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Journal of Organometallic Chemistry 577 (1999) 1–8

Journal of Organometallic Chemistry

Characteristic spectral studies, and antimicrobial and anti-inflammatory activities of diorganotin(IV) derivatives of dipeptides

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Received 12 June 1998

Abstract

New diorganotin(IV) complexes of general formula R_2SnL (R = *n*-Bu and Ph, and L = dianion of alanylphenylalanine (H_2L-1), phenylalanylleucine (H_2L-2), phenylalanylphenylalanine (H_2L-3), glycylleucine (H_2L-4) and glycylisoleucine (H_2L-5) have been prepared and characterised by elemental analyses, molar conductance, and the bonding in these complexes is discussed in terms of their IR, far-IR, ^{1}H-, ^{13}C- and ^{117}Sn-NMR, and ^{119}Sn Mössbauer spectral studies. The monomeric 1:1 complexes have distorted trigonal bipyramidal structure with *cis*-equatorial organic groups. The complexes, soluble in DMSO, have been screened against a wide spectrum of bacteria (*Escherichia coli*, *Rhizobium meliloti*, *Pseudomonas putida* and *Aeromonas formicans*) and fungi (*Aspergillus niger*, *Pencillium chrysogenum*, *Aureobasidium pullulans* and *Verticillium dahliae*) and are found to be active. The LD_{50} values (>500 mg kg^{-1}) have also been determined in the albino rats. Some of the complexes also exhibit very high anti-inflammatory activity.

Keywords: Organotin(IV); Dipeptides; Antimicrobial activity; Anti-inflammatory activity

1. Introduction

Trimethyltin(IV) derivatives of two terminally-protected dipeptides methyl-*N*-benzoyl-*l*-leucyl-*l*-histidine and *N*-benzoyl-*l*-histidyl-*l*-cysteine have been proposed as models for the interaction of trimethyltin with, respectively, the high-affinity site of ATPase (histidine only) and the low-affinity site of ATPase and haemoglobins (histidine and cysteine) [1]. Thereafter some tri- and diorganotin(IV) derivatives of terminally-protected dipeptides have been reported [2–5]. Studies have also been made of the trimethyl- and tricyclohexyltin(IV) and diorganotin(IV) [R_2Sn(IV)-, where R = Me, Bu, Oct and Ph] derivatives of glycylglycine [6–8] and of tributyltin derivatives of glutathione [9,10]. Recently some diorganotin(IV) derivatives of some other dipeptides have also been reported [11,12]. A few diorganotin(IV) derivatives of the dipeptides have shown antileukaemia and antitumour activities [13–15]. In view of this, we have reported herein the synthesis and structural studies of some diorganotin(IV) derivatives of dipeptides, viz. alanylpheylalanine (Ala–Phe), phenylalanylleucine (Phe–Leu), phenylalanylphenylalanine (Phe–Phe), glycylleucine (Gly–Leu) and glycylisoleucine (Gly–Ile). The antimicrobial and anti-inflammatory activities of the complexes along with the values of lethal dose (LD_{50}) have also been investigated and are reported in this paper.

2. Experimental

All the reactions were carried out under an anhydrous and oxygen-free nitrogen atmosphere. Solvents were purified, dried and stored under nitrogen.

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0022-328X/99/$ - see front matter
PII: S0022-328X(98)01017-1

Diphenyltin(IV) dichloride (E. Merck), dibutyltin(IV) oxide (Fluka), Ala–Phe, Phe–Leu, Phe–Phe, Gly–Leu and Gly–Ile (Sigma) were used as received.

2.1. Synthesis of diphenyltin(IV) complexes of dipeptides

The dipeptide (6.0 mmol) was dissolved in minimum amount (25 ml) of absolute methanol. To this was added sodium methoxide prepared by dissolving sodium (12.0 mmol) in absolute methanol (10.0 ml) under dry nitrogen and the resulting solution was refluxed for 2–3 h with constant stirring. The hot methanolic solution of diphenyltin(IV) dichloride (6.0 mmol) in a 1:1 molar ratio was added into the solution of the sodium salt of the dipeptide. The mixture was again refluxed with constant stirring for 5–6 h. It was centrifuged and filtered to remove sodium chloride, and the excess of solvent was removed under reduced pressure. The semi-solid product thus obtained was solidified by trituration with petroleum ether (b.p. 40–60°C). The complexes were recrystallised from a methanol and petroleum ether (b.p. 40–60°C) mixture (1:2, v/v).

2.2. Synthesis of dibutyltin(IV) complexes of dipeptides

The complexes were prepared under anhydrous conditions by dropwise addition of a dry, hot benzene–methanol (3:1, v/v 100 ml) solution of the dibutyltin(IV) oxide (6.0 mmol) in a 1:1 molar ratio to the dipeptide (6.0 mmol) in hot methanol (25 ml). The mixture was refluxed with constant stirring giving a clear solution in 10–30 min. Refluxing was continued for 9–10 h with an azeotropic removal of water. Any excess of the solvent was removed under reduced pressure. The oily product thus obtained was solidified by trituration with petroleum ether (b.p. 40–60°C), and recrystallised from a methanol and petroleum ether (b.p. 40–60°C) mixture (1:2, v/v).

Melting points were determined on a Toshniwal Capillary melting point apparatus and were uncorrected. Carbon and hydrogen analyses were performed on CHN analyzer. Carlo Erba 1108, Heracus, at the Central Drug Research Institute, Lucknow. Tin and nitrogen in the complexes were determined by gravimetric and Kjeldahl's methods. respectively [16]. IR (4000–400 cm^{-1} in KBr discs) and far-IR (600–200 cm^{-1} in CsI discs) spectra were recorded on an FTIR spectrophotometer model FTS 165 at the Institute of Petroleum Exploration, Dehradun and on a Perkin-Elmer 1600 series FTIR spectrophotometer, at the Chemistry Department, University of Roorkee (UOR), respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DRX-300 (300 MHz FT NMR) spectrophotometer at the Central Drug Research Institute, Lucknow, using $CDCl_3$ or DMSO-d_6 as solvent and TMS as the internal standard. ^{117}Sn-NMR spectra were recorded on a Bruker 250 MHz at the Indian Institute of Technology, Mumbai, India. The details of molar conductance, ^{119}Sn Mössbauer spectral studies were similar to those reported previously [17]. The antimicrobial activity of the complexes have been carried out by using a 2-fold serial dilution technique, at the Department of Biosciences and Technology, UOR: the LD_{50} values (mg kg^{-1}) were evaluated by using the reported method

Table 1
Characteristic properties of diorganotin(IV) complexes of dipeptides

Sl. No.	Complexes (empirical formula)	Yield (%)	M.p. (°C)	Analysis (%): Found (Calc.)				Molar conductance (ohm^{-1} cm^2 mol^{-1})
				Sn	N	C	H	
1	Bu_2SnL-1 $[C_{20}H_{32}N_2O_3Sn]$	92	72–75	25.40 (25.41)	5.95 (6.00)	51.40 (51.42)	6.85 (6.90)	50.0
2	Ph_2SnL-1 $[C_{24}H_{24}N_2O_3Sn]$	82	273–275	23.35 (23.40)	5.46 (5.52)	56.62 (56.84)	4.75 (4.77)	45.5
3	Bu_2SnL-2 $[C_{23}H_{38}N_2O_3Sn]$	90	92–95	23.30 (23.31)	5.48 (5.50)	54.23 (54.25)	7.50 (7.52)	63.5
4	Ph_2SnL-2 $[C_{27}H_{30}N_2O_3Sn]$	90	193–195	21.60 (21.61)	5.08 (5.10)	59.00 (59.05)	5.50 (5.51)	60.1
5	Bu_2SnL-3 $[C_{26}H_{36}N_2O_3Sn]$	80	115–118	21.82 (21.85)	5.12 (5.16)	57.45 (57.48)	6.65 (6.68)	35.6
6	Ph_2SnL-3 $[C_{30}H_{28}N_2O_3Sn]$	86	257–260	20.20 (20.35)	4.75 (4.80)	61.69 (61.78)	4.80 (4.84)	38.9
7	Ph_2SnL-4 $[C_{20}H_{24}N_2O_3Sn]$	92	276–279	25.76 (25.85)	6.05 (6.10)	52.30 (52.32)	5.25 (5.27)	70.0
8	Bu_2SnL-5 $[C_{16}H_{32}N_2O_3Sn]$	90	170–172	28.28 (28.32)	6.64 (6.68)	45.82 (45.85)	7.68 (7.70)	60.0
9	Ph_2SnL-5 $[C_{20}H_{24}N_2O_3Sn]$	90	237–240	25.78 (25.85)	6.08 (6.10)	52.30 (52.32)	5.25 (5.27)	71.2

Table 2
Characteristic IR frequencies (in cm^{-1}) of dipeptides and their diorganotin(IV) complexes

Ligand/complexes	νNH[a]	νCO_{amide}	ν_{as}(COO)	ν_s(COO)	$\Delta\nu$	ν_{as}Sn–C	ν_s(Sn–C)	ν(Sn–O)	νSn–N/νSn←N
H_2L-1	3264 m 3068 s	1676 s	1571 m	1388 s	183	–	–	–	–
Bu_2SnL-1	3203 m 3030 s 2957 s	1643 s	1619 s	1367 s	252	595 m	533 m	568 w	464 m 416 w
Ph_2SnL-1	3220 s 3056 m 2933 m	1633 w	1591 s	1311 s	280	262 s	227 vs	535 w	446 m 402 w 392 s
H_2L-2	3342 w 3262 s	1676 s	1600 s	1400 s	200	–	–	–	–
Bu_2SnL-2	3290 m 3031 m 2926 s	1651 s	1610 s	1338 s	272	605 m	545 w	555 m	480 w 420 w
Ph_2SnL-2	3309 w 3227 s 2954 m	1656 s	1610 s	1386 s	224	272 m .	228 m	555 m	419 w 409 w 392 s
H_2L-3	3274 m 3218 s	1680 s	1563 s	1386 s	177	–	–	–	–
Bu_2SnL-3	3200 m 3029 m 2957 ds 2925 ds	1639 vs	[b]	1364 s	275	618 w	553 m	559 m	491 m 463 w
Ph_2SnL-3	3253 m 3056 s 2965 m	1642 s	1587 vs	1327 s	260	265 s	227 s	530 m	452 m 405 w
H_2L-4	3250 m 3030 s	1680 s	1590 s	1403 m	187	–	–	–	–
Ph_2SnL-4	3204 m 3066 m 2970 m	1646 s	1598 s	1371 s	227	277 s	228 vs	551 s	432 s 413 s 391 s
H_2L-5	3246 s 3054 s 3029 s	1688 s	1566 s	1403 w	163	–	–	–	–
Bu_2SnL-5	3209 m 3150 m 2960 s	1640 m	1619 s	1395 s	224	600 m	534 s	570 m	478 m 437 m
Ph_2SnL-5	3157 m 3034 m 2970 w	1653 w	1600 w	1370 w	230	264 s	226 s	566 m	476 m 423 m 409 m

[a] vs, very strong; s, strong; m, medium; w, weak; ds, doublet strong.
[b] Merged with the νCO amide band.

[18]. Anti-inflammatory activity: A freshly prepared suspension of carrageenin, 0.05 ml (1.0% in 0.9% saline) was injected under the plantar aponeurosis of right palm of the rats by the method of Winter et al. [19]. One group of six rats was kept as a control and the animals of the other group of six, each was pretreated with the test drugs given orally 1 h before the carrageenin injection. The volume of the foot was measured before and after 3 h carrageenin treatment by the micropipette method described by Buttle et al. [20]. The mean increase in volume of palm in each group was calculated and % anti-inflammatory was calculated as given below:

$$\%\ \text{anti-inflammatory} = 1 - \frac{DT}{DC} \times 100$$

where DT and DC are the volumes of oedema in drug treated and control groups, respectively. Anti-inflammatory activity and LD_{50} values were determined at LLRM Medical College, Meerut, India.

3. Results and discussion

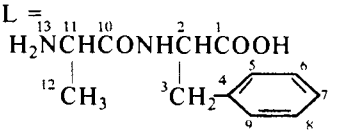
The reaction of Ph_2SnCl_2 with the sodium salt of the dipeptide in a 1:1 molar ratio led to the formation of the complexes according to Eq. (2). Dibutyltin(IV) oxide reacts with the dipeptide in equimolar ratio in benzene–methanol (3:1, v/v) mixture to give the complexes with an azeotropical removal of water (Eq. (3)).

$$H_2L + 2NaOMe \rightarrow Na_2L + 2MeOH \quad (1)$$

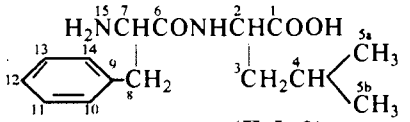
$$Ph_2SnCl_2 + Na_2L \xrightarrow{1:1} Ph_2SnL + 2NaCl \quad (2)$$

$$n\text{-}Bu_2SnO + H_2L \xrightarrow{1:1} n\text{-}Bu_2SnL + H_2O \quad (3)$$

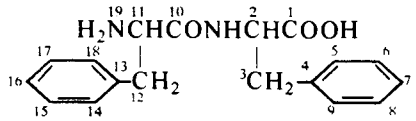
where, H_2L =



(H_2L-1)



(H_2L-2)



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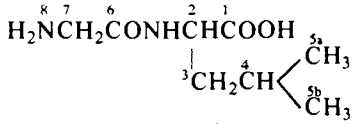
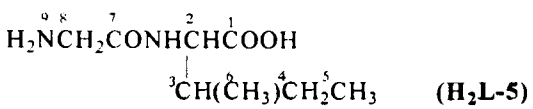


(H_2L-4)

and



(H_2L-5)

Table 3
^{1}H-NMR spectral data of the diorganotin(IV) complexes of dipeptides and ethyl ester of Phe–Leu

Sl. No.[a]	δ (ppm)[c]
1	4.16(t, 1H, 6, 8 Hz, H-2); 3.25(d, 2H, 6 Hz, H-3); 7.30(m, 5H, H-5 to H-9); 3.98(q, 1H, 7 Hz, H-11); 1.51(d, 3H, 7 Hz, H-12); 7.91(dbr, 2H, H-13); 1.29–1.41(m, 8H, H-α and H-β); 0.92(tq, 4H, 7, 7 Hz, H-γ); 0.80(t, 6H, 7 Hz, H-δ)
3	4.01(t, IH, 6, 8 Hz, H-2); 2.58(ddd, 2H, 6, 6, 6 Hz, H-3); 1.53(tq, 1H, H 4); 1.30(d, 3H, 7 Hz, H-5a); 0.87(d, 3H, 7 Hz, H-5b); 4.15(t, 1H, 6, 8 Hz, H-7); 3.24(d, 2H, 6 Hz, H-8); 7.30(m, 5H, H-10 to H-14); 7.93(d, 2H, H-15); 1.29–1.40(m, 8H, H-α and H-β); 1.02(tq, 4H, 7, 7 Hz, H-γ); 0.86(t, 6H, 7 Hz, H-δ).
4	4.00(t, 1H, 5, 8 Hz, H-2); 2.59(ddd, 2H, 6, 6, 6 Hz, H-3); 1.52(tq, 1H, H-4); 1.31(d, 3H, 7 Hz, H-5a); 0.87(d, 3H, 7 Hz, H-5b); 4.16(t, 1H, 6, 8 Hz, H-7); 3.26(d, 2H, H-8); 7.29(m, 5H, H-10 to H-14); 7.94(dbr, 2H, H-15); 7.43(d, 4H, 2 Hz, H-β); 7.39(dd, 4H, 2, 3 Hz, H-γ); 7.50(m, 2H, H-δ).
5	4.10(t, 1H, 6, 7 Hz, H-2); 3.25(d, 2H, H-3); 7.30(m, 5H, H-5 to H-9); 4.15(t, 1H, 6, 7 Hz, H-11); 3.02(d, 2H, H-12); 7.32(m, 5H, H-14 to H-18); 7.90(dbr, 2H, H-19); 1.30–1.41(m, 8H, H-α and H-β); 0.96(tq, 4H, 7, 7 Hz, H-γ); 0.79(t, 6H, 7 Hz, H-δ).
7	3.97(d, 1H, 6, 8 Hz, H-2); 1.31(ddd, 2H, 6, 6, 6 Hz, H-3); 1.56(m, 1H, H-4); 0.83(d, 6H, 6 Hz, H-5a and H-5b); 3.39(s, 2H, H-7); 8.32(s, 2H, H-8); 7.44(d, 4H, H-β); 7.35(dd, 4H, H-γ); 7.71(m, 2H, H-δ).
8	3.96(d, 1H, 3 Hz, H-2); 1.58(m, 1H, H-3); 1.40(m, 2H, H-4); 0.83–0.91(m, 10H, H-5, H-6 and H-γ); 4.89(s, 2H, H-8); 5.07(dbr, 2H, H-9); 1.25–1.40(m, 8H, H-α and H-β); 0.73(t, 6H, 7 Hz, H-δ).
EtL-2[b]	3.98(t, 1H, 5, 7 Hz, H-2); 2.01(ddd, 2H, 6,6, 6 Hz, H-3); 1.50(tq, 1H, H-4); 1.30(d, 3H, 7 Hz, H-5a); 0.85(d, 3H, 7 Hz, H-5b); 4.20(t, 1H, 6, 7 Hz, H-7); 3.26(d, 2H, H-8); 7.28(m, 5H, H-10 to H-14); 8.15(dbr coupled with peptide NH proton, 3H, H-15); 4.01(q, 2H, 7 Hz, H-α); 1.15(t, 3H, 7 Hz, H-β).

[a] Sl. no.—as indicated in Table 1.
[b] $CH_2(\alpha)CH_3(\beta)$ of ester; δ NH_2 are concentration dependent.
[c] s, singlet; d, doublet; t, triplet; q, quartet; tq, tripletquartet; dbr, doublet broad; m, multiplet.

Table 4
^{13}C-NMR spectral data of the diorganotin(IV) complexes of dipeptides and ethyl ester of Phe–Leu

Sl. no.[a]	δ (ppm)
1	C-1: 176.65; C-2: 56.86; C-3: 37.80; C-4: 136.72; C-5 and C-9: 130.05; C-6 and C-8: 129.95; C-7: 128.94; C-10: 171.78; C-11: 52.85; C-12: 20.30; C-α: 26.54; C-β: 27.82; C-γ: 26.58; C-δ: 14.45.
3	C-1: 178.67; C-2: 54.71; C-3: 43.52; C-4: 24.60; C-5a: 22.28; C-5b: 23.55; C-6: 171.91; C-7: 54.87; C-8: 37.87; C-9: 134.75; C-10 and C-14: 130.06; C-11 and C-13: 128.95; C-12: 127.61; C-α: 26.55; C-β: 27.12; C-γ: 26.82; C-δ: 13.47.
5	C-1: 176.19; C-2: 56.74; C-3: 37.98; C-4: 136.99; C-5 and C-9: 129.85; C-6 and C-8: 128.61;C-7: 126.94; C-10: 171.50; C-11: 55.25; C-12: 36.14; C-13: 135.57; C-14 and C-18: 129.57; C-15 and C-17: 127.39; C-16: 125.85; C-α: 25.90; C-β: 26.57; C-γ: 26.50; C-δ: 14.49.
9	C-1: 177.01; C-2: 62.50; C-3: 37.02; C-4: 27.25; C-5: 12.41; C-6: 15.72; C-7: 172.01; C-8: 47.50; C-α: 136.47; C-β: 135.92; C-γ: 127.80; C-δ: 128.30.
EtL-2[b]	C-1: 173.52; C-2: 52.51; C-3: 41.12; C-4: 24.51; C-5a: 21.81; C-5b: 22.91; C-6: 171.10; C-7: 55.22; C-8: 37.51; C-9: 135.10; C-10 and C-14: 129.50; C-11 and C-13: 128.90; C-12: 127.61; C-α: 61.51; C-β: 14.10.

[a] Sl. no.—as indicated in Table 1.
[b] $CH_2(\alpha)CH_3(\beta)$ of ester.

Table 5
^{117}Sn-NMR and ^{119}Sn Mössbauer data (80 K) of the diorganotin(IV) complexes of dipeptides

Complexes	δ (ppm)	QS (mm s^{-1})	IS (mm s^{-1})	ρ	τ_1	τ_2
Bu_2SnL-1	−197.8	–	–	–	–	–
Bu_2SnL-2	−201.8	3.08 ± 0.017	1.31 ± 0.004	2.35	0.914	1.166
Ph_2SnL-2	–	2.76 ± 0.016	1.19 ± 0.004	2.32	0.924	0.954
Bu_2SnL-3	−195.6	2.57 ± 0.016	1.20 ± 0.005	2.14	0.856	1.032
Ph_2SnL-4	–	2.78 ± 0.019	1.19 ± 0.005	2.34	0.920	0.948
Bu_2SnL-5	–	2.59 ± 0.015	1.22 ± 0.004	2.12	0.906	0.962

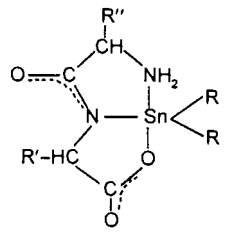
The above reactions are found to be quite facile and were complete within 9–10 h of refluxing. All of the newly synthesised complexes are cream coloured crystalline solids. The complexes are stable towards air and soluble in methanol but some of them, particularly diphenyltin(IV) derivatives are sparingly soluble in chloroform, dimethyl sulfoxide and dimethylformamide. The analytical data of the complexes are given in Table 1. In every instance the resulting complexes crystallised with 1:1 stoichiometry regardless of the proportions of the diorganotin moiety and dipeptide used. The low values of the molar conductances of 10^{-3} M solution of the complexes in methanol (35.6–71.2 ohm^{-1} cm^2 mol^{-1}) indicate their non-electrolytic nature. Several attempts to prepare dibutyltinglycylleucinate (Bu_2SnL-4) were failed even the reaction at high temperature (150°C) did not give the complex but yielded the unreacted reactants.

3.1. Infrared spectra

IR spectral data for the free dipeptides and their complexes are given in Table 2. All of the studied organotin(IV) derivatives of the dipeptides show very intense absorption bands in the range 2925–3309 cm^{-1} due to the νNH_2, which undergo a substantial lowering in comparison to the free dipeptides (3029–3342 cm^{-1}), indicating coordination by the amino group. The range of ν_{as}(COO) values shown by these amino-coordinated compounds (1587–1619 cm^{-1}) is substantially higher than in the free dipeptides (1563–1600 cm^{-1}) whereas the corresponding ν_s(COO) absorptions move to lower frequencies than in the dipeptides themselves, and (ν_{as}–ν_s)COO ($\Delta\nu$) is larger (224–280 cm^{-1}) in the complexes than in the dipeptides themselves (163–200 cm^{-1}). Strong interactions between the carboxylate carbonyl and the tin atom can thus be ruled out on this basis [6]. However the ν_{as}(COO) absorptions lie intermediate between those of the ionic and bridged structures (1578 cm^{-1} in the anionic sodium acetate [21]; 1576 cm^{-1} in the bridged trimethyltin acetate [22]) and those of the purely normal esters (1725 cm^{-1} in trimethylsilyl acetate [22]). Therefore the ionic bonding and also bridging or chelation can be excluded and the carboxylate group bonding unidentately to tin must be assumed. A strong intense band at 1682 ± 6 cm^{-1} in the free dipeptides undergoes a shift to a lower frequency (1633–1656 cm^{-1}) in the IR spectra of the organotin derivatives upon complex formation. This is probably due to the involvement of the peptide nitrogen in bonding with tin which lowers the bond order of C=O (amide) group due to the resonance stabilisation. It has been reported that σ donor power of peptide nitrogen is larger than that of amino nitrogen in $Ph_2Sn(^-OOCCH_2N^-COCH_2NH_2)$ for which a valence bonds structure is considered with resonance in peptide bonds only [7]. This is also in accordance with the crystallographic data of the coordinated glycylglycine in Ph_2Sn(Gly–Gly) with formal charges being QN_{pept}–$QO_{pept} = -0.50$ and bond orders 1.50 for $(C–N)_{pept}$ and $(C–O)_{pept}$ [8]. The conclusions drawn above are further supported by the presence of new bands in the far-IR spectra of all the complexes in the region 391–491 and at 550 ± 20 cm^{-1} which may be assigned to the ν(Sn–N)/ν(Sn←N) and νSn–O, respectively [17,23]. The ν_{as}(Sn–C) and the ν_s(Sn–C) in the dibutyl- and diphenyltin(IV) complexes are observed at 607 ± 12 and 543 ± 10 and at 270 ± 8 and 227 ± 1 cm^{-1}, respectively. This clearly indicates the existence of a bent C–Sn–C moiety in all of the organotin complexes [17,23].

3.2. NMR spectra

The ^{1}H-NMR spectra of the soluble complexes and ethyl ester of Phe–Leu (phenylalanylleucine) were recorded in deuterochloroform/deuterodimehtyl sulfox-



Where, R = n-Bu and Ph.

Fig. 1. Structure of diorganotin(IV) complexes of dipeptides.

Table 6
Results of antimicrobial activity of diorganotin(IV) complexes of dipeptides[a]

Complexes	Minimum inhibitory conce. (MIC) in μg ml^{-1} against:							
	Bacteria[b]				Fungi[c]			
	1	2	3	4	5	6	7	8
Bu_2SnL-1	25	50	12.5	12.5	50	12.5	12.5	25
Bu_2SnL-2	25	50	12.5	12.5	25	12.5	12.5	12.5
Ph_2SnL-2	50	50	12.5	50	50	25	12.5	25
Bu_2SnL-3	25	25	6.25	12.5	25	12.5	6.25	12.5
Bu_2SnL-5	12.5	25	6.25	12.5	25	12.5	6.25	25
Ph_2SnL-5	50	50	6.25	25	25	12.5	25	12.5
Bu_2SnO	50	50	12.5	50	25	25	25	25
Ph_2SnCl_2	12.5	50	12.5	12.5	12.5	12.5	25	25

[a] Solvent used DMSO.
[b] 1. *Escherichia coli*; 2. *Rhizobium meliloti*; 3. *Pseudomonas putida*; 4. *Aeromonas formicans*.
[c] 5. *Aspergillus niger*; 6. *Penicillium chrysogenum*; 7. *Aureobasidium pullulans*; 8. *Verticillium dahliae*.

Table 7
LD_{50} (in mg kg^{-1}) and antiinflammatory activity of diorganotin(IV) complexes of dipeptides

Sl. no.[a]	Compound	LD_{50} (mg kg^{-1})	Antiinflammatory activity (% inhibition) (50 mg kg^{-1} oral dose)
1	Bu_2SnL-1	>500	22.0
3	Bu_2SnL-2	>500	23.9
4	Ph_2SnL-2	>500	24.5
5	Bu_2SnL-3	>500	14.3
7	Ph_2SnL-4	>500	21.5
8	Bu_2SnL-5	>500	12.0
9	Ph_2SnL-5	>500	29.3
Phenyl butazone		–	38.4

[a] Sl. no.—as indicated in Table 1.

ide and the relevant data are given in Table 3. In ^{1}H-NMR spectra of the complexes studied, the CO(OH) resonance of the free dipeptide (δ 12.00–13.00 ppm) is absent, which suggests the replacement of the carboxylic proton by the organotin(IV) moiety. A broad resonance at δ 8.15 ppm in the spectrum of the ethyl ester of Phe–Leu due to the peptide –CONH– group coupled with the amino group is also not present for the complexes, indicating the involvement of the peptide nitrogen in bonding to tin. The –NCH< resonance is shifted upfield on complexation. The ^{13}C- and ^{117}Sn-NMR spectroscopy has been employed to obtain further information about the structure of compounds in solution. The value of δ (^{13}C) for the carboxylic carbon atom is shifted to larger δ value which is consistent with the presence of $C(O)O\cdots SnR_3$ interaction, whereas the peptide carbonyl carbon resonance is almost unchanged relative to that of the ethyl ester of dipeptide (such as C-6 in compound **3** relative to that of Phe–Leu ethyl ester (Table 4)) indicating non-involvement of the $C{=}O_{pept}$ group in the bonding. The tin shielding in ^{117}Sn-NMR spectra increases markedly with increase in coordination number, ca. $\delta = -50$ to -100 ppm for four-coordinate; $\delta \approx -200$ ppm for five-coordinate: $\delta \approx -330$ ppm for six-coordinate alkyltin compounds. Tin shifts are normally higher for phenyl compared with alkyl substituents [24]. The chemical shifts δ (^{117}Sn) of the dibutyltin(IV) derivatives are in the range $\delta = -195.6$ to -201.8 ppm (Table 5) which are the characteristic of the five-coordinated dialkyltin(IV) derivatives [21,24]. Due to insufficient solubilities of the diphenyltin(IV) derivatives in $CDCl_3$/DMSO-d_6 their ^{117}Sn-NMR spectra were not recorded.

3.3. ^{119}Sn Mössbauer spectra

Whether the coordination of the amino group nitrogen atom, bonding of the peptide nitrogen and the carboxylic oxygen to tin lead to chelation or polymerisation can be discussed with reference of the ^{119}Sn Mössbauer data listed in Table 5. The probable configuration of the solids studied is qualitatively inferred from Mössbauer data: all of the compounds exhibit doublet spectra centred in the 1.19–1.31 mm s^{-1} region. The isomer shifts (IS) are consistent with those of R_2Sn trid (where trid^{2-} are 'planar' ligands with ONO and SNO donor atoms) while quadrupole

splittings (QS) are in the range observed for complexes Me_2SnONO [25]. Earlier spectroscopic work leads us to assume a trigonal bipyramidal type configuration for R_2Sn trid, where C atoms of the organotin moiety and ligand N are lying in equatorial position and OO or OS ligand atoms are axial; ONO and ONS atoms would be located in a plane [25]. The crystal and molecular structures of R_2Sn(Gly–Gly), where R = Me, *n*-Bu and Oct, show that the actual configurations are consistently distorted from the ideal trigonal bipyramid [7]. The configuration (Fig. 1) may then be tentatively proposed for R_2SnL [which would then be alanylphenylalaninato-/phenylalanylleucinato-/phenylalanylphenylalaninato-/glycylleucinato-/glycylisoleucinato-O,N,N-(2−) diorganotin(IV)], mainly on the basis of the above mentioned similarity between QS values, taking also into account the symmetry of the coordinated ligand [26]. It has been reported that the equatorial N would release more negative charge into the neighbourhood of tin than the apical N, on coordination in R_2Sn(Gly–Gly) [8,27]. Besides the bond length Sn–N_{pept} is quite short [8], which suggests a consistent s character in that bond as well as its involvement into the π-delocalisation of the peptide group [8]. The latter circumstances would concentrate negative charge in the trigonal plane in the proximity of the tin nucleus, so that $\{N\}^{tbe}$ would be not very much different from $\{Ph\}^{tbe}$ [8]. The satisfactory agreement is reported between QS_{exp}[− 2.235 mm s^{-1} for Ph_2Sn(Gly–Gly) and 3.19–3.43 mm s^{-1} for R_2Sn(Gly–Gly), R = Me, *n*-Bu and Oct] and $QS_{calc.}$ [− 2.70 mm s^{-1} for Ph_2Sn(Gly–Gly) and − 3.09 mm s^{-1} for R_2Sn(Gly–Gly)], which confirms the assumed structure [8], but the sign of QS has inverted in R_2Sn(Gly–Gly) due to presence of the C–Sn–C angle greater than 120° [28,29]. In our compounds the sign of $QS_{obsd.}$ (2.57–3.08 mm s^{-1}) is also positive, which indicates the presence of the C–Sn–C angle greater than 120°. Further, the intermolecular hydrogen bonding between amino nitrogen and carbonyl oxygen taking place in Ph_2Sn(Gly–Gly) [8], is also found to the some extent in these compounds studied which is responsible for the low solubilities of the compounds in common organic solvents, particularly the diphenyltin(IV) derivatives. It is concluded that the structure of R_2SnL is probably distorted trigonal bipyramidal such as that of the R_2Sn(Gly–Gly) derivatives [7,8,27]. The ligating behaviour of the dipeptides towards R_2Sn (IV) moieties is then quite dissimilar from that of amino acids [30] owing to the acidity of the peptide hydrogen atom.

The results of antimicrobial activity of Ph_2SnCl_2 and Bu_2SnO and their complexes are compiled in Table 6. The results indicated that the complexes possess high bactericidal and fungicidal activities. Dibutyltin complexes are found to be more active than Bu_2SnO whereas diphenyltin derivatives are less active than Ph_2SnCl_2 except Ph_2SnL-5 against *P. putida* and *V. dahliae*, and Ph_2SnL-2 against *A. pullulans*. The most active compounds are Bu_2SnL-3 and Bu_2SnL-5. A quantitative structure–activity relationship (QSAR) for a series of triaryltin chloride derivatives indicated that the triaryltin cation was the causative agent for the biotoxicity [31], whereas Crowe made the assumption that toxicity of diorganotin compounds RR′SnXY is primarily determined by the $RR'Sn^{2+}$ moiety, the group XY influencing only the easiness of delivery of the active part $RR'Sn^{2+}$ into the cell [32]. An attempt is being made in the present study to corelate the mechanism of biotoxicity as a function of the leaving group present on the tin atom. The data shown in Table 6 indicated that dibutyltin cation imparts greater activity than diphenyltin cation (compounds **3** and **4**; **8** and **9** for comparison). Further, in order to see how effectively diorganotin cation is formed, we compare the dibutyltin complexes of all ligands except H_2L-4. Formation of the dipeptide anion (L^{2-}) is favoured by the presence of electron-withdrawing substituents and disfavoured when electron-donating substituents are present. The data given in Table 6 show the following order of increasing activity: Bu_2SnL-1 $\lesssim$ Bu_2SnL-2 $\lesssim$ Bu_2SnL-3 $\approx$ Bu_2SnL-5. This order is due to the electron-withdrawing effect of benzyl group and electron-donating effect of the substituents present in the dipeptide anion. The presence of benzyl group close to both (NH_2) and (COO^-) groups in Bu_2SnL-3 as well as the steric effect of the ligand make the formation of Bu_2Sn^{2+} easier and, therefore, the greater activity of Bu_2SnL-3 was observed. But in Bu_2SnL-5 steric factor as well as the presence of methylene group close to the NH_2 group and 1-methylpropyl at position-2 close to the COO^- group play an important role for its higher activity. The presence of methyl group close to the NH_2 group is responsible for the lower activity of Bu_2SnL-1.

The anti-inflammatory activity (% inhibition) of these compounds was conducted on adult albino rats (body weight 80–100 gm) of Froster charles species against carrageenin induced oedema in the doses of 50 mg kg^{-1} given orally and the toxicity (LD_{50}) was studied on mice (body weight 20–25 gm) of either sex and the pregnancy was excluded. The results are given in Table 7. The value of the standard drug, phenyl butazone, is 38.4% used for the comparison. Diphenyltin(IV) derivatives of dipeptides are found to be more active than the dibutyltin(IV) complexes. The compounds **1**, **3**, **4**, **7** and **9** exhibit high anti-inflammatory activity (> 20% inhibition). The compound **9**, which possesses methylene group close to NH_2 group and 1-methylpropyl group at position-2, has exhibited the most potent anti-inflammatory activity, and it was compared with the standard drug, phenyl butazone, and was found to be nearly equipotent. However compound **7** was found to possess less potent anti-inflammatory activity as compared to

compounds **9** and **4**, since in the compound **7**, 2-methylpropyl group at position-2 and methylene group close to the NH_2 group are present in comparison to 1-methylpropyl group in compound **9** and benzyl group in compound **4** at the respective positions. The reverse trend was found for the dibutyltin(IV) derivatives. The data indicated that when butyl group at tin is in close proximity with methylene group close to NH_2 group (compound **8**) the least activity has been found and the activity is going to increase when benzyl group/methyl group at position-11 and benzyl/2-methylpropyl group at position-2 are present in compounds **5**, **1** and **3**. Moreover, the LD_{50} of these compounds was found to be more than 500 mg kg^{-1} (the maximum dose tested) suggesting the safety margin of these compounds. Further, it has been observed that the toxicity of the diorganotin dipeptides is much lower than those of the di- and triorganotin derivatives of the amino acids (< 50 mg kg^{-1}) [33,34], indicating that the bigger biomolecules lower the toxicity but enhance the activity of the resulting organotin(IV) complexes.

Acknowledgements

This work was a part of a research project sponsored by UGC, New Delhi, India. The authors thank to the UGC for financial support. R. Yadav is thankful to CSIR, New Delhi for the award of a Senior Research Fellowship.

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