

Evolution of Dimethylselenide from Soils

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Alcohols, carbonyl compounds, and fatty acids were formed in two glucose-amended soils incubated under argon, but dimethylselenide was evolved under argon only from one, a selenium-rich clay, after the addition of selenite and glucose. Substantial quantities of dimethylselenide were released from the four soils tested when they were incubated with glucose and selenite in air. No dimethylselenide was produced in the selenium-rich clay soil in air if it received glucose but no selenite.

Fungi of several genera are able to form dimethylselenide in axenic culture, and species of *Scopulariopsis*, *Penicillium*, and *Aspergillus* have been demonstrated to be capable of synthesizing this product from inorganic selenium compounds (2, 3). At least one coryneform bacterium also can convert inorganic selenium to dimethylselenide in vitro (J. W. Doran and M. Alexander, unpublished data). Nevertheless, apart from the demonstration of the evolution of trace quantities from samples of amended sewage (3), nothing is known about dimethylselenide formation in natural ecosystems. The potential for such an activity is suggested by a report that volatile selenium is released from soil apparently as a result of microbial action (1), but the identity of the compound (or compounds) was not determined.

Selenium is present in many soils as selenate and selenite, and is probably present in organic compounds derived from plant tissues and microbial cells (6). In some regions, it is present in such high concentrations that plants accumulate the element to levels that are toxic to animals consuming the plants. On the other hand, the soils of many regions are deficient in this element, and, inasmuch as selenium is essential for livestock, its addition to the land is under consideration. Because volatilization would change the quantity in soil that might be available for assimilation by forage plants and also might lead to the presence of selenium in the atmosphere, possibly to account for its transport to remote areas such as Antarctica (8) and the ice sheets of Greenland (7), a study was initiated to assess whether this compound might be discharged from soil.

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MATERIALS AND METHODS

Samples of four soils were used: a selenium-rich clay soil from South Dakota containing approximately 30 ppm of selenium, 2.5% organic matter, and with a pH of 7.2; Honeoye silt loam from New York, with a pH of 5.5 and 5.6% organic matter; Croghan loamy sand from New York, with a pH of 5.9 and 4.6% organic matter; and a sandy loam with a pH of 5.9 and 0.5% organic matter from the Sonoran Desert near Tucson, Ariz. The soils were air-dried and passed through a 2-mm sieve prior to use. The Croghan loamy sand had been stored air dry for approximately 15 years, and the other soils had been stored for periods ranging from 6 months to 2 years.

A 10-g sample of soil was placed in a 50-ml glass bottle equipped with an inlet and an outlet tube. The soil was amended with water only or with a solution containing 100 mg of glucose or 100 mg of glucose and 10 mg of Na_2SeO_3 in sufficient water to bring the soil to field capacity. The inlet tube of the sample bottle was connected to a gas manifold, and the outlet was connected to a stainless steel tube 15 cm long by 3 mm outer diameter. The steel tube contained Porapak QS (100 to 120 mesh). A stream of high-purity grade argon or pure-grade air (Union Carbide, Linde Division) was passed through the sample bottles to sweep the volatile compounds present in the head space into the Porapak trap, where they were retained. The flow rate was regulated at 1 to 2 ml/min by means of a needle valve placed between the sample bottle and the Porapak trap. The needle valve and a clamp placed between the sample bottle and gas manifold allowed the sample to be isolated when the trap was removed for analysis.

The volatile metabolites retained in the trap were analyzed with a gas chromatograph-mass spectrometer, Perkin-Elmer model 270, at regular intervals. The trap was inserted into the injector port of the gas chromatograph and connected to the chromatographic column. The injector port was heated at 250 C to inject the contents of the trap into the chromatographic column (1.83 m by 3 mm outer diameter), which contained Chromosorb 101 (100 to 120 mesh). The column temperature was maintained at 25 C for 4

min, and then it was programmed to 100 C at a rate of 32 C/min and from 100 to 250 C at a rate of 6.5 C/min. The mass spectrum obtained for each compound was compared with the mass spectra of authentic compounds for the purposes of identification.

RESULTS AND DISCUSSION

Soils receiving water but no other amendment did not produce detectable amounts of volatile compounds under either air or argon. When the seleniferous clay soil was amended with glucose or glucose and sodium selenite and incubated under argon, however, ethanol, *n*-propanol, *n*-butanol, acetone, methyl ethyl ketone, methyl propyl ketone, acetic acid, and butyric acid were found at various times during a 48-day incubation period. In addition to these compounds, traces (<2 μg) of dimethylselenide were found when sodium selenite was included in the amendment. Similar experiments with the silt loam yielded ethanol, *n*- and *iso*-propanol, *n*-butanol, acetone, methyl ethyl ketone, ethyl acetate, ethyl butyrate, and butyl butyrate. Dimethylselenide was not detected, and the only significant difference in products between the silt loam receiving glucose alone and the same soil receiving glucose and selenite was the generation of *n*-hexanol when the selenium salt was added.

All four soils amended with glucose and Na_2SeO_3 and incubated under a stream of flowing air evolved dimethylselenide. The quantities of this metabolite evolved from the four soils during a 48-day incubation period are shown in Table 1. The initial rate of evolution was more rapid in the silt loam and the seleniferous clay than in the other soils, but a considerable amount of dimethylselenide appeared in the sandy loam late in the incubation period. Little evolution was evident from the loamy sand. The amount of selenium recovered as dimethylselenide was approximately 2% of that added with the seleniferous clay, sandy loam, and silt loam, and the recovery was about 0.3% with the loamy sand.

Ethanol and acetone were also found in the headspace over the silt loam, seleniferous clay, and loamy sand during the first 15 days, but only dimethylselenide was detected in the headspace thereafter. No volatile organic compounds other than dimethylselenide were detected in the sandy loam. When the selenium-containing South Dakota clay was amended with only glucose and incubated in air, ethanol, acetone, *iso*-propanol, and methyl ethyl ketone but no dimethylselenide were detected in the first 7 days, and no volatile compounds were evident in the next 53 days.

TABLE 1. *Dimethylselenide formation in soils incubated in air*

Soil	Dimethylselenide (μg) ^a			
	Day 0-7	Day 7-15	Day 15-31	Day 31-45
South Dakota clay	27	69	45	25
Honeoye silt loam	36	82	10	<1
Croghan loamy sand	0	19	<1	0
Arizona sandy loam	0	11	83	47

^a Total quantity per reaction vessel.

The results suggest that the microbial methylation of selenium is potentially widespread. This activity was found in the seleniferous soil and also in other soils when a readily available carbon source and sodium selenite were added. Because the seleniferous clay did not evolve the alkyl selenium product when amended with glucose only, the evolution must require that soils contain the element in a suitable concentration or in a form which is readily utilized by the microorganisms responsible for methylation. Volatile organic selenium compounds other than dimethylselenide were not detected by the procedures employed, yet they may be generated under certain circumstances.

The ecological significance of the finding that a volatile methylated selenium compound can be formed in soil is not clear. In this regard, it is noteworthy that selenium is found in the atmosphere above the geographic South Pole (8) and in the ice sheets of Greenland (7), and it is plausible to suggest that the element reaches these remote sites as a result of the discharge of dimethylselenide from soils in the tropics or temperate zone. Indeed, a parallel may be drawn with dimethylsulfide, which has recently been proposed to be the major volatile carrier of sulfur in the natural sulfur cycle (4). If dimethylselenide is, in fact, a common metabolite evolved from natural ecosystems, it would also be of great importance to learn more of its toxicity, especially in view of the known hazards of methylmercury and trimethylarsine. McConnell and Portman (5) reported that dimethylselenide had a low mammalian toxicity, the median lethal dose to mice and rats being greater than 1.0 g/kg when given intraperitoneally, but of significance in the context of the present investigation would be the toxicity of inhaled dimethylselenide. Furthermore, the identities of the organisms responsible for the formation of dimethylselenide in soil have yet to be determined, although it is known that fungi of diverse genera are able to bring

about selenium methylation in culture media. It is clear, therefore, that additional studies of the ecological significance, the microbial formation, and possibly the toxicology of dimethylselenide are warranted.

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