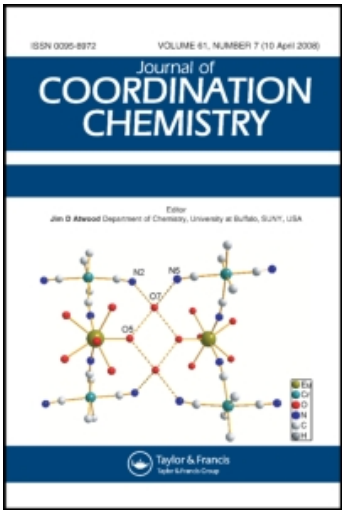

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Synthesis, characterization and initial biological studies of a new platinum(II) complex with deoxyalliin

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A new platinum(II) complex with deoxyalliin was synthesized and characterized by chemical and spectroscopic techniques. Elemental and mass spectrometry analyses of the solid complex fit to the composition $[Pt(C_6H_{11}NO_2S)Cl_2] \cdot H_2O$. ^{13}C NMR, ^{15}N NMR and infrared spectra of the complex are consistent with coordination of deoxyalliin to Pt(II) through the nitrogen and sulfur atoms forming a square-planar geometry. The complex is soluble in dimethylsulfoxide. Biological analysis for evaluation of a potential cytotoxic effect of the complex was performed using HeLa cells, a human cervix adenocarcinoma-derived cell line. The results were compared with those of a palladium(II) complex previously described.

Keywords: Platinum(II); Deoxyalliin; ^{15}N NMR; Cancer therapy

1. Introduction

Cisplatin or cis-diammindichloroplatinum(II), a square planar complex of platinum(II), has been extensively used for treatment of various types of cancer, mainly testicular, ovarian, bladder, head and neck cancers [1]. The anticancer properties of cisplatin were first observed in 1965 but only in 1978 did the compound become available for oncology practice [2]. Cisplatin is also a highly toxic drug and its major side effects are nephrotoxicity, neurotoxicity and ototoxicity [3]. Developing new anticancer drugs similar to cisplatin, but with reduced side effects, has stimulated the synthesis of new metal complexes of platinum(II), platinum(IV) and palladium(II). Carboplatin and nedaplatin are two of the cisplatin analogues, which have been used for treatment of ovarian, head, neck, testicular, bladder, and lung cancers [2].

Palladium(II) complexes containing S and N donor ligands have also been prepared and biological assays showed their activities against several tumor lines

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(HeLa, 3T3, JURKAT, Pam212 and Pam-ras) [4]. Platinum(II) and palladium(II) complexes with phenanthroline and amino acids were also prepared and displayed cytotoxic activities on Molt-4, a human leukemia cell line [5].

Deoxyalliin (sac, S-allyl-L-cysteine, $C_6H_{11}NO_2S$), a sulfur containing amino acid derived from cysteine, is a product of vegetal origin present in onion and garlic bulbs [6–8]. Recent studies have shown that deoxyalliin may be considered a biological antagonist of nitrosomorpholine, a substance responsible for the development of hepatic cancer in humans [9]. Deoxyalliin also exhibits capacity to inhibit *in vitro* proliferation of malignant cells from human nervous system and breast-cancer cells [10, 11]. Recently, a new palladium(II) complex with the amino acid deoxyalliin was prepared and analyzed in our laboratories. The complex shows antiproliferative and cytotoxic activities over HeLa and TM5 tumorigenic cells, as well as antitumoral activity against murine melanoma [12]. The present article describes the synthesis and preliminary biological study of a new platinum(II) complex with deoxyalliin.

2. Experimental

2.1. *Instrumentation*

L-deoxyalliin and potassium tetrachloroplatinate(II) of analytical grade were purchased from LKT and Acros laboratories, respectively. Elemental analyses for carbon, hydrogen and nitrogen were performed by using a CHNS-O EA1110 Analyzer, CE Instruments; cystine was used as a reference substance. Electrospray ionization mass spectrometry (ESI-MS) measurements were carried out using a Fissons VG Platform instrument. Infrared spectra were recorded on a FT-IR Spectrophotometer Perkin-Elmer Spectrum 2000, with samples prepared as CsI pellets. ^{13}C NMR and ^{15}N NMR spectra were recorded on a Varian 500 MHz Spectrometer; samples were analyzed in deuterium oxide and dimethylsulfoxide-d_6 solutions.

2.2. *Synthesis of the complex*

The complex was synthesized by adding 1.0×10^{-3} mol of a freshly prepared aqueous solution of K_2PtCl_4 to a solution of deoxyalliin hydrochloride containing 1.0×10^{-3} mol of the ligand (molar proportion Pt : deoxyalliin of 1 : 1). Final volume of the reaction mixture was about 15 mL. The reaction was carried out with stirring at room temperature. A pale yellowish solid of the complex slowly precipitated. After 10 h of constant stirring the solid complex was filtered, washed with cold water and dried in a desiccator under P_4O_{10}. Final yield was about 70%. Anal. Calcd for $[Pt(C_6H_{11}NO_2S)Cl_2] \cdot H_2O$ (%) C 16.2; H 2.95; N 3.15. Found (%): C 16.3; H 2.91; N 3.20. No single crystals of the complex were obtained, even after several attempts, in order to perform an X-ray structure determination.

2.3. *Cell culture and biological assays*

HeLa cells (human cervix adenocarcinoma; ATCC CCL-2) were cultured at 37°C in a humidified atmosphere containing 5% CO_2 using DMEM supplemented with 10% of Fetal Calf Serum (FCS). Penicillin (100 U mL^{-1}) and streptomycin (100 µg mL^{-1})

were used as antibiotics. All reagents were from Life Technologies-Invitrogen (Gaithersburg, MD). Culture flasks and 24-well plates were purchased from Costar (Corning Inc., NY). Cisplatin was purchased from Acros and MTT from Sigma.

A stock solution of the complex was prepared in phosphate buffered saline medium (PBS). Different concentrations of the complex were achieved by direct dilution of the stock solution into the cell medium. Cells were plated in a 24-well plate (2.5×10^4 cells/well) 24 h prior to the beginning of the experiment. Forty-eight hours after the addition of the complex or the vehicle, a MTT solution was added (aiming a final concentration of $0.50\,mg\,mL^{-1}$) and the cells were incubated for another period of 3 h. After washing twice with PBS, 200 µL of isopropanol was added and cell viability was determined by absorbance measurements at 570 nm.

3. Results and discussion

3.1. *Mass spectrometry*

An electrospray mass spectrum of the Pt(II)–deoxyalliin complex (ESI-30 V) exhibits one peak at m/z 426, which is assigned to the $[PtCl_2(sac)\text{-}H]^-$ molecular ion. ^{37}Cl isomer peaks are also observed at m/z 428 and 430. Other peaks observed are assigned to a recombination of Cl^- ions, free ligand (m/z 160) and the metallic complex.

3.2. *^{13}C and ^{15}N NMR spectroscopy*

^{13}C NMR and ^{15}N NMR spectra of the Pt(II)-deoxyalliin complex were analyzed in comparison to the spectra of deoxyaliin hydrochloride. The structure of the ligand with carbon numbering is shown in figure 1.

The ^{13}C NMR spectrum of the Pt(II)-deoxyalliin complex is consistent with coordination of the ligand to the metal through the N and S atoms. According to the ^{13}C NMR data, the chemical shift at 171 ppm in the spectrum of deoxyalliin hydrochloride is assigned to the carbon atom of the COOH group (C_1 in figure 1). In the spectrum of the complex only minor changes are observed for the chemical shift of the COOH, indicating that this group is not involved in coordination to Pt(II). Pronounced changes are observed for the chemical shifts of C_2, C_3 and C_4 (see figure 1) in the spectrum of the Pt(II)–complex when compared to the ligand. These changes are consistent with coordination of deoxyalliin to Pt(II) through the N and S atoms of the amino acid molecule. This proposition is reinforced by the results obtained for the Pd(II)–deoxyalliin complex, which also exhibits coordination though

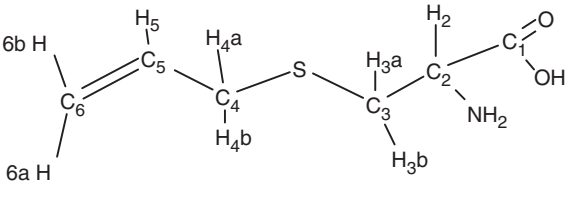

Figure 1. Schematic structure of deoxyalliin.

Table 1. ^{13}C chemical shifts for deoxyalliin hydrochloride (sac · HCl – deuterium oxide solution) and for $[Pt(sac)Cl_2] \cdot H_2O$ (dimethylsulfoxide deuterated solution).

	Isomer shift (ppm)					
Compound	C_1	C_2	C_3	C_4	C_5	C_6
sac · HCl	171.0	52.50	30.17	34.31	133.6	118.8
$[Pt(sac)Cl_2] \cdot H_2O$	168.6	61.56	37.85	39.08	130.7	121.9

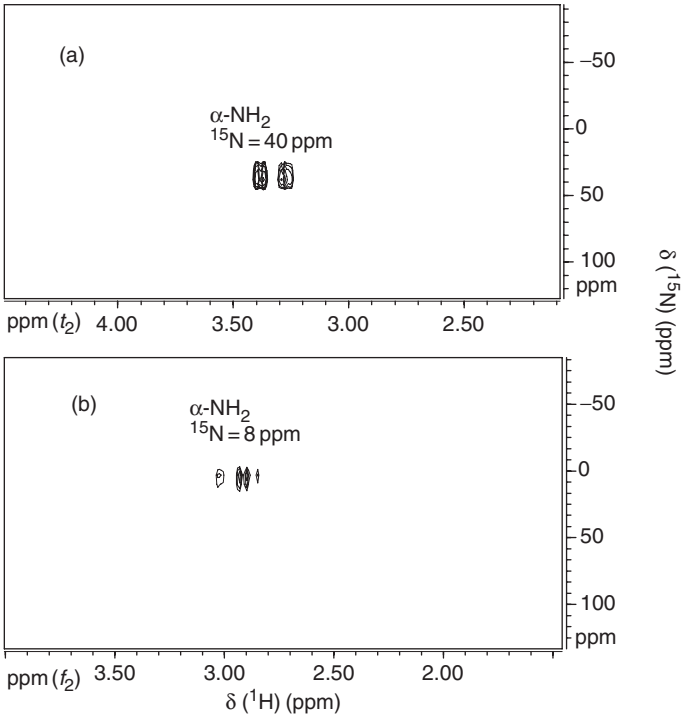

Figure 2. Amine region in ^1H–^{15}N HMBC spectra of deoxyalliin hydrochloride (a) and Pt(II)–deoxyalliin (b).

S and N atoms [12]. The ^{13}C NMR chemical shifts for deoxyalliin hydrochloride and the Pt(II)–deoxyalliin complex are given in table 1.

The ^{15}N chemical shifts for deoxyalliin hydrochloride and for the complex were indirectly obtained from the 2D spectra *via* the Heteronuclear [^1H–^{15}N] Multiple Bond Coherence technique (HMBC), as described for other metal complexes with amino acids [13, 14]. The assignment of the nitrogen resonance was performed by its correlation with protons H_{3a} and H_{3b} in figure 1. Analysis of the HMBC spectrum of deoxyalliin hydrochloride permitted identification of the ^{15}N chemical shift at 40.0 ppm while in the spectrum of the complex the ^{15}N chemical shift appears upfield at 8.0 ppm. The observed $\Delta\delta$ (δ complex–δ ligand) equal to -32 ppm confirms coordination through the NH_2 group. The HMBC spectra of deoxyalliin hydrochloride and of the platinum(II) complex are shown in figure 2.

3.3. *Infrared spectroscopy*

Pt(II)–deoxyalliin infrared spectrum was analyzed in comparison to the infrared spectrum of deoxyalliin hydrochloride. The IR spectrum of the Pt(II) complex exhibits two well resolved absorption bands at 3197 and 3103 cm^{-1}, which are assigned to the asymmetric and symmetric stretching modes of the coordinated NH_2 group [15]. The spectrum of the complex also exhibits a strong absorption band at 1741 cm^{-1}, which is assigned to the uncoordinated protonated carboxylic group. Moreover, the broad band with a maximum at 3437 cm^{-1} confirms the presence of hydration water in the complex composition [15].

The IR spectrum of the Pt(II)–deoxyalliin complex was also obtained in the region 700–150 cm^{-1} in order to identify frequencies related to M–S, M–Cl and M–N bonds. The IR spectrum shows vibrational absorption frequencies at 280, 321 and 577 cm^{-1}, which are assigned to ν(Pt–S), ν(Pt–Cl) and ν(Pt–N), respectively. These assignments are in agreement with the literature [15, 16].

3.4. *Biological analysis*

The complex was assayed in a range of concentrations varying from 2 μM up to 400 μM, using PBS as a negative control and cisplatin as a positive control for cytotoxicity. The results revealed that the complex was nontoxic to HeLa cells even when the highest concentration was tested. The palladium(II) complex with deoxyalliin, previously described, revealed antiproliferative and cytotoxic effects over HeLa and TM5 cells at concentrations varying from 100 to 400 μM [12].

4. Conclusions

Composition of the platinum(II) complex with L-deoxyalliin was found as 1 : 1 (metal : ligand). ^{13}C NMR, ^{15}N NMR and infrared data indicate coordination of the ligand to Pt(II) *via* nitrogen and sulfur atoms in a square planar geometry.

Based on the chemical and spectroscopic results, the proposed structure for the Pt(II)–deoxyalliin complex is shown in figure 3.

The biological evaluation for this complex showed that it was non cytotoxic to human cells derived from cervix adenocarcinoma. Based on this preliminary result, we believe that it might be of interest to further evaluate its biological effect

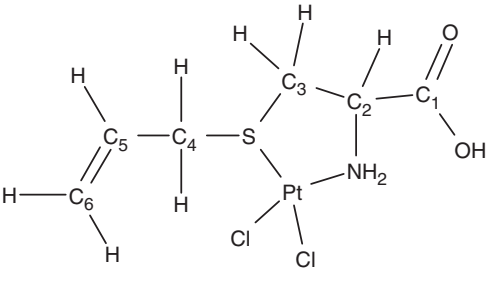

Figure 3. Structural formula proposed for the Pt(II)–deoxyalliin complex.

using cells from different organisms, such as bacteria and fungi, in order to provide information concerning a possible species-selective cytotoxic effect of the complex.

Acknowledgements

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References

[1] Y.S. Sohn, H. Baek, Y.H. Cho, Y. Lee, O. Jung, C.O. Lee, Y.S. Kim. *Int. J. Pharm.*, **79**, 153 (1997).
[2] D. Lebwohl, R. Canetta. *Eur. J. Cancer*, **34**, 1522 (1998).
[3] J.L. Butour, S. Wimmer, P. Castan. *Chem. & Biol. Int.*, **104**, 165 (1997).
[4] A.G. Quiroga, C.N. Ranninger. *Coord. Chem. Rev.*, **248**, 119 (2004).
[5] V.X. Jin, J.D. Ranford. *Inorg. Chim. Acta*, **304**, 38 (2000).
[6] H.E. Renis, R.E. Henze. *Food Res.*, **23**, 345 (1958).
[7] T. Suzuki, M. Sugii, T. Kakimoto, N. Tsuboi. *Chem. & Pharm. Bull.*, **9**, 251 (1961).
[8] Y. Kodera, A. Suzuki, O. Imada, S. Kasuga, I. Sumioka, A. Kanezawa, N. Taru, M. Fujikawa, S. Nagae, K. Masamoto, K. Maeshige, K. Ono. *J. Agr. Food Chem.*, **50**, 622 (2002).
[9] M.E. Dion, M. Agler, A. John. *Nut. Cancer*, **28**, 1 (1997).
[10] C. Welch, L. Wuarin, N. Sidell. *Cancer Lett.*, **63**, 211 (1992).
[11] G. Li, C.H. Quiao, R.I. Lin, J. Pinto, M.P. Osborne, R.K. Tiwari. *Oncol. Rep.*, **2**, 787 (1995).
[12] P.P. Corbi, A.C. Massabni, A.G. Moreira, F.J. Medrano, M.G. Jasiulionis, C.M. Costa-Neto. *Can. J. Chem.*, **83**, 104 (2005).
[13] P.P. Corbi, A.C. Massabni, C.M. Costa-Neto. *J. Coord. Chem.*, **58**, 1477 (2005).
[14] N. Juranic, S. Macura. *Inorg. Chim. Acta*, **217**, 213 (1994).
[15] K. Nakamoto. *Infrared and Raman Spectra of Inorganic and Coordination Compounds, Part B*, 5th Edn, p. 385, J. Wiley & Sons, New York (1997).
[16] Z. Velkov, R. Pelova, S. Stoev, E. Golovinsky. *J. Coord. Chem.*, **26**, 75 (1992).