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Organometallic Complexes with Biological Molecules. X: Dialkyltin(IV) and Trialkyltin(IV) Orotates: Spectroscopic and *in vivo* Investigations

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Several novel diorgano- and triorgano-tin(IV) derivatives of orotic acid, (2,6-dihydroxypyrimidine-4-carboxylic acid; H_3or) have been synthesized. In the diorganotin(IV) derivatives, the orotic acid behaved either as a monoanionic or as a dianionic ligand, yielding $R_2Sn(H_2or)_2$ and R_2SnHor (R = Me, Bu) species, respectively, while in the triorganotin(IV) orotates only monodeprotonation of the orotic acid occurred, giving R_3SnH_2or (R = Me, Bu) derivatives. Structural hypotheses are proposed and discussed for the solid state based on Mössbauer and IR spectroscopic data, and for solution on ^{1}H and ^{13}C NMR results. Finally, investigations have been carried out *in vivo*, showing the inhibitor properties of all of the newly synthesized derivatives towards *Ciona intestinalis* embryos. In particular, in order to test the cytotoxicity *in vivo* of Me_2SnHor, Bu_2SnHor, Me_3SnH_2or and Bu_3SnH_2or, exposure to these chemicals of *C. intestinalis* embryos at the 2–4-blastomere stage has been studied. The compound which exerts the highest cytotoxic effect is Bu_3SnH_2or at 10^{-5} M concentration because it blocks embryo development immediately. Me_3SnH_2or at 10^{-5} M concentration inhibits cell cleavage in the embryos at the 32-blastomere stage, while Bu_2SnHor at the same concentration gives rise to abnormal embryos. Me_2SnHor, is less toxic than the trimethyl, dibutyl and tributyl analogues, since 40% of the total number of treated embryos resulted in normal larvae. The ligand does not affect embryonic development significantly.

The results seem to indicate that the chemical species under investigation, especially Bu_3SnH_2or, interfere with polymerization of tubulin during the process of cell division in early embryo development. Copyright © 1999 John Wiley & Sons, Ltd.

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INTRODUCTION

6-Uracilcarboxylic acid (orotic acid; Fig.1) plays an important role in pyrimidine biosynthesis in mammalian systems. By way of example, *orotic aciduria*, a hereditary defect traced to a deficiency of the enzymes orotate phosphoribosyl transferase and orotidine 5′-phosphate decarboxylase, results in excretion of orotic acid in the urine, with consequent impairment of uridine triphosphate synthesis, and leads to megaloblastic anemia, leukopenia and retarded growth.[1]

In aqueous solution, orotic acid behaves prevalently as a diprotic acid with a pK_{a1}, of the exocyclic carboxylic group, of 2.09 and a pK_{a2}, of N(1)H, of 9.28.[2,3] As a consequence, at intermediate pH values (5–9) orotic acid is present as the H_2or^- anion in its derivatives. At $pH > 9.3$ it is

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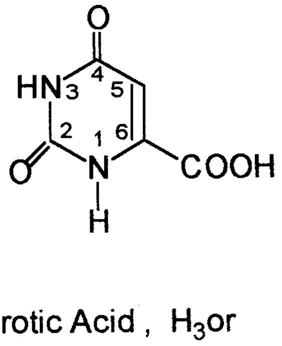

Figure 1 Structure of 2,6-dihydroxypyrimidine-4-carboxylic acid (orotic acid; H_3or).

conceivable that the Hor^{2-} anion is formed. Only at strongly alkaline pH or with very strong Lewis acids can further deprotonation of N(3)H ($pK_{a3} = 9.45$)[4] occur, with orotic acid behaving as a triprotic acid and forming orotates containing the or^{3-} anion.

Crystal structures of orotates of several metal ions, such as zinc(II), nickel(II), copper(II), calcium(II), palladium(II) and manganese(II) orotates, have been reported, in which the orotate behaves as a bidentate chelating ligand, binding through N(1) and an oxygen atom of the adjacent carboxylato group.[5–11]

However, in the case of the calcium(II) orotate system, the orotate behaves as a tridentate ligand in which, besides N(1), the carboxylato group chelates the metal through the two oxygen atoms.[8]

Lithium(I) and magnesium(II) orotates were shown to contain monoanionic bidentate orotate groups, in which coordination occurred through one oxygen atom of the carboxylato group and one uracil oxygen atom.[12]

Bidentate carboxylate groups bridging one Sn(II) and one Sn(IV) atom have been reported in the case of the reaction of $SnCl_2$ with the sodium salts of orotic and 2-thio-orotic acids, under aerobic conditions.[13] In several copper(II) and palladium(II) complexes of orotic acid and its derivatives, mono-, di- and full deprotonation occurred, showing the multiplicity of the binding sites.[14,15]

Two groups of workers[16,17] have also reported on the antitumor activity of platinum(II) and palladium(II) orotates and 3-methylorotates.

As organotin(IV) moieties have recently been widely studied for their cytotoxic properties,[18–21] it was of interest for us to synthesize organotin(IV) derivatives of orotic acid and examine their cytotoxic properties.

Previous reports have demonstrated that the complex network of ascidian embryonic development is regulated by a variety of multistep control processes of cellular interactions and could be impaired in one or more steps by pollutants.[18–21] Tunicates (*Urochordata, Ascidiacea*) are aquatic filter-feeding invertebrates that live in estuarine and coastal marine systems and are subject to chronic pollution. They seem to be a suitable material to test the effect of some pollutants. In this research we have studied the effects of Me_2SnHor, Bu_2SnHor, Me_3SnH_2or and Bu_3SnH_2or on the embryonic development of ascidian eggs.

The results obtained demonstrate that Bu_2SnHor, Me_3SnH_2or and Bu_3SnH_2or, at 10^{-5} M, inhibit mitosis. Bu_3SnH_2or is more toxic than Me_3SnH_2or

Table 1 Analytical data (calculated values in parentheses)

Compound	M.p. (°C)	Elemental analysis (%) C	H	N	Sn	H_2O	TG peak temperature (°C)
Me_2SnHor, **1**	> 350	27.53 (27.76)	2.59 (2.66)	9.06 (9.25)	38.60 (39.20)		
$Bu_2SnHor{\cdot}H_2O$, **2**	152 dec.	39.31 (38.55)	5.24 (5.47)	7.26 (6.91)	28.49 (29.31)	4.20 (4.44)	110
$Me_2Sn(H_2or)_2$, **3**	300 dec.	31.23 (31.40)	3.10 (2.63)	10.95 (12.21)	25.35 (25.86)		
$Bu_2Sn(H_2or)_2{\cdot}H_2O$, **4**	138 dec.	37.15 (38.60)	4.62 (4.50)	10.98 (10.00)	22.11 (21.20)	3.25 (3.20)	60
$Me_3SnH_2or{\cdot}H_2O$, **5**	180 dec.	28.76 (28.52)	4.12 (4.19)	8.02 (8.31)	37.11 (35.23)	4.66 (5.30)	109
Bu_3SnH_2or, **6**	140	46.00 (45.87)	6.76 (6.79)	6.48 (6.29)	26.00 (26.67)		

Table 2 Assignments of the more relevant absorption bands of $Sn(H_2or)_3 \cdot 0.5\ H_2O$, $R_2Sn(IV)$ and $R_3Sn(IV)$ orotates (R = Me, *n*Bu) in the 4000–250 cm^{-1} region[a–c]

H_3or	$Sn(H_2or)_3 \cdot$ 0.5 H_2O[d]	Me_2SnHor (**1**)	$Bu_2SnHor \cdot H_2O$ (**2**)	$Me_2Sn(H_2or)_2$ (**3**)	$Bu_2Sn(H_2or)_2 \cdot H_2O$ (**4**)	$Me_3SnH_2or \cdot H_2O$ (**5**)	Bu_3SnH_2or (**6**)	Assignment
3160 s,bd	3155 s,bd	3240 m	3164 s,bd	3268 s	3168 m,bd	3161 m,bd	3180 s,bd	$\nu(NH)$
3100 s	3101 s	3167 s,bd	3100 m	3178 s,bd	3101 s	3119 m,bd	3120 w	
1725 s	1720 s,bd	1710 s	1710 s	1728 s	1720 s	1705 s	1721 s	$\nu(C(2){=}O)$
1647 s	1665 s	1687 s	1683 s,bd	1673 s	1689 s	1657 s	1668 s	$\nu(C(4){=}O)$
	1616 s	1620 m	1634 s	1635 s	1634 s	1630 s	1637 s	$\nu_{as}(COO^-)$
1606 s		1610 s	1610 s	1607 s	1610 s	1613 s	1610 s	$\nu(C(5){=}C(6))$
	1494 m	1494 m	1488 m	1499 m	1474 m	1499 s	1498 s	$\delta(NH)$
	1435 m	1436 s	1462 m	1461 s	1452 s	1449 s	1460 m	$\nu_s(COO^-)$
1406 s	1382 s					1375 s	1369 s	δ_{NH}
		583 s		578 s		575 s	515 m	$\nu_{as}(SnC(2))$
		431 m		433 m		441 s		$\nu_s(SnC(2))$
	181	184	172	174	182	181	177	$\Delta\nu$ (cm^{-1})

[a] H_2or^- = orotate$^-$; Hor^{2-} = orotate^{2-}; or^{3-} = orotate^{3-}.
[b] Nujol and hexachlorobutadiene mulls; s, strong; w, weak; bd, broad; m, medium.
[c] $\Delta\nu = [\nu_{as}(COO^-) - \nu_s\ (COO^-)]$.
[d] see Ref. 13.

even at 10^{-7} M, and the latter is more toxic than Bu_2SnHor.

EXPERIMENTAL

All the complexes (Table 1) were prepared by refluxing methanolic solutions of the orotic acid with the corresponding stoichiometric amounts of dialkyltin(IV) oxides or trialkyltin(IV) hydroxides, freshly prepared by hydrolysis of the parent organotin(IV) chloride (gift from Witco GmbH, Bergkamen, Germany), according to standard procedures.[22] The white precipitates were recovered by filtration and recrystallized from absolute ethanol.

Thermogravimetric (TG) measurements (Table 1) were carried out with a Mettler TA-3000 system in a pure nitrogen atmosphere. The TG analysis carried out at up to 600 °C, according to most of the literature reports suggests, for the three derivatives containing water molecules (**2, 4** and **5**), no involvement of the water molecules in coordination with the tin atom. In fact, a single-step water loss occurring between 60 and 110 °C (Table 1) is observed in the thermograms of the above-mentioned compounds; these temperatures are lower than those reported in the case of loss of metal-coordinated water molecules.[23–27]

IR and Mössbauer spectra were obtained as previously reported.[28] The ^1H and ^{13}C NMR spectra were recorded with a Bruker AC250E spectrometer, operating at 5.87 T.

The solvent hexadeuterodimethyl sulfoxide (DMSO-d_6), used for the NMR measurements because the solubility of the compounds in non-coordinating solvents was too low, 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 samples were prepared immediately before the measurement.

Specimens of *Ciona intestinalis* used for the experiments were collected from the Gulf of Palermo. Male and female gametes were removed from the gonoducts of dissected animals and transferred to Syracuse dishes. The eggs were reared in Millipore-filtered sea-water while dry sperm were diluted before insemination to a final suspension of approximately 0.1% (v/v). The eggs were inseminated and allowed to hatch at 22 °C. Eggs were incubated in the organotin(IV)-containing solutions at the 2–4-blastomere stages. Control eggs, after fertilization, developed to the 2-, 4-, 8-cell (etc.), gastrula, neurula and larval stages.

RESULTS AND DISCUSSION

Infrared spectra

The coordination mode of the orotate ligand may be discussed on the basis of the comparison of the IR spectra of the free orotic acid[29] and of $Sn(H_2or)_3 \cdot 0.5H_2O$[13] with those of the newly synthesized dialkyltin(IV) and trialkyltin(IV) derivatives (Table 2).

The IR spectra of the alkyltin(IV) orotates **1**–**6** show characteristic frequencies in the ranges 3100–3300 cm^{-1} and 1474–1499 cm^{-1} (Table 2), which are attributable to NH stretching and deformation respectively.

Bands attributable to a further NH deformation mode are present at 1375 cm^{-1} in **5** and at 1369 cm^{-1} in **6** and are lacking in both R_2SnHor and $R_2Sn(H_2or)_2$ (R = Me, Bu; **1**–**4**) (Table 2).

The occurrence of one deformation band in the R_2SnHor and $R_2Sn(H_2or)_2$ derivatives, suggests in principle that only one of the two NH groups of the pyrimidine ring of the orotic acid is involved in coordination with the tin(IV) atom.

As far as the R_3SnH_2or complexes are concerned, the presence of deformation bands of both the NH groups of the orotic acid would exclude any involvement of these groups in the coordination of the organotin(IV) moieties.

Furthermore, in R_2SnHor, $R_2Sn(H_2or)_2$ and R_3SnH_2or complexes, bands attributable to ν_{as} (COO^-) and ν_s (COO^-) are present in the range 1616–1637 cm^{-1} and 1435–1462 cm^{-1}, respectively (Table 2). As previously reported, $\Delta\nu$ values [$\Delta\nu = \nu_{as}(COO^-) - \nu_s(COO^-)$] are indicative of the coordinating mode of the carboxylate.[30,31] Data in Table 2 clearly support the presence in all the orotate derivatives of bridging carboxylate groups [$\Delta\nu$ values vary from 172 cm^{-1} in **2** to 184 cm^{-1} in **1** (Table 2)].

Moreover, bands attributable to $\nu_{as}(SnC_2)$ and ν_S (SnC_2) have been identified for several dialkyltin(IV) and trialkyltin(IV) orotate derivatives in the ranges 515–583 cm^{-1} and 431–441 cm^{-1}. On the basis of the analytical and IR data, it would appear that the orotate group behaves as:

(1) a doubly deprotonated ligand linked through

N(1) and COO^- in R_2SnHor (R = Me, Bu) (by analogy with literature reports);[5–11]

(2) a monoanion in $R_2Sn(H_2or)_2$, presumably with one of the two orotate units coordinating the tin atom through the carboxylate group and the other through the deprotonated nitrogen atom of an NH group;

(3) a monoanion in R_3SnH_2or (R = Me, Bu) binding the organotin(IV) moieties only through COO^-.

Finally, as already mentioned, in the complexes **1**–**6** the carboxylato fragment of the orotate ligand behaves as a bidentate bridging group.

These bonding characteristics and consequent geometries at the tin in the organotin(IV) derivatives have been further investigated by tin-119m Mössbauer and NMR spectroscopy.

Mössbauer spectra

A better insight into the mode of coordination of the orotate ligand with tin and hence the geometry of the organotin(IV) derivatives may be obtained from the analysis and the deconvolution of the Mössbauer spectra of R_2SnHor, R_3SnH_2or and $R_2Sn(H_2or)_2$. Table 3 reports isomer shifts, δ ($mm s^{-1}$), experimental and calculated nuclear quadrupole splittings (respectively, Δ_{exp} and Δ_{calc}), together with full widths at half height of the resonant peaks, Γ ($mm s^{-1}$), and the proposed structures.[32–37] From the resolution of the Mössbauer spectra, and in particular from the Γ values it is evident that in R_2SnHor and R_3SnH_2or (R = Me, Bu), only one absorbing tin atom is present, while in $R_2Sn(H_2or)_2$ (R = Me, Bu), at least two different tin(IV) environments are present. In particular, the deconvolution of the Mössbauer spectra of $R_2Sn(H_2or)_2$ (R = Me, Bu) resulted in δ and Δ calculated for two different tin(IV) sites (Table 3). The first site would involve a trigonal-bipyramidal structure with an $R_2SnO_2N(1)$ environment around the tin(IV) atom (Fig. 2a), while the second would support an octahedral configuration with an R_2SnO_4 environment around the tin(IV) (Fig. 2b). The point-charge model formalism applied to the idealized structures reported in Fig. 2(a–c), would confirm the structural hypotheses based on IR data and on experimental Δ values. The molecular dynamics of $Bu_2SnHor{\cdot}H_2O$ have been investigated by variable-temperature ^{119}Sn Mössbauer spectroscopy. Preliminary results show that the absolute-recoil-free fraction is characteristic of Debye solids, while the calculated mean-square displacements of the tin nucleus suggest the occurrence of molecular association.[38]

NMR measurements

The ^1H and ^{13}C NMR spectral data of the complexes **1**–**6** and of the free ligand are collected in Tables 4 and 5, respectively. The literature values[39] of the ^{13}C chemical shifts of orotic acid are included in Table 5 for comparison purposes.

The presence of the N(3)H proton resonance in the spectra (Table 4) clearly shows that this nitrogen atom is not engaged in complex formation. The organotin(IV) moieties appear to bind the

Table 3 Experimental Mössbauer parameters,[a] isomer shift (δ) and nuclear quadrupole splittings (Δ_{exp}), measured at liquid N_2 temperature, and calculated nuclear quadrupole splittings according to the point-charge formalism applied to the idealized structures of Fig. 2(a–c).

Compound	δ^b (mm s^{-1})	$\Delta_{exp}{}^c$ (mm s^{-1})	Γ_1 (mm s^{-1})	Γ_2 (mm s^{-1})	Δ_{calc} (mm s^{-1})^d	Fig. 2
Me_2SnHor, **1**	1.16	3.24	1.12	1.10	−3.20	a
$Bu_2SnHor{\cdot}H_2O$, **2**	1.37	3.41	0.91	0.91	−3.20	a
$Me_2Sn(H_2or)_2$, **3**	1.52	3.73	1.07	1.07	3.62	a
	1.55	4.77	0.80	0.80	4.45	b
$Bu_2Sn(H_2or)_2{\cdot}H_2O$, **4**	1.32	3.44	0.93	0.93	3.62	a
	1.50	4.15	0.87	0.87	4.45	b
$Me_3SnH_2or{\cdot}H_2O$, **5**	1.38	3.77	0.92	0.92	−3.69	c
Bu_3SnH_2or, **6**	1.43	3.67	0.80	0.80	−3.69	c

[a] H_2or^-, orotate$^-$; Hor^{2-}, orotate^{2-}; or^{3-}, orotate^{3-}; sample thickness ranged between 0.50 and 0.60 mg $^{119}Sn\ cm^{-2}$.

[b] Isomer shift, $\delta \pm 0.03$, with respect to $BaSnO_3$ at room temperature.

[c] $\Delta_{exp} \pm 0.02$.

[d] Regular trigonal-bipyramidal or *trans*-R_2 octahedral structures of Fig. 2 (a–c) are assumed to estimate the nuclear quadrupole splittings according to the point-charge model. The pqs values used (mm s^{-1}) were:
(1) trigonal-bipyramidal structure: $\{Alk\}^{tbe} = -1.13$ (see Ref. 29); $\{N\}^{tbe}_{heter} = -0.21$ (calculated from $\{N\}^{tet}_{heter}$ (see Ref. 32); $\{COO\}^{tba}_{bridg} = 0.075$ (see Ref. 29).
(2) *trans*-R_2 octahedral structure: $([Alk] - [Cl]) = -1.03$ (see Ref. 27); $([COO] - [Cl])_{bridg} = 0.083$ (see Ref. 27).

orotic acid either at the carboxylato group and the deprotonated N(1) atom (compounds **1** and **2**) as reported for most orotato complexes,[5–10,12,14–16,40,41] or only at the carboxylato group (compounds **3, 4, 5** and **6**) or, more unusually, only at N(1) (compounds **3** and **4**), as discussed below.

The coupling constants $^2J(^{119}\text{Sn},^1\text{H})$ and $^1J(^{119}\text{Sn},\,^{13}\text{C})$ can give information on the bonding geometry at the tin atom, based on the empirical relationships given in Eqns[1] and [2]:[42,43]

$$\theta = 0.0161|^2J(^{119}\text{Sn},^1\text{H})|^2 - 1.32\,|^2J(^{119}\text{Sn},^1\text{H})| + 133.4 \tag{1}$$

$$^1J(^{119}\text{Sn},^{13}\text{C}) = 11.4\theta - 875 \tag{2}$$

where θ is the C–Sn–C bond angle.

For compound **1**, due to its limited solubility, only ^2J could be assessed from the ^{1}H NMR; the calculated θ value of 156° would suggest a distorted *trans*-octahedral geometry at tin. The discrepancy with the angle estimated from the solid-state Mössbauer data ($\theta = 126°$, calculated according to Sham and Bancroft[33] from the measured nuclear quadrupole splitting Δ, reported in Table 3) is rather large and could be ascribed to a solvent effect. A DMSO molecule could indeed bind to the metal and give rise to an equilibrium, fast on the NMR timescale, between a penta- and a hexa-coordinate tin complex. Spectral integration is in agreement with the 1:1 metal-to-ligand stoichiometry found in the solid state, and the ^{13}C chemical shifts of the orotato moiety appear to be typical of the Hor form of the ligand.

Compound **2** exists in solution mainly as the 1:1 Bu_2SnHor species (from the integrated intensities of the ^{1}H NMR spectra and from the ^{1}H and ^{13}C chemical shifts, Tables 4 and 5). However, together with this main product other species are present which have been identified by their ^{1}H and ^{13}C chemical shift values as the free ligand and the organometal moiety, and amount to 10–12% of the total, as deduced from the ratio of the integrated intensities of the signals. A precise ^2J(Sn,H) value cannot be extracted from the ^{1}H NMR spectrum because of the overlap of broad multiplet resonances; it is only possible to obtain a minimum value of 80 Hz, giving $\theta > 131°$. On the other hand, the $^1J(^{119}\text{Sn},\,^{13}\text{C})$ coupling of 782 Hz yields $\theta = 146°$.

These two results suggest that **2** behaves similarly to **1** in solution, giving rise to an equilibrium between a penta- and a hexa-coordinated species involving a DMSO molecule as the sixth ligand.

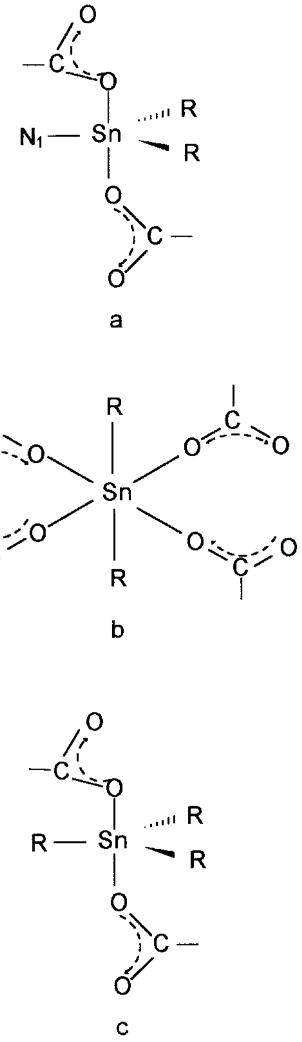

Figure 2 Proposed structures for R_2SnHor (a), $R_2Sn(H_2or)_2$ (a,b) and R_3SnH_2or (c) derivatives (R = Me, Bu).

For complex **3** the total integrated intensity of the proton spectrum corresponds to an organometal-to-ligand ratio of 1:2 and two sets of Sn–Me resonances are observed. The most abundant methyltin species, which accounts for approximately two-thirds of the total, exhibits the resonances of both the N(1)- and N(3)-bound protons; therefore the same formula $Me_2Sn(H_2or)_2$ that is found in the solid state and reported in Tables 4 and 5 can be proposed for this species. The ^{1}H and ^{13}C chemical shifts found for the minor species, also of 1:2 stoichiometry, suggest the presence of the

Table 4 ^{1}H NMR data for $R_nSn(IV)$ complexes with orotic acid (H_3or) in DMSO-d_6 solution (δ, ppm from TMS); coupling constants (Hz) to tin are in parentheses[a]

Compound	N(1)-H	N(3)-H	C(5)-H	CH_3 or α-CH_2	β-CH_2	γ-CH_2	δ-CH_3	θ (°C)
H_3or[b]	11.38	10.95	6.05					
	(11.72)	(11.23)	(6.25)					
Me_2SnHor, **1**	—	10.82	5.87	0.82				
				($^{2}J = 96.7$)				156
$Bu_2SnHor \cdot H_2O$, **2**	—	10.83	5.87	1.50 b,m	1.50 b,m	1.30 m	0.86	
				($^{2}J > 80$)				>131
$Me_2Sn(H_2or)_2$, **3**	11.29[c]	10.65[c]	5.99[c]	0.82[c]				
				($^{2}J = 94.1$)				152
	—	10.79	5.87	0.97				
				($^{2}J = 111.4$)				186
$Bu_2Sn(H_2or)_2 \cdot H_2O$, **4**	11.36	10.86	6.03	1.50 b,m	1.50 b,m	1.29	0.86	
	—	10.83	5.89					
$Me_3SnH_2or \cdot H_2O$, **5**	11.2 b	10.3 b	5.86	0.54				
				($^{2}J = 70.4$)				120
Bu_3SnH_2or, **6**	11.19	10.38	5.88	1.19 m	1.64 m	1.36 m	0.92 t	
				($^{2}J = 62.8$)				114

[a] The J value for the 119 isotope is reported, when ^{119}Sn and ^{117}Sn satellites are resolved; $B_0 = 5.87$ T; $T = 298$ K.
[b] Literature values for a tin(II, IV) mixed-valence complex, in parentheses, are reported for comparison (Ref. 13).
[c] Most abundant species.

ligand in the monoanionic form as required by stoichiometry, but this is associated with a deprotonated N(1) rather than with a deprotonated carboxylic group. A very broad proton resonance, almost lost in the baseline, at *ca* 4 ppm could indeed be indicative of free COOH groups rapidly exchanging their acidic proton with the water present in the solvent. For the two species, Eqn[1] yields θ values of 152° and 186° for the main and the secondary products, respectively. These NMR findings are in agreement with the existence of two different tin sites as observed in the solid state by ^{119}Sn Mössbauer (Table 3). However, while in the solid state one site is trigonal-bipyramidal (Fig. 2a) and the other *trans*-octahedral (Fig. 2b), in solution both species seem to have a more-or-less linear C–Sn–C skeleton. It is not unusual to find in solution a θ angle smaller than the theoretical 180° value,[44,45] as observed for the major complex, which would correspond to the structure of Fig. 2b in the solid state, with the ligand bound through bidentate bridging carboxylato groups. The minor

Table 5 ^{13}C NMR data for $R_nSn(IV)$ complexes with orotic acid (H_3or) in DMSO-d_6 solution (δ, ppm from TMS); (coupling constants (Hz) to tin are in parentheses[a]

Compound	C(4)	COO	C(2)	C(6)	C(5)	CH_3 or α-CH_2	βCH_2	γCH_2	δCH_2	θ (°C)
H_3or[b]	164.21	161.94	151.02	142.72	103.37					
	(164.10)	(161.80)	(150.90)	(142.60)	(103.30)					
Me_2SnHor, **1**	166.35	165.19	155.49	150.18	98.94	8.77				
$Bu_2SnHor \cdot H_2O$, **2**	166.39	165.48	155.70	150.72	98.76	27.31	26.70	25.71	13.66	
						($^{1}J = 782$)	($^{2}J = 44.0$)	($^{3}J = 123.8$)		146
$Me_2Sn(H_2or)_2$, **3**	164.46[c]	162.62[c]	151.04[c]	144.84[c]	102.37[c]	7.74[c]				
	166.40	165.20	155.48	150.10	98.74	13.37				
$Bu_2Sn(H_2or)_2 \cdot H_2O$, **4**	164.42	162.66	151.10	143.86	102.83	27.25	26.77	25.77	13.75	
	166.47	165.57	155.76	150.81	98.72					
$Me_3SnH_2or \cdot H_2O$, **5**	164.75	162.61	151.08	146.23	101.30	0.48				
						($^{1}J = 529.7$)				123
Bu_3SnH_2or, **6**	164.76	162.53	151.04	145.96	101.46	19.00	27.81	26.56	13.81	
						($^{1}J = 483.6$)	($^{2}J = 27.3$)	($^{3}J = 75.1$)		119

[a] The J value for the 119 isotope is reported, when ^{119}Sn and ^{117}Sn satellites are resolved; $B_0 = 5.87$ T; $T = 298$ K.
[b] Literature values in parentheses are (Ref. 39).
[c] Most abundant species.

Table 6 Results of development of fertilized *Ciona intestinalis* eggs incubated in a solution of H_3or, R_2SnHor or R_3SnH_2or (H_3or = orotic acid; R = Me, Bu) from the 2-cell stage[a]

		Development stages					
		Blastomeres					
Compound	Concentration (M)	2–4	32	Anomalous embryos	Early gastrulae	Normal larvae	Anomalous larvae
H_3or	10^{-5}			50		50	
	10^{-7}			20		80	
Me_2SnHor, **1**	10^{-5}					40	60
	10^{-7}					60	40
$Bu_2SnHor{\cdot}H_2O$, **2**	10^{-5}				100		
	10^{-7}					60	40
$Me_3SnH_2or{\cdot}H_2O$, **5**	10^{-5}		100				
	10^{-7}					45	55
Bu_3SnH_2or, **6**	10^{-5}	100					
	10^{-7}		80				20

[a] Data refer to five experiments and show the percentage of developed or arrested eggs and embryos. Controls developed 90% swimming larvae.

species would attain hexa-coordination by binding to the solvent. Because of the low solubility of **3** in DMSO, no tin satellites were observed in the carbon spectrum of the complex.

The ^{1}H and ^{13}C chemical shifts for the orotato moiety of complex **4** (Tables 4 and 5) suggest that the ligand is present partly as the carboxylato monoanion, and partly as the N(1)-deprotonated monoanion. Only one set of resonances is found for the tin-bound butyl groups. Though binding only through N(1) is rather unusual for the orotato ligand,[5–10,12,14–16,40,41] we have, as has been discussed, seen this possibility for complex **3**. However, for complex **4**, when the integrated intensity of the proton spectrum was considered, the surprising and intriguing result was obtained that the C(5)H resonances account for only half a proton per ligand unit. At present we have no explanation for this observation. The θ value obtained by means of Eqn(2) from ^1J ($\theta = 147°$) suggests a distorted *trans*-octahedral arrangement of bonds around the metal.

The organotin(IV) complexes **5** and **6** exhibit only one set of resonances, both in the ^{1}H and in the ^{13}C spectra, indicating the existence of single species. The integrated intensities of the proton spectra for **5** and **6** are in agreement with the stoichiometries derived by elemental analysis.

The θ values calculated by means of Eqns[1] or [2] are reported in Tables 4 and 5, respectively, and suggest for both complexes a spatial arrangement of the three alkyl groups in the equatorial plane of a trigonal bipyramid, as found in the solid state (Fig. 2c).

The vicinal tin–carbon couplings, when detected (Table 5), are larger than the geminal ^2J couplings, as has generally been reported,[46,47] and from their value (*ca* 75 Hz) a dihedral angle Sn–C_α–C_β–$^{13}C_\gamma$ of *ca* 180° can be evaluated from the existing Karplus-type relationship.[48]

Biological activity

The results are collected in Table 6. The eggs fertilized in seawater and used as controls develop regularly, resulting in swimming larvae (90%) with a trunk and a tail (Fig. 3). The eggs incubated in the ligand develop normally up to the larval stage at a percentage (80%) lower than that of the controls. Although at a very low percentage, normal larvae are obtained after treatment with Me_2SnHor at both 10^{-5} M (Fig. 4) and 10^{-7} M concentration, and after treatment with Bu_2SnHor at 10^{-7} M (Fig. 5). Bu_2SnHor at 10^{-5} M concentration blocks the development when embryos reach an advanced stage, giving rise to anomalous embryonic masses (Fig. 6). Me_3SnH_2or at 10^{-5} M concentration inhibits cleavage of the eggs at 16–32-blastomere stages (Fig. 7). Finally, Bu_3SnH_2or seems to be the most toxic compound, as the eggs incubated in 10^{-5} M solutions of this chemical do not cleave (Fig. 8) and in 10^{-7} M solutions they reach a larval stage that is not normal, since larvae appeared enveloped in their membrane (Fig. 9).

The main results obtained are:

(1) the ligand does not affect the embryonic development of *C. intestinalis* significantly;
(2) the compounds under investigation exert a toxic activity on *C. intestinalis* embryos at the

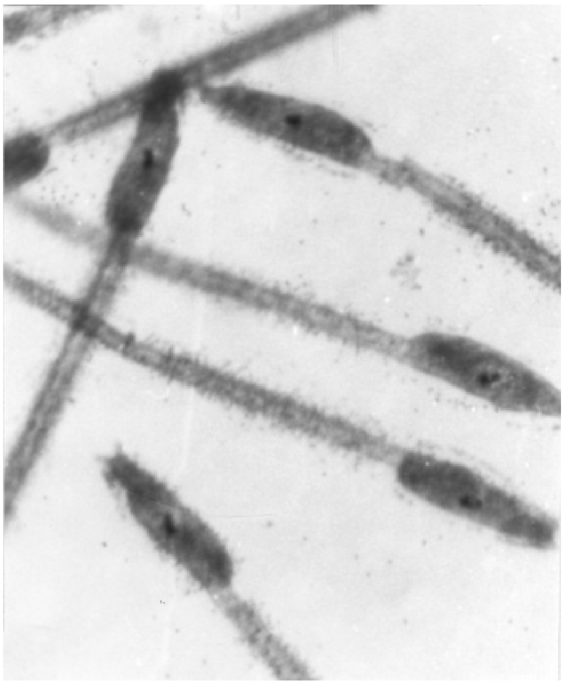

Figure 3 *Ciona intestinalis* larvae (magnification × 50).

Figure 4 10^{-5} M Me_2SnHor. Normal and anomalous larvae (magnification × 50).

Figure 5 10^{-7} M Bu_2SnHor. Normal and anomalous larvae (magnification × 50).

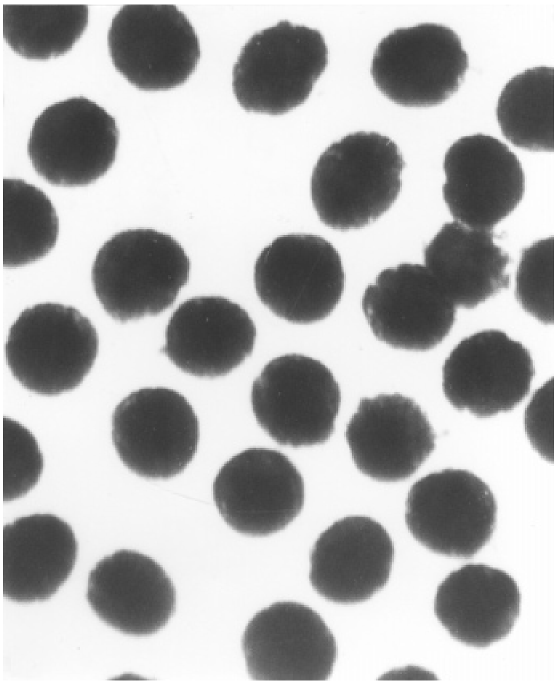

Figure 6 10^{-5} M Bu_2SnHor. Blocked anomalous embryos (magnification × 50).

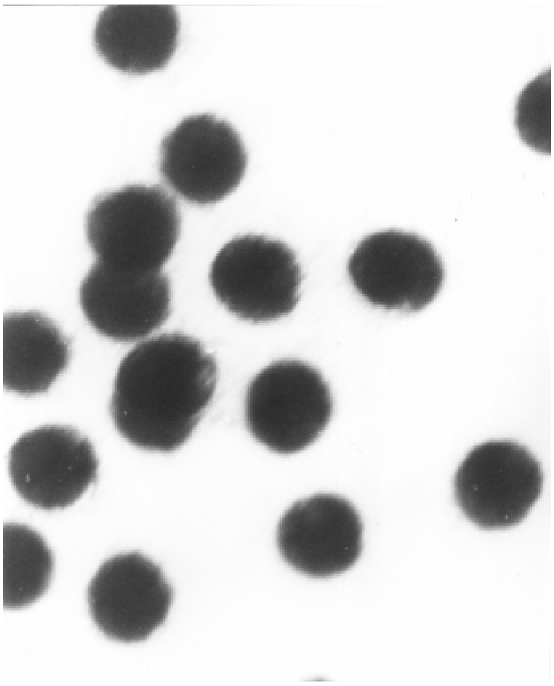

Figure 7 10^{-5} M Me_3SnH_2or. Blocked 16–32-cell stage (magnification × 50).

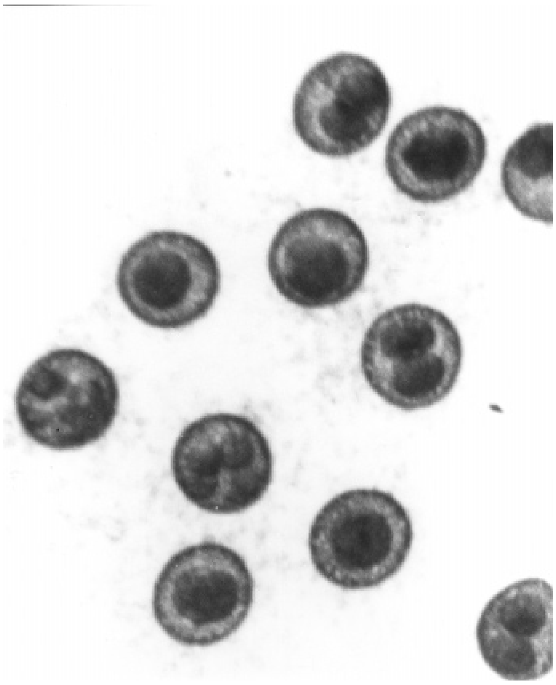

Figure 8 10^{-5} M Bu_3SnH_2or. The eggs stop developing at the 2–4 cell stage (magnification × 50).

first stages of development and the cytotoxicity observed seems to vary in the order: $Bu_3SnH_2or > Me_3SnH_2or > Bu_2SnHor$.

The results summarized above can be explained by suggesting that the chemicals tested here may interfere with the polymerization of tubulin, a protein which is implicated in the construction of the mitotic spindle during the process of cell division.[18–20] Presumably this is not the only cellular target of the cytotoxic action. In fact, a disorganization of mitochondria has also been observed in a previous study.[21] Moreover, Gianguzza *et al.*[21] found that anomalous muscle cells occurred in anomalous *C. intestinalis* larvae in response to tributyltin(IV) chloride treatments, which might be responsible for the loss of larval motility and for the tail elongation.

Considering the high toxic effect of TBT, data from the literature indicate that this chemical alters chemiluminescence responses,[49] phagocytosis by fish leucocytes[50] and natural immune reaction.[51,52]

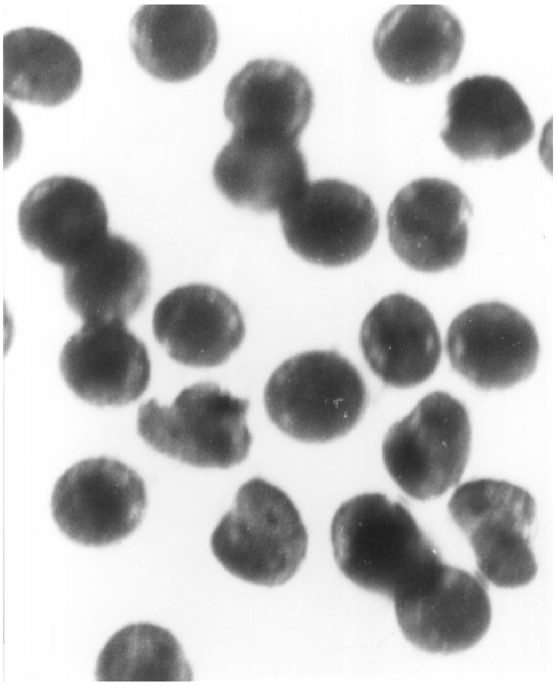

Figure 9 10^{-7} M Bu_3SnH_2or. Anomalous blocked early larvae (magnification × 50).

Conclusions

On the basis of IR (Table 2) and Mössbauer spectroscopy (Table 3), trigonal-bipyramidal environments around the tin(IV) atom are proposed for R_2SnHor and R_3SnH_2or complexes in the solid state, in which bridging carboxylate oxygen donor atoms in both the complexes, and deprotonated N(1) only in R_2SnHor, coordinate the tin(IV) atoms [originating from $R_2SnO_2N(1)$ and R_3SnO_2 environments, respectively]. On the basis of the Mössbauer data, two different tin(IV) sites are proposed in $R_2Sn(H_2or)_2$ derivatives: (1) octahedral, *trans*-R_2 with an R_2SnO_4 environment; and (2) trigonal-bipyramidal, *cis*-R_2 with an $SnO_2N(1)$ environment. The bond geometry around the metal is preserved in solution for R_3SnH_2or complexes as deduced from the NMR experimental results. An equilibrium, fast on the NMR timescale, between the penta-coordinated complex and a hexa-coordinated solvated one seems to occur in solutions of the R_2SnHor complexes; moreover, Bu_2SnHor appears also to be partially dissociated to give the free ligand and organometal moieties. For complexes $R_2Sn(H_2or)_2$, the NMR data show that two species are present in solution, as in the solid state; however, differences are observed in the bond geometry around the tin, and unusual binding through N(1), without involvement of the carboxylato group, appears to occur.

Biological activity tests of the complexes under study have shown that they all exert toxic activity towards the embryos of *C. intestinalis*, while the ligand itself does not affect the development of the embryos to any significant extent. The tested compounds seem to interfere with the polymerization of tubulin during the early stages of embryo development.

ADDED IN PROOF

Molecular dynamics

Molecular dynamics of $Bu_2SnHor{\cdot}H_2O$ have been investigated by variable temperature ^{119}Sn Mössbauer spectroscopy.[38]

The areas under the resonant peaks of the Mössbauer spectra of $Bu_2SnHor{\cdot}H_2O$ were determined as function of the temperature in the range 77.3–170 K and used to extract mulecular dynamics information, according to procedures reported previously,[53] and briefly summarized in the following.

From the relationship between the areas, A, and the absorber recoil-free fraction, f_a (Lamb-Mössbauer factor),[54] it is possible to obtain the relation for a 'thin' absorber (Eqn 3)[55]

$$\frac{d\ln A_{tot}}{dT} = \frac{d\ln f_a}{dT} = \frac{-3E_\gamma^2}{Mc^2K\vartheta_D^2} \qquad [3]$$

being E_λ the energy of the Mössbauer transition, M the effective vibrating mass, c the velocity of the light, K the Boltzmann constant, and θ_D the Debye temperature. From the experimental slopes of the function ln A_{tot}(T) and by solving Equation 3, it is possible to calculate relative values of the absorber recoil free fraction,[56] f_a^{rel}(T) and, as a consequence, values of Debye temperatures, θ_D.

According to (Eqn 4)

$$f_a = \exp(-k^2\langle x^2\rangle) \qquad [4]$$

k being the wave vector of γ-rays, the obtained data f_a^{rel}(T) may be used to determine the mean square displacements of the Mössbauer nuclei, $\langle x^2\rangle$(T).[57,58] Finally, the Debye cut-off frequencies, $\overline{\nu_D}$, are obtained from the solution of the relation (Eqn 3)[58]

$$\overline{\nu_D} = \frac{K\vartheta_D}{h} \qquad [5]$$

where h is the Planck constant.

related to the recoil-free fraction of the source, and for comparison absolute values of the absorber recoil-free fraction, f_a^{abs} may be calculated,[38] from which, again, data $\langle x^2\rangle$(T), θ_D and $\overline{\nu_D}$ may be obtained through Equations 3–5. The above mentioned procedure, effected by a suitable computer program, allows us obtain, for $Bu_2SnHor{\cdot}H_2O$, the following results (Eqn 5)

$$\frac{d\ln A_{tot}}{dT} = -1.013 \times 10^{-2}\,\mathrm{K}^{-1}$$

(correlation coefficient = 0.997)

$$\vartheta_D = 104.02\,\mathrm{K} \qquad [6]$$

$$\overline{\nu_D} = 72.07\,cm^{-1}$$

while the mean square displacement, $\langle x^2\rangle$, ranged from 0.172×10^{-2} Å^2 (77.3 K) up to 0.97×10^{-2} Å^2 (170 K). Functions ln($A_{tot}/A_{77.3}$) vs. T are shown in Figure 10(a), while sets of data $f_a^{rel,abs}$(T) and $\langle x^2\rangle$(T) are in Figures 10(b,c). It is worth to note that the above reported

1. low slope of function ln A(T),
2. high θ_D and $\overline{\nu_D}$ values, and
3. limited increments of $\langle x^2\rangle$ with increasing temperature,

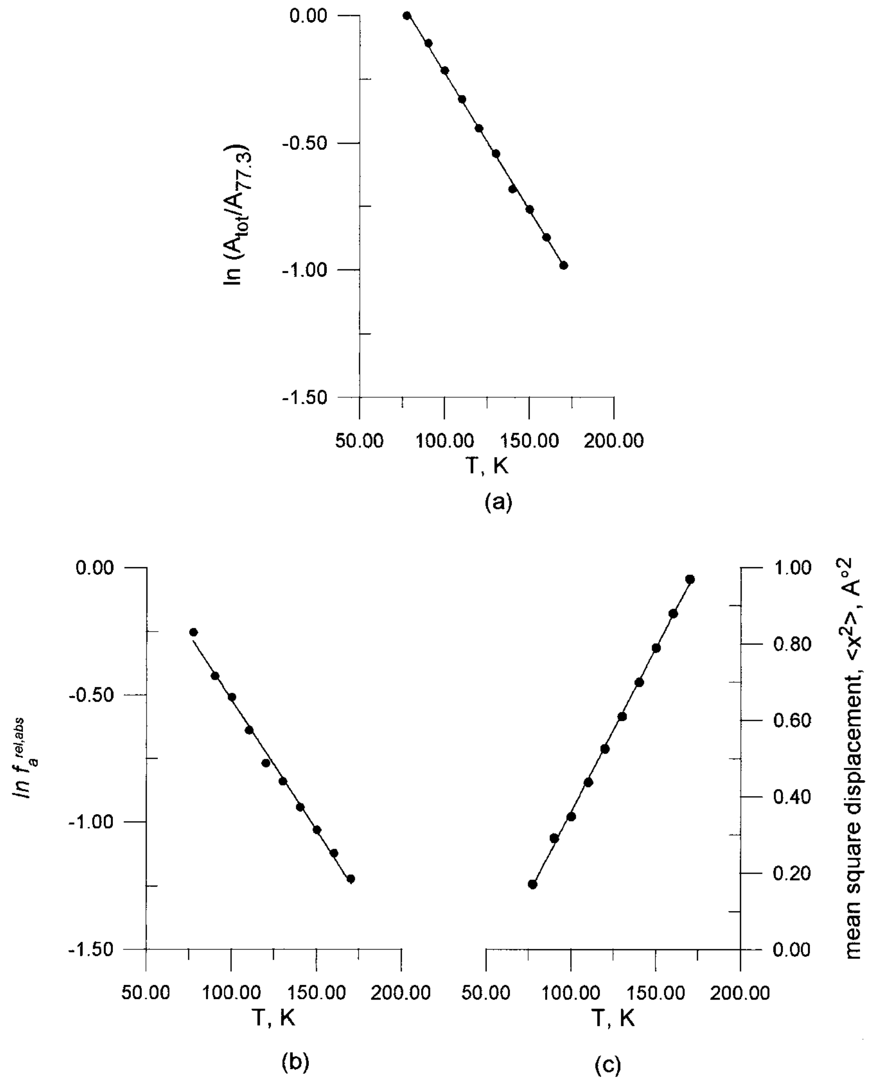

Figure 10 (a) Temperature dependence of the normalized total Lorentzian areas, A_{tot}, under the ^{119}Sn Mössbauer peaks for $Bu_2SnHor{\cdot}H_2O$ (●). Full line is the least square fit of the linear function to the experimental data points. The related equation is: $\ln(A_{tot}/A_{77.3}) = 0.854 - 1.08{\cdot}10^{-2}T$. (b) Recoil free fraction of the ^{119}Sn nuclei, f_a, as a function of temperature. Full line is the function $f_a^{rel}(T)$ obtained from the slope d(ln A)/dt, while data points are f_a^{abs} values. (c) Mean square displacement of the ^{119}Sn nuclei, $\langle x^2 \rangle$, as a function of temperature. Full line is the function $\langle x^2 \rangle(T)$ obtained from the slope d(ln A)/dt, while data points are the related $\langle x^2 \rangle$ values.

strongly characterizing, on the basis of fingerprint criteria,[38] the solid state polymeric nature of $Bu_2SnHor{\cdot}H_2O$.

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REFERENCES

1. L. Stryer, *Biochemistry*, 4th edn, W.H. Freeman, New York, 1995.
2. J. Maslowska and A. Dorabialski, *Polym. J. Chem.* **57**, 1089 (1983).
3. J. J. Kaneti and E. Golovinski, *Chem.–Biol. Interact.* **3**, 421 (1971).
4. E. R. Tucci, B. R. Doody and N. C. Li, *J.Phys. Chem.* **65**, 1570 (1961).

5. I. Mutikainen and P. Lumme, *Acta Crystallogr. Sect. B* **36**, 2233 (1980).
6. A. Karipides and B. Thomas, *Acta Crystallogr. Sect. C* **42**, 1705 (1986).
7. I. Mutikainen, *Inorg. Chim. Acta* **136**, 155 (1987).
8. O. Kumberger, J. Riede and H. Schmidbaur, *Chem. Ber.* **124**, 2739 (1991).
9. P. Castan, T. Ha, F. Nepveu and G. Bernardinelli, *Inorg. Chim. Acta*, **221**, 173 (1994).
10. F. Nepveu, M. Berkaoui, P. Castan and L. Walz, *Z. Krist.* **209**, 351 (1994).
11. I. Bach, O. Kumberger and H. Schmiedbaur, *Chem. Ber.* **123**, 2267 (1990).
12. F. Nepveu, N. Gaultier, N. Korber, J. Jaud and P. Castan, *J. Chem. Soc., Dalton Trans.* 4005 (1995).
13. S. P. Perlepes, V. Hondrellis, J. M. Tsangaris and U. Russo, *Inorg. Chim. Acta* **189**, 213 (1991).
14. P. Arrizabalaga, P. Castan and F. Dahan, *Inorg. Chem.* **22**, 2245 (1983).
15. P. Arrizabalaga, P. Castan and J. -P. Laurent, *J. Inorg. Biochem.* **20**, 215 (1984).
16. P. Castan, E. Colacio-Rodriguez, A. L. Beauchamp, S. Cros and S. Wimmer, *J. Inorg. Biochem.* **38**, 225 (1990).
17. A. D. Burrows, D. M. P. Mingos, A. J. P. White and D. J. Williams, *J. Chem. Soc., Dalton Trans.* 149 (1996).
18. C. Mansueto, L. Pellerito and M. A. Girasolo, *Acta Embryol. Morphol. Exper. (New Ser.)* **10**, 237 (1989).
19. C. Mansueto, M. Lo Valvo, L. Pellerito and M. A. Girasolo, *Appl. Organometal Chem.* **7**, 95 (1993).
20. C. Mansueto, M. Gianguzza, G. Dolcemascolo and L. Pellerito, *Appl. Organometal. Chem.* **7**, 391 (1993).
21. M. Gianguzza, G. Dolcemascolo, C. Mansueto and L. Pellerito, *Appl. Organometal. Chem.* **10**, 405 (1996).
22. M. A. Girasolo, G. Gulì, L. Pellerito and G. C. Stocco, *Appl. Organometal. Chem.* **9**, 241 (1995).
23. T. Pizzino, A. Fontana and F. Maggio, *Monatsh. Chem.* **121**, 3 (1990).
24. F. Hueso-Ureña, M. N. Moreno-Carretero, J. M. Salas-Peregrin and M. A. Romero-Molina, *Thermochim. Acta* **177**, 9 (1991).
25. F. Hueso-Ureña and M. N. Moreno-Carretero, *Thermochim. Acta* **182**, 119 (1991).
26. G. Maistralis, N. Katsarov, S. P. Perlepes and D. Kovala-Demertzi, *J. Inorg. Biochem.* **45**, 1 (1992).
27. E. J. Baran, R. C. Mercader, F. Hueso-Ureña, M. N. Moreno-Carretero, M. Quiros-Olozabal and J. M. Salas-Peregrin, *Polyhedron* **15**, 1717 (1996).
28. A. Pellerito, T. Fiore, A. M. Giuliani, F. Maggio, R. Vitturi, M. S. Colomba and R. Barbieri, *Appl. Organometal. Chem.* **7**, 601 (1997).
29. T. S. Hermann and J. M. Black, *Appl. Spectrosc.* **20**, 413 (1966).
30. G. B. Deacon and R. J. Phillips, *Coord. Chem. Rev.* **33**, 227 (1980).
31. G. B. Deacon, F. Huber and R. J. Phillips, *Inorg. Chim. Acta* **104**, 41 (1985).
32. G. M. Bancroft and R. H. Platt, *Adv. Inorg. Chem. Radiochem.* **15**, 59 (1972).
33. T. K. Sham and G. M. Bancroft, *Inorg. Chem.* **14**, 2281 (1975).
34. G. M. Bancroft, V. G. Kumar Das, T. K. Sham and M. G. Clark, *J. Chem. Soc., Dalton Trans.* 643 (1976), and references therein.
35. R. L. Collins and J. C. Travis, The electric field gradient tensor. In: *Mössbauer Effect Methodology*, Vol.3, Gruverman, I. J. (ed.), Plenum Press, New York, 1967, p. 123.
36. M. G. Clark, A. G. Maddock and R. H. Platt, *J. Chem. Soc., Dalton Trans.* 281 (1972).
37. L. Pellerito, G. Ruisi, N. Bertazzi, M. T. Lo Giudice and R. Barbieri, *Inorg. Chim. Acta* **17**, L9 (1976).
38. R. Barbieri, F. Huber, L. Pellerito, G. Ruisi and A. Silvestri, ^{119}Sn Mössbauer studies on tin compounds. Recent developments. In: *Chemistry of Tin*, Smith, P.J. (ed.), Blackie Glasgow, 1997, Ch.14, p.496.
39. M. V. Jovanovic and E. R. Biehl, *J. Heterocycl. Chem.* **24**, 191 (1987).
40. M. Sabat, D. Zglinska and B. Jezowska-Trzebiatowska, *Acta Crystallogr. Sect. B* **36**, 1187 (1980).
41. T. Solin, K. Matsumoto and K. Fuwa, *Bull. Chem. Soc. Jpn.* **54**, 31 (1981).
42. T. P. Lockhart and W. F. Manders, *Inorg. Chem.* **25**, 892 (1986).
43. T. P. Lockhart, W. F. Manders and J. J. Zuckerman, *J. Am. Chem. Soc.* **107**, 4546 (1985).
44. M. Camalli and F. Caruso, *Inorg. Chim. Acta* **169**, 189 (1990).
45. W. F. Howard, Jr, R. W. Crecely and W. H. Nelson, *Inorg. Chem.* **24**, 2204 (1985).
46. H. G. Kuivila, J. L. Considine, R. H. Sarma and R. J. Mynott, *J. Organometal. Chem.* **111**, 179 (1976).
47. B. Wrackmeyer, *Annu. Repts NMR Spectrosc.* **16**, 73 (1985).
48. D. Doddrell, I. Burfitt, W. Kitching, M. Bullpitt, C. -H. Lee, R. J. Mynott, J. L. Considine, H. G. Kuivila and R. H. Sarma, *J. Am. Chem. Soc.* **96**, 1640 (1974).
49. J. E. Warinner, E. S. Mathews and B. A. Weeks, *Mar. Environ. Res.* **24**, 281 (1988).
50. B. A. Weeka, P. I. Warinner, J. Mason and D. S. McGinnis, *J. Fish Biol.* **28**, 653 (1986).
51. N. Parrinello, V. Arizza, M. Cammarata and D. M. Parrinello, *Dev. Comp. Immunol.* **17**, 19 (1993).
52. D. Raftos and A. Hutchinson, *Biol. Bull.* **192**, 62 (1997).
53. R. Barbieri, A. Silvestri, A. Gennaro, M. Petrera, N. Burriesci, *J. Chem. Soc., Dalton Trans.* **1983** (1980).
54. P. Hannaford and J. W. G. Wignall, *Phys. Status Solidi* **35**, 809 (1969); and refs. therein.
55. R. H. Herber and M. F. Leahy, *Adv. Chem. Series* **157**, 155 (1976).
56. H. Sano and H. Yamamoto, *Chem. Lett.* **67** (1977).
57. H. A. Stockler and H. Sano, *J. Chem. Phys.* **50**, 3813 (1969).
58. V. I. Gol'danskii and E. F. Makarov, in *Chemical Application of Mössbauer Spectroscopy*, Gol'danskii, P. J. and Herber, R. H. (eds), Acad. Press, New York, 1968, Ch. 1, p. 1.