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Quantitation and Characterization of Arsenic Compounds in Vegetables Grown in Arsenic Acid Treated Soil

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Several vegetable crops were field grown in Matapeake silt loam soil treated with 100 ppm of arsenic as arsenic acid. Total arsenic (As) contents of the edible plant parts were generally low, ranging from 3.00 ppm for potato peel to trace quantities in cabbage and corn. The highest As concentrations in broccoli, cabbage, corn, green beans, lettuce, and potato peel were found in the methanol/water phase. Arsenic in the nonextractable or chloroform phases was predominant in beets, potato flesh, swiss chard, and tomato. Methylarsonic acid and/or arsenate were identified in the methanol/water phases of broccoli, lettuce, potato flesh, potato peel, and swiss chard. The quantity of arsenate and methylarsonic acid recovered by arsine generation was lower than the total As present in the methanol/water phases. However, digestion of the methanol/water phases in hot 2.0 N NaOH yielded total recovery as arsenate. Hence, most of the arsenic contained in the methanol/water phases appears to be a complex organic arsenic compound.

Arsenic is a ubiquitous element, and although trace quantities of arsenic occur throughout the lithosphere, concentrations may be significantly higher in certain locations as a result of weathering processes and anthropogenic activities such as metal refining and pesticide use (Schroeder and Balassa, 1966). Arsenic is rarely found as the free element in soil but is frequently present as a component of sulfidic minerals ("Arsenic: Medical and Biological Effects of Environmental Pollutants", 1977). Arsenates are naturally occurring in oxygenated environments, while arsenite is probably the dominant form under moderately reducing conditions, for instance, in flooded soils ("Arsenic: Medical and Biological Effects of Environmental Pollutants", 1977; Walsh and Keeney, 1975). Inorganic arsenic compounds found in the environment may be converted into organic arsenic compounds by microorganisms. For example, Challenger et al. (1933) found that arsenic trioxide is methylated to trimethylarsine by *Scopulariopsis brevicaule*.

Traditionally, concentrations of arsenic compounds found in environmental samples have been reported as total arsenic following digestion (Chapman, 1926). However, the toxicological properties of arsenic compounds are dependent on the chemical nature of the arsenic compound, as well as the quantity present. Therefore, it is essential to discern the various arsenic compounds present in environmental samples before rational decisions can be made regarding potential health problems. Although other methods have been reported recently (Braman et al., 1977; Lakso et al., 1979; Lunde, 1973) the advent of an interface to couple a liquid chromatograph to a graphite-furnace atomic absorption spectrophotometer allows one to separate and detect low concentrations of many arsenic compounds in both model and natural systems (Brinkman et al., 1977; Stockman and Irgolic, 1979).

Arsenobetaine and *O*-phosphatidyltrimethylarsoniumlactic acid (sic) have been found naturally occurring in lobster and algae, respectively, but information regarding the chemical nature of As compounds formed upon incorporation of arsenic into terrestrial plants is rare (Edmond et al., 1977; Cooney et al., 1978). This is possibly due to the low concentrations of arsenic sorbed by plants (typically less than 1 ppm) as compared with marine organisms. However, in this study, residues were characterized by solubility in methanol, water, and chloroform. The selectivity and sensitivity of liquid chromatography coupled with graphite-furnace atomic absorption spectroscopy were employed to determine the presence or absence of arsenate, arsenite, methylarsonic acid (MA), and dimethylarsinic acid (CA) in the methanol/water extracts of several vegetables. Residues were also characterized for solubility in chloroform and nonextractability.

EXPERIMENTAL SECTION

Reagents. All chemicals were reagent grade unless otherwise specified. Vineland Chemical Co. and The Ansul Chemical Co. supplied methylarsonic acid (97%), and dimethylarsinic acid (92%), respectively. Antifoam agent was purchased from Hodag Chemical Co., Chicago, IL, and a lecture bottle of trimethylarsine was obtained from Ventron-Alfa Products. Trimethylarsine oxide was prepared by combining stoichiometric quantities of trimethylarsine and 30% hydrogen peroxide and rotary evaporating the resulting solution to dryness at 80 °C.

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Instrumentation. The high-pressure liquid chromatographic system (HPLC) consisted of two Water Associates, Inc., solvent delivery systems (Model 6000A and Model 45, as the A and B pumps, respectively), a Model 660 solvent programmer, a WISP 710A automatic injection system, and an RCM-100 radial compression module. The radial compression module contained a cartridge packed with Bio-Rad Aminex A-27 anion-exchange resin. The graphite-furnace atomic absorption detection system (GFAA) consisted of a Perkin-Elmer 4000 atomic absorption spectrophotometer equipped with an HGA-400 graphite furnace, a PRS-10 printer, an automatic AS-1 sampling system, and a Model 76 recorder. The HPLC column output was connected to a Teflon flow-through sampling cup having the same exterior dimensions as a normal AS-1 sample cup. The interior dimensions were the same diameter, initially, but were tapered more sharply in the bottom $^1/_4$ of the cup. The volume of liquid in the Teflon cup was maintained at 50 μL by an overflow needle placed through the side of the cup. The inlet hole was drilled through the bottom to fit the plastic delivery tube from the HPLC.

The HPLC was programmed to initially deliver 1.2 mL/min of water, changing over a period of 15 min by program curve 9 to 100% 0.2 M ammonium carbonate. (As the age of the column increases, a program curve of 7 and an increased gradient period of 25 min were necessary.) The WISP 710A automatic injecting system was programmed for a run time of 30 min and an equilibrium delay of 12 min. The parameters for the HGA-400 graphite furnace were 7 s at 160 °C with 1-s ramp time for drying, 7 s at 1250 °C with 1-s ramp time for ashing, and 5 s at 2400 °C with 1-s ramp time for atomization. The atomic absorption spectrophotometer was programmed to monitor the As concentrations of the samples at 193.7 nm for 5 s and at a low slit height of 0.2 nm. Reading the peak height with a 20× scale expansion was normally used.

Arsenic triiodide was characterized by electron-impact mass spectrometry using a Du Pont 21-491 B mass spectrometer coupled to a Du Pont 21-094 data system. Methyldiiodoarsine was characterized on a Finnegan 4000 gas chromatograph–mass spectrometer (GC–MS). Both mass spectrometers were calibrated with a perfluorokerosene standard. Arsenic triiodide was introduced in the mass spectrometer by a heated probe (155 °C). Methyldiiodoarsine was injected on a 6-ft, 3% Dexsil 300 capillary column at 90 °C. The temperature of the column was increased 10 °C/min to 230 °C. The retention time of methyldiiodoarsine was 4.4 min.

Growth and Preparation of Vegetables. Several varieties of garden vegetables (beets, broccoli, cabbage, corn, green beans, lettuce, potatoes, swiss chard, and tomatoes) were grown in soil (Matapeake silt loam) treated with 100 ppm of arsenic applied as arsenic acid. At maturity, the edible portion of each crop was harvested and cleaned in a manner customary for food preparation. For instance, the outer leaves were removed from the cabbage and lettuce heads. The heads were then rinsed with copious quantities of water.

The plants were preserved prior to analysis. Beets, broccoli, green beans, and swiss chard were blanched and frozen. Similarly, the corn was blanched, removed from the cob, and frozen. The cabbage, lettuce, and tomatoes were dried for 24 h at 68 °C in an air circulating oven and then refrigerated.

Extraction and Total Arsenic Determination. Plant material was extracted by employing the procedure developed by Bligh and Dyer (1959), which utilizes 2, 4, and 0.5 mL of $CH_3OH/H_2O/CHCl_3$ per g of plant material, respectively. Vegetables that were not dried as a preserving measure were dried under the same conditions as the cabbage, lettuce, and tomatoes before extraction. The methanol/water phases were extracted with two additional 50-mL aliquots of chloroform. Aliquots of the methanol–water phase, the chloroform phase, and plant residue (nonextractable residual cellular material) were digested in a solution of nitric, perchloric, and sulfuric acids (Small and McCants, 1961). An aliquot of each digested sample was added to the AS-1 sample cup along with 20 μL of nickel nitrate and 1.0 mL of water and analyzed by the method of standard additions on the GFAA. The arsenic in the chloroform phase or nonextracted cellular material was not characterized further.

Arsenic Species in CH_3OH/H_2O Extract. Antifoam (4 mL) was added to an aliquot of the methanol/water phases of broccoli, lettuce, potato flesh, potato peel, and swiss chard. These aliquots were rotary evaporated in a 500-mL Florence flask at 54 °C to a brown oil. Next 0.9 M potassium iodide solution (20 mL) was swirled with the oil for 25 s and then 5.5 M HCl (200 mL) was added to the flask, which was swirled again until the brown material dissolved. Granular zinc (10 g) was added to the solution, and the resultant gases were dispersed, with moderate heating, into an aqueous trapping solution (Woolson et al., 1971) of 0.05 M ammonium iodine and 0.01 M iodine (35 mL). (**Caution**: The mixture forms ammonium triiodide, which may become explosive upon drying. However, we did not experience any difficulties with the procedure.) The trapping solution was rotary evaporated to dryness at 54 °C and the residue was dissolved in 2.0 M ammonium hydroxide (4–5 mL). An aliquot of the ammonium hydroxide solution was injected on the Bio-Rad Aminex A-27 anion-exchange resin for analysis. The remainder of the sample was added to a 100-mL pear flask and rotary evaporated. Hydroiodic acid (10 mL) was slowly added to the residue from a dropping funnel under nitrogen. The solution was stirred for 2 h and extracted with hexane (20 mL). The phases were separated and the hexane phase was reduced to approximately 0.1 mL. The hexane phase was analyzed by mass spectrometry.

Arsenic Species in Acidified CH_3OH/H_2O Extract. In order to determine if arsenite was present, we made the extract 8.0 M in HCl and extracted the acidic solution with benzene. Arsenite is extracted while arsenate is not (Tagawa, 1980).

Arsenic Species in Sodium Hydroxide Digest of CH_3OH/H_2O Extract. Aliquots of the methanol/water phases of broccoli, lettuce, potato flesh, potato peel, and swiss chard were added to a 500-mL Florence flask along with antifoam (4 mL). Each sample was rotary evaporated at 54 °C and the resulting oil was dissolved in 2.0 M sodium hydroxide (75 mL). The solution was heated gently for 17 h. After digestion, 2.0 M HCl (75 mL) and antifoam (4 mL) were added to the sodium hydroxide digest. The As compounds were characterized by generating and trapping the arsines as described above. A flow chart of the analysis scheme is presented in Figure 1.

RESULTS AND DISCUSSION

The total arsenic content of the vegetables grown on As-treated soil was low, ranging from 3.00 ppm for potato peel to trace amounts (<0.01 ppm) for corn and cabbage on a dry weight basis (Table I). These values are similar with values reported by other workers (Woolson, 1982). Corn and cabbage contained the least amount of As. Coefficients of variation ranged from 7 to 44% and averaged about 20%.

Table I. Arsenic Content of Vegetables (ppm of As, Dry Weight)

vegetable	CH_3OH/H_2O phase total	CH_3OH/H_2O phase speciated +5	CH_3OH/H_2O phase speciated MAA	$CHCl_3$ phase	nonextractable	total	normal levels[d]
broccoli	0.40	0.01	ND[a]	ND[b]	tr[b,c]	0.40	
beet	0.10			0.14	0.62	0.86	0.34
cabbage	tr[b]			tr[b]	tr[b]	tr	<0.1-0.4
corn	tr[b]			tr[b]	tr[b]	tr	0.01-0.05
green been	0.07			0.05	0.02	0.14	0.01-0.40
lettuce	0.41	0.09	tr	tr[b]	0.14	0.55	0.12
potato flesh	0.37	0.10	tr	0.11	0.70	1.19	0.01-0.2
potato peel	2.70	1.08	tr	0.25	0.20	3.00	0.02-2.4
swiss chard	0.17	0.08	0.02	0.01	0.79	1.00	0.01
tomato	tr[b]			0.11	tr[b]	0.11	0.01-0.08

[a] ND = not detectable. [b] KI reduced and benzene extracted. [c] tr = trace = detectable but less than 0.01 ppm. [d] Woolson (1982).

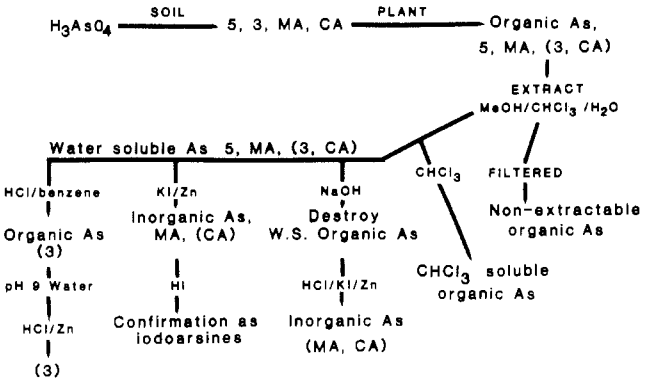

Figure 1. Flow chart of experimental scheme. Arsenic species are listed in order of decreasing concentrations. Species in parentheses were not detected.

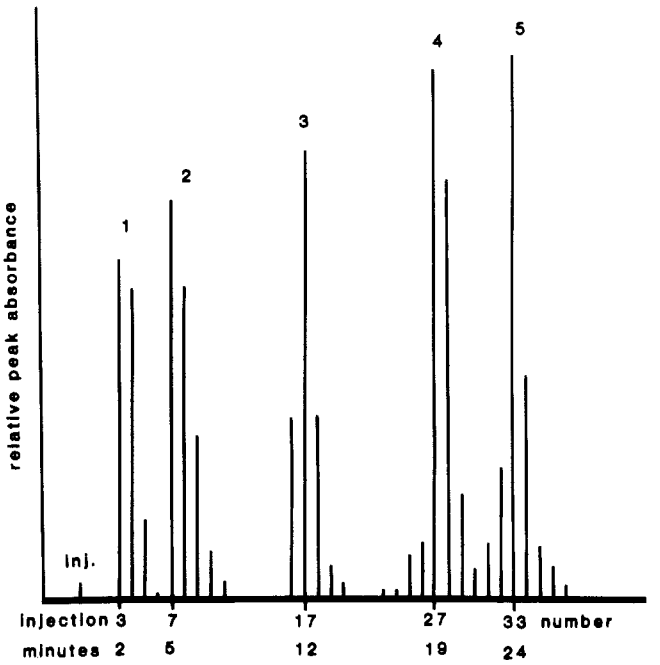

Figure 2. Elution pattern of five arsenicals from a radial compression column packed with Bio-Rad Aminex A-27 anion-exchange resin. The solvent program begins with water and becomes 100% 0.2 M ammonium carbonate after 15 min on a convex program. Peaks are as follows: 1, trimethylarsine oxide; 2, arsenite; 3, dimethylarsinate (CA); 4, methylarsonate (MA); 5, arsenate. Quantitation is by summation of furnace firings under each peak.

As shown in Table I, broccoli, cabbage, green beans, lettuce, and potato peel yielded most of their As to the methanol/water phase after extraction. Arsenic compounds expected to be found in this phase include the inorganic arsenic compounds, arsenate and arsenite, and the organoarsenical compounds, methylarsonic acid, dimethylarsinic acid, and trimethylarsine oxide. Upon reduction with hydrogen gas, all of these compounds form volatile arsines that may be trapped by an oxidizing solution.

A modification in the procedure reported by Woolson and Aharonson (1980) was necessary to separate arsenate, arsenite, methylarsonic acid, and dimethylarsinic acid in this study. Although the Dionex column yielded good selectivity and reproducibility for arsenate, methylarsonic acid (MA), and dimethylarsinic acid (CA) in soil extracts, it failed to distinguish between arsenite and trimethylarsine oxide. Moreover, the retention time of CA was extremely sensitive to the salt content of the matrix. Thus, for plant extracts, CA, arsenite, and trimethylarsine oxide had the same retention times. Some of these problems were alleviated when radial compression techniques were used with the Aminex A-27 resin. The combination of the radial compression technique, Aminex A-27 resin, and methanol-free solvents permitted the separation (Figure 2) of arsenite (peak 2), trimethylarsine oxide (peak 1), and CA (peak 3), as well as arsenate (peak 5) and MA (peak 4). Moreover, the radial compression column operated at a lower pump pressure. Unfortunately, as the salt content of the matrix reached 3–4%, CA still shortened its retention time.

The trapping solutions, containing the oxidized arsines, were analyzed by using the radial compression technique. Only arsenate was recovered from broccoli, whereas both arsenate and methylarsonic acid were detected in the potato peel, potato flesh, lettuce, and swiss chard. However, not all of the arsenic in the methanol/water phase was present as arsenate and methylarsonic acid. Only 40, 28, 3, 20, and 48% of the total As in the methanol/water phases of potato peel, potato flesh, broccoli, lettuce, and swiss chard, respectively, were recovered as arsenate. Methylarsonic acid accounted for 11% of the As in the methanol/water phase of swiss chard, while the potato peel, potato flesh, and lettuce yielded only trace quantities of methylarsonic acid. The As that was not recovered in the trap would therefore seem to be incorporated into a complex organic arsenic compound(s) that does (do) not readily lend its (their) arsenic to hydrogen reduction. However, upon digestion of an aliquot of each of the methanol/water phases with 2.0 M sodium hydroxide and repetition of the arsine generation, all the arsenic in the methanol/water phases was recovered as arsenate and/or MA. These results are in contrast to the CA obtained by

Table II. Electron-Impact MS Fragments of Arsenic Triiodide

fragments	m/e	% relative abundance
AsI_3^+	456	100
AsI_2^+	329	69
As_4^+	300	6
I_2^+	254	5
AsI^+	202	39
I^+	127	30
As^+	75	9

Crecelius (1977) when the urine of crab meat consumers was digested in 2.0 M sodium hydroxide.

Although the simple organic As compounds are recovered in their original form from the trapping solution, arsenate and arsenite are indistinguishable once both compounds are reduced. Since only arsenite is extracted into the benzene under acid conditions with no KI added and arsenate, methylarsonic acid, and dimethylarsinic acid remain in the aqueous phase (Tagawa, 1980), arsenite can be speciated in the original extract.

However, when the As extracted from the acidified methanol/water phases by benzene was analyzed by HPLC, both MA and arsenate were present. Since only arsenite is extracted from water under such conditions, it is possible that the plant material, in contrast to the aqueous standards, produced a salting out or paired ion effect and drove the acidified arsenate and MA into the benzene phase. Moreover, the benzene extracted colored material along with the As from the acidified methanol/water phase. In order to remove the colored material, a preacidification extraction of the methanol/water phase with benzene was conducted. Both colored material and As were recovered in the benzene phase. Extraction of the acidified preextracted methanol/water phase with benzene once again gave both colored material and As in the benzene phase. In spite of these problems, no arsenite was found in the methanol/water phases of any crop.

Arsenate and MA were initially identified in the methanol/water phase extracts by chromatographic techniques. Further evidence of their identity was obtained by forming the respective iodoarsines and analyzing them by electron-impact mass spectrometry. A standard sample of arsenic triiodide (Table II) produced a mass spectrum containing seven peaks at m/e 456, 329, 300, 202, 254, 127, and 75. The sample from the methanol/water extract produced the same fragmentation pattern when treated with hydriodic acid and analyzed by electron-impact mass spectrometry.

Mass spectrometry was also used to confirm the presence of MA in a like manner (Table III). However, methyldiiodoarsine generated from the methanol/water extracts could not be detected by mass spectrometry.

Although the plant material was washed extensively prior to extraction, the arsenate may arise as a result of contamination from the soil. If this is the case, then higher concentrations of arsenate should be present in roots taken from or leaves growing near the soil and lesser amounts of arsenate should appear in plant material growing higher above the soil surface. This trend can be observed in the potato and broccoli. The potato peel was in direct contact with the soil and hence contains the most arsenate. On the other hand, broccoli grew above the soil surface and the lower arsenic concentrations may reflect lower contamination from the soil. If the arsenic found in the broccoli methanol/water extract was a result of arsenate contamination from the soil, then all of the arsenic should be recovered by the arsine generation process. However,

Table III. Electron-Impact MS Fragments of Methyldiiodoarsine

fragments	m/e	% relative abundance
$CH_3AsI_2^+$	344	73
AsI_2^+	329	16
CH_3AsI^+	217	100
AsI^+	202	35
I^+	127	59
CH_3As^+	90	14
CH_2As^+	89	44
$CHAs^+$	88	22
CAs^+	87	3
As^+	75	18

only 3% of the arsenic was amenable to arsine formation. Furthermore, all of the arsenic present was recovered by digesting the extract with sodium hydroxide. This indicates that although some contamination may have occurred, arsenic compounds found in the methanol/water phase of broccoli are mainly complex organic arsenic compounds. Only after the organic arsenical complex is destroyed by sodium hydroxide digestion is the resulting inorganic arsenic amenable to arsine formation.

A source of this arsenate might be organic arsenic compounds in which arsenic replaces phosphorus in an arsenic analogue of a phosphate ester. In contrast to phosphate esters, arsenate esters are extremely unstable hydrolytically. During the extraction process, water from the extracting solution would undoubtedly hydrolyze any arsenate esters to inorganic arsenate and the inorganic arsenate would be recovered by arsine generation, which is in contrast to the experimental results. Therefore, the arsenic compounds formed by plants may be similar to water-soluble organoarsenicals isolated from marine organisms.

Traces of methylarsonic acid found in the methanol/water phase extracts were thought to have arisen from the methylation of the arsenic acid by soil microbes. Sachs and Michael found that Black Valentine beans absorb monosodium methylarsonate from soil (Sachs and Michael, 1971). Therefore, methylarsonic acid formed in the soil might be absorbed by the plants. In addition, any contamination of the plant material from the soil would include both arsenate and methylarsonic acid. Thus at the end of the growing season (approximately 6 months after the soil was treated with arsenic acid) the top 13 cm of soil from the garden plot was sampled and analyzed for arsenic compounds by techniques developed by Iadevaia et al. (1980). Although 65 ppm of arsenate was found in the soil sample, methylarsonic acid was not detected (detection limit 0.40 ppm).

Mammals, including man, have been shown to methylate arsenic (Crecelius, 1977; Tam et al., 1979). Since methylated As compounds are generally less toxic than inorganic arsenic compounds, methylation may act as a detoxifying mechanism. Plants may also possess the ability to render inorganic arsenic compounds less toxic by a similar mechanism. Hence, small quantities of methylarsonic acid may be present in the methanol/water phases as a plant metabolite even though it is not detectable in the soil.

The nonpolar lipid material was extracted into the chloroform phase (Table I). Concentrations of As in the chloroform phase ranged from 0.25 ppm for the potato peel to none detectable for broccoli. Only tomato contained higher arsenic concentrations in the chloroform phase than the methanol/water phase. The remainder of the vegetables gave similar values (within experimental error) for both the methanol/water and chloroform phases or much higher values for the methanol/water phases. Hence, the

major As compounds formed by tomatoes appear to be nonpolar lipid material, which is in direct contrast to the chemical nature of the arsenic acid originally applied to the field.

It is possible that the arsenic-containing lipid material in the chloroform decomposes into methanol/water-soluble products. However, if that were the case, the lipid material in tomatoes should decompose at a comparable rate to the lipid material in the other plant extracts if the compounds are similar. Since the ratio of As in the methanol/water phases to As in the chloroform phases is not similar in all the plant extracts, some lipid forms do not decompose to inorganic As.

Levels of As in the residual cellular material (residue) ranged from 0.79 ppm for swiss chard to trace amounts for tomato. Carbohydrate polymers and other large biomolecules that are neither methanol/water nor chloroform soluble are expected to remain in the residue. The nonextractable residue of the beet, corn, lettuce, potato flesh, and swiss chard contained relatively large As concentrations as compared to the nonextractable residue of the other crops.

Comparative residue levels are presented for crops grown on soil not treated with arsenic (Table I). Residues are higher in this study for some crops (lettuce, swiss chard, and potatoes), while for others, residues are not. Root and leaf crops have the highest residues. Residues that are present are primarily organic in nature.

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