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Methylated Forms of Arsenic in the Environment

Abstract. *Environmental samples were analyzed for arsenate and arsenite ions and the methylarsenic acids in nanogram amounts. Dimethylarsinic acid and methylarsonic acid were found in natural waters, bird eggshells, seashells, and human urine.*

Challenger (*1*) has reviewed his work and that of others on the biological methylation of arsenic up to 1944. He found that certain fungi are responsible for methylation but obtained negative results for bacteria (*2*). Indirect evidence for the methylation of arsenic in the environment by methanogenic bacteria has been provided in the work on laboratory cultures by McBride and Wolfe (*3*). Inorganic arsenic forms are reduced and methylated eventually to dimethylarsine by methanogenic bacteria. Dimethylarsinic acid and methylarsonic acid were intermediates. Nevertheless, the existence of these arsenic compounds in the environment has not heretofore been established.

A lack of suitable analytical chemical procedures has hampered environmental studies of arensic, especially the detection and determination of the inorganic arsenic ions and the methylarsenic acids at ambient environmental (parts per billion) concentrations. Although some work has been reported on the determination of arsenite and arsenate ions in seawater (*4*), we could find no reported work on determination of the methylarsenic acids at very low concentrations.

Most methods now used for the determination of arsenic in low concentrations are total elemental analysis procedures. Many depend on the reduction of inorganic arsenic ions to arsine and subsequent colorimetric analysis. The silver diethyldithiocarbamate (*5*) is an example. The lower limit of detection for this method is 0.2 ug or 2 ppb if 100-ml samples are analyzed. Neutron activation methods for arsenic have a limit of detection near 1 ng (*6*), but are comparatively time-consuming because of the separation steps, irradiation, and decay times involved. Atomic absorption methods for arsenic have limits of detection in the range of 0.5 to 1 part in 10^6 in the vaporized samples (*7*). Ando *et al.* (*8*) obtained a sensitivity of 6 ppb (1 percent deflection) with a 91-cm (path length) tube. Chu *et al.* (*9*) obtained a detection limit of near 0.01 μg in a flameless atomic absorption technique.

Starting with work reported by Braman, Justen, and Foreback (*10*) on the determination of arsenic as arsine in an electrical discharge, we have developed procedures that permit the determination of arsenite ion, arsenate ion, methylarsonic acid, and dimethylarsinic acid in aqueous solutions with high specificity and at lower limits of detection near 1 ng. These procedures depend on *p*H selective reduction reactions of the various arsenic forms with sodium borohydride and a separation of the volatile arsines produced by selective volatilization from a cold trap. We found that As(III) ion is the only arsenic form reduced to arsine by sodium borohydride at *p*H 4 to 9. As(V) ions must first be reduced to As(III) ions by sodium cyanoborohydride at *p*H 1 to 2 before they can be further reduced to arsine by sodium

Table 1. Analysis of environmental samples. The results are given in parts per billion (ppb) as arsenic; the precision is ± 10 percent relative or ± 0.01 ppb.

Sample	As(III)		As(V)		Methylarsonic acid		Dimethylarsenic acid		Total (ppb)
	ppb	%	ppb	%	%	qdd	%	qdd	
			Freshwater samples						
Hillsborough River	< 0.02	< 10	0.25	100	< 0.02	< 10	< 0.02	< 10	0.25
Withlacoochee River	< 0.02	< 5	0.16	38	0.06	14	0.30	48	0.42
Well water near Withlacoochee River	< 0.02	< 3	0.27	39.7	0.11	16	0.20	44	0.68
Remote Pond, Withlacoochee Forest	< 0.02	< 2	0.32	30	0.12	11	0.62	58.5	1.06
University Research Pond, USF	0.79	40.5	0.96	49	0.05	2.6	0.15	7.7	1.95
Lake Echols, Tampa	2.74	76.5	0.41	11.4	0.11	3.1	0.32	8.9	3.58
Lake Magdalene, Tampa	0.89	51	0.49	28	0.22	12.6	0.15	8.6	1.75
			Saline waters						
Bay, Causeway	0.12	6.8	1.45	81.9	< 0.02	< 1	0.20	11.3	1.77
Tidal flat	0.62	27	1.29	56.6	0.08	3.5	0.29	12.7	2.28
McKay Bay	0.06	4	0.35	23.6	0.07	4.7	1.00	68	1.48

Table 2. Analysis of human urine samples for arsenic compounds. The results are given in parts per billion (ppb) as arsenic. The precision of individual runs is ± 10 percent relative, or ± 0.1 ppb for small samples sizes. Four other samples not completely analyzed give: methlarsonic acid, 3.6 ± 2.4 (S.D.) ppb; dimethylarsenic acid, 15.5 ± 6.8 (S.D.) ppb.

Sample	As(III)		As(V)		Methylarsonic acid		Dimethylarsenic acid		Total (ppb)
	ppb	%	ppb	%	ppb	%	ppb	%	
M, age 28	< 0.1		0.84	8.1	0.61	5.9	8.9	86.5	10.4
M, age 27	5.1	20	7.9	30	2.5	9.7	10.4	40.2	25.9
M, age 42	< 0.5		2.4	7.8	2.4	8.1	25.2	84.0	30.0
F, age 40	2.4	10	4.3	18	1.8	7.6	15.5	64.5	24.0
Average	1.9	8.4	3.9	17	1.8	8.0	15.0	66	22.5

borohydride at *p*H 1 to 2. Methylarsonic acid, $CH_3AsO(OH)_2$, and dimethylarsinic acid, $(CH_3)_2AsO(OH)$, are reduced to methylarsine, CH_3AsH_2, and dimethylarsine, $(CH_3)_2AsH$, respectively, by sodium borohydride at *p*H 1 to 2. The *p*H requirements of the reduction reaction indicate that the various arsenic acids must be in the completely undissociated form before they can be reduced to the corresponding arsines.

Samples in aqueous solutions or water samples up to 50 ml in volume were placed in a volatilization chamber and buffered at the appropriate *p*H. Reduction of the arsenic forms was carried out by injection of the reducing agent through a side port in the chamber. The volatile arsines produced were scrubbed out of the sample by helium carrier gas and frozen out in a liquid nitrogen–cooled U trap half-packed with glass beads. Reduction was usually completed in 5 minutes; the liquid nitrogen was removed and the cold trap was allowed to warm to room temperature. The arsines were volatilized from the trap in order of their boiling points: arsine, b.p. −55°C; methylarsine, b.p. 2°C; and dimethylarsine, b.p. 55°C. They were then carried through an electrical discharge maintained in the carrier gas. Arsenic emission lines were produced as the arsines passed through the discharge, and the intensities were recorded (Heath scanning monochromator-photometric readout system). The appearance of atomic emission lines for arsenic at 234.9 nm or 228.8 nm was used for qualitative identification of the arsines. Since arsine, methylarsine, and dimethylarsine passed through the detector at different times, the analysis readout was similar in appearance to a gas chromatogram. A comparison of observed volatilization times for arsenic compounds volatilized from environmental samples to those of standard samples of methylarsonic acid and dimethylarsinic acid serves to identify the methylarsenic acids. Because of the high sensitivity inherent in emission detection systems, limits of detection were very low: 0.05 ng for As(III) and As(V), and 0.5 ng for the methylarsinic acids.

As(III) and As(V) ions may be determined separately in one analysis. The sample was buffered with potassium biphthalate to *p*H 4 to 5 and treated with 2 ml of 1 percent sodium borohydride. Only As(III) reacts under these conditions, and the arsine produced is a quantitative measure of As(III) only. After the arsine was volatilized its peak was seconded, the cold trap was again cooled; 4 ml of 10 percent oxalic acid solution was added, 1 ml of 1 percent sodium cyanoborohydride reagent was added, and the sample was allowed to stand for 2 minutes. Two milliliters of 1 percent sodium borohydride was then added, and the reaction was allowed to proceed for 5 minutes. The arsine produced in this step comes only from As(V) because the As(III) was already removed.

A second analysis was required to determine the methylarsenic acids. Hydrogen cyanide produced in the As(V) procedure above interferes in detection of the methylarsines, which were also produced. Samples were buffered to *p*H 1 to 2 with 10 percent oxalic acid solution and degassed to remove air and carbon dioxide, and 2 ml of 1 percent sodium borohydride was injected into the sample chamber. The methylarsines produced were trapped, separated in the half-packed U trap, and detected.

The analytical methods have been applied to the analysis of a variety of water samples, bird eggshells, a seashell, and limestone (*11*). Water samples can be analyzed without prior treatment other than degassing for 2 to 5 minutes to remove dissolved air and carbon dioxide. Water analysis must be made almost immediately after sampling to avoid concentration changes (Table 1). Both methylarsonic acid and dimethylarsinic acid were found in a wide range of natural waters in and around Tampa, Florida. Lakes and ponds were small, and all had much higher methylarsenic acid content than the rivers analyzed. All of the lakes and ponds except one were within 5 miles (8 km) of Tampa; most had homesites on the lakes. Tampa tap water contained only traces of arsenate ion. One sample of well water, at a remote camping area near (90 m) the Withlacoochee River, contained significant amounts of the methylarsenic acids. The methylarsenic acids were also found in saline water samples taken at several locations along the shores of Tampa Bay.

Samples of seashells, bird eggshells, and a sedimentary rock sample (Cretaceous limestone) were analyzed. These carbonate-containing solid samples were dissolved in HCl; the HCl solution was diluted with distilled water and 1- to 10-ml portions were analyzed. A large fraction of arsenic in the shell sample was found to be methylarsonic acid or dimethylarsinic acid. A limestone sample analyzed had only small traces, 0.005 ppb, of the methylated arsenic forms.

The methods were adapted to analysis of urine samples. Samples (1 to 5 ml) were diluted to 25 ml, treated with a few drops of Anti-Foam B (Technicon Corp.), degassed for 2 to 5 minutes, and carried through the analysis scheme (Table 2). In one case more than 40 to 90 percent of the arsenic being elaborated was in the form of methylarsonic acid or dimethylarsinic acid. This contrasts sharply with the report (*12*) that arsenic in urine was in the form of As(V) ions. Methylation of arsenic here is probably attributable to the methylcobalamin-methionene reactions in the body. Human urine is reported (*13*) to contain 2 to 10 mg of methionine per liter and thus the site at which arsenic methylation occurs is not completely understood.

The reported toxicity of As(III) is approximately 25 times greater than that of dimethylarsinic acid (*14*). Thus, detoxification of arsenic occurs as a consequence of the body methylation reactions. This may be a natural defense or simply an additional consequence of methylation reactions.

Attempts to detect dimethylarsine in various water samples and in urine by

cold trapping without reduction were unsuccessful. Since sensitivity to oxidation of this compound is a considerable problem, on-site environmental analysis may be required if dimethylarsine itself is to be detected.

Dimethylarsinic acid is a major and ubiquitous form of arsenic in the environment. It is particularly involved in biological systems. Methylarsonic acid, although found, was generally present in smaller concentrations than dimethylarsinic acid, a probable consequence of its being only an intermediate in the arsenic methylation sequence. Dimethylarsinic acid is very resistant to oxidation. It is not oxidized by bromine water, or, quantitatively, even by aqua regia. It could then have a considerable residence time in natural waters, unless subject to bacterial oxidation.

Both dimethylarsinic acid and methylarsonic acid are pesticides. Since they are identical with the biologically produced methylarsenic acids the detection of the effect of added methylarsenic pesticides will be difficult.

Finally, the introduction of arsenic compounds into the environment may eventually result in a general increase in their concentrations in water and air because of the bacterial mobilization of all the forms of arsenic. Information is needed on the effect of all the forms of arsenic on ecological systems.

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Hypertensive Action of 18-Hydroxydeoxycorticosterone

Abstract. *18-Hydroxydeoxycorticosterone is an adrenal steroid hormone causing salt and water retention and is secreted in greatly increased amounts in response to the pituitary hormone adrenocorticotropic hormone. Its production is abnormally high in some forms of hypertension in man and rat. Direct proof that 18-hydroxydeoxycorticosterone is capable of causing hypertension is present. Daily subcutaneous injections of 200 micrograms, a low physiological dose, significantly increase the blood pressure of unilaterally nephrectomized saline-treated rats after 2 weeks. This strengthens the hypothesis that 18-hydroxydeoxycorticosterone contributes to the etiology of hypertension, possibly by a mechanism involving stress-induced release of adrenocorticotropic hormone.*

18-Hydroxydeoxycorticosterone (18-OH-DOC) is an adrenal steroid native to several species including rat (*1*) and man (*2*). In both rat and man its secretion is increased greatly by adrenocorticotropic hormone (ACTH) (*2, 3*), a pituitary hormone liberated in response to stress. 18-OH-DOC is produced by the rat in quantities that may exceed those of corticosterone, the principal steroid affecting carbohydrate metabolism in this species (*4, 5*). It affects electrolyte and water excretion (*3, 6*) and has been implicated as a possible hypertensive agent in rats as well as man (*7*). However, the crucial experiment, namely to establish whether 18-OH-DOC can elicit hypertension, had to await its availability in sufficient quantity for biological assay. This experiment has now been performed with 18-OH-DOC prepared by a new organic synthesis (*8*). Weanling male Wistar rats were unilaterally nephrectomized and given 1 percent saline, a common procedure employed for the assay of hypertensive steroids (*9, 10*). One week after the operation the animals were separated into four groups of ten rats each. Three groups received subcutaneous injections, at 9 a.m., of steroid suspended in cottonseed oil, the fourth group received only cottonseed oil. Corticosterone (B) and deoxycorticosterone acetate (DOCA) were administered daily at a dosage of 200 μg for 21 days, 18-OH-DOC was given at a dosage of 200 μg for 20 days and of 140 μg on day 21, because of insufficient material. Blood pressure was determined in the afternoons by the tail-cuff method under light ether anesthesia (*11*), at the onset of the experiment and on days 2, 3, 9, 10, 16, 17, and 21. Body weights were checked on days 2, 9, 16, and 21. On day 21 the rats were decapitated, and the organs were removed and weighed.

The changes in blood pressure with time are depicted in Fig. 1. The blood pressures of the 18-OH-DOC–treated animals were elevated compared to those of the vehicle-treated animals on day 16 (155 ± 3 as compared to 137 ± 5 mm-Hg, $P < .01$), day 17 (160 ± 2 as compared to 143 ± 4 mm-Hg, $P < .01$), and day 21 (158 ± 3 as compared to 142 ± 5 mm-Hg, $P < .02$). On days 9 and 10 the increase was of marginal significance (140 ± 5 as compared to 128 ± 4 mm-Hg and 151 ± 3 as compared to 142 ± 4 mm-Hg, respectively, $.05 < P < .1$ by two-tailed t-test, $P < .05$ by one-tailed t-

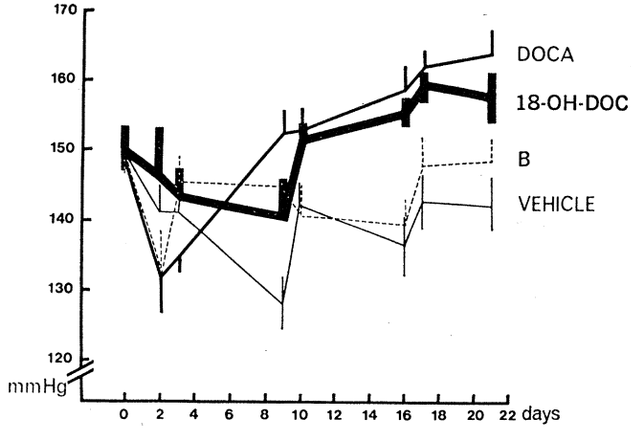

Fig. 1. Hypertensive action of 18-OH-DOC. Ten rats per group were injected subcutaneously with 200 μg of steroid daily for 21 days, except for the 18-OH-DOC – treated group, which received 200 μg for 20 days and 140 μg on day 21. One group was injected with vehicle only (0.1 ml of cottonseed oil). Blood pressures were measured by the tail-cuff method (*9*). Significant differences from the vehicle-injected rats occurred on days 16, 17, and 21 in the 18-OH-DOC–injected rats, on days 9, 16, 17, and 21 in the DOCA-injected rats, and on day 9 only in the corticosterone-injected rats. *B*, corticosterone.