Identification of Unknown Methyl Ketones in Volatile Flavor Components from Cooked Small Shrimp

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Two novel ketones in the aroma concentrate from cooked small shrimp, *Euphausia pacifica* Hansen, were isolated. They had a seafood-like aroma. Their structures were estimated by spectroscopic IR, NMR, and MS analyses and were confirmed as (5*Z*,8*Z*,11*Z*)- and (5*E*,8*Z*,11*Z*)-5,8,11-tetradecatrien-2-one by referring to the spectroscopic data of synthesized *all-Z* and *all-E* isomers. These two ketones were also found in the aroma concentrates from two other kinds of cooked small shrimps, *Euphausia superba* Dana and *Sergia lucens* Hansen. The partial structure of natural fatty acids was retained in the molecules, and no other geometrical isomer was found in the three kinds of cooked shrimps; therefore, we concluded that the stereostructures of these compounds were formed in living shrimps.

In a series of reports on the cooked shrimp flavor (Kubota et al., 1980a,b, 1982), the aroma concentrate from *Euphasia pacifica* Hansen produced a simple gas chromatographic aroma profile, the main component being identified as *N*,*N*-dimethyl-2-phenylethylamine (Kubota et al., 1980b). However, two other components in which we were especially interested remained unidentified. They were also found in gas chromatograms of the aroma concentrates extracted from cooked *Sergia lucens* Hansen (a small edible shrimp only found in Suruga Bay, Japan) and *Euphausia superba* Dana (krill from the Antarctic Ocean). Sniffing the effluents from a gas chromatograph of these two components revealed the characteristic aroma of seafood products; therefore, they seemed to be closely related with the flavor of cooked crustaceans. This report describes the identification of the chemical structures of these two previously unknown components.

EXPERIMENTAL SECTION

Sample Preparations. *E. pacifica*, *S. lucens*, and *E. superba* were used as samples. Two kinds of small shrimps, *E. pacifica* and *S. lucens*, were caught on the Japan coast, and the other one, *E. superba*, was brought from the Antarctic Ocean. All of these shrimps were frozen immediately after being caught and kept frozen below −20 °C. The aroma concentrates from cooked shrimps were prepared by using a modified Likens and Nickerson's apparatus (Nickerson and Likens, 1966). One kilogram of frozen samples with 1 L of deionized water was refluxed for 2 h, and 50 mL of purified diethyl ether was used for extraction. After being dried with anhydrous sodium sulfate, the solvent was distilled out at 40 °C to obtain an aroma concentrate. From 20 kg of *E. pacifica* was obtained 240 mg of aroma concentrate, which was used for separating and identifying the two unknown compounds.

Gas Chromatography (GC). *Analytical GC.* A Shimadzu Model 7A gas chromatograph equipped with a flame ionization detector was used for the purpose of analyzing. A fused silica capillary column (50 m × 0.25 mm (i.d.)), which had been coated with Carbowax 20M, was set. The oven temperature was programmed from 60 to 180 °C at 2 °C/min, the injector and detector temperatures being maintained at 220 °C. Nitrogen gas was used as the carrier with a flow rate at 1.2 mL/min, and the sample injection system was operated with an injection split ratio of 30:1.

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Preparative GC. A Shimadzu Model 4APF gas chromatograph equipped with a thermal conductivity detector was used to trap the effluents directly from the detector outlet. The glass column (3 m × 4 mm (i.d.)) was packed with 10% FFAP on Chromosorb W (60–80 mesh). The oven temperature was programmed from 60 to 180 °C at 4 °C/min, and the injector and detector temperatures were maintained at 200 °C. The flow rate of helium gas was 30 mL/min.

The unknown compounds were clearly separated under these conditions, each effluent being trapped in a U-shaped, 10 cm × 2 mm (i.d.) glass capillary tube kept below −70 °C.

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 5790A gas chromatograph. The gas chromatographic conditions were the same as those described in Analytical GC, except for using helium gas as the carrier instead of nitrogen gas. GC–MS was used under an ionization voltage of 70 eV and ion source temperature of 200 °C.

NMR Spectral Analyses. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on JEOL Model FX-90 (90 MHz) and Brucker Model AM 500 spectrometers, respectively. Samples were dissolved in $CDCl_3$ containing tetramethylsilane as the internal standard.

Infrared Spectral Analyses (IR). IR spectra were recorded using a Jasco Model IRA-1 infrared spectrometer, the samples being analyzed as KBr micropellets (5-mm diameter).

RESULTS AND DISCUSSION

Isolations of the Unknown Compounds. The unknown compounds (A and B) appearing on the gas chromatogram of the aroma concentrates obtained from *E. pacifica* are shown as peaks A and B, respectively, in Figure 1(I). The same compounds were identified on the gas chromatogram from *E. superba* (Figure 1(II)) and *S. lucens* (Figure 1(III)) from their GC retention times and mass spectra. The mass spectra of peaks A and B were almost the same (Table I), and they are assumed to have been stereoisomers; however, the information obtained from the GC–MS data was not enough to determine the structures of the unknown compounds. Therefore, further spectroscopic analyses were performed on pure samples of compounds A and B isolated by the preparative gas chromatography already described.

Identification of the Unknown Compounds. (*1*) *Compound A.* A colorless liquid with a fishy odor pro-

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Table I. Spectral Data of Peak A (Compound A) and Peak B (Compound B)

instrument	spectral data: peak A	peak B
MS, m/z (%)	M^+ 206 (0.6), 148 (19), 119 (16), 105 (18), 95 (21), 91 (26), 79 (66), 67 (26), 55 (16), 43 (100)	M^+ 206 (0.7), 148 (18), 119 (13), 105 (16), 95 (16), 91 (23), 79 (65), 67 (27), 55 (16), 43 (100)
^{1}H NMR (90 MHz, $CDCl_3$, δ)	0.96 (3 H, t, J = 7.2 Hz), 1.9–2.3 (4 H, m), 2.44 (2 H, br t, J = 5.4), 2.68–2.90 (4 H, m), 5.1–5.6 (6 H, m)	0.96 (3 H, t, J = 7.2 Hz), 1.9–2.4 (4 H, m), 2.44 (2 H, br t, J = 5.4), 2.64–2.90 (4 H, m), 5.1–5.7 (6 H, m)

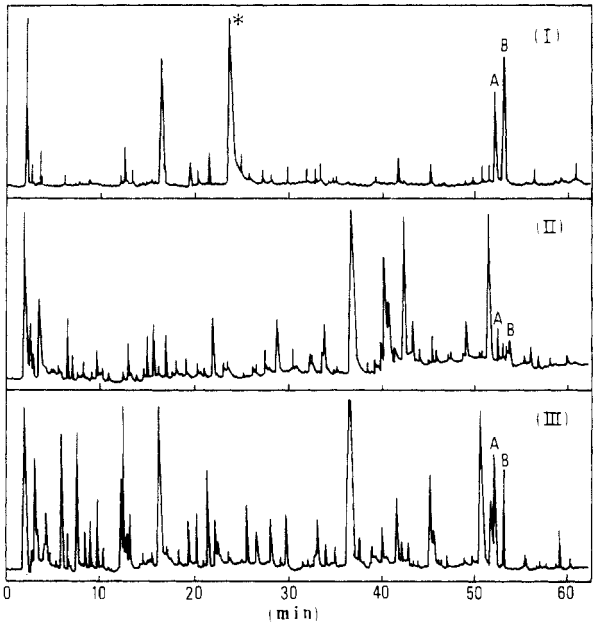

Figure 1. Gas chromatograms of the cooked aroma concentrates from *E. pacifica* Hansen (I), *E. superba* Dana (II), and *S. lucens* Hansen (III). See the Experimental Section for GC conditions. Key: A, peak A; B, peak B; *, *N,N*-dimethyl-2-phenylethylamine.

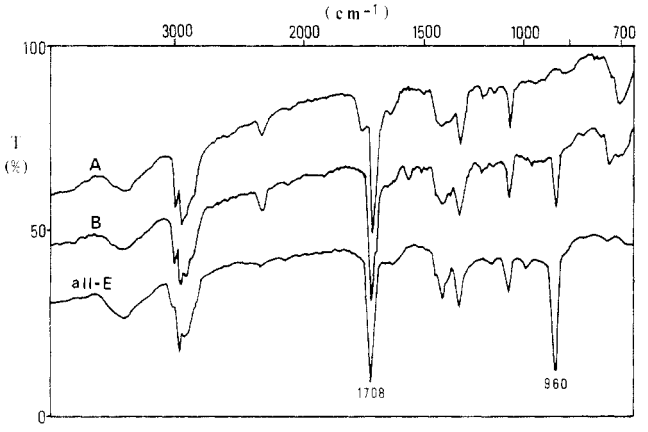

Figure 2. Infrared spectra of compound A (A), compound B (B), and synthetic (5*E*,8*E*,11*E*)-5,8,11-tetradecatrien-2-one (*all-E*).

duced only one sharp peak on the gas chromatogram, and its mass spectrum coincided with that of peak A from the aroma concentrates.

The IR spectrum of compound A is shown in Figure 2 with those of other compounds, and MS and ^{1}H NMR data are summarized in Table I. The absorption spectra of the $COCH_3$ (1708 and 1355 cm^{-1}) and =CH (3000 cm^{-1}) moieties were observed in IR. ^{1}H NMR indicated the following moieties: CH_2CH_3 δ 0.96 (3 H, t), $COCH_3$ δ 2.12 (3 H, s), $=CHCH_2CH=$ δ 2.80 (4 H, m), CH=CH δ 5.36 (6 H, m). The partial structure $(CH{=}CHCH_2)_3$ was elucidated by ^{1}H NMR spectra. Mass spectra indicated the presence of an acetyl group by the base peak at m/z 43. The molecular weight was calculated from the M^+ ion at m/z 206. In addition, the structure $CH{=}CHCH_2CH_2C$-

Table II. ^{13}C NMR Chemical Shifts of Compound A, Compound B, and Synthetic (5*E*,8*E*,11*E*)-5,8,11-Tetradecatrien-2-one (δ, 500 MHz, $CDCl_3$, Internal Me_4Si)

$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH_2COCH_3$

14 13 12 11 10 9 8 7 6 5 4 3 2 1

	A	B	*all-E*[a]
C-1	29.9	30.0	29.9
C-3	43.5	43.5	43.5
C-4	21.7	26.8	26.8
C-7	25.6	30.3	35.5
C-10	25.6	25.5	35.5
C-12	134.0	132.0	132.8
C-13	20.6	20.6	25.6
C-14	14.3	14.3	13.8
C-5,6,8,9,11	127.1	127.1	127.4
	127.9	127.6	128.8
	128.2	128.9	129.0
	128.6	129.4	129.7
	129.1		

OCH_3 was indicated by the (M^+ – CH_3COCH_3) peak at m/z 148, and compound A was elucidated as 5,8,11-tetradecatrien-2-one. Moreover, from the lack of IR absorption at around 960 cm^{-1}, it was estimated that all of the double bonds had a *Z* configuration. Conclusively, the structure of compound A was determined by referring to appropriate stereoisomers synthesized in our laboratory.

(2) *Compound B.* The IR spectrum of compound B (Figure 2) shows the absorption of the *E* double bond at 960 cm^{-1}. When the ^{1}H NMR spectrum was compared with that of compound A, it was observed that the methylene protons between the two double bonds were shifted to a lower field by 2.0 ppm and that the multiplet behaviors of the protons on the double bonds were different. Since these data indicated the presence of the *E* double bond in the molecule, the *all-E* isomer of 5,8,11-tetradecatrien-2-one was synthesized in this laboratory; however, the IR absorption of the *all-E* isomer at 960 cm^{-1} was stronger than that of compound B (Figure 2). These two compounds were separated from each other on the gas chromatogram.

Since these results suggested that compound B had both the *Z* and *E* double bonds in its structure, a comparison between the ^{13}C NMR spectrum (500 MHz) of compound B and those of synthetic (*all-E*)- and (*all-Z*)-5,8,11-tetradecatrien-2-one was made in order to determine the positions of the *E* and *Z* double bonds. The observed results are shown in Table II. An assignment from ^{13}C NMR was made by referring to the literature (Silverstein et al., 1981; Gunstone et al., 1977) and from the results of INEPT (insensitive nuclei enhanced by population transfer). The chemical shifts of the C-7 and C-10 methylene carbon atoms were the same in both the synthesized *all-E* (δ 35.5) and *all-Z* (δ 25.6) isomers; however, in the case of compound B, each chemical shift of C-10 (δ 25.5) and C-7 (δ 30.3) was clearly separated. The similarity between the δ values of C-10 in compounds A and B suggested that the C-10 methylene in the compound B molecule was placed between two *Z* double bonds. On the other hand, the C-7 methylene in compound B was not situated between either

two Z or two E double bonds. In addition, the chemical shift of C-13 (δ 20.6) of compound B coincided with that of the *all-Z* isomer, while the chemical shift of C-4 (δ 26.8) coincided with that of the *all-E* isomer. Conclusively, compound B was determined as the 5*E*,8*Z*,11*Z* isomer of compound A, i.e. (5*E*,8*Z*,11*Z*)-5,8,11-tetradecatrien-2-one.

The partial structure and configuration of compound A are same as those of linolenic acid, which could be a precursor of these ketones. However, if a methyl ketone is derived from a natural fatty acid, it is natural that an odd-numbered carbon ketone should be produced (Toda et al., 1982). Therefore, there might be some other metabolic pathway that can produce even-numbered methyl ketones. Compound B had an E double bound in its structure; however, it retained two Z configurations, and no other E isomers could be found through this study. We assume that compound B was originally present in raw shrimps and was not isomerized from compound A during the heating process. Through these discussions and results, we are interested in the occurrence of the ketones in crustaceans and in their contribution to the characteristic seafood flavor. Studies on the identification and formation of these new ketones are continuing.

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Effect of Nitrogen Source on Pyrazine Formation

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The role of the nitrogen source in pyrazine formation in model systems containing glucose and base was examined. The distribution of pyrazines formed in the reactions containing ammonium hydroxide, ammonium formate, ammonium acetate, glycine, and monosodium glutamate depends strongly on the nature of the nitrogen source. Pyrazines were identified by mass spectrometry and by means of Kovat's indices on polar and nonpolar fused silica capillary columns. A novel mechanism for a Strecker degradation and cleavage of glutamate of acetaldehyde and 2-hydroxyacetate is proposed.

The pyrazines have been recognized as important flavor constituents of a large number of cooked, roasted, and toasted foods (Maga, 1982). The latter discovery of naturally occurring pyrazines in a variety of biological systems further illustrates their ubiquity, remarkable physiological activity, and potency (Murray et al., 1970; Attygalle and Morgan, 1984).

The production of pyrazines from the reaction of carbohydrates and amine compounds has been studied extensively over the past several years. Hodge et al. (1972) proposed that amino acids and carbohydrates were important precursors for pyrazines formed during the nonenzymatic browning reaction. Koehler and Odell (1970) and Rizzi (1972) obtained pyrazines as products of lipid autoxidation. Ferretti et al. (1970) obtained pyrazines from the reaction of lactose with casein, while Wang and Odell (1973) demonstrated pyrazine formation from amino hydroxy compounds. Velisek et al. (1976) reported pyrazine formation from the reaction of glyoxal and glycine, and Davidek et al. (1977) reported dehydro-L-ascorbic acid reacted with ammonia or glycine to produce pyrazines.

Formation pathways for pyrazines and other heterocyclic compounds such as quinoxalines, imidazoles, and pyridines have been proposed by numerous researchers (Rizzi, 1972; Walradt et al., 1971; Shibamoto and Bernhard, 1977a,b, 1978). By far the most detailed and extensive formation pathways of alkylpyrazines have been proposed by Shibamoto and Bernhard (1977a,b): sugars react with amines with the formation of α-amino carbonyl intermediates, which condense to produce pyrazine compounds.

There is still much speculation as to the way in which the nitrogen atoms are incorporated into the pyrazine molecule. Free ammonia formed as a result of the decomposition of the amino acids may combine with sugars to form pyrazines. Another hypothesis suggests that nitrogen still bound to the amino acid may react with sugars to form pyrazines. Newell et al. (1967) reported that the same pyrazines formed regardless of the amino acid utilized. van Praag et al. (1968) reacted various amino acids with fructose and reached the same conclusion. Both groups assumed that free ammonia was the primary intermediate in pyrazine formation, thus resulting in the same series of pyrazines for all of the amino acids. Wilkins and Lin (1970) also assumed that free ammonia was nec-

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