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Identification of Arsenosugars at the Picogram Level Using Nanoelectrospray Quadrupole Time-of-Flight Mass Spectrometry

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State-of-the-art analytical methods for arsenic speciation rely typically on the availability of standards of defined structure, limiting the applicability of such methods to the determination of known compounds. Our previous high-energy tandem mass spectrometric studies demonstrated the strength of mass spectrometry for generating structurally diagnostic ions that allow for the identification of arsenic-containing ribofuranosides (arsenosugars) without the use of standards. We now report a more widely applicable and more sensitive approach, using negative-ion nano-electrospray low-energy tandem mass spectrometry for the generation of structurally useful product ions that allow for identification of arsenosugars at the picogram level. In the negative-ion mode, numerous product ions, suitable for characterizing naturally occurring dimethylated arsenosugars, were generated in high abundance. Application of the method to an algal extract unequivocally demonstrated the presence of a single dimethylated arsenosugar. In the positive-ion mode, characteristic tandem mass spectra were obtained for four trimethylarsonioribosides, allowing their identification without the need for standards. Overall it was demonstrated that nano-ES-MS/MS techniques can be used for characterizing arsenosugars on a routine basis, a necessary requirement for assessing potential health risks associated with consuming foods containing elevated levels of arsenosugars and for improving our understanding of arsenic biochemistry.

Arsenic-containing ribofuranosides (arsenosugars) were first identified in 1981 in the brown macroalga *Ecklonia radiata*.[1] Further investigations of other brown algae species, in addition to that of algae from other divisions, revealed the existence of 15 arsenosugar compounds.[2−6] The four major dimethylated arsenosugars (**1**−**4**) found in marine algae are presented in Figure 1. Because these compounds can occur at high concentrations (several milligrams of As per kilogram of wet weight) in marine organisms, including those used as human food, there is considerable interest regarding their toxicological behavior. Although studies suggest that arsenosugars exhibit no cytotoxicity or mutagenicity,[7] these arsenic compounds may be metabolized within humans to toxic forms. Recent studies[8−10] have reported elevated levels of dimethylarsinic acid, a suspected carcinogen,[11,12] in human urine following the consumption of seafood containing high levels of arsenosugars. Presumably, dimethylarsinic acid is a metabolite of arsenosugars in those studies. In addition to the human toxicological aspects, arsenosugars are of interest because of their pivotal role in the cycling and biotransformation of arsenic in marine systems.[2,13]

Trimethylated arsenosugars (trimethylarsonioribosides) have also been reported in marine samples.[4,14,15] Although these compounds occur as minor constituents compared with their dimethylated analogues, they are structurally more similar to arsenobetaine ($(CH_3)_3As^+CH_2COOH$), the major form of arsenic in marine animals, and can be transformed into arsenobetaine within shrimp.[16] Standard compounds of trimethylarsonioribosides (Figure 1; arsenosugars **5**−**8**) are not readily available, and their presence in marine samples may well be underestimated. An

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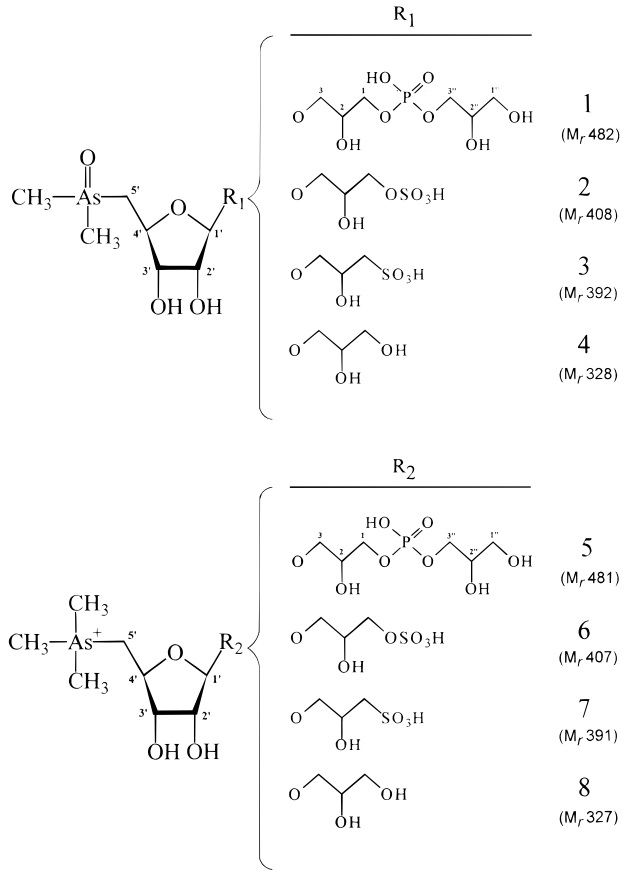

Figure 1. Structures of arsenosugar compounds investigated in this study. Note numbers **1**−**8** assigned to the arsenosugars.

analytical method allowing their identification without reference to standard compounds would be valuable.

Sensitive and selective analytical techniques are required for the rapid determination of arsenosugars and their potential transformation products in environmental and biological samples. The early work on the identification of arsenosugars adopted a natural products approach, whereby arsenosugars, extracted and purified in milligram quantities from kilograms of fresh alga, were identified by nuclear magnetic resonance spectroscopy and X-ray crystallographic analyses. Although this early approach does not lend itself to the routine analysis of arsenosugars, it has provided sufficient quantities of pure arsenosugars for use as standard compounds in the modern methods of determining arsenic species, e.g., high-performance liquid chromatography (HPLC) coupled on-line to arsenic-specific detectors such as an inductively coupled plasma mass spectrometer (ICPMS). The application of these new analytical techniques has allowed rapid examination of many more algal species for their arsenosugar content. The attractive features of the HPLC−ICPMS technique include high sensitivity and minimal sample preparation, e.g., sample extraction and dilution in most cases.[8,17−19] Limitations, however, include the possibility of arsenic compounds coeluting, which may lead to misidentifications, retention of arsenic species on-column, and the technique's inability to identify compounds for which reference compounds are not available. This inability to provide structural information for novel arsenic species greatly restricts the usefulness of atomic spectroscopic techniques for metabolic and transformation studies, in which new structures need to be characterized.

Recently, Corr and Larsen demonstrated the use of positive-ion electrospray (ES) MS for determining dimethylated arsenosugars at trace levels.[20] Fast-atom bombardment (FAB) tandem MS has also been reported for the structural characterization of arsenosugars at nanogram levels.[21] The main advantage of the latter approach is that the resulting tandem mass spectra of the arsenosugars contain several characteristic product ions that can be assigned to structural features of the compounds examined. As a result, tandem mass spectra are extremely useful for characterizing these compounds. Negative-ion detection of arsenosugars **1**−**4** provided superior sensitivity over their positive-ion detection. In addition, the negative-ion tandem mass spectra were found to contain a greater number of product ions compared with their corresponding positive-ion spectra and, thus, provide superior information regarding the structure of the arsenosugar compounds examined.

The objective of the present study was to develop an analytical method, based on negative-ion ES tandem MS, suitable for characterizing trace amounts of dimethylated arsenosugars in biological materials. In addition, the use of positive-ion ES tandem MS for characterizing cationic trimethylarsonioriboside species was explored. Finally, tandem mass spectra obtained under low-energy conditions (present study) and high-energy conditions[21] were compared.

EXPERIMENTAL SECTION

Instrumentation. Nano-ES Quadrupole Time-of-Flight (Q-TOF) MS. ES CID tandem mass spectra were obtained on a Micromass Q-TOF hybrid quadrupole time-of-flight tandem mass spectrometer (Wythenshawe) equipped with a ZSpray sample-introduction system in a nanoflow electrospray ion source. The mass spectrometer was operated in either the positive- or negative-ion mode with a source temperature of 20 °C. Cone voltage and skimmer off-set were set at 50 V and 1 V, respectively, with a capillary voltage of 3 kV, unless indicated otherwise. Argon was used as the collision gas, and the spectra were obtained using a range of collision energies from 5 to 40 eV. Spectra were acquired via the TOF analyzer and were integrated every 2.4 s over the m/z 50−500 range. Data were recorded and processed using the MassLynx software, version 3.1. Mass calibration was performed by multiple-ion monitoring of singly charged sodium and cesium iodide signals. Arsenosugars **1**−**4** were made up in methanol/water (1:1) solutions containing 50 mM ammonium acetate to the concentrations given in the corresponding figure legends. Arsenosugars **5**−**8** were made up in methanol/water (1:1) solutions to the concentrations given in the corresponding figure legends. A syringe pump was used to infuse these samples into the ES ion source via a fused silica capillary at a flow rate of 1 μL/min.

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ICPMS. A VG PlasmaQuad 3 ICPMS (VG Elemental, Winsford, Cheshire, U.K.) was used to determine As concentrations in the arsenosugar solutions. The quadrupole mass filter was operated in the single-ion monitoring mode (m/z 75) for the detection of arsenic. A displacement pump (Rheos 4000, Flux Instruments, Basel, Switzerland) was used to deliver carrier liquid (1% v/v nitric acid in 18 MΩ deionized water) into the ICPMS at a flow rate of 200 μL/min. Samples were injected into the carrier stream via a 1-μL internal chamber microinjection valve (Rheodyne 7520, Cotati, USA).[22] The ICPMS response was optimized for a maximum signal at m/z 75.

CHEMICALS AND MATERIALS

The dimethylarsinoylribosides were pure natural products isolated from algal sources.[3,14] Trimethylarsonioribosides were prepared by reduction of the analogous dimethylated arsenosugars with 2,3-dimercaptopropanol and methylation of the resultant arsine with methyl iodide.[23]

The partially purified *Sargassum laceriforium* extract had been preparatively chromatographed on an anion-exchange medium, and the strongly acidic fraction retained as previously reported.[3] HPLC−ICPMS analysis of this extract showed it contained only one arsenic compound, namely arsenosugar **2**.[21]

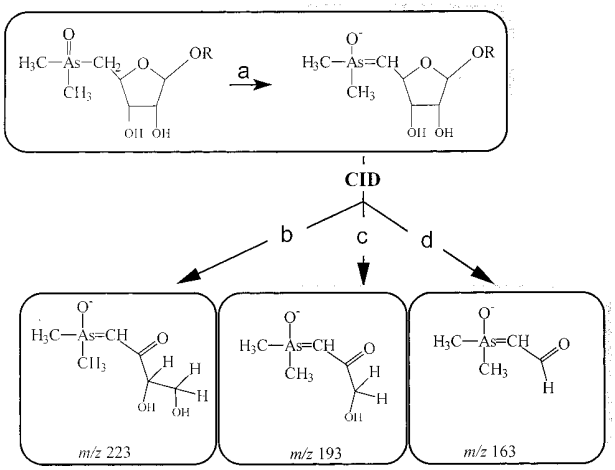
Inorganic arsenic standard solutions used for flow injection ICPMS were prepared by diluting 10 000 ppm As standards (VHG Lab, Inc., Manchester, U.K.) with 18 MΩ deionized water (Elga Ltd., U.K.) to make 0−160 ppb As solutions acidified to 1% v/v nitric acid (AnalaR, BDH, Poole, Dorset, U.K.).

RESULTS AND DISCUSSION

Negative-Ion ES-MS/MS of Dimethylarsinoylribosides. Our previous research analyzed the dimethylated arsenosugars **1**−**3** by FAB with a basic matrix and showed that they are readily deprotonated and thus were detected with high efficiency in the negative-ion mode.[21] The intensity of their $[M - H]^-$ ions, generated under negative-ion FAB conditions, was significantly higher than that obtained for the corresponding $[M + H]^+$ ions generated under positive-ion FAB conditions. This finding can be rationalized in terms of the presence of acidic functional groups such as sulfate, sulfonate, and phosphate in the arsenosugar aglycones. Also, from our previous work involving high-energy collision induced dissociation (CID) of negative and positive pseudomolecular ions originating from arsenosugars, we observed that the resulting negative-ion tandem mass spectra contain a larger number of informative product ions than do the corresponding positive-ion tandem mass spectra.[21]

Consequently, in the present study $[M - H]^-$ ions generated under ES conditions from each of the four dimethylated arsenosugars **1**−**4** were examined further using low-energy CID-MS/MS. Tandem mass spectra of $[M - H]^-$ ions originating from arsenosugar **1** were recorded at various collision energies. The ES cone voltage, which is known to affect ion fragmentation in the interface region of the ES and is also believed to influence the relative intensity of product ions formed in the collision cell,[24] was optimized and then kept constant at 50 V while varying the collision energies. Maximum signal intensities and numbers of product ions were obtained at collision energies of 30 eV (Figure 2A). Signal intensities were considerably lower for collision energies of 25, 35, and 40 eV. In addition, at a collision energy of 40 eV, fewer structurally informative product ions were observed. Proposed structural assignments for the product ions observed from arsenosugar **1** are presented in Figure 2. These assignments are similar to those made for the product ions of arsenosugar **1** obtained under high-energy CID conditions.[21] Most likely the product ions resulting from the arsenosugar **1** $[M - H]^-$ ions are generated by charge-remote fragmentation. As stated by Adams, charge-remote fragmentation is believed to result from ions containing localized charges that fragment in the gas phase to form products that are, in general, analogous to those formed upon gas-phase thermal decomposition of their corresponding neutral molecule.[25] In the case of arsenosugar **1**, the negative charge is located on the phosphate group.

Scheme 1. Proposed Formation Pathways for the Product Ions at *m/z* 223, 193 and 163



Overall, the negative-ion tandem mass spectrum obtained for arsenosugar **1** provides abundant structural information. A total of nine major product ions, all of which can be assigned to structural features of arsenosugar **1**, are observed. This compares well with the 11 structurally informative product ions observed under high-energy CID for the same compound.[21] Several additional ions of low relative intensity are also observed under low-energy CID conditions, i.e., m/z 359, 315, 287, and 273. The latter three are believed to form as a result of cross-ring cleavage of the ribose moiety. In addition, the low-energy tandem mass spectrum contains ions at m/z 193 and 223. To explain the presence of these ions, we propose the existence of a small population of gas-phase negative ions, formed under ES conditions, having a negative charge on their dimethylarsinoyl oxygen (Scheme 1a). Subsequent fragmentation of these species results in arsenic-containing ions at m/z 193 and 223 (Scheme 1b,c). The fact that little evidence for ion species containing a negative charge on the dimethylarsinoyl oxygen can be found in the high-energy tandem mass spectra obtained under FAB conditions, i.e., only the high-energy tandem mass spectrum of arsenosugar **3** exhibits ions at m/z 193,[21] suggests minor differences in the composition

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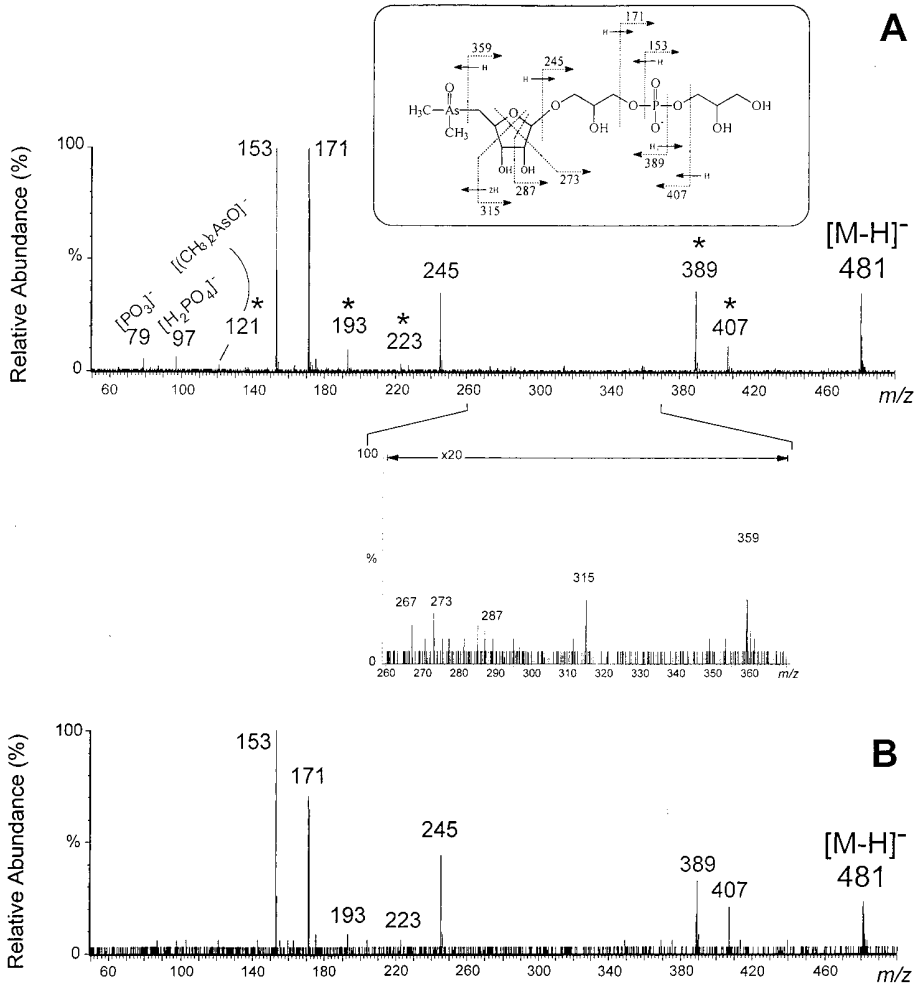

Figure 2. Negative-ion nano-ES Q-TOF mass spectra of the $[M - H]^-$ ion (*m/z* 481) originating from arsenosugar **1**: (A) acquired following the infusion of a solution containing approximately 120 pg/μL of As in the form of arsenosugar **1**; (B) acquired following the infusion of a solution containing 25 pg/μL of As in the form of arsenosugar **1**. Approximately two-minute acquisition time. Cone voltage and collision energy 50 V and 30 eV, respectively. Ion peaks labeled with an asterisk (*) are believed to contain arsenic.

of the gas-phase $[M - H]^-$ ion populations formed under ES and FAB conditions and/or that such ions once formed go on to fragment further and are therefore not observed under conditions of high-energy CID. Support for the hypothesis that such ions arise as a result of charge-remote fragmentation is presented later in this paper in the section describing positive-ion ES-MS/MS of trimethylarsonioribosides. For the sake of continuity, however, it is discussed briefly here. The trimethylated arsenosugars only differ from their corresponding dimethylated analogues in that a third methyl group is covalently bound to arsenic, and thus, they have a localized positive charge on the arsenic atom. It is expected that the trimethylated arsenosugars would afford the same type of ions as seen for the dimethylated compounds, i.e., *m/z* 223, 193, and 163, if, indeed, these product ions are formed in both cases as a result of charge-remote fragmentation. In fact, most of the tandem mass spectra recorded from the trimethylarsonium compounds (arsenosugars **5**−**8**) contain ion signals at *m/z* 193, 163, and/or 223.

In comparing the negative-ion ES tandem mass spectra obtained in this study with the positive-ion ES tandem mass spectrum of arsenosugar **1**, reported by Corr and Larsen,[20] it becomes clear that a significantly greater number of product ions relevant to structural features of arsenosugar **1** are present in the former. As discussed previously, under a single set of CID conditions, i.e., 30 eV collision energy, negative-ion tandem mass spectra contain a total of 14 product ions (9 of high relative intensity and 5 of low relative intensity), all of which can be assigned. Under positive-ion ES-MS/MS using a single set of CID conditions, a maximum of four product ions were observed and of these only two assigned to structural features of arsenosugar **1**.[20] However, positive-ion tandem mass spectra recorded at two different collision energies, i.e., 25 and 35 eV, gave a total of seven product ions, five of which were assigned.[20]

Another important aspect of the present study was to investigate the sensitivity afforded by negative-ion ES tandem MS for detecting arsenosugars. For this purpose, the tandem mass spectrum of a dilute solution of arsenosugar **1** was recorded (Figure 2B). The mass spectrum presented in Figure 2B corresponds to data collected over 2 min during which time a 15 pg of As per microliter of solution of arsenosugar **1** was infused into the ES-MS/MS at 1 μL/min. Thus, a total of 30 pg of As as arsenosugar **1** was consumed for the acquisition of this particular mass spectrum. The sensitivity afforded demonstrates a significant improvement over negative- and positive-ion FAB tandem mass

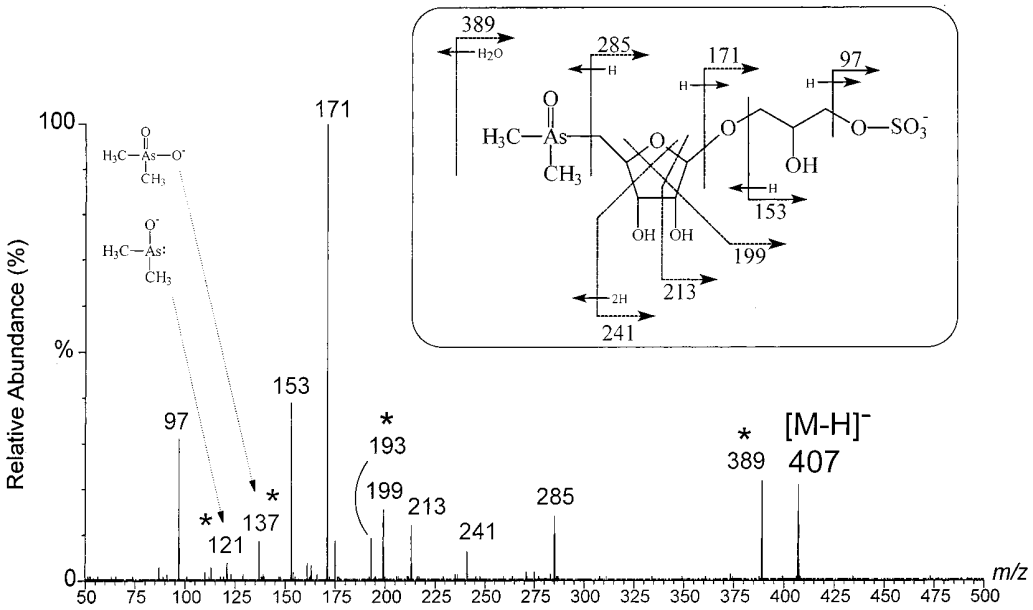

Figure 3. Negative-ion nano-ES Q-TOF mass spectrum of the $[M - H]^-$ ion (*m*/*z* 407) originating from arsenosugar **2**, acquired following the infusion of a solution containing approximately 70 pg/μL of As in the form of arsenosugar **2**. Approximately two-minute acquisition time. Cone voltage and collision energy were set to 50 V and 30 eV, respectively. Ion peaks labeled with an asterisk (*) are believed to contain arsenic.

spectrometry[21] and compares favorably with the apparent sensitivity afforded by positive-ion ES tandem MS for arsenosugars **1**−**4** as reported by Corr and Larsen.[20] In the latter case, good-quality (high signal-to-noise) tandem mass spectra were acquired following the introduction of 3.2 ng of arsenosugar **1**, 4.09 ng of arsenosugar **2**, 1.76 ng of arsenosugar **3**, and 0.16 ng of arsenosugar **4** into the ES source.

Tandem mass spectra of the $[M - H]^-$ ion originating from the sulfate-containing dimethylarsinoylriboside (arsenosugar **2**) were recorded also at various collision energies. Again, maximum ion intensity and number of structurally informative product ions were observed at a collision energy of 30 eV (Figure 3). We believe that product ions generated from the $[M - H]^-$ ions originating from arsenosugar **2** are also formed as a result of charge-remote fragmentation. Eleven product ions, all of which can be assigned to structural features of arsenosugar **2**, are observed under conditions of low-energy CID. This is superior to the nine structurally relevant product ions observed under high-energy CID from the same compound.[21] In the case of arsenosugar **2**, cross-ring cleavage of the ribose was observed under low-energy CID conditions. In fact, three product ions resulted from cross-ring cleavage, i.e., *m*/*z* 241, 213, and 199. The latter two ions are identical to those observed under high-energy CID conditions, except that they occur at significantly lower relative intensity. It is not immediately obvious why this type of cross-ring cleavage results in ions with significantly higher relative intensities in the case of arsenosugar **2** than in the case of arsenosugar **1**. One factor that may help explain these observations is that the relatively weak phosphodiester bonds (arsenosugar **1**) break under low-energy CID prior to the introduction of sufficient energy to cause cross-ring cleavage of the $[M - H]^-$ precursor ions. Support for this argument can be found in the tandem mass spectrum of arsenosugar **1** presented in Figure 2, i.e., four of the most intense product ions, *m*/*z* 171, 153, 407, and 389, seem to have formed following the cleavage of relatively weak phosphodiester bonds.

In comparing the negative-ion ES tandem mass spectra obtained in this study for arsenosugar **2** to the positive-ion ES tandem mass spectrum reported elsewhere for the same compound,[20] it becomes evident that a significantly larger number of product ions pertinent to structural features of arsenosugar **2** are found in the former. As already discussed, negative-ion tandem mass spectra contain a total of 11 structurally relevant product ions. Under positive-ion low-energy CID conditions, only three product ions were reported for arsenosugar **2**.[20]

The tandem mass spectrum of $[M - H]^-$ ions originating from arsenosugar **3** contained a number of characteristic ions (Figure 4) similar to those occurring under high-energy CID.[21] The main product ions were observed at *m*/*z* 373 $[M - H - H_2O]^-$; *m*/*z* 269 $[M - H - (CH_3)_2AsOH]^-$; *m*/*z* 155 $[M - H - (CH_3)_2As(O)$-ribose$]^-$; and *m*/*z* 137 $[M - H - (CH_3)_2As(O)$ribose $- H_2O]^-$. Only a single low-intensity ion occurring at *m*/*z* 197 is believed to have resulted from cross-ring cleavage of the ribose moiety similar to that described for high-energy CID.[21] The product ions observed at *m*/*z* 193 and 163 can be rationalized as corresponding to the ion species proposed in Scheme 1. The ion at *m*/*z* 175 may have formed from the ion species occurring at *m*/*z* 193 following loss of H_2O. These spectral features compare well with those observed under positive-ion ES tandem MS[20] for the same compound. In fact, under a single set of ES negative-ion CID conditions nine product ions were observed in the resulting tandem mass spectra, compared with eight obtained under two sets of positive-ion ES tandem mass spectrometry as reported elsewhere.[20] Since the assignments of the latter eight product ions[20] is largely speculative, more research is required for their clarification.

The tandem mass spectra acquired at various collision energies for $[M - H]^-$ ions originating from arsenosugar **4** are presented

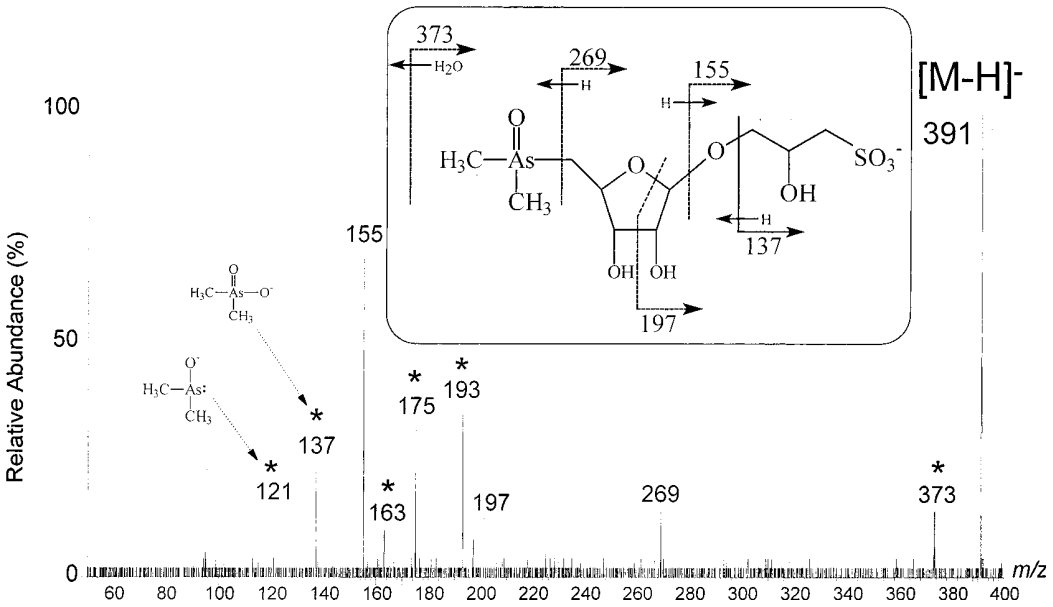

Figure 4. Negative-ion nano-ES Q-TOF mass spectrum of the $[M - H]^-$ ion (*m/z* 391) originating from arsenosugar **3**; acquired following the introduction of approximately 30 pg/μL of As in the form of arsenosugar **3**. Approximately two minute acquisition time. Cone voltage and collision energy were set to 30 V and 25 eV, respectively. Ion peaks labeled with an asterisk (*) are believed to contain arsenic.

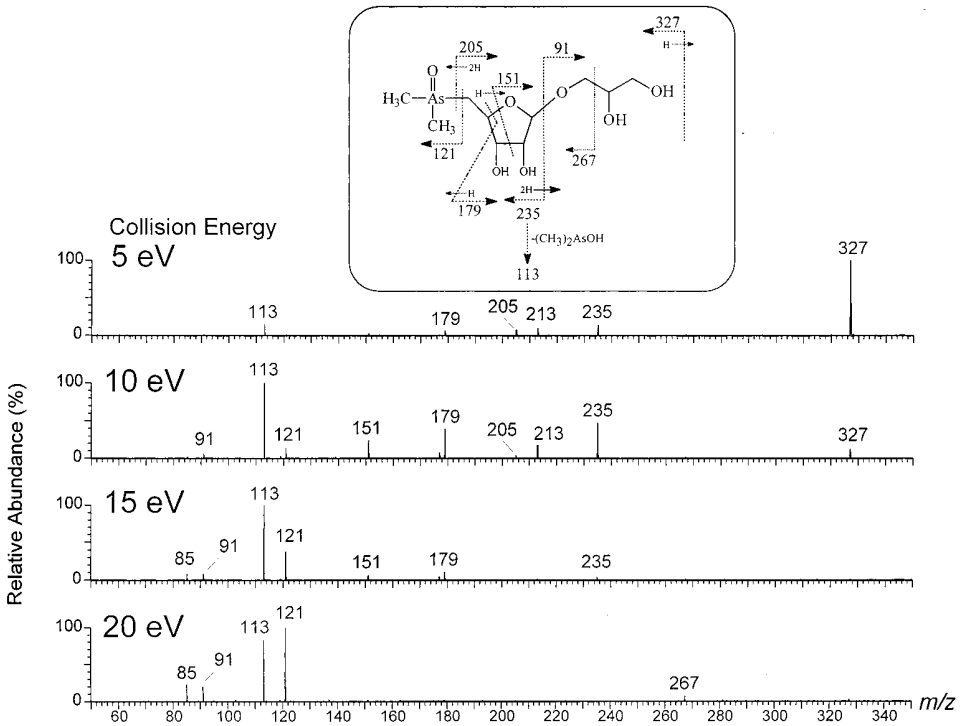

Figure 5. Negative-ion nano-ES Q-TOF mass spectra of the $[M - H]^-$ ion (*m/z* 327) originating from arsenosugar **4**, acquired following the introduction of approximately 1.4 ng/μL of As in the form of arsenosugar **4**. Approximately two-minute acquisition time. Cone voltage was set to 50 V; collision energy varied from 5 to 20 eV.

in Figure 5. The main product ions were observed at *m*/*z* 113, 121, 151, 179, 205, 213, and 235. The majority of these ions have been assigned to structural features of arsenosugar **4** as detailed in Figure 5. The tandem mass spectrum obtained using a collision energy of 10 eV most closely resembles the tandem mass spectrum obtained under high-energy conditions.[26] In fact, the majority of the product ions observed under low-energy CID conditions are also observed under high-energy[26] CID conditions. Under low-energy positive-ion CID conditions, only a single product ion was reported for this compound,[20] thus rendering this mode of tandem MS less than adequate for the identification of arsenosugar **4**.

In our previous work we noted that under high-energy CID conditions the tandem mass spectra resulting from arsenosugar pseudomolecular ions contained several product ions which formed following the loss of arsenic-containing neutral frag-

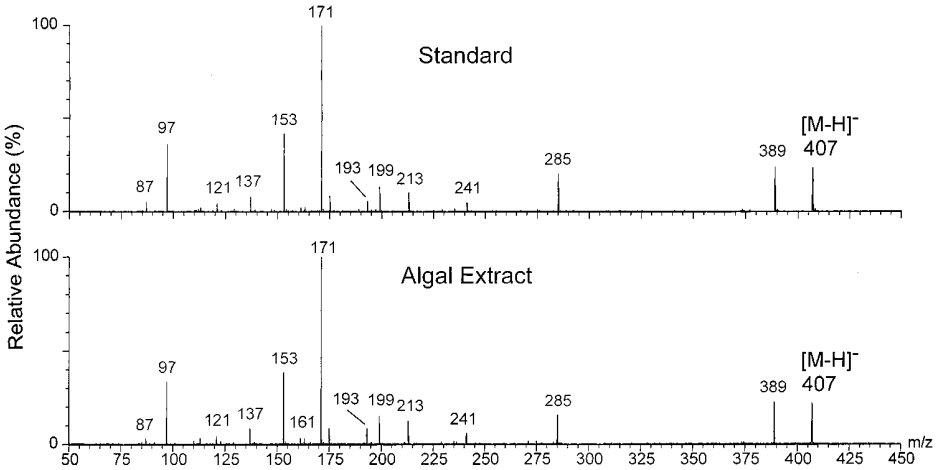

Figure 6. Negative-ion ES tandem mass spectra of precursor ion *m/z* 407 originating from standard arsenosugar **1** and algal (*Sargassum laceriforium*) extract.

ments.[21,26] In particular, the loss of a 122 amu fragment, corresponding to a $(CH_3)_2AsOH$ neutral moiety, gave intense $[M - H - 122]^-$ product ions. Because the same neutral loss is observed under low-energy CID conditions from all four dimethylarsinoyl sugars, monitoring for the neutral loss of 122 amu could be used to screen selectively for compounds containing dimethylarsinoyl moieties. However, the resulting $[M - H - 122]^-$ ion is of low relative intensity in the case of arsenosugar **1**—approximately 3% (normalized to the most intense product ion), as compared to 15% for arsenosugar **2**, 15% for arsenosugar **3**, and 35% for arsenosugar **4**. This low relative intensity from arsenosugar **1** unfortunately compromises to some extent the general usefulness of such a constant neutral-loss scanning approach, although it should still succeed well for detecting compounds **2**−**4**.

Identification of an Arsenosugar in an Algal Extract. To evaluate negative-ion ES tandem mass spectrometry for its suitability to assist in the identification of arsenosugars in biological samples, a partially purified algal (*S. lacerifolium*) extract[3] was analyzed. Several analytical techniques have already been used to conduct arsenic speciation on this material.[21,3] Data from these techniques have shown that a single arsenic species in the form of arsenosugar **2** is present. The purpose of further analysis of the algal extract, using ES tandem mass spectrometry, was to evaluate the technique's suitability to perform arsenic speciation on a biological matrix. The algal extract was treated as if its arsenosugar content was unknown. Following the introduction of the algal extract into the ES mass spectrometer, tandem mass spectra for precursor ions at *m/z* 481, 407, 391, and 327 were acquired. These particular ions were selected as precursors as they correspond to $[M - H]^-$ ions of arsenosugars **1**−**4**, respectively. Only the precursor at *m/z* 407 gave a tandem mass spectrum of significant intensity. The resulting tandem mass spectrum was identical to that obtained from arsenosugar **2**, thus suggesting the presence of arsenosugar **2** in the algal extract. For comparison purposes, the tandem mass spectra obtained from the precursor ion occurring at *m/z* 407 from both the algal extract and the purified reference compound (arsenosugar **2**) are presented together in Figure 6. The similarity between the two spectra, in terms of product ions present and their relative intensities, is excellent. As expected, no evidence was obtained suggesting the presence of arsenosugars **1**, **3**, or **4**.

Positive-Ion ES-MS/MS of Trimethylarsonioribosides. Trimethylarsonioribosides (arsenosugars **5**−**8**, Figure 1) occur as cations in solution and can thus be examined efficiently in the positive-ion mode by ES-MS/MS. The resulting ES mass spectra from these compounds contain intense M^+ species along with $[M - H + Na]^+$ and $[M - H + K]^+$ ions. The M^+ ions were selected as precursor ions and further examined under low-energy CID conditions. Tandem mass spectra of the M^+ ions, originating from arsenosugars **5**−**8**, were recorded. Upon examination of the resulting spectra, it was apparent that the product ions observed are formed as a result of charge-remote fragmentation with the positive charge localized on the arsenic atom. In fact, all product ions observed in these tandem mass spectra are thought to contain the arsenic atom.

The tandem mass spectrum obtained from the molecular ion of arsenosugar **5**, along with proposed assignments, are presented in Figure 7. Ions of low intensity present at *m/z* 175 and 193 suggest the occurrence of cross-ring cleavage. In general, the features of the low-energy CID mass spectrum of the M^+ ion originating from arsenosugar **5** are similar to those observed under high-energy CID conditions.[26] Of particular importance is the high intensity of the product ion occurring at *m/z* 235. This ion, which indicates the presence of a trimethylarsonioribose moiety, could be used in a precursor ion scan to screen for the presence of arsenosugar **5**.

For the M^+ ion originating from arsenosugar **6**, tandem mass spectra were recorded at different collision energies (Figure 8). At a collision energy of 10 eV, the M^+ ion fragments to form a single product ion at *m/z* 327 $[M - SO_3]^+$. At the higher collision energy of 30 eV, the relative intensity of the *m/z* 327 ion increases. At 40 eV, considerable fragmentation of the *m/z* 327 product ion occurs and cross-ring cleavage gives relatively high-intensity ions at *m/z* 163 and 193. Unfortunately, product ions at *m/z* 235 are of low relative intensity under these conditions so that if this particular ion is to be used as a precursor ion to screen samples for the presence of trimethylarsonioribosides, conditions for its formation will need to be optimized.

For arsenosugar **7**, both the M^+ ion and the $[M - H + Na]^+$ ions were examined under low-energy CID conditions (Figure 9).

(26) Pergantis, S. A.; Francesconi, K. A.; Wangkarn, S.; Thomas-Oates, J. E. In preparation for submission.

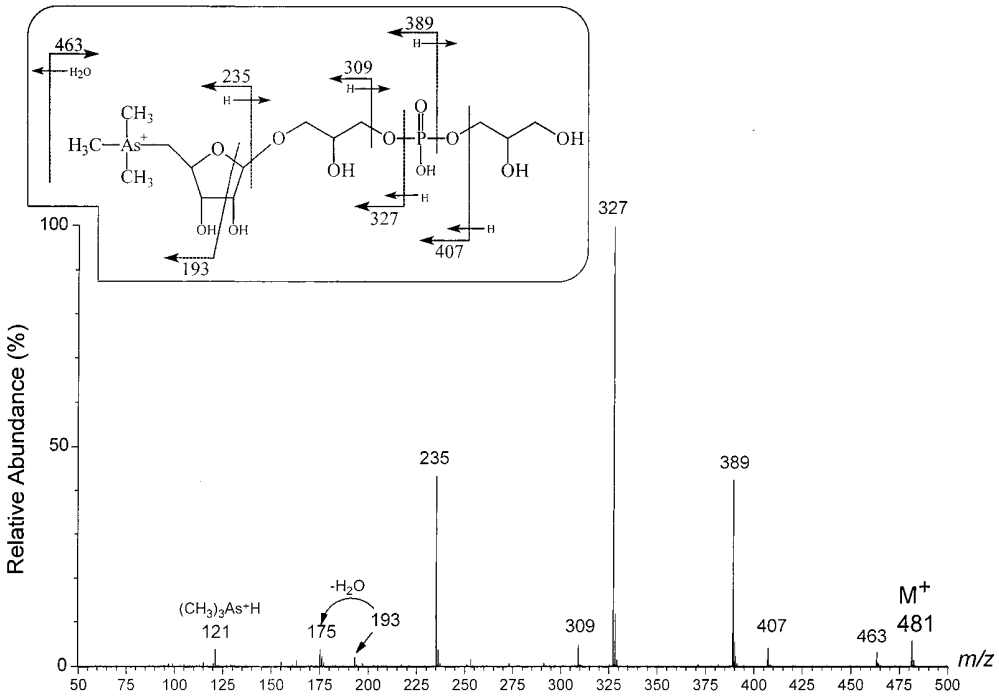

Figure 7. Positive-ion nano-ES Q-TOF mass spectrum of the $[M]^+$ ion (*m/z* 481) originating from arsenosugar **5**, acquired following the introduction of approximately 150 pg/μL of As in the form of arsenosugar **5**. Approximately two-minute acquisition time. Cone voltage and collision energy were set to 50 V and 30 eV, respectively.

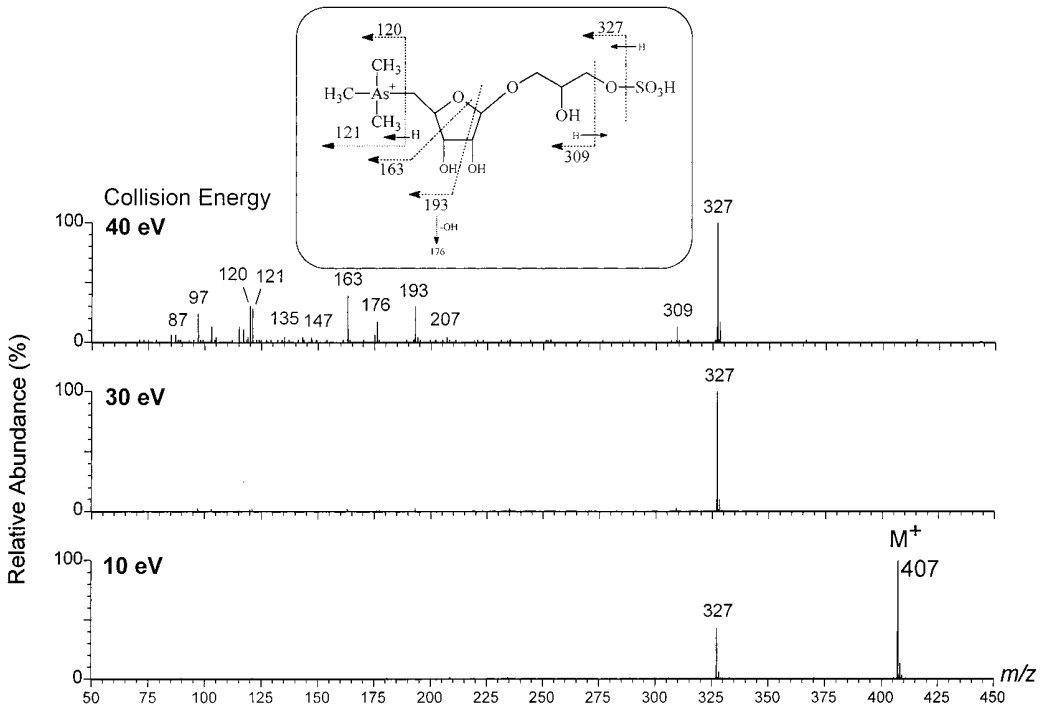

Figure 8. Positive-ion nano-ES Q-TOF mass spectra of the $[M]^+$ ion (*m/z* 407) originating from arsenosugar **6**, acquired following the introduction of approximately 170 pg/μL of As in the form of arsenosugar **6**. Approximately two-minute acquisition time. Cone voltage was set to 50 V; collision energies varied from 10 to 40 eV.

In Figure 9A, the tandem mass spectrum for M^+ is presented along with proposed assignments. Cross-ring cleavages are observed (*m/z* 163 and 193), as is an ion at *m/z* 235 indicating the presence of a trimethylarsonioribose moiety. A similar tandem mass spectrum was observed upon CID of the $[M - H + Na]^+$ precursor ion (Figure 9B). A major difference, however, is the presence of a high-intensity ion at *m/z* 295 resulting from the sodiated species, as opposed to an ion at *m/z* 293 from the nonsodiated species. The fact that the sodiated molecular ion also affords informative tandem mass spectra suggests that the technique can be used for the analysis of samples containing low levels of sodium.

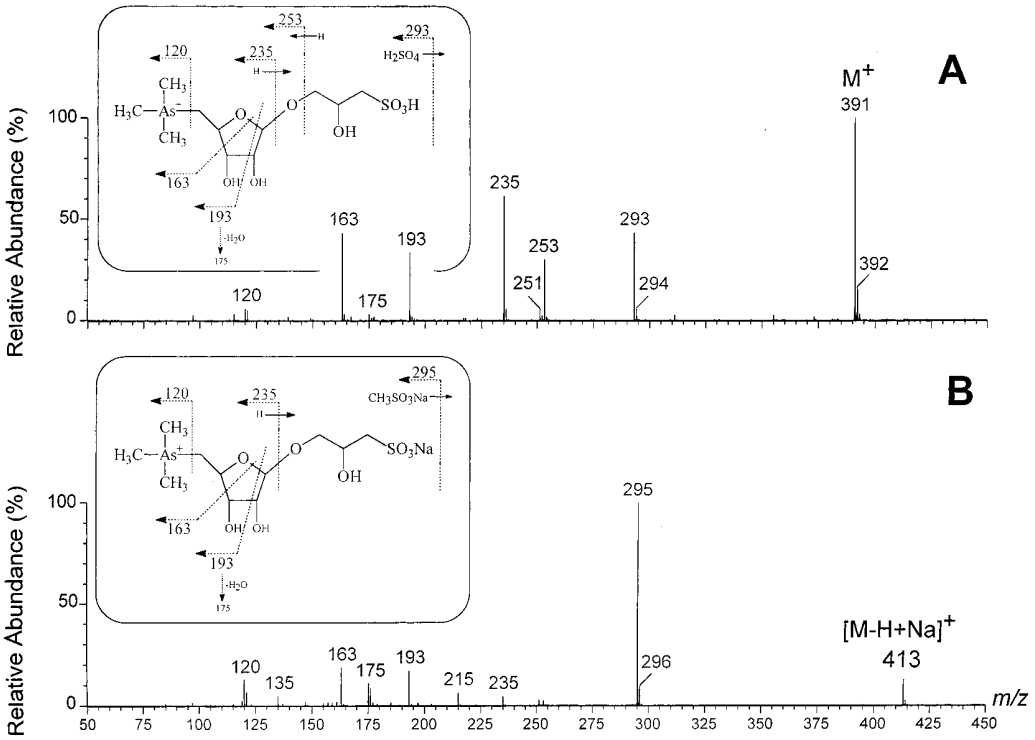

Figure 9. Positive-ion nano-ES Q-TOF mass spectra of: (A) $[M]^+$ (*m*/*z* 391) and (B) $[M - H + Na]^+$ (*m*/*z* 413) originating from arsenosugar **7**, acquired following the introduction of approximately 850 pg/μL of As in the form of arsenosugar **7**. Approximately two-minute acquisition time. Cone voltage and collision energy were set to 50 V and 35 eV, respectively.

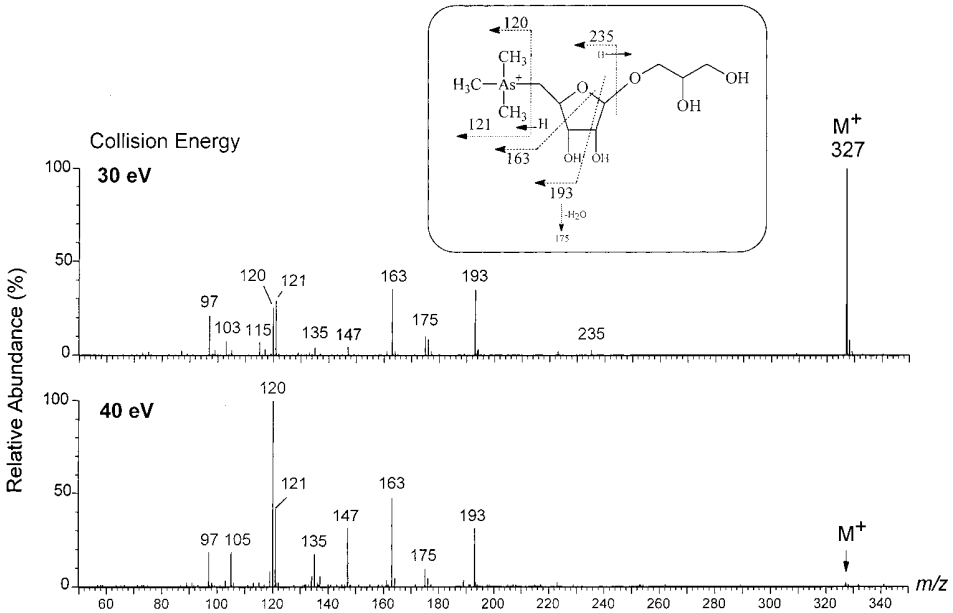

Figure 10. Positive-ion nano-ES Q-TOF mass spectra of the $[M]^+$ ion (*m*/*z* 327) originating from arsenosugar **8**, acquired following the introduction of approximately 170 pg/μL of As in the form of arsenosugar **8**. Approximately two-minute acquisition time. Cone voltage was set to 50 V; collision energies were 30 and 40 eV.

The tandem mass spectrum obtained for the M^+ ion originating from arsenosugar **8** (Figure 10) is almost identical to that obtained from arsenosugar **6**. This, of course, occurs because arsenosugar **6** predominantly loses a $-SO_3$ moiety, under low-energy CID conditions, yielding gas-phase species structurally equivalent to those of arsenosugar **8**. Thus, false identification of arsenosugar **8** is a potential problem in the presence of arsenosugar **6**. The structurally equivalent gas-phase species for the two arsenosugars (**6** and **8**) could arise if the ES cone voltage is sufficiently high to facilitate fragmentation of the arsenosugar **6** M^+ ion in the ES interface region to form ions at *m*/*z* 327. To avoid misidentifications, the ES cone voltage must be optimized to prevent such fragmentation of the molecular ion of arsenosugar **6** in the interface region of the ES mass spectrometer. Another way to avoid misidentifying these two compounds is to using HPLC to separate them prior to their introduction into the ES source.

CONCLUSIONS

Nano-ES quadrupole time-of-flight MS is one of the most sensitive mass spectrometric techniques for the structural characterization of arsenosugar compounds, requiring only picograms of material to obtain good-quality tandem mass spectra. It should be noted, however, that the sensitivity exhibited by nano-ES-MS/MS for detecting arsenosugars may degrade somewhat on analysis of nonpurified extracts if the sample matrix suppresses arsenosugar ionization. It is therefore necessary to evaluate further the technique's requirements for sample preparation. Efforts in our laboratories are currently ongoing to evaluate sample-preparation procedures that will allow the rapid identification of arsenosugars in biological extracts using nano-ES tandem mass spectrometry.

In comparison with the high-energy tandem mass spectra of arsenosugars, the low-energy tandem mass spectra provide at least as many structurally significant ions. This, in combination with the fact that instruments capable of performing low-energy CID (i.e., orthogonal time-of-flight, triple quadrupole, and ion trap instruments) are more readily available and generally offer higher sensitivity than the sector instruments required for high-energy CID, makes us believe that low-energy CID is likely to be of greater practical use for characterizing arsenosugars on a routine basis.

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NOTE ADDED AFTER ASAP POSTING

This article was inadvertently released ASAP on 12/01/99 before final corrections were made. There were five references omitted, which meant the remaining ones were cited incorrectly. The correct version was posted 12/13/99.

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