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Characterization of Arsenic Metabolites in Rice Plant Treated with DSMA (Disodium Methanearsonate)

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In this paper the results of a study of the metabolic products of disodium methanearsonate (DSMA) in hydroponically grown rice plant are reported. Gas chromatography with a multiple ion detection mass spectrometry (GC/MID/MS) and hydride generation–heptane cold trap (HG/HCT) technique was used for species determination of arsenicals. Most of the arsenic in rice plant was present as monomethylarsenic (MM-As) species. Relatively large amounts of transformed products such as inorganic arsenic and dimethyl- and trimethylarsenic species were detected in the nutrient solution through root exudation. Gel permeation and thin-layer chromatographic analyses revealed that the MM-As species accounting for nearly all of the arsenic in root were a mixture of nonconjugated methanearsonate, conjugated MM-As, and reduced methanearsonate (red-MA). The red-MA was further characterized by its being liable to be cleaved into arsenite.

INTRODUCTION

Organo arsenical pesticides such as methanearsonate monosodium salts (MSMA) and disodium salts (DSMA) are used as a selective preemergent contact herbicide to control weeds in cotton and uncropped land in USA. Methanearsonates (ferric salts) are also used as a fungicide to control sheath blight of rice in Japan.

Root uptake of methanearsonates by plants from nutrient solution is rapid, and the arsenic is translocated into all portion of the plants (Sckerl and Francs, 1969). The degree of absorption through leaves and stem depends upon a rate of application, temperature, and plant species (Arle and Hamilton, 1971; Keeley and Thullen, 1971). Basipetal and acropetal translocation of foliar- and stem-applied methanearsonates (Sckerl and Francs, 1969; Keeley and Thullen, 1971; Rumburg et al., 1960; Duble et al., 1969; Sachs and Michael, 1971) and exudation of the arsenic from roots into rhizosphere (Domir et al., 1976) have been observed.

There is no conclusive evidence that the C–As bond of methanearsonates is cleaved in plants. Methanearsonates are usually found unchanged or in a complex form (Sckerl and Francs, 1969; Duble et al., 1969; Sachs and Michael, 1971). Furthermore, there is no report that methanearsonates are converted into dimethyl- and trimethylarsenic compounds in plants. Recently, Knowles and Benson (1983) suggest in enzymatic study of Johnson grass that methanearsonate is reduced to arsenosomethane ($CH_3As{=}O$) which inhibits the malic enzyme.

The purpose of this study was to determine the metabolic products as arsenic component in rice plant on root-soak-treatment of DSMA.

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MATERIALS AND METHODS

Chemicals. DSMA [99.8% monomethylarsenic (MM-As), 0.2% inorganic arsenic (Inorg-As), <0.1% dimethylarsenic (DM-As), and <0.1% trimethylarsenic (TM-As), determined by the GC/MID/MS–HG/HCT method (see determination of arsenic)] was provided by Kumiai Chemical Ind. Co. Ltd. Sodium arsenite (>99.9% Inorg-As) and sodium arsenate (>99.9% Inorg-As) were purchased from Kanto Chemical Co. DMAA (98.2% DM-As, 1.4% MM-As, 0.4% Inorg-As, and <0.1% TM-As) was purchased by Sigma Chemical Co. TMA=O (96.9% TM-As, 3.1% DM-As, <0.1% MM-As, and <0.1% Inorg-As) was obtained by oxidizing trimethylarsine (TMA, Ventron Co.) with iodine (Odanaka et al., 1983). These arsenicals were diluted with water to prepare 1000 ppm As stock solutions.

Growth and Treatment. Rice plants (*Oryza sativa* L. var Nihonbare) were germinated and planted in Kasugai nutrient solution [NH_4Cl 6 mg, $Na_2HPO_4{\cdot}12H_2O$ 3 mg, KCl 4.5 mg, $CaCl_2{\cdot}2H_2O$ 0.6 mg, $MgCl_2{\cdot}6H_2O$ 0.9 mg, $FeSO_4{\cdot}7H_2O$ 1.0 mg/L, pH 5.5] (Kasugai, 1939). Three weeks after the germination, at the two- or three-leaf development stage, the seedlings were transferred to jars (two seedlings per jar) containing a 500 mL of the nutrient solution and DSMA (500 μg as arsenic). The jars wrapped with aluminum film shielding the light were kept in a greenhouse. The growing conditions included natural sunlight with temperatures from 18 to 32 °C. The treated plants were harvested 7 days after being transferred to the DSMA-contained nutrient solution. The roots were thoroughly rinsed with a vigorous flow of water to remove the arsenicals adhering to the root surface. Each plant was sectioned into shoots and roots. The tissue sections were

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dried in a desiccator under vacuum and stored at −20 °C until analyzed. The nutrient solution after removal of the rice seedlings was analyzed within 12 h (the remainder of nutrient solution was stored at −20 °C).

The plant samples obtained were used for the following experiments: (1) absorption and translocation, (2) fractionation of plant tissues, and (3) characterization of arsenic in methanol/water (M/W)-soluble extract.

In another experiment (root exudation) designed for determination of arsenic in the nutrient solution through root exudation, the rice seedlings were similarly exposed to the DSMA for 7 days as described above and then transferred to a fresh jar (two seedlings per jar) containing 500 mL of Kasugai nutrient solution. The day of transfer to DSMA-free solution was referred to as day 0; the nutrient solution was collected and renewed every seven day. The nutrient solution collected was stored at −20 °C until analyzed. The plants were harvested at day 7, 14, and 21, sectioned, dried, and stored as described above.

Analysis for Arsenic in Plant and Nutrient Solution. The roots (0.2–0.5 g) and shoots (0.3–1 g) of the rice seedlings were homogenized with a 50 mL of 10% methanol by Polytron (Kinematica, Switzerland). A 5 mL aliquot of 1 N NaOH was added to the homogenized samples and the mixture was shaken for 1 h. The solution was acidified with 5.5 mL of 1 N H_2SO_4, and 1–2 g of a mixture of active charcoal and Celite (1 + 1) was added. The mixture was filtered through a paper under suction and the residue was washed with 20–30 mL of water. Aliquots of the combined filtrate solution were assayed for As species with the GC/MID/MS–HG/HCT method.

Aliquots of the nutrient solution were directly analyzed for As species by the same GC/MID/MS–HG/HCT method.

Extraction for Fractionation of Plant Tissues. The analytical method used for fractionation of plant tissues was a modification of the procedure developed by Bligh and Dyer (1959). The root (0.2–0.5 g) and shoot (2–3 g) tissues were homogenized with a 150 mL of $CHCl_3$/CH_3OH/H_2O (1:1:1, v/v/v). The solvent was removed from the homogenate by vacuum filtration, and the residue was reextracted and filtered as described above. The combined filtrate was divided into chloroform and water layers by partition. The chloroform phase was further partitioned with water (50 mL), and the water layers were combined. After these extractions, the residue was extracted with a 50 mL of 0.1 N NaOH with shaking for 1 h. The mixture was centrifuged at 2000 rpm × 10 min and the supernatant liquid was removed.

Aliquots of the chloroform (C) phase, methanol/water (M/W) phase, aqueous NaOH phase, and unextractable residues (UR) were digested with 10 mL of a HNO_3–$HClO_4$–H_2SO_4 (6:2:2, v/v/v) mixture. These acid digestion samples were assayed for arsenic by use of the GC/MID/MS–HG/HCT method. A minor portion of the arsenic was detected in the C phase, aqueous NaOH phase, and UR (see Table V); therefore, the arsenic in these fractions were not characterized further.

Characterization of Arsenic in M/W Soluble Extract. The root (0.5–1.0 g) and shoot (2–3 g) were extracted with 100 mL of M/W (1:1, v/v) in a similar manner as described above. Only in the case of the shoot, the extract was further partitioned with chloroform in order to remove a large quantity of pigments. These M/W-soluble extracts of the root and shoot tissues were then concentrated on a rotavapor to ca. 3 mL, were filtered through a paper under suction, and were made up to 0.1 M NaCl (5–10 mL) solution. Aliquots of the sample solution were first subjected to gel permeation chromatography (GPC). Other aliquots of the sample solution were appropriated to measurement of total As [determined by the GC/MID/MS–HG/HCT after acid digestion (wet ashing); the results are represented in Figures 2 and 3] and As species (determined directly and after passing through the charcoal/Celite filter by the GC/MID/MS–HG/HCT; the results are represented in Figures 2 and 3).

A Sephadex G-10 chromatography column (100 × 2.0 cm) was used to separate As compounds from the other As compounds in conjugated form in the M/W soluble extract. The column was equilibrated and eluted with 0.1 M NaCl. The prepared sample solution was transferred onto the GPC column, and the column was eluted with 0.1 M NaCl. Dextran blue was used to determine the void volume (95 mL). A volume of 3–4 mL was collected at 0.5 mL/min. The only tube containing dextran blue and subsequent tubes were measured separately for sugar, amino acid and/or protein, total As, and As species. The sugar and amino acid/protein were determined by Anthrone (Drywood, 1946) and Ninhydrine (McGrath, 1972) methods, respectively. The As compounds in the tubes were monitored by the GFAA, and were also measured by the GC/MID/MS–HG/HCT method. The fractions containing As eluted from the GPC column were individually combined, were concentrated to dryness, and were subjected to TLC analysis.

A TLC method was ordinarily used for further separation or characterization of the arsenic obtained by the GPC analysis. The TLC was performed on a fine cellulose (Funakoshi, Japan). The following solvent systems were used: (A) ethyl acetate–acetic acid–water (3:2:1, v/v/v) (Odanaka et al., 1978), (B) phenol–water–ammonium hydroxide (400:99:1, v/v/v) (Sckerl and Francs, 1969). The prepared sample was spotted with four reference standards as arsenite, arsenate, DSMA, and DMAA. If necessary, for confirmation of As compounds developed on TLC, the As spots were scraped from the TLC plate, were extracted with distilled water, and then were determined for As species by the GC/MID/MS–HG/HCT method.

Determination of Arsenic. The combined method of gas chromatography with a multiple ion detection mass spectrometry (GC/MID/MS) system and hydride generation–heptane cold trap (HG/HCT) technique (Odanaka et al., 1983) was used for species specific determination of As compounds. Briefly, species of arsenic [inorganic arsenic (arsenite + arsenate), monomethyl-, dimethyl-, and trimethylarsenic compounds] in aqueous samples such as extracts of plant tissues or nutrient solutions were reduced with $NaBH_4$ to volatile arsines (arsine, methylarsine, dimethylarsine, and trimethylarsine, respectively) which were collected in a cold *n*-heptane (−80 °C) and were quantitatively determined by the GC/MS equipped with MID.

Absolute structure of As compounds in samples can not be defined only by this technique which is based on the determination of arsine derivatives from As compounds. So that, As compounds determined by this technique are named as inorganic arsenic (Inorg-As, arsenate or arsenite), monomethylarsenic (MM-As, the compounds having CH_3As as a basic structure), dimethylarsenic [DM-As, the compounds having $(CH_3)_2As$], and trimethylarsenic [TM-As, the compounds having $(CH_3)_3As$].

To test the applicability of this detection method for this study, spiked plant extracts (MeOH/NaOH) and nutrient solution with a mixture of sodium arsenate, DSMA, DMAA, and TMA=O were analyzed. As can be seen from Table I (interfering test), good recoveries for each As

Table I. Arsenic Speciation from Spiked and Unspiked Plant Extracts and Nutrient Solutions (Interfering Test)

sample	As species	μg of As unspiked	μg of As spiked	μg of As recovery	recovery, %
root extract[a] (40 mL/ 0.2 g)	Inorg-As	0.03	1.00	1.06	103
	MM-As	<0.01	1.00	0.99	99
	DM-As	<0.01	1.00	0.97	97
	TM-As	<0.01	1.00	0.94	94
shoot extract[a] (40 mL/ 0.5 g)	Inorg-As	0.02	1.00	0.99	97
	MM-As	<0.01	1.00	1.01	101
	DM-As	<0.01	1.00	0.97	97
	TM-As	<0.01	1.00	0.92	92
nutrient solution (50 mL)	Inorg-As	0.05	1.00	1.03	98
	MM-As	<0.01	1.00	1.02	102
	DM-As	<0.01	1.00	1.05	105
	TM-As	<0.01	1.00	0.96	96

[a] MeOH/NaOH extracts. Results are an average of two samples.

Table II. Arsenic Speciation from DSMA Spiked Plant Samples and Nutrient Solutions (Recovery Test)

sample	As species	μg of As unspiked	μg of As spiked	μg of As recovery	recovery, %
root (0.5 g)	Inorg-As	0.06		0.09	0.3[a]
	MM-As	<0.01	10.0	10.3	103
	DM-As	<0.01		<0.01	<0.1
	TM-As	<0.01		<0.01	<0.1
shoot (1.5 g)	Inorg-As	0.04		0.07	0.3[a]
	MM-As	<0.01	10.0	9.65	96.5
	DM-As	<0.01		<0.01	<0.1
	TM-As	<0.01		<0.01	<0.1
nutrient solution (50 mL)	Inorg-As	0.05		0.07	0.2[a]
	MM-As	<0.01	10.0	9.91	99.1
	DM-As	<0.01		<0.01	<0.1
	TM-As	<0.01		<0.01	<0.1

[a] The values may be derived from impurities in standard DSMA (see chemicals in Materials and Methods). Results are an average of two samples.

species were obtained. Therefore, the method of direct estimation from calibration curves was employed in every As species measurements.

A Hitachi Zeeman graphite furnace atomic absorption spectrometer (GFAA) was also used for determination of As compounds. By the GFAA procedure, sodium arsenite, sodium arsenate, disodium methanearsonate (DSMA), and dimethylarsinic acid (DMAA) (contained the same equivalent As atom) can be determined with a sensitivity similar to those of one another as described previously (Odanaka et al., 1979). In this study, trimethylarsine oxide (TMA=O) was found to have a sensitivity similar to those of the above four arsenicals by being coexisted with a salts such as NaCl.

Recovery Test. DSMA (10 μg as arsenic) was added to untreated rice plant (roots and shoots) and nutrient solution and was analyzed in the same manner as treated samples. The results are represented in Table II.

RESULTS AND DISCUSSION

Absorption and Translocation. The results of analyses for speciating As in the roots, shoots, and nutrient solution by the GC/MID/MS–HG/HCT method are represented in Table III. When rice seedling roots were immersed in the DSMA solution, the As compound was absorbed through the roots, and accounted for nearly a fourth of the applied As for 7 days. Translocation of arsenic from roots to shoots seemed to be very restricted; most (95%) of the arsenic absorbed by rice plant remained in the roots. MSMA, arsenate, and arsenite have been reported to accumulate similarly in the root of bean plant following absorption from nutrient solution (Sachs and Michael, 1971).

Table III. Chemicals Species of Arsenic in Shoots, Roots, and Nutrient Solution after Root-Soak-Treatment for 7 Days

sample	As species	μg of As at days 0	μg of As at days 7	ppm at days 0	ppm at days 7
shoots	Inorg-As	0.05	0.28	0.7[a]	0.87[a]
	MM-As	<0.01	5.74	<0.1	17.8
	DM-As	<0.01	0.06	<0.1	0.19
	TM-As	<0.01	0.01	<0.1	0.03
	subtotal	0.05	6.09 (1.2)		
roots	Inorg-As	0.05	0.83	1.3[a]	5.61[a]
	MM-As	<0.01	115	<0.3	777
	DM-As	<0.01	0.08	<0.3	0.54
	TM-As	<0.01	<0.01	<0.3	<0.07
	subtotal	0.05	116 (23.2)		
nutrient solution	Inorg-As	1.4	5.4	0.003	0.011
	MM-As	510	378	1.02	0.77
	DM-As	<0.5	1.5	<0.001	0.003
	TM-As	<0.5	<0.5	<0.001	<0.001
	subtotal	511	385 (77.0)		
total	Inorg-As	1.5	6.5		
	MM-As	510	499		
	DM-As	<0.5	1.6		
	TM-As	<0.5	0.01		
	total	512	507 (101)		

[a] Results are an average of two samples. Parentheses contain percent of the applied arsenic of DSMA (500 μg as arsenic). Dry weight basis.

Nearly all of the arsenic in roots was present in the form of monomethylarsenic (MM-As). Most of the arsenic in shoots was also the same MM-As species. Such a large quantity for the carbon–arsenic bond of methanearsonate in rice plant was in agreement with the finding of previous authors for several higher plants (Sckerl and Francs, 1969; Duble et al., 1969; Sachs and Michael, 1971; Domir et al., 1976).

Inorganic arsenic (Inorg-As) was detected in the roots, shoots, and nutrient solutions, and the overall yield of this species accounted for 1.3% (6.5 μg of As in Table III) of the applied As. Some of this species was probably impurities of standard DSMA present in the formulation (standard DSMA contained 0.2% Inorg-As, see chemicals in Materials and Methods), while others appear to have been true metabolite. Dimethylarsenic (DM-As), and trimethylarsenic (TM-As) species were also detected in several samples. The overall yield of these methylated products accounted for 0.3% (1.6 μg of As in Table III) of the applied As. DSMA spiked plant samples (root and shoot) were extracted and determined in the same manner as the treated plants, however, no other methylated products (below 0.1% of the applied As) were detected (Table II). So that, these methylated products were not artifact formed during the extraction and purification. However, such trace amounts of the products may be transformed by algae and/or microorganisms accidentally contaminated in the nutrient solution (see root exudation). Therefore, it is hard to judge whether these methylated products were true metabolite or not under only the present data.

Root Exudation. The rice seedlings grown in DSMA-containing nutrient solution for 7 days were transferred to DSMA-free solution. After several weeks, the plant and nutrient solutions were analyzed (see Materials and Methods). In this experiment, a significant amount of transformed products of DSMA was detected in the shoots, roots, and especially in the nutrient solutions (Table IV).

Table IV. Chemical Species of Arsenic in Shoots, Roots, and Nutrient Solution Obtained from Experiment for Root Exudation

		μg As at day				% of recovered As at day			
sample	As species	0	7	14	21	0	7	14	21
shoots	Inorg-As	0.20	0.48	0.60	1.04	0.4	1.0	1.0	1.6
	MM-As	3.36	2.84	3.58	3.58	5.9	6.0	6.1	5.5
	DM-As	0.09	0.12	0.12	0.36	0.2	0.3	0.2	0.6
	TM-As	0.01	0.04	0.06	0.08	<0.1	0.1	0.1	0.1
	subtotal	3.66	3.48	4.36	5.06	6.5	7.4	7.4	7.8
roots	Inorg-As	0.5	0.3	0.5	0.4	0.9	0.6	0.8	0.6
	MM-As	52.0	25.3	16.7	12.4	91.9	53.8	28.2	19.1
	DM-As	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.3
	TM-As	<0.1	0.5	0.8	0.8	<0.2	1.1	1.4	1.2
	subtotal	52.6	26.2	18.1	13.8	93.0	55.7	30.6	21.2
nutrient solution	Inorg-As	0.3	0.7	1.7	3.7	0.5	1.5	2.9	5.7
	MM-As	<0.1	12.8	27.2	30.4	<0.1	27.2	46.0	46.7
	DM-As	<0.1	2.6	5.8	8.5	<0.1	5.5	9.8	13.1
	TM-As	<0.1	1.3	2.0	3.6	<0.1	2.8	3.4	5.5
	subtotal	0.3	17.4[a]	36.7[b]	46.2[c]	0.5	37.0	61.9	71.0
total	Inorg-As	1.0	1.5	2.8	5.1	1.8	3.1	4.7	7.9
	MM-As	55.4	40.9	47.5	46.4	97.8	87.0	80.3	71.3
	DM-As	0.2	2.8	6.0	9.1	0.4	6.0	10.2	14.0
	TM-As	0.01	1.8	2.9	4.5	<0.2	4.0	4.9	6.8
	total	56.6	47.0	59.2	65.1	100	100	100	100

[a] Results are an average of two samples. The values of *a*, *b*, and *c* mean As content in the nutrient solutions obtained for 7, 14, 21 days, respectively [the volumes of nutrient solution obtained for 7 (*a*), 14 (*b*), and 21 (*c*) days were ca. 500, 1000, and 1500 mL, respectively].

The recovery of arsenic from the nutrient solution increased as a function of the harvest time. This increase (71.0–0.5 = 70.5% of recovered As for 21 days) was accompanied with a corresponding decrease (93.0 – 21.2 = 71.8%) in the root tissues. It appears as if the arsenic in root tissues were liable to be exudated into the rhizosphere rather than being translocated to shoots.

In the whole experimental system, the proportion of MM-As was steadily decreased during the course of the experiment. With this decline, the proportions of transformed products such as Inorg-As, DM-As, and TM-As species were increased; the sum of these products accounted for 28.7% (7.9 + 14.0 + 6.8) of the recovered As from the experimental system at 21 days {corresponded to 3.7% [18.7 (5.1 + 9.1 + 4.5) μg of As] of the originally applied As (500 μg)}.

Algae and/or other microorganisms accidentally contaminated in the nutrient solution or associated with the root surfaces were tested for their abilities to transform the DSMA to methylated/demethylated As compounds. The DSMA (50 μg as arsenic) was added to the nutrient solution which was previously prepared by dipping rice seedlings for one day in order to be contaminated intentionally, and incubated for 7 days under the same condition. DM-As as well as Inorg-As was found, but together both As species accounted for only 0.3% [0.1% (DM-As) + 0.2% (Inorg-As, it may be derived from impurities in standard DSMA)] of the applied As. Hence, it must be concluded that predominant amounts of the methylated and/or demethylated As products found in the experiment for root exudation are produced by rice plant seedlings.

There is little evidence of rupture of the C–As bond of methanearsonates in higher plants. Duble et al. (1969) found less than 0.1% of the ^{14}C applied as DSMA was released as volatile ^{14}C 10 days after the treatment of Coastal Bermuda grass, and they concluded that the C–As bond was stable in the plant. Sachs and Michael (1971) also reported that the C–As bond in ^{14}C–MSMA was not broken in bean leaves. Our data strongly indicate that the C–As bond of methanearsonate is ruptured by rice plant.

Table V. Fractionation of Plant Tissues[a]

plant tissues	chloroform extract	MeOH/water extract	NaOH extract	unextractable residues	total, %
shoot	0.3	72.7	26.5	0.5	100
root	0.2	97.7	2.1	0.1	100

[a] Results are an average of two samples.

There have been no reports on the methylation activity of organo arsenicals such as methanearsonates in higher plants. Only a few studies on the methylation of inorganic arsenic have been reported. Pyles and Woolson (1982) found methanearsonic acid in the broccoli, lettuce, potato, and swiss chard grown in silt loam soil treated with arsenic acid. However, it is unclear whether the methylated As compounds are produced in the plant, in the soil, or in both. Nissen and Benson (1982) reported that methanearsonic acid, methanearsinic acid, and dimethylarsinic acid were detected in the metabolic studies of inorganic arsenic in higher plants (tomato, corn, melon, and pea seedlings). However, such methylation products were not observed in the nutrient-sufficient plants but in the P- (and N-)-deficient plants.

Fractionation of Plant Tissues. The rice seedlings that were exposed to DSMA for 7 days were extracted successively with several solvents and were fractionated to chloroform (C) soluble, methanol/water (M/W) soluble, aqueous NaOH soluble, and unextractable residue. The distribution of arsenic in these fractions is shown in Table V. Methanol–water–chloroform have extracted a major portion accounting for three-fourths of the arsenic in the shoot tissues. Aqueous NaOH removed any remaining arsenic, leaving a very trace amount of the arsenic in the shoot residue. The roots yielded nearly all of their As to the M/W/C soluble extract. Moreover, very little As was partitioned from the M/W with chloroform which removes the nonpolar (lipid) metabolites; the arsenic in the chloroform accounted for one-two hundreth (shoots) ~one-five hundreth (roots) of the M/W soluble As from the plant tissues. Since formation of lipid soluble As compounds

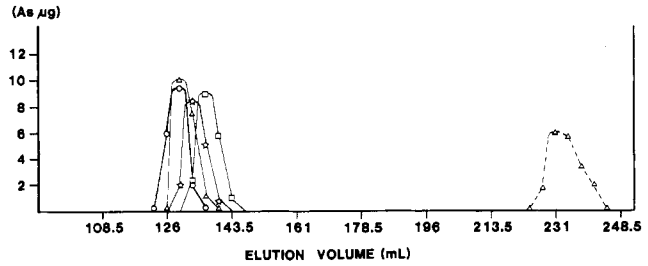

Figure 1. Gel permeation chromatograms of arsenic from standard samples. (O–O) Disodium methanearsonate. (□–□) Dimethylarsinic acid. (☆–☆) Trimethylarsine oxide. (△–△) Arsenate. (△--△) Arsenite.

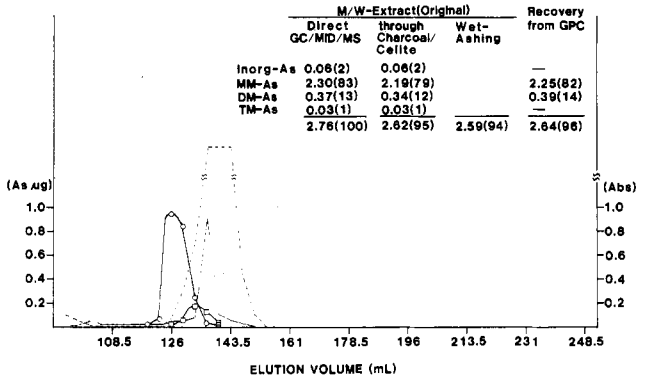

Figure 2. Gel permeation chromatograms of arsenic from methanol/water shoot extracts of DSMA-treated plants. (O–O) Monomethylarsenic (MM-As). (□–□) Dimethylarsenic (DM-As). (–) Amino acid. (--) Sugar.

(soluble in chloroform) from inorganic arsenic by marine phytoplankton (Lund, 1972; Irgolic et al., 1977; Bottino et al., 1978; Andreae & Klumpp, 1979; Sanders & Windom, 1980; Cooney, 1981; Benson & Cooney, 1981; Wrench & Addison, 1981; Klumpp & Peterson, 1981), freshwater algae (Baker et al., 1983), and freshwater plant (Nissen & Benson, 1982) has been reported, it is possible that As metabolism in rice plant is remarkably different from those in the above lower plants.

Characterization of Arsenic in M/W Soluble Extract. The M/W soluble extracts of the shoots and roots of the rice seedlings that were treated with a single dose of DSMA for 7 days were subjected to the GPC analysis. The GPC fractions were analyzed separately by the GFAA and the GC/MID/MS–HG/HCT method, and the results are shown in Figures 2 and 3. Detailed GC/MID/MS–HG/HCT analyses of arsenic in the M/W soluble extracts before passing through the GPC column, in the extracts after passing through charcoal/Celite filter, and in acid digestion (wet ashing) samples of the extracts are also shown in the same figures.

In the shoots, a single peak of MM-As and another single peak of DM-As, which accounted for 82% and 14% of the As originally present in the extract, respectively, were observed (Figure 2). These As peaks were partly overlapped by sugar or amino acid/protein peaks. However, the elution volume of the MM-As and DM-As were identical with that of the corresponding authentic standards as DSMA and DMAA (Figure 1), respectively. And also, charcoal/Celite adsorbable As in the extract before passing through the GPC column was only a small amount (5%) of the As originally present in the extract (see table in Figure 2). These results may be taken to indicate that the two As compounds in the M/W soluble extracts of the shoots are nonconjugated methanearsonate and dimethylarsinate, respectively.

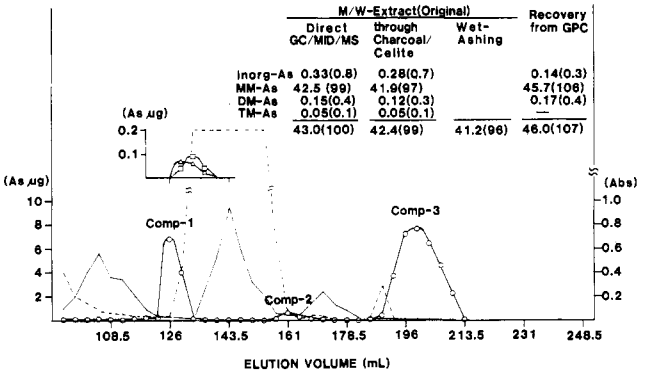

Figure 3. Gel permeation chromatograms of arsenic from methanol/water root extracts of DSMA-treated plants. (O–O) Monomethylarsenic (MM-As). (□–□) Dimethylarsenic (DM-As). (△–△) Inorganic arsenic (Inorg-As). (–) Amino acid. (--) Sugar.

Table VI. TLC Pattern of Arsenic from Standard Samples and GPC Elution Samples of M/W Root Extracts on TLC by Solvent System A [Ethyl Acetate–Acetic Acid–Water (3:2:1)]

	standards				root extracts (μg As)		
cm[a]	As(5)	As(3)	DSMA	DMAA	1	2	3
0–1					b	b	b
1–2					b	b	b
2–3					b	b	b
3–4		+++			b	b	b
4–5					b	b	b
5–6					b	b	b
6–7					b	b	b
7–8	+++				b	b	b
8–9					b	b	b
9–10					0.3	0.1	0.4
11–12			+++		7.5	2.2	5.8
12–13					0.1	b	b
13–14					b	b	b
14–15				+++	b	b	b
15–16					b	b	b

[a] Distances from origin (cm). [b] <0.1 μg of As. As(5) sodium arsenate. As(3) sodium arsenite.

In the roots, three separated peaks (compounds 1, 2, and 3 in Figure 3) of arsenic associated with MM-As were observed, other than a single peak of Inorg-As and another single peak of DM-As. The Inorg-As and DM-As, accounting for 0.3 and 0.4% of the As originally present in the extract (table in Figure 3), could be interpreted as noncomplexed arsenate and dimethylarsinate, respectively, from the results having the same elution pattern on GPC as that of corresponding standards of arsenate and DMAA (Figure 1).

The MM-As (compound 1 in Figure 3) with an elution volume of 115~130 mL, which accounted for 26% of the As originally present in the extract, was partly overlapped by sugar and amino acid/protein of plant materials. However, the elution volumes of both the compound 1 and standard DSMA were identical. The GPC fractions containing the arsenic of compound 1 were combined and were further characterized by the TLC method. In both solvent systems (Tables VI and VII), most of the MM-As was recovered at *Rf* values [0.72, solvent A (ethyl acetate–acetic acid–water); 0.34, solvent B (phenol–water–ammonium hydroxide)] corresponding almost exactly to expected values of the standard DSMA. Therefore, it seems probable that the compound 1 is a nonconjugated methanearsonate.

The MM-As (compound 2 in Figure 3) with an elution volume of 158~168 mL (accounting for 2.3% of the As

Table VII. TLC Pattern of Arsenic from Standard Samples and GPC Elution Samples of M/W Root Extracts on TLC by Solvent System B [Phenol-Water-Ammonium Hydroxide (400:99:1)]

	standards				root extracts (μg As)		
cm[a]	As(5)	As(3)	DSMA	DMAA	1	2	3
0–1					b	0.2	b
1–2	+++				b	0.2	b
2–3					b	0.5	b
3–4					b	0.6	b
4–5		+++			0.3	0.5	b
5–6			+++		7.7	0.3	1.8
6–7					b		0.5
7–8					b		0.1
8–9					b		0.2
9–10					b		0.2
10–11					b		0.2
11–12					b		0.3
12–13				+	b		0.3
13–14				++	b		1.4
14–15				+	b		0.8
15–16					b		b

[a] Distances from origin (cm). [b] <0.1 μg of As. As(5) sodium arsenate. As(3) sodium arsenite.

originally present in the extract) was different from DSMA standard in its elution volume. Furthermore, the compound 2 was eluted together with a yellow color component. With solvent system B (Table VII) on TLC, the compound 2 had a broad *Rf* value of 0~0.34 compared to a narrow band of 0.34 for the standard DSMA, and the major portion of the arsenic was present in *Rf* 0.22. Also, the wide band of compound 2 on TLC was exactly overlapped by that of the yellow color component. These results suggest that the compound 2 is a conjugate of the MM-As with a certain plant material. A finding of the As complex in plants treated with methanearsonates have been reported in Johnson grass (Sckerl and Francs, 1969), purple nutsedge (Duble et al., 1969), bean (Sachs and Michael, 1971), and wheat (Domir et al., 1976).

The MM-As (compound 3 in Figure 3) with an elution volume of 186~214 mL, which accounted for 75% of the As originally present in the extract, was greatly different from the standard DSMA in the elution volume. This compound was more similar to sodium arsenite than not otherwise there would be a change in the GPC pattern (Figure 1). The compound 3 seemed to be a free form, since the peak of compound 3 on GPC was little overlapped by the peaks of sugar or amino acid/protein, and since there were only trace amounts of charcoal/Celite adsorbable As in the original extract (see table in Figure 3). Compound 3 was further characterized by TLC. With the solvent system A (Table VI), the *Rf* values of both compound 3 and standard DSMA were identical. With the solvent system B (Table VII), compound 3 (MM-As) was separated into two major portions at *Rf* 0.34 and 0.84 (which corresponded to that of standard DSMA and DMAA, respectively), and a minor portion at a broad *Rf* value of 0.34~0.84. These results may be taken indicate that compound 3 is a less polar compound than methanearsonate and is easily converted to methanearsonate on TLC, especially with an acid solvent such as system A. Additional information about the character of this compound was obtained incidentally; compound 3 was decomposed into Inorg-As in a weak acid solution (e.g., acid concentration required for hydride generation reaction) after exposure to light. And the Inorg-As was identified as arsenite by TLC analysis. In consideration of these facts, it seems probable that compound 3 is reduced methanearsonate (red-MA).

DSMA was added to MeOH/water extracts of untreated plants (root tissues) and was then prepared in the same manner as the treated plants. However, only a single peak of MM-As which had a similar elution pattern as standard DSMA was observed in the GPC analysis. So that the red-MA found is not artifact formed during analytical preparation steps.

A similar reduced As compound has been observed by Nissen and Benson (1982) in a metabolic study of inorganic arsenic in a higher plant; a monomethyl As compound which was different from methanearsonate was detected in a paper chromatogram of the plant extract, and it was named as methanearsinic acid. These two As compounds (red-MA and methanearsinic acid), discovered separately, will probably be the same in structure, because both As compounds are liable to be oxidized to methanearsonate by drying (on TLC) and because both As compounds have a greater mobility than methanearsonate on TLC.

One other character of the red-MA was observed in our studies. As mentioned above, the red-MA was easily cleaved into arsenite. Also, this red-MA was completely converted (oxidized) to methanearsonate by filtration with active charcoal–Celite which was frequently used for cleanup of crude extracts from various samples. Therefore, special attention should be given to analysis of this As compound.

The red-MA comprised 75% of the arsenic in the root extract. However, the found values were variable depending upon extractive conditions presumably due to instability. Nearly all of the arsenic in the root will probably be composed of this red-MA compound. Hence, it follows that the major metabolic pathway of DSMA was reduction. Reduction (as major pathway) of arsenate to arsenite has been observed in higher plants (Nissen and Benson, 1982). These observations suggest that reduction of methanearsonates as well as arsenate may be common metabolic reaction to most higher plants including rice plants.

Registry No. MA, 124-58-3; MM-As, 83636-33-3; TM-As, 593-88-4; red MA, 593-58-8; DM-As, 83636-34-4; As, 7440-38-2.

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Degradation of Dimethoate and Pirimicarb in Asparagus

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Dimethoate at 0.25, 0.50, 1.0, and 1.12 kg AI/ha, and pirimicarb at 0.125, 0.25, and 0.50 kg AI/ha were applied as foliar sprays to control the European asparagus aphid, *Brachycolus asparagi*. Partial conversion of dimethoate to dimethoxon, and pirimicarb to (methylamino)pirimicarb and/or formyl(methylamino)pirimicarb occurred in the foliage (fern) as soon as 6–12 h after application. After applications the total residues of both compounds, including their toxic metabolites, decreased by about 90% in 7 days but at a slower rate thereafter. Only traces of pirimicarb (<0.01 ppm) remained 31 days after applications at 0.50 kg AI/ha, and up to 0.03 ppm of dimethoate remained 38 days after applications at 0.50 and 1.0 kg AI/ha. No residue was found above the limit of detection of 0.002 ppm in any asparagus spears following applications of dimethoate and pirimicarb for aphid control.

INTRODUCTION

The asparagus aphid, *Brachycolus asparagi* Mordvilko, is native to Europe and the Mediterranean region (Plant Pest Control Division, USDA, 1970) and was first found in North America in New York in 1969 (Leonard, 1971). Forbes (1981) identified this aphid from asparagus, *A. officinalis altilis*, in the Okanagan Valley of British Columbia in 1979 and suggested that a toxin injected by the aphid in feeding causes severe deformity (bonsai-type or witches' broom-type growth) and even death of the plants. Outbreaks of this pest in the Okanagan Valley of British Columbia and in western Washington state have been observed since 1979 and have caused considerable economic damage. Currently malathion, carbaryl, and mevinphos are the only insecticides registered for use on asparagus in Canada. A drawback to the use of these chemicals, however, is their short residual activity which requires that several sprays be applied during the growing season. This is undesirable since repeated applications in mature asparagus could cause physical damage to the tall foliage or ferns from tractor-mounted boom sprayers. Moreover these are broad-spectrum chemicals and could be detrimental to beneficial insects such as bees and aphid predators.

In 1982 field trials were conducted in Coldstream and Armstrong, British Columbia, to evaluate the effectiveness of dimethoate [*O,O*-dimethyl *S*-[(methylcarbamoyl)-methyl] phosphorodithioate] and pirimicarb (2-(dimethylamino)-5,6-dimethylpyrimidin-4-yl dimethylcarbamate) against the asparagus aphid, and to observe their degradation in asparagus plantings. These two aphicides were evaluated once more in 1983 in Summerland, British Columbia, and the results from both seasons are presented here.

MATERIALS AND METHODS

1982 Field Studies at Coldstream. Postharvest spray trials with dimethoate and pirimicarb were conducted in a 1-year-old stand of asparagus (commercial variety Mary Washington) at Coldstream, British Columbia. This stand was considered immature since the first marketable spears were not expected until 1983 or 1984. The experimental plots were 6 m long and 2 rows wide with treatments replicated 4 times in a randomized block design. End-to-end plots within blocks were separated by 1-m buffer strips of asparagus and adjacent rows were 1.5 m apart. A Solo manual backpack sprayer (Solo Leinkleinmotoren, Germany) was used to apply foliar sprays of dimethoate (Cygon 4E, an emulsifiable concentrate, at 1.12 kg AI/ha) and pirimicarb (Pirimor 50 WP, a wettable powder, at 0.50 kg AI/ha) in water at the rate of 2 L per 12 m row on July 24. Dimethoate was reapplied at the same rate on Sept 8 and pirimicarb on Aug 25. At intervals after spraying two plants from each treatment and the control plots were randomly selected and a sprig from the bottom, middle, and top of each plant was removed to form a composite sample for residue analysis. These samples were considered to be representative as the experimental plots were small and the insecticides were applied manually with a backpack sprayer which ensured even distribution of spray mists among plants. In the following spring, composite samples consisting of 10 randomly selected marketable spears were taken to ascertain if any residues had been carried over the winter and into the next crop.

1982 Field Studies at Armstrong. Postharvest spray trials similar to those at Coldstream were conducted in a 17-year-old stand of asparagus (commercial variety Mary Washington) at Armstrong, British Columbia. The treatment plots were 50 m long and 3 rows wide with treatments replicated 4 times in a randomized block design. Adjacent rows were 1.5 m apart. A tractor-mounted boom sprayer operating at 4.2 kg/cm^2 was used to apply dimethoate at 1.12 kg AI/ha and pirimicarb at 0.50 kg AI/ha at the rate of 675 L/ha on July 29. The application of dimethoate was repeated on Sept 17 and of pirimicarb on Sept 3.

1983 Field Studies at Summerland. Postharvest spray trials were again conducted in a 3-year-old stand of asparagus (commercial variety Mary Washington) at the Agriculture Canada Research Station in Summerland, British Columbia. Treatment plots were 4.5 m long and one row wide with treatments replicated 6 times in a randomized block design. End-to-end plots were separated

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