

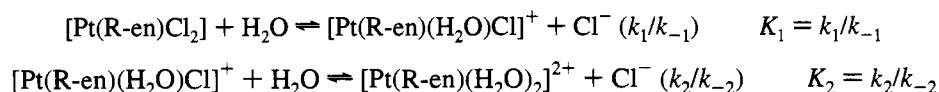
Mechanism of Aquation of Bicycloalkyl-Substituted (Ethylenediamine)dichloroplatinum(II) Complexes: A Search for the Understanding of Their Differences in Antitumor Activity

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This paper reports a mechanistic investigation of the aquation and anation reactions of substituted ethylenediamine platinum(II) complexes, which were shown to have very different *in vivo* antitumor activities. The general formula of the complexes is *rac*-[Pt(R-en)XY]ⁿ⁺ (X, Y = H₂O, Cl⁻; n = 0, 1, 2), where the substituted ethylenediamine (R-en) is (1*R*,2*R*,4*S*)-*exo*-2-(aminomethyl)-2-amino-7-oxabicyclo[2.2.1]heptane (R₁-en), (1*R*,2*S*,4*S*)-*endo*-2-(aminomethyl)-2-amino-7-oxabicyclo[2.2.1]heptane (R₂-en), or (1*S*,2*S*,4*S*)-*exo*-2-(aminomethyl)-2-amino-7-bicyclo[2.2.1]heptane (R₃-en). The two ligands X and Y are shown to be kinetically equivalent for X = Y within the accuracy of ¹⁵N, ¹⁹⁵Pt, ¹⁷O NMR, although the diamine is asymmetric. Using UV-vis spectrophotometry, it was possible to determine the rate constants (*k*₁, *k*₋₁, *k*₂, *k*₋₂) for the following two successive aquation/anation reactions:



The first aquation/anation rate constants for the complex *rac*-[Pt(R₃-en)Cl₂] have been found to be independent of pH in the range 2.2–5.8 and identical to those relative to R₁-en and R₂-en at pH = 2.2 within experimental error. The first and second aquation/anation rate constants obtained for *rac*-[Pt(R₁-en)Cl₂] can be compared with those of the literature for [Pt(en)Cl₂] (in parentheses): 10⁵*k*₁²⁹⁸/s⁻¹ = 3.2 ± 0.2 (3.4 ± 0.4); 10²*k*₋₁²⁹⁸/M⁻¹ s⁻¹ = 4.4 ± 0.2 (1.54 ± 0.03); 10⁵*k*₂²⁹⁸/s⁻¹ = 7.8 ± 1.0 (4.4 ± 0.6); *k*₋₂²⁹⁸/M⁻¹ s⁻¹ = 0.67 ± 0.01 (0.31 ± 0.04). The kinetic studies as a function of temperature for *rac*-[Pt(R₁-en)Cl₂] gave the activation and thermodynamic enthalpies and entropies. The high-pressure studies allowed the determination of the complete volume profile (values given in cm³ mol⁻¹): Δ*V*[‡]₁ = -9.4 ± 0.7; Δ*V*[‡]₋₁ = -4.0 ± 0.4; Δ*V*^o₁ = -5.3 ± 1.1; Δ*V*[‡]₂ = -6.6 ± 1.7; Δ*V*[‡]₋₂ = -4.4 ± 0.5; Δ*V*^o₂ = -2.2 ± 2.0. The volume profile obtained for the aquation of *rac*-[Pt(R₁-en)Cl₂] clearly precludes any dissociative mechanism that could have been correlated to a nucleophilic assistance of the 2-substituted norbornyl group. A common mechanism of aquation for [Pt(R-en)Cl₂] complexes involving a pentacoordinate intermediate is proposed. Thus, the rate constants for the aquation of [Pt(R-en)Cl₂] complexes, which is an essential step in the reaction with important biological nucleophiles like DNA cannot be used to establish a structure-activity relationship.

Introduction

cis-Diamminedichloroplatinum(II) (*cis*-DDP or cisplatin) is one of the most commonly used anticancer drugs.^{3,4} However there is a need for analogues that would induce less side effects, would be active on a larger range of tumors, and would not develop cross-resistance with *cis*-DDP.⁵ There are many arguments in favor of DNA being the target of *cis*-DDP at the origin of its antitumor activity.⁶ Therefore, in cooperation with Rhône-Poulenc-Rorer, we studied a new series of complexes, designed to give long-lived monoadducts on DNA,⁷ and tested their antitumor activity. Most of these compounds were found

to be very active on several tumors⁸ and deserved an investigation of their reaction with DNA. The binding of *cis*-DDP and *trans*-DDP to DNA is kinetically controlled, and the rate-limiting step for this reaction is the first aquation of the diamminedichloroplatinum(II) complex.^{9,10} Therefore we have investigated the aquation kinetics of the complexes shown in Chart 1.

According to the nature of the bicycloalkyl substituent on the ethylenediamine ligand, our complexes might undergo two types of chloride substitution. It could occur either by a classical associative mechanism that is the rule for square-planar platinum(II) complexes¹¹ or by a dissociative mechanism due to a

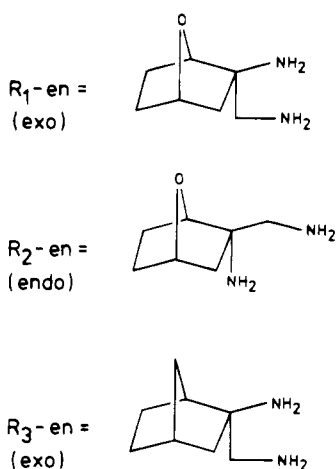
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Chart 1

rac-[Pt(R-en)Cl₂], with:



participation of the norbornyl or oxanorbornyl group similar to those clearly established for solvolytic reactions of 2-substituted norbornyl derivatives.¹² This hypothesis is schematically presented in Figure 1. There are precedents in the literature to

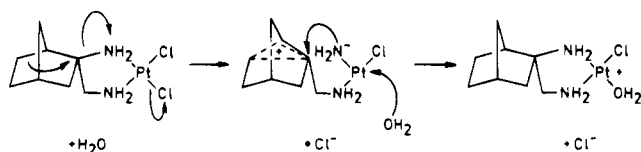


Figure 1. Schematic representation of a putative dissociative mechanism for the aquation of [Pt(R₃-en)Cl₂].

support the hypothesis of a formal amido ligand¹³ and of tricoordinated platinum(II) intermediates involved in nucleophilic substitution reactions.^{14,15} According to such a pathway, higher rates would be expected for the platinum complex of R₃-en compared to those of R₁-en and R₂-en.^{16,17} In order to determine unambiguously the mechanism of aquation of the three complexes, kinetic studies using UV spectrophotometry (for a potentiometric study, see the supplementary material, Appendix S1) have been made as a function of the concentration of the reactants, of the pH, of the temperature, and particularly of the pressure. The latter variable allows the determination of the activation volume of the reaction, an activation parameter which unambiguously allows to one draw a conclusion on the associative or dissociative nature of the pathway.¹⁸

Experimental Section

Chemicals and Solutions. Ethylenediaminedichloroplatinum(II) complexes [Pt(R-en)Cl₂] were synthesized by Rhône-Poulenc-Rorer.⁸ Solutions for the kinetic study of the first aquation step were obtained by dissolving [Pt(R-en)Cl₂] in HClO₄-acidified water and by adjusting, when needed, the chloride concentration with NaCl. Two [Pt(R-en)-

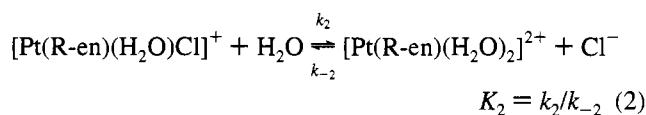
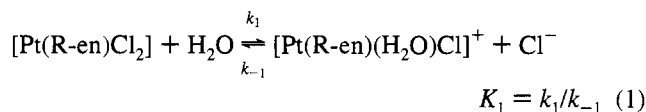
(H₂O)₂]²⁺ stock solutions (0.10 M for kinetic studies and 0.16 M for NMR measurements) were prepared: 2 equiv of AgClO₄ (Fluka) were added to [Pt(R-en)Cl₂] in 1 M HClO₄; the mixture was then stirred for 3 days at 318 K and finally filtered. The Pt concentration and the absence of residual Ag were checked by ICP (Perkin-Elmer 1000). Solutions for the study of the second aquation step were prepared by adding appropriate amounts of Cl⁻ and HClO₄ to these two solutions. Doubly distilled water was used in all cases. For the water exchange study, 7.5% ¹⁷O-enriched [Pt(R-en)(H₂O)₂]²⁺ was prepared by three successive exchanges and evaporations using 10% enriched water (Yeda). Concentrations were expressed in molarities at 0.1 MPa.¹⁹

NMR Measurements. NMR experiments were done on Bruker AM-400 (¹⁵N, ¹⁷O) and AC-200 (¹⁹⁵Pt) spectrometers. ¹⁵N{¹H} spectra (40.55 MHz) were obtained with 90° pulses of 19 μs over a total spectral width of 10 kHz, 8K data points, relaxation delay of 2s, and 80000–120000 scans. The spectra were referenced to NH₄⁺ (external) of a solution of 5 M NH₄NO₃ in 2 M HNO₃. ¹⁷O NMR spectra (54.2 MHz) were obtained with 90° pulses of 14 μs, over a total spectral width of 33 kHz, 2K data points, and an accumulation of 200–3000 scans. The spectra were referenced to the H₂O signal. A previously described fast-injection apparatus was used for the determination of the water exchange rate constants.²⁰ ¹⁹⁵Pt NMR spectra (42.9 MHz) were recorded with pulses of 15 μs, spectral widths of 83 kHz, 16K data points, and an accumulation of 1500 scans and were referenced to 1 M K₂PtCl₄ (external).

Kinetic Measurements. Absorption spectra were recorded by use of a Perkin-Elmer Lambda 19 spectrophotometer. The experimental set-ups for variable temperature and pressure kinetic experiments were as described previously.^{21,22} The errors quoted throughout the text and tables are ±1 standard deviation.

Results

NMR Study. The two steps aquation reaction of [Pt(R-en)-Cl₂] complexes, described by eqs 1 and 2, have been studied in detail for the derivative of R₁-en. The species present in solution



have been identified from their natural abundance ¹⁵N{¹H} spectra. A solution of [Pt(R₁-en)(H₂O)₂]²⁺ (0.16 M) in acidic medium (HClO₄ 1 M) shows two resonances: N₁ bound to the quaternary carbon at -29.8 ppm and N₂ bound to the methylene carbon at -55.7 ppm (reference NH₄⁺: 0 ppm). Six resonances were observed in a solution obtained by reacting [Pt(R₁-en)(H₂O)₂]²⁺ with an equimolar concentration of Cl⁻ in acidic medium (HClO₄ 1 M) (Figure 2). Two of them are assigned to the non-equivalent nitrogens of the remaining bis-aqua complex. The four other resonances at -11.3, -33.0, -37.5, and -58.6 ppm (intensities ca. 1:1:1:1) can be assigned to the two diastereoisomers *rac*-(A) and *rac*-(B) of [Pt(R₁-en)(H₂O)-Cl]⁺ (see Chart 2). For the assignment of the resonances the *cis* and *trans* influences of the chloro and aqua ligands were taken into account. Appleton et al.²³ and more recently Berners-

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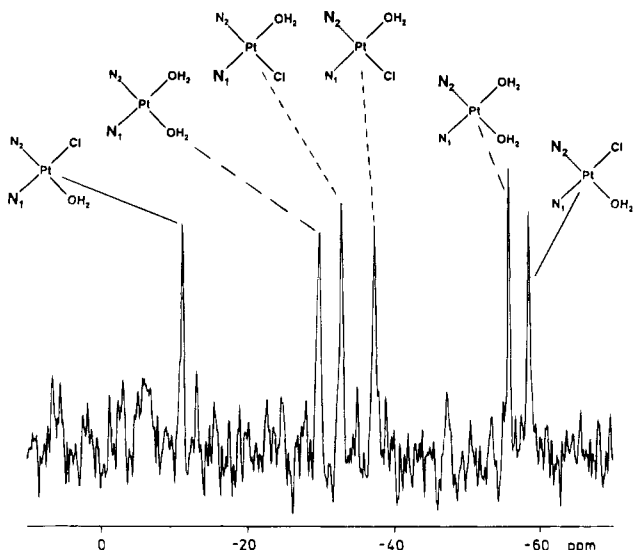
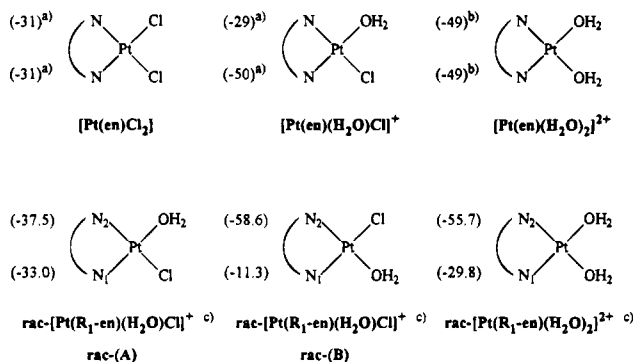


Figure 2. 40.55-MHz $^{15}\text{N}\{^1\text{H}\}$ spectrum of a 0.16 M $[\text{Pt}(\text{R}_1\text{-en})(\text{H}_2\text{O})_2]\text{-(ClO}_4)_2$ acid solution (HClO_4 1 M) after addition of an equimolar concentration of Cl^- and under equilibrium conditions (see text for the assignments).

Chart 2



^a See refs 18, 19. ^b Calculated with the additivity rule. ^c N^1 bound to the quaternary carbon; N^2 bound to the methylene carbon.

Price et al.²⁴ have determined these influences on series of $[\text{Pt}(\text{NH}_3)_2\text{XY}]$ compounds. The *trans* influence of H_2O compared to Cl^- was found to be -18.8^{23} and -21.2^{24} ppm. The smaller *cis* influence was found to be $+2.2^{23}$ and $+2.0^{24}$ ppm. For *rac*-(A) and *rac*-(B) we observe a *trans* influence of H_2O compared to Cl^- of -18.6 ppm and a corresponding *cis* influence of $+2.8$ ppm. Using these relationships, we can calculate the ^{15}N chemical shifts of $[\text{Pt}(\text{R}_1\text{-en})\text{Cl}_2]$, which could not be measured due to the low solubility of this neutral species: $\text{N}_1 = -14.1$ ppm and $\text{N}_2 = -40.4$ ppm. The ^{195}Pt spectrum (reference PtCl_4^{2-} : 0 ppm) of the same solution shows only a single peak at -447 ppm for both *rac*-(A) and *rac*-(B) isomers, apart from those at -227 ppm and -722 ppm for the diaqua and dichloro species, respectively (literature²⁵ for $\text{Pt}(\text{en})\text{Cl}_2$: -506 , -284 , -711 ppm, respectively, assuming $\delta(\text{PtCl}_6^{2-}) = +1630$ ppm). The line width at half-height of the three signals is approximately 500 Hz due to $\text{Pt-}^{14}\text{N}$ quadrupolar nuclei coupling²⁶ which is too large to distinguish the two isomers. The ^{195}Pt spectrum was obtained on a freshly prepared solution, oversaturated in the dichlorospecies; this species precipitated after

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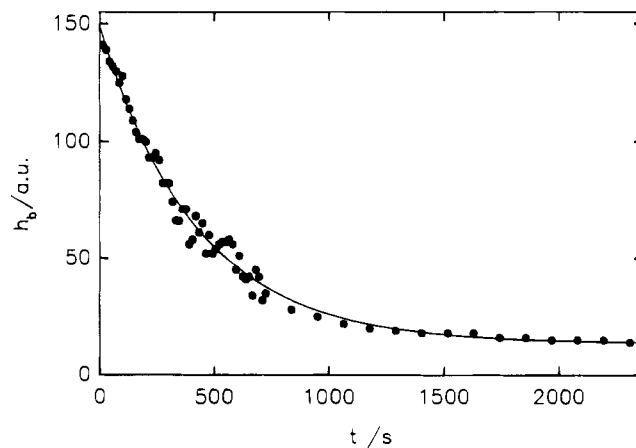
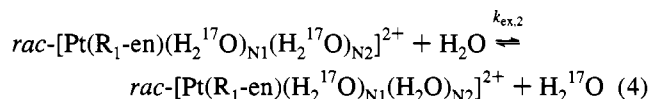
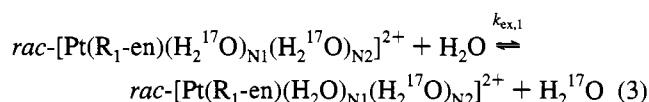


Figure 3. Kinetics of water exchange at 0.03 M $[\text{Pt}(\text{R}_1\text{-en})(\text{H}_2^{17}\text{O})_2]\text{-(ClO}_4)_2$ in 0.2 M HClO_4 , followed by the decrease in height h_b (arbitrary unit) of the ^{17}O -enriched (7.5%) NMR signal of coordinated water at 279.4 K and ambient pressure.

a while and could therefore not be detected in the natural abundance ^{15}N spectrum.

The equilibria in eqs 1 and 2, which will serve as model for our kinetic study, do assume that the lability of the two chloro and of the two aqua ligands in $[\text{Pt}(\text{R}_1\text{-en})\text{Cl}_2]$ and in $[\text{Pt}(\text{R}_1\text{-en})(\text{H}_2\text{O})_2]^{2+}$, respectively, are similar, and further that the concentrations of the diastereoisomers *rac*-(A) and *rac*-(B) are similar. This last point has been verified, within the accuracy of the $^{15}\text{N}\{^1\text{H}\}$ NMR integrations (see Figure 2). ^{17}O NMR is informative concerning the lability of the aqua ligands in $[\text{Pt}(\text{R}_1\text{-en})(\text{H}_2\text{O})_2]^{2+}$ through the study of the exchange reactions in (3) and (4), where the indices denote the water ligands *trans*



to the N_1 and N_2 nitrogens, respectively. The spectrum of 7.5% ^{17}O enriched $[\text{Pt}(\text{R}_1\text{-en})(\text{H}_2\text{O})_2]^{2+}$ shows only one signal at -87.3 ppm for bound water besides that of free water (0 ppm). The decrease of height h_b of this signal as a function of time, due to exchange with free water is shown in Figure 3. These data were first analyzed in terms of a single exponential with an exchange rate constant k_{ex} according to eq 5 ($x_\infty = \text{mole}$

$$h_b = h_{b,\infty} + (h_{b,0} - h_{b,\infty}) \exp[-k_{\text{ex}}t/(1 - x_\infty)] \quad (5)$$

fraction of coordinated water).²⁷ The fitting procedure gives an exchange rate constant of $(2.37 \pm 0.08) \times 10^{-3} \text{ s}^{-1}$ at 279.4 K. The data were then analyzed in terms of two parallel exchange pathways with two different rate constants. The more the $k_{\text{ex},1}/k_{\text{ex},2}$ ratio differs from unity, the worse is the quality of the fit. From a comparison of the fits versus this ratio it can be concluded that $k_{\text{ex},2}/k_{\text{ex},1}$ is smaller than 2 within experimental error.

Kinetic Analysis. The quantitative kinetic study of the two successive aquation/anation reactions (eqs 1 and 2) was performed by UV–vis absorption spectroscopy. The dichloro complexes $[\text{Pt}(\text{R-en})\text{Cl}_2]$ show similar maximum and minimum of absorption as observed for *cis*- $[\text{PtCl}_2(\text{NH}_3)_2]$ (maximum, 305

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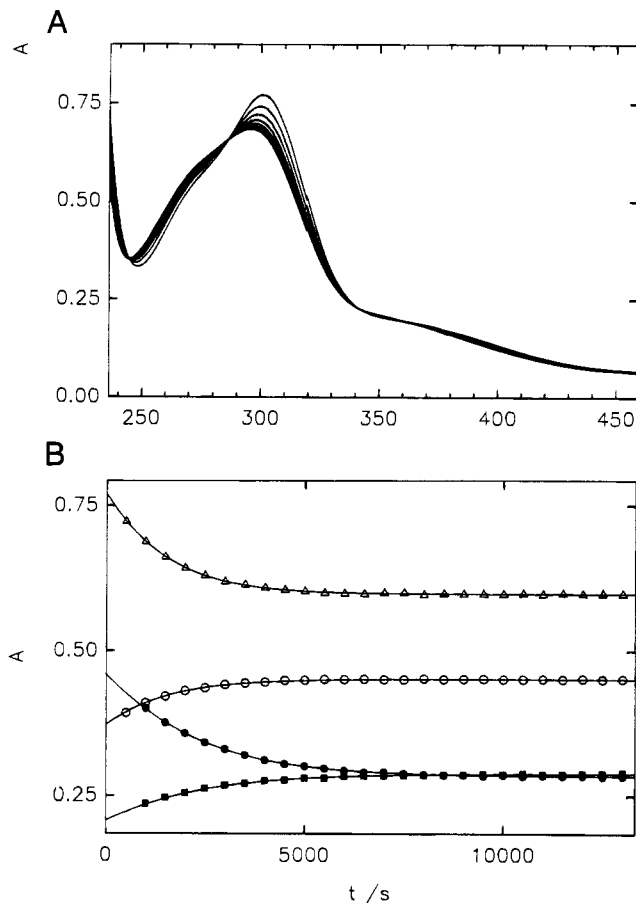


Figure 4. UV-vis kinetics for the first aquation step of $[\text{Pt}(\text{R}_1\text{-en})\text{Cl}_2]$ at $\text{pH} = 2.2$, $T = 311.3$ K, and $P = 101$ MPa with (experiment 1) and without added Cl^- (experiment 2). Initial conditions: (experiment 1) $[\text{complex}]_0 = 1.64 \times 10^{-3}$ M, $[\text{Cl}^-] = 1.21 \times 10^{-3}$ M; (exp 2) $[\text{complex}]_0 = 1.10 \times 10^{-3}$ M. Key: (A) spectra for experiment 1 with time intervals of 500s; (B) measured and calculated absorbance changes as a function of time at 257.0 nm (experiment 1, \circ ; experiment 2, \blacksquare) and at 307.7 nm (experiment 1, \triangle , experiment 2, \bullet).

nm; minimum, 246 nm);²⁸ $\text{R}_1\text{-en}$ (301.9, 249.0) (see Figure 4A), $\text{R}_2\text{-en}$ (301.9, 249.0), $\text{R}_3\text{-en}$ (300.5, 255.0). The same similitude is observed for the diaqua ions: *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})_2]^{2+}$ (255, 231),²⁹ $[\text{Pt}(\text{R}_1\text{-en})(\text{H}_2\text{O})_2]^{2+}$ (260.0, 234.5). The molar absorptions of the R-en complexes are very similar to those reported for the ethylenediamine complexes.³⁰

The first aquation/anation step (eq 1) was studied starting from the $[\text{Pt}(\text{R-en})\text{Cl}_2]$ complex. To better define the contributions of k_1 and k_{-1} to the measured absorbance changes, the kinetics was always performed twice: without and with added Cl^- . Further the observation was done at two wavelengths corresponding to maximum increase (257.0 nm) and decrease (307.7 nm) of the absorptions (Figure 4A). The measured absorbances were fitted to the well known integrated kinetic equations for first- and second order reversible reactions^{31,32} (see supplementary material: eq S2, UV-vis kinetic equations). During the fitting procedure six parameters (k_1 , k_{-1} , and absorbances of the dichloro and chloroaqua complexes at the two wavelengths) were used to adjust the calculated curves to

Table 1. Rate Constants for Aquation/Anation (k_1 , k_{-1}) of $[\text{Pt}(\text{R}_3\text{-en})\text{Cl}_2]$ (1.0×10^{-3} M) at 311.3 K, as a Function of pH (Medium: $\text{H}_2\text{O}/\text{DMF} = 90/10$).

pH	$10^4 k_1/\text{s}^{-1}$	$10k_{-1}/\text{M}^{-1} \text{s}^{-1}$
2.2	1.55 (0.02)	1.43 (0.05)
4.9	1.67 (0.05)	1.17 (0.08)
5.8	1.69 (0.03)	1.28 (0.09)

Table 2. Rate Constants for Aquation/Anation (k_1, k_{-1}) of $[\text{Pt}(\text{R-en})\text{Cl}_2]$ (1.0×10^{-3} M) in Acidic Medium ($\text{pH} = 2.2$) at 311.3 K ($\mu = 0.01$ M)

diamine	$10^4 k_1/\text{s}^{-1}$	$10k_{-1}/\text{M}^{-1} \text{s}^{-1}$
$\text{R}_1\text{-en}$	1.37 (0.01)	1.71 (0.02)
$\text{R}_2\text{-en}$	1.84 (0.01)	1.48 (0.01)
$\text{R}_2\text{-en}^a$	1.51 (0.04)	1.61 (0.02)
$\text{R}_3\text{-en}^a$	1.55 (0.02)	1.43 (0.05)

^a In $\text{H}_2\text{O}/\text{DMF} = 90/10$.

the four data sets of absorbencies versus time (Figure 4B). The reactions were followed for 3–4 half-lives as long as the isobestic points were maintained (no formation of the diaqua complex). The second aquation/anation step (eq 2) was studied in the reverse manner starting from the $[\text{Pt}(\text{R-en})(\text{H}_2\text{O})_2]^{2+}$ complex. Similarly two different concentrations of Cl^- were used and the observations made at two wavelengths (240.0 and 291.0 nm), and six parameters (k_2 , k_{-2} , and four absorbances) were used to adjust the calculated curves to the four data sets.

pH of the Kinetic Study. Near neutral pH, aqua ligands bound to platinum deprotonate to form hydroxo species.³³ The pK_a of the monacid $[\text{Pt}(\text{en})(\text{H}_2\text{O})\text{Cl}]^+$ (7.4) and of the diacid $[\text{Pt}(\text{en})(\text{H}_2\text{O})_2]^{2+}$ (5.8 and 7.6) are reported in parentheses.³⁴ Moreover the hydroxo species produced can dimerize at the concentrations used in the kinetic study or even oligomerize further.^{35,36}

During the kinetic study (pH fixed at 2.2) of the first aquation/anation step (eq 1), it was not necessary to take into consideration the formation of $[\text{Pt}(\text{R}_3\text{-en})(\text{HO})\text{Cl}]$ to account for the absorbance changes. In the pH range 2.2–5.8 the rate constant for the aquation k_1 and for the reverse anation k_{-1} , of $[\text{Pt}(\text{R}_3\text{-en})\text{Cl}_2]$ were found independent of pH (Table 1). Coley et al. made a similar observation for $[\text{Pt}(\text{en})\text{Cl}_2]$.³⁰

For the study of the second aquation/anation step (eq 2) the stock solution of $[\text{Pt}(\text{R-en})(\text{H}_2\text{O})_2]^{2+}$ (0.1 M) was prepared in 1 M HClO_4 to avoid the formation of the dinuclear species.³⁶ It was verified that the solution used for the kinetic study (pH = 1.4–1.6), obtained by dilution of the stock solution, did not contain dimers.

Kinetic Effect of the Diamine Ligand. The aquation (k_1) and anation (k_{-1}) rate constants of $[\text{Pt}(\text{R-en})\text{Cl}_2]$ are independent of the nature of the diamine $\text{R}_1\text{-en}$, $\text{R}_2\text{-en}$, or $\text{R}_3\text{-en}$ (Table 2). The aquation of $\text{Pt}(\text{R}_3\text{-en})\text{Cl}_2$ has been studied in $\text{H}_2\text{O}/\text{DMF}$ (90/10) due to the insolubility of this complex in water. As shown in Table 2, for $[\text{Pt}(\text{R}_2\text{-en})\text{Cl}_2]$, the aquation rate does not depend on whether water or the mixed solvent $\text{H}_2\text{O}/\text{DMF}$ (90/10) is used.

Determination of Thermodynamic and Activation Parameters. The similarity of the kinetic behaviour of the three $[\text{Pt}(\text{R-en})\text{Cl}_2]$ complexes allowed to restrict a full variable temperature and pressure study to the complex with the $\text{R}_1\text{-en}$ ligand. The

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Table 3. Rate Constants for the First (k_1 , k_{-1})^a and the Second (k_2 , k_{-2})^b Aquation/Anation Steps of [Pt(R₁-en)Cl₂] in Acidic Medium as Function of Temperature

T/K	10 ⁵ k ₁ /s ⁻¹	10 ² k ₋₁ /M ⁻¹ s ⁻¹	10 ⁴ K ₁ (=k ₁ /k ₋₁)/M
293.3	1.81 (0.01)	2.69 (0.03)	6.73
302.3	4.99 (0.05)	6.42 (0.09)	7.77
311.3	13.7 (0.1)	17.1 (0.2)	8.01
320.3	38.8 (0.5)	38.1 (0.7)	10.18

T/K	10 ⁶ k ₂ /s ⁻¹	10k ₋₂ /M ⁻¹ s ⁻¹	10 ⁵ K ₂ (=k ₂ /k ₋₂)/M
279.5	6.9 (1.0)	0.84 (0.03)	8.21
291.4	30 (4)	3.29 (0.09)	9.12
295.0	46 (1)	4.93 (0.21)	9.33
303.3	170 (17)	11.1 (0.5)	15.32

^a pH = 2.2; μ = 0.01 M. ^b pH = 1.4; μ = 0.04 M.

Table 4. Rate Constants, Activation Parameters, and Thermodynamic Parameters for the First and the Second Aquation/Anation Steps of [Pt(R₁-en)Cl₂] (This Work) and [Pt(en)Cl₂]^{a,b} in Acidic Water

	R ₁ -en	en ^{a,b}
k ₁ ²⁹⁸ /s ⁻¹	(3.2 ± 0.2) × 10 ⁻⁵	(3.4 ± 0.4) × 10 ^{-5 c}
k ₋₁ ²⁹⁸ /M ⁻¹ s ⁻¹	(4.4 ± 0.2) × 10 ⁻²	(1.54 ± 0.03) × 10 ⁻²
K ₁ ²⁹⁸ /M	(7.2 ± 0.3) × 10 ⁻⁴	(22 ± 1) × 10 ^{-4 c}
ΔH ₁ [‡] /kJ mol ⁻¹	86.0 ± 2.8	85 ± 10 ^c
ΔH ₋₁ [‡] /kJ mol ⁻¹	75.0 ± 2.6	73 ± 3
ΔH ₀ [‡] /kJ mol ⁻¹	+11.0 ± 2.5	+17 ± 4
ΔS ₁ [‡] /J K ⁻¹ mol ⁻¹	-42.8 ± 9	-42 ± 33 ^c
ΔS ₋₁ [‡] /J K ⁻¹ mol ⁻¹	-19.5 ± 9	-33 ± 8
ΔS ₀ [‡] /J K ⁻¹ mol ⁻¹	-23.3 ± 8	
ΔV ₁ [‡] /cm ³ mol ⁻¹	-9.4 ± 0.7	-9.2 ± 1.0
ΔV ₋₁ [‡] /cm ³ mol ⁻¹	-4.0 ± 0.4	
ΔV ₀ [‡] /cm ³ mol ⁻¹	-5.3 ± 1.1	

	R ₁ -en	en ^a
k ₂ ²⁹⁸ /s ⁻¹	(7.8 ± 1.1) × 10 ⁻⁵	(4.4 ± 0.6) × 10 ⁻⁵
k ₋₂ ²⁹⁸ /M ⁻¹ s ⁻¹	0.67 ± 0.01	0.31 ± 0.04
K ₂ ²⁹⁸ /M	(1.2 ± 0.1) × 10 ⁻⁴	(1.4 ± 0.1) × 10 ⁻⁴
ΔH ₂ [‡] /kJ mol ⁻¹	91.1 ± 9	34 ± 12
ΔH ₋₂ [‡] /kJ mol ⁻¹	74.3 ± 1.4	37 ± 12
ΔH ₀ [‡] /kJ mol ⁻¹	+16.8 ± 7	-4 ± 8
ΔS ₂ [‡] /J K ⁻¹ mol ⁻¹	-18.1 ± 31	-210 ± 42
ΔS ₋₂ [‡] /J K ⁻¹ mol ⁻¹	+0.9 ± 5	-130 ± 42
ΔS ₀ [‡] /J K ⁻¹ mol ⁻¹	-19.1 ± 26	
ΔV ₂ [‡] /cm ³ mol ⁻¹	-6.6 ± 1.7	
ΔV ₋₂ [‡] /cm ³ mol ⁻¹	-4.4 ± 0.5	
ΔV ₀ [‡] /cm ³ mol ⁻¹	-2.2 ± 2.0	

^a Reference 30. ^b Reference 37. ^c See also ref 38; k₁²⁹⁸/s⁻¹ = 3.20 × 10⁻⁵, K₁²⁹⁸/M = 1.5 × 10⁻³, ΔH₁[‡]/kJ mol⁻¹ = 97.1 ± 3, ΔS₁[‡]/J K⁻¹ mol⁻¹ = -5 ± 6.

temperature dependence of the rate constants for the first (293–320 K) and the second (280–303 K) aquation/anation steps were determined at four temperatures for both reactions (Table 3). The forward and reverse rate constants were independently least-squares analysed with ΔH[‡] and ΔS[‡] (or k²⁹⁸) as adjustable parameters in the Eyring equation. A similar procedure was applied for the thermodynamic parameters by using the kinetically obtained equilibrium constant K_i = k_i/k_{-i}, for i = 1, 2 (Table 4). The pressure dependence of the rate constants for the first (311.3K) and the second (279.0K) aquation/anation steps were determined up to 200 MPa for both reactions (Figure 5). The forward and reverse rate constants were independently least-squares analyzed by the linear eq 6, which assumes a pressure-independent volume of activation.¹⁸ The kinetically obtained K values, were analyzed by using eq 7.

$$\ln k = \ln k_0 - P\Delta V^\ddagger/RT \quad (6)$$

$$\ln K = \ln K_0 - P\Delta V^0/RT \quad (7)$$

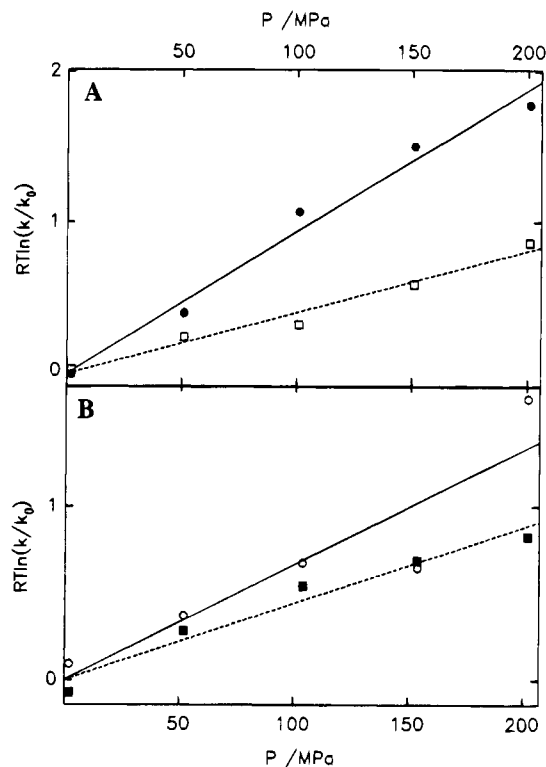


Figure 5. Pressure effect on normalized logarithm of the rate constants. (A) aquation k_1 (●) and anation k_{-1} (□) of [Pt(R₁-en)Cl₂] at pH = 2.2 and 311.3 K. Two experiments at each pressure, with 3 × 10⁻³ M Cl⁻ and without added Cl⁻. [complex]₀ = 1 × 10⁻³ M. (B) aquation k_2 (○) and anation k_{-2} (■) of [Pt(R₁-en)(H₂O)Cl]⁺ at pH = 1.6 and 279.0 K. At each pressure experiments were run with two Cl⁻ concentrations: 1 and 4 × 10⁻³ M. [complex]₀ = 3 × 10⁻³ M.

Discussion

This work was undertaken to establish a possible dissociative mechanism for the first aquation of [Pt(R₁-en)Cl₂], due to a possible specific effect of the norbornyl or oxanorbornyl group of the R-en ligand on the aquation rate constants and on the mechanism. The two chlorides are potentially not equivalent due to the asymmetry of the bicyclo substituted ethylenediamine ligand. However it could be shown by ¹⁵N, ¹⁹⁵Pt, and ¹⁷O NMR that the two chlorines are kinetically equivalent, as well as the two water oxygen in the analogue bis(aqua) complex. The quantitative kinetic study, performed by UV-vis spectrophotometry, could therefore be simplified by using eqs 1 and 2 as model for the data analysis. The obtained rate constants of the first aquation for the three diamine ligands studied are very close (Table 2) and the one for (R₁-en) equals that for ethylenediamine (en) within experimental errors (Table 4). This allows one to suggest the same mechanism for the first aquation step of these four Pt(II) diamine complexes. The temperature related activation parameters for the first aquation/anation step are also practically identical for [Pt(R₁-en)Cl₂] and [Pt(en)Cl₂]: small activation enthalpies and negative activation volumes, which are expected for the classical associative mechanism for square planar substitution. For the second aquation/anation step the rate and equilibrium constants are also close for both complexes, however the large differences in the ΔH[‡] and ΔS[‡] values, probably linked to the limitation of the experimental techniques, preclude any more detailed discussion of these two last activation parameters. The pressure-related activation parameters are much more accurately defined, and in the case of [Pt-(R₁-en)Cl₂] it has been possible to build up the complete reaction volume profile (Figure 6), which is nowadays recognized as a powerful mechanistic tool.³⁹ This profile shows two charac-

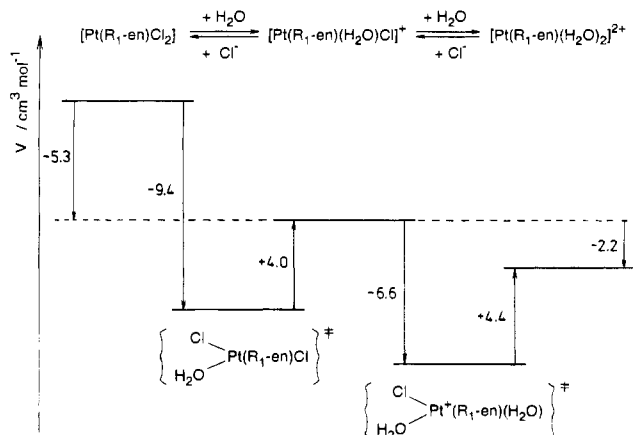


Figure 6. Volume profile for the two-step reversible aquation of $[\text{Pt}(\text{R}_1\text{-en})\text{Cl}_2]$ (step 1, 311.3 K; step 2, 279.0 K).

teristic minima for the transition states of the two successive aquation/anation steps. They are clear evidence for the existence of pentacoordinated transition states or intermediates for the classical I_a or A mechanisms of substitution on square planar Pt(II) complexes. The only possible close comparison is with the activation volumes of the first aquation step ΔV_1^\ddagger for $[\text{Pt}(\text{en})\text{Cl}_2]$ ($-9.2 \pm 1.0 \text{ cm}^3 \text{ mol}^{-1}$)³⁷ and for $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ ($-9.5 \pm 1.2 \text{ cm}^3 \text{ mol}^{-1}$)³⁷ which are equal to that for $[\text{Pt}(\text{R}_1\text{-en})\text{Cl}_2]$ within experimental error. For the second aquation step a ΔV_2^\ddagger value of $-10.5 \pm 1.0 \text{ cm}^3 \text{ mol}^{-1}$ has been obtained for the aquation of $[\text{Pt}(\text{dien})\text{Cl}]\text{Cl}$ ⁴⁰ and can be compared with our ΔV_2^\ddagger value of $-6.6 \pm 0.6 \text{ cm}^3 \text{ mol}^{-1}$. For $[\text{Pd}(\text{Me}_3\text{dien})\text{Cl}]\text{Cl}$ the whole volume profile for the aquation

has been obtained, and a comparison is also possible with the values obtained by Pienaar et al.⁴¹ given in parentheses: $\Delta V_2^\ddagger = -6.6 \pm 1.7$ (-11.6 ± 0.2) $\text{cm}^3 \text{ mol}^{-1}$, $\Delta V_{-2}^\ddagger = -4.4 \pm 0.5$ (-4.9 ± 0.4) $\text{cm}^3 \text{ mol}^{-1}$ and $\Delta V_{-2}^\circ = -2.2 \pm 2.0$ (-6.7 ± 1.0) $\text{cm}^3 \text{ mol}^{-1}$. The full volume profile obtained for the two steps aquation/anation reaction of *rac*- $[\text{Pt}(\text{R}_3\text{-en})\text{Cl}_2]$ demonstrates the occurrence for both steps of an associative activation mode; the transition state (I_a mechanism) or the intermediate (A mechanism) is probably a trigonal bipyramid in which the leaving chloride group and the entering water molecule occupy the equatorial positions. Any dissociative mechanism that could have been correlated to a nucleophilic assistance of the 2-substituted norbornyl group is therefore precluded.

This study shows that the comparison of the aquation steps of $[\text{Pt}(\text{R-en})\text{Cl}_2]$ complexes where the diamine varies cannot contribute to the understanding of their different biological properties. Further work is in progress to determine the next steps of DNA platination using UV and atomic absorption spectroscopies and the filter-binding assay with *Escherichia coli* UvrAB protein which binds to the DNA adducts.⁷ Parallel investigations with short oligonucleotides are also in progress.⁴² The aim of the whole work is to establish an eventual structure-activity relationship based on the substituted (ethylenediamine)-platinum complexes.

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Supplementary Material Available: Kinetic work with chloride specific electrodes (Appendix S1), UV-vis kinetic equations (eq S2), molar absorptivities of the complexes at the wavelengths used in the kinetic studies (Table S3), and rate constants for the two aquation steps (k_1 , k_2) of $[\text{Pt}(\text{R}_1\text{-en})\text{Cl}_2]$ in acidic medium as a function of pressure at 311.3 K for eq 1 and at 279.0 K for eq 2 (Table S4) (5 pages). Ordering information is given on any current masthead page.

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