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SIMULTANEOUS DETERMINATION OF BUTYLTIN AND PHENYLTIN COMPOUNDS IN OYSTERS BY CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

A method is described for the simultaneous determination of butyl- and phenyltin compounds in oyster samples. The organotin compounds were extracted (as chlorides) from oyster homogenates with hydrochloric acid and benzene in the presence of 0 05% tropolone. These compounds were converted into pentyl derivatives with pentyl Grignard reagent and then analysed by capillary gas chromatography with a flame photometric detector equipped with a 393-nm filter. The recoveries of six organotin compounds added to oyster samples ranged from 71 to 74%. The detection limits of butyl- and phenyltin compounds were in the 5–9 pg range as tin. We detected significant amounts of three organotin compounds (di- and tributyltin and triphenyltin) in oyster samples.

INTRODUCTION

World-wide production of organotin chemicals was ca. 35 000 tons in 1985 [1]. These compounds have been used for a variety of purposes as agricultural pesticides, antifoulants and stabilizers for vinyl chloride polymers, etc. However, a major environmental concern today is the use of tributyltin and triphenyltin compounds in antifouling marine paints [2]. Therefore, it is of utmost importance to determine the environmental distribution of organotin pollutants and their potential harmful effects on humans or other mammals [3,4]. As an integral part of our basic research on the toxicology of organotin compounds, we have tried to develop an analytical method for these com-

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pounds. By using capillary gas chromatography (GC) with flame photometric detection (FPD), we have obtained satisfactory results [5–7].

This report describes a method for simultaneous determination of butyl- and phenyltin compounds in oysters by using GC–FPD. This method is useful since it does not require any column chromatographic treatment of the samples before GC injection.

EXPERIMENTAL

Reagents

All chemicals were used without further purification. Triphenyltin chloride (Ph_3SnCl), tributyltin chloride (Bu_3SnCl) and dibutyltin dichloride (Bu_2SnCl_2) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Diphenyltin dichloride (Ph_2SnCl_2), phenyltin trichloride ($PhSnCl_3$) and butyltin trichloride ($BuSnCl_3$) were obtained from Alfa Products (Danvers, MA, U.S.A.). Pentylmagnesium bromide (PeMgBr, 2.0 *M* in diethyl ether) and tropolone were from Aldrich (Milwaukee, WI, U.S.A.). Other reagents and solvents used were commercial products of reagent grade.

Standard solution

Each organotin compound was dissolved in tetrahydrofuran (THF) at a concentration of 12.5–20 mg per 10 ml as a stock solution. Working standard solutions in the 0.5–4 μg/ml range were diluted with benzene (containing 0.05% tropolone as a complexing reagent [8]). Recovery experiments were carried out by adding each tin stock solution, diluted with distilled water, to oyster samples. The final THF concentration of both the benzene and water solutions was under 1.2%.

Oyster samples

Oyster samples were obtained from a source in Miyagi Prefecture.

Preparation of butyl- and phenyltin compounds from oyster

The samples for GC analyses of butyl- and phenyltin compounds from oyster were prepared as illustrated in Fig. 1. An oyster sample (5 g wet weight) was homogenized in 10 ml of distilled water. The homogenate was placed in a centrifuge tube with a glass stopper, and 1.5 g of sodium chloride and 0.5 ml of concentrated hydrochloric acid (35%) were added to the tube. The mixture was extracted twice with 10 ml of benzene (containing 0.05% tropolone) by agitating for 20 min in a mechanical shaker. After each shaking, the mixture was centrifuged for 10 min at 1800 *g*. Each benzene extract was removed. The benzene extracts were combined and dried with 0.5 g of anhydrous sodium sulphate. The extract was transferred to a separatory funnel, and 1 ml of pentylmagnesium bromide was added. The reaction mixture was allowed to stand

Oyster sample (5 g wet weight)
⊢Distilled water (10 ml)
Homogenate
⊢NaCl (1.5 g)
⊢HCl (0.5 ml)
⊢Benzene (0.05% tropolone) (2×10 ml)
Shaking (2×20 min)
|
Centrifugation (1800 *g*, 10 min)
|
Organic layer (dehydration with 0.5 g of Na_2SO_4)
⊢Pentylmagnesium bromide (1 ml)
Incubation (20 min)
⊢0.05 *M* HCl (25 ml)
Shaking (5 min)
|
Organic layer (rinse with 25 ml of distilled water)
|
Organic layer (dehydration with 0.5 g of Na_2SO_4)
|
Evaporation (just to dryness)
|
Residue (made up to 5 ml with toluene)
|
GC

Fig. 1. Preparation of butyl- and phenyltin compounds from oyster samples.

for 20 min at room temperature and subsequently extracted with 25 ml of hydrochloric acid (0.05 *M*) for 5 min. The separate organic layer was rinsed with 25 ml of distilled water and then evaporated just to dryness after drying with 0.5 g of anhydrous sodium sulphate. The residual material was made up to 5 ml with toluene for GC. A 0.5-μl volume of the sample solution was injected into the gas chromatograph.

Calibration curves

A 1-ml volume of standard mixtures (each organotin compound 0.5–4 μg/ml) used for calibration curves was pentylated as above, but without any extraction. The calibration curves were constructed by plotting the peak heights versus the amount of tin.

Apparatus

A Model 5890A (Hewlett-Packard, Avondale, PA, U.S.A.) gas chromatograph equipped with a flame photometric detector was operated in the sulphur mode (filter 393 nm). The column was an Ultra 1 capillary column (12.5 m×0.2 mm I.D., 100% dimethyl polysiloxane gum, 0.33 μm film thickness; Hewlett-

Packard). This packing was used because it is suitable for separating non-ionic organotin compounds [5–7].

RESULTS AND DISCUSSION

Gas chromatographic conditions

The FPD response for tin compounds is strongly dependent on the flame conditions, and the hydrogen-rich flame was found to enhance selectivity and sensitivity as well as the shape of the GC signals [9]. The oven temperature conditions for the butyltin compounds were preferable to those for the phenyltin compounds. Therefore, we used the experimental conditions listed in Table I.

Derivatization

A variety of derivatization techniques, such as hydrogenation [10], methylation [11] and ethylation [12], have been applied to the GC analysis of organotin compounds. However, these methods are not suitable for butyl- and phenyltin compounds as the derivatives are fairly volatile compared with benzene, and appreciable amounts of the derivatives are lost during routine concentration procedures [13]. On the other hand, the pentyl derivatives were sufficiently non-volatile to prevent loss, yet they were volatile enough to be analysed by GC [6]. In addition, pentyltin compounds are not industrial products. Thus, pentylation is a suitable derivatization technique for the analyses of various organotin compounds. Fig. 2 shows the gas chromatograms obtained with pentyl tributyltin ($PeBu_3Sn$, 42.1 pg), dipentyl dibutyltin (Pe_2Bu_2Sn, 39.1 pg), tripentyl butyltin (Pe_3BuSn, 36.5 pg), pentyl triphenyltin ($PePh_3Sn$, 55.0 pg), dipentyl diphenyltin (Pe_2Ph_2Sn, 48.3 pg) and tripentyl phenyltin (Pe_3PhSn, 49.3 pg as tin). The chromatogram shows good separation of the six tin compounds and sensitive detection. The detection limits of butyl- and phenyltin compounds were in the range 5–9 pg, expressed as tin.

TABLE I

GC CONDITIONS FOR DETERMINATION OF ORGANOTIN COMPOUNDS

Temperature	injection, 220 °C; detector, 250 °C;
Oven temperature programme	80 °C (2 min), 30 °C/min, 150 °C, 5 °C/min, 200 °C, 10 °C/min, 230 °C (20 min)
Flow-rate	H_2, 175 ml/min, air, 65 ml/min
Carrier gas	He, 2 ml/min
Purge gas	He, 50 ml/min
Make-up gas	N_2, 28 ml/min
Inlet type	splitless (purge time 2 min)
Detection	FPD (S filter 393 nm)
Column	100% dimethyl polysiloxane gum (12.5 m × 0.2 mm I.D., 0.33 μm film thickness)

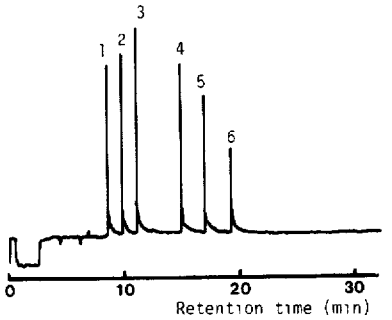

Fig. 2. Gas chromatogram of butyl- and phenyltin compounds. Peaks: 1 = $PeBu_3Sn$; 2 = Pe_2Bu_2Sn; 3 = Pe_3BuSn; 4 = Pe_3PhSn; 5 = Pe_2Ph_2Sn; 6 = $PePh_3Sn$.

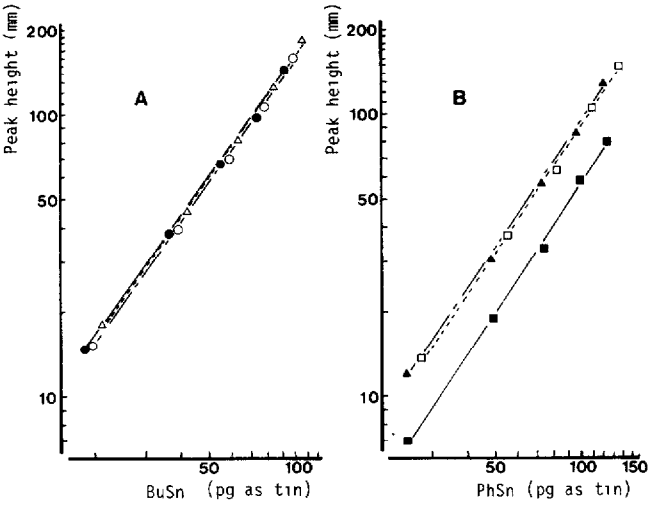

Fig. 3. Calibration curves of (A) butyltin and (B) phenyltin compounds. (△) Pe_3BuSn; (○) Pe_2B_2Sn; (●) $PeBu_3Sn$; (□) Pe_3PhSn; (▲) Pe_2Ph_2Sn; (■) $PePh_3Sn$.

Calibration

Standard mixtures were prepared containing various amounts of butyl- and phenyltin compounds in 20 ml of benzene (containing 0.05% tropolone) and determined by the above analytical procedure. The calibration data for each butyl- and phenyltin compound are shown in Fig. 3. The peak height on the log–log paper is linearly related to the concentration of each tin compound in the range 18–140 pg as tin.

Extraction and recoveries

We examined simultaneous extraction of six different organotins (mono-, di- and tributyltin and mono-, di- and triphenyltin) by using benzene (con-

TABLE II

RECOVERY OF BUTYLTIN COMPOUNDS FROM OYSTER TISSUE

$BuSnCl_3$			Bu_2SnCl_2			Bu_3SnCl		
Added (μg/5 g)	Found[a] (μg/5 g)	Recovery (%)	Added (μg/5 g)	Found[a] (μg/5 g)	Recovery (%)	Added (μg/5 g)	Found[a] (μg/5 g)	Recovery (%)
0	Not detected		0	0.23 ± 0.02		0	1.09 ± 0.10	
0.5	0 36 ± 0.02	72	0.5	0.59 ± 0.01	72	0.5	1.45 ± 0.01	72
1.0	0.72 ± 0.03	72	1.0	0.97 ± 0.02	74	1.0	1.82 ± 0.03	73
1.5	1.09 ± 0.05	73	1.5	1.32 ± 0.04	73	1.5	2.19 ± 0.04	73
2.0	1.42 ± 0.05	71	2.0	1.67 ± 0.04	72	2.0	2.52 ± 0.06	72

[a]Mean ± S.D., $n = 6$.

TABLE III

RECOVERY OF PHENYLTIN COMPOUNDS FROM OYSTER TISSUE

$PhSnCl_3$			Ph_2SnCl_2			Ph_3SnCl		
Added (μg/5 g)	Found[a] (μg/5 g)	Recovery (%)	Added (μg/5 g)	Found[a] (μg/5 g)	Recovery (%)	Added (μg/5 g)	Found[a] (μg/5 g)	Recovery (%)
0	Not detected		0	Not detected		0	1.19 ± 0.09	
0.7	0.52 ± 0.02	74	0.7	0.51 ± 0.02	73	0.8	1.78 ± 0.03	74
1.4	1.02 ± 0.03	73	1.4	1.01 ± 0.04	72	1 6	2.37 ± 0 04	74
2.1	1 52 ± 0.06	72	2.1	1.54 ± 0.07	73	2.4	2.93 ± 0.05	73
2.8	2.04 ± 0.07	73	2 8	2.04 ± 0 11	73	3.2	3.52 ± 0.10	73

[a]Mean ± S.D., $n = 6$.

taining 0.05% tropolone) as an extraction solvent from the oyster matrix. Each recovery of six organotins added to 5 g of homogenized oyster sample was evaluated. The results are shown in Tables II and III. Recoveries, which were corrected for organotin in the oyster, ranged from 71 to 74%. No fluctuation in recoveries was seen, either for different tin amounts or different tin species. All values of the yield were ca. 70%. The procedure consists of a simple extraction system and is able to determine butyl- and phenyltin compounds simultaneously. Corresponding gas chromatograms are shown in Fig. 4. These compounds, di- and tributyltin and triphenyltin, in oyster samples were well separated and not interfered from other substances.

Calculation of organotin compound concentrations in oyster samples

The relation between the peak height (y) and the amount of tin (x) can be expressed from the calibration graph as following equation:

$$y = ax^m \quad (1)$$

where y is the peak height of the standard, x is the amount of tin, a is a constant and m is the slope of calibration curve (the value of m was obtained by the

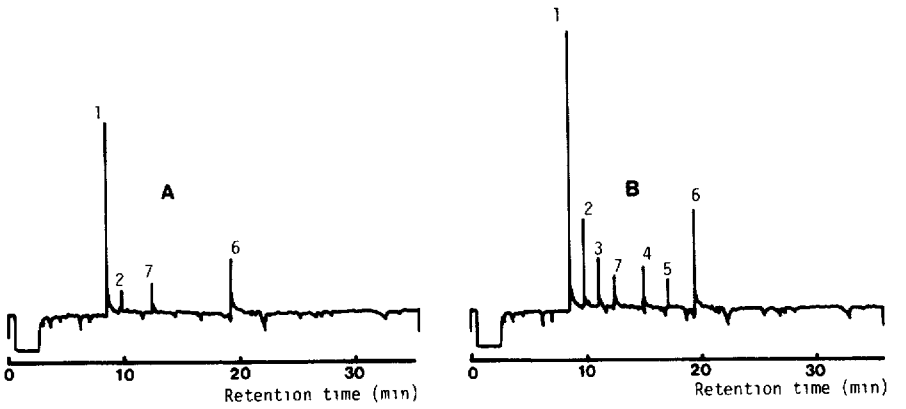

Fig. 4. Gas chromatograms for (A) oyster samples and (B) oyster samples spiked with butyl- and phenyltin compounds. Peaks: 1 = $PeBu_3Sn$; 2 = Pe_2Bu_2Sn; 3 = Pe_3BuSn; 4 = Pe_3PhSn; 5 = Pe_2Ph_2Sn; 6 = $PePh_3Sn$; 7 = unknown (probably Pe_4Sn).

TABLE IV

ESTIMATION OF ORGANOTIN CONCENTRATION IN OYSTER SAMPLES

Ionic species	Calculated concentration[a] (mean ± S.D., $n=6$) (ng/g as tin)	Estimated concentration[b] (ng/g as tin)
Bu_2Sn^{2+}	24.1 ± 1.3	24.6 ± 2.3
Bu_3Sn^+	110.9 ± 4.3	109.8 ± 10.1
Ph_3Sn^+	108 2 ± 7.1	99.7 ± 7.5

[a]Using eqns. 1, 2 and 3.

[b](Measured value/average of recovery) × 100 (from Tables II and III).

least-squares method). On this basis, the following relation may be used to determine the amount of organotin compounds in oysters:

$$y' = a(x' + b)^{m'} \tag{2}$$

where y' is the peak height of a sample obtained after addition of tin to an oyster sample, x' is the amount of tin added to oyster samples, a is a constant, m' is the slope of curve (we take the value of m' to be the same as slope m in eqn. 1, since the percentage recovery was independent of the amount of tin) and b is the amount of tin in the oyster sample. The point $(x_1,y_1),(x_2,y_2)$ satisfies the following equation:

$$b = \frac{x_2 y_1^{1/m} - x_1 y_2^{1/m}}{y_2^{1/m} - y_1^{1/m}} \tag{3}$$

where x_1 and x_2 are arbitrary amounts of tin added to the oyster samples, y_1 is the peak height for x_1 and y_2 is the peak height for x_2. These equations were used to obtain the amounts of tin compounds in oyster samples. The results are shown in Table IV. The calculated concentrations (dibutyltin 24.1, tributyltin 110.9, triphenyltin 108.2 ng/g as tin) agree approximately with the values estimated (dibutyltin 24.6, tributyltin 109.8, triphenyltin 99.7) from recovery data.

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