Flavor Components of Dried Squid

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The volatile components were produced by heating dried squid with glass beads and water. Two flavor concentrates (A and B), obtained by distillation at two temperature ranges, 125–190 and 215–230 °C, were analyzed by GC–MS. In addition to several headspace components, a total of 97 components were identified. Concentrate A gave a moderately roasted odor of dried squid and was characterized by the following many S,N heterocycles: nine 5,6-dihydro-2,4,6-trialkyl-4*H*-1,3,5-dithiazines (DT), four pyrrolidino[1,2-*e*]-2,4,6-dialkyl-4*H*-1,3,5-dithiazines (PD), and 3,4,5,6-tetrahydro-2,4,6-trimethyl-2*H*-1,3,5-thiadiazine (TD). Another concentrate having the strongly roasted odor contained fewer S,N heterocycles and more irritant compounds than A. The thermal environment where ammonia occurs prior to H_2S in the presence of aldehydes probably allows those heterocycles to be formed.

Dried squid, made simply by drying after removal of the internal organs, has been highly prized in the Far East for many centuries for its intense and characteristic flavor produced upon roasting. It extensively emits a perceptible odor when roasted, although it has only an ammonia-like and fishy odor before heating. Moreover, the roasted odor of dried squid is not only quite different from that of an undried one but also much stronger. Drying effects on seafood volatiles remain obscure other than that aldehydes from dried fish increased during drying (Yoshinaka, 1981). Some reports on gas chromatographic (GC) analysis of nondehydrated squid volatiles have already been published for canned (Yakush et al., 1987) and boiled squid (Ooyama, 1975; Lee et al., 1989). However, no information on dried squid volatiles has been available except for our previous paper concerning identification of some DT as the volatile components (Kawai and Ishida, 1989).

This paper reports overall volatile components arising from dried squid during roasting in the two temperature ranges 125–190 and 215–230 °C. After comparison between volatile constitution, we describe the contribution of identified heterocycles containing sulfur and nitrogen atoms in the same ring (S,N heterocycles) to a moderately roasted odor of dried squid. Further, we study the thermal environment that allows many S,N heterocycles to be formed in volatiles of dried squid and then discuss the mechanisms of their formation.

EXPERIMENTAL PROCEDURES

Preparation of Flavor Concentrates. No methods or techniques for generation and isolation of heated food volatiles have been established, probably because odors of heated foods are changeable upon heating. When some food is heated on a pan, for example, it gives off a boiled odor first and gradually produces a roasted odor. Stabilizing the odor quality of heated food was experimentally solved by a method using numerous glass beads. The method continuously produces the changeable odors from one sample as temperature elevates. The distillates fractionated are submitted to GC–MS analysis, measurement of pH, organoleptic evaluation, and other analyses of components. Such combination is considered to contribute more to the dynamic thermal study of heated food volatiles than only GC–MS analysis.

Dried squids (*Loligo edulis edulis*) made in Thailand were purchased from a wholesaler (Osaka, Japan). The hard muscle cut into 3-mm squares (120 g) and an equal weight of water were put into a 2-L round-bottom four-necked flask containing 876 g of dried glass beads (2.3-mm i.d.). The flask was equipped with a sealed bearing joint in a central neck to permit a propeller connected to a motor to stir moderately. In other necks, a condenser for distillation and a thermometer were inserted. The last neck was usually closed. The flask was heated with an electric mantle heater. The numerous glass beads enlarged a thermoconduction surface touching the sample more than 25 times as much area as the flask.

The time used for heating the dried squid–water–glass beads system from 25 to 230 °C was approximately 2 h. The apparatus was illustrated in our previous paper (Kawai and Ishida, 1989). The distillates, fractionated by the two temperature ranges according to temperatures of the beads, were extracted with ether and CH_2Cl_2 until the odors almost disappeared. The extracts were evaporated under reduced pressure until approximately 180 and 70 mg remained. The flavor concentrates were stored at −20 °C and then submitted for GC and GC–MS analyses.

Preparation of Headspace Sample. The headspace components, which were generated from the system held for 40 min at the temperature range 170–190 °C, were absorbed to Tenax GC (0.3 g, 60–80 mesh, Enka Research Institute, The Netherlands) from the residual neck of the flask under lightly reduced pressure, after passing through a small tube containing $CaCl_2$ (a drier). The temperature range was chosen because of the strong emission of the roasted odor. After prepurge by a nitrogen gas flow for 3 min without heating to remove moisture in the Tenax, the desorbed components purged on heating were directly injected to GC and GC–MS apparatus.

Identification of Components. Many compounds used as standards for identification were purchased commercially (Aldrich Chemical Co., Milwaukee; Tokyo Kasei Kogyo, Co., Tokyo; Wako Pure Chemical Co., Tokyo). The identical assignment of DT and 3,5-dialkyl-1,2,4-trithiolanes (TT) was based on agreement with MS and KI (Kovats indices) data and fragmentation patterns of their standards obtained from mixtures of aldehydes, ammonia, and H_2S (Kawai and Ishida, 1989). Pyrrolidino[1,2-*e*]-2,4-dimethyl-4*H*-1,3,5-dithiazine (peak 78 in Table I; $R^1 = R^2$ = methyl) synthesized by Kubota et al. (1989) was courteously presented by them as a standard. To identify some pyrrolidines (**20**, **31**, **59**, **62**, and **91**) and pyrroles (**23** and **63**), thermal reactions of proline with monosaccharides (glucose, rhamnose, and ribose, respectively) and reaction of proline with glycine were carried out in a manner similar to the methods described by Tressl et al. (1985b) but according to the glass beads method.

Estimation of H_2S Produced from Roasted Dried Squid. Each of the 11 distillates fractionated was used for measuring H_2S, pH, and odor evaluation. Half amounts or small portions were employed for reactions with 2 or 20% $ZnCl_2$ solutions. The

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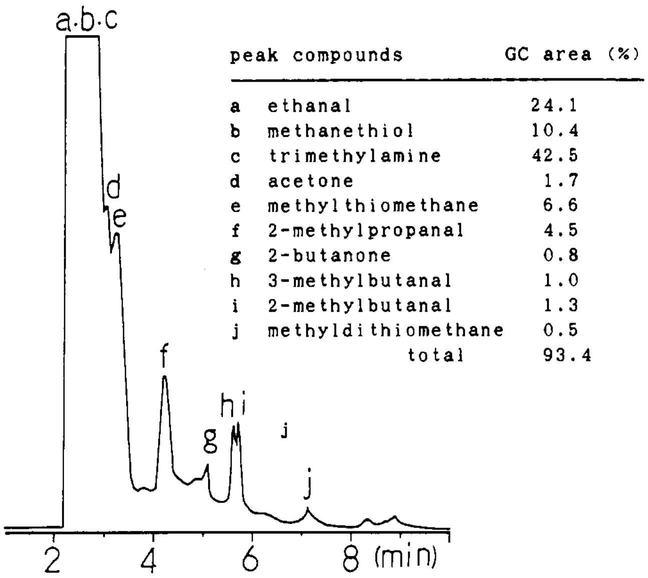


peak	compounds	GC area (%)
a	ethanal	24.1
b	methanethiol	10.4
c	trimethylamine	42.5
d	acetone	1.7
e	methylthiomethane	6.6
f	2-methylpropanal	4.5
g	2-butanone	0.8
h	3-methylbutanal	1.0
i	2-methylbutanal	1.3
j	methyldithiomethane	0.5
	total	93.4

Figure 1. Gas chromatogram of the headspace and the identified components of dried squid in the temperature range 170–190 °C held for 40 min.

precipitates of ZnS were repeatedly filtered until filtrates showed no white turbidity when more $ZnCl_2$ was added. They were washed with an excess of water, dried at room temperature, and weighed.

Apparatus for Analysis. The two flavor concentrates were analyzed by GC on a Hewlett-Packard 5890-A chromatograph equipped with a 0.25 mm (i.d.) × 60 m nonpolar and capillary column (0.25-μm film, DB-1 fused silica). Some detectors (flame ionization detector, flame thermionic detector, and flame photometric detector) were also used. Running conditions for GC and GC–MS, along with a GC–MS instrument, applied to this analysis, were the same as those described previously (Kawai and Ishida, 1989). For each flavor concentrate, 1.0-μL was injected into the GC.

RESULTS

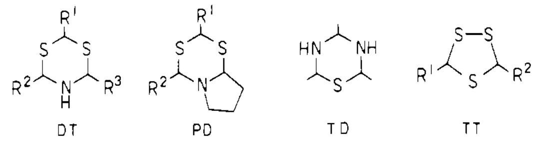
Headspace Components of Roasted Dried Squid. Figure 1 shows the gas chromatogram and the identified headspace components of roasted dried squid, where trimethylamine appeared the largest, followed by ethanal and methanethiol. The reconstitution gave little image for roasted dried squid. The GC resulted by not using solvents during the sample preparation, thus avoiding overlapping their peaks with those of solvents on GC charts. However, no H_2S or ammonia was detected by GC, probably because the $CaCl_2$ used as a drier caught them or the prepurge for removing the moisture from the Tenax eliminated them at the same time.

Characterization of Flavor Components. Two flavor concentrates (A and B), obtained from the two temperature ranges 125–190 and 215–230 °C, possessed moderately roasted and strongly roasted odors, respectively. Their gas chromatograms are shown in Figures 2 and 3. The different GC patterns between them probably reflect the difference between their odors.

Table I gives 94 identified compounds with their corresponding GC peak numbers, KI data, GC areas (percent) of total volatiles, and MS references. It also includes three major components that remained unidentified, alongside the capital letters (A–C) as in those figures.

The main component, C, in both concentrates is thought to be a pyrrolidine derivative as well as B, because its simple fragmentation and the huge base ion m/z 84 in the MS data (in Table I) resemble those of identifiable pyrrolidines. E.g., the MS data of 1-butylpyrrolidine (**41**) are as follows; 127 (M^+, 6), 85 (6), 84 (100), 70 (3), 55 (4), 42 (10). Since the MS data of C and B are almost the same (in Table I), they may be structural isomers.

Table II compiles the MS data of the little known compounds that appear as volatile components of foods. The identification of PD was based on MS fragment analysis of the authentic PD (**78**) together with the MS



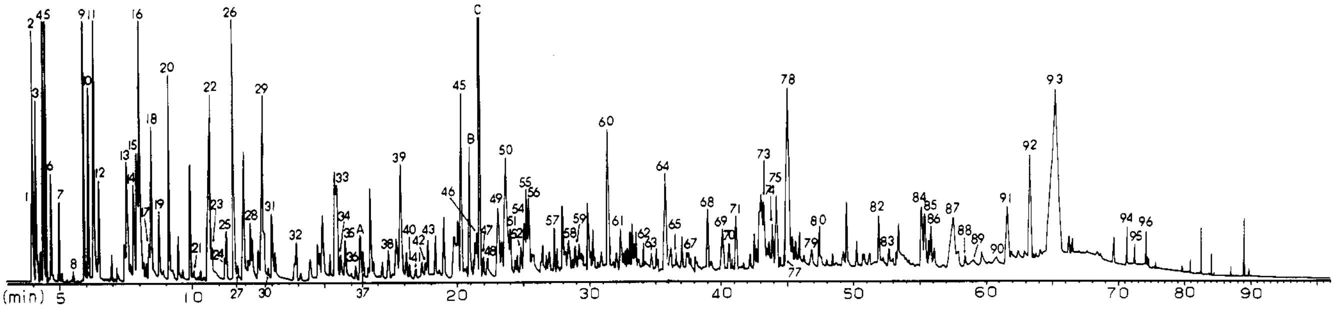


Figure 2. Gas chromatogram of the flavor concentrate (A) obtained from roasted dried squid in the temperature range 125–190 °C.

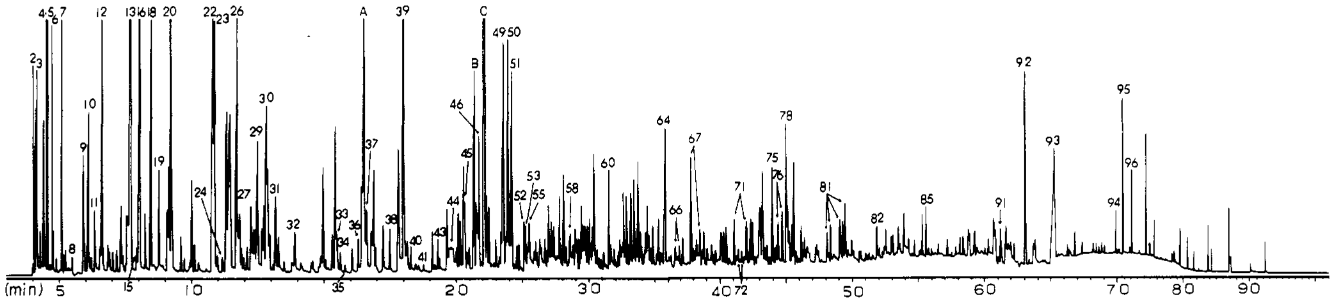


Figure 3. Gas chromatogram of the volatile concentrate (B) obtained from roasted dried squid in the temperature range 215–230 °C.

Table I. Volatile Compounds Identified from Roasted Dried Squid in the Two Temperature Ranges 125–190 and 215–230 °C[a]

		KI (DB-1)		GC peak area, %		
peak no.	compound[b]	unknown	known	125–190 °C	215–230 °C	MS ref
1	hydrogen sulfide	430	422			
2	ethanal	450	453			
3	methanethiol	460	470			
4	ethyl ether (solvent)	470	477			
5	dichloromethane (solvent)	504	510			
6	2-methylpropanal	523	520	0.33	0.38	
7	2-butanone	559	560	0.18	1.60	
8	oxolane	608	612	0.03	0.06	
9	3-methylbutanal	622	626	0.98	0.25	
10	2-methylbutanal	634	637	0.62	0.42	
11	1-pyrroline	645	651	2.41	0.27	
17	methyldithiomethane	723	725	0.14		
21	1,2-diethoxyethane	773	771	0.05		
29	furfuryl alcohol	832	830	2.03	0.54	
32	2-oxolanone	858	859	0.26	0.19	
42	methyltrithiomethane	949	949	0.10		
44	phenol	964	961		0.18	
47	2-acetyl-5-methylfuran	1013	1010	0.24		
53	4-methylphenol	1056	1052		0.26	
56	3,4,5,6-tetrahydro-2,4,6-trimethyl-2*H*-1,3,5-thiadiazine*	1068	1066	0.56		
61	benzothiazole	1198	1196	0.38		
64	indole	1265	1264	1.61	0.85	
70	3-methylindole	1365	1365	0.29		
80	pentadecane	1500	1500	0.33		
81	$C_{15}H_{32}O_3$ (probably 3,7,11-trimethyl-2,6,10-dodecatrienol; four isomers)	1525			0.18	
		1538			0.22	
		1546			0.26	
		1556			0.32	
85	methyl tetradecanoate	1707	1708	0.33	0.27	
87	tetradecanoic acid	1762	1758	0.50		
88	2-hexadecanone	1783	1787	0.05		
89	methyl pentadecanoate	1808	1805	0.13		
90	pentadecanoic acid	1854	1850	0.07		
92	methyl hexadecanoate	1908	1907	0.87	1.07	
93	hexadecanoic acid	1973	1981	2.30	0.67	
94	methyl octadecanoate	2109	2109	0.46	0.24	
	acyclic nitrogen compounds					
13	2-(dimethylamino)ethanol	706	708	0.61	2.90	
14	(dimethylamino)acetonitrile*	712	714	0.68		
19	*N,N*-dimethylformamide	738	735	0.42	0.36	
26	*N,N*-dimethylglycine methyl ester*	809	810	2.44	1.35	
95	hexadecanamide*	2144	2142	0.23	1.92	
96	*N*-methylhexadecanamide*	2178		0.33	0.64	
	imines					
22	*N*-ethylidene-2(or 3)-methylbutylamine	787	[790][c]	2.15	1.25	MS[c]
34	*N*-(2-methylpropylidene)-2-methyl-1-propenylamine[d,*]	892	892	0.16	0.11	
39	*N*-(2-methylpropylidene)-3-methylbutylamine	926	[927][c]	0.72	2.69	MS[c]
49	*N*-(2-methylbutylidene)-3-methylbutylamine	1026	[1026][c]	0.52	2.11	MS[c]
50	*N*-(3-methylbutylidene)-3-methylbutylamine	1034	[1033][c]	1.62	1.61	MS[c]
	acyclic unusual sulfur compounds					
28	1-(methylthio)ethane-1-thiol	822		0.23		MS[e]
46	1-(methylthio)-2-methylpropane-1-thiol	997		0.29	0.66	
57	3-(methylthio)hexanal*	1102		0.59		MS[f]
67	3-(methylethyl)-2,4,5-trithiahexane (an isomer of 67)	1300		0.32	0.79	MS[g]
		1315			0.21	
71	3-(2-methylpropyl)-2,4,5-trithiahexane (an isomer of 71)	1370		0.48	0.26	MS[g]
		1392			0.29	
	pyrazines					
25	2-methylpyrazine	798	798	0.40		
33	2,5-dimethylpyrazine	885	883	0.45	0.11	
35	2,3-dimethylpyrazine	894	895	0.21	0.06	
45	2,3,5-trimethylpyrazine	978	975	2.05	0.53	
52	2,5-dimethyl-3-ethylpyrazine	1055	1053	0.21	0.27	
54	2,6-dimethyl-3-ethylpyrazine	1061	1060	0.44		
55	2,3,5,6-tetramethylpyrazine	1067	1068	0.71	0.30	
	pyridines					
16	pyridine	718	714	1.80	2.68	
24	2-methylpyridine	795	798	0.08	0.06	
30	3-methylpyridine	835	834	0.07	0.65	
37	2,4-dimethylpyridine	908		0.15	0.30	MS[f]
38	2,3-dimethylpyridine	919	919	0.27	0.30	
40	3-ethylpyridine	932	932	0.07	0.11	
43	3,5-dimethylpyridine	951	951	0.25	0.17	
75	2-phenylpyridine	1432	1432	0.76	0.62	

Table I (Continued)

peak no.	compound[b]	KI (DB-1) unknown	KI (DB-1) known	GC peak area, % 125–190 °C	GC peak area, % 215–230 °C	MS ref
	pyrroles					
15	1-methylpyrrole	715	717	0.50	0.05	
18	pyrrole	726	727	0.86	1.14	
23	1-ethylpyrrole[h]	790	[790]	0.27	0.89	MS[f]
27	2-methylpyrrole	812	812	0.02	0.22	
36	2,5-dimethylpyrrole	900	902	0.10	0.13	
51	2-C_5H_{11}-pyrrole	1040		0.33	0.89	
63	1-(5-methylfurfuryl)pyrrole[i]	1247	[1248]	0.19		MS[j]
	pyrrolidines					
12	1-methylpyrrolidine	662	665	0.29	1.39	
20	1-ethylpyrrolidine[h,i,*]	750	[753]	1.67	2.29	MS[f]
31	1-propylpyrrolidine[h,*]	838	[839]	0.29	0.26	
41	1-butylpyrrolidine*	939	939	0.06	0.09	
48	1-methylproline methyl ester*	1017		0.19		MS[f]
58	1-acetylpyrrolidine	1128	1128	0.30	0.24	
59	1-furfurylpyrrolidine[i]	1134	[1134]	0.21		MS[k]
62	1-(5-methylfurfuryl)pyrrolidine[i]	1226	[1225]	0.21		MS[k]
91	proline anhydride[h,i]	1875	[1870]	0.69	0.31	MS[f]

5,6-Dihydro-2,4,6-trialkyl-4*H*-1,3,5-dithiazines

	R^1	R^2	R^3	KI (DB-1) unknown	KI (DB-1) known	GC peak area, % 125–190 °C	GC peak area, % 215–230 °C	MS ref
60	Me	Me	Me	1181	1181	1.00	0.52	
65	Et	Me	Me	1279	1282	0.24		
68	Me	Me	*i*-Pr	1330	1333	0.73		
69	*i*-Pr	Me	Me	1350	1352	0.59		
73	Me	Me	Bu	1415		0.43		MS[l]
74	*i*-Bu	Me	Me*	1423		0.39		MS[l]
77	Bu	Me	Me*	1453		0.17		MS[l]
79	*i*-Pr	Me	*i*-Pr*	1490	1492	0.32		
83	Bu	Me	Bu*	1641		0.12		MS[l]
		3,5-Dialkyl-1,2,4-trithiolanes						
66	Me	*i*-Pr		1279	1280		0.08	
		(an isomer of **68**)		1285	1286		0.11	
72	Et	*i*-Pr*		1377	1379		0.11	
		(an isomer of **72**)		1383	1385		0.09	
76	*i*-Pr	*i*-Pr*		1441	1444		0.25	
		(an isomer of **76**)		1448	1451		0.33	
		Pyrrolidino[1,2-*e*]-2,4-dialkyl-4*H*-1,3,5-dithiazines						
78	Me	Me		1462	1462	2.73	0.72	
82	C_3H_7	Me*		1621		0.51	0.15	
84	*i*-Bu	Me*		1703		0.63		
86	Bu	Me*		1723		0.19		
					total	49.19	41.89	

Major Compounds Remaining Unidentified and Their MS Data

		KI (DB-1)	125–190 °C	215–230 °C
A	126 (M^+, 9), 84 (7), 83 (100), 58 (28), 44 (2), 42 (6)	904	0.39	1.73
B	141 (M^+, 1), 85 (5), 84 (100), 70 (1), 55 (1), 42 (5)	989	0.86	1.05
C	141 (M^+, 5), 85 (6), 84 (100), 70 (2), 55 (2), 42 (4)	1004	4.04	4.14

[a] Abbreviations: R, alkyl; Me, methyl; Et, ethyl; *i*-Pr, methylethyl; Bu, butyl; *i*-Bu, 2-methylpropyl. [b] Compounds with KI (Kovats indices) in square brackets or with no KI are tentatively identified. [c] MS and KI are coincident with published data obtained from the same kind of GC column (DB-1) conditions (Flath et al., 1989). [d] MS and KI are identical with those of a reaction product obtained from 2-methylpropanal, ammonia, and H_2S (not isolated) [cf. Kawai et al. (1985)]. [e] Peer et al. (1971). [f] McLafferty and Stauffer (1989). [g] Dubs and Stussi (1978).[h,i] MS and KI are in agreement with those of reaction products of [h]*proline* with glycine and [i]*proline* with monosaccharide. [j] Tressl et al. (1985a). [k] Tressl et al. (1985b). [l] Hwang et al. (1986). * Asterisked compounds have not been reported previously as volatiles of food.

analytical references to DT (Ledl and Severin, 1974; Hwang et al., 1986; Kawai and Ishida, 1989). The fragmentation pattern of PD was almost the same as that of DT; e.g., cleavage on the molecular ion (M^+, 189) of PD (**78**) gave stepwise the *m/z* 129, 97, and 70 ions with loss of the parts of the structure through elimination of $SCHR^1$, $SCHR^1S$, and $SCHR^1SCR^2$. Among four PDs, two MS spectra of PD (**84** and **86**) are exactly the same as shown in Table II, so that PD **84** preceding PD **86** was identified as isobutyl-substituted PD and the latter as butyl-substituted PD, on the basis of the findings that the pair of MS spectra between DT with a branched-alkyl substituent and DT with a corresponding straight-chain alkyl at the same R^1 position in their formulas completely superimposed on each other, but their retention time values on GC were rather different as described by Kawai and Ishida (1989).

Variety of Components. As Table I represents, both flavor concentrates (A and B) consist of various sulfur-containing compounds (S compounds), nitrogen-containing compounds (N compounds), S,N heterocycles, and miscellaneous compounds as follows, where the first figure in parentheses indicates the number of identifiable components belonging to A: pyrrolidines (9, 6), DT (9, 1), pyridines (8, 8), pyrazines (7, 5), pyrroles (7, 6), imines (5, 5), acyclic unusual S compounds (5, 3), *N,N*-dimethylamino compounds (4, 3), PD (4, 2), usual S compounds (4, 2), TT

Table II. Mass Fragments and Identification of Volatile Compounds from Roasted Dried Squid[a,b]

N-methylhexadecanamide (**96**)	269 (M^+, 2), 240 (1), 142 (2), 128 (3), 100 (4), 86 (19), 73 (100)
N-(2-methylpropylidene)-2-methyl-1-propenylamine (**34**)	125 (M^+, 48), 110 (15), 82 (92), 55 (100), 41 (16), 29 (22)
1-(methylthio)-2-methylpropane-1-thiol (**46**)	136 (M^+, 16), 103 (62), 93 (25), 88 (14), 55 (100), 47 (14), 45 (30)
2-C_5H_{11}-pyrrole (**51**)	137 (M^+, 31), 94 (3), 82 (6), 81 (100), 80 (26), 67 (5), 54 (3), 53 (3)
1-propylpyrrolidine (**31**)	113 (M^+, 11), 112 (3), 85 (6), 84 (100), 70 (6), 55 (6), 42 (15)

		Pyrrolidino[1,2-*e*]-2,4-dialkyl-4*H*-1,3,5-dithiazines
R^1	R^2	
Me	Me (**78**)[c]	189 (M^+, 35), 129 (41), 128 (12), 97 (66), 96 (41), 70 (100), 69 (41), 60 (12)
C_3H_7	Me (**82**)	217 (M^+, 25), 129 (59), 128 (10), 97 (100), 96 (47), 70 (98), 69 (37), 60 (6)
i-Bu	Me (**84**)	231 (M^+, 26), 129 (65), 128 (10), 97 (100), 96 (42), 70 (87), 69 (36), 60 (10)
Bu	Me (**86**)	231 (M^+, 24), 129 (62), 128 (11), 97 (100), 96 (32), 70 (92), 69 (29), 60 (9)

[a] Abbreviations: the same as in Table I. [b] Principal fragments listed here. [c] Kubota et al. (1988).

Table III. Each GC Area (Percent) of S- and N-Containing Compounds and S,N Heterocycles Identified in the Flavor Concentrates (A and B)

	A (pH 9.0)	B (pH 8.0)	
S compounds	2.15	3.18	+1.0
N compounds	28.29	30.12	+1.8
S,N heterocycles	8.99	1.39	-7.6

(0, 3), and miscellaneous (26, 19). The wide variety of constitution shows that sufficient interactions obviously occurred among released low molecular weight compounds, such as H_2S, ammonia, methanethiol, and aldehydes, from dried squid in the flask during roasting. Also, many comparisons between the numbers in the classified components suggest that there is a tendency toward monotonous tone in the constitution from A to B. The tendency must have been caused by the more than high enough temperature range employed for B compared with that for A. It may organoleptically correspond with much experience in overheating foods.

Alteration of Components. The GC areas of three groups of S and N compounds and S,N heterocycles in the concentrates are given in Table III. Both areas of N compounds in A and B were 10 times as large as those of S compounds. This was probably influenced by the basic pH of the concentrates (pH 9.0 and 8.0).

From a comparison of each area in B to that in A, there was a large fall in S,N heterocycles (7.6%), although there were only small rises in S and N compounds (1 and 2%). Therefore, the difference between the odors in the concentrates was probably due to the fall of S,N heterocycles, while common roasted odors were ascribable to the unchanged amounts of S and N compounds. Further, from a comparison of each chemical group in B to that in A, pyrazines showed a 3% decrease (in Table I), whereas, there was no rise in every chemical group with the exception of TT, but some major compounds showed sharp rises, reaching 10% in total: 2-(dimethylamino)ethanol (**13**), imines (**39** and **49**), hexadecanamide (**95**), pyrrolidines (**12** and **20**), pyridine (**16**), and sulfide (**67**). These irritant flavor components, except for the imines, showed a total rise of 7%. Thus, the comparable fall of the S,N heterocycles with the rise of the irritant components must have influenced the odor of B to be strongly roasted. In other words, the S,N heterocycles contributed principally to the moderately roasted odor of A. The fall suggested that formation of the S,N heterocycles was hindered by decreasing pH and/or lack of their low molecular weight constituents during roasting.

Formation of H_2S and Alteration of pH during Heating. Figure 4 shows thermal effects on H_2S formation and pH alteration in the 11 fractionated distillates. As the temperature was elevated, H_2S increased with a gradual rise during heating up to 230 °C, and finally its total amount reached 750 mg/100 g of dried squid. The neutral pH (6.8) before heating became remarkably greater (9.8) at 101 °C after just the beginning of heating, indicating that some intensely basic compound with low bp occurred in this initial stage, and after that the pH decreased to 8.0. The occurrence of some basic compound evidently preceded appearance of H_2S below 103 °C (Figure 4).

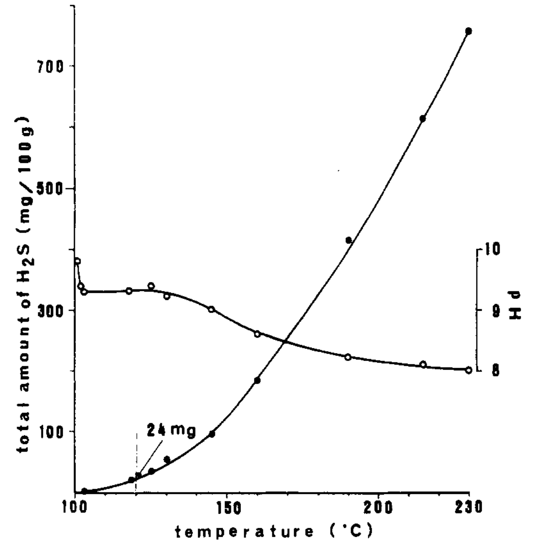


Figure 4. Thermal effects on H_2S formation and pH alteration upon heating dried squid to 230 °C. (●) H_2S; (○) pH.

Table IV. Recovery of Components (Grams from 100 g of Dried Squid)

	heating		loss on heating	gain from distillates	balance
	before	after[a]			
H_2O[b]	123.0[c]	1.6	121.4	129	+7
N	9.75	7.93	1.82	0.1[d]	-1.7
S[e]	1.70	0.8-0.9	0.8-0.9	0.7	-0.1-0.2
fat	4.00	3.91	0.09		-0.1
ash	8.00	4.73	3.27		
NaCl	3.80	1.83	1.97		

[a] 59 g. [b] Loss on drying at 105 °C for 4 h. [c] Moisture of the sample and water added to the system. [d] Calculated amount from $(NH_4)_2CO_3$ isolated. [e] Oxygen-flask method.

Recovery of Components on Roasting. A comparison between some components of dried squid before and after roasting was made, as given in Table IV. An excess amount of H_2O (7 g) was found in the distillates. It was probably produced by the thermal oxidation of organic substances in the dried squid.

The loss of N (1.8 g) was almost twice as large as that of S (0.8-0.9 g). It was likely caused by the release of ammonia because ammonium carbonate was isolated from the distillates. White masses, becoming large from white spots on the wall of the flask, condensed in the condenser above 200 °C. The resultant powder (270 mg) was identified as $(NH_4)_2CO_3$ [mp 82.8 °C dec; IR (KBr) 705, 835, 1280, 1600 cm^{-1}; H NMR (60 MHz) and GC showed neither signals nor peaks on their charts]. Therefore, a greater amount of ammonia released than H_2S must have made the distillates basic.

The loss of S (0.8–0.9 g) almost agreed with the gain of H_2S (0.75 g), indicating the amount of H_2S obtained was rather reliable. The precipitates of ZnS were not contaminated with zinc–ammonium complexes by N assay (ca. 20 mg in total). Also, the amount of $ZnCO_3$ as a general attendant on the precipitation of ZnS seemed little, because CO_2 generated could be considerably caught by ammonia under the basic conditions. Since nearly half the amount of S still remained in the residue (in Table IV), if water should be supplied to the residue above 230 °C, H_2S generation can continue until the S is depleted.

The crude fat also showed little loss. The lost ash and salt could be not ascertained, but they were probably scattered over the surface of the beads.

DISCUSSION

Flavor Components of Roasted Dried Squid. To well understand the difference between the odors of concentrates A and B, some odor profiles of identified compounds were inserted in parentheses after the corresponding peak numbers.

In concentrate A, the major component among the identified components, PD [**78**, a strong roasted aroma like DT 60 (Kubota et al., 1988)] has been recently synthesized from ethanal, ammonia, H_2S, and 1-pyrroline [**11**, fresh corn (Mills and Hodge, 1976)], so that its formation was confined to the amount of the pyrroline as described by Kubota et al. (1989). It supports the simultaneous decrease of the pyrroline and every PD in A as compared with those in B as shown in Table I. The PD and the pyrroline have been found from cooked shrimp (Kubota et al., 1988, 1989).

The next large component, *N,N*-dimethylglycine methyl ester (**26**, boiled dried squid odor with fishy note) could be one of the contributors, along with trimethylamine, found as the most dominant headspace component (in Figure 1). Among acyclic N compounds listed in Table I, merely two compounds (**13** and **19**) have been detected in food volatiles so far: cold-stored cod (McGill et al., 1977) and boiled shrimp (Kubota et al., 1986).

Many DTs have been recognized as having heated food flavors and as forming almost spontaneously from aldehydes, ammonia, and H_2S (Brinkman et al., 1972; Kubota et al., 1980; Kawai et al., 1985). Until now, a total of 47 DTs have been reported; nevertheless, all seven DTs other than parent DT [**60**, roasted shrimp (Kawai et al., 1985)] were detected only in two kinds of seafood volatiles: small shrimp (Kubota et al., 1988; Choi et al., 1983) and roasted dried squid (Kawai and Ishida, 1989). A mutually common factor in those seafoods is probably high pH; high pH in shrimp tissue was reviewed by Iida (1989) and the pH in A tested here is 9.0. TD [**56**, roasted cereals and roasted popcorn (Kawai et al., 1985)], a precursor of DT (**60**), has been recently found in the flavor concentrate obtained from a basic distillate (pH 8.8) of roasted short-necked clam (Kawai et al., 1990). The pH effect on volatiles will be discussed below.

Almost ordinary pyrazines are known to have roasted flavors. The decrease in pyrazines during roasting dried squid corresponded well to that described in the roasted volatiles of the clam as compared with the boiled ones (Kawai et al., 1990). Therefore, the pyrazines in proteinaceous food volatiles seem to contribute to boiled odors rather than roasted ones. A group of pyrazines (**25**, **33**, **35**, and **55**) with pyridines (**16** and **24**) and another group (**33**, **45**, and **52**) have been found from canned and boiled squid by Yakush et al. (1987) and Lee et al. (1989), respectively.

Some 1-alkylpyrrolidines (**12**, something dried; **20**; **31**; **41**, something roasted) increased or were unchanged. Furan-substituted pyrrolidines [**59** and **62**, both cereal plus sesame (Tressl et al., 1985b)] and pyrazine-substituted pyrrolidine [**91**, nut (Mills and Hodge, 1976)] decreased together with other furan compounds [**29**, **47**, **63**, nut (Mills and Hodge, 1976)]. Some pyrrolidines (**58**, **59**, and **91**) and all the pyrroles except **51** have been detected in volatiles of malt and roasted coffee (Tressl et al., 1981). Those pyrrolidines and the pyrroline were reported as Maillard reaction products of proline with monosaccharides by Tressl et al. (1985b). It is worth noting that proline has frequently occurred as the major free amino acid in the mantle muscle of fresh squids as reported by Suyama and Kobayashi (1980). These findings (Tressl et al., 1985b; Suyama and Kobayashi, 1980) suggest that proline plays a significant role in producing those components.

On the other hand, in concentrate B, two major components (**13** and **16**) possess only ammonia-like keen odors. Odors of imines listed in Table I possibly belong to a plant-like green note (Kawai et al., 1985). Those imines except **34** were found from hydrolyzed corn and reported to be formed from aldehydes with primary amines by Flath et al. (1989).

Acyclic unusual S compounds and TT in Table I were probably formed from aldehydes with H_2S (Boelens et al., 1974) and from that combination plus methanethiol (Peer et al., 1971; Dubs and Stussi, 1978). Reaction mixtures of the two types of combination were observed to have strong *Allium* plant-like or green odors by Boelens et al. (1974).

As described above, concentrate B evidently lost characteristic flavor components contributing to the moderately roasted odor as compared with A. Thus, overheating dried squid gave more irritant and more monotonous odors than moderate roasting.

Thermal Effects on H_2S Formation. The most characteristic and perceptible odor of roasted dried squid is probably due to the large quantity of H_2S (750 mg/100 g). Generally, H_2S formation from meat depends on three factors: pH, temperature, and heating time, as described by Hofmann and Hamm (1978). A comparison of the present H_2S amount with others in the literature was performed. Johnson and Vickery (1964) revealed that H_2S formation from domestic meats upon boiling was directly related to the high pH. According to their data, the H_2S amount at 100 °C for 2 h in pH 9.5 was 3 times as much as that in pH 7.5. The amount was ca. 5 mg/100 g of meat. Also, Hamm and Hofmann (1965) reported that H_2S formation from the protein (myofibrils) was influenced by high temperature. They described the H_2S amount at 120 °C for 0.5 h in pH 7.4 as 4 times larger than that at 100 °C, and the amount was nearly 2.0 mg/100 g. On the other hand, the H_2S amount from dried squid at 120 °C in pH 9.5 was 24 mg/100 g (Figure 4). When this amount was divided by 4 (a temperature factor), the result (6 mg) was almost in agreement with the value from meat (5 mg), whereas division of the amount into 3 (a pH factor) gave a result (8 mg) that was 4 times larger than the value from the protein. If the amount is divided by 3 × 4 (the factors multiplied), the figure agrees well with the value from the protein. Although there should be a gap between meat and protein, H_2S formation from dried squid is evidently influenced by both factors. The large total amount of H_2S must have been produced in this way. The high pH (9.8 at 101 °C) in the initial stage of heating dried squid was considered to have accelerated H_2S formation. No comparable information on H_2S formation above 120 °C has been available.

Mechanism of DT and TD Formation from Dried Squid. The compound that was mainly responsible for the high pH in distillates was probably ammonia. This was based on the isolation of $(NH_4)_2CO_3$ and the rapid increase in pH at the beginning of heating. Further, ammonia was detected as a major basic component of dried squid by Yamanishi and Miura (1956) and also of steam-boiled squid by Ooyama (1975). Low fatty aldehydes in Figures 1–3 are common components of dried squid. They are always found even in a low temperature range (98–125 °C) not only from dried squid but also from foods overall (Maarse and Visscher, 1988), suggesting that their occurrence has no relationship to temperature. Josephson and Lindsay (1987) revealed that low fatty aldehydes are derived from higher ones through retroaldol condensation.

The above thermal environment of roasted dried squid is considered to allow many S,N heterocycles (nine DT, four PD, and TD in Table I) to be formed, where ammonia arose prior to H_2S in the presence of aldehydes, and also accelerated generation of H_2S. Ammonia can react with aldehydes before participation of H_2S under basic conditions. Probably, elimination of ammonia and addition of H_2S occur stepwise, and then those compounds are accumulated. This situation agrees well with the formation mechanism for DT and TD as proposed from the model systems by Kawai et al. (1985).

However, in spite of the simultaneous presence of aldehydes, ammonia, and H_2S in the two concentrates, a gap between DT in A and B occurred (Table I). This was probably due to the decrease in pH (pH 9.0 and 8.0) based on a decrease in ammonia; it continued to increase during roasting up to 230 °C, but it must have been more consumed in reaction with increasing CO_2 than it increased, missing formation of the S,N heterocycles and pyrazines. Pyrazine formation depended on an ammonia concentration as reported by Shibamoto and Russell (1977).

Foods that produce in a stepwise manner large quantities of ammonia and H_2S on heating seem to be limited. Neither high pH nor a remarkable increase in pH during boiling domestic meats and chicken muscle was reported by Johnson and Vickery (1964) and Mecchi et al. (1964). The behavior of pH suggests that DT, especially DT substituted with higher alkyl groups, does not much arise from such food volatiles. The S,N heterocycles, as described above, can be formed easily in basic volatiles of seafoods, such as dried squid, small shrimp, and crab, where pH facilely elevates upon a little heating.

Supplementary Material Available: Effects of heating on dried squid, spectra of isolated and authentic $(NH_4)_2CO_3$, effects of pH and temperature on H_2S formation, and chromatogram of dried squid (4 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

Boelens, M.; van der Linde, L. M.; de Valois, P. J.; van Dort, H. M.; Takken, H. J. Organic Sulfur Compounds from Fatty Aldehydes, Hydrogen Sulfide, Thiols, and Ammonia as Flavor Constituents. *J. Agric. Food Chem.* **1974**, *22*, 1071–1076.

Brinkman, H. W.; Copier, H.; de Leuw, J. J. M.; Tjan, S. B. Components Contributing to Beef Flavor. Analysis of the Headspace Volatiles of Beef Broth. *J. Agric. Food Chem.* **1972**, *20*, 177–181.

Choi, S. H.; Kobayashi, A.; Yamanishi, T. Odor of Cooked Small Shrimp, *Acetes japonicus* Kishinouye; Difference between Raw Material and Fermented Product. *Agric. Biol. Chem.* **1983**, *47*, 337–342.

Dubs, P.; Stussi, R. Investigation of the Heated-Space of Roasted Meat. II. Synthesis of Substituted 2,4,5-Trithiahexanes. *Helv. Chim. Acta* **1978**, *224*, 2351–2359.

Flath, R. A.; Matsumoto, K. E.; Binder, R. G.; Cunningham, R. T.; Mon, T. R. Effects of pH on the Volatiles of Hydrolyzed Protein Insect Baits. *J. Agric. Food Chem.* **1989**, *37*, 814–819.

Hamm, R.; Hoffmann, K. Changes in the Sulphydryl and Disulphide Groups in Beef Muscle Protein during Heating. *Nature* **1965**, *207*, 1269–1271.

Hofmann, K.; Hamm, R. Sulfhydryl and Disulfide Groups in Meat. *Adv. Food Res.* **1978**, *24*, 1–111.

Hwang, S. S.; Carlin, J. T.; Bao, Y.; Hartman, G. J.; Ho, C. T. Characterization of Volatile Compounds Generated from the Reactions of Aldehydes with Ammonium Sulfide. *J. Agric. Food Chem.* **1986**, *34*, 538–542.

Iida, H. Odor of Shellfish. In *Odor of Marine Products*; Koizumi, C., Ed.; Koseishakoseikaku: Tokyo, 1989; pp 42–52.

Johnson, A. R.; Vickery, J. R. Factors Influencing the Production of Hydrogen Sulfide from Meat during Heating. *J. Sci. Food Agric.* **1964**, *15*, 695–701.

Josephson, D. B.; Lindsay, R. C. Retro-Aldol Degradation of Unsaturated Aldehydes: Role in the Formation of *c*4-Heptenal from *t*2,*c*6-Nonadienal in Fish, Oyster, and Other Flavors. *JAOCS, J. Am. Oil Chem. Soc.* **1987**, *64*, 132–138.

Kawai, T.; Ishida, Y. Comparison of Volatile Components of Dried Squid to Reaction Products Formed from the Mixtures of Hydrogen Sulfide, Ammonia, and Aldehydes. *J. Agric. Food Chem.* **1989**, *37*, 1026–1031.

Kawai, T.; Irie, M.; Sakaguchi, M. Degradation of 2,4,6-Trialkyltetrahydro-1,3,5-thiadiazines during Storage. *J. Agric. Food Chem.* **1985**, *33*, 393–397.

Kawai, T.; Ishida, Y.; Kakiuchi, H.; Ikeda, N.; Tsuneya, T. Volatile Components of Boiled and Roasted Short-necked Clam *Tapes philippinarum*. *Nippon Suisan Gakkaishi* **1990**, *56*, 795–802.

Kubota, K.; Kobayashi, A.; Yamanishi, T. Some Sulfur Containing Compounds in Cooked Odor Concentrate from Boiled Antarctic Krills (*Euphausia superba* Dona). *Agric. Biol. Chem.* **1980**, *44*, 2677–2682.

Kubota, K.; Shijimaya, H.; Kobayashi, A. Volatile Components of Roasted Shrimp. *Agric. Biol. Chem.* **1986**, *50*, 2867–2873.

Kubota, K.; Watanabe, K.; Kobayashi, A. Novel Dithiazine Compounds in Volatile Components from Cooked Sakuraebi (*Sergia lucens* Hansen). *Agric. Biol. Chem.* **1988**, *52*, 1537–1540.

Kubota, K.; Takeuchi, M.; Kobayashi, A.; Kitahara, T. Structural Conformation of Pyrrolidino[1,2-*e*]-2,4-dimethyl-4*H*-1,3,5-dithiazine and Its Natural Precursors. *Nippon Nogeikagaku Koenyoshisyu* **1989**, 235.

Ledl, F.; Severin, T. Sulfur Compounds from Cysteine and Xylose. *Z. Lebensm.-Unters. Forsch.* **1974**, *154*, 29–31.

Lee, J. H.; Choi, B. D.; Lee, K. H.; Ryu, H. S. Flavor Components in the Squid Processing. *Bull. Korean Fish. Soc.* **1989**, *22*, 370–374.

Maarse, H.; Visscher, C. A. In *Volatile Components in Food. Qualitative Data. Supplement 5*; TNO-CIVO Food Analysis Institute: Zeist, The Netherlands, 1988.

McGill, A. S.; Hardy, R.; Gunstone, F. D. Further Analysis of the Volatile Components of Frozen Cold Stored Cod and the Influence of these on Flavor. *J. Sci. Food Agric.* **1977**, *28*, 200–205.

McLafferty, F. W.; Stauffer, D. B. In *The Wiley/NBS Registry of Mass Spectra Data*; Wiley & Sons: New York, 1989; Vol. 1.

Mecchi, E. P.; Pippen, E. L.; Lineweaver, H. Origin of Hydrogen Sulfide in Heated Chicken Muscle. *J. Food Sci.* **1964**, *29*, 393–399.

Mills, F. D.; Hodge, J. E. Amadori Compounds: Vacuum Thermolysis of 1-Deoxy-1-L-proline-D-fructose. *Carbohydr. Res.* **1976**, *51*, 9–21.

Ooyama, S. Studies on the Odor of Canned Fish (Part 3), Volatile Components of Steam-Boiled Fish. *Kaseishi* **1975**, *26*, 470–473.

Peer, H. G.; van der Heijden, S. B.; van Orden, T. L. Synthesis of Dithiohemiacetals. *Synth. Commun.* **1971**, *1*, 277–284.

Shibamoto, T.; Russell, G. F. A Study of the Volatiles Isolated from a D-Glucose–Hydrogen Sulfide–Ammonia Model System. *J. Agric. Food Chem.* **1977**, *25*, 109–112.

Suyama, M.; Kobayashi, H. Free Amino Acids and Quaternary Ammonium Bases in Mantle Muscle of Squids. *Nippon Suisan Gakkaishi* **1980**, *46*, 1261–1264.

Tressl, R.; Grunewald, K. G.; Kamperschroer, H.; Silwar, R. Formation of Pyrroles and Aroma Contributing Sulfur Components in Malt and Roasted Coffee. *Prog. Food Nutr. Sci.* **1981**, *5*, 71–79.

Tressl, R.; Grunewald, K. G.; Kersten, E.; Rewicki, D. Formation of Pyrroles and Tetrahydroindolizin-6-ones as Hydroxyproline-Specific Maillard Products from Glucose and Rhamnose. *J. Agric. Food Chem.* **1985a**, *33*, 1137–1142.

Tressl, R.; Rewicki, D.; Helak, B.; Kamperschroer, H. Formation of Pyrrolidines and Piperidines on Heating L-Proline with Reducing Sugars. *J. Agric. Food Chem.* **1985b**, *33*, 924–928.

Yakush, E. V.; Misharina, T. A.; Golovnya, R. V. Heterocyclic Organic Bases in the Flavor of Canned Squid *Ommastrephes bartrami*. *Prinkl. Biokhim. Mikrobiol.* **1987**, *23*, 131–134.

Yamanishi, T.; Miura, K. Studies on Characteristic Odor of Squid-III. On the Unpleasant Odorous Substance of Rain Spoilt Dried Squid. *Nippon Suisan Gakkaishi* **1956**, *22*, 486–489.

Yoshinaka, R. Volatile Components. In *Microcomponents of Fish and Shellfish* (in Japanese); Ikeda, S., Ed.; Koseisha-koseikaku: Tokyo, 1981; pp 110–142.

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Registry No. DT ($R^1 = R^2 = R^3 = Me$), 86241-90-9; DT ($R^1 = Et, R^2 = R_3 = Me$), 54717-14-5; DT ($R^1 = R^2 = Me, R^3 = i$-Pr), 104691-41-0; DT ($R^1 = i$-Pr, $R^2 = R^3 = Me$), 104691-40-9; DT ($R^1 = R^2 = Me, R^3 = Bu$), 101517-79-7; DT ($R^1 = i$-Bu, $R^2 = R^3 = Me$), 101517-87-7; DT ($R^1 = Bu, R^2 = R^3 = Me$), 101517-81-1; DT ($R^1 = R^3 = i$-Pr, $R^2 = Me$), 121098-13-3; DT ($R^1 = R^3 = Bu, R^2 = Me$), 101517-78-6; TT ($R^1 = Me, R^2 = i$-Pr), 121098-28-0; TT ($R^1 = Et, R^2 = i$-Pr), 121098-29-1; TT ($R^1 = R^2 = i$-Pr), 54934-99-5; PD ($R^1 = R^2 = Me$), 116505-60-3; PD ($R^1 = C_3H_7, R^2 = Me$), 132344-95-7; PD ($R^1 = i$-Bu, $R^2 = Me$), 132344-96-8; PD ($R^1 = Bu, R^2 = Me$), 132344-97-9; hydrogen sulfide, 7783-06-4; ethanol, 75-07-0; methanethiol, 74-93-1; 2-methylpropanal, 78-84-2; 2-butanone, 78-93-3; oxolane, 109-99-9; 3-methylbutanal, 590-86-3; 2-methylbutanal, 96-17-3; 1-pyrroline, 5724-81-2; methyldithiomethane, 624-92-0; 1,2-diethoxyethane, 629-14-1; furfuryl alcohol, 98-00-0; 2-oxolanone, 96-48-0; methyltrithiomethane, 3658-80-8; phenol, 108-95-2; 2-acetyl-5-methylfuran, 1193-79-9; 4-methylphenol, 106-44-5; 3,4,5,6-tetrahydro-2,4,6-trimethyl-2*H*-1,3,5-thiadiazine, 53897-63-5; benzothiazole, 95-16-9; indole, 120-72-9; 3-methylindole, 83-34-1; pentadecane, 629-62-9; 3,7,11-trimethyl-2,6,10-dodecatrienol, 4602-84-0; methyl tetradecanoate, 124-10-7; tetradecanoic acid, 544-63-8; 2-hexadecanone, 18787-63-8; methyl pentadecanoate, 7132-64-1; pentadecanoic acid, 1002-84-2; methyl hexadecanoate, 112-39-0; hexadecanoic acid, 57-10-3; methyl octadecanoate, 112-61-8; 2-(dimethylamino)ethanol, 108-01-0; (dmethylamino)acetonitrile, 926-64-7; *N*,*N*-dimethylformamide, 68-12-2; *N*,*N*-dimethylglycine, 7148-06-3; hexadecanamide, 629-54-9; *N*-methylhexadecanamide, 7388-58-1; *N*-ethylidene-2(or 3)-methylbutylamine, 132344-98-0; *N*-(2-methylpropylidene)-2-methyl-1-propenylamine, 5339-41-3; *N*-(2-methylpropylidene)-3-methylbutylamine, 41807-57-2; *N*-(2-methylbutylidene)-3-methylbutylamine, 54518-97-7; *N*-(3-methylbutylidene)-3-methylbutylamine, 35448-31-8; 1-(methylthio)ethane-1-thiol, 31331-53-0; 1-(methylthio)-2-methylpropane-1-thiol, 132374-41-5; 3-(methylthio)hexanal, 38433-74-8; 3-(methylethyl)-2,4,5-trithiahexane, 69078-81-5; 3-(2-methylpropyl)-2,4,5-trithiahexane, 69078-82-6; 2-methylpyrazine, 109-08-0; 2,5-dimethylpyrazine, 123-32-0; 2,3-dimethylpyrazine, 5910-89-4; 2,3,5-trimethylpyrazine, 14667-55-1; 2,5-dimethyl-3-ethylpyrazine, 13360-65-1; 2,6-dimethyl-3-ethylpyrazine, 13925-07-0; 2,3,5,6-tetramethylpyrazine, 1124-11-4; pyridine, 110-86-1; 2-methylpyridine, 109-06-8; 3-methylpyridine, 108-99-6; 2,4-dimethylpyridine, 108-47-4; 2,3-dimethylpyridine, 583-61-9; 3-ethylpyridine, 536-78-7; 3,5-dimethylpyridine, 591-22-0; 2-phenylpyridine, 1008-89-5; 1-methylpyrrole, 96-54-8; pyrrole, 109-97-7; 1-ethylpyrrole, 617-92-5; 2-methylpyrrole, 636-41-9; 2,5-diethylpyrrole, 625-84-3; 2-C_5H_{11}-pyrrole, 1551-12-8; 1-(5-methylfurfuryl)pyrrole, 13678-52-9; 1-methylpyrrolidine, 120-94-5; 1-ethylpyrrolidine, 7335-06-0; 1-pyrrolepyrrolidine, 7335-07-1; 1-butylpyrrolidine, 767-10-2; 1-methylproline methyl ester, 27957-91-1; 1-acetylpyrrolidine, 4030-18-6; 1-furfurylpyrrolidine, 61893-12-7; 1-(5-methylfurfuryl)pyrrolidine, 61480-99-7; proline anhydride, 19943-27-2.