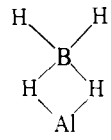
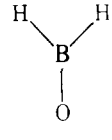
for bidentate species (between 2375 and 2550 cm^{-1});[6] yet the presence of more than two peaks, as well as the observation of four features in the B-H_b stretching region (at 2142, 2177, 2230, and 2252 cm^{-1}), indicates that there is either more than one type of surface species or strong intramolecular vibrational coupling among BH_4 groups attached to the same Zr atom. The former explanation is consistent with the assignments of features at 693 and 910 cm^{-1} to Zr-O stretching modes of multiply (bulk-like) and singly (or, at least, less highly) coordinated Zr atoms.[8,9] Additionally, some of the BH_4 ligands displaced during adsorption might remain on the surface, forming complexes A and B. Similar surface species have been



A



B

observed for diborane (B_2H_6) adsorption on Al_2O_3[10] and could also be expected to produce additional structure in the B-H stretching regions. Modes appearing at 1106, 1130, 1173, 1220, and 1260 cm^{-1} (and possibly 1030 cm^{-1}) can be assigned to deformations of the metal-BH_4 structure.[7] An AlH_2BH_2 deformation might also contribute to structure near 1457 cm^{-1}.[10] The 1378-cm^{-1} peak has been previously assigned to a B-O stretch, and a B-O vibration might also contribute to the intensity of the 1260-cm^{-1} peak.[10] Unresolved structure between 480 and 600 cm^{-1} can be assigned to Zr-B skeletal stretching modes,[11] with possible contributions from additional Zr-O vibrations.[8] Low energy features near 264 and 323 cm^{-1} probably arise from BH_4-Zr-BH_4 bending and Zr-BH_4 torsional modes.[7]

Although this is by no means a complete characterization of alumina-supported $Zr(BH_4)_4$ (the details of more extension results characterizing the supported complex as a function of temperature, as well as its interaction with D_2, D_2O, H_2O, C_2H_4, C_3H_6, and C_2H_2 will be reported later), this preliminary communication does indicate the versatility of IETS and clearly demonstrates the value of information that can be obtained by applying IETS to the study of supported complexes. This work represents an important extension of IETS beyond the study of adsorption either on oxide surfaces[3] or on reduced metallic particles evaporated onto oxide supports.[12-15]

Acknowledgment. We thank Dr. F. N. Tebbe and Dr. L. E. Firment both for useful correspondence as well as for supplying the $Zr(BH_4)_4$. This research was supported by the National Science Foundation under Grant No. ENG78-16927. Additional support from the Camille and Henry Dreyfus Foundation in the form of a Teacher-Scholar award to W.H.W. is also greatly appreciated.

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Enhanced CD Spectra in *cis*-Bis(nucleotide)platinum(II) Complexes. Comparison with the CD Enhancement Induced in DNA by the Anti-Tumor Agent, *cis*Pt

Sir:

We have observed a marked enhancement of the CD spectrum of 5'-GMP or 5'-dGMP on coordination of two molecules of either nucleotide to cis positions of complexes which are derivatives of *cis*Pt (≡*cis*$Pt^{II}(NH_3)_2Cl_2$).[1] The latter species is gaining widespread clinical use as an anti-tumor agent.[2] After assessing numerous biological and biochemical studies on *cis*Pt, Roberts and Thomson[3] have concluded that DNA is the important molecular target for the anti-tumor activity of *cis*Pt and have suggested that the effective interaction of *cis*Pt with DNA is an intrastrand cross-link. Numerous lines of evidence indicate that guanosine moieties in DNA are attacked first by Pt(II) complexes such as *cis*Pt[4] when Pt/DNA ratios are low. Several studies on small molecules, the first of which was on bis(guanosine)ethylenediamineplatinum(II) nitrate,[5] verify that two adjacent guanine bases can coordinate to Pt(II) via N-7.[4] Other lines of evidence demonstrate that intrastrand cross-linking between adjacent guanosine moieties does occur in Pt-DNA.[6] However, a clear structural relationship between well-defined N-7-bound *cis*-bis(6-oxopurine)platinum(II) complexes and the possible intrastrand cross-link has not been established because of the difficulty involved in either crystallizing nucleic acids or of obtaining NMR spectra on Pt-DNA.[1]

A dramatic effect of low concentrations of *cis*Pt on the CD of polynucleotides was reported by Eichhorn and his coworkers[7,8] and later confirmed by others.[9] At low Pt/base ratios, there is a marked enhancement in the CD spectrum of the polynucleotide and, for calf thymus DNA, there is a positive increase in the CD at ~275 nm. No such effect is observed for the trans isomer of *cis*Pt[7,8] (or for other Pt(II) complexes which do not have cis leaving groups[9]). The trans isomer is not an anti-tumor agent. The magnitude of the enhancement [$(\epsilon_L - \epsilon_R) \sim 5$-$7.5$ M^{-1} cm^{-1} per mole of affected nucleotide] increases with increasing GC content of the polynucleotide.[8,9] The guanosine moieties are probably playing a role in the enhancement.

In Figure 1, we present the CD spectrum of the complex sodium bis(5'-GMP)tnPt^{II}. The maximum at 284 nm has a value of $(\epsilon_L - \epsilon_R)$ 4.6 M^{-1} cm^{-1} per nucleotide. The strength of this band closely approximates the magnitude of the effect of *cis*Pt and other Pt(II) compounds with cis leaving groups on the CD of DNA in the 275-nm region of DNA, when allowance is made for the low Pt/base ratios used to observe the enhanced CD.

The two tn complexes, for which spectra are presented in Figure 1, were obtained as microcrystalline powders and were characterized by ^1H and ^{13}C NMR (Table I), by UV as well as CD spectroscopy (Table II), and by elemental analysis (Table I). The shift pattern obtained for resonances assignable to the heterocyclic ring closely resembles that found for Pt(II)

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Table I. NMR Spectral Data for Guanosine Derivatives[a]

	^{1}H NMR		^{13}C NMR				
	H-8	Δ^b	C-6	C-4	C-2	C-8	C-5
5′-GMP	4.42		92.18	87.31	84.83	70.89	49.33
bis(5′-GMP)tnPt^{2-} [c]	4.93	0.51	90.26	88.41	83.98	73.09	47.33
(5′-GMP)dienPt[d]	5.12	0.70					
bis(Guo)tnPt^{2+} [e]	4.72		90.14	87.89	83.92	73.14	47.64
(Guo)dienPt^{2+} [f]	4.79		90.28	87.79	84.02	73.36	48.23
5′-dGMP	4.49						
bis(5′-dGMP)tnPt^{2-} [d]	4.99	0.50					

[a] In D_2O, parts per million downfield from dioxane. ^{1}H NMR and ^{13}C NMR spectra were obtained on JEOL MH100 and Varian CFT20 instruments, respectively. [b] Difference in shift between coordinated and free ligand. Guo insufficiently soluble for spectral measurement. [c] Anal. Calcd for $Na_2[(5'\text{-GMP})_2tnPt]\cdot 11H_2O$ ($C_{25}H_{56}N_{12}Na_2O_{27}P_2Pt$): C, 22.37; H, 4.57; N, 13.60; P, 5.01. Found: C, 22.44; H, 4.63; N, 13.64; P, 5.10. [d] Did not crystallize. [e] Anal. Calcd for $[(Guo)_2tnPt](NO_3)_2\cdot 3.5H_2O$ ($C_{23}H_{43}N_{14}O_{19.5}Pt$): C, 27.02; H, 4.24; N, 19.17. Found: C, 26.94; H, 4.22; N, 19.19. [f] Guo is known to be coordinated via N-7; see ref 14.

Table II. UV and CD Spectral Data for Guanosine and Guanosine Derivatives[a]

	UV, $10^{-4}\epsilon$ M^{-1} cm^{-1} (λ_{max})	CD,[b] ($\epsilon_L - \epsilon_R$) M^{-1} cm^{-1} (λ_{max})
5′-GMP	1.2 (252)	0.12 (284), −0.8 (245), 2.0 (212)
bis(5′-GMP)tnPt^{2-}	1.2 (257)	4.6 (284), −3.4 (253), 3.0 (223)
(5′-GMP)dienPt[c]	1.1 (258)	−0.64 (282), −0.9 (246)
Guo	1.0 (252)	−0.8 (248), 2.2 (211)
Bis(Guo)tnPt^{2+}	1.0 (260)	−0.76 (233)
(Guo)dienPt^{2+}	1.1 (258)	
5′-dGMP	1.4 (251)	−0.2 (283), −1.8 (248), 2.8 (208)
bis(5′-dGMP)tnPt^{2-} [c]	1.1 (257)	3.8 (283), −5.2 (250), 3.1 (224)

[a] Cary 14 and Cary 60 instruments (see Figure 1). [b] Maxima or minima except for long wavelength value of free ligand, where values at enhancement wavelengths are also quoted. [c] Intensities approximate since these species did not crystallize.

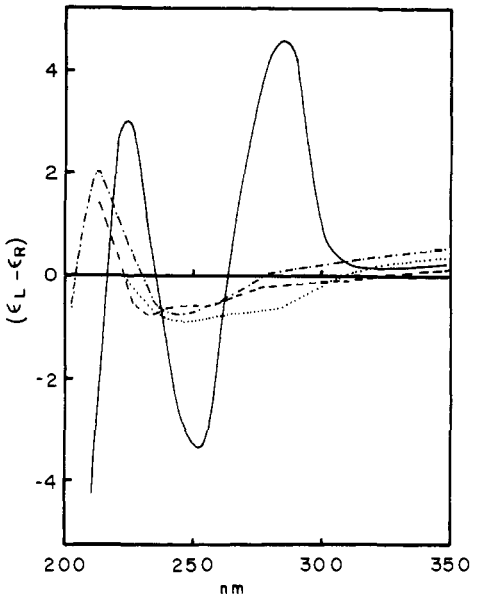

Figure 1. Aqueous solution CD spectra (pH 7) of bis(5′-GMP)tnPt^{2-} (—), bis(Guo)tnPt^{2+} (- - -), (5′-GMP)dienPt (• • •), and 5′-GMP (- • -). Solutions used for determining ($\epsilon_L - \epsilon_R$) were 2.0×10^{-4} M in nucleoside(tide). The values for the bis complexes would be twice as great if expressed in terms of Pt molarity. Spectra were obtained at 25 °C with 1-cm cells on a Cary 60 spectropolarimeter equipped with a Model 6002 CD attachment.

complexes of 6-oxopurines in which the purine is known from X-ray crystallography[10-14] to coordinate to Pt(II) via N-7 (Table I). The NMR spectra provide good evidence that the two GMP fragments in sodium bis(5′-GMP)tnPt^{II} are in identical environments (on the NMR time scale) since only one resonance is observed for each NMR active nucleus of the nucleotide. Preliminary studies with nonstoichiometric Pt^{II}–IMP complexes (which have been characterized by X-ray crystallography[10-13] as containing two identical N-7-bound *cis*-IMP molecules) indicate similar, although somewhat diminished, CD effects to that shown in Figure 1 for 5′-GMP. In this series, the $(NH_3)_2Pt^{II}$ derivative[12] and the tnPt^{II} derivative[13] have similar spectra.

Two complexes, which could not be crystallized but which can be shown to exhibit N-7 bonding by NMR, are of interest. The complex (5′-GMP)dienPt^{II} does not have an enhanced CD spectrum (Table II), whereas the complex ion (5′-dGMP)$_2$tnPt$^{II\ 2-}$ has an enhanced CD spectrum closely similar to that shown in Figure 1 for the 5′-GMP analogue, Table II. The value of ($\epsilon_L - \epsilon_R$) = 3.8 M^{-1} cm^{-1} at λ_{max} 283 nm is somewhat lower for the 5′-dGMP complex. As shown in Figure 1, the bis(guanosine)tn complex also does not exhibit an enhanced CD. Preliminary studies now in progress with 6-oxopurine derivatives have revealed enhanced CD spectra for cis-bis(nucleotide) complexes only.

The compounds studied here are the first examples of inert, isolated, metal–nucleotide complexes exhibiting these effects. In the only other report of enhancement of Cotton effects of metal nucleotide complexes,[15] it was observed that solutions of Zn(II) or Pb(II) salts and certain adenosine nucleotides (but not adenosine itself) exhibit enhanced CD spectra, some of which are analogous to those reported here. In these 1:1 probably dimeric complexes, the phosphate group is coordinated to the metal ion unlike in the 1:2 monomeric Pt compounds studied here. The specificity of the effect[15] is emphasized by the absence of enhancement with other nucleotides and other labile metal ions. High concentrations of guanosine nucleotides exhibit enhanced Cotton effects.[16]

The appearance of enhanced CD spectra for both the platinum(II)–cis-bis(nucleotide) complexes and platinum(II)–polynucleotide species suggests that structural relationships may exist between well-defined small molecular complexes and the geometry about the Pt(II) in Pt–DNA. In the absence of transition metals, the observation of enhanced CD for dinucleotides, oligonucleotides, and polynucleotides has generally been attributed to base stacking.[17] The Pt(II) center

is expected to restrict the possible relative orientations of the purine bases. More detailed analysis of the CD spectra of these and other nucleotide species coordinated to Pt(II) may provide better insight into the structure of the Pt–DNA complex which promotes the enhanced CD effect, and these compounds may be of value in theoretical studies of the effect of base stacking or metal binding on the CD spectra of nucleotide species.

Acknowledgments. The authors thank the NIH (GM 20544) for support and Matthey-Bishop, Inc., for a loan of K_2PtCl_4.

References and Notes

(1) Abbreviations used: dien = diethylenetriamine; GC = guanine cytosine; 5′-GMP and 5′-dGMP = 5′-guanosinemonophosphate and the 2′-deoxy analogue, respectively; Guo = guanosine; Pt–DNA = complex formed between *cis*Pt and DNA at low Pt/DNA; and tn = trimethylenediamine.
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^{252}Cf Plasma Desorption Mass Spectrometry of Palytoxin

Sir:

Palytoxin is the most powerful toxin among those obtained from marine animals.[1] There has been considerable interest in elucidating the structure of this molecule because of its unusual physiological and molecular properties.[2,3] Despite its high molecular weight (estimated to be ~3300),[4] there do not appear to be any repeating units of sugars and amino acids and its toxicity can be markedly reduced by subtle changes in its chemical structure. Recently, larger quantities of highly purified fractions of the toxin have become available and studies on structural details of pieces of the molecule are now being carried out using this material.[5,6] Previous attempts to determine the molecular weight by field desorption were hampered by sample purity problems.[7] Because of its high mass, low volatility, and thermal instability, this molecule represents one of the most difficult problems ever encountered for a mass spectrometric measurement of a natural product. We report here the molecular weight determination of highly purified palytoxin (M_1) by ^{252}Cf plasma desorption mass spectrometry (^{252}Cf PDMS). We also report molecular weight determinations for *N*-acetylpalytoxin (M_2) and *N*-acetylperhydropalytoxin (M_3).

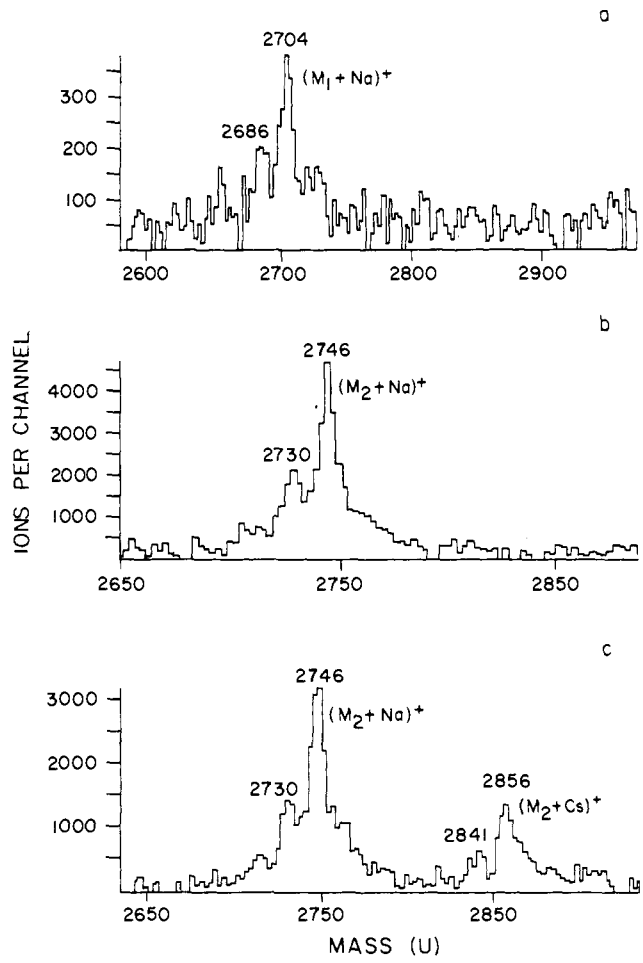

Figure 1. ^{252}Cf plasma desorption positive ion mass spectra of palytoxin and derivatives: (a) palytoxin, T = 10 000 s; (b) *N*-acetylpalytoxin, T = 112 000 s; (c) *N*-acetylpalytoxin deposited over a film of CsI, T = 50 000 s.

Details of the ^{252}Cf PDMS method are given in an earlier paper.[8] The system was optimized for high mass ion analysis by reducing the flight path of the time-of-flight mass spectrometer to 45 cm and operating the CEMA electron multiplier detectors at high gain for improved detection efficiency of high-mass ions. Palytoxin was isolated from *palythoa tuberculosa* and purified according to a new method published elsewhere.[5] Acetylation of palytoxin was carried out with *p*-nitrophenyl acetate in water containing a trace amount of pyridine giving *N*-acetylpalytoxin,[9] a species with considerably reduced toxicity. *N*-acetylperhydropalytoxin was formed by catalytic hydrogenation of the monoacetate with platinum oxide in aqueous ethanol. Thin deposits of palytoxin (~10 μg/cm^2) were prepared by direct evaporation of a 2-propanol–methanol–water solution onto a Ni foil (10^{-3} mm thick). More uniform deposits of the two derivatives which were much less toxic were made by an electrospray method[10] and were of an equivalent thickness. The samples were irradiated with a ^{252}Cf source giving a fission fragment (FF) flux in the samples of 2500 FF/(cm^2 s) for periods of up to 31 h. The mass region covered was from 0 to 5000 u. Positive and negative ion mass spectra were recorded, but only the positive ion spectra gave significant results.

Figure 1a shows part of the positive ion spectrum of palytoxin in the region of M = 2650–2900 u where a significant peak was detected at M = 2704 u. There was also evidence of a second smaller peak at M = 2686 u. No peaks were observed above M = 2900 u. The lower mass region showed a complex pattern of fragment ions extending to ~1600 u with few prominent lines. Figure 1b shows the spectrum obtained for

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