STUDIES ON POTENTIALLY BIODYNAMIC HETEROCYCLIC ORGANOTIN(II) MACROCYCLIC COMPLEXES

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Abstact: Antibacterial and antifertility aspects of organotin(II) macrocycles derived from 1,10-phenanthroline and dicarboxylic acids are described with a support of micro estimations, IR, ^{1}H NMR, ^{13}C NMR and ^{119}Sn NMR spectroscopy. The complexes have been characterized on the basis of molecular weight determinations and conductivity measurements. The isolated products are coloured solids, soluble in dimethylsulfoxide, dimethylformamide and methanol. Conductivity measurements in dry DMF show them to be non-electrolytes. An octahedral geometry has been suggested for these complexes. The organotin complexes were tested *in vitro* to evaluate their activity against the bacteria *Escherichia coli* and *Staphylococcus aureus*. The studies demonstrate that the concentrations reached levels sufficient to inhibit and kill these bacteria. Emphasis has been given to the antifertility activity. The testicular sperm density sperm morphology, sperm motility, density of cauda epididymis, spermatozoa and fertility in mating trial and biochemical parameters of the reproductive organs of the male albino rats were examined.

Introduction

Organotin compounds, a front area of inorganic and metal-organic chemistry, have been receiving increasing attention due to the important industrial[1] (including pesticides, anti-fouling paints and fire retardants[2]), pharmacalogical[3-5] and environmental applications. Organotin chelates with nitrogen, oxygen and sulfur donor ligands have received much attention during the last few years.[6] The considerable developments over recent decades in the use of organotin compounds as reagents or intermediates in organic synthesis prompted the preparation of many new organotin compounds.[7]

Many organotin compounds exhibit interesting antitumour activity against several human cancer cell lines. Their intensive investigation has led to the discovery of compounds with excellent *in vitro* antitumour activity, but in many cases there is disappointingly low *in vivo* toxicity.[8,9] The use of organotin(IV) halides as anti-inflammatory agents against different types of oedema in mice has been reported.[10] This work stemmed from our interest in the development of a systematic synthetic methodology for the preparation of organotin(II) macrocycles. Organotin(II) macocycles exhibit a broad spectrum of biological activity that includes bactericidal, fungicidal and antitumour effects. Our on going work with tin(II) derivatives involving such systems led us to describe the synthetic and biochemical features of some organotin(II) macrocyclces. The biochemistry of synthetic organometallics has generated active research relating to their biochemical significance.

An objective of the present work is to highlight a systematic study of the synthesis, spectroscopic characterization and biochemical aspects of the organotin(II) macrocycles of 1,10-phenanthroline and dicarboxylic acids. All the complexes have been tested *in vitro* against bacteria and *in vivo* against male albino rats for their antifertility activity.

Experimental

All solvents used were of high purity and distilled before use. $SnCl_2$ (BDH), malonic acid, succinic acid, glutaric acid and adipic acid (Fluka) and 1,10-phenanthroline (E. Merck) were used as obtained.

Synthetic Procedure

Synthesis of the complex [Sn(Mac^1)Cl$_2$]
The reaction was carried out in 1:2:2 molar ratios. A magnetically stirred solution of $SnCl_2$ in methanol was added to a solution of 1,10-phenanthroline. The reaction mixture was stirred for 30 minutes and then the methanolic solution of malonic acid was added. The resultant mixture was stirred over night to yield solid product which was removed by filtration, washed several times with same solvent and vacuum dried. These compounds were recrystallized in benzene. The purity of the products was checked by TLC on silica gel-G.

Same procedure has been used for the synthesis of [Sn(Mac^2)Cl$_2$], [Sn(Mac^3)Cl$_2$] and [Sn(Mac^4)Cl$_2$]. The reagents used were succinic acid, glutaric acid and adipic acid respectively, in place of malonic acid.

Synthesis of the organotin(II) macrocycle [CH$_3$Sn(Mac)C$_5$H$_5$N]
The reaction was carried out in 1:1 molar ratio. In a Shlenk tube, a saturated methanolic solution of [Sn(Mac^1)Cl$_2$] was taken, stirred and pyridine was added. The stirred suspension was then cooled to -5°C and stirred for 35 minutes. The sodium hydroxide (0.006 mol) followed by CH_3I (0.003 mol) were added. The solution was gradually warmed to 20°C and stirred further for 40 minutes. The solution was refluxed to half the volume, stirred, filtered and dried *in vacuo*. The physical properties of the complexes are given in Table-1.

Same procedure has been used for the synthesis of [CH$_3$Sn(Mac^2)C$_5$H$_5$N], [C$_2$H$_5$Sn(Mac^3)C$_5$H$_5$N] and [C$_2$H$_5$Sn(Mac^4)C$_5$H$_5$N] complexes. The reagents used were C_2H_5Br in place of CH_3I with [Sn(Mac^2)Cl$_2$], [Sn(Mac^3)Cl$_2$]and [Sn(Mac^4)Cl$_2$], respectively.

Table-1: Physical properties and analytical data of the organotin(II) macrocycles.

Compound	M.P. °C and Colour	Analysis, Found (Calcd.) %					Mol. Wt. Found (Calcd.)
		C	H	N	Cl	Sn	
[Sn(Mac^1)Cl$_2$]	230	52.86	2.48	8.02	10.12	17.28	692
	Light Yellow	(52.52)	(2.35)	(8.17)	(10.33)	(17.30)	(686.08
[Sn(Mac^2)Cl$_2$]	235	53.64	2.63	7.68	9.72	16.41	705
	Light yellow	(53.82)	(2.82)	(7.85)	(9.93)	(16.62)	(714.13)
[Sn(Mac^3)Cl$_2$]	220	54.86	3.11	7.46	9.44	15.82	736
	Light yellow	(55.03)	(3.26)	(7.55)	(9.55)	(15.99)	(742.18)
[Sn(Mac^4)Cl$_2$]	232	55.94	3.28	7.10	8.99	15.04	762
	Light yellow	(56.14)	(3.66)	(7.27)	(9.21)	(15.41)	(770.24)
[CH$_3$Sn(Mac^1)C$_5$H$_5$N]	241	60.76	3.03	8.99	-	16.21	686
	Yellow	(60.98)	(3.14)	(9.87)		(16.73)	(709.0)
[CH$_3$Sn(Mac^2)C$_5$H$_5$N]	247	61.78	3.64	8.65	-	15.65	711
	Off white	(61.91)	(3.82)	(9.50)		(16.10)	(737.19)
[C$_2$H$_5$Sn(Mac^3)C$_5$H$_5$N]	259	61.00	4.28	8.07	-	14.79	750
	Off white	(63.19)	(4.39)	(8.98)		(15.23)	(779.26)
[C$_2$H$_5$Sn(Mac^4)C$_5$H$_5$N]	238	60.64	4.40	7.71	-	14.30	778
	Light yellow	(63.97)	(4.71)	(8.67)		(14.70	(807.32)

Physical measurements and analytical methods

The molecular weights were determined by the Rast Camphor Method. Conductivity measurements in dry DMF were performed with a conductivity Bridge type 305. Nitrogen and chlorine were estimated by the Kjeldahl's and Volhard's method, respectively. Tin was estimated as tin oxide gravimetrically. Infrared spectra of the precursors and the complexes were recorded in the range 4000-200 cm^{-1} with the help of a Nicolet-Magna FTIR-550 spectrophotometer as KBr pellets. ^1H NMR spectra were recorded in methanol, using TMS as the standard and ^{119}Sn NMR spectra were recorded on a JEOL FX - 90Q spectrometer at 33.35 MHz. Carbon and hydrogen analyses were performed at Regional Sophisticated Instrumentation Center, Central Drug Research Institute, Lucknow.

Biochemical Procedure

Antibacterial Activity

The organotin(II) macrocycles have been tested for the *in vitro* growth inhibitory activity against bacteria *Escheriehia coli* and *Staphylococcus aureus*. The bacteria were cultured in nutrient agar medium and used as inoculums for the study. The percentage of bacterial growth inhibition produced by the complexes was estimated by the serial dilution technique[11] employing a 10^{-6} M solution of the complexes prepared in dimethylformamide.

Antifertility Activity

The antifertility activity of the complexes has been studied on male albino rats. Healthy, adult male albino rats of sprague dawley stain were used in the present investigations. The rats were divided into five groups of six animals each. The group A served as vehicle (olive oil) treated control. In the groups B, C and D 30 mg kg^{-1} body weight was suspended in 0.2 mL olive oil and receive same dose of the compounds orally for a period of 60 days $[CH_3Sn(Mac^1)C_5H_5N]$, $[CH_3Sn(Mac^2)C_5H_5N]$, $[C_2H_5Sn(Mac^3)C_5H_5N]$ and $[C_2H_5Sn(Mac^4)C_5H_5N]$, respectively. The animals were screened for fertility test and autopsied for detailed pathological and biochemical studied. Reproductive organs were excised, blotted free of blood, weighed and fixed in Bouin's fluid for histological studies.

The sperm motility and density of *Cauda epididymal* spermatozoa was assessed. The protein, sialic acid[12] and fructose were determined.[12] The activity of acid phosphatase was estimated. The vaginal smear or vaginal plug confirmed the positive mating. The females were checked for implantation after 16 days of pregnancy. The results were analysed with the help of student 't' test.

Results and Discussions

The products formed are solids and soluble in methanol, benzene, carbontetrachloride, dimethylsulphoxide and dimethyl formamide. The conductivity values measured for 10^{-3} M solutions in anhydrous DMF are in the range 19 – 25 $ohm^{-1}cm^2mol^{-1}$, showing them to be non-electrolytes.

Spectral Aspects

IR spectra

The IR spectra of the starting materials and their organotin macrocycles were recorded and their comparative studies confirmed the formation of the complexes with the prepared coordination pattern.[13,14] The significant band in the compounds observed at (1600-1610 cm^{-1} is assigned to the ν(C=N) vibrations. This suggest the bidentate (NN) coordination of phenanthroline in the compounds. This has been further confirmed by the appearance of new bands at 398 – 438 cm^{-1} in the spectra of all the complexes due to Sn←N. Bands in the region 262-289 cm^{-1} are due to Sn-Cl bond.

^1H NMR spectra

^1H NMR spectra do not show any signal corresponding to the hydroxyl group. Proton signals due to 1,10-phenanthroline appear at δ 7.45 – 9.15 ppm. In the spectra of the complexes, a singlet appearing in the regions δ 2.93 – 2.98 and δ 3.14 – 3.17 ppm is assigned to the methylene protons of malonic and succinic acid respectively, while a multiplet observed in the regions δ 3.23 – 3.27 and δ 3.28 – 3.30 ppm is ascribed to the methylene protons of glutaric and adipic acid respectively (Table-2).

Table-2: ^{1}H NMR spectral data of the organotin(II) macrocycles.

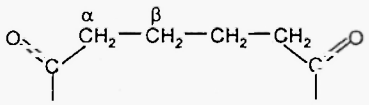
Compounds	Phenanthroline moiety	CH_2	$-(CH_2)_2-$	$-(CH_2)_3$	$-(CH_2)_4$
$[Sn(Mac^1)Cl_2]$	8.50 – 9.10	2.93	-	-	-
$[Sn(Mac^2)Cl_2]$	7.53 – 9.15	-	3.14	-	-
$[Sn(Mac^3)Cl_2]$	7.45 – 9.10	-	-	3.21	-
$[Sn(Mac^4)Cl_2]$	7.48 – 9.05	-	-	-	3.27
$[CH_3Sn(Mac^1)C_5H_5N]$	7.45 – 9.10	2.98	-	-	-
$[CH_3Sn(Mac^2)C_5H_5N]$	7.55 – 9.16	-	3.17	-	-
$[C_2H_5Sn(Mac^3)C_5H_5N]$	7.46 – 9.12	-	-	3.23	-
$[C_2H_5Sn(Mac^4)C_5H_5N]$	7.45 – 9.15	-	-	-	3.30

^{13}C NMR spectra

The conclusions drawn from the IR and ^{1}H NMR spectra are in agreement with the ^{13}C NMR spectral data (Table-3) regarding the authenticity of the proposed structures.

Table-3: ^{13}C NMR spectral data of the organotin(II) macrocycles.

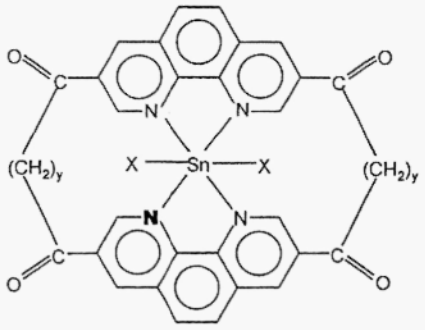
Compound	C=O	Phenanthroline moiety	C_α	C_β
$[Sn(Mac^1)Cl_2]$	176.40	C_2 138.4; C_3 125.2; C_4 122.4; C_5 128.3; C_6 148.2	33.51	-
$[Sn(Mac^2)Cl_2]$	177.45	C_2 135.8; C_3 125.4; C_4 124.2; C_5 129.2; C_6 150.4	33.43	-
$[Sn(Mac^3)Cl_2]$	175.92	C_2 134.2; C_3 124.3; C_4 121.0; C_5 127.1; C_6 148.8	33.98	26.47
$[Sn(Mac^4)Cl_2]$	177.33	C_2 136.6; C_3 126.5; C_4 123.4; C_5 128.4; C_6 149.9	33.20	25.33
$[CH_3Sn(Mac^1)C_5H_5N]$	175.76	C_2 136.8; C_3 125.8; C_4 123.6; C_5 127.9; C_6 149.6	32.85	-
$[CH_3Sn(Mac^2)C_5H_5N]$	176.84	C_2 138.4; C_3 126.4; C_4 123.4; C_5 129.0; C_6 149.2	32.54	-
$[C_2H_5Sn(Mac^3)C_5H_5N]$	176.31	C_2 138.4; C_3 125.2; C_4 122.8; C_5 128.2; C_6 148.9	33.70	23.20
$[C_2H_5Sn(Mac^4)C_5H_5N]$	177.10	C_2 135.6; C_3 124.3; C_4 121.2; C_5 129.1; C_6 148.8	33.74	25.69



^{119}Sn NMR spectra

^{119}Sn NMR spectra of the complexes give signals at -δ 538-589 ppm, indicating coordination number six in these complexes around tin atoms.[15]

On the basis of the above results the structure shown in Fig.1 is proposed for the organotin(II) macrocycles.



Where, X = Cl, CH_3, C_2H_5 and C_5H_5N

Biochemical Aspects

Antibacterial Activity

The antibacterial activity of the complexes were screened using the serial dilution technique. The results (*Table IV*) showed that the organotin complexes are more toxic against gram positive organism namely *staphylococcus aureus*, than the gram negative organism *Escherichia coli*. The organotin(II) macrocycles produced *ca.* 88.4 – 100% growth inhibition against the gram positive organism. However, in the antibacterial activity against *Escherichia coli* the complexes are of the order of *ca* 37-68%.

Table-4: Percentage of inhibition to bacterial growth by the organotin(II) macrocycles.

Compounds	0 h	4 h	8 h	24 h
Against *Staphylococcus aureus*				
$[Sn(Mac^1)Cl_2]$	10.3	30.1	50.1	77.4
$[Sn(Mac^2)Cl_2]$	10.5	30.8	50.6	90.4
$[Sn(Mac^3)Cl_2]$	11.6	33.7	52.7	88.4
$[Sn(Mac^4)Cl_2]$	12.3	35.8	54.9	-
$[CH_3Sn(Mac^1)C_5H_5N]$	12.5	37.3	56.7	88.9
$[CH_3Sn(Mac^2)C_5H_5N]$	18.1	38.9	56.8	90.0
$[C_2H_5Sn(Mac^3)C_5H_5N]$	18.8	40.6	-	99.7
$[C_2H_5Sn(Mac^4)C_5H_5N]$	20.2	40.9	58.2	100.0
Against *Escherichia coli*				
$[Sn(Mac^1)Cl_2]$	13.2	31.3	33.2	36.9
$[Sn(Mac^2)Cl_2]$	12.6	35.4	42.3	46.4
$[Sn(Mac^3)Cl_2]$	-	-	46.0	-
$[Sn(Mac^4)Cl_2]$	15.6	34.9	51.1	54.9
$[CH_3Sn(Mac^1)C_5H_5N]$	22.2	28.1	-	55.3
$[CH_3Sn(Mac^2)C_5H_5N]$	25.3	25.8	-	58.7
$[C_2H_5Sn(Mac^3)C_5H_5N]$	22.9	29.0	63.2	-
$[C_2H_5Sn(Mac^4)C_5H_5N]$	27.8	30.4	65.4	67.9

Antifertility Activity

The testicular morphology, testicular sperm density, sperm motility, density of cauda epididymal spermatozoa and fertility in mating trials and biochemical parameters of reproductive organs with the heterocyclic organotin(II) macrocyclic complexes *in vivo* were examined and discussed.

The results show that the complexes are able to inhibit fertility due to the synergistic effects of the tin. The results are grouped under the following headings:

Body and Organ Weights

Body weights of rats were not affected after their tin complexes were administrated. However, the weights of testes, epididymis, seminal vesicle and ventral prostate were significantly decreased (Table-5).

Table-5: Effects of the organotin(II) macrocycles on body weight and organ weight of male rats.

Group	Treatment	Body weight		Organ weight (mg)			
		Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate
A	Control	120.0 ± 15.6	222 ± 14.3	1340.0 ± 40.0	490.0 ± 17.3	510.0 ± 19.3	475.0 ± 20.8
B	$[CH_3Sn(Mac^1)C_5H_5N]$	225.5 ± 13.4	237 ± 17.4	1108 ± 20.5	402.5 ± 15.4[a]	418.0 ± 20.5[b]	405.0 ± 18.5[b]
C	$[CH_3Sn(Mac^2)C_5H_5N]$	230.0 ± 18.4	245.0 ± 14.3	980.0 ± 18.9[b]	385.3 ± 13.7[b]	405.7 ± 18.4[b]	485.0 ± 17.3[b]
D	$[C_2H_5Sn(Mac^3)C_5H_5N]$	210.0 ± 14.9	228.0 ± 13.7	930.5 ± 20.5[b]	350.4 ± 14.8[b]	370.5 ± 30.5[b]	330.0 ± 10.3[b]
E	$[C_2H_5Sn(Mac^4)C_5H_5N]$	218.0 ± 17.4	229.0 ± 18.5	850.0 ± 30.1[b]	290.5 ± 13.5[b]	310.5 ± 17.5[b]	305.0 ± 13.4[b]

Groups B, C, D and E compared with Group A
Mean ± SEM of six determinations
a = $P \leq 0.01$
b = $P \leq 0.001$

Fertility Test

The sluggish motile spermatozoa was unable to fertilize normal cyclic females. The test was 50 to 97% negative in rats treated with the compounds.

Sperm Motility

The sperm motility declined significantly after treatment with compounds.

Sperm Density

The sperm density in testes and cauda epididymis were declined significantly after the treatment (Table-6).

Table-6: Sperm dynamics and fertility test after treatment with the organotin(II) macrocycles

Group	Treatment	Sperm motility Cauda epididymis (%)	Sperm density (million/ml)		Fertility test (%)
			Testes	Cauda epididymis	
A	Control	85.0 ± 4.8	5.1 ± 0.8	60.1 ± 3.5	100% + ve
B	$[CH_3Sn(Mac^1)C_5H_5N]$	62.0 ± 6.5[a]	4.1 ± 0.4[a]	48.1 ± 4.6[a]	50% - ve
C	$[CH_3Sn(Mac^2)C_5H_5N]$	50.0 ± 6.8[b]	3.5 ± 0.3[b]	38.1 ± 3.9[b]	70% - ve
D	$[C_2H_5Sn(Mac^3)C_5H_5N]$	35.0 ± 6.4[b]	2.1 ± 0.4[b]	31.2 ± 4.6[b]	85% - ve
E	$[C_2H_5Sn(Mac^4)C_5H_5N]$	30.5 ± 3.4 [b]	1.5 ± 0.3[b]	25.1 ± 3.4[b]	95% - ve

Groups B, C, D and E compared with Group A
Mean ± SEM of six determinations
a = $P \leq 0.01$
b = $P \leq 0.001$

Biochemical Changes

Total protein and sialic acid contents of testes, epididymis, ventral prostate and seminal vesicle were depleted significantly after treatment with the complexes. The acid phosphatase levels of testes, epididymis and ventral prostate were also reduced significantly. A significant decrease in seminal vascular fructose contents was also noticed whereas the testicular cholesterol contents were increased significantly after the treatment with various compounds (Tables-7).

Table-7: Testicular biochemistry of organotin(II) macrocycles after treatment on rats.

Group	Treatment	Glycogen (mg/g)	Total protein (mg/g)	Total cholesterol (mg/g)	Sialic acid (mg/g)	Phosphatase (mg/ip/g/hr)	
						Acid	Alkaline
A	Control	3.50 ± 0.27	220.0 ± 15.6	5.7 ± 0.21	5.4 ± 0.40	3.20 ± 0.19	10.40 ± 0.90
B	$[CH_3Sn(Mac^1)C_5H_5N]$	4.30 ± 0.15[a]	180.0 ± 8.6[a]	6.8 ± 0.31 [a]	4.2 ± 0.50 [a]	4.10 ± 0.15[a]	13.0 ± 0.85[a]
C	$[CH_3Sn(Mac^2)C_5H_5N]$	4.80 ± 0.17[b]	150.0 ± 17.5[b]	7.3 ± 0.41[b]	3.8 ± 0.40[b]	4.90 ± 0.11[b]	15.0 ± 0.75[b]
D	$[C_2H_5Sn(Mac^3)C_5H_5N]$	5.10 ± 0.15[b]	140.0 ± 13.4[b]	8.3 ± 0.3[b]	3.1 ± 0.35[b]	5.40 ± 0.15[b]	15.8 ± 0.91[b]
E	$[C_2H_5Sn(Mac^4)C_5H_5N]$	5.25 ± 0.19[b]	128.0 ± 10.5[b]	8.7 ± 0.4[b]	2.1 ± 0.25[b]	5.80 ± 0.17	16.80 ± 0.95[b]

Discussions

The present study revealed that administration of the heterocyclic organotin(II) macrocyclic complexes caused a significant reduction in the weights of testes and other sex accessory glands. The prostate and seminal vesicle are well documented androgen dependent parameters.[16] Sperm motility is considered as an important parameters in evaluating the fertility potential.[17] The present tin(II) complexes significantly reduce the fertility of male rats. Since a number of androgen sensitive parameters (protein, sialic acid, fructose, acid phosphatase and total cholesterol) in target organs were found to be altered by these complexes, it is probably that the structure and function of epididymis and other sex accessory organ are changed.

Our findings indicate that $[C_2H_5Sn(Mac^1)C_5H_5N)]$ complex has more pronounced effect of fertility on various biochemical parameters of the reproductive organs as compared to the control.

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