Formation of Pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine in the Volatiles of Boiled Short-Necked Clam, Clam, and Corbicula

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The characteristic odor constituents from boiled short-necked clam, clam, and corbicula were investigated. Some distinctions in the composition of sulfur- and/or nitrogen-containing substances were observed among the shellfish species. Pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine (I), which is a common component from cooked small shrimp, was also found in clam and corbicula. The presence of a large quantity of compound I should contribute a roasted odor to the taste of boiled corbicula. Compound I possesses an extremely low odor threshold. 1-Pyrroline and compound I were produced at the same time during heating and were increased by longer heating. It is postulated that compound I was formed secondarily from 1-pyrroline with ethanal and hydrogen sulfide in the shellfish during heating.

INTRODUCTION

Various kinds of shellfish are used as food materials throughout the world. Such shellfish as oysters (Ronald and Thomson, 1964; Josephson et al., 1985) and ascidian (Fujimoto et al., 1982) are preferred for their characteristic fresh aroma, but many shellfish are eaten after being cooked, because they give a pleasant and appetizing aroma when heated. In Japanese dishes, corbicula, short-necked clam, and clam are often used as ingredients of soup. Among them, corbicula is usually cooked with miso, which is a fermented product from soy beans, to mask the earthy odor of corbicula. As these bivalves each present a characteristic aroma of their own when heated, details of their cooked volatiles have been published by Nishibori et al. (1972), Kawai et al. (1990), Gadbois et al. (1967), and Mendelsohn and Brooke (1968). However, no direct comparison among them can be drawn because the heating conditions were quite different. In this paper, the volatiles of boiled corbicula, short-necked clam, and clam were separated under almost the same conditions, and the compositions of the volatiles among them were compared. The formation of pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine, which has been previously found in the volatiles of cooked small shrimps (Kubota et al., 1988), was also investigated to learn fundamental data about its formation mechanism in seafood.

EXPERIMENTAL PROCEDURES

Sample Preparation. Three kinds of live bivalves, corbicula (*Corbicula japonica*, Lake Shinji, Japan), short-necked clam (*Tapes phillppinarum*, Lake Hamana, Japan), and clam (*Meretrix lusoria*, Mie coast, Japan), were used as samples. The bodies of the short-necked clams and clams were isolated along with the juice by hand-shucking. Corbicula was used without sucking the body from its shell. One kilogram of the edible part of samples with 1 L of deionized water was refluxed for 2 h in a modified Likens–Nickerson apparatus (Likens and Nickerson, 1964), and 50 mL of purified diethyl ether was used for extraction. After being dried with anhydrous sodium sulfate, the solvent was distilled at 39 °C to obtain the volatiles. The effect of heating time on the volatile components of corbicula was also investigated under the same conditions. Methyl decanoate (0.032 mg) was added to the ether extract after distillation as the internal standard.

Gas Chromatography (GC). *General Analytical GC.* Shimadzu Model 7A gas chromatographs equipped with a flame photometric detector (FPD) or a flame thermionic detector (FTD) and with a flame ionization detector (FID) were used for analysis purposes. A fused silica open tubuler column (50 m × 0.25 mm i.d.), which had been coated with PEG 20M or OV-1 (Gasukuro Kogyo) was set up. The oven temperature was programmed from 60 to 180 °C at 2 °C/min, the injector and detector temperatures being maintained at 200 °C. Nitrogen gas was used as the carrier at a flow rate of 1.2 mL/min. The OV-1 column was used for quantitative analyses because it had been observed that one compound was partially decomposed in the polar column.

Enantiomer Separation GC. A Hewlett-Packard 5890 (Series II) gas chromatograph equipped with FID was used to separate the optical isomers. A fused silica open tubuler column (25 m × 0.25 mm i.d.), which had been coated with CP-cyclodextrin-2,3,6,-M-19 (Chrompack), was set up. The oven temperature was held at 60 °C for 8 min and then programmed to 170 °C at 1 °C/min, the injector and detector temperatures being maintained at 200 °C. Helium gas was used as the carrier at a flow rate of 0.9 mL/min.

Gas Chromatography–Mass Spectrometry (GC–MS). GC–MS spectra were recorded on a JEOL Model DX-300 mass spectrometer, which was combined with a Hewlett-Packard Model 5790 A gas chromatograph. The gas chromatographic conditions were the same as those described under Gas Chromatography. GC–MS was used under an ionization voltage of 70 eV and ion source temperature of 200 °C.

Odor Threshold Determination. The threshold value of pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine was determined by 10 expert panelists who work with perfumes at Takasago Corp. A synthetic sample was dissolved in purified water in a small beaker, and the concentration was reduced step by step at 10^{-1} intervals. The threshold concentration is that at which a significant difference was detected by more than 50% of the panel by using the triangular test method.

RESULTS AND DISCUSSION

Characterization of Volatiles. Table I shows the change of pH values during heating and the yield of volatile concentrate from each boiled bivalve calculated on the basis of the wet weight of edible parts. There is no significant difference in the pH values among the samples, which were maintained in almost the neutral range. The yields of volatiles were less than those of small shrimps (Kubota and Kobayashi, 1987). The odor of the short-necked clam was very weak, and the clam and corbicula presented nutty and roasted odors, respectively. Gas chromatograms of their volatiles are shown in Figure 1. The

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Table I. Change of pH during Heating and the Yield of Volatiles from Boiled Bivalves

sample	pH[a]	volatiles: yield,[b] mg/100 g	volatiles: odor profile
short-necked clam	6.82–7.15	0.34	oily
clam	6.86–7.78	0.30	nutty
corbicula	7.53–7.56	0.82	stimulating, roasted

[a] Before heating–after heating. [b] Wet weight of edible portions.

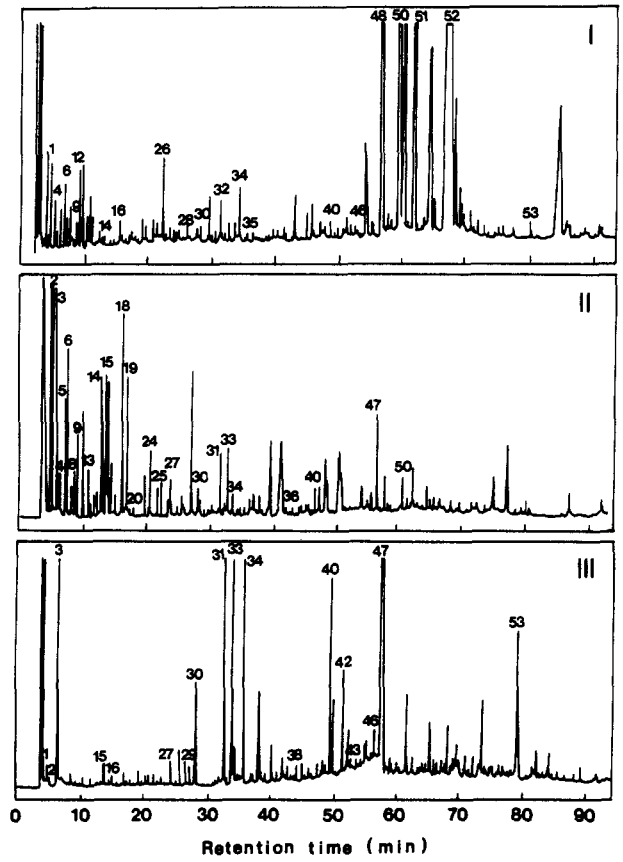

Figure 1. Gas chromatograms of the boiled volatile concentrates from short-necked clam (I), clam (II), and corbicula (III). See Experimental Procedures for the GC conditions.

chromatograms demonstrated quite different profiles. Most peaks were identified by comparing their mass spectra and retention indices from GC with those of reference compounds and are summarized in Table II. The concentrate of the short-necked clam consisted of acyclic carbonyl compounds and alcohols as major components and a trace amount of nitrogen- and/or sulfur-containing compounds. Among them, long-chain aldehydes occupied about 40% of the peak area on GC, although they were only tentatively identified because authentic samples were unavailable. As these compounds have high boiling points, they do not have a large impact on the odor of the short-necked clam. Kawai et al. (1990) have also described that no characteristic impact compounds was detected in the volatile concentrate of short-necked clams heated at 98–101 °C for 65 min. The volatiles of clams consisted of such compounds as carbonyls, alcohols, and nitrogen- and/or sulfur-containing compounds. Among them, it seems that a greater quantity of methylpyrazines than others contributed to the roasted aroma of clam. The major part of the volatiles in corbicula consisted of nitrogen- and/or sulfur-containing compounds. Predominant among these were 1-pyrroline, indole, 3,5-dimethyl-1,2,4-trithiolane, acetylthiazole, and

Table II. Identified Compounds in the Volatiles from Short-Necked Clam (I), Clam (II), and Corbicula (III)

no.[a]	compd	KI (PEG 20M)	peak area % on GC: I	II	III
	Aldehydes				
	ethanal[b]			+	
1	pentanal	909	1.06	0.18	0.20
6	hexanal	1067	0.69	0.51	nd[d]
11	heptanal	1186	0.04	0.09	nd
28	(*E*,*E*)-2,4-heptadienal	1465	0.18	nd	nd
39	(*E*,*E*)-2,4-decadienal	1782	0.08	nd	nd
48	hexadecanal[c]	2098	5.01	nd	nd
50	C_{17} aldehyde[c]	2159	6.35	nd	nd
52	octadecanal[c]	2308	28.0	nd	nd
30	benzaldehyde	1490	0.22	0.69	0.91
49	phenylacetaldehyde	2140	nd	0.26	2.06
	Ketones				
5	2,3-pentanedione	1046	nd	0.04	nd
16	3-hydroxy-2-butanone	1256	0.17	0.01	0.10
17	2-octanone	1262	nd	0.01	0.05
23	2-nonanone	1373	0.20	0.12	nd
29	2-decanone	1479	nd	nd	0.05
32	2-undecanone	1585	0.39	nd	nd
41	2-tetradecanone	1855	nd	nd	0.02
35	acetophenone	1615	0.05	nd	nd
42	β-ionone	1901	nd	nd	1.42
	Alcohols				
2	ethanol	915	0.65	0.11	0.14
4	propanol	1022	0.42	0.44	nd
7	butanol	1121	0.34	0.01	nd
14	pentanol	1238	0.08	1.37	nd
22	hexanol	1343	0.64	0.03	nd
25	heptanol	1310	0.57	0.30	nd
8	1-penten-3-ol	1130	0.31	0.45	nd
26	1-octen-3-ol	1417	0.41	0.13	nd
38	2-phenyl-2-propanol	1779	nd	nd	0.10
40	benzyl alcohol	1829	0.27	0.43	1.96
	Esters				
45	methyl tetradecanoate	1987	0.14	nd	nd
51	methyl hexadecanoate	2203	0.11	nd	nd
	N- and/or S-Containing Compounds				
3	1-pyrroline	990	nd	1.10	12.10
9	pyridine	1168	0.40	0.55	0.19
10	pyrazine	1180	nd	0.01	nd
15	2-methylpyrazine	1241	0.08	2.37	1.18
18	2,5-dimethylpyrazine	1297	nd	1.33	nd
19	2,6-dimethylpyrazine	1303	0.11	1.53	0.37
20	2,3-dimethylpyrazine	1314	0.12	0.17	nd
24	2,3,5-trimethylpyrazine	1382	nd	0.66	nd
53	indole	2398	0.40	nd	2.89
	hydrogen sulfide[b]		+	+	+
31	*trans*-3,5-dimethyl-1,2,4-trithiolane	1568	nd	1.28	3.08
33	*cis*-3,5-dimethyl-1,2,4-trithiolane	1588	nd	1.31	3.52
36	*trans*-3-ethyl-5-methyl-1,2,4-trithiolane	1652	nd	0.79	nd
37	*cis*-3-ethyl-5-methyl-1,2,4-trithiolane	1675	nd	0.38	nd
44	3,6-dimethyl-1,2,4,6-tetrathiane[c]	1961	nd	1.21	0.79
34	acetylthiazole	1597	0.61	0.65	6.13
43	benzothiazole	1903	nd	1.30	0.48
47	pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine	1990	nd	1.33	13.02
	Others				
13	2-pentylfuran	1203	0.08	0.71	nd
12	limonene	1193	0.30	nd	0.06
27	acetic acid	1417	0.20	0.21	0.24
46	phenol	1988	0.09	0.46	1.11

[a] Numbers refer to Figure 1. [b] This was only detected by GC–MS. [c] Tentative identification by MS spectrum. [d] nd, not detected.

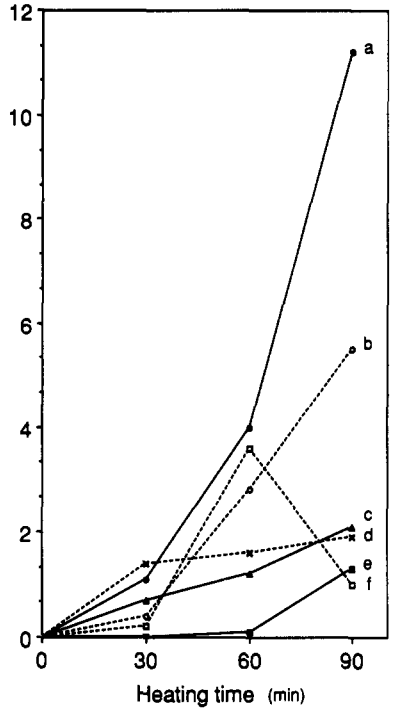

Figure 2. Effect of heating time on the formation of the main volatiles from corbicula. The concentration of each compound is presented as the ratio of the peak area percent from GC to that of the internal standard. (a) Pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine; (b) 1-pyrroline; (c) β-ionone; (d) benzaldehyde; (e) 3,5-dimethyl-1,2,4-trithiolane (cis and trans); (f) acetylthiazole.

pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine (I). In some seafoods, like small shrimp and dried squid, 2,4,6-trimethyl-5,6-dihydro-1,3,5-dithiazine (thialdine) has been found as one of the main products of cooked volatiles (Kawai and Ishida, 1989). However, in the shellfish now reported, thialdine was scarcely detectable, and if it was detected, the concentration was very small. This indicates that little ammonia is formed from these shellfish by heating, and this is one of the differences between the shellfish used here and other seafood. Bicyclic dithiazine (I) was recently found from small shrimp as a novel compound (Kubota et al., 1988). As this compound occupied the greatest part (13.0% on GC) of the volatiles of corbicula, it is likely that this could have contributed to the corbicula flavor. The odor threshold was determined by a trained panel consisting of 10 judges. A threshold of 1.0×10^{-11} ppb of water was given, and this is an extremely low value. An odor description was also carried out, and the odor was described most often as being meaty and onion-like. Its concentration, calculated from the yield and the peak area percent on GC of the volatiles, was about 1.06 ppm in the edible material. These facts indicate that compound I was a significant contributor to the stimulating roasted odor of corbicula. 1-Pyrroline was also identified as another main component of corbicula. Although this compound has been found in the volatiles from dried squid (Kawai et al., 1991), wine (Almy et al., 1983), and white bread crust (Folkes and Gramshaw, 1977), it was scarcely identified in the volatiles of boiled seafood. Since 1-pyrroline has a strong corn-like odor (Yoshizawa et al., 1965), it seems that this is also another strong impact constituent in the odor of corbicula.

Effect of Heating Time on the Volatile Compound Formation. The quantities of the main volatile components of corbicula formed with the increase of heating time were investigated. The quantity was calculated as the ratio of the peak area percent on GC to that of the internal standard and is shown in Figure 2. The nonpolar column was used for this investigation because part of compound I was degraded in the polar column. Acetylthiazole was produced in quantity after 1 h, but some degradation was observed during longer heating. This obviously indicates that as the sample was heated longer, the greater were the quantities of compound I, 1-pyrroline, and 3,5-dimethyl-1,2,4-trithiolane produced. Among them, the increase of compound I was the most marked during 60–90 min of heating.

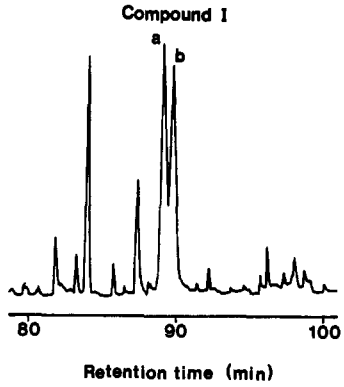

Figure 3. Gas chromatogram of the enantiomeric separation of pyrrolidino[1,2-*e*]-4*H*-dimethyl-1,3,5-dithiazine (I) in the volatiles of corbicula. See Experimental Procedures for the GC conditions.

	KI	
	a	b
synthetic I	1617	1623
compound I	1616	1623

Formation Mechanism for Compound I. Compound I has several stereoisomers because of its three asymmetric carbons in the molecule. In corbicula, one peak was observed on the gas chromatogram, and its retention index coincided with that of the synthetic compound in a similar manner to that with small shrimp (Kubota et al., 1991). In this paper, the volatile concentrate from corbicula boiled for 2 h was analyzed by GC, using a permethylated β-cyclodextrin column (Takeoka et al., 1990), and the gas chromatogram is shown in Figure 3. Compound I was resolved into two enantiomers with a peak area ratio of 51.0:49.0%. The retention indices of each coincided well with those of the racemic synthesized I. From these results, it was concluded that compound I in corbicula was a racemic mixture like that of the synthetic compound. In addition, it was confirmed in our previous study (Kubota et al., 1991) that compound I was synthesized predominantly from hydrogen sulfide, ethanal, and 1-pyrroline. In this paper, both 1-pyrroline and compound I were detected as major components in corbicula at the same time, and these compounds were also identified in clam, although their concentrations were far less. On the other hand, neither 1-pyrroline nor compound I was found in short-necked clam. It is known that 1-pyrroline was the Strecker degradation product from proline and ornithine (Yoshizawa et al., 1965).

From these results, it was postulated that, in the natural product, 1-pyrroline was initially formed from such precursors as amino acids during heating, and that compound I was produced secondarily from 1-pyrroline with ethanal and hydrogen sulfide in the same manner as with the synthetic method. Further details of the precursor are under investigation.

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