Synthesis, Characterization, and Antitumour Activity of Palladium(II) Complex of 5-Fluorouracil-1-acetic Acid and Ammine

J. Q. QU, J. HUANG, L. F. WANG*, and G. C. SUN

National Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, 730000, P. R. China
e-mail: qujq@netease.com

Received 24 November 2000

Palladium(II) complexes of 5-fluorouracil-1-acetic acid (HL) and ammine have been prepared and characterized by means of elemental analysis, molar conductivity, IR, UV, ^{1}H NMR spectra, and TG-DTA. The general formula for the complex is $PdL_2(NH_3)_2$. The IR and ^{1}H NMR spectra show that HL and ammine coordinate to the metal ion through the ammine nitrogen and carboxyl oxygen. The complex and HL have been studied for their antitumour activity against HL-60 human leukemia cells *in vitro*.

cis-Diamminedichloroplatinum(II) (cisplatin) has been found as anticancer agent against testicular tumour, ovarian carcinomas, squamous cell carcinomas, and a variety of sarcomas [1]. Its discovery has stimulated considerable interest in the search for more potential anticancer platinum and other metal complexes [2—4]. A large number of analogues of cisplatin have been tested. The structurally analogous palladium complexes tested have either little or marginal antitumour activity [2]. *Gill* [5] reported several palladium complexes with bidentate amine ligands, which showed anticancer activity comparable to or greater than cisplatin. *Puthraya et al.* [6, 7] discovered seventeen palladium complexes of 2,2′-bipyridine and amino acid. Many of those complexes have shown anticancer activity comparable to or greater than cisplatin.

On the basis of the studies on the metal complexes of 5-fluorouracil-1-acetic acid (HL) [8—11] and in view of the importance of palladium complexes as potential anticancer drugs, we report here on the synthesis, characterization, and antitumour activity of mixed-ligand palladium(II) complex of 5-fluorouracil-1-acetic acid and ammine.

EXPERIMENTAL

The reagents used included $AgNO_3$ purchased from Beijing General Factory of Chemical Reagents. HL and $Pd(NH_3)_4Cl_2$ were prepared according to the literature method [12, 13]. All the solvents and reagents used were of anal. grade.

The C, H, N data of the complex were determined using a Varian EL elemental analyzer. Electrolytic conductance measurement was made with a DDS-11A digital conductometer with DMSO as solvent ($\approx 10^{-3}$ mol dm^{-3} solution) at 25 °C. IR spectra were recorded on a Nicolet 170SX FTIR spectrophotometer using KBr discs in the range $\tilde{\nu}$ = 200—4000 cm^{-1}. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer using DMSO as solvent. ^{1}H NMR spectra were measured with an FT-80A nuclear magnetic resonance instrument using DMSO-d_6 as solvent and TMS as internal reference. TG-DTA measurement was made in a nitrogen atmosphere at room temperature and 800 °C using a Dupont 1090-B thermal analyzer.

$Pd(NH_3)_4(NO_3)_2$

$Pd(NH_3)_4Cl_2$ (1.0 mmol) was added to a solution of $AgNO_3$ (2.0 mmol in 30 cm^3 of H_2O) in a beaker covered with black paper and the contents were stirred continuously for 24 h on ice-water bath. The AgCl precipitate obtained was removed by filtration and the colourless liquid was added into ethanol, whereupon white precipitate formed. The precipitate was filtered off, washed with ice water and ethanol several times and dried in a vacuum desiccator. Yield: 34 %.

Complex

HL (0.6 mmol) was added to a solution of $Pd(NH_3)_4(NO_3)_2$ (0.3 mmol) in 30 cm^3 of water. The solution was continuously stirred for 5 h at room temperature, and then the yellow precipitate formed was collected by filtration, washed with ice water several times and dried in a vacuum desiccator to constant mass. Yield: 40 %.

*The author to whom the correspondence should be addressed.

Table 1. IR Data of HL and its Complex

Compound	$\tilde{\nu}/cm^{-1}$								
	$\nu_{as}(NH_3)$	$\nu(OH)$	$\nu_s(NH_3)$	$\delta(NH_3)$	$\nu_{as}(COO^-)$	$\nu_s(COO^-)$	$\rho(NH_3)$	ν(Pd—N)	ν(Pd—O)
HL		3196							
Complex	3246		3187	1643	1581	1403	801	493 457	280 270

MTT Assay

HL-60 human leukemia cells were propagated continuously in culture and grown in RPMI 1640 medium with 10 % inactivated fetal calf serum (FCS) and antibiotics. Cells harvested from exponential phase were seeded equivalently into 96 well plates and incubated for 24 h, then compounds studied were added in concentration gradient. The final concentrations were maintained at $c/(\mu mol\ dm^{-3})$ 100, 75, 50, and 25, respectively. The plates were maintained at 37 °C in a humidified 5 % CO_2—90 % N_2—5 % O_2 atmosphere and incubated for 48 h, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added, the following procedure referred to literature [14]. The measurements of absorption of the solution concerned with the number of live cells were performed on ELISA spectrophotometer at 570 nm. Palladium dichloride was examined at the same conditions.

The survival ratio (SR) is expressed as $A/A_0 \times 100$ %, where A is average absorbance of the experimental wells and A_0 is average absorbance of the control wells. The logarithm of concentration numerical values of the compounds and SR were treated by linear regression analysis and IC_{50} values were obtained from the equations. IC_{50} was defined as drug concentration required for reducing the number of living cells by 50 %.

RESULTS AND DISCUSSION

The elemental analyses for the newly prepared complex: w_i(found): 27.65 % C, 2.96 % H, 16.51 % N, w_i(calc.): 28.02 % C, 2.70 % H, 16.34 % N. The complex is air-stable and insoluble in methanol, ethanol, ether, and tetrahydrofuran, slightly soluble in water, soluble in DMSO. The molar conductivity value of the complex in DMSO at 25 °C is 12.0 S cm^2 mol^{-1}, indicating that it is nonelectrolyte in DMSO [15].

The important IR frequencies of HL and the complex, along with their relative assignments are given in Table 1. The strong band at 3196 cm^{-1} in the free ligand assigned to $\nu(OH)$ vibration disappears, but two new bands corresponding to $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ vibrations are observed at 1581 cm^{-1} and 1403 cm^{-1} in the complex. They show that the carboxylate group of HL coordinates to the metal ion through the OH oxygen. $\Delta\tilde{\nu} = \tilde{\nu}(\nu_{as}(COO^-)) - \tilde{\nu}(\nu_s(COO^-)) = 178\ cm^{-1}$, which strongly suggests unidentate coordination of the ligand carboxyl group with palladium ion [16]. The complex displays characteristic vibrations at 3246 cm^{-1}, 3187 cm^{-1}, 1643 cm^{-1}, and 801 cm^{-1}, which are assigned to $\nu_{as}(NH_3)$, $\nu_s(NH_3)$, $\delta(NH_3)$, and $\rho(NH_3)$, these facts indicate that ammine is involved in the complex formation. By comparison of the far-IR spectra of the complex with those of HL, new peaks appear at 497 cm^{-1}, 457 cm^{-1}, 280 cm^{-1}, and 270 cm^{-1}, and are attributed to ν(Pd—N) and ν(Pd—O) [17]. These bands confirm the coordination of metal ion through nitrogen and oxygen atom, all of them indicate that the complex exists in *cis*-form [18].

The UV absorptions of DMSO solutions of HL and the complex were measured. For HL and the complex, λ_{max}/nm ($\log\{\varepsilon\}$) are 270 (4.45) and 267 (5.02), respectively. After forming the complex, the λ_{max} shifts a little. The $\log\{\varepsilon\}$ value of the complex should be about 4.75 if the additional rule could be applied. The abnormal result is probably due to special steric properties of the complex.

The ^1H NMR spectra of HL and its complex are shown in Figs. 1 and 2. HL exhibits four signals at δ

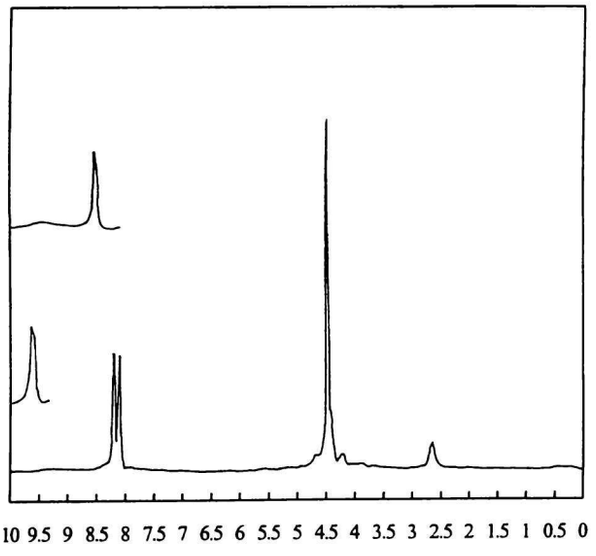


Fig. 1. The ^1H NMR spectrum of HL.

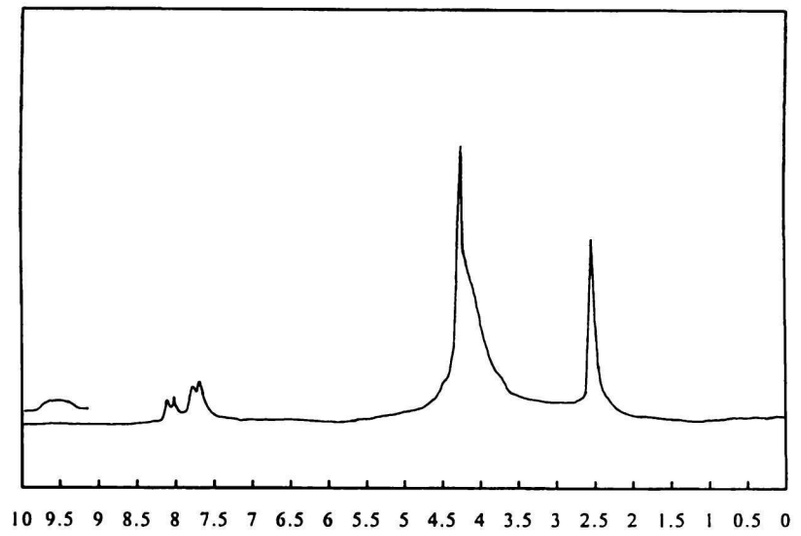
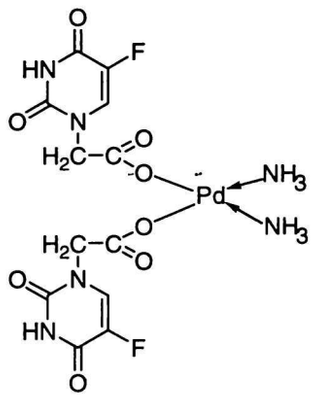


Fig. 2. The ^{1}H NMR spectrum of the complex.



Formula 1. Suggested structure of the complex.

Table 2. Antitumour Activity of Studied Compounds against HL-60 Cells

Compounds	$IC_{50}/(\mu mol\ dm^{-3})$
HL	88.17
Complex	30.47
5-FU	72.92

= 11.95 (1H, s), 11.80 (1H, s), 8.18 (1H, d, J = 7 Hz), and 4.50 (2H, s) assigned to —COOH, N_3—H, C_6—H, and —CH_2 groups, respectively. When coordinated to the metal ion in the complex, the band at δ = 11.95 disappears, and the δ values of 11.80, 8.18, and 4.50 are shifted upfield by 0.02, 0.33, 0.23. These results indicate the coordination of metal ion with oxygen atom of carboxyl group. Since the signal of NH_3 is overlapped with that of —CH_2 group, the band of the complex at δ = 4.27 turns broad and the proton number turns to be 5, indicating the involvement of NH_3 in the complex formation.

The thermal decomposition of the complex has been studied as well. The endothermic peak of the complex indicates the beginning of decomposition at 235 °C and occurs through the following three continuous stages: 260 °C, 315 °C, 419 °C. The complex has not melting point. Heating to about 750 °C, the residue mass corresponds to the value calculated for PdO.

According to the aforementioned data, for the complex prepared we propose the structure shown in Formula 1.

The data of the antitumour activity of HL, complex, and 5-fluorouracil (5-FU) are given in Table 2. The concentration of DMSO was controlled under 1 % to assure not to affect the result [19]. The results showed that the complex produces more inhibitory effect compared to the free ligand and 5-FU. Although palladium dichloride has some cytotoxicity effect at high concentration, the effect is not evident at IC_{50} dosage of the complex (SR > 90 %).

Acknowledgements. This project was supported by the Natural Science Foundation of Gansu Province (ZS991-A23-059-Y) and China Innovation Centre for Life Science (JB 00980462).

REFERENCES

1. Rosenberg, B., in *Metal Ions in Biological Systems*, Vol. *11*. (Sigel, H., Editor.) P. 127. Dekker, New York, 1980.
2. Cleare, M. J. and Hyde, P. C., in *Metal Ions in Biological Systems*, Vol. *11*. (Sigel, H., Editor.) P. 1. Dekker, New York, 1980.
3. Roberts, J. J., in *Metal Ions in Genetic Information Transfer*. (Eichhorn, G. L. and Marzilli, L. G., Editors.) P. 273. Elsevier, Amsterdam, 1981.
4. Sadler, P. C., Nasr, M., and Narayan, V. L., in *Platinum Coordination Complexes in Cancer Chemotherapy*. (Hacker, M. P., Douple, E. B., and Krakoff, I. H., Editors.) P. 290. Nijhoff, Boston, 1984.
5. Gill, D. S., in *Platinum Coordination Complexes in Cancer Chemotherapy*. (Hacker, M. P., Douple, E. B., and Krakoff, I. H., Editors.) P. 267. Nijhoff, Boston, 1984.
6. Puthraya, K. H., Srivastava, T. S., Amonkar, A. J., Adwankar, M. K., and Chitnis, M. P., *J. Inorg. Biochem. 25*, 207 (1985).
7. Puthraya, K. H., Srivastava, T. S., Amonkar, A. J., Adwankar, M. K., and Chitnis, M. P., *J. Inorg. Biochem. 26*, 45 (1985).
8. Wang, L. F., Yang, Z. Y., Peng, Z. R., Cheng, G. Q., Guo, H. Y., Sun, A. L., Wang, Q., and He, F. Y., *J. Coord. Chem. 28*, 167 (1993).

9. Yang, Z. Y., Yang, R. D., Wang, L. F., and Yang, X. P., *Bull. Soc. Chim. Belg. 104*, 129 (1995).
10. Yang, Z. Y., Wang, L. F., Wu, J. G., Li, X. Y., and Wang, Q., *Sci. Bull. 9*, 183 (1992).
11. Yang, Z. Y., Wang, L. F., Wu, J. G., Yang, K. W., and Zhu, Y., *Acta Chem. 51*, 115 (1993).
12. Tada, M., *Bull. Chem. Soc. Jpn. 48*, 3427 (1975).
13. Wendlandt, W. W. and Funes, L. A., *J. Inorg. Nucl. Chem. 26*, 1879 (1964).
14. Geary, W. J., *Coord. Chem. Rev. 7*, 81 (1971).
15. Patricia, P. and Trevor, J. M., *Cancer Res. 50*, 1392 (1990).
16. Nakamoto, K., *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, p. 232. Wiley, New York, 1978.
17. Vadde, R., Jagannatha, S. S., Somu, S., and Puri, L., *Trans. Met. Chem. 9*, 106 (1984).
18. Balice, V. and Theophanides, T., *J. Inorg. Nucl. Chem. 32*, 1237 (1970).
19. Dodoff, N., Grancharow, K., and Gugova, R., *J. Inorg. Biochem. 54*, 212 (1994).