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Unprecedented Water Addition to the α,β-Unsaturated Enone Bond, Mediated by the Combination of Carbonate and Platinum(II)

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Abstract: The cytostatic compounds *cis*-[Pt(A9pyp)(dmso)Cl_2] (**1**) and [Pt(A9pyp)(dmso)(cbdca)] (**2**) (A9pyp = (*E*)-[1-(9-anthryl)-3-(2-pyridyl)-2-propenone) as carrier ligand; cbdca = cyclobutane dicarboxylate) have been found to add water across the enone C=C bond of the ligand A9pyp. The water addition occurs in the presence of carbonate buffer, and has been followed in detail using NMR and ESI-MS spectroscopy. The spectroscopic data clearly indicate that the platinum(II) ion, the carbonate species, and the proximity of the enone C=C bond to the metal ion, are all required for this unusual hydration. A difference in kinetics is observed between chloride and cbdca, showing that the Pt–ligand dissociation plays an important role in the hydration kinetics.

Keywords: carbonates · enones · platinum · water chemistry

Introduction

Catalytic reactions involving platinum-mediated activation of alkenes across a carbon–carbon double bond have been recently reviewed.[1] In general, the coordination of an alkene bond to an electrophilic platinum center appears to activate the alkene towards nucleophilic attack, whereby the formed Pt–C bond is cleaved, regenerating in this way the catalyst. In particular, alkene bonds conjugated to a carbonyl group, so-called α,β-unsaturated enones, display a more electrophilic C=C bond. These electrophilic C=C bonds are known to react with nucleophiles at the β-carbon of the enone. For instance, malonate addition to α,β-unsaturated enones (such as chalcone) or the peroxidation of α,β-unsaturated enones with an organic catalyst have been more recently reported.[2] However, to the best of our knowledge, nucleophilic attack to α,β-unsaturated enones mediated by platinum has not been reported.

Carbonate and phosphate ions are present in relatively high concentration in the cellular medium and also in the extra and intracellular fluids (carbonate is present in much higher concentration (ca 24 mM) than phosphate (around 4 mM) in external fluids). Recent studies have indicated the formation of new platinum species from cisplatin in the medium, due to the presence of carbonate or phosphate ions; subsequently, influencing the uptake, the DNA binding and even the cytotoxicity of these drugs.[3] Consequently, the effect of carbonate and phosphate ions towards the hydrolysis of platinum-based anticancer compounds has been investigated in detail.[3,4] Recently, we have reported that two new compounds, that is, *cis*-[Pt(A9pyp)(dmso)Cl_2] (**1**) and [Pt(A9pyp)(dmso)(cbdca)] (**2**) (A9pyp = (*E*)-[1-(9-anthryl)-3-(2-pyridyl)-2-propenone; cbdca = cyclobutane dicarboxylate), designed and synthesized in our laboratories,[5,6] display interesting cytotoxic activity against the human ovarian carcinoma (A2780) cells and the cisplatin-resistant counterpart (A2780R). These two compounds differ only in their leaving groups (see Figure 1).

Due to possible implications of carbonate ions towards activation of platinum-based drugs, the hydrolysis of **1** and **2** in the presence of such buffer ions has now been investigated in detail, using different spectroscopic techniques. Surprisingly, the NMR studies show an interesting reactivity of the C(8)=C(9) bond of the A9pyp ligand for both Pt compounds, and which appears to be unprecedented. When three equivalents of carbonate buffer are added to solutions of **1** or **2** in DMF, water addition across the C(8)=C(9) bond rapidly occurs and clearly identified, as presented in detail below.

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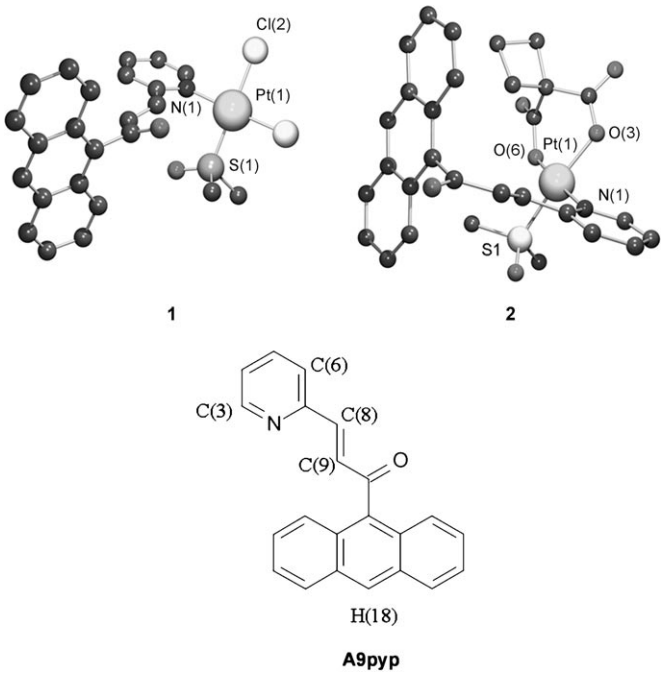


Figure 1. PLATON/POVRAY view of of *cis*-[Pt(A9pyp)(dmso)Cl_2] (**1**) and [Pt(A9pyp)(dmso)(cbdca)] (**2**). Hydrogen atoms have been omitted for clarity with the exception of H(8) and H(9) from the C(8)=C(9) bond.[5,6] At the bottom part the chemical structure of the carrier ligand A9pyp and indication of the atom numbers.

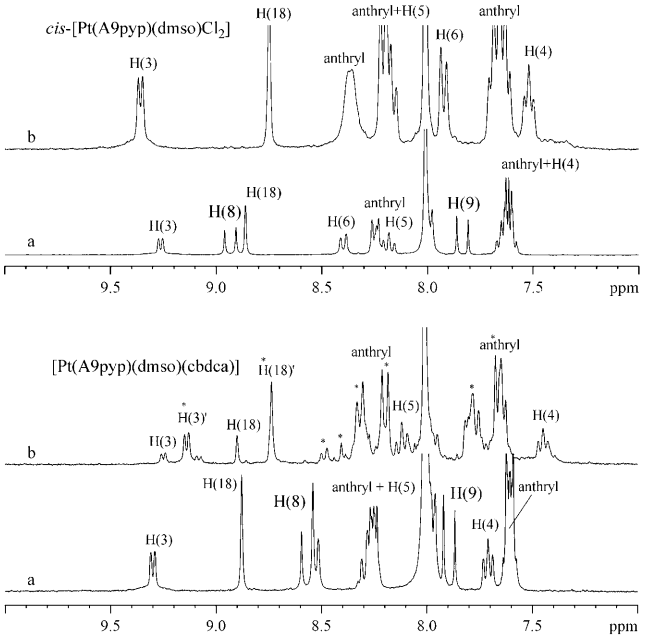
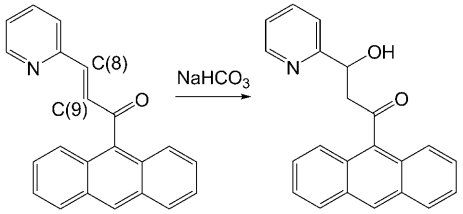


Figure 2. Spectroscopic changes of *cis*-[Pt(A9pyp)(dmso)Cl_2] (**1**) (top spectra) and [Pt(A9pyp)(dmso)(cbdca)] (**2**) (bottom spectra) in the aromatic region of the ^{1}H NMR (300 MHz) 24 h after addition of carbonate buffer (spectra b) to the original [D_7]DMF solution (spectra a). The reaction of **2** is slower, displaying several additional peaks of possible intermediate species.

Results and Discussion

The ligand A9pyp consists of a fully conjugated system (see Figure 1), and the H(8) and H(9) protons of the carbon–carbon double bond give rise to two doublets in the aromatic region on the ^{1}H NMR spectrum with a coupling of 15 Hz, which is typical for a *trans* configuration.[5,6] Upon addition of three equivalents of carbonate buffer (in D_2O) to a solution of **1** or **2** in [D_7]DMF, the disappearance of these two doublets is easily observed within a number of hours, as clearly visible in the spectra displayed in Figure 2. Detailed NMR studies indicate that the C(8)=C(9) bond undergoes a chemical water addition upon addition of carbonate to the solution (Scheme 1). The evidence is discussed in more detail below, using advanced NMR techniques.

Together with the changes in the aromatic region in the ^{1}H NMR spectrum of both compounds (Figure 2), three new peaks appear during the course of the reaction. These peaks appear at 6.02, 3.67, and 3.62 ppm in the ^{1}H NMR spectrum of *cis*-[Pt(A9pyp)(dmso)Cl_2] (**1**) in [D_7]DMF carbonate buffer (details are in Figure S1 in the Supporting Information). Likewise, three new peaks at 6.04, 3.56, and 3.49 ppm are observed for [Pt(A9pyp)(dmso)(cbdca)] (**2**) (see Figure S2 in the Supporting Information). Compound **1** reacts almost immediately (products already visible 15 min after addition of carbonate buffer) yielding only a single species in solution, while compound **2** reacts significantly slower (products visible after 15 min (Figure S2 in the Supporting Information) together with unreacted **2**).



Scheme 1. Chemical structure of the hydration of C(8)=C(9) in the presence of carbonate.

These observations suggest an important role for the initial ligand exchange (chloride ligands versus cbdca ligand) towards the nucleophilic addition across the double bond. As expected for a competitive ligand substitution, addition of an excess of NaCl together with the carbonate buffer to the solution containing compound **1**, resulted in slower reactions (3 days after the carbonate addition the water addition across C(8)=C(9) bond is not completed). This observation confirms that the displacement of the chloride ligands must play an important role in the kinetics of the water addition to the C(8)=C(9) enone bond.

To determine the nature of the new species observed after the carbonate addition to compounds **1** and **2**, the ^{13}C {^{1}H} NMR spectra of the reaction mixture (24 h after mixing) were recorded. The ^{13}C {^{1}H} NMR spectra of both compounds (see Figures S3 and S4 in the Supporting Information) show that the peaks at 6.02 (**1**) and 6.04 ppm (**2**) belong to a CH group (80 ppm), while the peaks at 3.62 (**1**)

and 3.56 ppm (**2**) correspond to a CH_2 group (56 ppm). Peaks at 3.67 (**1**) and 3.49 ppm (**2**) are easily assigned to a CH_3 group from the dmso ligand. Therefore, the new peaks appearing in the NMR spectra are due to the formation of a new product, in which the ligand has lost its conjugation. Even though the proton of the OH group cannot be observed in the ^{1}H NMR spectra of both compounds, the ^{13}C NMR spectra clearly shows the peak of the corresponding CH at 80 ppm, and which has been assigned to a −CH(OH)− group.

To investigate the conformations of compounds **1** and **2** after the hydration of the C(8)=C(9) bond, 2D NOESY NMR studies were performed 24 h after the carbonate addition. Both compounds show a cross-peak between the peak at 6.02 ppm (**1**) (and 6.04 ppm for **2**) and H(6) from the pyridine ring (see Figures S5 and S6 in the Supporting Information). These cross-peaks indicate that the C(8) from the original carbon–carbon double bond has undergone an addition of an OH group, as would be expected, since it is in the β position to the conjugated keto group of the A9pyp ligand. Similarly, the reaction of compound **2** with carbonate reveal a cross-peak between the new peak at 6.04 (CH group) and that at 3.56 ppm (CH_2) (see Figure S6 in the Supporting Information). The cross-peaks observed between the peak at 3.56 ppm (the H(3) proton in *ortho* to the nitrogen of the pyridine ring) and the peak of anthryl protons, suggest that the CH_3 protons of the coordinated dmso ligand are quite close to the pyridine ring and the anthryl ring. This is further evidence that, during the exchange process, the dmso ligand remains coordinated for both compounds.

The 2D NOESY spectrum of **1** confirms that the water addition across the C(8)=C(9) bond is comparable to that observed for **2**; in addition, a cross-peak between the new peak of the CH_2 group and the anthryl protons of **1** in the 2D NOESY was observed. Therefore, both compounds must form a similar reaction product; albeit that different conformations are present. In fact such different conformations would be expected, given the different size and bulkiness of the leaving groups.

To investigate the possible effect of the leaving groups on the carbonate addition in compounds **1** and **2**, in more detail, time-dependent studies using ^{195}Pt NMR spectroscopy were performed. The ^{195}Pt NMR spectrum in [D_7]DMF of *cis*-[Pt(A9pyp)(dmso)Cl_2] (**1**) displays a single peak at −2832 ppm. Changes after addition of three equivalents of carbonate (Pt:carbonate = 1:3) are observed; the generation of a new peak at −2712 ppm appears after 2.5 h, together with another small peak at −2435 ppm (Figure S7 in the Supporting Information). As shown in the ^{1}H NMR spectrum, **1** reacts almost immediately, and the peak corresponding to the unreacted **1** is rapidly disappearing. These new peaks indicate that the chloride ligands in compound **1** dissociate from platinum(II) in the presence of carbonate. To investigate the species formed when the chloride ligands are deliberately removed in the presence of water, two equivalents of $AgClO_4$ in water were added to the solution of **1** in DMF. After 24 h the precipitated AgCl was filtered off and the ^{195}Pt NMR spectrum of the clear solution was recorded giving a peak at −2408 ppm (spectrum not shown). Therefore, the peak observed at −2435 ppm may correspond to a new species in which both chloride ligands are substituted, yielding a species with a [$PtNSO_2$] environment. In addition, the peak at −2712 ppm is likely to correspond to the substitution of a single chloride ligand, yielding a species with [PtNSClO] environment.

The ^{195}Pt NMR spectrum of compound **2** displays a single peak at −2490 ppm when dissolved in [D_7]DMF. When three equivalents of carbonate buffer are added to a solution of **2**, a new peak at −2422 ppm (see Figure S8 in the Supporting Information) gradually appears, with a peak remaining at −2488 ppm corresponding to the unreacted **2**. The difference in the platinum chemical shift of these two species is small, suggesting that the peak at −2422 ppm may also correspond to a donor set [$PtNO_2S$] around the platinum.[7] So when compound **2** reacts in the presence of carbonate species in solution, it is likely that the chelating cbdca ligand might become monodentate within the coordination sphere of the platinum. This hypothesis is supported by the ^{1}H NMR spectrum of compound **2** in the presence of three equivalents of carbonate in 600 MHz, in which alterations in the cbdca chemical shifts (Figure S9) are observed. Compound **2** in [D_7]DMF shows the cbdca ligand chemical shifts at 2.48, 1.83, and 1.50 ppm.[5] After the addition of carbonate buffer, splitting of the peaks is observed in this region, clearly indicating modifications in the cbdca ligand symmetry.

ESI-MS measurements of both compounds with $NaHCO_3$ were performed. A peak at *m*/*z* 657 (Figure S10 in the Supporting Information) and at *m*/*z* 765 (Figure S11 in the Supporting Information) were observed for **1** and **2**, respectively, each containing a typical monoplatinum isotopic pattern. From NMR spectroscopic studies hydration of the C(8)=C(9), chloride and cbdca displacement, and a CH_3 group of the dmso ligand closer to the H(3) proton of the pyridine ring in the reactions of **1** and **2** with $NaHCO_3$ have been observed. When a $KHCO_3$ buffer instead of a $NaHCO_3$ buffer is used, peaks at *m*/*z* 673 and at *m*/*z* 781 for **1** and **2**, respectively, were obtained. The observed difference in the ESI-MS spectrum between the addition of $NaHCO_3$ or $KHCO_3$ corresponds to a *m*/*z* of 16, indicating that the species formed contain a Na and a K ion, respectively. This observation may be explained when it is assumed that Pt^{II} forms a five-membered chelate ring between the nitrogen atom of the pyridine ring and the oxygen atom of a deprotonated OH group from the hydration of the C(8)=C(9) bond (Figure 3). The coordination sphere of these Pt species is completed by a dmso ligand, and by a chloride ligand in the case of **1** and by the monoanionic Hcbdca ligand for **2**, giving a neutral species. The cationic species observed by ESI-MS are therefore ascribed to the presence of Na^+ or K^+ from the carbonate buffer. It should be noted that the dmso ligand remains coordinated to the platinum as observed in both compounds using NMR spectroscopy.

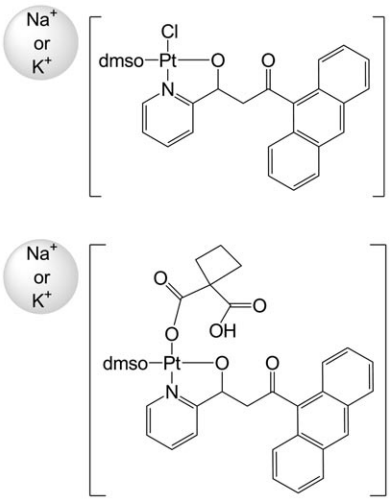


Figure 3. Proposed chemical structures of compounds **1** (top) and **2** (bottom) in DMF after the reaction with carbonate buffer ($NaHCO_3$ or $KHCO_3$).

To further investigate the mechanism of this C(8)=C(9) bond hydration, a number of additional studies were performed. Firstly, a control test using a solution of the free A9pyp ligand in [D_7]DMF was studied upon addition of carbonate buffer. No spectroscopic changes at all were observed in the peaks of the C(8)=C(9) bond of A9pyp up to 24 h after the addition. Moreover, when *cis*-[$Pt(dmso)_2Cl_2$] and free A9pyp are mixed in situ (1:1) in [D_7]DMF, and three equivalents of carbonate buffer are added, water addition across the C(8)=C(9) bond was observed by ^{1}H NMR spectroscopy, starting within the first hour after the addition of carbonate (see Figure S12 in the Supporting Information). Time-dependent studies of this reaction show no changes on the integral of the new peak assigned to the water addition to C(8) 8 h after the carbonate addition (Figure S13 in the Supporting Information). Therefore, the platinum(II) coordination to the ligand plays a crucial role in this water addition. Moreover, no water addition across the C(8)=C(9) bond is observed by ^{1}H NMR spectroscopy when 40% water in the absence of carbonate is added to the DMF solution of compounds **1** and **2**. Thus, it can be concluded that the modification of the C(8)=C(9) bond occurs also only when carbonate is present in the solution and under basic conditions (pH above 8). Finally, similar water addition across the C(8)=C(9) bond was observed when three equivalents of carbonate buffer are added to a solution of **1** and **2** in [D_6]acetone or [D_3]acetonitrile.(data not shown)

To study the possible role of the A9pyp ligand in the mechanism of the water addition across the C(8)=C(9) bond, a modified ligand was used with a *meta*-substitution of the enone instead of *ortho* {(*E*)-[1-(9-anthryl)-3-(3-pyridyl)-2-propenone], abbreviated as *meta*-A9pyp, Figure S14 in the Supporting Information} was synthesized (see Experimental Section). In the presence of *cis*-[$Pt(dmso)_2Cl_2$] (1:1) and three equivalents of carbonate buffer, this ligand shows no water addition at all across the C=C bond, confirming the requirement of the Pt–pyridine coordination to be close to the double bond.

Finally, we have used phosphate instead of carbonate; to see whether water addition across the C=C bond. Indeed the same reactions occur, albeit slower.

Conclusion

All together, the data presented above clearly indicate that the platinum(II) ion, the carbonate species, and the *ortho* substitution and consequently the proximity to the metal ion, are all required for the hydration of the C(8)=C(9) enone bond. The fact that compound **1** reacts faster than **2** towards the hydration, suggests that the initial ligand dissociation plays also an important role in the kinetics of this reaction.

Electrophilic acitivation of alkenes using platinum(II) have been largely explored.[1] Nevertheless, an OH^- attack to α,β-unsaturated enones mediated by platinum has not been reported. Modifications of the carrier ligand may have implications towards the in vitro activity of these compounds; therefore, additional studies in this field would be worthwhile. Given the fact that in recent years several new platinum compounds with different carrier ligands from the classical platinum-based drugs have been largely reported, attention for possible side reactions in carbonate solution such platinum compounds is recommended.

Experimental Section

All NMR spectra were recorded using a Bruker DPX300 or DPX600 spectrometer using a 5 mm multi-nucleus probe. The temperature was kept constant at 20 °C or 37 °C by a variable-temperature unit. ^{195}Pt chemical shifts were referenced to $Na_2[PtCl_6]$ ($\delta = 0$ ppm).

Due to the poor solubility of both compounds in aqueous media, solutions containing *cis*-[Pt(A9pyp)(dmso)Cl_2] (**1**) or [Pt(A9pyp)(dmso)-(cbdca)] (**2**) in [D_7]DMFwere prepared in situ and mixed with a carbonate buffer. Carbonate buffer was prepared dissolving $NaHCO_3$ (1.7 mg, 0.02 mmol) in D_2O (pH 8.5). In a NMR tube the solutions of the compounds were mixed with carbonate buffer, so that the final concentration of carbonate was 25 mM, and the Pt:carbonate ratio was 1:3. Spectroscopic changes were followed over time using ^{1}H NMR spectroscopy at 37 °C. In addition, 2D NMR and ^{195}Pt NMR spectroscopy were performed in order to investigate the reaction products from both compounds **1** and **2** and carbonate.

Phosphate buffer in D_2O (pH 7.8) was also used to investigate its reaction with compounds **1** and **2** in solution. Phosphate buffer was prepared by dissolving Na_2HPO_4 (7.06 mg, 0.05 mmol) and KH_2PO_4 (6.78 mg, 0.05 mmol) in D_2O. ^{1}H NMR, 2D NMR and ^{195}Pt NMR spectroscopy were also performed to study the reactions of both compounds **1** and **2** with phosphate, under the same conditions as described for hydrogencarbonate experiments.

Synthesis of (*E*)-1-(9-anthryl)-3-(3-pyridyl)-2-propenone (*meta*-A9pyp): A warm solution of 9-acetylanthracene (2 g, 9.08 mmol) in ethanol (40 mL) was added to a stirred solution of 3-pyridinecarboxaldehyde (0.9 mL, 9 mmol) and NaOH (1.5 M, 8 mL, 12 mmol) in ethanol (50 mL). The reaction mixture was stirred for 4 h at room temperature. The orange precipitate was collected and recrystallized from ethanol. An orange-yellow solid was obtained in a yield of 70%. ^{1}H NMR (300 MHz,

[D_7]DMF, 25 °C): $\delta = 8.85$ (s, 1H), 8.80 (s, 1H), 8.60 (d, $J = 3.6$ Hz, 1H), 1H), 8.23 (m, 3H), 7.88 (m, 2H), 7.65 (d, $J = 16$ Hz, 1H), 7.56 (m, 4H), 7.45 (d, $J = 16$ Hz, 1H), 7.43 ppm (t, $J = 7.8$, 3.9 Hz, 1H); elemental analysis calcd (%) for $C_{22}H_{15}NO{\cdot}H_2O$: C 80.7, H 5.2, N 4.3; found: C 81.8, H 4.7, N 4.2.

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