Carbohydrate Research, 166 (1987) 47–58
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

NEW N.M.R.-SPECTROSCOPIC APPROACHES FOR STRUCTURAL STUDIES OF POLYSACCHARIDES: APPLICATION TO THE *Haemophilus influenzae* TYPE a CAPSULAR POLYSACCHARIDE

R. ANDREW BYRD*, WILLIAM EGAN, MICHAEL F. SUMMERS*,
Biophysics Laboratory, Center for Drugs and Biologics, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892 (U.S.A.)

AND AD BAX
National Institute of Diabetes and Digestive and Kidney Diseases, The National Institutes of Health, Bethesda, MD 20892 (U.S.A.)
(Received June 23rd, 1986; accepted for publication in revised form, January 26th, 1987)

ABSTRACT

The extension of several modern nuclear magnetic resonance (n.m.r.) spectroscopic techniques to polysaccharides is discussed and illustrated, using the native *Haemophilus influenzae* type a capsular polysaccharide. These techniques provide for the unambiguous assignment of all n.m.r. resonances (^{1}H, ^{13}C, and ^{31}P) *via* high-sensitivity homonuclear and ^{1}H-detected heteronuclear correlations, and they are capable of locating the intersaccharide linkages (both *O*-linked and phosphoric diester-linked) and appended groups (*e.g.* *O*-acetyl groups). To illustrate the power and sensitivity of these methods, a 10-mg sample of the *H. Influenzae* type a polysaccharide (repeat unit mol. wt. = 376) was studied. The combined acquisition time for the two-dimensional ^{1}H–^{13}C correlation data (one-bond and multiple-bond), the ^{1}H–^{31}P correlation data, and the ^{1}H–^{1}H (homonuclear Hartmann–Hahn) data was ~18 h.

INTRODUCTION

^{1}H- and ^{13}C-n.m.r. spectroscopy are important tools for the structural characterization of carbohydrates and their derivatives (for recent reviews, see refs. 1–6). Although less utilized, ^{31}P-n.m.r. spectroscopy can also provide valuable structural information for phosphorus-containing polysaccharides[6]. Structural elucidation has been achieved through identification of heteronuclear scalar couplings and resonance assignments based on model compounds and empirically derived rules[1–5]. Two-dimensional (2D) chemical-shift correlation maps are extremely useful in the structural analysis of polysaccharides, as they provide greatly enhanced

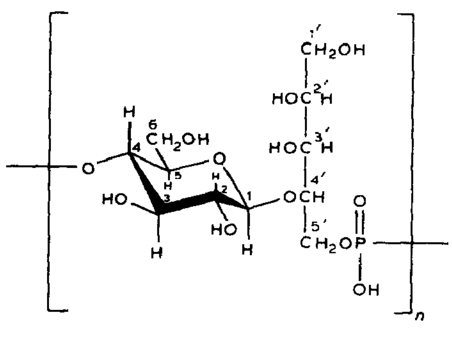
*To whom correspondence should be addressed.

0008-6215/87/$ 03.50

resolution of the usually crowded regions of the conventional 1D spectra. Recently, a procedure employing two-dimensional COSY and ^{13}C-detected C–H correlation spectroscopy combined with 1D selective INEPT spectroscopy[7] was shown to provide unambiguous resonance assignments and structural information for polysaccharides, without the need for chemical methods. Although complete, these methods require relatively large quantities of pure materials for such an analysis.

In contrast, there have been introduced new n.m.r. methods for homonuclear and heteronuclear correlations, involving ^{1}H-detection, that provide equivalent correlations but at much improved sensitivity. The heteronuclear shift correlation methods all involve creation and detection of heteronuclear multiple-quantum coherence. The power of these indirect-detection methods has been demonstrated for small molecular systems; for example, the coenzyme[8] B_{12}, and the application to a trisaccharide as discussed by Lerner and Bax[9]; however, there have been no similar studies of polymers, where slow molecular-correlation times can enhance ^{1}H relaxation and preclude the creation and detection of heteronuclear multiple-quantum correlations.

Herein, we examine the procedure for complete resonance assignment and structural analysis of native polysaccharides *via* these new n.m.r. methods. We also illustrate the high-sensitivity n.m.r. determination of phosphoric diester linkage-sites in polysaccharides, which are not amenable to conventional chemical methylation analysis[10]. Two of the pulse sequences utilized in this work are described in a previous paper[9], and the other two pulse sequences are described elsewhere[11–18]. The *H. influenzae* type a polysaccharide[6,19] was chosen, as its structure is known, but, despite its simplicity, the structure is not readily determined solely by chemical methods[6,10]. The n.m.r. procedure consists of determining monosaccharide composition (including resonance assignments and anomeric configuration) and intersaccharide linkages, and identification and location of appended groups.



RESULTS AND DISCUSSION

Monosaccharide composition. — Identification of monosaccharide units is approached by first analyzing the ^1H homonuclear shift correlation spectra. The conventional approach involves use of the COSY experiment, which identifies direct J-couplings (*e.g.*, geminal and vicinal). Although the anomeric-proton resonances are usually well resolved from the other ^1H resonances, and provide a good starting point for assigning the remaining resonances, the overlap of other proton resonances often leads to ambiguities or failure of this approach. Even for the relatively simple *H. influenzae* type a polysaccharide, ambiguities arise in the COSY spectrum (see Fig. 1). This situation is best resolved by use of the two-dimensional phase-sensitive

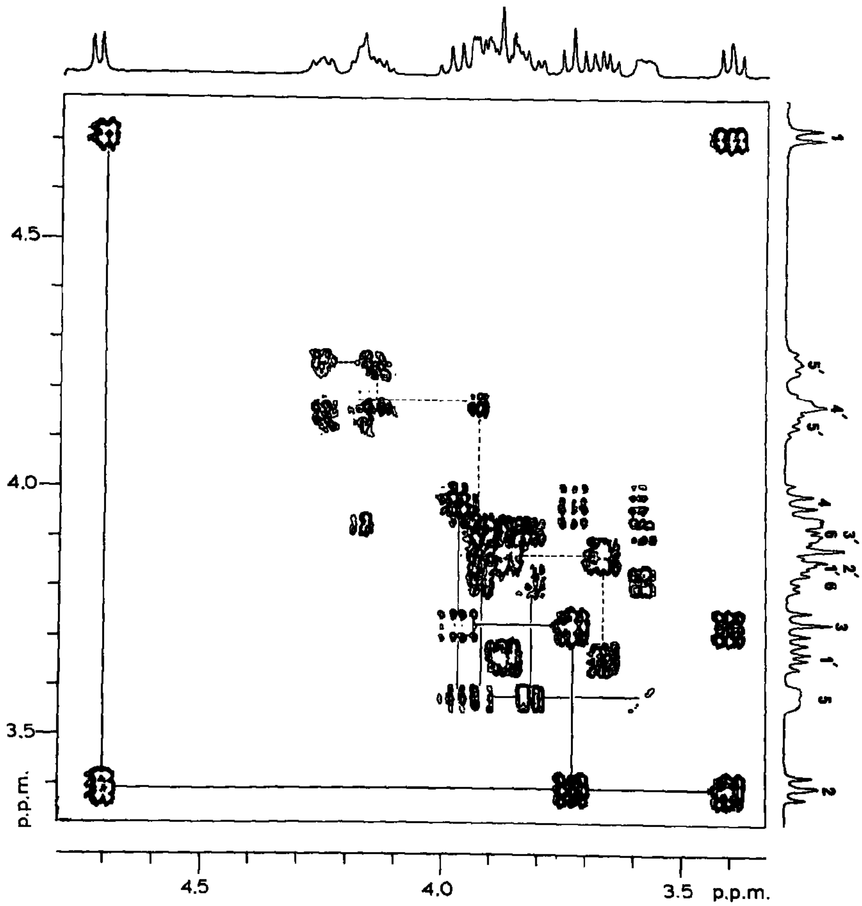


Fig. 1. Two-dimensional COSY spectrum of *H. influenzae* type a capsular polysaccharide. Solid lines and dashed lines connect signals from the glucosyl and ribitol residues, respectively. A conventional 1D proton spectrum with signal assignments added is given to the right of the spectrum.

HOHAHA experiment, which offers improved resolution and sensitivity, and reveals both direct and relayed J-coupling information[11-15]. From a HOHAHA spectrum, "J-networks" can be determined, where a J-network is defined as a group of protons that are serially linked *via* ^1H–^1H J (scalar) coupling. For example, all of the protons of a single saccharide unit belong to the same J-network. Magnetization transfer occurs in this experiment during the mixing period, τ, in which a composite spin-lock pulse is applied. Magnetization is transferred from one proton (A) to another (M) at a rate proportional to the inverse of the coupling constant (J_{AM}). If proton M is coupled to another proton, X, the magnetization of A can be relayed to X *via* the M nucleus. For intermediate to long mixing periods (50–80 ms), magnetization from one proton can be distributed over most of the other protons of the same J-network. For the *H. influenzae* type a polysaccharide, a typical mixing period of 75 ms resulted in magnetization transfer across a network of four to five ^1H nuclei. This is seen clearly in the HOHAHA spectrum in Fig. 2, where magnetization is transferred from the glucosyl H-1 atom to the H-2, H-3, H-4, and H-5 atoms. The transfer and propagation through the J-network is oscillatory in nature, at rates proportional to the respective coupling-constants[14,15]. This leads to inequivalence in the intensity of cross peaks as a function of the mixing period τ, which is seen for the cross peaks between the D-glucosyl H-5 and H-6 atoms in Fig. 2. Any ambiguity in determination or assignment of the J-network is generally accounted for in the COSY experiment. Other relevant parameters for the

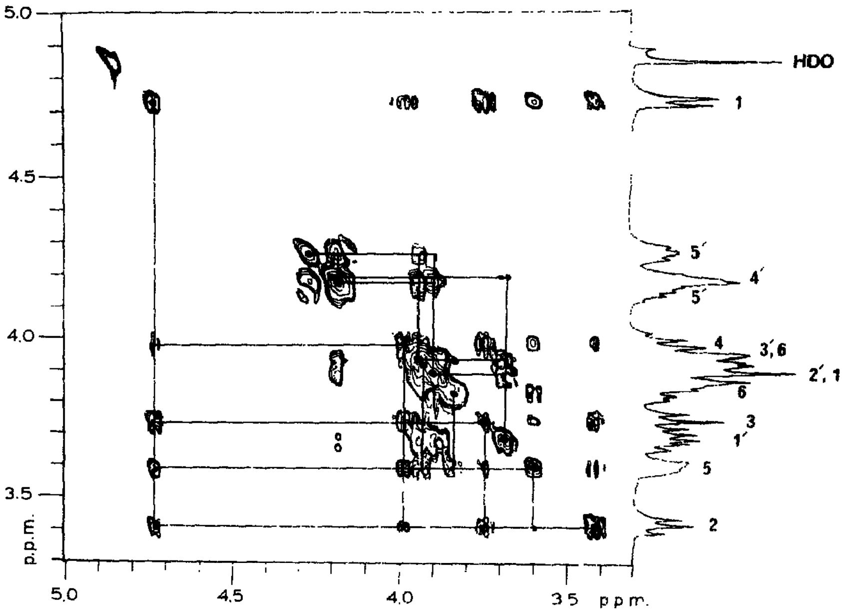


Fig. 2. Two-dimensional homonuclear Hartmann–Hahn (HOHAHA) spectrum of *H. influenzae* type a capsular polysaccharide. As in Fig. 1, solid and dashed lines connect signals from the glucosyl and ribitol moieties, to the left and right of the diagonal, respectively.

TABLE I

^{1}H- AND ^{13}C-N.M.R.-SPECTRAL CHEMICAL SHIFTS AND SIGNAL ASSIGNMENTS FOR THE *H. influenzae* TYPE a CAPSULAR POLYSACCHARIDE[a]

Assignment	*^{13}C Shift*	*^{1}H Shift*
C-1	104.5	4.71
C-2	75.7	3.40
C-3	77.0	3.74
C-4	76.2	3.97
C-5	77.2	3.58
C-6	62.9	3.82, 3.91
C-1′	65.0	3.66, 3.87
C-2′	74.0	3.94
C-3′	73.8	3.91
C-4′	81.5	4.17
C-5′	67.4	4.26, 4.13

[a]T, 20°; 10 mg of sample in 0.5 mL of D_2O. ^{13}C-Chemical shifts referenced to external, aqueous TSP, and ^{1}H shifts referenced to internal, residual HDO (4.84 p.p.m.).

HOHAHA experiment are given in the Experimental section. From the HOHAHA and COSY spectra, all of the ^{1}H-n.m.r. resonances for the *H. influenzae* type a polysaccharide are readily assigned; they are listed in Table I.

When appended groups, such as *O*-acetyl groups, are present in the structure, the foregoing procedures will not identify these groups as part of a *J*-network, and hence, as belonging to a particular saccharide unit. However, the heteronuclear experiments to be described account for these groups and eliminate any ambiguity.

Having established the *J*-networks in the ^{1}H spectra, it is possible to identify the monosaccharide unit that corresponds to this network by determining the associated ^{13}C spectra. This is achieved by a one-bond, ^{1}H–^{13}C correlation map obtained from a ^{1}H-detected, BIRD-modified, HMQC experiment[9,16]. The group of ^{13}C resonances that correlate with all of the members of a *J*-network will consequently represent one monosaccharide unit. Then, by comparison of this group of resonances with known assignments of monosaccharides and model compounds, it is a straightforward matter to identify the monosaccharide and to establish the furanoid or pyranoid form of the sugar. It should, however, be emphasized that these methods can be used independently in order to achieve this assignment if such reference data do not exist or are not totally consistent due to sample conditions.

Figure 3 shows the ^{1}H–^{13}C HMQC spectrum obtained for the *H. influenzae* type a sample. In this study, ^{13}C decoupling was not used during the acquisition period. Thus, correlation peaks in the 2D spectrum appear minimally as doublets in the ^{1}H dimension. The separation between the signals is equal to the ^{1}H–^{13}C coupling constant, and this coupling constant is useful in determining the anomeric stereochemistry of the monosaccharide unit[3]. Proton–proton coupling is also retained; hence, a triplet in the normal, 1D proton spectrum will appear as a doublet

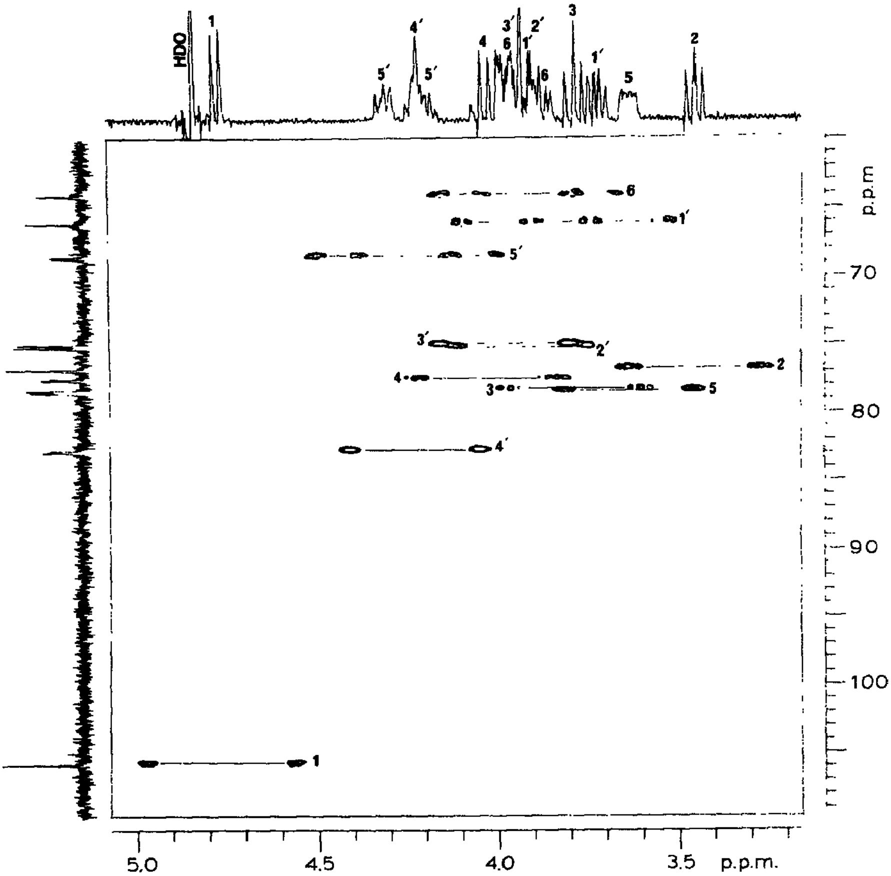


Fig. 3. ^{13}C-Coupled, ^{1}H-detected proton–carbon multiple-quantum correlation (HMQC) spectrum of *H. influenzae* type a capsular polysaccharide. The conventional 1D proton and carbon spectra are given at the top and left-hand side, respectively, of the Figure. The horizontal lines connect the correlation peaks for each ^{13}C signal; as discussed in the text, the separation (in Hz) between these signals directly gives the ^{1}H–^{13}C coupling constant.

($J_{H,C}$) of triplets ($J_{H,H}$) in the 2D spectrum. To avoid phase distortions due to ^{1}H–^{1}H coupling, the acquisition time in the t_1 dimension is kept much shorter than $1/J_{HH}$. We typically used an acquisition time of 20–30 ms in the t_1 dimension, which normally provides adequate resolution in the ^{13}C frequency dimension. Other relevant parameters are given in the Experimental section. The total acquisition time was 2.2 h. From these data, all of the ^{13}C signals could be readily assigned by correlation with the ^{1}H signals previously assigned. For example, the C-1 chemical shift was assigned from correlation of the two doublets at 104.5 p.p.m. (in the ^{13}C dimension) with H-1 (4.71 p.p.m. in the 1D proton spectrum at the top of Fig. 3). From these data, the glucose and ribitol moieties were identified, and all ^{1}H resonances and J-networks were accounted for. Additionally, the value of the J_{CH} coupling identified the anomeric stereochemistry of the D-glucosyl group to be β. The remaining ^{13}C assignments were made similarly; they are given in Table I.

Inter-saccharide linkages, and appended groups. — In order to continue the structural analysis, it was necessary to determine the types and sites of linkages between the monosaccharide units identified. This was readily achieved by examining chemical-shift correlations based on two- or three-bond couplings, *e.g.*, the coupling between a proton and a carbon nucleus across an *O*-linked glycosidic linkage. This is best achieved with the 2D heteronuclear multiple-bond correlation (HMBC) experiment[18]. The method is similar to the 1D-selective INEPT experiment in that it relies on long-range (~3–10 Hz) scalar coupling interactions. Because HMBC is a ^{1}H-detected experiment, it is very sensitive, and the entire 2D spectrum can generally be obtained in less (or at least equal) time than is required to obtain a single selective INEPT spectrum. All of the correlations are obtained in a single HMBC spectrum; hence, this method is clearly superior, especially for polysaccharides containing multiple glycosidic linkages and appended groups (such as acetyl groups). Previously, the HMBC experiment had been applied exclusively to low molecular weight (<2000) molecules, and we were concerned that rapid $^{1}H,T_2$ relaxation for the polymer would preclude detection of HMBC connectivity, as the experiment utilizes a relatively long Δ_2 delay period. The HMBC spectrum of *H. influenzae* type a is shown in Fig. 4. The total data-acquisition time was 14 h. One of the linkage sites is immediately assigned, as H-1 of the D-glucose gives an HMBC correlation peak with C-4 of the ribitol. A similar correlation peak between H-4′ and C-1 is not observed at the contour levels shown in Fig. 4, but it can be observed at very low contour levels. The low intensity of this signal relative to the H-1–C-4′ signal may be due to differences in coupling constants[20]; on the other hand, the H-1 signal is a sharp doublet (coupled only to H-2), whereas that of H-4′ is a broad, low-intensity multiplet (coupled to H-3′,H-5′a, and H-5′b), and, in general, more-extensive $J_{H,H}$ coupling for a particular proton signal leads to weaker HMBC correlation peaks[‡] for the signal[8,21,22]. All of the remaining correlation peaks in this spectrum (see Fig. 4) are due to expected intra-saccharide multiple-bond correlations. These are summarized in Table II. Discrimination between intra- and inter-saccharide correlations is made by reference to the *J*-networks previously established. Clearly, in complex systems, iterative comparison of the one-bond and multiple-bond correlations will resolve any discrepancies[9].

‡The correlation depends on creation of heteronuclear multiple quantum coherence, whose intensity is determined by the quantity sin ($\pi J_{CH}\Delta$), which is optimized (for a simple two-spin case) by setting Δ_1 in Figure 2C (ref. 9) or Δ_2 in ref. 17 to ($1/2J_{CH}$). However, for cases where one of the spins is coupled to other spins, *e.g.*, the protons, the intensity is a product of the above sine term and a cosine term involving all coupled protons. This cosine term lessens the effective intensity of heteronuclear multiple quantum coherence. This is the case for the correlation of the H-4′ of ribitol to glucose C-1, as H-4′ is coupled to three protons (H-5′a, H-5′b, and H-3′), whereas the correlation of glucose H-1 to ribitol C-4′ is affected only by coupling of H-1 to a single proton, H-2.

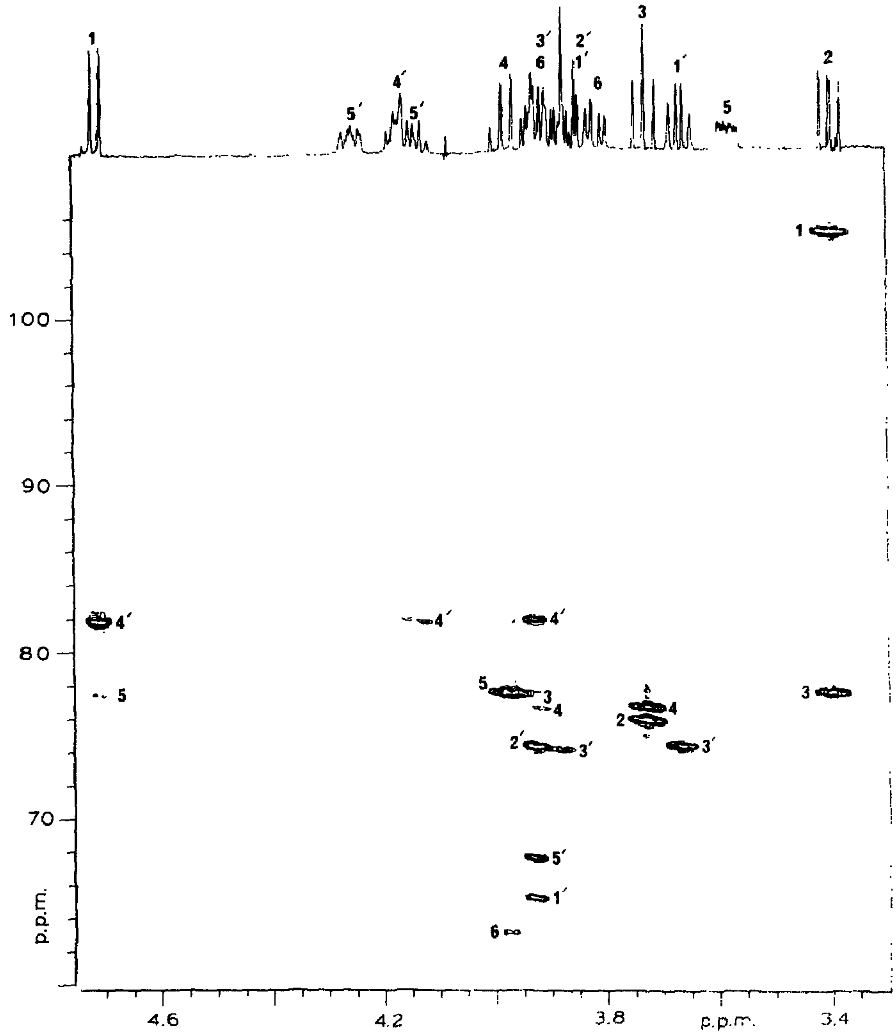


Fig. 4. ^{1}H-Detected, ^{1}H–^{13}C multiple-bond, multiple-quantum correlation (HMBC) spectrum of *H. influenzae* type a capsular polysaccharide. The strongly resolution-enhanced 1D proton spectrum with labeled signals is given at the top of the Figure, and ^{13}C frequencies are labeled in the 2D spectrum.

The location of any appended groups present will be determined in an analogous manner in the HMBC experiment. For acetyl groups, the proton at the site of attachment will exhibit a three-bond correlation to the carbonyl group of the acetyl group, *and* the methyl protons of the same group will correlate with this carbonyl group. Similarly for pyruvic acetalated saccharides (commonly occurring in *Klebsiella*), the proton at each attachment site will exhibit a three-bond correlation to the acetal carbon atom. Hence, all appended groups that exhibit a three-bond

TABLE II

SUMMARY OF OBSERVED CONNECTIVITIES IN THE N.M.R. SPECTRA OF THE *H. influenzae* TYPE a CAPSULAR POLYSACCHARIDE[a]

Proton	*COSY*	*HOHAHA*	*HMBC*
H-1	H-2	H-2, H-3, H-4, H-5	C-5, C-4′
H-2	H-1, H-3	H-1, H-3, H-4, H-5	C-1, C-3
H-3	H-2, H-4	H-1, H-2, H-4, H-5	C-2, C-4
H-4	H-3, H-5	H-1, H-2, H-3, H-5	C-3, C-5, C-6
H-5	H-4, H-6a, H-6b	H-1, H-2, H-3, H-4, H-6a, H-6b	
H-6a	H-5, H-6b	H-5, H-6b	
H-6b	H-5, H-6a	H-5, H-6a	
H-1′a	H-1′b, H-2′	H-1′b, H-2′, H-3′, H-4′	C-3′
H-1′b	H-1′a, H-2	H-1′a, H-2′, H-3′, H-4′	C-3′
H-2′	H-1′a, H-1′b, H-3′	H-1′a, H-1′b, H-3′, H-4′	
H-3′	H-2′, H-4′	H-1′a, H-1′b, H-2′, H-4′, H-5′a, H-5′b	C-1′, C-2′, C-4′, C-5′
H-4′	H-3′, H-5′a, H-5′b	H-1′a, H-1′b, H-2′, H-3′, H-5′a, H-5′b	C-1
H-5′a	H-5′b, H-4′	H-3′, H-4′, H-5′b	
H-5′b	H-5′a, H-4′	H-3′, H-4′, H-5′a	C-4′

[a]All protons gave one-bond correlation signals with their attached carbon atoms.

^{1}H-^{13}C coupling, as well as the ^{1}H and ^{13}C resonance assignments, may be accounted for *via* the HMBC experiment.

The ^{1}H–^{13}C heteronuclear correlation experiments, previously described, permit the assignment of all of the ^{1}H and ^{13}C resonances, *and* define the glucosyl-(1→4)-ribitol linkage. However, there is no information provided for the phosphoric diester linkage between disaccharide units. The previous n.m.r. methods for determining the phosphoric diester linkage sites had been designed (*i*) to identify ^{1}H–^{31}P scalar couplings in the ^{31}P spectrum by performing selective ^{1}H decoupling experiments, or (*ii*) to assign the ^{13}C resonances and determine which resonances exhibit ^{31}P–^{13}C scalar couplings[6]. The first method relies on a resolved ^{1}H spectrum, which is not likely in the general case. In the latter method, comparison of the chemical shifts with those of model compounds, and analysis of the scalar couplings, were used in order to determine the linkage sites. The major difficulties encountered with this approach are that (*i*) the values of two- and three-bond coupling-constants are quite similar, resulting in some ambiguities, and (*ii*) there must be a sufficient quantity of material to provide a high-quality ^{13}C-n.m.r. spectrum. In order to circumvent these problems, we have utilized the ^{1}H-detected, ^{1}H–^{31}P HMQC two-dimensional experiment[17,18], which correlates protons with phosphorus *via* long-range ^{1}H–^{31}P scalar coupling. Other 1D methods are available, such as ^{1}H–^{31}P spin-echo-difference spectroscopy[23,24]; however, HMQC is a more-general method, as it allows assignment for molecules that contain more than one ^{31}P nucleus. The efficiency of this correlation is related to the efficiency of the creation

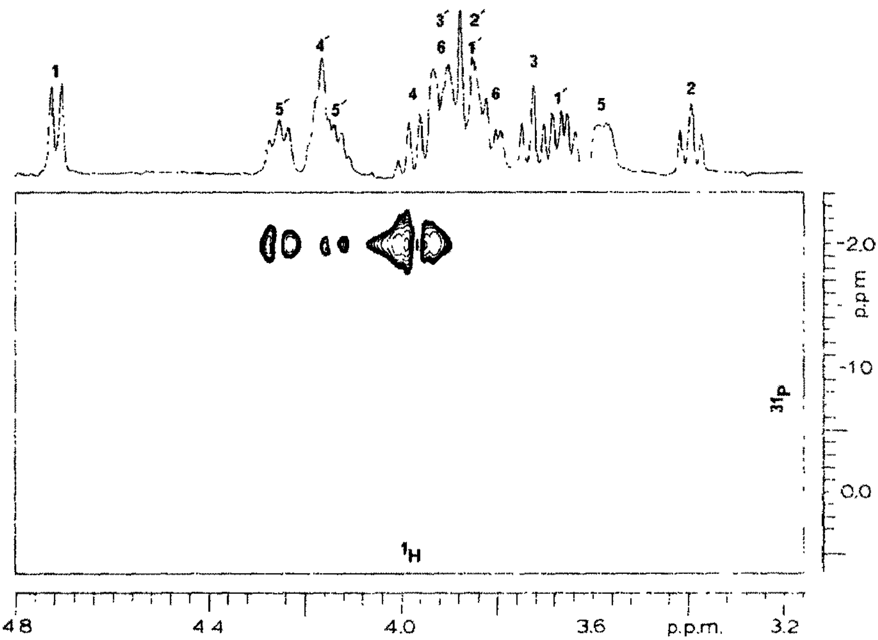


Fig. 5. ^{1}H-Detected, proton-phosphorus multiple-quantum correlation (HMQC) spectrum of *H. influenzae* type a capsular polysaccharide. The conventional 1D proton spectrum with labeled signals is given at the top of the Figure, and the ^{31}P frequencies given are referenced to external phosphate.

of heteronuclear multiple-quantum coherence at the end of the time Δ. This is maximized by choosing Δ to be approximately $1/(^2J_{HP})$. In practice, a shorter duration is often chosen in order to prevent cancellation of the signal due to H–H coupling. This experiment correlates the protons at each linkage site with the phosphorus chemical shift, thus determining the phosphoric diester linkage. The efficacy of this experiment can be seen in Fig. 5, where the linkage between C-4 of D-glucosyl and C-5′ of ribitol, *via* the phosphoric diester, is unambiguously established. The total data-acquisition required only 12 min, which is extremely favorable compared to that needed for the recording of a one-dimensional ^{13}C-n.m.r. spectrum. Furthermore, we found this HMQC experiment to be significantly more efficient for determining phosphoric diester linkages in polysaccharides[25] than an alternative, 2D heteronuclear relay experiment[26,27].

SUMMARY

The n.m.r. experiments described herein provide a novel and rapid approach for unambiguous assignment of ^{1}H, ^{13}C, and ^{31}P signals in the spectra of native polysaccharides. The combined acquisition time for the two-dimensional ^{1}H–^{13}C correlation data (one-bond and multiple-bond), the ^{1}H–^{31}P correlation data, and

the ^1H–^1H (homonuclear Hartmann–Hahn) data was ~18 h. In addition, this approach allows determination of the anomeric configuration (based on correlations involving the anomeric J_{CH} values[3,4]), the linkage sites, and the sites of side-group attachment to be confidently determined. All of these experiments rely on through-bond scalar couplings; hence, the data are unambiguous, and are uncomplicated by assumed or implied tertiary structures, as would be required for utilization of the nuclear Overhauser effect for determination of linkage sites[28]. The redundancy that exists in the combined heteronuclear correlation experiments provides for confident analysis of even extremely complicated and overlapping spectra. Because the heteronuclear correlation experiments rely on ^1H-detection, rather than detection of the low-γ nucleus, the data are obtained in much shorter time-periods, and allow analysis of much smaller amounts of sample than previously possible. The results presented confirm the applicability to large-molecular systems. The instrumental requirements that are necessary in order to implement these indirect-detection methods[9] are greatly outweighed by the advantages that they provide. Although it is, of course, necessary to establish the absolute configuration of the constituent sugars by conventional procedures, the approach outlined herein can subsequently yield a complete structural analysis of an unknown polysaccharide with very small amounts of pure material in a reasonably short time. We therefore expect the approach outlined here to be widely used in the future.

EXPERIMENTAL

A sample (10 mg) of *H. influenzae* type a (refs. 6, 10, 19) was dissolved in 0.5 mL of D_2O (99.9%; Merck); this sample was kindly provided by Drs. J. B. Robbins and R. Schneerson, NICHD, Bethesda, MD. The solution was then lyophilized, and the residue dissolved in 0.5 mL of D_2O (99.96%; Merck). All of the n.m.r. experiments were conducted with a JEOL GX-400 NMR spectrometer, except for the HMBC experiment, which was performed with a Nicolet NT-500 spectrometer. Both spectrometers were equipped with a temperature-control unit that maintained the samples at 20.0 ±0.2°. The NT-500 instrument was equipped with a Cryomagnet Systems ^1H-probe having a broad-band decoupling coil for irradiation of ^{13}C. The GX-400 ^1H-probe was modified to contain a broad-band decoupling coil for irradiation of ^{13}C and ^{31}P. All two-dimensional experiments were carried out without sample spinning. The ^{13}C chemical shifts were referenced to external sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate (TSP), and the ^1H chemical shifts were referenced to HDO (4.84 p.p.m.). Details on parameter optimization have already been described here (and in previous work[17,21]). The parameters used in this work were as follows: *HOHAHA:* $2 \times 256 \times 1024$ data matrix size, 1 kHz × 1 kHz spectral width, 16 scans per t_1 value, 1.5-s recycle delay, 6-kHz spin-lock RF field, 77.6-ms mixing time, and 3-Hz and 6-Hz Gaussian filtering in t_2 and t_1, respectively; *COSY:* 256×1024 data matrix size, 16 scans per t_1 value, 2-s recycle delay, 90° pulses throughout, unshifted sine-bell filtering in t_1 and t_2;

^{1}H–^{13}C HMQC: 2 × 128 × 512 data matrix size, zero-filled to 256 data points in t_1, 32 scans per t_1, 0.8-s pulse delay (including the 250-ms Δ_2 period), 12-μs 90° ^{1}H pulse-width, 45-μs 90° ^{13}C pulse-width, 6-Hz and 20-Hz Gaussian filtering in t_2 and t_1; *HMBC:* 256 × 512 data matrix size, 128 scans per t_1, 0.9-s pulse delay, 70-μs (82°) ^{13}C pulse-width, 17-μs (90°) ^{1}H pulse-width, Δ_1 and Δ_2 durations of 3.4 and 55 ms, respectively, sine-bell filter, and 20-Hz Gaussian broadening in t_2 and t_1, respectively; *^{1}H–^{31}P HMQC:* 64 × 1024 data matrix size, zero-filled to 128 data points in t_1, 8 scans per t_1 value, 1.25-s recycle delay, 12.5-μs 90° ^{1}H pulse-width, 40-μs 90° ^{31}P pulse-width, sine-bell, and 10-Hz Gaussian filters in t_2 and t_1.

REFERENCES

1 K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 3–10.
2 E. Barreto-Bergter and P. A. J. Gorin, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 67–103.
3 K. Bock and H. Thogersen, *Annu. Rep. NMR Spectrosc.*, 13 (1982) 1–57.
4 K. Bock, C. Pedersen, and H. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 193–226.
5 J. F. G. Vliegenthart, L. Dorland, and H. van Halbeek, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 209–274.
6 W. Egan, in J. S. Cohen (Ed.), *Magnetic Resonance in Biology*, Vol. 1, Wiley, New York, 1980, pp. 197–258.
7 A. Bax, W. Egan, and P. Kováč, *J. Carbohydr. Chem.*, 3 (1984) 593–611.
8 A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, 108 (1986) 2093–2094.
9 L. Lerner and A. Bax, *Carbohydr. Res.*, 166 (1987) 47–58.
10 P. Branefors-Helander, C. Erbing, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 56 (1977) 117–122.
11 L. Braunschweiler and R. R. Ernst, *J. Magn. Reson.*, 53 (1983) 521–528.
12 D. G. Davis and A. Bax, *J. Am. Chem. Soc.*, 107 (1985) 2820–2821.
13 D. G. Davis and A. Bax, *J. Am. Chem. Soc.*, 107 (1985) 7197–7198.
14 A. Bax and D. G. Davis, *J. Magn. Reson.*, 65 (1985) 355–360.
15 A. Bax and D. G. Davis, in N. Nicolai and G. Valensin (Eds.), *Advanced Magnetic Resonance Techniques in Systems of High Molecular Complexity*, Birkhauser, Basel, in press.
16 A. Bax and S. Subramanian, *J. Magn. Reson.*, 67 (1986) 565–569.
17 R. A. Byrd, M. F. Summers, G. Zon, C. S. Spellmeyer, and L. G. Marzilli, *J. Am. Chem. Soc.*, 108 (1986) 504–505.
18 M. H. Frey, W. Leupin, O. W. Sorensen, W. A. Denny, R. R. Ernst, and K. Wuthrich, *Biopolymers*, 24 (1985) 2371–2380.
19 W. Egan, R. Schneerson, K. E. Werner, and G. Zon, *J. Am. Chem. Soc.*, 104 (1982) 2898–2910.
20 A. Parfondry, N. Cyr, and A. S. Perlin, *Carbohydr. Res.*, 59 (1977) 299–309.
21 M. F. Summers, L. G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, 108 (1986) 4285–4294.
22 A. Bax and L. Lerner, *Science*, 232 (1986) 960–967.
23 R. Freeman, T. H. Mareci, and G. A. Morris, *J. Magn. Reson.*, 42 (1981) 341–345.
24 J. S. Cohen, C. Chen, and A. Bax, *J. Magn. Reson.*, 59 (1984) 181–187.
25 R. A. Byrd, unpublished results.
26 M. A. Delsuc, E. Guittet, N. Trotin, and J. Y. Lallemand, *J. Magn. Reson.*, 56 (1984) 163–166.
27 D. Neuhaus, G. Wider, G. Wagner, and K. Wuthrich, *J. Magn. Reson.*, 57 (1984) 164–168.
28 J.-R. Brisson and J. P. Carver, *J. Biol. Chem.*, 258 (1983) 1431–1434.