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Research Article

SYNTHESIS, CHARACTERIZATION, ANTIFERTILITY AND BIOCHEMICAL STUDIES OF ORGANOSILICON COMPLEX DERIVED FROM SCHIFF BASE LIGAND

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ABSTRACT

The present investigation is an attempt to synthesize and characterize the ligand (N ′ -[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester) and its organosilicon complex and also to determine their possible antifertility potentials. For this, ligand was prepared by the condensation of S-benzyl dithiocarbazate with 3-acetyl coumarin and this ligand reacts with organosilicon (IV) chloride to yield particular complex. The structure of the compound has been elucidated by physicochemical and spectral (IR, ^{1}H NMR, ^{13}C NMR, ^{29}Si NMR) studies. In male rats, administration of ligand and its organosilicon complex at a dose level of 20 mg/kg b.wt./day for 45 days caused a significant decline in the weight of reproductive organs. The treatment also diminished the sperm motility and density significantly. Fertility test showed negative fertility in treated rats. A significant reduction in glycogen, protein and sialic acid and an increase in cholesterol content of testes were noticed. Present study indicated that ligand (N ′ -[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester) and its organosilicon complex showed antifertility effects on male reproductive functions.

Keywords: Organosilicon complex, Spectral Studies, Biochemical studies.

INTRODUCTION

Control of fertility constitutes a global health issue, as over population have both major personal and societal impact and it is necessary to control it on the time. As we know the entire available contraceptive in the market are not safe, mostly they are steroid in nature and they have more or little hazardous side effect. Attention has now been focused on safe chemicals for possible contraceptive effect[1-3]. Chemicals can interfere with hormonal control, the male reproductive tract and directly alter the male reproductive tract function[4]. The chemical control of fertility in the male has received attention since quite some time and a large number of synthetic compounds have been tested for their antispermatogenic and antiadrogenic effects. Organosilicon compounds of sulfur containing ligands have attracted much attention recently due to their biological importance. The sulfur containing ligands are well known for their anticarcinogenic, antibacterial, and antifungal effect. It has been reported that the activity of sulfur-containing ligands increases on complexation[5-7]**.** The aim of present study was to evaluate the antifertility effects of the **organosilicon** complex in male albino rats.

EXPERIMENTAL

In view of the moisture sensitive nature of the starting materials, the synthetic reaction was performed under moisture free conditions. The chemicals used were of reagent grade. Solvents (E

Merck) were dried by standard methods before use. In the present investigation, ligand and its complex have been synthesized in our laboratory.

Synthesis of Ligand

Preparation of the S-benzyldithiocarbazate

A cold solution of KOH (11.4 g) in 90% ethanol (70 mL) added hydrazine hydrate (10 g) with constant stirring. A solution of CS_2 was added drop wise with continuous stirring over a period of one hour and temperature of the reaction mixture was kept below 10 °C. During the addition, the oily layer so formed was separated and dissolved in cold 40% ethanol (60 mL). The solution was cooled in a freezing mixture and benzyl- chloride (25 g) was added drop wise while stirring for two hours. The white solid was separated by filtration, washed with water and dried in air. The crude product was recrystallized from benzene (M.P.-125° C).

Preparation of the 3-acetyl coumarin s - benzyldithiocarbazate

For the preparation of the ligand, 3-acetylcoumarin was mixed with **S-benzyldithiocarbazate** in 1:1 molar ratio and refluxed on a water bath for five-six hours. Alcohol was used as the solvent. The solution then concentrated under reduced pressure. On cooling overnight, crystals separated out which were further purified by washing with ethanol and finally recrystallized with acetone. (Fig. 1)

Synthesis of the complexes

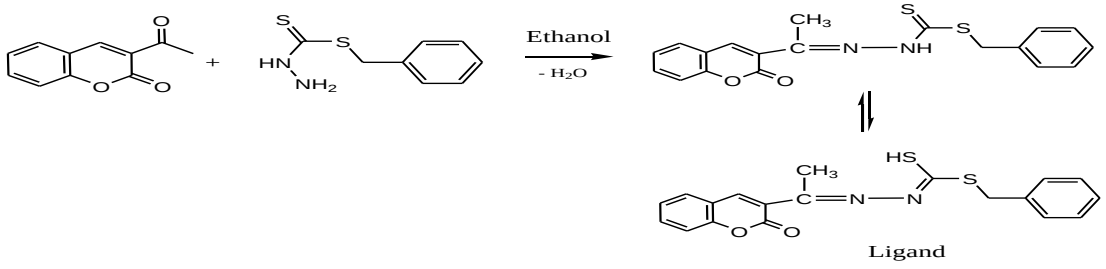
For the preparation of the complexes, methanolic solution of Ph_2SiCl_2 was mixed with the corresponding sodium salt of the ligand in equimolar ratio using methanol as a solvent. The solution was refluxed for a period of 15–17 hours. The white precipitate of sodium chloride formed during the course of the reaction was removed by filtration, and the filtrate was dried under reduced pressure. The resulting product was repeatedly washed with a mixture of methanol and *n*-hexane (1:1) and then finally dried under vacuum. The purity was further checked by thin layer chromatography with silica gel-G using DMSO as a solvent.

Analytical methods and physical measurements

Nitrogen and sulfur were estimated by the Kjeldahl's and Messenger's methods[8], respectively. Silicon was determined gravimetrically as SiO_2. The conductance was measured with a conductivity bridge type 304 Systronics model, and the molecular weights were determined by the Rast Camphor method. Infrared (IR) spectra were recorded on a Perkin-Elmer 577 Grating Spectrophotometer in the range 4000-200 cm^{-1}, as Nujol mulls using CsI Cell. ^1H NMR spectra were recorded in DMSO-d_6 alongwith ^{13}C and ^{29}Si NMR spectra, using TMS as the internal/ external standard. The physiochemical properties and analytical data of the ligand and its complex are listed below

[1-(2-oxo-2H-chrome-3-yl-ethylidene] hydrazine carbthionicacid benzyl ester (LH).

Compound	Colour	M.P.	Found (Calcd.) (%)					Mol.Wt Found (Calcd.)
			C	H	N	S	Si	
Ligand ($C_{19}H_{16}N_2O_2S_2$)	Reddish orange	155°C	60.91 (61.93)	4.19 (4.38)	6.56 (7.60)	16.54 (17.40)	-	366.85 (368.47)
Complex	Brown	170°C	68.56 (71.91)	3.91 (4.86)	4.23 (5.41)	10.47 (12.38)	4.28 (5.42)	581.36 (585.23)



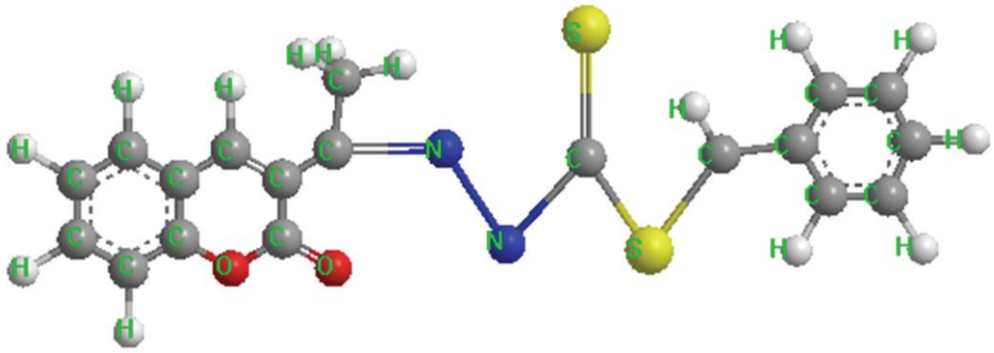


Fig. 1: Structure of the Ligand

Animal Model Used

Fifteen healthy adult male albino rats (*Rattus norvegicus*) of Wistar Strain of an average body weight 150-200 gms with proven fertility have been employed for experimentation. The animals were kept in clean polypropylene cages covered with chrome plates grills and maintained under controlled environmental conditions (12-h light: 12-h dark). The animals were mostly maintained on standard pellet diet procured from Ashirwad Industries, Chandigarh and occasionally on germinated/ sprouted gram and wheat seeds as an alternative feed. They were given clean water *ad libitum*. Animal procedures were approved by the Institutional Ethical Committee and conducted in compliance with the Guidelines for Care and Use of Animals for Scientific Research[9].

Calculation of Median Lethal Dose (LD_{50})

In the present study, ligand and its organosilicon complex were given orally with the help of hypodermic syringe having pearl point needle. Five-five animals were tested for ligand and complex. Control rats were given equivalent amount of vehicle. Poisoning symptoms and mortality were observed daily for three days following the treatment. Results of the toxicity were analyzed statistically for the determination of LD_{50} values of the compound[10].

Treatment Protocol

Animals were divided into three groups having 5 animals each. Group I animals were kept as control and were administered olive oil only. Animals of Group II received ligand emulsified in olive oil at a dosage of 20 mg/kg b. wt./day for 45 days and Group III were administered organosilicon complex emulsified in olive oil at a dosage of 20 mg/kg b. wt./day for 45 days.

Sacrification schedule and body & organ weight measurements

At the end of the experimentation, the rats were weighed and sacrificed under light ether anesthesia. The initial and final body weights of the animals were recorded. The male reproductive organs were removed, weighed and processed for detailed biochemical and histopathological studies.

Sperm Dynamics

The sperm motility in cauda epididymis and density of testicular and cauda epididymis was determined[11].

Haematological Profiles

Haemoglobin[12], Haematocrit[13] , Total erythrocyte count(TEC), Total leukocyte count (TLC)[14] and Blood urea[15] were determined from the blood collected directly from the heart at the time of sacrification according to standard methods.

Biochemical Parameters

The total protein[16], sialic acid[17], glycogen[18] and cholesterol[19] were assessed in testes.

Statistical Calculations

The data obtained from the above experiments were subjected to statistical analysis. All the values of body and organs weights, sperm dynamics, blood toxicity profiles and tissue biochemistry were expressed in terms of mean ± SEM. The data were analyzed statistically by using Student's "t" test and the significance of differences was set at $P < 0.01$ and $P < 0.001$[20].

RESULTS

Spectral Analysis

Electronic Spectra

The bands in the electronic spectra of the ligand N′-[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazine carbodithionic acid benzyl ester and its complex appear at 260 and 324 nm, assigned to π - π * electronic transitions within the benzene ring. Another band observed at 365 nm in the spectrum of the ligand is due to the n- π * transitions of the azomethine (> C = N) group which undergoes a blue shift in the complex due to the polarization within the >C=N chromophore caused by the silicon–ligand electron interaction during the chelation. The shift of this band in the spectrum of the complex suggests the coordination of nitrogen to silicon atom.

IR Spectra

The IR spectra of the ligand displays two sharp bands around 3450 – 3300 cm^{-1} and 3550 – 3400 cm^{-1}, assignable to ν sym and ν asym vibrations of the NH_2 group respectively. These bands remain unchanged in the silicon (IV) complex. Furthermore, strong bands at 3250 cm^{-1} due to ν (NH) vibrations are observed. These bands disappear in the complex. A sharp and strong band at 1625 cm^{-1} is due to the azomethine group of the ligand. In the IR spectrum of the complex this showed a lower shift of the order 20 cm^{-1} indicating the coordination of the azomethine nitrogen to the silicon atom. One strong band located at 1050 cm^{-1} in the ligand was attributed to ν (C = S) moiety, which disappears in the case of complexes. These data on comparison with the spectrum of the ligand suggested that the azomethine nitrogen and thiolic sulfur atom of the ligand are involved in coordination with the silicon ion. A doublet at ~ 2950 and ~ 2900 cm^{-1} is assigned to symmetric and asymmetric vibrations of S-CH_2-C_6H_5 grouping.

^{1}H NMR Spectra

The ^{1}H NMR spectral data of the ligand and its corresponding organosilicon (IV) complex were recorded in DMSO-d 6 with TMS as an internal standard. The ^{1}H NMR spectrum of the ligand exhibits -CH_2 -proton signals at δ 4.15 – 4.16 ppm and aromatic proton signals at δ 6.38 – 7.50 ppm, and these remain at the same position in the spectrum of the metal complex. The proton of the -NH group of the ligand gives a signal at δ 10.20 ppm, which is absent in the spectrum of silicon complex indicating the chelation of the ligand moiety to silicon with the sulfur atom. The proton signals of the methyl groups appear at δ 1.15 – 1.19 ppm in the organosilicon (IV) complex.

^{13}C NMR Spectra

^{13}C NMR spectra were recorded in dry methanol using TMS as the internal standard and these spectra also support the authenticity of the proposed structures. The considerable shifts in the positions of carbons of the silicon complex attached to N and S, respectively, clearly indicate that the nitrogen and sulfur of the ligand group participate in the complexation reaction. The signals due to the carbon atoms attached to the thionic and azomethine groups in the ligand appear at 176.20 ppm and 164.25 ppm, respectively. However, in the spectrum of the corresponding silicon (IV) complex, these appear at ~ 168 ppm (thionic group) and at ~ 160 ppm (azomethine group), respectively. The considerable shifts in carbons attached to S and N indicate the involvement of sulfur and nitrogen atoms in coordination.

^{29}Si NMR Spectra

The signal at δ 93 ppm is indicative of pentacoordinated state of the silicon atom in the ^{29}Si NMR spectrum of the complex of $Ph_2SiCl(L)$[21].

Effects of ligand and complex administration on body and organ weight measurements

The results presented in Table 1 clearly revealed that the weight of testis and epididymis of the treated rats decreased significantly and non significant changes in **Vas deferens & Seminal vesicle** weight ($P < 0.01$ and $P < 0.001$) in comparison to the control group and a normal decrease in the body weight was found in both the treated as well as control groups.

Effects of ligand and complex administration on sperm dyanamics

A significant decrease in the sperm density in testes and cauda epididymis was observed ($P < 0.01$ and $P < 0.001$) after ligand and complex treatment (Table 2). Also the sperm motility in cauda epididymis was severely impaired ($P < 0.01$ and $P < 0.001$). The fertility test showed 70% and 95% negative fertility after administration of ligand and complex respectively (Table 2).

Effects of ligand and complex administration on the blood analysis

Haematocrit value, Total erythrocyte count (TEC) and Blood urea of treated groups were found to be decreased significantly ($p \leq 0.01$ and $p \leq 0.001$)

while Total leukocyte count (TLC) & Haeomoglobin concentration, showed non-significant changes after treatment (Table 3).

Effects of ligand and complex administration on the testicular biochemistry

A marked reduction in sialic acid, protein and glycogen content of testes was observed ($P < 0.01$ and $P < 0.001$) whereas testicular cholesterol increased ($P < 0.01$ and $P < 0.001$) significantly in dose dependent fashion (Table 4).

DISCUSSION

The present study revealed that administration of ligand (3-acetyl coumarin S-benzdithiacarbazate) and its organosilicon complex at dose level of 20 mg/kg/b.wt./day for 45 days to male rats resulted in antifertility. The weight of testes is largely dependent on the mass of differentiated spermatogenic cells and reduction in weight of testes, as revealed in this study may be due to reduced tubule size, decreased number of germ cells, and elongated spermatids[22]. The decreased weight of testes may also be due to spermatogenic arrest and inhibition of steroid synthesis by Leydig cells [23-25]. The reduction in weight of accessory sex organs may be due to low availability of androgens or antiandrogenic activity of ligand and complex[26-28].

Sperm motility is considered one of the most important parameters evaluating the sperm fertilizing ability. The motility of sperm in cauda epididymis indicates less ability of sperm to interact with the oocyte plasma membrane[29]. Decreased sperm density in the epididymis is an indicator of reduced spermatogenesis as a result of the antispermatogenic nature of any agent[30,31]. Low caudal epididymal sperm density may be due to alteration in androgen metabolism[32-34]. The physiological and biochemical integrity of epididymis are dependent on androgens. The negative fertility may be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical milieu of cauda epididymis[35].

Administration of ligand and complex results into the reduction of erythrocytes count which may be due to inhibition of production of red blood cells in bone marrow[36]. The decline in erythrocyte counts also may be due to the disruptive action of the ligand and complex on the erythropoietic tissues as a result of which the viability of the cells might be affected[37]. Haematocrit percent may be reduced due to decrease in the size of RBC[38]. Increase in the rate of erythrocyte destruction could be the possible reason for reduction in the number and size of erythrocytes[39]. Treatment with ligand and complex also changes the biochemical parameters of the reproductive tract. A fall in glycogen level may be due to interference in glucose metabolism, which may affect the maturational process of spermatozoa and their motility. Inhibition of glycogen synthesis eventually decreases spermatogenesis process[40-42]. Decrease in the testicular sialic acid and protein concentration is due to the antispermatogenic activity or reduced androgen production[43] or may be due to the impairment in the functional ability of spermatozoa[44].

Increased concentration of testicular cholesterol in testes may be the result of its non-utilization leading to the reduction of the production of Testosterone, the main hormone involved in the control of fertility of animals including rats[45].

Table 1: Body and organ weight measurements of ligand and complex treated male rats

Treatment	Body Weight		Organ weight			
	Initial	Final	Testis	Vas deferens	Seminal vesicle	Epididymis
	gm.		mg/100g body wt.			
Group I Control	208±12.7	180±16.4	999.60±2.71	139±1.9	621.40±17.3	360.80±7.7
Group II Ligand 20 mg/kg b.wt./day	206±19.4^{ns}	169±11.5^{ns}	982.13±1.46*	134±1.1^{ns}	577.65±11.2^{ns}	315.47±5.8**
Group III Complex 20 mg/kg b.wt./day	201±10.4^{ns}	164±13.5^{ns}	978.21±1.80*	130±0.9^{ns}	556.60±12.5^{ns}	319.32±5.76*

Mean ±SEM of 5 Animals) Groups II and III compared with group I

ns = non-significant

* = significant (P<0.01)

** = highly significant (P<0.001)

Table 2: Sperm motility and fertility test of ligand and complex treated male rats

Treatment	Sperm motility (%)	Sperm density (million/ml)	Fertility test (%)
Group I Control	73.60±4.34	6.03±.51	100%(+)ve
Group II Ligand 20 mg/kg b.wt./day	53.31±4.76*	3.94±.70*	71%(-)ve
Group III Complex 20 mg/kg b.wt./day	38.29±4.15**	2.26±.43**	95%(-)ve

Mean ±SEM of 5 Animals) Groups II and III compared with group I
ns = non-significant
* = significant (P<0.01)
** = highly significant (P<0.001)

Table 3: Blood analysis of ligand and complex treated male rats

Treatment	TEC	TLC	Hb	Hcrit	Blood urea
	million/mm³	cells/mm³	gm%	%	mg/dl
Group I Control	6.25±.19	8503±6.04	12.97±.87	49.54±2.31	50.61±3.34
Group II Ligand 20 mg/kg b.wt./day	4.71±.15**	8480±5.14ns	10.45±.83ns	38.31±1.71*	65.52±3.09**
Group III Complex 20 mg/kg b.wt./day	3.57±.23**	8473±3.73ns	8.71±.82ns	30.12±1.67*	79.93±3.67*

Mean ±SEM of 5 Animals) Groups II and III compared with group I
ns = non-significant
* = significant (P<0.01)
** = highly significant (P<0.001)

Table 4: Biochemical changes in testes after ligand and complex treatment

Treatment	Cholesterol	Glycogen	Sialic acid	Protein
	mg/g			
Group I Control	8.01±.04	2.99±.20	4.92±.24	358.4±6.67
Group II Ligand 20 mg/kg b.wt./day	10.19±0.19*	2.08±.03**	3.53±.22**	292.2±6.81*
Group III Complex 20 mg/kg b.wt./day	10.84±0.12*	1.78±.08**	3.03±.18**	195.6±2.27*

Mean ±SEM of 5 Animals) Groups II and III compared with group I
ns = non-significant
* = significant (P<0.01)
** = highly significant (P<0.001)

CONCLUSION

On the basis of the spectral studies, the ligand coordinate to metal monobasic bidentate giving five-coordinate geometries around the metal. The synthesized compound was characterized by elemental analysis, UV-visible, IR and NMR spectroscopy, Thus, it may be concluded that orally administered ligand and its organosilicon complex produced antifertility effects or may be a potential source for the development of an antifertility drug for males because of their antispermatogenic nature and some antifertility effects on reproductive organs.

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