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Reactions of Methylcobalamin with Tin and Lead Compounds

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Abstract

Reactions of $CH_3[Co]$ with $(CH_3)_nM^{(4-n)+}$ (n = 2, 3; M = Sn, Pb) at concentrations high enough to detect $(CH_3)_4M$ in the head space (yields 7.08×10^{-5}–2.06×10^{-5}%), indicate that dismutation is the major route of production. Similarly, kinetic reactions at lower concentrations show that no demethylation of $CH_3[Co]$ by $(CH_3)_3M^+$ (M = Sn, Pb) occurs after 60 days. From the methylation of $SnCl_2$ by $CH_3[Co]$ at pD 1.0 and under aerobic conditions, the following hydrolysis species were observed in the 400 MHz ^1H NMR spectrum: $CH_3Sn(OH)Cl_2 \cdot 2H_2O$ (63.6%), $[CH_3Sn(OH)(H_2O)_4]^{2+}$ (17.6%) and $CH_3Sn(OH)_2Cl \cdot nH_2O$ (18.8%). No methylation products were observed from similar reactions with Pb(II) salts.

Introduction

Concern about methylation of metals arises from increased toxicity of organometallic species in comparison with their inorganic counterparts [1, 2]. Use of organolead gasoline anti-knock additives and the increase in use of organotin compounds from 5,550 tons in 1965 to 33,000 tons in 1980 [3] renders the study of environmental cycling of these metals, via methylation reactions, important. Electrophilic attack by Pb(IV) and Sn(IV) compounds on CH_3^- is the obvious pathway for their methylation, whilst a similar electrophilic attack by Sn(II) or Pb(II) would require a preceding oxidising step to Pb(IV) and Sn(IV).

It has been pointed out that naturally occuring $CH_3[Co]$ (a vitamin B_{12} coenzyme) is the only biochemical methylating agent capable of transferring CH_3^- to an oxidised metal (M^{n+}) [4–8]. The other main natural methylating systems, S-adenosylmethionine and ^5N-methyltetrahydrofolate, essentially transfer methyl groups as CH_3^+. The CH_3^- transfer step to mercury(II), shown in eqn. 1, may occur both enzymatically or non-enzymatically. There is some evidence that Me[Co] does play a part in natural methylations [6, 9] but there is no decisive evidence for an enzymatic role in such processes.

$$CH_3[Co] + Hg^{2+} \xrightarrow{H_2O} CH_3Hg^+ + H_2O[Co]^+ \qquad (1)$$

Under different conditions $CH_3[Co]$ may transfer a methyl group as $CH_3\cdot$ or CH_3^+. This raises the possibility of free radical transfer to reduced metals (Pb(0), Sn(0)), lower oxidation metals (Sn(II), Pb(II)), or complexed metals. The way in which standard electrode potentials may control methylation (CH_3^- or $CH_3\cdot$) has been discussed [4]. The problem has to date been discussed as though most metals were present in the environment as free cations or aquocations, but this is not often the case. Metals in soils or sediments may be complexed by natural organic ligands, often containing sulphur. Mercury is known to be present in sediment as Hg(0) as well as complexed Hg(II). In these circumstances it is not easy to predict the electronic environment of metals. If they are present in an 'electron-rich' situation, then CH_3^+ or $CH_3\cdot$ transfer may become possible. Reactions for methylation of a variety of metals by $CH_3[Co]$ in aqueous abiotic media have not produced conclusive results.

In kinetic studies of $CH_3[Co]$, with $(CH_3)_3M^+$ (M = Sn, Pb) demethylation of $CH_3[Co]$ occurred, but volatile $(CH_3)_4M$ products were not determined. Rate reactions were also incorrectly derived [10]. In contrast it has been reported that a number of inorganic Sn(IV) salts under different conditions of ionic strength and pH do not react with $CH_3[Co]$ [8]. Demethylation of $^{14}CH_3[Co]$ by inorganic Pb(IV) compounds was also reported to occur, but methylmetal products were not detected [11]. Studies in aqueous abiotic systems show that methylation of Sn(II) by $CH_3[Co]$ may occur via formation of $CH_3\cdot$ from reductive homolytic cleavage of the CH_3–Co bond, methylation of Sn(II) to $CH_3Sn\cdot^{III}$ and oxidation by a weak oxidant such as O_2 to form CH_3Sn^{IV} (eqns. 2 and 3).

$$CH_3[Co] + Sn^{II} \rightleftharpoons [Co^{II}] + CH_3Sn\cdot^{III} \qquad (2)$$

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$$CH_3Sn\cdot^{III} \overset{O_2}{\rightleftharpoons} CH_3Sn^{IV} \tag{3}$$

However the identity of the CH_3Sn^{IV} and the value of J $Sn^{117,119}$–^1H constant were not clearly established by ^1H NMR [12]. Reports on similar reactions with Pb(II) do not exist but its methylation to $(CH_3)_4Pb$ by a $CH_3[Co]$ model compound ($(CH_3)_2Co(N_4)ClO_4\cdot H_2O$) in acetonitrile and in water [13, 14] indicate that such a process may be feasible. Although other naturally occurring biochemical metabolites such as CH_3I or $(CH_3)_3\overset{+}{N}(O\bar{H})(CH_2)_n$-COOH may methylate Pb(II) and Sn(II) by CH_3^+ oxidative addition reactions [15], in view of the conflicting reports methylation reactions of the CH_3^- donating naturally-occurring $CH_3[Co]$ need further investigation.

In the present study experiments using $CH_3[Co]$ and $(CH_3)_3M^+$ or $(CH_3)_2M^{2+}$, at high enough concentrations to detect any volatile $(CH_3)_4M$ (M = Sn, Pb), were performed in aqueous abiotic media. To confirm demethylation kinetics of $CH_3[Co]$ by these compounds, pseudo first order reaction kinetics were carried out at lower concentrations. Reactions of Sn(II) and Pb(II) salts with $CH_3[Co]$ were also studied by ^1H 400 MHz NMR to establish the identity of the CH_3Sn^{IV} species, measure the J $Sn^{117,119}$–^1H constant, and to determine the feasibility of similar processes taking place for Pb(II).

Experimental

Reactions of $(CH_3)_nM^{(4-n)+}$ (n = 2, 3; M = Sn, Pb) for the detection of $(CH_3)_4M$ were performed in 30 cm^3 deoxygenated aqueous solutions, buffered at pH 4.5 (pthalate), made to 0.1 M in HOAc and NaOAc ($(CH_3)_3PbOAc$ reaction only) or 0.1 M in HCl and NaCl ($(CH_3)_3SnCl$, $(CH_3)_2SnCl_2$, $(CH_3)_3PbCl$ and $(CH_3)_2PbCl_2$ reactions). All solutions were made 4.6×10^{-3} M in $CH_3[Co]$ and 8.3×10^{-3} M in $(CH_3)_2M^{2+}$ or $(CH_3)_3M^+$, with blanks containing 8.3×10^{-3} M of $(CH_3)_3M^+$ or $(CH_3)_2M^{2+}$ only. $CH_3[Co]$ was used at the above concentrations because it has been reported that at higher concentrations it forms a less reactive dimer [12]. Reactants were placed in 50 cm^3 vials, sealed with 'crimp on' teflon-lined silicon-rubber septa, and were left in a darkroom for 60 days. Head space gases were analysed by direct sampling with a gas tight syringe and injecting in a gc-fid system (PYE 104). A glass column (1.8 m length, 4 mm i.d.) packed with 10% SP-2100 on Chromosorb W 80/100 mesh was used for all such analyses. Conditions included a carrier gas flow of 30 cm^3 min^{-1}, injector temperature 100 °C, oven and detector temperatures 60 °C, retention time for Me_4Sn of 2.4 min (limit of detection 0.15 μg dm^{-3}). Under similar conditions but with oven and detector temperatures of 70 °C Me_4Pb eluted after 5.1 min (limit of detection 0.13 μg dm^{-3}).

Quantitation was carried out by the method of standard additions, using peak height measurements. Identity of the products was confirmed by gc-ms (PYE 204 gc–d coupled to magnetic deflection VG-micromass 16 F; ionisation mode, electron impact; emission current 20 mA; electron energy 70 eV, accelerating voltage 4 KV; resolution 1500 at 10% valley). A 50 m 1% SP-2100 (WCOT) capillary column was used for all gc-ms analyses. For He carrier gas flow of 2 cm^3 min^{-1}, injector temperature 100 °C, oven temperature 80 °C and interface temperature 250 °C, Me_4Sn eluted after 3.55 min (limit of detection 2.0 μg dm^{-3}) whilst under similar conditions but with an oven temperature of 120 °C, Me_4Pb eluted after 3.96 min (limit of detection 3.5 μg dm^{-3}).

Kinetic reactions were carried out in deoxygenated, phthalate buffered aqueous solutions at pH 4.5, containing $(CH_3)_3SnCl$ or $(CH_3)_3PbOAc$ and $CH_3[Co]$. They were also made either 0.1 M in HCl and NaCl ($(CH_3)_3SnCl$ reactions) or 0.1 M HOAc and NaOAc ($(CH_3)_3PbOAc$ reactions). Pseudo first-order reaction conditions were followed with four $(CH_3)_3M^+$ solutions made 15, 10, 7.5 and 5×10^{-3} M. Concentrations of $CH_3[Co]$ in all cases were kept constant at 5×10^{-5} M. The rate laws for these conditions take the form:

$$-\frac{d[CH_3[Co]]}{dt} = k[(CH_3)_3M^+]^n[CH_3[Co]] \tag{4}$$

for large excess of $(CH_3)_3M^+$ (where $[(CH_3)_3M^+]_t \sim [(CH_3)_3M^+]_o$) its concentration remains virtually constant and may be incorporated into the rate coefficient to give a new coefficient $k_1' = k\,[(CH_3)_3M^+]_o$. The rate law then simplifies to:

$$-\frac{d[CH_3[Co]]}{dt} = k_1'[CH_3[Co]] \tag{5}$$

because

$$\begin{aligned} -\frac{d[CH_3[Co]]}{dt} &= k[(CH_3)_3M^+]^n[CH_3[Co]] \\ &= k_1'[CH_3[Co]] \end{aligned} \tag{6}$$

$$k_1' = k[(CH_3)_3M^+]^n \tag{7}$$

and

$$\log k_1' = \log k + n \log [(CH_3)_3M^+] \tag{8}$$

Plotting $\log k_1'$ *vs.* $\log [(CH_3)_3M^+]$ would give n as slope and k as the intercept. k_1' values could initially be obtained after integrating eqn. 5 to:

$$\ln\left(\frac{[CH_3[Co]]_t}{[CH_3[Co]]_o}\right) = -k_1't \qquad (9)$$

Plotting ln ($[CH_3[Co]]_t/[CH_3[Co]]_o$) *vs.* t for each $(CH_3)_3M^+$ initial concentration would yield k_1' as the slope of the graph. For the above experiments reactants were placed in 5 cm^3 matched sets of quartz cells, covered with aluminium foil, and placed in a darkroom. Analysis by UV–Vis spectrophotometry (Perkin–Elmer 555) was carried out every 7 days for 60 days using a 5 × 10^{-5} M $CH_3[Co]$ solution in the reference position. Absorbance increase at 351 nm (absorbance maximum for $H_2O[Co]^+$) and absorbance decrease at 538 nm (absorbance maximum of $CH_3[Co]$) were followed. Experiments were performed twice with two different batches of $CH_3[Co]$ (Sigma) which had the correct extinction coefficient ((I) 11.23 × 10^{-4} cm^{-1} M^{-1} and (II) 12.05 × 10^{-4} cm^{-1} M^{-1} [16]).

Reactions of Sn(II) and Pb(II) salts with CH_3-[Co] were performed according to a literature method [12]. Reactants were placed in flasks covered with aluminium foil and solutions in D_2O were made 15 × 10^{-3} M in $SnCl_2$ and 0.1 M in DCl or 15 × 10^{-3} M in $Sn(AOc)_2$ and 0.2 M in CD_3COOD. For Pb(II) salts, solutions in D_2O were made 15 × 10^{-3} M in $PbCl_2$ and 0.3 M in DCl or 15 × 10^{-3} M in $Pb(OAc)_2$ and 0.2 M in CD_3COOD. All solutions contained $CH_3[Co]$ at 1.5 × 10^{-3} M concentrations. Blanks of 1.5 × 10^{-3} M $CH_3[Co]$ in D_2O were also made containing the appropriate amounts of DCl or CD_3COOD, and were treated in a similar manner as the rest of the samples. After bubbling air for 10 min, flasks were kept in a darkroom for 7 days prior to analysis by ^1H NMR (Bruker 400 MHz F.T. NMR; S.E.R.C. facility at Warwick University, U.K.).

Results and Discussion

Results from reaction of $(CH_3)_nM^{(4-n)+}$ with $CH_3[Co]$, at 8.3 × 10^{-3} and 4.6 × 10^{-3} M respectively, are listed in Table I. Yields are higher in blanks than in vials containing $CH_3[Co]$, suggesting that a dismutation reaction of $(CH_3)_nM^{(4-n)+}$ species (eqns. 10 and 11) is the predominant pathway for production of volatile $(CH_3)_4M$.

$$4(CH_3)_2M^{2+} \rightleftharpoons 2(CH_3)_3M^+ + 2CH_3^+ + 2M^{2+} \qquad (10)$$

$$2(CH_3)_3M^+ \rightleftharpoons (CH_3)_4M + (CH_3)_2M^{2+} \qquad (11)$$

Disproportionation and dismutation of $(CH_3)_2M^{2+}$ to $(CH_3)_4M$ would proceed via eqns. 10 and 11 whilst dismutation of $(CH_3)_3M^+$ to $(CH_3)_4M$, via reaction 11. However $(CH_3)_4M$ products could not be detected from $(CH_3)_2M^{2+}$ reactions because of diminishing yields and relatively low initial concentrations. $CH_3[Co]$ will not methylate $(CH_3)_3M^+$, and yields due to dismutation from such reactions (($(CH_3)_3M^+$ + $CH_3[Co]$; pH 4.5, 60 days) are diminished. At pH 7 it is expected that an equilibrium between the 'base on' (benzimidazole co-ordinated to cobalt) and the 'base off' (uncoordinated benzimidazole) form of $CH_3[Co]$ exists. Concentration of the 'base off' fraction increases with lowering the pH. It can only be assumed that the $(CH_3)_3M^+$ moiety is coordinated to the N3 nitrogen of the 5,6-dimethyl benzimidazole (Fig. 1), as in the case of the slow reaction of Hg^{2+} with $CH_3[Co]$ [4].

TABLE I. Reactions of $CH_3[Co]$ with $(CH_3)_3M^+$ and $(CH_3)_2M^{2+}$ Salts (M = Sn, Pb).[a]

Compound	$(CH_3)_4M$ products (ng)	Yield (%)[c]
(1) $(CH_3)_3SnCl$	4.0	2.38 × 10^{-5}
(2) $(CH_3)_3SnCl$[b]	8.5	7.08 × 10^{-5}
(3) $(CH_3)_2SnCl_2$	–	
(4) $(CH_3)_2SnCl_2$[b]	–	
(5) $(CH_3)_3PbOAc$	9.8	3.36 × 10^{-5}
(6) $(CH_3)_3PbOAc$[b]	14.4	4.95 × 10^{-5}
(7) $(CH_3)_3PbCl$	6.0	2.06 × 10^{-5}
(8) $(CH_3)_3PbCl$[b]	9.8	3.36 × 10^{-5}
(9) $(CH_3)_2PbCl_2$	–	
(10) $(CH_3)_2PbCl_2$[b]	–	

[a] Concentration of $(CH_3)_3M^+$ and $(CH_3)_2M^{2+}$ was 8.33 × 10^{-3} M. [b] Samples did not contain any $CH_3[Co]$. [c] Assuming maximum yield that could be producted by methylation from 1.4 × 10^{-4} mol of $CH_3[Co]$ (the limiting reagent) is 1.4 × 10^{-4} mol of $(CH_3)_4M$.

This could be a plausible explanation for the lower yields of $(CH_3)_4M$ from dismutation of $(CH_3)_3$-

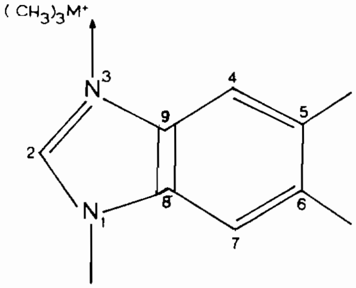

Fig. 1. $(CH_3)_3M$ coordinated to N3 of the $CH_3[Co]$ benzimidazole ring.

TABLE II. NMR Spectra of Reactions of Sn(II) Salts with CH_3[Co].

Compound	δ(CH_3)	J $Sn^{117,119}-{}^1H$ (Hz)	% Abundance
$CH_3Sn(OH)Cl_2 \cdot 2H_2O$	−4.1315[a] −2.5765[b]	126	63.6
$[CH_3Sn(OH)(H_2O)_4]^{2+}$	−4.1584[a] −2.6034[b]	128	17.6
$CH_3Sn(OH)_2Cl \cdot nH_2O$	−4.1742[a] −2.6292[b]	c	18.8
$CH_3Sn(OAc)_3$	−4.3744[a] −2.8194[b]	d	100

[a]Values quoted downfield from H_2O. [b]Values quoted downfield from dioxan. [c]Very low intensity could not be calculated. [d]Very low intensity satellite peak; spectra stored with the Editors-in-Chief.

M^+ in the presence of CH_3[Co] (eqn. 11), although conditions that would promote this reaction are unknown. Only S^{2-}, sulphur containing amino acids and oxygen ligands have been reported to enhance yields of $(CH_3)_4M$ from dismutation reactions of $(CH_3)_3M^+$ [17–20].

Reactions analysed by UV–Vis spectrophotometry every 7 days for 60 days did not indicate any increase in absorbance at 351 nm or any decrease at 538 nm. For this time span and with reference CH_3[Co] and samples treated similarly a significant variation of the absorbance values at the above mentioned wavelengths should have occurred. Repetition of the experiment with a different batch of CH_3[Co] gave similar results.

Reactions of CH_3[Co] with $SnCl_2$ yielded three CH_3Sn^{IV} species at 0.9375δ, 0.9466δ and 0.9308δ (Fig. 2). For greater accuracy their chemical shifts were recorded downfield from both dioxan and water (Table II). Peak areas were used for calculations of their relative abundance and J $Sn^{117,119}-{}^1H$ constants were found to be in the range of 126–128 Hz (literature values: 65 Hz [12]; 121–132 Hz [21]). After comparing coupling constants and chemical shifts with literature values, it was established that the species present were $CH_3Sn(OH)Cl_2 \cdot H_2O$, $[CH_3Sn(OH)(H_2O)_4]^{2+}$ and $CH_3Sn(OH)_2Cl \cdot nH_2O$. Their identity has been explained in terms of %S character of the Sn–C bond and comparison of theoretical with observed J values [21]. It appears that either the product from the above reaction is not CH_3SnCl_3, or that any CH_3SnCl_3 formed hydrolyses during 7 days to three hydrolysis products. Data from the reaction of $Sn(OAc)_2$ with CH_3[Co] suggest that CH_3Sn^{IV} may have been formed but coupling constants could not be calculated. Chemical shifts agree with CH_3Sn^{IV} values from corresponding $SnCl_2$ reactions; however formation of CH_3Sn^{IV} from this reaction cannot be certain.

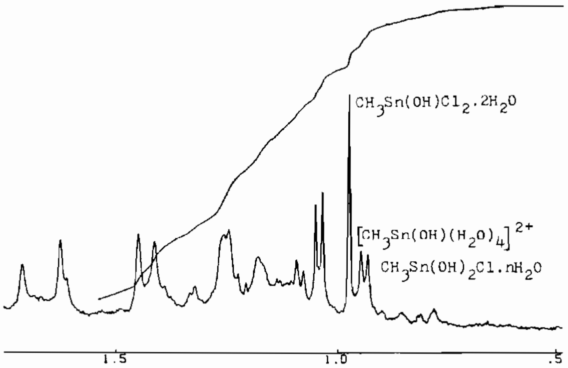

Fig. 2. ^1H NMR spectrum of CH_3Sn^{IV} species ($SnCl_2$ + CH_3[Co]).

Monomethyllead products were not observed from either $PbCl_2$ or $Pb(OAc)_2$ reactions, possibly because it is difficult to oxidise Pb(II) to Pb(IV) via a $Pb^{III}\cdot$ using O_2 as an oxidant. Standard electrode potentials indicate that a much stronger oxidising agent is needed to effect such a process (E^θ Pb^{4+}/Pb^{2+} = 1.46 V; E^θ $O_2 + 2H^+/H_2O_2$ = 0.68 V). However, use of stronger oxidising agents may have resulted in breaking of the CH_3–Co bond via oxidation of the metal (Co^{3+}/Co^{2+} = 1.81 V). Direct CH_3^- transfer to Pb(II) as in the case of a CH_3[Co] model ($(CH_3)_2Co(N_4)ClO_4 \cdot H_2O$) could have resulted in formation of CH_3Pb^+ or $(CH_3)_2Pb^{(II)}$ [14]. Their disproportionation (eqns. 12, 13) would have yielded $(CH_3)_2Pb^{2+}$ and $(CH_3)_4Pb$ but neither dissolved species could be detected by NMR.

$$2CH_3Pb^+ \longrightarrow CH_3Pb^{2+} + Pb^0 \qquad (12)$$

$$2(CH_3)_2Pb \longrightarrow (CH_3)_4Pb + Pb^0 \qquad (13)$$

Conclusions

Studies of reactions of $(CH_3)_nM^{(4-n)+}$ (n = 2, 3; M = Sn, Pb) with CH_3[Co] and analysis for volatile

$(CH_3)_4M$ indicated that the main process for its production is dismutation reactions and not methylation. Instead the benzimidazole ring of $CH_3[Co]$ may coordinate $(CH_3)_nM^{(4-n)+}$ and hinder dismutation reactions. Kinetic studies of $(CH_3)_3M^+$ showed that no reaction takes place in 60 days (60 days ~ $t_{1/2}$ for corresponding reaction with CH_3HgOAc [10]). It has also been established that $SnCl_2$ can be methylated by $CH_3[Co]$ to $MeSn^{IV}$ and the identity, relative abundance and coupling constants of the three hydrolysis products have been established. It is however difficult to envisage that such specialised conditions could be met in the natural abiotic or enzymatic environment.

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