

Thiopropenal S-Oxide: A Lachrymatory Factor in Onions

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The lachrymatory factor in onions was isolated by gas chromatography of a raw onion extract. By means of spectral analysis, the isolate was identified as thiopropenal S-oxide. Synthesis confirmed this

identification. Thioethanal, thioacetone, thiobutanal, and thiohexanal S-oxides were also synthesized and their lachrymogenic properties were evaluated.

The lachrymatory factor (L.F.) and its precursors in onions have been the subject of extensive studies over the past several years. Kupiecki and Virtanen (1960) and Schwimmer *et al.* (1960) demonstrated that *Allium cepa* contains an enzyme, allinase, capable of cleaving alkyl cysteine sulfoxides. The manner by which the cysteine sulfoxide was split appeared to parallel that observed by Stoll and Sebeck (1949) in garlic, in that ammonia and pyruvic acid were formed by the reaction. Studies by Virtanen and Matikkala (1961) and Virtanen and Spare (1961) indicated that L.F. was also formed enzymatically during the cleavage of S-(1-propenyl)cysteine sulfoxide. This L.F. does not occur in garlic. Based on mass spectral analysis, Virtanen's group (Moisio *et al.*, 1962) determined that the L.F. had a molecular weight of 90, and suggested that it might be 1-propenylsulfenic acid.

Previously, two other structures of molecular weight 90 had been suggested for volatile components of onions. Niegisch and Stahl (1956) suggested β -hydroxypropanthial as the possible structure for an isolate of raw onions obtained by them. Wilkens (1961) proposed thiopropenal S-oxide as a possible structure for the L.F. None of the compounds were verified by synthesis.

The present work was aimed at the isolation, identification, and synthesis of the L.F. in onions.

EXPERIMENTAL

Isolation of the Lachrymator from Onions. Two kilograms New York State Yellow Globe onions frozen at -20°C were chopped in a prechilled Hobart grinder. The onions were transferred to a 3 l. flask and placed on a Buchi rotary evaporator. The evaporator had a 2 l. two neck round-bottomed receiver which was cooled in a dry-ice isopropyl alcohol bath. A trap cooled with liquid nitrogen connected the receiver flask to a Welsh Disto-Pump. Generally, with 1350 g batches of onion, the distillation time averaged 2 hr. With the rotary flask immersed in a 20°C water bath, a vacuum of 0.7–1.0 mm mercury was maintained in the system. The distillate collected from the flask and the liquid nitrogen trap was extracted with redistilled monofluorotrichloromethane. Removal of the solvent in a Kuderna Danish concentrator gave the product used in the study.

The lachrymator was isolated from the oil on a 8 ft \times $\frac{1}{8}$ in. o.d. stainless steel gas chromatographic (glc) column packed with H.P. Chromosorb W coated with 20% SE-30. The column was maintained at 60°C .

Synthesis of Thioalkanal S-Oxide. Thioalkanal S-oxides were synthesized from their corresponding alkylsulfinyl chlorides by the method of Sheppard and Diekmann (1964). The alkylsulfinyl chloride was added dropwise to equimolar

amounts of a triethylamine dissolved in trichlorofluoromethane at -20°C . The addition was completed in 30 min. The reaction mixture was stirred at -40 to -20°C for 1 hr after the addition had been completed. The trichlorofluoromethane was removed from the rotary evaporator under 100 mm Hg pressure. The residue was centrifuged at 10,000 rpm at -20°C for 1 hr. The solution was filtered and placed on the rotary evaporator to remove the residual solvent at 90 mm Hg pressure, leaving the desired product.

Analytical Methods. For mass spectral (ms), infrared absorption (ir), or nuclear magnetic resonance (nmr) analysis, the sample was collected from the end of the gas chromatographic column in glass tubes.

Mass spectral analyses were carried out on a Perkin-Elmer Hitachi model RMU-6E mass spectrometer. Samples were analyzed directly through the liquid introducer.

Ir spectra were obtained on 0.098 mm thick sodium chloride cells on samples dissolved in carbon disulfide or carbon tetrachloride. A Perkin-Elmer Model 621 recording infrared spectrophotometer was used in these analyses. Nmr spectra were obtained using a Varian HR100 spectrometer. Compounds for nmr analysis were diluted in deuterated chloroform using tetramethylsilane as an internal standard.

RESULTS AND DISCUSSION

A typical chromatogram of the concentrated extract obtained from raw onions is shown in Figure 1. A compound possessing strong lachrymogenic properties was noted at a retention time of 9 min.

The mass spectrum of the lachrymator contained a strong

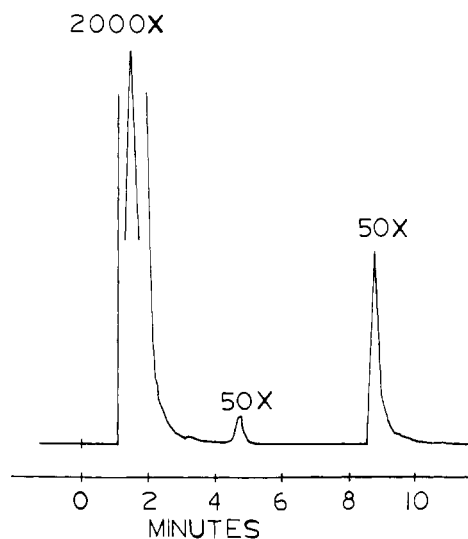


Figure 1. Gas chromatograph of onion distillate, $\frac{1}{8}$ in. \times 8 ft SE-30 column maintained at 60°C

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Table I. Mass Spectral Data of S-Oxides

<i>m/e</i>	(Relative Intensity)					
	Natural Thiopropanal S-Oxide	Synthetic Thiopropanal S-Oxide	Virtanen Natural Lachrymator	Thioacetone S-Oxide	Thiobutanal S-Oxide	Thiohexanal S-Oxide
26	12	6	8			
27	35	22	21		35	87
28					9	
29	45	9	15	45	30	68
31	2	2	2			
32	11				2	
34			2	8		
36		10				
37	6	4	5			
38	10	5	8		2	4
39	56	38	39	3	18	53
40	11	10	12	15	2	30
41	100	100	100	100	39	100
42	36	30	32	30	7	12
43	12	10	16	11	16	23
44	4	3	6	7	2	11
45	30	19	25	9	11	21
46	7	3	6	3	4	2
47	6	4	7		7	7
48	14	8	12		6	8
49	2	3	3	3		2
50	2	2	3		3	3
51					2	
53		2			6	10
54					4	4
55	5	6			100	60
56	2	3			11	15
57	8	2	7			13
58	17	3	6	3	4	4
59	4	3	3		5	7
60						6
61	3	2	3			3
62	3	2	4			
63	6	3	7		11	11
64	8	4	2		4	2
65			3			
67		2	2			9
69	3	2	2		2	10
70						5
71	4	3	4			4
72	3	2	3			2
73	11	10	14	2		5
74	7	3	2			
75	2	3	2			2
76		2			9	16
77						4
79						2
81						4
82						7
83						15
84						3
85						2
86				2		
88				3		
89						3
90	50	50	50	18		
91	2	2	2			
92	2	2	3			
104					17	
115						13

peak of mass 90, and fragments of mass 73 and 45, as well as sulfur isotope peaks at mass 91 and 92 (Table I). Niegisch and Stahl (1956) reported a similar peak pattern for one of the compounds observed in their studies of onion volatiles. β -Hydroxy propanthial



was suggested as the possible structure. Spare and Virtanen (1963) obtained a similar spectrum from the volatile products

of the action of an onion allinase on isolated *S*-(1-propenyl)-cysteine sulfoxide. 1-Propenylsulfenic acid [$\text{CH}_3\text{—CH=CHS(=O)H}$] was suggested as the structure of this lachrymatory factor. The mass spectrum of this material reported by Spare and Virtanen (1963) was very similar to the pattern of the natural lachrymator and the synthetic thiopropanal *S*-oxide prepared in this study.

An infrared spectrum of the isolated lachrymator diluted in carbon disulfide showed characteristic absorption bands at

8.83 and 9.00 μ (Table II). These absorption bands corresponded to the stretching vibration of C=S=O function of sulfoxes found by Sheppard and Diekmann (1964) in aromatic sulfoxes. Similar spectra were reported by Wilkens (1961) for the onion lachrymator isolated by him.

Nmr spectra of the isolated lachrymator contained bands exhibiting chemical shifts of 1.18, 2.80, and 8.14 (Table III). The triplet at 8.14 ppm was far downfield, apparently because of the interaction of the —CH= group with the π clouds of the S=O and C=S bonds. The integral ratio of 3:2:1 indicating that the presence of single —CH₃, —CH₂, and —CH= groups, respectively, was incompatible with the structures proposed by Niegisch and Stahl (1956) and Spare and Virtanen (1963). Only the structure of thiopropanal S-oxide CH₃CH₂CH=S=O, suggested by Wilkens (1961), could be reconciled with all the spectral observations. Comparison of the spectra obtained from isolated lachrymator with those of synthetic thiopropanal S-oxide (Tables I, II, III) indicated that the two were essentially identical, confirming that the lachrymatory factor in onion is indeed thiopropanal S-oxide.

As shown by Spare and Virtanen (1963), the lachrymatory factor in onions is formed by the action of the enzyme allinase

on S-(1-propenyl)cysteine sulfoxide. In slightly alkaline medium this sulfoxide has been shown, by Virtanen and Spare (1961), to form cycloalliin by the reaction of the amino group with the double bond. Based on the structure of the lachrymatory factor confirmed by us, the following mechanism for its formation in a natural system is proposed.

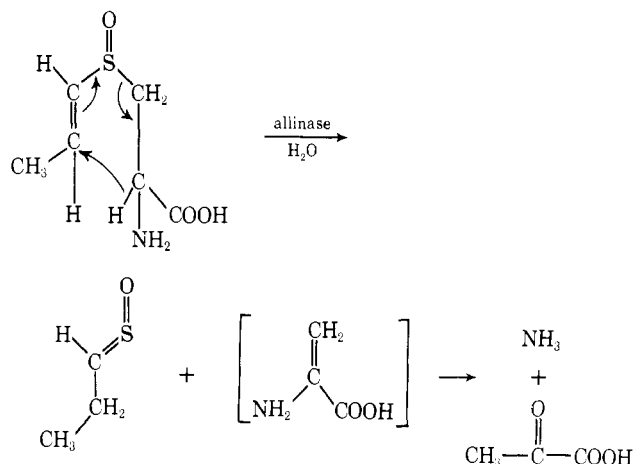


Table II. Infrared Absorptions of S-Oxide (μ) Synthetic S-Oxides

Thioacetone S-Oxide	Thioacetanal S-Oxide	Thiohexanal S-Oxide	Thiopropanal S-Oxide	Natural Thiopropanal S-Oxide (Wilkens, 1961)
3.35–3.50 (M) 7.75 (M)	3.35–3.50 (S) 7.5 (W) 8.2 (W)	3.35–3.50 (S)	3.35–3.50 (S) 7.57 (M) 5.9 (M) 7.3 (W)	3.35–3.50 (S) 5.8 (W)
9.1 (S) ^a	8.75 (S) 8.95 (S) 9.6 (M) 9.8 (M)	8.77 (S) 8.93 (S) 9.63 (M) 10.3 (W)	8.83 (S) 9.00 (S) 9.82 (M) 10.5 (W)	8.75 (S) 9.00 (S) 9.8 (M)

^a S—Strong Intensity; M—Medium Intensity; and W—Weak Intensity.

Table III. Proton Nmr Band Center Locations (δ , TMS) of Various Thioalkanal S-Oxides

Alkyl	—CH ₃	—(CH ₂)—	—CH ₂	H —C=
I CH ₃ CH ₂ —C= (syn.)	1.18 (T)	...	2.76 (M)	8.22 (T)
II CH ₃ CH ₂ —C= (natural)	1.18 (T)	...	2.80 (M)	8.14 (T)
III CH ₃ —CH ₂ —CH ₂ —C=	0.98 (T)	1.55 (M)	2.74 (M)	8.16 (T)
IV CH ₃ —(CH ₂) ₃ —CH ₂ —C=	0.89 (T)	1.39 (M)	2.75 (M)	8.13 (T)
V CH ₃ —C=	2.22 (D)	8.22 (M)
VI	2.34 (S) 2.10 (S)

T—Triplet; S—Singlet; D—Doublet; M—Multiplet.

Proton Nmr Data of the Natural vs. the Synthetic Thiopropanal S-Oxide, Coupling Constants, *J*

	—CH ₃ Triplet	—CH ₂ — Quintet	H —C= Doublet
Natural	7 cps	7 cps	8 cps
Synthetic	8 cps	7 cps	8 cps

In addition to *S*-(1-propenyl)cysteine sulfoxide, Virtanen and his coworkers also synthesized *S*-vinyl- and *S*-(1-butenyl)-cysteine sulfoxide. Addition of an enzyme preparation isolated from onions to these sulfoxides produced, in all cases, volatile lachrymators. From *S*-vinyl-cysteine sulfoxide, Dabritz and Virtanen (1964) obtained a compound of molecular weight 76, to which they assigned the basis of mass spectra $\text{CH}_2=\text{CH}-\text{S}(=\text{O})\text{H}$ (vinylsulfenic acid). A lachrymator identical to that formed in raw onions was obtained by allinase action on *S*-(1-propenyl)cysteine sulfoxide (Spare and Virtanen, 1963). Later, Muller and Virtanen (1966) obtained a lachrymator of mass 104 from *S*-(1-butenyl)cysteine sulfoxide. By analogy, the structure of butenylsulfenic acid was assigned to this lachrymator. These three compounds represent the only aliphatic sulfenic acids whose existence has been claimed, yet none of them had been confirmed by synthesis.

Synthesis of a series of straight chain thioalkanal *S*-oxides showed them to possess lachrymogenic properties. Thioacetone *S*-oxide, the only nonaromatic sulfine whose synthesis had been previously reported (Sheppard and Diekmann, 1964) was, however, found not to be a lachrymator. Apparently, by analogy to the onion lachrymator, the sulfenic acids reported by Virtanen's group (Dabritz and Virtanen, 1966) were in fact the thioalkanal *S*-oxides of ethane, propane, and butane. Analytical data for the synthesized *S*-

oxides are summarized in Tables I, II, and III. All the synthesized lachrymators were found to be stable at room temperature for less than 1 hr.

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