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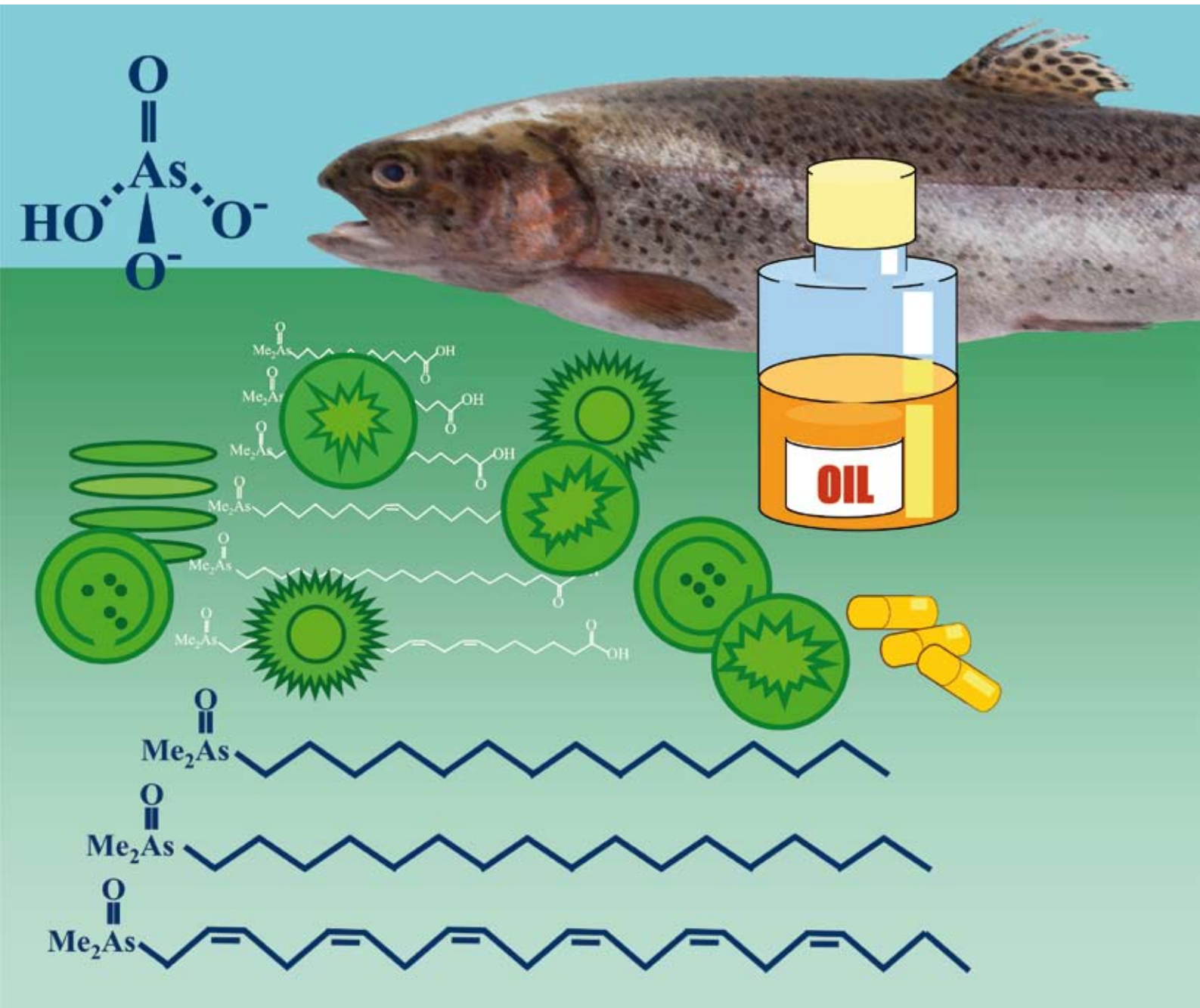

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Arsenic-containing hydrocarbons: natural compounds in oil from the fish capelin, *Mallotus villosus*†

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Arsenic-containing hydrocarbons have been identified for the first time as natural components of fish oil.

The study of naturally occurring arsenic compounds has both chemical interest, because of the novelty of the compounds present, and human health interest because the arsenicals occur in many common foods. The highest arsenic concentrations are found in seafoods, and many studies have reported on the type of arsenic compounds present in such foods.[1] The vast majority of this work has dealt with water-soluble compounds and it is only recently that structures have been reported for lipid-soluble species (arsenolipids), namely dimethylarsinoyl fatty acids found in cod liver oil.[2] We now report the isolation from a fish oil of three arsenic-containing hydrocarbons; surprisingly these compounds contain the equivalent of an even number of carbon atoms which raises intriguing questions about their biogenesis.

Major arsenic compounds in oil from the plankton-feeding fish capelin (*Mallotus villosus*) from the north Atlantic were isolated by solvent partitioning, preparative ion-exchange chromatography, and reversed-phase HPLC.[3] High resolution accurate mass spectrometry,[4] performed on the purified HPLC fractions, showed the presence of three arsenic compounds with the following molecular formulae: $C_{17}H_{37}AsO$ (calcd for $[M + H]^+$ 333.2134; found 333.2139; Δm = 1.5 ppm); $C_{19}H_{41}AsO$ (calcd for $[M + H]^+$ 361.2446; found 361.2450; Δm = 1.1 ppm); $C_{23}H_{37}AsO$ (calcd for $[M + H]^+$ 405.2134; found 405.2149; Δm = 3.7 ppm). These mass spectrometric data, together with the chromatographic properties of the compounds, indicated that the arsenic was present as a homologous pair of dimethylarsinoyl-alkanes and a dimethylarsinoyl-alkene possessing six double bonds (Fig. 1). We synthesized compound A,[5] and showed that the chromatographic and mass spectrometric properties of the synthesized product were identical with those of the natural product, thereby confirming the assignment for compound A, and, by analogy, for its homolog compound B. Although the mass spectrometric data provided an essentially unequivocal molecular formula for compound C, we cannot presently assign the positions or geometries of the double bonds with certainty; the compound is drawn by analogy to *all-cis*-4,7,10,13,16,19-docosahexaenoic acid [DHA, 22:6 (n = 3)], the most abundant and frequently occurring 22:6 acid in fish.[6] We estimate that these three compounds constitute at least 70% of the total arsenic originally in capelin oil; the remaining arsenic appears to comprise less polar compounds, the structures of which are currently unknown.

All three compounds contained the equivalent of an even number of carbon atoms. However, naturally occurring hydrocarbons usually contain an odd number of carbon atoms because they are derived, by overall decarboxylation[7] (decarbonylation of the aldehyde[8]) from long-chain fatty acids that predominantly possess even-numbered carbon chains.[9] This is reflected in various environmental samples. For example, sediments contributed to by senescent microalgae contain fatty acids with a strong predominance of even-numbered chain lengths and also contain hydrocarbons dominated by odd chain length compounds.[10] Capelin oil is no different from other fish oils in containing mostly long-chain fatty acids that contain an even number of carbon atoms;[11] yet the arsenic-containing hydrocarbons reported here all contain the equivalent of even-numbered carbon chains. This suggests either that the hydrocarbons are formed by the loss of two carbons from a long-chain

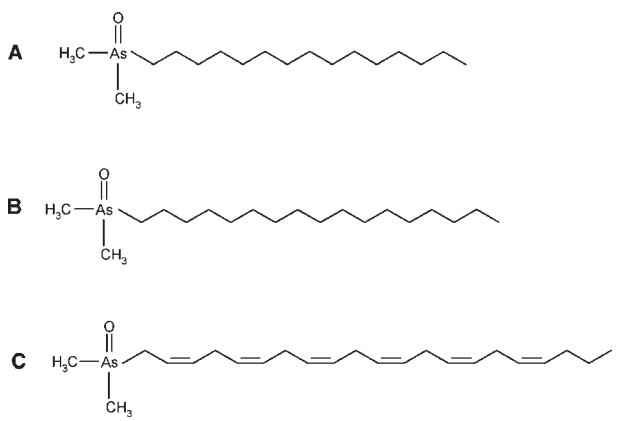

Fig. 1 Arsenic-containing hydrocarbons identified in oil from capelin. Assignments were based on high resolution accurate mass spectrometry, and, for compound A, confirmed by chemical synthesis. The position and geometry of the double bonds in compound C were not determined; they have been assigned by analogy to *all-cis*-4,7,10,13,16,19-docosahexaenoic acid [DHA, 22:6 (n = 3)], a common fatty acid in fish.

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fatty acid,[12] or that none is lost.[13] The loss of two carbons in the biosynthesis of hydrocarbons has been demonstrated for a higher plant (*Pisum sativum*); one carbon is removed by α-oxidation and the second by decarbonylation of the aldehyde formed by reduction of the resulting carboxylic acid.[12] A mechanism involving reduction of long-chain fatty acids to hydrocarbons *via* the alcohols, and therefore without the loss of any carbons, has been reported in a study using a bacterium (*Vibrio furnissii*),[13] although such a mechanism has never been indicated for fish or for the algae on which they feed. Examination of the three arsenic-containing hydrocarbons against the profile of normal fatty acids in capelin oil[11] indicated that the latter mechanism (involving no loss of carbon) is more likely than the former. For example, DHA, 22:6, the analog of compound C in terms of chain length and number of double bonds is abundant in capelin oil, but acids that might yield compound C by loss of one or two carbon atoms are absent. Thus, DHA is the likely precursor of compound C.

Whether the arsenic-containing hydrocarbons have a function or are made through inaccuracies in biosynthetic processes remains to be determined. Information on the fidelity of biochemical transformations (except for those involving nucleic acids) is sparse. The sensitivity and specificity of analytical techniques for determining arsenic compounds means that very small quantities of compounds, that might even be formed essentially accidentally, can be detected and characterized. It is, though, generally accepted that organisms, particularly marine organisms, must biochemically modify the arsenic that they are obliged to absorb (because of the similarity of arsenate to phosphate) so that it cannot decouple oxidative phosphorylation or interfere with enzyme function. The incorporation of an arsenic-containing group into an alkane might be thought to be the ultimate stage in a detoxification process with the arsenic placed out of all metabolic involvement. On the other hand, such molecules, with the arsenic-containing polar head and a hydrophobic tail, could have some specific but currently unknown membrane function.

We will explore these questions by investigating the distribution and biosynthesis of the arsenic-containing lipids in other fish oils and in organisms, particularly in view of the clear differences found for the two oils (cod liver and capelin) examined so far. The human metabolism of arsenolipids will also be of interest; the consumption of fish oils and oily fish is encouraged by nutritionists, primarily because of the high levels of polyunsaturated lipids that they contain.

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Notes and references

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3 Oil from capelin (100 g containing 11.7 μg As g^{-1}), obtained by heating and pressing whole fish, was partitioned between hexane (250 mL) and water–methanol (1 : 9; 150 mL). The aqueous methanol fraction was evaporated to yield an oil (0.42 g, 0.52 mg As) which was dissolved in methanol–chloroform–water (60 : 30 : 8 v/v/v) and applied to a column containing DEAE Sephadex A-25 (26 × 240 mm). Chromatography was performed with the above solvent mixture but with increasing concentrations of sodium acetate (from 0 to 1.0 M) dissolved in the water portion. The arsenic was monitored by inductively coupled plasma mass spectrometry (ICPMS). Most of the arsenic eluted near the void volume of the column (the non-acidic fraction); this fraction was evaporated to yield an oil (74 mg, 0.34 mg As). The remaining arsenic was distributed over more than 50 fractions (each *ca.* 20 mL), but no clear peak was obtained. Reversed-phase HPLC/electrospray mass spectrometry (Waters Atlantis dC_{18} column with ethanol–aqueous 5 mM ammonium acetate pH 6 as mobile phase; gradient elution from 50 to 90% ethanol) of the non-acidic fraction showed the presence of three arsenic compounds. A portion of this non-acidic material (20% ≡ *ca.* 70 μg As) was then subjected to preparative HPLC under the above reversed-phase conditions. Fractions (10 mL) were collected and individually measured for arsenic content by ICPMS and by electrospray MS. The arsenic was contained in three partly overlapping bands which were individually analyzed by high resolution accurate mass spectrometry.

4 Accurate mass was obtained by matrix assisted laser desorption ionization (MALDI) Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICRMS) using an IonSpec mass spectrometer equipped with a 4.7 Tesla magnet. The fractions from HPLC were re-dissolved in methanol (*ca.* 200 μL), and tiny amounts (nL) were deposited on a matrix of crystallized 2,5-dihydroxybenzoic acid. Spectra were obtained from 5 to 10 laser shots and recalibrated using a matrix ion (*m*/*z* 273) as an internal standard. The MALDI spectra (see ESI†) for compounds B and C showed, besides the ions from the protonated arsenolipid, also ions arising from a sodiated species. Mass resolution (full width at half maximum height) for the protonated species of the three compounds ranged from 21 500 to 25 400.

5 1-Pentadecanol was converted to the triflate which was then treated with sodium dimethylarsenide and the resultant arsine oxidized to yield the desired product 1-dimethylarsinoylpentadecane as a waxy solid. (^{13}C NMR, 90 MHz, $CDCl_3$, δ 14.1, 14.5 ($(CH_3)_2As$; CH_2CH_3)); 22.4, 22.6 (CH_2As, CH_2CH_3); 29.0–31.9 (CH_2).

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