for the conformational preference of piperidine with NH equatorial are unknown, and various explanations advanced[2,33,37] in favor of either conformer remain, as yet, unproven speculation.

Carbon-13 Nuclear Magnetic Resonance and the Conformations of Biological Molecules[1]

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Carbon-13 is not an abundant isotope; it represents only 1.11% of the carbon in natural abundance. Its magnetic moment is smaller than that of ^1H; in a magnetic field of 23 kG it resonates at 25 MHz, compared to 100 MHz for ^1H. For equal numbers of nuclei, its intrinsic sensitivity is only 1.59% of that of ^1H. Thus, at natural abundance it is roughly 10^4 times harder to detect than ^1H. Nevertheless, with the development of Fourier transform techniques[2] and large sample tubes it has become possible to detect ^{13}C routinely in compounds of carbon in natural abundance at concentrations as low as 10^{-2} M.

Due to the larger number of surrounding electrons, the chemical shift range of ^{13}C is roughly 200 ppm, compared to 10 ppm for protons.[3-9] Couplings to ^1H make ^{13}C spectra very complex, but they can be removed by broad-band irradiation at the ^1H resonance frequency (noise decoupling). The decoupling operation gives an increase in signal-to-noise ratio by collapse of multiplets, as well as by the ^1H–^{13}C cross-relaxation mechanism known as the nuclear Overhauser effect.[10] Carbon-13 resonances are comparable in width to those of ^1H in the same compound—they appear narrower due to the wider chemical shift range and due to the simplification gained by decoupling all protons. As molecular weights increase, the rate of molecular reorientation decreases and the ^{13}C resonances become broader. If a high degree of local motion is present, the broadening will not be as great

as expected from consideration of the molecular size alone; this has been found to occur for a wide variety of polymers.

Carbon-13 nuclear magnetic resonance has become almost an everyday tool in the hands of the practicing chemist. Large amounts of data on an enormous variety of compounds are available,[3-9] and can be effectively used as a means of identification or location of substituents.

Reliable information on conformation has been somewhat slower in coming, but chemical shifts are now used routinely to determine anomers, epimers, and other geometric isomers. Rotational isomers about single bonds are presently being investigated by means of ^{13}C spin–spin coupling to ^1H,[11] ^{13}C,[12] and ^{31}P.[13,14] Such information is also apparently present in the ^{13}C chemical shifts of carbohy-

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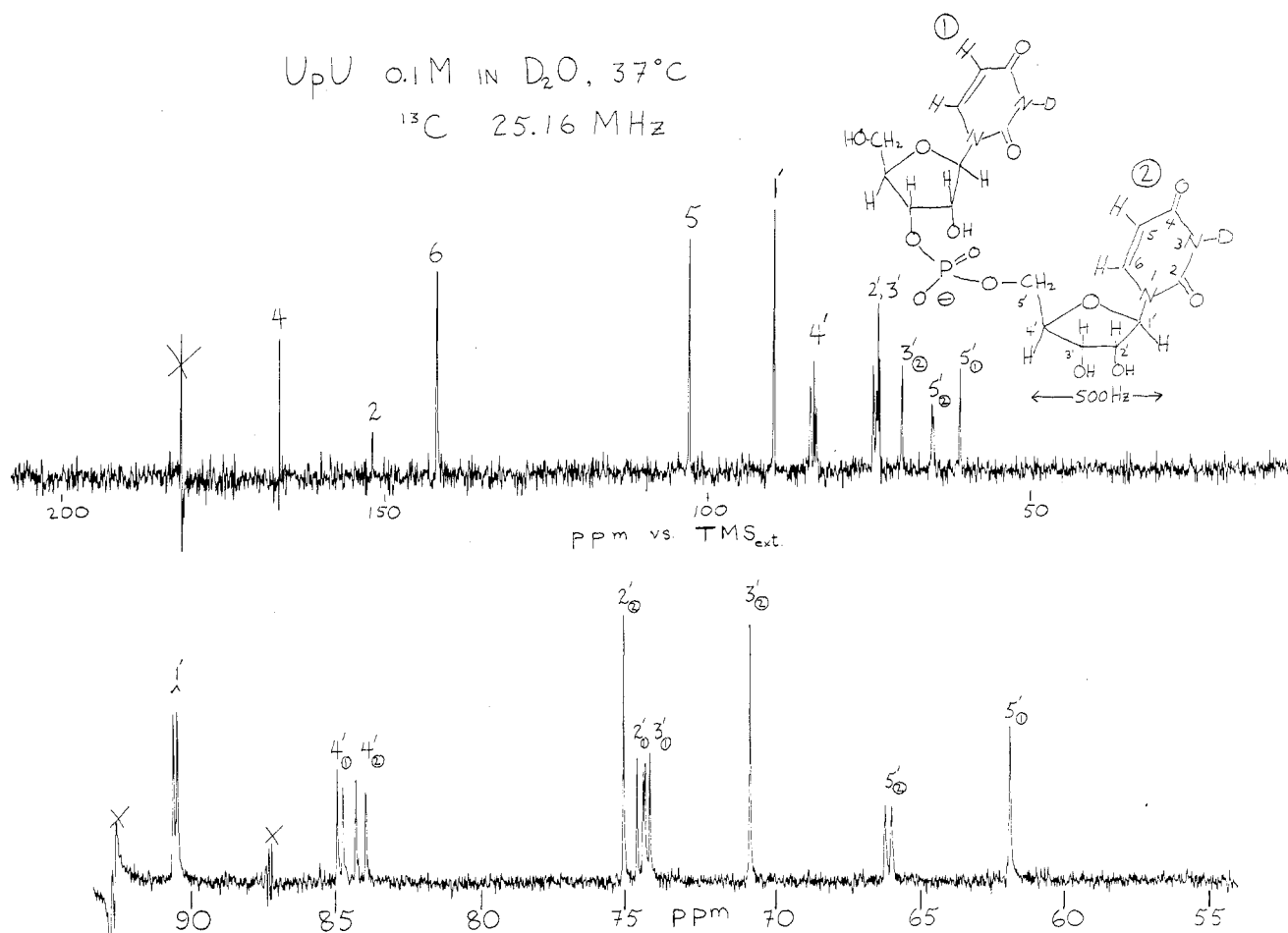
Figure 1. Carbon-13 NMR spectrum of uridylyl-(3′–5′)-uridine, 0.14 M in D_2O, pD 7.2, 37°, Varian XL-100-15, spectral width 5 kHz with 8000 accumulations (upper) and 1 kHz with 34,000 accumulations (lower).[18a] The chemical shifts are expressed with respect to $(CH_3)_4Si$ contained in a tube of 5 mm o.d. concentric with the 12-mm sample tube. Peaks marked X are due to spurious spectrometer frequency (upper spectrum) or resonances "folded" from the high-frequency end of the spectrum. Resonances due to individual nucleoside units are indicated by ① and ②.

drates.[15,16] Spin–lattice relaxation times are currently showing promise for detailed investigation of the relative mobilities of groups in complex molecules.[3b,4b,5,8] Thus, during the last few years it has become feasible to apply the techniques of ^{13}C NMR to more complex molecules of biological interest.[4b,6–8] This has been our main concern, and we shall outline some of our techniques and successes in three principal areas: nucleic acids, carbohydrates, and peptide hormones.

Nucleic Acids

Assignment of Spectra. The ^{13}C NMR spectrum of uridylyl-(3′–5′)-uridine is shown in Figure 1. Most of the resonances can be readily assigned on the basis of the spectra of the isolated base and ribose moieties and consideration of substituent effects.[17] For C-2′ and C-3′ of the monomers, which have very similar substituents and environments, assignment was difficult.[17a,b] It was finally established by consideration of the influence of a phosphate group at either C-2′ or C-3′ [14] and by single-frequency proton decoupling.[17c] The carbon atom to which the phosphate is linked

shows an increased chemical shift (decreased shielding) of 2.5 ± 0.2 ppm and a spin–spin coupling to ^{31}P of 5.0 ± 0.5 Hz. In addition, the neighboring carbon atoms experience no appreciable change in chemical shift due to phosphorylation, but couple to ^{31}P by 2–9 Hz. These effects can also be used to assign unequivocally the resonances of both ribofuranose moieties of the dinucleoside monophosphate with the exception of the C-1′, which are too far removed from the phosphorus atom to be spin-coupled to it. The distinction between the two C-4′ is made on the basis that a large coupling through three bonds is usually observed from a phosphate attached at C-5′.[14]

[13]C–[31]P Spin–Spin Coupling. A noteworthy characteristic of the [13]C–[31]P couplings through three bonds in a wide variety of nucleotides is that they vary over a wide range, 2–10 Hz.[14,18] This is suggestive of the well-known variation in couplings between ^{1}H and ^{1}H,[19a] and ^{1}H and [31]P[19b,c] that have been used successfully to determine dihedral angles. Na-

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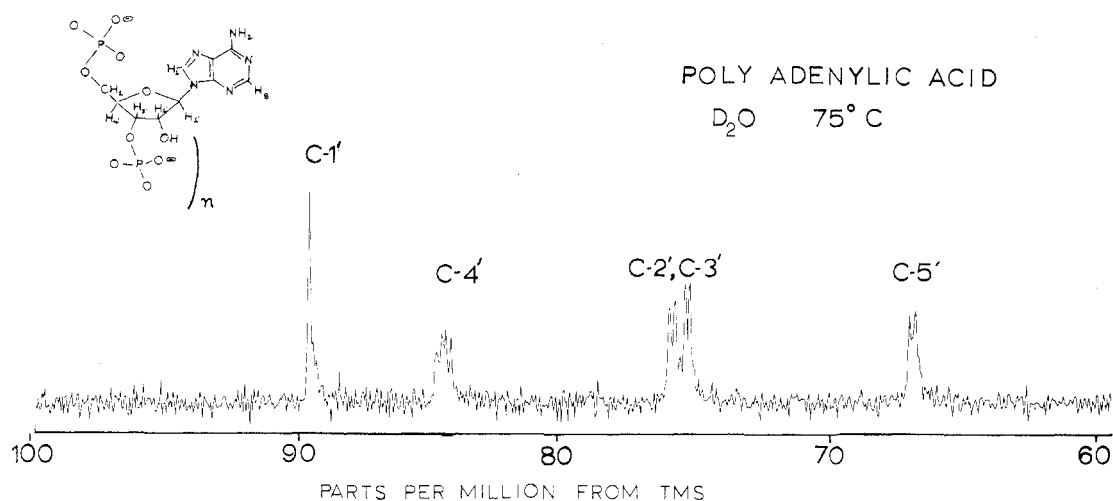
Figure 2. The ribofuranose carbon region of the ^{13}C NMR spectrum of polyadenylic acid, 40 mg/ml in D_2O, pD 7, 0.01 M phosphate, 75°, 49,000 accumulations. The resonance of C-4′ is a quartet due to interaction with phosphate groups at C-3′ and C-5′.[24b]

ture has provided an excellent model compound in cAMP (adenosine 3′,5′-cyclic phosphate). The analogs of thymine, uracil, cytosine, and guanine are also readily available. X-Ray crystallographic studies have indicated that these compounds adopt a conformation in which the phosphorus atom has a trans orientation relative to C-2′ and a gauche orientation relative to C-4′.[20] Thus, if this conformation were maintained in aqueous solution, one might expect a large $^{31}P-^{13}C$ coupling to C-2′ and a considerably smaller one to C-4′ (two pathways are possible).
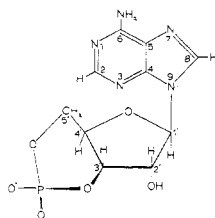
By means of ^1H NMR[21] the conformations of the 3′,5′-cyclic nucleotides have been established as consistent with the X-ray data. The trans coupling between the ^{31}P and ^{13}C-2′ was 8.0 ± 0.3 Hz for a series of six such compounds.[13c,18] The couplings to C-4′ were 4.5 ± 0.5 Hz, leading to an estimate for the coupling through a single pathway in a gauche arrangement of 2.3 Hz. Such couplings could then be used, with appropriate caution as they were determined from a limited number of model compounds, to estimate the preferences for rotational isomers in the nonrigid nucleotides.

In 5′-nucleotides the large couplings to C-4′ are indicative of a C-4′-trans location of the phosphorus atom, whereas in 3′-nucleotides the couplings to C-2′ and C-4′ are indicative of a more complex blend of conformers, with a slight preference for C-4′-trans.[14,18] The latter are expected to vary more from one nucleotide to another since the nature and extent of pucker of the ribose ring depend upon whether the base is a purine or a pyrimidine.[18,22,23]

Conformational Transitions in Polynucleotides. The greater dispersion of ^{13}C chemical shifts and the removal of couplings to ^1H by broad-band irradiation give the possibility for detailed studies on polynucleotides of high molecular weight which were inaccessible or difficult by ^1H NMR. In particular, one could hope to use the $^{13}C-^{31}P$ couplings for analysis of backbone conformations.

Figure 2 shows the ribofuranose resonances of polyadenylic acid under conditions where the polymer is highly disordered and has a high degree of mobility for the constituent nucleotides. All resonances but that of C-1′ are split due to coupling with ^{31}P; that of C-4′ is coupled to one phosphate via C-5′ by 8.5 Hz, and to another phosphate via C-3′ by 4.6 Hz. Similar spectra are obtained under corresponding conditions for poly(cytidylic acid) and poly(uridylic acid).[14,18,24] The couplings for the "disordered" forms of both polyadenylic acid and polyuridylic acid indicate that in fact a strong conformational preference is manifest. In particular, the large couplings to C-4′ from the phosphate attached to C-5′ show that the C-4′-trans rotamer with respect to rotation about the O–C-5′ bond is completely preferred; this leads to an extended structure avoiding contact between monomer units. Thus, although the populations of rotamers about some bonds may be equalized in this disordered state, there seems to be little diversity in rotamers about O–C-5′ and O–C-3′. This is in line with current concepts of nucleic acid structure based on analysis of a large volume of crystallographic data for nucleotides[25] and theoretical calculations,[26] which indicate that the principal source of flexibility in nucleotides is rotation about O–P bonds.

Detailed studies of the order–disorder transitions of poly(uridylic acid)[14,18,24a] and poly(adenylic acid)[24b] have been made by ^{13}C NMR. In both cases the resonances of the ordered forms were too broad to yield $^{13}C-^{31}P$ couplings, but plots of chemical shift

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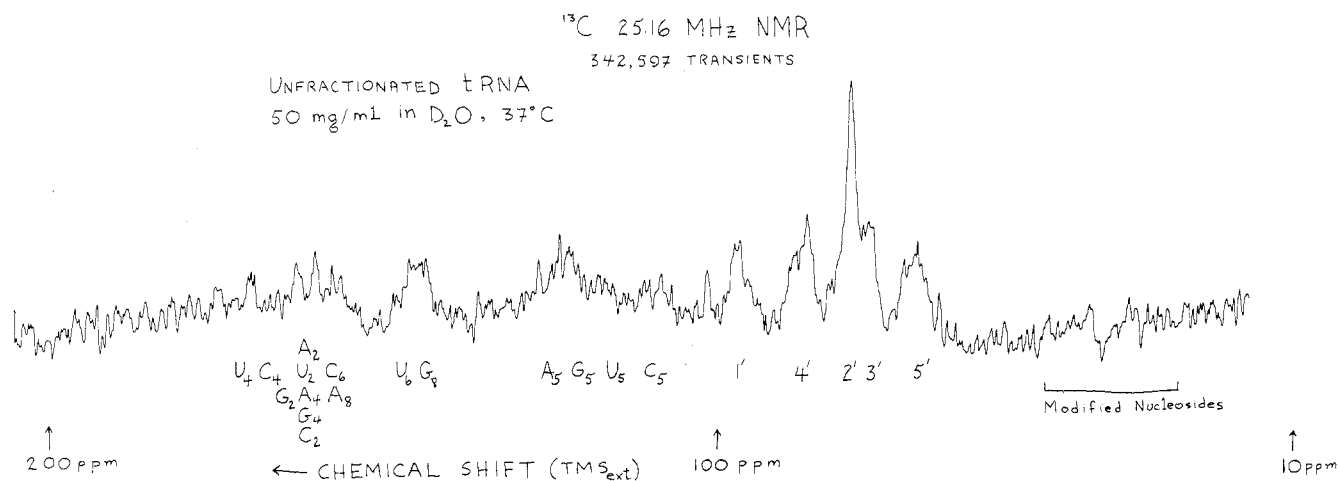
Figure 3. The carbon-13 NMR spectrum of a mixture of transfer RNA species from yeast, 50 mg/ml in D_2O, pD 7.2 37°, 340,000 transients.[18a]

vs. temperature gave clear indications of the transition to a less ordered state.

The ^{13}C NMR spectra of the poly(adenylic acid)–poly(uridylic acid) complex were studied[24b] as a model for double-stranded RNA. Under conditions where an excess of one component is present, relatively narrow ^{13}C resonances for that component can be observed. However, under conditions of stoichiometric combination of the two to form a double-stranded complex, the ^{13}C resonances are too broad to be observed. Thus, in the uncomplexed homopolynucleotides a high degree of local mobility is maintained. The resultant rapid modulation of ^{13}C–^1H dipole–dipole interactions leads to relatively narrow resonances. In the complementary duplex, and in highly ordered natural polynucleotides (vide infra), this mobility is lost and the motion of the constituent monomers becomes more like that of the complex itself, resulting in very broad resonances.

Polynucleotides of Biological Origin. The naturally occurring polynucleotides present a difficult problem for ^{13}C NMR. This is because one expects a wide variety of sequences of only four principal nucleotides and a rather high degree of organization leading to low mobility. Thus, the resonances of one nucleotide will lie over a range of several parts per million due to sequence-specific effects, and they will be of widths comparable to the differences in chemical shift. This leads to broad envelopes of resonances, Figure 3, from which it is difficult to extract conformational information.

The transfer RNA species contain approximately 80 nucleotides and have molecular weights in the range of 25,000. They are thought to contain highly ordered as well as less-ordered regions in their overall structures. Figure 3 is a spectrum from a mixture of such species, but an equally uninformative spectrum was obtained with 50 mg of the purified transfer RNA for phenylalanine in D_2O.[27a] Even in a mixture of dimethyl sulfoxide and water, in which denaturation of a considerable amount of secondary structure is expected, the ^{13}C NMR spectrum of the purified RNA was no more promising. The limitation of only

four major components is overwhelming. Recently it has been shown that the so-called minor nucleosides of transfer RNA (dihydrouridine, ribothymidine, pseudouridine) have resonances sufficiently different from those of the common nucleosides that they can be distinguished in ^{13}C NMR spectra of unfractionated transfer RNA,[27b] as was found in the ^1H NMR spectra.[27c]

Only one report of a ^{13}C NMR spectrum of DNA has appeared so far,[18a] and this was obtained on a highly denatured form. With native DNA the conformational rigidity and slow molecular motion lead to ^{13}C resonances too broad for detection.[24b] However, by careful sonication to lower molecular weight and separation by membrane filtration, ^{13}C spectra can be obtained.[24b] They are of a quality comparable to that of Figure 3, and therefore no information on the backbone conformation is easily extracted.

Hydrogen Bonding of Nucleosides. It is well known that the ^{13}C chemical shifts of carbonyl carbons are sensitive to the formation of hydrogen bonds.[3a] The situation with biological compounds is more difficult than those previously studied because one wishes to compare the chemical shifts of a component in aqueous solution, where it can form hydrogen bonds to water, with those of that component in a more complex molecule in aqueous solution, where it can form hydrogen bonds to other components as well as to water. Thus far no definitive evidence for an intramolecular hydrogen bond in a biological compound in aqueous solution has been obtained by ^{13}C NMR. To test for the extreme value to be expected, we studied the system cytidine–guanosine in dimethyl sulfoxide.[28] The changes in chemical shift of the carbonyl carbons of each residue caused by formation of the hydrogen-bonded dimer were small (cytidine +0.66 ppm; guanosine +0.27 ppm). As these are reasonable estimates of the limits that one could expect in aqueous solution, ^{13}C NMR will not be an accurate method for studies of hydrogen bonds in large complex molecules in water.

Base Stacking and Ring Currents. It has been known for some time that in the presence of an exter-

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nal magnetic field the mobile π electrons of conjugated systems circulate anisotropically so as to create an opposing internal magnetic field.[29a] Calculations of these ring-current effects have been made for the bases occurring in nucleic acids.[29b] The influence of such fields on a neighboring nucleus can be treated simply as a reduction or enhancement of the external field, and will be the same in parts per million for any magnetic nucleus. These stacking shifts have been well documented in ^{1}H NMR studies of the association of mononucleotides in solution.[30] Parallel studies have now been reported by ^{13}C NMR of mono- and dinucleotides.[18] Two immediate problems become apparent from the ^{13}C data: the changes in ^{13}C chemical shift are much larger than predicted from the corresponding ^{1}H NMR values, and they are up to twice as large as the maximum predicted theoretically for a nucleus positioned 3.4 Å away.[29b] Thus, structural models cannot be constructed from the ^{13}C data until a satisfactory explanation for the discrepancy arises. One possible source is alteration of molecular wave functions and energies due to close approach of the conjugated π systems on complex formation, leading to alterations in ^{13}C chemical shift. It is hoped that calculations of these effects can soon be made.

Carbohydrates

Assignment and Conformational Sensitivity. Carbohydrates provide excellent examples of the power of the ^{13}C NMR technique. The chemical shifts are so sensitive to substitution, configuration, and conformation that assignment of resonances is often quite difficult.[31] An unequivocal way to resolve doubtful assignments is to obtain the ^{13}C spectrum of a specifically deuterated compound.[32] This removes the nuclear Overhauser enhancement from the resonance of the deuterated carbon and results in an approximately ninefold diminution in the intensity of the resonance of the deuterated carbon.

As well as the obvious conformational sensitivity, some very subtle effects can be distinguished on comparing the chemical shifts of C-1 in four compounds of glucose linked α,1→4: amylose (linear polymer, 100.9 ppm), maltotriose (linear trisaccharide, central residue, 101.0 ppm), cyclohexaamylose (cyclic hexamer, 102.5 ppm), cycloheptaamylose (cyclic heptamer, 102.9 ppm).[16] The difference of up to 2.0 ppm is clearly due to restriction of the allowed rotamers about O–C bonds in the cyclic compounds. As a tool for conformational analysis this will only become useful, however, when data for compounds of well-defined conformation become available.

Determination of the Composition and Sequence of Complex Glucans. The ^{13}C NMR spectra of many complex carbohydrates of high molecular weight often have remarkably high resolution. Figure 4 shows the spectrum of an extracellular glucan from the organism *Tremella mesenterica*.[15,16] Chemical
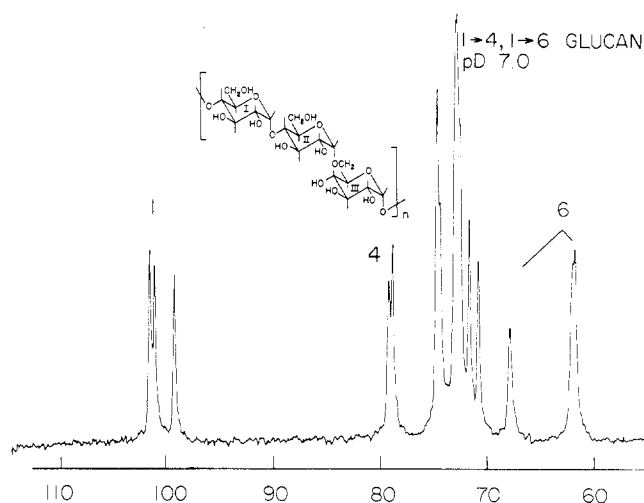
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Figure 4. Carbon-13 NMR spectrum of a glucan from *Tremella mesenterica* in D$_2$O, pD 7, 100 mg/ml, 37°, 48,000 acquisitions.[15,16] The repeating sequence of the polymer, as determined from the ^{13}C data, is shown above the spectrum.

procedures had established that the polymer contained glucose linked α,1→4 and α,1→6 in a ratio of approximately 2:1. By comparison with model di- and trisaccharides, it was possible to assign all resonances in the spectrum. The doublet centered at 101 ppm is due to C-1 in an α,1→4 link, whereas the resonance at 99 ppm is due to C-1 in an α,1→6 link. The relative areas under these two groups are 2:1, confirming the original composition. Further confirmation comes from consideration of the relative areas due to a linked (68 ppm) or unlinked C-6 (62 ppm). The doublet structure of the α,1→4 resonance at 101 ppm is attributable to the sensitivity of the chemical shift to whether that glucose moiety is linked to the previous one by an α,1→4 or an α,1→6 link. A similar argument applies to the doublet at 79 ppm (linked C-4 sensitive to whether linkage at C-1 is α,1→4 or α,1→6), and at 62 ppm (better resolved at pD 14, unlinked C-6 sensitive to nature of linkage at C-1 of the same residue). From these doublets the sequence of the glucan is concluded to be . . . α,1→4; α,1→4; α,1→6 . . . .

Composition and Conformation of Polysaccharide Antigens from Pathogenic Organisms. A principal use of ^{13}C NMR in our laboratory is as a rapid nondestructive technique for the identification of polysaccharide antigens.[33,34] It is particularly advantageous in this case because the polysaccharides are relatively unstable under chemical treatment, and the ^{1}H NMR spectra are hopelessly complex. The structures of these immunogens are intimately related to their effectiveness as vaccines.

Neisseria meningiditis elaborates a polysaccharide based on *N*-acetylmannosamine α-1-phosphate. The ^{13}C NMR spectrum of the antigen is complex (Figure 5).[33] Chemical evidence indicated the presence of *O*-acetyl substituents at a level of less than 1 mol/mol of hexapyranose. Obvious are the *O*-acetyl methyl resonance at 21.6 ppm, the *N*-acetyl methyl resonances at 23.3 ppm, and the C-1 resonance at 96 ppm (with an approximate 5-Hz coupling to phosphorus).

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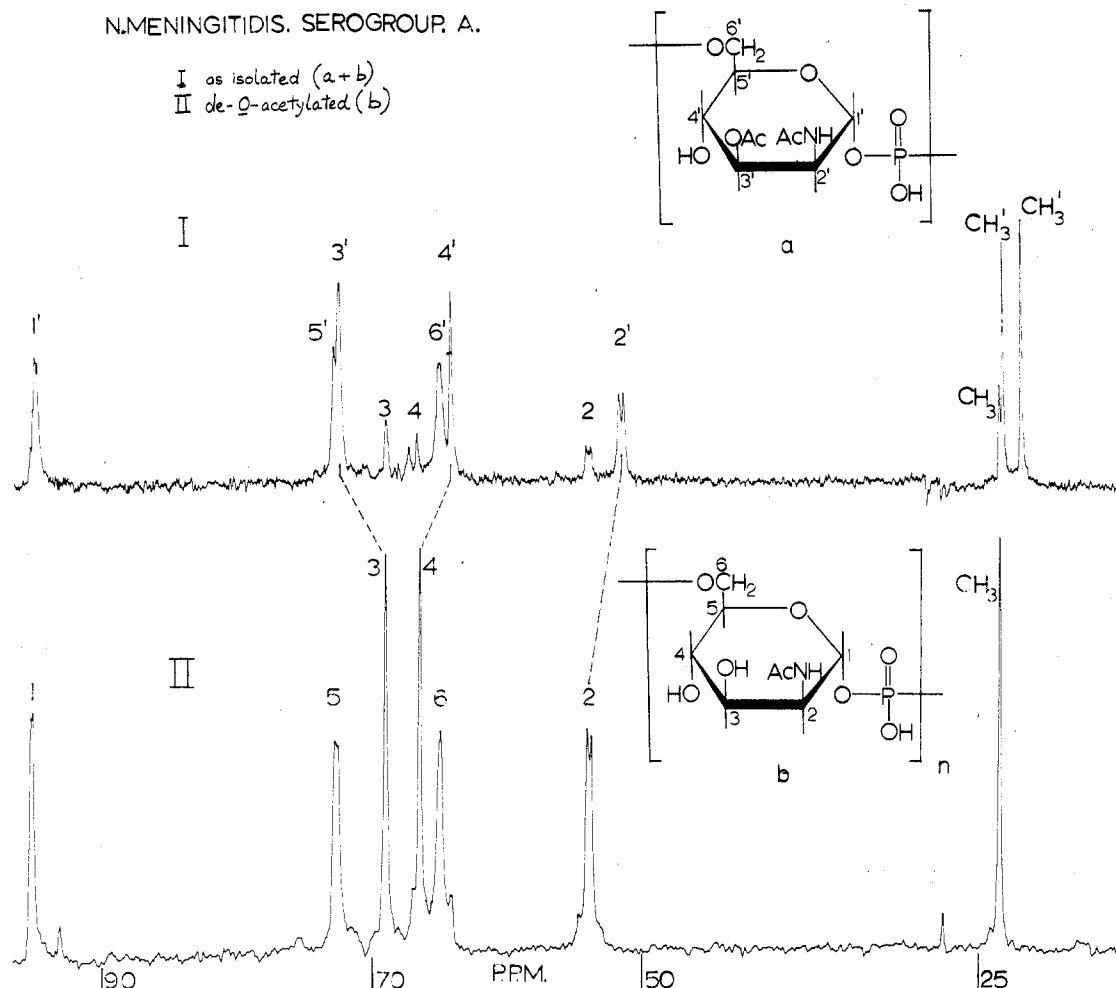
Figure 5. Carbon-13 NMR spectrum of a polysaccharide antigen from *Neisseria meningiditis* (upper) and its de-O-acetylated derivative (lower), both 100 mg/ml in D_2O, pD 7, 32°, 70,000 accumulations. The upper spectrum is due to a mixture of species a and b, whereas the lower spectrum is due to b alone.[33]

The group of peaks at 51.9 and 54.3 ppm with apparent spin–spin couplings to ^{31}P are expected for the acetamido-bearing carbon, C-2. A suggestion that the O-acetyl groups might be located on C-3 comes from the splitting of the N-acetyl methyl resonance, and the two apparent groups of resonances for C-2.

The remainder of the spectrum becomes amenable to analysis only after considering that of the de-O-acetylated polymer, the lower spectrum of Figure 5. Its simple form suggests only a single repeating unit. The chemical shift and 5-Hz coupling to ^{31}P of the C-1 resonance confirm an α linkage to phosphorus at this position. The other attachment point for the phosphate is determined by noticing that neither C-3 nor C-4 is coupled to ^{31}P, whereas C-5 and C-6 are,[35] and the chemical shift of C-6 is ca. 2.5 ppm greater than that of an unphosphorylated N-acetylmannosamine residue. Thus, the other attachment point is C-6.

Returning to the upper spectrum of Figure 5 we must now determine which of the possible sites is (are) O-acetylated. A study of O-acetylated monomers[32] indicated that O-acetylation results in an *increase* in the chemical shift of the O-acetylated car-

bon by ca. 3 ppm, and a *decrease* in the chemical shift of the neighboring carbons by ca. 2 ppm. In the upper spectrum we see that one of the C-2 groups has a chemical shift 2 ppm less than that of C-2 in the unacetylated polymer. Similarly a resonance of chemical shift slightly lower than that of C-6 is apparently due to C-4 displaced by a neighboring O-acetyl group.

Finally, in the native polymer there is a resonance of chemical shift 3 ppm greater than that of C-3 in the unacetylated polymer, and this must be due to an O-acetylated C-3. No unexplained resonances are present in the spectrum of the native polymer, so that the only species present are the unacetylated polymer (unprimed resonances) and the 3-O-acetylated polymer (primed resonances). Comparison of the relative areas under the various resonances yields a composition of 18 mol % of the former and 72 mol % of the latter. The ^{13}P–^{13}C coupling to C-2 is 8.6 Hz, indicative of an extended conformation with respect to rotation about the O–C-1 bond.

Peptide Hormones

Assignment of Resonances. The large number of possible constituent amino acids and sequences of attachment suggests that ^{13}C NMR should be of particular value in studies of peptides and proteins. It is in fact possible to resolve a large number of resonances

(35) Note that in the lower spectrum the coupling to C-5 is barely resolved and that to C-6 is unresolved. The couplings are manifest in this case by the greater widths and decreased vertical intensities of these resonances relative to those of C-3 and C-4.
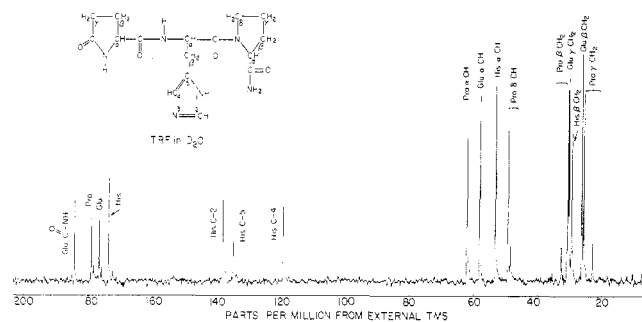
Figure 6. Carbon-13 NMR spectrum of thyrotropin-releasing factor in D_2O, 80 mg/ml, pD 8.9, 37°, 7,379 accumulations.[36a]

Figure 7. NT_1 values (msec) for the carbons of oxytocin, 100 mg/ml in D_2O, pD 3.9, 32°.[40e]

from such compounds, but assignments are as tricky as with other compounds. A general approach to assignments is to use chemical shifts calculated from monomer and oligopeptide data, assuming a random conformation. Deviations from these values are usually indicative of specific conformations. A study of smaller constituent peptides, or of hormones with one amino acid substituted, are valuable in ambiguous cases.[36] In some cases substitution by ^2H[37] or ^{13}C[38] must be done for complete certainty. A comprehensive review of applications of ^{13}C NMR in this field will appear shortly.[39]

The Conformations of Proline in Peptides. Although a considerable number of conformational inferences have been made from the ^{13}C chemical shifts of peptides, the data for proline (amide terminal residue in Figure 6) in peptides stand out as the clearest and most immediately useful.[39] In considering the ^{13}C spectrum of oxytocin (see Figure 7), its substituted analogs, and its constituent peptides, it became evident from the chemical shifts that the populations of the cis and trans conformers (with respect to rotation about the X–Pro amide bond) of the proline residue were very dependent on the nature of the N-acyl residue.[28,36,40] The results of a large number of studies[39] suggest that the resonance of the γ carbon is the most reliable indicator of the conformation about the X–Pro bond.[41]

Spin–lattice relaxation times provide a new approach to the study of conformational dynamics.[4b,5,8] Generally speaking, for protonated carbons in small molecules tumbling rapidly, the longer is NT_1, where N is the number of directly attached hydrogens, the more mobile is the carbon atom.[5,8] The NT_1 values for proline in peptides demonstrate this sensitivity to motion. That of C-γ has been found to be as much as three times the value for C-α. This was taken to indicate rapid intracyclic motion of proline at a rate as high as 10^{11} sec^{-1}. Interestingly, in some compounds both C-γ and C-β show this pronounced mobility,

demonstrating that the mode and rate of ring puckering depend on the nature of the neighboring aminoacyl residues.[36,40b,e,42]

Thyrotropin-Releasing Factor (TRF). The sensitivity of ^{13}C chemical shifts and spin–lattice relaxation times to the state of the proline residue in a peptide hormone is well exemplified by the data for TRF,[36a,43] whose formula and spectrum are shown in Figure 6. Notice first that the proline β, γ, and δ resonances have satellites of relative intensity approximately 0.2. These are due to the cis conformer. Changing the solvent to pyridine reduces the population of this conformer to a negligible value, whereas raising the temperature of an aqueous solution increases it. This represents the first observation of the cis conformer in a biologically active hormone. The variation with pH of the ^{13}C chemical shifts of the histidine residue indicates that the major tautomer of the imidazole residue is that with the proton on N-3, suggesting a possible explanation for the hyperactivity and hypoactivity of the 3- and 1-methylhistidine derivatives of TRF, respectively.[43b]

The T_1 values of corresponding carbons in TRF are very similar at pH 9.9 and 4.7.[43b] This obviates any model for the hormone involving a hydrogen bond to the imidazole residue ($pK = 6.2$). The NT_1 values of the imidazole carbons are longer than that of the α-carbon, implying independent rapid motion of the side chain. Rapid intracyclic motion in both the pyroglutamate and prolyl rings is indicated by the nonequivalence of the NT_1 values (for Pro at pH 4.7: α, 0.38; β, 0.81; γ, 0.79; δ, 0.46 sec).

Oxytocin and Vasopressin. These neurohypophyseal hormones have been extensively studied by ^{13}C chemical shifts and spin–lattice relaxation times (Figure 7). Chemical shift assignments were based on consideration of those of peptide fragments[40a] and deuterium[37] and amino acid substituted[40d] analogs and on variations of chemical shifts with pH,[40c] and confirmed in some cases by ^{13}C substitution.[38] The prolyl chemical shifts indicated that this residue has an absolute preference for the trans isomer with respect to rotation about the Cys–Pro bond. Most

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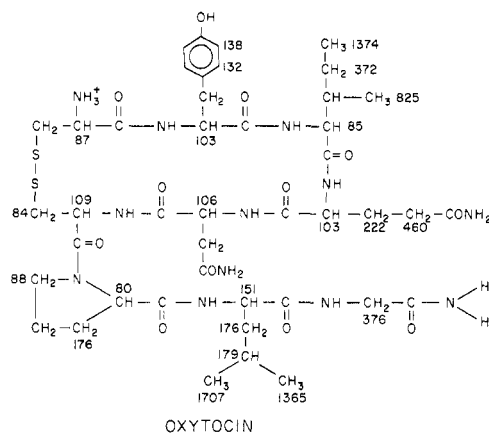(41) D. Dorman and F. A. Bovey, *J. Org. Chem.*, **38**, 2379 (1973).

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chemical shifts were in agreement with those predicted from the data for amino acids and peptides, and therefore apparently contained very little information of conformational origin. However, monitoring the response of the ^{13}C chemical shifts to titration of the α-amino group of cystine provided evidence for a conformational transition at the isoleucyl residue due to alteration of the disulfide dihedral angle.[40c]

The NT_1 values of Figure 7 provide a dynamical map of almost every carbon in the molecule. The near-equality of those of the backbone α carbons in the cyclic portion demonstrate that no rapid torsional oscillations occur; the rapid increase in those of the α carbons of the terminal tripeptide with increasing distance from the point of attachment to the ring are indicative of rapid segmental motion. These data eliminate any model for the overall conformation which has the terminal glycinamide residue bound rigidly to a residue in the cyclic portion, in which case the segmental motion would be severely restricted. The NT_1 value of the prolyl β carbon is twice as large as that of either the α or δ carbons due to rapid internal motion in the prolyl ring. The mobilities of the methyl groups of isoleucine and valine depend upon the particular environment of each group. Finally, some evidence for rotation of the aromatic moiety of tyrosine about either of the aryl–C-β or the C-α-C-β bonds is found in the larger NT_1 values for the aromatic carbons relative to that of C-α.

Similar conclusions have been drawn for lysinevasopressin[40e] and for oxytocin in $(Me)_2SO$.[40b] More recently a detailed analysis of the corresponding data for angiotensin II has been made in terms of models with isotropic or anisotropic overall motion of the oligopeptide with and without internal segmental motion.[42a] In this case conclusive evidence for rapid internal motion of the aromatic ring of phenylalanine about the aryl–C-β bond was obtained. By use of a 68-MHz spectrometer, a similar dynamical analysis for the decapeptide hormone luteinizing-hormone-releasing hormone has been completed.[44]

Conclusion

There is little doubt that ^{13}C NMR provides an invaluable source of conformational information for large molecules of biological interest. Its principal advantages are the wide range of chemical shifts and the possibility for proton decoupling. Spin–lattice relaxation times provide an extremely powerful monitor of molecular dynamics, but are at present limited in the detail they can provide due to lack of rigorous but convenient theoretical models. After a quiet period, considerable contemporary interest in the more theoretical aspects of the problem is evident,[42a,45] and we have every reason to believe that the immediate future will be very exciting.

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Use of Carbon-13 Magnetic Resonance Spectroscopy for Biosynthetic Investigations[†]

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NMR spectroscopy offers many advantages when applied to biosynthetic problems. It is the only technique which can separately detect the presence of

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isotopes of all biosynthetically useful elements (^1H, ^2H, ^3H, ^{13}C, ^{15}N, ^{17}O, etc.), and the only nondestructive method capable of directly determining the locations and concentrations of isotopic labels in a metabolite. Spin–spin coupling effects can be used to provide *direct* evidence for the incorporation of intact biogenetic units, and for biosynthetic processes involving bond formation and cleavage, whereas such information can only be deduced indirectly by other methods.

The first applications[1,2] of NMR to biosynthetic problems in the late sixties traced the fate of ^{13}C-enriched precursors by increases in the intensi-

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