Nuclear Magnetic Resonance in Carbohydrate Chemistry

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Contents

I. Scope of the Review

Nuclear magnetic resonance has had a revolutionary effect on the practice of carbohydrate chemistry since its inception, about 15 years ago, in the form of proton magnetic resonance spectroscopy. Although the primary uses of the method were for elucidation of structure and conformational preferences, routine applications for analysis of the composition of mixtures and the monitoring of reactions have become indispensable. As the result, the theory of carbohydrate chemistry has been greatly strengthened. This review is intended to display these important advances and to indicate how current advances in instrumentation are influencing the further progress.

Although carbohydrate chemists have made numerous ingenious applications of nmr spectroscopy and, indeed, have made a number of observations basic to the development of the theory of nmr, these contributions, in turn, depended on the available instrumentation. Therefore, it seemed most appropriate to organize this review in terms of the nmr experiment. Thus, the full potential of the method is best displayed, and the requirements for effective planning and making of experiments are best delineated. However, a discussion of the theory behind the nmr phenomenon as well as details of the extensive and immensely sophisticated instrumentation used is beyond the scope of this review. These topics are extensively covered in a number of textbooks.[1-8]

This review does not deal with all applications of nmr that have appeared in the carbohydrate literature. Instead, the authors have selected contributions which are considered to well illustrate the significance of the tool in its many facets to carbohydrate chemistry. Contributions from 1968 are given special attention. However, the construction of a coherent presentation required the inclusion of earlier basic contributions. Several reviews,[9-13b] a section in "Rodd's Chemistry of Carbon Compounds,"[14] as well as a section on configurational analysis in carbohydrate chemistry[15] cover earlier publications. A yearly review of the literature on carbohydrate chemistry which includes a chapter on nmr spectroscopy is available for the years 1967–1969.[16] Applications of nmr to oligosaccharides and polysaccharides are not included as recent advances have now been well reviewed.[13b]

II. The Nmr Experiments

A. The Nmr Signals

1. The Nuclear Probes

All nuclei that have been used as probes in nmr experiments on carbohydrates have a spin of ½. The magnetic parameters for these nuclei are summarized in Table I.[17]

2. Field Strength

The earliest work was at 40 MHz,[18,19] but most published proton magnetic resonance experiments of carbo-

(1) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N. Y., 1959.

(2) J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. I–II, Pergamon Press, Oxford, 1965.

(3) A. Carrington and A. D. McLachlan, "Introduction to Magnetic Resonance," Harper Row, New York, N. Y., 1967.

(4) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, Oxford, 1969.

(5) F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy," Academic Press, New York, N. Y., 1969.

(6) E. D. Becker, "High Resolution NMR," Academic Press, New York, N. Y., 1969.

(7) R. M. Lynden-Bell and R. K. Harris, "Nuclear Magnetic Resonance Spectroscopy," Thomas Nelson and Sons, Ltd., London, 1969.

(8) T. C. Farrar and E. D. Becker, "Pulse and Fourier Transform NMR. Introduction to Theory and Methods," Academic Press, New York, N. Y., 1971.

(9) R. U. Lemieux and D. R. Lineback, *Annu. Rev. Biochem.*, **32**, 155 (1963).

(10) L. D. Hall, *Advan. Carbohyd. Chem.*, **19**, 51 (1964).

(11) T. D. Inch, *Annu. Rev. NMR (Nucl. Magn. Resonance) Spectrosc.*, **2**, 35 (1969).

(12) L. D. Hall and J. F. Manville, *Advan. Chem. Ser.*, **No. 74**, 228 (1968).

(13) (a) B. Coxon, *Methods Carbohyd. Chem.*, **6**, 513 (1972); (b) *Advan. Carbohyd. Chem. Biochem.*, **27**, 7 (1972).

(14) S. Coffey, Ed., "Rodd's Chemistry of Carbon Compounds," Vol. I, Part F, 2nd ed, Elsevier, London, 1967, pp 139–165.

(15) R. J. Ferrier, *Progr. Stereochem.*, **4**, 43 (1969).

(16) "Carbohydrate Chemistry," Vol. I–III, Specialist Periodical Reports, The Chemical Society, London, 1968–1970.

(17) NMR Tables, Varian Associates, Palo Alto, Calif., 1968.

(18) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Amer. Chem. Soc.*, **80**, 6098 (1958).

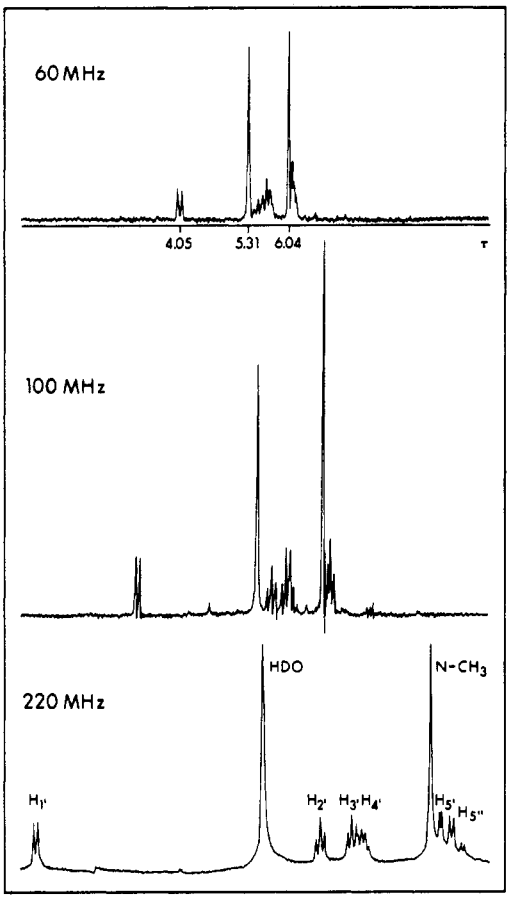


Figure 1. The partial proton magnetic resonance spectra of 3-methyl-1-(β-D-ribofuranosyl)imidazolium iodide (1) in D_2O solution at 60, 100, and 220 MHz.[22]

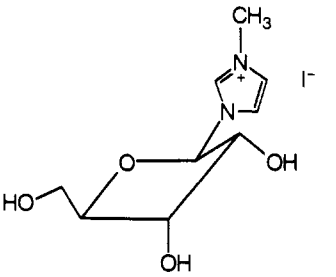
hydrate derivatives are at 60 or 100 MHz. Although carbohydrate substances are as a group particularly amenable to pmr studies, because of the substantial chemical shifts encountered, the advent of spectrometers operating at much higher frequencies (up to 300 MHz), with homonuclear and heteronuclear spin decoupling as well as with homonuclear and heteronuclear field-frequency lock systems, is of great significance to the field. Advances in the manufacture of wide-gap electromagnets permit high-resolution studies not only in 5-mm diameter sample tubes but also of larger volumes. This increases the sensitivity of the measurements and is most important for ^{13}C nmr. For protons, the sensitivity at 100 MHz is 2.7 times that at 60 MHz.[20] With the advent of Fourier transform nmr spectroscopy,[8,21] sensitivity enhancement has been greatly increased and the accumulation of data on the ^{13}C nucleus with a natural abundance of 1.1% has been greatly simplified.

There is a further advantage to obtaining nmr spectra at the highest possible magnetic field, namely resolution. Since a chemical shift between two nuclei is directly proportional to the magnitude of the external magnetic field, a complex, second-order spectrum at 60 MHz will very likely become first order at 220 MHz. As an example, consider the pmr spectra of 3-methyl-1-(β-D-ribofuranosyl)imidazolium iodide (1) in D_2O solution at 60, 100, and 220 MHz[22] (Figure 1). Only the ribofuranosyl proton region is shown, and the increase in resolution and simplification of the spectrum are readily seen.

TABLE I. Nuclei Used as Probes in Carbohydrate Chemistry[17]

Isotope	Nmr frequency, MHz at 23,487 G	Natural abundance, %	Relative sensitivity[a]
^{1}H	100.00	99.985	1.00
^{13}C	25.144	1.108	1.59×10^{-2}
^{15}N	10.133	0.37	1.04×10^{-3}
^{19}F	94.077	100	0.833
^{31}P	40.481	100	6.63×10^{-2}

[a] For equal number of nuclei at constant field.



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3. Field-Frequency Lock Systems

Most spectrometers today are equipped with a "field-frequency" stabilization (lock) system. Pmr spectra on a Varian HA-100 spectrometer may be obtained either in the locked "field-sweep" or the locked "frequency-sweep" mode.[23] The former mode of operation has the advantage in that it allows easy integration of the spectrum which is very difficult, if not impossible, to perform in the "frequency-sweep" mode. Double and triple resonance experiments, on the other hand, are performed using "frequency-sweep."[23]

For solvents such as $CDCl_3$ and DMSO-d_6, tetramethylsilane (TMS) is normally used for the internal lock and the reference signal. For D_2O solutions, water-soluble internal references DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate), TSP (sodium 3-trimethylsilyltetradeuteriopropionate), or pyrazine are normally used. External references, either a small, sealed capillary placed in the solution under study or a coaxial capillary system, are also used. Reference compounds include pure TMS, cyclopentane, or hexamethyldisiloxane or solutions of TMS in CCl_4 or $CDCl_3$. It is important to note that the chemical shift of the hydrogen in the reference compound normally changes with solvent. For example, there exists a 0.33-ppm shift between pure tetramethylsilane and the compound as a 5% solution in $CDCl_3$. When working with the coaxial capillary, both the inner tube and the standard 5-mm nmr tube must be of high precision to minimize spinning side bands. To eliminate bulk susceptibility corrections to the chemical shifts, it is necessary to have only a small quantity of an internal standard in solution. Then the external reference is used for the lock signal and the shifts are calibrated with respect to the internal standard. This is especially significant for variable-temperature pmr studies.[24]

^{19}F and ^{31}P nmr measurements have been carried out using mainly homonuclear lock systems. For ^{19}F studies, $CDCl_3$ solutions with internal $CFCl_3$ are usually used.[25] In

(19) R. U. Lemieux, R. K. Kullnig, and R. Y. Moir, *J. Amer. Chem. Soc.*, **80**, 2237 (1958).

(20) Reference 1, p 39.

(21) R. R. Ernst and W. A. Anderson, *Rev. Sci. Instrum.*, **37**, 93 (1966).

(22) R. U. Lemieux, S. S. Saluja, and T. L. Nagabhushan, unpublished results.

(23) "Varian HA-100 NMR Spectrometer Operating Manual," Varian Associates, Palo Alto, Calif.

(24) B. J. Blackburn, F. E. Hruska, and I. C. P. Smith, *Can. J. Chem.*, **47**, 4491 (1969).

^{31}P nmr, a capillary of P_4O_6 or 85% H_3PO_4 has served as the external reference.[26]

With the "new generation" of spectrometers, data accumulation has been further simplified. These contain lock, observing and decoupling channels so that an internal lock on ^1H, ^2H, or ^{19}F may be used while any nucleus is observed (for example, ^1H, ^{11}B, ^{13}C, ^{19}F, ^{31}P).[27,28] The decoupling channel allows for ready homo- and heteronuclear decoupling. Using these instruments it is possible to use the ^2H of a solvent as the lock signal and observe ^{13}C while decoupling ^1H. This eliminates the need for external references for most measurements. However, an internal standard should always be used as a reference for accurate calibration of chemical shifts even when a solvent heteronuclear lock is used. The larger range of sample-tube sizes allows for an increase in the signal-to-noise factor for all nuclei and is especially important for ^{31}P and natural abundance ^{13}C nmr. As an additional feature involving signal-to-noise enhancement, the spectrometers have the Fourier transform pulse capability for several nuclei. These spectrometers are interfaceable with small, digital computers which accumulate the time-domain signal from the pulse experiment, transform it to a standard "frequency domain" absorption spectrum, and allow the spectrum to be recorded in the conventional manner.

The 220-MHz Varian spectrometer operates in the HR (high resolution, no field-frequency stabilization) mode. A lock signal is not required and an internal standard is used.

4. *Nuclear Magnetic Double Resonance*

Nuclear magnetic double resonance refers to a spectroscopic experiment in which a system is simultaneously irradiated at two different frequencies. The experiments have had widest application as an aid in the analysis of nmr spectra. The multiplet structure arising from the coupling of certain nuclei can be made to disappear since the spins of these nuclei are decoupled from the remainder of the spin system.

Nuclear magnetic double resonance experiments including "selective irradiation"[29-32] and "spin tickling"[32-34] have been extensively applied to carbohydrate chemistry. Two excellent reviews have appeared on this subject,[35,36] and its applications to carbohydrate chemistry have been well documented.[12,37-40]

Double resonance experiments can be subdivided into two categories,[35,36] namely, "homonuclear" and "heteronuclear." In "homonuclear" double resonance the irradiated and the observed nuclei belong to the same nuclear species, whereas in "heteronuclear" double resonance two different nuclei are involved. A convenient notation for describing these experiments is A-{X},[35] where the nuclear species being observed is designated first while the decoupled nucleus is written as {X}.

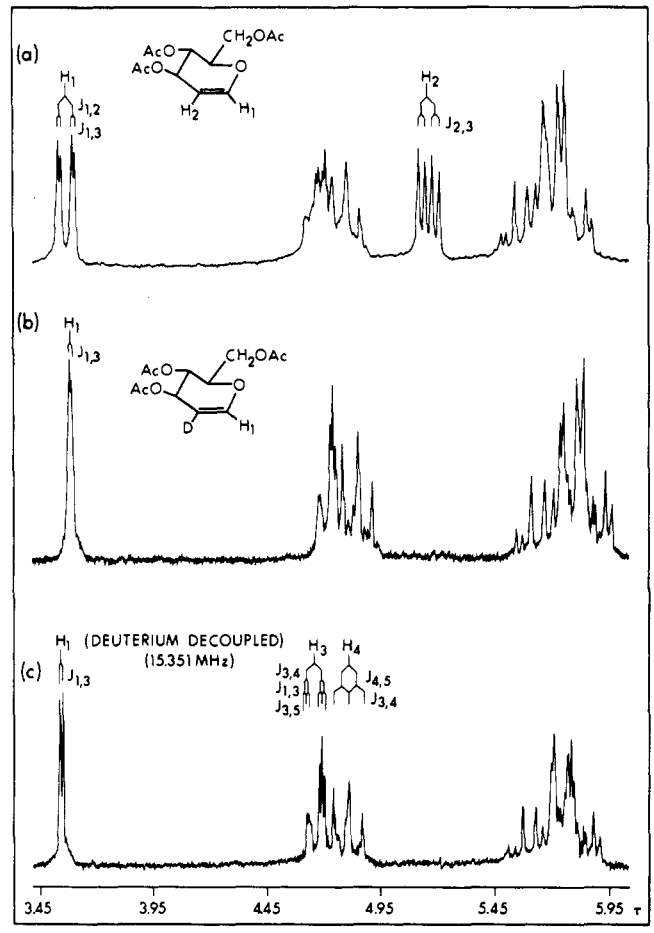

Figure 2. The 100-MHz partial proton magnetic resonance spectrum of tri-*O*-acetyl-2-deoxy-1,5-anhydro-D-*arabino*-hex-1-enitol in D_2O (a). The spectra of the 2-deuterio analog are given in b (undecoupled) and c (deuterium decoupled).[43]

In a "selective irradiation" experiment the transitions involved in a particular spectral splitting are irradiated simultaneously. This results in the collapse of associated transitions in another region of the spectrum. This method is most useful in the analysis of weakly coupled systems. In applications to systems with intermediate or strong coupling, it is usually not possible to prevent more than one spin coupling from being disturbed by the strong irradiation field. For tightly coupled spectra, "spin-tickling" is performed.[33]

Besides the application of the above techniques to the analysis and simplification of nmr spectra, double resonance methods have been applied to the measurement of chemical shifts[12,37-39] and to the determination of the relative signs of coupling constants.[30,33,34,37] Relative signs of coupling constants can only be determined on systems having at least three mutually coupled spins, such as an ABC system, and "spin-tickling" techniques are normally used.[33]

Heteronuclear double resonance techniques are also used.[35,41] A heteronuclear decoupler was developed for use with the Varian HA-100 spectrometer[42] for the fol-

(25) L. D. Hall, J. F. Manville, and N. S. Bhacca, *Can. J. Chem.*, **47**, 1 (1969).
(26) M. M. Crutchfield, C. H. Dungan, J. H. Letcher, V. Mark, and J. R. Van Wazer, "^{31}P Nuclear Magnetic Resonance," Interscience Publishers, New York, N. Y., 1967, Chapter 1.
(27) "Bruker HFX NMR Spectrometer Operating Manual," Bruker Scientific, Inc., Elmsford, N. Y.
(28) "Varian XL-100 NMR Spectrometer Operating Manual," Varian Associates, Palo Alto, Calif.
(29) J. P. Maher and D. F. Evans, *Proc. Chem. Soc., London*, 208 (1961).
(30) R. Freeman and D. H. Whiffen, *Mol. Phys.*, **4**, 321 (1961).
(31) R. Freeman and D. H. Whiffen, *Proc. Phys. Soc., London*, **79**, 794 (1962).
(32) W. A. Anderson and R. Freeman, *J. Chem. Phys.*, **37**, 85 (1962).
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(37) R. U. Lemieux and J. W. Lown, *Can. J. Chem.*, **42**, 893 (1964).
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(39) R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, **44**, 249 (1966).
(40) L. D. Hall and J. F. Manville, *Carbohyd. Res.*, **8**, 295 (1968).
(41) W. McFarlane, *Annu. Rev. NMR (Nucl. Magn. Resonance) Spectros.*, **1**, 135 (1968).
(42) R. Burton and L. D. Hall, *Can. J. Chem.*, **48**, 59 (1970).

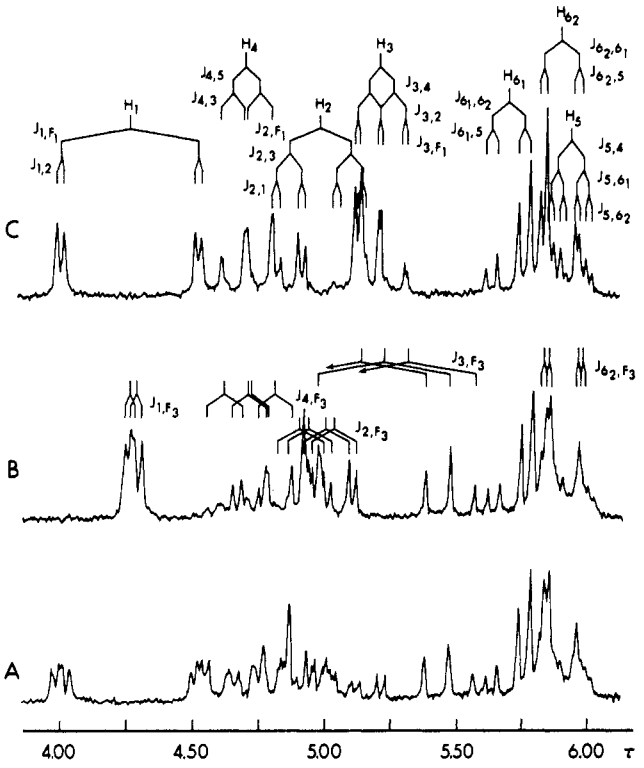
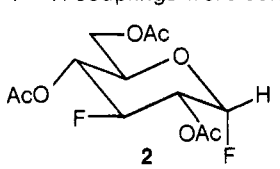


Figure 3. (A) The partial 100-MHz proton magnetic resonance spectrum of **2**; (B) the change in the spectrum on decoupling F1; (C) the observed spectrum with simultaneous decoupling of F3.[44]

lowing experiments: observe ^{1}H at 100 MHz; irradiate ^{2}H, ^{19}F, or ^{31}P; observe ^{19}F at 94 MHz; irradiate ^{1}H, ^{2}H, or ^{31}P. With the advent of the new spectrometers, heteronuclear decoupling experiments have become routine and include additional observing channels (^{2}H, ^{13}C, and ^{31}P).

The advantage of H-{^{2}H} decoupling is illustrated in Figure 2.[43] Note the disappearance of the small $^{1}H1$ to $^{2}H2$ coupling. Hall and coworkers[44] obtained an excellent ^{1}H spectrum (Figure 3) of 2,4,6-tri-*O*-acetyl-3-deoxy-3-fluoro-α-D-glucopyranosyl fluoride (**2**). By means of ^{1}H-{^{19}F} decoupling experiments, the source and magnitude of the individual ^{19}F-^{1}H couplings were confirmed.



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5. *Line Broadening*

An unsaturated nmr resonance has the familiar Lorentz line shape as predicted by the Bloch equations.[45] However, when the applied rf field becomes too strong, the spins do not have sufficient time to relax and a saturation of the signal takes place. Saturation produces a broadening of the spectrum and decreases the intensity of the central portion of the resonance absorption. Hence the experimentally imposed conditions under which a spectrum is recorded can affect sensitivity, resolution, and the relative intensities of signals.

Paramagnetic ions such as Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, and Cu^{2+} may readily be chelated by carbohydrate substances and hence affect the nmr spectra by leading to line broadening.[46] The magnetic fields associated with the unpaired electrons in paramagnetic substances provide an efficient relaxation mechanism for the nuclei because the electron magnetic moment is of the order of 10^3 times as large as the moment of a nucleus.[46] When line broadening is observed, the sample should be checked for traces of paramagnetic ions by passing it through an ion-exchange column.[47]

Broadening of the nmr resonances by paramagnetic Cu^{2+} ions has, on the other hand, aided the interpretation of the spectra of thioethers and related carbohydrate derivatives[48] owing to the selective broadening of signals from protons geminal to the sulfur atom. For example, diethyl sulfide exhibits a recognizable triplet for the methyl group upon the addition of $CuCl_2$, while the CH_2 resonance is broadened so as to be indistinguishable from the base line. The resonances of methyl 6-*S*-methyl-6-thio-α-D-glucopyranoside triacetate corresponding to the $-SCH_3$ (τ 7.82) and $-SCH_2-$ (τ 7.39) protons were found to be greatly broadened owing to specific interactions with the cupric ions while the other peaks in the spectrum remained sharp.[48]

Paramagnetic metal ion interactions with nucleosides, nucleotides, and related molecules have been extensively studied using nmr techniques, and this work has been well reviewed.[49-51] Most of these studies were carried out using ^{1}H and ^{31}P nmr. For example, a binuclear 2:2 Cu^{2+}–adenosine monophosphate complex has been proposed for both the 5′ and 3′ adenosine monophosphate isomers. The nmr data for adenosine 2′-monophosphate can be interpreted in terms of an intramolecular complex involving N3 and the phosphate group.[52] The Co^{2+}, Mn^{2+}, and Ni^{2+} ion complex with adenosine triphosphate involves the binding of the metal to the phosphate groups and simultaneously, *via* a water molecule bridge, to the N7 position of the ring.[53] Recently,[54] specific line broadening effects were observed in the natural abundance ^{13}C nmr spectra and can be used in determining the nature of the paramagnetic ion binding site. The influence of paramagnetic Mn^{2+} ions on the proton-decoupled ^{13}C spectrum of adenosine 5′-monophosphate is shown in Figure 4. The metal ion is held near the N7 position of the base since the C5 and C8 base resonances are broadened preferentially to the other eight carbon resonances.[54] Kotowycz and Suzuki[54a] have extended these studies to complexes of Mn^{2+} with a series of nucleosides and nucleotides and have shown[54b] that both 5′ and 2′ adenosine monophosphate form 2:1 complexes with Cu^{2+}.

B. The Nmr Parameters

Applications of nmr to problems of carbohydrate chemistry involve measurements of four nmr parameters.

1. *Relative Intensities of the Signals*

a. Pmr Spectra

The property that, under proper operating conditions,

(43) R. U. Lemieux and T. L. Nagabhushan, unpublished results.
(44) L. D. Hall, R. N. Johnson, A. B. Foster, and J. H. Westwood, *Can. J. Chem.*, **49**, 236 (1971).
(45) Reference 3, p 182.
(46) Reference 1, p 207.
(47) M. Cohn and T. R. Hughes, Jr., *J. Biol. Chem.*, **237**, 176 (1962).
(48) E. V. E. Roberts, J. C. P. Schwarz, and C. A. McNab, *Carbohyd. Res.*, **7**, 311 (1968).
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(50) U. Weser, *Struct. Bonding (Berlin)*, **5**, 41 (1968).
(51) R. M. Izatt, J. J. Christensen, and J. H. Rytting, *Chem. Rev.*, **71**, 439 (1971).
(52) N. A. Berger and G. L. Eichhorn, *Biochemistry*, **10**, 1847 (1971).
(53) T. A. Glassman, C. Cooper, L. W. Harrison, and T. J. Swift, *Biochemistry*, **10**, 843 (1971).
(54) G. Kotowycz and K. Hayamizu, *Biochemistry*, **12**, 517 (1973).
(54a) G. Kotowycz and O. Suzuki, *Biochemistry*, **12**, 3434 (1973).
(54b) G. Kotowycz and O. Suzuki, *Biochemistry*, in press.

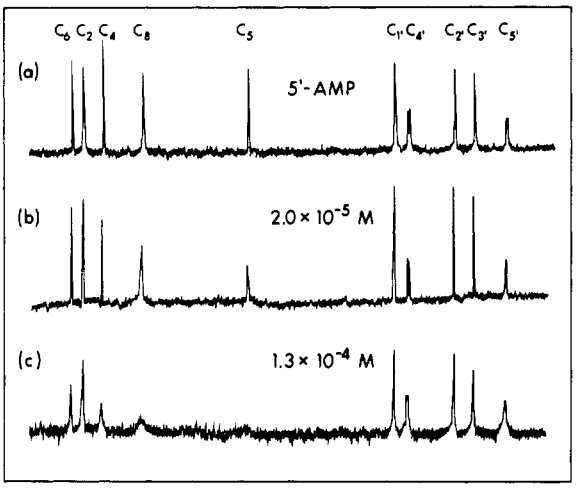
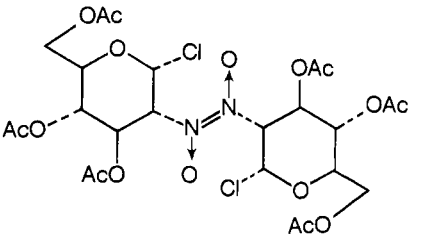


Figure 4. The natural abundance ^{13}C proton-decoupled nmr spectra of adenosine 5′-monophosphate in D_2O: (a) the spectrum of the metal-free solution; (b and c) the effect of Mn^{2+}. The metal ion concentrations are indicated in the figure.[54]

the relative intensities of absorption signals for different hydrogens is equal to the relative amounts of the hydrogens producing the signals has been particularly important to analytical carbohydrate chemistry. Appropriate chemical shifts are often present for signals arising from components of a mixture, and rapid quantitative analysis is possible. A nonreacting internal standard is often necessary to provide a reference signal, and this reference should be as near as possible to the resonances under consideration. High-precision sample tubes are required for precise measurements. Thus, crude reaction mixtures can often be conveniently analyzed prior to separation and actual yields determined. Carbohydrate chemistry abounds with examples of molecular rearrangements,[55] and the technique offers a particularly attractive means for assessing such phenomena. For example, the proportions of anomers, including furanose and pyranose forms, for sugars in aqueous solution have been determined.[39,56,57] A recent publication on the solution properties of 2-deoxy-D-*erythro*-pentose (2-deoxyribose) well illustrates the potential of such measurements.[58] Thus, the equilibria achieved between the α and β anomers of the sugar in both the furanose and pyranose forms could be determined at a variety of temperatures, and the kinetics of the changes in composition leading to the mutarotation phenomena at 0° were obtained. The method is particularly useful for following the solution kinetics of complex reactions and has received many applications especially for the detection of reactive intermediates. Thus, Lemieux and coworkers[59] were able to achieve evidence that the formation of alkyl tri-*O*-acetyl-2-oximino-α-D-*arabino*-hexopyranosides (**7**) on reacting the dimeric form tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (**3**) with an alcohol in dimethylformamide involves dissociation of the dimer as the overall rate-controlling stage and that the next stage is the conversion of the monomeric form (**4**) to tri-*O*-acetyl-2-oximino-α-D-*arabino*-hexopyranosyl chloride (**5**). This latter compound then enters into a reversible conversion to tri-*O*-acetyl-2-deoxy-2-nitroso-D-glucal (**6**) which is the precursor of the α-glycoside **7**. Pmr made possible under appropriate conditions the detection of the highly reactive nitrosoalkene intermediate **6** and also provided a convenient means for measuring the relative amounts of α-glycosides formed under competitive conditions. Furthermore, the formation of a third labile product in low yield was detected which remains to be characterized but may be the β anomer **8**. Such investigations could not be accomplished prior to the advent of pmr and comprise perhaps the most important single *practical* use of the method to carbohydrate chemistry. The number of applications made are too numerous to mention, but all amount to conducting a reaction in the nmr tube under temperature control. In view of the wide range of deuterated solvents presently commercially available, there exists little limitation, on this account, to the reaction systems available for study.



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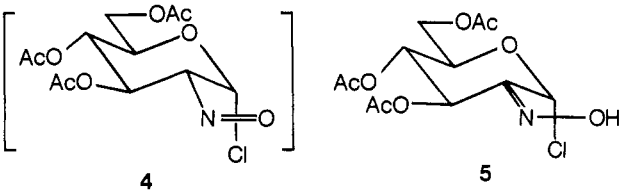

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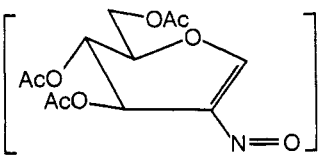

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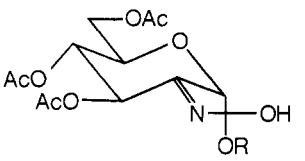

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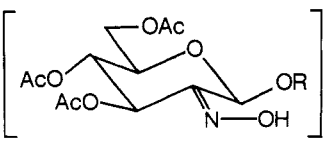

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b. Carbon-13 Nmr Spectra

Carbon-13 nmr has been amply described in two recent monographs.[60a,b] Natural abundance ^{13}C nmr spectra are at present most commonly obtained in the Fourier transform mode.[21] The relative intensities of lines which arise from the same number of nuclear spins may be influenced by several phenomena. Differences in the relaxation time T_1 between the different nuclei must be considered.[8] With efficient proton noise decoupling,[61] all splittings due to the ^{13}C–H coupling constants are eliminated and the ^{13}C resonances are enhanced not only by the collapse of multiplets but also by the nuclear Overhauser effect.[62,63] If dipole–dipole relaxation pro-

(55) R. U. Lemieux, "Molecular Rearrangements," Part 2, P. de Mayo, Ed., Interscience, New York, N. Y., 1964, p 709.

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(59) R. U. Lemieux, Y. Ito, K. James, and T. L. Nagabhushan, *Can. J. Chem.*, **51**, 7 (1973).

(60) (a) J. B. Stothers, "Carbon-13 NMR Spectroscopy. Organic Chemistry—A Series of Monographs," Vol. 24, A. T. Blomquist and H. Wasserman, Ed., Academic Press, New York, N. Y., 1972; (b) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, N. Y., 1972.

(61) R. R. Ernst, *J. Chem. Phys.*, **45**, 3845 (1966).

(62) K. F. Kuhlmann and D. M. Grant, *J. Amer. Chem. Soc.*, **90**, 7355 (1968).

(63) K. F. Kuhlmann, D. M. Grant, and R. K. Harris, *J. Chem. Phys.*, **52**, 3439 (1970).

vides the dominant mechanism between directly bonded carbon and hydrogen atoms, an enhancement of nearly three[62,63] may be observed for the ^{13}C nuclei of methyl, methylene, or methine groups.[63] But for appreciable contributions from other relaxation mechanisms, variations in the magnitude of the enhancement *can*[63] be observed depending on the number of nearest-neighbor protons. Experimental conditions also give rise to phase and intensity anomalies.[21,64] Integrated ^{13}C spectra have been reported[65] for sucrose and adenosine 5′-monophosphate. However, relative intensities of ^{13}C resonances should be interpreted very cautiously.

2. Coupling Constants

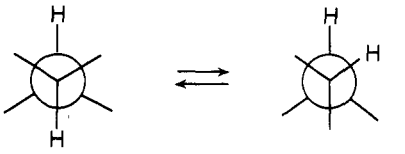
Nuclear spin–spin coupling constants, to be referred to herein simply as coupling constants, are designated by J and are expressed either as cycles per second (cps) or as hertz (Hz). The nuclei involved (N1 and N2) are represented in parentheses as J(N1,N2).

Until 1968, virtually all nmr measurements involved proton magnetic resonance, herein designated as pmr, and were concerned with measuring the coupling constant and chemical shift parameters and interpreting their values. Coupling constants over two chemical bonds are designated as ^2J(N1,N2), over three bonds ^3J(N1,N2), etc., and this nomenclature is used in this review.

When a first-order spectrum is observed, the magnitudes of the coupling constants may be determined directly from the spectrum. Otherwise, the determination of the true coupling constant is rarely accomplished except by a complete analysis of the spectrum. Computer techniques in the analysis of nmr spectra have been described,[66] and a variety of computer programs for nmr analyses are available.[67] Theoretical spectra have been published,[68,69] and these also serve as an aid in determining values of the true chemical shifts and coupling constants.

Besides the magnitudes, a knowledge of the signs of coupling constants is necessary in the study of carbohydrate configurations whenever theoretical spectra are to be calculated. Indeed, that the signs of the coupling constants for geminal and vicinal hydrogens are opposite was first discovered in attempts to calculate the theoretical spectra for the ABC system of 4-substituted dioxolanes.[70] Relative signs of coupling constants are often determined using double resonance techniques.[29-34]

Many factors affect the magnitudes of observed coupling constants and must be considered. If the coupling undergoes a change with temperature, this likely results from a conformational equilibrium. Thus, determination of nmr spectra at two widely different temperatures can be useful to establish the conformational purity of a compound. A time-averaged nmr spectrum for conformers is normally observed and the chemical shifts as well as the coupling constants are intermediate between those expected for the separate conformers. For example, the simplest situation involves appreciable populations of only two conformers **9a** and **9b**.



9a **9b**

If P_a and P_b are the relative populations (mole fractions) of the two conformers ($P_a + P_b = 1$), the observed time-averaged coupling constant is given by the equation

$$J_{obsd} = J_{av} = P_aJ_a + P_bJ_b$$

where J_a is the value of the specific coupling in **9a** and J_b is the value in **9b**. If chemical shift data (see section II.B.3) are available, the time-averaged chemical shift is related to δ_a and to δ_b by the relationship

$$\delta_{av} = P_a\delta_a + P_b\delta_b$$

where δ_a and δ_b are the chemical shifts for either specific nuclei or groups of nuclei in conformers **9a** and **9b**, respectively.

On occasions, the coupling constants or chemical shifts in specific conformers can be determined by nmr measurements at very low temperatures when interconversion is slow on the nmr time scale. Normally, however, the coupling constants are estimated from model compounds possessing a conformationally rigid structure. For example, the demonstration that coupling constants for vicinal hydrogens defining a torsion angle of about 180° was three to four times larger than when the torsion angle is about 60° was obtained from a study of the epimers of 4-*tert*-butylcyclohexyl compounds.[18] Once the relative populations of the conformers are known at equilibrium, the standard free energy may be calculated for the process using the expression

$$\Delta G^\circ = -RT\ln (P_b/P_a)$$

P_a and P_b may be estimated from the spectra in two additional ways. At temperatures where resonances are observed for the separate conformers, integration of these resonances (see section II.B.1) will yield P_a and P_b. Secondly, δ_a and δ_b may be measured indirectly from related compounds known to exist in a specific conformation and then used to compute P_a and P_b (see section III).

Conformational equilibria have been estimated by assuming, in most cases, values for J(60°) and J(180°). Although such practice has provided much useful insight on conformational equilibria, it must be realized that unless these are the only important conformers and the true values of J_a and J_b are known, such conclusions are susceptible to considerable error. This source of error arises because only under rather unusual circumstances will the torsion angles actually be 60 and 180°, especially for open-chain compounds, and only small changes from the 60° torsion angle can cause large changes in coupling.

For a so-called first-order spectrum, the width of the signal for a given hydrogen is the sum of all the coupling constants. When the hydrogen is coupled to several protons, a band may be observed wherein the lines are not resolved. In such cases, a half-band width is measured. This half-band width is related to the sum of all the coupling constants involved.

Several other factors influence the determination of coupling constants. If the proton under study is coupled with an exchangeable proton in a solvent such as DMSO as are the methylene protons in the $-CH_2OH$ fragment, $J(CH_2,OH)$ may be measured. However, in water the hy-

(64) R. Freeman and H. D. W. Hill, *J. Mag. Resonance*, **4**, 366 (1971).

(65) A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.*, **55**, 189 (1971).

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(67) "Quantum Chemistry Program Exchange, Catalog and Procedures," Vol. VII, Chemistry Department, Indiana University, Bloomington, Ind., 1971.

(68) K. B. Wilberg and B. J. Nist, "The Interpretation of NMR Spectra," W. A. Benjamin, New York, N. Y., 1962.

(69) Reference 5, Appendix D, p 275.

(70) R. R. Fraser, R. U. Lemieux, and J. D. Stevens, *J. Amer. Chem. Soc.*, **83**, 3901 (1961).

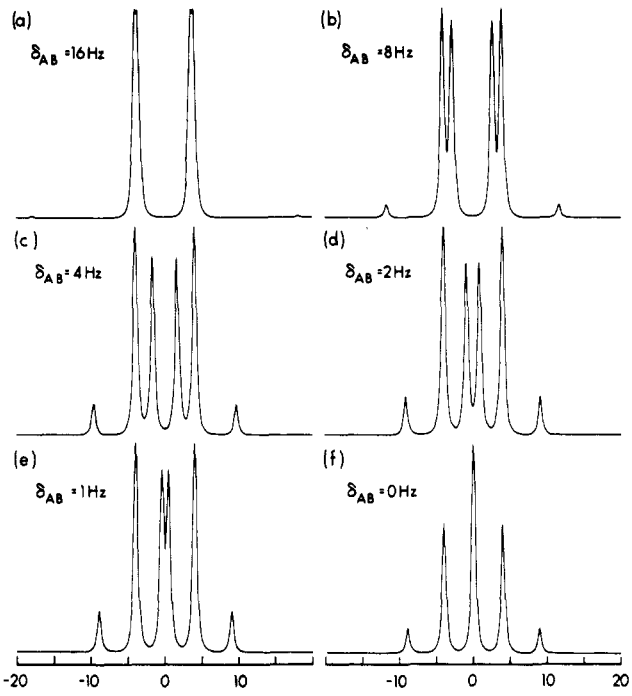


Figure 5. The computed spectrum for the X region of an ABX system. The spectra are calculated as a function of the relative chemical shift δ_{AB}. The coupling constants used for this calculation are J_{AB} = 8 Hz, J_{BX} = 8 Hz, and J_{AX} = 0 Hz.[74]

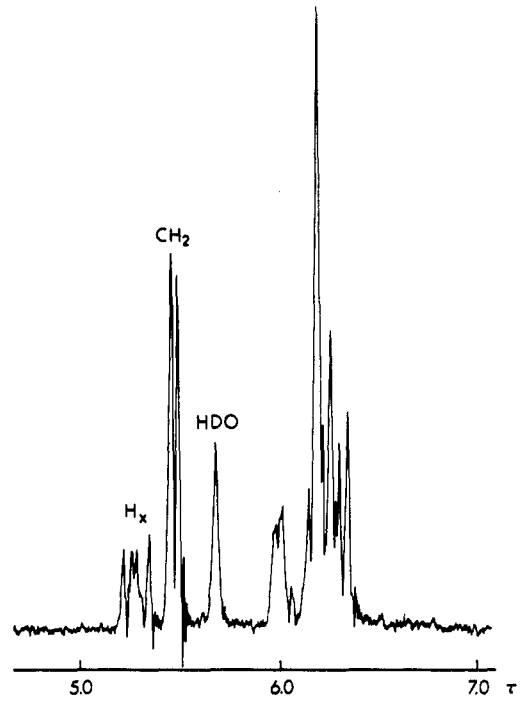
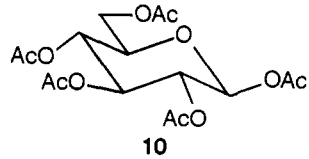


Figure 6. The 60-MHz proton magnetic resonance spectrum of trichloroethyl β-D-galactopyranoside in D_2O at 70°.[75] Note the similarity between the anomeric proton (H_x) resonance and the computed spectrum shown in Figure 5e.

droxyl proton undergoes rapid exchange and the coupling cannot be observed.

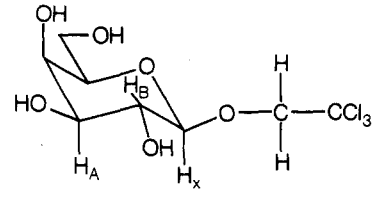
Another factor which must be considered involves second-order effects arising out of virtual long-range coupling.[71,72] This phenomenon arises in the system of protons $AB(X)_n$ where $\delta_A - \delta_B$ is not at least an order of magnitude larger than J_{AB} and has the result of producing a signal for X which is greatly more complex than the doublet expected from the coupling of X only with B regardless of how great $\delta_X - \delta_A$ may be. The spacing found for the "rough" doublets observed for the anomeric proton of most sugars is less than the actual coupling constant. For example, the spacing of the "doublet" signal for the anomeric proton of penta-*O*-acetyl-β-D-glucopyranose (**10**) is 6.7 Hz in chloroform at 60 MHz, 6.9 Hz



10

in chloroform at 100 MHz, and 7.5 Hz in pyridine at 60 MHz.[38] Obviously, the chemical shift between H2 and H3 is greater in pyridine than in chloroform, and this was at once apparent from the spectrum. The shift between H2 and H3 was made very large by introducing a methyl group for the acetyl group at the 3 position. The spacing of the doublet corresponding to the anomeric proton now increased to 8.0 Hz, and this is probably the value for the coupling constant in the pentaacetate. Partial virtual coupling has been studied in detail in a four-spin system ABMX.[73]

Caution must be exercised in assigning coupling constants to spectra that are subject to virtual long-range coupling.[38] As in Figure 5,[74] virtual coupling can profoundly affect the structure of the signal for the X hydrogen of an ABX system where A is not appreciably coupled to X. This situation is typical of the pmr signal for the anomeric hydrogen of sugars and related compounds. An example is the spectrum shown for trichloroethyl β-D-galactopyranoside (**11**) in Figure 6.[75] The chemical shift δ_{BX} is about 60 Hz (at 60 MHz). Very good agreement is obtained from a comparison of the anomeric proton (H_x) spectrum with the calculated spectrum shown in Figure 5e with δ_{AB} = 1 Hz and J_{AB} = J_{BX} = 8 Hz.



11

a. Proton–Proton

i. Geminal Coupling Constants $^2J(H,H)$. Certain trends have been established[76-78] for geminal $^2J(H,H)$ coupling constants, and the factors influencing it are:[76,78] (a) $^2J(H,H)$ increases (becomes more positive) as the hybridization on the carbon atom adopts more s character (*i.e.*, the coupling constant increases with an increase in the H–C–H angle); (b) for both sp^2 and sp^3 methylene groups, introduction of a more electronegative atom α to the hydrogens increases the geminal coupling constant;

(71) F. A. L. Anet, *Can. J. Chem.*, **39**, 2262 (1961).

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(74) The spectra were calculated by Dr. J. S. Martin, Department of Chemistry, University of Alberta, Edmonton, Canada.

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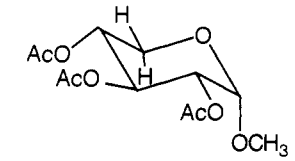
(78) G. E. Maciel, J. W. McIver, Jr., N. S. Ostlund, and J. A. Pople, *J. Amer. Chem. Soc.*, **92**, 4151 (1970).

(c) substitution of a more electronegative atom β to the hydrogens leads to a decrease in $^2J(H,H)$; and (d) a π-electron system adjacent to a CH_2 group generally leads to a decrease in $^2J(H,H)$.

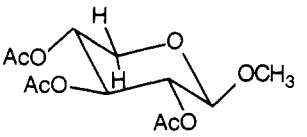
Geminal coupling constants can have either sign (ref 70, 76, 79) namely, negative in saturated systems[40,80] and positive in unsaturated carbohydrate derivatives.[40]

The following examples[81] illustrate the suggestion[76] that geminal coupling depends in part upon the relative orientation of an electronegative vicinal substituent (X) with the coupling being numerically larger when the substituent is gauche to only one of the geminal hydrogens than when it is in gauche relationship to both the geminal hydrogens. Coxon has observed examples with ribopyranose derivatives,[82] and Hall and Manville have observed examples with 2-deoxyhexopyranose derivatives.[83] This phenomenon promises to become of increasing utility in conformational analysis. The above geminal coupling constants may be compared with the value measured for cyclohexane; from 1,1,2,2,3,3,4,4-octadeuteriocyclohexane $^2J(H,H) = -13.05$ Hz.[84]



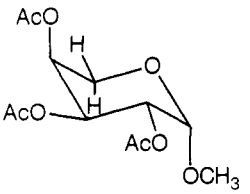
12

$J_{5a,5e}$ −10.7



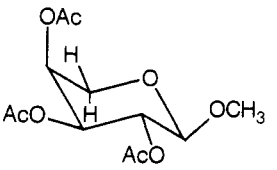
13

−11.8



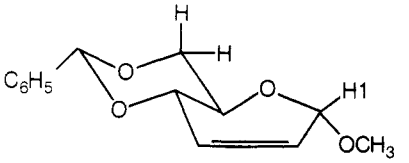
14

$J_{5a,5e}$ −13.2



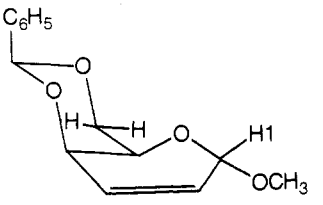
15

−13.1 Hz



16

$J_{6a,6e} = -9.9$ Hz



17

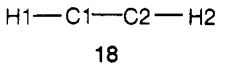
$J_{6a,6e} = -12.5$ Hz

ii. Vicinal Coupling Constants $^3J(H,H)$ in a Saturated System. As early detected,[18,19] coupling constants for vicinal hydrogens bear a relationship to torsion angle, and this has made possible the determination of configuration and/or conformation almost routine. This led to the proposal by Karplus[85-87] that there exist relationships of the form

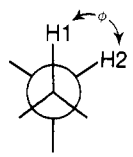
$$^3J(H1, H2) = a\cos^2\phi + b \quad \text{(ref 85)}$$

$$^3J(H1, H2) = A + B\cos\phi + C\cos 2\phi \quad \text{(ref 87)}$$

in saturated systems (**18**), where ϕ is the angle between the H1-C1-C2 and the H2-C2-C1 planes (**19**), and J_0 and b are constants for the particular system under study. In the latter equation the constants are $A = 4.22$, $B = -0.5$, and $C = 4.5$ Hz with a C-C bond length of 1.543 Å.



18



19

The magnitude of the coupling is dependent on a variety of other parameters besides the torsion angle ϕ.[87] These include the electronegativity of substituents[88a] and substituent induced changes in hybridization of the carbon atoms, the H1-C1-C2 and C1-C2-H2 bond angles, and the C1-C2 bond length as well as other molecular properties.[87] For example, a recent publication[88b] on the X-ray structure of tri-*O*-acetyl-β-D-arabinopyranosyl bromide has provided proton-proton torsion angles which bear only a modest correlation with the observed coupling constants in solution.

The original calculations by Karplus[85] predicted the values for a of 8.5 Hz for $0° \leq \phi \leq 90°$ and 9.5 Hz for $90° \leq \phi \leq 180°$ with $b = -0.28$ Hz. These results were soon verified by Anet.[89] However, it became apparent that the general shape of the Karplus curve may be subject to a displacement depending on the nature of the compounds studied. It was necessary to displace the curve upward by 2.2 Hz to account for the couplings in 1,3-dioxolane.[88a] Hence the application of the Karplus curve for conformational analysis requires a knowledge of the constants for the structures under consideration. Coxon[13a] has tabulated observed values of vicinal coupling constants for chair forms of a variety of carbohydrate derivatives. The couplings between vicinal diaxial protons range from 8.6 to 11.5 Hz with a mean value of 9.63 Hz, whereas between diequatorial protons the couplings are smallest and in the range 0.6-3.5 Hz (mean, 1.54 Hz). The coupling constants between an axial proton and a vicinal equatorial proton fall in the range 1.5-5.8 Hz with a mean value of 3.66 Hz.

Williams and Bhacca[90] and Booth[91] have studied the variation of the vicinal coupling constants with the *orientation* of a given electronegative substituent. This configurational dependence of the coupling is quite large and for the nondistorted chair conformers (**20** and **21**), the magnitudes of the coupling constants are $^3J_{ae} = 5.5 \pm 1.0$ Hz and $^3J_{ea} = 2.5$-3.2 Hz, respectively.[90] This effect has been discussed in greater detail.[12]

(79) B. D. Nageswara Rao, J. D. Baldeschwieler, and J. I. Musher, *J. Chem. Phys.*, **37**, 2480 (1962).
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(89) F. A. L. Anet, *Can. J. Chem.*, **39**, 789 (1961).
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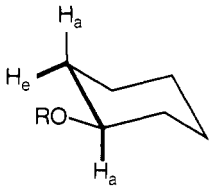
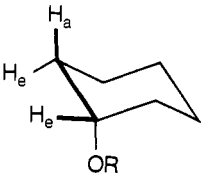
(91) H. Booth, *Tetrahedron Lett.*, 411 (1965).



20, R = H or Ac
J_{ae} = 5.5 ± 1.0 Hz



21, R = H or Ac
J_{ea} = 2.5–3.2 Hz

Karplus[87] pointed out that a structural analysis based on the relationship between the magnitude of the coupling constant and the angle ϕ will yield the most reliable results by comparing closely related molecules. As well, solutions to a structural problem should depend *not* on the exact values of the coupling constants but on the fact that certain couplings are "large" rather than "small" or *vice versa*. Hence torsion angles calculated using the former equation or similar relationships are only very approximate due to the difficulties in determining values for the constants for any one specific kind of structure.[87] Values from 8 to 16 Hz[92] have been assumed for $a = J(0°)$ in the Karplus relationship.

The Karplus type of relationship is most valuable in the study of conformational differences between closely related compounds. For these purposes the constant "b" can be neglected and the equation takes the form[93]

$$^3J(H1, H2) = K \cos^2 \phi \ (i.e., \ 4J_{60°} = J_{180°})$$

where K depends on the precise nature of the H1–C1–C2–H2 fragment. The constant must take account of all the other factors affecting the magnitude of the coupling in addition to the torsion angle ϕ.

A consequence of the $\cos^2 \phi$ relationship is that, when ϕ is between 50 and 70° or between 110 and 130°, small variations in ϕ cause large changes in the magnitude of the coupling and provide a sensitive probe for the study of conformational changes.[18,85] However, the magnitude of the coupling is not very sensitive to small variations in ϕ near 0, 90, and 180°.[93]

Experimental determinations of the signs of vicinal coupling constants have shown that these couplings are absolutely positive[94] but, according to Karplus,[85] may be negative with $\phi \simeq 90°$. Many theoretical calculations have also been carried out on vicinal proton–proton coupling constants, and these have been summarized by Maciel, *et al.*[95]

In carbohydrate chemistry, the determination of $^3J(H,H)$ values has been used to determine points of configuration as well as conformational preferences for pyranose,[96–133] furanose,[134–148] and acyclic[149–160] sugars. A noteworthy example is the demonstration of the so-called "re-

(92) S. Sternhell, *Quart. Rev., Chem. Soc.*, **23**, 236 (1969).

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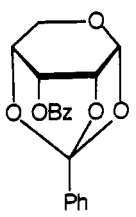
TABLE II. Calculated Values for the Proton-Proton Torsion Angles ϕ (Deg) in 23[119]

Angle	Orientation of the protons[a]	Calcd from molecular models	Calcd from ^3J(H,H)
$\phi_{1,2}$	e,q	22	36 (139)[b]
$\phi_{2,3}$	q,a	50	47 (129)
$\phi_{3,4}$	a,e	72	63 (113)
$\phi_{4,5}$	e,q	100	103 (74)
$\phi_{4,5'}$	e,q	20	44 (132)

[a] Symbols used are: e, equatorial; a, axial; q, quasi. [b] The alternative values of ϕ calculated from the Karplus equation are given in parentheses.

verse anomeric effect" by Lemieux and Morgan.[161] The driving force for the pyridinium ring to achieve the equatorial orientation forces the pyranose ring into the boat conformation ($^{2,5}B$) as established by X-ray crystallographic analysis.[162] The vicinal coupling constants found for the compound in solution support the maintenance of this unusual conformation since $J_{1,2}$ = 2.8, $J_{2,3}$ = 3.1, $J_{3,4}$ = 3.2, and $J_{4,5}$ = 5.7 Hz.[161]

Coxon[119] has analyzed the 100-MHz pmr spectrum of 3-*O*-benzoyl-1,2,4-*O*-benzylidine-α-D-ribopyranose (**22**). Since the molecule is locked in an inflexible, skew conformation of defined geometry (**23**), it was chosen for a study of the correlation between geminal, vicinal, and long-range coupling constants with conformation (see Table II). The vicinal coupling constants were calculated using the Karplus equation.[87] A long-range coupling J(H2,H4) of 2.45 Hz over four saturated bonds was also observed for this molecule.[119] Monosaccharides with nitrogen-containing rings have also been studied,[163-166] and the Karplus relationship has been extended to vicinal coupling constants in the system H1-C1-NH2.[167]



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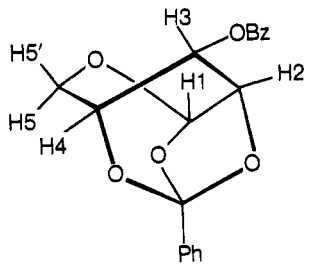

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Well-resolved signals have been observed for the hydroxyl proton resonances of alcohols dissolved in DMSO, thus allowing the measurement of the coupling constant ^3J(CH,OH).[168] Rader[169] studied the pmr spectra of cyclohexanols in DMSO and found the axial OH proton to higher field (0.21-0.27 ppm) than the equatorial proton for epimeric sets of molecules. A correlation of ^3J(CH,OH) with conformation was also observed with the equatorial epimer exhibiting a larger coupling by 1.0-1.5 Hz than the axial epimer. For example, in *trans*-2-methylcyclohexanol, J = 5.3 Hz, where, for the cis epimer, J = 4.0 Hz. The magnitudes of the coupling were related to the effect of torsion angle and the populations of rotational isomers about the C-O bond.[169]

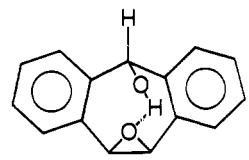
The proton of the anomeric hydroxyl of sugars resonates at lower field than those of the other hydroxyl groups.[168,170] As well, the anomeric hydroxyl proton of glucose and related sugars shows characteristic splittings of ^3J(H,OH) = 4.0-4.5 Hz for α anomers (OH axial) and ^3J(H,OH) = 6.0-7.0 Hz for β anomers (OH equatorial).[170]

Perlin[171a] has studied hydroxyl pmr spectra of sugars in DMSO and found that some sugars exist as furanoses to a greater extent in this solvent than in water. This difference is very marked for D-arabinose. Cyclic and acyclic forms of certain sugars could also be distinguished by the hydroxyl proton signals. Values of ^3J(H,OH) have now been measured in a large variety of pyranoses.[171b]

Fraser and coworkers[172] have carried out an ABC analysis of the pmr spectra of the $-CH_2OH$ group of 3β-acetoxy-5β,6β-oxidocholestan-19-ol in $CDCl_3$, benzene, and a 2:1 mixture of acetone and $CDCl_3$. Two vicinal ^3J(CH,OH) coupling constants were obtained involving the same hydroxyl proton for torsion angles of 80 and 160° (±10°). A least-squares analysis of these data together with several literature values was applied to the Karplus function to evaluate the coefficients, and the following equation was obtained.

$$^3J(\mathrm{CH,\ OH}) = 10.4\cos^2\phi - 1.5\cos\phi + 0.2$$

Karplus behavior thus accounts for the variation of ^3J(CH,OH) with ϕ. For ϕ = 80 and 160°, the observed couplings are 0.3 and 10.5 Hz, whereas those calculated using this equation are 0.2 and 10.8 Hz, respectively.[172] In **24**, the OH group is internally hydrogen bonded and



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J(CH,OH)^{trans} = 12.5 Hz (ϕ = 180°).[173] For ϕ = 167°, the observed coupling constant is 11.4 Hz.[174] The calculated values for these coupling constants are 12.1 and 11.5 Hz, respectively.[172] The observed coupling constants of sugar-ring hydroxyl protons of several nucleosides dissolved in mixtures of dry DMSO-d_6 and C_6D_6 have been analyzed using this equation.[175] The preferred conformations of the OH bonds of the sugar were thus calculated.

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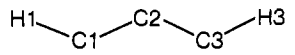
TABLE III.[a] Calculated Coupling Constants ^4J(H,H) for Propane[182]

ϕ, deg	ϕ', deg	^4J(H,H), Hz	ϕ, deg	ϕ', deg	^4J(H,H), Hz
0	0	−1.04	120	120	−0.12
	60	−0.58		180	0.45
	120	−0.25		240	−0.29
	180	−0.29		300	−0.62
	240	−0.25			
	300	−0.58	180	180	1.44
				240	0.45
60	60	−0.71		300	−0.49
	120	−0.46			
	180	−0.49	240	240	−0.12
	240	−0.62		300	−0.46
	300	−0.32			
			300	300	−0.71

[a] In Figure 7, the dihedral angles ϕ and ϕ' are defined.

iii. Long-Range Coupling Constants ^4J(H,H), ^5J(H,H) in Saturated Systems. It was early detected that long-range coupling over four bonds was sensitive to orientation effects.[176] Indeed, the now-extensive studies of a variety of systems where coupling is observed between nuclei separated by four or more bonds have shown these couplings to be very sensitive to the relative orientations of the bonds. These have been measured in both saturated and unsaturated systems as well as systems involving heteroatoms and have been well reviewed.[92,177,178]

In saturated systems, the largest four-bond couplings J(H1,H3) (**25**) have been observed for protons separated



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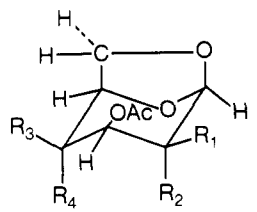
in the planar zig-zag or W arrangement.[92] For example, consider systems **26** to **28**[179] and **29**.[180] These first-order coupling constants follow the W arrangement for long-range couplings between hydrogen substituents in 1,3-diequatorial positions on the pyranose chair conformer. In molecule **29**, a coupling between H1 and H5 across the C5 ring oxygen atom, a coupling between H4 with H6 (exo), and a possible long-range coupling of H6 (exo) with proton H1 across the oxygen atom of the anhydro bridge were also observed.[180]



	J(H1,H3), Hz	
26, $R_2 = R_4 = OAc$; $R_1 = R_3 = H$	1.3	(179)
27, $R_1 = R_4 = OAc$; $R_2 = R_3 = H$	1.4	(179)
28, $R_2 = R_3 = OAc$; $R_1 = R_4 = H$	1.8	(179)
29, $R_1 = R_3 = OAc$; $R_2 = R_4 = H$	J(H1,H3) ≃ J(H3,H5) = 1.1	(180)

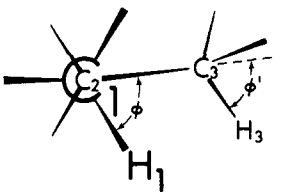


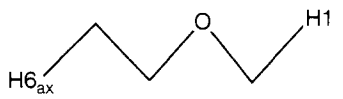
Figure 7. The dihedral angles ϕ and ϕ' in propane are depicted and are measured from the C1–C2–C3 plane.[182]

Barfield has carried out theoretical studies of the conformation and substituent dependence of four-bond, long-range ^{1}H–^{1}H coupling constants.[181,182] The major contribution to four-bond couplings in saturated systems is the indirect σ-electron mechanism.[181] The coupling constants are strongly conformation and substituent dependent and can be either positive or negative in sign. Calculations of ^4J(H1,H3) for couplings in propane were evaluated for 60° intervals of the torsion angles ϕ and ϕ' (see Figure 7).[182] It is seen that maximum coupling (+1.44 Hz) corresponds to the W arrangement ($\phi = \phi' = 180°$) (Table III).[182]

The signs of four-bond, long-range coupling constants have been determined and ^4J (diequatorial) couplings are positive (0.8 to 1.6 Hz) and ^4J (equatorial, axial) couplings are negative in sign (−0.2 to −0.7 Hz) for the chair conformations of pyranoid sugars.[40] For example, in 1,6-anhydro-β-D-mannopyranose triacetate, $J_{1,3}$ = +1.6 Hz and $J_{3,5}$ = +1.5 Hz.[40]

The very large long-range coupling ^4J(H2,H4) = 2.45 Hz was observed for compound **23**. The torsion angles are both about 170° and very close to the planar W arrangement that is optimal for long-range coupling.[119] Four-bond couplings of 0.4 Hz were observed between both H1 and H5 and H1 and H6 (exo) in 1,6:2,3-dianhydro-4-deoxy-β-D,L-*ribo*-hexopyranose and well display the W arrangement for the coupling through a bridging oxygen atom.[183]

Five-bond coupling constants along single bonds also show a stereochemical dependence. Lemieux, Fraga, and Watanabe[81] observed ^5J(H1,H6ax) = ±0.6 Hz for compound **17**. In this regard, it is of interest to note the antiperiplanar arrangements of the alternate σ bonds and that all six atoms must be near in one plane (**30**). Coxon[184] has shown that ^5J varies from 0.1 to 0.9 Hz and



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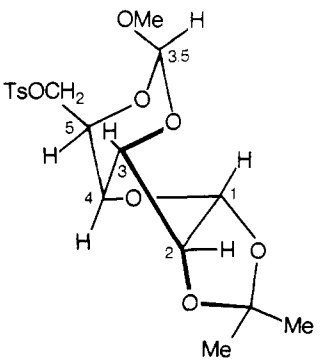

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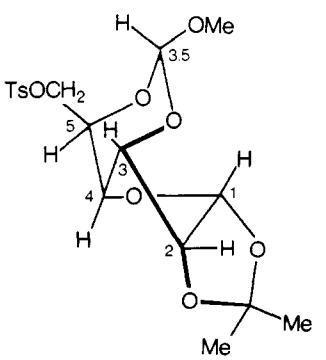

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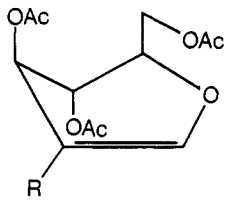
confirmed that the favored arrangement for five-bond coupling occurs when protons in a 1,4 relationship are diequatorial in a saturated, six-membered, oxygen heterocycle. Thus, a long-range coupling of 0.42 Hz was observed between proton H3,5 with proton H4 in 1,2-*O*-isopropylidene-3,5-*O*-[(*endo*-methoxy)methylidene]-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (**31**). This coupling was not observed for the *exo*-methoxy isomer (**32**).[184]

iv. Long-Range Coupling Constants $^4J(H,H)$, $^5J(H,H)$ in Unsaturated Systems. Long-range allylic coupling ^4J(H,H) (H—C—C=C—H) and homoallylic coupling ^5J(H,H) (H—C—C=C—C—H) constants are very sensitive to conformation.[92,177,178] Garbisch[185] found that the relationship between the allylic coupling and the torsion angle ϕ between the protons is given by

$$^4J\,(H,H) = 1.3\cos^2\phi - 2.6\sin^2\phi \qquad (0^\circ \leq \phi \leq 90^\circ)$$

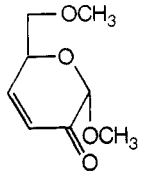
Allylic couplings can have either sign,[92,178] and in cyclic compounds experimental values range from +1.6 to −3.5 Hz.[178] An inversion of the sign of the coupling constants is observed for calculated values of the allylic coupling in propene as a function of the dihedral angle.[182] Homoallylic coupling constants are positive.[92,178]

Lemieux and O'Neill[186] have measured allylic coupling constants in 3,4,6-tri-*O*-acetyl-D-glucal (**33a**) and in the 2-substituted derivatives. The ^4J(H1,H3) coupling constants are 1.3 Hz for the glucal (**33a**), 1.1 Hz for the 2-chloroglucal (**33b**), and ≤0.3 Hz for the 2-nitro derivative (**33c**).

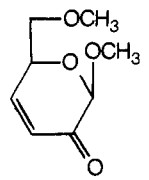


33a, R = H
b, R = Cl
c, R = NO_2

Allylic coupling constants have also been reported in hex-2-enosides[81,187] and hex-3-enopyranosiduloses (**34** and **35**).[187]



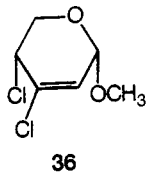
34, J(H3,H5) = |2.7| Hz



35, J(H3,H5) = |2.6| Hz

Coxon and coworkers have determined some of these long-range coupling constants using double resonance techniques in D-*glycero*-pent-2-enopyranosyl derivatives.[188] In **36**, J(H1,H4) = 1.3 Hz and J(H2,H4) = −1.2 Hz.

The negative sign for allylic couplings was further confirmed[12,40] and J(H1,H3) = −1.3 Hz in **37** and J(H4,H6a) = −1.4 Hz and J(H4,H6b) = −1.6 Hz in **38**.



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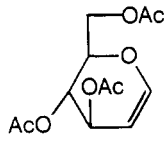

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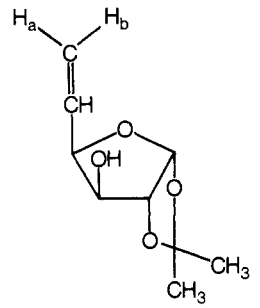

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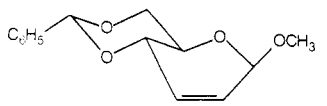
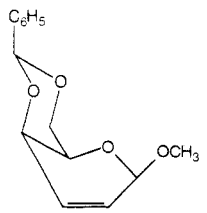
Lemieux and coworkers studied methyl 4,6-*O*-benzylidene-D-hex-2-enopyranosides (Table IV).[81] This work was extended to 2,3-unsaturated methyl pentosides (Table V).[189]

Measurement of coupling constants in unsaturated carbohydrate derivatives has been extended to heterocyclic *N*-glycosides[124] and to purine derivatives (Table VI).[190]

b. ^{13}C–Proton

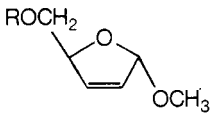
Vicinal Coupling Constants $^3J(^{13}C,H)$ in the System ^{13}C–C–X–H. The possible value of this coupling to studies of rotational isomerism was examined by Karabatsos and coworkers.[191-194] The dependence of $^3J(^{13}C,H)$ on torsion angle was established since the values of J(trans) and J(gauche) were estimated to be 3.5 (**49**) and 0.2 Hz (**50**) respectively for propionaldehyde-3-^{13}C and 7.8 (**51**) and 0.7 (**52**) Hz for propionaldehyde-3-^{13}C oxime *O*-

TABLE IV. Long-Range Coupling Constants Observed in Methyl 4,6-O-Benzylidene-D-hex-2-enopyranosides[81]

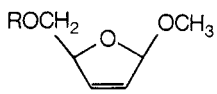
	J (H1,H3)	J (H1,H4)	J (H2,H4)
 **39** α-Erythro isomer (**16**)	−1.2	2.6	−2.5
	<0.3	1.5	−2.2
 **40** α-Threo isomer (**17**)	−0.8	1.7	~0
	<0.3	<0.3	<0.3

(185) E. W. Garbisch, Jr., *J. Amer. Chem. Soc.*, **86**, 5561 (1964).
(186) I. K. O'Neill with R. U. Lemieux, Ph.D. Thesis, University of Alberta, Edmonton, Canada, 1966.
(187) E. F. L. J. Anet, *Carbohyd. Res.*, **1**, 348 (1966).
(188) B. Coxon, H. J. Jennings, and K. A. McLauchlan, *Tetrahedron*, **23**, 2395 (1967).
(189) R. U. Lemieux, K. A. Watanabe, and A. A. Pavia, *Can. J. Chem.*, **47**, 4413 (1969).
(190) R. J. Ferrier and M. M. Ponpipom, *J. Chem. Soc. C*, 560 (1971).
(191) G. J. Karabatsos, *J. Amer. Chem. Soc.*, **83**, 1230 (1961).
(192) G. J. Karabatsos, J. D. Graham, and F. M. Vane, *J. Amer. Chem. Soc.*, **84**, 37 (1962).
(193) G. J. Karabatsos and C. E. Orzech, Jr., *J. Amer. Chem. Soc.*, **87**, 560 (1965).
(194) G. J. Karabatsos, C. E. Orzech, Jr., and N. Hsi, *J. Amer. Chem. Soc.*, **88**, 1817 (1966).

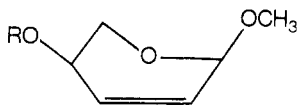
TABLE V. Allylic Coupling Constants for 2,3-Unsaturated Methyl Pentosides[189]



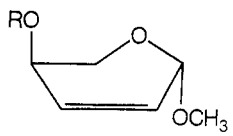
41a, R = Bz
b, R = Ac
c, R = H



42a, R = Bz
b, R = Ac
c, R = H



43a, R = Bz
b, R = Ac
c, R = H

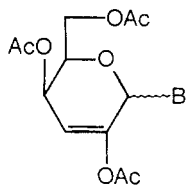


44a, R = Bz
b, R = Ac
c, R = H

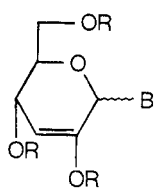
Compound	Approximate coupling constants,[a] Hz J(H1,H3)	J(H2,H4)
α-D-Furanoside		
41a	−1.2	−1.2
41b	−1.2	−1.2
41c	−1.2	−1.2
β-D-Furanoside		
42a	−1.2	−1.2
42b	−1.2	−1.2
42c	−1.2	−1.2
α-L-Pyranoside		
43a	−0.9	−1.2
43b	−1.2	−1.2
43c	−1.1	−2.0
β-L-Pyranoside		
44a	<0.3	0
44b	<0.3	0
44c	−0.8	<0.5

[a] The signs are assumed for these coupling constants.

TABLE VI. Coupling Constants (Hz) for Purine Derivatives[190]



45, B = theophyllin-7-yl
46, B = 2,6-dichloropurin-9-yl

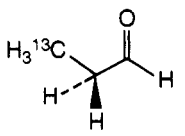


47, R = Ac; B = theophyllin-7-yl
48, R = Bz; B = theophyllin-7-yl

	Isomer	J(H1′,H3′)	J(H1′,H4′)
45	α	1.0	0
	β	<0.5	<0.5
46	α	1.0	0
	β	1	1.7
47	α	0.7	0.7
	β	1.7	2.0
48	α	0.7	1.5
	β	1.7	~3

methyl ether. This was also observed more recently in the spectra of ^{13}C-enriched α-D-glucopyranose.[195]

Lemieux and coworkers[196] observed a torsion angle coupling constant relationship by measuring coupling



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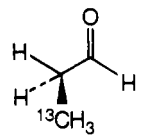

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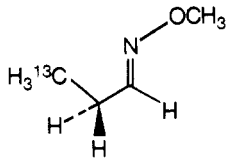
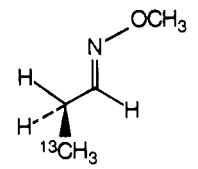


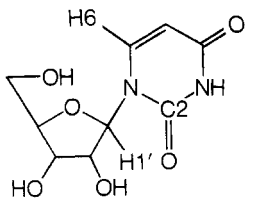
51



52

constants in ^{13}C-enriched (at the 2 position) uridine (**53**) and the cyclonucleoside structures **54** and **55** and related structures of known conformation.[197] The results are given in Table VII and are plotted in Figure 8[196] with the

TABLE VII. Relationship between Torsional Angle and Vicinal ^{13}C–^{1}H Coupling Constants[196]



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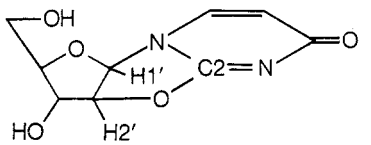
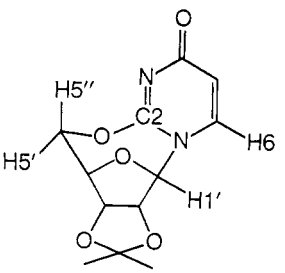


54



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Vicinal atoms	$^{3}J(^{13}C,H)$, Hz	Torsion angle φ, deg
C2–H1′	2.4 (**53**)	−45
	3.6 (**54**)	−125
	6.6 (**55**)	170
C2–H2′	2.0 (**54**)	120
C2–H5′	8.7 (**55**)	175
C2–H5″	2.0 (**55**)	50
C2–H6	8.0 (uracil)	180
	8.0 (**53**)	180
	7.2 (**55**)	~175

assumption that the signs of the coupling constants are positive throughout the range of the torsion angles. The torsion angles (see Table VII) were originally estimated from molecular models[196] and recently determined by X-ray crystallographic analysis.[197] Except for φ(C2–H5′) and φ(C2–H5″), the agreement was very good. For **55**, an interesting bonding electron density distribution was observed[197] and could account for the exceptionally strong coupling [J(C2,H5′) = 8.7 Hz] for the size of the torsion angle [φ(C2–H5′) = 158°]. Hence, information on conformational preferences of molecules may be usefully obtained from the measurement of vicinal ^{13}C to H coupling constants.[196] This conclusion is now supported by similar observations reported by Schwarz and Perlin.[198] Also, recently, Wasylishen and Schaefer[199] calculated that the

(195) A. S. Perlin and B. Casu, *Tetrahedron Lett.*, 2921 (1969).

(196) R. U. Lemieux, T. L. Nagabhushan, and B. Paul, *Can. J. Chem.*, **50**, 773 (1972).

(197) L. T. J. Delbaere, M. N. G. James, and R. U. Lemieux, *J. Amer. Chem. Soc.*, **95**, 7866 (1973).

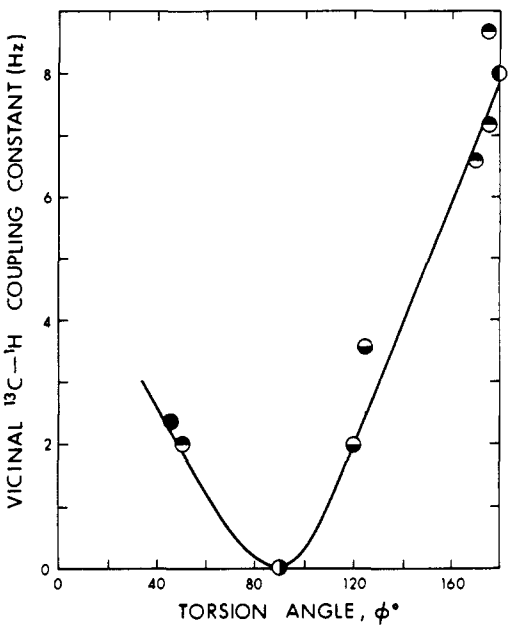


Figure 8. The plot of the vicinal $^{13}C-^{1}H$ coupling constants *vs.* the torsion angles for compounds **53** (●), **54** (◒), **55** (◓), uracil (◐), and α-D-glucopyranose triacetate 1,2-(methyl orthoacetate) (◑).[196]

torsion angle dependence of $^{3}J(^{13}C,H)$ in propane may be represented by

$$^{3}J(^{13}C,H) = 4.26 - 1.00 \cos \phi + 3.56 \cos 2\phi$$

where ϕ is the torsion angle. The magnitudes of the coupling constants determined using this equation are in remarkable agreement with the data obtained for the cyclonucleosides.[196]

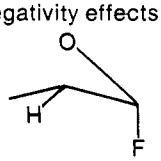
c. ^{31}P-Proton

Vicinal Coupling Constants $^{3}J(^{32}P,H)$ in the System $^{31}P-O-C-H$. Tsuboi and coworkers[200-202] have studied the conformation of the P-O-C-H group in nucleotides and dinucleotide phosphates by analyzing the $^{3}J(^{31}P,H)$ coupling constants, which were also considered to be sensitive to the torsion angle. The values for $^{3}J(^{31}P,H)$ of 4.5, 1.9, 22.3, and 23.5 Hz were assigned to torsion angles of 60, 65, 175, and 180°, respectively, for the system (PO_3^{2-})-O-C-H in aqueous solution. The investigation was extended to 1,2,3-dioxaphosphorinanes.[203]

d. ^{19}F-Proton

i. Geminal Coupling Constants $^{2}J(H,F)$ in the System H-C-F. Hall and coworkers have extensively studied specifically fluorinated carbohydrates,[25,204,205] observing both the proton and fluorine resonances. The measurements were extended to geminal $^{19}F-^{1}H$ couplings of a series of hexopyranosyl fluoride derivatives,[25] and these couplings were found to depend on the orientation of the substituent at C2. In the case of an equatorial substituent, $J(H,F)$ is *ca.* 53 Hz. When the C2 substituent is axial, $J(H,F)$ appears somewhat weaker, *ca.* 49 Hz. Heteronuclear decoupling experiments on 3,4,5-tri-*O*-acetyl-2-bromo-2-deoxy-α-D-mannopyranosyl fluoride[206] showed this coupling, 50.2 Hz, to be positive.

ii. Vicinal Coupling Constants $^{3}J(H,F)$ in the System F-C-C-H. Vicinal $^{19}F-^{1}H$ coupling constants parallel the general stereospecificity of vicinal $^{1}H-^{1}H$ couplings and are positive in sign.[207] Hall and associates (ref 25, 44, 83, 204-206, 208-216) later examined this relationship in a large variety of fluorinated sugar derivatives and indeed found that the vicinal coupling, $^{3}J(^{19}F,H)$, is stronger for the atoms in antiparallel orientations, $J(180°)$ = 24 Hz, than when the atoms are in *gauche* relationship. A very high dependence of coupling on torsion angle in the range about 50-70° seems evident from the observation that the vicinal coupling in structures of type **56** (axial fluorine, equatorial hydrogen) is in the range 1-1.5 Hz but in the range 7.5-12.6 Hz for compounds of type **57** (equatorial fluorine, axial hydrogen). Ihrig and Smith[217] have suggested from a study of a series of fluoro-substituted bicyclo compounds that, although a Karplus-type relationship exists between the torsion angle and $^{3}J(^{19}F,H)$, the phenomenon is highly sensitive to substituent electronegativity effects.



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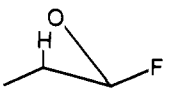

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Recently, Phillips and Wray[218] examined a number of deoxyfluoro-D-glucopyranoses, and an attempt was made to rationalize the $^{3}J(^{19}F,H)$ coupling constants on the basis of conformational electronegativity effects. Foster and coworkers[219] have applied the use of ^{19}F resonance data to probe the mutarotational equilibrium for 3-deoxy-3-fluoro-L-idose and considered the implications of $^{3}J(^{19}F,H)$ couplings on the conformational properties of the components.

iii. Long-Range Coupling Constants $^{4}J(H,F)$ and $^{5}J(H,F)$. The long-range $^{19}F-^{1}H$ coupling constants across four saturated bonds parallel the corresponding $^{1}H-^{1}H$ couplings;[220] *i.e.*, the long-range $^{19}F-^{1}H$ coupling constants are largest when the five nuclei are coplanar, for example, in a 1,3-diequatorial relationship. In this orientation for the nuclei, the ^{4}J coupling is positive, but the

(198) J. Schwarz and A. S. Perlin, paper presented at the 55th Chemical Conference and Exhibition, Chemical Institute of Canada, June 5-7, 1972, Quebec City, Canada, Paper 162.

(199) R. Wasylishen and T. Schaefer, *Can. J. Chem.*, **50**, 2710 (1972).

(200) M. Tsuboi, F. Kuriyagawa, K. Matsuo, and Y. Kyogoku, *Bull. Chem. Soc. Jap.*, **40**, 1813 (1967).

(201) M. Tsuboi, M. Kainosho, and A. Nakamura in "Recent Developments of Magnetic Resonance in Biological Systems," S. Fugiwara and L. H. Piette, Ed., Hirokawa Publishing Co., Tokyo, 1968, p 43.

(202) M. Tsuboi, S. Takahashi, Y. Kyogoku, H. Hayatsu, T. Ukita, and M. Kainosho, *Science*, **166**, 1504 (1969).

(203) M. Kainosho, A. Nakamura, and M. Tsuboi, *Bull. Chem. Soc. Jap.*, **42**, 1713 (1969).

(204) L. D. Hall and J. F. Manville, *Chem. Ind. (London)*, 991 (1965).

(205) L. D. Hall and J. F. Manville, *Can. J. Chem.*, **47**, 19 (1969).

(206) L. D. Hall and J. F. Manville, *Carbohyd. Res.*, **9**, 11 (1969).

(207) K. L. Williamson, Y.-F. Li, F. H. Hall, and S. Swager, *J. Amer. Chem. Soc.*, **88**, 5678 (1966).

(208) L. D. Hall and J. F. Manville, *Chem. Ind. (London)*, 468 (1967).

(209) L. D. Hall and J. F. Manville, *Chem. Commun.*, 37 (1968).

(210) A. D. Barford, A. B. Foster, J. H. Westwood, and L. D. Hall, *Carbohyd. Res.*, **11**, 287 (1969).

(211) J. Adamson, A. B. Foster, L. D. Hall, R. N. Johnson, and R. H. Hesse, *Carbohyd. Res.*, **15**, 351 (1970).

(212) L. D. Hall and P. R. Steiner, *Can. J. Chem.*, **48**, 451 (1970).

(213) L. D. Hall and P. R. Steiner, *Can. J. Chem.*, **48**, 2439 (1970).

(214) A. B. Foster, R. Hems, and L. D. Hall, *Can. J. Chem.*, **48**, 3937 (1970).

(215) J. S. Brimacombe, A. B. Foster, R. Hems, J. H. Westwood, and L. D. Hall, *Can. J. Chem.*, **48**, 3946 (1970).

(216) L. D. Hall, P. R. Steiner, and C. Pedersen, *Can. J. Chem.*, **48**, 1155 (1970).

(217) A. M. Ihrig and S. L. Smith, *J. Amer. Chem. Soc.*, **92**, 759 (1970).

(218) L. Phillips and V. Wray, *J. Chem. Soc. B*, 1618 (1971).

(219) A. B. Foster, R. Hems, J. H. Westwood, and J. S. Brimacombe, *Carbohyd. Res.*, in press.

(220) A. B. Foster, R. Hems, L. D. Hall, and J. F. Manville, *Chem. Commun.*, 158 (1968).

sign can change when four of the nuclei are in one plane, for example, when the fluorine and the hydrogen are not both in equatorial orientation. However, the long-range, four-bond coupling constants between an axial fluorine and an axial proton were found to be positive in 2-deoxy-2-fluorohexopyranosyl fluorides.[221] Stereospecific $^4J(F,H)$ coupling constants have been established in 3-fluorinated glucose derivatives and follow these patterns.[214,215] Five-bond $^{19}F-^1H$ coupling constants have also been studied in pyranose derivatives.[220,222]

Long-range $^{19}F-C-O-C-^1H$ four-bond coupling constants have been observed in furanose derivatives.[216] In a series of seven pentofuranosyl fluoride derivatives this coupling has a magnitude of 5.5–7.9 Hz when the atoms are in trans relationship. In the cis relationship, the coupling is between 1.0 and 1.8 Hz.[216] Smaller ^4J values were observed for the couplings between the fluorine and H3, namely, 1.7 to 2.4 Hz for the cis relationship and 0.7 Hz for the trans relationship.[216] These studies were extended to several hexofuranosyl fluoride derivatives.[213] In 5-fluoropyrimidine nucleosides, a long-range ^5J coupling is observed between ^{19}F and the anomeric proton. The magnitude of this coupling is dependent upon the anomeric configuration of the nucleoside and is >1.5 Hz for the β isomers and <1.5 Hz for the α isomers.[223]

e. Fluorine–Fluorine

Vicinal Coupling Constants $^3J(^{19}F,^{19}F)$ in the System $^{19}F-C-C-^{19}F$. The vicinal $^{19}F-^{19}F$ coupling constants of the α and β anomers of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl fluoride and of the corresponding D-manno derivatives were measured.[221] The trans coupling is −20.0 Hz while the *gauche* couplings range from −13.5 to −18.8 Hz.

3. Chemical Shift

a. Proton Magnetic Resonance

The chemical shift has proven of great value to carbohydrate investigations. The early observations[224] that the chemical shift of a proton depends on its environment in the molecule was demonstrated in 1958 by Lemieux and coworkers[18,19] to apply to diastereoisomeric compounds. That pmr chemical shifts of a molecule are strongly dependent upon substitutional, orientational, and electronegativity effects of neighboring and distant groupings has since become of broad utility to organic chemistry.[93,225-228] Booth,[93] in an excellent review article on conformational analysis of cyclic compounds, summarizes those effects which are well recognized.

i. Substitutional Effects. One of the most interesting stereospecific dependences found in the original study[18,19] of O-acetylated pyranose derivatives was that an equatorial proton normally resonates at lower field than the chemically similar but axially orientated proton. Specific structural and configurational features can, however, cause exceptions to this general rule.[39] The shielding effect of a neighboring carbonyl group[229] on the equatorial proton and the deshielding of an axial proton by a syn-axial group[37] are the more common sources of exceptions to the rule, but the nature and orientation of neighboring groups also can have an important influence.

TABLE VIII.[a] The Effects of Configuration on the Proton Magnetic Resonance Chemical Shifts of Hexopyranose Pentaacetates[38]

Carbohydrate	Chemical shift[b] H-1 Obsd	H-1 Calcd[c]	H-5 Obsd	H-5 Calcd[c]
β-D-*gluco*	5.76	5.75	3.9	3.9
β-D-*manno*	5.93	5.95	3.9	3.9
β-D-*allo*	6.00	6.00	4.2	4.15
β-D-*galacto*	5.74	5.75	4.1	4.1
β-D-*gulo*	6.00	6.00	4.3	4.35
α-D-*gluco*	6.34	6.35	4.1	4.15
α-D-*manno*	6.09	6.15	4.1	4.15
α-D-*galacto*	6.36	6.35	4.4	4.35
α-D-*altro*	6.02	5.95	4.5	4.4
α-D-*gulo*	6.25	6.15	4.6	4.6

[a] The spectra are observed in $CDCl_3$. [b] The chemical shift is in ppm. [c] The empirical rules from ref 38 were used to calculate a value for the chemical shift.

By considering the dependence of ring proton shifts upon the orientation of a given proton with respect to the ring and upon the orientation of other electronegative substituents attached to the ring, Lemieux and Stevens[38] established simple empirical rules to correlate the chemical shifts of the ring protons in acetylated glycopyranoses. Using the chemical shifts of the ring protons of β-D-glucopyranose pentaacetate and β-D-xylopyranose tetraacetate in $CDCl_3$ as the references for the acetylated hexo- and pentopyranoses, the effects on changing the sugar configuration could be expressed to a good approximation as follows.

(1) If the proton under consideration has remained axial, (a) add 0.20 ppm for an axial acetoxy group at a neighboring position (*i.e.*, the shielding of the axial proton by a neighboring equatorial acetoxy group is greater than by a neighboring axial acetoxy group); (b) add 0.25 ppm for an opposition of the proton by an axial acetoxy group (*i.e.*, the syn-axial acetoxy group has a deshielding effect on the axial proton).

(2) If the proton under consideration has achieved the equatorial orientation, (a) add 0.60 ppm because of the change from the environment of an axial hydrogen to that of an equatorial hydrogen; (b) subtract 0.20 ppm for an axial acetoxy group at a neighboring position (*i.e.*, the effect is opposite to that noted above for the axial proton); (c) subtract 0.20 ppm for an axial acetoxy group at the next to neighboring position (an apparent long-range effect).

The agreement found for the H1 and H5 protons in a series of hexopyranose acetates is seen in Table VIII.

The above studies were extended to free sugars in aqueous solutions, and empirical rules for calculating proton chemical shifts in pyranoses were also presented.[39] The chemical shifts of the ring protons of β-D-xylopyranose and β-D-glucopyranose in D_2O served as the reference compounds. These are as follows.

(1) If the proton under consideration is in an equatorial orientation, add 0.60 ppm.

(2) If the proton under consideration is in an axial orientation, (a) add 0.30 ppm for each neighboring axial hydroxyl group; (b) add 0.35 ppm for each axial hydroxyl group which is in opposition to the axial proton.

The procedure provides the following results for compounds in the conformations shown in Table IX.[39] Similar agreement was obtained when the empirical rules were

(221) L. D. Hall, R. N. Johnson, J. Adamson, and A. B. Foster, *Can. J. Chem.*, **49**, 118 (1971).

(222) A. B. Foster, R. Hems, and J. H. Westwood, *Carbohyd. Res.*, **15**, 41 (1970).

(223) R. J. Cushley, I. Wempen, and J. J. Fox, *J. Amer. Chem. Soc.*, **90**, 709 (1968).

(224) J. T. Arnold, S. S. Dharmatti, and M. E. Packard, *J. Chem. Phys.*, **19**, 507 (1951).

(225) Reference 2, Chapters 3, 4, and 10.

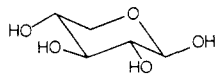
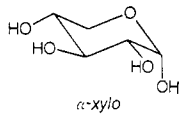
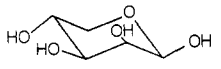
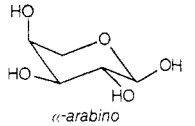
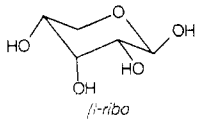
(226) Reference 6, Chapter IV.

(227) Reference 4, Parts 2 and 3.

(228) Reference 5, Chapter III.

(229) A. Nickon, M. A. Castle, R. Harada, C. E. Berkoff, and R. O. Williams, *J. Amer. Chem. Soc.*, **85**, 2185 (1963).

TABLE IX. Pmr Chemical Shifts[a] for Pentopyranoses in D_2O[39]

		H_1	H_2	H_3	H_4	H_{5a}	H_{5e}
 Reference *β-xylo*		4.65	3.26	3.49	3.58	3.36	4.00
 *α-xylo*	Obsd	5.26	3.63				
	Calcd[b]	5.25	3.56				
 *β-lyxo*	Obsd	4.94	4.04			3.32	4.09
	Calcd[b]	4.95	3.86			3.36	4.00
 *α-arabino*	Obsd	4.60	3.58	3.75	4.04		
	Calcd[b]	4.65	3.61	3.79	4.18		
 *β-ribo*	Obsd	4.99	3.60	4.13	3.79		
	Calcd[b]	5.00	3.56	4.09	3.88		

[a] In ppm from external tetramethylsilane. [b] The calculated values are obtained using the empirical rules from ref 39.

TABLE X. Pmr Chemical Shifts[a] for Hexopyranoses in D_2O[39]

Proton	Glucose α	Glucose β	Mannose α	Mannose β	Allose[b] α	Allose[b] β	Galactose α	Galactose β	Talose α	Talose β
H1 obsd	5.32	4.74	5.25	4.97	...	5.00	5.34	4.68	5.37	4.88
calcd	5.34	...	5.34	5.04		5.09	5.34	4.74	5.34	5.04
H2 obsd	3.60	3.32	4.01	4.03	...	...	3.87	3.55	...	...
calcd	3.62	...	3.92	3.92			3.97	3.67		

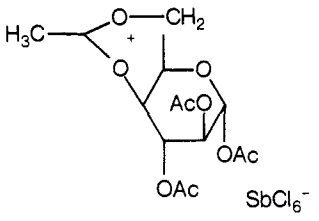
[a] Chemical shifts are in ppm from external tetramethylsilane. [b] The chemical shift for H_3^{β} was 4.28 ppm.

applied to hexopyranoses using the chemical shifts for the ring protons of β-D-glucopyranose as reference (Table X).

These studies can be of considerable use in the establishment of points of configuration especially at the anomeric center. For example, it follows that, for an anomeric pair of aldopyranoses, (a) if the anomeric protons are shifted by about 0.60 ppm then the substituents at C2 and C3 are both in equatorial orientation; (b) if the chemical shift is only about 0.2–0.3 ppm, then one of the substituents on either C2 or C3 is axial (if one form shows H1 to be strongly (about 8 Hz) coupled to H2, then H3 is axial); (c) if there is little chemical shift, then both the substituents on C2 and C3 may be in axial orientation; (d) in the case of anomers with H3 and/or H5 in axial orientation, the form which has the axial proton(s) to lower field is the form that has the substituent at C1 in axial orientation (this rule is of particular value for acetylated structures since, for these, the signal for H5 is normally readily observed).

Stevens and Fletcher have reported chemical shifts for protons in a large number of derivatives of pentofuranoses.[230]

Paulsen and coworkers[231] have studied carbohydrate carbonium ions. For example, the nmr spectrum of tri-*O*-acetyl-α-D-idopyranose-4,6-acetoxonium hexachloroantimonate (**58**) in nitromethane at −25° showed the methyl signal of the acetoxonium group to be chemically shifted 0.76 ppm downfield from the *O*-acetyl signals. Similar chemical shifts for the methyl signals of 2-methyl-1,3-dioxolenium tetrafluoroborate (2.67 ppm) and 2-methyl-*cis*-4,5-tetramethylene-1,3-dioxolenium tetrafluoroborate (2.72 ppm) had been observed by Winstein and coworkers.[232]



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ii. Solvent Effects. The solvent effect on the chemical shift is, by definition, the change in the resonance position of some given proton of the solute between solvents A and B (at infinite dilution) measured from some common reference.[233] Solvent effects arise as a consequence of intermolecular forces and interactions. Their importance to nmr measurements arises from the relatively high concentrations of solute that are necessary, compared with other spectroscopic methods, to observe a signal. The deliberate use of solvent shifts may help in

(230) J. D. Stevens and H. G. Fletcher, Jr., *J. Org. Chem.*, **33**, 1799 (1968).

(231) H. Paulsen, W. P. Trautwein, F. G. Espinosa, and K. Heyns, *Chem. Ber.*, **100**, 2822 (1967).

(232) C. B. Anderson, E. C. Friedrich, and S. Winstein, *Tetrahedron Lett.*, 2037 (1963).

(233) P. Laszlo, *Progr. Nucl. Magn. Resonance Spectrosc.*, **3**, 231 (1967).

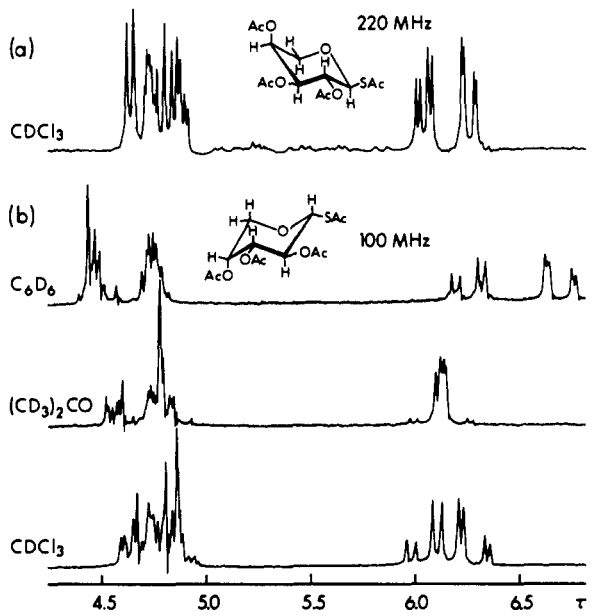

Figure 9. (a) The partial 220-MHz proton magnetic resonance spectrum of 1-thio-α-L-arabinopyranose tetraacetate in $CDCl_3$ to be compared with (b), the partial 100-MHz spectrum of 1-thio-α-D-arabinopyranose tetraacetate in C_6D_6, $(CD_3)_2CO$, and $CDCl_3$.[241]

the determination of molecular conformations and structures by simplification of spectra due to the characteristics of particular solvents.[234] A voluminous literature exists on solvent effects in nmr and has been reviewed by several authors.[233,235]

Most carbohydrate derivatives are studied in $CDCl_3$ with TMS as internal standard. To study particular solvent effects, spectra are observed in polar solvents such as $(CD_3)_2CO$, D_2O, CD_3CN, $(CD_3)_2SO$, or aromatic solvents like C_6D_6 and C_5D_5N. In a given solvent, resonances corresponding to two or more different nuclei in the same molecule may have nearly identical chemical shifts, but a change in the solvent will often separate these resonances and thus simplify the spectrum.[38] Solutes with exchangeable protons normally arising from OH or NH groups may provide anomalous chemical shifts for these protons because of rapid exchange with trace impurities (usually water) so that an averaged chemical shift is observed. If it is desired to examine the chemical shifts and fine structure of the signals for exchangeable protons, the use of a strong hydrogen-bonding solvent such as $(CD_3)_2SO$ will reduce the rate of proton exchange. Thus, coupling constants and chemical shifts for the protons in groups such as H-C-N-H and H-C-O-H can be readily measured.[150,168-175,236-239] In the case of amines, the use of a strong acid such as trifluoroacetic acid can decrease the rate of exchange so that the fine structure of the protons attached to nitrogen is readily observed.[167] On the other hand, the addition of a solvent with exchangeable deuterium such as D_2O or CD_3OD leads to exchange of the proton by a deuteron and simplifies the spectrum since the active hydrogen signal will no longer appear and ^3J(D,H) couplings are very weak.[240]

(234) A. D. Buckingham, T. Schaefer, and W. G. Schneider, *J. Chem. Phys.*, **32**, 1227 (1960).

(235) J. Ronayne and D. H. Williams, *Annu. Rev. NMR (Nucl. Magn. Resonance) Spectrosc.*, **2**, 83 (1969).

(236) J. T. Marvel, S. K. Sen, J. W. Berry, and A. J. Deutschman, Jr., *Carbohyd. Res.*, **8**, 148 (1968).

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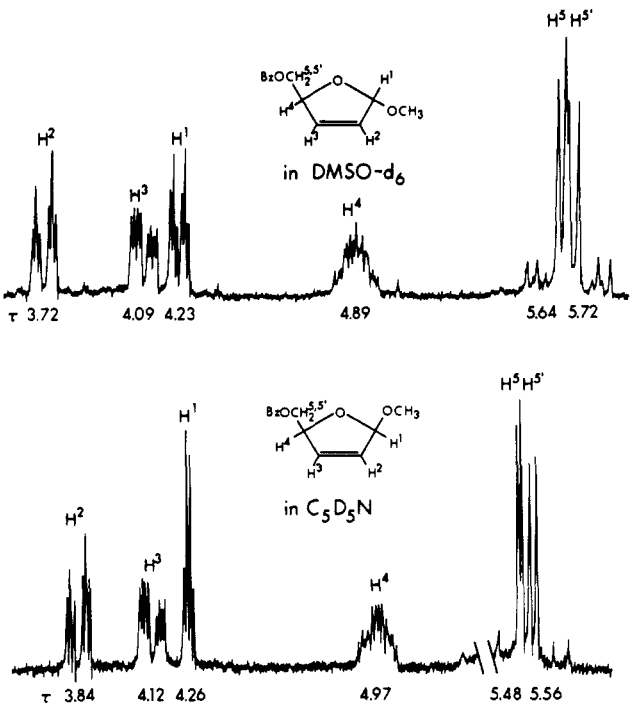


Figure 10. The 100-MHz proton magnetic resonance spectra of methyl 5-*O*-benzoyl-2,3-dideoxy-α-D-*glycero*-pent-2-enofuranoside and its β anomer.[189]

Aromatic solvents induce a solvent shift on solute protons[233,235] owing to the magnetic anisotropy of the aromatic ring. This implies large upfield shifts for protons situated above the aromatic plane (shielding) and large downfield shifts (deshielding) for protons in the plane of the aromatic ring.[234] Aromatic solvents may consequently be useful to better approximate first-order spectra.

Holland and coworkers reported a study of the pmr spectra of a series of acetylated 1-thioaldopyranoses.[241] The use of a solvent effect to simplify spectra is well demonstrated by the results reported in Figure 9. It is seen that the spectrum for 1-thio-α-D-arabinopyranose tetraacetate in $(CD_3)_2CO$ at 100 MHz is highly unrevealing as compared to those in $CDCl_3$ and C_6D_6. Although the change in applied field from 100 to 220 MHz brought about further resolution, the best resolution was achieved at 100 MHz using C_6D_6 as solvent.[241] Lemieux, Watanabe, and Pavia[189] could distinguish methyl 5-*O*-benzoyl-2,3-dideoxy-α-D-*glycero*-pent-2-enofuranoside from its β anomer only by using solvent techniques. For the α anomer, of seven solvents used only $(CD_3)_2SO$ brought about a complete separation of the signals for H1 and H3. The specific solvent effect on the nmr spectrum is seen in Figure 10. It is noteworthy here that the desired resolution resulted from a nonaromatic solvent which may have been expected to have an effect similar to that of chloroform.

It was early noted[18] that axial acetoxy groups tend in general to produce their methyl resonance to lower field than equatorial acetoxy groups with chloroform as solvent. This tendency can be assigned, as a first approximation, to the preferred orientation of acetoxy groups[242] and, as a consequence, the methyl group of an equatorial acetoxy group is shielded as a result of the diamagnetic anisotropy of the carbonyl group of a neighboring acetoxy group; compare, for example, partial structures **59**

(240) Reference 5, p 132.

(241) C. V. Holland, D. Horton, M. J. Miller, and N. S. Bhacca, *J. Org. Chem.*, **32**, 3077 (1967).

(242) J. S. Martin, J. Hayami, and R. U. Lemieux, *Can. J. Chem.*, **46**, 3263 (1968).

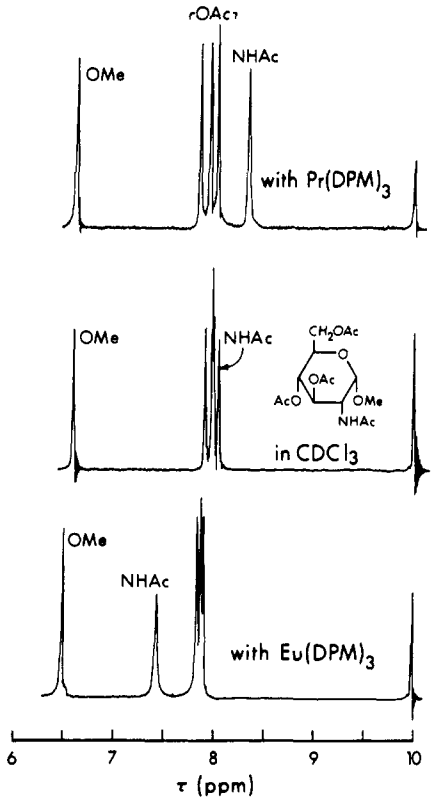
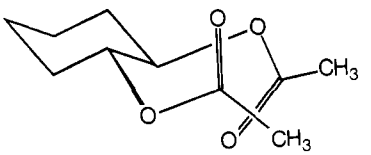


Figure 11. The 60-MHz proton magnetic resonance spectra of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside in $CDCl_3$ solution. The normal spectrum is shown in the middle, the upper spectrum is observed with $Pr(DPM)_3$, whereas the lower spectrum is observed with $Eu(DPM)_3$.[253]

and **60**. However, as pointed out by Lemieux and Stevens,[38] there is no regularity to the chemical shift involved and, indeed, exceptions to the rule exist with chloroform as solvent and the shifts are strongly solvent dependent. For example, the chemical shifts of axial acetoxy groups were not readily distinguishable from those of equatorial acetoxy groups when pyridine was the solvent.[38]



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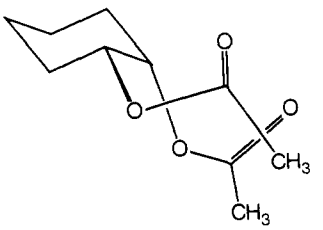

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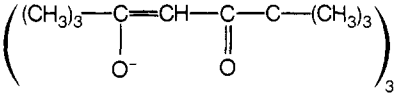
The aromatic solvent-induced shifts of a variety of acetylated monosaccharides have also been studied.[243-247] Freemantle and Overend[245] showed that the proton shift of the acetoxy methyl resonance occurs to higher field in C_6D_6 (0.3–0.45 ppm) than in $CDCl_3$. The absolute values of the solvent-induced shifts cannot be rationalized quantitatively and, therefore, the use of chemical shifts for acetoxy groups to determine points of configuration and conformation can only serve as a guide.[38]

Perlin,[171a] Casu and coworkers,[170] and Marvel and coworkers[236] have studied the hydroxyl proton resonances of sugars in dimethyl sulfoxide. The anomeric hydroxyl of glucose and related sugars gives a proton resonance at the lowest field, its chemical shift and splitting being characteristic of the configuration of the anomeric linkage [τ 3.70–4.05 ppm, ^3J = 4.0–4.5 Hz for α anomers (OH axial) and τ 3.40–3.68 ppm, ^3J = 6.0–7.0 Hz for β anomers (OH equatorial)].[170] Nonanomeric hydroxyls of glucose and most sugars related to glucose give nmr peaks in the range τ 5–6, taken as characteristic of hydroxyl groups free to associate with dimethyl sulfoxide by hydrogen bonding.[170] Dimethyl sulfoxide was also used in the studies of seven osotriazoles,[150] and the chemical shifts and coupling constants were interpreted in terms of preferred conformations of the molecules.

iii. Shift Reagents. Improvements in resolution have been obtained by the use of nmr shift reagents, namely europium[248-251] and praseodymium[252] chelates of dipivaloylmethane ($Eu(DPM)_3$ and $Pr(DPM)_3$). These have produced remarkable shifts and simplification of the pmr spectra of carbohydrates[253-255] when the structural requirement for complexing is present, normally an hydroxyl or carbonyl group, and the material is soluble in an appropriate aprotic solvent.[256,257] Butterworth, *et al.*,[253] measured the pmr spectrum of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside in $CDCl_3$ at 60 MHz, shown in Figure 11. Comparison with the spectra of the same solution after the addition of $Eu(DPM)_3$ (**61**) and $Pr(DPM)_3$ (**62**) demonstrates the



M

61, M = Eu^{3+}

62, M = Pr^{3+}

utility of nmr shift reagents for spectral simplification and definitive peak assignments. It can be seen from Figure 11 that characteristic downfield and upfield shifts of the *N*-acetyl methyl signal can serve as a diagnostic tool for the assignment of *N*-acetyl signals in the presence of acetoxy signals by utilizing shift reagents. *N*-Acetyl resonances may also be differentiated from *O*-acetyl signals by inspection of the spectrum as they show relative line

(243) R. F. Butterworth, M. H. Freemantle, and W. G. Overend, *Chem. Ind. (London)*, 1485 (1968).

(244) M. H. Freemantle and W. G. Overend, *Chem. Commun.*, 503 (1968).

(245) M. H. Freemantle and W. G. Overend, *J. Chem. Soc. B*, 547 (1969).

(246) M. H. Freemantle and W. G. Overend, *J. Chem. Soc. B*, 551 (1969).

(247) K. D. Carlson, C. R. Smith, Jr., and I. A. Wolff, *Carbohyd. Res.*, **13**, 403 (1970).

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(250) P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *J. Amer. Chem. Soc.*, **92**, 5734 (1970).

(251) P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *J. Amer. Chem. Soc.*, **92**, 5737 (1970).

(252) J. Briggs, G. H. Frost, F. A. Hart, G. P. Moss, and M. L. Staniforth, *Chem. Commun.*, 749 (1970).

(253) R. F. Butterworth, A. G. Pernet, and S. Hanessian, *Can. J. Chem.*, **49**, 981 (1971).

(254) I. Armitage and L. D. Hall, *Chem. Ind. (London)*, 1537 (1970).

(255) P. Girard, H. Kagan, and S. David, *Bull. Soc. Chim. Fr.*, 4515 (1970).

(256) J. K. M. Sanders and D. H. Williams, *J. Amer. Chem. Soc.*, **93**, 641 (1971).

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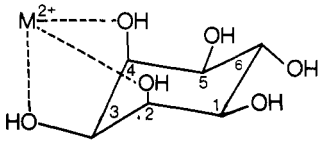
broadening arising from the presence of the nitrogen quadrupole moment provided that these resonances do not overlap.

iv. Intermolecular Interactions. Intermolecular interactions as well as conformational and solvent effects influence the chemical shift. Tetra-*O*-acetyl-β-D-glucopyranosyl halides and phenoxides in acetonitrile solutions show a specific deshielding of H1, H3, and H5 on addition of tetraethylammonium halides due to the formation of anion–molecule complexes.[242] The spectra of the nitrophenoxide derivative on addition of tetraethylammonium chloride are shown in Figure 12. On a simple electrostatic model the complexes may be regarded as involving the anion in an interaction with the molecule as a whole and not with any atom or functional group.[242]

Complexing of sugars with diamagnetic ions was studied by Angyal and Davies.[258] Addition of $CaCl_2$ to a solution of *epi*-inositol in D_2O causes a large downfield shift (0.32 ppm in 2*M* $CaCl_2$) of the H3 proton, whereas the other protons shift to a lesser extent. A possible complex is shown in **63** in which an axial–equatorial–axial se-



63

quence of three oxygen atoms in a six-membered ring is favored for complex formation.[258] These studies have now been extended to include a variety of carbohydrates and salts.[259]

b. ^{13}C Magnetic Resonance

The potential of ^{13}C studies in conformational analysis is now well recognized since the ^{13}C chemical shift is very sensitive to the conformational change.[260-264] Hence ^{13}C magnetic resonance studies have now been extensively applied to the study of carbohydrates,[65,195,262-272] nucleotides,[273,274] and nucleosides.[275-277]

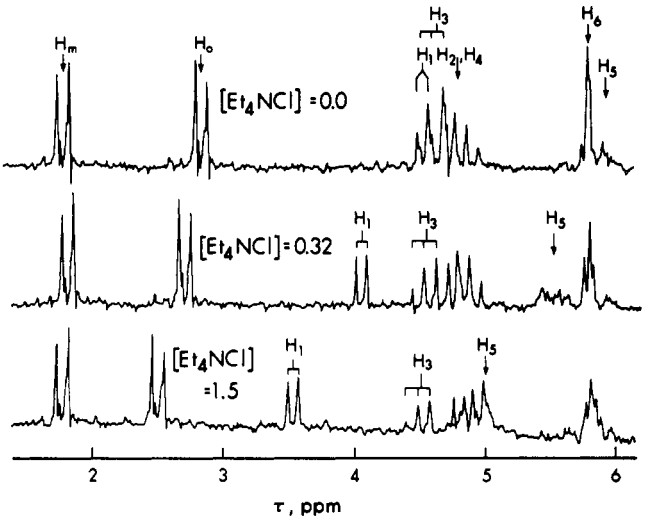


Figure 12. The effect of adding tetraethylammonium chloride on the 100-MHz spectrum of tetra-*O*-acetyl-β-D-glucopyranosyl *p*-nitrophenoxide in acetonitrile.[242]

Roberts and coworkers[278] have interpreted substituent effects of hydroxyl groups in alkyl-substituted cyclohexanols as a combination of steric and electronic effects. 1,3-Diaxial interactions were also found to have a significant effect on the ^{13}C chemical shifts. These measurements were extended to the inositols in an attempt to isolate and measure the steric effect apart from the inductive effects arising from the hydroxyl groups.[262,263] This is possible because the substituent at each carbon is the same. An empirical series of substituent parameters were derived for the unsubstituted inositols by assuming that the ^{13}C chemical shift differences are associated with changes in the steric perturbation accompanying the epimerization of any center in the molecule. The chemical shift of the carbon to which the hydroxyl group is directly attached (the α carbon) is chosen as the reference. A change in its chemical shift is then correlated with changes of the hydroxyl groups on the β, γ, and δ carbons from the equatorial to an axial configuration. As the hydroxyl group on the α carbon can adopt either an axial (a) or equatorial (e) conformation, the ^{13}C chemical shifts in two molecules were chosen as a reference. The single ^{13}C resonance in *scyllo*-inositol, which has all OH groups equatorial, served as one such reference and, for molecules with an axial hydroxy group, the C2 resonance of *myo*-inositol, which has five equatorial and one axial hydroxyls, was chosen. It was found that for the carbon with an α-equatorial hydroxyl, the change of orientation of one of the β-hydroxyl groups from equatorial to axial produced a shielding of about 1.7 ppm. The change in orientation from equatorial to axial of both β-hydroxyls caused a shielding of about 3.6 ppm. Thus, the effect appeared near additive. A change of one of the γ-hydroxyl groups from equatorial to axial orientation caused a shielding of about 2.8 ppm and, when both γ-hydroxyl groups were thus changed, the shift was nearly doubled, 6.8 ppm, and, therefore, again the effect seemed cumulative. The effect of changing the orientation of the δ-hydroxyl was very small and near the experimental error as would be expected from attenuation of the steric effects that would accompany increasing distance between the

(258) S. J. Angyal and K. P. Davies, *Chem. Commun.*, 500 (1971).
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(260) G. W. Buchanan, D. A. Ross, and J. B. Stothers, *J. Amer. Chem. Soc.*, **88**, 4301 (1966).
(261) D. K. Dalling and D. M. Grant, *J. Amer. Chem. Soc.*, **89**, 6612 (1967).
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interacting groups. The effect on the chemical shift of the reference carbon (with α-hydroxyl equatorial) of changing the orientation of both the β- and γ-hydroxyls from equatorial to axial orientation was thus expected to be about 1.7 + 2.8 = 4.5 ppm, but the observed shift to higher field was only 3.2 ppm. The similar effects on the chemical shift of a carbon with an α-hydroxyl in axial orientation indicated a slight shielding effect (0.6 ppm) for the change at the β position but a deshielding effect of about 2.3 ppm for the change at the γ position. The fact that both of the γ effects are largest was considered to reflect the importance of syn-axial interactions.

^{13}C nmr techniques were applied to some pentose and hexose aldopyranoses,[195,264-267] and chemical shift values are tabulated.[264,266] The sugars were studied mainly as equilibrium mixtures of mutarotational isomers. As would be expected, the resonances of the anomeric carbons are always to lower field than those of the remaining pyranose carbons. Also, the resonances corresponding to the C6 carbon on the unsubstituted hexoses occur to highest field. These resonances for an anomeric pair are always at least partially superimposed, agreeing with the observation that steric or proximity effects greatly diminish with distance.[264] Assignments were made based on spectra of D-glucose enriched with ^{13}C (D-glucose-^{13}C),[195] from the ^{13}C nmr spectrum of D-glucose-3-*d* and D-glucose-5,6,6'-d_3[267] and from measurements on other derivatives and structurally related compounds.[266] Dorman and Roberts[264] assigned many resonances by correlating observed chemical shift differences with changes in the chemical shift that would be expected from configurational and conformational effects. Comparison of the proton decoupling frequencies with the pmr spectra of these compounds served to corroborate these assignments.

Perlin, Casu, and Koch examined the ^{13}C chemical shifts of a number of aldoses and their methyl glycopyranosides.[266] The spectra of the anomeric methyl D-glycopyranosides were found to be close counterparts of those of the parent sugar except, of course, for the C1 signals. The anomeric carbons are deshielded by about 7 ppm. It was pointed out that the electron density at a given carbon nucleus is a major factor in determining the chemical shifts of ^{13}C nuclei, and, therefore, the chemical shifts were compared with electron densities estimated by molecular orbital calculations. Generally good correspondence was achieved. The chemical shifts could be related to changes in nonbonded interactions with changes in configuration. Thus, the overall shielding states of the ^{13}C nuclei of the different isomeric compounds appeared to reflect variations in the magnitude of repulsive interactions in the molecules. It was pointed out that the polarizing effects leading to deshielding of protons (as reflected by the empirical rules presented by Lemieux and Stevens[39]) leads to shielding of the ^{13}C nuclei. The effect of changes in relative orientation of vicinal bonds and their diamagnetic anisotropies must also have an effect as is considered to be the case for hydrogen atoms[279] since these are complementary to the polarization effects.

Perhaps the most convenient manner to present the data which have accumulated on the ^{13}C spectra of carbohydrates is by way of approximate empirical rules. Although the application of the rules provides neither appreciable theoretical insight nor high precision, the trends are delineated with few exceptions and could be useful for provisional assignments.

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TABLE XI. Application of the Empirical Rules for the ^{13}C Nmr Spectra of Inositols[a]

Inositol		Carbon atom 1	2	3	4	5	6
scyllo- (ref)		118.8	118.8	118.8	118.8	118.8	118.8
myo-	Obsd	120.1	120.3	120.1	121.4	118.2	121.4
	Calcd[b]	119.8	120.0	119.8	120.8	118.2	120.8
chiro-	Obsd	120.9	122.0	119.7	119.7	122.0	120.9
	Calcd	121.0	122.0	120.8	120.8	122.0	121.0
epi-	Obsd	120.8	118.0	122.4	118.0	120.8	125.7
	Calcd	120.0	118.1	121.2	118.1	120.0	122.8

[a] The observed chemical shifts are from ref 263 and are in ppm to high field of CS_2. [b] The chemical shifts are calculated using the empirical rules presented in the text.

Empirical rules for the ^{13}C spectra of inositols[263] are: (a) use the chemical shift of the ^{13}C atoms of the all-equatorial *scyllo*-inositol as reference (addition corresponds to shielding); (b) if the hydroxyl is axial, add 1.0 ppm (the bonded hydrogen is deshielded by 0.6 ppm[39]); (c) if an hydroxyl group on a geminal carbon is axial, add 1.2 ppm (the bonded hydrogen is deshielded by 0.3 ppm[39]); (d) if the attached hydroxyl is equatorial and an hydroxyl group is axial at the next to geminal position, add 2.0 ppm (the bonded hydrogen is deshielded by 0.35 ppm[39]); (e) if the attached hydroxyl is axial and an hydroxyl group is axial at the next to geminal position, subtract 1.7 ppm (the effect on the bonded equatorial hydrogen was not obtained[39]).

Using the above rules, the ^{13}C nmr spectra for the inositols listed in Table XI are accounted for as shown.

Substantial discrepancy only occurs for C6 of *epi*-inositol, but the rules correctly order the chemical shifts. It must be kept in mind that conformational purity is assumed but this must be only approximately so.

Similar empirical rules can be drawn up for sugars and their methyl glycopyranosides. The above-presented empirical rules for inositols provide a 1.0-ppm shift for the change from equatorial to axial orientation of the hydroxyl group bonded to the ^{13}C atom. In the case of sugars and their glycopyranosides, this rule does not apply at the anomeric center; for example, C1 of β-glucopyranose is shifted from C1 of the α anomer by 3.9 ppm. This is not surprising since the anomeric effect[55,167,280,281] requires a substantial change in bonding phenomena for a substituent at the anomeric center.[282a] The marked deshielding of C3 has been commented on[266] and appears related to the presence of the ring oxygen across the pyranose ring. The addition of about 2.0 ppm to the chemical shift otherwise calculated for the C3 atoms of pyranoses for the presence of at least one axial hydroxyl group in the molecule produces a result in much better agreement with the observed chemical shift. A possible special effect of this kind is perhaps not surprising since the changes in configuration should affect transannular polarization effects. Otherwise, the rules are the same except that β-D-glucopyranose is the reference compound for hexopyranoses

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TABLE XII. Application of the Empirical Rules for the ^{13}C Nmr Spectra of the Pentopyranoses[a]

Pento-pyranose		Carbon atom 1	2	3	4	5
		β Anomers				
xylo	(266)	95.5	118.0	116.1	122.8	127.2
(ref)	(264)	95.9	118.4	116.7	123.3	129.5
lyxo	(266)	98.2	122.1	119.6	125.5	128.3
	(264)	98.5	122.7	119.8	125.9	128.6
	Calcd[b]	96.7	119.0	119.3	124.8	127.2
ribo	(266)	98.4	121.1	121.1	123.7	129.4
	(264)	98.6	121.4	124.0	125.1	129.6
	Calcd	97.5	119.2	119.1	124.0	129.2
arabino	(266)[c]	95.3	120.1	119.7	123.8	126.1
	(264)[c]	95.8	120.6	120.1	124.0	124.0
	Calcd	95.5	120.0	119.3	123.8	128.4
		α Anomers				
xylo	(266)	100.0	120.6	119.2	123.1	131.1
	(264)	100.4	119.6	121.0	123.1	131.6
	Calcd	99.5	119.2	120.1	122.8	129.2
lyxo	(266)	98.2	122.1	121.6	124.7	129.4
	(264)	98.5	122.0	122.4	125.0	129.6
	Calcd	100.7	120.2	121.3	124.8	129.2
ribo[d]	(266)	98.9	123.6	122.0	124.7	129.4
arabino	(266)[e]	99.4	123.6	123.6	123.8	129.9
	(264)[e]	100.0	126.4	126.4	123.8	130.2
	Calcd	99.5	121.4	121.3	123.8	130.4

[a] The observed chemical shifts are from ref 264 and 266 and are in ppm to high field of CS_2. [b] The chemical shifts are calculated using the empirical rules presented in the text. [c] The chemical shifts were obtained for α-L-arabinose. [d] Conformational equilibrium unknown. [e] The chemical shifts were obtained for β-L-arabinose.

and β-D-xylose for the pentopyranoses. The agreement between the observed ^{13}C chemical shifts[264,266] with those calculated using the above empirical rules is seen in Tables XII and XIII.

The natural abundance ^{13}C nuclear magnetic resonance spectra of complex carbohydrate structures promises to have a revolutionary effect on characterization and structure determination[282b,c] Dorman and Roberts[282b] showed that the ^{13}C nmr spectra of disaccharides (cellobiose, lactose, maltose, and sucrose) could be anticipated to a useful approximation from the spectra of the model hexoses and their methyl glycosides, taking into consideration the effects of the substitutional changes on the chemical shifts of the aglyconic carbon atoms. The procedures developed promise to find widespread application both for the study of oligosaccharides and polysaccharides. This important advance is well illustrated by the spectrum for kanamycin A which is reproduced in Figure 13. Thus one could readily see the presence of 18 carbon atoms (see Table XIV), two of which could have been expected to be anomeric (δ 96–100 ppm), likely four carbons attached to nitrogen (δ 40–56 ppm) and one hydrocarbon center (δ 28.1 ppm). The remainder of the signals have chemical shifts in the regions typical for carbohydrate structures,[264,266] and it could have, for example, been immediately suspected that the signal at 60.7 ppm was from a carbon in a primary hydroxymethyl group. Many other such conclusions could have been made in the event that the structure was unknown. The assignments made in Table XIV are to an extent based on such considerations but also through comparisons made with simple model compounds.[282d] The data in Table XIV show that ^{13}C nmr spectra are probably the best single physical characterization of such complex structures so

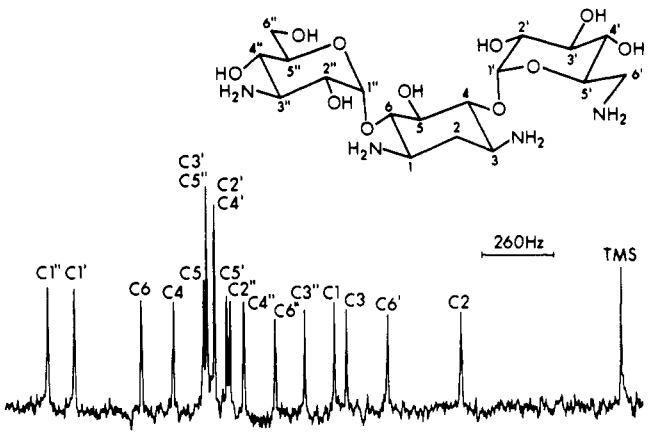


Figure 13. The natural abundance proton-decoupled ^{13}C Fourier transform nmr spectrum of Kanamycin A in D_2O at pH 3.6.[282d]

TABLE XIII. Application of the Empirical Rules for the ^{13}C Nmr Spectra of Hexopyranoses[a]

Hexo-pyranose		Carbon atom 1	2	3	4	5
		β Anomers				
gluco	(266)	96.5	118.2	116.6	122.8	116.6
(ref)	(264)	96.7	118.3	116.8	122.9	116.8
manno	(266)	98.2	120.8	118.7	125.2	115.9
	(264)	99.1	121.5	119.4	125.9	116.8
	Calcd[b]	97.7	119.2	119.8	124.8	116.6
allo	(266)	99.0	121.2	121.4	125.6	119.0
	(264)	99.1	119.1	121.4	125.7	121.2
	Calcd	98.5	119.4	119.6	123.8	118.6
galacto	(266)	95.8	120.2	119.5	123.1	117.3
	(264)	96.0	120.5	119.7	123.8	117.6
	Calcd	96.5	120.2	119.8	123.8	117.6
		α Anomers				
gluco	(266)	100.4	120.9	119.5	122.8	121.2
	(264)	100.5	119.7	121.1	122.9	121.1
	Calcd	100.5	119.4	123.7	122.8	118.6
manno	(266)	97.6	121.0	121.5	124.9	119.8
	(264)	98.6	121.8	122.2	125.6	120.3
	Calcd	101.7	120.4	124.7	124.8	118.6
galacto	(266)	100.0	123.5	123.5	123.8	122.0
	(264)	100.3	123.3	124.1	123.3	122.3
	Calcd	100.5	121.4	124.7	123.8	119.8

[a] The observed chemical shifts are from ref 264 and 266 and are in ppm to high field of CS_2. [b] The chemical shifts are calculated using the empirical rules presented in the text.

far developed. Indeed, in connection with pmr spectra, it can be anticipated that the structure of a new such compound could be so well delineated as to allow ready detailed chemical proof of structure. Comparison of the spectra for kanamycins A and B obviously shows their close structural relationships with the presence of an additional amino group in kanamycin B. The shift of the carbon assigned to C3′ would confirm the conclusions based on the appearance of five signals in the region 40–56 ppm and assigned to carbons bonded to amino groups. Table XIV lists the major changes in chemical shift with change in pH from acid to alkaline conditions for both kanamycin A and B. It is seen that the marked shifts occur for carbons geminal to the amino group (β carbons). The shifts of the carbons attached to the amino group are relatively very small. Thus, the measurement of the spectra of such compounds at two different pH's can provide much useful information. For example, the shifts assigned to C5′ would strongly support the presence of a

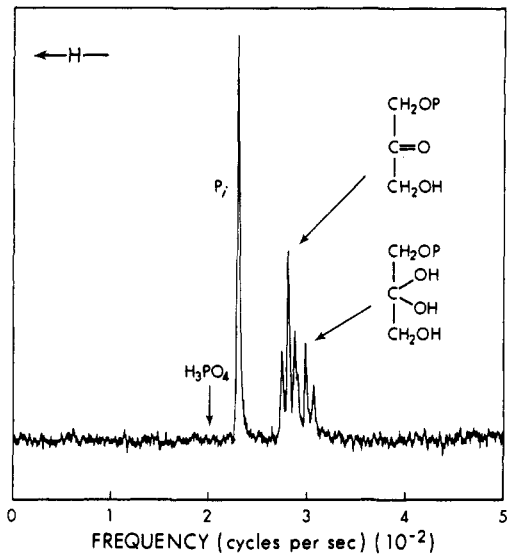


Figure 14. The ^{31}P nmr spectrum of a solution of dihydroxyacetone monophosphate at pH 6.5.[293]

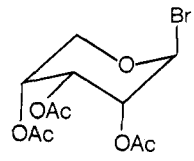
CH_2NH_2 group (the signal at 41–42 ppm) in these antibiotics.

^{13}C natural abundance nmr can also be very useful to gain information on the structure for carbohydrates as complex as polysaccharides. Thus, Perlin and coworkers[282e] were able to achieve evidence for a biose repeating sequence in heparin, a substance long known for its intractability. Another example is the demonstration by Jennings and Smith[282f] that a glucan elaborated by *Tremella mesenterica* has the sequence (1→4, 1→4, 1→6)$_n$ as had been earlier determined by chemical means. It may also be noted that pmr spectroscopy can shed information on polysaccharide structures.[282g]

c. ^{19}F Magnetic Resonance

The effects of configuration, substitution, and solvent on the chemical shift of the anomeric fluorine of hexopyranosyl fluorides was investigated by Hall, Manville, and Bhacca.[25] Empirical rules were formulated to account for the shifts observed with chloroform-*d* as solvent. A *gauche* oxygen at C2 causes a shielding of about 11 ppm with the substituent on the oxygen being acetyl, benzoyl, or methyl. A syn-axial acetoxy group shielded the fluorine of an α anomer by about 4 ppm. The effect on the axial α-fluorine by inversion at C4 to provide an axial acetoxy group was only about 1 ppm but about 4 ppm when the fluorine at C1 was in equatorial orientation (β anomer).

The anomeric effect[55,167,280,281] favors the axial orientation for a polar substituent such as fluorine at the anomeric center. As was previously found for tri-*O*-acetyl-β-D-ribopyranosyl bromide (**64**),[283] the fluorine analog



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also exists in the $^1C_4$ conformation.[205] Recent results by Lemieux and Pavia[284] indicate that the nonbonded interaction between syn-axial acetoxy groups is relatively weak. It may be noted that, in fact, the syn-axial relationship for an acetoxy group and a small halogen such as fluorine may be very weakly repulsive if not attractive. This could arise since the ether oxygen of esters is known to be electron deficient as the result of resonance stabilization.

TABLE XIV. The ^{13}C Nmr Shifts[a] of Kanamycins A and B[282d]

	Kanamycin A pH 3.6	pH 9.6	Δ	Kanamycin B pH 5.5	pH 10.6	Δ
C1	50.4	51.0		50.2	50.7	
C2	28.1	36.1	8	28.9	35.8	6.9
C3	48.3	49.6		48.9	49.6	
C4	78.8	87.6	8.8	78.7	86.8	8.1
C5	73.4	74.8		74.7	74.7	
C6	84.3	88.4	4.1	84.2	88.2	4.0
C1′	96.1	99.9		96.3	100.5	4.2
C2′	71.4	72.4		54.2	55.6	
C3′	72.8	73.5		68.9	73.8	4.9
C4′	71.4	71.7		71.2	71.7	
C5′	69.3	72.9	3.6	69.6	73.0	3.4
C6′	41.0	42.0		40.7	41.9	
C1″	100.7	100.4		100.8	100.1	
C2″	68.7	72.4	3.6	68.5	72.1	3.6
C3″	55.7	54.9		55.5	54.5	
C4″	66.2	70.0	3.8	66.1	69.6	3.5
C5″	72.8	72.9		73.4	72.4	
C6″	60.7	61.1		60.5	60.7	

[a] The chemical shifts are in ppm to low field of TMS.

d. ^{31}P Magnetic Resonance

As previously mentioned,[202] $^3J(^{31}P,H)$ coupling constants may be of value in describing the geometry of the structure. However, the measurement of ^{31}P chemical shifts for phosphorus-containing compounds can be useful in checking purity and establishing structure.

The availability of Fourier transform ^{31}P nmr was used by Gray[293] to examine the relative amounts of tautomers in an aqueous solution of D-fructose 1,6-diphosphate. The compound was found, as was previously concluded,[294] to exist predominantly (~90%) in the β-furanose form. The proportion of the α-furanose form was about 8% and the keto form about 1.7%. The method was also used to examine the hydration of dihydroxyacetone monophosphate. It was concluded that the low-field triplet (see Figure 14) arose from the hydrated keto form and was present to the extent of 45%.

Ho and coworkers[295] examined the variation of the ^{31}P chemical shifts with pH for several molecules of biological interest. The ^{31}P chemical shifts in compounds with

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phospho diester and symmetrically substituted pyrophosphate linkages remained essentially constant, whereas for phospho monoesters there were relatively large changes (about 4 ppm) with change of pH from 3 to 9. For glucose 1-phosphate, there was a 3.7-ppm shift to low field over the pH range 3.0–8.6. The signal was a doublet with spacings of 6.0, 6.5, and 7.0 Hz at pH 3.0, 5.8, and 8.6, respectively.

^{31}P nmr measurements were used in the assignment of the structures for dimethyl (1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose) 3-*C*-phosphonate and its 3-epimer, dimethyl (1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose) 3-*C*-phosphonate[296] and two isomers of inosine 5′-thiophosphate.[297]

4. Relaxation Times

Measurements of the relaxation times of individual nuclei in a molecule are mainly concerned with the ^{13}C nuclei and are discussed in the section on Fourier transform nmr.

C. Special Techniques

1. Deuteration

Deuteration techniques have received extensive applications in carbohydrate chemistry. The isotopic substitution has such a very minor effect on the electronic structure of the molecule[298] that the chemical shifts of the remaining nuclei are practically unaltered. Deuterons have a spin of one, and the value of the magnetogyric ratio is much smaller than that for protons. Indeed, an H–D coupling is smaller than the corresponding H–H coupling by a factor of 6.54. The deuterium spectrum, of course, does not appear in the region of the proton spectrum.

Fully deuterated solvents are extensively used and the ready availability of heavy water (D_2O) is of particular importance to carbohydrate chemistry. In order to minimize the obscuring of the nmr signals arising from the nonexchangeable hydrogens of a sugar, it is advantageous to freeze-dry the carbohydrate one or two times from D_2O. If the HDO peak still creates difficulties, it may be shifted away from the region of interest by changing the temperature of the measurement. To replace exchangeable hydrogens from solutions in water-immiscible solvents, the practice of adding a drop of D_2O is commonly used.

Feeney and Roberts[299] have applied a double resonance technique for the removal of the ^1H absorption signal of HDO. Figure 15 shows the ^1H spectrum of isoprenaline sulfate. Strong irradiation at 8.2 ppm not only decreases the intensity of the HDO signal but also provides the chemical shift for the NH_2^+ protons. Other labile protons in isoprenaline have very broad resonances and therefore the collapse of the HDO resonance could also be achieved by double irradiation in the region 3.5–6.5 ppm. Almost a total collapse of the HDO absorption was observed for irradiation at 4.0 ppm.[299]

Many applications of specific deuteration have been made in carbohydrate chemistry. These include studies concerned with the assignment of coupling constants. For example, Lemieux and Levine[300] found the deuterolysis of methyl 2-deoxy-2-iodo-β-D-glucopyranoside (**65**)

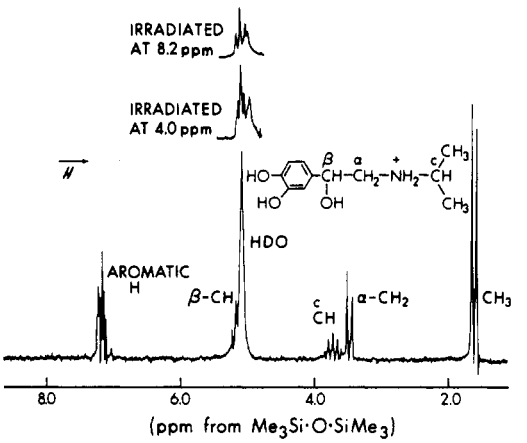
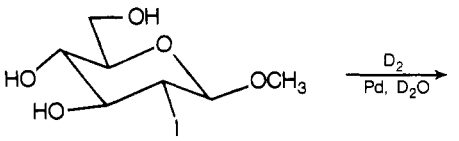


Figure 15. The 100-MHz proton magnetic resonance spectrum of isoprenaline sulfate in D_2O.[299]

proceeded with near complete retention of configuration. The coupling of the anomeric hydrogen with H2 in the product (**66**) was 9.9 Hz and therefore there could be no



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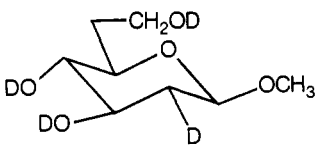
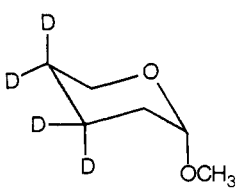
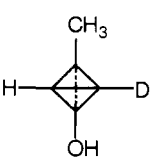


66

doubt about the configuration at C2. Anomerization of **66** to the α form showed $J_{1,2}$ = 3.9 Hz, necessarily the value for $J_{1e,2a}$. Thus the coupling constant for $J_{1e,2e} \simeq$ 1.4 Hz in the methyl 2-deoxy-α-D-glucopyranoside could be assigned.[300] Specific deuteration can also be effectively used to eliminate virtual coupling.[37,71,72] Thus, for example, the signal for H2 in 2-methoxytetrahydropyran is strongly affected by virtual coupling with the axial H4[301] since the protons at the 4 position are only weakly chemically shifted from those at the 3 position. To obtain nmr data that would enable the assessment of the coupling constants of H2 with the geminal hydrogens at the 3 position, the virtual coupling of H2 with the hydrogens at the 4 position was accomplished by replacement of the two C4 hydrogens by deuterium. In 4,4′,5,5′-tetradeuterio-2-methoxytetrahydropyran (**67**), sharp signals were obtained for the hydrogens at the 2, 3, and 6 positions.[301] The assignment of the distribution of a deuterium atom at C5 of tetra-*O*-acetyl-β-D-xylopyranose by nmr allowed the determination of the absolute configuration of optically active 1-deuterioethanol (**68**) since it was possible to degrade the monodeuterated xylose to dextro-



67



68

(296) L. Evelyn, L. D. Hall, P. R. Steiner, and D. H. Stokes, *Chem. Commun.*, 576 (1969).

(297) K. Haga, M. Kainosho, and M. Yoshikawa, *Bull. Chem. Soc. Jap.*, **44**, 460 (1971).

(298) Reference 1, pp 161–163.

(299) J. Feeney and G. C. K. Roberts, *Chem. Commun.*, 205 (1971).

(300) R. U. Lemieux and S. Levine, *Can. J. Chem.*, **42**, 1473 (1964).

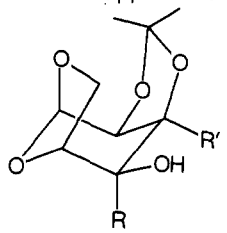
(301) R. U. Lemieux, A. A. Pavia, J. C. Martin, and K. A. Watanabe, *Can. J. Chem.*, **47**, 4427 (1969).

rotatory **68** of about 30% optical purity.[302] Thus it is seen that deuteration in conjunction with nmr spectroscopy can be useful for determining stereochemical routes of reaction as well as points of configuration.

The use of specific deuteration is well demonstrated by the following controversy. Hall, Manville, and Bhacca[25] proposed that the rotamer for the acetoxymethyl group of both tetra-*O*-acetyl-α-D-gluco- and galactopyranosyl fluoride which has the acetoxy group antiparallel to C4 is particularly unfavored for both configurations at C4. The interpretation was reported as being at variance with that previously preferred by Lemieux and Stevens[38] who in fact made no rotamer assignment for related glucose and mannose derivatives. Lemieux and Martin[303a] have more recently commented on the problems inherent to the use of vicinal proton–proton coupling constants in studies of these rotamer populations where there is no basis for anticipating the torsion angles involved. Holland, Horton, Miller, and Bhacca[241] did assign the conformer which has C4 and the C6-acetoxy group of 1-thio-β-D-glucopyranose pentaacetate in antiparallel relationship as making a major contribution to the rotamer population. More recently,[303b] Gagnaire, Horton, and Taravel have confirmed this conclusion by measuring *J*(H5,H6) for methyl tetra-*O*-acetyl-6-deuterio-α-D-glucopyranoside of known absolute configuration.

Deuteration is also useful in the simplification of spectra. Thus, the preparation of 6,6′-dideuteriopenta-*O*-acetyl-β-D-glucopyranose simplified the multiplet corresponding to the C5 and C6 hydrogens, and the resultant signal was a one-proton doublet arising from the H5 proton.[38] The anomeric hydrogen of penta-*O*-acetyl-β-D-glucopyranose was assigned to the doublet at lowest field by showing that the signal was absent in the spectrum of the C1-deuterated derivative.[38] This approach was extended in the assignment of spectra of aldoses in D_2O solutions.[39] 2,5,5′-Trideuterio-β-D-arabinopyranose, obtained by exchange of 2-*O*-benzyl-5,5′-dideuterio-D-arabinose in D_2O –NaOD solution followed by hydrogenolysis, allowed the examination of the coupling between H3 and H4.

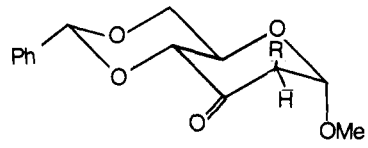
Many other experiments have been carried out in which deuterium atoms have been attached directly to the carbon skeleton.[73,267,304-308] The 100-MHz nmr spectrum of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose (**69**) was interpreted completely[307] by comparison of its spectrum with those of the deuterated analogs (**70–72**). Alterations in the appearance (but not position)



69, R = R′ = H
70, R = D; R′ = H
71, R = H; R′ = D
72, R = D; R′ = D

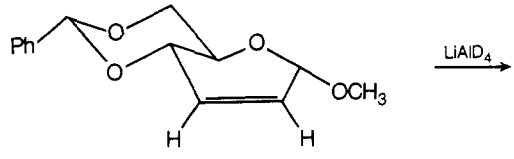
of various signals of the nmr spectrum of **69** as a function of isotopic substitution permitted the complete determination of chemical shifts and coupling constants for all protons in the molecule. Assignment of ring proton signals of compound **69** would have been difficult using normal double resonance techniques owing to the small chemical shift separation between some of the ring protons.

The observation[309] that the axial proton at C2 in methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose (**73**) resonates at lower field than the equatorial C2 proton was confirmed by the preparation of the deuterated derivative **74**.[306]



73, R = H
74, R = D

Fraser-Reid and Radatus[310] used deuterium labeling to establish the course of the reaction of **75** with lithium aluminum deuteride. That the deuterium was incorporated from the face of the molecule from which the methoxy departed was indicated by the magnitude of *J*(H2,H3) = 6.0 Hz. The β anomer of **75** provided the C3 epimer of **76** for which *J*(H2,H3) = 1.7 Hz.



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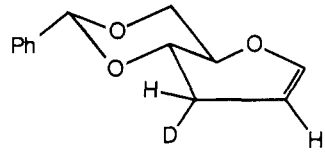

76

Horton and coworkers[311-313] have carried out specific deuteration experiments of individual acetyl groups in carbohydrate derivatives. For example, each of the acetyl group signals in the nmr spectrum of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose[312] and methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside[313] was identified.

Assignment of *N*-acetyl signals in the presence of acetoxy groups in carbohydrates has been resolved only recently by specific N- and O-deuterioacetylation[311,312] by solvent-induced shift techniques[314,315] and by an application of shift reagents $Eu(DPM)_3$ and $Pr(DPM)_3$.[253]

The above deuteration techniques have been applied[316] to octa-*O*-acetylsucrose. Individual acetoxy

(302) R. U. Lemieux and J. Howard, *Can. J. Chem.*, **41**, 308 (1963).
(303) (a) R. U. Lemieux and J. C. Martin, *Carbohyd. Res.*, **13**, 139 (1970); (b) D. Gagnaire, D. Horton, and F. R. Taravel, *ibid.*, **27**, 363 (1973).
(304) W. Mackie and A. S. Perlin, *Can. J. Chem.*, **43**, 2921 (1965).
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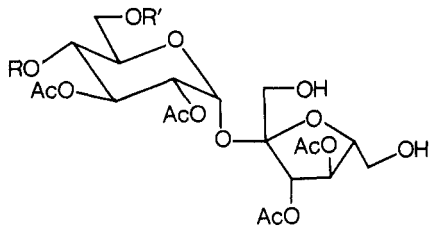
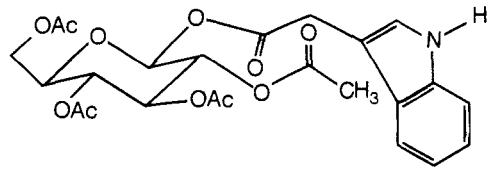
(311) D. Horton, J. B. Hughes, J. S. Jewell, K. D. Philips, and W. N. Turner, *J. Org. Chem.*, **32**, 1073 (1967).
(312) D. Horton, W. E. Mast, and K. D. Philips, *J. Org. Chem.*, **32**, 1471 (1967).
(313) D. Horton and J. H. Lauterbach, *J. Org. Chem.*, **34**, 86 (1969).
(314) F. W. Lichtenthaler and H. P. Albrecht, *Chem. Ber.*, **99**, 575 (1966).
(315) F. W. Lichtenthaler, G. Bambach, and P. Emig, *Chem. Ber.*, **102**, 994 (1969).
(316) T. Suami, T. Otake, S. Ogawa, T. Shoji, and N. Kato, *Bull. Chem. Soc. Jap.*, **43**, 1219 (1970).

group signals in the pmr spectrum of this molecule were assigned by direct comparison with the spectra of specifically deuterated octa-*O*-acetylsucrose. These measurements served to confirm the structures of 2,3,6,3′,4′-penta-*O*-acetylsucrose (**77**) and 2,3,4,3′,4′-penta-*O*-acetylsucrose (**78**). The structures of molecules **77**[317] and **78**[318] were assigned previously on the basis of their chemical behavior.



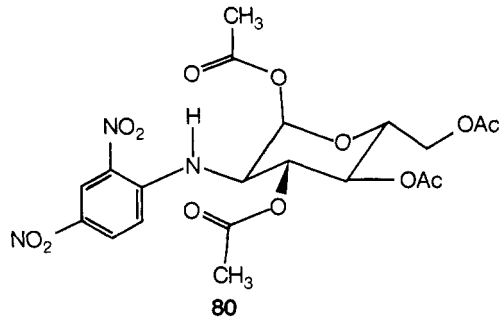
77, R = H; R′ = Ac
78, R = Ac; R′ = H

Pravdic and Keglevic[319] found an unusually high-field (τ 8.42) acetyl group resonance for 2,3,4,6-tetra-*O*-acetyl-1-*O*-(indol-3-ylacetyl)-D-glucopyranose (**79**). The signal disappeared on replacing the 2-*O*-acetyl group by a trideuterio acetyl group. Considering the orientation for *O*-acetyl groups relative to the pyranose ring,[242] the conformation of **79** is expected to be that shown. Thus, a



79

shielding by the aromatic nucleus is not surprising.[320] Similar effects were noted for related aryl derivatives and occur, for example, for compounds such as 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2′,4′-dinitroanilino)-α-D-glucopyranose (**80**).[321] The methyl resonance of the 3-*O*-acetyl group appears at τ 8.13. As expected, the methyl resonance of the anomeric *O*-acetyl group is deshielded and is at τ 7.73.

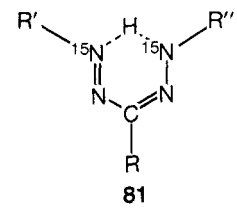


80

Deuteration techniques have also been used in the study of the degree of methylation at each position in mixtures of partially methylated methyl glycopyranosides.[322,323] The chemical shifts corresponding to each of the ten methyl groups of methyl 2,3,4,6-tetra-*O*-methyl-α- and β-D-glucopyranoside were determined by systematic selective deuteration of the methyl groups.[322] Spectral assignments for *O*-methyl groups in monomethylated D-hexoses have now been recorded.[324]

2. *Specific Labeling Using* ^{15}N

From a study of the pmr spectrum of ^{15}N-labeled 6-acetamido-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose, Coxon[325] showed the coupling of ^{15}N with the proton to be about 1.5 Hz over three bonds and 0.5 Hz over two bonds. The coupling of the directly bonded nuclei was much greater, 91.3 Hz. This value was near the 94 Hz observed by Mester and coworkers[326] for ^{15}N–H groupings in sugar osazones. In the case of sugar formazans, the coupling was only 46.5 Hz.[327,328] The occurrence of the signal for the N–H proton as a triplet with this spacing in the doubly labeled compound **81**[327] was interpreted in terms of a rapid tautomerization. However, this result also provides evidence for a strong hydrogen bond between the two nitrogens as depicted in structure **81**. A similar structure was observed in the case of FHF^- by Martin and Fujiwara[329] with $J(^1H,^{19}F)$ equal to 120.5 Hz, about one-fourth of that in the HF molecule, 521 Hz.[330]



81

It is considered that the magnitudes of coupling constants between directly bonded ^{15}N and a proton are determined mainly by Fermi contact interaction and hence proportional to the amount of s character of the nitrogen-bond orbital.[325,331]

3. *Nuclear Overhauser Effect*

The nuclear Overhauser effect[332] is observed during nuclear magnetic double resonance experiments. The effect involves saturation of the signal due to a nucleus X (A-{X}) while an enhancement of the absorption intensity of a second nucleus A is observed. This allows the detection of nuclear–nuclear relaxation processes. When dipole–dipole interactions are the dominant relaxation mechanism, the maximum nuclear Overhauser enhancement for ^{13}C–{^1H} is 2.988[62,63] and has been used to great advantage for the enhancement of natural abundance ^{13}C spectra. If A and X are both protons, complete saturation of the X proton will result in a 50% enhancement of the integrated intensity of A.[333] Since the relaxation has a $1/r^6$ dependence on the separation between

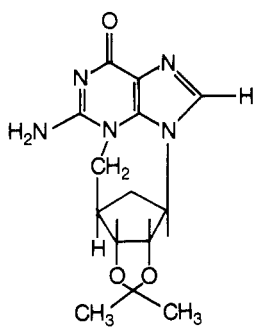
(317) R. U. Lemieux and J. P. Barette, *J. Amer. Chem. Soc.*, **80**, 2243 (1958).
(318) H. Bredereck, H. Zinner, A. Wagner, G. Faber, W. Greiner, and W. Huber, *Chem. Ber.*, **91**, 2824 (1958).
(319) N. Pravdic and D. Keglevic, *Carbohyd. Res.*, **12**, 193 (1970).
(320) C. E. Johnson, Jr., and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).
(321) R. U. Lemieux and G. Lee, unpublished data.
(322) D. Gagnaire and L. Odier, *Carbohyd. Res.*, **11**, 33 (1969).
(323) E. B. Rathbone and A. M. Stephen, *Tetrahedron Lett.*, 1339 (1970).
(324) E. G. Gros, I. O. Mastronardi, and A. R. Frasca, *Carbohyd. Res.*, **16**, 232 (1971).
(325) B. Coxon, *Carbohyd. Res.*, **11**, 153 (1969).
(326) L. Mester, G. Vass, A. Stephen, and J. Parello, *Tetrahedron Lett.*, 4053 (1968).
(327) L. Mester, A. Stephen, and J. Parello, *Tetrahedron Lett.*, 4119 (1968).
(328) L. Mester and G. Vass, *Tetrahedron Lett.*, 3847 (1969).
(329) J. S. Martin and F. Y. Fujiwara, *Can. J. Chem.*, **49**, 3071 (1971).
(330) E. L. Mackor, C. MacLean, and C. W. Hilbers, *Recl. Trav. Chim. Pays-Bas*, **87**, 655 (1968).
(331) G. Binsch, J. B. Lambert, B. W. Roberts, and J. D. Roberts, *J. Amer. Chem. Soc.*, **86**, 5564 (1964).
(332) (a) A. Abragam, "The Principles of Nuclear Magnetism," Oxford University Press, London, 1967, Chapter VIII; (b) J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect. Chemical Applications," Academic Press, New York, N. Y., 1971.

TABLE XV. Nuclear Overhauser Study of the Equilibrium Process 83 ⇄ 84[167]

Expt	Signal obsd	Signal irradiated	Increase in area, %
1	H_{2e}	CH_3 (4-axial)	2.0
2	H_{2a}	CH_3 (4-axial)	8.8
3	H_{5a}	N-CH_3	6.0

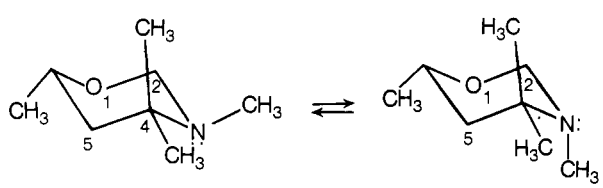
the observed and irradiated nuclei, nuclear Overhauser studies have been of considerable stereochemical and conformational interest.[167,184,333-337]

Schirmer, *et al.*,[336] illustrated the quantitative use of the nuclear Overhauser effect in conformational studies by determining the relative distances between protons H8, H1′, and H2′ in 2′,3′-isopropylidene-3,5′-cycloguanosine (**82**) in solution while Hart and Davis[334,335] have studied the conformation of purine and pyrimidine derivatives about the glycosidic bond.



82

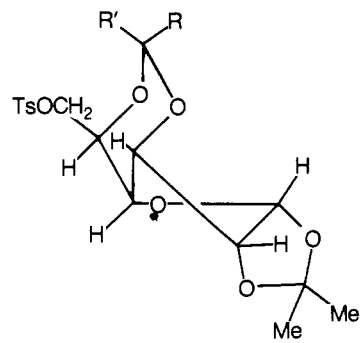
Booth and Lemieux[167] have carried out nuclear Overhauser experiments in their study of rapidly interconverting mixtures of conformers. In tetrahydro-3,4,4,6-tetramethyl-1,3-oxazine (**83**), the N-CH_3 signal (τ 7.80 in benzene) and the H_{5a} signal (τ 8.58) are well shifted, and in both conformations (**83**, **84**) the axial methyl group at position 4 is in a 1,3-diaxial relationship to the axial hydrogen at position 2. Hence the magnitude of the enhancement for H_{2a} on irradiation of the axial CH_3 at position 4 forms a convenient standard on which to base any quantitative treatment of the enhancement observed for H_{5a} on irradiation of the N-CH_3. The results of the nuclear Overhauser experiments on the conformational equilibrium **83** ⇄ **84** are given in Table XV.



83 **84**

From Dreiding models the distance between H_{2a} and the closest hydrogen of the axial CH_3 at position 4 is almost the same as that between H_{5a} and the closest hydrogen of the axial N-CH_3, assuming staggered conformations. Hence it might be expected that the nuclear Overhauser enhancement observed in experiments 2 and 3 would be the same if the compound possessed conformation **84** only. Qualitatively the data indicate that the contribution made by conformation **84** is appreciable and may dominate the equilibrium. The results may indicate that the anomeric effect[55,280,281] has an influence on the conformational equilibria. However, since the enhancement is very sensitive to small differences in molecular dimensions, and relaxation of H_{5a} may occur by protons other than those of the N-CH_3 group, a quantitative analysis of the data was not feasible.[167]

It is expected on conformational grounds that the methyl group of a methoxy attached to a six-membered ring will be *gauche* to the geminal hydrogen, and this is supported by the optical rotation of methylated carbohydrates.[303a] That the methyl group and vicinal hydrogen are strongly compressed in structures **85** and **86** was shown by Coxon[184] who found a 25% enhancement of the integrated intensity of the proton resonances on irradiating the methoxyl protons. A calculation of the van der Waals interaction energies was made.



85, R = H; R′ = OCH_3
86, R = OCH_3; R′ = H

Internuclear double resonance (indor)[338] techniques have been developed.[338-340] An indor experiment can be carried out between nuclei of the same or of different species and offers a means of locating hidden resonances as well as information concerning the structure of the energy level diagram and the relative signs of coupling constants.[341,342] These results are of great value in the assignment of observed lines to theoretical transitions in computerized, iterative analyses of spectra.[343] A simple modification of a Varian HA-100 spectrometer for ^{1}H-{^{1}H}[344] and ^{1}H-{^{13}C}[272] indor experiments has been described together with several applications of this technique in carbohydrate chemistry.

4. *Fourier Transform Nmr Spectroscopy*

In a conventional high-resolution nmr experiment the spectra are scanned by sweeping the field (or the frequency), and the absorption signals are observed in the continuous-wave mode in which the rf field is allowed to run continuously. In the Fourier transform nmr experiment a sequence of short, equally spaced, rf pulses are applied to the sample.[8,21,345] These pulses simultaneously excite all spins with resonance frequencies within a certain region. The resultant free induction decay

(333) F. A. L. Anet and A. T. R. Bourn, *J. Amer. Chem. Soc.*, **87**, 5250 (1965).
(334) P. A. Hart and J. P. Davis, *J. Amer. Chem. Soc.*, **91**, 512 (1969).
(335) P. A. Hart and J. P. Davis, *Biochem. Biophys. Res. Commun.*, **34**, 733 (1969).
(336) R. E. Schirmer, J. H. Noggle, J. P. Davis, and P. A. Hart, *J. Amer. Chem. Soc.*, **92**, 3266 (1970).
(337) P. A. Hart and J. P. Davis, *J. Amer. Chem. Soc.*, **93**, 753 (1971).
(338) E. B. Baker, *J. Chem. Phys.*, **37**, 911 (1962).
(339) R. Kaiser, *J. Chem. Phys.*, **39**, 2435 (1963).
(340) E. B. Baker, *J. Chem. Phys.*, **45**, 609 (1966).
(341) R. Freeman and W. A. Anderson, *J. Chem. Phys.*, **39**, 806 (1963).
(342) V. J. Kowalewski, *Progr. Nucl. Magn. Resonance Spectrosc.*, **5**, 1 (1969).
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(344) R. Burton, L. D. Hall, and P. R. Steiner, *Can. J. Chem.*, **48**, 2679 (1970).
(345) R. R. Ernst, *Advan. Magn. Resonance*, **2**, 1 (1966).

signals in the time domain are then added coherently by a time-averaging computer, and Fourier transformation of the signal then gives the ordinary high-resolution spectrum. This is possible since the free induction signal contains all the information available in the corresponding continuous wave spectrum and the two modes of display are related by a Fourier transformation.[346] As the free induction signals are produced very rapidly, it is possible to obtain spectra in a much shorter time than in the continuous wave mode where one resonance is observed at a time and this facility has been used to follow the mutarotation of sugars.[271,282c] Consequently the sensitivity is much higher. The signal to noise ratio improves by a factor of $(F/\Delta)^{1/2}$ for spectra obtained in the *same given time* on the same spectrometer. F is the spectral width and Δ the line width.[21] This factor holds for spectral widths of about 1000 Hz and corresponds to a signal to noise enhancement of at least one order of magnitude. Alternatively, the time required to obtain a spectrum with a given signal-to-noise ratio is a factor of 100 shorter in a Fourier transform experiment than in the conventional one.

The experimental requirements for Fourier transform nmr experiments have been discussed by Ernst and Anderson,[21] Freeman and coworkers,[347,348] Farrar,[349] and Horsley, *et al.*[350] Technically, several basic requirements must be considered and satisfied.[347,348] These include pulse generation, detection of the signal and digitization, signal accumulation, the Fourier transformation, and then the display of the nmr absorption signal. In this mode of operation new techniques are applied and certain questions arise that never have to be considered in continuous wave nmr. One of these is folding of spectra. Extra lines appear in the final spectrum when experimentally the carrier frequency is not set correctly with respect to the nuclear precession frequencies. Digitization of the data may also introduce folding of spectra when the spectral width F is not wide enough to include all the signals from the nuclei in the sample under study. Since a large number of free induction decays are accumulated, high-field-frequency stability is required. A suitable time-averaging computer must be available with a capacity large enough for sufficient resolution, data storage, and Fourier transformation.

Fourier transform applications now range from ^1H measurements of long-range coupling constants of 0.05 Hz,[351] measurements on dilute aqueous solutions with an overwhelmingly large H_2O signal[352] to the measurement of a natural abundance ^{13}C spectrum of 0.02 M ribonuclease A,[353] and natural abundance ^{15}N nmr.[354]

The technique has been extended to the measurement of partially relaxed Fourier transform spectra of individual ^1H[355,356] and ^{13}C[65,270,271,357-361] lines. In this experiment the magnetization is first inverted by means of a

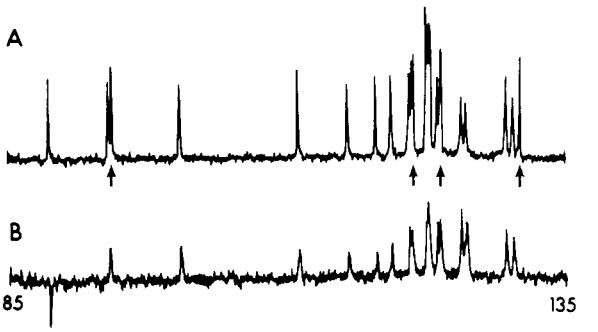

Figure 16. The proton-decoupled ^{13}C nmr spectra of 0.5 M aqueous stachyose obtained at 15.08 MHz using Fourier transform techniques: (A) normal spectrum, (B) partially relaxed Fourier transform spectrum with τ = 0.34 sec. The terminal galactose resonances identified using the partially relaxed Fourier transform technique are identified with the arrows in (A).[270]

180° rf pulse. This inverts the distribution of spin populations across the energy-level diagram. After an interval τ, a 90° pulse induces the free induction signal which is recorded and then Fourier transformed. After thermal equilibrium has been established, the measurement is repeated with a different value of τ. A series of such measurements yields a series of spectra which may be grouped together to form a three-dimensional diagram. The intensity of each resonance may then be studied as a function of τ. For τ much shorter than T_1, a negative peak will appear. As τ increases, the negative peak decreases in amplitude, goes through a null, and then becomes positive. For values of τ much larger than T_1, a normal resonance is observed. Intensities obtained from these spectra are related to longitudinal relaxation times by the equation[362]

$$A = A_0 [1 - 2 \exp(-\tau/T_1)]$$

where A is the observed intensity in a spectrum with a given value of τ and A_0 is the equilibrium intensity in a normal Fourier transform spectrum.

Partially relaxed Fourier transform spectra are interesting since they yield information concerning the spin–lattice relaxation times of individual nuclei in the molecule. The measurement of partially relaxed spectra can be used to resolve overlapping peaks.[65] For two carbons with identical chemical shifts but different relaxation times, a partially relaxed spectrum may show both a positive and a negative peak and hence both resonances may be identified. This technique is especially useful as an aid in assigning ^{13}C resonances of complex molecules when internal motion contributes to the T_1 of some of the carbons.

The nonreducing tetrasaccharide stachyose **87** has been studied in the above manner.[270] The normal proton-decoupled natural abundance ^{13}C Fourier transform spectrum of 0.5 M aqueous stachyose is shown in Figure 16A. From the proton-decoupled partially relaxed ^{13}C Fourier transform spectrum (Figure 16B), the galactose resonances could be divided into two sets with appreciably different T_1 values. The first corresponds to $T_1 \simeq 0.5$

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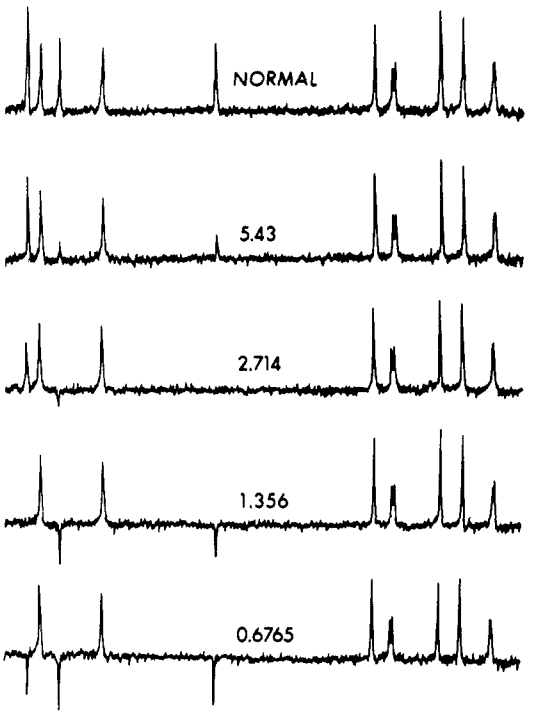


Figure 17. *The proton-decoupled ^{13}C Fourier transform nmr spectra of 1 M adenosine 5′-monophosphate in H_2O obtained at 15.08 MHz. The normal spectrum is shown at the top, whereas the rest are partially relaxed Fourier transform spectra. The τ values (seconds) are indicated in the figure.*[65]

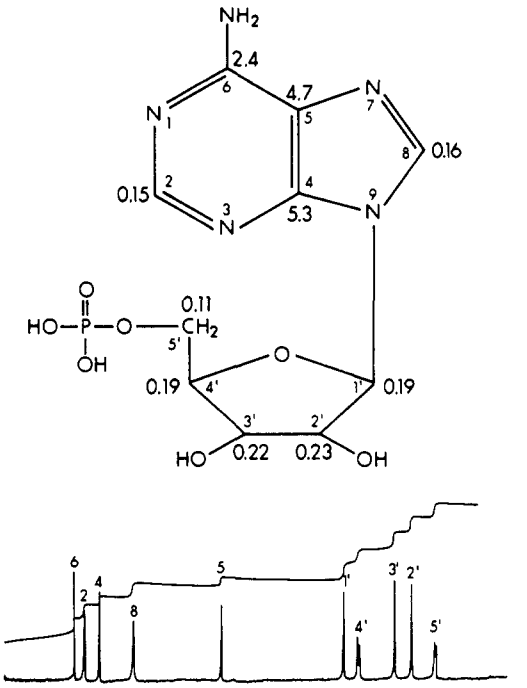
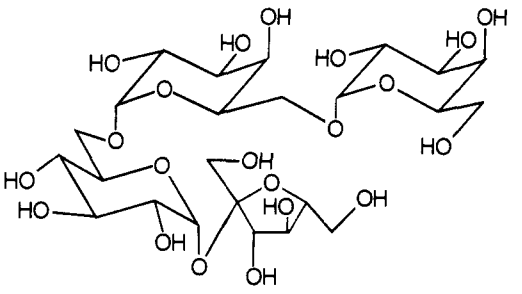


Figure 18. *The ^{13}C T_1 values of aqueous 1 M adenosine 5′-monophosphate at 42° are given in large digits in the top part of the figure. The proton-decoupled ^{13}C Fourier transform nmr spectrum of this solution is shown at the bottom.*[65] *In a recent publication*[274] *the 2′ and 3′ ribose resonances have been reassigned from those shown.*

sec, while for the other set $T_1 < 0.5$ sec. Since one galactopyranose ring is terminal with possibly a faster internal reorientation rate than the other galactose ring, the set of resonances corresponding to T_1 values of 0.5 sec was assigned to the terminal galactose.



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These experiments have been extended to studies of sucrose, adenosine 5′-monophosphate (5′-AMP), and cholesteryl chloride.[65] A series of partially relaxed ^{13}C Fourier transform spectra obtained for 5′-AMP are shown in Figure 17, and the measured T_1 values are given in Figure 18.[65] The protonated carbons have T_1 values of less than 1 sec, while the nonprotonated carbons have spin–lattice relaxation times greater than 1 sec. From measured T_1 values of protonated carbons rotational correlation times are determined.[65]

Finally, Patt and Sykes[363] have carried out a series of water eliminated Fourier transform nmr experiments which should prove very useful in ^1H nmr studies of carbohydrates. ^1H Fourier transform nmr experiments in D_2O solutions have been hampered by a large, residual HDO resonance which arises from a variety of problems associated with the study of weak resonances in the presence of a strong resonance. The experiment is carried out with a pulse sequence of the form $T-\pi-t_n-\pi/2$, where T is a relatively long initial waiting time and t_n is the time required for the HDO resonance to attain zero net longitudinal magnetization. If there is a large difference in T_1 values between the ^1H resonances of the molecule under study and that of HDO, the above pulse sequence will null the HDO resonance while a normal spectrum will be observed for the other resonances.[363]

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III. Conformational Equilibria

Conformational analysis[55,57,364a,364b] of sugars makes the assumption that the geometry of the pyranoses can be compared with that of cyclohexane while that of the furanoses can be compared with that of cyclopentane.[57,364a] Two chair forms or the less stable skew and boat forms are possible for the pyranoid ring, while the furanoid ring may adopt one of the envelope or twist forms. These conformations coexist in equilibrium in solution with one conformation usually predominating. The predominant conformations for many carbohydrates have been compiled and discussed.[57] The procedures used to estimate conformational equilibria were presented in section II.B.2.

Durette, Horton, and Bhacca[365] have recently reviewed the application of these nmr methods to conformational equilibria problems in carbohydrate chemistry and have compared these critically.

A. Pyranose Derivatives

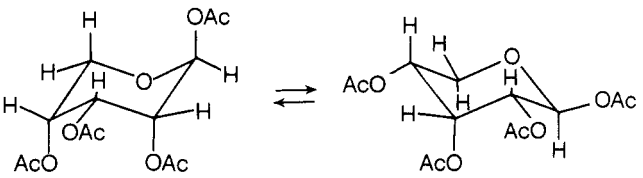
The conformational equilibria of β-D-ribopyranose tetraacetate (**88** ⇄ **89**) were studied[365,366] by applying the

(364) (a) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Interscience, New York, N. Y., 1965, p 351; (b) P. L. Durette and D. Horton, *Advan. Carbohyd. Chem. Biochem.*, **26**, 49 (1971).

(365) P. L. Durette, D. Horton, and N. S. Bhacca, *Carbohyd. Res.*, **10**, 565 (1969).

(366) N. S. Bhacca and D. Horton, *J. Amer. Chem. Soc.*, **89**, 5993 (1967).

methods of averaging of spin coupling, averaging of chemical shifts, and direct integration. The temperature dependence of the nmr spectrum is shown in Figure 19.[365] The data indicate that β-D-ribopyranose tetraacetate undergoes rapid interconversion at room temperature in acetone-d_6 and is a 9:11 mixture of the *1C*(D) and *C1*(D) conformers. The method of averaging of chemical shifts was found to be unreliable as the chemical shifts of the protons under consideration in the two conformers may not be independent of temperature.[365] Using the spectral method of averaging of spin coupling, the conformational equilibria of a large series of peracylated D-aldopentopyranosyl derivatives have been established.[367,368]



1C *C1*

88 **89**

Durette and Horton[369] have continued their conformational studies on pyranoid sugar derivatives by nmr to include the tri-*O*-acetyl-D-aldopentopyranosyl bromides and chlorides having the α-xylo, α-lyxo, β-ribo, and β-arabino configurations and the β-xylo chloride isomer. As well the tri-*O*-benzoyl-α- and β-D-xylopyranosyl chlorides and tri-*O*-benzoyl-β-D-ribopyranosyl bromide derivatives were studied. The conformation favored at room temperature tends to become the exclusive form at low temperatures.[369] The conformational studies have been extended to methyl, ethyl, isopropyl, and *tert*-butyl tribenzoylated β-D-ribopyranosides and the corresponding triacetates,[370] the 2,3,4-tri-*O*-acetyl-D-aldopentopyranosyl benzoates and 2,3,4-tri-*O*-benzoyl-D-aldopentopyranosyl acetates,[371] the eight methyl D-aldopentopyranoside triacetates and some corresponding tribenzoates,[372] and the 1-thio-D-aldopentopyranose tetraacetates.[373] Conformational equilibria have also been studied in heterocyclic *N*-glycosides[124] and α-glycopyranosides and glycosylamine derivatives.[97]

Solvent effects have a marked effect on conformational equilibria. Large changes occur in the optical rotation and the nmr spectra of methyl 2-deoxy-α-L- and methyl 3-deoxy-β-L-*erythro*-pentopyranoside (**90**) when dissolved in a variety of solvents including chloroform, acetone, acetonitrile, pyridine, dimethyl sulfoxide, and water.[374] These changes result almost exclusively from changes in conformational equilibria. The nmr spectrum of **90** in D_2O required the compound to exist largely in the *1C* conformation since $J_{1,2}$ = 6.0, $J_{2,3'} \simeq J_{3',4} \simeq$ 8.9, $J_{2,3} \simeq J_{3,4}$ = 4.5, and $J_{3,5}$ = 1.9 Hz. In this conformation a specific rotation of +95° was observed (expected specific rotation: 100°[375]). In $CDCl_3$ solutions, $J_{1,2}$ decreased to 2.2 Hz and $H_{3'}$ and H_3 were weakly (<3 Hz) coupled with both H_2 and H_4. Similarly, H_4 was weakly coupled with both $H_{5'}$ and H_5. In chloroform, therefore, compound **90** exists largely in the *C1* conformation and a specific rotation of 142° is observed (expected, 137°). Thus changes

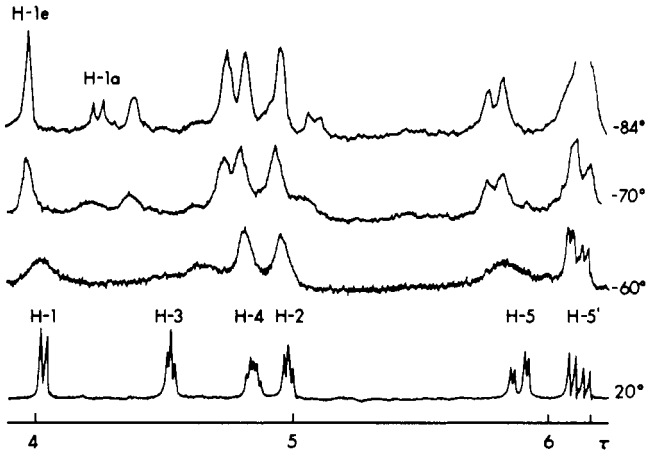
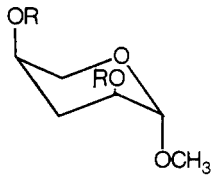


Figure 19. The 220-MHz nmr spectra of β-D-ribopyranose tetraacetate in $(CD_3)_2CO$ at −84, −70, −60, and 20°. Only the low-field region is shown.[365]

in conformation occur with changes in the solvent environment. A linear relationship is obtained between the coupling constant $J_{1,2}$ (measured from the spacing of the doublet for H1) and the specific rotation. The value for $J_{1,2}$ is considered to be a measure of the relative populations of the *1C* and *C1* conformations for **90**.[374]



90, R = H
91, R = CH_3CO
92, R = p-$O_2NC_6H_4CO$
93, R = CH_3SO_2
94, R = CH_3

Derivatives of methyl 3-deoxy-β-L-*erythro*-pentopyranoside (**91** to **94**) were also studied.[284] Compounds **91**, **92**, and **93** exist largely in the *C1* conformation with both R groups in axial and opposing orientation. Since this situation is maintained for diverse solvents such as CCl_4, $CDCl_3$, CD_3CN, and, in the case of **91**, D_2O, the preference for the *C1* conformation is not appreciably affected by the dielectric constant of the solvent. As for the dimethoxy compound **94**, the conformational equilibrium favors the 2,4-diequatorial conformer *1C*, in CCl_4. These data indicate that a meaningful quantitative conformational analysis of molecules that contain interacting atoms having unshared electron pairs must consider the substituents on these atoms. The electrostatic repulsion between the oxygen atoms at the 2 and 4 positions is minimized by the presence of strongly electron-attracting substituents such as the acyl group in compound **91**. The *C1* conformation is then favored. As for compound **94**, the more electron-rich oxygen atoms of the methyl ether groupings exert a stronger repulsion than in compounds **91**, **92**, or **93**, thus favoring the *1C* conformer. Similarly, compound **90** in $CDCl_3$ has both the 2- and 4-hydroxy groups axial (*C1*). In D_2O, hydrogen bonding of both hydroxyls with solvent (S) (COH···S) increases the negative charge on the oxygen atoms through polarization of the O–H bond (CO←H←S). This occurs to such an extent that the repulsion of the C–O bond dipoles in opposing axial orientation becomes adequately large to force the compound from the *C1* to the *1C* conformation.

Further evidence that specific solvation effects can alter conformational equilibria was obtained from a study

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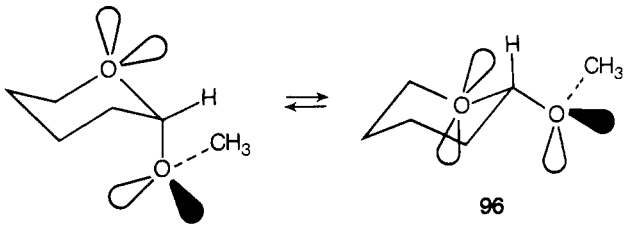
of the equilibrium process **95** ⇄ **96**.[301] The conformational



95 **96**

equilibria were studied by measuring the coupling constant of H2 with the geminal protons at the 3 position. Optical rotatory data were used to provide information on the effect of solvent on the orientation of the methoxy grouping relative to the pyranose ring. The results indicate an increase in the more polar equatorial form with increasing solvent polarity. Solvent molecules capable of donating a hydrogen atom to form hydrogen bonds with the oxygens of the acetal linkage also stabilized the equatorial conformation (**96**).[301]

B. Furanose Derivatives

A computer analysis of the entire 100-MHz nmr spectrum of β-pseudouridine (β-ψ) (**97**) and α-pseudouridine (α-ψ) in aqueous solution has been carried out.[376-378] The observed coupling constants are given in Table XVI.

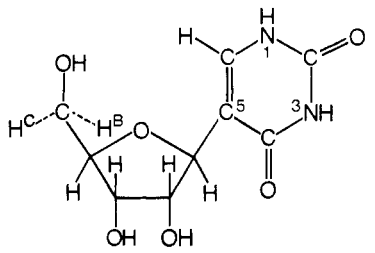


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TABLE XVI. Coupling Constants (Hz) for β- and α-Pseudouridine at 30°[378]

Coupling constant	β-ψ	α-ψ
$J_{61'}$	0.8	1.3
$J_{1'2'}$	5.0	3.3
$J_{2'3'}$	5.0	4.2
$J_{3'4'}$	5.2	7.9
$J_{4'5'B}$	3.2	2.4
$J_{4'5'C}$	4.6	5.7
$J_{5'B5'C}$	−12.7	−12.7

By an application of the modified Karplus equation[379] an equilibrium between several puckered forms of the ribose is proposed. These studies have been extended to uridine,[380] β-cyanuric acid riboside,[381] orotidine,[382] and dihydrouridine.[383]

Acknowledgments. Appreciation is expressed to Dr. J. S. Martin for carrying out the calculated spectra shown in Figure 5, to Drs. A. Allerhand, M. Barfield, J. Feeney, G. R. Gray, L. D. Hall, S. Hanessian, D. Horton, and J. S. Martin, *Biochemistry, The Canadian Journal of Chemistry, Carbohydrate Research, Chemical Communications, The Journal of the American Chemical Society, The Journal of Chemical Physics,* and *The Journal of Organic Chemistry* for permission to reproduce Figures, and to the National Research Council of Canada for financial support.

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