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The antispermatogenic activity of some phenylbismuth(III) *O,O′*-dialkyldithiophosphates

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Ten dialkyldithiophosphate derivatives of phenylbismuth(III) of the type, $Ph_{(3-n)}Bi[S(S)P(OR)_2]_n$ [where $n = 1$; R = Me(1), Et(2), Pri(3), Pr^n(4) and Bu^n(5); $n = 2$; R = Me(6), Et(7), Pr^i(8), Pr^n(9) and Bu^n(10)] have been synthesized by the reactions of triphenylbismuth(III) with corresponding dialkyldithiophosphoric acids in 1:1 and 1:2 stoichiometric ratios, respectively, in stirred benzene solution. The newly synthesized brown colored compounds, 1–10 have been characterized by elemental analyses, molecular weight measurements, IR and NMR (^1H, ^{13}C and ^{31}P) spectral studies. The ligand diethyldithiophosphoric acid, $[(C_2H_5O)_2P(S)SH]$, and its organobismuth(III) derivatives, compounds 2 and 7 were administered to adult male rats by oral gavage at the dose of 25 mg per kg body weight per day, for 60 days, and their effects were evaluated and compared for changes in testicular morphology, circulatory concentrations of testosterone, FSH and LH, sperm dynamics, fertility index and testicular cell population dynamics. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: *O,O′*-dialkyldithiophosphoric acid; organobismuth(III) derivatives; bidentate ligands; tetra and penta coordinated bismuth; sperm; FSH; LH; testosterone; fertility

INTRODUCTION

Bismuth, despite being a heavy metal, finds many applications in medicine.[1,2] Known as 'Magisterium bismuti' the bio-utility of bismuth and its compounds has a history of about 350 years and a vast array of biological and medicinal activity has been reported for bismuth-containing compounds.[1,2] Mostly used in the treatment of gastrointestinal disorders,[3] bismuth compounds have also been examined for the treatment of a number of diseases[4–8] and in various other areas[9–14] of medical science.

A fairly deducible idea is to introduce bismuth, for which a vast array of biological activity has been established, and dialkylditiophosphate moieties, which are also known for their potent biocidal effects,[15–20] into the same compound. This type of combination of the two kinds of biological activity in a single molecule could produce better potency and longer lasting effect.

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We report herein the synthesis and characterization of phenylbismuth(III) dialkyldithiophosphate derivatives, **1–10**. In this communication a comparative study of the effects of *O,O′*-diethyldithiophosphoric acid ligand, $(C_2H_5O)_2P(S)SH$, and corresponding organobismuth(III) derivatives, **2** and **7**, on the reproductive systems of male albino rats vs control animals has also been presented.

RESULTS AND DISCUSSION

O,O′-Dialkyldithiophosphate derivatives of phenylbismuth (III), **1–10** have been synthesized by the reactions of Ph_3Bi with corresponding *O,O′*-dialkyldithiophosphoric acids, $(RO)_2P(S)SH$, in 1:1 and 1:2 molar ratios, respectively, first by stirring for ~6 h and finally by warming for ~30 min in benzene solution.

$$Ph_3Bi + n[(RO)_2P(S)SH] \xrightarrow[\text{Reflux}]{C_6H_6} \underset{\mathbf{1-10}}{Ph_{(3-n)}Bi[S(S)P(OR)_2]_n} + nC_6H_6$$

where, $n = 1$; R = Me (**1**), Et(**2**), Pr^i(**3**), Pr^n(**4**) and Bu^n(**5**); and $n = 2$; R = Me (**6**), Et (**7**), Pr^i (**8**), Pr^n (**9**) and Bu^n (**10**).

During the reaction, the cleavage of the Ph–Bi bond takes place[21] and corresponding organobismuth(III) derivatives are formed. These reactions are quite facile and quantitative. All the new derivatives, **1–10**, are dark-brown colored solids and soluble in common organic solvents. Molecular weight measurements reveal their monomeric nature in freezing benzene solution (Table 1). It is worth mention here that compounds **1–3** and **6–8** have already been reported in the literature.[22]

IR spectra

The tentative assignments of the important characteristic bands in the IR spectra of these new organobismuth(III) derivatives (summarized in Table 2) were made by comparing them with the IR spectral data reported earlier.[23–29]

The medium intensity broad bands observed in the regions 790–850 and 955–1000 cm^{-1} were assigned to [P–O–(C)][23,24] and [(P) –O–C],[23,24] respectively. IR spectra of these compounds **1–10** exhibit strong bands in the region 630–655 cm^{-1} with shifts towards lower wave numbers[25–27] as compared with their positions in IR spectra of free ligands (640–690 cm^{-1}), which may be due to the bidentate chelation of the dialkyldithiophosphate ligand moieties[25] with bismuth(III) center in these derivatives. Medium to weak intensity bands present in the region 535–565 cm^{-1} may be due to P–S stretching vibrations.[26–28] Bi–Ph vibrations (Y-mode)[29] were observed in the region 430–455 cm^{-1}.

^{1}H NMR spectra

^{1}H NMR spectra of these new derivatives, **1–10** (Table 3), exhibit the signals of alkyl protons of ligand moieties at their expected positions. Splitting due to coupling between α-protons and phosphorus was observed in the signals of these alkyl groups attached to the OP(S)S group. The phenyl group (attached to Bi) protons were observed as a complex pattern in the range 7.32–7.80 ppm.

^{13}C NMR spectra

In the ^{13}C NMR spectra (Table 4) of these new derivatives, **1–10**, ^{13}C–^{31}P NMR coupling was observed up to three bond lengths. No further splitting of the ^{13}C signals of the alkyl groups, attached to the P–O group, was observed. A downfield shift was observed in the positions of the signals of different alkyl group carbons of dialkyldithiophosphate moieties as compared with their positions in free ligands (Table 5). This downfield shift indicates strong bidentate chelation of dithiophosphate moieties with bismuth(III) center. The phenyl group (attached to Bi) carbons appear as a set of four signals in the spectra of all new derivatives, **1–10,** in the range 127.09–140.70 ppm.

Table 1. Synthetic, physical and analytical data of the compounds, **1–10**, $Ph_{(3-n)}[S(S)P(OR)_2]_n$

Sample no.	Compound R	n	Reactants g (mmol) Ph_3Bi	$(RO)_2P(S)SH$	Color/physical state	Empirical formula, % yield (m.p./°C)	Molecular weight found (calcd)	Elemental analysis (%), found (calcd) Bi	S	C	H
1	Me	1	1.75 (3.97)	0.63 (3.98)	Light brown/solid	$C_{14}H_{16}O_2PS_2Bi$, 91 (160)	517 (520.37)	40.13 (40.16)	12.29 (12.33)	32.27 (32.31)	3.10 (3.11)
2	Et	1	2.15 (4.88)	0.91 (4.87)	Brown/sticky solid	$C_{16}H_{20}O_2PS_2Bi$, 89 (–)	546 (548.42)	38.12 (38.11)	11.65 (11.69)	35.01 (35.04)	3.65 (3.68)
3	Pr^i	1	1.78 (4.04)	0.87 (4.06)	Light brown/solid	$C_{18}H_{24}O_2PS_2Bi$, 88 (175)	572 (576.47)	36.24 (36.25)	11.14 (11.13)	37.48 (37.50)	4.16 (4.20)
4	Pr^n	1	1.47 (3.34)	0.72 (3.36)	Brown/solid	$C_{18}H_{24}O_2PS_2Bi$, 90 (181)	573 (576.47)	36.23 (36.25)	11.10 (11.13)	37.47 (37.50)	4.19 (4.20)
5	Bu^n	1	1.44 (3.27)	0.79 (3.26)	Brown/solid	$C_{20}H_{28}O_2PS_2Bi$, 88 (–)	605 (604.52)	34.52 (34.57)	10.60 (10.61)	39.70 (39.73)	4.65 (4.68)
6	Me	2	1.17 (2.66)	0.84 (5.31)	Light brown/solid	$C_{10}H_{17}O_4P_2S_4Bi$, 87 (180)	598 (600.43)	34.72 (34.80)	21.34 (21.36)	20.01 (20.00)	2.84 (2.86)
7	Et	2	2.08 (4.72)	1.76 (9.45)	Light brown/solid	$C_{14}H_{25}O_4P_2S_4Bi$, 89 (183)	653 (656.54)	31.80 (31.83)	19.54 (19.54)	25.67 (25.61)	3.84 (3.85)
8	Pr^i	2	1.33 (3.02)	1.29 (6.02)	Light brown/sticky solid	$C_{18}H_{33}O_4P_2S_4Bi$, 85 (–)	710 (712.64)	29.28 (29.32)	17.98 (18.00)	30.33 (30.33)	4.68 (4.68)
9	Pr^n	2	1.20 (2.72)	1.17 (5.46)	Reddish brown/sticky solid	$C_{18}H_{33}O_4P_2S_4Bi$, 83 (–)	713 (712.64)	29.35 (29.32)	17.95 (18.00)	30.31 (30.33)	4.66 (4.68)
10	Bu^n	2	1.20 (2.72)	1.32 (5.45)	Dark brown/solid	$C_{22}H_{41}O_4P_2S_4Bi$, 87 (194)	765 (768.74)	27.18 (27.18)	16.64 (16.69)	34.36 (34.37)	5.36 (5.39)

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Table 2. IR spectral data (cm^{-1}) of new organobismuth(III) dialkyldithiophosphate derivatives, $Ph_{(3-n)}Bi[S(S)P(OR)_2]_n$

Sample no.	Compound R	Compound *n*	ν(P–S)	ν(P═S)	ν[(P)–O–C]	ν[P–O–(C)]	ν(Ph–Bi) (Y-mode)
1	Me	1	555, m	637, str	955, m, br	805, m	440, m
2	Et	1	540, m	648, str	960, m, br	820, m	430, m
3	Pr^i	1	539, m	638, str	970, m, br	800, m	450, m
4	Pr^n	1	535, m	646, str	980, m, br	848, m	450, m
5	Bu^n	1	565, m	655, str	995, m, br	850, m	435, m
6	Me	2	550, m	630, str	960, m, br	840, m	450, m
7	Et	2	538, m	640, str	975, m, br	850, m	445, m
8	Pr^i	2	536, m	642, str	965, m, br	790, m	455, m
9	Pr^n	2	530, m	650, str	1000, m, br	845, m	455, m
10	Bu^n	2	558, m	653, str	998, m, br	842, m	450, m

Abbreviations: m = medium; br = broad; str = strong.

Table 3. ^1H NMR spectral data for the compounds, **1–10,** $Ph_{(3-n)}Bi[S(S)P(OR)_2]_n$

Sample No.	Compound R	Compound n	^1H NMR Chemical Shifts (δ, ppm)* and coupling constants (J, Hz)	
1	Me	1	3.60, d, 6H; ^3J (POCH) = 15.59;	($POC\mathbf{H}_3$)
			7.35, m, 6H, (*meta* + *para*); 7.72, m, 4H, (*ortho*)	($BiC_6\mathbf{H}_5$)
2	Et	1	1.26, t, 6H; ^3J (HCCH) = 6.97;	($POCH_2C\mathbf{H}_3$)
			3.96, dq, 4H, ^3J (POCH) = 9.73, ^3J(HCCH) = 7.15;	($POC\mathbf{H}_2CH_3$)
			7.36, m, 6H, (*meta* + *para*); 7.75, m, 4H, (*ortho*)	($BiC_6\mathbf{H}_5$)
3	Pr^I	1	1.25, d, 12H; ^3J (HCCH) = 6.25;	($POCHC\mathbf{H}_3$)
			4.58, m, 2H, ^3J (POCH) = 10.25, ^3J(HCCH) = 6.25;	($POC\mathbf{H}CH_3$)
			7.36, m, 6H, (*meta* + *para*); 7.75, 4H, (*ortho*)	($BiC_6\mathbf{H}_5$)
4	Pr^n	1	0.89, t, 6H; ^3J (HCCH) = 7.33;	($POCH_2CH_2C\mathbf{H}_3$)
			1.68, m, 4H, ^3J (HCCH) = 7.33,	($POCH_2C\mathbf{H}_2CH_3$)
			3.92, dt, 4H, ^3J(POCH) = 8.50; ^3J (HCCH) = 7.33;	($POC\mathbf{H}_2CH_2CH_3$)
			7.36, m, 6H, (*meta* + *para*); 7.76, m, 4H, (*ortho*)	($BiC_6\mathbf{H}_5$)
5	Bu^n	1	0.93, t, 6H; ^3J(HCCH) = 7.33;	($POCH_2CH_2CH_2C\mathbf{H}_3$)
			1.43, m, 4H, ^3J(HCCH) = 7.33;	($POCH_2CH_2C\mathbf{H}_2CH_3$)
			1.72, m, 4H, ^3J(HCCH) = 7.33;	($POCH_2C\mathbf{H}_2CH_2CH_3$)
			2.99, dt, 4H, ^3J(POCH) = 8.41, ^3J (HCCH) = 7.33;	($POC\mathbf{H}_2CH_2CH_2CH_3$)
			7.34, m, 6H, (*meta* + *para*); 7.75, m, 4H, (*ortho*)	($BiC_6\mathbf{H}_5$)
6	Me	2	3.67, d, 12H, ^3J(POCH) = 15.56;	($POC\mathbf{H}_3$)
			7.32,m, 3H, (*meta* + *para*); 7.80, m, 2H, (*ortho*)	($BiC_6\mathbf{H}_5$)
7	Et	2	1.25, t, 12H, ^3J(HCCH) = 7.15;	($POCH_2C\mathbf{H}_3$)
			3.96,dq, 8H, 3J (POCH) = 9.34, ^3J (HCCH) = 7.15;	($POC\mathbf{H}_2CH_3$)
			7.33, m, 3H(*meta* + *para*); 7.63, m, 2H, (*ortho*)	($BiC_6\mathbf{H}_5$)
8	Pr^I	2	1.25, d, 24H; ^3J (HCCH) = 5.86;	($POCHC\mathbf{H}_3$)
			4.56, m, 4H, ^3J (POCH) = 10.46, ^3J(HCCH) = 5.86;	($POC\mathbf{H}CH_3$)
			7.38, m, 3H, (*meta* + *para*); 7.74, m, 2H, (*ortho*)	($BiC_6\mathbf{H}_5$)
9	Pr^n	2	0.87, t, 12H; ^3J (HCCH) = 7.33;	($POCH_2CH_2C\mathbf{H}_3$)
			1.62, m, 8H, ^3J (HCCH) = 7.33;	($POCH_2C\mathbf{H}_2CH_3$)
			3.84, dt, 8H, ^3J(POCH) = 8.98; ^3J (HCCH) = 7.33;	($POC\mathbf{H}_2CH_2CH_3$)
			7.40, m, 3H, (*meta* + *para*); 7.74, m, 2H, (*ortho*)	($BiC_6\mathbf{H}_5$)
10	Bu^n	2	0.92, t, 12H; ^3J (HCCH) = 7.33;	($POCH_2CH_2CH_2C\mathbf{H}_3$)
			1.44, m, 8H, ^3J (HCCH) = 7.33;	($POCH_2CH_2C\mathbf{H}_2CH_3$)
			1.71, m, 8H, ^3J (HCCH) = 7.33;	($POCH_2C\mathbf{H}_2CH_2CH_3$)
			2.98, dt, 8H, ^3J(HCCH) = 8.59; ^3J (HCCH) = 7.33;	($POC\mathbf{H}_2CH_2CH_2CH_3$)
			7.39, m, 3H, (*meta* + *para*); 7.74, m, 2H, (*ortho*)	($BiC_6\mathbf{H}_5$)

* Abbreviation: d = doublet; m = complex pattern; t = triplet; dq = doublet of quartets; dt = doublet or triplets.

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Table 4. ^{13}C and ^{31}P NMR spectral data for the compounds, **1–10**, $Ph_{(3-n)}Bi[S(S)P(OR)_2]_n$

Sample no.	Compound R	Compound *n*	^{13}C NMR chemical shifts (δ, ppm)[a] and coupling constants (J, Hz)		δ' (= δCp − δCm)	σR^o (= $\delta'/22.06$)	^{31}P NMR chemical shift (δ, ppm)
1	Me	1	53.64, d, $^2J_{PC} = 5.58$	(PO**C**H$_3$)	−2.75	−0.125	101.45
			140.22(i), 137.47(o)	(Bi**C**$_6$H$_5$)			
			130.43(m), 127.68(p)				
2	Et	1	15.85, d, $^3J_{PC} = 9.31$	(POCH$_2$**C**H$_3$)	−2.73	−0.124	99.10
			63.11, d, $^2J_{PC} = 6.20$	(PO**C**H$_2$CH$_3$)			
			140.67(i), 137.44(o)	(Bi**C**$_6$H$_5$)			
			130.42(m), 127.69 (p)				
3	Pr^i	1	23.63, d, $^3J_{PC} = 4.35$	(POCH**C**H$_3$)	−2.75	−0.125	92.78
			73.02, d, $^2J_{PC} = 5.58$	(PO**C**HCH$_3$)			
			140.20(i), 137.52(o)	(Bi**C**$_6$H$_5$)			
			130.47(m), 127.72 (p)				
4	Pr^n	1	10.08	(POCH$_2$CH$_2$**C**H$_3$)	−3.82	−0.173	96.73
			23.26, d, $^3J_{PC} = 8.68$	(POCH$_2$**C**H$_2$CH$_3$)			
			69.00, d, $^2J_{PC} = 6.20$	(PO**C**H$_2$CH$_2$CH$_3$)			
			140.26(i), 137.53(o),	(Bi**C**$_6$H$_5$)			
			132.14(m), 128.00(p)				
5	Bu^n	1	13.56	(POCH$_2$CH$_2$CH$_2$**C**H$_3$)	−0.6	−0.027	93.98
			21.87	(POCH$_2$CH$_2$**C**H$_2$CH$_3$)			
			31.84, d, $^3J_{PC} = 5.58$	(POCH$_2$**C**H$_2$CH$_2$CH$_3$)			
			34.90, d, $^2J_{PC} = 3.72$	(PO**C**H$_2$CH$_2$CH$_2$CH$_3$)			
			137.53(i), 130.47(o),	(Bi**C**$_6$H$_5$)			
			128.32(m), 127.72(p)				
6	Me	2	53.80, d, $^2J_{PC} = 5.58$	(PO**C**H$_3$)	−2.27	−0.103	101.57
			140.27(i), 137.48(o)	(Bi**C**$_6$H$_5$)			
			130.44(m), 128.17(p)				
7	Et	2	15.87, d, $^3J_{PC} = 8.68$	(POCH$_2$**C**H$_3$)	−2.72	−0.123	99.22
			63.20, d, $^2J_{PC} = 5.58$	(PO**C**H$_2$CH$_3$)			
			140.70(i), 137.43(o),	(Bi**C**$_6$H$_5$)			
			130.41(m), 127.69 (p)				
8	Pr^i	2	23.70, d, $^3J_{PC} = 7.44$	(POCH**C**H$_3$)	−2.75	−0.125	92.78
			73.02, d, $^2J_{PC} = 6.20$	(PO**C**HCH$_3$)			
			140.18(i), 137.50(o)	(Bi**C**$_6$H$_5$)			
			130.45(m), 127.70 (p)				
9	Pr^n	2	10.12	(POCH$_2$CH$_2$**C**H$_3$)	−0.33	−0.015	96.65
			23.31, d, $^3J_{PC} = 8.68$	(POCH$_2$**C**H$_2$CH$_3$)			
			69.22, d, $^2J_{PC} = 6.82$	(PO**C**H$_2$CH$_2$CH$_3$)			
			140.26(i), 132.16(o)	(Bi**C**$_6$H$_5$)			
			128.33(m), 128.00 (p)				
10	Bu^n	2	12.91	(POCH$_2$CH$_2$CH$_2$**C**H$_3$)	−0.57	−0.026	94.14
			21.14	(POCH$_2$CH$_2$**C**H$_2$CH$_3$)			
			31.11, d, $^3J_{PC} = 5.58$	(POCH$_2$**C**H$_2$CH$_2$CH$_3$)			
			34.15, d, $^2J_{PC} = 4.35$	(PO**C**H$_2$CH$_2$CH$_2$CH$_3$)			
			136.82(i), 129.81(o)	(Bi**C**$_6$H$_5$)			
			127.66(m), 127.09 (p)				

[a] Abbreviation: d = doublet.

In view of the possibility of dπ–pπ conjugation between Ph–Bi, corrected chemical shift values (δ') for phenyl carbons were calculated[30] by the relation $\delta' = \delta$Cp − δCm (where δCp and δCm are the chemical shift values of *para* and *meta* carbons of the phenyl ring). The δ' values were found to be negative (ranging from −0.33 to −3.82 ppm), indicating the

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Table 5. ^{1}H, ^{13}C and ^{31}P NMR spectral data (δ, ppm) of the *O,O′*-dialkylphosphorodithioic acid ligands, $[(RO)_2P(S)SH]$

Sample no.	Ligand	NMR	SH	$^{1}H/^{13}C$ NMR Alkyl (R)[a]				^{31}P NMR
				H_α/C_α	H_β/C_β	H_γ/C_γ	H_δ/C_δ	
1	R = Me	^{1}H NMR	3.81, s	3.40, d	—	—	—	90.21
		^{13}C NMR		51.77	—	—	—	
2	R = Et	^{1}H NMR	3.35, s	4.20, dq	1.42, t	—	—	84.87
		^{13}C NMR		64.24	15.89	—	—	
3	R = Pr^i	^{1}H NMR	3.03, s	4.88, m	1.41, d	—	—	81.72
		^{13}C NMR		68.89	13.14	—	—	
4	R = Pr^n	^{1}H NMR	2.48, s	3.93, m	1.68, m	0.99, t	—	82.34
		^{13}C NMR		64.15	28.67	12.86	—	
5	R = Bu^n	^{1}H NMR	2.30, s	2.60, m	1.70, m	1.46, m	0.88, t	80.09
		^{13}C NMR		62.95	34.08	20.96	12.65	

[a] H_α, H_β, H_γ and H_δ refer to $\{HS(S)P[O(CH_\alpha)_P\ (CH_\beta)_q\ (CH_\gamma)_r(CH_\delta)_s]_2\}$ and C_α, C_β, C_γ and C_δ refer to carbons attached to H_α, H_β, H_γ and H_δ, respectively.
Abbreviations: s = singlet; d = doublet; t = triplet; m = complex pattern, dq = doublet of quartets.

dπ–pπ conjugation in these derivatives. The Hammet–Taft constants[31] σR^o (calculated by the equation $\sigma R^o = \delta'/22.06$) were also found to be negative (in the range −0.015 to −0.173; Table 4), which shows the poor dπ–pπ conjugation in these derivatives.

^{31}P NMR spectra

The proton decoupled ^{31}P NMR chemical shift values (92.78–99.83 ppm) for these new derivatives, **1–10** (Table 4), occur in the region expected for the bidentate mode[32] of attachment of the dilakyldithiophosphate ligands.

On the basis of the above physicochemical and spectral evidence and the presence of a lone pair of electrons, the following structures (Figs 1 and 2), in which the bismuth(III) center acquires pseudo trigonal bipyramidal and pseudo octahedral coordinations, have been proposed for these new **1–5** and **6–10** derivatives, respectively.

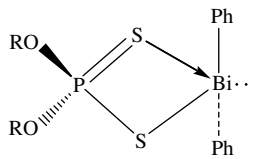

Figure 1. Proposed structures of compounds (**1–5**).

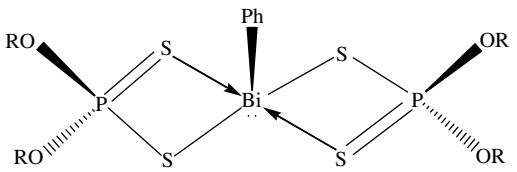

Figure 2. Proposed structures of compounds (**6–10**).

In Fig. 2 the alkyl groups attached to P–O group may be non-equivalent as there is a possibility that the two alkyl groups (which are above the basal plane defined by the four sulfur atoms of two dialkyldithiophosphate ligands) may be in the vicinity of the phenyl group, and they are expected to be magnetically non-equivalent to the remaining two alkyl groups (which are below the basal plane). However, only one set of signals was observed for alkyl group protons (in ^{1}H NMR spectra) and alkyl group carbons (in ^{13}C NMR spectra), which indicates that the intramolecular interaction between phenyl group and alkyl groups (which are above the basal plane) is not significant, probably due to the larger size of bismuth. Similar results were observed in the case of $PhSb[S(S)P(OR)_2]_2$ compounds,[28] in which all alkyl groups are equivalent. However, in analogous arsenic compounds,[28] $PhAs[S(S)P(OR)_2]_2$, the splitting in the NMR signals of these alkyl groups (both in ^{1}H and ^{13}C) was observed. Since the size of Bi is larger than As and even with Sb, it is not surprising that the splitting of signals does not take place in the present case.

Antispermatogenic activity

Treatment of the test compounds [*O,O′*-diethyldithiophosphoric acid ligand, $(C_2H_5O)_2P(S)SH$ (group II) and its organobismuth(III) compounds, **2** (group III) and **7** (group IV)], did not affect the body weights of male rats during the period of exposure. The body weights of the experimental rats increased at the same rate as in the control rats (group I), except for the animals in group IV (compound **7**-treated rats). However, testicular mass decreased significantly ($p < 0.001$), in all the groups, as compared with control rats (Table 6). Weights of accessory sex glands were also reduced significantly (Table 6). In the ligand and compound **7**-treated rats (groups II and IV), reduction in the accessory sex glands was more than that of control group and compound **2**-treated group (Table 6). Sperm motility was decreased by 62.42, 56.77 and 81.18% in groups II, III and IV, respectively, in

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Table 6. Effects of the test compounds on the body and organ weights

Treatment	Final body weight (g)	Testes (mg per 100 g body weight)	Epididymides (mg per 100 g body weight)	Seminal vesicles (mg/100 g body weight)	Ventral prostates (mg/100 g body weight)
Group I (control)	258.50 ± 3.95	1458.80 ± 15.42	720.15 ± 3.33	736.82 ± 3.65	459.38 ± 8.90
Group II (diethyldithio-phosphoric acid), 25 mg per kg body weight per day	250.00^{ns} ± 4.90	708.40^{**} ± 8.33	233.60^{**} ± 2.83	341.60^{**} ± 3.39	160.80^{**} ± 3.76
Group III (compound **2**), 25 mg per kg body weight per day	245.00^{ns} ± 3.35	628.99^{**a+} ± 3.66	263.70^{**a+} ± 2.09	622.61^{**a+} ± 12.05	194.67^{**a} ± 6.07
Group IV (compound **7**), 25 mg per kg body weight per day	237.50^{*} ± 2.79	478.00^{**a+b+} ± 8.12	254.86^{**} ± 5.91	321.15^{**b+} ± 18.65	136.55^{**ab+} ± 5.04

Values are expressed as mean ± SEM ($n = 10$); ns = non-significant.
Levels of significance: $^{*}p < 0.01$; $^{**}p < 0.001$ compared with group I; $^{a}p < 0.01$; $^{a+}p < 0.001$ compared with group II; $^{b}p < 0.01$; $^{b+}p < 0.001$ compared with group III.

comparison to control animals (Table 7). Sperm density was highly suppressed in the testes and cauda epididymides of all the treated rats (Table 7), when compared with the control group. The number of spermatozoa, in group IV animals, was lower than that in groups II and III (Table 7). The fertility index was decreased by 100% in group IV-treated rats, in comparison with control animals (Table 7). Concentration of testosterone and LH in the serum of group II, III and IV rats decreased significantly ($p < 0.001$). FSH concentration was also decreased significantly in all the treated groups in comparison with control animals (Table 7). In the transverse section of control rat testis, showing active spermatogenesis, interstitial cells are conspicuous (Fig. 3).

The testes of rats (treated with ligand, compound **2** and **7**) showed marked histopathological changes and spermatogenesis was disturbed. Testes showed disorganized seminiferous tubules. The tubular lumen had clear irregular spaces devoid of sperm. A microphotograph of testes of rats exposed to the ligand showed damaged seminiferous tubules. Normal architecture was distorted and disorganized (Fig. 4). The seminiferous tubules became irregular in shape and size, and were reduced in diameter. The lumen was occupied by damaged spermatogenic cells (Fig. 5). Treatment of compound **7** caused degeneration in spermatogenic cells and in Sertoli cells (Fig. 6).

The count of Sertoli cells decreased significantly ($p < 0.001$) in all the treated groups. The number of spermatogonia, primary and secondary spermatocytes and rounded spermatids was also decreased significantly ($p < 0.001$), following the

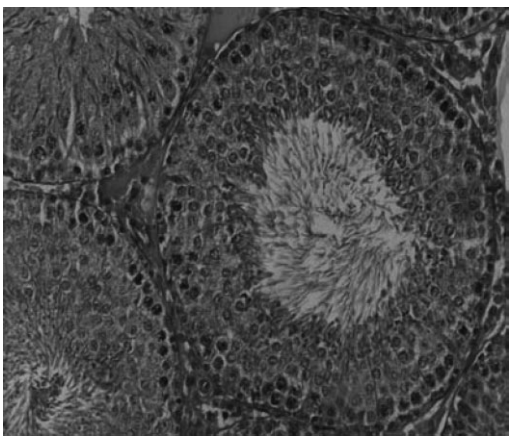

Figure 3. Transverse section of control rat testis showing active spermatogenesis. Interstitial cells are conspicuous. HEX 200.

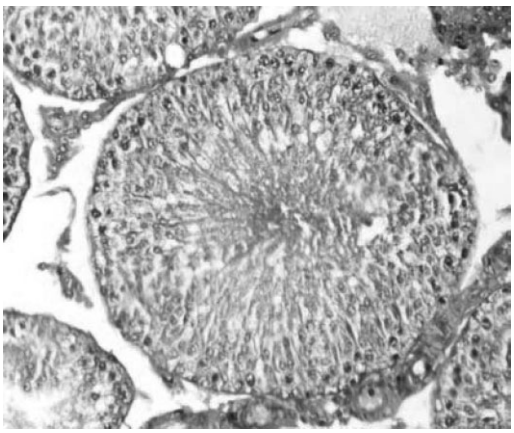

Figure 4. Microphotograph of ligand-treated rat testis illustrating decreased diameter of seminiferous tubule. A reduction could be seen in the number of germ cells. The lumen is filled with cellular debris. HEX 200.

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Table 7. Effects of the test compounds on sperm dynamics, fertility index and serum hormonal assay

Treatment	Sperm motility (%), cauda epididymides	Sperm density (million ml^{-1})		Fertility index (%)	Serum testosterone (mg ml^{-1})	LH (mIU ml^{-1})	FSH (mIU ml^{-1})
		Testes	Cauda epididymides				
Group I (control)	75.90 ± 1.58	6.32 ± 0.74	56.89 ± 1.20	97.15 (+ve)	5.52 ± 0.14	8.00 ± 0.28	20.20 ± 0.49
Group II (diethyldithio-phosphoric acid), 25 mg per kg body weight per day	28.52** ± 0.76	1.75** ± 0.22	35.96** ± 2.80	56.07 (−ve)	1.90** ± 0.16	3.47** ± 0.06	2.99** ± 0.58
Group III (compound **2**), 25 mg per kg body weight per day	32.81**^{a} ± 0.54	3.05** ± 0.38	36.27** ± 3.02	49.53 (−ve)	1.10**$^{a+}$ ± 0.02	1.90**$^{a+}$ ± 0.16	1.80** ± 0.34
Group IV (compound **7**), 25 mg per kg body weight per day	14.28**$^{a+b+}$ ± 2.70	1.25**^{ab} ± 0.12	12.92**^{ab} ± 4.86	100 (−ve)	1.06**$^{a+}$ ± 0.08	1.58**^{a} +0.10	1.63** ± 0.25

Values are expressed as mean ± SEM ($n = 10$).
Level of significance: * $p < 0.01$; ** $p < 0.001$ compared with group I; ^{a} $p < 0.01$, $^{a+}$ $p < 0.001$ compared with group II; ^{b} $p < 0.01$, $^{b+}$ $p < 0.001$ compared with group III.

administration of ligand, compound **2** and **7** in comparison with the control group (Table 8). Diameter of seminiferous tubules reduced significantly ($p < 0.001$), when compared with control animals (Table 8).

The pivotal role of androgens (mainly testosterone) in spermatogenesis, male fertility and phenotype is well established.[33,34] The quantitative production of sperm generally requires the presence of FSH, LH and testosterone.[35] The regulation of spermatogenesis depends primarily on an interaction between FSH and testosterone.[36] FSH plays a key role in the development of the immature testis by stimulating Sertoli cell proliferation and later progression of spermatogenesis.[37] Testosterone alone can maintain complete spermatogenesis, but the synergistic action of FSH is necessary to normalize quantitative aspects

Figure 5. Microphotograph of compound **2**-treated rat testis showing abnormal seminiferous tubule with vacuolization and arrest of spermatogenesis. The interstitial space is increased. Leydig cells are not prominent. HEX 200.

Figure 6. Microphotograph showing severely affected histoarchitecture of testis of compound **7**-treated rats. Vacuoles are visible in seminiferous tubule. Spermatogenesis is disturbed. Leydig cells are less conspicuous. HEX 200.

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Table 8. Effects of the test compounds on testicular cell population dynamics and diameter of seminiferous tubules

Treatment	Testicular cell counts (number/10 cross sections)						Seminiferous tubules diameter (μm)
	Sertoli cell	Spermatogonia	Primary spermatocytes		Secondary spermatocytes	Rounded spermatids	
			Preleptotene	Pachytene			
Group I control	3.95 ± 0.05	10.26 ± 0.20	18.92 ± 0.22	35.68 ± 0.26	58.88 ± 0.54	34.25 ± 0.17	250.58 ± 10.46
Group II (diethyldithio-phosphoric acid) 25 mg per kg body weight per day	$1.09^{**} \pm 0.04$	$7.54^{**} \pm 0.08$	$9.33^{**} \pm 0.04$	$10.86^{**} \pm 0.09$	$15.77^{**} \pm 0.25$	$23.98^{**} \pm 0.18$	$199.25^{**} \pm 3.95$
Group III (compound **2**), 25 mg per kg body weight per day	$1.06^{**} \pm 0.09$	$5.54^{**a+} \pm 0.12$	$6.28^{**a+} \pm 0.10$	$7.56^{**a+} \pm 0.08$	$16.43^{**} \pm 0.36$	$22.96^{**} \pm 0.45$	$145.00^{**a+} \pm 4.04$
Group IV (compound **7**), 25 mg per kg body weight per day	$1.07^{**} \pm 0.10$	$4.80^{**a+b+} \pm 0.08$	$6.30^{**+} \pm 0.28$	$4.81^{**a+b+} \pm 0.20$	$11.76^{**a+} \pm 0.28$	$22.24^{**a} \pm 0.57$	$140.25^{**a+} \pm 3.93$

Values are expressed as mean ± SEM ($n = 10$).
Level of significance: ** $p < 0.001$ compared with group I; ^{a} $p < 0.01$, $^{a+}$ $p < 0.001$ compared with group II; ^{b} $p < 0.01$ compared with group III.

of spermatogenesis.[38] LH stimulates the production of testosterone in Leydig cells.[39]

Administration of the test compounds decreased the serum testosterone concentration significantly in male rats, which might be due to the altered hormonal milieu of the testes,[40] and simultaneously testosterone inhibition affected the growth, maintenance and development of male reproductive organs. Significant reduction in the relative weights of the testes indicates low biosynthesis of androgens, which would be not sufficient to maintain the structural and functional integrity of male gonads and accessory organs.

Seminiferous tubular diameter is one of the most important indications of testosterone level.[41] Decrease in diameter of seminiferous tubules reflects tubular shrinkage. Seminiferous tubules contain Sertoli cells and germ cells. Spermatogenesis appears to be particularly dependent on the interaction between germ cells and Sertoli cells.[42] Therefore, reduced number of Sertoli cells could affect the production of sperm.

Reduced count of germ cells, i.e. spermatogonia, primary and secondary spermatocytes and spermatids, indicates low concentration of FSH and LH.[43] Decreased sperm production correlates well with decrease in germ cells, testicular weight[44] and disturbed testicular microenvironment.

Treatment of adult male rats with the test compounds caused a reduction in the sperm motility in the cauda epididymides. The epididymis contributes to the initiation of sperm motility by providing them with a unique environment along the length, by secreting proteins and ions.

Inhibition in sperm motility resulted in abnormal sperm functions, which ultimately gave rise to complete sterility.

EXPERIMENTAL

Care was taken to exclude moisture throughout the experimental manipulations. Solvents (E. Merck) were carefully dried by standard methods before use. Ph_3Bi (Aldrich) was used as received. *O*,*O*′-dialkyldithiophosphoric acids were prepared by the literature method.[45] Bismuth and sulfur were estimated by complexometric and Massenger's methods, respectively.[46]

Molecular weights were determined cryoscopically in benzene solution using a Beckmann's thermometer. Elemental analyses (C and H) were carried out on a Perkin Elmer Series II 2400 Shimadzu FT IR spectro-photometer on KBr optics as Nujol mull in the range 4000–400 cm^{-1}. NMR spectra were recorded in $CDCl_3$ solution in 5 mm NMR tubes on a Jeol FT AL 300 spectrometer operating at 300.40, 75.45 and 121.50 MHz for ^{1}H, ^{13}C and ^{31}P NMR spectra, respectively. ^{31}P NMR spectra were recorded using 85% H_3PO_4 as external reference.

Since all the new derivatives, **1–10**, have been prepared using similar methods, the synthetic method employed for one representative compound (**1**) is given in detail and the synthetic, physical and analytical data of all new derivatives, **1–10**, have been summarized in Table 1.

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Synthesis of derivative 1

A benzene solution (~30 ml) of Ph_3Bi (1.75 g, 3.97 mmol) was mixed with a benzene solution (~25 ml) of dimethyldithiophosphoric acid, $(CH_3O)_2P(S)SH$ (0.63g, 3.98 mmol), and the resulting reaction mixture was stirred for ~6 h. Thereafter, it was warmed gently for ~30 min to ensure the completion of reaction. After the completion of the reaction, the excess solvent was removed under reduced pressure and the residue was recrystallized using petroleum benzene (40–60 °C) to yield the analytically pure derivative (**1**) [1.88g (92%) yield].

Colony-bred male albino rats of Wistar strain (90–100 days old), weighing 200 g, were used for the study. Animals were acclimatized under standard laboratory conditions (12 h light/12 h dark cycle, 70 ± 10 °F and 30–70% relative humidity) and had *ad libitum* access to certified rodent chow and water for the duration of the study.

All the procedures involving animals were performed according to the guidelines of the Indian National Science Academy (2000) and the study was approved by the Institutional Animal Ethical Committee of University of Rajasthan, Jaipur, India.

Each test compound was dissolved in olive oil (vehicle) and was given by oral gavage, employing the 25 mg per kg body weight per day dose level for similar durations. The duration of treatment was set for 60 days. Control animals received a similar volume of vehicle (0.5 ml olive oil per day).

Proven fertile male rats were divided into four groups of 10 animals in each group. In group I, rats received vehicle only; in group II, rats were given diethyldithiophosphoric acid ligand [i.e. $(C_2H_5O)_2P(S)SH$] (25 mg per kg body weight per day); in group III, rats were given compound **2** (25 mg per kg body weight per day); and in group IV, rats were given compound **7** (25 mg per kg body weight per day).

The fertility test of each experimental animal was done before and on the 55th day of treatment, by a natural mating exposure test. The male rats were cohabitated with proestrous females in 1 : 2 ratios. Vaginal smears were checked for positive mating. The inseminated females were separated and the number of litters delivered recorded.

Individual body weight data were recorded on the first day of the treatment, weekly thereafter and on the day of autopsy. Experimental male rats were autopsied on day 61 using light ether anesthesia. Blood was collected from cardiac puncture; serum was separated at 3000 rpm and stored at −20 °C for the serum hormonal assay.

Sperm motility in cauda epididymides and sperm density in testes and cauda epididymides were observed by the method of Prasad *et al*.[47] Reproductive organs, i.e. testes, epididymides, seminal vesicles and ventral prostates, were removed, trimmed free from fat and connective tissues and weighed. Testes were placed into Bouin's fixative for 48 h. After immersion in the fixative, slabs, perpendicular to the longest axis of the organ, were then cut and dehydrated in graded series of alcohol and embedded in paraffin and bee's wax; 5 μm-thick sections were cut and stained with hematoxylin and eosin.

Histological changes were studied at microscopic level and were supplemented by histometric study.[48] Histometric study of seminiferous tubules and germ cells was performed using a Camera lucida (eye piece graticules) and calibrated with a micrometer. The relative number of each variety of germ cells of the seminiferous epithelium cycle was ascertained for the histomorphological changes, according to the standard method.[49] The group count of all the germ types was designated[50] by Abercrombie.

Results are expressed as arithmetic means with their standard error (±SEM). Results were analyzed by one-way analysis of variance (ANOVA). The limit of significance was set at $p < 0.05$. ANOVA was followed by unpaired Student's *t*-test.

CONCLUSIONS

The results of this study indicate that the test compounds are capable of suppressing the process of spermatogenesis by inhibiting the serum testosterone levels and the concentration of FSH and LH. The fertility of male animals was suppressed by 56.07, 49.53 and 100% in diethyldithiophosphoric acid ligand, compound **2** and compound **7** treated rats, respectively.

Compound **7** is the most potent compound to produce sterility in male rats among the test compounds used in this comparative study.

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