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Isolation and Identification of a Red Pigment from *Allium* Subgenus *Melanocrommyum*

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Three new sulfur-containing compounds were identified in *Allium* L. species belonging to the subgenus *Melanocrommyum* as the first examples of sulfur-containing pyrrole derivatives in nature. Some of these species are traditionally used in Southwest and Central Asia as vegetables and herbal drugs. A hypothetical biogenetic scheme is proposed in which L-(+)-*S*-(3-pyrrolyl)cysteine sulfoxide is enzymically degraded. The resulting 2-lactyl-3′-pyrrolyl sulfoxide is condensed readily to the red pigment 3,3′-dithio-2,2′-dipyrrole. All compounds are chemically unstable, rendering the analysis extremely difficult. Correlation NMR in combination with diffusion NMR allowed the identification of these low molecular weight compounds. For the first time, the compounds involved in the coloring process of *Allium* plant material have been identified from native plant material.

KEYWORDS: ***Allium*; *Melanocrommyum*; pinking; discoloration; dithiodipyrrole; 3′-pyrrolyl cysteine sulfoxide; structure elucidation; biogenesis**

INTRODUCTION

The genus *Allium* L. contains more than 700 species worldwide. They occur mainly in the semiarid regions of Europe, North America, North Africa, and Asia. Species such as garlic (*A. sativum* L.) and onion (*A. cepa* L.) have been commonly used since ancient times not only as a spice and vegetable but also as a medicinal plant. The beneficial antibiotic (*1*) and antidiabetic (*2*) activities of garlic are well-known and related to a great number of bioactive sulfur-containing compounds (*3, 4*). Despite their long history in traditional medicine, especially in Asia, some *Allium* species are almost unknown and not well characterized, mostly due to geopolitical reasons.

Among these, species of the subgenus *Melanocrommyum* Webb et Berth. Rouy are only known in the Western world as ornamental plants, the so-called “drumstick” onions. However, more than 200 species of this subgenus exist worldwide, growing mainly in Southwest and Central Asia. Ethnobotanical studies revealed a broad spectrum of uses for representatives of the subgenus *Melanocrommyum* (*5, 6*). In native populations, most species are used as vegetables or also as spicy vegetables such as *A. stipitatum* Regel. Others are used for medicinal purposes: for instance, *A. motor* Kamelin et Levichev is used as a tonic after long winters and *A. rosenorum* R. M. Fritsch is used for wound healing (*7*). Further species of interest are *A. macleanii* J. G. Baker and *A. giganteum* Regel.

Most of the species of the subgenus *Melanocrommyum* used in a traditional manner by native populations are characterized by a deep orange to red ichor (fluid discharge) occurring after damage of the cells. From local reports it can be assumed that plants producing this red dye were also traditionally used for staining of textiles. The formation of this red dye requires only about 30 s after plant material has been wounded. A slow discoloration of plant material over hours and days is also well-known for *Allium* species that are widely used in Europe and America (*8, 9*). Onion (*A. cepa* L.) and garlic (*A. sativum* L.) develop intensive discolorations under certain conditions. Onion purees and powders turn slowly red when acidified and stored at room temperature, more quickly under increased temperature. Garlic, on the other hand, develops a green-blue color under equivalent conditions, posing a problem in product processing. In contrast, this discoloration is desired for the traditional Chinese product “laba”, which is homemade with garlic and vinegar in the winter and eaten at the Chinese New Year’s Eve (*10*). Despite 50 years of research on the sulfur chemistry of *Allium*, little is known about the formation of these dyes in the plants themselves, whereas the compounds and the mechanisms of formation of flavors have been thoroughly analyzed (*11, 12*). Model systems showed that probably a reaction of isoalliin or alliin with alliinase could be the first step of this reaction (*12–15*). However, none of the described structures was ever directly isolated from fresh plant samples. The only information about color formation in *Allium* species is available from model reaction systems. The Imai group (*15*) mixed purees of onion

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(*A. cepa*) and garlic (*A. sativum*) and isolated two pigment precursors (PPs) and a reddish purple pigment (PUR-1) from these mixtures. PPs were isolated from a heat-treated solution containing color developer and either L-valine or L-alanine, and their structures were determined as 2-(3,4-dimethylpyrrolyl)-3-methylbutanoic acid (PP-Val) and 2-(3,4-dimethyl-1*H*-pyrrolyl)propanoic acid (PP-Ala), respectively. The structure of PUR-1 was determined as (1*E*)-1-[1-((1*S*)-1-carboxy-2-methylpropyl)-3,4-dimethyl-1*H*-pyrrole-2-yl]-prop-1-enylene-3-[1-((1*S*)-1-carboxy-2-methylpropyl)-3,4-dimethyl-1*H*-pyrrole-2-yl]idenium.

Another example of the diversity of natural dyes based on pyrrole structures is the prodiogine alkaloids, which are not related to *Allium*. A variety of reports about the "bleeding" of bread and other starch-containing foods are known, particularly from the Middle Ages (*16*). Cases such as these in more recent times were proved to be caused by *Serratia marcescens.* Several microorganisms similar to *Serratia* and S*treptomyces* species form deep red colonies (*16, 17*). Until today, a wide range of substances of the chemically diverse class of prodiogines have been isolated, among them the linear tripyrrolyl prodigiosin (2-methyl-3-amyl-6-methoxyprodigiosene) produced by *S. marcescens* (*18*). This class of substances received attention due to their anticancer and immunosuppressant activities (*18, 19*).

In this study we investigated species of the subgenus *Melanocrommyum* that produced a red dye. Besides structure elucidation of pyrroles isolated from *A.* subg. *Melanocrommyum*, a hypothetical biogenetic scheme for the formation of the red dye is presented.

MATERIALS AND METHODS

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

Plant Material. The plant material (*Allium giganteum* Regel, *Allium rosenorum* R.M. Fritsch, and *Allium jesdianum* Boiss. et Buhse, closely related species of the genus *Allium*, subgenus *Melanocrommyum*) was collected in Tajikistan and Iran. Voucher specimens were deposited in the living plant collection at IPK Gatersleben, Germany.

Plant Processing. *Extraction Procedure for L-(+)-S-(3-Pyrrolyl)-cysteine Sulfoxide (***1***).* Bulbs (10.5 g fresh weight, *A. rosenorum*) were homogenized under liquid nitrogen and extracted three times with methanol at 4 °C for enzyme inactivation and then three times at 4 °C for 3–4 h with water. The combined water fractions were lyophilized and stored at –20 °C. Further purification was performed using preparative RP-HPLC.

*Extraction Procedure for 2-Lactyl-3′-pyrrolyl Sulfoxide (***3***).* Bulbs (19.8 g fresh weight, *A. giganteum*) were cleaned, peeled, chopped into small pieces, and put immediately into methanol to inhibit enzymic activity. After 20 min, the pieces were homogenized in methanol and extracted with methanol for 45 min. The filtered and evaporated extract was further separated using LC followed by HPLC.

*Extraction Procedure for 3,3′-Dithio-2,2′-dipyrrole (***5***).* Bulbs (258.4 g fresh weight, *A. giganteum*) were cleaned, peeled, and chopped in a mixer (Braun KSM1) to give a homogeneous matter. To avoid dryness, 60 mL of water was added, and the matter was incubated for 16 h at room temperature. The color turned orange-red. The residue was filtered through linen and extracted first with 500 mL of dichloromethane and then with 800 mL of ethyl acetate. After filtration and evaporation, the extracts were separated using LC.

LC Separation. LC separations were performed using a cylindrical glass column (420 × 35 mm) with a small delivery tube wand and a stopcock at its end. As stationary phase (absorbent), silica gel 60 was used. Mobile phases and separation procedures are described in detail for the single substances.

*Separation Procedure for L-(+)-S-(3-Pyrrolyl)cysteine Sulfoxide (***1***).* Preparative HPLC was performed on a 250 × 25 mm i.d., 5 μm, Lichrospher RP Select B column (Merck, Darmstadt, Germany). A methanol/water gradient was used (A, H_2O; B, MeOH): isocratic 100% A for 15 min, 27 mL/min; 100–5% A over 10 min, 39 mL/min; isocratic 5% A for 15 min, 39 mL/min; UV detection at 254 and 200 nm. The precursor **1** eluted at 7.0 min. Compound **5** could be detected at 46 min using the same system. The collected fractions of **1** (57 mg, 0.013% related to fresh weight of bulbs) were instantly dried under vacuum and stored at – 20 °C.

*Separation Procedure for 2-Lactyl-3′-pyrrolyl Sulfoxide (***3***).* Seventy-five grams of silica gel 60 was suspended in 250 mL of *n*-hexane and poured into the chromatography column. Elution of the extract was performed using a stepwise gradient of *n*-hexane/ethyl acetate/methanol/water (100 mL each). Fractions containing pyrrole compounds were combined, and solvent was removed under vacuum. Further purification was performed by HPLC on a 250 × 4 mm i.d., 5 μm Spherimage ODS 2 RP column with integrated guard column (Knauer, Berlin, Germany). Detection was performed at 254 nm. Water (pH 6) was used as solvent (1 mL/min). The pyrrole sulfoxide eluted at 5.8 min. The same fractions were combined (0.7 mg, mixture with various sugars) and directly used for further investigations.

*Separation Procedure for 3,3′-Dithio-2,2′-dipyrrole (***5***).* Seventy grams of silica gel was suspended in petroleum ether (40–60 °C). Elution of the extract was performed using a stepwise gradient of petroleum ether/ethyl acetate/methanol. Fractions exhibiting a red color (8 mg) were combined and used for further investigation. Purity could be controlled by the HPLC system described for compound **1**.

Analytical Measurements. ESI-MS measurements were conducted using a Shimadzu LC 20 HPLC system containing an autosampler, a high-pressure mixing pump, a column oven, and a UV detector in combination with a QTrap 2000 equipped with a TurboIonspray ion source (Applied Biosystems/MDS Sciex, Toronto, Canada). The ESI-MS operating conditions were as follows: positive ion mode or negative ion mode (compound **3**), scan range, 30–1700 amu; source temperature, 200 °C; ion spray voltage, 44.5 kV; curtain gas, 10; declustering potential, 80 or 190 V; entrance potential, 10 V; flow rate, 10 μL/min (direct infusion), 0.2 mL/min (HPLC separation).

NMR experiments were conducted on a Bruker Avance 400 spectrometer. By the aid of standard correlation experiments (COSY, TOCSY, HSQC, HMBC) and NOESY/ROESY experiments, structure elucidation was performed. Diffusion experiments were run for the analysis of 2-lactyl-3′-pyrrolyl sulfoxide with standard Bruker pulse sequences (*20*). Structure elucidation of compound **5** was also performed with a Bruker AMX 300 spectrometer.

The measurement of optical rotation was performed on a Jasco DIP-370 digital photometer at 589 nm and 20 °C. The sample was dissolved in water.

Analytical Data of the Identified Compounds. *L-(+)-S-(3-Pyrrolyl)cysteine sulfoxide (***1***, **Figure 1**):* ^{13}C NMR (100.61 MHz, D_2O) δ 51.3 (C2), 53.9 (C3), 110.3 (C4′), 115.9 (C5′), 124.7 (C3′), 126.5 (*C*2′), 174.3 (C1); ^{1}H NMR (400.13 MHz, D_2O) δ 3.64 (dd, 1, $J = 6.7$, 5.7 Hz, H3), 3.83 (dd, 1, $J = 7.8$, 5.7 Hz, H3), 4.23 (dd, 1, $J = 7.8$, 6.7 Hz, H2), 6.35 (dd, 1, $J = 3.0$, 3.9 Hz, H4′), 6.92 (dd, 1, $J = 1.0$, 3.9 Hz, H5′), 7.22 (dd, 1, $J = 1.0$, 3.0 Hz, H2′); FT-IR (ν_{max}, KBr) 3200, 1620, 1410, 1380, 1000, 750; UV (λ_{max}, H_2O) 220, 250; HR-ESI-MS, 203.044761; molecular formula, $C_7H_{10}N_2O_3S$; MS calculated ($M + H^+$), 203.0490. Dimer sodium adduct $C_{14}H_{20}N_4O_6S_2Na$: HR-ESI-MS, 427.074966; MS calculated, 427.072198; specific optical rotation [α_D^{20}], +53.3 mL g^{-1} dm^{-1} (589 nm, in water).

*2-Lactyl-3′-pyrrolyl sulfoxide (***3***, **Figure 2**):* ^{13}C NMR (100.61 MHz, DMSO-d_6) δ 22.0 (C3), 76.6 (C2), 108.1 (C4′), 111.9 (C2′), 123.1 (C5′), 130.3 (C3′), 170.2 (C1); ^{1}H NMR (400.13 MHz, DMSO-d_6) δ 1.97 (s, 3, H3), 6.14 (dd, 1, $J \approx 1$, 3 Hz, H4′), 6.55 (dd, 1, $J \approx 1$, 3 Hz, H2′), 7.06 (dd, 1, $J \approx 1$, 3 Hz, H5′); molecular formula, $C_7H_9O_4NS$; MS calculated ($M - H^+$), 202.0174; ESI-MS measured, 201.816.

*3,3′-Dithio-2,2′-dipyrrole (***5***, **Figure 3**):* ^{13}C NMR (75 MHz, CD_3OD) δ 108.0 (C5, C5′), 120.3 (C4, C4′), 125.5 (C2, C2′), 132.0 (C3, C3′); ^{1}H NMR (300 MHz, MeOD) δ 6.43 (d, 2, $J = 3.66$ Hz, H5, H5′), 6.28 (d, 2, $J = 3.66$ Hz, H4, H4′); FT-IR (ν_{max}, KBr) 3370, 3108, 2922, 2851, 2360, 2341, 1697, 1524, 1455. 1392, 1186, 1073, 1046, 1038, 768; UV (λ_{max}, MeOH) 519, 297; molecular formula, $C_8H_6N_2S_2$; MS calculated [M^+], 193.9996; HR-EI-MS, 193.9984.

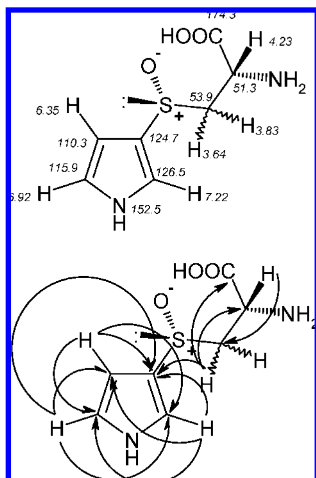

Figure 1. Formula and ^{1}H,^{13}C HMBC correlations of L-(+)-*S*-(3-pyrrolyl)cysteine sulfoxide (**1**).

RESULTS AND DISCUSSION

Red Coloring of Plant Material. During botanical expeditions in Central and Southwest Asia, a red pigmentation of plant material directly after harvest was observed. It was assumed that this pigmentation was caused either by air oxidation or by an enzymic reaction. However, exclusion of oxygen also led to formation of a red dye. In further experiments, plant material of *A. giganteum* Regel developed a red color under several conditions: heating of the material; alkaline pH; and incubation with alliinase from garlic. Likewise, a crude or enzyme extract of the same species catalyzed the formation of a red pigment when incubated with a methanolic, enzyme-free extract of the same species. These findings led to the assumption that an alliinase-like enzyme is involved in the formation of this red dye.

Isolation and Identification of the Precursor L-(+)-*S*-(3-Pyrrolyl)cysteine Sulfoxide. To elucidate molecules involved in the formation of the red dye, three different purification procedures (aqueous/methanolic extracts and lipophilic extract) allowed the identification of two precursor substances, **1** and **3**, and a red pigment, **5**. MS data of **1** indicated a compound with two nitrogen atoms and a molecular mass of 202 g/mol. The structure of this new substance was mainly elucidated by NMR. Correlation experiments (HSQC, HMBC, COSY) allowed structure elucidation, but gave only limited information about relative stereochemistry; HMBC correlations are shown in **Figure 1**. Particularly useful was the correlation between the methylene protons (3.64 ppm) and the quaternary carbon atom (C3′) of the pyrrole ring (124.7 ppm), which established the connection between these two parts of the molecule. The complete pyrrole ring is characterized by proton signals at 7.22 ppm (H2′), 6.35 ppm (H4′), and 6.92 ppm (H5′). The corresponding carbon atoms were found at 126.5 ppm (C2′), 110.3 ppm (C4′), and 115.9 ppm (C5′).

The cysteine moiety of compound **1** revealed signals that strongly correlate with those found for a set of synthetic cysteine sulfoxides (*21*). For L-(+)-alliin, proton signals at 4.18 ppm (H2), 3.21 ppm (H3), and 3.45 ppm (H3) as well as at 174.0 ppm (C1), 53.7 ppm (C2), and 57.9 ppm (C3) were found. For compound **1**, the corresponding chemical shifts are 4.23 ppm (H2), 3.64 ppm (H3), 3.83 ppm (H3), 174.3 ppm (C1), 51.3 ppm (C2), and 53.9 ppm (C3). This indicates that this substance is derived from L-cysteine. Comparison with ^{1}H NMR spectra of synthetic (+) and (−)-cysteine sulfoxides gave evidence that compound **1** is also a (+)-isomer. This can be deduced from chemical shifts of protons H3. For (+)-cysteine sulfoxides, the difference between these two protons at C3 is 0.2 ppm. For (−)-cysteine sulfoxides, these proton signals collapse to one signal (*21*). For compound **1**, the difference is clearly 0.2 ppm, indicating a (+)-cysteine sulfoxide. This was verified by the determination of the optical rotation of the substance. The resulting optical rotation of [α_D^{21}] +53.3 mL g^{-1} dm^{-1} (589 nm, in water) is very close to the published optical rotation of L-(+)-alliin given as [α_D^{21}] +62.8 mL g^{-1} dm^{-1} (*22*). The difference might be due to the higher molecular weight of the precursor substance [L-(+)-*S*-(3-pyrrolyl)cysteine sulfoxide, 202 g/mol; L-(+)-*S*-2-(propenyl)cysteine sulfoxide (L-(+)-alliin), 177 g/mol]. The pyrrole ring of compound **1** might also influence the value for optical rotation.

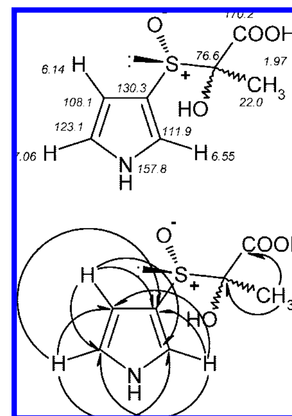

Figure 2. Formula and ^{1}H,^{13}C HMBC correlations of 2-lactyl-3′-pyrrolyl sulfoxide (**3**).

Identification of the Precursor 2-Lactyl-3′-pyrrolyl Sulfoxide. As a second precursor, the highly unstable thiosemiketal **3** was identified (**Figure 2**). During the purification procedure, it remained associated with various monosaccharides. Due to its instability, we were not able to isolate this compound in pure form. It can be assumed that the sulfur pyrrole moiety of the compound also forms thioketals with saccharides of the obtained extract. Therefore, analytical arguments leading to the formula shown in **Figure 2** are mainly based on various NMR experiments. Due to the low amount of this substance, its reactivity, and its mixture with traces of unidentified sugars, UV and IR spectroscopic measurements were not useful for structure elucidation. Therefore, the given structure is based mainly on various NMR experiments, especially diffusion experiments allowing assignments of signals, which belong to the same molecule.

ESI-MS analysis gave a molecular mass of 203 amu (deprotonated molecular ion peak at *m/z* 202, [M − H^{+}] in negative mode, protonated molecular ion peak at *m/z* 204 [M + H^{+}] in positive mode). This indicated a low molecular weight compound with one nitrogen atom. The ^{1}H,^{15}N HSQC spectrum of the DMSO-d_6 solution of this fraction did not show any signal originating from a NH correlation. However, a ^{1}H,^{15}N HMBC experiment yielded a correlation peak of exactly one nitrogen resonance at 157.8 ppm (unsubstituted pyrrole at 149 ppm) with three hydrogens. The chemical shift and correlation pattern indicated a pyrrole ring substituted at position 3. All ring carbons were assigned on the basis of ^{1}H,^{13}C HSQC and ^{1}H,^{13}C HMBC experiments. For comparison, unsubstituted pyrrole exhibits proton resonances of 6.62 and 6.05 ppm at positions 2 and 3 and carbon resonances of 118.5 and 108.2 ppm, respectively (*23*). The proton spin system appeared to be isolated because no correlations outside the ring were found by TOCSY, ROESY/

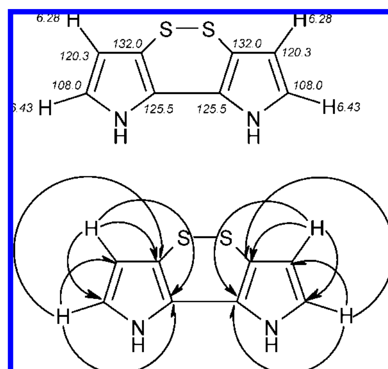

Figure 3. Formula and $^{1}H,^{13}C$ HMBC correlations of 3,3′-dithio-2,2′-dipyrrole (**5**).

NOESY, and HMBC experiments. We therefore expected a heteroatom at position 3 of the pyrrole ring. Because the mass spectrum exhibited a fragment of 114*m*/*z* and additional fragments showed the loss of two neutral water molecules and a carbonyl fragment indicating the presence of a carboxy function and an additional hydroxyl group ($M^+ - 18$, 186*m*/*z*; $M^+ - 18 - 18$, 168*m*/*z*; $M^+ - 18 - 28$, 158*m*/*z*), we concluded that the substituent at position 3 starts with a sulfoxide. The resonance of the carbonyl carbon was detected in the 1D ^{13}C spectrum at 170.2 ppm. Only one correlation was found to a proton resonance at 1.97 ppm, which was attached to a carbon resonating at 22.0 ppm in the $^{1}H,^{13}C$ HSQC. Because no other resonances could be correlated with these two fragments, only the formula of **Figure 2** fulfills all analytical data.

To support the proposed structure, an intact porphyrin ring system and porphyrin aggregation products were ruled out by mass spectrometry and diffusion NMR experiments, which showed that the separated LC fraction contained pyrrole resonances belonging to a low molecular weight species. Furthermore, the typical *meso* proton and carbon resonances of porphyrin derivatives were missing. However, a number of further condensation products with sugars must be assumed because 2-lactyl-3′-pyrrolyl sulfoxide was always accompanied with traces of unidentified sugars.

Isolation and Identification of the Red Dye 3,3′-Dithio-2,2′-dipyrrole. A purified red dye could be obtained from an extract of bulbs from *A. giganteum*. The chopped bulb material was extracted several times with ethyl acetate/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. High-resolution mass spectrometry gave a molecular weight of 193.9984, which fits the molecular formula of $C_8H_6N_2S_2$. The IR spectra showed some characteristic absorptions, which led to the assumption that a thiopyrrole structure is involved in this molecule.

In the ^{1}H NMR, only two protons with chemical shifts of 6.43 and 6.28 ppm (doublet, $J = 3.66$ Hz) were visible. In the ^{13}C NMR, a total of four carbons were observed (108.0, 120.3, 125.5, and 132.0 ppm). A $^{1}H,^{13}C$ HMBC experiment allowed the conclusion that all carbons had long-distance couplings to the two protons and that the proton with a chemical shift of 6.43 ppm is attached to the carbon at 108.0 ppm; also, the proton with 6.28 ppm is attached to the carbon at 120.3 ppm (shifts and allocation verified using the ACD calculator). These results led to the assumption that the protons were at neighboring 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. This could be also confirmed

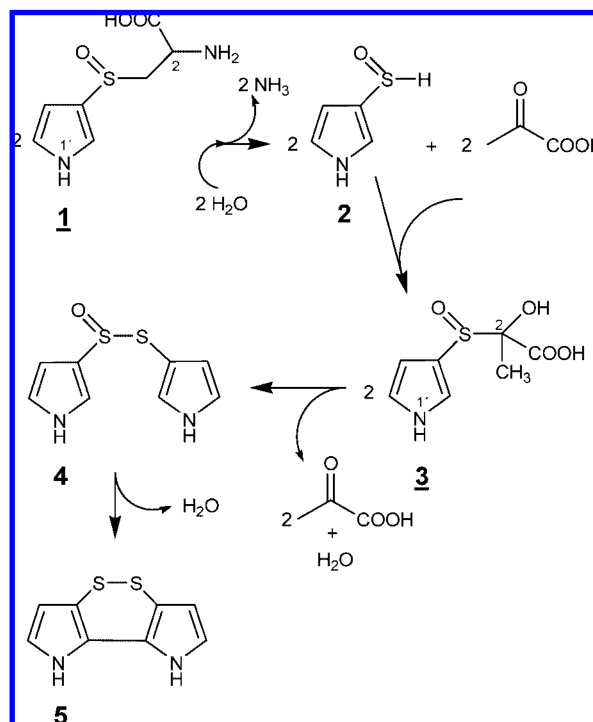

Figure 4. Proposed biogenetic scheme leading to dithiodipyrrole derivatives in *Allium* (identified compounds are underlined).

by HMBC experiments as depicted in **Figure 3**. However, the position of the two sulfur atoms could not be clarified with the existing data material. Therefore, all possibilities containing two pyrrole rings and two sulfur atoms were simulated with the ACD calculator. It turned out that the only possibility would be the 3,3′-dithio-2,2′-dipyrrole as depicted in **Figure 3**.

3,3′-Dithio-2,2′-dipyrrole represents the first isolated and identified dye from *Allium* plant material; furthermore, it is a new compound that differs from pyrroles produced in model systems (*15*). The latter were N-substituted, in contrast to the 3-substitution found in the plant constituents of this study.

It must be pointed out that the stability of 3,3′-dithio-2,2′-dipyrrole is rather low. Even during long-period NMR measurements, the proton signal at 6.43 ppm vanished, suggesting that a polymerization at the carbon directly linked to the pyrrole nitrogen took place. The initial red color turned to deep red and then to blackish colors. Another observation showed that the color switched to dark green if the substance was dissolved in $CDCl_3$. It is not clear which reaction might be responsible for this effect.

An interesting observation is that cells surrounding plant vessels have higher concentrations of the red dye than other parts of the plant, which is easily visible under the microscope. The plant vessels have important functions in transportation of nutrients in the plant. It is possible that the production of the red dye after damage of the cell has a function to protect the important transportation vessels. After cell disruption, the tissue is normally very vulnerable to infections by bacteria and fungi, which can be propagated through the vessel and spread systematically through the plant. The cytotoxic potential of 3,3′-dithio-2,2′-dipyrrole and its precursor 3-pyrrolylcysteine sulfoxide will be tested because structural similarities to the family of prodigiosines exist. At least partially, prodigiosines exert their cytotoxic acitivities via the formation of copper(II) complexes coordinated to three nitrogen atoms (*24*). The plant protection theory is supported by the fact that the pyrroles are mainly located in the outer parts of the bulbs as shown in **Figure 5**. In

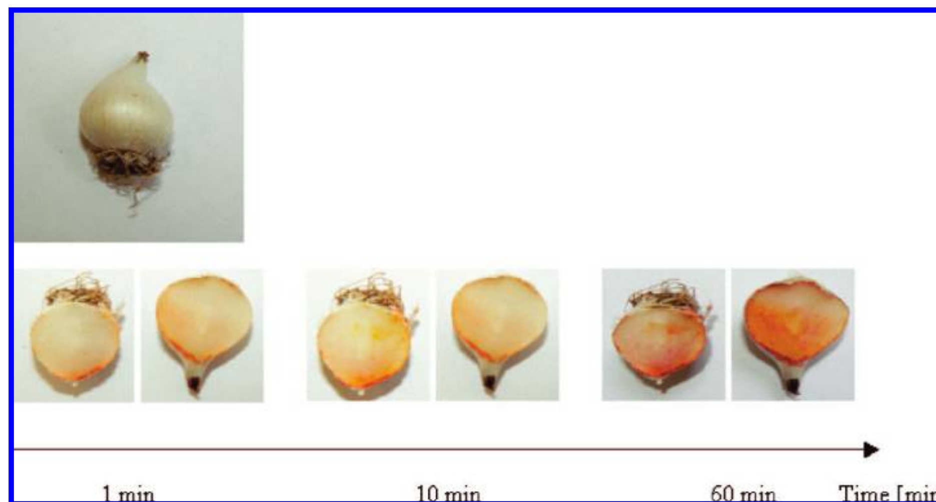

Figure 5. Natural formation of the red dye depending on time after cutting the bulb (*A. macleanii* subgenus *Melanocrommyum*).

the bulbs as also in leaves and stems the highest concentrations are in cells surrounding vessels. The total amount of compound **1** related to the fresh weight of whole bulbs was found to be about 0.01% but should be locally much higher (at least 10-fold higher in cells surrounding vessels).

The impact of isolated substances on the nutritional value of plants is also still unknown. A number of pyrrole-containing substances were already identified in nutritional plants, including tetrapyrroles such as chlorophyll, heme, and vitamin B_{12} (*17*). Their biological functions are manifold; one of the main functions is the coordination of metal ions in the planar ring system. The resulting metallotetrapyrroles are involved in many bioenergetic processes. Formation of metal complexes seems to be also possible for the here described dipyrrole. This compound as well as related substances might be also of importance because of their antioxidative properties and eventually cancer protective effects. Further investigations on these effects are necessary.

Proposed Biogenetic Pathway of 3,3′-Dithio-2,2′-dipyrrole. The hypothetical biogenetic pathway for the formation of the red dye is shown in **Figure 4**. After disruption of the cell, an enzyme with a C-S lyase activity (alliinase reaction) converts the precursor L-(+)-*S*-(3-pyrrolyl)cysteine sulfoxide **1** into the sulfenate **2**. In the case of *N*-oxides, a related thermal reaction type is known as the Cope elimination, proceeding via a five-membered cyclic intermediate (*25, 26*). Subsequently, the sulfenate **2** could condensate with pyruvate to the semithioketal **3**, which was identified as a reaction product. The elucidation of this putative intermediate was possible due to the incomplete inactivation of the enzymes during the whole extraction, beginning with the disruption of cell material. Therefore, small amounts of the semithioketal can be traced under the described conditions. Further intermediates (**Figure 4**) are supposed to be present, but could not be identified until now. However, the proposed intermediate **3** is involved in the formation of the red dye, because incubation of this compound with alliinase leads to a fast red discoloration. Therefore, we assume that the entire enzymatic cascade is much more complex as described for garlic. Compound **1** seems to be related to cysteine sulfoxides such as alliin, which are responsible for the formation of volatile sulfur compounds of *Allium* species. Alliin, for example, reacts by the catalysis of the enzyme alliinase to allylsulfenic acid (comparable to compound **2**), which condenses spontaneously to alliicin (comparable to compound **4**).

Because L-(+)-*S*-(3-pyrrolyl)cysteine sulfoxide **1**seems to be stable in intact cells, the precursor and the enzyme must be located in different cell compartments. Compound **1** might be located in the cytosol of the cell like the closely related alliinase substrates alliin, isoalliin, methiin, and propiin, whereas the enzyme alliinase itself is found in the vacuole. However, the location of the precursor **1** is as yet unclear. The semithioketal **3** might react with the thiosulfinate **4** substituted by two pyrrole rings. Compound **4** is in full accordance with the alliinase reaction model starting from cysteine sulfoxides such as compound **1** (*12*). Loss of water would lead spontaneously to the isolated dithiodipyrrole **5**. This is in accordance with the chemical instability of chemically related phenoxymethyl phenyl sulfoxide, which rearranges to the corresponding sulfenate and finally forms a thiosulfinate (*27*). This final reaction step is probably enzyme catalyzed but can also be induced by heat or alkaline treatment. Furthermore, the pyrrole-containing compounds are not N-substituted as suggested in the model reactions of Imai et al. (*15*), emphasizing a completely different origin for reddish colored compounds in *Allium* extracts. The speculation about the precursors for the dye formation in *Allium* species (*13, 15*) is now substantiated with structural data, complementing our knowledge about flavors in *Allium* species (*11, 28*).

However, it must be pointed out that a red discoloration of plant material containing compound **1** can be also achieved under strong alkaline conditions or by heat. Therefore, a pathway similar to that depicted in **Figure 4** is possible under certain chemical conditions. However, in living plants, we never observed a red discoloration without wounding of the plant material, thus starting the enzymatic reaction cascade.

ABBREVIATIONS USED

COSY, correlated spectroscopy; TOCSY, total correlated spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple-bond coherence; NOESY, nuclear Overhauser effect spectroscopy; ROESY, rotating frame Overhauser effect spectroscopy; DMSO, dimethyl sulfoxide.

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