The Reactions of (–)-(R)-2-Aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II), Carboplatin and Cisplatin with Guanosine: Kinetic Comparison of the Reactivities

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The reaction of a new antitumor platinum complex, (–)-(R)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) (**1**), with guanosine (Guo) in an aqueous solution was kinetically compared with the reactions of carboplatin (**2**) and cisplatin (**3**) with Guo using high performance liquid chromatography (HPLC) analyses and proton nuclear magnetic resonance (¹H-NMR).

Reactions of **1** and **2** with Guo proceeded according to second order kinetics; the 1,1-cyclobutanedicarboxylate (CBDCA) group of **1** and **2** was directly substituted with two N-7 of Guo.

The reaction of **3** followed a two-step course through the intermediate, *cis*-Pt(NH₃)₂Cl(Guo).

The reaction rates of the three platinum complexes with Guo were related to 3 ≫ 1 > 2. This result indicated that the CBDCA ligand (chelate ligand, bidentate ligand) coordinated to platinum more strongly than the Cl ligand (monodentate ligand), and the trans effect of the 2-aminomethylpyrrolidine ligand was stronger than that of the ammine ligand.

Keywords kinetics; antitumor platinum complex; guanosine; 2-aminomethylpyrrolidine Pt(II) complex; 1,1-cyclobutanedicarboxylato Pt(II) complex; bis(N₇-guanosine) Pt(II) complex; carboplatin; cisplatin

The antitumor platinum(II) complexes are represented by the general formula PtL₂X₂, where X₂ represents either two monodentate ligands or one bidentate anionic ligand, usually called leaving groups, and L₂ represents two monodentate ligands or one bidentate ammine ligand, usually called carrier ligands.

The activity of these complexes is believed to be due to bifunctional crosslinking with deoxyribonucleic acid (DNA). A number of fundamental studies have been carried out on the interaction of these complexes with DNA, especially with guanine bases, and on the structure elucidation of the reaction products.¹⁾

In the preceding paper,²⁾ we reported that (–)-(R)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) (**1**) reacts with 2 eq of guanosine (Guo) at room temperature to form [(–)-(R)-2-aminoethylpyrrolidine]-bis(N₇-guanosine)platinum(II).

In order to explore the characteristic features of this reaction, we carried out reactions using other two antitumor platinum(II) complexes, *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin, **2**) and *cis*-diamminedichloroplatinum(II) (cisplatin, **3**), with Guo under comparable conditions, and kinetically compared the reactivities of **1**, **2** and **3**. Thus, **1** and **2** were allowed to react with 2 eq Guo in an aqueous solution separately at 40 °C and 60 °C, **3** was similarly treated in a 0.9% sodium chloride injection at the same temperatures, and the respective reactions were followed by means of proton nuclear magnetic resonance (¹H-NMR) spectroscopy and high performance liquid chromatography (HPLC).

Chart 1

Experimental

Materials Guo was obtained from Kanto Chemical Co., Inc.

(–)-(R)-2-Aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) (**1**), *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin, **2**) and *cis*-diamminedichloroplatinum(II) (cisplatin, **3**) were prepared according to the respective published methods.^{3–5)}

Reaction of **1, **2** or **3** with Guo in Aqueous Solution** A solution of 20 mg (67 μmol) of Guo in 20 ml of water was treated with **1** (15 mg, 33 μmol) or **2** (12 mg, 33 μmol), and the mixture was allowed to stand separately at 40 °C or 60 °C in a water bath. The reaction of **3** was carried out in a similar way using 10 mg (33 μmol) of **3** and 20 mg of Guo in 20 ml of a 0.9% sodium chloride injection. Each of the reaction mixtures was analyzed at intervals by HPLC and ¹H-NMR spectroscopy. The ¹H-NMR spectra were measured with samples prepared by adding 5 ml of D₂O to 1 ml aliquots.

Measurements HPLC analyses were performed on a Shimadzu LC-6A liquid chromatograph with detection at 235 nm (in the case of **3**, 210 nm), on a Tosoh TSKgel G-Oligo-PW column (7.8 × 300 mm), using 0.1 M Na₂SO₄ aqueous solution as an eluent. The flow rate was 1 ml/min.

¹H-NMR spectra were recorded on a JEOL JNM-GX400 spectrometer operating in the Fourier transform mode with sodium 4,4-dimethyl-4-silapentanesulfonate (DSS) as an internal standard, and the chemical shifts are given in ppm units.

Results and Discussion

The ligand substitution in square planar platinum(II) complexes has been shown to follow the S_N2 mechanism.⁶⁾

The rate laws are consistent with both solvent-assisted and second-order substitution pathways, and the reactions

Chart 2

under consideration may be conveniently schematized as Chart 2.

Pathway (A) is a direct nucleophilic substitution of X_2 in platinum(II) complexes with two Guo. Pathway (B) proceeds through the solvent-assisted substitution pathway, in which the first reaction is a nucleophilic substitution of X_2 with H_2O , and the second reaction is that of the aqua complexes with Guo.

Since the leaving group, 1,1-cyclobutanedicarboxylate (CBDCA), of **1** and **2** is not two monodentate (anion) ligands but a bidentate ligand chelating to the platinum, it appears likely to be stable enough not to be substituted with water. In fact, we checked the aqueous solution of **1** or **2** stored at 40 °C for 7 d by 1H -NMR spectroscopy and HPLC, and confirmed that the liberation of CBDCA was not noticed at all, suggesting that the solvent (H_2O)-assisted pathway can be neglected.

Consequently, the reactions of **1** or **2** with Guo are reasonably presumed to follow pathway (A), and the equation to be considered is:

Further, the checking of the reaction mixture of **1** or **2** with Guo by 1H -NMR spectroscopy and HPLC demonstrated that the only detectable product was always that derived from 1 eq **1** or **2** and 2 eq Guo, and the formation of the product containing one Guo could not be observed at all (Figs. 1 and 2).

Accordingly, the binding of the second Guo may be a very rapid reaction ($k_1 \ll k_2$), and the reaction of **1** or **2** with Guo can be considered to proceed according to second order kinetics.

Fig. 1. HPLC Chromatogram of the Reaction Mixture of **1** (C), **2** (B) and **3** (A) with Guo

A: the residual ratio of **3** was 71.2% at 60 °C for 2 h. B: the residual ratio of **2** was 67.9% at 60 °C for 25 h. C: the residual ratio of **1** was 76.7% at 60 °C for 6 h. a: $cis-[Pt(NH_3)_2Cl(Guo)]$. b: $cis-[Pt(NH_3)_2(Guo)_2]$, c: $[(2\text{-aminomethylpyrrolidine})Pt(Guo)_2]$

$$-d[Pt \text{ complex}]/dt = k[Pt \text{ complex}][2(Guo)] \\ = k_{obs}[Pt \text{ complex}]^2$$

Residual ratio (α) is defined:

$$\alpha = [Pt \text{ complex}]/[Pt \text{ complex}]_0$$

$$1/\alpha = k_{obs}[Pt \text{ complex}]_0 t + 1$$

Table I shows the residual ratios of **1** and **2** remaining in the reaction mixtures kept separately at 40 °C and 60 °C by HPLC at intervals. The residual ratios of **1** and **2** are in good proportion to those of Guo in both cases.

Plots of time vs. reciprocal of the residual ratios of **1** and **2** are found to be linear for all reactions as illustrated in Fig. 3. Thus, it can be concluded that the reactions of **1**

Fig. 2. Parts of 1H -NMR Spectra of the Reaction Mixture of **1** (C), **2** (B) and **3** (A) with Guo at 60 °C for 24 h, Showing from Left to Right Guanine H_8 and the Sugar H_1 .

a and c: free Guo, b and d: bound Guo to Pt through the N-7 atom.

TABLE I. Residual Ratios of the Reaction Mixtures of **1**, **2** and **3** with Guo at 40 °C and 60 °C

Time (h)	1		2		3	
	40 °C	60 °C	40 °C	60 °C	40 °C	60 °C
2	97.4% (98.0%)	90.1% (91.2%)	99.9% (100.9%)	97.2% (97.4%)	94.0% (96.7%)	71.2% (81.9%)
4	96.4% (97.5%)	82.9% (84.2%)	98.6% (98.9%)	93.8% (94.2%)	89.4% (95.0%)	52.4% (69.8%)
6	91.4% (92.9%)	76.7% (78.3%)	97.4% (98.1%)	90.9% (91.4%)	84.9% ^{a)} (92.1%)	39.9% (59.9%)
24	79.3% (80.2%)	40.4% ^{b)} (41.9%)	92.8% (—)	67.9% ^{c)} (68.7%)	52.2% (70.0%)	10.5% ^{b)} (26.0%)
k_{obs}^d	4.56	34.6	2.16	11.8	—	—

Residual ratios of Guo corresponding to each of the Pt complexes respectively are in parentheses. a) 5 h, b) 26 h, c) 25 h, d) Second-order rate constant ($M^{-1} h^{-1}$).

Fig. 3. Time vs. $1/[\text{Residual Ratio}]$ Profile of the Reaction Mixtures of **1**, **2** and **3** with Guo at 40 °C and 60 °C

—●—, **1**; —■—, **2**; —▲—, **3**; at 40 °C. —○—, **1**; —□—, **2**; —△—, **3**; at 60 °C.

and **2** with Guo proceed according to second order kinetics as anticipated.

Contrary to the above cases, it has been known that one or two chloride molecules of the leaving groups of **3** are replaceable with H_2O to give the corresponding aqua-complexes.⁷⁾ Therefore, both pathways (A) and (B) in Chart 2 should be considered in the reaction of **3** with Guo. Nevertheless, taking into account the high chloride ion concentration in a 0.9% sodium chloride injection, it appears likely that pathway (B) is negligible⁸⁾ and the reaction proceeds through pathway (A) in this case, too.

The HPLC chromatogram of the reaction mixture of **3** with Guo (2 eq) in a 0.9% sodium chloride injection at 60 °C for 2 h exhibited a peak due to the intermediate,⁹⁾ *cis*- $\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{Guo})$, in addition to that of *cis*- $\text{Pt}(\text{NH}_3)_2(\text{Guo})_2$ (Fig. 1). This observation evidently indicates that the reaction of **3** with Guo follows a two-step course ($k_1 > k_2$).¹⁰⁾ The shelf-life of *cis*- $\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{Guo})$ is comparatively long, and a prolonged reaction time or the use of excess Guo is required to complete the reaction.

Table I also shows the residual ratios of **3** remaining in the reaction mixtures kept separately at 40 °C and 60 °C by HPLC at intervals. The residual ratios of **3** are not in proportion to those of Guo in this case. As illustrated in Fig. 3, plots of time vs. reciprocal of the residual ratios of **3** are not linear in accord with the above observation. Thus, it can be concluded that the reaction of **3** with Guo in a

0.9% sodium chloride injection does not proceed according to second order kinetics, but is a stepwise reaction, different from the reaction of **1** and **2** with Guo in aqueous solution.

As seen from Fig. 3, the reaction rates of the three platinum complexes with Guo were found to follow a decreasing order: $3 \gg 1 > 2$.

The large difference in the reactivities between **3** and **2**, having the same carrier ligands (NH_3), should be considered to be due to the different nature of the leaving groups, and it may be ascribed not only to the difference in binding-energies between $\text{Pt}-\text{Cl}$ and $\text{Pt}-\text{OCO}-$, but also to the stabilizing effect owing to the chelation of the leaving group (bidentate ligand, CBDCA) to Pt in **2**.

On the other hand, comparison of the reactivities of **1** and **2** must be done by taking into account the nature of their carrier ligands, since the leaving group is the same (CBDCA). The difference in the reaction rates between **1** and **2** can be ascribed to the trans effect^{6,11)} of the carrier ligand. It may be assumed that the trans effect of the 2-aminomethylpyrrolidine ligand is stronger than that of ammine ligand (NH_3).

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