Contribution from the Faculty of Pharmaceutical Sciences,
Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

Characterization of (1,2-Cyclohexanediamine)platinum(II) Isomers and Their d(GpG) Adducts by Means of ^{1}H NMR Spectroscopy. A Minor Structural Change Induced by the Isomers

Kenji Inagaki,* Hideaki Nakahara, Maarten Alink,[1] and Yoshinori Kidani

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Diastereoisomers obtained from the reaction of d(GpG) with the three isomers of Pt(1,2-cyclohexanediamine)$^{2+}$ have been investigated by means of high-resolution ^{1}H NMR spectroscopy. The reaction of d(GpG) with each Pt(*R,R*/*S,S*/*R,S*-dach)$^{2+}$ gave a G-N7, G-N7 adduct (dach = 1,2-cyclohexanediamine), abbreviated as Pt(*R,R*/*S,S*/*R,S*-dach)(d(GpG)-*N7,N7*), with an anti-anti configuration of the two guanines. Comparison of the NMR spectra of Pt(*R,R*-dach)(d(GpG)) and Pt(*S,S*-dach)(d(GpG)) indicates that a difference in the conformations (λ and δ) of the five-membered chelate ring (Pt(*R,R*/*S,S*-dach) moiety) induces a minor change in the structure of the bound d(GpG) moiety. Two isomers produced from the reaction of d(GpG) with Pt(*R,S*-dach)$^{2+}$ could be separated by means of HPLC and were characterized by NMR spectral analysis. The structures were compared with those of earlier reported adducts with *cis*-Pt$(NH_3)_2^{2+}$. The steric effect of the cyclohexane and the chelate rings—being expected in the case of a reaction with the -GpG- sequence in DNA—will be discussed.

Introduction

It is generally accepted that the cytotoxicity of antitumor-active platinum compounds is related to their binding with DNA.[2] It has been well-known that *cis*-Pt$(NH_3)_2Cl_2$ selectively binds to a guanine base to generate an intrastrand cross-linking between adjacent guanines on the same strand of DNA.[3] DNA second-strand synthesis by using *cis*-Pt$(NH_3)_2^{2+}$-treated template strand is blocked at $(dG)_n$ ($n \geq 2$) sequences in the template strand;[4] i.e., platination at the $(dG)_n$ sequences is expected to lead to an inhibition of DNA replication. A second-generation platinum compound—being produced by changing the nonleaving ligands of *cis*-Pt$(NH_3)_2Cl_2$—indicates antitumor activities comparable to, or more than, that of *cis*-Pt$(NH_3)_2Cl_2$.[5] One of the most promising compounds is a platinum complex involving 1,2-cyclohexanediamine (abbreviated as dach) as a nonleaving group.[6] The ligand dach has three isomeric forms (so-called *R,R*-dach, *S,S*-dach, and *R,S*-dach)[6] (see Figure 1). Generally, antitumor activity is considered to be influenced by an uptake into cells and a reactivity with biological substances (e.g., target DNA) and so on. Pt(*R,R*-dach)Cl_2 and Pt(*S,S*-dach)Cl_2 are optical isomers. An intriguing observation is that certain differences are observed in their in vivo activities though the difference is minor.[6] This may be attributed to diastereomeric interactions between Pt(dach)$^{2+}$ and a certain biological substance in living cells. One of the possible candidates clearly is the DNA molecule because DNA is a chiral compound. Binding of optically active Pt complexes to DNA is expected to result in diastereoisomers as shown in the previous communication.[7] The purpose of the present paper is to explore the presence of such a diastereomeric interaction, being determined by means of NMR spectral analysis.

Binding of *cis*-Pt$(NH_3)_2^{2+}$ to DNA results in Pt-induced conformational changes for the DNA double helix.[8] Similar conformational changes are expected to be induced by coordination of Pt(dach)$^{2+}$ to DNA. In fact, monoclonal antibodies elicited from *cis*-Pt$(NH_3)_2^{2+}$-DNA—induced by using *cis*-Pt$(NH3)_2^{2+}$-modified DNA as an antigen—also recognize a Pt(dach)-modified DNA.[9] This suggests that a DNA bending induced by a binding of Pt(dach)$^{2+}$ to DNA might be very similar to that induced by binding of *cis*-Pt$(NH_3)_2^{2+}$ to DNA. In the present work, we use d(GpG) (Figure 1) as a model DNA molecule, and the structural changes induced after the binding of the Pt(dach)$^{2+}$ isomers to d(GpG) are studied by NMR spectroscopy and are compared with those of *cis*-Pt$(NH_3)_2^{2+}$. Our efforts are also focused on the reactivity of Pt(dach)$^{2+}$ with DNA.

Table I. H NMR Data for Pt(*R,R*/*S,S*-dach)(d(GpG)) Adducts (pH = 5-6, 300 K)[a]

	chem shift, ppm			
	H8(5′)	H8(3′)	H1′(5′)	H1′(3′)
Pt(*R,R*-dach)(d(GpG))	8.18	8.52	overlap	
Pt(*S,S*-dach)(d(GpG))	8.09	8.51	6.21 (d)	6.24 (t)
cis-Pt$(NH_3)_2$(d(GpG))[b]	8.27	8.57	6.19	6.22
cis-Pt$(NH_3)_2$(r(GpG))[b]	8.54	8.32	6.06 (s)	5.90 (d)
Pt(*R,R*-dach)(r(GpG))	8.47	8.24	6.04 (s)	5.89 (d)
Pt(*S,S*-dach)(r(GpG))	8.38	8.22	6.03 (s)	5.88 (d)

[a] Abbreviations: s, singlet; d, doublet; t, triplet. [b] From ref 10a.

Experimental Section

Materials and Purification of the Reaction Products. The dinucleotides and DNA (calf thymus DNA) used in the present work were

(1) Present address: Leiden University, Leiden, The Netherlands.
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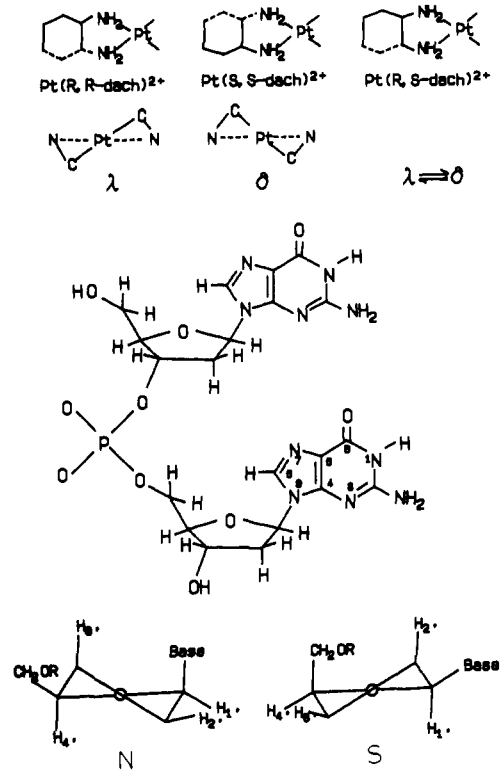

Figure 1. Schematic representations of Pt(dach)$^{2+}$ isomers, d(GpG), and *N*-type and *S*-type conformations of the deoxyribose moieties: (top) Pt(*R*,*R*/*S*,*S*/*R*,*S*-dach)$^{2+}$ and the conformations of the five-membered chelate ring; (middle) 2′-doexyguanylyl(3′-5′)guanosine (d(GpG)); (bottom) conformations of the 2′-deoxyribose ring [(left) *N*-type (C3′-endo; (right) *S*-type (C2′-endo)].

commercially available. The platinum complexes Pt(*R*,*R*/*S*,*S*/*R*,*S*-dach)Cl_2 were prepared according to the method described before.[6b] For the purpose of characterization of the reaction products, the nucleotide 5′-dGMP, d(GpG), r(GpG), or d(ApG) was allowed to react with a stoichiometric amount of Pt(*R*,*R*/*S*,*S*/*R*,*S*-dach)Cl_2 in water at 37 °C for 1 day and the reaction products were purified by reverse-phase HPLC (Cosmosil $5C_{18}$). A 1%/min linear gradient of methanol (from 0 to 30% of methanol) against 0.05 M phosphate buffer (pH 4.5) was employed as eluent. Enzymatic digestion of the platinum-modified DNA was carried out according to the method described previously.[3f,8] The enzymatic digestion products were quantified by using HPLC (at 260 nm).

NMR Measurements. The NMR spectra were recorded at 27 °C on a JEOL GX-400 spectrometer with use of standard Fourier transform techniques. A trace amount of TSP-d_4 was added as an internal reference. NMR samples were prepared by lyophilizing the reaction product three times from 99.7% D_2O and were finally dissolved in 99.95% D_2O. The pH (uncorrected meter readings in D_2O) of the NMR sample was adjusted by adding small amounts of a D_2O solution of 1 M $DClO_4$ and/or 1 M NaOD. Trapezoidal window techniques were applied to improve spectral resolution. Typical conditions for recording the NMR spectra include 200–300 scans, 45° pulse width, 32K data points, and 0.6-mL sample volume. The peak due to the residual HOD was suppressed by the WEFT pulse sequence when necessary. The NOE was measured in the difference spectral mode after carefully degassing the sample, and the preirradiation time, 5 s, was used for complete saturation. The 2D-COSY experiments were performed by using the sequence (90–t_1–90–acq)$_n$. The data were collected with an accumulated data matrix of 1024 × 512 points, and the resulting matrix was zero-filled to a final matrix of 1024 × 1024 points; a symmetrical contour map was used.

Results

Pt(*R*,*R*/*S*,*S*-dach)(d(GpG)). Table I indicates the chemical shift data of the several Pt(*R*,*R*/*S*,*S*-dach)d(GpG) adducts in comparison with *cis*-Pt$(NH_3)_2$d(GpG). Each of these adducts was isolated by using preparative HPLC. Resonances in the 8–5 ppm range of the NMR spectra were assigned on the basis of the literature values.[10,11] The G-H8 protons (free d(GpG); 7.76 ppm

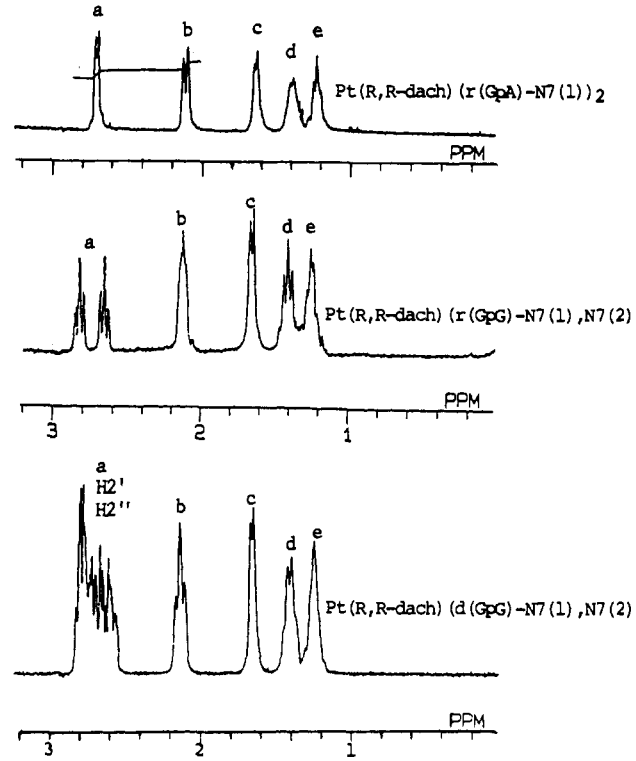

Figure 2. Cyclohexane proton region of the NMR spectra of Pt(*R*,*R*-dach)–nucleotide adducts. Assignments: a, C-H1$_{ax}$ + C-H2$_{ax}$; b, C-H3$_{eq}$ + C-H6$_{eq}$; c, C-H3$_{ax}$ + C-H6$_{ax}$; d, C-H4$_{eq}$ + C-H5$_{eq}$; e, C-H4$_{ax}$ + C-H5$_{ax}$.

for the 3′G-H8 and 8.02 ppm for the 5′G-H8) shift downfield upon platination. Such a downfield shift is good evidence of platination at the G-N7 sites because of a polarizing effect of the G-H8 bond toward the purine rings. The NMR–pH titration curve of Pt(*R*,*R*/*S*,*S*-dach)(d(GpG)) also gives evidence of platination at the N7 sites of the both guanines (supplementary material Figure S1). That is, no protonation was observed at the G-N7 site and the pK_a values (being 8.5) at G-N1 agreed well with the pK_a value at G-N1 of an N7-platinated guanine.[10]

Since Pt(*R*,*R*-dach)X_2 and Pt(*S*,*S*-dach)X_2 (X = Cl, OH_2, NH_3) are optical isomers, the NMR technique cannot discriminate between the isomers; i.e., both complexes should give the same spectrum. On the other hand, the reactions of d(GpG) with Pt(*R*,*R*-dach)$^{2+}$ and Pt(*S*,*S*-dach)$^{2+}$ gave Pt(*R*,*R*-dach)(d(GpG)-*N7*,*N7*) and Pt(*S*,*S*-dach)(d(GpG)-*N7*,*N7*), respectively, and both adducts are theoretically diastereomers of one aother.[7,12] In Table I, one can see that the chemical shift of the 5′G-H8

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(11) The chemical shifts of the H8 protons of Pt(*R*,*R*/*S*,*S*-dach)(d/r(GpG)) are in good agreement with the corresponding data of *cis*-Pt$(NH_3)_2$(d/r(GpG)). The signal of the 5′G-H8 proton of Pt(*R*,*R*/*S*,*S*-dach)(r(GpG)) is broader, compared with that of the 3′G-H8 proton. In the NMR–pH titration curve, the chemical shift change of the 5′G-H8 proton is more sensitive to the conformational change induced upon deprotonation at N(1), compared with that of the 3′G-H8 proton. These features are also in good agreement with the data of *cis*-Pt$(NH_3)_2$(d/r(GpG)).[10c]

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Table II. H NMR Data for Pt(*R,S*-dach)$^{2+}$ Compounds[a]

	a	b	c	d
Pt(*R,S*-dach)$(NH_3)_2$	3.09 (2 H) (13 Hz)	1.85 (2 H), 1.80 (2 H)	1.60 (2 H) (23 Hz)	1.42 (2 H) (23 Hz)
Pt(*R,S*-dach)$(dGMP)_2$	3.22 (2 H) (30 Hz)	1.93 (4 H) (20 Hz)	1.69 (2 H) (30 Hz)	1.45 (2 H) (30 Hz)
RS-1	3.26 (2 H) (25 Hz)	1.95 (4 H) (18 Hz)	1.66 (2 H) (26 Hz)	1.48 (2 H) (25 Hz)
RS-2	3.22 (2 H) (30 Hz)	2.06 (1 H), 1.98 (3 H)	1.69 (2 H) (40 Hz)	1.50 (2 H) (40 Hz)

[a] Values in ppm. The numbers in parentheses indicate the integrated proton number and the line width of the signals at half-height (see also Figure 4). Assignment: a, C-H1 + C-H2; b, C-H3 + C-H6; c and d, C-H4 + C-H5.

proton of Pt(*R,R*-dach)(d(GpG)) differs from that of Pt(*S,S*-dach)(d(GpG)). That is, the d(GpG) moiety experiences a difference between the λ conformation (for Pt(*R,R*-dach)$^{2+}$) and δ conformation (for Pt(*S,S*-dach)$^{2+}$) of the five-membered chelate ring. The protons of the sugar moieties of both adducts also show a slightly diferent NMR spectral pattern. These results clearly indicate that the conformational difference between λ and δ induces a minor change for the structure of the bound d(GpG) moiety. Unfortunately, the cyclohexane ring protons of Pt(*R,R*-dach)(d(GpG)) severely overlap with the H2′/H2″ protons of the sugar moieties so that further conformational analysis of the bound d(GpG) moiety could not be achieved.

Since Pt(*R,R*/*S,S*-dach)$(NH_3)_2$ have a C_2 axis through Pt and the middle of the two amino groups, the C-H1 proton of the cyclohexane ring should be equivalent to the C-H2 proton. Figure 2 shows a 1–3 ppm region of the NMR spectrum (the cyclohexane proton region) for r(GpA), r(GpG), and d(GpG). Each peak was assigned with the aid of the 2D-COSY technique (data not shown). Evidently, the axial and the equatorial protons of the cyclohexane ring have a different chemical shift, indicating the presence of the fixed cyclohexane and chelate rings, like in *trans*-decalin. In Pt(*R,R*-dach)(r(GpG)-*N7,N7*), the C-H1 and the C-H2 protons experience a different magnetic field, in which each of the peaks shows three doublets, as shown in Figure 2. A splitting pattern of the peak is consistent with that expected from two $^3J_{ax-ax}$ and $^3J_{ax-eq}$. This agrees well with the fact that a C_2 symmetry of Pt(*R,R*-dach)X_2 become C_s symmetry after binding with GpG. The NMR spectrum of Pt(*R,R*-dachc)(r(GpA)-*N7*)$_2$ (top of Figure 2) is similar to that of Pt(*R,R*/*S,S*-dach)$(NH_3)_2$; i.e., the two C-H1 and C-H2 protons of the cyclohexane ring show the same chemical shift. Furthermore, a single peak was observed for each G-H8, A-H8, and A-H2 in the purine proton region. These results are to be expected when the GpA molecules exhibit fast rotation about the Pt–N7 bond.

Pr(*R,S*-dach)$(NH_3)_2$. ^{13}C NMR spectrum of the reference compound Pt(*R,S*-dach)$(NH_3)_2$ shows only three signals due to the cyclohexane ring; i.e., δ(C(1)) and δ(C(2)) = 59.12, δ(C(3)) and δ(C(6)) = 27.46, and δ(C(4)) and δ(C(5)) = 21.91 ppm. The peak due to C(1) and C(2) is accompanied by ^{195}Pt satellite peaks, having a $^3J_{Pt-C}$ value of 25 Hz. This is a good evidence for rapid conformational puckering of the chelate ring.[13] That is, two λ and δ conformations of the chelate ring are interconverting, with a simultaneous inversion of the cyclohexane ring.

Table II shows ^{1}H NMR data of the Pt(*R,S*-dach)$^{2+}$ moiety. The protons C-H1 and C-H2 of [Pt(*R,S*-dach)$(NH_3)_2$]$^{2+}$, being assigned to peak a, are equivalent under a rapid interconversion on the NMR time scale between the limiting conformations of λ and δ. In fact, the half-height width of peak a (13 Hz) is almost equal to that expected from time-averaged vicinal constants, $0.5(^3J_{ax-ax} + 4(^3J_{ax-eq}) + {}^3J_{eq-eq})$. The result is in quite good agreement with that obtained from the above ^{13}C NMR data. Four protons C-H3 and C-H6—being assigned to peak b—gave two splitting peaks even under the rapid interconversion because the two protons C-H3 and C-H6 cis to the amino groups (both protons are equivalent) are not equivalent to those trans to the amino groups. This is also the case for the assignment of the four protons at C-H4 and C-H5.

Pt(*R,S*-dach)(dGMP-*N7*)$_2$. For the square-planar complex

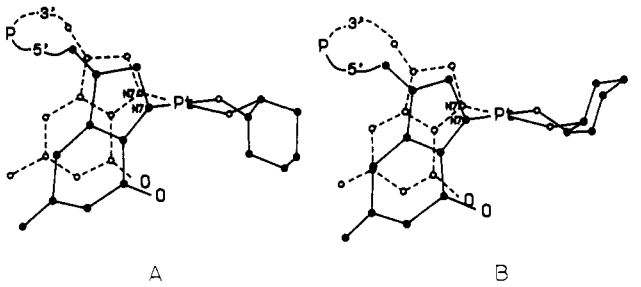

Figure 3. Proposed structures of *RS*-2 and *RS*-1. The sugar–phosphate–sugar structures were omitted.

Pt(*R,S*-dach)(dGMP-*N7*)$_2$, there is no mirror plane because the bound dGMP contains a chiral element (deoxyribose moiety). Therefore, the two G-H8 peaks due to the dGMP are expected to appear at fast rotation about the Pt–purine bond.[12] One is H8 of dGMP at the same side of the *R*-carbon atom, and the other is H8 at the same side of the *S*-carbon atom. Four G-H8 peaks should be expected in the case of the slow rotation. The H NMR spectrum of Pt(*R,S*-dach)(dGMP-*N7*)$_2$ shows two G-H8 peaks with same intensity (at 8.49 and 8.46 ppm at pH = 6), suggesting that the rotation about the Pt–N7 is indeed fast.

Since binding of the chiral ligand dGMP to Pt(*R,S*-dach)$^{2+}$ breaks the mirror symmetry, the proton C-H1 of Pt(*R,S*-dach)$(dGMP)_2$ is no longer equivalent to the proton C-H2. This is likely to lead a greater half-height width of peak a. The agreement of the chemical shifts of the four protons (C-H3 and C-H6), being assigned to peak b, is accidental. From these results, we can draw the conclusion that Pt(*R,S*-dach)$(dGMP)_2$ is a flexible compound, with rapid inversion of the chelate and the cyclohexane rings and fast rotation of dGMP about the Pt–N7 bond. If the inversion of the chelate ring (λ and δ) is slow on the NMR time scale, four G-H8 peaks should be observed even in the case of fast rotation about the Pt–N7 bond.

Pt(*R,S*-dach)(d(GpG)-*N7,N7*), *RS*-1 and *RS*-2 Isomers. Reaction of Pt(*R,S*-dach)$^{2+}$ with d(GpG) results in two isomers with Pt(*R,S*-dach)$^{2+}$ cross-linked between the N7 sites of the two guanines. One has a configuration such that the *R* (*S*) carbon atom on the cyclohexane ring exists on the same side of the 3′G (5′G) base, and the other has a configuration of just the opposite sense. The two isomers have been separated by preparative HPLC and are called *RS*-1 and *RS*-2 according to their HPLC elution order. From the NMR spectra of *RS*-1 and *RS*-2, the binding ratio of Pt(*R,S*-dach)$^{2+}$ to d(GpG) was found to be 1:1; i.e., integration of the H1′ protons (2 H) of d(GpG) was the same as that of the methine protons of the cyclohexane ring. The G-H8 resonances for *RS*-1 and *RS*-2 were observed to shift downfield (0.8 ppm for the 3′G-H8 and less than 0.3 ppm for the 5′G-H8), compared to those for free d(GpG). These values are in good agreement with the literature values for *cis*-Pt$(NH_3)_2$(d(GpG)-*N7,N7*).[10] The pK_a values obtained from NMR–pH titration curves also support that *RS*-1 and *RS*-2 are the Pt(*R,S*-dach)(d(GpG)-*N7,N7*) adducts, respectively (supplementary material Figure S2). NOE difference spectra of *RS*-1 and *RS*-2 were observed after irradiation of the 3′G-H8 proton. NOE difference peaks were observed between 3′G-H8 and 5′G-H8, H2′/H2″, and H3′, indicating that the two guanines are in an anti-anti configuration. From these data, the schematic structures of *RS*-1 and *RS*-2 are shown in Figure 3. However, we cannot say which adduct (*RS*-1 or *RS*-2) corresponds to which structure (A or B in Figure 3). The H NMR data of *RS*-1 and *RS*-2 (Figure 4 and Table II) show that all the half-height widths of

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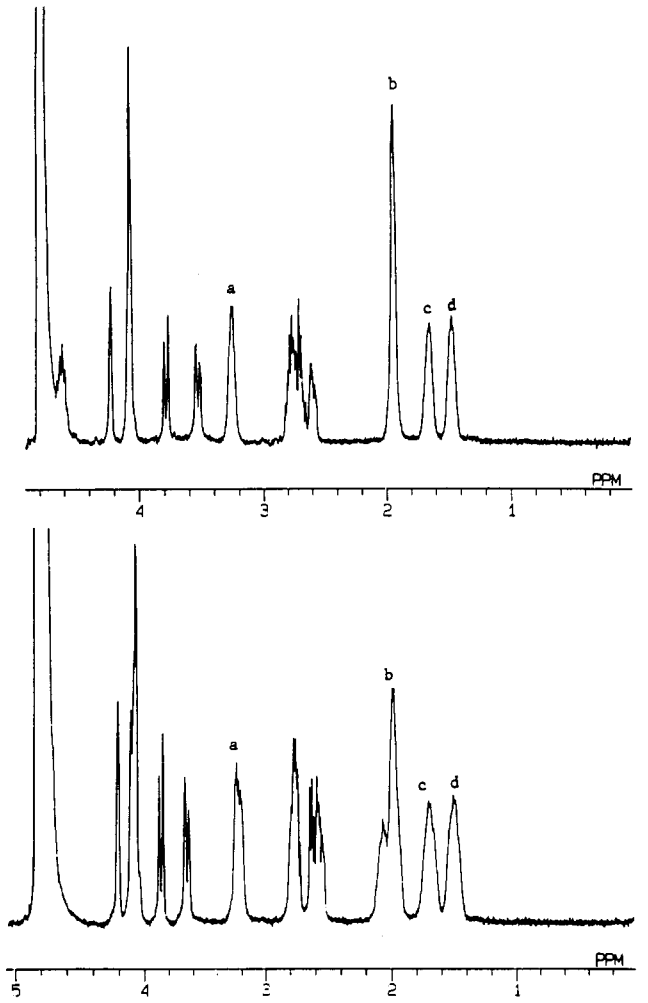

Figure 4. H NMR spectra of *RS*-**1** and *RS*-**2** in the 1–5 ppm region. Cyclohexane protons: a, C-H1 + C-H2; b, C-H3 + C-H6; c and d, C-H4 + C-H5. For the other signals, see Table III.

the cyclohexane protons of *RS*-**2** are larger than those of *RS*-**1**, suggesting that the rate of the interconversion between λ and δ conformations is a relatively slow in the case of *RS*-**2**. It is particularly intriguing that peak b is split into two peaks with the intensity ratio of 1:3 (see Table II). Inspection of a molecular model of structure A shows that one of the four protons (C-H2 and C-H6), i.e., one of the two protons being cis to the amino groups, experiences an anisotropic effect due to the carbonyl groups at C(6) of the guanines. In such a case, the proton under consideration is expected to resonate at lower field than the other three protons. Furthermore, structure A seems to be more sterically crowded compared to structure B. This is likely to slow down the inversion rate between the λ and δ conformations and may lead to an increase of the half-height width of the cyclohexane protons. From these considerations, structure A is most likely to be assigned to *RS*-**2**. On the other hand, structure B seems to have less steric hindrance for the interconversion between the λ and δ conformations of the chelate ring. The NMR spectral data of the cyclohexane moiety of *RS*-**1** are very similar to that of Pt(*R*,*S*-dach)(dGMP)$_2$. In the latter compound, the inversion between the two conformations is rapid on the NMR time scale. The assigments of structures A and B to *RS*-**2** and *RS*-**1**, respectively, seem to be reasonable on the basis of the considerations mentioned above.

The conformation of the sugar ring is generally presented by a rapid pseudorotational equilibrium between the *S*-type (C2′-endo) and the *N*-type (C3′-endo) conformers.[14] For the purpose of the conformational analysis, a resolution enhancement spectrum was simulated with the aid of a LAOCOON-type microcomputer program.[15] Table III shows the coupling constants of the sugar

Table III. H NMR Data for *RS*-**1** and *RS*-**2**

RS-**1**			*RS*-**2**		
	δ, ppm	*J*, Hz		δ, ppm	*J*, Hz
5′G-H8	8.12		5′G-H8	8.14	
5′G-H1′	6.25	$J_{1'-2'} < 0.1$	5′G-H1′	6.21	$J_{1'-2'} < 0.1$
5′G-H2′	2.68	$J_{2'-2''} = -14.0$	5′G-H2′	2.62	$J_{2'-2''} = -14.0$
5′G-H2″	2.74	$J_{1'-2''} = 6.2$	5′G-H2″	2.75	$J_{1'-2''} = 7.6$
5′G-H3′	4.60	$J_{2'-3'} = 7.0$	5′G-H3′	4.69	$J_{2'-3'} = 7.6$
		$J_{2''-3'} = 10.6$			$J_{2''-3'} = 10.5$
		($J_{3'-P} = 7.4$)			($J_{3'-P} = 6.7$)
5′G-H4′	4.08	$J_{3'-4'} = 8.0$	5′G-H4′	4.10	$J_{3'-4'} = 8.0$
5′G-H5′	3.78	$J_{4'-5'} = 2.6$	5′G-H5′	3.86	$J_{4'-5'} = 2.6$
5′G-H5″	3.52	$J_{4'-5''} = 3.3$	5′G-H5″	3.63	$J_{4'-5''} = 3.2$
		$J_{5'-5''} = -12.8$			$J_{5'-5''} = -12.8$
3′G-H8	8.45		3′G-H8	8.54	
3′G-H1′	6.20	$J_{1'-2'} = 6.8$	3′G-H1′	6.23	$J_{1'-2'} = 6.9$
3′G-H2′	2.79	$J_{2'-2''} = -13.8$	3′G-H2′	2.79	$J_{1'-2'} = -13.9$
3′G-H2″	2.59	$J_{1'-2''} = 6.8$	3′G-H2″	2.56	$J_{1'-2''} = 6.4$
3′G-H3′	4.77	$J_{2'-3'} = 6.6$	3′G-H3′	4.75	$J_{2'-3'} = 6.9$
		$J_{2''-3'} = 3.2$			$J_{2''-3'} = 3.6$
3′G-H4′	4.23	$J_{3'-4'} = 3.3$	3′G-H4′	4.21	$J_{3'-4'} = 3.6$
		($J_{4'-P} = 1.5$)			($J_{4'-P} = 1.5$)
3′G-H5′	4.07	$J_{4'-5'} = 2.6$	3′G-H5′	4.06	$J_{4'-5'} = 3.2$
3′G-H5″	4.07	$J_{4'-5''} = 2.6$	3′G-H5″	4.06	$J_{4'-5''} = 5.2$

moieties obtained after the simulation. The H1′ proton of the 5′-sugar of *RS*-**1** and *RS*-**2** shows a sharp doublet. The 5′-sugar of both adducts has a pure *N*-type conformer, being calculated from the $\sum 2''$ ($=J_{1'-2''} + J_{2''-3'} + J_{2'-2''}$) or $\sum 1'$ ($=J_{1'-2'} + J_{1'-2''}$).[14] The H1′ proton of the 3′-sugar of both adducts shows a triplet with an intensity of 1:2:1, indicating about two-thirds *S* conformation. Orientation about the C4′–C5′ bond of the sugar could be calculated from the values of $J_{4'-5'}$ and $J_{4'-5''}$ ($J_{gauche} = 2.0$ Hz and $J_{trans} = 10.7$ Hz were used as the limiting coupling constants).[16] The results indicate 80% gauche-gauche conformation for the 5′-sugar of *RS*-**2**, 55% for the 3′-sugar of *RS*-**2**, 80% for the 5′-sugar of *RS*-**1**, and 87% for the 3′-sugar of *RS*-**1**. The values obtained for *RS*-**1** are in good agreement with the literature values for *cis*-Pt(NH$_3$)$_2$(d(GpG)-*N7*,*N7*) (78% for the 3′-sugar and 81% for the 5′-sugar),[10a] but the conformation of the 3′-sugar of *RS*-**2** differs somewhat from that of *RS*-**1**. These results suggest that conformational difference between *RS*-**1** and *RS*-**2** is attributed to a conformational difference of the phosphodiester bond but not of the five-membered furanose ring.

Discussion

Relative Reactivity of d(GpG) Sequence in DNA. Since platination of DNA is controlled kinetically,[2] the two end products with Pt(*R*,*S*-dach)$^{2+}$, *RS*-**1** and *RS*-**2**, are determined by the binding site of the first platination step, i.e., the monofunctional adduct. Binding of 3′G to the same side of the *R*-carbon atom results in *RS*-**1** as the end product and that to the same side of the *S*-carbon atom should give *RS*-**2**. The ratio of the two end products might be controlled by steric factors of both Pt(*R*,*S*-dach)$^{2+}$ and the incoming ligand d(GpG). The cyclohexane ring of Pt(*R*,*S*-dach)$^{2+}$ is almost perpendicularly oriented with respect to the platinum coordination plane so that an approach of the incoming ligand d(GpG) from the one side might be impeded by the cyclohexane ring. This is likely to slow down the reaction rate. In the reaction between 5′-GMP and Pt(1*R*,3*S*-dach)$^{2+}$, the cyclohexane ring somewhat retarded the approach of 5′-GMP.[12d]

A monofunctional binding of Pt complex to dinucleotides is influenced by the nature of the unplatinated base. The reaction of monofunctional platinum complex Pt(diethylenetriamine)$^{2+}$ (abbreviated as dien), with XpG and GpX (X = A, C, T) gave the adducts for which Pt(dien)$^{2+}$ binds only to the guanine base.[17]

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Competition reactions of $Pt(dien)^{2+}$ with XpG (X = A, C, T) indicated that the reactivity of $Pt(dien)^{2+}$ with the guanine base is influenced by the unplatinated X-bases.[17] The results suggest that the first platination step is controlled by the unplatinated base adjacent to the platinated guanine. In the present work, no difference was observed for the two isomers (*RS*-**1** and *RS*-**2**) produced from the reaction of d(GpG) with $Pt(R,S\text{-dach})^{2+}$, whereas a significant difference was observed between the two isomers produced from the reaction of $Pt(R,S\text{-dach})^{2+}$ with d(ApG).[18] The result of competition reactions between $Pt(R,R\text{-dach})Cl_2$ and $Pt(R,S\text{-dach})Cl_2$ with a common reactant d(GpG) shows that $Pt(R,R\text{-dach})$(d(GpG)-*N7*,*N7*) appears to be a slightly predominant species, compared to $Pt(R,S\text{-dach})$(d(GpG)-*N7*,*N7*) (data not shown). However, no difference was observed between the amounts of *RS*-**1** and *RS*-**2**. These results suggest that the cyclohexane ring of $Pt(R,S\text{-dach})^{2+}$ hardly impedes the approach of d(GpG). This may be attributed to the flexibility of both $Pt(R,S\text{-dach})^{2+}$ and d(GpG).

Since the -GpG- sequence in DNA exists in a restricted environment compared to that of the dinucleotide, d(GpG), a significant difference might be provided for the reaction products between $Pt(R,S\text{-dach})^{2+}$ and the -GpG- sequence in DNA. In fact, the ratio of the two isomers (*RS*-**1** and *RS*-**2**)—which are obtained from the enzymatic digestion[3f,g] of the reaction solution of $Pt(R,S\text{-dach})^{2+}$ with DNA—is not equal (*RS*-**1**:*RS*-**2** = 0.63:0.37).[7] Molecular model inspection suggests that $Pt(R,S\text{-dach})^{2+}$ after the first platination is situated inside of the DNA helix when the N7 site of the 3'G base binds to the same side of the *S*-carbon atom. Then, the 1:1 intermediate forms a chelate with the 5'G base (17-membered chelate ring) to yield *RS*-**2**. When the N7 site of the 3'G base binds to the same side of the *R*-carbon atom, $Pt(R,S\text{-dach})^{2+}$ is oriented to the outside of the DNA helix. On the other hand, when the *S*-side of $Pt(R,S\text{-dach})^{2+}$ binds to the 5'G base of the -GpG- sequence in DNA, *RS*-**1** is expected as the end product. In the reaction of $Pt(R,S\text{-dach})^{2+}$ with -GpG- sequence in B-DNA, if the first platination to the 3'G base is preferred to that to the 5'G base, formation of *RS*-**1** might be superior to that of *RS*-**2**. The cyclohexane ring in the 1:1 intermediate of *RS*-**2** being situated inside of the DNA helix—seems to experience greater steric hindrance through the rotation about the Pt-N7 bond, and this may be a reason why the amounts of *RS*-**2** are less than those of *RS*-**1** in the case of the reaction of DNA. However, it remains unresolved which side of the guanine residue (3'G or 5'G) is preferred for the first platination. Whereas the dinucleotide d(GpG) is a considerably flexible molecule, consequently, the same ratio of both isomers (*RS*-**1** and *RS*-**2**) is likely to be observed in the case of the reaction of $Pt(R,S\text{-dach})^{2+}$ with d(GpG).

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Supplementary Material Available: Figures S1 and S2, showing the pH-NMR titration curves of $Pt(R,R/S,S/R,S\text{-dach})$(d(GpG)-*N7*,*N7*) (2 pages). Ordering information is given on any current masthead page.

(18) The reaction gave two $Pt(R,S\text{-dach})$(d(ApG)-*N7*,*N7*) adducts, as is the case of the reaction between d(GpG) and $Pt(R,S\text{-dach})^{2+}$. The ratio of the two adducts was 65:35, being calculated from the integration of the NMR spectrum.

Contribution from the Departament de Química Inorgànica, Universitat de Barcelona, Diagonal 647, 08028 Barcelona, Spain, Departament de Química Inorgànica, Facultat de Química, Universitat de València, Dr. Moliner 50, 46100 Burjassot, València, Spain, and Laboratoire de Chimie Inorganique, URA 420 CNRS, Université de Paris-Sud, 91405 Orsay, France

Oxalato-Bridged and Related Dinuclear Copper(II) Complexes: Theoretical Analysis of Their Structures and Magnetic Coupling

Santiago Alvarez,*[1a] Miguel Julve,*[1b] and Michel Verdaguer*[1c]

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A theoretical analysis of the structural variations found for dinuclear Cu(II) complexes with oxalato and related polynuclear bridging ligands and their influence on the magnitude of the magnetic exchange interactions is presented in this paper. The family of compounds studied can be represented by the general formula $[(AA)Cu(\mu\text{-}C_2O_4)Cu(AA)]X_n$, where AA can be a chelating ligand like 2,2'-bipyridine (bpy) or tetramethylethylenediamine (tmen), and X is a counteranion or a solvent molecule. Three types of distortions from an ideal square-planar geometry around the copper atoms are considered: (1) the removal of the copper ions from the ligands' plane; (2) a twist of the square planar A_2CuO_2 cores toward a tetrahedral geometry by rotation of the AA ligand; (3) folding of the $A_2Cu\text{-ox-}CuA_2$ skeleton through the O--O hinges and axial coordination of X. An evaluation of second-order Jahn-Teller distortions through the analysis of orbital topologies and atomic electronegativities is presented, which might be helpful in predicting how stable a distorted molecule is relative to the undistorted one, as well as the relative extent for such distortions in a series of related structures.

In the last few years, there has been a great deal of interest in the synthesis and magnetic coupling of transition-metal atoms with polyatomic bridging ligands.[2] A sound theoretical interpretation of the factors contributing to the magnetic coupling in dinuclear complexes is now available thanks to the classical work of Hay, Thibeault, and Hoffmann[3] and later contributions from Kahn and co-workers.[4] However, the wide range of structural variations found for dinuclear Cu(II) complexes with oxalato and similar bridging ligands and their influence on the magnitude of the magnetic interactions have not been considered in detail previously, and we feel a theoretical analysis of such features is in order.

A number of dinuclear copper complexes with oxalato bridge, which can be represented by the general formula $[(AA)Cu(\mu\text{-}C_2O_4)Cu(AA)X_{\cdot}n$, have been structurally characterized in the last few years.[5-8] In these complexes, AA is a chelating ligand,

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