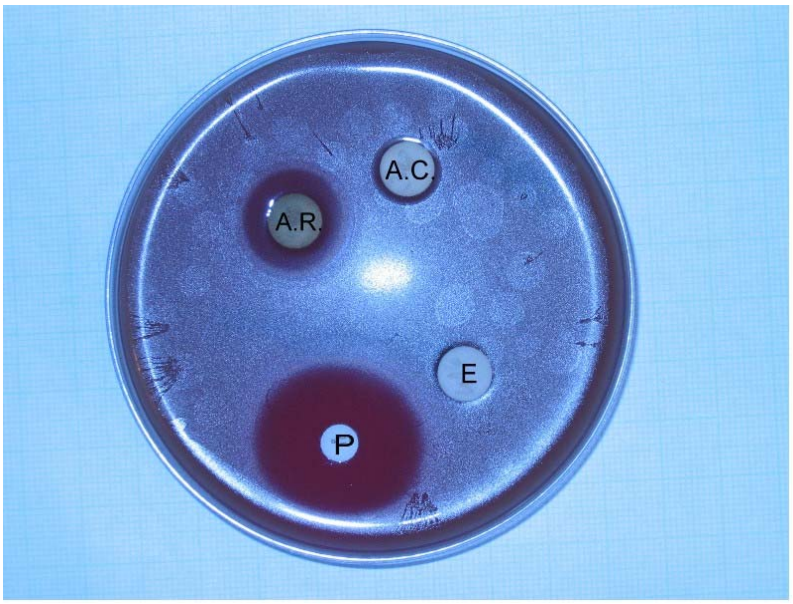


Figure 4.49: Example of a plate inoculated with *Streptococcus pyogenes*.

P Penicillin

E Ethyl acetate

A.R. Extract obtained from *A. rosenorum*

A.C. Extract obtained from *A. cristophii*

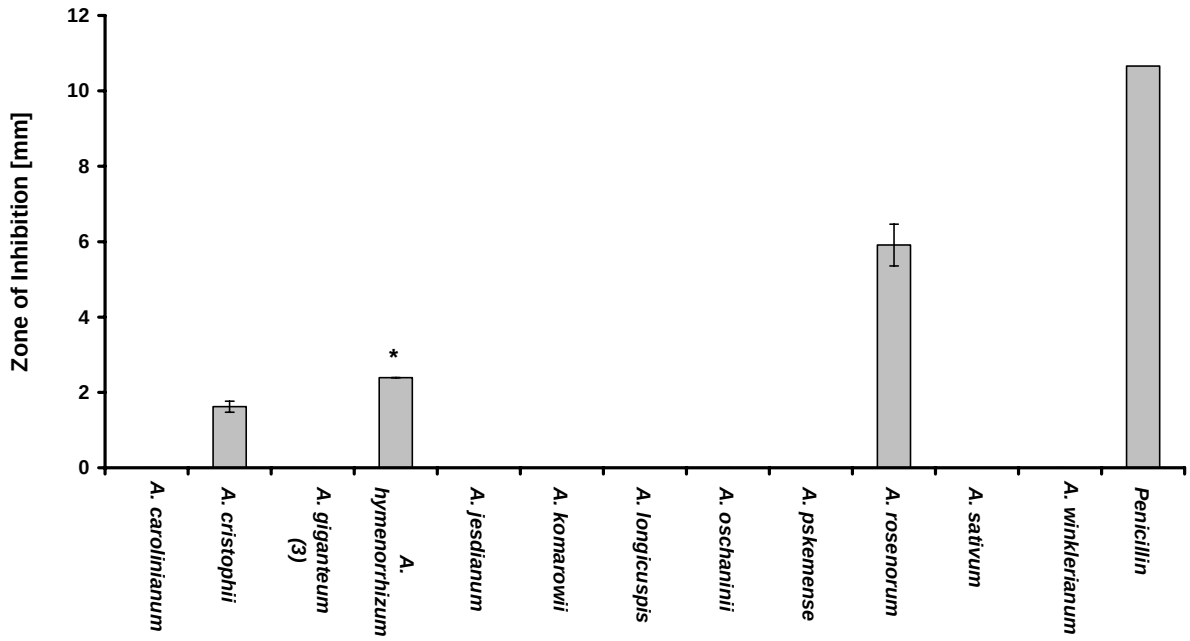


Figure 4.50: Zones of inhibition of particular *Allium*-extracts tested on inhibitory activity towards *Streptococcus pyogenes.* Standard deviations are given by error bars.

Effectiveness towards *Streptococcus agalactiae*

In the first experiment it has been shown that extracts obtained from *A. hymenorhizum*, *A. pskemense*, *A. rosenorum* and *A. oschaninii* possessed an inhibitory activity towards *Streptococcus agalactiae*. Also these results could be confirmed in the second experiment (Fig. 4.52). Apart from these extracts, extract obtained from *A. longicuspis* exhibited an appreciable inhibitory effect (inhibition zone was found to be 2.08 ± 0.04 mm). The largest inhibition zone was observed for extract obtained from *A. pskemense* (2.35 ± 0.34 mm), the smallest for extract obtained from *A. oschaninii* (1.49 ± 0.24 mm). At distinct from the first experiment, the values of inhibition zones considerably differed in the case of *A. hymenorrhizum* (4.32 mm in the first experiment, 1.74 mm in the second experiment). Other results did not differ significantly. The bacterium exhibited susceptibility towards penicillin, which was used as a positive control.

Tab. 4.10: Zones of inhibition of particular *Allium* extracts tested on inhibitory activity towards *Streptococcus agalactiae.* Standard deviations of penicillin were not determined because of limited amounts of the antibiotic.

Acc.-No.	Botanical Name	Zone Of Inhibition [mm]	SD [mm]
Tax 0514	*A. pskemense*	2.35	± 0.34
6163	*A. hymenorrhizum*	1.74	± 0.35
Tax 1125	*A. longicuspis*	2.08	± 0.04
Cz001	*A. sativum longicuspis* type	0.00	± 0.00
6912	*A. giganteum* (3)	0.00	± 0.00
6110	*A. rosenorum*	1.80	± 0.06
6135	*A. oschaninii*	1.49	± 0.24
6145	*A. winklerianum*	0.00	± 0.00
6150	*A. carolinianum*	0.00	± 0.00
Tax 3666	*A. jesdianum*	0.00	± 0.00
6142	*A. komarowii*	0.00	± 0.00
1016	*A. cristophii*	0.00	± 0.00
	Penicillin	10.73	*

* SD not determined

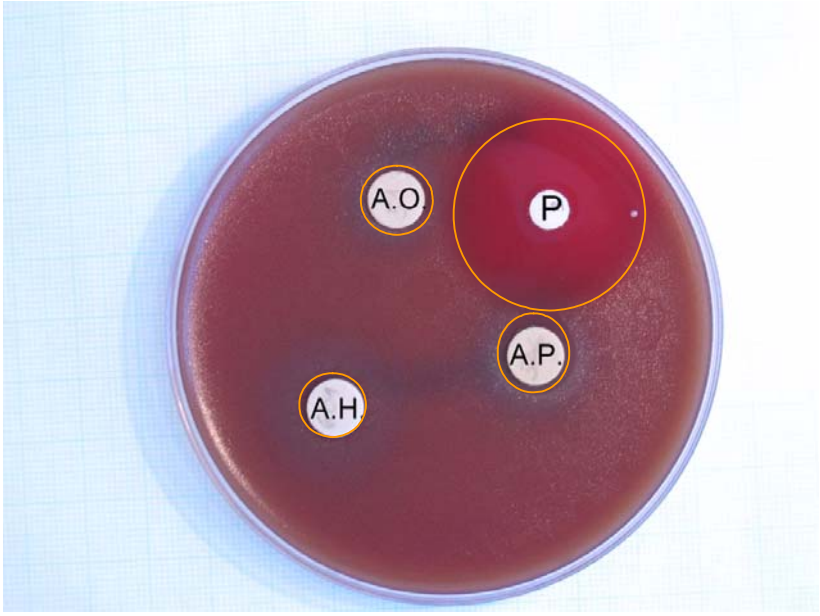


Figure 4.51: Example of a plate inoculated with *Streptococcus agalactiae.*

P Penicillin

A. O. Extract obtained from *Allium oschaninii*

A. H. Extract obtained from *Allium hymenorrhizum*

A. P. Extract obtained from *Allium pskemense*

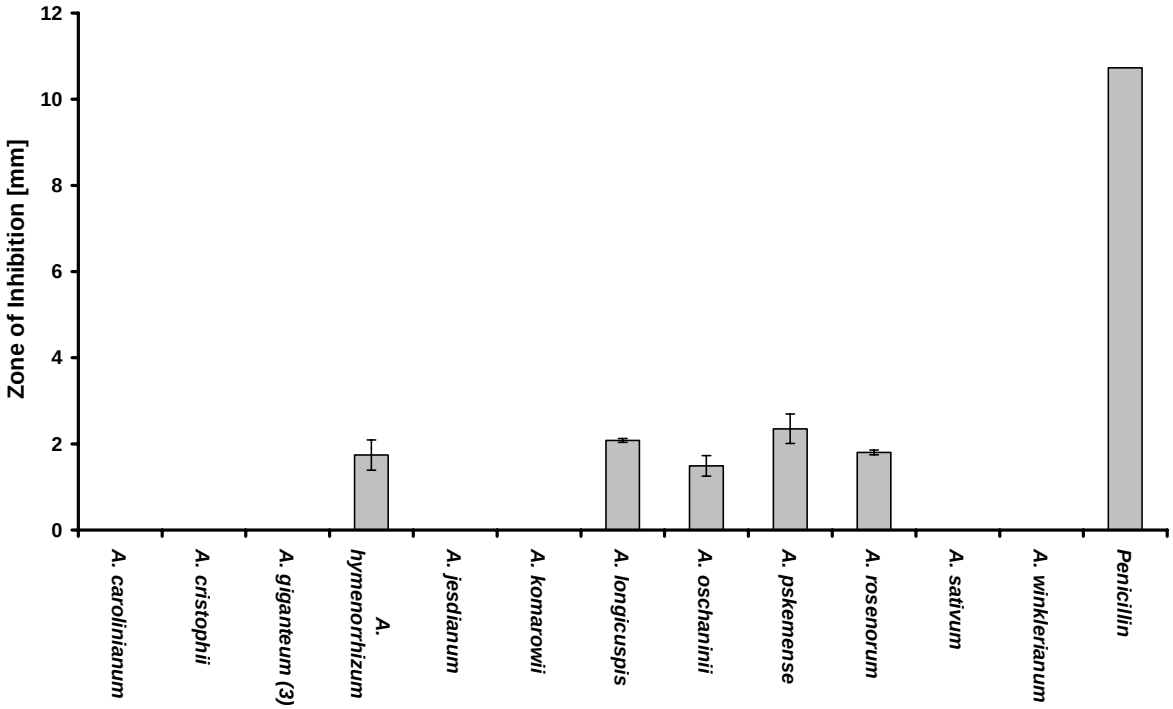


Figure 4.52: Zones of inhibition of particular *Allium* extracts tested on inhibitory activity towards *Streptococcus agalactiae.* Standard deviations are given by error bars.

Effectiveness towards *Staphylococcus aureus*

In the first experiment, it has been shown that extracts obtained from *A. pseudobodeanum*, *A. carolinianum*, *A. giganteum* (3), *A*. *hymenorrhizum*, *A. jesdianum* (2), *A. komarowii*, *A. longicuspis*, *A. macleanii*, *A. pskemense*, *A. rosenorum*, *A. sativum*, *A. winklerianum*, and *A*. *oschaninii* posses an inhibitory effect towards the bacterium *Staphylococcus aureus*. These results were not confirmed completely in the second experiment (Fig. 5.54). Extracts obtained from *A. pskemense*, *A. hymenorrhizum*, *A. longicuspis*, *A. sativum*, *A. giganteum* (3), *A. rosenorum* and *A. oschaninii* showed a significant inhibition activity. Other extracts were found to be ineffective. The largest inhibition zones were observed for extracts obtained from *A*. *longicuspis* (5.61 ± 0.23 mm) and *A. hymenorrhizum* (4.51 ± 0.31 mm), the smallest for *A. giganteum* (3) (0.66 ± 0.10 mm). The values of the inhibition zones in first experiment differed considerably from the values acquired in the second experiment. Deviations ranged from 0.58 to 2.41 mm. Furthermore, in the case of the positive control (oxacillin), the deviation between the first and the second experiment was even 6.32 mm. Nevertheless, *Staphylococcus aureus* showed a considerable susceptibility towards oxacillin.

Table 4.11: Inhibition zones of particular *Allium* extracts tested on inhibitory activity towards *Staphylococcus aureus.* Standard deviations of oxacillin were not determined because of limited amount of the antibiotic.

Acc.-No.	Botanical Name	Zone Of Inhibition [mm]	SD [mm]
Tax 0514	*A. pskemense*	3.79	± 0.75
Tax 2218	*A. macleanii*	0.00	± 0.00
6163	*A. hymenorrhizum*	4.51	± 0.31
Tax 1125	*A. longicuspis*	5.61	± 0.23
Cz001	*A. sativum longicuspis* type	2.98	± 0.51
6912	*A. giganteum* (3)	0.66	± 0.10
6110	*A. rosenorum*	3.45	± 0.63
6135	*A. oschaninii*	2.71	± 0.04
6145	*A. winklerianum*	0.00	± 0.00
6150	*A. carolinianum*	0.00	± 0.00
Tax 3666	*A. jesdianum*	0.00	± 0.00
6142	*A. komarowii*	0.00	± 0.00
1016	*A. cristophii*	0.00	± 0.00
1035	*A. giganteum*	0.00	± 0.00
1024	*A. pseudobodeanum*	0.00	± 0.00
	Oxacillin	7.08	*

* SD not determined

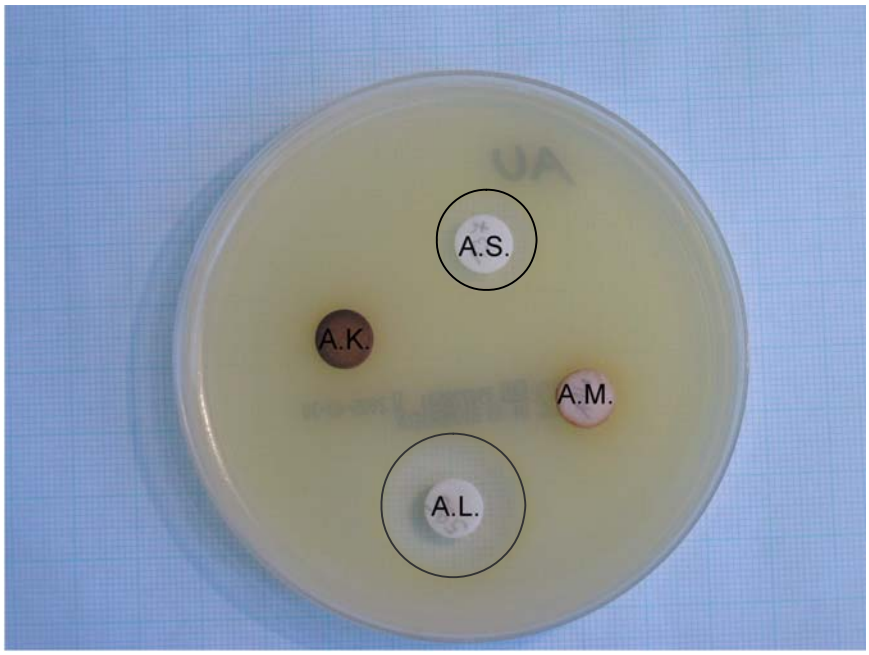


Figure 4.53: Example of a plate inoculated with *Staphylococcus aureus.*

A. S. Extract obtained from *Allium sativum*

A. K. Extract obtained from *Allium komarowii*

A. L. Extract obtained from *Allium longicuspis*

A. M. Extract obtained from *Allium macleanii*

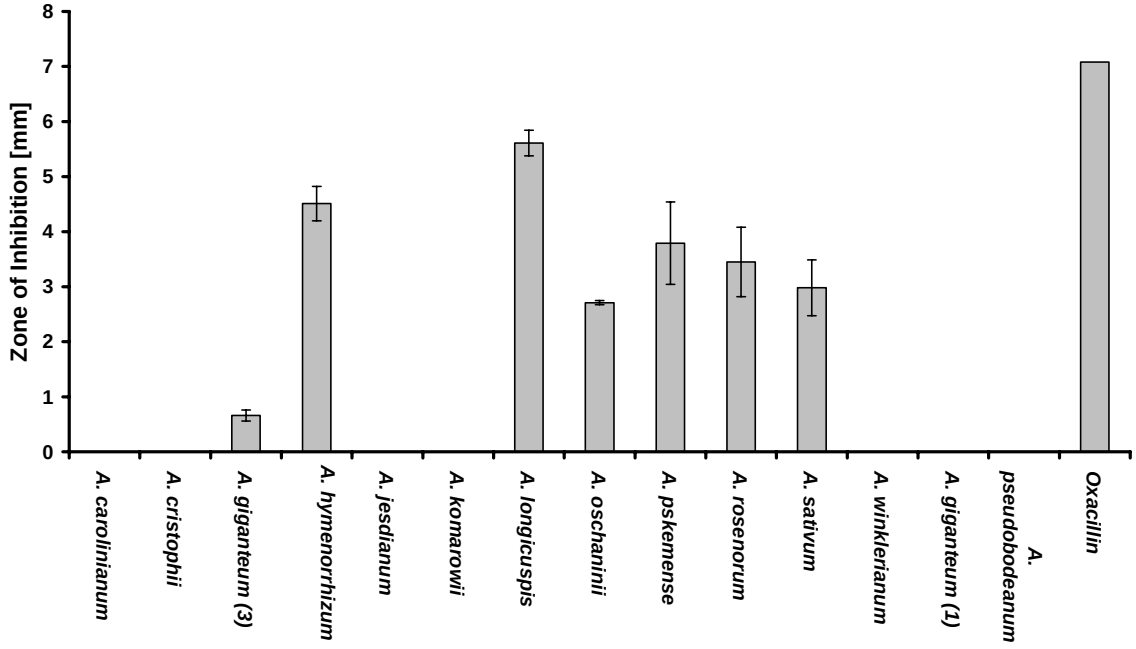


Figure 4.54: Inhibition zones of *Allium* extracts tested on inhibitory activity towards *Staphylococcus aureus*. Standard deviations are given by error bars.

Effectiveness towards *Staphylococcus epidermidis*

In the first experiment, it has been shown that extracts obtained from *A. carolinianum*, *A*. *giganteum* (3), *A. hymenorrhizum*, *A. jesdianum* (1) and (2), *A. komarowii* (2), *A. longisuspis*, *A. pskemense*, *A. rosenorum*, *A. sativum*, *A. winklerianum* and *A. oschaninii* exhibit a significant inhibitory activity towards *Staphylococcus epidermidis*. In the second experiment, this fact could not be confirmed in the case of *A. jesdianum* (1), *A. giganteum* (3) and surprisingly *A. hymenorrhizum* (Fig. 4.56). Extracts obtained from these *Allium* species did not show any inhibitory effect. The largest inhibition zone was observed for extracts obtained from *A. sativum* (6.79 ± 0.37 mm), *A. longicuspis* (4.79 ± 0.42 mm) and *A. rosenorum* (3.83 ± 0.53 mm). Inhibition zone surrounding the disc soaked with the extract obtained from *A. sativum* was almost as large as the inhibition zone surrounding oxacillin (6.83 mm). *Staphylococcus epidermidis* was found to be susceptible towards the positive control. Some values of inhibition zones obtained in the first experiment differed significantly from the results acquired in the second experiment. The deviation was in the range between 0.06 and 2.29 mm and in the case of *A. hymenorrhizum* even 7.02 mm.

Tab.4.12: Zones of inhibition of particular *Allium* extracts tested on inhibitory activity towards *Staphylococcus epidermidis.* Standard deviations of oxacillin were not determined because of limited amount of the antibiotic.

Acc.-No.	Botanical Name	Zone Of Inhibition [mm]	SD [mm]
Tax 0514	*A. pskemense*	3.16	± 0.74
6163	*A. hymenorrhizum*	0.00	± 0.00
Tax 1125	*A. longicuspis*	4.79	± 0.42
Cz001	*A. sativum longicuspis* type	6.79	± 0.37
6912	*A. giganteum* (3)	0.00	± 0.00
6110	*A. rosenorum*	3.83	± 0.53
6135	*A. oschaninii*	1.49	± 0.31
6145	*A. winklerianum*	1.77	± 0.03
6150	*A. carolinianum*	3.50	± 0.39
Tax 3666	*A. jesdianum* (2)	2.11	± 0.97
6142	*A. komarowii* (2)	2.40	± 0.58
1033	*A. jesdianum* (1)	0.00	± 0.00
1016	*A. cristophii*	0.00	± 0.00
	Oxacillin	6.83	*

* SD not determined

Figure 4.55: Example of a plate inoculated with *Staphylococcus epidermidis.*

A. Ca Extract obtained from *Allium carolinianum*

A. S. Extract obtained from *Allium sativum*

A. R. Extract obtained from *Allium rosenorum*

A. K. Extract obtained from *Allium komarowii*

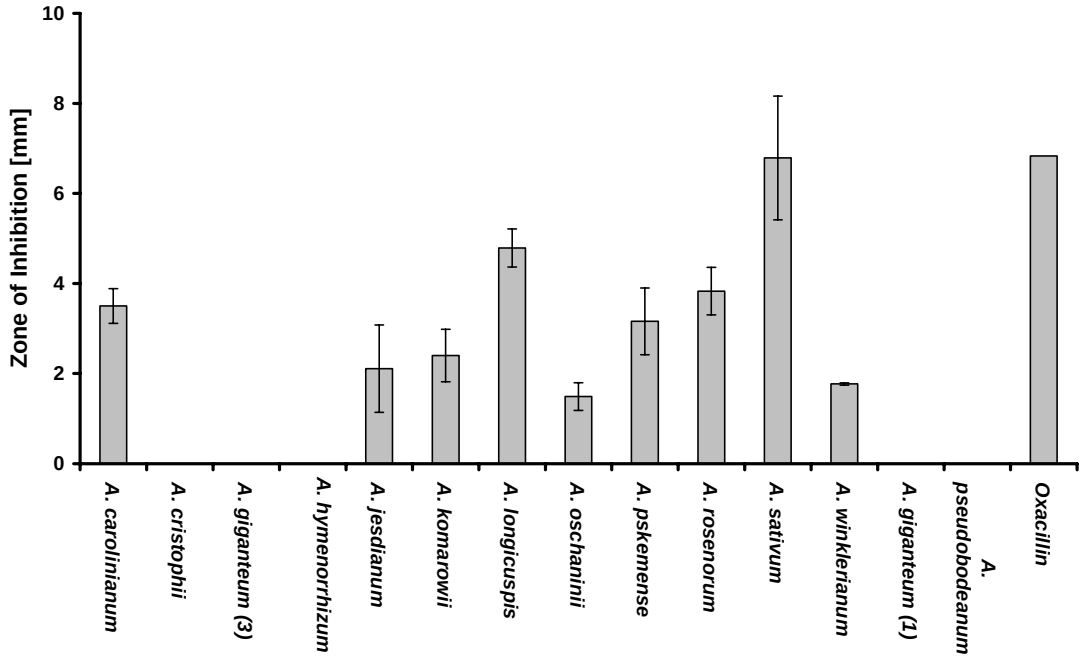


Figure 4.56: Zones of inhibition of particular *Allium* extracts tested on inhibitory activity towards *Staphylococcus epidermidis.* Standard deviations are given by error bars.

4.5. *Radical Scavenger Activity of Allium Extracts*

Radical scavenger Activity was measured with two different plate readers. First plate reader (SLT Rainbow, Labinstrument) allowed measurements at 517 nm. The following Table 4.13 and Figure 4.57 show the acquired results.

Table 4.13: The relative radical scavenger activity of extracts obtained from wild *Allium* species. Absorption was measured at 517 nm.

Acc.-Number	Botanical Name	Relative Scavenger Activity [%]	SD [%]
4125	*A. alaicum*	115.4	±2.7
4134	*A. talassicum* (1)	27.5	± 0.8
4142	*A. pskemense* (1)	100.6	± 0.1
4143	*A. turkestanicum*	85.9	± 8.0
4156	*A. margaririferum*	6.4	± 2.4
4165	*A. giganteum* (1)	109.1	± 6.4
4170	*A. komarowii* (1)	110.1	± 3.6
4188	*A. jodanthum*	44.0	± 5.8
4190	*A. filidens*	18.5	± 2.1
4193	*A. karataviense*	52.7	± 2.5
6031	*A. griffithianum*	21.2	± 13.6
6050	*A. rosenbachianum*	33.9	± 3.9
6054	*A. bucharicum*	42.9	± 7.1
6074	*A. darwasicum*	21.1	± 3.8
6084	*A. oschaninii* (1)	23.6	± 1.7
6085	*A. barsczewskii*	21.1	± 3.3
6094	*A. schugnanicum*	9.7	± 3.2
6101	*A. stipitatum* (1)	15.6	± 2.2
6106	*A. hissaricum*	44.7	± 8.5
6110	*A.rosenorum*	121.9	± 3.6
6113	*A. verticillatum*	25.5	± 0.4
4900	*A. sativum*	45.0	± 6.4

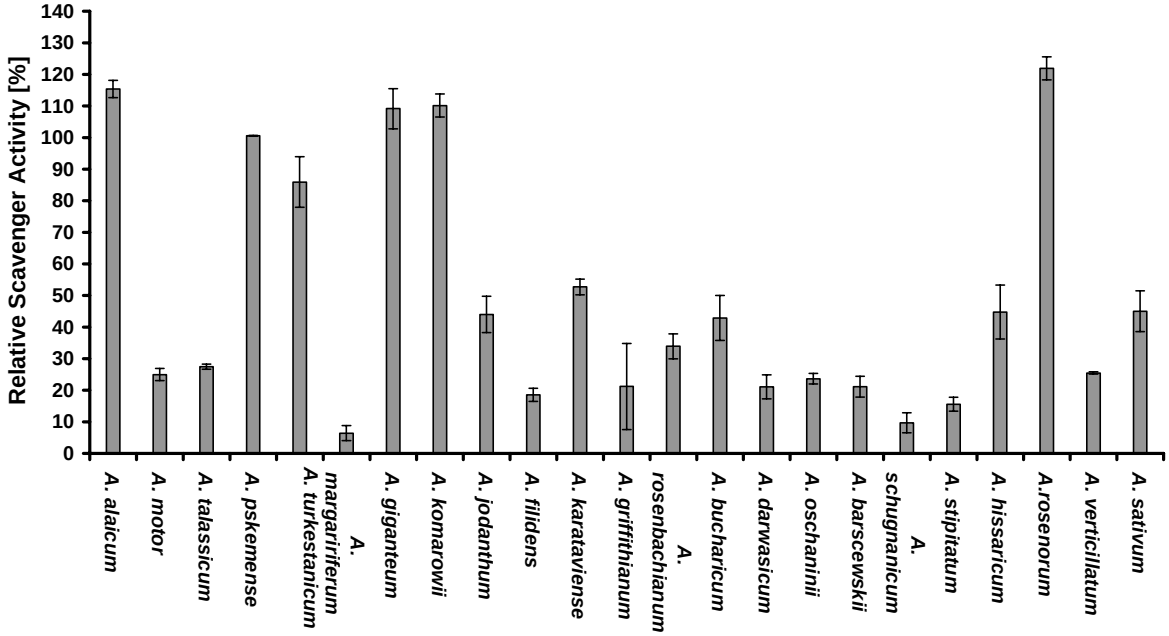


Figure 4.57: The relative scavenger activity of extracts obtained from wild *Allium* species at 517 nm. The relative activity of the positive control (BHT) was set to lie at 100%. Standard deviations are given by error bars.

A second plate reader (Multiscan EX, Thermo) allowed only measurements at 540 nm. Calculated results of the relative scavenger activity are listed in the following Table 4.14 and Figure 4.58. The relative scavenger activity of the positive control (BHT) was set to lie at 100%.

Table 4.14: The relative radical scavenger activities of extracts obtained from wild *Allium* species. Absorption was measured at 540 nm.

Acc.-Number	Botanical Name	Relative Scavenger Activity [%]	SD [%]
1006	*A. chelotum*	100.3	± 0.1
1016	*A. cristophii*	55.7	± 4.7
1017	*A. paradoxum*	19.3	± 3.8

Acc.-Number	Botanical Name	Relative Scavenger Activity [%]	SD [%]
1022	*A. iranicum*	13.0	± 4.9
1023	*A. oschaninii* (2)	10.7	± 3.0
1024	*A. pseudobodeanum*	91.6	± 10.8
1033	*A. jesdianum* (1)	112.8	± 12.8
1035	*A. giganteum* (2)	52.5	± 7.0
4133	*A. motor*	110.4	± 3.4
4135	*A. talassicum* (2)	4.1	± 6.1
6103	*A. stipitatum* (2)	16.2	± 11.4
6110	*A. rosenorum*	109.4	± 0.7
6112	*A. suworowii*	4.4	± 5.2
6135	*A. oschaninii*	51.7	± 3.0
6136	*A. alexeianum*	9.9	± 4.1
6142	*A. komarowii* (2)	115.8	± 0.7
6145	*A. winklerianum*	115.8	± 1.0
6150	*A. carolinianum*	95.4	± 1.7
6163	*A. hymenorrhizum*	35.8	± 1.9
6166	*A. caeruleum*	-3.8	± 5.9
Tax 1125	*A. longicuspis*	84.0	± 1.8
Tax 1211	*A. aflatunense*	16.6	± 0.0
Tax 1678	*A. altaicum* (1)	18.2	± 1.4
Tax 2218	*A. macleanii*	120.7	± 7.7
Tax 2724	*A. pskemense* (2)	27.7	± 2.3
Tax 2746	*A. altaicum* (2)	44.4	± 2.0
Tax 3666	*A. jesdianum* (2)	109.2	± 3.4
Tax 3951	*A. jesdianum* (3)	108.9	± 2.1
Tax 5394	*A. hybrid*	104.3	± 5.3
Tax 0382	*A. oleraceum*	84.9	± 2.7
Tax 0514	*A. pskemense* (3)	52.7	± 12.7
6912	*A. giganteum* (3)	121.1	± 1.3
4900	*A. sativum*	49.4	± 3.4

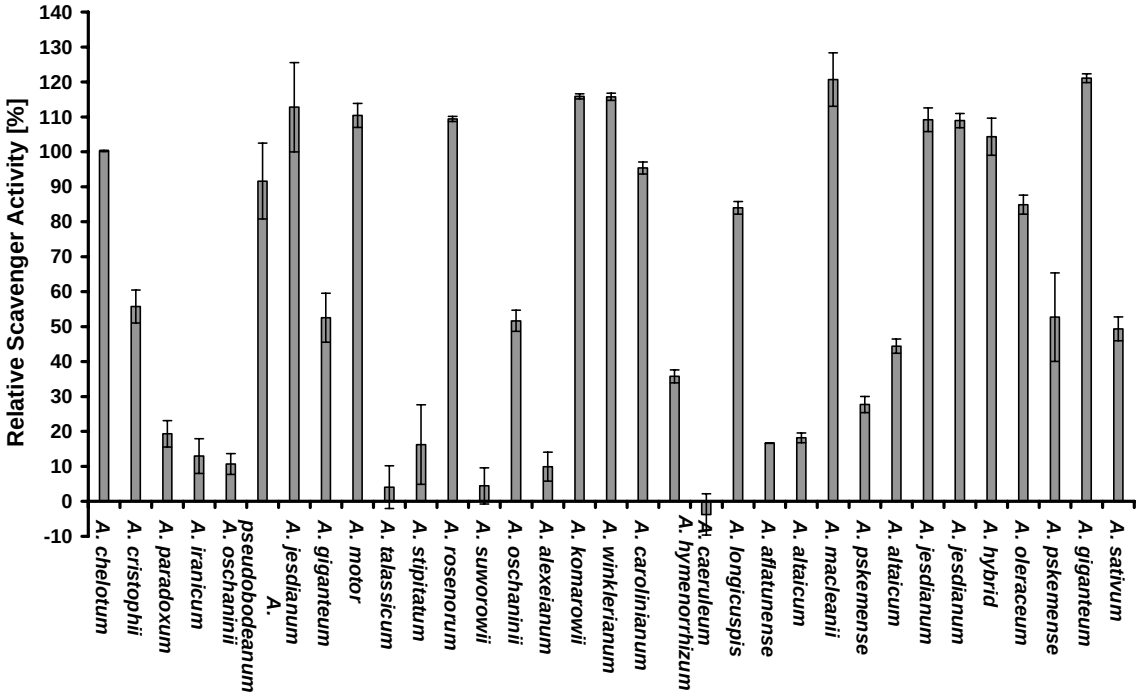


Figure 4.58: The relative radical scavenger activity of extracts obtained from wild *Allium* species. Relative activity of positive control (BHT) was set to 100%. Standard deviations are given by error bars.

As already mentioned, scavenger activity was measured with two different equipments at two different wavelengths. In order to determine possible differences between these two measurements, two samples obtained from the same *Allium* species possessing equal accession numbers were measured with both equipments. Measurements with an extract obtained from *A. sativum* at 517 nm showed the relative activity of 45.0 ± 6.4 % and at 540 nm 49.4 ± 3.4 %. Experiments with extract obtained from *A. rosenorum* at 517 nm exhibited a relative activity of 121.9 ± 3.6 % and at 540 nm 109.4 ± 0.7 %. Relative differences between these two experiments seem to be approximately 10%, which is in the range of observed standard deviations of a number of measurements. Furthermore, BHT was used for calibration for all experiments and its relative scavenger activity was set to lie at 100%.

Results acquired in this experiment could be divided into four groups. The first group include samples where the relative scavenger activity reached more than 100 %. It means that the relative activity is higher than the activity measured for the positive control (BHT). The relative scavenger activity over 100 % was determined for extracts obtained from

A. rosenorum (121.9 ± 3.6 %), *A. giganteum* (1) (109.1 ± 6.4 %) and (3) (121.1 ± 1.3 %), *A. macleanii* (120.7 ± 7.7 %), *A. winklerianum* (115.8 ± 1.0 %), *A. alaicum* (115.4 ± 2.7 %), *A. komarowii* (1) (110.1 ± 3.6 %), *A. jesdianum* (for all tested accession numbers, the values ranged from 108.9 to 112.8 %), *A. motor* (110.4 ± 3.4 %), *A. pskemense* (1) (100.6 ± 0.1 %) and *A. chelotum* (100.3 ± 0.1 %). Extracts obtained from *A. rosenorum*, *A. macleanii* and *A. giganteum* (3) exhibited the highest relative scavenger activity. These *Allium* species belong mostly to the subgenus *Melanocrommyum*; only *A. pskemense* belongs to the subgenus *Cepa*.

The second group includes samples where the relative activity ranged from 80 to 100 %. This activity was determined for extracts obtained from *A. turkestanikum* (85.9 ± 8.0%), *A. pseudobodeanum* (91.6 ± 10.8 %), *A. carolinianum* (95.4 ± 1.7%), *A. longicuspis* (84.0± 1.8 %) and *A. oleraceum* (84.9 ± 2.7 %). These *Allium* species belong to the subgenera *Allium*, *Polyprason* and *Melanocrommyum*.

The third group includes samples where the relative scavenger activity ranged between 40 and 80%. Extracts obtained from *A. jodanthum* (44.0 ± 5.8 %), *A. karataviense* (52.7 ± (2.5 %), *A. bucharicum* (42.9 ± 7.1 %), *A. hissaricum* (47.7 ± 8.5 %), *A. sativum* (45.0 ± 6.4 %), *A. cristophii* (55.7 ± 4.7 %), *A. giganteum* (2) 52.5 ± 7.0 %), *A. altaicum* (2) (44.4 ± 2.0 %) and *A. pskemense* (3) (52.7 ± 12.7 %) belong to this group. These *Allium* species are members of the subgenera *Melanocrommyum*, *Allium*, *Reticulobulbosa* and *Cepa*.

The fourth group includes samples with relative scavenger activity smaller than 40 %, which is not of practical relevance. These are *A. motor*, *A.talassicum* (1), *A. margaritiferum*, *A.filidens*, *A. griffithianum*, *A. rosenbachianum*, *A. darwasicum*, *A. oschaninii* (1), *A. barsczewskii*, *A. schugnanicum*, *A. stipitatum* (1), *A. verticillatum*, *A. paradoxum*, *A. iranicum*, *A. oschaninii* (2), *A. talassicum*, *A. stipitatum* (2), *A. suworowii*, *A. alexeianum*, *A. hymenorrhizum*, *A. aflatunense*, *A. pskemense* (2) and *A. caeruleum*.

Interesting results were acquired from experiments with *A. caeruleum*. Relative scavenger activity of extracts obtained from this species was calculated in a negative range (-3.8 ± 5.9 %). This means that this species supports the formation of free radicals.

In some species belonging to the subgenus *Melanocrommyum*, a reddish liquid coming out of the damaged tissue was observed. This red dye was determined as dithiodipyrrole (see chapter 4.3.). The substance was isolated from extract obtained from *A. giganteum* and purified using LC separation. Obtained fractions were screened for antioxidant activity. In some screened fractions, remarkably high radical scavenger activities were found. In following Table 4.15, the relative scavenger activity of investigated fractions is listed.

Table 4.15.: The relative scavenger activity of some fractions obtained from LC separation of an *A. giganteum* extract.

Fraction-Number	Relative Scavenger Activity [%]
3	12.6
4	78.1
5	109.3
6	137.5
7	102.6
8	125.8
9	85.1
10	116.1

Significantly high relative scavenger activity was observed in fractions numbers 5,6,7,8, and 10 (the activity was found to be higher than 100%). The substance dithiodipyrrole was obtained from fraction number 4, where the relative scavenger activity was found to be 78.1%. Also fractions 3 and 5 contained small amounts of this compound. The chemical composition of fractions 3 and 5-10 were not further investigated.

5. Discussion

5.1. *Methodology*

5.1.1. *HPLC-Method*

HPLC-Methods for the determination of cysteine sulphoxide have been intensively examined in the group of Keusgen since 1995. Most recent publications about this method are Krest et al. [2000], Fritsch and Keusgen [2006] and Schmitt [2004]. Especially Schmitt put a lot of emphasis on the validation of the method. The limit of detection was determined to be 0.036% and the limit of quantitation was at 0.059% cysteine sulphoxides, related to the fresh weight of bulbs. For the determination of these values, an evaporated extract of *Allium* (0.2-1.0 g fresh weight of bulbs) was solved in 5.00 ml of OPA derivatization reagent and UV detection was carried out at 334 nm. However, if the concentration of cysteine sulphoxides was below this range, less than 5 ml of OPA reagent was used for derivatization and/or amount of extracted bulbs was increased. Taking these modifications into account, the limit of quantitation is at about 0.01%, which is sufficient for this kind of investigation. Additionally, sensitivity of method can be increased by fluorescence detection, because formed isoindole derivatives of cysteine sulphoxides also exhibit a strong fluorescence.

The recovery rate was also determined. For this task, ethiin was used. Ethiin was also observed in some *Allium* species, but only in trace amounts [Kubec et al. 2002]. Therefore, ethiin can be used as an internal standard for quantitative analysis or for determination of the recovery rate. By this method, Schmitt observed a recovery rate of 92.2 %, but with variations between 64.1% and 119.3%. The recovery rate was therefore re-determined and found to be at 102.7%. In conclusion, used methods for extraction and sample preparation leads to negligible losses in cysteine sulphoxides.

5.1.2. *FIA-Method*

The FIA-method was intended as a rapid method for a pre-screening of samples. One of the disadvantages of this method was that no differentiation between individual cysteine sulphoxides was possible. Therefore, samples with a high amount of cysteine sulphoxides had been re-determined using HPLC.

Each sample could be analyzed within approximately 8 min. However, the most time consuming step was the sample preparation. The samples had to be extracted in the same manner as for HPLC analysis and diluted several times. Because of this procedure, sample throughput was restricted to a maximum of three bulbs and/or leaves per day (threefold

measurement) including re-calibration steps. The throughput of this method could be increased by a paralleled, automatic extraction module, which was, however, not available.

The method was found to be highly sensitive. In this case, fluorescence detection of isoindole derivatives was used. Alliin standards could be detected down to a concentration of 3 µM and the limit of quantitation was at about 10 µM. Because several dilution steps were involved in the sample preparation, which could be easily reduced leading to an increased sensitivity. Obtained sensitivity was found to be absolutely sufficient for a screening program of wild *Allium* species.

A problem was the correlation between FIA and HPLC, which was found to be 75%. Results obtained by FIA were averagely 25% lower than those obtained by HPLC. These differences can be easily explained by substrate specificity of the alliinase, which was used for the FIA-method. Alliinase showed the highest sensitivity towards alliin and isoalliin. All other cysteine sulphoxides were digested by the enzyme in much lower rates. Especially those species having high amounts of methiin would lead to reduced values of cysteine sulphoxides by the FIA-method (substrate specificity of alliinase towards methiin was about 40 %, see Figure 4.10). On the other hand, valuable wild *Allium* species leading a red dye were also covered by the FIA method, because the unknown precursor of the red dye was also accepted by the alliinase.

In conclusion, both methods are sufficient for a screening program on cysteine sulphoxides in wild *Allium* species. The long time needed for a single analysis (about 70 min per run) and the complex sample preparation are the major disadvantages of the used HPLC-method. The FIA method allows a rapid determination of the total content of cysteine sulphoxides, but the used apparatus and the extraction equipment have to be more automated. The disadvantage was that found values are mostly lower as those obtained by HPLC.

5.2. *Intraspecific Comparison of Cysteine Sulphoxides and Possible Pharmaceutical Use*

Amounts of cysteine sulphoxides were investigated in various wild *Allium* species, which were collected in Iran, Turkmenistan, Uzbekistan and Tajikistan. Additional samples were obtained from the IPK Gatersleben, Germany. Total amounts as well as relative amounts of cysteine sulphoxides were determined by means of HPLC. Amounts of cysteine sulphoxides of identical *Allium* species possessing different Acc. numbers and Tax. numbers, respectively, were compared and acquired results depicted in the following figures. In some cases, compared samples of the same species were collected at different places at different times. These details were conclusively given in Tab.3.1 - 3.5. It has to be taken into account

that botanical field trips in Middle Asia were performed at different times (April to July) and also in different areas (Georgia, Iran, Turkmenistan, Uzbekistan, Tajikistan), mostly in semi-dry mountainous areas. But plants were also collected in deserts (*e.g.*, *A. caspium*), in forests (*e.g.*, *A. paradoxum*) or even in boggy meadows (*e.g.*, *A. fedschenkoanum*). Plant material was collected at altitudes between normal sea level and up to 3600 m.

5.2.1. *Allium barsczewskii*

In bulbs of *A. barsczewskii,* significantly dissimilar values of total amount of cysteine sulphoxides were found. In bulbs of **Acc.-No. 4166**, 0.94% were detected whereas in bulbs of **Acc.-No. 6168**, 5.72% of cysteine sulphoxides were found (Fig. 5.1). Such dissimilarity could be explained by the anatomy of examined plant material. The bulb of *A. barsczewskii* is very small and can dry out rather fast. Correct storage without any lost in weight by drying is rather difficult. Therefore, when the sample was not prepared immediately after collection or if the plant was stored over several months, the concentration of cysteine sulphoxides could significantly increase, because of loss of water.

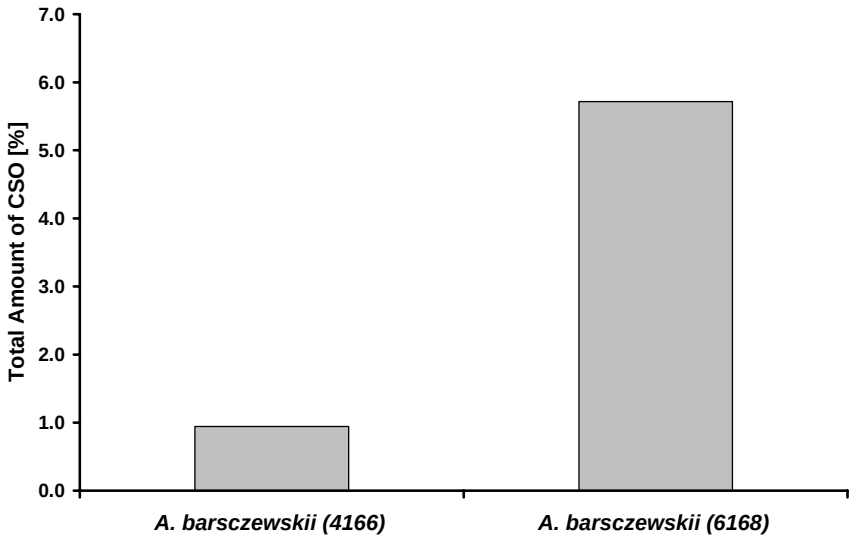


Figure 5.1: Total amounts of cysteine sulphoxides in bulbs of *A. barsczewskii*. Concentrations are related to fresh weight (average of n=4).

Another explanation for observed differences in concentrations gives the place of collection. Bulbs of **Acc.-No. 4166** were collected in Uzbekistan at an altitude of 1750 meter in rocky clefts, whereas bulbs of **Acc.-No. 6168** were collected in Tajikistan at altitude of

2130 meter on a loamy slope. Therefore, grows conditions of **Acc.-No. 6168** were probably much better.

Time of collection plays also an important role; previous investigations showed that the values of cysteine sulphoxides could vary during ontogenesis [Lancaster et al. 1986; Lancaster et al. 1984; Randle et al 1993; Schmitt 2004]. Bulbs of **Acc.-No. 4166** were collected at the end of May, when the ontogenesis was not finished, whereas bulbs of **Acc.-No. 6168** were collected at the end of June, meaning that cysteine sulphoxides were already stored in the bulb for upcoming dormancy leading to higher amounts of these compounds in the storage compartment.

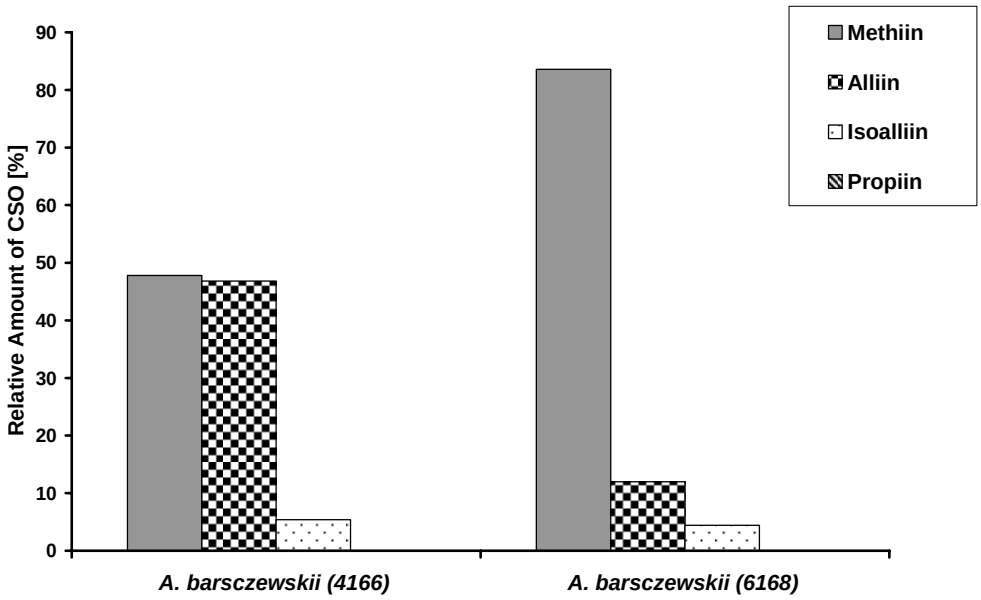


Figure 5.2: Relative amounts of cysteine sulphoxides in bulbs of *A. barsczewskii* (total cysteine sulphoxides = 100%).

The pattern of flavour precursors of different bulbs of *A. barsczewskii* corresponded rather well; in both Acc.-numbers, methiin, alliin, and isoalliin were found (Fig. 5.2). Nevertheless, the values of particular cysteine sulphoxides were found to be different; in bulbs of **Acc.-No. 4166**, methiin and alliin were the most abundant cysteine sulphoxides. In the case of **Acc.-No. 6168,** methiin (83.59%) was found to be the most abundant cysteine sulphoxide. In both cases, isoalliin was detected in small amounts. As already mentioned above, dissimilar values of cysteine sulphoxides could be explained by different ontogenetic stages of collected plants [Lancaster et al. 1986; Randle et al 1993; Schmitt 2004], as well as different environmental conditions, *e.g.*, availability of light, water, and nutrition. It has to be noticed, that the most health benefits of *Allium* are related to alliin and isoalliin. High levels of methiin lead to an unpleasant taste of plant material. The only remarkable health benefit for methiin is an antidiabetic effect [Keusgen 2002a].

5.2.2. *Allium oschaninii*

A. oschaninii is a close relative of common onion (*A. cepa*). The total amounts as well as patterns of cysteine sulphoxides were therefore expected to be similar to common onion. Bulbs of *A. oschaninii* were, again, collected at different places in different time; bulbs of **Acc.-No. 4123** were collected in Uzbekistan at the end of May 2003, whereas bulbs **Acc.-No. 6135** were collected in Tajikistan at the end of June 2004. *A. oschaninii* typically grows on stony, steep slopes. Values of the total amount of flavour precursors nearly corresponded; in the bulbs of **Acc.-No. 4123**, 0.38% and in the bulbs of **Acc.-No. 6135** 0.27% were detected (Fig. 5.3). The slightly higher amount of cysteine sulphoxides in the bulbs of **Acc.-No. 4123** could be explained by a long period between plant collection and sample preparation. Previous investigations showed that a long storage could be responsible for higher amounts of cysteine sulphoxides [Bacon et al.1999; Kopsell and Randle 2001; 2002]. The bulbs of **Acc.-No. 4123** were collected in May 2003, but their extraction was performed in April 2004.Therefore, the value of the total amount of flavour precursors in the bulbs of **Acc.-No. 4123** could increase in this time (dry out of bulb material and perhaps liberation of cysteine sulphoxides out of the corresponding γ-glutamyl derivatives).

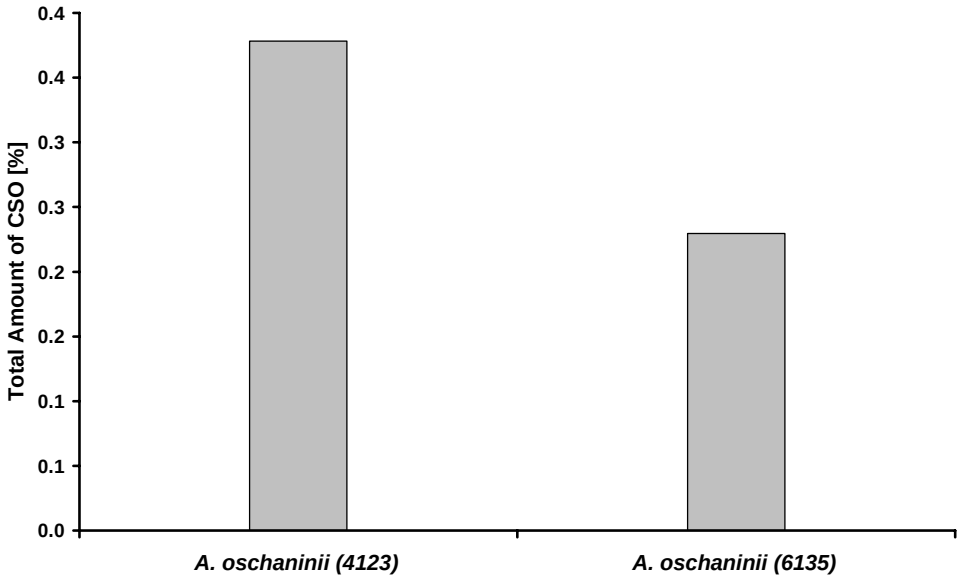


Figure 5.3: Total amounts of cysteine sulphoxides in bulbs of *A. oschaninii*. Concentrations are related to fresh weight (average of n=4).

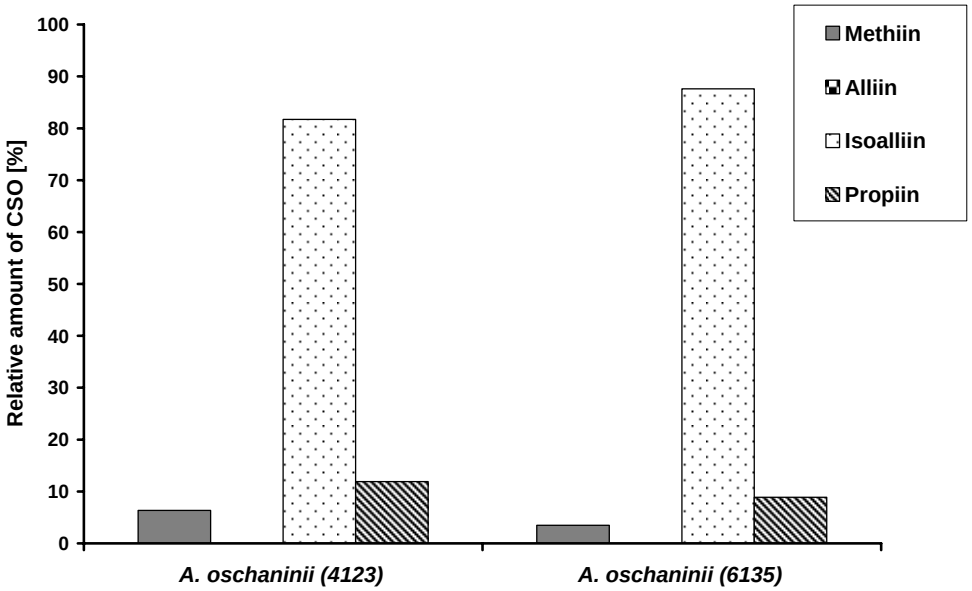


Figure 5.4: Relative amounts of cysteine sulphoxides in bulbs of *A. oschaninii* (total cysteine sulphoxides = 100%).

The composition of cysteine sulphoxides in investigated samples was found to be nearly identical (Fig. 5.4). In both samples methiin, isoalliin and propiin were detected. Isoalliin was determined to be the most abundant flavour precursor (over 80%), methiin and propiin were found in trace amounts. This composition of flavour precursors corresponds with the cysteine sulphoxide pattern of common onion. *A. oschaninii* is therefore highly estimated by the local population as a substituent of common onion.

5.2.3. *Allium paradoxum var. normale*

Allium paradoxum var. normale is a small plant possessing round bulbs with a characteristic smell. This plant typically grows at shadow places in forests. In the compared bulbs of A. *paradoxum var. normale*, dissimilar total amounts of cysteine sulphoxides were observed; in bulbs of **Acc.-No. 1017** (collected in Iran), 0.82% was detected, whereas in bulbs of **Acc.-No. 0135** (collected in Turkmenistan), merely 0.16% were found (Fig. 5.5). The reason for such dissimilarity could be a different ontogenetic stage, as well as different environmental conditions, *e.g.*, diverse kind of soil or light and water exposition. Plants of **Acc.-No. 1017** grew on rich soil in a rather wet forest under nearly ideal conditions. As already explained for *A. barsczewskii* (see above), a good nutrition may lead to higher concentrations of cysteine sulphoxides.

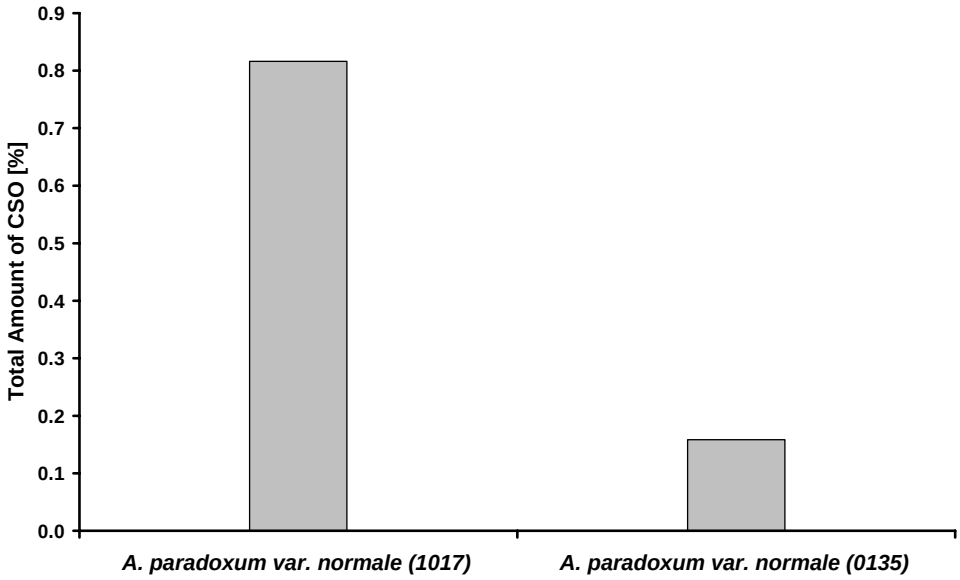


Figure 5.5: Total amounts of cysteine sulphoxides in bulbs of *A. paradoxum var. normale.* Concentrations are related to fresh weight (average of n=4).

The pattern of cysteine sulphoxides in examined samples was found to be rather similar. In both samples, methiin, isoalliin and propiin were found (Fig. 5.6). In bulbs of **Acc.-No. 1017**, nearly the same relative amount of methiin and isoalliin was found (methiin 46.58% and isoalliin 48.79%). In bulbs of **Acc.-No. 0135**, methiin was detected to be the most abundant flavour precursor (70.01%). This dissimilarity could be probably explained by different nutrition of the plants like explained above [Coolong and Randle 2003] and perhaps by different ontogenetic stages. Altitude plays in this case also a role; plants of **Acc.-No. 1017** were collected at an altitude of 1450 meters, whereas plants of **Acc.-No. 0135** were found at an altitude of 700m. In both samples, propiin was detected only in trace amounts. *A. paradoxum* is also highly estimated by local populations and is mostly used for flavouring reasons. The specific ratio between methiin, isoalliin and propiin gives the plant a very special taste and smell.

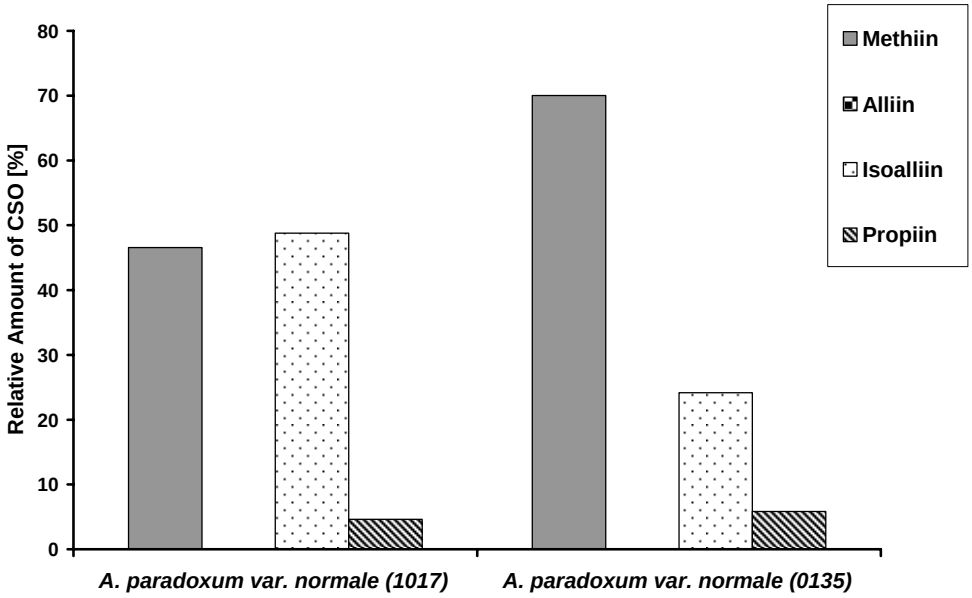


Figure 5.6: Relative amounts of cysteine sulphoxides in bulbs of *A. paradoxum var. normale* (total cysteine sulphoxides = 100%).

5.2.4. *Allium caspium*

Both compared plants of *A. caspium* were collected in sandy regions, but in different states. Bulbs of **Acc.-No. 4021** were collected in Uzbekistan and bulbs of **Acc.-No. 030** were collected in Turkmenistan. In examined samples, the total amounts of flavour precursors were found to be rather similar. In the bulbs of **Acc.-No. 4021**, 0.1% and in the bulbs of **Acc.-No. 030**, 0.15% of total cysteine sulphoxides were detected (Fig. 5.7). *A. caspium* is a typical desert plant and the ecology was nearly identical for both plants. Therefore, differences in the amount of cysteine sulphoxides were rather small.

The composition of cysteine sulphoxides in examined samples concurred significantly well; in both samples, methiin and isoalliin were found in a nearly identical ratio (Fig. 5.8). As explained above, the ecology of both places where the plants were collected was rather similar.

There was only little information available, if this plant is used by the local population or not. In some cases, the plant is believed to be poison, probably because of the content of saponins. But there were also some rare oral reports that the bulbs are eatable. Observations of Keusgen in 2003 showed, that leaves of the plants were only seldomly damaged by animals [Keusgen 2006, personal communication].

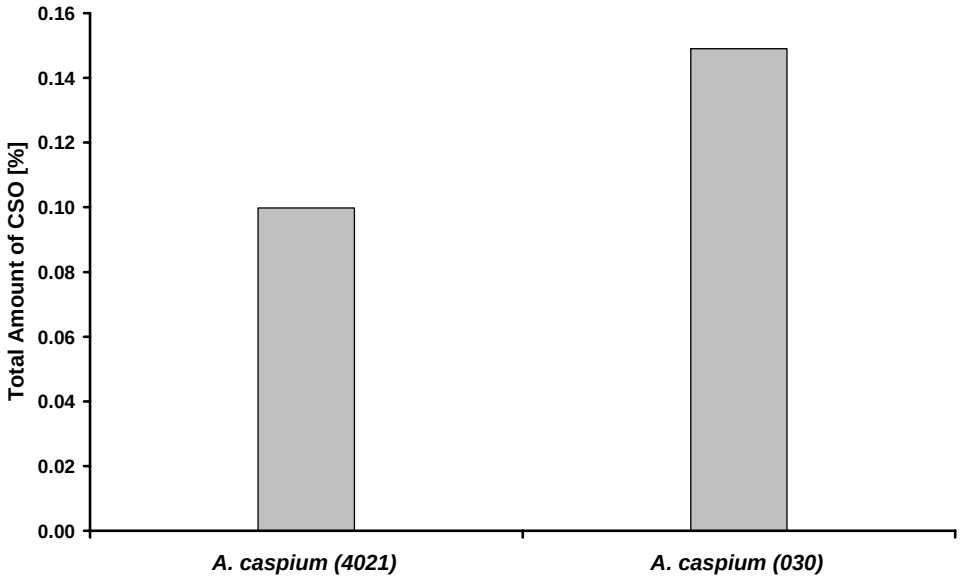


Figure 5.7: Total amounts of cysteine sulphoxides in bulbs of *A. caspium.* Concentrations are related to fresh weight (average of n=4).

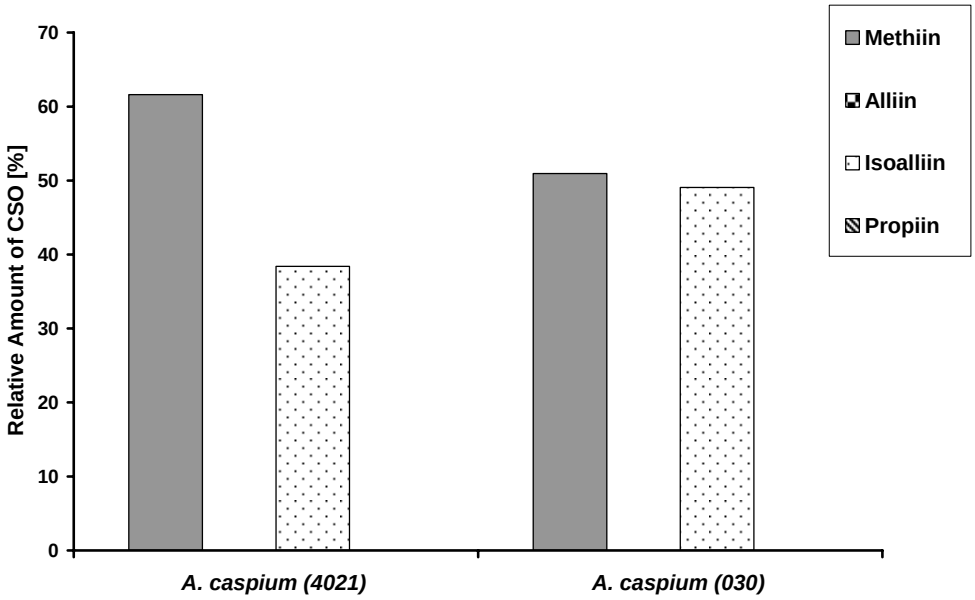


Figure 5.8: Relative amounts of cysteine sulphoxides in bulbs of *A. caspium* (total cysteine sulphoxides = 100%).

5.2.5. *Allium komarowii*

A. komarowii was the only species where the bulbs and leaves of two different Acc.-numbers were analysed. Therefore, the bulbs and leaves of this species could be compared with each other. The next two Figures 5.9 and 5.10 show the comparison of the bulbs of different origins.

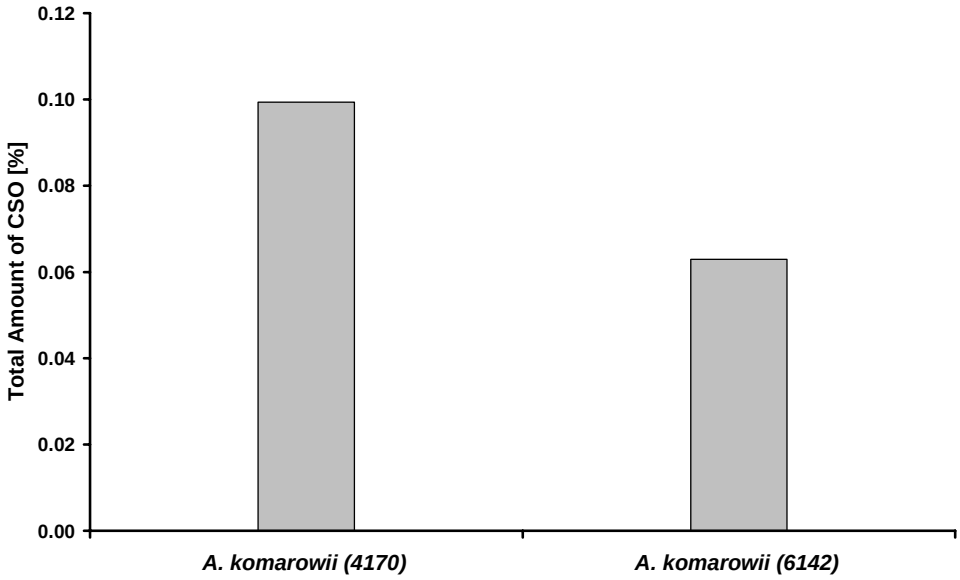


Figure 5.9: Total amounts of cysteine sulphoxides in bulbs of *A. komarowii.* Concentrations are related to fresh weight (average of n=4).

In investigated samples, the total amounts of cysteine sulphoxides were found to be relatively congruent. In bulbs of **Acc.-No. 4170** (collected in Uzbekistan), 0.1% and in bulbs of **Acc.-No. 6142** (collected in Tajikistan), 0.06% were detected. The slight dissimilarity could be probably explained by different time of collection (May and June, respectively). This plant typically grows on rubble slopes.

Interestingly, the composition of cysteine sulphoxides in investigated samples was found to be dissimilar. In the bulbs of **Acc.-No. 4170**, methiin, isoalliin, and propiin were determined, whereas in the bulbs of **Acc.-No. 6142**, methiin and isoalliin were detected. Additionally, the ratio of single flavour precursors in examined samples was significantly different. In the bulbs of **Acc.-No. 4170**, methiin was detected to be the most abundant cysteine sulphoxide (83.84%); isoalliin and propiin were found in small amounts. In the bulbs of **Acc.-No. 6142**, isoalliin was detected to be the main cysteine sulphoxide (55.12%). These curious results could not be explained by various ontogenetic stages of investigated samples. Although the bulbs of **Acc.-No. 4170** were collected at the end of May (usually full flower) and the bulbs **Acc.-No. 6142** at the end of June (usually begin of fruiting stage), both plants were found in nearly the same ontogenetic stage. Therefore, different nutrition as well as different environmental conditions were probably responsible for these divergent results.

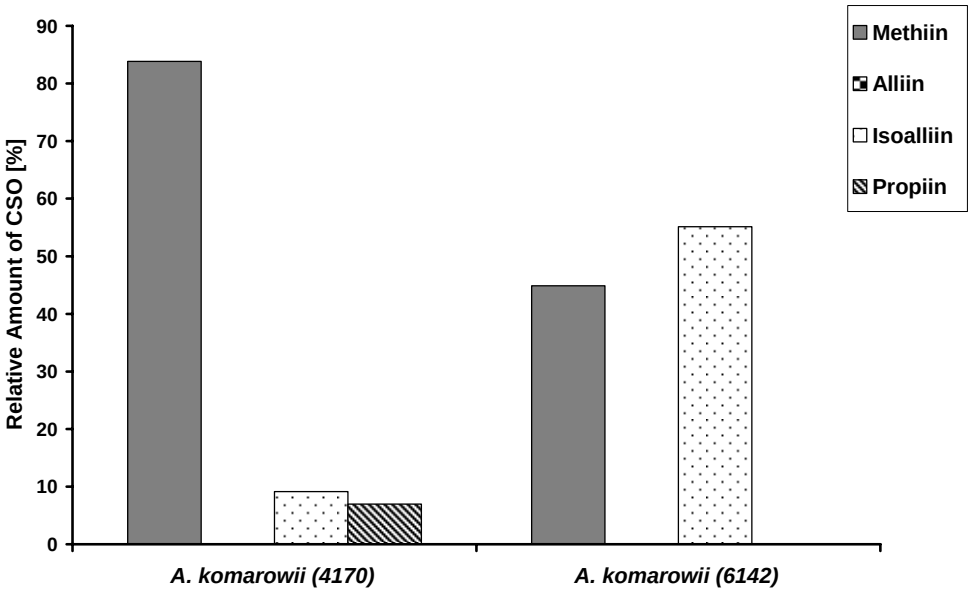


Figure 5.10: Relative amounts of cysteine sulphoxides in bulbs of *A. komarowii* (total cysteine sulphoxides = 100%).

The following two Figures 5.11 and 5.12 show the total amount as well as the composition of cysteine sulphoxides in leaves of two different samples of *A. komarowii*.

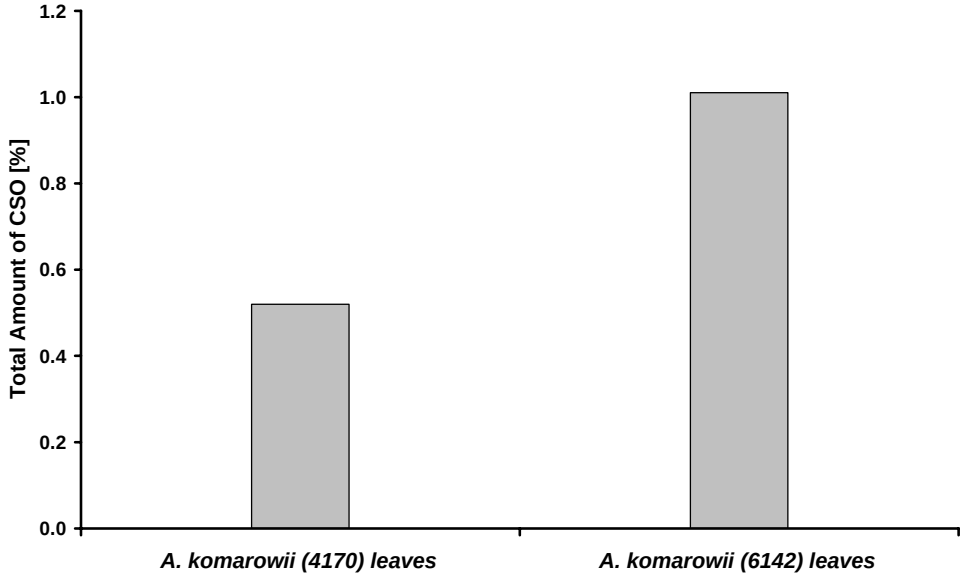


Figure 5.11: Total amounts of cysteine sulphoxides in leaves of *A. komarowii*. Concentrations are related to dry weight (average of n=4).

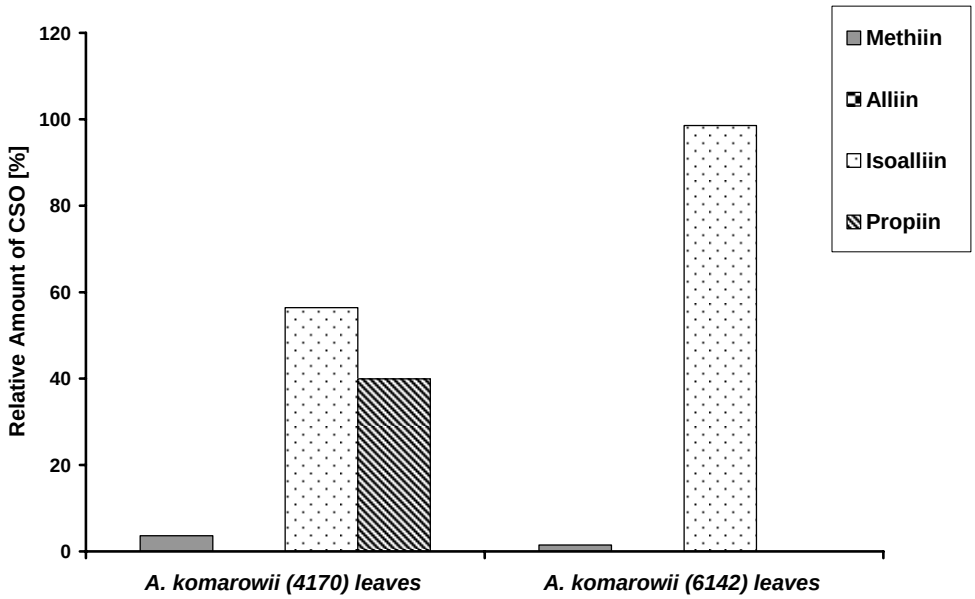


Figure 5.12: Relative amounts of cysteine sulphoxides in leaves of *A. komarowii* (total cysteine sulphoxides = 100%).

In investigated leaves of *A. komarowii*, the total amounts of cysteine sulphoxides were found to be relatively similar. Nevertheless, obtained results contrasted with results gained from investigations with the bulbs of *A. komarowii*. In the leaves of **Acc.-No. 6142**, a higher total amount (1.01%) was detected, whereas in the leaves of **Acc.-No. 4170**, a lower amount (0.52%) was found. In the bulbs, the opposite outcome was observed. In collected plants of **Acc.-No. 4170**, some plant parts were missing (some leaves and inflorescences); they were probably eaten by animals (goads; see Fig. 8.5 in the Appendix). However, plants of **Acc.-No. 6142** were found complete. Therefore, the suggestion that cysteine sulphoxides are produced to defend the plant against animals could be supported by this fact (leaves with higher amount of cysteine sulphoxides were not eaten by animals).

In examined samples, the pattern of cysteine sulphoxides was found to be different. In the leaves of **Acc.-No. 4170**, methiin, isoalliin, and propiin were detected, whereas in the leaves of **Acc.-No. 6142**, only methiin and isoalliin were found. In both cases, isoalliin was detected to be the main cysteine sulphoxide. Also a high amount of isoallin leads to a hot taste of leaves as found for **Acc.-No. 6142**. Both Acc.-numbers were easily accessible for animals**.** The plants of **Acc.-No. 6142** grew directly at the boarder of a road.

5.2.6. *Allium giganteum*

Three different samples of *A. giganteum* were compared. Plants of **Acc.-No. 4165** were collected in Uzbekistan, **Acc.-No. 6912** in Tajikistan and **Acc.-No. 1028** in Iran. The total amount of cysteine sulphoxides differed significantly (Figure 5.13); in bulbs of **Acc.-No.**

4165, a very small total amount was found (0.02%), whereas in bulbs of **Acc.-No. 6912** and **Acc.-No. 1028**, a relative high amount of flavour precursors could be determined (over 0.3%). Elucidation for these dissimilar results could give the place of collection. The bulbs of **Acc.-No. 4165** were collected at a distant place on gypsous-limestone subsoil, which is not typical for *A. giganteum*. Additionally, the plants of **Acc.-No. 4165** did not look like a typical *A. giganteum*. These plants were firstly determined as *A. majus* and later re-determined as *A. giganteum*. Furthermore, the plants of **Acc.-No. 4165** were collected in full flower, whereas the other two samples were collected in an earlier ontogenetic stage before flowering.

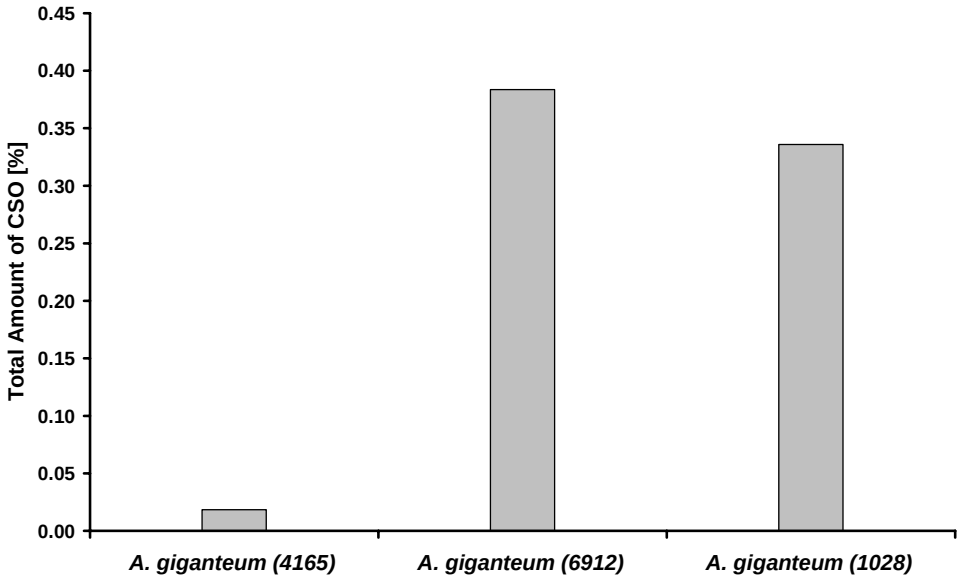


Figure 5.13: Total amounts of cysteine sulphoxides in bulbs of *A. giganteum*. Concentrations are related to fresh weight (average of n=4).

In the case of **Acc.-No. 4165** and **Acc.-No. 6912**, the composition of cysteine sulphoxides concurred rather well. In both investigated samples, methiin and isoalliin were found (Figure 5.14). In the bulbs of **Acc.-No. 6912**, methiin was determined to be the main cysteine sulphoxides (89.11%) and isoalliin was found in small amounts (10.89%). In the bulbs of **Acc.-No. 4165**, the ratio of methiin and isoalliin was found to be relatively identical. Surprisingly, methiin was found to be the only cysteine sulphoxide in the bulbs of **Acc.-No. 1028**. This plant was collected at a rather wet loamy slope in a limestone area. Storage of these bulbs made some difficulties, because the bulbs did not have a hard outer shell.

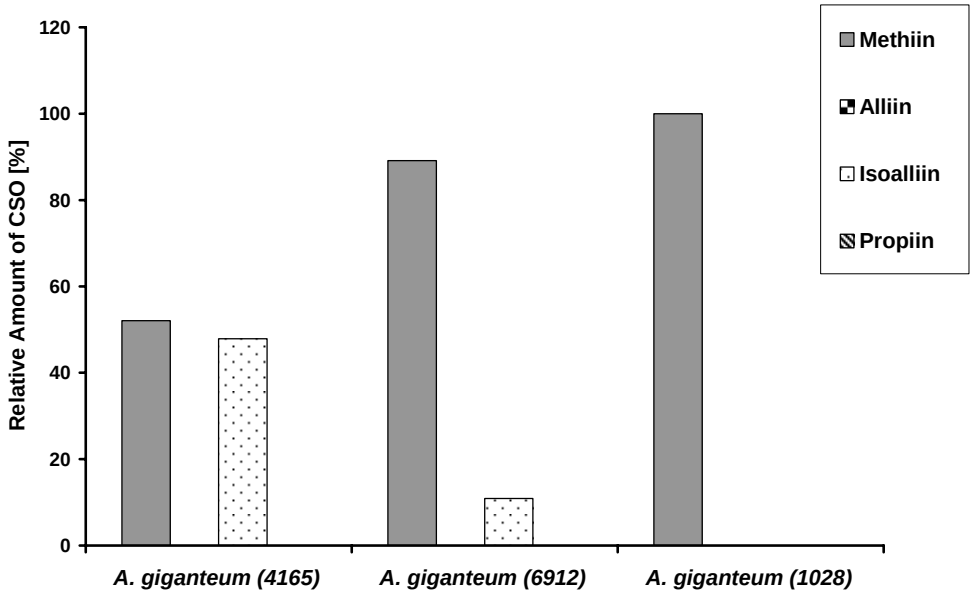


Figure 5.14: Relative amounts of cysteine sulphoxides in bulbs of *A. giganteum* (total cysteine sulphoxides = 100%).

5.2.7. *Allium pskemense*

Two of three examined samples of *A. pskemense* (**Tax-No. 0514** and **Tax-No. 1297**) were acquired from the IPK collection in Gatersleben, Germany, samples of **Acc.-No. 4142** were collected in Uzbekistan. In all three samples, the values of total amounts of cysteine sulphoxides were found to be relatively different (Fig. 5.15). In bulbs of **Acc.-No. 4142**, 0.19%, in bulbs of **Tax-No. 0514**, 0.38% and in bulbs of **Tax-No. 1297**, even 0.65% were detected. Logically, the smallest amount was found in the bulbs of **Acc.-No. 4142**, because they were collected at the place of origin and the nutrition as well as the environmental conditions there were completely different from the conditions in Germany. Additionally, the bulbs of **Acc.-No. 4142** were collected at the end of May, meaning that they were in a different ontogenetic stage then the other two investigated samples, which were collected at the end of September. It has to be mentioned, that *A. pskemense* is also cultivated in house gardens in Uzbekistan. This plant is a close relative of common onion and also used in a very similar manner.

The patterns of flavour precursors of **Acc.-No. 4142** and **Tax-No. 0514** were found to be relatively identical. Methiin, alliin, isoalliin, and propiin were detected in all samples. In both cases, isoalliin was determined to be the main cysteine sulphoxide (43.44% and 84.30%, respectively). The other flavour precursors were detected in minor amounts. In the bulbs of **Tax-No. 1297**, methiin, isoalliin and propiin were detected whereas alliin was not found (Fig. 5.16). Isoalliin was also determined to be the main cysteine sulphoxide (82.94%).

It has to be taken into account that the bulbs of *A. pskemense* were remarkably big and it was very difficult to examine representative parts of this bulb.

Schmitt [2004] investigated the total amount as well as the composition of cysteine sulphoxides in different parts of common onion. It was found out that both, the total amount and the pattern of cysteine sulphoxides, were not distributed homogenously over the bulb. *A. pskemense* is a close relative of common onion and in the case of **Tax-No. 1297**, presumably another part of the bulb was investigated as for the other, smaller samples. This could be an explanation for such dissimilar results.

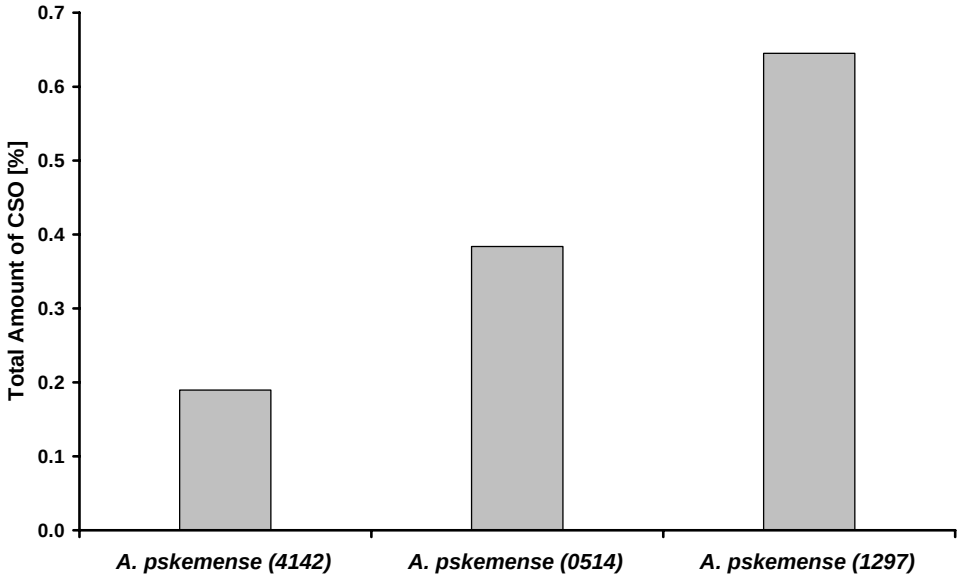


Figure 5.15: Total amounts of cysteine sulphoxides in bulbs of *A. pskemense*. Concentrations are related to fresh weight (average of n=4).

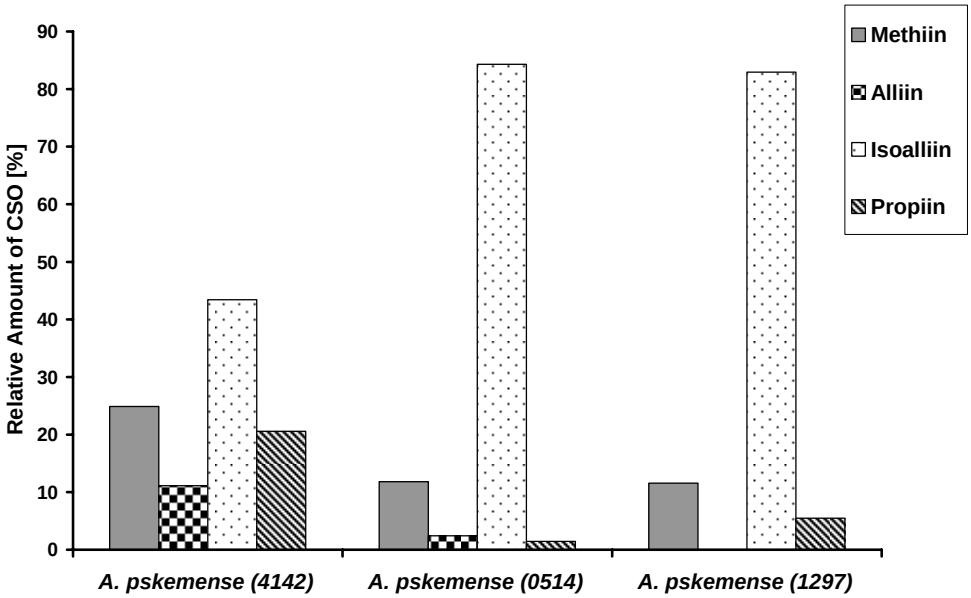


Figure 5.16: Relative amounts of cysteine sulphoxides in bulbs of *A. pskemense* (total cysteine sulphoxides = 100%).

5.2.8. *Allium suworowii*

The samples of *A. suworowii* were collected in different regions of Tajikistan. The total amounts of cysteine sulphoxides in investigated samples were found to be significantly different (Fig. 5.17). The values ranged from 0.05% to 1.82%. In bulbs of **Acc.-No. 6076** and **Acc.-No. 6090**, similar results were found (approximately 0.4%) whereas in bulbs of **Acc.-No. 6083** and **Acc.-No. 6112** completely different results were observed. In the case of **Acc.-No. 6112**, the significantly higher total amount of flavour precursors could be explained by a dissimilar ontogenetic stage because these samples were collected at the begin of July (dormancy of the plant!), whereas the other samples were collected at the end of April (begin of the flowering stage). For the remarkably small total amount in bulbs of **Acc. No. 6083** are probably environmental conditions responsible (no detailed information available).

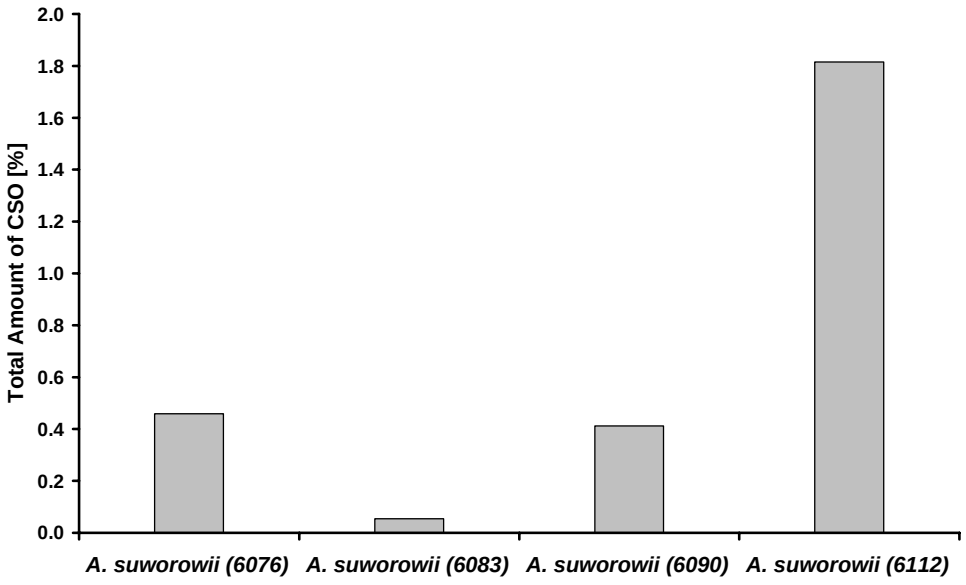


Figure 5.17: Total amounts of cysteine sulphoxides in bulbs of *A. suworowii*. Concentrations are related to fresh weight (average of n=4).

The composition of cysteine sulphoxides was found to be rather similar. In all examined samples, methiin and isoalliin were found (Fig. 5.18). In the bulbs of **Acc.-No. 6076** and **Acc.-No. 6112**, methiin was determined to be the main cysteine sulphoxide, whereas in the bulbs of **Acc.-No. 6083** and **Acc.-No. 6090**, isoalliin was found to be the dominant compound. Interestingly, the samples of **Acc. No. 6083** and **Acc.-No. 6090** were first

determined as *A. aflatunense* and *A. giganteum*, respectively (probably because of their different shapes). Later, they were re-determined as *A. suworowi*. It was told that *A. suworowii* was highly estimated by local population and was intensively collected. This can be explained by the relatively high amount of isoalliin. Later on, the plant became rare in nature and people started to collect *A. stipitatum* instead of *A. suworowii* [Keusgen 2006, personal communication]. However, *A. stipitatum* has a slightly different pattern of cysteine sulphoxides (contains higher amounts of methiin, compare Fig. 4.41).

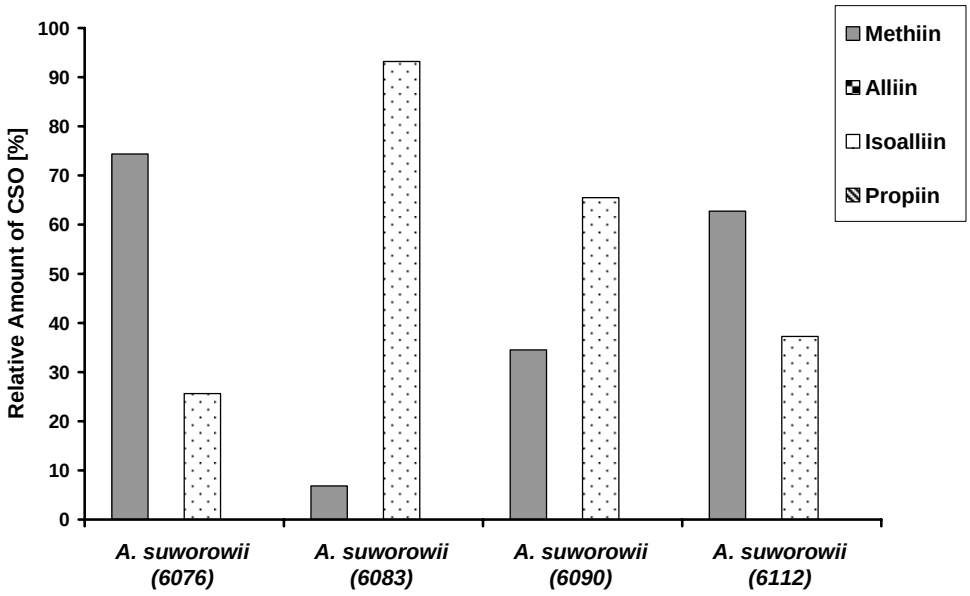


Figure 5.18: Relative amounts of cysteine sulphoxides in bulbs of *A. suworowii* (total cysteine sulphoxides = 100%).

5.2.9. *Allium jesdianum*

As in the case of *A. pskemense*, two of three examined samples (**Tax.-No. 1222** and **Tax.-No. 3666**) were acquired from the IPK collection, Gatersleben, Germany. Samples of **Acc.-No. 1033** were collected in Iran.

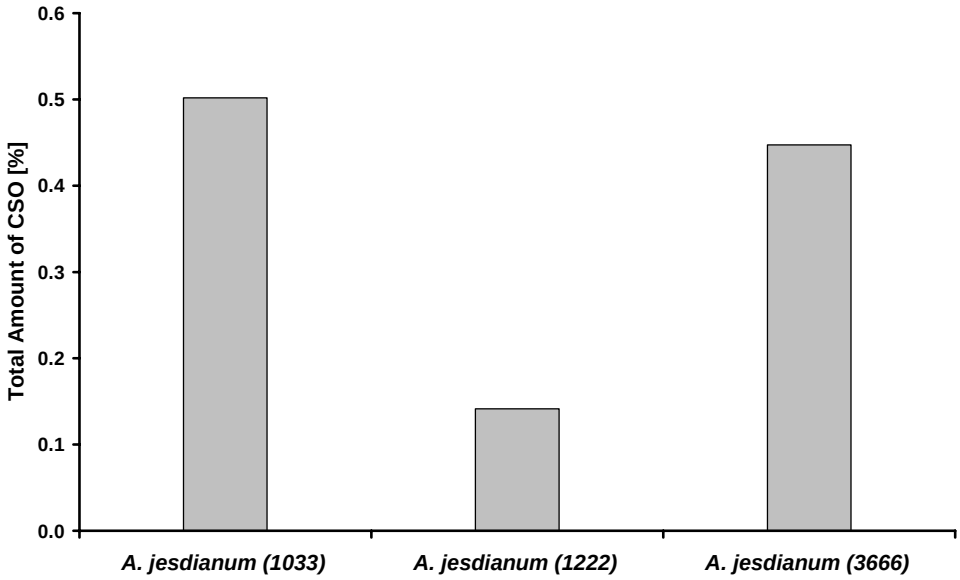


Figure 5.19: Total amounts of cysteine sulphoxides in bulbs of *A. jesdianum*. Concentrations are related to fresh weight (average of n=4).

In bulbs of **Acc.-No. 1033** and **Tax-No. 3666**, relatively identical values of total amounts of cysteine sulphoxides were found (approximately 0.50%, Fig 5.19). However, in bulbs of **Tax-No. 1222**, only 0.14% were detected. The composition of flavour precursors was found to be identical in bulbs of **Acc.-No. 1033** and **Tax-No. 3666**. In both samples, methiin and isoalliin were found. Methiin was determined to be the main cysteine sulphoxide, whereas isoalliin was found in small amounts. In the bulbs of **Tax-No. 1222**, methiin and propiin were detected. As already mentioned, the samples of **Tax-No. 1222** and **Tax-No. 3666** were obtained from the IPK collection, Gatersleben in Germany. The environmental conditions for both Tax.-numbers were presumably similar. Nevertheless, the patterns of cysteine sulphoxides were found to be different. Samples of **Tax-No. 1222** were collected in year 2003 whereas samples of **Tax-No. 3666** were collected in year 2004. Thus, weather conditions may have an influence on the pattern of cysteine sulphoxide. It has to be also taken into account that the **Tax-No. 1222** is probably a different subspecies of *A. jesdianum* or even a different species. As also demonstrated in these investigations, the total amount of cysteine sulphoxides sometimes showed huge intra-specific variations. In contrast, the pattern of cysteine sulphoxides is much more stable (Fig. 5.20). Because **TaxNo. 1222** contained propiin instead of isoalliin, this plant is significantly different from the other two plants.

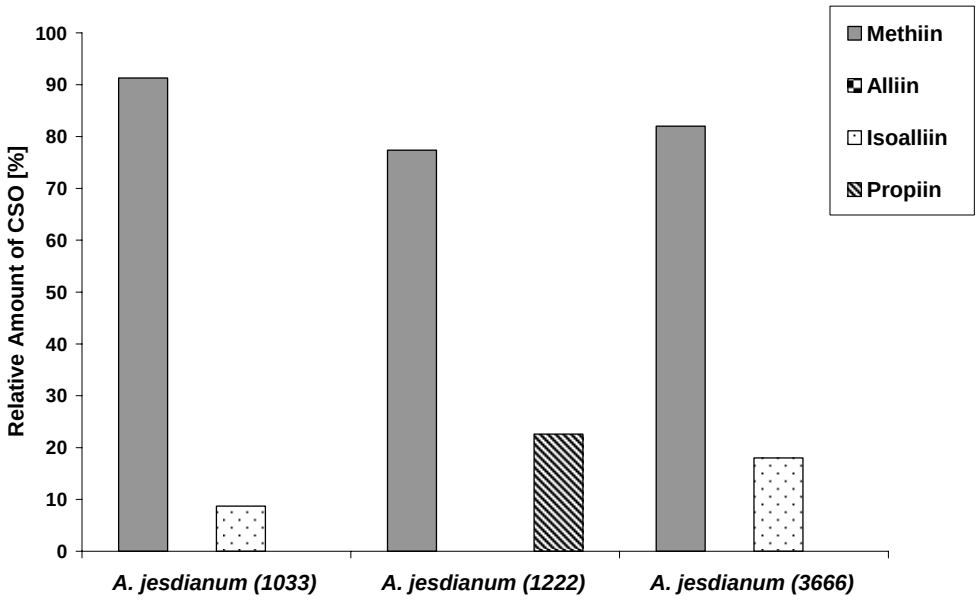


Figure 5.20: Relative amounts of cysteine sulphoxides in bulbs of *A. jesdianum* (total cysteine sulphoxides = 100%).

5.2.10. *Allium aflatunense*

Samples of **Acc.-No. 4101** (*A. aflatunense*) were collected in Uzbekistan, whereas samples of **Tax-No. 2657** were obtained from the IPK collection in Gatersleben, Germany. The values of the total amount of cysteine sulphoxides were found to be remarkably congruent; in both samples, approximately 0.6% cysteine sulphoxides were found (Fig. 5.21).

In investigated samples, both methiin and isoalliin were detected. In the bulbs of **Acc.-No. 4101**, methiin was determined to be the most abundant cysteine sulphoxide and isoalliin was found in small amounts. In the case of **Tax.-No. 2657**, isoalliin was found to be the main flavour precursor and methiin was detected in small amounts (Fig. 5.22). Such unequal results could be explained by different environmental conditions of investigated samples. The plants collected in Uzbekistan were exposed to a completely different climate in comparison to the plants cultivated in Germany. Additionally, the samples of **Acc.-No. 4101** were collected in an early ontogenetic stage (at the beginning of June), whereas the samples of **Tax-No. 2657** were collected in a late ontogenetic stage (at the end of September).

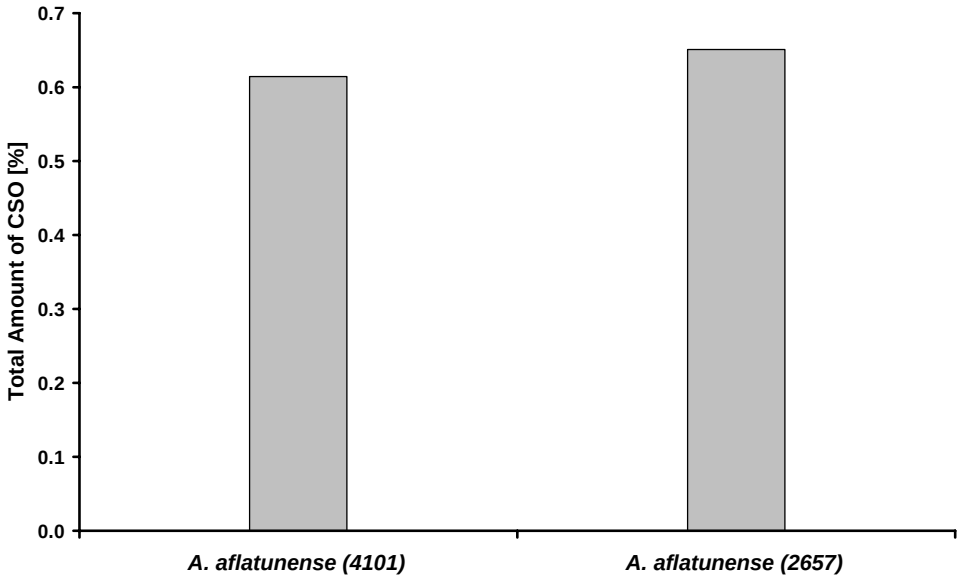


Figure 5.21: Total amounts of cysteine sulphoxides in bulbs of *A. aflatunense*. Concentrations are related to fresh weight (average of n=4).

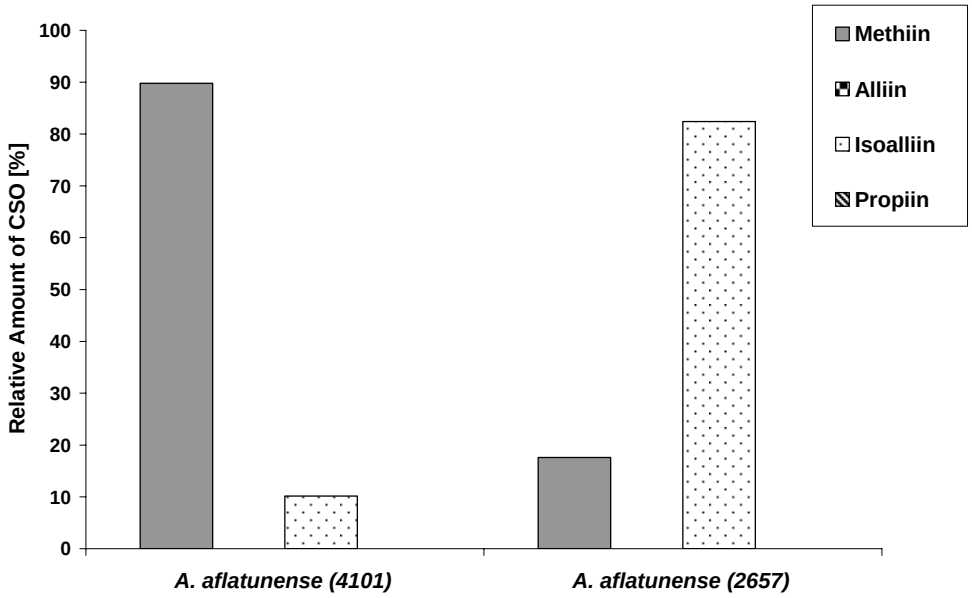


Figure 5.22: Relative amounts of cysteine sulphoxides in bulbs of *A. aflatunense* (total cysteine sulphoxides = 100%).

5.2.11. *Allium filidens*

Compared samples of *A. filidens* were collected in Uzbekistan (**Acc.-No. 4144**) and in Tajikistan (**Acc.-No. 6164**, Fig. 5.23).

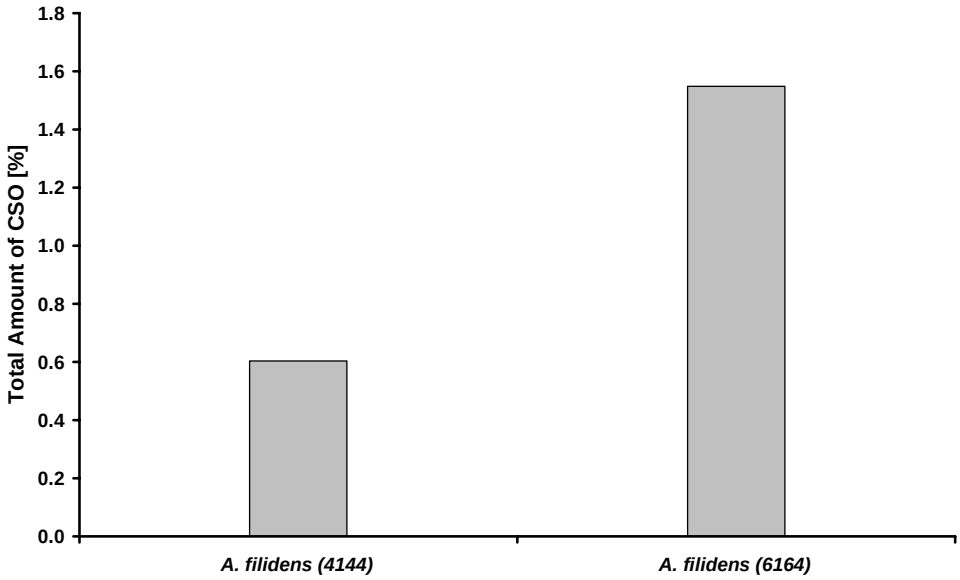


Figure 5.23: Total amounts of cysteine sulphoxides in bulbs of *A. filidens*. Concentrations are related to fresh weight (average of n=4).

The total amount of cysteine sulphoxides of **Acc.-No. 6164** was found to be more as double as high as that of **Acc.-No. 4144** (0.60 % and 1.55 %, respectively). Interestingly, **Acc.-No. 6164** was collected in a garden in the surrounding of a village. An *Allium* species leading such a high amount of cysteine sulphoxides could be interesting for cultivation. Initially, the taxonomic name of this species could not be determined. Then, the name *A. filidens* ssp. *mogianense* R.M. Fritsch et F.O. Khass. was given for **Acc.-No. 6164**.

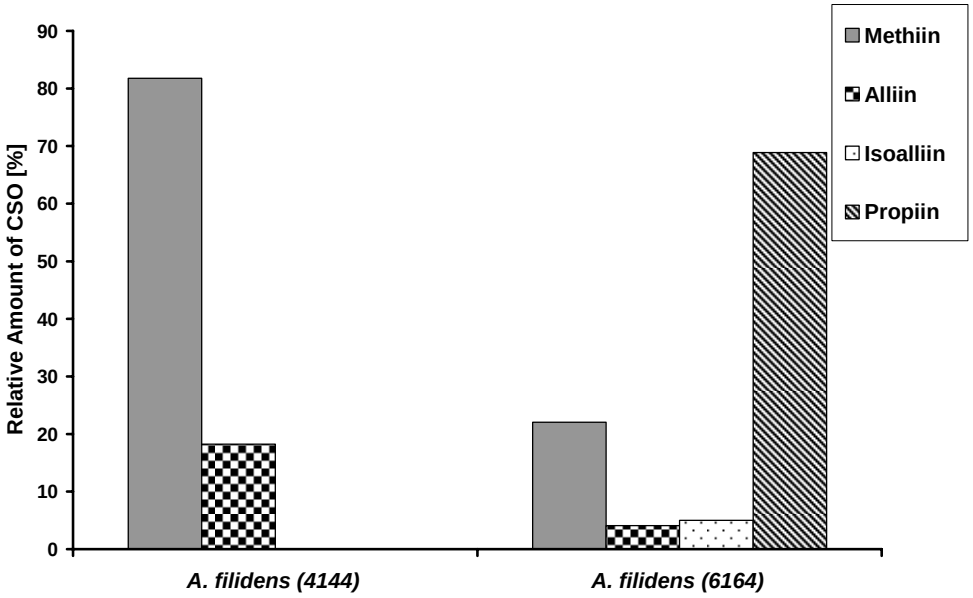


Figure 5.24: Relative amounts of cysteine sulphoxides in bulbs of *A. filidens* (total cysteine sulphoxides = 100%).

Comparison of the relative amounts of cysteine sulphoxides gave significant variations between both samples (Fig. 5.24). Methiin (81.79 %) besides alliin was the most abundant for **Acc.-No. 4144**. This distribution of cysteine sulphoxides can be typically expected for the subgenus *Allium* to which *A. filidens* belongs. In contrast, **Acc.-No. 6164** showed all four cysteine sulphoxides where propiin was found to be the main constituent (68.87 %). Propiin is also usual for the subgenus *Allium*. However, such large amounts are unique. The **Acc.-No. 6164** is therefore presumably not *A. filidens* in a broad sense. These findings demonstrate that chemical composition may contribute to the final determination of plant material. The botanical name of **Acc.-No. 6164** has to be reconsidered.

5.2.12. *Allium ophiophyllum*

The plant samples of **Acc.-No. 4155** (*A. ophiophyllum*) were collected in Uzbekistan and of **Acc.-No. 082** in Turkmenistan (Fig 5.25). The values of the total amounts of cysteine sulphoxides were found to be relatively similar. In bulbs of **Acc.-No. 4155**, 0.039% and in bulbs of **Acc.-No. 082**, 0.084% were found.

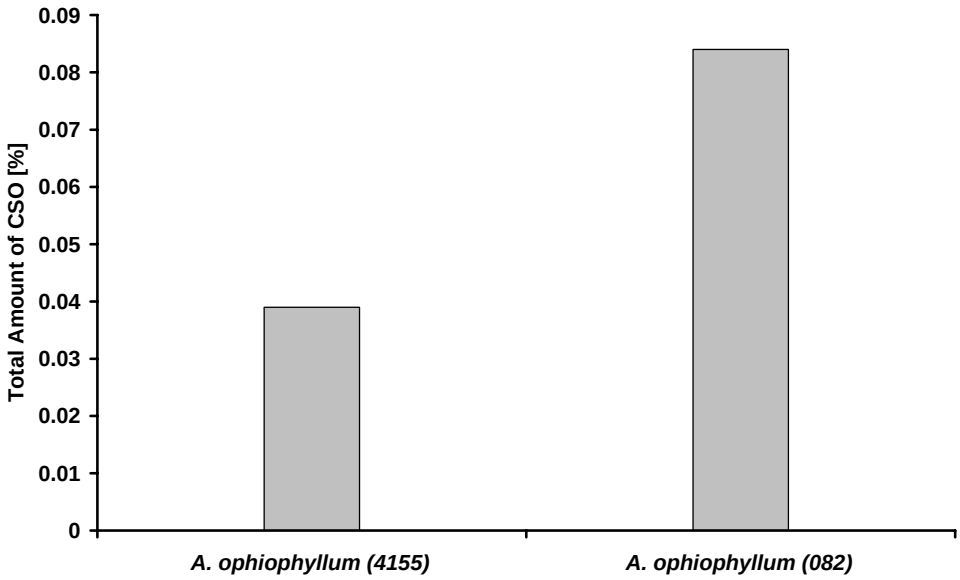


Figure 5.25: Total amounts of cysteine sulphoxides in bulbs of *A. ophiophyllum*. Concentrations are related to fresh weight (average of n=4).

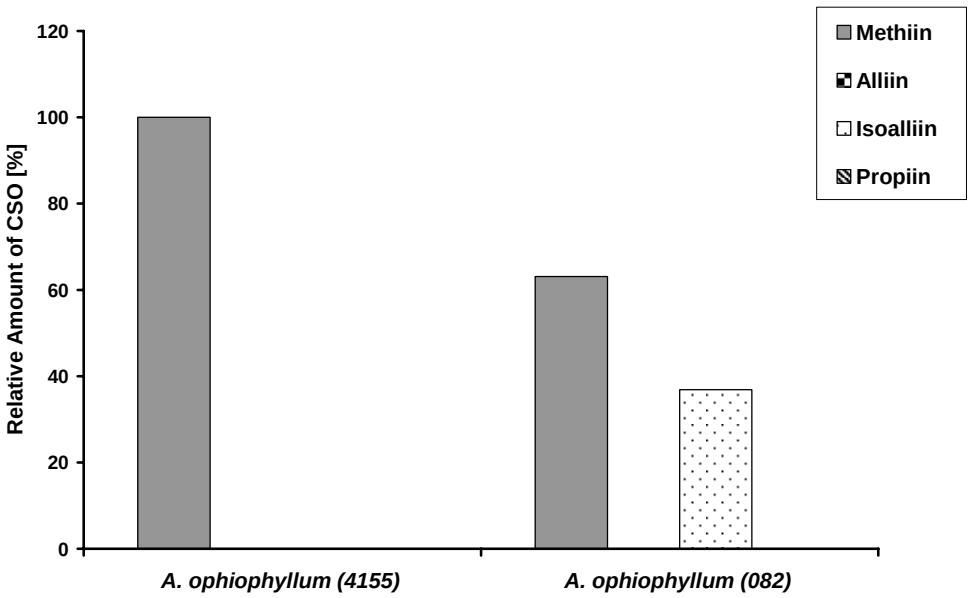


Figure 5.26: Relative amounts of cysteine sulphoxides in bulbs of *A. ophiophyllum* (total cysteine sulphoxides = 100%).

The composition of cysteine sulphoxides was found to be rather different (Fig. 5.26). In investigated samples obtained from **Acc.-No. 4155**, only methiin was detected whereas in samples obtained from **Acc.-No. 082** methiin and isoalliin were found. This dissimilarity could be probably explained by different ontogenetic stage of investigated plants. Samples of **Acc.-No. 4155** were collected in July at the end of flowering whereas samples of **Acc.-No. 082** were collected in earlier ontogenetic stage (in April). In this case, altitude does not play any role; both samples were collected at a nearly identical altitude (approximately 650 meters).

5.2.13. *Allium cristophii*

Compared samples of *A. cristophii* were collected in Iran (**Acc.-No. 1016)** and in Turkmenistan (**Acc.-No. 011**). Both samples were found at an altitude of approximately 1700 meters and they grew under nearly identical conditions. The total amount of cysteine sulphoxides was found to be relatively congruent (Fig. 5.27). In bulbs of **Acc.-No. 1016**, 0.34% and in bulbs of **Acc.-No. 011**, 0.20% were detected.

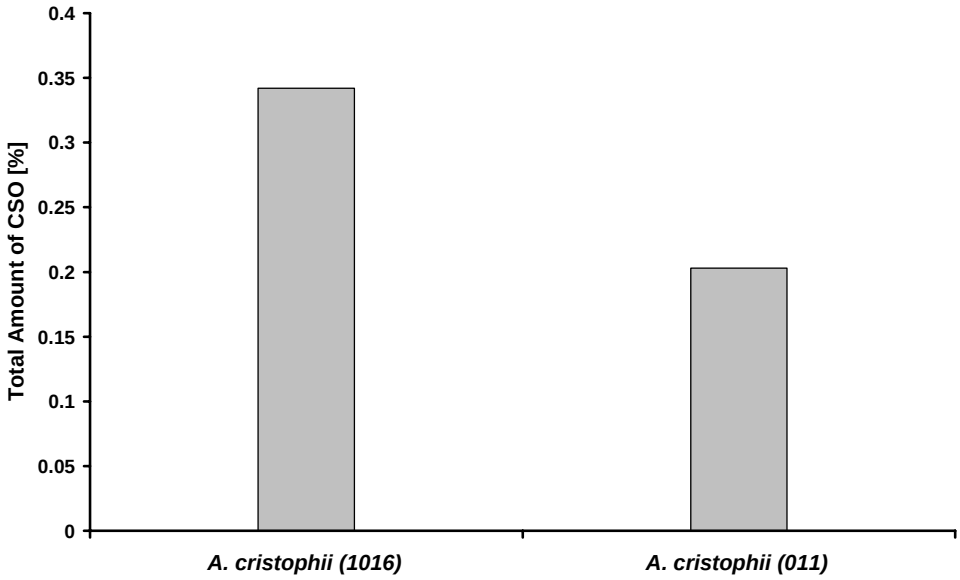


Figure 5.27: Total amounts of cysteine sulphoxides in bulbs of *A. cristophii*. Concentrations are related to fresh weight (average of n=4).

In the investigated samples, different patterns of cysteine sulphoxides were observed. In the samples obtained from **Acc.-No. 1016**, methiin and isoalliin were found to be the most abundant cysteine sulphoxides (71.35% and 25.73%, respectively). Alliin was found in trace amounts and propiin could not be detected. In the samples obtained from **Acc.-No. 011**, methiin and propiin were the most abundant cysteine sulphoxides (84.24% and 15.27%, respectively). Isoalliin was detected in trace amounts and alliin could not be detected (Fig. 5.28). As already mentioned, investigated plants grew under nearly identical conditions. Therefore, there must be another explanation for such dissimilarities.

The plants of **Acc.-No. 011** did not look like a typical *A. cristophii* at the place of collection in Turkmenistan. These plants were firstly determined as *A. eugenii* and later re-determined as *A. cristophii*. Therefore, it is possible that **Acc.-No. 011** is not *A. cristophii* or it belongs to a different subspecies. The botanical name of **Acc.-No. 011** should be reconsidered.

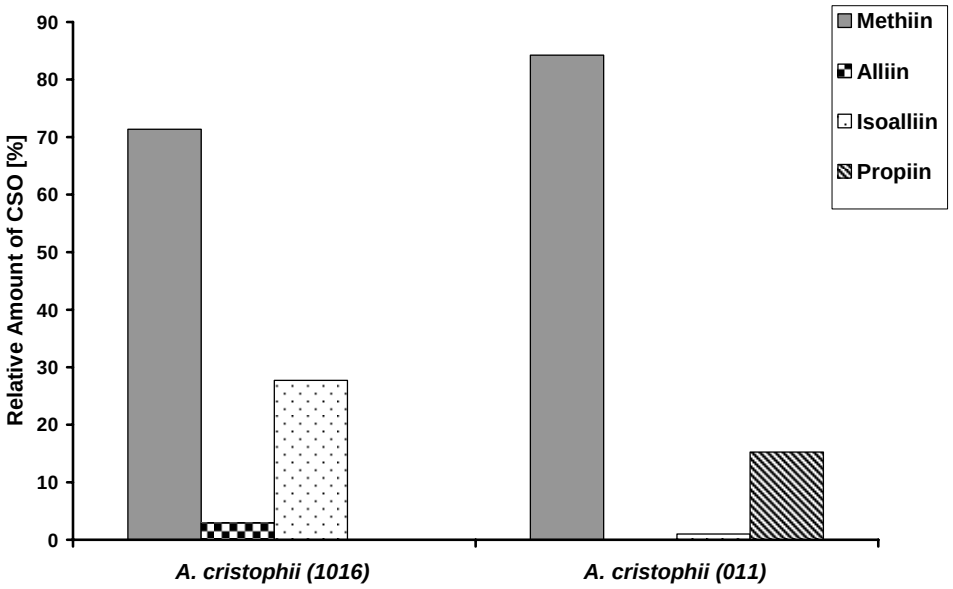


Figure 5.28: Relative amounts of cysteine sulphoxides in bulbs of *A. cristophii* (total cysteine sulphoxides = 100%).

5.3. *Categorization of Wild Allium Species According to Altitude*

Wild *Allium* species need specific conditions for their growth. Some of them grow better at dry places others at wet places and also altitude plays an important role. But as a general rule, most species need a certain amount of water. Places not even having sufficient precipitation in winter and spring are rare in *Allium* species. There are only a few species, which are adapted to dessert or to semi-dessert climate.

Investigation showed that some of the wild *Allium* species could be found at a certain altitude *e.g.*, *A. carolinianum* grows mainly at an altitude above 2500 meters and *A. caspium* typically below 500 meters [Keusgen 2006, personal communication]. The following figures are categorized according to the altitude (below 1000 meters, 1000-2000 meters and above 2000 meters). The wild *Allium* species listed on the X-axis are lined up in order to increasing humidity of the natural place of growth. Furthermore, the relative amount of cysteine sulphoxides is depicted to find possible correlations between the increase in humidity as well as in the altitude and the cysteine sulphoxide composition. It has to be mentioned that only species collected in the wild with known place (ecology) and altitude of collection were respected in this investigation.

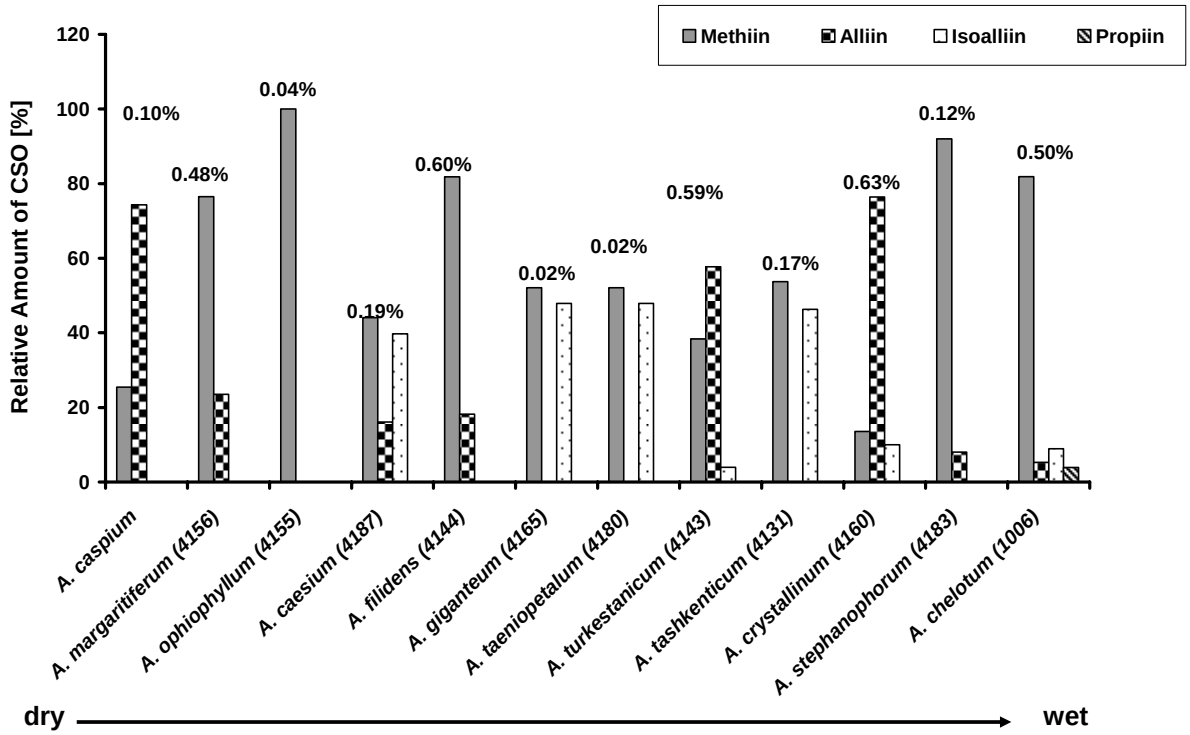


Figure 5.29: Relative amounts of cysteine sulphoxides in bulbs of wild *Allium* species, which grew at an altitude below 1000 meters (total cysteine sulphoxides = 100%). Species are lined up in order to increasing humidity.

In the Figure 5.29, *Allium* species collected below an altitude of 1000 m are summarized. On the left side of the graph, species collected at dry places are given (*e.g.*, desert). The humidity of the place of collection increased from the left to the right side of the graph. For instance, *A. chelotum* was collected at shady places in a wet forest.

All depicted species show a huge variety in their absolute amount and relative composition of cysteine sulphoxides. There are only weak trends visible concerning the relative composition of cysteine sulphoxides. Species collected at more dry places and at medium dry places display the highest variety in cysteine sulphoxides (*e.g.*, *A. caesium*, *A. turkestanicum*, *A. crystallinum*), whereas for species collected at wet places methiin is the dominant cysteine sulphoxide *(e.g.*, *A. stephanophorum*, *A. chelotum*). However, it has to be taken into account, that species belonging to the subgenus *Allium* are concentrated in the middle part of the Figure 5.29. The membership of a species to different subgenera might have a stronger influence on the pattern of cysteine sulphoxides than environmental conditions (for details see below).

In the next two Figures 5.30 and 5.31, the composition of cysteine sulphoxides of *Allium* species growing between 1000 m and 2000 m is depicted. *Allium* species at drier places show a huge variety of different cysteine sulphoxides. Interestingly, the absolute

amount of cysteine sulphoxides increases from the left (dry places) to the right (semi-dry places) of the Figure 5.30, which means that a certain amount of water is probably necessary for a sufficient production of these compounds. The highest amounts were found in samples obtained from *A. suworowii* (1.81%), which was collected at a southwest exposed grassy slope with some trees. This place has nearly full sun during summertime (hot and dry), but sufficient humidity during the rest of the year.

The *Allium* species collected between 1000 m and 2000 m at more wet places, do all have methiin as the main cysteine sulphoxide (Figure 5.31). But again, most of theses species belong to the same subgenus *Melanocrommyum*. In conclusion, the membership to a certain subgenus might have a bigger influence on the composition of cysteine sulphoxides than the place of growth.

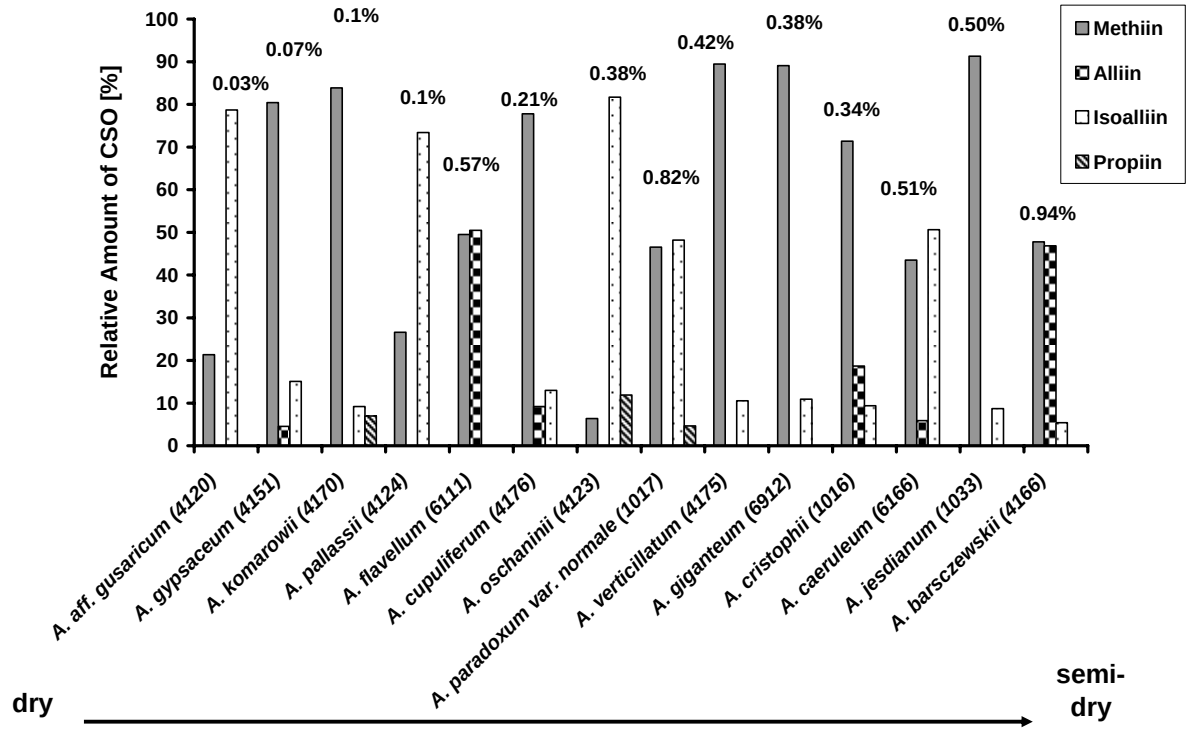


Figure 5.30: Relative amounts of cysteine sulphoxides in bulbs of wild *Allium* species, which grew at an altitude between 1000 and 2000 meters (total cysteine sulphoxides = 100%). Species are lined up in order to increasing humidity (here from dry to semi-dry).

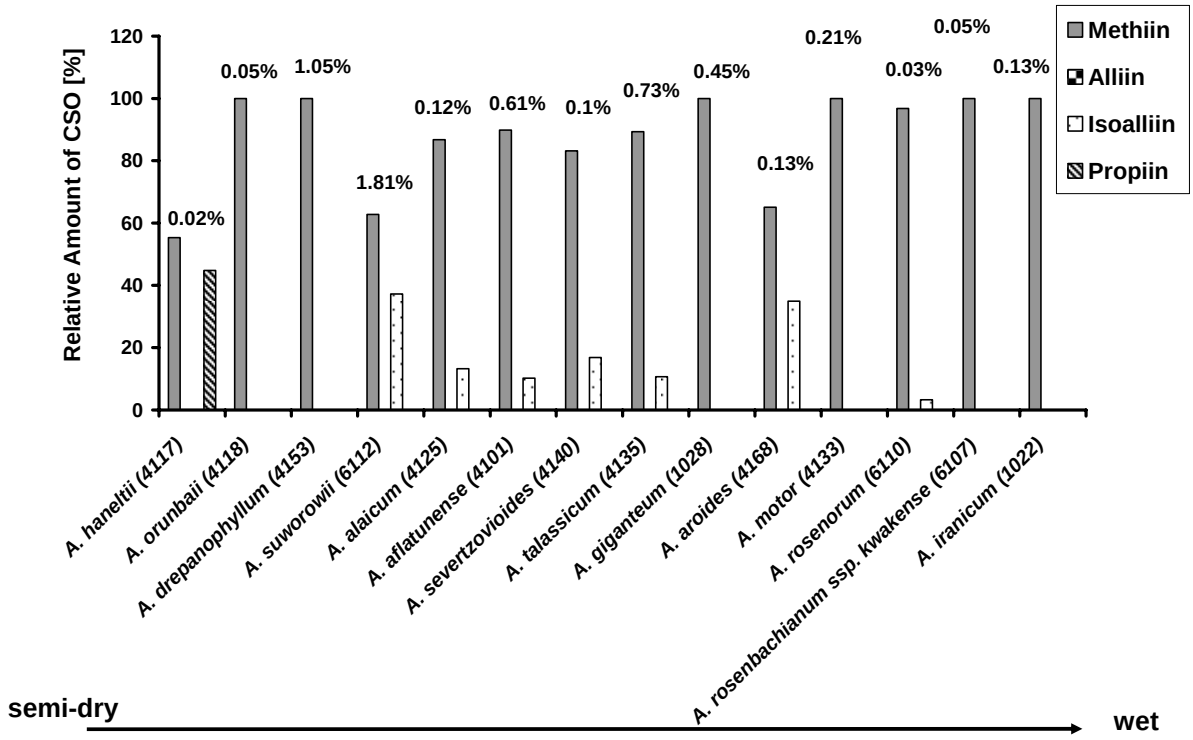


Figure 5.31: Relative amounts of cysteine sulphoxides in bulbs of wild *Allium* species, which grew at an altitude between 1000 and 2000 meters (total cysteine sulphoxides = 100%). Species are lined up in order to increasing humidity (here from semi-dry to wet).

In the next Figure 5.32, the composition of cysteine sulphoxides of species collected above 2000 m is depicted. At this altitude, no *Allium* species at really dry places could be found. With the exception of *A. oschaninii* and *A. komarowii*, which typically grow on rubble slopes, all other species contain methiin as main cysteine sulphoxide. The given species belong to a number of different subgenera (*e.g.*, *Cepa*, *Melanocrommyum*, *Polyprason*).

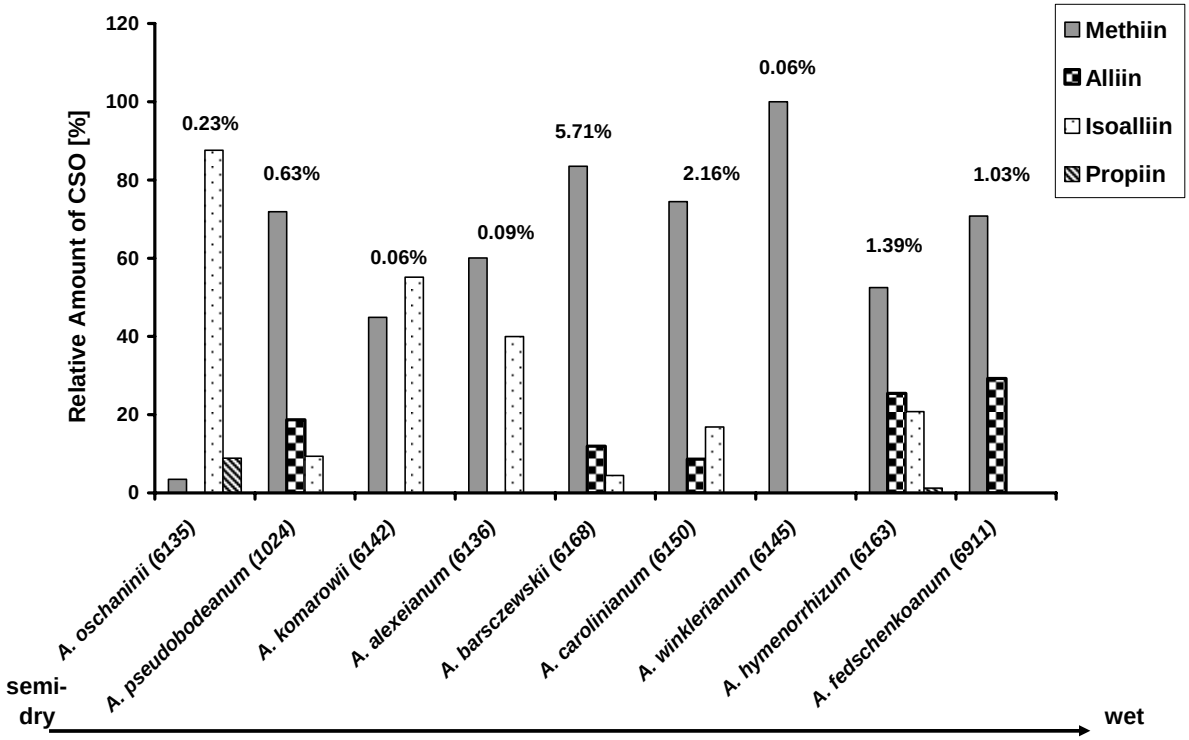


Figure 5.32: Relative amounts of cysteine sulphoxides in bulbs of wild *Allium* species, which grew at altitude above 2000 meters (total cysteine sulphoxides = 100%). Species are lined up in order to increasing humidity (here from semi-dry to wet).

The results listed above lead to the assumption that there is a possible correlation between humidity and altitude regarding certain subgenera. This correlation is depicted in the next Figure 5.33. For the most important subgenera, circles mark centres of distributions. Generally, *Allium* does not like dry habitats in altitudes higher than 2000 m and wet habitats in low lands. Most species grow between 500 m and 2000 m at semi-dry places.

It is clearly visible, that the subgenus *Melanocrommyum* is probably best adapted to the climate and biotopes of Central Asia, where it is dominant. This subgenus can be found even at very dry places at low altitudes (*A. caspium*) as well as at wet places at high altitudes (*A. winklerianum*). Most of the species prefer more humid places at altitudes between 1000 m and 2000 m.

In contrast, the subgenus *Allium* has more specific growth conditions. All listed species were found between an altitude of 500 and 2000 m. Species of this subgenus most likely prefer dry places with sufficient precipitation in winter and spring. The subgenus *Reticulatobulbosa* can be found at similar, but somewhat drier places. This subgenus also can be found at altitudes between 2000 m and 2500 m. The subgenus *Polyprason* was typically found at altitudes higher than 1500 m and at places, which are more humid as those, which were preferred by the subgenus *Allium*. There is only one exception

(*A. ophiophyllium*), which was found on pestrotsvet soil (rather salty and dry) in Uzbekistan. However, this species was formerly a member of the subgenus *Allium*, which would perfectly fit to typical biotopes of this subgenus [Friesen et al. 2006]. For the subgenus *Cepa*, only two species could be respected in this investigation (*A. oschaninii* and *A. fedschenkoanum*). A tendency that plants growing at higher altitudes need more humidity could be observed.

In conclusion, subgenera seem to have a bigger influence on the pattern of cysteine sulphoxides (see chapter "Chemotaxonomy" below) than specific biotopes. However, the best growth conditions leading to the highest amounts of cysteine sulphoxides are below 2000 m at semi-dry places in full sun.

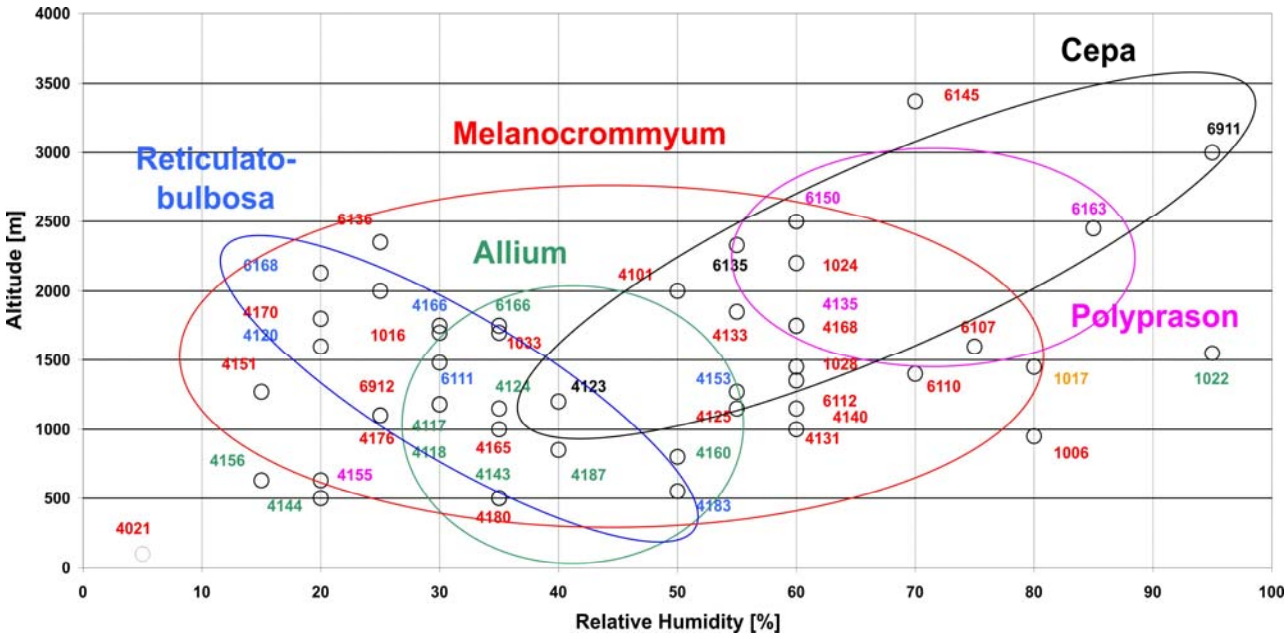


Figure 5.33: Correlation between relative humidity (biotope at the place of collection) and altitude of collected wild *Allium* species. Individual Acc.-numbers are marked by spots. The relative humidity was given according to the following list of biotopes: desert (5%), rock and rocky slope (5-25%), granitic soil (30-35%), limestone with grass (35-40%), grass (40-50%), perennials (50-60%), shrubs and trees (60-70%), forest (70-80%), river, bog (80-95%).

5.4. *Chemotaxonomy and Pharmaceutical Use of Certain Subgenera and Sections*

As mentioned in Chapter 1.1.2., genus *Allium* is taxonomically divided into several subgenera, which are again divided into sections. For the following discussion, the classification of Friesen et al. [2006] was used. Wild *Allium* species investigated in this work belonged to the subgenera *Allium*, *Reticulatobulbosa*, *Polyprason*, *Cepa*, *Amerallium* and

Melanocrommyum. Furthermore, the relative amount of cysteine sulphoxides (pattern) is shown and correlations between the composition of cysteine sulphoxides and the taxonomic classification will be discussed. Also possible medicinal used is discussed in this section.

5.4.1. *Allium Species Belonging to the Subgenus Allium*

Over 300 species belong to subgenus *Allium* (one typical representative is garlic). Subgenus *Allium* is further divided into 15 sections. In the first Figure 5.34 below, species belonging to various sections of subgenus *Allium* are presented, in the second Figure 5.35, species belonging to section *Allium* L. are shown.

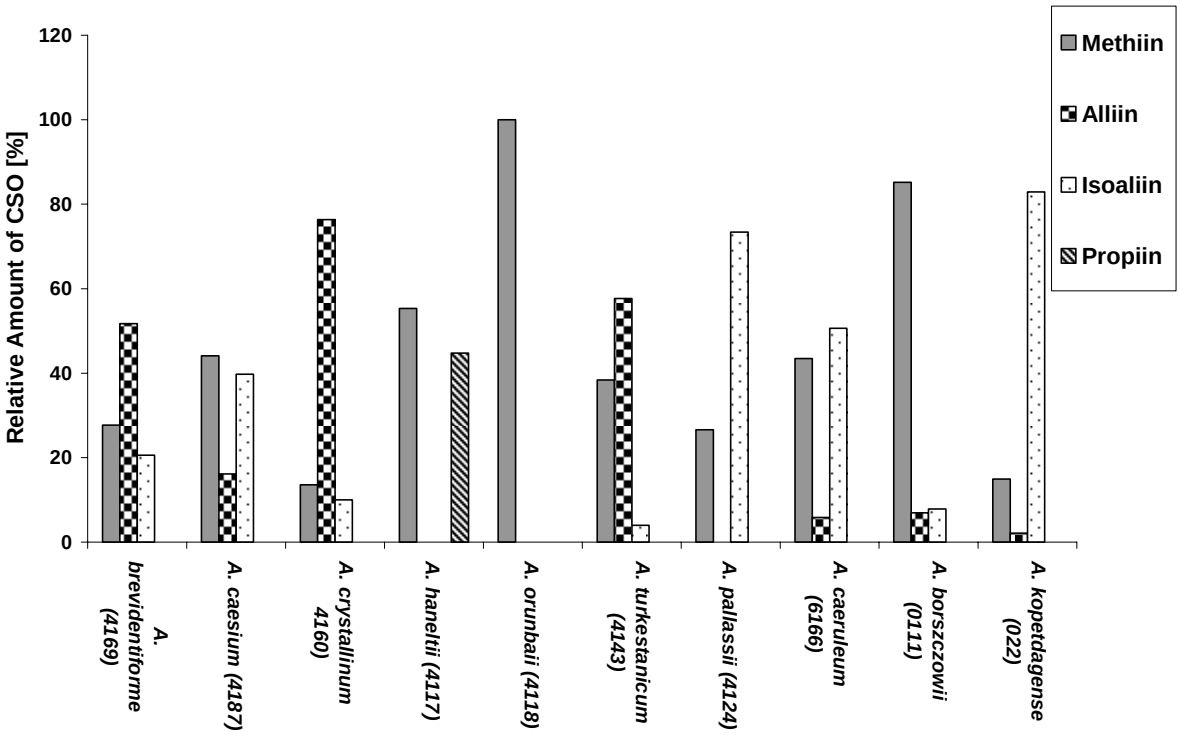


Figure 5.34: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Allium* (total cysteine sulphoxides = 100%).

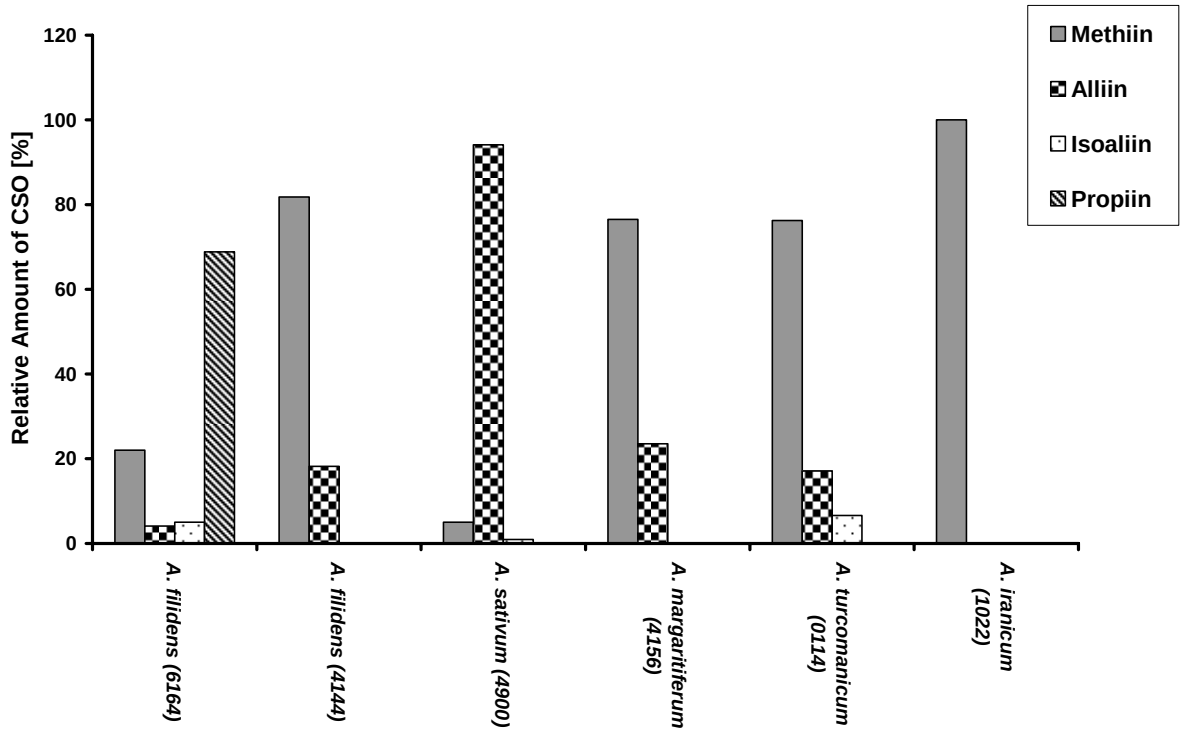


Figure 5.35: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Allium* sect. *Allium* (total cysteine sulphoxides = 100%).

Methiin was detected in all investigated species. Several species contained alliin and isoalliin, but in strongly varying concentrations (see *A. brevidentiforme* and *A. sativum*). Typically, only one of these three cysteine sulphoxides dominated and the other were found in small amounts. Propiin occurred only in two cases, but in remarkably high amounts in bulbs of *A. haneltii* 44.76% and in bulbs of *A. filidens* (Acc.-No. 6164) 68.87%. In the case of *A. iranicum*, methiin was the only cysteine sulphoxide found. This is not typical for species belonging to subgenus *Allium* sect. *Allium*. For this section, typically high amounts of methiin are combined with small amounts of alliin. The explanation for such different pattern can be given through the place of collection. *A. iranicum* was collected at a very wet place, which is not a typical location of this species. Furthermore, collected plants were very young. Therefore, the relative amount of cysteine sulphoxides was presumably different from plants, which had already finished their ontogenesis [Randle et al 1993; Schmitt 2004]. In bulbs of *A. filidens* (Acc.-No. 6164) and *A. sativum*, the pattern of cysteine sulphoxides were found to be untypical for the section *Allium*. Bulbs of A. *filidens* (Acc.-No. 6164) contained small amounts of methiin, trace amounts of alliin and isoalliin and a significantly high amount of propiin. Bulbs of wild *A. sativum* contained remarkably high amounts of alliin and trace amounts of methiin and isoalliin. It has to be mentioned that both species were probably used by native populations.

The values of total cysteine sulphoxides ranged between 0.02% (bulbs of *A. haneltii*) and 1.69% (bulbs of wild *A. sativum*). Remarkably high amounts were detected in bulbs of *A. filidens* Acc.-No. 6164 (1.55%) and *A. turcomanicum* (1.07%).

In summary, the subgenus *Allium* showed high variations in the pattern of cysteine sulphoxides. In addition, occurrence of alliin and isoalliin is high in this subgenus. Both cysteine sulphoxides are mainly responsible for the spicy taste and health benefits of *Allium*. Therefore, a number of species belonging to this subgenus might be considered as medicinal plant as well as a "natural" substituent for garlic and leek. Remarkable are *A. brevidentiforme*, *A. crystallinum*, *A. turkestanicum*, *A. caeruleum* and *A. kopetdagense*. A*. iranicum* is also estimated by native population and has to be re-investigated. This species is closely related to *A. ampeloprasum*.

Species leading high amounts of methiin can be considered as anti-diabetic drugs [Keusgen 2002a]. However, it must be taken into consideration, that methiin is also converted by alliinase activity if plant material is disrupted. Therefore, species rich in methiin seem to be of less medicinal value. Methiin could be found in nearly all *Allium* species in more or less high amounts.

5.4.2. *Allium Species Belonging to the Subgenus Reticulatobulbosa*

Eight of the investigated species belonged to subgenus *Reticulatobulbosa.* This subgenus contains approximately 80 species. All investigated species belonged to section *Campanulata* Kamelin. In all examined plants methiin was found (Fig.5.36). Its relative concentrations were mostly higher than 50%. Apart from *A. drepanophyllum* and *A. aff. gusaricum*, all species contained alliin. Isoalliin dominated only in bulbs of *A. aff. gusaricum*; in other cases, it was either not detected or detected in trace amounts. In none of examined species, propiin was found. The highest value of total cysteine sulphoxides was found in bulbs of *A. barsczewskii* Acc.-No. 6168 (5.71%) and the lowest value was detected in bulbs of *A. aff. gusaricum* (0.03%). Remarkably high amounts were also found in bulbs of *A. jodanthum* (1.54%) and *A. drepanophyllum* (1.05%).

Plants leading high amounts of alliin and isoalliin can be also considered as medicinal plants. However, bulbs of these plants are usually very slender and hard. They easily dry out during storage and will get a wooden consistence. This might be the reason, that subgenus is seldomly used by native populations.

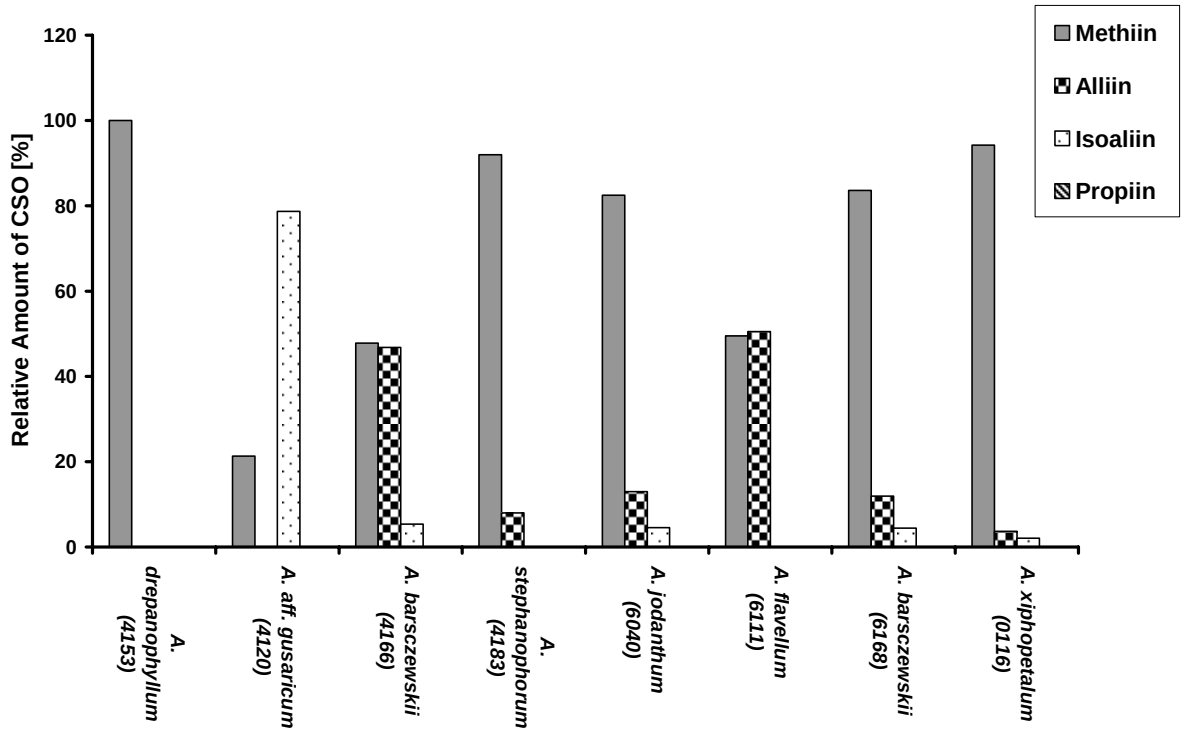


Figure 5.36: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Reticulatobulbosa* (total cysteine sulphoxides = 100%).

5.4.3. *Allium Species Belonging to the Subgenus Polyprason*

The subgenus *Polyprason* contains approximately 50 species, which are divided into 4 sections. In all examined samples, methiin was determined to be the main cysteine sulphoxide and alliin as well as isoalliin were found in small concentrations. Bulbs of *A. hymenorrhizum* contained also propiin, but only in trace amounts. The values of total cysteine sulphoxides ranged between 0.04% (bulbs of *A. ophiophyllum* Acc.-No. 4155) and 2.16% (bulbs of *A. carolinianum*). A High value of total cysteine sulphoxides was also detected in bulbs of *A. hymenorrhizum* (1.39 %). The latter two species can be considered as medicinal plants.

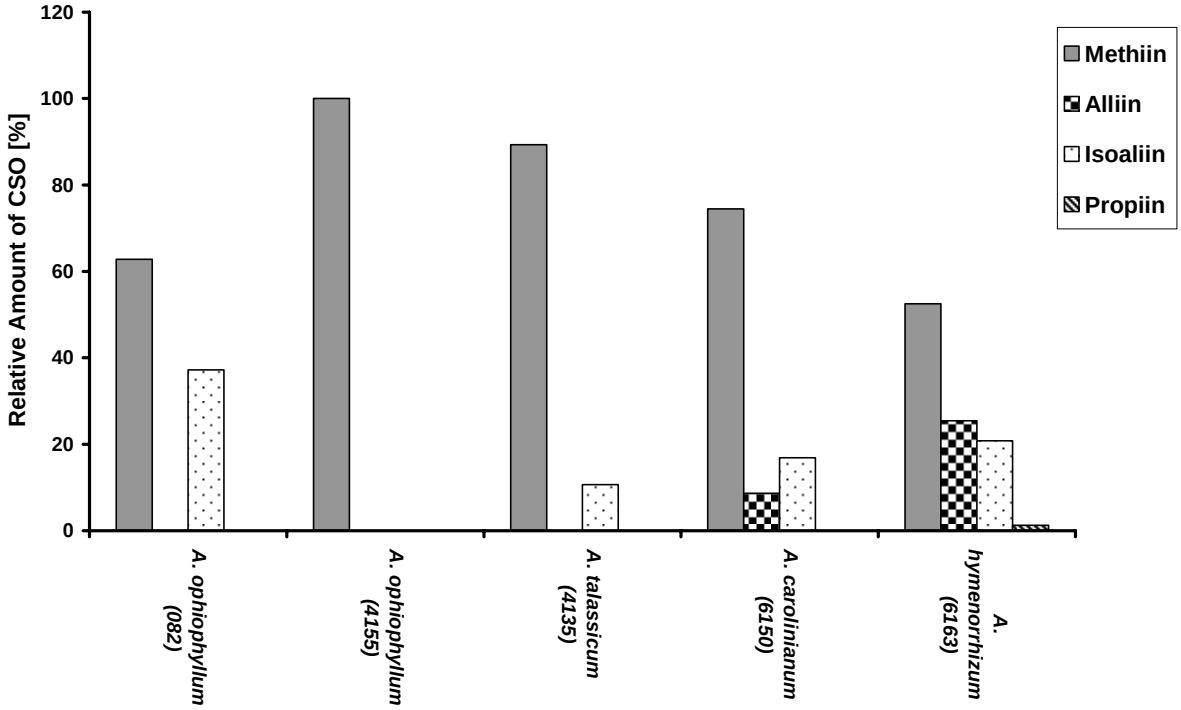


Figure 5.37: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Polyprason* (total cysteine sulphoxides = 100%).

5.4.4. *Allium Species Belonging to the Subgenus Cepa*

Subgenus *Cepa* contains approximately 30 rhizomatous species. The typical representative of this subgenus is common onion. In investigated species, relatively high values of total amount of cysteine sulphoxides were found (in bulbs of *A. fedschenkoanum* even 1.03%). The composition of cysteine sulphoxides differed remarkably; in all samples, methiin was found (Fig. 5.38). Its concentration varied from 3.50% to 70.78%. Relatively high amount of isoalliin was found in species *A. oschaninii* and *A. pskemense*. Bulbs of *A. fedschenkoanum* contained relatively high amounts of alliin (29.22%). With the exception of *A. fedschenkoanum*, propiin was found in small amounts in all examined samples. The highest value of total cysteine sulphoxides was detected in bulbs of *A. fedschenkoanum* (0.7%) and the lowest value in bulbs of *A. pskemense* (0.19%).

All investigated species of this subgenus can be considered as vegetable, spice or medicinal plant. With the exeption of *A. fedschenkoanum*, all species lead relative high amounts of isoalliin. Therefore, species belonging to this subgenus are highly estimated by native populations, mostly as a substituent of common onion.

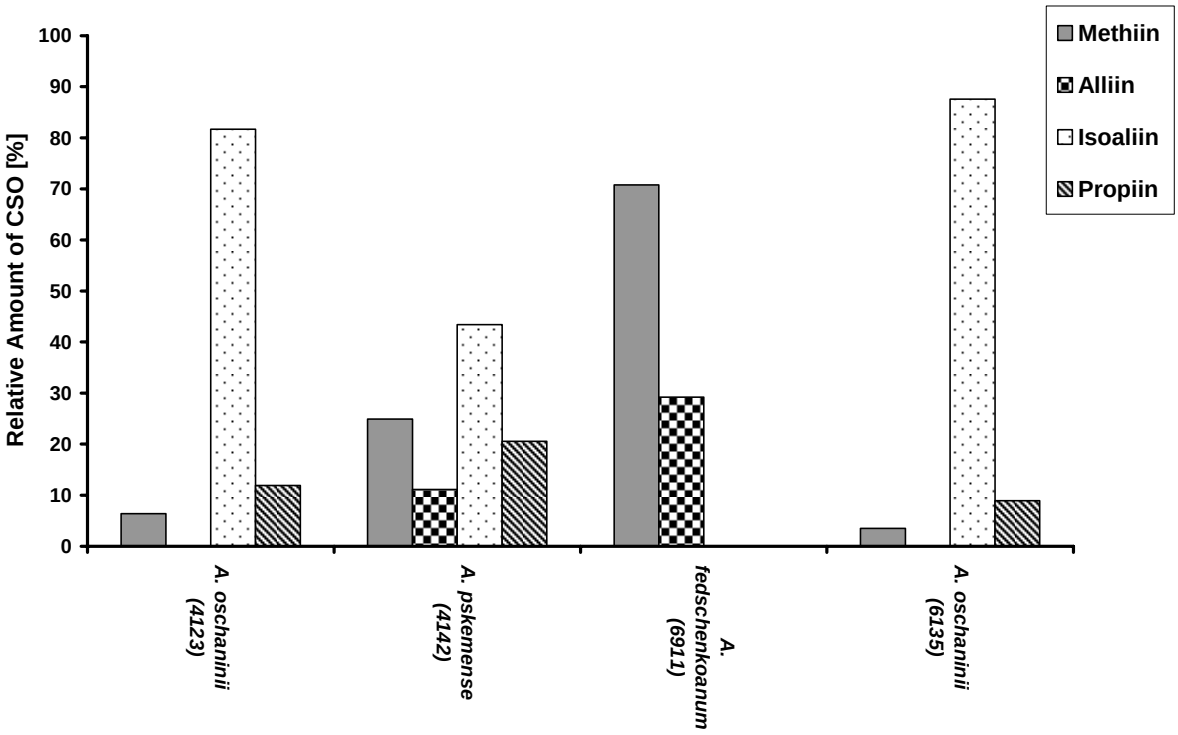


Figure 5.38: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Cepa* (total cysteine sulphoxides = 100%).

5.4.5. *Allium Species Belonging to the Subgenus Amerallium*

Only two samples of identical species belonging to the subgenus *Amerallium* were investigated (*A. paradoxum var. normale*). The relative amount of cysteine sulphoxides is already presented and discussed above (Figure 5.6). *A. paradoxum* is heavily used by native populations, mostly as spicy vegetable.

5.4.6. *Allium Species Belonging to the Subgenus Melanocrommyum*

Subgenus *Melanocrommyum* is a very large subgenus containing 140 species, which are divided into 15 sections. The majority of species belonging to the subgenus *Melanocrommyum* do not have the typical garlic or onion smell and most of them contain only trace amounts of cysteine sulphoxides (below 0.1%) [Fritsch 2006].

However, the smell of crunched bulbs is sometimes very strong and unpleased. It must be suggested, that this subgenus contains further substances with unknown chemical composition, which also contribute to the sensoric properties of these plants.

Species investigated in this work belonged to sections *Megaloprason*, *Kaloprason*, *Acmopetala*, *Compactoprason*, *Regeloprason*, *Popovia*, *Verticillata*, *Brevicaule*, *Acanthoprason* and *Aroidea*. A big quantity of investigated samples belonged to the first five mentioned sections; in following Figures 5.39 - 5.44 they are presented in detail.

Section *Megaloprason*

Examined species belonging to the section *Megaloprason* displayed the characteristic cyteine sulphoxide pattern of the subgenus *Melanocrommyum*; in all samples, methiin was found in significantly high amounts (Fig. 5.39). In bulbs of *A. rosenbachianum ssp. kwakense*, methiin was even the only cysteine sulphoxide found. In remaining samples, also other cysteine sulphoxides were detected, but only in trace amounts. The values of total cysteine sulphoxides ranged between 0.03% (*A. rosenorum*) and 0.61% (*A. aflatunense*). Remarkably high amount of cysteine sulphoxides were detected in bulbs of *A. jesdianum* and *A. chelotum*. In both cases, 0.5% were found.

Bulbs of these species were usually not used by native populations. However, leaves were often collected in springtime, especially of those species leading a red dye (*e.g.*, *A. rosenorum*). Leaves were used as a general tonic after wintertime, for preparation of soups and some special national dishes.

A. stipitatum also belongs to the section *Megaloprason*. In our investigations only samples obtained from IPK Gatersleben, Germany, were examined. The total amount of cysteine sulphoxides was found to be 0.96%. Methiin was determined to be the main cysteine sulphoxide (79.81%); isoalliin was detected in small amounts. Bulbs of A. stipitatum are extensively used by the population of Central Asia. The plant does not lead a red dye.

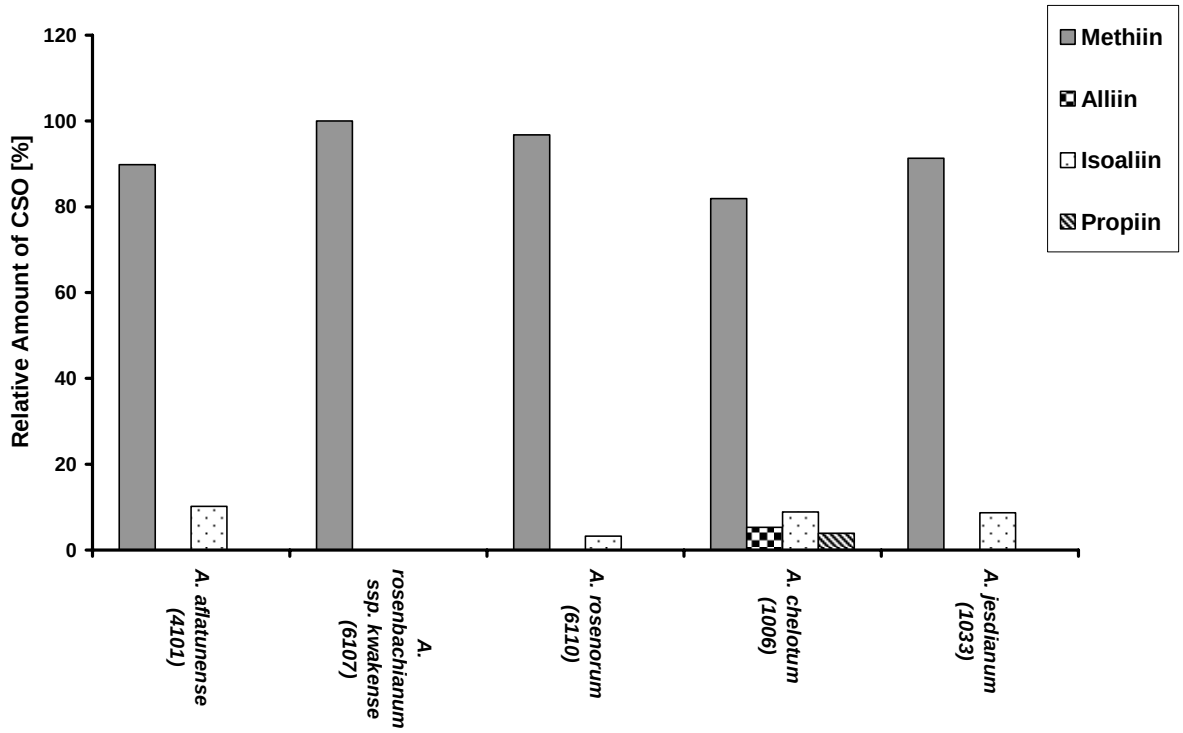


Figure 5.39: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Melanocrommyum* section *Megaloprason* (total cysteine sulphoxides = 100%).

Section *Kaloprason*

The pattern of cysteine sulphoxides in examined species belonging to section *Kaloprason* does not correspond with results obtained from the section *Megaloprason*. In all investigated species, methiin was found, but other cysteine sulphoxides were detected in significantly high amounts *e.g.*, *A. caspium ssp baissunense* and *A. alexeianum* (Fig. 5.40). Interestingly, bulbs of *A. caspium ssp. baissunense* contained alliin as the main flavour precursor. The lowest value of total cysteine sulphoxides was found for *A. caspium ssp. baissunense* (0.08%) and the highest value for *A. cristophii* (0.63%). Furthermore, bulbs of *A. cristophii* contained propiin.

Like described for the section *Megaloprason*, bulbs were seldomly used. Only leaves and flowers of *A. pseudobodeanum* (*A. akaka s.l.* later redetermined as *A. ellisii*) were strongly collected during springtime. This plant also leads a red dye.

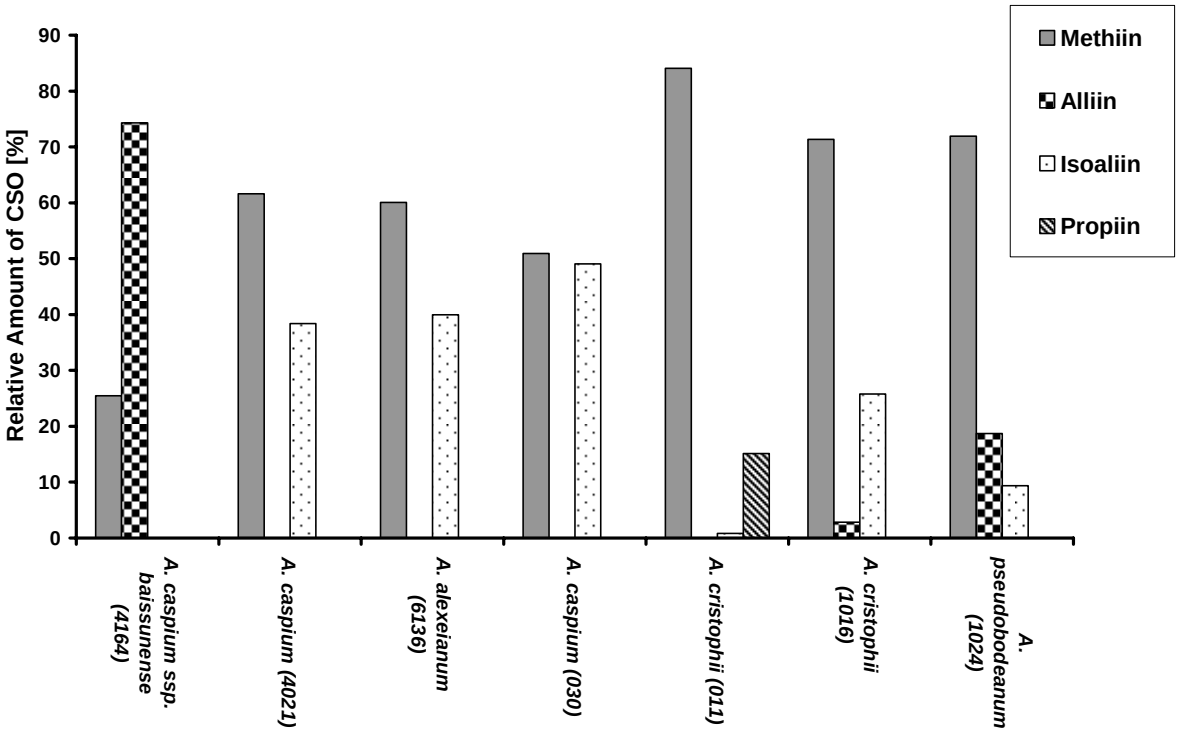


Figure 5.40: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Melanocrommyum* section *Kaloprason* (total cysteine sulphoxides = 100%).

Section *Acmopetala*

Section *Acmopetala* seems to be characterised by significant amounts of isoalliin besides methiin. The only exception is *A. motor*, which only contains methiin (Fig. 5.41) In the cases of *A. suworowii* (Acc.-No. 6090 and 6083), isoalliin dominated. In bulbs of *A. tashkenticum* and *A. taenipetalum*, the ratio of methiin and isoalliin was found to be rather equal. In the remaining samples, methiin dominated. In three of four samples obtained from *A. suworowii* species, significantly high values of total cysteine sulphoxides were found (bulbs of **Acc.-No.** 6112 contained even 1.81%). In other investigated species, much lower values (below 0.21%) were detected.

As already mentioned above, bulbs of *A. suworowii* were originally collected by native populations. However, this species got rare in our days. Besides *A. suworowii*, leaves of *A. motor* are highly estimated. The term “motor” means “health”. Again, this species leads a red dye and is probably therefore used.

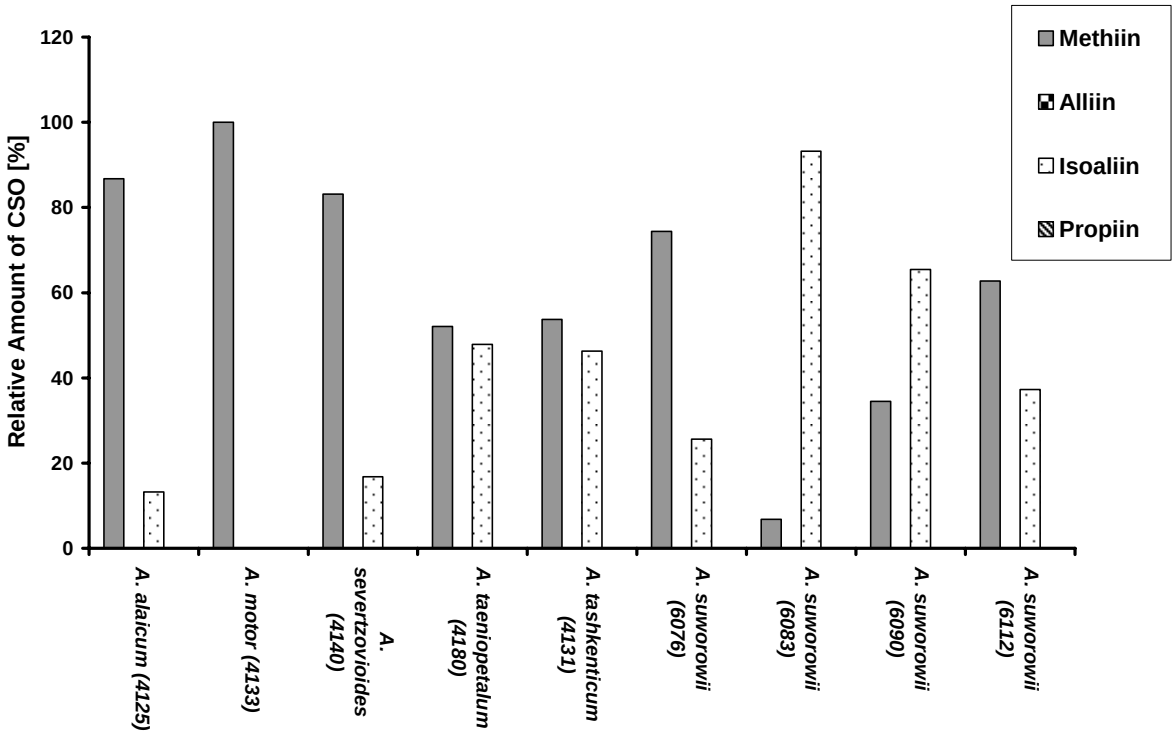


Figure 5.41: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Melanocrommyum* section *Acmopetala* (total cysteine sulphoxides = 100%).

Section *Regeloprason*

In all examined samples, methiin was found as the main cysteine sulphoxide (Fig. 5.42). Bulbs of *A. winklerianum* and *A. hissaricum* contained methiin as the only cysteine sulphoxide. Furthermore, in all further species isoalliin was detected. Small amounts of alliin were found in bulbs of *A. cupuliferum*, in bulbs of *A. yatei*, where also small amounts of propiin could be detected. The values of total cysteine sulphoxides were found to be rather low; the highest value was detected in bulbs of *A. regelii* (0.34%) and the lowest value in bulbs of *A. winklerianum* (0.06%). These species were not typically used by native populations.

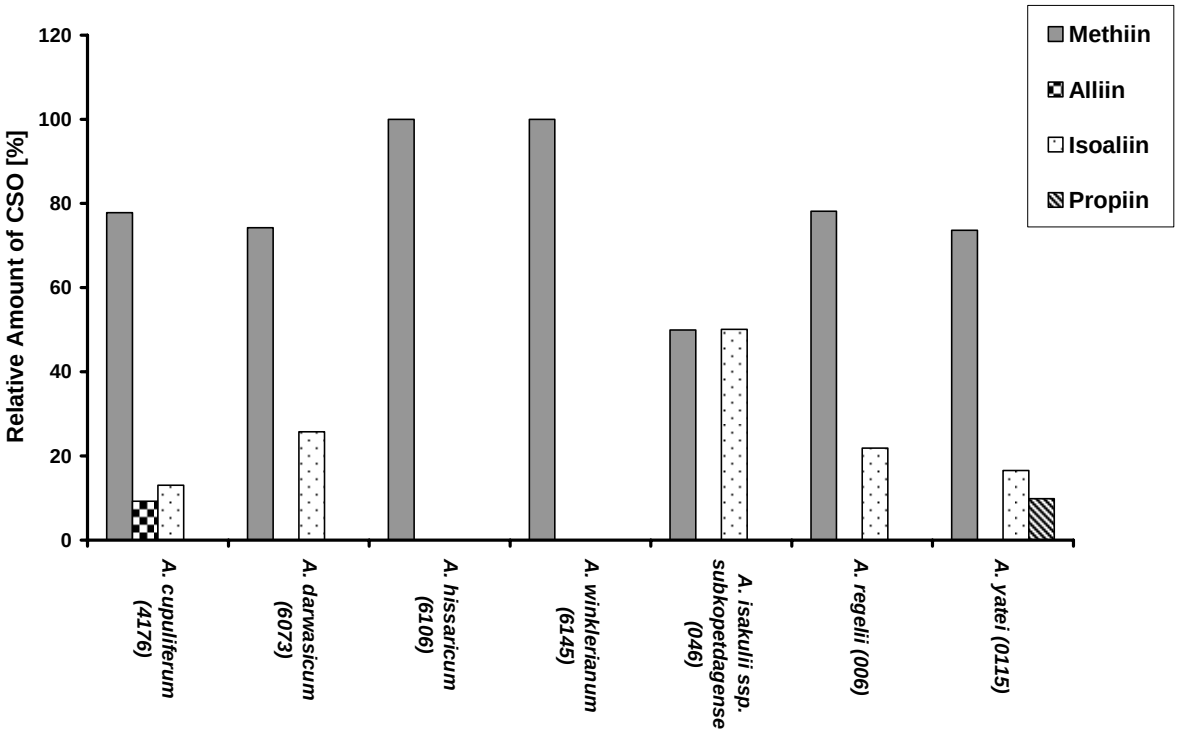


Figure 5.42: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Melanocrommyum* section *Regeloprason* (total cysteine sulphoxides = 100%).

Section Compactoprason

Section *Compactprason* seems to be characterized by significant amounts of methiin combined with more or less high amounts of isoalliin. Values of total amount of cysteine sulphoxides ranged from 0.02% (bulbs of *A. giganteum* **Acc.-No.** 4165) to 0.45% (bulbs of *A. giganteum* **Acc.-No.** 1028). It has to be mentioned that leaves of these two species (Fig. 5.43) are highly estimated by native population of Central Asia, probably because of high amount of isoalliin. Investigated plants produced a red dye.

A. macleanii also belongs to the section *Compactoprason*. In our investigations only samples obtained from IPK Gatersleben, Germany, were examined. The value of total amount of cysteine sulphoxides was found to be 1.34%. Methiin was determined to be the most abundant cysteine sulphoxide (52.81%); alliin and isoalliin were found in smaller amounts (24.99% and 20.99%, respectively). Propiin was detected in trace amounts (1.32%). The plant also produces a red dye.

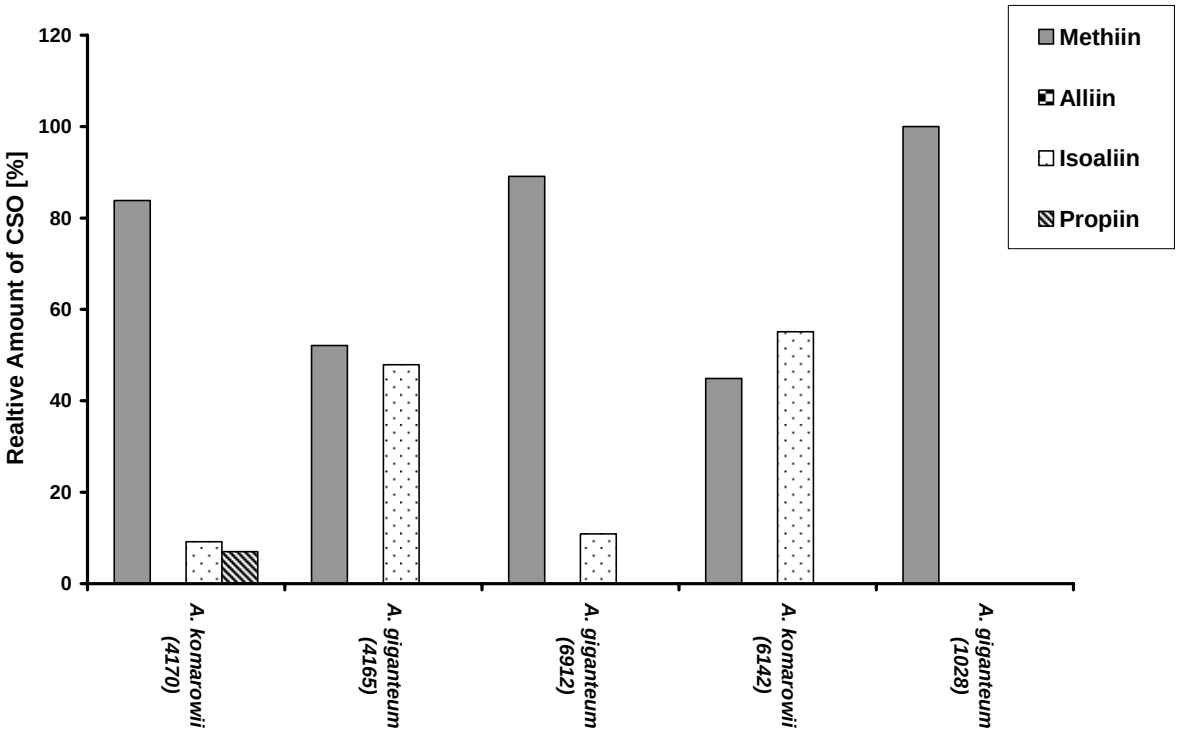


Figure 5.43: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Melanocrommyum* section *Compactoprason* (total cysteine sulphoxides = 100%).

Other Investigated Sections Belonging to Subgenus *Melanocrommyum*

In the Figure 5.44 below, results obtained from investigations of other species belonging to subgenus *Melanocrommyum* are depicted. Also here, methiin was detected in significantly high amounts. Besides methiin, other cysteine sulphoxides were found, but only in trace amounts. The highest value of total cysteine sulphoxides was detected in bulbs of *A. brachyscapum* (0.44%) and the lowest value in bulbs of *A. gypsaceum* (0.07%). These species were typically not used as vegetable, spice or medicinal plant.

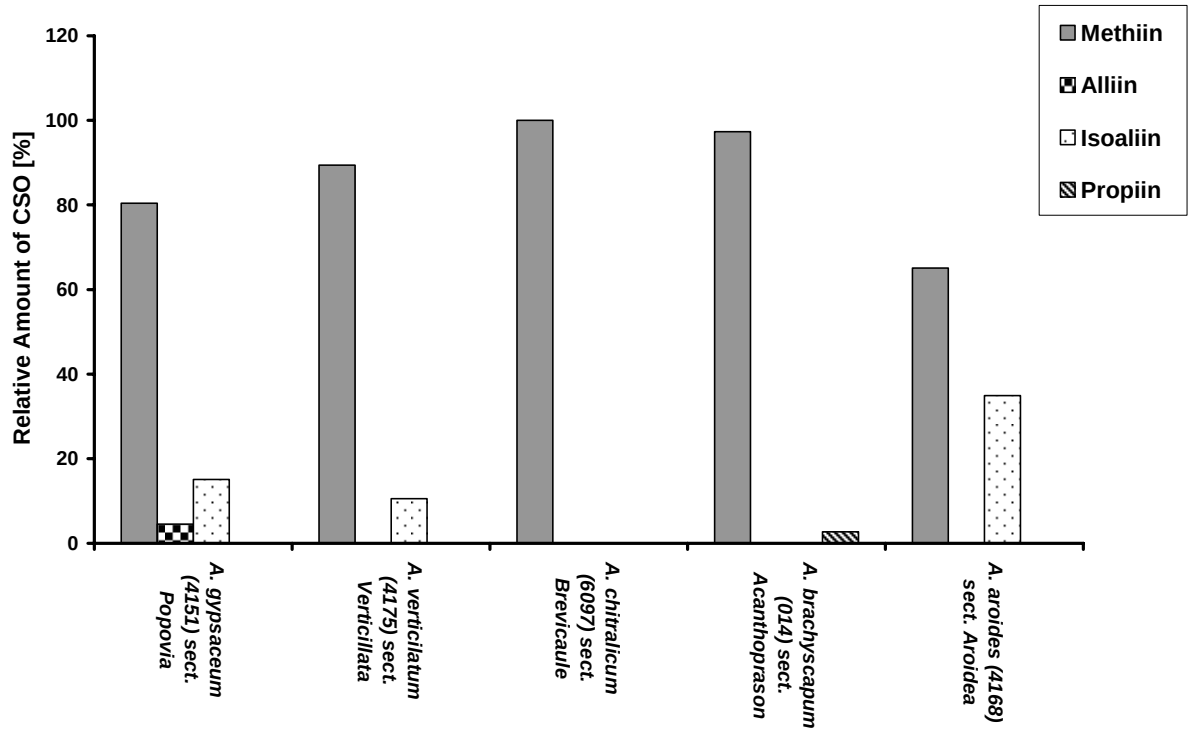


Figure 5.44: Relative amounts of cysteine sulphoxides in bulbs of the subgenus *Melanocrommyum* sections *Popovia*, *Verticillata*, *Brevicaule*, *Acanthoprason* and *Aroidea* (total cysteine sulphoxides = 100%).

5.5. *Antibiotic Activity*

There are many reports of medically significant antimicrobial properties of garlic. Activity against Gram-positive and Gram-negative bacteria, antifungal and antiprotozoal activity has been reported by Ankri et Mirelman [1999]. Kabelík [1970] described the strong activity against pathogenic yeasts, especially *Candida* species. There are also reports about inhitory effects of garlic on plant pathogenic microbes [Curtis et al. 2004; Fry et al. 2005]. The main antibacterial principle was identified by Cavalito in 1944 as diallylthiosulphinate (allicin). Later, a number of other sulphur containing substances derived from garlic were found to have an antibacterial activity, *e.g.*, thiosulphinates {2-propenesulfinothioic acid S-methyl ester and 2-propenesulfinothioic acid S-(Z,E)-1-propenyl ester} as well as ajoene [Naganawa et al. 1996; Yoshida et al. 1999].

As already mentioned, the antimicrobial properties are well described for garlic. There are also some reports about antimicrobial activity of common onion and some further *Allium* species [Yin and Tsao 1999]. In contrast, we focused our investigation on undescribed *Allium* species. In pre-screening experiments, six *Allium* extracts were tested against non-human pathogenic microorganisms belonging to bacteria, fungi and microalgae. Results showed that an extract obtained from *A. longicuspis* possessed the highest inhibitory effect towards all

examined microorganisms. *A. longicuspis* is a close relative of garlic; therefore, such results were expected. Furthermore, the amount of cysteine sulphoxide especially of alliin was very high (total amount = 2.98% and alliin= 2.54%, respectively). Hence, acquired results are in agreement with results described in literature.

Extracts obtained from *A. pskemense* and *A. oleraceum* strongly inhibited *Mycothypha microspora*, *Microbotryum violaceum* and *Chlorella fusca*, whereas *Bacillus megaterium* and *Eurotium rubrum* were only moderate inhibited. *A. altaicum* extract showed a high inhibitory activity towards *Micotypha microspora* and *Mycrobotryum violaceum*; effects towards other tested microorganisms were found to be rather weak.

Although the amounts of cysteine sulphoxides in bulbs of *A. jesdianum* and *A. giganteum* were found to be relatively high (0.45% and 0.38%, respectively), extracts obtained from these plants did not show any remarkable inhibition effect towards tested microorganisms. An explanation for this phenomenon could be given by the composition of cysteine sulphoxides. For *A. jesdianum* and *A*. *giganteum*, a significantly high amounts of methiin were found. Other cysteine sulphoxides occured only in trace amounts.

In the broad screening experiment, human pathogenic bacteria were tested towards a higher number of *Allium* extracts including an extract obtained from garlic. As already mentioned, garlic possess a significant inhibitory activity against Gram-positive as well as Gram-negative bacterial strains. Nagawana [1996] showed that ajoenes, sulphur-containing compounds derived from garlic, possess an antimicrobial activity against *Klebsiella pneumoniae* and *Escherichia coli*. Ankri and Mirelman [1999] reported about antimicrobial effects of allicin towards *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and a number of other Gram-negative bacterial strains.

In our investigation, none of examined extract (including extract obtained from garlic) exhibited inhibitory effects against Gram-negative bacteria tested. Such disagreement could be explained by the concentration of active compounds in the tested extracts, which was probably too low. The above-mentioned active principles are all based on instable and volatile sulphur compounds. Here, ethyl acetate extracts were tested, which had to be concentrated by a carefully evaporation of the solvent under reduced pressure. This would lead to a loss of active compounds. Further on, extracts had to be stored over some days before testing could be started. Storage would lead to an increased amount of di-, tri-, and polysulphides [Keusgen 2002a], which are probably less active. Nevertheless, extracts prepared in the same manner were used in pre-screening experiments and the inhibitory effect was found to be significantly high.

In experiments with Gram-positive bacterial strains, more favourable results were obtained. Some *Allium* extracts possessed a significant inhibitory activity against examined bacterial strains. Considerable inhibition was observed in extract obtained from

A. rosenorum. All tested Gram-positive bacterial strains exhibited certain sensitivity towards this extract. Interestingly, young fresh or dried leaves are used by the native population of Tajikistan for the national soup dishes, which are much esteemed for strong tonic properties [Keusgen et al. 2006; Fritsch et al. 2007]. Furthermore, fresh leaves of *A. rosenorum* are applied to wounds to promote quick healing. The total amount of cysteine sulphoxides was found to be rather low (0.03%). Therefore, other compounds than cysteine sulphoxides are probably responsible for such antimicrobial activity. Thiosulphinates should be considered for this.

Remarkable inhibitory effect was observed for extract obtained from *A. hymenorrhizum*. Three bacterial strains (*Streptococcus pyogenes*, *Staphylococcus aureus* and *Streptococcus agalactiae*) showed certain sensitivity towards this extract. The total amount of cysteine sulphoxides in bulbs of *A. hymenorrhizum* was found to be relative high (1.39%). Moreover, the most abundant flavour precursors were alliin and isoalliin.

Inhibitory effect against *Staphylococcus aureus*, *Streptococcus agalactiae* and *Staphylococcus epidermidis* was observed for extract obtained from *A. pskemense*, which is a close relative of common onion. The total amount of cysteine sulphoxides was found to be 0.38% and isoalliin was determined to be the most abundant flavour precursor. Extract obtained from garlic as well as *A. longicuspis* (a close relative of garlic) possessed inhibitory effect only against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Noteworthy antimicrobial activity was observed for extract obtained from *A. cristophii*, which inhibited only *Streptococus pyogenes*; other examined bacterial strains showed none susceptibility toward this extract.

5.6. *Radical Scavenger Activity*

Free radicals are atoms or group of atoms with an unpaired number of electrons. They are very unstable and react quickly with other compounds trying to capture the needed electron to gain higher stability. These compounds become a free radical itself, starting a chain reaction, which can finally result in disruption of living cell. Antioxidants neutralize free radicals by donating one of their own electrons ending the chain reaction. Antioxidants are stable, they act as scavenger of free radicals. Natural antioxidant (free radical scavenger) as L-ascorbic acid and α-tocoferol are widely used because they are seen as being safer and causing fewer adverse reactions than synthetic antioxidants.

In our investigation, methanolic extracts obtained from bulbs of *Allium* species were examined. To evaluate the radical scavenger activity, a simple method based on the use of stable free radical diphenylpicrylhydrazil (DPPH) was used. As a control of effectiveness, a synthetic antioxidant butyrated hydroxytoluene (BTH) was used. The relative scavenger activity of BHT was determined to be 100%.

Remarkably high relative radical scavenger activities were found for extracts obtained from *A. rosenorum* (121.9% and 109.4%, respectively), *A. giganteum* (109.1%), *A. macleanii* (120.7%), *A. winklerianum* (115.8%), *A. alaicum* (115.4%), *A. komarowii* (110.1%), *A. motor* (110.4%), and *A. jesdianum* (108.9% and 112.8%). Interestingly, all remarked *Allium* species belong to subgenus *Melanocrommyum*. As already mentioned, total amount of cysteine sulphoxides of species belonging to subgenus *Melanocrommyum* is usually rather low. Furthermore, methiin is normally the main cysteine sulphoxide. Therefore, other compounds are presumably responsible for the significantly high scavenger activity. Some of remarked *Allium* species are highly estimated by native population in Central Asia. The medicinal use of *A. rosenorum* has been already mentioned in chapter 5.5. Moreover, fresh or cooked leaves and bulbs of *A. komarowii* are applied against anemia and bad circulation and beyond they are used as anabolic drugs for horses [Keusgen et al. 2006]. Furthermore, fresh leaves of *A. jesdianum* are used as a general tonic and against rheumatism. Also leaves of *A. motor* (the term “motor” means “health”) are used as a tonic by native population in Uzbekistan.

As mentioned in chapter 4.5, in some species belonging to the subgenus *Melanocrommyum*, a reddish liquid coming out of the damaged tissue was observed. This red dye was determined as dithiodipyrrole and presumed to be responsible for the significantly high scavenger effect. The relative scavenger activity of the isolated red dye was found to be 78.1 %. Acquired results showed that the remarkably high scavenger activity of extracts obtained from *Allium* species forming the red dye cannot be explained only due to this single compound (dithiodipyrrole). There must be other substances, which are responsible for such high scavenger activity. This assumption is supported by results acquired from “not red” *Allium* species belonging to subgenus *Melanocrommyum*. Extracts obtained from *A. alaicum* as well as *A. chelotum* exhibited also significantly high relative scavenger activities (115.4 and 100.3%, respectively). Nevertheless, there was no colour reaction found.

For extracts obtained from *Allium* species, which do not belong to subgenus *Melanocrommyum*, lower scavenger activities were observed. A relatively high scavenger activity was found in extracts obtained from *A. carolinianum* (95.4%), *A. longicuspis* (84.0%) and *A. oleraceum* (84.9%). Štajner et al. 2002, described an antioxidative activity in all plant parts of *A. pskemense*. In our investigation, the relative scavenger activity of *A. pskemese* ranged between 27.7% and 100.6%, in dependence on the examined Acc.-No; the highest scavenger activity was found for samples collected at the place of origin (Uzbekistan).(see chapter 4.5.).

5.7. *Biogenesis of the Red Dye*

It must be assumed that the red dye, a dithiodipyrrole, is a new colour principle in nature. However, biogenesis of this compound is not clear yet. Formation of the red dye was observed by I) heating of plant material and enzyme free extracts, II) alkaline conditions and III) incubation with alliinase isolated from garlic. Also, a crude enzyme extract of *e.g.*, *A. giganteum*, which was incubated with a methanolic extract of the same species, catalysed the formation of this red dye. In contrast, no alliinase, which is comparable to the alliinase of garlic, could be found in *A. giganteum*. Therefore, it must be assumed that colour formation is catalysed by an enzyme, which is related to alliinase, but does not have the same properties (no isoenzyme). This assumption is supported by the fact that a partially purified protein fraction having the capability to form the red dye also exhibited a catechol oxidase activity.

A hypothetic pathway for the formation of the dithiodipyrrole is given in Figure 5.45. Because of the susceptibility of precursors to alliinase, the cysteine sulphoxide **1** must be assumed, but this substance could not be isolated until now. However, the following enzymatic reaction is a type of C-S lyase reaction (alliinase reaction), but reaction products are not the same as for garlic (thiosulphinates). Instead of these thiosulphinates (allicin for garlic), the thioketal **2** could be isolated. NMR analysis giving a full proof of the proposed structure was performed by Dr. Uwe Reinscheid, MPI Göttingen. In addition to this structure, also a number of further condensation products with sugars must be assumed. The sulphoxypyrrole moiety of compound **2** was always accompanied with traces of unidentified sugars.

The rather instable compound **2** is probably accumulated either in the vacuole or the in the cytosol of the plant cell. Damage of cell leads to formation of the red dye **3** even in seconds. A still unidentified enzyme might catalyse this reaction. But also alkaline conditions cause a intensive red colour within seconds. Alternatively, even slight heating of an enzyme-free, methanolic extract leads to a red colour of this extract. This behaviour supports the structure of **2** and that this compound is not an artefact but a storage-form of thiopyrroles.

The dithiodipyrrole **3** itself is also instable and has a tendency to polymerisation reactions. Repeated NMR measurements of a sample of this compound gave evidence to dimerisation because an additional proton signal belonging to the pyrrole ring system rose over time supporting the structure of compound **4**. Old samples gave only one significant NMR proton signal, which can be explained by structure **5**. For the latter structures, no full structure elucidation could be performed.

The physiological function of the red dye is unclear. It can be assumed that this compound has an antibiotic activity. As an interesting fact, cells surrounding plant vessels

have rather high concentration of the dye (observation by microscopy). This may prevent from infections, which are caused by bacteria or fungi propagating through these vessels.

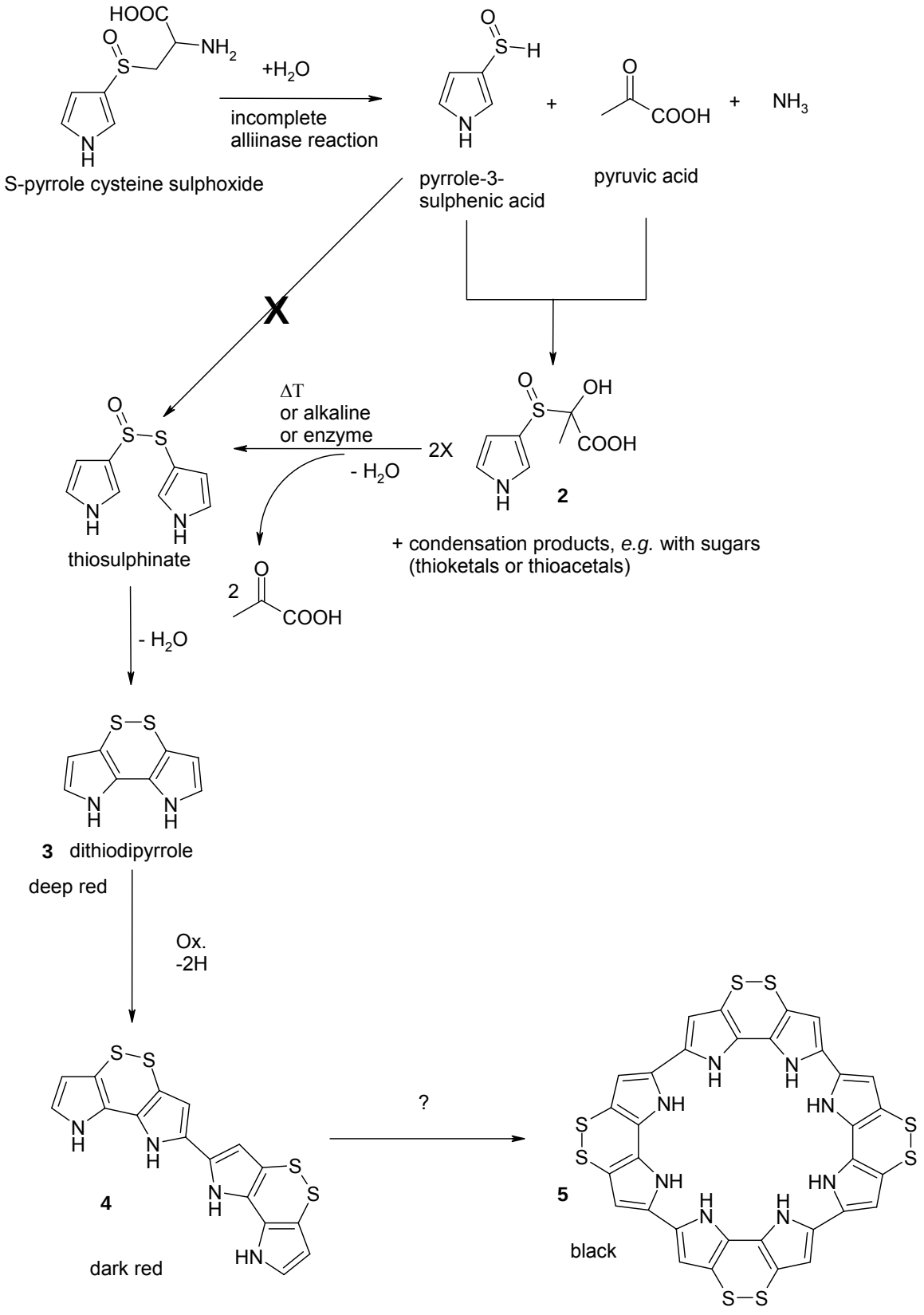


Figure 5.45. The hypothetic biogenesis of the red dye **3**. This compound is highly instable and might be oxidized to further thiopyrrole derivatives. The precursor of the whole reaction cascade is substance **1**. Full structure elucidation could be obtained for compounds **2** and **3**.

6. Summary

Since ancient times, onions, garlic and some other species of the genus *Allium* L. (onions) have been used as phyto-pharmaceutics, seasonings, and vegetables. Carvings in pyramid walls of the Old Kingdom of Egypt and written sources of the ancient cultures of the Greece and Rome mentioned the importance of common onion (*A. cepa* L.) and garlic (*A. sativum* L.) in the daily diet of man. The medicinal benefits of these two species were intensely investigated during the last decades and lipid lowering, antibiotic, anti-atherosclerotic and anti-diabetic effects were described. Also, a canceroprotective effect was proven by a number of ethnic studies. The health benefits of *Allium* vegetables are mainly related to sulphur containing compounds as well as saponins.

The species-rich genus *Allium* has a main centre of distribution reaching from Southwest Asia to the high mountains of Middle Asia. In this area, several wild species are used by the local population, as one can be concluded from casual remarks in some floras. The so-called cysteine sulphoxides of these plants are believed to be mainly responsible for these health benefits. These compounds are converted to thiosulphinates like allicin, when plant material is disrupted. This reaction is catalysed by the action of the enzyme alliinase.

In this investigation, 98 *Allium* samples with different Accession numbers belonging to 77 wild species were investigated. Most of the investigated species belonging to the subgenera *Allium*, *Melanocrommyum* and *Reticulatobulbosa* were chemically analysed and bioactivity was tested. For many species, a remarkable radical scavenger activity and an antibiotic activity, especially against fungi, were observed. It can be concluded, that traditionally used wild *Allium* species do have a real biological effect.

In the first step of the investigation, the total amount of cysteine sulphoxides of various species was determined by a biosensoric method, which was based on immobilized alliinase. Besides thiosulphinates, ammonia is produced by this reaction, which can be used for quantification of these cysteine sulphoxides. It was found that this method was suitable for a rapid pre-screening of these compounds. A disadvantage is that the sample preparation is rather time consuming. In comparison to HPLC, this method gave averagely lower amounts of total cysteine sulphoxides, which can be explained by the substrate specificity of immobilized alliinase. Methiin is converted slower as alliin or isoalliin leading to lower total amounts of cysteine sulphoxides of wild species, which are usually rich in methiin.

Further on, methiin, alliin, isoalliin and propiin were determined by HPLC. Most species (34) belonged to the subgenus *Melanocrommyum*. Out of this, 5 samples (species) were obtained from IPK collection in Gatersleben, 5 samples from Iran, 6 from Turkmenistan, 15 from Uzbekistan and 10 from Tajikistan. The most remarkable species were *A. suworowii* (Tajikistan, total amount of cysteine sulphoxides up to 1.81%), *A. macleanii* (Tajikistan and

IPK collection, up to 1.34%), *A. paradoxum* (Iran, 0.82%) and *A. pseudobodeanum*, re-determined as *A. ellisii* Bak (Iran, 0.63%). Out of the other subgenera, the most important ones are *A. barsczewskii* (Tajikistan, 5.71% total cysteine sulphoxides), *A. jodanthum* (Tajikistan, 1.54%), *A. filidens* (Tajikistan, 1.55%), *A. hymenorrhizum* (Tajikistan, 1.39%), *A. turcomanicum* (Turkmenistan, 1.07%) and *A. fedschenkoanum* (Tajikistan, 1.03%). From great interest is also *A. longicuspis* (IPK collection), a close relative of *A. sativum*, for which a total amount of 2.98% cysteine sulphoxides was determined.

The highest amount of methiin was found for *A. barsczewskii* (4.78%), the highest amount of alliin for *A. longicuspis* (2.54%), followed by *A. barsczewskii* (0.68%), the highest amount of isoalliin was determined for *A. suworowii* (0.67%) and the highest amount of propiin was found for *A. filidens* (1.07%). Species, which are rich in alliin and isoalliin can be considered as medicinal plants. Besides the above mentioned species, *A. paradoxum* var. *normale* (0.40% isoalliin), *A. turcomanicum* (0.18% alliin), *A. fedschenkoanum* (0.30% alliin), *A. carolinianum* (0.19% alliin and 0.36% isoalliin), *A. hymenorrhizum* (0.35% alliin and 0.29% isoalliin), *A. turkestanicum* (0.34% alliin), *A. pskemense* (0.32% isoalliin), *A. oschaninii* (0.20% isoalliin) and *A. macleanii* (0.33% alliin and 0.28% isoalliin) are from great interest and might be considered for medicinal use.

Besides these known cysteine sulphoxides, also a new sulphur compound containing two pyrrole ring systems and a disulpho-bridge was isolated. This dithiodipyrrole is typical for a number of members of the subgenus *Melanocrommyum* like *A. macleanii*, *A. giganteum*, *A. jesdianum*, *A. rosenorum*, *A. winklerianum* and *A. rosenbachianum* and has a deep red colour, which occurs after heating or wounding of plant material. It must be assumed that an alliinase-like enzyme is also involved in the formation of this compound. Many of these species leading the dithiodipyrrole are used as traditional medicinal plants. In our investigation, a high radical scavenger activity could be found for this substance.

In total, 39 species were tested on radical scavenger activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. All species showing a significantly higher radical scavenger activity as 100%, related to butylated hydroxytoluene (BHT), belonged to the subgenus *Melanocrommyum*, mostly leading the dithiodipyrrole. Most remarkable were *A. rosenorum* (121.9%), *A. macleanii* (120.7%) and *A. alaicum* (115.4%). From the other subgenera, *A. pskemense* (up to 100.6%), *A. carolinianum* (95.4%) and *A. longicuspis* (84.0%) have to be mentioned.

Also antifungal, antialgal and antibacterial activity of some selected *Allium* species were tested. It can be assumed, that *Allium* species are most potent against algae and fungi (*e.g.*, *A. longicuspis*). However, the found results for activity against human pathogenic bacteria were not in accordance with literature data. 17 Different *Allium* species were tested, which were collected in Tajikistan (7), Uzbekistan (4), Iran (5) and obtained from the IPK collection

(4). Extracts did not show any activity against Gram-negative bacterial strains and only a moderate activity against Gram-positive bacterial strains. These differences to literature data can be explained by the fact that most of the active volatile sulphur compounds got lost during sample preparation. But nevertheless, the highest activity was found for *A. rosenorum*, a species with only a low content of cysteine sulphoxides (0.03%). This means, that besides the volatile sulphur compounds there must exist another active principle probably related to the dithiodipyrrole. Also extracts obtained from *A. hymenorrhizum*, *A. longicuspis* and *A. pskemense* showed a moderate antibiotic activity. Interestingly, an extract obtained from *A. cristophii* was selectively active towards *Streptococcus pyogenes*.

By this investigation it could be demonstrated that the genus *Allium* contains a fairly high number of wild species, which have to be considered as medicinal plants, mostly belonging to the subgenus *Melanocrommyum*. This subgenus it only used in the western world for ornamental plants. In contrast, many species of the subgenus *Melanocrommyum* are traditionally used in Central Asia. Also the chemistry of *Allium* does not seem to be clarified yet. Biogenesis of dithiodipyrroles is totally unknown. In addition, some unknown compounds exposing a rather strong radical scavenger activity must be considered. Further studies are necessary to answer these open questions.

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8. Appendix

8.1. *Foto Documentation*

Fig. 8.1: Plant collectors near Sarimarguzor, Tajikistan

Fig. 8.2: A*. karataviense* (Acc.-No. 6087)

Fig. 8.3: *A. caspium ssp. caspium* (Acc.-No. 0038)

Fig. 8.4: *A. barsczewskii* (Acc.-No. 4177)

Fig. 8.5: Tajikistan, Pamir-range near Vanch valley

Fig. 8.6: Tajikistan, Hissar-range near Karakul river

Fotos were kindly provided by M. Keusgen.

Fig. 8.7: *A. oschaninii* (Acc.-No. 4123)

Fig. 8.8: A*. alaicum* (Acc.-No. 4125)

Fig. 8.9: *A. komarowii* (Acc.-No. 4170)

Fig. 8.10: *A. rosenorum* (Bot. Garden, Dushanbe)

Fig. 8.11: *A. stipitatum* (Bot. Garden, Dushanbe)

Fig. 8.12: *A. cristophii* (Bot. Garden, Dushanbe)

Fotos were kindly provided by M. Keusgen

8.2. *Spectral Data of the Red Dye*

IUPAC name: 3,3'-dithio-2,2'-dipyrrole

MS (Kratos MS 50, Department of Chemistry, University of Bonn, Dr. G. Eckardt)
Molecular formula: $C_8H_6N_2S_2$
HR-MS: 193.9984 amu; MS calculated: 193.9996 amu

Ion (m/z)	rel. Intensity	Description
196	71 %	m^++2H
194	**100 %**	**M^+**
164	80 %	m^++2H-S
161	29 %	m^+-H-S
150	32 %	
136	36 %	
131	29%	m^++H-2S
119	13 %	
114	11 %	
104	23 %	
96	63 %	½ m^+-H
81	26 %	
69	53 %	
64	11 %	S_2^+
57	24 %	
45	17 %	

NMR-Data (Bruker AMX 300, measured by Dr. Stefan Kehraus):

^{1}H-NMR (300 MHz, solvent: MeOD)

H at Carbon No.	Shift δ (ppm)	Spin System
2, 7	6.43	d, *J* = 3.66 Hz
3, 6	6.28	d, *J* = 3.66 Hz

^{13}C-NMR (+ HMBC, 75 MHz, solvent: MeOD):

Carbon No.	Shift δ (ppm)
2	107.98
3	120.29
3a	131.97
5a	131.97
6	120.29
7	107.98
8a	125.53
8b	125.53

NMR-Data [ACD predictor, default settings, calculated (Advanced Chemistry Development, Inc., 110 Yonge Street, 14th floor, Toronto, Ontario, Canada M5C 1T4)]:

^{1}H-NMR (300 MHz, Solvent: MeOD)

H at Carbon No.	Shift δ (ppm)	Spin System
2, 7	6.58	d, *J* = 6.75 Hz
3, 6	6.43	d, *J* = 6.75 Hz

^{13}C-NMR (40 MHz, Solvent: MeOD):

Carbon No.	Shift δ (ppm)
2	102.75
3	124.86
3a	136.64
5a	136.64
6	124.86
7	102.75
8a	126.76
8b	126.76

UV: In MeOH, 0.08 mg/mL

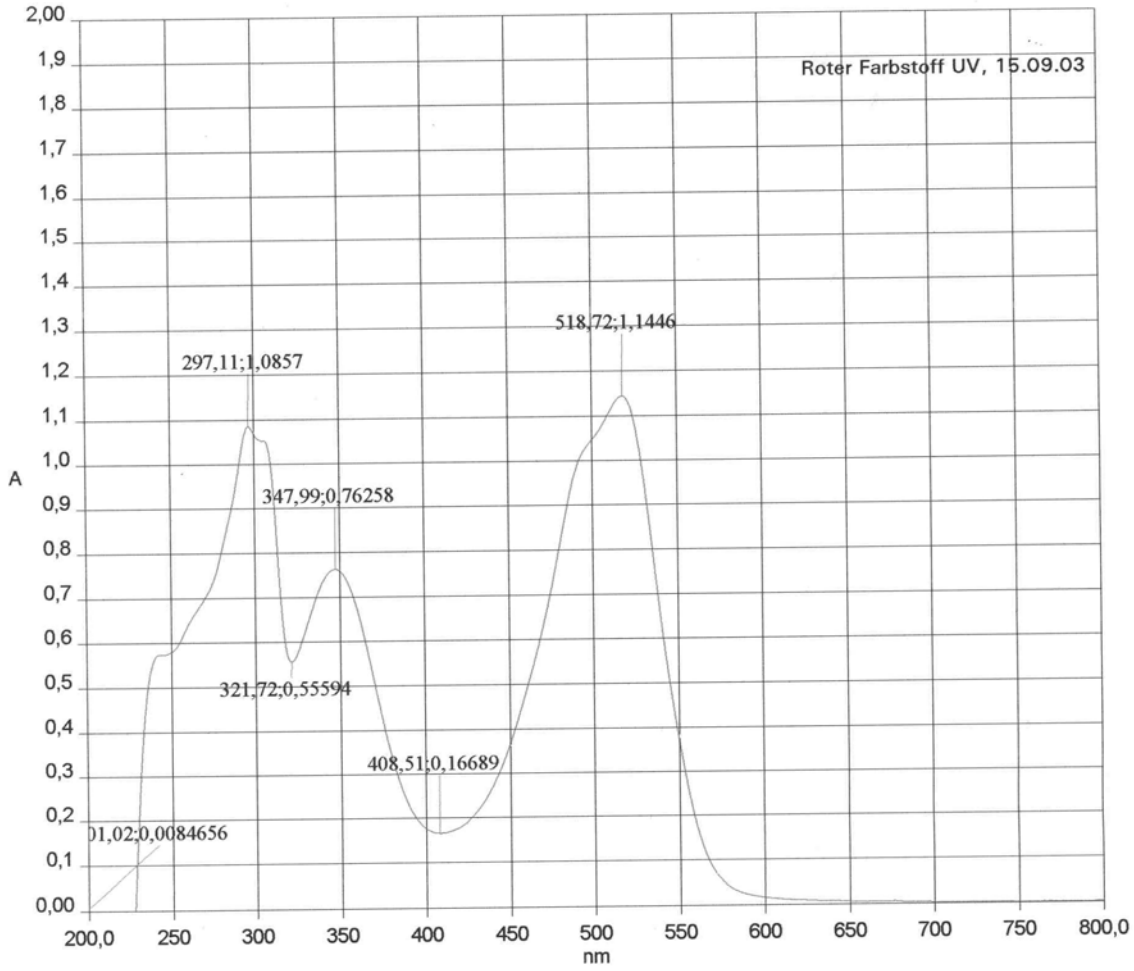
Max_1: 519 nm (Au=1,1446)
Max_2: 297 nm

IR: FT-IR

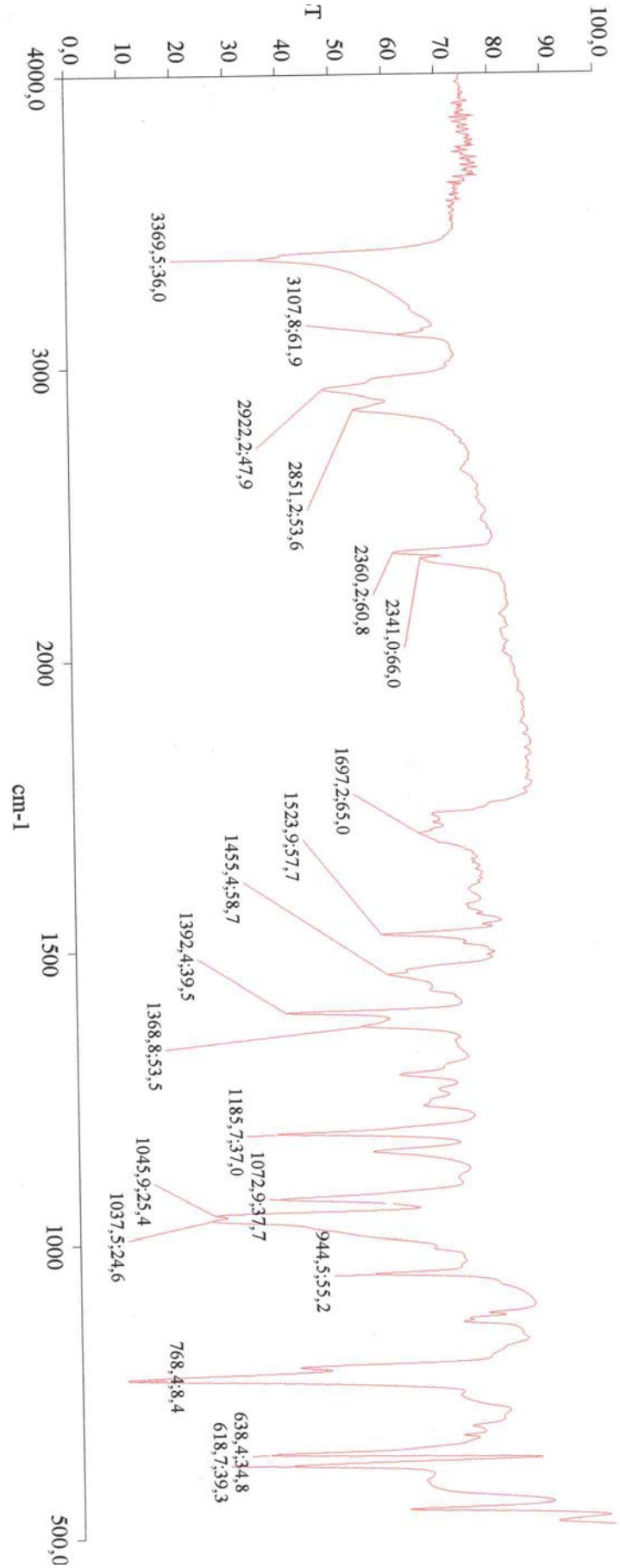
Wavenumber (cm^{-1})	Intensity	Functionality
3370	S	ν NH
3108	W	ν C=C-H (aryl)
2922	M	solvent
2851	M	solvent
2360	M	ν S-S
2341	M	ν S-S
1697	W	ν C=N
1524	M	ν C-S, δ NH, δ C=C (aryl)
1455	M	
1392	S	
1369	M	
1186	S	
1073	S	
1046	S	
1038	S	
945	M	
768	S	two neighbouring C=C-H (aryl)
638	S	
619	S	

Melting point: Decomposition at approximately 170 °C

UV/VIS-Spectrum



IR-Spectrum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80
70
60
50
40
30
20
10
0,0
T
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3000
2000
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1000
500,0
cm-1
3369,5;36,0
3107,8;61,9
2922,2;47,9
2851,2;53,6
2360,2;60,8
2341,0;66,0
1697,2;65,0
1523,9;57,7
1455,4;58,7
1392,4;39,5
1368,8;53,5
1185,7;37,0
1072,9;37,7
1045,9;25,4
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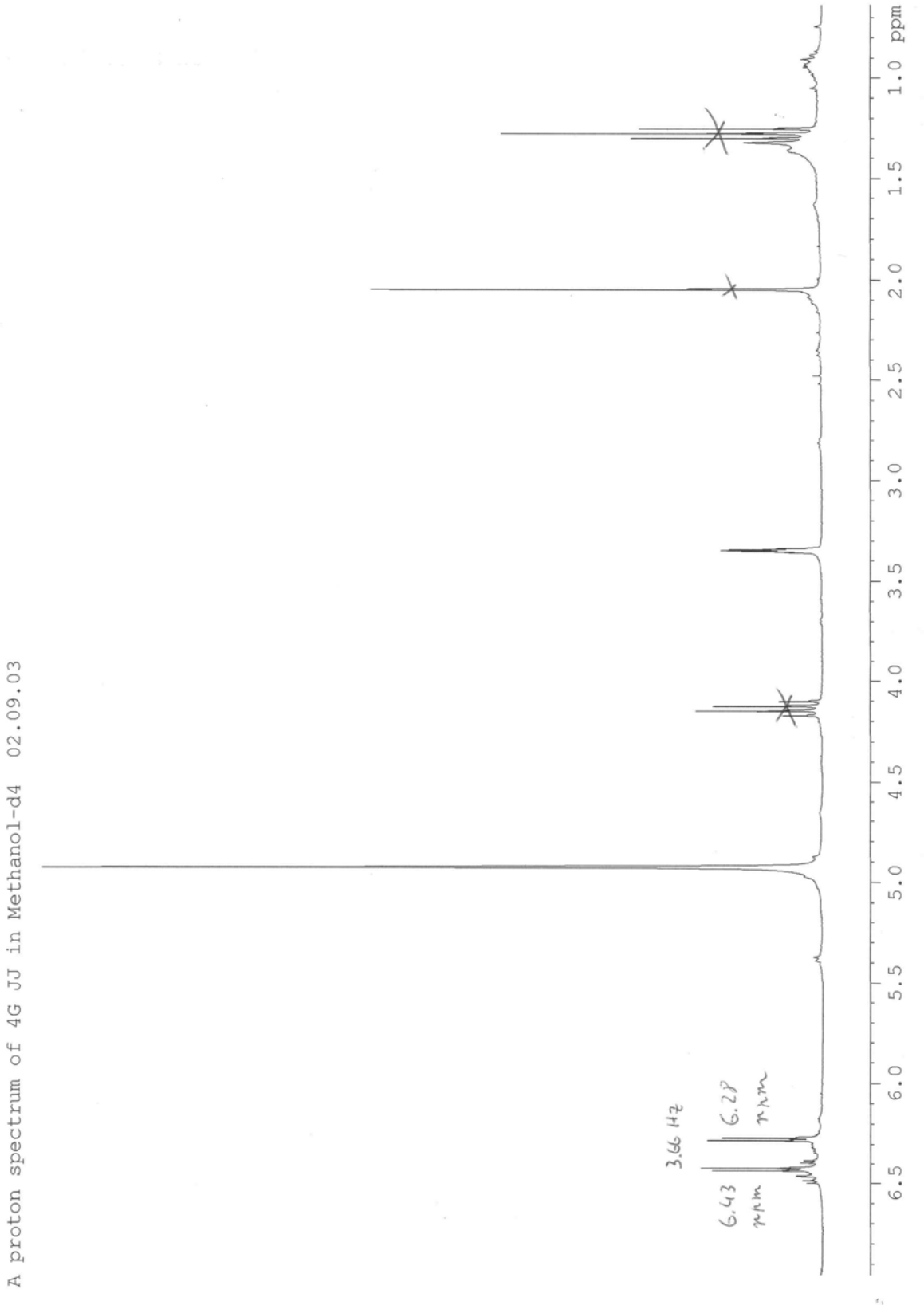
768,4;8,4
638,4;34,8
618,7;39,3

1H-NMR-Spectrum



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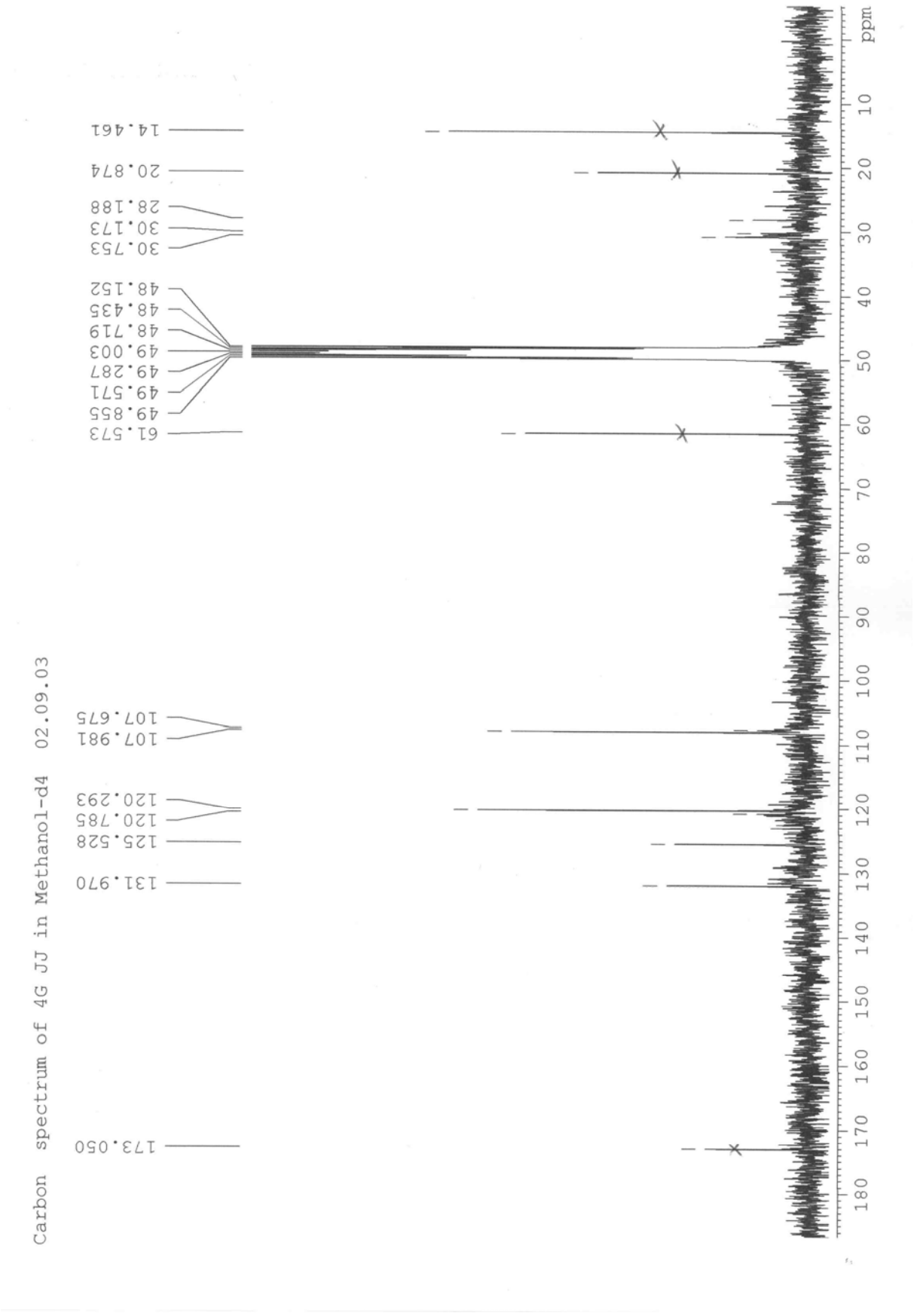

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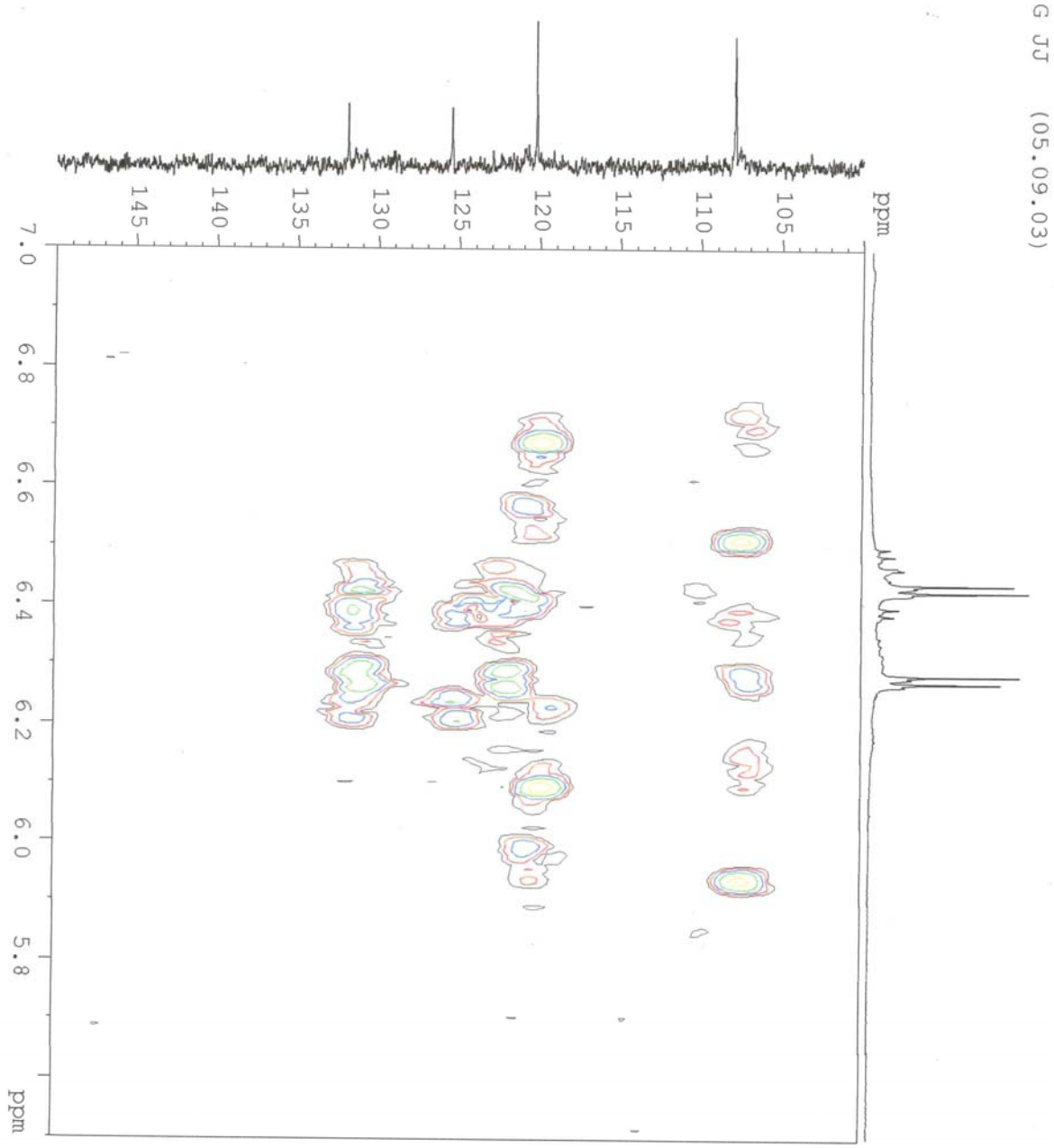
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140
135
130
125
120
115
110
105
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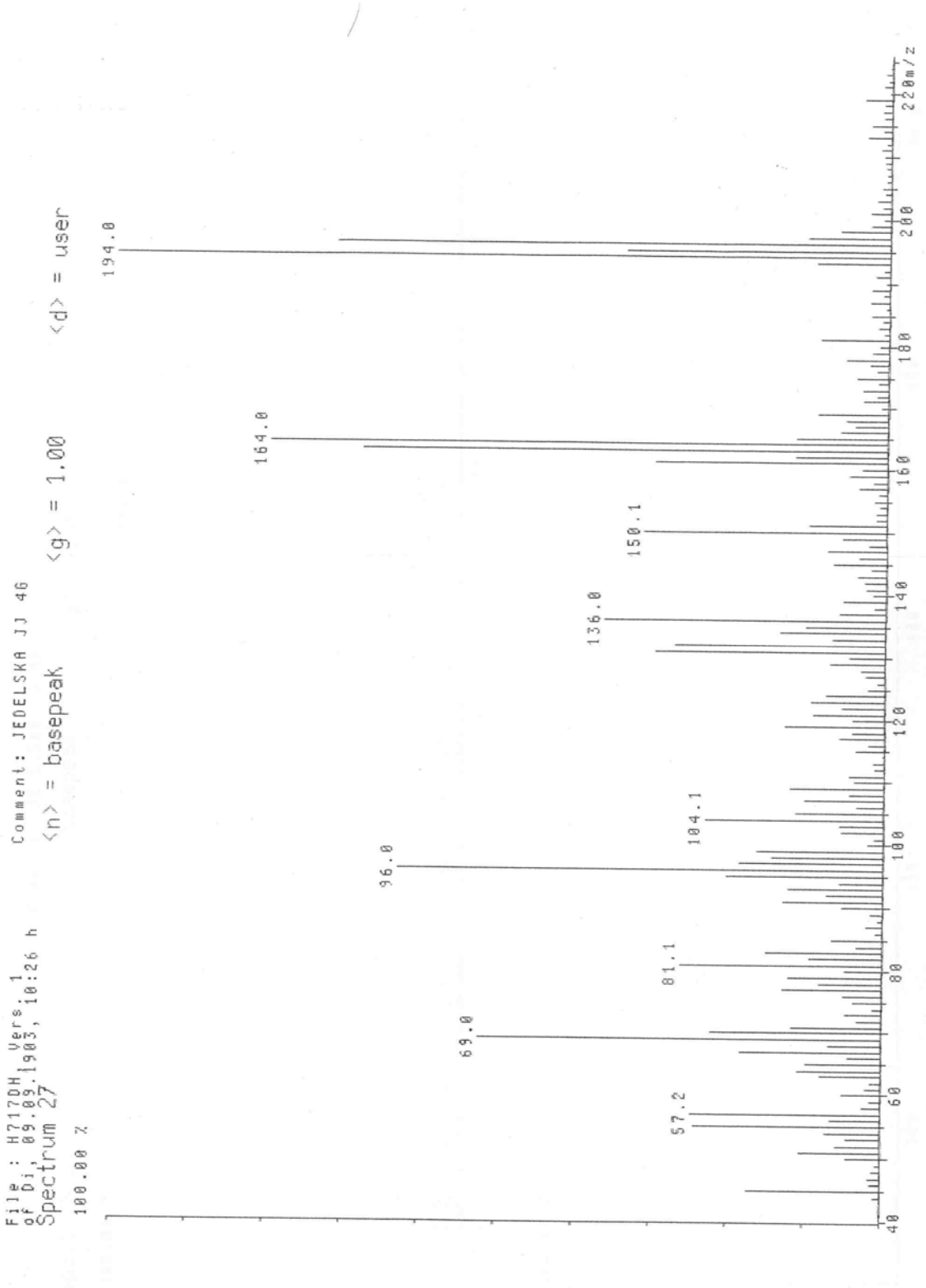
6.6
6.4
6.2
6.0
5.8
ppm

Mass-Spectrum



List of Publications

Poster with Abstract

Jarmila Jedelska, Reinhard M. Fritsch, Michael Keusgen:
Schwefelpyrrole - eine neue Naturstoffklasse in arzneilich genutzten zentralasiatischen *Allium*-Arten
***Abstractband Arznei- und Gewürzpflanzentagung, Jena**, 2004, 34*

Nilüfer Pekgöz, Klaus Pistrick, Jarmila Jedelska, Maia Akhalkatsi, George Nakhutsrishvili, Michael Keusgen:
Aroma-Präkursoren von georgischen *Allium*-Arten aus Wildsammlungen
***Abstractband Arznei- und Gewürzpflanzentagung, Jena,** 2004, 102*

Jarmila Jedelska, Helena Koblihova, Furkat O. Khassanov, Hikmat Hisoriev, Parvina A. Kurbonova, Reinhard M. Fritsch, Michael Keusgen:
Aroma-Präkursoren und Scavenger-Aktivität von zentralasiatischen *Allium*-Arten
***Abstractband Arznei- und Gewürzpflanzentagung, Jena,** 2004, 104*

N. Pekgöz, K. Pistrick, J. Jedelska, M. Akhalkatsi, G. Nakhutsrishvili, M. Keusgen:
Aroma Precursors of Georgian Wild *Allium* Species
Abstract 53rd GA Annual Congress (2005), 135.

J. Jedelska, R. Fritsch, M. Keusgen:
Antibiotic Activity of Some Wild *Allium* L. Species
Abstract 53rd GA Annual Congress (2005), 193.

J. Jedelska, R.M. Fritsch, H. Hisoriev, P.A. Kurbonova, F.O. Khassanov, M. Keusgen:
Aroma Precursors and Scavenger Activity of *Allium* Species from Central Asia
Abstract 53rd GA Annual Congress (2005), 250

A. Vogt, J. Jedelska, R.M. Fritsch, M. Keusgen:
Sulphurpyrroles- a New Class of Substances of Medicinally Used *Allium* Species Growing in Central Asia
Abstract 53rd GA Annual Congress (2005), 294.

H. Schulz, M. Baranska, J. Jedelska, M. Keusgen:
Mapping of *Allium* Plants by NIR FT Raman Microspectroscopy
Abstract 53rd GA Annual Congress (2005), 381.

J. Jedelská, M. Keusgen, R. M. Fritsch:
Sulphur Chemistry of Drumstick Onions (*Allium* Subgenus *Melanocrommyum*)
***Planta Medica* 72** (2006), 1029.

Short Lecture:

J. Jedelská, R. M. Fritsch, M. Keusgen:
Bioactivity of some wild Allium L. species from Central Asia
Abstractband DPhG Joinet Meeting 2006 (2006), 79.

Publications:

J. Jedelska, R.M. Fritsch, M. Keusgen:
Schwefelpyrrole – eine neue Naturstoffklasse in arzneilich genutzten, zentralasiatischen *Allium*-Arten
Tagungsband Fachtagung für Arznei- und Gewürzpflanzen 2004 (2005), 66-70

N. Pekgöz, K. Pistrick, J. Jedelska, A. Akhalkatsi, G. Nakhutsrishvili, M. Keusgen:
Aroma-Präkursoren von georgischen *Allium*-Pflanzen
Tagungsband Fachtagung für Arznei- und Gewürzpflanzen 2004 (2005), 210-215.

J. Jedelska, H. Koblihova, F.O. Khassanov, H. Hisoriev, P.A. Kurbonova, R.M. Fritsch, M. Keusgen:
Aroma-Präkursoren und Scavenger-Aktivität von zentralasiatischen *Allium*-Arten
Tagungsband Fachtagung für Arznei- und Gewürzpflanzen 2004 (2005), 219-224.

R. M. Fritsch, M. Gurushidze, J. Jedelská, M. Keusgen:
Better than only nice - ornamental "drumstick onions" of *Allium* subg. *Melanocrommyum* are also potential medicinal plants.
Herbertia (2007), submitted for publication.

J. Jedelská, U.M. Reinscheid, M. Keusgen:
Sulfurpyrroles – a new colour principle in nature.
In preparation.

Curriculum Vitae

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