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Detection of arsenic-containing hydrocarbons in canned cod liver tissue

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ABSTRACT

Arsenic is a metalloid well known to be potentially toxic depending of its species. Lipid-soluble arsenicals (arsenolipids) are present in a wide range of biological samples in which they could play a role in the biosynthesis of organoarsenic compounds from inorganic arsenic compounds. Arsenolipids have recently attracted considerable interest. In order to gain deeper insights into the impact of arsenolipids new analytical approaches for reliable determination of this class of arsenic-containing hydrocarbons in various matrices are needed.

High concentrations of arsenolipids were found in seafood which served as sample material in this study. We report the investigation of three arsenolipids found in canned cod liver from which they were extracted and purified by solid phase extraction (SPE) using a silica gel column and ethyl acetate/methanol as eluent. Analytical studies were conducted by means of gas chromatography coupled with ICP-MS, MIP-AES and EI-qMS and by TOF-MS. The results obtained by GC-ICP-MS and GC-MIP-AES showed the existence of numerous arsenic compounds in the SPE fractions collected. Three major peaks were found within a retention time window between 10 and 25 min. The presence of arsenic compounds in the fish tissue could be confirmed using GC-EI-qMS analysis. Corresponding information of the molecular weights of the major arsenic species were provided by TOF-MS which allows highly accurate mass determinations. The results showed the presence of the arsenic-containing hydrocarbons with the following molecular formulas: $C_{17}H_{37}AsO$ (calculated for $[M+H]^+$ 333.2133; found 333.2136; $\Delta m = 0.90$ ppm); $C_{19}H_{41}AsO$ (calculated for $[M+H]^+$ 361.2446; found 361.2446; $\Delta m = 0.00$ ppm); $C_{23}H_{37}AsO$ (calculated for $[M+H]^+$ 405.2133; found 405.2145; $\Delta m = 2.96$ ppm). Suggestions for the corresponding structures are discussed.

1. Introduction

Expect a multiplicity of water-soluble arsenic species lipid-soluble forms of arsenic compounds are present in the environment in a wide range, particularly in marine organisms [1–4]. In order to investigate the relevance of long-chain arsenic-containing hydrocarbons (in the following called 'arsenolipids') in organisms, new analytical approaches for reliable determination of this class of arsenic compounds in various matrices are necessary.

More than 40 years ago Lunde [5–7] reported that lipids extracted from various marine organisms contain elevated concentrations of arsenic as organic compound. Total concentrations of arsenic in oil and fatty acid fraction extracted from marine fishes and invertebrates were found to be between 4.7 and 84 mg As kg^{-1}, and from seaweed between 5.7 and 221 mg As kg^{-1}, respectively [7]. A fractionation of fish lipids on silica gel column was also performed using different polar eluents. Investigations on lower and higher freshwater plants based on isotope labelled experiments with ^{74}As indicated the presence of a high percentage (52–80%) of arsenolipids beside arsenite and water-soluble, lipid-related compounds like anionic glycosidic derivatives of trimethylarsoniumlactate [8].

The distinction in water-soluble and lipid-soluble arsenic-containing compounds which plays an important role in marine organisms was also made by Maher [9,10] and by Kaise et al. [11]. An unidentified, lipid-soluble dimethylated arsenic species was the major component analysed in marine organisms [11]. The fractionation in lipid-soluble and water-soluble forms was carried out with water, methanol, chloroform, or mixtures of these solvents. Recently, such fractionations were also applied for the determination of organoarsenicals in seafood products [12] and in Japanese flying squid tissues [13].

Benson et al. [14] suggested that o-phosphatidyltrimethylarsoniumlactate is a major arsenic-containing product in marine

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organisms, similar in structure to lecithin. In 1988 a phosphatidylarsenosugar was detected and characterized in brown algae (Wakame) by Morita and Shibata [15] by ^{1}H NMR. Edmonds et al. [16] determined phosphatidylarsenocholine and phosphatidyldimethylarsinylribose in digestive gland of lobster using HPLC-ICP-MS.

Several investigations were focused on the extraction of arsenolipids by alkaline solutions to separate them in alkaline-stable and alkaline-labile fractions [17,18] analysed by using HPLC-ICP-MS. Neutral and polar organoarsenicals were also found and determined in fish oil after acid digestion [19] using IC-ICP-MS.

Recently, intact natural occurring arsenic-containing long-chain compounds were detected in fish oil [20] using liquid chromatography. Long-chain hydrocarbons containing arsenic could be analysed in oil of capelin [21] and Sashimi tuna [22]. Arsenic-containing long-chain fatty acids were found in cod liver oil by using HPLC-ICP-MS and HPLC-ESI-MS and identified by high-resolution MS [23].

Also in organometalloid analysis the gas chromatographic methods offer an excellent option combining effective separation and a variety of detection features. Table 1 depicts some examples of the application of gas chromatography to the analysis of organoarsenicals in environmental and food samples. Furthermore, the optimization of the preceding extraction and/or derivatization can lead to a significant improvement on the resulting complete analytical methodology.

The aim of the present work was to investigate the occurrence of recently discovered arsenolipids in fish tissues and to identify as much as possible arsenolipids by combination of comprehensive methods. Therefore, two sensitive and element-specific detectors (ICP-MS and MIP-AES) combined with gas chromatography to identify As (m/z 75, λ = 189 nm) were employed. The capabilities of both techniques for sensitive determination of the arsenolipids should be investigated and compared with results obtained by GC-EI-qMS as a molecule selective method. Complementarily, flow injection electrospray ionization high-resolution mass spectrometry in combination with interpretation of isotopic pattern should be applied for identification of arsenolipids without any chromatographic separation.

2. Experimental

2.1. Instrumentation

2.1.1. GC-ICP-MS:

The GC-ICP-MS setup consists of a gas chromatograph (HP 6890) coupled online with an ICP-MS 7500ce via a heated transfer line (both Agilent Technologies, Wilmington, DE, USA). The conditions for separation and detection are listed in Table 2.

2.1.2. GC-MIP-AES

A gas chromatograph (HP 6890, Agilent) was coupled with a microwave induced plasma atomic emission detector (G 2350 AED, jas, Moers, Germany). The conditions for separation and detection are presented in Table 2.

2.1.3. GC-MS

GC-EI-qMS consisting of Network GC System 6890N, mass selective detector 5973 and injector 7683 Series served as a method for the identification of the metalloid species based on their molecular mass. Also, chromatographic as well as detection conditions are summarized in Table 2.

2.1.4. TOF-MS:

For high-resolution mass spectrometry, a micrOTOF (Bruker Daltonics, Bremen, Germany), equipped with an Agilent CE-ESI-MS sprayer kit (G1607A), was used. A mixture of iso-propanol and water (50:50, v/v) containing 0.2% formic acid served as sheath-liquid and was pumped with a syringe pump model KDS 601553 (KDScientific, Holliston, MA, USA) selecting a flow rate of 3 μL min^{-1}. Sample introduction was performed applying pressure to the sample vial which was connected with the coaxial sheath-liquid sprayer via a short piece of fused silica capillary (50 μm I.D., 360 μm O.D.).

2.2. Sample preparation

2.2.1. Extraction of arsenolipids from canned cod liver (CCL)

Cod (*Gadus morhua*) liver (60 g of canned fish liver) was cleaned from the surrounding oil, cut in small pieces, and mixed with methanol (250 mL). Afterward, the slurry consisting of the hackled cod liver in methanol was sonicated for 24 h at room temperature. The supernatant (MeOH + arsenolipids) was transferred to plastic tubes (15 mL), and centrifuged at 5000 rpm for 10 min. The yellow liquid was collected from the Eppendorf tubes and the excess of solvent was removed under reduced pressure to yield a cream-yellow liquid (10 mL). This liquid was transferred to tubes and centrifuged again at 5000 rpm for 10 min. The solvent was evaporated under reduced pressure resulting in about 1 mL of yellowish oil. This oil was fractionated by preparative column chromatography on silica gel. The column was packed with 24 g of silica gel (0.063–0.200 mm). The eluent was a mixture of ethyl acetate/MeOH with changing compositions added in the order described in Table 3.

Between 12 and 13 fractions of 20 mL each were collected. Afterwards, the excess of solvent in each fraction was evaporated under reduced pressure to dryness. Then, each dry fraction was dissolved with MeOH (1 mL) and finally analysed. Canned cod liver from different brands were analysed with the result that the main volatile arsenic components under investigation were identically. However, the concentrations of these As-compounds were quite different depending on many factors not specified on the can.

3. Results and discussion

Derived from the aim of the present work the occurrence of the known long-chain arsenolipids in cod liver tissue as an up to now not investigated sample will be investigated by the combination of comprehensive gas chromatographic methods. With only GC-EI-qMS a detection of the organoarsenicals is impossible even if their molecular masses are known. This method lacks on selectivity for the detection of arsenic that m/z interferences can occur always. Therefore, two sensitive and element-specific detectors (ICP-MS and MIP-AES) were combined with gas chromatography to identify the element arsenic in the compounds under investigation. Both detectors are able to be employed element-specifically using independent principles of determination. The molecular masses of the organoarsenicals should be also monitored with high-resolution MS and isotopic pattern.

3.1. Optimization of GC-ICP-MS conditions

The ICP-MS conditions were optimized with respect to maximum sensitivity using Xe m/z 124 added to the Ar-carrier gas flow. The carrier gas flow rate of the ICP-MS and the Ar/O_2 pressure as well had the main influence on peak height and shape. Optimal conditions were achieved using 0.6–0.7 L min^{-1} carrier gas and an Ar/O_2 gas pressure of 138 kPa. To avoid dead volumes in the system the separation capillary column was conducted from the injector up to the tip in the torch.