Mitomycin Antitumor Compounds. 2.† Interaction of Transition Metal Ions with Mitomycin C. Solution Structure and Biological Activity of a Pd^{2+}−MMC Complex‡

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Interactions of Cu^{2+}, Zn^{2+}, and Pd^{2+} ions with the antitumor compound mitomycin C (MMC) have been investigated by UV−vis, circular dichroism, and ^{13}C NMR spectroscopy. While Zn^{2+} and Cu^{2+} neither interacted with MMC nor catalyzed the formation of mitosenes, Pd^{2+} induced strong MMC spectral modifications, suggesting the formation of a major complex, in which MMC acted as a bidentate ligand through N(1) and N(4) atoms. The coordination mode in this complex was solvent dependent: in MeOH, the NH_2 of the carbamate function was also involved as a third coordination site whereas, in H_2O, Pd^{2+} hydrolysis was more effective, leading to the replacement of the carbamoyl NH_2 function with either H_2O or OH^- ligands. Although coordination of the indoline nitrogen prevented methanol elimination and consequent aziridino ring opening, Pd^{2+} complexation maintained MMC biological activity against cancer cells, as shown by IC_{50} values. This suggests that alternative mechanisms in the biological activity of MMC should be explored.

Introduction

Mitomycin C (MMC, **1**) (Figure 1) is an important antitumor antibiotic isolated from *Streptomyces caespitosus*. The cytostatic effect of MMC is attributed to the bioreductive alkylation of DNA.[2] Its activation mechanism has been extensively studied[3] and shown to proceed via reduced mitosene[4] intermediates. The C(1) site, upon its reductive activation, is the most reactive site of the MMC molecule whereas the C(10) site is activated only after the opening of the aziridine ring.[5] In the absence of external nucleophiles, reductively activated MMC preferentially acts as a trapping agent for electrophiles, whereas in the presence of nucleophiles (e.g., DNA), nucleophilic substitution reactions occur at the C(1) and C(10) sites.[5]

Several ways of reduction, e.g., chemical,[6−9] electrochemical,[10] and enzymatic,[11,12] are used for in vitro MMC activation. Metal ions such as Cr^{2+} are also used in the chemical activation of MMC.[9] In particularly, Cu^{2+} ions are known to strongly promote the strand scission of bacteriophage ΦX174 by MMC and sodium hydrosulfite.[13] Recently, some metal complexes of Cu^{2+} have been reported to exert a synergistic effect with MMC against tumor cell lines,[14] and the combination of various Pt^{2+} complexes with MMC has been shown to determine a remarkable enhancement in cell killing, specially under hypoxic conditions.[15]

However, the mechanisms of the influence of metal ions on the biological activity of MMC remain unclear; for instance, the putative complexes of the reduced form of MMC with Cr^{3+}, involved in Cr^{2+} activation, have never been demonstrated.[9]

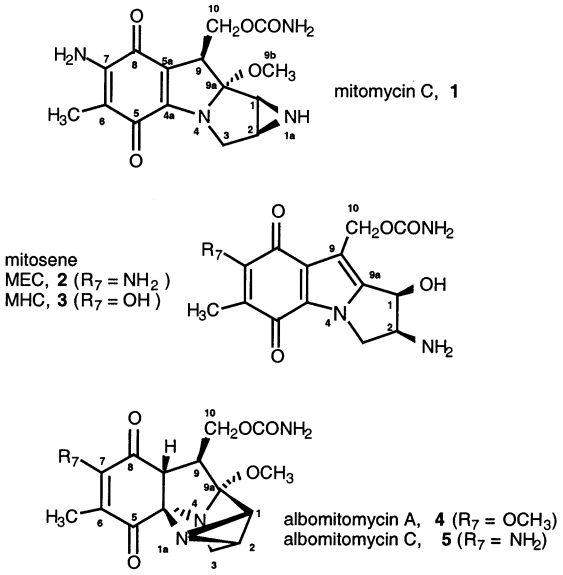

Figure 1. Structures of mitomycin C (**1**), 2,7-diamino-1-hydroxymitosene (**2**), 2-amino-1,7-dihydroxymitosene (**3**), and albomitomycin C (**5**).

Previous studies report that MMC does not form stable complexes with Zn^{2+}, Cu^{2+}, or Pt^{2+}, but rearranges to 2,7-diamino-hydroxymitosene (MEC, **2**) (Figure 1), capable of complex formation.[16,17] On the basis of these findings, it was suggested that these metal ions might either interact with the aziridino ring or acidify the solution medium through metal ion hydrolysis, thereby inducing MMC hydrolysis. A possible effect of the pH on the opening of the aziridino ring[8] has also

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Table 1. Spectroscopic Features of the Pd^{2+}−MMC System, in Water, and in Methanol at 1/1 Metal-to-Ligand Molar Ratio ([MMC] = 10^{-4} M; Δt = 24 h)

compound	solvent		$\pi\rightarrow\pi^*$ $^1A\rightarrow{}^1B_b$	n$\rightarrow\pi^*$ $^1A\rightarrow{}^1U''$ C(11)=O	$\pi\rightarrow\pi^*$ $^1A\rightarrow{}^1L_a$	$\pi\rightarrow\pi^*$ $^1A\rightarrow{}^1L_b$	n$\rightarrow\pi^*$ $^1A\rightarrow{}^1U'$ C(8)=O	n$\rightarrow\pi^*$ $^1A\rightarrow{}^1U$ C(5)=O
MMC	H_2O	UV	218 (24600)	-	253 sh (8700)	360 (22000)	-	570 (215)
		CD	216 (5.8)	235 (−3.8)	252 sh (−0.9)	357 (−2.3)	393 (5.0)	570 (−0.3)
+ Pd^{2+}	H_2O	UV	n.d.[a]	-	254 (8930)	361 (10900)	-	450 (2900)
		CD	n.d.[a]	240 (−2.8)	278 (1.0)	314 (−0.3) 343 (1.8)	405 (−0.4)	456 (0.4)
+ Pd^{2+}	MeOH	UV	n.d.[a]	-	n.d.[a]	313 sh (8200) 353 (14000)	-	493 (1200)
		CD	n.d.[a]	250 (−5.0)	276 (−2.7)	314 sh (3.0) 334 (3.4)	387 (2.2)	493 (1.0) 612 (−0.3)

[a] n.d = not determined.

been recently argued;[18] however, the assumption that the aziridino ring is protonated only at acidic pH,[19,20] favoring the nucleophilic attack of water to C(2), no longer holds since the recent pK_a assignment of the MMC functional groups. [18]

Our interest in the biological effects of metal complexes of antitumor compounds[21,22] prompted us to investigate the possibility that MMC forms stable metal complexes. As a part of a project aimed at the characterization of the interaction between antitumor compounds and metal ions by CD spectroscopy,[21] the interaction of MMC with some metal ions was reinvestigated. Cu^{2+} and Pd^{2+} were selected for this study because of their well-recognized role in the activation of streptonigrin.[22] The formation of a complex of a 1/1 metal-to-ligand stoichiometry between Pd^{2+} and MMC has been hypothized. The structure of this complex in solution has finally been described, based on the full assignment of the electronic transitions in the CD spectrum of MMC.[1]

Results and Discussion

The MMC structure is shown in Figure 1. Its electronic spectrum, either in water or in methanol, has three distinct regions of absorption above 200 nm (Table 1). The absorption band observed at 218 nm, with a shoulder at 253 nm, is accompanied by another at 360 nm that is characteristic of the aminobenzoquinone chromophore. MMC comprises an aziridinopyrroloindole unit that contains asymmetric carbons at C(1), C(2), C(9), and C(9a). The X-ray structure of this molecule shows that the aziridino ring is bent over the benzoquinone ring,[23] which increases the total dissymmetry of the molecule.

In the CD spectrum, two positive bands, centered at 216 and 393 nm, and two negative bands at 235 and 357 nm, are observed. A negative shoulder is present at 253 nm (Figure 2).

Cu^{2+}−MMC Systems in H_2O and in MeOH. The addition of either Cu^{2+} or Zn^{2+} to aqueous solutions of MMC did not result in spectral modifications, and the light blue color did not change for the next 24 h. Similar experiments performed with different MMC concentrations (ranging from 10^{-4} to 10^{-3} M) and various metal-to-ligand molar ratios (ranging from 0.5 to 2), either in water or in methanol, resulted in similar findings. No changes in the spectra, indicating the formation of mitosene (MEC, **2**, or MHC, **3**)[1] (Figure 1) or metal ion coordination, were observed. However, as previously reported,[16] a solution of MMC in methanol turned to slight green 24 h after adding Cu^{2+} at the 1/1 metal-to-ligand molar ratio. A reduced CD signal revealed MMC degradation, but no formation of mitosene, such as MEC, was detected.

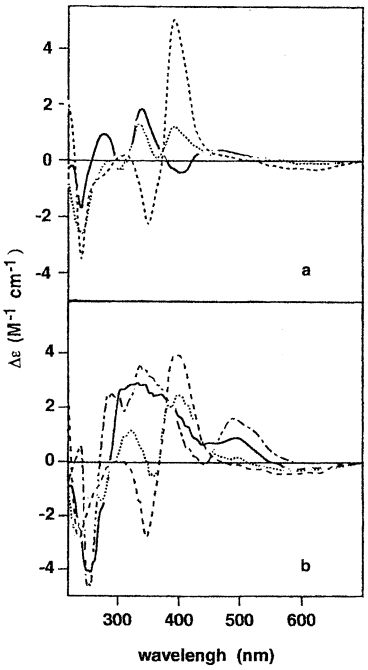

Figure 2. CD spectra of Pd^{2+}−MMC systems in water (a) and in methanol (b), as a function of the metal-to-ligand molar ratio: 0:1 (- - -), 0.5:1 (····), 1:1 (—), 2:1 (—·—); [MMC] = 10^{-4} M, [KCl] = 0.1 M; Δt = 24 h.

Pd^{2+}−MMC Systems in H_2O. When $[PdCl_4]^{2-}$ was added to an aqueous solution of MMC (10^{-4} M) at neutral pH at the 1/1 metal-to-ligand molar ratio, the light blue solution immediately turned yellow. In the UV−vis spectra, the hyperchromic effect observed in the 200−250 region a few minutes after the mixing is due to the superposition of the MMC absorption bands with the charge-transfer bands of the Pd^{2+}−Cl bonds. However, the concomitant hypochromic effect of the band at 360 nm is associated to the interaction between drug and metal ions (Table 1). The insignificant modifications of vis-UV absorption spectra that were observed as a function of metal-to-ligand molar ratio, time, and experimental conditions (Table 1) are not detailed here.

In the CD spectra, the modifications observed as a function of the experimental conditions were more significant. In a first set of experiments, the evolution of the CD spectra was followed as a function of metal-to-ligand molar ratio and of time (Figure 2a). After 2 h, both bands at 357 (negative) and 393 nm (positive) were reduced in intensity with inversion of their CE sign. The

Table 2. ^{13}C-NMR[a] Data for Mitomycin C, in the Absence and in the Presence of Pd^{2+}, in Methanol-d_4 at 50.327 MHz (δ in ppm relative to internal solvent) ([MMC] = 10^{-2} M; Δt = 24 H)

	C(1)	C(2)	C(3)	C(6a)	C(9)	C(9b)	C(10)
MMC	37.50	33.56	50.80	8.16	44.72	50.79	62.99
+ Pd^{2+}	43.45	41.01	27.90	8.14	44.47	50.80	62.98

[a] The acquisition sequence (see Experimental Section) did not allow to identify the signals of quaternary carbon atoms, e.g., C(4), C(5), C(6), C(7), C(8), C(9a).

band at around 235 nm had a small hypochromic shift at 240 nm whereas the shoulder at 253 nm turned positive. Finally, the broad negative band centered at 570 nm underwent an ipsochromic shift to 510 nm with change of the CE. Isodichroic points were observed at 350 and 448 nm. After 24 h, at 1/1 metal-to-ligand molar ratio, the CD spectrum showed negative bands at 240 and 405 nm and positive bands at 278, 343 and 456 nm (Figure 2a).

When Pd^{2+} was added at low metal-to-ligand molar ratio (1/10), the CD spectrum of MMC was slightly modified, suggesting that the interaction between Pd^{2+} and MMC was not catalytic but stoichiometric.

Adding EDTA to an aqueous solution of the 1/1 Pd^{2+}–MMC system to remove the metal ion resulted in spectral modifications in the 300–550 nm region. As the EDTA concentration was raised from 1/2 to 2/1 metal-to-ligand molar ratios, the negative band at 405 nm and the positive at 341 nm decreased in intensity. However, the MMC original CD spectrum was never observed.

Pd^{2+}–MMC Systems in MeOH. In a second set of experiments, Pd^{2+} ions were added to MMC solutions in methanol as a function of the metal-to-ligand molar ratio and as a function of time (Figure 2b). The resulting UV–vis absorption spectra were similar to those observed in water (Table 1). In the CD spectra, however, the pattern was totally different. After 2 h, while the band at 393 nm did not change, the CE of the band at 357 nm and the shoulder at 253 nm were modified, and the intensity of the band at 240 nm increased with a small bathochromic shift. After 24 h, negative bands at 245 and 612 nm with a shoulder at 265 nm and positive bands at 334, 384, and 551 nm were observed.

^{13}C NMR Studies. In the NMR experiments, $PdCl_4^{2-}$ was added to methanol-d_4 solutions of MMC and the spectra recorded 24 h later. Because of the limited solubility of K_2PdCl_4 in MeOH (see Experimental Section), only a small amount of Pd^{2+} reacted with MMC. Under these conditions, the coordination was incomplete and a large fraction of free MMC remained in solution. The ^{13}C NMR data are reported in Table 2.

The chemical shifts of the carbon atoms in free MMC were in agreement with the data reported in the literature.[24] The addition of the metal ion resulted in a downfield shift of the C(1) and C(2) signals and in an upfield shift of the C(3) signal. The benzoquinone ring was not affected by the interaction with the metal ions, as shown by the absence of shift for the peak associated to C(6a) in the aromatic ring. The signal relative to the methoxy group at C(9b), partially obscured by the solvent, did not change and the C(10) signal broadened.

Chromatographic Analysis. The Pd^{2+}–MMC systems were checked by TLC analysis at different metal-to-ligand molar ratios in order to determine if any chemical modification of the drug occurred through the interaction with Pd^{2+}. When the metal ion was almost equimolar to the drug, only one spot was present with R_f equal to zero indicating the presence of a highly polar compound. By contrast, when the metal-to-ligand molar ratio was lower than 1, one additional spot was present with R_f equal to 0.84 indicating the presence of free MMC. However, when the complex was formed in MeOH, the R_f was equal to 0.91 indicating the presence of a nonpolar compound. No mitosenes were formed during the reaction (MEC R_f = 0.41 and MHC R_f = 0.65).

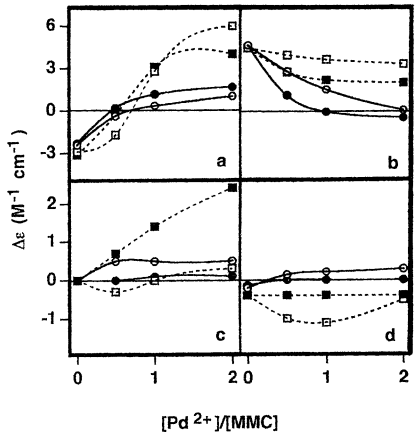

Figure 3. Variations of $\Delta\epsilon$ at 357 (a), 393 (b), 498 (c), and 570 nm (d) in water (circle) and in MeOH (square) as a function of the $[Pd^{2+}]$/[MMC] molar ratio and as a function of time; Δt = 2 h (open signet) and Δt = 24 h (closed signet); [MMC] = 10^{-4} M, [KCl] = 0.1 M.

Spectroscopic Analysis. Starting from a previous paper reporting the assignment of the UV–vis and CD bands to the electronic transitions of MMC,[1] identification of the species resulting from the interaction between MMC and Pd^{2+} was attempted.

In the MMC UV–vis spectrum, the two intense absorption bands at 218 and 360 nm and the shoulder at 253 nm have been assigned to $\pi\rightarrow\pi^*$ transitions $^1A\rightarrow{}^1B_b$, $^1A\rightarrow{}^1L_b$, and $^1A\rightarrow{}^1L_a$, respectively, where a is the short and b the long axis of the molecule. In the CD spectrum the bands at 216 (positive), 357 nm (negative) and the negative shouder at 253 nm correspond to the bands observed in the absorption spectra.[1] Similarly, the additional bands present at 235, 393, and 570 nm have been assigned to the $n\rightarrow\pi^*$ transitions of the carbamoyl function at C(11)=O and the carbonyl functions at C(8)=O and C(5)=O, respectively.[1] No significant difference was observed between the MMC spectra in water and in methanol.[1]

To simplify the spectroscopic analysis of the Pd^{2+}–MMC system, the CD spectra of MMC were analyzed in two parts: the 220–400 nm region, which corresponds to the domain of the $\pi\rightarrow\pi^*$ transitions, and the 400–600 nm region for the $n\rightarrow\pi^*$ transitions of the carbonyl functions. The $n\rightarrow\pi^*$ transitions of the carbamate function are considered apart at 235 nm.

In the UV–vis spectrum, the interaction of MMC with the metal ions resulted in a strong hypochromic effect of the band at 357 nm, suggesting that the $\pi\rightarrow\pi^*$ transitions were modified by the metal ion (Table 1). Spectroscopic modifications observed in the CD spectra were strongly dependent on the solvent (water or methanol) as shown in Figure 3. The evolution of the

bands at 357, 393, 498, and 570 nm was found to be a function of metal-to-ligand molar ratio, time, and solvent used. While the CD band at 357 nm related to the $\pi\rightarrow\pi^*$ transitions, those at 393 and 570 nm related to the C═O functions of C(8) and C(5), respectively, and the one at 498 nm can be considered a combination of the last two. The band at 357 nm ($\pi\rightarrow\pi^*$) decreased in intensity as a function of time and metal concentration until inversion of the Cotton effect in both solvents, water and methanol (Figure 3a). In the carbonyl region, the modification of the band at 393 nm (C(8)═O function) was stronger in water than in methanol, whereas the $n\rightarrow\pi^*$ transitions of C(5)═O function (associated to the signals at 498 and 570 nm) were more affected in methanol than in water (Figure 3). Furthermore, in MeOH, 2 h after mixing the reagents, the band at 570 nm (associated to the $n\rightarrow\pi^*$ transitions of C(5)═O) increased in intensity whereas the band at 393 nm ($n\rightarrow\pi^*$ transitions of C(8)═O) underwent only a small hypochromic effect.

Concerning the other bands (and the related transitions), the sign of the CE of the $^1A\rightarrow{}^1L_a$ transitions was reversed in water but not that of the $n\rightarrow\pi^*$ transition of the C(11)═O function. The same transitions underwent a small bathochromic shift with a hyperchromic effect in methanol.

Solution Structure of the Complex. Basic coordination chemistry helps us to assign the possible MMC coordination sites. MMC has four nitrogen atoms, all of which could be hypothetic coordination sites. N(7), an aromatic amine, can be immediately excluded because of its low basicity[18,19] and because the related C(6a) NMR signal was not modified during the interaction with Pd^{2+} (Table 2).

N(11), an amide nitrogen, also displays a low basicity.[18,19] However, the orientation of the carbamoyl group in the molecular plane is very important in determining the CE sign of several bands;[1] thus, its implication could not be totally ruled out. It has recently been reported[25] that indole-3-acetamide binds Pd^{2+} as a bidentate ligand through metalation of the β-carbon in the heteroaromatic ring and the amide oxygen atom, via previous formation of indolenine. For MMC, this possibility can be excluded under our experimental conditions because the presence of water inhibited the metalation of the cycle via the suggested indolenine intermediate.

Concerning the aziridino ring, recent studies[18] consider N(1) as much more basic than previously reported[8,20] and therefore possibly involved in the metal ion binding. The downfield shift of the resonances of C(1) and C(2) (from 36.75 and 32.81 ppm in free MMC to 42.72 and 40.26 ppm in the complex) would support N(1) metal ion binding. However, because of its distance from the chromophore, the N(1) simple coordination cannot account for the strong spectral modifications observed in the CD spectra.

Finally N(4), the indoline nitrogen, displays a pK_a of 2.7.[18] This value is at the high end of the aminoquinone pK_a values. This was previously attributed to the electronic delocalization between N(4) and O(8) that allows an internal hydrogen bonding between N(7) and O(8), which would stabilize the protonated function.[18]

Other clues in the interpretation of the CD spectra and, consequently, in the elucidation of the solution structure of this complex come from a survey of the literature. Albomitomycin A (**4**, Figure 1) is a natural product extracted from fermentation broths of mitomycin A by *Streptomyces caespitosus*.[26] In this molecule, N(1) is bound to C(4a) and a H atom to C(8a). The quinone ring of mitomycin A is not present in albomitomycin A (**4**), the dihydroquinone ring taking a half-chair conformation, and the significant deviation of the six-membered ring from planarity is the main reason for its achromaticity. Mitomycin A can be transformed to albomitomycin A in a protic solvent and the transformation may occur through an intramolecular Michael reaction, which is not only governed by the geometrical prerequisite in the molecule but also by the electronic characteristic of the C(4a) atom.[27]

The X-ray crystal structures of several mitomycins show that the aziridino moiety is bent over the pyrrole ring.[23] Because of the flexibility of the mitomycin skeleton, the rearrangement is possible also in the case of MMC, even if the lack of stability of albomitomycin C (**5**) does not allow X-ray characterization.[28] However, the inspection of the X-ray structure of MMC revealed that the distance between N(1a) and N(4) was only 3.075 Å, confirming the viability of metal coordination.[23]

The stoichiometry of the Pd^{2+}−MMC complex cannot be directly determined. In fact, if the coordination seemed to be more stable in methanol, the limited solubility of the K_2PdCl_4 in this solvent (see Experimental Section) did not allow isolation of the adduct. Attempts to dissociate the complex by competition with another ligand such as EDTA were not conclusive, and the protonation of the ligand by acidification was not attempted to avoid the opening of the aziridino ring. However, the addition of EDTA to the 1/1 Pd^{2+}−MMC complex in water revealed a partial inversion in the CE of the $n\rightarrow\pi^*$ transitions of C(8) and of the $^1A\rightarrow{}^1L_a$ transitions, suggesting the formation of a ternary species rather than the dissociation of the Pd^{2+}−MMC complex. It is known that the steric effect of the aziridine ring prevents a high coordination number and the strength of the metal-to-nitrogen bond in the aziridine complexes is lower than that of the corresponding ammonia complexes, because of its lower basicity.[29,30] Recent papers on the coordination of Pd^{2+} to aziridine[31,32] show that this molecule can act as a bidentate ligand depending on the ring substituents. On these grounds and because of the steric hindrance of MMC as a ligand, the formation of a complex of 1/1 metal-to-ligand stoichiometry between Pd^{2+} and MMC can reasonably be suggested.

Concerning the coordination mode of MMC, it was observed that the coordination of Pd^{2+} to MMC in methanol was different from that in water, as revealed by the different evolution of the 235 nm band. This band, associated to the $n\rightarrow\pi^*$ transition of the carbamoyl group, was not affected in water (Figure 2) whereas its CE sign changed in methanol (Figure 3). This result could be explained by the interaction of Pd^{2+} with the NH_2 of the carbamate in methanol. In agreement with this suggestion, in methanol all CD bands had a hyperchromic effect compared to water, because of the enhanced dissymmetry of the molecule. On the other hand, the C(8)═O function was not affected in MeOH whereas the sign of its CE changed in H_2O. This could

be related to the different orientation of the carbamoyl group in water and in methanol, it being involved in coordination in methanol only. Furthermore, the TLC experiments were in agreement with the different coordination modes proposed. In water, the mitomycin acted as a bidentate ligand and the presence of the metal ion increased the polar interaction with the silica gel. However, when the complex was formed in MeOH, the MMC acted as a tridentate ligand, masking in part the positive charge of the metal ion, and the behavior of this species was less polar compared to free MMC.

In the X-ray structure of free MMC, the N(1) and N(4) point in opposite sides of the plane of the molecule.[23] When the metal ion coordinates, the configuration of N(4) is presumed to be inverted in order that the lone pair can be oriented for coordination, inducing the change of sign relative to the position of C(5) in the plane. This was effectively observed in both cases, in water and in methanol. The change of the substituent orientation also influences the CE of $\pi\rightarrow\pi^*$ transitions. In the absence of X-ray crystal structure of the Pd^{2+}−MMC complex, this interpretation remains speculative. However, it is in agreement with the results of our previous spectroscopic studies[1] and with molecular mechanistic studies (Wieczorek R., Fiallo M. M. L., unpublished results).

Other clues in the interpretation of our spectroscopic results came from recent pK_a assignments.[18] It is described that the C(8)═O function is electronically related to N(4), depending on its protonation state, in agreement with the CD spectral modifications observed. On the other hand, the C(5)═O could be affected by the coordination of N(4) through steric effects. Further, in the free MMC it is suggested that the quinone ring has a chairlike conformation,[1] whereas in the bound MMC the conformation of the quinone seems related to the solvent used, and consequently, to the different coordination mode. In fact, in agreement with the CE signs of the two carbonyl functions, in water the quinone would have an inverted chairlike conformation, whereas in methanol the boatlike conformation seems to be preferred to compensate the rotation of the carbamoyl function occurring in this coordination mode.

In the absence of potentiometric measurements, because of the fragility of MMC in acidic medium, the expected low stability of the Pd^{2+}−MMC complex is a working hypothesis. However, the inertia of Pd^{2+}, compared to other labile metals, allows to identify this complex spectroscopically.

It is reported that the hydrolysis effect of Cu^{2+} and Zn^{2+} ions and MMC begins with weak interactions. NMR spectra suggest a coordination with both N(7) and N(1).[16,17] In our experimental conditions, MEC was not formed and the coordination with an hydrolyzed form of MMC could be excluded because, in the former case, the CD spectroscopic pattern was totally different (Fiallo, M. M. L., unpublished results).

Antitumor Activity. The 1/1 Pd^{2+}−MMC complex was studied for its biological activity in vitro against K562 leukemia cells by measuring the inhibition of cell growth. MMC was used as a positive reference compound.

The 1/1 Pd^{2+}−MMC complex maintained an antitumor activity ($IC_{50} = 15.8 \pm 5.3$ nM) compared to free MMC ($IC_{50} = 1.0 \pm 0.3$ nM); when EDTA was added to the solution to remove the metal ion, the mixture was still active. A control of the antitumor activities of EDTA and K_2PdCl_4 under the same experimental conditions revealed biological activity only at higher concentration ($IC_{50} \gg 1\ \mu$M) and excluded any extra effect of the ligand or the metal ion on the antitumor activity of the complex. Because usually mitosenes are reported to show antitumor activity lower than MMC,[33] MEC[1] was checked and found to be less active at the same concentration values ($IC_{50} = 33.0 \pm 3.2$ nM). It has been reported that the binding of the indoline nitrogen prevents methanol elimination and the consequent aziridino ring opening.[18] On the other hand, the presence of metal ions does not cause a significantly more rapid opening of the aziridino ring.[29] The biological effect of the Pd^{2+}−MMC complex seem to occur through a different mechanism from the reductively activated opening of the aziridino ring.

Conclusion

Reaction of Pd^{2+} with MMC afforded a metal complex. Various CD and ^{13}C NMR spectra revealed bidentate coordination of the ligand via N(1) and N(4). The X-ray crystallographic analysis of free MMC suggested that the coordination of the metal ion between N(1) and N(4) could occur because the aziridine ring bent over the benzoquinone ring, shortening the distance between N(1a) and N(4) to 3.075 Å. The amide group could coordinate Pd^{2+} only in methanol solution because of the metal ion hydrolysis in water.

A recent paper[34] has shown that the biological activity of a derivative of the antitumor compound CC-1065 could be tuned by the interaction with metal ions, thus confirming that the implication of the metal ions in these systems has not been yet totally explored.

Molecular mechanistic calculations are actually underway to verify the solution structures of the metal complexes of Pd^{2+} with MMC and other derivatives.

Experimental Section

Mitomycin C was purchased from Aldrich. Solution concentrations were determined by using $\epsilon = 22000\ M^{-1}\ cm^{-1}$ at 360 nm.[4] *cis*-2,7-Diamino-1-hydroxymitosene (MEC) and *cis*-2-amino-1,7-dihydroxymitosene (MHC) were prepared following the published procedures.[20]

$K_2[PdCl_4]$ was obtained from Johnson Matthey and the concentration of the Pd^{2+} standard solution (containing KCl 0.2 M) was determined by using $\epsilon = 150\ M^{-1}\ cm^{-1}$ at 450 nm. $CuSO_4 \times 5\ H_2O$ and $ZnSO_4 \times 7\ H_2O$ were obtained from Aldrich. All samples were assayed against serial dilutions of reference standards. EDTA disodium salt was purchased from Aldrich.

TLC analysis was performed on silica plates Alugram SIL G/UV_{254} silica gel 60 (Macherey-Nagel, Germany). The developing solution used was 3:1 (v/v) chloroform:methanol. All products were identified by cospotting of authentic samples (MMC, $R_f = 0.84$; MEC, 0.41; MHC, 0.65). Identification of the spots has been done with a UV lamp.

Potentiometric measurements were obtained with a Metrohm Model E603 pHmeter at 25 °C using a Metrohm EA147 combined glass electrode.

Absorption spectra were recorded on a Varian Cary 219 and a Hewlett-Packard 8453 diode array spectrophotometers. Circular dichroism measurements were made on a Jobin Yvon Model Mark V dichrograph. The temperature was controlled at 25 °C using a cryostat. The instrument was calibrated using a standard solution of epiadrosterone (3.4×10^{-3} M) in a 1

cm cell ($\Delta\epsilon$ = 3.3 M^{-1} cm^{-1} at 304 nm). All spectra were recorded using a 0.2 cm cell and the following parameters: λ = 220−700 nm, step 1 nm, speed 0.3 nm s^{-1}, response 0.3 s, spectral bandwidth 1.4 nm, number of cycles 2. MeOH and water blanks were used as references, depending on the experimental conditions. Results were expressed as ϵ (molar absorption coefficient) and $\Delta\epsilon$ (differential molar absorption coefficient). The values of ϵ and $\Delta\epsilon$ were expressed as molar concentration of mitomycin.

^{13}C NMR spectra (50.327 MHz) were recorded with a Bruker DPX200 at room temperature. Dpt 135 sequence was used (4600 scans). Chemical shifts were referenced to internal solvent signal and reported in parts per million (ppm) downfield to TMS. To avoid the hydrolysis of mitomycin C due to the presence of hydrolyzed metal ions, MMC (2 mg) was dissolved in methanol-d_4 (0.5 mL) and $K_2[PdCl_4]$ added as a solid (2 mg). After 24 h, because of the limited solubility of the palladium salt in methanol, the undissolved material has been filtered and the spectra recorded. Methanol-d_4 (49.30 ppm) was obtained from CEA (Saclay, France).

Cell Cultures. K562 is a human leukemia cell line, established from a patient with a chronic myelogeneous leukemia in blast transformation.[35] K562 cells were cultured in vivo in RPMI 1640 medium (Sigma) supplemented with 9% heat-inactivated fetal calf serum (Biomedia, France) and containing L-glutamine (2 mM), $NaHCO_3$ (23 mM), streptomycin (0.1 mg mL^{-1}), and penicillin (100 U ml^{-1})(Sigma).

In Vitro Inhibition of K562 Leukemia Cell Growth. For the growth studies, Petri dishes are seeded with 4 mL of cells (approximately 1×10^5 cells/ml) then incubated in the presence of mitomycin C and its derivatives for 3 days at 37 °C in an humidified incubator in aerobic conditions with 5% CO_2. Samples were added in the growth medium to the final dilution to from 0.1 to1000 nM. Cells viability was assessed by tripan blue exclusion, and cell counting was made with a Coulter counter.

Drug effect was expressed by the inhibitory concentration (IC_{50}), obtained by plotting the logarithms of drug concentration against percent inhibition of cell growth and extrapolating the concentration required to inhibit 50% of cell growth. Each IC_{50} determination was repeated at least three times using different preparations of the metal complexes.

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Appendix

Abbreviations used: CD, circular dichroism; CE, Cotton effect; EDTA, ethylenediaminotetraacetic acid; MMC, mitomycin C; MEC, *cis*-2,7-diamino-1-hydroxymitosene; MHC, *cis*-2-amino-1,7-dihydroxymitosene; TMS, tetramethylsilane.

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