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Palladium(II) Complex derived from Harmaline; an Alkaloid Isolated from *Peganum harmala* Seeds: Synthesis, Characterization and Cytotoxic Activity

TALAL A.K. AL-ALLAF* and LUAY J. RASHAN†
Department of Chemistry
Faculty of Science, Applied Science University, Amman-11931, Jordan

The 1 : 1 molar ratio reaction of *trans*-[Pd(DMSO)$_2$Cl$_2$] (**I**) with harmaline, the naturally occuring alkaloid, isolated from *Peganum harmala* seeds, in non-aqueous solvent, was found to give, mainly, the monomer *trans*-[Pd(harmaline)(DMSO)Cl$_2$] (**II**) as a major component and the dimer [Pd(harmaline)Cl$_2$]$_2$ (**III**) as a minor one. The monomer (**II**) can be formed, as a single product, either by the direct reaction between **I** and harmaline in DMSO or by dissolving **II** in DMSO. In both cases the pure monomer (**II**) can be precipitated from its DMSO solution by the addition of 0.05 M HCl. The complexes have been characterized by their C, H, N analysis, ^{1}H, ^{13}C, IR and mass spectroscopic methods. The monomer (**II**) has been tested for its cytotoxic activity against several tumour cell lines, *i.e.*, P_{388}, L_{1210}, K_{562}, Raji, HeLa, RD and Hep-2. This activity was compared with those of reference standards, cisplatin, carboplatin and 5-fluorouracil (5-FU).

INTRODUCTION

In our recent work on the palladium(II) complexes, we have given a brief survey about the importance of platinum complexes as antitumour agents and reported the first antileukaemic *trans*-palladium(II) complex with 7-methoxy-1-methyl-9H-pyrido[3,4-b]indole (harmaline); a naturally occurring β-carboline alkaloid, isolated from *Peganum harmala* seeds.[1] As a continuation of our comprehensive interest in the biological evaluation of metal cmplexes with *e.g.*, β-carboline alkaloids,[2–5] we are presenting here another new *trans*-palladium(II) complex derived from 4,9-dihydro-7-methoxy-1-methyl-3H-pyrido[3,4-b]indole (harmaline) as another naturally occurring β-carboline alkaloid, isolated from *Peganum harmala* seeds. The present study also includes the examination of this complex against several tumour cell lines, both the fluid suspension cell lines (P_{388}, K_{562}, L_{1210} and Raji) and the solid ones (HeLa, RD and Hep-2).

Both authors are on leave from the University of Mosul, Iraq.
†Department of Pharmacology, Faculty of Pharmacy, Applied Science University Amman-11931, Jordan.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded at the University of Jordan, Amman, Jordan, on a Burker-DPX 300 MHz spectrometer using DMSO-d_6 as a solvent with TMS as an internal standard. IR spectra were recorded on Nicolet FT-IR (Impact 400) spectrometer, using KBr discs in the range 4000–400 cm^{-1} or CsI discs in the range 4000–200 cm^{-1} on SP 2000 Pye Unicam spectrophotometer. Elemental analyses were performed at the Atlantic Microlab, Inc., Norcross, Georgia-30091 (USA). Mass spectrum of complex (**II**) was performed at Sussex University, Brighton, UK, using FAB technique with cold inlet and 3-nitrobenzoyl alcohol (3-NBA) as a solvent.

Starting Materials

The compounds K_2PdCl_4, $PdCl_2$ and harmaline were commerical products (Fluka) and used without further purification. The complex *trans*-$[Pd(DMSO)_2Cl_2]$ was prepared as described in our previous article.[1]

Preparation of *trans*-[Pd (DMSO)(harmaline) Cl_2]

The complex *trans*-$[Pd(DMSO)_2Cl_2]$ (0.5 g, 1.5 mmol) was suspended in acetone (40 mL) and placed in a 150 mL two-necked round-bottomed flask fitted with water condenser. A suspension of harmaline (0.32 g, 1.5 mmol) in acetone (40 mL) was gradually added to the above supension. The reaction mixture was immediately turned clear orange; it was left under reflux for *ca.* 3 h, then filtered through celite. The clear orange solution was taken to dryness to give an orange residue. This was redissolved in a small amount of DMSO (to destroy the dimeric product if there is any) and the product was precipitated by 0.05 M hydrochloric acid solution. The solid was collected by filtration, washed with water and air dried first, then under vacuum at 60°C for several hours. The yield was 0.55 g (78%).

This complex can also be prepared by dissolving the complex *trans*-$[Pd(DMSO)_2Cl_2]$ in DMSO with gentle heating and after cooling the solution to room temperature it was treated with 0.05 M HCl until complete precipitation. The solid thus formed was treated as above. In both cases, the solid can be recrystallized from $CHCl_3$/n-hexane to give fine orange crystals.

Biological Methods

(1) *Complex:* The complex (**II**) was dissolved in 10% DMSO. Serial dilutions of 0:1, 1.0 and 10.0 μg/mL were used and millipore (0.2 μm) filtered under laminar flow conditions. Reference standards (cisplatin, carboplatin and 5-FU) were purchased from Bristol Myers (USA).

(2) *Cell lines:* Several cell lines were used in the present study; these include both the fluid suspension cell lines (*e.g.*, P_{388}, K_{562}, L_{1210} and Raji) and solid cell lines (*e.g.*, HeLa, RD and Hep-2). The fluid suspension cell lines were maintained in RPMI-1640 and supplemented with 5% fetal calf serum, L-glutamine and antibiotics, whereas the solid cell lines were maintained in Dulveccos minimum

essential mdium (DEME) and supplemented with 5% fetal calf serum, L-glutamine and antibiotics.

(3) *Cytotoxicity test*: MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was performed in a 96-well plate[6,7]. The above cell lines (2×10^4 cells/mL) were seeded in each well with 100 μL of the growth medium and antibiotics, using the method described in our previous work.[3]

RESULTS AND DISCUSSION

The preparation of the starting material *trans*-[$Pd(DMSO)_2Cl_2$] and its reaction with harmaline are summarized in Scheme 1. The complex *trans*-[Pd harmaline) (DMSO) Cl_2] (**II**) was isolated from its DMSO solution and purified as described in the experimental part. The physical properties of **II** are listed in Table-1.

SCHEME 1
THE PREPARATIVE ROUTE FOR PURE (II)

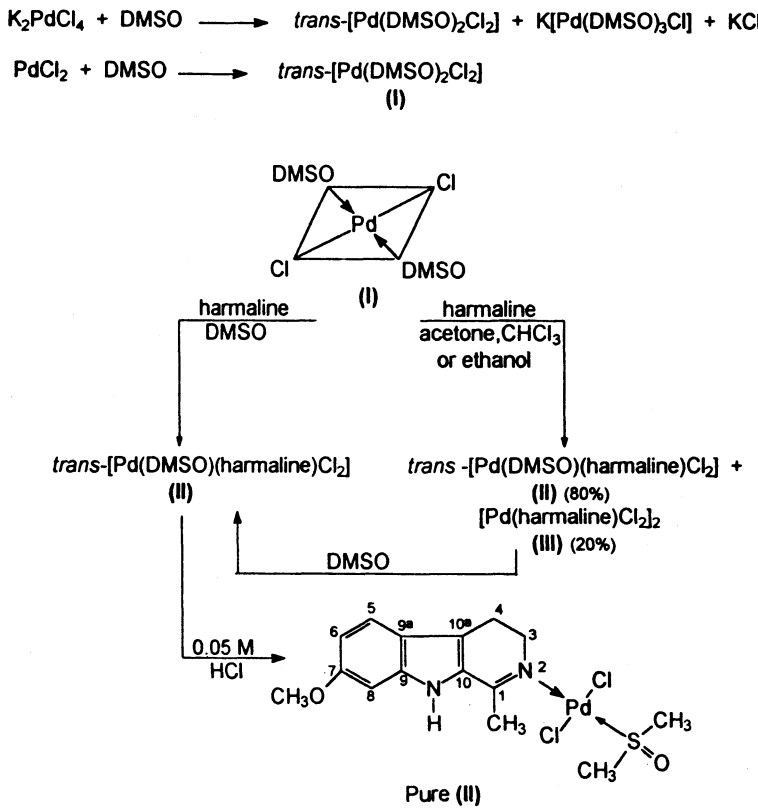

TABLE-1
PHYSICAL PROPERTIES AND ANALYSES OF COMPLEX (II)

Method of analysis	Results
Colour	Orange
m.p. (°C)	> 146 with decomposition
IR (cm^{-1})*	ν(N—H) = 3311m; ν(C—H) = 2996, 2919m; ν(C=C) = 1633s; ν(C=N) = 1556s; ν(S=O) = 1121s; ν(Pd—Cl) = 320m
Elemental analysis	Found (%): C = 38.6, H = 4.5, N = 6.1; calculated (%) for $C_{15}H_{20}N_2O_2Cl_2SPd$: C = 38.5, H = 4.3 N = 6.0
Mass spectrum†	m/z = 470, 460, 435, 391, 370, 215, 136, 69
^1H NMR‡	δ(CH_3) = 3.2s (3H); δ(CH_3O)=3.8s (3H) δ(NH)=11.6b (1H); δ(HC–3) = 3.0d (1H), (J = 31) and 3.9m (1H); δ(HC–4) = 2.9b (2H); δ(HC–5) = 6.8dd (1H) (J = 1.8); δ(HC–6) = 7.5dd (1H) (J = 2.8); δ(HC–8) = 6.9b(1H).
^{13}C NMR‡	δ(C-1) = 164.4; δ(C-3) = 52.9, δ(C-4) = 19.8, δ(C-5) = 118.3, δ(C-6) = 112.5, δ(C-7) = 159.3, δ(C-8) = 94.7, δ(C-9) = 139.7, δ(C-9a) = 119.0, δ(C-10) = 128.5, δ(C-10a) = 122.1, δ(CH_3) = 24.6, δ(CH_3O) = 55.8

*Selected IR bands; s. strong; m. medium bands.

†FAB technique used with cold inlet, using 3-NBA as a solvent; molecular ion m/z = 469.4.

‡Downfield from internal TMS at room temperature, using DMSO-d_6 as a solvent; s, singlet; dd, doublet of doublet; m, multiplet; b, broad signals

We have previously reported that the reaction of *trans*-[Pd(DMSO)$_2$Cl$_2$] with β-carboline alkaloids isolated from *Peganum harmala* seeds leads to the formation of various palladium(II)-alkaloid complexes, both the monomer and the dimer.[2] Later studies of ours on such complexes, but with platinum metal, using the FAB-mass spectroscopic technique, showed that two products could be detected the monomer (with one coordinated DMSO molecule) and the dimer (without DMSO).[8] Recently, we have found that palladium metal gave almost certainly similar products when *trans*-[Pd(DMSO)$_2$Cl$_2$] was treated with harmine[1], the monomer *trans*-[Pd(harmine)(DMSO)Cl$_2$] (30% proportion) and the dimer [Pd(harmine)Cl$_2$]$_2$ (70% proportion). In the present study, the reaction of *trans*-Pd(DMSO)$_2$Cl$_2$ with harmaline was found to give, again, the monomer and the dimer (Scheme-1) but with *ca.* 80% and 20% proportions, respectively, *i.e.*, opposite trend to that of harmine one. This may be due to the fact that harmaline possesses some saturation on C-3 and C-4, which in turn allows some structural modification and this prefers the formation of the monomer, in contrary to harmine which does not possess such a structural flexibility, *i.e.*, planar molecule.

However, the characterization data are clearly assigned to the formal *trans*-[Pd(harmaline)(DMSO)Cl$_2$] (**II**). The DMSO molecule has been coordinated *via* sulphur and this is very clear from the strong IR band appearing at 1121 cm^{-1}. The rest of other spectral data obtained from ^1H, ^{13}C and mass techniques are in a good agreement with those expected for complex **II** and compare very well with those obtained for the harmine analogue complex.[1]

The cytotoxic activity of complex **II** (Scheme-1) against the different cell lines

compared with those of the reference standards are shown in Table-2. It appears from these results that complex **II** exhibited different cytotoxic activities against all the cell lines. However, this complex showed certain selectivity especially towards the fluid suspension cell lines (P_{388}, K_{562}, L_{1210} and Raji), where the IC_{50} values were ranging from 0.25–0.45 μg/mL. This activity is somewhat approaching to the IC_{50} values of cisplatin against the same cell lines and superior to those of carboplatin where the IC_{50} values were greater than 10 μg mL against the same cell lines. Furthermore, complex **II** showed no cytotoxic activities against the solid cell lines (HeLa, RD and Hep-2) at concentrations ≤ 10 μg/mL.

TABLE-2
CYTOTOXIC ACTIVITIES OF COMPLEX II WITH STANDARD REFERENCES AGAINST DIFFERENT TUMOUR CELL LINES

Compound	IC_{50} (μg/mL)						
	P_{388}	L_{1210}	K_{562}	Raji	HeLa	RD	Hep-2
(II)	0.35	0.45	0.40	0.25	>10	> 10	>10
Cisplatin	0.15	6.00	0.25	NT	5.5	> 10	1.8
Carboplatin	> 10	> 10	> 10	> 10	> 10	> 10	>10
5-FU	0.15	NT	0.15	NT	NT	NT	NT

NT: Not tested.

Preliminary, the selectivity of complex **II** against the leukaemic cell lines (P_{388}, K_{562} and L_{1210}) and the Buerkitts lymphoma cell lines (Raji) is very interesting and promising. Nevertheless it is premature to conclude about such activity unless otherwise confirmed by *in vivo* tests in animal models. Therefore, further studies are necessary to confirm the above activity.

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