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On the First Polyarsenic Organic Compound from Nature: Arsenicin A from the New Caledonian Marine Sponge *Echinochalina bargibanti*

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Abstract: Reported here is the first polyarsenic compound ever found in nature. Denominated arsenicin A, it was isolated along a bioassay-guided fractionation of the organic extract of the poecilosclerid sponge *Echinochalina bargibanti* collected from the north-eastern coast of New Caledonia. In defining an adamantine-type polyarsenic structure for this compound, deceptively simple NMR spectra were complemented by extensive mass spectral analysis. However, it was only the synthesis of a model compound that provided the basis to discriminate structure **4** from other spectrally compatible structures for arsenicin A; to this end, a comparative ab initio simulation of IR spectra for the natural and the synthetic compounds was decisive. Arsenicin A is endowed with potent bactericidal and fungicidal activities on human pathogenic strains. All this may revive pharmacological interest in arsenic compounds while prompting us to rethink the arsenic cycle in nature.

Keywords: ab initio calculations · antibiotics · arsenic · mass spectrometry · natural products

Introduction

Arsenic is ubiquitous on earth, both on the crust and in the sea, as well as in the atmosphere. Inorganic forms of this element are alkylated by marine organisms to give monoarsenic compounds, which are present in brown algae, mollusks, arthropods and vertebrates.[1–4] Besides volatile compounds, like alkyl arsines,[4] more commonly found are nonvolatile methyl arsine oxides, methylarsonic acid and dimethylarsinic acid, alongside water-soluble polar betaines, cholines, as well as carbohydrate and lipid derivatives.[1–7] As for sulfur derivatives, thio-organoarsenates are rarely encountered,[8] while thioarsenic acid is known to be derived from the metabolism of cod-liver arsenolipids in humans.[9]

Marine sponges seldom appeared in these reports and, to the best of our knowledge, only for water-soluble and lipid-soluble raw fractions from "Demospongia",[4] which we understand to mean the class Demospongia. Therefore, it was exciting to find in our laboratories that a poecilosclerid sponge from the north-eastern coast of New Caledonia, *Echinochalina bargibanti* Hooper and Lévi, 1993, overthrows the limits of the above classes of natural compounds containing arsenic, providing the first polyarsenic compounds from nature. Here we describe one of them, constituted of C, H, O and As only, while sulfur analogues also present in the sponge have so far defied any attempt at conclusive molecular characterization.

Deceptively simple NMR spectra for this oxygenated polyarsenic compound were corroborated by extensive mass spectrometric analysis. Compatible structures from such study were discriminated against a synthetic analogue of reliable structure, relying on comparative ab initio quantum chemical calculations of IR spectra.

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Results and Discussion

Raw dichloromethane extracts of the residue from ethanol extraction of *E. bargibanti* showed the most potent antibacterial and antifungal activities ever observed for marine organisms from New Caledonia in the ORSTOM/IRD laboratories at Noumea. The active fractions were subjected to reverse-phase flash chromatography, setting apart sterols and carotenoids from the active compounds, which showed up as bioactive and UV-active spots on TLC. Pure arsenicin A was separated through elution by preparative HPLC (Experimental) from minor related metabolites that proved labile under the normal workup conditions. Solvent evaporation carried out under a nitrogen atmosphere to minimize oxidation also abated the yield of arsenicin A from a new batch of freeze-dried *E. bargibanti*, while a HPLC trace peak appeared that can be attributed to the corresponding compound with an oxygen atom less by MS analysis. This elusive precursor of arsenicin A was collected in tiny amounts, insufficient for a complete spectral assignment. Otherwise, the workup of a collection of *E. bargibanti* stored for a longer time gave arsenicin A as the major compound, as expected from easy oxidation of As^{III} compounds. On the latter observation one might question whether arsenicin A is an artefact compound. We rule out this hypothesis on the basis that extraction of the sponge and isolation of compounds under an atmosphere of N_2 still affords arsenicin A as the main compound.

Spectral analysis: "Mass defective" EIMS signals for arsenicin A pointed to the presence of heteroatoms. Observation of only a $[M+1]^+$ peak at mass above the molecular ion restricted the choice to heteroatoms that do not give isotopic clusters, while its 3% relative intensity with respect to the $M^{\bullet+}$ peak agreed with NMR evidence for three carbon atoms. Detection of a strong signal at m/z: 75 in the inductively coupled plasma mass spectrum (ICP-MS) of pure arsenicin A confirmed the presence of arsenic, which has a single stable isotope. HR-EIMS experiments revealed the composition $C_3H_6As_4O_3$ of the molecular ion 389.7177 ± 0.0020 (calcd: 389.7181), while showing that fragment ions m/z: 360 and 330 derive from the molecular ion by loss of one or two formaldehyde molecules, respectively, and that m/z: 300, 225, 150 and 75 are contributed by four, three, two and one arsenic atom, respectively. Tandem fragmentation experiments carried out by soft-ionization APCI(+)-MS^n, in conjunction with HR-EIMS experiments on the corresponding fragments, brought to light the cascade m/z: 391 $[M+H]^+ \rightarrow 361$ $[M+H-H_2CO]^+ \rightarrow 253$ $[C_2H_4As_3]^+ \rightarrow 225$ $[As_3]^+$ (see Experimental Section).

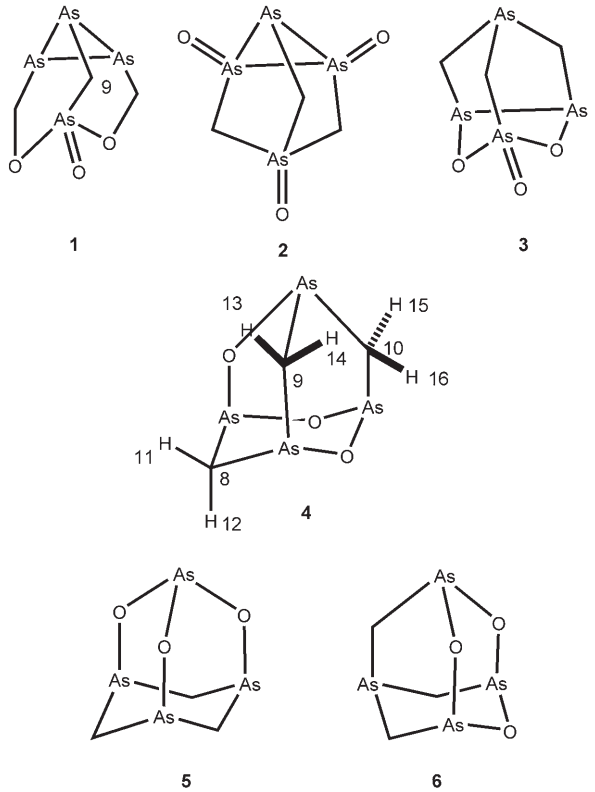
^1H NMR spectra of arsenicin A in $CDCl_3$ suggested the presence of three methylene groups, two of which are magnetically equivalent (Table 1). In accordance, ^{13}C NMR spectra showed three methylene carbons, two of which are magnetically equivalent (Table 1). The optical inactivity of arsenicin A and the absence of a Cotton effect are in line with these elements of symmetry. As-containing groups were also apparent from diagnostic[10] FTIR absorption bands at 674, 722 and 765 cm^{-1}.

Table 1. NMR spectral data for arsenicin A (**4**).[a]

C-Atom	δ_H [ppm] (J [Hz])	δ_C [ppm]	HMBC correlation with	NOE interaction at
8,10	2.42 dt (13.8, 0.9) H12 = H15	23.05 t[b]	C9	H_{gem}
	1.37 d (13.8) H11 = H16			H_{gem}, H13, H14
9	2.23 t (0.9) H13 and H14	17.03 t[b]	C8 = C10	H11, H16

[a] $CDCl_3$ (^1H and HMBC spectra at 400 MHz, ^{13}C at 75.4 MHz). [b] From coupled ^{13}C NMR experiment, $^1J = 132$ Hz.

Our aim at a diffraction analysis was defeated by the difficulty of obtaining suitable crystals of either arsenicin A itself or a derivative from reactions of unequivocal mechanism. This posed us the challenging problem of assembling all spectral information to arrive at a defined structure for arsenicin A. The symmetric structure **1** (No absolute configuration meaning is attributed to any of the representations **1**–**6**), based on a three-membered ring formed by As



atoms,[11] fits the MS observation of loss of two formaldehyde molecules and formation of fragment ions that contain arsenic atoms. Structure **1** also explains the NMR spectroscopic homo and heterocorrelations. The high field ^{13}C NMR spectroscopic resonances are more problematic to rationalize in the absence of data for analogues; however,

the dominance of the low As electronegativity[12] and the contribution of resonance structures of type $-H_2C-O^{(+)}=As-(-OCH_2-)-O^{(-)}$ which impoverish the electron density at the cyclic oxygen atoms might be advocated as the responsible factors.

Structures **2** and **3** for arsenicin A, which better fit the observed ^{13}C NMR chemical shifts, are inconsistent with the tangled rearrangements that would have to be invoked to explain the MS fragmentations.

Adamantane-type structures **4–6** for arsenicin A fit better than **1–3** as a result of the known tendency of $As^{III}-As^{III}$ bonds to undergo oxidation by insertion of an oxygen atom between two As atoms.[13] Of these, structures **5** and **6** can be ruled out on the basis of their C_{3v} symmetry, which implies single ^1H and ^{13}C NMR spectroscopic resonances. Structure **4** deserves close scrutiny. Its C_2 symmetry and minimized geometry (see in the following) rationalize the NMR observations (Table 1) as to: 1) the presence of only two ^1H NMR doublets ($\delta_H = 2.42$ and 1.37 ppm), both heterocoupled to the same triplet ($\delta_C = 23.05$ ppm) according to HMQC experiments, and a triplet ($\delta_H = 2.23$ ppm) heterocoupled to a triplet ($\delta_C = 17.03$ ppm), with a ^1H integration ratio doublet/triplet 2:1; 2) the single W long-range coupling between H12 and H14 (or the equivalent H13 and H15), in agreement with the small H12-C8-C9-H14 dihedral angle (or the equivalent H13-C9-C10-H15), 4.4° in the B3LYP-minimized structure (see in the following) and 3) the NOE enhancement between H11 and H13 (or the equivalent H14 and H16), as suggested from a calculated small separation through space of 3 Å (Figure 1).

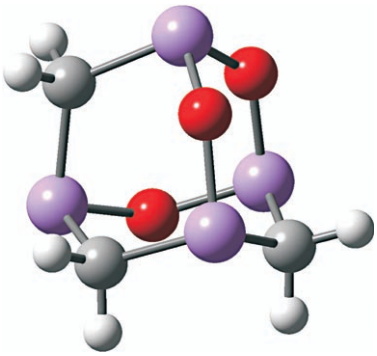
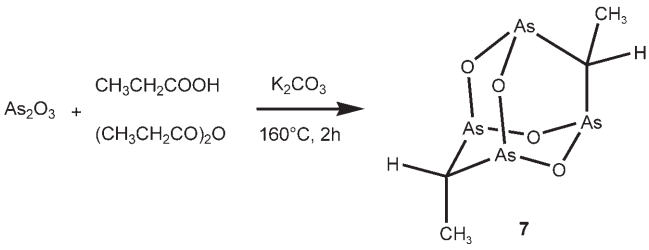


Figure 1. Energy-minimized geometry of arsenicin A (**4**) from ab initio quantum chemical calculations (DFT/B3LYP/6-311G**).

Vibrational analysis by ab initio quantum mechanical calculations: Geometries **1–3**, from minimization by ab initio density functional theory calculations B3LYP with 6-311G** basis set, were subjected to vibrational analysis at the same level of theory, indicating that the strongest IR absorption bands, including As=O stretching, lie above 900 cm^{-1} (Table 2). A similar analysis for geometries **5** and **6** gave a single intense normal mode arising essentially from As–O stretching between 730 and 780 cm^{-1}, which is the frequency range for IR active absorptions of arsenicin A. This single normal mode is in disagreement with observations for arsenicin A (Table 2).

To our pleasure, geometry **4** found no inconsistency from similar calculations. The low-frequency IR active transitions (647, 705 and 750 cm^{-1}) were in fact calculated in the same number as, and at values close to, the observed spectrum (674, 722 and 765 cm^{-1}). The lowest frequency band must stem from overall skeleton deformation, while the two normal modes of vibrations at higher frequency must substantially derive from As–O bond stretching (Table 2). This approach and conclusion received confirmation from the experiments and calculations described in the next section.

Synthesis of a model compound: From spectral analysis, as shown above, we have provided strong, albeit indirect, support to structure **4** for arsenicin A. Given the situation, a direct proof for structure **4** could only be arrived at by total synthesis. Because of the deceptively simple NMR spectra for these compounds, what was really needed was the synthesis of a close structural analogue that finds support from direct structural correlation or diffraction analysis. The latter situation is provided by compound **7**,[14] which was obtained as indicated in Scheme 1. The mass spectrometric



Scheme 1. Synthesis of model compound **7**.[14]

fragmentation for **7** shows close similarities to that of arsenicin A. HR-EIMS analysis of fragment ions *m*/*z*: 377 and 333 for **7** suggest the loss from the molecular ion of one and two acetaldehyde molecules, respectively. Moreover, fragment ions *m*/*z*: 300, 225, 150 and 75 imply the contribution of four, three, two and one arsenic atoms, respectively. Tandem fragmentations by APCI(+)-MS^n experiments, coupled to the HR-EIMS data for the corresponding fragments, revealed the cascade *m*/*z*: 421 $[M+H]^+ \rightarrow 377$ $[M+H-CH_3CHO]^+ \rightarrow 333$ $[M+H-2\,CH_3CHO]^+ \rightarrow 225$ $[As_3]^+$ (see Experimental Section), which is in full agreement with related observations for arsenicin A. These mass spectral fragmentations could have, a priori, suggested structures that, like **1**, bear CH_2O and catenated arsenic units. This occurred to us in a preliminary presentation of arsenicin A at a meeting.[15] It was only the present comparative study of **4** and **7**, providing firm ground for the unusual nature of the fragmentations that allowed us to point firmly to structure **4** for arsenicin A. To our great pleasure, a nice agreement was observed between experimental and theoretically calculated IR spectra—along the methodology de-

Table 2. Experimental IR data and calculated frequencies from DFT/B3LYP/6-311G** ab initio vibrational analysis for compounds **1**–**7**.

IR frequency [cm^{-1}] Calcd	Experim.	Relative intensity	Assignment	Minimized structure
			Structure **1**	
965		v strong	As=O stretching	
1042		v strong	out of plane C–H bending	
1055		v strong	out of plane C–H bending	
1284		weak	in plane C–H bending	
			Structure **2**	
903		v strong	As=O asym stretching	
909		v strong	As=O asym stretching	
1069		medium	out of plane C–H bending	
			Structure **3**	
713		v strong	out of plane ring bending	
962		v strong	As=O stretching	
1152		weak	out of plane C–H bending	
			Structure **4**	
448		medium	rings deformations	
647	674	strong	ring deformations	
705	723	v strong	As–O stretching	
750	765	v strong	As–O stretching	
1143	1105	medium	out of plane C–H bending	
			Structure **5**	
648		medium	rings deformations	
670		medium	rings deformations	
777		v strong	As–O stretching	
1162		medium	out of plane C–H bending	
			Structure **6**	
633		medium	As–C sym stretching	
735		v strong	As–O stretching	
1152		medium	out of plane C–H bending	
			Compound **7**	
701	726	strong	As–O asym stretching	
769	794	v strong	As–O sym stretching	
1144	1112	weak	CH bending	

scribed in the previous section—for compound **7** (Table 2), which definitely supports structure **4** for arsenicin A.

Biological activity: Arsenic compounds, apart from their bad reputation as poisons, are best known for inorganic forms, which find a specialized role in the treatment of certain diseases.[12] These include severe pathologies like sleeping sickness and syphilis. Arsenic trioxide in particular has found use in the therapy of leukaemia,[16] recently getting approval by the FDA to treat acute promyelocytic leukaemia.[17] Organoarsenical compounds have also been pharmacologically evaluated and in particular organoarsenicals of marine origin were tested on mice.[2] Still none has entered clinical use,[12] in spite of their lower general toxicity than inorganic forms of arsenic. These prospects, and the strong antibacterial and antifungal activity of extracts of our sponge, stimulated us to carry out a preliminary assay of arsenicin A. Antibacterial activity was evaluated by the standard microdilution plate test[25] with human pathogenic *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* at 10 μg/disc for a disc of 6 mm diameter. Activity was evaluated as inhibition diameter (mm): (*Sa*/*Ec*/*Ca*) = (24/28/26) for arsenicin A and (22/30/22) for gentamycin, used as a control test. These experiments revealed that both arsenicin A and gentamycin are strongly active at the concentrations tested.

Are these observations of potential pharmacological interest? Although the stability of arsenicin A is not very high, the answer is yes, if it is recalled that inorganic forms of arsenic show far weaker antibiotic activity against clinical strains of *S. aureus* than kanamycin,[18] itself far less active than gentamycin,[19] and that stability and activity might be improved on structural modification. These constitute alluring prospects for arsenicin A related antibiotics, provided that toxicity can be minimized.

Conclusion

We have disclosed here the existence of polyarsenic compounds in nature. The molecular structure of one of them, arsenicin A, could be fully elucidated despite deceptively

simple NMR spectra and failure to get suitable crystals for diffraction analysis. This result could have not been achieved before the advent of tandem mass spectrometry. Following dependencies, this technique allowed us to reconstruct the whole edifice for arsenicin A. However, selection among compatible structures was only made possible by the comparison with a synthetic analogue of proven structure. Successful comparative simulation of IR spectra from ab initio calculations for the natural and the synthetic compounds provided final confirmation for structure **4**.

This novel polyarsenic compound stimulates theoretical and biosynthetic work to understand the mechanism of the poorly known biosynthesis of organic compounds of arsenic.[1,5] However, a prerequisite involves unravelling the organism responsible for the synthesis of arsenicin A: sponge cells, microbial symbionts or both in cooperation?

From the applied side, this compound, not defied by such harsh pathogenic strains as resistant *Staphylococcus aureus*, may open new pharmacological perspectives, paving the way to much demanded new antibacterial drugs to combat the rising resistant strains. At any event, the polyarsenic nature of this compound calls for a rethinking of the recycling of arsenic in nature.

Experimental Section

General methods: Propionic acid, propionic anhydride and arsenic (III) oxide (*CAUTION*) were used as received from Aldrich. NMR spectra were taken with an Avance 400 Bruker spectrometer and (when stated) a Varian XL-300 spectrometer with ^{1}H at 400 MHz in $CDCl_3$ and ^{13}C at 75.43 MHz, respectively. δ values are in ppm relative to $SiMe_4$ ($\delta=0$ ppm) and J values in Hz; ^{1}H/^{1}H correlations were taken from one-bond and long-range correlated spectroscopy (COSY) experiments and selective decoupling irradiations, ^{1}H/^{13}C assignments from heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) techniques. NOE data were obtained from both differential NOE 1D, with 5 s preirradiation, and bidimensional NOESY. ^{13}C NMR data were taken from heteronuclear bidimensional experiments at 400 MHz or 1D (XL-300) at 75.43 MHz, multiplicity from APT experiments. EIMS (m/z, rel.%) and HR-EIMS spectra were taken with a Kratos-MS80 mass spectrometer equipped with a home-built computerized acquisition system. APCI-MS and tandem (MS/MS)^n were taken with a Bruker Esquire-LC mass spectrometer equipped with an atmospheric pressure chemical ionization ion source used in positive ion mode. The sample was injected into the source from a methanolic solution. Inductively coupled plasma mass spectrometry (ICP-MS) spectra were recorded with a ICP-MS HP-4500 series apparatus, equipped with autosampler ASX-500 (CETAC Technologies, Omaha, USA) and software HP-4500 Chemstation by using a Sc 1 mg L^{-1}, Rh 0.5 mg L^{-1} solution as an internal standard introduced online. IR spectra (cm^{-1}) were recorded with a FTIR Equinox 55 Bruker apparatus by using a film obtained by evaporation of a solution of compound in CH_2Cl_2. Cotton effects were deduced by circular dichroic (CD) spectra, recorded by a Jasco J-710 spectropolarimeter.

Ab initio calculations: All the energy calculations and geometry optimizations were performed with the Gaussian 03 program suite[20] utilizing unrestricted hybrid density functional/molecular orbital theory with a Lee–Yang–Parr correlation functional,[21] a Becke 3-parameter exchange functional (for example, B3LYP)[22] and basis sets of 6-311++G(d,p).[23] Vibrational frequencies were computed at the same level of theory.[24] All structures were characterized through frequency calculations as true minima (number of imaginary frequency$y=0$). Calculations were carried out by using Intel Pentium 4 (CPU 3.06 GHz, RAM 512 Mb) under Microsoft Windows XP operating system. The Gaussian checkpoint files were converted by using the freqcheck utility in the Gaussian program. The converted files were then used to view the animated vibrational motions of the molecules in Gauss View 3.0 (Gaussian, Pittsburgh, PA).

Collections and isolation: The sponge (R1858/881 M) was collected along the north-eastern coast of New Caledonia at 18–25 m depth during the program "SMIB" (Substances Marines d'Intérêt Biologique). The sponge (6.2 Kg moist) was immediately frozen and then freeze-dried (480 g dry weight). A small amount of freeze-dried sponge was extracted (EtOH), evaporated, CH_2Cl_2/H_2O partitioned and the residue from the evaporation of the organic phase was subjected to gradient flash chromatography (Si-60, *n*-hexane/AcOEt), monitoring both antimicrobial (*Staphylococcus aureus*) and antifungal (*Candida albicans*) activities. This procedure was then carried out on the active fractions from the whole freeze-dried material. The CH_2Cl_2 extract (20 g) was subjected to flash chromatography collecting 26 fractions of 0.1 L each. Combined fractions 6–15 were subjected to reversed-phase flash chromatography (RP-18, MeCN/H_2O), setting free the organoarsenicals (UV spots) from sterols and carotenoids. The solvent, MeCN, was evaporated at room temperature and the remaining aqueous residue was extracted with AcOEt and evaporated to yield a mixture of arsenic compounds containing arsenicin A (**4**) as the major component. The residue was subjected to preparative HPLC purification (Lichrosorb CN, 7 mm, UV detection at $\lambda=254$ nm) with *n*-hexane/AcOEt 96:4 to give pure **4** (5.2 mg, 0.001%) and minor related metabolites.

Arsenicin A (2,4,6-trioxa-1,3,5,7-tetrarsa-tricyclo[3.3.1.13,7]decane 4): Optically inactive at 589 and 577 nm ($c=1$ mg mL^{-1} in *n*-hexane); ^{1}H and ^{13}C NMR data: in Table 1; FTIR (film from evaporation of a CH_2Cl_2 solution): 2921 (weak), 1105 (medium), 765 (very strong), 723 (very strong), 674 cm^{-1} (strong); UV/Vis ($CHCl_3$): λ_{max} (ε) = 314 (2100), 285 (1600), 258 (3900), 240 nm (5300 mol^{-1} dm^3 cm^{-1}); CD (MeOH, $c=2\times10^{-5}$ M): no Cotton effect; MS (70 eV, EI): m/z (%): 391 (3) $[M+1]^+$, 390 (92) $[M]^+$, 360 (57) $[M-CH_2O]^+$, 330 (8) $[M-2(CH_2O)]^+$, 316 (1), 301 (2), 300 (1), 287 (16), 273 (21), 271 (35), 257 (12), 254 (9), 253 (2), 252 (4), 241 (4), 225 (6), 199 (23), 182 (13), 166 (10), 163 (18), 150 (7), 109 (8), 91 (37), 89 (14), 88 (3), 75 (3); HR-EIMS: m/z: 389.7177 ± 0.0020 (calcd for $C_3H_6As_4O_3^+$: 389.7181), 359.7073 ± 0.0010 (calcd for $C_2H_4As_4O_2^+$: 359.7075), 329.6977 ± 0.0010 (calcd for $CH_2As_4O^+$: 329.6969), 300.7796 ± 0.0020 (calcd for $C_2H_4As_3O_3^+$: 300.7808), 300.6945 ± 0.0010 (calcd for HAs_4^+: 300.6942), 299.6858 ± 0.0010 (calcd for As_4^+: 299.6864), 286.7651 ± 0.0010 (calcd for $CH_2As_3O_3^+$: 286.7652), 272.7498 ± 0.0010 (calcd for $As_3O_3^+$: 272.7495), 270.7702 ± 0.0010 (calcd for $CH_2As_3O_2^+$: 270.7703), 256.7545 ± 0.0010 (calcd for $As_3O_2^+$: 256.7546), 252.7953 ± 0.0010 (calcd for $C_2H_4As_3^+$: 252.7961), 251.7878 ± 0.0010 (calcd for $C_2H_3As_3^+$: 251.7882), 240.7595 ± 0.0010 (calcd for As_3O^+: 240.7597), 224.7644 ± 0.001 (calcd for As_3^+: 224.7648), 198.8357 ± 0.001 (calcd for $HAs_2O_3^+$: 198.8357), 181.8328 ± 0.0010 (calcd for $As_2O_2^+$: 181.8330), 165.8380 ± 0.0010 (calcd for As_2O^+: 165.8381), 162.8511 ± 0.0010 (calcd for $CHAs_2^+$: 162.8510), 149.8432 ± 0.0010 (calcd for As_2^+: 149.8432), 108.9273 ± 0.0010 (calcd for $H_2AsO_2^+$: 108.9271), 90.9167 ± 0.0010 (calcd for AsO^+: 90.9165), 88.9374 ± 0.0010 (calcd for CH_2As^+: 88.9372), 74.9215 ± 0.0010 (calcd for As^+: 74.9216); APCI(+)-MS: m/z: 391 $[M+H]^+$; APCI-(MS)^n: m/z: 391→361→253→225.

Synthesis and structural characterization of model compound 7: A mixture of arsenic(III) oxide (38.8 mg, 0.19 mmol), K_2CO_3 (27.2 mg, 0.19 mmol), propionic acid (0.05 mL, 0.68 mmol) and propionic anhydride (0.2 mL, 1.56 mmol) was heated at 160 °C whilst stirring for 2 h. H_2O (0.08 mL) was then added and the mixture was heated at 80 °C for 1 h. Additional water was added and the mixture was extracted with CH_2Cl_2 (3 mL × 3). The combined organic phases were treated with Na_2SO_4 and evaporated to give a residue that was purified by flash chromatography on silica gel, eluting with CH_2Cl_2. Evaporation of fractions 2 and 3 gave pure compound **7** (102 mg, 80%).

9,10-Dimethyl-2,4,6,8-tetraoxa-1,3,5,7,-tetraarsa-tricyclo[3.3.1.13,7]decane (7): ^{1}H and ^{13}C NMR spectroscopic data matched reported values.[14] FTIR (film from evaporation of a CH_2Cl_2 solution): 2954 (medium), 1445 (weak), 1112 (weak), 1014 (weak), 794 (very strong), 726 cm^{-1} (strong).

MS (70 eV, EI): *m*/*z* (%): 421 (4) $[M+1]^+$, 420 (75) $[M]^+$, 376 (79) $[M-CH_3CHO]^+$, 332 (40) $[M-2(CH_3CHO)]^+$, 300 (3), 289 (44), 273 (68), 257 (33), 225 (3), 199 (17), 182 (45), 166 (24), 150 (11), 109 (4), 91 (100), 75 (5); HR-EIMS: *m*/*z*: 419.7272 ± 0.002 (calcd for $C_4H_8As_4O_4^+$: 419.7286), 375.7025 ± 0.0020 (calcd for $C_2H_4As_4O_3^+$: 375.7024), 331.7544 ± 0.0010 (calcd for $As_3O_2^+$ 331.7546), 224.7645 ± 0.0020 (calcd for As_3^+: 224.7648); APCI(+)-MS: *m*/*z*: 421 $[M+H]^+$; APCI-(MS)^n: *m*/*z*: 421→377 $[M+H-CH_3CHO]^+$→333 $[M+H-2(CH_3CHO)]^+$→225.

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