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M.A. Carvalho [a], B.C. Souza [a], R.E.F. Paiva [a], F.R.G. Bergamini [a], A.F. Gomes [a], F.C. Gozzo [a], W.R. Lustri [b], A.L.B. Formiga [a], G. Rigatto [a] & P.P. Corbi [a]

[a] Institute of Chemistry, University of Campinas - UNICAMP, P.O. Box 6154, 13083-970, Campinas-SP, Brazil

[b] Biological and Health Sciences Department - UNIARA, 14801-320, Araraquara-SP, Brazil

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Synthesis, spectroscopic characterization, DFT studies, and initial antibacterial assays *in vitro* of a new palladium(II) complex with tryptophan§

M.A. CARVALHO†, B.C. SOUZA†, R.E.F. PAIVA†, F.R.G. BERGAMINI†, A.F. GOMES†, F.C. GOZZO†, W.R. LUSTRI‡, A.L.B. FORMIGA†, G. RIGATTO† and P.P. CORBI*†

†Institute of Chemistry, University of Campinas – UNICAMP, P.O. Box 6154, 13083-970, Campinas-SP, Brazil
‡Biological and Health Sciences Department – UNIARA, 14801-320, Araraquara-SP, Brazil

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A new palladium(II) complex with the amino acid L-tryptophan (Pd-TRP) was synthesized in aqueous solution and characterized by chemical and spectroscopic methods. Elemental, ESI-QTOF mass spectrometric, and thermal analyses of the solid compound permitted proposing the $[Pd(C_{11}H_{11}N_2O_2)_2]\cdot 2H_2O$ composition. Infrared, Raman, and UV-Vis spectroscopic data indicate the coordination of the ligand to Pd(II) through monodentate oxygen of carboxylate and through nitrogen of the amino group. The ^{1}H and ^{13}C NMR spectroscopic data confirm coordination through the carboxylate, while ^{15}N NMR data confirm coordination of nitrogen of NH_2. Density functional theory studies confirmed nitrogen and oxygen coordination to palladium(II) as a minimum of the potential energy surface with calculations of the hessians showing no imaginary frequencies. Biological studies were performed to provide information concerning the antibacterial activities of the compound. The Pd-TRP complex was shown to be active against Gram-positive and Gram-negative pathogenic bacterial strains.

Keywords: Palladium(II); L-Tryptophan; ESI-QTOF-MS; Molecular modeling; Infrared; Antibacterial agent

1. Introduction

Metal complexes have been considered as therapeutic agents for a long time. Experiments with metallopharmaceutical agents were first performed based on the knowledge of the toxic properties of metal ions in biological systems [1]. One of the most well-known applications of metal compounds as pharmaceutical agents is the use of silver compounds as bactericides. Silver complexes, such as silver-sulfadiazine, have been used clinically as antibacterial agents since the 1950s. Silver-sulfadiazine is an insoluble polymeric compound that slowly releases Ag(I), being applied topically as a

*Corresponding author. Email: ppcorbi@iqm.unicamp.br; pedrocorbi@yahoo.com
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cream to prevent bacterial infections in the cases of severe burns [2]. Another remarkable example of use of metal compounds as pharmaceutical agents is cisplatin, or *cis*-diamminedichloroplatinum(II), used worldwide as an anticancer agent since 1978 [3]. The first studies dealing with cisplatin were related to its antibacterial activities toward *Escherichia coli* bacterial strains [4]. Despite its wide use as an anticancer drug, cisplatin possesses toxic side effects such as nephrotoxicity, ototoxicity, hematotoxicity, and gastrointestinal toxicity, including nausea and vomiting [3]. To find anticancer drugs with reduced side effects when compared to cisplatin, second generation compounds based on cisplatin structure were synthesized. The most well-known example is carboplatin, which has been used worldwide for treatment of ovarian, head, neck, testicular, bladder, and lung cancers, with an improved therapeutic index when compared to cisplatin [1, 3].

Based on the similarities of coordination geometry and on thermodynamic parameters of the palladium(II) complexes compared to platinum(II) analogs, synthesis and application of palladium-based compounds in medicinal chemistry is examined; palladium compounds were reported to be kinetically more reactive than the platinum analogs [5].

In the past decade, extraordinary increase of syntheses and biological assays of new metallopharmaceutical agents has been observed. Specifically, due to the growth of multi-resistant bacterial strains, syntheses of new antibacterial agents of silver(I), gold(I), and also platinum(II) and palladium(II) for treatment of infectious diseases has been reported. Kazachenko *et al.* [6] investigated the synthesis and antibacterial activities of silver complexes with the amino acids histidine and tryptophan. Both compounds showed good antibacterial activity against Gram-negative and Gram-positive bacterial strains and low toxicity. In addition, antibacterial activities of palladium(II) complexes of tetracyclines (tetracycline, doxycycline, and chlortetracycline) have been reported [7]. The palladium(II) complex of tetracycline is as efficient as free tetracycline in inhibiting the growth of two *E. coli* sensitive bacterial strains and 16 times more potent than free tetracycline against *E. coli* HB101/pBR322, a bacterial strain resistant to tetracycline [7].

In our research group, new metal compounds of platinum(II), palladium(II), gold(I), and silver(I) have been synthesized and evaluated as antibacterial and antitumor agents. Abbehausen *et al.* [8, 9] described the synthesis and biological activities of two new silver(I) and gold(I) complexes with N-acetyl-L-cysteine and mercaptothiazoline, respectively. Both compounds were effective against Gram-positive and Gram-negative bacterial strains. The gold(I) compound with mercaptothiazoline was also shown to be cytotoxic to HeLa tumorigenic cells, inducing 85% cell death at a concentration of $2.0\,\mu mol\,L^{-1}$. Spera *et al.* [10] described the antibacterial activities of a palladium(II) complex with deoxyalliin, of composition $[Pd(C_{12}H_{22}O_4N_2S_2)]$. The compound was shown to be effective against *Staphylococcus aureus* (Gram-positive), *E. coli* and *Pseudomonas aeruginosa* (Gram-negative) bacterial cells. Also, a new dimeric platinum(II) complex with methionine sulfoxide, of coordination formula $[(C_5H_{10}NO_3S)Pt(\mu\text{-}Cl)_2Pt(C_5H_{10}NO_3S)]\cdot 2.5H_2O$ and a polymeric gold(I) compound with N-acetyl-L-cysteine, of composition $AuC_5H_8NO_3S\cdot 0.75H_2O$ were synthesized and characterized by spectroscopic techniques. Preliminary antibacterial studies show the antibacterial activities of the compounds against pathogenic bacterial strains [11, 12]. More recently, a dimeric Pd(II) complex with mercaptothiazoline, named Pd-MTZ, was synthesized. Coordination to Pd(II) was shown to occur through the N and S in a

square-planar geometry. The compound was insoluble in water and in common organic solvents. Antibacterial studies showed the non-activity of the compound against *E. coli*, *P. aeruginosa*, and *S. aureus* pathogenic bacterial cells [13].

Tryptophan ($C_{11}H_{12}N_2O_2$, M 204.22 g mol^{-1}, TRP, figure 1) is an amino acid used as a precursor of serotonin, a neurotransmitter responsible to control appetite, sleep patterns, and mood [14]. This essential amino acid contains at least three coordination sites to metals: the oxygen of carboxylate and also the nitrogen of NH_2 and the indolic ring, which makes TRP a versatile ligand in coordination chemistry. A silver(I) complex with tryptophan of composition $Ag_3C_{22}H_{22}O_7N_5$ has already been reported [5], and a copper(II) complex with tryptophan, of coordination formula $[Cu(TRP)_2]$, is described in the literature [15]. The Cu(II) complex was shown to possess anti-inflammatory, antiulcer, and anti-convulsive activities [15].

Çakir and Biçer [16] described the synthesis and electrochemical analyses of a new vanadium-TRP complex, $Na_4[V_3O_9(TRP)]$. No biological applications were described by the authors. A mixed palladium complex with tryptophan and diethylenetriamine, $[Pd(dien)TRP]^{2+}$, was described in the literature. Coordination of tryptophan to Pd(II) occurs through nitrogen of NH_2, while the diethylenetriamine coordinates tridentate to Pd(II) [17]. Synthesis, characterization, and biological studies of a palladium(II) complex with tryptophan, with emphasis on its potential application as an antibacterial agent, are described in this article.

2. Experimental

2.1. *Reagents and equipments*

L-Tryptophan (TRP, 98%) and potassium tetrachloropalladate(II) (K_2PdCl_4, 98%) were purchased from Sigma-Aldrich Laboratories. Potassium hydroxide (85%) was obtained from Fluka. Elemental analyses for carbon, hydrogen, and nitrogen were performed using a CHNS/O Perkin Elmer 2400 Analyzer. Infrared (IR) spectra from 4000 cm^{-1} to 400 cm^{-1} of TRP and the Pd(II)-TRP complex were measured using an ABB Bomen MB Series Model B100 with resolution of 4 cm^{-1}; samples were prepared as KBr pellets. Raman spectrum was recorded using a Jobin-Yvon T64000 single spectrometer system, equipped with a confocal microscope and a nitrogen-cooled charge coupled device (CCD) detector. The spectrum was collected using a 514.5 nm

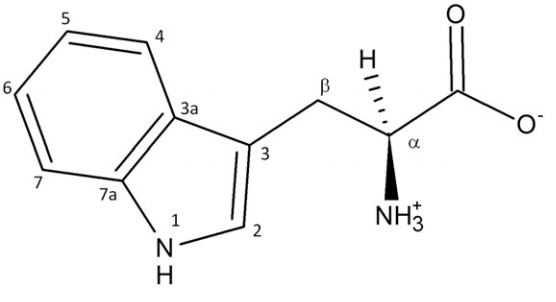

Figure 1. Structural formula of TRP showing carbon and hydrogen numbering.

(2 mW) line of an Ar/Kr laser at room temperature and using a solid sample of the complex. The UV-Vis spectroscopic measurements and kinetic studies of the Pd-TRP complex were carried out using a Hewlett-Packard 8453A diode array spectrophotometer. A dimethylsulfoxide solution of the complex (7.80×10^{-5} mol L^{-1}) was prepared and measured in 10.00 mm quartz cuvettes. The measurements were performed over 42 h at intervals of 1h and the temperature was maintained constant at 298.0 ± 0.1K using an Agilent 89090A temperature control device. The ^{1}H NMR spectra of TRP and Pd-TRP were recorded on a Bruker Avance 250 MHz spectrometer operating at 250.1 MHz. The ^{13}C NMR spectrum of TRP was also recorded on a Bruker Avance 250 MHz operating at 62.9 MHz while the ^{13}C Pd-TRP spectrum was recorded on a Bruker Avance 400 MHz operating at 100.6 MHz; samples were prepared in deuterated dimethylsulfoxide solutions. The ^{15}N NMR chemical shifts for TRP and the Pd-TRP complex were indirectly detected in the solution-state by a heteronuclear [^{1}H–^{15}N] multiple bond coherence (HMBC) experiment. The ^{1}H–^{15}N NMR data were acquired on a Bruker Avance 400 MHz spectrometer using a 5-mm probe at 303 K. Electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-QTOF-MS) measurements were carried out in a Waters Synapt HDMS instrument. A sample of Pd-TRP was dissolved in DMSO at a concentration of 1.0 mg mL^{-1} and then further diluted in 50 : 50 H_2O/MeCN (0.1% formic acid v/v) to 2 μmol L^{-1}. The resulting solutions were directly infused into the instrument's ESI source at a flow rate of 15 μL min^{-1}. ESI(+) mass spectra (fullscans) and fragment ion spectra for quadrupole-isolated ions (QTOF-MS/MS) were acquired in reflectron W-mode at a scan rate of 1 Hz. For fragment ion spectrum experiments, by collision-induced dissociation (argon as collision gas), the desired ion was isolated in the mass-resolving quadrupole, and the collision energy of the trap cell was increased until sufficient fragmentation was observed. Prior to all analyses, the instrument was externally calibrated with phosphoric acid oligomers (H_3PO_4, 0.05% v/v in 50 : 50 H_2O/MeCN) ranging from m/z 99 to 980. Thermogravimetric and differential thermal analysis (TGA/DTA) were performed on a simultaneous TGA/DTA Seiko EXSTAR 6000 thermoanalyzer using the following conditions: synthetic air, flow rate of 50 cm^3 min^{-1}, and heating rate of 10°C min^{-1}, from 25°C to 1000°C. The residue of the thermal treatment was analyzed on a Shimadzu XRD-6000 diffractometer (Cu-Kα radiation, $\lambda = 1.5406$ Å) with a graphite monochromator at room temperature. The sample was scanned over the 2θ range from 4° to 70°.

2.2. *Synthesis of the complex*

The palladium(II) complex with L-tryptophan (Pd-TRP) was synthesized by the reaction of 1.0×10^{-3} mol of a freshly prepared aqueous potassium tryptophanate solution (10.0 cm^3, pH = 10) with 5.0×10^{-4} mol of an aqueous solution of K_2PdCl_4 (5.0 cm^3). The synthesis of the complex was carried out with stirring at room temperature. After 90 min of constant stirring, the orange solid obtained was collected by filtration, washed with cold water, and dried in a desiccator with P_4O_{10}. The yield was 74%. Anal. Calcd for $[Pd(C_{11}H_{11}N_2O_2)_2] \cdot 2H_2O$ (%): C, 48.2; H, 4.00; N, 10.2. Found: C, 48.6; H, 3.65; N, 9.84. The complex is soluble in DMSO, but insoluble in water, chloroform, ethanol, methanol, acetone, and hexane. No single crystals were obtained for a detailed X-ray crystallographic study. The aqueous potassium

tryptophanate solution used in the synthesis of the Pd-TRP complex was prepared *in situ* by reaction of equivalent amounts of TRP and potassium hydroxide.

2.3. *Molecular modeling*

Geometric optimizations were carried out using GAMESS software [18] with a convergence criterion of 10^{-4} a.u. in a conjugate gradient algorithm. The LANL2DZ [19] effective core potential was used for palladium and the atomic 6-31G (d) basis set [20–23] for all other atoms. Density functional theory (DFT) calculations were performed using the B3LYP [24, 25] gradient-corrected hybrid to solve the Kohn–Sham equations with a 10^{-5} a.u. convergence criterion for the density change. The final geometries were confirmed as minima of the potential energy surface (PES) with calculation of the hessians showing no imaginary frequencies.

The harmonic vibrational frequencies and intensities were calculated at the same level of theory with analytical evaluation of second derivatives of energy as a function of atomic coordinates. Frequencies were scaled by a factor of 0.9614, as recommended by Scott and Radom [26] and analyzed using the software Molden-4.7 [27].

2.4. *Antibacterial assays*

Four referenced bacterial strains: *E. coli* – ATCC 25922, *P. aeruginosa* – ATCC 27853, *Enterococcus faecalis* – ATCC 7080, and *S. aureus* – ATCC 25923 were used for antibacterial experiments. The antibiogram assay was performed by the disc diffusion method [28, 29]. The sensitivity of Pd-TRP complex was tested in Mueller–Hinton (MH) agar. The microorganisms were transferred to separate test tubes containing 5.0 cm^3 of sterile brain heart infusion (BHI) medium and incubated for 18 h at 35–37°C. Sufficient inocula were added in new tubes until the turbidity equaled 0.5 McFarland ($\sim 1.5 \times 10^8$ CFU cm^{-3}). The bacterial inocula diluted with BHI (McFarland standard) were uniformly spread using sterile cotton swabs on sterile Petri dishes containing MH agar.

Sterile filter paper discs (10 mm diameter) were aseptically impregnated with 800 μg of Pd-TRP according to the following procedure: 20.0 mg of Pd-TRP were suspended in 1000 μL of distilled water, homogenized in a vortex, and 40 μL of the suspension were collected with a micropipette and transferred to the paper discs. Sterile discs impregnated with 800 μg of pure TRP were used as a negative control.

Discs impregnated with the Pd-TRP complex or with TRP were dried and sterilized in a vertical laminar flow under UV radiation for 45 min before the experiment. The impregnated discs were placed on the surfaces of the solid agar. The plates were incubated for 18 h at 35–37°C and examined thereafter. Clear zones of inhibition around the discs were measured and the complex sensitivity was assayed from the diameter of the inhibition zones (in millimeters). The observed results were compared to discs with the standard antibiotics gentamicin (GEN) and ceftriaxone (CRO), which were considered our positive controls, and also with discs impregnated with lithium tetrachloropalladate(II) and pure tryptophan. The obtained data were also compared with other palladium(II) complexes described earlier [6, 9].

3. Results and discussion

3.1. *^{1}H and ^{13}C NMR spectroscopic measurements*

Following literature recommendations [30], the structural formula of TRP with hydrogen and carbon numbering is shown in figure 1.

The ^{1}H NMR spectrum of TRP shows hydrogen atoms of methylene (CH_2) in the range 2.9–3.4 ppm and the hydrogen bonded to α-carbon at 3.5 ppm. The multiplet at 6.9–7.6 ppm represents the aromatic protons in TRP. The observed data match with those described in the literature for the TRP molecule [31]. After coordination, the methylene protons are in the range 2.9–3.2 ppm, while hydrogen of α-carbon are at 3.4–3.5 ppm. Since the complex contains water, the signal at 3.33 ppm is observed in the ^{1}H NMR spectrum of the complex, making difficult the attribution of the ^{1}H signal of the hydrogen bound to the α-carbon. No changes were observed for aromatic protons of the indole group of TRP, indicating no coordination through N–H of the indole ring. The ^{1}H NMR spectra of TRP and Pd-TRP are shown as "Supplementary material."

The Pd-TRP complex was also studied by ^{13}C NMR. ^{13}C chemical shifts for the complex, in comparison to free L-TRP, are presented in table 1. C2, C3, C3a, C7a, C4, C5, and C6 of the Pd-TRP complex show only minor differences when compared to the chemical shifts of free TRP, which indicates no coordination through the N-aromatic ring. On the other hand, shifts observed for the carbon of carboxylate, and also for carbons Cα and Cβ in the ^{13}C NMR spectrum of the Pd-TRP complex, when compared to the free ligand, show coordination of the ligand to Pd(II) through nitrogen of NH_2 (H_2N–Pd) and oxygen of the carboxylate group (COO–Pd). The ^{13}C NMR spectra are shown in "Supplementary material."

3.2. *^{1}H–^{15}N NMR spectroscopic measurements*

The ^{1}H–^{15}N HMBC spectra of TRP and Pd-TRP are shown as "Supplementary material." It is possible to confirm that one of the coordination sites of the ligand to Pd(II) is nitrogen of NH_2. In the ligand spectrum, ^{15}N chemical shift of NH_2 is at 39.8 ppm while for the complex, this signal is shifted upfield to −18.3 ppm. The observed $\Delta\delta$ (δ complex–δ ligand) of −58.1 ppm confirms coordination through NH_2. It is also possible to state that no coordination occurs through nitrogen of the aromatic ring since the ^{15}N chemical shift of the nitrogen of the indole group is observed at 130.4 ppm in the ligand spectrum and the complex.

Table 1. Solution-state $^{13}C\{^{1}H\}$ and ^{15}N NMR data for TRP and Pd-TRP.

	Chemical shifts (ppm)												
Compounds	COO^-	Cα	Cβ	C2	C3	C3a	C7a	C4	C7	C5	C6	NH_2	NH
TRP	170.9	55.20	27.55	124.6	110.0	127.7	136.8	118.8	111.8	118.7	121.3	39.8	130.4
Pd-TRP	181.2	58.75	29.63	124.5	109.7	127.6	137.0	118.8	112.0	118.8	121.7	−18.3	130.4

3.3. *IR and Raman spectroscopic data*

The Pd-TRP IR spectrum was analyzed in comparison to the ligand spectrum (Supplementary material). The characteristic $\nu(NH_3^+)$ and $\delta(NH_3^+)$ bands and the carboxylate stretching vibrations in the free ligand confirm its existence in the zwitterionic form [32]. In the infrared spectrum of TRP, a strong band at 3403 cm^{-1} is assigned to ν(N–H) of the N-aromatic ring and two poorly resolved bands between 3090 cm^{-1} and 2980 cm^{-1} correspond to asymmetric and symmetric vibrations of NH_3^+. The poor resolution is attributed to the presence of intermolecular hydrogen bonds. The angular deformation of the NH_2 group is observed at 1590 cm^{-1}. Bands at 1668 and 1414 cm^{-1} are attributed to asymmetric and symmetric stretching modes of free COO^-. In Pd-TRP complex the asymmetric and symmetric stretching frequencies of NH_2 group shift to high energies at 3228–3107 cm^{-1}, while symmetric and asymmetric stretches for COO^- appear in the region 1377 and 1651 cm^{-1}, indicating coordination through NH_2, and COO^- groups.

Raman spectroscopic measurements at 150–700 cm^{-1} were performed to identify frequencies related to Pd–O and Pd–N bonds. Two bands are observed at 400 cm^{-1} and at 540 cm^{-1}, assigned to Pd–O and Pd–N bonds. Similar results were observed for Pd(II) complexes with glycine [33] and methionine sulfoxide [34].

3.4. *UV-Vis spectroscopic measurements*

The UV-Vis spectrum of the Pd-TRP complex exhibits absorptions at 283 nm ($\varepsilon = 2.0 \times 10^4\,dm^3\,mol^{-1}\,cm^{-1}$) and at 293 nm ($\varepsilon = 2.2 \times 10^4\,dm^3\,mol^{-1}\,cm^{-1}$). Also, a shoulder is observed at 275 nm. According to the literature, these bands are assigned to π–π^* transitions in the indole group of tryptophan [35]. The magnitude of the molar absortivities (ε) is consistent with intra-ligand transitions. The UV-Vis spectra of TRP and the Pd-TRP complex are also shown as "Supplementary material."

3.5. *Mass spectrometric measurements*

ESI-QTOF-MS analysis of Pd-TRP shows the monoprotonated ions ($[Pd\text{-}TRP_2 + H]^+$, m/z 513.07) as the most abundant species in solution (figure 2a). An isotope pattern comparison for the singly charged $[Pd\text{-}TRP_2 + H]^+$ ion (figure 2b) shows good agreement with theoretical predictions. The mass error was +0.78 ppm for $[Pd\text{-}TRP_2 + H]^+$ ($C_{22}H_{23}N_4O_4Pd^+$, Calcd m/z 513.0760, exp. m/z 513.0764), considering the monoisotopic ion.

The $[Pd\text{-}TRP_2 + H]^+$ ion was further characterized by collision-induced dissociation experiments. The fragment ion spectrum (figure 3) of $[Pd\text{-}TRP_2 + H]^+$ shows the loss of a neutral L-TRP ligand (204 Da) from the precursor, as well as losses due to fragmentation of the ligand, thus confirming the proposed composition of the Pd-TRP complex.

3.6. *Thermal analysis*

The TGA data confirmed the composition of the complex formulated as $[Pd(C_{11}H_{11}N_2O_2)_2] \cdot 2H_2O$. Water content is lost from 25°C to 120°C. Oxidation of

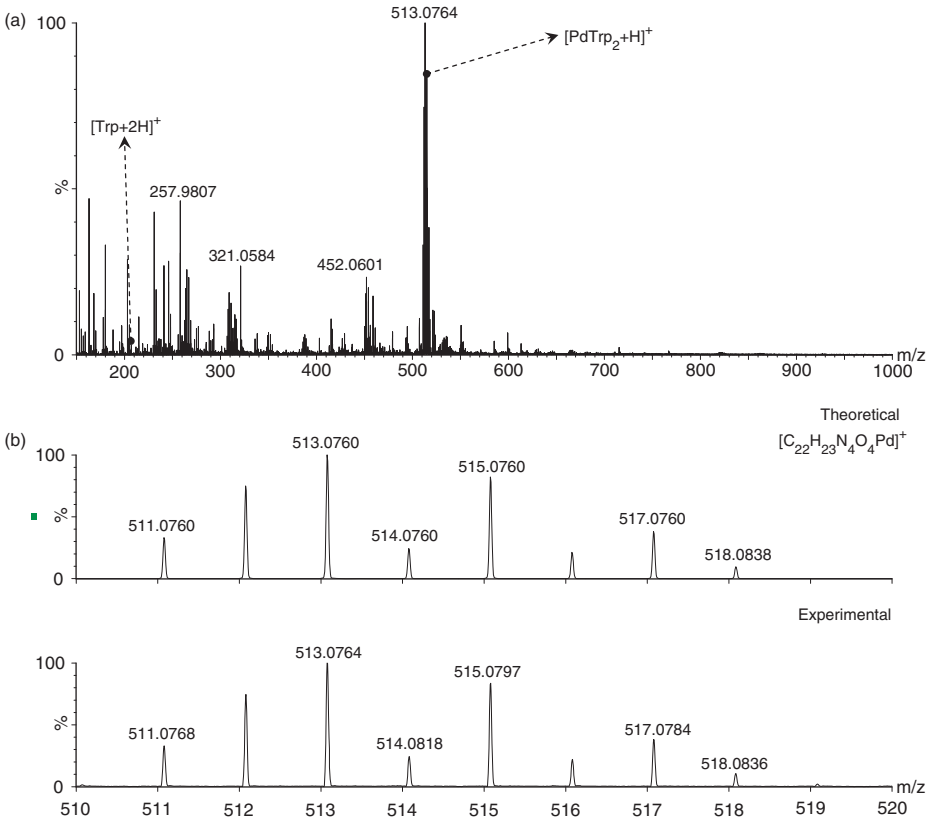

Figure 2. Mass spectra for the Pd-TRP complex. (a) ESI(+)-QTOF mass spectrum from m/z 150 to 1000, demonstrating $[Pd(TRP)_2 + H]^+$ ions. The notation TRP in this case refers to the tryptophan ligand minus one hydrogen ($C_{11}H_{11}N_2O_2$, 203.0820 Da). (b) Isotope pattern comparison for the monoprotonated complex, $[Pd(TRP)_2 + H]^+$ of m/z 513.08.

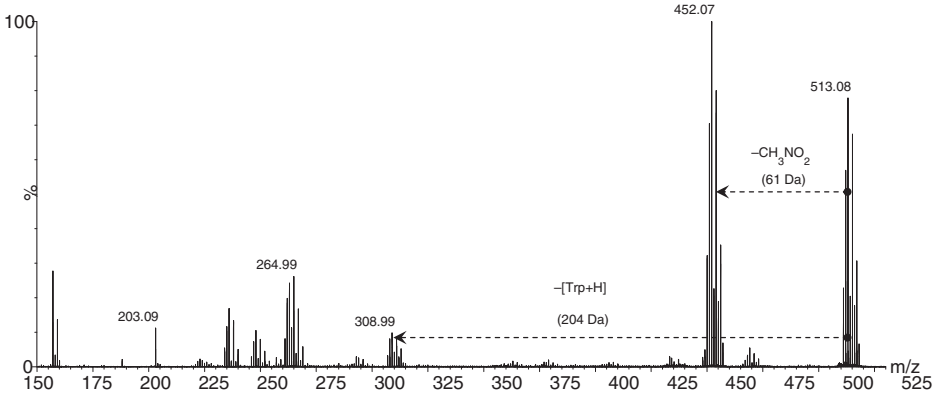

Figure 3. Fragment ion mass spectrum (collision-induced dissociation) for the Pd-TRP monoprotonated ion, $[Pd(TRP)_2 + H]^+$ of m/z 513.08.

the ligand starts at 215°C and continues until 477°C. The residue of the thermal decomposition matches palladium oxide (PdO). In the DTA we can see exothermic peaks at 230°C and 477°C related to ligand oxidation, and also a small endothermic peak at 826°C related to reduction of PdO to Pd. The residue of the thermal treatment was identified by powder X-ray diffraction studies as metallic Pd [36]. The TG/DTA curves are also presented as "Supplementary material."

3.7. *Kinetic studies*

In order to provide information concerning the stability of the Pd-TRP complex in solution, preliminary kinetic studies in dimethylsulfoxide were performed. These studies were carried out using UV-Vis spectroscopic measurements. It was possible to observe that after 42 h no differences were observed, which indicates the stability of the compound in solution under the conditions.

3.8. *Molecular modeling*

Theoretical studies with Pd-TRP were performed in order to provide additional information concerning the N, O coordination of the ligand to Pd(II). According to DFT studies, coordination through nitrogen of NH_2 and the oxygen of the COO^- group, in a monodentate form, was shown to be a minimum of the PES, with calculation of the hessians showing no imaginary frequencies. As expected for Pd(II) complexes, the coordination sphere is square-planar except for small deviations. The calculated Pd–N bond distance is 2.08 Å, Pd–O 2.03 Å, while the calculated N–Pd–O angle is 81.6°. These results are comparable to experimental X-ray diffraction data observed for a mixed Pd(II) complex with the amino acid benzoylvaline (Bzval), of coordination formula [Pd(bipy)(Bzval-N,O)], where the Pd–N bond distance is 2.02 Å, Pd–O 1.99 Å and N–Pd–O angle is 82.0° [37]. The analysis of the theoretical vibrational spectrum showed the main IR bands observed in the experimental spectrum of the complex, e.g., calculation predicts one strong band at 1703 cm^{-1} and other at 1305 cm^{-1} for the asymmetric and symmetric COO^- stretching, respectively. Other calculated bands are 3536 cm^{-1} for the indolic N–H stretching and 3309 cm^{-1} and 3387 cm^{-1} for the amine NH_2 symmetric and asymmetric stretchings, respectively. All these calculated frequencies confirm the previous assignments of the experimental IR spectrum. Moreover, the calculated frequencies revealed that the mode at 393 cm^{-1} is consistent with a symmetric Pd–O stretch and other at 509 cm^{-1} originates from a symmetric Pd–N stretching. The same pattern is observed in the experimental Raman spectrum and is consistent with a *trans* configuration.

3.9. *Antibacterial assays*

Antibiotic sensitivity profiles of bacterial strains demonstrate the antibacterial activity of the palladium(II) complex with TRP against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus*) microorganisms, as observed by the disc diffusion method. The complex showed no activity against *E. faecalis*. Paper discs impregnated with the Pd-TRP complex exhibited inhibition zones for *E. coli*,

Table 2. Antibiotic sensitivity profiles of bacterial strains against Pd-TRP, pure TRP (L-tryptophan), K_2PdCl_4, and the standard antibiotics GEN and CRO.

	Results			
Compounds	*S. aureus* Inhibition zone diameter (mm)	*E. faecalis* Inhibition zone diameter (mm)	*E. coli* Inhibition zone diameter (mm)	*P. aeruginosa* Inhibition zone diameter (mm)
Pd-TRP (800 μg)	12.0 (±0.1)	0.0	13.0 (±0.1)	15.0 (±0.1)
TRP (800 μg)	0.0	0.0	0.0	0.0
K_2PdCl_4 (800 μg)	15.0 (±0.1)	17.0 (±0.1)	20.0 (±0.1)	19.0 (±0.1)
GEN (40 μg)	28.0 (±0.1)	20.0 (±0.1)	26.0 (±0.1)	25.0 (±0.1)
CRO (40 μg)	19.0 (±0.1)	0.0 (±0.1)	30.0 (±0.1)	0.0 (±0.1)

P. aeruginosa, and *S. aureus* of 13.0 ± 0.1 mm, 15.0 ± 0.1 mm, and 12.0 ± 0.1 mm, respectively. The inhibition zones for *E. coli* and *P. aeruginosa* indicated that these bacterial strains are sensitive to the palladium(II) complex, being similar to the results obtained for the Pd(II) complex with S-allyl-L-cysteine recently described by us [10]. The observed results are comparable to mixed palladium(II) complexes with aminomethylbenzimidazole (AMBI) and the amino acids (AA) glycine, alanine, cysteine, methionine, and serine. Most of the compounds, of composition $[Pd(AMBI)(AA)]^{n+}$, have antibacterial activities against Gram-negative *E. coli* bacterial strains in the concentration of $5.0\,mg\,cm^3$ [38]. Also, the results obtained for the Pd-TRP complex are comparable to the platinum(II) and palladium(II) complexes with Schiff bases 1*H*-indol-2,3-dionethiosemicarbazone and 1*H*-indol-2,3-dionesemicarbazone against the *E. coli* bacterial strains [39].

For *S. aureus*, the inhibition zone of 12.0 ± 0.1 mm indicates the sensitivity of this bacterial strain to the palladium(II) complex, being comparable to the recently published antibacterial activity of the gold(I) complex with N-acetyl-L-cysteine [12]. Pure TRP did not exhibit antibacterial activity against the considered bacterial strains under the same experimental conditions, while discs impregnated with lithium tetrachloropalladate(II) also exhibited clear inhibition zones for the bacterial strains similar to the Pd-TRP complex, as observed in table 2. These results indicate that the antibacterial activity of the palladium(II) complex is most probably due to the Pd(II) ions, being similar to the described mechanisms of action of some Ag(I) compounds used as antibacterial agents and also to the Pd(II) complex with deoxyalliin already reported [10].

The antibacterial activity of the Pd-TRP complex, as well as the stability of the compound in solution reported in section 3.7, is of significance in medicinal chemistry. The stability of the Pd-TRP complex leads us to consider the preparation of novel antibacterial wound dressings, which have shown to possess some intrinsic benefits like stimulation of healing in indolent wounds, prophylactic applications, and treatment of critically colonized wounds [40].

4. Conclusion

A new palladium complex with tryptophan (Pd-TRP) with a 1 : 2 molar composition (metal : ligand) was obtained and structurally characterized. The ^1H, ^{13}C, and ^{15}N

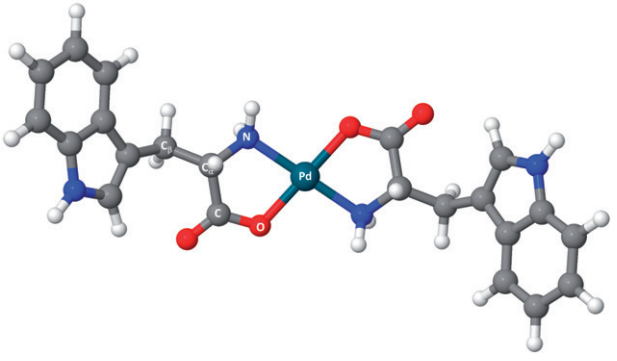

Figure 4. Optimized structure for the Pd-TRP complex.

NMR data, as well as ESI-QTOF, IR, Raman, and UV-Vis spectroscopic measurements, support coordination of the ligand to Pd(II) *via* the nitrogen of the NH_2 group and the oxygen of carboxylate group. Based on the chemical, spectroscopic and DFT results a schematic structure for the Pd-TRP complex is shown in figure 4.

Biological studies using the disc diffusion method revealed antibacterial activity of the Pd-TRP complex against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus*) microorganisms, which reinforces the potential of application of Pd(II) complexes with amino acids as antibacterial agents. Further studies concerning the preparation of wound dressings with the Pd-TRP complex are envisaged in order to evaluate the potential of application of the compound in skin infections.

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