differential lability of the diastereomers.[14] This then requires that under reductive alkylation conditions, some reaction other than cobalt-to-cobalt alkyl group transfer allows the interconversion of the diastereomers but does not come to equilibrium. Studies to demonstrate conclusively the applicability of Schemes I and II to the isomerization processes discussed here, as well as to further probe the steric effects on these isomerizations, are currently in progress.

Acknowledgment. This research was supported by the National Science Foundation, Grant CHE 89-96104. The authors are grateful to Professor R. G. Finke (University of Oregon) for communicating data on the thermolysis of β-CH_3Cbl prior to its publication.

Contribution from the Department of Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, Tennessee 37235

Rates of Substitution by Sulfur Nucleophiles in *cis*-Diamminebis(guanosine)platinum(II) Chloride

Julie A. Beaty and Mark M. Jones*

Received November 19, 1991

The rates of displacement of guanosine from *cis*-$[(NH_3)_2Pt(Guo)_2]^{2+}$ by six sulfur-containing nucleophiles have been measured at several temperatures and several concentrations of nucleophile by using ^{13}C NMR spectroscopy. *cis*-Diamminebis(guanosine)platinum(II) chloride [(**1**)Cl] reacts with the nucleophiles sarcosine-*N*-carbodithioate, dimethyldithiocarbamate, diethyldithiocarbamate, thiourea, 1-methyl-2-thiourea, and 1-ethyl-2-thiourea to form products in which a guanosine is removed in the initial step and additional ligands may be removed in subsequent steps if the nucleophile is added in excess. The rate of guanosine displacement in the first step of these reactions was found to be slower than that for the corresponding reaction with cyanide; the calculated enthalpies and entropies of activation are consistent with these findings, also. Other sulfur-containing nucleophiles investigated which did not have a significant effect on the displacement of guanosine from **1** over a 24-h period include NaSCN, $Na_2S_2O_3$, L-methionine, thiobarbituric acid, DMSO, glutathione, L-cysteine, and thiocarbohydrazide.

Introduction

The reaction of *cis*-diamminedichloroplatinum(II) (*cis*-DDP or cisplatin) with DNA, in which the platinum complex can bind both mono- and bifunctionally at the N-7 position to form an intrastrand cross-link between two adjacent guanine bases in the DNA helix, is generally accepted to be the basis for its antineoplastic activity.[1] Previous reports have determined the exact mode of binding, including the dihedral angles and orientation of the guanines, from X-ray crystallographic data and from NMR spectroscopic studies.[2] The use of cisplatin as an antitumor drug is somewhat limited by its concentration-dependent nephrotoxicity[3] and a variety of other side effects.[4] The control of these adverse effects via the administration of compounds which possess nucleophilic sulfur atoms, such as thiosulfate,[5] diethyldithiocarbamate,[6] glutathione,[7] WR-2721,[8] and the like has been reported in some detail. These compounds not only can react directly with cisplatin but also may react with platinum bound to DNA, in which case they would have a tendency to reduce the antineoplastic activity of the cisplatin. The present study was undertaken to obtain rate data on the reactions of sulfur-containing nucleophiles with the model compound *cis*-$[(NH_3)_2Pt(Guo)_2]^{2+}$ (**1**). Previous studies have found that such model compounds mirror many of the more significant reactions of platinum with DNA.[9] In order to displace a nucleoside from a platinum complex, a nucleophile capable of competing with the nucleoside for the coordination site on the platinum center must be present. The nucleophiles selected for these studies incorporated structural features of or are identical to compounds that (1) have been used to control the adverse effects of cisplatin in vivo, (2) have been used experimentally in vitro to remove platinum from DNA, or (3) are important nucleophiles within the cell. With these criteria in mind, the following nucleophiles were examined: NaSCN, $Na_2S_2O_3$, L-methionine, thiobarbituric acid, DMSO, glutathione (GSH), L-cysteine, thiocarbohydrazide, cyanide, sarcosine-*N*-carbodithioate (Sar-DTC), thiourea (Tu), diethyldithiocarbamate (DiEt-DTC), dimethyldithiocarbamate (DiMe-DTC), 1-methyl-2-thiourea (MeTu), and 1-ethyl-2-thiourea (EtTu). Of these nucleophiles, only the last seven provided data acceptable for kinetic analysis. The first compound investigated was cyanide since it has been shown to be the most effective at removing

(1) (a) Cardonna, J. P.; Lippard, S. J.; Gait, M. J.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 5793. (b) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. *Biochemistry* **1985**, *24*, 707. (c) Basch, H.; Krauss, M.; Stevens, W. J.; Cohen, D. *Inorg. Chem.* **1986**, *25*, 684. (d) van Hemelryck, B.; Guittet, E.; Chottard, G.; Girault, J.-P.; Herman, F.; Huynh-Dinh, T.; Lallemand, J.-Y.; Igolen, J.; Chottard, J.-C. *Biochem. Biophys. Res. Commun.* **1986**, *138*, 758. (e) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; van der Marel, G. A.; Haasnoot, C. A. G.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 1528. (f) Marzilli, L. G.; Kline, T. P.; Live, D.; Zon, G. In *Metal-DNA Chemistry*; Tullius, T. D., Ed.; American Chemical Society: Washington, DC, 1989; and references therein.

(2) (a) Sherman, S. E.; Gibson, D.; Wand, A. H.-J.; Lippard, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 7368. (b) Admiraal, G.; van der Veer, J. L.; de Graaff, R. A. G.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1987**, *109*, 592. (c) Reedijk, J.; Fichtinger-Schepman, A. M. J.; van Oosterom, A. T.; van de Putte, P. *Struct. Bonding* **1987**, *67*, 53. (d) Lippert, B. *Prog. Inorg. Chem.* **1989**, *37*, 1.

(3) (a) Krakoff, I. H.; *Cancer Treat. Rep.* **1979**, *63*, 1523. (b) Dentino, M.; Luft, F. C.; Yum, M. N.; Williams, S. D.; Einhorn, L. H. *Cancer* **1978**, *41*, 1274.

(4) von Hoff, D. D.; Schilsky, R.; Reichert, C. M.; Reddick, R. L.; Rozencwerg, M.; Young, R. C.; Muggia, F. M. *Cancer Treat. Rep.* **1979**, *63*, 1527.

(5) (a) Howell, S. B.; Taetle, R. *Cancer Treat. Rep.* **1980**, *64*, 611. (b) Ishizawa, M.; Taniguchi, S.; Baba, T. *Japan J. Pharmacol.* **1981**, *31*, 883.

(6) (a) Borch, R. F.; Katz, J. C.; Leider, P. H.; Pleasants, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5441. (b) Jones, M. M.; Basinger, M. A.; Mitchell, W. M.; Bradley, C. H. *Cancer Chemother. Pharmacol.* **1986**, *17*, 38.

(7) Zunino, F.; Pratesi, G.; Micheloni, A.; Cavalletti, E.; Sala, F.; Tofanetti, O. *Chem. Biol. Interact.* **1989**, *70*, 89.

(8) Glover, D.; Glick, J. H.; Weiter, C.; Fox, K.; Guerry, D. *J. Clin. Oncol.* **1987**, *5*, 574.

(9) (a) de Castro, B.; Kistenmacher, T. J.; Marzilli, L. G. *Agents Actions Suppl.* **1981**, *8*, 434. (b) Reily, M. D.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 4916. (c) Miller, S. K.; Marzilli, L. G. *Inorg. Chem.* **1985**, *24*, 242. (d) Fouts, C. S.; Reily, M. D.; Marzilli, L. G.; Zon, G. *Inorg. Chim. Acta* **1987**, *137*, 1.

0020-1669/92/1331-2547$03.00/0

cisplatin from DNA[10] and from oligonucleotides.[11] Although cyanide is not practical for use in vivo, it provides a standard to which other potentially useful nucleophiles may be compared. We have previously reported the rate studies on the reaction of cyanide with **1**,[12] while the rate studies for the remaining six sulfur-based nucleophiles are reported here.

Experimental Section

The preparation and characterization of *cis*-diamminebis(guanosine)platinum(II) chloride dihydrate [(**1**)Cl·2H$_2$O] has been reported previously.[11] Thiourea, sarcosine, sodium diethyldithiocarbamate, L-methionine, thiobarbituric acid, glutathione, L-cysteine, and thiocarbohydrazide were obtained from Sigma Chemical Co. 1-Methyl-2-thiourea, 1-ethyl-2-thiourea, sodium dimethyldithiocarbamate, sodium thiocyanate, and dimethyl-d_6 sulfoxide were purchased from Aldrich Chemical Co. The sodium thiosulfate was obtained from Fisher Scientific Co.

Preparation of Sarcosine-*N*-carbodithioate. A 6.00-g (0.15-mol) sample of NaOH was dissolved in 60 mL of ethanol in a round-bottomed flask in an ice-water bath. To this solution was added 6.68 g (0.075 mol) of sarcosine, and the mixture was stirred until solution was complete. The reaction flask was cooled in a ice-water bath and an excess of carbon disulfide (12 mL, 0.195 mol) in ethanol was added dropwise from an addition funnel. The drop rate was adjusted to maintain a temperature below 10 °C. The solid that accumulated after 2 days of stirring was collected by vacuum filtration and washed with cold acetone. After being dried in vacuo overnight, the product was gound with a mortar and pestle to give a white powder in 83% yield (as the dihydrate). Anal. Calcd[13] for $C_4H_5NO_2S_2Na\cdot 2H_2O$: C, 19.59; H, 3.67; N, 5.71; S, 26.12. Found: C, 19.77; H, 3.81; N, 5.40; S, 25.87.[13] ^{1}H NMR (D_2O): δ 3.33 (s, 3 H, CH_3), 4.54 (s, 2 H, CH_2). ^{13}C NMR (D_2O): δ 45.09 (CH_3), 61.52 (CH_2), 177.31 (C=O), 200.84 (C=S).

Preparation and Sodium Sarcosinate. A 1.0-g (0.112-mol) sample of sarcosine was dissolved in a small amount of water. To this solution was added 0.112 mol of $NaHCO_3$. The resulting solution was stirred for a couple of hours and then lyophilized to yield a white solid which was used without further purification.

^{13}C NMR Spectra. The rates of displacement of guanosine by the six sulfur-containing nucleophiles were studied in unbuffered solutions[14] containing various molar ratios of complex to nucleophile at 308, 312, 316, and 320 K. The samples were dissolved in a mixture of 90% H_2O and 10% D_2O and referenced externally to DMSO at 39.5 ppm. Spectra were obtained at 50.3 MHz on a Bruker 200-MHz instrument using a 10-mm probe and WALTZ ^{1}H decoupling[15] to prevent the sample from overheating during irradiation. The solutions were 2.77×10^{-2} M in complex and 2.77×10^{-1} M in nucleophile for a 1:10 ratio; collection of data began immediately after mixing the solutions. The acquisition time for each spectrum was 10 min with a 15–65-min interval (depending on ratio, temperature, and nucleophile) between data points. The temperature was kept constant throughout the experiments by using a variable temperature unit which was calibrated with a 4% MeOH in methanol-d_4 solution to ensure that the displayed temperature was accurate. The reaction was monitored by following the change in the ribose ^{13}C NMR peak heights measured as signal to noise ratios for the disappearance of **1** as a function of time.

^{1}H NMR Spectra. The hydroxy protons in **1** were "deuterated" by dissolving the solid in D_2O and lyophilizing it; this procedure was repeated three times. The complex and respective nucleophile were mixed in the desired ratio in a glovebag under nitrogen using D_2O (minimum isotopic purity of 99.996 atom % D from Aldrich Chemical Co.) as the solvent. The spectra were obtained at 200.13 MHz on an IBM 200-MHz NMR spectrometer and referenced to the HDO peak at 4.60 ppm. Presaturation was utilized to reduce the intensity of the residual HDO resonance.

Pseudo-first-order rate constants for the disappearance of **1** were calculated from the integrated form of the first-order rate equation (1),

$$\ln [C]_t = -kt + \ln [C]_0 \quad (1)$$

where $[C]_t$ is the concentration of complex at time t and $[C]_0$ is the initial concentration of complex. The rate constants were determined by measuring the peak heights (at constant peak width and fixed number of scans) of the ^{13}C and ^{1}H NMR resonances for the ribose carbons in **1**. From the peak heights, the percent reactant remaining at a given time was calculated. The natural logarithm of the percent reactant was graphed against time (in hours), producing a pseudo-first-order curve, the slope of which yields the rate constant. This procedure was carried out for all six sulfur-containing nucleophiles using 1:5, 1:7.5, 1:10, 1:11.25, 1:12.5, and 1:15 mole ratios at 308, 312, 316, and 320 K. Subsequently, the activation energies, E_a, were determined from a plot of ln k vs $1/T$. The E_a values were used to calculate the enthalpies, ΔH, and entropies, ΔS, of activation for all six nucleophiles from standard equations.[16]

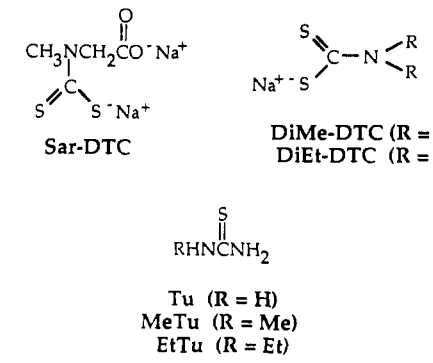

Figure 1. Structures of the six nucleophiles for which kinetic data were obtained.

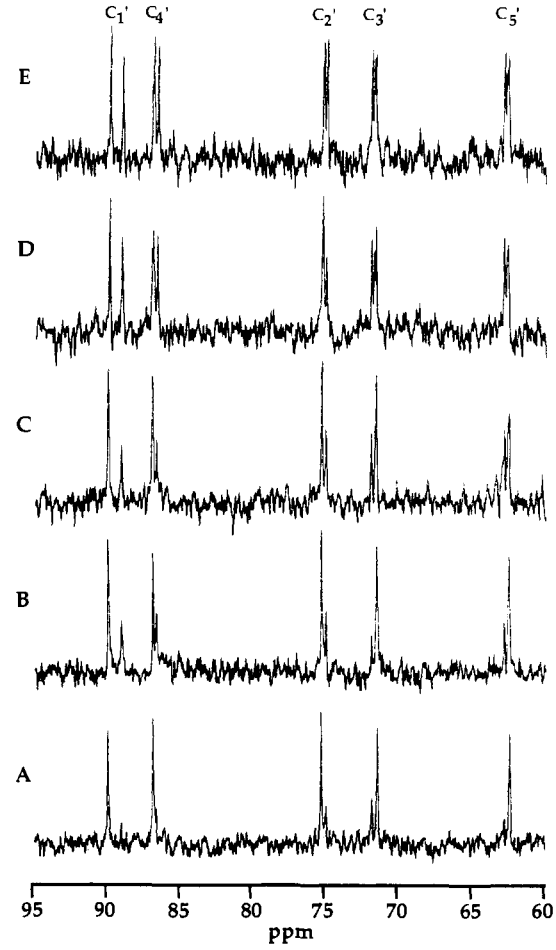

Figure 2. Representative ^{13}C NMR spectra (with peak assignments) showing the decrease in reactant and increase in product with time at 316 K for a 1:10 ratio of **1** to 1-methyl-2-thiourea: (A) t = 215 min; (B) t = 355 min; (C) t = 495 min; (D) t = 775 min; (E) t = 915 min.

Results

When **1** is reacted with Sar-DTC, DiMe-DTC, DiEt-DTC, Tu, MeTu, and EtTu (structures shown in Figure 1), the displacement

(10) Raudaschl-Sieber, G.; Lippert, B. *Inorg. Chem.* **1985**, *24*, 2426.
(11) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153.
(12) Jones, M. M.; Beaty, J. A. *Inorg. Chem.* **1991**, *30*, 1584.
(13) Analyses were done at Vanderbilt University.
(14) The pH values were as follows: sar-DTC ≈9.5, DiMe-DTC ≈7.4, DiEt-DTC ≈7.8, Tu ≈4.45, MeTu ≈3.8, and EtTu ≈3.4.
(15) Shaka, A. J.; Keeler, J.; Freeman, R. *J. Magn. Reson.* **1983**, *53*, 313.
(16) March, J. *Advanced Organic Chemistry*; John Wiley & Sons: New York, 1985: p 197.

Table I. Pseudo-First-Order Rate Constants (s^{-1}) for Various Mole Ratios of Nucleophile at 316 K

	rate const					
ratio	Sar-DTC	DiMe-DTC	DiEt-DTC	Tu	MeTu	EtTu
1:5	6.7×10^{-5}	4.5×10^{-5}	3.6×10^{-5}	9.5×10^{-6}	7.3×10^{-6}	1.0×10^{-5}
1:7.5	1.0×10^{-4}	7.7×10^{-5}	5.2×10^{-5}	1.3×10^{-5}	1.1×10^{-5}	1.4×10^{-5}
1:10	1.2×10^{-4}	1.0×10^{-4}	6.2×10^{-5}	1.6×10^{-5}	1.8×10^{-5}	1.8×10^{-5}
1:12.5	1.57×10^{-4}	1.5×10^{-4}	7.7×10^{-5}	1.9×10^{-5}	2.2×10^{-5}	2.2×10^{-5}
1:15			9.4×10^{-5}	2.2×10^{-5}	2.9×10^{-5}	2.6×10^{-5}

Table II. Pseudo-First-Order Rate Constants (s^{-1}) for 1:10 Molar Ratios of Nucleophile at Various Temperatures

	rate const					
T, K	Sar-DTC	DiMe-DTC	DiEt-DTC	Thiourea	MeTu	EtTu
308	6.3×10^{-5}	4.8×10^{-5}	2.1×10^{-5}	7.9×10^{-6}	9.6×10^{-6}	8.6×10^{-6}
312	9.1×10^{-5}	7.1×10^{-5}	3.4×10^{-5}	9.9×10^{-6}	1.3×10^{-5}	1.4×10^{-5}
316	1.2×10^{-4}	9.8×10^{-5}	6.0×10^{-5}	1.5×10^{-5}	1.8×10^{-5}	1.9×10^{-5}
320	1.8×10^{-4}	1.4×10^{-4}	9.2×10^{-5}	2.2×10^{-5}	2.5×10^{-5}	2.4×10^{-5}

of guanosine by the respective nucleophile can be monitored by using ^{13}C NMR spectroscopy. As the reaction proceeds, the ribose carbon resonances in the starting complex, **1**, decrease in intensity as new ribose carbon peaks emerge and gradually increase in intensity (see Figure 2). Data were collected at various time intervals until a white solid began to precipitate from solution, as evidenced by a decrease in the lock signal. At this point, data acquisition was halted to assure that data pertaining to subsequent reaction(s) were not included in the data used for calculating the rates for the initial step of the reaction (eq 2). The lack of any

$$\underset{\mathbf{1}}{\textit{cis}\text{-}[(NH_3)_2Pt(Guo)_2]^{2+}} + \text{nuc} \rightarrow \underset{\mathbf{2}}{\textit{cis}\text{-}[(NH_3)_2Pt(Guo)(nuc)]^{+}} + \text{Guo} \quad (2)$$

peaks other than those corresponding to **1** and **2** in either the ^1H or the ^{13}C NMR spectra, at this time, confirmed that the reactions had not proceeded beyond the formation of **2**.

Precipitation was expected to occur since according to eq 2, one of the products of the reaction is the displaced guanosine, which has a very low solubility in water.[17] The precipitates obtained from the 1:10 reaction mixtures of **1** with each of the six nucleophiles were isolated and characterized by melting point and UV, IR, and ^1H and ^{13}C NMR spectroscopy and the results were identical to those for free guanosine. The filtrates remaining after the precipitates were removed from the 1:10 reaction mixtures were lyophilized and analyzed by using ^1H and ^{13}C NMR spectroscopy. The analyses were done *several days after initiation of reaction* in order to ensure complete precipitation of the guanosine. The ^{13}C NMR spectra of these filtrates showed no resonances in the aliphatic carbon region which were characteristic of those for **1**, **2**, or free guanosine. Instead, only peaks corresponding to the methyl and/or methylene carbons of the respective nucleophile added are evident. Similarly, the ^1H NMR spectra of the 1:10 reaction filtrates contained major peaks corresponding to the respective methyl and/or methylene protons with other very minor peaks present only upon extreme magnification of the spectra.

In an effort to isolate, characterize, and confirm the product of the reaction in eq 2, each of the nucleophiles was mixed with **1** in a 1:1 ratio. As with the 1:10 ratio reactions, a white solid (guanosine) eventually precipitated from solution and was removed by filtration. The ^{13}C NMR spectra of the freeze-dried filtrates from the 1:1 reaction mixtures with Sar-DTC and Tu contained a peak pattern similar to that seen for **1** and for free guanosine, except with different chemical shifts. These new chemical shifts matched those observed for the product peaks in the 1:10 ratio reactions. The spectra contained no other resonances. Carbon and hydrogen elemental analyses on the freeze-dried filtrates from the 1:1 ratio reactions of **1** with Sar-DTC, Tu, MeTu, and EtTu are consistent with the formation of **2** as the product. The amount of filtrate from the 1:1 DiMe-DTC and DiEt-DTC reactions was not sufficient for characterization; however, results analogous to those above are likely.

(17) The solubility of guanosine in water is 1 g in 1320 mL at 18 °C. (Merck Index)

Table III. Activation Parameters for the Nucleophiles at 316 K

nucleophile	$\Delta H^{\ddagger}$, kJ/mol	$\Delta S^{\ddagger}$, kJ/mol
Sar-DTC	63 ± 6	−121 ± 19
DiMe-DTC	64 ± 5	−121 ± 17
DiEt-DTC	94 ± 2	−29 ± 5
thiourea	62 ± 3	−140 ± 10
MeTu	58 ± 3	−152 ± 9
EtTu	63 ± 5	−136 ± 15

Table IV. Second-Order Rate Constants at 316 K

nucleophile	rate const, L/(mol·sec)
Sar-DTC	$(4.7 \pm 0.2) \times 10^{-4}$
diMe-DTC	$(3.7 \pm 0.3) \times 10^{-4}$
diEt-DTC	$(2.4 \pm 0.2) \times 10^{-4}$
thiourea	$(5.9 \pm 0.6) \times 10^{-5}$
1-methyl-2-thiourea	$(6.0 \pm 0.6) \times 10^{-5}$
1-ethyl-2-thiourea	$(6.6 \pm 0.4) \times 10^{-5}$

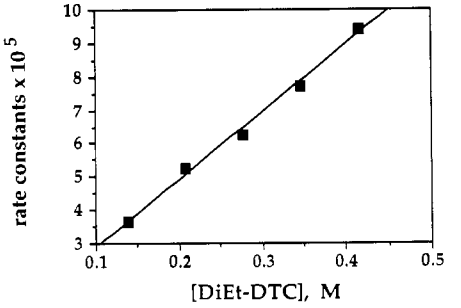

Figure 3. Representative graph of the pseudo-first-order rate constants versus initial DiEt-DTC concentration for 1:5, 1:7.5, 1:10, 1:12.5, and 1:15 molar ratios at 316 K.

The pseudo-first-order rate constants for 1:5, 1:7.5, 1:10, 1:12.5, and 1:15 molar ratios of all six sulfur-containing nucleophiles at 316 K are presented in Table I. In addition, the pseudo-first-order rate constants at 308, 312, 316, and 320 K (averaged over at least three trials at each temperature) are shown in Table II. A representative set of data for the enthalpies and entropies of activation, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ (at 316 K) is given in Table III. The overall order of each of the reactions was determined from a graph of the pseudo-first-order rate constant for various mole ratios of **1** to nucleophile plotted against initial nucleophile concentration (see Figure 3). The straight line obtained indicated a second-order process overall. Table IV lists the second-order rate constants obtained at 316 K for all six nucleophiles.

In order to confirm the ^{13}C NMR rate data, the rates of displacement of guanosine with 1:10 ratios of **1** to the same nucleophiles used in collecting the ^{13}C NMR kinetic data were determined by using ^1H NMR spectroscopy. The H-8 peak in

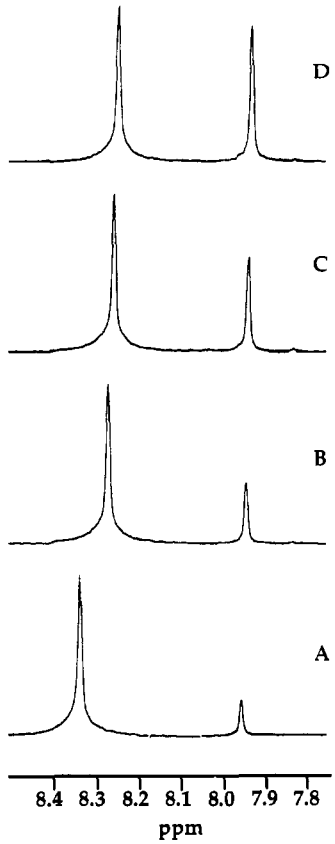

Figure 4. Representative ^{1}H NMR spectra showing the decrease in reactant (left peak) and increase in product (right peak) with time at the H-8 proton for a 1:10 ratio of **1** to Sar-DTC: (A) t = 10 min; (B) t = 35 min; (C) t = 75 min; (D) t = 135 min.

Table V. Comparison of Pseudo-First-Order Rates Determined by ^{13}C NMR and ^{1}H NMR Spectroscopy

nucleophile	T, K	rate, s^{-1}	
		^{13}C NMR	^{1}H NMR
Sar-DTC	308	6.3×10^{-5}	5.6×10^{-5}
DiMe-DTC	308	4.8×10^{-5}	5.1×10^{-5}
DiEt-DTC	308	2.1×10^{-5}	2.0×10^{-5}
thiourea	316	1.5×10^{-5}	1.5×10^{-5}
MeTu	316	1.8×10^{-5}	1.5×10^{-5}
EtTu	316	1.8×10^{-5}	2.0×10^{-5}

the proton spectrum was studied since its chemical shift is affected to the greatest extent by a change in the environment at the platinum center. In addition, the H-8 proton stands out clearly since its chemical shift is significantly downfield from the ribose proton region (where changes are indistinguishable due to the cluttered nature of that region). Analogous to the ^{13}C NMR spectra, the ^{1}H NMR spectra show the reactant H-8 peak decreasing as the product H-8 peak increases (see Figure 4). The rates of displacement were calculated from the ^{1}H NMR data and were found to be extremely close to those obtained from the ^{13}C NMR data (see Table V).

Several other sulfur-containing nucleophiles (structures shown in Figure 5) were investigated but proved unsatisfactory for use in these studies for several reasons. The reactions of **1** with NaSCN and $Na_2S_2O_3$ did not progress significantly over a 24-h period. Thiobarbituric acid was not soluble at the appropriate concentrations until it was made very basic; however this extreme basicity appeared to result in a destruction of the platinum–guanosine complex itself. The reactions of **1** with L-methionine, DMSO, GSH, and L-cysteine showed no evidence of reaction after a week, even at elevated temperatures and higher mole ratios; thus, these nucleophiles were deemed not feasible for a kinetic study on the NMR time scale. Thiocarbohydrazide was not useful because it was not soluble at the concentrations used in these studies.

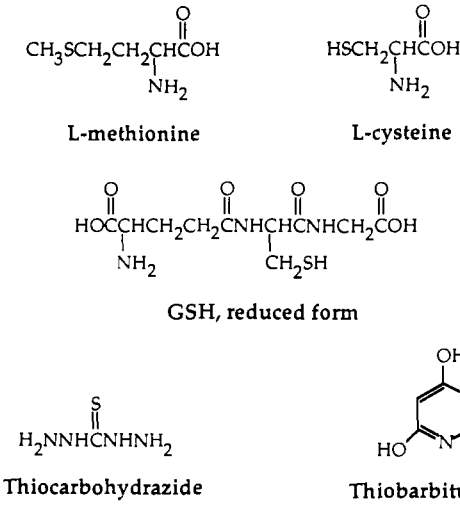

Figure 5. Structures of some of the other nucleophiles investigated.

Discussion

On the basis of the previous success of using ^{13}C NMR spectroscopy to obtain rate data on the displacement of guanosine from *cis*-$[(NH_3)_2Pt(Guo)_2]^{2+}$ with KCN,[20] ^{13}C NMR spectroscopy was employed to obtain similar rate data for six sulfur-containing nucleophiles: three dithiocarbamates and three thioureas. Unbuffered solutions were used in order to eliminate any competition between the nucleophile under study and potential nucleophiles furnished by the buffer system. The actual pH values, which did not vary substantially during the course of the reaction, are listed in ref 14. The rate data were determined by using the ribose carbon atoms on **1** since these carbons relax most readily and thus require a minimum acquisition time. The relaxation times of the ribose carbons remained constant (linear plots for the relative ^{13}C peak height versus concentration analogous to that seen with KCN[12]) over the ranges of concentration (1.38×10^{-2} to 5.54×10^{-2} M) and temperature (308–320 K) used in this study. Because the FID represents the average of the changes in a spectrum over a period of time, a short acquisition time is especially important for kinetic studies. Moreover, the fact that different carbons relax at different rates makes quantitative integration of the ^{13}C NMR resonances complicated. If the spin–lattice relaxation time (T_1) is too short, some of the carbon atoms may not have a chance to completely return to a Boltzmann distribution between pulses; this results in a ^{13}C NMR signals that are considerably weaker than expected based on the number of carbons responsible for these signals. For this reason, the data presented here consider only the relative intensities of a the peaks in a single spectrum; thus it is essential only that the T_1 and T_2 (spin–spin relaxation time) of the peak being measured remain constant during the course of the reaction. The T_2 is kept constant by halting data acquisition once precipitation is evidenced (by a decrease in lock signal); this ensures that the homogeneity of the sample is preserved throughout the time of data collection.

After the collection of kinetic data was complete, the reactions of **1** with an *excess* of the respective nucleophiles were allowed to continue in order to aid in the identification of subsequent reaction products which might appear in the NMR spectrum at these extended times and to ensure that the data used for the rate determinations were collected prior to the formation of such products. It should be stressed that although displacement of the second guanosine does inevitably occur, it is removed in a step subsequent to that used to determine the pseudo-first-order rate constants. Moreover, reliable estimates of the time of appearance of a *cis*-$[(NH_3)_2Pt(nuc)_2]$ type of species neither were desired from this study nor could be obtained via the NMR method employed here, due to the interference of precipitated guanosine. On the basis of the results from the elemental analyses and ^{1}H and ^{13}C NMR spectra the products resulting from mixtures of excess nucleophile with **1** can be identified as free guanosine and $Pt(nuc)_4^{+2-4x}$ (**3**) (where x is the charge on the nucleophile).

Platinum complexes similar to **3** result from the strong trans-labilizing effect of a sulfur ligand; the compound $[Pt(tu)_4]Cl_2$ has been isolated and characterized by spectroscopic[18] and crystallographic[19] means.

The ^1H and ^{13}C NMR spectra and elemental analyses of the filtrates from the 1:1 reactions confirm the presence of a product such as **2**. The fact that the only product observed in these filtrates is **2** seems logical since according to Lippert et al. the reaction in eq 2 is not likely to proceed in the reverse direction due to the low concentration of guanosine in solution and the high thermodynamic stability of the platinum–ammine linkage.[20] The presence of a monoadduct species has been reported previously in reactions between cyanide and a platinated double-strand oligonucleotide which give the adduct *cis*-$[Pt(NH_3)_2(dGuo\text{-}N7)(CN)]^+$ as an intermediate before subsequent reaction yields unplatinated double-strand oligonucleotide (comparative to the non-guanosine-containing product observed in the 1:10 ratio reactions).[21]

Although none of the sulfur-containing nucleophiles are as strongly basic as cyanide (compare second-order rate constants in Table IV with that for cyanide, 7.00×10^{-4} L mol^{-1} s^{-1} at 316 K[22]), the reactions do eventually proceed beyond **2** to a complex reminiscent of the final complex formed in the reactions with cyanide, $Pt(CN)_4^{2-}$. From the data in Table II, Sar-DTC has the fastest rate of displacement of the first guanosine, while Tu has the slowest. The substitution of a methyl or ethyl group on thiourea has a rather modest effect on the rate constants and corresponding activation parameters. It was considered that the Sar-DTC reactions might be more rapid due to a reaction with the carboxylic acid group rather than with the dithiocarbamate group. This idea was investigated by reacting **1** with sodium sarcosine in a 1:10 ratio to see if a (guanosine) precipitate formed; since no precipitate was evident even after 5 months, it was concluded that the dithiocarbamate group and not the carboxylic acid group was the site of reaction. However, the reaction of Sar-DTC with **1** may be facilitated by the more favorable charge interaction of their two ions. The relatively slow rates of reaction observed overall for the six sulfur-containing nucleophiles suggest that the removal of platinum from inter- and intrastrand cross-links with DNA does not play a significant role in the various processes which occur in vivo subsequent to the reactions of cisplatin with DNA and thus do not affect the antineoplastic activity of the cisplatin.

The activation parameters presented for Sar-DTC, DiMe-DTC, Tu, MeTu, and EtTu (see Table III) compare well with values found for the displacement of similar platinum(II) complexes[23] and to those reported previously for cyanide.[12] The negative entropies of activation are in accord with the associative mechanism by which substitution processes in square-planar complexes commonly proceed. Moreover, the magnitudes of the entropy of activation values suggest that a considerable degree of steric rearrangement (i.e., the movement of guanosine during the substitution process) is necessary to attain the transition state.

Although DiEt-DTC follows the same general trend of a negative ΔS^* and a large positive ΔH^*, its values for these activation parameters are curiously different from those of the other sulfur-containing nucleophiles. DiEt-DTC has a much larger E_a, owing to the larger ΔH^* and less negative ΔS^* values; in addition, it has a more positive temperature coefficient in that there is more than a 4-fold increase in the pseudo-first-order rate constants as the temperature increases from 308 to 320 K compared to a 3-fold or less increase in rate constants with temperature for the other five nucleophiles. Borch and his co-workers have reported that platinum–guanosine bisadducts are unreactive toward 10 mM DiEt-DTC at 37 °C.[21] Our data, extrapolated to allow the extent of reaction to be calculated under analogous conditions, support the above observation. However, if much higher concentrations of starting platinum complex and DiEt-DTC are used, a reaction occurs (albiet a rather sluggish one), suggesting that the rearrangement(s) required to reach the transition state must require more energy for DiEt-DTC than for the other nucleophiles studied.

The result of its intermediate reactivity makes DiEt-DTC potentially useful for application as a chemoprotective drug against cisplatin renal toxicity, especially compared to Tu, for example. Because of its high affinity for platinum complexes, Tu is capable of disrupting the cisplatin–DNA complex in vitro; however, it is not useful as an inhibitor of nephrotoxicity due to its carcinogenicity. Thiourea has been shown to reverse platinum–DNA cross-links in solution[25] and to inhibit platinum–DNA cross-links in cultured cells.[26] Although DMSO has been shown to react with several ammine-containing platinum compounds to form complexes such as $[Pt(en)(Me_2SO)Cl]Cl$ and *cis*-$[Pt(NH_3)_2(Me_2SO)Cl]Cl$[27] which can undergo subsequent reaction with DNA, our results suggests that if the platinum–ammine complex is already linked to two adjacent guanosines (as it is in DNA), reaction with DMSO is very slow at best. The lack of evidence of any reaction of **1** with glutathione at room temperature over a 24-h period is noteworthy since GSH is the major sulfhydryl compound found in cells and is known to react with clinically utilized platinum(II) and platinum(IV) antineoplastic agents;[28] reaction may occur at a significant rate with higher temperatures and longer reaction times. Moreover, the reaction of GSH with a single-bonded cisplatin DNA adduct would be expected to occur more rapidly.

Chemoprotective drugs must protect against the adverse effects of cisplatin without reversing the reaction that gives rise to its antitumor properties. The relatively slow rates of reactivity of **1** with the nucleophiles investigated here suggest that a reversal of the reaction of cisplatin with DNA is not a probable consequence of the delayed treatment with such nucleophiles, since most such nucleophiles are rapidly excreted via the kidneys. In addition, the results of this study suggest that fragments resulting from the repair of platinated DNA will be quite stable to platinum(II) substitution processes in vivo and that their subsequent metabolism may result in somewhat different final products than uric acid, which is the normal ultimate product of guanosine metabolism. The rates of reaction between platinated DNA and the sulfur-containing nucleophiles investigated here (via an associative mechanism like that found with our model complex) are likely to be slower due to the more limited access of the nucleophile to the platinum.

Acknowledgment. We wish to acknowledge, with thanks, the support received for this work from the National Institutes of Health (Grant CA-38997-05) and from the Center in Molecular Toxicology at Vanderbilt (Grant ES-00267).

(18) Woollins, J. D.; Woollins, A.; Rosenberg, B. *Polyhedron* **1983**, *2*, 175.
(19) Arpalahti, J.; Lippert, B.; Schollhorn, H.; Thewalt, U. *Inorg. Chem.* **1988**, *153*, 51.
(20) Arpalahti, J.; Lippert, B.; *Inorg. Chem.* **1990**, *29*, 104.
(21) Schwartz, A.; Sip, M.; Leng, M. J. *J. Am. Chem. Soc.* **1990**, *112*, 3673.
(22) The second-order rate constant for the 1:10 mole ratio reaction of **1** with KCN at 316 K was estimated from data in ref 10.
(23) (a) Miller, S. E.; House, D. A. *Inorg. Chim. Acta* **1989**, *161*, 131. (b) Evans, D. J.; Ford, N. R.; Green, M. *Inorg. Chim. Acta* **1986**, *125*, L39. (c) Eapen, S.; Green, M. *J. Inorg. Biochem.* **1985**, *24*, 233. (d) Evans, D. J.; Green, M. *Inorg. Chim. Acta* **1987**, *128*, 27.
(24) Bodenner, D. L.; Dedon, P. C.; Keng, P. C.; Borch, R. F. *Cancer Res.* **1986**, *46*, 2745.
(25) Filipski, J.; Kohn, K. W.; Prather, R.; Bonner, W. M. *Science* **1979**, *204*, 181.
(26) (a) Zwelling, L. A.; Filipski, J.; Kohn, K. W. *Cancer Res.* **1979**, *39*, 4989. (b) Micetich, K.; Zwelling, L. A.; Kohn, K. W. *Cancer Res.* **1983**, *43*, 3609.
(27) (a) Sundquist, W. I.; Ahmed, K. J.; Hollis, L. S.; Lippard, S. J. *Inorg. Chem.* **1987**, *26*, 1524. (b) Lempers, E. L. M.; Bloemink, M. J.; Reedijk, J. *Inorg. Chem.* **1991**, *30*, 201.
(28) Smith, E.; Brock, A. P. *Br. J. Cancer* **1988**, *57*, 548.