References and Notes

(1) Muetterties, E. L. *Science* **1977**, *196*, 839.
(2) Thomas, M. G.; Beier, B. F.; Muetterties, E. L. *J. Am. Chem. Soc.* **1976**, *98*, 1296.
(3) Grubbs, R.; Lau, C. P.; Cukier, R.; Brubaker, C., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 4517.
(4) Wender, I.; Pino, P. "Organic Synthesis Via Metal Carbonyls"; Interscience: New York, 1968; Vol. 1, Chapter 1.
(5) Vannice, M. A. *J. Catal.* **1975**, *37*, 449.
(6) The most active cluster complex, $Ir_4(CO)_{12}$, had a turnover frequency of $N = 1 \times 10^{-5}$ s^{-1} at 140 °C.[2]
(7) C_2H_4 and H_2 (0.5 atm each) were added to an ampule containing 2 cm^3 of toluene and 50 mg of complex before heating; the turnover frequency was $<10^{-9}$ s^{-1}. CO(g) may inhibit reaction. However, activation of $Mo(CO)_6$ in toluene near 140 °C with removal of CO followed by treatment at 90 °C also gave no reaction (turnover frequency $<10^{-8}$ s^{-1}).
(8) Wrighton, M. S.; Schroeder, M. A. *J. Am. Chem. Soc.* **1973**, *95*, 5764. The turnover frequency for the hydrogenation of monoolefins is apparently $<10^{-5}$ s^{-1}.
(9) Wrighton, M. S.; Ginley, D. S.; Schroeder, M. A.; Morse, D. L. *Pure Appl. Chem.* **1975**, *41*, 671.
(10) Brenner, A.; Hucul, D. A.; Hardwick, S. J. *Inorg. Chem.* **1979**, *18*, 1478.
(11) Brenner, A.; Hucul, D. A. *Inorg. Chem.* **1979**, *18*, 2836.
(12) Brenner, A. *J. Mol. Catal.* **1979**, *5*, 157.
(13) Brenner, A.; Hucul, D. A. *J. Catal.*, **1980**, *61*, 216.
(14) Turnover frequencies are between 0.1 and 5 s^{-1} at 25 °C for the hydrogenation of ethylene.
(15) A "traditional" catalyst is made by impregnation with an aqueous solution of the appropriate metal salt followed by calcination and reduction.
(16) Massoth, F. E. *J. Catal.* **1973**, *30*, 204.
(17) Brenner, A.; Burwell, R. L., Jr. *J. Catal.* **1978**, *52*, 353.
(18) Even at subambient temperatures and several hundred Torr of H_2, the hydrogenation of ethylene remains about first order in H_2.[19]
(19) Bond, G. C. "Catalysis by Metals"; Academic: New York, 1962; Chapter 11.
(20) Experiments were done as those given in Table I except to avoid possible complications from hydrogenolysis of residual pentane all runs [except for $V(CO)_6$ and $Fe(CO)_5$] were done as dry mixes and neither $Ni(CO)_4$ nor $Co_4(CO)_{12}$ have been run in H_2.
(21) Burwell, R. L., Jr.; Brenner, A. *J. Mol. Catal.* **1976**, *1*, 77.
(22) Brenner, A.; Burwell, R. L., Jr. *J. Am. Chem. Soc.* **1975**, *97*, 2565.
(23) Anderson, R. B. *Catalysis* **1956**, *4*, 29.
(24) Hydrocarbon synthesis has been reported for several carbonyl cluster complexes supported on hydroxylated alumina and heated for several hours at ~300 °C in closed ampules.[25] The H_2(g) is supplied by the water gas shift reaction [the H_2O(g) is produced by heating the hydroxylated Al_2O_3]. In this system the long contact times do not allow one to either identify the primary reaction products or discriminate between hydrogenation of subcarbonyl species or reaction with CO(g).
(25) Smith, A. K.; Theolier, A.; Basset, J. M.; Ugo, R.; Commereuc, D.; Chauvin, Y. *J. Am. Chem. Soc.* **1978**, *100*, 2590.
(26) Brenner, A., unpublished results.
(27) Dalla Betta, R. A.; Shelef, M. *J. Catal.* **1977**, *49*, 383.
(28) Direct measurements of oxidation state for several supported carbonyls have established that for each mole of CH_4 formed during TPDE in He the metal is oxidized by six units [letting the oxidation state of C be 2, this is equivalent to the oxidation state of the four hydrogens changing from +1 (in σ-OH) to −0.5 (in CH_4)].[10,11,13,17]
(29) At 250 °C, $H_2/CO = 3$, and $P = 1$ atm; the initial turnover frequency for $Mo(CO)_6/Al_2O_3$ was 3×10^{-2} s^{-1} and $E_a = 92$ kJ/mol, giving an extrapolated activity of $N = 5 \times 10^{-5}$ s^{-1} at 140 °C.[30,26] For $W(CO)_6/Al_2O_3$, $N = 2 \times 10^{-3}$ s^{-1} at 250 °C.[28]
(30) Brenner, A.; Hucul, D. A. *Proc. Int. Conf. Chem. Uses Molybdenum, 3rd, 1979*, in press.

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Sequence Dependent Binding of *cis*-Dichlorodiammineplatinum(II) to DNA

Sir:

cis-Dichlorodiammineplatinum(II) (*cis*-DDP) is an anticancer drug of clinical importance.[1] The site of cytotoxic action of *cis*-DDP is generally believed to be DNA.[2] We previously reported that covalent binding of *cis*-DDP to DNA unwinds and shortens the double helix.[3] Here we present evidence that the drug binds selectively to the $(dG)_n \cdot (dC)_n$, $n \leq 4$, sequence in DNA and discuss a possible recognition site for this interaction.

Closed and nicked circular pSM1 DNAs and *cis*-DDP were incubated at 37 °C in 5 mM NaCl, 1 mM sodium phosphate buffer (pH 7.4), as described previously.[3] The final concentration of *cis*-DDP was 0.023 mM and the formal ratio (r_f) of Pt to DNA phosphate was 0.075. At various times, samples were removed from the reaction mixture, brought to 0.2 M in NaCl, and frozen to stop the reaction,[3] and spin dialyzed[4] for subsequent analysis by restriction endonuclease cleavage, gel electrophoresis, and atomic absorption (AA) spectroscopy. The spin dialysis step removes unbound platinum and excess salt to facilitate restriction endonuclease cleavage and the determination of the mole ratio of platinum bound per DNA phosphate (r) by carbon arc AA spectroscopy.

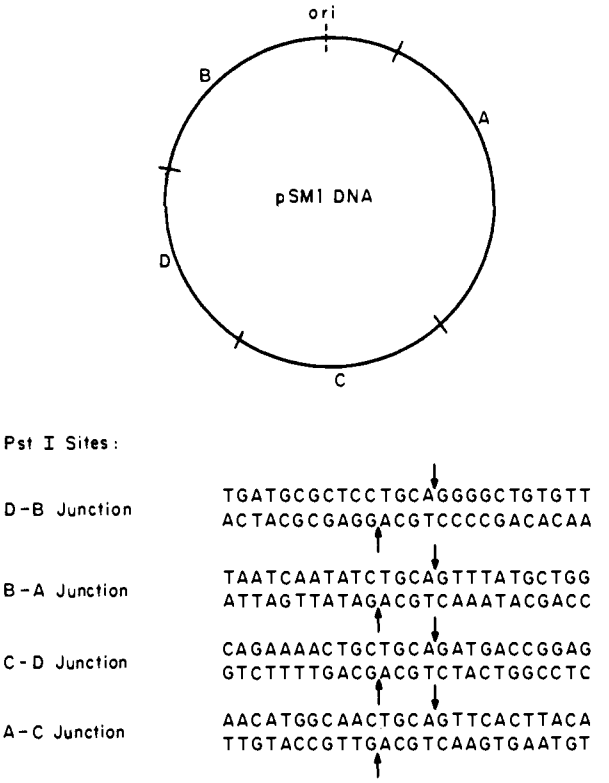

Figure 1. Map of bacterial plasmid pSM1 DNA showing the origin of replication (ori) and cleavage pattern by the restriction endonuclease Pst I. Fragments A, B, C, and D are 1.80, 1.60, 1.19, and 1.09 kilobases in length, respectively. The sequence of bases at each of the four cutting sites is also shown.

The restriction endonuclease Pst I (New England Biolabs) cleaves the circular pSM1 DNAs at four sequence specific loci to produce four fragments designated A–D in order of decreasing size (Figure 1). The fragments are separable by agarose gel electrophoresis (Figure 2, control channels C). Cleavage of DNA that had been incubated with *cis*-DDP produces partially fragmented pieces that are readily identified on the gels because of their greater length. These partials include the four "dimers" BA, AC, DB, and CD and the four "trimers" DBA, BAC, ACD, and CDB (Figure 1), all of which eventually appear over the time course of the experiment (Figure 2). Since *cis*-DDP unwinds the double helix, it is not surprising that the Pst I enzyme would be unable to cut the platinated DNA. It is significant, however, that fragments B and D are the first to disappear over the incubation time course, concomitant with the appearance of the DB dimer on the gels (Figure 2). This result indicates that, at low levels of platination (t = 20 min, r = 0.004), the restriction endonuclease Pst I can cleave the DNA at three of the four normal cutting sites but not at the D–B junction.[5] At comparable binding levels, *trans*-dichlorodiammineplatinum(II) did not produce this effect.

Since all four cutting sites have the same six-base-pair se-

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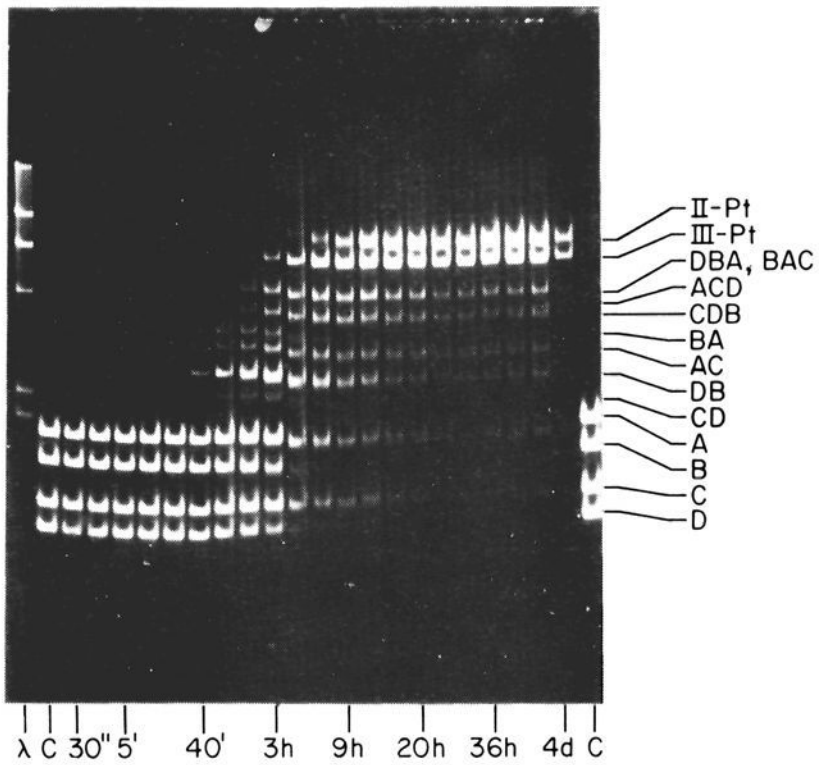

Figure 2. Electrophoresis in 1% agarose gels of a mixture of closed and nicked circular pSM1 DNAs incubated with *cis*-DDP for various time intervals and treated with Pst I restriction endonuclease. Channels 2–24 correspond to incubation times of 0 (C, control) and 30 s, 1.5, 5, 10, 20, and 40 min, 1.3, 2, 3, 5, 7, 9, 12, 16, 20, 24, 30, 36, 48, 72, 96, and 0 (C) h. The amount of platinum bound per nucleotide for channels 3–23 was determined by AA to rise from 3.63×10^{-3} after 20 min to 0.070 near the end of the incubation period. Labels refer to fragments identified in Figure 1; the symbols II-Pt and III-Pt refer to nicked circular and linear DNAs, respectively, produced following long time incubation with *cis*-DDP (see ref 15). Channel 1 contains λ DNA cut with Hind III as an internal standard for sizing the pSM1 DNA fragments. Further experimental details and results will be reported elsewhere.

quence, the selective inhibition by *cis*-DDP of cleavage at the D–B junction must involve base pairs adjacent to the restriction sequence. Figure 1 displays the sequences[6] of pSM1 DNA surrounding the four Pst I restriction sites. Examination of the region around the D–B junction reveals the occurrence of a unique $(dG)_4(dC)_4$ cluster to which we ascribe the selectivity of cleavage inhibition.[7] The other three cutting sites are not adjacent to an oligo(dG)·(dC) sequence. Studies of the reaction of *cis*-DDP with DNAs of varying (G + C)/(A + T) ratios have shown that the extent of binding increases with the (G + C) content; moreover, binding to poly(dG)·poly(dC) is substantially greater than to poly(dG·dC).[8] A study of *cis*-DDP with various nucleotides showed the rate of reaction with 5′-GMP to be the most rapid.[9] A previous investigation of the effect of *cis*-DDP on the Bam H1 restriction enzyme digest of λ DNA was interpreted as evidence for binding to adjacent guanine bases, although this was not a unique interpretation.[10] The present results are the first demonstration of selective binding of *cis*-DDP to a specific sequence in a naturally occurring DNA. It is also noteworthy that the effect occurs with as little as four bound platinum atoms per thousand nucleotides.

An examination of a CPK space filling model of $(dC)_4(dG)_4$ reveals that *cis*-DDP can bind two adjacent guanine bases at N-7, or two adjacent cytosine bases at N-3, if base pairing is disrupted. The models indicate that such binding could produce a twofold shortening of the DNA measured along the original helix axis, as found by electron microscopy.[3,11] Numerous X-ray diffraction studies of guanine or cytosine containing DNA fragments coordinated to *cis*-DDP support the likelihood of N-7 (G) or N-3 (C) coordination.[2,12] It is interesting that the two guanine N-7 atoms of the d(pGpG) unit cannot bind the inactive *trans*-diammineplatinum(II) moiety as revealed by studying CPK models of the dinucleotide and the platinum complex. The difficulty of coordinating a *trans*-diammineplatinum(II) complex to adjacent guanine bases on a DNA strand was noted previously.[8b] Thus, of the various candidates for the *cis*-DDP recognition site on DNA,[2,13] intrastrand cross-linking of nearest neighbor guanine[10,14] or cytosine bases by the drug is strongly supported by the present results.

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References and Notes

(1) (a) Rosenberg, B.; Van Camp, L.; Trosko, J. E.; Mansour, V. H. *Nature (London)* **1969,** *222,* 385–386. (b) For recent reports on the clinical status of the drug, see *Biochimie* **1978,** *60,* 915–965.
(2) Roberts, J. J.; Thompson, A. J. *Prog. Nucleic Acid Res. Mol. Biol.* **1979,** *22,* 71–133.
(3) Cohen, G. L.; Bauer, W. R.; Barton, J. K.; Lippard, S. J. *Science* **1979,** *203,* 1014–1016.
(4) Neal, M.; Florini, J. *Anal. Biochem.* **1973,** *55,* 328–330.
(5) Platinum binding inhibits cleavage at the four Pst I cutting junctions in the order DB ≫ AC > BA ~ CD as shown by a more detailed analysis to be reported elsewhere.
(6) Rosen, J., unpublished results from the laboratory of E. Ohtsubo, SUNY—Stony Brook.
(7) Alternatively, it may be the $(dG)_2(dC)_2$ clusters at both ends of the junction that together produce the selectivity. Sequencing studies are currently in progress to identify which bases are platinated near the D–B junction.
(8) (a) Stone, P. J.; Kelman, A. D.; Sinex, F. M. *Nature (London)* **1974,** *251,* 736–737. (b) Stone, P. J.; Kelman, A. D.; Sinex, F. M.; Bhargava, M. M.; Halvorson, H. O. *J. Mol. Biol.* **1976,** *104,* 793–801.
(9) Mansy, S.; Chu, G. Y. H.; Duncan, R. E.; Tobias, R. S. *J. Am. Chem. Soc.* **1978,** *100,* 607–616.
(10) Kelman, A. D.; Buchbinder, M. *Biochimie* **1978,** *60,* 893–899.
(11) Macquet, J.-P.; Butour, J.-L. *Biochimie* **1978,** *60,* 901–914.
(12) Gellert, R. W.; Bau, R. *Met. Ions Biol. Syst.* **1979,** *8,* 1–55.
(13) Barton, J. K.; Lippard, S. J. *Ann. N.Y. Acad. Sci.* **1978,** *313,* 686–700.
(14) (a) Kelman, A. D.; Peresie, H. J.; Stone, P. J. *J. Clin. Hematol. Oncol.* **1977,** *7,* 440–451. (b) Cramer, R. E.; Dahlstrom, P. L.; Seu, M. J. T.; Norton, T.; Kashiwagi, M. *Inorg. Chem.* **1980,** *19,* 148–154.
(15) Howe-Grant, M.; Wu, K. C.; Bauer, W. R.; Lippard, S. J. *Biochemistry* **1976,** *15,* 4339–4346.

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Mechanism of Oxidative Cleavage of α-Hydroxyalkylchromium Complexes

Sir:

One key intermediate in the Fischer–Tropsch process may be a metal-bound α-hydroxyalkyl group.[1,2] Transition metal complexes containing this group[3–5] are relatively rare and often quite unstable, and thus their chemistry has not been extensively explored. Hydroxymethyl complexes of cobalt(III) macrocycles,[3,4] for example, undergo unimolecular decomposition in aqueous solution by an internal two-electron process (eq 1) preventing an examination of their chemical reactions.

$$[Co^{III}(N_4chel)CH_2OH]^{n+} + H_2O \rightarrow [Co^{I}(N_4chel)]^{n-1} + HCHO + H_3O^+ \quad (1)$$

The closely related chromium(III) analogues[6,7] such as $(H_2O)_5CrCH_2OH^{2+}$ and other $(H_2O)_5CrCRR'OH^{2+}$ cations are stable to decomposition in this manner.[8] We have found that these complexes, prepared by the published methods,[6] are very powerful but selective reducing agents. They react with

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