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Allium Discoloration: The Color-Forming Potential of Individual Thiosulfinates and Amino Acids: Structural Requirements for the Color-Developing Precursors

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Structural requirements for compounds involved in *Allium* discoloration have been investigated in detail. The abilities of all 20 protein amino acids and six naturally occurring 1-propenyl-containing thiosulfinates to form the pigments have been studied. Furthermore, several analogues of these thiosulfinates were prepared by synthesis, and their color-forming abilities were evaluated, together with those of various amino compounds. It has been found that an unsubstituted primary amino group and a free carboxyl group are essential structural features required for amino compounds to be able to generate the pigments. Out of the thiosulfinate analogues tested, only those containing at least a three-carbon chain with the β-carbon bearing a hydrogen atom yielded the pigments after reacting with glycine. Thiosulfonates, sulfoxides, sulfides, and disulfides did not form any colored products when mixed with glycine. The pH optimum for pigment formation has been found to be between 5.0 and 6.0 for all thiosulfinates tested.

KEYWORDS: ***Allium*; onion; leek; garlic; pinking; reddening; greening; discoloration; pigment; thiosulfinate**

INTRODUCTION

Undesirable discoloration often occurs during the processing of garlic (*Allium sativum* L.), onion (*Allium cepa* L.), and leek (*Allium porrum* L.). In the case of garlic, intensely green, blue-green, or blue pigments are generated, whereas onion and leek homogenates may turn pink or red (*1*−*8*). The formation of these pigments significantly lowers the organoleptic quality of the product and causes great economic losses to producers. Thus, effective controlling of this discoloration process is apparently of great economic importance for the food industry. In contrast, the greening is a desirable and required process in the preparation of the traditional Chinese homemade garlic product called "Laba" (*9*, *10*).

Several explanations of this puzzling phenomenon have been suggested. The pinking of onion was once believed to be caused by some pink fungi or bacteria (*4*) or by formation of a betanine type pigment (*2*). Körner and Berk (*5*) suggested that the pinking of leek was a result of enzymatic oxidation of phenolic substances to quinones, which in turn reacted with amino acids. On the other hand, Hong and Kim (*11*) concluded that the greening of garlic was a physiological disorder occurring temporarily as a consequence of external stress to which the garlic had been exposed. In the nonscientific literature, this phenomenon is popularly explained by the reaction of sulfur-containing compounds of garlic with copper ions to yield blue cupric sulfate.

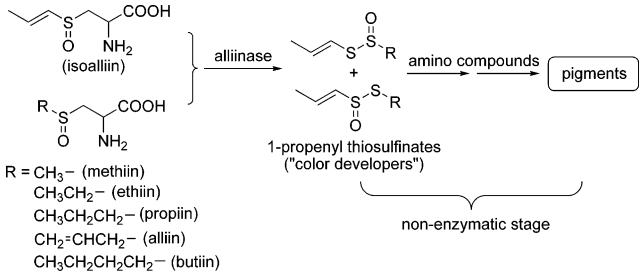

Figure 1. Formation of pigments in *Allium* species.

Shannon et al. (*6*, *7*) and Lukes (*8*) were the first to recognize the pivotal role of (*E*)-*S*-(1-propenyl)cysteine sulfoxide (isoalliin) in the pinking of onion and the greening of garlic, respectively. Recently, we showed that the discoloration of both onion and garlic is of a very similar nature, with isoalliin being the primary precursor (*12*). The discoloration is a very complex multistep process, consisting of enzymatic and nonenzymatic stages. Isoalliin, together with other *S*-alk(en)ylcysteine sulfoxides (mainly methiin, propiin, and alliin), is enzymatically cleaved upon disruption of the tissue, yielding 1-propenyl-containing thiosulfinates [(*E*)-CH_3CH═$CHS(O)SR$ and (*E*/*Z*)-CH_3CH═

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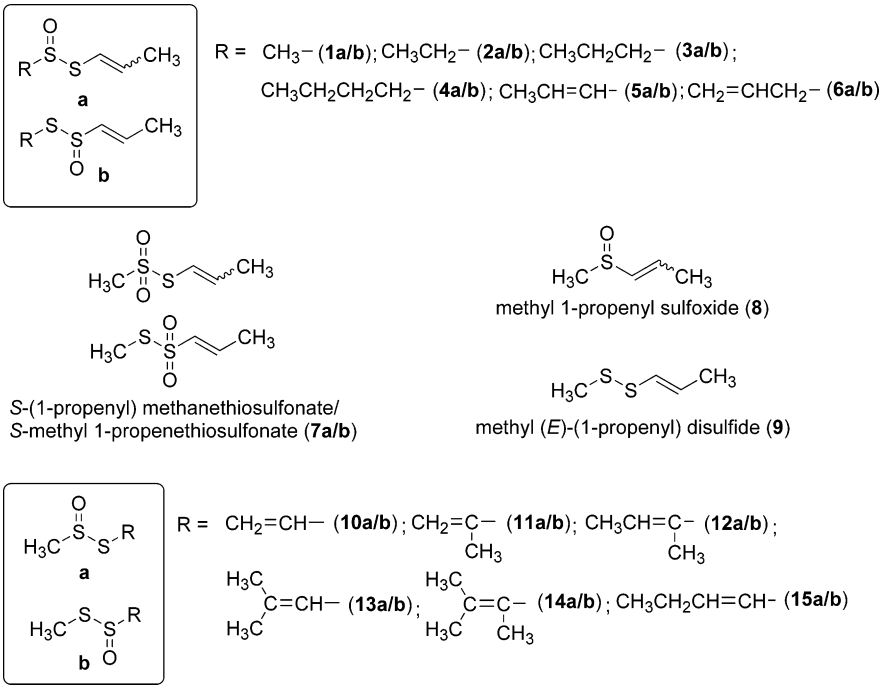


Figure 2. Structures of the thiosulfinates and their analogues tested.

CHSS(O)R]. The thiosulfinates subsequently react in the nonenzymatic stage with amino acids to produce the pigments (**Figure 1**).

Recently, Imai et al. (*13*) identified several reddish compounds generated in a model system consisting of isoalliin, alliin, alliinase, and alanine/valine to be various *N*-substituted 3,4-dimethylpyrrole oligomers. However, the chemical structure of the pigments formed in onion and garlic homogenates is likely to be far more complex. For example, Lee et al. (*14*) isolated a green-colored compound from a garlic homogenate. Although the structure of this sulfur-containing compound remains unknown, it is obviously of a different type than the dimethylpyrrole oligomers reported by Imai et al. (*13*).

Here, we describe our investigations into the relative contribution of individual amino compounds and 1-propenyl-containing thiosulfinates to discoloration of *Allium* species. Structural requirements for the color-developing precursors and possible reaction pathways involved in the discoloration process are also discussed.

MATERIALS AND METHODS

Synthesis of Reference Compounds. Alk(en)yl (*E*)-(1-propenyl) disulfides [alk(en)yl = methyl, ethyl, propyl, allyl, (*E*)-(1-propenyl), and butyl] were synthesized following the methods reported in our previous study (*12*). 1-Alkenyl methyl disulfides [vinyl, isopropenyl, (*E*/*Z*)-(1-methyl-1-propenyl), 2-methyl-1-propenyl, and (*E*/*Z*)-(1-butenyl) methyl disulfides] were prepared analogously using the appropriate alkenylmagnesium bromides and methyl thiocyanate. The yields typically ranged between 25 and 40%, and a purity of 60 and 80% was usually achieved after distillation at reduced pressure, with dimethyl disulfide and trisulfide being the major impurities. MS data of the synthesized disulfides can be found in the Supporting Information.

S-Allylcysteine, *S*-methylcysteine, alliin, methiin, and γ-glutamyl-*S*-allylcysteine were prepared according to the methods described in Kubec et al. (*15*, *16*). All other amino compounds were purchased from Lachema, Fluka, Merck, or Aldrich and were ≥99% pure. Arginine, lysine, histidine, and glycine methyl ester were in the form of hydrochlorides.

Thiosulfinates (**1a/b**−**6a/b** and **10a/b**−**15a/b**) were obtained by oxidation of the corresponding disulfides with 3-chloroperoxybenzoic acid (*m*-CPBA) as described previously (*12*). Methyl (*E*/*Z*)-(1-propenyl)sulfide and its sulfoxide (**8**) were prepared according to Block et al. (*17*). A mixture of (*E*/*Z*)-*S*-(1-propenyl)methanethiosulfonate and *S*-methyl (*E*)-1-propenethiosulfonate (**7a/b**) was prepared by oxidation of **1a/b** with $NaIO_4$ (*18*). No attempt was made to separate the individual thiosulfinate or thiosulfonate regiomers.

Model Experiments. A thiosulfinate (50 μmol, 10% solution in CH_3-OH) was mixed with 2.5 mL of an amino acid solution (0.1 M in 0.5 M KH_2PO_4 buffer) in 8 mL glass vials. The vials were capped, briefly sonicated (1 min), and kept for 6 h at 45 °C. After they were cooled to room temperature, the solutions were filtered (0.45 μm) and their UV−vis spectra were recorded. Because of the limited solubility of tryptophane, tyrosine, cystine, anthranilic acid, and 3-aminobenzoic acid, saturated solutions of these amino acids in 0.5 M KH_2PO_4 buffer were used. In all cases, at least two parallel experiments were conducted. Experiments with the thiosulfinate analogues (**7a/b**−**15a/b**) were performed analogously. After recording the absorption spectra, the model solutions were poured into clean glass vials. The vials were placed in a white surrounding, and their pictures were taken by a digital camera (Olympus C-770 UZ). The pictures were subsequently processed using Adobe Photoshop to create the tables attached in the Supporting Information.

Instrumentation. An Agilent 6890N chromatograph (Agilent Technologies) equipped with a 5973 Agilent MS detector and an HP-5 fused silica capillary column (30 m × 0.25 mm i.d.; film thickness of 0.25 μm; Agilent Technologies) was used for gas chromatography/mass spectrometry analyses. Mass spectra were obtained by electron impact (EI) ionization at 70 eV over the range of 30−425 mass units. NMR spectra were recorded on a Varian Gemini 300 HC spectrometer, and UV−vis spectra were measured on a Varian 100 BIO spectrophotometer.

RESULTS AND DISCUSSION

In the first stage of the study, the influence of pH on the pigment formation was investigated. Naturally occurring thiosulfinates (*E*/*Z*)-RS(O)SCH=$CHCH_3$ and (*E*)-RSS(O)CH=$CHCH_3$ [R = methyl, ethyl, propyl, butyl, (*E*)-(1-propenyl), and allyl, **1a/b**−**6a/b**] (**Figure 2**) were mixed with a glycine aqueous solution at pH between 2.0 and 9.0 (45 °C, 6 h). As can be seen in **Table 1**, the pH value had an enormous effect on the pigment formation. Besides, not only the intensity of the color

Table 1. Effect of pH on Pigment Formation in Model Mixtures Consisting of Glycine and Thiosulfinates (45 °C, 6 h)

	relative intensity (%) and λ_{max} (nm)								
						6a/b[a]			
pH	**1a/b**	**2a/b**	**3a/b**	**4a/b**	**5a/b**	380–470	470–620	B/Y[b]	total
2.0	4 (512)	0	0	1 (510)	20 (524)	14 (394)	7 (582)	2.1	10 (582)
2.5	8 (512)	0	0	2 (510)	19 (524)	13 (394)	11 (582)	3.5	13 (582)
3.0	14 (512)	3 (520)	0	3 (510)	22 (524)	9 (396)	13 (582)	5.8	14 (582)
3.5	25 (520)	6 (520)	6 (522)	10 (514)	27 (526)	7 (396)	15 (582)	9.1	15 (582)
4.0	48 (526)	14 (520)	8 (522)	45 (518)	35 (526)	9 (400)	21 (582)	9.2	21 (582)
4.5	99 (532)	38 (520)	14 (516)	88 (532)	43 (528)	20 (396)	45 (582)	9.5	45 (582)
5.0	99 (534)	100 (520)	23 (538)	81 (538)	75 (530)	29 (396)	80 (582)	11.6	79 (582)
5.5	100 (538)	77 (526)	100 (520)	100 (542)	100 (534)	44 (396)	100 (582)	9.5	100 (582)
6.0	78 (538)	62 (526)	53 (524)	64 (538)	89 (534)	66 (398)	63 (582)	4.0	71 (582)
6.5	52 (532)	41 (520)	66 (522)	49 (534)	58 (530)	96 (398)	46 (582)	2.0	63 (582)
7.0	26 (526)	21 (520)	54 (520)	41 (532)	34 (528)	100 (398)	27 (524)	1.1	47 (398)
7.5	16 (520)	16 (514)	27 (514)	25 (526)	18 (526)	71 (396)	19 (524)	1.1	33 (396)
8.0	8 (512)	0	22 (512)	22 (524)	9 (524)	47 (398)	14 (522)	1.2	23 (398)
8.5	0	0	0	16 (518)	3 (524)	21 (400)	8 (524)	1.7	12 (400)
9.0	0	0	0	6 (514)	0	0	1 (522)		1 (522)

[a] Intensities are expressed separately for the regions of 380–470 and 470–620 nm. [b] The ratio of integrated areas in the regions of 470–620 ("blue", B) and 380–470 nm ("yellow", Y).

but also its shade was dramatically affected by pH, especially in the case of the allyl derivatives (**6a/b**) (Supporting Information). Because the model solutions exhibited different absorption maxima λ_{max}, comparison of the absorbance values $A(\lambda_{max})$ would have been quite meaningless. In this case, the integrated areas over the whole visible region (380−620 nm) represent a more meaningful and comparable expression of the extent of discoloration. In the case of glycine, the pH optimum for the pigment formation was found to be close to the value of 5.5 for all thiosulfinates studied. A very similar pH dependence was also observed for alanine (Supporting Information). Although an anomalous behavior of some amino acids cannot be ruled out, it is reasonable to assume that most of them exhibit the pH optimum for pigment formation near to 5.5 as well. Apparently, the observed optimal pH is very close to the natural pH values of onion or garlic homogenates. For example, we found the pH of onion and garlic homogenates to be 5.6 and 6.1, respectively, whereas other researchers reported the pH of onion juice to be 5.2−5.4 and that of garlic to be 6.2−6.3 (*19*−*21*).

Our results obtained with model systems correspond quite well with the data reported previously for onion, leek, and garlic homogenates. For example, Joslyn and Sano (*1*) found that the extent of garlic greening was greatest at pH 4.0−5.0, whereas Kim and Kim (*22*) reported the maximum at pH 5.5. Slightly lower pH values were observed for the pinking of onion and leek homogenates. Lee and Parkin (*19*) found a pH optimum of 4.0−5.0 for pinking of an onion homogenate, while Shannon et al. (*6*) determined a sharp optimum at pH 4.6−4.8 for the pigment formation in a mixture of onion juice, glycine, and formaldehyde. Körner and Berk (*5*) observed that the leek pinking was maximal at pH 4.0−4.5 within the temperature range of 45−50 °C.

On the basis of visual observations, no considerable differences were found in the pigment-forming abilities of the four thiosulfinate compounds with a saturated side chain (methyl, ethyl, propyl, and butyl, **1a**/**b**−**4a**/**b**). On the other hand, the two thiosulfinate derivatives containing an unsaturated side chain (1-propenyl **5a**/**b** and allyl **6a**/**b**) exhibited a significantly enhanced tendency to generate colored products after mixing with amino acids. The absorption spectra of model mixtures prepared from Gly or Ala and **1a**/**b**−**5a**/**b** at pH 2.0−9.0 always exhibited only a single maximum in the visible region (380−620 nm). The absorption maxima λ_{max} varied between 510 and 542 nm, being pH-dependent with slight shifts toward lower wavelengths outside the pH region of 5.0−6.0 (the optimal pH for pigment formation) (**Table 1**). In contrast, the absorption spectra of pigments obtained from the allyl derivatives **6a**/**b** usually contained several local maxima (**Figure 3**). Typically, the color of the model mixtures prepared from Gly/Ala and **6a**/**b** was blue or dark blue at pH ≤ 6.0, showing a maximum near 580 nm with a significant shoulder around 530 nm. However, at pH > 6.0, the resulting color tended to be reddish, and at pH > 8.0, yellowish tones predominated (λ_{max} near 396 and 440 nm) (Supporting Information).

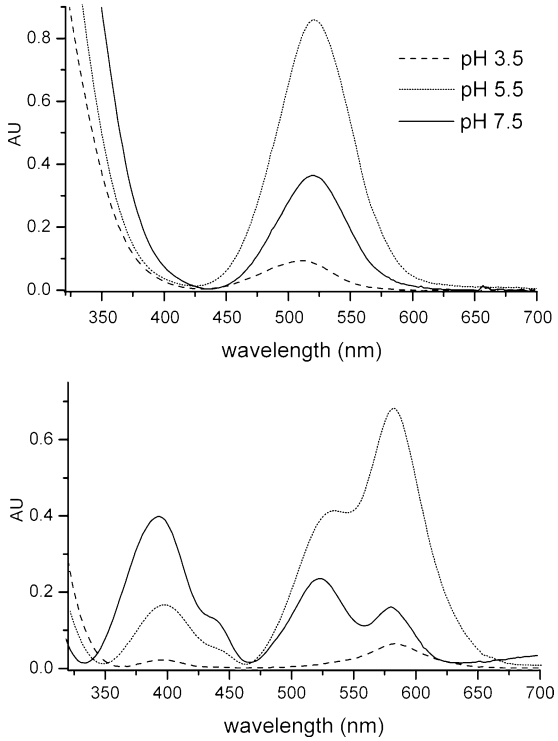


Figure 3. Absorption spectra of model mixtures consisting of alanine and **5a/b** (top) or **6a/b** (bottom) at different pH values.

We have also elucidated the pigment-forming abilities of other protein amino acids. It has been found that all of them except for cysteine and proline were able to form colored products when mixed with 1-propenyl-containing thiosulfinates (**1a**/**b**−**6a**/**b**)

Table 2. Relative Intensities and Absorption Maxima of the Pigments Formed from Individual Amino Acids and Thiosulfinates (45 °C, 6 h, pH 5.5) (Relatively to Glycine)[a]

	relative intensity (%) and λ_{max} (nm)						refs				
	1a/b	**2a/b**	**3a/b**	**4a/b**	**5a/b**	**6a/b**	*4*[b]	*5*[c]	*6*[d]	*23*[e]	*24*[f]
Gly	100 (534)	100 (524)	100 (530)	100 (538)	100 (530)	100 (584)	+	+	100	100	100
Ala	172 (520)	145 (518)	173 (528)	99 (534)	119 (524)	121 (582)	+	NP	109	66	64
Val	63 (512)	97 (512)	74 (518)	20 (518)	57 (516)	69 (582)	+	+	107	NP	84
Leu	22 (514)	52 (514)	41 (524)	7 (522)	40 (518)	37 (580)	+	NP	96	NP	55
Ile	19 (512)	68 (512)	34 (518)	4 (518)	24 (516)	39 (580)	+	NP	83	NP	65
Phe	10 (512)	54 (512)	24 (516)	4 (514)	25 (514)	50 (588)	+	+	172	NP	62
Tyr	243 (512)	32 (516)	84 (530)	17 (530)	43 (518)	71 (586)	+	+	40	NP	23
Ser	0	0	0	0	0	78 (582)	+	+	28	NP	83
Thr	13 (446)	1 (448)	1 (448)	0	2 (450)	47 (582)	–	NP	26	NP	54
Cys	0	0	0	0	0	0	–	NP	0	0	0
Met	6 (518)	4 (518)	6 (526)	4 (526)	23 (518)	38 (582)	+	NP	103	99	79
Pro	0	0	0	0	0	0	–	+	0	0	0
Trp	0	0	0	0	9 (518)	7 (586)	–	NP	little (+)	NP	18
His	0	0	0	0	0	5 (446)	–	NP	27	NP	8
Lys	168 (520)	60 (518)	87 (524)	69 (526)	81 (522)	74 (582)	+	NP	59	77	48
Arg	122 (518)	69 (518)	72 (520)	68 (524)	66 (520)	124 (590)	+	NP	116	42	72
Asp	123 (516)	24 (518)	72 (528)	58 (534)	76 (520)	163 (582)	+	+	44	43	NP
Asn	108 (516)	13 (514)	33 (518)	41 (522)	55 (516)	61 (586)	–	NP	95	NP	98
Glu	141 (516)	73 (516)	121 (524)	90 (526)	95 (520)	181 (584)	+	+	53	74	70
Gln	128 (516)	46 (514)	83 (520)	63 (526)	74 (518)	99 (586)	NP	NP	NP	NP	94

[a] NP, not performed. [b] An Et_2O extract of white onion, pH 5.6, 40 °C, a few hours. [c] Leek juice, no pH control, 45 °C, 24 h. [d] $A_{520\ nm}$, an Et_2O extract of white onion + formaldehyde, pH 5–6, 50 °C, 90 min. [e] $A_{520\ nm}$, an Et_2O extract of garlic + formaldehyde, 40 °C, 24 h. [f] $A_{590\ nm}$, isoalliin + alliinase + allicin, pH 5.6, 37 °C, 3 days.

at pH 5.5 (the pH optimum for Gly and Ala). However, the intensity of the color formed as well as the λ_{max} values varied considerably (**Table 2**). The greatest color-generating ability exhibited alanine, followed by glycine and glutamic acid. On the other hand, histidine and tryptophane showed only a limited tendency to yield colored products after mixing with the thiosulfinates. Interestingly, serine and threonine formed only little or no pigment with **1a**/**b**−**5a**/**b**, but they produced a significant amount of a blue pigment with **6a**/**b**. As with Gly and Ala, most of the other amino acids yielded pink, red, or magenta products upon mixing with thiosulfinates **1a**/**b**−**5a**/**b**, with only a single λ_{max} between 512 and 530 nm. In contrast, the allyl derivatives **6a**/**b** usually generated blue or violet pigments at pH 5.5 (λ_{max} of 580−590 nm with other local maxima near 400, 440, and 518−530 nm). Only methionine, histidine, and tryptophane yielded greenish pigments under the experimental conditions (Supporting Information). None of the model mixtures, however, exhibited any significant absorption near 560 nm. Hence, the greenish tones observed in several model mixtures were only a consequence of parallel formation of blue and yellow compounds.

Several authors have reported data on the color-forming potential of amino acids (*4*−6, *23*, *24*). However, the data published are fragmentary and, in many cases, quite controversial, being obtained under different experimental conditions (e.g., different pH, time, and temperature). Besides, various thiosulfinate sources (leek, onion, or garlic extracts) were used, hence showing very different thiosulfinate profiles. On the other hand, here, we report for the first time data obtained with individual naturally occurring 1-propenyl thiosulfinates. As can be seen in **Table 2**, our data correspond quite well with the above-mentioned studies.

The pigment-forming abilities of several nonprotein amino acids and dipeptides have also been studied (**Table 3**; Supporting Information). In agreement with previous reports (*6*, *24*), no pigment was formed with betaine, sarcosine, and glycine methyl ester upon mixing with **5a**/**b** or **6a**/**b**. Apparently, both an unsubstituted primary amino group and a free carboxyl group

Table 3. Intensity and Absorption Maxima of the Pigments Formed from Various Amino Acids and Dipeptides (45 °C, 6 h, pH 5.5) (Relatively to Glycine)[a]

	relative intensity (%) and λ_{max} (nm)		refs	
	5a/b	**6a/b**	*6*[b]	*24*[c]
glycine methyl ester	2	4	0	NP
N-methylglycine (sarcosine)	0	0	0	NP
N,*N*-dimethylglycine (betaine)	0	0	0	NP
β-alanine	86 (526)	68 (580)	107	NP
α-methyl alanine	15 (524)	19 (580)	NP	NP
4-aminobutyric acid	58 (524)	28 (580)	79	31
5-aminovaleric acid	25 (526)	13 (580)	NP	NP
1-aminocyclohexane carboxylic acid	6 (510)	4 (582)	NP	NP
(*Z*)-2-aminocyclohexane carboxylic acid	7 (512)	12 (580)	NP	NP
anthranilic acid	0	0	NP	NP
3-aminobenzoic acid	0	0	NP	NP
4-hydroxyproline	3	0	NP	NP
cysteic acid	22 (516)	39 (588)	23	NP
cystine	9 (526)	24 (584)	17	NP
S-methylcysteine	33 (522)	60 (588)	126	NP
methiin	14 (514)	90 (446)	61	NP
S-allylcysteine	4 (518)	23 (584)	NP	NP
alliin	28 (448)	43 (448)	NP	NP
γ-glutamyl-*S*-allylcysteine	30 (512)	6 (472)	NP	NP

[a] NP, not performed. [b] $A_{520\ nm}$, an Et_2O extract of white onion + formaldehyde, pH 5–6, 50 °C, 90 min. [c] $A_{590\ nm}$, isoalliin + alliinase + allicin, pH 5.6, 37 °C, 3 days.

are essential structural features of color-developing amino compounds. Unlike the alicyclic amino compounds studied, the aromatic ones did not form colored products upon the reaction with **5a**/**b** or **6a**/**b**. On the other hand, the straight-chain 3-, 4-, and 5-amino acids showed a significant pigment-forming potential, which gradually decreased with the increasing distance between the amino and the carboxyl groups. Unlike cysteine, various cysteine derivatives, which lack a free sulfhydryl group −SH (cystine, cysteic acid, *S*-alkylcysteines, and their sulfoxides), were capable of forming colored products when mixed with **5a**/**b** or **6a**/**b**. The inability of cysteine to form the pigment

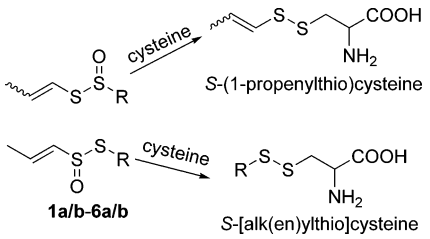


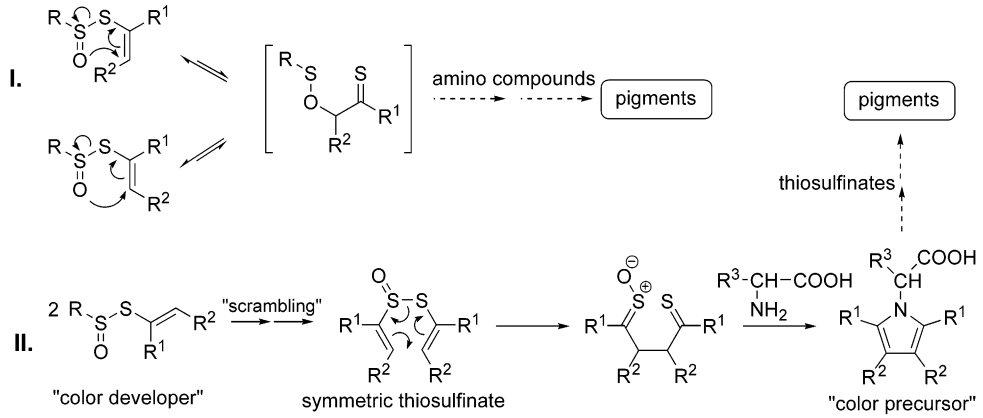
Figure 4. Inhibition effect of cysteine on *Allium* discoloration.

can be easily explained by its reaction with thiosulfinates to yield *S*-[alk(en)ylthio]cysteine derivatives (*25*) (**Figure 4**). γ-Glutamyl-*S*-allylcysteine dipeptide, an abundant component of garlic, also formed colored products with both **5a**/**b** and **6a**/**b**.

Data on the free amino acid composition of *Allium* vegetables are surprisingly scarce in the literature. Although the composition varies significantly, Gln, Glu, Asn, Asp, Arg, and Lys are typically the most abundant free protein amino acids in onion, leek, and garlic (*26*−*31*). Wünsch et al. (*32*) reported γ-aminobutyric acid (GABA) to be present in substantial amounts in leek. All of these amino acids also exhibit a very high color-forming potential (**Table 2**). However, the predominant free amino acids in intact *Allium* species are various *S*-alk(en)-ylcysteine sulfoxides (alliin in garlic and isoalliin in onion and leek). Even though these sulfur amino acids are rapidly enzymatically cleaved upon tissue disruption, a substantial amount of these compounds remains undecomposed even in finely minced homogenates (*33*, *34*). It can therefore be concluded that Glu, Gln, Asn, Asp, Arg, Lys, GABA, and the undecomposed portion of *S*-alk(en)ylcysteine sulfoxides are the major free amino acid contributors to the discoloration. However, γ-glutamyl-*S*-alk(en)ylcysteines and their sulfoxides together with other oligopeptides are very abundant components found in the tissue. Although we tested only one member of this oligopeptide group, γ-glutamyl-*S*-allylcysteine, it can be assumed that also other γ-glutamyl dipeptides possess the color-forming ability. Thus, most likely, these compounds can also significantly participate in the discoloration process.

To get a better insight into the reaction pathways involved in the formation of the pigments, we also decided to study the pigment-forming abilities of selected 1-propenyl thiosulfinate analogues. Several structural analogues of naturally occurring thiosulfinates **1a**/**b** were prepared by synthesis and allowed to react with glycine. These included the corresponding thiosulfonates (**7a**/**b**), sulfoxide (**8**), disulfide (**9**), and various thiosulfinate analogues (**10a**/**b**−**15a**/**b**) (**Figure 2**). Out of the compounds tested, only those containing at least a three-carbon chain with unsubstituted β-position, i.e., the 1-methyl-1-propenyl (**12a**/**b**) and (*E*/*Z*)-(1-butenyl) (**15a**/**b**) analogues, formed colored products (λ_{max} of 506 and 530 nm, respectively). None of the other compounds were able to form a pigment under the experimental conditions (pH 5.5, 45 °C, 6 h). Most likely, the two active thiosulfinates can undergo a [2,3]-sigmatropic rearrangement similar to that described by Block et al. (*18*) for **1a**−**6a**. This rearrangement would yield the corresponding thials as highly reactive intermediates, perhaps capable of reacting with amino compounds (**Figure 5**). On the other hand, the sulfide, sulfoxide, thiosulfonate (**7**−**9**, respectively), and the thiosulfinates without the color-forming ability (**10a**/**b**, **11a**/**b**, **13a**/**b**, and **14a**/**b**) cannot undergo this rearrangement. Another possible explanation of the color-forming ability of **12a**/**b** and **15a**/**b** lies in their transformation into symmetric α,β-unsaturated thiosulfinates by the well-documented scrambling reaction. The latter compounds would consequently follow the mechanism proposed by Imai et al. (*13*) for **5a**/**b**, consisting of their rearrangement into dithial *S*-oxides, which could react with amino acids to yield *N*-substituted pyrrole derivatives (**Figure 5**). However, the pigment-forming ability of **12a**/**b** is rather surprising. If the mechanism proposed by Imai et al. (*13*) was followed, the reaction of **12a**/**b** with an amino acid would lead to the formation of *N*-substituted 2,3,4,5-tetramethylpyrrole as an intermediate (the color precursor). However, this pyrrole would not be able to form any oligomeric structures by cross-linking at C-2 and C-5 without losing its aromaticity. Further research is therefore needed to clarify this observation.

It can be stated that the extent of discoloration depends on many factors, some of them being quite unpredictable and difficult to control. Apparently, the key factor affecting the discoloration process is the level of isoalliin in the tissue. The content and relative proportions of the other *S*-alk(en)ylcysteine sulfoxides seem to be less important. Isoalliin serves as the precursor of 1-propenyl-containing thiosulfinates (**1a**/**b**−**6a**/**b**) (the color developers), and it is also the major nonvolatile precursor of the onion and leek pungency. A maximal content



$R^1 = H-, R^2 = CH_3-, R = CH_3-$ (**1a**); $R^1 = H-, R^2 = CH_3-, R = CH_3CH_2-$ (**2a**);

$R^1 = H-, R^2 = CH_3-, R = CH_3CH_2CH_2-$ (**3a**); $R^1 = H-, R^2 = CH_3-, R = CH_3CH_2CH_2CH_2-$ (**4a**);

$R^1 = H-, R^2 = CH_3-, R = CH_3CH{=}CH-$ (**5a**); $R^1 = H-, R^2 = CH_3-, R = CH_2{=}CHCH_2-$ (**6a**);

$R^1 = CH_3-, R^2 = CH_3-, R = CH_3-$ (**12a**); $R^1 = H-, R^2 = CH_3CH_2-, R = CH_3-$ (**15a**)

Figure 5. Thiosulfinate rearrangements possibly involved in *Allium* discoloration.

of isoalliin is thus desirable and highly prized in these two *Allium* species. Selecting onion/leek cultivars with a reduced isoalliin content (and thus with a lower tendency to pinking) would therefore cause lowering of both their pungency and their pharmaceutical values. On the other hand, being only a minor component in garlic [typically <5% of the *S*-alk(en)ylcysteine sulfoxide pool] (*15*), the effect of isoalliin on organoleptic/pharmaceutical properties of garlic is far less important. Hence, selecting garlic varieties with suppressed isoalliin biosynthesis may be an elegant way to completely prevent the greening without the need of using any additives or special technological treatment. The level of isoalliin may also be quite efficiently governed by postharvest storage. For example, Kopsell et al. (*35*) reported a significant increase in the isoalliin content in onion stored at 5 °C. A several-fold increase was observed in the formation of isoalliin-derived thiosulfinates (the color developers) in homogenates prepared from garlic stored at 4 °C (*36*). As a consequence, Lukes (*8*) found that garlic stored at 3 °C for 2−4 weeks was much more susceptible to greening than that stored at regular temperature. Excessive low-temperature storage of garlic and onion intended for industrial processing should therefore be avoided to eliminate their tendency for discoloration.

Another important factor affecting the extent of discoloration is the overall conversion of isoalliin into 1-propenyl-containing thiosulfinates **1a**/**b**−**6a**/**b**. The total content of these color-developing thiosulfinates depends mostly on (i) the degree of homogenization, (ii) the pH value during homogenization, and, in the case of onion, (iii) the relative activities of alliinase and lachrymatory synthase (*37*). The higher the lachrymatory synthase activity is, the less 1-propenyl-containing thiosulfinates are formed, with proportionally higher amounts of propanethial *S*-oxide being generated instead. The latter compound (the onion lachrymatory factor) was found not to possess the color-developing ability (*12*). Such onion would therefore be more pungent with a lower potential for pinking.

As shown in this and several previous studies, the pH value can dramatically affect not only the formation of the color-developing thiosulfinates (**1a**/**b**−**6a**/**b**) but also their subsequent reactions with amino compounds. It was demonstrated that a maximal amount of 1-propenyl-containing thiosulfinates in homogenized garlic is formed between a pH of 4.5 and 5.0 (*38*). It is also an optimal pH range for the pigment formation. Therefore, lowering the pH of the homogenate below 4.0 as quickly as possible is highly important to minimize the extent of discoloration.

A lot of novel information about *Allium* discoloration has recently been obtained. The natures of both garlic greening and onion/leek pinking have been clarified, together with well-proved identification of the key precursors and intermediates (the color developers) (*12*). In this study, we have thoroughly examined the color-forming potential of various amino compounds and thiosulfinates. Moreover, Imai et al. (*13*) revealed the structure of one group of colored compounds generated in model mixtures simulating discoloration in garlic homogenates. However, continuous research in our laboratory clearly shows that the discoloration is an immensely complex process, yielding hard to separate mixtures of many colored compounds even when only simple model mixtures are studied. Further research is aimed at identification of major colored compounds formed during the reaction of thiosulfinates **1a**/**b**−**6a**/**b** with amino compounds and determination of the reaction pathways involved.

ABBREVIATIONS USED

A, absorbance; *m*-CPBA, 3-chloroperoxybenzoic acid; GABA, γ-aminobutyric acid.

Supporting Information Available: EI MS data of the newly synthesized 1-alkenyl methyl disulfides, data showing the effect of pH on pigment formation in model mixtures of alanine and thiosulfinates, and data showing the resulting color of model mixtures of various amino compounds and thiosulfinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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