

Preparation of Pd(II) complexes of cyclohexanone anthranilic acid, cyclohexanone5-bromoanthranilic acid, characterization, and study of their antitumor ability

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Abstract - The cyclohexanone forms bidentate ligands with anthranilic acid and 5-bromoanthranilic acid. The ligands are CHAA (cyclohexanone anthranilic acid) and CHBrAA (Cyclohexanone5-bromoanthranilic acid). CHAA and CHBrAA coordinate to Pd(II) ion through – O of –COO group and -N of -C=N group. The structures of the Pd(II) complexes with CHAA and CHBrAA are found to be square planar from characterization techniques. The complexes and ligands were tested for the antitumor activity by the in vitro method, tissue culture, and percentage increase in the life span of Swiss Albino mice.

Index Terms - Cyclohexanoneanthranilic acid, Cyclohexanone5-bromoanthranilic acid, square planar Pd(II) complexes, antitumor studies of [Pd(CHAA)₂], antitumor studies of [Pd(CHBrAA)].

I.INTRODUCTION

The ligands [1] are synthesized by refluxing equimolar concentrations of cyclohexanone and anthranilic acid or 5-bromoanthranilic acid for 12 hours, cooling, and are recrystallized from alcohol. Using the elemental analysis and spectral studies, the structures of ligands have arrived at. The complexes were prepared and analyzed for their structure. The complexes are tested for antitumor studies following in vitro cytotoxicity assay [2], tissue culture [3], and tumor reduction [4] studies.

II. EXPERIMENTAL

Preparation of CHAA AND CHBrAA

The ligands cyclohexanone anthranilic acid (CHAA) and cyclohexanone5-bromoanthranilic acid (CHBrAA) were prepared by refluxing a mixture 0.98gm(0.01M) of cyclohexanone and 1.37gm(0.01M) or 2.15gm(0.01M) 5-bromoanthranilic acid for 12hrs. and cooling.

Structural determination of ligand and complexes.

The CHN analysis, halogen, and metal determination by standard methods [5] are employed to study the composition of complexes. Molar conductance of 10⁻⁴ M solution of complexes in nitrobenzene or acetone is found out.

Magnetic susceptibilities of complexes were found out using the Guoy balance with Hg[Co(NCS)₄] as standard.

The infrared spectra of ligands and complexes are recorded in arrange of 4000-400cm⁻¹ by the KBr disc technique.

UV-visible spectra of all ligands and complexes were studied to confirm the conclusions arrived at from magnetic susceptibility and other techniques.

The ligands and the complexes were tested for their antitumor ability by invitro cytotoxic assay, tissue culture experiments, and tumor reduction studies.

[2]The DLA or Ehrlich cells aspirated from tumor-bearing mice are washed with saline solution and was suspended in phosphate buffer solution(PBS) to get the concentration of 10x10⁶ 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 0.1 ml of 1%, trypan blue was added and the percent of age cell death was determined using a Neubauer hemocytometer.

[3]10 ml of L929 cells trypsinized and made the single-cell suspension of concentration 50,000 is added to bottles containing 10 ml MEM with 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 days. After washing the cell surfaces with

PBS-EDTA are collected by trypsinization and centrifuged. The pellets obtained are suspended in 1ml Phosphate buffer solution and the cell count is determined.

[4] The tumor cells aspirated from Ehrlich ascites tumor-bearing mouse washed, suspended in phosphate buffer solution, and one million tumor cells injected to Swiss albino mice intraperitoneally. Five doses of the drug at a concentration of 50µg./animal were given intraperitoneally on alternate days. The percentage of animal survival is calculated.

III. RESULTS AND DISCUSSION

The complexes formed are recrystallized from alcohol. The melting point of CHAA is 125°C and that of CHBrAA is 215°C. The elemental data of ligands are as follows.

Elements	CHAA	CHBrAA
C%	72.16(71.86)	52.81(52.71)
H%	7.23(6.95)	4.91(4.76)
N%	6.52(6.44)	4.86(4.73)
The calculated values are given in the parenthesis.		

The conductances of the complexes in nitrobenzene were negligible indicating nonelectrolytic nature. The Pd(II) complexes of CHAA and CHBrAA are found to be diamagnetic.

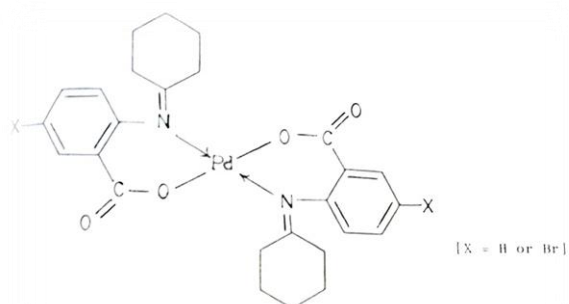
The infrared spectral data of the ligand and the Pd(II) complexes are analyzed for structural determination. The assigned peak and the frequency of absorption are reported in the following table.

The assigned peak	Absorption frequency (cm ⁻¹)			
	CHAA	[PdL ¹ ₂]	CHBrAA	[PdL ² ₂]
v OH	3390	-	3390	-
v _{sy} H ₂ O	-	3100-3300	-	3100-3300
v _{asy} COO	1680	1600	1670	1660
v _{sy} COO	1440	1400	1440	1410
v C=N	1610	1590	1610	1590
v M-N	-	510	-	510
v M-O	-	380	-	380
L ¹ H – CHAA, L ² H – CHBrAA				

The band at 3390 cm⁻¹ present in ligand disappeared in complexes which may be due to the -OH group. The -COOH group is coordinated to the Pd(II) ion by removing -H by satisfying one of the primary valencies. The broadband in the range 3100-3300[6]

indicates the coordinated water molecule. In the complexes, the energy gap between the asymmetric and symmetric carboxylate being in the range 170-200cm⁻¹ [7] indicates the monodentate character of the carboxylate group. The frequencies at 510cm⁻¹ and 380cm⁻¹ are due to Pd-N and Pd-O bonds following the reports of N.S. Biradar et al.[8] about the M-N and M-O bonds.

The band recorded in electronic spectra of ligands at 44100cm⁻¹ and 27650cm⁻¹ are red shifted in Pd(II) complexes indicating the coordination to metal ions. Based on the above studies the Pd(II) complexes of the ligands cyclohexanone anthranilic acid and cyclohexanone5-bromoanthranilic acids are assigned square planar geometry as shown below.



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. The complexes have more antitumor activity than the ligands.

In vitro cytotoxic effect of CHAA, CHBrAA, and their Pd(II) complexes.		
Drug µg./ml	Tumor cell line & percentage of dead cells	
	Dalton's Lymphoma Ascites (DLA)	Ehrlich Ascites (EA)
CHAA (L ¹ H) 50	9	-
CHAA (L ¹ H) 25	9	-
CHAA (L ¹ H) 10	9	-
CHAA (L ¹ H) 5	9	-
CHAA (L ¹ H) 1	9	-
[PdL ¹ ₂]	100	100
L ¹ H=CHAA 50	100	100
[PdL ¹ ₂] 25	100	100
[PdL ¹ ₂] 10	100	90
[PdL ¹ ₂] 5	100	88
[PdL ¹ ₂] 1	60	58
CHBrAA (L ² H) 50	23	10
CHBrAA (L ² H) 25	22	9
CHBrAA (L ² H) 10	21	8
CHBrAA (L ² H) 5	20	8
CHBrAA (L ² H) 1	Not done	6

[PdL ₂]	50	100	93
[PdL ₂]	25	85	80
[PdL ₂]	10	72	55
[PdL ₂]	5	70	40
[PdL ₂]	1	54	Not done

Two Pd(II) complexes were selected for the growth inhibition test of L929 cells in culture. 50,000 lung fibroblast cells after one day of incubation in 10 ml of minimum essential medium containing 10% serum are treated with different concentrations of ligand and kept for 6 days more in incubation. With the help of a hemocytometer, the number of viable cells counted. The live-cell percentage in the sample is compared with the control of the experiment.

Effect of growth inhibition of L929 cells in culture by Pd(II) complexes of CHAA(L ¹) and CHBrAA (L ²).			
	Live cells (% of control)		
Drug	25µg/ml	10µg/ml	5µg/ml
[PdL ¹]	1	9	28
[PdL ²]	20	25	41

The Pd (II) complex of Cyclohexanone anthranilic acid (CHAA) is more active than cyclohexanone 5-bromoanthranilic acid (CHBrAA) in the growth inhibition of L929 cells. The complex [Pd(CHAA)₂] is tested for the increase in the life span of Swiss Albino mice having tumors in the intraperitoneal cavity. Groups of 6 mice were investigated by giving 5 doses of the drug at a rate of 50µg/dose/animal. Survival days were 23.5 ± 1.50 with a percentage increase in life span by 23.70.

IV. CONCLUSION

Cyclohexanone anthranilic acid and cyclohexanone 5-bromoanthranilic acid form stable complexes with Pd(II) which are diamagnetic. Solutions of these complexes in nitrobenzene are non-electrolytes. The spectral studies confirm that the complexes are square planar in structure. The complexes are more active against tumor cells than the ligands. Pd(II) complex of cyclohexanone anthranilic acid possesses more antitumor ability than that of 5-bromoanthranilic acid.

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