

# Antithrombotic Organosulfur Compounds from Garlic: Structural, Mechanistic, and Synthetic Studies<sup>1</sup>

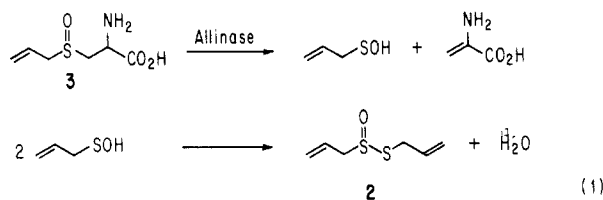
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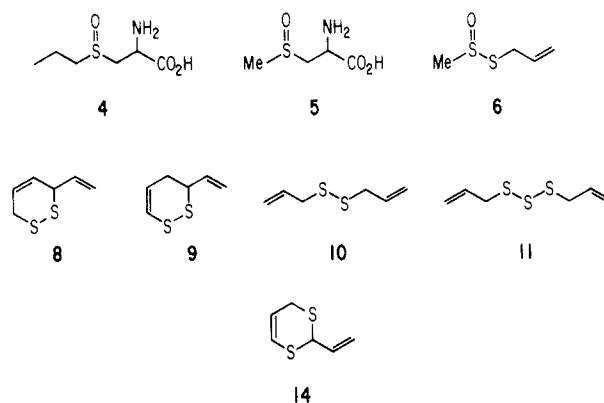
**Abstract:** Garlic (*Allium sativum*) extracts contain a compound termed ajoene, which, among other compounds from garlic, is a potent inhibitor of platelet aggregation. The structure of ajoene was determined by spectroscopic methods to be (*E* and *Z*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide. Ajoene can be readily synthesized by decomposing alliin in acetone-water. A detailed mechanism is presented for the thermal decomposition of alliin. Ajoene could be oxidized to its 9,9-dioxide, 4,9,9-trioxide, and 4,4,9,9-tetraoxide. An attempted synthesis of the ajoene isomer (*E*)-4,5,9-trithiadodeca-1,7,11-triene 9-oxide led instead to a sulfine (thial *S*-oxide) by way of the sulfoxide thio-Claisen rearrangement. Decomposition of the garlic component *S*-allyl methanethiosulfinate led to various homologues of ajoene. A variety of ajoene homologues in which the 6,7-double bond has been replaced by an *o*-phenylene group have been prepared from *o*-thiosalicylic acid. Data on the antithrombotic activity of a variety of structures related to ajoene have been used to explain the molecular basis for antithrombotic activity of ajoene, which is attributed to its ability to alter platelet membranes by capturing sulfhydryl groups.

Garlic (*Allium sativum*) has been used worldwide as a folk medicine for prevention of stroke, coronary thrombosis, and atherosclerosis, as well as for treatment of various diseases including infections and vascular disorders.<sup>3</sup> Garlic extracts are also reputed to reduce serum cholesterol levels and increase blood coagulation time.<sup>4</sup> In the course of research on physiologically active sulfur compounds in plants of the *Allium* family<sup>5</sup> we have investigated garlic-derived compounds with antithrombotic activity (e.g., preventing aggregation of platelets) and have discovered an organosulfur compound of unusual structure, (*E,Z*)-ajoene **1**. We describe here isolation and characterization of **1** from garlic extracts, a biogenetically modelled synthesis of **1** from the garlic antibacterial constituent alliin (allyl 2-propenethiosulfinate, CH<sub>2</sub>=CHCH<sub>2</sub>S(O)SCH<sub>2</sub>CH=CH<sub>2</sub>, **2**), and preparation of various antithrombotic organosulfur compounds related to **1** of new structural types.<sup>6</sup> Development of a synthesis of **1** from **2** led to an improved understanding of the chemical transformations of **2** while investigation of alternative syntheses of **1** and its homologues led to the discovery of the sulfoxide thio-Claisen rearrangement.<sup>7</sup>

Reports from the 19th century describe diallyl (from "Allium") disulfide, trisulfide, and tetrasulfide in steam-distilled garlic oil.<sup>8</sup> In 1944 Cavallito described the isolation of the odoriferous antibacterial substance alliin (**2**) from extraction of garlic with ethanol at room temperature.<sup>9</sup> Four years later Stoll and Seebeck<sup>10</sup> reported that intact garlic cloves contain 0.24% by weight *S*-allylcysteine *S*-oxide (alliin) (**3**), a colorless odorless solid, and an enzyme allinase which converts **3** into **2** (eq 1). Subsequent



research revealed that the cysteine sulfoxide fraction of garlic consists of 85% **3** along with 2% *S*-propylcysteine sulfoxide **4** and 13% *S*-methylcysteine sulfoxide **5**.<sup>11</sup> Action of allinase on the mixture of **3**-**5** affords allyl methanethiosulfinate, MeS(O)-SCH<sub>2</sub>CH=CH<sub>2</sub> (**6**), methyl methanethiosulfinate, MeS(O)SMe (**7**), and other mixed or symmetrical thiosulfonates RS(O)SR' (R



and R' are variously methyl, propyl, and allyl) found in garlic extracts in addition to **2**.<sup>11</sup> Analysis of garlic extracts by Brodnitz and co-workers using GC-MS revealed the presence of 79% of a 2.4:1 mixture of two compounds A and B claimed to be 3-

(1) (a) Portions of the material covered in this paper are the subjects of U.S. Patent Applications filed by the Research Foundation of the State University of New York. (b) The Chemistry of Alkyl Thiosulfonate Esters. 9. (c) Part 8: Block, E.; Ahmad, S.; Jain, M. K.; Crecey, R. W.; Apitz-Castro, R.; Cruz, M. R. *J. Am. Chem. Soc.* **1984**, *106*, 8295-8296.

(2) (a) SUNY-Albany. (b) New York State Department of Health. (c) University of Delaware. (d) IVIC.

(3) Block, E. In *Folk Medicine: The Art and Science*; Steiner, R. P., Ed.; American Chemical Society: Washington, DC, 1986; pp 125-137. Fenwick, G. R.; Hanley, A. B. *CRC Crit. Rev. Food Sci. Nutr.* **1985**, *22*, 199-271, 273-377; **1986**, *23*, 1-74. Lau, B. H. S.; Adetumbi, M. A.; Saez, A. *Nutr. Res. (N.Y.)* **1983**, *3*, 119-128. Bolton, S.; Null, G.; Troetel, W. M. *Am. Pharm.* **1982**, *NS22*, 40-43 and references therein.

(4) (a) Sodimu, O.; Joseph, P. K.; Augusti, K. T. *Experientia* **1984**, *40*, 78-80. (b) Kamanna, V. S.; Chandrasekhara, N. *Lipids* **1982**, *17*, 483-488. (c) Chi, M. S. *Proc. Soc. Exp. Biol. Med.* **1982**, *171*, 174-178. (d) Bordia, A. *Atherosclerosis (Shannon, Irel.)* **1978**, *30*, 355-360.

(5) Block, E. *Sci. Am.* **1985**, *252*, 114-119.

(6) Preliminary communication, ref 1c; also, see: Ahmad, S. Ph.D. Thesis, State University of New York at Albany, 1986.

(7) Block, E.; Ahmad, S. *J. Am. Chem. Soc.* **1985**, *107*, 6731-6732.

(8) Wertheim, T. *Justus Liebigs Ann. Chem.* **1844**, *51*, 289-315; **1845**, *55*, 297-304. Semmler, F. W. *Arch. Pharm.* **1892**, *230*, 434-443.

(9) Cavallito, C. J.; Bailey, J. H. *J. Am. Chem. Soc.* **1944**, *66*, 1950-1951. Cavallito, C. J.; Buck, J. S.; Suter, C. M. *J. Am. Chem. Soc.* **1944**, *66*, 1952-1954.

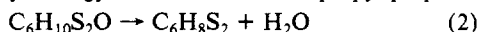
(10) Stoll, A.; Seebeck, E. *Helv. Chim. Acta* **1948**, *31*, 189-210. Stoll, A.; Seebeck, E. *Adv. Enzymol. Relat. Subj. Biochem.* **1951**, *11*, 377-400.

(11) Freeman, G. G.; Whenham, R. J. *J. Sci. Fd. Agric.* **1975**, *26*, 1869-1886. Whitaker, J. R. In *Advances in Food Research*; Chichester, C. O., Mrak, E. M., Stewart, G. F., Eds.; Academic Press: New York, 1976; Vol. 22.

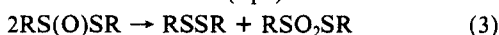
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† Fellow of the John Simon Guggenheim Memorial Foundation 1984-1985.

vinyl-6*H*-1,2-dithiin (**8**) and 3-vinyl-4*H*-1,2-dithiin (**9**), respectively, on the basis of analysis by <sup>1</sup>H NMR, IR, Raman, and mass spectrometry.<sup>12</sup> Compounds **8** and **9** were said to be dehydration products of allicin (**2**) formed during gas chromatography according to eq 2 by analogy to the conversion of propyl propane-

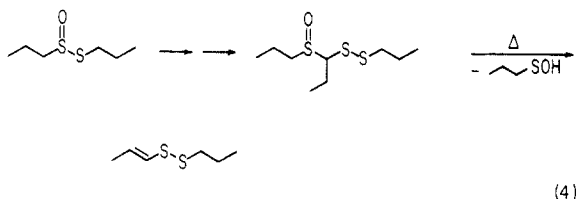


thiosulfinate to 1-propenyl propyl disulfide at 150 °C.<sup>12</sup> The other volatile components of garlic extract were determined to be allyl alcohol (5.4%), methyl allyl disulfide (1.2%), diallyl disulfide **10** (5.7%), dimethyl trisulfide (2.4%), allyl methyl trisulfide (1.5%), diallyl trisulfide **11** (1.0%), and sulfur dioxide.<sup>12</sup> Brodnitz further observed that **2** underwent nearly complete decomposition at 20 °C after 20 h giving as major products **10** (66%), **11** (9%), diallyl sulfide (14%), sulfur dioxide, and traces of **8** and **9**. Thio-sulfonates, typically produced along with disulfides upon disproportionation of most thiosulfonates (eq 3) were not formed from



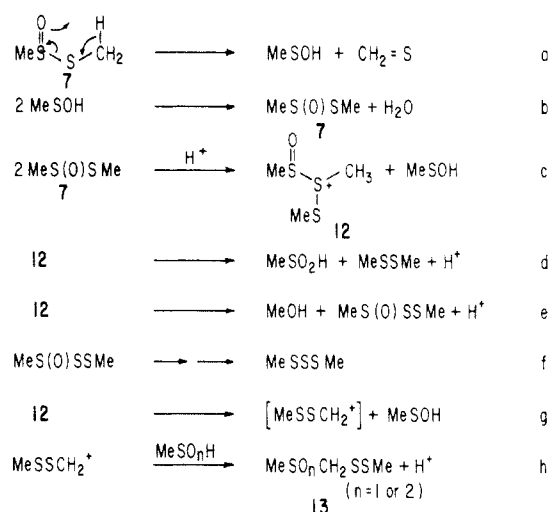
**2**.<sup>12</sup> In his discussion of the decomposition of **2** Brodnitz does not mention the observation Cavallito made in 1944<sup>9</sup> that "[neat samples]... of allicin undergo a chemical change on standing at room temperature, yielding an inactive viscous liquid which is no longer water soluble and cannot be distilled... The antibacterial agent appears to have undergone an intermolecular reaction involving three molecules." The ability of various components of garlic oil to inhibit aggregation of blood platelets<sup>13</sup> has been ascribed to **2**, allyl methyl trisulfide or diallyl trisulfide **11**.<sup>14</sup> Recently two of us<sup>15</sup> reported that an unknown garlic-derived polar compound, now named ajoene **1**, is a particularly potent anti-thrombotic agent. Unlike several other inhibitors of platelet aggregation, this compound has been found to inhibit aggregation induced by all known inducers.<sup>15</sup>

In an effort to better understand the chemistry of allicin (**2**) and related alkyl alkanethiosulfonates one of us began in 1972 a systematic investigation of this unusual class of compounds focussing initially on methyl methanethiosulfinate **7** and saturated homologues. These studies demonstrated that the disproportionation of **7** indicated by eq 3 is in fact a complex process (see Scheme I)<sup>16</sup> involving (1) as an initiating step a β-elimination reaction, giving an alkanesulfenic acid and thioaldehyde; (2) as the second key step acid-catalyzed intramolecular thioalkylation to a sulfonium intermediate **12**; (3) partitioning of the sulfonium intermediate to (a) alkanesulfenic acid, the precursor of thio-sulfonate and disulfide, (b) to trisulfide, or (c) by a second β-elimination to an α-disulfide carbonium ion (alkyl dithiocarbocation); (4) the latter ion can give α-alkylsulfenyl or α-alkylsulfenyl disulfide **13** by readdition of alkanesulfenic or alkanesulfenic acid, respectively, to the carbonium ion. In the case of higher alkyl alkanethiosulfonates, e.g., PrS(O)SPr, β-elimination of an alkanesulfenic acid from an α-alkylsulfenyl disulfide affords an alkyl 1-alkenyl disulfide (eq 4). Our findings suggest that the earlier mechanistic proposals for decomposition of allicin require modification.



(4)

Scheme I



## Results and Discussion

**Isolation of (E,Z)-Ajoene from Garlic Extracts.** Extension of our investigation to allicin itself was initiated by correspondence between several of us (E.B., M.K.J., and R.A.C.) over the identity of antithrombotic substances isolated from garlic extracts<sup>15</sup> and led to the collaborative studies described herein. Isolation and separation of the garlic constituents was monitored with bioassay for inhibition of platelet aggregation (see Experimental Section). Chopped garlic pieces were soaked in methanol for 3 days. The concentrate was suspended in water and extracted with ether. The ether extract was concentrated, and the residue was stored at 25 °C for 4 days as a 10% solution in methanol, diluted with water, and repeatedly extracted with hexane to separate the less polar compounds from the more polar ones. The aqueous methanolic layer was then extracted with methylene chloride. Concentration of the hexane and methylene chloride extracts separately afforded yellow oils.

Repeated HPLC (silica gel/hexane) and preparative thin-layer chromatography of the hexane extract gave several divalent sulfur compounds which were characterized by direct comparison of their spectral data and GC retention times with data on authentic material. The major nonpolar components were thereby identified as diallyl disulfide **10**, diallyl trisulfide **11**, diallyl tetrasulfide, allyl methyl trisulfide, 2-vinyl-4*H*-1,3-dithiin, **14**, 3-vinyl-4*H*-1,2-dithiin, **9**, and allicin, **2**. Careful comparison of the <sup>1</sup>H NMR and other spectroscopic data on our compounds **14** and **9** with Brodnitz's compounds A and B, respectively, showed **14** and A to be identical and **9** and B to also be identical, requiring that Brodnitz's structural assignments be revised.

Analysis of the methylene chloride extract by HPLC (silica gel/hexane-*i*-propanol 92:8) indicated the presence of two major fractions which could be separated from each other either by flash chromatography (silica gel/ethyl acetate) or by preparative HPLC. Elemental analysis as well as the molecular ion peak under chemical ionization with tetramethylsilane or methane suggested a molecular formula C<sub>9</sub>H<sub>14</sub>S<sub>3</sub>O for both polar fractions. Further structural elucidation of these compounds was performed by spectroscopic methods as follows.

**Fraction 1 (minor fraction).** The IR spectrum displayed a sulfoxide (C–S(O)–C) stretch at 1045 cm<sup>−1</sup> in addition to strong C=C absorption at 1650 cm<sup>−1</sup>. The <sup>13</sup>C NMR spectrum indicated nine nonequivalent carbons (six olefinic and three saturated). The <sup>1</sup>H NMR spectrum was quite complex suggesting the presence of several diastereotopic CH<sub>2</sub> groups and a (Z)-CH=CHS– group (δ 6.55 ppm doublet, *J* = 9 Hz, integrating for one proton). The above data are consistent with the structures (Z)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide, CH<sub>2</sub>=CHCH<sub>2</sub>S(O)CH<sub>2</sub>CH=CHSSCH<sub>2</sub>CH=CH<sub>2</sub>, (Z)-**1**, or (Z)-4,5,9-trithiadodeca-1,7,11-triene 9-oxide, CH<sub>2</sub>=CHCH<sub>2</sub>S(O)CH=CHCH<sub>2</sub>SSCH<sub>2</sub>CH=CH<sub>2</sub>, **15**. Structures such as CH<sub>2</sub>=CHCH<sub>2</sub>SCH<sub>2</sub>CH=CHS(O)SCH<sub>2</sub>CH=CH<sub>2</sub> possessing a –S(O)S– group instead of a

(12) Brodnitz, M. H.; Pascale, J. V.; Van Derslice, L. J. *Agric. Food Chem.* **1971**, *19*, 273–275.

(13) Samson, R. R. *Atherosclerosis (Shannon, Irel.)* **1982**, *44*, 119–120. Gaffen, J. D.; Tavares, I. A.; Bennett, A. J. *Pharm. Pharmacol.* **1984**, *36*, 272–274. Srivastava, K. C. *Biomed. Biochim. Acta* **1984**, *43*, S335–S346; also, see: ref 4d.

(14) Ariga, T.; Oshiba, S.; Tamada, T. *Lancet* **1981**, *1*, 150–151. Augusti, K. T.; Mathew, P. T. *Experientia* **1974**, *30*, 468–470.

(15) Apitz-Castro, R.; Cabrera, S.; Cruz, M. R.; Ledezma, E.; Jain, M. K. *Thromb. Res.* **1983**, *32*, 155–169. Apitz-Castro, R.; Escalante, J.; Vargas, R.; Jain, M. K. *Thromb. Res.* **1986**, *42*, 303–311.

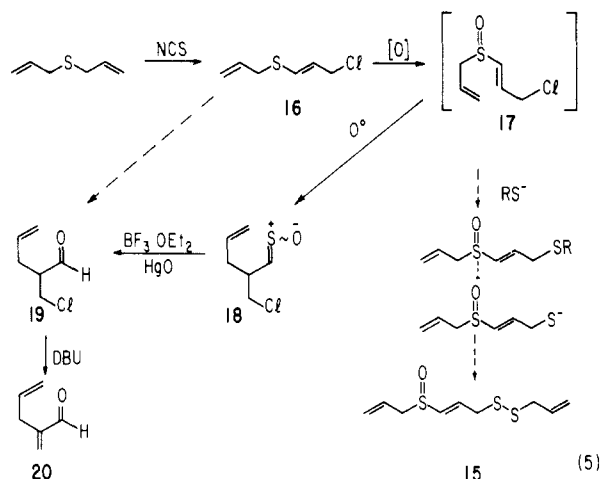
(16) Block, E.; O'Connor, J. J. *Am. Chem. Soc.* **1974**, *96*, 3929–3944.

C-S(O)-C group are ruled out by the IR spectrum since the former thiosulfinate group possesses a characteristic band at 1100  $\text{cm}^{-1}$ . Of the two alternative structures, **1** and **15**, we favored the former since the  $^{13}\text{C}$  NMR spectrum showed *two* deshielded saturated carbons at  $\delta$  54.5 and 53.1 and one more shielded saturated carbon at  $\delta$  41.4 which is more in keeping with a pair of saturated carbon atoms flanking a sulfinyl group<sup>17</sup> than a disulfide function (see below for additional NMR data on this point). Structure (*Z*)-**1** was termed (*Z*)-ajoene ("ajo", pronounced "aho", is garlic in Spanish).

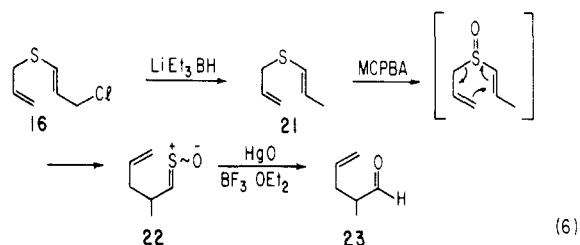
Fraction 2 (major isomer): the major fraction was characterized as (*E*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide (*E*)-**1** on the basis of the explanation given for the *Z* isomer (i.e., fraction 1). The *E* stereochemistry was established by analysis of the  $^1\text{H}$  NMR spectrum, namely appearance of a doublet at 6.38 ppm ( $J = 15$  Hz) corresponding to one hydrogen.

In spite of the accumulation of a variety of spectroscopic information on polar fractions 1 and 2 the choice between isomers **1** and **15** was not unequivocal. It seemed possible to resolve our dilemma through unambiguous total synthesis of the two isomers, through clarification of the mechanisms leading to **1** or **15** to determine if the most reasonable mechanism clearly favored one isomer or through study of chemical transformations of ajoene. All three approaches have been used as will be described below.

**Attempted Synthesis of (*E*)-4,5,9-Trithiadodeca-1,7,11-triene 9-Oxide ((*E*)-**15**). The Sulfoxide Thio-Claisen Rearrangement.** It is known that treatment of allyl sulfides with *N*-chlorosuccinimide affords 3-chloro-1-propenyl sulfides.<sup>18</sup> Application of this procedure to diallyl sulfide afforded allyl 3-chloro-1-propenyl sulfide **16** in quantitative yield (eq 5). Our initial strategy was

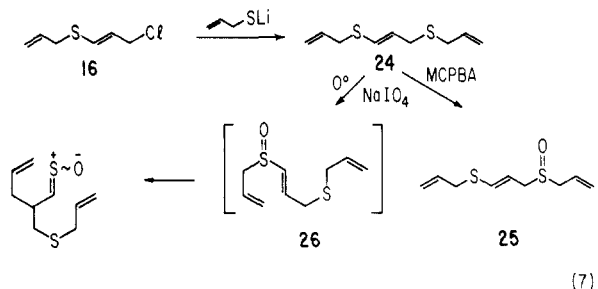


to convert **16** to the corresponding sulfoxide **17** and then replace the chlorine with a sulfur function such as the thioacetate or 2-tetrahydropyranythio group which could become part of a disulfide group (eq 5). To our surprise MCPBA oxidation of sulfide **16** at 0 °C led not to **17** but rather to (*Z*)-2-chloromethyl-4-pentenethial *S*-oxide **18** in 90% isolated yield.<sup>7</sup> To determine if the sulfoxide-accelerated thio-Claisen reaction was general, sulfide **16** was reduced to allyl (*E*)-1-propenyl sulfide **21** with lithium triethylborohydride (eq 6). Oxidation of **21** under the same conditions used for **16** afforded in 76% yield (*Z*)-2-methyl-4-pentenethial *S*-oxide **22**. To demonstrate the utility of our discovery, sulfine **18** was desulfurized by using boron trifluoride etherate-mercuric oxide<sup>19</sup> affording 2-chloromethyl-4-pentenal **19** in 94% crude yield; this was treated with an equivalent of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) affording a 63% distilled yield of 2-methylene-4-pentenal **20** as a colorless oil (eq 5).



Attempts to achieve the same transformation by way of sulfide **16** were unsuccessful due to the instability of this compound and its transformation products under the conditions required for the normal thio-Claisen reaction (heat, mercuric oxide). In a similar manner sulfine **22** gave 2-methyl-4-pentenethial **23** in 92% isolated yield upon treatment with mercuric oxide-boron trifluoride etherate.

Since we were unsuccessful in utilizing 3-chloro-1-propenyl allyl sulfoxide **17** in nucleophilic substitution reactions we examined the use of the precursor **16** in reaction with a thiolate nucleophile. We found that lithium 2-propenethiolate reacted cleanly with **16** in ethanol at 0 °C giving (*E*)-4,8-dithiaundeca-1,5,10-triene **24** in 90% yield (eq 7). Oxidation of bissulfide **24** with 1 molar equiv



of MCPBA gave exclusively (*E*)-4,8-dithiaundeca-1,6,10-triene 4-oxide **25** in 88% yield. On the other hand oxidation of **24** using sodium metaperiodate gave a mixture of **25** and a sulfine (as indicated by NMR analysis), presumably arising from sulfoxide thio-Claisen rearrangement of (*E*)-4,8-dithiaundeca-1,6,10-triene 8-oxide **26**. Compounds **24** and **25** are of interest for comparison of antithrombotic activity (see below) and  $^{13}\text{C}$  NMR chemical shifts with those for **1**. The saturated carbons of bissulfide **24** were found at  $\delta$  35.6, 33.39, and 32.8 ppm while those for sulfoxide sulfide **25** appeared at  $\delta$  54.1, 53.9, and 35.3 ppm, providing support for the NMR arguments for the structure of **1** advanced above.

Further aspects of the sulfoxide thio-Claisen reaction will be presented elsewhere. Our discovery of the significant facilitating effect of the sulfinyl oxygen on the thio-Claisen reaction made it apparent that structure **1** was to be preferred to **15** for (*E*)-ajoene since there was no indication of rearrangement of this material to a sulfine upon heating.

**Mechanism for Formation of (*E*)-Ajoene. Decomposition of Allicin 2.** Application of "Occam's razor" to the problem at hand suggests that the source of *all* of the sulfur-containing products isolated from garlic should be allicin and that the mechanism for formation of these products should be similar to that proposed for decomposition of alkyl alkanethiosulfonates (i.e., Scheme I). Thus  $\beta$ -elimination of allicin should afford 2-propenesulfenic acid and thioacrolein. The latter compound is reported to dimerize to structures **9** and **14** (Scheme IIb).<sup>20</sup> *S*-Allylthiolation of allicin should give sulfonium ion **27**, which could undergo  $\beta$ -elimination to carbocation **28** followed by  $\gamma$ -addition of 2-propenesulfenic acid giving (*E*)-ajoene (Scheme IIc,d), or could undergo hydrolysis giving allyl alcohol and diallyl trisulfide **11** (Scheme IIe,g). Hydrolysis of allicin should give 2-propenesulfenic acid (Scheme IIf);  $\beta,\gamma$ -unsaturated sulfenic acids are known to readily lose sulfur dioxide by retroene type reactions

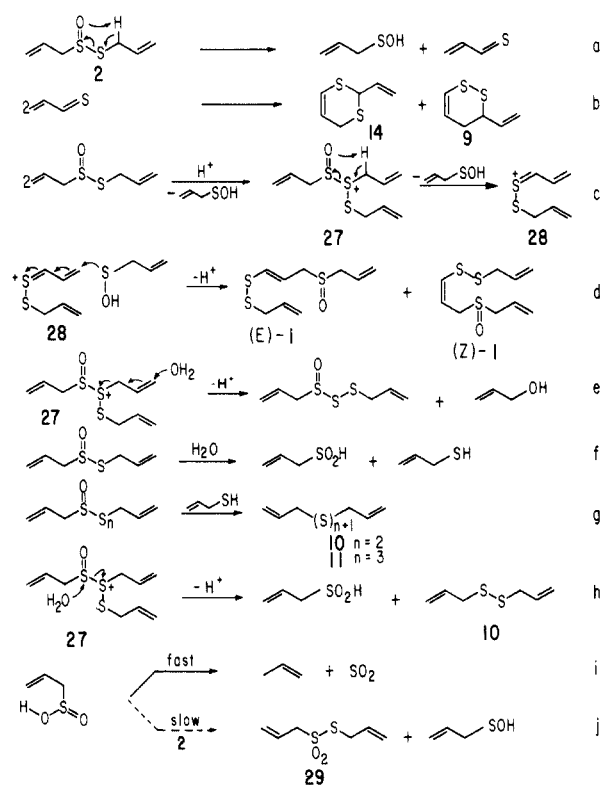
(17) Block, E.; Bazzi, A. A.; Lambert, J. B.; Wharry, S. M.; Andersen, K. K.; Dittmer, D. C.; Patwardhan, B. H.; Smith, D. J. H. *J. Org. Chem.* **1980**, *45*, 4807-4810.

(18) Mura, A. J., Jr.; Bennett, D. A.; Cohen, T. *Tetrahedron Lett.* **1975**, 4433-4436. Vilsmaier, E.; Schutz, J.; Zimmerer, S. *Chem. Ber.* **1979**, *112*, 2231-2242.

(19) Vedejs, E.; Fuchs, P. L. *J. Org. Chem.* **1971**, *36*, 366-367.

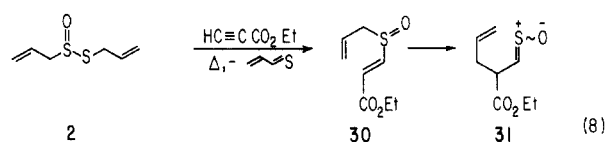
(20) Bock, H.; Mohmand, S.; Hirabayashi, T.; Semkow, A. *Chem. Ber.* **1982**, *115*, 1339-1348. Vedejs, E.; Eberlein, T. H.; Varie, D. L. *J. Am. Chem. Soc.* **1982**, *104*, 1445. Beslin, P. J. *Heterocycl. Chem.* **1983**, *20*, 1753-1754.

Scheme II



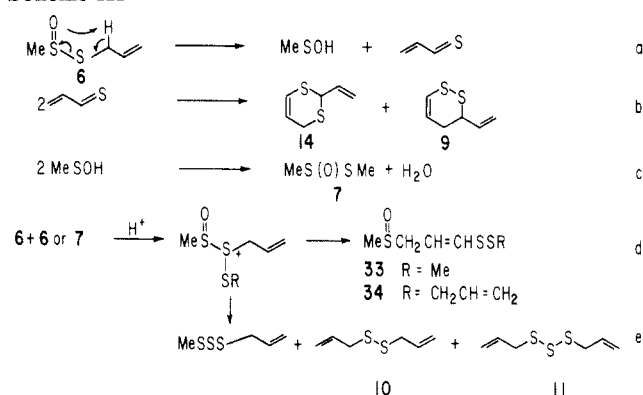
(Scheme III).<sup>21</sup> Diallyl disulfide **10** could arise via attack of 2-propenethiol on allcin (Scheme IIg). The absence of allyl 2-propenethiosulfonate **29** (Scheme IIj) can be understood if the rate of loss of sulfur dioxide from 2-propenesulfinic acid is more rapid than its rate of nucleophilic attack on allcin.

Can we substantiate this apparently self-consistent mechanism for formation of **1** from **2**? We were delighted to find that we could! When a solution of allcin, prepared in high yield by peracetic acid oxidation of diallyl disulfide, was refluxed as a 10% solution in 3:2 acetone/water for 4 h, diluted with methanol and repeatedly extracted with pentane to remove nonpolar materials and then extracted with methylene chloride and concentrated, (*E,Z*)-ajoene **1** (4:1 *E:Z*) could be isolated in 34% yield (0.34 g from 1 g of **2**). Synthetic **1** was identical in all respects with material isolated from garlic extracts. GC analysis of the pentane-soluble fraction (0.52 g from 1 g of **2**) indicated a 21:17:50:12 mixture of **10/11/14/9**, respectively. This ratio changed to 4:4:75:17 when **2** was decomposed in the same solvent mixture at 37 °C for 2 days or at 25 °C for 7 days, reflecting partial decomposition of some compounds at the higher temperature and/or different temperature dependence of the reactions of Scheme II. The near identity of the 4.4:1 ratio of **14** to **9** observed in the 37 and 25 °C decomposition of **2** and the 4.5:1 ratio of **14** to **9** found from dimerization at -180 °C of thioacrolein from flash vacuum pyrolysis of diallyl sulfide<sup>20</sup> provide support for steps a and b. When allcin was distilled into a liquid nitrogen cooled trap the previously noted deep blue color of thioacrolein was observed. 2-Propenesulfinic produced upon heating allcin could be trapped with methyl propiolate; the initial 1-alkenyl 2-alkenyl sulfoxide adduct **30** underwent the sulfoxide thio-Claisen rearrangement to sulfine **31** (eq 8).<sup>22</sup> When allcin was decomposed



(21) Corey, E. J.; Engler, T. A. *Tetrahedron Lett.* **1984**, 25, 149–152 and references therein.

Scheme III



by boiling with water, propene could be isolated and identified by IR spectroscopy, providing support for step i.

In another experiment a drop of allcin on IR salt plates was allowed to decompose by standing at room temperature for several days. Periodic analysis by IR spectroscopy indicated the gradual disappearance of the sulfinyl band of allcin at 1090  $\text{cm}^{-1}$  along with the appearance of a new C-S(O)-C band at 1045  $\text{cm}^{-1}$ ; sulfonyl absorption (1320, 1120  $\text{cm}^{-1}$ ) was not detected. TLC analysis of the material remaining on the salt plate after 4 days showed a complex mixture of compounds including a spot with the same  $R_f$  value as **1**. Heating allcin neat, in pure acetone, or in 80:20 benzene/water afforded a product in which the **1E:1Z** ratio was ca. 9:1; the reaction product was not as clean as in the case of the 3:2 acetone/water solution. In one decomposition of allcin we isolated a small amount of a partially reduced homologue of ajoene, 4,5,9-trithiadodeca-1,11-diene 9-oxide **32** [IR 1040  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR  $\delta$  5.8–6.0 (2 H), 5.1–5.4 (4 H), 3.2–3.5 (4 H), 2.6–2.8 (4 H), 2.1 (quin, 2 H,  $\text{CH}_2\text{CH}_2\text{CH}_2$ );  $^{13}\text{C}$  NMR  $\delta$  133.2, 125.6, 123.5, 118.6, 56.0, 49.1, 42.1, 37.2, 21.9; CI-MS indicated  $\text{C}_9\text{H}_{16}\text{S}_3\text{O}$ ]. A search was made for the presence of allyl 2-propenethiosulfonate **29** ("pseudoallicin"<sup>23</sup>) among the decomposition products of allcin by using an authentic sample of **29** to establish analytical conditions. None was found.

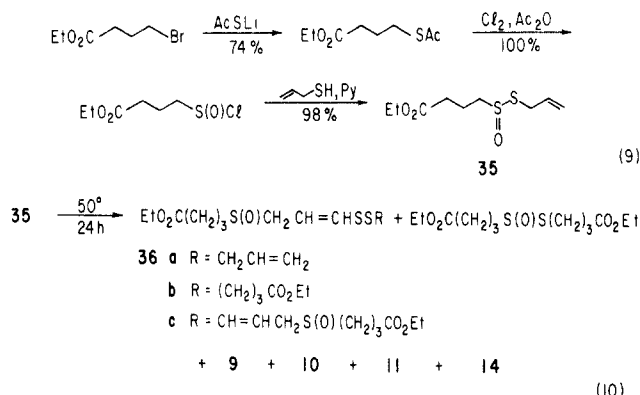
#### Decomposition of Garlic Component Allyl Methanethiosulfinate and Related Compound Allyl 3-Carbethoxypropanethiosulfinate.

An authentic sample of the garlic component and allcin homologue allyl methanethiosulfinate **6** was prepared in 95% yield by condensation of methanesulfinyl chloride with 2-propenethiol in the presence of pyridine. A homogeneous 10% solution of **6** in 3:2 acetone/water was stirred at 37 °C for 36 h and then worked up as in the case of decomposition of **2**. The nonpolar fraction consisted of 98% of a 3.1:1 mixture of heterocycles **14** and **9** along with trace amounts of diallyl disulfide **10**, diallyl trisulfide **11**, and allyl methyl trisulfide. The polar fraction consisted of ca. 80% methyl methanethiosulfinate **7**, 10% 2,3,7-trithioocta-4-ene 7-oxide **33** (4:1 *E:Z*), and 6% 4,5,9-trithiadeca-1,6-diene 9-oxide **34** (2:1 *E:Z*). The spectroscopic properties of **33** and **34** are similar to those of ajoene **1** (see Experimental Section); the biological properties are discussed below. The formation of these various products from **6** (Scheme III) is consistent with, and supportive of, the general mechanism for decomposition of allcin given in Scheme II. Although we did not search for them, compounds **33** and **34** are presumably present in low concentration in garlic extracts.

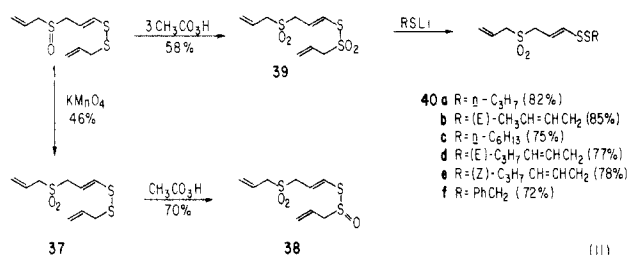
We have also synthesized allyl 3-carbethoxypropanethiosulfinate **35** (eq 9). By analogy to the decomposition of **6**, compound **35** should afford ajoene analogues containing carboxylate groups **36**, whose antithrombotic activity we sought to measure. The decomposition products of **35** are shown in eq 10.

(22) For comparison, see: Bell, R.; Cottam, P. D.; Davies, J.; Jones, D. N.; Meanwell, N. A. *Tetrahedron Lett.* **1980**, 21, 4379–4382.

(23) Belous, M. A.; Postovskii, I. Ya. *Zhur. Obshchei Khim.* **1950**, 20, 1701–1710. Bodyrev, B. G.; Zakharchuk, A. T. *Doklady Akad. Nauk S.S.S.R.* **1954**, 94, 877–879.

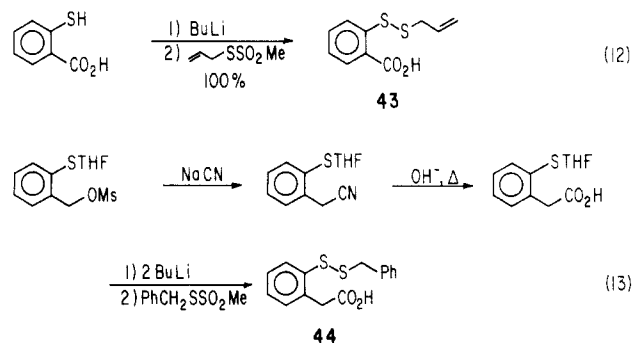


**Oxidation of (*E,Z*)-Ajoene. Preparation of Homologues for Structure-Activity Study.** In order to further characterize ajoene (1) as well as to determine those structural features essential for antithrombotic activity, the oxidation of ajoene was examined. Oxidation of (*E,Z*)-1 with potassium permanganate in acetone at  $-20^\circ\text{C}$  gave (*E,Z*)-4,5,9-trithiadodeca-1,6,11-triene 9,9-dioxide 37 in 46% yield. The *E* isomer, easily isolated in pure form by HPLC showed sulfone bands in the IR at 1320 and 1120  $\text{cm}^{-1}$  and showed  $^{13}\text{C}$  NMR bands for saturated carbons at  $\delta$  56.4, 54.7, and 41.4 ppm. Further oxidation of 37 with peracetic acid at  $0^\circ\text{C}$  gave (*E,Z*)-4,5,9-trithiadodeca-1,6,11-triene 4,9,9-trioxide 38 in 70% yield after flash chromatography. The *E* isomer, easily isolated by HPLC, showed sulfone bands at 1320 and 1130  $\text{cm}^{-1}$ , a thiosulfinate sulfinyl band at 1095  $\text{cm}^{-1}$ , and  $^{13}\text{C}$  NMR bands for saturated carbon atoms at  $\delta$  60.1, 56.8, and 55.1 ppm. Oxidation of (*E*)-1 with 3 equiv of peracetic acid at  $-20^\circ\text{C}$  gave the unstable (*E*)-4,5,9-trithiadodeca-1,6,11-triene 4,4,9,9-tetraoxide 39 in 58% yield after flash chromatography. The above chemical studies and spectroscopic data provide further support for the validity of the structural assignment for 1. Since we found that oxidized (sulfonyl) derivatives of ajoene also displayed antithrombotic activity (see below) we took advantage of the electrophilic reactivity of tetraoxide 39 at the sulfur at position 5 to prepare a series of homologues 40 via reaction with various thiolates (eq 11). The biological activity of these compounds will be discussed below.



***o*-Phenylene Sulfinyl Disulfides.** Since the central double bond of ajoene and its various analogues proved essential for biological activity (see below) and since *Z* isomers were somewhat more active than the *E* isomers, it was of interest to determine if compounds related to (*Z*)-1 with the central double bond replaced by an *o*-phenylene group would also possess antithrombotic activity. Starting from the commercially available *o*-thiosalicylic acid and using a straightforward series of steps (Scheme IV and eq 12 and 13) it proved possible to synthesize in good yields a series of crystalline *o*-phenylene alkanethio disulfide 41, *o*-phenylene alkanesulfinyl disulfides 42, and related compounds which were tested for antithrombotic activity.

**Comparative Antithrombotic Activity of (*E,Z*)-Ajoene and Homologues.** Various pure components of garlic extracts or the related synthetic structures described above were tested for antithrombotic activity as summarized in Table I. Under carefully standardized conditions<sup>15</sup> (see Experimental Section) the ID<sub>50</sub> (concentration of suspected antithrombotic agent necessary to reduce by 50% the extent of platelet aggregation induced by agonist ADP or collagen compared to the control) of 40 different compounds was determined in vitro by using human platelet



suspensions. As indicated in the table five different types of variations were made to the structure of the reference compound (*Z*)-ajoene, (*Z*)-1, namely variation of the sulfinyl-terminal allyl group ("a"), variation of oxidation state at the isolated sulfur ("b"), variation of the central double bond ("c"), variation of the disulfide group ("d"), and variation of the disulfide-terminal allyl group ("e"). The results indicate that (1) saturation of the central carbon-carbon double bond results in loss of activity (entry 8), (*Z*)-1 is more active than (*E*)-1 (entry 2; also entries 3/4, 5/6, 29/30), and that antithrombotic activity is retained upon replacement of the central double bond with an *o*-phenylene group (entry 32); (2) the disulfide group is essential for activity (entry 9); (3) activity is retained upon oxidation of the sulfinyl to sulfonyl function (entries 3, 4) or the disulfide to thiosulfinate function (entries 5, 6) but is lost upon reduction of the sulfinyl group (entry 31, 33) to a sulfide group, unless the latter group bears a 19-crown-6 group<sup>24</sup> (entry 38); (4) oxidation of the disulfide group to a thiosulfonate function gives a compound which induces rather than inhibits platelet aggregation; (5)  $\alpha,\beta$ -unsaturated disulfides without sulfinyl or sulfonyl groups are inactive even if other groups such as carboxyl functions are present (entries 16, 35–37); activity is retained upon replacement of the disulfide-terminal allyl group with benzyl, *n*-propyl, 2-butenyl, or (*Z*)-2-hexenyl (entries 24, 25, 28, 29) but not with (*E*)-2-hexenyl or *n*-hexyl (entries 26, 27); (6) replacement of the sulfinyl-terminal alkyl group with methyl or 3-carbethoxypropyl (entries 19–22) but not benzyl or *n*-propyl (39, 40) reduces or eliminates activity; (7) other known components of garlic extracts, including some previously claimed to be the active antithrombotic factor of garlic (entries 10–14, 17, 18), show significantly less activity than 1.

On the basis of the data in Table I as well as a variety of biochemical studies involving platelets,<sup>25</sup> we postulate that platelet membrane sulphydryl groups<sup>26</sup> participate in a disulfide exchange reaction with ajoene and its homologues. The resultant alteration in the membrane prevents aggregation. We further suggest that coordination of the sulfinyl group of ajoene, or sulfonyl or crown ether group of homologues, with calcium ions assists in the binding of these compounds to the platelet membranes.

## Experimental Section

**General Procedures and Materials.** General procedures and selected syntheses of ajoene homologues are given below. For many of the final products and intermediates obtained, whose identity is readily discerned by the usual analytical techniques, reaction, workup conditions, physical data, and analytical methods are provided as supplemental data. Some of the products, such as sulfines, thiosulfonates, ajoene-derived thiosulfonates, and some unsaturated aldehydes, proved too reactive or unstable for satisfactory elemental analysis.

**Extraction and Separation of Garlic Components.** Chopped garlic pieces (600 g) were soaked in methanol (1000 mL). The methanol was replaced 3 times in 72 h (by using a total of 3 L methanol). The methanol extract was concentrated in vacuo to about 100 mL, diluted

(24) Prepared by using the corresponding mercaptomethyl 19-crown-6: Rastetter, W. H.; Phillion, D. P. *J. Org. Chem.* **1980**, *45*, 1535–1538. We thank Dr. Rastetter for a sample of the mercaptomethyl 19-crown-6.

(25) Jain, M. K.; Apitz-Castro, R.; Catalfamo, J. L., unpublished results.

(26) For the role of sulphydryl groups in platelet activation, see: Graff, G.; Gellerman, J. L.; Sand, D. M.; Schlenk, H. *Biochim. Biophys. Acta* **1984**, *799*, 143–150. Silk, S. T.; Wong, K. T. H.; Marcus, A. J. *Biochemistry* **1981**, *20*, 391–397. Bell, R. L.; Kennerly, D. A.; Stanford, N.; Majerus, P. W. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 3238–3241.

Table I. ID<sub>50</sub> Values for (Z)-Ajoene and Analogues

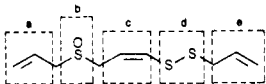
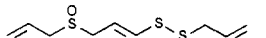
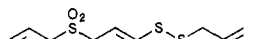
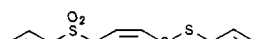
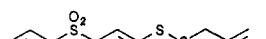


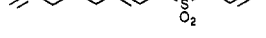
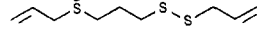
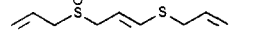
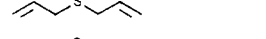
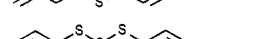
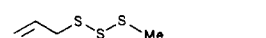
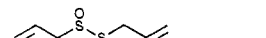
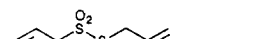
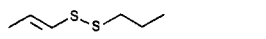



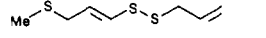
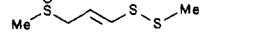
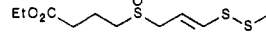
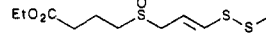
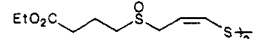
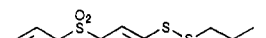



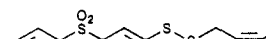
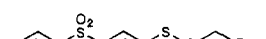
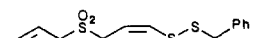
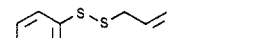
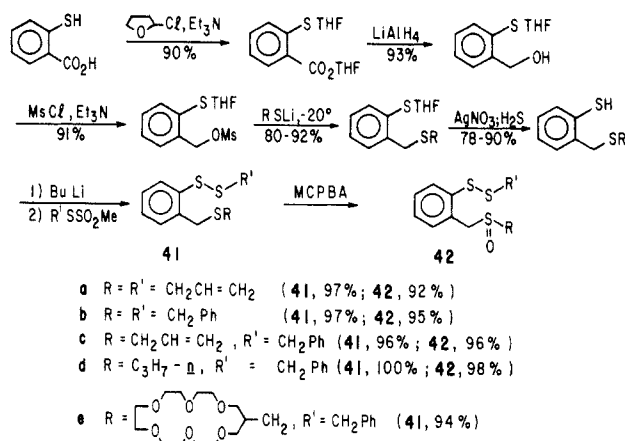
| entry no. | compd no. | structure   | structrl variatn | ID <sub>50</sub> (μM) |              |
|-----------|-----------|---|------------------|-----------------------|--------------|
|           |           |   |                  | ADP                   | collagen     |
| 1         | (Z)-1     |    | none             | 166 ± 38 (5)          | 196 ± 64 (7) |
| 2         | (E)-1     |    | c                | 213                   | 243 ± 90 (4) |
| 3         | (E)-37    |    | b, c             | 299                   | 128 ± 81 (3) |
| 4         | (Z)-37    |    | b                | 209                   | 214          |
| 5         | (E)-38    |    | b, c, d          | 259                   | 234          |
| 6         | (Z)-38    |    | b, d             | 182                   | 140          |
| 7         | (E)-39    |    | b, c, d          | causes aggregatn      |              |
| 8         | 32        |    | c                | >400                  | >400         |
| 9         | 25        |    | c, d             | >400                  | >400         |
| 10        |           |    | b-e              | >>400                 | >>400        |
| 11        | 10        |    | a-d              | >400                  | >400         |
| 12        | 11        |    | a-d              | >400                  | >400         |
| 13        |           |   | a-d              | >>400                 |              |
| 14        | 2         |  | b-e              | >400                  | >400         |
| 15        | 29        |  | a-d              | 384                   | 312          |
| 16        |           |  | a-c, e           | >>400                 |              |
| 17        | 9         |  | a-e              | >400                  | >400         |
| 18        | 14        |  | a-e              | >400                  | >400         |
| 19        | 34        |  | a, c             | >400                  | 374          |
| 20        | 33        |  | a, c, e          | >400                  | 332          |
| 21        | 36a       |  | a, c             | 388                   | 388          |
| 22        | 36b       |  | a, c, e          | >400                  | 362          |
| 23        | 36c       |  | a-e              | >400                  | >400         |
| 24        | 40a       |  | b, c, e          | 210                   |              |
| 25        | 40b       |  | b, c, e          | 208                   |              |
| 26        | 40c       |  | b, c, e          | >400                  |              |
| 27        | 40d       |  | b, c, e          | >400                  |              |
| 28        | 40e       |  | b, c, e          | 280                   |              |
| 29        | (E)-40f   |  | b, c, e          | 184                   |              |
| 30        | (Z)-40f   |  | b, e             | 76                    |              |
| 31        | 41a       |  | b, c             | >>400                 |              |
| 32        | 42a       |  | c                | 98                    |              |

Table I (Continued)

| entry no. | compd no. | structure | ID <sub>50</sub> (μM) |       |          |
|-----------|-----------|-----------|-----------------------|-------|----------|
|           |           |           | structrl variatn      | ADP   | collagen |
| 33        | 41c       |           | b, c, e               | >>400 |          |
| 34        | 42c       |           | c, e                  | 96    |          |
| 35        | 43        |           | a-c                   | >>400 |          |
| 36        |           |           | a-c                   | >>400 |          |
| 37        | 44        |           | a-c, e                | >>400 |          |
| 38        | 41e       |           | a-c, e                | 263   |          |
| 39        | 42b       |           | a, c, e               | 102   |          |
| 40        | 42d       |           | a, c, e               | 85    |          |

## Scheme IV



with water (100 mL), and extracted with ether (3 × 300 mL). The combined ether extract was dried (magnesium sulfate) and concentrated in vacuo affording 3.2 g of yellow residue which was kept in methanol (15 mL) for 4 days at room temperature. The methanol solution was filtered to remove any solid material, and the filtrate was concentrated in vacuo giving 2 g of a yellow oil which was suspended in 50% aqueous methanol (200 mL) and extracted with pentane (75 × 3 mL) and methylene chloride (50 × 3 mL). The pentane and methylene chloride extracts were dried (MgSO<sub>4</sub>) and concentrated separately in vacuo, giving 0.72 and 0.58 g of oily residues, respectively. Repeated preparative TLC (silica gel/pentane) and preparative HPLC (silica gel/hexane) of the pentane concentrate afforded 0.1 g of diallyl disulfide (10), 0.04 g of diallyl trisulfide (11), 0.02 g of 3-vinyl-4*H*-[1,2]-dithiin (9), and 0.09 g of 2-vinyl-4*H*-[1,3]-dithiin (14). These compounds were characterized by comparison of their IR, proton, and carbon NMR spectroscopic data with data for authentic samples.

The methylene chloride concentrate was subjected to flash chromatography (silica gel/ethyl acetate) affording 0.065 g of (*E,Z*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide (ajoene) (1). Preparative HPLC (silica gel/hexane-isopropanol 92:8) gave pure *E* and *Z* isomers. (*E*)-4,5,9-Trithiadodeca-1,6,11-triene 9-oxide: IR (neat) 3080 (w), 3000–2850 (s), 1632 (w), 1610 (w), 1435 (m), 1050 (s, sulfoxide) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.38 (dt, 1 H, *J* = 14.8, 1 Hz, *E* SSCH=CHCH<sub>2</sub>), 5.98 (m, 3 H), 5.4 (m, 2 H), 5.2 (m, 2 H), 3.5 (m, 4 H), 3.36 (d, 2 H, *J* = 7.2 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 134.75, 132.59, 125.68, 123.74, 119.26, 116.94, 54.48, 53.08, 41.37. (*Z*)-4,5,9-Trithiadodeca-1,6,11-triene 9-oxide: IR (neat) 3080 (w), 3025–2850 (s), 1635 (m), 1600 (w), 1420 (m), 1400 (m), 1045 (s, CS(O)C), 922 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ

6.55 (d, 1 H, *J* = 9 Hz, *Z* SSCH=CH), 6.14–4.97 (m, 7 H), 3.62–3.26 (m, 6 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 138.58, 132.64, 125.74, 123.85, 119.32, 118.13, 55.02, 49.73, 42.18. Anal. Calcd for C<sub>9</sub>H<sub>14</sub>S<sub>3</sub>O: C, 46.12; H, 6.02; S, 41.03. Found: C, 45.80; H, 5.90; S, 40.80.

**Allyl 2-Propenethiosulfinate (Allicin) (2).**<sup>9</sup> The procedure developed by Cavallito et al.<sup>9</sup> was used with some modification. Commercial diallyl disulfide (K & K) was kept at a vacuum of 0.05 mm at 0 °C until traces of diallyl sulfide were removed, as indicated by gas chromatography. Peracetic acid (35%; 258 g, 1.187 mol) was added dropwise via an addition funnel to a solution of disulfide (165 g, 1.13 mol) in chloroform (1800 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, and anhydrous sodium carbonate (400 g) was added in small portions during 30 min with vigorous stirring. The mixture was stirred for an additional 30 min at 0 °C and then filtered through a pad of Celite and anhydrous magnesium sulfate. The filtrate was concentrated under vacuum, ultimately by using a vacuum of 0.05 mm for 1 h to remove the last traces of acetic acid to give 165 g (90% yield) of crude allicin (2) which could be easily purified by preparative HPLC (silica gel/hexane-tetrahydrofuran 90:10): IR (neat) 3075 (m), 3025–2900 (m), 1635 (m), 1425 (m), 1403 (m), 1230 (m), 1080 (s, S(O)S), 990 (m), 830 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.30–5.04 (m, 6 H), 3.94–3.65 (m, 4 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 133.02, 125.95, 124.07, 119.10, 59.98, 35.06.

**Allyl 3-Chloro-1-propenyl Sulfide (16).** The procedure developed by Vilsmaier et al.<sup>18</sup> was followed. *N*-Chlorosuccinimide (12.28 g, 92.1 mmol) was added in small portions to a stirred solution of allyl sulfide (10 g, 87.7 mmol) in carbon tetrachloride (100 mL) at 0 °C. The reaction mixture was then stirred at 4 °C for 10 h, filtered, and concentrated in vacuo giving 13 g (100% yield) of the title compound exclusively as the *E* isomer: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.31 (d, 1 H, *E* SCH=CH), 6.02–4.99 (m, 4 H), 4.12 (d, 2 H, *J* = 7 Hz), 3.32 (d, 2 H, *J* = 6.5 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 133.13, 129.84, 122.83, 118.02, 45.15, 35.17.

**(*Z*)-2-Chloromethyl-4-pentenethial S-Oxide (18).** Peracetic acid (35%, 19.3 g, 88.9 mmol) was added dropwise via constant addition funnel to a stirred solution of 16 (12 g, 80.8 mmol) in methylene chloride (120 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 30 min then diluted with ether (30 mL), washed with saturated sodium bicarbonate solution (30 × 3 mL) and saturated sodium chloride solution (30 mL), dried (magnesium sulfate), and concentrated in vacuo giving 12 g (90% yield) of 18 as a colorless oil: IR (neat) 3075 (m), 3000–2850 (s), 1640 (s), 1440 (s), 1130 (s), 930 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.11 (d, 1 H, *J* = 9 Hz, CH=SO), 5.74 (m, 1 H), 5.12 (m, 2 H), 3.85 (m, 3 H), 2.42 (m, 2 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 176.4 (CH=SO), 133.3, 118.7, 45.6, 38.1, 35.5; MS, (rel intensity) 129 (M<sup>+</sup> - Cl), 117, 115, 97, 89, 88, 87, 79 (100); ca. 5% of the (*E*)-sulfine was also present.

**Attempted Preparation of 2-Chloromethyl-4-pentenal (19) from Allyl 3-Chloro-1-propenyl Sulfide (16).** A mixture of 16 (0.1 g, 0.67 mmol), *n*-nonane (0.05 g as an internal VPC standard), and mercuric chloride



(0.201 g, 0.74 mmol) in tetrahydrofuran (3 mL) and water (1 mL) was stirred at room temperature for 30 min. Workup afforded 0.08 g of a thick oil which was subjected to VPC analysis, indicating no detectable quantities of **16** or aldehyde **19**.

**2-Chloromethyl-4-pentenal (19).** Freshly distilled boron trifluoride etherate (0.203 g, 1.43 mmol) was added to a vigorously stirred suspension of red mercuric oxide (0.309 g, 1.43 mmol) in 15% aqueous tetrahydrofuran (2.5 mL) under argon. The sulfine **18** (0.25 g, 1.36 mmol) was dissolved in tetrahydrofuran (1 mL) and was added via a dropping funnel during 15 min. Stirring was continued for 30 min at 25 °C and 2 h at 50 °C. The red mercuric oxide gradually dissolved, and a black precipitate of mercuric sulfide appeared. The reaction mixture was diluted with ether (15 mL), the precipitated salts were filtered, and the filtrate was washed to pH 10 with saturated sodium carbonate solution (10 × 2 mL) and to neutrality with saturated sodium chloride solution (10 × 3 mL). The aqueous layers were extracted with ether (10 mL). The combined organic extract was dried (magnesium sulfate) and concentrated in vacuo at 0 °C giving a 94% yield of crude oil which was purified by flash distillation at room temperature (at 0.05 mm) giving **19** in 55% yield as colorless oil: IR (neat) 3075 (m), 3000–2850 (s), 2725 (s), 1730 (s), 1640 (s), 1440 (s), 1300 (m), 1130 (m), 1000 (m), 930 (s)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.78 (s, 1 H,  $\text{CH}=\text{O}$ ), 6.10–5.38 (m, 1 H), 5.33–4.96 (m, 2 H), 3.77 (d, 2 H,  $J = 5$  Hz,  $\text{ClCH}_2$ ), 2.89–2.28 (m, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  194.00, 134.90, 117.78, 100.45, 44.14, 42.90; MS, (rel intensity) 97 ( $\text{M}^+ - \text{Cl}$ ), 95, 91, 90, 83, 79, 77, 75, 69, 68, 67, 65, 56, 55 (100).

**2-Methylene-4-pentenal (20).** Distilled **19** (0.15 g, 1.13 mmol) was dissolved in methylene chloride (5 mL). To this solution was added slowly 1,5-diazabicyclo[5.4.0]undec-5-ene (0.19 g, 1.24 mmol) with stirring and cooling to keep the temperature below 30 °C. The reaction mixture was stirred for 15 min after the addition was complete. It was then diluted with pentane (10 mL), washed with water (2 × 10 mL), and dried over magnesium sulfate. The solvent was then removed by distillation by using a Vigreux column affording 0.104 g (96% yield) of **20** as a VPC homogeneous colorless oil: IR (neat) 3075 (s), 3000–2850 (s), 2700 (m), 1690 (m), 1640 (m), 1430 (m), 1340 (m), 1240 (m)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.57 (s, 1 H,  $\text{CH}=\text{O}$ ), 6.27 (s, 1 H), 6.05 (s, 1 H), 6.04–5.48 (m, 1 H), 5.11–5.05 (m, 2 H), 2.98 (br d, 2 H,  $J = 6$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  194.01, 148.42, 134.56, 134.16, 117.27, 31.93; MS, (rel intensity) 96 ( $\text{M}^+$ ), 95, 81, 78, 77, 69, 68, 67 (100).

**(*E,Z*)-Allyl 1-Propenyl Sulfide (21).** Lithium triethylborohydride (7.4 mL, 7.4 mmol, 1 M in tetrahydrofuran) was added dropwise to a stirred solution of chlorosulfide **16** (1 g, 7.81 mmol) in tetrahydrofuran (10 mL) at –20 °C. The reaction mixture was stirred for 25 min, diluted with cold pentane (15 mL), and quenched with moist sodium sulfate. The reaction mixture was then washed with water (10 × 2 mL), saturated with sodium chloride solution (10 mL), dried (magnesium sulfate), and concentrated in vacuo to give 0.65 g (82% yield) of the title compound as a colorless oil:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.10–5.38 (m, 3 H), 5.27–4.91 (m, 2 H), 3.20 (br d, 2 H), 1.79–1.60 (m, 3 H, 2 methyl groups).

**2-Methyl-4-pentenethial S-Oxide (22).** Lithium triethylborohydride (40.7 mL, 40.7 mmol, 1 M in tetrahydrofuran) was added dropwise to a stirred solution of **16** (5.5 g, 37.0 mmol) in ether (50 mL) at –20 °C. The reaction mixture was stirred for 30 min and quenched with ice-cold water. The organic layer was washed with cold saturated sodium chloride solution (20 × 3 mL), dried (magnesium sulfate), and cooled to 0 °C. Peracetic acid (35%, 8.44 g, 39.9 mmol) was added dropwise with stirring to the reaction mixture. Stirring was continued at 0 °C for 15 min. The reaction mixture was then diluted with ether (30 mL), washed with saturated sodium bicarbonate solution (30 × 3 mL) and saturated sodium chloride solution (30 mL), dried (magnesium sulfate), and concentrated in vacuo giving 4.0 g (83% yield, 2 steps from **16**) of the crude sulfine **22**: IR (neat) 3070 (m), 3000–2850 (s), 1635 (s), 1450 (s), 1120 (s), 920 (s)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.98 (d, 1 H,  $J = 9.5$  Hz,  $\text{CH}=\text{SO}$ ), 6.17–4.84 (m, 3 H), 3.88–3.40 (m, 1 H), 2.31–1.86 (m, 2 H), 1.17 (d, 3 H,  $J = 7$  Hz); ca. 5% of (*E*)-sulfine was also present.

**2-Methyl-4-pentenal (23).**<sup>28</sup> Boron trifluoride etherate (0.109 g, 0.77 mmol) was added to a stirred suspension of red mercuric oxide (0.167 g, 0.77 mmol) in 15% aqueous tetrahydrofuran (1.0 mL) under argon followed by slow addition of sulfine **22** (0.1 g, 0.77 mmol) and *n*-nonane (0.05 g, as VPC internal standard) in tetrahydrofuran (0.5 mL). The reaction mixture was stirred at room temperature for 30 min (the reaction was monitored by VPC). Workup as in the case of **19** followed by

quantitative VPC analysis indicated a 92% yield of aldehyde **23**.

**(*E*)-4,8-Dithiaundeca-1,5,10-triene (24).** A solution of 2-propenethiol (1 g, 13.5 mmol) in ethanol (5 mL) was added at 0 °C with stirring to a solution of sodium ethoxide prepared from sodium metal (0.33 g, 14.35 mmol) and absolute ethanol (50 mL). To this solution was then added dropwise at 0 °C with vigorous stirring (*E*)-allyl 3-chloro-1-propenyl sulfide **18** (13.5 mmol) in tetrahydrofuran (10 mL). A thick white precipitate formed, and the mixture was stirred at room temperature for 2 h. Hexane (100 mL) was added, and the mixture was washed with water (3 × 50 mL), dried (magnesium sulfate), and concentrated in vacuo giving (*E*)-4,8-dithiaundeca-1,6,10-triene (**24**) (2.25 g, 90% yield) homogeneous by NMR spectroscopy:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.20–4.91 (m, 8 H), 3.33 (d, 2 H,  $J = 6$  Hz), 3.20–2.99 (m, 4 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  134.31, 133.67, 125.79, 125.20, 117.54, 117.05, 35.60, 33.39, 32.80.

**(*E*)-4,8-Dithiaundeca-1,6,10-triene 4-Oxide (25).** To a solution of **24** (1 g, 5.38 mmol) in methylene chloride (50 mL) was added dropwise with stirring at 0 °C a solution of metachloroperoxybenzoic acid (80–85%, 1.16 g, 5.4 mmol) in methylene chloride (25 mL). The reaction mixture was stirred at 4 °C for 12 h, washed with 5% aqueous sodium bicarbonate solution (4 × 50 mL) and water (3 × 50 mL), dried, and concentrated in vacuo giving (*E*)-4,8-dithiaundeca-1,6,10-triene 4-oxide (**25**) (0.95 g, 88% yield): IR (neat) 3075–2880 (m), 1630 (m), 1605 (m), 1040 (s), 930 (s)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.21 (d, 1 H,  $J = 15$  Hz,  $\text{E}$   $\text{SSCH}=\text{CH}$ ), 6.24–5.02 (m, 7 H), 3.50–3.22 (m, 6 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  133.18, 132.91, 125.79, 123.58, 118.13, 114.25, 54.05, 53.89, 35.33.

**Pyrolysis of Allicin (2).** (*E,Z*)-4,5,9-Trithiadodeca-1,6,11-triene 9-Oxide (**Ajoene**) (**1**). Crude allicin (**2**) (5.8 g, 36 mmol) was dissolved in 40% aqueous-acetone (58 mL), and the homogeneous solution was heated at 63–64 °C for 4 h. The reaction mixture was diluted with 50% aqueous-methanol (200 mL) and washed with pentane (5 × 50 mL). The aqueous-methanolic layer was then saturated with ammonium sulfate and extracted with methylene chloride (50 × 2 mL). The methylene chloride extract was dried (magnesium sulfate) and concentrated in vacuo affording 2.0 g (37% yield) of crude (*E,Z*)-4,5,9-trithiadodeca-1,6,11-triene (**1**) which could be purified further by flash chromatography (silica gel/ethyl acetate) giving 1.0 g (17% yield) of pure **1**. HPLC analysis (silica gel/hexane-isopropanol 92:8) indicated a 90:10 *E/Z* ratio. Synthetic **1** was identical in all respects with the natural material.

In a separate attempt a mixture of allicin (12.5 g, 77.2 mmol), water (12.5 mL), and benzene (112.5 mL) was stirred at 37 °C for 48 h. The reaction mixture was then concentrated in vacuo, suspended in 50% aqueous-methanol (600 mL), and washed with pentane (100 × 4 mL). The aqueous-methanolic layer was saturated with sodium chloride and extracted with methylene chloride (100 × 3 mL), the methylene chloride extract was dried (magnesium sulfate) and concentrated in vacuo giving 3.5 g (28% yield) of crude (*E,Z*)-ajoene **1** (*E/Z* ratio 10:90) which was purified by flash chromatography (silica gel/EtOAc) affording 2 g (17%) of (*E,Z*)-**1**. In another attempt a solution of 0.2 g of **2** in  $\text{CDCl}_3$  (1 mL) was kept at 25 °C for 6 days (disappearance of starting material was monitored by NMR spectroscopy). Repeated preparation of HPLC and TLC (silica gel/hexane) afforded 0.03 g of diallyl disulfide **10**, 0.01 g of diallyl trisulfide **11**, 0.008 g of 3-vinyl-4*H*-1,2-dithiin (**9**), and 0.015 g of 2-vinyl-4*H*-1,3-dithiin (**14**). These compounds were characterized by direct comparison of their spectral data (IR,  $^1\text{H}$ , and  $^{13}\text{C}$  NMR spectra) with data on authentic samples.

**Formation of 4,5,9-Trithiadodeca-1,11-diene 9-Oxide (32).** A sample of 15 g of **2** containing ca. 10% acetic acid was decomposed at 25 °C for 5 days. Column chromatography (silica gel) gave 3.5 g of nonpolar material, eluted with hexane, and 10 g of polar material, eluted with 80%  $\text{CH}_2\text{Cl}_2$ /20% MeOH. Preparation of HPLC (1:1, hexane/THF) on 0.350 g of the polar material gave 0.024 g of pure (*E*)-**1** and 0.220 g of a second, more polar material (retention time 38 min compared to 30 min under the same conditions for (*E*)-**1**), identified as the title compound: IR (neat) 1640 (m), 1420 (m), 1040 (s), 995 (m), 930  $\text{cm}^{-1}$  (m);  $^1\text{H}$  NMR  $\delta$  5.8–6.0 (2 H), 5.1–5.4 (4 H), 3.2–3.5 (4 H), 2.81 (t, 2 H,  $J = 6.8$  Hz), 2.72 (mult, 2 H), 2.1 (quin, 2 H,  $\text{CH}_2\text{CH}_2\text{CH}_2$ );  $^{13}\text{C}$  NMR  $\delta$  133.23 (d), 125.63 (d), 123.53 (t), 118.62 (t), 55.97 (t), 49.07 (t), 42.06 (t), 37.20 (t), 21.94 (t); CI-MS ( $\text{CH}_4$ ) indicated  $\text{C}_9\text{H}_{16}\text{S}_3\text{O}$ .

**Pyrolysis of Allyl 2-Propenethiosulfinate (2) in Ethyl Propiolate. Trapping of 2-Propenesulfenic Acid.** A solution of **2** (60 mg, 0.37 mmol) in ethyl propiolate (1 mL) was kept overnight at room temperature. Concentration in vacuo afforded 100 mg of oily residue.  $^1\text{H}$  NMR analysis revealed the presence of 2-carbethoxy-4-pentenethial S-oxide (i.e., sulfoxide thio-Claisen rearrangement of the 2-propenesulfenic acid-ethyl propiolate adduct) exhibiting a characteristic doublet at  $\delta$  8.66,  $J = 9.5$  Hz. Unreacted starting materials and various impurities were also present.

(27) Block, E.; Corey, E. R.; Penn, R. E.; Renken, T. L.; Sherwin, P. F.; Bock, H.; Hirabayashi, T.; Mohmand, S.; Solouki, B. *J. Am. Chem. Soc.* **1982**, *104*, 3119–3130.

(28) Montgomery, L. K.; Matt, J. W.; Webster, J. R. *J. Am. Chem. Soc.* **1967**, *89*, 923–934.



**Generation of Propene by Thermolysis of Allicin (2).** A mixture of allicin (2) (4 g, 24.7 mmol) and water (4 mL) was placed in a 25-mL, round-bottom flask. The flask was connected via an adapter to a U-shaped glass tube leading into an inverted burette (50 mL) filled with water. The lower end of the burette was immersed in a water-filled crystallizing dish while the upper end was connected to a potassium bromide IR gas cell via a drying tube containing calcium sulfate and phosphorus pentoxide. The IR cell and drying tube were evacuated to remove air. The allicin-containing flask was then heated at 110 °C (bath temperature), and the gas evolved was collected in the burette. After the evolution of gas had ceased (70 min), the gas was allowed to enter the IR cell via the drying tube. The IR spectrum of the gas was identical with the published spectrum of propene (Sadler #43990P). Because of its solubility in water, no sulfur dioxide could be detected. To find the exact volume of propene evolved from allicin (2), a control experiment was performed by using silicone oil (4 g) and water (4 mL). The actual amount of propene generated by the decomposition of allicin (2) (i.e., 33.5 mL, 1.5 mmol, 6.1% yield from 45) was calculated by subtracting the volume of gases generated by the control (16 mL) from that given by the experiment with allicin (2) (49.5 mL).

**Allyl Methanethiosulfinate (6).** The procedure of Block and O'Connor<sup>16</sup> was used. To a solution of 2-propanethiol (3.7 g, 50 mmol) and anhydrous pyridine (5 mL) in anhydrous ether (100 mL) was added a solution of methanesulfinyl chloride (4.9 g, 50 mmol) in anhydrous ether (50 mL) at 0–2 °C with stirring during 1 h. A heavy white precipitate appeared during this period. The cold reaction mixture was treated with chilled 1 M sulfuric acid (3 × 25 mL) and ice water (8 × 25 mL). The ether layer was separated, and the combined aqueous layer was saturated with ammonium sulfate and extracted with methylene chloride (4 × 50 mL). The ether and methylene chloride extracts were combined, dried (magnesium sulfate), and concentrated in vacuo giving allyl methanethiosulfinate (6) (6.43 g, 95% yield): IR (neat) 3080–2900 (m), 1630 (m), 1420 (m), 1090 (s, S(O)S), 930 (m) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.36–5.64 (m, 1 H), 5.48–5.12 (m, 2 H), 3.77 (d, 2 H, *J* = 6 Hz), 3.03 (s, 3 H).

**(*E,Z*)-4,5,9-Trithiadeca-1,6-diene 9-Oxide (34) and (*E,Z*)-2,3,7-Trithiaocta-4-ene 7-Oxide (33).** Allyl methanethiosulfinate (6) (5 g, 36.8 mmol) in a homogeneous acetone/water solution (30 mL and 20 mL, respectively) was heated with stirring at 37 °C for 36 h. The reaction mixture was then diluted with methanol (100 mL) and water (100 mL) and extracted with hexane (3 × 100 mL), the aqueous layer was saturated with sodium chloride and extracted with methylene chloride (4 × 100 mL), and the methylene chloride extract was dried (magnesium sulfate) and concentrated in vacuo giving 2.4 g of an oil. Repeated flash chromatography (silica gel/ethyl acetate) and HPLC (silica gel/hexane-isopropanol) gave methyl methanethiosulfinate (7) (1 g), 0.045 g of (*E*)-4,5,9-trithiadeca-1,6-diene 9-oxide, 0.0225 g of (*Z*)-4,5,9-trithiadeca-1,6-diene 9-oxide, 0.1 g of (*E*)-2,3,7-trithiaocta-4-ene 7-oxide, and 0.015 g of (*Z*)-2,3,7-trithiaocta-4-ene 7-oxide. Methyl methanethiosulfinate (7): IR (neat) 3000 (m), 2920 (m), 1425 (s), 1300 (m), 1087 (s), 942 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.03 (s, 3 H), 2.69 (s, 3 H).

**(*E*)-4,5,9-Trithiadeca-1,6-diene 9-Oxide:** IR (neat) 3100–2900 (m), 1635 (m), 1607 (m), 1420 (m), 1410 (m), 1040 (s, S=O), 945 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.37 (d, 1 H, *J* = 15 Hz, *E* SSCH=CH), 6.28–5.45 (m, 2 H), 5.30–4.94 (m, 2 H), 3.50 (d, 2 H, *J* = 7 Hz), 3.36 (d, 2 H, *J* = 7 Hz), 2.60 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 134.91, 132.53, 119.21, 116.73, 56.64, 41.37, 37.43.

**(*Z*)-4,5,9-Trithiadeca-1,6-diene 9-Oxide:** IR (neat) 3100–2900 (m), 1640 (w), 1420 (m), 1055 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.80 (d, 1 H, *J* = 10 Hz, *Z* SSCH=CH), 6.15–5.54 (m, 2 H), 5.32–4.96 (m, 2 H), 3.65 (d, 2 H, *J* = 8 Hz), 3.37 (d, 2 H, *J* = 6 Hz), 2.62 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 138.85, 132.64, 119.32, 118.08, 52.92, 42.18, 37.76.

**(*E*)-2,3,7-Trithiaocta-4-ene 7-Oxide:** IR (neat) 3000 (m), 2900 (m), 1602 (m), 1410 (m), 1040 (s, S=O), 940 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.45 (d, 1 H, *J* = 15 Hz, *E* SSCH=CH), 6.35–5.65 (m, 1 H), 3.53 (d, 2 H, *J* = 7 Hz), 2.67 (s, 3 H), 2.44 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 133.72, 116.51, 56.53, 37.38, 22.12.

**(*Z*)-2,3,7-Trithiaocta-4-ene 7-Oxide:** IR (neat) 3100–2900 (m), 1600 (m), 1420 (m), 1040 (s), 940 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.62 (d, 1 H, *J* = 9.5 Hz, *Z* SSCH=CH), 6.18–5.56 (m, 1 H), 3.60 (d, 2 H, *J* = 8 Hz), 2.57 (s, 3 H), 2.47 (s, 3 H).

**(*E,Z*)-4,5,9-Trithiadodeca-1,6,11-triene 9,9-Dioxide (37).** The procedure of Block et al.<sup>27</sup> was used. A solution of potassium permanganate (0.7 g, 4.43 mmol) in acetone (100 mL) was added dropwise during a period of 1.5 h to a solution of (*E,Z*)-ajoene (0.5 g, 2.1 mmol) and suspended magnesium sulfate (6 g) in acetone (70 mL) maintained at –20 to –23 °C. The disappearance of the more polar ajoene was monitored by liquid chromatography. The reaction mixture was warmed to room temperature, filtered through Celite, and concentrated in vacuo giving the title compound (0.5 g) in 46% yield. The structure of the

isomers, easily separated by HPLC (2:98 isopropanol–hexane) as above, was established by spectroscopic analysis. (*E*)-4,5,9-Trithiadodeca-1,6,11-triene 9,9-dioxide (37): IR (neat) 3065–2900 (m), 1630 (m), 1610 (m), 1320 (s), 1120 (s), 935 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.44 (d, 1 H, *J* = 14 Hz, *E* SSCH=CH), 6.17–5.00 (m, 7 H), 3.77 (d, 2 H, *J* = 7 Hz), 3.70 (d, 2 H, *J* = 6 Hz), 3.36 (d, 2 H, *J* = 7 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 136.42, 132.37, 124.98, 124.77, 119.32, 114.79, 56.42, 54.70, 41.37.

**(*Z*)-4,5,9-Trithiadodeca-1,6,11-triene 9,9-Dioxide (37):** IR (neat) 3050–2900 (m), 1630 (m), 1595 (m), 1315 (s), 1125 (s), 935 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.58 (d, 1 H, *J* = 9 Hz, *Z* SSCH=CH), 6.20–5.00 (m, 7 H), 3.87 (d, 2 H, *J* = 8 Hz), 3.69 (d, 2 H, *J* = 6 Hz), 3.38 (d, 2 H, *J* = 7 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 139.55, 132.53, 125.14, 124.50, 119.43, 116.89, 57.02, 51.94, 42.24.

**(*E,Z*)-4,5,9-Trithiadodeca-1,6,11-triene 4,9,9-Trioxide (38).** Peracetic acid (35%, 0.287 g, 1.32 mmol) was added slowly at 0 °C with stirring to a solution of (*E,Z*)-4,5,9-trithiadodeca-1,6,11-triene 9,9-dioxide (37) (0.3 g, 1.2 mmol) in chloroform (15 mL). After stirring the reaction mixture for 1 h at 0 °C, solid anhydrous sodium carbonate (1 g) and magnesium sulfate (1 g) were added, and stirring was continued for 15 min. The mixture was then filtered and concentrated in vacuo giving (*E,Z*)-4,5,9-trithiadodeca-1,6,11-triene 4,9,9-trioxide (38) (0.32 g, 94% yield). Further purification of the crude product could be achieved by flash chromatography (silica gel/ethyl acetate) giving 0.24 g (70% yield) of pure 38. The structures of the isomers easily separated by preparative HPLC (silica gel/hexane-isopropanol, 90:10) was established by the following spectral data. *E* Isomer: IR (neat) 3100–2850 (m), 1640 (m), 1320 (s), 1130 (s), 1095 (s), 942 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.82 (d, 1 H, *J* = 15 Hz, *E* SSCH=CH), 6.38–5.24 (m, 7 H), 3.94–3.62 (m, 6 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 127.95, 127.09, 126.82, 125.25, 124.82, 60.14, 56.75, 55.13. *Z* Isomer: IR (neat) 3100–2850 (s), 1635 (m), 1320 (s), 1125 (s), 1090 (s), 990 (m), 940 (m) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.94 (d, 1 H, *J* = 9.5 Hz, *Z* SSCH=CH), 6.43–5.24 (m, 7 H), 3.96–3.63 (m, 6 H).

**(*E*)-4,5,9-Trithiadodeca-1,6,11-triene 4,4,9,9-Tetraoxide (39).** Peracetic acid (35%, 1.624 g, 7.48 mmol) was slowly added at –20 °C with stirring to a solution of (*E*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide (0.5 g, 2.14 mmol) in chloroform (30 mL). After stirring the solution for 2 h at –20 °C and then warming to room temperature during 10 h, solid anhydrous sodium carbonate (3 g) and magnesium sulfate (2 g) were added, and stirring was continued for 15 min. The mixture was filtered through Celite, sodium carbonate, and magnesium sulfate and concentrated in vacuo giving (*E*)-4,5,9-trithiadodeca-1,6,11-triene 4,4,9,9-tetraoxide (0.51 g, 85% yield). The crude product was further purified by flash chromatography (silica gel/ethyl acetate) giving 0.35 g (58% yield) of pure tetraoxide 39: IR (neat) 3100–2850 (m), 1635 (w), 1320 (s), 1130 (s), 947 (m), 870 (m) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.70 (d, 1 H, *J* = 16 Hz, *E* SO<sub>2</sub>SCH=CH), 6.46–5.27 (m, 7 H), 4.05 (d, 2 H, *J* = 6 Hz), 3.83 (d, 2 H, *J* = 6 Hz), 3.74 (d, 2 H, *J* = 6 Hz).

**(*E*)-4,5,9-Trithiadodeca-6,11-diene 9,9-Dioxide (40a).** *n*-Butyllithium (1.55 M) in hexane (75.5 μL, 0.106 mmol) was added dropwise to a stirred solution of 1-propanethiol (8.66 mg, 0.114 mmol) in dry tetrahydrofuran (1.5 mL) under argon at 0 °C. The resulting solution of lithium 1-propanethiolate was added via syringe to a solution of (*E*)-tetraoxide 39 (30 mg, 0.106 mmol) in dry tetrahydrofuran (5 mL) at –20 °C with stirring under argon. The reaction mixture was stirred at –20 °C for 15 min, allowed to warm to room temperature, treated with saturated ammonium chloride solution (5 mL), and diluted with methylene chloride (15 mL). The organic layer was separated, washed with water (5 mL), dried (magnesium sulfate), and concentrated in vacuo to give 26 mg (97% yield) of 40a as a yellow oil. The crude product was further purified by preparative HPLC (silica gel/hexane isopropanol 98:2) affording 2.2 mg (82% yield) of 40a homogeneous by NMR spectroscopy [IR (neat) 3000–2850 (s), 1640 (m), 1610 (m), 1320 (s), 1120 (s), 940 (m), 760 (m) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.46 (d, 1 H, *J* = 15 Hz, *E* SSCH=CH), 6.18–5.20 (m, 4 H), 3.78 (d, 2 H, *J* = 6.5 Hz), 3.71 (d, 2 H, *J* = 6.5 Hz), 2.72 (t, 2 H, *J* = 6.5 Hz), 2.06–0.82 (m, 5 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 136.66, 125.01, 124.74, 114.34, 56.41, 54.79, 40.50, 22.49, 13.05]. In another attempt a mixture of (*E,Z*)-tetraoxide 39 was used yielding (*E,Z*)-dioxide 40a. The isomers could be separated easily by preparative HPLC as above. (*Z*)-4,5,9-Trithiadodeca-6,11-diene 9,9-dioxide: IR (neat) 3050–2850 (s), 1610 (m), 1320 (s), 1130 (s), 945 (m) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.49 (d, 1 H, *J* = 9 Hz, *Z* SSCH=CH), 6.09–5.21 (m, 4 H), 3.87–3.58 (m, 4 H), 2.79–2.52 (m, 2 H), 1.86–1.14 (m, 2 H), 0.96 (t, 3 H, *J* = 6 Hz).

**2-Tetrahydrofuranyl *o*-(2-Tetrahydrofuranyltio)benzoate (45).** The procedure of Kruse et al.<sup>29</sup> was used with some modification. Sulfuryl

(29) Kruse, C. G.; Broekhof, N. L. J. M.; van der Gen, A. *Tetrahedron Lett.* 1976, 1725–1728.

chloride (6.75 g, 50 mmol) was added to tetrahydrofuran (50 mL) at  $-30^{\circ}\text{C}$  followed by the addition of a few crystals of  $\alpha,\alpha'$ -azoisobutyronitrile. A greenish solution was obtained which was irradiated at  $-30^{\circ}\text{C}$  for 15 min with a Hanovia 450-W mercury lamp. The resulting colorless solution of 2-chlorotetrahydrofuran was added slowly via a dropping funnel to a stirred solution of *o*-thiosalicylic acid (2.5 g, 16.2 mmol) and triethylamine (17.0 g, 168.3 mmol) in tetrahydrofuran (70 mL) at  $-78^{\circ}\text{C}$ . A thick white precipitate appeared during the addition. The reaction mixture was allowed to warm to room temperature. Stirring was continued for 2.5 h. The mixture was then filtered, and the residue was washed with ether (200 mL). The combined filtrate was dried (magnesium sulfate) and concentrated in vacuo. The residue was redissolved in methylene chloride (100 mL), washed with water ( $50 \times 2$  mL), dried (magnesium sulfate), and concentrated in vacuo giving 4.3 g (90% yield) of **45** as a thick colorless oil homogeneous by NMR spectroscopy: IR (neat) 3050–2850 (s), 1715 (s), 1590 (m), 1565 (m), 1465 (s), 1440 (m), 1280–1250 (s), 1085 (s), 1040 (s), 905 (s), 745 (m)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.02–7.03 (m, 4 H), 6.62–6.46 (m, 1 H), 5.84–5.62 (m, 1 H), 4.24–3.82 (m, 4 H), 2.33–1.71 (m, 8 H).

***o*-(2-Tetrahydrofuran-2-ylthio)benzyl Alcohol (46).** Lithium aluminum hydride (0.753 g, 19.8 mmol) was added in small portions to a stirred solution of **45** (2.85 g, 9.7 mmol) in ether (100 mL) at  $-20^{\circ}\text{C}$ . The reaction mixture was then allowed to warm to room temperature, stirred for 8 h, quenched carefully with moist sodium sulfate, and filtered. The filtrate was washed with water ( $50 \times 5$  mL), dried (magnesium sulfate), and concentrated in vacuo affording 1.9 g (93% yield) of **46** as a colorless oil homogeneous by NMR spectroscopy: IR (neat) 3400 (s), 3050–2850 (s), 1590 (w), 1480–1430 (m), 1040 (s), 750 (m)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.75–7.13 (m, 4 H), 5.51–5.24 (m, 1 H), 4.75 (br q, 2 H), 4.09–3.78 (m, 2 H), 3.77–3.34 (br s, 1 H), 2.48–1.76 (m, 4 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  143.86, 135.02, 133.40, 129.41, 128.65, 128.49, 88.36, 67.64, 63.65, 32.74, 25.03. Anal. Calcd for  $\text{C}_{11}\text{H}_{14}\text{O}_2\text{S}$ : C, 62.83; H, 6.71. Found: C, 62.55; H, 6.74.

***o*-(2-Tetrahydrofuran-2-ylthio)benzyl Methanesulfonate (47).** The procedure developed by Crossland and Servis<sup>30</sup> was used. A solution of methanesulfonyl chloride (0.5 g, 2.6 mmol) in methylene chloride (1 mL) was added dropwise to a stirred solution of **46** (0.5 g, 2.4 mmol) and triethylamine (0.36 g, 3.56 mmol) in methylene chloride (25 mL) at  $-10^{\circ}\text{C}$ . Stirring was continued at  $-10^{\circ}\text{C}$  for 10 min. The mixture was then washed with water ( $25 \times 7$  mL), and all the aqueous layers were extracted with methylene chloride (20 mL). The combined methylene chloride extract was dried (magnesium sulfate) and concentrated in vacuo giving 0.64 g (91% yield) of **47** as a thick oily product homogeneous by NMR spectroscopy: IR (neat) 3050–2850 (s), 1590 (w), 1480–1450 (m), 1350 (s), 1170 (s), 1040 (s), 920 (s)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.81–7.17 (m, 4 H), 5.63–5.36 (m, 1 H), 5.33 (s, 2 H), 4.10–3.78 (m, 2 H), 2.93 (s, 3 H,  $\text{CH}_3$ ), 2.46–1.74 (m, 4 H). Mesylate **47** is a highly reactive liquid and was washed immediately in the subsequent steps.

**Allyl *o*-(2-Tetrahydrofuran-2-ylthio)benzyl Sulfide (48).** *n*-Butyllithium (1.5 mL, 2.32 mmol, 1.55 M) in hexane was added dropwise to a stirred solution of 2-propenethiol (0.175 g, 2.36 mmol, freshly distilled) in tetrahydrofuran (5 mL) at  $0^{\circ}\text{C}$  under argon. The resulting solution of 2-propenethiolate was added slowly to a vigorously stirred solution of the above mesylate **47** (0.62 g, 2.15 mmol) in tetrahydrofuran (15 mL) at  $-20^{\circ}\text{C}$  under argon. The reaction mixture was allowed to warm to room temperature and stirred for 30 min. Methylene chloride (30 mL) was then added to the reaction mixture which was then washed with water ( $20 \times 3$  mL), dried (magnesium sulfate), and concentrated in vacuo, giving 0.5 g (87% yield) of **48** as a yellow oil homogeneous by NMR spectroscopy: IR (neat) 3065 (w), 3025–2850 (s), 1635 (w), 1590 (w), 1470 (m), 1440 (m), 1430 (m), 1050 (s), 920 (m), 750 (m)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.82–7.08 (m, 4 H), 6.15–5.44 (m, 2 H), 5.30–4.92 (m, 2 H), 4.17–3.82 (m, 4 H,  $\text{ArCH}_2\text{S}$  singlet overlapping the  $\text{OCH}_2$  multiplet), 3.10 (d, 2 H,  $J = 7$  Hz), 2.48–1.75 (m, 4 H). Anal. Calcd for  $\text{C}_{14}\text{H}_{18}\text{OS}_2$ : C, 63.12; H, 6.81. Found: C, 63.27; H, 6.90.

**Allyl (*o*-Mercaptophenyl)methyl Sulfide (49).** The procedure of Missakian et al.<sup>31</sup> was used. A solution of silver nitrate (0.69 g, 4.06 mmol) in water (3 mL) was added to a solution of **48** (0.48 g, 1.8 mmol) in methanol (25 mL). A white precipitate appeared immediately. The reaction mixture was heated gently on a steam bath for 10 min and filtered. The residue was washed with hexane and dissolved in methylene chloride (25 mL). Hydrogen sulfide was bubbled gently for 5 min through the solution giving a black precipitate of silver sulfide. The mixture was filtered through a pad of Celite–magnesium sulfate and concentrated in vacuo, giving 0.33 g (93% yield) of **49** as a yellow oil, homogeneous by NMR spectroscopy and VPC analysis: IR (neat)

3050–2850 (m), 2525 (m, SH), 1635 (m), 1470 (s), 1440 (s), 1430 (m), 920 (s), 740 (s)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.55–6.99 (m, 4 H), 6.17–5.50 (m, 1 H), 5.30–4.94 (m, 2 H), 3.91 (s, 1 H, SH), 3.81 (s, 2 H,  $\text{ArCH}_2\text{S}$ ), 3.09 (d, 2 H,  $J = 6.5$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  136.42, 134.10, 131.94, 131.24, 130.48, 127.79, 126.11, 117.48, 34.68, 34.52; MS, (rel intensity) 196 ( $\text{M}^+$ , 100), 154, 153, 135, 121, 77.

**Allyl *o*-(Allylthiomethyl)phenyl Disulfide (41a).** A solution of *n*-butyllithium (0.415 mL, 0.64 mmol, 1.55 M) in hexane was added dropwise to a stirred solution of thiophenol **49** (0.12 g, 0.61 mmol) in tetrahydrofuran (5 mL) at  $-10^{\circ}\text{C}$  under argon. The resulting solution was added dropwise to a solution of allyl methanethiosulfonate<sup>23</sup> in tetrahydrofuran (5 mL) with stirring under argon at  $-20^{\circ}\text{C}$ . The reaction mixture was allowed to warm to room temperature and stirred for 30 min. It was then diluted with methylene chloride (15 mL), washed with water ( $10 \times 3$  mL), dried (magnesium sulfate), and concentrated in vacuo affording 0.16 g (97% yield) of **41a** as a yellow oil: IR (neat) 3075 (s), 3000–2900 (s), 1630 (s), 1585 (m), 1460 (s), 1440 (s), 1420 (s), 1220 (s), 985 (s), 915 (s), 740 (s)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.02–7.05 (m, 4 H), 6.21–5.45 (m, 2 H), 5.35–4.89 (m, 4 H), 3.89 (s, 2 H), 3.36 (d, 2 H,  $J = 7$  Hz,  $\text{SSCH}_2$ ), 3.12 (d, 2 H,  $J = 6.5$  Hz,  $\text{SCH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  137.53, 136.76, 134.21, 132.67, 129.85, 129.60, 127.80, 126.84, 118.94, 117.38, 41.68, 34.70, 33.13.

**Allyl *o*-(Allylsulfinylmethyl)phenyl Disulfide (42a).** Sulfide **41a** (0.07 g, 0.26 mmol) was dissolved in methylene chloride (10 mL) and cooled to  $-20^{\circ}\text{C}$ . A solution of MCPBA (0.056 g, 0.26 mmol, 80%) in methylene chloride (3 mL) was added dropwise, and the reaction mixture was allowed to warm to room temperature, stirred for 10 min, and washed with 5% sodium bicarbonate solution ( $10 \times 5$  mL) and water (10 mL). The methylene chloride phase was dried (magnesium sulfate) and concentrated in vacuo giving 0.068 g (92% yield) of **42a** as a thick oil which was purified by preparative HPLC (silica gel/hexane–isopropanol, 92:6), giving 0.06 g (81% yield) of **42a** as a colorless oil: IR (neat) 3100–2900 (m), 1635 (m), 1590 (w), 1470 (m), 1440–1400 (m), 1040 (s), 920 (m)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.02–7.20 (m, 4 H), 6.33–4.89 (m, 6 H), 4.19 (AB quartet, 2 H,  $J_{\text{AB}} = 13.0$  Hz), 3.60–3.22 (m, 4 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  136.84, 131.99, 131.15, 130.17, 130.05, 128.60, 127.40, 125.41, 123.16, 118.73, 55.13, 54.83, 40.93. Anal. Calcd for  $\text{C}_{13}\text{H}_{16}\text{OS}_2$ : C, 54.89; H, 5.67. Found: C, 54.66; H, 5.90.

**Platelets.** Blood was drawn from the anticubital vein of healthy adults into plastic syringes containing 1/10 final volume of 3.8% trisodium citrate. Prior to blood collection informed consent was obtained under an approved protocol from the Human Studies Research Committee of the Office of Public Health, New York State Department of Health. The donor denied having taken any medication for at least 10 days prior to testing. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared at room temperature by centrifuging the blood as detailed elsewhere.<sup>15</sup>

**Aggregation Studies and  $\text{ID}_{50}$  Determination.** Platelet aggregation was measured turbidimetrically in a dual-channel aggregometer (Payton Associates, Buffalo, New York) equipped with an OmniScribe recorder (Houston Instruments, Austin, TX). PPP was used to represent 100% aggregation. In a typical experiment 0.45 mL of PRP (250,000  $\mu\text{L}$ ) was pipetted into each 1-mL siliconized glass cuvette and warmed to  $37^{\circ}\text{C}$  for 1 min with stirring. A known amount of methanolic solution (0.2–3  $\mu\text{L}$ ) of the suspected antithrombotic agent was then added and allowed to react for 2 min followed by the addition of 50  $\mu\text{L}$  of ADP (100  $\mu\text{M}$ ) or collagen (1  $\mu\text{g}$ ). Not more than 3  $\mu\text{L}$  of methanol (vehicle) or solution of compound were added to the platelet suspension. The extent of platelet aggregation was measured after 3 min.  $\text{ID}_{50}$  values were calculated in relation to the vehicle control.

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(30) Crossland, R. K.; Servis, K. L. *J. Org. Chem.* **1970**, *35*, 3195–3196.

(31) Missakian, M. G.; Ketcham, R.; Martin, A. R. *J. Org. Chem.* **1974**, *39*, 2010–2012.

104228-59-3; (*E*)-**40a**, 104228-60-6; (*Z*)-**40a**, 104324-43-8; **40b**, 104228-62-8; **40c**, 104324-53-0; **40d**, 104228-65-1; **40e**, 104228-64-0; **40f**, 104228-63-9; **41a**, 104324-49-4; **41b**, 104324-60-9; **41c**, 104324-49-4; **41d**, 104324-61-0; **41e**, 104324-62-1; **42a**, 104324-50-7; **42b**, 104324-63-2; **42c**, 104324-50-7; **42d**, 104324-64-3; **42e**, 104324-65-4; **43**, 104324-66-5; **43-Na**, 104324-67-6; **44**, 104324-68-7; **45**, 104324-44-9; **46**, 104324-45-0; **47**, 104324-46-1; **48a**, 104324-47-2; **48b**, 104324-54-1; **48d**, 104324-55-2; **48e**, 104324-56-3; **49a**, 104324-48-3; **49b**, 104324-57-4; **49d**, 104324-58-5; **49e**, 104324-59-6;  $\text{H}_2\text{C}=\text{CHCH}_2\text{SSMe}$ , 34135-85-8;  $\text{CH}_3\text{CH}=\text{CHSSPr}$ , 5905-46-4;  $\text{EtO}_2\text{C}(\text{CH}_2)_3\text{Br}$ , 2969-81-5;  $\text{AcSLi}$ , 84434-88-8;  $\text{EtO}_2\text{C}(\text{CH}_2)_3\text{Ac}$ , 104228-51-5;  $\text{EtO}_2\text{C}(\text{CH}_2)_3\text{S}(\text{O})\text{Cl}$ , 104228-52-6;  $\text{EtO}_2\text{C}(\text{CH}_2)_3\text{S}(\text{O})\text{S}(\text{CH}_2)_3\text{CO}_2\text{Et}$ , 104228-54-8; (*E*)- $\text{CH}_3\text{CH}=\text{CHCH}_2\text{SH}$ , 58688-79-2;  $\text{CH}_3(\text{CH}_2)_3\text{SH}$ , 111-31-9; (*E*)-

$\text{PrCH}=\text{CHCH}_2\text{SH}$ , 89222-69-5; (*Z*)- $\text{PrCH}=\text{CHCH}_2\text{SH}$ , 104108-89-6;  $\text{PhCH}_2\text{SH}$ , 100-53-8;  $\text{PhCH}_2\text{SSO}_2\text{Me}$ , 7559-62-8; allyl sulfide, 592-88-1; 2-propenethiol, 870-23-5; ethyl propiolate, 623-47-2; 2-carbethoxy-4-pentenethial *S*-oxide, 104324-39-2; propene, 115-07-1; methanesulfinyl chloride, 676-85-7; 1-propanethiol, 107-03-9; lithium 1-propanethiolate, 16203-40-0; 2-chlorotetrahydrofuran, 13369-70-5; *o*-thiosalicylic acid, 147-93-3; allyl methanethiosulfonate, 14202-77-8; 18-mercaptomethyl-1,4,7,10,13,16-hexaoxacyclononadecane, 77661-77-9.

**Supplementary Material Available:** Syntheses and IR,  $^1\text{H}$  NMR, and  $^{13}\text{C}$  NMR data for **29**, **35**, **36**, **40-43**, **50-52**, **54-58**, and **60-66** (23 pages). Ordering information is given on any current masthead page.

## One-Step Stereochemical Determination of Contiguous Four Acyclic Chiral Centers on the Steroidal Side Chain: A Novel Synthesis of Brassinolide

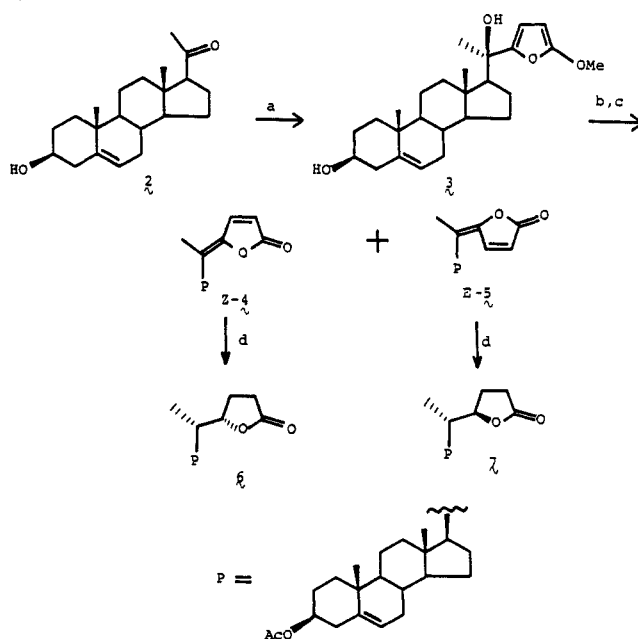
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**Abstract:** The stereoselective synthesis of brassinolide and its enantiomer (22*S*,23*S*,24*R*)-24-epibrassinolide was accomplished. The key feature of this synthesis is based on the stereoselective reduction of the 5-ylidenetetronate derivative to control the stereochemistry of the contiguous four acyclic chiral centers on the steroid side chain in one-step, wherein the stereochemically determinative step was carried out in a cyclic system. The addition reaction of the dianion of the tetronate derivative to the 20-oxo steroid afforded the adduct, whose syn-dehydration reaction brought about the formation of the desired (*Z*)-5-ylidenetetronate. The stereoselective reduction of the (*Z*)-5-ylidenetetronate afforded the key intermediate for the synthesis of brassinolide. On the other hand, the (*E*)-5-ylidenetetronate could also be prepared from the same adduct by manipulation of the dehydration reaction, and this approach led to the synthesis of (22*S*,23*S*,24*R*)-24-epibrassinolide.

Naturally occurring steroids,<sup>1</sup> with their wide range of structural and stereochemical features, continue to provide challenging synthetic targets.<sup>2</sup> In particular, stereoselective construction of steroidal side chains has been the subject of extensive synthetic efforts,<sup>3</sup> because their physiological activity has been reflected in the stereochemistry of the side chain.<sup>1</sup> In conjunction with the

Scheme 1<sup>a</sup>



<sup>a</sup>Steps: (a) 5-lithio-2-methoxyfuran, THF,  $-78^\circ\text{C} \rightarrow$  room temp., 1 h; (b) *p*-TsOH, acetone, room temp., 5 h; (c)  $\text{Ac}_2\text{O}$ , py, room temp., 6 h; (d) 10% Pd-C,  $\text{H}_2$ , EtOAc, 1 atm, room temp., 6 h.

synthesis of physiologically active steroids, we have been interested in the stereocontrolled construction of the polyhydroxylated steroid side chains, and here report a novel synthesis of brassinolide (**1**).<sup>4</sup>

(4) Grove, M. D.; Spencer, G. F.; Rohwedder, W. K.; Mandava, N.; Worley, J. F.; Warthen, J. D., Jr.; Steffens, G. L.; Flippen-Anderson, J. L.; Cook, J. C. *Nature (London)* **1979**, *281*, 216.

(1) Reviews: (a) Ecdysones: Bergamasco, R.; Horn, D. H. S. *Progress in Ecdysone Research*; Hoffmann, J. A., Ed.; Elsevier/North Holland Biomedical Press: Amsterdam, 1980; p 299. (b) Vitamin D<sub>3</sub> metabolites: Norman, A. W.; Schaefer, K.; Herrath, D. V., Grigoleit, H.-G., Eds.; *Vitamin D Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism*; Gruyter: Berlin, 1982. (c) Withanolides: Glotter, E.; Kirson, I.; Lavie, D.; Abraham, A. *Bio-Organic Chemistry*; van Tamelen, E. E., Ed.; Academic Press: New York, 1978; Vol. II, p 57.

(2) For a review, see: (a) Piatak, D. M.; Wicha, J. *Chem. Rev.* **1978**, *78*, 199. (b) Redpath, J.; Zeelen, F. *J. Chem. Soc. Rev.* **1983**, *12*, 75.

(3) (a) Trost, B. M.; Matsumura, Y. *J. Org. Chem.* **1977**, *42*, 2036. (b) Trost, B. M.; Verhoeven, T. R. *J. Am. Chem. Soc.* **1978**, *100*, 3435. (c) Schow, S. R.; McMorris, T. C. *J. Org. Chem.* **1979**, *44*, 3760. (d) Koreeda, M.; Tanaka, Y.; Schwartz, A. *Ibid.* **1980**, *45*, 1172. (e) Tanabe, M.; Hayashi, K. *J. Am. Chem. Soc.* **1980**, *102*, 862. (f) Temple, J. S.; Schwartz, J. *Ibid.* **1980**, *102*, 7381. (g) Dauben, W. G.; Brookhart, T. *Ibid.* **1981**, *103*, 237. (h) Midland, M. M.; Kwon, Y. C.; *J. Org. Chem.* **1981**, *46*, 229. (i) Marino, J. P.; Abe, H. *J. Am. Chem. Soc.* **1981**, *103*, 2097. (j) Takahashi, T.; Yanada, H.; Tsuji, J. *Ibid.* **1981**, *103*, 5259; *Tetrahedron Lett.* **1982**, *23*, 233. (k) Stork, G.; Atwal, K. S. *Ibid.* **1982**, *23*, 2073. (l) Midland, M. M.; Kwon, Y. C. *Ibid.* **1982**, *23*, 2077. (m) Stevens, R. V.; Gaeta, F. C. S.; Lawrence, D. S. *J. Am. Chem. Soc.* **1983**, *105*, 7713. (n) Schmuft, N. R.; Trost, B. M. *J. Org. Chem.* **1983**, *48*, 1404. (o) Desmaële, D.; Ficini, J.; Guingant, A.; Kahn, Ph. *Tetrahedron Lett.* **1983**, *24*, 3079. (p) Ziegler, F. E.; Mencil, J. J. *Ibid.* **1983**, *24*, 1859. (q) Midland, M. M.; Kwon, Y. C. *J. Am. Chem. Soc.* **1983**, *105*, 3725. (r) Kametani, T.; Tsubuki, M.; Nemoto, H. *Tetrahedron Lett.* **1981**, *22*, 2373; *J. Chem. Soc., Perkin Trans. 1* **1983**, 3077. (s) Kametani, T.; Tsubuki, M.; Furuyama, H.; Honda, T. *Ibid.* **1985**, 557. (t) Sardina, F. J.; Mourino, A.; Castedo, L. *Tetrahedron Lett.* **1983**, *24*, 4477. (u) Catalan, C. A. N.; Kokke, W. C. M. C.; Doque, C.; Djerassi, C. *J. Org. Chem.* **1983**, *48*, 5207. (v) Kyler, K. S.; Watt, D. S. *J. Am. Chem. Soc.* **1983**, *105*, 619. (w) Castedo, L.; Granja, J. R.; Mourino, A. *Tetrahedron Lett.* **1985**, *26*, 4959. (x) Takahashi, T.; Ueno, H.; Miyazawa, M.; Tsuji, J. *Tetrahedron Lett.* **1985**, *26*, 4463. (y) Midland, M. M.; Gabriel, J. *J. Org. Chem.* **1985**, *50*, 1143. (z) Mikami, K.; Kawamoto, K.; Nakai, T. *Tetrahedron Lett.* **1985**, *26*, 5799.