REPORT

Complexation of β-sitosterol with tris (dibenzylideneacetone) dipalladium and its anti-microbial activity

Talat Mahmood[1*], Yasmeen Bibi[1], Raana Zafar[2], Aneela Wahab[1], Iffat Mahmood[1], Nuzhat Arshad[1] and Sikandar Khan Sherwani[1]

[1]Federal Urdu University of Arts, Sciences and Technology, Gulshan-e-Iqbal Campus, Karachi, Pakistan
[2]Karachi Medical and Dental College, Karachi, Pakistan

Abstract: β-sitosterol is a naturally occurring plant sterol (phytosterol) present in many fruits and vegetables. Scientific research has proven that β-sitosterol is helpful in maintaining the proper functioning of our body. Previously we described the complexation of β-sitosterol with trace metals (Mahmood *et al*., 2013). Trace metals after the formation of complex unable to absorb in the body and hence eliminated out from the body thus reducing metal toxicity (Marsha, 1996). The present article describes the complexation of β-sitosterol with Palladium (Pd) metal. Palladium is a toxic metal and due to polluted and hazardous environment traces of this metal can be transferred into the body, which is harmful for human health. Our aim is to make Pd-sterol complex so that this toxic metal (Pd) does not absorb in the body and hence excreted out from the body in the complex form. In order to form this complex β-sitosterol (Ib) is reacted with Tris (dibenzylideneacetone) dipalladium or [Pd_2 $(DBA)_3$] (Ia) in 2:1 ratio in an inert atmosphere and dimethylformamid (DMF) added as a solvent. The resulting complex [Pd_2 $(DBA)_3$.(β-sitosterol) (**Ic**) was identified by various spectroscopic techniques such as IR, Mass and ^{1}H-NMR. This new organo metallic complex (**Ic**) also showed significant antibacterial and antifungal activity. The present work revealed that Pd-sterol complex does not only reduce metal toxicity but also helpful in minimizing bacterial and fungal infections present in the body. Our research also concluded that we must take plenty of fruits and vegetables in our diet so that natural plant sterol such as β-sitosterol can enhance our defense mechanism and maintain other functions of our body.

Keywords: β-sitosterol; tris (dibenzylideneacetone) dipalladium; organometallic; antibacterial; antifungal

INTRODUCTION

The organo metallic reagent Tris (dibenzylideneacetone) dipalladium or [Pd_2 $(DBA)_3$] is a zerovalent palladium complex discovered in 1970 (Takahsashi *et al*., 1970). It is prepared from dibenzylideneacetone and sodium tetrachloropalladate. The complex has a dark violet color and it is commonly recrystallized from chloroform. The C=C bonds of dibenzylideneacetone ligand coordinate separately to two Pd atoms. In Pd_2 $(DBA)_3$ the Pd-Pd distance is 3.245 Å (Ukai *et al*., 1974). This reagent is used as a catalyst for different types of coupling reactions such as Negishi coupling, Suzuki coupling also used for asymmetric allylic alkylation and for Buchwald-Hartwig amination (Sheng *et al*., 2008). β-sitosterol is a naturally occurring plant sterol (phytosterol). It is present in fruits, vegetables, nuts and seeds. Foods such as rice bran, wheat germ corn oils, soybeans and peanut butter also contain β-sitosterol in its natural form. β-sitosterol is effective in lowering cholesterol, also use to boost the immune system and lessen the effect of the flu or common cold (Matsuoka *et al*., 2008). HIV/AIDS patients use it in the hope that it slow down the disease. It is also used to reduce the swelling in arthritis sufferers. Many people use β-sitosterol for relieving the symptoms of menopause, certain allergies, migraine, headache, tuberculosis. It also acts as an antimicrobial, anticancer, antidiabetic, anti-inflammatory and antipyretic agent (Normen *et al*., 2001). The remarkable pharmacological properties of β-sitosterol initiate us to start working on it and for this purpose we decide to make organometallic compound of it, as β-sitosterol is present in plants and vegetables it transfer in the body so if any toxic metal present in the body form complex with it and hence excreted out from the body in the complex form (Marsha, 1996).

In this paper we are presenting the preparation of $Pd_2(DBA)_3$.(β-sitosterol) complex (**Ic**) and its structure elucidation with different spectroscopic techniques such as IR, Mass and ^{1}H-NMR Furthermore we are also presenting the antibacterial and antifungal activity of this newly formed complex (**Ic**).

MATERIAL AND METHOD

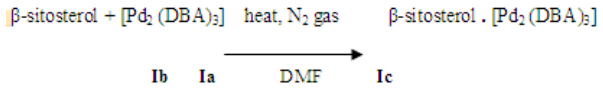
The reagents [Pd_2 $(DBA)_3$], β-sitosterol and anhydrous dimethyl form amide (DMF) of analytical grade (Merck) were used without further purification. All glassware was

*Corresponding author: e-mail: talatmhmd@gmail.com

washed properly and then rinsed with distilled deionized water and finally dried in oven before used. Melting point was determined with a Gallenkamp melting point apparatus and is uncorrected. Hot plate stirrer (lab Tech) with bead was used for stirring. Thin layer chromatography (TLC) was performed on pre-coated silica gel GF-254. IR spectra were recorded on a Jasco-302-A spectrophotometer. The mass spectrum was scanned on a Jeol-JMS HX-110 mass spectrometer. The ^{1}H-NMR spectrum was recorded on a Brucker spectrometer operating at 500MHz. The chemical shift values are reported in δ (ppm) relative to $SiMe_4$ (TMS) as an internal standard. The coupling constant (*J*) is given in Hz.

Procedure for the formation of Pd_2 (DBA)$_3$(β-sitosterol) complex

In order to make complex (**Ic**) different ratios of β-sitosterol (Ib) and [Pd_2 (DBA)$_3$] (Ia) were taken (1:1,1:2, 1:3 and 2:1, 2:2). At all ratios complex (**Ic**) occurred but the stable complex was formed only in 2:1 ratio. The solution of β-sitosterol (Ib) (1x 10^{-3} moles /50ml) and [Pd_2 (DBA)$_3$] (Ia) (5x10^{-4}moles/25ml) were taken in 2:1 ratio in a conical flask and mixed with magnetic stirrer and then 1 ml anhydrous dimethyl formamide (DMF) was added as a solvent. During the reaction to provide an inert atmosphere nitrogen gas pass into the reaction. The mixture was stirred upto10-15 minutes until it starts boiling and its color changes from violet to dark brown. The dark brown precipitate was filtered and then washed with water and acetone and then dried in vacuo. After drying the precipitate was dissolved in hot chloroform and then filtered to give dark brown crystals of (**Ic**).



Biological Assay

Preparation of media for antimicrobial activity

Muller Hinton agar and Muller Hinton broth were used as the media for culturing bacterial strains and Sabourd dextrose agar (SDA) (Smyth *et al*., 2011) was used as the media for fungal strains.

Screening of antibacterial activity

Antibacterial activity was determined by using the agar-well method. The Autoclaved Muller Hinton broth was used to refresh the bacterial culture, later well were punched into Muller Hinton Agar and 10 micro liters of culture were poured into the wells (Perez and Bazerque 2009). The 10 mg/ml of (**Ic**) was tested for *antibacterial active.* All plates were incubated at 28±2^{o}C for 24-48 h and after the incubation diameter of zone of inhibition was noted by Vernier caliper. Gentamicin antibiotic was used as a standard.

Screening of antifungal activity

Antifungal activity was determined by using the agar-well method. Autoclaved distilled water was used for the preparation of fungal spore suspension and transfer aseptically into each SDA plates (Wuthi-udomlert and Vallisuta 2011). 10mg/ml of (**Ic**) was tested for antifungal activity. All plates were incubated at 28±2oC for 24-48h and after the incubation diameter of zone of inhibition was measured by vernier caliper. Gresiofulvin antifungal agent was used as a standard.

RESULTS

The reagent Pd_2 (DBA)$_3$ itself is violet in color complexation of β-sitosterol with Pd_2 (DBA)$_3$ was indicated by observing the color change from violet to brown. M.p. and elemental analysis of the newly formed Pd_2 (DBA)$_3$.(β- sitosterol) complex (**Ic**) is presented in table 1.

This complex appears as brown needles, fairly stable in air in the solid state soluble in $CHCl_3$ and hexane. The complex formation was also confirmed by TLC on which it showed a pure single spot (TLC system hexane: ethylacetate 8:2). The infrared (IR) (table 1), mass, and ^{1}H-NMR (table 2) spectrum of complex (**Ic**) also confirmed its formation. The results of antibacterial and anti-fungal activity of the complex (**Ic**) are presented in table 3.

DISCUSSION

The IR spectrum of the complex (**Ic**) showed the characteristic peaks of OH (broad), C=O, olefinic (C=C) and aromatic (C=C). Whereas the IR spectrum of β-sitosterol (Ib) showed only the characteristic peaks of OH (broad) and olefinic (C=C). This IR data indicate that DBA is present in the complex (**Ic**).

The mass spectrum of the complex (**Ic**) does not show the molecular ion peak however it clearly indicate the presence of DBA in the complex (**Ic**) by showing the fragment of (C_6H_5- CH=CH)$_2$ CO^+ at *m/z* 234 (100%) (Ukai *et al*., 1974).

The presence of DBA in the complex (**Ic**) is also confirmed by its ^{1}H-NMR spectrum which showed the multiplet of the aromatic protons in the region δ 7.40- δ 7.60 whereas the olefinic protons appear as a doublet at δ 7.10 and δ 7.70 with the *J* value of 16 Hz showing its trans configuration the other ^{1}H-NMR data is similar to that of β-sitosterol (Ib) (Wahab *et al*., 2012). ^{1}H-NMR data of β-sitosterol (Ib) and the complex (**Ic**) are presented in table 2.

The above results indicate that Pd_2 (DBA)$_3$ is attached to β-sitosterol in the form of adduct that is Pd_2 (DBA)$_3$.(β-sitosterol).

The results of biological activity indicate that the complex (**Ic**) is highly active against Gram positive

Table 1A: Physical parameters of the complex (**Ic**)

Complex	Colour	Yield (%)	M.P. (^oC)	Elemental analysis (Calcd) (%)		
				C	H	O
Ic	Brown	85	135-137	65.05 (65.10)	5.05 (5.00)	5.08 (5.01)

Table 1B: Infrared vibrational frequencies (cm^{-1}) of (Ib) and (**Ic**) (KBr)

Ib ν (cm^{-1})	Ic ν (cm^{-1})	Functional group
3400	3350	OH (broad)
1650	1650	C=C
	Peaks of DBA	
	1625	C=C (conjugation)
	1590 & 1475	C=C (aromatic)
	1650	C=O (conjugation)
	970	CH (trans)

Table 2: ^{1}H-NMR data of (Ib) and (**Ic**) at 500 MHz in $CDCl_3$: δ in ppm *J* in Hz

Proton	Ib	**Ic**
H-3	3.51 (1H, m)	3.50 (1H, m)
H-6	5.30 (1H, br, s)	5.33 (1H, br, s)
CH_3-18	0.67 (3H, s)	0.66 (3H, s)
CH_3-19	0.99 (3H, s)	0.98 (3H, s)
CH_3-21	0.92 (3H, d, *J*=6.2 Hz)	0.90 (3H, d, *J*=6.0 Hz)
CH_3-26	0.81 (3H, d, *J*=6.5 Hz)	0.80 (3H, d, *J*=6.7 Hz)
CH_3-27	0.78 (3H, d, *J*=6.2 Hz)	0.77 (3H, d, *J*=6.5 Hz)
CH_3-29	0.84 (3H, t, *J*=7.2 Hz)	0.83 (3H, t, *J*=7.0 Hz)
	-	Peaks of DBA:
Aromatic protons	-	7.40-7.60 (10H, m)
Olefinic protons	-	7.10 (2H, d, *J*=16Hz)
Olefinic protons	-	7.70 (2H, d, *J*=16Hz)

Table 3: *In vitro* Antibacterial and Antifungal Activity of complex (**Ic**) values are zone of inhibition (mm and an average of triplicate)

Gram positive bacteria	Zone of inhibition in mm (mean ± S.D)		Fungal isolates	Zone of inhibition in mm (mean ± S.D)	
	Ic	Standard Gentamicin		**Ic**	Standard Gresiofulvin
Bacillus cereus	11 ±0.6	>15	*Aspergillus flavus*	18±0.6	>12
Staphylococcus aureus	15±0	>15	*Aspergillus niger*	15±0.6	>12
Staphylococcus aureus AB 188	17±0	>15			
Staphylococcus epidermidis	15±0.6	>15			
Gram negative bacteria					
Enterobacter aerogenes	nil	>15			
Escherichia coli	nil	>15			
Proteus mirabilis	nil	>15			
Pseudomonas aeroginosa	nil	>15			
Salmonella typhi	nil	>15			
Shigella dysenteriae	nil	>15			

bacteria and it showed remarkable activity against two fungal strains (*Aspergillus flavus* and *Aspergillus niger*).

CONCLUSION

All the above discussion conclude that metal-sterol complex is beneficial to human health because it does not

only reduce the metal toxicity but also help in minimizing the bacterial and fungal infection so it is suggested that fruits and vegetables must be taken in diet so that β-sitosterol enters in the body and have activate our defense mechanism.

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