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Allium chemistry: Use of new instrumental techniques to "see" reactive organosulfur species formed upon crushing garlic and onion*

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Abstract: Three different instrumental methods have been used to examine the organosulfur chemistry of intact and cut garlic and onions: X-ray fluorescence spectroscopic imaging (XFS), direct analysis in real time (DART) mass spectrometry, and ultra-performance liquid chromatography-(Ag^+)-coordination ion spray mass spectrometry (UPLC–(Ag^+)CIS–MS). The first technique has been used to map the location of different chemical forms of sulfur in intact and damaged onion cells, the second technique, to identify the reactive, volatile sulfur compounds formed on cutting the plants, and the third technique, to identify members of families of polysulfides found in the distilled oil of garlic.

Keywords: direct analysis in real time; garlic; mass spectrometry; onion; sulfur compounds; ultra-performance liquid chromatography; X-ray fluorescence spectroscopy.

INTRODUCTION

Un-bruised bulbs of garlic (*Allium sativum*) and onion (*Allium cepa*) have little odor, but instantly make their presence known when they are cut or crushed. The odors are associated with mixtures of reactive organosulfur compounds formed by enzymatic action on odorless precursors in the intact plant, e.g., **1** and **12** for garlic and **16** and **22** for onion, as shown in Scheme 1 [1].

Thus, *S*-alk(en)ylcysteine sulfoxide precursors **1** (alliin), **12** (methiin), **16** (isoalliin), and **22** (propiin) are cleaved by the alliinase enzymes splitting off aminoacrylic acid, which rapidly hydrolyzes to ammonium pyruvate. Sulfenic acids **2**, **13**, **17**, and **23** self- or cross-condense, giving thiosulfinates **3** (allicin), **14**, **15**, **18**, and **24–26**. Thiosulfinate **18** is highly reactive and spontaneously rearranges to zwiebelane **21**. In onion, a second enzyme, LF synthase, converts sulfenic acid **17** to onion lachrymatory factor (LF) **19**, which can be further transformed to bis-sulfine **20**. Further processing the garlic- and onion-derived organosulfur compounds, for example, by steam distillation, leads to surprisingly complex families of nonpolar polysulfides (e.g., **11**) that are both difficult to separate by chromatography and hard to characterize by more common mass spectrometric methods. There are significant analytical problems at all stages of the above-described procedures. Efforts to determine the con-

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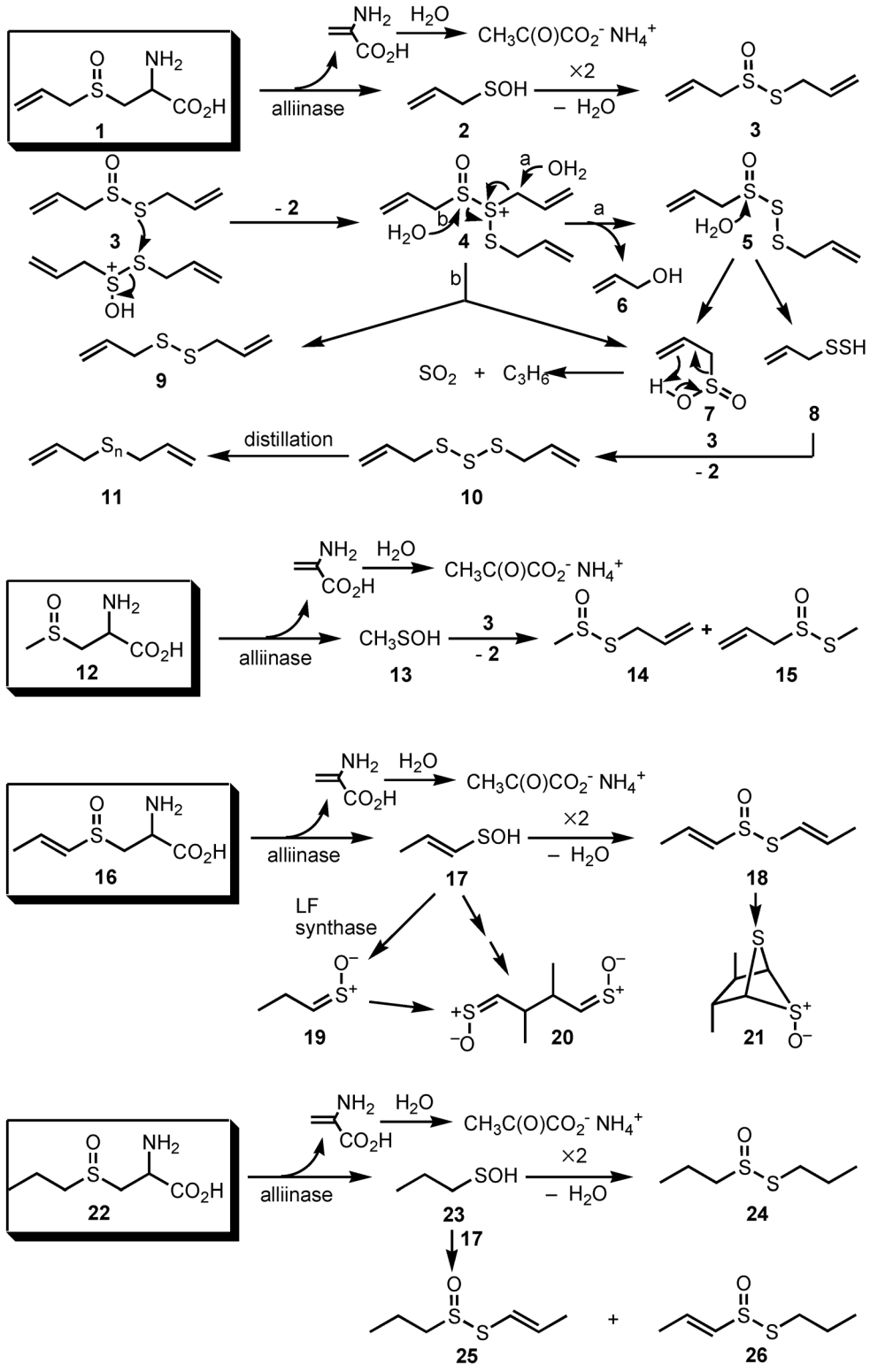

Scheme 1 Transformations that occur upon cutting genus *Allium* plants.

stituents of the intact plant require that the plant tissue be probed, which initiates decomposition of the sought-for precursors, unless the enzymes are first deactivated. Enzymatic cleavage of precursors is sufficiently fast so as to thwart efforts to characterize the intermediates involved. Finally, the complexity, nonpolarity, and thermal fragility of the final products make full characterization highly challenging. We describe here our collaboratively developed solutions to these analytical problems as relevant to *Allium* chemistry.

RESULTS AND DISCUSSION

X-ray fluorescence spectroscopic imaging

In situ information on sulfur biochemistry as it occurs in *Allium* species is difficult to obtain because of a lack of biophysical techniques that have sufficient sensitivity to molecular form. Sulfur lacks a well-established spectroscopic probe and is often called a spectroscopically silent element. For example, the low natural abundance, weak magnetic moment, and significant nuclear electric quadrupole moment of ^{33}S combine to make ^{33}S NMR challenging, and it is infrequently used. Fortunately, sulfur K-edge X-ray absorption spectroscopy (XAS) can be used as a direct probe of the sulfur biochemistry of living cells to generate maps of different chemical forms of sulfur. In brief, the cell samples are scanned in a microfocus X-ray beam at a number of different incident energies, providing sensitivity to different sulfur chemical forms. The sulfur X-ray fluorescence is monitored, and with information about the spectra of standard solution species, the data is converted to quantitative maps of the different chemical forms, e.g., organic disulfides at 2469.88 eV, organic sulfides at 2470.55 eV, organic sulfoxides at 2473.59 eV, and sulfate at 2479.58 eV. The sulfur K-edge XAS of a pure sample of propanethial *S*-oxide, **19**, the onion LF, was also measured. In intact onion cells from a green onion bulb and a red onion leaf (near the transport vessels), XFS imaging using an X-ray microprobe in parallel with an optical microscope showed elevated levels of sulfoxides (e.g., the LF-precursor **16**) in the cytosol and elevated levels of reduced sulfur in the central transport vessels and bundle sheath cells. XAS of onion sections showed increased levels of LF **19** and thiosulfinates **24–26**, along with decreased levels of LF-precursor **16**, following cell breakage [2,3]. Related techniques, microscopic X-ray absorption near-edge structure spectroscopy (μ-XANES), and confocal microscopic X-ray fluorescence analysis, have been used to study the distribution and local speciation (chemical form) of selenium in growing onion roots and leaves exposed to selenite and selenate [4].

DART mass spectrometry

Direct analysis in real time (DART), one of several popular methods used for ambient ionization mass spectrometry, has attracted attention because of its ability to directly analyze samples under open air conditions without prior treatment [5]. The DART source is generally used with high-resolution (HR) time-of-flight mass spectrometers. DART is a "soft ionization" method which for most compounds gives simple mass spectra that are easily obtained by momentarily holding the sample in the gas stream, either manually or using an automatic sampling device. Ionization under positive ion (PI-DART) or negative ion (NI-DART) conditions gives species formed when analytes collide with protonated water clusters $[(H_2O)_n+H]^+$ or with oxygen anion radicals $[O_2]^{\bullet-}$, respectively [6]. The PI mode produces an $[M+H]^+$ ion for analytes that have high proton affinities while the NI mode produces an $[M–H]^-$ ion for analytes containing an acidic functional group. The ability to perform HR-DART mass spectrometry under PI or NI conditions, without the need for sample preparation or solvent, presents unique opportunities in food and natural products chemistry [5], allowing the direct observation of the complex cascade of enzymatically induced flavor-releasing processes that rapidly occur when plants are cut. For example, capsaicin was measured by slicing open a red pepper pod and passing the different parts of the pod in front of the ionizing beam. In this manner, capsaicin and related compounds were detected as

their $[M+H]^+$ species, with elemental composition confirmed through HR-MS. Notably, these measurements were made within a few seconds and without any sample preparation other than cutting open the pod [5]. Other recent applications of DART-MS in natural products chemistry include identification of volatile organic compounds from eucalyptus [7] and relatively nonvolatile compounds, such as curcuminoids and alkaloids, from tumeric [8] and *Atropa acuminata* [9], respectively.

When a garlic clove was sampled, PI-DART showed strong signals for protonated allicin (**3**), weaker signals for the isomeric allyl methyl thiosulfinates **14** and **15** and allyl alcohol (**6**), as well as a very weak peak for diallyl trisulfides *S*-oxide **5**. Analysis of a freshly cut garlic clove by NI-DART showed significant signals corresponding to SO_2 and pyruvate $[CH_3C(O)CO_2^-]$ as well as those corresponding to 2-propenesulfenic acid (**2**) and 2-propenesulfinic acid (**7**). The NI-DART signal for **2** is very short-lived, disappearing in less than 1 s. The SO_2 likely arises from decomposition of **7** to propene, C_3H_6, which was detected in trace amounts [10].

When an onion bulb was sampled as above, the PI-DART mass spectrum showed strong signals for the protonated LF **19** (a sulfine), seen as a set of a set of three intense ions at *m/z* 91 ($[C_3H_6SO + H]^+$), *m/z* 108 ($[C_3H_6SO + NH_4]^+$), and *m/z* 181 ($[(C_3H_6SO)_2 + H]^+$). In these spectra, **19** reacts with a proton on a 1:1 or 2:1 basis or with ammonium ions, formed from the decomposition of aminoacrylic acid. Minor peaks are identified as **20**, **21**, and **24–26**, while NI-DART analysis showed SO_2 and pyruvate. All of the above peaks were characterized by HR-MS.

Application of PI-DART methods to *Allium siculum*, an ornamental *Allium*, led to the identification of a series of C_8 thiosulfinates containing butyl and/or 1-butenyl groups, along with a new lachrymatory organosulfur compound, (*E*)/(*Z*)-butanethial *S*-oxide (**27**), whose identity was further established by NMR spectroscopy and independent synthesis [11]. Compound **27** is only the fourth sulfine known to occur naturally. The likely precursor of **27**, namely *(R_S,R_C,E)*-*S*-(1-butenyl)cysteine *S*-oxide (homoisoalliin), was isolated from homogenates of *A. siculum*, and a closely related species *Allium tripedale*, and fully characterized. Because compounds containing the 1-butenyl group have not been previously identified in genus *Allium* species, this work extends the range of known *Allium* sulfur compounds.

Ultra-performance liquid chromatography-(Ag^+)-coordination ion spray-mass spectrometry (UPLC–(Ag^+)CIS–MS)

While HPLC–(Ag^+)CIS–MS, employing post-separation infusion of a $AgBF_4$ solution, has proven useful, e.g., for analysis of nonpolar and poorly ionized substances such as polyolefins [12a], lipid peroxidation products [12b–d], glycosides [12e] and saponins [12f], application of this powerful technique to organosulfur compounds has been very limited [13]. UPLC, employing smaller chromatographic support particle size and higher pressures, has an advantage over HPLC in significantly reducing the elution times with a resultant sharpening of peaks, important for MS analysis of late-eluting trace components [14].

Application of the technique of UPLC–(Ag^+)CIS–MS to a sample of garlic oil led to rapid separation (13 min) of a series of peaks identified by selective ion monitoring as the $^{107}Ag/^{109}Ag$ adducts of diallyl disulfide through nonasulfide. In this work, immediately following chromatographic separation, a solution of $AgBF_4$ is introduced into the liquid sample ($AgBF_4$ is preferable to $AgNO_3$, since the nitrate ion can lead to oxidation processes; furthermore, $AgBF_4$ is a stronger Lewis acid). This same technique was used to characterize other families of polysulfides in garlic oil, allowing identification of several previously unknown compounds [15].

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