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Organometallic Complexes with Biological Molecules: XII. Solid-state and Solution Studies on Dialkyltin(IV)– and Trialkyltin(IV)–thiaminepyrophosphate Derivatives

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Dialkyltin(IV) and trialkyltin(IV) derivatives of the coenzyme thiaminepyrophosphate (H_2TPP) have been synthesized with general formula $R_2Sn(HTPP)_2{\cdot}nH_2O$ (Alk = Me, n = 2; Alk = Bu, n = 4) and $R_3SnHTPP{\cdot}nH_2O$ (R=Me, n = 2; R = Bu, n = 1), respectively. The solid-state structure of the complexes has been investigated through infrared and Mössbauer spectroscopy.

The infrared data suggest the involvement of only phosphate oxygen atoms in the coordination of both dialkyl- and trialkyl-tin(IV) moieties, with phosphate anions behaving as monoanionic bidentate bridging or chelating groups, with the tin(IV) involved in six- and five-fold coordination geometries, respectively, in $R_2Sn(HTPP)_2{\cdot}nH_2O$ (R = Me, n = 2; R = Bu, n = 4) and $R_3SnHTPP{\cdot}nH_2O$ (R = Me, n = 2; R = Bu, n = 1). The ^{119}Sn Mössbauer data, and in particular rationalization of the experimental nuclear quadrupole splittings, Δ, through the point-charge model formalism, suggests the occurrence of an octahedral trans-R_2 structure in $R_2Sn(HTPP)_2{\cdot}nH_2O$ (R = Me, n = 2; R = Bu, n = 4) and a trigonal-bipyramidal structure in $R_3SnHTPP{\cdot}nH_2O$ (R = Me, n = 2; R = Bu, n = 1). ^{1}H and ^{13}C NMR spectra, in D_2O, suggested that the soluble derivatives, at room temperature, in solution, maintained the solid-state structure.

The interactions of dibutyltin(IV)–thiaminepyrophosphate (DBTPP) and tributyltin(IV)–thiaminepyrophosphate (TBTPP) complexes with Bluescript KS(+) plasmid and immortalized 3T3 fibroblasts were studied.

Both compounds have a clear inhibitory effect on the growth of immortalized mouse embryonal fibroblasts (NIH-3T3), TBTPP being the much more active. No evidence was found, however, for DNA cleavage by the compounds at molar ratios as high as 1:10 (DBTPP, TBTPP/DNA base pairs). According to our observations, the cytotoxicity of TBTPP does not seem to be based on direct interaction with DNA, but in the presence of TBTPP (1:10, TBTPP/DNA bp), plasmid DNA seems to be more susceptible to cleavage by UV. Copyright © 1999 John Wiley & Sons, Ltd.

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INTRODUCTION

Many molecules present in living systems contain phosphate groups involved in the coordination of metal ions, naturally or accidentally occurring in the cells or in the biological fluids. If other, different, donor atoms are present in such molecules, competition may occur between the oxygen atoms of the phosphate groups and the other donor atoms, which are mostly nitrogen.

An interesting phosphate-containing molecule is represented by thiaminepyrophosphate (H_2TPP)

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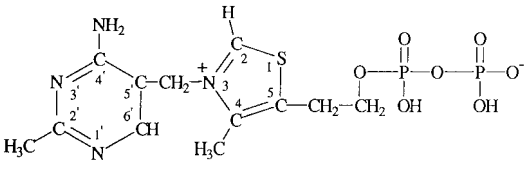

Figure 1 Thiaminepyrophosphate (H_2TPP), with the numbering scheme referred to in NMR assignments (according to Ref. 31).

(cocarboxylase), Fig. 1, a characteristic cofactor for several enzymes, which, in the presence of divalent metal ions [magnesium(II), manganese(II) etc.], functions as a covalent catalyst in enzymic reactions involving the apparent formation of acyl carbanions.[1] As an example, in the conversion of pyruvate to carbon dioxide and acetaldehyde, the intermediate formed from the addition of pyruvate to H_2TPP is decarboxylated to give the adduct of acetaldehyde, 2-(1-hydroxyethyl)thiaminepyrophosphate (H_2ETPP).[2,3]

In order to understand the mechanism of the enzymic reaction, it is important to establish with which part of the thiaminepyrophosphate the interaction with the metal ions occurred.

Recently, Lindqvist[4] and coworkers reported the X-ray crystal structure of calcium–thiaminepyrophosphate, according to which the metal ion coordinates the pyrophosphate group and does not react with other parts of the coenzyme

Rhodium(III) complexes of thiaminepyrophosphate and of 2-(1-hydroxyethyl) thiaminepyrophosphate were characterized by ^{1}H, ^{13}C and ^{31}P NMR.

Doublets in ^{31}P NMR spectra indicate bidentate coordination to rhodium(III) at the pyrophosphate moiety, while the ^{1}H and ^{13}C NMR signals for the pyrimidine group are essentially the same as those of the same group in uncomplexed H_2TPP, establishing that the pyrimidine position N(1′) is not coordinated to the metal ion.[5]

The broadening of resonance lines in the NMR of thiaminepyrophosphate by manganese(II) has been used to calculate values for the transverse relaxation times T_2. Subsequently, the longitudinal relaxation times T_1 were determined by using progressive power saturation, also by FT NMR.

Since the paramagnetic contribution to T_2^{-1} is always larger than to T_1^{-1}, T_1 was free of scalar quantities and therefore it was used to evaluate internuclear distances according to the Solomon–Bloembergen[6,7] relationship and hence to establish, the folded structure, from which, according to Grande *et al.*, manganese(II) binds thiaminepyrophosphate (H_2TPP) through its pyrophosphate group.[8]

The same kind of coordination was claimed by Williams and Baran to exist[9] in VO^{2+}TPP complexes, in the pH range 3–4, using spectrophotometric techniques, while at higher pH, coordination through N(1′) of the pyrimidine ring of the thiamine residue was suggested by the same authors.[9]

Khan and Rao,[10] studying copper(II)-catalyzed hydrolysis of H_2TPP to thiaminemonophosphate and phosphate, proposed coordination of both the phosphates of the pyrophosphate group together with N(1′) of the pyrimidine ring.

EXPERIMENTAL

Chemical syntheses

$R_2Sn(HTPP)_2{\cdot}nH_2O$ and $R_3SnHTPP{\cdot}nH_2O$ complexes (Table 1), were obtained by refluxing methanolic suspensions of the thiaminepyrophosphate ($H_2TPP{\cdot}4H_2O$) (4 mmol in 50 cm^3) and

Table 1 Analytical data (calculated values in parentheses) of thiaminepyrophosphate–organotin(IV) complexes

Compound[a]	C (%)	H (%)	N (%)	S (%)	Peak temperature, (°C)	Total H_2O loss (%)
$Me_2Sn\ (HTPP)_2{\cdot}2H_2O$	30.21	4.09	10.44	6.16	62	2.38
	(30.60)	(4.05)	(10.98)	(6.28)		(2.60)
$Bu_2Sn\ (HTPP)_2{\cdot}4H_2O$	34.22	4.67	9.85	5.30	68	6.26
	(33.43)	(5.08)	(9.75)	(5.57)		(6.78)
$Me_3SnHTPP{\cdot}2H_2O$	28.60	4.38	8.91	4.92	47; 69	5.78
	(28.91)	(4.85)	(8.99)	(5.15)		(6.13)
$Bu_3SnHTPP{\cdot}H_2O$	40.17	6.53	8.04	4.39	30; 50; 75	3.79
	(39.41)	(6.34)	(7.66)	(4.38)		(2.46)

[a] Thiaminepyrophosphate = $H_2TPP{\cdot}4H_2O$: peak temperature 122 °C, experimental (calculated) water loss = 15.18% (14.51%).

Table 2 Assignment of relevant absorption bands of thiaminepyrophosphate H_2TPP dialkyltin(IV)–thiaminepyrophosphate $R_2Sn(HTPP)_2{\cdot}nH_2O$ (R = Me, n = 2; R = Bu, n = 4), and trialkyltin(IV)–thiaminepyrophosphate $R_3SnHTPP{\cdot}nH_2O$ (R = Me, n = 2; R = Bu, n = 1) derivatives in the 4000–250 cm^{-1} region[a]

Assignment	$H_2TPP{\cdot}4H_2O$	$Me_2Sn(HTPP)_2{\cdot}2H_2O$	$Bu_2Sn(HTPP)_2{\cdot}4H_2O$	$Me_3SnHTPP{\cdot}2H_2O$	$Bu_3SnHTPP{\cdot}H_2O$
NH_2 bending or ring	1646 s	1652 s	1652 s	1652 s	1652 s
stretching coupling	1616 s	1598 s	1594 s	1597 s	1595 m, bd
Skeletal C=C, C=N stretchings	1588 w	1561 s		1560 s, bd	
	1537 m	1535 m	1542 s, bd	1541 s, bd	1540 m, bd
$\nu(PO_3)$ (deg)	1090 s, bd	1105 vs	1100 s, bd	1105 s	1105 m, bd
		1059 s	1054 s, bd	1061 s, bd	1072 s, bd
$\nu(PO_3)$	970 s	995 s	1010 m, bd	995 s, bd	998 m, vbd
		938 s	935 s, vbd	940 s, bd	940 m, vbd
ν(P—O—C)	800 m	810 m	810 m	800 m	810 m
PO_3 sym. def	578 m	587 m	575 m, bd	585 m, bd	590 m, bd
PO_3 deg. def. + PO_3 rock + ν_{as} SnC_2	543 m, bd	533 s, bd	535 s, bd	552 s, bd	545 m, bd
PO_3 (sym. def + deg. def) + PO_3 rock + $\nu_{sym}SnC_2$	504 m, bd	508 s, bd	508 s, bd	509 s, bd	508 s, bd

[a] Nujol and hexachlorobutadiene mulls; s = strong, m = medium, w = weak, bd = broad; v = very.

Table 3 Experimental Mössbauer parameters, isomer shift, δ (mm s^{-1}), and nuclear quadrupole splittings, Δ_{exp} (mm s^{-}), measured at liquid-N_2 temperature, and nuclear quadrupole splittings, Δ_{calcd}, calculated according to the point charge formalism applied to the idealized structures of Figs 2(A and B)[a]

Compound	δ[b]	Δ_{exp}[c]	Γ_1	Γ_2	C-Sn-C angle ± 13 (deg)	Δ_{calcd}[d]	Idealized structure
$Me_2Sn(HTPP)_2{\cdot}2H_2O$	1.25	4.28	0.98	0.92	180	−3.98	Fig. 2(A)
$Bu_2Sn(HTPP)_2{\cdot}4H_2O$	1.38	3.53	0.92	0.95	143	−3.98	Fig. 2(A)
$Me_3SnHTPP{\cdot}2H_2O$	1.27	3.58	0.97	0.94		−3.26	Fig. 2(B)
$Bu_3SnHTPP{\cdot}H_2O$	1.42	3.54	0.95	0.95		−3.26	Fig. 2(B)

[a] Sample thickness ranged between 0.50 and 0.60 mg $^{119}Sn\ cm^{-2}$.
[b] Isomer shift, $\delta \pm 0.03$ mm s^{-1} with respect to room-temperature $BaSnO_3$.
[c] $\Delta_{exp} \pm 0.02$, mm s^{-1}.
[d] Regular trigonal-bipyramidal or *trans*-R_2 octahedral structures of Fig. 2(A, B) are assumed to estimate the nuclear quadrupole splittings according to the point-charge model. The p.q.s. values used (mm s^{-1}) were (see refs. 28, 29): (1) trigonal-bipyramidal structure: $\{R\}^{tbe} = -1.13$ (R = Me, Bu; Ref. 28); $\{P—O\}^{tba} = -0.0325$ (calculated from Ref. 29); $\{P—O\}^{tbe} = 0.015$ (calculated from Ref. 29); (2) *trans*-R_2 octahedral structure: $([R]—[Cl])^{oct} = -1.03$ (R = Me, Bu; Ref. 28); $(\{P—O]—[Cl])^{oct} = -0.036$ (calculated from Ref. 29).

dimethyltin(IV) oxide (2 mmol), dibutyltin(IV)oxide (2 mmol), bis[tributyltin(IV)]oxide (2 mmol) and trimethyltin(IV)hydroxide (4 mmol), respectively, according Eqns [1]–[3]

$$2H_2TPP + R_2SnO \rightarrow R_2Sn(HTPP)_2 + H_2O \quad (R = Me, Bu) \quad [1]$$

$$2H_2TPP + (Bu_3Sn)_2O \rightarrow 2Bu_3SnHTPP + H_2O \quad [2]$$

$$H_2TPP + Me_3SnOH \rightarrow Me_3SnHTPP + H_2O \quad [3]$$

R_2SnO (R = Me, Bu), $(Bu_3Sn)_2O$ and Me_3SnOH were freshly prepared by hydrolysis of aqueous solutions of the parent organotin(IV) chlorides R_2SnCl_2 and R_3SnCl (R = Me, Bu) which were gifts from Witco GmbH, Bergkamen, Germany, according to standard procedures.[11]

The white precipitates were recovered by filtration and recrystallized from absolute ethanol.

Analytical methods

TG measurements (Table 1), were performed using a TA-3000 Mettler system in a pure nitrogen atmosphere. The results of TG analysis, carried out up to 600 °C, suggest the presence of water molecules not involved in coordination of tin(IV) atoms,[12–17] in all the investigated thiaminepyrophosphate–alkyltin(IV) complexes, namely $R_2Sn(HTPP)_2{\cdot}nH_2O$ and $R_3SnHTPP{\cdot}nH_2O$.

IR spectra were recorded, as Nujol and hexachlorobutadiene mulls, on a Perkin-Elmer grating spectrometer model 983G, between Csl windows.

The spectra were analyzed through a Perkin-Elmer 3600 data station with Perkin-Elmer PE983 software (Table 2).

^{119}Sn Mössbauer spectra (Table 3) were measured with a multichannel analyzer (TAKES model 639, Ponteranica, Bergamo, Italy) and Wissenschaftliche Elektronik system (MWE, München, Germany) consisting of an MR250 driving unit, an FG2 digital-function generator and an MA250 velocity transducer, moved at linear velocity and constant acceleration, in a triangular waveform. The liquid-nitrogen temperature spectra were obtained using a DN700 liquid-nitrogen cryostat with a DN1726 sample holder and a model ITC 502 temperature controller from Oxford Instruments, Oxford, UK. The temperature control was better than ±0.1 K. The multichannel calibration was performed with an enriched iron foil (^{57}Fe 95.2%, thickness 0.06 mm; Dupont, MA, USA), at room temperature, using a ^{57}Co–Pd source (10 mCi; Dupont, MA, USA), while the zero point of the Doppler velocity scale had been determined, at room temperature, through absorption spectra of natural $CaSnO_3$ ($^{119}Sn = 0.5$ mg cm^{-2}) and a Ba $^{119}SnO_3$ source (10 mCi; Amersham, UK).

The ^1H and ^{13}C NMR spectra were recorded with a Bruker AC250E spectrometer, operating at 5.87 T.

The D_2O, used for the NMR measurements was a Merck (Darmstadt, Germany) UVASOL reagent (>99.9% deuteriation) and was used for field-frequency lock and as a reference (^1H, $\delta = 2.57$ ppm; ^{13}C, $\delta = 39.7$ ppm). The spectra were recorded at room temperature (298 K) and the

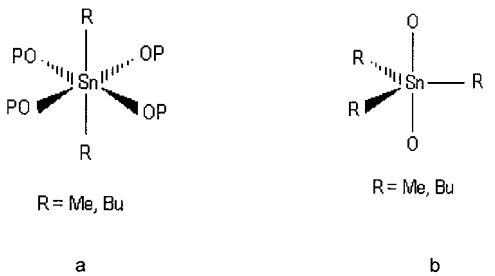

Figure 2 Regular structures of tin assumed to estimate the nuclear quadrupole splittings according to the point-charge model formalism (Table 3) for $R_2Sn(HTPP)_2{\cdot}nH_2O$ (R = Me, $n = 2$; R = Bu, $n = 4$) and $R_3SnHTPP{\cdot}nH_2O$ (R = Me, $n = 2$; R = Bu, $n = 1$), respectively. The partial quadrupole splittings (pqs) (mm s^{-1}) used in the calculations are reported in Table 3.

samples were prepared immediately before the measurement (Tables 4, 5)

Biological studies

Cell cultures and effects of organotin(IV) derivatives on cell growth

Cell culture conditions were essentially as described previously,[18] with minor modifications. In detail, cultured mouse embryonic fibroblasts (NIH 3T3) were harvested and detached from subconfluent culture by successive rinsing with PBS^0 (Ca^{2+}/Mg^{2+}-free phosphate-buffered saline), 1.0×10^{-6} mol dm^{-3} EDTA in PBS^0 and 1.0×10^{-6} mol dm^{-3} EDTA in PBS^0 containing 5 mg cm^{-3} trypsin.

Cells were counted and plated at a concentration of $(1.0–2.0) \times 10^5$ cells/25 cm^2 flask in DME (Dulbecco's modified Eagle's medium; Sigma, MO, USA), supplemented with antibiotics (40 mg dm^{-3} penicillin, 8 mg dm^{-3} ampicillin, 90 mg dm^{-3} streptomycin; Sigma) and 10% fetal calf serum (Gibco, Italy).

Cells were cultured at 37 °C in a humid 5% CO_2 atmosphere. After 24 h (the optimal time for the cells to adhere to the flask surface), filter-sterilized dialkyltin(IV)– and trialkyltin(IV)–thiaminepyrophosphate derivative solutions (DMSO 0.7%) were added to the medium at different concentrations (ranging from 1.0×10^{-9} to 1.0×10^{-6} mol dm^{-3}).

After incubation (ranging from 0.5 to 24 h), the medium was changed and cells were grown for additional 48 h. Untreated cells, and cells treated only with the solvent (DMSO 0.7%) or with the parent chemical compounds [i.e. thiaminepyrophosphate or dialkyltin(IV) and trialkytin(IV) chlorides] were used as controls.

To measure the cell toxicity of the compounds, cells, treated as described above, were harvested by trypsinization, pelleted and lyzed in 0.1 mol dm^{-3} NaOH at 50 °C for 90 min, and the absorbance of the solution was then measured at 260 nm using a DU-65 spectrophotometer (Beckman, CA, USA), as described by Di Liegro *et al.*[19]

Electrophoretic analysis of $DNA/H_2TPP{\cdot}4H_2O$, $Bu_2Sn(HTPP)_2{\cdot}4H_2O$ and $Bu_3SnHTPP{\cdot}H_2O$ mixtures

Either the $Bu_2Sn(HTPP)_2{\cdot}4H_2O$, $Bu_3SnHTPP{\cdot}H_2O$, or the control $H_2TPP{\cdot}4H_2O$ itself, was mixed with Bluescript plasmid DNA (Stratagene, CA, USA), at three different molar ratios (1:10, 1:50 and 1:100 butyltin(IV)–thiaminepyrophosphate complex/DNA base pairs, bp). After 30–60 min of preincubation at room temperature, the mixtures were analyzed by gel electrophoresis on 1% agarose in 1×10^{-2} mol dm^{-3} Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid], pH 7.4.

In some experiments, before running the gel, DNA/butyltin(IV)–thiaminepyrophosphate complexes were exposed to a Spectroline UV (254 nm) lamp (Aldrich Chemical Co., Inc.) for 15–30 min, at room temperature, to introduce nicks into the DNA double helix.

After running at 10–20 mA for 4–6 h, the gel was stained in ethidium bromide (2 mg cm^{-3} in distilled water) for 20 min, rinsed with water and photographed under uv illumination through a red filter.

RESULTS AND DISCUSSION

IR data

In the IR spectra of all the dialkyltin(IV)–and trialkytin(IV)–thiaminepyrophosphate derivatives, very strong and broad bands were present in the range 3400–3100 cm^{-1} which could be ascribed to NH_2 stretching with partial contributions of OH stretching of H-bonded H_2O molecules,[20] in agreement with the TGA information.

The biggest changes in the IR spectra on coordination compared with free thiaminepyrophosphate occurred in the regions 1100–900 and 600–500 cm^{-1}, ranges in which PO_3 stretching of the pyrophosphate groups normally occur[21,22] (Table 2). In the latter range variations occurred also for the presence of symmetric and asymmetric

Table 4 ^{1}H NMR data for thiaminepyrophosphate H_2TPP, dialkyltin(IV)–thiaminepyrophosphate $R_2Sn(HTPP)_2{\cdot}nH_2O$ (R = Me, n = 2; R = Bu, n = 4), and trimethyltin(IV)–thiaminepyrophosphate $Me_3SnHTPP{\cdot}2H_2O$ derivatives in D_2O solution[a]

Assignment/Compound	$H_2TPP{\cdot}4H_2O$[b]	$Me_2Sn(HTPP)_2{\cdot}2H_2O$	$Bu_2Sn(HTPP)_2{\cdot}4H_2O$	$Me_3SnHTPP{\cdot}2H_2O$
C2—H	9.60	9.60	9.60	9.58
C6′—H	7.86	7.94	7.94	7.96
C5′—CH_2	5.50	5.53	5.53	5.53
O—CH_2	4.07(q)	4.21(q)	4.19(q)	4.16(m)
C5—CH_2	3.21(t)	3.34(t)	3.33(t)	3.33(t)
C4—CH_3	2.60	2.58	2.59	2.57(t)
C2′—CH_3	2.50	2.56	2.56	2.39
Sn—Bu			1.64–0.88	
Sn—CH_3		0.93		0.55
$^{2}J(^{119}Sn^{1}H)$		98.4		68.7
C—Sn—C		159.4		117.8
$^{2}J(^{117}Sn^{1}H)$				66.1
C—Sn—C				116.2

[a] Coupling constants to tin, in parentheses, are in Hz; B_0 = 5.87 T; T = 298 K; δ, ppm from TMS.
[b] Thiaminepyrophosphate = $H_2TPP{\cdot}4H_2O$; abbreviations: t = triplet; q = quartet; m = multiplet. The numbering of the atoms is according to Ref. 31.

Table 5 ^{13}C NMR data for thiaminepyrophosphate H_2TPP, dialkyltin(IV)–thiaminepyrophosphate $R_2Sn(HTPP)_2{\cdot}nH_2O$ (R = Me, n = 2; R = Bu, n = 4), and trimethyltin(IV)thiaminepyrophosphate $Me_3SnHTPP{\cdot}2H_2O$ derivatives in D_2O solution[a]

Assignment/Compound	$H_2TPP{\cdot}4H_2O$[b]	$Me_2Sn(HTPP)_2{\cdot}2H_2O$	$Bu_2Sn(HTPP)_2{\cdot}4H_2O$	$Me_3SnHTPP{\cdot}2H_2O$
C2′	167.72	167.54	167.23	168.26
C4′	165.43	165.33	165.43	165.70
C2	157.42	157.42	151.05	153.07
C6′	150.69	150.55	149.76	150.15
C4	145.78	146.08	146.02	146.36
C5	138.35	138.27	138.45	138.90
C5′	109.00	108.65	108.79	108.94
5β CH_2	62.14	67.67	67.53	67.75(q)
Bridged CH_2	52.36	52.93	52.85	53.15(t)
5α CH_2	25.05	30.20	30.18	30.85(q)
2′CH_3	24.45	24.53	24.35	26.14(q)
4′CH_3	13.59	13.85	13.85	13.23(q)
Sn-R		0.95	28.90, 28.66, 23.83, 15.64	3.04

[a] B_0 = 5.87 T; T = 298 K; δ, ppm from TMS.
[b] Thiamine pyrophosphate = $H_2TPP{\cdot}4H_2O$; abbreviations. t = triplet; q = quartet. The numbering of the atoms is according to Ref. 31.

SnC_2 vibrations. The above mentioned evidence strongly supports the involvement of only the pyrophosphate groups in coordinating to the tin(IV) atom of the dialkyltin(IV) and trialkyltin(IV) moieties.

Mössbauer spectra

The coordination polyhedra for the complexes was inferred from Mössbauer spectra at liquid-nitrogen temperature and by extracting the experimental quadrupole splittings Δ_{exp}, mm s^{-1} (Table 3), together with the isomer shifts, calculated C-Sn-C angles for the dialkyltin(IV) derivatives and calculated Δ_{calc} for both dialkyltin(IV) and trialkyltin(IV) complexes, by the rationalization of the experimental Δ_{exp} according to the point-charge model formalism applied to the idealized structures

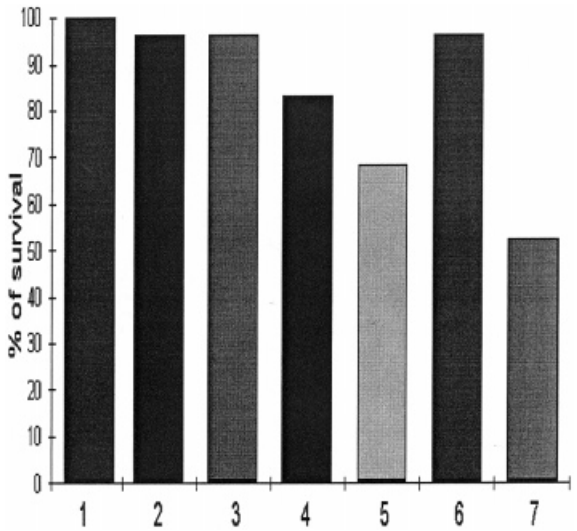

Figure 3 Effects of 6 h of incubation of 3T3 cells with different compounds, (1) untreated cells; (2) cells treated with dimethyl sulfoxide; (3) H_2TPP; (4) $Me_2Sn(HTPP)_2 \cdot H_2O$; (5) $Bu_2Sn(HTPP)_2 \cdot 4H_2O$; (6) $Me_3SnHTPP \cdot 2H_2O$; (7) $Bu_3SnHTPP \cdot H_2O$. In samples 4–7, the various compounds were used at a concentration of 1.6×10^{-6} mol dm^{-3}.

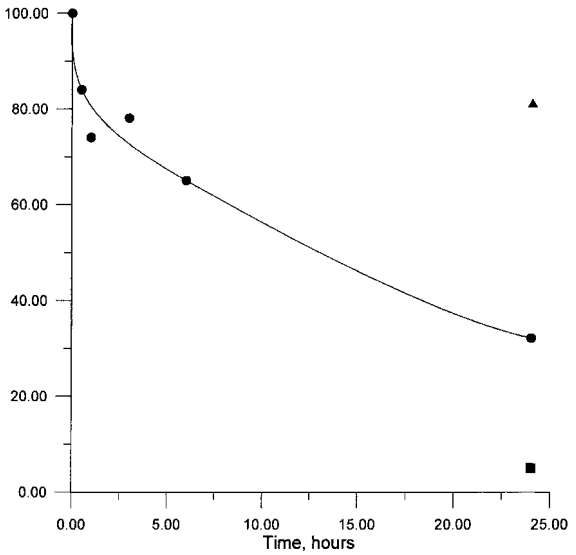

Figure 5 Time dependence of the effects on 3T3 cell growth of 1.00×0^{-6} mol dm^{-3} solutions of $Bu_3SnHTPP \cdot H_2O$ (●), Bu_3SnCl (■) and H_2TPP (▲) (in these two latter cases only the effect of 24 h of treatment is reported).

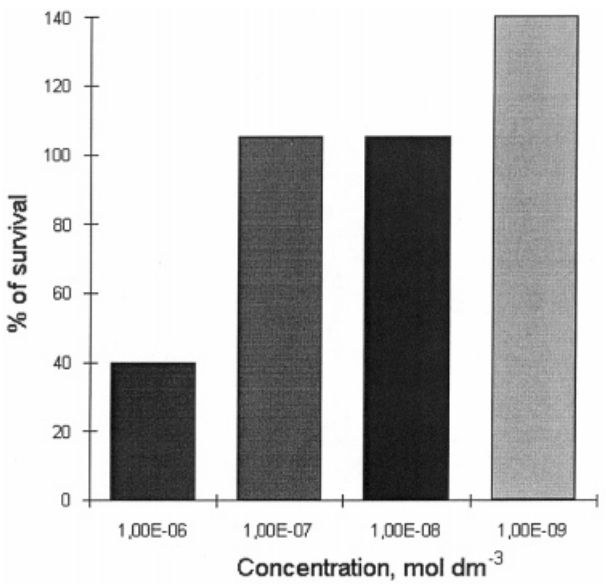

Figure 4 Dose dependence of the effects on the growth of 3T3 cells treated for 6 h with $Bu_3SnHTPP \cdot H_2O$ solutions.

of Figs 2(A and B).[23–28] The comparison of experimental and calculated nuclear quadrupole resonances, Δ_{exp} and Δ_{calc}, reported in Table 3 shows that their difference was within the tolerance limit of the method, ± 0.4 mm s^{-1}), suggesting *trans*-R_2 octahedral configurations. The isomer shifts are characteristic of covalent bonds and reflect the meaning of the parameter.

^{1}H and ^{13}C NMR

The ^{1}H and ^{13}C NMR measurements were carried out in D_2O (Tables 4 and 5). The ^{1}H and ^{13}C data in the complexes did not show significant shifts of the atoms (H or C) of the pyrimidine and of the thiazole groups,[30,31] which excluded any involvement of both groups in coordinating the tin(IV) atoms of the alkytin(IV) moieties. On the other hand, the above findings are in agreement with the involvement of the oxygen atoms of the phosphate groups in coordination of the alkytin(IV) moieties, which would rule out electronic effects on both the hydrogen and carbon atoms of the pyrimidine and of the thiazole groups.

The methyltin(IV) moiety of both $Me_2Sn(HTPP)_2 \cdot 2H_2O$ and $Me_3SnHTPP \cdot 2H_2O$ showed coupling constants $^2J(^1H–^{119}Sn)$, Table 4, which may be related to the coordination number of the tin atom and to the C-Sn-C angles of the organometallic moiety.

The equations of Lockhart and Mander,[32]

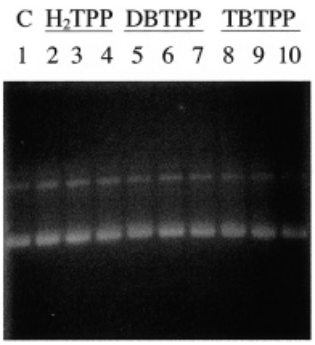

Figure 6 Electrophoretic analysis of Bluescript plasmid mixed with different amounts of H_2TPP (lanes 2–4), DBTPP (lanes 5–7) or TBTPP (lanes 8–10), at molar ratios (DNA bp/compound) of 100:1 (lanes 2, 5 and 8), 50:1 (lanes 3, 6 and 9) or 10:1 (lanes 4, 7 and 10). In lane 1, untreated plasmid was electrophoresed as control (C).

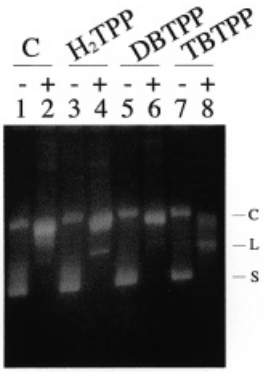

Figure 7 Electrophoretic analysis of Bluescript plasmid mixed with H_2TPP (lanes 3, 4), DBTPP (lanes 5, 6) or TBTPP (lanes 7, 8), at molar ratios of 10:1 DNA bp/compound. After 30 min of DNA compound interaction at room temperature, mixtures were incubated for an additional 15 min, at room temperature, with (+) or without (−) UV irradiation (254 nm). Naked DNA, treated (+) or not (−) with UV, was electrophoresed as control (C: lanes 1, 2). C, relaxed circular plasmid DNA; L, linear plasmid DNA; S, supercoiled plasmid DNA.

relating $^2J(^1H–^{119}Sn)$ and the geometry of the organometallic moiety were therefore used to evaluate the C-Sn-C angles in $Me_2Sn(HTPP)_2{\cdot}2H_2O$ and $Me_3SnHTPP{\cdot}2H_2O$, which were found to be 159.4° and 117.8°, respectively (Table 4). These results tend to confirm that both $Me_2Sn(HTPP)_2{\cdot}2H_2O$ and $Me_3SnHTPP{\cdot}2H_2O$ maintain in D_2O their solid-state configurations, in particular slightly distorted octahedral *trans*-Me_2 geometry

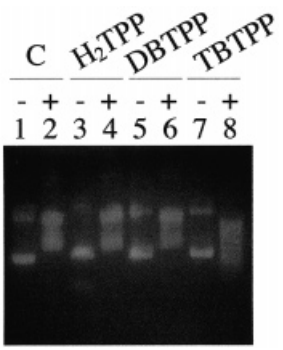

Figure 8 Electrophoretic analysis of Bluescript plasmid mixed with H_2TPP (lanes 3, 4), DBTPP (lanes 5, 6) or TBTPP (lanes 7, 8), at molar ratios of 10:1 DNA bp/compound. After 30 min of DNA/compound interaction at room temperature, mixtures were incubated for an additional 30 min, at room temperature, with (+) or without (−) UV. irradiation (254 nm). Naked DNA, treated (+) or not (−) with UV, was electrophoresed as control (C, lanes 1, 2). C, relaxed circular plasmid DNA; S, supercoiled plasmid DNA.

$Me_2Sn(HTPP)_2{\cdot}2H_2O$ and eq-Me_3 trigonal-bipyramidal around the central tin atom in $Me_3SnHTPP{\cdot}2H_2O$.

Finally, ^1H and ^{13}C NMR spectra of $Bu_3SnHTPP{\cdot}H_2O$ were not recorded, owing to low solubility of this complex in D_2O.

Effects of organotin(IV) derivatives of H_2TPP on cell growth

3T3 cells were treated as described under 'Biological studies' in the Experimental section. Figure 3 shows the results of control experiments run with the solvent (DMSO), or with thiaminepyrophosphate (H_2TPP), compared with organotin(IV) derivatives of H_2TPP. The first two compounds (DMSO and H_2TPP) did not have any effect on cell growth. We did not notice any effects even after 24 h of incubation. Among the alkyltin(IV)–thiaminepyrophosphate compounds tested, after 6 h of incubation $Me_2Sn(HTPP)_2{\cdot}H_2O$ and $Me_3SnHTPP{\cdot}2H_2O$ did not have any effect, while $Bu_2Sn(HTPP)_2{\cdot}4H_2O$ and $Bu_3SnHTPP{\cdot}H_2O$, at 1.0×10^{-6} mol dm^{-3} concentration, inhibited cell growth by 30% and 50%, respectively. The dose dependence of 6 h of incubation of 3T3 cells with the most active compound, i.e. $Bu_3SnHTPP{\cdot}H_2O$, is shown in Fig. 4. A significant inhibition of cell growth occurred only at 1.0×10^{-6} mol dm^{-3}. Figure 5 shows the time dependence of cell growth on $Bu_3SnHTPP{\cdot}H_2O$ at a concentration of

1.0×10^{-6} mol dm^{-3}. A significant effect was already evident after 1 h of incubation, reaching the maximum inhibitory effect at 24 h. As a consequence, the percentage of surviving cells was rather low (32%). Comparison with parent compounds (i.e. Bu_3SnCl and H_2TPP, at 24 h) reveals that while H_2TPP did not have any effect on cell growth, Bu_3SnCl was extremely toxic, as already described in the literature.[17,18] The inhibitory activity of the tributyltin(IV) derivative is modulated by the presence of the H_2TPP.

Electrophoretic analysis of DNA/$H_2TPP{\cdot}4H_2O$, $Bu_2Sn(HTPP)_2{\cdot}4H_2O$ and $Bu_3SnHTPP{\cdot}H_2O$ mixtures

Either dibutyltin(IV)–thiaminepyrophosphate (DBTPP) or tributyltin(IV)–thiaminepyrophosphate (TBTPP), or the control thiaminepyrophosphate, H_2TPP, itself, was mixed with plasmid DNA; after at least 30 min of incubation, the mixtures were analyzed by agarose gel electrophoresis. Figure 6 shows one of these gels.

No evidence of DNA cleavage by the compounds was found, even at molar ratios as high as 1:10 (H_2TPP derivative/DNA bp). Interestingly, however, in the presence of TBTPP (1:10 TBTPP/DNA bp), plasmid DNA seemed to be more susceptible to cleavage by UV. As shown in Fig. 7, the supercoiled form of plasmid DNA (S) disappeared, as expected,[33] almost entirely after 15 min of UV treatment (lanes marked by even numbers), whereas, as a result of nicking, the relaxed circular form (C) increased significantly; a high frequency of nicking on both filaments of the DNA double helix should induce also the appearance of relaxed linear DNA (L); this was indeed the case for all the samples. However, the relative ratio of linear/circular DNA was clearly higher for the TBTPP/DNA complex (lane 8). The increased sensitivity of the TBTPP/DNA complex (lane 8) with respect to either of the other complexes (lanes 4 and 6) or naked DNA (lane 2) was even more evident after 30 min of UV treatment (Fig. 8). Sensitivity to UV was clearly dose-dependent for all the samples; as a consequence the linear/circular DNA ratio was higher after 30 min than after a 15 min treatment. In the case of TBTPP/DNA complexes (lane 8), no discrete band was clearly visible after electrophoresis. This finding may be attributed to a higher degree of nicking inducing a continuous smear of DNA fragments.

In conclusion, the data presented in this paper demonstrate cytotoxicity of TBTPP. Although cytotoxicity does not seem to depend on direct cleavage of DNA by the compound, our results suggest that TBTPP may enhance the effects of other DNA-cleaving treatments, through unknown molecular mechanisms that will deserve attention in the future.

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