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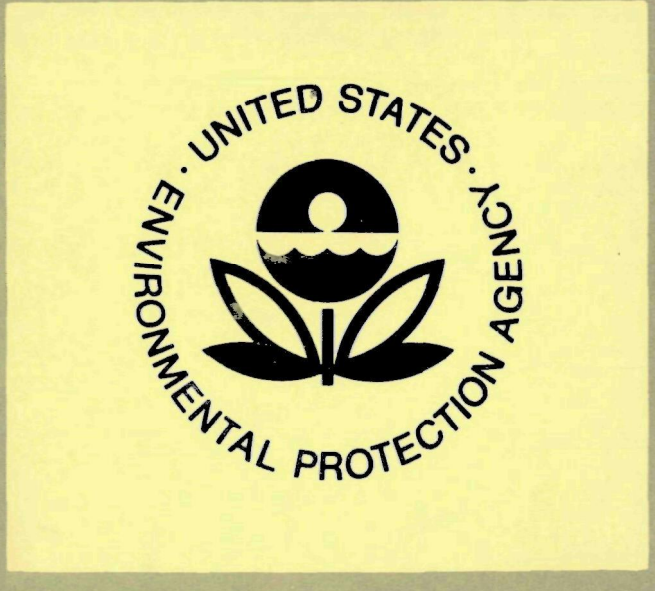
Environmental Health Effects Research Series

COMPARATIVE METHYLATION CHEMISTRY OF PLATINUM, PALLADIUM, LEAD, AND MANGANESE



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COMPARATIVE METHYLATION CHEMISTRY OF PLATINUM, PALLADIUM, LEAD, AND MANGANESE

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ABSTRACT

A study was carried out to evaluate the potential for platinum, palladium, lead, and manganese salts and oxides to be biochemically methylated. Methylation is an important, well recognized determinant of metal toxicity, the striking example being the extreme health hazard of methylated mercury. The possible biological methylation of the metals which are associated with emissions arising from the use of automotive fuels, fuel additives, and catalytic control devices is of special concern to the Environmental Protection Agency Fuel and Fuel Additives Program.

Salts of platinum, palladium, and lead and oxides of lead all containing the metal in a 4^+ valence were observed to demethylate methylcobalamin, a biologically active form of vitamin B-12. Inorganic salts and oxides of manganese were unreactive. No evidence for a stable monomethyl-metal derivative was found using palladium and lead compounds as reactants. However, salts of platinum 4^+ do result in the formation of stable methylation products. The reaction product formed from methylcobalamin and hexachloroplatinate was shown definitively to be a monomethyl-platinum compound. It is sufficiently stable in aqueous solutions under a variety of conditions to exist in freshwater ecosystems and to exhibit toxic effects on mammalian cells.

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The technical assistance of Mr. Richard Ryon and Mr. James A. Happe of the Lawrence Livermore Laboratory General Chemistry Division were invaluable aids in this study. Mr. Ryon performed the analytical determinations for platinum using X-ray fluorescence, while Mr. Happe obtained the NMR spectrum of our [Me-^{14}C]Pt compound. I also wish to acknowledge that Mr. Robert E. Elson (Lawrence Livermore Laboratory Organic Materials Division) demonstrated the release of methyl groups from MeB-12 as MeCl under certain conditions.

SECTION I

CONCLUSIONS

Methylcobalamin (MeB-12), a biologically active form of vitamin B-12 is readily demethylated at 22°C under slightly acid conditions by halogen platinate salts and platinic sulfate. At pH 1.0 in the presence of 1.0 M sodium chloride (NaCl) and potassium hexachloroplatinate (K_2PtCl_6) almost half of the methyl groups appear as MeCl. This is indicative of an overall transfer from MeB-12 to ionic platinum and a subsequent transfer to chloride ions. At pH 2.0 in the absence of high concentrations of NaCl a mono-MePt compound is formed from MeB-12 and K_2PtCl_6. This methylated Pt compound has characteristic UV light absorption and NMR spectra, it is quite soluble in water, and it carries a net negative charge at pH 7.0. Time-storage experiments indicate that our Me-Pt product is sufficiently stable with respect to temperature, salt, and pH to exist in freshwater systems and to exhibit biological activity.

Salts containing ionic palladium (Pd) can also demethylate MeB-12 at pH 2.0 but the rates are much slower than are observed with Pt^{4+} salts. The three Pd compounds shown to be reactive were $K_2PdCl_6 > PdSO_4 > K_2PdCl_4$.

No significant demethylation was detected when MeB-12 was incubated at 22°C for as long as 24-48 hrs with various lead salts containing Pb^{2+} ions. However, salts and insoluble oxides containing lead in the 4+ valence state slowly demethylated MeB-12 to extents of 40-100% after 24 hrs at 22°C. Demethylation by Pb compounds was correlated with a parallel volatilization of the MeB-12 methyl group.

No reactivity was detected between MeB-12 and several manganous salts at any pH for periods up to 24-48 hrs at 22°. Manganese dioxide suspensions were also unreactive.

SECTION II

RECOMMENDATIONS

We have demonstrated that MeB-12 can be demethylated by three metals of interest to the EPA Fuel and Fuel Additives Program. Also, we have definitively shown that one platinum salt (K_2PtCl_6) reacts to form a rather stable mono-MePt compound that has never been described previously in the literature. As a consequence of these findings many new questions have been raised about the biochemical methylation of platinum and palladium in particular. It is recommended that additional research be carried out to characterize adequately the new MePt compound that we have purified as well as any stable methylation products that may be formed from platinic sulfate under neutral as opposed to acidic conditions. It should also be carefully determined whether the halogen palladate salts react with MeB-12 to generate small amounts of stable MePd. Furthermore, if it is determined in the near future that catalyst attrition products contain ionic forms of Pt and Pd, then the reactivity of small emission particles with MeB-12 should be examined. It would lend considerable credence to our in vitro studies on the methylation of Pt-salts if emission particles from catalyst equipped cars were found to demethylate MeB-12. However, even if such particles contain only unreactive, non-ionic Pt and Pd, this does not preclude their subsequent oxidation to reactive, ionic forms in the environment.

As soon as methylated forms of Pt and Pd have been characterized chemically, it will be imperative to carry out research on their biological effects. A logical approach would be to compare the cellular and molecular toxicity of the methylated compound with the parent metal salt used as a reactant with MeB-12. Some comparative studies should be feasible in the near future using our newly isolated mono-MePt material and K_2PtCl_6. Since the amounts of MePt available will be small, the biological test systems would be restricted to small animals (rodents) and cultured mammalian cells. The types of studies that I would recommend are as follows: 1) chronic low dose administration to rodents with subsequent monitoring of organ and subcellular organelle distribution; this could be coupled with assays of key enzymes known to be sensitive to SH reagents; 2) acute effects on cultured mammalian cells such as cell killing, growth inhibition, depression of macromolecular synthesis, and induction of DNA strand scissions; 3) induction of chromosome breakage and aberrations in cultured mammalian cells or human lymphocytes; and 4) mutagenicity in mammalian test systems using several established phenotypic markers.

SECTION III

INTRODUCTION

GENERAL

Methylation is an important determinant of metal toxicity, particularly for metals such as Hg, Pb, and Sn (1). There was little impetus for studying the possible biological methylation of heavy metals, however, until several investigators (1967-1970) described the conversion of $HgCl_2$ to both mono- and di-MeHg by lake bottom sediments, homogenates of decaying fish, methanogenic (sewage) anaerobes, and aerobic microorganisms. Wood et al. demonstrated that the Me donor in sewage bacteria was in fact MeB-12 (2). Several laboratories have since shown that free MeB-12 even in the absence of any cellular system will react to form first mono- and then di-MeHg (3-5).

In addition to acquiring preformed $MeHg^{1+}$ from the environment, evidence is accumulating that mammalian tissues containing the Hg^{2+} ion can convert it to $MeHg^{1+}$. This has been demonstrated with fish liver homogenates and again the methylating agent was shown to be MeB-12 (6). Based on several biological and chemical studies, MeB-12 seems to be generally responsible for the environmental methylation of not only Hg but also selenium and tellurium. No other known biological Me donor has yet been shown to alkylate these metals. These processes are biological in that they require a ubiquitous, biologically active compound (i.e. MeB-12) and they are mediated by cells which supply the Me group donor. However, there is no definitive evidence that any cell contains an enzyme that will accelerate the chemical reaction between MeB-12 and Hg^{2+} or any other metal ion. Consequently, we prefer to term the formation of $MeHg^{1+}$, or any other methylated metal, in the presence of MeB-12 as "biochemical methylation".

Because of the experience with $MeHg^{1+}$, the EPA Fuel and Fuel Additives Program continues to be concerned about the possible biochemical transformation of the oxides and soluble salts of Pt, Pd, Pb, and Mn. These four metals are associated with emissions arising from the use of automotive fuels, fuel additives, and hydrocarbon control devices. Pb and Mn are present in fuel additives, while Pt and Pd are used as smog control catalysts. These latter two metals are of special concern because they are novel pollutants with which mankind has limited biological experience (7,8). Soluble platinum compounds (Pt^{4+}) are more toxic to experimental animals than compounds of other metals of interest (Mn, Pb) when administered orally; however, palladium salts are more toxic than these metals when administered intravenously. Analyses of the impact of these control devices on the future use and demand for Pt and Pd indicate that these metals will appear at readily detectable levels in the environment by the end of the decade. Yet, there is no information as to the likely biotransformation (methylation) of Pt and Pd or the relative hazards to expect from any organo-Pt or Pd compounds.

OBJECTIVE

The objective of this project is to compare the methylation chemistry of Pt, Pd, Pb, and Mn using MeB-12 as a biological alkylating agent. The ability to demethylate MeB-12 in vitro is being used to indicate whether ionic forms of these four metals are potentially methylatable either in the environment or within mammalian tissues. Any of these metal ions which demethylate MeB-12 will then be studied in detail to determine whether such reactions yield stable Me-metal compounds. Once any newly discovered Me-metal compounds have been adequately characterized, their toxic effects on mammalian cells will have to be evaluated. Estimation of their effects at the cellular or macromolecular level is not within the scope of this methylation chemistry project.

We were prompted to suggest a thorough study of the biochemical methylation of Pt, in particular, because of an unconfirmed communication in 1971 which merely stated (no data given) that Pt^{4+} can demethylate MeB-12 (9). As will become apparent under RESULTS and DISCUSSION (SECTION V) we have obtained firm data that Pt can be biochemically methylated, yielding a rather stable organo-Pt compound. Furthermore, we have demonstrated chemical reactivity of MeB-12 with Pd and Pb ions for the first time. In view of our findings and recent reports (10,11) that microorganisms in lake sediments can form tri- and tetra-Me lead, continued research on the biochemical methylation of selected metals should be of vital interest to the EPA.

SECTION IV

MATERIALS and METHODS

All metal salts and oxides were purchased from either Chemical Procurement Labs, Research Organic/Inorganic Chem. Co., or the J.T. Baker Chem. Co. and were of the highest purity commercially available. Vitamin B-12 (cyanocobalamin) was obtained from Calbiochem; methyl iodide and other unlabeled alkyl iodides from Eastman Chem. Co.; [Me-^{3}H]methyl iodide (300 μCi/μmole) from International Chemical and Nuclear Corp.; and [Me-^{14}C]methyl iodide (10 μCi/μmole) from the New England Nuclear Corp.

MeB-12, [Me-^{14}C]MeB-12, and [Me-^{3}H]MeB-12 were synthesized in the dark from vitamin B-12 and either unlabeled or labeled methyl iodide following reduction of the vitamin with sodium borohydride (12). Ethyl B-12 and propyl B-12 were prepared in the same manner. Alkyl cobinamides were synthesized from the corresponding alkyl iodide and diaquocobinamide (13). The concentration of each alkyl corrinoid solution was determined from the absorbance of its α-band and published molar extinction coefficients (14). All light absorption spectra were taken at 22°C with a Cary Model 15 recording spectrophotometer using 1.0 ml solution volumes and a 1-cm light path. Complete phytolysis of any unreacted MeB-12 in solution was achieved by a 30 min exposure at 22°C to a 40 W tungsten lamp at a distance of 10 cm. Rates of absorbance change at 350 nm were monitored with a Gilford Model 240 spectrophotometer equipped with a Honeywell recorder. All pH measurements were made with a Beckman Model 1019 research pH meter. Radioactivity determinations were made at 5°C in a Packard Model 3320 liquid scintillation spectrometer using a water-miscible counting fluid. The platinum content of small segments of paper (chromatography and electrophoresis runs) and of unknown solutions was determined by X-ray fluorescence. The method used was energy dispersive analysis (Si detector, pulse height analysis) and the detection limit was about 0.1 nmole with a counting time of 15 min. Proton nuclear magnetic resonance spectra were taken at 5°C with a spectrometer designed in the General Chemistry Division of Lawrence Livermore Laboratory. It operates at 60 MHz and utilizes a Varian magnet and power supply. The spectrometer was locked to water solvent and signal to noise improvement was achieved by ensemble averaging methods. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal Me group reference.

Except where specifically stated otherwise, reaction-mixtures contained 40 μM concentrations of unlabeled or Me-labeled MeB-12 and they were carried out in the dark at 22°C at pH 2.0 in 0.01 M HCl. Other essential experimental details are given in legends to the corresponding figures and tables.

SECTION V

RESULTS and DISCUSSION

PLATINUM

We confirmed that in the presence of K_2PtCl_6 (+K_2PtCl_4) micromolar concentrations of MeB-12 are rapidly demethylated. The characteristics of this process were then studied in more detail. As seen in Fig. 1A, MeB-12 is converted to aquoB-12 with no other discernible corrinoid intermediates accumulating on the reaction pathway. Evidence for this is the presence of isosbestic points at 490 nm, 367 nm, and 335 nm (Fig. 1A) which almost exactly match the spectral cross-over wavelengths when MeB-12 is photolyzed with light directly to aquoB-12 (Fig. 1C). Figure 1B shows that K_2PtCl_4 alone is unreactive, while K_2PtCl_6 added alone results in a small amount of demethylation after 5 min. Based on the initial rates of 350 nm absorbance increase, the optimal pH for the reaction with K_2PtCl_6 (+K_2PtCl_4) is about 2 (Fig. 2). Reactivity diminishes rapidly below and above pH 2. Demethylation is neglibible at pH 7 even after 2 hrs of incubation. The initial reaction rate is influenced somewhat by the acid solvent used, but for any acid chosen the fastest rate occurs at pH 2. The inset in Fig. 2 shows that very small concentrations of K_2PtCl_4, far less than the MeB-12 and the K_2PtCl_6 levels, markedly accelerate the initial reaction rate. Initial reaction rates were measured on a recorder within seconds after rapid mixing of the components.

The stoichiometry of the demethylation by chloroplatinate clearly involves a 1:1 consumption of MeB-12 and the Pt^{4+} salt K_2PtCl_6 (Fig. 3). When the ratio of K_2PtCl_6 to MeB-12 is reduced to 0.25, the MeB-12 is demethylated to the extent of 25% and complete conversion to aquoB-12 can only be achieved by photolysis of the Me-cobalt bond with light (Fig. 3). The Pt^{2+} salt K_2PtCl_4 acts only catalytically to speed up the rate of the reaction. The initial reaction rate depends on the concentration of all three components (MeB-12, K_2PtCl_6, and K_2PtCl_4), but MeB-12 can be quantitatively demethylated after 2 hrs by the addition of K_2PtCl_6 alone (Fig. 4). An initial lag in the reaction when K_2PtCl_4 is omitted may represent the time required for traces of Pt^{2+} to be formed in the system. A lag followed by eventual complete demethylation is consistent with the suggestion (9) that the Pt^{2+} ion plays an essential role in the reaction mechanism as depicted below.

$$\text{MeB-12} + Pt^{2+} \rightarrow Pt^{2+}\cdot\text{MeB-12} \qquad (1)$$

$$Pt^{2+}\cdot\text{MeB-12} + {}^{*}Pt^{4+} \rightarrow Pt^{4+}\cdot\text{Me} + {}^{*}Pt^{2+} + \text{aquoB-12} \qquad (2)$$

The net overall reaction is then

$$\text{MeB-12} + Pt^{4+} \rightarrow Pt^{4+}\cdot\text{Me} + \text{aquoB-12} \qquad (3)$$

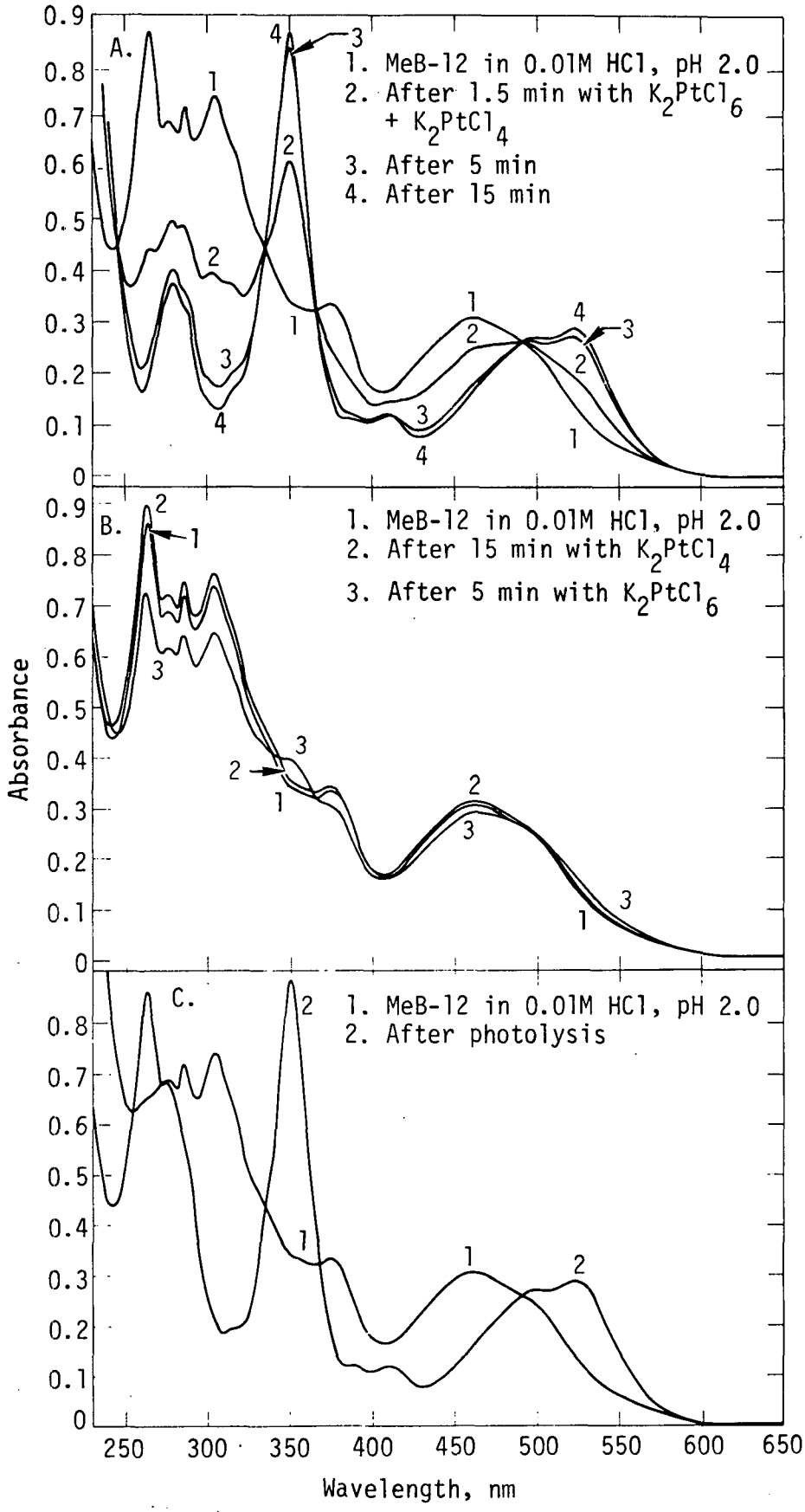

Figure 1. Demethylation of MeB-12 with K_2PtCl_6 in the presence of K_2PtCl_4. Each platinum salt was present at a concentration of 100 μM.

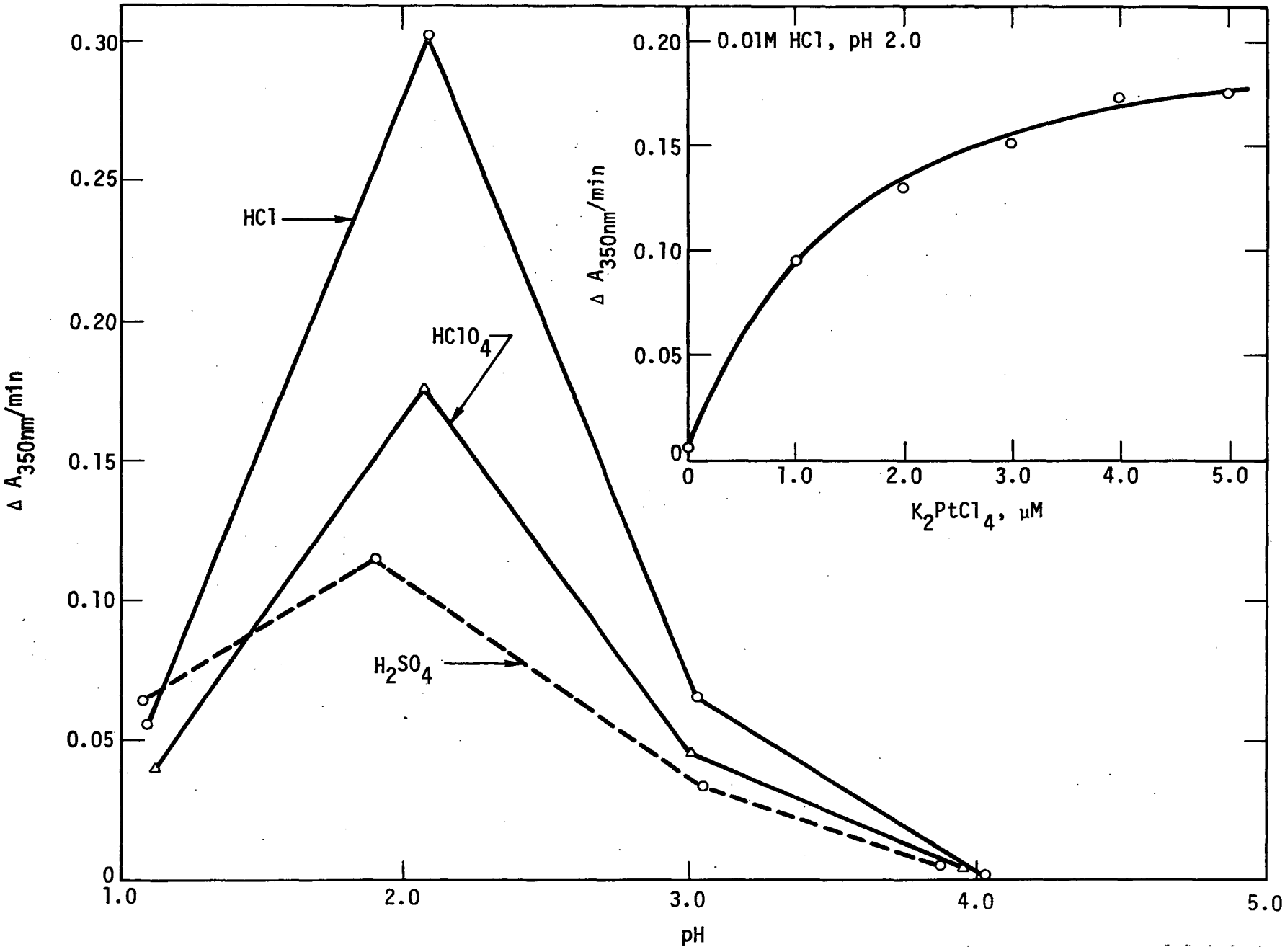

Figure 2. pH Dependence of the demethylation of MeB-12 with K_2PtCl_6 (100 μM) in presence of K_2PtCl_4 (100 μM). Inset - catalytic effect of low concentrations of K_2PtCl_4.

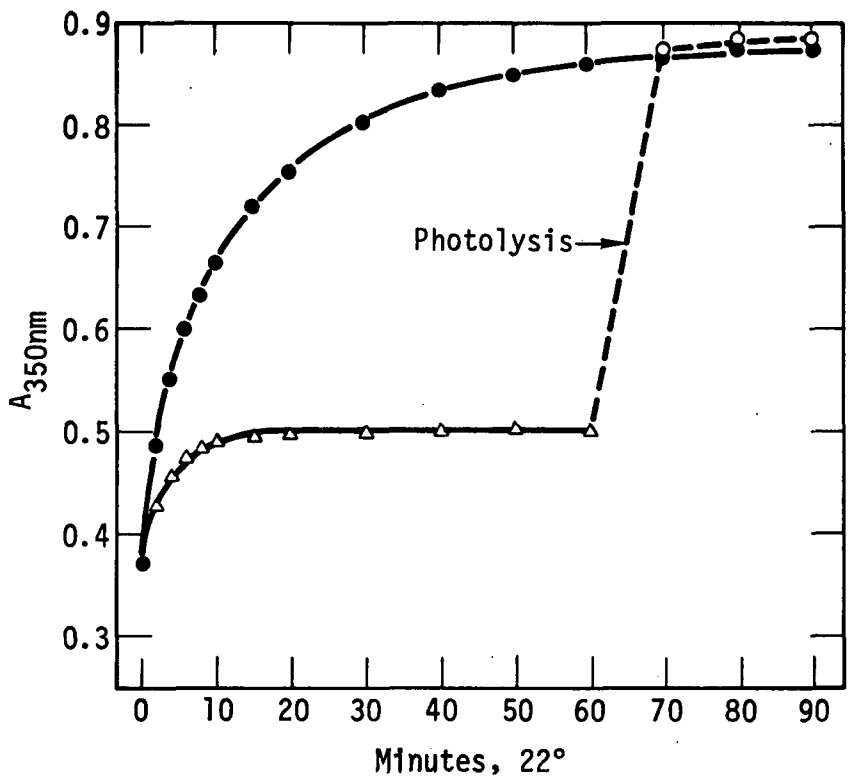

Figure 3. Stoichiometry of MeB-12 demethylation with the amount of K_2PtCl_6 added. Closed circles - 40 μM MeB-12 + 40 μM K_2PtCl_6 + 10 μM K_2PtCl_4; open triangles - 40 μM MeB-12 + 10 μM K_2PtCl_6 + 40 μM K_2PtCl_4; open circles - photolysis for 10, 20, and 30 min of the partially reacted mixture.

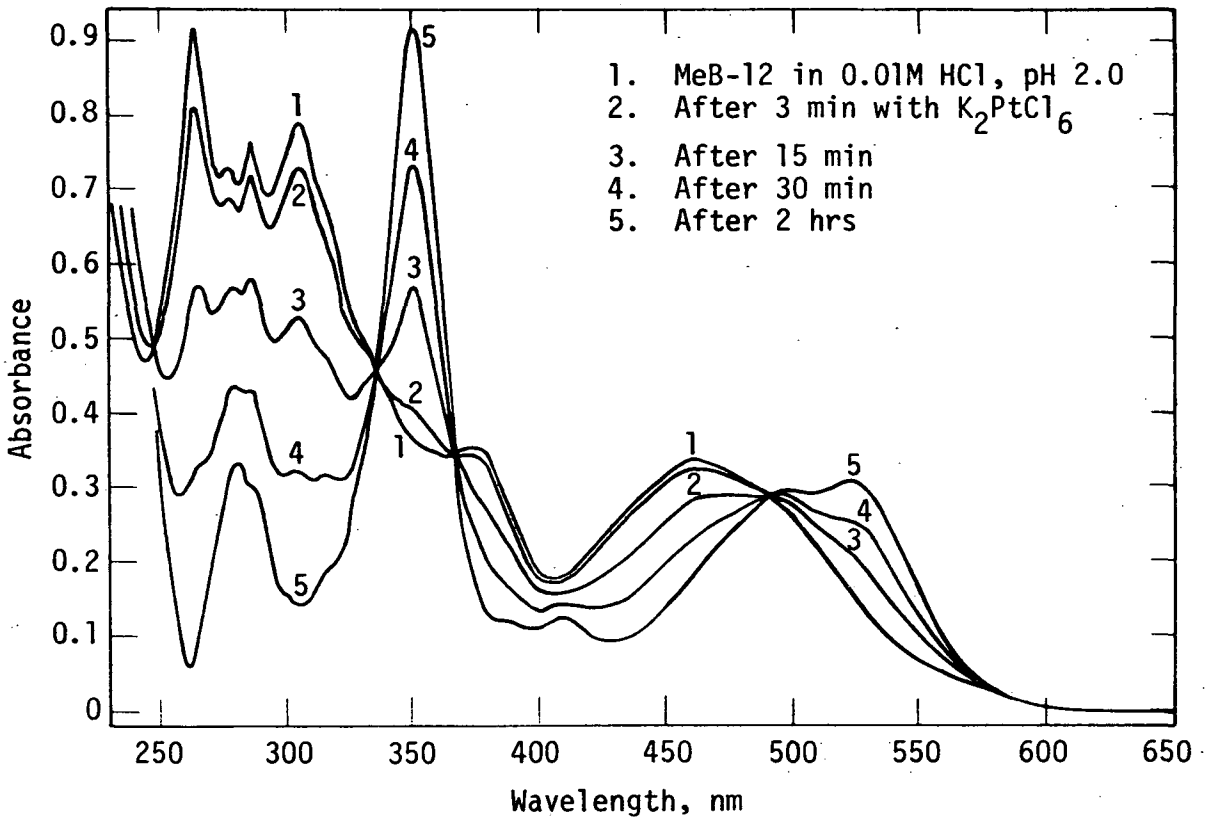

Figure 4. Time-dependent quantitative demethylation upon the addition of 100 μM K_2PtCl_6 alone.

and the methyl transfer is actually a 2-electron redox switch. In reaction 3, the stoichiometry does not reflect the pivotal function of the Pt^{2+} ion. Thus, K_2PtCl_4 would be required only in catalytic (trace) amounts. Its concentration would influence the initial reaction rate as in Fig. 2 (inset), but not the ultimate final extent of demethylation (Fig. 1A versus Fig. 4).

Relative to other alkylcorrinoids MeB-12 is by far the most reactive alkyl group donor. Ethyl B-12 and Mecobinamide are demethylated only 1-2% as rapidly as MeB-12 at pH 2.0 (Table 1). This specificity pattern

Table 1. ALKYLCORRINOID SPECIFICITY OF THE DEMETHYLATION BY K_2PtCl_6[a]

Alkylcorrinoid, 40 μM	Δ A_{350nm}/min	Relative demethylation rate
MeB-12	0.336	1.0
EthylB-12	0.004	0.012
PropylB-12	0.002	0.006
Mecobinamide	0.007	0.02
Propylcobinamide	0.0004	0.001

[a]Reaction mixtures contained 100 μM K_2PtCl_6 + 100 μM K_2PtCl_4.

is similar to what was observed several years ago in the reactivity of alkylcorrinoids with $HgCl_2$. It indicates that just as in the reaction with Hg^{2+}, the Me group of MeB-12 is being transferred to Pt as a carbanion (i.e. $CH_3^{\ominus}$). Among other halogen Pt^{4+} compounds 100 μM levels of K_2PtBr_6, K_2PtI_6, and chloroplatinic acid demethylated MeB-12 100%, 85%, and 65%, respectively, as fast as K_2PtCl_6. When reactions between MeB-12 and K_2PtCl_6 were carried out at pH 1.0 in 0.1 M HCl + 1.0 M NaCl, 37% of the Me groups were released as MeCl. This indicates an overall transfer from MeB-12 to Pt^{4+} to $Cl^{\ominus}$ under very high acidic-salt conditions.

While the reaction with the hologen platinates requires slightly acidic conditions, the demethylation of MeB-12 by $Pt(SO_4)_2$ does not. Raising the pH from 2.0 to 7.0 instead increased the reaction rate by two fold (Table 2). Moreover, the reaction rate at pH 2.0 in both $HClO_4$ (Table 2) and dilute H_2SO_4 (Fig. 5) was not markedly dependent on the simultaneous addition of the Pt^{2+} ion added as K_2PtCl_4. Depending on the acid

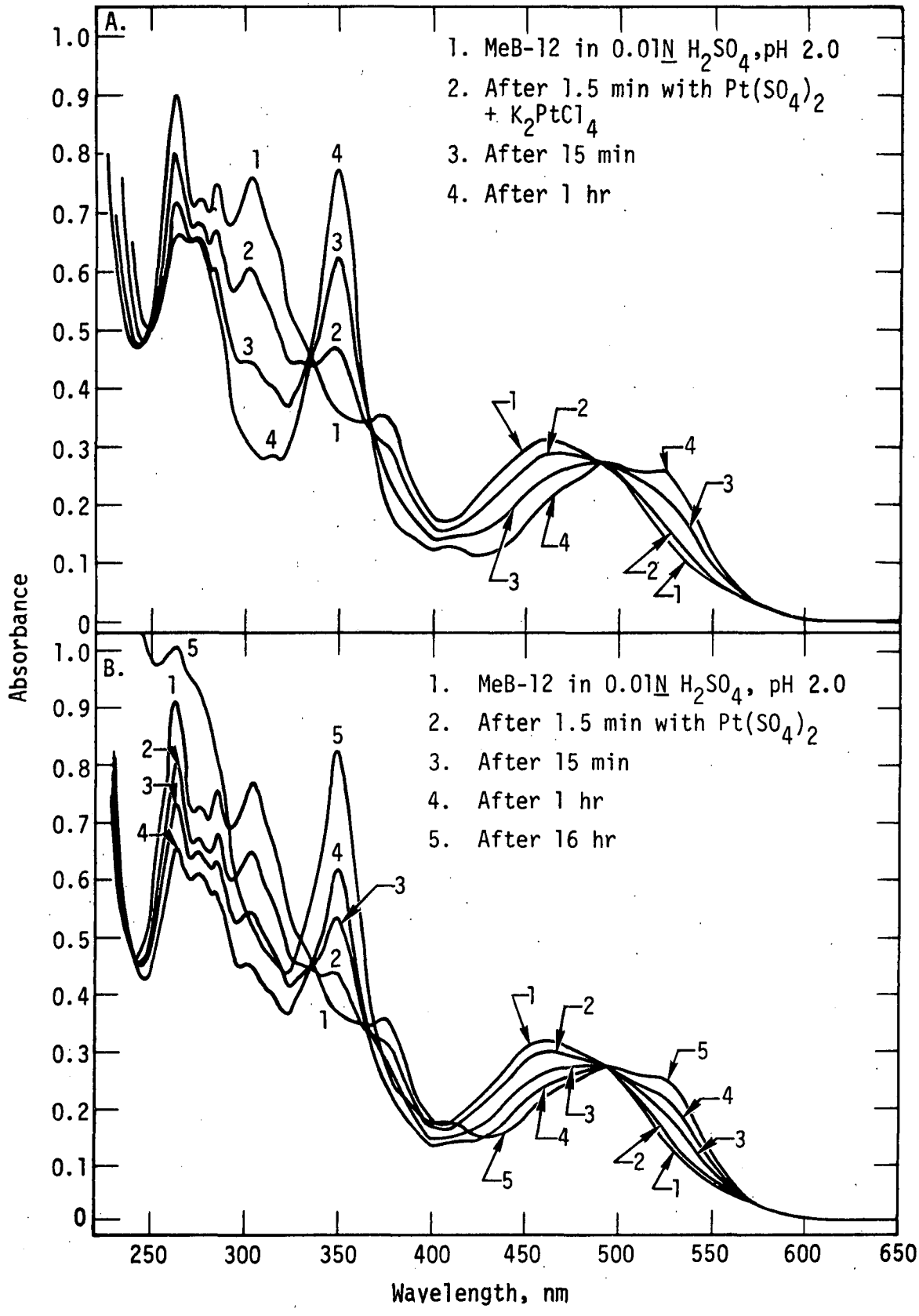

Figure 5. Demethylation of MeB-12 by 100 μM $Pt(SO_4)_2$.

Table 2. RELATIVE RATES OF MeB-12 DEMETHYLATION BY K_2PtCl_6 VERSUS $Pt(SO_4)_2$

Pt^{4+} Compounds, 100 µM	pH	Δ A_{350nm}/min	Relative demethylation rate
K_2PtCl_6 + K_2PtCl_4	2.0[a]	0.033	1.0
K_2PtCl_6	2.0[a]	0.001	0.03
$Pt(SO_4)_2$ + K_2PtCl_4	2.0[a]	0.029	0.88
$Pt(SO_4)_2$	2.0[a]	0.015	0.46
$Pt(SO_4)_2$	2.0[b]	0.024	0.73
$Pt(SO_4)_2$	3.0[b]	0.037	1.11
$Pt(SO_4)_2$	4.5[c]	0.033	1.0
$Pt(SO_4)_2$	7.0[d]	0.045	1.4

[a]Incubation solvent was $HClO_4$ and K_2PtCl_4 when present was 1.0 µM.

[b]Incubation solvent was 0.01 N H_2SO_4.

[c]Incubation buffer was 0.1 M Na-acetate.

[d]Incubation buffer was 0.1 M K-phosphate.

solvent selected, $Pt(SO_4)_2$ will demethylate MeB-12 at 46-73% of the rate obtained with 100 µM K_2PtCl_6 + 1.0 µM K_2PtCl_4. In contrast, finely divided suspensions of PtO_2 did not demethylate MeB-12 at any pH even after 48 hrs of incubation.

Most of our effort with Pt has been directed at the fate of the Me group when equimolar levels of MeB-12 and K_2PtCl_6 react at pH 2.0 in the presence of low $Cl^{\ominus}$ concentrations. To facilitate this study, extensive use was made of the [Me-^{14}C]MeB-12 and the [Me-^{3}H]MeB-12 that we synthesized. When either 40 µM [Me-^{14}C]MeB-12 or [Me-^{3}H]MeB-12 are reacted with 40 µM K_2PtCl_6 about 75% of the label is retained in the incubation residue after lyophilization to near dryness. Upon subsequent paper chromatography (Fig. 6) and paper electrophoresis (Fig. 7) much of the radioactivity coincides with a major zone of Pt. There was no selective loss of ^{3}H relative to ^{14}C in mixed labeled reaction mixtures (Fig. 7). Large amounts of ^{14}C-Pt product were then prepared by reacting 50 µmoles each of [Me-^{14}C]MeB-12 and K_2PtCl_6. Purification of the ^{14}C-Pt product was achieved by three successive column chromatography steps (Table 3), the material being monitored by its radiolabel and its UV absorbance. The isolated product has a ^{14}C/Pt ratio of 1.21 and is

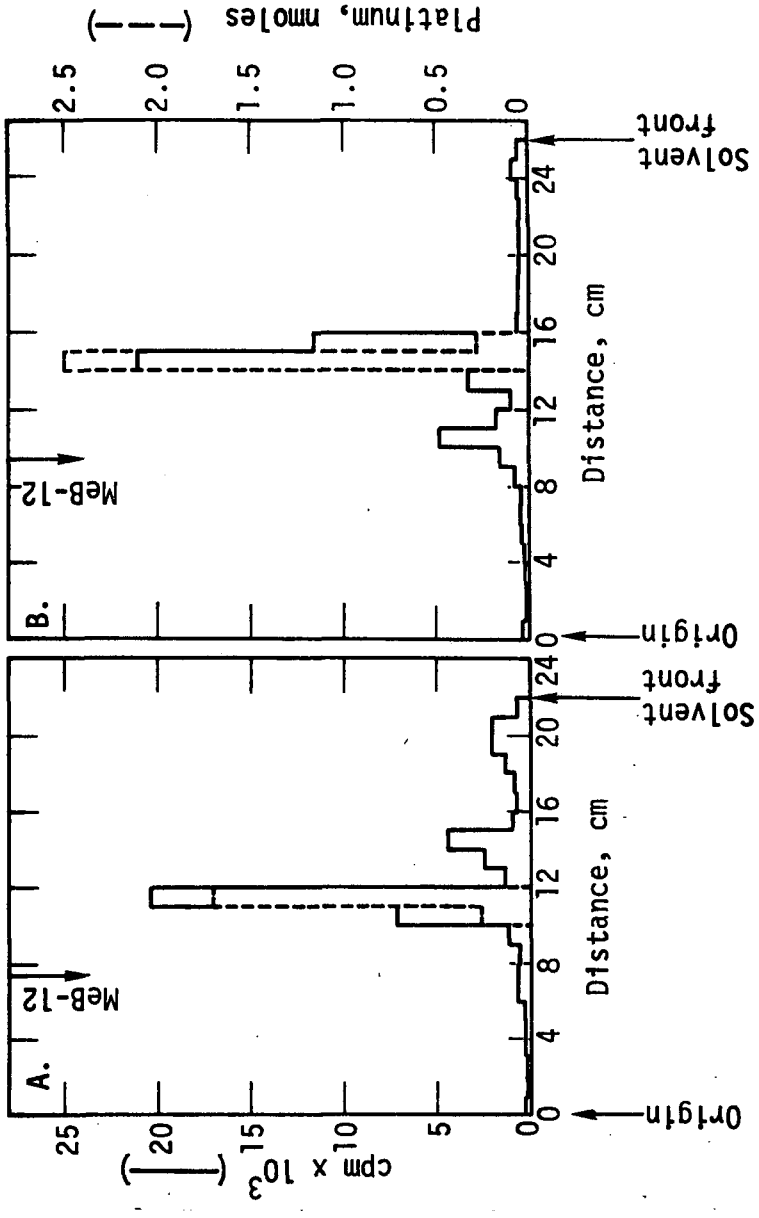

Figure 6. Ascending paper chromatography of the reaction products from a 2 hr incubation of 40 μM [Me-^{14}C]MeB-12 + 40 μM K_2PtCl_6. After lyophilization the residue was dissolved in 0.1 ml of water and aliquots were subjected to paper chromatography in the dark in two solvent systems. A. H_2O:n-butanol:isopropanol:acetic acid (100:100:70:1) and B. n-butanol:ethanol:H_2O (50:15:35). The chromatograms were then cut into 1 cm sections for radioactive counting and Pt analysis.

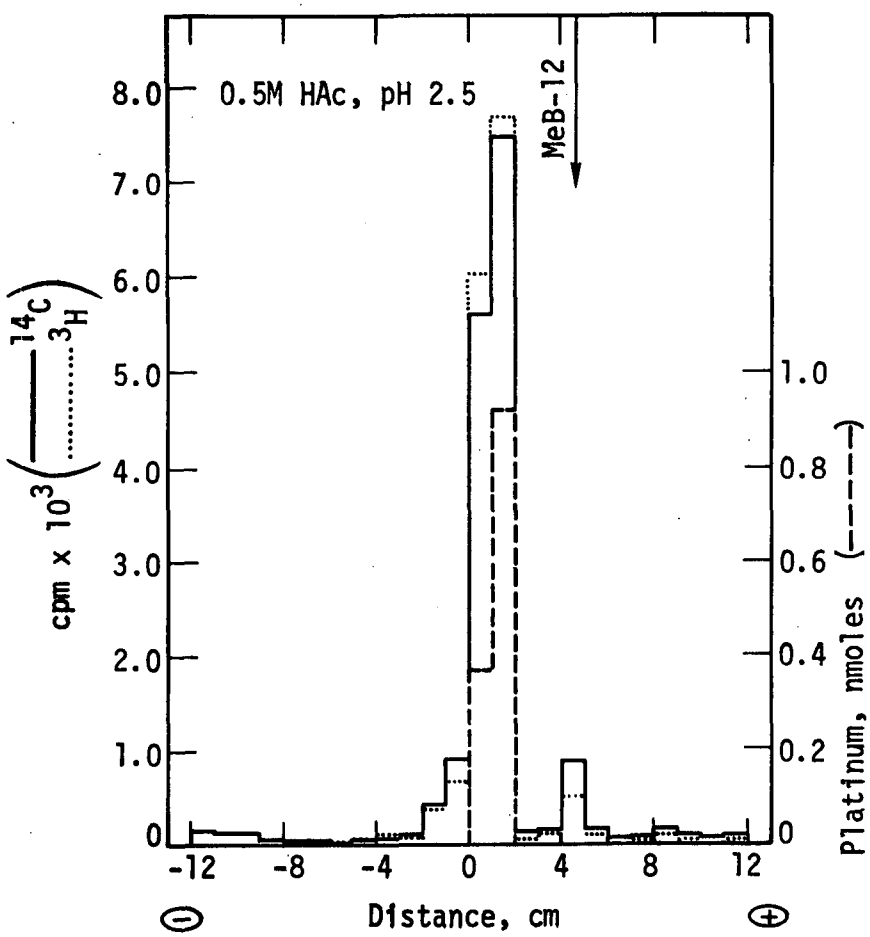

Figure 7. Paper strip electrophoresis of the reaction products from mixed labeled [Me-^{14}C]MeB-12 + [Me-^{3}H]MeB-12 + K_2PtCl_6. Reaction mixtures contained 20 nmoles of each type of Me-labeled MeB-12 (both with specific radioactivities of 13,000 cpm/nmole) + 40 nmoles of K_2PtCl_6. After a 2 hr incubation the mixture was processed as in Figure 6 and subjected to paper electrophoresis in the dark in 0.5 M acetic acid. The paper strips were then sectioned and analyzed.

Table 3. PURIFICATION SUMMARY OF THE [Me-^{14}C]Pt PRODUCT

Step	^{14}C (cpm)	^{14}C (μmoles)	Yield (%)
Original reaction mixture	13.2×10^6	50	100
Lyophilized incubation mixture	9.14×10^6	34.5	69
1st Sephadex G-15 column	7.49×10^6	28.5	57
2nd Sephadex G-15 column	5.39×10^6	20.5	41
CM-cellulose column	4.80×10^6	18	36

characterized by absorption maxima at 260 nm and 208 nm with a minimum at 240 nm (Fig. 8). Spectrally, the ^{14}C-Pt product resembles a Pt^{4+} salt (K_2PtCl_6) rather than a Pt^{2+} salt (K_2PtCl_4) (Fig. 8B), but the actual Pt valence state remains to be determined definitively. Generally, the overall recovery of ^{14}C relative to the original incubation mixture was 36-42% (Table 3). Similarly, a purified ^{3}H-Pt compound was obtained in the same overall yield starting from 50 μmoles of [Me-^{3}H]MeB-12. It exhibited an identical absorption spectrum and the ^{3}H/Pt ratio was 1.3. This information, in combination with our paper chromatographic-electrophoretic results (Figs. 6 and 7) and the release of MeCl under extreme acid-salt conditions, proves that the isolated ^{14}C-Pt product contains intact Me groups.

To determine the nature of the bonding between carbon and Pt, the isolated compound was studied by proton-NMR spectroscopy (Fig. 9). It yields a three banded spectrum with a J (coupling constant for ^{1}H, ^{195}Pt) of 78.2 Hz and a τ for ^{194}Pt-Me + ^{196}Pt-Me of 6.956. The NMR spectrum confirms the presence of an H-C-Pt covalent bonding pattern in the product. When considered with other data that the carbon is present in intact Me groups, the NMR spectrum (Fig. 9) provides definitive evidence that the product is an Me-Pt compound.

The stability of our purified [Me-^{14}C]Pt product with respect to its 260 nm absorbance was studied under a variety of conditions. Under each condition tested the loss of absorbance at 260 nm plotted as a first order decomposition process with respect to time (e.g. Fig. 10). In Table 4 we have summarized the times required for 50% decay of 180 μM solutions of the [Me-^{14}C]Pt compound. These half-lives indicate that although our Me-Pt derivative is moderately light-sensitive, it is sufficiently stable with respect to temperature, NaCl, and pH to exist

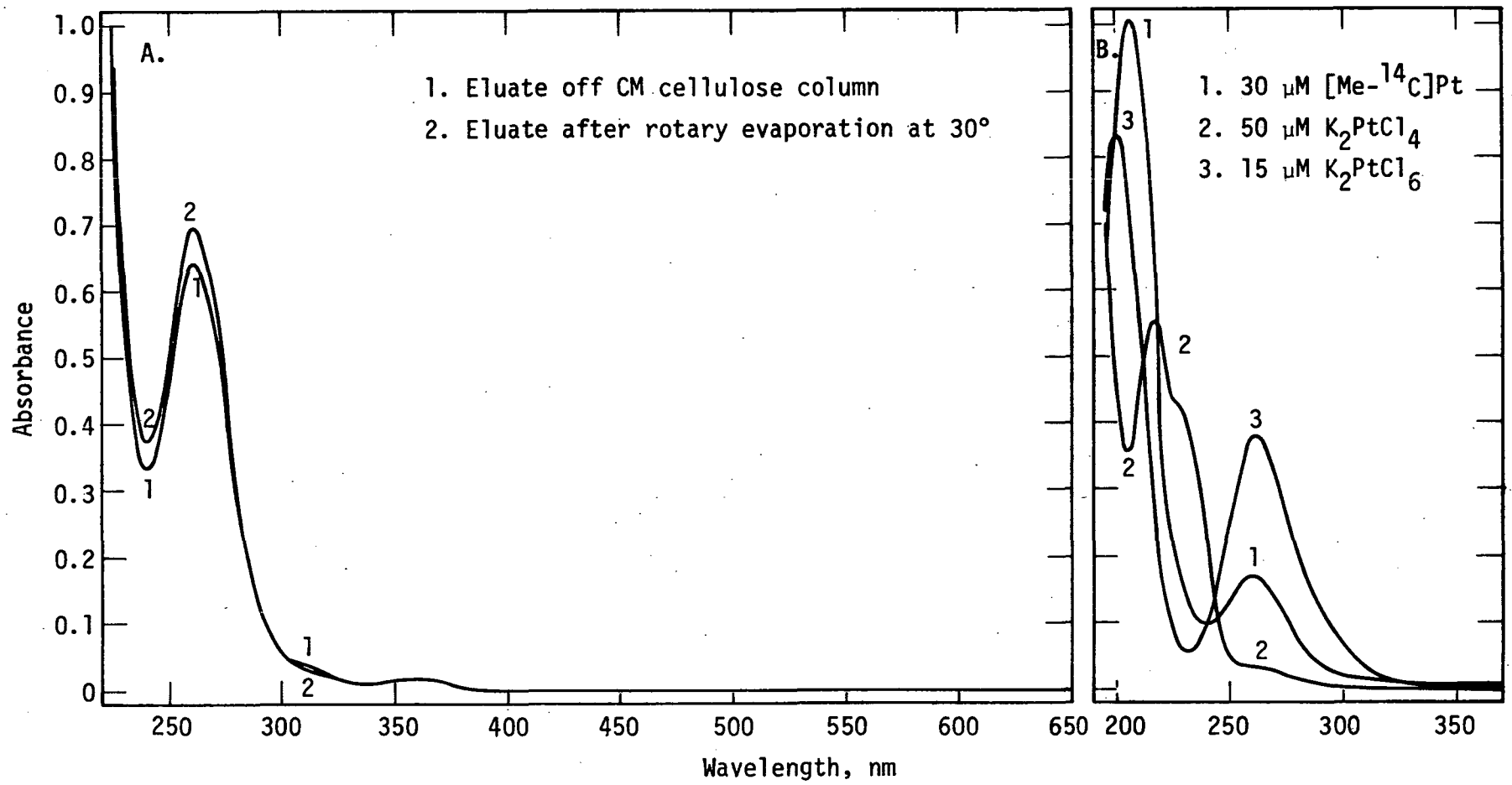

Figure 8. Absorption spectrum of the [Me-^{14}C]Pt reaction product formed from [Me-^{14}C]MeB-12 and K_2PtCl_6. A. Spectrum after CM-cellulose column purification step and a subsequent flash evaporation step to concentrate the compound in solution. B. Comparison of the light absorption spectrum to two chloroplatinates which contain Pt^{2+} and Pt^{4+}, respectively. All spectra were taken at pH 2.0 in 0.01 M HCl.

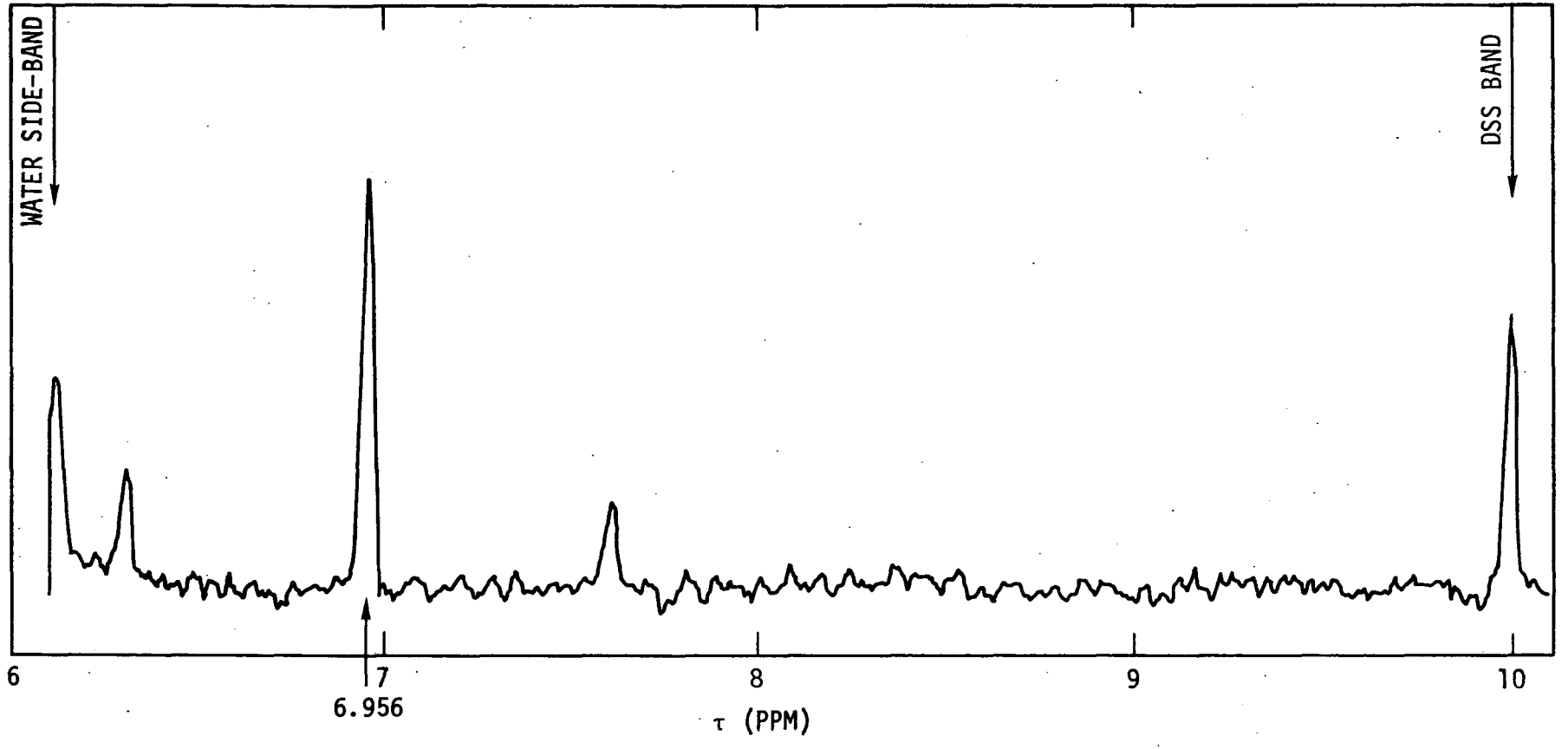

Figure 9. Proton NMR spectrum of the [Me-^{14}C]Pt product dissolved in water at a ^{14}C concentration of 33 mM and containing a 5 mM level of DSS internal standard.

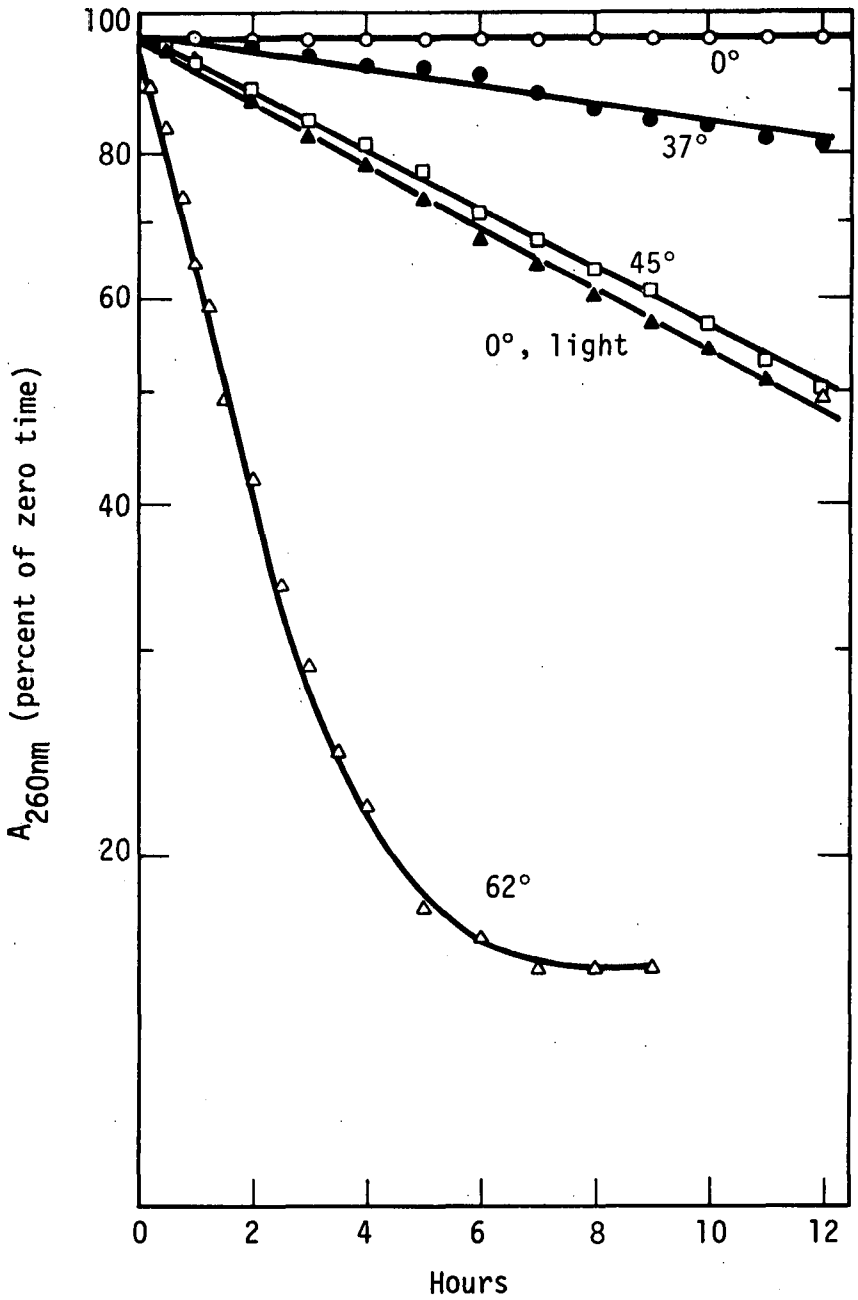

Figure 10. Effect of light and temperature on the UV absorption of the [Me-^{14}C]Pt product. Temperatures shown are in °C. Light is a 40 W tungsten lamp at a distance of 10 cm.

Table 4. STABILITY OF THE [Me-^{14}C]Pt PRODUCT UNDER VARIOUS CONDITIONS

Storage condition in aqueous solution	Half-life[b]
0°C, Dark, H_2O	>>21 days
22°C, Dark, H_2O	10.2 days
37°C, Dark, H_2O	1.3 days
45°C, Dark, H_2O	9.7 hrs
62°C, Dark, H_2O	1.3 hrs
0°C, Light, H_2O[a]	9.0 hrs
37°C, Light, H_2O[a]	6.0 hrs
22°C, Dark, 4 mM NaCl	10.2 days
22°C, Dark, 0.12 M NaCl	2.8 days
22°C, Dark, 0.1 M HCl	2.8 days
22°C, Dark, 0.01 M HCl	9.0 days
22°C, Dark, 0.1 M acetic acid	6.2 days
22°C, Dark, 0.1 M Na-acetate (pH 4.5)	7.0 days
22°C, Dark, 0.1 M K-phosphate (pH 7.0)	3.3 days
22°C, Dark, 0.1 M Na-pyrophosphate (pH 8.4)	2.3 days

[a]Light = 40 W tungsten lamp at a distance of 10 cm.

[b]Half-life is the time required for the 260 nm absorbance peak of a 180 μM solution of [Me-^{14}C]Pt to decrease by one-half of the maximal possible amount.

in freshwater ecosystems. It would also appear to be stable enough to have biological activity.

PALLADIUM

When 40 μM MeB-12 is mixed with 40-100 μM K_2PdCl_6 at pH 2.0, a complex forms immediately. It has absorption maxima at 350 nm, 495 nm, and 525 nm and is stable for about 30 min in the dark. No evidence for a spectrally distinct complex between 40 μM MeB-12 and 40-100 μM K_2PdCl_6 was observed at pH 7.0 and no complex with K_2PdCl_4 was detectable at any pH. Spectrophotometric titration at pH 2.0 revealed that two equivalents of K_2PdCl_6 were necessary to convert MeB-12 completely into a complex.

Upon extensive incubation of the complex the absorbance at 350 nm slowly increased and its spectrum became identical to that of aquoB-12. However, since both the complex and aquoB-12 have maxima at 350 nm, it is difficult to determine spectrophotometrically the rate of complex demethylation. Using 40 μM [Me-^{14}C]MeB-12 we estimated that after 24 hrs at 22°C at least 80% of the MeB-12 was demethylated by 100 μM K_2PdCl_6 and 23% was demethylated in the presence of K_2PdCl_4. Thus, Pd^{4+} and Pd^{2+} can react with MeB-12 at pH 2.0, but the rates are much slower than for 100 μM Pt^{4+}. Also, Pd^{2+} does not significantly increase the rate of complex formation between K_2PdCl_6 and MeB-12 or its rate of breakdown to aquoB-12.

In view of the reactivity of $Pt(SO_4)_2$ we tested the effect of 100 μM $PdSO_4$ on 40 μM MeB-12. Incubations were made at pH 2.0, 4.5, and 7.0. After 48 hrs the extents of demethylation were 72%, 32%, and 0%, respectively. Since K_2PdCl_6 reacts faster than K_2PdCl_4, it is possible that the demethylation observed with Pd^{2+} salts is due to their slow oxidation to Pd^{4+}.

LEAD

A study was made of the reactivity between 40 μM MeB-12 and various Pb compounds at pH 2.0 (0.01 M HCl) and at pH 4.5 (0.1 M Na-acetate). The increase in absorbance at 350 nm served as a measure of the extent of MeB-12 demethylation to aquoB-12. No significant reaction occurred at either pH between MeB-12 and 100 μM $PbCl_2$, $PbBr_2$, and $Pb(Ac)_2$ after 24 hrs at 22°C. However, $Pb(Ac)_4$ altered the spectrum of MeB-12 and formed a complex over a period of 0-30 min. Upon prolonged incubation for 24 hrs, the conversion of MeB-12 to aquoB-12 was 57-64% at pH 2.0 (Fig. 11). The extent of demethylation at pH 4.5 was 31% for $Pb(Ac)_4$; at pH 7.0 it was 0%. Since Pt^{2+} stimulates the reaction between Pt^{4+} and MeB-12, we examined the possibility that Pb^{2+} might behave similarly with respect to Pb^{4+}. In contrast, no evidence was found that $Pb(Ac)_2$ promotes a faster reaction between MeB-12 and $Pb(Ac)_4$.

Both $Pb(Ac)_4$ and PbF_4 are rapidly hydrolyzed by water forming a brown precipitate of Pb_3O_4 and PbO_2. At 100-200 nmole/ml levels these fine suspensions of Pb_3O_4 and PbO_2 are barely visible so that the reaction solutions still appear quite clear. For comparison, 40 μM solutions of MeB-12 were incubated with 100 nmole/ml fine suspensions of Pb_3O_4 and PbO_2 at pH 2.0 (Table 5). After 24 hrs the extents of demethylation were 57% and 37%, respectively. At pH 4.5 they were both 25% and at pH 7.0 they were 0%. Thus, from the similar extents of demethylation, it is likely that when one adds $Pb(Ac)_4$ or PbF_4 to aqueous solutions the actual Pb compounds being tested for reactivity are Pb_3O_4 and PbO_2. Apparently, suspensions of Pb oxide are in equilibrium with traces of Pb^{4+} that are reactive with MeB-12. The rate of MeB-12 demethylation is quite slow due to the extremely low solubility of Pb_3O_4 and PbO_2 in water; nonetheless, it is significant over an extended period of time. It should be noted in Table 5 that a close correspondence was observed

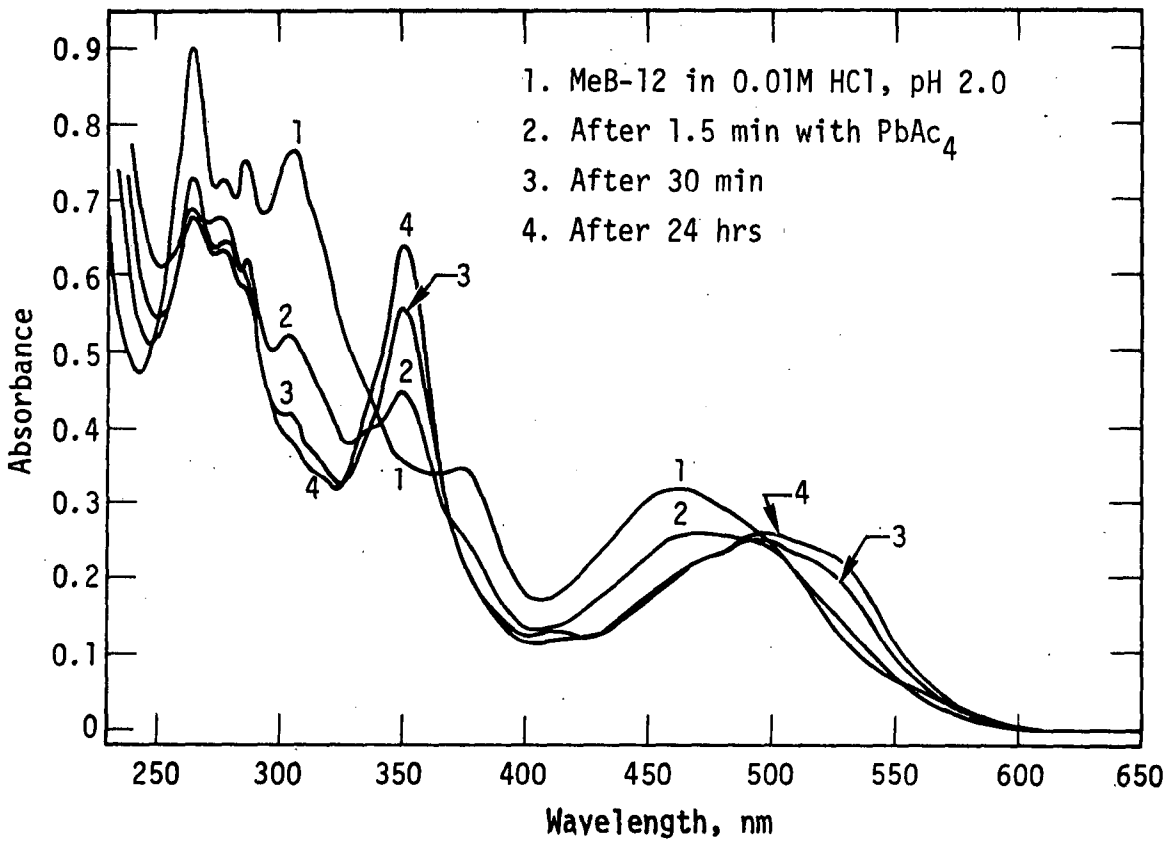

Figure 11. Partial demethylation of MeB-12 with $Pb(Ac)_4$ at a concentration of 100 nmoles/ml.

Table 5. EXTENTS OF [Me-^{14}C]MeB-12 DEMETHYLATION AND THE RECOVERY OF ^{14}C AFTER PROLONGED INCUBATION WITH VARIOUS LEAD SALTS AND OXIDES[a]

Compound	Concentration	% Demethylation	% of the initial ^{14}C recovered
$PbCl_2$	100 μM	0-2	97
$PbBr_2$	100 μM	0-2	97
$Pb(Ac)_2$	100 μM	0-2	98
$Pb(Ac)_4$	100 nmoles/ml	59	40
$Pb(Ac)_4$	200 nmoles/ml	100	2
Pb_3O_4	100 nmoles/ml	57	33
Pb_3O_4	200 nmoles/ml	90	2
PbO_2	100 nmoles/ml	37	61
PbO_2	200 nmoles/ml	61	31

[a]Incubations were for 24 hrs at 22°C in the dark at pH 2.0.

[b]After the incubation the reaction mixtures were lyophilized to dryness in the dark and then reconstituted in water to determine the recovery of non-volatile ^{14}C.

between the extents of demthylation by several lead compounds and the disappearance (volatization) of ^{14}C. Several paper electrophoresis runs of the lyophilized Pb reaction mixtures (analogous to Fig. 7) revealed the presence of ^{14}C associated only with the remaining unreacted [Me-^{14}C]MeB-12.

MANGANESE

We also examined the reactivity of several Mn^{2+} salts and MnO_2 with MeB-12. After incubation of 40 μM MeB-12 with 100 μM $MnCl_2$, $MnBr_2$, $MnSo_4$, and a fine suspension (100 nmoles/ml) of MnO_2 for 24-48 hrs, we could detect no demethylation. No reactivity was observed at pH 2.0, 4.5, or 7.0.

SECTION IV

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16. ABSTRACT

A study was carried out to evaluate the potential for platinum, palladium, lead, and manganese salts and oxides to be biochemically methylated. Methylation is an important, well recognized, determinant of metal toxicity; the striking example being the extreme health hazard of methylated mercury. The possible biological methylation of the metals which are associated with emissions arising from the use of automotive fuels, fuel additives, and catalytic control devices is of special concern to the Environmental Protection Agency's Catalyst Research Program.

Salts of platinum, palladium, and lead, and oxides of lead all containing the metal in a 4^+ valence were observed to demethylate methylcobalamin, a biologically active form of vitamin B-12. Inorganic salts and oxides of manganese were unreactive. No evidence for a stable monomethyl-metal derivative was found using palladium and lead compounds as reactants. However, salts of platinum 4^+ do result in the formation of stable methylation products. The reaction product formed from methylcobalamin and hexachloroplatinate was shown definitively to be a monomethyl-platinum compound It is sufficiently stable in aqueous solutions under a variety of conditions to exist in freshwater ecosystems and to exhibit toxic effects on mammalian cells.

17. KEY WORDS AND DOCUMENT ANALYSIS

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