Full Paper

Applied Organometallic Chemistry

Received: 16 July 2008 Revised: 6 January 2009 Accepted: 6 January 2009 Published online in Wiley Interscience

(www.interscience.com) DOI 10.1002/aoc.1484

Ecofriendly synthesis, antimicrobial and antispermatogenic activity of triorganotin(IV) complexes with 4′-nitrobenzanilide semicarbazone and 4′-nitrobezanilide thiosemicarbazone

Pratibha Chaudhary, Monika Swami, D. K. Sharma and R. V. Singh*

New series of triorganotin(IV) complexes with 4′-nitrobenzanilide semicarbazone (L^1H) and 4′-nitrobenzanilide thiosemicarbazone (L^2H) of the type $[R_3Sn(L)]$ (R = -CH_3, -C_6H_5 and *n*-C_4H_9) were synthesized under microwave irradiation. All the complexes were characterized by elemental analysis, conductance measurements, molecular weight determinations and spectral data, viz., IR, UV–vis, ^1H, ^{13}C and ^{119}Sn NMR. The central tin atoms of these complexes are all five-coordinated with trigonal bipyramidal geometry. In order to assess their growth inhibitory potency semicarbazone, thiosemicarbazone and their triorganotin(IV) complexes were tested *in* vitro against some pathogenic fungi and bacteria. Also the ligands and their organotin(IV) complexes were studied to assess the effects of long-term ingestion of these compounds on fertility, body and reproductive organ weights. The biochemical analyses were also performed on blood samples and reproductive organs of male rats. The findings have been presented in this paper. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: semicarbazone; thiosemicarbazone; spectral studies; antimicrobial activity; fertility; triorganotin(IV) complexes

Introduction

In modern chemistry microwave synthesis represents one of the important dimensions. The main advantage of microwave heating is the almost instantaneous 'in-core' heating of materials in a homogenous and selective manner, coupled with the significantly shorter reaction times that can be achieved. This implies a considerable saving in energy.[1,2] The synthesis of a number of tin metal compounds has been accomplished in pressure vessels similar to those used in high pressure organic synthesis. The chemistry of organotin compounds recently has developed not only as reagents[3] but also as intermediates in organic synthesis.[4] Semicarbazones and thiosemicarbazones are versatile ligands in both neutral and anionic forms.[5] Chelates of organotin(IV) moieties with N, O and S donor ligands[6,7] have received much attention during the last few years. Organotins have been used in industrial and agricultural applications as plastic stabilizers and catalysts, antifouling paints, molluscicides, fungicides[8] and disinfectants.[9] The tributyltin chloride is a membrane-active molecule[10] and its action mechanism appears to be dependent on organotin lipophilic behavior.[11] The toxicity spectrum of organotin compounds is very broad and they may provoke immunotoxicity, hepatotoxicity, teratogenicity and neurotoxicity in animals and humans.[12,13] Semicarbazones[14,15] and thiosemicarbazones[16,17] are biologically important nitrogen and oxygen/sulfur donor ligands and their organotin(IV) complexes show significant activity.[18,19] Organotin compounds having the general formulae R_nSnX_{4-n} are biologically active.[20–23] The nature of the alkyl group is of prime importance in determining their toxicity towards particular living species.[24] Several reports have appeared on the complexes of triorganotin halides with various nitrogen and oxygen/sulfur-containing ligands.[25–27] Encouraged by these findings and our interest in the field of organotin complexes, two ligands (4′-nitrobenzanilide semicarbazone and 4′-nitrobenzanilide thiosemicarbazone) and their corresponding triorganotin(IV) complexes have been prepared and characterized and their biological aspects have been studied.

Experimental

The reagents 4′-nitrobenzanilide,[28] trimethyltin(IV) chloride, triphenyltin(IV) chloride and tri-*n*-butyltin(IV) chloride were prepared according to the literature methods.[29] Semicarbazide hydrochloride (Merck) and thiosemicarbazide (Merck) were commercial products and used as such. All the reagents and the solvents used were dried, distilled and purified by the standard methods[30,31] and purity was checked by thin-layer chromatography. The reactions were carried out under strict anhydrous conditions and adequate care was taken to keep the organotin(IV) complexes, chemicals and glass apparatus free from moisture.

* *Correspondence to: R. V. Singh, Department of Chemistry, University of Rajasthan, Jaipur-302 055, India. E-mail: rvsjpr@hotmail.com*

Department of Chemistry, University of Rajasthan, Jaipur-302 055, India

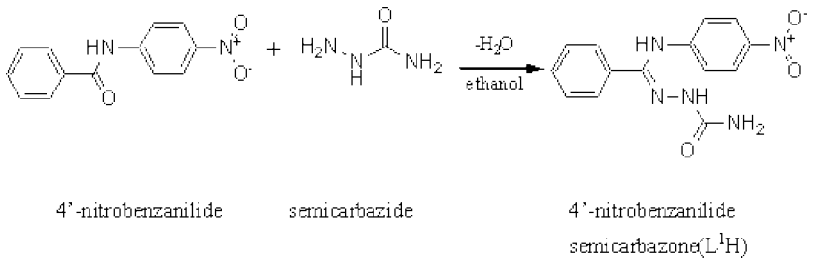


Figure 1. Synthesis of 4′-nitrobenzanilide semicarbazone (L^1H): $C_{14}H_{13}N_5O_3$.

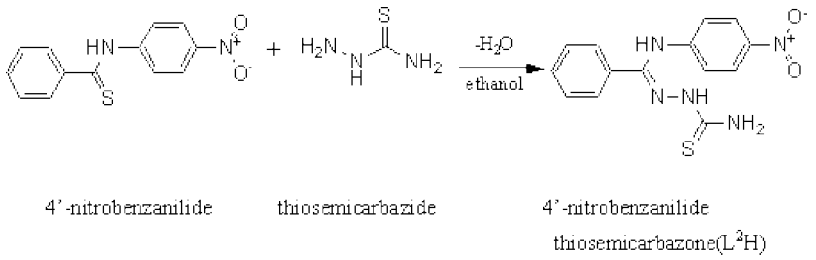


Figure 2. Synthesis of 4′-nitrobenzanilide thiosemicarbazone (L^2H): $C_{14}H_{13}N_5O_2S$.

Preparation of the Ligands

Two different routes were employed for the synthesis of the ligands for comparison purposes. These are the eco-friendly microwave-assisted synthesis and the conventional thermal method. The microwave irradiations were carried out in a Samsung domestic microwave oven, model G2719N at 160 W. The progress of the reaction was monitored by TLC on silica gel-G.[32] A comparison between thermal method and microwave methods is given in Table 1.

Analytical Methods and Physical Measurements

Nitrogen and sulfur were determined by the Kjeldahl's[33] ($14 \times V_1N_1 \times 100/1000 \times w$) and Messenger's[34] methods ($32 \times w_1 \times 100/w$), respectively. Tin was estimated gravimetrically as SnO_2 (118.69 × wt of oxide × 100/150.69 × wt of comp. taken) and chlorine was estimated volumetrically by Volhard's[35] method ($35.5 \times Z \times 100/170 \times w$). Carbon and hydrogen were determined on an Elementar Analysensysteme GmbH Varion EL *III*, Germany at University Scientific Instrumentation Center, Delhi University, Delhi. The Rast Camphor Method[36] was used to carry out the molecular weight determinations. The conductivity of the resulting complexes was determined at room temperature in dry DMF by the Systronics conductivity bridge (model 305) using a cell having a cell constant (0.5 cm^{-1}). The electronic spectra of the ligands and their complexes were recorded in methanol on a Varian Cary 100 UV–vis spectrophotometer in the range 190–900 nm. IR spectra were recorded on a Perkin–Elmer model 577 spectrophotometer in the range 4000–200 cm^{-1} on KBr and polyethylene discs. The ^1H and ^{13}C NMR spectra were recorded at 270.13 and 67.93 MHz respectively, on a Bruker AM 270 instrument. The ^{119}Sn NMR spectra were recorded at 186.50 MHz on a Bruker WM 500 instrument. Chemical shifts are quoted in ppm downfield from TMS for ^1H and relative to tetramethyltin for ^{119}Sn and referenced to residual protons of $CDCl_3$ ($\delta = 7.24$) for ^1H NMR at Delhi University, Delhi.

Antimicrobial Activity

Antifungal Studies

Bio-efficacies of the synthesized compounds were checked *in vitro*. The *in vitro* antifungal activities of the ligands and their complexes were evaluated against two pathogenic fungi, *Rhizopus oryzae* (causes zygomycosis) *and Aspergillus flavus* (causes aspergillosis of lungs) by the agar plate technique.[37] In order to compare the results obtained, the *Bavistin* was used as standard drug. The compounds were directly mixed with the medium in 0.01 and 0.1% (in methanol) concentrations. The medium was then poured into Petri plates and a small disc (0.7 cm) of the fungus culture was cut

Figure 3. Synthetic route of tin complexes.

Table 1. Comparison between conventional and microwave methods of synthesis

Compound	Yield (%)		Solvent (ml)		Time	
	Thermal	Microwave	Thermal	Microwave	Thermal (h)	Microwave (min)
L^1H	82	90	100	3	6	4.5
$Me_3Sn(L^1)$	80	90	40	2	12	5
$Ph_3Sn(L^1)$	79	88	30	3	12	7
$n\text{-}Bu_3Sn(L^1)$	78	90	25	2	11	5
L^2H	78	91	100	3	5	4
$Me_3Sn(L^2)$	78	87	40	3	13	6
$Ph_3Sn(L^2)$	79	86	35	2	12	5
$n\text{-}Bu_3Sn(L^2)$	79	88	25	3	13	6

Table 2. Physical properties and analytical data of the ligands and their corresponding organotin(IV) complexes

Compound	Colour	Melting point (°C)	Analysis (%) found (calcd)					Molecular weight, found (calcd)
			C	H	N	S	Sn	
$L^1H\ C_{14}H_{13}N_5O_3$	Turmeric yellow	130–132	56.08(56.18)	4.25(4.38)	23.29(23.40)	–	–	299.02(299.29)
$Me_3Sn(L^1)\ C_{17}H_{21}N_5O_3Sn$	Off white	211–214	44.05(44.19)	4.43(4.58)	15.01(15.16)		25.60(25.69)	462.38(462.07)
$Ph_3Sn(L^1)\ C_{32}H_{27}N_5O_3Sn$	Wheatish yellow	210–212	59.16(59.29)	4.08(4.20)	10.68(10.80)	–	18.19(18.31)	648.42(648.29)
$n\text{-}Bu_3Sn(L^1)\ C_{26}H_{39}N_5O_3Sn$	Off white	223–224	52.99(53.08)	6.54(6.68)	11.81(11.90)	–	20.12(20.17)	588.54(588.32)
$L^2HC_{14}H_{13}N_5O_2S$	Mustard yellow	136–138	53.23(53.32)	4.07(4.16)	22.09(22.21)	10.05(10.17)	–	315.47(315.35)
$Me_3Sn(L^2)\ C_{17}H_{21}N_5O_2SSn$	Light green	206–208	42.58(42.70)	4.31(4.43)	14.52(14.65)	6.64(6.71)	24.70(24.82)	478.34 (478.14)
$Ph_3Sn(L^2)\ C_{32}H_{27}N_5O_2SSn$	Light green	182–184	57.73(57.85)	3.99(4.10)	10.46(10.54)	4.70(4.83)	17.75(17.87)	664.24(664.35)
$n\text{-}Bu_3Sn(L^2)\ C_{26}H_{39}N_5O_2SSn$	Light red	275–278	51.56(51.67)	5.41(6.50)	11.48(11.59)	5.17(5.30)	19.50(19.64)	604.17(604.38)

with a sterile cork borer and transferred aseptically into the centre of a Petri dish containing the medium, with a certain amount of the compound. Suitable checks were kept where the culture discs were grown under the same conditions on PDA without the compound. These Petri dishes were wrapped in polythene bags containing a few drops of alcohol and were placed in an incubator at 25 ± 2 °C. Controls were also run and three replicates were used in each case. The linear growth of the fungus was obtained by measuring the diameter of the fungal colony after four days. The amount of growth inhibition in each of the replicate was calculated by percentage inhibition $= (C - T) \times 100/C$, where C = diameter of the fungus colony in the control plate after 96 h and T = diameter of the fungus colony in tested plates after the same period.

Antibacterial Activity

For the evaluation of degree of inhibitory effects on the growth of a wide spectrum of microorganisms, antibacterial activity was performed against one Gram-positive (*Staphylococcus aureus*) and three Gram-negative (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria. In order to compare the results obtained the Imipinem was used as standard drug. Determination of the antibacterial activity was carried out by the paper-disc plate method.[38] The compounds were dissolved in DMF at 500 and 1000 ppm concentrations. The Whattman no. 1 papers with a diameter 5 mm were soaked in these solutions. These discs were placed on the appropriate nutrient medium (0.5% peptone, 0.15% yeast, 0.15% beef extract, 0.35% sodium chloride and 0.13% KH_2PO_4 in 1000 cm^3 distilled water which was autoclaved for 20 min at 15 psi before inoculation), previously seeded with organisms, in Petri dishes and stored in an incubator at 28 ± 2 °C. The inhibition zone thus formed around each disc was measured (in mm) after 96 h.

Antifertility Activity

The activity of synthetic products towards biological systems is an important feature of current research and S and N donor ligands play a significant role in this direction. A large number of organotin(IV) complexes have been shown to cause atrophy of the testis, prostate and epididymis in male albino rats. In view of the potential interest in these biologically active compounds, the antifertility activity of both the ligands and their corresponding organotin (IV) complexes was studied in male albino rats.

Animals and Treatment

Male albino rats of Sprague–Dawley strain (180–200 g) were provided by the animal house of the Delhi University. Animals were housed in plastic cages under controlled temperature of 21 ± 1 °C and 12 : 12 h light/dark cycles and were maintained on standard rat pellets (Hindustan Lever Ltd, India) and tap water *ad libitum*. Male rats of proven fertility were randomly divided into 10 groups of 10 animals each. Only nine compounds were used separately and each compound was administered at a dose level of 2 mg day^{-1} kg^{-1} body weight orally by gavage tube for 60 days. One group served as control and each animal of this group received 0.5 ml olive oil per day orally.

After 55 days of the treatment, male rats were cohabitated with pro-oestrous females in the ratio 1 : 4. The presence of sperm in the morning vaginal smear was the evidence of mating. Females were separated and resultant pregnancies were noted when dams gave birth. The number and weights of litters were recorded. Fertility

Table 3. IR spectral data (cm^{-1}) of the ligands and their organotin (IV) complexes

Compound	ν (NH)	ν (C=N)	ν (NH_2)	ν (C=O)/ν (C=S)	ν (OH)/ν (SH)	ν (Sn–O)/ν (Sn–S)	ν (Sn ← N)	$\nu(Sn-C)_{as}$, $\nu(Sn-C)_s$
L^1H	3128–3300	1608	3340, 3418	1686	3300	–	–	–
$Me_3Sn(L^1)$	–	1632	3395, 3128	1665	–	578	430	560, 535
$Ph_3Sn(L^1)$	–	1628	3480, 3116	1662	–	580	428	–
n-$Bu_3Sn(L^1)$	–	1630	3490, 3122	1660	–	574	426	565, 540
L^2H	3142–3246	1612	3355, 3431	1024	2700	–	–	–
$Me_3Sn(L^2)$	–	1634	3475, 3136	1002	–	364	420	530, 504
$Ph_3Sn(L^2)$	–	1626	3470, 3124	997	–	362	422	–
n-$Bu_3Sn(L^2)$	–	1632	3466, 3130	994	–	358	416	528, 502

Table 4. ^1H NMR spectral data (δ, ppm) of the ligands and their corresponding organotin(IV) complexes

Compound	-NH (bs)	$-NH_2$ (bs)	φ-NH (s)	Aromatic protons (m)	$(CH_3)_3Sn/(n\text{-}C_4H_9)_3Sn$
L^1H	10.00 (1H)	2.74 (2H)	10.46 (1H)	7.22–8.74	–
$Me_3Sn(L^1)$	–	2.78 (2H)	10.65 (1H)	6.88–8.60	1.22
$Ph_3Sn(L^1)$	–	2.82 (2H)	10.59 (1H)	6.96–8.60	–
n-$Bu_3Sn(L^1)$	–	2.78 (2H)	10.59 (1H)	6.98–8.59	0.62–1.70
L^2H	10.32 (1H)	3.20 (2H)	10.60 (1H)	6.90–8.76	–
$Me_3Sn(L^2)$	–	3.32 (2H)	10.88 (1H)	7.32–8.86	1.18
$Ph_3Sn(L^2)$	–	3.28 (2H)	10.86 (1H)	7.26–8.80	–
n-$Bu_3Sn(L^2)$	–	3.27 (2H)	10.81 (1H)	7.22–8.80	0.60–1.72

Table 5. J values in Hz for tin complexes

Compound	^1H (^{119}Sn, ^{13}C)	^2J (^{119}Sn, ^{13}C)	^2J (^{119}Sn,H)
$Me_3Sn(L^1)$	555	–	53.51
$Ph_3Sn(L^1)$	531	41.00	–
$Me_3Sn(L^2)$	527	–	51.00
$Ph_3Sn(L^2)$	554	40.00	–

was calculated in control as well as in treated groups. On day 61, i.e. 24 h after the last dose, 10 animals from each group were autopsied under light ether anesthesia and reproductive organs were dissected out, freed from adherent tissues and weighed up to the nearest milligram. Blood was collected by cardiac puncture and serum was separated by centrifugation at 3000 rpm and stored at −20 °C. The sperm motility in cauda epididymis and sperm count in cauda epididymis and testes were measured by using Neubaur's haemocytometer. The data were analysed statistically by Student's *t*-test.

Body Weight, Sperm Motility and Sperm Density

Body weights of animals were recorded before the experiment and every 2 weeks thereafter. Sperm motility and sperm density were assessed in cauda epididymis by the method of Prasad *et al*.[39]

Tissue Biochemistry

Once testis freed from each rat was kept at −20 °C until assayed for cholesterol,[40] glycogen[41] and proteins.[42] Protein was estimated in epidicymides.[42]

Blood and Serum Analysis

Whole blood was analyzed for RBC and WBC count, haemoglobin,[43] haematocrit, sugar[44] and urea,[45] and serum was analyzed to estimate cholesterol,[46] total protein[42] and phospholipids.[47]

Results and Discussion

The observation was that in microwave method the reaction time was drastically reduced with improved yield as compared to the conventional method.[48] The difference was observed due to strong microwave effect, and the high enhancement of reaction rate. In conclusion we have developed an easy and convenient synthetic procedure for organotin(IV) compounds by using microwave technique.

Microwave-assisted Synthesis of L^1H

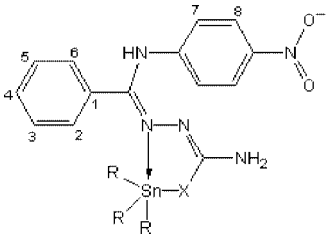
The ligand was prepared by the condensation of semicarbazide hydrochloride (2.7 g, 24 mmol) and sodium acetate (2.0 g, 24 mmol) with 4′-nitrobenzanilide (5.8 g, 24 mmol) in the presence of few drops of ethanol (~3 ml) and irradiated by microwave irradiation for ~4.5 min. The resulting yellow coloured precipitate was recrystallized with alcohol and dried under vacuum (Fig. 1).

Eco-friendly Microwave-assisted Synthesis of L^2H

The ligand L^2H was prepared by the condensation of thiosemicarbazide (2.0 g, 22 mmol) with 4′-nitrobenzanilide (5.3 g, 22 mmol) in presence of a few drops of ethanol (~3.0 ml) and irradiated by the microwave irradiation for nearly ~4 min. The resulting yellow coloured precipitate was then recrystallized with alcohol and dried under *vacuum*. The analytical results came in good consistence with the proposed formula (Fig. 2).

Table 6. ^{13}C NMR spectral data (δ, ppm) of the ligands and their corresponding organotin(IV) complexes

Compound	>C=O/>C=S	>C=N	Aromatic carbon			$(CH_3)_3Sn/(C_6H_5)_3Sn/(n\text{-}C_4H_9)_3Sn$	^{119}Sn NMR
			C_1 C_2 C_6	C_8 C_4 C_7	C_3 C_5		
L^1H	168.60	157.72	157.6 129.4 125.4	156.8 128.9 119.1	132.8 127.9	–	–
$Me_3Sn(L^1)$	163.72	153.86	156.4 130.1 125.4	153.86 128.7 118.8	132.9 127.4	15.56	– 178.6
$Ph_3Sn(L^1)$	165.68	154.56	156.2 128.9 125.4	154.56 128.4 118.8	133.0 127.6	141.12 C(i), 139.42 C(o), 138.12 C(m),132.14 C(p)	– 243.6
$n\text{-}Bu_3Sn(L^1)$	162.98	152.97	156.2 129.1 125.4	152.97 128.4 118.4	133.0 127.6	26.77 C(1), 26.58 C(2), 26.10 C(3), 13.46 C(4)	– 224.2
L^2H	175.82	162.91	158.2 129.8 124.3	157.9 128.4 118.4	132.4 127.0	–	–
$Me_3Sn(L^2)$	167.74	156.25	156.9 128.4 124.2	156.25 128.6 118.0	132.6 128.0	14.98	– 182
$Ph_3Sn(L^2)$	168.32	157.18	157.2 129.4 124.1	157.18 128.0 118.2	132.2 127.6	140.84 C(i), 139.22 C(o), 137.99 C(m), 131.94 C(p)	– 248.4
$n\text{-}Bu_3Sn(L^2)$	166.29	156.48	157.1 129.0 124.2	156.48 128.2 118.0	132.6 127.4	26.48 C(1), 26.28 C(2), 26.08 C(3), 13.06 C(4)	– 227.6



Conventional Thermal Method

A similar procedure was followed for the synthesis by the thermal method where instead of few drops of alcohol ~100 ml were taken and the contents were refluxed for 5–6 h. The solution was then concentrated under reduced pressure, which on cooling gave yellow crystalline precipitate. These were recrystallized twice in alcohol.

Preparation of the Complexes

Microwave method

The reations of trimethyltin(IV) chloride, triphenyltin(IV) chloride and tri-*n*-butyltin(IV) chloride with corresponding sodium salts of the ligands (L^1H and L^2H) were carried out in equimolar ratio, using 2–3 ml of methanol as a solvent in the microwave oven for 5–7 min. The products recovered from the microwave oven were dissolved in few millilitres of dry methanol. The white precipitate of NaCl formed during the course of the reaction was recovered by filtration and the filtrate was dried under reduced pressure. The resulting products were repeatedly washed with *n*-hexane and petroleum ether and finally dried at 60 °C/0.5 mmHg for 3–4 h (Fig. 3).

Thermal method

In the thermal method instead of 5–7 min reactions were completed in 11–13 h and the reaction mixture was refluxed over a distillation assembly fitted with quick fit interchangeable joints. The white precipitate of sodium chloride obtained was removed by filtration. The mother liquor was concentrated by removing the excess of solvent. Compounds were dried under reduced pressure for 3–4 h. These were purified by the same process as described in the above method. The purity was further checked by thin-layer chromatography using silica gel-G.

Table 7. Fungicidal screening data of the ligands and their corresponding complexes

	Average percentage inhibition after 96 h			
	Rhizopus oryzae		*Aspergillus flavus*	
Compound	Concentration (0.01%)	Concentration (0.1%)	Concentration (0.01%)	Concentration (0.1%)
L^1H	34	45	40	51
Me_3SnCl	40	48	44	54
$Me_3Sn(L^1)$	54	63	60	72
$Ph_3Sn(L^1)$	58	70	66	80
n-$Bu_3Sn(L^1)$	63	76	72	84
L^2H	38	50	44	56
$Me_3Sn(L^2)$	60	71	66	78
$Ph_3Sn(L^2)$	64	75	70	85
n-$Bu_3Sn(L^2)$	68	85	78	90
Bavistin	82	96	84	98

Table 8. Antibactericidal bioassay results for the ligands and their corresponding organotin(IV) complexes (concentration, ppm)

	Diameter of inhibition zone (mm) after 24 h							
	Escherichia coli (−)		*Pseudomonas aeruginosa* (−)		*Klebsiella pneumoniae* (−)		*Staphylococcus aureus* (+)	
Compound	500	1000	500	1000	500	1000	500	1000
L^1H	8	10	8	11	10	13	12	13
Me_3SnCl	10	12	11	14	12	14	14	15
$Me_3Sn(L^1)$	13	16	16	19	15	19	16	20
$Ph_3Sn(L^1)$	15	17	14	17	17	23	19	24
n-$Bu_3Sn(L^1)$	16	19	30	40	19	25	21	26
L^2H	9	12	9	12	12	15	13	15
$Me_3Sn(L^2)$	15	18	15	16	17	21	17	21
$Ph_3Sn(L^2)$	18	20	16	19	19	25	20	26
n-$Bu_3Sn(L^2)$	19	23	18	21	20	27	21	28
Imipinem	28	32	30	40	30	38	34	46

Elemental Analysis and Electronic Spectra

Satisfactory results of elemental analysis and spectral studies reveal that the complexes are of good purity. The complexes obtained were yellow to green in colour, showed a range of melting points and were powdery. The complexes were soluble in chloroform and some of them also in dimethylsulfoxide as well as in methanol, acetone and dimethylformamide. The analytical data for all the complexes are presented in Table 2.

The electronic spectra of the organic moieties exhibit three intense maxima at 235–240, 272–277 and 330–355 nm. The bands in the regions 235–240 nm and 272–277 nm are assignable to $\pi-\pi^*$ transitions of C═O/C═S and azomethine groups. The considerable hypsochromic shifting of the third band ($n-\pi^*$) in the spectra of the metal complexes may be attributed to the coordination of the azomethine nitrogen to the metal atom. The resulting complexes are non-electrolyte in nature, as indicated by their molar conductance in the range 10–15 $ohm^{-1}\,cm^2\,mol^{-1}$ in 10^{-3} M solution in dry DMF.

IR Spectra

The IR spectra of the complexes were compared with the ligands and from the shifts in frequency and/or from the intensity lowering, the coordination sites were ascertained. The IR spectrum of the ligand (L^2H) shows a band at 2700 cm^{-1} assignable to ν (-SH), which disappears in the corresponding complexes, indicating possible deprotonation on complexation. Ligands L^1H and L^2H show sharp and strong bands at 1608 and 1612 cm^{-1} respectively due to (> C═N),[49] which are shifted to the higher frequency side by 14–30 cm^{-1} in the spectra of the corresponding metal complexes, indicating the coordination of the azomethine nitrogen to the tin atom. In the case of L^1H the bands due to ν (NH/OH) mode in the region 3300–3128 cm^{-1} disappear in the organotin(IV) complexes, thereby indicating complexation through the azomethine nitrogen and ketonic oxygen atoms. The bands at 1686 and 1024 cm^{-1}, due to ν (>C═O) and ν (>C═S) are shifted to lower frequencies in the complexes compared with the ligands. The band observed in the region 3340–3431 cm^{-1} attributed to asymmetric and symmetric modes of $-NH_2$ group remains at nearly the same position in the spectra of the complexes, suggesting non-involvement of this amino group in bonding. This view is corroborated by the appearance of new bands in the regions 574–580, 358–364, 416–430 and 502–565 cm^{-1} ascribable to the (Sn–O),[50] (Sn–S),[51] (Sn ← N),[52] $(Sn-C)_{asym}$ and $(Sn-C)_{sym}$ [53] vibrations respectively (Table 3).

^{1}H NMR Spectra

The proton magnetic resonance spectra of the ligands as well as their corresponding metal complexes were recorded in DMSO-d_6 (Tables 4 and 5). The spectra of the ligands (L^1H and L^2H) show signals at δ 10.00 and δ 10.32 ppm, respectively, which are due to the NH proton. These NH proton signals disappear in the complexes, indicating the bond formation through an oxygen/sulfur atom after enolization/thioenolization of the ligand molecules and coordination of the azomethine nitrogen to the tin atom. The azomethine proton undergoes deshielding in the organotin(IV) complexes due to the donation of a lone pair of electrons by nitrogen to the tin atom. The NH of the ring appears at δ 10.46 ppm in case of the ligands (L^1H) and δ 10.60 ppm in the case of (L^2H), which remains unchanged in the tin complexes, showing non-involvement of this group in complexation. The

Table 9. Changes in the body weight and weights of reproductive organs after treatment with the ligands and their organotin(IV) complexes (values are expressed as mean ± SEM)

		Body weight (g)		Organ weight (mg/100 g body weight)			
Group	Treatment	Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate
A	Control	210 ± 12	225 ± 20	1325 ± 40	480 ± 40	445 ± 20	310 ± 20
B	L^1H	206 ± 14[b]	214 ± 17[b]	910 ± 30[b]	405 ± 40[b]	340 ± 13[b]	250 ± 12
C	$Ph_3Sn(L^1)$	190 ± 15[a]	212 ± 12[b]	700 ± 20[a]	285 ± 19[b]	210 ± 18[a]	170 ± 14
D	$n\text{-}Bu_3Sn(L^1)$	188 ± 12[a]	208 ± 15[b]	688 ± 20[a]	260 ± 16[a]	195 ± 16[a]	145 ± 15
E	L^2H	192 ± 15[b]	202 ± 13[b]	850 ± 50[b]	380 ± 18[b]	265 ± 14[b]	210 ± 16
F	$Ph_3Sn(L^2)$	182 ± 13[b]	200 ± 11[b]	695 ± 60[b]	274 ± 10[a]	200 ± 15[b]	165 ± 13
G	$n\text{-}Bu_3Sn(L^2)$	180 ± 13[a]	180 ± 15[a]	669 ± 40[b]	252 ± 14[a]	170 ± 12[a]	136 ± 16

All figures ± SEM.
[a] $p \leq 0.001$ highly significant; [b] $p \leq 0.01$ significant; [c]non-significant.
Groups B and E compared with Group A. Groups C and D compared with Group B. Groups F and G compared with Group E.

Table 10. Effect of ligands and organotin(IV) complexes on sperm dynamics and fertility of male rats (values are expressed as mean ± SEM)

			Sperm density (million ml^{-1})		
Group	Treatment	Sperm motality (cauda epididymis) (%)	Testes	Cauda epididymis	Fertility (%)
A	Control	85.86 ± 3.9	6.15 ± 0.52	64.29 ± 2.9	98 (+)
B	L^1H	58.90[b] ± 2.4	4.31[b] ± 0.23	53.16[b] ± 2.6	46 (−)
C	Me_3SnCl	46.64[c] ± 1.6	2.95[c] ± 0.35	42.14[c] ± 2.8	58 (−)
D	$Me_3Sn(L^1)$	35.16[b] ± 1.7	1.98[b] ± 0.26	30.16[b] ± 2.6	86 (−)
E	$Ph_3Sn(L^1)$	34.46[a] ± 1.4	1. 46[a] ± 0.82	29.72[a] ± 2.4	90 (−)
F	$n\text{-}Bu_3Sn(L^1)$	33.32[a] ± 2.2	1. 12[a] ± 0.24	28.38[a] ± 2.1	92 (−)
G	L^2H	46.76[b] ± 2.5	4.23[b] ± 0.41	50.28[b] ± 3.6	50 (−)
H	$Me_3Sn(L^2)$	34.12[a] ± 2.4	1.24[a] ± 0.24	28.99[a] ± 3.2	91 (−)
I	$Ph_3Sn(L^2)$	33.56[a] ± 2.8	1. 02[a] ± 0.82	27.68[a] ± 2.7	92 (−)
J	$n\text{-}Bu_3Sn(L^2)$	32.26[a] ± 2.6	0. 78[a] ± 0.64	26.12[a] ± 2.5	94 (−)

Means ± SEM of 10 animals.
[a] $p \leq 0.001$ highly significant; [b] $p \leq 0.01$ significant; [c] non-significant.
Dose = 2 mg kg^{-1} per day for 60 days.

appearance of a signal due to NH_2 (δ 2.74–3.32 ppm) at almost the same position in the ligands and their corresponding tin complexes shows its non-involvement in complex formation. The aromatic proton signals appeared in the range δ 6.88–8.86 ppm in the spectra of the ligands and complexes. Some additional signals were due to CH_3Sn and n-C_4H_9-Sn in the ranges δ 1.22–1.18 and δ 0.60–1.72 ppm respectively.

^{13}C NMR Spectra

^{13}C NMR spectral data (Table 6) also support the authenticity of the proposed structures. The considerable shifts in the positions of carbon atoms adjacent to the azomethine nitrogen (157.72–162.91 ppm) and thiolic sulfur/enolic oxygen (δ 168.60–175.82 ppm) support the penta-coordinated geometry of the complexes. The shifts in the positions of carbon atoms adjacent to the coordinating atoms clearly indicate the bonding of azomethine nitrogen to the central metal atom. The peaks were assigned by comparing standard data and data reported for the similar complexes.[54,55]

^{119}Sn NMR Spectra

^{119}Sn NMR spectra of all compounds were recorded and exhibit a sharp ^{119}Sn resonance in the region δ −178.6 to −248.4 ppm, which is compatible with a penta-coordinated geometry.[56]

Antimicrobial Screening

Antifungal activity

It is clear from the biological screening data given in Table 7 that all of the triorganotin(IV) complexes were more toxic than their parent ligands but slightly lower than the standard drug. The increase in the activity of organotin(IV) complexes may be due to the effect of central metal ion in the normal cell process. The antimicrobial activity of these ligands and their complexes can be ascribed to the hydrogen bond formation between the (→C═N) atom of the compound and some bioreceptors in the cells of microorganisms, which in turn block the synthesis of protein by inhibiting the movement of ribosome along with RNA. This inhibits the synthesis of DNA in the cell nucleus. The greater toxicity of the complexes than the bases can also be explained by the greater lipophilic character of the complexes.[57] It has also been observed

Table 11. Biochemical changes in the tissues of male rats after treatment with the ligands and their organotin(IV) complexes (values are expressed as means ± SEM)

Group	Treatment	Sialic acid (mg g^{-1})			Protein (mg g^{-1})			Testicular glycogen (mg g^{-1})	Testicular cholesterol (mg g^{-1})
		Testes	Epididymis	Seminal vesicle	Ventral prostate	Testes	Epididymis	Seminal vesicle	Ventral prostate		
A	Control	8.12 ± 0.54	9.05 ± 0.20	8.72 ± 0.22	8.65 ± 0.16	259.8 ± 7.82	228.0 ± 6.50	217.0 ± 6.20	210.0 ± 4.46	6.50 ± 0.40	8.40 ± 0.68
B	L^1H	7.64^b ± 0.12	8.9^b ± 0.14	7.60^a ± 0.18	7.94^b ± 0.26	200.4^b ± 5.34	195.0^b ± 6.50	187.0^b ± 5.60	176.0^b ± 3.12	6.08^b ± 0.42	8.24 ± 0.22
C	$Me_3Sn(L^1)$	6.22^b ± 0.12	6.20 ± 0.20	6.12^b ± 0.18	6.26^b ± 0.10	134.0^b ± 2.24	143.0^b ± 2.10	140.0 ± 4.60	126.0^b ± 2.64	5.38^b ± 0.24	12.50 ± 0.45
D	$Ph_3Sn(L^1)$	6.10^b ± 0.16	6.00^b ± 0.12	5.82^b ± 0.12	5.94^b ± 0.10	132.9^b ± 4.22	141.0^b ± 3.30	139.0^a ± 2.24	125.0^a ± 3.51	5.22^b ± 0.20	13.64 ± 0.86
E	n-$Bu_3Sn(L^1)$	5.42^a ± 0.10	5.64^b ± 0.14	5.16^a ± 0.14	5.36^b ± 0.14	130.6^a ± 2.44	138.0^a ± 4.20	137.0^a ± 3.24	123.0 ± 3.44	5.08^b ± 0.40	13.80 ± 1.18
F	L^2H	7.30^b ± 0.14	8.6^b ± 0.10	7.60^b ± 0.18	7.80^a ± 0.68	198.5^b ± 4.65	192.0^b ± 7.70	183.0^b ± 7.45	171.0^b ± 4.52	5.94^b ± 0.28	9.96 ± 0.64
H	$Me_3Sn(L^2)$	6.12^b ± 0.18	6.16^b ± 0.14	5.95^a ± 0.18	5.96^b ± 0.12	132.6^b ± 2.14	140.0^b ± 4.10	136.0 ± 3.40	124.0 ± 4.60	5.24^b ± 0.20	13.80 ± 0.36
I	$Ph_3Sn(L^2)$	5.94^a ± 0.14	5.82^a ± 0.10	5.46^a ± 0.17	5.58^a ± 0.14	130.0^a ± 3.82	138.0^a ± 4.20	135.0^b ± 4.16	122.0^a ± 2.30	5.19^b ± 0.21	14.10 ± 1.28
J	n-$Bu_3Sn(L^2)$	5.28^a ± 0.12	5.40^a ± 0.16	5.08^a ± 0.16	5.18^a ± 0.10	129.0^a ± 3.26	136.0^b ± 5.10	134.0^a ± 5.12	120.0 ± 4.60	4.95^a ± 0.26	14.60 ± 0.98

Groups B and F compared with Group A. Groups C, D and E compared with Group B. Groups H, I and J compared with Group F.
$^a p \leq 0.001$ highly significant; $^b p \leq 0.01$ significant; ^{c}non-significant.

that the toxicity of the ligands and their complexes decreases on lowering the concentration. From the data obtained it is evident that some of these complexes exhibit a good activity against the tested organisms but specially significant is the complex [(n-C_4H_9-)$_3$Sn(L^2)], which showed the highest activity among the complexes possibly due to the difference in sulfur content and highest lipophilic nature of tri-n-butyltin(IV) complex.[58]

Antibacterial Activity

The antibacterial activity results presented in Table 8 show clearly that the newly synthesized ligands and their metal complexes containing organotin (IV) possess good biological activity. Eight chemically synthesized compounds viz. L^1H, L^2H, [Me_3SnL^1], [Me_3SnL^2], [Ph_3SnL^1], [Ph_3SnL^2], [n-Bu_3SnL^1] and [n-Bu_3SnL^2] were tested *in vitro* for their antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at two different concentrations in methanol. All the synthesized compounds show higher activity than the ligands but slightly lower than the standard drug. Among all the compounds the most toxic complex is [n-Bu_3SnL^2] since it was found that the toxicity of the organotins varied significantly according to their substition.[59] The order of toxicity is tri $\gg$ di $\geq$ mono-organotins. Within the same substituent series the toxicity depends on the properties of R group. The larger and the more lipophilic the R group, the more toxic is the organotin.[60] From the bactericidal activity, it is apparent that the complexes were more toxic towards Gram-positive strains than Gram-negative strains. The reason is the difference in the structures of the cell walls. The walls of Gram-negative cells are more complex than those of Gram-positive cells. Lipopolysacharides form an outer lipid membrane and contribute to the complex antigenic specificity of Gram-negative cells. The enhanced bactericidal activity of the ligand on complexation with organotin (IV) halide may be explained by chelation theory,[61] according to which chelation reduces the polarity of the central metal atom because of partial sharing of its positive charge with the donor groups and possible π-electron delocalization within the whole chelate ring. This chelation increases the lipophilic nature of the central atom, which favours the permeation of the complexes through the lipid layer of the cell membrane. Compounds inhibit the growth of bacteria to greater extent as concentration increased. Also, the complexes of thiosemicarbazone were found to possess higher activity than corresponding semicarbazone. The compounds showed good antibacterial activity against *S. aureus* and moderate activity against *K. pneumoniae* and poor activity against *E*. coli and *P. aeruginosa*.

Antifertility Activity

Male rats exposed to ligands L^1H and L^2H and their corresponding triorganotin(IV) complexes (2 mg kg^{-1} body weight per day) for 60 days showed following alternation in reproductive function of male rats.

Body weight, fertility and sperm dynamics

During the period of experiment the rats remained healthy, growing at normal growth rate. Their body weights gain was similar to that of control animals (Table 9). Treatment of different compounds at 2 mg kg^{-1} per day for 60 days caused a 94%

Table 12. Effects on whole blood and blood serum of rats after treatment with the ligands and their organotin(IV) complexes (values are expressed as mean ± SEM)

Group	Treatment	RBC count (million mm^{-3})	WBC count (million mm^{-3})	Haemoglobin (g%)	Haematocrit (%)	Blood sugar (mg dl^{-1})	Blood urea (mg dl^{-1})	Serum cholesterol (mg dl^{-1})	Serum phospholipids (l)	Serum protein (mg dl^{-1})
A	Control	4.8 ± 0.4	8652 ± 78	13.9 ± 0.6	44.6 ± 4.4	86.4 ± 1.8	35.8 ± 2.8	90 ± 4	87 ± 9	13640 ± 682
B	L^1H	5.7 ± 0.2^a	8538 ± 81^a	12.2 ± 0.4^a	42.1 ± 4.2^a	76.9 ± 6.4^a	42.0 ± 3.9^a	82 ± 5	82 ± 4	13100 ± 546
C	$Ph_3Sn(L^1)$	5.3 ± 0.8^a	8524 ± 100^a	13.6 ± 0.2^a	43.9 ± 0.8^a	75.4 ± 8.4^a	40.6 ± 3.0^a	70 ± 8^A	56 ± 6^a	11758 ± 685
D	$n\text{-}Bu_3Sn(L^1)$	5.2 ± 0.4^a	8512 ± 96^a	13.8 ± 0.4^a	44.8 ± 0.6^a	75.0 ± 9.2^a	40.2 ± 3.6^a	68 ± 9^a	55 ± 2^a	11682 ± 487
E	L^2H	5.4 ± 0.6^a	8530 ± 75^a	12.9 ± 0.5^a	42.9 ± 5.6^a	76.1 ± 2.9^a	41.8 ± 3.2^a	81 ± 9	81 ± 1	13094 ± 756
F	$Ph_3Sn(L^2)$	4.9 ± 0.2^a	8498 ± 120^a	14.0 ± 0.2^a	44.6 ± 0.6^a	74.9 ± 9.2^a	40.2 ± 2.8^a	68 ± 4^a	54 ± 9^c	11600 ± 816
G	$n\text{-}Bu_3Sn(L^2)$	4.7 ± 0.8^a	8450 ± 140^a	14.5 ± 0.8^a	45.4 ± 1.8^a	74.5 ± 3.4^a	39.8 ± 2.6^a	67 ± 8^a	53 ± 2^c	11472 ± 917

$^a p > 0.05$; $^c p < 0.01$ vs control.

reduction in the fertility (Table 10). Sperm motility was decreased by 58.90–32.26%. Significant reduction ($p < 0.01$) was observed in sperm density after all the treatments (Table 10).

Biochemical findings

Protien and sialic acid contents of testes, epididymis, seminal vesicle and ventral prostate were reduced significantly after in all experimental groups when compared with control. Testicular glycogen contents were decreased while the testicular cholesterol increased significantly in all the experimental groups when compared with control (Table 11).

Blood and serum analysis

Blood variables, i.e. RBC and WBC counts, haemoglobin, haematocrit, sugar and urea were within the normal range. Cholesterol and protein did not changed significantly in any of the treatment but phospholipids were decreased only in the [Ph_3SnL^2] and [$n\text{-}Bu_3SnL^2$] (Table 12).

Conclusion

The antibacterial activity maximizes for the tri-*n*-butyltin(IV) complex in line with other studies in this area,[62] although the activity of all the organotin complexes is reasonable. The antifungal properties of all the compounds are broadly excellent, and far exceed the levels for the ligand. The influence of the metal is most clearly visible in this area. The weights of the testes, epididymis, seminal vesicle and ventral prostate were significantly decreased in the treated male rats when compared with the control group. The principal cells of epididymis synthesize proteins which have important role for maturation of spermatozoa,[63] and alternation in secretion and function of these proteins causes incomplete maturation of spermatozoa with a decline in sperm motility. In the present study the epididymal protein was also decreased by organotin(IV) complex feeding. Reduced glycogen reflects a decreased number of post-meiotic germ cells, which are thought to be the sites of glucose metabolism.[64] Cholesterol is involved in steroidogenesis in testes. It is the most important precursor in the synthesis of steroid hormones and its level is related to the fertility of individuals.[65] Increased level of cholesterol may be due to decreased androgen production, which resulted in accumulation of cholesterol in testes, and hence impaired spermatogenesis.[66]

It has been observed that the blood and serum parameters were within a normal range, indicating the non-toxicity of the compounds on general body metabolism. Our results reflect the antispermatogenic effects of organotin(IV) complexes in male rats, without affecting general body metabolism. In conclusion our results confirm that the long-term ingestion of the compounds produces adverse effects on fertility and reproductive system in adult male rats.

Acknowledgement

The authors are grateful to UGC, New Delhi, India for financial assistance through grant F. no. 34-324/2008 (S.R.).

References

[1] K. P. Srivastava, *Res. J. Chem. Environ.* **2001**, *5*, 77.
[2] K. P. Srivastava, *Chem.:Ind. J.* **2003**, *1*, 215.
[3] T. N. Michell, Organotin reagents in cross-coupling. In *Metal Catalysis Cross-coupling Reactions* (Eds.: F. Diederich, P. J. Stang). Wiley-VCH: Weinheim, **1998**, p. 157.
[4] D. Marton, R. Russo, D. Stivanello, G. Tagliavini, *Organometallics* **1996**, *15*, 1645.
[5] L. Labib, J. E. Khali, M. F. Iskander, L. S. Refaat, *Polyhedron* **1996**, *15*, 349.
[6] D. K. Dey, M. K. Saha, M. K. Das, N. Bhartiya, R. K. Bansal, *Polyhedron* **1999**, *18*, 2687.
[7] M. Nath, R. Yadav, *Bull. Chem. Soc. Jpn.* **1997**, *70*, 1331.
[8] R. Singh, N. K. Kaushik, *Spectrochim. Acta A Mol. Biomol. Spectrosc. (available online)* **2008**, *71*, 669.
[9] C. J. Evans, *Industrial Uses of Tin Chemicals, In Chemsitry of Tin*, 2nd edn (Ed.: P. J. Smith). Blackie Academic & Professional: Glasgow **1998**, pp. 388–428.
[10] J. J. Chicano, A. Ortis, J. A. Teruel, F. Aranda, *J Biochem. Biophys. Acta* **2001**, *1510*, 330.
[11] J. Kuczera, J. Gabrielska, T. E. Kral, S. Przstalski, *Appl. Organomet. Chem.* **1997**, *11*, 591.
[12] Y. Arakawa, Biological actions of di- and trialkyltin compounds. In *Chemistry of Tin*, 2nd edn (Ed.: P. J. Smith). Blackie Academic & Professional: Glasgow **1998**, p. 388.
[13] P. J. Smith, Health and safety aspects of tin chemicals. In *Chemistry of Tin*, 2nd edn (Ed.: P. J. Smith). Blackie Academic & Professional: Glasgow **1998**, p. 429.
[14] M. Das, S. E. Livingstone, *Inorg. Chim. Acta* **1976**, *19*, 5.
[15] F. D. Popp, W. Krisch, *J. Org. Chem.* **1961**, *26*, 1566.
[16] C. Shipman Jr., S. H. Smith, J. C. Drach, D. L. Klayman, *Antimicrob. Agents Chemother.* **1981**, *19*, 682.
[17] A. Saxena, J. P. Tandon, *J. Antifung. Antibact. Agents Japan* **1981**, *9*, 413.
[18] M. Jain, K. Poonia, M. Agarwal, R. V. Singh, *Main Group Met. Chem.* **2006**, *29*, 127.

[19] R. V. Singh, P. Chaudhary, K. Poonia, S. Chauhan, *Spectrochim. Acta, Inorg. Biochem.* **2008**, *33*, 377.
[20] P. J. Smith, *Toxicological Data on Organotin Compounds*. International Tin Research Institute: London, **1978**.
[21] H. L. Singh, S. Varshney, A. K. Varshney, *Appl. Organomet. Chem.* **2000**, *14*, 212.
[22] S. G. Teoh, *J. Organomet. Chem.* **1999**, *580*, 1.
[23] S. Nicklin, M. W. Robson, *Appl. Organomet. Chem.* **1988**, *2*, 487.
[24] P. P. Singh, K. K. Sharma, *Indian J. Chem.* **1993**, *32B*, 551.
[25] R. Singh, N. K. Kaushik, *Main Group Met.Chem.* **2008**, *31*(1), 333.
[26] R. Singh, N. K. Kaushik, *Spectrochim. Acta A, Mol. Biomol. Spectrosc.* **2006**, *65*, 950.
[27] R. Singh, N. K. Kaushik, *Main Group Met. Chem.* **2004**, *27*(6), 327.
[28] S. Paul, P. Nanda, R. Gupta, *Molecules* **2003**, *8*, 374.
[29] S. H. L. Thoonen, B. J. Deelman, G. Koten, *J. Organomet. Chem.* **2004**, *689*, 2145.
[30] W. L. F. Armarego, D. D. Perrin, *Purification of Laboratory Chemicals*, 4th edn. Butterworth-Heinemann: Oxford.
[31] B. S. Furniss, A. J. Hannaford, P. W. G. Smith, A. R. Tatchell, *Vogel's Text Book of Practical Organic Chemistry*, 5th edn. Longman: Harlow, **1989**.
[32] J. Basset, R. C. Denney, G. H. Jeffery, J. Mendham, *Vogel's Text Book of Qualitative Inorganic Analysis*, 4th edn. Longman: Harlow, **1978**.
[33] A. I. Vogel, *A Text Book of Quantitative Inorganic Analysis*. Longman: London **1962**.
[34] S. P. Mittal, R. V. Singh, J. P. Tandon, *Indian J. Chem.* **1981**, *20A*, 199.
[35] J. Volhard, *J. Prakt. Chem.* **1874**, *9*, 217; Cf. *Ann. der Chem.* **1878**, *190*, 1.
[36] K. Rast, *Ber. Chem. Ges., Iv B* **1922**, *1051*, 3727.
[37] J. G. Horsfall, *Bot. Rev.* **1945**, *11*, 419.
[38] H. H. Thornberry, *Phytopathology* **1950**, *40*, 419.
[39] M. R. N. Prasad, N. J. Chinoy, K. M. Kadama, *Fertil. Steril.* **1972**, *23*, 186.
[40] B. L. Oser, *Hawk's Physiological Chemistry*, 14th edn. McGraw Hill: New York, **1965**, p. 246.
[41] R. Montgomery *Arch Biochem Biophys.* **1957**, *67*, 378.
[42] O. H. Lowry, D. J. Rosenburg, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **1951**, *193*, 265.
[43] W. H. Crossby, J. I. Munn, F. W. Furth, *US Armed Force Med. J.* **1954**, *5*, 695.
[44] A. Astoor, E. J. King, *Meth. Biochm. J* **1954**, *56*, XLIV.
[45] H. Varley, *Practical Biochemistry*, 4th edn. White Herrers Press: London, **1969**, p. 158.
[46] A. Zlatkis, B. Zak, A. J. Boyle, *J. Lab Clin. Med.* **1953**, *41*, 486.
[47] D. B. Zilversmit, A. K. Davis, M. T. Hamphestern, *J. Lab. Clin. Invest* **1950**, *35*, 155.
[48] K. D. Raner, C. R. Strauss, F. Vyskoc, M. Mokbel, *J. Org. Chem.* **1993**, *58*, 950.
[49] K. C. Satpathy, B. B. Jal, R. Mishra, *Indian. J. Chem.* **1985**, *24A*, 147.
[50] M. S. Singh, M. D. Raju, K. Tawade, A. K. Singh, *Main Group Met. Chem* **1998**, *21*, 489.
[51] D. K. Dey, M. K. Saha, M. Gielen, M. Kemmer, M. Biesmans, R. Willem, *J. Organomet. Chem.* **1999**, *590*, 88.
[52] M. S. Singh, M. D. Raju, A. K. Singh, P. Narayan, *Synth. React. Inorg. Met.-Org. Chem.* **1999**, *29*, 73.
[53] C. Ma, J. Zhang, R. Zhang, *Polyhedron* **2004**, *23*, 1981.
[54] R. K. Dubey, S. Pathak, *Main Group Met. Chem.* **2008**, *31*, 29.
[55] R. M. Silverstein, F. X. Webster, *Spectrometric Identification of Organic Compounds*, 6th edn. Wiley: Chichester, **2005**.
[56] J. Wang, *Prog. Nat. Sci.* **1998**, *8*, 180.
[57] R. Mash, *Ann. Appl. Biol.* **1954**, *41*, 652.
[58] A. Kalsoom, M. Mazhar, S. Ali, M. F. Mahon, K. C. Molloy, M. I. Chaudry, *Appl. Organomet. Chem.* **1997**, *11*, 47.
[59] G. E. Walsh, L. L. Mclaughlan, E. M. Lores, M. K. Louie, C. H. Deans, *Chemosphere* **1985**, *14*, 383.
[60] G. Huang, S. Dai, H. Sun, *Appl. Organomet. Chem.* **1996**, *10*, 377.
[61] R. S. Srivastava, *Inorg. Chim. Acta.* **1981**, *56*, 65.
[62] B. Wrackmeyer, *Annu Rep NMR Spectrosc.* **1985**, *16*, 73.
[63] M. Ksturi, B. Manivannan, R. N. Ahmad, P. D. Shaikh, K. M. Pathan, *Ind J. Exp. Biol.* **1995**, *33*, 725.
[64] V. P. Dixit, S. C. Joshi, *Indian. J. Exp. Biol.* **1982**, *20*, 534.
[65] K. B. Eik-Nes, P. F. Hall, *Proc. Soc. Exp. Biol. Med.* **1962**, *III*, 280.
[66] R. S. Bedwal, M. S. Edwards, M. Katoch, A. Bahuguna, R. Dewan, *Indian. J. Exp. Biol.* **1994**, *32*, 243.

