

Flavor Components of Garlic Extract

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Diallyl thiosulfinate was found to be a major constituent of solvent extracted garlic. Upon gas chromatography, diallyl thiosulfinate undergoes dehydration, leading to the formation of two isomeric disulfides. At room temperature diallyl

thiosulfinate undergoes a rearrangement. After 24 hr, sulfur dioxide, diallyl mono-, di-, and trisulfides are the major products of this reaction. The role of thiosulfates in the formation of flavor components of garlic and other *Alliums* is discussed.

The pioneering studies of Semmler (1892) established the importance of diallyl disulfide and diallyl trisulfide in the flavor of garlic distillates. Cavallito *et al.* (1944a,b, 1945) and Stoll and Seebeck (1948) showed that *Allium sativum* (garlic) contains allyl-S-cysteine sulfoxide (alliin) and an enzyme, allinase. By action of allinase on alliin, diallyl thiosulfinate (allicin) $\text{CH}_2=\text{CH}-\text{CH}_2\text{S}(=\text{O})-\text{SCH}_2-\text{CH}=\text{CH}_2$ is formed. By analogy with the work of Barnard (1957) on the disproportionation of aryl thiosulfates, Carson (1967) suggested that thiosulfates in *Alliums* disproportionate to disulfides and thiosulfates. Such disproportionation would account for the origin of the disulfides. The presence of trisulfides has been demonstrated in *Allium* distillates (Carson and Wong, 1961a; Semmler, 1892) but their origin has not been established. This paper attempts to clarify the role of allicin in the formation of the flavor components of garlic oil.

EXPERIMENTAL

Isolation of Flavor Components from Garlic. One kilogram of domestic garlic cloves, of unknown origin, was purchased locally. Ten portions of 0.1 kg of cloves suspended in 0.2 l. of redistilled trichlorofluoromethane were chopped in a Waring Blendor. The total slurry was stirred in a 2-l. reaction flask for 3 hr. The solvent was decanted and the bulk of the volatiles were removed in a rotary evaporator at 90 Torr and 20°C.

Fractionation and Identification. Garlic extract was separated into individual components by a 10 ft \times 1/4 in. o.d. stainless steel gas chromatographic column packed with Chromosorb W, HP, 80/100 mesh, coated with 5% Carbowax 20M. The column oven temp was programmed from 70° to 170°C at 4° per min. The injection port temperature was maintained at 100°C to minimize sulfide disproportionation. Fractions were rechromatographed on two 200 ft \times 0.02 in. bore Golay columns, one containing Apiezon-L and the other coated with Carbowax 20M. Only chromatographic fractions found to contain less than 2% impurities on both columns were analyzed further.

Mass spectral analyses of the garlic extract were carried out

on a Perkin-Elmer Hitachi Model RMU 6E mass spectrometer. Samples were analyzed either through a liquid introducer or by injection into an F&M 5750 gas chromatograph equipped with a support coated open tubular (SCOT) column, 50 ft \times 0.02 in. bore containing Carbowax 20M, coupled through a Biemann helium separator to the mass spectrometer (gc-ms). High resolution mass spectral analyses were carried out on an AEI Model M.S.-9 unit. Infrared spectra (ir) were obtained using potassium bromide discs, using a Beckman Model IR 5A infrared spectrophotometer. Nuclear magnetic resonance (nmr) spectra were obtained using a Varian HA-100 spectrometer. Compounds were analyzed in deuterated chloroform, using tetramethylsilane as an internal standard. Raman spectra were obtained on a Spex-1401 double beam spectrometer.

Synthesis of Diallyl Thiosulfinate. Diallyl thiosulfinate was synthesized by the action of perbenzoic acid on diallyl disulfide by the method of Small *et al.* (1947).

RESULTS AND DISCUSSION

Analysis of the garlic extract by gas chromatography (glc) on the SCOT Carbowax column indicated that eight components accounted for 96% of the total peak area. Six of the components were identified by gc-ms and have been reported previously in garlic (Table I). Two components, each with a molecular weight of 144, accounted for 78% of the total peak area. Both mass spectra were similar (Table III). Fragments of M-33 (111), M-41 (103), and M-72 (72) appeared characteristic of these spectra. Glc on an SE-30 column did not separate these components from each other.

Approximately 25 mg each of the two unknown components was trapped from the 10 ft \times 1/4 in. Carbowax 20M column. The earlier eluting peak was designated as 144-I and the latter as 144-II. High resolution mass spectrometry indicated a most probable molecular formula of $\text{C}_8\text{H}_8\text{S}_2$ for both compounds. Ir analysis showed the presence of a vinyl group in both unknowns, while Raman spectroscopy ruled out the presence of a mercaptan or thiol group. The absence of absorption in the ir spectra at 9.25 μ confirmed that no thiosulfinate grouping was present in the molecule. From the aforementioned data, it is apparent that the two peaks are closely related, a supposition that is backed up by glc and ms data. Nmr assignments for the garlic trappings are sum-

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Table I. Components of Garlic Extract

Component	Relative Retention Time ^{a,b}	Percentage
Allyl alcohol	0.33	5.4
Methyl allyl disulfide	0.63	1.2
Diallyl disulfide	1.00	5.7
Dimethyl trisulfide	1.08	2.4
Methyl allyl trisulfide (144-I)	1.21	1.5
Diallyl trisulfide (144-II)	1.46	23.5
	1.56	1.0
	1.65	55.4

^a Diallyl disulfide = 1.00. ^b SCOT Carbowax 20M column programmed at 4° C per min from 70° to 170° C.

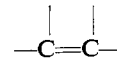
Table II. Nmr Assignments for Garlic Trappings, 144-I and 144-II

Chemical Shift, δ	Pattern (J)	Integral Value	Assignment
Peak 144-I			
2.50	multiplet	2	
3.69	apparent doublet (7 cps)	1	
5.16-5.32	multiplet	2	
5.72-6.24 6.01	broad multiplet, unresolved doublet (11 cps)	2	
6.37	doublet (11 cps)	1	
Peak 144-II			
3.31	apparent triplet (5.5 cps) 2nd order splitting (2 cps)	2	
4.72	doublet (7 cps)	1	
5.24-5.47	multiplet	2	
5.82-6.16 5.96	broad multiplet, unresolved doublet (11 cps)	2	
6.31	doublet (11 cps)	1	

marized in Table II. Since fraction 144-II gave a stronger spectrum, it was studied by nmr first and the conclusions drawn applied to fraction 144-I.

First, the multiplet at 5.24 to 5.47 ppm and the multiplet at 5.82 to 6.15 ppm are typical of a vinyl group CH₂=CH—. The remaining olefinic signals, two doublets with a coupling of 11

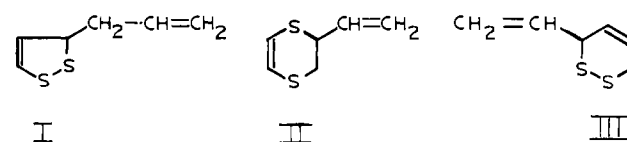
cps, suggest a cis double bond $\begin{matrix} \text{H} & \text{H} \\ | & | \\ -\text{C} & = & \text{C}- \end{matrix}$. The signal at 3.31 ppm



suggests S—CH₂—C=C, although the splitting pattern is not normal. (In a reference spectrum of methyl allyl disulfide, the S—CH₂—C=C is a doublet centered at 3.40 ppm.) No reference values could be found with which to associate the doublet at 4.72 ppm. It is tentatively assigned as a >CH— in a deshielded environment. At this point, it is helpful to note the absence of normal alkyl methyl or methylene protons, or even normal allylic methylenes. This information indicates a preponderance of electronegative groups, relative to alkyl hydrogens. The nmr data, plus the ms data indicating a molecular weight of 144 and an empirical formula of C₆H₈S₂, suggest a ring structure with a terminal vinyl group. Working within the empirical formula given, possible ring systems were taken from Patterson *et al.* (1960). The three and four membered ring systems were ruled out because none could fit the basic nmr signals observed. Of the six possible five- or six-membered rings, two could be ruled out because there is no signal indicative of either —S—CH₂—S— or S—CH—S— in the spectrum. A third ring possibility



would necessitate the presence of —SH, or —S—CH=CH₂, neither of which is observed. This leaves three possible ring systems that will fit the basic nmr spectrum.

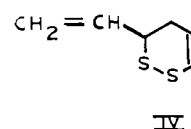


Both the methylene and methine signals in I and II would not be expected as low as 3.3 and 4.7 ppm, respectively.

However, the olefinic signals of the unknown are consistent with reference values for systems similar to III. The methine proton, influenced by two double bonds and the sulfur atoms, would be expected as far down as 4.7 ppm. Lastly, as mentioned earlier, a methylene between a sulfur atom and a double bond resonates in the 3.3 ppm region. The triplet observed could be rationalized as nonequivalence of the two hydrogens due to the cyclic structure. The nmr spectrum suggests III as a structure worthy of consideration.

Results of decoupling experiments done on compound 144-II substantiated the presence of the postulated cis double bond and vinyl group, and were fully compatible with the postulated structure.

If the reasoning used to arrive at this postulate is applied to fraction 144-I, we observe that our entire olefinic region remains basically unchanged. The remaining signals appear to correspond to the methylene and methine protons in the postulated structure III, but they are both approximately 1 ppm farther upfield, indicating less influence by electronegative groups. The isomer of III



is postulated for fraction 144-I of the natural garlic trap.

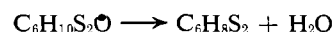
Table III. Mass Spectral Data for Garlic Trappings

	144-I Relative Intensity	144-II Relative Intensity
37	6	5
38	12	8
39	52	32
40	6	5
41	25	16
42	19	16
44	16	12
45	71	60
46	9	7
47	9	6
50	9	4
51	12	8
52	6	3
53	15	9
54	6	2
57	6	6
59	11	8
60	5	3
61	4	3
64	7	3
65	11	10
66	8	7
67	16	13
69	15	12
70	4	4
71	66	45
72	70	42
73	15	10
74	8	5
77	40	27
78	16	12
79	50	24
80	7	4
84	12	8
85	22	15
95	12	7
97	63	50
98	6	6
99	6	10
103	66	28
104	11	4
105	8	3
109	5	5
110	8	22
111	100	100
112	14	11
113	7	6
141	16	13
144	78	66
145	8	7
146	12	8

Based on nmr data and in agreement with the other analytical information obtained on the trapped unknowns, structure IV is proposed for compound 144-I, and structure III for 144-II.

It was previously observed (Brodnitz, 1969), that at temperatures above 150° C, propyl propane thiosulfinate undergoes a dehydration reaction leading to the formation of cis and trans propenylpropyl disulfides. By analogy, it appeared likely that compounds 144-I (3-vinyl-1,2-dithi-5-ene) and 144-II (3-vinyl-1,2-dithi-4-ene) might be formed by a similar reaction from diallyl thiosulfinate. Analysis of the crude garlic extract indicated that its ir and nmr spectra were very similar to those obtained on synthetic diallyl thiosulfinate. The ir spectrum of the garlic extract showed strong absorption at 9.25 μ, characteristic of thiosulfonates, and the absence of absorption at 8.92 μ, indicative of thiosulfonates (Carson and Wong, 1961b).

The major components occurring in gas chromatography of synthetic diallyl thiosulfinate on columns coated with Carbowax 20M were shown by gc-ms to be compounds 144-I and 144-II. In addition, diallyl disulfide and diallyl trisulfide were also identified in the chromatographed sample. Oaks *et al.* (1964) gas chromatographed hexane extracts of garlic cloves. Two unknown compounds, numbered 19 and 21, appear to be compounds 144-I and 144-II. These assignments are supported by the identification by Oaks *et al.* (1964) of diallyl trisulfide as the component which eluted between the two unknowns. These observations show that compounds 144-I and 144-II as well as peaks 19 and 21 reported by Oaks *et al.* (1964) are products of diallyl thiosulfinate decomposition during glc and are not components of garlic. The following dehydration reaction is suggested for their formation:



As determined by ir, a nearly complete decomposition of synthetic diallyl thiosulfinate held at 20° C occurred within 20 hr. The major products of this degradation were identified by gc-ms as diallyl disulfide (66%), diallyl sulfide (14%), and diallyl trisulfide (9%). Sulfur dioxide was observed in the headspace above the aged thiosulfinate sample. When this sample of synthetic diallyl thiosulfinate was gas chromatographed under the same conditions used in studying natural allicin, only traces of compounds 144-I and 144-II were observed. A similar degradation was observed when a garlic extract was held at room temperature for 20 hr. With both the garlic extract and the synthetic allicin, no indications were found by glc, ir, or nmr spectroscopy to support the hypothesis that thiosulfonates were formed in this reaction. Based on these observations, it appears that the breakdown of thiosulfinate plays a major role in the formation of di- and trisulfide found as components of garlic. This suggests that steam distillation at atmospheric pressure, as proposed by Cavallito *et al.* (1945), is not essential for the formation of diallyl disulfide from allicin. The specific composition of disulfides in a species of *Allium* appears to be related to the composition of S-alkyl or alkenyl cysteine sulfoxides which occur in the plant. In garlic, allyl- and methylcysteine sulfoxide are enzymatically converted to the corresponding thiosulfonates. Upon mild heating, or even at room temperature, the thiosulfonates undergo nonenzymatic rearrangement leading to the formation of symmetrical and mixed mono-, di-, and trisulfides, as well as sulfur dioxide. These allyl and methyl sulfides are main components of commercial garlic oils.

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