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Complexes of Tryptophan Dipeptides with the $R_2Sn(IV)^{2+}$ Ion (R = Me, Ph): Spectroscopic Studies, Solution Properties and Structural Implications

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Tryptophan dipeptides such as L-tryptophyl-L-alanine (H_2TrpAla), L-tryptophyl-L-tyrosine (H_2TrpTyr), L-tryptophyl-L-tryptophan (H_2TrpTrp), along with L-histidyl-L-tyrosine (H_2HisTyr), were reacted with R_2SnO (R = Me, Ph) yielding the corresponding complexes. The complexes have been characterized by IR and ^{119}Sn Mössbauer spectroscopy in the solid state and by ^{1}H and ^{13}C NMR in CD_3OD solutions.

Monomeric species were detected, with the tin atom arranged in a pentacoordinated trigonal-bipyramidal structure. The dipeptides are coordinated via the terminal amino group, deprotonated peptide nitrogen and terminal carboxylate group.

No side-chain appears to be involved in bonding. Determination of rotamer populations for selected compounds was accomplished by vicinal coupling constant analysis and side-chain orientations are interpreted. In this connection, the potential applications of these compounds as drugs is discussed.

Keywords: diorganotin(IV); tryptophan dipeptides; IR; ^{1}H and ^{13}C NMR spectra; Mössbauer spectra; structure and potential applications

INTRODUCTION

Amino acids and peptides containing aromatic side-chains, such as histidine, tyrosine and tryptophan, besides being involved in metal ion complexation via the amino and carboxylate groups, via peptide nitrogen and, in some cases, through donor atoms located in the aromatic ring, show additional features such as intramolecular ring stacking and metal–aromatic ring interactions which exert a strong influence on the conformation of the peptide ligand.[1]

Molecular recognition is often promoted in proteins by the presence of such side-chains[2] and activity heavily depends on it as in the case of bleomycin, a glycopeptide showing antitumor activity, which contains a DNA binding domain, possibly acting by intercalating the bithiazole moiety into double-stranded DNA helices, and a metal binding domain, which also participates in DNA unwinding and in determining the sequence and strand selectivity of DNA cleavage.[3–5]

In general, this mode of action of a drug molecule raises the possibility that small complexes of metal ions possessing pharmacological activity with peptides bearing aromatic side-chains might synergically interact with DNA. The peptide ligand may bring the DNA base pairs into close contact with the site of the biologically active metal domain, the ligand and metal ion moieties acting in a concerted fashion on DNA and the complex behaving as a pseudo-bifunctional adduct.

Diorganotin(IV) derivatives are known to possess antitumor activity[6] although their mode of action is not well understood, and tryptophan, which contains an indole ring, is known to act as a corepressor ligand which activates the trpR protein in the trp repressor/operator system.[7,8] In this context, tryptophan bound to the repressor is acting as a structural component providing a conformation that recognizes the appropriate DNA sequence and thereby enhances operator-specific binding to it. Molecules containing a functionalized indole ring, such as 4′,6′-diamino-2-phenylindole (DAPI), have been reported to

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interact by intercalation to GC-rich regions as well as to sites in the minor groove,[9] similarly to the distamycin–AT complex.[10,11]

EXPERIMENTAL

L-Dipeptides (H_2TrpAla · EtOH, H_2TrpTyr · 0.5H_2O, H_2TrpTrp · 2H_2O, H_2HisTyr · H_2O) were purchased from Bachem Feinchemikalien AG (Switzerland) and used without further purification, except that excess 2,2-dimethoxypropane was added during the syntheses to remove all water molecules.

Me_2SnO and Ph_2SnO were obtained by hydrolysis of Me_2SnCl_2 and Ph_2SnCl_2 dissolved in water by treatment with 25% (w/w) aqueous ammonia solution.

The solvent (methanol; MeOH) was dried by standard methods and syntheses were carried out under exclusion of moisture. Some of the complexes contain one water molecule, since the complexes, as well as the free ligands, are fairly hygroscopic. We attempted to remove the water molecules from the complexes by drying under vacuum at about 80 °C. Apparently the process is reversible, and analytical data are consistent with the presence of water, which is confirmed by IR spectra.

Elemental microanalyses were performed by Dipartimento di Chimica Organica ed Industriale, University of Milan, and are reported, along with the molecular weight osmometrically determined for a selected compound, in Table 1. Infrared spectra were recorded as split mulls on a Perkin–Elmer model 983G instrument.

Table 1 Analytical data for $R_2Sn(IV)^{2+}$ complexes with dipeptides

Compound	C[a]	H[a]	N[a]	M.p. (°C)
Me_2SnTrpAla · 0.5H_2O	44.95 (44.58)	5.64 (5.14)	9.10 (9.75)	196
Ph_2SnTrpAla · H_2O	55.58 (55.35)	5.00 (4.82)	8.09 (7.45)	147[c]
Me_2SnTrpTyr · H_2O	50.08 (49.65)	5.26 (5.11)	7.86 (7.90)	198[c]
Me_2SnTrpTrp[b]	53.48 (53.66)	5.10 (4.88)	10.12 (10.43)	194[c]
Me_2SnHisTyr	43.79 (43.90)	5.01 (4.77)	11.72 (12.05)	196[c]

[a] Found (Calcd) (%); [b] MW = 509 (537) osmometrically measured in methanol, at 25 °C. [c] Decomposition.

^{119}Sn Mössbauer spectra were measured with a Laben 8001 multichannel analyzer, an MWE velocity transducer (Wissenschaftliche Elektronik GmbH, Munchen, Germany), an FG2 digital function generator (Wissenschaftliche Elektronik GmbH) and an MA250 velocity transducer (Wissenschaftliche Elektronik GmbH) moved at linear velocity and constant acceleration in a triangular waveform. A DN700 Oxford cryostat with DTC2 temperature controller was used to maintain the absorber samples (absorber concentration, 0.5–0.6 mg ^{119}Sn cm^{-2}) at the temperature of liquid nitrogen.

^{1}H and proton-decoupled ^{13}C NMR spectra were recorded in CD_3OD and D_2O on a Bruker AC250E instrument, operating at 250.1 MHz for ^{1}H and at 62.89 MHz for ^{13}C, and a Varian VXL 5300S A11.700 TESLA instrument, operating at 500 MHz for ^{1}H and at 125.69 MHz for ^{13}C. Tetramethylsilane (TMS) or 3-(trimethylsilyl)-1-propanesulphonic acid sodium salt (DSS) were used as internal standards. The resonances appear to be somewhat dependent on concentration, which for the title compounds was 0.02 M. To improve confidence in the assignment of the spectra, simulated spectra were obtained by use of the PANIC (Bruker) program.

Synthesis of the complexes

The compounds were synthesized by refluxing, for 4 h, 2 mmol of R_2SnO and 2 mmol of the appropriate dipeptide in 50 cm^3 of dry MeOH. The solvent was reduced under vacuum to a small volume and the solid precipitated upon cooling, as in the case of Me_2SnTrpAla (the solid was recrystallized from anhydrous MeOH, and colorless crystals were obtained for which the X-ray structure determination is in progress; preliminary results confirm Me_2SnTrpAla to be monomeric), or by addition of diethyl ether. The white solids were collected on a sintered glass filter, washed with diethyl ether and small amounts of cold dry MeOH, and stored in a vacuum desiccator over P_4O_{10}. Solubility in MeOH for the R_2SnL complexes was reasonably good, while it was distinctly less so in D_2O.

RESULTS AND DISCUSSION

$R_2Sn(IV)^{2+}$ complexes with dipeptides in the solid state

Infrared spectra of the complexes are reported in Table 2. Vibrational modes such as ν(NH) (amino terminal or peptide bond NH), $\nu(COO^-)$ and ν(CO) groups are expected to be sensitive to the binding of the dipeptide moiety to the metal ion. The broad bands due to amino group vibrations, $\nu(NH_2)$, are clearly distinguishable from indole and imidazole NH sharper vibrations. The latter are almost unshifted following coordination of the peptide to the organotin(IV) moiety, while those attributed to the amino group are shifted upon coordination and are located in the 3100–3300 cm^{-1} region. The amide (I) band (involving the peptide carbonyl group) is consistently shifted to lower wavelengths.

The vibrations relative to $\nu(COO^-)$ consistently show $\Delta\nu$ values $[\Delta\nu = \nu_{as}(COO^-) - \nu_{sym}(COO^-)]$ equal to or higher than 200 cm^{-1}, which suggests that carboxylate groups are coordinating in a monodentate fashion.[12] It is noteworthy that the amide (II) band, which involves the peptide NH groups and is located in the 1550–1590 cm^{-1} region, disappears upon coordination.[13] As for the organometallic moiety, $\nu(SnC_2)$, asymmetric and symmetric, have been located in the 500–600 cm^{-1} region.

The IR data support the notion that the dipeptides chelate the metal ion, bonding through the terminal amino and carboxylate groups and a deprotonated peptide nitrogen, yielding pentacoordinated tin(IV) complexes, forming five-membered rings, while no evidence is offered for the involvement of the side-chain donor atoms in bonding.

^{119}Sn Mössbauer data (Table 3) give an insight into the structure of the complexes in the solid state. The experimental nuclear quadrupole splitting parameters, ΔE (mm s^{-1}), suggest a trigonal-bipyramidal geometry around the tin(IV) atom.[14]

ΔE values vary from 2.46 mm s^{-1} for $Ph_2SnTrpAla$ to 2.87 mm s^{-1} for $Me_2SnHisTyr$ and are in the range of those reported for several $R_2Sn(IV)$ complexes of dipeptides.[15]

Mössbauer data have been rationalized through a literal version of a point-charge model calculation of ΔE to give C–Sn–C angles for each of the R_2SnL complexes.[16,17] The partial quadrupole splitting values, p.q.s. (mm s^{-1}) used throughout the calculations are: $\{Alk\}^{tbe} = 1.13$; $\{NH_2\}^{tba} = +0.01$; $\{N_{pept}\}^{tbe} = -0.30$; $\{COO^-\}^{tba} = +0.075$.

Regular trigonal-bipyramidal structures are considered, except for C–Sn–C angles that are allowed to vary. The dimethyltin(IV) moiety and deprotonated peptide nitrogen lie on the equatorial plane, while the amino and carboxylate groups are located at apical positions (Fig. 1).

$R_2Sn(IV)^{2+}$ complexes with dipeptides in solution

^{13}C NMR spectra

^{13}C NMR chemical shifts for the dipeptides and their complexes were measured in CD_3OD and

Table 2 Some relevant IR frequencies (cm^{-1}) for $R_2Sn(IV)^{2+}$ complexes with dipeptides

Compound	ν(NH)	Amide I	ν_{as} (COO^-)	$\nu_{sym}(COO^-)$	$\nu_{as}(SnC_2)$	$\nu_{sym}(SnC_2)$
$Me_2SnTrpAla \cdot 0.5H_2O$	3650m[c] 3408 bd[b] 3270 bd	1620 s	1607 vs	1403 m	561 m	528 m
$Ph_2SnTrpAla \cdot H_2O$	3405 bd[b] 3220 bd	1618 s	1618 s	1396 m		
$Me_2SnTrpTyr \cdot H_2O$	3620 m, sh[e] 3410 vbd[c] 3270 bd	1611 bd[d]	1611 bd[d]	1396 m	567 m	533 m
$Me_2SnTrpTrp$	3620 m, sh[e] 3410 vbd 3270 bd	1611 bd[d]	1611 bd[d]	1396 m	567 m	533 m
$Me_2SnHisTyr$	3322m[f] 3260m[g]	1625 vs	1583 s	1389 m	561 m	526 m

[a] sh, shoulder; w, weak; m, medium; s, strong; vs, very strong; b, broad; as, asymmetric; sym, symmetric. [b] ν(OH) of water present in the formula. [c] ν(OH(Tyr) + H_2O present in the formula. [d] Overlap of amide (I) and $\nu_{as}(COO^-)$ bands. [e] ν(NH) indole. [f] ν(OH) Tyr. [g] ν(NH) imidazole, superimposed on $\nu(NH_2)$.

Table 3 ^{119}Sn Mössbauer parameters for $R_2Sn(IV)^{2+}$ complexes with dipeptides at liquid-nitrogen temperature

Compound	δ[a] (mm s^{-1})	ΔE[b] (mm s^{-1})	Γ_1[c] (mm s^{-1})	Γ_2[c] (mm s^{-1})	C–Sn–C angle[d] (degrees)
$Me_2SnTrpAla \cdot 0.5H_2O$	1.13	2.73	0.84	0.84	113
$Ph_2SnTrpAla \cdot H_2O$	1.06	2.46	0.84	0.91	116
$Me_2SnTrpTyr \cdot H_2O$	1.15	2.79	0.91	0.93	115
$Me_2SnTrpTrp$	1.16	2.81	0.87	0.91	115
$Me_2SnHisTyr$	1.15	2.87	1.02	1.03	117

[a] Isomer shift relative to room-temperature $Ca^{119}SnO_3$. [b] Nuclear quadrupole splitting. [c] Full width at half-height of the resonant peaks. [d] C–Sn–C angle values calculated for $R_2Sn(IV)^{2+}$ complexes with dipeptides according to point-charge model.

are reported in Table 4. In this solvent, the occurrence of unique ^{13}C signals confirms that the complexes exist in the form of undissociated monomers, except in the case of $Me_2SnTrpTrp$, where the major signals relative to the complex species are flanked by minor ones which are found at the same δ values of the free ligand. This prompted us to evaluate osmometrically the molecular weight of the dipeptide complex $Me_2SnTrpTrp$ (Table 1), which gave satisfactory results indicating that in CD_3OD dissociation is negligible. The shifts of the resonances, which are recorded relative to the free ligands, are more pronounced for carboxylate, peptide and methine carbon atoms (>1 ppm), while for HisTyr and TrpAla, due to their limited solubility, solutions were obtained in CD_3OD by addition of stoichiometric amounts of NaOD (D_2O solution); hence the observed shifts are distinctly smaller. The assignments were made according to Bradbury and Norton[18] and London.[19] A cross-comparison among all the ligands and complexes enables us to assign the ^{13}C resonances of αCH, α'CH and βCH_2, $\beta' CH_2$. In $Me_2SnGlyTrp$,[20] where Trp is the C terminus, Trp αCH are found at 58.98 and βCH_2 at 27.10 ppm; in free Trp (DMSO-d_6, pH = 6.7)[19] the resonances are located at 56.31 and 27.63 ppm.

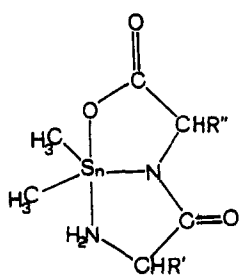


Figure 1 Structure of the complexes, in which R' = $(\beta)CH_2Trp$, R'' = $(\beta')CH_2H$; R' = $(\beta)CH_2Trp$, R'' = $(\beta')CH_2Trp$; R' = $(\beta)CH_2Trp$, R'' = $(\beta')CH_2Tyr$; or R' = $(\beta)CH_2His$, R'' = $(\beta')CH_2Tyr$; and Trp, Tyr and His are the same as in Table 4.

In $Me_2SnTrpAla$ (this work), where Trp is the amino terminus, the resonances are found at 56.19 and 29.74 ppm.

In $Me_2SnTrpTrp$, where two Trp moieties are involved as both N and C termini we assign the resonances at 56.43 and 27.73 ppm to the N-terminus while those at 58.97 and 30.09 ppm have been assigned to the C-terminal moiety ($C\alpha', \beta'$).

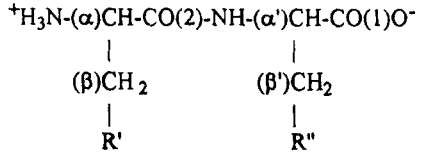
According to the observations made by Bradbury and Norton[18] for the aromatic carbon resonances, the major shifts relative to L-tryptophan are expected for the Trp moiety which is involved in peptide bonding via the amino group;[18] hence the higher field resonances have been assigned to the N-teminal Trp moiety.

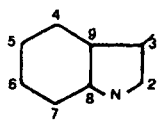
^{1}H NMR spectra

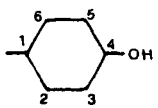
The resonances for the individual amino acids were assigned according to Wüthrich.[21] Some caution must be exercised when assigning resonances to protons belonging to the same amino acid in dipeptides, since an altered spectrum is obtained, depending on the sequence of the amino acid. The simplest spectrum was obtained in the case of TrpAla which was in agreement with the work by McDonald and Phillips.[22] For the remaining peptides and their complexes reference was made to our previous work[23] and to the reports by Wüthrich,[21,24] Rabenstein *et al.*,[25] Martin[26,27] and Huber and co-workers.[20]

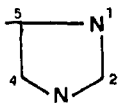
Apparently, aliphatic αCH and βCH_2 and aromatic side-chain protons belonging to the same amino acid, but present in the context of different dipeptides, have different chemical shifts.

Table 4 ^{13}C chemical shifts[a] of $R_2Sn(IV)^{2+}$ complexes with dipeptides and free dipeptides in CD_3OD









Compound	CO-1	CO-2	αCH	βCH$_2$	α'CH	β'CH$_2$	R' = Trp CH-2	C-3	CH-4	CH-5	CH-6	CH-7	C-8	C-9	R" = Tyr C-1	CH-2,6	CH-3,5	C-4	R_2Sn (R = Me, Ph)
H_2TrpAla[c]	179.82	176.34	56.74	31.61	51.63	19.36	124.78	111.15	119.52	119.73	122.38	112.22	138.14	128.87					
Me_2SnTrpAla · 0.5H_2O	181.16	175.71	56.19	29.74	53.29	19.95	125.39	109.73	119.43	120.28	122.95	112.57	138.39	128.58					−0.15
																			−0.23
Ph_2SnTrpAla · H_2O	180.75	175.46	56.21	29.12	53.24	19.79	125.68	108.60	119.45	120.36	123.00	112.58	137.88	128.47					C_1 = 138.37–138.26
																			$C_{2,6}$ = 135.87–136.69
																			$C_{3,5}$ = 130.28–130.26
																			C_4 = 131.82–131.67
H_2TrpTyr	177.46	169.87	55.04	28.94	58.11	38.23	125.71	108.40	119.28	120.27	122.82	112.59	138.36	128.42	116.05	131.48	130.23	157.02	
Me_2SnTrpTyr · H_2O	179.66	175.27	56.32	29.88	58.50	36.49	125.63	109.68	119.41	120.37	123.05	112.68	138.43	128.57	116.08	132.32		157.37	−0.63
																			−1.38
							R', R" = Trp												
H_2TrpTrp	177.92	170.07	54.88	28.79	57.38	29.01	124.57	108.26	119.29	120.19	122.19	112.18	137.88	128.31					
							125.96	108.26	119.64	120.19	122.75	112.51	138.21	129.18					
Me_2SnTrpTrp[d]	180.37	175.34	56.43	27.73	58.97	30.09	125.08	110.19	119.33	120.20	122.24	112.28	137.74	128.66					−0.98
							125.32	110.91	119.66	120.26	122.92	112.65	138.45	129.92					−2.37
							R' = His								R" = Tyr				
							C-2	CH-4	C-5						C-1	CH-2,6	CH-3,5	C-4	
H_2HisTyr[c]	179.04	175.74	55.72	32.63	57.57	38.46	136.48	127.92	121.45						117.43	131.16	132.23	160.68	
Me_2SnHisTyr	179.54	174.59	56.19	30.70	58.70	36.52	136.94	128.67	b						116.14	132.18		157.28	−0.85
																			−1.43

[a] In ppm from TMS. [b] Not observed. [c] Solvent: CD_3OD + NaOD. [d] $|^1J(^{119}Sn, ^{13}C)| = 631.8$ Hz, $|^1J(^{117}Sn, ^{13}C)| = 604.5$ Hz, which appears to be identical for both CH_3 groups in the organometallic moiety. C–Sn–C angle = 132°.

In the case of the $Me_2SnTrpTrp$ complex, the protons of the C-terminal amino acid have been assigned to the lower field resonances while those referring to the N-terminal moiety (which are expected to be more shielded) are assigned to the higher field frequencies (Table 5). In all tryptophan-containing complexes, the N-terminal Trp moiety gives rise to αCH resonances in the range 3.83–3.92 ppm, while the diastereotopic βCH_2 protons fall in the range 2.96–3.56 ppm (the case of $Me_2SnTrpTrp$).

The only C-terminal Trp moiety is present in $Me_2SnTrpTrp$ and an α'CH resonance is detected at 4.51 ppm while $\beta' CH_2$ resonances are in the 3.35–3.63 ppm range. A similar trend was assumed to be present for the aromatic 3-indolyl protons, i.e. the lower field resonances were attributed to the C-terminal moiety. αCH and α'CH protons are shifted upfield in the case of $Me_2SnTrpTrp$ and $Me_2SnTrpTyr$. βCH_2 protons are generally shifted downfield for all complexes, except for the case of $Me_2SnTrpTrp$, where the N-terminal Trp moiety shows one βCH_2 proton shifted downfield and the other in the opposite direction. For $Me_2SnTrpAla$ and $Me_2SnHisTyr$ the shifts are downfield for αCH and upfield for α'CH relative to the free dipeptide in its monoanionic form (NaOD added). In the absence of reliable vicinal coupling constant values, no firm evaluation of the steric hindrance exerted by the phenyl groups of $Ph_2SnTrpAla$ relative to the less bulky dimethyltin(IV) group can be made. The αCH resonances of Trp and α'CH of Ala are shifted more downfield in the diphenyltin(IV) than in the dimethyltin(IV) complex.

It is well established that aromatic rings are capable of inducing large, through-space chemical shift effects such as current shifts, and chemical shift differences may occur in the observed side-chain protons without stacking and without positive interactions with the neighbouring aromatic group. Additionally, side-chain intramolecular interactions of the hydrophobic type may exert a strong influence towards the preferred side-group conformation.

Cohen[28] presented pH-dependent ^1H NMR spectra for the 3-indolyl side-chain protons in TrpAla, AlaTrp and TrpTrp. In $Me_2SnTrpTrp$ we observe two distinct sets of resonances for each aromatic proton, apparently referring to each Trp moiety, albeit separated in the case of CH-5 and CH-6 by just 1 Hz. Larger differences are observed for CH-2, CH-4 and CH-7 protons.

Solution behavior and conformation

The solution behavior of the complexes (stability and/or dissociation in CD_3OD and D_2O solvents) has been monitored and structural details, namely C–Sn–C angles, obtained by evaluating $|^1J(^{119}Sn, ^{13}C)|$ and $|^2J(^{119}Sn, ^1H)|$. In order to determine C–Sn–C angles for the organometallic moieties in solution, Lockhart and Manders' relationship[29] between C–Sn–C bond angles and $|^1J|$ and $|^2J|$ was applied: the $|^1J|$ coupling constant for $Me_2SnTrpTrp$ is reported in Table 4; $|^2J|$ coupling constants for all complexes are reported in Table 5, along with calculated C–Sn–C angles. In general, for the complexes in solution, the calculated C–Sn–C angles are slightly larger than those calculated for the complexes in the solid state by use of the literal point-charge model (see Table 3). It is noteworthy that, in both ^{13}C and ^1H NMR spectra, the chirality of the complexes is responsible for the appearance of two magnetically nonequivalent $CH_3(Sn)$ resonances.

While in CD_3OD there is little (if any) indication of dissociation of the complexes, in D_2O solution the signals which are present in CD_3OD are generally doubled, following the partial hydrolysis of the complex. For instance, in $Me_2SnTrpTrp$ the extent of hydrolysis was evaluated by observing that in D_2O the Me_2Sn moiety gives rise to resonances at 0.49 and −0.63 ppm, corresponding to those at 0.46 and −0.55 ppm in CD_3OD with the addition of just one lower field signal at 0.64 ppm, whose integrated intensity corresponds to *ca* 15% of the total amount of complex. The lack of chirality of the newly formed species gives support to the view that upon hydrolysis of Me_2Sn^{2+} complexes of dipeptides[30] the apical carboxylate and amino groups have been removed from tin, with water molecules presumably taking their place, while bonding through N_{pept} is preserved.

Conformation-dependent effects have been stressed as prevailing in Trp-containing peptides.[21] It must be remembered that while in the free ligand the peptide backbone is predominantly in a flexible extended form, in these complexes chelation by amino, peptide nitrogen and carboxylate groups provides a stiff backbone; therefore it is only attached side-chain interactions which play a crucial role in determining which conformation is preferred. An estimate of solution conformation of nonchelated side-chains can be accomplished by ^1H NMR analysis of vicinal proton coupling constants about the $\alpha CH–\beta CH_2$ bond.[27,31]

Table 5 ^{1}H chemical shifts[a] of $R_2Sn(IV)^{2+}$ complexes with dipeptides and free dipeptides in CD_3OD

Compound	αCH	βCH$_2$	α′CH	β′CH$_2$	CH-2[g,i] CH-2′[k]	CH-4[g,i] CH-4′[k] CH-2,6[h]	CH-5[i] CH-5′[k] CH-3,5[h]	CH-6[i] CH-6′[k]	CH-7[i] CH-7′[k]	R$_2$Sn (R = Me, Ph)	$\lvert^2J(^{119}Sn, ^1H)\rvert$[b] $\lvert^2J(^{117}Sn, ^1H)\rvert$[b]		Me–Sn–Me angle	
$H_2Trp^qAla^d$	3.64 dd	3.23 dd 2.96 dd	4.20 q	1.25 d	7.10 s[i]	7.32 d[i]	6.98 t[i]	7.07 t[i]	7.62 d[i]					
$Me_2SnTrp^sAla \cdot 0.5H_2O$	3.84 dd	3.47 dd 3.17 dd	4.18 q	1.32 d	7.17 s[i]	7.37 d[i]	7.03 t[i]	7.13 t[i]	7.62 d[i]	+0.41 +0.63	79.2 76.3	81.9 78.2	130	133
$Ph_2SnTrpAla \cdot H_2O$	3.92 dd	[m] [m]	4.35 q	1.37 d	7.01 s[i]	7.39 d[i]	7.12 t[i]	7.29 t[i]	7.56 d[i]	$H_{2,6}$ = 7.88 $H_{3,5}$ = 7.64 H_4 = 7.42				
$H_2Trp^tTyr^{t'}$	4.03 dd	3.41 dd 3.13 dd	4.43 dd	3.11 dd 2.90 dd	7.18 s	7.36 d[i] 6.99 d[h]	7.04 t[i] 6.63 d[h]	7.13 t[i]	7.66 d[i]					
$Me_2SnTrp^zTyr^{z'} \cdot H_2O$	3.87 dd	3.44 dd 3.21 dd	4.39 dd	3.20 dd 3.08 dd	7.24 s	7.43 d[i] 6.49 s[h]	7.07 t[i]	7.16 t[i]	7.69 d[i]	−0.16 +0.53	80.3 77.4	78.3 76.0	131	128
$H_2Trp^nTrp^{n'}$	3.97 dd	3.34 dd 3.01 dd	4.62 dd	3.38 dd 3.20 dd	7.05 s[i] 7.08 s[k]	7.26 d[i] 7.39 d[k]	7.01 t[i] 7.01 t[k]	6.92 t[i] 6.92 t[k]	7.57 d[i] 7.60 d[k]					
$Me_2SnTrp^pTrp^{p'}$	3.83 dd	3.56 dd 2.96 dd	4.51 dd	3.63 dd 3.35 dd	6.78 s[i] 7.16 s[k]	7.24 d[i] 7.29 d[k]	7.03 t[i] 7.04 t[k]	7.11 t[i] 7.12 t[k]	7.39 d[i] 7.63 d[k]	−0.55 +0.46	80.2 77.3	77.3 74.7	131	127
$H_2HisTyr^d$	3.50 t	3.11 dd [e]	4.40 dd	[e] 2.82 dd	7.54 d[g]	6.73 s[g] 6.58 d[h]	6.93 d[h]							
$Me_2SnHisTyr$	3.77 t	[f] [f]	4.35 dd	[f] 3.06 dd	7.75 d[g]	7.06 s[g] 6.51 dd[h]	6.56 dd[h]			−0.086 +0.578	81.5 78.3	78.5 75.5	133	129

Abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet.

[a] In ppm from TMS. [b] In Hz. [c] In degrees, from Ref. 29. [d] Solvent: CD_3OD + NaOD. [e] Multiplet extended from 3.18 to 3.11. [f] Multiplet extended from 2.90 to 2.83. [g] His; [h] Tyr; [i] Trp; [k] Trp′; [m] Multiplet extended from 3.40 to 3.28, some resonances being obscured by CD_3OD signals. [n] $|J_{AB}| = 15.0$ Hz; [n′] $|J_{AB}| = 14.6$ Hz. [p] $|J_{AB}| = 14.2$ Hz; [p′] $|J_{AB}| = 15.0$ Hz. [q] $|J_{AB}| = 14.3$ Hz. [s] $|J_{AB}| = 14.8$ hz. [t] $|J_{AB}| = 15.0$ Hz. [t′] $|J_{AB}| = 13.9$ Hz. [z] $|J_{AB}| = 14.8$ Hz; [z′] $|J_{AB}| = 13.4$ Hz.

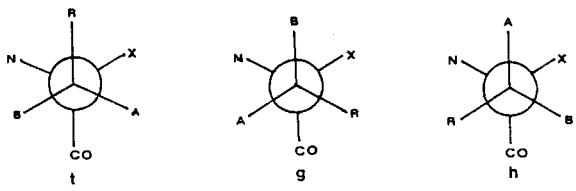


Figure 2 Side-group rotamers where A, B and X are protons: **t**, *trans* rotamer; *g*, *gauche* rotamer; **h**, *gauche*, most-hindered, rotamer.

The amino acids under investigation, His, Tyr, and Trp, give rise for the αCH and diastereotopic βCH_2 protons to a three-spin ABX system. Their spectra are time-averaged over three predominant staggered rotamers, as illustrated in Fig. 2

Carboxylate (or carbonyl for N-terminal amino acids in dipeptides) are *anti* (*trans*) relative to the side-chain group in the **t** rotamer, *gauche* in the **g** rotamer and also *gauche* in the most hindered **h** rotamer. Only when ^{1}H NMR spectra exhibited a sufficient number of lines could proton spin coupling constants J_{AX} and J_{BX} be determined and related to the mole fractions of each of the three rotamers according to the procedure followed by Martin and co-workers.[26,27]

Such unambiguous assignments were possible only in the case of free and dimethyltin(IV)-coordinated TrpAla, TrpTyr and TrpTrp peptides and are reported in Table 6.

In L,L-dipeptides such as ours, side-chains occur at the same side of the chelate plane; therefore, nonbonded interactions are expected to be the main determinant for side-chain conformation. In the free dipeptides apparently the rotamer with *anti* carbonyl and side-chain groups predominates amongst the three staggered ethanic rotamers (Fig. 2). A comparison between free and coordinated peptides presented in this work gives the following indications.

For H_2TrpAla and Me_2SnTrpAla there is no relevant change in rotamer population for the aromatic side-chain in the N-terminal amino acid (Trp) going from the free dipeptide to the complex. This finding is in agreement with Martin's[32] report on PhePhe and its Pd^{2+} complex, where the N-terminal phenylalanine shows little change in rotamer population between free and coordinated dipeptide.

For H_2TrpTyr and Me_2SnTrpTyr, where both amino acids bear an aromatic side-chain, a slight decrease in the mole fraction of *trans* rotamer (**t**) is observed in the complex relative to the free dipeptide for the N-terminal Trp moiety, along with a sizable increase in the **h** rotamer population at the expense of the other *gauche* rotamer (**g**).

A striking difference is observed for the C-terminal Tyr moiety. The population of the most hindered rotamer **h** by far exceeds those of **t** and **g**, reversing the trend observed in the free dipeptide, where the least hindered rotamer **t** accounted for half of the rotamer population.

For H_2TrpTrp and Me_2TrpTrp, the N-terminal Trp shows a slight increase in **t** rotamer population in going from the free to the coordinated dipeptide, along with a minor increase for the **h** rotamer and decrease for the **g** rotamer. The C-terminal moiety, similarly to C-terminal Tyr, again shows a dramatic increase of the **h** rotamer following complexation.

From the conformational studies, the following conclusions can be drawn concerning the conformations adopted by Trp complexes in CD_3OD solutions relative to the free dipeptides.

(a) The amino-terminal Trp moiety, even in its complexed form, tends to adopt a set of conformations which do not differ appreciably from

Table 6 Coupling constants and rotamer populations (as mole fraction)

			Population		
	J_{AX}[a] (Hz)	J_{BX}[a] (Hz)	**t**[a]	**g**[a]	**h**[a]
H_2TrpAla	4.6	7.7	0.48	0.20	0.32
Me_2SnTrpAla	4.3	7.6	0.47	0.18	0.35
H_2TrpTyr	5.2–4.8	8.4–7.9	0.55–0.50	0.26–0.22	0.19–0.28
Me_2SnTrpTyr	3.8–5.0	7.8–2.8	0.49–0.04	0.13–0.24	0.37–0.72
H_2TrpTrp	5.0–4.8	8.3–7.4	0.54–0.46	0.24–0.22	0.22–0.32
Me_2SnTrpTrp	3.6–4.7	9.2–2.7	0.62–0.03	0.11–0.21	0.27–0.76

[a] J_{AX}, J_{BX} and rotamer fractions are ordered according to the sequence of amino acids in the dipeptides.

those shown by free dipeptides. The N-terminal amino acid apparently retains a high degree of mobility of the side-chain group even in its coordinated form. It must be stressed that all available evidence suggests that neither 3-indolyl nor 5-imidazolyl groups appear to be coordinated to the (organo)metallic moieties.

(b) The C-terminal aromatic amino acids (Trp, Tyr), in their complexed form, appear to favor a conformation which directs the aromatic side-chain towards the (organo)metallic moiety. ^1J and ^2J NMR coupling constant values relative to the Me_2Sn^{2+} moieties and ^{119}Sn Mössbauer data all point to C–Sn–C angles indicative of pentacoordinated tin, which rules out additional coordination by nitrogen or oxygen donor atoms located in the side-chain groups. X-ray crystal structure determinations of dimethyltin(IV) and diethyltin(IV) complexes with AlaHis and GlyTyr dipeptides[33,34] show a similar orientation of the 5-imidazolyl and phenolate groups where neither side-group is coordinated.

Transition-metal ions such as Pd^{2+} and Cu^{2+} form Pd(L-tyrosinate)$_2$[35] and Cu(glycyl-L-tryptophanate)[36] complexes where the side-chain aromatic ring occupies the space above the coordination plane. The latter, in particular, show the Cu^{2+} ion to be sandwiched between two indole rings, one from the same complex molecule and the other from a neighbouring one.

Ligand–ligand and metal–ligand stacking interactions have been recently investigated[37] in ternary complexes of transition-metal ions (Pd^{2+}, Cu^{2+}) with tyrosine- and tryptophan-containing dipeptides and aromatic bipyridyl or 1,10-phenanthroline (phen).

The general conclusion is that the conformation of the C-terminal side-chain group affects the stabilization of the complexes, L-Trp (as C-terminus) in an L-Tyr-L-Trp complex being favored relative to the corresponding L-Tyr-D-Trp complex, since in the former the aromatic phenanthroline ligand can be sandwiched by both Trp indole and tyr phenolate groups, while in the latter, the side-chains being located on different sides of the coordination plane, only one of them is capable of creating the conditions for stacking interactions with 1,10-phenanthroline. In this connection, interactions between transition-metal ions and aromatic side-chains are deemed to be responsible for the orientation which disposes the C-terminal aromatic side-chain over the metal ion. The observation that, even in some of the (organo)tin(IV) complexes presented in this work, a similar conformation is favored, strongly suggests that hydrophobic interactions and inter- and/or intra-molecular hydrogen bonding may also play a crucial role in defining the preferred conformation.

Potential applications of the complexes

It is tempting to suggest that these complexes may act as bifunctional drugs towards DNA: the aromatic side-chain groups, which are acting as pendant arms on the exterior part of the complex molecule, may interact with DNA as intercalators and/or via hydrogen bonding.[38] Moreover, organotin(IV) compounds have been reported to interact with DNA[39] via phosphodiester groups[40] and are known to possess antitumor activity although their mode of action has not been fully established. A synergic combination of DNA recognition (intercalator) and DNA fixation (organometallic ion) could ultimately lead to a better affinity of the complexes towards DNA, inducing more relevant and specific cytotoxic effects.

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