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DETERMINATION OF BUTYLTIN AND PHENYLTIN COMPOUNDS IN BIOLOGICAL AND SEDIMENT SAMPLES BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY

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SUMMARY

A method is described for the gas chromatographic determination of nanogram amounts of mono-, di- and tributyltin compounds and mono-, di- and triphenyltin compounds in biological and sediment samples. These compounds are converted into the corresponding chlorides with hydrochloric acid, extracted with ethyl acetate and hydrogenated with sodium tetrahydroborate. The corresponding hydrides, mono-*n*-butyltin hydride, di-*n*-butyltin hydride, tri-*n*-butyltin hydride, monophenyltin hydride, diphenyltin hydride triphenyltin hydride, are detected by electron-capture gas chromatography after clean-up by silica gel column chromatography. The detection limits are 10–20 ng/g for mono-*n*-butyltin chloride, 0.5–1 ng/g for di-*n*-butyltin chloride, 1–2 ng/g for tri-*n*-butyltin chloride and diphenyltin chloride and 2.5–5 ng/g for monophenyltin chloride and triphenyltin chloride, in biological and sediment samples.

INTRODUCTION

In recent years, organotin compounds have been used for a variety of purposes. Triorganotin and diorganotin compounds are mainly used in the chemical industry and agriculture, *e.g.*, tributyltin compounds are used as industrial biocides and dibutyltin compounds as catalysts or stabilizer for poly (vinyl chloride), and triphenyltin and tricyclohexyltin compounds are used as agricultural fungicides and miticides, respectively.

A variety of analytical methods, such as atomic absorption spectrometry[1–3], gas chromatography (GC)[4–12] and high-performance liquid chromatography[13], have been applied to the determination of organotin compounds. To reveal environmental contamination by organotin compounds and their degradation or metabolism, a rapid and sensitive method applicable to various organotin compounds is required.

In the above methods, alkyltin, phenyltin and tricyclohexyltin compounds are separately analyzed, and the operations are time-consuming. We have already developed a GC method for the simultaneous determination of dibutyltin and tribu-

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tyltin compounds in biological and sediment samples after hydrogenation and silica gel clean-up[14]. Here we describe the application of the method to the determination of monobutyltin and phenyltin compounds.

EXPERIMENTAL

Apparatus

The gas chromatograph (Model G180; Yanagimoto, Kyoto, Japan) was equipped with a ^{63}Ni electron-capture detector. Two 1.5 m × 2 mm I.D. glass column packed with 20% PEG 20M and 10% OV-1 on Chromosorb W AW DMCS (80–100 mesh) were used.

GC conditions

With 20% PEG 20M and mono-*n*-butyltin hydride ($BuSnH_3$) as sample: injection port temperature, 120°C; oven temperature, 60°C; carrier gas (nitrogen) flow-rate, 30 ml/min. With 10% OV-1: (1) samples monophenyltinhydride ($PhSnH_3$) and di-*n*-butyltin hydride (Bu_2SnH_2); injection port temperature, 150°C; oven temperature, 100°C; carrier gas (nitrogen) flow-rate, 40 ml/min; (2) samples tri-*n*-butyltin hydride (Bu_3SnH) and diphenyltin hydride (Ph_2SnH_2); injection port temperature 200°C; oven temperature, 170°C; carrier gas (nitrogen) flow-rate, 40 ml/min; (3) sample triphenyltin hydride (Ph_3SnH); injection port temperature 275°C; oven temperature, 250°C; carrier gas (nitrogen) flow-rate, 40 ml/min.

Reagents

Di-*n*-butyltin dichloride (Bu_2SnCl_2), tri-*n*-butyltin chloride (Bu_3SnCl) and triphenyltin chloride (Ph_3SnCl), each analytical grade, were obtained from Tokyo Chemical Industry (Tokyo, Japan). Mono-*n*-butyltin trichloride ($BuSnCl_3$), monophenyltin trichloride ($PhSnCl_3$) and diphenyltin dichloride (Ph_2SnCl_2), each analytical grade, were obtained from Strem Chemicals (Newburyport, MA, U.S.A.). The 5% water-deactivated silica gel (Kieselgel 60, 70–230 mesh; Merck) was used for column clean-up. Pesticide-grade solvents and analytical grade chemicals were used throughout.

Standard solution

Standard stock solutions (1000 μg/ml) were prepared by dissolving 100 mg of each butyltin chloride and phenyltin chloride in 100 ml of ethanol. Working standards (0.0625–2.0 μg/ml) were prepared by diluting these standard stock solutions in ethanol prior to use.

Determination of butyltin and phenyltin in biological samples

A biological sample was homogenized in a commercial meat grinder. About 10g of the homogenate were placed in a separating funnel, 100 ml of water, 15 g of sodium chloride and 10 ml of concentrated hydrochloric acid (35%) were added and the mixture extracted with 50 ml ethyl acetate for 30 min in a mechanical shaker. After centrifugation of the mixture at 1100 *g* for 5 min, a measured amount (30 ml) of the organic layer was transferred to a 50 ml round-bottom flask. The solution was rotary-vacuum evaporated to dryness (1–2 min) at 40°C. (Caution: this step should

not be prolonged.) The residue was dissolved in 1 ml ethanol and then hydrogenated with 2 ml of 2.5% sodium tetrahydroborate in ethanol for 10 min. To the reaction mixture were added 15 ml of water and 5 g of sodium chloride and this was extracted with 5 ml of hexane for 5 min. The hexane layer was passed through a 8 cm × 1 cm I.D. glass clean-up column containing about 3 g of hexane-rinsed silica gel. The column was eluted with hexane. The first 5 ml were discarded, the next 5–15 ml were collected for $BuSnH_3$, Bu_2SnH_2, Bu_3SnH, $PhSnH_3$, Ph_2SnH_2 and the next 15–40 ml for Ph_3SnH. For the analysis of $BuSnH_3$, the eluant (10 ml) was injected into the gas chromatograph without concentration. For the butyltin and phenyltin hydrides, except $BuSnH_3$, the eluents were rotary-vacuum evaporated to about 2 ml, transferred to a graduated test-tube (rinsing the flask with hexane) and adjusted to 1–5 ml under a stream of nitrogen or air at 40°C. A 5 μl volume of each sample solution was injected into gas chromatograph for analysis. Peak heights obtained from the sample injection were evaluated by the use of calibration curves.

Determination of butyltin and phenyltin in sediment

About 20 g of wet sediment were placed in a separating funnel, 50 ml of water and 5 ml of concentrated hydrochloric acid (35%) were added and the mixture extracted with 50 ml of ethyl acetate for 30 min in a mechanical shaker. After centrifugation at 1100 *g* for 5 min, a measured amount (30 ml) of the organic layer was analyzed as described for biological samples.

Calibration curves

A 1-ml volume of each standard (0.0625–2.0 μg/ml) was hydrogenated and extracted with hexane as described above. A 5-μl volume of the hexane layers was injected into the gas chromatograph, and calibration curves were constructed by plotting the peak heights against the concentrations for $BuSnCl_3$ (0.05–0.4 μg/ml), Bu_2SnCl_2 (0.0125–0.1 μg/ml), Bu_3SnCl (0.025–0.2 μg/ml), $PhSnCl_3$ (0.05–0.4 μg/ml), Ph_2SnCl_2 (0.025–0.2 μg/ml) and Ph_3SnCl (0.05–0.4 μg/ml).

RESULTS AND DISCUSSION

Analysis of $BuSnH_3$ by electron-capture GC

The separation of $BuSnH_3$ by GC was investigated on the following column packings: (1) 2–10% OV-1; (2) 3% SE-30; (3) 2% OV-17; (4) 15% DEGS; (5) 20% PEG 20M, each on Chromosorb W AW DMCS; (6) 5% OV-225 on Gas-Chrom Q. The $BuSnH_3$ peak could be detected only when using 20% PEG 20M on Chromosorb W AW DMCS. The calibration line for $BuSnH_3$ (0.05–0.4 μg/ml), determined in this way deviated from linearity at low concentrations. This was probably due to slight adsorption of $BuSnH_3$ on the column packing, which could be avoided by injecting an high concentration of $BuSnH_3$ (100 μg/ml) into the gas chromatograph before analysis.

Extraction of organotin from biological samples

In our previous study[14], ethyl acetate–hexane (3:2) was found efficiently to extract Bu_3SnCl and Bu_2SnCl_2 from fish samples. However, for $BuSnCl_3$ or phenyltin chlorides, the extraction efficiency was not good, especially at low levels (0.1

μg/g). For example, the average recovery of Ph_3SnCl after silica gel clean-up was 75.9% (n = 3) for 1.0 μg/g spiked, but 33.3% (n = 3) at 0.1 μg/g.

Here ethyl acetate was again tried for extraction. The average recovery was 81.5% (n = 5) for samples spiked at a level of 1.0 μg/g and 86.3% (n = 5) at 0.1 μg/g. Ethyl acetate, which extracted more impurities than ethyl acetate–hexane (3:2), was used because of the high extraction efficiency and satisfactory silica gel clean-up.

Extraction of organotin from sediment

In our previous study[14], hexane efficiently extracted Bu_3SnCl and Bu_2SnCl_2 from sediment samples. However, for $BuSnCl_3$ or phenyltin chlorides, the extraction efficiency was not as good as that obtained for a fish sample. Here ethyl acetate, the same solvent as for the fish sample, was again used for extraction, as it gave a better recovery than hexane. Sodium chloride was used to increase the extraction efficiency further. However, this resulted in interference with the hydrogenation and the recovery was not improved, because more impurities were extracted by adding sodium chloride. Consequently, subsequent extractions were carried out without sodium chloride.

Silica gel clean-up

The elution patterns of butyltin and phenyltin hydrides are shown in Fig. 1 for 5% water-deactivated silica gel. All the hydrides except Ph_3SnH (15–30 ml) were eluted from 5 to 15 ml. As shown in Fig. 2, interference was observed at the Ph_3SnH peak position in the gas chromatogram of the eluent (0–15 ml), particularly for fish

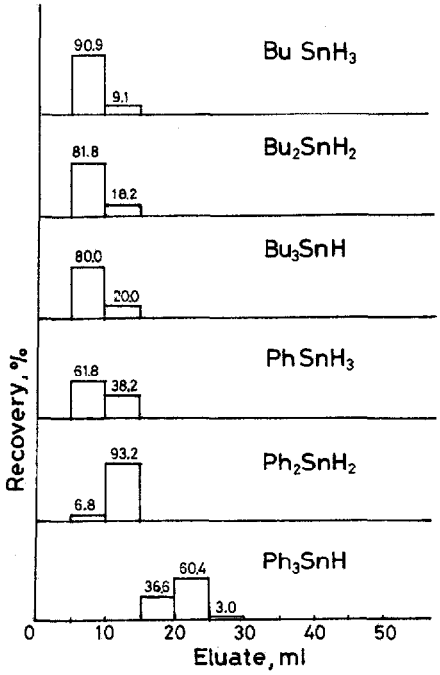

Fig. 1. Elution patterns of organotin hydrides from a 5% water-deactivated silica gel column.

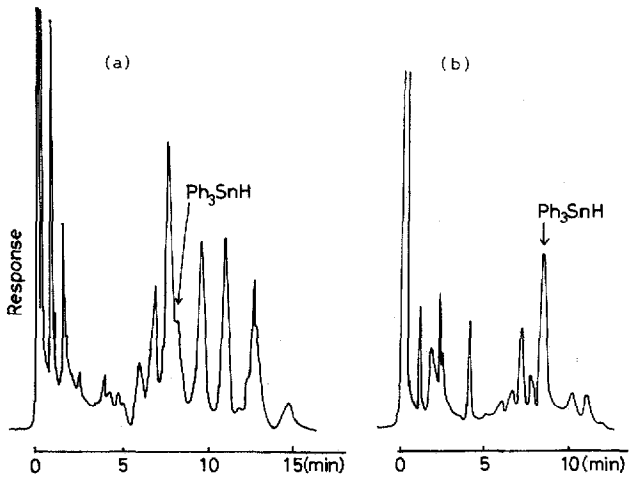

Fig. 2. Gas chromatograms of Ph_3SnH after silica gel clean-up. Crucian carp muscle was spiked with 10 μg of Ph_3SnCl. (a) Column temperature, 250°C; sample, 5 ml; eluate, 0–15 ml; (b) column temperature, 250°C; sample, 5 ml; eluate, 15–40 ml.

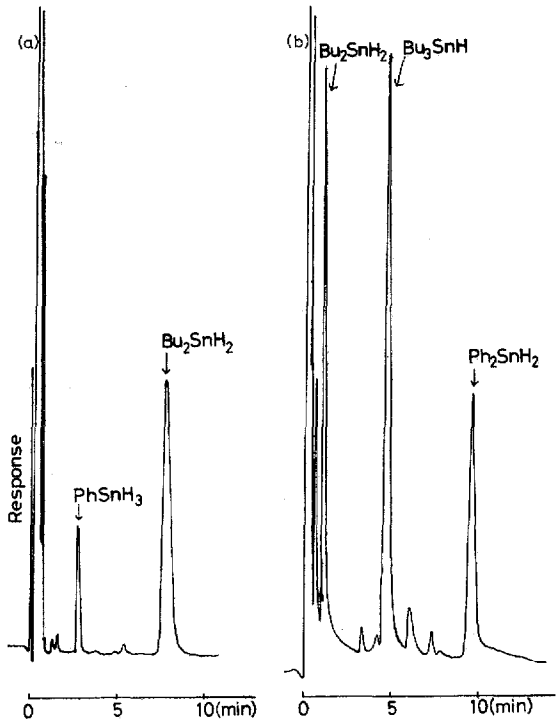

Fig. 3. Gas chromatograms of organotin hydrides recovered from crucian carp muscle spiked with 10 μg each of organotin chlorides. GC column, 10% OV-1. (a) Column temperature, 100°C; sample, 5 ml; (b) column temperature, 170°C; sample, 5 ml.

TABLE I

RECOVERY OF BUTYLTIN AND PHENYLTIN COMPOUNDS FROM BIOLOGICAL AND SEDIMENT SAMPLES

Sample	*Amount of tin compound added (μg)*	*Recovery* (%)*					
		$BuSnCl_3$	*Bu_2SnCl_2*	*Bu_3SnCl*	*$PhSnCl_3$*	*Ph_2SnCl_2*	*Ph_3SnCl*
Fish**	1	86.6 ± 4.7	86.2 ± 5.6	88.5 ± 4.7	75.0 ± 4.1	86.0 ± 3.3	86.3 ± 3.7
	10	95.3 ± 3.7	99.3 ± 3.1	99.8 ± 1.1	85.3 ± 4.4	88.0 ± 1.6	81.5 ± 1.8
Sediment***	1	70.3 ± 3.7	90.0 ± 3.2	90.1 ± 3.3	71.7 ± 2.4	76.7 ± 3.3	74.1 ± 3.1
	10	73.3 ± 2.4	96.6 ± 2.1	95.9 ± 2.1	85.7 ± 3.5	85.2 ± 2.6	80.8 ± 3.0

* Mean ± standard deviation, $n = 5$.
** Round crucian carp muscle, 10 g.
*** Wet sediment, 20 g; water, 46.2%; ignition loss, 10.0%.

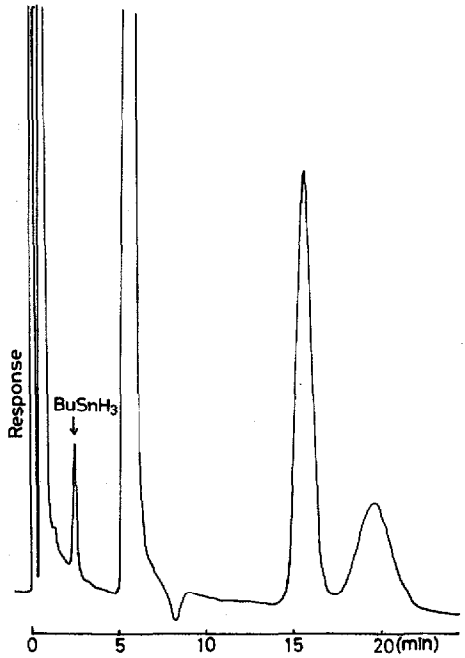

Fig. 4. Gas chromatogram of $BuSnH_3$ recovered from crucian carp muscle spiked with 10 μg of $BuSnCl_3$. GC column, 20% PEG 20M; column temperature, 60°C; sample, 10 ml.

samples. So eluent was separately collected from 5 to 15 ml for the organotin hydrides except Ph_3SnH, and from 15 to 40 ml for Ph_3SnH.

Recoveries of organotin from biological and sediment samples

The recoveries of butyltin and phenyltin chlorides added to fish muscle and sediment were evaluated. Corresponding gas chromatograms are shown in Figs. 3 and 4, and the results are given in Table I. The recoveries varied from 86.2 to 99.8% for butyltin chlorides and from 75.0 to 88.0% for phenyltin chlorides spiked at the levels of 1 and 10 μg per 10 g of fish sample. For sediment, they varied from 70.3 to 96.6% for butyltin chlorides and from 71.7 to 85.7% for phenyltin chlorides spiked at the levels of 1 and 10 μg per 20 g of sediment. These results indicate that there were no serious interferences from the sample matrix. The precision of the method was also evaluated by replicate analysis ($n = 5$) of fish and sediment samples spiked with 1 and 10 μg each of butyltin and phenyltin chlorides. For the fish sample, the reproducibility varied from 1.1 to 5.6% expressed as the standard deviation at the levels of 1 and 10 μg. For sediment, it varied from 2.1 to 3.7% at the same levels (better reproducibility was obtained).

Detection limit

The absolute detection limits for butyltin and phenyltin hydrides were 0.02–0.05 and 0.05–0.1 ng which gave signals twice the baseline noise. The detection limits were dependent on the volume of sample solution injected into the gas chromatograph. The eluent from column chromatography could be evaporated at 40°C in a

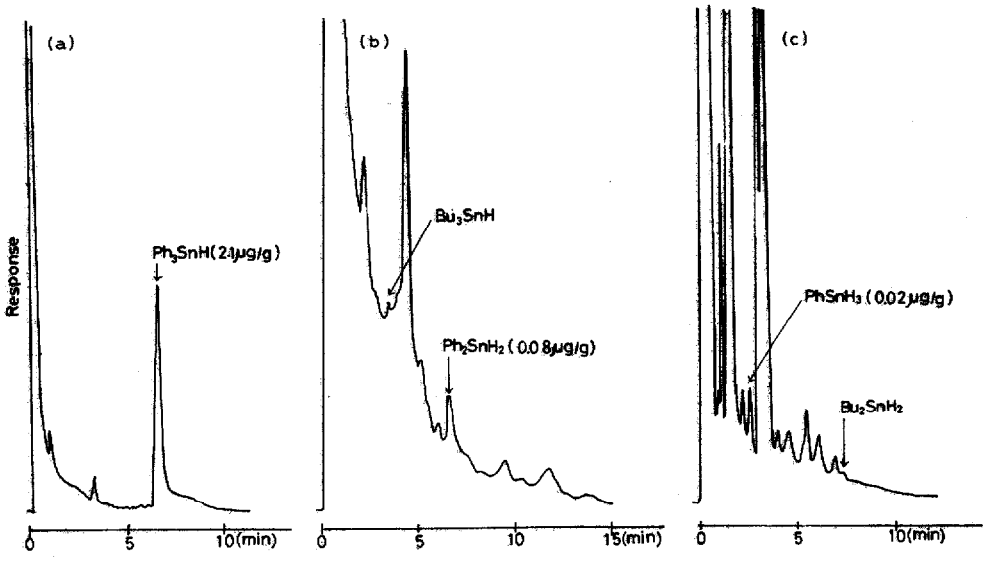

Fig. 5. Gas chromatograms of Ph_3SnCl and its metabolites, Ph_2SnCl_2 and $PhSnCl_3$, as their hydrides. GC column, 10% OV-1. (a) Column temperature, 250°C; sample, 10 ml; (b) column temperature, 170°C; sample, 1 ml; (c) column temperature, 100°C; sample, 1 ml.

rotary evaporator to 1–2 ml without loss of the organotin hydrides, except $BuSnH_3$. Further, the GC sample solution, except for $BuSnH_3$, could be adjusted to 1 ml without loss of the hydrides by evaporation under a stream of nitrogen or air at 40°C. The eluent or the sample solution should not be evaporated to dryness, because the hydrides are easily evaporated under these conditions. The detection limits were 10–20 ng/g for $BuSnCl_3$ (GC sample: 10 ml), 0.5–1.0 ng/g for Bu_2SnCl_2, 1–2 ng/g for Bu_3SnCl and Ph_2SnCl_2 and 2.5–5 ng/g for $PhSnCl_3$ and Ph_3SnCl (GC sample: each 1 ml) for biological and sediment samples.

Application to bioconcentration and metabolism studies

The proposed method was applied to studies of the bioconcentration and metabolism of Ph_3SnCl by carp. This experiment was carried out by exposing carp to 0.006 μg/ml Ph_3SnCl for 10 days. Fig. 5 shows typical gas chromatograms of phenyltin chlorides as their hydrides in carp muscle exposure for 7 days. For Ph_2SnH_2 and $PhSnH_3$, the GC sample solution was concentrated to 1 ml, but no interferences were observed on the gas chromatograms.

Application to environmental samples

Environmental samples were analyzed by the proposed method. Figs. 6 and 7 show typical gas chromatograms of organotin hydrides in fish and sediment from Lake Biwa (Shiga Prefecture, Japan). For round crucian carp, the Bu_3SnH peak was further identified on 3% SE-30 on Gas-Chrom Q (60–80 mesh). At high concentrations, gas chromatography–mass spectrometry (GC–MS) can probably be applied to the identification of Bu_3SnH or other hydrides.

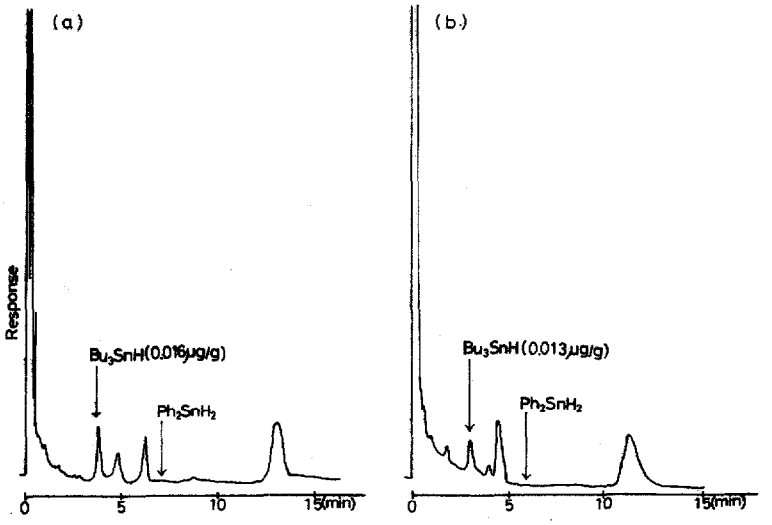

Fig. 6. Analysis of round crucian carp obtained from Lake Biwa. (a) GC column, 10% OV-1; column temperature, 170°C; sample, 2 ml. (b) GC column, 3% SE-30; column temperature, 125°C; sample 2 ml.

Interfering substances

The influence of IS (ionic strength), PM (hydrous iron oxide) and FA (fulvic acid) on the extraction and hydrogenation of organotin chlorides was investigated by reference to Donard and Weber's work[15]. The experiments were carried out as follows. (1) Amounts of sodium chloride, PM and FA in 50 ml water were prepared as in Table II. (2) Butyltin and phenyltin chlorides, each 1 μg, were added to the solutions, and recovery tests were carried out by the same procedure as for sediment.

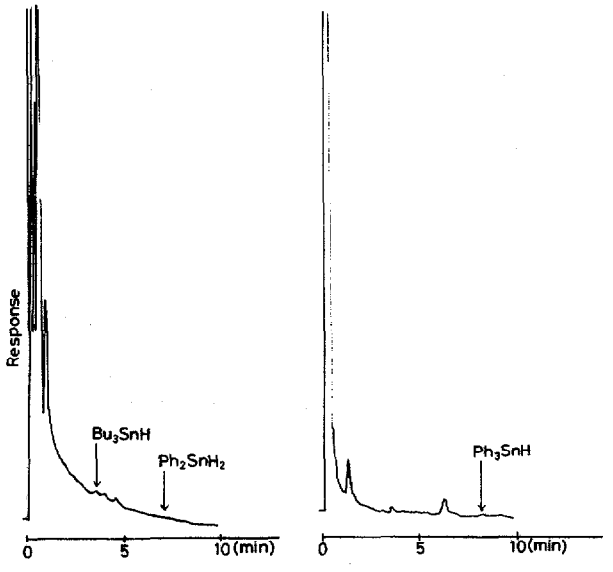

Fig. 7. Analysis of sediment obtained from Lake Biwa. GC column, 10% OV-1; sample, 5 ml.

TABLE II

INFLUENCE OF IS, PM AND FA ON THE EXTRACTION AND HYDROGENATION OF ORGANOTIN CHLORIDES

No.	*IS (NaCl g)*	*PM (mg)*	*FA (mg)*	*Recovery (%)*					
				$BuSnCl_3$	*Bu_2SnCl_2*	*Bu_3SnCl*	*$PhSnCl_3$*	*Ph_2SnCl_2*	*Ph_3SnCl*
1	0	0	0	100	100	100	100	100	100
2	10	0	0	114	115	100	123	105	92
3	0	50	0	60	71	70	73	75	86
4	0	0	5	71	129	113	105	110	95
5	0	50	5	60	97	95	70	87	80
6	0	100	10	50	110	84	70	96	96
7	2	50	5	65	96	100	83	90	82
8	10	50	5	–	–	–	–	–	–

Recoveries were shown at the basis of experiment 1 (not adding sodium chloride, PM and FA) in which each recovery of these organotin chlorides was expressed as 100%. Sodium chloride and FA tended to enhance the recoveries, but the opposite was observed for PM from experiments 2–4. A mixture of PM and FA tended to decrease the recoveries of $BuSnCl_3$ and $PhSnCl_3$, but had little influence on those of other organotin chlorides in experiments 5, 6. A mixture of sodium chloride, PM and FA had little influence at low salt concentration (experiment 7). At high salt concentration (experiment 8), much PM and FA were extracted into ethyl acetate, so interfering with the hydrogenation. Thus the influence of PM should be considered in sediment analysis.

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