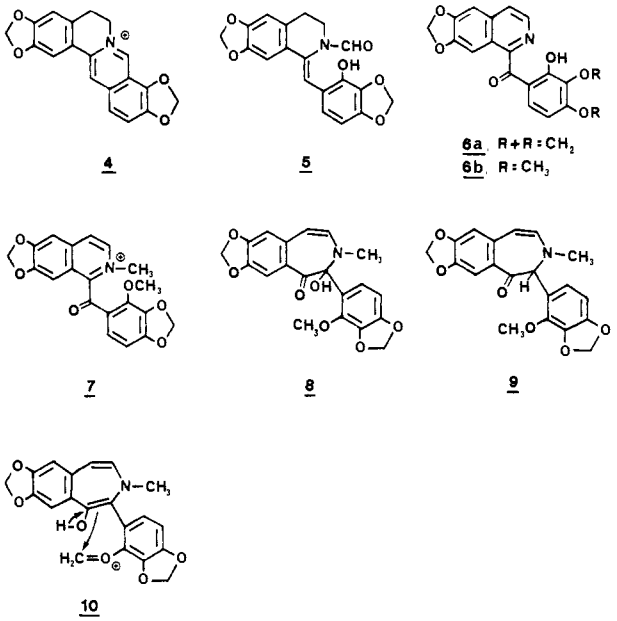
by 2.9% and H-12 (δ 6.73) by 15.3%. The latter NOE was critical in confirming that the ring-D methylenedioxy was located at C-9,10 rather than at the alternate C-11,12 site. Turning now to the minor compound **3**, $[\alpha]^{23}_D$ +409° (c 0.0007, $CHCl_3$), four NOE's were recorded following irradiation of the pseudoaxial H-14. The stronger two concerned the acidic C-14 alcoholic hydrogen at δ 3.12 (10.7%) and the N-methyl singlet at δ 2.62 (7.1%). Weaker effects were observed for the H-1 singlet at δ 6.94 (3.5%) and the H-15β doublet at δ4.15 (0.9%).[7]

The biogenesis of (+)-turkiyenine (**1**) appears to be radically different from that of any other isoquinoline-derived alkaloid. The origin of turkiyenine may hypothetically be traced to the pseudobenzylisoquinoline **5** which could be obtained biogenetically from the protoberberinium alkaloid coptisine (**4**).[8] Hydrolysis and



oxidation of **5** would yield pseudobenzylisoquinoline **6a**, structurally related to the known rugosinone (**6b**).[9] O,N-Dimethylation of **6a** would provide salt **7**, which through base-catalyzed isomerization could lead to pseudobase **8**. Reduction of **8** would give rise to ketone **9**. Enolization of this ketone, and oxidation of the aromatic methoxyl group to an oxonium ion as in **10**, would then set the stage for the formation of the methylenoxy bridge[10] and the alkaloid (+)-turkiyenine (**1**).

It has been previously suggested that the intermediacy of pseudobenzylisoquinolines could be one of several possible ways by which 8,9,10-oxygenated aporphines may be formed in nature.[8] It is now apparent that pseudobenzylisoquinolines may also be implicated in the biogenesis of the unusual base (+)-turkiyenine (**1**).

Acknowledgment. This research was supported by NSF Grant CHE-8210699.

(7) (-)-Dihydroturkiyenine (**2**): ν_{max} ($CHCl_3$) 3350 cm^{-1}; λ_{max} MeOH 223, 288, 309 sh, 319 nm (log ϵ 4.47, 3.92, 3.83, 3.76); MS, m/z 365 $(M-2)^+$ (0.4), 364 (0.4), 351 (49), 323 (45), 322 (96), 320 (100), 308 (87). (+)-Epidihydroturkiyenine (**3**): ν_{max} ($CHCl_3$) 3550 cm^{-1}; λ_{max} MeOH 226, 286, 313 nm (log ϵ 4.44, 3.86, 3.79); MS, m/z 365 $(M-2)^+$ (0.3), 363 (0.3), 362 (1), 323 (46), 322 (100), 320 (47), 308 (96). NMR spectra for **2** and **3** are in CD_3CN.

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Synthesis and DNA Binding and Photonicking Properties of Acridine Orange Linked by a Polymethylene Tether to (1,2-Diaminoethane)dichloroplatinum(II)

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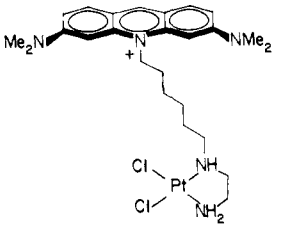
Received June 1, 1984

There is much current interest in the binding of the antitumor drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP) to its putative target in the cancer cell, DNA.[1] Both the position and mode of binding of *cis*-DDP to DNA can be altered by the presence of the intercalating dye ethidium bromide in the incubation medium.[2] Moreover, prior coordination of *cis*-DDP or $[Pt(en)Cl_2]$ (en = 1,2-diaminoethane) is known to affect the binding of intercalators to DNA.[3] We were therefore interested to construct a molecule in which both an intercalating functionality and a diamine-coordinated $\{PtCl_2\}$ moiety are connected by an appropriate linker chain and to study its DNA binding and cleaving properties. There is precedence for compounds containing both intercalator and metal-binding functionalities in the naturally occurring antitumor antibiotic bleomycin,[4] in a family of synthetic molecules designed as footprinting agents,[5] and in certain metallointercalation reagents such as [Pt(terpy)Cl]Cl.[3a] Here we report the synthesis, characterization, DNA binding, and photoactivated DNA cleaving (nicking) properties of cation **1**, in which acridine orange is linked by a hexamethylene chain to (1,2-diaminoethane)dichloroplatinum(II).

Compound **1** was synthesized in overall 18% yield by the following nine-step procedure. The hydroxyl group of 6-chloro-1-



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hydroxyhexane was protected with dihydropyran,[6] following which the chloro group was converted to an iodo group by a Finkelstein reaction.[7] Alkylation of acridine orange free base[8] by refluxing in xylene with a trace of $NaHCO_3$, deprotection of the alcohol,[9]

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and recrystallization from isopropyl alcohol/methanol gave dark red needle crystals of intermediate **2**, 3,6-bis(dimethylamino)-10-(6-hydroxyhexyl)acridinium iodide. By treatment of **2** with 48% HBr at 95 °C, the hydroxyl group was replaced with a bromo group.[10] Addition of 1,2-diaminoethane in dry methanol under nitrogen[11] introduced the chelating chain into the cation. Recrystallization from HCl-saturated dry ethanol yielded intermediate **3**, 3,6-bis(dimethylamino)-10-[6-((2-aminoethyl)amino)-hexyl]acridinium chloride–tetrahydrochloride, as a bright red microcrystalline solid.[12] Platinum was next introduced into the chelate ring by a modification of Dhara's procedure (K_2PtI_4/DMF/H_2O)[13] followed by methathesis ($AgNO_3$/dilute HCl) to produce the chloride salt of **1**.[14] Solubility tests revealed this salt to be highly water soluble, >20 mg/mL.

Examination of CPK models of **1** and the DNA double helix indicated that, with platinum bound to the bases, the acridine ring is capable of intercalating at a distance of one to two base pairs from the platinum binding site. Superhelical DNA was used to study the DNA binding properties of **1** since this assay is known to be a sensitive monitor of alterations in the double helix produced both by intercalators[15] and by covalent binding platinum complexes.[3a,16] Figure 1a,b displays the results of standard gel electrophoretic analyses[16b] of the binding of **1** and [Pt(en)Cl_2], respectively, to a mixture of supercoiled (form I) and nicked (form II) closed circular pBR322 DNAs.[17] Both platinum complexes unwind and rewind form I DNA by binding covalently to the bases in a manner similar to that observed for *cis*- and *trans*-DDP.[16] The coalescence point, where the nicked and superhelical DNAs comigrate in the electrophoresis gel, corresponds to a drug/nucleotide (D/N) ratio of 0.052 (9) for **1**, nearly half the D/N value of 0.11 (3) for [Pt(en)Cl_2]. This result clearly establishes that covalent binding of platinum to DNA does not interfere with the ability of the tethered acridine orange moiety to interact with the DNA and cause additional duplex unwinding. This interaction is probably intercalative, but since acridine orange stacks with single-stranded polynucleotides,[18] further experiments are required to establish the binding mode.

Figure 1a,b also reveals that the mobility of the nicked DNA band increases with increased platinum binding for DNA-containing bound [Pt(en)Cl_2] but not **1**. This increased mobility of form II DNA in the presence of [Pt(en)Cl_2] is indicative of shortening of the DNA helix.[16] The failure of **1** to shorten the DNA is consistent with the previous observation[2b] that addition of ethidium bromide to the incubation mixture during platination of DNAs with *cis*-DDP diminishes the shortening effect. The presence of a nearby, tethered intercalator in **1** prevents the platinum binding mode responsible for the shortening.

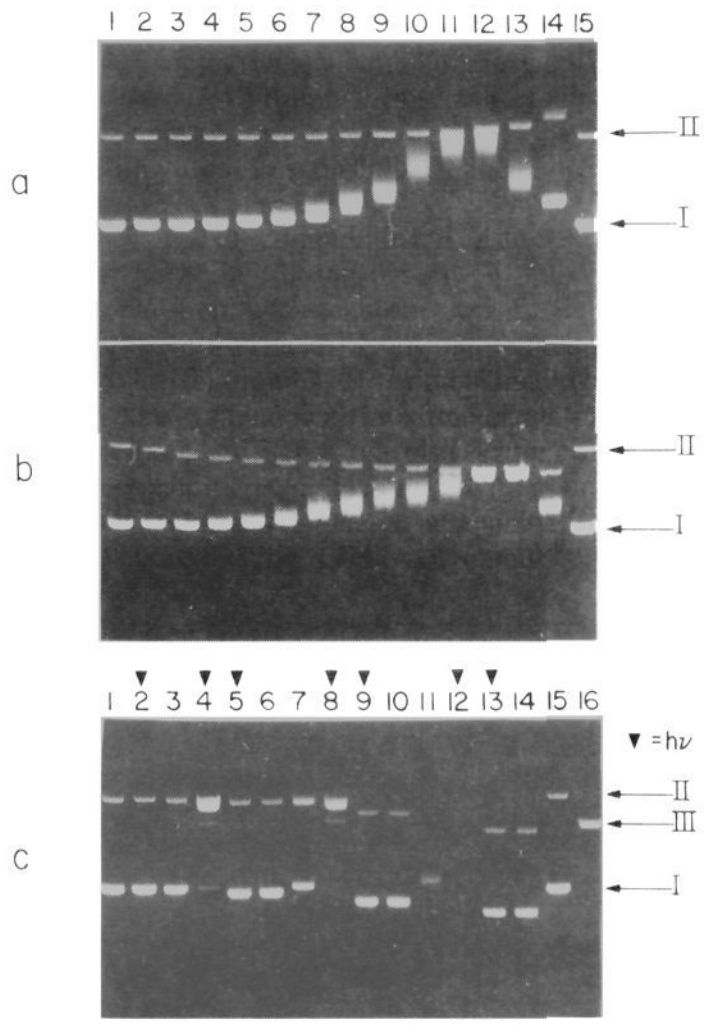

Figure 1. (a) Titration of a mixture of nicked (II) and closed (I) circular pBR322 DNAs with **1**. D/N levels for lanes 1–15: 0.0, 0.0026, 0.0054, 0.0061, 0.014, 0.019, 0.019, 0.023, 0.027, 0.035, 0.043, 0.061, 0.087, 0.190, and 0.0, respectively. Gel electrophoresis was performed in the dark using 50 mM Tris, 50 mM boric acid, and 2.5 mM EDTA pH 8.0 as the buffer and 1% agarose gels at 8 °C and at 60 V for 22 h; 0.25 μg of DNA was loaded in each lane. Gels were stained with a 0.5 μg/mL solution of ethidium bromide before photographing. (b) Titration of forms I and II pBR322 DNAs with [Pt(en)Cl_2]. D/N levels for lanes 1–15: 0.0, 0.0085, 0.018, 0.023, 0.030, 0.036, 0.043, 0.051, 0.064, 0.062, 0.080, 0.092, 0.141, 0.198, and 0.0, respectively. Gel electrophoresis conditions are the same as in (a) except that light was not excluded. (c) Demonstration of DNA nicking by **1** following visible-light irradiation. Lane identification: 1, 2, 15, control DNAs I and II; 3, 4, **1** at D/N = 0.023; 5, 6, [Pt(en)Cl_2] at D/N = 0.016; 7, 8, **1** at D/N = 0.091; 9, 10, [Pt(en)Cl_2] at D/N = 0.045; 11, 12, **1** at D/N = 0.228; 13, 14, [Pt(en)Cl_2] at D/N = 0.121; 16 linear (form III) pBR322 DNA, produced by EcoRI digestion. Gel conditions are as in (a) except 0.3 μg/mL of ethidium bromide were added to both the gel and the running buffer. Samples in the lanes marked by an arrowhead were illuminated as described in the text.

Acridine orange is known to nick DNA in the presence of light.[19] As shown in Figure 1c, compound **1** is also capable of nicking DNA upon irradiation. Using a focused 300-W visible-light source[20] we found that a 45-s exposure was sufficient to convert most of the supercoiled to nicked DNA and to produce some linear DNA at the relatively low D/N ratio of 0.023 (lane 4). More extensive cleavage occurs upon irradiation of samples with higher D/N ratios (lanes 8 and 12). Controls (Figure 1c) indicate that, under identical conditions, no nicking occurs upon irradiation of platinum-free DNA or DNA containing bound [Pt(en)Cl_2]. Given the efficiency of the nicking reaction, it should be possible to use

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the acridine orange moiety of **1** as an internal, photoactivated "molecular scissors" to map platinum binding sites on ^{32}P-end-labeled DNA restriction fragments, in a manner complementary to our previous mapping of *cis*-DDP binding to DNA using exonuclease III.[2a,21]

In summary, the intercalator-linked platinum complex **1** has been synthesized in good yield. From its DNA binding and light-activated nicking properties, **1** should prove to be a useful probe of the regioselectivity and stereospecificity of diaminedichloroplatinum(II) binding to DNA. The similarity of **1** to the antitumor drug *cis*-DDP makes it potentially useful for probing aspects of the biological mechanism of action of anticancer platinum compounds.

Acknowledgment. This work was supported by U.S. Public Health Service Grants CA 15826 (at Columbia University) and CA 34992 (at MIT) awarded by the National Cancer Institute, Department of Health and Human Services. B.E.B. is a Postgraduate Fellow of the Canadian Natural Sciences and Engineering Research Council. We thank Engelhard Industries for a loan of K_2PtCl_4 from which the platinum compounds were synthesized.

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Observation of 1000-fold Enhancement of ^{15}N NMR via Proton-Detected Multiquantum Coherences: Studies of Large Peptides

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The severe sensitivity limitations associated with the observation of ^{15}N NMR signals, especially at natural abundance, make any improvement in sensitivity of considerable importance.[2,3] The potential advantages of using indirect detection via protons for nuclei like ^{15}N and ^{13}C over conventional direct detection methods have been recognized,[4-8] but the enhancement has not been explicitly measured.[8] It is important to know if the theoretical enhancement over simple direct detection $(\gamma_{^1H}/\gamma_{^{15}N})^3$, about 1000-fold, can be achieved, and if so, whether this can be done routinely on samples of interest at natural abundance.

We report here the quantitative determination of the enhancement under typical conditions for biological macromolecules using a 50 mM solution of 2-pyrrolidinone (I) in water and also demonstrate the application of this method to an experimentally demanding case, showing chemical shift correlation of amide protons and nitrogen resonances in the 28-residue peptide thymosin α_1 at 30 mM in water. Both samples were at natural abundance for ^{15}N.[9]

The spectra of I with ^{15}N detection using INEPT[2] and with indirect ^1H detection of ^{15}N satellites using multiquantum co-

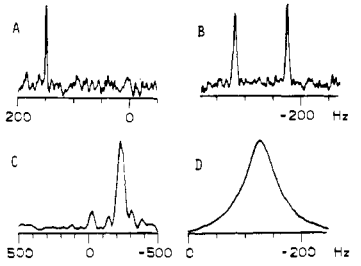

Figure 1. Comparison[9] of direct ^{15}N and indirect proton-detected spectra of pyrrolidinone (I) (50 mM, 95%H_2O/5%D_2O). (A) Directly detected INEPT[2,3] spectrum of 20-mm o.d. sample, active volume 10 mL, 2180 scans in 28 min, $S/N = 9$. (B) ^1H-detected ^{15}N spectrum of I taken in a 12-mm tube in a modified 12-mm ^1H probe, active volume 1 mL, 128 scans in 0.8 min, $S/N = 11$. The signals are at the position of the ^{15}N satellites. (C) ^{15}N projection of a complete two-dimensional data set for I, as in B. Total accumulation time 13 min (16 blocks), $S/N = 70$. (D) ^1H spectrum of I using Redfield pulse acqusition.

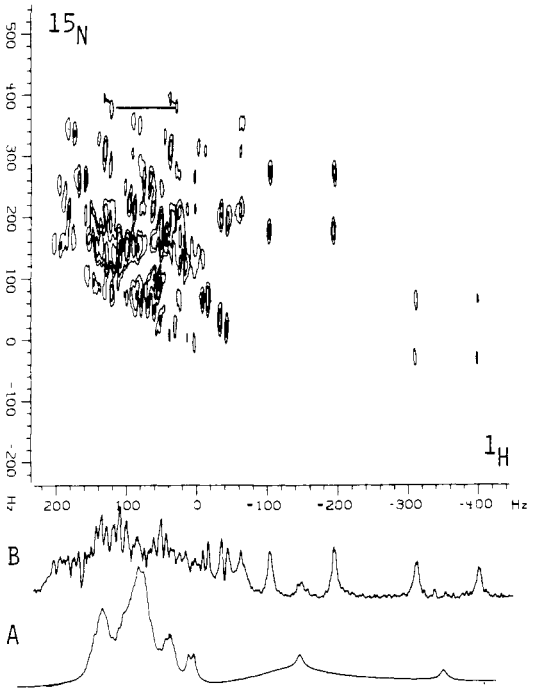

Figure 2. Two-dimensional $^1H/^{15}N$ chemical shift correlation for thymosin α_1, via proton-detected double-quantum (nonconservative) coherence. Sample was 30 mM in 95% H_2O/5%D_2O, at pH 5.4. Total accumulation time about 12 h. The zero-frequency points are the instrumental synthesizer frequencies and correspond to 8.04 ppm (from Me_4Si, ^1H) and 125.3 ppm (from NH_3, ^{15}N). Chemical shifts in the ^{15}N direction are given by $(\Delta_H - \Delta_N)$, cf. ref 8, for this cycling scheme, and the negative value of γ_N. Each amide proton gives a set of four peaks in the contour plot arising from the large ^1H-^{15}N coupling and the smaller coupling of amide to H^α. One such set is connected by a line in the upper left. (A) Redfield pulse ^1H spectrum of the amide region. (B) ^1H (f_2) projection of the two-dimensional data set and a contour plot of the data set. Each amide appears as four lines from couplings to $^1H^\alpha$ and to ^{15}N.

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