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Musty Odor in Chronically Off-Flavored Channel Catfish: Isolation of 2-Methylenebornane and 2-Methyl-2-bornene

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Chronically off-flavored fish from six commercial channel catfish ponds were sampled during October–December for volatile compounds. Two compounds were consistently isolated from these fish at greater than 0.1 ppm concentrations. The compounds were identified as 2-methylenebornane (1) and 2-methyl-2-bornene (**2**). They were present both with (one pond) and without (five ponds) detectable (2 ppb) concentrations of the known muddy odor producing compound 2-methylisoborneol (MIB). The mass spectra of the compounds isolated from fish tissue are reported and are identical with those of reference standards of 1 and 2. The dehydration products of MIB, 1 and 2, both impart a musty odor and are believed to be the cause of off-flavor in these instances.

Commercially cultured channel catfish (*Ictalurus punctatus*) are often afflicted with a musty odor in their edible flesh that renders the fish unmarketable. This odor has been associated with the presence of 2-methylisoborneol (MIB) (Martin et al., 1987) and geosmin (Lovell et al., 1986). Both compounds are odorous secondary metabolites of certain species of cyanobacteria (Izaguirre et al., 1983; Slater and Blok, 1983; Sugiura et al., 1986) and actinomycetes (Sivonen, 1982; Gerber, 1983).

Although the musty odors produced by these two compounds are similar, they have been differentiated by processor taste panels. The catfish industry has applied the term "blue green" (associated with dense algal blooms) to describe the MIB type of off-flavor and "woody" (decaying woodlike odor) to the geosmin type of off-flavor (Marshall, 1987). During the summer months when off-flavor problems are most severe, these two major types of off-flavor afflict 50–70% of the ponds involved in commercial channel catfish culture in Western Mississippi (Marshall, 1987). We have used the difference in odor to study MIB, the more commonly encountered type of off-flavor.

Several species of cyanobacteria (Slater and Blok, 1983) and actinomycetes (Gerber, 1983) produce volatile excretion products under appropriate environmental conditions (Persson, 1982; Sivonen, 1982). The off-flavor phenomenon is most commonly associated with high water temperatures, high fish-stocking densities, and heavy-feeding regimes consistent with the production of a eutrophic environment (Brown and Boyd, 1982; Persson, 1982; Juttner, 1984). In a previous summer study (Martin et al., 1987) 2-methylisoborneol was isolated from mud, water, and fish at concentrations capable of causing off-flavor. The onset of off-flavor was correlated with the isolation of greater than 10 ppb concentrations of 2-methylisoborneol in the water and sediment and 100–200 ppb in fish muscle tissue. In all cases the phytoplankton population was dominated by a dense population of *Oscillatoria agardhii* undergoing senescence and fragmentation. In the winter months, phytoplankton communities are usually not dominated by cyanobacteria and conceivably the musty flavors are not directly attributable to production of metabolites by aquatic microorganisms. Dense phytoplankton populations are often present when fish are judged to be off-flavor. However, off-flavor in channel catfish has been inconsistently associated with dense algal blooms (Armstrong et al., 1986).

The objective of this investigation was to isolate odorous compounds that could be responsible for off-flavor in cultured channel catfish during winter months. In particular, investigations were conducted on six commercial culture ponds that contained fish that were known to be off-flavor due to MIB in the summer months, but remained off-flavor throughout the winter.

MATERIALS AND METHODS

Isolation and Quantitation of Off-Flavor Volatiles from Water and Sediment. Water samples (4 L) were collected bimonthly from six commerical channel catfish culture ponds in west-central Mississippi from a single point approximately 10 cm below the surface. To each, 40 mg of mercury(II) chloride and 60 mL of a 10 ppm solution of camphor (in acetone) were added as a bacterial inhibitor and internal standard, respectively. A subsample of the water (600 mL) was extracted in duplicate by closed-loop stripping for 2 h to isolate volatile organic compounds (Hwang et al., 1984). Volatile organics were separated and quantitated by capillary gas chromatography (GC) and further characterized by gas chromatography/mass spectroscopy (GC/MS). Five known musty odor producing compounds were used as standards: 2-methylisoborneol (MIB), geosmin, 2-isopropyl-3-methoxypyrazine, 2-isobutyl-6-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine.

Sediment samples (approximately 100 g wet weight) were collected from a point 10 m from the shoreline by excavating the upper 1–2 cm of sediment for a distance of 5–10 m. Mercury(II) chloride (10 mg) and camphor (50 ng) (1 ppb solution in acetone) were then added. The sediment samples (50 g wet weight) were blended with 550 mL of distilled water and subjected to closed-loop stripping as used with the water samples. The volatile extract from the sediment was then analyzed by capillary gas chromatography. The sediment was dried and the concentration of the volatiles reported on a dry-weight basis.

Isolation of the Volatile Fraction from Channel Catfish. Six fish from each of the culture ponds were caught bimonthly by hook and line and transported at 0 °C to the laboratory. Four of the channel catfish were subjected to microwave distillation to isolate the volatile compounds (Martin et al., 1987). After the distillation was complete, the volatiles were thawed. The hexane layer was separated and concentrated to 100 μL under a constant flow of nitrogen.

In addition, two of the channel catfish were subjected to organoleptic analysis by a trained industrial taste panel. In this method the fish fillets are sealed in brown paper bags and cooked in a microwave oven at high power for

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Table I. Concentration of 2-Methyl-2-bornene (1) and 2-Methylenebornane (2) in Off-Flavored Channel Catfish Collected in December from Commercial Catfish Ponds in Mississippi

pond	concn, μg/kg 1	 2	duration of off-flavor, months
T12	3860 ± 810	1050 ± 160	18–20
T21	534 ± 46	91 ± 18	8–9
T22	2620 ± 510	337 ± 46	24
F31	267 ± 31	35 ± 4	7
F22	241 ± 36	25 ± 1	4
F1	155 ± 28	5.4 ± 1.0	5

5 min. Subsequently, the bags were opened and the aroma and taste characterized. The fish samples were designated acceptable or unacceptable, and the off-flavor was described.

Dehydration of 2-Methylisoborneol. The procedure of Lapalme et al. (1979) was used to prepare authentic samples of 2-methylenebornane (1) and 2-methyl-2-bornene (2) by the dehydration of MIB using thionyl chloride in dry pyridine. The MS obtained for 2 (see Figure 3b) was identical with the MS of 2, prepared from (+)-camphor (tolylsulfonyl)hydrazone and methyllithium, as reported by Burgstahler et al. (1976). The MS of 1 (Figure 4B) was also the same as that previously reported by Takai et al. (1980) and Walter et al. (1983).

Instrumentation. Quantitative measurements of MIB, geosmin, the three pyrazine isomers, and dehydration products 2-methyl-2-bornene and 2-methylenebornane were conducted on a Perkin-Elmer gas chromatograph, Model Sigma 3, equipped with a flame ionization detector and a commercially available capillary column DB-5 [J&W Scientific; 30 m × 0.32 mm (i.d.) fused silica; 5% phenyl methyl silicone]. The operating temperatures were as follows: column, 40–250 °C, programmed at 8 °C/min with a 2-min initial hold; injector, 275 °C; detector, 325 °C. Helium at a flow rate of 10 mL/min was used as the carrier gas. Two microliters of the fish, sediment, or water volatile extracts was injected and split 3:1.

The identities of MIB and products 1 and 2 were confirmed on a Hewlett-Packard 5970 GC-MS system (Hewlett-Packard, Palo Alto, CA) equipped with a commercially available (HP1) 25 m × 0.25 mm (i.d.) flexible fused silica capillary column coated with cross-linked methyl silicone (Hewlett-Packard). The operating temperatures were as follows: column, 40–200 °C, programmed at 8 °C/min; injector, 200 °C. Sample volume was 0.5 μL, splitless injection. Helium at a flow rate of 1 mL/min was used as the carrier gas.

RESULTS AND DISCUSSION

Ponds selected for the study had been producing off-flavored fish for at least 4 months based on organoleptic testing records (Delta Pride Inc., Indianola, MS). The off-flavor was described as intense and of the MIB type. The dehydration products had been detected in several other chronically off-flavor culture ponds in the summer and fall months, but appreciable concentrations of MIB (2 ppb) were also present. Presumably, if 1 and 2 were capable of causing off-flavor, they would be detectable in chronically off-flavored fish sampled during the winter months when MIB was absent.

Off-flavored fish sampled during October, November, and December contained elevated concentrations of 1 and 2, whereas MIB was absent from fish, water, and sediment (Table I). In one pond water sample (T-12), an MIB concentration of 0.28 ± 0.06 ppb was initially detected (Figure 1, Oct 15 sample). However, no MIB was isolated from the T-12 (Oct 15) fish samples. These fish contained ppm concentrations of the MIB dehydration products (Figure 2). Detectable concentrations of MIB or the other four volatile off-flavor-producing standards were not found in subsequent sediment, water, and fish samples. In all the ponds GC analysis of volatiles from the off-flavored fish resulted in the isolation of 1 and 2 at concentrations of 0.005–1 and 0.1–4 ppm, respectively (Figure 1).

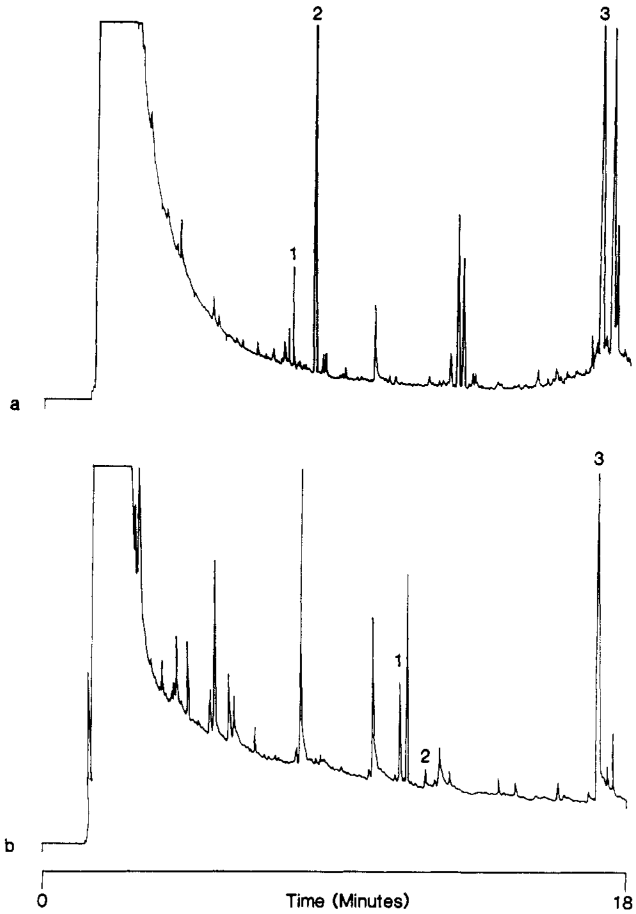

Figure 1. (a) Capillary gas chromatogram of off-flavor fish extract: (1) 2-methylenebornane; (2) 2-methyl-2-bornene; (3) 1-chlorododecane. (b) Capillary gas chromatogram of extract of pond water producing off-flavor fish: (1) camphor (internal standard); (2) 2-methylisoborneol; (3) 1-chlorododecane (time marker).

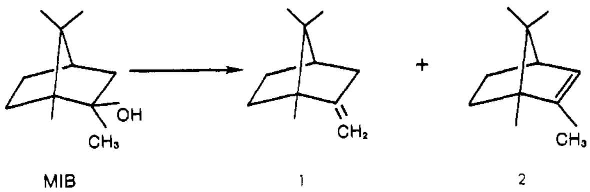

Figure 2. Dehydration products of MIB.

The two major components of the GC of winter off-flavor catfish (peaks 1 and 2, Figure 1a) had identical relative retention times on both DB5 and HP1 fused silica capillary columns as authentic samples of 1 and 2. Also, their MS were identical with those reported in the literature (Takai et al., 1980; Burgstahler et al., 1976) and matched the MS of authentic samples of 1 and 2 prepared as described in the Experimental Section (Figures 3 and 4).

Control experiments were done to correlate concentrations of the products isolated with the musty off-flavor in fish flesh. Additional experiments were designed to quantitate the concentrations of 1 and 2 isolated from fish

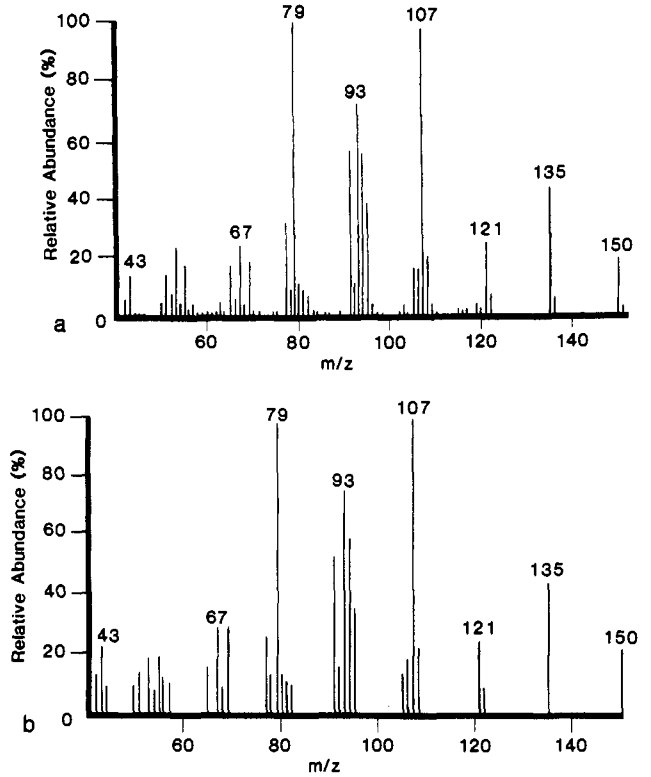

Figure 3. Mass spectra (EI): (a) peak 2 of Figure 1a; (b) authentic 2-methyl-2-bornene (2).

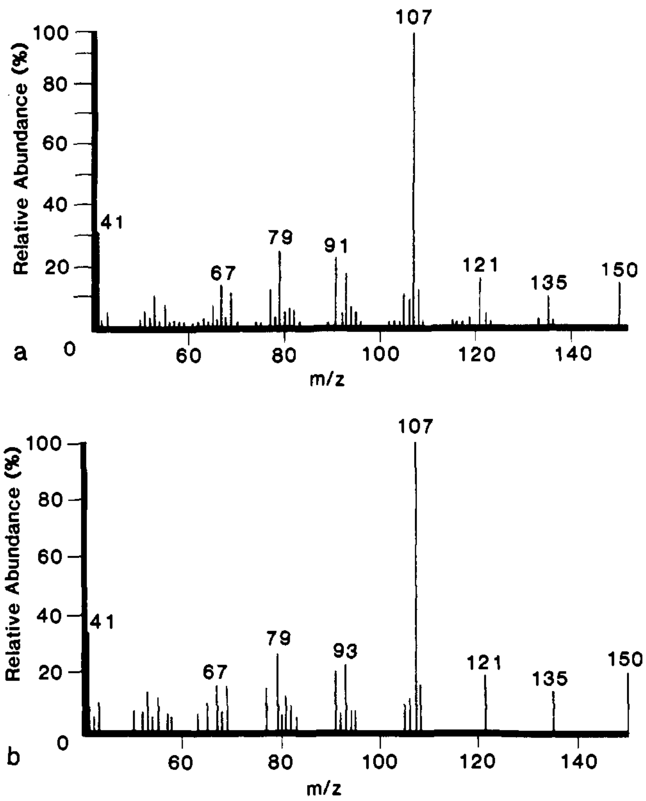

Figure 4. Mass spectra (EI): (a) peak 1 of Figure 1a; (b) authentic 2-methylenebornane (1).

flesh and determine whether these products were produced during the microwave distillation process. The compounds produced by the reaction of 2-methylisoborneol with thionyl chloride in pyridine imparted a musty odor in dilute 0.1 ppm aqueous solution and have been reported as odorous compounds (Burgstahler et al., 1976). When on-flavor catfish (50 g) enriched with a mixture of 1 and 2 was subjected to microwave distillation, a 56 ± 4% recovery of the products was achieved. Also when channel catfish without objectionable flavor were enriched with 500 ng of MIB and subjected to microwave distillation, no dehydration products were observed in the GC. This latter experiment shows the MIB did not dehydrate in our extraction procedure or instrumental analysis method. These control experiments clearly establish that 1 and 2 are present in off-flavor catfish and are not formed in the microwave distillation procedure or in the GC.

It has been reported that the mass spectrum (shown in Figure 4) is actually the spectrum of 2-methylisoborneol when the mass spectrometer "source and rods are dirty" (McGuire, 1985). It is possible that 2-methylisoborneol underwent dehydration in acidic media prior to mass spectroscopic analysis in this report. Earlier references (Burgstahler et al., 1976; Takai et al., 1980) correctly identify the mass spectra with the MIB dehydration products and not the parent compound.

It is our contention that when fish are exposed to MIB for long periods, 2-methylisoborneol undergoes dehydration in fish flesh and is stored as the hydrocarbon products (Figure 2). These compounds appear to impart a musty odor to fish flesh and may be more slowly eliminated than the parent compounds (2-methylisoborneol), possibly due to a slower metabolism at the colder water temperatures encountered during the winter months and the more hydrophobic nature of the products. It is probable that when MIB is present in fish flesh, off-flavor is due not only to 2-methylisoborneol but also to 1 and 2. However, these compounds (1 and 2) were also isolated from off-flavored fish in the apparent absence of MIB during the winter months. Management practices to limit the severity and duration of off-flavor during the summer and fall months may be desirable in certain cases to circumvent protracted cases of off-flavor caused by MIB dehydration products in fish tissue during the cooler months of the year.

Registry No. 1, 27538-47-2; 2, 72540-93-3; MIB, 2371-42-8.

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Stereoisomeric Flavor Compounds. 18. Enantiodiscrimination of Chiral Flavor Compounds by Diastereomeric Derivatization

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The stereodifferentiation of chiral secondary alcohols, 4(5)-alkyl-substituted $\gamma(\delta)$-lactones via corresponding 1,4(1,5)-diols, chiral 1,3-diols, and chiral 1,3-monothioglycols was carried out by diastereomeric derivatization with (*S*)-*O*-acyllactyl chlorides as chiral auxiliaries. The acetyl, propionyl, isobutyryl, and hexanoyl moieties, respectively, were used as protecting groups of the alcoholic function of the chiral auxiliary lactyl chloride. These methods allow evaluation of the enantiomeric composition of the above-mentioned naturally occurring flavor components by HRGC in an inexpensive, convenient, and reliable manner.

1. INTRODUCTION

"Chiral recognition" has been found one of the most exciting principles in biological activity. Intensive efforts were started in pharmacology, pharmacy, and medicine, since one has recognized that enantiomeric drugs exhibit pharmacodynamic and pharmacokinetic differences and metabolic interactions (Williams and Lee, 1985; Testa, 1986). In the field of flavor chemistry too, it is well established that chiral discrimination is an important principle of odor perception (Ohloff, 1986).

Therefore, research on structure-activity relationships can be connected with the following targets: biogenesis and evaluation of the optical purity of naturally occurring chiral flavor compounds; analytical differentiation between natural and nature identical chiral flavor components; development of efficient and reliable analytical methods for the determination of enantiomeric (diastereomeric) volatiles by HRGC, HRGC/MS, and HPLC with chiral and achiral phases (Mosandl et al., 1987).

Referring to recently published results (Deger et al., 1986; Mosandl et al., 1987), this paper demonstrates that optically pure references with definite chirality are indispensable for reliable interpretation of chiral stereodifferentiation in flavor chemistry.

The enantiodifferentiation of chiral secondary alcohols, 4(5)-alkyl-substituted $\gamma(\delta)$-lactones via corresponding 1,4(1,5)-diols, alkane-1,3-diols, and chiral 1,3-monothioglycols by diastereomeric esters of (*S*)-*O*-acetyl, propionyl-, isobutyryl-, and hexanoyllactic acid esters, was proved to be a convenient method for chirality evaluation by HRGC on the achiral phase DB 210-30W.

2.0. EXPERIMENTAL SECTION

Synthesis and Separation of Compounds. *2.1. Diastereomeric Carbamates from (R)-1-(1-Naphthyl)ethyl Isocyanate and Octan-2-ol (Nonan-2-ol).* (*R*)-(−)- (70%) and (*S*)-(+)-alkan-2-ol (30%) were mixed to yield alkan-2-ol solutions of definitive ee values. These solutions were used for synthesis of diastereomeric esters of (*R*)-2-phenylpropionic acid (HTS) and for synthesis of diastereomeric carbamates from (*R*)-1-(naphthyl)ethyl isocyanate (Deger, 1988). The elucidation of absolute configuration of alkan-2-ols from their corresponding diastereomeric HTS esters is a well-established method (Helmchen and Schmierer, 1976).

Therefore, ^1H NMR spectroscopy of mixtures of diastereomeric HTS alkan-2-yl esters is an unambiguous method for the interpretation of the HRGC behavior of their corresponding diastereomeric carbamates from (*R*)-1-(naphthyl)ethyl isocyanate.

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