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A 2-*O*-methylriboside unknown outside the RNA world contains arsenic

Ronald A. Glabonjat, Georg Raber, Kenneth B. Jensen, Nikolaus Guttenberger, Klaus Zangger, and Kevin A. Francesconi*

Abstract: Lipid-soluble arsenic compounds, also called arsenolipids, are ubiquitous marine natural products of currently unknown origin and function. In our search for clues about the possible biological roles of these compounds, we investigated arsenic metabolism in the unicellular green alga *Dunaliella tertiolecta*, and discovered an arsenolipid fundamentally different from all those previously identified; namely, a phytyl 5-dimethylarsinoyl-2-*O*-methyl-ribofuranoside. The discovery is of particular interest because 2-*O*-methylribosides have, until now, only been found in RNA. We briefly discuss the significance of the new lipid in biosynthesis and arsenic biogeochemical cycling.

The biological chemistry of arsenic is highly topical, with reported human health issues ranging from detrimental effects[1] from arsenic exposure to arsenic's beneficial use as a therapeutic agent.[2] Arsenic has even been reported to have an essential role in extremophile biology, in that it could replace phosphorus in DNA[3], a claim that has been vigorously refuted.[4] We now report the discovery of an unusual arsenic-containing lipid from a unicellular marine alga that incorporates a 2-*O*-methylriboside bound to phytol. Methylation of the ribose ring in the 2-*O* position has never been found in simple natural carbohydrates, yet is found in all major classes of RNA,[5,6] and has been hypothesized as the evolutionary link between RNA and DNA.[5,7] The discovery of this new arsenylated 2-*O*-methylriboside could again stimulate speculation about a biological role for arsenic in early life.

We first detected the new arsenolipid in extracts of the marine unicellular alga *Dunaliella tertiolecta* and in extracts from oceanic phytoplankton. HPLC/elemental mass spectral analysis revealed that *Dunaliella* contained small amounts of several arsenolipids including two known compounds,[8-10] and one major arsenic-containing lipid with properties quite different from previously reported compounds (Figure S1). By combining mass spectral data with data from degradation and derivatization experiments, partial chemical synthesis, and NMR spectroscopy on a trace of isolated natural product, as detailed below, we identified the new compound as phytyl 5-dimethylarsinoyl-2-*O*-methyl-ribofuranoside (Figure 1).

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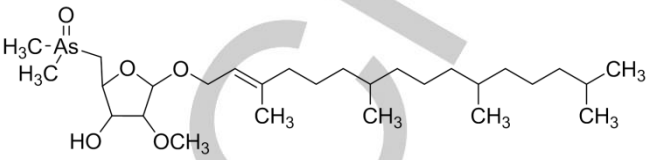


Figure 1. A phytyl 5-dimethylarsinoyl-2-O-methyl-ribofuranoside from the unicellular alga *Dunaliella tertiolecta*.

High resolution electrospray mass spectrometry of an ethanol/dichloromethane extract of the alga showed an ion with m/z 547.3337 $[M+H]^+$ corresponding to a molecular formula of $C_{28}H_{56}AsO_5^+$ (Δm = -0.26 ppm) (Figure 2). Tandem MS analysis of this molecular ion yielded an arsenic-containing product with $[M+H]^+$ of m/z 269.0366 resulting from a neutral loss of the lipid moiety corresponding to a $C_{20}H_{38}$-unit (m/z 278.2972; see below) The mass spectrum also revealed fragment ions at m/z 123 and 137, which are characteristic of the dimethylarsinoyl (Me_2AsO) group. Treatment of the extract of *D. tertiolecta* with H_2S or MeI/DTT produced, respectively, thioxo and trimethylated derivatives of the natural product, which supported this dimethylarsinoyl assignment (Figure S2 & Table S1). Acid hydrolysis of the arsenolipid quantitatively produced a water-soluble arsenical with m/z 269 $[M+H]^+$, identical with the MS fragmentation product, whereas base hydrolysis produced no change suggesting that the lipid contained a glycosidic bond (Figure S2 & Table S1).

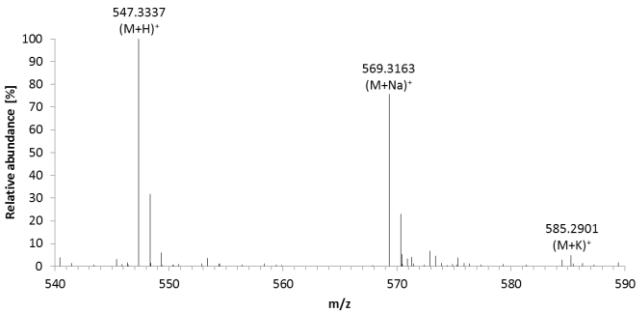
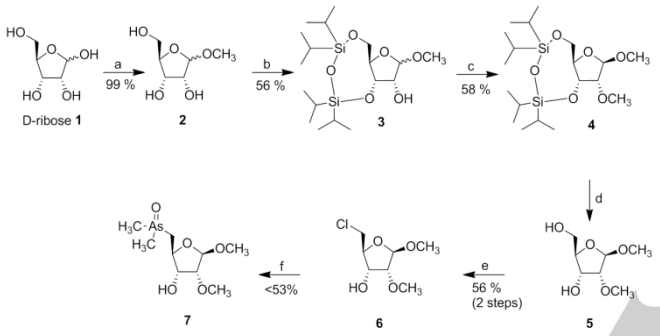


Figure 2. HR-ESMS of the phytyl 5-dimethylarsinoyl-2-*O*-methyl-ribofuranoside from the unicellular alga *Dunaliella tertiolecta*.

Further tandem mass spectral analysis of the m/z 269 ion from the original arsenolipid revealed a fragmentation pattern similar to that shown by dimethylarsinoylribosides[11] except that some characteristic fragment-ions had an additional 14 Da. This pattern was consistent with a methoxy substitution of one of the sugar ring hydroxyl groups (Figure S9); *O*-methylation of the intact lipid with HBF_4/$TMSCHN_2$ gave a product (m/z 561.3494) with an additional 14 Da, which supported the presence of a

single methoxy in the original lipid, and its location at the 2-*O*-position was derived from tandem MS data before and after 3-*O*-methylation (Figure S2 & Table S1).

Encouraged by the preliminary results showing similarities between our compound and arsenoribosides, while remaining sceptical about the 2-*O*-methoxy motif, we then synthesized the model methyl 5-dimethylarsinoyl-2-*O*-methyl-β-D-ribofuranoside (**7**) for comparison with the natural product (Scheme 1). On electrospray ionization in the tandem mass spectrometer, the model compound obligingly produced the 5-dimethylarsinoyl-2-*O*-methyl ribose, which then fragmented in a manner identical with that of the natural product (Figures 3, S3 & S4). In contrast, two previously synthesized non-*O*-methylated arsenic-containing cyclic ethers, with exact masses identical with that of 5-dimethylarsinoyl-2-*O*-methyl ribose, did not match this fragmentation pattern.[12]



Scheme 1. Synthesis of methyl 5-dimethylarsinoyl-2-*O*-methyl-β-D-ribofuranoside. Reagents and conditions: (a) cat. H_2SO_4, MeOH, 0 °C → rt, overnight; b) 1.1 eq 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, anhydrous pyridine, 0 °C → rt, 60 min; (c) 3.1 eq NaH, MeI/1,3-dimethyl-2-imidazolidinone, rt, 40 min; (d) 3.0 eq TBAF·3H_2O, THF, rt, 30 min; (e) PPh_3, CCl_4, anhydrous pyridine, 50-55 °C, 30 min; (f) Me_2AsI/Na, THF, rt overnight, then H_2O_2 (for full details see Supporting Information).

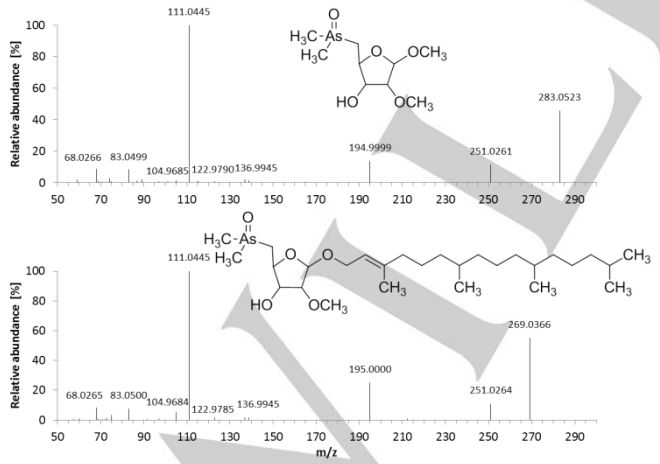
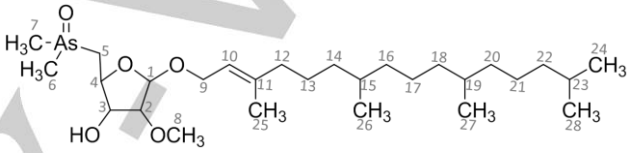


Figure 3. Tandem mass spectral comparison between synthetic methyl 5-dimethylarsinoyl-2-*O*-methyl-β-D-ribofuranoside (**7**), and the phytyl 5-dimethylarsinoyl-2-*O*-methyl-ribofuranoside from the unicellular alga *Dunaliella tertiolecta*.

Concurrent with our attempts to arrive at a viable structure by mass spectrometry and synthesis, we cultured more *D. tertiolecta* in a medium enriched with arsenate with a view to obtaining sufficient material for NMR analysis (Figure S7). In this way, we produced about 2 g of dry cells containing ca 20 µg As as the new arsenolipid, which was purified (Figure S8) by solvent partitioning, silica column chromatography, and preparative reversed-phase HPLC to provide a material weighing ca 0.1 mg and containing 15 µg As. Full spectra assignment of all ^1H and ^{13}C signals (Table 1) corroborated the structure shown in Figure 1. ^1H NMR clearly showed the signals corresponding to the proposed dimethylarsinoylriboside structure, including the 2-*O*-methyl group. Equally informative was the hydrophobic portion of the molecule, which revealed a branched hydrocarbon chain consistent with phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol), an algal natural product commonly incorporated into lipids including chlorophyll-a and vitamin K_1.

Table 1. ^1H and ^{13}C NMR results for phytyl 5-dimethylarsinoyl-2-*O*-methyl-ribofuranoside extracted from *Dunaliella tertiolecta*.



Position	δ(^1H) [ppm]	δ(^{13}C) [ppm]	Position	δ(^1H) [ppm]	δ(^{13}C) [ppm]
1	5.03	103.7	15	1.41	32.7
2	3.73	84.2	16	1.09/1.29	37.3
3	4.23	75.7	17	1.22/1.33	24.5
4	4.23	77.9	18	1.09/1.29	37.4
5	2.45	37.2	19	1.41	32.8
6, 7	1.83	16.2	20	1.09/1.29	37.4
8	3.56	58.7	21	1.30	24.8
9	4.05/4.20	64.4	22	1.17	39.4
10	5.31	119.3	23	1.56	28.0
11	---	141.9	24, 28	0.90	22.6/22.7
12	2.03	40.0	25	1.68	16.4
13	1.44	25.2	26	0.89	19.7
14	1.13/1.31	36.8	27	0.89	19.7

The presence of phytol in the arsenolipid was also consistent with the previous tandem MS observation of a neutral loss of a $C_{20}H_{38}$-unit (m/z 278.2972; Δm = -0.40 ppm). We then acid-hydrolyzed the purified lipid and quantified the two products; the data showed excellent agreement with a 1:1 molar ratio of arsenosugar/phytol (Figure S5). On the basis of the experiments reported here, the new arsenolipid was identified as ((3-hydroxy-2-methoxy-1-((3,7,11,15-tetramethylhexadec-2-en-1-yl)oxy)tetrahydrofuran-4-yl)methyl)dimethylarsine oxide (Figure 1).

Algae are responsible for about 50 % of the total global primary production of carbon, and most of the algal productivity is attributed to unicellular algae (phytoplankton).[13] It is likely that the new arsenolipid will prove to be widespread among unicellular algae, and thus its biosynthesis and degradation will play a pivotal role in the biogeochemical cycling of arsenic. Macroalgae, however, do not appear to synthesize this

arsenolipid, but rather they produce lipids incorporating an arsenoriboside.[8,14] The transformation of arsenate by methylation is a common process in biology, possibly invoked as a detoxification step. This process takes place through the universal methyl donor *S*-adenosylmethioneine, which can also be responsible for 2'-*O*-methylation of RNA,[5] and in the case of macroalgae, is further thought to serve as the source of the ribose ring itself.[15] Whether the 2-*O*-methyl analogue of *S*-adenosylmethioneine can act in a similar manner is unknown. The fundamental difference in the arsenolipids produced by unicellular compared with macroalgae might reflect an early evolutionary change in their ability to handle or use arsenic in an evolving world still exploring possible elemental substrates.

While there are no data that clearly demonstrate a biological role for arsenic, it is increasingly being reported in molecules that play an important role in biology, for example bound into phosphatidylcholines as constituents of membrane lipids.[10] The hydrophobic phytol part of the arsenolipid described here might also be relevant to arsenic's role in lipid chemistry by anchoring the molecule in the membrane of the chloroplast, a role similar to phytol in chlorophyll-a. We hope the report of this unusual new natural product will stimulate research into arsenic's biological chemistry.

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Keywords: arsenic • arsenolipid • phytol • arsenosugar • 2-*O*-methyl-riboside

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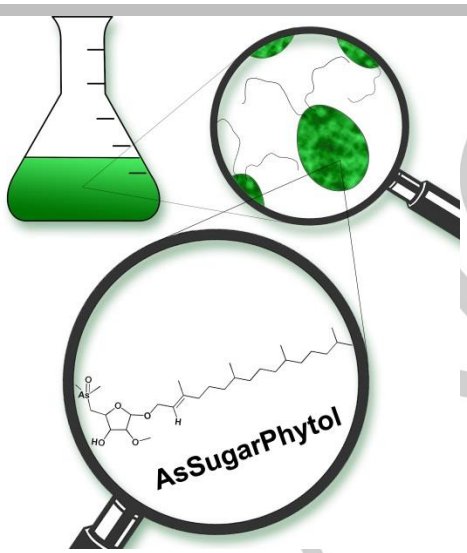

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