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Characterization and Properties of Monoammine Nitroimidazole Complexes of Platinum $[PtCl_2(NH_3)(NO_2Im)]$. Crystal and Molecular Structure of *cis*-Amminedichloro(1-{(((2-hydroxyethyl)amino)carbonyl)methyl}-2-nitroimidazole)platinum(II)

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The characterization of monoammine(nitroimidazole)platinum(II) complexes of structure $[PtCl_2(NH_3)(NO_2Im)]$ (NO_2Im = 1-{(((2-hydroxyethyl)amino)carbonyl)methyl}-2-nitroimidazole, Etanidazole (I), 1-{2-nitro-1-imidazolyl}-3-methoxy2-propanol, Misonidazole (II), and 1-{2-hydroxyethyl}-2-methyl-5-nitroimidazole, Metronidazole (III)) is reported. Both cis and trans isomers may be isolated for II and III. The crystal structure of *cis*-amminedichloro(1-{(((2-hydroxyethyl)amino)carbonyl)methyl}-2-nitroimidazole)platinum(II) has been determined by X-ray diffraction. The crystals are orthorhombic, space group *Pnab* with cell dimensions a = 14.867 (7) Å, b = 9.915 (5) Å, c = 19.015 (9) Å, and Z = 8. The structure was refined to R = 0.062 and R_w = 0.052. Platinum has the expected square-planar coordination. The Pt–Cl bond trans to the nitroimidazole ligand is shorter (2.269 (3) Å) than normal. The dihedral angle between the platinum plane and the imidazole ring is 111°, while the nitro group makes an angle of 31° with the imidazole ring plane. Electrochemistry and ^{195}Pt NMR data are also reported. The relevance of the chemical properties to their biological properties as radiosensitizers and hypoxic cytotoxins is discussed.

Monoammine(nitroimidazole)platinum(II) complexes of structure $[PtCl_2(NH_3)(NO_2Im)]$ (NO_2Im = substituted nitroimidazole) are of interest both as radiosensitizers and as hypoxic cytotoxins.[1-3] Hypoxic toxicity refers to inherent cytotoxicity in the absence of O_2. Radiosensitization refers to enhancement of radiation damage by compounds, preferably under hypoxic (oxygen-deficient) conditions.[4-6] The biological properties of the mixed ammine nitroimidazole complexes are, in many ways, intermediate between those of the parent *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) and the bis(nitroimidazole) complexes $[PtCl_2(NO_2Im)_2]$.[7-9] Thus, the monoammine nitroimidazole series binds to DNA faster than the analogous bis(nitroimidazoles), possibly due to the presence of the H-bonding NH_3 group.[1] Unlike cisplatin, they show higher toxicity in hypoxic than in aerobic cells. This latter property may be initially explained by toxicity stemming from the reduction of the nitroimidazole group.[10] This paper reports on some chemical properties of this class of complexes and the molecular structure of a representative example, *cis*-amminedichloro(1-{(((2-hydroxyethyl)amino)carbonyl)methyl}-2-nitroimidazole)platinum(II).

Experimental Section

Starting Materials and Physical Methods. The ligands were obtained as gifts from the National Cancer Institute, Bethesda, MD (I, Etanidazole, 1-{(((2-hydroxyethyl)amino)carbonyl)methyl}-2-nitroimidazole), Roche (II, Misonidazole, 1-{2-nitro-1-imidazolyl}-3-methoxy-2-propanol), and Rhone-Poulenc (III, Metronidazole, 1-{2-hydroxyethyl}-2-methyl-5-nitroimidazole) and were used without further purification. The platinum complex $K[PtCl_3(NH_3)]$ was prepared by a published procedure.[11] IR spectra were obtained as KBr disks on Nicolet FT6000 series and Perkin-Elmer 1430 spectrophotometers. UV/visible spectra were run in phosphate buffer solution, pH 7.0, on a Perkin-Elmer Lambda 6B instrument. ^1H and ^{195}Pt NMR spectra were run on a Bruker 250 MHz spectrometer in either DMF-d_7 or D_2O with TSP (^1H) as internal reference or Na_2PtCl_6 in D_2O (^{195}Pt) as external reference. ^{195}Pt NMR samples were run at a pulse width of 10 μs with a relaxation delay of 0.5 s. Usually a sweep width of 30 kHz was used and 5000–10000 scans were adequate. All shifts are positive to lower shielding. Polarography measurements were made on a PAR 173 instrument in H_2O, phosphate buffered to pH 7 versus a Ag/AgCl electrode. Elemental analyses were performed by Robertson Laboratories, Madison, NJ.

Preparation of Complexes. The general method involves addition of 1 equiv of an aqueous solution of the ligand to an aqueous solution of $K[PtCl_3(NH_3)]$. Reaction time and workup are dependent on the ligand employed and solubility of complex formed.

***cis*-[$PtCl_2(NH_3)$(Etanidazole)] (Ic)** was prepared by addition of a solution of $K[PtCl_3(NH_3)]$ (0.18 g, 0.5 mmol) dissolved in 3 mL of water to an aqueous solution (3 mL) of 0.5 mmol of the 2-nitroimidazole, and the solution left overnight at room temperature. The next day the yellow compound had crystallized and suitable crystals were chosen for diffraction studies. The solution was decanted, and the crystals were washed with cold water. Yield: 50%. The decanted solution was concentrated, and a second crop of product could be isolated. IR spectroscopy showed that the two products were identical. Total yield: 75%. Dec pt: 215 °C (brown). Anal. Calcd for $C_7H_{13}N_5Cl_2O_4Pt$: C, 16.90; H, 2.62; N, 14.09; Cl, 14.29. Found: C, 17.08; H, 2.53; N, 14.32; Cl, 14.28.

***cis*-[$PtCl_2(NH_3)$(Misonidazole)] (IIc)** was similarly prepared. Overnight stirring gave a deep orange solution, which was evaporated to dryness to give an orange oil. The oil was dissolved in acetone and filtered to remove KCl, and then the acetone solution was dried over $CaSO_4$. The solution was filtered and evaporated to minimum volume. Upon addition of ether a yellow-orange solid was obtained, which was filtered off, washed with ether, and dried in vacuo. Yield: 65%. Anal. Calcd for $C_7H_{14}N_4Cl_2O_4Pt$: C, 17.35; H, 2.89; N, 11.6. Found: C, 17.56; H, 2.88; N, 11.38.

***trans*-[$PtCl_2(NH_3)$(Misonidazole)] (IIt)** was prepared by heating a solution of IIc in EtOH for 2–4 h until a clear yellow solution was obtained. Evaporation to half-volume and cooling to −3 °C yielded a bright yellow precipitate, which was filtered off and washed with Et_2O. Yield: 70%. Anal. Calcd for $C_7H_{14}N_4Cl_2O_4Pt$: C, 17.35; H, 2.89; N, 11.6. Found: C, 17.62; H, 3.01; N, 11.32.

***cis*-[$PtCl_2(NH_3)$(Metronidazole)] (IIIc).** The complex was prepared by the same procedure. Overnight the pale yellow product precipitated from solution and was washed with H_2O, acetone, and ether. Yield: 75%. Anal. Calcd for $C_6H_{12}N_4Cl_2O_3Pt$: C, 15.86; H, 2.64; N, 12.34.

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(1) Farrell, N.; Skov, K. A. *J. Chem. Soc., Chem. Commun.* **1987**, 1043.
(2) Skov, K. A.; Farrell, N. P.; Adomat, H. *Radiat. Res.* **1987**, *112*, 273.
(3) Skov, K. A.; Adomat, H.; Chaplin, D. J.; Farrell, N. P. *Anti-Cancer Drug Des.* **1990**, *5*, 121.
(4) Farrell, N. Transition Metal Complexes as Drugs and Chemotherapeutic Agents. In *Catalysis by Metal Complexes*; James, B. R., Ugo, R., Eds.; Reidel-Kluwer: Dordrecht, The Netherlands, 1989; Chapter 8, pp 183–207.
(5) Skov, K. A. *Radiat. Res.* **1987**, *112*, 217.
(6) Farrell, N. *Prog. Clin. Biol. Med.* **1989**, *10*, 90.
(7) Farrell, N.; Gomes Carneiro, T. M.; Einstein, F. W. B.; Jones, T. R.; Skov, K. A. *Inorg. Chim. Acta* **1984**, *92*, 61.
(8) Bales, J. R.; Coulson, C. J.; Gilmour, D. W.; Mazid, M. A.; Neidle, S.; Kuroda, R.; Peart, B. J.; Ramsden, C. A.; Sadler, P. J. *J. Chem. Soc., Chem. Commun.* **1983**, 432.
(9) Bales, J. R.; Mazid, M. A.; Sadler, P. J.; Aggarwal, A.; Kuroda, R.; Neidle, S.; Gilmour, D. W.; Peart, B. J.; Ramsden, C. A. *J. Chem. Soc., Dalton Trans.* **1985**, 795.
(10) Rauth, A. M. *Int. J. Radiat. Oncol. Biol. Phys.* **1984**, *10*, 1293.
(11) Abrams, M. J.; Giandomenico, C. M.; Vollano, J. F.; Schwartz, D. A. *Inorg. Chim. Acta* **1987**, *131*, 3.

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Table I. Crystallographic Data for *cis*-[$PtCl_2(NH_3)$(Etanidazole)] (Ic)

formula	$C_7H_{13}N_5Cl_2O_4Pt$
fw	497.21
space group	orthorhombic *Pnab*
a, Å	14.867 (7)
b, Å	9.915 (5)
c, Å	19.015 (9)
α, deg	90.00
β, deg	90.00
γ, deg	90.00
V, Å^3	2803.0 (2)
Z	8
D_c, Mg m^{-3}	2.356
diffractometer	Syntex P1
radiation	Mo Kα (0.710 69)
scan mode $2\theta/\theta$, deg	2θ = 60
μ(Mo Kα), mm^{-1}	10.51
T, °C	295
tot. no. of reflcns	4096
no. with $I > 2.5\sigma(I)$	2030
R, %	6.2
R_w, %	5.2

Table II. Positional Parameters (×10^4) with Their Esd's and Temperature Factors $U_{eq} = {}^1/_3\sum_i\sum_j U_{ij}a_i^*a_j^*\bar{a}_i\bar{a}_j$ for *cis*-[$PtCl_2(NH_3)$(Etanidazole)] (Ic)

atom	x	y	z	U_{eq}, Å^2
Pt	1697.5 (2)	1598.6 (4)	2007.9 (2)	429
Cl(1)	2932 (2)	2767 (4)	2356 (2)	658
Cl(2)	1689 (2)	2656 (4)	929 (2)	691
N(1)	1682 (7)	651 (9)	2986 (5)	566
N(2)	586 (5)	594 (9)	1731 (5)	437
N(3)	-426 (5)	-614 (8)	1176 (4)	392
N(4)	-1026 (9)	-4031 (13)	754 (9)	1411
N(5)	1138 (6)	-832 (9)	783 (5)	460
O(1)	1897 (5)	-926 (10)	1041 (5)	803
O(2)	934 (6)	-1126 (9)	198 (4)	703
O(3)	148 (5)	-3187 (8)	1307 (4)	578
O(4)	-2258 (8)	-6021 (12)	715 (6)	1319
C(1)	446 (7)	-300 (11)	1226 (5)	432
C(2)	-254 (7)	860 (12)	2000 (6)	533
C(3)	-868 (7)	98 (14)	1668 (6)	519
C(4)	-868 (7)	-1670 (12)	753 (5)	493
C(5)	-539 (7)	-3037 (12)	964 (6)	516
C(6)	-909 (16)	-5469 (21)	1009 (15)	2145
C(7)	-1404 (22)	-6448 (21)	748 (11)	2139

Found: C, 16.12; H, 2.84; N, 12.58.

***trans*-[$PtCl_2(NH_3)$(Metronidazole)] (IIIt).** The cis isomer was suspended in acetone and left under reflux for 24 h, after which time the pale yellow solution was filtered, evaporated to half-volume, and left in the freezer. A small amount of the yellow trans isomer precipitated, was filtered off and dried. Anal. Calcd for $C_6H_{12}N_4Cl_2O_3Pt$: C, 15.86; H, 2.64; N, 12.34. Found: C, 16.24; H, 2.93; N, 12.72.

Crystallographic Measurements and Structure Resolution. Crystal data: $Pt(NH_3)(C_7H_{10}N_4O_4)Cl_2$, fw = 497.21, orthorhombic, *Pnab* (nonstandard group for *Pbcn*, No. 60), a = 14.867 (7) Å, b = 9.915 (5) Å, c = 19.015 (9) Å, V = 2803(2) Å^3, $F(000)$ = 1872, D_{calcd} = 2.356 Mg m^{-3}, Z = 8, λ(Mo Kα) = 0.710 69 Å, μ(Mo Kα) = 10.51 mm^{-1}, and T = 295 °C (Table I). The crystal chosen for X-ray diffraction analysis, after examination under a polarizing microscope for homogeneity, was mounted roughly along the a axes and had the following distances (mm) between the indicated faces: 0.520 (100–100), 0.480 (001–001), 0.442 (011–011), and 0.452 (011–011). The cell parameters were obtained from the refined angles of 15 well-centered reflections on a Syntex P1 diffractometer using graphite-monochromatized Mo Kα radiation. The intensity data were collected as described earlier.[12] A set of 4096 independent reflections (h,k,l) were measured up to 2θ = 60° by the $2\theta/\theta$ scan technique. On the basis of the criterion $I > 2.5\sigma(I)$, a set of 2030 observed reflections were used for the structure resolution. The data were corrected for absorption from the equations of the crystal faces (transmission factors varied from 0.017 to 0.055) and for Lorentz and polarization effects.

The coordinates of the Pt atom were determined from the three-dimensional Patterson map. The positions of all the other non-hydrogen

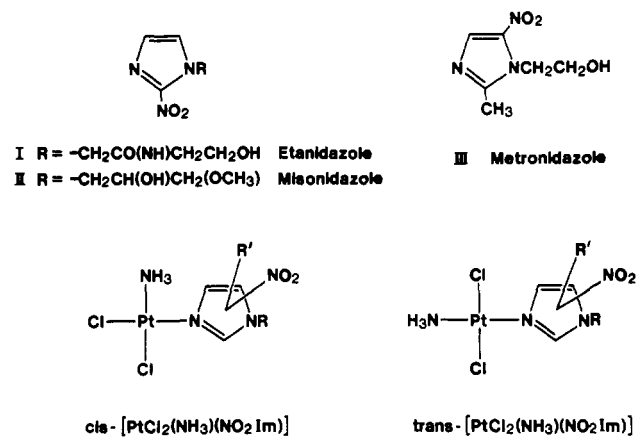

Figure 1. Structures of specific nitroimidazoles studied and the cis and trans isomers of [$PtCl_2(NH_3)(NO_2Im)$]. In the latter case the nitroimidazole is represented as a general structure.

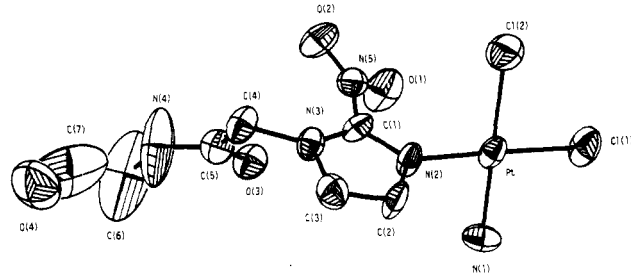

Figure 2. ORTEP diagram for *cis*-[$PtCl_2(NH_3)$(Etanidazole)] (Ic).

atoms were obtained by structure factor and difference-Fourier calculations. The refinement was done by using full-matrix least-squares calculations minimizing $\sum w(F_o - F_c)^2$. Isotropic secondary extinction corrections were included in the calculations. The H atoms, except those in NH_3 and OH, were fixed at the calculation positions with isotropic B = 6.0 Å^2 (C–H = 0.95 Å, N–H = 0.85 Å). Individual weights $w = 1/\sigma^2(F)$ were applied. The refinement of the scale factor, coordinates, and anisotropic temperature factors of all atoms converged to $R = \sum||F_o| - |F_c||/\sum|F_o| = 0.062$ and $R_w = [\sum w(|F_o| - |F_c|)^2/\sum|F_o|^2]^{1/2} = 0.052$. The refined coordinates are listed in Table II. The scattering curves of Cromer and Waber[13] were used, except for hydrogen.[14] The anomalous dispersion terms of Pt and Cl[15] were included in the structure factor calculations. The calculations were performed on a Cyber 830 computer with programs already described.[12]

Results and Discussion

The representative 2- and 5-substituted nitroimidazoles used and the general structures of their platinum complexes are shown in Figure 1. The 5-nitroimidazole, Metronidazole (also known as Flagyl, III), is used in the treatment of anaerobic bacterial infections. Misonidazole (II) has received considerable attention as a clinical candidate as a radiosensitizer, but toxic side effects at the concentrations required for efficient radiosensitization have limited its efficacy and application. Presently, Etanidazole (I, SR-2508) is considered the most likely analogue to replace Misonidazole and is currently in clinical trials.[16]

The complexes are prepared by the reaction

$$K[PtCl_3(NH_3)] + NO_2Im \rightarrow [PtCl_2(NH_3)(NO_2Im)]$$

The cis configuration is expected to be the initial product, but we have found in our work on these systems that the reaction chemistry is quite different depending on the substitution position of the NO_2 group. Nitroimidazoles are less reactive than imidazole due to the lower basicity of the coordinating nitrogen (pK_a values

(12) Melanson, R.; Rochon, F. D. *Can. J. Chem.* **1975**, *53*, 1139.
(13) Cromer, D. T.; Waber, J. T. *Acta Crystallogr.* **1965**, *18*, 104.
(14) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175.
(15) Cromer, D. T. *Acta Crystallogr.* **1965**, *18*, 17.
(16) Coleman, C. N.; Noll, L.; Howes, A. E.; Harris, J. R.; Zakar, J.; Kramer, R. A. *Int. J. Radiat. Oncol. Biol. Phys.* **1989**, *16*, 1085.

Table III. Bond Lengths (Å) and Angles (deg) for *cis*-[$PtCl_2(NH_3)$(Etanidazole)] (Ic)

Pt–Cl(1)	2.269 (3)	N(4)–C(5)	1.29 (2)
Pt–Cl(2)	2.304 (3)	N(4)–C(6)	1.52 (3)
Pt–N(1)	2.084 (9)	N(5)–O(1)	1.24 (1)
Pt–N(2)	2.000 (8)	N(5)–O(2)	1.19 (1)
N(2)–C(1)	1.32 (1)	C(5)–O(3)	1.22 (1)
N(2)–C(2)	1.38 (1)	C(7)–O(4)	1.34 (3)
N(3)–C(1)	1.34 (1)	C(2)–C(3)	1.34 (2)
N(3)–C(3)	1.34 (1)	C(4)–C(5)	1.50 (2)
N(3)–C(4)	1.48 (1)	C(6)–C(7)	1.32 (3)
N(5)–C(1)	1.43 (1)		
Cl(1)–Pt–Cl(2)	91.8 (1)	C(1)–N(5)–O(2)	117.3 (9)
Cl(1)–Pt–N(1)	88.8 (3)	C(2)–C(3)–N(3)	107 (1)
Cl(1)–Pt–N(2)	178.0 (3)	C(1)–N(2)–C(2)	104.8 (9)
Cl(2)–Pt–N(1)	179.0 (3)	C(1)–N(3)–C(4)	129.5 (9)
Cl(2)–Pt–N(2)	89.3 (3)	C(3)–N(3)–C(4)	122.3 (9)
N(1)–Pt–N(2)	90.1 (4)	N(3)–C(4)–C(5)	110.5 (9)
Pt–N(2)–C(1)	130.8 (7)	C(4)–C(5)–O(3)	122 (1)
Pt–N(2)–C(2)	123.9 (7)	N(4)–C(5)–O(3)	123 (1)
N(2)–C(1)–N(3)	111.1 (9)	C(4)–C(5)–N(4)	115 (1)
N(2)–C(1)–N(5)	124.2 (9)	C(5)–N(4)–C(6)	124 (2)
N(2)–C(2)–C(3)	110 (1)	O(1)–N(5)–O(2)	125.9 (9)
C(1)–N(3)–C(3)	107.7 (9)	N(4)–C(6)–C(7)	121 (2)
C(1)–N(5)–O(1)	116.8 (9)	C(6)–C(7)–O(4)	108 (2)

are as follows: Im, 7.0; 2-MeIm, 7.85; 4(5)-NO_2Im, –0.15; Metronidazole, 2.3). The deactivating nature of the nitro group is also reflected in the slower reactions of the 2-substituted ligands compared to the 5-substituted isomer. Further, whereas with K_2PtCl_4, 5-nitroimidazoles such as metronidazole give initially *cis*-[$PtCl_2(NO_2Im)_2$], the only product isolated from the 2-nitroimidazole Misonidazole is the complex *trans*-[$PtCl_2(NO_2Im)$]. To confirm the configuration of the initial product [$PtCl_2(NH_3)(NO_2Im)$], an X-ray crystal structure determination was carried out on the product with Etanidazole, which gave suitable crystals.

Description of the Structure. A labeled diagram of the compound is shown in Figure 2. The bond angles and distances are listed in Table III. The coordination around the Pt atom is square planar. The deviations from the weighted best plane are as follows (Å): Pt, 0.0003 (4); Cl(1), –0.007 (3); Cl(2), –0.003 (3); N(1), –0.025 (9), N(2), –0.0.053 (8). The angles around the Pt atom are close to the expected 90 and 180°.

The compound is the cis isomer, expected since the trans effect of chloride is larger than that of NH_3. The Pt–Cl(1) bond in the trans position to the nitroimidazole N(3) is significantly shorter (2.269 (3) Å) than normal, while the Pt–Cl(2) distance is normal (2.304 (3) Å), reflecting the lower trans influence of the nitroimidazole group with respect to chloride. The Pt–Cl bond is also shorter than in related complexes of *cis*-[$PtCl_2L_2$]; cf. 2.296 (2) Å, L = N-MeIm,[17] and 2.289 (2) Å, L = Metronidazole.[9] This shortening may further reflect the weak donor strength of a 2-nitroimidazole in comparison to a 5-nitroimidazole. The shorter Pt–Cl bond might also be caused by the presence of π bonds between Pt atom and the organic ligand. The imidazole ring probably has some empty π^* orbitals which are capable of accepting electron density from the Pt atom. Similar Pt–Cl distances (2.264 (4) and 2.263 (3) Å) were observed in the structure *cis*-[$PtCl_2(CH_3CN)_2$].[18] Acetonitrile is also expected to accept electron density from the metal into its empty π^* orbitals. The Pt–NH_3 distance is 2.084 (9) Å, while the Pt–N(imidazole) is shorter, 2.000 (8) Å. The latter value agrees well with the values observed in Pt–imidazole derivatives.[7-9,17,19] For example Pt–N(substituted imidazole) bond lengths are 2.016 (9) Å in *trans*-[$PtCl_2$(Misonidazole)$_2$][7] and 2.015 Å in *cis*-[$PtCl_2$(*N*-MeIm)$_2$].[17] The longer Pt–NH_3 bond might be attributed to the fact that NH_3 is involved in extensive hydrogen bonds.

The imidazole ring is planar, and its dihedral angle with the platinum coordination plane is 111.3°. The N–O distance (1.24 (1) and 1.19 (2) Å) are close to the published values for nitroimidazole compounds. One of the oxygen atoms, O(1), of the nitro group is located at 3.12 (1) Å from the Pt atom. The nitro group forms a dihedral angle of 30.7° with the imidazole ring. The corresponding angle for *trans*-[$PtCl_2$(Misonidazole)$_2$] is 45.6°[7,8] whereas both *cis*- and *trans*-[$PtCl_2$(Metronidazole)$_2$] have the nitro group coplanar with the imidazole ring.[8,9] In the *trans*-bis(Misonidazole) complex steric effects might explain the lack of coplanarity, but this would not be expected to be as critical with only one nitroimidazole. An interesting possibility is that the subsequent loss of coplanarity could reduce conjugation between the nitro group and the imidazole ring thereby increasing the donor strength of the weak N(3) nitrogen. The bond lengths and angles in the imidazole ring are normal, but the last four atoms on the side chain are very disordered, as shown by the high temperature factors of N(4), C(6), C(7), and O(4). For this reason the bond distances between these atoms have very high standard deviations (0.03 Å) which are probably underestimated. Attempts to refine two different positions for these four atoms were made but without success.

The packing of the molecules (Figure S1) consists of layers of molecules parallel to the *bc* plane. The molecules are held together by hydrogen bonds. The NH_3 ligand forms three hydrogen bonds with O(3) N(1)---O(3) = 2.89 (1) Å), O(4) (N(1)---O(4) = 2.95 (2) Å), and Cl(1) (N(1)---Cl(1) = 3.47 (1) Å), and the angles Pt–N(1)---O(3) = 104.2 (4), Pt–N(1)---O(4) = 133.2 (5), and Pt–N(1)---Cl(1) = 101.4 (3)°. The hydroxyl groups are also hydrogen bonded to each other. The O(4)---O(4) distance is 2.81 (2) Å, and the angle C(7)–O(4)---O(4) = 107 (1)°.

Chemical Properties and Isomerization of [$PtCl_2(NH_3)(NO_2Im)$]. Characterization data are given in Table IV. The very facile isomerization observed for bis(nitroimidazole) complexes such as [$PtCl_2$(Metronidazole)$_2$][8,9] is somewhat surprisingly not general for monoammine nitroimidazole complexes, and the isomerization is very dependent on the nature of NO_2Im. For the Misonidazole complex the initial cis isomer is readily isomerized to the trans complex upon reflux in EtOH. Attempts to induce isomerization of the Etanidazole derivative in EtOH or DMF were not successful, and no solids could be isolated pure from the deep red solutions.

Similarly, attempts to induce isomerization of *cis*-[$PtCl_2(NH_3)$(Metronidazole)] (IIIc) by heating in EtOH led to decomposition. Prolonged heating in acetone or in DMF (followed by evaporation, extraction with acetone, and recrystallization) gave small amounts of a yellow solid characterized as the trans isomer, IIIt. The different behavior between [$PtCl_2$(Metronidazole)$_2$] and [$PtCl_2(NH_3)$(Metronidazole)] in this case may be explained by the fact that the bis complex may readily dissociate one ligand, thus setting up exchange and isomerization reactions, and this may not occur so readily with the mono complex. Attempts to prepare IIIt by formation in situ of the cations [$PtCl(NH_3)$(Metronidazole)$_2$]$^+$ (from *cis*-[$PtCl_2(NH_3)$(Metronidazole)] and excess Metronidazole) or [$PtCl(NH_3)_2$(Metronidazole)]$^+$ (from *cis*-[$PtCl_2(NH_3)_2$] and excess Metronidazole) followed by reaction with HCl did not yield good results. No clean products were obtained. These results underline the problem that no general methods to these complexes are readily available and isolation is very dependent on the nature of the nitroimidazole. Indeed, good synthetic routes to *trans*-[$PtCl_2$(L)(L')] complexes in general are not common.

Spectroscopic Data. The principal characterization data are collected in Table IV. The IR spectra in the ν(Pt–Cl) region confirm the cis geometry of the initial isolated products and the isomerization to the trans isomer. For II and III, distinct differences in the ν(OH) and ν(NH) region from 3500 to 3000 cm^{-1} assist in further differentiation.

The range of ^{195}Pt NMR chemical shifts found is expected for a [$PtCl_2N_2$] coordination sphere, and the values confirm the low donor ability of these ligands in comparison to closely related

(17) Graves, B. J.; Hodgson, D. J.; van Kralingen, C. G.; Reedijk, J. *Inorg. Chem.* **1978**, *17*, 3007.
(18) Rochon, F. D.; Melanson, R.; Howard-Lock, H. E.; Lock, C. J. L.; Turner, G. *Can. J. Chem.* **1984**, *62*, 860.
(19) Carmichael, J. W.; Chan, N.; Cordes, A. W.; Fair, C. K.; Johnson, D. A. *Inorg. Chem.* **1972**, *11*, 1117.

Table IV. Spectroscopic and Electrochemical Data for Platinum–Nitroimidazole Complexes $[PtCl_2(NH_3)(NO_2Im)]$

NO_2Im Complexes[a]	UV/vis[b] λ_{max}, nm (log ϵ)	IR, cm^{-1}[c] ν(NH,OH)	IR, cm^{-1}[c] ν(Pt–Cl)	NMR, ppm[d] $\delta(^1H)$ ring	NMR, ppm[d] $\delta(^1H)$ other	NMR, ppm[d] $\delta(^{195}Pt)$	polarography[e] $E^{1/2}$, mV
Ic	311 (3.88)	3560, 3460, 3340, 3275*, 3140, 3120	340, 324	7.75, 7.38	5.36, 3.58 (t), 3.33 (t)		−0.204 (−0.384)
IIc	310 (4.08)	3310*, 3115, 3110	338, 332	7.88, 7.44	4.80 (m), 4.52 (m), 4.10 (m), 3.31	−2000	−0.245 (−0.384)
IIt	311 (3.75)	3480, 3260*, 3180, 3110	337	7.75 (d), 7.69 (d)	4.75 (m), 4.44 (m), 4.05 (m), 3.31	−1978	−0.240 (−0.384)
IIIc	300 (3.93)	3440, 3270*, 3180, 3140	336, 330	8.41	4.64 (t), 3.87 (t), 3.04	−2078	−0.320 (−0.480)
IIIt	307 (4.08)	3480*, 3300, 3210, 3150	342	8.14	4.61 (t), 3.89 (t), 3.00		−0.470 (−0.480)

[a] The abbreviations c and t refer to the geometry of the complex as cis and trans, respectively. See Figure 1. [b] In phosphate buffer, pH 7.0. [c] As KBr disks. Asterisk denotes strongest peak in this region. [d] Nonexchangeable protons in DMF-d_7. ^1H relative to TMS, ^{195}Pt relative to Na_2PtCl_6 in D_2O. Singlets except where stated. d = doublet, m = multiplet, and t = triplet. [e] 1 mM in phosphate buffer, pH 7.0, versus Ag/AgCl. Values in parentheses refer to free ligand.

species (cf. *cis*-$[PtCl_2(N\text{-MeIm})_2]$, −2185 ppm). The 2-$NO_2$Im complexes give more positive chemical shifts than their 5-NO_2Im counterparts, indicative of decreased shielding, a point also observed for $[PtCl_2(NO_2Im)_2]$.[9] This may be purely electronic in nature, as the 2-NO_2 group is one bond closer to the Pt atom. A large steric effect of the ortho substituent on the Pt chemical shift has been noted in complexes of substituted pyridines,[20] and steric effects here of the 2-nitro group may also contribute to the deshielding. In ^1H NMR spectroscopy, the chemical shifts of the ring protons are shifted to high frequency, with respect to free ligand, upon platination. A significant shielding (0.27 ppm) is observed for the unique C(4) proton in *trans*-$[PtCl_2(NH_3)$(Metronidazole)] (IIIt) in comparison to the cis isomer. At the field strength employed (250 MHz) no satellites from the expected Pt–H coupling were seen on the ring protons.

Reduction Potential of Nitro Group. There is generally an increase in reduction potential of the nitroimidazole of approximately 0.15–0.25 V upon platination. A correlation between the polarographic reduction potential and electron affinity has been reported for free nitroimidazoles,[21] whose electron affinity also correlates with radiosensitizing efficiency.[22] To a first degree, the more positive reduction potentials may therefore make for more effective radiosensitization. There is an interesting difference between the cis and trans isomers with Metronidazole (III) in that the potential of the trans isomer (NO_2Im with trans NH_3) is hardly affected compared to free ligand whereas the cis isomer (NO_2Im with trans Cl) shows the usual increase in reduction potential. This change is consistent with the shifts observed in the ^1H NMR spectra. The increased back-bonding to the nitroimidazole expected in the trans complex, because of the trans NH_3 group, may compensate for the loss of electron density due to Pt–N bond formation. Complexes IIc and IIt show essentially the same reduction potential, but delocalization may not be so important in this case due to the proximity of the nitro group to the metal center and the fact that the group is no longer coplanar with the imidazole ring. That both geometry and trans ligand influence the reduction potential is relevant because radiosensitization and hypoxic toxicity will depend to some degree on this property.

Biological Activity. There is general agreement that DNA is the target of radiation damage with an enhancement of strand breaks being produced by interaction of radiation-damaged DNA and O_2.[23] The classic oxygen mimetic radiosensitizers such as nitroimidazoles are believed to act by the interaction of the "electron-affinic" nitro group with a potentially damaged site on DNA (e.g. charge separation). Electron transfer from that site (i.e. sugar or base) to the NO_2 group prevents charge recombination (repair) and produces the radical anion. The subsequent irreversible chemical reactions on the now positively charged DNA site eventually results in a strand break (fixation). The mechanistic requirements for radiosensitizers such as a redox-active site and DNA affinity are inherent properties of many metal complexes.[4-6] Indeed, radiosensitization is formally a novel way to produce strand breaks using metal complexes because radiation-damaged DNA acts as a reducing agent toward either the metal ion or ligand.

The series $[PtCl_2(NH_3)(NO_2Im)]$ was first designed in attempts to target radiosensitizing nitroimidazoles to DNA.[1,2] During the studies on these complexes their inherent toxicity, independent of combination with radiation, has become of increasing interest.[3] The factors affecting radiosensitization and hypoxic toxicity may not be the same. In principle, the properties which affect radiosensitization and toxicity include reduction potential of the nitroimidazole group, binding to DNA, and complex geometry. The integrity of the complexes inside cells and the effect of DNA binding on the stability and reduction potential of any purported ternary species Pt–NO_2Im–DNA will also be important in determining biological activity. Interestingly, the complexes $[PtCl_2(NH_3)(NO_2Im)]$ are more efficient radiosensitizers[2] and bind better to DNA than the closely related $[PtCl_2(NO_2Im)_2]$.[24] Since the reduction potentials in both sets of complexes are similar for the same nitroimidazole, the improved DNA binding is implied as the source of differentiation. However, on a molar basis the complexes discussed here are not in general significantly better than free ligand, one exception being $[PtCl_2(NH_3)(5\text{-}NO_2Im)]$.[25] Efficient radiosensitization requires that electron transfer occur from a DNA molecule damaged by radiation to the redox-active group of the ligand (or metal). DNA coordination may result in relative restriction of the NO_2Im group to a limited number of sites—unless the potential radiation damage is very close to the metal binding site, chemical repair may occur.

Unlike cisplatin, the series $[PtCl_2(NH_3)(NO_2Im)]$ shows higher toxicity in hypoxic than in aerobic cells. This property may be initially explained by the Pt–DNA binding resulting in aerobic toxicity with the increased hypoxic toxicity stemming from the reduction of the nitroimidazole group. In this case the conformational changes upon Pt–DNA binding will be important.[3,24] Further, the enhancement of hypoxic cytotoxicity emphasizes the fact that relatively simple changes on the parent molecule (i.e. substitution of an NH_3 ligand in *cis*-DDP by NO_2Im) results in a significant alteration of antitumor activity. There is not the same clear-cut difference in toxicity between the cis and trans isomers of $[PtCl_2(NH_3)(NO_2Im)]$ as with those of $[PtCl_2(NH_3)_2]$.[3] The presence of a planar ligand has been shown to dramatically affect toxicity of *trans*-$[PtCl_2L_2]$ in both murine[26,27] and human tumor[28]

(20) Marzilli, L. G.; Hayden, Y.; Reily, M. D. *Inorg. Chem.* **1986**, *25*, 974.
(21) Greenstock, C. L.; Ruddock, G. W.; Neta, P. *Radiat. Res.* **1976**, *66*, 472.
(22) Adams, G. E.; Flockhart, I. R.; Smithen, C. E.; Stratford, I. J.; Wardman, P.; Watts, M. E. *Radiat. Res.* **1976**, *67*, 9.
(23) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987.
(24) Skov, K. A.; Adomat, H.; Konway, D. C.; Farrell, N. P. *Chem.-Biol. Interact.* **1987**, *62*, 117.
(25) Skov, K. A.; Farrell, N. P. *Int. J. Radiat. Biol.* **1990**, *57*, 947.
(26) Farrell, N.; Tam, T. B. Ha; Souchard, J.-P.; Wimmer, F. L.; Cros, S.; Johnson, N. P. *J. Med. Chem.* **1989**, *32*, 2240.

cell lines. The complexes discussed here may represent a further example of this effect.

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(27) van Beusichem, M.; Farrell, N. Presented at the Sixth International Symposium on Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy, San Diego, CA, Jan 1991; Abstract P-186.
(28) Farrell, N.; Qu, Y.; Van Beusichem, M.; Kelland, L. R. Reference 27, Abstract P-96.

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Supplementary Material Available: Anisotropic temperature factors (Table S1), fixed coordinates of the hydrogen atoms (Table S2), weighted least-squares planes (Table S3), distances and angles of possible hydrogen bonds (Table S4), and a stereoscopic view of packing in the crystal *cis*-[$PtCl_2(NH_3)$(1-{(((2-hydroxyethyl)amino)carbonyl)methyl}-2-nitroimidazole] (Figure S1) (5 pages); observed and calculated structure factor amplitudes (Table S5) (15 pages). Ordering information is given on any current masthead page.

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Syntheses and Characterization of Complexes Derived from α-Aminomalonate and *trans*-[$CoCl_2$(2,3,2-tet)]$^+$ (2,3,2-tet = 1,9-Diamino-3,7-diazanonane)

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The reaction of α-aminomalonate (AM^{2-}) with *trans*-[$CoCl_2$(2,3,2-tet)]ClO_4 (2,3,2-tet = 1,9-diamino-3,7-diazanonane) (**1**) in MeOH in the presence of Et_3N under aerobic conditions gave an AM^{2-} complex (**2**), unusually stabilized α-diamine complexes (**3**, **4**), and a carbinolamine complex (**5**). The X-ray crystallographic study was undertaken on [Co(AM)(2,3,2-tet)]ClO_4·H_2O (**2**) and the α-diamine complexes [Co(*N*-(2-aminoethyl)-*N*-(6-amino-4-azahexyl)-α,α-diaminomalonato)]ClO_4 (**3**) and [Co(*N*-(9-amino-3,7-diazanonyl)-α,α-diaminomalonato)]ClO_4·H_2O (**4**), and the following data were obtained. For compound **2**: $C_{10}H_{23}N_5O_4CoClO_4$·$H_2O$, monoclinic, space group *Cc*, a = 13.856 (2) Å, b = 11.466 (2) Å, c = 11.898 (3) Å, β = 107.32 (1)°, Z = 4, R = 0.057, R_w = 0.066. For compound **3**: $C_{10}H_{21}N_5O_4CoClO_4$, monoclinic, space group $P2_1/c$, a = 7.399 (5) Å, b = 23.199 (5) Å, c = 9.452 (4) Å, β = 92.02 (5)°, Z = 4, R = 0.050, R_w = 0.052. For compound **4**: $C_{10}H_{21}N_5O_4CoClO_4$·$H_2O$, space group $P2_1/c$, a = 7.360 (2) Å, b = 13.571 (3) Å, c = 17.370 (6) Å, β = 91.02 (2)°, Z = 4, R = 0.067, R_w = 0.070. The C–N bond formation between AM^{2-} and the amine ligand was promoted by dioxygen, Co(III) ion, heat, and MeOH and was enhanced photoinductively. The carbinolamine complex **5** was also obtained from the reaction of ketomalonate anion (KM^{2-}) with **1** in MeOH in the presence of NEt_3. X-ray crystallographic study on the carbinolamine complex, [Co(*N*-(9-amino-3,7-diazanonyl)-α-amino-α-hydroxylmalonato)]ClO_4·H_2O (**5**) was also performed: $C_{10}H_{20}N_4O_5CoClO_4$·$H_2O$, orthorhombic, space group $P2_12_12_1$, a = 7.291 (1) Å, b = 13.408 (2) Å, c = 17.143 (3) Å, Z = 4, R = 0.061, R_w = 0.066. The results of X-ray crystallography and pK_a measurements indicated that the uncoordinated NH_2 or OH groups of **3**, **4**, and **5** had some properties which were similar to those of an anilinic amino group or a phenolic hydroxy group in spite of being bound to the sp^3 carbon atoms.

Introduction

Recently, much attention has been focused on the reactivities of bioactive molecules toward transition-metal complexes in the light of the chemistry of metalloenzymes.

α-Aminomalonic acid (AMH_2) has not been found in nature; however, it is known that AMH_2 can act as an inhibitor of enzymes which include pyridoxal phosphate (PLP) as a cofactor and for which glycine can be a substrate.[1] Especially, it inhibits δ-aminolaevulate synthetase in the biosynthesis of porphyrin of heme proteins.[2] In those reactions, AMH_2 becomes tightly bound to PLP in the enzymes. In contrast, AMH_2 is converted to a chiral glycine, (*S*)-[2-^{3}H]glycine, by L-aspartate β-decarboxylase (PLP as the cofactor) in ^3H_2O.[3] Thus the reactivities of AMH_2 have been intensively investigated in biology; however, those toward transition-metal complexes have not been interpreted.

In the course of our research of the asymmetric syntheses of α-amino acids from the thermal decarboxylation of prochiral α-amino-α-alkylmalonate anion (ARM^{2-}) with cobalt(III)–tetraamine complexes under acidic conditions,[4,5] we adopted AM^{2-} as a precursor for the synthesis of chiral glycine in which one of the methylene protons is substituted by a deuterium. Contrary to our expectation, the reaction of AM^{2-} did not lead to chiral induction. However, it gave a variety of interesting complexes.

It is well-known that high-valent transition-metal complexes are capable of stabilizing unstable organic compounds with substantial electron-withdrawing effects of the metal ions, so that they can be available as reaction probes for ligand reactions. Previously, Sargeson and co-workers developed the inter- and intramolecular C–N bond formation between substrates containing unsaturated bonds, such as >C=O,[6] –C≡N,[7] and >C=C<,[8] and

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(1) (a) Thanassi, J. W.; Fruton, J. S. *Biochemistry* **1962**, *1*, 975. (b) Paleker, A. G.; Tate, S. S.; Meister, A. *Ibid.* **1971**, *10*, 2180. (c) Paleker, A. G.; Tate, S. S.; Meister, A. *J. Biol. Chem.* **1973**, *248*, 1158.
(2) Matthew, M.; Neuberger, A. *Biochem. J.* **1963**, *87*, 601.
(3) Paleker, A. G.; Tate, S. S.; Meister, A. *Biochemistry* **1970**, *9*, 2310.
(4) Ajioka, M.; Yano, S.; Matsuda, K.; Yoshikawa, S. *J. Am. Chem. Soc.* **1981**, *103*, 2459.
(5) Yashiro, M.; Ajioka, M.; Yano, S.; Toriumi, K.; Ito, T.; Yoshikawa, S. *Inorg. Chem.* **1986**, *25*, 1709.
(6) (a) Harrowfield, J. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1979**, *101*, 1514. (b) Gainsford, A. R.; Pizer, R. D.; Sargeson, A. M.; Whimp, P. O. *Ibid.* **1981**, *103*, 792. (c) Harrowfield, J. M.; Sargeson, A. M.; Springborg, J.; Snow, M. R.; Taylor, D. *Inorg. Chem.* **1983**, *22*, 186. (d) Engelhardt, L. M.; Gainsford, A. R.; Gainsford, G. J.; Golding, B. T.; Harrowfield, J. M.; Herlt, A. J.; Sargeson, A. M.; White, A. H. *Ibid.* **1988**, *27*, 4551.
(7) (a) Buckingham, D. A.; Foxman, B. M.; Sargeson, A. M.; Zanella, A. *J. Am. Chem. Soc.* **1972**, *94*, 1007. (b) Gainsford, G. J.; Jackson, W. G.; Sargeson, A. M. *Ibid.* **1979**, *101*, 3966.

0020-1669/91/1330-4535$02.50/0