Synthesis and Antitumor Activity of a Series of 1,2-Diaminocyclohexanepalladium(II) Dicarboxylate Complexes

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Abstract

A series of complexes of the type [Pd(O⌒O)(DACH)] (O⌒O = dicarboxylate ligand, DACH = 1,2-diaminocyclohexane) has been prepared. These complexes have been characterized by elemental analysis and infrared spectroscopy. The complexes have been screened *in vitro* for antitumor activity against the L1210 leukemia cell line. These palladium complexes lack antitumor activity, which may be due to (1) lack of solubility and/or (2) lack of stability of the complexes in solution.

Introduction

In recent years a great deal of effort has been devoted to developing transition metal antitumor agents which have better therapeutic properties than the prototype drug *cis*-$[PtCl_2(NH_3)_2]$ (CDDP) [1]. The bulk of the work to date has involved investigations of platinum complexes as potential antitumor agents, however some investigations involving palladium have been done [2]. For the most part, palladium complexes have shown little or no antitumor activity. This has been attributed to the higher lability of palladium(II) complexes compared to platinum(II) complexes. Thus, whereas platinum compounds such as CDDP maintain their structural integrity *in vivo* long enough to reach their cellular target(s), analogous palladium compounds undergo hydrolysis and/or various substitution reactions too quickly to be effective as antitumor agents. Therefore, if an antitumor palladium drug is to be developed, it must somehow be stabilized so that it can reach the cancerous cells intact.

In an effort to solve this problem we have adopted the approach proposed by Gill [2e]. This approach involves the use of chelating ligands to stabilize the palladium complex. Because platinum antitumor agents containing the bidentate ligand 1,2-diaminocyclohexane (DACH) tend to have very high therapeutic indexes, we have chosen this ligand as a starting point for our palladium(II) complexes. We report here the synthesis of a series of 1,2-diaminocyclohexanepalladium(II) complexes containing bidentate dicarboxylate ligands§. These complexes have been tested *in vitro* against the L1210 murine leukemia cell line. These tests have shown the complexes to be inactive against this particular cell line. These results are presented here.

Experimental

Citraconic acid, 1,1-cyclobutanedicarboxylic acid, 3,4-furandicarboxylic acid, disodium epoxysuccinate, disodium maleate, DL-malic acid, disodium malonate, methylmalonic acid, phenylmalonic acid, tartronic acid, *cis*/*trans*-1,2-diaminocyclohexane, and (±)-*trans*-1,2-diaminocyclohexane were purchased from Aldrich Chemical Company (Milwaukee, Wis.) and were used as received. K_2PdCl_4 was obtained from Johnson Matthey (Seabrook, N.H.) The dicarboxylic acids were converted to their disodium salts by treatment with sodium hydroxide, or to their barium salts by treatment with barium hydroxide. Elemental analyses were performed by Robertson Laboratories (Madison, N.J.). Infrared spectra were recorded for KBr pellets on a Beckman Microlab 250 MX infrared spectrometer. Conductivity measurements were made using a YSI Model 32 conductance meter. The cell used was a Fisher-Brand dip-type glass conductivity cell, which utilizes platinum electrodes.

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§The following abbreviations are used throughout the text: CDDP = *cis*-$[PtCl_2(NH_3)_2]$; DACH = 1,2-diaminocyclohexane (a mixture of *cis* and *trans* isomers); *trans*-DACH = (±)-*trans*-1,2-diaminocyclohexane; citracon = citraconate, CBDCA = 1,1-cyclobutanedicarboxylate; FDC = 3,4-furandicarboxylate, ESC = epoxysuccinate; mal = malonate; Me-mal = methylmalonate; Ph-mal = phenylmalonate; tar = tartronate; O⌒O = general representation of a dicarboxylate ligand.

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Preparation of [Pd(Me-mal)(DACH)]

Silver sulfate (0.139 g, 0.45 mmol) was dissolved in 50 ml of water. The flask containing this solution was wrapped with foil, then [$PdCl_2$(DACH)] (0.131 g, 0.45 mmol) was added. After stirring overnight the mixture was filtered giving a yellow solution of [$Pd(SO_4)$(DACH)], which was subsequently concentrated to 10 ml on a rotary evaporator at 40 °C. A solution of disodium methylmalonate (0.067 g, 0.41 mmol) in 20 ml of water was added dropwise with stirring. After stirring for 2 h, a yellow precipitate had formed. The solution volume was reduced to 5 ml to maximize precipitation, then the product was filtered, washed with water, and dried *in vacuo* to give [Pd(Me-mal)(DACH)] in 58% yield.

[Pd(FDC)(DACH)] was prepared in similar manner.

Preparation of [Pd(Ph-mal)(DACH)]

[$Pd(OH_2)_2$(DACH)]$(NO_3)_2$ was prepared by reacting [$PdCl_2$(DACH)] with two mole equivalents of silver nitrate in water. Filtration of AgCl gave a yellow solution which could be evaporated to give [$Pd(OH_2)_2$(DACH)]$(NO_3)_2$. This compound was invariably contaminated with palladium metal which formed due to decomposition during the evaporation process. The palladium metal could be removed by treatment with activated charcoal (*vide infra*).

[$Pd(OH_2)_2$(DACH)]$(NO_3)_2$ (0.52 g, 1.37 mmol) was dissolved in 75 ml of water and was treated with activated charcoal. A golden yellow solution was obtained upon filtration. To this solution was added a solution of disodium phenylmalonate (0.34 g, 1.51 mmol) in 20 ml of water. After stirring for 1 h, the mixture was concentrated to 3 ml and was then stored overnight at 4 °C. The crude product was filtered, washed with water, then acetone, and was dried *in vacuo*. In this way [Pd(Ph-mal)(DACH)] was obtained as a yellow solid in 32% yield. This compound was purified by recrystallization from 1-propanol.

Preparation of [$Pd(SO_4)$(DACH)]

[$PdCl_2$(DACH)] (0.951 g, 3.26 mmol) was suspended in 20 ml of methanol. To this suspension was added an aqueous solution of silver sulfate (1.016 g, 3.26 mmol). The flask was wrapped with foil and the mixture was stirred overnight. After this time, the mixture was filtered and the solvent evaporated under reduced pressure to give [$Pd(SO_4)$(DACH)] in 87% yield. *Anal.* Found: C, 22.85; H, 5.11; N, 8.88. Calc. for $C_6H_{14}N_2O_4PdS$: C, 22.78; H, 5.10; N, 8.84%.

Preparation of [Pd(tar)(DACH)]

Tartronic acid (0.121 g, 1.01 mmol) was dissolved in 30 ml of water and this solution was neutralized with 1 N NaOH (as indicated by phenolphthalein). The resulting solution of disodium tartronate was added to a solution of [$Pd(SO_4)$(DACH)] (0.319 g, 1.01 mmol) in 75 ml of water. After stirring for 2 h, the yellow precipitate was filtered and washed successively with water, acetone, and ether. Drying *in vacuo* gave [Pd(tar)(DACH)] in 50% yield.

Preparation of [Pd(maleato)(DACH)]·H_2O

Disodium maleate monohydrate (0.130 g, 0.73 mmol) was dissolved in 75 ml of water. To this solution was added [$Pd(SO_4)$(DACH)] (0.231 g, 0.73 mmol). After stirring for 2 h the solvent was evaporated under reduced pressure. The pale yellow solid was washed with water, then acetone, and was dried *in vacuo*. [Pd(maleato)(DACH)]·H_2O was obtained as a pale yellow solid in 33% yield.

The complexes [Pt(mal)(*trans*-DACH)] and [Pd(CBDCA)(*trans*-DACH)]·H_2O were prepared in similar manner.

Preparation of [Pd(ESC)(DACH)]

Disodium epoxysuccinate (0.175 g, 0.99 mmol) was dissolved in 40 ml of water. This solution was stirred over REXYN 101 (H-form) for 40 min to convert the disodium salt to the acid. After filtration, the solution was treated with $Ba(OH)_2 \cdot 8H_2O$ (0.314 g, 0.99 mmol). This solution of barium epoxysuccinate was added to a solution of [$Pd(SO_4)$(DACH)] (0.310 g, 0.98 mmol) in 50 ml of water. After stirring for 3 h, the reaction mixture was filtered. Evaporation of the solvent gave [Pd(ESC)(DACH)] in 52% yield.

Preparation of [Pd(citracon)(DACH)]

Citraconic acid (0.066 g, 0.50 mmol) was dissolved in 50 ml of water and $Ba(OH)_2 \cdot 8H_2O$ (0.159 g, 0.50 mmol) was added. This solution was added to a solution of [$Pd(SO_4)$(DACH)] (0.145 g, 0.49 mmol) in 20 ml of water. After stirring for 2 h the reaction mixture was filtered. Evaporation of the solvent gave [Pd(citracon)(DACH)] in 77% yield.

[Pd(DL-malato)(DACH)]·H_2O was prepared in similar manner.

Cytotoxicity Studies

L1210 murine leukemia cells are routinely cultured in McCoy's SA medium supplemented with glutamine, penicillin, streptomycin, and 10% horse serum. When grown at 37 °C in a humidified atmosphere consisting of 90% air:10% CO_2, these cells have a doubling time of approximately 15 h. To determine the cytotoxicity of the palladium complexes, 4 ml of the cell suspension (10^5 cells/ml) were added to culture tubes in triplicate, and the test complex was added at final concentrations of either 0.01, 0.1, 1.0, or 10 μg/ml. After 72 h of incubation, the cell concentration of non-treated control cultures and drug-treated cultures were determined using a Coulter counter (Coulter Electronics, Hialeah, Fla.).

The ID_{50} value (defined as that concentration of drug required to inhibit cell growth by 50%) was calculated by extrapolation of the line defined by drug concentration *versus* percent inhibition of growth.

Results and Discussion

A series of 1,2-diaminocyclohexanepalladium(II) dicarboxylate complexes has been prepared. Figure 1 illustrates the ten dicarboxylate ligands used in this study. Three general synthetic methods have been utilized to prepare these complexes (eqns. 1–3), depending on the solubility characteristics of the products. For example, because [Pd(mal)(DACH)] is sparingly soluble in water, the route illustrated in eqn. (1) proves most useful in this case. The sodium sulfate can be easily separated from the [Pd(mal)-(DACH)] by washing with water. On the other hand, the complex [Pd(ESC)(DACH)] is water soluble. Thus the route shown in eqn. (2) is more convenient in this instance. Reaction of $[Pd(SO_4)(DACH)]$ with Ba(ESC) in water yields an aqueous solution of [Pd(ESC)(DACH)] and a white precipitate of $BaSO_4$. The barium sulfate can be separated by filtration. The route shown in eqn. (3) has been used only in the preparation of [Pd(Ph-mal)(DACH)]. Again this complex is sparingly soluble in water, so the sodium nitrate can be separated by washing with water. It should be noted that the route depicted in eqn. (1) is probably equally as useful in the preparation of [Pd(Ph-mal)(DACH)].

$$[Pd(SO_4)(DACH)] + Na_2(O\frown O) \longrightarrow [Pd(O\frown O)(DACH)] + Na_2SO_4 \quad (1)$$

$$[Pd(SO_4)(DACH)] + Ba(O\frown O) \longrightarrow [Pd(O\frown O)(DACH)] + BaSO_4 \quad (2)$$

$$[Pd(OH_2)_2(DACH)](NO_3)_2 + Na_2(O\frown O) \longrightarrow [Pd(O\frown O)(DACH)] + 2NaNO_3 + 2H_2O \quad (3)$$

The (DACH)palladium(II) dicarboxylate complexes have been characterized by elemental analysis and infrared spectroscopy. The elemental analysis data (Table I) confirms the stoichiometry of one dicarboxylate ligand per palladium atom. In all cases the infrared spectra (Table II) display patterns which are typical of dicarboxylate ligands which are bound in a bidentate fashion to a metal center. The carbonyl region displays either one or two bands depending upon the degeneracy of the $\nu_a(COO)$ band. All the dicarboxylatopalladium(II) complexes display one $\nu_s(COO)$ band in the region 1350–1410 cm^{-1}. The exception to this is [Pd(mal)(*trans*-DACH)] which displays two bands in this region. The reason for this is not clear.

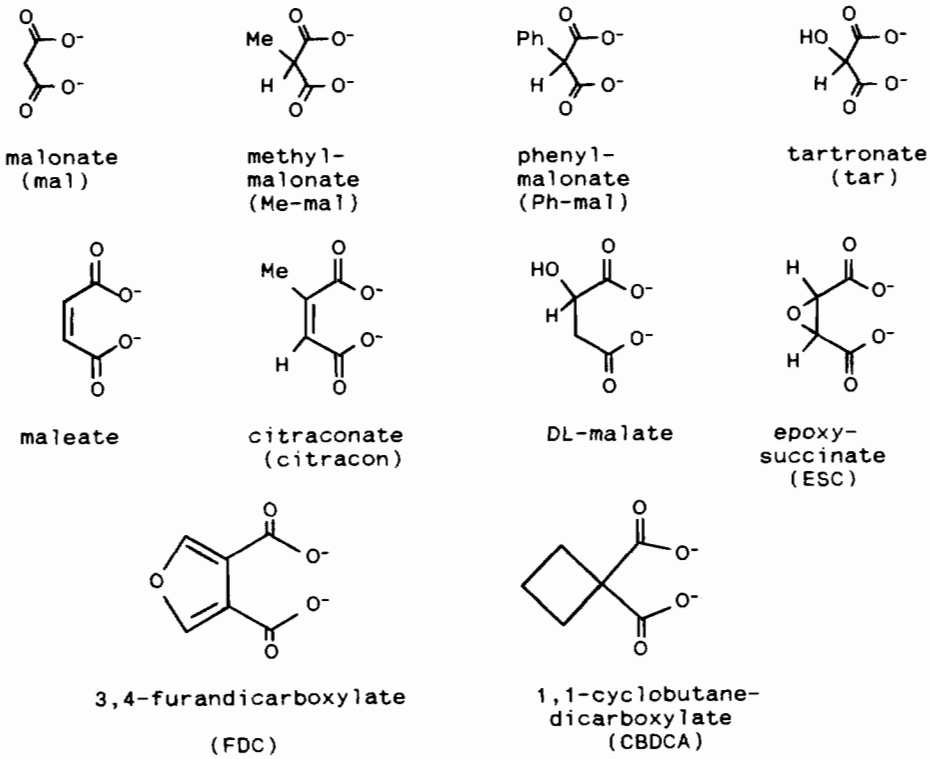

Fig. 1. Structures of the dicarboxylate ligands.

TABLE I. Elemental Analyses of 1,2-Diaminocyclohexanepalladium Dicarboxylate Complexes

	Experimental (%)			Calculated (%)		
	C	N	H	C	N	H
[Pd(Me-mal)(DACH)]	35.54	5.24	8.14	35.67	5.40	8.32
[Pd(Ph-mal)(DACH)]	44.96	5.32	6.89	45.18	5.07	7.02
[Pd(FDC)(DACH)]	38.28	4.23	7.28	38.46	4.31	7.47
[Pd(ESC)(DACH)]	34.19	4.82	8.05	34.25	4.61	7.98
[Pd(tar)(DACH)]	32.05	4.81	8.53	31.92	4.77	8.27
[Pd(citracon)(DACH)]	38.04	5.50	8.32	37.89	5.21	8.03
[Pd(DL-malato)(DACH)]·H_2O	33.02	5.99	8.40	32.40	5.45	7.55
[Pd(mal)(DACH)]	33.08	4.67	7.32	33.5	5.01	8.68
[Pd(CBDCA)(*trans*-DACH)]·H_2O	38.27	5.62	7.32	37.85	5.84	7.35
[Pd(maleato)(DACH)]	34.71	4.57	7.95	34.05	5.15	7.94

TABLE II. Infrared Data[a] for 1,2-Diaminocyclohexanepalladium(II) Dicarboxylate Complexes

	ν_a(COO)	ν_s(COO)
[Pd(Me-mal)(DACH)]	1647	1409
	1614	
[Pd(Ph-mal)(DACH)]	1630	1380
[Pd(FDC)(DACH)]	1595	1389
[Pd(ESC)(DACH)]	1625	1357
[Pd(tar)(DACH)]	1675	1362
	1630	
[Pd(citracon)(DACH)]	1651(sh)[b]	1381
	1611(sh)	
	1580	
[Pd(DL-malato)(DACH)]	1600	1390
[Pd(mal)(*trans*-DACH)]	1654	1404
	1619	1389
[Pd(CBDCA)(*trans*-DACH)]	1619(sh)	1362
	1609	
[Pd(maleato)(DACH)]	1659[b]	1398
	1595	
	1577	

[a]All spectra were recorded for KBr pellets. Band positions are given in cm^{-1}. [b]One of these bands is due to the carbon–carbon double bond of the dicarboxylate ligand, however this band cannot be specifically assigned.

TABLE III. Molar Conductivities of 1,2-Diaminocyclohexanepalladium(II) Dicarboxylate Complexes

	Λ_M (T = 0 h)	Λ_M (T = 24 h)
[Pd(ESC)(DACH)]	97.5	98.2
[Pd(citracon)(DACH)]	53.9	49.8
[Pd(DL-malato)(DACH)]	99.9	88.1
[Pd(CBDCA)(*trans*-DACH)]	4.9	5.3

The *in vitro* cytotoxicity of the (DACH)palladium(II) dicarboxylate complexes against the L1210 leukemia cell line has been investigated. A compound with an ID_{50} value of 10 μg/ml or less is generally considered to have significant antitumor activity [3]. For all the compounds investigated here the ID_{50} values are greater than 20 μg/ml. Thus none of these complexes display antitumor activity. The lack of antitumor activity for the (DACH)palladium(II) dicarboxylate complexes may be due to two factors: (1) Lack of solubility and/or (2) lack of stability of the complexes.

Many of the (DACH)palladium(II) dicarboxylate complexes investigated in this study are not appreciably soluble in water; a property which may inhibit their antitumor activity from the start. Furthermore, most of the complexes which are water soluble display rather high molar conductivities (Table III), the exception being [Pd(CBDCA)(*trans*-DACH)]. Although the molar conductivities are not so high as to consider the compounds to be 1:1 electrolytes [4], they are high enough to suggest the existence of some hydrolysis products which are charged. In retrospect this result is not surprising. It is well established that the most stable chelate rings are those which are five-membered, with the stability of the chelate ring decreasing with increasing ring size [5]. Thus the complex [Pd(CBDCA)(*trans*-DACH)] with its six-membered chelate ring should be more stable than [Pd(ESC)(DACH)], [Pd(citracon)(DACH)], and [Pd(DL-malato)(DACH)] which all contain seven-membered chelate rings. This is consistent with our results in which [Pd(CBDCA)(*trans*-DACH)] displays the lowest molar conductivity. However, even [Pd(CBDCA)(*trans*-DACH)] does not display antitumor activity. Thus the lack of antitumor activity does not necessarily correlate with the lack of complex stability.

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