Synthesis and Physicochemical, Antimitogenic, and Antiviral Properties of a Novel Palladium (II) Coordination Compound

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Abstract □ The synthesis, identification, and analytical characterization of a newly synthesized coordination complex, $PdCl_2$(2,6-diaminopyridine)·H_2O, are reported. The solubility of the compound in water and in different aqueous and basic solutions ranged between 7.5 and 14.2 × 10^{-4} *M*. Almost complete suppression (99.7%) of uptake of tritiated thymidine by phytohemagglutinin-induced transforming lymphocytes was produced with 50 μg of the drug. Furthermore, there was a good dose–response relationship between the amount of the drug used and its ability to suppress DNA synthesis. No lymphocytotoxic effect was produced with 50 μg of the drug after 1 and 72 hr of incubation at 37°. The antiviral activity of the complex was measured against vaccinia virus in Hela and chicken embryo fibroblast monolayer cultures. Complete inhibition of plaque-forming units was produced in both cell cultures.

Keyphrases □ Palladium (II) coordination compound—synthesis and physicochemical, antimitogenic, and antiviral properties of $PdCl_2$(2,6-diaminopyridine) □ Coordination complexes—synthesis and physicochemical, antimitogenic, and antiviral properties of $PdCl_2$(2,6-diaminopyridine), effect on DNA synthesis □ Antiviral agents, potential—synthesis and physicochemical properties of palladium (II) 2,6-diaminopyridine complex □ Antimitogenic agents, potential—synthesis and physicochemical properties of palladium (II) 2,6-diaminopyridine complex

Antineoplastic and immunosuppressive agents could be chemically classified as organic or organometallic compounds. The most recent compounds are the platinum coordination complexes (1–3). The mode of action of these complexes is still under investigation (2, 4).

One problem that has faced investigators in the field of the metal coordination complexes is formulating such complexes into pharmaceutically and biologically acceptable forms. The present article briefly outlines some preliminary experiments concerning the synthesis, physicochemical parameters, and antimitogenic and antiviral activities of a novel palladium (II) 2,6-diaminopyridine complex.

EXPERIMENTAL

Synthesis—To a solution of 0.072 g (0.66 × 10^{-3} mole) of 2,6-diaminopyridine in 10 ml methanol, 0.256 g (0.66 × 10^{-3} mole) of *cis*-$PdCl_2(C_6H_5CN)_2$ (5) in 15 ml methanol was added. A red precipitate formed immediately. The reaction mixture was continuously stirred for 3 hr and was then filtered, washed with methanol, and dried in a vacuum oven at room temperature. The product turned brown at 200° and melted at 245°. The IR spectrum (KBr) showed ν 3490 (OH), 3340 (NH), and 1620 and 1575 (C=N and C=C) cm^{-1}. The UV spectrum of the compound showed a peak at λ_{max} 280 nm with a molar absorptivity ($\epsilon^{M}{}_{1\,cm}$) of 4520, and another at λ_{max} 337 nm with a molar absorptivity ($\epsilon^{M}{}_{1\,cm}$) of 3860. The compound is formulated as $PdCl_2$(2,6-diaminopyridine)·H_2O.

Anal.—Calc. for $C_5H_9Cl_2N_3Pd$: C, 19.85; H, 3.01; Cl, 23.44; N, 13.90. Found: C, 20.23; H, 3.02; Cl, 23.15; N, 13.30.

Solubility Measurement—With a rotating-bottle disintegration apparatus[1], the following procedure was used to determine the intrinsic solubility at 30° of the metal complex in water at different pH's. Aliquots (5 ml) of the solvent were placed in six 10-ml screw-capped test tubes. Excess solid drug was placed into each test tube which was then very tightly capped. The test tubes were attached with rubberbands onto the shaft of the apparatus. The whole apparatus, including the attached test tubes, was then put into a stainless-steel tank which was filled with water thermostatically controlled at 30°. The test tubes should be well below the water level. As the motor rotated, mixing of the suspension in each test tube allowed single crystals of the suspended drug to expose maximum surface area for complete and fast equilibrium solubility measurements. Sampling was done at 2, 4, 8, 12, 24, and 48 hr. Each test tube was considered a sample and was randomly picked up from among the six. The contents of the test tube were immediately transferred into a preheated (around 35°) 10-ml disposable hypodermic syringe[2]. On the tip of the syringe, a preheated swinny adapter was attached[3] containing a white, plain, 13-mm, 0.45-μm filter[3]. The suspension was then forced through the filter and the clear filtrate was collected into a preheated clean test tube. The required dilutions were immediately made and the solutions were assayed spectrophotometrically at λ_{max} 280 and/or 337 nm.

Table I—Solubility of Palladium (II) 2,6-Diaminopyridine Complex in Different Aqueous Media

Stock Medium	pH of Stock Medium	pH of Saturated Solution of Stock Medium[a]	Solubility, moles
0.1 *M* HCl	1.00	1.15	Unstable[b]
0.001 *M* HCl	2.85	2.75	7.5 × 10^{-4}
0.0001 *M* HCl	4.00	3.00	8.25 × 10^{-4}
Boiled distilled water	7.00	3.10	12.5 × 10^{-4}
0.00001 *M* NaOH	8.80	4.50	14.2 × 10^{-4}
0.001 *M* NaOH	10.82	4.70	10.8 × 10^{-4}

[a] At time of sampling; the pH of solution was checked after filtration. [b] The λ_{max} 280 and 337 nm disappeared. New peaks appeared at λ_{max} 212, 233 (sh), and 330 nm.

Antimitogenic Activity—The antimitogenic effect was measured with phytohemagglutinin-induced blastogenic transformation of human lymphocytes. The cell cultures were prepared as described for lymphocytes (6, 7); the lymphocytes were isolated from heparinized blood[4,5]. Cell suspensions of 1 × 10^6 cells/ml were prepared in Medium 199[6], supplemented with 4% *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid[7] buffer solution (1.0 *N*, pH 8.1), 20% human AB serum, penicillin G (100 units/ml), and streptomycin (100 μg/ml). The cell suspensions were distributed into screw-capped tissue culture glass tubes (15 × 150 mm), each receiving 1.0 ml. Blastogenic transformation was initiated with 0.02 ml of phytohemagglutinin-M[8]. To test the antimitogenic effect of the palladium compound, separate solutions containing 25–500 μg drug/1 ml tissue culture medium were prepared and filtered through membrane filters[9]. Next, 0.1 ml of each solution was

[1] Ernest D. Menold, Lesler, Pa.
[2] Jintan Terumo Co., Ltd., Japan.
[3] Millipore Corp., Bedford, Mass.
[4] Ficoll gradient, Pharmacia, Uppsala, Sweden.
[5] Isopaque gradient, Nyco, Oslo, Norway.
[6] Catalog No. 118EE, Gibco, Grand Island, N.Y.
[7] Calbiochem, Switzerland.
[8] Difco Labs., Detroit, Mich.
[9] Sartorius, Gottingen, West Germany.

Table II—Effect of Palladium (II) 2,6-Diaminopyridine on Tritiated Thymidine Incorporation by Phytohemagglutinin-Induced Lymphocytes

Lymphocyte Donors	Normal Cells	Cells with Phytohemagglutinin	Concentration of Drug in Culture, μg/ml				
			2.5	12.5	25.0	37.0	50.0
	Uptake of Tritiated Thymidine in Counts Per Minute (cpm)						
1	641[a]	130,463	119,163	90,321	45,127	13,659	369
2	400	135,866	120,096	63,447	45,440	14,799	640
3	325	150,678	139,285	134,053	75,766	10,479	374
4	436	105,000	95,000	82,053	30,278	2,416	121
Mean cpm	450	130,345	118,386	92,467	49,152	10,338	376
Mean percent suppression			9.2[b]	29.2	62.3	92.1	99.7

[a] Mean of duplicate values. [b] $100 - \frac{100 \times \text{cpm of cells containing phytohemagglutinin and drug}}{\text{cpm of control cells containing only phytohemagglutinin}}$.

added to each of the duplicate culture tubes subsequent to the addition of phytohemagglutinin and incubated at 37°. At 56 hr after the addition of phytohemagglutinin and the drug, tritiated thymidine, 2 μCi (specific activity[10] 2.0 Ci/mmole) was added to each culture tube and further incubated. Eighteen hours later the cells were washed with cold 0.9% NaCl solution and the nucleic acids were precipitated with cold 5% trichloroacetic acid. The precipitate was then washed with cold absolute methanol. The acid-insoluble fraction was then processed for liquid scintillation counting.

Antiviral Activity—The antiviral activity of the drug was measured against vaccinia virus in Hela and chicken embryo fibroblast monolayer cultures. The Hela cells have been maintained in this laboratory for the last 12 years[11]. The chicken embryo fibroblast monolayers were prepared as described in Ref. 8. The medium used for the growth of both cell cultures was the same as used for lymphocytes except that 10% fetal calf serum[12] was used instead of human serum. Plastic tissue culture flasks[13] of 25 cm^2 were used for preparation of the cell monolayers. Vaccinia virus[14] was subcultured in each cell monolayer until 50–100 plaque-forming units/flask were produced with 10^{-4} viral dilution. The plaque assays were prepared as described by Garabedian and Scott (9).

To measure the antiviral activity, 0.3-ml aliquots of the drug (230 μg/ml in medium) were mixed separately with 0.3 ml of 2×10^{-3} and 2×10^{-4} dilutions of the virus and mixed thoroughly. Immediately, 0.3 ml of each mixture was then inoculated into each of duplicate flasks of Hela and chicken embryo fibroblast cultures and incubated for adsorption at 37°. Two hours later the inocules were decanted and the monolayers were covered with 2.0 ml of chilled methylcellulose medium overlays and reincubated at 37°. Seventy-two hours later the overlays were washed out with a chilled solution of 0.9% NaCl and the monolayers were stained with 0.5% gentian violet solution.

RESULTS AND DISCUSSION

A plot for the amount of complex in solution per milliliter *versus* time was drawn. The plateau region was considered the equilibrium state and was attained after 4 hr in all cases. The asymptote to the plateau intercepting the concentration axis was considered to be the average intrinsic solubility of the metal complex in the respective solvent (Table I).

A pure sample of the ligand (2,6-diaminopyridine) when dissolved in water showed a UV spectrum with λ_{max} 212, 233 (sh), and 330 nm, which is identical to the spectrum of the complex when dissolved in 0.1 *M* HCl. This suggests that the complex decomposes at pH 1. The solubility of the complex over a 2–11 pH range is almost the same. This is because the pH of all of these solutions in the presence of the metal complex ranges between 2 and 5 (Table I).

Almost complete suppression (99.7%) of uptake of tritiated thymidine by the phytohemagglutinin-induced transforming lymphocytes was produced with 50 μg of the drug. Furthermore, there was a good dose–response relationship between the amount of the drug used and its ability to suppress DNA synthesis (Table II). Finally, no lymphocytotoxic effect was produced with 50 μg of the drug after 1 or 72 hr of incubation at 37°. The trypan blue exclusion test for viability was used for measuring the toxic effect (10).

Table III—Effect of Palladium (II) 2,6-Diaminopyridine on Vaccinia Virus Plaque Assay in Hela and Chicken Embryo Fibroblast Monolayers

Cell Culture	Plaque-Forming Units					
	Drug Control	Cell Control	Virus Control		Virus plus Drug[a]	
			1×10^{-3}[b]	1×10^{-4}	1×10^{-3}	1×10^{-4}
Hela (3)[c]	0	0	392[d]	39	0	0
Chicken embryo fibroblasts (3)	0	0	470	80	0	0

[a] See text for concentration. [b] The 10^{-3} and 10^{-4} indicate the virus dilutions used. [c] Figure in parentheses is the number of experiments. [d] Represents the average values of three experiments.

Complete inhibition of plaque-forming units was produced in both cell cultures (Table III). In the control flasks, the compound did not produce any visible harmful effect on fully grown or newly prepared cultures.

The $PdCl_2$(2,6-diaminopyridine)·H_2O is a novel palladium complex which has shown antimitogenic and antiviral activities. However, it is very poorly soluble in water and aqueous acidic or basic media, which makes it difficult to formulate into a dosage form. Derivatives of this complex with high water solubilities are currently being synthesized.

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Detection of Thioamides: Determination of Ethionamide with 2,3-Dichloro-1,4-naphthoquinone

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Abstract □ 2,3-Dichloro-1,4-naphthoquinone is reacted with thioamides in microquantities in the presence of ammonia in an alcoholic medium to give colored products showing absorbance maxima between 530 and 540 nm. The reaction is specific for thioamides and forms the basis of its spot test. The reaction is also used for the quantitative determination of ethionamide in tablets. The results compare favorably with those obtained by the official BP method. The method is simple, accurate, and precise.

Keyphrases □ Ethionamide—determination using 2,3-dichloro-1,4-naphthoquinone □ Thioamides—determination of ethionamide using 2,3-dichloro-1,4-naphthoquinone □ 2,3-Dichloro-1,4-naphthoquinone—colorimetric determination of ethionamide □ Colorimetry—determination of ethionamide using 2,3-dichloro-1,4-naphthoquinone

Thioamides are used widely as antitubercular agents (1). They are usually detected by fluorescein-1,3,6,8-tetramercuritetraacetate (2), Dragendorff reagent (3), sodium nitroferricyanide (4), sodium pentacyanoamine ferroate (5), and ammoniacal copper sulfate solution (6). Hydrogen sulfide, evolved on reacting thioamides with hydrazine hydrate, is detected with lead acetate paper (7).

Recently, 2,3-dichloro-1,4-naphthoquinone has been used in the detection and determination of sulfur-containing compounds such as thiosemicarbazones, thiosemicarbazides, and thioureas (8, 9). Therefore, it was of interest to use this reagent in the detection of thioamides and the determination of drugs containing the thioamide moiety. In the present work, reaction conditions were sought for a spot test for thioamides with 2,3-dichloro-1,4-naphthoquinone. The absorption maxima of colored products, obtained on reacting various thioamides with 2,3-dichloro-1,4-naphthoquinone, were determined.

Several methods for the estimation of ethionamide include iodometric (10, 11), acidimetric–alkalimetric (12–14), gravimetric (15), complexometric (16, 17), polarographic (18, 19), and spectrophotometric (20–29) procedures. However, most of them are not specific for the thioamide moiety of the molecule. In view of the specificity and sensitivity of this color reaction, 2,3-dichloro-1,4-naphthoquinone reagent is used for the quantitative estimation of ethionamide BP, a well-known antitubercular drug. Optimum conditions for the reaction have been studied.

EXPERIMENTAL

Apparatus—All spectral measurements were made with a spectrophotometer[1] having four matched 10-ml cells of 1-cm light path.

Reagents and Materials—Ethionamide BP, thioacetamide[2], thionicotinamide[3], and absolute alcohol (Ind.P.) were used. Thiobenzamide (30), thioisonicotinamide (31), and 2,3-dichloro-1,4-naphthoquinone (32) were synthesized by known methods. All other reagents were of analytical grade. Various brands of ethionamide tablets were obtained from the market.

Preparation of 2.5% Ethanolic Ammonia—Dry ammonia was passed into absolute alcohol at −5° until the weight had increased by about 20%. The solution was diluted with absolute alcohol to obtain 2.5% (w/v) of ammonia and was stored in a refrigerator.

2,3-Dichloro-1,4-naphthoquinone Reagent Solution—The concentration used was 0.026% (w/v) in absolute alcohol.

Standard Ethionamide Solution—A 0.03% (w/v) solution in absolute alcohol was prepared.

Detection of Thioamides—*Plate Method*—About 3 drops of ethanolic solution of thioamide (0.1 mg/ml) were spotted on a

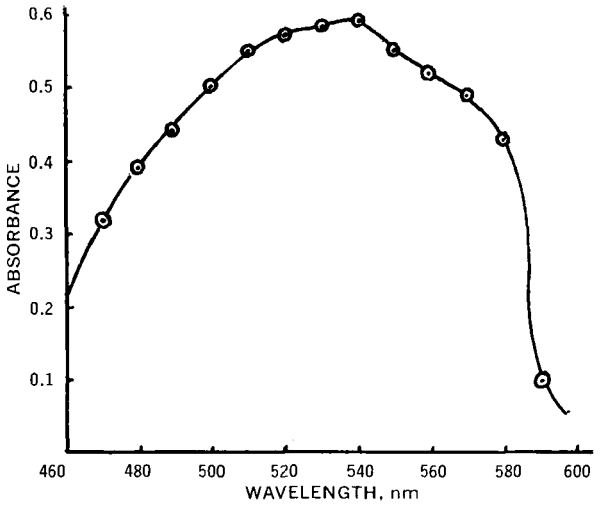

Figure 1—*Visible spectrum of the colored product obtained on reacting ethionamide with 2,3-dichloro-1,4-naphthoquinone reagent.*

[1] Spectronic 20, Bausch & Lomb.
[2] British Drug Houses.
[3] Aldrich.