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Antifertility and antimicrobial studies of pharmaceutically important organolead(IV) complexes of phenanthrolines

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The present study deals with a brief description of antifertility and antimicrobial aspects along with the spectral characterization of lead(IV) complexes. The testicular sperm density, testicular sperm morphology, sperm motility, density of cauda epididymal spermatozoa and fertility in mating trails and biochemical parameters of reproductive organs of an interesting class of biologically potent complexes on male albino rats at the dosages have been examined and discussed. Lead(IV) complexes have been synthesized using amino acids and 1,10-phenanthroline, 4,7-phenanthroline or 1,7-phenanthroline. A series of di- and tri-organolead(IV) [LPbR$_n$L′] and [LPbClR$_n$L′] complexes where, L = amino acid (tyrosine and phenylalanine) and L′ = 1, 10-phenanthroline, 4,7-phenanthroline or 1,7-phenanthroline and $n = 2$ or 3 have been prepared by the conventional methods. Structure elucidation has been done by IR, UV, ^{1}H, ^{13}C and ^{207}Pb NMR spectroscopy. On the basis of spectral evidences, it has been concluded that the carboxylic acid of the amino acid is behaving as a monodentate ligand in all these complexes and the complexes are octahedral in shape with a coordination number six around the lead atom. The complexes have been screened against a number of fungi and bacteria to assess their growth inhibiting potential. Lead complexes incorporating the chelating 1,10-phenanthroline ligand showed a range of activities. The metal free non-chelating ligands 1,7-phenanthroline and 4,7-phenanthroline were inactive and the complexes derived from 1,7-phenanthroline displayed only marginal activity. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: organolead(IV) complexes; NMR spectra; antimicrobial activity; antifertility activity

INTRODUCTION

Asymmetric synthesis has been a well-investigated area of organic chemistry.[1] Metal-based chiral complexes have invoked interest in many researchers,[2–4] primarily due to their use as catalysts,[5,6] and has led to a challenging new subarea of inorganic asymmetric synthesis. Synthetic routes to asymmetric complexes are still very important and require a new approach which includes the choice of chiral auxiliary.[7–9] There is an increased interest in the synthesis of the tin-based antitumour drugs, and the activity of these complexes is closely related to their structure.[10,11] The chiral complexes have wide applications in the field of medicine as antitumour and anti-HIV agents,[12] as catalysts[13,14] and also as enzyme model systems.[15] 1,10-Phenanthroline (1,10-Ph), 2,2′-bipyridine and their substitutes, both in the metal-free state and as ligands coordinated to transition metals, disturb the functioning of a wide variety of biological systems.[16] When the metal-free *N*,*N*′-chelating bases are found to be bioactive, it is usually assumed that the sequestration of trace metals is involved, and that the resulting metal complexes are the actual active species.[17,18]

The *in vitro* antibacterial action of 1, 10-Ph has been demonstrated on several species of bacteria, whereas, phenanthroline metal complexes can be bacteriostatic[19] and bacteriocidal[20] towards many Gram-positive bacteria. However, they are relatively ineffective against Gram-negative organisms. On the other hand, *m*-and *p*-substituted phenanthrolines are less effective than 1,10-Ph

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Table 1. Physical properties and analytical data of the complexes

Compound formed	Reactants (g, mmol)			Colour and m.p.(°C)	Analysis found (calcd), %					Mol. wt, found (calcd)
					C	H	N	Cl	Pb	
(**1**) [Ph_3Pb(1,10-Ph)(Tyrosine)]	Tyrosine 0.45, 2.47	1,10-Phenanthroline 0.45, 2.47	Ph_3PbCl 1.17, 2.47	Yellow 211	77.9 (78.1)	4.1 (4.3)	4.2 (5.3)	—	25.4 (25.9)	776 (799)
(**2**) [Ph_3Pb(4,7-Ph)(Tyrosine)]	Tyrosine 0.46, 2.52	4,7-Phenanthroline 0.45, 2.52	Ph_3PbCl 1.20, 2.52	Yellow 195	77.8 (78.1)	4.2 (4.3)	3.8 (5.3)	—	25.3 (25.9)	773 (799)
(**3**) [Ph_3Pb(1,7 -Ph)(Tyrosine)]	Tyrosine 0.46, 2.52	1,7-Phenanthroline 0.45, 2.52	Ph_3PbCl 1.20, 2.52	Light yellow 189	78.0 (78.1)	4.1 (4.3)	4.3 (5.3)	—	25.4 (25.9)	770 (799)
(**4**) [Ph_3Pb(1,10-Ph)(Phenylalanine)]	Phenylalanine 0.45, 2.47	1,10-Phenanthroline 0.45, 2.47	Ph_3PbCl 1.17, 2.47	Yellow 217[a]	59.8 (59.9)	4.3 (4.4)	4.4 (5.4)	—	26.0 (26.5)	767 (782)
(**5**) [Ph_3Pb(4,7 -Ph) (Phenylalanine)]	Phenylalanine 0.46, 2.52	4,7-Phenanthroline 0.45, 2.52	Ph_3PbCl 1.20, 2.52	Yellow 224[a]	59.7 (59.9)	4.27 (4.4)	4.5 (5.4)	—	26.1 (26.5)	765 (782)
(**6**) [Ph_3Pb(1,7 -Ph)(Phenylalanine)]	Phenylalanine 0.45, 2.46	1,7-Phenanthroline 0.45, 2.46	Ph_3PbCl 1.17, 2.46	Light yellow 203	59.8 (59.9)	4.3 (4.4)	4.5 (5.4)	—	26.0 (26.5)	757 (782)
(**7**) [Me_2Pb(1,10-Ph)(Tyrosine)Cl]	Tyrosine 0.44, 2.41	1,10-Phenanthroline 0.44, 2.41	Me_2PbCl_2 0.74, 2.41	Light yellow 191	57.7 (58.0)	3.9 (4.1)	5.3 (6.6)	5.1 (5.6)	32.1 (32.6)	606 (635)
(**8**) [Bu^t_2Pb(1,10-Ph)(Tyrosine)Cl]	Tyrosine 0.44, 2.41	1,10-Phenanthroline 0.44, 2.41	$Bu^t_2PbCl_2$ 0.95, 2.41	Light yellow 184	64.3 (64.6)	5.1 (5.3)	4.4 (5.9)	4.5 (4.9)	28.3 (28.9)	692 (718)

[a] Decomposition temperature.

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at preventing fungal growth, and 2,9-dimethyl-1,10-phenanthroline (dmphen) was the most potent inhibitor.[18] Therefore, these findings have prompted us to synthesize such types of compounds with an aim of characterizing them structurally and biologically and to find out which part of the molecule is actually responsible for its physicological activity.

Fertility is an important issue of global concern. At the beginning of the twentieth century, the rate of increase in population was about 10 million per year. It is now increasing at much faster rate of 100 million per year. The rapid increase of population has an adverse effect on national economies. For the last few decades scientific research has been conducted into population control. The dramatic success of oral contraceptives in women and the lack of a pill for men have stimulated research into male fertility control. In other ways many contraceptive methods have been developed for males, but they involve many disadvantages, both mechanical (intra vas devices, use of condoms) and surgical (vasectomy). The male, an integral part of the family unit, has largely been ignored by family planners and the development of a new and improved contraception agent for men has lagged behind the development of female contraceptives. Currently, efforts are being made to develop a male contraceptive agent, which would inhibit fertility without affecting sexual function and libido. Therefore, this approach may form the basis for clinical regulation of male fertility in the future. Inorganic compounds have also been investigated and applied for antifertility activity only and have not been screened for toxicological effect. Therefore, in the present investigation an effort has been made to develop a male contraceptive using different doses of the ligand and its lead (IV) complex orally, tested on the reproductive organs of male albino rats.

EXPERIMENTAL

Glass apparatus with standard quick fit joints was used during the experiment. The chemicals and solvents used were dried and purified by the standard methods.[21]

Synthesis of the complexes

To a solution of tyrosine (0.45 g; 2.47 mmol) in dry methanol (15 ml) was added a solution of 1,10-phenanthroline (0.44 g; 2.44 mmol) in the same solvent. The resultant mixture was refluxed for 20 h after adding a solution of triphenylleadchloride (1.17 g; 2.46 mmol) in hot methanol. The mixture was allowed to stand overnight in refrigerator. The solid product obtained was isolated by filtration, washed with ether and dried *in vacuo*. Similarly, other complexes were synthesized by the reactions of 1,10-phenanthroline, 4,7-phenanthroline or 1,7-phenanthroline with tyrosine or phenylalanine in 1 : 1 : 1 molar ratio. Safety precautions were employed using the standard safety methods.[22]

Analaytical methods and physical measurements

The molecular weights were determined by the Rast Camphor method. Conductivity measurements in dry dimethylformamide were performed with a conductivity bridge type 305 (Systronics). Infrared spectra were recorded on a Nicolet Magna FT-IR 550 spectrophotometer in KBr pellets. The far infrared spectra of the complexes were recorded on the same spectrophotometer in Nujol Mulls using CsI cell. ^{1}H NMR spectra were recorded on a Jeol FX 90Q spectrometer. ^{13}C NMR spectra were recorded on the said instrument using TMS as the internal standard at 22.49 MHz using DMSO-d_6 as the solvent. The electronic spectra were recorded on a Perkin Elmer UV–vis spectrophotometer in the range 200–600 nm, using dry methanol as the solvent. The ^{207}Pb spectra were recorded in dry methanol using $PbMe_4$ as the internal standard. The physical properties and analytical data of the metal complexes are listed in Table 1.

Antimicrobial assay

Antifungal activity

The antifungal activity was evaluated against several fungi by the radial growth method.[23] The compounds were directly mixed with the medium in 25, 50, 100 and 200 ppm (methanol) concentrations. 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 4 days. The amount of growth inhibition in each of the replicates was calculated by the equation $(d_c - d_t) \times 100/d_c$,

Table 2. IR Spectral data (cm^{-1}) of the organolead complexes

Compound	ν(Pb–O)	ν(Pb–N)	ν(Pb–Cl)	Pb–Me/Pb–Ph	ν(Pb–C) Asymmetric	ν(Pb–C) Symmetric
[Ph_3Pb(1,10-Ph)(Tyrosine)]	515	380	366	259	575	527
[Ph_3Pb(4,7-Ph)(Tyrosine)]	544	387	340	280	590	530
[Ph_3Pb(1,7 -Ph)(Tyrosine)]	520	390	345	260	595	533
[Ph_3Pb(1,10-Ph)(Phenylalanine)]	541	392	345	265	576	532
[Ph_3Pb(4,7 -Ph) (Phenylalanine)]	555	383	356	278	587	531
[Ph_3Pb (1,7 -Ph)(Phenylalanine)]	536	381	351	275	590	530
[Me_2Pb (1,10-Ph)(Tyrosine)Cl]	558	390	363	1178	589	529
[Bu^t_2Pb(1,10-Ph)(Tyrosine)Cl]	530	395	349	—	586	538

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Table 3. ^{1}H NMR and ^{207}Pb NMR spectral data (δ, ppm) of the organolead compounds

Compound	Phenanthroline moiety	Tyrosine/ phenylalanine CH	CH_2	Pb–Ph/Pb–Me	^{207}PbNMR
[Ph_3Pb(1,10-Ph)(Tyrosine)]	7.41–9.10	6.33–7.19	3.02	7.71	−2210
[Ph_3Pb(4,7-Ph)(Tyrosine)]	7.53–9.15	6.60–6.90	3.05	7.75	−2218
[Ph_3Pb(1,7 -Ph)(Tyrosine)]	7.55–9.16	7.02–7.19	3.12	7.52	−2215
[Ph_3Pb(1,10-Ph)(Phenylalanine)]	8.50–9.10	6.63–6.94	3.18	7.54	−2230
[Ph_3Pb(4,7 -Ph)(Phenylalanine)]	7.48–9.05	7.02–7.12	3.18	7.00	−2227
[Ph_3Pb(1,7-Ph)(Phenylalanine)]	7.41–9.17	7.05–7.18	3.15	7.55	−2236
[Me_2Pb(1,10-Ph)(Tyrosine)Cl]	7.46–9.12	6.66–6.94	3.09	7.22	−2245
[Bu^t_2Pb(1,10-Ph)(Tyrosine)Cl]	7.45–9.10	6.62–6.98	3.10	—	−2241

Table 4. ^{13}C NMR spectral data (δ, ppm) of the compounds

Compound	Phenanthroline moiety	Tyrosine/ phenylalanine	CH	CH_2	C═O	Pb–Ph
[Ph_3Pb(1,10-Ph)(Tyrosine)]	C_1,142.50; C_2,129.01; C_3,148.42	C_{34},132.81; C_{35},129.30; C_{36},115.66; C_{37},154.21	63.22	39.01	177.30	136.60, 137,20, 139,51, 140.85
[Ph_3Pb(4,7-Ph)(Tyrosine)]	C_1,142.52; C_2,129.04; C_3,148.40	C_{34},132.92; C_{35},129.46; C_{36},115.83; C_{37},154.38	63.38	39.28	177.84	136.60, 137.24, 139.53, 140.82
[Ph_3Pb(1,7 -Ph)(Tyrosine)]	C_1,142.57; C_2,129.06; C_3,148.52	C_{34},132.40; C_{35},129.58; C_{36},115.68; C_{37},154.44	64.02	38.96	177.96	137.01, 137.52, 139.84, 141.06
[Ph_3Pb(1,10-Ph)(Phenylalanine)]	C_1,143.01; C_2,128.80; C_3,149.00	C_{34},132.20; C_{35},129.30; C_{36},115.76; C_{37},154.58	63.48	38.90	177.66	137.00, 137.55, 139.87, 141.03
[Ph_3Pb(4,7 -Ph) (Phenylalanine)]	C_1,143.02; C_2,128.41; C_3,149.20	C_{34},140.50; C_{35},127.06; C_{36},128.70; C_{37},125.40	64.08	39.36	177.78	137.03, 137.50, 139.81, 141.00
[Ph_3Pb(1,7-Ph)(Phenylalanine)]	C_1,143.06; C_2,128.40; C_3,149.01	C_{34},140.58; C_{35},127.26; C_{36},128.78; C_{37},125.48	64.12	39.12	177.56	136.67, 137.29, 139.52, 140.84
[Me_2Pb(1,10-Ph)(Tyrosine)Cl]	C_1,143.02; C_2,128.10; C_3,149.02	C_{34},140.58; C_{35},127.48; C_{36},28.96; C_{37},125.6	63.88	39.08	177.40	—
[Bu^t_2Pb(1,10-Ph)(Tyrosine)Cl]	C_1,142.20; C_2,128.32; C_3,148.62	C_{34},140.52; C_{35},127.38; C_{36},128.88; C_{37},125.42	63.76	39.16	177.58	—

where d_c is the diameter of control plate and d_t is the diameter of the fungal colony on the test plate.

Antibacterial activity

In vitro bacterial activity of the complexes was tested using the paper disc diffusion method.[24] The chosen strains were *Escherichia coli* and *Xanthomonas compestris* (strains were chosen keeping in view their economic importance). The liquid medium containing the nutrient agar medium was auto claved for 20 min at 15 lb pressure before inoculation and was poured onto a Petri plate and allowed to solidify. The bacteria were cultured for 24 h at 36 °C in an incubator. The test compounds were added dropwise to 10 mm diameter papers discs placed in the centre of the agar plates. The plates were then kept at 5 °C for 1 h and transferred to an incubator maintained at 36 °C. The width of the growth inhibition zone around the disc was measured after 24 h of incubation. Four replicates were taken for each treatment. The susceptibility of certain strains of bacterium towards the complexes was judged by measuring the diameter of growth inhibitor zone around the each disk.

Antifertility activity

In the present investigations, healthy adult male albino rats (*Rattus norvegicus*) each weighing between 200 and 250 g and of proven fertility were used. These were preferred over other laboratory mammals because of their medium size, easy of handling and maintenance, covertly observable sex and libido and relatively short gestation period of 23–30 days. Animals were regularly checked for any disease and if found infected were isolated and treated. Animals were fed on a diet of rat feed pellets obtained from Hindustan Lever Ltd, Mumbai. Water was provided *ad libitum*.

Fertility test

In these investigations doses of the compounds mixed in vehicle (olive oil) were given orally using a hypodermic syringe with a pearl point needle for 60 days and withdrawn

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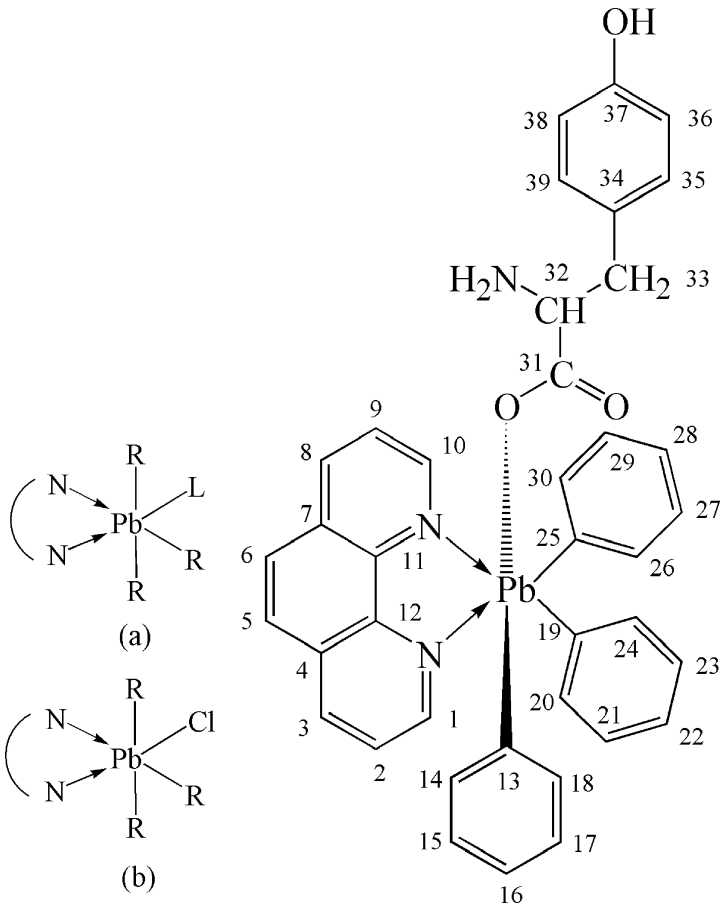

Figure 1. Proposed structures for the complexes. N∩N is the donor system of the phenanthroline R = Ph, Me or Bu^t, L = $C_9H_{11}NO_3$ and $C_9H_{11}NO_2$.

Table 5. Fungicidal screening data of the starting materials and their lead complexes

Average (%) inhibition after 96 h	Concentration in ppm	Compound[a]						*Bavistin* (standard)	Ph_3PbCl
		(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
	25	46.5	12	36	50	11	36	84	34
	50	60	22	71	68	19	48	87	45
Alternaria alternata	100	73	55	b	80	49	73	100	54
	200	90	70	b	95	b	88	100	61
	25	52	15	35.4	55	50	38	82	32
	50	64	29	58	b	61	43	91	41
Alternaria brassica	100	77	53	71	84	55	68	100	56
	200	98.7	65	80	100	a	79	100	60
	25	43	11	25	45	13	28	83	17
	50	54	18	39	57	42	51	86	26
Fusarium oxysporum	100	67	45	47	69	53	62	100	35
	200	78	b	68	78	71	60	100	44
	25	41	17	23	33	37	35	82	19
	50	52	33	34	39	50	47	82	31
Macrophomina phaseolina	100	77	71	41	54	73	65	100	39
	200	92	88	69	74	b	74	100	48

[a] Compounds as in Table 1.
[b] Fungicidal activity could not be measured.

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(recovery) for 30 days. After the completion of the treatment, the fertility test was done. On day 61 the animals were autopsied and blood was extracted from the heart. The serum was separated and used for serum biochemistry. Reproductive tissues (testis, epididymis, vas deferens, seminal vesicle and ventral prostate) and vital organs (liver, kidney, heart and adrenal) were blotted free of blood, weighed and used for tissue biochemistry and histopathology.

Body and organ weights

The body weight of each animal was measured both before and after the treatment.

Spermatozoa motility and count

The spermatozoa motility was determined according to the method of Prasad *et al*.[25] using a WBC counting Neubauer

Table 6. Bactericidal screening data of the precursors and their corresponding lead complexes

	Diameter of inhibition zone (mm) after 24 h (concentration in ppm)					
	Escherichia coli			*Xanthomonas compestris*		
Compound	500	1000	2000	500	1000	2000
Ph_3PbCl	4	6	8	5	6	9
1,10-Phenanthroline	6	8	10	6	—	—
1,7-Phenanthroline	2	3	6	2	4	6
4,7-Phenanthroline	2	4	6	2	—	5
[Ph_3Pb(1,10-Ph)(Tyrosine)]	11	13	16	10	13	17
[Ph_3Pb(4,7-Ph)(Tyrosine)]	5	7	—	5.5	7.7	11
[Ph_3Pb(1,7-Ph)(Tyrosine)]	8	10	12	8	11	14
[Ph_3Pb(1,10-Ph)(Phenylalanine)]	10	13	15	10	14	20
[Ph_3Pb(4,7-Ph)(Phenylalanine)]	5	7	9	6	8	10
[Ph_3Pb (1,7-Ph)(Phenylalanine)]	9	11	14	10	11	14
Standard (*Streptomycin*)	17	18	20	3	5	6

Table 7. LD_{50} for the organolead(IV) complexes

Sample no.	Dose	Animal	Death
1	100	30	30
2	50	30	30
3	25	30	20
4	12.5	30	15

Table 8. Changes in body weight and weight of testis, epididymides, seminal vesicle, prostate gland, adrenals and kidneys following the administration of [Ph_3Pb(1,7-Ph)(Tyrosine)]

Treatment	Body weight, g		Testis, mg/100 g body wt	Epididymides, mg/100 g body wt	Seminal vesicle, mg/100 g body wt	Prostate gland, mg/100 g body wt	Adrenals, mg/100 g body wt	Kidneys, mg/100 g body wt
	Initial	Final						
Control, group I	185 ± 8.1	204.0 ± 7.2	1320.4 ± 60.2	422.6 ± 21.56	580.41 ± 26.56	279.5 ± 12.37	24.95 ± 1.2	699 ± 31.4
2.5 mg/day for 60 days, group II	179 ± 9.0	160.7^{ns} ± 12.11	1255.42^{ns} ± 36.7	360.0^{ns} ± 30.89	542.29^{ns} ± 25.6	264.4 ± 20.2	25.2 ± 1.89^{ns}	789.69 ± 35.1^{ns}
5 mg/day for 60 days, group III	187 ± 10.2	206.0 ± 14.4	1195.3^{ns} ± 4.9	330.26* ± 18.94	464.16* ± 18.2	233.1 ± 17.2	22.27 ± 2.43	800 ± 38.4^{ns}
12 mg/day for 60 days, group IV	198 ± 9.2	178.8^{ns} ± 6.9	1080.0 ± 29.5	295.5** ± 10.9	431.2 ± 16.4	206.46 ± 4.8	20.6 ± 2.58^{ns}	764.59 ± 22.1^{ns}

Mean ± SEM of six animals.
^{ns} Non-significant.
* $p \leq 0.01$, significant.
** $p \leq 0.001$, highly significant.

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Table 9. Changes in sperm motility, cauda epididymides, sperm density of testis and cauda epididymides and fertility rate after the treatment with [Ph_3Pb(1,7-Ph)(Tyrosine)]

Treatment	Sperm motility (%) (cauda epididymides)	Sperm density (million/ml)		Fertility rate (%)
		Testis	Cauda epididymides	
Control, group I	68.65 ± 2.2	4.13 ± 0.4	35.70 ± 2.90	98 (+ve)
2.5 mg/day for 60 days, group II	50.13* ± 3.6	2.89 ± 0.9	23.69* ± 2.4	40 (−ve)
5 mg/day for 60 days, group III	48.65* ± 5.2	2.18* ± 0.3	17.21* ± 3.3	50 (−ve)
12 mg/day for 60 days, group IV	39.45** ± 1.9	1.91** ± 0.01	14.05** ± 0.9	65 (−ve)

Mean ± SEM of six animals.
ns Non-significant.
* $p \leq 0.01$, significant.
** $p \leq 0.001$, highly significant.

chamber of a haemocytometer and were expressed as million spermatozoa/ml suspension.

Bio-chemical studies

Protein was estimated by the procedure given by Lowry[26] method reported earlier. Sialic acid was estimated by the procedure given by Warren.[27] Cholesterol was done as per the method of Zlatkis *et al*.[28] Glycogen was estimated by the method of Montgomery.[29] Fructose was done by the method of Foreman *et al*.[30] Ascorbic acid was estimated by the method of Roe and Kuether.[31] Acid phosphatase and alkaline phosphatase were measured by the methods of Fiske and Subbarow.[32] The values for the body weight, organ weight, sperm dynamics and biochemical estimations were averaged, standard error of the mean values were calculated and Student's *t*-test was applied for the standard comparisons.

In these investigations doses of the compounds mixed in vehicle (olive oil) were given orally using a hypodermic syringe having pearl point needle, for 60 days and withdrawn (recovery) for 30 days.

The LD_{50} is the statistically derived single dose of a substance that can be expected to cause death in 50% of the animals. In a prohibited analysis method of LD_{50}, the selected dose levels should bracket the expected LD_{50} value with at least one dose level higher than the expected LD_{50} but not causing 100% mortality and one dose level below the expected LD_{50} but not causing 0% mortality. The toxicity of the complexes was determined by calculating the LD_{50} values. Symptoms of poisoning and mortality were observed and the results of toxicity LD_{50} values of the complexes are given in Table 7.

RESULTS AND DISCUSSION

The 1:1:1 molar reactions of amino acid, and different phenanthrolines with organolead chlorides (Ph_3PbCl, $Bu^t_2PbCl_2$ and Me_2PbCl_2) in dry methanol proceeded smoothly. The resulting products were filtered and washed several times with the same solvent. All the products were coloured solids and were completely soluble in most of the organic solvents. All these complexes were purified by recrystallization. Their purity was further checked by thin-layer chromatography using silica gel-G. It was observed that the spot moved as such for a particular type of compounds. The molecular weight determinations show these compounds to be monomers. The molar conductivity in dry dimethylformamide was found to be in the range 23–33 ohm^{-1} cm^2 mol^{-1}, indicating the non electrolytic behaviour.

Spectral aspects

The coloured solids were characterized by the IR, UV, ^1H NMR, ^{13}C NMR and ^{207}Pb NMR spectroscopy. The IR spectra of the starting materials and their complexes supported the formation of the complexes with the proposed coordination mode. The amino acids exhibit a ν(OH) band of the carboxylate group[33] at ca. 3200 cm^{-1}. However, the IR spectra of the complexes do not show this band, indicating the deprotonation of the carboxylic group. This is further supported by the appearance of a new medium intensity band in the far IR region 515–558 cm^{-1}, attributed to Pb–O stretching vibrations, indicating the coordination of the metal through the oxygen atom.[34] No splitting was observed in the band at ca. 1650 cm^{-1} due to $(COO)_{asym}$ and $(COO)_{sym}$ vibrations. In the free phenanthroline molecule, strong interactions between C═C and C═N gave rise to two groups of doublets (1550–1580 and 1445–1490 cm^{-1}). These bands undergo remarkable changes due to coordination and new bands are found to appear in the spectra of the complexes at 1600–1610 and 1560–1565 cm^{-1}, confirming the bidentate (NN) coordination of phenanthroline.[35] A medium to sharp intensity band observed in the far IR region of the metal complexes at (340–366) cm^{-1} was assigned to ν(Pb–Cl) mode. The medium to sharp intensity bands were observed at 575–595 and 527–538 cm^{-1}, which may be assigned to the asymmetric and symmetric modes of Pb–C stretching

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Table 10. Biochemical changes in cholesterol, glycogen, protein and sialic acid contents of testis, epididymides, seminal vesicle and prostate gland following the administration of [Ph_3Pb(1,7-Ph)(Tyrosine)]

Treatment	Cholesterol, mg/g		Glycogen, mg/g		Protein, mg/g				Sialic acid, mg/g			
	Testis	Liver	Testis	Liver	Testis	Epididy-mides	Seminal vesicle	Prostate gland	Testis	Epididy-mides	Seminal vesicle	Prostate gland
Control, group I	10.13 ± 0.37	15.96 ± 0.18	2.58 ± 0.13	6.90 ± 0.39	192.4 ± 7.6	208.00 ± 6.7	204.7 ± 5.8	170.0 ± 6.7	5.3 ± 0.10	4.8 ± 0.60	5.4 ± 0.4	5.27 ± 0.30
2.5 mg/day for 60 days, group II	10.09^{ns} ± 0.3	15.4^{ns} ± 2.01	2.47^{ns} ± 0.10	6.03^{ns} ± 0.8	164.0^{ns} ± 5.5	199.6^{ns} ± 8.0	197.6^{ns} ± 4.7	163.3^{ns} ± 6.1	5.0^{ns} ± 0.20	4.2^{ns} ± 0.26	4.78 ± 1.5	4.91^{ns} ± 1.2
5 mg/day for 60 days, group III	9.96^{ns} ± 0.51	15.39^{ns} ± 0.26	2.31^{ns} ± 0.19	5.65^{ns} ± 2.3	148.1* ± 7.2	180.1* ± 4.6	180.5* ± 4.9	140.1* ± 6.0	4.9^{ns} ± 0.3	4.06^{ns} ± 0.7	4.05 ± 0.40	4.2^{ns} ± 0.09
12 mg/day for 60 days, group IV	9.0* ± 0.02	14.91^{ns} ± 0.44	2.01* ± 0.14	5.10^{ns} ± 2.2	113.1* ± 15.3	145.6* ± 14.09	168.6* ± 8.3	127.2* ± 10.2	4.2^{ns} ± 0.30	3.7^{ns} ± 0.62	3.3* ± 0.02	3.64^{ns} ± 0.4

Mean ± SEM of six animals.
^{ns} Non-significant.
* $p \leq 0.01$, significant and ** $p \leq 0.001$, highly significant.

Table 11. Changes in body weight and weight of testis, epididymides, seminal vesicle, prostate gland adrenal and kidney following the administration of the compound [Ph_3Pb(1,10-Ph)(Tyrosine)]

Treatment	Body weight, g		Testis, mg/100 g body wt	Epididymides, mg/100 g body wt	Seminal vesicle, mg/100 g body wt	Prostate gland, mg/100 g body wt	Adrenal, mg/100 g body wt	Kidney, mg/100 g body wt
	Initial	Final						
Control, group I	185.0 ± 8.1	204.0 ± 7.2	1311.5 ± 70.2	402.0 ± 21.56	504.1 ± 30.5	369.0 ± 14.6	24.6 ± 1.2	723.5 ± 31.4
2.5 mg/day for 60 days, group II	198.0 ± 10.11	173.8^{ns} ± 4.9	1036.1* ± 42.01	359.2^{ns} ± 19.20	462.0^{ns} ± 42.9	256.2** ± 19.9	27.0^{ns} ± 2.6	620.7^{ns} ± 27.4
5 mg/day for 60 days, group III	182.0 ± 6.9	164.0^{ns} ± 5.0	918.1** ± 28.02	290.0* ± 22.0	378.03* ± 22.28	193.3** ± 9.5	26.23^{ns} ± 1.48	673.2^{ns} ± 38.9

Mean ± SEM of six animals.
^{ns} Non-significant.
* $p \leq 0.01$, significant.
** $p \leq 0.001$, highly significant.

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Table 12. Changes in sperm motility, cauda epididymides, sperm density of testis and cauda epididymides and fertility rate after [Ph_3Pb(1,10-Ph)(Tyrosine)] administration

Treatment	Sperm motility (%) (cauda epididymides)	Sperm density (million/ml)		Fertility rate (%)
		Testis	Cauda epididymides	
Control, group I	70.16 ± 0.22	4.95 ± 0.01	39.32 ± 0.59	100+ve
2.5 mg/day for 60 days, group II	37.91** ± 1.3	2.91** ± 0.2	18.97** ± 1.07	75−ve
5 mg/day for 60 days, group III	19.68** ± 4.9	0.99** ± 0.06	9.75** ± 1.1	90−ve

Mean ± SEM of six animals.
ns Non-significant.
* $p \leq 0.01$, significant.
** $p \leq 0.001$, highly significant.

vibrations. One strong intensity band in the spectrum of the complex at 1178 cm^{-1} can be assigned to Pb–CH_3 stretching vibrations. A new band observed at 259–280 cm^{-1} may be assigned to (Pb–Ph).[36,37] The IR spectral data of the complexes are given in Table 2.

The electronic spectra of the complexes were recorded in carbon tetrachloride. Two prominent peaks were observed at 215–225 and 250–267 nm in the UV region and assigned to the $\pi-\pi^*$ electronic transitions.

The ^{1}H NMR spectra were recorded in DMSO-d_6. The chemical shift values relative to the TMS peak are shown in Table 3. A comparative study of the ^{1}H NMR spectra of the starting materials and their complexes confirmed the proposed skeleton. A doublet was observed in the high field at δ3.02–3.18 ppm due to $-CH_2$ protons of tyrosine or phenylalanine. A complex pattern in the region δ3.64–3.80 ppm was assigned to NH_2–CH. The phenyl proton signals appeared in the region δ6.33–7.19 ppm. Proton signals due to 1,10-phenanthroline, 4,7-phenanthroline and 1,7-phenanthroline appeared at δ7.44–9.17 ppm. Since the –COOH proton signal was absent in the ^{1}H NMR spectra, this confirms the coordination through the carbonyl group which was already supported by the Pb–O peak in the far IR spectra. ^{1}H NMR spectral data of the complexes are given in Table 3.

^{13}C NMR spectra of the complexes recorded in methanol exhibit the carboxylic carbon signal at δ 4.19 ppm (showing an upfield shift from the free amino acid) attributed to the monodentate nature of the carboxylic group. The ^{13}C NMR spectral data of the complexes are listed in Table 4. On the basis of the spectral evidence, it may be inferred that the carboxylic acid of the amino acid (tyrosine or phenylalanine) is behaving as monodentate in these complexes and the complexes are octahedral in shape with a coordination number six around the lead atom.

The ^{207}Pb NMR spectra of the complexes gave signals at δ2209–2245 ppm, indicating coordination number six in the complexes around lead atom.[38] These results are in accordance with the results by West *et al*.[36,39] The most suitable structures for these derivatives considering their physical measurements, analytical data and spectral evidences are depicted in Fig. 1.

Biological evaluation

Antimicrobial assay

The results described in Tables 5 and 6 reveal that all the compounds are active against these organisms, even at low concentrations, and the inhibition of the growth of microorganism was found to be dependent on the concentration of the compounds. The results of the biological screening indicated that the metal chelates are more active than the starting materials.

The bioactivity is enhanced after chelation. The chelation reduces the polarity of the central atom mainly because of partial sharing of its positive charge with the donor groups and delocalization within the whole chelate ring. The antifungal activity of these compounds may well be explained in the light of modern electronic theory, as resonating rings also affect fungitoxicity. Resonating structures such as benzene and other conjugated systems may serve as powerhouses to activate potentially reactive groupings.

Antifertility activity

Effect on animal body weights

No significant changes were noted in the body weights after the treatment of the complexes [Ph_3Pb(1,7-Ph)(Tyrosine)] and [Ph_3Pb(1,10-Ph)(Tyrosine)] at 2.5, 5 and 12 mg dose levels per day for 60 days (Tables 8 and 11).

Effect on organs weights

Oral administration of ligand and complexes caused significant reduction ($p \leq 0.01$) in the weights of testis and accessory sex organs whereas no changes were observed in the weights of kidney and adrenal glands (Tables 8 and 11).

Effect on fertility

The male rats were kept for fertility test after 55 days of [Ph_3Pb(1,10-Ph)(Tyrosine)] and [Ph_3Pb(1,7-Ph)(Tyrosine)] administration and showed remarkable results. A 40–90% negative fertility was observed at different dose levels of [Ph_3Pb(1,7-Ph)(Tyrosine)] and [Ph_3Pb(1,10-Ph)(Tyrosine)] (Tables 9 and 12).

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Table 13. Biochemical changes in cholesterol, glycogen, protein and sialic acid contents of testis, epididymides, seminal vesicle and prostate gland following the administration of [Ph_3Pb(1,10-Ph)(Tyrosine)]

Treatment	Cholesterol, mg/g		Glycogen, mg/g		Protein, mg/g				Sialic acid, mg/g			
	Testis	Liver	Testis	Liver	Testis	Epididymides	Seminal vesicle	Prostate gland	Testis	Epididymides	Seminal vesicle	Prostate gland
Control, group I	11.13 ± 0.37	16.59 ± 0.18	2.53 ± 0.13	6.29 ± 0.39	199.57 ± 12.0	240.0 ± 11.11	214.2 ± 7.4	180.1 ± 13.9	5.02 ± 0.06	5.94 ± 0.07	4.86 ± 0.09	4.6 ± 0.15
2.5 mg/day for 60 days, group II	9.06 ± 1.19^{ns}	16.02 ± 0.29	1.19** ± 0.19	5.91^{ns} ± 0.19	158.0* ± 4.2	213.04^{ns} ± 16.6	182.01* ± 6.6	111.0* ± 10.3	3.82** ± 0.13	4.79* ± 0.2	3.9** ± 0.05	3.5** ± 0.04
5 mg/day for 60 days, group III	8.7** ± 0.82	14.26* ± 0.44	1.11** ± 0.028	4.56* ± 0.09	136.0** ± 5.9	160.0** ± 10.9	138.0** ± 11.8	106.0** ± 5.0	3.67** ± 0.05	4.18** ± 0.19	3.58** ± 0.04	3.30** ± 0.07

Mean ± SEM of six animals.
^{ns} Non-significant.
* $p \leq 0.01$, significant.
** $p \leq 0.001$, highly significant.

Antispermatogenic effects

Sperm motility of the cauda epididymides was decreased significantly ($p \leq 0.01$, $p \leq 0.001$) after oral administration at all the dose levels (Tables 9 and 12). Sperm density of the testis and cauda epididymides was decreased significantly ($p \leq 0.001$) in rats treated with the ligand and its complexes at all the dose levels (Tables 9 and 12).

Tissue biochemistry

Total protein contents and sialic acid concentration of testis and accessory sex organs were significantly reduced after the treatment with both the complexes. Reduced levels of testicular glycogen and cholesterol were noticed following the administration of [Ph_3Pb(1,10-Ph)(Tyrosine)] and [Ph_3Pb(1,7-Ph)(Tyrosine)] (Tables 10 and 13).

Histopathology

The histopathology of testes treated with different doses of ligand and its complexes exhibited drastic changes. Most of the tubules showed more or less spermatogenic arrest. However, the damage was not uniform. Residual sperm and cell debris were present in the lumen of some tubules. Interstitial stroma had slight atrophy and nacrotic nuclei. The epididymis showed normal epithelium. The intertubular stroma appeared to be degenerated. The lumen had lower numbers of sperm.

Haematology

Total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin concentration, haematocrit and blood urea values were in the normal range for treatment with [Ph_3Pb(1,10-Ph)(Tyrosine)] and [Ph_3Pb(1,7-Ph)(Tyrosine)]. No change was observed in TEC, haemoglobin concentration and haematocrit at the entire dose levels (Table 14).

CONCLUSIONS

The testis weight of treated rats declined as a result of degenerative changes, which were indicated by the inhibition of spermatogenesis, obliteration of lumen of the seminiferous tubules and few viable Leydig cells, owing to the hormonal imbalances caused by complexes. This was reported earlier.[40]

The weight of epididymides, seminal vesicle and prostate glands were reduced significantly ($p \leq 0.001$), which may be due to the absence of sperm in the lumen of epididymides tubules. The epididymides provides a suitable environment for morphological and biochemical changes in the spermatozoa under the influence of androgen. Motility and density of cauda epididymides sperms decreased significantly ($p \leq 0.001$), which reflected the anti androgenic nature of complexes. This was also reported earlier.[41,42]

The decline in protein, sialic acid, glycogen and cholesterol contents of testis, epididymides, seminal vesicle and prostate glands suggests the interference in androgen level.[43]

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Table 14. Haematological data of organolead (IV) complexes

Serial no.	Group	Dose, mg/kg	Haemoglobin	Haematocrit	Urea, mg/dl	TEC, million/mm^3	TLC, mm^3
1	Control	Vehicle treated	14.00 ± 0.02	47.0 ± 0.4	32.0 ± 1.26	6.56 ± 0.26	53.00 ± 253.34
2	[Ph_3Pb(1,7-Ph)(Tyrosine)]	2.5	13.85 ± 0.03	46.0 ± 0.2	32.0 ± 1.15	6.50 ± 0.15	53.00 ± 250.34
3	[Ph_3Pb(1,10-Ph)(Tyrosine)]	2.5	14.00 ± 0.4	47.0 ± 0.3	32.4 ± 1.05	6.55 ± 0.20	53.00 ± 240.14

Haematological studies, viz. TEC, LEC, PCV and haemoglobin, showed no adverse effect on general metabolism as these values were in the normal range.

The complexes affect testes and sex accessory glands histopathologically as well as biochemically without dysfunction of their physiological mechanism.

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