

# Bond-Selective Photodissociation of Aliphatic Disulfides

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The photochemistry of aliphatic disulfides is presented. The photolysis products are photoionized with coherent vacuum ultraviolet radiation and analyzed by time-of-flight mass spectrometry. With 248-nm excitation, the predominant dissociation pathway is S—S bond cleavage. With 193-nm excitation, S—S bond cleavage, C—S bond cleavage, and molecular rearrangements are all observed as primary processes. The branching ratio for S—S bond cleavage relative to C—S bond cleavage is typically 1–2 orders of magnitude greater at 248 nm than 193 nm. This wavelength dependence cannot be explained readily by photodissociation from the ground electronic state. The ground state S—S bond energy,  $\sim 280$  kJ/mol, is much larger than the C—S bond energy,  $\sim 235$  kJ/mol. If dissociation occurred from the ground state, higher wavelength radiation would be expected to favor the lower energy process, but the opposite effect is observed. Thus, excited state photochemistry is indicated. These results are discussed with respect to the differences between low and high energy collision-induced dissociation of peptides that contain disulfide linkages and to the possibility of achieving bond-selective photodissociation of such ions. (*J Am Soc Mass Spectrom* 1995, 6, 872–876)

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We have studied the photochemistry of disulfide linkages in simple aliphatic molecules to better understand the structural information that may be obtained by photodissociation of similar linkages in peptides. Recent studies of peptide ion photodissociation with ultraviolet radiation (typically 193 nm) have been performed in sector [1], time-of-flight [2, 3], and Fourier transform [4, 5] mass spectrometers. Although the instrumental modifications are often more complex with photodissociation than with collision-induced dissociation (CID) or surface-induced dissociation (SID), photodissociation has the potential advantage that energy deposition is well defined with respect to the amount of energy deposited and the internal mode(s) that are initially excited. In specific situations, this latter characteristic may permit targeted bond cleavage in a molecule. For example, many small molecules are known to photodissociate directly from an excited electronic state. If these processes result in preferential bond cleavage at the chromophore and if similar processes occur in large molecules, then site-selective bond cleavage may be achieved when the appropriate electronic state is excited.

Unfortunately, it is hard to predict the occurrence of such processes in large molecules because most photo-

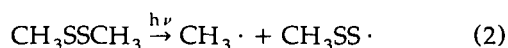
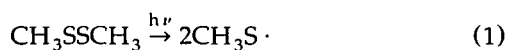
chemical studies are limited to small ( $C_1$ – $C_3$ ) aliphatic compounds. The primary photodissociation pathways typically are inferred from the distribution of stable molecules produced by secondary reactions. Larger molecules cannot be studied owing to the increasing complexity of secondary reactions. We have used photodissociation–photoionization mass spectrometry to extend photochemical investigations of aliphatic molecules into the  $C_3$ – $C_{10}$  size range [6]. In these experiments, molecular photodissociation is performed with a pulsed laser beam. After an appropriate time delay, the products are softly photoionized with coherent vacuum ultraviolet radiation [7] and then mass analyzed. Photodissociation–photoionization permits larger molecules to be studied because the primary products are detected and secondary reactions are minimized. Our most recent studies involved molecules that photodissociate directly from an excited electronic state. Indeed, we have found that the site of bond cleavage in “large” molecules can be controlled by the wavelength of excitation. For example, a dissociative electronic state is populated when iodoalkanes are excited with 248-nm radiation. Dissociation from this state results exclusively in C—I bond cleavage and this selectivity persists as the molecular size increases [8]. In contrast, a different electronic state is populated when iodoalkanes are excited with 193-nm radiation and this state results in nonselective C—I, C—C, and C—H bond cleavages. Alkanethiols exhibit nonselective C—C, C—S, and S—H bond cleavages

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when excited with 193-nm radiation, but the S—H dissociation channel is strongly enhanced when excitation is performed with 248-nm radiation [9]. This behavior is opposite what would be expected based upon ground state bond energies, but is consistent with the excited state electronic configurations of these molecules, which indicate a greater contribution of  $\sigma_{\text{SH}}^*$  to the state populated at 248 nm than at 193 nm.

There is evidence in the literature to suggest that similar selectivity may be observed with disulfides. Photodissociation of dimethyl and diethyl disulfide with far ultraviolet radiation (< 200 nm) has been shown to yield products that correspond to both C—S and S—S bond cleavage [10, 11], as indicated in reactions 1 and 2 that use dimethyl disulfide as an example:



Both of these reactions are observed in thermal decomposition studies as well [12]. In contrast, other experiments have suggested that photodissociation of dimethyl and diethyl disulfide with mid-ultraviolet radiation (> 230 nm) yields exclusively S—S bond cleavage [13]. It should be noted, however, that one study with radiation in the mid-ultraviolet region gave products that could be ascribed to C—S bond cleavage although the authors attributed them to secondary reactions of the S—S bond cleavage products [14].

We report here a systematic study of the photodissociation mechanisms of several alkyl disulfides in the  $\text{C}_2$ – $\text{C}_8$  size range. Our immediate goal is to explore the selectivity of S—S bond dissociation in these molecules. The results also are discussed with respect to the differences between low and high energy CID of peptides that contain disulfide linkages and to the possibility of achieving bond-selective photodissociation of such ions.

## Experimental

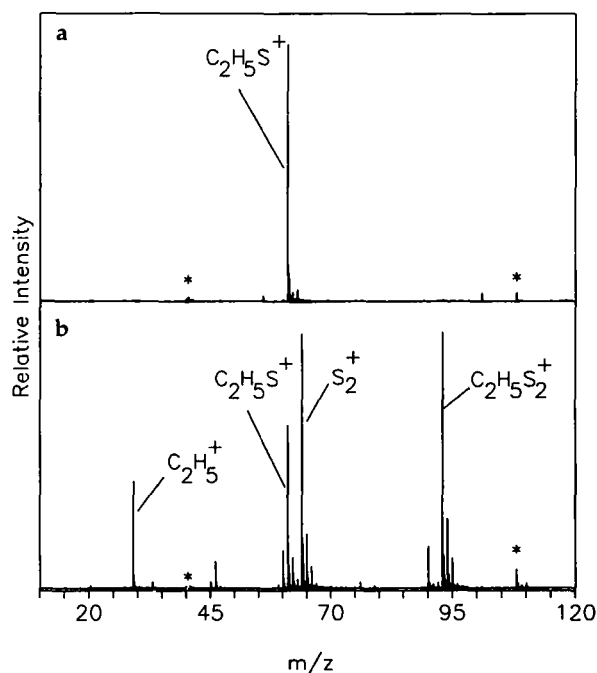
Molecular photodissociation was performed in a supersonic jet expansion. Analyte molecules were seeded into the expansion by bubbling the argon carrier gas through the liquid sample. The sample vapor pressure was adjusted in the 0.1–5-torr range by heating the sample reservoir and transfer line to an appropriate temperature. No evidence of thermal decomposition was observed in the photoionization mass spectra. The carrier gas and analyte vapor were sent through a pulsed valve (General Valve, Fairfield, NJ; 0.76-mm diameter orifice) into the primary expansion chamber. A lightly focused excimer laser beam at 193 or 248 nm crossed the expansion ~ 5 mm downstream from the pulsed valve exit. Laser fluences on the order of 10–30  $\text{mJ}/\text{cm}^2$  were found to give adequate photodissociation yields without induction of multiphoton pro-

cesses. Collisional cooling of the primary products in the expansion greatly reduced the amount of secondary fragmentation relative to our previous experiments [6, 8, 9]. The timing and location of the excimer laser pulse relative to the pulsed valve determined the cooling efficiency. This dependence is discussed elsewhere [15].

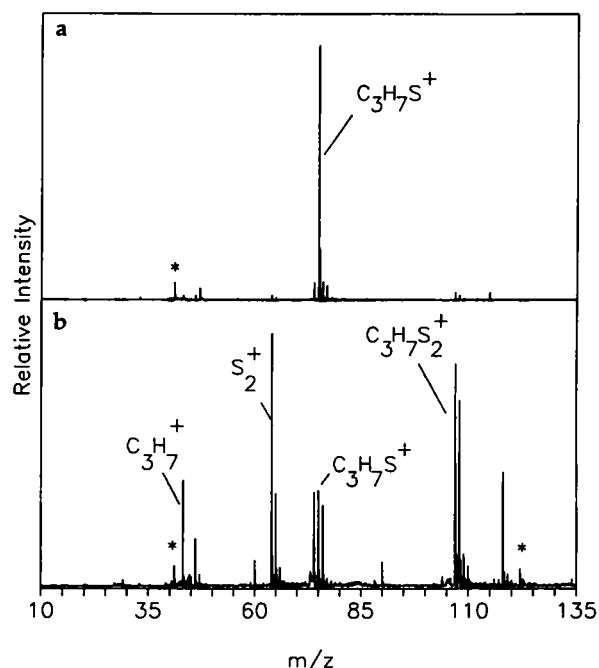
The core of the jet expansion was skimmed 20 mm downstream of the pulsed valve exit. The resulting molecular beam was sent into a second vacuum chamber where it crossed a coherent vacuum ultraviolet beam in the source region of a reflecting-field time-of-flight mass spectrometer. The vacuum ultraviolet radiation permitted soft ionization of the photodissociation products and any remaining undissociated parent molecules. Photoionization was performed with 128.1-nm (9.68-eV) radiation produced by nonresonant frequency tripling of 384.3-nm radiation from a Nd:YAG pumped dye laser system [7]. Physical separation of the ultraviolet and vacuum ultraviolet beams was accomplished by off-axis focusing of the ultraviolet radiation into the frequency tripling cell [7].

## Results and Discussion

Figures 1–3 show the 9.68-eV photoionization mass spectra of three representative compounds that have been photodissociated with 248-nm (Figures 1a–3a) and 193-nm (Figures 1b–3b) radiation: diethyl, di-*n*-propyl, and di-*n*-butyl disulfide. For clarity, the photoionization mass spectra of the undissociated samples (i.e., photodissociation laser beam blocked) have been

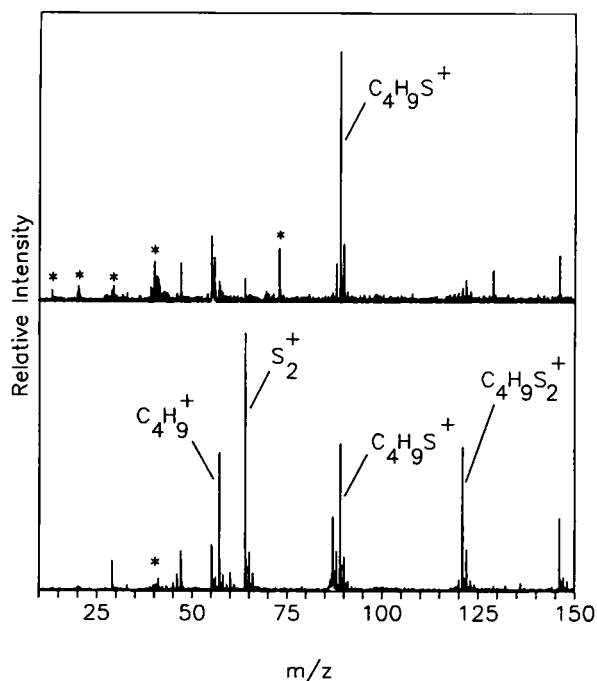


**Figure 1.** Photoionization mass spectra of diethyl disulfide photodissociated with (a) 248- and (b) 193-nm radiation. Background peaks are indicated by an asterisk (\*).



**Figure 2.** Photoionization mass spectra of di-*n*-propyl disulfide photodissociated with (a) 248- and (b) 193-nm radiation. Background peaks are indicated by an asterisk (\*).

subtracted from the photodissociation-photoionization mass spectra to remove or reduce spurious peaks produced by impurities and by ionic fragmentation of the respective molecular ions. (Peaks that arise from semivolatile impurities are not completely removed by background subtraction because their signal intensities



**Figure 3.** Photoionization mass spectra of di-*n*-butyl disulfide photodissociated with (a) 248- and (b) 193-nm radiation. Background peaks are indicated by an asterisk (\*).

change with time.) Because the impurity peaks generally do not overlap the main photodissociation peaks, background subtraction does not cause significant distortion of the photodissociation-photoionization signal. A broad, intense peak centered at  $m/z$  40 is present in all spectra. This peak is removed by background subtraction, but elevated noise in the baseline often is observed. This peak is thought to arise from electron ionization of the argon carrier gas by photoelectrons generated by the ultraviolet pump radiation.

The 248-nm photodissociation spectra in Figures 1a-3a are dominated by photoionized  $RS\cdot$  radicals that are produced by S-S bond cleavage (reaction 1). These ions include  $C_2H_5S^+$  ( $m/z$  61) for diethyl disulfide,  $C_3H_7S^+$  ( $m/z$  75) for dipropyl disulfide, and  $C_4H_9S^+$  ( $m/z$  89) for dibutyl disulfide. Peaks also are observed 40 mass units higher than the photoionized  $RS\cdot$  radicals. We attribute these ions to photoionized clusters of the corresponding  $RS\cdot$  radical with an argon atom. Photoionized  $RS_2\cdot$  and  $R\cdot$  radicals produced by C-S bond cleavage (reaction 2) are very weak or absent from these spectra. These ions include  $C_2H_5S_2^+$  ( $m/z$  93) and  $C_2H_5^+$  ( $m/z$  29) for diethyl disulfide,  $C_3H_7S_2^+$  ( $m/z$  107) and  $C_3H_7^+$  ( $m/z$  43) for di-*n*-propyl disulfide, and  $C_4H_9S_2^+$  ( $m/z$  121) and  $C_4H_9^+$  ( $m/z$  57) for di-*n*-butyl disulfide. The spectra in Figures 1a-3a show that S-S bond dissociation is strongly preferred at 248 nm.

The 193-nm photodissociation spectra (Figures 1b-3b) are much more complex and show products of several processes. Cleavage of the S-S bond is indicated by the presence of photoionized  $RS\cdot$  radicals. Cleavage of the C-S bond is indicated by the presence of photoionized  $RS_2\cdot$  and  $R\cdot$  radicals and by photoionized  $S_2$  ( $m/z$  64), which is an expected secondary decomposition product of the  $RS_2\cdot$  radical. Rearrangements also are observed. One such process is indicated by the presence of photoionized  $RS_2H$  ions in each of the spectra ( $m/z$  94 for diethyl disulfide,  $m/z$  108 for di-*n*-propyl disulfide, and  $m/z$  122 for di-*n*-butyl disulfide). Another is indicated by the presence of photoionized  $R_2S$  ions ( $m/z$  90 for diethyl disulfide,  $m/z$  118 for di-*n*-propyl disulfide, and  $m/z$  146 for di-*n*-butyl disulfides). A third is indicated by a series of ions that correspond to photoionized  $CH_2S$  ( $m/z$  46),  $C_2H_4S$  ( $m/z$  60),  $C_3H_6S$  ( $m/z$  74), and  $C_4H_8S$  ( $m/z$  88). For each compound, all of the ions in this series are observed up to the maximum number of carbon atoms in the alkyl chain. For example, di-*n*-propyl disulfide yields  $m/z$  46, 60, and 74.

Interestingly, there is no direct evidence for C-C bond cleavage as a primary process. Photodissociation products larger than  $RS_2\cdot$  are absent from the spectra. Although  $C_2H_5^+$  ( $m/z$  29) in the spectrum of di-*n*-butyl disulfide could be interpreted as a primary product of C-C bond cleavage, the complementary product that corresponds to photoionized  $C_4H_9S_2C_2H_4\cdot$  ( $m/z$  149) is not observed. More likely  $C_2H_5^+$  and  $C_4H_7^+$  are secondary decomposition products of  $C_4H_9\cdot$  pro-

duced by C—S bond cleavage. These secondary fragmentations have been observed in previous photodissociation-photoionization experiments [6, 9]. Based upon our experience with C—C bond cleavage in other systems [8, 9], one would also expect to see C—C bond cleavage at other locations in the molecule. For example, photoionized  $C_3H_7\cdot$  ( $m/z$  43) and its complement,  $C_4H_7S_2CH_2\cdot$  ( $m/z$  135) also would be produced if nonselective C—C bond cleavage was a primary process for di-*n*-butyl disulfide. However, neither product is observed in Figure 2b.

Because the photoionization cross sections of the photodissociation products are not known, the relative yield of S—S versus C—S bond cleavage cannot be obtained. However, the change in this ratio when going from 248- to 193-nm photodissociation can be quantitatively determined. For a given compound, the relative yield of S—S versus C—S bond cleavage at 248 nm is given by:

$$R_{248} = \frac{\sigma_{RS}}{2\sigma_{RSS}} \frac{A_{RS,248}}{A_{RSS,248}}$$

where  $\sigma_{RS}$  and  $\sigma_{RSS}$  are the photoionization cross sections for the  $RS\cdot$  and  $RS_2\cdot$  radicals, and  $A_{RS,248}$  and  $A_{RSS,248}$  are the peak areas of the respective ions in the photoionization mass spectra. Similarly, the relative yield of S—S versus C—S bond cleavage at 193 nm is given by

$$R_{193} = \frac{\sigma_{RS}}{2\sigma_{RSS}} \frac{A_{RS,193}}{A_{RSS,193}}$$

The change in the relative yield of S—S versus C—S bond cleavage when going from 248 to 193 nm photodissociation is given by

$$\frac{R_{248}}{R_{193}} = \frac{A_{RS,248}}{A_{RSS,248}} \frac{A_{RSS,193}}{A_{RS,193}}$$

This ratio is independent of the photoionization cross sections and therefore can be determined directly from the mass spectra. These equations are valid only if secondary decomposition does not occur. However, Figure 2 shows that many of the  $RS_2\cdot$  radicals produced by 193-nm photodissociation undergo secondary decomposition to  $S_2$ . Thus,  $A_{RSS,193}$  underestimates the fraction of molecules that undergo C—S bond cleavage and  $R_{248}/R_{193}$  is a lower limit for the true change in the branching ratio. Table 1 gives values for  $R_{248}/R_{193}$  for several disulfides. These values show that the branching ratio for S—S bond cleavage relative to C—S bond cleavage is typically 1–2 orders of magnitude higher at 248 nm than 193 nm.

This wavelength dependence cannot be explained readily by photodissociation from the ground electronic state. The ground state S—S bond energy,  $\sim 280$  kJ/mol in these molecules, is much larger than the

**Table 1.** Relative yield of S—S to C—S bond cleavage at 248 nm versus 193 nm

Compound	$R_{248}/R_{193}$ <sup>a</sup>
Dimethyl disulfide	13
Diethyl disulfide	240
Di- <i>n</i> -propyl disulfide	96
Di- <i>n</i> -butyl disulfide	13
Di- <i>s</i> -butyl disulfide	34
Di- <i>t</i> -butyl disulfide	6

<sup>a</sup>See text for definition.

C—S bond energy,  $\sim 235$  kJ/mol. If dissociation occurred from the ground state, decreasing the photon energy (i.e., going from 193 to 248 nm) would be expected to favor the lower energy process, but the opposite effect is observed. Also, dissociation from the ground state would be expected to mirror thermal decomposition pathways because the latter clearly proceed through the ground electronic state manifold. However, thermal decomposition studies of disulfides are nonselective; they show both C—S and S—S bond cleavage [12]. Thus, the selectivity of S—S bond cleavage with 248-nm photodissociation indicates that dissociation occurs through an excited electronic state.

The photodissociation characteristics of simple disulfides give insight into the CID behavior of peptides that contain intermolecularly bonded disulfide linkages. High energy CID yields a "triplet" of ions that correspond to C—S and S—S bond cleavages along the disulfide linkage [16]. These ions are usually sufficient to identify the component peptides on either side of the linkage. In contrast, low energy CID does not produce these ions [16]. It is well known that low energy CID proceeds through vibrationally excited intermediates whereas high energy CID can proceed through electronically excited intermediates as well [17]. With low energy CID, protonated peptide molecules undergo charge-mediated fragmentations that have threshold energies as small as a few tenths of an electronvolt [18]. These low energy processes compete favorably with higher energy C—S or S—S bond cleavages, so dissociation of the disulfide linkage is not favored. This picture is consistent with the thermal decomposition chemistry of simple disulfides, which gives no indication of bond-selective dissociation along the ground state potential surface. In contrast, high energy CID may proceed through an excited electronic state of the disulfide functionality, which promotes C—S and S—S bond cleavage over other processes. In this case, the relative yields of S—S and C—S bond cleavage would be dependent on the energy of excitation as indicated by the 248- and 193-nm photochemistry of simple disulfides.

Nonselective dissociation, whether induced by photons or collisions, may not be practical for structural analysis of large ( $> 10$ -ku) precursor ions. If the product ion current is spread over many mass-to-charge ratio peaks, then the signal-to-noise ratio at any single

mass-to-charge ratio will be low and the mass resolution may not be sufficient to separate adjacent peaks. In principle, bond-selective photodissociation overcomes these problems by limiting the precursor ion to a few, specific dissociation channels. The photochemical behavior of simple aliphatic disulfides suggests that such selectivity is possible in larger systems. Future work will investigate this possibility.

## Acknowledgment

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