construction of a variety of peptides.

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DNA Binding Specificity of the Gold(III) Complex $(C_2H_5)_3PAuBr_3$

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Considerable attention has recently been focused on the antitumor agent *cis*-diaminedichloroplatinum(II) (cisplatin, **1**), which binds to purine sites located within the major groove of DNA. The origin for the antitumor effects of the compound lies in its ability to form an intrastrand cross-link between bases located on the same strand of DNA.[1] Compounds of gold have also been shown to have antitumor effects.[2] Studies attempting to uncover the chemical and biochemical events which may underlie the cytotoxicities of these agents have found that Au(I) and Au(III) complexes can bind to DNA in vitro and apparently can cleave DNA in cell culture.[3] In light of the close chemical similarity between the compounds of platinum and gold and the possibility that both may use DNA as a biological target site, we studied the binding of the square-planar Au(III) complex $(C_2H_5)_3PAuBr_3$ (**2**) to a *Hind III/NciI*, 139 base pair restriction fragment of pBR-322 DNA using DNA sequencing methodology.[4,5]

The sites of gold binding on the 3'-end-labeled restriction fragment[4] were uncovered by treating the metalated DNA with either dimethyl sulfate (DMS) or formic acid under mild conditions (37 °C for 10 min), followed by heating in the presence of excess piperidine to produce a strand break in the polymer.[6,7] The sites of strand scission in the absence and presence of various amounts of **2** were in turn determined via separation of the resulting oligonucleotide products by using high-resolution polyacrylamide gel electrophoresis and scanning of the resulting autoradiogram with microdensitometry.[5]

Densitometric scans of autoradiograms resulting from cleavage of the gold-bound restriction fragment with DMS/piperidine and HCO_2H/piperidine are shown in Figures 1 and 2. As is evident from the difference scan shown in Figure 1, the concentrations of oligomers resulting from DMS/piperidine treatment of the fragment decrease in the presence of **2**. This effect appears to be independent of sequence (all guanines are affected) and to increase with increasing concentration of **2**. Since the reaction of DMS with DNA results in methylation of the N-7 position of guanine, the most likely explanation for the observed gold-induced inhibition is that **2** is bound directly to N-7 of the heterocycle and is blocking alkylation of this site. A similar blocking effect has been previously observed for $[Pt(dien)]^{2+}$, where dien is diethylenetriamine, bound to the N-7 position of the nucleoside, guanosine.[8] This conclusion, binding of **2** to N-7 of guanine, was confirmed by examining the acid-catalyzed depurination of the fragment in the presence of the gold compound. As is evident from the difference scan shown in Figure 2, the gold complex

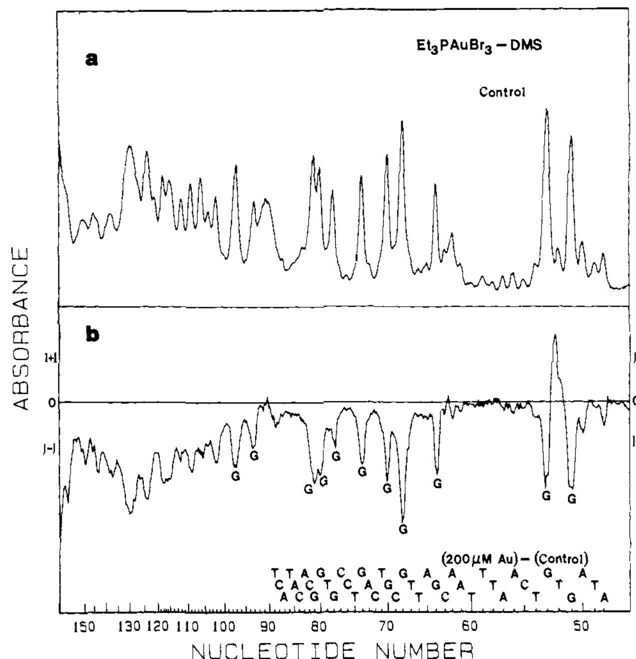

Figure 1. Microdensitometric scans of autoradiographic data involving dimethyl sulfate/piperidine treatment of the restriction fragment in the presence and absence of **2** are shown. Absorbance scan of the control (**a**), no **2**, and difference scan of 200 μM **2** – control (b). The small amount of background cleavage at all sites of the polymer, determined from controls, was due to DNA cleavage in the presence of only hot piperidine (Ambrose, B. J. B.; Pless, P. C. *Biochemistry* **1985**, *24*, 6194). The broad peak at position ~90 and the positive peak in the difference scan at position 52 were due to defects on the autoradiogram.

(1) (a) Lippard, S. J. *Science (Washington, D.C.)* **1982**, *218*, 1075. (b) Marcelis, A. T. M., Reedijk, *J. Recl. Trav. Chem. Pays-Bas* **1982**, *103*, 121. (c) Roberts, J. J.; Pera, M. P., Jr. In *Molecular Aspects of Anticancer Drug Action*; Neidle, S.; Waring, M. J., Eds., Academic: New York, 1983, p 183. (d) Dabrowiak, J. C.; Bradner, W. T. *Prog. Med. Chem.* **1987**, in press.

(2) (a) Lorber, A.; Simon, T. M. Loeb, J.; Peter, A.; Wilcox, S. A. *J. Rheumatol.* **1979**, *6* (suppl 5), 82. (b) Finkelstein, A. E.; Burrone, O. R.; Walz, D. T.; Misher, A. *J. Rheumatol.* **1977**, *4*, 245. (c) Mirabelli, C. K.; Johnson, R. K.; Hill, D. T.; Faucette, L. F.; Girard, G. R.; Kuo, G. Y.; Sung, C. M.; Crooke, S. T. *J. Med. Chem.* **1986**, *29*, 218.

(3) (a) Blank, C. E.; Dabrowiak, J. C. *J. Inorg. Biochem.* **1984**, *21*, 21. (b) Mirabelli, C. K.; Sung, S.-M.; Zimmerman, J. P.; Hill, D. T.; Mong, S.; Crooke, S. T. *Biochem. Pharm.* **1986**, *35*, 1427. (c) Mirabelli, C. K.; Zimmerman, J. P. Bartus, H. R.; Sung, C.-M.; Crooke, S. T. *Ibid.* 1435. (d) Pillai, C. K. S.; Nandi, U. S. *Biopolymers* **1978**, *17*, 709.

(4) Lown, J. W.; Sondhi, S. M.; Org, C.-W.; Skorobogaty, A.; Kishikawa, H.; Dabrowiak, J. C. *Biochemistry* **1986**, *25*, 5111.

(5) (a) Ward, B.; Skorobogaty, A.; Dabrowiak, J. C. *Biochemistry* **1986**, *25*, 7827. (b) Dabrowiak, J. C.; Skorobogaty, A.; Rich, N.; Vary, C. P. H.; Vournakis, J. N. *Nucleic Acids Res.* **1986**, *14*, 489. (c) Ward, B.; Rehfuss, R.; Dabrowiak, J. C. *J. Biomol. Struct. Dynam.* **1987**, *4*, 685.

(6) Maxam, A.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499.

(7) Metalation of the restriction fragment with **2** was carried out in a 50 mM Tris-Cl, pH 7.5 (0.1 mM EDTA) buffer in a total volume of 6 μL containing ~3 μM base pairs of the restriction fragment and **2** at four different concentrations in the range (2×10^{-4})–(2×10^{-7}) M. The stock solutions of **2** were freshly prepared in a 1:1 DMF/buffer solution. After incubation for 3 h at 37 °C, the gold-DNA solutions were treated with either 2 μL of 0.2% DMS in a 1:1 DMF/H_2O solution or 2 μL of a 20% aqueous solution of HCO_2H for 10 min at 37 °C. The reactions were terminated by addition of 12 μL of 20% aqueous piperidine and heating to 90 °C for 30 min. The resulting mixtures were frozen (−78 °C), lyophilized to dryness, and washed twice with 10 μL of methanol and the residue was taken up in 20 μL of the electrophoresis loading buffer,[5a] containing 100 mM mercaptoethanol. The 1:1 correspondence between the cleavage products and those produced by DNase I digests of the fragment indicated that the mercaptoethanol removed **2** from DNA. Control reactions in the absence of **2** confirmed that the aforementioned conditions resulted in ~80% uncleaved DNA, indicating that the products were statistically the result of a single cleavage on the full-length fragment. Electrophoresis, autoradiography, microdensitometry, and establishment of sequence were as earlier described.[4,5] The observed changes in oligonucleotide concentration were significant and well outside the limits of error of the measurement.[5b,c]

(8) Johnson, N. P.; Macquet, J. P.; Wiebers, J. L.; Monsarrat, B. *Nucleic Acids Res.* **1982**, *10*, 5255.

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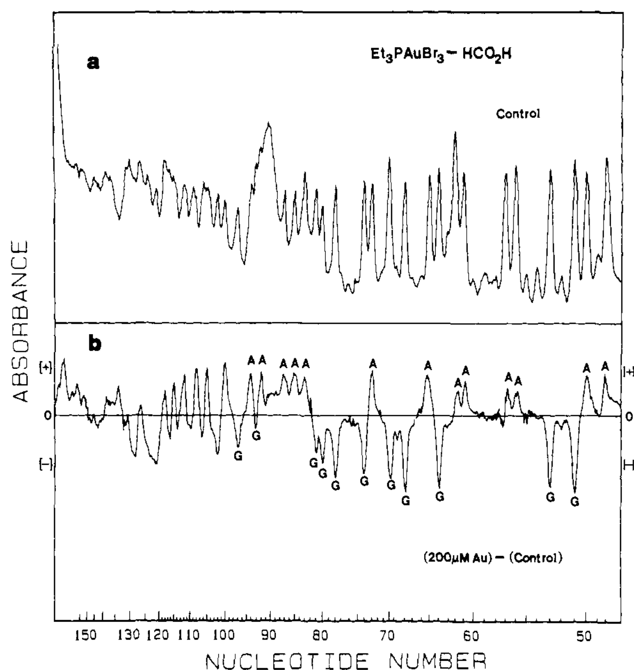

Figure 2. Microdensitometric scans of autoradiographic data involving HCO_2H/piperidine treatment of the restriction fragment in the presence and absence of **2** are shown. Absorbance scan of the control (a), no **2**, and the difference scan, 200 μM **2** − control (b).

inhibits formic acid induced cleavage at all of the guanine residues of the fragment. Since acid depurination initially involves protonation at the most basic site of the purine, in this case N-7,[9] gold binding to this site would inhibit protonation and thus the strand scission process. Figure 2 also shows that while cleavage at G is inhibited by **2**, the gold compound *enhances* cleavage at the adenine residues of the fragment. This phenomenon can be explained by gold binding to adenine and/or possibly thymine in such a manner which disrupts the Watson and Crick hydrogen bonding between A and T. Disruption of hydrogen bonding between these bases would expose adenine to facile protonation, ultimately resulting in a strand break in the polymer. A parallel can be found with the Au(III) complex $HAuCl_4$ which is believed to bind to all of the bases of DNA but shows an affinity for AT sites.[3d,10] This complex causes increased hyperchromicity upon binding to DNAs having a high (A + T) content, suggesting that base unpairing at the interaction site may be occurring.

The groups that are lost from **2** upon the binding of the complex to DNA are presently unknown. However, a single-crystal X-ray analysis of the compound revealed that the Au–Br bond *trans* to the phosphine ligand is longer (2.47 Å) than the remaining Au–Br bonds (2.41 Å) of the compound.[11] Thus, on the basis of the kinetic trans effect, the trans bromide should be the ligand which is most readily lost in the binding of **2** to DNA. However, a recent study[12] has shown that **2** reacts with deprotonated phthalimide (ptm) in nonaqueous media to yield as the major product the unexpected *cis* isomer, i.e., *cis*-$[AuBr_2(ptm)P(C_2H_5)_3]$. Ascertaining which ligands are lost from **2** and, if and to what extent the complex acts in a mono or bidentate fashion toward DNA will require further investigation.

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(9) Izatt, R. M.; Christenzen *Chem. Rev.* **1971**, *71*, 439.
(10) Gibson, D. W.; Beer, M.; Barrnett, R. J. *Biochemistry* **1971**, *10*, 3669.
(11) Eggleston, D. S.; Chodosh, D. F.; Hill, D. T.; Girard, G. R. *Acta Crystallogr., Sect. C* **1984**, *C40*, 1357.
(12) Price, S. J. B.; DiMartino, M. J.; Hill, D. T.; Kuroda, R.; Mazid, M. A.; Sadler, P. J. *Inorg. Chem.* **1985**, *24*, 3425.

A Diazirine Precursor for a Dioxacarbene: Generation and Reactions of Methoxyphenoxycarbene

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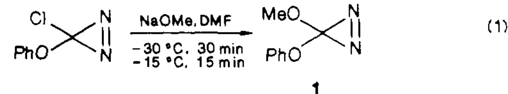
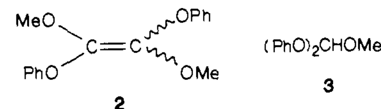
The archetypal nucleophilic carbene[2] dimethoxycarbene has been generated by pyrolyses of 7,7-dimethoxynorbornadienes[3] or hexamethoxycyclopropane[4] and by diisopropylethylamine deprotonation of the carbene's conjugate acid, dimethoxymethyl cation.[5] Much has thus been learned about the carbene's chemistry, but these generative methods require either elevated temperature or nonneutral conditions, precluding studies at low or ambient temperatures or in alkenic solvents, respectively. We now report the first instance of dioxacarbene generation from a diazirine precursor under mild and neutral conditions, as well as preliminary results of reactivity and theoretical studies that accord with anticipated nucleophilic properties.

In analogy to other halodiazirine exchange reactions,[6] reaction of 3-bromo-3-methoxydiazirine[7] with methoxide should furnish 3,3-dimethoxydiazirine, an ideal precursor for dimethoxycarbene. Preliminary studies suggested that this exchange did proceed but that the dimethoxydiazirine was unstable under the generative conditions.[8] Accordingly, we targeted 3-methoxy-3-phenoxydiazirine (**1**) as a more accessible dioxacarbene precursor. 3-Chloro-3-phenoxydiazirine[9] was converted[6] to **1** by stirring with 5-fold excess of fresh NaOMe in dry DMF, eq 1.



Diazirine **1** was extracted with cold pentane from a crushed ice/water quench of the reaction mixture, dried ($CaCl_2$, SiO_2), filtered, and concentrated under vacuum at −30 °C. Yields of yellow **1** ranged from 60% to 90%; λ_{max} (pentane) 362 nm; IR (CCl_4) 1545 cm^{-1} (N=N);[7] ^1H NMR (δ_{CCl_4}) 3.50 (OMe), 7.06 (C_6H_5). Monitored by UV, **1** in pentane decayed thermally at 25 °C with first-order kinetics, presumably to methoxyphenoxycarbene (MeOCOPh); $k = 1.81 \times 10^{-4}$ s^{-1}, $\tau_{1/2} = 64$ min, $E_a \sim 20$ kcal/mol ($20 < T < 50$ °C). The decomposition products (90%) were the cis and trans MeOCOPh dimers **2** and diphenyl methyl orthoformate (**3**) in a 45:45:10 distribution.



(1) Visiting Scientist on leave from the Politechnika, Warsaw.

(2) Moss, R. A. *Acc. Chem. Res.* **1980**, *13*, 58.
(3) Hoffmann, R. W. *Acc. Chem. Res.* **1985**, *18*, 248. Hoffmann, R. W.; Reiffen, M. *Chem. Ber.* **1976**, *109*, 2565. Hoffmann, R. W.; Lillienblum, W.; Dittrich, B. *Ibid.* **1974**, *107*, 3395. Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 529. Lemal, D. M.; Gosselink, E. P.; McGregor, S. D. *J. Am. Chem. Soc.* **1966**, *88*, 582. Lemal, D. M.; Lovald, R. A.; Harrington, R. W. *Tetrahedron Lett.* **1965**, 2779. Hoffmann, R. W.; Hauser, H. *Tetrahedron* **1965**, *21*, 891.
(4) Moss, R. A.; Cox, D. P. *Tetrahedron Lett.* **1985**, *26*, 1931.
(5) Olofson, R. A.; Walinsky, S. W.; Marino, J. P.; Jernow, J. L. *J. Am. Chem. Soc.* **1968**, *90*, 6554.
(6) Cox, D. P.; Moss, R. A.; Terpinski, J. *J. Am. Chem. Soc.* **1983**, *105*, 6513. Moss, R. A.; Terpinski, J.; Cox, D. P.; Denney, D. Z.; Krogh-Jespersen, K. *Ibid.* **1985**, *107*, 2743. Moss, R. A.; Fedorynski, M.; Terpinski, J.; Denney, D. Z. *Tetrahedron Lett.* **1986**, *27*, 419
(7) Graham, W. H. *J. Am. Chem. Soc.* **1965**, *87*, 4396.
(8) Fedorynski, M.; Moss, R. A., unpublished work. Further elaboration of these studies is planned.
(9) Moss, R. A.; Perez, L. A.; Włostowska, J.; Guo, W.; Krogh-Jespersen, K. *J. Org. Chem.* **1982**, *47*, 4177.

0002-7863/87/1509-3811$01.50/0 © 1987 American Chemical Society