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Organotin(IV) chloride complexes with phosphocholine and dimyristoyl-L-α-phosphatidylcholine

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Several complexes of R_nSnCl_{4-n} (R = Me, Ph, n = 1–3; R = nBu, n = 2, 3) with phosphocholine and dimyristoyl-L-α-phosphatidylcholine (phospholipid) have been synthesized and characterized by means of Mössbauer spectroscopy and NMR. Triorganotin chlorides form complexes of $(R_3SnCl)_2 \cdot L$ stoichiometry with a trigonal bipyramidal pentacoordinate tin environment, while the others form 1:1 complexes with an octahedral hexacoordinate tin environment, with the ligands coordinating through anionic phosphodiester moieties in all cases. Copyright © 2000 John Wiley & Sons, Ltd.

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INTRODUCTION

Organotin compounds are widely used products possessing toxicity towards biological targets which depends substantially on the structure of organotin toxicants.[1] On the basis of the known electron-acceptor properties of these compounds it can be proposed that their toxicity is related to their interaction with electron-donor groups in biologically important molecules. Phosphoryl-containing fragments, e.g. anionic phosphodiester groups, are considered to be important from this point of view. This is supported by the prevalence of these fragments in different kinds of biomolecules: phospho- and phosphono-lipids, ATP, nucleic acids etc. The reactions of organotin compounds with mono-nucleotides and DNA have been extensively investigated.[2–5] The effects of organotin compounds on model biological membranes[6–8] and inhibition of ATP synthesis have been studied.[9] It can be suggested that the interaction of organotin compounds with cellular phosphorus-containing molecules, resulting in inhibition of phospholipid synthesis and intracellular phospholipid transport and metabolism finally leading to inhibition of DNA synthesis, is responsible for antiproliferative activity of organotin compounds.[10,11]

This work deals with the possible modes of interaction of organotin compounds with phospholipids which, being the main structural components of cell membranes, are the first possible target of organotin biocidic activity. This has been suggested earlier, during the investigations of trialkyltin antimicrobial activity,[12] and the reaction products of the trialkyltin chlorides with phospholipids have been obtained[13] but their structures have not been studied in detail. On the other hand, previous investigation of organotin chloride complexation with *O*-ethyl(*N*-ethyl-*N*,*N*-dimethylammoniomethyl)phosphonate[14] revealed the strong donor activity of the anionic phosphonomonoester moiety towards organotin chlorides. It could be proposed that related phosphodiester anionic fragments contained in phospholipids will interact with organotin chlorides in an analogous way, leading to the formation of molecular complexes with Sn–O–P coordination bonds. In order to study this interaction we have synthesized several molecular complexes of mono-, di- and triorganotin chlorides with dimyristoyl-L-α-phosphatidylcholine (DMPC) and phosphocholine (PC)—the short-chain phospholipid analogue (Scheme 1)

The following complexes have been obtained.

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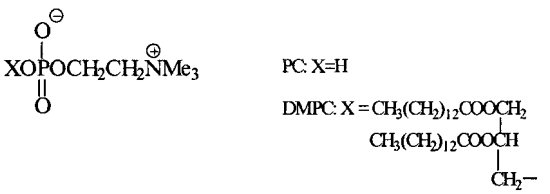


Scheme 1 PC and DMPC.

$(R_3SnCl)_2$·PC	R = Me (**1**), Ph (**2**)
R_2SnCl_2·PC	R = Me (**3**), nBu (**4**)
$RSnCl_3$·PC	R = Me (**5**), Ph (**6**)
$(R_3SnCl)_2$·DMPC	R = Me (**7**), nBu (**8**), Ph (**9**)
R_2SnCl_2·DMPC	R = Me (**10**), nBu (**11**), Ph (**12**)
$RSnCl_3$·DMPC	R = Me (**13**), Ph (**14**)

Their structures have been studied in the solid state and in solution by means of Mössbauer spectroscopy and NMR.

EXPERIMENTAL

All organotin chlorides used were purchased or synthesized and purified by normal methods to purity of not less than 98%. Dimyristoyl-L-α-phosphatidylcholine was purchased from Sigma and used as delivered. Phosphocholine was prepared from phosphocholine chloride calcium salt (Sigma) as described[15] with the exception that the Amberlite CG-50 resin was used in the Ca^{2+} ion-exchange step.

Preparation of the phosphocholine (PC) complexes

A solution of PC (1 mmol) in a minimal amount of dry methanol was mixed with the calculated amount of organotin chloride dissolved in dry chloroform or methanol. The following isolation steps were used:

$(Me_3SnCl)_2$·PC (1)
After evaporation of the solvents the oily colourless residue was washed with petroleum ether and vacuum-dried. The product crystallized upon standing: m.p. 99–102 °C. Analysis: Found: C, 22.74; H, 5.29; N, 2.12. Calcd for: $C_{11}H_{32}Cl_2NO_4PSn_2$: C, 22.70; H, 5.50; N 2.41%. ^{31}P NMR (CD_3OD): $\delta = -7.6$ ppm. ^{119}Sn NMR (CD_3OD): $\delta = 19$ ppm.

$(Ph_3SnCl)_2$·PC (2)
The product precipitated immediately as a viscous mass and crystallized slowly. The white solid was filtered, washed subsequently with chloroform and petroleum ether and vacuum-dried. Decomposition took place at 230 °C. Analysis: Found: C, 51.50; H, 4.76; N, 1.90. Calcd for $C_{41}H_{44}Cl_2NO_4PSn_2$: C, 51.58; H, 4.61; N, 1.47%. ^{31}P NMR (DMSO-d_6): $\delta = -3.7$ ppm. The ^{119}Sn NMR chemical shifts have not been determined due to the poor solubility of the adduct.

Me_2SnCl_2·PC·MeOH (3)
The product was precipitated upon standing. The solution was diluted with chloroform and an additional portion of a white solid precipitated. The product was decanted, washed with petroleum ether and vacuum-dried. The complex crystallized with one molecule of methanol: m.p. 155–160 °C. Analysis: Found: C, 22.25; H, 5.48; N, 3.35. Calcd for $C_8H_{24}Cl_2NO_5PSn$: C, 22.08; H, 5.52; N, 3.22%. ^{31}P NMR (D_2O): $\delta = -7.2$ ppm. ^{119}Sn NMR (D_2O): $\delta = -313$ ppm.

nBu_2SnCl_2·PC (4)
After evaporation of the solvents, the oily residue was washed with petroleum ether and vacuum-dried. The product became a viscous mass upon standing. Analysis: Found: C, 32.19; H, 6.46; N, 1.99%. Calcd for $C_{13}H_{32}Cl_2NO_4PSn$: C, 32.04; H, 6.57; N, 2.88%. ^{31}P NMR (CD_3OD): $\delta = -8.0$ ppm. ^{119}Sn NMR (CD_3OD): $\delta = -169$ ppm (bz).

$MeSnCl_3$·PC (5) and $PhSnCl_3$·PC (6)
The products were precipitated immediately as white solids. Both decomposed at 220 °C. Analysis:

Found (**5**): C, 16.62; H, 4.05; N, 3.23. Calcd for $C_6H_{17}Cl_3NO_4PSn$: C, 17.00; H, 4.01; N, 3.31%. Found (**6**): C, 27.12; H, 3.97; N, 2.84%. Calcd for $C_{11}H_{19}Cl_3NO_4PSn$: C, 27.19; H, 3.91; N, 2.88%. ^{31}P NMR (DMSO-d_6): $\delta = -4.2$ ppm (**5**); -4.1 ppm (**6**). ^{119}Sn NMR chemical shifts have not been determined due to poor solubility of the complexes.

Preparation of the dimyristoyl phosphatidylcholine (DMPC) complexes

A solution of DMPC (1 mmol) in dry chloroform was mixed with the calculated amount of organotin chloride dissolved in the same solvent. After evaporation of chloroform the residue was washed with petroleum ether and vacuum-dried. The complexes were obtained as white solids. In the case of Ph_2SnCl_2 the complex separated immediately as a white gel, which was isolated upon addition of acetone. The complex was decanted and vacuum-dried, and appeared as a thin white powder. The complexes **13** and **14** were precipitated as amorphous white solids from chloroform solution upon standing overnight. The products had the following analytical and spectroscopic data:

$(Me_3SnCl)_2$·DMPC (7)
M.p. 48–50 °C. Analysis: Found: C, 47.23; H, 8.41; N, 1.19. Calcd for $C_{42}H_{90}Cl_2NO_8PSn_2$: C, 46.82; H, 8.36; N, 1.30%. ^{31}P NMR ($CDCl_3$): $\delta = -9.1$ ppm. ^{119}Sn NMR ($CDCl_3$): $\delta = 79$ ppm (br).

$(nBu_3SnCl)_2$·DMPC (8)
M.p. 55–58 °C. Analysis: Found: C, 54.15; H, 9.79; N, 1.08. Calcd for $C_{60}H_{126}Cl_2NO_8PSn_2$: C, 54.18; H, 9.48; N, 1.05%. ^{31}P NMR ($CDCl_3$): $\delta = -6.0$ ppm. ^{119}Sn NMR ($CDCl_3$): $\delta = 133$ ppm (br).

$(Ph_3SnCl)_2$·DMPC (9)
M.p. 108–110 °C. Analysis: Found: C, 59.74; H, 7.35; N, 0.99. Calcd for $C_{72}H_{102}Cl_2NO_8PSn_2$: C, 59.63; H, 7.04; N, 0.97%. ^{31}P NMR ($CDCl_3$): $\delta = -11$ ppm. ^{119}Sn NMR ($CDCl_3$): $\delta = -296$ ppm (br).

Me_2SnCl_2·DMPC (10)
M.p. 103–108 °C. Analysis: Found: C, 50.19; H, 8.88; N, 1.38. Calcd for $C_{38}H_{78}Cl_2NO_8PSn$: C, 50.80; H, 8.69; N, 1.56%. ^{31}P NMR ($CDCl_3$): $\delta = -12.0$ ppm. ^{119}Sn NMR ($CDCl_3$): extremely broadened, not detectable.

nBu_2SnCl_2·DMPC (11)
M.p. 74–76 °C. Analysis: Found: C, 53.71; H, 9.51; N, 1.38. Calcd for $C_{44}H_{90}Cl_2NO_8PSn$: C, 53.78; H, 9.17; N, 1.43%. ^{31}P NMR ($CDCl_3$): $\delta = -9.7$ ppm. ^{119}Sn NMR ($CDCl_3$): $\delta = -126$ ppm (br).

Ph_2SnCl_2·DMPC (12)
M.p. 143–145 °C. Analysis: Found: C, 56.43; H, 7.89; N, 1.34. Calcd for $C_{48}H_{82}Cl_2NO_8PSn$: C, 56,38; H, 8.03; N, 1.37%. NMR spectra have not been obtained due to poor solubility.

$MeSnCl_3$·DMPC (13)
M.p. 140–145 °C. Analysis: Found: C, 47.11; H, 8.15; N, 1.26. Calcd for $C_{37}H_{75}Cl_3NO_8PSn$: C, 48.34; H, 8.17; N, 1.52%. NMR spectra are discussed below.

$PhSnCl_3$·DMPC (14)
Decomposes above 120 °C. Analysis: Found: C, 50.41; H, 7.92; N, 1.31. Calcd for $C_{42}H_{77}Cl_3NO_8PSn$: C, 51.41; H, 7.85; N, 1.43%. NMR spectra are discussed below.

Spectroscopic measurements

^{119m}Sn Mössbauer spectra were measured with a multichannel analyser (TAKES Model 269; Ponteranica, Bergamo, Italy, and an MWE Wissenschaftliche Electronik system, Munchen Germany) consisting of an MR 250 driving unit, an FG 2 digital function generator and an MA 250 velocity transducer moved at linear velocity, constant acceleration in a triangular waveform. The liquid-nitrogen temperature spectra were obtained using a Cryo Industries of America Model NRD-1238-DMB (Atkinson, NH, USA) liquid-nitrogen cryostat with sample holder and 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 by using a ^{57}Co–Pd source (10 mCi; DuPont, MA, USA), while the zero point of the Doppler velocity scale was determined at room temperature through the absorption spectra of natural $CaSnO_3$ (^{119}Sn = 0.5 mg cm^{-2}) and a $Ba^{119}SnO_3$ source (10 mCi; Amersham, UK).

NMR spectra (^{1}H, ^{13}C, ^{31}P, ^{119}Sn) were obtained at 300 K using a Varian VXR-400 instrument operating at 400.0, 100.6, 161.9 and 149.1 MHz respectively.

Table 1 Mössbauer parameters of tri- and diorganotin chloride adducts with PC (**1**–**4**) and DMPC(**7**–**12**)

Compound	IS[a] (mm s^{-1})	QS[b] (mm s^{-1})	Γ_1[c] (mm s^{-1})	Γ_2[c] (mm s^{-1})
1	1.27	3.58	0.93	0.93
2	1.28	3.08	0.90	0.90
3	1.22	3.99	1.00	1.00
4	1.45	3.60	0.91	1.16
7	1.36	3.71	0.89	0.91
8	1.52	3.57	0.90	0.87
9	1.23	3.22	0.89	0.89
10	1.41	4.33	1.02	0.88
11	1.54	4.22	0.90	0.80
12	1.22	3.81	0.82	0.83

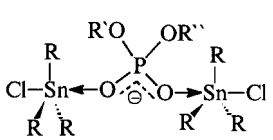
[a] Isomer shifts with respect to $CaSnO_3$, ± 0.03 mm s^{-1}.
[b] Nuclear quadrupole splitting, ± 0.03 mm s^{-1}.
[c] Full width at half-height of the resonant peaks, ± 0.05 mm s^{-1}.

RESULTS AND DISCUSSION

Phosphocholine and phospholipid readily form complexes with organotin halides since their molecules contain the anionic phosphodiester (OPO^-) moiety possessing a potentially strong donor ability. As the complexes obtained contain charged fragments, their solubility in non-polar solvents is significantly lowered. Nevertheless the phospholipid complexes having long hydrocarbon chains are readily soluble in common solvents. Triorganotin chlorides form 2:1 complexes with the ligands while di- and monoorganotin chlorides form 1:1 complexes even when mixed at a 2:1 component ratio. Phosphocholine does not react with nBu_3SnCl under the conditions used. The common procedure leads to a separable mixture of starting components. The reaction of diphenyltin dichloride and phosphocholine gave an unreproducible mixture of unstable products according to NMR, Mössbauer spectroscopic and analytical data.

Mössbauer spectra

The ^{119m}Sn Mössbauer parameters (isomer shifts IS and quadrupole splittings QS) of the tri- and diorganotin complexes with phosphocholine and phospholipid are given in Table 1. The QS values for triorganotin chloride complexes (**1**, **2**, **7**–**9**) suggest a trigonal-bipyramidal tin environment with an equatorial R_3Sn moiety,[16] the phospholigands being bridging bidentate and connecting two tin centres through OPO fragments (Scheme 2).

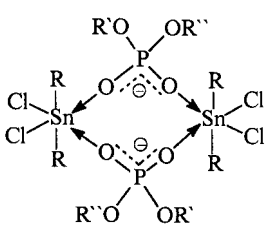


Scheme 2 Structure of triorganotin complexes **1**, **2** and **7**–**9**.

Relatively high QS values for the diorganotin chloride complexes (**3**, **4**, **10**–**12**) suggest an octahedral *trans*-R_2SnX_4 tin coordination environment[16] for all these adducts. It can be proposed that the adjacent tin atoms are connected by bidentate bridging OPO anionic moieties forming an oligomeric structure (Scheme 3; the possible cyclic dimeric structure is shown). The QS values for phosphocholine complexes are lower than the corresponding values for phospholipid ones, indicating the greater extent of distortion of the coordination octahedra in the phosphocholine complexes.

NMR spectra

The ^{1}H and ^{13}C NMR spectra of the complexes display a significant increase of ^{119}Sn–^{1}H and ^{119}Sn–^{13}C spin coupling values compared with the



Scheme 3 A possible cyclic dimeric structure of diorganotin chloride complexes **3**, **4** and **10**–**12**.

Table 2 ^{31}P and ^{119}Sn NMR parameters of $MeSnCl_3$·DMPC (**13**) and $PhSnCl_3$·DMPC (**14**), $CDCl_3$, +30 °C

Complex	δ (^{31}P) (ppm)	δ(^{119}Sn) (ppm)	^2J(^{119}Sn–^{31}P) (Hz)
13	−11.0	−507 (t)[a]	233
14	−9.7	−571 (t)	210

[a] t, triplet.

organotin chlorides, due to the existence of penta- and hexacoordinated tin species in solution. This fact also causes the high-field shift and broadening of ^{119}Sn signals (see the Experimental section). In the case of phosphocholine complexes soluble only in polar solvents, this effect is strongly enhanced by involving donor solvent molecules in coordination equilibria and an increased population of penta- and hexacoordinate tin complexes.

The ^{31}P and ^{119}Sn NMR spectra of the adducts except for **13** and **14** display no ^{119}Sn–^{31}P coupling at room temperature. This indicates high lability of these complexes in solution leading to fast (on the NMR timescale) exchange processes involving species with different tin coordination numbers. In contrast, the phospholipid complexes with monoorganotin trichlorides, $MeSnCl_3$·DMPC (**13**) and $PhSnCl_3$·DMPC (**14**), exhibit ^{119}Sn–^{31}P coupling in chloroform solution even at room temperature (Table 2). The ^{31}P NMR spectrum of both **13** and **14** consists of a broad singlet with overlapping $^{119/117}Sn$ satellites, while a triplet with a corresponding ^{119}Sn–^{31}P coupling constant appears in the ^{119}Sn NMR spectrum.

The δ(^{119}Sn) values correspond to hexacoordinated methyl- and phenyltin trichloride.[17–19] The ^2J(^{119}Sn–^{31}P) values suggest an octahedral tin coordination environment with PO groups in a *trans* position relative to each other or relative to chlorine atoms.[17,18] The multiplicity of ^{119}Sn NMR signals corresponds to a structure of the type shown in scheme 3 with one of the R groups at each tin atom replaced by a chlorine atom. The observed spectra suggest that the phospholipid complexes of methyl- and phenyltin trichlorides exist in chloroform solution in hexacoordinated form, even at room temperature. It indicates the strong donor ability of anionic phosphodiester moieties of phospholipids towards organotin compounds compared with analogous neutral phospho- and phosphono-ester groups, and the remarkable kinetic stability of the complexes obtained in chloroform solution.

On the basis of an NMR study of the interaction of trialkyltin derivatives with synthetic phospholipid membranes in aqueous media, an electrostatic type of interaction between trialkyltin cations and anionic phosphodiester groups has been proposed.[6] Taking into account the above results, coordination between trialkyltin compounds and the phosphodiester moieties of membrane lipids cannot be completely ruled out. Indeed, in aqueous media the complexes arising are involved in ligand-exchange equilibria with water molecules and other donor groups, resulting in the observed loss of spin coupling and broadening of the NMR signals.

CONCLUSIONS

Organotin(IV) chlorides readily participate in donor–acceptor interactions with phospholipids through anionic OPO fragments. These interactions can serve as models for the effect of organotin compounds on phospholipids contained in biological membranes, with the restriction that the medium used in this study is not biological. Nevertheless the information obtained suggests that the key step in the interaction of organotin compounds with cell membranes is their complexation with phosphodiester fragments of the lipid bilayers. The strength of these interactions is governed by the Lewis acidity of organotin compounds, which decreases as the number of organyl substituents at tin increases. However, the known trends in organotin toxicity suggest an opposite dependence on this parameter.[1] The explanations below can be offered, providing that one of the following possibilities is taking place.

(a) The interaction between organotin compounds with membrane phospholipids and the changes in membrane properties caused by these interactions provide an insignificant contribution to toxicity. In this case the membrane phospholipids play a protective role, preventing organotins from penetrating the cells. However, organotin compounds possessing relatively low Lewis acidity and

high lipophilicity, such as triorganotins, appear to be able to penetrate the cells more easily. Inside the cells they interact with intracellular phosphoryl-containing molecules, resulting in the observed organotin toxicity trends. At this stage the Lewis acidity is still significant since tetraorganotins are much less active than triorganotins.

(b) The toxicity of organotin compounds depends strongly on their ability to interact with cell membranes; therefore mono-organotins and, hypothetically, tin tetrahalides are the most toxic amongst organotin compounds. (Attempt to prepare phospholipid complexes of $SnCl_4$ led to destruction of phospholipid molecules.) However, their high ability to undergo hydrolysis in biological conditions leading to non-active and insoluble forms decreases their activity towards membrane phospholipids, resulting in the observed reversed order of toxicity of the organotin compounds. Further studies should help to make a definite choice between these possibilities.

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