APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 6, 173–178 (1992)

Chemical methylation of germanium(II) in model aqueous solutions

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Inorganic germanium(II) in micromolar concentrations was reacted with methyl iodide (CH_3I) and methylcobalamin (CH_3-CoB_{12}) at various pH values and with different salt matrices. In all experiments monomethylgermanium was the only product. The reaction with CH_3-CoB_{12} at pH 1 yielded approximately 1.3% of the added germanium, whereas no methylation occurred at pH 7. Reaction yields with CH_3I were lowest at pH 1 in 0.1 mol dm^{-3} KCl (1.6%) and highest at pH 7.6 in artificial seawater (6%). For the reaction of CH_3-CoB_{12} with germanium(II) a free-radical mechanism is assumed, whereas methylation by CH_3I is most likely an oxidative addition mechanism.

Keywords: Methylation, germanium, methyl iodide, methylcobalamin

INTRODUCTION

Two methylated germanium compounds, monomethylgermanium (MMGe) and dimethylgermanium (DMGe), are found in natural waters. In contrast to inorganic germanium (Ge_i) which resembles silicon in its biogeochemical cycle (in the case of silicon, uptake into siliceous organisms and dissolution during sedimentation), the methylgermanium species (Me_xGe) display a conservative behaviour in estuaries and oceans.[1] High-precision vertical profiles of Me_xGe in open ocean waters indicate that there is no production or consumption of these compounds even in biologically highly active zones.[2] Continents have been identified as the source of Me_xGe, and rivers as the major transport mechanism to the oceans. From the input flux of natural rivers, a residence time of more than 1 million years was calculated, which makes these species uniquely persistent among the naturally occurring organometals.

Biological and chemical mechanisms which have been found to methylate other elements such as arsenic, mercury, lead, selenium and tin failed to produce Me_xGe from GeO_2. The organisms tested included diatoms, dinoflagellates, blue–green algae and aerobic bacteria and fungi.

From the standard redox potential for the Ge^{IV}/Ge^{II} couple a free-radical mechanism for the methylation of GeO_2 by methylcobalamin (CH_3-CoB_{12}) was assumed. Attempts to methylate GeO_2 with CH_3-CoB_{12} under conditions found to work for mercury and platinum failed. However, the production of MMGe, DMGe and TMGe (trimethylgermanium) in anaerobic sewage digesters and polluted rivers indicates a biological methylation of Ge_i under reduced anaerobic conditions.

In contrast to CH_3-CoB_{12}, which is able to transfer methyl groups as carbanions, radicals or carbonium ions, most of the other naturally occurring methylating agents such as methyl iodide (CH_3I), S-adenosylmethionine and betaine function as carbonium-ion donors. For these compounds, an oxidative addition mechanism of CH_3^+ to low-oxidation-state metals such as M^0 or M^{II} (M = Sn, Pb) is feasible.[3] Attempts to methylate M^0 and M^{II} by the carbonium-ion donor CH_3I have been successful.[4] Whereas all previous attempts to carry out a methylation of Ge_i in aqueous model systems were made with Ge^{IV} (GeO_2), in this study we attempt to identify a methylation of Ge^{II} (GeI_2) by CH_3I using the methods of the successful experiments with tin and lead. Of course, the known chemical synthetic reactions of CH_3I with Ge^{II} have not been carried out on systems directly relevant to the environment.

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0268–2605/92/020173–06 $05.00

Received 27 July 1991
Accepted 30 November 1991

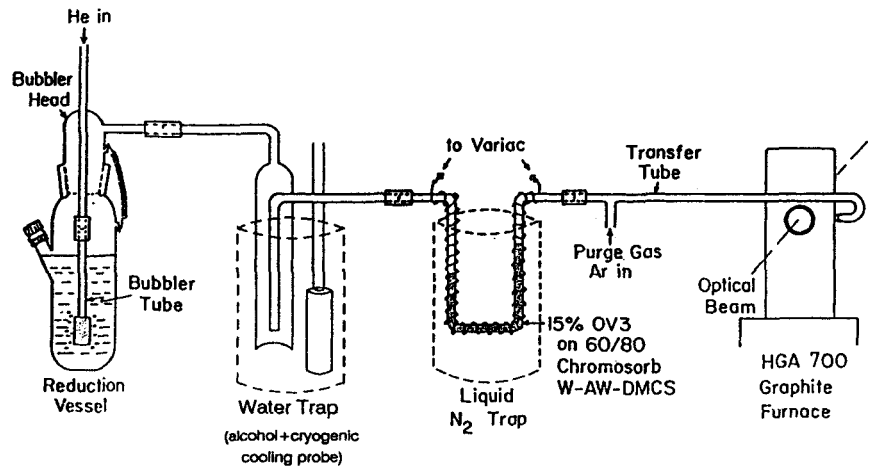

Figure 1 Apparatus for determination of inorganic and methylgermanium species.

EXPERIMENTAL

Standards and reagents

Inorganic germanium standards were made by serial dilution of a commercially available 1000 ppm germanium standard solution in the form of sodium hexafluorogermanate (Johnson Matthey, Seabrook, NH, USA). The working standard had a concentration of 100 ppb germanium. Monomethylgermanium trichloride, dimethylgermanium dichloride and trimethylgermanium chloride (Alfa products, Karlsruhe, Germany) were used to make the Me_xGe standards. They were prepared by serial dilution of the pure compound in deionized water down to concentrations of 13–18 ppb.

All other reagents were commercially obtained and were of analytical grade purity.

Apparatus

We used a slight modification of the gas chromatography–atomic absorption spectroscopy (GC AA) combination described by Hambrick *et al.*[5] for the analyses of Me_yGe compounds ($Me_yGe = Ge_i + Me_xGe$). This system consisted of a hydride generation vessel, a water trap, a U-shaped glass tube filled with chromatographic material and a heating wire coiled round it. An atomic absorption spectrometer (AA) was used as detector (Fig. 1). The Me_yGe compounds were reduced in the hydride generation vessel, by addition of sodium borohydride, to the corresponding volatile germanes. The germanes and other volatile substances were stripped from the aqueous matrix by a helium stream (40 $cm^3 min^{-1}$). After being dried in the water trap, all volatile compounds condensed on the chromatographic column, which was cooled by liquid nitrogen to −196 °C. The column was connected to the left internal argon inlet of the graphite furnace. To reduce the negative influence of helium on the sensitivity and the lifetime of the graphite tubes, the helium carrier stream was mixed with argon (100 $cm^3 min^{-1}$) prior to entering the furnace.[6] After reaction and stripping was complete the liquid nitrogen was removed, the column was slowly heated by the heating wire and all compounds eluted according to their boiling points. The eluting substances were purged to the graphite furnace and were analysed for germanium by AA. The different Me_yGe compounds were identified by their retention times. Full details of the apparatus, reaction conditions and glassware handling are described elsewhere.[5,6]

We used this system with modifications as described below. The U-shaped water trap was changed to a cylindrical one (Fig. 1). This trap has a smaller size, it is easier to handle and it is more stable against damage. Instead of the Perkin–Elmer 5000 AA spectrometer, we used a Perkin–Elmer 1100 B AA spectrometer equipped with a specially made analogue output, which is connected to an integrator. The graphite furnace programme cycle was changed so that the temperature of 2700 °C during analysis was decreased to prevent disintegration of the graphite tubes and contact rings. We have chosen a temperature of 2400 °C because the sensitivity for all Me_yGe decreases sharply below 2300 °C. The following furnace cycle was used. Step 1: temp. 30 °C; ramp 1 s, hold 5 s; step 2: temp. 2900 °C, ramp 2 s, hold

3 s; step 3: temp. 2400 °C, ramp 1 s, hold 10 s; step 4: temp. 2400 °C, ramp 1 s, hold 60 s; step 5: temp. 30 °C, ramp 10 s, hold 5 s. We found that cysteine increases the sensitivity of MMGe about 10% and for DMGe about 300%. Ge_i and TMGe are not influenced. Therefore we add cysteine–HCl (Sigma) at a final concentration of 0.5 g dm^{-3} to the hydride generation vessel prior to borohydride reduction.

Analytical procedure

Depending on the Ge_i concentration used in a given experiment, 10–100 µl of the sample was transferred to the hydride generation vessel, and deionized water (25 cm^3), 2 mol dm^{-3} Tris–buffer solution adjusted to pH 6.0 (1.25 cm^3), 0.2 mol dm^{-3} EDTA (0.2 cm^3) and 0.23 mol dm^{-3} cysteine–HCl (0.5 cm^3) were added. The generation vessel was attached to the apparatus with a clamp and the solution was purged with helium for at least 3 min. Then the chromatographic column was immersed in liquid nitrogen and the hydride generation was started by injecting 1.25 cm^3 of 20 % aqueous $NaBH_4$ solution (Fluka) with a syringe. After the solution was stripped for 10 min, the liquid nitrogen was removed and the power supply of the heating wire, the furnace cycle, as well as the integrator were started. The Me_yGe compounds elute within 1 min. Quantification was done by the method of standard additions.

Experimental procedure

Three single-factor experiments were conducted to evaluate the methylation efficiency of $Ge^{II}I_2$ by CH_3I at one pH and by CH_3-CoB_{12} at two pH values (Table 1). Conditions similar to those of Fanchiang and Wood[7] in their experiments on methylation of tin(II) chloride by CH_3-CoB_{12} were used in these experiments.

In addition, two three-way factorial experiments (Table 2) were carried out to test the influence of combinations of the three independent factors CH_3I, CH_3-CoB_{12} and the oxidizing agent MnO_2. All reactions were carried out in darkness and at room temperature, in 120-cm^3 serum bottles sealed with grey butyl rubber stoppers. The volume of the reaction solution in the serum bottles was always 50 cm^3.

Final concentrations and final matrices for the single-factor experiments were obtained by mixing double-concentrated, freshly prepared stock solutions directly in the aluminium-foil-covered serum bottles to a final volume of 50 cm^3. The 600 µmol dm^{-3} methylcobalamin stock solution was prepared by dissolving 160 mg of CH_3CoB_{12} in 200 cm^3 of deionized water in an aluminium-foil-covered flask. For the different experiments, GeI_2 stock solutions were made in different matrices. Deionized water was used for the pH 6.5 and 0.2 mol dm^{-3} HCl (double the final concentration) for the pH 0.9 experiment. To avoid oxidation of the Ge^{II} during the dissolution, the different matrices were de-aerated by boiling them for 10 min and cooling them under a stream of nitrogen. Then the amount of GeI_2 required to obtain a double-concentrated stock solution was added and dissolved by ultrasonication. Methyl iodide, as the pure compound, was injected through the rubber stopper. Blanks were made by adding 25 cm^3 of de-aerated deionized water (DDW), prepared in the same way as the GeI_2 matrices, to 25 cm^3 of each single-component

Table 1 Experimental design and yields of single-factor experiments

		Stock solution					Yield (%)		
Expt	Compound	Concn (µmol dm^{-3})	Volume added (cm^3)	Final concn (µmol dm^{-3})	Final matrix	pH	MMGe	DMGe	TMGe
1	GeI_2	200	25	100	0.1 mol dm^{-3} HCl	0.9	1.6	0	0
	CH_3I	Pure	5×10^{-3}	1600					
	CH_3-CoB_{12}	0	0	0					
2	GeI_2	300	25	150	0.1 mol dm^{-3} HCl	0.9	1.3	0	0
	CH_3I	0	0	0					
	CH_3-CoB_{12}	600	25	300					
3	GeI_2	300	25	150	Deionized water	6.5	0	0	0
	CH_3I	0	0	0					
	CH_3-CoB_{12}	600	25	300					

Table 2 Experimental design and yields of factorial experiments

Factorial assay	DDW (cm^3)	MnO_2[a]	CH_3-CoB_{12}[a]	CH_3I[a]	Yield (MMGe) (%) KCl	ASW
1	25.0	–	–	–	0	0
2	12.5	+	–	–	0	0
3	12.5	–	+	–	0	0
4	—	+	+	–	0	0
5	25.0	–	–	+	1.9	6.3
6	12.5	+	–	+	1.2	1.9
7	12.5	–	+	+	2.6	6.5
8	—	+	+	+	0	0.7
Final concn ($\mu mol\ dm^{-3}$)		10	10	320		

[a]+, 12.5 cm^3 of 40 $\mu mol\ dm^{-3}$ stock solution (CH_3-CoB_{12} or MnO_2) added; or 1 µl of pure CH_3I added.
–, Compound not added

stock solution. For the methyl iodide blank, 5 µl of CH_3I was injected into 50 cm^3 of DDW. All three methylation experiments with blanks were carried out in triplicate.

The methylation experiment with methyl iodide (experiment 1 in Table 1) was carried out under nitrogen. From the double-concentration GeI_2 stock solution, 25 cm^3 were transferred with a volumetric pipette to a serum bottle which was prepurged with nitrogen. During this transfer, the Ge^{II} stock solution was continuously mixed with a magnetic stirrer, because even at concentrations as low as 20 $\mu mol\ dm^{-3}$ GeI_2 does not dissolve completely. To obtain the final concentration of Ge^{II}, 25 cm^3 of DDW were added and the serum bottle was closed. Then 5 µl of pure methyl iodide was injected through the rubber stopper.

In experiments 2 and 3, 25 cm^3 of CH_3-CoB_{12} stock solution and 25 cm^3 of GeI_2 stock solution, either in deionized water (pH 6.5) or in 0.2 $mol\ dm^{-3}$ HCl (pH 0.9), were added to the serum bottles. The transfer of the CH_3-CoB_{12} solution was made in a darkened room using a volumetric pipette. After mixing, the assays were purged for 1 min with synthetic air before being closed.[7]

Experimental design and yields of the factorial experiments are recorded in Table 2. To obtain all possible combinations of three independent factors, $2^3 = 8$ different assays are necessary. Each assay was prepared in aluminium-foil-covered serum bottles similar to the single-factor experiments. After prepurging with nitrogen, each of the eight serum bottles was filled with 25 cm^3 of a 20 $\mu mol\ dm^{-3}$ GeI_2 stock solution which was prepared in de-aerated 0.2 $mol\ dm^{-3}$ KCl for the first, and in de-aerated, double-concentrated artificial seawater (ASW) for the second experiment. Single-composition ASW is: $MgSO_4.7H_2O$ (14 $g\ dm^{-3}$), NaCl (32 $g\ dm^{-3}$) and $NaHCO_3$ (0.2 $g\ dm^{-3}$); pH 7.6.[8] The volume of the reaction solution in the serum bottles was brought up to 50 cm^3 by adding different volumes of DDW or the MnO_2 or CH_3-CoB_{12} stock solutions according to Table 2. CH_3I was added by injecting 1 µl of the pure compound through the rubber stopper and was not considered to change the volume in the serum bottle. A 40 $\mu mol\ dm^{-3}$ stock solution of CH_3-CoB_{12} was prepared by dissolving 27 mg of the compound in 500 cm^3 of DDW in an aluminium-foil-covered flask. The 40 $\mu mol\ dm^{-3}$ stock solution of MnO_2 was prepared by diluting a 4 $mmol\ dm^{-3}$ solution of hydrous MnO_2 in DDW. The hydrous MnO_2 solution was made from $MnCl_2$ and $KMnO_4$ according the method of Murray.[9]

RESULTS AND DISCUSSION

Experimental design and yields of the single-factor experiments are recorded in Table 1. The yields are mean values of triplicates with standard deviations of 6.3% for the CH_3-CoB_{12} and 24% for the CH_3I experiments. Analyses were made after three and nine weeks of incubation. There are no differences in the yields of these two

incubation times. No Me_yGe-blanks have been found in assays containing CH_3I or CH_3-CoB_{12} on its own, nor Me_xGe-blanks in assays containing only GeI_2. The results show that methylation by CH_3-CoB_{12} occurs only at pH 1 and that MMGe is the only methylation product. The methylation mechanism of tin(II) chloride by CH_3-CoB_{12} is thought to be a free-radical mechanism according to Eqns [1]–[3]:[7]

$$(CH_3)Co^{III}B_{12} + Sn^{II} \rightarrow Co^{II}B_{12r} + (CH_3)Sn^{III\cdot} \quad [1]$$

$$(CH_3)Sn_2^{III\cdot} + O_2 \rightarrow (CH_3)Sn^{IV+} + O_2^- \quad [2]$$

$$Co^{II}B_{12r} + H_2O + O_2 \rightarrow H_2O\text{-}Co^{III}B_{12+} + O_2^- \quad [3]$$

The redox potential of Ge^{IV}/Ge^{II} (−0.13 V) makes substitution by a carbonium ion unlikely. Hence, one would predict a similar mechanism for the methylation of germanium.[3] In this case the attack of Ge^{II} results in a homolytic cleavage of the Co–C bond and the production of a $(CH_3)Ge^{III\cdot}$ radical by the transfer of a $CH_3^\cdot$ radical. The produced $(CH_3)Ge^{III\cdot}$ would then be oxidized by O_2 to the stable end-product $(CH_3)Ge^{IV}$. Evidence for this mechanism is given by the fact that no DMGe or TMGe is observed. If an electrophilic attack followed by a heterolytic cleavage of the Co–C bond and a carbanion transfer occurred, the first methylation product would be $(CH_3)Ge^{II}$. This could either be further methylated to $(CH_3)_2Ge^{II}$ or disproportionate to $(CH_3)_2Ge^{IV}$ and Ge^0 (Eqn [4]) according to a similar reaction involving tin.[10]

$$2(CH_3)Ge^{II} \rightarrow (CH_3)_2Ge^{IV} + Ge^0 \quad [4]$$

Both would lead to DMGe and TMGe [carbanion transfer to $(CH_3)_2Ge^{IV}$]. The absence of the higher methylated germanium species indicates two things, first, that the most feasible methylation mechanism of Ge^{II} by CH_3-CoB_{12} is a free-radical mechanism (see Eqns [1]–[3] for tin) and second, that MMGe is, in contrast to monomethyltin,[10] stable against disproportionation and dismutation (Eqns [4], [8]) under the conditions tested. At pH values below 1, CH_3-CoB_{12} exists mainly in the base-off form (uncoordinated 5,6-dimethylbenzimidazole[7]). The finding that no methylation occurred at pH 7 indicates that the base-off form is responsible for the methylation of Ge^{II} by CH_3-CoB_{12}.

Methylation of Ge^{II} by CH_3I to MMGe (Table 1) could proceed by the mechanism of oxidative addition suggested for tin according to the following equations ([5]–[8]) Ref. 4 and refs therein) (Y = ligand):

$$Y_2\overset{\curvearrowright}{Ge}: + \overset{\curvearrowright}{CH_3I}: \rightarrow Y_2Ge^{+\cdot} + CH_3^\cdot + I^- \quad [5]$$

$$Y_2Ge^{+\cdot} + I^- \rightarrow IY_2Ge^\cdot \quad [6]$$

$$IY_2Ge^\cdot + CH_3^\cdot \rightarrow CH_3GeY_2I \quad [7]$$

The oxidation state of germanium in the MMGe produced is +IV. Further methylation could be envisaged to occur by the following dismutation reaction:

$$2(CH_3)Ge^{IV} \rightarrow (CH_3)_2Ge^{IV} + Ge^{IV} \quad [8]$$

The lack of DMGe and TMGe strengthens our views on the stability of MMGe against dismutation according to the results on the CH_3-CoB_{12} experiments.

To confirm the single-factor results, we performed two factorial experiments under conditions more closely related to the environment. We lowered the concentrations of GeI_2 and methylating agents (Table 2) and used neutral pH. The first experiment was done in 0.1 mol dm^{-3} KCl (pH = 7.2) to maintain ionic strength and the second one in ASW (pH = 7.6) to simulate oceanic conditions. A factorial design was chosen to investigate the effect of all combinations of three independent factors on the methylation of Ge^{II}. The reaction solution was kept under nitrogen to hinder oxidation of Ge^{II}. We tested the factors CH_3I, CH_3-CoB_{12} and the oxidizing agent MnO_2. The MnO_2 was used to replace oxygen in Eqns [2] and [3] so that methylation by CH_3-CoB_{12} could occur under anaerobic conditions. Analyses of the Me_xGe produced were made after an incubation time of four weeks. Results are shown in Table 2. No DMGe or TMGe was observed under any combination. The response of the different combinations in KCl and ASW is qualitatively the same, but the yields of MMGe are higher by a factor of 3 in ASW. A clear positive effect was given by CH_3I alone, whereas CH_3-CoB_{12} does not methylate germanium on its own or together with MnO_2. The combination of CH_3I with CH_3-CoB_{12} gives an increase in response from 1.9% to 2.6% in KCl but not a significant increase in ASW. The lack of higher methylated germanium species in this case indicates that CH_3-CoB_{12} does not function as a carbanion donor for the $(CH_3)Ge^{IV}$ produced through oxidative addition

by CH_3I. A negative influence was found for MnO_2, probably due to absorption of Ge^{II} to its surface as was observed for tin.[10] Methyl iodide is a naturally occurring methylating agent, which was found in concentrations of up to 3 μmol dm^{-3} above Laminaria beds in south-west Ireland.[11] The successful methylation experiments with 10 μmol dm^{-3} CH_3I in ASW indicate that a methylation of Ge^{II} to MMGe could occur in the oceans. However, germanium is considered to exist in the +IV state in the oceans.[12] A methylation of Ge^{IV} is not possible by oxidative addition of CH_3I.

CONCLUSIONS

At low pH values, where the base-off form is dominant, CH_3-CoB_{12} is able to methylate Ge^{II} forming MMGe as the primary product. The most feasible mechanism for this is a free-radical attack rather than an electrophilic attack, due to the fact that no DMGe or TMGe is observed. At neutral pH, CH_3-CoB_{12} appears not to play an important role, whereas the highest production of MMGe (6% of Ge^{II}) is obtained from the reaction with CH_3I in artificial seawater. The mechanism of methylation of Ge^{II} by CH_3I is assumed to be an oxidative addition of a CH_3^+ to the lone pair of electrons of Ge^{II}. Methyl iodide found in the oceans can possibly drive the methylation reaction of Ge^{II}.* However, because germanium is most likely in the +IV state in the oceans, methylation by CH_3I is not very plausible. Because MMGe appears to be stable against dismutation, methylation by CH_3I would only be an additional source for the oceanic MMGe and not for the DMGe. Although the experiments described above have shown that a chemical methylation of germanium to MMGe by CH_3I is possible, it is not clear whether this reaction contributes to the methylgermanium compounds found in natural waters.

* Other methyl carbonium ion sources also occur, e.g. DMSP (dimethyl sulphopropiothetin).

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