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Identification of Polyhalogenated Anisoles and Phenols in Oysters Collected from Tokyo Bay

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For several years, we have monitored trace organic contaminants in aquatic biota and reported previously that the herbicide CNP [1,3,5-trichloro-2-(4-nitrophenoxy)benzene](YAMAGISHI et al. 1978) and the insecticide chlordane (MIYAZAKI et al. 1979, 1980) were confirmed in fish and shellfish collected from Tokyo Bay. During the gas chromatographic analysis with an electron capture detector (ECD) of oyster samples from Tokyo Bay, several unknown peaks were found near that of hexachlorobenzene on a 3% OV-1 column. These compounds were much more sensitive to an ECD than a flame ionization detector (FID) and were almost unchanged by treatment with conc. H_2SO_4. Then, these compounds were extracted from the oyster, cleaned up and identified as polyhalogenated anisoles by gas chromatography/mass spectrometry (GC/MS). However, they are known to be the compounds formed through microbiological methylation from polyhalogenated phenols. Thus, the presence of these halogenated phenols in the sample were also confirmed by GC/MS. This paper describes the identification of polyhalogenated anisoles and phenols in oyster collected from Tokyo Bay.

MATERIALS AND METHODS

Oysters (Crassostrea gigas) were collected at the seashore of Keihinjima along Tokyo Bay, on July 25, 1980. The shucked sample (500 g) was homogenized and extracted three times with acetonitrile (1, 0.2, 0.1 L). The acetonitrile was poured into 2L of 2% NaCl solution. After adjusting to pH 2-3 with 2N HCl, the aqueous solution was extracted with hexane (200 mL x 3). The combined hexane was extracted with 0.1N K_2CO_3 solution (200 mL x 3). The hexane was dried (Na_2SO_4) and concentrated. The residue was dissolved in hexane (30 mL) and extracted with acetonitrile saturated with hexane (30 mL x 3). The acetonitrile was poured into 2% NaCl solution (1 L), and the aqueous solution was extracted with hexane (100 mL x 3). The hexane was dried (Na_2SO_4) and concentrated to ca. 3 mL. Further purification was carried out on a Florisil column (30 g, Floridin Co., 60-100 mesh). After washing the column with 120 mL of

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hexane, the eluate (F-A) with 5% dichloromethane in hexane (200 mL) was concentrated and submitted to GC/MS analysis. On the other hand, the extract with 0.1N K_2CO_3 solution described above was adjusted to pH 2-3 with 2N HCl and extracted with hexane (200 mL x 3). The hexane was dried (Na_2SO_4) and concentrated to ca. 0.5 mL. Ethylation of the residue was carried out in a similar manner to the method of WU & PEARSON (1977). Briefly, the residue, 100 mg of anhydrous potassium carbonate and 2 mL of ethyl bromide-ethanol-acetone (1 + 1 + 1) were added into a reaction vessel. The vessel was sealed and heated for 15 min at 150°C. After cooling, the reaction mixture was poured into 100 mL of 2% NaCl solution and extracted with hexane (50 mL x 3). The hexane was dried (Na_2SO_4) and concentrated. Further purification was carried out on a Florisil column (25 g). After washing the column with 120 mL of hexane, the eluate (F-B) with 20% dichloromethane in hexane (200 mL) was concentrated and submitted to GC/MS analysis.

GC/MS analysis was performed on a JEOL JMS D-300 mass spectrometer equipped with a Hewlett Packard 5710A gas chromatograph and a JEOL JMS 2000 data system under the following conditions: column, 3% OV-1 on 80-100 mesh Chromosorb W(AW-DMCS), 1.8 m x 2 mm (i.d.); column temp. 170°C; injection and separator temp. 250°C; He 30 mL/min ; EI 70 eV. Mass fragmentography with a 30 m OV-17 or a 10 m OV-101 glass capillary column equipped with the mass spectrometer was carried out at base peak for accurate assignment under the following conditions: splitless mode, EI 20 eV, injection and separator temp. 250°C; the oven temp. with OV-17 column was programmed from 80 to 180°C with 4°C/min, then kept at 180°C; the oven temp. with OV-101 column was programmed from 70 to 150°C with 4°C/min, then kept at 150°C. GC analysis was carried out using a gas chromatograph equipped with an ECD (^{63}Ni) and a 3 m x 3 mm (i.d.) glass column paked with 3% OV-1 on 80-100 mesh Chromosorb w(AW-DMCS); column temp, 190°C; injection and detector temp. 250°C, N_2 70 mL/min.

Pentachloroanisole (PCA), 2,3,4,6-tetrachloroanisole (m-TCA), 2,3,5,6-tetrachloroanisole (p-TCA) and 2,4,6-tribromoanisole (2,4,6-TBA), reference compounds, were prepared from the corresponding halogenated phenols by methylation with diazomethane. Pentachlorophenetole (PCT), 2,3,4,6-tetrachlorophenetole and 2,4,6-tribromophenetole, reference compounds, were prepared from the corresponding halogenated phenols by ethylation described above.

RESULTS AND DISCUSSION

Fig. 1 shows the reconstructed ion chromatogram and the mass spectra of F-A fraction obtained from oyster. The fragmentation patterns of weak peaks A-1, A-2, A-3 and A-4 in the F-A fraction gave the characteristic halogen clusters of the molecular ions $(M)^+$, M-15($-CH_3$), and M-43($-CH_3$, -CO) ions except for $(M-43)^+$ ion of peak A-3. These mass spectra are similar to those of chloroanisoles (WILLIAMS 1973, BURLINGAME 1977, TULP & HUTZINGER 1977, WU et al. 1978). The number of halogens included in these compounds could be revealed from isotope ratios around these clusters. Thus, the compounds of A-1, A-2, A-3 and A-4 were tentatively identified as trichloroanisole, bromodichloroanisole, trichloroanisole and chlorodibromoanisole, respectively. Also, the spectrum of peak A-5 is similar to that of chloroanisoles (Fig. 1). The high resolution measurement of the compound gave the elemental composition of $C_7H_4OCl_4$ $(M)^+$ (observed mass 245.8984, error 4.0 mmu, as $C_7H_4O^{35}Cl_3{}^{37}Cl_1$) and C_6HOCl_4 $(M-15)^+$ (observed mass 228.8786, error 0.5 mmu, as $C_6HO^{35}Cl_4$). Thus, A-5 was tentatively identified as tetrachloroanisole (TCA); however, three isomers are theoretically possible. Then, mass fragmentography with OV-17 or OV-101 glass capillary column showed that the peaks of m- and p-TCA could not be separated and the retention times of TCA in the sample were equal to those of the isomers. On the other hand, the retention time of o-isomers distinctly differed from those of m- and p-isomers (IDE et al. 1972, ROTT et al. 1979) and the difference between m- and p-isomers was shown in relative intensity on mass spectra (IDE et al. 1972). On the basis of these mass spectra, A-5 was assumed to be the mixture consisting of m- and p-isomers (ca. 8 : 2)(Table 1).

Table 1. Mass Spectral Data for m-, p-TCA and A-5(TCA in oyster)

Compound	Relative Intensity		
m/z	244$(M)^+$	299$(M-15)^+$	201$(M-43)^+$
p-TCA	100	45	52
m-TCA	96	100	48
A-5(TCA in sample)	100	93	49

Intensity values are normalized to all chlorine-35 peak

A-6 was tentatively identified as 2,4,6-tribromoanisole (TBA) by comparing with the mass spectrum and retention time of an authentic sample; Peak A-6 agreed with that of the specimen on OV-1 column, and on both glass capillary columns of OV-17 and OV-101, though six isomers are theoretically possible.

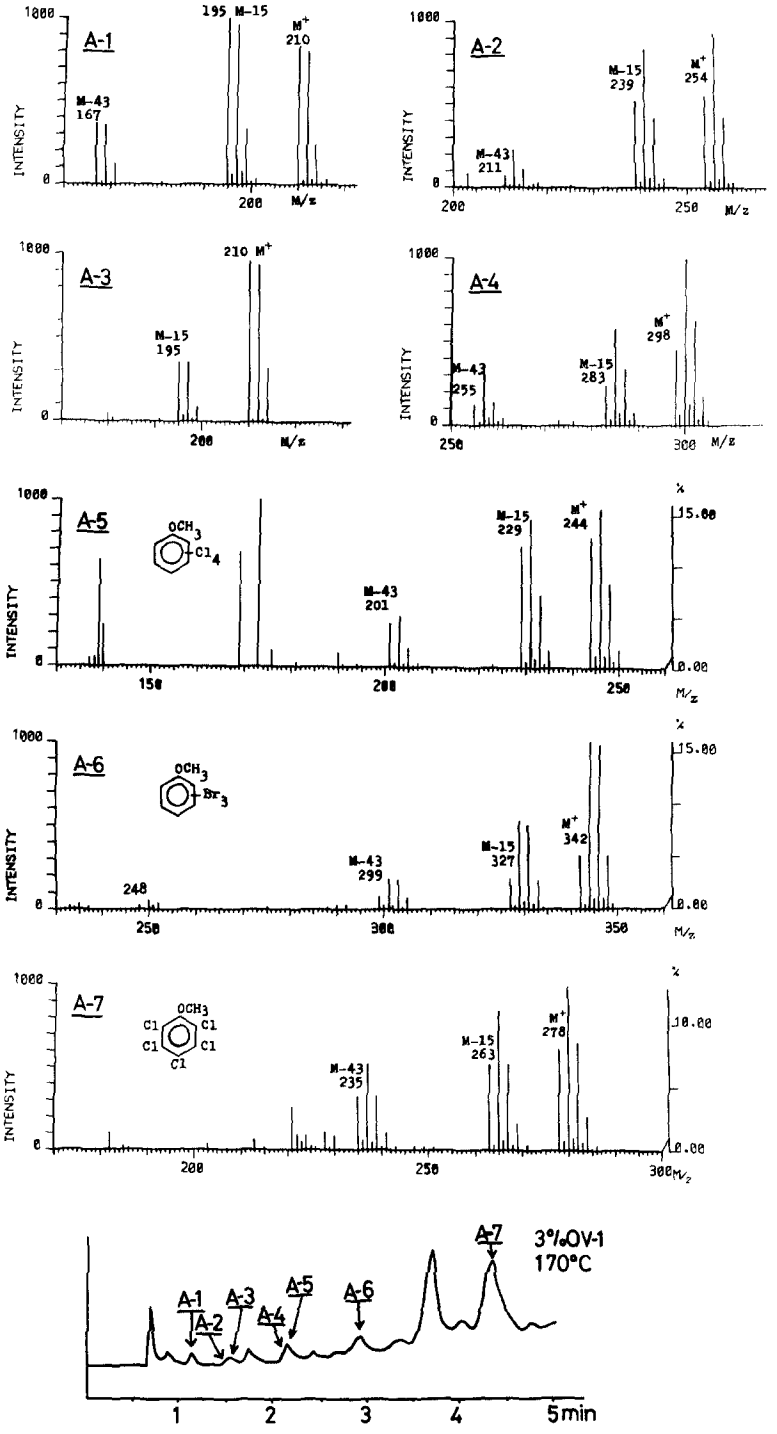

Fig. 1. Reconstructed Ion Chromatogram and Mass Spectra of F-A Fraction Obtained from Oyster

The fragmentation pattern of peak A-7 shows $(M)^+$ at m/z 278, $(M-15)^+$ at m/z 263 and $(M-43)^+$ at 235 (Fig. 1). The isotopic distribution of each cluster indicated the presence of five chlorine atoms in the molecule. The high resolution measurement of the compound led to the tentative elemental compositions of $C_7H_3OCl_5$ $(M)^+$, C_6OCl_5 $(M-15)^+$ and C_5Cl_5 $(M-43)^+$, respectively (Table 2). Thus, A-7 was identified as pentachloroanisole by comparing with an authentic sample in mass spectrum, and also in retention time on OV-1 column.

Table 2. High Resolution Measurement of A-7 in Oyster

Observed mass(m/z)	Error(mmu)	Assignment	
277.8657	3.0	$C_7H_3O^{35}Cl_5$	
279.8610	1.2	$C_7H_3O^{35}Cl_4{}^{37}Cl_1$	$(M)^+$
283.8537	0.0	$C_7H_3O^{35}Cl_2{}^{37}Cl_3$	
262.8404	1.2	$C_6O^{35}Cl_5$	
264.8362	0.0	$C_6O^{35}Cl_4{}^{37}Cl_1$	$(M-15)^+$
266.8371	3.8	$C_6O^{35}Cl_3{}^{37}Cl_2$	
234.8459	1.6	$C_5{}^{35}Cl_5$	
236.8395	-1.6	$C_5{}^{35}Cl_4{}^{37}Cl_1$	$(M-43)^+$
238.8394	1.0	$C_5{}^{35}Cl_3{}^{37}Cl_2$	
240.8375	1.2	$C_5{}^{35}Cl_2{}^{37}Cl_3$	

PCA residues were found in industrial and municipal waste water effluents (BURLINGAME 1977), shellfish, fish, canned fish products (KOIDU & ROSEN 1976) and broiler tissues (PEEL et al. 1972, HARPER & BALNAVE 1975). It is also known to be formed by microbial transformation from pentachlorophenol (PCP)(SUZUKI & NOSE 1971, KUWATSUKA &IGARASHI 1975, ROTT et al. 1979, MURTHY et al. 1979). Therefore, the presence of halogenated phenols in the sample were assumed from this information.

Fig. 2 shows the reconstructed ion chromatogram and the mass spectra of F-B fraction obtained from the oyster. The fragmentation patterns of peaks B-1, B-2, B-3 and B-4 gave the characteristic halogen clusters of molecular $(M)^+$ and intense $(M-28)^+$ ions. These mass spectra are similar to that of PCT (WU et al. 1978). The number of halogen atoms in each compound could be revealed from isotopic ratios around these halogen cluster ions. Thus, B-1, B-2, B-3 and B-4 were tentatively identified as dichlorophenetole, trichlorophenetole, dibromophenetole, and chlorodibromophenetole, respectively.

B-5 and B-6 were tentatively identified as tetrachlorophenetole and tribromophenetole, respectively, by comparing with authentic 2,3,4,6-tetrachlorophenetole

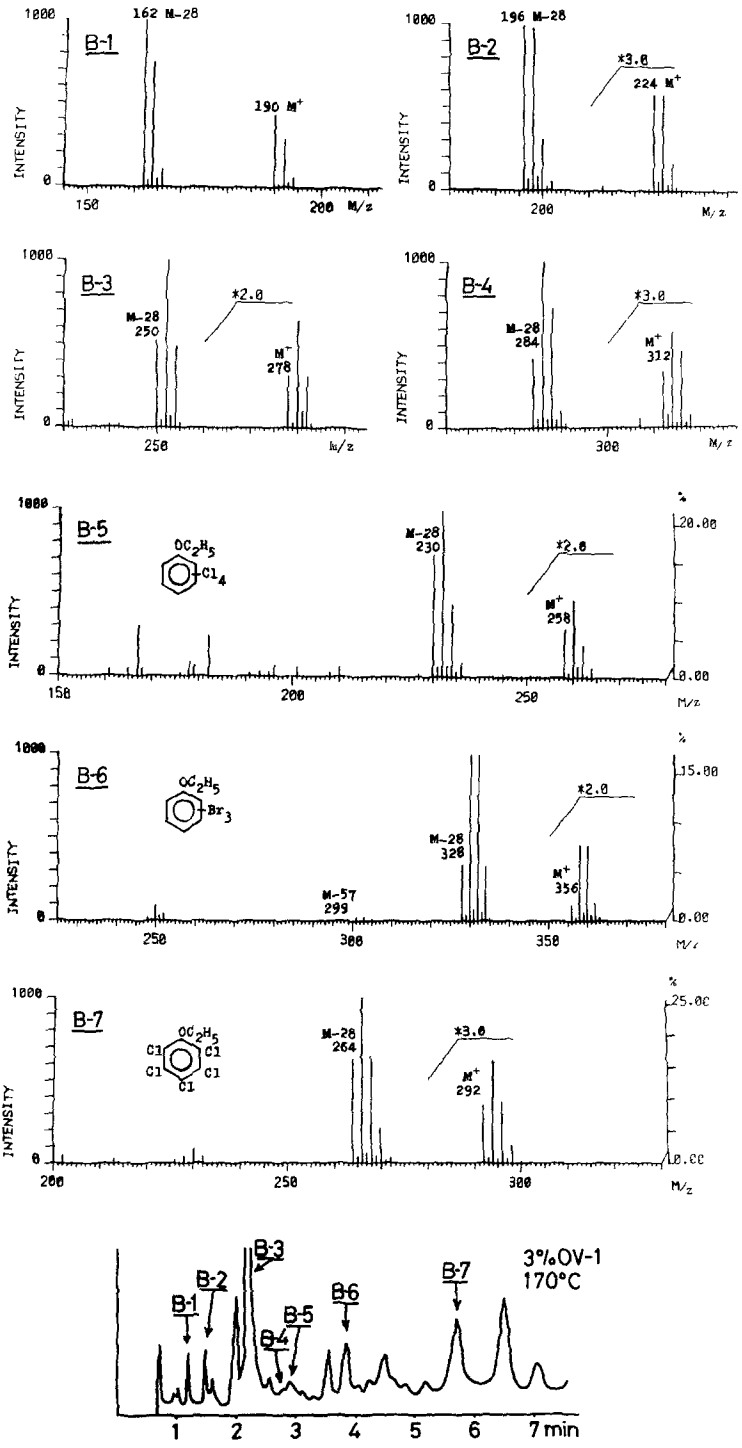

Fig. 2. Reconstructed Ion Chromatogram and Mass Spectra of F-B Fraction Obtained from Oyster

and 2,4,6-tribromophenetole in mass spectra, and also in retention times on OV-1 column. Peak B-7 shows characteristics of chlorine clusters (Cl_5) at m/z 292 $(M)^+$ and of intense $(M-28)^+$ ion. The high resolution measurement of B-7 led to the tentatively elemental compositions of $C_8H_5OCl_5$ $(M)^+$ and of C_6HOCl_5 $(M-28)^+$ as shown in Table 3. Thus, B-7 was identified as PCT by comparing with an authentic sample in mass spectrum, and in retention time on OV-1 column.

Table 3. High Resolution Measurement of B-7 from Oyster

Observed mass(m/z)	Error(mmu)	Assignment	
291.8816	3.2	$C_8H_5O^{35}Cl_5$	
293.8751	0.0	$C_8H_5O^{35}Cl_4{}^{37}Cl_1$	$(M)^+$
295.8692	-3.0	$C_8H_5O^{35}Cl_3{}^{37}Cl_2$	
263.8475	0.5	$C_6HO^{35}Cl_5$	
265.8435	-0.3	$C_6HO^{35}Cl_4{}^{37}Cl_1$	$(M-28)^+$
267.8417	0.6	$C_6HO^{35}Cl_3{}^{37}Cl_2$	
269.8405	2.3	$C_6HO^{35}Cl_2{}^{37}Cl_3$	

Thus, the identification of these halogenated phenetoles indicated the presence of the corresponding phenols. The residues accompanying halogenated anisoles and the corresponding phenols may be formed through methylation of the respective halogenated phenols. Quantitation by ECD-GC showed that approximate residual levels of these compounds in the oyster were as follows: PCA 20 ppb, TBA 4 ppb (as 2,4,6-TBA), TCA 3 ppb (as m-TCA), PCP 2 ppb in the edible portion.

GLICKMAN et al. (1977) reported that PCA is more persistent than PCP in rainbow trout. This persistent property seems to be one reason that the residual level of PCA in the sample is higher than that of PCP. The occurrence of both PCP and PCA was assumed to indicate environmental contamination derived from PCP used in fields. PCP had been widely used as a herbicide in rice fields in Japan about twenty years ago, but its high toxicity to aquatic organisms was revealed. Although PCP has been prohibited from paddy-field use at present, it is applied to wood as a termiticide, fungicide or bactericide. The residues of tri- and tetrachlorophenols and respective anisoles may be derived from PCP, its metabolites in fields, or its impurities and intermediates of industrial and agricultural chemicals. However, the origin of the bromophenol derivatives found in the oyster is unclear.

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