Identification of Dimethylated Arsenic by Gas Chromatography–Mass Spectrometry in Blood, Urine, and Feces of Rats Treated with Ferric Methanearsonate

Dimethylarsinate [salt of dimethylarsinic acid ($(CH_3)_2AsO_2H$; DMAA)] was detected as a metabolite in the blood, urine, and feces of male Wistar rats, which had been administered orally with a 100 mg/kg dose of ferric methanearsonate [$(CH_3AsO_3)_3Fe_2$; MAF]. Following separation with inorganic arsenic and methanearsonate [salt of methanearsonic acid ($CH_3AsO_3H_2$; MAA)] by cellulose thin-layer chromatography (TLC), dimethylarsinate was converted to dimethylarsine ($(CH_3)_2AsH_2$) by reduction and identified by gas chromatography–mass spectrometry (GC–MS).

MAF (ferric methanearsonate) is a fungicide widely used for controlling Sheath blight of rice in Japan. Therefore, it is likely that animals are exposed to this compound or its metabolites either directly or indirectly through feedstuffs, and knowledge concerning its fate in animals is of environmental importance.

Information concerning the methylated form of arsenic in the environment has been scant until recently. Braman and Foreback (1973) detected the trace of following arsenic in natural water, bird eggshells, seashells, and human urine: dimethylarsinic acid (DMAA), methylarsonic acid (MAA), arsenate (As^{5+}), and arsenite (As^{3+}). They suggested that DMAA was the major and ubiquitous form of arsenic, and MAA was also present, but in lower concentration in the environment. Edmonds and Francesconi (1977) reported the presence of dimethylated and trimethylated arsenic in marine fauna.

In microorganisms, methylation of arsenic has been generally known (McBride and Wolfe, 1971; Cox and Alexander, 1973, 1974). However, there has been a little evidence that this phenomenon occurs in higher animals. Lakso and Peoples (1975) observed that the methylated form of arsenic was excreted in the urine of cows and dogs treated with inorganic form of arsenic. Organoarsenic compounds were measured by Crecelius (1975) in human urine after drinking inorganic arsenic-rich wine. There has seldom been reports on the methylation of arsenic in mammals except for the above two reports, and even in these reports the methylated form of arsenic compounds in the urine were not identified in detail. Furthermore, no published work is available on the methylation of monomethyl organoarsenic compounds such as MAF or MAA in animals. This paper describes an identification of arsenic metabolite by TLC and GC–MS in the blood, urine, and feces of the rat treated with MAF.

EXPERIMENTAL SECTION

Apparatus. A spectrophotometer (Hitachi 323) was used for characterization of arsenic. Identification of arsenic was performed with a gas chromatograph–mass spectrometer (Shimadzu LKB-9000). The operating conditions of GC–MS are summarized in Table I.

Reagents. Reference compounds were obtained from the following sources: MAF and disodium methanearsonate ($CH_3AsO_3Na_2{\cdot}6H_2O$; DSMA), Kumiai Chemical Co.; arsenic acid (H_3AsO_4) and sodium arsenite ($NaAsO_2$), Kanto Chemical Co.; dimethylarsinic acid (cacodylic acid; DMAA), Nakarai Chemical Co. MAF was 87.7% of purity, and other arsenic compounds were not contained. Glass plate (20 × 20 cm) coated with a 0.25-mm layer of fine cellulose powder was used in thin-layer chromatography. A mixture of active charcoal and Celite (1:3) was washed by 6 N HCl. Silver diethyldithiocarbamate (AgDDC)–pyridine solution was prepared by dissolving 0.5 g of AgDDC in 100 mL of pyridine. All other chemicals were reagent grade and solvents were pesticide residue analysis grade or equivalent.

Table I. Operating Conditions of Gas Chromatograph-Mass Spectrometer

Model	Shimadzu LKB-9000
Ionization	70 eV
Accel. voltage	3400 V
Chamber temp.	270 °C
Column	5% PEG-20 M on Chromosorb 101 (80/100), 2 m × 3 mm glass column
Column Temp.	155 °C
Injection temp.	200 °C
Carrier gas (flow rate)	Helium (30 mL/min)

Treatment of Animals. A male Wistar rat (SPF, weight 290–320 g) was given a single oral dose of MAF at 100 mg/kg. MAF was suspended in water with the aid of 10 mL of sodium carboxymethyl cellulose (CMC; 0.5%) and administered by a stomach tube. Water and feed (Oriental Yeast, MF) were provided ad libitum. Feces and urine were separately collected every day after dosing. After 5 days the animals were anesthetized with nembutal, and the blood samples were collected from the right ventricle.

Extraction and Isolation. The modified procedures of Abe were used. Aliquots of the samples (1–5 g) were mixed with 20 mL of 0.2 N NaOH solution and shaked for 1 h. The mixture was acidified with 4 mL of 1 N H_2SO_4, and 5–10 g of a mixture of active charcoal and Celite was added. The mixture was filtered through a paper under suction and residue was washed with 50–100 mL of water. The combined filtrate was condensed to about 40 mL under vacuum and filled up to 50 mL with water. To the solution 16.6 g of KI and 50 mL of HCl were added, and the solution was washed twice with 50 mL of benzene. The benzene layer was back-washed twice with 40 mL of water. The water layers were combined and added 0.5 mL of 0.01 N NaOH and condensed to about 0.5 mL under vacuum and spotted as a band on thin-layer plate. The plate was developed for 13 cm with a mixture of ethyl acetate, acetic acid, and water (3:2:1, v/v). The location of arsenic compounds were determined by spraying 5% $SnCl_2$ solution and 15% KI solution. The R_f values of each spots were compared with those of authentic compounds.

Measurements and Identification of Arsenic. Determination of arsenic content of each sample was performed utilizing the AgDDC method following wet ashing with 1 mL of H_2SO_4, 3 mL of $HClO_4$, and 5 mL of HNO_3. Reductive volatilization method for identification of arsenic was a modification of that of Talmi (1975). An aqueous sample (1–3 mL) in a reaction vessel (a long test tube) was acidified with 1 N HCl (0.5 mL) and filled up to 4 mL with distilled water. One milliliter of toluene was

0021-8561/78/1426-0505$01.00/0

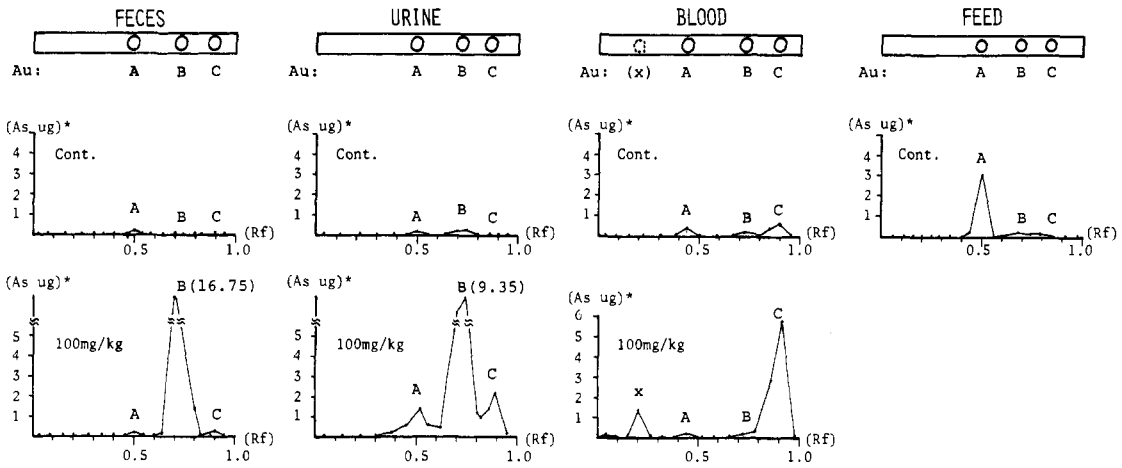

Figure 1. Thin-layer chromatograms of arsenic in urine, feces, blood, and feed extract: Au, authentic compounds; A, arsenic acid; B, DSMA; C, DMAA; (X, unknown). Feces and urine are the second-day samples and blood is the fifth-day sample after treatment of MAF. The asterisk indicates the values in the feces and blood are As μg/g of sample weight and in the urine and feed are As μg/10 g of sample weight.

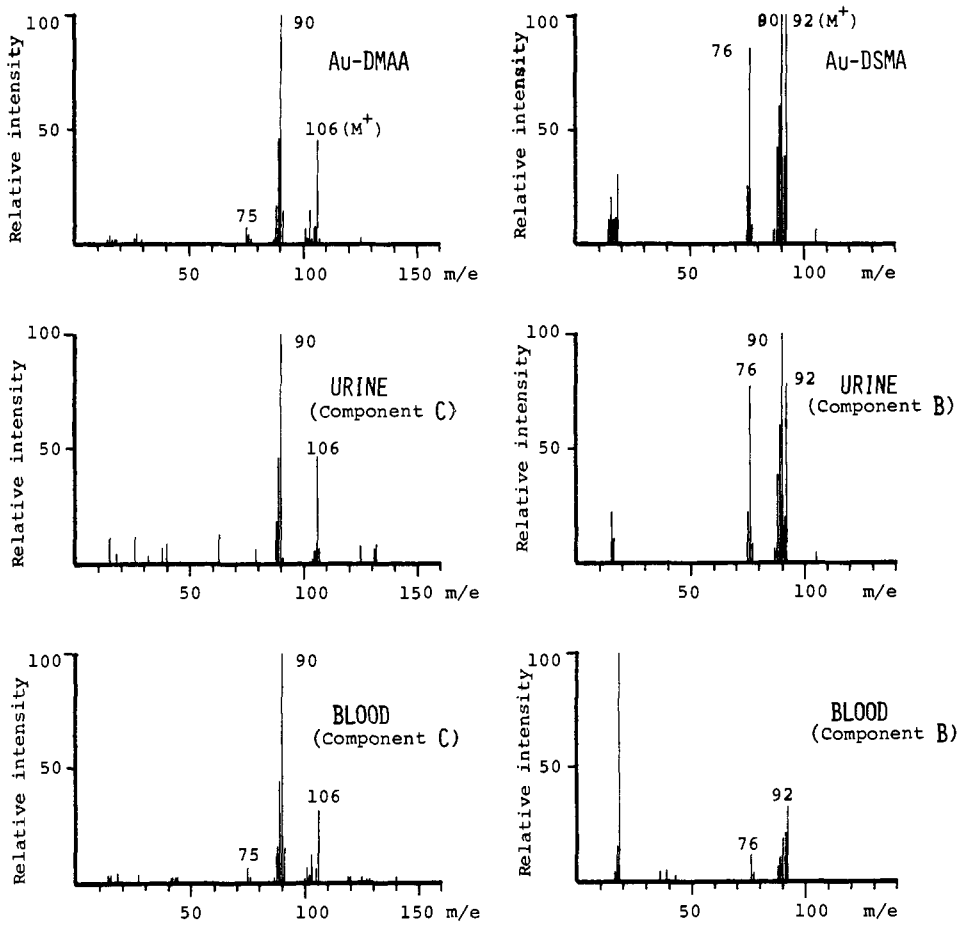

Figure 2. Mass spectrum of reduction derivation of authentic arsenic and arsenic obtained from TLC development of urine and blood extract (Figure 1): left, corresponds to compound C (DMAA) on the TLC plate; right, corresponds to compound B (DSMA) on the TLC plate; upper, authentic compounds; middle, urine of the second day; lower, blood of the fifth day.

then added and reduction was initiated by dropping 20% $NaBH_4$ solution (1 mL) in the aqueous sample. The vessel was allowed to stand throughout the reaction (30–45 s). Immediately after the reaction, the upper organic layer was removed for subsequent analysis by the GC–MS.

RESULTS AND DISCUSSION

To check the stability of MAF, 3000 μg of MAF was added to 10 mL of control sample of urine and analyzed by the described method. Any breakdown products were not observed on thin-layer plate, provided that MAF was all detected as the form of methanearsonate such as DSMA on thin-layer plate.

The rate of the intestinal absorption of orally administered MAF was usually lower, but the arsenic when absorbed in the rat body remained in the blood for a long term, and the arsenic levels in the blood was highest at the fifth day. Therefore the blood of the fifth-day sample was used for analysis of arsenic products. In order to detect effectively the arsenic metabolite, the samples of urine and

feces at the second day were analyzed. Thin-layer chromatographic analysis of the extracts of these samples indicated the presence of three major components, as shown in Figure 1. Component A, B, and C had a mobility similar to the authentic compounds: arsenic acid, DSMA, and DMAA respectively. The component A in the urine was identified as arsenate by comparing its absorption spectrum of AgDDC method with that of an authentic sample. By their comparative GC–MS spectra (Figure 2) the components B and C in the blood and urine were distinctly identified as methanearsonate and dimethylarsinate. The other component X which was observed in the blood could not be identified. These results indicated that a major portion of arsenic in feces was methanearsonate, and as a minor portion there was dimethylarsinate, the metabolite of MAF. According to the undetectability of arsenic in the bile, this metabolite might be due to the methylation slightly occurred in the intestinal tract by bacterial flora. On the other hand, the presence of dimethylarsinate as the predominant component in the blood indicates that the methylation largely occurred in the rat body. The major urinary elimination form was identified as methanearsonate, and dimethylarsinate and arsenate were minor. These results indicate that methylation occurs with organoarsenic compound such as methanearsonate. In addition, the fact that the presence of dimethylarsinate was detected predominantly in the blood of the control rats fed the control feed which contained arsenate but no dimethylarsinate as a normal component indicates that methylation also occurs with inorganic arsenic compound.

The arsenic metabolites in mammals were recently reported by Lakso and Peoples (1975) and Crecelius (1975). Lakso and Peoples analyzed the urine for inorganic arsenic (IA) and methylated arsenic (MA) in the cows (rumen) and the dogs (animal body) treated orally with arsenate and arsenite. The AgDDC colorimetric analysis revealed MA as the metabolite, and they suggested that methylation of arsenic occurred not only in rumen by the microorganisms but also in animal body. Crecelius detected DMAA, MAA, arsenate, and arsenite in human urine after drinking arsenite-rich wine, using the analytical procedures based on $NaBH_4$ reduction of the arsenic to the separating gaseous arsine by controlled pH method and the detection of the arsenic by emission spectrometry.

In these two reports, the urine of the mammals which had been treated with the inorganic form of arsenic was analyzed, and MA in cows and dogs and DMAA and MAA in human were detected as the metabolite of inorganic arsenic. However, these metabolites were not identified in detail. In our studies, the analysis was performed on the urine, the feces, and the blood of the rats which were treated before with MAF (monomethyl organic form of arsenic), and the dimethylated form of arsenic was identified as the metabolite by combining TLC and GC–MS. It was confirmed that methylation of monomethyl arsenic compound to dimethylated arsenic compound could occur in the rat. Therefore, it is conceivable that the biological methylation of arsenic in mammals occurs with inorganic arsenic and monomethyl organoarsenic compound, and dimethylated arsenic compound may be the final methylated form of arsenic in mammals.

ACKNOWLEDGMENT

The authors are grateful to Y. Shirasu, Division of Toxicology, Institute of Environmental Toxicology, for his help, advice, and encouragement, and to Y. Kabasawa for technical assistance during this investigation. The ferric methanearsonate and disodium methanearsonate used in the investigation were provided by Kumiai Chemical Co. The authors express appreciation for this support.

LITERATURE CITED

Abe, H., private communication, 1973.
Braman, R. S., Foreback, C. C., *Science* **182**, 1249 (1973).
Cox, D. P., Alexander, M., *Bull. Environ. Contam. Toxicol.* **9**, 84 (1973).
Cox, D. P., Alexander, M., *Microb. Ecol.* 1 (1974).
Crecelius, E. A., "Chemical Changes in Arsenic Following Ingestion by Man", U.S. Energy Research and Development Administration Contract No. E(45-1):1830, 1975.
Edmonds, J. S., Francesconi, K. A., *Nature (London)* **3**, 436 (1977).
Lakso, J. U., Peoples, S. A., *J. Agric. Food Chem.* **23**, 674 (1975).
McBride, B. C., Wolf, R. S., *Biochemistry* **10**, 4312 (1971).
Talmi, Y., Bostick, D. T., *Anal. Chem.* **47**, 2145 (1975).

Yoshitsugu Odanaka*
Osami Matano
Shinkō Gotō

Institute of Environmental Toxicology
Suzuki-cho 2-772
Kodaira-shi
Tokyo, 187, Japan

Received for review May 16, 1977. Accepted September 23, 1977.

Effect of Grain Moisture Content on the Degradation Rate of Methyl Phoxim in Corn, Sorghum, and Wheat

Residue data were obtained at five intervals over a 30-day period from wheat, corn, and sorghum containing eight levels of moisture, following 10 ppm applications of methyl phoxim emulsion spray. High-moisture content reduces the effectiveness and persistence of methyl phoxim in stored grain. Degradations differed significantly (P 0.05) at each moisture level. After 30 days of storage, 10, 3, and 22.9% of the initial residue deposit remained on the 20% moisture sorghum, wheat, and corn respectively. The highest residue deposits remained in the 6% grain moisture levels. After 30 days of storage, 71, 63, and 68% of the initial residue deposit remained on sorghum, wheat, and corn, respectively.

Methyl phoxim (phenylglyoxylonitrile oxime (*O*)-*O*,*O*-dimethyl phosphorothioate) (Bay SRA 7660) is a promising grain protectant. At 5 ppm, it was effective for 9 months against the rice weevils, *Sitophilus oryzae* (L.); for 2 months against red flour beetles, *Tribolium castaneum* (Herbst) and confused flour beetles, *T. confusum* (Jacquelin duVal); and for 6 months against lesser grain borers, *Rhyzopertha dominica* (F.) (Alnaji et al., 1977). It pro-

0021-8561/78/1426-0507$01.00/0