Degradation of Monosodium Methanearsonic Acid by Soil Microorganisms

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Monosodium methanearsonic acid (MSMA) was chosen as the model compound for a study of the degradation of organic arsenicals in soil. Comparison of evolved $^{14}CO_2$ from four sterile and nonsterile soils 60 days after treatment with MSMA-^{14}C showed that from 1.7 to 10.0% of the MSMA-^{14}C was degraded in nonsterile soil, as compared with 0.7% in steam-sterilized controls. Four soil microorganisms isolated in pure culture degraded from 3 to 20% of the MSMA-^{14}C to $^{14}CO_2$ when grown in liquid culture containing 10 p.p.m. of MSMA and 1 gram per liter of yeast extract. Thin-layer chromatography (TLC) on silica gel G-coated plates effected the separation of MSMA, arsenate, and arsenite. Only arsenate and MSMA were detected after TLC of extracts from the soil and microbial growth experiments. These data indicate that soil microorganisms are at least partly responsible for MSMA degradation in soil.

Inorganic arsenicals such as arsenic trioxide, sodium arsenite, lead arsenate, calcium arsenate, and Paris Green have been used for many years as insecticides, herbicides, soil sterilants, and silvicides. In recent years, organic arsenical herbicides, in which the organic group is bonded directly to the arsenic atom, have been used extensively for postemergence control of weeds in cotton. Several of the more important herbicides are sodium cacodylate (monosodium dimethylarsinic acid) and sodium salts of methanearsonic acid. The latter compounds exist in two principal forms: the monosodium salt (MSMA) at pH 6.4 and the disodium salt (DSMA) at pH 10.2 (Baetke, 1966). The organic arsenicals are particularly effective against the perennial weed Johnson grass [*Sorghum halepense* (L.) Pers.] which infests many areas of the cotton growing region. McWhorter (1966) has reviewed the usage of DSMA for Johnson grass control in the cotton growing regions of the southeastern United States.

Recently, Schweizer (1967) has pointed out that information on the persistence of DSMA in soils is extremely meager. Only two detailed studies of this problem are at present known to exist. In one study, the initial and residual phytotoxicity of DSMA to cotton has been measured over a broad range of concentrations in three soils (Schweizer, 1967). Toxicity decreased with time, particularly during the first 16 weeks after soil incorporation. Growth of cotton planted immediately after incorporation of DSMA in Bosket silt loam was reduced significantly by concentrations of 50 to 80 p.p.m. In these same soils, other plants were shown to have different degrees of susceptibility to DSMA concentrations. Rice was extremely sensitive to soil concentrations of 50 p.p.m., while corn, cotton, and wheat were little affected.

In a more recent study (Dickens and Hiltbold, 1967), the oxidation of the methyl carbon of methanearsonate was associated with the oxidation of soil organic matter in a number of soils. Additions of organic matter to a Norfolk loamy sand greatly increased the decomposition of methanearsonate. In three of the soils, there was no evidence of microbiological adaptation to methanearsonate. In Norfolk loamy sand, however, increasing decomposition of methanearsonate relative to soil organic matter occurred with time of incubation.

Because of the wide usage of organic arsenicals, and because little information exists on the fate of these compounds in soils, the simplest organic arsenical, MSMA, was selected as a model for studying the metabolism of this class of compounds by soil microorganisms.

MATERIALS AND METHODS

Monosodium hydrogen methanearsonate hexahydrate (MSMA·$6H_2O$) and MSMA-^{14}C (specific activity 0.91 μc. per mg. in a 10-ml. water solution) were generously supplied by the Ansul Co., Marinette, Wis. Nonlabeled MSMA was recrystallized once from hot water. The radioactive material appeared as a single spot on a thin-layer chromatogram in several solvent systems and consequently was used without further purification. Inorganic arsenicals, including sodium arsenate and sodium arsenite, were purchased as the analytical reagents and used without further purification.

Metabolic Studies. Experiments involving the release of radioactive CO_2 from MSMA-^{14}C treated soils were conducted in a system consisting of two test tubes connected in series. One tube contained 5 grams of treated soil (at 10 and 100 p.p.m. of MSMA) while a second tube contained a CO_2 trapping mixture, 2-methoxyethanol and monoethanolamine (7 to 10, v./v.). Carbon dioxide-free air was passed over the soil and metabolic $^{14}CO_2$ was collected in the trapping solution. The soils studied were Sharkey clay, Hagerstown silty clay loam, Cecil sandy loam, and Dundee silty clay loam. All soils were initially adjusted to field capacity and maintained at 28–30° C.; the evolved $^{14}CO_2$ was sampled periodically. Some properties of these soils are shown in Table I.

Experiments involving the release of radioactive CO_2 from MSMA-^{14}C from pure cultures of soil microorganisms were conducted in Roux bottles. Sterile mineral salt solutions containing 3% agar and 100 p.p.m. MSMA (MSMA plus 0.5 μc. of MSMA-^{14}C) were poured into tilted Roux bottles and allowed to solidify. The composition of the salt solution has been described (Kearney *et al.*, 1964). Isolates of soil microorganisms from enrichment culture

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Table I. Physical Properties of Soils Used in MSMA Decomposition Studies

Soil	pH	Organic Matter, %	Clay (0.002 Mm.), %	Field Capacity, %
Sharkey clay	6.2	3.90	67.1	38.4
Hagerstown silty clay loam	6.8	2.50	39.5	25.8
Cecil sandy clay loam	5.3	1.89	20.1	17.8
Dundee silty clay loam	5.0	1.67	29.0	24.3

solutions were transferred to the agar and evolved $^{14}CO_2$ was collected as previously described. Soil enrichment techniques were employed to isolate soil microorganisms capable of metabolizing MSMA. Fifty grams of Hagerstown silty clay loam was added to 100 ml. of water containing 100 p.p.m. of MSMA and then agitated on a mechanical shaker at 27° C. for 2 weeks. Dilution plates containing 3% nutrient broth agar and 100 p.p.m. of MSMA were then prepared. After 3 to 5 days, organisms were isolated in pure culture and used for metabolism studies.

Metabolic studies with pure cultures of microorganisms were done in shake flasks maintained at 30° C. in a shaker-incubator. The medium consisted of the same mineral salt solution used in the Roux bottle studies. Solutions were amended with 1% yeast extract, MSMA, and 1 μc. of MSMA-^{14}C to give a final concentration of 10 p.p.m.

All determinations of radioactivity were carried out in a Nuclear-Chicago Mark I liquid scintillation spectrometer (1965). Composition of the counting solutions used to monitor aqueous (1 ml. of sample to 15 ml. of solution) and nonaqueous (1 ml. of sample to 17 ml. of solution) samples were as follows: aqueous counting solution, 1,4-dioxane (1 liter), 2-ethoxyethanol (100 ml.), PPO (6.0 grams), POPOP (0.3 gram), naphthalene (90.0 grams); nonaqueous counting solution, toluene (1 liter), PPO (5 grams), POPOP (150 mg.). All counting systems were optimized to 100% efficiency by the channels ratio–external standard method before comparison.

Thin-Layer Chromatography. Thin-layer chromatography was carried out on 20 × 20 cm. glass plates coated 0.25 mm. thick with a suitable support and dried overnight. Silica gel G, silica gel H, and cellulose were examined as the solid phases for chromatography of methanearsonate, arsenite, and arsenate. Several sprays for the visualization of the arsenicals on plates were tested. Three of the more successful reagents and the color produced with final product are shown in Table II.

Table II. Spray Reagents Used to Detect Arsenite, Arsenate, and MSMA on Thin-Layer Chromatograms

Spray	Color and Detection Limit: Na arsenite	Na arsenate	MSMA
2*N* HCl–12% $(NH_4)_2S$ (1 to 1)	Yellow 1.5 μg.	...	...
1% $(NH_4)_6$ Mo_7O_{24}[a]	...	Blue 1.5 μg.	Blue 1.5 μg.
1% $SnCl_2$ in 10% HCl			
12% $(NH_4)_2S$–H_2O (1 to 1)			
1% Et_2NCS_2 in 50% H_2O–acetone	...	Orange 2.5–3.0 μg.	Orange 2.5–3.0 μg.
0.1% dithizone in benzene			

[a] Stahl (1965).

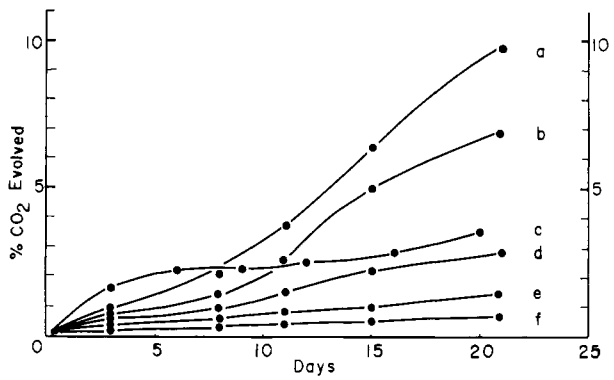

Figure 1. MSMA decomposition: two rates applied to four soils

a. Sharkey clay + 10 p.p.m. MSMA
b. Sharkey clay + 100 p.p.m. MSMA
c. Hagerstown silty clay loam + 10 or 100 p.p.m. MSMA
d. Cecil sandy loam + 10 or 100 p.p.m. MSMA
e. Dundee silty clay loam + 10 or 100 p.p.m. MSMA
f. Steam-sterilized controls

RESULTS AND DISCUSSION

Preliminary studies indicated that MSMA decomposition to CO_2 was a slow process that did not involve a lag phase. Hagerstown silty clay loam slowly evolved $^{14}CO_2$ from MSMA-^{14}C applied at a rate of 100 p.p.m. Only 7% decomposition was observed after 60 days. In another experiment involving four soils and two rates of MSMA application, the rate of decomposition was again a slow process (Figure 1). After 3 weeks' incubation, all soils had evolved radioactive CO_2 to a degree proportional to the amount of organic matter in the soil. The rate of evolution ranged from 10% decomposition for the highest organic matter soil (Sharkey clay), to 1.7% decomposition in the lowest organic matter soil (Dundee silty clay loam). Only in Sharkey clay was there a difference in CO_2 production between the high (100 p.p.m.) and low (10 p.p.m.) application rates of MSMA. The initial rapid rate of $^{14}CO_2$ release from Hagerstown silty clay loam probably occurs because it was a fresh soil (the others were air-dried) with an active microbial population already established. Steam-sterilized soils produced essentially no $^{14}CO_2$; therefore, soil microorganisms appear to play some role in the decomposition process.

Using the soil enrichment technique, a fungus, several actinomycetes, and several bacteria were isolated in pure culture. When the fungus was transferred as a spore suspension to agar in Roux bottles containing 100 p.p.m. of MSMA-^{14}C as the sole carbon source, 2% of the radioactive carbon was released after a 1-week incubation

period. Cultured in the same manner, one of the bacterial species released 1% of the carbon as $^{14}CO_2$. In shake flasks containing 1 gram per liter of yeast extract and 10 p.p.m. of MSMA-^{14}C, all the soil isolates were tested for their ability to utilize MSMA as evidenced by a reduction of the total radioactivity in the solution culture (Figure 2). The bacterial species metabolized about 20% of the organic arsenical after 3 days of incubation. Growth and decomposition apparently leveled off after the exogenous energy source was exhausted. The fungus and two actinomycetes degraded 3, 13, and 9%, respectively, of the total supplied MSMA. In the absence of an energy source, microbial metabolism of the organic arsenical herbicide was nonexistent.

Since there is only one carbon to utilize in MSMA, any conceivable metabolism of this compound must produce some form of inorganic arsenic. To identify the breakdown products of MSMA (other than CO_2) the thin-layer chromatographic systems (Table III) were employed to separate MSMA from the two inorganic arsenicals, arsenate and arsenite.

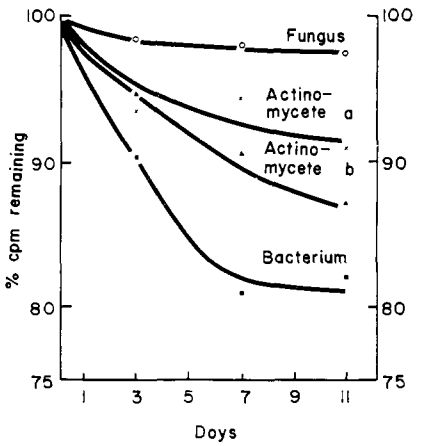

Figure 2. MSMA decomposition by four soil microorganisms

Table III. Separation of Sodium Arsenite, Sodium Arsenate, and MSMA Using Several Thin-Layer Chromatographic Systems

Support	Solvent	R_f Na arsenite	R_f Na arsenate	R_f MSMA
MN cellulose 300 G	MeOH–BuOH–H_2O (3:1:1)[a]	0.80	0.55	0.60
	Pyridine–ethyl acetate–H_2O (2:5:5) upper layer[b]	0.43	...	...
Silica gel H	MeOH–NH_4OH–10% TCA–H_2O (50:15:5:30)[c]	0.45	0.27–0.60	0.67
Silica gel G	As above	0.57	0.00–0.53	0.73

[a] Kawanabe *et al.* (1964).
[b] Siuda (1965).
[c] Stahl (1965).

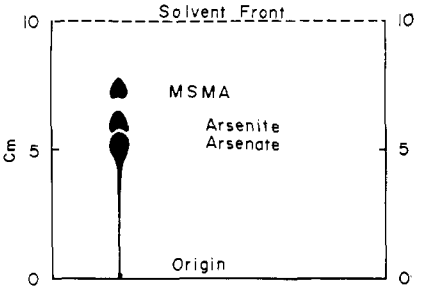

Figure 3. Thin-layer chromatography of three arsenic compounds

Several acidic solvent systems and types of supports work with varying efficiency (Table III). Cellulose and silica gel H (with organic binder) allow the movement of arsenite, but arsenate and MSMA remain on the origin. Although $CaSO_4$ binder is reported to prevent the efficient separation of phosphates (Stahl, 1965), this is not so in the case of the arsenicals under study. MSMA, arsenate, and arsenite separate best on thin-layer plates coated with silica gel G (calcium sulfate binder).

Figure 3 illustrates the separation which may be expected when microgram quantities of three arsenic compounds are cochromatographed. Silica gel G–coated glass plates were spotted with approximately 1.5 μg. of each of the three compounds. The plate was then developed in a methanol-ammonia-trichloroacetate-water solvent system (Stahl, 1965). Arsenate (valence +5) migrates 6/10 of the distance to the front with tailing. Though several supports and solvents were examined, streaking of arsenate was never completely eliminated. Arsenite (valence +3) migrates 8/10 of the distance and MSMA to R_f 8.5. Arsenite was visualized by using the ammonium sulfide spray, arsenate and MSMA by using the ammonium molybdate–stannous chloride spray (Table II). All spraying was done in a hood and the stannous chloride solution made fresh daily.

Using the TLC system developed, aliquots from bacterial growth media were concentrated, spotted, and developed. Soils were extracted three times with hot water, concentrated, and spotted. In both cases, arsenate and MSMA were the only products discovered. No arsenite was detected. Autoradiography of these plates showed only radioactive MSMA to be present.

Metabolism of most organic herbicides leads to the formation of less toxic products. Metabolism of DSMA (LD_{50} 700 to 1000) in soils yields the inorganic arsenate (LD_{50} 40 to 100)—a compound that is 10 to 25 times more toxic. Schweizer (1967) has calculated that at the normal rates of application of DSMA in Mississippi (equivalent to 6 kg. per hectare per year in the area actually treated) more than 50 years would elapse before residues from DSMA would reach toxic levels in cotton. In contrast, rice is very susceptible to DSMA residue and phytotoxic

levels may be reached in the time range of 3 to 80 years depending on soil type.

A number of factors, however, govern the behavior of arsenic in soil. Its availability to plants is in part mediated by soil pH, arsenic being less available for plant uptake as soil pH increases with the formation of insoluble calcium arsenates. Soils of high clay content with increased iron and aluminum concentrations are more able to complex arsenate than soils of lighter texture. Since arsenic and phosphorus are chemically similar, the availability of arsenic for plant uptake is proportional to the phosphorus content of the soil (Everett, 1962). Also, as previously mentioned, the susceptibility of the individual plant species is an important factor in an assessment of the toxicity of arsenic in soils.

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