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Heteropolymetallic Complexes of 1,1′-Bis(diphenylphosphino)ferrocene (dppf). IV. Solvolytic Behavior and Cytostatic Properties Towards the KB Cell-line of dppf and 1,2-Bis(diphenylphosphino)ethane *cis*-Complexes of Pt(II) and Pd(II)

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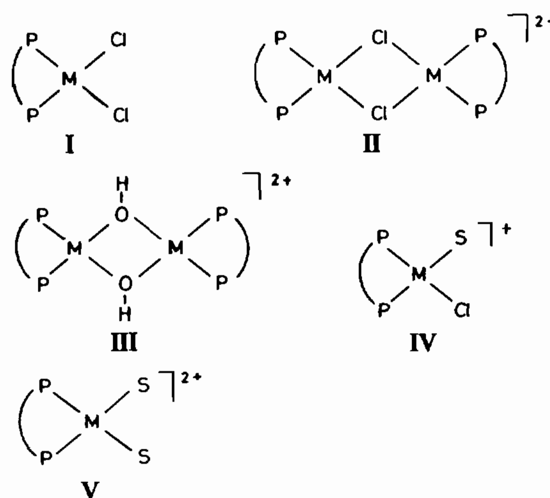
Abstract

The novel *cis*-platinum(II) complexes [(dppe)-Pt(μ-OH)]$_2$(BF$_4$)$_2$ and [(dppe)Pt(DMF)$_2$](BF$_4$)$_2$ have been prepared and characterized by ^{31}P NMR, together with *cis*-[(dppe)Pt(μ-Cl)]$_2$(BF$_4$)$_2$, both in poorly and strongly coordinating solvents (dppe = 1,2-bis(diphenylphosphino)ethane). All these complexes and their dppf analogs (dppf = 1,1′-bis(diphenylphosphino)ferrocene) as well as (dppf)PtCl$_2$, (dppe)PtCl$_2$, (dppf)PdCl$_2$, [(dppf)Pd(μ-Cl)]$_2$(BF$_4$)$_2$ and [(dppf)Pd(μ-OH)]$_2$(BF$_4$)$_2$ have been tested as antiproliferating agents towards Eagle's KB cell-line. Their activity is compared with that of free diphosphine ligands. For Pt(II) complexes, the ID_{50} figures are found to be higher than those observed for free dppf and dppe. On the contrary, the activity of the palladium dppf complexes is substantially identical to that of free diphosphine.

Introduction

Platinum(II) complexes of the type *cis*-[(dppf)-Pt(X)(Y)] (X = or ≠ Y; dppf = 1,1′-bis(diphenylphosphino)ferrocene) may be expected to exhibit antitumoral activity as a consequence of three features: (i) they contain a stable *cis*-Pt(II)P$_2$ moiety, which possesses very high reactivity towards nucleosides [1–3]; (ii) they embody a ferrocenyl moiety which is a powerful antitumor agent in its oxidated form [4]; (iii) they contain a diphosphine ligand which can be related to 1,2-bis(diphenylphosphino)-ethane (dppe), which is known to possess remarkable antitumor activity both *in vitro* and *in vivo* [5].

We have been investigating in recent years the solution chemistry of complexes of type I–V as well as their reactivity with DNA-relevant biomolecules [1–3]:

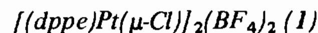


We report here on the synthesis of some *cis*-halido dppe platinum(II) complexes of type II–V. Moreover, we report also on the cytostatic activity on Eagle's KB cell-line of species I–V (in which the diphosphine is dppf or dppe and M is Pt or Pd).

Results and Discussion

Synthesis of Platinum(II) Complexes with dppe

These were prepared by procedures described for the synthesis of the related complexes with dppf [6].



[(dppe)Pt(μ-Cl)]$_2$(BF$_4$)$_2$ (1)

This complex was prepared according to ref. 7 from *cis*-(dppe)PtCl$_2$ by reaction with 1 mole of AgBF$_4$ in anhydrous acetone. It is soluble and stable in non-coordinating solvents, while undergoing in-

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stantaneous and quantitative solvolysis in acetonitrile, dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) to give the corresponding monosolvato species of type **IV** (**4**). In fact, the ^{31}P NMR spectra (Table I) of **1** turn out to be quite useful for monitoring this behavior, which closely resembles that observed for the related dppf complex [1–3, 6].

TABLE I. ^{31}P NMR Data for Complexes 1–4 in Various Solvents

Species	δ^a	J^b	Solvent
1	47.3(s)	3784	$CDCl_3/CH_2Cl_2$ (1:1 *v/v*)
2	34.0(s)	3558	DMSO-d_6
2	34.5(s)	3558	DMF-d_7
3	36.4(s)	3901	DMF
3	36.8(s)	3902	DMSO-d_6
4	45.3(d)	3620	
	34.6(d)	3909	DMF

[a]Chemical shifts are in ppm from external H_3PO_4 (85% *w/w*). [b]Coupling constants (Hz) of $^{195}Pt-^{31}P$.

[(dppe)Pt(μ-OH)]$_2$(BF$_4$)$_2$ (2)

Addition of 2 moles of $AgBF_4$ in methanol to (dppe)$PtCl_2$ affords complex **2** (in good yield), which is characterized by ^{31}P and ^{1}H NMR spectra (Table I). In DMF-d_7, the hydrogen atom of the hydroxo ligand is clearly detected as a singlet (δ = 6.37) flanked by the ^{195}Pt satellites (J = 17 Hz), while this resonance appears as a singlet at δ = 5.53 in DMSO. In fact, the signal appears slightly broadened, the two satellite peaks being therefore undetectable. The dimeric nature of **2** is preserved both in coordinating and non-coordinating solvents, as inferred by its ^{31}P NMR spectrum (Table I).

[(dppe)Pt(DMF)$_2$](BF$_4$)$_2$ (3)

By reaction of (dppe)$PtCl_2$ with 2 moles of $AgBF_4$ in anhydrous DMF, complex **3** can be obtained in good yield. The ^{31}P NMR spectrum in DMF (Table I) displays a simple resonance at 36.4 ppm, flanked by the expected satellite peaks with J = 3901 Hz.

The whole of the data show that isostructural dppf and dppe *cis*-platinum(II) complexes possess quite similar spectral properties and solvolytic behavior, thus making the comparison of their *in vitro* activity towards the KB cell-line reliable [8].

Cytostatic Activity of Complexes 1–4 and of their dppf Pd and Pt Analogs

The complexes tested *in vitro* are listed in Table II together with the relevant biological activity parameters (ID_{50}, *i.e.* the compound concentration at which the cells showed 50% growth inhibition in relation to the control values). All compounds were added to cell cultures as DMSO solutions so that the final culture medium contained 0.5% (*v/v*) of the organic solvent. It appears that all complexes and ligands are active as antiproliferating agents, but only free dppe exhibits activity which is comparable to that of *cis*-$(NH_3)_2PtCl_2$ (cisplatin). Moreover, both free diphosphines are significantly more active than their platinum complexes. On the other hand, it is also found that dimeric palladium complexes are about twice as active as free dppf.

TABLE II. Cytostatic Activity of dppf, dppe and of some Complexes of type I–V as Tetrafluoroborate Salts

Entry species	ID_{50} (μg/ml MEM)	ID_{50}[a] (μM)[c]
dppf	9.4	17.0
(dppf)$PtCl_2$	50.6	61.7
$[(dppf)Pt(\mu\text{-}Cl)]_2^{2+}$[b]	296.7	170.0
$[(dppf)Pt(\mu\text{-}OH)]_2^{2+}$	23.4	13.7
$[(dppf)Pt(DMF)_2]^{2+}$	183.0	171.2
dppe	0.35	0.9
(dppe)$PtCl_2$	25.0	37.6
$[(dppe)Pt(\mu\text{-}Cl)]_2^{2+}$[b]	68.9	48.1
$[(dppe)Pt(\mu\text{-}OH)]_2^{2+}$	20.4	14.6
$[(dppe)Pt(DMF)_2]^{2+}$	20.8	22.8
(dppf)$PdCl_2$	11.9	16.3
$[(dppf)Pd(\mu\text{-}Cl)]_2^{2+}$[b]	13.9	8.9
$[(dppf)Pd(\mu\text{-}OH)]_2^{2+}$	9.2	6.0
Cisplatin	0.11	0.37

[a]Control experiments carried out with ferrocene and $NaBF_4$ (10^{-2} M) show that these species are practically inactive. [b]These complexes dissociate quantitatively in DMSO to give *cis*-$[(dppf)MCl(DMSO)]^+$. [c]Analytical molar concentration.

The observation of the strong cytostatic ability of dppe and dppf is not unexpected in that dppe was already found to be active towards Eagle's KB (0.25 μM) [5] and towards a wide range of tumors at much lower doses *in vivo* [9].

The cytoxic potency of the free ligand could be invoked for interpreting the ID_{50} figures observed for the palladium complexes. In fact, in view of the known higher lability of palladium(II) complexes with respect to those of platinum(II), our results could be just the consequence of the different ability of the administered metal complexes to release the active free dppf ligand, inside the cytoplasm. Thus, the ID_{50} values observed for $[(dppf)Pd(\mu\text{-}Cl)]_2^{2+}$ and $[(dppf)Pd(\mu\text{-}OH)]_2^{2+}$ (8.9 and 6.0, respectively) are practically identical to those observed for dppf (17.0) and for (dppf)$PdCl_2$ (16.3), after making allowance for the dimeric nature of the previous species.

In conclusion, the results herein described confirm previous observations on gold diphosphine complexes [5]; *i.e.*, although the metal center may play

a specific biological role, it is the diphosphine which is the major determinant of activity.

Experimental

All chemicals used were reagent grade. The solvents were dried over molecular sieves. Literature methods were used for the preparation of complexes (dppf)$PtCl_2$ [10], (dppe)$PtCl_2$ [11], [(dppf)-$Pt(\mu$-$X)]_2(BF_4)_2$ ($X = Cl^-$, OH^-) and [(dppf)Pt-$(DMF)_2](BF_4)_2$ [6]. Infrared spectra were recorded on a Perkin-Elmer 599B spectrometer. NMR spectra were obtained with a Jeol FX 90Q spectrometer at 27 °C with the residual solvent peak as an internal reference for the proton spectra. The $^{31}P\{^1H\}$ NMR spectra in DMF were obtained by using a coaxial capillary containing D_2O for deuterium lock. All chemical shifts are reported positive to lower shielding.

Synthesis of [(dppe)Pt(μ-Cl)]$_2$(BF$_4$)$_2$ (1)

To a suspension of (dppe)$PtCl_2$ (0.500 g, 0.752 mmol) in anhydrous acetone (30 ml), a solution of $AgBF_4$ (0.149 g, 0.752 mmol) in anhydrous acetone was added dropwise. The reaction mixture was stirred for 15 min and filtered. Addition of Et_2O to the filtrate gave a white microcrystalline precipitate which was recovered by filtration. The yield of pure product was 0.37 g (71%). *Anal.* Calc. for $C_{26}H_{24}BClF_4P_2Pt$: C, 43.63; H, 3.38. Found: C, 42.71; H, 3.46%. IR (Nujol): ν_{PtCl} at 280 and 290 cm^{-1}. ^{31}P NMR in CD_3Cl/CH_2Cl_2, 1:1 mixture: δ 47.3, singlet flanked by ^{195}Pt satellites with $J_{PtP} =$ 3784 Hz.

Synthesis of [(dppe)Pt(μ-OH)]$_2$(BF$_4$)$_2$ (2)

Addition of (dppe)$PtCl_2$ (0.500 g, 0.752 mmol) in 25 ml of CH_3OH to a solution of $AgBF_4$ (0.293 g, 1.504 mmol) in CH_3OH (5 ml) led to the immediate precipitation of AgCl. The reaction mixture was stirred for 2 h and filtered. The filtrate was concentrated under vacuum to *ca.* 5 ml and, by addition of Et_2O, a white solid was formed which was purified by crystallization from CH_3OH/Et_2O. The yield was 0.367 g (70%). *Anal.* Calc. for $C_{26}H_{25}BF_4OP_2Pt$: C, 44.78; H, 3.61. Found: C, 43.59; H, 3.53%. IR (Nujol): ν_{OH} at 3560 cm^{-1} (w). ^{31}P NMR in DMSO-d_6: δ 34.0, singlet flanked by ^{195}Pt satellites ($J_{PtP} =$ 3558 Hz). ^1H NMR in DMF-d_7: δ 7.8 (complex multiplet, 20 H, C_6H_5), 6.37 (singlet flanked by ^{195}Pt satellites ($J_{PtH} = 17$ Hz), 1 H, OH), 2.5 (complex multiplet, 4 H, C_2H_4).

Synthesis of [(dppe)Pt(DMF)$_2$](BF$_4$)$_2$ (3)

A solution of $AgBF_4$ (0.179 g, 0.903 mmol) in DMF (2 ml) was added to a solution of (dppe)$PtCl_2$ (0.300 g, 0.451 mmol) in 3 ml of DMF. The reaction mixture was stirred for 1 h; the solvent was removed under vacuum and the residue was triturated with Et_2O (30 ml). A white powder was formed which was recovered by filtration. The solid was dissolved in CH_2Cl_2 (5 ml), filtered to eliminate AgCl and reprecipitated by addition of Et_2O. The white microcrystalline precipitate, collected by filtration, was washed with Et_2O and dried under vacuum. The yield was 0.371 g (90%). *Anal.* Calc. for $C_{32}H_{38}B_2F_8N_2O_2P_2Pt$: C, 42.08; H, 4.19; N, 3.07. Found: C, 42.77; H, 4.30; N, 2.97%. ^{31}P NMR in $CDCl_3$: δ 35.67, singlet with $J_{PtP} = 3928$ Hz.

In Vitro Cytostatic Activity Evaluation

An established cell-line of human oral epidermoid carcinoma (KB cells) (Flow Laboratoires Ltd.) [8] was employed for the cytostatic assay using the method of ref. 12. The cells were grown in 25-cm^2 tissue culture flasks with Eagle's Minimal Essential Medium (MEM)(Difco) supplemented with 10% newborn calf serum (Gibco), 1% non-essential amino acids (Gibco) and glutamine (2 mM) and buffered with TES (*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid) (3 mM), BES (*N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid) (3 mM), HEPES (*N*-2-hydroxyethylpiperazine *N'*-2-ethanesulfonic acid) (3 mM) and TRICINE (*N*-tris(hydroxymethyl)methylglycine) (3 mM) [13]. For the *in vitro* cytostatic assay the cells in logarithmic growth phase which were re-fed 24 h before testing were used. The cells were treated for 5 min at 37 °C with 0.05% 1:250 trypsin solution and then suspended in MEM to obtain a concentration of 10^5 cells/ml. Aliquots (1 ml) were seeded in each Leighton tube (Bellco) and incubated at 37 °C. After 24 h the viable cells were attached to the bottom of the tubes. The tubes were regrouped at random and the base line was evaluated in five of these by counting with a Bürker chamber the cells detached from the glass surface by trypsin solution [14]. The culture medium of the other Leighton tubes was changed and the cells were fed with 4 ml MEM (control tubes) and with 4 ml MEM containing the compounds to be tested (treated tubes). The compounds were dissolved immediately before use in sterile dimethyl sulfoxide. The final solvent concentration in MEM (0.5%) did not show any cytotoxic effect. At least five concentration levels were used for each compound and each concentration value was tested in triplicate. The incubation was carried out at 37 °C for 72 h, the time interval in which exponential growth occurs. As a positive control cisplatin was always included. Cell growth was estimated by counting the viable cells (Tryphan blue exclusion test), as previously described. The cytostatic activity was evaluated as percentage of growth inhibition in the treated tubes with respect to the controls on the basis of the formula: $100 - [(T - B)/(C - B) \times 100]$, where

B is the base line and T and C are the number of viable cells, respectively, in the treated and the control tubes after 72-h incubation. The significance of these results was evaluated by use of the classical Student's t-test ($p < 0.01$). The inhibition values were plotted against $\log D$, where D is the drug concentration in μg/ml of MEM. From these curves the ID_{50} values were obtained.

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