Studies on the antitumor activity of complexes of $R_2Sn^{(IV)}$ with penicillamine enantiomers and with 3-thio-propanoic acid, and correlation with structural aspects

R. Barbieri, A. Silvestri
Dipartimento di Chimica Inorganica, Università di Palermo, I-90123 Palermo (Italy)

S. Filippeschi, M. Magistrelli
Istituto di Ricerche Farmacologiche M. Negri, I-20157 Milan (Italy)

and **F. Huber**
Fachbereich Chemie, Universität Dortmund, O-4600 Dortmund 50 (F.R.G.)

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Abstract

The activity of diorganotin(IV) derivatives, with Sn–S bonds, against murine leukemia P-388, has been investigated. The water-soluble penicillamine ($PenH_2$) complexes, Me_2Sn(D-Pen), Me_2Sn(L-Pen), Me_2Sn(D-$PenH)_2$ and Me_2Sn(L-$PenH)_2$, are essentially inactive, and do not show any dependence of the antitumor activity from the configuration about the chiral centre. The complexes with 3-thiopropanoic acid (H_2Tpr), $R_2Sn(Tpr)$, are active for R = Et and Ph, in line with the general behaviour of $R_2Sn^{(IV)}$ derivatives. The nature of the organotins in the vehicle of administration is investigated by ^{119}Sn Mössbauer spectroscopy. The structures of solid state polymers, occurring in suspensions, are discussed. In aqueous phase, the skeleton C_2SnS (equatorial, in trigonal bipyramidal species) and C_2SnS_2 (tetrahedral) are maintained in freshly prepared specimens. The compounds investigated insert into classes R_2SnSR' and $R_2Sn(SR')_2$, for both antitumor activity and structure, irrespective of the presence, and the eventual configuration, of a chiral centre of an amino acid tail in the ligand.

Introduction

In the context of studies on the antitumor activity of organotin(IV) compounds [1], we have reported on complexes of $R_2Sn^{(IV)}$ moieties characterized by the occurrence of $Sn–S_{1,2}$ bonds [2, 3]. The L-cysteinates $R_2Sn[SCH_2CH(NH_2)COO]$, R_2Sn(L-Cys), have been determined to be active against murine leukemia P-388 for R = Et, Ph, while inactive for R = Me, Bu^n [2, 3], in line with general findings on organotins [1]; DL-penicillaminates, $R_2Sn[SC(CH_3)_2CH(NH_2)COO]$, R_2Sn(DL-Pen), were instead moderately active for R = Me, Bu^n, at very large and low doses, respectively, while Ph_2Sn(DL-Pen) was inactive [2]. A possible contribution of the chirality of the ligands to the antitumor effect was consequently supposed [2]. We then planned to investigate the anti-leukemia action of the L- and D-enantiomers of the penicillaminates, Me_2Sn(L-Pen) and -(D-Pen), as well as of $Me_2Sn[SC(CH_3)_2CH(NH_3^+)COO^-]_2$, Me_2Sn(L-$PenH)_2$ and -(D-$PenH)_2$, which are soluble in water and allow a facile attribution of the nature of the species in the vehicle of the administration for the antitumor tests. Moreover, the related complexes with 3-thiopropanoic acid $R_2Sn(SCH_2CH_2COO)$ [$R_2Sn(Tpr)$; R = Me, Et, Bu^n, Ph] have been tested, in order to establish the role of the amino group and of the chiral carbon in this congeneric series of compounds. ^{119}Sn Mössbauer spectra of solids and frozen aqueous solutions have been measured, and the results have been employed in discussing possible structure–activity correlations.

Experimental and treatment of data

The synthesis of solid samples of Me_2Sn-(D-Pen), -(L-Pen), -(D-$PenH)_2$, -(L-$PenH)_2$, Bu_2^nSn(DL-Pen) and Me_2Sn(L-Cys), was effected according to the literature [2,4]. The compounds $R_2Sn(Tpr)$ were obtained by reaction of R_2SnO with H_2Tpr in n-

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hexane under reflux, according to the procedure reported for the synthesis of $Bu_2^nSn[S(CH_2)_3COO]$ [5]. In the preparation of the aqueous solutions (described in footnote g to Table 2), the following materials were used. Me_2SnCl_2 was a gift from Schering A.G., Bergkamen (B.R.D.); L-$CysH_2$, L-$PenH_2$ and H_2Tpr were from Fluka A.G., Buchs (Switzerland); the phosphate buffer from Sigma, St. Louis (U.S.A.); 2-hydroxypropylcellulose (for Klucel), from Ega Chemie, Steinheim (B.R.D.); Hepes from Calbiochem, La Jolla (CA, U.S.A.). Other reagents and solvents were from C. Erba, Milan (Italy). The pH values were measured with a Crison 2002 instrument.

The activity of the complexes towards P-388 lymphocytic leukemia in mice was determined according to U.S. National Cancer Institute protocols for primary screening [6], at the Istituto M. Negri. The results obtained are in Table 1, where details are reported on the implantation of the tumor and on the administration of the drugs (footnotes).

The Mössbauer spectra were measured at 77.3 K with the apparatus, and data reduction procedures, reported earlier [7]; the sources were moving at r.t. with linear velocity, constant acceleration, in a triangular waveform. The results are reported in Table 2, where the nature of solid and frozen solution absorbers is detailed (footnote g).

The Mössbauer parameters nuclear quadrupole splitting, ΔE, concerning particular aqueous systems, have been rationalized by the point-charge model formalism [8]; calculations have been performed as described earlier [7], and the results are reported in Fig. 1 for the nominal (regular) tin environments in **I** and **II**. The partial quadrupole splitting parameters, *pqs*, employed in the calculations were from the literature [7, 9]; the *pqs* value for trigonal bipyramidal axial phosphate has been taken as +0.13 mm s^{-1} [10]. Structures **I** and **II** respect the Muetterties rule for tbp configurations [11], the most electronegative ligands being allocated in axial position.

TABLE 1. The role of the chirality of the amino acid, as well as of the presence of the amino group, in the ligand, in the effect of their complexes with $R_2Sn^{(IV)}$ moieties against leukemia P-388 in mice[a]

Compound[b]	Dose[c] (mg/kg/inj)	T/C[d] (%)
Me_2Sn(D-Pen)	50	95
	25; 12.5	118
Me_2Sn(L-Pen)	50; 12,5	114; 118
	25	136
Me_2Sn(Tpr)	50	Toxic
	25; 12.5	110; 105
Et_2Sn(Tpr)	25	Toxic
	12.5	155
Bu^n_2Sn(Tpr)	12.5	Toxic
Ph_2Sn(Tpr)	25	Toxic
	12.5	130
Me_2Sn(D-$PenH)_2$	50–12.5	100–104
Me_2Sn(L-$PenH)_2$	50–12.5	109–100

[a]10^6 cells were inoculated intraperitoneally (I.P.) on day zero in CD_2F_1 mice. [b]$PenH_2$ = penicillamine, $HSC(CH_3)_2CH(NH_3^+)COO^-$; H_2Tpr = 3-thio-propanoic acid, $HSCH_2CH_2COOH$. [c]The drugs were administered daily on days 1 to 5, for a total of 5 injections (I.P.), as solution in phosphate buffer saline, PBS (the Pen complexes; Na_2HPO_4 8.06 mM, KH_2PO_4 1.47 mM, NaCl 0.137 mM, KCl 2.68 mM, pH = 7.0–7.3); and as suspension in Klucel (the Tpr complexes; Klucel is formed by the surfactant 2-hydroxypropylcellulose (0.3 g) and NaCl (0.9 g) in 100 ml of aqueous solution). [d]Median survival time of the test mice group divided by that of the control group. Reproducible $T/C \geq 120\%$ is required to demonstrate activity [6].

Discussion

The data in Table 1 clearly indicate that, in the $Me_2Sn^{(IV)}$ derivatives, the chirality of penicillaminate in both 1:1 and 1:2 complexes, as well as the eventual occurrence of an amino acid tail in the ligand, do not give any significant contribution to the effect against murine leukemia P-388 shown by these compounds. In fact, there are some peculiarities such as the moderate activity of one sample of Me_2Sn(L-Pen), and, more important, the decrease of the effect by the 1:2 complexes with respect to the 1:1 derivatives (due to the formation of the second Sn–S bond). The latter will be discussed in the context of the general evaluation of the antitumor activity of organotins, which is in preparation in our laboratory and will be hopefully published in the near future. The congeneric R_2Sn(Tpr) compounds exhibit the usual trend, where ethyl and phenyl radicals bound to the metal induce antitumor activity [1, 2]; besides,

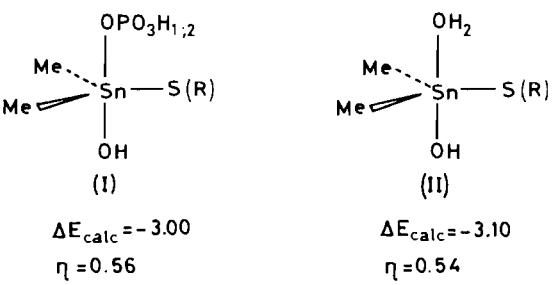

Fig. 1. The possible tin environments in: Me_2Sn(Pen) (**I**); Me_2Sn(Tpr) (**II**), (symbols: Table 1, footnote b), in the respective aqueous phases in the vehicles of administration, PBS and Klucel (see the Tables and text). ΔE_{calc} is the ^{119}Sn Mössbauer nuclear quadrupole splitting, and $\eta = (V_{xx} - V_{yy})/V_{zz}$ is the asymmetry parameter [8, 9], both calculated according to the point-charge model [8, 9].

TABLE 2. ^{119}Sn Mössbauer parameters of $R_2Sn^{(IV)}$ complexes in the solid state and in frozen aqueous solution[a]

No.	Absorber samples	δ[b] (mm s^{-1})	ΔE[c] (mm s^{-1})	Γ_1[d] (mm s^{-1})	Γ_2[d] (mm s^{-1})
Solids[e]					
1	Me_2Sn(D-Pen)	1.19	2.55	0.85	0.82
2	Me_2Sn(L-Pen)	1.19	2.54	0.94	0.82
3	Bu^n_2Sn(DL-Pen)	1.29	2.69	0.91	0.89
4	Me_2Sn(Tpr)	1.31 1.33[f]	3.16 3.20[f]	0.85	0.81
5	Et_2Sn(Tpr)	1.40	3.10	0.87	0.92
6	Bu^n_2Sn(Tpr)	1.40 1.42[f]	3.19 3.21[f]	0.85	0.85
7	Ph_2Sn(Tpr)	1.28	3.14	0.87	0.88
Frozen aqueous solutions[g]					
8	Me_2Sn(L-Pen)	1.23	2.70	0.86	0.78
9	Me_2Sn(L-Pen)-phosphate	1.24	2.88	0.74	0.86
10	Me_2Sn(Tpr)	1.17	2.65	1.11	1.08
11	Me_2Sn(Tpr)-Klucel	1.23	2.72	0.83	1.06
12	Me_2Sn(Tpr)-phosphate	1.27	3.18	0.97	1.16
13	Me_2Sn(L-PenH)$_2$-phosphate	1.24	2.71	0.77	1.02
14	Me_2Sn(L-Pen)-Hepes	1.19	2.72	0.91	0.81
14'	id., measured after storage at r.t. for 4 months[h]	1.15	2.87	0.94	1.01
15	Me_2Sn(L-Cys)-Hepes[i]	1.24	2.86	0.91	0.82
15'	id., measured after storage at r.t. for 7 months[l]	1.13	3.16	1.05	0.90

[a]Parameters have been determined at liquid N_2 temperature. Symbols are as in Table 1, footnotes; Cys = cysteine, $HSCH_2CH(NH_3^+)COO^-$. Average data are reported for multiple measurements. [b]Isomer shift with respect to r.t. $Ca^{119}SnO_3$. [c]Nuclear quadrupole splitting (+0.02 mm s^{-1}) [7]. [d]Full width at half height of the resonant peaks at larger and lesser velocity than the spectrum centroid, respectively. [e]The absorber thickness was in the range 0.5–0.6 mg^{119}Sn/cm^2. [f]Ref. 5. [g]1–2 ml of aqueous solutions 10 mM in $Me_2SnL_{1,2}$ in polythene holders, frozen by immersion into liquid N_2 soon after preparation [7], unless otherwise stated. The solutions were: (i) in H_2O adjusted to pH = 7.0–7.4 with NaOH; (ii) in phosphate buffer 0.1 M ($KH_2PO_4 + Na_2HPO_4$), pH = 7.30; (iii) in Klucel (see Table 1, footnote c); (iiii) in Hepes buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) 0.2 M, pH = 7.4. The aqueous solutions were also obtained from 10 mM Me_2SnCl_2 + 10–20 mM ligand in the appropriate buffer, and the pH was adjusted to the given values. [h]No solid reaction products were formed. [i]Average data from ref. 7, Table 3, nos. 6 and 7. [l]Solid products were formed.

the $Bu^n_2Sn^{(IV)}$ derivative is toxic (in the sense of the definition in ref. 6), analogously to Bu^n_2Sn(DL-Pen) [2].

The role of the ligands in the determination of the environment of tin atoms in the compounds here investigated (which would help in order to attempt antitumor activity–structure correlations) is extracted from the Mössbauer parameters reported in Table 2 and discussed on the basis of literature reports. In this context the chirality of Pen^{2-} is irrelevant, as well as the nature of the alkyl radical bound to tin, as shown by the invariance of the Mössbauer parameters of compounds **1–3** in Table 2. These complexes are polymeric in the solid state, with a trigonal bipyramidal configuration where the organic radicals and thiol sulfur, bound to the metal, lie in the equatorial plane, being axially bridged by amino nitrogen and monodentate carboxyl [12]. Essentially the same structure, with bridging carboxylate, holds for R_2Sn(Tpr) [13], as suggested by Okawara *et al.* [14], and this is in line with the invariant ΔE parameters of **4–7**, Table 2. In the antitumor screening summarized in Table 1, Me_2Sn(Pen) have been administered in aqueous solution, so that their solid state structures have a minor relevance in the present context; on the other hand, R_2Sn(Tpr) were injected as suspensions in Klucel, so that these polymers (such as the flat-ring hexamer Bu^n_2Sn(Tpr) [13]) could eventually interact with hydrophobic sites of biological systems, besides dissolving in aqueous phases.

The complex Me_2Sn(L-Pen) is aqueous solution at neutral pH, **8** in Table 2, shows Mössbauer parameters quite similar to those of the related L-cysteinate [3], so that both species would have the

same structure, i.e. a trigonal bipyramid with equatorial methyl radicals and thiol sulfur, and axial hydroxyl and amino nitrogen [3]. Phosphate would coordinate tin yielding the species **I**, Fig. 1, as suggested by the agreement of ΔE_{calc} with ΔE_{exp} (**9**, Table 2); then, **I** would be injected in mice in the present screening (Table 1, footnotes). In any case, the skeleton C_2SnS would characterize this drug in aqueous media, owing to the large stability constants of tin–thiol sulfur bonds [15], in line also with a number of Mössbauer studies on frozen aqueous solutions of $Me_nSn(SR)_{4-n}$ ($n=2$, 3) [3, 7, 16–18].

A trigonal bipyramidal tin environment is also likely to be maintained in $Me_2Sn(Tpr)$ in neutral aqueous solution, i.e. **II** of Fig. 1, which accounts satisfactorily for the magnitude of ΔE_{exp} of **10** and **11** of Table 2. In fact, in this aqueous context the carboxyl group does not seem to be coordinating the metal centre, as indicated by infrared and ^1H NMR studies [19]. Moreover, it appears that Klucel does not play any role in structure **II**, since 2-hydroxypropylcellulose does not bind to organotins [16]. Phosphate would eventually coordinate tin in $Me_2Sn(Tpr)$ analogously to Me_2Sn(L-Pen) (**9** and **11**, Table 2).

The environment of tin in the species $Me_2Sn(PenH)_2$ in aqueous solution is C_2SnS_2, possibly distorted from the regular tetrahedral geometry [17], which turns to trigonal bipyramidal in Hepes buffer, due to an axially coordinated nitrogen atom [17]. In the present context, the phosphate ligands in the vehicle of administration of the drug (PBS, Table 1) are expected to yield the analogous complex $Me_2Sn(PenH)_2(H_nPO_4^{(3-n)-})$, $n=1$ and 2, where the environment of tin would be $C_2SnS_2O_{(axial)}$; on the other hand, the latter gives the point-charge parameter $\Delta E_{calc} = -2.14$ mm s^{-1} ($\eta = 0.82$), which is inconsistent with ΔE_{exp} (**13**, Table 2) [20]. The drug injected in mice would be then characterized by the tetrahedral tin environment referred to previously.

Me_2Sn(L-Pen), and the analogous complex Me_2Sn(L-Cys), decompose upon prolonged storage in aqueous Hepes buffer at room temperature, as shown by the changes of Mössbauer parameters of systems **14–15** in Table 2; the same desulfuration reactions seem to occur [16, 17] which have been detected for the corresponding bis-ligand complexes [17] (see also Table 2, footnotes h and i). It follows that the drugs employed in the present screening maintain tin–thiol sulfur bonds in the freshly prepared solutions in the vehicles of administration.

It is concluded that $Me_2Sn(Pen)$, $R_2Sn(Tpr)$ and $Me_2Sn(PenH)_2$ insert into the respective congeneric series R_2SnSR' and $R_2Sn(SR')_2$ for both the activity of each member with respect to leukemia P-388 in mice and the nature of tin environment in aqueous systems [2, 3, 16, 17], irrespective of the chirality of the ligand molecule.

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