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Homoarsenocholine – A novel arsenic compound detected for the first time in nature

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ABSTRACT

The arsenic speciation was determined in macrofungi of the *Ramaria* genus with HPLC coupled to inductively coupled plasma mass spectrometry. Besides arsenic species that are already known for macrofungi, like arsenobetaine or arsenocholine, two compounds that were only known from marine samples so far (trimethylarsoniopropanate and dimethylarsinoylacetate) were found for the first time in a terrestrial sample. An unknown arsenical was isolated and identified as homoarsenocholine. This could be a key intermediate for further elucidation of the biotransformation mechanisms of arsenic.

1. Introduction

Macrofungi are well known for their ability to accumulate enormous amounts of various elements, depending on the fungal species. One of these elements is arsenic, where more than 1000 mg kg^{-1} dry mass (dm) can be taken up by certain species [1–3]. In addition to this, macrofungi are known to be able to contain a remarkable variety of organoarsenicals. Terrestrial organisms usually only contain inorganic arsenic (iAs), methylarsonic acid (MA) and/or dimethylarsinic acid (DMA) [4–6], but in macrofungi, arsenic species typically attributed to the marine environment can be found as well. The most prominent is arsenobetaine (AB), which is, like iAs and DMA, the major arsenic compound in many macrofungi [7,8]. Trimethylarsine oxide (TMAO), arsenocholine (AC), the tetramethylarsonium ion (TETRA) and arsenosugars have been detected in macrofungi as well, but usually only at low or trace concentrations [7]. Up to now, it is unclear why the arsenic speciation in macrofungi can vary so much between different species. It is also unknown if the macrofungi are metabolizing arsenic to the different compounds themselves, if it is induced by microorganisms or if they are just accumulating it from the surrounding environment. *In vitro* studies by Nearing et al. have shown that AB is not present during the vegetative life stage (mycelium) of *Agaricus* spp., but can be found in all parts of the fungi during fruit-body formation (reproductive life stage), including the mycelium [9,10]. Another important, yet unanswered question is, why terrestrial macrofungi contain these various arsenic species. Concerning AB, it has been speculated that it might serve as an osmolyte and help maintaining the structure of the fruit-bodies [7]. In a recent publication it has been shown that AB can protect against osmotic and temperature-induced stress, similar to its nitrogen-analogue, glycine betaine [11].

One unusually looking group of terrestrial macrofungi are the so-called clavarioid fungi (coral fungi) of the genus *Ramaria*. Some species, like *Ramaria flava*, are edible, but there are also *Ramaria* fungi that can be poisonous to animals [12]. Until now, total arsenic concentrations have been investigated in very few individual samples and range from 0.2 to 11 mg As kg^{-1} dm [8,13–19]. Only one sample of *Ramaria pallida* has been investigated for its arsenic speciation so far [8]. The main arsenic species was AB (81%), followed by 13% AC and small amounts of DMA, MA and iAs. In our study, we aimed to look into the arsenic speciation of these bizarre mushrooms to broaden the current knowledge and understanding of arsenic speciation in the environment.

2. Materials and methods

We investigated the arsenic speciation of six collections of the genus *Ramaria*. Three samples were collected and identified by J. Borovička (one in Slovakia in 2014, and two in Czech Republic in 2011 and 2016), and three samples were collected and identified by W. Goessler in Austria in 2017. In order to characterize the collections we performed ITS rDNAsequencing; the sequences were submitted to the GenBank

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database under the accession numbers MH366531–MH366536. For storage, the samples were freeze-dried. Sample preparation and determination of the total arsenic concentration as well as of the most common water-soluble arsenic species in aqueous extracts is described in detail elsewhere [20]. Briefly, the freeze-dried fungal samples were digested with nitric acid and then investigated with inductively coupled plasma triple quadrupole mass spectrometry (ICPQQQMS, 8800, Agilent Technologies, Waldbronn, Germany) for the determination of total arsenic concentrations (Appendix A, Table S1). The Standard Reference Materials® 1573a (Tomato Leaves, NIST, Gaithersburg, USA) and SRM® 1640a (Trace Elements in Natural Water, NIST) were prepared and measured together with the samples for quality control. The results were in good accordance with the certificates (Appendix A, Table S2). For speciation analysis, dried fungal samples were extracted with ultrapure water and then investigated with high performance liquid chromatography (HPLC, 1200, Agilent Technologies) coupled to ICP-QQQMS. Anion-exchange and cation-exchange chromatography were used to detect and quantify arsenate [As(V)], MA, DMA, AB, TMAO, AC and TETRA. A Q-Exactive Hybrid Quadrupole-Orbitrap MS (Thermo Fisher Sci., Erlangen, Germany) was used for high-resolution electrospray ionization mass spectrometry (HR ES-MS) measurements (Appendix A, Table S3). It was coupled to an HPLC with a LC cation-exchange column (Zorbax 300-SCX, Agilent Technologies) and 30 mM ammonium formate, pH 2.3% and 8% methanol as mobile phase. The flow rate of 1.5 mL min^{-1} was split with a T-piece after the column to reduce the input to the MS (split ratio: approximately 1 + 1).

3. Results and discussion

The total arsenic concentrations in the six investigated samples ranged from 1.7 to 61 mg kg^{-1} dm, with a median of 18 mg kg^{-1} dm (Table 1). Extraction with water resulted in an extraction efficiency of 90 ± 10%, and a column recovery of 93 ± 5%.

The main arsenic species in the extracts was unambiguously AB, accounting for 84 ± 9% of the extracted arsenic. We also detected small amounts of As(V), MA, DMA, TMAO, AC, TETRA, trimethylarsoniopropanate (TMAP or AB2) and dimethylarsinoylacetate (DMAA) in all six samples. Their identity was confirmed with spiking experiments and co-chromatography. The most important results are given in Table 1. Concentrations of all detected arsenic species can be found in Appendix A, Table S4. TMAP and DMAA are known compounds from the marine environment [21–23], and DMAA has been identified as urinary metabolite of arsenosugars [24], but they have never been found in natural terrestrial samples before.

Further, we found several unassigned peaks in the anion- and also cation-exchange chromatograms (Fig. 1). With spiking experiments, we excluded dimethylarsinoylethanol (DMAE), dimethylarsinoylpropionate (DMAP), dimethylarsinoylbutanate (DMAB) and the glycerol-, phosphate-, sulfate- and sulfonate- arseno-riboses as possible candidates. Oxidation experiments proved that no known thio-arsenic compounds were present.

One of the detected unknown compounds (UNK A in Fig. 1b) was attracting our attention, because it was eluting from the cation-exchange column very late, even after the permanent cation TETRA.

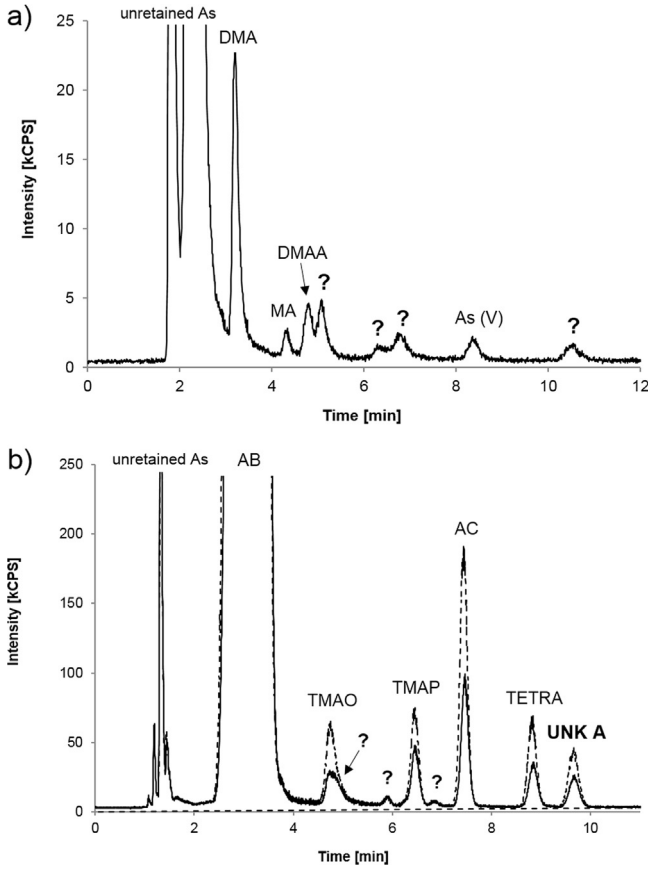

Fig. 1. Exemplary anion-exchange (a) and cation-exchange (b) chromatograms of a Ramaria extract. Dotted line: extract spiked with TMAO, TMAP, AC, TETRA and UNK A (= AC2).

Thus, UNK A was isolated by injecting an aqueous fungal extract multiple times onto the cation-exchange column and collecting the respective fractions.

The mobile phase was removed by freeze-drying, and the residue was dissolved in a small amount of ultrapure water. The presence and concentration of UNK A was controlled with HPLC-ICPMS. Next, the isolate was subjected to HPLC single quadrupole ES-MS to get an idea on the molecular mass of the compound. At the elution time of UNK A (as specified with HPLC-ICPMS), we found a signal with *m/z* 179. With this information, we started the investigation of UNK A with HR ES-MS. We were able to detect a molecule with an exact *m/z* of 179.0411 and a sum formula of $C_6H_{16}OAs$. Fragmentation experiments revealed characteristic fragments of *m/z* 161, 121, 105 and 59, as shown in Fig. 2.

The molecular mass of 179 and the corresponding fragments have already been reported by McSheehy et al. [25] There, the authors subjected a solution of inorganic arsenic and acetic acid to UV irradiation, and then investigated the solutions with ES-MS. They found several products, including a molecule with *m/z* 179. We agree with

Table 1
Total As and extracted As concentrations in *Ramaria* samples [mg kg^{-1} dm] and detected As species [% of extracted As]. Other As species that were detected in small amounts (< 5%) are: MA, DMA, As (V), TMAO, TETRA, DMAA and several unknown As species.

Sample ID	Species	Origin	Total As [mg kg^{-1}]	Extr. As [mg kg^{-1}]	AB [%]	AC [%]	TMAP [%]	AC2 [%]
ASP-017	*R. subbotrytis*	Slovakia	25 ± 2	22 ± 2	92 ± 7	1.6 ± 0.3	0.16 ± 0.02	0.27 ± 0.04
ASP-023	*R. subbotrytis*	Czech Republic	61 ± 5	66 ± 4	91 ± 9	2.7 ± 0.4	0.3 ± 0.1	0.47 ± 0.02
ASP-068	*R. subbotrytis*	Czech Republic	44 ± 4	39 ± 1	80 ± 20	1.7 ± 0.2	0.58 ± 0.01	0.47 ± 0.04
STM-107	*R.* aff. *largentii*	Austria	1.7 ± 0.1	1.4 ± 0.1	67 ± 2	4.1 ± 0.2	1.26 ± 0.1	0.82 ± 0.08
STM-108	*R.* cf. *pallida*	Austria	11.7 ± 0.2	9.5 ± 0.1	89 ± 1	1.57 ± 0.02	0.46 ± 0.02	0.37 ± 0.01
STM-109	*R.* cf. *pallida*	Austria	8.3 ± 0.3	6.9 ± 0.2	87 ± 2	2.4 ± 0.1	0.83 ± 0.03	0.93 ± 0.01