142. Ruthenium(II) Complex of the Alkaloid 2-Bromoleptoclinidinone. Preparation and Interaction with Double-Stranded DNA

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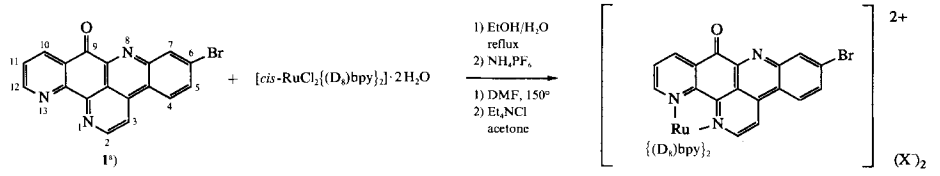
The fused pentacyclic alkaloid 2-bromoleptoclinidinone (**1**) isolated from an ascidian possesses a bidentate chelating site. Complexation of **1** with $[cis\text{-}RuCl_2\{(D_8)bpy\}_2] \cdot 2H_2O$ forms an octahedral complex which was isolated both as hexafluorophosphate **2** and chloride **3** salts. Intercalation of **3** in calf-thymus DNA was shown to occur as well as photoactivated cleavage of double-strand supercoiled DNA pBR322 under visible-light irradiation.

The fused pentacyclic, antileukemic alkaloid 2-bromoleptoclinidinone (**1**) isolated from an ascidian [1] possesses a 1,10-phenanthroline moiety. It may, therefore, be expected to form bidentate transition-metal chelate complexes. Although complexation with Fe^{II} in a standard colourimetric test was not observed with **1** [1] or its debromo analog (ascididemin) [2], we report here the preparation of an octahedral Ru^{II} complex incorporating ligand **1** (*Scheme*) and a study of its interaction with DNA.

1. Preparation of the Ru^{II} Complex of 1. – In order to simplify the NMR spectrum of the target complex which would contain the unsymmetrical ligand **1**, we prepared a deuterated ruthenium precursor $[cis\text{-}RuCl_2\{(D_8)bpy\}_2] \cdot 2H_2O$ [3]. Treatment of **1** with this complex in aqueous EtOH, followed by addition of ammonium hexafluorophosphate afforded the green complex **2** in excellent yield (*Scheme*). The hexafluorophosphate **2** thus obtained is soluble in MeCN and other organic solvents but not in H_2O.

Similar treatment of **1** with $[cis\text{-}RuCl_2\{(D_8)bpy\}_2] \cdot 2H_2O$ in DMF followed by addition of a saturated solution of tetraethylammonium chloride in acetone provided the

Scheme



2 X = PF_6^- (97%)
3 X = Cl^- (76%)

a) Systematic numbering.

[1]) U.R.A. 422 du C.N.R.S.

green complex **3** in good yield. Chloride **3** is H_2O-soluble and suitable for DNA binding studies.

2. Spectroscopic Properties. – The (D_{16})-complexes **2** and **3** were characterised by their spectroscopic data.

Table 1. *^{1}H-NMR Chemical Shifts* (CD_3CN, 200 MHz) *and Coupling Constants of Complex* **2**. δ in ppm rel. to TMS, J in Hz.

δ [ppm]		δ [ppm]	
H–C(11)	7.72 (*dd*, $J(10,11) = 8.02$, $J(11,12) = 5.59$)	H–C(3)	8.59 (*d*, $J(2,3) = 6.64$)
H–C(2)	8.07 (*d*, $J(2,3) = 6.60$)	H–C(4)	8.72 (*d*, $J(4,5) = 8.71$)
H–C(12)	8.08 (*dd*, $J(11,12) = 5.43$, $J(10,12) = 1.39$)	H–C(10)	8.74 (*dd*, $J(10,11) = 8.01$, $J(10,12) = 1.36$)
H–C(5)	8.19 (*dd*, $J(4,5) = 8.77$, $J(5,7) = 2.08$)	H–C(7)	8.75 (*d*, $J(5,7) = 1.84$)

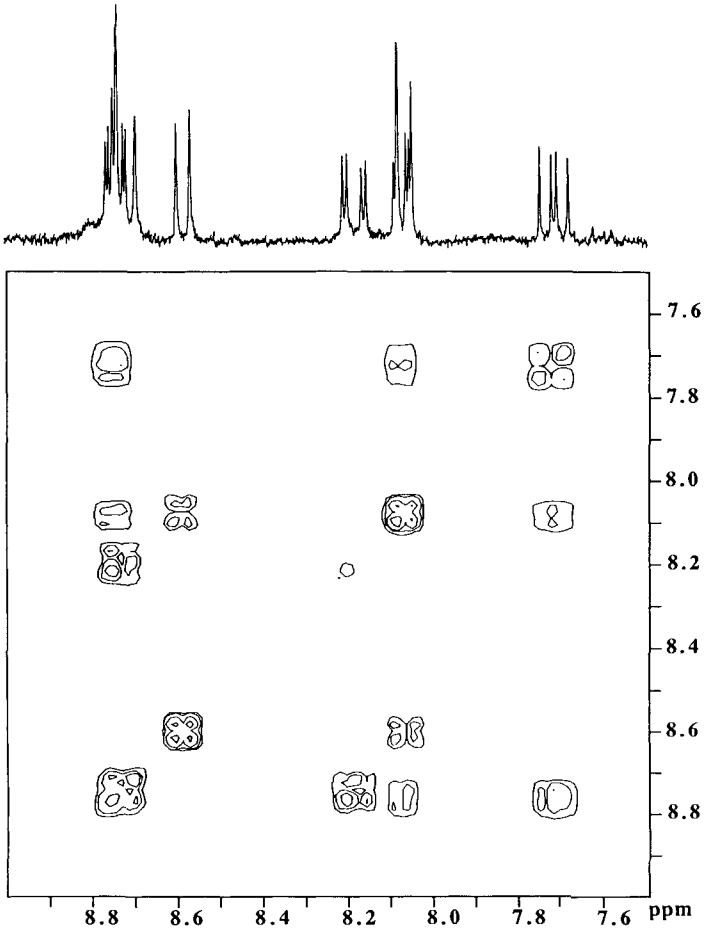


Fig. 1. *H,H COSY Spectrum of complex* **2** *in CD_3CN at 200 MHz*. Spectrum width in F_1 and F_2, 1602.56 Hz; size in F_2, 1 K; number of experiments, 256; number of acquisitions, 128; window function in F_1 and F_2; cosine bell; zero-filling in F_1; relaxation delay, 1s.

In the ^{1}H-NMR spectra of **2** (*Table 1* and *Fig. 1*) and **3**, only the aromatic resonances of **1** are observable. In the free ligand **1**, the aromatic protons of the bipyridine unit appear at lower field than in 2,2′-bipyridine due to the electron-withdrawing nature of both the ketone group and the Br-atom [1] [2]. In complex **2**, the aromatic resonances of **1** are clearly differentiated thereby allowing assignment of each proton. Upon complexation, the H–C(2) and H–C(12) signals are considerably shifted upfield ($\Delta\delta$ = 1.16 and 1.07 ppm, resp.) appearing at 8.07 and 8.08 ppm, respectively, due to the shielding effect of the aromatic ring of the (D_8)bipyridine groups. The other protons only experience slight downfield shifts due to charge depletion resulting from the coordination of the Ru^{II} ion. The ^{1}H-NMR spectrum of chloride **3** is different from that of **2**. The aromatic protons of **1** appear in the same range (7.6–8.7 ppm) but some of them are shifted. In addition, diluting the sample (D_2O) induced more changes in the spectrum, possibly due to π-stacking of the extended planar pentacyclic ligand **1**. Exchanging the Cl^- ions of **3** for PF_6^- ions provided a green compound, the ^{1}H-NMR spectrum of which was identical to that of **2**.

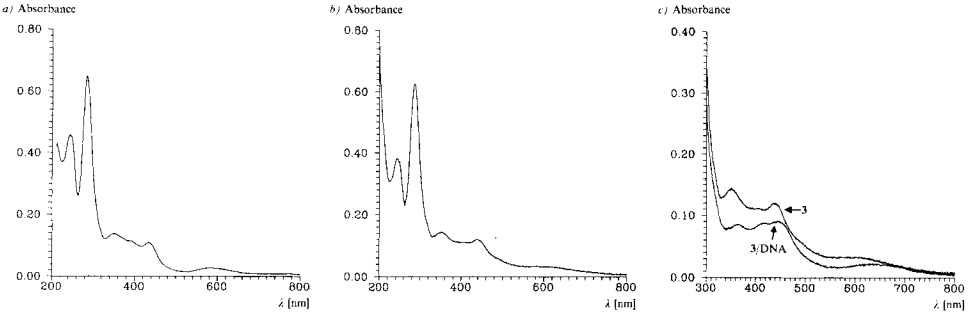


Fig. 2. *UV/VIS Absorption spectra of* a) **2** *in MeCN,* b) **3** *in H_2O, and* c) **3** (10 μM)*/calf-thymus DNA* (100 μM nucleotides) *in a buffered aqueous solution* (50 mM NaCl, 5 mM *Tris*, pH 7.4) *as compared to* **3** (10 μM) *in H_2O*

Table 2. *UV/VIS Absorption Data for Complexes* **2** *and* **3** (10 μM), *and for* **3** *in Presence of DNA*

2 (MeCN)		**3** (H_2O)		**3**/DNA[a])	
λ [nm]	ε	λ [nm]	ε	λ [nm]	ε
246	32100	244	38400	244	26800
284	42800	283	62300	283	47000
351	11464	352	14100	366	6200
394	8917			411	6600
436	7643	441	11800	450	6900
592	2641	600	3300	630	2500

[a]) **3** (10 μM) and calf-thymus DNA (100 μM nucleotides) in buffered aqueous solution (50 mM NaCl, 5 mM *Tris*, pH 7.4).

The UV/VIS spectra of **2** and **3** were measured in MeCN and H_2O, respectively (*Fig. 2, Table 2*). The spectrum of **3** in H_2O differs from that of **2** in MeCN in that the absorption band at 394 nm in **2** is not observed in **3**. The bands at 436 and 441 nm for **2** and **3**, respectively, may be attributed to metal to ligand transitions (metal-to-ligand charge transfer (= MLCT)) by analogy with the MLCT nature of the 452-nm band of $[Ru(bpy)_3]^{2+}$.

No fluorescence is observed for **2** or **3** upon excitation in the charge-transfer bands above 320 nm. This may be due to internal quenching of the well-known luminescence

of the excited state $[Ru(bpy)_3]^{2+}$*, possibly by generation of an excited state $[Ru^{III}(bpy)_2(\mathbf{1}^-)]^{2+}$* by electron transfer to the ligand **1** which may be expected to be a significantly stronger electron acceptor than bpy itself. Similar intracomplex quenching has, *e.g.*, been observed in the $[Ru(bpy)_2(dppz)]^{2+}$ (dppz = dipyridophenazine) complex in aqueous solution [4] and in complexes containing pyridinium acceptor groups linked to a $[Ru(bpy)_3]^{2+}$ unit [5–7].

3. Binding to Double-Stranded DNA. – Complex $[Ru\{(D_8)bpy\}_2(\mathbf{1})]^{2+}$ appeared to be a good candidate for binding to double-helical DNA due to the planarity of the extended pentacyclic ligand **1**.

As was pointed out above, $[Ru\{(D_8)bpy\}_2(\mathbf{1})]^{2+}$ (10 μM) shows no detectable luminescence in MeCN and in buffered aqueous solutions. It also remains nonfluorescent in the presence of calf-thymus DNA (100 μM nucleotides).

Significant perturbations are observed in the UV/VIS spectra that are in favour of intercalation into the calf-thymus DNA double helix [8]. Complex **3** in the presence of calf-thymus DNA in a buffered aqueous solution shows hypochromism and a shift to longer wavelength for the CT bands (above 320 nm) as compared to **3** in H_2O (*Table 2* and *Fig. 2*). Interestingly, the π–π* transitions at 244 and 283 nm are also decreased in intensity but do not experience any bathochromic shift in the presence of DNA like the CT bands do. An absorption band at 411 nm which is not present in the spectrum of **3** appears upon mixing with DNA.

Intercalation is also supported by 1% agarose gel electrophoresis (*Fig. 3*). Addition of increasing amounts of **3** (0.7–10 μM) to a buffered solution of pBR322 DNA (30 μM

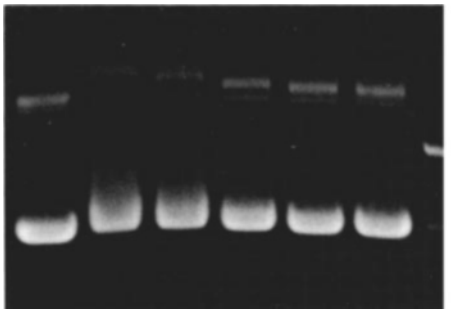


Fig. 3. Electrophoresis gel showing results of intercalation of complex **3** *into pBR322 DNA.* Control experiment in the absence of **3**, *Line 1; Lines 2–6* represent decreasing concentrations of **3** (10, 7, 3, 1, and 0.7 μM, resp.) in the presence of pBR322 DNA (30 μM nucleotides). The three bands observed from bottom to top in each line are the supercoiled, linear, and nicked (closed circular) forms, respectively, of the plasmid DNA.

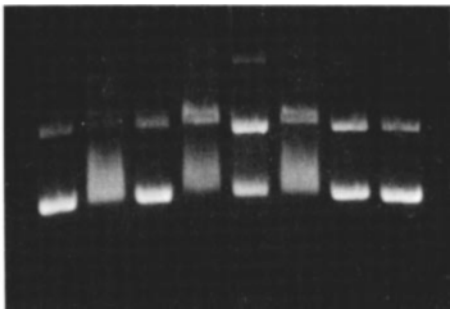


Fig. 4. *Electrophoresis gel showing results of photochemical cleavage of double-strand supercoiled circular DNA pBR322 into nicked DNA mainly, by irradiation in the presence of complex* **3** *or of* $[RuCl_2(bpy)_3]$. Control experiments show pBR322 DNA in the absence of any reagent without irradiation (*Line 1*) and after irradiation (*Line 8*); *Line 2:* pBR322 DNA and **3** without irradiation; *Line 3:* pBR322 DNA and $[RuCl_2(bpy)_3]$ without irradiation. *Lines 4–7* represent irradiation experiments of pBR322 (30 μM nucleotides) in the presence of **3** (*Line 4*, 10 μM; *Line 6*, 7 μM) or of $[RuCl_2(bpy)_3]$ (*Line 5*, 10 μM; *Line 7*, 7 μM).

nucleotides, 50 mM NaCl, 5 mM *Tris*, pH 7.4) results in an increased streaking and retardation of DNA (*Lines 6–2*). This change in electrophoretic mobility of DNA is probably due to lengthening and unwinding of the helix upon intercalation of complex **3** [8]. In contrast, $[RuCl_2(bpy)_3]$ itself does not lead to the same effects (compare *Lines 2* and *3* in *Fig. 4*). One may note that the parent ligand **1** itself might display DNA-intercalating properties, which could be related to its antileukemic activity [1]. Its insolubility in H_2O prevented a direct study of such an interaction.

The complex $[Ru(bpy)_2(dppz)]^{2+}$ has also been found to interact with DNA with, however, restoration of its fluorescence [9].

4. Photochemical Cleavage of DNA. – Complex **3** was found to effect photocleavage of pBR322 DNA under irradiation with VIS light. The experiments were performed by illumination for 1 h with a mercury lamp (glass-water cut-off filter; 1000 W) of a solution containing the supercoiled circular double-strand DNA pBR322 (30 μM nucleotides) as well as **3** (10 μM) in buffered aqueous solutions (50 mM NaCl, 5 mM *Tris*, pH 7.4). DNA cleavage was analysed by 1% agarose gel electrophoresis, monitoring the conversion of supercoiled DNA into nicked DNA and linear DNA (*Fig. 4*). At the concentrations used, the supercoiled pBR322 DNA gives nicked circular DNA by cleavage of one strand (*Line 4*). At lower concentrations of **3**, less and less cleavage occurs, the transformation into the nicked circular form decreasing from 10 to 7 μM (*Lines 4* and *6*, resp.). *Line 8* shows that no DNA cleavage occurs upon irradiation in the absence of complex **3**. As already mentioned above, the migration of pBR322 is retarded by the intercalation of **3** (*Line 2*). On the contrary, the migration of pBR322 is not affected by the presence of 10 μM $[RuCl_2(bpy)_3]$ (*Line 3*) which indicates a different way of binding. The photoactive cleavage of 30 μM supercoiled pBR322 by $[RuCl_2(bpy)_3]$ gives nicked circular DNA [10]. Thus, complex **3** is a moderately efficient DNA photocleavage reagent, comparable to $[RuCl_2(bpy)_3]$ upon irradiation with VIS light.

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Experimental Part

General. UV/VIS spectra: MeCN and H_2O solns.; *Cary-3* spectrometer. ^{1}H-NMR spectra: CD_3CN or D_2O solns.; *Bruker-AC-200* spectrometer operating at 200 MHz; δ in ppm; Fast atom bombardment (FAB)-MS: matrix, 3-nitrobenzyl alcohol; *ZAB-HF-VG* spectrometer.

*Materials. 6-Bromo-9*H*-quino[2,3,4-*de*][1,10]phenanthrolin-9-one* (**1**) was obtained from natural sources [1]. In view of the very small quantity available (*ca.* 4 mg), **2** and **3** were characterised by their spectral properties; microanalytical data could not be obtained. The synthesis of **1** has been described recently [11]. (D_8)Bpy [3], $[cis\text{-}RuCl_2\{(D_8)bpy\}_2] \cdot 2H_2O$ [3] [12], and $[RuCl_2(bpy)_3]$ were prepared according to literature procedures. Type-I calf thymus DNA and pBR322 were obtained respectively from *Sigma* and *Boehringer Mannheim*.

Bis[2,2'-($^2H_8$*)bipyridine](6-bromo-9*H*-quino[2,3,4-*de*][1,10]phenanthrolin-9-one)ruthenium(II) Hexafluorophosphate* (**2**). A mixture of 1 mg ($2.8 \cdot 10^{-6}$ mol) of **1** and 1.9 mg ($3.1 \cdot 10^{-6}$ mol) of $[cis\text{-}RuCl_2\{(D_8)bpy\}_2] \cdot 2H_2O$ in 1 ml of EtOH/H_2O 1:1 was refluxed for 15 min. After cooling and addition of 2.2 equiv. of NH_4PF_6 dissolved in a minimum amount of H_2O, filtration provided 2.9 mg (97%) of pure green solid **2**. ^{1}H-NMR and H,H COSY (CD_3CN): *Fig. 1* and *Table 1*. FAB-MS: 938, 792, 627, 428.

Chloride **3**. A mixture of 2 mg ($5.6 \cdot 10^{-6}$ mol) of **1** and 3.8 mg ($6.2 \cdot 10^{-6}$ mol) of $[cis\text{-}RuCl_2\{(D_8)bpy\}_2] \cdot 2H_2O$ in 1 ml of DMF was heated at 150° for 3 h. After cooling and concentration of the mixture, a sat. soln. of Et_4NCl

was added until precipitation of a green solid. Filtration and abundant washing with acetone afforded 3.6 mg (76%) of **3**. FAB-MS: 794, 629, 465, 428.

Exchanging the Cl^- ions of **3** for PF_6^- ions by addition of 2 equiv. of NH_4PF_6 dissolved in a minimum amount of H_2O to an aq. soln. of **3** provided a green precipitate. ^{1}H-NMR: identical to that of **2**.

Intercalation Assay. A 30 μM nucleotides soln. of pBR322 with various concentrations of **3** in 15 μl of 50 mM NaCl/5 mM *Tris* pH 7.4 was submitted to 1% agarose gel electrophoresis followed by staining with ethidium bromide.

Photocleavage Assay. Photo-activated cleavage of pBR322 (30 μM nucleotides) with various concentrations of **3** (or $[RuCl_2(bpy)_3]$ in 15 μl of 50 mM NaCl/5 mM *Tris* pH 7.4 was conducted by irradiation for 1 h at 4° with a mercury lamp (glass-water cut-off filter, 1000 W). Samples were subjected to 1% agarose gel electrophoresis followed by staining with ethidium bromide.

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