I. REACTIONS OF BICYCLO[3.3.0]OCTENYL TOSYLATES

II. NITROGEN-15 NUCLEAR MAGNETIC RESONANCE INVESTIGATIONS OF ORGANIC REACTIONS

Thesis by

Michael Wei-Kuo Nee

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To Amy

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# ABSTRACT

## PART I

### A. Elimination Reactions of 6-Bicyclo[3.2.0]-2-heptenyl Tosylates

The 6- and 7-bicyclo[3.3.0]-2-octenyl tosylates with 2,4,6-trimethylpyridine have been reported to yield mixtures of bicyclo-[3.3.0]octa-2,6-diene and bicyclo[3.3.0]octa-2,7-diene. Under the same conditions, the stereoisomeric 6-bicyclo[3.2.0]-2-heptenyl tosylate gives ring-opened products, 1,3,5-cycloheptatriene and 5-ethylidene-1,3-cyclopentadiene.

### B. Acetolysis of Some Bicyclo[3.3.0]-2-octenyl Tosylates

The products of the acetolysis of the stereoisomeric 6-,7-, and 8-bicyclo[3.3.0]-2-octenyl tosylates are reported. These tosylates were themselves stable to skeletal rearrangements, but were found to undergo 1,2-hydride shifts and elimination solvolysis.

## PART II

### Assignment of the Nitrogen-15 Nuclear Magnetic Resonances of Biotin and Unequivocal Synthesis of (+)-[1-$^{15}$N]Biotin

The $^{15}$N NMR spectra of biotin, desthiobiotin, and 2-imidazolidinone were measured. Assignment of the resonances of biotin and desthiobiotin was achieved by off-resonance decoupling. The biotin assignment was confirmed by measurement of biotin specifically labeled with $^{15}$N at N1.

## PART III

### A $^{15}$N Nuclear Magnetic Resonance Study of the Base-Catalyzed -$NH_2$ Exchange Reactions of Acetamide and Thioacetamide

The base-catalyzed -$NH_2$ exchange reactions of acetamide and thioacetamide were studied by $^{15}$N nuclear magnetic resonance spectroscopy by use of line-shape analysis. The $^{15}$N NMR spectra of these primary amides at intermediate exchange rates were broad doublets, which indicated that the two amide protons were exchanging at different rates. The line-shape analysis indicated that the ratio of exchange rates was $6 \pm 1$ for acetamide and $3 \pm 1$ for thioacetamide.

PART IV

Determination of the Binding Interactions of cis-$[Pt(NH_3)_2Cl_2]$ with Nucleosides by $^{15}N$ Nuclear Magnetic Resonance Spectroscopy

The $^{15}N$ NMR chemical shifts and $^{15}N$-$^{195}Pt$ coupling constants of several platinum(II)-ammine complexes were measured. The magnitude of the coupling constants were dependent on the trans ligand. A similar dependence on the trans ligand was found for the $^{15}N$ chemical shifts of the coordinated ammonia ligands. The magnitude of the $^{15}N$ platinum coordination shift was proportional to the $^{15}N$ protonation shift of the ligand. Cis-$[Pt(NH_3)_2Cl_2]$ was found to bind to guanosine through N7 and another nitrogen site. The drug also binds to N3 of cytidine and to all four of the nitrogen sites of adenosine. No evidence was found to support chelate binding of nucleosides by cis-$[Pt(NH_3)_2Cl_2]$.

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# Part I

## A. Elimination Reactions of 6-Bicyclo[3.2.0]-2-heptenyl Tosylates

# ELIMINATION REACTIONS OF 6-BICYCLO[3.2.0]-2-HEPTENYL TOSYLATES [1]

Michael Nee, William F. Gorham, and John D. Roberts *

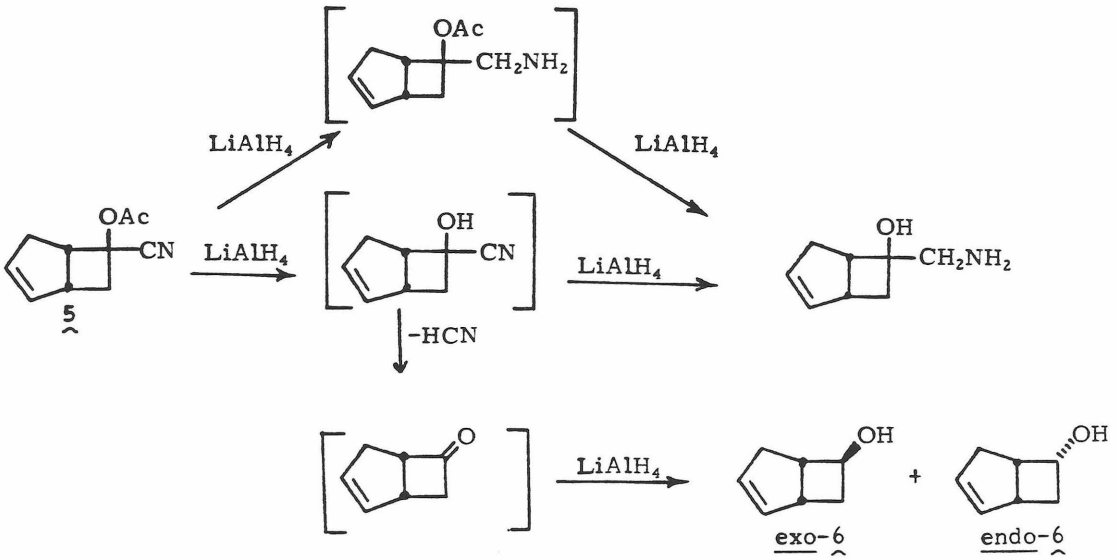
Gates and Crellin Laboratories of Chemistry

California Institute of Technology
Pasadena, California 91125

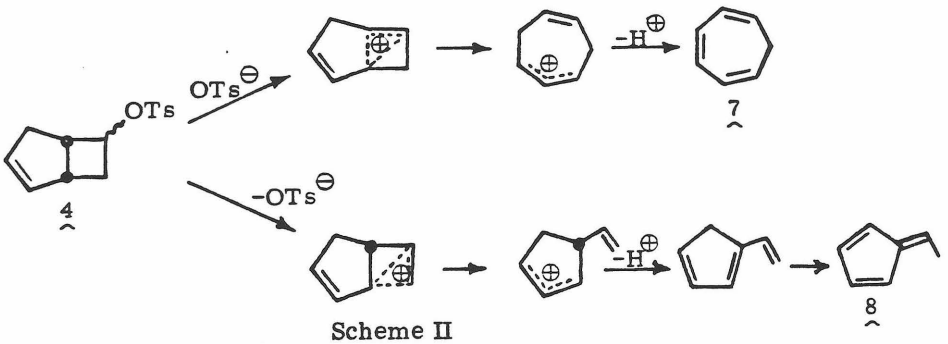
As part of an early attempted synthesis of pentalene, it was reported in 1951 [2] that the reaction of a mixture of stereoisomeric 6- and 7-bicyclo[3.3.0]-2-octenyl tosylates (1 and 2) with collidine (2,3,5-trimethylpyridine) and other tertiary amine bases appeared to form variable amounts of a yellow polyene, 3. This material had an ultraviolet absorption maximum at 260 nm (log ε 3.65) and a shoulder at 250 nm (log ε 3.62). On quantitative hydrogenation, the material absorbed more than 3, but less than 4, equiv of hydrogen and seemed best considered as an impure octatetraene. Variable and small yields in the preparation and lack of the powerful, later-developed separation and structural analysis techniques prevented resolution of the structural problem at the time.

There are seven possible $C_8H_{10}$ tetraenes with conjugated or cross-conjugated double bonds. Of these, the linear trans,trans[3] and cis, trans[4] have been synthesized and have ultraviolet maxima about 40 nm toward shorter wavelengths than those of 3, and this fact clouds the earlier assignment of octatetrene formation.

In a related investigation,[5] pure samples of the exo and endo isomers of 1 and 2 were prepared and subjected individually to elimination conditions with tertiary amines. However, despite a slight yellow cast occasionally observed for the elimination products, the infrared, proton NMR, and carbon-13 NMR spectra indicated formation of only bicyclo[3.3.0]-2,6-octadiene and bicyclo[3.3.0]-2,7-octadiene. That none of the tetraene was found suggested that the polyene arose from an impurity in the original tosylate mixture and, in fact, the culprits were ultimately found to be the stereoisomeric 6-bicyclo[3.2.0]-2-heptenyl tosylates, 4. These tosylates were derived from the corresponding alcohols, 6 (Scheme I), formed in varying amounts in the reduction of the cyanohydrin acetate, 5, which was a key intermediate in the formation of 1 and 2 in the earlier synthesis of these substances.[2] Reduction of the acetate group of 5 in competition with reduction of the cyano group would lead to the isomers of 6 along with the desired aminomethyl alcohol. Subsequent contamination of the tosylate mixture of 1 and 2 with the stereoisomeric tosylates, 4, could lead to ring-opened $C_7$ trienes. Thus, acetolysis of 6-bicyclo[3.2.0]-2-heptenyl mesylate yields better than 30%, 1,3,5-cycloheptatriene, 7[6] and pyrolysis of the methyl xanthates of 6 also yields 7.[7] However, 7 is colorless with a $\lambda_{max}$ of 261 nm. A possible yellow $C_7$ triene is the fulvene, 5-ethylidene-1,3-cyclopentadiene, 8, which can readily be envisioned as being formed from 4 by a mechanism similar to that postulated for formation of 7 (Scheme II).




Scheme I




Scheme II

Reduction of bicyclo[3.2.0]-2-hepten-6-one with lithium aluminum hydride at refluxing ether temperatures yielded a 70:30 mixture of *endo*- and *exo*-6, respectively. A corresponding mixture of the tosylates 4, heated with collidine, gave an intensely yellow product mixture which, before distillation, was found to be comprised of 70% 7, 15% 8, and 15% *exo*-4. The individual epimeric tosylates, *exo*-4 and *endo*-4, behave differently when heated with collidine. The *endo*-4 isomer undergoes clean elimination to yield only 7, while *exo*-4 did not react under the same conditions. However, when the ionizing power of the medium was increased by addition of 1.9 equivs of p-toluenesulfonic acid, then the exo isomer underwent elimination to produce a 1:4 mixture of 7 and 8, respectively. In the presence of p-toluenesulfonic acid epimerization can occur along with elimination [5] and *endo*-4, so formed from *exo*-4, could be the precursor of the 7 formed under these conditions. The substantially greater reactivity of *endo*-4 here compared to that of *exo*-4 is in full accord with the acetolysis rates. [8] Steric strain associated with having the bulky tosyl group in the endo position probably facilitates the ionization of *endo*-4.

The formation of both 7 and 8 accounts for the quantitative hydrogenation data when recalculated on the basis of a $C_7$ triene. Furthermore, the absorption maximum at 260 nm with a shoulder at 250 nm is reasonable, because 7, as mentioned, has a maximum at 261 nm (log $\varepsilon$ 3.54) [9] and 5-ethylidene-1,3-cyclopentadiene has a maximum at 254 nm (log $\varepsilon$ 4.16) [10] which tails off well into the visible region of the spectrum.

## EXPERIMENTAL SECTION

The $^1$H NMR spectra were taken on a Varian EM-390 spectrometer operating at 90 MHz.

The tosylate elimination reactions in collidine were carried out as previously described.[5]

Bicyclo[3.2.0]-2-hepten-6-one was prepared as previously described.[5]

*exo*- and *endo*-Bicyclo[3.2.0]-2-hepten-6-ol, 6, was prepared by lithium aluminum hydride reduction of bicyclo[3.2.0]-2-hepten-6-one in refluxing ether. From 14.4 g of bicyclo[3.2.0]-2-hepten-6-one was obtained 11.5 g of a 70:30 mixture of *endo*-6 and *exo*-6, respectively. The epimers were separated by preparative gas chromatography on a 3/8 in x 16 ft Carbowax 20M column. *exo*-Bicyclo[3.2.0]-2-hepten-6-ol, *exo*-6: $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.78-3.29(m, 6 H), 3.50-3.82 (m, 1 H), 4.90 (d, 1 H, $J$ = 6 Hz), 5.50-5.85 (m, 2 H). *endo*-Bicyclo[3.2.0]-2-hepten-6-ol, *endo*-6: $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.30-3.28 (m, 6 H), 4.12-4.50 (m, 1 H), 4.68 (d, 1 H, $J$ = 5 Hz), 5.73 (s, 2 H).

The *exo*- and *endo*-bicyclo[3.2.0]-2-hepten-6-yl tosylates, 4, were obtained individually or as a mixture from treatment of the corresponding alcohols for 1 h with 1.1 equiv of p-toluenesulfonyl chloride in the presence of excess pyridine at 0 °C. The reaction mixtures were stirred at room temperature for 15 h, diluted with ether, and then washed with 1 N hydrochloric acid, 5% sodium bicarbonate solution, and water. The ethereal layer was dried over potassium carbonate and the ether removed under reduced pressure. *endo*-Bicyclo[3.2.0]-2-hepten-6-yl

tosylate, *endo*-4: $^1$H NMR ($CDCl_3$) δ 1.62-3.30 (m, 6 H), 2.41 (s, 3 H), 4.90-5.18 (m, 1 H), 5.76 (br s, 2 H), 7.56 (d of d, 4 H). *exo*-Bicyclo-[3.2.0]-2-hepten-6-yl tosylate, *exo*-4: $^1$H NMR ($CDCl_3$) δ 1.95-3.28 (m, 6 H), 2.41 (s, 3 H), 4.37-4.60 (m, 1 H), 5.68 (br s, 2 H), 7.56 (d of d, 4 H).

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# Part I

## B. Acetolysis of Some Bicyclo[3.3.0]-2-octenyl Tosylates

# ACETOLYSIS OF SOME BICYCLO[3.3.0]-2-OCTENYL TOSYLATES [1]

Michael Nee and John D. Roberts *

Gates and Crellin Laboratories of Chemistry

California Institute of Technology
Pasadena, California 91125

A wide variety of mono-, bi-, and tricyclooctane derivatives undergo solvolysis in polar solvents to form products with skeletal rearrangement. [2,3] Formation of bicyclo[3.3.0] octane derivatives is not uncommon in such rearrangements. In connection with the solution to a different problem relating to such ring systems, we have investigated the acetolysis of the stereoisomeric 6-, 7-, and 8-bicyclo[3.3.0]-2-octenyl derivatives with which, in addition to carbon and hydrogen migrations, there is also the possibility of double-bond participation.

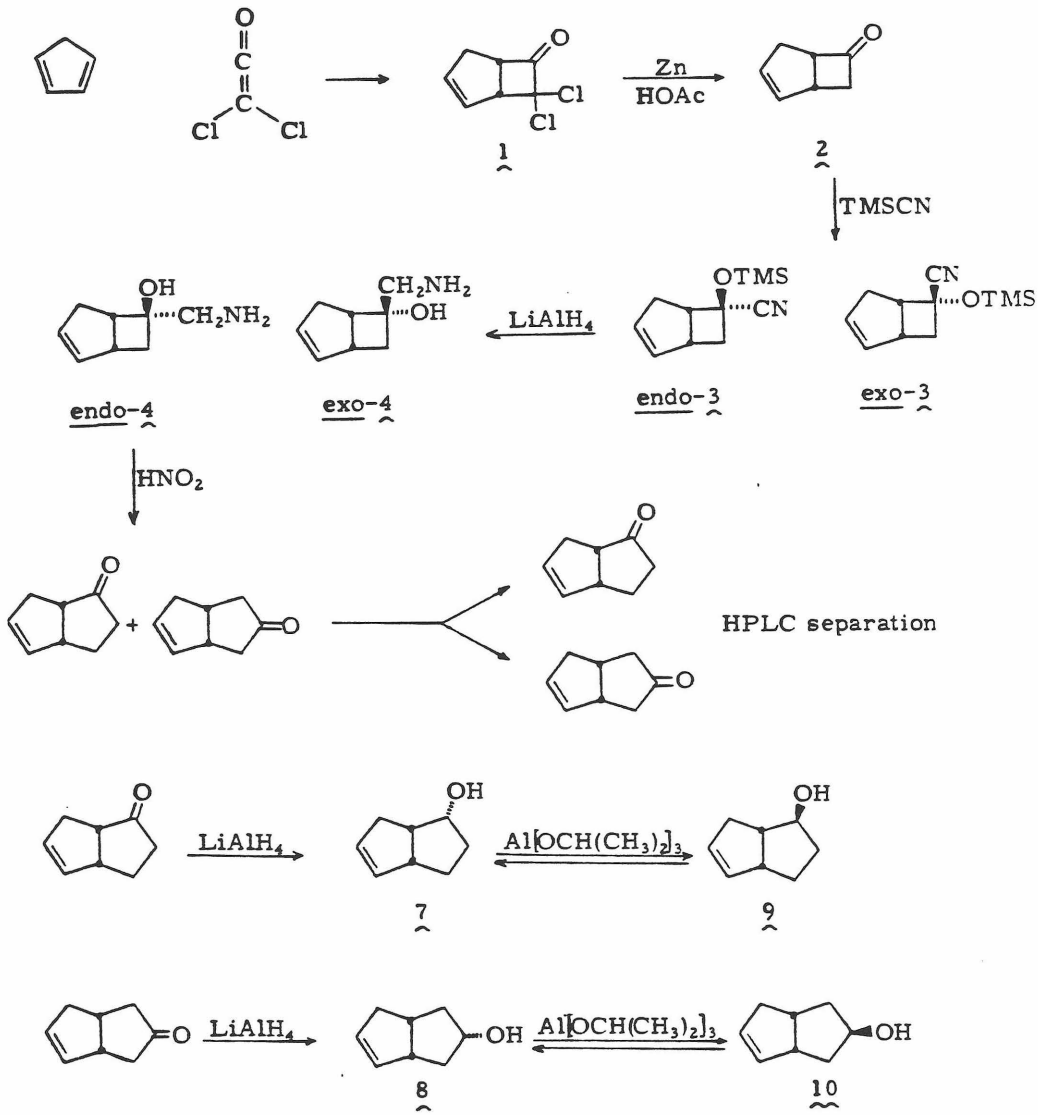
## RESULTS AND DISCUSSION

Acetolysis products of the saturated parent system 2-bicyclo[3.3.0] octanyl tosylate have previously been reported [4] and are given in Table I. A small amount of the carbon-migration product, *anti*-8-bicyclo[3.2.1] octanyl acetate, was obtained from the *exo*-2-bicyclo[3.3.0] octanyl tosylate, and some of the 1,2-hydride migration product, 1-bicyclo[3.3.0]

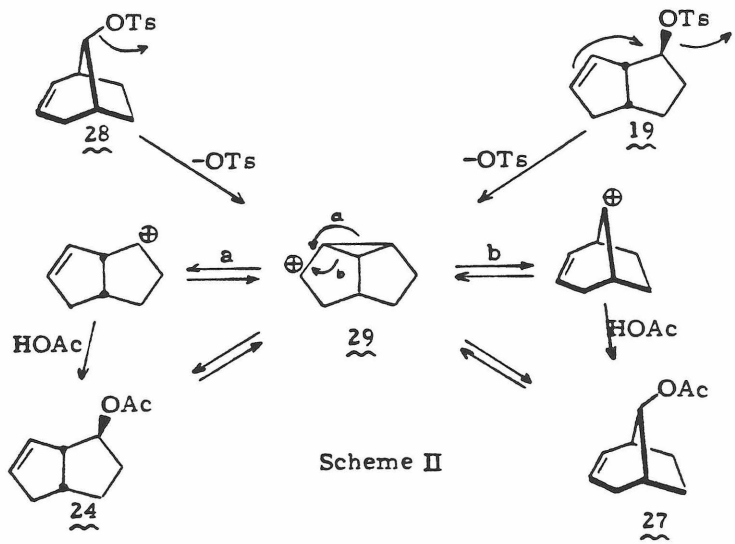
octanyl acetate, was observed from both *endo*- and *exo*-2-bicyclo[3.3.0] octanyl tosylates, along with substantial amounts of the elimination product, bicyclo[3.3.0]-2-octene. The unrearranged acetates formed are the result of inversion with the *endo*-tosylate, and mixed inversion and retention with the *exo*-tosylate.

The acetolysis products of the stereoisomeric 6- and 7-bicyclo [3.3.0]-2-octenyl tosylates derived from the alcohols prepared by Scheme I as well as the corresponding 8-tosylates are given in Table II. The acetolysis of the *endo*-tosylates 11, 13, and 17 leads predominantly to the substitution products 20, 22, and 24, respectively, resulting from an $S_N$ 2-type mechanism and the elimination products 25 and 26. Some 1,2-hydride shift products were also observed, which included shifts from C6 to C7 and vice versa as well as shifts from C8 to C7. The 1,2-hydride shifts do not occur with equal facility in either direction. *exo*-6-Bicyclo[3.3.0]-2-octenyl tosylate (12) solvolyzes to yield a relatively large amount (~60% of the ester formed) of the 1,2-hydride shift products 22 and 23. On the other hand, *exo*-7-bicyclo[3.3.0]-2-octenyl tosylate (14) yields only a small amount of hydride-shift product 20. Inversion of stereochemistry is usual, except with 8-*exo*-bicyclo[3.3.0]-2-octenyl tosylate (19),[5] which yields mostly the retention product 24 and what appears to be *anti*-8-bicyclo[3.2.1]-2-octenyl acetate (27).[6]

Very few carbon skeletal rearrangements take place in the acetolysis of bicyclo[3.3.0]-2-octenyl tosylates, except with 19. This indicates a greater degree of stability of bicyclo[3.3.0]octanes relative to the




Scheme I




Scheme II

other ring systems which might be produced by rearrangement. This stability has a thermodynamic rationalization. Schleyer et al.[7] have calculated that the equilibrium between the bicyclo[3.2.1] octane system and the bicyclo[3.3.0] system should favor the [3.2.1] system at room temperature. However, at higher temperatures, >373 K, the equilibrium shifts in favor of the [3.3.0] system because of a more favorable entropy. Some formation of the [3.2.1] products might be expected at our solvolysis temperatures (368 K) as is seen in the solvolysis of *exo*-2-bicyclo[3.3.0] octanyl tosylate and *exo*-8-bicyclo [3.3.0]-2-octenyl tosylate (19). It is interesting that 19 yields a rearranged product; the very similar 12 yields none. The difference is between a vinyl and an allyl migration, as can also be seen in the acetolysis of *anti*-8-bicyclo[3.2.1]-2-octenyl tosylate (28),[3] where vinyl migration occurs when either vinyl or allyl migration is possible (Scheme II), as expected from formation of a tricyclic cation, 29. One ring opening of 29 leads to 8-bicyclo-[3.3.0]-2-octenyl acetate (24), while the other leads to 8-bicyclo[3.2.1]-2-octenyl acetate (27).

1,2-Hydride shifts appear to occur somewhat more readily with unsaturated tosylates than with the corresponding saturated tosylates, while each series gives essentially the same amount of 1,2-elimination products. However, no consistent pattern is obvious to permit rationalization of the results.

In conjunction with another study to be described later, the elimination products and stabilities of some of the bicyclo[3.3.0]-2-enyl tosylates in collidine, 2,4,6-trimethylpyridine, were investigated,

and the results are summarized in Table III. Some epimerization occurs faster than elimination when tosylate 11 is heated at 90°C for 16 h in neat collidine. However, no 1,2-hydride shift was observed, and, at higher temperatures (125°C), elimination appears to give only the diene, bicyclo[3.3.0]-2,6-octadiene (25). The *exo*-tosylate eliminates less readily, and some is still left after heating at 125°C for 15 h. If 0.1 equiv of p-toluenesulfonic acid is added to the collidine to provide either an acid catalyst or a higher ionic strength, such as would be present after 10% elimination has taken place, then epimerization occurs quite easily in competition with elimination. Thus, after 24 h at 95°C, the product mixture consists of 36% each of the *exo*- and *endo*-6-bicyclo[3.3.0]-2-octenyl tosylates, 11 and 12, in addition to diene 25. Similar results were obtained with *endo*-7-bicyclo[3.3.0]-2-octenyl tosylate (13). It is clear that when elimination is attempted in neat collidine, as soon as some p-toluenesulfonic acid is formed the ionic character of the solvent changes, and epimerization can occur at a significant rate. When the temperature is low, epimerization can be faster than elimination. The importance of p-toluenesulfonic acid in facilitation of epimerization suggests an internal-return-type mechanism. [8]

## EXPERIMENTAL SECTION

*endo*- and *exo*-Bicyclo[3.3.0]-2-octen-6-ol and *endo*- and *exo*-bicyclo[3.3.0]-2-octen-7-ol were prepared as summarized in Scheme I. The procedure was a modified version of that described by Roberts and Gorham. [9] The *endo*- and *exo*-bicyclo[3.3.0]-2-octen-8-ols were pre-

pared by the procedure of Fujita. [10]

7,7-Dichlorobicyclo[3.2.0]-2-hepten-6-one (1) was prepared as described by Grieco. [11] From 143.5 g (0.97 mol) of dichloroacetyl chloride, 375 g (5.7 mol) of cyclopentadiene, and 100 g (0.99 mol) of triethylamine was obtained 124.3 g (72%) of 1: bp 60-65°C (2.5 mm) [lit. [11] bp 49-50°C (0.3 mm)]; $^1$H NMR ($CDCl_3$) δ 2.30-2.90 (m, 2H), 3.90-4.35 (m, 2H), 5.65-6.10 (m,2H).

Bicyclo[3.2.0]-2-hepten-6-one (2) was also prepared as described by Grieco, [11] and from 124.3 g (0.70 mol) of 1, 261.7 g (4.00 mol) of zinc dust, and 400 mL of glacial acetic acid there was obtained 61.5 g (81%) of 2: bp 25°C (2.0 mm) [lit.[11] bp 60°C (∿15 mm)]; $^1$H NMR ($CDCl_3$) δ 2.30-2.80 (m,3H), 3.10-3.55 (m,2H), 3.65-3.95(m, 1H), 5.60-5.85 (m, 2H).

6-Cyano-6[(trimethylsilyl)oxy]bicyclo[3.2.0]-2-heptene (3) was prepared by the method of Evans. [12] From 16.2 g (0.15 mol) of 2, 16.5 g of trimethylsilyl cyanide (TMSCN, 0.17 mol) and a catalytic amount of zinc iodide was obtained 22.0 g (71%) of 3 (approximately a 50:50 mixture of the *endo*-3 and *exo*-3 isomers): bp 54-55°C (0.6 mm); IR (neat) 1600, 1255 (Si-C), 1147, 882, 845 $cm^{-1}$; $^1$H NMR ($CDCl_3$) δ 0.23 (split s, 9 H, $Si(CH_3)_3$), 1.85-3.50 (m, 6 H),5.70 (br s, 2 H); mass spectrum, *m/e* 207 (molecular ion).

Anal. Calcd for $C_{11}H_{17}NOSi$: C, 63.72; H, 8.26; N, 6.76. Found: C, 63.55; H, 8.10; N, 6.49.

6-(Aminomethyl)bicyclo[3.2.0]-2-hepten-6-ol (4) was prepared by the method of Evans. [12] From 87.3 g (0.42 mol) of 3 and 26.0 g of

lithium aluminum hydride (0.69 mol) was obtained 48.0 g (82%) of 4 (approximately a 50:50 mixture of *endo*-4 and *exo*-4 isomers): bp 67°C (1 mm) [lit. [9] bp 83-84°C (1.5 mm)]; $^1$H NMR ($CDCl_3$) δ 1.5-3.3 (m, 11 H), 5.65 and 5.75 (2 s, 2 H).

Bicyclo[3.3.0]-2-octen-6-one (5) and bicyclo[3.3.0]-2-octen-7-one (6) were prepared by a Tiffeneau-Demjanov ring expansion of 4, as described previously. [9] From 10.8 g (∿78 mmol) of crude 4, 4.5 mL (78 mmol) of glacial acetic acid, and 5.35 g (78 mmol) of sodium nitrite was obtained 8.75 g (92%) of a 62:38 mixture of 5 and 6. The isomeric ketones were separated on a Waters 500 Prep HPLC by using a 95:5 hexane-ethyl acetate solvent mixture and a flow rate of 0.4 L/min. Isomer 5 has a retention volume of about 2.2 L and 6 of about 2.7 L (column volume of 1 L). For bicyclo[3.3.0]-2-octen-6-one (5): $^1$H NMR ($CDCl_3$) δ 1.40-2.33 (m, 4 H), 2.33-3.10 (m, 3 H), 3.30-3.63 (m, 1 H), 5.47-5.77 (m, 2 H). For bicyclo[3.3.0]-2-octen-7-one (6): $^1$H NMR ($CDCl_3$) δ 1.70-3.17 (m, 7 H), 3.17-3.63 (m, 1 H), 5.50-5.80 (m, 2 H).

*endo*-Bicyclo[3.3.0]-2-octen-6-ol (7) was prepared by lithium aluminum hydride reduction of 5 at 0°C: bp 46-47°C (0.5 mm); $^1$H NMR ($CDCl_3$) δ 1.07-1.83 (m 4 H), 1.83-2.87 (m, 3 H), 2.90-3.43 (m, 2 H), 3.77-4.30 (m, 1 H), 5.40-5.73 (m, 2 H).

*endo*-Bicyclo[3.3.0]-2-octen-7-ol (8) was prepared by lithium aluminum hydride reduction of 6 at 0°C: bp 94-95°C (1.5 mm); $^1$H NMR ($CDCl_3$) δ 1.10-2.88 (m, 8 H), 2.88-3.38 (m, 1 H), 4.11 (p, $J \approx 6$ Hz, 1 H), 5.47-5.89 (m, 2 H).

*exo*-Bicyclo[3.3.0]-2-octen-6-ol (9) was obtained by equilibration of the endo isomer, 7, with aluminum isopropoxide by using the Cope [13] procedure. The exo isomer, 9, was separated from the endo isomer by preparative high-performance LC under the same conditions as those for the separation of 5 and 6; $^1$H NMR ($CDCl_3$) δ 1.1-3.0 (m, 9 H), 3.8-4.0 (m, 1 H), 5.4-5.8 (m, 2 H).

*exo*-Bicyclo[3.3.0]-2-octen-7-ol (10) was also obtained by equilibration of the endo isomer, 8, with aluminum isopropoxide. [13] The exo isomer, 10, was separated from the endo isomer by preparative high-performance LC: $^1$H NMR ($CDCl_3$) δ 1.21-2.18 (m, 6 H), 2.40-3.06 (m, 2 H), 3.23 (m, 1 H), 4.24 (p, $J \approx 5.5$ Hz, 1 H), 5.52 (s, 2 H).

*endo*-6-Bicyclo[3.3.0]-2-octenyl tosylate (11) was obtained from treatment of the alcohol 7 with 1.1 equiv of p-toluenesulfonyl chloride in the presence of excess pyridine at 0°C for 1 h and stirring of the mixture at room temperature for 15 h. The mixture was diluted with ether and then washed with 1 N hydrochloric acid, 5% sodium bicarbonate solution, and water. The ethereal layer was dried over potassium carbonate. The crude tosylate obtained by evaporation of the ether was not further purified: $^1$H NMR ($CDCl_3$) δ 1.05-1.85 (m, 4 H), 1.85-2.92 (m, 3 H), 2.42 (s, 3 H), 2.92-3.36 (m, 1 H), 4.52-4.92 (m, 1 H), 5.32-5.66 (m, 2 H), 7.54 (dd, 4H).

*exo*-6-Bicyclo[3.3.0]-2-octenyl tosylate (12) was prepared as described for 11 by starting from 9: $^1$H NMR ($CDCl_3$) δ 1.65-3.50 (m, 8 H), 2.40 (s, 3 H), 4.53-4.68 (m, 1 H), 5.43 (br s, 2 H), 7.55 (dd, 4 H).

*endo*-7-Bicyclo[3.3.0]-2-octenyl tosylate (13) was prepared from 8 in the same manner as described for 11: $^1$H NMR ($CDCl_3$) δ 1.34-1.77 (m, 3 H), 1.77-2.82 (m, 4 H), 2.40 (s, 3 H), 2.82-3.21 (m, 1 H), 4.77 (p, J ≈ 6.4, 1 H), 5.50 (br s, 2 H), 7.52 (dd, 4 H).

*exo*-7-Bicyclo[3.3.0]-2-octenyl tosylate (14) was prepared from 10 in the same manner as described for 11: $^1$H NMR ($CDCl_3$) δ 1.13-3.32 (m, 8 H), 2.44 (s, 3 H), 4.91 (p, J ≈ 4.8 Hz, 1 H), 5.50 (br s, 2 H), 7.56 (dd, 4 H).

3,4-Epoxycyclooctene (15) was prepared by oxidation of 1,3-cyclooctadiene with m-chloroperbenzoic acid in dichloromethane. From 6.1 g (56 mmol) of 1,3-cyclooctadiene and 12.0 g of m-chloroperbenzoic acid (80-90%) was obtained 5.5 g (79%) of 15: bp 46-50°C (3.5 mm) [lit. [14] bp 94-97°C (41 mm)]; $^1$H NMR ($CDCl_3$) δ 1.10-2.53 (m, 8 H), 2.93-3.20 (m, 1 H), 3.35-3.49 (br d, 1 H), 5.45-5.91 (m, 2 H).

*endo*-Bicyclo[3.3.0]-2-octen-8-ol (16) was prepared as described by Crandall. [14] From 5.6 g (45 mmol) of 15 and 2.5 equiv of lithium diethylamide was obtained 4.0 g (71%) of 16: bp 69-70 °C (5.5 mm) [lit.[14] bp 93-96 °C (20 mm)]; $^1$H NMR ($CDCl_3$) δ 0.67-2.88 (m, 7 H), 3.00-3.37 (m, 1 H), 4.04-4.31 (m, 1 H), 5.50-5.94 (m, 2 H).

*endo*-8-Bicyclo[3.3.0]-2-octenyl tosylate (17) was prepared from 16 in the same manner as described for 11: $^1$H NMR ($CDCl_3$) δ 1.29-2.89 (m, 7 H), 2.45 (s,3H), 3.04-3.36 (m, 1 H), 4.69-5.00 (m, 1 H), 5.36-5.83 (m, 2 H), 7.55 (dd, 4 H).

*exo*-Bicyclo[3.3.0]-2-octen-8-ol (18) was prepared as described by Fujita,[10] except that the acetate was not isolated. The tosylate 17 was allowed to react with tetraethylammonium acetate tetrahydrate in acetone for 24 h to yield *exo*-bicyclo[3.3.0]-2-octen-8-yl acetate. The acetate was then saponified in a solution of potassium hydroxide and methanol to yield 17. From 3.7 g of 16 was obtained 1.6 g (43%) of 18: bp 66-70°C (3 mm); $^1$H NMR ($CDCl_3$) δ 1.03-3.00 (m, 10 H), 3.89-3.97 (m, 1 H), 5.30-5.60 (m, 2 H).

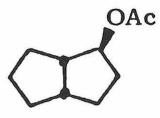
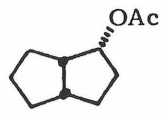
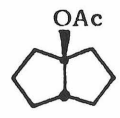
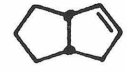
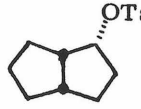
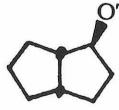
*exo*-8-Bicyclo[3.3.0]-2-octenyl tosylate (19) was prepared from 18 in the same manner as described for 11: $^1$H NMR ($CDCl_3$) δ 1.17-2.98 (m, 7 H), 2.41 (s, 3 H), 3.07-3.33 (m, 1 H), 4.57-4.92 (m, 1 H), 5.25-5.72 (m, 2 H), 7.52 (dd, 4 H).

General Procedure for Acetolysis Reactions. The tosylate and 2 equiv of anhydrous sodium acetate were dissolved in anhydrous glacial acetic acid, and nitrogen was bubbled through the solution for 5 min. The container was sealed, immersed in a thermostated oil bath at 95 ± 0.5°C for 18 h, and then cooled in an ice bath. Ether and water were added to the solution, and the excess acetic acid was removed by washing with saturated sodium carbonate solution. The ethereal layer was washed with water and then dried over potassium carbonate. The products were analyzed by gas chromatography. Samples for structural analysis were obtained by preparative gas chromatography. The stereoisomeric 7-substituted acetates had identical reaction times. The acetate mixtures were then reduced to the alcohols with lithium

aluminum hydride and the alcohols analyzed. Proton NMR spectroscopy was used to identify the acetolysis products.

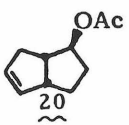
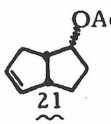
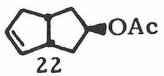
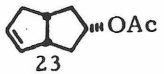
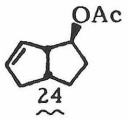
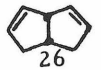
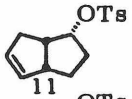
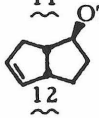
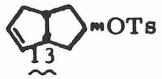
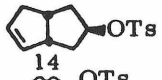
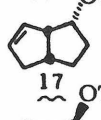
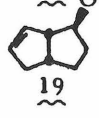
Stability of Tosylates in Collidine. In a round-bottomed flask equipped with a reflux condenser, drying tube, and magnetic stirrer was placed a solution of the tosylate in collidine. The flask was immersed in a preheated oil bath at the prescribed temperature for 15-24 h, at which time either the reaction mixture was worked up or a 1-mL aliquot was removed for analysis and the remainder heated further at a higher temperature. The product isolations involved taking up the cooled mixture in ether, washing the ethereal solution with iced 10% sulfuric acid and water, and drying over magnesium sulfate. The products were analyzed by proton NMR spectroscopy.

Table I. Acetolysis Products of Endo- and Exo-2-Bicyclo[3.3.0]octyl Tosylates

| |  |  |  |  |  |
|---|---|---|---|---|---|
|  [a] | 51.3 | - | 1.7 | 0 | 47 |
|  [a] | 24.6 | 14.7 | 0.9 | 7.9 | 52 |

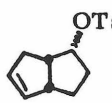
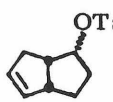
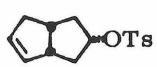
[a] Reference 4

Table II. Acetolysis Products of 2-Bicyclo[3. 3. 0]octenyl Tosylates

| |  20 |  21 |  22 |  23 |  24 |  25 |  26 |  27 | Other products |
|---|---|---|---|---|---|---|---|---|---|
|  11 | 50 | - | 5 | 2 | - | 41 | - | - | 2 |
|  12 | 3 | 10 | 5 | 14 | - | 58 | 9 | - | 1 |
|  13 | 2 | - | 45 | 10 | - | 30 | 10 | - | 3 |
|  14 | 2 | - | 5 | 38 | - | 21 | 25 | - | 5 |
|  17 | - | - | 7. 5 | 1 | 63 | 4 | 21 | - | 3. 5 |
|  19 | - | - | - | - | 69 | 3 | 19 | 8[a] | 1 |

[a]Tentative assignment

Table III. Products of 6- and 7-Bicyclo[3. 3. 0]-2-octenyl Tosylates in 2, 4, 6-Trimethylpyridine

| Tosylate | Concentration, M | Temp, time | Product, % 25 | 26 | 11 | 12 | 13 | 14 | 10 |
|---|---|---|---|---|---|---|---|---|---|
|  | 0.88 | 80°, 16.5 h | - | - | 93 | 7 | - | - | - |
| | | 90°, 16.3 h | - | - | 93 | 7 | - | - | - |
| | | 90-120°, 3.5 h | - | - | 83 | 17 | - | - | - |
| | | 120°, 15 h | 81 | - | - | 19 | - | - | - |
|  + 0.093 M p-TsOH | 0.91 | 95°, 24 h | 20 | - | 36 | 36 | - | - | - |
|  + 0.084 M p-TsOH· $H_2O$ | 0.86 | 95°, 25.5 h | 31 | 22 | - | - | 12 | 19 | 16 |

1. Supported by the National Science Foundation.

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5. A previous solvolysis study done on *endo*- and *exo*-bicyclo-[3.3.0]-2-octen-8-yl brosylates in 70% aqueous dioxane found no rearrangement products. Chien, C.-C. Ph.D. Dissertation, University of Illinois, 1974.

6. This product is tentatively identified; however, it was definitely a rearrangement product and not one of the bicyclo[3.3.0]-2-octenyl acetates.

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13. Cope, A. C.; Brown, M.; Petree, H. E. J. Am. Chem. Soc. 1958, 80, 2852-2855.

14. Crandall, J. K.; Chang, L. H. J. Org. Chem. 1967, 32, 532-536.

# Part II

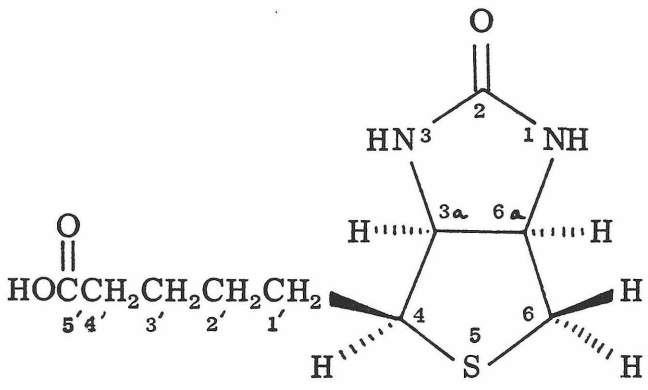
## Assignment of the Nitrogen-15 Nuclear Magnetic Resonances of Biotin and Unequivocal Synthesis of (+)-[1-$^{15}$N]Biotin

# ASSIGNMENT OF THE NITROGEN-15 NUCLEAR MAGNETIC RESONANCES OF BIOTIN AND UNEQUIVOCAL SYNTHESIS OF (+)-[1-$^{15}$N]BIOTIN[1]

Amy Abe, Michael Nee, and John D. Roberts*
The Gates and Crellin Laboratories of Chemistry†
California Institute of Technology
Pasadena, California 91125

Wayne L. Wittenberg and Nelson J. Leonard*
Roger Adams Laboratory, School of Chemical Sciences
University of Illinois, Urbana, Illinois 61801

(+)-Biotin (1) is an essential cofactor for several enzymes involving carboxylation and carbonyl-exchange reactions.[2] It participates in the fixation, activation, and transfer of carbon dioxide, forming an N-carboxybiotin in which N1 was established as the point of attachment.[3] The chemical reactivity of the two unreido nitrogens of 1 is strikingly

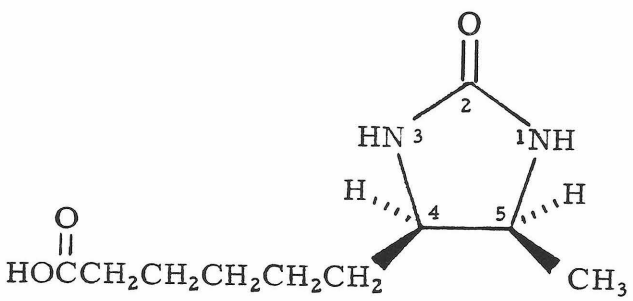



1

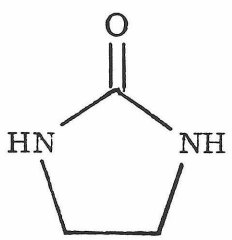
different. Thus, N1- and N3-acylated products in an 100:7 ratio resulted from the reaction of methyl chloroformate with biotin methyl ester. [4a] The low proportion of the N3 product was attributed to steric hindrance to the approach of the reagent to N3 because of the presence of the carboxylbutyl side chain. A similar argument was employed [4b] to account for the difference in exchange rates for the unreido protons with ethanol.

Because of the lack of sensitive physical methods to distinguish between the two nitrogens of 1 in solution, we have examined the $^{15}N$ NMR spectra in the hope of providing a useful probe for determining how protein systems interact with biotin.

The $^{15}N$ chemical shifts[5] and one-bond NH coupling constants for biotin and two model compounds, desthiobiotin (2), and 2-imidazolidinone (3), are given in Table I. For both biotin and desthiobiotin, two




2




3

well-resolved $^{15}N$ resonances are observed in the proton-decoupled spectra. The two resonances in desthiobiotin were assigned on the basis of selective decoupling. The coupled spectrum shows two broad doublets corresponding to one-bond NH splittings of 90 and 91 Hz. Selective irradiation of the protons of the methyl group at C5 changed the downfield $^{15}N$ doublet into a broad doublet of doublets. This behavior is consistent with the assignment of the downfield resonance to N1, with a three-bond coupling of the methyl protons (typical[6,7] $^3J_{NH}$ = 2-4 Hz) to N1 which can be removed by the selective decoupling, and also with the residual splitting due to the C4 proton.

The two resonances of biotin were assigned both by off-resonance decoupling and by synthesis of biotin specifically labeled with $^{15}N$ at N1. Explicit assignments of the biotin proton chemical shifts were necessary for the proton off-resonance decoupling experiments. The ureido proton resonances are observed at 220 MHz as two broad singlets at $\delta$ 6.45 and 6.37. On the basis of homonuclear decoupling experiments, the downfield ureido resonance was assigned to H3.

On irradiation with a coherent decoupling field ($\gamma H_2$), the biotin one-bond NH couplings ($J$) are reduced and the long-range splittings effectively removed. The residual coupling ($J^r$), which is linearly dependent on the difference ($\Delta\nu$) between the resonance frequency of the directly bonded proton and the decoupler frequency, goes to zero when the decoupler frequency coincides with the proton resonance.[8] Thus, $J^r = J\Delta\nu/(\gamma H_2)$. As the proton decoupler was moved to higher field for

biotin, the residual coupling of the nitrogen resonance centered at 294.7 ppm went to zero before the residual coupling of the resonance centered at 285.6 ppm. Therefore, the resonance centered at 294.7 ppm corresponds to the nitrogen directly coupled to the downfield ureido proton, H3. Thus, the chemical shift of N3 is 294.7 and of N1 is 285.6 ppm.

Synthesis of (+)-[1-$^{15}$N] biotin was achieved by the multistep procedure of Vasilevskis et al.,[9] starting with the (-)-α-methylbenzylamine salt of methyl 5-(2,5-dihydro-4-hydroxy-3-nitrothien-2-yl)-pentanoate (4) kindly provided by Hoffmann-LaRoche, Inc., and using $^{15}$N-labeled KNCO[10] with the reduced nitro ketone 5 to introduce the label unequivocally into the eventual N1 position of (+)-biotin (7). The proton-decoupled spectrum of the N1-labeled biotin in 0.1 N $NaHCO_3$ gave a single resonance at 283.5 ppm (see Figure 1). This result confirms the off-resonance decoupling assignment.

The nitrogen chemical shift range of ureas[11] is between 270 and 320 ppm. The equilibrium concentration of the imino tautomer is usually small. Comparison of the chemical shifts of *O*-methylisourea[12] and tetramethylurea indicates that the imine nitrogen should be downfield from urea nitrogens by about 80 ppm. The range of nitrogen chemical shifts observed for 1-3 is consistent with all of these nitrogens being of the urea type. Furthermore, the differences in nitrogen shifts among the homologous ureas 1-3 are consistent with known β-, γ-, and δ-

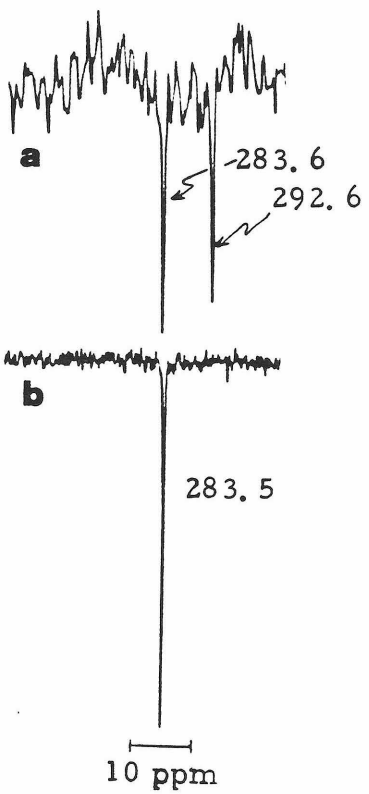
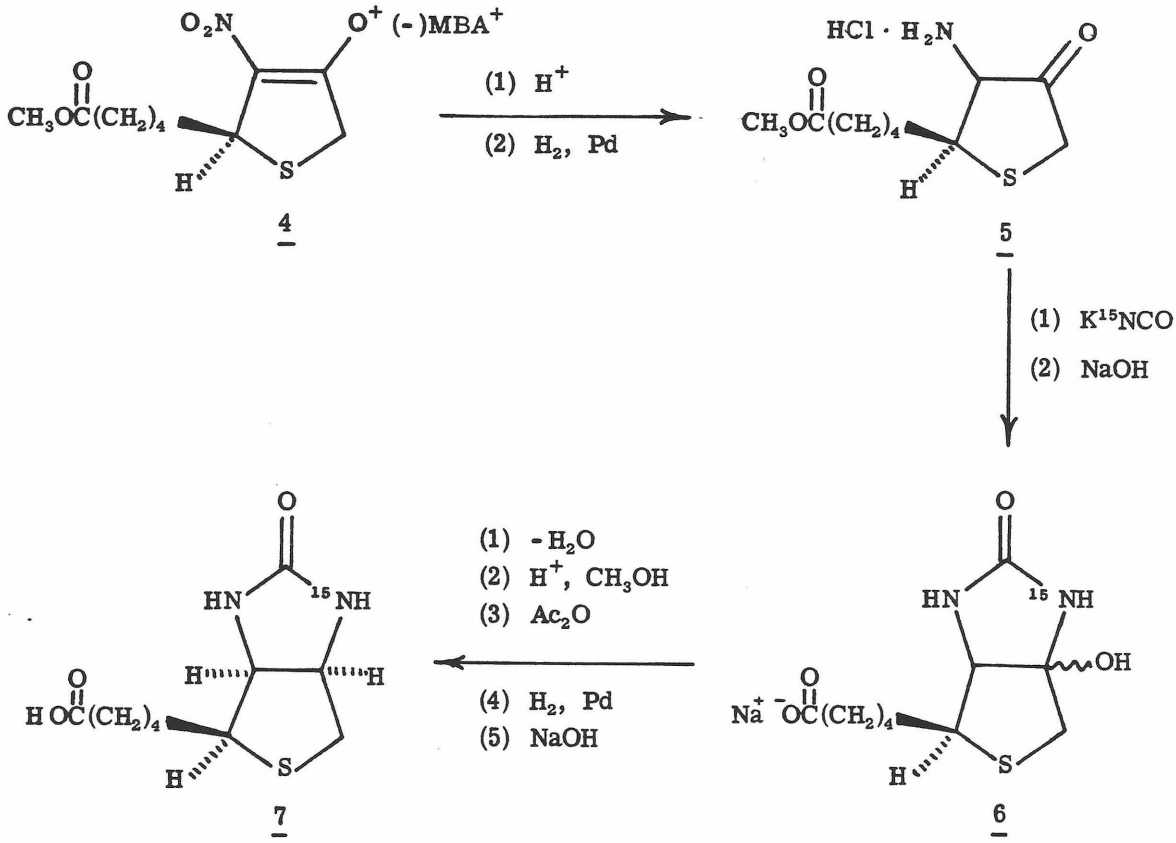



Figure 1. (a) Natural-abundance $^{15}N$ NMR spectrum of (+)-biotin in 0.1 N $NaHCO_3$. (b) $^{15}N$ NMR spectrum of (+)-biotin, specifically labeled with $^{15}N$ (93%) at N1, in 0.1 N $NaHCO_3$.




substituent effects on ureas.[13,14] Downfield shifts in the range 12-19 ppm result from replacing a hydrogen on the α carbon by a methyl group in acrylic ureas.[13] Similar β-substitutent effects can account for most of the shift differences observed between 2-imidazolidinone (3) and desthiobiotin (2).

γ-Substituent effects are sensitive to molecular conformation and increase with steric crowding at the nitrogen induced by the γ substituent. For cyclic compounds, where the relationship between the nitrogen and γ carbon is fixed, gauche effects are pronounced. Koch and co-workers[14] reported that the substituent effects for sterically

hindered urea nitrogens are increased by 10-12 ppm (upfield) for $\gamma$ substituents and 4 ppm (downfield) for $\delta$ substituents.[15]

The 5.7-ppm difference between the desthiobiotin resonances falls within the range expected for $\gamma$ and $\delta$ effects on N3 arising from the carboxypentyl side chain at C4. For biotin molecules in the crystal,[16] the sulfur is in the endo position forming 84 and 86° dihedral angles with N3 and N1 which constrains C1' to a pseudoequatorial position and a 43° dihedral angle to N3. The solution conformation is similar as indicated by the proton-coupling constants.[17,18] The difference between the interactions of N1 and N3 with C1', combined with the presence of a $\gamma$-sulfur atom, could reasonably cause the N3 nitrogen shift of biotin to be only 2 ppm downfield from that of 2-imidazolidinone (3) and yet 9 ppm upfield from that of N1.

The steric hindrance which causes these $^{15}$N-shift differences is in accord with the observed retardation of chemical reactions at N3 relative to N1.[2,4] The availability of (+)-biotin unequivocally labeled at N1 suggests its use as a mechanistic probe for following the biological carboxylation and transcarboxylation of biotin and investigating the interaction between biotin and avidin, inter alia.

Table 1. $^{15}N$ NMR Chemical Shifts and Coupling Constants for Biotin and Model Compounds

| compd (concn, M) | | solvent | $\delta^{15}N$ ($^1J_{NH}$, Hz) N1 | $\delta^{15}N$ ($^1J_{NH}$, Hz) N3 |
|---|---|---|---|---|
| 2-imidazolidinone (3) | (1) | $H_2O$ | 294.0 | 294.0 |
| | (1.8) | $H_2O$ | 295.5 | 295.5 |
| | (1) | 0.1 N $NaHCO_3$ | 294.3 | 294.3 |
| | (2) | $(CH_3)_2SO$ | 296.7 | 296.7 |
| | | | (93) | (93) |
| desthiobiotin (2) | (saturated soln) | 0.1 N $NaHCO_3$ | 276.8 | 282.5 |
| | (0.7) | $(CH_3)_2SO$ | 279.3 | 285.0 |
| | | | (90) | (91) |
| biotin (1) | (saturated soln) | 0.1 N $NaHCO_3$ | 283.6 | 292.6 |
| | (0.3) | $(CH_3)_2SO$ | 285.6 | 294.7 |
| | | | (94) | (92) |

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† Contribution No. 6345 from these laboratories.

(1) This work was supported at the University of Illinois by Research Grants CHE-76-23543 and CHE-79-22001 and at the California Institute of Technology by the National Science Foundation and the Public Health Service, Research Grant GM-11072 from the Division of General Medical Sciences.

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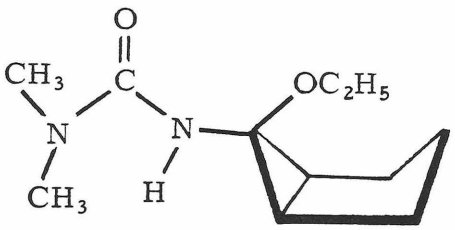
(15) For example, the NH shift in 9 is 10.6 ppm upfield from the corresponding shift in 8.[14]



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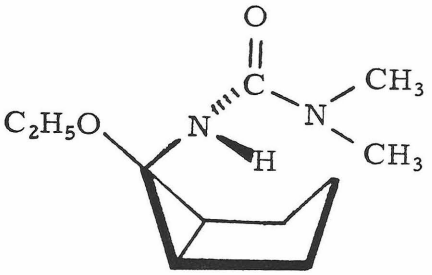

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# Part III

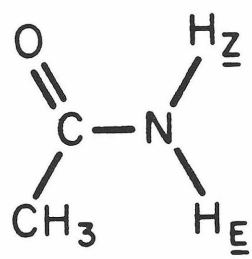
## A $^{15}N$ Nuclear Magnetic Resonance Study of the Base-Catalyzed $-NH_2$ Exchange Reactions of Acetamide and Thioacetamide

# A $^{15}N$ NUCLEAR MAGNETIC RESONANCE STUDY OF THE BASE-CATALYZED $-NH_2$ EXCHANGE REACTIONS OF ACETAMIDE AND THIOACETAMIDE[†]

Michael Nee, Yu Chun ,[‡] Michael E. Squillacote,
and John D. Roberts[*]

Gates and Crellin Laboratories of Chemistry
California Institute of Technology
Pasadena, California 91125

Nuclear magnetic resonance (NMR) spectroscopy has been very fruitful for the study of barriers to rotation about the C-N bond[1,2] of amides as well as the relative acid and base-catalyzed exchange rates of the NH protons.[3,5] For primary amides, the barrier to internal rotation about the C-N bond is high[2] and, as a result, the two amide protons are chemically and magnetically nonequivalent. Thus, for both acetamide (1) and thioacetamide (2), the $^1J_{^{15}N-^1H}$ coupling constants are unequal.[6,7] Of special interest is the fact that the exchange rates are also unequal.[4,5]

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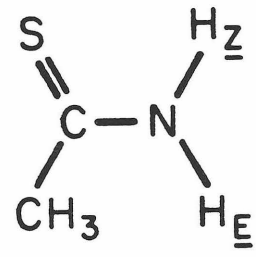



2

Differences in the exchange rates are expected to be observable in the

coupled $^{15}N$ NMR spectrum of a primary amide when the protons are undergoing intermediate rates of exchange on the NMR time scale. The coupled $^{15}N$ NMR spectrum of a primary amide in water at neutral pH is a close doublet of doublets which is sufficiently broadened by the three-bond coupling to the methyl group to make it appear as a triplet. As the pH is increased, one coupling of the doublet of doublets is expected to collapse to a doublet; then, with further increases in pH, change to a singlet.

Proton-coupled $^{15}N$ spectra of acetamide and thioacetamide at intermediate base-catalyzed exchange rates are shown in Fig. 1. Attempts to determine the proton exchange rate ratios of acetamide and thioacetamide with $^{15}N$ spectra taken at the natural-abundance level of the isotope (0.37%) were unsuccessful. Although the doublet was observed, the signal-to-noise ratios were too poor to give conclusive results. Consequently, acetamide and thioacetamide, both 99% enriched in $^{15}N$, were used in exchange-rate determinations.

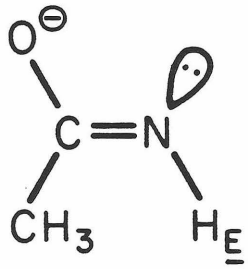
As the overall exchange rate increases, the triplet for acetamide broadens, Fig. 1A(b,c), collapses to an ill-defined peak, Fig. 1A(d), and becomes a broad doublet, Fig. 1A(e). This doublet narrows, sharpens, Fig. 1A(f-h), and finally coalesces to a singlet, Fig. 1A(i,j). These spectra clearly show that one of the amide protons of acetamide is exchanging faster than the other, and this can be presumed to be the proton trans to the carbonyl.[4] Thioacetamide behaves similarly to acetamide, except that the doublet, although clearly observable, never

sharpens, Fig. 1B(g-i), which indicates that the difference in the E/Z amide proton exchange rates with thioacetamide is less than with acetamide.

Line shapes of the proton-coupled $^{15}N$ spectra in the intermediate exchange region were calculated, using a program developed by Anet for six different values for the ratios of the base-catalyzed E and Z amide proton exchange rates, $r_{-OH}$. The calculated spectra for $r_{-OH}$ = 1,2,3,4,6 and 8 are shown in Fig. 2. When $r_{-OH}$ = 1, the protons are chemically equivalent and the triplet collapses directly to a singlet. When $r_{-OH}$ = 2, there is very little change in the appearance of the spectra when compared to those for $r_{-OH}$ = 1, except that, as the singlet evolves, it is somewhat broader initially, as can be seen by comparing Fig. 2A(g,h) with Fig. 2B(h,i). With $r_{-OH}$ = 3 or 4, the shape of the expected intermediate doublet is clear, Fig. 2C(g-i) and Fig. 2D(g-i). With larger values of $r_{-OH}$, $r_{-OH}$ = 6 and 8, the doublet becomes still better defined, Figs. 2E and 2F.

Close comparison of experimental spectra and calculated spectra shows that the ratio of exchange rates, $r_{-OH}$, for acetamide and thioacetamide is 6 $\pm$ 1 and 3 $\pm$ 1, respectively. The former value is in good agreement with that reported by Perrin[4,5] from line-shape analysis and NMR saturation-transfer methods. The fact that the base-catalyzed $r_{-OH}$ ratio for thioacetamide is somewhat smaller than that for acetamide may be due to the higher electronegativity and shorter bond distance of oxygen compared with sulfur, which would be expected to result in larger lone-pair repulsions for the E anion (3) of acetamide than

for the E anion of thioacetamide (4).



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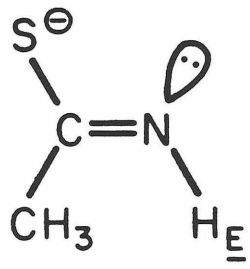

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Thus, it appears that the measurement of relative exchange rates of the E and Z protons of primary amides can be measured with reasonable precision by $^{15}N$ NMR, provided, of course, that the C-N rotational barrier is high.

## EXPERIMENTAL

The $^{15}N$ NMR spectra were obtained with a Bruker WH-180 FT NMR spectrometer operating at 18.25 MHz. The spectra were taken with a 30° flip angle, 7000-Hz spectral width, 8K data points, and a delay of 1 sec. The number of transients per spectrum varied from 400 to 3200. To take advantage of the nuclear Overhauser effect and still retain the proton couplings, the spectra were taken in the gated-decoupling mode. The proton-proton and proton-nitrogen coupling constants for thioacetamide in acetone-$d_6$ [7] were measured at 500 MHz with a Bruker WM-500 FT NMR spectrometer.

The concentrations of $^{15}$N-labeled acetamide and thioacetamide used for taking the spectra were 2.4 and 0.5 M, respectively. The exchange rates were varied by changing the pH with 0.1 N NaOH or 0.1 N HCl, and by changing the sample temperature. Intermediate rates of exchange were observed at about pH 8-9 for acetamide and pH 7-8 for thioacetamide, at room temperature.

Acetamide, 99% $^{15}$N-enriched, was obtained from Stohler Isotope Chemicals. The $^{15}$N-labeled thioacetamide was prepared by the method of Gilbert.[8] Hydrogen sulfide was bubbled for 30 min into a mixture of 0.52 g of diethylamine and 0.8 g of 99% $^{15}$N-labeled acetonitrile (Prochem Corp.) maintained at 50° C. The reaction mixture was held at 50-60° C for 3 h, then the solvent was removed under reduced pressure. The residue was added to dichloromethane. Part of the thioacetamide was collected by filtration and another part crystallized on concentration of the filtrate. The yield was 268 mg (m.p. 109-111° C, literature[8]: 111-113° C).

Line shapes for the intermediate exchange regions were calculated for acetamide, using the Anet program with coupling constants of $^{1}J_{\underline{N}\text{-}H(\underline{E},\underline{Z})}$ = 90.9 Hz and 88.4 Hz, respectively, and a natural line width of 4 Hz. The corresponding coupling constants for thioacetamide are about 2 Hz larger, but the differences do not affect the line shapes in the intermediate exchange regions.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation, and by the Public Health Service, Research Grant No. GM-11072 from the Division of General Medical Sciences. The authors are indebted to Prof. Charles Perrin for his suggestion that the $^{15}N$ spectra could be used to determine these rate ratios. We are also grateful to Prof. F. A. L. Anet for help with the use of his site-exchange program.

* Author to whom correspondence should be addressed.

† Contribution No. 6500

≠ On leave from the Institute of Photographic Chemistry, Academia Sinica, Peking, China.

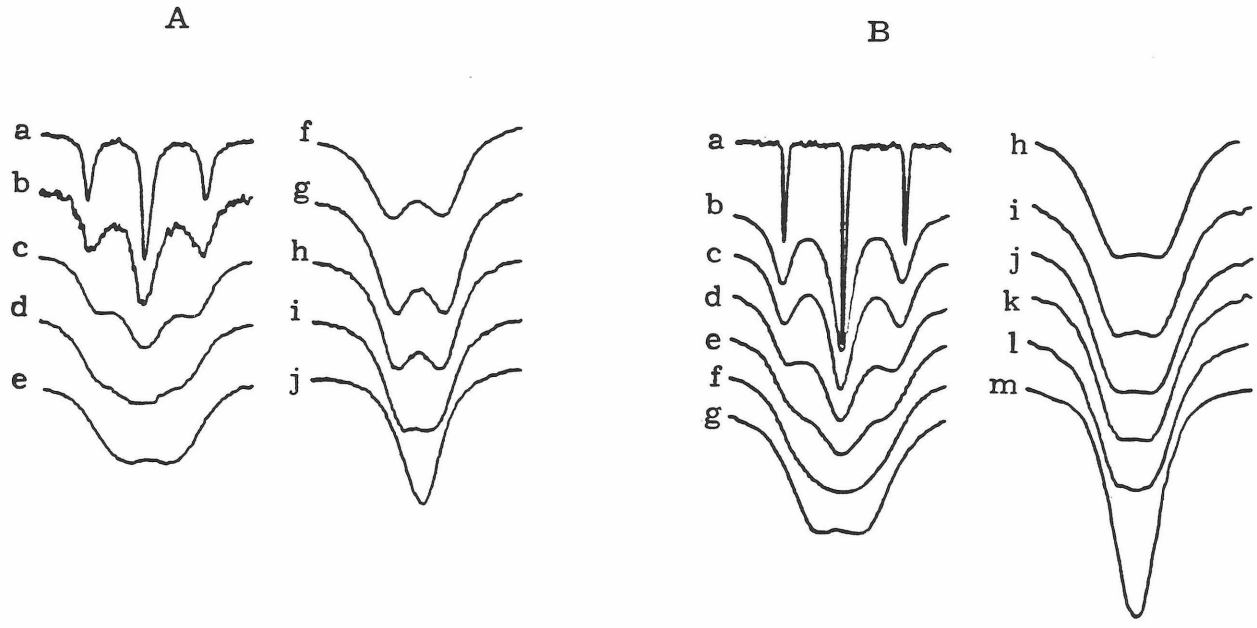


Figure 1. Experimental spectra of 2.4 M $^{15}N$ labeled acetamide (A) and of 0.5 M $^{15}N$-labeled thioacetamide (B).

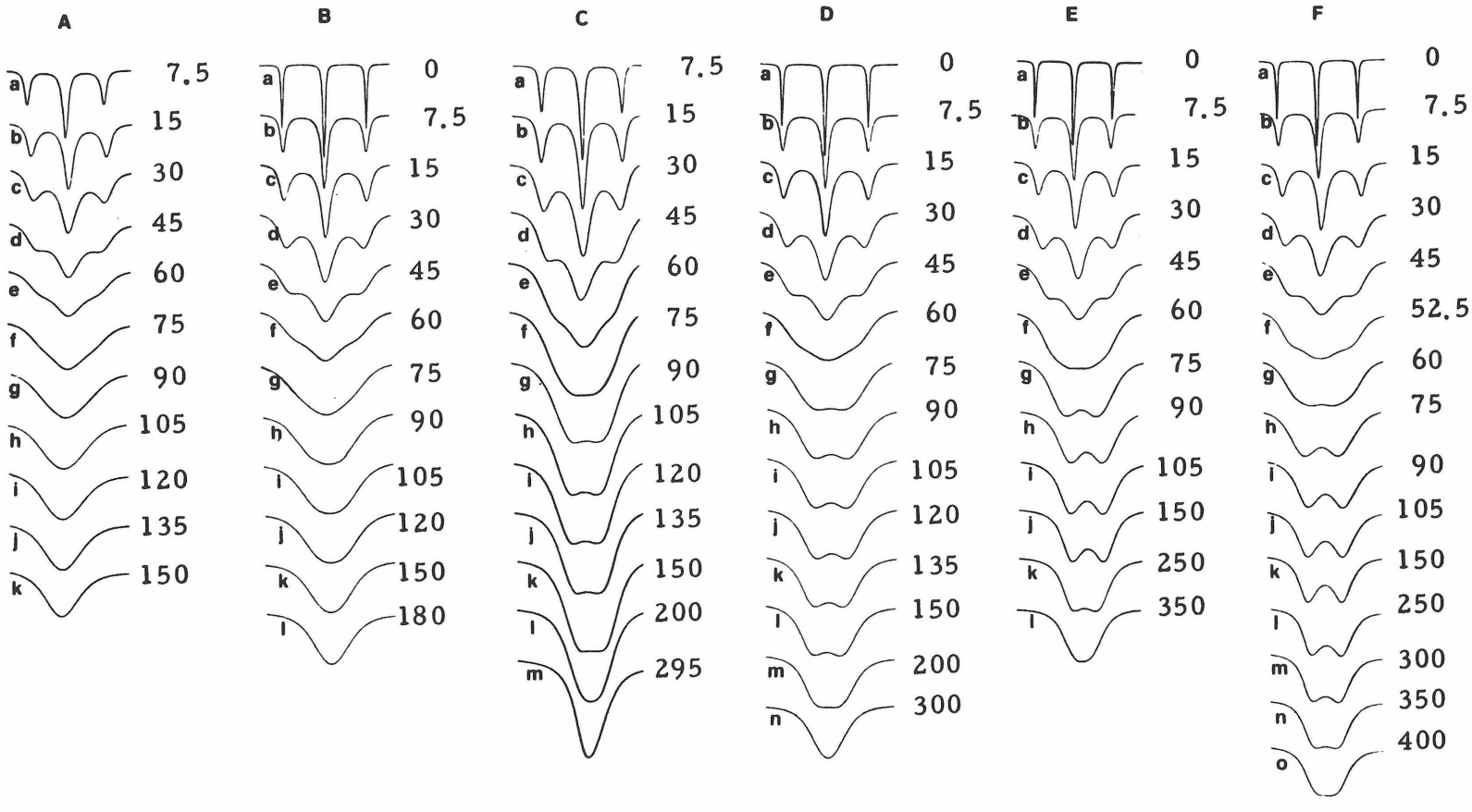


Figure 2. Calculated spectra, with average rate values, $\underline{k}_{av}$ ($sec^{-1}$), given on the right side of each spectrum. A, $\underline{r}_{OH}$ = 1; B, $\underline{r}_{OH}$ = 2; C, $\underline{r}_{OH}$ = 3; D, $\underline{r}_{OH}$ = 4; E, $\underline{r}_{OH}$ = 6; F, $\underline{r}_{OH}$ = 8.

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7. The coupling constants of thioacetamide in acetone-$d_6$ are:

$$^{1}J_{N\text{-}H(Z)} = 90.8 \text{ Hz}, \quad ^{1}J_{N\text{-}H(E)} = 89.2 \text{ Hz},$$

$$^{2}J_{H(Z)\text{-}H(E)} = 3.75 \text{ Hz}, \quad ^{3}J_{N\text{-}H} = 2.0 \text{ Hz}.$$

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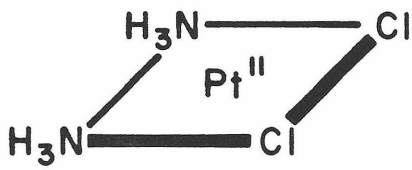
# Part IV

## Determination of the Binding Interactions of cis-$[Pt(NH_3)_2Cl_2]$ with Nucleosides by $^{15}N$ Nuclear Magnetic Resonance Spectroscopy

INTRODUCTION

Intense interest has been focused on the chemistry of platinum complexes since the discovery of the antitumor activity of cis-diammine-dichloroplatinum(II), (cis-$[Pt(NH_3)_2Cl_2]$),I, by Rosenberg, et al. [1] Cis-$[Pt(NH_3)_2Cl_2]$ has proven to be a very effective anticancer agent, especially for cancers of genitourinary origin. [2] The drug has recently been approved by the Food and Drug Administration for use in the treatment of testicular and ovarian cancers.

Systematic studies have been made of structure-activity relationships in the anticancer effects of platinum complexes. [3] Most of these studies have concentrated on neutral square planar complexes of the type $[Pt\ A_2\ X_2]$, where $A_2$ is either two monodentate amine ligands or one bidentate amine ligand and $X_2$ is either two monodentate anionic ligands or one bidentate anionic ligand. Several structural requirements have become evident for antitumor activity. A particularly important requirement was determined early on, [1,4] when it was found that both cis-$[Pt(NH_3)_2Cl_2]$,I, and cis-diamminetetrachloroplatinum(IV),III, were effective antitumor agents, but the corresponding trans isomers, II and IV, were inactive. In general, two moderately labile cis-leaving groups are necessary for activity. In addition, two inert amine ligands are also required. Although several of the platinum complexes tested had properties which allowed for easy administration, only a few were more effective than cis-$[Pt(NH_3)_2Cl_2]$. Research with other metal ions has shown that anticancer activity is not limited to platinum complexes. [2]

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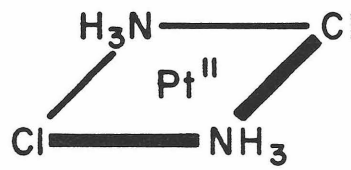


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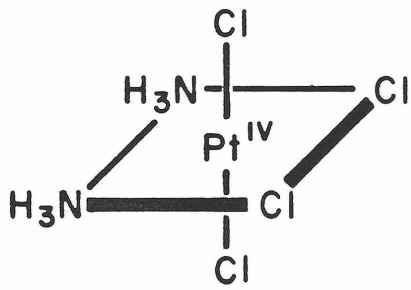


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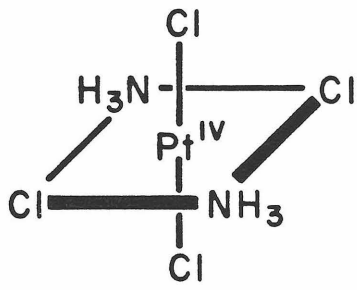



IV

The most effective complexes with other metals seem to be rhodium (II) complexes; however, these still are not as active as cis-$[Pt(NH_3)_2Cl_2]$.

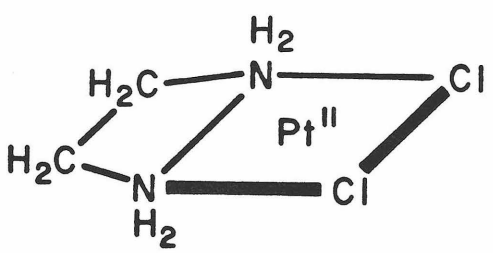
A mode of action of cis-$[Pt(NH_3)_2Cl_2]$ was suggested by Rosenberg and coworkers [1,5] on the basis of the observation that cis-$[Pt(NH_3)_2Cl_2]$ induced filamentous growth in the bacteria, E. coli. Filamentous growth in bacteria is usually indicative of an agent interacting with DNA, resulting in the selective inhibition of DNA replication but not affecting other growth processes, such as RNA or protein synthesis. This response in common to UV and X-irradiation and cytotoxic alkylating agents, all of which act by damaging DNA. In support of the view that cis-$[Pt(NH_3)_2Cl]$ acts on DNA, is the fact that platinum

compounds can induce lysogenic E. coli to enter the lytic cycle. [6] Normally, release of phage DNA is a rare event; however, agents reacting with DNA can induce this release. The antitumor activity of the platinum compounds and their ability to induce lysogenic E. coli correlate well.

Biochemical studies confirmed the interaction of cis-$[Pt(NH_3)_2Cl_2]$ with DNA. The rate of DNA synthesis in vitro is inhibited by cis-$[Pt(NH_3)_2Cl_2]$ relative to RNA and protein synthesis. [7,8,9] This selective effect on DNA synthesis is analogous to that produced by an agent such as mustard gas. [10] The DNA is modified so that it can no longer operate as a template for replication, but transcription and translation are not affected.

An alternative to direct action on DNA is inhibition of DNA synthesis through inactivation of one or more enzymes involved in DNA replication. This possibility is not very likely because protein synthesis is not affected by cis-$[Pt(NH_3)_2Cl_2]$. In addition, cis-$[Pt(NH_3)_2Cl_2]$ does not inactivate DNA polymerase in vitro, [11] and reversibly interacts with a variety of other enzymes. [12]

A somewhat puzzling result was obtained when DNA treated with

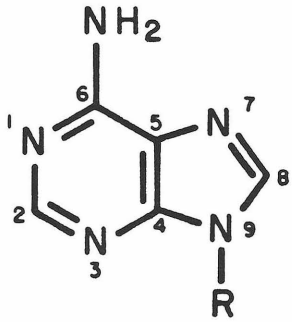



V

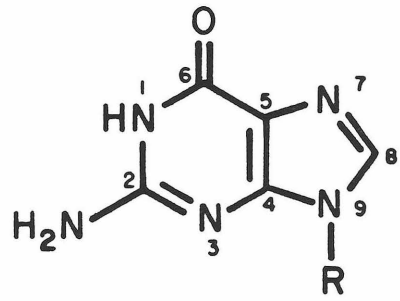
$[Pt(en)Cl_2]$, V, was used as a substrate for DNA and RNA polymerases *in vitro*. [13] Both DNA and RNA synthesis were reduced, which is in conflict with the *in vivo* cell-culture studies.

Several mechanisms have been proposed for the selective interaction of *cis*-$[Pt(NH_3)_2Cl_2]$ with DNA resulting in the inhibition of DNA synthesis. Because two labile sites are required for activity in platinum complexes, interstrand cross-linking was proposed as the cytotoxic event, in analogy to bifunctional alkylating agents, such as nitrogen mustards. However, although interstrand cross-linking was shown to be possible, [8] the number of cross-links per platination reaction is very low. [9] The inactivation dose is much lower than that required for significant cross-linking. [14] The inactivation of bacteriophage by *cis*- and *trans*-$[Pt(NH_3)_2Cl_2]$ also indicates that interstrand cross-linking in DNA is not the important cytotoxic event. [15] While only five molecules of the platinum complex bound to the phage are required for activity, an average dose of 35 molecules of platinum per phage was required to form one cross-link in the phage DNA. It seems that interstrand cross-linking is not an important cytotoxic event in the action of platinum complexes. [15,16]

The complexation sites of *cis*-$[Pt(NH_3)_2Cl_2]$ are on the bases of DNA. There are four bases in DNA, two purine bases: adenine, VI, and guanine, VII, and two pyrimidine bases: cytosine, VIII, and thymine, IX.



VI



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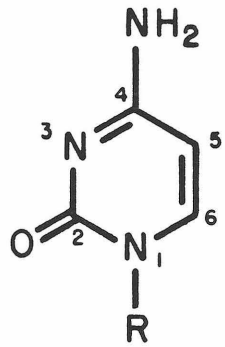
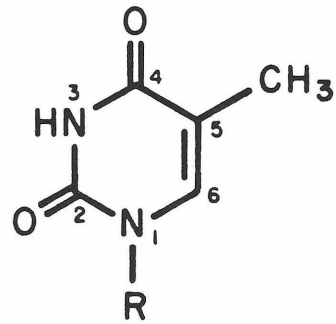


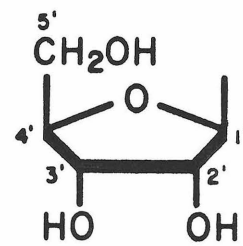
VIII



IX

BASE : R = H

NUCLEOSIDE : R =



Each base has several potential metal-binding sites.

With the individual bases, a kinetic selectivity is observed, where the rates of reaction are in the order: guanine > adenine > cytosine >> thymine.[17,18,19] The same kinetic selectivity is observed in polynucleotides.[20] Studies with different DNA's have shown that the amount of platinum binding is dependent on the (G+C)/(A+T) ratio of the DNA.[21,22,23] More platinum binding is observed in DNA's with higher G+C content.

The platination sites of the purine and pyrimidine bases have been determined by a variety of techniques including UV spectroscopy, proton and carbon NMR spectrometry, Raman spectrometry, and x-ray diffraction. Mansy, et al[24] used protonation and selective methylation to block specific binding sites on the bases and determine the most likely sites for platinum coordination by cis and trans-$[Pt(NH_3)_2Cl_2]$. They concluded that cytidine is bound at N3, but might also be chelate binding by cis-$[Pt(NH_3)_2Cl_2]$ between N3 and C2 or N3 and the $NH_2$ at C4. Adenosine is bound by cis-$[Pt(NH_3)_2Cl_2)$ at both N1 and N7, with chelate binding between N1 or N7 and the $NH_2$ at O6 also possible for the cis compound. At low pH, cis-$[Pt(NH_3)_2Cl_2]$ binds to guanosine at N7, while at high pH, binding at N1 becomes more important.

X-ray structural studies of platinum complexes bound to cytidine derivatives show that binding occurs at N3 with no evidence for chelate binding.[25] Proton NMR studies of the binding of $[Pt(dien)Cl]^+$

(dien = $HN(CH_2CH_2NH_2)_2$) to cytidine [26] and cytidine 5'-monophosphate [27] and of the binding of $[Pt(en)(H_2O)_2]^{2+}$ to cytidine [28], along with Raman and carbon NMR studies of cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and $[Pt(en)(H_2O)_2]^{2+}$ binding to cytidine [28], also indicate N3 as the reaction site in solution.

With 9-methyladenine, X-ray studies have shown binding of [Pt(diisopropyl sulfoxide) $Cl_3]^-$ to both N1 and N7. [29] Binding of $[Pt(dien)Cl]^+$ to adenosine [26] and adenosine 5'-monophosphate [27] in solution was detected by proton NMR. The results were consistent with platinum binding to N1 and N7 of the adenine base. Carbon NMR studies also indicated binding of platinum to N1 and N7. [30]

Guanosine derivatives have received major attention because of the apparent selectivity of cis$[Pt(NH_3)_2Cl_2]$ binding to guanine bases. Crystal structures have been determined for $[Pt(en)\ (Guanosine)_2]^{2+}$ [31] and cis-$[Pt(NH_3)_2(Guanosine)_2]^{2+}$. [32] In both cases, the guanosines are bound to platinum through N7. The N7 binding to different platinum complexes also occurs in solution. [27,33,34]

In general, platinum compounds bind nucleic acid bases at the same sites as other heavy metal ions. [35] The difference in the activity of cis- and trans- $[Pt(NH_3)_2Cl_2]$ in vivo cannot be attributed to binding site selectivity, because all platinum complexes have similar selectivity, while differing greatly in antitumor activity. Indeed, trans-$[Pt(NH_3)_2Cl_2]$ shows a higher selectivity for guanosine than does cis-$[Pt(NH_3)_2Cl_2]$. [19] Most of the current proposals for the interaction

of cis[$Pt(NH_3)_2Cl_2$] with DNA are based on the chelating ability of the cis compound.

One of the more controversial proposals is that cis-[$Pt(NH_3)_2Cl_2$] forms a chelate complex with a single guanine base through N7 and 06. [36] (see Figure 1) Chelation in this manner would disrupt hydrogen

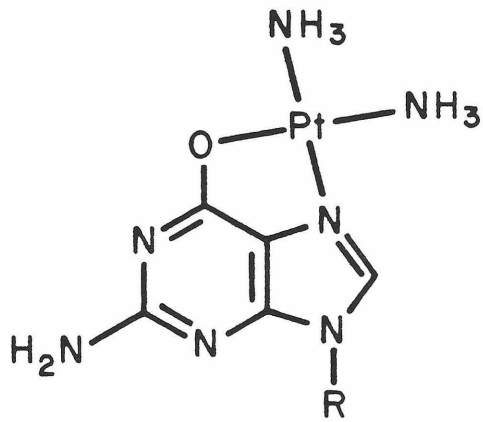



Figure 1

bonding with 06 and allow for the possibility of mispairing and base-substitution mutations. No unambiguous evidence for this mode of binding has yet been provided. On the other hand, several authors have argued that such a chelate is unlikely because of steric constraints.[34,37] Chelation in a similar manner has been observed in a palladium complex with 6-mercaptopurine, [38] however this complex would be expected to be rather strained because of angle distortions in the purine ring. The high affinity of palladium for sulfur must stabilize this complex.

Perhaps the most attractive proposal at this time is intrastrand

cross-linking, [39,40,41] wherein cis-$[Pt(NH_3)_2Cl_2]$ binds adjacent bases on the same strand. (see Figure 2) The distance between the two chlorides in cis-$[Pt(NH_3)_2Cl_2]$ is about 3 Å, which is close to the interplanar base separation of 3.4 Å in DNA. Binding of two adjacent guanines by cis-$[Pt(NH_3)_2Cl_2]$ would lead to distortion of base stacking which has, in fact, been observed. [40] Intercalation of 9-aminoacridine into DNA is inhibited by treatment of the DNA with $[Pt(en)Cl_2]$, a result which indicates the presence of intrastrand cross-links. [41] Further evidence for intrastrand cross-linking is provided by the fact that $[Pt(en)Cl_2]$ binding prevents cleavage of the DNA by the restriction enzyme BAM-1, which is specific for the adjacent guanines in the sequence GGATCC. [42] Dinucleoside monophosphates have been shown to react with cis-$[Pt(NH_3)_2Cl_2]$ to yield chelates involving binding to adjacent bases. [43] Thus, the GpG pair was chelated by cis-$[Pt(NH_3)_2Cl_2]$ at N7 of each base. Chelates were also formed with ApA and GpC.

The methods employed to determine the binding interactions of cis-$[Pt(NH_3)_2Cl_2]$ with nucleic-acid bases have provided only indirect evidence for the drug's binding sites. X-ray diffraction studies are limited to crystalline nucleoside-platinum (II) complexes. Proton NMR can only be used to determine binding at sites with adjacent protons. Because these methods have been limited to observation of the most favorable nucleoside-platinum (II) complexes, it has generally been assumed that cis-$[Pt(NH_3)_2Cl_2]$ binds selectively to only one site on

cytidine and guanosine and to two sites on adenosine.

The purpose of the present research was to use $^{15}N$ NMR to monitor binding interactions of cis-$[Pt(NH_3)_2Cl_2]$ with nucleosides in solution. The binding interactions were observed indirectly through the ammonia ligand resonances of 99% $^{15}N$ enriched cis-$[Pt(NH_3)_2Cl_2]$ and directly through the nitrogen resonances of the complexed nucleosides.

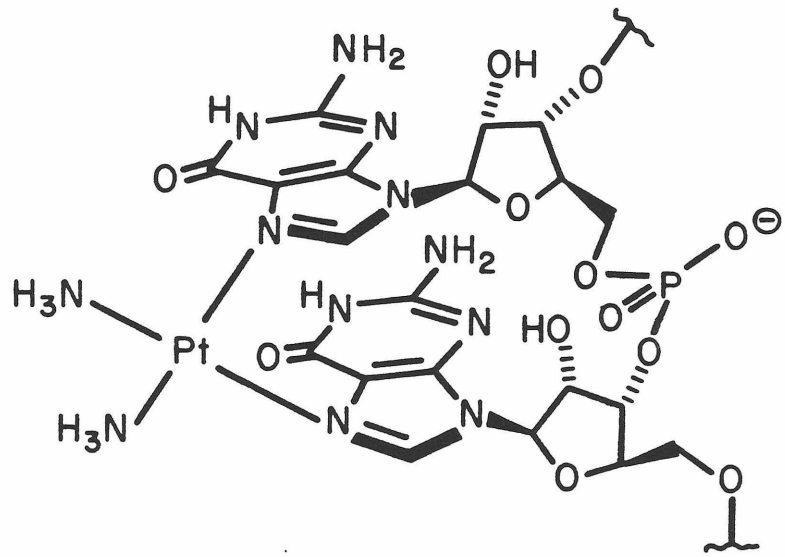



Figure 2

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EXPERIMENTAL

The $^{15}N$ NMR spectra were taken on a Bruker WH-180 FT NMR spectrometer operating at 18.25 MHz and a Bruker WM-500 FT NMR spectrometer operating at 50.69 MHz. The spectral conditions for the measurement of the $^{15}N$-labeled ammonia complexes were: 9000-Hz spectral width, 8K data points, and broad-band proton noise-decoupling. The spectral conditions for the measurements of the nucleoside-platinum complexes at the natural-abundance level of nitrogen-15 were: 9000 Hz (WM-500: 30,000 Hz) spectral width, 8K (WM-500:16K) data points, 30° pulse angle, 20-second delay time, and gated decoupling (during acquisition only). The sample temperature was maintained at approximately 30°C during the measurements. The chemical shifts are reported in ppm upfield of external 1M $DNO_3$ and are accurate to ±0.1 ppm. The coupling constants are accurate to ±1 Hz.

cis-$[Pt(NH_3)_2Cl_2]$: The $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ was prepared as described previously [1] using 99% $^{15}N$-enriched ammonium acetate [2] and potassium tetrachloroplatinate (II). [3] Unlabeled cis-$[Pt(NH_3)_2Cl_2]$ was prepared by the method of Dhara. [4]

$K[Pt(NH_3)Cl_3]$: The $^{15}N$-labeled $K[Pt(NH_3)Cl_3]$ was prepared from 99% $^{15}N$-enriched ammonium acetate and potassium tetrachloroplatinate (II). To 900 mg of potassium tetrachloroplatinate (II) dissolved in 5 ml of water was added 250 mg of 99% $^{15}N$-enriched ammonium acetate. The solution was boiled for 1.5 hours, then cooled to room temperature and filtered to remove the cis-$[Pt(NH_3)_2Cl_2]$ formed. About 5 ml of ethanol was added

to precipitate the excess ammonium acetate. The $^{15}N$-labeled $K[Pt(NH_3)Cl_3]$ was not isolated, but was characterized by means of its $^{15}N$ spectrum.

$[Pt(NH_3)_3Cl]Cl$: The $^{15}N$-labeled $[Pt(NH_3)_3Cl]Cl$ was prepared from $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$. To 50 mg of 99% $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ dissolved in 20 ml of water was added 28 mg of silver nitrate. The solution was heated to about 60°C for 10 minutes, then filtered to remove the precipitated silver chloride. Formation of cis-$[Pt(NH_3)_2(H_2O)]^+$ was confirmed by the $^{15}N$ spectrum of the solution. To this solution was added 13 mg of 99% $^{15}N$-labeled ammonium acetate and then the solution was boiled for one hour. The formation of $^{15}N$-labeled $[Pt(NH_3)_3Cl]^+$ was confirmed by the $^{15}N$ spectrum of the solution. No attempt was made to isolate the product.

Monopyridine Complexes: A mixture of the $^{15}N$-labeled chloro-ammine complex and an equimolar amount of pyridine in water was allowed to stand at room temperature for approximately one day before taking the $^{15}N$ spectra.

Aquo-Pyridine Complexes: To the solution of the monopyridine complex was added two equivalents of silver nitrate. The mixture was heated to 50-60°C for approximately 15 minutes, then filtered before taking the spectra.

Bis- and Tripyridine Complexes: To the solution of either the monopyridine or aquo-pyridine complex was added an excess of pyridine. The solution was allowed to stand at room temperature for about one day before taking the spectra.

cis-$[Pt(NH_3)_2anCl]^+$: To 50 mg of 99% $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ in 20 ml of $H_2O$ was added an equimolar amount, 16 mg, of 95% $^{15}N$-labeled aniline.[5] The solution used for the spectra was heated to 70-80°C for approximately 15 minutes.

cis-$[Pt(NH_3)_2an_2]^{2+}$: To the solution of $^{15}N$-labeled cis-$[Pt(NH_3)_2anCl]^+$ were added two additional equivalents (32 mg) of $^{15}N$-labeled aniline. The solution was heated to 70-100°C for approximately 30 minutes. The $^{15}N$ spectrum of this solution showed that only 60% of the cis-$[Pt(NH_3)_2anCl]^+$ was converted to cis-$[Pt(NH_3)_2an_2]^{2+}$.

Monochloro-Mononucleoside Complexes: To 100 mg of 99% $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ in 25 ml of water was added one molar equivalent of the nucleoside. The solution used for the $^{15}N$ spectra was heated to dissolve the nucleoside, then allowed to stand at room temperature or heated to 70-80°C for 30 minutes.

Monoaquo-Mononucleoside Complexes: To the solution of the monochloro-mononucleoside complex was added about 2.2 molar equivalents of silver nitrate. The solution was heated to 50-60°C for about 15 minutes, then filtered to remove the solid silver chloride. With some of the samples, the complete removal of silver chloride was not possible because of the fine particle size.

Bisnucleoside Complexes: To the solution of either the monochloro- or monoaquomononucleoside complex was added a second molar equivalent of the nucleoside. The mixture was allowed to stand at room temperature

for one day or was heated to 70-80°C for 30 minutes before taking the $^{15}N$ spectra.

Unlabeled Bisnucleoside Complexes: A mixture of 1.9 g of cis-$[Pt(NH_3)_2Cl_2]$ and two molar equivalents of the nucleoside in 500 ml of water was heated to 50-60°C for approximately 12 hours. The solution used for taking the spectra were concentrated to about 25 ml on a rotary evaporator.

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RESULTS AND DISCUSSION

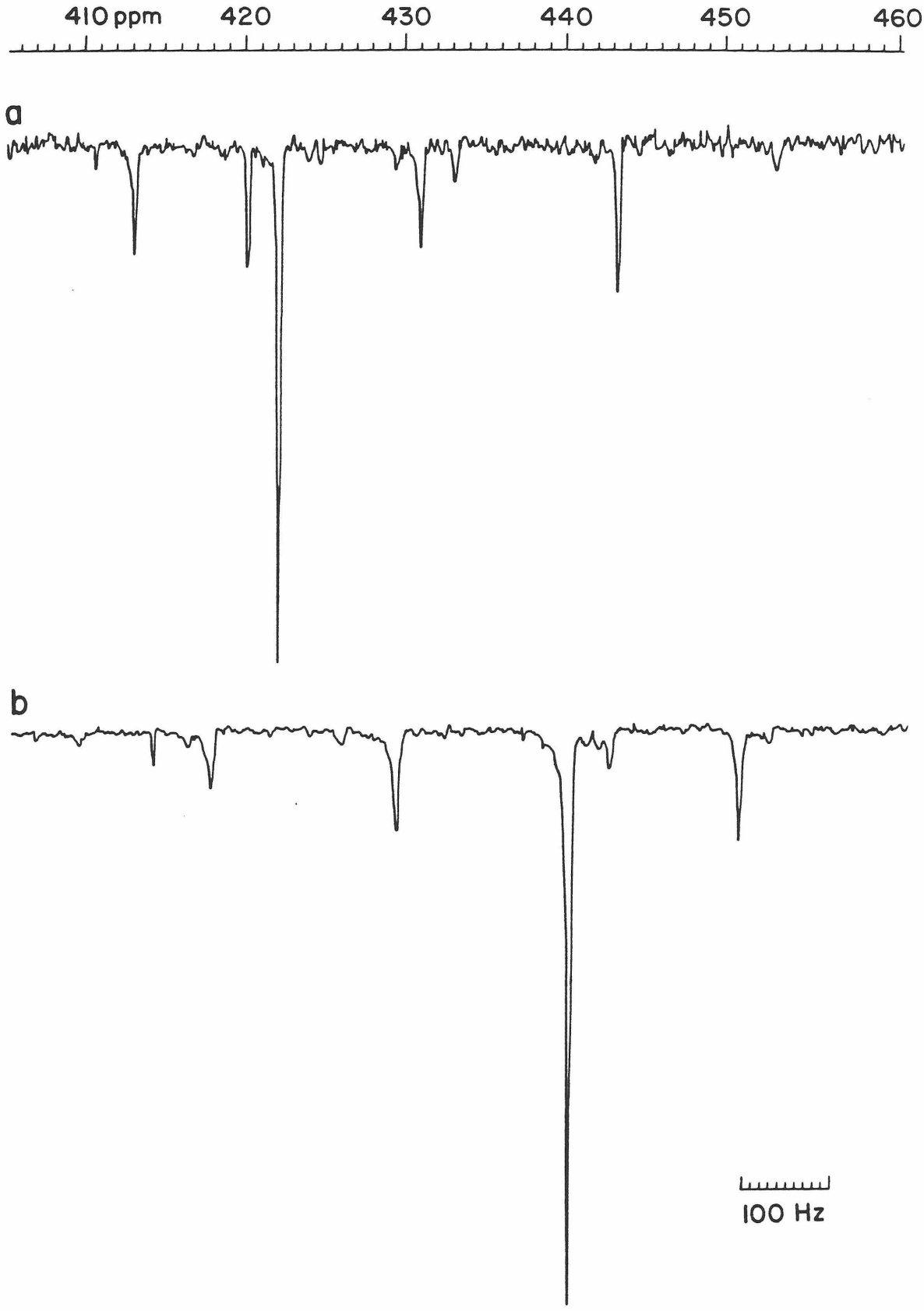
A. cis-$[Pt(NH_3)_2Cl_2]$ and Aquation Products

The proton-decoupled $^{15}N$ NMR spectrum of an aqueous solution of cis-$[Pt(NH_3)_2Cl_2]$, 99% $^{15}N$ enriched, is shown in Figure 3a. The $^{15}N$ enrichment was necessary because of the low solubility of cis-$[Pt(NH_3)_2Cl_2]$ in water, 8.9 mM. [1] Three major resonances are shown in the spectrum. The largest resonance at 422.1 ppm arises from the equivalent ammonia nitrogens of cis-$[Pt(NH_3)_2Cl_2]$. Flanking this resonance is a pair of signals separated by 325 Hz which result from coupling of the nitrogen with platinum-195 (33.8% natural abundance, spin $\frac{1}{2}$). The other two major resonances at 420.2 and 443.2 ppm arise from the non-equivalent ammonias of the cis-$[Pt(NH_3)_2Cl_2]$ mono-aquation product, cis-$[Pt(NH_3)_2(H_2O)Cl]^+$. The peaks also show satellites corresponding to one-bond spin couplings with platinum-195 of 343 Hz and 368 Hz, respectively. Upon addition of two equivalents of silver nitrate, which removes the chloride ligands and allows water molecules to be complexed in their place, the $^{15}N$ NMR spectrum shows only one resonance at 440.2 ppm with $^1J_{^{15}N-^{195}Pt}$ of 388 Hz. (see figure 3b) The chemical shift and coupling constant are in agreement with the values previously reported for cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. [2] The large upfield shift of the ammonia resonances upon changing from a trans chloride to a trans aquo ligand allows assignment of the two resonances of cis-$[Pt(NH_3)_2(H_2O)Cl]^+$. The downfield resonance corresponds to the ammonia resonance trans to chloride and the upfield

Figure 3.

(a) The $^{15}N$ spectrum of 100 mg (0.33 mmole) of $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ dissolved in 20 ml of water. The spectrum was taken with proton decoupling, 3700 scans, a 45° pulse width, and a 2 second repetition rate.

(b) The $^{15}N$ spectrum of 100 mg (0.33 mmole) of $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ dissolved in 20 ml of water, which has been treated with 113 mg (0.67 mmole) of silver nitrate. The spectrum was taken with proton decoupling, 29,700 scans, a 45° pulse width, and a 2 second repetition rate.


410 ppm
420
430
440
450
460
a
b
100 Hz

resonance to the ammonia trans to water. The sizes of the coupling constants, $^{1}J_{^{15}N-^{195}Pt}$, are also in accord with this assignment. An ammonia trans to water has a larger coupling constant than an ammonia trans to chloride. The identity of the monoaquation product was confirmed by generating this species independently from the reaction of cis-$[Pt(NH_3)_2Cl_2]$ with one equivalent of silver nitrate.

The intensities of the $^{15}N$ resonances of cis-$[Pt(NH_3)_2Cl_2]$ and cis-$[Pt(NH_3)_2(H_2O)Cl]^+$ allow the percent aquation to be determined in the sample of aqueous cis-$[Pt(NH_3)_2Cl_2]$ shown in Figure 3a. Approximately 34% of the cis-$[Pt(NH_3)_2Cl_2]$ was monoaquated. This value is in agreement with the percentage calculated from the aquation equilibrium constants for cis-$[Pt(NH_3)_2Cl_2]$ ($K_1=3.63\times10^{-3}$M, $K_2=1.11\times10^{-4}$M at 25°C) [3] of 63% of cis-$[Pt(NH_3)_2Cl_2]$, 36% of cis-$[Pt(NH_3)_2(H_2O)Cl]^+$, and 1% of cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. Apparently, the aquation equilibrium occurred when the sample was heated to dissolve the solid cis-$[Pt(NH_3)_2Cl_2]$. At 25° C, the exchange is slow [3b] on the NMR time scale, so the resonances of the cis-$[Pt(NH_3)_2Cl_2]$ and cis-$[Pt(NH_3)_2(H_2O)Cl]^+$ are sharp.

The protons on the coordinated waters of the bisaquated product, cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ are acidic. The pKa of these protons can be measured by determining the $^{15}N$ shift of the ammonias as a function of pH. Table 1 shows the pH dependence of the ammonia resonances of cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. Above pH 5.2, a precipitate begins to form, presumably because of formation of oxygen-bridged dimers and trimers. [4]

Despite being unable to measure the limiting shift for the monohydroxide complex, cis-$[Pt(NH_3)_2(OH)(H_2O)]^+$, the first acid dissociation constant, pKa(1), can be calculated from the chemical-shift dependence at acidic pH's. [5] Figure 4 shows the calculated dependence of the ammonia $^{15}N$ shift on pH. The calculated pKa(1) value of 5.2±0.1 is comparable to the value of 5.6 [6] obtained by potentiometric methods.

It has generally been assumed that cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ is the species which actually reacts with DNA. In extracellular fluids, the chloride concentration is relatively high, about 100 mM, [7] while the intracellular concentration averages about 3 mM. [7] So it has been suggested that in the blood, cis-$[Pt(NH_3)_2Cl_2]$ remains intact, until it passes through the cell membrane, where it hydrolyzes to cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, cis-$[Pt(NH_3)_2\ OH(H_2O)]^+$ and cis-$[Pt(NH_3)_2(OH)_2]$.[8] Table 2 shows the relative distribution of cis-$[Pt(NH_3)_2Cl_2]$ aquation products at different chloride concentrations. It can be seen that the aquation products are very dependent on the chloride concentration. The species which reacts with DNA could be any of these compounds; however, the totally aquated species would be most abundant.

TABLE 1

pH Dependence of the Ammonia $^{15}N$ Chemical Shift of

cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$

| pH | $\delta^{15}N$, ppm |
|---|---|
| 1.95 | 440.2 |
| 3.56 | 440.1 |
| 3.90 | 440.0 |
| 4.33 | 439.8 |
| 4.80 | 438.2 |
| 5.22 | 437.1 |

Figure 4.

The plot of the ammonia $^{15}N$ chemical shift dependence of $^{15}N$-labeled cis-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ on pH. The calculated titration curve is shown in the plot.

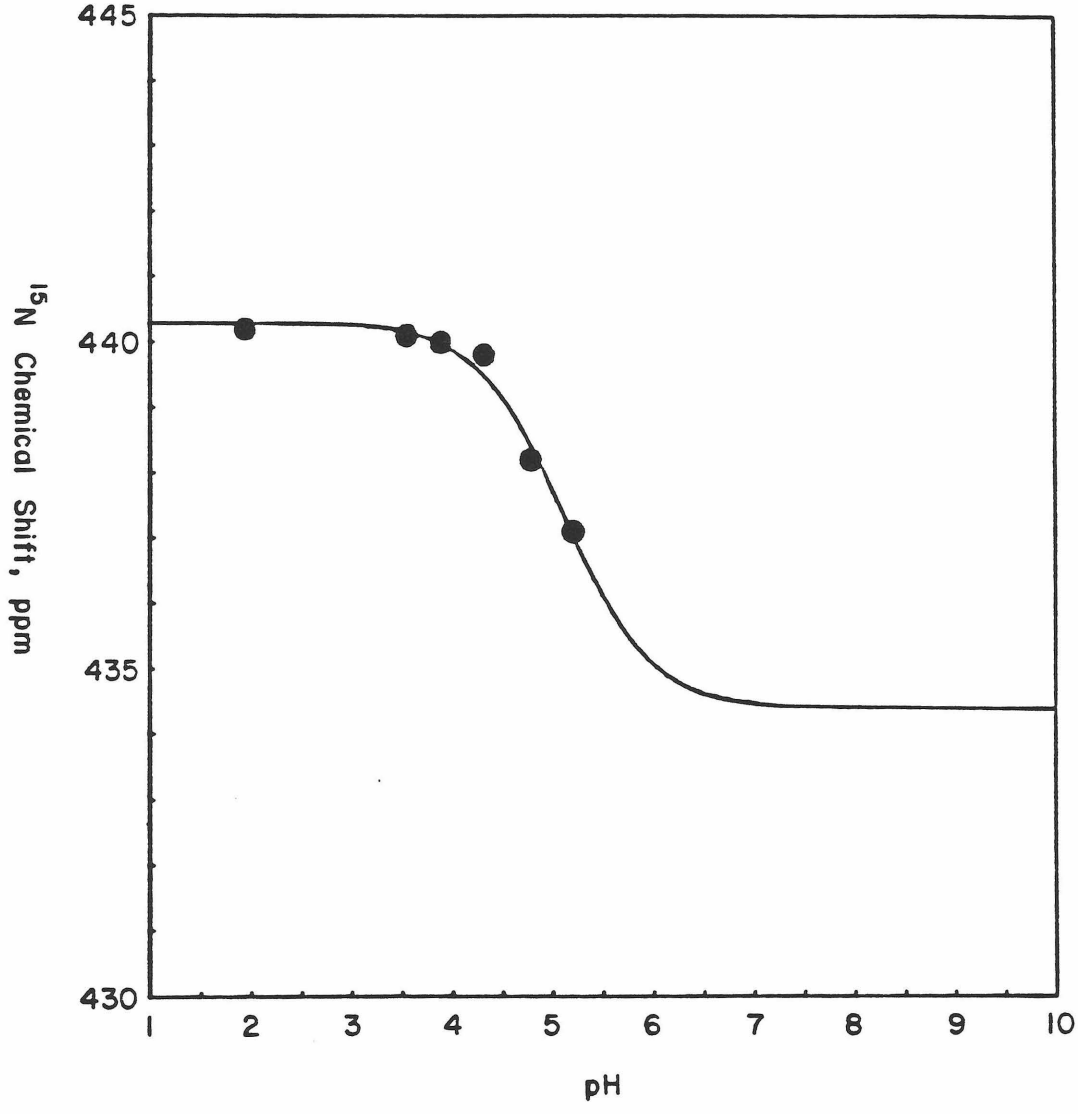
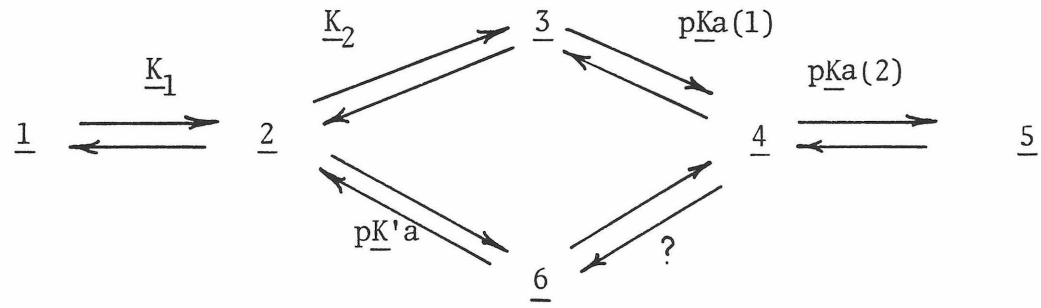

445
440
435
430
$^{15}N$ Chemical Shift, ppm
1
2
3
4
5
6
7
8
9
10
pH

TABLE 2

Distribution of cis-$[Pt(NH_3)_2Cl]$ Aquation Products at pH7 and 35°C.




| | Relative Concentrations, mM (% total Pt) | | | | | |
|---|---|---|---|---|---|---|
| $[Cl^-]$, mM | 1 | 2 | 3 | 4 | 5 | 6 |
| 100 (Blood) | 4.28 (86) | 0.19 (4) | 0.004 (0) | 0.11 (2) | 0.05 (1) | 0.37 (7) |
| 3 (Cytoplasm) | 0.04 (1) | 0.16 (3) | 0.10 (2) | 3.08 (62) | 1.31 (26) | 0.32 (6) |

cis-$[Pt(NH_3)_2Cl_2]$, 1; cis-$[Pt(NH_3)_2(H_2O)Cl]^+$, 2;

cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, 3; cis-$[Pt(NH_3)_2(OH)(H_2O)]^+$, 4;

cis-$[Pt(NH_3)_2(OH)_2]$, 5; cis-$[Pt(NH_3)_2(OH)Cl]$, 6

$K_1 = 4.37 \times 10^{-3}$ M [a]; $K_2 = 1.88 \times 10^{-3}$ M [a]; $pK_a' = 6.7$ [b]

$pK_a(1) = 5.6$ [c]; $pK_a(2) = 7.3$ [c]

(a) Reference 3a.

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RESULTS AND DISCUSSION

B. Platinum-ammine Complexes

Binding of cis-$[Pt(NH_3)_2Cl_2]$ with nucleosides could lead to a complex mixture of products because of the number of potential metal binding sites on each nucleoside. [1] The $^{15}N$ chemical shifts and coupling constants of model systems were measured to determine the effects of nitrogen ligand binding to cis-$[Pt(NH_3)_2Cl_2]$. Pyridine was chosen as a model for unsaturated nucleoside ring nitrogens, such as N7 of guanosine or adenosine. Aniline was chosen to model the binding of the exocyclic amines, such as the $NH_2$ at C6 of adenosine or the $NH_2$ at C2 of guanosine.

The reaction of one equivalent of pyridine with cis-$[Pt(NH_3)_2Cl_2]$, 99% enriched in $^{15}N$, yielded two pyridine complexes. The $^{15}N$ spectrum of the reaction mixture showed four resonances, each with corresponding satellites from coupling to platinum-195. The resonances at 417.6 and 423.5 ppm correspond to the nonequivalent ammonias of the monopyridine complex, cis-$[Pt(NH_3)_2pyCl]^+$. The third resonance at 419.6 ppm is of the bispyridine complex, cis-$[Pt(NH_3)_2py_2]^{2+}$. Formation of the bispyridine complex is consistent with the presence of the last peak at 422.1 ppm from unreacted cis-$[Pt(NH_3)_2Cl_2]$. The peak intensities indicate that the ratio of monopyridine to bispyridine complex in the reaction mixture was about 3:2.

That the monopyridine complex has a chloride ligand is confirmed by the large, 19.2 ppm, upfield shift of the ammonia trans to the

chloride upon removal of the chloride with silver nitrate and substitution of a water in its place. The resonance of the ammonia trans to the pyridine shifts only 2.2 ppm downfield upon reaction of the monopyridine complex with silver nitrate. At the same time, the ammonia resonances of the bispyridine complex are unchanged which confirms that for this substance there are no chlorides directly bound to the platinum.

The one-bond coupling constant, $^{1}J_{^{15}N-^{195}Pt}$, also indicates the nature of the ligand trans to the ammonia and helps in assigning the resonances. The affect of the trans ligand on $^{1}J_{^{15}N-^{195}Pt}$ will be discussed in the next section.

Addition of a second equivalent of pyridine to either the 1:1 product mixture or the 1:1 aquo product mixture results in formation of just the bispyridine complex. The $^{15}N$ spectrum shows only one resonance at 419.6 ppm, which again confirms the identity of the bispyridine complex. The $^{15}N$ chemical shift of the coordinated pyridines in the bispyridine complex, cis-$[Pt(NH_3)_2py_2]^{2+}$ was 174.6 ppm, which is 115 ppm upfield from the shift of neat pyridine. Because the pyridine nitrogen has a long $T_1$ and an unfavorable nuclear Overhauser enhancement (NOE)[2], the $^{15}N$ spectrum of the coordinated pyridine did not have a large enough signal-to-noise ratio to allow measurement of the one-bond coupling with platinum-195, even though the pyridines were 37% enriched in $^{15}N$.

Aniline binding to cis-$[Pt(NH_3)_2Cl_2]$ gives similar ammonia

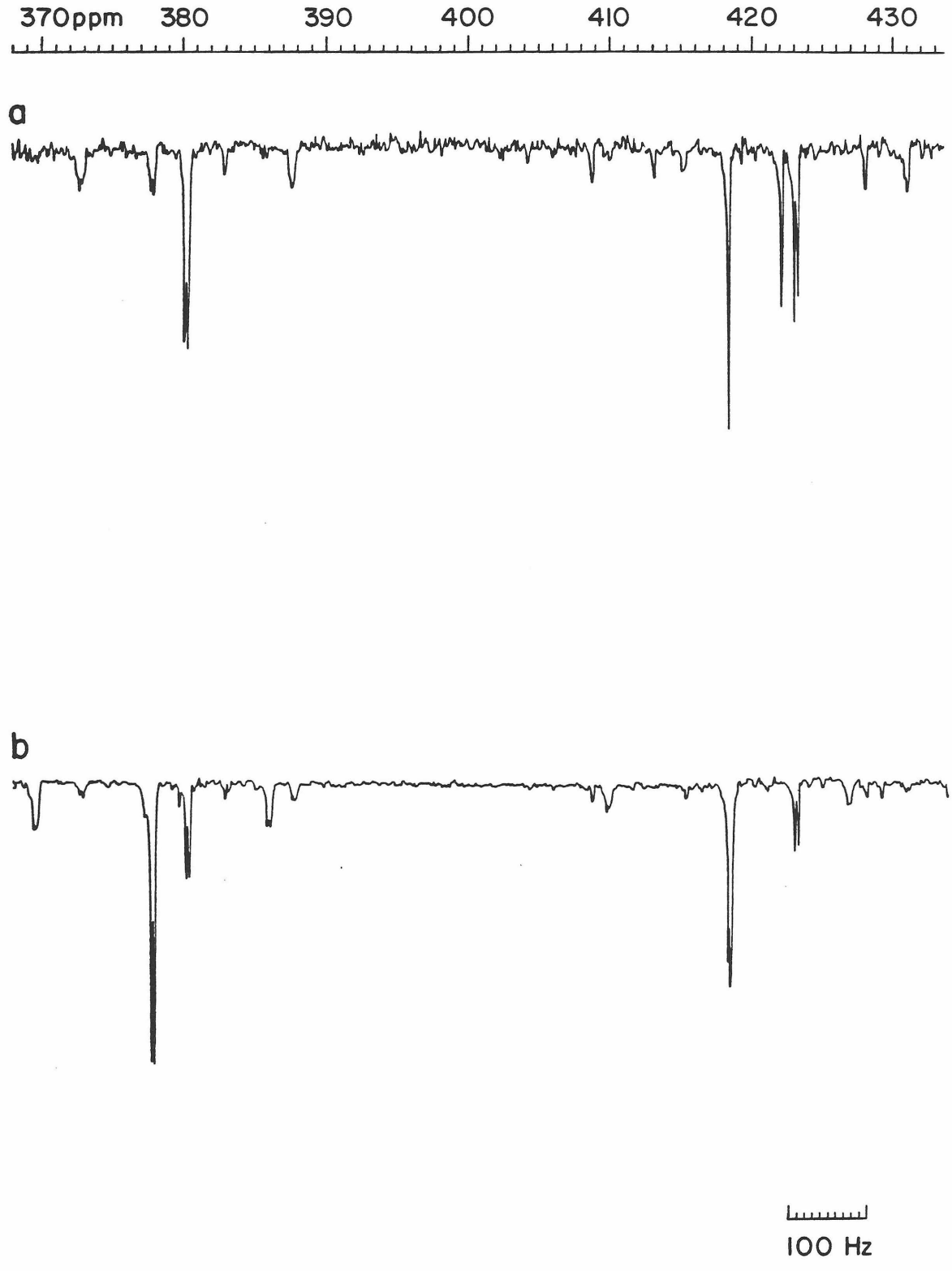
chemical shifts as pyridine binding. The monoaniline complex, cis-$[Pt(NH_3)_2$ an Cl$]^+$, has two resonances at 418.4 and 423.1 ppm, where the downfield resonance corresponds to the ammonia trans to chloride and the upfield resonance to the ammonia trans to aniline. The $^{15}N$ chemical shift of the aniline, which was 95% $^{15}N$ enriched, coordinated to platinum in the monoaniline complex was 378.2 ppm, 59 ppm upfield of free aqueous aniline. Because both the ammonia and aniline ligands were $^{15}N$-labeled, the $^{15}N$-$^{15}N$ couplings through platinum were observed in the $^{15}N$ spectrum (see Figure 5a.) The coupling between the aniline nitrogen and the trans ammonia nitrogen was 4.5 Hz. Two-bond $^{15}N$-$^{15}N$ couplings of about 5 Hz have been reported for 1-methylimidazole-platinum(II) complexes, where all of the nitrogens were 100% $^{15}N$-labeled. [3] These couplings constants are in the normal range for two-bond $^{15}N$-$^{15}N$ couplings. [2] It is interesting to note that there was no detectable coupling between cis ligands.

Addition of approximately two equivalents of additional $^{15}N$-labeled aniline to the monoaniline complex resulted in the slow formation of the bisaniline complex, cis-$[Pt(NH_3)_2$ $an_2]^{2+}$ (see Figure 5b.) Even after heating the mixture to near boiling for 30 minutes, only about 60% of the cis-$[Pt(NH_3)_2$an Cl$]^+$ was converted to cis-$[Pt(NH_3)_2an_2]^{2+}$. The slow formation of the bisaniline complex is in contrast to the rather facile formation of the bispyridine complex. The bisaniline complex had resonances at 380.6 and 418.4 ppm, from the aniline and ammonia ligands, respectively. Again the two-bond $^{15}N$-$^{15}N$ couplings can be observed and have a value of 3.5 Hz.

Figure 5.

(a) The $^{15}N$ spectrum of 50 mg (0.17 mmole) $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ and 16 mg (0.17 mmole) of $^{15}N$-labeled aniline in 3 ml water after heating at 70-80°C for about 15 minutes. The spectrum was taken with proton decoupling, 2000 scans, a 36° pulse width, and a 4 second repetition rate.

(b) The $^{15}N$ spectrum of the same sample as (a) except that an additional 32 mg of $^{15}N$-labeled aniline was added and heated to 90-100°C for about 30 minutes. The spectrum was taken under the same conditions as (a) except that the number of scans was 5498.


370ppm
380
390
400
410
420
430
a
b
100 Hz

In addition to cis-$[Pt(NH_3)_2Cl_2]$, other platinum(II)-ammine complexes were measured. Included were $[Pt(NH_3)Cl_3]^-$, $[Pt(NH_3)_3Cl]^+$, and $[Pt(NH_3)_4]^{2+}$ as well as the aquation and pyridine substitution products of the first two of these complexes. The chemical shifts and coupling constants, $^1J_{^{15}N-^{195}Pt}$, for all of the platinum(II)-ammone complexes measured are listed in Table 3.

Assignment of the ammonia resonances in mixtures of platinum complexes is aided by the resonances of ammonias trans to chloride moving about 20 ppm upfield on substitution of water for chloride and the one-bond couplings to platinum-195 increasing with the trans ligand in the order: N < Cl < $H_2O$. The resonances for compounds with no chloride ligands show no change if reacted with silver nitrate or excess nitrogen ligand.

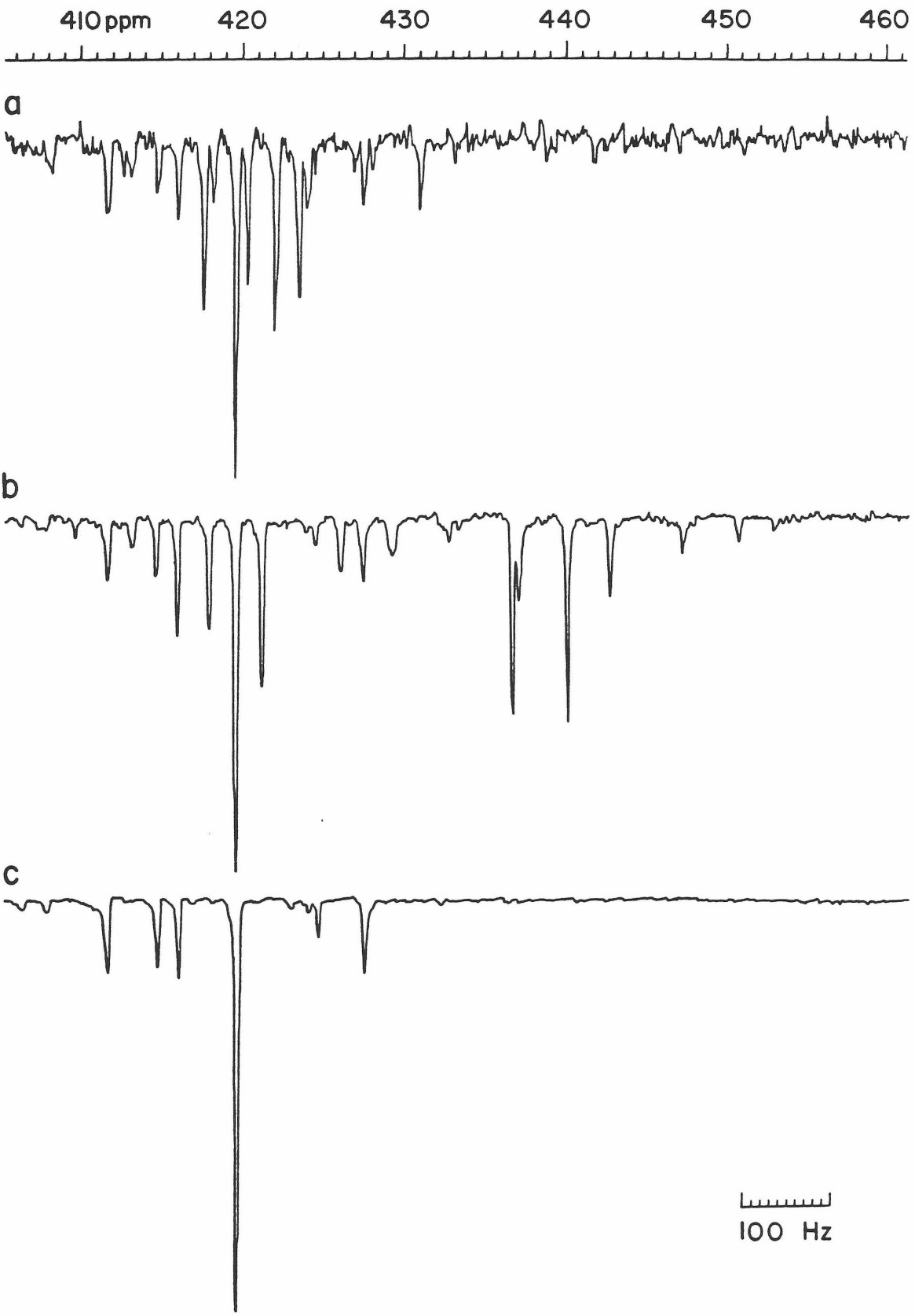
An extreme example of a $^{15}N$ spectrum of a complex mixture of platinum compounds is shown in Figure 6a. This spectrum is of a mixture of platinum compounds obtained when a preparation of cis-$[Pt(NH_3)_2Cl_2]$, contaminated with $[Pt(NH_3)Cl_3]^-$ and $[Pt(NH_3)_3Cl]^+$, was allowed to react with one equivalent of pyridine. When this mixture was treated with two equivalents of silver nitrate, to remove the chloride ligands, several of the resonances shift upfield, as shown in Figure 6b. Finally, addition of a second equivalent of pyridine gave the spectrum shown in Figure 6c, where only three compounds are present: cis-$[Pt(NH_3)_2py_2]^{2+}$, $[Pt(NH_3)py_3]^{2+}$, and $[Pt(NH_3)_3py]^{2+}$. Only a small amount of $[Pt(NH_3)Cl_3]^-$ and $[Pt(NH_3)_3Cl]^+$ was present in the prepara-

Figure 6.

(a) The $^{15}N$ spectrum of 100 mg (0.33 mmoles) of $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ and 26 mg (0.33 mmoles) of pyridine in 20 ml of water after standing at room temperature for 24 hours. The spectrum was taken with proton decoupling, 6075 scans, a 45° pulse width, and a 3 second repetition rate.

(b) The $^{15}N$ spectrum of the same sample as in (a) after treatment with 113 mg (0.67 mmoles) silver nitrate. The spectral conditions were the same as in (a) except that 19,140 scans were taken.

(c) The $^{15}N$ spectrum of the same sample as in (b) except an additional 26 mg (0.33 mmoles) of pyridine was added. The spectral conditions were the same as in (a) except that 23,728 scans were taken.


410 ppm
420
430
440
450
460
a
b
c
100 Hz

tion of cis-$[Pt(NH_3)_2Cl_2]$, but still the reaction with one equivalent of pyridine resulted in an almost uninterpretable spectrum.

TABLE 3

COORDINATED AMMONIA $^{15}N$ CHEMICAL SHIFTS AND COUPLING CONSTANTS

| Compound | $\delta^{15}N$, ppm | | | $^1J_{^{15}N-^{195}Pt}$, Hz | | |
|---|---|---|---|---|---|---|
| | trans Cl | trans N | trans $H_2O$ | trans Cl | trans N | trans $H_2O$ |
| cis$[Pt(NH_3)_2Cl_2]$ | 422.1 | | | 325 | | |
| cis$[Pt(NH_3)_2(H_2O)Cl]^+$ | 420.2 | | 443.2 | 343 | | 368 |
| cis$[Pt(NH_3)_2(H_2O)_2]^{2+}$ | | | 440.2 | | | 388 |
| $[Pt(NH_3)Cl_3]^-$ | 420.4 | | | 324 | | |
| $[Pt(NH_3)(H_2O)_3]^{2+}$ | | | 442.4 | | | 387 |
| $[Pt(NH_3)_3Cl]^+$ | 424.2 | 420.4 | | 329 | 282 | |
| $[Pt(NH_3)_4]^{2+}$ | | 421.2 | | | 286 | |
| cis$[Pt(NH_3)_2pyCl]^+$ | 417.6 | 423.5 | | 343 | 273 | |
| cis$[Pt(NH_3)_2py(H_2O)]^{2+}$ | | 421.3 | 436.8 | | 290 | 384 |
| cis$[Pt(NH_3)_2py_2]^{2+}$ | | 419.6 | | | 288 | |
| $[Pt(NH_3)py_3]^{2+}$ | | 414.7 | | | 302 | |
| | | $NH_3$ | py | | $NH_3$ | py |
| $[Pt(NH_3)_3py]^{2+}$ | | 416.1 | 424.7 | | 295 | 277 |
| cis$[Pt(NH_3)_2anCl]^+$ | 418.4 | 423.1 | | 351 | 287 | |
| cis$[Pt(NH_3)_2an_2]^{2+}$ | | 418.4 | | | 307 | |

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RESULTS AND DISCUSSION

C. $^{15}N$-$^{195}Pt$ Coupling Constants

The $^{15}N$ resonance of a nitrogen bound directly to the platinum nucleus appears as a triplet comprised of a central peak flanked by satellites arising from couplings to platinum-195. Because platinum-195 has a natural abundance of only 33.8% and a spin of $\frac{1}{2}$, the coupling satellites should be one-fourth the intensity of the central resonance, if the linewidths are the same. However, the satellites were usually less than one-fourth the central peak intensity and broader because the relaxation times of platinum-195 are very short. At 50.69 MHz, the coupling satellites were much broader than at 18.25 MHz. This shows that the chemical-shift anisotropy relaxation mechanism becomes more efficient in platinum-195 at higher field strengths and reduces the relaxation times still further. [1]

The $^{195}Pt$ satellites complicate the $^{15}N$ spectra of platinum complexes, especially for mixtures. However, the $^{15}N$-$^{195}Pt$ coupling constants, $^{1}J_{^{15}N-^{195}Pt}$, when obtainable from the satellites are very useful in the assignment of the various coordinated nitrogen resonances. A general trend was observed in the magnitude of the coupling constants of the platinum complexes listed in Table 3 as a function of the trans ligand. Ammonia ligands trans to a nitrogen ligand showed couplings ranging between 270-310 Hz. Ammonias with a trans chloride ligand had coupling constants ranging between 320-355 Hz, and those with a trans water ligand, between 365-390 Hz.

Some discussion of the theory of spin-spin couplings is necessary to rationalize the trends. The one-bond coupling constant, $^{1}J_{^{15}N\text{-}^{195}Pt}$, has been interpreted assuming dominance of the Fermi contact term [2,3]:

$$^{1}J_{^{15}N\text{-}^{195}Pt} \propto \gamma_N \gamma_{Pt} |\Psi_{N_{2s}}(0)|^2 |\Psi_{Pt_{6s}}(0)|^2 \alpha_{Pt}^2 \alpha_N^2 (\Delta E)^{-1} \qquad (1)$$

where $\gamma_N$ and $\gamma_{Pt}$ are the magnetogyric ratios of $^{15}N$ and $^{195}Pt$, respectively; $|\Psi(0)|^2$ is the valence shell s-electron density for nitrogen and platinum; $\alpha^2$ represents the percentage s-character in the orbitals used by nitrogen and platinum atoms to form the Pt-N bond; and $\Delta E$ is the mean triplet excitation energy. It has been assumed that the $|\Psi(0)|^2$ terms do not significantly change in a series of related compounds. [3] Also, it has been found that $\Delta E$ does not correlate with the coupling constant. [2] Therefore, it was argued that the coupling constant was directly proportional to the s-character of the bonding orbitals. [2]

The *trans* effect has been the subject of great interest in the transition-metal chemistry. A considerable amount of experimental evidence has been accumulated on the "*trans* effect" in square-planar platinum(II) complexes. [4,5] The *trans* effect is a measure of the ability of a ligand to labilize the position trans to itself. It is thus a kinetic effect which has been explained in terms of two factors: 1) a *trans* influence and 2) stabilization of the trigonal

bipyramidal transition state.[5,6] The trans influence is a ground-state effect where a ligand weakens the ligand-metal bond trans to itself. With sigma-bonding ligands, the trans influence order is similar to that for the kinetic trans effect.[7] The qualitative experimental order for the kinetic trans effect is as follows:[8]

$$CO, C_2H_4, CN^- > PR_3, AsR_3, H^- > CH_3^-, S\,C(NH_2)_2$$
$$> C_6H_6^-, NO_2, SCN^-, I^- > Br^- > Cl^- > py, NH_3, F^-, OH^-, H_2O \quad .$$

Several methods have been used to provide a measure of the relative trans influence of ligands in square-planar platinum complexes, including infrared,[9] NMR,[10] and X-ray structural[11] methods. The one-bond coupling constant between phosphorus-31 and platinum-195 has been shown to be dependent on the ligand trans to the phosphorus.[12] The general trend is for smaller values of $^1J_{^{31}P-^{195}Pt}$ when the group opposite to the phosphorus has a high trans influence. Recently, the one-bond coupling constant between nitrogen-15 and platinum-195 was also shown to have a similar dependence on the trans ligand.[3,13] In fact, a linear relationship between $^1J_{^{15}N-^{195}Pt}$ and $^1J_{^{31}P-^{195}Pt}$ was found and was attributed to a dependence of both coupling constants on the percentage s-character of the platinum bond with either nitrogen or phosphorus.[3]

The trans and cis ligand effects on the magnitude of the coupling constants were determined from the coupling constants of the platinum complexes in Table 3 and these are listed in Table 4. The relative

trans influence of the ligands, obtained from the $^{15}N$-$^{195}Pt$ coupling constants, decreases in the order:

$$py > NH_3 > an > Cl^- > H_2O \quad .$$

This order is in agreement with metal-ligand σ-overlap integrals, [7,11a] and the trans influence order obtained from equilibrium constants of platinum(II)-dimethyl sulfoxide complexes. [14] However, it is slightly different from the experimental trans effect series, where chloride proceeds nitrogen ligands. The overall anionic charge of the complexes could have an effect on the electronic nature of the bond, [6] thus the chloride ligand, which is the only anionic ligand in this series, might have a charge effect in addition to the bonding effect.

There also is a substantial cis ligand effect on the magnitude of the coupling constant. (see Table 4) The relative cis influence of the ligands decreases in the order:

$$Cl^- > NH_3 > py > H_2O > an \quad .$$

From MO calculations, it has been predicted that the cis influence of a ligand should be of comparable magnitude to its trans influence. [7,15] However, the cis influence of a ligand, as determined from these coupling constants, does not seem to be related to its trans influence. A similar result has been reported for $^{31}P$-$^{195}Pt$ coupling constants. [16]

The trans- and cis-ligand effects can be used to predict the value of $^{1}J_{^{15}N-^{195}Pt}$ for other platinum-ammine complexes. For example, $[Pt(NH_3)py_3]^{2+}$, which was not used to determine the ligand effects, has one trans pyridine and two cis pyridine ligands. The base value for the coupling constant is 324 Hz, the coupling constant of $[Pt(NH_3)Cl_3]^-$. The trans pyridine effect would be -53 Hz (see Table 4) and the two cis pyridines effect would be 2x(+16 Hz). The predicted coupling constant for $[Pt(NH_3)py_3]^{2+}$ of 303 Hz is in good agreement with the measured value of 302 Hz.

TABLE 4

RANGE OF RELATIVE LIGAND EFFECT

ON $^{1}J_{^{15}N-^{195}Pt}$, Hz (AVERAGE)

| | Position | |
|---|---|---|
| Ligand | trans | cis |
| $H_2O$ | +41 +45 (43) | +17 +20 (18) |
| $Cl^-$ | 0 | 0 |
| aniline | -44 -38 (-41) | +21 +26 (+24) |
| $NH_3$ | -48 -43 (-44) | +1 +4 (+3) |
| pyridine | -55 -52 (-53) | +13 +18 (+16) |

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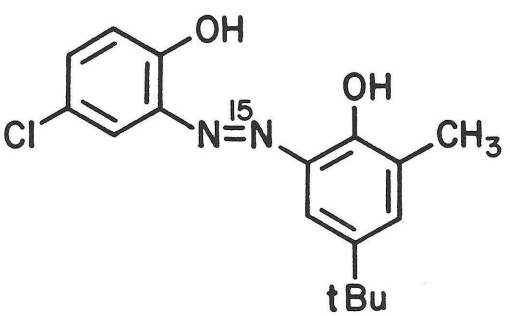
RESULTS AND DISCUSSION

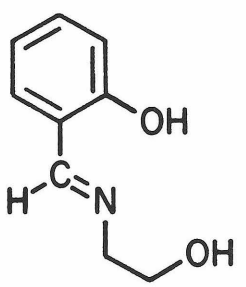
D. $^{15}N$ Chemical Shifts

The $^{15}N$ chemical shifts of the ammonia ligands in the platinum complexes listed in Table 3 are all in the range of 410-445 ppm. Platinum coordination induces a 38-68 ppm upfield shift of the ammonia $^{15}N$ resonance relative to liquid ammonia. The magnitude of the upfield shift is dependent on the ligand trans to the ammonia. The $^{15}N$ chemical shift of the coordinated pyridines in cis-$[Pt(NH_3)_2py_2]^{2+}$ is 115 ppm upfield from the shift of neat pyridine. Platinum coordination of aniline causes an upfield shift in the aniline resonance of approximately 60 ppm relative to free aqueous aniline.

In general, platinum coordination to a nitrogen causes a large upfield shift of the $^{15}N$ resonance. In addition to the upfield shifts observed with ammonia, pyridine, and aniline, platinum coordination causes upfield shifts in other nitrogen compounds. The nitrogen resonances of 1,2-diaminoethane, [1] 1-methylimidazole, [1] the singly $^{15}N$-labeled diarylazo-ligand, [2] X, and the Schiff's Base, [3] XI, all




X




XI

shifted upfield upon coordination with platinum. The coordination shift ranged from 30 to 180 ppm. These coordination shifts were a function of the nature of the ligand trans to the nitrogen. [1,3,4] In the case of 1-hexanamine, [4] the coordination shift was actually slightly downfield when the trans ligand was a phosphine.

The magnitude of the complexation shifts appear to be dependent both on the nitrogen structural type and the type of ligand opposite to the nitrogen. A plot of the magnitude of the coordination shift as a function of the chemical shift of the free ligand is shown in Figure 7. For a constant trans ligand, the coordination shift increases with decreasing chemical shift of the free ligand.

The nature of the ligand trans to the nitrogen has a marked influence on the coordination shift. The trans- and cis-ligand effects on the ammonia chemical shifts in Table 5 were determined from the chemical shifts of the platinum-ammine complexes listed in Table 3. The relative order of the trans-ligand induced downfield shift is:

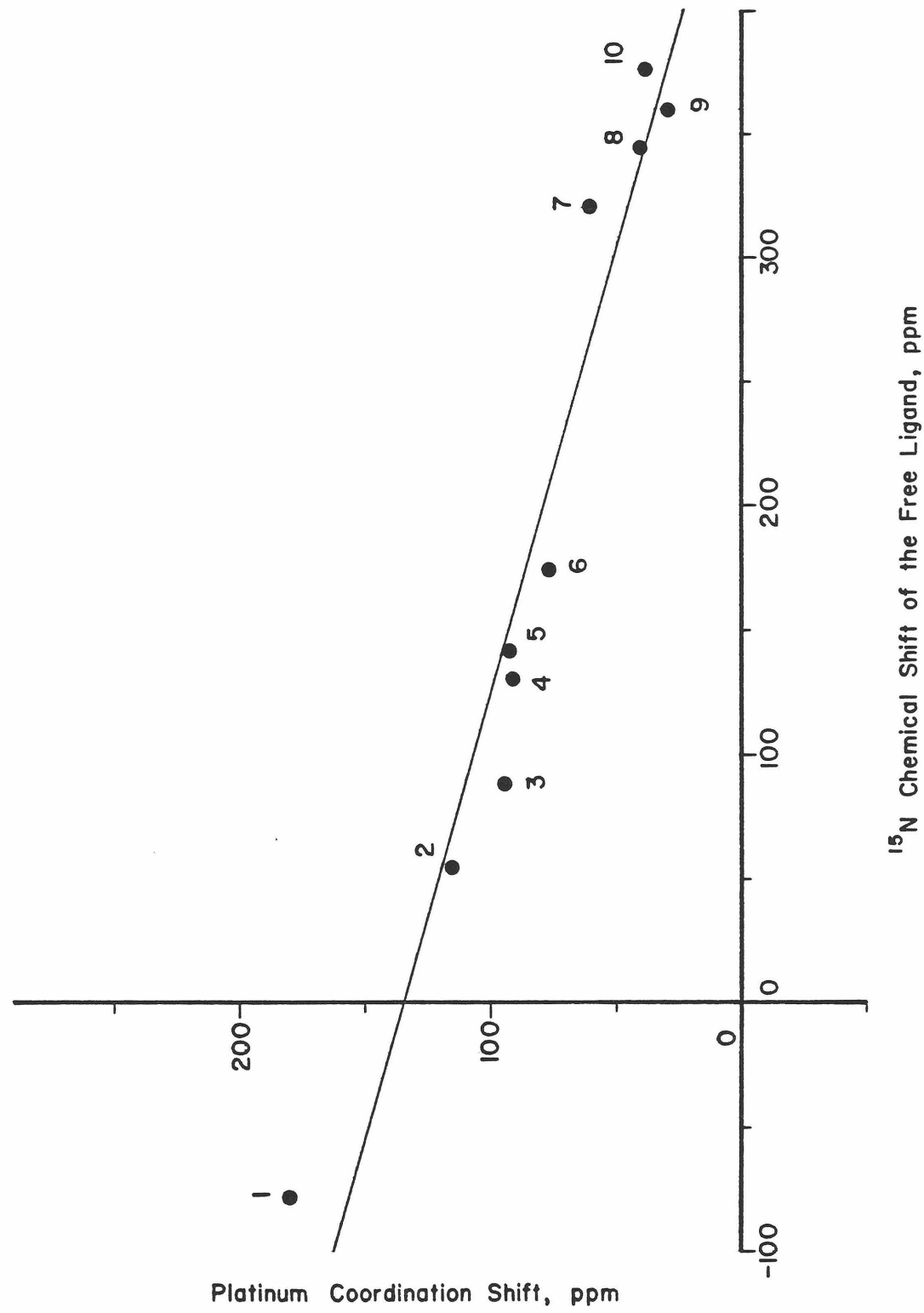
$$NH_3 > Cl^- \sim an \sim py > H_2O \quad .$$

This order is qualitatively similar to the trans influence of these ligands. From these data and other studies, [3,4] a general trend was observed: trans ligands with low trans influences tend to shift the nitrogen resonances upfield. The range of the trans-ligand effect on the chemical shift is about 65 ppm, from phosphine ligands which have

Figure 7.

A plot of the $^{15}N$ platinum coordination shift versus the chemical shift of the free ligand. The coordination shifts correspond to platinum complexes with a nitrogen ligand, either pyridine or ammonia, trans to the measured ligand. A least squares fit of the points yields a slope of -0.28, an intercept of 132 ppm, and a correlation coefficient, r, of -0.957. The shifts are from the present work and papers cited in the text.

| No. | Ligand |
|---|---|
| 1 | X |
| 2 | Pyridine |
| 3 | XI |
| 4 | 1-Methylimidazole |
| 5 | Guanosine, N7 |
| 6 | Cytidine, N3 |
| 7 | Aniline |
| 8 | 1-Hexanamine |
| 9 | 1,2-Diaminoethane |
| 10 | Ammonia |


200
100
0
-100
0
100
200
300
1
2
3
4
5
6
7
8
9
10
Platinum Coordination Shift, ppm
$^{15}N$ Chemical Shift of the Free Ligand, ppm

high trans influences to water with low trans influence. Cis ligands have a small, but significant effect on the nitrogen shift. This effect is opposite in sign to the trans-ligand effect and the order is slightly different.

To gain an understanding of the effects of platinum coordination on the $^{15}N$ chemical shifts, it is necessary to outline the factors influencing $^{15}N$ chemical shifts. Nuclear magnetic shieldings can be regarded as the sum of three terms: [5,6,7]

$$\sigma^A = \sigma_d^A + \sigma_p^A + \sum_{B \neq A} \sigma^{AB} \qquad (2)$$

where $\sigma^A$ is the shielding constant of the nucleus concerned, $\sigma_d^A$ is the local diamagnetic term, $\sigma_p^A$ is the local paramagnetic term, and the $\sum_{B \neq A} \sigma^{AB}$ term (which is normally small for first-row elements and is usually neglected) takes into account the shielding effects of other atoms in the molecule and medium effects.

The diamagnetic contribution has been approximated from the following equation [8,9] (in SI units):

$$\sigma_d^k = \sigma_d(\text{free atom/free ion}) + \frac{e^2}{30m} \sum_{\alpha}' (Z_\alpha / r_\alpha) \qquad (3)$$

where $\sigma_d^k$ is the diamagnetic contribution to the shielding of the $k$ th nucleus in a molecule, $\sigma_d$(free atom/free ion) is the diamagnetic shielding of the approximate free atom or ion (depending upon whether the atom

in question bears a formal charge), $Z_\alpha$ is the atomic number of the $\alpha$th nucleus, $\underline{r}_\alpha$ is the distance between nuclei $\underline{k}$ and $\alpha$, and the summation runs over all atoms directly bound to nucleus $\underline{k}$. This equation has been used to calculate the diamagnetic contribution to the nitrogen shieldings in organic molecules [8,10] and metal-EDTA complexes. [11] The contribution of the platinum nucleus to the nitrogen diamagnetic shielding, calculated from Equation 3, is about 350 ppm. Overall, the calculated diamagnetic shielding for the nitrogen of a platinum coordinated ammonia is greater than 700 ppm. If the third term in Equation 2 is, as usual, ignored, then the paramagnetic deshielding of the nitrogen from platinum coordination must be on the order of 250-300 ppm to account for the observed chemical shieldings. Such a cancelling of a large diamagnetic term and a large opposing paramagnetic term has been proposed to account for the small complexation shifts observed in metal-EDTA complexes. [11] However, the diamagnetic shielding equation assumes the bonding between the nitrogen and neighboring nuclei is essentially covalent, which is probably not true for coordination complexes. In addition, it has been argued that the paramagnetic term cannot be as large as was suggested for the metal-EDTA complexes, because in a series of rhodium (III) complexes the chemical shieldings did not correlate with the position of the ligands in the spectrochemical series. [12] Thus, the diamagnetic shielding term is probably much less than the 700 ppm calculated from Equation 3 for the nitrogen of a platinum coordinated ammonia.

The paramagnetic contribution is usually expressed in the following relationship: [5]

$$\sigma_p^A \alpha (\underline{\Delta E})^{-1} \langle \underline{r}^{-3} \rangle_{2p} \sum_B Q_{AB} \qquad (4)$$

where $\underline{\Delta E}$ is the average excitation energy, $\langle \underline{r}^{-3} \rangle_{2p}$ is the mean value of the inverse cube of the 2p orbital radius, and the $Q_{AB}$ term (where B is the neighbor of A) contain the elements of the charge-density/bond order matrix. These terms are not easy to calculate; however, qualitative arguments about the magnitude of the paramagnetic contribution can be made from Equation 4. In organic molecules, the paramagnetic deshielding comes mainly from the electronic circulations on the resonant atom. However, in coordination complexes, interactions of the ligand and the metal, such as circulations on the ligand atom which use the unoccupied d-orbitals of the metal, can also make a contribution to the deshielding and should be sensitive to the ligand-field splittings. [13] Electronic circulations, primarily on the metal, such as $d\pi \rightarrow d\sigma^*$, make smaller contributions which may shield or deshield the ligand atom. [13]

In the case of square planar platinum-ammine complexes, where ammonia is the ligand, the ammonia is a sigma donor and no strong interaction is expected with the metal d-orbitals. Hence, the ligand-field splittings of these complexes are small and the ligand substitution patterns should have a small effect on the paramagnetic deshieldings.

However, the coordination of platinum to the nitrogen lone pair should have a similar effect on the paramagnetic deshielding as protonation. The protonation shift has been explained in terms of a second-order paramagnetic effect. Protonation of simple amines usually results in a downfield shift of the nitrogen resonance and has been explained as the removal of the shielding effects of the lone-pair electrons. The low-field shift of aniline and conjugated nitrogen compound is partially due to the delocalization of the lone pair into the pi-electron system, thereby lowering the $n \rightarrow \pi^*$ transition energy, $\Delta E$, and increasing the magnitude of the paramagnetic deshielding. Protonation in these compounds "coordinates" the lone-pair electrons, which results in the observed upfield shifts. There is a correlation between the magnitude and direction of the protonation shifts and the chemical shift of the neutral compound [5] (see Figure 8). The platinum coordination shift correlates well with the protonation shift (see Figure 9). This relationship indicates that part of the upfield coordination shift is from the complexation of the lone-pair electrons. The same relationship was reported for a series of rhodium (III) complexes. [12]
A least-squares fit of the points yields a shape of 0.7 and an intercept of 46 ppm. This indicates that the diamagnetic contribution to the upfield coordination shift is about 45 ppm and the change in the paramagnetic contribution on coordination to platinum is about 70% of the corresponding change on protonation.

Figure 8.

A plot of the $^{15}N$ protonation shift versus the chemical shift of the free ligand. The points correspond to ligands with reported platinum coordination shifts (or similar ligands). A least squares fit of the points yields a slope of -0.40, an intercept of 131 ppm, and a correlation coefficient, r, of -0.995.

| No. | Ligand |
|---|---|
| 1 | Diazobenzene |
| 2 | Pyridine |
| 4 | 1-Methylimidazole |
| 5 | Guanosine, N7 |
| 6 | Cytidine, N7 |
| 7 | Aniline |
| 8 | 1-Hexanamine |
| 9 | 1,2-Diaminoethane |
| 10 | Ammonia |

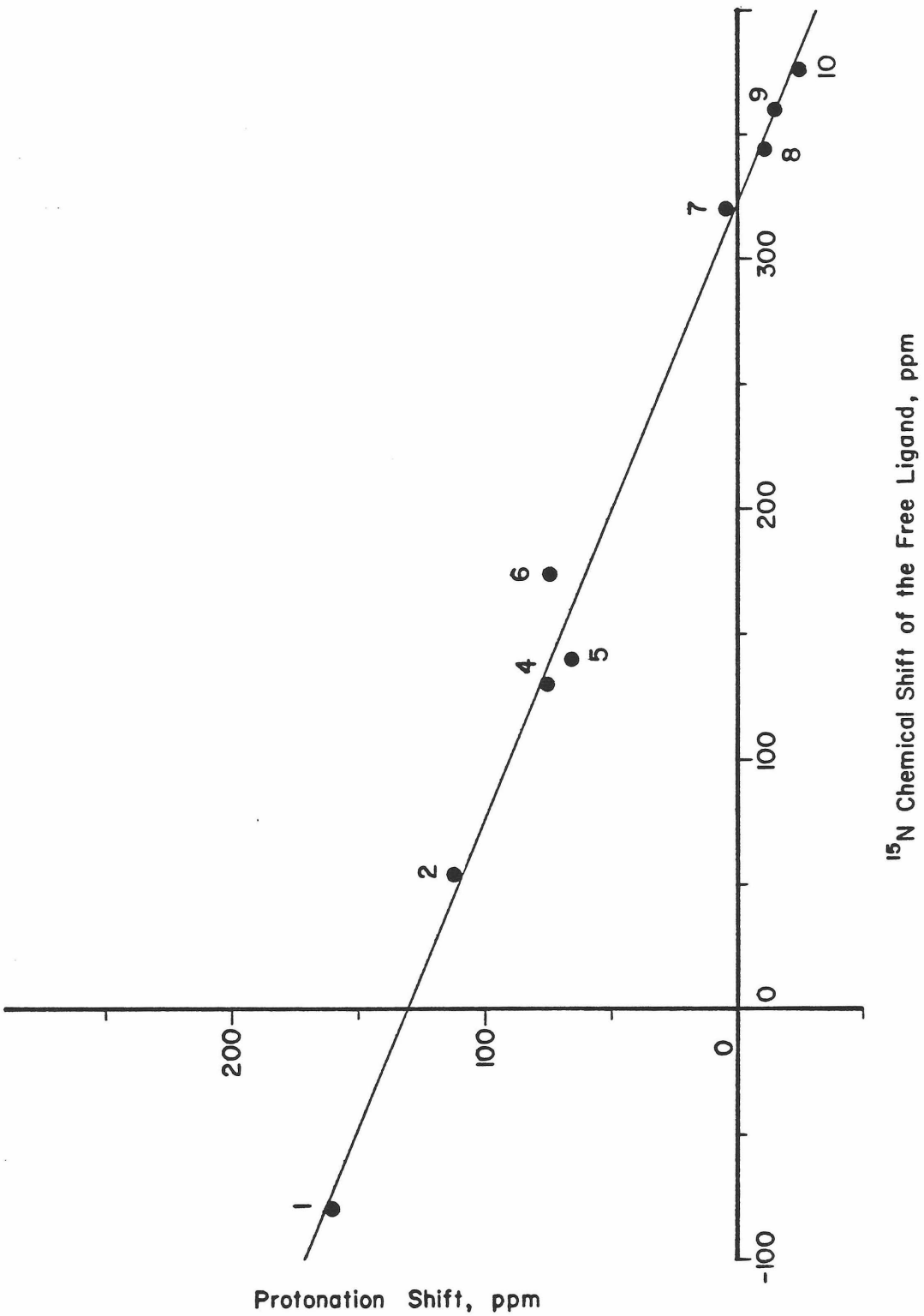
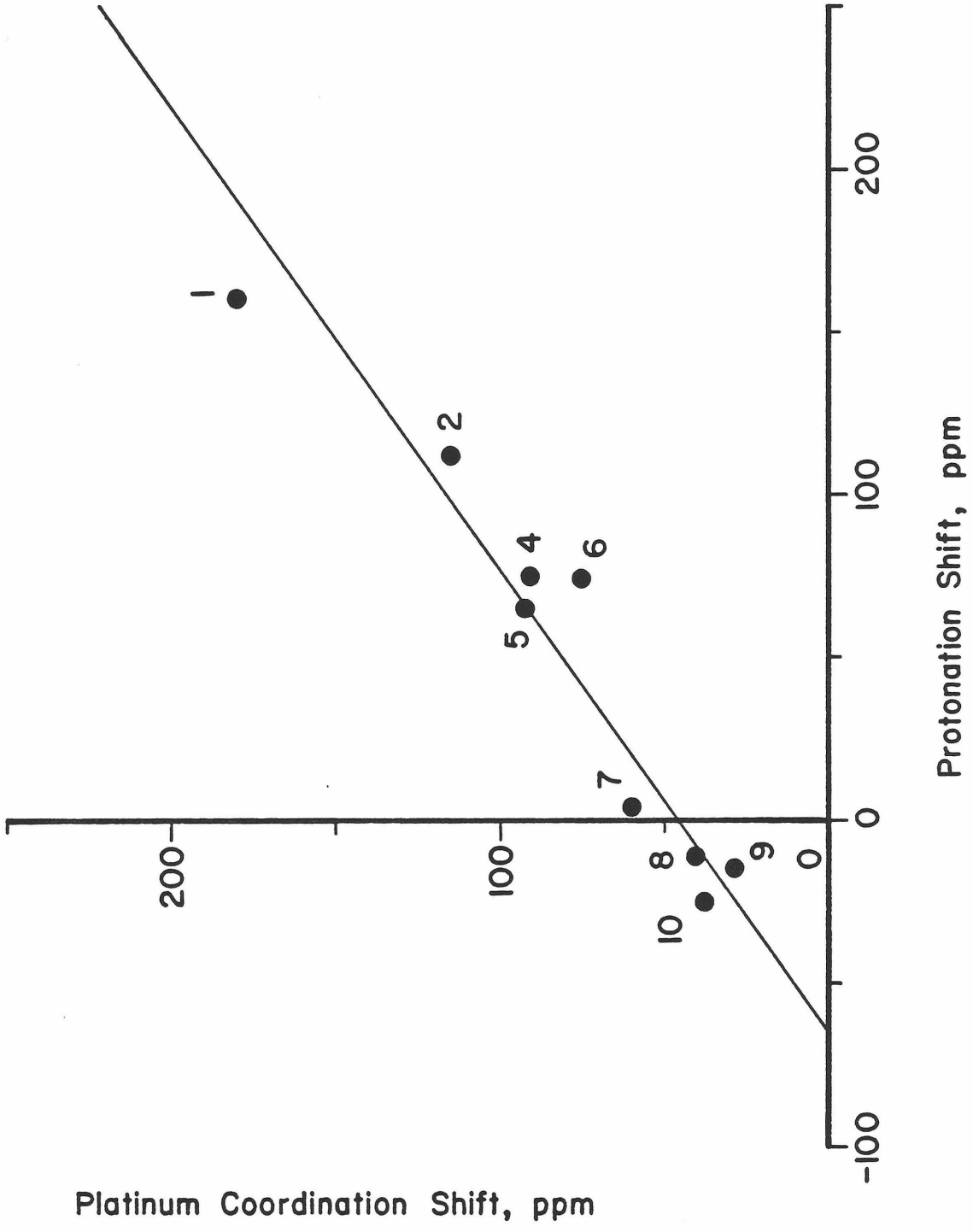

200
100
0
-100
0
100
200
300
1
2
4
5
6
7
8
9
10
Protonation Shift, ppm
$^{15}$N Chemical Shift of the Free Ligand, ppm

Figure 9.

A plot of the $^{15}N$ platinum coordination shift versus the $^{15}N$ protonation shift of the free ligand. A least squares fit of the points yields a slope of 0.71, an intercept of 46 ppm and a correlation coefficient, r, of 0.961. The compounds are numbered the same as in Figure 8.


200
100
0
-100
Protonation Shift, ppm
200
100
0
Platinum Coordination Shift, ppm
1
2
4
5
6
7
8
9
10

As pointed out earlier, the trans-ligand has a large effect on the magnitude of the coordination shift. The diamagnetic shielding should be inversely related to the bond distance between the nitrogen and neighboring nuclei. Because the trans influence of the trans-ligand has an effect on the length of the nitrogen-platinum bond, the diamagnetic shielding should also reflect the trans influence of the trans-ligand. In fact, the observed shielding increases with trans-ligands of low trans influence, which is the expected trend.

The bonding of the nitrogen to platinum must also affect the paramagnetic deshielding. As indicated by the one-bond coupling constant, $^{1}J_{^{15}N-^{195}Pt}$, the s-character of the nitrogen-platinum bond increases with trans-ligands of low trans influence. Thus, the nitrogen 2p orbital radius increases with trans-ligands of low trans influence and the paramagnetic deshielding decreases because of the inverse cubed relationship to the 2p orbital radius.

TABLE 5

RANGE OF RELATIVE $^{15}N$ CHEMICAL SHIFT LIGAND EFFECTS, ppm (AVERAGE)

| Ligand | Position | |
|---|---|---|
| | trans | cis |
| $H_2O$ | +19.2 +21.1 (+20.1) | -3.0 -0.9 (-2.0) |
| pyridine | + 0.5 + 2.0 (+ 1.2) | -6.4 -3.9 (-4.8) |
| aniline | 0.0 +1.0 (+ 0.5) | -4.7 -3.7 (-4.2) |
| $Cl^-$ | 0 | 0 |
| $NH_3$ | -3.0 -0.7 (- 1.7) | +0.8 +2.1 (+1.4) |

D. References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RESULTS AND DISCUSSION

E. Platinum-Nucleoside Complexes

Cytidine, when allowed to react with an equimolar amount of $^{15}N$-labelled cis-$[Pt(NH_3)_2Cl_2]$, formed a monocytidine complex, cis-$[Pt(NH_3)_2(Cy)\ Cl]^+$. The $^{15}N$ spectrum of this complex gave two resonances at 420.7 and 423.9 ppm, with couplings to platinum-195 of 339 and 300 Hz, respectively. The resonance assignments listed in Table 6 were made on the basis of the observed chemical shifts and coupling constants. A comparison of the model complexes shows that a $^{15}N$ nucleus of an ammonia ligand trans to a chloride ligand comes into resonance at lower field than one trans to a nitrogen ligand. In addition, $^{15}N$ of ammonia ligands trans to a chloride have coupling constants in the range of 320-350 Hz, while those trans to a nitrogen have smaller couplings, usually in the range of 270-310 Hz. [1] Thus, the resonance at 420.7 ppm can be assigned to the ammonia trans to the chloride ligand and the upfield resonance at 423.9 ppm to the ammonia ligand trans to the coordinated cytidine. Cytidine reacts relatively slowly with cis-$[Pt(NH_3)_2Cl_2]$. After one day at room temperature, only 69% of the cis-$[Pt(NH_3)_2Cl_2]$ had reacted with cytidine and after nine days, some cis-$[Pt(NH_3)_2Cl_2]$ was still detectable.

When a second equivalent of cytidine reacts with the monochloromonocytidine complex, a new resonance appeared in the $^{15}N$ spectrum at 421.5 ppm, with concommitant decreases in the resonances of the monochloromonocytidine complex. The new resonance had a platinum-

195 coupling of 304 Hz. That only one resonance was formed when two equivalents of cytidine were added to cis-$[Pt(NH_3)_2Cl_2]$ indicates formation of a symmetric biscytidine complex, where both cytidines are coordinated to platinum, each at the same cytidine nitrogen. The biscytidine complex also formed very slowly. Even after one week, there was still the monochloromonocytidine complex predominantly in the solution. The relatively slow reaction of cytidine with cis-$[Pt(NH_3)_2Cl_2]$ is in agreement with the reported reactivity of cis-$[Pt(NH_3)_2Cl_2]$ with the different nucleosides. [2]

The $^{15}N$ chemical shifts and coupling constants of the platinum-cytidine complexes listed in Table 6 indicate cytidine acts only as a monodentate ligand. No evidence was obtained for chelate binding of cytidine to cis-$[Pt(NH_3)_2Cl_2]$ through either N3 and the $NH_2$ at C4, or N3 and the oxygen at C2. (see Figure 10) The monocytidine complex clearly had a chloride complexed to the platinum, as evidenced by the $^{15}N$ ammonia-to-platinum-195 coupling of 339 Hz. If chelate binding had occurred between N3 and the $NH_2$ at C4, both of the coupling constants would have been in the range of 270-310 Hz. Also, if chelation involved N3 and the oxygen at C2, the coupling constant of the ammonia ligand trans to the carbonyl would have been greater than 350 Hz. [1,3,4] Therefore, cytidine must be a monodentate ligand in both the mono- and biscytidine complexes, with coordination occurring through a nitrogen site. From the $^{15}N$ spectra of the ammonia ligands alone, it is not possible to determine whether N3 or the $NH_2$ at C4 is involved.

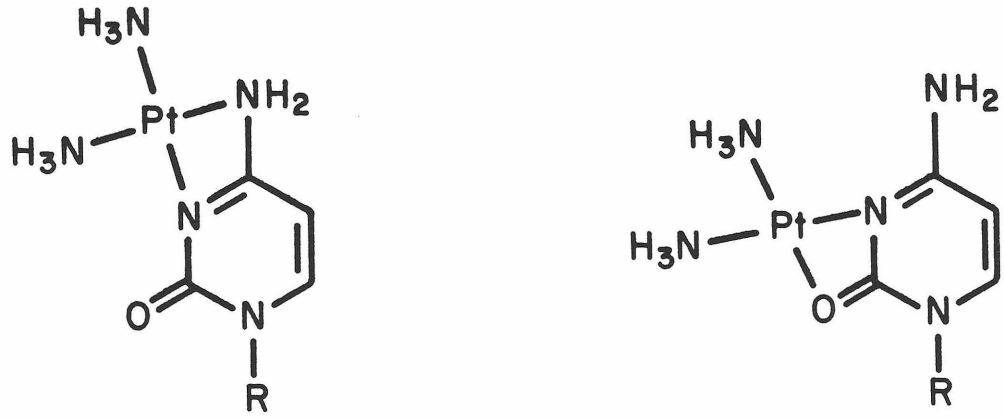



Figure 10. Cytidine chelation complexes

The nitrogens of coordinated cytidine can be observed directly by $^{15}N$ NMR at the natural-abundance level. The chemical shifts for the biscytidine complex are listed in Table 7. Comparison of the $^{15}N$ chemical shifts of the biscytidine complex with those of free cytidine 5'-monophosphate shows that the resonance for N1 is unchanged in the coordinated cytidine relative to the free, while N3 shifts 76 ppm upfield and that of the $NH_2$, 11 ppm downfield relative to the uncoordinated base. The large upfield shift of N3 upon coordination indicates that N3 is the site of cis-$[Pt(NH_3)_2Cl_2]$ binding to cytidine. The platinum coordination shift of cytidine has the same magnitude and direction as the protonation shift. [5,6]

When guanosine reacts with $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$, complex spectra were obtained, which indicate that a mixture of guanosine

complexes was formed. (see Figures 11 and 12) The spectra of the products formed when guanosine reacts with an equimolar amount of $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ are shown in Figures 11a and 12a. Six peaks at 419.7, 420.1, 420.8, 422.1, 422.8, and 424.1 ppm, were present in both spectra, although the relative intensities of the peaks were different for each sample. Another difference between the two spectra is a peak at 423.9 ppm in the spectrum shown in Figure 11a. The resonance at 422.1 ppm in these spectra can be assigned to unreacted cis-$[Pt(NH_3)_2Cl_2]$. The 1:1 products, shown in Figure 11a, when allowed to react with a second equivalent of guanosine gave the 2:1 spectrum of Figure 11b. The other 1:1 sample was treated with two equivalents of silver nitrate to replace any chloride ligands with aquo ligands and the resulting spectrum is shown in Figure 12b.

Comparison of the chemical shifts, coupling constants, and peak areas of the resonances of the 1:1 products, with the 2:1 products and 1:1 aquo products, allows assignment of a majority of the resonances of the different guanosine complexes. One resonance at 420.8 ppm appears in each of the spectra. Because this resonance is the main peak in the 2:1 product spectrum, this peak can be assigned to a symmetric bisguanosine complex. Formation of a bisguanosine complex is consistent with unreacted cis-$[Pt(NH_3)_2Cl_2]$ in the 1:1 products. Four other major peaks appear to come in pairs. The reason is that the intensities of the ones at 419.7 and 424.1 ppm are high in the first spectrum (Figure 11a) and low in the second (Figure 12a), while the intensities of the peaks at 420.1 and 422.8 ppm remain relatively constant in both

Figure 11.

(a) The $^{15}N$ spectrum of 100 mg (0.33 mmoles) of $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ and 94 mg (0.33 mmoles) of guanosine in 25 ml of water after standing at room temperature for 10 days. Acquisition parameters: 2043 scans, a 45° pulse width, and a 2 second repetition rate.

(b) The $^{15}N$ spectrum of the same sample as (a) after an additional 94 mg of guanosine was added and the sample was left standing at room temperature of 3 days. Acquisition parameters: 20,000 scans, a 77° pulse width, and a 3 second repetition rate.

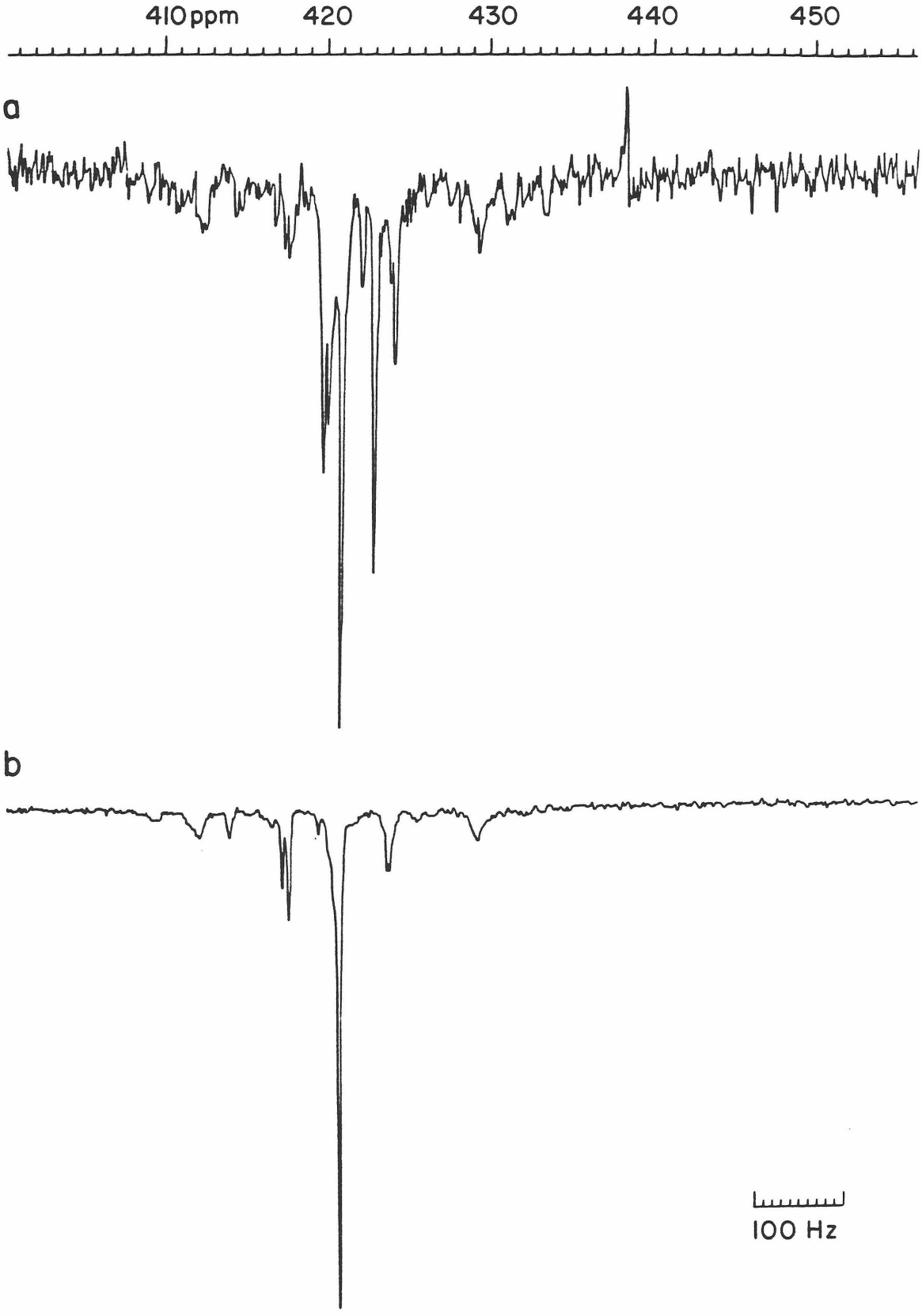
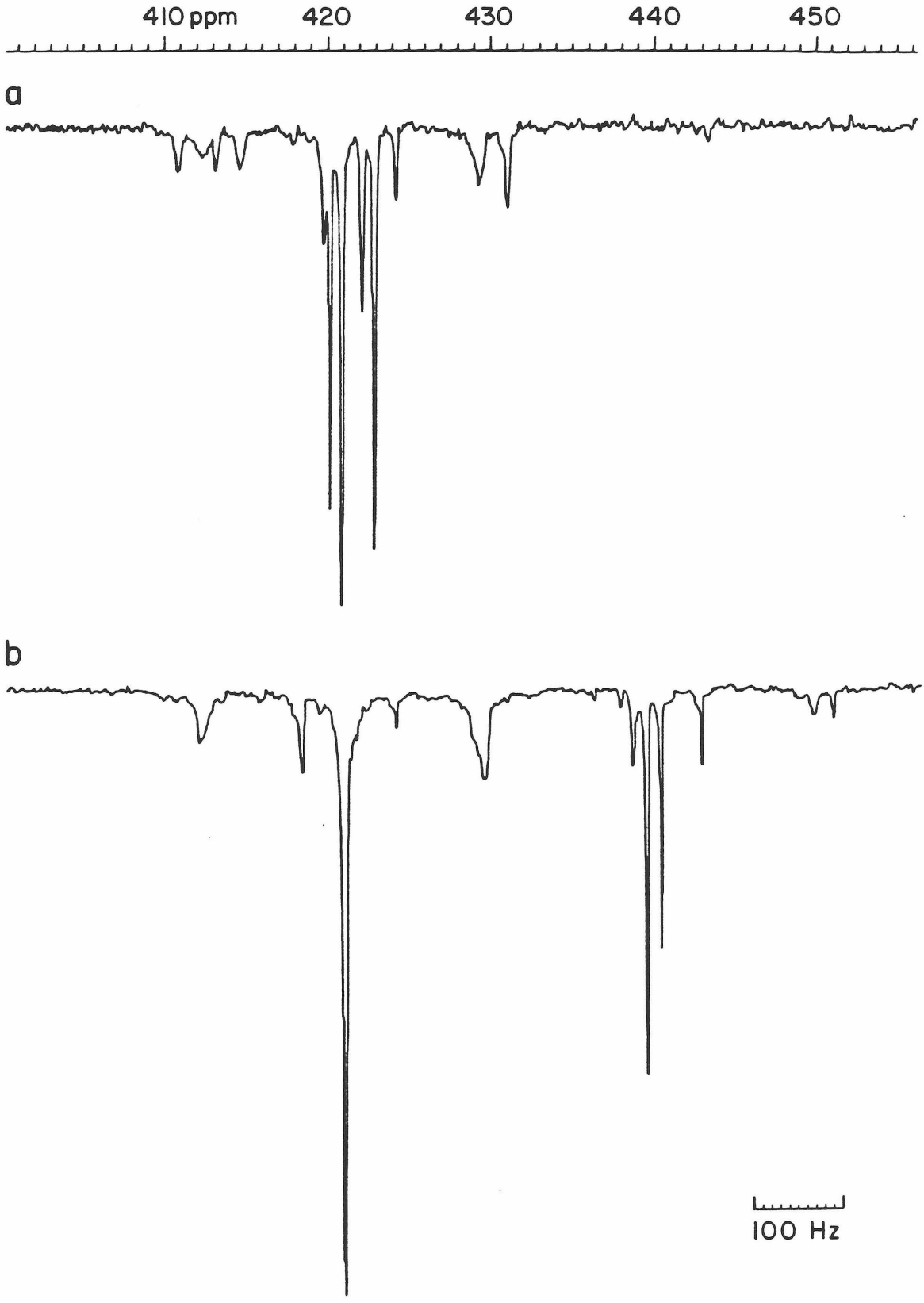

410 ppm
420
430
440
450
a
b
100 Hz

Figure 12.

(a) The $^{15}N$ spectrum of 100 mg (0.33 mmole) of $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ and 94 mg (0.33 mmole) of guanosine in 20 ml of water after heating at 50-60°C for one hour. Acquisition parameters: 8200 scans, a 45° pulse width, and a 2 second repetition rate.

(b) The $^{15}N$ spectrum of the same sample as (a) except after treatment with 113 mg (0.67 mmole) of silver nitrate. Acquisition parameters: 22,750 scans, 45° pulse width, and a 2 second repetition rate.


410 ppm
420
430
440
450
a
b
100 Hz

spectra. Silver chloride treatment causes changes in shift for all of the peaks: those at 419.7 and 420.1 ppm go upfield to 438.5 and 439.3 ppm, respectively, while those at 422.8 and 424.1 ppm go downfield to 420.9 and 418.0 ppm, respectively. Each pair can be assigned to monochloromonoguanosine complexes. The ratio of the monochloromonoguanosine complexes in the two preparations is 2:1 in the first (Figure 11a) and 4:1 in the second (Figure 12a). The resonance at 420.1 ppm has a coupling constant of 338 Hz, which indicates that it arises from an ammonia trans to a chloride ligand. The other resonance at 422.8 ppm has a coupling constant of 298 Hz, consistent with an ammonia trans to a guanosine bound through one of its nitrogens. The shift behavior of all four peaks upon substitution of an aquo ligand for chloride also argues for the assignment of these peaks to a pair of monochloromonoguanosine complexes. The monochloromonoguanosine complex with resonances at 420.1 and 422.8 ppm and the major symmetric bisguanosine complex at 420.8 ppm can be assumed to have guanosines coordinated to platinum through the same nitrogen sites. There remains one resonance at 423.9 ppm in the first 1:1 products spectrum which also appears in the 2:1 products spectrum. This resonance could correspond to a second symmetric bisguanosine complex or else to a monochloro-monoguanosine complex, where the second peak is hidden under another peak.

The spectrum of the 2:1 products (Figure 11b) shows seven peaks, other than coupling satellites. The peaks at 420.8 and 423.9 have been

assigned to symmetric bisguanosine complexes. Three resonances are of approximately the same intensities. One pair of these resonances is probably from an unsymmetric bisguanosine complex or an unsymmetric bisguanosine complex, where the other ammonia resonance overlaps with the large peak at 420.8 ppm.

Seven peaks can be seen in the spectrum of the 1:1 aquo complexes (Figure 12b). Again the symmetric bisguanosine complex gives a resonance at 420.8 ppm. The peaks at 420.9 and 439.3 ppm are from the monoaquomonoguanosine complex corresponding to the major monochloromonoguanosine complex formed in the 1:1 products. The peaks at 418.0 and 438.5 are assigned to a second aquated monoguanosine complex which comes from the monochloromonoguanosine complex with resonances at 419.7 and 424.1 ppm. A third monoaquomonoguanosine complex was also formed, as indicated by the small resonances at 424.1 and 442.7 ppm. Finally, the resonance at 440.2 ppm is from cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. The assignments of the ammonia resonances of the platinum-guanosine complexes are listed in Table 6.

It is clear from the spectra of the $^{15}N$-labeled ammonia ligands that cis-$[Pt(NH_3)_2Cl_2]$ reacts with guanosine at more than one site on the base. At least two monochloromonoguanosine complexes are formed along with the corresponding symmetric bisguanosine complexes. However, as stated before, the $^{15}N$ spectra of the ammonia ligands cannot be used to determine the guanosine coordination sites. Probably, the primary coordination site is N7, as has been indicated by other studies.[7,8]

The other sites could be N1, N3, or the $NH_2$ at C2.

To determine the coordination site of cis-$[Pt(NH_3)_2Cl_2]$ on guanosine, it was necessary to take the $^{15}N$ spectrum of the guanosine nitrogens. The chemical shifts of cis-$[Pt(NH_3)_2Guo_2]^{2+}$ are listed in Table 7. A comparison of the coordinated guanosine shifts with those of free guanosine 5'-monophosphate shows that the guanosines are bound to platinum through N7, because the N7 resonance shifts upfield by 92 ppm while the other resonances shift slightly downfield or remain unchanged. Again the platination shifts are similar in direction and magnitude to the protonation shifts in guanosine.[5] The relative amount of the symmetric bisguanosine complex, bound through N7, in a 2:1 product mixture can be estimated from the areas of the ammonia peaks in Figure 11b. About 66% of the 2:1 mixture was the bisguanosine complex. The other complexes individually were at most 15% of the total mixture. The total of the other complexes was at most one-fourth of the major symmetric bisguanosine complex and probably less because guanosines are not equivalent in the unsymmetric bisguanosine complexes. The signal-to-noise ratio of the spectrum of the bisguanosine complexes at the natural-abundance level was not high enough to detect these other complexes.

It should be noted that, as with cytidine, chelate binding of guanosine by cis-$[Pt(NH_3)_2Cl_2]$ does not appear to be important. If chelate binding through the $NH_2$ at C6, and at N1, or N3 (see Figure 13) had occurred, then a pair of resonances from the nonequivalent ammonias

should have been observed in all the spectra. The chelate complex should not be affected by the silver nitrate treatment or addition of guanosine. The controversial chelate binding through N7 and the oxygen at $C_6$ [9] (see Figure 13) was also not observed. Complexation of the oxygen would have shifted the <u>trans</u> ammonia resonance about 20 ppm upfield. There were no resonances in this region in either the

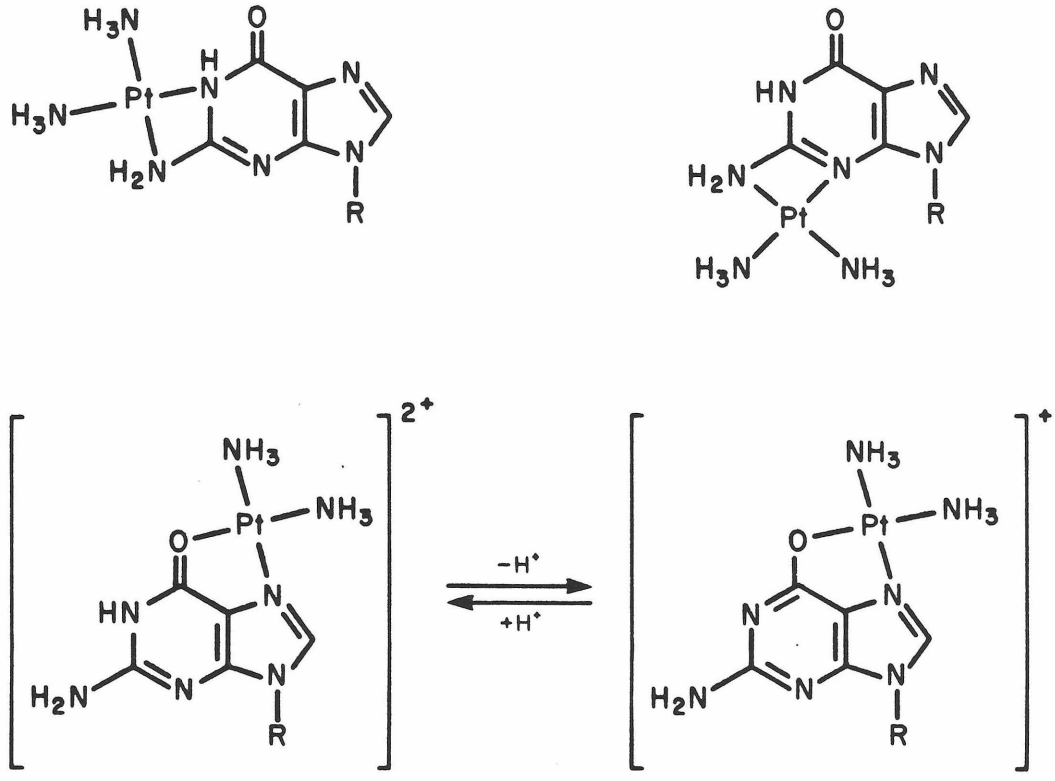


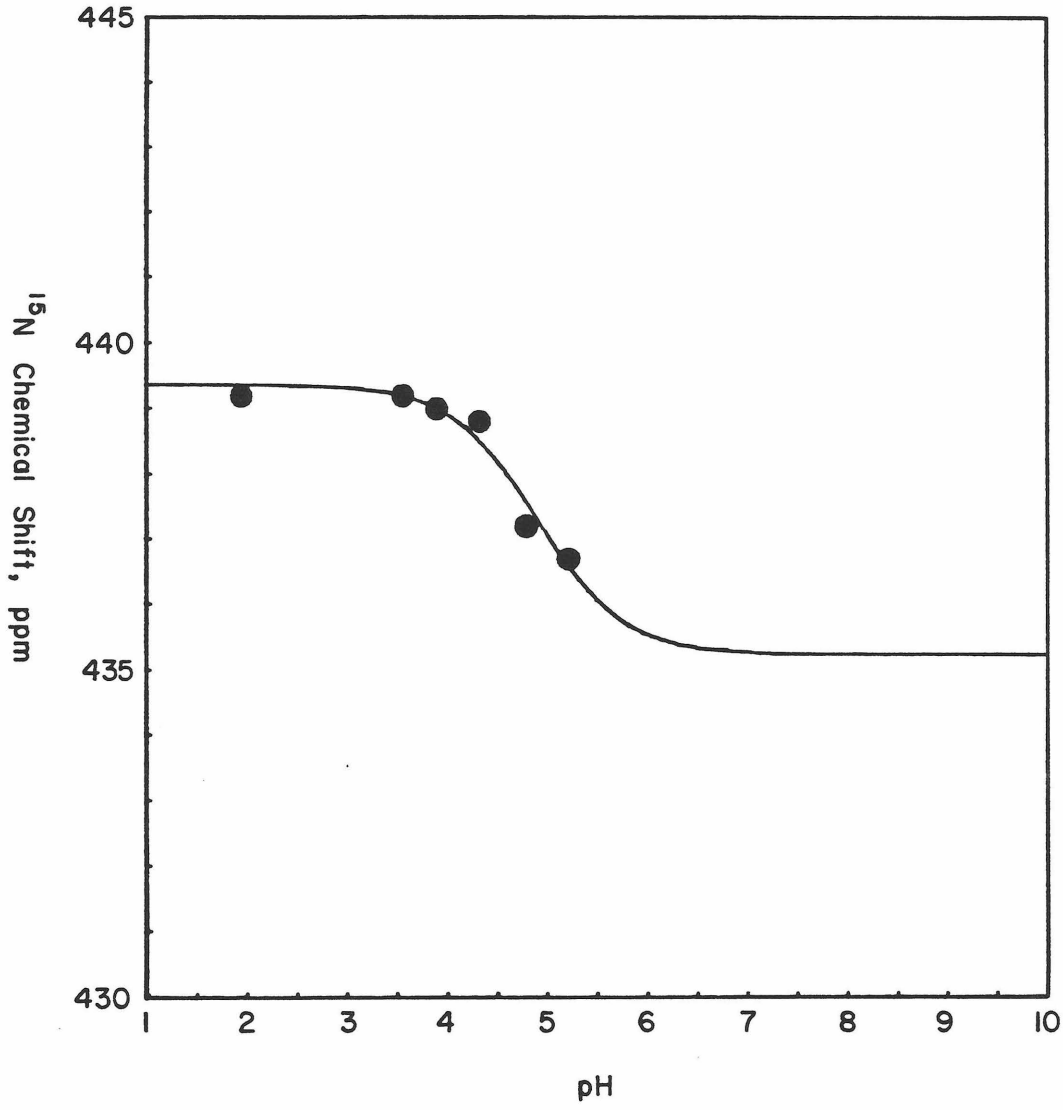

Figure 13

1:1 or 2:1 products. In the 1:1 aquo-products spectrum, the chemical shifts of the resonances assigned here to cis-$[Pt(NH_3)_2Guo^{N7}(H_2O)]^+$ might also be consistent with the chelate complex. It is certainly conceivable that the carbonyl oxygen is not a strong enough ligand to displace a coordinated chloride, but is able to displace a more weakly bound water. Thus the chelate would be formed only after the chloride ligand was removed. The ammonia resonance trans to the oxygen ligand is pH dependent. The chemical-shift dependence of this resonance with pH is listed in Table 8 and shown in Figure 14. This dependence indicates that the ligand trans to the ammonia has acidic protons as expected for an aquo ligand (Figure 15) or the amide proton at N1 of guanosine (Figure 13). However, the $pK_a$ value of this acidic group, calculated from the chemical-shift data, of 4.9 is more reasonable for a platinum-coordinated water. [10] The $pK_a$ of the amide proton of free guanosine is about 9.2 and it is highly unlikely that platinum coordination to the carbonyl oxygen at C6 would increase the acidity of this proton by more than 4 $pK_a$ units. The $pK_a$ value indicates a somewhat greater acidity than usual for a coordinated water, [10] but it could be that hydrogen bonding involving the carbonyl oxygen (Figure 15) stabilizes the conjugate base more than the acid.

Formation of the bisguanosine complex with both guanosines bound through N7 is a facile process. Neither cytidine nor adenosine (see later) show any tendency to form bis complexes in a 1:1 mixture.

Figure 14.

The plot of the ammonia $^{15}N$ chemical shift dependence of $^{15}N$-labeled cis$[Pt(NH_3)_2Guo(OH_2)]^{2+}$ on pH. The calculated titration curve is shown on the plot.


445
440
435
430
$^{15}N$ Chemical Shift, ppm
1
2
3
4
5
6
7
8
9
10
pH

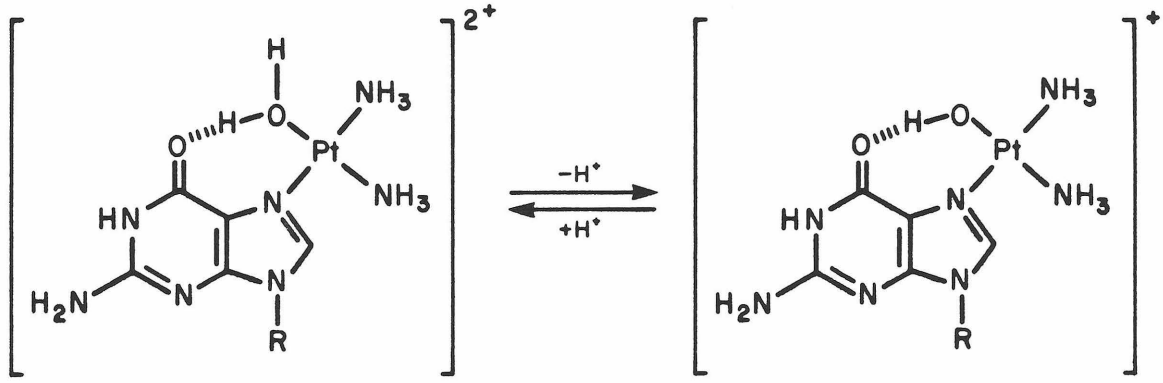



Figure 15

However, both guanosine and pyridine form bis complexes in competition with mono complexes in the 1:1 mixtures. It is possible that base-stacking interactions in these complexes makes them favorable.

The $^{15}N$ spectra of the products from the reaction of adenosine with one equivalent of $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ are deceptively simple. In one preparation, the spectrum showed only two broad peaks centered at 417.6 and 420.5 ppm. (see Figure 16a) The second preparation gave a spectrum with three broad peaks, at 417.6, 418.2, and 420.5 ppm. (see Figure 17a) Each of these peaks is actually composites of several overlapping resonances. This was evident when the 1:1 mixture was treated with an excess of silver nitrate. The upfield portion of the spectrum of the resulting 1:1 aquo products (Figure 17b), clearly shows five separate peaks at 436.1, 437.5, 438.9, 439.5, and 440.2 ppm. The upfieldmost peak is from cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$.

Figure 16.

(a) The $^{15}N$ spectrum of 100 mg (0.33 mmole) of $^{15}N$-labeled cis[$Pt(NH_3)_2Cl_2$] and 89 mg (0.33 mmole) of adenosine in 25 ml of water after standing at room temperature for 9 days. Acquisition parameters: 1635 scans, a 77° pulse width, and a 3 second repetition rate.

(b) The $^{15}N$ spectrum of the same sample as (a) after an additional 89 mg of adenosine was added and was left standing at room temperature for 7 days. Acquisition parameters: 7820 scans, a 45° pulse width, and a 3 second repetition rate.

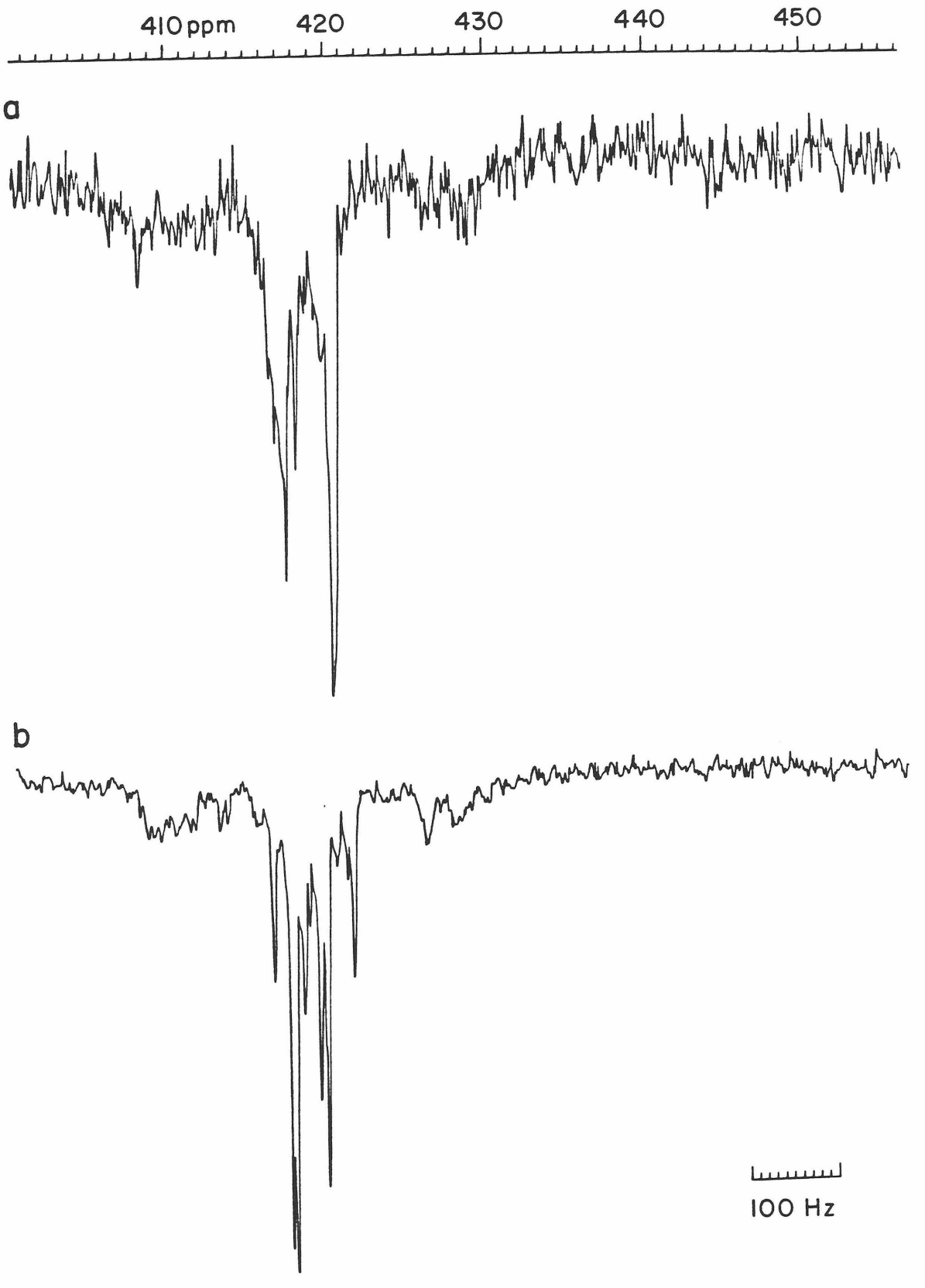
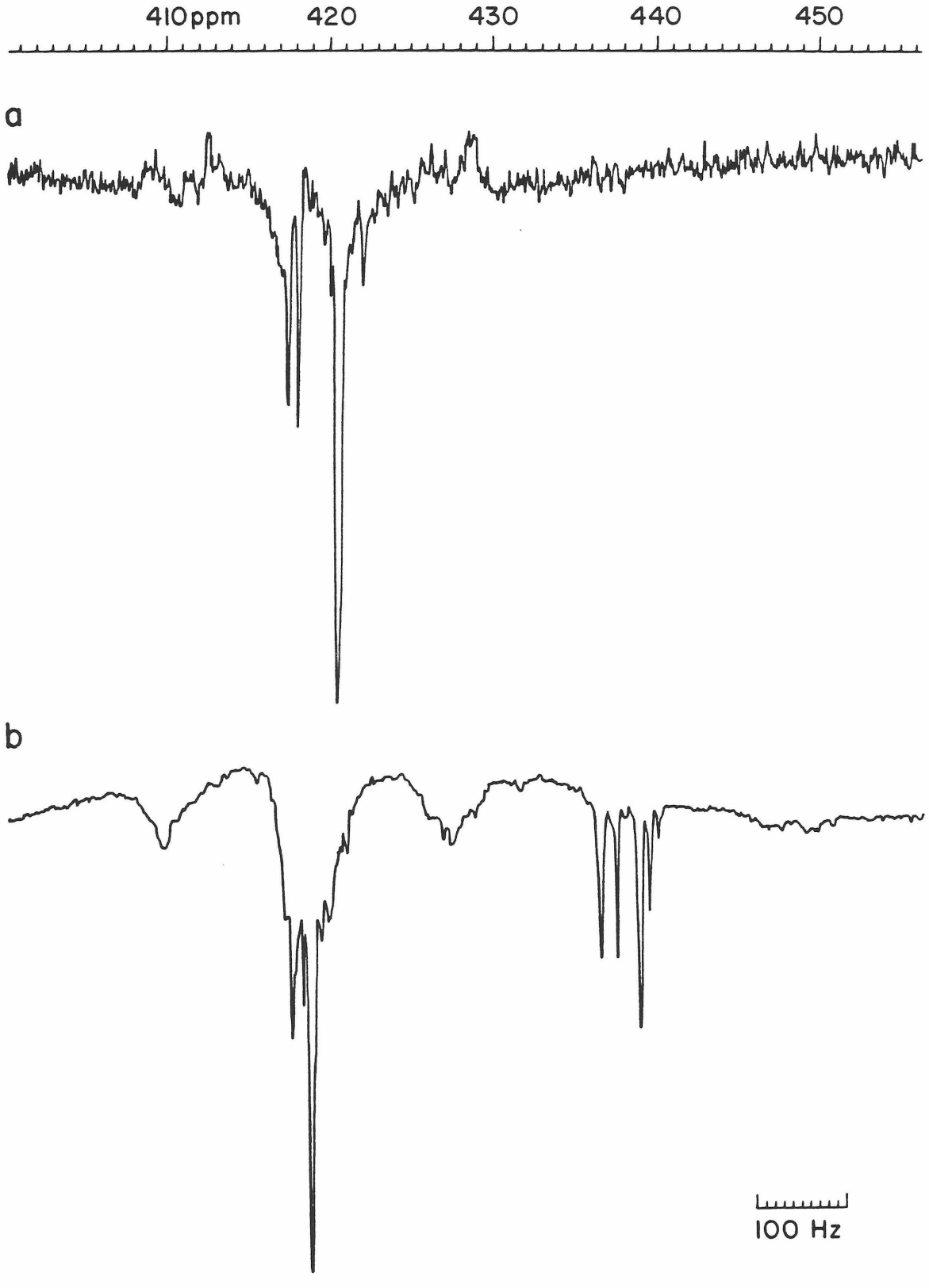

410 ppm
420
430
440
450
a
b
100 Hz

Figure 17.

(a) The $^{15}N$ spectrum of 100 mg (0.33 mmole) of $^{15}N$-labeled cis-$[Pt(NH_3)_2Cl_2]$ and 89 mg (0.33 mmole) of adenosine in 20 ml of water after heating to 50-60°C for one hour. Acquisition parameters: 613 scans, a 60° pulse angle, and a 5 second repetition rate.

(b) The $^{15}N$ spectrum of the same sample as (a) except after treatment with 150 mg (0.88 mmole) of silver nitrate. Acquisition parameters: 11,740 scans, a 60° pulse angle, and a 5 second repetition rate.


410ppm
420
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b
100 Hz

The remaining peaks must be from the ammonia ligands trans to water in the four possible monoaquomonoadenosine complexes. The coordination sites on the adenosine are N1, N3, the $NH_2$ at C6, and N7. In the downfield region of the spectrum, the peaks from the ammonia ligands trans to adenosine are not well resolved [11] and identification of individual resonances is not possible.

Addition of a second equivalent of adenosine to the original mixture of 1:1 products results in the formation of a complex mixture. The spectrum of the 2:1 mixture (Figure 16b) shows at least ten resolvable peaks. There are ten possible different bisadenosine complexes possible and these complexes would have a total of sixteen nonequivalent ammonia ligands. The spectrum of the 2:1 products is too complex to assign the resonances of individual bisadenosine complexes.

The binding of adenosine by cis-$[Pt(NH_3)_2Cl_2]$ must be relatively unselective. Apparently, cis-$[Pt(NH_3)_2Cl_2]$ can coordinate with adenosine at any of the four possible sites. Also there is no preferential formation of the symmetric over the unsymmetric bisadenosine complexes. This fact is in contrast to the bisguanosine complexes. Most, if not all, of the possible bisadenosine complexes are formed in the 2:1 mixture. Chelate binding through the $NH_2$ at C6 and N7 or N1 are possibilities which cannot be excluded on the basis of the ammonia resonance chemical shifts.

Because the 2:1 mixture of adenosine and cis-$[Pt(NH_3)_2Cl_2]$ forms such a large number of different complexes, rather than one dominant

complex, the concentration of each coordinated adenosine is too low to observe their nitrogens at the natural-abundance level with presently available instrumentation.

In summary, the $^{15}N$ spectra of the products from cis-$[Pt(NH_3)_2Cl_2]$ with the various nucleosides clearly show that more sites are involved than previously reported. [12] Thus, adenosine has been reported to bind cis-$[Pt(NH_3)_2Cl_2]$ only at N1 and N7. [13,14] The present work indicates binding at all of the nitrogen sites, except N9. Guanosine complexes are bound to platinum through N7 and at least one other nitrogen. Previous reports have only determined the cis-$[Pt(NH_3)_2Cl_2]$ binding site for the isolated bisguanosine complex. [7,8] Liquid chromatography of the products of the 1:1 mixture of cis-$[Pt(NH_3)_2Cl_2]$ and guanosine does suggest that several complexes are formed, but the identity of these complexes was not determined. [15] No evidence was obtained in the present record for the formation of a chelate complex of guanosine with cis-$[Pt(NH_3)_2Cl_2]$ and on this basis, cis-$[Pt(NH_3)_2Cl_2]$ probably does not inhibit DNA synthesis by involving intrabase chelation of guanosine. However, cis-$[Pt(NH_3)_2Cl_2]$ does seem to preferentially coordinate to two guanosines through N7. This fact supports an intra-strand chelation mechanism for interaction of cis-$[Pt(NH_3)_2Cl_2]$ on DNA.

$^{15}N$ NMR spectroscopy is clearly an excellent method for investigating the reactions of cis-$[Pt(NH_3)_2Cl_2]$ with nucleic acids. The binding of cis-$[Pt(NH_3)_2Cl_2]$ to nucleosides can be monitored either indirectly

through the ammonia ligands or directly through the base nitrogens.

TABLE 6

AMMONIA LIGAND $^{15}N$ CHEMICAL SHIFTS AND COUPLING CONSTANTS OF PLATINUM-NUCLEOSIDE COMPLEXES

| Compound | Trans Ligand | $\delta\ ^{15}N$, ppm ($^{1}J_{^{15}N-^{195}Pt}$, Hz) |
|---|---|---|
| cis-$[Pt(NH_3)_2Cy\ Cl]^+$ | $Cl^-$ | 420.7 (339) |
| | Cy,N3 | 423.9 (300) |
| cis-$[Pt(NH_3)_2Cy_2]^{2+}$ | Cy,N3 | 421.5 (304) |
| cis-$[Pt(NH_3)_2GuoCl]^+$ | $Cl^-$ | 420.1 (338) |
| | Guo,N7 | 422.8 (298) |
| cis-$[Pt(NH_3)_2Guo_2]^{2+}$ | Guo,N7 | 420.8 (310) |
| cis-$[Pt(NH_3)_2GuoH_2O]^{2+}$ | Guo,N7 | 420.9 (321) |
| | $H_2O$ | 439.3 (376) |
| cis-$[Pt(NH_3)_2GuoCl]^+$ | $Cl^-$ | 419.7 |
| | Guo,N? | 424.1 |
| cis-$[Pt(NH_3)_2GuoH_2O]^{2+}$ | Guo,N? | 418.0 |
| | $H_2O$ | 438.5 |
| cis-$[Pt(NH_3)_2Guo_2]^{2+}$ | Guo,N? | 423.9 |

TABLE 7

$^{15}N$ CHEMICAL SHIFTS OF NUCLEOSIDES AND PLATINUM-NUCLEOSIDE COMPLEXES

| | $\delta_{^{15}N}$, ppm | | | | |
|---|---|---|---|---|---|
| Compound | N1 | N3 | N7 | N9 | $NH_2$ |
| Cytidine 5'-monophosphate[5] | 223.5 | 175.2 | | | 283.2 |
| cis-$[Pt(NH_3)_2Cy_2]^{2+}$ | 223.6 | 251.0 | | | 272.4 |
| Guanosine 5'-monophosphate[5] | 229.1 | 211.3 | 141.2 | 207.2 | 303.5 |
| cis-$[Pt(NH_3)_2Guo_2]^{2+}$ | 228.1 | 211.4 | 233.5 | 202.7 | 300.3 |

TABLE 8

$^{15}N$ CHEMICAL-SHIFT DEPENDENCE ON pH OF AMMONIA LIGAND TRANS TO WATER FOR cis-$[Pt(NH_3)_2Guo^{N7}(H_2O)]^{2+}$

| pH | $\delta_{^{15}N}$, ppm |
|---|---|
| 1.95 | 439.2 |
| 3.56 | 439.2 |
| 3.90 | 439.0 |
| 4.33 | 438.8 |
| 4.80 | 437.2 |
| 5.22 | 436.7 |

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