*Environ. Sci. Technol.* **1998**, *32,* 2238−2243

# Determination of Arsenic Compounds in Earthworms

ANITA GEISZINGER,[†] WALTER GOESSLER,[†] DORIS KUEHNELT,[†] KEVIN FRANCESCONI,[‡] AND WALTER KOSMUS*,[†]

*Institute for Analytical Chemistry, Karl-Franzens-Universitaet Graz, Universitaetsplatz 1, A-8010 Graz, Austria, and Institute of Biology, Odense University, DK-5230 Odense M, Denmark*

Earthworms and soil collected from six sites in Styria, Austria, were investigated for total arsenic concentrations by ICP-MS and for arsenic compounds by HPLC−ICP-MS. Total arsenic concentrations ranged from 3.2 to 17.9 mg/kg dry weight in the worms and from 5.0 to 79.7 mg/kg dry weight in the soil samples. There was no strict correlation between the total arsenic concentrations in the worms and soil. Arsenic compounds were extracted from soil and a freeze-dried earthworm sample with a methanol/water mixture (9:1, v/v). The extracts were evaporated to dryness, redissolved in water, and chromatographed on an anion- and a cation-exchange column. Arsenic compounds were identified by comparison of the retention times with known standards. Only traces of arsenic acid could be extracted from the soil with the methanol/water (9:1, v/v) mixture. The major arsenic compounds detected in the extracts of the earthworms were arsenous acid and arsenic acid. Arsenobetaine was present as a minor constituent, and traces of dimethylarsinic acid were also detected. Two dimethylarsinoylribosides were also identified in the extracts by co-chromatography with standard compounds. This is the first report of the presence of dimethylarsinoylribosides in a terrestrial organism. Two other minor arsenic species were present in the extract, but their retention times did not match with the retention times of the available standards.

## Introduction

Earthworms are important in agricultural soils as well as in pastures and natural grassland (*1*) where they play a significant role in the development of soil structure and fertility (*2*) by enhancing decomposition processes, aeration, and water permeability. Earthworms can also bioaccumulate elements from the soil, which in turn can be transferred to predators of worms such as birds, amphibians, shrews, and moles (*3*). Gut-associated processes and physical nutrient-enrichment processes of earthworms can influence the bioavailability of a particular element to plants (*4*). These processes both in the earthworm itself and in the soil via the worms' casts might also affect other organisms.

There have been many studies on the bioaccumulation of metals from soil by earthworms. Only a few of these studies have examined arsenic. There are no literature data available concerning arsenic compounds in earthworms. The bioavailability and toxicity of arsenic is highly dependent on its chemical form. In general, the inorganic forms of arsenic are much more toxic than the organoarsenic forms. Organoarsenic species are common in marine organisms where they are thought to represent metabolic end products of a process for detoxifying inorganic arsenic found in seawater and marine sediments. The major arsenic compound in marine animals is arsenobetaine. The tetramethylarsonium ion and arsenocholine ion are minor arsenic compounds frequently found in marine animals. In marine algae, dimethylarsinoylribosides (arsenosugars) predominate (*5*).

There have been former studies on the arsenic species in terrestrial organisms where, generally, arsenic concentrations are much lower than in marine samples. Nevertheless, recent work has also demonstrated the presence of arsenobetaine and other organoarsenic compounds in terrestrial organisms although their occurrence has been so far restricted to fungi and ants (*6*, *7*). The present study aims to expand out knowledge of arsenic in terrestrial organisms by examining the naturally occurring arsenic compounds in earthworms collected from sites high in arsenic.

## Experimental Section

**Reagents.** Deionized water was distilled twice in a quartz still and purified with a cartridge deionization system (Nanopure, Barnstead). The resulting Nanopure water was used for preparation of samples as well as of standards and had a resistivity of 18.2 MΩ·cm. All the reagents used were of analytical grade or better.

**Sample Collection and Preparation.** In May 1995, earthworm (*Lumbricidae*) samples (including all representative species of a sampling point) were collected from six sites in Styria (Austria) with soil arsenic concentrations above 5 mg/kg (Table 1). The soil was turned with a spade to a depth of 20 cm, and worms were collected by hand; only adult worms were used. They were transported (4 °C) to the laboratory in Graz where they were immediately washed free of adhering soil particles, placed in Petri dishes, and maintained for 4 days at 4 °C in order to empty their guts.

The worms were washed three times in Nanopure water and deep-frozen in small plastic bags. The samples were dried in an Alpha 1-4 freeze-drier (Christ, Osterode am Harz, Germany) and ground in a steel mill (Janke & Kunkel, Germany) at room temperature.

Soil samples were taken with a soil auger and were separated into two portions. One part was dried in a drying oven at 105 °C for 24 h for the determination of total arsenic concentration; the other part was freeze-dried for the determination of the arsenic compounds. All soil samples were sieved with a plastic screen (pore size < 1 mm).

**Determination of Total Arsenic in Earthworms.** Aliquots (∼0.2 g) of freeze-dried powdered worm tissue were weighed to 0.1 mg into the Teflon digestion vessels (90 mL) of an MLS-1200 Mega (MWS, Leutkirch, Germany) microwave digestion system. Subboiled concentrated nitric acid (3 mL) and 30% hydrogen peroxide (0.5 mL) were added. After 15 min, the vessels were closed and placed into the microwave oven. The samples were then mineralized with the following microwave heating program: 2 min at 250 W, 30 s at 0 W, 5 min at 300 W, 30 s at 0 W, 10 min at 400 W, 30 s at 0 W, 5 min at 500 W, and 4 min at 600 W. The resulting digests were quantitatively transferred into 50 mL volumetric flasks. The elements Ga, In, and Re were used as internal standards to correct for instrumental instabilities. Solutions of these elements were added to the analytical solutions so as to reach

* Corresponding author phone: +43-316-380 5304; fax: +43-316-380-9895; e-mail: walter.kosmus@kfunigraz.ac.at.
[†] Karl-Franzens-Universitaet Graz.
[‡] Odense University.


S0013-936X(98)00018-2 CCC: $15.00 
Published on Web 06/24/1998

**TABLE 1. Locations, the Nature of Their Soil Including pH of Soil, Concentrations of Arsenic in Soil and Earthworms, and the Ratio of Arsenic Concentration in Earthworms to That in Soil at Six Sites of Styria**

| | | | As concentration (mg/kg dry mass) | | |
|---|---|---|---|---|---|
| location | type of soil | pH | soil | earthworms | ratio e/s |
| 1 (Graz) | Fluvisol | 5.7 | 22.0 | 4.0 | 0.18 |
| 2 (Veitsch) | Cambisol | 7.7 | 66.4 | 6.4 | 0.10 |
| 3 (Admont) | Leptosol | 6.0 | 48.8 | 4.8 | 0.10 |
| 4 (Leibnitz) | Rigosol | 6.5 | 5.0 | 3.2 | 0.64 |
| 5 (Bruck) | Planosol | 5.8 | 45.7 | 8.2 | 0.18 |
| 6 (Knittelfeld) | Cambisol | 5.6 | 79.7 | 17.9 | 0.22 |

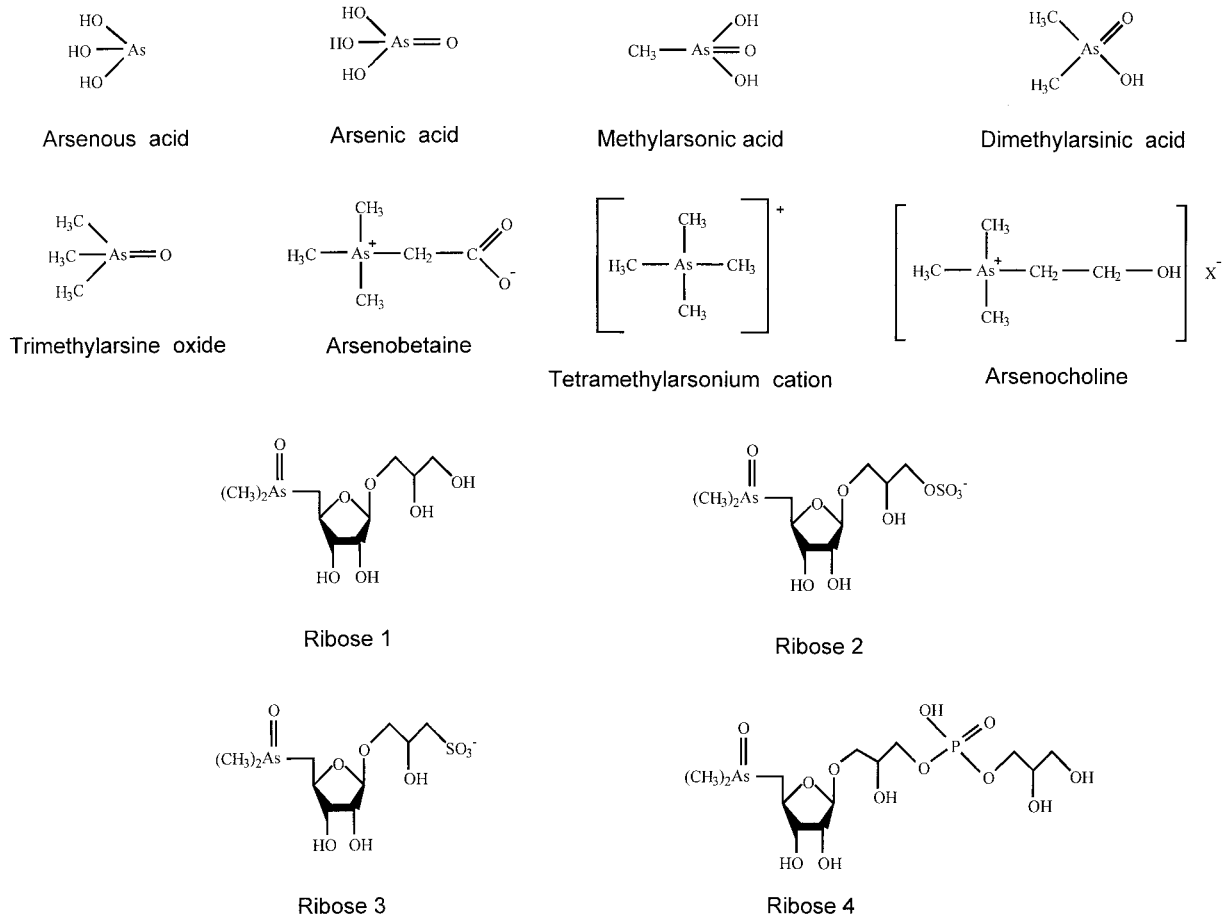


**FIGURE 1. Structures of investigated arsenic compounds.**

a final concentration of 50 $\mu$g/L each. The flasks were filled up to the mark with Nanopure water, closed, and well shaken.

**Determination of Total Arsenic in Soil Samples.** Aliquots (~2 g) of the dried soil were weighed to 0.1 g into 100 mL round-bottomed flasks, and 20 mL of aqua regia was added. The samples were allowed to stand at room temperature for 24 h. Then these mixtures were refluxed for 1 h. The extracts were filtered through folded filter papers (*8*). The filtrates were diluted (1:20), and nitric acid (2%) and the internal standard were added as mentioned before.

Total arsenic concentrations were determined in soil samples and in earthworms with a VG Plasma Quad 2 Turbo Plus inductively coupled argon-plasma mass spectrometer (VG Elemental Ltd., Winsford, U.K.). Pine needles (SRM 1575, National Bureau of Standards NIST, Gaithersburg, MD) and olive leaves (*Olea europea*, SRM-BCR 62, Community Bureau of Reference, Brussels, Belgium) served as standard reference materials. The results obtained were in good agreement with the certified values, 234 ± 20 $\mu$g/kg (210 ± 40 $\mu$g/kg certified) and 212 ± 35 $\mu$g/kg (0.2 mg/kg recommended), respectively.

**Preparation of Arsenic Standards.** The following compounds were used as arsenic standards in the HPLC separations: As(III) as $NaAsO_2$ and As(V) as $Na_2HAsO_4 \cdot 7H_2O$ were purchased from Merck; methylarsonic acid (MA) and dimethylarsinic acid (DMA) were gifts from the Vineland Chemical Company (Vineland, NJ); arsenobetaine bromide (*9*), arsenocholine bromide (*9*, *10*), trimethylarsine oxide (*11*), and tetramethylarsonium iodide (*9*) were synthesized according to published procedures. Ribose 1 (glycerol ribose) was synthesized by Dr T. Kaise (Tokyo University of Pharmacy and Life Science,1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan). Ribose 2 (sulfate ribose), ribose 3 (sulfonate ribose), and ribose 4 (phosphate ribose) were isolated from marine algae (*12*, *13*) (Figure 1).

**Extraction of Arsenic Compounds.** Aliquots (~0.2 g) of the earthworm powder or the freeze-dried soil sample were weighed into 50-mL polyethylene centrifuge tubes with screw caps and conical base. A methanol/water mixture (30 mL, 9:1, v/v) was added; the closed tubes were fastened to a cross-shaped rotating device and turned top over bottom for 14

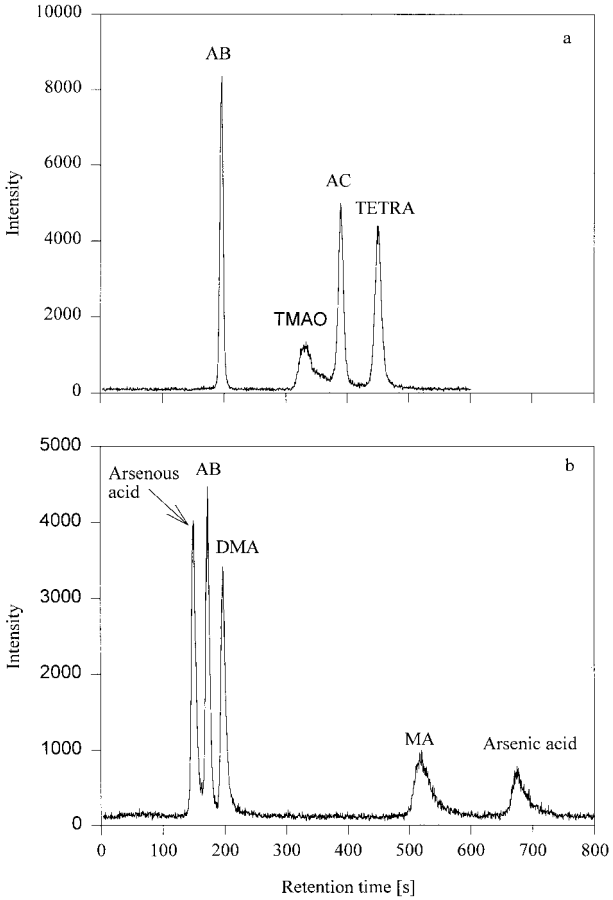


**FIGURE 2. (a) Chromatogram of a solution containing 5 $\mu$g of As/L of arsenobetaine (AB), trimethylarsine oxide (TMAO), arsenocholine ion (AC), and the tetramethylarsonium cation (TETRA) on the Supelcosil LC-SCX cation-exchange column (aqueous 20 mM pyridine solution at pH 3,0; column temperature 30 °C; flow rate 1.5 cm$^3$/min; 100 mm$^3$ injected). (b) Chromatogram of a solution containing 5 $\mu$g/L arsenous acid, arsenobetaine (AB), dimethylarsinic acid (DMA), methylarsonic acid (MA), and arsenic acid on the Supelcosil LC-SAX 1 anion-exchange column (aqueous 20 mM ammonium phosphate buffer at pH 4.16; column temperature 30 °C; flow rate 1.5 cm$^3$/min; 100 mm$^3$ injected).**

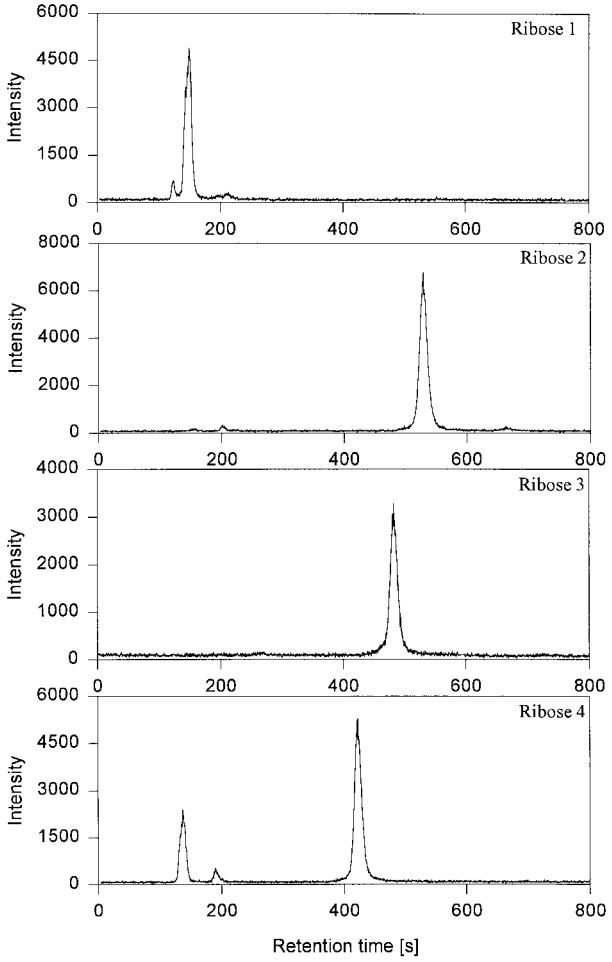


**FIGURE 3. Chromatograms of standard solutions of arsenoriboses on the Supelcosil LC-SAX 1 anion-exchange column (conditions see Figure 2b). Ribose 4 contains impurities of ribose 1 (signal at 180 and 195 s) as a degradation product.**

h. The extracts were centrifuged (2600 rpm, 10 min) and decanted into a 250-mL round-bottomed flask, and the residue was washed with methanol/water (30 mL, 9:1, v/v) and centrifuged (2600 rpm, 10 min), and the supernatant was decanted into the 250-mL round-bottomed flask. The washing was repeated three times. The combined supernatants were gently evaporated (rotary evaporator) at room temperature to dryness. The residue was redissolved in water (10 mL). The samples were then centrifuged in an ultracentrifuge (Hettich, Germany) for 15 min at 9500 rpm and filtered through 0.2 $\mu$m cellulose nitrate filters (Sartorius, Goettingen, Germany). Aliquots (100 $\mu$L) of these solutions were then directly chromatographed (*6*, *7*).

**Chromatographic Separation of Arsenic Compounds.** The separations were performed on a Supelcosil LC-SAX 1 (Supelco, Bellefonte, PA) anion-exchange column (25 cm × 4.6 mm i.d., 5-$\mu$m silica-based particles with quaternary aminopropyl exchange sites) and a Supelcosil LC-SCX cation-exchange column (25 cm × 4.6 mm i.d., 5-$\mu$m silica-based particles with propylsulfonic acid exchange sites). Both columns were operated at 30 °C.

The mobile phase for the anion-exchange HPLC was 20 mM phosphate at pH 4.16 prepared by combining 2.3 g of ammonium dihydrogen phosphate, 10 g of methanol, and Nanopure water (final volume 1 L) and adjusting the pH with *o*-phosphoric acid.

The mobile phase for the cation-exchange chromatography was 20 mM pyridine at pH 3.0 prepared by dissolving 1.58 g of pyridine to 1 L and adjusting the pH of this solution by the addition of formic acid. For checking the stability of the ICP-MS, rubidium chloride (RbCl) was added to all mobile phases to achieve a concentration of 50 ng/mL.

The HPLC system consisted of a Hewlett-Packard 1050 solvent delivery unit (Hewlett-Packard, Waldbronn, Germany) and a Rheodyne 9125 six-port injection valve (Rheodyne, Cotati, CA) with a 100-$\mu$L injection loop. The outlet of the HPLC column was connected via a 60-cm, 1/16-in. PEEK (polyether-ether-ketone) capillary tubing (0.25 mm i.d.) to a hydraulic high-pressure nebulizer (HHPN) (Knauer, Berlin, Germany). A VG Plasma Quad 2 Turbo Plus inductively coupled plasma mass spectrometer served as the arsenic-specific detector. The ion intensity at $m/z$ 75 ($^{75}$As) was monitored using the "time-resolved" analysis software version 1a (Fisons Scientific Equipment Division, Middlesex, U.K.). Additionally, the ion intensity at $m/z$ 77 ($^{40}$Ar$^{37}$Cl, $^{77}$Se) was monitored to detect possible argon chloride ($^{40}$Ar$^{35}$Cl) interferences on $m/z$ 75. Throughout the whole work, no ArCl interference was observed. Prior to each HPLC−ICP-MS run, the ion intensity at $m/z$ 87 (Rb added to the mobile phases) was adjusted at the rate meter of the instrument. Instrumental settings used throughout this work have been published in former works (*6*, *7*).

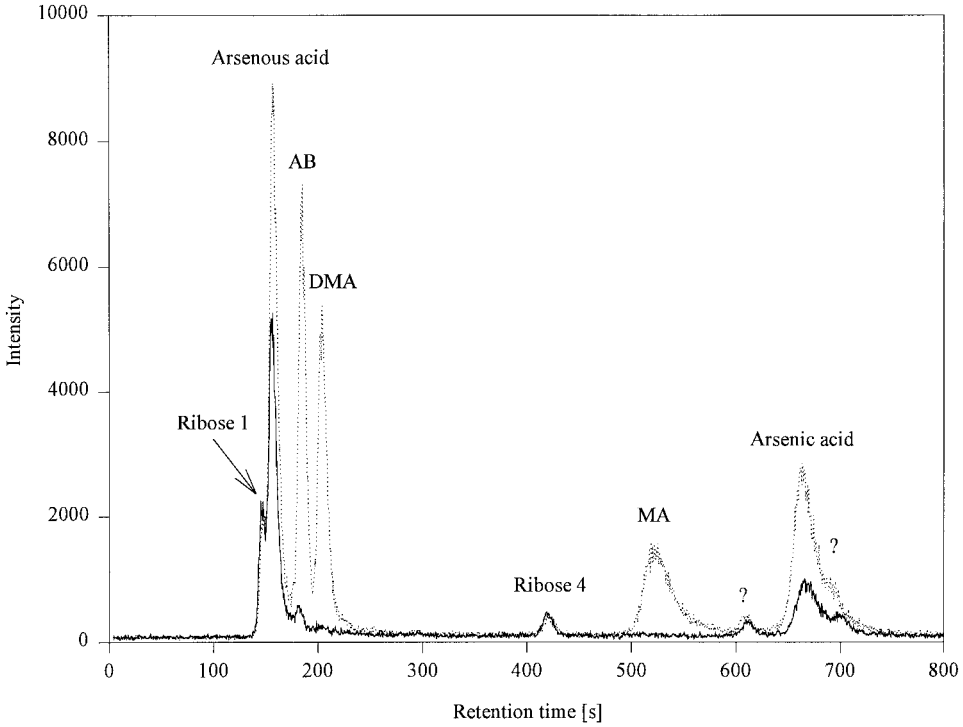


**FIGURE 4. Chromatogram of earthworm extract on the anion-exchange column (conditions see Figure 2b). Dotted line: Chromatogram of the same extract spiked with 10 $\mu$g of As/L of arsenous acid, AB, DMA, MA, and arsenic acid.**

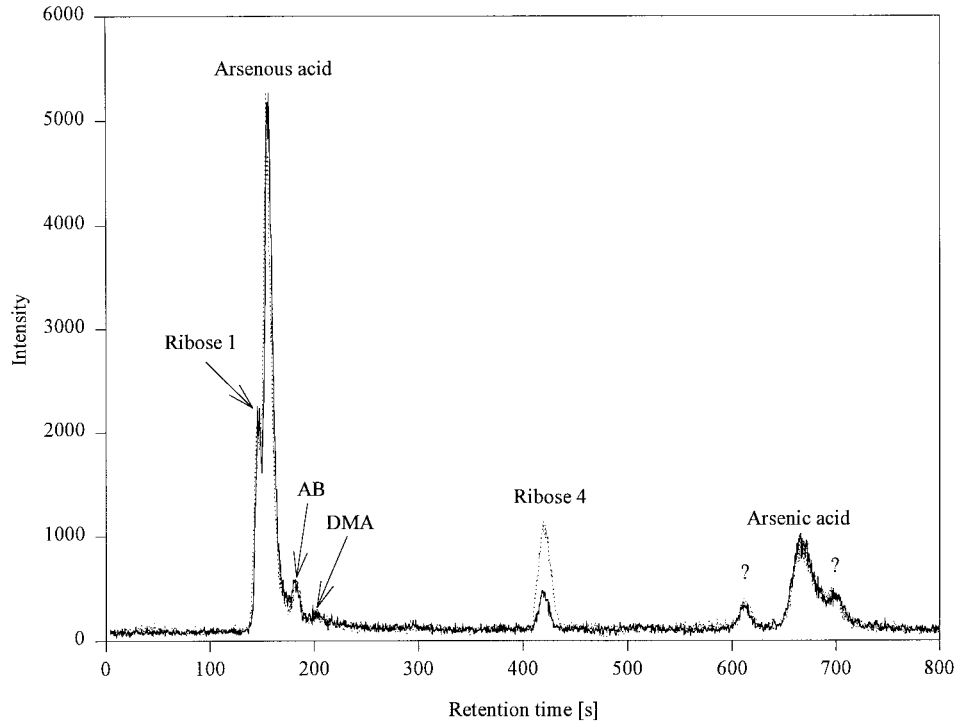


**FIGURE 5. Chromatogram of earthworm extract on the anion-exchange column (conditions see Figure 2b). Dotted line: Chromatogram of the same extract spiked with ribose 4.**

## Results and Discussion

**Total Arsenic Concentrations.** The total arsenic concentration in earthworms ranged from 3.2 to 17.9 mg/kg dry weight. The total arsenic concentration in soil ranged from 5.0 to 79.7 mg/kg (Table 1). No strict correlation between the arsenic concentration in the worm and the soil was observed, but all results were found in a narrow range with the exception of the sampling site 4, the one with the lowest arsenic concentration in soil. The concentration factors (arsenic concentration in earthworms divided by the arsenic concentration in soil) of the five sampling sites high in arsenic contents showed variations ranging from 0.1 to 0.22, while the concentration factor of the uncontaminated site was much higher, 0.64 respectively (Table 1). This high ratio could suggest bioaccumulation at a larger scale in soils with low arsenic levels. Earthworms might be able to partially regulate concentrations of contaminants, and possibly the

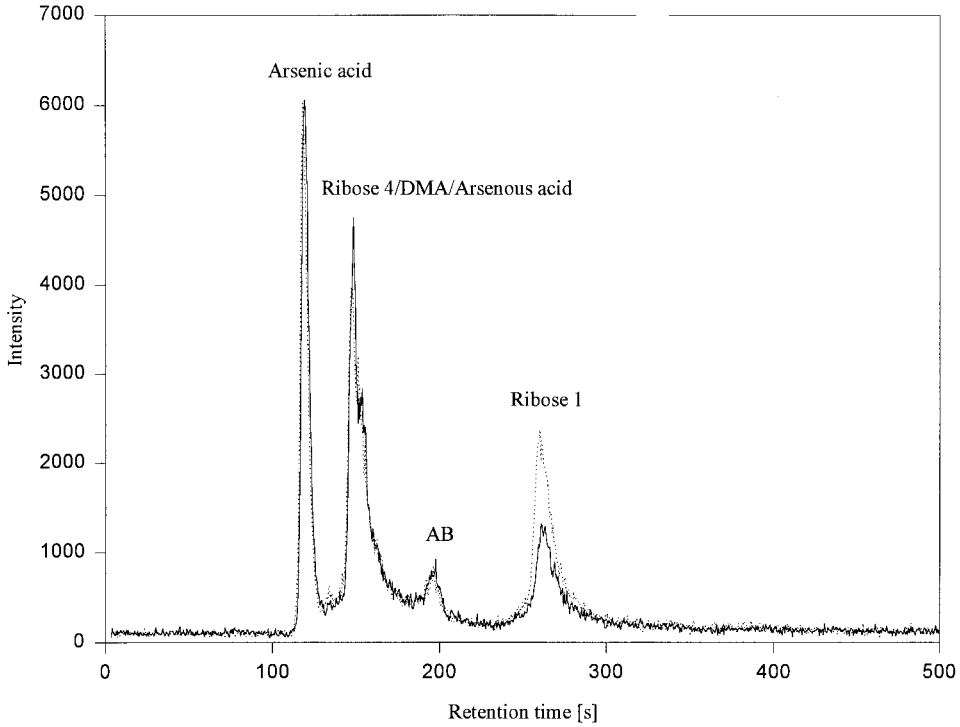


**FIGURE 6. Chromatogram of earthworm extract on the cation-exchange column (conditions see Figure 2a). Dotted line: Chromatogram of the same extract spiked with ribose 1.**

elemination rate of a toxicant is increased at higher contamination levels.

The arsenic concentrations found in soil and earthworms (Table 1) indicate that earthworms do not accumulate arsenic. Although the different sampling places revealed a wide range of arsenic concentrations in the earthworms, the arsenic concentrations found in earthworms never exceeded the arsenic concentrations found in soil.

**Chromatographic Separation of Arsenic Compounds.** Due to the different chemical properties of the investigated arsenic compounds, it is not readily possible to separate all the investigated arsenic compounds with one chromatographic run (*14*−*16*). Thus, anion-exchange as well as cation-exchange chromatography was employed to separate the arsenic compounds. The Supelcosil LC-SCX cation-exchange column allows the separation of arsenobetaine, trimethylarsine oxide, arsenocholine ion, and the tetramethylarsonium cation with a 20 mM aqueous pyridine solution at pH 3.0 (Figure 2a). Only arsenosugar 1 is retained (∼280 s) on this chromatographic system. Arsenosugars 2−4 coelute at ∼170 s with dimethylarsinic acid. Arsenous acid, arsenobetaine, dimethylarsinic acid, methylarsonic acid, and arsenic acid can be successfully separated on a Supelcosil LC−SAX1 with a 20 mM $NH_4H_2PO_4$ at pH 4.16 as mobile phase (Figure 2b). All arsenosugars can also be successfully separated on this column (Figure 3). Arsenosugar 1, however, elutes almost at the same retention time with arsenous acid. Therefore the identification of this arsenosugar on the cation-exchange column is preferable because no other arsenic compound is eluting at 280 s. The identification of the arsenic compounds present in soil and earthworm samples was based on the comparison of the retention times with synthetic standards and confirmed by spiking experiments.

**Arsenic Compounds in Soil.** Only traces of the total arsenic in the soil were extractable with the methanol/water mixture (9:1, v/v). The arsenic in the extract was present entirely as arsenic acid. The low extraction efficiency may be because arsenic is bound as insoluble inorganic compounds in the mineral phases of the soil. Such bound arsenic is only extracted with strong reagents: For example, concentrated nitric acid was necessary to efficiently extract arsenic from estuarine sediments; milder reagents such as dilute hydrochloric acid or acetic acid extracted only 60% and 22%, respectively, of the total arsenic present (*17*). Such forcing conditions may change the arsenic compounds present in the soil samples and are therefore not suitable for preparing extracts for analysis of arsenic species.

**Arsenic Compounds in Earthworms.** Arsenic compounds were determined in aqueous extracts of the earthworm samples from all five sites. The results were essentially the same; only those obtained from the site at Admont (site 3) will be presented here.

In the anion-exchange chromatograms (Figures 4 and 5) of the earthworm extracts, eight signals were obtained. Arsenous acid and arsenic acid were the two major arsenic compounds. Figure 4 shows a chromatogram of an aqueous extract of earthworm powder spiked with arsenous acid, arsenobetaine, dimethylarsinic acid, methylarsonic acid, and arsenic acid (1 ng of As each). Overlapping signals for arsenous acid, arsenobetaine, and arsenic acid were obtained. The retention time of two signals (605 and 700 s) did not match with the retention times of any of the available standards. These signals were not evident in the cation-exchange chromatogram, presumably because they were unresolved from other (known) arsenic compounds.

Although arsenobetaine is the major arsenic compound in marine animals such as fish, crustaceans, and molluscs, reports of its presence in terrestrial organisms have so far been restricted to fungi and ants (*18*, *6*, *7*). The origin of arsenobetaine in the earthworm is not known; it may be selectively accumulated from trace levels below detection limit in the soil, which is likely to contain fungal material, or it might be metabolized by the worms themselves or some internal microbial partners.

A signal with a retention time of 282 s identical with the retention time for the ribose 1 (the glycerol derivative) was detected with cation-exchange chromatography. Spiking the earthworm extract with synthetic arsenosugar 1 produced a

single peak with a retention time of 282 s (Figure 6). On anion exchange, this peak from the earthworm extract chromatographed near the void volume; this behavior, although identical with that shown by the arsenosugar 1, can be taken as supporting evidence only because many other cationic arsenic compounds also elute at or near the void volume on anion exchange under these conditions. The shoulder at the low retention time site of arsenous acid in the anion-exchange chromatogram of the earthworm extract can additionally be taken as supporting evidence.

The arsenic compound with a retention time of 418 s in the anion-exchange chromatogram was clearly separated from all other arsenic compounds in the extract, and its retention time matched that for ribose 4. When the earthworm extract was spiked with ribose 4, a single peak with a retention time of 418 s was obtained. On the cation-exchange chromatography ribose 2, ribose 3, ribose 4, DMA, arsenous acid, and MA would elute with the same retention time. The signal at ~140 s can give no support for the presence of ribose 4 because arsenous acid is present at much higher concentrations than ribose 4. It is significant that in the large body of work concerning arsenic compounds in algae, arsenosugars 1 and 4 are almost always reported as occurring together (*19*, *20*). Thus, the identification of the arsenosugars in earthworms, based primarily on co-chromatography with authentic material on anion exchange (arsenosugar 4) or on cation exchange (arsenosugar 1), is consistent with the previous results from marine algae where both these compounds occur together.

Until recently, arsenosugars have only been reported from algae or from animals feeding on algae. The study of Larsen et al. (*21*), however, has shown the presence of arsenosugars in deep-sea vent organisms where the food chain is based on bacteria, and living algae play no part. These authors suggested that the production of arsenosugars is also carried out by nonphotosynthetic microorganisms. The results reported here support that view and suggest that the production of arsenoriboses is a more widespread process than previously thought.

Arsenosugars, unlike arsenobetaine and dimethylarsinic acid, have not previously been reported in terrestrial organisms. Their presence in the earthworms may arise from three possible sources. First, biotransformation of inorganic arsenic by the worm itself. This, however, seems to be unlikely since laboratory studies have detected only simple methylated arsenic metabolites in animals exposed to inorganic arsenic (*22*), but cannot be excluded because the cited mammals are not related to annelida. Second, microbial alkylation of inorganic arsenic in the ingested soil by the gut microflora of the worm. Earthworms are known to provide an advantageous environment to microorganisms. Many different bacterial communities occur there that may elicit such transformation. Third, selective bioaccumulation from trace concentrations in the soil that cannot be detected with the analytical methods developed so far. Organoarsenic compounds were not detected in our soil samples, although the extraction procedure was repeated with much larger sample weights. Additional investigations of soil water, which might be considered as a natural extractant of the soil, collected at the same site as the worms revealed also low total arsenic concentrations (<2 $\mu$g/L) (unpublished data). This supports the hypothesis that earthworms did not take up organic arsenic compounds from the soil, although water or methanol are not expected to have the same efficiency as the fluids of the intestinal environment. However, water and methanol are known to be efficient extractants for these arsenic compounds.

The present study shows the presence of arsenous acid, arsenic acid, arsenobetaine, dimethylarsinic acid, and two arsenoriboses in extracts of earthworms. The detection of arsenobetaine and the arsenosugars is of particular interest since it extends the reported presence of these compounds to earthworms. Our study examined mixtures of unidentified worm samples, and we cannot comment on whether the arsenic compounds were present in all individual worm species contained in the samples. Future work will examine single species samples under field and laboratory conditions. Results from the work carried out so far on arsenic compounds in terrestrial organisms such as mushrooms and earthworms suggest that further work in this area may contribute significantly to the elucidation of arsenic biotransformation processes.

## Acknowledgments

The authors are grateful to Dr. Toshikazu Kaise for providing arsenosugar 1 and Lamona Sommerhuber for the assistance in soil analysis.

## Literature Cited

(1) Stockdill, S. M. J. *Pedobiologia* **1982**, *24*, 29−35.
(2) Shaw, C.; Pawluk, S. *Pedobiologia* **1986**, *29*, 327−339.
(3) Dunger, W. *Tiere im Boden*; Neue Brehm Bücherei No. 327; Ziemsen Verlag: Wittenberg, 1983; pp 63−86.
(4) Devliegher, W.; Verstraete, W. *Soil Biol. Biochem*. **1996**, *28*, 489−496.
(5) Francesconi, K. A.; Edmonds, J. S. *Adv. Inorg. Chem*. **1997**, *44*, 147−189.
(6) Kuehnelt, D.; Goessler, W.; Irgolic, K. J. *Appl. Organomet. Chem*. **1997**, *11*, 289−296.
(7) Kuehnelt, D.; Goessler, W.; Irgolic K. J. *Appl. Organomet. Chem*. **1997**, *11*, 859−867.
(8) ÖNORM L 1085. *Chemical Analysis of Soils*; Determination of Mineral Nutritive and Toxic Elements in Acid Digests; Österreichisches Normungsinstitut: 1989.
(9) McShane, W. J. Ph.D. Dissertation, Texas A&M University, 1982.
(10) Irgolic, K. J.; Junk, T.; Kos, K.; McShane, W. S.; Pappalardo, G. C. *Appl. Organomet. Chem*. **1987**, *1*, 403−412.
(11) Merijanin, A.; Zingaro, R. A. *Inorg. Chem*. **1966**, *5*, 187−191.
(12) Edmonds, J. S.; Francesconi, K. A. *J. Chem. Soc., Perkins Trans.* **1983**, *1*, 2375−2382.
(13) Edmonds, J. S.; Francesconi, K. A. *Nature* **1981**, *289*, 602−604.
(14) Larsen, E. H.; Pritzl, G.; Hansen, S. H. *J. Anal. Atom. Spetrom*. **1993**, 557−563.
(15) Gailer, J.; Irgolic, K. J. *J. Chromatogr. A* **1996**, *730*, 219−229.
(16) Gailer, J.; Irgolic, K. J. *Appl. Organomet. Chem*. **1994**, *8*, 129−140.
(17) Langston, W. J. *J. Mar. Biol. Assoc. U.K.* **1980**, *60*, 869−881.
(18) Byrne, A. R.; Slejkovec, Z.; Stijve, T.; Fay, L.; Goessler, W.; Gailer, J.; Irgolic, K. J. *Appl. Organomet. Chem*. **1995**, *9*, 305−313.
(19) Morita, M.; Shibata, Y. *Appl. Organomet. Chem*. **1990**, *4*, 181−190.
(20) Francesconi, K. A.; Edmonds, J. S. *Oceanogr. Mar. Biol. Annu. Rev*. **1993**, *31*, 111−151.
(21) Larsen, E. H.; Quetel, C. R.; Munoz, R.; Fiala-Medioni, A.; Donard, F. X. *Mar. Chem*. **1997**, *57*, 341−346.
(22) Vather, M.; Marafante, E. In *The Biological Alkylation of Heavy Elements*; Craig, P. J., Glockling, F., Eds.; Special Publication 66; Royal Society of Chemistry: London, 1988; pp 105−119.

*Received for review January 12, 1998. Revised manuscript received May 1, 1998. Accepted May 13, 1998.*

ES980018Y