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Interactions of Diorganolead(IV) with 3-(2-Thienyl)-2-sulfanylpropenoic Acid and/or Thiamine: Chemical and in Vitro and in Vivo Toxicological Results

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The reactions of $PbR_2(OAc)_2$ (R=Me, Ph) with 3-(2-thienyl)-2-sulfanylpropenoic acid (H_2tspa) in methanol or ethanol afforded complexes $[PbR_2(tspa)]$ that electrospray ionization-mass spectrometry (ESI-MS) and IR data suggest are polymeric. X-ray studies showed that $[PbPh_2(tspa)(dmso)]\cdot dmso$, crystallized from a solution of $[PbPh_2(tspa)]$ in dmso, is dimeric, and that $[HQ]_2[PbPh_2(tspa)_2]$ (Q=diisopropylamine), obtained after removal of $[PbPh_2(tspa)]$ from a reaction including Q, contains the monomeric anion $[PbPh_2(tspa)_2]^{2-}$. In the solid state the lead atoms are O,S-chelated by the $tspa^{2-}$ ligands in all these products, and in the latter two have distorted octahedral coordination environments. NMR data suggest that $tspa^{2-}$ remains coordinated to PbR_2^{2+} in solution in dmso. Neither thiamine nor thiamine diphosphate reacted with $PbMe_2(NO_3)_2$ in D_2O. Prior addition of H_2tspa protected LLC-PK1 renal proximal tubule cells against $PbMe_2(NO_3)_2$; thiamine had no statistically significant effect by itself, but greatly potentiated the action of H_2tspa. Administration of either H_2tspa or thiamine to male albino Sprague−Dawley rats dosed 30 min previously with $PbMe_2(NO_3)_2$ was associated with reduced inhibition of δ-ALAD by the organolead compound, and with lower lead levels in kidney and brain, but joint administration of both H_2tspa and thiamine only lowered lead concentration in the kidney.

Introduction

As is well-known, "inorganic lead" [lead(II)] is highly toxic. It probably affects every mammalian organ system but has particularly dire effects on the nervous system, especially in children.[1] The toxicity of organolead(IV) compounds ("organic lead") is also well documented, mainly in regard to the use of tetraalkylleads (TALs) as antiknocking agents for gasoline. Though now completely banned in the U.S. and the E.U., TALs are still added to automobile fuel in more than 70 countries around the world.[2] These environmentally widespread organometals, which cause a variety of neurological and behavioral deficits,[3] are readily absorbed via the gastrointestinal tract, the respiratory tract, and the skin, and are metabolized basically by dealkylation.[4] In rabbits, for example, tetramethyllead is metabolized to $PbMe_2^{2+}$ (73%), $PbMe_3^{+}$ (19%), and Pb^{2+} (6%), only 2% remaining as $PbMe_4$.[5] The toxicity of TALs therefore seems to derive mainly from their "organic" and "inorganic" ionic metabolites. For aquatic organisms, these forms can be ranked by toxicity in the order $PbR_3^{+} > PbR_2^{2+} > Pb^{2+}$, and toxicity also seems to increase with alkyl chain length;[5] these trends may also apply to non-aquatic mammals, although in this respect data are scarce.[4]

3-Aryl-2-sulfanylpropenoic acids (3-aryl-2-mercaptoacrylic acids, H_2L, I, Scheme 1) are an interesting group of compounds that can easily be prepared from 5-(arylmethylene)rhodanines by alkaline hydrolysis.[6] Though initially believed to exist in solution as an equilibrium mixture of thione and ene-thiol forms, their chemical and physical

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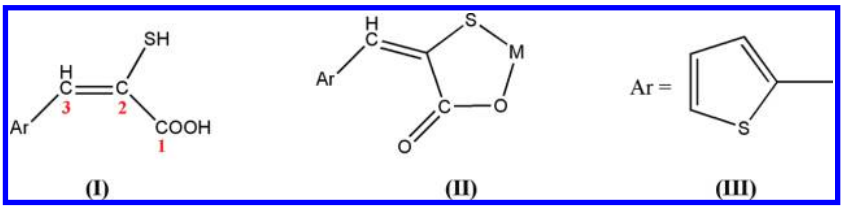
Scheme 1



properties were soon found to be consistent only with the sole presence of the latter.[7] This is probably also the form present in the solid state, although evidence for this is scarce[8] because of the formation of disulfides upon crystallization of H_2L.[9] H_2L are more easily deprotonated than α-mercaptoacetic acid, the ease of deprotonation depending on the aryl substituent on C(3), especially in the case of the -SH group.[10,11] Both HL^- and L^{2-} form very stable complexes with metal ions. Usually (see, e.g., ref 12), though not always,[9] L^{2-} is O, S-coordinated to the metal ion in a five-membered chelate ring (II, Scheme 1).

3-Aryl-2-mercaptoacrylic acids can have valuable pharmacological properties, including the prevention of kainate-receptor-mediated motor neuron death and the inhibition of neuraminidase (which makes them potential antivirals[13]) and of calpains, a family of cytosolic cysteine proteases that seem to be involved in cell degeneration following events such as myocardial, cerebral, or renal ischemia. 3-Aryl-2-mercaptoacrylic acids with anticalpain activity are neuroprotective, and can partially protect rat kidneys against ischemia-reperfusion injury.[14,15] H_2L also inhibit alkaline phosphatase, carboxypeptidase A, ceruloplasmin, and thermolysin[10] (probably by extracting the active Zn^{2+} or Cu^{2+} ions from the catalytic centers of these enzymes), and can significantly influence the distribution of biometals in rat kidneys.[16] The possibility of exploiting their coordination properties to remove toxic lead(II) from the body is suggested by the propensity of both L^{2-} anions and Pb^{II} for O,S-coordination, and was explored by Tandon et al.,[17] who showed that 3-(2-furyl)-2-sulfanylpropenoic acid induced significant urinary and faecal excretion of the metal by male Wistar rats that had been administered lead(II) acetate intragastrically for several days.

As part of a project on the coordination of H_2L and PbR_n^{2+} (n = 2,3) and the influence of H_2L on the toxicity of organolead(IV) cations, we present here the results of reacting dimethyl- or diphenyllead(II) with 3-(2-thienyl)-2-sulfanylpropenoic acid (H_2tspa, Ar- = III, Scheme 1), with or without diisopropylamine (which was included with a view to obtaining anionic complexes that would be more soluble in the usual solvents). Since the beneficial effects of administrating thiamine (vitamin B_1) in cases of lead(II) poisoning, though known for years,[18] have not been fully explained,[19] we also investigated the possible interactions of both thiamine and thiamine diphosphate (TDP) with $PbMe_2(NO_3)_2$. Finally, we performed in vitro and in vivo biological assays of the influence of H_2tspa and thiamine, alone or in combination, on the toxicity of $PbMe_2(NO_3)_2$ (as far as we know, this is the first exploration of the possible effects of thiamine on the evolution of organolead(IV) derivatives in living cells or organisms).

Experimental Procedures

Material and Measurements. H_2tspa was prepared by condensation of 2-thiophenecarboxaldehyde (Aldrich) with rhodanine (Aldrich), subsequent hydrolysis in an alkaline medium, and acidification with HCl.[20] $PbPh_2(OAc)_2$ was obtained by mixing diphenyllead chloride (ABCR) with silver acetate (Aldrich) in methanol; after stirring for 5 h, the silver chloride was filtered out, and the solution was concentrated. The reactions of dimethyllead(IV) bromide[21] with silver acetate and silver nitrate afforded $PbMe_2(OAc)_2$ and $PbMe_2(NO_3)_2$, which were isolated after stirring for 2 h. Thiamine chloride monohydrate ($TCl \cdot H_2O$) was prepared from $TCl \cdot HCl$ (Aldrich) by the method of Pletcher et al.[22] Thiamine nitrate, TNO_3, was obtained by reacting $TCl \cdot H_2O$ with silver nitrate.

Elemental analyses were performed in a Carlo-Erba 1108 microanalyzer. Melting points were determined with a Büchi apparatus and are uncorrected. IR spectra were recorded from KBr pellets on a Bruker IFS66 V FT-IR spectrophotometer. Mass spectra were recorded in methanol using positive or negative electrospray ionization on a Hewlett-Packard 1100 LC/MSD spectrometer. pD measurements of a D_2O solution of $PbMe_2(NO_3)_2$ at 25 °C were carried out with a Crison micropH 2000 instrument. NMR studies in D_2O of the interaction of $PbMe_2(NO_3)_2$ with thiamine or thiamine diphosphate were carried out in 5 mm o.d. tubes on Varian Unity Inova 300 spectrometer operating at 300.05 MHz (^{1}H) and 121.44 MHz (^{31}P). ^{1}H spectra were referenced to the methyl group of internal DSS and ^{31}P spectra to an external 85% solution of H_3PO_4. NMR experiments in dmso-d_6 were carried out in 5 mm o.d. tubes on Varian Unity Inova 300 or Bruker AMX 500

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instruments operating at 300.05 and 500.14 MHz (^{1}H) and 75.40 and 125.77 (^{13}C). The internal references for chemical shifts (ppm) were the solvent signal ($\delta = 2.50$ ppm) for ^{1}H spectra and the central peak of deuterated dmso ($\delta = 39.50$ ppm) for ^{13}C spectra. Signals are described as follows: chemical shift; multiplicity (for ^{1}H; s = singlet, d = doublet, t = triplet, *ps*t = *pseudo*triplet, sept = septuplet, br = broad signal); relative integral (for ^{1}H); and coupling constants J in Hz. The NMR spectra of [$PbMe_2$(tspa)] were recorded using freshly prepared solutions because spectra recorded more than 24 h later showed changes indicative of evolution to $(Htspa)_2$. The dissociation of the $tspa^{2-}$ ligand may have been secondary to the observed slow evolution of the organometallic moiety to trimethyllead(IV) and (probably) Pb^{II}, a process in keeping with the known tendency of dialkyllead(IV) compounds to undergo redistribution reactions.[23]

[$PbMe_2$(tspa)]. A solution of 0.04 g (0.23 mmol) of H_2tspa in 15 mL of absolute ethanol was neutralized with sodium hydroxide to avoid protodemetalation reactions, and was then added to a solution of 0.08 g (0.23 mmol) of $PbMe_2(OAc)_2$ in 15 mL of the same solvent that had previously been placed in an ethanol/liquid nitrogen bath. After 4 h of stirring, the mixture was concentrated to one-third of its volume, and the resulting solid was separated out by centrifugation and vacuum-dried. Yield 45%. Anal. Calcd for $C_9H_{10}S_2O_2Pb$, C 25.65, H 2.39, S 15.21%; found, C 25.27, H 1.86, S 15.15%. IR (cm^{-1}): $\nu_a(COO^-)$ 1520vs; $\nu_s(COO^-)$ 1361vs. Main (>10% abundance) metalated ESI-MS (+) peaks at *m*/*z* (%): 549.1 (100) [$C_3H_3O_2Pb_2S_2$]; 423.0 (11) [M+H]; 393.0 (83) [Pb(tspa)+H]. ^{1}H NMR (dmso-d_6, ppm): δ C(3)H 7.81 (s, 1), C(5)H 7.31 (brs, 1), C(6)H 7.08 (*ps*t, 1), C(7)H 7.51 (brs, 1), Me 1.98 (s, 6; $^2J = 128$ Hz). ^{13}C NMR (dmso-d_6, ppm): δ C(1) 173.2, C(2) 136.3, C(3) 129.8, C(4) 142.4, C(5) 128.7, C(6) 126.6, C(7) 127.3, Me 32.8.

[$PbPh_2$(tspa)]. A solution of 0.2 g (1.07 mmol) of H_2tspa in 25 mL of ethanol was added to a suspension of 0.52 g (1.07 mmol) of $PbPh_2(OAc)_2$ in 35 mL of methanol. The mixture immediately afforded a yellow solid that after 1 h of stirring was separated out by centrifugation and vacuum-dried. Yield 91%. Anal. Calcd for $C_{19}H_{14}O_2S_2Pb$, C 41.82, H 2.59, S 11.75%; found, C 41.42, H 2.48, S 11.22%. IR (cm^{-1}): $\nu_a(COO^-)$ 1521vs; $\nu_s(COO^-)$ 1363vs. Main (>10% abundance) metalated ESI-MS (+) peaks at *m*/*z* (%): 1093.1 (6) [2M+H]; 547.0 (100) [M+H]; 407.1 (29) [$PbPh_2CO_2$+H]. ^{1}H NMR (dmso-d_6, ppm): δ C(3)H 7.90 (s, 1), C(5)H 7.37 (d, 1), C(6)H 7.10 (*ps*t, 1), C(7)H 7.59 (d, 1), Ph: H_o 7.91 (d, 4; $^3J = 170$ Hz); H_m 7.58 (t, 4; $^4J = 74$ Hz); H_p 7.43 (t, 2). ^{13}C NMR (dmso-d_6, ppm): δ C(1) 171.7, C(2) 137.6, C(3) 129.5, C(4) 141.3, C(5) 128.3, C(6) 126.5, C(7) 127.5, Ph: C_o 134.2 $^2J = 120$ Hz, C_m 130.1, C_p 129.9. Single crystals of [$PbPh_2$(tspa)(dmso)]·dmso were grown by slow evaporation of a dmso-d_6 solution.

[HQ]$_2$[$PbPh_2$(tspa)$_2$]. A 0.2 g portion (1.07 mmol) of H_2tspa and 0.15 mL (1.07 mmol) of diisopropylamine (Q) in 25 mL of ethanol were added to a suspension of 0.26 g (0.54 mmol) of $PbPh_2(OAc)_2$ in 30 mL of methanol. After 2 h of stirring the solution afforded a solid that was separated out by centrifugation and identified as [$PbPh_2$(tspa)]. The mother liquor then afforded a second solid that was likewise separated out by centrifugation and vacuum-dried. Yield 17%. Anal. Calcd for $C_{38}H_{50}N_2S_4O_4Pb$, C 48.85, H 5.39, N 3.00, S 13.73%; found, C 48.62, H 5.56, N 2.94, S 13.25%. IR (cm^{-1}): $\nu_a(COO^-)$ 1546vs; $\nu_s(COO^-)$ 1336vs; $\delta(NH_2)$ 1610s. Main (>10% abundance) metalated ESI-MS (-) peaks at *m*/*z* (%): 731.0 (60) [$PbPh_2$(Htspa)(tspa)]; 594.9 (82) [$PbPh_2$(tspa)SOH]; 392.9 (100) [Pb(tspa)+H]. ^{1}H NMR (dmso-d_6, ppm): δ C(3)H 7.79 (s, 1), C(5)H 7.23 (brs, 1), C(6)H 7.04 (*ps*t, 1), C(7)H 7.42 (d, 1), Q: CH_3 1.12 (d, 24), CH 3.20 (sept, 4); Ph: H_o 8.00 (d, 4; $^3J = 167$ Hz); H_m 7.32 (t, 4); H_p 7.23 (brs, 2). ^{13}C NMR (dmso-d_6, ppm): δ C(1) 171.7, C(2) 138.9, C(3) 127.3, C(4) 142.9, C(5) 126.0, C(6) 122.8, C(7) 125.4, Q: CH_3 19.1, CH 45.9; Ph:Ci 166.6, Co 134.9, $^2J = 114$ Hz, *Cm* 128.7, Cp 128.0. Crystals suitable for X-ray diffractometry were obtained from the mother liquor after several days' storage.

X-ray Crystallography. X-ray data for [$PbPh_2$(tspa)(dmso)]·dmso were collected at 293 K at the São Carlos Institute of Physics, University of São Paulo, Brazil, using a Nonius Kappa CCD diffractometer, and X-ray data for [HQ]$_2$[$PbPh_2$(tspa)$_2$] at 100 K at the RIAIDT Services, University of Santiago de Compostela, Spain, using a Bruker APEXII automatic diffractometer. In both cases Mo Kα radiation ($\lambda = 0.71073$ Å) was used. Crystal data, experimental details and refinement results are summarized in Supporting Information, Table S1. Corrections were made for Lorentz effects, polarization[24] and absorption.[25] Structures were solved by direct methods.[26] The uncoordinated dmso molecule of [$PbPh_2$(tspa)(dmso)].dmso is disordered between two positions with occupancies of 60% and 40%. The structure of [HQ]$_2$[$PbPh_2$(tspa)$_2$] was determined following use of the SQUEEZE function[27] of PLATON[28] to eliminate the contribution of disordered solvent molecules to the intensity data; this strategy afforded slightly better refinement results than an attempt to model the solvent atoms. In the refinements, non-H atoms were treated anisotropically.[26] Hydrogen atoms were refined as riders at geometrically calculated positions, except H(3) and H(10) in [HQ]$_2$[$PbPh_2$(tspa)$_2$], which were located from a difference Fourier map and refined isotropically. Scattering factors were taken from the International Tables for Crystallography.[29] The main calculations were performed with SHELXL-97,[28] and figures were plotted with ORTEP-3 and Mercury.[30]

In Vitro Studies. Phosphoric acid, formic acid, acetic acid, and crystal violet were supplied by Panreac (Barcelona, Spain); glutaraldehyde, 2-(*N*-morpholino)ethanesulfonic acid (MES), L-glutamine, fetal bovine serum (FBS) and Medium 199 by Sigma (Madrid, Spain); and LLC-PK1 renal proximal tubule cells and Eagle's Essential Medium (EMEM) by the American Tissue Culture Collection (ATCC). Solutions of $PbMe_2(NO_3)_2$, H_2tspa, and thiamine were made up in deionized water and sterilized by passage through a 0.20 μm filter. H_2tspa solutions were brought to neutral pH by addition of $NaHCO_3$. These solutions were diluted to appropriate concentrations with fresh medium (see below). Frozen LLC-PK1 cells were thawed, in a 75 cm^2 cell culture flask, in Medium 199 supplemented with 3% FBS and 1.5 g/L $NaHCO_3$, and were maintained at 37 °C in a 5% CO_2 atmosphere with twice-weekly replacement of the medium.

The effects of H_2tspa and thiamine ($TCl \cdot H_2O$) on the inhibition of cell proliferation by $PbMe_2(NO_3)_2$ were measured by crystal violet staining[31] as follows, after verification (by the same method) that neither had a significant effect on cell viability at a concentration of 40 μM. Cells were seeded at $1 \cdot 10^4$ cells/well in a

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96-well microplate with 100 μL of growth medium per well, and were kept for 24 h at 37 °C in a 5% CO_2 atmosphere. The medium was replaced with fresh medium alone (controls) or containing H_2tspa, thiamine or both at concentrations of 40 μM; after 24 h $PbMe_2(NO_3)_2$ was added at concentrations of 1−1000 μM (except for negative controls), four wells being used for each concentration and pretreatment; and after a further 24 h the cells were fixed to the plate with 10 μL of 11% glutaraldehyde for 15 min at room temperature (RT), the medium was removed, and the cells were washed four times with deionized water and stained for 15 min at RT with 100 μL of a 0.1% crystal violet solution (0.1 g of crystal violet in 100 mL of 200 mM phosphoric acid, 200 mM formic acid and 200 mM MES; pH 6). Then the staining solution was removed, the plate was washed four times with deionized water and dried, and the uniformity of coloration within each well was promoted by treatment with 100 μL of 10% acetic acid and gentle shaking at RT for 15 min. Absorbance was read at 595 nm in a BioRad model 680 microplate reader, and cell viability was expressed as IC_{50}, the concentration of $PbMe_2(NO_3)_2$ required for 50% inhibition of cell growth with respect to controls.

In Vivo Studies. The experiment was conducted with 24 male albino Sprague−Dawley rats weighing 110−150 g, which were randomly divided into four groups of six rats each. The animals were housed individually in metabolic cages and maintained on a standard pellet diet with water *ad libitum*. δ-Aminolevulinic acid hydrochloride (Aldrich) and the emulsifier polyoxyethylene sorbitan monooleate (Tween 80, from Analema) were used as received. $PbMe_2(NO_3)_2$ and $TCl\cdot H_2O$ were dissolved in Milli-Q water, and H_2tspa in a 10% (*v*/*v*) solution of Tween 80 in Milli-Q water; all were administered i.p., at the dosages specified below, in a constant volume of 10 mL/kg. Thirty minutes after administration of 36.1 mg/kg of $PbMe_2(NO_3)_2$ to all four groups, one group received an 18.6 mg/kg dose of H_2tspa, and two groups a 48.1 mg/kg dose of $TCl\cdot H_2O$, to one of which an 18.6 mg/kg of H_2tspa was given after a further 30 min. After 7 days the animals were sacrificed under light CO_2 anesthesia. Blood was collected by cardiac puncture into heparinized tubes that were kept in liquid nitrogen[32] until the determination of lead concentration by atomic absorption spectrometry (AAS) at 283.3 nm in a Perkin-Elmer 1100 B spectrometer with electrothermal atomization. Livers, kidneys, and brains were removed, lyophilized, powdered, and digested with a mixture of nitric acid and H_2O_2 in a microwave oven, and their lead concentrations were determined by AAS at 217 nm in a Perkin-Elmer 3110 flame spectrometer. All determinations were performed in triplicate.

δ-ALAD Activity. δ-ALAD activity in whole blood was assayed as per Berlin and Schaller.[33] The concentration of the salt formed by porphobilinogen and modified Ehrlich's reagent was calculated from absorbance measured at 555 nm against a reagent blank using a molar absorption coefficient of 6.2×10^4 L M^{-1} cm^{-1}. Results are expressed as (μmol ALA) min^{-1} (L erythrocytes)$^{-1}$.

Statistical Analyses. Results are expressed as means ± SEMs. The statistical significance of differences between means was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test. The criterion of statistical significance was $p < 0.05$.

Results and Discussion

The ^{1}H NMR spectrum of a 10 mM solution of $PbMe_2(NO_3)_2$ in D_2O, pD = 5.6, showed the expected signals for the methyl protons at 2.36 ppm (J = 138 Hz), and also a weak signal at 1.36 ppm (J = 77 Hz) that is attributed to $PbMe_3^+$ formed by redistribution of the methyl groups.[23] Addition of the stoichiometric amount of solid thiamine simply superimposed this spectrum on that of thiamine, without modifying any signals in either; and the only metalated species represented in the ESI-MS spectrum of an aqueous solution of $PbMe_2(NO_3)_2$, TNO_3, and H_2tspa in 1:1:1 mol ratio (H_2tspa was previously dissolved in aqueous $NaHCO_3$) were derived from [$PbMe_2$(tspa)]. It may be concluded that thiamine and "organic lead" do not interact, at least under these experimental conditions. Furthermore, similarly negative results were obtained in ^{1}H and ^{31}P NMR experiments on the possible interaction of $PbMe_2^{2+}$ with TDP, even though TDP is known to chelate $SnMe_2^{2+}$ both in the solid state and in D_2O.[34] This difference between $SnMe_2^{2+}$ and $PbMe_2^{2+}$ may be due to the "softer" nature of the latter.

The reactions of H_2tspa with dimethyl- and diphenyllead(IV) acetate give the 1:1 complexes [PbR_2(tspa)] (R = Me, Ph), which were also obtained, albeit with lesser purity, by reacting H_2tspa with the corresponding diorganolead(IV) nitrates. These light yellow complexes decompose without melting and are insoluble in all common organic solvents except dmf and dmso, in which they are only slightly soluble.

The reaction of a mixture of $PbPh_2(OAc)_2$, the acrylic acid, and diisopropylamine (Q) in 1:2:2 mol ratio afforded both [$PbPh_2$(tspa)] and, after removal of this product, $[HQ]_2[PbPh_2(tspa)_2]$, but it was not possible to isolate the methyl analogue from the analogous reaction with $PbMe_2(OAc)_2$. $[HQ]_2[PbPh_2(tspa)_2]$ is more soluble than [PbR_2(tspa)], dissolving in dmso, dmf, acetone, acetonitrile, and, though with greater difficulty, chloroform and ethanol.

Noteworthy in the ESI-MS spectra of [$PbMe_2$(tspa)] and [$PbPh_2$(tspa)] in methanol, apart from the [M+H] signal (see Figure 1 for the methyl derivative), is a small peak corresponding to a dimetalated fragment {$[C_3H_3O_2Pb_2S_2]$ for the methyl derivative, [2M+H] for the phenyl derivative} that probably reflects the polymeric nature of both these compounds (vide infra). The ESI-MS spectrum of $[HQ]_2[PbPh_2(tspa)_2]$ shows signals only for monometalated species, including an intense (60%) signal for the monoanion $[PbPh_2(tspa)_2+H]$.

The molecular structures of $[PbPh_2(tspa)(dmso)]\cdot$dmso and $[HQ]_2[PbPh_2(tspa)_2]$ are shown with the corresponding numbering schemes in Figures 2 and 3, respectively, and selected bond lengths and angles are listed in the Supporting Information, Table S2.

In the crystal of $[PbPh_2(tspa)(dmso)]\cdot$dmso, [$PbPh_2$(tspa)-(dmso)] monomers are linked in dimers by two asymmetric Pb−O···Pb bridges [Pb−O(1) = 2.313 Å; Pb−O(1)^i = 2.607 Å]. Although both metal−oxygen distances are longer than the sum of the covalent radii [2.12 Å[35]], they are well inside the usual range for Pb−O distances in lead compounds.[36] The long Pb···Pb distance, 4.217 Å, rules out any significant metal−metal interaction. Each Pb atom has a distorted octahedral environment defined by two apical C atoms belonging to its two phenyl groups, the chelating O and S atoms of its $tspa^{2-}$ ligand, the O atom of its coordinated dmso molecule and, at a greater distance, the O^i atom of the

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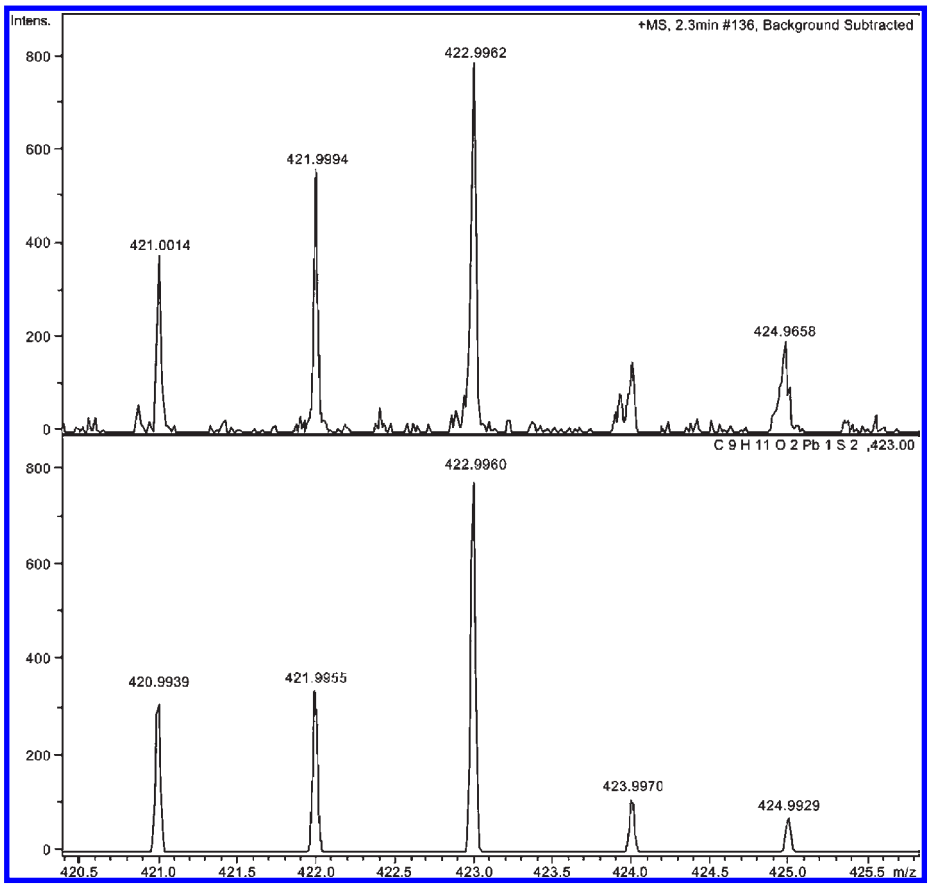

Figure 1. Comparison of the [M+H] signals in experimental (upper trace) and simulated (lower trace) ESI-MS spectra of $[PbMe_2(tspa)]$.

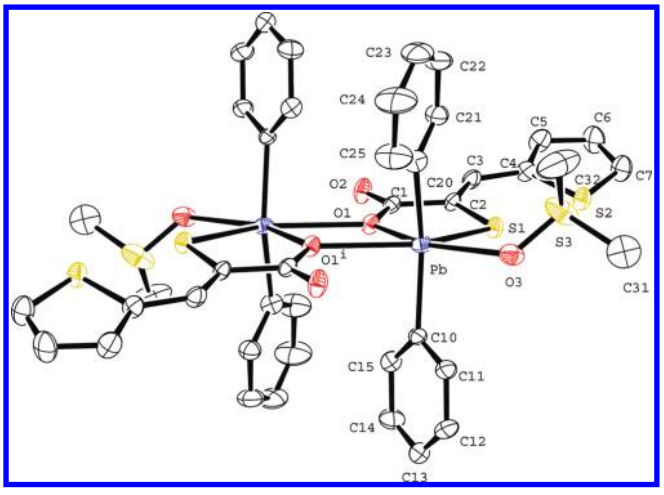

Figure 2. Dimeric structure of $[PbPh_2(tspa)(dmso)]\cdot dmso$, with the numbering scheme used.

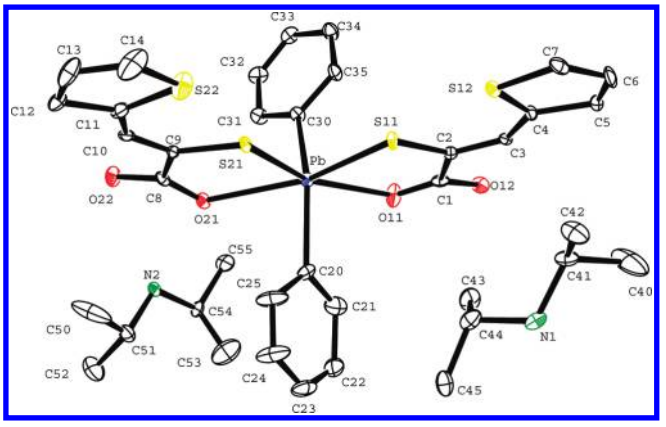

Figure 3. Structure of $[HQ]_2[PbPh_2(tspa)_2]$, with the numbering scheme used.

other $tspa^{2-}$ anion of the dimer. The four angles around the metal in the equatorial plane add up almost exactly to 360° but are heterogeneous: O(1)−Pb−O(1)^i, O(1)−Pb−S(1) and S(1)−Pb−O(3) are all less than 90°, which leaves a wide O(1)−Pb−O(3) angle (139.99°) toward which the phenyl groups bend to reduce steric hindrance [C(10)−Pb−C(20) = 151.7°]. The dihedral angle between the phenyl ring planes, 55.29°, may optimize packing.

There are two intradimeric hydrogen bonds in $[PbPh_2(tspa)(dmso)]\cdot dmso$: C(11)−H(11)···O(3), between the coordinated dmso molecule and a phenyl C−H; and C(25)−H(25)···O(2)^i, between a C−H of the other phenyl and the uncoordinated carboxylate O of the other monomer (see Supporting Information, Table S3 for details). In addition, the highly disordered uncoordinated dmso molecule is weakly linked to a $[PbPh_2(tspa)(dmso)]$ monomer by another hydrogen bond, C(5)−H(5)···O(4).

$[HQ]_2[PbPh_2(tspa)_2]$ consists of diisopropylammonium cations and $[PbPh_2(tspa)_2]^{2-}$ anions. In the latter the Pb atom has a distorted octahedral environment defined by the coordinated C atoms of its phenyl groups and the chelating O and S atoms of its $tspa^{2-}$ ligands. One of these ligands is slightly farther away than the other [Pb−O(11) = 2.484 Å, Pb−O(21) = 2.563 Å; Pb−S(11) = 2.5992 Å, Pb−S(21) = 2.616 Å], but both are practically flat, with rms deviations from

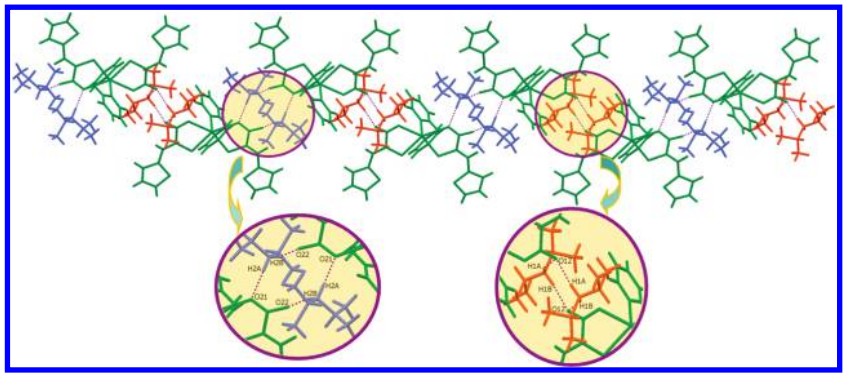

Figure 4. Chain of anions and cations linked by hydrogen bonds in $[HQ]_2[PbPh_2(tspa)_2]$. The chain runs parallel to the *y* axis.

the least-squares planes of only 0.1147 Å for [S(21) O(21) O(22) C(8) C(9) C(10) C(11) C(12) C(13) C(14) S(22)] and 0.0441 Å for [S(11) O(11) O(12) C(1) C(2) C(3) C(4) C(5) C(6) C(7) S(12)]. They make a dihedral angle of 5.77° with each other (in spite of which the rms deviation from the least-squares plane through all their atoms is only 0.1100 Å and the sum of the four equatorial angles around Pb is practically 360°), and adopt a *cis* arrangement in which the S−Pb−S angle is only 81.47°. Together with the narrow bite of the $tspa^{2-}$ ligands [O(11)−Pb−S(11) = 73.31°, O(21)−Pb−S(21) = 71.69°], this latter feature leaves a wide O−Pb−O angle of 133.5°, toward which the phenyl groups bend to reduce steric hindrance (C−Pb−C = 144.5°) while forming a dihedral angle of 89.69° between their planes.

The two diisopropylammonium cations are each hydrogen bonded to two anions (Supporting Information, Table S3), N(1) forming bonds with two uncoordinated oxygens [both of type O(12)] and N(2) with one uncoordinated and one coordinated oxygen [of types O(22) and O(21), respectively]. These interactions link the anions and cations in chains parallel to the *y* axis (Figure 4), leaving each unit cell with four symmetry-related 195 $Å^3$ cavities, each with an electron density integrating to 66 e^- that probably corresponds to an average of about 2.5 disordered molecules of ethanol.

The IR spectra of $[PbR_2(tspa)]$ and $[HQ]_2[PbPh_2(tspa)_2]$ lack the ν(S−H) band located at 2567 cm^{-1} in the spectrum of H_2tspa, and the COOH bands at 1662 cm^{-1} [ν(C=O)], 1408 cm^{-1} [δ(O−H)], and 1269 cm^{-1} [ν(C−O)] in the latter spectrum are replaced by typical carboxylate bands. The positions of these bands in $[PbMe_2(tspa)]$ (ν_a at 1520 cm^{-1} and ν_s at 1361 cm^{-1}) and $[PbPh_2(tspa)]$ (ν_a at 1521 cm^{-1} and ν_s at 1363 cm^{-1}) afford practically identical values of $\Delta\nu = \nu_a - \nu_s$, 159 and 158 cm^{-1}. This similarity suggests that the carboxylate group has the same coordination mode in both compounds; and the observed values, which are within the accepted range for bridging carboxylate[37] and close to the values found for $[SnR_2(xspa)]$ [*x*spa = 3-(2-pyridyl)-2-sulfanylpropenoato], in which X-ray studies show carboxylate bridges to create a polymeric structure,[38] suggest that $[PbMe_2(tspa)]$ and $[PbPh_2(tspa)]$ are likewise polymeric, in keeping with their solubilities and ESI-MS data.

The IR spectrum of $[HQ]_2[PbPh_2(tspa)_2]$ has a band at 1610 cm^{-1} that can be attributed to the diisopropylammonium $\delta(NH_2)$ vibration.[39] Its carboxylate bands (ν_a at 1546 cm^{-1}, ν_s at 1336 cm^{-1}) afford $\Delta\nu = 210$ cm^{-1}, a value similar to those found for mercury(II)-tspa complexes in which, as in the case of $[HQ]_2[PbPh_2(tspa)_2]$, X-ray studies have shown the carboxylate group to be monodentate and hydrogen bonded to the diisopropylammonium ion.[12]

The ^1H NMR signals of all the complexes integrate to values in keeping with the solid-state stoichiometry of these compounds; the absence of the carboxylic and thiol proton signals of H_2tspa confirm that the ligand is not protonated in dmso solution; and in the spectrum of $[HQ]_2[PbPh_2(tspa)_2]$ the high-field septuplet and doublet show that the isopropylammonium acts as counterion. The coordination of $tspa^{2-}$ via C(2)−S is supported by the shift of the C(3)H signal to higher field;[9,12,40] and indeed, the similarly upfield shifts of all the thienyl ring protons are compatible with O,S-coordination. Nevertheless, certain changes in the coordination spheres are indicated by the ^1H-^{207}Pb coupling constants. Specifically, the value of $^2J(^1H\text{-}^{207}Pb)$ in $[PbMe_2(tspa)]$, 128.5 Hz [smaller than for most dimethyllead(IV) complexes in dmso[41]], must reflect the reduction in coordination number upon dissociation of the polymer, in which the coordination number is five, even if dissociation is not complete (vide infra); while the reduction in $^3J(^1H\text{-}^{207}Pb)$ from 205.5 Hz in $PbPh_2(OAc)_2$ to 170 Hz in $[PbPh_2(tspa)]$ and 167 Hz in $[PbPh_2(tspa)_2]^{2-}$, values intermediate between those corresponding to coordination numbers of five and six,[42−44] suggests partial dissociation of $[PbPh_2(tspa)_2]^{2-}$. The small coordination number of $[PbMe_2(tspa)]$ is particularly interesting in relation to the observed redistribution of methyl groups in this compound (see the Experimental Section), for since this process doubtless requires that the participating lead centers draw close to each other,[23] it must be facilitated by the uncrowding of their coordination spheres.

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The predominant O,S-coordination of all the complexes in solution in dmso is supported in their ^{13}C spectra by the deshielding of C(1) and C(2) upon coordination to the organometallic moiety. The downfield shift of the carboxylate signal, from 166.3 ppm in the spectrum of H_2tspa to 171.1−173.2 ppm in the complexes, may be due to the inductive effect of the Pb−O···Pb bridges of the presumed minor product $[PbPh_2(tspa)(dmso)]_2$ and/or, in the case of $[PbR_2(tspa)]$, to the major product existing as oligomers with bridging carboxylate groups, a situation that has been related to the deshielding of the carboxylate signals of diorganotin(IV) complexes of sulfanylcarboxylic acids.[45]

In general, neither the ^1H nor the ^{13}C NMR signals of the organometallic moieties underwent significant shifts upon coordination, the only exceptions being the shift of the Pb-bound carbon signals to lower frequencies than in the corresponding acetates. The $^2J(^{13}C\text{-}^{207}Pb)$ values of $[PbPh_2(tspa)]$ and $[HQ]_2[PbPh_2(tspa)_2]$, 120.0 and 114.0 Hz, respectively, are in keeping with the analysis of the corresponding $^3J(^1H\text{-}^{207}Pb)$ values.[43]

Since preliminary experiments showed $PbMe_2^{2+}$ to be rather more toxic to LLC-PK1 cells than $PbPh_2^{2+}$, we present and discuss here only the results obtained with the former. Renal cells were chosen as in vitro test culture because, of the organs in which most lead accumulates (liver and kidney), it is the kidney in which the interaction of $PbMe_2^{2+}$ with therapeutic or prophylactic agents is likely to be most observable: whereas hepatic alkyllead is apparently largely metabolized to inorganic species (these being the main forms observed in faeces), the predominance of dialkyllead in urine suggests that metabolization processes are less active in the kidney.[46,47]

As Figure 5 shows, thiamine had no statistically significant protective effect in LLC-PK1 cells, but H_2tspa did ($p < 0.05$), and the combination of H_2tspa and thiamine was almost twice as effective as H_2tspa alone: with this latter treatment, 50% inhibition of the cells required about 2.5 times as much $PbMe_2(NO_3)_2$ as with no treatment ($p < 0.001$). H_2tspa seems likely to act by chelating the lead, thereby forestalling or reducing its toxic activity. The coadjuvant effect of thiamine may be partly due to reinforcement of some of its normal biological roles: first, the promotion of cell replication as the cofactor of transketolase, an enzyme involved in ribose synthesis, following metabolization to TDP upon entry into the cell;[48] and second, the reduction of lead-induced oxidative stress[49] through the radical-scavenging activity of both thiamine and TDP.[50]

Some 99% of blood-borne Pb^{II} is found in erythrocytes, over 80% of it bound to and inactivating δ-aminolevulinic acid dehydratase (δ-ALAD), a cytosolic metalloprotein that catalyzes the second step of heme biosynthesis. δ-ALAD activity in blood is accordingly regarded as a useful

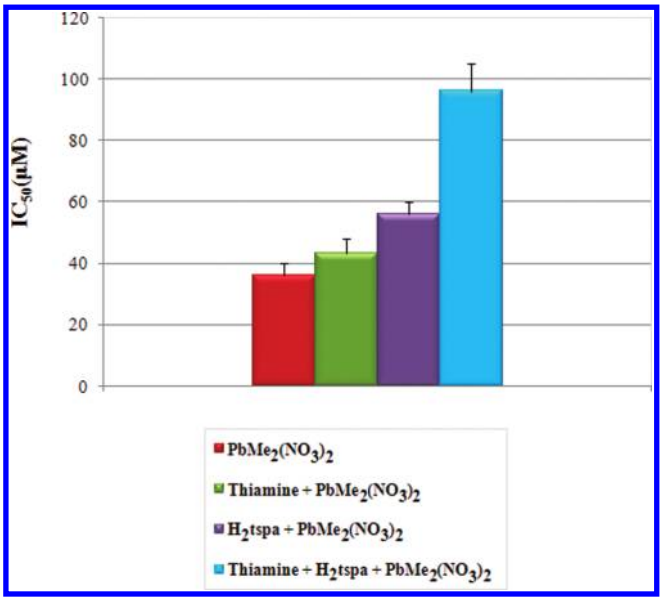

Figure 5. Protective effects of thiamine and H_2tspa, alone and in combination, on the concentration of $PbMe_2(NO_3)_2$ required for a 50% reduction in the viability of LLC-PK1 cells.

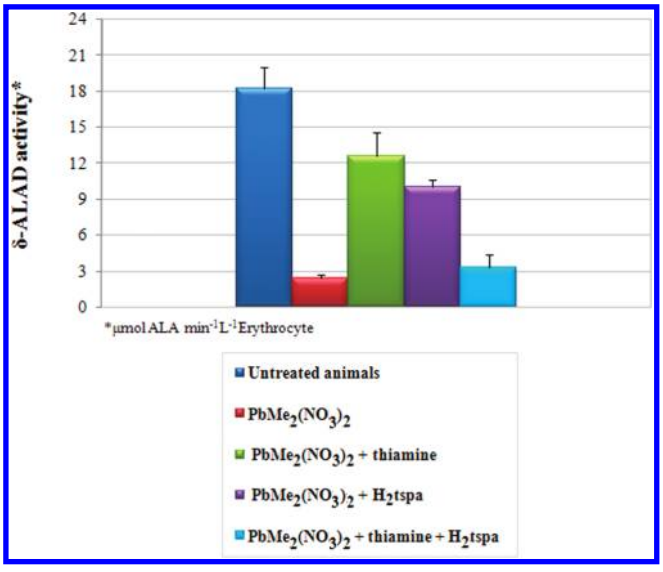

Figure 6. Blood δ-ALAD activity in Sprague−Dawley rats 1 week after i.p. administration of $PbMe_2(NO_3)_2$, with or without thiamine, H_2tspa, or both.

biomarker of lead intoxication.[51,52] In this study, i.p. administration of a 36.1 mg/kg dose of $PbMe_2(NO_3)_2$ to Sprague−Dawley rats reduced blood δ-ALAD activity from 18.2 ± 1.9 to 2.4 ± 0.3 (μmol ALA) min^{-1} (L erythrocytes)$^{-1}$ within a week of administration (Figure 6).

The inhibition of δ-ALAD by "inorganic lead" is attributed to its replacing zinc in the native enzyme, in which Zn^{II} is coordinated to three cysteine residues and a water molecule or hydroxide ion. The zinc atom may act not only by holding this H_2O or OH^- molecule ready to accept the proton taken from the substrate but also by coordinating to the carbonyl oxygen of the latter.[53] The replacement of Zn^{II} by the larger

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Table 1. Total Lead Concentrations in Rat Blood and Tissues Following Various Treatments (means ± SEMs)

group	blood[a]	liver[b]	kidney[b]	brain[b]
$PbMe_2(NO_3)_2$	30.6 ± 1.9	71.0 ± 5.6	105.2 ± 18.6	21.6 ± 1.3
$PbMe_2(NO_3)_2$+thiamine	17.6 ± 5.4	47.9 ± 6.4	35.4 ± 7.2	8.8 ± 1.1
$PbMe_2(NO_3)_2$+H_2tspa	57.7 ± 5.1	78.2 ± 9.6	56.6 ± 9.9	13.4 ± 0.5
$PbMe_2(NO_3)_2$+thiamine+H_2tspa	52.7 ± 9.5	84.0 ± 17.0	58.2 ± 9.5	19.2 ± 2.7

[a] mg/L. [b] μg/g dry tissue.

Pb^{II} gives rise not only to the logical lengthening of the three cysteine-metal distances but also to the reorientation of a serine residue to form a serine-metal bond. Furthermore, analysis of a molecular model of Pb—δ-ALAD suggests that a stereochemically active lone pair on the metal may reduce its electrophilicity and hinder the approach of the substrate.[54] Though this latter effect cannot operate in the case of the lead(IV) derivative $PbMe_2^{2+}$, its methyl groups might likewise hinder the approach of the substrate. Alternatively, the inhibition of δ-ALAD observed in this study may have been due to the partial metabolization of $PbMe_2^{2+}$ to Pb^{II}.[46,47]

When thiamine or H_2tspa were administered 30 min after $PbMe_2(NO_3)_2$, significantly larger residual activities were observed, 12.5 ± 2.0 (μmol ALA) min^{-1} (L erythrocytes)$^{-1}$ ($p < 0.001$) and 10.0 ± 0.6 (μmol ALA) min^{-1} (L erythrocytes)$^{-1}$ ($p < 0.05$), respectively; the difference between these residual activities is not statistically significant. However, by contrast with its in vitro efficiency, combination therapy[19] (in this case successive administration of thiamine and H_2tspa at half-hour intervals) achieved a residual activity of only 3.3 ± 1.0 (μmol ALA) min^{-1} (L erythrocytes)$^{-1}$, a level that is not statistically different from that of the animals treated with $PbMe_2(NO_3)_2$ alone. We have no explanation of this anti-synergistic effect, which requires further study.

The administration of thiamine to rats, cattle, freshwater fish and sheep poisoned with "inorganic lead" has been reported to lower total lead concentrations in all tissues.[55−58] In the present study of "organic lead" poisoning, total lead concentrations in blood, liver, kidney, and brain were all lower in thiamine-treated rats than in untreated animals (Table 1), but the reduction was only statistically significant for kidney and brain ($p < 0.01$ in both cases).

These reductions may have been due either to prevention of the deposition of lead in these organs or to promotion of its elimination. The sequestration of lead by thiamine or TDP through direct interaction appears to be ruled out by the NMR results described in the first paragraph of this Results and Discussion section. It is also unlikely that the lead is complexed by hydrolyzed "yellow" or "thiol" forms of the vitamin,[59] as has been suggested for "inorganic lead" poisoning (ref 58 and references therein), since the presence of these forms is negligible at pH < 9.[60] It therefore seems possible that the mechanism of the beneficial effects of thiamine in lead poisoning is not complexation, as has usually been assumed, but a general reinforcement of aspects of cell metabolism that are involved in the natural elimination of the metal (vide supra).

Like thiamine, H_2tspa brought about a statistically significant reduction in the lead content of kidney and brain ($p < 0.05$ in both cases), but lead levels in blood and liver were higher after H_2tspa treatment than in animals treated only with $PbMe_2(NO_3)_2$, although the difference in these cases was not statistically significant. By contrast, H_2tspa treatment of Pb^{II}-poisoned rats reduces lead levels in all four locations.[61] This difference may be due partly to the use of different administration routes[62] (gastric gavage for Pb^{II}, i.p. in this study), but probably derives largely from the different toxicokinetics of Pb^{II} and $PbMe_2^{2+}$.

Lead levels in blood, liver, kidney, and brain following administration of both H_2tspa and thiamine exhibited a pattern similar to that observed following administration of H_2tspa alone, except that only the reduction in kidney was statistically significant ($p < 0.05$).

In relation to the lowering of lead levels in kidney by thiamine and H_2tspa, it may be noted that urinary excretion is the main route for the elimination of $PbMe_2^{2+}$ by $PbMe_4$-poisoned rabbits,[47] and may therefore be similarly important for all mammals.

Conclusions

The reactions of $PbMe_2^{2+}$ and $PbPh_2^{2+}$ with 3-(2-thienyl)-2-sulfanylpropenoic acid (H_2tspa) give [$PbMe_2$(tspa)], [$PbPh_2$(tspa)] (which in dmso evolves to [$PbPh_2$(tspa)(dmso)], and [HQ]$_2$[$PbPh_2$(tspa)$_2$]. X-ray studies of dimeric [$PbPh_2$(tspa)(dmso)] and ionic [HQ]$_2$[$PbPh_2$(tspa)$_2$] (HQ = diisopropylammonium) show that the Pb atom has a distorted octahedral environment, and the $tspa^{2-}$ ligand is S,O-bidentate. NMR and ESI-MS studies confirm that $tspa^{2-}$ remains coordinated to the metal in dmso and water, respectively, which suggests that $tspa^{2-}$ may similarly coordinate diorganolead(IV) cations in biological media.

Pretreatment with H_2tspa protects LLC-PK1 renal proximal tubule cells against strong inhibition of their growth by $PbMe_2^{2+}$. This effect of H_2tspa is increased by addition of thiamine, but thiamine by itself has no protective effect.

Administration of $PbMe_2^{2+}$ to Sprague−Dawley rats reduces δ-ALAD activity in blood, but this reduction is partially prevented by administration of either thiamine or H_2tspa (but not both) shortly after $PbMe_2^{2+}$. Similarly, the accumulation of lead in kidney and brain is partially prevented by post-$PbMe_2^{2+}$ administration of either thiamine or H_2tspa, but joint administration of both compounds is only effective in the kidney.

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Whereas both solid-state and solution studies show interaction between $PbMe_2^{2+}$ and H_2tspa, NMR experiments show no interaction in either dmso or water between $PbMe_2^{2+}$ and either thiamine or thiamine diphosphate. Accordingly, the influence of thiamine on the toxicity of organolead(IV) that was observed in vitro and in vivo in this study is ascribed to some indirect biochemical mechanism rather than to direct vitamin-metal interaction.

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Note Added after ASAP Publication. This paper was published on January 20, 2010, with the incorrect artwork for Figure 1. The corrected version was reposted on January 25, 2010.

Supporting Information Available: Tables S1−S3 and CIF files {CCDC numbers 748232 and 748233 for $[HQ]_2[PbPh_2(tspa)_2]$ and $[PbPh_2(tspa)(dmso)]\cdot$dmso, respectively}. This material is available free of charge via the Internet at http://pubs.acs.org.