by filtration. Solvent removal at reduced pressure gave **8b** (50 mg, 85%) as a crystalline solid. Recrystallization from pentane gave the X-ray sample[8] (mp 81-82 °C). The product was not stable to silica gel chromatography and gave almost complete hydrolysis to 1,15-anhydrothromboxane B_2 on attempted analytical or preparative chromatography: ^{1}H NMR (C_6D_6) δ 6.29 (1 H, dd, J = 16.1, 6.3 Hz), 6.10 (1 H, dd, J = 16.1, 8.6 Hz), 5.60 (1 H, dd, J = 3.9, 3.3 Hz), 5.37-5.52 (2 H, m), 5.36 (1 H, m), 4.50 (1 H, t, J = 8.2 Hz), 4.48 (1 H, dd, J = 6.5, 3.9 Hz), 2.73 (1 H, ddd, J = 10.3, 6.5, 3.6 Hz), 1.2-2.4 (17 H), 1.38 (1 H, d, J = 10.3 Hz), 0.95 (3 H, t, J = 6.7 Hz); ^{13}C NMR (C_6D_6) δ 172.71, 134.99, 132.20, 130.96, 128.17, 106.11, 82.19, 77.46, 71.53, 51.12, 42.28, 35.14, 32.92, 32.23, 26.79, 26.67, 26.29, 25.00, 23.21, 14.58; IR (CH_2Cl_2) cm^{-1} 3010, 2959, 2931, 1730, 1243, 1111, 1032; MS (CI-CH_4), m/e 335 (M + 1), 317 (M + 1 - H_2O); HRMS calcd for $C_{20}H_{31}O_4$ (M + 1) 335.2222, found 335.2192; TLC dec to 1,15-anhydro-TXB_2.

Sodium and Potassium Thromboxane A_2 (1). A. Hydrolysis in Methanol/Water. To a vial containing 1 mg (0.003 mmol) of **8a** in 0.050 mL of methyl-d_4 alcohol was immediately added 0.025 mL of a 0.12 M solution of NaOH(D) in (D)H_2O under nitrogen. After stirring at 25 °C for 30 min, ^{1}H NMR and biological assay showed the saponification to sodium TXA_2 to be complete. Such solutions were diluted and used for biological testing and could be stored for at least a week of -20 °C without significant loss in biological activity.

B. Aprotic Hydrolysis with Me_3SiOK. To 5 mg (0.015 mmol) of **8a** was added 0.10 mL (0.03 mmol) of a 0.3 M solution of Me_3SiOK (Petrarch) in anhydrous tetrahydrofuran (distilled from Ph_2CO/Na) or ether at 25 °C under argon. The solution slowly turned yellow, and the reaction proceeded to completion over the course of 5 h. The tetrahydrofuran or ether saponification solutions were used after dilution for biological testing. Saponification for NMR analysis was carried out in dry THF-d_8.

In a few instances, the methanol/water procedure gave substantial decomposition of the product sodium TXA_2 during the saponification. The Me_3SiOK/THF hydrolysis procedure on the other hand is highly reproducible and is thus preferred: ^{1}H NMR THF-d_8) δ (potassium salt) 5.87 (2 H, br t, J = 9 Hz, H13, H14), 5.73 (1 H, t, J = 4 Hz, H11), 5.45-5.70 (2 H, m, H5, H6), 4.72 (1 H, dd, J = 4, 6 Hz, H9), 4.53 (1 H, br t, J = 6 Hz, H12), 4.18 (1 H, br q, J = 6 Hz, H15), 3.22 (1 H, m, H10a), 1.4-2.5 (18 H, m), 1.05 (3 H, br t, H20). All peaks were broadened (w/2 ca. 2 Hz) possibly due to slow exchange of the salt aggregates. Further physical characterization was not possible due to the lability of the TXA_2 salts.

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Mechanism of the Reaction between *cis*-[$PtCl_2(NH_3)_2$] and DNA in Vitro†

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Abstract: Products of the reaction between *cis*-[$PtCl_2(NH_3)_2$] and salmon sperm DNA in vitro have been purified. These adducts were compared with synthesized model compounds of known structures and identified as *cis*-[$Pt(NH_3)_2(Gua)_2]^{2+}$, *cis*-[$Pt(NH_3)_2(Gua)(Ade)]^{2+}$, and *cis*-[$Pt(NH_3)_2(Gua)(H_2O)]^{2+}$. Kinetics of the reaction indicate that the platinum compound binds initially at the N(7) position of Gua, the majority of these monofunctional lesions rapidly chelate to another purine base (preferentially but not exclusively Gua), and the remaining monofunctional lesions react slowly, primarily with an Ade base.

There is good evidence that the fixation of *cis*-[$PtCl_2(NH_3)_2$] (*cis*-DDP)[3] on DNA is the cellular event responsible for the antitumor activity of this drug.[1] However, the trans isomer which is not antitumoral[2,3] also enters the cell and covalently binds to DNA.[4-6] Several studies have quantitated the chemical and biological effects of the DNA damage caused by these compounds. When equal amounts of *cis*- or *trans*-DDP fixed on the DNA are compared, lesions formed by *cis*-DDP are more toxic[4-6] and more mutagenic.[5] They also inhibit DNA synthesis[6-8] and undergo DNA repair[6,9] to a greater extent than DNA lesions formed by *trans*-DDP. Physical chemical studies indicate that *cis*-DDP and *trans*-DDP bind differently to DNA in vitro. Their effects on the secondary structure and the stability of DNA have been compared when 5-50 platinum atoms are bound per 1000 nucleotides. Under these conditions, both isomers form interstrand cross-links, shorten the DNA, and prevent the intercalation of ethidium bromide. However *cis*-DDP destabilizes the DNA while *trans*-DDP stabilizes the polymer, and only the cis isomer causes an increase in the circular dichroism spectrum of DNA at these levels of DNA binding (ref 10, and references therein). The structures of the platinum-DNA adducts which are responsible for the different biochemical and physical chemical effects of these compounds have not yet been determined.

Evidence has accumulated for several years that *cis*-DDP binds to the N(7) position of guanine (Gua) bases in oligonucleotides (ref 11 and 12 and references therein), but platinum-containing adducts have only recently been isolated from DNA, and their quantitation is an active area of research. We have previously developed a method to separate platinum-DNA adducts from

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DNA by means of acid-catalyzed depurination followed by electrophoresis and paper chromatograpy.[13] Subsequently, this method was used to determine the mode of fixation of [PtCl(dien)]Cl on DNA by comparing the platinum–DNA adducts with acid-hydrolyzed platinum–nucleoside complexes of known structures.[14] In the present work, we report the structures of the principal adducts which are isolated from DNA treated with *cis*- or *trans*-DDP. Platinum-containing adducts have also recently been isolated from platinum–DNA complexes by enzymatic digestion.[15-18] Results from these two different analytical methods are comparable and permit a preliminary quantitative description of the main steps of the reaction between *cis*-DDP and DNA in vitro.

Materials and Methods

cis- and *trans*-DDP were prepared from K_2PtCl_4 (Johnson Matthey) by published methods.[19,20] Salmon sperm DNA was purchased from Worthington Chemical Co., nucleosides and other DNAs from Sigma Chemical Co. or Calbiochem, KH_2PO_4 and LiCl from Merck, $NaClO_4$ from Fluka, and [^{14}C(8)]Gua from Commissariat â L'Energie Atomique, Saclay, France. All other chemicals were purchased from Prolabo.

Platinum–DNA complexes were synthesized and adducts were cleaved from the complex by acid-catalyzed depurination as previously described.[13,14] Briefly, 10 vol of hot formic acid was added to a 1 mg/mL solution of platinated DNA, and the mixture was reacted for 15 min at 100 °C. Formic acid was evaporated, the solid residue was resuspended in distilled water, and the products were separated by high-voltage electrophoresis at pH 2. In some experiments, additional purification in a second dimension was performed by means of ascending paper chromatography perpendicular to the direction of electrophoresis. The paper was cut into 1-cm strips which were eluted in distilled water and the profile of platinum concentration was determined by flameless atomic absorption.

In order to identify the nucleobase ligands in these adducts, they were eluted from the paper in distilled water and reacted 5–7 h at 100 °C in 0.1 M thiourea (see below). The reaction mixture was concentrated by lyophilization and subjected to high-voltage electrophoresis at pH 2 followed by ascending paper chromatography perpendicular to the direction of electrophoresis. Bases were identified by their electrophoretic and chromatographic mobilities and by HPLC.[14]

The extinction coefficients of the adducts or model compounds were calculated from their UV absorbance in H_2O, pH 6, divided by the molar concentration of platinum as determined by flameless atomic absorption. IR spectra of samples which had been freshly prepared in KBr or CsBr pellets were recorded on a Perkin-Elmer 557 spectrophotometer. NMR spectra of 0.1 M solutions in D_2O were recorded on a Varian EM-360 spectrophotometer with DSS as an internal standard.

cis- and *trans*-[$Pt(NH_3)_2(dGuo)_2$]Cl_2 were synthesized by the method of Kong and Theophanides,[21] and their purity was verified by electrophoresis and paper chromatography. IR spectroscopy of these molecules showed a complete absence of Pt–Cl stretching, the extinction coefficient, $\epsilon_{255} = 2.1 \times 10^4$ L mol^{-1} cm^{-1}, was consistent with a complex containing two nucleobase ligands per platinum atom, and a Pt–H(8) coupling constant of 26 Hz was observed, indicating platium fixation at N(7)–dGuo.[21] Elemental analysis indicated the presence of two molecules of water in the cis complex and four water molecules in the trans complex.

In a typical synthesis of *cis*-[$PtCl(NH_3)_2(dGuo)$]Cl, 500 mL of equimolar *cis*-DDP and dGuo (6×10^{-3} M) was reacted for 7 h at 37 °C in the dark. The solution was then evaporated to dryness, resuspended in 20 mL of water, and filtered. The filtrate was concentrated to 3–4 mL and left overnight at 5 °C. The product was precipitated at 5 °C from the filtrate of this solution by the addition of 200 mL of acetone. Electrophoresis and paper chromatography showed that the product was pure. UV absorption, $\epsilon_{250} = 9 \times 10^3$ L mol^{-1} cm^{-1}, indicated a complex with one nucleoside per platinum atom. The Pt–Cl stretching of an inner-shell chloride was observed. Proton NMR revealed a 26-Hz Pt–H(8) coupling constant which is characteristic of platinum fixation at N(7)–Gua.[21] Elemental analysis was consistent with the formula *cis*-[$PtCl(NH_3)_2(dGuo)$]Cl·$2H_2O$.

cis-[$Pt(NH_3)_2(dGuo)(dAdo)$]Cl_2 was synthesized by reacting 6×10^{-3} M *cis*-[$PtCl(NH_3)_2(dGuo)$]Cl with a 10-fold excess of dAdo. The reaction was followed by thin-layer chromatography (PEI cellulose developed with 0.5 M LiCl) and allowed to continue for 6 days at 37 °C at which time the initial platinum complex had completely disappeared. The sample was evaporated to dryness, resuspended in a minimum volume of water, and filtered to remove the unreacted dAdo. This procedure was repeated until no solid material was observed and the product was precipitated with acetone at 5 °C. IR spectra revealed that the Pt–Cl stretching had disappeared during the reaction, and the extinction coefficient, $\epsilon_{255} = 2.2 \times 10^4$ L mol^{-1} cm^{-1}, indicated two nucleoside ligands per platinum atom. Elemental analysis was consistent with the formula *cis*-Pt[$(NH_3)_2(dGuo)(dAdo)$]Cl_2·$2H_2O$. However electrophoresis of this product revealed two peaks, 10% of the platinum-containing material migrating ahead of the major product, and the NMR spectrum in the region of H(8) was complex. These products, which were not further characterized, may represent platinum fixation at the N(7) and N(1) positions of Ado[22,23] or partial hydrolysis of the glycosyl linkage during the reaction (see below).

[$Pt(tu)_4$]Cl_2 was synthesized by reacting K_2PtCl_4 with stoichiometric thiourea for 2 h at 50 °C, and the product was precipitated by concentration. IR spectroscopy revealed that the inner-shell chloride had disappeared during the reaction. The product was pure judging by electrophoresis and paper chromatography, and its formula was verified by elemental analysis.

trans-[$Pt(NH_3)(dGuo)(tu)_2$]Cl_2 was synthesized by reacting 8×10^{-3} M *cis*-[$PtCl(NH_3)_2(dGuo)$]Cl with a 10-fold excess of thiourea for 15 min at 60 °C. Electrophoresis of the reaction mixture revealed that dGuo was not released. After precipitation from acetone, the purity of the product was verified by electrophoresis and paper chromatography. Elemental analysis was consistent with the formula [$Pt(NH_3)(dGuo)(tu)_2$]Cl_2·$2H_2O$. The IR spectrum showed no inner-shell chloride. We assume that the chloride was replaced by an initial thiourea ligand which then labilized the NH_3 trans to this position, resulting in two thiourea ligands having a trans geometry in the final product.

The corresponding Pt–base complexes of these model compounds were synthesized by hydrolysis with formic acid as described above.

Table I. Characterization of the Products Formed by Reacting *cis*-[$Pt(NH_3)_2(Gua)_2$]$^{2+}$ with Thiourea

prod	electroph[a]	R_f[b]
cis-[$Pt(NH_3)_2(Gua)_2$	1.28	0.14
trans-[$Pt(NH_3)(Gua)(tu)_2$]$^{2+}$	1.29	0.44
[$Pt(tu)_4$]$^{2+}$	1.30	0.52
c	0.45	0.20
c	0.45	0.40

[a] Distance migrated during electrophoresis relative to Gua. [b] Ascending paper chromatography, 1 M ammonium acetate/ethanol 35/65 (v/v). [c] Not identified.

Results

Several types of evidence indicate that platinum–base complexes formed with DNA are stable during acid hydrolysis. First, hydrolysis of *cis*-[$Pt(NH_3)_2(dGuo)_2$]$^{2+}$ did not liberate Gua judging by the absence of guanine in the electrophoresis profile of the hydrolyzed compound. This result is consistent with previous mass spectral and NMR studies of [$Pt(dien)(N(7)–dGuo)$]$^{2+}$ which revealed that acid hydrolysis did not break the platinum–base bond of this compound.[14] Second, the following experiment indicates that the exchange of nucleobase ligands does not occur during acid hydrolysis. *cis*- or *trans*-[$Pt(NH_3)_2(dGuo)_2$]Cl_2 was hydrolyzed in the presence of [^{14}C]Guo, the hydrolysate was separated by electroporesis, and the profiles of platinum and radio-

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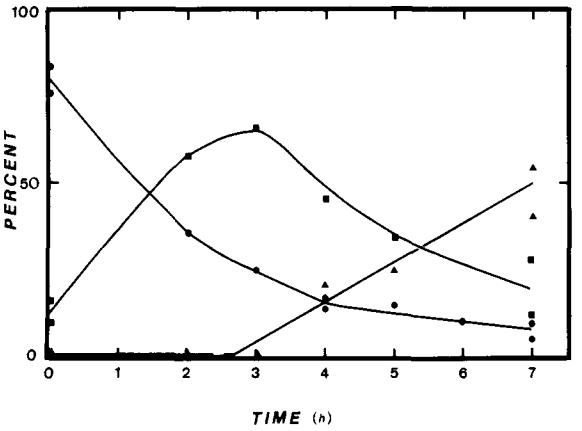

Figure 1. Relative concentrations of the principal platinum-containing products which were formed during the reaction of 1 mg/mL *cis*-$[Pt(NH_3)_2(Gua)_2]^{2+}$ with 0.1 M thiourea at 100 °C. (●) *cis*-$[Pt(NH_3)_2(Gua)_2]^{2+}$, (■) *trans*-$[Pt(NH_3)(Gua)(tu)_2]^{2+}$, (▲) $[Pt(tu)_4]^{2+}$.

activity were determined. No platinum compounds which contained radioactivity were observed. Identical results were found after acid hydrolysis of *cis*- or *trans*-DDP-DNA complexes, r_b = 0.1, in the presence of [^{14}C]Guo. We conclude from these experiments that platinum–base complexes were neither destroyed nor rearranged during acid hydrolysis.

Control experiments were performed to determine the reaction conditions necessary to quantitatively remove the base from the platinum–base complex by ligand substitution with thiourea. The reaction between *cis*-$[Pt(NH_3)_2(Gua)_2]^{2+}$ and 0.1 M thiourea at 100 °C produced four platinum-containing products which were characterized by electrophoresis and paper chromatography (Table I). *trans*-$[Pt(NH_3)(Gua)(tu)_2]^{2+}$ and $[Pt(tu)_4]^{2+}$ were identified by comparing their migration with that of model compounds. The kinetics of the reaction is shown in Figure 1. In this experiment, a 1 mg/mL solution of acid-hydrolyzed *cis*-$[Pt(NH_3)_2(dGuo)_2]Cl_2$ was reacted with 0.1 M thiourea at 100 °C. At various times, aliquots were frozen, lyophilized, and resuspended in $^1/_{10}$ vol of distilled water in order to concentrate them prior to electrophoresis and paper chromatography. In the absence of thiourea, hydrolyzed starting material gave one spot; hence, data in Figure 1 show that some reaction with thiourea apparently took place during sample handling prior to electrophoresis. Nevertheless, the results permit a qualitative characterization of the reaction and justify our choice of reaction conditions which are required to separate platinum from the nucleobase.

cis-$[Pt(NH_3)_2(Gua)_2]^{2+}$ disappeared during the reaction with a half-life of about 1.5 h. *trans*-$[Pt(NH_3)(Gua)(tu)_2]^{2+}$ appeared during the reaction as an intermediate which reached a maximum concentration after 3 h and then disappeared with a half-life of 2 h to form $[Pt(tu)_4]^{2+}$. Two additional products occurred after 3 h of reaction (Table I) which did not contain Gua judging from their low UV absorbance and accounted for 20% of the recovered material after 7 h. These products were not further characterized, but we note that platinum-containing products with similar migration were observed after extensive reaction of thiourea with *cis*-DDP alone.

These kinetic results show that Gua is displaced from *cis*-$[Pt(NH_3)_2(Gua)_2]^{2+}$ by thiourea in two steps and that 7–8 h of reaction with 0.1 M thiourea at 100 °C is required to remove 95% of the base. In contrast, after 24 h at 37 °C in 1 M thiourea (typical conditions found in the literature to remove platinum from nucleobases[24]), 20–30% of the *cis*-$[Pt(NH_3)_2(Gua)_2]^{2+}$ had not reacted and only 10–20% of the recovered platinum was in the form of $[Pt(tu)_4]^{2+}$.

The electrophoresis profile of hydrolyzed *cis*-DDP-DNA contained three major peaks which migrated toward the catohde (Figure 2). Two-dimensional separation using electrophoresis

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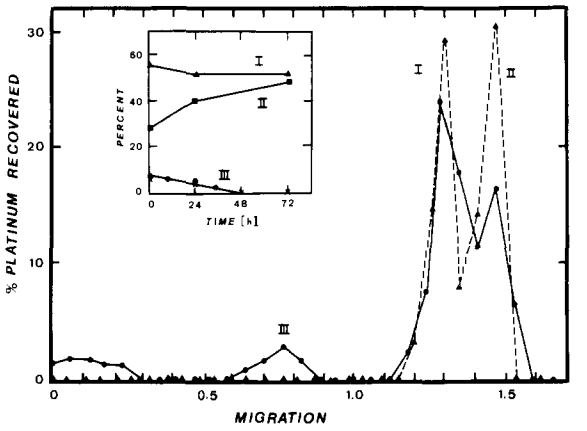

Figure 2. Electrophoresis profile of the complex formed between *cis*-PDD and DNA. *cis*-DDP and DNA (r_i = 0.1) were reacted in 0.01 M $NaClO_4$ for 2 h at 37 °C. In order to remove the unreacted *cis*-DDP, the complex was precipitated by 2 vol of ethanol at −70 °C, the pellet was washed with cold ethanol and resuspended in 0.01 M $NaClO_4$, and the r_b was determined by UV absorption and atomic absorption spectroscopy, r_b = 0.04. Aliquots of this solution were hydrolyzed either immediately (●) or after 72 h at 37 °C (▲) and subjected to paper electrophoresis for 45 min at 100 mA (7000 V), pH 2. The origin is at zero and the abscissa is the distance migrated toward the cathode relative to guanine which migrated 18 cm from the origin in these experiments. Insert: the relative concentrations of each adduct during incubation of the Pt–DNA complex at 37 °C in the absence of unreated *cis*-DDP.

Table II. Characterization of the Platinum–DNA Adducts Formed by *cis*-DDP in Vitro

electroph[a]	R_f[a]	adduct
0.75	0.25	III
1.3	0.14	I
1.5	0.28	II

[a] See Table I.

and paper chromatography revealed that each of these peaks was a single compound which could be characterized by its eletrophoretic mobility and R_f (Table II). In addition, about 5% of the material remained near the origin, and no platinum-containing species were observed which migrated toward the anode. No other peaks were observed in the electrophoresis profile from r_b = 0.0005 to 0.4. Above this level of binding, a fifth peak occurred at 1.8 units. The relative sizes of the peaks at 1.3 units (I) and 1.5 units (II) varied from approximately equal intensities at $0.1 \leq r_b \leq 0.4$ to a ratio of I/II = 3.5 ± 1 at r_b = 0.0005 as previously reported.[13]

Platinum–DNA adduct I liberated Gua after reaction with thiourea. Its extinction coefficient, $\epsilon_{250} = 2 \times 10^4$ L mol^{-1} cm^{-1}, was consistent with a complex containing two Gua per platinum atom. The electrophoresis profile of hydrolyzed *cis*-$[Pt(NH_3)_2(dGuo)_2]Cl_2$ (Figure 3a) was identical with the migration of the platinum–DNA adduct corresponding to peak I (Figure 2). Furthermore, after paper chromatography, the hydrolyzed model compound had an R_f of 0.16 which was the same as the R_f of adduct I (Table II). We therefore conclude that adduct I is *cis*-$[Pt(NH_3)_2(Gua)_2]^{2+}$.

The extinction coefficient of adduct II, $\epsilon_{255} = 1.9 \times 10^4$ L mol^{-1} cm^{-1}, indicated that two bases were bound to each platinum atom in this complex. Reaction of the adduct with thiourea liberated Gua and Ade which were identified by electrophoresis, paper chromatography, and HPLC. The electrophoresis profile of hydrolyzed *cis*-$[Pt(NH_3)_2(dGuo)(dAdo)]Cl_2$ gave a peak which migrated 1.5 units (Figure 3b), and paper chromatography revealed that this peak contained a single compound with R_f = 0.32. The electrophoretic and chromatographic migration corresponded to adduct II (Table II), and hence this adduct was identified as *cis*-$[Pt(NH_3)_2(Gua)(Ade)]^{2+}$.

Adduct III released Gua after reaction with thiourea. The electrophoresis profile of the model compound, hydrolyzed *cis*-$[PtCl(NH_3)_2(dGuo)]Cl$, is shown in Figure 3c. The R_f of this compound was 0.2. Both the electrophoretic and chromatographic

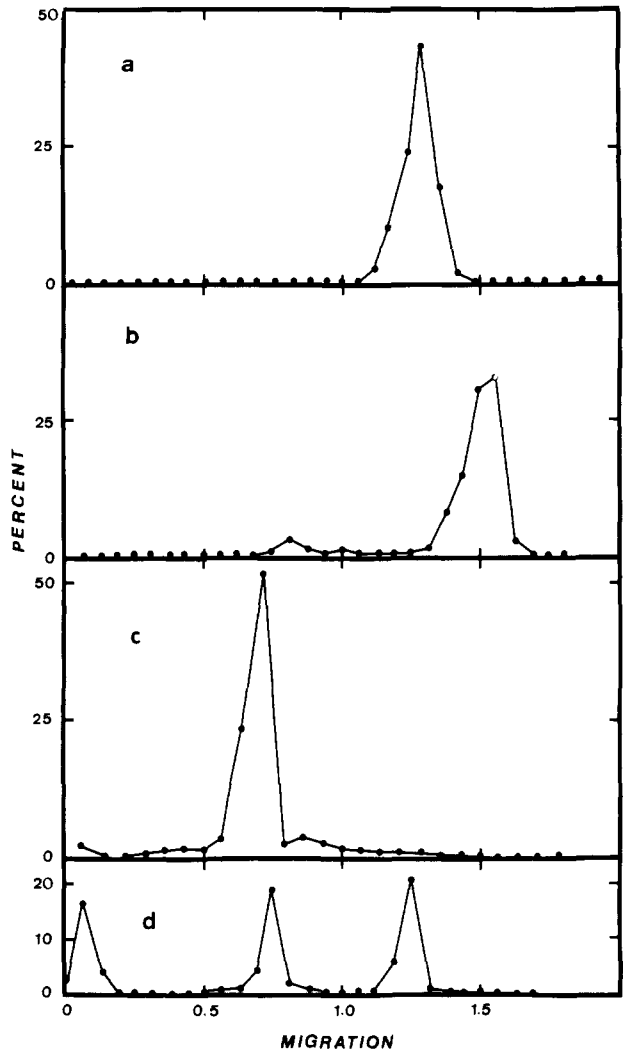

Figure 3. Electrophoresis profiles of hydrolyzed model compounds (abscissa as in Figure 2). (a) *cis*-$[Pt(NH_3)_2(Gua)_2]^{2+}$; (b) *cis*-$[Pt(NH_3)_2(Gua)(Ade)]^{2+}$; (c) *cis*-$[Pt(NH_3)_2(Gua)Cl]^{2+}$; (d) hydrolyzed *cis*-DDP-poly(dG)·poly(dC).

migration of this model compound corresponded to adduct III (Table II). The identification of this platinum–DNA adduct as monofunctional fixation on Gua is strongly supported by its reactivity. When the freshly prepared platinum–DNA complex was allowed to incubate in the absence of unreacted *cis*-DDP, adduct III disappeared, indicating that it is an unstable lesion which further reacts with DNA. The kinetics of this reaction are shown in the insert of Figure 2. As a control experiment, *cis*-$[PtCl(NH_3)_2(dGuo)]Cl$ was reacted with DNA, r_b = 0.1, and the products were isolated by acid hydrolysis. The electrophoresis profile revealed that the resulting Pt–DNA complex contained the same bis adducts as found for the reaction of *cis*-DDP with DNA, but with different frequencies: 85% Gua_2 adduct and 15% hydrolyzed *cis*-$[Pt(NH_3)_2(dAdo)]^{2+}$. Taken together, this evidence strongly suggests that adduct III is identical with hydrolyzed *cis*-$[PtCl(NH_3)_2(dGuo)]Cl$.

Some preliminary experiments were performed to characterize the stability of the monofunctional adduct. After 2-h reaction of *cis*-DDP with DNA at 37 °C, this adduct represented about 10% of the total lesions (Figure 2). After reaction at 25 °C, the relative concentration of the adduct was 20–30%,[25] suggesting that the initial concentration of adduct III may depend on the temperature of the reaction between *cis*-DDP and DNA. Similar results were found after removing unreacted *cis*-DDP by alcohol

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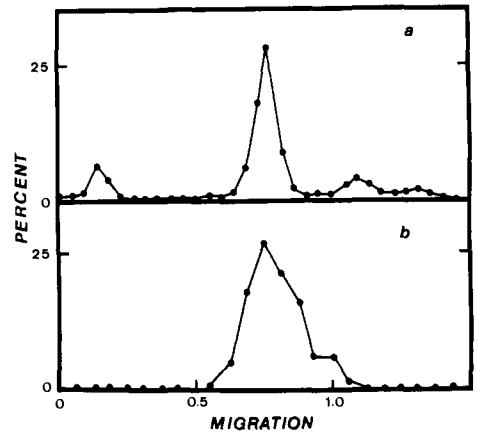

Figure 4. Electrophoresis profiles of hydrolyzed *trans*-DDP-DNA (a) and *trans*-$[Pt(NH_3)_2(Gua)]^{2+}$ (b) (abscissa as in Figure 2).

precipitation or by dialysis against 0.1 NaCl. Hence, the slow reaction can apparently be blocked by NaCl or by precipitating the platinum–DNA complex.

The structures of the *cis*-DDP-DNA adducts formed in vitro seem to be independent of G–C content and DNA secondary structure. All three adducts were found after the reaction of *cis*-DDP with purified DNA from *Escherichia coli*, *Micrococcus lysodeikticus*, and *Clostridium perfringens*, r_b = 0.05. Furthermore, the same adducts were also observed in DNA which had been denatured by heating and rapidly cooling. The electrophoresis profile of poly(dG)·poly(dC) which had reacted with *cis*-DDP for 24 h at 37 °C, r_b = 0.2, had three peaks of equal intensity (Figure 3d). The material at 0.75 and 1.3 units in the electrophoresis profile was subjected to paper chromatography. These two compounds had R_f values of 0.26 and 0.2 and were consequently identified as hydrolyzed *cis*-$[PtCl(NH_3)_2(dGuo)]^{2+}$ and *cis*-$[Pt(NH_3)_2(dGuo)_2]^{2+}$, respectively.

Hydrolyzed *trans*-$[Pt(NH_3)_2(dGuo)_2]Cl_2$ comigrated during electrophoresis with the major hydrolysis product of *trans*-DDP-DNA (Figure 4). Ascending paper chromatography (5% formic acid/ethanol, 25/75, v/v) resolved two platinum-containing peaks at R_f = 0.2 and 0.4 which chromatographed identically for the adduct and the model compound. This evidence suggests that the major adduct in the *trans*-DDP-DNA complex is identical with hydrolyzed *trans*-$[Pt(NH_3)_2(dGuo)_2]Cl_2$.

Discussion

In contrast to alkylating agents, fixation of platinum compounds at the N(7) position of Gua does not weaken the sugar–base glycosyl bond.[14] Heating the platinum–DNA complex does not release the adduct and thereby create apurinic sites.[26] Hence, it was not possible to separate adducts from DNA by heating at neutral pH. On the other hand, acid-catalyzed depurination appears to be a nondestructive technique for separating the platinum–base complex from platinated polynucleotides. Previous studies have indicated that platinum–DNA bonds are stable in acid,[14,27,28] and we have verified that the complex *cis*-$[Pt(NH_3)_2(Gua)_2]^{2+}$ is neither destroyed nor rearranged in our acid hydrolysis conditions.

During the preparation of this manuscript, Rahn reported the purification of Pt–DNA adducts by acid hydrolysis followed by cation-exchange chromatography at basic pH.[29] He found platinum–base complexes containing one guanine, two guanines, and one guanine plus one adenine. However, the bis(guanine) adduct decomposed in his conditions, perhaps due to reaction with the eluant, and it is difficult to quantitatively compare our results.

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Platinum-DNA adducts have recently been isolated by using enzymatic hydrolysis of the phosphodiester bond.[15-18] This method gives information about the base sequence of the DNA binding site which is lost by acid hydrolysis of the glycosyl linkage. However, the platinum-base adducts which are observed by the two methods can be compared. Both methods give similar results for the abundance of three types of platinum-base adducts and their kinetics of formation.

The initial fixation of *cis*-DDP on an oligonucleotide has recently been observed by X-ray crystallography.[30] The platinum atom binds to the N(7) position of Gua and, by means of a water bridge between the platinum and O(6), forms a seven-membered ring (N(7)-Pt-O-H-O(6)-C(6)-C(5)) which appears to destabilize the double helix of the DNA. After 2 h of reaction at 37 °C, this adduct accounts for about 10% of the recovered platinum. Apparently a majority of the monofunctional adduct reacts rapidly with DNA while about 10% disappears with a half-life of 24 h (Figure 2). These results show that the monofunctional adduct, *cis*-$[Pt(NH_3)_2(dGuo)(H_2O)]^{2+}$, chelates with DNA by two kinetically distinct reactions. The product of the first reaction is primarily a Gua_2 adduct. The product of the second reaction is preferentially, but not exclusively, *cis*-$[Pt(NH_3)_2(Gua)(Ade)]^{2+}$. Both reactions form Gua-Gua and Gua-Ade adducts and so the kinetic differences are probably not a consequence of different reactivity of the purine bases. Rather the monofunctional platinum-DNA adduct may react in two steps whose kinetics are determined by the relative positions of the bases at the binding site. A rapid reaction probably occurs with an adjacent purine base, preferably Gua. If an adjacent purine binding site is not available, a slower reaction may occur with a distant purine base.

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Registry No. *cis*-DDP, 15663-27-1; *trans*-DDP, 14913-33-8; dGuo, 961-07-9; dAdo, 958-09-8; tu, 62-56-6; *cis*-$[Pt(NH_3)_2(dGuo)_2]Cl_2$, 98064-86-9; *trans*-$[Pt(NH_3)_2(dGuo)_2]Cl_2$, 98168-18-4; *cis*-$[PtCl(NH_3)_2(dGuo)]Cl$, 98064-87-0; *cis*-$[Pt(NH_3)_2(dGuo)(dAdo)]Cl_2$, 98087-59-3; *trans*-$[Pt(NH_3)_2(dGuo)(tu)_2]Cl_2$, 98064-88-1; *trans*-$[Pt(NH_3)(Guo)(tu)_2]Cl_2$, 98064-89-2; *cis*-$[Pt(NH_3)_2(Gua)_2]^{2+}$, 81628-84-4; *cis*-$[Pt(NH_3)_2(Gua)(Ade)]^{2+}$, 98064-91-6; *cis*-$[Pt(NH_3)_2(Gua)(H_2O)]^{2+}$, 98064-92-7; $[Pt(tu)_4]Cl_2$, 14552-88-6.

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(31) Abbreviations used are as follows: DDP, $PtCl_2(NH_3)_2$; r_b, bound platinum per nucleotide; r_i, initial platinum per nucleotide; en, ethylenediamine; dien, diethylenetriamine; DDP-DNA, the complex formed between DDP and DNA; tu, thiourea.

Biosynthesis of Riboflavin. Incorporation of Multiply ^{13}C-Labeled Precursors into the Xylene Ring

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Abstract: The biosynthetic origin of the two 4-carbon units which give rise to the eight carbon atoms of the *o*-xylene moiety of riboflavin has been studied in stable isotope labeling experiments with cultures of the fungus *Ashbya gossypii*. Administration of precursors carrying multiple ^{13}C labels followed by NMR analysis of the enrichment and ^{13}C-^{13}C coupling patterns in the resulting riboflavin samples established unequivocally that each of the identical 4-carbon units arises from the intact carbon chain of a pentose derivative by an intramolecular rearrangement process involving excision of C4 and reconnection of C3 and C5.

Knowledge on the biosynthesis of riboflavin (**3**) has expanded considerably in recent years (Figure 1).[2,3] The first committed biosynthetic step consists of the opening of the imidazole ring of GTP by the enzyme GTP cyclohydrolase II.[4] Two subsequent enzymatic steps lead to the formation of 5-amino-6-(ribitylamino)-2,4-(1*H*,3*H*)-pyrimidinedione 5′-phosphate (**1**).[5-10] The conversion of this compound to 6,7-dimethyl-8-ribityllumazine (**2**) requires the addition of four carbon atoms, the origin of which is so far incompletely understood. A variety of potential precursors such as acetoin,[11,12] diacetyl,[13] pyruvate,[14] a tetrose,[15,16] a pentose,[17] and the ribityl moiety of 5-amino-6-(ribitylamino)-2,4-(1*H*,3*H*)-pyrimidinedione[18,19] have been discussed in the past. A detailed review of earlier work has been presented.[20]

Valuable evidence has been obtained by incorporation of isotopically labeled precursors, which is conveniently studied at the biosynthetic level of riboflavin rather than 6,7-dimethyl-8-ribityllumazine.[1,17,20-24] Since the formation of riboflavin by a dismutation of **2** is well understood,[25-27] the labeling pattern of the latter compound can be easily inferred from the distribution

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