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PREFACE

The continuing growth of the use of NMR spectroscopy in chemistry is clearly reflected both in the disparate nature of the areas covered in Volume 13 of Annual Reports and in the amount of information contained in each report presented.

Drs Bock and Thøgerson have reviewed the NMR of carbohydrates which is an area showing considerable expansion since it was last reviewed in Volume 5A. Recent developments in the NMR of alkaloids are covered by Professor Crabb who builds on his previous reports in Volumes 6A and 8. For the first time in this series I am happy to include reports from Professor Hinton and Drs Metz and Briggs on Thallium NMR, and from Professor Kidd and Dr Boeré on rotational correlation times in nuclear magnetic relaxation.

It is a pleasure to be able to express my thanks to all of the contributors for the careful preparation, and prompt submission, of their manuscripts. These efforts, in no small way, facilitate the continuing success of Annual Reports on NMR Spectroscopy.

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G. A. WEBB
March 1982

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Rotational Correlation Times in Nuclear Magnetic Relaxation

RENÉ T. BOERÉ AND R. GARTH KIDD

Nuclear Magnetic Resonance Spectroscopy in the Study of Mono- and Oligosaccharides

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I. INTRODUCTION

Since this subject was last reviewed in this series[1] the importance of NMR spectroscopy in the study of carbohydrates has increased tremendously. This has occurred primarily because the introduction of pulsed Fourier transform (FT) NMR spectrometers has made the measurement of ^{13}C NMR spectral parameters easy, which is particularly important for the study of carbohydrates in aqueous solutions. Furthermore, pulsed NMR instruments have increased the sensitivity of ^{1}H NMR spectra by several orders of magnitude and facilitated the measurement of relaxation times and nuclear Overhauser enhancement (NOE) factors. Computer control of the spectrometers has made new experiments possible such as two-dimensional NMR spectra, and simplified other experiments. Magnet technology has improved and 500 MHz ^{1}H NMR spectrometers are commercially available with the associated high dispersion and sensitivity, and today multinuclear spectrometers are routine tools in many chemical laboratories.

This review covers the period 1973–1980, particularly the last part of the period. It is primarily concerned with a description of how to assign NMR parameters and how to use these values in the study of carbohydrates. Special emphasis is given to the ^{1}H NMR parameters because the application of ^{13}C NMR spectroscopy in the study of monosaccharides and oligo- and polysaccharides has recently been reviewed.[2–5]

The NMR parameters of nucleotides, nucleosides and aminoglycoside antibiotics are not discussed in the present review. The NMR parameters of the former compounds have been discussed extensively in a recent review.[6]

No attempt has been made to cover all applications in which NMR data have been used to establish carbohydrate structures or to study carbohydrates in solution. The yearly reports from The Chemical Society on carbohydrate chemistry[7] and nuclear magnetic resonance spectroscopy[8] are excellent references in this respect. Several general reviews on NMR spectroscopy of carbohydrates have appeared during the period.[9–11] A

description of how NMR parameters are obtained is beyond the scope of the present review, but readers are referred to general monographs.[12–15]

II. ASSIGNMENT TECHNIQUES

The assignment of the NMR signals is a necessary prerequisite for the application of NMR spectroscopy in structural investigations of carbohydrates. Since assignment techniques have been described in many reviews and monographs (e.g. references 12, 13), special emphasis is given to the problems associated with the assignment of signals in the NMR spectra of carbohydrates and their derivatives. The assignment techniques for ^{1}H NMR data and ^{13}C NMR data are described separately.

A. ^{1}H NMR assignments

The following points will be discussed:

1. Comparison with model compounds
2. Isotopic substitution
3. Double resonance experiments
4. Relaxation experiments
5. Two-dimensional spectroscopy
6. Paramagnetic shift reagents
7. Protonation shifts
8. Miscellaneous

1. Comparison with model compounds

With modern high field NMR spectrometers the ^{1}H NMR spectra of most monosaccharides can be analysed on a first-order basis. Mutarotated mixtures of carbohydrates in aqueous (D_2O) solutions give well resolved spectra when measured on a high field spectrometer.

The ^{1}H chemical shifts and coupling constants for the most predominant anomers of aldohexoses and aldopentoses and the corresponding methylglycosides together with those of the most common methyldeoxyhexopyranosides and methyl-2-acetamido-2-deoxyhexopyranosides are given in Section IV. Assignment techniques based on comparison with model compounds are important when analysing spectra of complex oligosaccharides.[16,17] Difficulties will often arise because the protons are located at the surface of the molecules (in contrast to the ^{13}C nuclei) making interunit shielding and deshielding effects important.[18]

De Bruyn, Anteunis and coworkers have in a series of papers[19–26] described the 300 MHz ^{1}H NMR spectra in D_2O of a series of mono- and oligosaccharides. They conclude that shift increments can be used in the

identification of individual proton resonances and to assess the position of glycosidic linkages.

The chemical shifts of protected carbohydrate derivatives (e.g. acetates) have been discussed in previous reviews[9–11] and follow the general rules for ^{1}H NMR chemical shifts.[27] The chemical shifts of common protecting groups used in carbohydrate chemistry are given in reference 11.

When comparing ^{1}H chemical shifts with literature data it is important, particularly in aqueous solutions, to measure the spectra at the same temperature and in the same solvent.

2. *Isotopic substitution*

If the molecules of interest contains spin $\frac{1}{2}$ nuclei other than protons (e.g. ^{19}F, ^{31}P or ^{13}C (enriched)) heteronuclear spin–spin couplings will appear in the ^{1}H NMR spectrum. This can be valuable in the assignment of the proton spectra, particularly if heteronuclear decoupling facilities are available. A recent review has discussed results obtained for fluorinated carbohydrate derivatives.[28]

Deuterium substitution in carbohydrates causes the substituted proton to disappear in the ^{1}H NMR spectrum and also reduces the spin–spin couplings by approximately a factor of six. The result of this substitution is generally that spin–spin couplings, to all neighbouring protons from the site where the deuterium substitution has taken place, are removed. The reduced couplings can of course be removed by deuterium decoupling.

Figure 1 shows the ^{1}H NMR spectrum of octa-*O*-acetyl-*β*-D-gentiobiose together with two deuterated derivatives which clearly illustrates the points discussed above.

Protons which are neighbouring to the substituted site will in addition to the reduction of spin–spin couplings also experience an isotope effect and resonate at lower frequencies, as demonstrated recently.[29] The preparation of deuterated derivatives normally requires several more or less laborious synthetic steps,[30] but a convenient method for the preparation of glycosides labelled with deuterium on hydroxy-bearing carbon atoms has recently been developed as described in Section II.B.2.

3. *Double resonance experiments*

(a) *Homonuclear decoupling* (^1H–$\{^1H\}$ *experiments*). Homonuclear decoupling is probably the single most widely used experiment to assist in the assignment of proton spectra. With computer-controlled FT instruments this experiment can be performed in the difference mode, as described by Gibbons *et al.*,[31] and applied in the analysis of the spectra of oligosaccharides.[17]

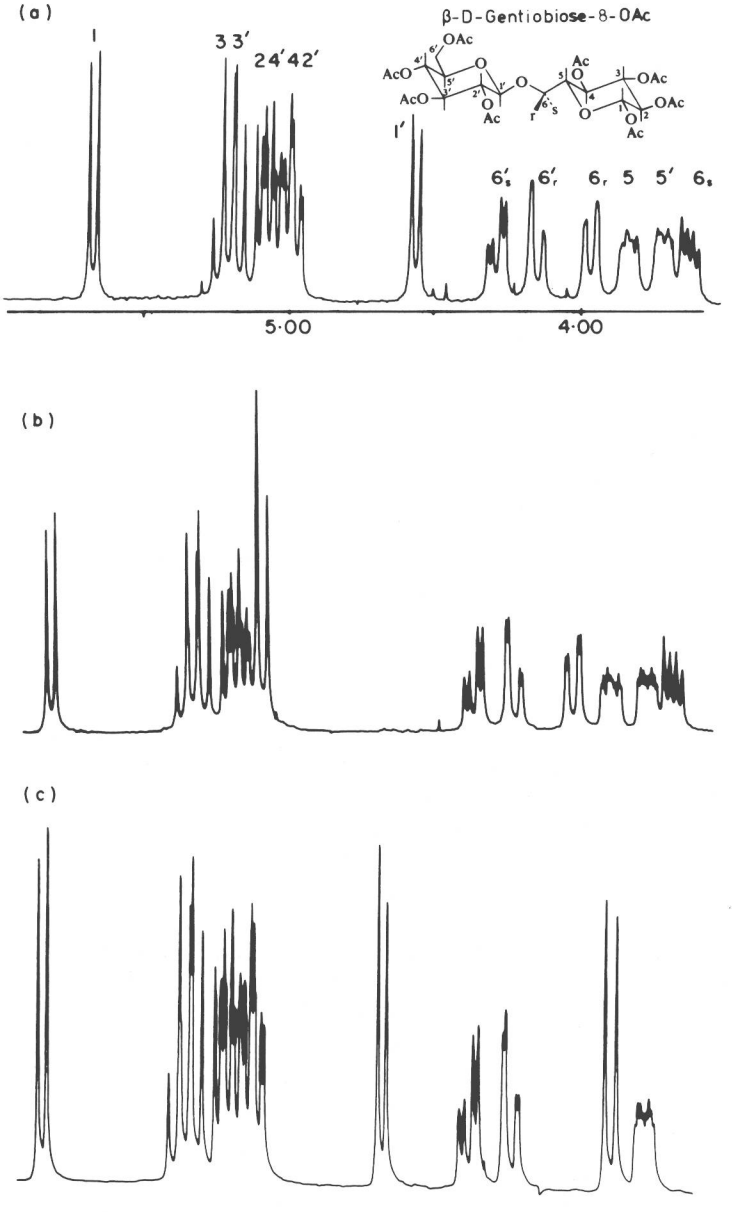


FIG. 1. Partial ^1H 270 MHz spectrum of octa-*O*-acetyl-*β*-D-gentiobiose and deuterated derivatives in deuteriochloroform. (a) Spectrum of normal compound. (b) Spectrum of [1′-^2H] derivative. (c) Spectrum of [6_r,6_s-$^2H_2$] derivative.

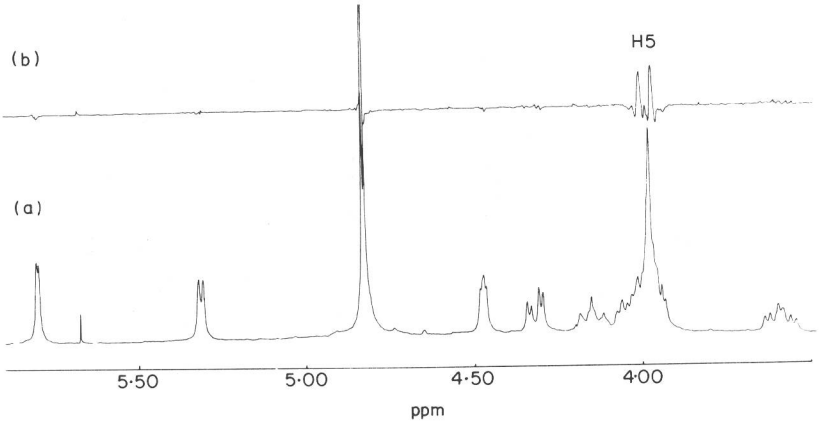


FIG. 2. Partial 270 MHz ^{1}H spectrum of *p*-trifluoroacetamidophenyl-3-*O*-(3,6-dideoxy-α-D-*ribo*-hexopyranosyl)-α-D-mannopyranoside in D_2O at 310 K. (a) Normal spectrum. (b) Difference decoupling experiment with saturation of the H-6 resonances at 1·2 ppm. The chemical shift of H-5 is easily determined from the experiment and it is also seen that H-5 and H-4 are spin–spin coupled with a large coupling constant (10 Hz).

This is illustrated in Fig. 2 which shows how this technique makes it possible to obtain both chemical shift and coupling information from a "hidden resonance".

The only limitation to this experiment is that Block–Siegert shifts are induced when the chemical shift difference between the saturated proton(s) and the observed proton(s) becomes too small. This makes it more difficult to interpret the difference spectrum.

A recent version of a multi-homodecoupling experiment, the two-dimensional scalar coupling experiment (SECSY), has been developed by Ernst *et al.*[32]

In this experiment the data points are collected in a data matrix as a function of t_1 and t_2, where t_1 and t_2 are the times in a 90°–t_1–90°–FID(t_2) pulse sequence. The data are then Fourier-transformed with respect to both directions in the data matrix. The results can then be displayed as shown in reference 33. A modification of the pulse sequence, i.e. 90°–t_1–90°–t_1–FID(t_2), where the half echo is sampled, results after data manipulation in a spectrum, as shown in Fig. 3.

Figure 3 shows the SECSY experiment at 400 MHz of a disaccharide, methyl-2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside. Figure 3(b) shows the two-dimensional scalar coupled spectrum as a contour diagram with the normal spectrum appearing along the horizontal line in the middle. Resonances which are spin–spin coupled give rise to signals off this line and are connected by parallel lines (as shown in Fig. 3(b)), i.e. the high

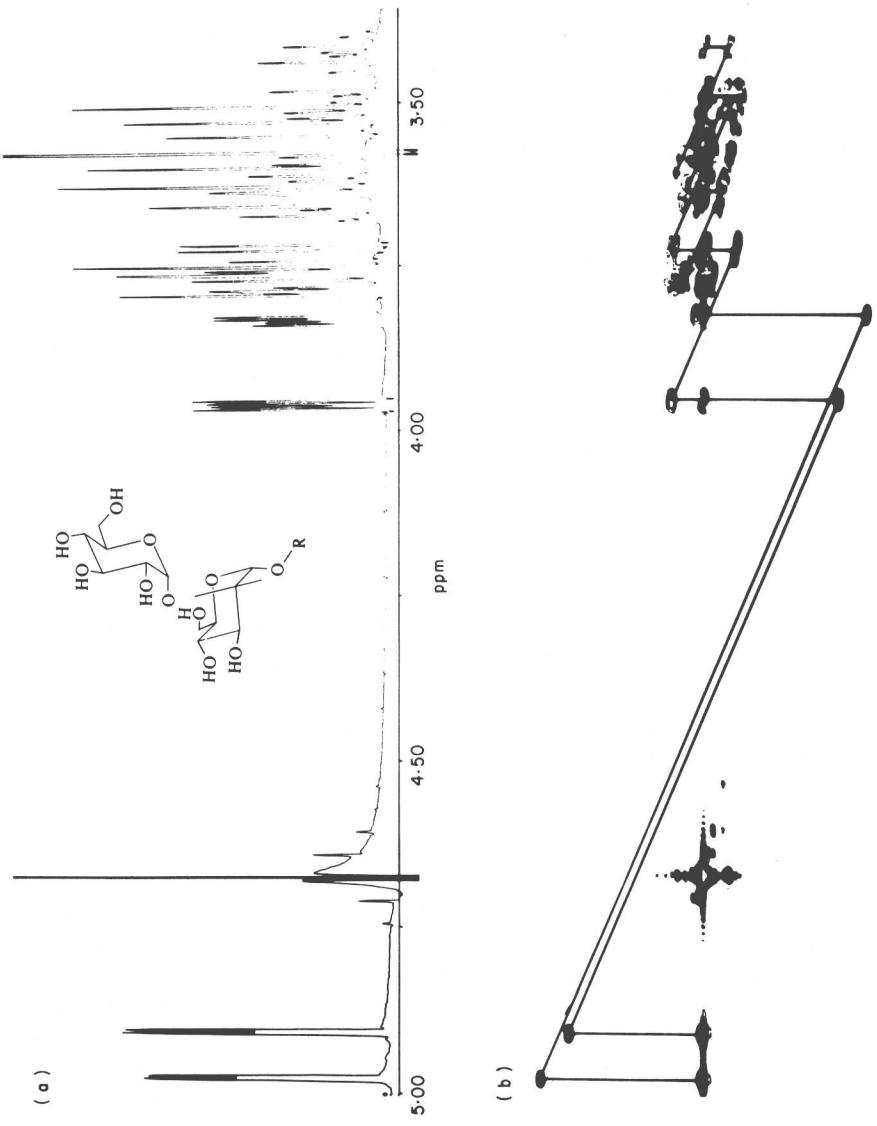
frequency H-1 is spin–spin coupled to H-2 resonating at 3·85 ppm and H-1 resonating at 4·90 ppm is spin–spin coupled to H-2 resonating at 3·95 ppm. This is a very powerful experiment in the analysis of the spectra of complex oligosaccharides and makes it possible to perform "homodecoupling" experiments without the use of a homodecoupler, i.e. avoiding the problems with off-resonance effects and other difficulties associated with this experiment. The disadvantage is that the experiment is rather time consuming in acquisition, processing and plotting time and normally requires *c.* 20 h of instrument time.

(b) *INDOR experiments.* INDOR experiments[34] have been used extensively in the spectral analysis of carbohydrate derivatives in continous wave experiments.[20–26] It is also possible to perform these experiments on FT instruments[35] by applying selective pulses[36] to the resonance lines. An example of the application of this technique in the analysis of the ^{1}H NMR spectrum of methylhepta-*O*-acetyl-β-D-cellobioside is shown in Fig. 4 using the difference technique. In order to obtain good results it is important to have a very stable magnetic field, which may be obtained with a superconducting magnet.

(c) *Nuclear Overhauser experiments.* Nuclear Overhauser experiments[37,38] have become a useful tool in the assignment of ^{1}H NMR spectra of complex oligosaccharides,[16,18,39,40] particularly when performed in the difference mode.[41] In Fig. 5 is shown the result of saturation of H-1 in methylhepta-*O*-acetyl-β-D-cellobioside. Protons H-3′ and H-5′ have their signals enhanced because of the 1–3 diaxial relationship to H-1′; also H-4 is enhanced due to its closeness in space to H-1′ in the preferred conformation of the oligosaccharide. H-6b is also enhanced because H-6a has the same chemical shift as H-1. H-4′ experiences a negative NOE because it is very strongly relaxed by H-5′, H-3′ and H-2′, all of which are relaxed by the saturated H-1′. This second-order effect has been discussed in detail by Noggle and Schirmer.[37]

The numerical values of the enhancements can furthermore be used in a conformational analysis of oligosaccharides.[40] However, the NOE values are dependent not only on the correlation times of the molecules (T_c) (i.e. dependent on the size of the molecule, the viscosity of the solution and the temperature) but also on the applied magnetic field strength, as shown in Fig. 6. For a 0·1 M sample of a heptasaccharide in D_2O at 300 K the NOE values are zero for some atoms at 400 MHz, but positive at, for example, 270 MHz. Larger molecules, i.e. polysaccharides, may show negative enhancements, as illustrated in Fig. 7.

For larger molecules spin diffusion[42] may be a problem and the transient method[43] may be preferred. Alternatively, a two-dimensional FT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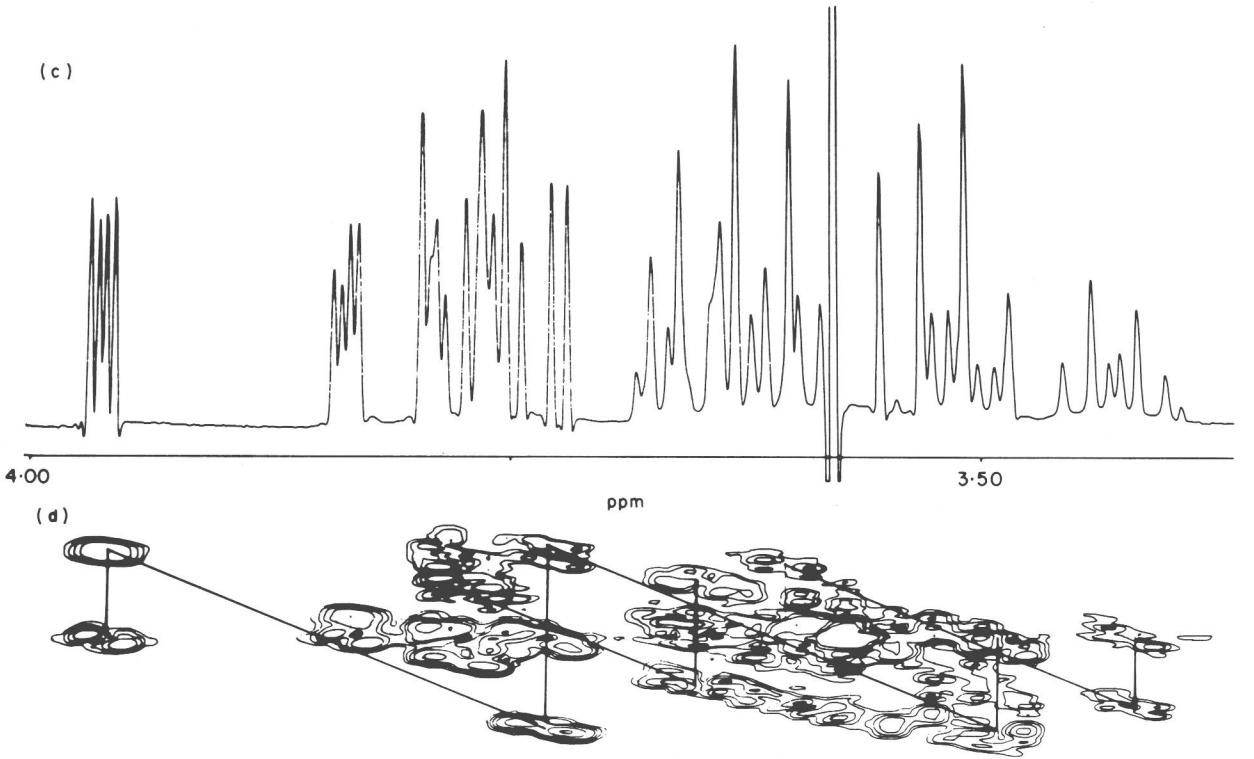

FIG. 3. Partial 400 MHz ^{1}H spectrum of 8-methoxycarbonyloctyl-2-*O*-(α-D-manno-pyranosyl)-α-D-mannopyranoside in D_2O at 300 K. (a) Normal one-dimensional spectrum. (b) Contour diagram of a two-dimensional SECSY experiment. The normal spectrum, seen from the top, is displayed along the centre line. The spin–spin connectivities are indicated on parallel diagonal lines. Thus H-2 resonating at 3·95 ppm is coupled to H-1 at 4·90 ppm and H-3 at 3·74 ppm. (c) Enlargement of part of the normal spectrum. (d) Enlargement of part of the contour diagram.