

6. Bioinorganic Therapy



PS6.1 — MO

RUTH MARGALIT

HARRY B. GRAY

Arthur Amos Noyes Laboratory
California Institute of Technology
Pasadena, California 91125
U.S.A.

MICHAEL CLARKE

Department of Chemistry
Boston College
Chestnut Hill, Massachusetts
U.S.A.

S.C. SRIVASTAVA

Department of Chemistry
Brookhaven National Laboratory
Upton, New York
U.S.A.

BIOLOGICAL TISSUE DISTRIBUTION OF $\text{Ru}(\text{NH}_3)_5\text{-BLEOMYCIN}$

The reaction of $\text{Ru}(\text{NH}_3)_5\text{H}_2\text{O}^{2+}$ with the chemotherapeutic agent bleomycin at pH 7.2 gives a Ru-modified derivative. The modified bleomycin has been purified and characterized (by ^1H NMR and differential pulse electrochemistry) as $\text{Ru}(\text{NH}_3)_5^{3+}\text{-(pyrimidine)-BLM}$.

A model compound, $\text{Ru}(\text{NH}_3)_5\text{His}^{3+}$, was found to be effective in the scission of DNA strands, *in vitro*, when combined with a reducing agent. The activity of $\text{Ru}(\text{NH}_3)_5\text{BLM}$ in DNA strand scission will be presented and discussed. The distribution of the radioactive drug $^{106}\text{Ru}(\text{NH}_3)_5\text{BLM}$ in normal and tumor-bearing mice has been studied.



PS6.2 — TU

M.G. BASALLOTE

E.J.G. CONEJERO

R. VILAPLANA

F. GONZÁLEZ-VÍLCHEZ

Departamento de Química Inorgánica
Facultad de Ciencias
Apto. 40, Puerto Real, Cádiz
Spain

PLATINUM COMPLEXES OF EDDA AND ITS ETHYL ESTER: SYNTHESES AND ANTITUMOR PROPERTIES

Structure-activity relationship for antitumor properties of platinum complexes is still not clear, although *cis*- $[\text{PtA}_2\text{X}_2]$ compounds seem to be active when A ligands are inert and X ligands can be easily replaced in biological media. The nature of A is of great importance for the antitumor activity of the complex, with little changes in its structure leading sometimes to main changes in activity. Therefore we are testing complexes of this type with new structures in order to obtain *cis*-DDP analogs with higher therapeutic indexes.

We have shown recently activity against Ehrlich ascites tumor for some platinum complexes with aminopolycarboxylic ligands [1]. However, $[\text{Pt}(\text{EDDA-H}_2)\text{Cl}_2]$ was showed to be inactive in a previous study by SPEER *et al.* [2]. One interesting problem in structure-activity relationship is that related to the effect of the charge of the complex, active compounds being normally neutral complexes. So esterification of carboxylic groups in ligands must lead to an increase in the activity of the complexes. However, the results of previous studies with similar ligands [3,4] by other authors are contradictory and new data must be obtained for solving the problem. In this sense, we report in this paper the results of a new test of this compound and of its analog with the ethyl ester of the ligand.

SYNTHESIS AND CHARACTERIZATION

[Pt(EDDA-H₂)Cl₂] was synthesized from K₂PtCl₄ by the method of LIU [5]. The compound was obtained as yellow crystals with a yield of 55%. Elemental analysis results were: C:16.18 (16.30); H:2.71 (2.73); N:6.38 (6.33); Cl:16.79 (16.04) where theoretical values are shown in parenthesis. The infrared spectrum shows bands at 3110 cm⁻¹ (N-H), 1700 cm⁻¹ (COOH) and 330 cm⁻¹ (Pt-Cl). A band is observed in the electronic spectrum of this compound in aqueous solution (262 nm), its position being similar to the one observed for platinum complexes with EDTA [6]. The value for COOH in the ¹³C-NMR spectrum is also similar (169.64 ppm) to those of EDTA complexes [1].

EDDA-Et₂·2HCl was obtained by refluxing a solution of EDDA-H₂ in ethanol, and with HCl stream. After three hours, the solution was concentrated and precipitation of a white solid occurred. The solid was washed with water and ethanol and dried at 60°C. The yields were variable, ranging from 27 to 71%. Elemental analysis results were: C:38.85 (39.35); H:7.95 (7.96); N:8.68 (9.17); Cl:22.78 (23.23). The bands due to N-H and C=O stretching appear in the IR spectrum at 3400 cm⁻¹ and 1740 cm⁻¹. In the ¹H-NMR spectrum, the ethyl groups give signals at 1.24 ppm (CH₃) and 4.17 ppm (CH₂) (J = 7 Hz). Two significant signals in the ¹³C spectrum are those of C=O (166.12 ppm) and CH₃ (13.86 ppm).

[Pt(EDDA-Et₂)Cl₂] was obtained from K₂PtCl₄ and EDDA-Et₂ in dilute HCl solution. The solution was heated and stirred for about 2 hours. Concentrated HCl was added to cause precipitation, and then a pale yellow solid was obtained. It was washed with water and ethanol and dried at 60°C (yield = 30%). The elemental analysis results were the following: C:23.43 (24.00); H:4.10 (4.04); N:5.67 (5.02); Cl:15.22 (14.23).

In the IR spectrum, the N-H band is displaced to 3110 cm⁻¹ while the C=O band appears also at 1730 cm⁻¹. The Pt-Cl band appears in this case at 325 cm⁻¹. The electronic spectrum of this compound shows bands at 282 and 306 nm. This displacement to longer wavelengths is due to the HCl used for the dissolution of the complex and has been observed for Pt-EDTA complexes [6]. The NMR spectra also show changes with coordination.

ANTITUMOR ACTIVITY

Antitumor tests were performed against Ehrlich ascites tumor in Swiss mice and the results are shown in Table I. [Pt(EDDA-H₂)Cl₂] was dissolved in saline, and [Pt(EDDA-Et₂)Cl₂] in saline-DMSO 1:1. Control and test groups were of 10 and 8 animals respectively. The compounds were injected i.p. in day 1, and the experience was finished in day 90. T/C values have been calculated from the median survival times of the test and control groups.

Table I

Compound	Dose (mg/Kg)	T/C
[Pt(EDDA-H ₂)Cl ₂]	100	85
	50	100
	25	98
[Pt(EDDA-Et ₂)Cl ₂]	100	122
	50	91
	25	109

The values of Table I show no activity for both compounds. This result is in agreement with that of SPEER [2] for the case of the EDDA complex. Moreover, for the EDDA-Et₂ complex no significant increase in activity is observed.

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PS6.3 — TH

J.P. ALBERTINI

A. GARNIER-SUILLEROT

Laboratoire de Chimie bioinorganique
U.E.R. de Médecine et Biologie Humaine
Université Paris Nord
93012 Bobigny Cedex
France

**INTERACTION OF BLEOMYCIN
WITH Pd(II) COMPLEXES
([PdCl₄]²⁻, [cis-Pd(NH₃)₂Cl₂],
[Pd(en)Cl₂]) AND WITH [cis-Pt(NH₃)₂Cl₂].
ANTITUMOR ACTIVITY OF THE BLM-
[cis-Pt(NH₃)₂Cl₂] SYSTEM**

Bleomycin (BLM) and *cis*-diamminodichloroplatinum(II) (*cis*-DDPt) are used in combination chemotherapy to treat malignant tumors [1-3]. The two drugs exhibit synergism. The biological target for both *cis*-DDPt and bleomycin is believed to be DNA.

In this communication we address the question of whether prior covalent binding of *cis*-DDPt to BLM might alter the interaction of these two drugs with DNA and their antitumor activity.

Because of the slowness of Pt(II) ligand exchange reactions, parallel studies were conducted on the corresponding Pd(II) complexes which react 10⁵ times faster.

In this communication we report experiments showing that [PdCl₄]²⁻ as well as [cis-Pd(NH₃)₂Cl₂] and [Pd(en)Cl₂] reacts with bleomycin in a three steps process forming a 1:1 BLM.Pd(II) complex. In the same way a similar complex is obtained between BLM and [cis-Pt(NH₃)₂Cl₂]; its antitumor activity has been tested.

Interaction of BLM with Pd(II) complexes

The addition of [PdCl₄]²⁻ to an aqueous BLM solution gives rise to the immediate formation of

a first complex (**I**). The most striking features of this formation are i) the release of two protons, ii) the quenching of the pyrimidine fluorescence, iii) the appearance of a CD band at 367 nm which can be assigned to d-d transition. As time elapses this complex evolves to a second one (complex **II**); this occurs without any modification of the pH value but with noticeable change in the CD spectrum. The half life time of complex **I** is about 7 minutes. The last step, giving rise to the ultimate complex (**III**), is slower. Here again this occurs without modification of the pH value and, at pH 3, about three days are necessary to reach its complete formation. However if the pH is raised to about 7 the transformation of complex **II** to **III** occurs at once (it should be noticed that complex **III** is obtained directly by addition of [PdCl₄]²⁻ to BLM in a pH 7 Hepes buffer).

When either [cis-Pd(NH₃)₂Cl₂] or [Pd(en)Cl₂] are substituted for [PdCl₄]²⁻ one still observes a three steps process (complexes **I'**, **II'** and **III'**).

On the contrary when [PdCl₄]²⁻ is added to depyruvamide bleomycin (depBLM) one still observes the release of two protons but no evolution with time: only one complex (**d**) is observed.

The striking feature is that the CD spectra of complexes **III**, **III'** and **d** are similar strongly suggesting that the same four ligands are involved in the coordination square, most probably the secondary amine nitrogen, the pyrimidine nitrogen and the two peptide nitrogens. It must be pointed out that these ligands are different from those usually found in metal bleomycin complexes (the metal being copper, iron and cobalt) [4,5].

Interaction of BLM.Pd(1:1) complex (III) with DNA

The addition of DNA to complex **III** gives rise to a quenching of the bithiazole fluorescence suggesting that it is still able to intercalate between the base pairs of DNA. Moreover an immediate modification in the 320-340 nm region (d-d transition) of the CD spectrum is observed which can be assigned to a modification of the ligand field around the Pd(II) ion. However no release of Pd(II) from the complex could be detected.