In geometric isomerization of olefins we consider the reaction coordinate as the C=C torsion, which becomes nonrestorative at the transition state. The isotope dependent mode which is most affected during the reaction is the out-of-plane C-H (C-D) bend (Figure 2). Its frequency in the tranistion state should be very low, behaving analogously to the C-H(D) stretch in H(D) transfers. Assuming an 820-cm^{-1} C-H bending frequency for a trisubstituted alkene[11] and the same factor of 1.36 diminution for C-D bends as for stretches (from the square root of the ratio of the reduced masses) predicts a reduction of the value of about 7 for the primary isotope effect expected for a symmetrical H(D) transfer transition state (without substantial tunnelling) to $7^{(820/2950)} = 1.7$ for the torsional case.[12] For the geometric isomerization transition state of an acyclic olefin, the perpendicularly twisted structure, this should be a reasonable estimate. However, since the vinyl carbons in *trans*-cyclohexene are probably pyramidal[13] and the reactant bending frequency therefore somewhat higher than 820 cm^{-1}, and since there seems to be a modest tunnelling correction, this is a close lower bound. The observation is in excellent accord with this simple model.

The model has been more quantitatively justified by semiempirical molecular orbital calculations. We have examined the prototypical but artificial olefin M_2C=CMH(D), where M is a hydrogen of mass 15 (to approximate a methyl group), by MNDO[14] calculations, previous results with which have shown good success in estimating vibrational frequencies[15] as well as deuterium isotope effects on rate.[16] The molecule chosen demonstrates the effect for an acyclic case and avoids the complication of coupling of essential modes with, e.g., internal rotations of the methyl groups were the real case of trimethylethylene used. We find a ZPE contribution to the isotope effect of 1.48 and a total nontunnelling isotope effect of 1.51. In accord with the arguments above, the out-of-plane C-H(D) bending mode of reactant (H, 961 cm^{-1}; D, 812 cm^{-1} calcd) dominates. Other stretches and bends involving the C-H(D) bond are well compensated between reactant and transition state. The imaginary frequency of the activated complex corresponds to the torsion of the M_2C and CMH(D) groups (H, 588i cm^{-1}; D, 481i cm^{-1}) and affords estimated tunnelling corrections to the rate of 1.55 (H) and 1.27 (D) under the usual assumption of a truncated parabolic barrier. The net isotope effect predicted is 1.85, in excellent accord with the observation as well as with the rationalization above.

This is the first SDIE reported for alkene geometric isomerization. It might better be described as "quasiprimary". Just as the entire stretching frequency of a C-H (C-D) oscillator may be "lost" at the transition state for atom transfer, the entire C-H (C-D) out-of-plane bending frequency may be lost in an alkene geometric isomerization. Most importantly, the arguments here presented are not at all unique to the unusual **2**, and large isotope effects should be found for other alkenes as well.

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(10) Tunnelling components to SDIE in hydride transfer and E2 elimination have previously been suggested: Huskey, W. P.; Schowen, R. L. *J. Am. Chem. Soc.* **1983**, *105*, 5704. Saunders, W. H., Jr., *J. Am. Chem. Soc.* **1984**, *106*, 2223. Saunders, W. H., Jr. *J. Am. Chem. Soc.* **1985**, *107*, 164.

(11) Bellamy (Bellamy, L. J. *The Infrared Spectra of Complex Molecules*; John Wiley and Sons: New York, 1958; p 51) reports a range of 800-840 cm^{-1}.

(12) Loss of a bending and not a stretching degree of freedom was suggested many years ago to explain the low primary isotope effects observed when the activated complex is nonlinear. See: Hawthorne, M. F.; Lewis, E. S. *J. Am. Chem. Soc.* **1958**, *80*, 4296. Also: see, More O'Ferrall, R. A. *J. Chem. Soc. B* **1970**, 785.

(13) Allinger, N. L.; Sprague, J. T. *J. Am. Chem. Soc.* **1972**, *94*, 5734.

(14) The AMPAC program is available from Quantum Chemistry Program Exchange, Indiana University Department of Chemistry, as QCPE 506.

(15) Dewar, M. J. S.; Ford, G. P.; McKee, M. L.; Rzepa, H. S.; Thiel, W.; Yamaguchi, Y. *J. Mol. Struc.* **1978**, *43*, 135 and references therein.

(16) Brown, S. B.; Dewar, M. J. S.; Ford, G. P.; Nelson, D. P.; Rzepa, H. S. *J. Am. Chem Soc.* **1978**, *100*, 7832.

A New Antitumor Complex: Bis(acetato)bis(imidazole)copper(II)

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The great success of *cis*-diamminedichloroplatinum(II) (*cis*-DDP) in the clinical treatment of human malignancies[1] has stimulated research in the area of so-called second generation platinum compounds[2] and other metal-based antitumor compounds such as titanium,[3] vanadium,[4] gold,[5] germanium,[6] and copper[7] complexes. We have sought new antineoplastic metal complexes based on the following strategy: (i) coordination sphere of the square-planar type, (ii) zero net charge of the complex, and (iii) selection of the moderate leaving ligand. Copper was also chosen as the central metal because of high affinity for nucleic bases of DNA.

Among various copper complexes tested, we found strong antitumor activity in the bis(acetato)bis(imidazole)copper(II) complex, $[Cu(AcO)_2(HIm)_2]$, in which the crystal structure has been clarified.[8] In the 50% inhibition dose (ID_{50})[9] of cell growth using the mouse cancer cell line B16 melanoma,[5] indeed, the cytotoxic effect (20 ng/mL) of $[Cu(AcO)_2(HIm)_2]$ was comparable to that (8 ng/mL) of the excellent therapeutic drug *cis*-DDP and was superior to that (100 ng/mL) of mitomycin C.

Figure 1 shows the ESR spectra of $[Cu(AcO)_2(HIm)_2]$ and the 1:2 Cu(II) complex-deoxyguanosine (dG) systems at 77 K. Both the ESR spectra exhibit a typical Cu(II) hyperfine pattern and are characteristic of pseudo-square-planar Cu(II) system in the local environment of C_{2v} and D_{4h} symmetries.[10,11] In the case

(1) Bajetta, E.; Rovej, R.; Buzzoni, R.; Vaglini, M.; Bonaclonna, G. *Cancer Treat. Rep.* **1982**, *66*, 1299-1302. Perry, D. J.; Weltz, M. D.; Brown, A. W.; Henderson, R. L.; Neglia, W. J.; Berenberg, J. L. *Cancer* **1982**, *50*, 2257-2259.

(2) Vollano, J. F.; Blatter, E. E.; Dabrowiak, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 2732-2733. Barnard, C. F. J.; Cleare, M. J.; Hydes, P. C. *Chem. Britain* **1986**, *22*, 1001-1004.

(3) Köpf, H.; Köpf-Maier, P. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 477-478.

(4) Toney, J. H.; Brock, C. P.; Marks, T. J. *J. Am. Chem. Soc.* **1986**, *108*, 7263-7274.

(5) 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*, 218-223.

(6) Schein, P. S.; Slavik, M.; Smythe, T.; Hoth, D.; Smith, F.; Macdonald, J. S.; Woolley, P. V. *Cancer Treat. Rep.* **1980**, *64*, 1051-1056.

(7) Basosi, R.; Trabalzini, L.; Pogni, R.; Antholine, W. E. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 151-159. Agrawal, S.; Singh, N. K.; Aggarwal, R. C.; Sodhi, A.; Tandon, P. *J. Med. Chem.* **1986**, *29*, 199-202.

(8) Henriksson, H.-A. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1977**, *B33*, 1947-1950. Imai, H.; Ohono, H.; Tamura, H. *Nippon Kagaku Kaishi* **1987**, 672-677.

(9) Burchenal, J. H.; Kalaher, K.; Dew, K.; Lokys, L. *Cancer Treat. Rep.* **1979**, *63*, 1493-1498.

(10) Sugiura, Y.; Hirayama, Y. *J. Am. Chem. Soc.* **1977**, *99*, 1581-1585. Sugiura, Y. *Inorg. Chem.* **1978**, *17*, 2176-2182.

0002-7863/87/1509-6870$01.50/0

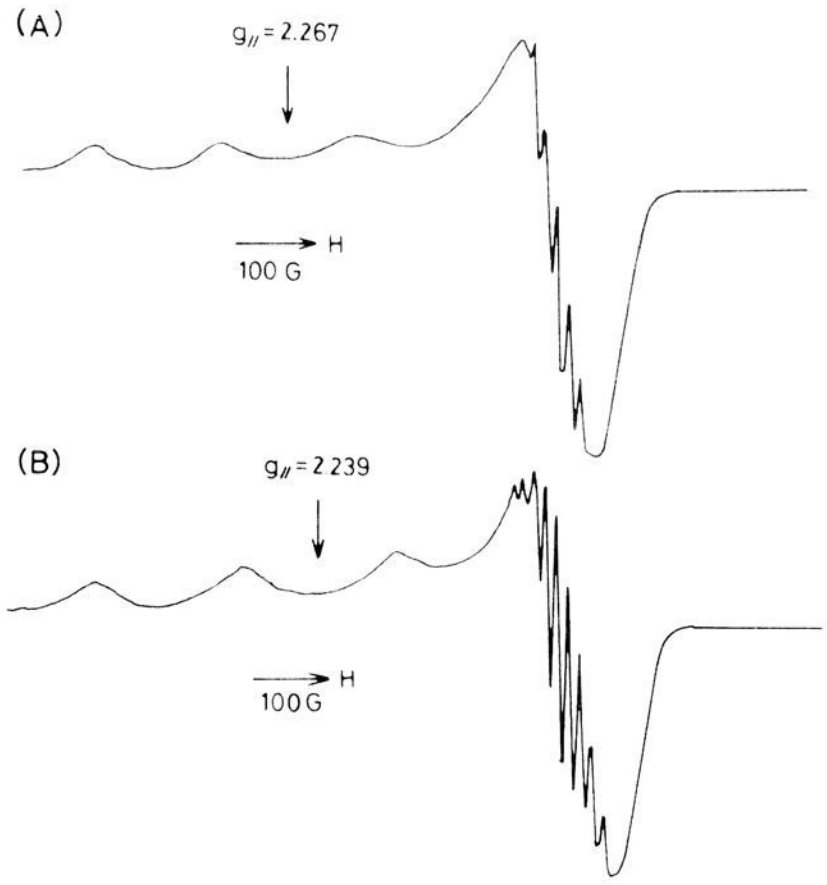

Figure 1. ESR spectra for $[Cu(AcO)_2(HIm)_2]$ (A) and $[Cu(AcO)_2(HIm)_2]$–dG (B) systems measured at 77 K. Each sample contained 5 mM $[Cu(AcO)_2(HIm)_2]$. The $[Cu(AcO)_2(HIm)_2]$–dG (1:2) complex was prepared by addition of 10 mM dG to (A) in water and incubated for 48 h at 37 °C.

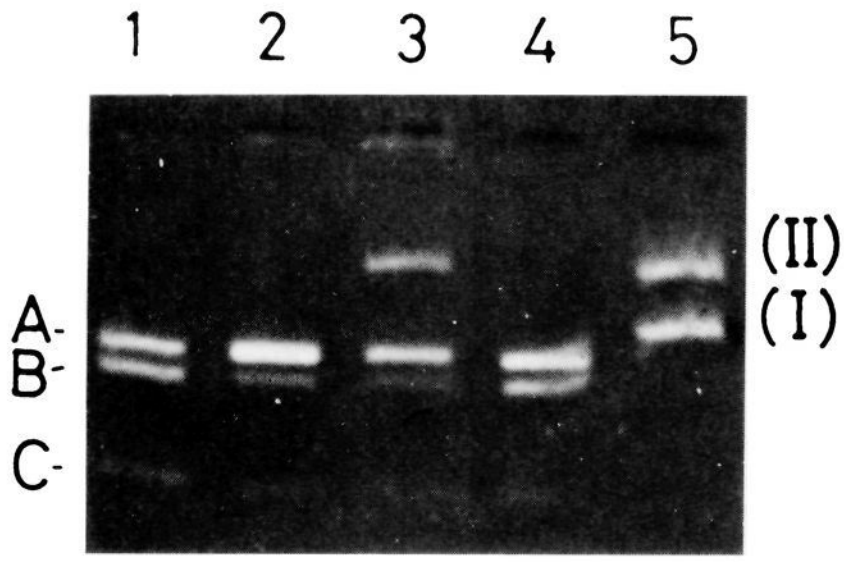

Figure 2. Agarose (1%) gel electrophoretic patterns of ethidium bromide stained mixtures of closed and open circular ϕX174 RF DNAs incubated with $[Cu(AcO)_2(HIm)_2]$ and treated with *Ban*I. Each sample contained 0.3 μg of ϕX174 RF DNA and 100 μM Tris-HCl buffer (pH 8.0). The samples of lanes 2 and 3 were incubated with 6 μM and 30 μM $[Cu(AcO)_2(HIm)_2]$, respectively. The samples of lanes 1–4 were digested with *Ban*I, and lane 5 shows the intact DNA alone.

of $[Cu(AcO)_2(HIm)_2]$ alone, the well-defined five lines of nitrogen (^{14}N, $I = 1$) nuclear superhyperfine splitting (hfs) were observed at the perpendicular region. The estimated ESR parameter ($g_{\|}$ = 2.267 and $A_{\|}$ = 152.8 G) indicates that the Cu(II) site is in the square-planar environment with the $(N_{HIm})_2$ donor set. When $[Cu(AcO)_2(HIm)_2]$ was reacted with 2 equiv of deoxynucleosides (dNs) for 48 h at 37 °C, the ESR feature of the dG system was altered the most significantly. Nine well-defined lines of N-hfs and ESR parameter ($g_{\|}$ = 2.239 and $A_{\|}$ = 183.3 G) demonstrate the coordination of two additional dG ligands via the nitrogen atom to $[Cu(AcO)_2(HIm)_2]$. This fact was also supported by the Job plot experiments,[12] where the mole ratio of dG/$[Cu(AcO)_2(HIm)_2]$ in the ternary complex is approximately 2. By contrast, the ESR characteristic of the dT system was almost identical with that of the original complex $[Cu(AcO)_2(HIm)_2]$, indicating no coordination of dT to the present Cu(II) complex. The reaction of $[Cu(AcO)_2(HIm)_2]$ with 2 equiv of several amines also gave ESR parameters (HIm, $g_{\|}$ = 2.237 and $A_{\|}$ = 186.1 G; pyridine, $g_{\|}$ = 2.239 and $A_{\|}$ = 177.8 G), whereas the addition of aniline induced no significant ESR alteration ($g_{\|}$ = 2.262 and $A_{\|}$ = 155.6 G) of the original Cu(II) complex. The present ESR results suggest that the binding site of dG is not the N(2) exocyclic nitrogen atom but rather the N(7) ring nitrogen atom. In $[Cu(9\text{-methylguanine})_2(H_2O)_3]SO_4\cdot 3H_2O$[13] the guanine N(7) coordination has been demonstrated.

Furthermore, we investigated the effect of $[Cu(AcO)_2(HIm)_2]$ on the digestion of restriction enzyme *Ban*I which cleaves the sequence specific loci (G↓GPyPuCC).[14] Figure 2 shows the typical gel electrophoretic patterns of a mixture of covalently closed circular (form I) and open circular (form II) ϕX174 RF DNAs cleaved with *Ban*I after incubation with $[Cu(AcO)_2(HIm)_2]$.[15] The molar ratios (r) of $[Cu(AcO)_2(HIm)_2]$/DNA were 0.1 and 0.5. The enzyme *Ban*I cuts the circular ϕX174 RF DNAs at the nucleotide sequence-specific loci to produce the three fragments (A–C in Figure 2). The gel electrophoretic pattern at low r value of 0.1 (lane 2) was nearly the same as the control (lanes 1 and 4) and hence $[Cu(AcO)_2(HIm)_2]$ dose not inhibit the cleavage activity of *Ban*I. In the experiment the r value was 0.5 (lane 3); remaining DNA at the origin indicates the decrease in mobility with the binding of $[Cu(AcO)_2(HIm)_2]$ to ϕX174 RF DNA. The appearance of band II, the disappearance of band C, and the paleness of band B suggest blocking of the cleavage activity of *Ban*I. Probably, the binding of the present Cu(II) complex to the guanine bases in the recognition sites of *Ban*I contributes to this inhibition. This is supported by the fact that both the site of the copper binding of N(7) guanine and the recognition site of *Ban*I for DNA are located in the major groove of DNA. The trans geometry of $[Cu(AcO)_2(HIm)_2]$[8] and the CPK space-filling model of a 1:2 ratio of the Cu(II) complex–dG system suggest that the binding mode of $[Cu(AcO)_2(HIm)_2]$ to DNA may be an interstrand cross-link rather than an intrastrand cross-link as shown in *cis*-DDP.[16] Moreover, a monofunctional adduct with no cross-linking also seems to be possible. The prevention of *Ban*I is not caused by direct binding of $[Cu(AcO)_2(HIm)_2]$ to the restriction enzyme, because the unreacted $[Cu(AcO)_2(HIm)_2]$ is removed by drop dialysis, the concentration of the Cu(II) complex is low (30 μM), and the added *Ban*I is found in sixfold excess compared to ϕX174 RF DNA. Moreover, if *Ban*I is inactivated by $[Cu(AcO)_2(HIm)_2]$, the gel electrophoretic pattern should be the same as that of lane 5.

In conclusion, the complex $[Cu(AcO)_2(HIm)_2]$ showed high cytotoxic activity for the mouse B16 melanoma cell. The Cu(II)–ESR and restriction enzyme *Ban*I–cleaving experiments indicate that the target of $[Cu(AcO)_2(HIm)_2]$ may be the guanine residues of the DNA helix. Research of the chemistry and molecular biology for the action mode of the present new antitumor Cu(II) complex is now underway.

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(11) Sugiura, Y.; Kuwahara, J.; Suzuki, T. *Biochim. Biophys. Acta* **1984**, *782*, 254–261.
(12) Spinelli, M.; Dabrowiak, J. C. *Biochemistry* **1982**, *21*, 5862–5870.
(13) Sletten, E.; Flogstad, N. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1976**, *B32*, 461–466.
(14) Sugisaki, H.; Maekawa, Y.; Kanazawa, S.; Takanami, M. *Nucleic Acids Res.* **1982**, *10*, 5747–5752.
(15) The complex $[Cu(AcO)_2(HIm)_2]$ was incubated with 60 μM ϕX174 RF DNA for 24 h at 37 °C in 100 μM Tris-HCl buffer (pH 8.0). Unreacted $[Cu(AcO)_2(HIm)_2]$ was removed by drop dialysis against 2 mM Tris-HCl buffer (pH 8.0) with use of the 0.05 μm pore size of the millipore membrane. $[Cu(AcO)_2(HIm)_2]$-treated DNAs were cleaved with 1.8 units of *Ban*I in 10 mM Tris-HCl buffer (pH 8.0) containing 7 mM $MgCl_2$ and 7 mM 2-mercaptoethanol for 2 h at 50 °C. The DNAs (0.3 μg) were subject to electrophoresis together with the reference sample on a 1% agarose slab gel which contained ethidium bromide (0.5 μg/mL).
(16) Cohen, G. L.; Ledner, J. A.; Bauer, W. R.; Ushay, H. M.; Caravana, C.; Lippard, S. J. *J. Am. Chem. Soc.* **1980**, *102*, 2487–2488. Tullius, T. D.; Lippard, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 4620–4622. Lippard, S. J. *Science (Washington, D. C.)* **1982**, *218*, 1075–1082.