Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy of Some Nucleosides and Nucleotides[1a]

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Abstract: High-resolution nitrogen-15 NMR spectra of six nucleosides and six nucleotides have been obtained at the natural-abundance level by high-resolution NMR spectroscopy. All of the nitrogen resonances have been assigned on the basis of comparison of their chemical shifts and nitrogen–hydrogen coupling constants with those of related compounds. Studies of the effect of protonation on the nitrogen chemical shifts of four of these compounds enabled the site of protonation to be detected directly.

Nuclear magnetic resonance spectroscopy of carbon-13 and protons has proved to be one of the most powerful methods for providing information on electronic structure and conformations of nucleosides[2] and nucleotides.[3] Carbon and proton NMR spectra fail to give direct information about the nitrogens of these substances, which clearly play a very important role in their biological activity. We can therefore expect that nitrogen NMR spectra could provide more detailed insight into nucleoside and nucleotide structures. However, with the exception of one nitrogen-15 enriched nucleotide,[4] nitrogen NMR spectra have not so far been reported on these substances. The reason is the difficulty in detecting nitrogen-15 NMR signals because of its low gyromagnetic ratio (−0.101 relative to protons)[5] and its low natural abundance (0.37% in naturally occurring nitrogen). Because we are now able to detect nitrogen-15 at the natural-abundance level on a routine basis, we have investigated some of the naturally occurring nucleosides and nucleotides.[6]

Experimental Section

The nucleosides and nucleotides in this study were all commercially available and were used without further purification. The nucleoside spectra were taken in dimethyl sulfoxide as solvent and those of the nucleotides in neutral, aqueous solution, except when noted otherwise. The concentrations were 0.5–1.0 M. The pH values of the aqueous solutions were determined by means of a Radiometer pH meter.

The nitrogen spectra were obtained at a frequency of 18.25 MHz with a Bruker WH-180 pulse spectrometer, which has been described in some detail elsewhere.[6] A 0.1 M $D^{15}NO_3$ solution in D_2O provided both the reference standard and the external field-frequency lock. The chemical shifts reported are in ppm, upfield from the resonance of external $D^{15}NO_3$. The reproducibility was about 0.1 ppm. The normal operating conditions employed a pulse width of 30 μs (30° flip angle) and a pulse delay of 2 s. With these conditions, useful spectra could usually be obtained with accumulation time of 3–6 h. The samples were run at about 30°, when gated proton decoupling or no decoupling was used, and at about 60°, when full proton decoupling was used. To determine the changes in nitrogen shifts on protonation, trifluoroacetic acid was added to the DMSO solutions, while the aqueous solutions were acidified with concentrated hydrochloric acid. Typical spectra which illustrate what is possible and the kind of problems involved are shown in Figure 1.

Results and Discussion

The nitrogen-15 chemical shifts obtained for six nucleosides and six nucleotides are shown in Table I. Most of the chemical shifts had to be obtained from proton-coupled spectra, because the azine-like nitrogens (N1, N3, and N7) have a very unfavorable nuclear Overhauser effect (NOE), on the order of −1 to −2.[7] As a result, it is almost impossible to detect these nitrogen resonances with the proton decoupler turned on. The lack of signal enhancement and the splittings produced by the protons required longer accumulation times for reasonable signal-to-noise ratios, but this was usually compensated by the important additional information obtained from the nitrogen–hydrogen coupling constants. It is interesting that N9, which binds the sugar residue, has a larger NOE than the azine-like nitrogens of perhaps −2 to −3. This estimate is based on comparison with the NOE of the exocyclic amino nitrogen which is close to the theoretical value of −4.93, as judged from an adenine sample labeled with 95% ^{15}N.[8] Apparently N9 undergoes dipolar relaxation, to some degree, under the influence of the hydrogens on the pentose ring.

Assignments of resonances to specific nitrogens have been made on the basis of nitrogen–hydrogen coupling constants and the ^{15}N studies of related compounds. In adenosine, the resonance at higher field can be assigned to the N6 amine nitrogen atom, because it falls within the limits for nitrogen bound to an aromatic ring system. Also, the one-bond nitrogen–hydrogen coupling constant of 90.3 Hz is close to the value calculated for sp^2-hybridized nitrogen atoms.[9] The resonance assigned to N9 shows a proton coupling constant of 5 Hz, similar to the 4-Hz two-bond coupling constant observed for pyrrole.[10] The proton spectra of ^{15}N-labeled adenine derivatives have shown that two-bond nitrogen–hydrogen coupling constants are about 16 Hz if the nitrogen atoms are in the pyrimidine ring, and about 10 Hz if in the imidazole ring.[11] These results permit the resonance of N7 to be identified by its proton coupling constant (11.0 Hz), which is smaller than the N1 (15.8 Hz) and N3 (17.1 Hz) couplings in the six-membered ring. Further support comes from exchange of H8 by heating adenosine in D_2O at 100 °C.[12] This results in a broad singlet for N7 because of the two-bond ^{15}N–D splittings. The two remaining resonances were assigned by comparison with model compounds. In quinazoline, N1 (different numbering order than in the purine system), which is adjacent to the bridgehead carbon, has its resonance 11.4 ppm at higher field than N3. The difference is still greater in purine, where N3 is 19.1 ppm at higher field. The nitrogen shifts of 2- and 4-aminopyridine given in Table I help in estimating the influence of the amino group on the N1 and N3 of adenine derivatives. It will be seen that the ring nitrogen *in* the 2-amino compound is 8.8 ppm toward higher field than in the 4-amino compound. Accordingly, we can predict a 19.1–8.8 = ~10 ppm shift of N3 to higher field than N1. The observed difference is 13.1 ppm, which is quite good considering the possible uncertainties in shift because of tautomeric equilibria involving N7 and N9 in purine.[13] If N9 of purine carried a substituent similar to that on N9 of adenosine, this could well make the shift difference between N1 and N3 in purine comparable to that observed for adenosine, because an amino group shifts a N2 nitrogen more to higher field than a N3 or N4 nitrogen.[14] On these bases, we assign the higher field resonance to N3 and

Table I. Nitrogen-15 Chemical Shifts of Nucleosides and Related Compounds in Dimethyl Sulfoxide (ppm upfield from external $D^{15}NO_3$)

Nucleoside[a]	N1	N3	N7	N9	NH_2
Adenosine	139.6	152.7	134.7	205.6	293.8
Guanosine	228.0	209.5	128.6	205.3	302.0
Inosine	200.7	161.2	126.7	200.7	
Uridine	231.6	217.5			
Thymidine	231.3	219.5			
Cytidine	222.2	166.1			281.0
Cytidine + 1.5 equiv CF_3CO_2H	221.1	230.9			269.0
2′-Deoxyadenosine	139.1	152.1	134.3	202.1	293.7
Quinazoline	92.2	80.8			
Purine	94.5	113.6	187[b]	187[b]	
2-Aminopyridine	109.8				301.6
4-Aminopyridine	101.0				306.6

[a] The structures and numbering are shown for reference in Figure 2. [b] Only one very broad signal for N7 and N9 could be observed, presumably because of slow hydrogen exchange.

Table II. Dependence of Adenosine Nitrogen-15 Chemical Shifts on Trifluoroacetic Acid Concentration (ppm upfield from external $D^{15}NO_3$) in Dimethyl Sulfoxide[a]

Mol equiv of acid	N1	N3	N7	N9	C6 NH_2
0	139.6	152.7	134.7	205.6	293.8
0.16	147.0	153.0	135.4	205.0	293.4
0.31	155.6	152.9	135.3	204.4	292.2
1.6	211.3	150.8	131.4	197.4	284.9

[a] See Figure 2 for structures and numbering.

the lower field resonance to N1, and this is a reversal of the previous assignments made for adenosine 5′-triphosphate.[4]

The question of where adenosine prefers to accept a proton is a very important problem which has been investigated by x-ray diffraction[15,16] and spectroscopic[17] techniques. Although N1 seems commonly accepted as the most basic nitrogen of adenine and its derivatives, it seemed very worthwhile to investigate the behavior of the nitrogen resonances on protonation, because these should be capable of giving direct insight as to the preferred position of protonation.

The changes in ^{15}N shift on protonation can be dramatic, especially for azine nitrogens. Thus, the nitrogen resonance of pyridine shifts upfield on protonation by about 97 ppm, as judged from the ^{15}N shift of a 3.5 M solution of pyridinium trifluoroacetate in dimethyl sulfoxide. This change in shift is usually ascribed to changes in the paramagnetic contribution to the nitrogen screening constant.[18,19]

On addition of acid to an adenosine solution, the resonance line of one nitrogen, the one we have assigned to N1, moved 71.7 ppm to higher field, whereas all of the other nitrogen resonances only shift slightly downfield (Table II). That this shift is somewhat less than is observed for the pyridine nitrogen may mean that N1 is not the exclusive site of protonation or that the formal positive charge on the N1 nitrogen is distributed partly by resonance to the exocyclic amino nitrogens (N6 and N9). The shifts of both of these nitrogens move downfield on protonation, which is consistent with either partial protonation of these nitrogens or with their involvement in conjugation with a protonated N1 nitrogen.[18] These shift changes are similar to those observed for 2-aminopyridine where the protonation shifts are 105 ppm upfield for N1 and 9.4 ppm downfield for the NH_2. The resonances of N3 and N7 for adenosine shift downfield by only 1.9 and 2.7 ppm, respectively, which is rather small for introduction of a positively charged

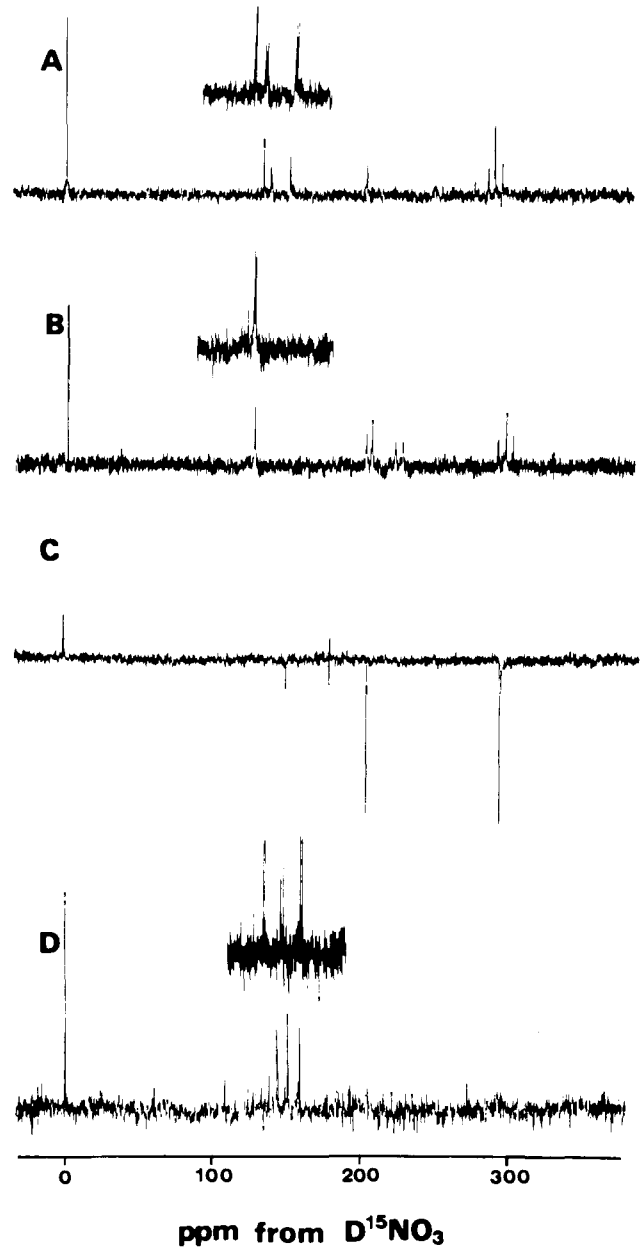

Figure 1. Illustrative nitrogen-15 spectra at the natural-abundance level of some nucleosides and nucleotides. The insets show proton couplings to the nitrogens where applicable. The strong peak on the far left is $D^{15}NO_3$ in an external capillary; A, adenosine in dimethyl sulfoxide, proton coupled; B, guanosine in dimethyl sulfoxide, proton coupled; C, adenosine 5′-monophosphate in H_2O, proton decoupled; D, same as C, proton coupled.

atom in the ring system. As one possible calibration shift, the ring nitrogen of 3-nitropyridine is fully 7 ppm downfield from pyridine.[18] One possible explanation is that N3 and N7 are themselves protonated just sufficiently to give an upfield shift component, more or less cancelling the expected downfield component produced by protonation of N1. Under the conditions we used, there was no evidence for significant amount of diprotonation, as has been observed in trifluoroacetic acid.[20] Arguments in support of protonation at other than N1 have been adduced from the fluorescence spectra[21] of adenine and its derivatives. Our results are decisive in showing that protonation in solution takes place mainly at N1 but do not exclude lesser amounts of protonation at the other nitrogens. Here, as in the following case, the resonance line of the protonated nitrogen was considerably broadened upon protonation, which may be due to relatively slow hydrogen exchange.

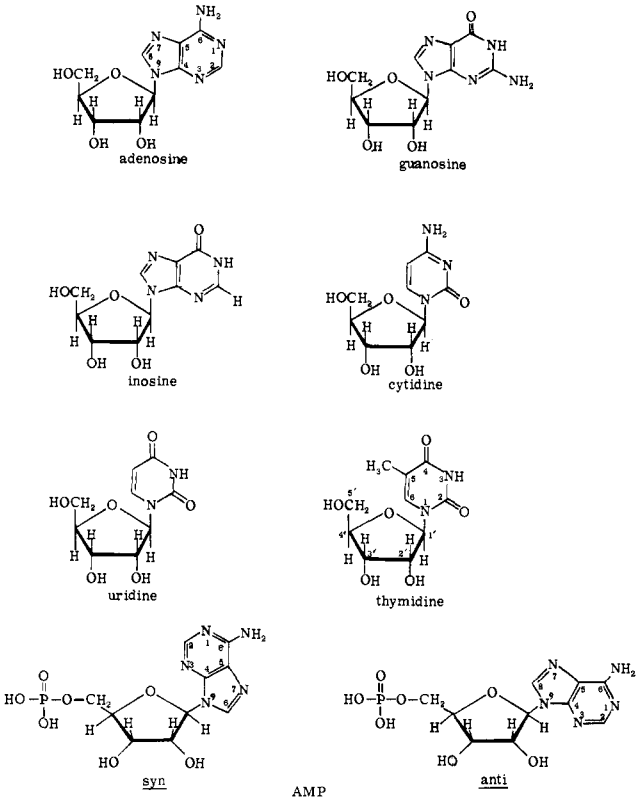

Figure 2. Structures and numbering systems.

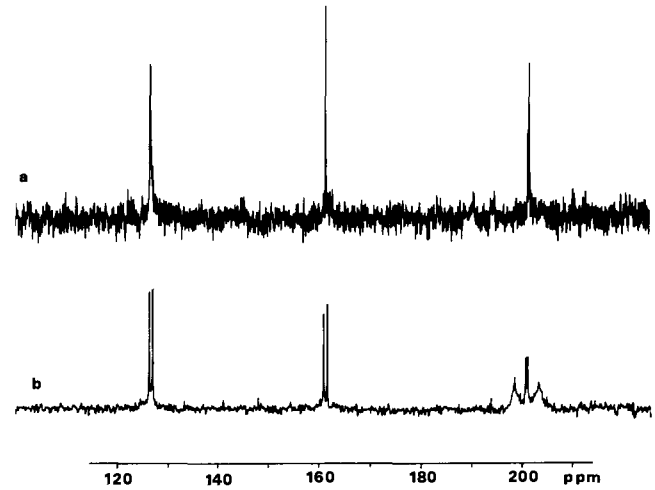

Figure 3. Natural-abundance ^{15}N NMR spectra of inosine in dimethyl sulfoxide: (a) with proton decoupling and no NOE, (b) without proton decoupling.

Table III. Dependence of Guanosine Nitrogen-15 Chemical Shifts in Dimethyl Sulfoxide on Acid Concentration (ppm upfield from external $D^{15}NO_3$)[a]

Mol equiv acid	N1	N3	N7	N9	C2 NH_2
0	228.0	209.5	128.6	205.3	302.0
0.17	227.5	209.3	143.6	203.6	300.5
0.36	227.2	209.9	150.5	203.0	300.0
1.86	226.0	211.0	194.9	199.4	296.8

[a] See Figure 2 for structures and numbering.

For guanosine, assignment of the nitrogen resonances to particular nitrogens can be made very easily (Table I). Only one nitrogen atom will be expected to exhibit no significant coupling to hydrogen and, therefore, the singlet resonance in the coupled ^{15}N NMR spectrum has to be assigned to N3. The C6 carbonyl in guanosine shifts the N3 resonance 56.8 ppm to higher field compared to N3 in adenosine, and this shift is indicative of a smaller degree of aromatic character of the pyrimidine ring system. N1 is easily identified by the doublet splitting associated with the directly bonded hydrogen which incidentally shows that the intermolecular hydrogen exchange is slow in dimethyl sulfoxide. The ^{15}N-H coupling constant of 85.4 Hz is intermediate between the value of a sp^2 and a sp^3 hybridized nitrogen. The resonance of N3 is just at the lower limit of the range of ^{15}N amide shifts.[18] The remaining resonance in the aromatic nitrogen region is attributed to N7 because of its coupling constant of 12.2 Hz. The resonance assigned to N9 shows a small splitting of 3.7 Hz, typical for a pyrrole-like nitrogen.

Both theory[22] and experiment[17] indicate that N7 is the most basic nitrogen atom in guanosine. As the data of Table III show, this conclusion is fully supported by the fact that the resonance of N7 moved upfield by 66.3 ppm on addition of acid. The protonation shift of N7 is smaller than that for N1 of adenosine which may indicate that protonation also occurs to some degree on the other guanosine nitrogens. This is particularly so for N3 whose resonance moved 2.4 ppm to higher field than for the unprotonated species after the addition of 1.86 equiv of acid. Assuming no dication formation under these conditions, the results suggest that, although the predominant site of protonation is N7, there also must be considerable formation of the form with a proton on N3. That the other guanosine nitrogens all move downfield on protonation indicates that these are not significantly protonated.

For inosine, only *three* signals were observed when the spectrum was taken with proton decoupling and with no NOE (Figure 3a). However, without decoupling at a sample temperature of 20 °C, the spectrum shown in Figure 3b was obtained. This spectrum shows that the signal at 200.7 ppm in the proton-decoupled spectrum is actually comprised of two ^{15}N resonances, one with a proton-nitrogen coupling constant of 85 ± 5 Hz and the other with a coupling constant of 8.0 Hz. The signal with the one-bond nitrogen-hydrogen coupling constant of 85 Hz clearly arises from N1. The 85-Hz coupling constant also shows that inosine exists predominantly in the keto form.[23] The signal at 200.7 ppm with an 8.0 Hz coupling constant can be assigned to N9, because its chemical shift is quite comparable to the N9 of adenosine and guanosine. It should be noted that this 8.0-Hz coupling is the largest we have observed for N9 in the purine nucleosides and nucleotides we have investigated so far. The ^{15}N resonance at 161.2 ppm can be attributed to N3 because of its 13.2-Hz coupling to the C2 hydrogen. This coupling is somewhat larger than the corresponding 11.8 Hz observed for N7.

The nitrogen resonances in the pyrimidine nucleosides (Table I) can be very easily assigned on the basis of the observed hydrogen couplings. For both uridine and thymidine, N3 has N-H couplings of 89.1 and 91.5 Hz, respectively. Once assigned, it is interesting to note the different influences of the 5-methyl group on the two nitrogen resonances of thymidine. Although the methyl is a γ substituent for both nitrogen atoms of thymidine, only the resonance of N1 is shifted 2.0 ppm to higher field; that of N3 does not change at all.

The assignments for cytidine are based on the assumption that the azine-like N3 must give a resonance at lower field than that of N1. Proton NMR results with ^{15}N-labeled cytosine derivatives indicate N3 to be the most basic nitrogen.[24,25] This is apparently also true for cytidine, because on adding acid the resonance of N3 shifts 64.8 ppm upfield, which is somewhat less than the upfield shifts observed for protonation of adeno-

Table IV. Nitrogen-15 Chemical Shifts of Nucleotides in Water Solution (ppm upfield from external $D^{15}NO_3$)[a]

Nucleotide	N1	N3	N7	N9	NH_2
Adenosine 5′-monophosphate	152.1	160.4	144.8	207.0	297.0
Adenosine 5′-triphosphate	151.7	160.3	145.0	207.2	297.0
Adenosine 5′-triphosphate (pH 2.5)	214.3	154.4	139.5	200.1	288.8
Guanosine 5′-monophosphate	229.1	211.3	141.2	207.2	303.5
Uridine 5′-monophosphate	230.0	216.5			
Thymidine 5′-monophosphate	229.2	219.5			
Cytidine 5′-monophosphate	223.5	175.2			283.2

[a] See Figure 2 for structures and numbering.

sine and guanosine. On the other hand, the C4 amine nitrogen shifts 20.8 ppm to lower field, which indicates strong delocalization of the unshared pair on the amino nitrogen to N3. The very small change in the resonance position of N1 either indicates that this nitrogen atom plays no significant role in the stabilization of the cation, or else, that there is a fortuitous cancellation of delocalization and protonation shifts.

The ^{15}N resonance assignments for the nucleotides summarized in Table IV were based on those for the nucleosides. Water was the solvent, and because of rapid hydrogen exchange, no one-bond nitrogen–hydrogen coupling constant could be observed. Comparisons between the nucleosides in dimethyl sulfoxide and the nucleotides in water provide information as to the combined effects on the ^{15}N shifts of the phosphate group and the influence of a strong hydrogen-bonding solvent.

The resonances of the azine-like nitrogens of adenosine 5′-monophosphate (AMP) and adenosine 5′-triphosphate (ATP) are between 7.6 and 12.2 ppm at higher field than in adenosine, while the resonance of N9 shows no considerable change. This is consistent with considerable evidence that the nitrogen resonances of substituted pyridines are shifted strongly upfield in water compared to what is observed for nonprotic solvents.[18,26] These shift changes can be attributed to hydrogen-bond formation in water which produces a change in the paramagnetic shielding term analogous to that postulated for the protonated species mentioned earlier. The electron pair of N9 is strongly conjugated with the aromatic ring system and is unlikely to form strong hydrogen bonds with the solvent. This is supported by the small chemical-shift change of N9 between water and dimethyl sulfoxide compared to the 7 to 12 ppm changes observed for N1, N3, and N7.

Comparison of Tables I and IV shows that the change of shift of N3 is the smallest of the three azine-like nitrogens. One possibility is that N3 is already considerably hydrogen bonded in dimethyl sulfoxide with the 2′-hydroxyl hydrogen on the sugar residue. This possibility appears to be ruled out by a comparison of the chemical shifts of 2′-deoxyadenosine and adenosine (Table I). The chemical-shift difference for N3 of these compounds is only 0.6 ppm, indicating that intramolecular hydrogen bonding between N3 and the 2′-hydroxyl of adenosine does not take place to any major extent, at least in dimethyl sulfoxide solution. The largest shift difference between these two compounds was for N9 and probably is best attributed to the γ effect which the 2′-hydroxyl group has on the chemical shift of adenosine (in other words, removal of the 2′-hydroxyl results in a downfield ^{15}N shift of 3.5 ppm).

There is no significant difference in the ^{15}N chemical shifts of AMP and ATP. Because AMP is only slightly soluble in acidic solution, the change of the nitrogen resonances, on addition of acid, was only investigated for ATP. It was found that, as in dimethyl sulfoxide, N1 is the most basic nitrogen atom. The total upfield shift of 62.6 ppm of N1 on protonation is less than observed for adenosine but because of the hydrogen bonding of N1 with water (which produces an upfield shift), the overall shift for N1 is expected to be less in water than in dimethyl sulfoxide. This conclusion agrees with the finding that, when protonated, the shift of N1 is almost the same in both solvents.

For guanosine 5′-monophosphate (GMP), the azine-like N7 resonance moves fully 12.6 ppm upfield on phosphorylation and changing the solvent from dimethyl sulfoxide to water, which strongly indicates increased hydrogen bonding in water. On the other hand, the resonance of the amide-like N1 hardly changes. Similar effects were noted for the C2 amine nitrogen and N9. The most important result for guanosine and GMP is that there is no substantial change (<2 ppm) in the shielding of the resonance of N3 between dimethyl sulfoxide and water. As with adenosine and AMP, this indicates that N3 is already extensively hydrogen bonded in dimethyl sulfoxide. If a hydrogen bond between N3 and the C2′-hydroxyl group is responsible, this bond should be more favorable than for adenosine. As a result, the anti conformer of guanosine will be favored more over the syn conformers than will be the case for adenosine.

The chemical shifts of the pyrimidine nucleotides are almost identical to those of the nucleosides in dimethyl sulfoxide with the exception of N3 in cytidine 5′-monophosphate (CMP). This nitrogen is not well placed to form intramolecular hydrogen bonds to the sugar residue, and indeed, its resonance is shifted 9.1 ppm to higher field on phosphorylation and change to water solution. Because of low solubility, nitrogen signals could not be observed for CMP in acidic solution.

It should be clear that ^{15}N NMR is capable of giving further evidence on the structure conformations and sites of protonation of nucleosides and nucleotides. Of special interest is the rather direct insight which can be obtained on hydrogen bonding through changes in shifts with changes in solvent.

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References and Notes

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Comparisons of ^1H and ^{13}C NMR Chemical Shifts for Low Spin d^6 Complexes of Pyridine and Substituted Pyridines as Probes of π Back-Bonding

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Abstract: Effects of metal-to-ligand π back-bonding in pentaammineruthenium(II) and pentacyanoferrate(II) complexes have been investigated by changes in ^1H and ^{13}C nuclear magnetic resonance chemical shifts. For purposes of comparison, corresponding complexes of other low spin d^6 metal atoms, Co(III) and Rh(III), as well as the protonated ligands, have been studied. ^1H shift changes from free ligand positions upon protonation and complexation reflect current concepts of Lewis acid charge withdrawal (H^+, Co(III), Rh(III)) and π back-bonding (Ru(II) and Fe(II)) when comparisons are made for β and γ protons. The shift changes at the α positions of pyridine and substituted pyridines as ligands are not explained in this manner. Solvent effects on ^1H chemical shifts are of the same order of magnitude as changes caused by complexation (0.2 ppm vs. 0.1–1.0 ppm). ^{13}C shift changes on complexation, however, are an order of magnitude larger than the ^1H changes while solvent effects are similar. The ^{13}C shifts at the β and γ positions are consistent with π back-bonding concepts and are a much more sensitive probe than the ^1H shift changes. On an empirical basis, the γ position ^{13}C shifts of aromatic ligands appear to be a sensitive and reliable probe of π back-bonding.

Development of a means for obtaining quantitative information concerning the nature of metal-to-ligand π bonding is a matter of current interest. ^{19}F NMR results on a series of *trans*-bis(triethylphosphine)fluorophenyl complexes of nickel(II), palladium(II) and platinum(II) have demonstrated the ^{19}F nucleus, especially when in the para position, is a reasonable probe of electron density changes.[1] ^1H NMR chemical shift changes have been studied in a series of related ruthenium(II) complexes[2] and ^1H and ^{19}F changes have been compared for a series of ruthenium(II) and rhodium(III) complexes.[3] ^1H and ^{13}C NMR results have been obtained for several pentacyanoferrate(II) complexes.[4] Coulson has recently reported the results of linear least-squares multiple regression analysis of ^{13}C and ^{19}F NMR data for a series of aryl platinum complexes with a range of substituents on the phenyl ligand.[5] These studies demonstrate that chemical shift changes, especially for ^{19}F and ^{13}C nuclei, are a promising means of directly observing π back-bonding effects which have been previously deduced by other means.[6] The nature of the mechanism by which electron density alterations cause chemical shift changes in transition metal complexes is not yet clear.[7,8] Further empirical evidence is required that would compare complexes of similar structure which include metal centers that have been shown to be non-π-back-bonding as well as those which are known to participate in π back-bonding.

In the case of the ^{19}F study by Parshall,[1] a closely related series of diamagnetic d^8 complexes allows a comparison of the Ni(II), Pd(II), and Pt(II) centers but, of necessity, included no non-π-back-bonding metal centers. Coulson's study[5] included a large number of substituents but was restricted to platinum complexes. The other studies cited compared the effects of various ligands, but each was quite limited in the number and range of examples reported. In this study we report the ^1H and ^{13}C NMR spectra of diamagnetic, substitution inert d^6 complexes of Co(III), Rh(III), Fe(II), and Ru(II) with pyridine, γ-picoline and 4-benzoylpyridine. γ-Picoline and 4-benzoylpyridine were chosen to test the effect of ligands with Hammett σ constants of opposite sign (−0.17 for para methyl and +0.46 for para benzoyl) on apparent degree of back-bonding. One might expect that the more electron withdrawing substituent, para benzoyl, could cause a greater degree of back-bonding than the electron donating para methyl substituent. Spectra of the protonated ligands and of the ligands in various solvents are included as an aid in interpretation of the spectra of the complexes.

Experimental Section

Reagents. CD_3OD, CH_3OD, and D_2O (Stohler Chemical Co.), reagent grade CCl_4 (Fisher), and 4-benzoylpyridine (Aldrich) were used without further purification. Pyridine (Fisher) and γ-picoline (City Chemical Co.) were distilled and stored over molecular sieves. Methanol (Fisher) was distilled over sodium and stored over molecular sieves. Argon was bubbled through chromous ion scrubbing towers and passed through a calcium sulfate drying tower. Amalgamated zinc was prepared by adding mercuric chloride (5% by weight) to acid-washed 20-mesh zinc. Sodium trifluoromethanesulfonate was prepared by neutralization of sodium carbonate with trifluoromethanesulfonic acid (3M Co.) that was doubly distilled from all glass apparatus.

Cobalt Complexes. Aquopentaamminecobalt(III) perchlorate was used as the starting material. It was synthesized by the method of Gould and Taube[9] and gave the appropriate visible absorption spectrum (ϵ at 474 nm = 64, literature value 66). The aquopentaammine complex was converted to the dimethylformamide–pentaammine complex by addition to dimethylformamide at 95 °C.[10] Preparation of each complex was then accomplished by addition of a fivefold excess of the ligand and heating it 95 °C for 20–30 min.[10,11] Isolation was by crystallization from saturated aqueous $NaClO_4$. Extinction coefficients were within 5% of literature values.[10,11] Pyridine-*2-d*-pentaamminecobalt(III) iodide was prepared as outlined above except that the product was obtained by precipitation of the iodide salt by addition of saturated methanolic NaI. The precipitate was filtered,