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# Arsenic compounds in terrestrial organisms. IV. Green plants and lichens from an old arsenic smelter site in Austria

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**Two lichens and 12 green plants growing at a former arsenic roasting facility in Austria were analyzed for total arsenic by ICP–MS, and for 12 arsenic compounds (arsenous acid, arsenic acid, dimethylarsinic acid, methylarsonic acid, arsenobetaine, arsenocholine, trimethylarsine oxide, the tetramethylarsonium cation and four arsenoriboses) by HPLC–ICP–MS. Total arsenic concentrations were in the range of 0.27 mg As (kg dry mass)$^{-1}$ (*Vaccinium vitis idaea*) to 8.45 mg As (kg dry mass)$^{-1}$ (*Equisetum pratense*). Arsenic compounds were extracted with two different extractants [water or methanol/water (9:1)]. Extraction yields achieved with water [7% (*Alectoria ochroleuca*) to 71% (*Equisetum pratense*)] were higher than those with methanol/water (9:1) [4% (*Alectoria ochroleuca*) to 22% (*Deschampsia cespitosa*)]. The differences were caused mainly by better extraction of inorganic arsenic (green plants) and an arsenoribose (lichens) by water. Inorganic arsenic was detected in all extracts. Dimethylarsinic acid was identified in nine green plants. One of the lichens (*Alectoria ochroleuca*) contained traces of methylarsonic acid, and this compound was also detected in nine of the green plants. Arsenobetaine was a major arsenic compound in extracts of the lichens, but except for traces in the grass *Deschampsia cespitosa*, it was not detected in the green plants. In contrast to arsenobetaine, trimethylarsine oxide was found in all samples. The tetramethylarsonium cation was identified in the lichen *Alectoria ochroleuca* and in four green plants. With the exception of the needles of the tree *Larix decidua* the arsenoribose (2′*R*)-dimethyl[1-*O*-(2′,3′-dihydroxypropyl)-5-deoxy-*β*-D-ribofuranos-5-yl]-arsine oxide was identified at the low μg kg$^{-1}$ level or as a trace in all plants investigated. In the lichens an unknown arsenic compound, which did not match any of the standard compounds available, was also detected. Arsenocholine and three of the arsenoriboses were not detected in the samples. Copyright © 2000 John Wiley & Sons, Ltd.**

**Keywords: Arsenic; terrestrial environment; lichens; green plants; arsenobetaine; arsenoriboses; HPLC–ICP–MS**

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## INTRODUCTION

During the past two decades a variety of arsenic compounds have been identified in the marine environment.[1] The determination of arsenic compounds offers the possibility of elucidating the cycling and metabolic pathway of arsenic in the environment. Additionally, it is indispensable for assessing risks posed by arsenic-containing material, since the toxicities of the arsenic compounds differ significantly. Although seawater contains only about 2 μg As dm$^{-3}$, marine organisms are known to be rich in arsenic. Arsenic concentrations in the mg kg$^{-1}$ range easily allow the identification and quantification of arsenic compounds even when the extraction step, which is necessary to make these compounds available for analysis, is not quantitative. Arsenobetaine was identified as the major arsenic compound in marine animals such as fish, crustaceans and molluscs. Marine algae were found to contain arsenoriboses as the major arsenic compounds.[1] Since these arsenic compounds are

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less toxic than inorganic arsenic, no health risks are posed by the consumption of seafood.

Arsenic concentrations in terrestrial organisms are generally much lower than in the marine environment. Uncontaminated terrestrial plants usually contain about 0.2 to 0.4 mg As $kg^{-1}$.[2] Exceptions are arsenic-accumulating plants, for instance the mushroom *Laccaria amethystina*,[3] as well as plants grown in arsenic-contaminated areas. Arsenic concentrations in these organisms can be similar to those in marine organisms. For a long time arsenic compounds in terrestrial organisms seemed to be restricted to arsenous acid, arsenic acid and the 'simpler' methylated compounds methylarsonic acid, dimethylarsinic acid and trimethylarsine oxide.[2] The determination of arsenic compounds in these organisms was impeded by the low arsenic concentrations, non-quantitative extraction procedures and the analytical instruments available.

Although nowadays the separation power of HPLC in combination with the low detection limits achievable by ICP–MS allow the identification and quantification of arsenic compounds in terrestrial plants, only a few data on arsenic compounds in the terrestrial environment are available. Among the non-aquatic terrestrial plants, mushrooms were investigated most intensively. Dimethylarsinic acid was reported to be the major arsenic compound in the arsenic-accumulating mushroom *Laccaria amethystina* collected in Slovenia.[4] Methylarsonic acid and arsenate were minor arsenic compounds. In 1995 arsenobetaine was detected for the first time in the terrestrial environment.[5] It was the major arsenic compound in the arsenic-accumulating mushrooms *Agaricus haemorrhoidarius* and *Agaricus placomyces* from Slovenia and *Sarcodon imbricatum* from Switzerland. Additionally *Sarcodon imbricatum* contained the tetramethylarsonium cation. The presence of these two arsenic compounds in the terrestrial environment indicated that terrestrial organisms also contain the complex arsenic compounds formerly believed to exist only in the marine ecosystem. Two years later arsenocholine was detected for the first time in the terrestrial environment.[6,7] Arsenocholine was identified as a minor arsenic compound in the mushroom *Amanita muscaria* collected from a former arsenic smelter site in Austria. Arsenobetaine, inorganic arsenic, dimethylarsinic acid, the tetramethylarsonium cation and at least seven unidentified arsenic compounds, which did not coelute with any of the standard compounds available, were also detected in methanol/water extracts of this mushroom. The presence of arsenobetaine, arsenocholine and the tetramethylarsonium cation in a variety of higher fungi was confirmed by Slejkovec *et al.*[8] Larsen and co-workers reported inorganic arsenic, methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide and traces of arsenobetaine to be present in *Laccaria amethystina* collected from contaminated soil in Denmark.[9] Koch *et al.* investigated three mushroom species (*Tarzetta cupularis*, *Pluteus cervinus*, *Fomitopsis pinicola*) growing at a hot-spring environment in Canada showing naturally elevated arsenic concentrations in the water.[10] Besides inorganic arsenic, dimethylarsinic acid was found in *Tarzetta cupularis* and *Pluteus cervinus. Tarzetta cupularis* also contained a trace of (2′*R*)-dimethyl[1-*O*-(2′,3′-dihydroxypropyl)-5-deoxy-*β*-D-ribofuranos-5-yl]arsine oxide (glycerol-ribose, Fig. 1).

In 1997 an arsenoribose was detected for the first time in an algal sample of terrestrial origin.[11] Lai and co-workers reported the glycerol-ribose as the major arsenic compound in a methanol/water extract of the freshwater alga *Nostoc* sp. The presence of arsenoriboses (glycerol-ribose, phosphate-ribose) in freshwater algae was confirmed by Koch *et al.*[10] Almost no data on the arsenic compounds in non-aquatic green plants and lichens are available. Tomato leaves were shown to contain methylarsonic acid and dimethylarsinic acid.[2] Carrots grown in four soil samples with total arsenic concentrations of 6.5, 30.0, 93.3 and 338 mg As (kg dry mass)$^{-1}$, respectively, contained only inorganic arsenic.[12] Recently, arsenobetaine, trimethylarsine oxide and the tetramethylarsonium cation were reported in the green plants *Dactylis glomerata*, *Trifolium pratense* and *Plantago lanceolata* from Gasen, Styria, Austria, a site with a soil arsenic concentration of up to 4000 mg As $kg^{-1}$. Additionally, a trace of arsenocholine was found in *Plantago lanceolata*.[13] Koch *et al.* investigated methanol/water (1:1) extracts of a variety of terrestrial green plants and lichens collected from a hot spring environment in Canada showing naturally elevated arsenic concentrations in the water.[10] Three different lichens (*Bryoria* sp., *Alectoria* sp. and *Cladonia* sp.) were analyzed. Besides inorganic arsenic, the phosphate-ribose was detected in two samples of *Alectoria* sp. and in one sample of *Bryoria* sp. *Cladonia* sp. contained inorganic arsenic, a trace of dimethylarsinic acid and a trace of the glycerol-ribose. Inorganic arsenic was also found in the green plants investigated (*Scirpus* sp., *Thuja plicata, Fumaria hygrometrica, Erigeron* sp., *Mimulus* sp.). Additionally, one

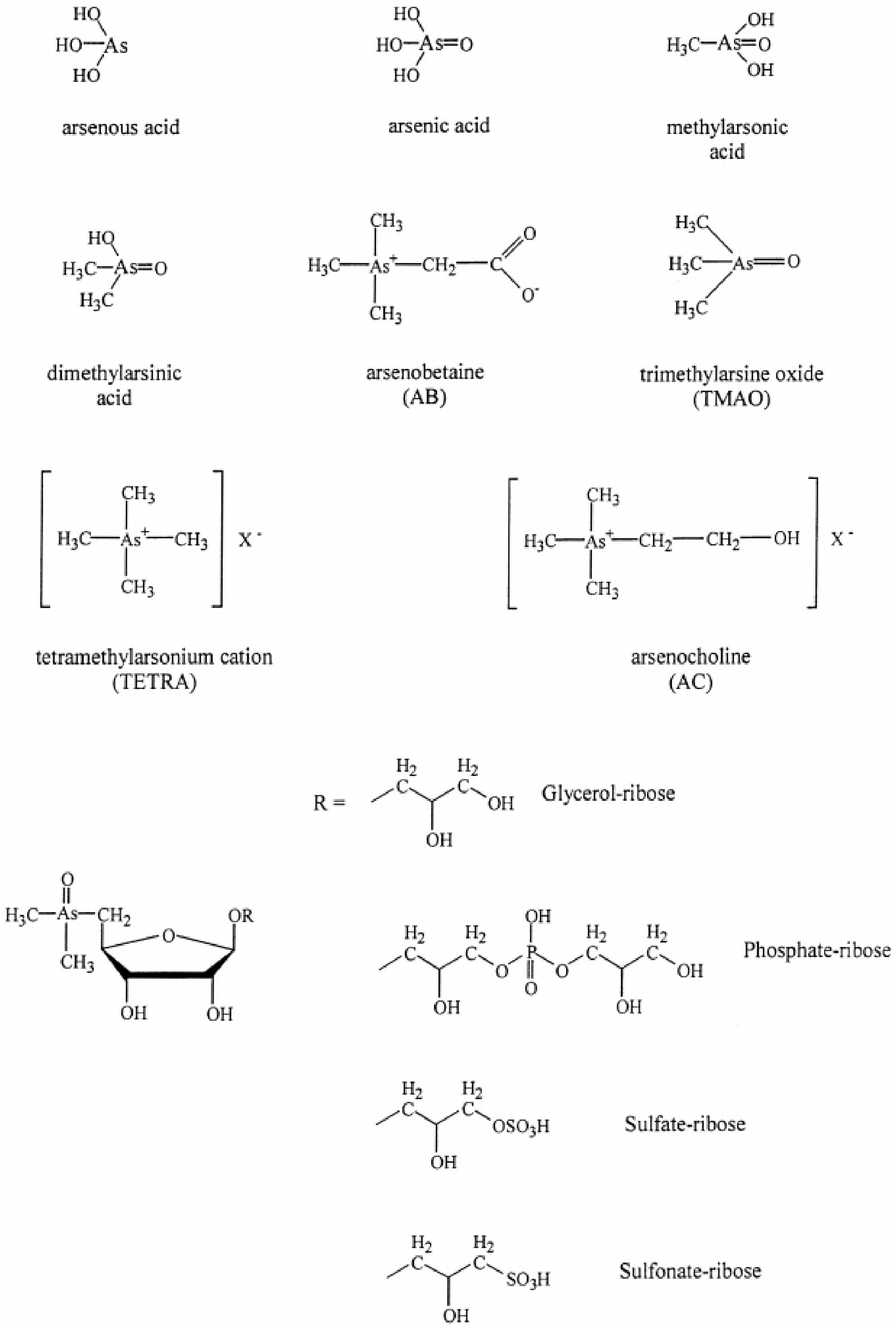

**Figure 1** Arsenic compounds investigated in green plants and lichens from an old arsenic smelter site in Austria.

sample of *Scirpus* sp. contained methylarsonic acid and another contained dimethylarsinic acid. These compounds were also detected in *Thuja plicata*. Methylarsonic acid was found in one and dimethylarsinic acid in two samples of *Fumaria hygrometrica*. In contrast to the glycerol-ribose, which was present in all samples of *Fumaria hygrometrica*, the phosphate-ribose was only detected in one sample of this plant. The only organic arsenic compound in the extracts of *Erigeron* sp. was dimethylarsinic acid. In *Mimulus* sp. the tetramethylarsonium cation was found in addition to inorganic arsenic.[10] These results indicate that organic arsenic compounds formerly attributed only to marine organisms and mushrooms are also present in terrestrial green plants and lichens.

In this work a variety of green plants and two different kinds of lichens collected at a former arsenic smelter site were analyzed for their total arsenic concentrations by ICP–MS, and for 12 arsenic compounds [arsenous acid, arsenic acid, dimethylarsinic acid (DMA), methylarsonic acid (MA), arsenobetaine (AB), arsenocholine (AC), trimethylarsine oxide (TMAO), the tetramethylarsonium cation (TETRA) and four arsenoriboses] (Fig. 1) by cation- and anion-exchange HPLC–ICP–MS.

## EXPERIMENTAL

### Reagents, solutions and samples

All solutions were prepared with Milli-Q (18.2 MΩ cm) water. Nitric acid (Merck, Darmstadt, Germany; p.a.) was further purified in a quartz sub-boiling distillation unit. Pyridine (p.a.), 30% hydrogen peroxide (suprapur), ammonium dihydrogen phosphate (p.a) and 25% aqueous ammonia (suprapur) were purchased from Merck, formic acid (puriss. p.a) and methanol (puriss. p.a) from Fluka (Buchs, Switzerland). The glycerol-ribose was synthesized by Dr Toshikazu Kaise (Laboratory of Environmental Chemistry, School of Life Science, University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachijoji, Tokyo 192-03, Japan). The phosphate-, the sulfonate- and the sulfate-riboses were isolated from marine algae by Dr Kevin A. Francesconi (Institute of Biology, Odense University, DK-5230 Odense M, Denmark).[14,15] The lichens *Alectoria ochroleuca* and *Usnea articulata*, leaves of the green plants *Achillea millefolium*, *Alnus incana*, *Asplenium viride*, *Dryopteris dilata*, *Equisetum pratense*, *Fragaria vesca*, *Rubus idaeus*, *Vaccinium myrtilis* and *Vaccinium vitis idaea*, needles of the green plants *Picea abies* and *Larix decidua*, and the grass *Deschampsia cespitosa* were collected in the area of a former roasting facility for arsenopyrite in Poellatal, Carinthia, Austria.[7] The samples were cleansed of soil, rinsed with tapwater, frozen at −20 °C, and freeze-dried for 24 h at −10 °C and for 24 h at 10 °C at 0.1 mbar.

### Instrumentation

The freeze-dried samples were pulverized in a Retsch ZM 1000 mill (Retsch, Haan, Germany) equipped with a titanium rotor and a 0.25-mm sieve. For determination of total arsenic concentrations the samples were digested with an MLS-1200 Mega microwave system (MLS, Leutkirch, Germany). Total arsenic was determined with an HP4500 ICP–MS (Hewlett Packard, Waldbronn, Germany) inductively coupled argon-plasma mass spectrometer (ICP–MS) equipped with a Babington-type nebulizer.

The chromatographic analysis of sample extracts for arsenic compounds was performed with an Hewlett Packard 1100 chromatographic system including solvent delivery unit, autosampler and column heater. The arsenic compounds were separated on a Hamilton (Reno, USA) PRP-X100 anion-exchange column (25 cm × 4.1 mm i.d.; 10-μm styrene–divinylbenzene particles with trimethylammonium exchange sites) and a Zorbax 300-SCX cation-exchange column (15 cm × 4.6 mm i.d.; porous silica microspheres with chemically bonded aromatic sulfonic acid exchange sites). The outlet from the HPLC column was connected via 100 cm of 1/16-inch (0.16-cm) PEEK (polyether-ether-ketone) capillary tubing (0.25 mm i.d.) to the Babington-type nebulizer of the HP4500 ICP–MS. The ion intensity at *m/z* 75 ($^{75}$As) was monitored. Additionally, *m/z* 77 ($^{40}$Ar$^{37}$Cl, $^{77}$Se) was monitored to detect possible $^{40}$Ar$^{35}$Cl interferences on *m/z* 75. Instrumental settings are given in Table 1. The chromatograms were smoothed with the Hewlett Packard ICP–MS Chromatographic Software G1824C, version C.01.00, and the peak areas were determined using software written in-house.[16] The arsenic compounds were quantified with external calibration curves established with arsenous acid, arsenic acid, methylarsonic acid and dimethylarsinic acid on the Hamilton PRP-X100 column, and with arsenobetaine, arsenocholine, trimethylarsine oxide and the tetramethylarsonium

**Table 1** Operating conditions for the HP4500 ICP-MS

| | |
|---|---|
| *Instrumental settings* | |
| RF power forward | 1350 W |
| RF power reflected | $< 1$ W |
| Plasma gas | 14.6 $\mathrm{dm^{-3}\ min^{-1}}$ |
| Auxiliary gas | 0.95 $\mathrm{dm^{-3}\ min^{-1}}$ |
| Nebulizer gas | 1.15 $\mathrm{dm^{-3}\ min^{-1}}$ |
| Nebulizer | Babington type |
| Spray chamber | Peltier cooled, 2 °C |
| Sampling cone | Nickel, 1.0-mm orifice |
| Skimmer cone | Nickel, 0.4-mm orifice |
| Sampling depth | 5.8 mm |
| *Aquisition parameters* | |
| Determination of total concentrations | |
| Detector mode | Auto |
| Monitored masses | $^{75}$As, $^{77}$Se or $^{40}$Ar$^{37}$Cl |
| Integration time/point | 3 s |
| Repetitions | 5 |
| Rinse time | 60 s at 2 $\mathrm{cm^3\ min^{-1}}$ |
| Stabilization | 60 s at 0.4 $\mathrm{cm^3\ min^{-1}}$ |
| Sample consumption during measurement | 0.4 $\mathrm{cm^3\ min^{-1}}$ |
| HPLC | |
| Detector mode | Pulse |
| Monitored masses | $^{75}$As, $^{77}$Se or $^{40}$Ar$^{37}$Cl |
| Points/mass | 1 |
| Integration time/mass | 0.300 s |
| Total analysis time | 360 s (Zorbax 300-SCX)<br>1200 s (PRP-X100) |

cation on the Zorbax 300-SCX column. The glycerol-ribose was quantified with the calibration curve for arsenobetaine considering that all arsenic compounds have the same response for arsenic when ICP–MS is used for detection.

## Total arsenic concentrations in green plants and lichens

Aliquots of the freeze-dried material (*ca* 0.2 g) were weighed to 0.1 mg into Teflon digestion vessels. Concentrated nitric acid (5.00 $\mathrm{cm^3}$) and 30% hydrogen peroxide (1.00 $\mathrm{cm^3}$) were added to each vessel. The vessels were closed, secured in the rotor and placed in the microwave oven. The samples were digested with the following program (W/min): 250/2, 0/0.5, 300/5, 0/0.5, 450/5, 0/0.5, 600/5, 500/7, 0/2 (ventilation). The digests of the leaves of *Alnus incana* and *Vaccinium vitis idaea* were transferred quantitatively to 10-$\mathrm{cm^3}$ volumetric flasks; the digests of the other plants were transferred to 50-$\mathrm{cm^3}$ volumetric flasks. The flasks were filled to the mark. Total arsenic concentrations were determined in these solutions by ICP–MS with an external calibration curve established with aqueous solutions of arsenic acid containing 1.00, 5.00, or 10.0 μg As $\mathrm{dm^{-3}}$.

## Arsenic compounds in green plants and lichens

The freeze-dried and pulverized samples (0.5 g of *Alectoria ochroleuca*, *Achillea millefolium*, *Asplenium viride*, *Deschampsia cespitosa*, *Dryopteris dilata*, *Equisetum pratense*, *Fragaria vesca*, *Larix decidua* or *Rubus idaeus*, 0.8 g of *Vaccinium myrtilis*, or 1 g of *Alnus incana*, *Picea abies*, *Usnea articulata* or *Vaccinium vitis idaea*) were weighed to 0.1 mg into screw-capped 50-$\mathrm{cm^3}$ polyethylene vials. Water (10 $\mathrm{cm^3}$) or methanol/water (9:1) (10 $\mathrm{cm^3}$) was added to each vial. Arsenic compounds were extracted on a cross-shaped rotor by turning the vials at 45 rpm for 14 h at 25 °C. The water extracts were centrifuged and the supernatants were filtered through cellulose ester filters (Millipore, Bedford, MA, USA). The methanol/

water extracts were centrifuged. Aliquots (2.0 $cm^3$) were evaporated to dryness in a Maxi Dry Lyo (Heto-Holten, Allerod, Denmark) at 50 mbar at room temperature, then the residues were redissolved in 2.00 $cm^3$ or 1.00 $cm^3$ water, respectively, and filtered through cellulose ester filters.

The undiluted extracts were analyzed for arsenobetaine, arsenocholine, trimethylarsine oxide, the tetramethylarsonium cation and the glycerol-ribose by cation-exchange chromatography on the Zorbax 300-SCX column with an aqueous solution of 20 mM pyridine at pH 2.6 (adjusted with formic acid) as mobile phase, and for arsenous acid, arsenic acid, dimethylarsinic acid, methylarsonic acid and the phosphate-, sulfate- and sulfonate-riboses by anion-exchange chromatography on the Hamilton PRP-X100 column with an aqueous solution of 20 mM $NH_4H_2PO_4$ at pH 5.6 [adjusted with aqueous ammonia (25%)] as mobile phase.

# RESULTS AND DISCUSSION

## Total arsenic concentrations in green plants and lichens

The green plants and lichens were collected at a former arsenic smelter site, which ceased operation more than 100 years ago.[7] All samples investigated grew on soil with an elevated arsenic concentration (730 mg As $kg^{-1}$)[7]. Total arsenic concentrations determined in green plants and lichens from the smelter site are summarized in Table 2. No correlations between the total arsenic concentrations in related plants were observed. The leaves of the fern *Asplenium viride* contained 7 mg As (kg dry mass)$^{-1}$, which is about 3.5 times more than the leaves of the fern *Dryopteris dilata* [2 mg As (kg dry mass)$^{-1}$]. The total arsenic concentration in the leaves of *Vaccinium myrtilis* [1.4 mg As (kg dry mass)$^{-1}$] is almost five times higher than in the leaves of *Vaccinium vitis idaea* [0.3 mg As (kg dry mass)$^{-1}$].

Most of the plants accumulated arsenic. The highest arsenic concentration was detected in the leaves of *Equisetum pratense* [8 mg As (kg dry mass)$^{-1}$]. This concentration is still not high compared with reported arsenic concentrations in other terrestrial plants grown on contaminated soil. For example, the foliage of *Agrostis tenuis* grown on mine waste was found to contain up to 3170 mg As (kg dry mass)$^{-1}$.[2] The leaves of *Vaccinium vitis idaea* [0.3 mg As (kg dry mass)$^{-1}$] as well as the leaves of *Alnus incana* [0.4 mg As (kg dry mass)$^{-1}$] contained arsenic at concentrations commonly reported for uncontaminated terrestrial plants.[2] Total arsenic concentrations in all samples were sufficiently high for the determination of arsenic compounds.

## Arsenic compounds in green plants and lichens

Arsenic compounds were extracted with water or methanol/water (9:1) from the green plants and lichens. The extracts of the green plants and lichens were subjected to anion- as well as to cation-exchange HPLC–ICP–MS. Standard solutions of 12 arsenic compounds (Fig. 1) were chromatographed to identify and quantify the arsenic compounds in the extracts. Arsenous acid, dimethylarsinic acid, methylarsonic acid, the phosphate-ribose, arsenic acid, the sulfonate-ribose and the sulfate-ribose can be separated within 20 min on the PRP-X100 anion-exchange column with 20 mM aqueous $NH_4H_2PO_4$ at pH 5.6 as mobile phase.[17] Under these conditions arsenocholine, the tetramethylarsonium cation, arsenobetaine, trimethylarsine oxide and the glycerol-ribose co-elute with arsenous acid at the solvent front. The determination of low concentrations of dimethylarsinic acid in the extracts is hampered when high amounts of arsenous acid, arsenocholine, the tetramethylarsonium cation, arsenobetaine, trimethylarsine oxide and/or the glycerol-ribose are present. The high signals from these compounds can completely overlap the signal of dimethylarsinic acid, or the signal of dimethylarsinic acid appears as a shoulder on the broad tailing signal of these compounds. Arsenobetaine, the glycerol-ribose, trimethylarsine oxide, arsenocholine and the tetramethylarsonium cation can be identified and quantified within 6 min on the Zorbax 300-SCX column with 20 mM aqueous pyridine at pH 2.6 as mobile phase. These five arsenic compounds are well separated from arsenous acid, arsenic acid, methylarsonic acid, dimethylarsinic acid and the phosphate-, sulfate- and sulfonate-riboses, which elute with or close to the solvent front on this column. Therefore arsenic acid, methylarsonic acid, dimethylarsinic acid, arsenobetaine, arsenocholine, trimethylarsine oxide, the tetramethylarsonium cation and the four arsenoriboses can be determined directly with these two chromatographic separations. Arsenous acid has to be calculated by subtraction of the sum of arsenobetaine, arsenocholine, trimethylarsine oxide, the tetramethylarsonium cation and the

**Table 2** Total arsenic concentrations and concentrations of arsenic compounds [extracted with methanol/water (9:1) or water] of green plants and lichens (mean of two determinations)

| | | Concentration [mg As (kg dry mass)$^{-1}$] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | | Arsenous acid | Arsenic acid | DMA | MA | AB | TMAO | TETRA | Glycerol-ribose | Unknown | Sum of species | Total arsenic[d] | Percentage of total |
| *Usnea articulata* | M[a] | 0.028 | <0.005 | <0.005 | <0.005 | 0.046 | 0.096 | <0.001 | 0.024 | 0.006 | 0.20 | 1.17, 1.07 | 18 |
| | W[b] | <0.005 | 0.014 | <0.005 | <0.005 | 0.065 | 0.12 | <0.001 | 0.077 | 0.006 | 0.28 | | 25 |
| *Alectoria ochroleuca* | M | 0.025 | <0.010 | <0.010 | Trace | 0.10 | 0.015 | 0.007 | 0.012 | 0.012 | 0.17 | 4.30, 4.28 | 4 |
| | W | <0.010 | 0.085 | <0.010 | Trace | 0.12 | 0.022 | 0.006 | 0.031 | 0.013 | 0.28 | | 7 |
| *Asplenium viride* (leaves) | M | 0.18 | 0.13 | 0.052 | 0.049 | <0.002 | 0.032 | 0.009 | 0.004 | <0.002 | 0.46 | 6.92, 7.21 | 7 |
| | W | 3.00 | 1.00 | <0.010 | 0.065 | <0.002 | 0.028 | 0.008 | 0.009 | <0.002 | 4.11 | | 58 |
| *Dryopteris dilata* (leaves) | M | 0.022 | 0.10 | <0.010 | <0.010 | <0.002 | 0.014 | 0.006 | 0.005 | <0.002 | 0.15 | 2.22, 1.83 | 7 |
| | W | 0.43 | 0.39 | Trace | <0.010 | <0.002 | 0.014 | 0.005 | 0.004 | <0.002 | 0.84 | | 41 |
| *Deschampsia cespitosa* | M | 0.039 | 0.33 | 0.017 | 0.021 | Trace | 0.011 | <0.002 | 0.008 | <0.002 | 0.43 | 2.05, 1.87 | 22 |
| | W | 0.19 | 0.71 | 0.024 | 0.027 | Trace | 0.012 | <0.002 | 0.005 | <0.002 | 0.97 | | 49 |
| *Picea abies* (needles) | M | 0.032 | 0.10 | 0.009 | <0.005 | <0.001 | 0.015 | <0.001 | 0.005 | <0.001 | 0.16 | 0.90, 0.92 | 18 |
| | W | 0.25[c] | 0.11 | Trace | Trace | <0.001 | 0.015 | <0.001 | Trace | <0.001 | 0.38 | | 42 |
| *Larix decidua* (needles) | M | 0.32 | 0.30 | <0.010 | <0.010 | <0.002 | 0.11 | <0.002 | <0.002 | <0.002 | 0.73 | 3.81, 3.61 | 20 |
| | W | 0.99 | 0.15 | <0.010 | <0.010 | <0.002 | 0.093 | <0.002 | <0.002 | <0.002 | 1.23 | | 33 |
| *Alnus incana* (leaves) | M | <0.005 | 0.011 | Trace | 0.012 | <0.001 | 0.043 | <0.001 | Trace | <0.001 | 0.07 | 0.37, 0.46 | 17 |
| | W | 0.049[c] | 0.025 | Trace | 0.018 | <0.001 | 0.042 | <0.001 | <0.001 | <0.001 | 0.13 | | 31 |
| *Fragaria vesca* (leaves) | M | <0.010 | 0.091 | 0.022 | 0.016 | <0.002 | 0.046 | 0.010 | 0.009 | <0.002 | 0.19 | 3.60, 3.90 | 5 |
| | W | 1.01[c] | 0.40 | Trace | 0.037 | <0.002 | 0.043 | 0.013 | 0.008 | <0.002 | 1.51 | | 40 |
| *Vaccinium myrtilis* (leaves) | M | 0.12[c] | 0.026 | Trace | 0.008 | <0.001 | 0.013 | <0.001 | Trace | <0.001 | 0.17 | 1.36, 1.38 | 12 |
| | W | 0.49 | 0.13 | <0.006 | Trace | <0.001 | 0.014 | <0.001 | <0.001 | <0.001 | 0.63 | | 46 |
| *Vaccinium vitis idaea* (leaves) | M | <0.005 | 0.011 | <0.005 | 0.019 | <0.001 | 0.004 | 0.003 | Trace | <0.001 | 0.04 | 0.27, 0.30 | 14 |
| | W | 0.078 | 0.018 | <0.005 | 0.020 | <0.001 | 0.004 | 0.004 | <0.001 | <0.001 | 0.12 | | 42 |
| *Rubus idaeus* (leaves) | M | 0.030[c] | 0.10 | Trace | <0.010 | <0.002 | 0.048 | <0.002 | Trace | <0.002 | 0.18 | 2.40, 2.81 | 7 |
| | W | 0.96 | 0.30 | <0.010 | <0.010 | <0.002 | 0.044 | <0.002 | <0.002 | <0.002 | 1.30 | | 50 |
| *Achillea millefolium* (leaves) | M | 0.086 | 0.081 | 0.069 | 0.040 | <0.002 | 0.005 | <0.002 | 0.011 | <0.002 | 0.29 | 2.06, 2.04 | 14 |
| | W | 0.31 | 0.29 | 0.065 | 0.062 | <0.002 | 0.008 | <0.002 | <0.002 | <0.002 | 0.74 | | 36 |
| *Equisetum pratense* (leaves) | M | 0.47 | 0.44 | <0.010 | 0.037 | <0.002 | 0.16 | <0.002 | Trace | <0.002 | 1.11 | 8.09, 8.45 | 13 |
| | W | 3.10 | 2.50 | <0.010 | 0.080 | <0.002 | 0.16 | <0.002 | Trace | <0.002 | 5.84 | | 71 |

[a] M, methanol/water (9:1) extract.
[b] W, water extract.
[c] Sum of arsenous acid and DMA
[d] Two individual determinations.

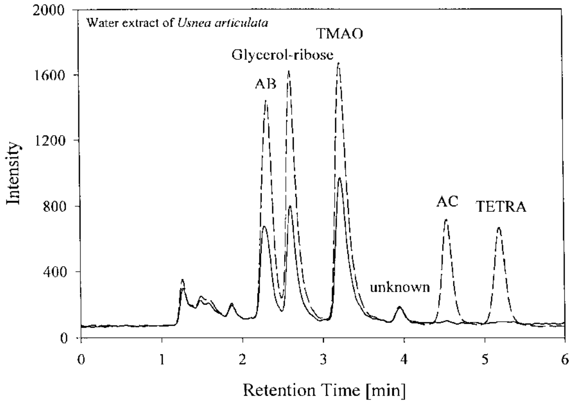

**Figure 2** Chromatogram of a water extract of *Usnea articulata* on the Zorbax 300-SCX column (mobile phase, 20 mM pyridine pH 2.6; flow, 1.5 $cm^3 min^{-1}$; temperature, 30 °C; injection volume, 50 $mm^3$). Broken line: 45 $mm^3$ of extract spiked with 5 $mm^3$ of a standard solution containing arsenobetaine, trimethylarsine oxide, arsenocholine, the tetramethylarsonium cation and the glycerol-ribose (50 ng As $cm^{-3}$ each).

glycerol-ribose from the front peak in the anion-exchange chromatogram.

Arsenic compounds were extracted with two different extractants, water or methanol/water (9:1), from the green plants and lichens. Methanol/water (9:1) extracted 4% (*Alectoria ochroleuca*) to 22% (*Deschampsia cespitosa*) of the total arsenic from the plants (Table 2). Extraction yields obtained with water [7% (*Alectoria ochroleuca*) to 71% (leaves of *Equisetum pratense*)] were generally higher than those achieved with methanol/water (9:1). Compared with methanol/water (9:1), water extracted at least twice as much of the total arsenic from the green plants (exception: needles of *Larix decidua*), and almost ten times as much from the leaves of *Asplenium viride*. Extraction yields obtained with water were low for the lichens [7% (*Alectoria ochroleuca*), 25% (*Usnea articulata*)]. For the green plants the higher extraction yields achieved with water compared to methanol/water (9:1) can be explained by better extractability of inorganic arsenic.[5] The glycerol-ribose contained in the lichens is also better removed by water than by methanol/water (9:1).

Besides inorganic arsenic, various organic arsenic compounds were detected in the extracts of the green plants and lichens (Table 2). Since the existence of dimethylarsinic acid and methylarsonic acid in the terrestrial environment is well established,[2] the presence of these arsenic compounds in the extracts of the green plants and lichens is not discussed in detail in this work. The organic arsenic compounds in extracts of the lichens *Alectoria ochroleuca* and *Usnea articulata* were similar. A chromatogram of a water extract of *Usnea articulata* is presented in Fig. 2. The extracts of *Alectoria ochroleuca* and *Usnea articulata* contained arsenobetaine, trimethylarsine oxide, the glycerol-ribose and an unknown arsenic compound, which did not co-elute on the Zorbax 300-SCX column with any of the standard arsenic compounds available. In the extracts of *Alectoria ochroleuca* a small amount of the tetramethylarsonium cation was detected. This compound was not detected in the extracts of *Usnea articulata*. Arsenobetaine, one of the major arsenic compounds in the extracts of *Usnea articulata* and the major arsenic compound in the extracts of *Alectoria ochroleuca*, has been identified in several terrestrial mushrooms,[5–9] some of which contain it as their major arsenic compound. The glycerol-ribose is a major constituent in the extracts of *Usnea articulata* and a minor constituent in the extracts of *Alectoria ochroleuca*. Arsenoriboses are the major arsenic compounds in marine algae,[1] and have been previously detected in the terrestrial environment in freshwater algae,[10,11] earthworms,[18] microbial mats, lichens and green plants.[10] A trace of the glycerol-ribose was reported in the mushroom *Tarzetta cupularis*.[10] In contrast to our results, Koch *et al.* found the phosphate-ribose in some samples of *Alectoria* sp., whereas the glycerol-ribose was not detected.[10] The glycerol-ribose, which we found in the extracts of the lichens, was identified as the major arsenic compound in an extract of the freshwater alga *Nostoc* sp.[11] It was also detected in extracts of green algae, which additionally contained the phosphate-ribose, from a hot spring environment in Canada.[10] An extract of the lichen *Cladonia* sp. from the same area was reported to contain traces of this arseno-ribose.[10] Lichens are symbiotic organisms of fungi, algae and/or cyanobacteria.[19] The presence of arsenobetaine can be attributed to the fungal component, and the glycerol-ribose to the algal component, of the organism.

All extracts of the green plants contained inorganic arsenic. Inorganic arsenic was dominant in all water extracts. Trimethylarsine oxide was also detected in all extracts in a concentration range of 4 μg As (kg dry mass)$^{-1}$ (leaves of *Vaccinium vitis idaea*) to 160 μg As (kg dry mass)$^{-1}$ (leaves of *Equisetum pratense*). The tetramethylarsonium cation was only identified in the extracts of the

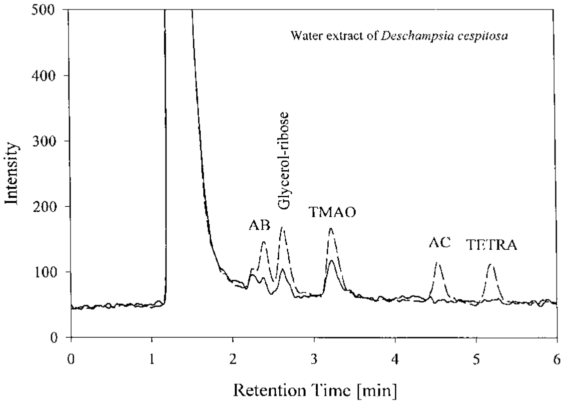

**Figure 3** Chromatogram of a water extract of *Deschampsia cespitosa* on the Zorbax 300-SCX column (mobile phase, 20 mM pyridine pH 2.6; flow, 1.5 cm$^3$ min$^{-1}$; temperature, 30 °C; injection volume, 50 mm$^3$). Broken line: 45 mm$^3$ of extract spiked with 5 mm$^3$ of a standard solution containing arsenobetaine, trimethylarsine oxide, arsenocholine, the tetramethylarsonium cation and the glycerol-ribose (5 ng As cm$^{-3}$ each).

leaves of the ferns (*Dryopteris dilata*, *Asplenium viride*) and in the extracts of the leaves of *Fragaria vesca* and *Vaccinium vitis idaea* at low concentrations [3 µg As kg$^{-1}$ (*Vaccinium vitis idaea*) to 13 µg As (kg dry mass)$^{-1}$ (*Fragaria vesca*)]. All green plants with the exception of *Larix decidua* contained the glycerol-ribose in at least one of the two different extracts. The concentration of this arsenic compound never exceeded 11 µg As (kg dry mass)$^{-1}$ (methanol/water extract of the leaves of *Achillea millefolium*). In most of the extracts only traces were detected. The fact that the glycerol-ribose is present at very low concentrations in the green plants could have hampered its detection in many samples of terrestrial origin until now because of the lack of methods with sufficiently low detection limits. The chromatographic separation on the Zorbax 300-SCX cation-exchange column with 20 mM pyridine at pH 2.6 as mobile phase allows the quantification of arsenic compounds at a concentration of 0.2 ng As cm$^{-3}$ extract. Assuming that the concentration of the arsenic compounds in the extract is ten times lower than the concentration in the biological material (1 g extracted with 10 cm$^3$ of extractant), arsenic compounds present at the low µg kg$^{-1}$ level in the material can be quantified, provided that the extraction procedure is close to quantitative. Koch *et al.* reported extracts of three samples of the moss *Fumaria hygrometrica* to contain the glycerol-ribose at a concentration of 0.18–0.31 mg As (kg dry mass)$^{-1}$,[10] which is more than ten times higher than the concentrations of the glycerol-ribose found in our green plant samples, but the authors could not exclude the possibility that this high amount of arsenoriboses derived from microbial mats observed in and around the moss samples. The phosphate-ribose, which was detected in one sample of *Fumaria hygrometrica* [0.03 mg As (kg dry mass)$^{-1}$],[10] was not detected in any of our samples. Traces of arsenobetaine were only detected in the extracts of *Deschampsia cespitosa* (Fig. 3). Geiszinger[13] reported small amounts of arsenobetaine [0.5–3.5 $\mu$g As (kg dry mass)$^{-1}$] in the terrestrial green plants *Dactylis glomerata, Trifolium pratense* and *Plantago lanceolata* grown on soil with elevated arsenic concentrations. These results indicate that arsenobetaine, thought to be present mainly in marine animals and terrestrial mushrooms, is more widespread than expected. However, whether arsenobetaine and the glycerol-ribose are formed by the green plants themselves, or by microorganisms on the plant or in the soil and then taken up by the plant, is not certain yet. Arsenocholine and the phosphate-, the sulfate- and the sulfonate-riboses were not detected in any of the samples.

Parallels between related plants exist for the two fern samples. Extracts of the leaves of *Asplenium viride* and *Dryopteris dilata* contained almost the same arsenic compounds. Besides inorganic arsenic, which was dominant in the water extracts of both plants, dimethylarsinic acid [methanol/water (9:1) extract of the leaves of *Asplenium viride*, water extract of the leaves of *Dryopteris dilata*], and methylarsonic acid (all extracts of the leaves of *Asplenium viride*), trimethylarsine oxide and small amounts of the tetramethylarsonium cation [5–9 µg As (kg dry mass)$^{-1}$] and the glycerol-ribose [4–9 µg As (kg dry mass)$^{-1}$] were detected. The leaves of the related green plants *Vaccinium myrtilis* and *Vaccinium vitis idaea* contained the same arsenic compounds (inorganic arsenic, methylarsonic acid, trimethylarsine oxide; traces of the glycerol-ribose in the methanol/water extracts), with the exception of a trace of dimethylarsinic acid, which was only found in the methanol/water extract of the leaves of *Vaccinium myrtilis*, and the tetramethylarsonium cation, which was only detected at the low µg kg$^{-1}$ level in all extracts of the leaves of *Vaccinium vitis idaea*.

The detection of the glycerol-ribose in a variety of green plants and two lichen species and the presence of traces of arsenobetaine in the grass *Deschampsia cespitosa* support the assumption that most of the arsenic compounds detected in the marine environment are also present in the terrestrial environment. The much lower arsenic concentrations in the terrestrial environment require analytical methods with detection limits at the sub-ng cm$^{-3}$ level to identify minor arsenic compounds and to complete the knowledge about arsenic compounds in the terrestrial ecosystem.

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