On the Origin of Cytotoxicity of the Natural Product Varacin. A Novel Example of a Pentathiepin Reaction That Provides Evidence for a Triatomic Sulfur Intermediate

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Abstract: A density functional theoretical study is presented, which implicates a novel S_3-cleavage in the decomposition of a pentathiepin. This study predicts an interconversion between a pentathiepin and an open-chain polysulfur ion intermediate from which a key determinant in the chemistry then follows. Expulsion of diatomic sulfur, S_2, is unlikely from the unimolecular collapse of the open-chain polysulfur ion. Instead, S_3 can dissociate due to an unusually long and weak sulfur−sulfur (S4−S5) bond. A mechanistic picture now emerges which predicts that the novel S−S cleavage reaction and the unanticipated S_3 fragmentation are a result of delocalization of the negative charge within the remaining carbon−sulfur fragment. The computed results presented here reveal a new aspect to the chemistry of pentathiepins, that of S_3 unit transfer, which is proposed to have significance in the mechanism of cytotoxicity of the natural product varacin, **1**.

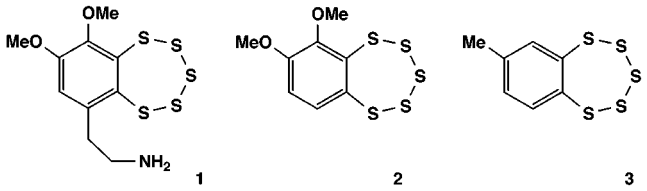
Introduction

Ten years ago a report appeared describing the natural product pentathiepin, varacin (**1**) (Scheme 1).[1] Ireland and co-workers isolated **1** from a marine ascidian and discovered that it exhibited cell-killing (cytotoxic) activity.[1] It was suggested that the cytotoxic activity of **1** was derived from DNA damage.[1,2] Structurally related pentathiepins have been characterized, which also exhibit cytotoxicity,[3−7] for example, 6,7-dimethoxybenzopentathiepin (**2**) yielded an IC_{50} value of 6.1 μg/mL against human tumor cell line HeLa S3.[3] Pentathiepins are structurally unique molecules with interesting biological properties; however, insight is needed to uncover the factors that underlie the bioactivity.

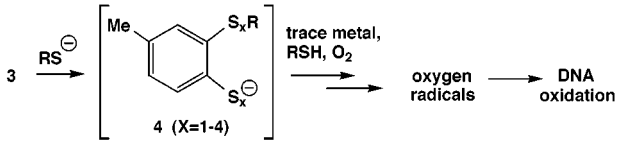
Information is limited on the intermediates involved in biochemical reactions of pentathiepins. A recent in vitro study provided insight. 7-Methylbenzopentathiepin (**3**) cleaved plasmid DNA in the presence of thiol by a mechanism suggested to involve polysulfide ion (**4**) (Scheme 2).[8] Evidence suggested that the thiol-triggered DNA damage by **3** involved the conversion of molecular oxygen to oxygen radicals from a trace metal-dependent Fenton reaction.[8] Information on the organic chemistry of pentathiepins may also help reveal the factors related to the bioactivity.

Chenard and co-workers[9] reported that diethylamine reacted with (trifluoromethyl)benzopentathiepin (**5**) to establish an equilibrium with the benzotrithiole (**5′**) and cyclic sulfur S_8 (K_{eq} = **5**:**6** = 46:54) (Scheme 3). Such amine-dependent chemistry may be biologically relevant because Behar and Danishefsky suggested that the polysulfur ring of **1** decomposed from an intramolecular reaction with the amine free-base.[10] Varacin **1** is stable as a trifluoroacetate salt.[4,10−13] It is noteworthy that an

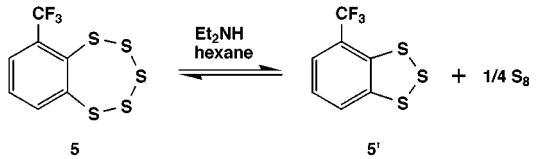
Scheme 1



Scheme 2



Scheme 3



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(2) Barrows, L. R.; Paxton, M. B.; Kennedy, K. A.; Thompson, L. H. *Carcinogenesis* **1991**, *12*, 805.
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adjacent amine group confers an enhanced bioactivity on pentathiepin molecules.[3] However, the chemical pathway for activation remains unknown.

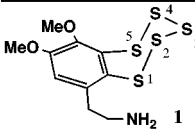
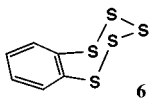
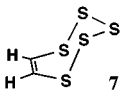
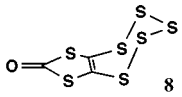
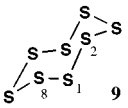
Little is known of the intermediates involved in the biochemistry of pentathiepins. Pentathiepins have been used as reagents in organic synthesis,[14−18] but it has not been possible to detect the reactive species spectroscopically. Even elucidating aspects of the reaction mechanism by product identification has presented problems. Polysulfur compounds often form as mixtures of isomers or can equilibrate, which complicate analyses.[19] For example, results from mass spectrometry can be misleading because the compounds can isomerize producing false parent peaks.[6,7,20] Reversed-phase HPLC analyses revealed that polysulfur reactions previously reported to yield homogeneous products often yield complicated mixtures.[21] Low-temperature methods can help maintain homogeneous products,[22] but X-ray crystallography represents the reliable method of establishing polysulfur structure.[9,23,24] It remains a challenging task to determine the structural assignment of intermediates in pentathiepin reactions.

Since direct experimental observations of the intermediates in reactions of pentathiepins have not been possible, theoretical calculations were conducted to predict the geometries and energetics of the intermediates. Calculations were performed to discover the factors that are likely to influence pentathiepin decomposition with the aim of evaluating the viability of the intermediates. The detailed pathway for the formation of the reactive intermediates is described. Computational methods have been used in the past to study questions related to pentathiepin rearrangement[14,25−27] but did not focus on intermediates that potentially contribute to the biochemistry. We report calculations that suggest a new reaction scheme. The key feature is the presence of a new intermediate, triatomic sulfur, S_3. The energetics are consistent with S_3 playing an important role in the reaction mechanism. It has not been possible to locate a low-energy pathway for production of diatomic sulfur, S_2. The computational data given here provide new insight to the chemistry of pentathiepins, which is relevant to the natural product **1** under physiological conditions.

Computational Details

Density functional thoeretical (DFT) calculations were performed to probe the structural features and reactions of pentathiepins. Standard computational notations have been used.[28] Geometries were optimized by using DFT with the exchange-correlation of B3LYP along with the 6-31G*, 6-311+G*, or 6-311G** basis set.[29] B3LYP/6-31G*, B3LYP/6-311+G*, and B3LYP/6-311G** theoretical calculations are found to reproduce X-ray crystal structures of sulfur heterocycles in addition to molecules which contain hypervalent sulfur centers.[30] The qualities of the geometry optimizations were reasonable as assessed by comparison of calculated and experimentally available geometries of pentathiepins (Table 1, vide infra).[24,31] Transition structures have been confirmed by frequency calculations, and in most cases the corresponding two minima have been established by tracing the internal reaction coordinate (IRC). This study examines gas-phase and solution-phase reactions. Solvent effects were calculated with the polarized continuum model (PCM)[32,33] and self-consistent reaction field (SCRF) single-point calculations at the B3LYP/6-31G* level. The solvent calculations employ a parametrized cavity radius with 100 points per interlocking sphere and a dielectric constant of 80.10 to simulate an aqueous environment.

Table 1. Calculated and Experimental Values of Pentathiepin Structural Parameters[a−d]

Compd	S1-S2	C1-S1-S2	S8-S1-S2	S1-S2-S3-S4[h]
 **1**	2.111	104.0		72.0
 **6**	(2.041)[b,e]	(104.5)[b,e]		(74.8)[b,e]
	2.100	105.0		73.0
	2.110[f]	104.2[f]		73.0[f]
 **7**	2.094	106.0		76.0
 **8**	(2.053)[g]	(104.4)[g]		(74.0)[g]
 **9**	(2.060)[e]		(108.0)[e]	(98.8)[e]
	2.100		109.2	97.8

[a] Distances in Å; angles in deg. [b] Experimental values in parentheses. [c] B3LYP/6-31G* optimized geometries unless otherwise noted. [d] Numbering of the pentathiepin ring shown in **1**. [e] Reference 31. [f] B3LYP/6-311G** optimized geometry. [g] Reference 23. [h] The dihedral angle θ = S1−S2−S3−S4 is positive for a clockwise movement from S1 to S4 as you look from S2 to S3.

Results and Discussion

We thought that the research should focus on the mechanism of pentathiepin decomposition since spectroscopic observations of the intermediates have not been possible. In the present study,

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(15) Kimura, T.; Hanzawa, M.; Horn, E.; Kawai, Y.; Ogawa, S.; Sato, R. *Tetrahedron Lett.* **1997**, *38*, 1607−1610.
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(23) Galloway, C. P.; Doxsee, D. D.; Fenske, D.; Rauchfuss, T. B.; Wilson, S. R.; Yang, X. *Inorg. Chem.* **1994**, *33*, 4537−44.
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(25) Davidson, B. S.; Ford, P. W.; Wahlman, M. *Tetrahedron Lett.* **1994**, *35*, 7185−8.
(26) Buemi, G.; Zuccarello, F.; Raudino, A. *J. Mol. Struct. THEOCHEM* **1988**, *44*, 245−51.
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(29) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian-94*; Gaussion: Pittsburgh, PA, 1994.
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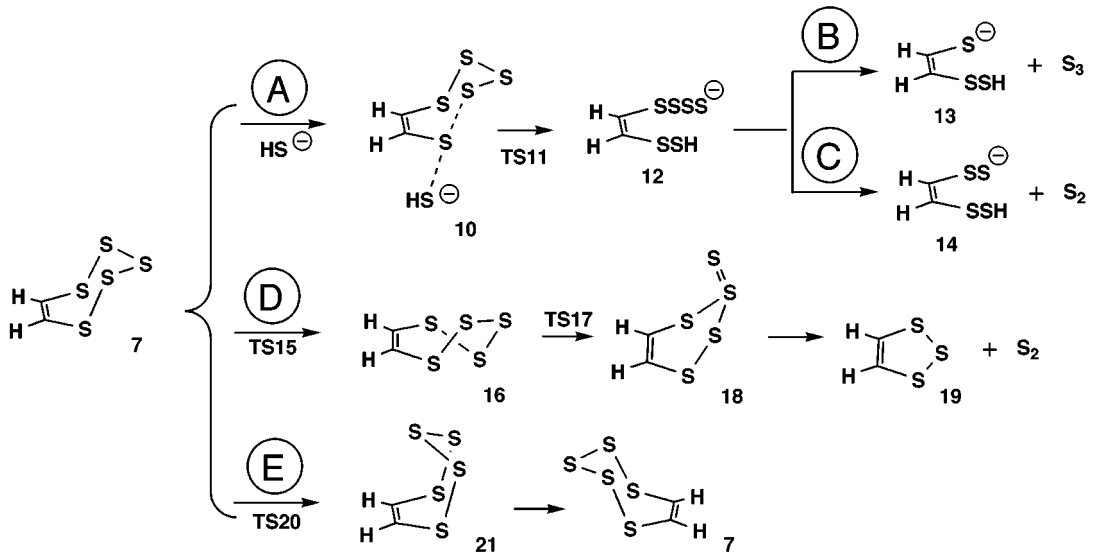
(33) Miertus, S.; Tomasi, J. *J. Chem. Phys.* **1982**, *65*, 239−245.

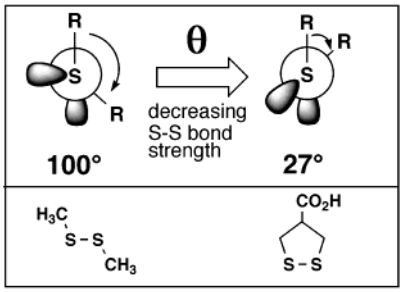
Scheme 4



theoretical calculations have been conducted for predicting geometries and energetics of the intermediates produced in pentathiepin reactions. The proposed reaction scheme is shown in Scheme 4 and the calculated structures are shown in Figures 1, 3, and 4. The calculated structures are shown in Figures 1, 3, and 4. The structural features of pentathiepins will be discussed first, followed by the structural and energetic features of the possible decomposition pathways A−E. Discovery of a facile low-energy route to pentathiepin decomposition (Pathway A then B, Scheme 4) and the corresponding S_3 intermediate derived from this pathway may provide a clue to explain the factors significant for biological activity.

Pentathiepin Structure. The molecular structure of pentathiepins has been previously established by X-ray crystallography.[24] The DFT method employed here is found to reproduce the experimental geometry of benzopentathiepin **6**.[9,31] Table 1 shows that the B3LYP/6-31G* level describes well **6** when compared to the X-ray crystal data and a B3LYP calculation with a triple-ζ (6-311G**) basis set. Experimental S−S bond lengths are generally 2.05 Å; while the longest S−S bonds known fall in the range 2.11−2.12 Å. DFT geometry optimizations provide reasonable agreement with a ∼0.06 Å discrepancy. The chair conformation of pentathiepin is the most stable, representing the global minimum.[14,25−27] We find that pentathiepins **1** and **6**−**8** display similar structural features to each other and display a gross similarity to stable cyclic S_8 (**9**) (Table 1). The B3LYP/6-31G* calculated structures for **1**, **6**, and **7** correlate with the X-ray structures of **8** and **9** in the S1−S2 bond lengths [**1** (2.111 Å), **6** (2.041 Å), **7** (2.094 Å), **8** (2.053 Å), and **9** (2.060 Å)]. However, the C1−S1−S2 bond angles in **1** (104.0°), **6** (104.5°), **7** (106.0°), and **8** (104.4°) are reduced compared to the S2−S1−S8 bond angle of **9** (108.0°). The main unit composed of five contiguous sulfurs shows a similarity between the pentathiepins, dihedral angle θ (S1−S2−S3−S4), **1** (72.0°), **6** (74.8°), **7** (76.0°), and **8** (74.0°), but is reduced when compared to cyclic S_8, **9** (θ = 98.8°). Pentathiepins may be less stable than cyclic S_8 given their reduced dihedral angle θ. For example, reduced dihedral angles have been linked with enhanced lone-pair interactions on adjacent sulfurs in disulfide molecules (Scheme 5). This is known to be destabilizing from antibonding overlap in the π^* molecular orbital,[34] which can

Scheme 5



reduce the sulfur−sulfur bond energy.[35] To determine the factors that underlie pentathiepin decomposition, two mechanistic extremes were considered. One emanates from a bimolecular reaction (Pathway A−C) and the other from unimolecular (Pathway D,E) reactions (Scheme 4).

Bimolecular Reaction. To explore the bimolecular pathway (A in Scheme 4), we have located intermediates on the DFT potential energy surface for the reaction of pentathiepin **7** + HS^- (Figure 1). The reaction of thiolate ion (RS^-) or thiol (RSH) with polysulfides is a known facile process (Scheme 6).[8,36] Our model focused on the deprotonated forms of RSH and RSSH since these groups are expected to exist largely as the corresponding anions (RS^- and RSS^-) at physiological pH. RSH and RSSH possess relatively low pK_a values, ∼12 and 6, respectively.[37,38] In addition, the proton affinity of H_2S decreases by ∼30 kcal/mol when it approaches the S1 position of pentathiepin **7** at a distance of 5.0 Å compared to 2.5 Å (**10**+**H**, Scheme 6). A B3LYP/6-31G* calculation predicts that intermediate **10** forms near equivalent S1−S2 and HS−S1 bond distances (2.505 and 2.502 Å, respectively). This structural feature is corroborated at the B3LYP/6-311+G* level (Figure 1). We suggest that the unanticipated equivalency of the S−S bonds is a result of the increasing importance of the apical sulfurs (S2 and HS^-) to share the negative charge. The S−S−S

(34) Steudel, R. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 655−664.

(35) Oae, S.; Doi, J. T. *Organic Sulfur Chemistry: Structure and Mechanism*; CRC Press: Boca Raton, FL, 1991.

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(37) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*; Harper & Row: New York, 1987.

(38) Everett, S. A.; Folkes, L. K.; Wardman, P.; Asmus, K.-D. *Free Radical Res.* **1994**, *20*, 387−400.

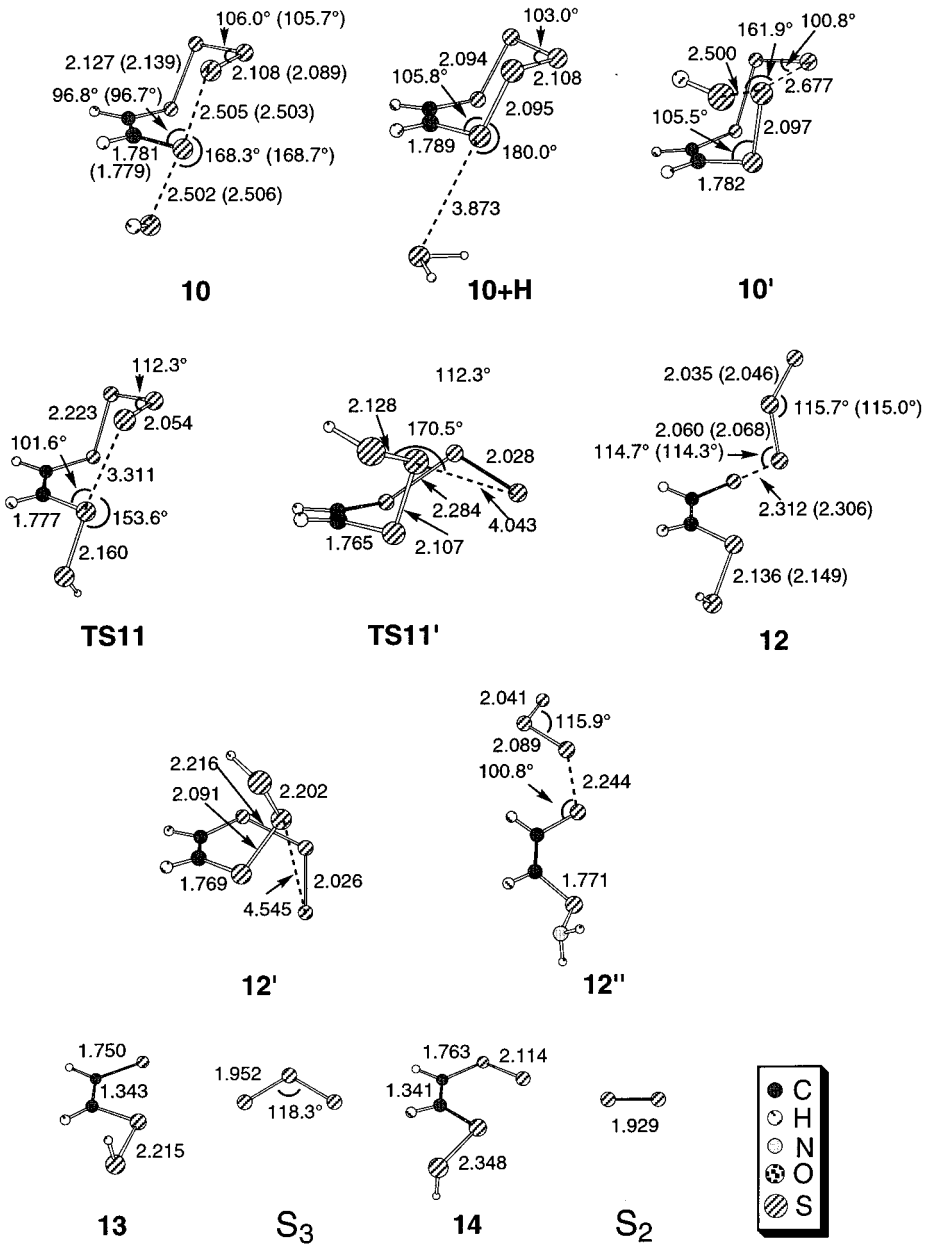
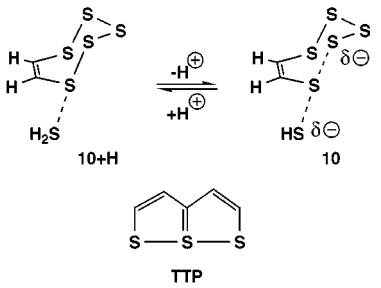


Figure 1. B3LYP/6-31G* optimized geometries (bond distances are in Å, angles are in deg). Values in parentheses are from B3LYP/6-311+G* calculations.

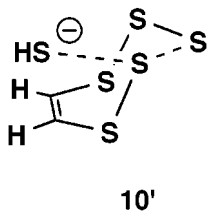
Scheme 6



linkage in ground state **10** bears a gross similarity with the known symmetric S−S−S linkage of trithiapentalene (TTP, Scheme 6), although the latter system involves the π-delocalization of a neutral system.

We have investigated several axial/equatorial approaches of HS^- to the polysulfur ring. The nucleophile seems to prefer the apical position on the sulfur atom (S1) adjoining the ethylene

Scheme 7



of **7**. Attack of the nucleophile at the S1 position (**10**, Scheme 6) is favored compared to attack at S2 (**10′**, Scheme 7) (vide infra). Sato et al. noted that the sulfur adjoining the benzene of **6** was a possible focal point of carbanion attack.[17] Similarly, Mulhearn and Bachrach calculated a 2−5 kcal/mol preference for attack of thiolate ion on the terminal sulfur of a trisulfide, although their system was a linear acyclic compound.[39]

(39) Mulhearn, D. C.; Bachrach, S. M. *J. Am. Chem. Soc.* **1996**, *118*, 9415−9421.

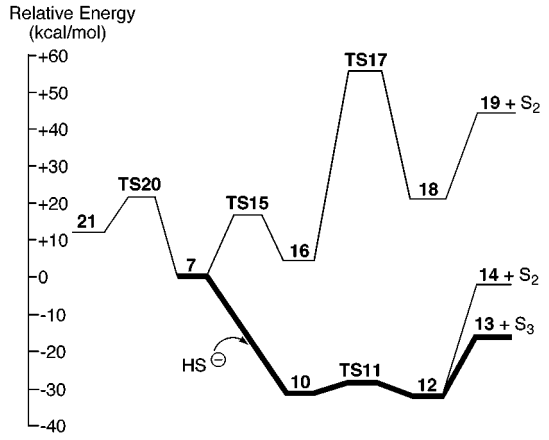
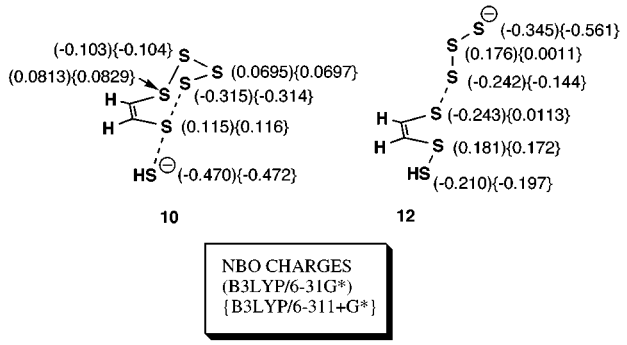


Figure 2. B3LYP/6-31G* calculated potential energy surface. The gas-phase energies are shown, and the solution-phase energies are discussed in the text. Energies of **14**+S2 and **13**+S3 were optimized with the reagents separated at a 5 Å distance.

We find that pentathiepin **7** forms an intermediate structure with HS^- (**10**) in an exothermic process in the gas phase (−33.3 kcal/mol, B3LYP/6-31G*). The B3LYP/6-31G* optimized structure **10** possesses a strong S1−ligand interaction, where HS^- engages in coordination opposite to the S1−S2 bond in the apical direction. Structure **10′** has HS^- engaged opposite the S2−S3 bond. The S1−SH^- bond distance in **10** is 2.502 Å and in **10′** the S2−HS^- bond distance is 2.500 Å. The apical S1−S2 distance is longer in **10** (0.411 Å) than the pentathiepin precursor, **7**, as is the S2−S3 bond distance in **10′** (0.570 Å) (compare Table 1 and Figure 1). This is anticipated for ligands which can interact with the LUMO of pentathiepin S−S to form a 3-centered-4-electron (3c-4e) bond.[40−42] The pentathiepin−ligand contact is accompanied by a transfer of electron density as revealed by an increase in the negative natural bond order (NBO)[43] charges at apical pentathiepin sulfur and a decrease in the charge at HS^-. The transfer of electron density is reminiscent of charge-transfer interactions known to accompany S_8−amine reactions.[44−46]

A transition state has been located (**TS11**), which predicts that open-chain polysulfide ion **12** is derived from **10**. The activation barrier of **TS11** is 1.9 kcal/mol with gas-phase B3LYP/6-31G* calculations. A transition state (**TS11′**) with an activation barrier of 4.5 kcal/mol has also been located representing an attack of HS^- at S2 and the connection of minima **10′** and **12′**. The reactions of **7** + HS^- → **12/12′** are best described as an addition−elimination. Since the activation barrier to **12** is reduced compared to **12′**, the potential for a unimolecular rearrangement of open-chain **12** was examined, the energetic features of which are given in Figure 2. The negative charge is not localized on any one sulfur in structures **10** and **12**. For example, the NBO charge on HS^- (−0.470) is similar to the charge on S2 (−0.315). This may explain why PCM calculations indicate little effect on the energetics of **10** → open-chain **12** interconversion when increasing the dielectric constant from the gas-phase ($\epsilon = 0$) value to that of water ($\epsilon = 80.1$). However, it should be noted that PCM can have difficulties with sulfur anions since it assumes an even charge distribution. We believe that Scheme 4 is qualitatively reasonable because the charges involved are comparable by B3LYP calculations using the 6-31G* basis set and also the 6-311+G* basis set with diffuse functions (Scheme 8).

Scheme 8



Triatomic Sulfur (S_3). Two fragmentation pathways of **12** were examined on the DFT potential energy surface. One involves cleavage of the S4−S5 bond of **12** to give **13** + S_3 (B in Scheme 4) and the other cleavage of the S3−S4 bond to give **14** + S_2 (C in Scheme 4). Several rotational isomers have been identified as competing minima for structures **13** and **14** (Figure 1, Scheme 9A,B). While generally not regarded as a strong H-bond acceptor, such a species can serve this role because of an association of hydrogen with the terminal anion (e.g., **13′**, Scheme 9A). The resulting six-membered ring structure **14‴** is the most stable local minimum of the latter series (Scheme 9B).

Although few reports have appeared on the existence of sulfur-transfer, as S_3,[47−49,60] compared to S_2,[50−54] the computational results here argue that S_3 dissociation provides the favored low-energy route to pentathiepin decomposition. Pathways B and C were optimized with the resulting species isolated at a fixed distance of 5.0 Å (Scheme 4). The B3LYP/6-31G* calculation reveals an unusually long S4−S5 bond distance for **12** (2.312 Å), a structural feature that is corroborated at the B3LYP/6-311+G* level (2.307 Å, Figure 1). A similarly long S4−S5 bond distance is found in **12″**, which represents a reaction involving **7** with NH_2^-. The structural features of **12**

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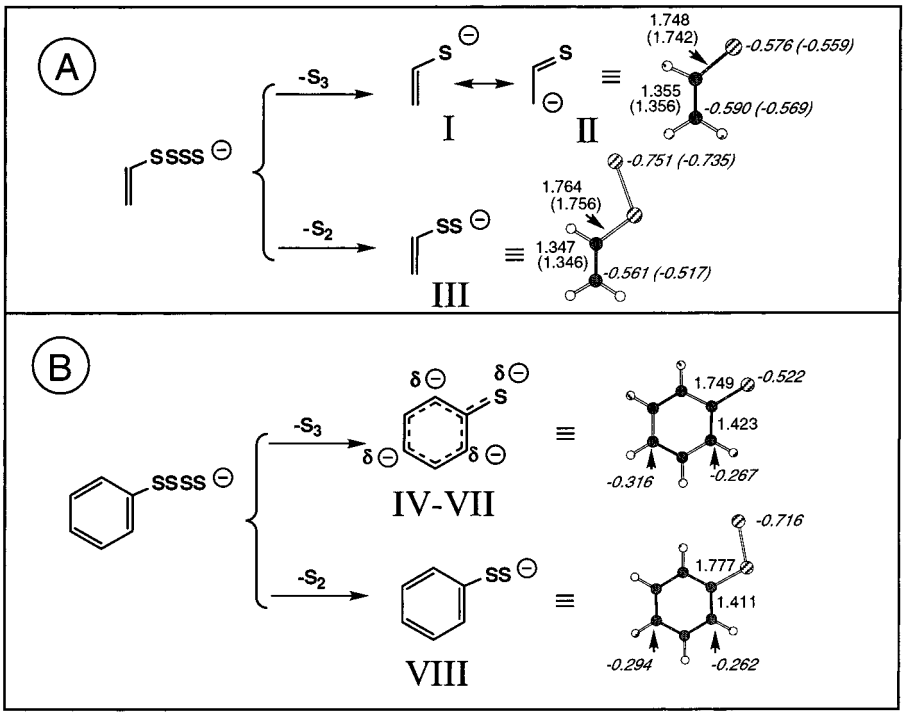
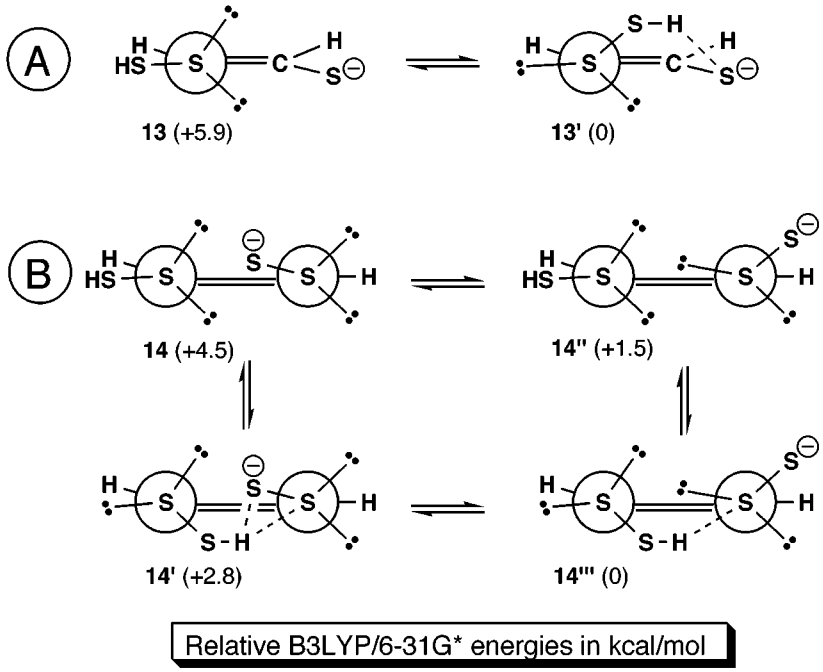


Figure 3. B3LYP/6-31G* optimized geometries. Values in parentheses are B3LYP/6-311+G* optimized geometries. Distances are in Å, natural bond order, NBO, charges are in italics.

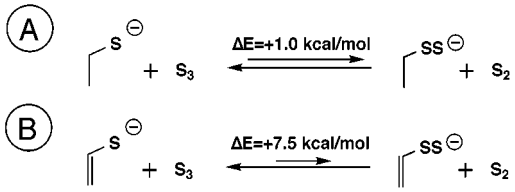
Scheme 9



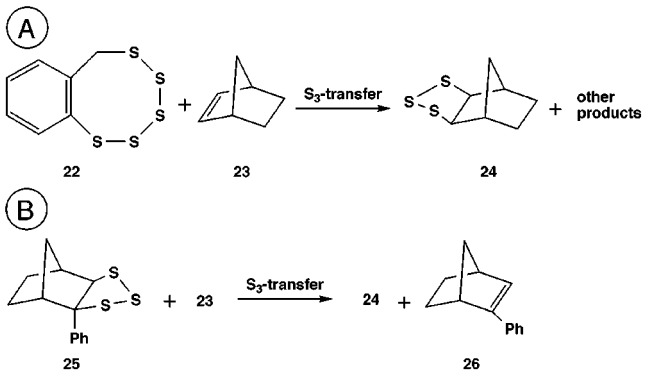
and **12″** predict a potential for S_3 cleavage. Ordinary sulfur−sulfur bond distances are short in comparison (∼2.05 Å); for example, the crystal structure of linear S_6 reveals S−S bond distances of 2.027−2.080 Å.[55] We find that the driving force to fragmentation of the S4−S5 bond in **12** or **12″** is based upon delocalization of the negative charge in the sulfur-substituted carbon fragment. To illustrate this point a computational analysis involving charge redistribution and resonance is presented, which implicates S_3-cleavage in a pentathiepin decomposition (structures **I**−**VIII**, Figure 3).

Electronic redistribution plays an important role in determining the potential for S−S fragmentation of a polysulfur ion (Figure 3A,B). Figure 3A shows that the departure of S_3 results in a resonance stabilized allyl anion (**I** ↔ **II**). Departure of S_2 yields a carbon−sulfur fragment (**III**) with a reduced capacity for resonance stabilization. The terminal sulfur in structure **III** has an enhanced negative NBO (−0.751) charge compared to **I** ↔ **II** (−0.576). In addition, structure **III** has a shorter C−C bond length (1.347 Å) as anticipated for increased double-bond character compared to **I** ↔ **II** (1.355 Å). Figure 3B shows a similar structural characteristic upon departure of S_3, which yields a resonance stabilized benzyl anion (**IV**−**VII**). Departure of S_2 produces a carbon−sulfur fragment **VIII**, which possesses an enhanced negative charge at terminal S and a reduced

Scheme 10



Scheme 11



capacity for delocalization. A mechanistic picture now emerges that indicates the importance of sulfur−sulfur (S4−S5) bond cleavage. The unanticipated potential for S_3 fragmentation reflects an importance for the delocalization of the negative charge in the remaining carbon−sulfur fragment. A reduced energy gap materializes for a decomposition yielding S_3 compared to S_2 (Figure 2). The delocalization plays a determining role in the stability of **13** over **14** since the trend is in large part nullified in a saturated system (Scheme 10). The relative energies indicate that sulfur cleavage is largely insensitive to S_2 versus S_3 fragmentation in the saturated system (Scheme 10A). However, the energetic preference for S_3 fragmentation is ascribed to delocalization of the negative charge in the unsaturated "allyl" system (Scheme 10B). Preference for attack of the nucleophile at the S1 position of pentathiepin underlies the predicted favorability of S_3 versus S_2 discussed here. Interestingly, the higher barrier calculated for attack at the S2 position of pentathiepin precludes a pathway that favors S_2, which could conceivably lead to an anion that can delocalize (Figure 1).

These data predict that pentathiepin **7** derives reactivity from a S_3-fragmentation (Pathway A then B, Scheme 4). Triatomic sulfur is detectable by transient absorption spectroscopy,[56] but has not been considered previously in a pentathiepin reaction. A related sulfur-transfer reaction may shed light on the mechanism of pentathiepin decomposition. Upon heating of 6H-benzo[*g*]-1,2,3,4,5-pentathiocin (**22**) with norbornene (**23**) a S_3-transfer reaction occurs giving *exo*-3,4,5-trithiatricyclo-[5.2.1.$0^{2,6}$]decane (**24**, Scheme 11A).[60] Ghosh and Bartlett[47] also suggested that S_3 transfer may occur via the unequal bond-breaking of *endo*-2-phenyl-*exo*-3,4,5-trithiatricyclo[5.2.1.$0^{2,6}$]-decane (**25**) giving **24** and 2-phenyl norbornene (**26**) and in a bimolecular concerted process (Scheme 11B).[47] Our computational data predict that open-chain **12** is a candidate for loss of S_3; however, further insight on alternative fragmentation pathways was needed.

Diatomic Sulfur (S_2). Our computational data indicate that the energetics are inconsistent with diatomic sulfur, S_2, playing a role in the reaction mechanism via Pathway C (Scheme 4). However, thiosulfoxide species have been implicated previously in S_2 release (e.g., **27**, Scheme 12).[50−52] Thus the rearrangement

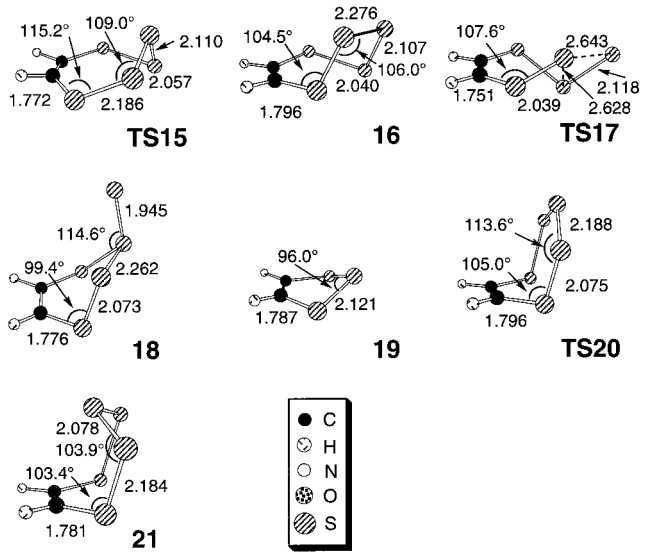
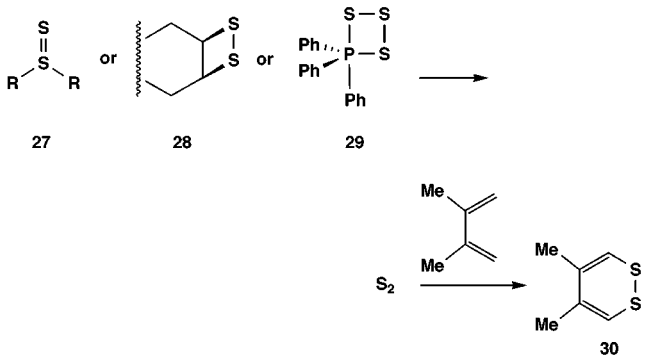


Figure 4. B3LYP/6-31G* optimized geometries. Distances are in Å, angles are in deg.

Scheme 12



of **7** to thiosulfoxide **18** was examined as a possible pathway for S_2 production (Pathway D, Scheme 4). We find that twist-boat pentathiepin (**16**, Figure 4) can be formed directly from the chair form **7** via transition state **TS15**, with the outer sulfur (S4) rotated down relative to the S1−S2 bond. Chair-form pentathiepin **7** has C_s symmetry and **TS15** has no symmetry, therefore this involves a reaction path bifurcation occurring before the transition state. The barrier separating **7** and **16** is calculated as 17.0 and 16.3 kcal/mol, respectively, with gas-phase B3LYP/6-31G* and solution-phase PCM//B3LYP/6-31G* calculations. Unimolecular conversion of **16** to thiosulfoxide **18** is predicted via **TS17**. The geometry of **TS17** shows a shorter S3−S4 bond (2.118 Å) compared to S2−S3 (2.643 Å), which indicates a developing S═S thiosulfoxide bond. The transition state for interconversion between the two species (**16**, **18**) is highly energetically costly (53.6 kcal/mol) and endothermic (15.9 kcal/mol), which predicts that a thiosulfoxide would be experimentally inaccessible. We find that increasing the dielectric constant from $\epsilon = 0$ (gas-phase) to 80.1 (water) has little effect on the energetics of this interconversion.

A pathway for diatomic sulfur formation has thus far not been established for pentathiepins. However, the idea of a "S_2 fragment" departing from pentathiepin **5** during its equilibration with trithiolane **5′** was inferred by Chenard,[9] but has yet no experimental support since the nature of the chemical intermediates responsible for equilibrium has not been defined. Chenard's[9] pentathiepin studies did not involve attempts to trap the S_2 intermediate. We show that the predicted energetics are

inconsistent with S_2 playing a role in the mechanism. Our calculations predict that the conversion of pentathiepin **7** to trithiolane **19** + S_2 is endothermic by 45.4 kcal/mol (Figure 2). Undoubtedly, S_2-transfer is significant in chemical systems separate from the pentathiepins (Scheme 12). The decomposition of dithietane (**28**) yields a Diels−Alder adduct (**30**) from the addition of the S_2 unit to the diene.[52] In addition, the decomposition of intermediate R_3PS_3 (**29**) is similar to the established method for $^1O_2$ generation from triphenyl phosphite ozonide (R_3PO_3).[50−54] However, our computational data on pentathiepin contrast these examples and indicate that the cleavage of S_2 via Pathway C or D has a higher energetic cost in comparison to S_3-cleavage (Pathway B, Scheme 4). It should be noted that pentathiepins may cleave S_2 in a mass spectrometer, but that this high-energy process provides little information regarding species relevant in biochemistry.[6,7,20] Interestingly, Sato et al.[60] suggested a possible equilibrium between S_3 and $S_2 + S_X$. Unfortunately, the computational data collected thus far cannot corroborate this suggestion since S_2 may be expected to attack S_X and lead to a polysulfur species, which exhibits no particular bias toward S_3 cleavage. A challenging aspect of structure determination is the assignment of reactive intermediates, such as organic anions[57] and polysulfur species.[22,58−61]

Chair−Chair Interconversion. There are a number of experimental[25] and theoretical[14,25−27] studies on chair−chair interconversions of pentathiepins (represented as Pathway E in Scheme 4). Previous experimental data show that pentathiepins have significant barriers to rearrangement, ∼24−29 kcal/mol, so that chair−chair interconversion is not observed.[14,15,27,62] We find that pentathiepin **7** can rearrange to the boat form (**21**) via transition structure **TS20** on the DFT potential energy surface. The magnitude of the calculated barrier is 26.6 and 23.8 kcal/mol, respectively, with gas-phase B3LYP/6-31G* and solution-phase PCM//B3LYP/6-31G* calculations (Figure 4), which corroborate similar experimental findings. We are able to evaluate that pentathiepin decomposition must exist separate from Pathway E. In this paper, the preference for pentathiepin decomposition is predicted based upon a bimolecular reaction, Pathway A then B (Scheme 4).

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Conclusion

This study has opened up a new aspect in polysulfur chemistry. Density functional theoretical calculations predict the formation of triatomic sulfur, S_3, in the decomposition of pentathiepin. The complicated product mixtures associated with pentathiepin reactions have hampered previous experimental attempts to elucidate aspects of the reaction mechanism. Our theoretical study provides evidence for an open-chain polysulfur ion intermediate, **12**, from which a key determinant in the chemistry then follows. Expulsion of diatomic sulfur is unlikely from a unimolecular collapse of **12** or from a reaction path involving thiosulfoxide **18**. Instead, S_3 can dissociate from **12** due to an unusually long and weak S4−S5 bond. Aside from the fundamental interest in the area, it is of special significance that the S_3 unit transfer may be at the basis of the biological activity of pentathiepins. How the S_3 intermediate may modulate the antitumor activity of pentathiepins is yet unknown. Oxygen radicals appear to play a role in the DNA-cleaving activity in vitro.[8] Reactive sulfur and oxygen species would be expected to exhibit distinct chemistry. When compared to other polysulfur-containing natural products that induce DNA-damaging activity, such as calicheamicin and esperamicin, pentathiepins have received little attention. The computed results presented here predict that a novel S−S cleavage reaction to generate S_3 is related to the process from which pentathiepins decompose.

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Supporting Information Available: Descriptions of the geometries of all stationary points and absolute energies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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