Pure & Appl. Chem., Vol. 49, pp. 1151 - 1168. Pergamon Press, 1977. Printed in Great Britain.

FOURIER TRANSFORM NMR SPECTROSCOPY NITROGEN-15 STUDIES OF AMINO SUGARS

Bruce Coxon

Institute for Materials Research, National Bureau of Standards, Washington, D.C. 20234, USA

Abstract - The synthesis and nuclear magnetic resonance (n.m.r.) spectroscopy of ^{15}N-labeled derivatives of 6-amino-6-deoxy-D-glucose, 6-amino-6-deoxy-D-galactose, and 5-amino-5-deoxy-D-ribose are reviewed. The use of these derivatives in the measurement of ^{15}N coupling constants, ^{15}N nuclear Overhauser effects, and ^{15}N spin-lattice relaxation times is described. Integration of the natural abundance ^{15}N n.m.r. spectra of aqueous solutions of the common 2-amino sugar hydrochlorides and their N-acetyl derivatives has given ratios of α and β anomers for the equilibrated solutions that are in good agreement with published data from proton n.m.r., with the exception of 2-amino-2-deoxy-D-galactose hydrochloride, for which a discrepancy was noted. The equilibria of this amino sugar in aqueous solution have been reinvestigated by proton n.m.r. spectroscopy at 60 and 220 MHz, and by ^{13}C n.m.r., and the α-pyranose anomer found to be predominant. Correlations of the ^{15}N chemical shifts with molecular structure and stereochemistry have been made.

INTRODUCTION

For the practising chemist, the development and wide utilization of the pulse-Fourier transform method (1) of nuclear magnetic resonance (n.m.r.) spectroscopy have had greatest impact on ^{13}C n.m.r. studies, so that spectra of good quality are now routinely and rapidly obtained from ^{13}C nuclei at natural abundance in a wide range of molecular species. The application of this greatly improved method of data acquisition to the nitrogen n.m.r. spectroscopy of bioorganic materials has been much slower than for ^{13}C, due partly, perhaps, to the fact that the n.m.r. sensitivity of the ^{14}N and ^{15}N nuclei at constant field is some 15 times smaller than that of an equal number of ^{13}C nuclei.

^{14}N resonances are often broadened by the electric quadrupole moment of this nucleus to the extent of 50-1000 Hz (2), and although a detailed theoretical analysis of the line-broadening phenomenon can yield useful information (2), the low resolution ^{14}N spectra obtained have usually had little appeal for the organic chemist. On the other hand, the low natural abundance (0.365%) of the ^{15}N nucleus causes the relative n.m.r. sensitivity of this nucleus at natural abundance to be ∿46 times smaller than that of ^{13}C, so that in a sense, ^{15}N n.m.r. can be said to be 46 times more difficult than ^{13}C n.m.r. Fortunately, recent improvements in instrumentation have tended to alleviate the sensitivity disadvantage of ^{15}N. This nucleus (spin 1/2) has no quadrupole moment, and extremely narrow lines, for example, of half-line width less than 0.1 Hz, may be obtained under suitable conditions. For diamagnetic nitrogen compounds, the range of nitrogen chemical shifts observed is approximately 900 p.p.m., and it has been stated that these shifts are particularly valuable for distinction of isomeric organic structures (2). There is available a large body of data on the nitrogen magnetic resonance parameters of simple organic and inorganic compounds (2), that has been obtained by many different experimental techniques, including direct observation of ^{14}N and ^{15}N signals in the continuous wave or pulse-Fourier transform modes (using compounds in which ^{15}N is present at either natural abundance, or in high enrichment), and also indirect observation of nitrogen spectra by means of various double resonance techniques. The ^{14}N and ^{15}N chemical shift scales are now thought to be identical for all practical purposes, and the remarkable discrepancies between ^{14}N and ^{15}N shifts reported earlier have been explained as being due to non-uniformity of experimental conditions or

simply to experimental errors (2).

From a general point of view, it is of interest to determine the utility of ^{15}N magnetic resonance parameters in the structural, conformational, and quantitative analysis of nitrogenous carbohydrates. These substances are important components of many biological systems, including the aminoglycoside antibiotics (3,4) and certain immunochemical and antitumor polysaccharides (5). The ^{15}N parameters that are potentially available include chemical shifts, coupling constants, spin-lattice (T_1) and spin-spin (T_2) relaxation times, nuclear Overhauser effects, and n.m.r. signal intensities. Relaxation times and nuclear Overhauser effects may be subdivided into contributions from various sources that provide further molecular information. Because of experimental difficulties, measurements of T_2 have not been performed as frequently as those of T_1.

Quantitative analysis of organic materials by pulse-Fourier transform ^{13}C n.m.r. spectroscopy has sometimes been attended by difficulties caused by widely differing ^{13}C relaxation times, different nuclear Overhauser effects, and inadequate digital definition of narrow resonances. Similar problems may be expected in the quantitative application of ^{15}N n.m.r. spectroscopy, although methods are available for overcoming the difficulties. Relaxation times can be diminished and nuclear Overhauser effects removed (6-10), by the addition of paramagnetic reagents to solutions, and, by suitable gating of decoupling frequencies, the nuclear Overhauser effect may be either retained in coupled spectra (11), or discarded from decoupled spectra (12). However, in cases of marginal signal:noise ratio, deliberate loss of the nuclear Overhauser effect may be undesirable.

We have applied ^{15}N n.m.r. spectroscopy to the quantitative analysis of the pyranose anomers of amino sugars with the recognition that they probably represent the most favorable case, in that the structures of these anomers are extremely similar, and, therefore, their ^{15}N nuclei may be expected to possess relaxation times that are of the same order, and also similar nuclear Overhauser effects. Additionally, the similarity of the chemical shifts of the ^{15}N nuclei of the anomers would cause these nuclei to experience equal perturbations by the radiofrequency pulse.

^{15}N n.m.r. spectroscopy has recently been applied (13) to quantitative analysis of the biosynthetic incorporation of L-valine-^{15}N into penicillin G by a high-producing strain of *Penicillium chrysogenum*.

SYNTHESIS AND SPECTROSCOPY OF ^{15}N LABELED AMINO SUGAR DERIVATIVES

In 1969, before equipment for implementation of the pulse-Fourier transform n.m.r. method was available to us, it appeared that one way of diminishing the sensitivity problem inherent in observation of the n.m.r. of ^{15}N at natural abundance would be to synthesize derivatives labeled in high enrichment with the ^{15}N isotope. Few examples of highly enriched amino sugar derivatives were known, possibly because many of the synthetic methods available had employed a large excess of the nitrogen-introducing reagent. Symmetrical multi-nitrogen species such as hydrazine and azide ion are unsuitable, as double labeling would be necessary. An amino sugar synthesis of high-yield was desired that would require only one molecular equivalent of a commercially available, but relatively inexpensive intermediate labeled in high enrichment with ^{15}N. For this reason, the nucleophilic substitution reactions of terminal deoxyhalo and p-tolylsulfonyl derivatives of carbohydrates with potassium phthalimide-^{15}N were investigated. Reactions that give 70-85% yields of crystalline deoxyphthalimido carbohydrate derivatives by the use of up to 1.3 molecular equivalents of potassium phthalimide-^{15}N have been developed in the D-glucofuranose (14,15), D-galactopyranose (16), and D-ribofuranoside series.

6-Amino-6-deoxy-D-glucose-6-^{15}N derivatives

Reactions of 1,2:3,5-di-O-isopropylidene-6-O-p-tolylsulfonyl-α-D-glucofuranose or its 6-deoxy-6-iodo derivative with potassium phthalimide-^{15}N in hexamethylphosphorictriamide (HMP) afforded (14,15) 6-deoxy-1,2:3,5-di-O-isopropylidene-6-phthalimido-α-D-glucofuranose-6-^{15}N (^{15}N enrichment 99%) in yields of 81-84%. The N-phthaloyl group was smoothly removed by treatment of the phthalimido derivative with hydrazine hydrate giving 6-amino-6-deoxy-1,2:3,5-di-O-isopropylidene-α-D-glucofuranose-6-^{15}N, which was characterized as its N-acetyl and N-(trifluoroacetyl) derivatives (14,15). These ^{15}N-labeled derivatives and their unlabeled (^{14}N) analogs were studied initially

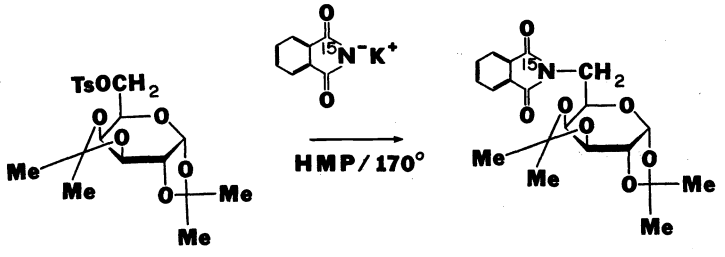
by continuous wave proton, ^{13}C, and ^{19}F n.m.r. spectroscopy, and by ^{13}C Fourier transform techniques, and characteristic proton, ^{13}C, and ^{19}F chemical shifts, and also ^{1}H-^{1}H, ^{1}H-^{15}N, ^{1}H-^{19}F, ^{13}C-^{15}N, and ^{15}N-^{19}F coupling constants were reported and discussed in terms of molecular structure (17). By the use of empirical expressions (18) relating ^{15}N coupling constants to bond orbital s-character, the magnitudes of the ^{1}H-^{15}N and ^{13}C-^{15}N coupling constants of the amide derivatives of 6-amino-6-deoxy-1,2:3,5-di-O-isopropylidene-α-D-glucofuranose were used to infer a predominant contribution from the polarized canonical form of the amide bond in these derivatives (17). The availability of ^{15}N-labeled 6-amino-6-deoxy-D-glucose derivatives allowed detailed elucidations of the mass fragmentation pathways of these derivatives (15).

Indirect observation of ^{15}N n.m.r. spectra

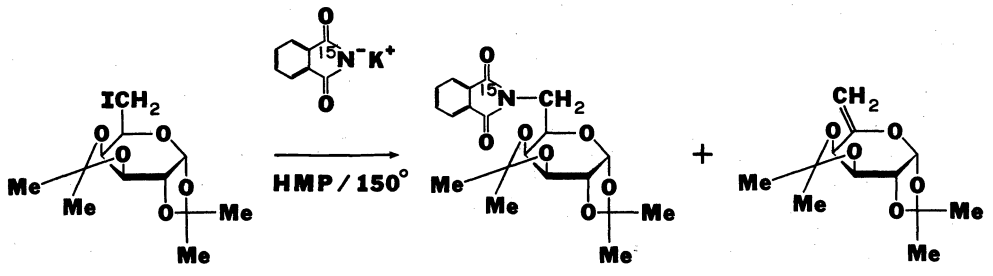
In cases where there is interaction in the form of spin-spin or dipole-dipole coupling between the ^{15}N nucleus and a more sensitive nucleus (such as the proton), the difficulties of direct observation of ^{15}N spectra may be avoided by using internuclear double resonance methods to observe these spectra indirectly, by monitoring the transitions of the more sensitive nucleus. This method was applied (17) to 6-deoxy-1,2:3,5-di-O-isopropylidene-6-(trifluoroacetamido)-α-D-glucofuranose-6-^{15}N by holding the proton observing frequency constant at the frequency of one of the strong lines of the NH proton multiplet, whilst the double resonance frequency was swept through the ^{15}N region. The experiment was actually performed under both double and triple resonance conditions (17); in the latter case, a small coupling between the ^{19}F nuclei and the NH proton was removed by simultaneous irradiation at the ^{19}F frequency. Under the conditions of triple resonance, the ^{15}N spectrum (obtained indirectly as the response at the frequency of the NH proton transition) consisted of a wide doublet that contained the ^{15}N-^{1}H coupling constant of ∿90 Hz. Good agreement between experimental and theoretical spectra was obtained (17). The key advantage of the indirect method is that the n.m.r. sensitivity of the more sensitive nucleus is utilized. However, this method is not general, in that it cannot be used unless the ^{15}N nucleus is coupled to another nucleus, by either spin-spin or dipole-dipole interaction.

6-Amino-6-deoxy-D-galactose-6-^{15}N derivatives

Reaction of 1,2:3,4-di-O-isopropylidene-6-O-p-tolylsulfonyl-α-D-galactopyranose with 1.3 molecular equivalents of potassium phthalimide-^{15}N in HMP at 170° gave a 70% yield of 6-deoxy-1,2:3,4-di-O-isopropylidene-6-phthalimido-α-D-galactopyranose-6-^{15}N. Almost the same yield (64%) of 6-deoxy-6-phthalimido derivative was obtained from reactions of commercially available

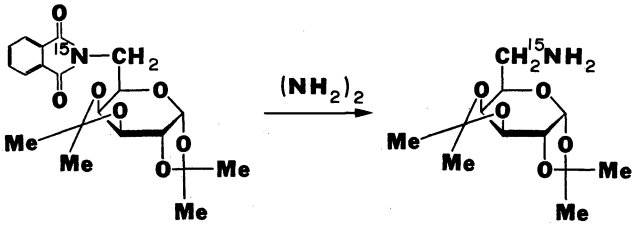


6-deoxy-6-iodo-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose with potassium phthalimide in HMP at 150°, although integration of the proton n.m.r. spectrum (90 MHz) of the total product of the reaction indicated a 7:3 ratio, respectively, of the deoxyphthalimido derivative and a second compound that proved to be the elimination product, 6-deoxy-1,2:3,4-di-O-isopropylidene-L-arabino-hex-5-enopyranose. In order to effect complete conversion of the



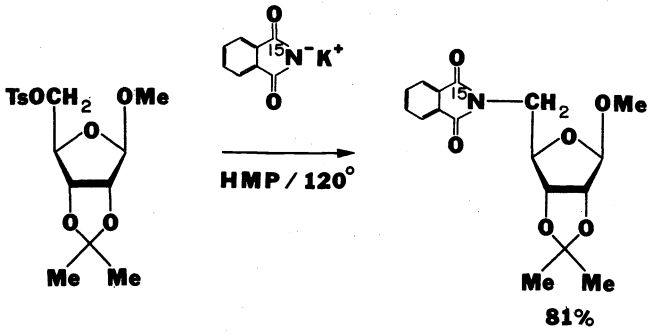
6-p-tolylsulfonyl- and 6-deoxy-6-iodo-D-galactopyranose precursors, significantly higher temperatures and a slightly higher proportion of potassium phthalimide were necessary than for the D-glucofuranose analogs. The diminished reactivity of 6-O-sulfonylgalactopyranose derivatives towards nucleophiles other than phthalimide ion has been noted previously (19-21) and explained in terms of polar, repulsive forces in the transition state (22).

Treatment of 6-deoxy-1,2:3,4-di-O-isopropylidene-6-phthalimido-α-D-galactopyranose-6-^{15}N with hydrazine hydrate yielded 6-amino-6-deoxy-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose-6-^{15}N, the non-enriched analog of which has been synthesized previously by other methods (23,24).



5-Amino-5-deoxy-D-ribose-5-^{15}N derivatives

In work designed to extend the synthesis of ^{15}N-labeled amino sugars to the pentose series, reactions of methyl 2,3-O-isopropylidene-5-O-p-tolylsulfonyl-β-D-ribofuranoside with potassium phthalimide-^{15}N in HMP at 120° have given methyl 5-deoxy-2,3-O-isopropylidene-5-phthalimido-β-D-ribofuranoside-5-^{15}N in 81% yield. Comparison of the ^{13}C n.m.r. spectra of the ^{14}N and ^{15}N analogs



of the 5-deoxy-5-phthalimido derivative allowed measurement of the ^{13}C-^{15}N coupling constants $^1J_{^{13}CH_2,^{15}N}$ 9.8, $^1J_{^{13}C=O,^{15}N}$ 13.4, and $^2J_{^{13}C\text{-}1',^{15}N}$ 8.5 Hz.

These values are similar to those (9.8, 14.6, and 7.3 Hz, respectively) measured for 6-deoxy-1,2:3,5-di-O-isopropylidene-6-phthalimido-α-D-glucofuranose-6-^{15}N, and afford confirmatory evidence for assignments of the ^{13}C spectra, in addition to that provided by off-resonance decoupling or gated irradiation of protons.

Direct observation of ^{15}N n.m.r. spectra

The availability of the ^{15}N-labeled amino sugar derivatives permitted the pulse power and pulse width of our n.m.r. spectrometer to be accurately optimized for the rapid pulsing mode prior to the more difficult direct observation of n.m.r. spectra of ^{15}N at natural abundance (16). This was especially important, as before this work was commenced, the range of ^{15}N spin-lattice relaxation times likely to be encountered in amino sugars was unknown. In our earlier work (16), pulse-Fourier transform ^{15}N n.m.r. spectra were obtained at 9.1 MHz from solutions of blocked amino sugar derivatives in 9:1 chloroform-d:hexafluorobenzene contained in 10 m.m. sample tubes, using heteronuclear field-frequency stabilization on the ^{19}F signal of the hexafluorobenzene. Only a small perturbation (flip angle) of the macroscopic magnetization vector was used, with no delay between successive data acquisitions (rapid pulsing mode). Under the conditions of proton decoupling at 90 MHz, ^{15}N n.m.r. spectra of excellent signal:noise ratio could be obtained by application of a single pulse to the fully blocked, ^{15}N-enriched amino sugar derivatives (16). However, appreciable signal averaging was required for spectra of ^{15}N at natural abundance. For example (16), 0.75 g of non-enriched 6-deoxy-1,2:3,4-di-O-isopropylidene-6-phthalimido-α-D-galactopyranose required 8,192 pulses (112 min) to give a ^{15}N signal:noise ratio of

13:1. The ^{15}N chemical shifts measured for a group of blocked amino sugar derivatives are reported in Table 1, and it is apparent that the major differences in shifts are caused by the substituents on the nitrogen atoms. For example, the shifts of the phthalimido derivatives are all quite similar to each other, but are markedly downfield of the amino nitrogen resonances. In these terminal amino sugar derivatives, the nitrogen nuclei are somewhat remote from the points of stereochemical and ring size differences in the molecules.

TABLE 1. ^{15}N chemical shifts of blocked amino sugar derivatives

Derivative	^{15}N chemical shifts[a] (p.p.m. upfield from $NH_4{}^{15}NO_3$)
6-Deoxy-1,2:3,5-di-O-isopropylidene-6-phthalimido-α-D-glucofuranose	220.8
6-Deoxy-1,2:3,4-di-O-isopropylidene-6-phthalimido-α-D-galactopyranose	220.3
Methyl 5-deoxy-2,3-O-isopropylidene-5-phthalimido-β-D-ribofuranoside	220.2[b]
2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose	264.1[c]
6-Amino-6-deoxy-1,2:3,5-di-O-isopropylidene-α-D-glucofuranose	361.8
6-Amino-6-deoxy-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose	361.2
2,3,4-Tri-O-acetyl-β-D-xylopyranosyl cyanide	117.8[d]

[a]Measured from solutions in 9:1 v/v $CDCl_3$:C_6F_6 unless stated otherwise [b]In $(CD_3)_2CO$ [c]In $CDCl_3$ [d]In $CDCl_3$ containing 0.05M CrAcAc

^{15}N NUCLEAR OVERHAUSER EFFECTS

Natural abundance ^{15}N n.m.r. spectroscopy is often aided by a substantial negative nuclear Overhauser effect (n.o.e.) (10,16,25,26) produced by irradiation of the protons at their resonance frequency. This is true of even the terminal deoxyphthalimido derivatives, in which the ^{15}N atoms are not bonded directly to protons. Integration of the ^{15}N spectra of 6-deoxy-1,2:3,4-di-O-isopropylidene-6-phthalimido-α-D-galactopyranose-6-^{15}N obtained with and without irradiation of the protons indicated (16) that this irradiation inverts the ^{15}N signal and increases its intensity by a factor of 3.4, which, therefore, corresponds to a total n.o.e. of -4.4. The maximum theoretical n.o.e. for the ^{15}N nucleus is given by $\gamma_{^1H}/2\gamma_{^{15}N} = -4.93$ where $\gamma_{^1H}$ and $\gamma_{^{15}N}$ are the magnetogyric ratios of the proton and the ^{15}N nucleus, respectively.

The almost maximal value of the n.o.e. observed experimentally for the 6-deoxy-6-phthalimido derivative indicates that the predominant mechanism for spin-lattice relaxation of the ^{15}N nucleus in this compound is by nuclear-nuclear, magnetic dipole-dipole interaction with the protons. In order to characterize the n.o.e. more fully, a study was made of the effect of the chromium(III) acetylacetonate complex on the ^{15}N spectrum of the 6-deoxy-6-phthalimido-D-galacto derivative. This metal complex (CrAcAc) was used originally for shortening the long relaxation times of the carbonyl ^{13}C nuclei in metal carbonyls (9), thus permitting the ^{13}C n.m.r. spectra of these compounds to be acquired readily by the pulse-Fourier transform method. The relaxation reagent-CrAcAc is known (10,27) to have at least two major effects: (a) it removes the n.o.e. by replacing nuclear-nuclear dipole-dipole interaction by electron-nuclear dipole-dipole interaction due to the unpaired electron density in the complex. Irradiation of the protons then produces no

additional polarization of the ^{15}N nuclei; (b) spin-lattice relaxation times are drastically diminished because dipolar relaxation by the electron-nuclear mechanism is much more efficient than that by the nuclear-nuclear mechanism, owing to the large magnetic moment associated with the unpaired electron density. The effect of CrAcAc on the ^{15}N spectrum of the 6-deoxy-6-phthalimido-D-galacto derivative is illustrated in Fig. 1 and it may be seen that gradual increase of the CrAcAc concentration from zero (Fig. 1a) to 0.05M (Fig. 1e) first causes the initial negative intensity of the ^{15}N resonance to diminish (Fig. 1b) and invert (Fig. 1c), which is followed then by an increase of the positive signal:noise ratio to a maximum limiting value. The change in signal:noise ratio from -94:1 to 24:1 corresponds to a n.o.e. of -4.9, and the experiment represents an alternative, although slightly less accurate method, for measurement of the n.o.e. The presence of the CrAcAc reagent did not cause any shift of the ^{15}N resonance.

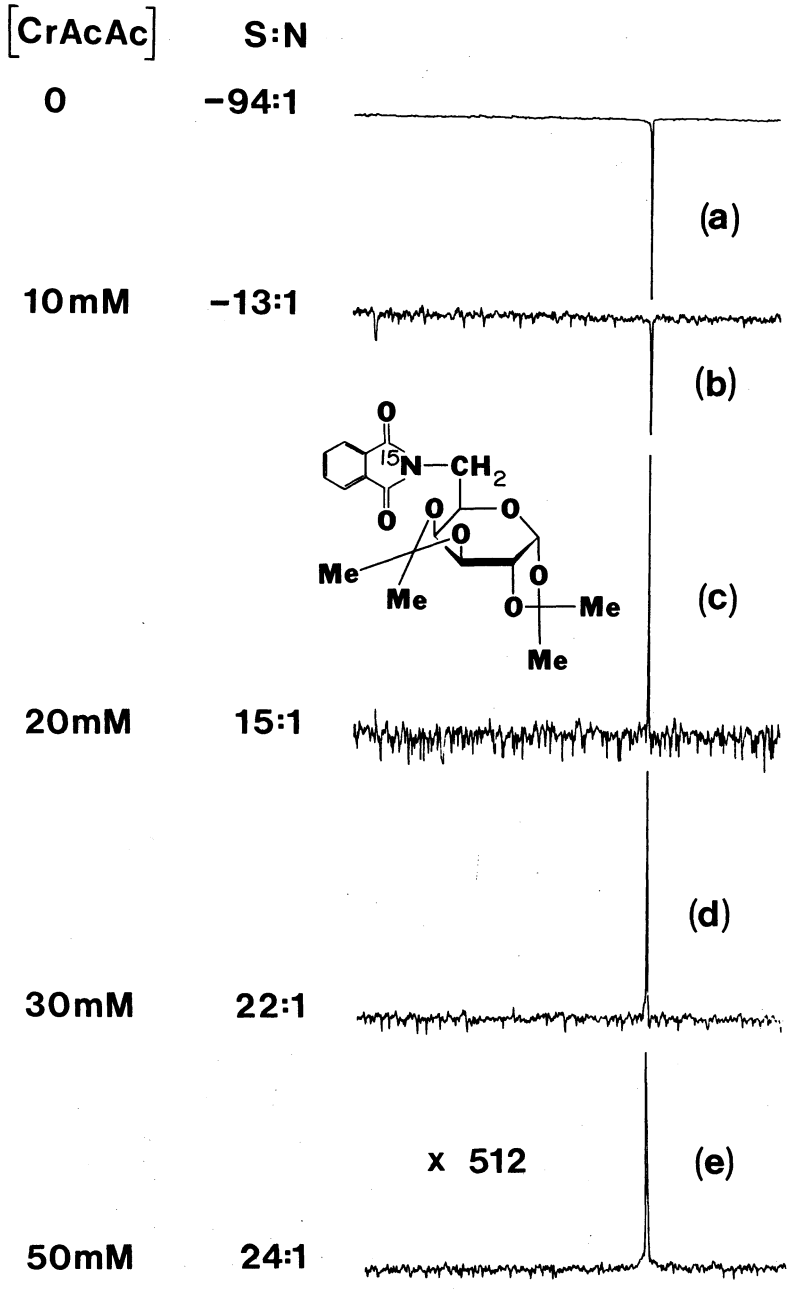


Fig. 1 Effect of increasing concentration of chromium (III) acetylacetonate (CrAcAc) on the proton decoupled ^{15}N n.m.r. spectrum of 6-deoxy-1,2:3,4-di-O-isopropylidene-6-phthalimido-α-D-galactopyranose-6-^{15}N in 9:1 v/v chloroform-d:hexafluorobenzene at 9.12 MHz.

The use of a relaxation reagent such as CrAcAc may be indispensable in the natural abundance ^{15}N n.m.r. spectroscopy of ^{15}N nuclei that have long relaxation times. For example, under the conditions used for the natural abundance ^{15}N n.m.r. spectroscopy of amino sugars (~90° pulse with a 10 second delay between pulses), no spectrum could be observed for ^{15}N at natural abundance in a 1.4M solution of 2,3,4-tri-O-acetyl-β-D-xylopyranosyl cyanide in chloroform-d. However, a ^{15}N spectrum was readily obtained from this solution after the addition of CrAcAc to a concentration of 0.05M. In this application, any n.o.e. that might have been present was sacrificed in the interest of obtaining a much shorter ^{15}N relaxation time. The chemical shift (117.8 p.p.m.) of the nitrile ^{15}N nucleus is compared in Table 1 with the ^{15}N shifts of other blocked amino sugar derivatives, including 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose. The nitrile ^{15}N resonance is at lower field than that of any other nitrogen-substituted carbohydrate measured in the present study.

^{15}N SPIN-LATTICE RELAXATION TIMES

Of the ^{15}N-enriched amino sugar derivatives discussed thus far, the N-phthaloyl derivatives might be expected to display the longest ^{15}N spin-lattice relaxation times (T_1), since for these derivatives, it would be anticipated that dipolar relaxation of the ^{15}N nucleus would be less efficient than for derivatives in which this nucleus is directly bonded to one or more protons. From the practical point of view of recording n.m.r. spectra, it is important to be aware of the likely range of T_1 values, so that the performance of the spectrometer may be optimized. Such optimization is particularly important for studies of less sensitive nuclei at low abundance. It would also be of interest to determine if ^{15}N T_1's can be correlated with molecular structure. Accordingly, the ^{15}N T_1's of 6-deoxy-1,2:3,5-di-O-isopropylidene-6-phthalimido-α-D-glucofuranose-6-^{15}N and 6-deoxy-1,2:3,4-di-O-isopropylidene-6-phthalimido-α-D-galactopyranose-6-^{15}N have been measured by the inversion-recovery Fourier transform method (28), which is thought (29) to be one of the more accurate methods available. The method is illustrated by the stacked plots shown in Fig. 2, in which may be seen the recovery of the ^{15}N magnetization that has occurred during each of a series of delay times t, following inversion of the magnetization vector by a 180° pulse. In this particular example, the free induction decay (f.i.d.) signal was sampled 25 times (with signal averaging) for each t value, although good results were also obtained from measurements in which a single f.i.d. was acquired for each value of t. The values of T_1 were computed automatically using a least squares fit, and the values obtained under

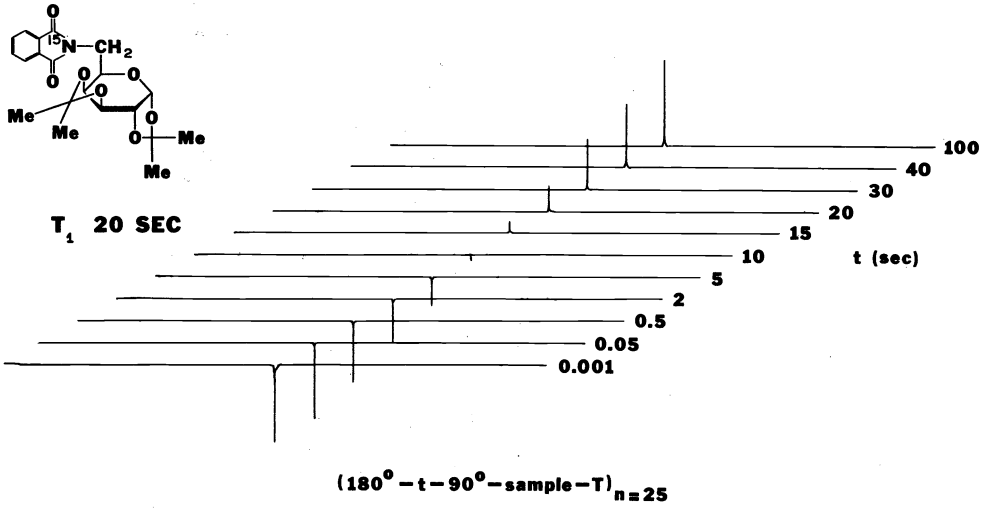


Fig. 2 Measurement of the ^{15}N spin-lattice relaxation time (T_1) of 6-deoxy-1,2:3,4-di-O-isopropylidene-6-phthalimido-α-D-galactopyranose-6-^{15}N by the inversion-recovery method.

various conditions are shown in Table 2. The ^{15}N T_1 values of the 6-deoxy-6-phthalimido-D-gluco derivative were measured at two different concentrations in the presence of atmospheric oxygen and were found to be significantly longer (71 sec) at a concentration of 0.28M, than at 0.85M (31 sec). The solution of higher concentration was noticeably viscous, and the larger value of T_1

TABLE 2. ^{15}N spin-lattice relaxation times of enriched, blocked amino sugar derivatives

Derivative	Concentration[a] (M)	O_2 present?	Number of determinations	Mean T_1[b] (s)
6-Deoxy-1,2:3,5-di-O-isopropylidene-6-phthalimido-α-D-gluco-furanose	0.85	Yes	1	31
	0.85	No	4	37
	0.28	Yes	5	71
6-Deoxy-1,2:3,4-di-O-isopropylidene-6-phthalimido-α-D-galacto-pyranose	1.0	Yes	3	20
	1.0	No	1	20

[a]In 9:1 v/v $CDCl_3:C_6F_6$ [b]Measured by inversion-recovery method

observed on dilution of this solution by a factor of three undoubtedly reflects the decrease in the viscosity of the solution. The component of relaxation time T_1^{DD} due to the dipolar interaction between the ^{15}N nucleus and neighboring protons is given by $T_1^{DD} = r^6/h^2\gamma_H^2\gamma_{^{15}N}^2\tau_c$ where r is the internuclear distance, and τ_c a molecular correlation time that is known (31) to be proportional to viscosity. Thus increased viscosity of the solution increases the correlation time and thereby decreases the dipolar contribution to T_1.

Removal of paramagnetic oxygen from the solutions of the phthalimido derivatives by saturation with nitrogen had little or no effect on their ^{15}N T_1 values (see Table 2). These results indicate that correlation of the T_1 values with molecular structure requires careful definition of solution concentrations. The rather long T_1 values (20-71 sec) observed for the phthalimido derivatives suggest that the dipolar contributions to relaxation from protons not bonded to the ^{15}N nucleus are relatively inefficient, as may be predicted from the theoretical dependence of these contributions on the sixth power of the nitrogen-proton internuclear distances.

The advantages of using highly enriched ^{15}N-labeled amino sugar derivatives for studies of ^{15}N magnetic resonance parameters may be summarized as follows: (a) the pulse parameters of the spectrometer may be optimized rapidly, (b) proton-coupled ^{15}N spectra may be obtained rapidly without using gated irradiation techniques, (c) quantitative measurements of the n.o.e. may be performed rapidly and accurately because of the excellent signal:noise ratio of the ^{15}N spectra of enriched derivatives, (d) proton-^{15}N coupling constants may be measured readily from the proton n.m.r. spectra and ^{13}C-^{15}N coupling constants from the ^{13}C n.m.r. spectra of the ^{15}N-enriched derivatives (the alternative method of measuring these coupling constants from the ^{15}N satellites in the proton and ^{13}C spectra of non-enriched derivatives is difficult because of the low intensity of the satellite signals due to ^{15}N at natural abundance), and (e) measurements of T_1 by the inversion-recovery method may be performed within a reasonable period of time (<24 hr), even for T_1 values as long as 70 sec.

In its simple form, this method requires a waiting period (T, see Fig. 2) of 3-5 times T_1 between each pulse sequence to permit re-equilibration of the magnetization (29). With ^{15}N at natural abundance in the amino sugars, it is expected that the existing sensitivity of our spectrometer would require inordinately long data acquisition times for measurements of T_1, because of the necessity of performing extensive signal averaging for each of 10-20 delay times t. Some savings in time are possible by use of other methods (29) for measurement of T_1.

In many cases, the synthesis of ^{15}N-labeled amino sugar derivatives is tedious, and is less applicable to biological problems unless organisms are grown on enriched substrates. Therefore, the development of more routine methods for recording the n.m.r. spectra of ^{15}N in natural abundance was of great interest.

NATURAL ABUNDANCE ^{15}N NMR SPECTROSCOPY OF AMINO SUGARS

Techniques

For studies of the natural abundance ^{15}N n.m.r. spectroscopy of the common amino sugars, we have extended the sensitivity of our spectrometer (a) by use of 15 m.m. sample tubes instead of the 10 m.m. tubes used previously and (b) by installation of a quadrature phase detection system designed and built at the National Bureau of Standards (NBS). The use of the larger sample tube corresponds to an improvement of 2.3 in the filling factor, after allowance has been made for the volume of a concentric capillary tube (o.d. 1.8 m.m.) of saturated aqueous $NH_4{}^{15}NO_3$ solution which served as an external ^{15}N reference. It has been known for some years that application of the quadrature phase detection technique increases the sensitivity of Fourier transform n.m.r. spectroscopy (32). The method depends on the simultaneous acquisition of two f.i.d. signals which differ in phase by 90°, and hence correspond to the absorption and dispersion components of magnetization. This method may be contrasted with conventional single phase detection in which only one of these components of magnetization is sampled. In the quadrature method, the acquisition of a second f.i.d. signal in a separate area of computer memory provides additional information which when combined with the first f.i.d. signal by means of a complex Fourier transformation yields a $\sqrt{2}$ improvement in signal:noise ratio, which corresponds to a time saving factor of 2 in signal averaging. Wider use of the quadrature method has been inhibited until the last two years by the frequent occurrence in the n.m.r. spectra of undesirable images that are caused by the two f.i.d. signals having unequal amplitudes, and phases that are not exactly 90° apart. Methods for cancellation of images have been proposed (33,34) and implemented in recent commercial instrumentation that are based on shifting the phase of the radio-frequency pulses by 0°, 90°, 180°, or 270° together with exchange of the two data memory areas and addition and subtraction of data. In a software method developed at NBS, the errors in the phases and amplitudes of the dual, f.i.d. signals are corrected arithmetically by means of a special algorithm, before Fourier transformation (35). This method has the advantage that it does not require calibration of 90° and 180° phase shifts at each radio-frequency, as may the hardware method described above. Also, unlike quartz crystal radiofrequency filters, the software method is not frequency selective, and we have therefore used it for quadrature detection of ^{1}H, ^{13}C, and ^{15}N n.m.r. signals in the Fourier transform mode.

Natural abundance ^{15}N n.m.r. spectra (usually with proton decoupling) have been obtained from a variety of nitrogenous carbohydrates. Hydrochlorides of the 2-amino-2-deoxy derivatives of D-glucose, D-mannose, and D-galactose, and the corresponding N-acetyl derivatives have been studied as approximately 1.4M solutions in 9:1 water:methyl sulfoxide-d_6, with field-frequency stabilization on the deuterium signal of the methyl sulfoxide-d_6. The use of deuterium oxide as solvent was avoided because of the likelihood that exchange of the NH protons of the amino sugars for deuterons would remove any n.o.e. resulting from irradiation at the proton frequency, and also because dipolar relaxation of the ^{15}N nucleus by directly attached deuterons would be expected to be much less effective than that due to directly bonded protons. Measurement of the ^{15}N n.m.r. spectra of aqueous solutions of single anomers of the amino sugars was quite difficult because the rates of mutarotation were generally greater than the rate of data acquisition. Fully mutarotated, aqueous solutions of the amino sugars each displayed two ^{15}N resonances of unequal intensities (see Fig. 3a, for example) which are assumed to represent pyranose anomers.

Quantitative analysis

Anomeric equilibria in derivatives of amino sugars have been studied previously (36,37), by proton n.m.r. spectroscopy at 60 MHz. The ^{15}N signals of the anomers have in most cases been assigned initially by comparison of the ^{15}N intensities with the proton integrals obtained previously, which were supported by calculations of anomeric proportions based on optical rotatory data (37). ^{13}C n.m.r. data have been reported for 2-amino-2-deoxy-D-glucose hydrochloride (38,39), 2-amino-2-deoxy-D-mannose hydrochloride (39), and for the 2-acetamido-2-deoxy derivatives of D-glucose, D-mannose, and D-galactose (40), in connection with structural studies of the polysaccharide antigens of Neisseria Meningitidis. Estimates of the dominant anomers in equilibria of the acetamido sugars from their ^{13}C resonances were stated (40) to be in qualitative agreement with the proton results reported earlier (37).

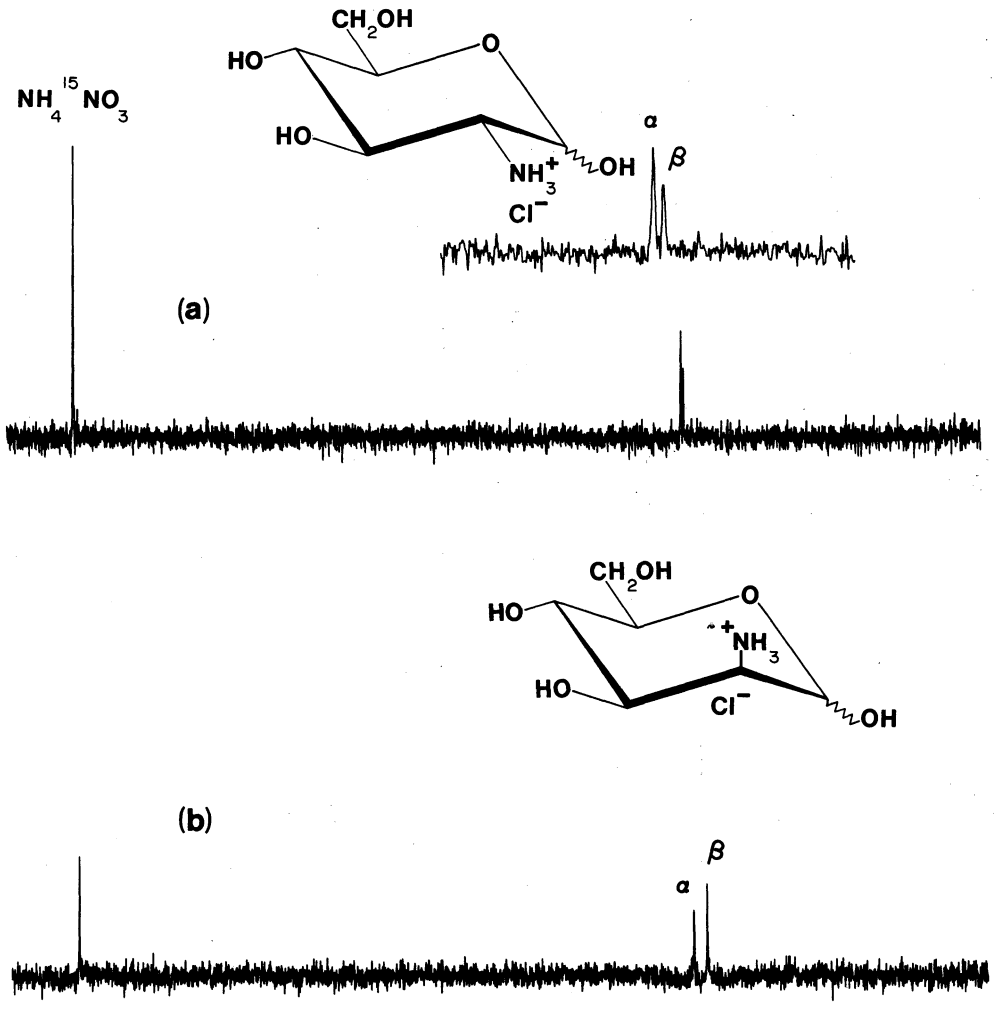


Fig. 3 Proton decoupled, natural abundance ^{15}N n.m.r. spectra at 9.12 MHz of equilibrated solutions in 9:1 v/v water: methyl sulfoxide-d_6, (a) 2-amino-2-deoxy-D-glucose hydrochloride, and (b) 2-amino-2-deoxy-D-mannose hydrochloride.

2-Amino-2-deoxy-D-hexose hydrochlorides. The natural abundance ^{15}N n.m.r. spectra of the mutarotated 2-amino-2-deoxy hydrochloride derivatives of D-glucose, D-mannose, and D-galactose are shown in Fig. 3a, 3b, and 4c, respectively. The ^{15}N chemical shift difference between the anomers of 2-amino-2-deoxy-D-mannose hydrochloride was found to be considerably greater than that of the anomers of the D-gluco or D-galacto analogs. The assignments of the ^{15}N signals of the α and β anomers of 2-amino-2-deoxy-D-glucose hydrochloride shown in Fig. 3a were confirmed by comparison of a spectrum of principally the α-anomer obtained from a solution of crystalline 2-amino-2-deoxy-α-D-glucose hydrochloride in methyl sulfoxide-d_6 with the spectrum of a similar solution of mixed anomers that was prepared by equilibration of the hydrochloride in water, freeze-drying of the solution, and dissolution of the dried residue in methyl sulfoxide-d_6. The ^{15}N spectra of mutarotated solutions of 2-amino-2-deoxy-D-glucose hydrochloride and its D-galacto epimer are so similar (see Fig. 3a and 4c) that it seems probable that the anomeric assignments are the same, that is, that the stronger resonance at lower field should in each case be assigned to the α-anomer. However, the proton data reported earlier (37) had indicated an α:β ratio of 63:37 for mutarotated 2-amino-2-deoxy-D-glucose hydrochloride, but an α:β ratio of 47:53 for the D-galacto epimer. The predominance of β-anomer in solutions of the D-galacto epimer appeared (37) to be supported by calculations of an α:β ratio of 46.4:53.6 from the specific rotations +121° and +44.5° of the crystalline α and β anomers, and their equilibrated mixture

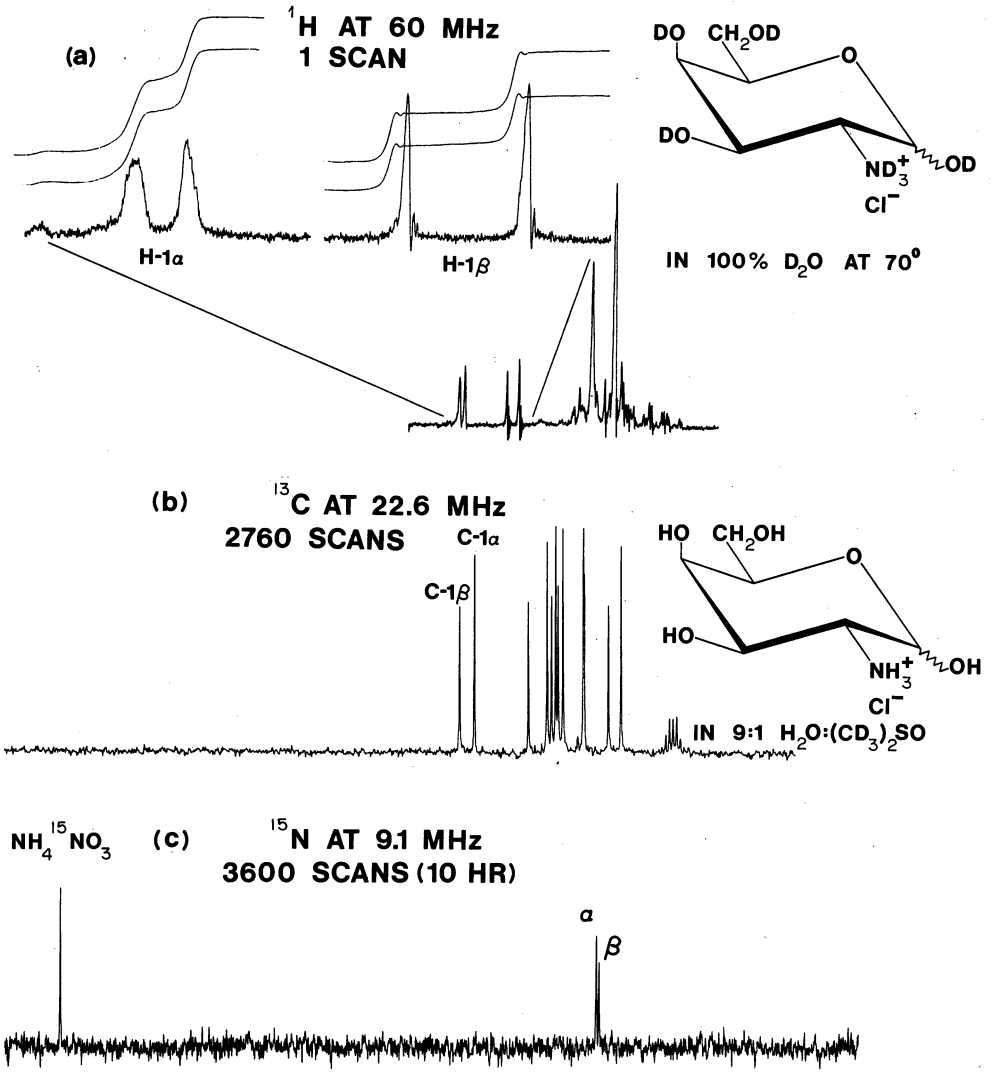


Fig. 4 Comparison of ^{1}H, ^{13}C, and ^{15}N n.m.r. spectra of equilibrated solutions of 2-amino-2-deoxy-D-galactose hydrochloride (the ^{13}C and ^{15}N n.m.r. spectra were recorded with proton decoupling at 90 MHz).

(+80°). We have reinvestigated the proton n.m.r. spectroscopy of equilibrated solutions of 2-amino-2-deoxy-D-galactose hydrochloride at 60 MHz (continuous wave mode) and at 220 MHz (continuous wave and Fourier transform modes), and the results indicate a predominance of the α-anomer over the temperature range 17-70°. The proton n.m.r. studies published previously (37) were conducted at 70° so as to displace the HOD resonance to higher field than at ambient temperature. This resonance is an analytical interference in quantitative analysis of the amino sugar anomers, and because the resonance is often broad, the intensity in its base may still make some contribution to the proton integral of 2-amino-2-deoxy-α-D-galactopyranose hydrochloride, even at 70°. We have attempted to minimize this interference by freeze-drying the salt twice with 99.8% deuterium oxide, once with 100% deuterium oxide, followed by preparation of the analytical solution from the latter solvent. The integrals of the H-1α and H-1β proton signals of 2-amino-2-deoxy-D-galactopyranose hydrochloride in 100% deuterium oxide at 60 MHz and 70° clearly indicate a preponderance of the α-pyranose anomer (see Fig. 4a). The peak heights of the H-1α and H-1β doublets are quite misleading, as the H-1β doublet (which displays excellent ringing) is inherently a much sharper signal than that of H-1α. The broadness of the latter signal (H-1e) persists at 220 MHz (see Fig. 5), which tends to suggest that the broadening is not due to virtual coupling produced by strong coupling of

TABLE 3. Analysis of aqueous solutions of 2-amino-2-deoxy-D-galactose hydrochloride by ^{1}H, ^{13}C, and ^{15}N n.m.r. spectroscopy

Nucleus	Freq. (MHz)	Mode	Solvent	Temp. (°C)	Ratio at equilibrium: Pyranose α	Pyranose β	Furanose α	Furanose β
^{1}H	60	CW[a]	100% D_2O	30	56	44		
				70	58	42		
			9:1 D_2O: $(CD_3)_2SO$	70	58	42		
	220	CW	100% D_2O	17	57	43		
		FT[b]		17	59	41		
		CW		70	59(54%)	41(38%)	(5%)	(3%)
		FT		71	57(53%)	43(41%)	(4%)	(2%)
^{13}C	22.6	FT	9:1 H_2O: $(CD_3)_2SO$	30	60	40		
^{15}N	9.1	FT		30	63	37		

[a]Continuous wave [b]Pulse-Fourier transform

H-2 and H-3,but to an unresolved long-range coupling, possibly of H-1e with H-4e. The ratios of pyranose anomers measured at 17°, 30°, and 70° (see Table 3) are not significantly different. Neither are the ratios measured at 70° for solutions of the 2-amino-2-deoxy-D-galactose hydrochloride in either 100% deuterium oxide or in 9:1 deuterium oxide:methyl sulfoxide-d_6. At 70°, the 220 MHz proton n.m.r. spectrum (Fig. 5) of this amino sugar displayed two additional doublets of minor intensity at lowest field (spacings 5 and 2 Hz), that were assigned as the H-1 signals of α and β furanose forms. The minor doublet of smaller spacing ($J_{1,2}$ 2 Hz) was assigned as the H-1 signal of 1,2-trans 2-amino-2-deoxy-β-D-galactofuranose hydrochloride, on the basis that the H-1 signal of penta-O-acetyl-β-D-galactofuranose in chloroform-d at 60 MHz appeared as a broad singlet that indicated a small value (<0.6 Hz) for the coupling constant $J_{1,2}$. A small value of $J_{1,2}$ (2.8 Hz) has been reported for trimethylsilyl 2,3,5,6-tetra-O-(trimethylsilyl)-β-D-galactofuranoside, the isolation of which indicated the presence

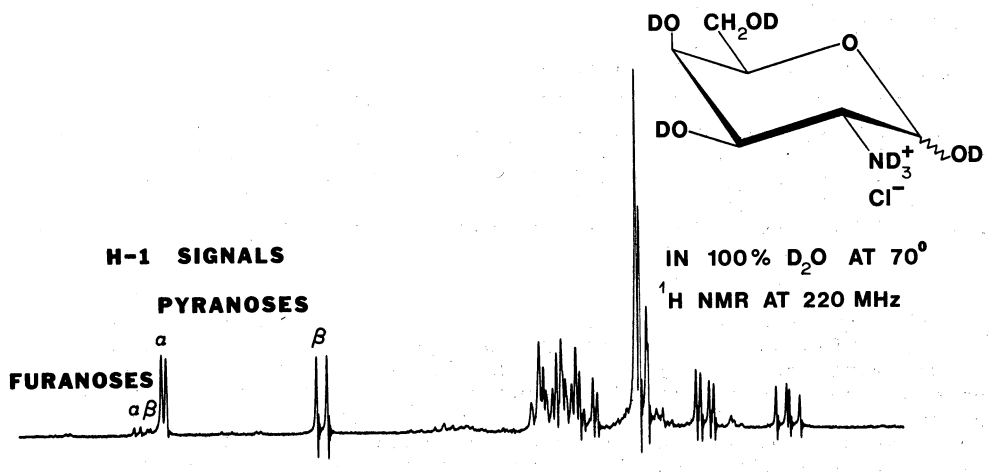


Fig. 5 220 MHz proton n.m.r. spectrum of an equilibrated solution of 2-amino-2-deoxy-D-galactose hydrochloride in 100% deuterium oxide.

of 14% of β-D-galactofuranose in an equilibrated solution of D-galactose (41). The proportions of anomeric pyranose and furanose forms that were measured at 220 MHz are shown in Table 3. The α-pyranose form predominates over the β-pyranose form and the α-furanose form over the β-furanose form. The continuous wave and Fourier transform modes at 220 MHz were found to give similar quantitative results (Table 3). Comparison of the 220 MHz (Fig. 5) and 60 MHz (Fig. 4a) spectra affords an excellent example of the much greater ease with which minor components of a mixture may be detected and measured at the high field strengths that are available in a superconducting solenoid.

The predominance of the α-pyranose anomer was confirmed by the ^{13}C n.m.r. spectrum (Fig. 4b) of an equilibrated solution of 2-amino-2-deoxy-D-galactose hydrochloride in 9:1 water:methyl sulfoxide-d_6 obtained at 22.6 MHz and 30°. Integration of the well separated ^{13}C-1 signals in this spectrum yielded an α:β ratio of 60:40 for pyranose forms, which may be compared with the ratio of 63:37 that was obtained by integration of the ^{15}N spectrum (Fig. 4c) of this solution (see Table 3). The assignment of the ^{13}C-1 resonance at lower field as that of the β-anomer was confirmed by observation of the discrete spectrum of a solution of this crystalline anomer in methyl sulfoxide-d_6. In this solvent, mutarotation is slow, and the spectrum obtained after signal averaging for one hour displayed only the six ^{13}C resonances of the β-anomer. Mutarotation was then accelerated by the addition of 10% (by volume) of water, and the appearance and gradual increase in intensity of the six ^{13}C signals of the α-anomer were observed.

TABLE 4. Analysis of aqueous solutions of amino sugars by ^{1}H and ^{15}N n.m.r. spectroscopy

Compound	Ratios of pyranose anomers at equilibrium			
	From ^{1}H[a]		From ^{15}N[b]	
	α	β	α	β
2-Amino-2-deoxy-D-glucose hydrochloride	63	37	63	37
2-Amino-2-deoxy-D-mannose hydrochloride	43	57	43	57
2-Amino-2-deoxy-D-galactose hydrochloride	58[c]	42[c]	63	37
2-Acetamido-2-deoxy-D-glucose	68	32	64	36
2-Acetamido-2-deoxy-D-mannose	57	43	63	37
2-Acetamido-2-deoxy-D-galactose	65	35	64	36

[a]For solutions in deuterium oxide at 60 MHz; literature data (ref. 37), unless noted otherwise [b]From peak areas computed for solutions in 9:1 v/v water: methyl sulfoxide-d_6. Approximately 90° pulses (30 μsec) were used with a 10 second delay between each pulse. Line-broadening (0.5 Hz) was applied to the spectrum by exponential filtering of the free induction decay signal. [c]This work.

The assignment of the less intense ^{15}N resonance at higher field in the ^{15}N spectrum (Fig. 4c) of 2-amino-2-deoxy-D-galactose hydrochloride equilibrated in 9:1 water:methyl sulfoxide-d_6 as that of the β-anomer was confirmed by the observation of a single ^{15}N resonance (having the same chemical shift) for a non-mutarotated solution of the crystalline β-anomer in methyl sulfoxide-d_6. Thus the predominance of the α-pyranose anomer in equilibrated aqueous solutions of 2-amino-2-deoxy-D-galactose hydrochloride seems to be established. Most literature values (42-47) for the specific rotations of these solutions fall in the range +92-98°, and if the proportions of pyranose anomers in the equilibrated solution are recalculated from the values (47) $[\alpha]_D$ + 125° → +93° for the α-anomer, and $[\alpha]_D$ + 44° → 93° for the β-anomer, by neglecting the small proportions of furanose forms, then an α:β ratio of 60:40 is obtained. This ratio agrees especially well with that found by integration of ^{13}C n.m.r. spectra (Table 3). The calculation of anomeric proportions from optical rotations is not unequivocally applicable unless both pyranose

forms are known in the crystalline state, and indeed, the general unavailability of crystalline furanose forms of the amino sugars prohibits the quantitative analysis of these forms by polarimetry. It is possible that the previous authors were misled by a value of +80° for the optical rotation of equilibrated 2-amino-2-deoxy-D-galactose hydrochloride which appears to have been abstracted incorrectly (48-50).

Integration of the ^{15}N spectra (Fig. 3) of 2-amino-2-deoxy-D-glucose hydrochloride and its D-manno epimer gave anomeric proportions that are in excellent agreement with those determined (37) earlier by proton n.m.r. spectroscopy (see Table 4).

2-Acetamido-2-deoxy-D-hexoses. The ^{15}N n.m.r. spectra of equilibrated solutions of 2-acetamido-2-deoxy-D-glucose and of its D-galacto analog were found to be very similar and each displayed two closely spaced resonances (Fig. 6). The more intense resonance at lower field was assigned to the α-anomer in each case, in agreement with the predominance of α-anomer determined earlier by proton n.m.r. (37), and indicated by ^{13}C n.m.r. (40). Good agreement was obtained between the anomeric proportions measured by integration of the ^{15}N spectra and the ^{1}H spectra (37) of the equilibrated solutions (see Table 4).

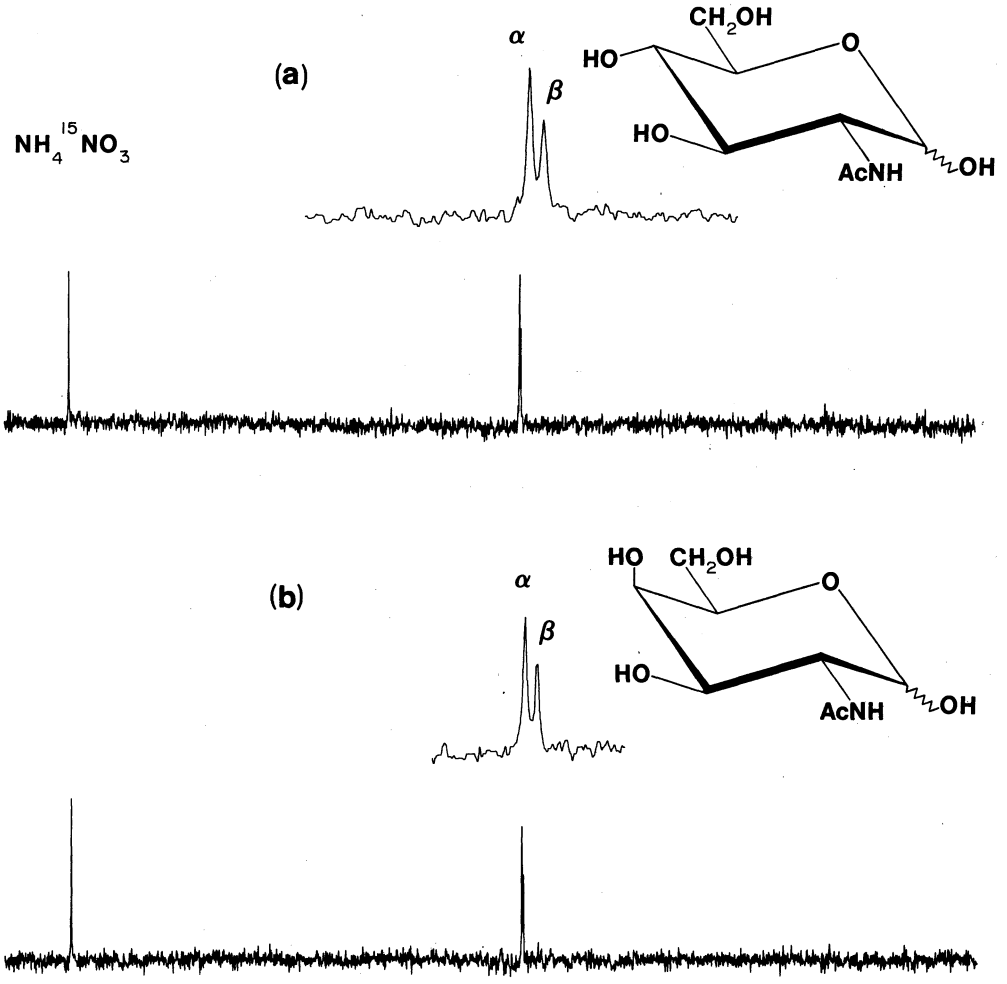


Fig. 6 Proton decoupled ^{15}N n.m.r. spectra at 9.12 MHz of equilibrated solutions in 9:1 v/v water:methyl sulfoxide-d_6, (a) 2-acetamido-2-deoxy-D-glucose, and (b) 2-acetamido-2-deoxy-D-galactose.

The ^{15}N n.m.r. spectrum (see Fig. 7a) of an equilibrated solution of 2-acetamido-2-deoxy-D-mannose displays two well separated resonances, and the more intense peak at lower field has been assigned to the α-anomer. This

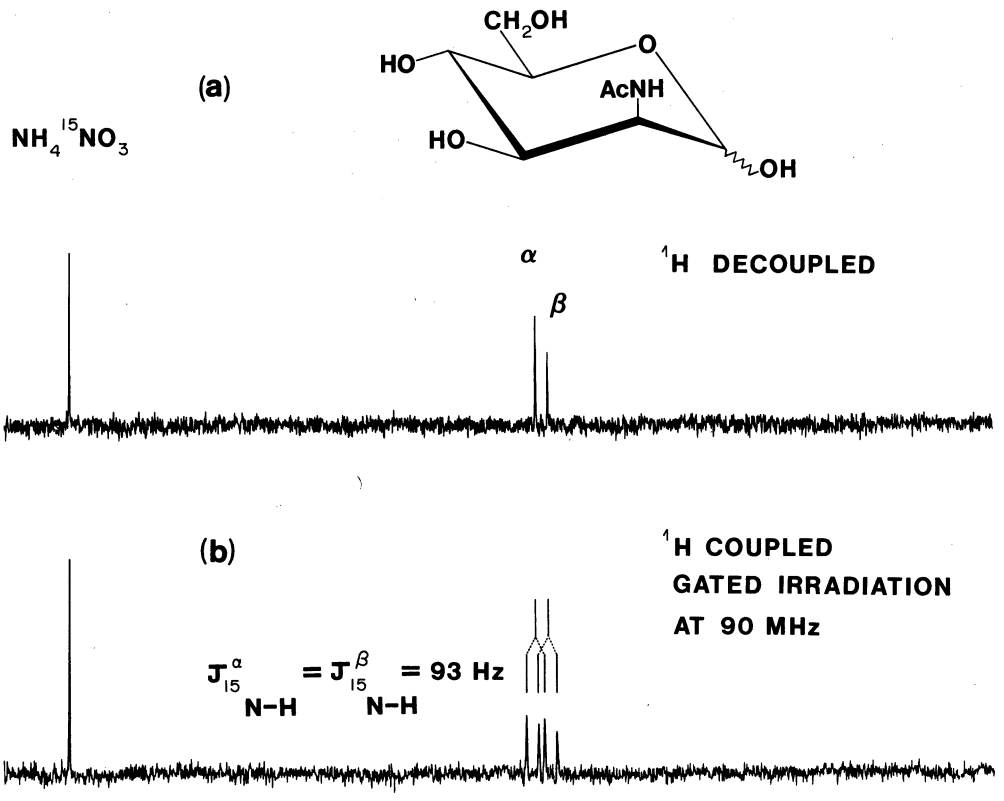


Fig. 7 ^{15}N n.m.r. spectra at 9.12 MHz of an equilibrated solution of 2-acetamido-2-deoxy-D-mannose in 9:1 v/v water:methyl sulfoxide-d_6, (a) protons decoupled, and (b) protons coupled.

assignment was confirmed by observation of the rather rapid mutarotation that occurred when crystalline 2-acetamido-2-deoxy-β-D-mannose monohydrate was dissolved in methyl sulfoxide-d_6. The ^{15}N spectrum that was obtained by scanning from 1-32 hr after dissolution of the sugar still displayed a slight majority of the β-anomer, but after seven weeks, integration of the spectrum indicated an α:β ratio of 91:9. Similar results were obtained from a methyl sulfoxide-d_6 solution of the solid residue prepared by freeze-drying of an equilibrated solution of 2-acetamido-2-deoxy-D-mannose in water. Evidently the α-anomer is even more stable than the β-anomer in methyl sulfoxide, than in water. Only fair agreement was obtained between the α:β ratio 57:43 determined for an equilibrated solution of 2-acetamido-2-deoxy-D-mannose in deuterium oxide by proton n.m.r. (37) and the α:β ratio of 63:37 measured by ^{15}N n.m.r. spectroscopy of a solution of this derivative in 9:1 water:methyl sulfoxide-d_6, perhaps owing to the sensitivity of this α:β equilibrium to solvent.

A proton-coupled ^{15}N spectrum (see Fig. 7b) was obtained for equilibrated 2-acetamido-2-deoxy-D-mannose by irradiation of the protons at 90 MHz during the periods between data acquisitions. This gated irradiation technique (11) allows the acquisition of an 'undecoupled' spectrum with the retention of most of the n.o.e. In this instance, the ^{15}N spectrum consists of two overlapping doublets (Fig. 7b) that indicate that the ^{15}N-H coupling constants of the α and β anomers of 2-acetamido-2-deoxy-D-mannose are each equal to 93 Hz. This magnitude of ^{15}N-H coupling constant generally indicates an important contribution from an sp^2 hybridized nitrogen atom to the structure of the acetamido group, although in this work, no evidence has been adduced for the existence of *cis* and *trans* forms about the amide bond of acetamido-deoxy sugars.

^{15}N chemical shifts

The ^{15}N chemical shifts of the amino sugar hydrochlorides and their N-acetyl derivatives are summarized in Table 5. Mono-acylation of the nitrogen atom causes a substantial downfield shift (∿90 p.p.m.) of the ^{15}N resonance. This

TABLE 5. ^{15}N chemical shifts of amino sugars

Compound	^{15}N chemical shifts of anomers[a]		Difference
	α	β	(β-α)
2-Amino-2-deoxy-D-glucose hydrochloride	341.1	342.5	1.4
2-Amino-2-deoxy-D-mannose hydrochloride	344.5	352.0	7.5
2-Amino-2-deoxy-D-galactose hydrochloride	342.1	343.9	1.8
2-Acetamido-2-deoxy-D-glucose	252.8	253.6	0.8
2-Acetamido-2-deoxy-D-mannose	256.5	263.3	6.8
2-Acetamido-2-deoxy-D-galactose	253.1	253.9	0.8

[a]P.p.m. upfield (±0.2 p.p.m.) from external $NH_4{}^{15}NO_3$, for solutions in 9:1 v/v water:methyl sulfoxide-d_6 at 9.12 MHz.

shift may be compared with that (∿141 p.p.m.) produced by di-N-acylation in the form of N-phthaloylation of the 6-amino-6-deoxy-di-O-isopropylidene derivatives (see Table 1.).

Correlation of the ^{15}N chemical shifts (Table 5) of the anomeric and epimeric amino sugars with their $^4C_1$(D) conformations (37) allows the following generalizations for the compounds studied:

(a) An equatorial nitrogen nucleus resonates at lower field than a corresponding axial nitrogen nucleus (by 3.4-9.5 p.p.m. for hydrochlorides and 3.7-9.7 p.p.m. for N-acetyl derivatives).

(b) The nitrogen nucleus of α-anomers resonates at lower field than that of β-anomers.

(c) The nitrogen chemical shift difference of anomers (see Table 5) is substantially greater (6.8-7.5 p.p.m.) when the nitrogen nucleus at C-2 is axial (D-manno configuration) than when the nitrogen nucleus is equatorial (D-gluco and D-galacto configurations, differences in ^{15}N shifts of anomers, 0.8-1.8 p.p.m.).

(d) The effect of inversion of configuration at C-4 on the chemical shift of an equatorial nitrogen nucleus at C-2 is small (0.3-1.8 p.p.m.) and comparable with the effect (0.8-1.8 p.p.m.) of inversion at C-1.

The occurrence of the ^{15}N resonance of an equatorial nitrogen nucleus at lower field than that of an axial nitrogen nucleus is reminiscent of the well known chemical shift difference shown by axial and equatorial protons (in the absence of syn-axial substituents) which has often been interpreted in terms of angular effects of the diamagnetic anisotropies of carbon-carbon, or carbon-oxygen bonds (51). Many of the earlier theoretical treatments (51,52) were derived on the assumption that the anisotropy of the C-H bond is negligible, an assumption that has subsequently proved to be invalid (53).

Most theories of ^{15}N chemical shifts (2,54) have been concerned principally with explanation of the major differences within the large range (∿900 p.p.m.) of nitrogen shifts, in terms of the diamagnetic and paramagnetic shielding contributions from the electrons associated with the nitrogen atoms in different structures. The shielding contributions from other electrons in the molecule have usually been considered to be unimportant, and the effects of shielding anisotropy, ring currents, and solvents have been ignored (2). It is clear, however, that interpretation of the stereochemical dependence of nitrogen shifts observed in the present work requires assessment of the shielding contribution of electrons and bonds other than those associated directly with the nitrogen atom.

Consideration of the ^{15}N chemical shifts of the amino sugars (Table 5) reveals that, from an empirical point of view, the ^{15}N resonance moves to lower field as the number of carbon-carbon or carbon-oxygen bonds in trans-coplanar relationship with the nitrogen atom increases. Thus the nitrogen nuclei of 2-amino-2-deoxy-β-D-mannose hydrochloride and of 2-acetamido-2-deoxy-β-D-mannose resonate at highest field in each of their respective groups of anomeric and epimeric amino sugars (Table 5). In compounds having the β-D-manno configuration, and $^4C_1$(D) conformation, there are no C-C or C-O bonds in trans-coplanar relationship with the axial nitrogen atom. In terms of the point-dipole approximation (51), this corresponds to an absence of deshielding C-C and C-O bonds, the electrical centres of gravity of which subtend a small angle (<55°) between the bond axis and the nucleus of interest. The observation of ^{15}N deshielding effects (Table 5) in the α-D-manno and D-gluco derivatives, in which there are C-O, and C-C and C-O bonds, respectively, in trans-coplanar orientation to the nitrogen nucleus, suggests that the diamagnetic anisotropy of the C-C and C-O bonds is numerically greater than that of the C-H bonds. However, complete interpretation of such deshielding effects in terms of anisotropies is difficult because of the inadequacies of the point-dipole approximation (53). For protons, at least, the use of a modified treatment in which the dipole was assigned a finite length did not substantially improve the calculated anisotropies (53,55).

Acknowledgements - Thanks are due Dr. Cheri Fisk of the National Institutes of Health for the 220 MHz proton n.m.r. spectra, and Drs. R.B. Johannesen, S. Parks, and R. Schaffer for their help and support.

REFERENCES

1. R.R. Ernst and W.A. Anderson, Rev. Sci. Instrum. 37, 93 (1966).
2. G.A. Webb and M. Witanowski in Nitrogen NMR (editors M. Witanowski and G.A. Webb) Plenum Press, London (1973).
3. S. Umezawa, Advan. Carbohyd. Chem. Biochem. 30, 111 (1974).
4. H. Umezawa, Advan. Carbohyd. Chem. Biochem. 30, 183 (1974).
5. R.L. Whistler, A.A. Bushway, P.P. Singh, W. Nakahara, and R. Tokuzen, Advan. Carbohyd. Chem. Biochem. 32, 235 (1976).
6. G.N. LaMar, J.Amer. Chem.Soc. 93, 1040 (1971); Chem. Phys. Lett. 10, 230 (1971).
7. D.F.S. Natusch, J.Amer.Chem.Soc. 93, 2567 (1971).
8. R.Freeman, K.G.R. Pachler, and G.N. LaMar, J.Chem. Phys. 55, 4586 (1971).
9. O.A. Gansow, A.R. Burke, and W.D. Vernon, J. Amer. Chem. Soc. 94, 2550 (1972).
10. L.F. Farnell, E.W. Randall, and A.I. White, Chem. Commun. 1159 (1972).
11. O.A. Gansow and W. Schittenhelm, J. Amer. Chem. Soc. 93, 4294 (1971).
12. R. Freeman, H.D.W. Hill, and R. Kaptein, J. Magn. Resonance, 7, 327 (1972).
13. H. Booth, B.W. Bycroft, C.M. Wels, K. Corbett, and A.P. Maloney, Chem. Commun. 110 (1976).
14. B. Coxon, Carbohyd. Res. 11, 153 (1969).
15. B. Coxon, Carbohyd. Res. 19, 197 (1971).
16. B. Coxon, Carbohyd. Res. 35, C1 (1974).
17. B. Coxon and L.F. Johnson, Carbohyd. Res. 20, 105 (1971).
18. G. Binsch, J.B. Lambert, B.W. Roberts, and J.D. Roberts, J. Amer. Chem. Soc. 86, 5564 (1964).
19. R.S. Tipson, Advan. Carbohyd. Chem. 8, 107 (1953).
20. J.M. Sugihara and W.J. Teerlink, J. Org. Chem. 29, 550 (1964).
21. S. Nadkarni and N.R. Williams, J. Chem. Soc. 3496 (1965).
22. A.C. Richardson, Carbohyd. Res. 10, 395 (1969).
23. V.I. Veksler, L.N. Kovalenko, and A.V. Markovich, Zh. Obshch, Khim. 34, 704 (1964).
24. W.A. Szarek and J.K.N. Jones, Can. J. Chem. 43, 2345 (1965).
25. J.M. Briggs, L.F. Farnell, and E.W. Randall, Chem. Commun. 680 (1971).
26. P.S. Pregosin, E.W. Randall, and A.I. White, Chem. Commun. 1602 (1971).
27. G.C. Levy and J.D. Cargioli in Nuclear Magnetic Resonance Spectroscopy of Nuclei Other Than Protons (editors T. Axenrod and G.A. Webb), Wiley & Sons, New York (1974).
28. R.L. Vold, J.S. Waugh, M.P. Klein, and D.E. Phelps, J. Chem. Phys. 48, 3831 (1968).
29. G.C. Levy and I.R. Peat, J. Magn. Resonance, 18, 500 (1975).
30. I. Solomon, Phys. Rev. 99, 559 (1965).

31. J.W. Emsley, J.Feeney, and L.H. Sutcliffe, High Resolution Nuclear Magnetic Resonance Spectroscopy, volume 1, Pergamon Press, Oxford (1965).
32. A.G. Redfield and R.K. Gupta, Advan. Magn. Resonance, 5, 81 (1971).
33. E.O. Stejskal and J. Schaefer, J. Magn. Resonance, 13, 249 (1974).
34. E.O. Stejskal and J. Schaefer, J. Magn. Resonance, 14, 160 (1974).
35. S.I. Parks and R.B. Johannesen, J. Magn. Resonance, 22, 265 (1976).
36. D. Horton, J.S. Jewell, and K.D. Philips, J. Org. Chem. 31, 3843 (1966).
37. D. Horton, J.S. Jewell, and K.D. Philips, J. Org. Chem. 31, 4022 (1966).
38. A.S. Perlin, N.M.K. Ng Ying Kin, and S.S. Bhattacharjee, Can. J. Chem. 50, 2437 (1972).
39. D.R. Bundle, I.C.P. Smith, and H.J. Jennings, J. Biol. Chem. 249, 2275 (1974).
40. D.R. Bundle, H.J. Jennings and I.C.P. Smith, Can. J. Chem. 51, 3812 (1973).
41. R.S. Shallenberger and T.E. Acree, Carbohyd. Res. 1, 495 (1966).
42. P.A. Levene and F.B. LaForge, J. Biol. Chem. 18, 127 (1914).
43. P.A. Levene, J. Biol. Chem. 57, 337 (1923).
44. R. Kuhn, W. Bister, and H. Fischer, Ann. 617, 109 (1958).
45. R. Brossmer, Methods Carbohydrate Chem. 1, 216 (1962).
46. R.W. Jeanloz and P.J. Stoffyn, Methods Carbohydrate Chem. 1, 221 (1962).
47. D. Horton in The Amino Sugars, volume 1A (editor R.W. Jeanloz) Academic Press, New York (1969).
48. A.B. Foster and M. Stacey, Advan. Carbohyd. Chem. 7, 247 (1952).
49. A.B. Foster and D. Horton, Advan. Carbohyd. Chem. 14, 213 (1959).
50. D. Horton, Advan. Carbohyd. Chem., 15, 159 (1960).
51. L.M. Jackman, Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, Pergamon Press, London (1959).
52. L.D. Hall, Tetrahedron Letters, 1457 (1964).
53. L.M. Jackman and S. Sternhell, Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, 2nd edition, Pergamon Press, Oxford (1969).
54. G.A. Webb in reference 27.
55. J.W. ApSimon, W.G. Craig, P.V. Demarco, D.W. Mathieson, L. Saunders, and W.B. Whalley, Chem. Commun. 359 (1960).