

Comparison of Volatile Components of Dried Squid to Reaction Products Formed from the Mixtures of Hydrogen Sulfide, Ammonia, and Aldehydes

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Reaction products formed from the mixtures of hydrogen sulfide, ammonia, and three aldehydes (ethanal, propanal, and 2-methylpropanal or butanal) were analyzed by GC-MS. Characterization of them revealed 30 5,6-dihydro-2,4,6-trialkyl-4*H*-1,3,5-dithiazines as major products. In their mass spectral data, there were some fragment ions that distinguished among the kinds of branched- and straight-chain alkyl-substituted dithiazines. According to those data and the gas chromatographic retention time data, two volatile components of dried squid were newly identified as 5,6-dihydro-2,4-dimethyl-6-isopropyl-4*H*-1,3,5-dithiazine (4) and 5,6-dihydro-4,6-dimethyl-2-isopropyl-4*H*-1,3,5-dithiazine (5).

Volatile constituents of many foods frequently include hydrogen sulfide, ammonia, and lower fatty aldehydes such as ethanal, propanal, 2-methylpropanal, and butanal (van Straten and Maarse, 1983). The compounds are well-known as degradation products of amino acids and lipids during cooking of proteinous foods (Obata and Tanaka, 1965; Fujimaki et al., 1969; Josephson and Lindsay, 1987) and also as natural precursors of heated flavor components (Wilson, 1975; Takken et al., 1976).

Mixtures of the compounds produce principally 5,6-dihydro-2,4,6-trialkyl-4*H*-1,3,5-dithiazines when H₂S occurs in large amounts (Kawai et al., 1985, 1986), and the formation mechanisms have been proposed by Shu et al. (1985) and by Kawai et al. (1985). Dithiazines are known to be powerful, characteristic flavor substances and contribute to heated food flavors (Brinkman et al., 1972; Kubota et al., 1980; Shu et al., 1985; Hwang et al., 1986). Sources and odor profiles of dithiazines identified in natural products and in model reactions were reviewed by Kawai et al. (1986).

Dithiazine 1 (R alkyl; R¹ = R² = R³ = methyl) has been found in various foods since it was isolated from the volatiles of heated beef broth (Brinkman et al., 1972). However, the three analogues, 2, 3, and 11, with ethyl substituents have been detected merely in antarctic krill and small shrimp (Kubota et al., 1980, 1986; Choi et al., 1983) in spite of the expectation based on the findings of more dithiazines from the model reactions (Ledl and Severin, 1974; Ledl, 1975; Shu et al., 1985; Hwang et al., 1986). This is probably due to the lack of comparable mass spectral data (MS) together with gas chromatographic retention time (R_t) data of dithiazines substituted with branched alkyl groups or with straight alkyl ones. On our analysis of the volatile components of edible dried squid (Kawai et al., 1987), several compounds regarded as dithiazines substituted with more than an ethyl group have also remained unidentified for the same reason. Quite recently, dithiazine 20 and 5,6-dihydro-4-butyl-2,6-dimethyl-4*H*-1,3,5-dithiazine have been isolated from small shrimp by preparative GC separation procedures (Kubota et al., 1988).

This paper presents the characterization of reaction products formed from the mixtures of H₂S, ammonia, and three aldehydes (ethanal, propanal, and 2-methylpropanal or butanal). We also describe some differences between

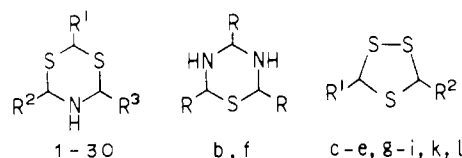
MS fragmentation patterns of isopropyl- and propyl-substituted dithiazines. Further, this paper reports the identification of two novel dithiazines found in volatile components of dried squid.

EXPERIMENTAL SECTION

Preparation of Reaction Mixtures A and B. I. Mixture A. Three aldehydes (ethanal, propanal, 2-methylpropanal) were mixed in a 1:1:1 molar ratio below 5 °C. The mixture was added dropwise into 3.0 mol of the concentrated (25–28%) aqueous ammonium hydroxide (400 mL) under 0 °C. H₂S gas was bubbled moderately through the mixture of the aldehydes and ammonia with stirring, keeping the temperature of the mixture in the range –5 to –15 °C until the reaction temperature ceases to rise. After approximately 2 times as much as the theoretical weight of H₂S (210 g, 6.2 mol) was consumed, the reaction mixture was allowed to stand overnight at room temperature. Then, the organic layer was separated, washed with water, dried over sodium sulfate, and distilled twice in the temperature range 30–136 °C (4 mm). The distillate (160 g) was stored at –20 °C until it was used.

II. Mixture B. This mixture was prepared in the same manner as that of mixture A except butanal was used instead of 2-methylpropanal.

Preparation of Substances. Dithiazines 1, 11, 18, and 30 and 3,4,5,6-tetrahydro-2,4,6-trialkyl-2*H*-1,3,5-thiadiazines **b** and **f** were prepared by the procedures described by Kawai et al. (1985, 1986), respectively. 3,5-Dialkyl-1,2,4-trithiolanes **e** and **i** were



synthesized by the methods of Asinger et al. (1959). 3,6-Dimethyl-1,2,4,5-tetrathiane **j** was isolated from distillation of trithiolane **i** and recrystallized from dichloromethane: colorless crystals; mp 87.4 °C.

Dithiazine 18: colorless crystals; mp 49.7 °C; bp 114–115 °C (1 mm); NMR δ 0.36–0.79 (br, 1 H), 1.02 (d, 12 H, *J* = 6 Hz), 1.09 (d, 6 H, *J* = 6 Hz), 1.48–2.45 (m, 3 H), 3.88 (dd, 2 H, *J* = 5 Hz), 4.27 (d, 1 H, *J* = 5 Hz); IR (KBr, cm⁻¹) 2960, 2930, 2870, 1465, 1385, 1365, 1180, 1155, 1135, 785, 770; odor, pleasant roasted leek odor having meaty and slightly floral note. Anal. Calcd for C₁₁H₂₀NS₂: N, 5.66; S, 25.91. Found: N, 5.57; S, 26.6.

Dithiazine 30: pale yellow liquid; bp 133–135 °C (3 mm); NMR δ 0.72–0.87 (br, 1 H), 0.94 (t, 6 H, *J* = 7 Hz), 1.07 (t, 3 H, *J* = 7 Hz), 1.17–2.12 (m, 12 H), 2.16 (t, 2 H, *J* = 7 Hz), 4.22 (t to d, 1 H, *J* = 7 Hz); IR (liquid, cm⁻¹) 2985, 2950, 2880, 1460, 1380, 1175, 1135, 780, 775; odor, unpleasant roasted garlic odor with green vegetable note. Anal. Found: N, 5.16; S, 23.8.

Preparation of Volatile Concentrate of Dried Squid. Dried squids are made simply by drying them after taking out their internal organs. The dried squids (*Loligo edulis edulis*), highly prized for their intense and characteristic flavors produced upon heating, were purchased from a local supermarket and employed for preparation. Forty-seven grams of dried squid cut into 5-mm

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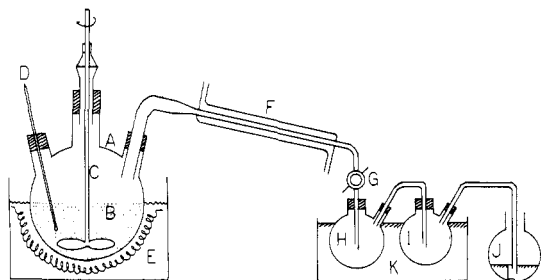


Figure 1. Heating apparatus: A, flask; B, glass beads; C, propeller; D, thermometer; E, mantle heater; F, condenser; G, cock; H-J, flasks for trap; K, salt and ice; L, water.

squares and the equal weight of water were put into a 1-L flask containing 380 g of small glass beads (2.3-mm i.d.) cleaned with solvents and water. The contents were stirred with a propeller connected with a motor and were heated with an electric mantle heater. The apparatus is shown in Figure 1. A fraction distilled in the temperature range 120–180 °C (temperature of the beads) was extracted with CH_2Cl_2 . The extract was concentrated under reduced pressure and immediately submitted to GC and GC-MS analyses. The concentrate had a flavor of roasted dried squid.

By the use of numerous glass beads, a thermoconduction surface touching the sample increased more than 17 times as much area as the flask. The enlarged surface enhanced the generation of heated flavor volatiles from the sample. The odor quality of the

heated dried squid was stabilized in this way.

Apparatus for Analysis. The preparations were analyzed by gas chromatography (GC) on a Hewlett-Packard 5840A chromatograph equipped with a 0.25 mm (i.d.) \times 30 m capillary column (0.25- μm film, DB-1 fused silica). The column temperature was held at 50 °C for 5 min and programmed to increase 3 °C/min from 50 to 220 °C. The compounds containing sulfur and nitrogen were also recorded on a gas chromatograph: Hitachi 163 equipped with a flame photometric detector (FPD) and a flame thermionic detector (FTD) together with a flame ionization detector (FID). Gas chromatography-mass spectrometry data (GC-MS) were recorded on a Hitachi M-80A mass spectrometer combined with a 0.25 mm (i.d.) \times 50 m capillary column (0.25- μm film, DB-1 fused silica). The column temperature was held at 75 °C for 5 min and programmed to increase 3 °C/min to 240 °C. The ionization energy was 20 eV. Many peaks were identified by comparison of their MS spectral and R_t data with those of the authentic compounds synthesized as described above.

Infrared (IR) and nuclear magnetic resonance (NMR) spectra were recorded on a Jasco IR-810 instrument and on a Hitachi Model R-24B (60-MHz) instrument, respectively. In the latter, the solvent was CDCl_3 and tetramethylsilane was used as an internal standard. They were used for the determination of the synthetic compounds.

RESULTS AND DISCUSSION

Characterization of Reaction Products. Figures 2 and 3 represent the GC chromatograms of the reaction

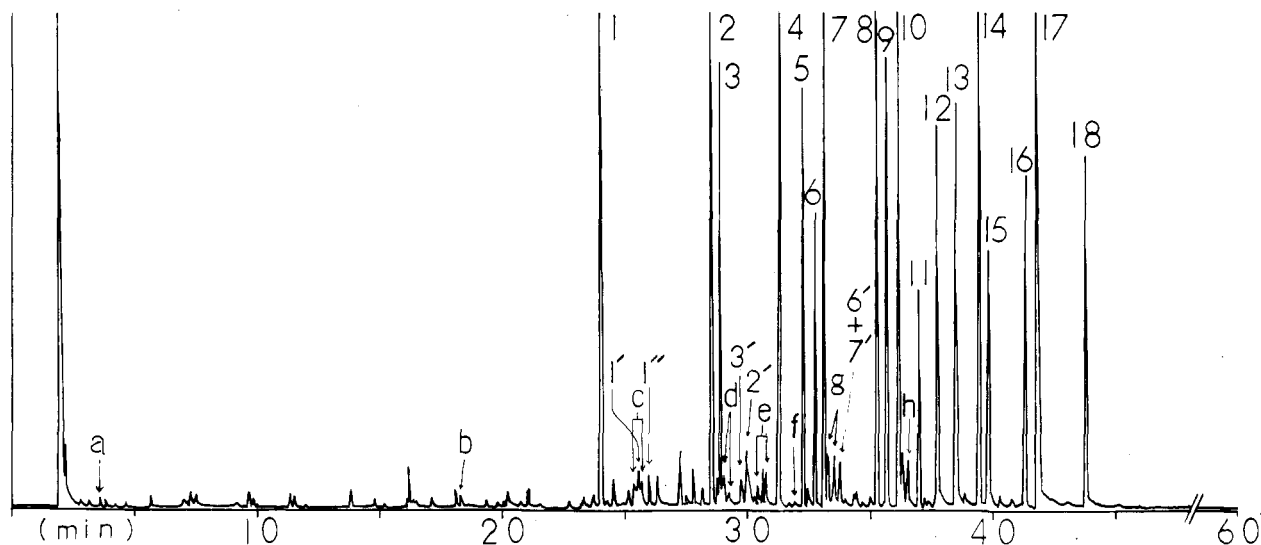


Figure 2. Gas chromatogram of the reaction products formed from the mixture of H_2S , ammonia, ethanal, propanal, and 2-methylpropanal.

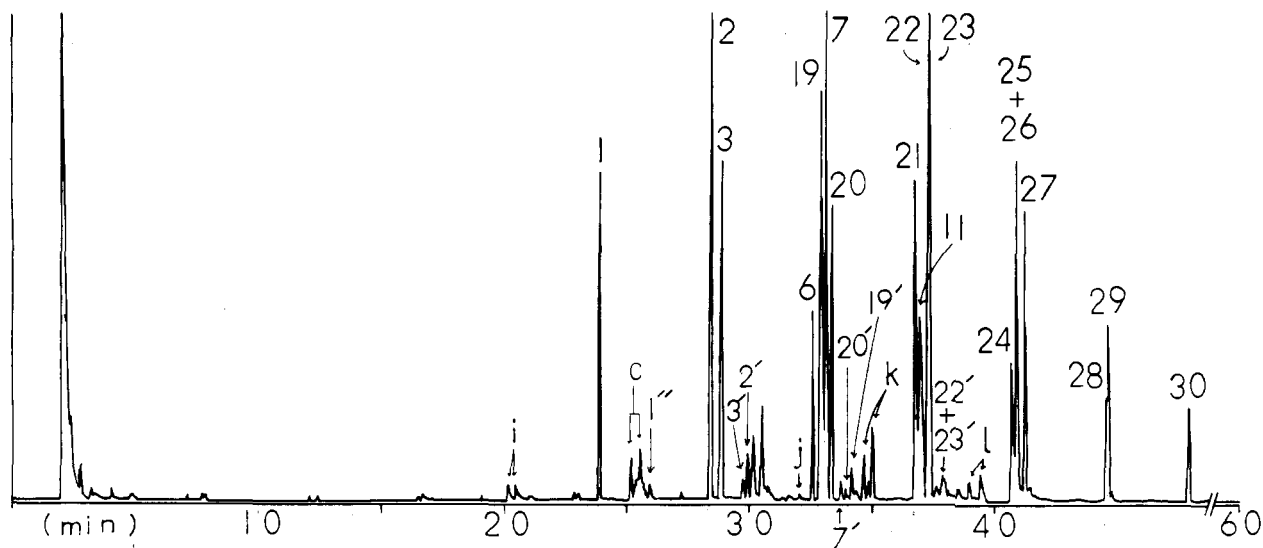


Figure 3. Gas chromatogram of the reaction products formed from the mixture of H_2S , ammonia, ethanal, propanal, and butanal.

Table I. MS Fragments and Identification of Reaction Products Formed from the Mixture of Hydrogen Sulfide, Ammonia, Ethanal, Propanal, and 2-Methylpropanal^a

compd (peak no.)	R ¹	R ²	R ³	MW	R _t	GC peak area, %	mass spectral data
5,6-Dihydro-2,4,6-trialkyl-4H-1,3,5-dithiazines 1-18							
1	Me	Me	Me ^{c,d}	163	24.0	6.27	163 (12), 103 (7), 71 (20), 70 (14), 60 (15), 56 (10), 44 (100), 42 (5)
1'			an isomer of 1		25.6	0.19	163 (10), 103 (6), 71 (17), 70 (11), 60 (14), 56 (10), 44 (100), 42 (4)
1''			an isomer of 1		26.0	0.26	163 (17), 103 (7), 71 (18), 70 (8), 60 (16), 56 (7), 44 (100), 42 (3)
2	Me	Me	Et ^{c,d}	177	28.5	6.42	177 (18), 117 (10), 85 (22), 84 (23), 74 (8), 70 (28), 60 (12), 58 (80), 44 (100), 41 (12)
2'			an isomer of 2		30.0	0.73	177 (16), 117 (9), 85 (18), 84 (26), 74 (9), 70 (31), 60 (15), 58 (78), 44 (100), 41 (12)
3	Et	Me	Me ^{c,d}	177	28.9	3.90	177 (10), 103 (9), 74 (8), 71 (25), 70 (16), 60 (6), 56 (9), 44 (100), 41 (6)
3'			an isomer of 3		29.8	0.40	177 (8), 103 (9), 74 (7), 71 (23), 70 (16), 60 (5), 56 (8), 44 (100), 41 (6)
4	Me	Me	<i>i</i> -Pr ^b	191	31.3	5.09	191 (16), 131 (10), 99 (15), 98 (21), 84 (26), 72 (77), 60 (13), 55 (20), 44 (100)
5	<i>i</i> -Pr	Me	Me ^b	191	32.3	3.85	191 (10), 103 (13), 88 (4), 71 (34), 70 (25), 60 (4), 56 (8), 55 (6), 44 (100)
6	Me	Et	Et ^c	191	32.7	2.43	191 (10), 131 (6), 99 (10), 98 (17), 84 (12), 74 (10), 70 (5), 58 (100), 56 (4), 41 (10)
7	Et	Me	Et ^c	191	33.1	4.65	191 (15), 117 (12), 85 (31), 84 (30), 74 (16), 70 (25), 58 (80), 56 (4), 44 (100), 41 (18)
6' + 7'			isomers of 6 and 7 (mixed)		33.8	1.27	191 (10), 131 (5), 99 (8), 98 (16), 85 (8), 84 (17), 74 (18), 70 (16), 58 (100), 44 (30)
8	Me	Et	<i>i</i> -Pr ^b	205	35.3	4.50	205 (16), 145 (10), 113 (14), 112 (32), 98 (25), 72 (88), 58 (100), 55 (20), 41 (10)
9	Et	Me	<i>i</i> -Pr ^b	205	35.7	3.98	205 (14), 131 (12), 99 (18), 98 (27), 84 (24), 74 (7), 72 (76), 55 (15), 44 (100), 41 (11)
10	<i>i</i> -Pr	Me	Et ^b	205	36.2	4.99	205 (14), 117 (16), 85 (50), 84 (50), 70 (22), 58 (80), 55 (10), 44 (100), 41 (14)
11	Et	Et	Et ^c	205	37.0	2.07	205 (9), 131 (6), 99 (12), 98 (20), 84 (12), 74 (12), 70 (6), 58 (100), 41 (17)
12	Me	<i>i</i> -Pr	<i>i</i> -Pr ^b	219	37.7	2.87	219 (7), 159 (6), 127 (5), 126 (24), 112 (12), 84 (4), 72 (100), 60 (7), 55 (19)
13	<i>i</i> -Pr	Me	<i>i</i> -Pr ^b	219	38.5	4.26	219 (13), 131 (15), 99 (26), 98 (34), 88 (12), 84 (24), 72 (85), 60 (6), 55 (24), 44 (100)
			BHT		39.3		internal standard
14	Et	Et	<i>i</i> -Pr ^b	219	39.5	4.05	219 (16), 145 (14), 113 (18), 112 (44), 98 (23), 74 (15), 72 (94), 58 (100), 55 (16), 41 (23)
15	<i>i</i> -Pr	Et	Et ^b	219	39.8	3.31	219 (10), 131 (9), 99 (16), 98 (28), 84 (11), 74 (5), 70 (6), 58 (100), 55 (6), 41 (11)
16	Et	<i>i</i> -Pr	<i>i</i> -Pr ^b	233	41.4	2.89	233 (9), 159 (7), 127 (10), 126 (30), 112 (11), 88 (5), 72 (100), 55 (19), 41 (12)
17	<i>i</i> -Pr	Et	<i>i</i> -Pr ^b	233	41.9	6.13	233 (15), 145 (16), 113 (23), 112 (54), 98 (25), 88 (14), 72 (98), 58 (100), 55 (35), 41 (16)
18	<i>i</i> -Pr	<i>i</i> -Pr	<i>i</i> -Pr ^b	247	43.8	4.76	247 (7), 159 (9), 127 (12), 126 (35), 112 (13), 88 (9), 72 (100), 55 (25)
3,5-Dialkyl-1,2,4-trithiolanes c-e, g, h							
c	Me	Et ^c		166	25.3	0.37	166 (66), 106 (23), 102 (51), 101 (27), 92 (35), 74 (25), 73 (29), 69 (63), 64 (40), 60 (77), 59 (50), 45 (42), 41 (100)
		(two isomers)			25.6	0.45	
d	Me	<i>i</i> -Pr		180	29.0	0.28	180 (34), 137 (9), 115 (61), 92 (19), 87 (12), 74 (15), 64 (13), 60 (91), 59 (63), 55 (100), 45 (17), 41 (39)
		(two isomers)			29.3	0.27	
e	Et	Et ^f		180	30.2	0.24	
		(two isomers)			30.7	0.38	
g	Et	<i>i</i> -Pr ^b		194	33.4	0.47	194 (23), 151 (5), 129 (42), 120 (7), 106 (7), 87 (18), 74 (74), 73 (30), 55 (100), 45 (18), 41 (41)
		(two isomers)			33.6	0.56	
h	<i>i</i> -Pr	<i>i</i> -Pr ^f		208	36.5	0.57	
N-Ethylidene-1,1'-diaminoethane ^g							
a				86	3.6	0.10	86 (3), 70 (18), 59 (100), 54 (3), 43 (3), 42 (10), 31 (16), 29 (16)
3,4,5,6-Tetrahydro-2,4,6-trimethyl-2H-1,3,5-thiadiazine ^h							
b				146	18.5	0.16	146 (2), 103 (16), 70 (44), 60 (10), 44 (100), 42 (16), 34 (14), 28 (31)
3,4,5,6-Tetrahydro-2,4,6-triethyl-2H-1,3,5-thiadiazine ^h							
f				188	32.1	tr	131 (21), 99 (18), 98 (62), 74 (32), 70 (17), 58 (100), 56 (44), 43 (20), 41 (41), 34 (28)
					83.12		
					total		

^a Abbreviations: Me, methyl; Et, ethyl; *i*-Pr, isopropyl. ^b Not previously reported. ^c Ledl, 1975. ^d Hwang et al., 1986. ^e Kubota et al., 1980. ^f Tjan et al., 1972. ^g Kawai et al., 1985.

products formed from mixtures A and B, respectively. Eighteen dithiazines, 1-18, which were structurally predicted, appeared as sharp peaks as shown in Figure 2. Also the same number of dithiazines occurred in Figure 3, where two pairs of dithiazines (22 and 23, 25 and 26) overlapped on two peaks, but each dithiazine was differentiated from the other on the basis of its mass spectral data obtained by the continuous-scanning MS procedures (1 spectrum/2 s). Tables I and II give the identified compounds along with the corresponding peak numbers (dithiazines) and letters (other compounds) as in those figures, including their MS fragments and *R_t* data. The identical assignment of every dithiazine and trithiolane having different alkyl substituents in the structural formula was based on MS fragment analysis of authentic compounds together with

MS references (Ledl and Severin, 1974; Ledl, 1975; Kubota et al., 1980; Hwang et al., 1986).

Most reaction products in Tables I and II are expected to be found in foods. The aldehydes tested here not only are provided from Strecker reaction and retro-aldol degradation during cooking or storage (Fujimaki et al., 1969; Josephson and Lindsay, 1987) but also are found in volatile components of many seafoods (Ikeda, 1980; Kasahara and Nishibori, 1985). Ammonia and H₂S are general constituents of their flavors (Ikeda, 1980). Further, the above-mentioned dithiazines identified in foods have been considered to be important contributors to heated food flavors, because of their spontaneous formation from H₂S, ammonia, and aldehydes in addition to their flavor characteristics (Brinkman et al., 1972; Kubota et al., 1980).

Table II. MS Fragments and Identification of Reaction Products Formed from the Mixture of Hydrogen Sulfide, Ammonia, Ethanal, Propanal, and Butanal^a

compd (peak no.)	R ¹	R ²	R ³	MW	R _t	GC peak area, %	mass spectral data
5,6-Dihydro-2,4,6-trialkyl-4H-1,3,5-dithiazines 1-3, 6, 7, 11, 19-30							
1	Me	Me	Me	163	24.0	4.55	
1''	an isomer of 1				26.0	0.30	
2	Me	Me	Et	177	28.5	7.69	
2'	an isomer of 2				30.0	0.55	
3	Et	Me	Me	177	28.9	4.87	
3'	an isomer of 3				29.8	0.40	
6	Me	Et	Et	191	32.7	3.28	
19	Me	Me	Pr ^b	191	33.0	6.65	191 (20), 131 (8), 99 (15), 98 (31), 84 (14), 72 (56), 70 (35), 60 (14), 55 (10), 44 (100)
19'	an isomer of 19				34.2	0.76	191 (23), 131 (12), 99 (23), 98 (43), 84 (7), 72 (86), 70 (42), 60 (22), 55 (15), 44 (100)
7	Et	Me	Et	191	33.1	5.99	
7'	an isomer of 7				33.8	0.46	191 (16), 117 (22), 85 (31), 84 (39), 74 (22), 70 (27), 58 (89), 44 (100), 41 (19)
20	Pr	Me	Me ^c	191	33.4	3.77	191 (9), 103 (9), 88 (3), 71 (29), 70 (19), 60 (6), 56 (8), 55 (6), 44 (100)
20'	an isomer of 20				34.0	0.32	191 (12), 103 (13), 88 (5), 71 (40), 70 (36), 60 (7), 56 (11), 55 (10), 44 (100)
21	Me	Et	Pr ^b	205	36.9	5.00	205 (22), 145 (6), 113 (14), 112 (46), 98 (13), 84 (26), 72 (70), 58 (100), 55 (8), 41 (17)
11	Et	Et	Et	205	37.0	2.18	
22	Et	Me	Pr ^b	205	37.3	9.38	205 (27), 131 (8), 99 (20), 98 (47), 84 (19), 74 (13), 72 (61), 70 (41), 55 (16), 44 (100), 41 (31)
23	Pr	Me	Et ^b	205			205 (6), 117 (6), 85 (22), 84 (22), 70 (23), 58 (66), 55 (12), 44 (100), 41 (13)
22' + 23'	isomers of 22 and 23 (mixed)				37.8	1.04	205 (11), 131 (3), 117 (9), 99 (11), 98 (16), 85 (21), 84 (24), 72 (28), 70 (22), 58 (51), 55 (12), 44 (100)
24	Me	Pr	Pr ^b	219	40.7	2.39	219 (19), 159 (4), 127 (16), 126 (76), 112 (9), 98 (27), 84 (14), 72 (100), 60 (10), 55 (22), 43 (11)
25	Et	Et	Pr ^b	219	40.9	5.55	219 (22), 145 (6), 113 (16), 112 (49), 98 (23), 84 (30), 74 (13), 72 (88), 58 (100), 55 (20), 41 (22)
26	Pr	Et	Et ^b	219			219 (16), 131 (4), 99 (9), 98 (22), 84 (25), 74 (11), 70 (8), 58 (100), 55 (10), 41 (22)
27	Pr	Me	Pr ^b	219	41.2	5.36	219 (22), 131 (14), 99 (38), 98 (74), 88 (16), 84 (17), 72 (100), 70 (38), 60 (14), 55 (25), 44 (82)
28	Et	Pr	Pr ^b	233	44.4	7.15	233 (17), 159 (4), 127 (22), 126 (80), 112 (26), 98 (28), 88 (11), 84 (21), 72 (100), 55 (27), 41 (22)
29	Pr	Et	Pr ^b	233	44.5		233 (18), 145 (9), 113 (26), 112 (77), 98 (13), 88 (12), 84 (37), 72 (100), 58 (61), 55 (25), 41 (15)
30	Pr	Pr	Pr ^b	247	47.9	2.22	247 (9), 127 (24), 126 (100), 98 (26), 88 (16), 72 (69), 55 (24)
3,5-Dialkyl-1,2,4-trithiolanes c, i, k, l							
i	Me	Me		152	20.3	0.32	
	(two isomers)				20.6	0.33	
c	Me	Et		166	25.3	0.73	
	(two isomers)				25.6	1.10	
k	Et	Pr		194	34.8	0.82	194 (26), 130 (16), 129 (16), 106 (16), 101 (14), 87 (25), 74 (25), 73 (17), 55 (100), 45 (23), 41 (46)
	(two isomers)				35.1	1.44	
l	Pr	Pr ^d		208	39.0	0.55	208 (17), 144 (12), 143 (12), 120 (7), 115 (7), 101 (6), 88 (15), 87 (20), 73 (12), 55 (100), 45 (12)
	(two isomers)				39.4	0.69	
3,6-Dimethyl-1,2,4,5-tetrathiane ^e							
j				184	32.0	0.10	
						85.94	
						total	

^a Abbreviations: Me, methyl; Et, ethyl; Pr, propyl. ^b Not previously reported. ^c Kubota et al., 1988. ^d Kruse and Anger, 1981. ^e Nixon et al., 1979.

Under the present reaction conditions where large amounts of H₂S were used to complete the reactions, the GC peak areas in Tables I and II showed dithiazines as the major products (79-80%) and trithiolanes as the minor products (3-6%) in both reaction mixtures. Besides, they represented that each dithiazine was produced in the range 2-8%. These indicated that almost all alkyl groups of the aldehydes were converted impartially into the substituents of all dithiazines. This wide distribution of alkyl groups suggests that C₃H₇-substituted dithiazines also can occur in volatile components of foods as well as the dithiazines already found in them. However, reported as model reaction products, many other dithiazines with the following substituents remained undetected: H (Ledl and Severin, 1974), ethyl (Ledl, 1975), 2-methylpropyl (Shu et al., 1985; Hwang et al., 1986), butyl, and pentyl (Hwang et al., 1986). This is likely still caused by little accumulation of MS

spectral data and, further, by lack of sufficient interaction of released H₂S, ammonia, and aldehydes from heated foods during isolation procedures of natural volatiles because the formation of dithiazines requires some amount of H₂S, as examined here and also as described by Kawai et al. (1985). Incidentally, the apparatus shown in Figure 1, for example, seemed adequate to generate heated flavor components including dithiazines.

Minor isomers of dithiazines, which clearly appeared on GC, are listed in Tables I and II. Among them, the minor isomers of dithiazine 1 occupied less than 10% area of a corresponding major one. The dithiazine has three geometrical isomers as reported by Butenko et al. (1972). In order to characterize the formation of the minor isomers, thiadiazine b, a precursor of dithiazine 1, was loaded on the gas chromatograph under the same analytical conditions; three isomers occurred on the gas chromatograph

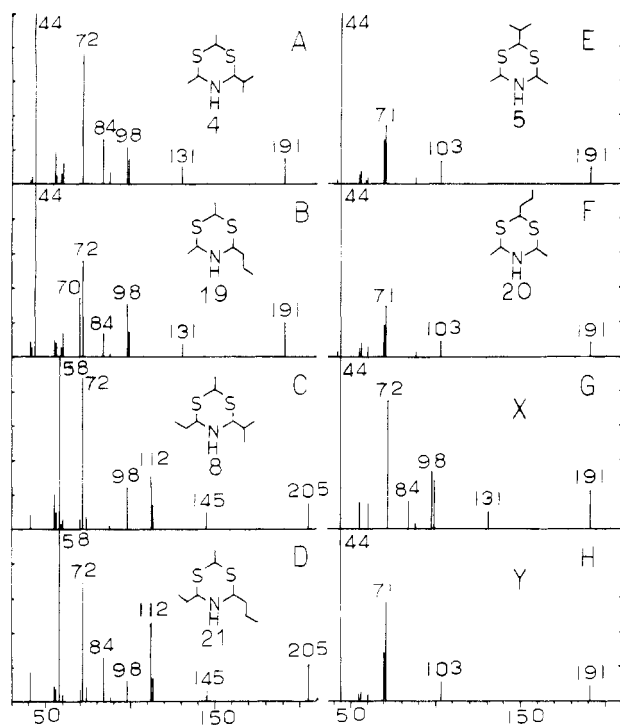


Figure 4. Mass spectra of C_3H_7 -substituted dithiazines (parts A-F) and two dried-squid components (X, Y): A-D, C_3H_7 group at the R^3 position; E and F, C_3H_7 group at the R^1 position.

along with a GC survivor of the thiadiazine, showing a similar ratio of their GC peak areas as given in Table I. As discussed in our previous paper (Kawai et al., 1985), an acyclic unsaturated imine, which must be produced by elimination of ammonia from thiadiazine **b**, has been proposed as an intermediate. The imine, $CH_3CH=NCH(CH_3)SCH=CH_2$, can theoretically produce three isomers when it reacts with H_2S . Thus, the minor isomers were derived from the thiadiazine and were not degradation products of the major isomer. Those isomers interest us because of the differences among their flavor characteristics.

Dithiazines **1** and **11** were mutually common products in mixtures A and B. Their R_t data in Tables I and II were useful internal standards in comparing the two kinds of C_3H_7 -substituted dithiazines; a R_t value for an isopropyl-substituted dithiazine was smaller than that for the corresponding propyl-substituted one. A R_t datum for

BHT (2,6-di-*tert*-butyl-4-methylphenol) was also added in Table I for the same reason, because the agent was often detected in volatiles of seafoods wrapped with plastic sheets or packed in such containers (e.g., dried squid, short-necked clam, salted cod roe). It is probably used in plastic manufacture for an antioxidant. Trithiolanes as well as dithiazines have strong and characteristic flavors. Their occurrence in foods was reviewed by Ohloff and Flament (1978), and formation mechanisms from H_2S and aldehydes were described by Boelens et al. (1974), Nixon et al. (1979), and Shu et al. (1985).

Mass Spectra of C_3H_7 -Substituted Dithiazines. Isopropyl-substituted dithiazines and propyl-substituted ones were respectively divided into four groups. The C_3H_7 substituent position order is as follows: $R^1 = C_3H_7$, R^2 and $R^3 =$ methyl or ethyl; $R^2 =$ methyl, $R^3 = C_3H_7$; $R^2 =$ ethyl, $R^3 = C_3H_7$; $R^2 = R^3 = C_3H_7$. They were once more arranged in molecular weight order in their respective groups. Moreover, structurally corresponding dithiazines were paired for comparison of their mass spectral data, as follows, where the first number indicates the isopropyl-substituted dithiazine: 5-20, 10-23, 15-26; 4-19, 9-22, 13-27; 8-21, 14-25, 17-29; 12-24, 16-28, 18-30.

In the first group, since the mass spectral data of the pair were almost the same, it was impossible to distinguish one from the other except by using their R_t data. E.g., the mass spectra of the pair 5 and 20 shown in Figure 4E,F were exactly the same; however, their R_t values were rather different (Tables I and II). In the second group, the mass spectrum of the latter in the pair represented the distinctive fragment ion, 70 (C_4H_8N), but that of the former did not. E.g., the mass spectra of the pair 4 and 19 are given in Figure 4A,B. In the third group, only the MS datum of the latter gave the increased ion by addition of a CH_2 group, 84 ($C_5H_{10}N$): e.g., mass spectra of the pair 8 and 21 in Figure 4C,D. Finally, in the fourth group, the ion 98 ($C_6H_{12}N$) occurred only in the mass spectrum of the latter (Tables I and II).

Those fragment ions (70, 84, 98) were produced by decomposition of the molecular ions (M^+) of dithiazines having propyl substituents at the R^2 and/or R^3 position. The m/e 70 ion, for example, occurred as follows: Cleavage on M^+ of dithiazine 19 (Figure 4B) gave the m/e 98 ion, indicating $CH_2=CHN^+H=CHCH_2CH_2CH_3$ with loss of the upper part of the structure $R^1CH(S)S$, and fragmentation of the ion yielded the two ions 84 ($CH_2=N^+HCH=CHCH_2CH_3$) and 70 through elimination of two

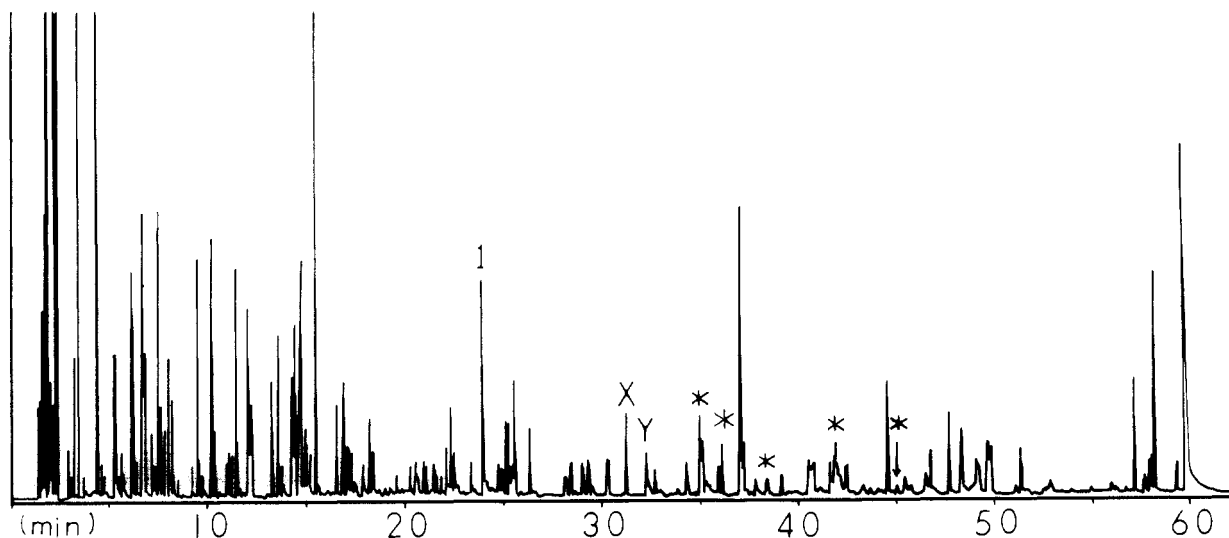


Figure 5. Gas chromatogram of the flavor concentrate prepared from heated dried squid. Peaks 1, X, and Y: dithiazines identified. Peaks marked with asterisks: dithiazines remained unidentified.

CH₂ groups. On the other hand, decomposition of the corresponding isopropyl-substituted dithiazines showed different fragmentation patterns: E.g., M⁺ of dithiazine 4 (Figure 4A) yielded a similar ion, 98 [CH₂=CHN⁺H=CHCH(CH₃)₂], with loss of the upper part, but fragmentation of this ion gave only ion 84 [CH₂=N⁺HCH=C(CH₃)₂] with removal of the CH₂ group, and it ceased so that the *m/e* 70 ion was absent in the mass spectrum.

As described above, those characteristic ions and the exact *R_t* data can contribute to the characterization of dithiazines possessing higher alkyl substituents. This finding was also supported by several mass spectral data of C₄H₉-substituted dithiazines reported by Hwang et al. (1986) and Kubota et al. (1988).

Identification of Two Components of Dried Squid as Dithiazines. The GC chromatogram of the flavor concentrate of dried squid is shown in Figure 5. It represents several peaks regarded to be dithiazines. One of them was identified as dithiazine 1, two (X, Y) were thought to be C₃H₇-substituted dithiazines, and the others remained contaminated. In comparison of the mass spectral and *R_t* data of the two (X, Figure 4G, *R_t* = 31.3; Y, Figure 4H, *R_t* = 32.3) with those of dithiazines 4 and 5 (Figure 4A,E; *R_t*, Table I), the two components were identified as 5,6-dihydro-2,4-dimethyl-6-isopropyl-4*H*-1,3,5-dithiazine (4) and 5,6-dihydro-4,6-dimethyl-2-isopropyl-4*H*-1,3,5-dithiazine (5), respectively.

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Registry No. 1, 86241-90-9; 2, 54717-13-4; 3, 54717-14-5; 4, 104691-41-0; 5, 104691-40-9; 6, 54717-15-6; 7, 54717-16-7; 8, 121098-09-7; 9, 121098-10-0; 10, 121098-11-1; 11, 54717-17-8; 12, 121098-12-2; 13, 121098-13-3; 14, 121098-14-4; 15, 121098-15-5; 16, 121098-16-6; 17, 121098-17-7; 18, 115152-65-3; 19, 104691-39-6; 20, 104691-38-5; 21, 121098-18-8; 22, 121098-19-9; 23, 121098-20-2; 24, 121098-21-3; 25, 121098-22-4; 26, 121098-23-5; 27, 121098-24-6; 28, 121098-25-7; 29, 121098-26-8; 30, 121098-27-9; a, 54140-09-9; b, 53897-63-5; c, 116505-59-0; d, 121098-28-0; e, 54644-28-9; f, 95465-59-1; g, 121098-29-1; h, 54934-99-5; i, 23654-92-4; j, 67411-27-2; k, 121098-30-4; l, 92658-66-7; hydrogen sulfide, 7783-06-4; ammonia, 7664-41-7; ethanal, 75-07-0; propanal, 123-38-6; 2-methylpropanal, 78-84-2; butanal, 123-72-8.

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