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Identification of Musk Xylene and Musk Ketone in Freshwater Fish Collected from the Tama River, Tokyo

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Musk xylene(5-tert-butyl-2,4,6-trinitroxylene) and musk ketone(2-acetyl-5-tert-butyl-4,6-dinitroxylene), artificial perfumes widely used in Japan, have been identified in environmental biota for the first time. These compounds were isolated from freshwater fish(Carassius auratus langsdorfii) collected from the Tama River in the Tokyo area and confirmed by gas chromatography-mass spectrometry(GC-MS). The levels of musk xylene and musk ketone in a pooled sample were approximately 0.2 and 0.05 ppm on wet weight basis, respectively.

MATERIALS AND METHODS

Samples. Fish samples(C. auratus langsdorfii)were collected at Noborito downstream in the Tama River on August 28, 1980.

Procedure. A pooled sample(600 g) was homogenized, dehydrated with sodium sulfate and extracted three times(2.0,1.0,0.5 L) with an n-hexane-dichloromethane mixture(1:1). After filtration with a glass filter, the extract was concentrated in a Kuderna-Danish evaporator. The residue was dissolved in n-hexane(150 mL). Purification of the hexane extract was conducted according to the following three cleanup procedures: partition, saponification, and column and thin-layer chromatography.

In the first step, the hexane from sample extracts was partitioned with acetonitrile(150 mL x 3). After evaporation to 50 mL, the acetonitrile was poured into 2% NaCl solution(500 mL), and in turn, the aqueous solution was extracted with dichloromethane(100 mL x 3). The solution was dried over sodium sulfate and concentrated almost to dryness.

In the second steps, the residue from partition was refluxed for 1 h with 50 mL of 1-N methanolic KOH(YAMAGISHI et al. 1979). The solution was poured again into 2% NaCl solution; it was extracted with dichloromethane(50 mL x 3) and concentrated to near dryness.

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In the third steps, the residue was chromatographed on a Florisil column with an n-hexane-dichloromethane mixture(90:10) and an n-hexane-dichloromethane-acetonitrile mixture(90:9:1). The Florisil column was packed with 22 g Florisil(PR grade 60-100 mesh, activated at 650°C for 18 h) in a glass column(2.3 x 10 cm). After the elution with 200 mL of an n-hexane-dichloromethane mixture(Fraction I), the substances were recovered by elution with 200 mL of an n-hexane-dichloromethane-acetonitrile mixture(Fraction II). Fraction II was concentrated to approximately 0.5 mL. Further, the sample was cleaned up by thin-layer chromatography on silica gel(hexane-dichloromethane, 90:10) and submitted to GC-MS analysis.

All solvents and reagents were pesticide grade or equivalent. Musk xylene and musk ketone, the reference compounds, were obtained commercial product(INOUE MFG. Co., Ltd., Japan).

Apparatus. GC analysis was conducted using an electron capture with a ^{63}Ni(ECD) and a dual flame ionization detection(FID): columns; 3% OV-1 on 80-100 mesh, Shimalite AW BW DMCS, at 190 °C, N_2 60 mL/min; 3% OV-17 on 60-80 mesh, Gas Chrom Q, at 200 °C, N_2 75 mL/min; OV-17+OV-210; 1.5% and 1.95% on 80-100 mesh, Chromosorb W AW DMCS, at 190 °C, N_2 75 mL/min; column sizes; glass columns, 3 mm ID x 2 m; injection and detector temperature; 240 °C.

GC-MS analysis was performed on a JEOL JMS-D300 JMA 2000 Disc system; 70 eV; CI; CH_4 at 1 torr; column; 3% OV-1 on 100-120 mesh Chromosorb W AW DMCS, at 210 °C 2 mm ID x 1.8, He 30 mL/min, injection and separator temperature; 250 °C.

RESULTS AND DISCUSSION

An ECD gas chromatogram of two unknown compounds obtained from the fish sample is shown in Fig.1. In addition to the unknown compounds P-1 and P-2, a group of polar compound such as dieldrin, heptachlor epoxide, and CNP(1,3,5-trichloro-2-(4-nitrophenoxy)benzene) were present in fraction II. Retention times of P-1 and P-2 were, respectively, at 1.9 and 2.6 min on a 3% OV-1 column(Table 1).

As preliminary tests to characterize these substances, three experiments were conducted. First, it was observed, indicating that both substances of P-1 and P-2 were not effected by refluxing with methanolic KOH. Second, when treated with concentrated sulfuric acid, these substances were decomposed. Third, in a column chromatography on Florisil under the condition of this

TABLE 1. Retention Times(min) on Unknown Compounds of P-1 and P-2, and Relative to Aldrin and Other Organic Compounds.

Compound	OV-1	OV-17	OV-17+OV-210
P-1	1.90	3.34	3.00
P-2	2.67	5.89	5.20
Musk Xylene	1.90	3.34	3.00
Musk Ketone	2.67	5.89	5.20
Heptachlor Epoxide	3.65	6.74	5.20
Dieldrin	5.20	10.63	8.06
NIP[1)]	5.67	15.10	11.94
CNP[2)]	7.45	20.04	15.36
Aldrin	2.90	4.55	3.48

1). 1,5-dichloro-2-(4-nitrophenoxy)benzene.
2). 1,3,5-trichloro-2-(4-nitrophenoxy)benzene.

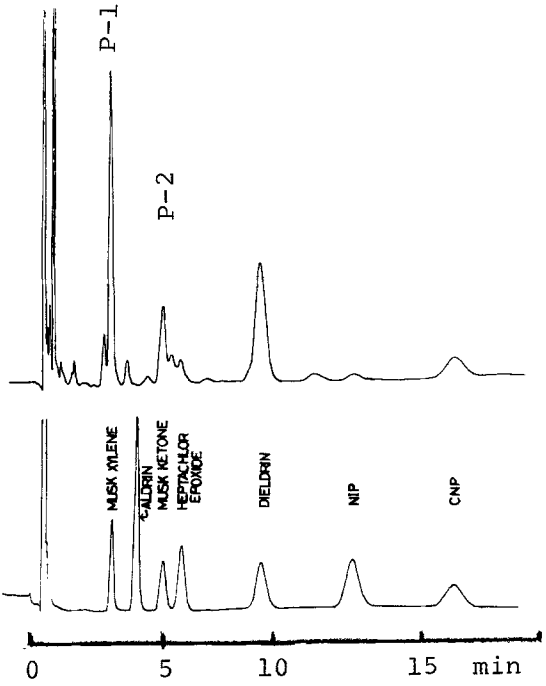

Fig. 1. A Typical ECD Gas Chromatogram of the Unknown Compounds obtained from Fish Sample.

3% OV-1
190°C
N_2 75 mL/min

study, these substances were not eluted by an n-hexane-dichloromethane mixture(90:10).

But P-1 and P-2, which adsorbed by the support in the same column were partially recovered by elution with a solvent mixture of n-hexane-dichloromethane-acetonitrile(90:9:1). In the case of this fraction, if the sample was not saponified, it was observed that fatty material from sample extracts are present in large excess and mask the peaks of these substances on FID gas chromatographic analysis. However, it was possible to confirm the presence of both substances in the fraction by two cleanup procedures of saponification and thin-layer chromatography for GC-MS analysis.

The GC-MS fragmentation pattern of P-1, found in the fish sample, was characterized by molecular ion $(M)^+$ at m/z 297 and intense $(M-15)^+$ at m/z 282, as showed in Fig. 2. The molecular ion indicates that the compound contained ann odd number of nitrogen atoms in the molecule on the basis of the "nitrogen rule".

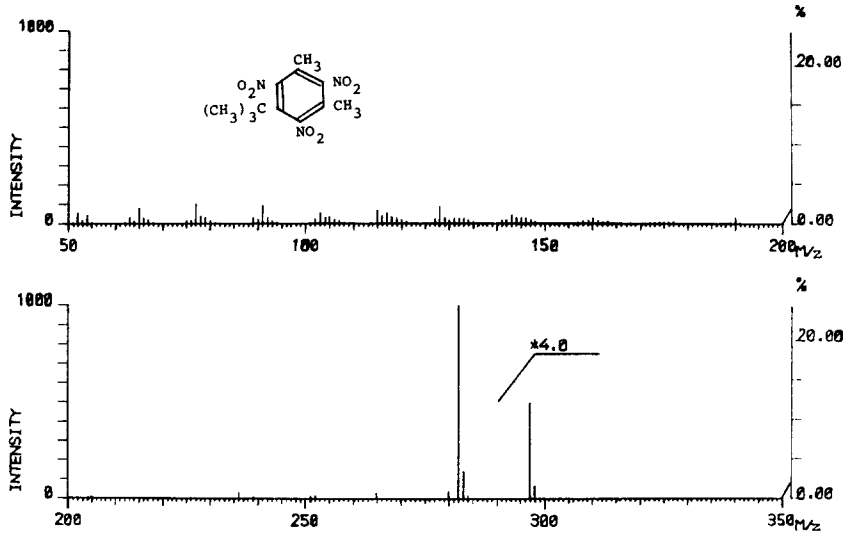

Fig. 2. EI Mass Spectrum of Musk Xylene Isolated from Fish Sample.

The high resolution measurement of the compound afforded the elemental compositions of $C_{12}H_{15}N_3O_6$ as the molecular ion, and of $C_{11}H_{12}N_3O_6$ as $(M-15)^+$ ion (Table 2). Thus, this unknown compound was identified as musk xylene by comparison with an authentic sample in mass spectrum and in retention times. The gas chromatographic comparisons using an ECD, made on the

substance, authentic musk xylene, and the mixture of the both, gave also the same retention times: 1.9, 3.3, and 3.0 min on OV-1, OV-17, and OV-17+OV-210 columns, respectively.

TABLE 2. High Resolution Measurements of the Unknown Substance(P-1).

Observed Mass (m/z)	Error (mmu)	Assignment	
282.0684	-4.0	$C_{11}H_{12}N_3O_6$	$(M-15)^+$
297.0980	2.0	$C_{12}H_{15}N_3O_6$	$(M)^+$

The mass spectrum of P-2 isolated from the fish sample is shown in Fig. 3. The fragmentation pattern of P-2 was characterized molecular ion $(M)^+$ at m/z 294 and intense $(M-15)^+$ at m/z 279. As shown in Table 3, the high resolutions measurments of the substance afforded the elemental compositions of $C_{14}H_{18}N_2O_5$ as the molecular ion, and of $C_{13}H_{15}N_2O_5$ as $(M-15)^+$ ion. Thus, this unknown compound was identified as musk ketone by comperison with authentic sample in mass spectrum and in retention times.

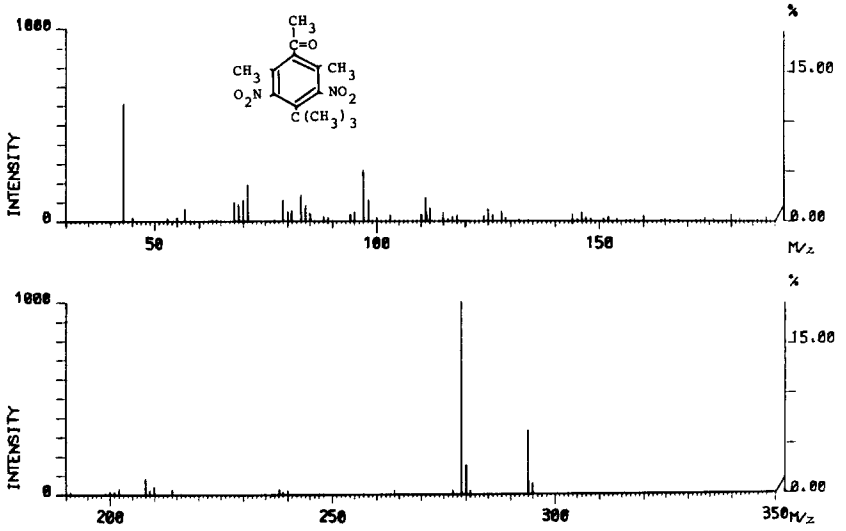

Fig. 3. EI Mass Spectrum of Musk Ketone Isolated from Fish Sample.

TABLE 3. High Resolution Measurements of the Unknown Substance(P-2).

Observed Mass (m/z)	Error (mmu)	Assignment	
279.0979	0.0	$C_{13}H_{15}N_2O_5$	$(M-15)^+$
294.1219	0.3	$C_{14}H_{18}N_2O_5$	$(M)^+$

The acute toxicity of musk xylene to mammals is relatively low. The acute oral LD_{50} in rats is reported as $>$ 10 g/kg and the acute dermal LD_{50} in rabbits as $>$ 15 g/kg(FOGLEMAN 1970a). The acute toxicity of musk ketone is reported to be 10 g/kg in both rats and rabbits (FOGLEMAN 1970b). However, it is noted that a study on rabbits with musk ketone had been observed to increase glutamic-pyruvic transaminase in serum on both the high level and the low level(POWERS 1971, 1972).

In Japan, the production of musk xylene and musk ketone is estimated to be no less than 100 tons each per year(AKIYAMA 1980). However, there is little known about the fate and effects of musk xylene and musk ketone in the aquatic environment.

In the present study, the results suggest that both musk xylene and musk ketone may exist as bioaccumulation type pollutant in the aquatic or marine environment. This may also give a clue to explaining the fact that chemical substances having a nitro group, for example, the herbicide CNP is greatly accumulated by fish and shellfish in Tokyo Bay and its surrounding river after application in the agricultural ground of a rice paddy field(YAMAGISHI et al. 1978, YAMAGISHI & AKIYAMA 1981).

The levels of musk xylene and musk ketone in freshwater fish samples examined here were 0.20 and 0.05 ppm on wet weight basis, respectively. These levels seem remarkably high, compared with the levels of dieldrin(0.011 ppm) and p,ṕ-DDE(0.015 ppm) in the same samples. Therefore, it may be concluded that more detailed monitoring on the contamination in living organisms and aquatic environment is need for musk xylene and musk ketone.

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