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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC IDENTIFICATION OF PHENOLIC ACIDS IN RECENT SEDIMENTS

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SUMMARY

Saponification extraction of sediment samples was performed for the analysis of phenolic acids. After separation of phenolic acids by silica gel column chromatography, *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric (*cis* and *trans*), ferulic (*cis* and *trans*) and protocatechuic acids were identified, and the presence of *o*- and *m*-hydroxybenzoic acids was indicated by gas chromatography–mass spectrometry in lake, river and sea sediments. The content of each phenolic acid was less than 110 μg per gram of dry sample. These phenolic acids are mainly derived from vascular plants and their detritus.

INTRODUCTION

Although it is well known that a series of phenolic acids (*p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic and protocatechuic acids) are widely distributed in vascular plants[1–7], very little attention has been paid to their environmental and geochemical study. The occurrence of a series of phenolic acids in soils has been reported[8,9], and they are considered to play a major role in humus formation[9]. It is also known that these acids are widely distributed in natural and polluted waters at concentrations between nanogram and microgram per litre levels[10–12]. *p*-Coumaric and ferulic acids are thought to be two of the cause materials of so-called Kashin–Beck disease[13]. However, the occurrence of *p*-coumaric, ferulic and protocatechuic acids in bottom sediments has not yet been reported, although *p*-hydroxybenzoic acid has been identified in lake sediment[14] and *p*-hydroxybenzoic, vanillic and syringic acids, *etc.*, have been found in sea sediments[15,16]. We report here the quantitative analysis of *o*-, *m*- and *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric (*cis* and *trans*), ferulic (*cis* and *trans*) and protocatechuic acids in lake, river and sea sediments.

EXPERIMENTAL

Apparatus

Care was taken to minimize contamination of samples by using glass treated overnight with chromic acid mixture. Glassware was also heated to 500° for *ca.* 3 h before use.

Homogenization of sediment was carried out with an Ace Homogenizer (Nihon Seiki). Centrifuging was done using a Type H-100 centrifuge (Kokusan Ensinki).

The gas chromatographic–mass spectrometric (GC–MS) measurements were performed using a Shimadzu LKB 9000 instrument. A silanized glass column (2 m × 3 mm I.D.) was packed with 1% silicone OV-1 on Chromosorb W AW DMCS (80–100 mesh). The flow-rate of carrier gas (helium) was 30 ml/min. The column temperature was programmed from 100 to 200° at 6°/min. The injection block, molecular separator and ion source were maintained at 250, 300 and 330°, respectively. The chromatograms were recorded with the total ion current monitor (TICM) at 20 eV. The mass spectra were taken at 70 eV with an accelerator voltage of 3.5 kV.

Chemicals

All the organic solvents were guaranteed reagent grade and distilled. Silica gel (100 mesh) was ignited at 500° for *ca.* 3 h and deactivated with 5% water after cooling in a desiccator. Potassium hydroxide was melted by heating at *ca.* 300° for 6 h to remove organic contaminants. N,O-Bis(trimethylsilyl)acetamide–acetonitrile solution (25%, TMS–BA) was used as a silylation reagent. Authentic compounds were purchased from Wako (Osaka, Japan).

Samples

We studied five diverse sediments collected from: Lake Haruna (mesotrophic lake; altitude, 1084 m; area, 1.23 km^2; maximum depth, 13 m) in Gunma Prefecture near the Tokyo area; Tama River (Chohfu, *ca.* 15 km from Tokyo Bay, highly polluted) in the Tokyo area; Komagari Reservoir (volume, 23,900 m^3, not polluted); Yatsuse River (small stream, not polluted) in Chichi-jima Island, the Ogasawara (Bonin) Islands, which is *ca.* 1000 km south of Tokyo Bay; and the Gulf of Mexico near Galveston Bay, which was collected as a part of US–Japan co-operative study on environmental problems. The surface sediments were stored at temperatures below 4° until analysis.

Analysis of sediments

To determine phenolic acids, wet sediment (*ca.* 10 g) was first homogenized by stirring for 10 min ($1.0 \cdot 10^4$ rpm). Homogenized sediment was refluxed for 2 h to extract organic matter with 0.5 *M* potassium hydroxide–methanol solution and was then centrifuged ($1.8 \cdot 10^3$ *g*). The alkaline methanol extract and residue were acidified (pH <2) separately with concentrated hydrochloric acid, and the residue was extracted three times with 100 ml of ethyl acetate by stirring and centrifugation. The ethyl acetate extracts and the methanol extract were combined and washed five times with 30 ml of distilled water. The ethyl acetate layer was then concentrated to 10.0 ml under reduced pressure at temperatures below 30°. Two- or five-tenths of the concentrate was evaporated to dryness, redissolved in 50 μl of benzene–ethyl acetate (1:1), and chromatographed through a silica gel column (180 × 4 mm I.D.). After elution with three column volumes of benzene–ethyl acetate (95:5), four column volumes of benzene–ethyl acetate (1:1) eluate (phenolic acid fraction) was trimethylsilylated with TMS–BA. The trimethylsilyl derivatives of phenolic acids were determined by GC–MS. Based on four replicate addition experiments, the percentage recoveries of *o*-, *m*- and *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic and

protocatechuic acids were 44 (standard deviation, S.D., 27), 82 (S.D., 5.1), 73 (S.D., 8.5), 83 (S.D., 5.3), 72 (S.D., 1.5), 72 (S.D., 2.9), 90 (S.D., 14) and 13 (S.D., 1.8), respectively.

A blank test to check the contamination throughout the procedure showed that *o*-, *m*- and *p*-hydroxybenzoic acids are possible contaminants. However, the amounts were so small that their presence did not affect the analytical results for the sediment samples.

RESULTS AND DISCUSSION

A typical gas chromatogram and mass fragmentogram of the phenolic acid fraction obtained from the sediment of Yatsuse River are shown in Fig. 1, together

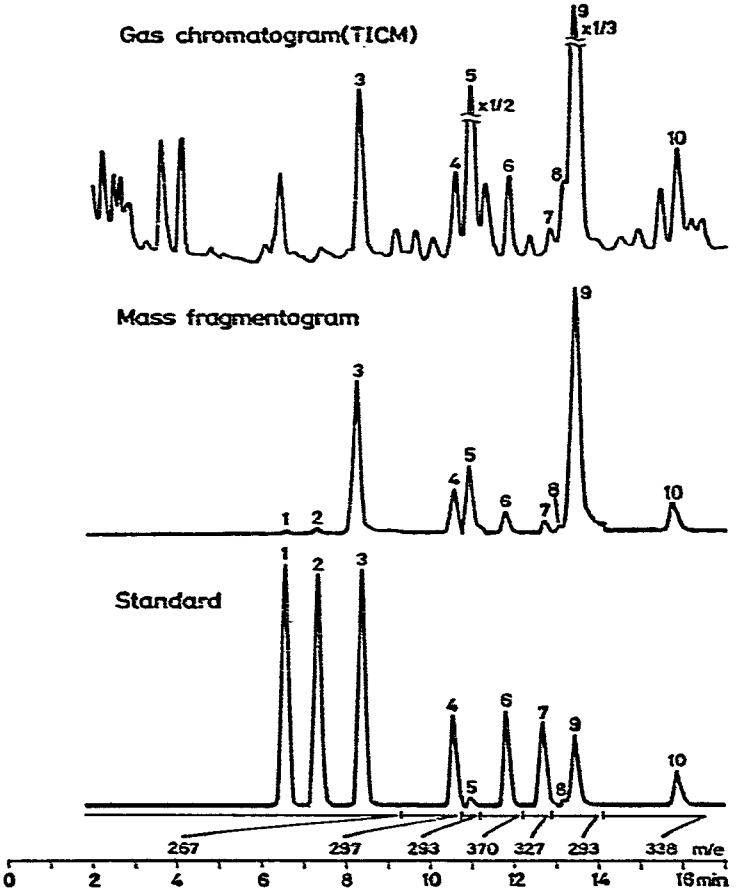

Fig. 1. Gas chromatogram and mass fragmentogram of the phenolic acid fraction obtained from the sediment of Yatsuse River, and mass fragmentogram of authentic compounds. The mass fragments at *m/e* 267, 297, 293, 327, 370 and 338 correspond to molecular ion (*M*) − 15 of *o*-, *m*- and *p*-hydroxybenzoic, vanillic, *p*-coumaric and syringic acid and *M* of protocatechuic and ferulic acid TMS derivatives, respectively. Peaks: 1 = *o*-hydroxybenzoic acid; 2 = *m*-hydroxybenzoic acid; 3 = *p*-hydroxybenzoic acid; 4 = vanillic acid; 5 = *p*-coumaric acid (*cis*); 6 = protocatechuic acid; 7 = syringic acid; 8 = ferulic acid (*cis*); 9 = *p*-coumaric acid (*trans*); 10 = ferulic acid (*trans*).

with the reference mass fragmentogram of authentic compounds. On the chromatogram, *o*-, *m*- and *p*-hydroxybenzoic, vanillic, syringic, protocatechuic, *p*-coumaric (*cis* and *trans*) and ferulic (*cis* and *trans*) acids were found, but other peaks were not identified. The mass spectra of *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric (*trans*), ferulic (*trans*) and protocatechuic acid trimethylsilyl derivatives obtained from the sediment of Yatsuse River are shown in Fig. 2, but those of *o*- and *m*-hydroxybenzoic acids are not shown because no clear mass spectra were obtained from the small amounts present. The mass spectra of the *cis*- and *trans*-forms of *p*-coumaric and ferulic acids are very similar, as reported by Hartley and Jones[17], and thus only those of the *trans*-forms are shown in Fig. 2. These mass spectra are identical with those of authentic compounds. The analytical results for the phenolic acids are summarized in Table I. A series of phenolic acids related to vascular plants and *o*- and *m*-hydroxybenzoic acids were found, with *p*-coumaric acid predominating

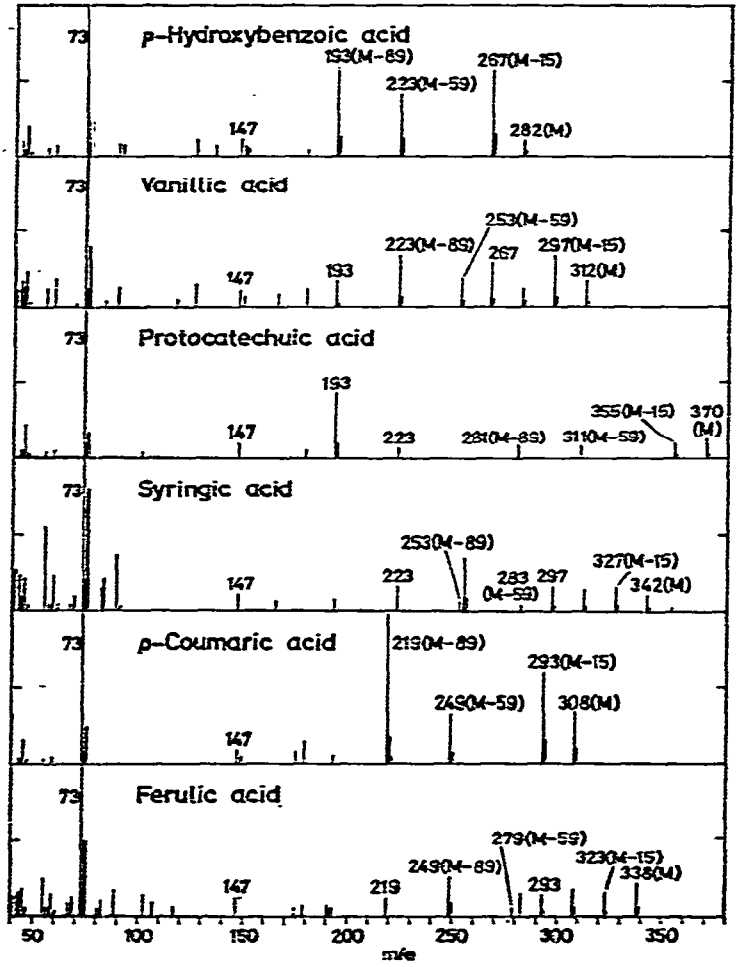

Fig. 2. Mass spectra of phenolic acid trimethylsilyl derivatives obtained from the sediment of Yatsuse River.

TABLE I

PHENOLIC ACIDS FOUND IN LAKE, RIVER AND SEA SEDIMENTS (μg/g DRY SEDIMENT)

Compound	*Lake Haruna*	*Tama River*	*Komagari Reservoir*	*Yatsuse River*	*Gulf of Mexico*
o-Hydroxybenzoic acid	0.6	0.1	0.06	0.2	0.02
m-Hydroxybenzoic acid	4.9	0.25	0.26	0.47	0.071
p-Hydroxybenzoic acid	14	1.2	7.9	16	0.51
Vanillic acid	4.8	0.77	5.6	9.2	0.18
Syringic acid	1.6	0.32	3.1	4.0	0.045
p-Coumaric acid*	23	11	86	110	1.1
Ferulic acid*	9.5	6.5	22	13	0.35
Protocatechuic acid**					

* *cis* + *trans*.

** Present but not quantified.

in all the samples studied so far. The content of each phenolic acid is less than 110 μg per gram of dry sediment, and somewhat lower in the sea sediment than in lake and river sediments.

When the saponification procedure was omitted, the contents of these phenolic acids decreased by an order of magnitude. This suggests that most of these acids are present in the sediments in combined forms, such as esters, rather than as free acids. This is consistent with the fact that a proportion of the phenolic acids, such as *p*-coumaric acid, is present as esters with lignin[5] and carbohydrates in the cell walls of *Lolium multiflorum*[7], and would be liberated by alkaline methanol hydrolysis.

Degens *et al.*[15] have suggested that phenolic acids found in sea sediments are due to terrestrially derived lignin. Lignin compounds occur in the support structure of vascular land plants but are apparently absent from marine organisms, and lignin oxidation products, including *p*-hydroxybenzoic, vanillic and syringic acids, and $^{13}C/^{12}C$ ratios have been investigated as indicators of land-derived organic matter in surface sediments from the western Gulf of Mexico[16]. Further, very little is known about the wide occurrence of this series of phenolic acids except for vascular plants and their detritus, although human urine contain many kinds of phenolic acid, including this series[18]. Therefore, most of the series of phenolic acids found in our samples are derived from widely occurring vascular plants and their detritus. However, human urine might affect the sediment of Tama River, which is highly polluted by sewage. In addition, *o*-, *m*- and *p*-hydroxybenzoic acids are produced by some micro-organisms, such as fungi and bacteria[19–21]. The source of *o*- and *m*-hydroxybenzoic acids found in the sediments may, therefore, be these micro-organisms. Furthermore, the phenolic acids should be distributed widely in sedimentary environments as in the case of natural and polluted waters[9–11].

These results show that saponification extraction and this analytical method are useful for the quantitative identification of phenolic acids in sediments down to the 10 ng level.

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