Physiochemical and Biochemical Studies of Organotin(IV) Complexes of 2-[(3- Chloroanilinocarbonyl)] benzoic acid Containing Peptide Linkage

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Summary: A series of new metal complexes of tin(IV) with 2-[(3-chloroanilinocarbonyl)] benzoic acid containing peptide linkage have been synthesized and characterized by IR, multinuclear NMR (^{1}H,^{13}C,^{119}Sn) and mass spectrometry. Two types of such complexes have been synyhesized R_2SnL_2 and R_3SnL, where R = Me, n-Bu and Ph and L = 2-[(3-chloroanilinocarbonyl)] benzoic acid. IR and NMR spectral data agree with the coordination of the tin center through oxygen donor atoms. Biological screening results shows that reported complexes exhibits significant antibacterial and antifungal activity against the standard bacterial and fungal strains.

Introduction

The increasing use of organotin compounds has produced an ubiquitous contamination in aquatic ecosystem. Their presence in the environment is a consequence of their use in agriculture (as fungicides, preservative biocides, etc.) and in industry [as wood preservatives, marine antifouling paints (tributyltin; TBT); etc; and as stabilizers for PVC (dibutyltin; DBT; monobutyltin; MBT)] [1]. Interaction of organotin compounds with living organisms and finally, with man will surely increase within the next few years giving rise to pollution and toxicological problems. The solution of these problems will require an in-depth knowledge of the mechanisms of cell detoxification, a process that probably involves a reaction with biologically important ligands such as glutathione reduced, amino acids and peptides. These compounds may adopt a variety of structural modes depending on the nature of organic substituent on the Sn atom and/or the carboxylate ligand [2,3].

The biochemical and toxicological properties of these compounds have been studied extensively [4-6], and their toxicity can be related to the number and the nature of organic substituents on tin(IV)

Our purpose was to synthesize and characterize some organotin(IV) complexes of 2-[(3-chloroanilinocarbonyl)] benzoic acid derived from amidation of 3-chloroaniline and toxilic anhydride.

In different M/L ratios, two kinds of complexes of the title carboxylic acid were obtained, R_2SnL_2 and R_3SnL, where L is the carboxylic acid mentioned above. These complexes were characterized by IR, multinuclear NMR (^{1}H,^{13}C,^{119}Sn) and mass spectrometry.

Results and Discussion

All complexes are slighty soluble in cold methanol and *n*-hexane but freely soluble in DMSO and $CHCl_3$. The complexes have sharp melting points. The metal derivatives are stable at room temperature and non- hygroscopic. The physical data is given in Table-1.

Table-1: Physical Data of Organotin(IV) Derivatives of 2-[(3 Chloroanilinocarbonyl)] benzoic acid

Comp. No.	General Formula	Molecular Formula	M.Wt.	M.P. (°C)	Yield (%)
(1)Me_2	Me_2SnL_2	$C_{30}H_{24}N_2O_6Cl_2Sn$	697	165	62
(2)Bu_2	Bu_2SnL_2	$C_{36}H_{36}N_2O_6Cl_2Sn$	781	110	92
(3)Me_3	Me_3SnL	$C_{17}H_{27}NO_3ClSn$	439	67	80
(4)Bu_3	Bu_3SnL	$C_{26}H_{37}NO_3ClSn$	565	97	85
(5)Ph_3	Ph_3SnL	$C_{32}H_{24}NO_3ClSn$	624	122	70

Infrared spectral data

In order to clarify the manner of the ligand coordination at the tin center IR spectra in the range of 4000-250 cm^{-1} as KBr disc were recorded and

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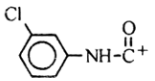
Table-2: IR Spectral Data[a] (cm^{-1}) of Organotin(IV) Derivatives of 2-[(3-Chloroanilinocarbonyl)] benzoic acid

Comp. No.	ν_{NH}	$\nu_{C=O}$	ν_{COO} (Asym)	ν_{COO} (Sym)	$\Delta\nu$	ν_{Sn-C}	ν_{Sn-O}
HL	3352s	1722s	1598s	1377w	221	–	–
(1)Me_2	3369s	1719s	1578s	1422s	156	532s	420s
(2)Bu_2	3342m	1722s	1565s	1420s	145	568s	452m
(3)Me_3	3329m	1721s	1578s	1400m	178	553m	412s
(4)Bu_3	3334s	1722s	1591m	1431s	160	526s	430m
(5)Ph_3	3349s	1720m	1589s	1415m	174	-	445m

[a] s, strong; m, medium; w, weak

Table-3: Mass Spectral Data[a] of Organotin(IV) Derivatives of 2-[(3-Chloroanilinocarbonyl)] benzoic acid

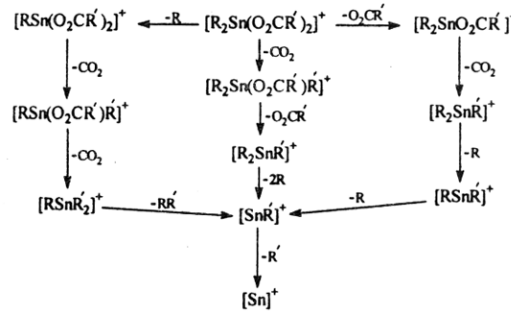
Fragment Ion	**(1)**Me_2	**(2)**Bu_2	**(3)**Me_3	**(4)**Bu_3	**(5)**Ph_3
$R_2Sn(OOCR')_2$	697(n.o)	781(n.o)	–	–	–
$R_3SnOOCR'$	–	–	439(2)	565(5)	624(4)
$RSnOOCR'$	409(n.o)	451(n.o)	409(3)	451(10)	471(2)
$SnOOCR'$	394(n.o)	394(16)	394(n.o)	394(16)	394(4)
R_3Sn^+	–	–	164(20)	290(17)	347(n.o)
R_2Sn^+	149(n.o)	233(28)	149(24)	233(4)	271(4)
$C_6H_5^+$	76(36)	76(54)	76(39)	76(34)	76(19)
Sn^+	120(9)	120(11)	120(21)	120(7)	120(14)
$C_6H_2ClCOSn^+$	257(100)	257(100)	257(100)	257(100)	257(n.o)
	154(10)	154(17)	154(17)	154(3)	154(100)

[a] not observed

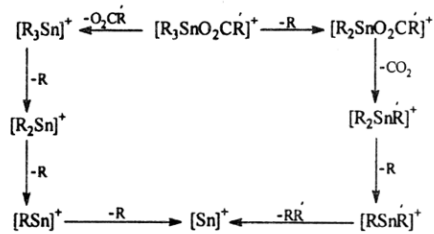
important bands for structural elucidation are given in Table-2. The absorption of interest are those of ν(CO), ν(Sn–C), ν(Sn–O) and ν(NH). In the spectra, bands in the range of 452-412 cm^{-1} are assigned to Sn–O [7] and those in the range of 568-526cm^{-1} are assigned to the Sn–C [7]. ν(C=O) of the peptide group is unaffected which indicates that peptide group is not involved in coordination. The difference $\Delta\nu$ is important in prediction of behaviour of ligand. In all compounds this difference is less than 200 cm^{-1} which indicates that ligand acts as bidentate [8,9].

Mass Spectral data

The main fragment ions observed for all synthesized compounds are listed in Table-3. The molecular ion peak is observed in all triorganotin(IV) carboxylates, while it is absent in all diorganotin(IV) carboxylates [10]. The fragments ions are in good agreement with the expected structure of the compounds. The base peak in compounds (1)-(4) is due to $[C_6H_2ClCOSn]^+$ fragment at m/z 257. While in compound (5), base peak is due to $[C_6H_5ClNCO]^+$ fragment at m/z 154. In diorganotin(IV) carboxylates the primary fragmentation is due to loss of the R group, where R is methyl, butyl and phenyl while same pattern is observed for triorganotin(IV) derivatives. However, the secondary and tertiary fragmentation occurs by loss of R group in triorganotin(IV) derivatives, while diorganotin(IV) derivatives exhibit slightly different patterns. The general mass fragmentation patterns for both di- and triorganotin(IV) compounds are given in Scheme 1 and 2.

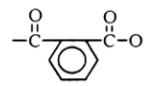


Scheme 1: Fragmentation Pattern for Diorganotin dicarboxylates.



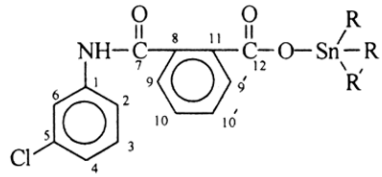
Scheme 2: Fragmentation Pattern for Triorganotin Carboxylates.

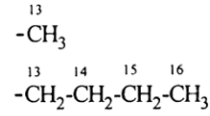
Table-4. ^{1}H NMR Data[a] of Organotin(IV) Derivatives of 2-[(3-Chloroanilinocarbonyl)] benzoic acid

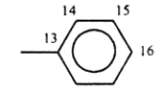
Comp. No.	Cl–C_6H_4	–NH		R
HL	7.37-7.41m	7.28s	7.80-7.85 d,d[8.59] 7.95-7.98 d,d[8.59]	–
(**1**)Me_2	7.38-7.43m	7.28s	7.80-7.84 d,d[8.76] 7.96-7.99 d,d[8.76]	1.27s
(**2**)Bu_2	7.37-7.45m	7.28s	7.80-7.87 d,d[8.72] 7.97-7.99 d,d[8.72]	0.87t, 1.27s, 1.34-1.47m, 1.52-1.74m
(**3**)Me_3	7.38-7.46m	7.28s	7.82-7.87 d,d[8.65] 7.95-7.99 d,d[8.65]	0.90s
(**4**)Bu_3	7.36-7.48m	7.28s	7.79-7.83 d,d [8.74] 7.94-7.98 d,d[8.74]	0.93t, 1.27s, 1.30-1.44m, 1.60-1.69m
(**5**)Ph_3	7.41-7.47m	7.28s	7.85-7.89 d,d [8.80] 7.96-8.00 d,d[8.80]	7.50-7.59m

[a] s, singlet; dd, doublet of doublet; t, triplet; m, multiplet

Table-5: ^{13}C NMR Data of Organotin(IV) Derivatives of 2-[(3-Chloroanilinocarbonyl)] benzoic acid







R′ = R for triorganotin

R′ = L for diorganotin

Carbon No.	HL	(**1**)Me_2	(**2**)Bu_2	(**3**)Me_3	(**4**)Bu_3	(**5**)Ph_3
1	134.6	134.5	135.5	134.6	134.5	134.6
2	132.7	132.7	132.7	132.7	132.8	132.8
3	134.0	134.0	134.0	134.0	134.2	134.2
4	130.4	130.0	130.0	130.4	130.6	130.0
5	128.1	128.1	128.1	128.1	128.1	128.2
6	129.3	129.6	129.5	129.6	129.4	129.7
7	166.7	166.7	166.7	166.8	166.7	166.8
8	131.4	131.5	131.5	131.5	131.4	131.5
9,9′	116.8	116.5	116.4	116.7	116.8	116.9
10,10′	123.8	123.8	123.8	123.8	123.8	123.8
11	131.6	131.8	131.7	131.6	131.9	131.8
12	172.5	172.6	172.8	172.9	172.7	172.8
13	–	29.6	29.6	-1.2	29.6	137.3
14	–	–	27.5	–	27.3	136.1 [47.6]
15	–	–	26.8	–	26.8	130.4
16	–	–	13.6	–	13.5	129.2 [61.3]

^{1}H NMR Spectral data

^{1}H NMR spectral data of di- and triorganotin(IV) derivatives of 2-[(3-chloroanilino-carbonyl)]benzoic acid are given in Table-4. Different protons were assigned on the basis of multiplicity, intensity pattern. Aromatic protons of chlorophenyl group and the benzoate group were assigned with difficulty due to the narrow range on the NMR scale. Alkyl groups attached to tin were assigned in their characteristic range.

^{13}C NMR Spectroscopy

^{13}C NMR data are given in Table-5. The aromatic resonances were assigned by comparison with values calculated from incremental method [11]. The involvement of the carboxylate group in bonding

Table-6: ^{119}Sn NMR Data of Organotin(IV) Derivatives of 2-[(3-Chloroanilinocarbonyl)] benzoic acid

Comp. No.	δ(^{119}Sn)ppm
(1) Me_2	41.8
(2) Bu_2	-88.4
(3) Me_3	–
(4) Bu_3	155.9
(5) Ph_3	-46.9

to tin is confirmed by the resonance ascribed to C(12), which exhibits a shift after coordination. The carbon of phenyl and alkyl groups attached to tin are observed at almost similar positions in the experimental data as calculated from incremental method[11] and reported in the literature [12-17].

^{119}Sn NMR Spectroscopy

The value of δ ^{119}Sn define the region of various coordination numbers of central tin atom [18]. The results are given in Table 6. In all complexes, ^{119}Sn spectra show only a sharp singlet indicating the formation of single species. In general ^{119}Sn chemical shifts move to lower frequency with increasing coordination number. Although the shift ranges are somewhat dependent on the nature of the substituents at the tin atom. In all the complexes, ^{119}Sn chemical shifts values lie in the tetrahedral environment around the tin.

Biological activity

The synthesized compounds are screened for antibacterial activity by agar well diffusion method [19]. All of the compounds show significant antibacterial activity against the tested bacteria. The data is given in Table-7. The ligand was found to be active against *stephlococcus aurues* and its organotin carboxylates showed more significant antibacterial effects.

When the reported compounds were screened against different fungi using tube diffusion method [20], it was observed that higher antifungal activity was shown by triphenyltin compound. The order of increase in reactivity is as follows: $Me_2 < Bu_2 < Me_3 < Bu_3 < Ph_3$. The reason is that reactivity is associated with the length of R group. Miconazole and ketoconazole were used as standard drugs. The antifungal data is reported in Table-8.

Experimental

All the glass apparatus with standard quick fit joints was used throughout the work after cleaning and drying at 120^{0}C. All the di- and triorganotin compounds were purchased from Aldrich. All the solvents were dried before use by reported methods [21].

Instrumentation

Melting points were determined by using MP–D Mitamura Riken Kogyo (Japan) electrothermal melting point apparatus and are uncorrected. Infrared spectra were recorded as KBr Pellets on a Bio-Rad FTIR spectrometer. ^{1}HNMR spectra were recorded on a Brucker AM 250 spectrometer (Germany), using $CDCl_3$ as an internal reference. Mass spectra were

Table-7: Antibacterial Activity[a] for Organotin(IV) Derivatives of 2-[(3-Chloroanilinocarbonyl)] benzoic acid

Name of Bacteria	Zone of inhibition (mm)					
	HL	(1) Me_2	(2) Bu_2	(3) Me_3	(4) Bu_3	(5) Ph_3
Escherichia coli	–	–	10	–	10	5
Bacillus subtilis	–	–	–	12	5	–
Shigella flexenari	–	–	–	–	5	–
Stephlococcus aureus	10	–	5	10	5	–
Pseudomonoas aeruginosa	–	–	–	–	–	–
Salmonella typhi	–	–	–	–	5	–

[a] Standard drug; Ampicilline $(H_2O)_3$, Cephalexin Na

Table-8: Antifungal Activity for Organotin(IV) Derivatives of 2-[(3-Chloroanilinocarbonyl)] benzoic acid

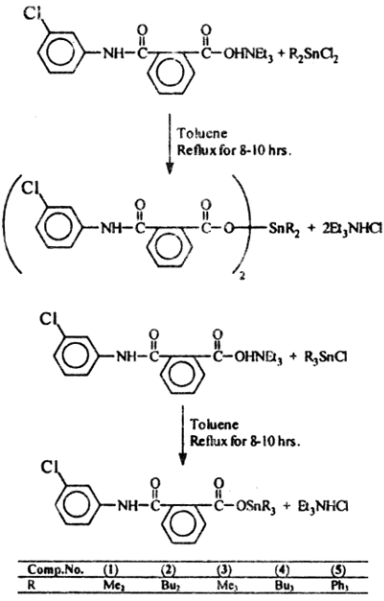
Name of Fungi	Percent inhibition						
	HL	(1) Me_2	(2) Bu_2	(3) Me_3	(4) Bu_3	(5) Ph_3	Standard Drug
Trichophyton longiusus	13	30	65	60	90	90	Miconazole Ketoconazole
Candida albicans	0	0	0	0	0	100	Miconazole Ketoconazole
Aspergillus flavis	0	0	0	0	0	85	Amphotericin-B, Flucytosine
Microsporum canis	50	30	70	70	90	85	Miconazole Ketoconazole
Fusarium solani	0	0	40	0	0	85	Miconazole
Candida glaberata	0	0	0	0	0	100	Miconazole

recorded on MAT8500 Finnigan mass spectrometer (Germany).

General procedure for synthesis

A solution of phathalic anhydride (10 gm, 0.067 mole) in HOAc (300 mL) was added to a solution of 3-chloroaniline (7.1ml, 0.067 mole) in HOAc (150 mL) and the mixture was stirred at room temperature overnight. The white precipitates were filtered, washed with cold distilled H_2O (200 mL) and air dried. 2-[(3-chloroanilinocarbonyl)]benzoic acid (1gm, 0.0036mole) was suspended in dry toluene (100 mL) and treated with Et_3N (0.51mL, 0.0036 mole).The mixture was refluxed for 2-3 hours. To a solution of triethylammonium 2-[(3-chloroanilinocarbonyl)]benzoate in dry toluene, diorganotindichloride (0.0018mole) or triorganotinchloride (0.0036mole) was added as solid with constant stirring and was refluxed for 8-10 hours. The reaction mixture contained Et_3NHCl was filtered off and the solvent from filterate was removed through rotary apparatus. The mass left behind was recrysta-llized from $CHCl_3$ and *n*-hexane mixture (1:1). General chemical equations are given as below:

General Equation



Comp.No.	(1)	(2)	(3)	(4)	(5)
R	Me_2	Bu_2	Me_3	Bu_3	Ph_3

Conclusions

It was concluded that in solid state organotin (IV) carboxylates have five or six coordination around tin, due to bidentate nature of carboxylates group. All the complexes shows the primary fragmentation due to loss of the alkyl group followed by the elimination of the CO_2 and the remaining part of the ligand, which leaves Sn^+ as the end product. Organotin (IV) carboxylates are found to be active against tested bacteria and fungi. From the NMR data, absence of OH signal (^1H NMR) and shifting of C(12) signal (^{13}C NMR) confirms the binding of carboxylate group to central tin atom. ^{119}Sn data confirms the 4-coordination around the central tin atom in solution state.

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