

Preparation, Characterisation, and Antitumour Studies of 1,3-Cyclohexanebis(2-Aminobenzoic Acid) and its Palladium Complex.

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Abstract

1,3-cyclohexanedione reacts with 2-aminobenzoic acid (anthranilic acid, abbreviated as AA) in a 1:2 ratio forming a tetradentate ligand 1, 3-Cyclohexanebis (2-aminobenzoic acid)(abbreviated as 1,3CHAA).. The ligand forms a square planar complex with Pd(II) ion, in the ratio 1:1. The structure of the ligand and its palladium(II) complex (Pd1,3CHAA) were, characterized by standard analytical methods. The percentage of elements is calculated by CHN analysis and the amount of Palladium by standard method. Magnetic susceptibility is determined by the Guoy method. In addition, the infrared spectra, electronic spectra, and conductance measurements are investigated for structural elucidation of the compound. The complex is found to be a diamagnetic square planar complex. 1, 3-Cyclohexanebis(2-aminobenzoic acid) and its Pd (II)chelates are analyzed for anticancer activity by in-vitro cytotoxic assay using Daltons Lymphoma Ascites, and Ehrlich Ascites tumor cells. Both ligand and complex tested for antitumor activity by tissue culture experiments using L929 cells. The ligand and complex showed antitumor activity in in-vitro studies, the complex being ten times more active than the ligand. The Pd (1,3CHAA) destroyed the L929 tumor cells in culture, but the 1,3CHAA failed to show this activity.

Keywords: Palladium complex of 1,3-cyclohexane(2-aminobenzoic acid), infrared spectrum, square planar complex, in-vitro cytotoxic assay, Dalton's lymphoma ascites, Ehrlich ascites, L929 cells.

1. Introduction

The Schiff base ligand 1,3cyclohexanebis(anthranilic acid) (1,3CHAA) and the Pd(II) complex are prepared by refluxing equimolar concentrations of 1,3cyclohexanedione and anthranilic acid and cooling. Based on the elemental analysis and spectral studies, the structure of the ligand and the complex are arrived at. The complex [Pd(1,3CHAA)] is prepared and analyzed for its structure. The complex and the ligand are tested for antitumor activity following in vitro cytotoxicity assay and tissue culture methods.

2. Experimental

2.1. Preparation of 1, 3-Cyclohexanebis(2-Aminobenzoic Acid) – 1,3CHAA.

The ligand 1, 3-cyclohexanebis(2-aminobenzoic acid) (1,3CHAA) is prepared by refluxing a mixture of 1.4g(0.01M) of 1,3cyclohexanedione and 2.74g.(0.02M) anthranilic acid for 24hrs. The resultant solution is concentrated to half its volume and cooled when the ligands are separated. Washed with a minimum quantity of alcohol and dried in a desiccator. The melting point is 108°C.

2.2. Preparation Of Palladium (II) Complex of 1,3CHAA-[Pd(1,3CHAA)]

Aqueous solution of Pd(II) chloride (0.53g; 0.003 M) is mixed with an alcoholic solution of 1,3CHAA (0.003M) and boiled in presence of sodium acetate. The pink-colored [Pd (1,3CHAA)] complex formed, filtered, washed with ethanol, and dried in vacuum.

2.3. Structural Determination of 1,3CHAA and [Pd(1,3CHAA)].

The percentage composition of elements is calculated by CHN analysis and that of palladium by standard methods [1]. Molar conductance of 10^{-4} M solution of complex in nitrobenzene is determined. Magnetic susceptibilities of the complex is measured using the Guoy balance with Hg [Co(NCS)₄] as standard. The infrared spectra of ligands and complexes are recorded in a range of 4000-400cm⁻¹ by the KBr disc technique. UV-visible spectra of ligand and complex were studied for further confirmation of structure arrived at from magnetic susceptibility and other techniques. 1,3CHAA and the complex [Pd(1,3CHAA)] were tested for their antitumor ability by in-vitro cytotoxic assay and tissue culture experiments.

[2]The DLA or Ehrlich cells aspirated from tumor-bearing mice are washed with saline solution and suspended in phosphate buffer solution(PBS) to get the concentration of 10×10^6 cells per ml. Different concentrations of drugs ranging from 1µg to 50µg are taken in different tubes and 1 million tumor cells are added. After making the final volume to 1 ml with PBS, all the tubes are incubated at 37°C for 3 h along with a control tube without adding the drug. After incubation, 0.1ml of 1%, trypan blue was added and the percent of cell death was determined using a Neubauer hemocytometer.

[3]10 ml of L929 cells trypsinized and made the single-cell suspension by pipetting up and down. A concentration 50,000 cells is added to bottles containing 10 ml minimum essential medium (MEM) with 10% goat serum and antibiotics. The resulting solution is incubated for 24 h.at 37°C. After adding different concentrations of drugs, incubation is continued for the next six more days. After incubation the medium is poured off, the cell surfaces was washed three times with PBS-EDTA and the attached cells collected by trypsinization and centrifuged. The pellets obtained are suspended in 1ml Phosphate buffer solution and the cell count is determined.

3. Results and discussion.

The complex formed is recrystallized from alcohol. The physicochemical characteristics obtained are given in Table 1.

Table 1-Physicochemical Characteristics of 1,3CHAA and [Pd(1,3CHAA)]

Characteristics	C%	H%	N%	Pd%	Conductance $\text{Ohm}^{-1}\text{mol}^{-1}\text{cm}^2$	Magnetism (B.M)
1,3CHAA	69.55 (68.57)	4.91 (5.14)	7.32 (8.00)	0	0	0
[Pd(1,3CHAA)]	53.01 (52.81)	3.89 (3.98)	6.78 (6.16)	23.98 (23.39)	5.38	D

Note – calculated values are given in the parenthesis., D- diamagnetic.

The conductance of the chelate [Pd(1,3CHAA)] in nitrobenzene is negligible indicating a nonelectrolyte nature. The complex is diamagnetic indicating the absence of unpaired electrons in the complex.

The infrared spectral data of the ligand and the Pd(II) complexes are compared for structural determination. The assigned peak and the frequency of absorption are reported in the following table.

TABLE 2. Important Infrared Spectral Frequencies of 1,3CHAA and [Pd(1,3CHAA)]

Frequency(cm^{-1})	ν OH	ν_{asy} COO	ν_{sy} COO	ν C=N	ν M-N	ν M-O
1,3CHAA	3390	1664	1440	1593	--	--
[Pd(1,3CHAA)]	-	1580	1380	1570	510	310

The band due to –OH of the free carboxylic acid group disappears in the complex confirming the removal of –H from two –COOH groups of 1,3CHAA. Both the primary valences of Pd(II) ion are satisfied by two –COOH groups. The energy gap between the asymmetric and symmetric carboxylate group of the complex is in the range $170\text{-}200\text{cm}^{-1}$ [4] indicating the monodentate character of the carboxylate group. The frequencies at 510cm^{-1} and 310cm^{-1} are due to Pd-N and Pd-O bonds following the reports of K. Nakamoto.[5] about the M-N and M-O bonds. The absence of a band in $3100\text{-}3300\text{cm}^{-1}$ confirms the absence of coordinated water molecules in the complex.

The band recorded in electronic spectra of ligands at 44000cm^{-1} and 27500cm^{-1} are red shifted in Pd(II) complexes indicating the coordination to metal ions. Based on the above studies [Pd(1,3CHAA)] is assigned a square planar geometry as shown below.

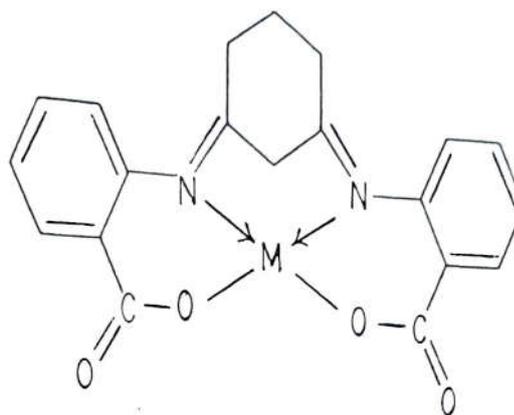


Figure1. M (1,3CHAA) where M=Pd(II)

The results obtained by the trypan blue exclusion method after incubating one million tumor cells in different concentrations of the ligand and complexes in one ml are tabulated below (Table.3.). The complex has more antitumor activity than the ligands.

Table.3. Antitumour Activity of the Ligand 1,3CHAA and [Pd(1,3CHAA)]

Tumour cell line	Lymphoma Ascites (DLA)					Ehrlich Ascites (EA)				
	50	25	10	5	1	50	25	10	5	1
Drug($\mu\text{g./ml}$)	50	25	10	5	1	50	25	10	5	1
	Percentage of dead cells									
1,3CHAA	9	9	9	9	9	8	6	6	6	6
[Pd(1,3CHAA)]	100	100	100	100	52	86	84	82	66	64

50,000 lung fibroblast (L929) cells after one day of incubation in 10 ml of minimum essential medium containing 10% serum are treated with different concentrations of ligand and complex separately and kept for 6 days more in incubation. With the help of a hemocytometer, the number of viable cells was counted. The live-cell percentage in the sample is compared with the control of the experiment and tabulated in Table.4. The [Pd(1,3CHAA)] is found to be more active than 1,3CHAA.

Table.4. Growth inhibition of L929 tumour cells in culture of the Ligand 1,3CHAA and [Pd(1,3CHAA)]

Tumour cell line	L929 live cells (% of control)		
Drug($\mu\text{g./ml}$)	25	10	5
1,3CHAA	100	100	100
[Pd(1,3CHAA)]	15.47	32.14	43.40

4. Conclusion

1,3-Cyclohexanebis(2-aminobenzoic acid) is a stable tetradentate ligand formed from 1,3 cyclohexanedione and 2-aminobenzoic acid. The ligand forms a stable neutral

diamagnetic square planar chelate with Pd(II) ion. The Pd(II) complex [Pd(1,3CHAA)] is more active against tumor cells than the ligand 1,3CHAA.

5. References

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