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REVIEW

Chemical form of arsenic in marine macroalgae

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Chemical forms of water-soluble and lipid soluble arsenic compounds in marine macroalgae, including edible algae, are reviewed. Major water-soluble arsenic compounds in algae are arsenic-containing ribofuranosides (ACRs). Red and green algae contain mainly a glycerophospho derivative at the glycerol terminal while brown algae contain a sulfate or sulfonate derivatives at the glycerol terminal together with a glycerophospho derivative. Brown algae which belong to the Sargasso species contain significant amounts of inorganic arsenic in the form of arsenate. Lipid-soluble arsenic identified in a brown algae (*Undaria pinnatifida*) was an acylated derivative of an ACR. Thirty-eight marine algae were analyzed by HPLC/ICP/MS and their arsenic compounds are characterized. The characterization of arsenic compounds may show their role in the chemical taxonomy of algae species in addition to giving an understanding of the toxicological and geochemical implication of the element.

Keywords: Arsenic speciation, marine algae, arsenosugar, arsenolipid, HPLC/ICP, methylarsenic

INTRODUCTION

Arsenic is contained in seawater at a concentration of 2 ppb (parts per billion, 10^9) and it is bioconcentrated in marine algae. Brown algae contain higher concentrations of arsenic, at several tens of ppm (on a dry weight basis) while green algae and red algae contain a lesser amount of several ppm on a dry weight basis.[1,2]

The chemical form of arsenic in marine water is the pentavalent inorganic form (arsenate) but that in marine algae is known to be not the same form. For example, Lunde reported that 97% of arsenic is present in organic form in *Laminaria hyperborea*. Sanders reported that arsenic is present in organic form at 53%, 78% and 57% in chlorophyceae, phaeophyceae and rhodophyceae respectively, through the examination of 56 algae species.[1]

Marine algae have been eaten as foodstuffs by the people of marine nations, especially by the Japanese. Marine algae have been recommended as a healthy food for the nutritional aspect of mineral supplementation. It has been applied also for cosmetic purposes in Japan.

Since arsenic is known as a toxic element, it has been a public concern whether algal arsenic is toxic or not. Daily intake of arsenic through the ingestion of marine algae in the average Japanese is estimated about 100 μg/day. It is important to know the chemical form of arsenic in edible marine algae and to understand its toxicological implication. In the present paper, we deal with the chemical form of arsenic in marine macroalgae, including edible algae.

Algae is a primary accumulator of arsenic in the marine environment and is an important stage of arsenic metabolism through the food chain. Therefore, it is also important to know its chemical form in order to understand the geochemical cycle of the element.

WATER-SOLUBLE ARSENIC COMPOUNDS IN MARINE ALGAE

1. Brown Algae

Preliminary work on the characterization of arsenic in brown algae has been published. Combined methods such as hydride generation/atomic emission[3] and HPLC/ICP emission spectrometry[4] have been applied for speciation work.

Yasui *et al.* reported that arsenic extracted with 6 mol dm^{-3} HCl from algae is in the organic form at 83–97% in Kajime, Arame and Wakame and at 30% in Hijiki.[5] Characterization has been continued by several other workers and revealed that

Arsenic containing ribofuranosides and other arsenic compounds found in the marine environment.
(I) AsO_4^{3-}. (II) $(CH_3)_2AsO_2^-$. (III) As-I. (IV) As-II. (V) As-III. (VI) As-IV. (VII) As-V. (VIII) As-VI.

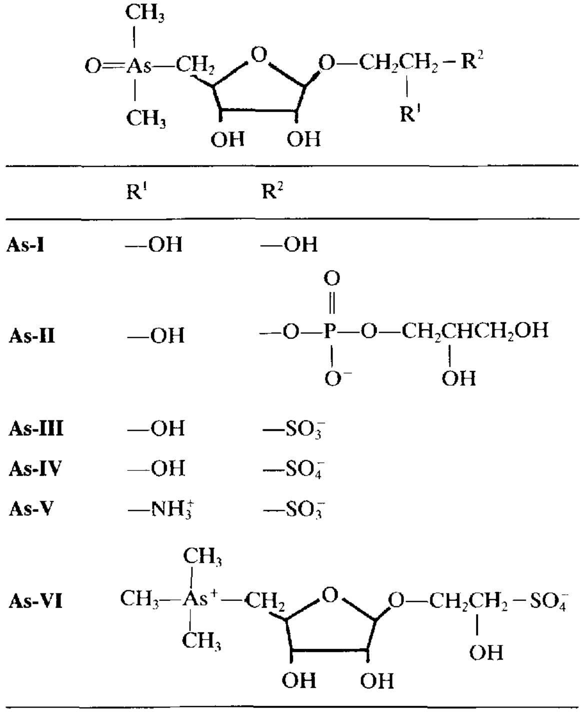

Figure 1 Structure of arseno-sugars and simple arsenic compounds identified in the marine environment.

the arsenic contained in Laminariales species is mostly in organic form with small molecular weights (less than 1000).[6] Those studies, however, have given limited information on the chemical structure of arsenicals.

The first identification of water-soluble organic arsenic compounds in marine macro-algae was done by Edmonds and Francesconi.[7] Methanol extraction of a brown Kelp (*Ecklonia radiata*) sample gave three main arsenic containing fractions. The major arsenicals were isolated and established to be arsenic containing ribofuranosides (ACR: designated As-I, As-II, As-III) on the bases of microanalysis, ^1H and ^{13}C NMR, and field desorption mass spectrometry. Structure of ACRs are shown in Figure 1) together with some simple arsenic compounds found in the marine environment.

Two compounds of the same class, As-I and As-IV, were found in the giant clam *Tridacna maxima*.[8] A Single crystal X-ray structure determination confirmed that As-IV was formulated correctly. The chirality of the ribose system in the compound is D. A synthesis of As-I has been developed by Mcadam and Stick from tribenzoyl-2-chloro-D-ribose[9] and gave exactly the same ^1H NMR spectrum As-I isolated from algae.

The brown marine alga *Laminaria Japonica* (Makonbu in Japanese) is one of the most favored and mass marketed in Japan. It is also known that glutamic acid was first isolated as the tasty component from Makonbu. Methanol extracts of *Laminaria Japonica* have been fractionated by using Sephadex LH20, DEAE sephadex and HPLC columns. Three components were isolated and these were shown to be the ribofuranoside derivatives (As-I, As-II, As-III) that had been previously isolated from *Ecklonia radiata*. By using an HPLC-ICP method, three derivatives were quantitatively determined and gave the amounts As-I (3%), As-II (17%) and As-III (80%) in a methanol extract.[10]

During the separation procedure, it was found that the compound As-II is rather easily decomposed to give As-I by losing a glyceryl-phosphate group.

Although those three ACRs was the same as those for *Ecklonia radiata*, a minor difference was found in the dominant arsenical (As-III). When the fraction was purified and subjected to ^1H NMR spectrometry, a minor arseno-sugar compound was found to be present. Main and minor components were not well resolved by conventional chromatography; the minor ^1H NMR spectrum is quite similar whereas that of the main component was identical to that of As-III. From the similarity in physico-chemical properties to As-III and ^1H NMR spectroscopic considerations, it was assumed that the minor component of the fraction was a diastereomeric pair of As-III isomers, epimeric at the CHOH group of the side chain. The presence of a diastereomeric pair may indicate the presence of two different biosynthetic pathways for the As-III compounds.

The brown alga *Undaria pinnatifida* (Wakame in Japanese) is in the same order as *Ecklonia radiata* and *Laminaria Japonica* and these three species are similar. Wakame is cultured and mass marketed in Japan. Water-soluble arsenicals in *Undaria pinnatifida* were also isolated by the same procedures as *Laminaria Japonica*. Mainly, the three arseno ribofuranosides (As-I, II, III) that are identical to those in *Ecklonia radiata* and *Laminaria Japonica* were isolated. A diastereomeric pair was also found in the compound As-III.

Eisenia bicyclis (Arame in Japanese) is also an edible algae although the consumption is limited. In an earlier report, it has been suggested that similar arsenicals may be present in the algae.[5] HPLC-ICP-AES analysis gave a characterization of water soluble arsenicals that are identical to the compounds As-I, II, III. *Ecklonia radiata, Undaria pinnatifida, Laminaria radiata* and *Eisenia bicyclis* all belong in the order Laminariales and apparently contain the same arsenosugars in their tissue.

Another edible brown alga, *Hizikia fusiforme* (Hijiki in Japanese) belongs to the order Fucales. Arsenicals were extracted with methanol from fresh tissues and separated on Sephadex LH-20 and Sephadex DEAE by column chromatography with an eluant of tris-buffer. The major arsenical was identified as inorganic arsenate (50%) and compound As-IV[11] (Fig. 1).

Minor arsenicals are ribofuranoside derivatives of different side chain (As-I, III, V). Two diastereomers are again present as compound As-III.

It has been suggested in an earlier report that inorganic arsenic, (arsenate) is present as a major arsenical in *H. fusiforme* by hydride-generation-atomic spectrometric and other methods.[12] It has also been indicated through feeding experiments that the effects of arsenic in *H. fusiforme*, when fed to rats, is more similar to those of inorganic arsenic than organic arsenic found in fish.[13] These findings were confirmed by rigorous identification of arsenate after separation and isolation from the alga.

The localization of arsenicals was examined in *H. fusiforme* by dissecting the algae in various parts and subjecting it to analysis by HPLC-ICP-AES after extraction with methanol. At both stems and leaves, the total arsenic concentration gradually rises from top to root. This may reflect that older positions (near the root) are exposed to arsenic for a longer period than newer position (near the top). It is also noted that arsenic concentration is higher at the surface layer than at the center layer. By speciation analysis, using HPLC-ICP-AES, it was found that inorganic arsenic (arsenate) is present only in the surface layer and arsenic-ribofuranosides distribute rather evenly in each tissue. The reason why *H. fusiforme* accumulates arsenate and why arsenate is only distributed at the surface is not clear. One may speculate that inorganic arsenic at the surface tissue may play a role of an anti-bacterial effect but this aspect needs more evidence.

Sargassum thunbergii (Umitoranoo in Japanese) is not a popular food in Japan but it is known as a folk medicine for anthelmintic problems. Essentially, a similar purification procedure was applied to the extract of *Sargassum thunbergii* and five fractions containing arsenic were separated and characterized.[14]

The major arsenical was identical with As-IV and the others were characterized as As-I, II and a new ribofuranoside As-VI. The known compounds As-I, II, IV were characterized on the basis of their spectroscopic and chromatographic properties. The assignment of compound As-VI was made by ^{1}H NMR (cosy) and chromatographic analysis. Compound As-VI gives a strong singlet CH_3 peak at 1.96 ppm in the NMR which corresponds to three methyl groups in one molecule and shows a strong basic character due to the presence of a quarternary arsonium ion. Further evidence was given by the synthesis of the compound which was derivatized by reduction of compound As-III followed by methylation.

The compound As-VI is quite unique because it is the only quarternery substituted arsenical identified in marine algae and also it is close to arsenobetaine which is the most abundant arsenic species in marine animals. The metabolic pathway from compound As-VI to arsenobetaine via arsenocholine is possible if we consider that dimethyl-arsinoyl-ribose derivatives are converted to dimethylarisinoylethanol under anaerobic condition.[15] This speculation, however, may need further validation because compound As-VI is a minor compound in the algae.

Another interesting point is the occurrence of As-V in *H. fusiform* and *S. thunbergii*. As shown in Figure 1, the three carbon side chain of As-III terminates not in oxygen, but in sulfonate. The side chains of As-I, II, and IV on the other hands, are all terminated in oxygen. This means that the three carbon side chains of As-I, As-II and As-IV may be derived from glycerol while that of As-III arises from another compound. One possible origin of the three carbon side chain containing a sulfonate group is an amino acid, cysteine. The structure of As-V is quite interesting because its side chin contains an amino group instead of a hydroxyl group at the center of the three carbon chain. One possible speculation on the biosynthetic pathway based on the above data may be summarized as follows; the thiol group of cysteine is oxidized to a sulfonate group and the carboxyl group is reduced to hydroxyl group and makes a covalent ether bond to the 1-carbon of the ribose ring, while the amino group is converted to a keto group through the action of a transaminase and is finally reduced to a hydroxyl group. Based on this hypothesis, the occurrence of two forms of As-III may be explained by the stereospecific reduction of the keto group to a hydroxyl group. Edmonds and Francesconi noted the presence of free 2,3-dihydroxy-1-propane sulfonate ($HOCH_2CH(OH)CH_2SO_3^-$) in some algae.[16] It is interesting that *D*-cysteinolic acid ($HOCH_2CH(NH_3^+)CH_2SO_3^-$) is also identified in various seaweeds, especially in green and brown algae.[17] The metabolic relationship between these compounds and arseno-sugars should to be a target of future study.

Sphaerotirchia divaricata (Ishimozuku in Japanese) is also an edible algae. The typical concentration of arsenic in the algae is rather low (about 2 ppm on a wet weight basis) compared with other brown algae. About 25% of the arsenic extracted with methanol from the algae was lipid soluble and the rest was water soluble. Water soluble arsenic was chromatographed on Sephedex columns and six arsenicals were shown to be present, four being in major to medium amounts and two being present in small amounts. The four arsenicals were identified to be in the form of arsenic-containing ribofuranosides As-I, II, III, V. In addition to ACRs, two other arsenic species in small amount (less than 5%) were present. Their chromatographic behavior showed that they had a smaller molecule size than the ACRs and were negatively charged but further purification was not made. Other brown algae were analyzed by HPLC-ICP-MS and the result of arsenic characterization is given in Table 1 and Table 2.

2. Red Algae [Rhodophyta]

The only red alga whose arsenicals are well characterized is *Porphyra tenera*, (Asakusanori in Japanese). The algae is cultured in a massive scale and much eaten in the form of a sheet (Nori) in Japan. Major arsenic is present in the form of compound As-II. A minor component is compound As-I but no other arseno-sugar compounds are detected. In Yakinori, a baked sheet of *Porphyra tenera* or other *Porphyra* sp., a similar composition of arseno-sugar was detected. Details are described in other parts of this publication. Other red algae were analyzed by HPLC/ICP/MS and the result are given in Table 2.

3. Green Algae [Chlorophyta]

The concentration of arsenic in green algae is comparatively low (typically several ppm on a dry weight basis). In an earlier report, it was shown that the chemical forms of arsenic in green algae (16 species are 47% in the inorganic form and 53% in the organic form.[1] However it should be pointed out that the amount of organic arsenic is underestimated because arsenic species that are not converted o arsine derivatives by the hydrine generation method are not counted. Only one report is available at present on the rigorous identification of arsenicals in green algae.

Odium fragile (MIRU in Japanese) is an edible green algae distributed in the northern part of Japan. A fresh sample (12 kg wet weight, ca.7 mg As) was extracted with methanol and subjected to further purification using Sephadex G-15, DEAE Sephadex, CM-Sephadex, and DEAE Toyopearl column chromatography. The major arsenicals were identified as the compounds As-I and As-II by ^1H NMR spectroscopy. Neither sulfate ester type nor sulfonic acid type ACRs were detected. Together with ACRs, a small portion of arsenic (5%) was identified to be present in the form of dimethylarsinic acid by ^1H NMR, titration (pka 6.30) and HPLC retention time. Although the presence of dimethylarsinic acid in algae has been suggested in an earlier report,[2]) this report was the first isolation of the compound from natural marine algae.

Five other green algae species were analyzed by HPLC-ICP-MS method and the result is given in Table 2 and references therein. All samples analyzed had a similar pattern of ACRs; mainly As-II and As-I and no As-III, IV. In some species, significant amount of arsenic is present in unidentified chemical form.

It seems probable that the chemical form of arseno-sugar is specific to individual species. The structure of arseno-sugars in red algae and green algae is rather simple; As-III and As-I are dominant. On the other hand, the structure of arseno-sugars in brown algae is more complicated; sulfur containing sugars become dominant togethe with As-II and As-I. This may be reflecting the path of evolution where brown algae diversed later in the algae family tree. It is also pointed out that the analysis of arseno-sugars in algae may give a clue to the taxonomy of the species.

Table 1 List of marine algae and plant

		⟨⟨SEAWEED⟩⟩	Sampling Dates*	Number†
[CHLOROPHYTA]				
Ulvales	Ulvaceae	*Ulva pertusa* Kjellman ⟨ANAAOSA⟩	(A)	(1)
		Ulva arasakii Chihara ⟨NAGAAOSA⟩	(A)	(2)
Siphonales	Bryopsidaceae	*Bryopsis maxima* Okamura ⟨OOHANEMO⟩	(A)	(3)
	Caulerpaceae	*Caulerpa brachypus* Harvey ⟨HERAIWAZUTA⟩	(C)	(4)
	Codiaceae	*Codium fragile* (Suringar) Hariot ⟨MIRU⟩	(C)	(5)
[RHODOPHYTA]				
⟨Florideae⟩				
Gelidiales	Gelidiaceae	*Gelidium divaricatum* Martens ⟨HIMETENGUSA⟩	(B)	(6)
Cryptonemiales	Corallinaceae	*Corallina pilulifera* Postels et Ruprecht ⟨PIRIHIBA⟩	(B)	(7)
	Grateloupiaceae	*Grateloupia ramosissima* Okamura ⟨SUZIMUKADE⟩	(C)	(8)
		Grateloupia okaurai Yamada ⟨KYOUNOHIMO⟩	(C)	(9)
		Grateloupia turuturu Yamada ⟨TURUTURU⟩	(C)	(10)
		Cyrtymenia sparsa Okamura ⟨HIDIRIMEN⟩	(A)	(11)
		Carpopeltis flabellata (Holmes) Okamura ⟨KOMENORI⟩	(B)	(12)
		Carpopeltis crispata Okamura ⟨TOSAKAMATU⟩	(C)	(13)
	Endocladiaceae	*Gloipoeltis furcata* Postels et Rupecht ⟨HUKUROHUNORI⟩	(B)	(14)
Gigartinales	Nemastomaceae	*Schizymenia dubyi* (Chauvin) J. Agardh ⟨BENISUNAGO⟩	(A)	(15)
	Hypneaceae	*Hypnea charoides* Lamouroux ⟨IBARANORI⟩	(C)	(16)
		Hypnea japonica Tanaka ⟨KAGIIBARANORI⟩	(C)	(17)
		Hypnea variabilis Okamura ⟨TACHIIBARANORI⟩	(C)	(18)
	Phyllophoraceae	*Ahnfeltia paradoxa* (Suringar) Okamura ⟨HARIGANE⟩	(A)	(19)
	Gigartinaceae	*Gigartina intermedia* Suringar ⟨KAINORI⟩	(B)	(20)
		Chondrus ocellatus Holmes ⟨TUNOMATA⟩	(B)	(21)
		Chondrus verrucosus Mikami ⟨IBOTUNOMATA⟩	(A)	(22)
		Chondrus sp. ⟨OOBATUNOMATA⟩	(C)	(23)
Rhodymeniales	Champiaceae	*Coeloseira pacifica* Dawson ⟨ISOMATU⟩	(A)	(24)
		Lomentaria catenata Harvey ⟨HUSITUNAGI⟩	(C)	(25)
Ceramiales	Ceramiaceae	*Psilothallia dantata* (Okamura) Kylin ⟨BENIHIBA⟩	(A)	(26)
		Campylaephora crassa (Okamura) Nakamura ⟨HUTOIGISU⟩	(A)	(27)
		Centroceras clavulatum (Agardh) Montagne ⟨TOGEIGISU⟩	(C)	(28)
	Rhodomelaceae	*Chondria crassicaulis* Harvey ⟨YUNA⟩	(A)	(29)
		Laurencia okamurai Yamada ⟨MITUDESOZO⟩	(A)	(30)

[PHAEOPHYTA]				
Dictyotales	Dictyotaceae	*Spathoglossum pacificum* Yendo ⟨KOMON GUSA⟩	(C)	(31)
		Pachydictyon coriaceum ⟨Holmes⟩ Okamura ⟨SANADAGUSA⟩	(C)	(32)
		Dictyopteris prolifera ⟨Okamura⟩ Okamura ⟨HERAYAHAZU⟩	(C)	(33)
		Padina arborescens Holmes ⟨UMIUCHIWA⟩	(C)	(34)
Chordariales	Choradariaceae	*Heterochordaria abietina* (Ruprecht) Setchell et Gardner ⟨MATUMO⟩	(A)	(35)
Punctariales	Asperococcaceae	*Myelophycus caespitosus* (Harvey) Kjellman ⟨IWAHIGE⟩	(B)	(36)
Laminariales	Alariaceae*	*Undaria pinnatifida* (Harvey) Suringar ⟨WAKAME⟩	(A)	(37)
Fucales	Sargassaceae	*Sargassum* sp. ⟨HONDAWARA-ZOKU⟩	(A)	(38)
		⟨⟨HIGHER PLANT⟩⟩		
[MONOCOTYLEDONEAE]				
	Zosteraceae	*Phyllospadix japonica* Makino ⟨EBIAMAMO⟩	(B)	(39)

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* Identification of each species was kindly performed by Dr. T. Hori (Univ. Tsukuba) Dr. M. Yoshizaki (Toho Univ.). The above classification is based on the reference (seaweeds) and the reference (higher plant) with a modification based on the reference

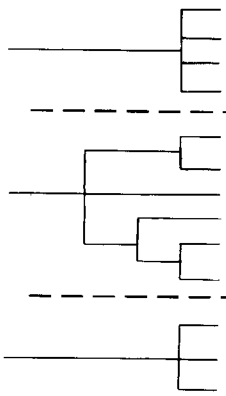
* Sampling sites for algae and plants and dates are as follows;

(A): Cho-shi, Chiba Pref., May 26, 1987
(B): Hiraiso, Nakaminato, Ibaraki Pref., June 17, 1987
(C): Inamuragasaki, Kamakura, Kanagawa Pref., July 11, 1987.

† Number—this gives key to Table 2

Table 2 Arsenic species in marine algae and plant

	Total As (μg/g dry)	Lipid-phase (μg/g As)	Water-phase (μg/g As)	(X)	(XI)	(XII)	(XIII)	(XIV)	(XV)	(I)	(IV)	Unidentified species					
												U2	U3	U4	U5	U6	Others
[CHLOROPHYTA]																	
(1)	17.1	3.3	6.9	★	⋆								★				
(2)	15.5	4.4	4.2	★	⋆								⋆				
(3)	19.4	9.7	3.9	⋆	★												★
(4)	11.6	3.5	3.7														★
(5)	18.2	9.1	3.1	⋆	⋆												
*1	0.6**	—	0.4**	⊙	○						○						
[RHODOPHYTA]																	
(6)	33.1	0.6	7.0	★	★												
(7)	21.6	1.3	3.3	⋆	★												⋆
(8)	16.9	0.6	4.4	⋆	★							⋆					
(9)	16.1	0.6	7.6	★	★												
(10)	7.1	0.6	2.8	⋆	★									⋆			
(11)	44.8	1.6	31.1	⋆	★							★					
(12)	14.9	1.0	7.1	⋆	★												⋆
(13)	13.4	—	8.9	★	★							★		★			
(14)	25.0	1.2	19.1	★	★												⋆
(15)	12.0	1.0	8.3	⋆	★									★			
(16)	16.7	1.2	4.0	⋆	★												⋆
(17)	9.9	1.0	2.3		★												⋆
(18)	6.0	0.4	2.5	⋆	★									⋆			
(19)	11.7	1.2	6.8	⋆	★		⋆					★					
(20)	19.8	0.9	10.6	⋆	★							⋆					⋆
(21)	13.2	0.6	12.4	⋆	★												
(22)	17.9	2.0	10.0	⋆	★									⋆			⋆
(23)	7.9	0.3	3.6	⋆	★									⋆			
(24)	23.1	4.8	8.0	⋆	★							★		⋆			
(25)	6.6	0.3	1.8	⋆	⋆												
(26)	22.4	4.0	6.3	★	★							★					
(27)	11.5	2.4	4.5	★	⋆		⋆					⋆					
(28)	8.8	0.3	2.6	⋆	★					⋆							
(29)	22.5	2.5	5.6	⋆	⋆		⋆					★					⋆
(30)	19.2	1.8	9.0	★	⋆		★					⋆					



[PHAEOPHYTA]															
(31)	16.3	4.8	11.3	★	★	★	★								★
(32)	16.7	2.8	12.0	★	★	★									★
(33)	8.1	0.9	11.7	★	★	★							★		
(34)	17.6	0.9	11.7	★	★	★									★
(35)	56.8	2.1	36.3	★	★	★									●
*2	1.8**	0.4**	0.9**	⊙	○	○		⊙							
(36)	33.3	3.0	10.3	★	★	★						★			
*3	—		4.0**	○	○	⊙									
(37)	33.8	8.4	13.9	★	★	★								★	
*4	2.8**	0.7*	2.0**	○	○	⊙									
(38)	71.6	1.9	59.2	★	★	★	★			★				★	
*5	10*	—	4.2**		○	○	⊙	○		⊙					
*6	7.4**	—	3.8**	○	○	○	⊙	○	○	○					
⟨⟨HIGHER PLANT⟩⟩															
(39)	4.5	—	0.7								★				

*1 *Codium fragile* (MIRU);
*2 *Sphaerotrichia divaricata* ⟨ISHIMOZUKU⟩
*3 *Laminaria japonica* ⟨MAKONBU⟩;
*4 *Undaria pinnatifida* ⟨WAKAME⟩;
*5 *Hizikia fusiforme* ⟨HIJIKI⟩;
*6 *Sargassum thunbergii* ⟨UMITORANOO⟩
** Wet base.

The content of each arsenic species in the samples is designated by the free marks. 0.1–1 μg/g As (dry) ★; 1–10 μg/g As ★; more than 10 μg/g As ★. Identified compounds by the purification studies are marked by open circles ○ (⊙ indicates the most abundant species).

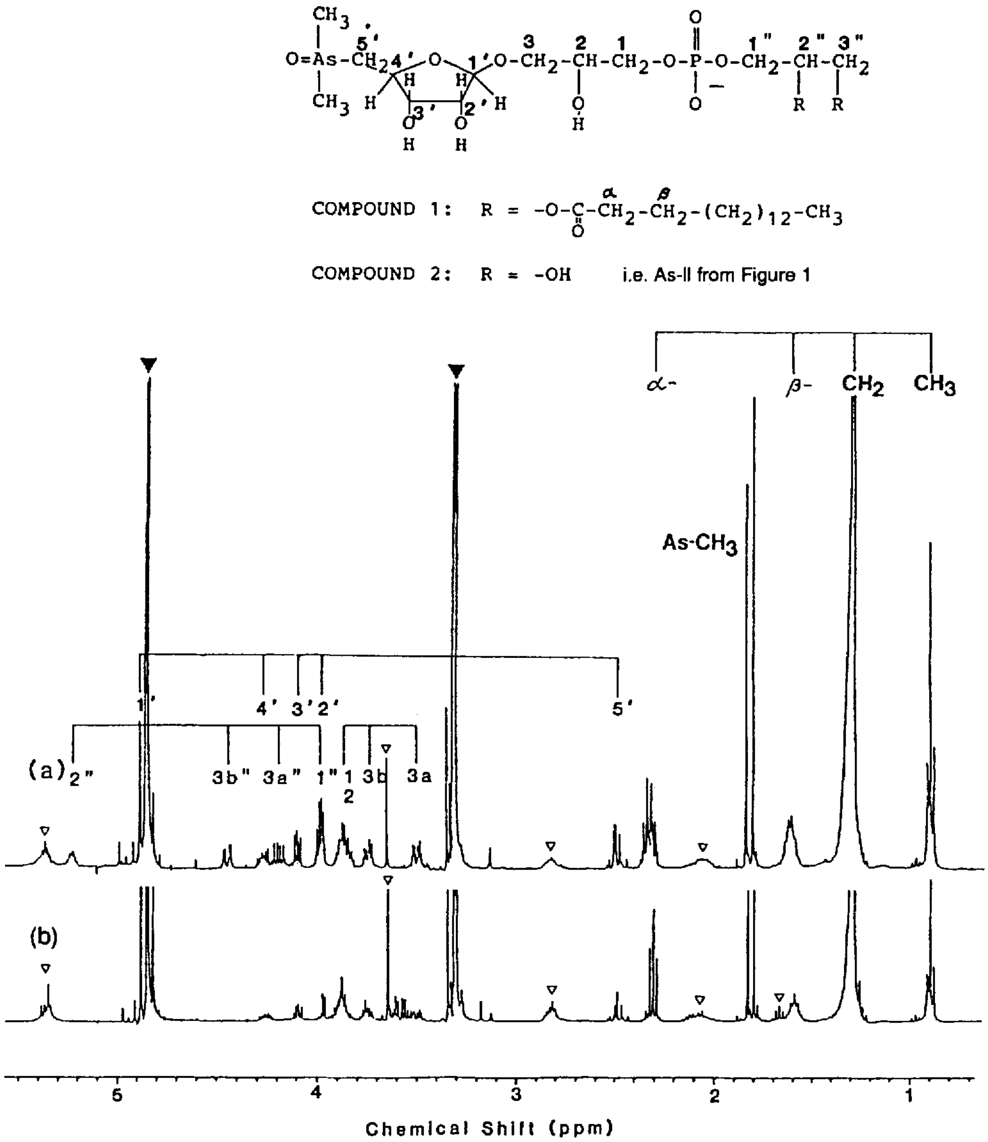


Figure 2 The structure of lipid-soluble (1) and water-soluble (2) arsenic compounds from *U. pinnatifida* (upper) and 1H NMR spectra of compound 1(a) and its degradation product 2 (b). Large triangles (▼) denote the residual proton resonances of the solvent. Small triangles (▽) represent the resonances of impurities.

LIPID-SOLUBLE ARSENIC

It has been reported that algae also contain various amounts of lipid-soluble arsenic. In fact it was a main target of arsenic study in late 1970s. Some researchers speculated that arsenic might occur in lecithin type lipids by replacement of nitrogen.[18,19] Arsenic shows similar chemical properties to nitrogen and phosphorus, and might be expected to be metabolized in place of them.

After the discovery of the arseno-sugar derivative containing a glycerophosphoryl moiety (AS-II in Figure. 1), a diacylated form of AS-II was proposed as the structure of lipid soluble arsenic by Edmonds and Francesconi[7]) and Knowles and Benson.[20]) The proposed structure reasonably explained the results of the earlier report[21]) performed by this group.

The first isolation and identification was made for a brown alga *Undaria Pinnatifida* which contains 25% of arsenic in the lipid-soluble form.

Lipid soluble arsenic was extracted in chloroform/methanol mixture (1:1) from the algae. After removing solvent by evaporation under reduced pressure, lipid-soluble arsenic was purified by hexane/acetonitrile partition, Sephadex LH-20 column chromatography and a silicagel HPLC. Structure determination was made by ^{1}H NMR (cosy) and GC/MS.

The lipid-soluble arsenic identified was fairly unstable and is easily decomposed even in methanol. During ^{1}H NMR measurement to obtain a cosy spectrum, it was noticed that some of the resonances lost intensity and several new resonances appeared. The new resonances were identical to those of compound As-II. In the solution, a deutero methyl ester of a fatty acid (mainly palmitic acid) was identified by GC/MS analysis. From the analysis of ^{1}H NMR spectra, the structure of lipid-soluble arsenic was determined as a diacylated derivative of compound As-II. (Fig. 2). The fatty acid was mostly saturated acid; 90% was in the form of palmitic acid.

The marine macro alga *Fucus spiralis* assimilates arsenate to form lipid-soluble compounds that account for 60% of a radioactive label.[22]) The green alga Plalymonas cf. Suecica was found to incorporate arsenate into chloroform soluble compounds (49%).[23] There is a possibility that these lipid-soluble arsenic compounds are the compounds as described above. Further work on separation and identification of individual compounds is necessary for elucidating lipid-soluble arsenic in more detail.

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