Determination of the Structure and Conformation of Bacterial Polysaccharides by Carbon 13 Nuclear Magnetic Resonance

STUDIES ON THE GROUP-SPECIFIC ANTIGENS OF NEISSERIA MENINGITIDIS SEROGROUPS A AND X*

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SUMMARY

The application of carbon 13 nuclear magnetic resonance to analysis of some phosphorylated acetamido-hexose-containing meningococcal polysaccharides is described. A complete assignment of the spectra of both the serogroup A and X polysaccharides has been made. In addition the spectrum of a structurally related 1-6-α-linked 2-acetamido-2-deoxy-d-glucose phosphate polysaccharide from Staphylococcus lactis NCTC 2102 has also been completely assigned. This has resulted in the structural elucidation of the serogroup X polysaccharide as a homopolymer of 2-acetamido-2-deoxy-D-glucopyranose, linked by 1-α-phosphodiester bonds. In addition, the O-acetyl substituents of the serogroup A polysaccharide have been located at C-3 of approximately 70% of the repeating units (1-6-α-linked 2-acetamido-2-deoxy-D-mannopyranose phosphate) of the homopolymer. Preliminary conformational information was also obtained from the large three-bond couplings between 31P and 13C (V31P-13C), indicative of highly extended backbone formations.

Carbon 13 NMR has been applied extensively to monosaccharides (1-4) and oligosaccharides (3-6) and only recently has its application to problems associated with polysaccharide structure been investigated (3, 5-7). It has been shown that the high molecular weight homoglucan, amylase, gives only six well separated signals associated with the 6 individual carbon atoms of the monomeric α-D-glucopyranose unit (3, 9). Pullm and co-workers have demonstrated further that the spectrum of heparin, a more complex heteropolysaccharide, yields information on the structure and the disaccharide sequence of the two different component sugar residues (7). In addition it has recently been established that this technique can be employed to determine the structure and the trisaccharide sequence of a glucan containing only two different linkages. The elucidation of this sequence was made possible by the presence of multiplet resonances of conformational origin (6).

The application of this technique to the meningococcal polysaccharides was carried out to explore further the potential of this technique in elucidating the structure and sequence of phosphorylated polysaccharides. The serogroup A polysaccharide has been shown to be essentially a 1-6-linked homopolymer of partially O-acetylated 2-acetamido-2-deoxymannosyl phosphate (8) and that of serogroup X to be a homopolymer of 2-acetamido-2-deoxy-d-glucosyl phosphate linked either 1-3 or 1-4 (9). Due to its close structural similarities with the hexosamine-containing meningococcal polysaccharides, the previously isolated 1-6-linked 2-acetamido-2-deoxyglucosyl phosphate homopolymer obtained from Staphylococcus lactis NCTC 2102 (10) was also examined by this technique. It was hoped that in addition to structural and sequence information this technique would also yield conformational information from the 3-bond couplings between 31P and 13C (V31P-13C). Studies on the cyclic nucleotides have shown that the 3-bond 31P-13C couplings are dependent on the dihedral angle between these bonds (11, 12). Some preliminary work on monomeric units related to the meningococcal polysaccharides has been published. This includes the measurement of 31P-13C couplings of the 2-acetamido-2-deoxyglucosyl-1-phosphates and the chemical shift assignments for the 2-acetamido-2-deoxyhexoses (4).

In this paper we have confirmed the basic structure and sequence of the S. lactis and serogroup A polysaccharides and also determined that the individual 2-acetamido-2-deoxyglucopyranose units of the serogroup X polysaccharide are linked 1-4-α by phosphodiester bonds. It has been further demonstrated that the serogroup A polysaccharide contains both monoacetylated and unacetylated residues of 2-acetamido-2-deoxymannopyranose in the approximate ratio of 7:3, the O-acetyl substituents being located at C-3 of these residues. The large couplings between 31P-13C (V31P-13C) in the spectra of these polysaccharides have also indicated that they all have highly extended backbone conformations.

MATERIAL AND METHODS

The cells of the different serogroups of Neisseria meningitidis were obtained from the culture collection of the Canadian Communicable Disease Center and were grown in 18-hour cultures in NCDM medium as previously described (13). The strains used

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STAPHYLOCOCCUS LACTIS N.C.T.C. 2102.

FIG. 1. $^{13}$C NMR spectra of the polysaccharide from Staphylococcus lactis (Staph. lactis) with spectral windows of 5.0 KHz and 2.5 KHz (inset), both 12,506 accumulations.

N. MENINGITIDIS SEROGR0UP. X.

FIG. 2. $^{13}$C NMR spectra of the serogroup X polysaccharide with spectral windows of 2.5 KHz, 13,292 accumulations, and 1.0 KHz (inset), 29,133 accumulations.

and their corresponding serogroups were 604A and 247X, and their group-specific polysaccharide antigens were both isolated and purified by a procedure previously described (9). The 1→6-linked 2-acetamido-2-deoxyglucosyl phosphate polymer was obtained from a cell wall preparation of S. lactis 2102 by extraction with dilute alkali as described by Archibald and Stafford (10). De-O-acetylation of the serogroup A polysaccharide was carried out by dissolution of the polysaccharide (100 mg) in water with the subsequent adjustment of the pH of the solution to 11.0 using dilute ammonia solution. After 1 hour the solution was concentrated to dryness, redissolved in water, and lyophilized to yield 75 mg of the de-O-acetylated polysaccharide. The synthesis of 2-acetamido-2-α-D-glucopyranose 4-phosphate is described elsewhere.1

Carbon 13 NMR spectra were recorded in 12-mm tubes at 37° on a Varian XL-100-15 spectrometer operating at 25.16 MHz in the pulsed Fourier transform mode with complete proton decoupling. The computers used in these studies (Varian 620-1 and Varian 620-L) allowed acquisition of 4096 and 8192 input data points, respectively. Chemical shifts are reported in parts per million downfield from external tetramethylsilane (5-mm concentric tube in 12-mm sample tube), and the deuterium oxide 2H resonance was used for field-frequency lock. All of the polysaccharides were run as fairly viscous solutions in deuterium oxide, at concentrations of 100 mg per ml. Proton NMR spectra at 220 MHz were obtained from the Canadian 220-MHz NMR Center, Sheridan Park, Ontario.

RESULTS AND DISCUSSION

Assignment of Signals—The $^{13}$C NMR spectrum of the cell wall homopolymer from S. lactis is shown in Fig. 1. It had been previously deduced by chemical procedures that it was a 1→6-linked homopolymer of 2-acetamido-2-deoxyglucosyl phosphate (10), and the spectrum is entirely consistent with this structure. Except for the C-6 signal at 65.3 p.p.m., which was assigned to dryness, redissolved in water, and lyophilized to yield 75 mg of the de-O-acetylated polysaccharide. The synthesis of 2-acetamido-2-α-D-glucopyranose 4-phosphate is described elsewhere.2

1 D. R. Bundle and H. J. Jennings, manuscript in preparation.

REFERENCE

1 D. R. Bundle and H. J. Jennings, manuscript in preparation.
TABLE I
Carbon-13 chemical shifts* of polysaccharides and relevant monomers

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<tr>
<th>Polysaccharide</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>CH3 (NHOCH3)</th>
<th>C=O (NHOCH3)</th>
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<tr>
<td>S. lactis 2102</td>
<td>95.2</td>
<td>55.0</td>
<td>71.5</td>
<td>70.3</td>
<td>73.0</td>
<td>65.3</td>
<td>23.0</td>
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<td>54.8</td>
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<td>α-ᴅ-GlcNAc 4-phosphate, NH₂⁺</td>
<td>91.6</td>
<td>54.9</td>
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<td>71.7</td>
<td>61.5</td>
<td>23.3</td>
</tr>
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<td>β-ᴅ-GlcNAc 4-phosphate, NH₂⁺</td>
<td>96.0</td>
<td>57.5</td>
<td>74.4</td>
<td>74.8</td>
<td>76.2</td>
<td>61.7</td>
<td>23.5</td>
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<td>N. meningitidis serogroup A</td>
<td>96.4</td>
<td>54.3</td>
<td>69.7</td>
<td>67.3</td>
<td>73.6</td>
<td>65.6</td>
<td>23.3</td>
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<tr>
<td>N. meningitidis serogroup A, de-O-acetylated</td>
<td>96.4</td>
<td>54.3</td>
<td>69.7</td>
<td>67.1</td>
<td>73.5</td>
<td>65.6</td>
<td>23.3</td>
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* In parts per million from external tetramethylsilane.

† Signals assigned to the 3-0-acetyl moiety of the A polysaccharide.

§ Signals assigned to the O-acetyl group.

also previously shown by chemical procedures to be a 2-acetamido-2-deoxyglucosyl phosphate homopolymer (9), can be similarly assigned. In this case an even more accurate assignment can be made by comparison with the spectrum of the S.
la&tis polymer where the only significant differences in chemical shift (Table I) were exhibited by a downfield shift of the C-6 resonance of S. lactis of 4.0 p.p.m. and of the C-4 resonance of the serogroup X polymer by 4.8 p.p.m. The significance of these shifts caused by the substituent effect of the phosphodiester group is discussed later under “Position of Linkages” and “Conformation.” The 13C NMR spectrum of the native serogroup A polymer is shown in Fig. 3a and exhibits a higher degree of complexity. This is due to the presence of O-acetyl groups, which has been previously determined by analytical procedures (8). It has also been established that the principal structural feature of the polysaccharide is a repeating unit of 1→6-linked 2-acetamido-2-deoxymannopyranose (8). The 13C NMR spectrum of the de-O-acetylated serogroup A polymer (Fig. 3b) is consistent with this basic structure. The signals were assigned by methods similar to those employed for the S. lactis polymer, using the previously assigned chemical shifts of 2-acetamido-2-deoxymannopyranose (4) for comparison. The downfield shift of the C-6 resonance in this case was 3.9 p.p.m.

Composition—Having previously established the chemical shifts characteristic of each carbon atom of the monomers (4), these data can now be utilized to determine the composition of the polysaccharides. Thus the large upfield shift of the C-2 resonances (17.6 p.p.m.), coupled with the presence of low field signals (17.6 to 17.8 p.p.m.) and high field signals (23.0 to 23.3 p.p.m.), are indicative of the presence of 2-acetamido-2-deoxyhexoses (4). The utility of the chemical shift data to determine the configuration of the hexosamine is illustrated by a comparison...
of the chemical shifts of the epimeric polysaccharides of S. lactis and de-O-acetylated serogroup A (Table I). These indicate that differences in chemical shift, particularly those of C-1, C-2, C-3, and C-4, are sufficiently large to differentiate between the α-gluc and α-manno configuration for the 2-acetamidohexose units. These differences have been attributed to shielding effects due to the axial acetamido group at C-2 of the α-manno epimer (4). Similar effects have also been reported for the hexoses (2).

The 13C NMR spectra of the native polysaccharides also suggest that they are all completely N-acetylated. An examination of the chemical shift differences between the hexosamines and their N-acetylated derivatives (Table II) indicates that significant differences (up to 2.8 p.p.m.) are apparent, especially in the C-1, C-2, and C-3 resonances. These signals characteristic of the hexosamines would have been readily detectable in any of the spectra unless the free hexosamine constituted less than 5% of the content of polysaccharide.

Peculiar to the spectrum of the native polysaccharide of serogroup A are the complexity of the signals and the introduction of a high field (21.6 p.p.m.) and a low field (175.1 p.p.m.) signal in addition to those associated with the acetamido group. These resonances are indicative of an O-acetyl group, the presence of which had previously been established by analytical procedures (8). The spectrum of the serogroup A polysaccharide also manifests two acetamido signals at 23.0 and 23.3 p.p.m.; this splitting of the acetamido resonance and the further complexities in the spectrum are due to the introduction of the O-acetyl group and are discussed under the section on location of O-acetyl substituent. Provided that the polysaccharide (serogroup A) is completely N-acetylated then a comparison of the integrals of the CH₃ signals of the two acetamido groups with that of the O-acetyl group should give a fairly accurate method of estimating the degree of O-acetylation of the polysaccharide. The legitimacy of this determination is based on the knowledge that carbon atoms of both these groups are in the same chemical environment (attached to 3 hydrogen atoms) and should experience identical Overhauser enhancements (14). Calculated from the spectrum of the serogroup A polysaccharide the O-acetyl content is 0.72 mole per acetamido-mannosyl phosphate unit. This value is consistent with that obtained by 220 MHz 1H NMR spectroscopy, where integration of the resonances of the O-acetyl and acetamido groups gave a value of 0.74 mole of O-acetyl per acetamido-mannosyl phosphate unit. This result compares favorably with that previously found for the serogroup A polysaccharide by chemical procedures, although considerable variability in the O-acetyl content was observed in different preparations of this polysaccharide (8). Finally, the presence of phosphate ester groups can be confirmed by the characteristic doublets in all the 13C NMR spectra of the polysaccharides. These are due to coupling between 31P and 13C (2 to 9 Hz) (11, 12).

Anomeric Configuration and Sequence—The high positive specific rotations given by the S. lactis (10) and the serogroup X (9) polysaccharides had indicated that they were α-linked. An examination of their spectra (Figs. 1 and 2) shows that the chemical shifts of their anomeric carbons are identical (95.2 p.p.m.); the presence of only one resonance indicates complete anomeric homogeneity. However, it was not possible to determine the anomeric configuration simply by comparison with the chemical shifts of the anomeric carbons of 2-acetamido-2-deoxy-α- and β-glucopyranose 1-phosphate (4), because the chemical shifts of the polysaccharides were intermediate between those of the α- and β-1-phosphate derivatives. This lack of correlation of chemical shifts is due to the different influences of the phosphonomonooester and phosphodiester groups on the anomeric center. However, it has been previously established in the hexoses (2) and the hexosamines (4) that the chemical shifts of carbons other than those of the anomeric centers are also dependent on the anomeric configuration, this dependence being significantly large in the ring carbons (C-2, C-3, and C-5). On this evidence the anomeric configuration of all the polysaccharides can be assigned as α, the S. lactis and serogroup X polysaccharides by correlation of these resonances with those of 2-acetamido-2-deoxy-α-D-glucopyranose or its 1-phosphate derivative (4) and those of the serogroup A polysaccharide with 2-acetamido-2-deoxy-α-D-mannopyranose (4).

In previous studies on the 13C NMR of polysaccharides it has been demonstrated that the sequence of the polysaccharides can be determined from the spectra (6, 7). This can be interpreted most readily from the number and intensities of the anomeric signals (6, 7), although in some cases other signals can be used to support this determination provided the spectra are not too complex (6). The simplest example of this determination is that of the α-linked homogalcan amyllose which gives six signals representing each carbon of its α-D-glucopyranose unit (3, 6). The S. lactis and serogroup X polysaccharides can be included in this category, because although they are complicated by phosphorous to carbon (13P-13C) couplings, they each give one basic signal for the 8 carbon atoms of the simple repeating unit of 2-acetamido-2-deoxyglucopyranosyl α-1-phosphate. Although the spectrum of the serogroup A polysaccharide is complicated by the presence of O-acetyl groups, the de-O-acetylated polysaccharide also gives a spectrum which is consistent with the polysaccharide having a repeating unit of 2-acetamido-2-deoxy-mannopyranosyl α-1-phosphate. It has been suggested (8) that the serogroup A polysaccharide does contain linkages other than those formed by the 1→6-phosphoester bonds. We could find no evidence in the spectrum of the native polysaccharide (Fig. 30) for any significant amount of these linkages in this preparation of the serogroup A polysaccharide. A few extraneous signals of low intensity are present in the spectrum of the de-O-acetylated serogroup A polysaccharide (Fig. 30), and these could be attributed to the partial degradation of the polysaccharide during the alkaline de-O-acetylation procedure. However, although some of these signals cannot be detected in the spectrum of the native polysaccharide, their structural significance cannot be completely ignored. This is due to the increased signal-to-noise ratio obtained on the de-O-acetylated

### Table II

<table>
<thead>
<tr>
<th>Compound</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>C-6</th>
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<tbody>
<tr>
<td>α-D-Glucosamine HCl</td>
<td>+1.8</td>
<td>−0.2</td>
<td>+1.0</td>
<td>+0.5</td>
<td>0.0</td>
<td>+0.4</td>
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<td>β-D-Glucosamine HCl</td>
<td>+2.3</td>
<td>+0.1</td>
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<td>+0.2</td>
<td>−0.1</td>
<td>+0.5</td>
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<tr>
<td>α-D-Mannosamine HCl</td>
<td>+2.8</td>
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<td>+0.2</td>
<td>+0.1</td>
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<td>β-D-Mannosamine HCl</td>
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<td>−1.5</td>
<td>+2.5</td>
<td>+0.6</td>
<td>+0.3</td>
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</table>
polysaccharide compared to that of the native polysaccharide (Fig. 3). One could say with certainty that this additional structural feature could only represent no more than 10% of the total linkages in the polysaccharide.

Position of Linkages—As mentioned previously in the assignment of the 13C resonances (“Assignment of Signals”), the downfield shifts of resonances due to carbons attached to the phosphodiester group can be used to determine the position of the linkage. This effect caused by the introduction of substituents on the hydroxyls of sugar rings has been well documented for phosphate (4, 11, 12), acetate (4), methoxy (2, 3, 15), and even occurs where the substituent is another sugar ring (6). However, the position of the phosphodiester linkage can also be determined independently by examination of the pattern of coupling between 13C and 31P. The spectrum of the S. lactis polysaccharide manifests four such couplings, although to resolve the C-6 signal into a doublet did require instrument conditions of higher resolution (Fig. 1). This coupling is known to occur on the α-carbon atom to the phosphate group and only to extend to the β carbon atom (11, 12). Therefore this pattern of coupling is only consistent with a structure represented by 2-acetamido-2-deoxyglucopyranose units linked 1→6 by phosphodiester bonds. The spectrum of the de-O-acetylated serogroup A polysaccharide gave a very similar phosphate coupling pattern to that of S. lactis polysaccharide, thus confirming its 1→6-phosphodiester linkage (10). In this case the C-6 coupling could not be resolved but the reduced height and breadth of the resonance compared to those of C-3 and C-4 indicates that it is coupled.

The serogroup X polysaccharide gave a coupling pattern different from those of the other polysaccharides, indicating a linkage to C-3 or C-4. This was subsequently supported by the observation that the polysaccharide was resistant to periodate oxidation (9). The spectrum indicates five couplings to 31P, on the C-1, C-2, C-3, C-4, and C-5 resonances. The five doublets are only consistent with a C-4 phosphodiester linkage as the alternative C-3 linkage would not give coupling to C-5, while the C-2 resonance would be coupled to two phosphorus atoms of the phosphodiester backbone. A C-6 link is ruled out by the chemical shift of C-6 (61.3 p.p.m.), which is very similar to that in the α anomer of 2-acetamido-2-deoxyglucopyranose (4) (61.9 p.p.m.), and by the observed 31P coupling to C-4.

Further confirmation of the C-4 linkage in the serogroup X polysaccharide was also obtained by taking the 13C NMR spectrum of its autohydrolysis product. The assigned chemical shifts of this monophosphate ester derivative were identical to those of the synthetic 4-phosphate derivative (Table 1). The spectrum showed both α and β anomeric resonances free of coupling to 31P (91.8 p.p.m. and 96.1 p.p.m.) due to the normal equilibration process following the hydrolysis of the phosphodiester group. Dependencies on the anomeric configuration were also found in all the other resonances (as in the synthetic compound, Table 1); the C-6 resonance was not coupled to 31P, consistent with a 3- or 4-phosphate derivative. The salient feature of the spectrum was that the α and β signals of C-2 were not coupled to 31P, thus eliminating the possibility of C-3 substitution. In addition, the spectrum shows a complex multiplet of 12 overlapping resonances, 71.3 to 76.4 p.p.m., which correspond to those of C-3, C-4 and C-5 of the synthetic 4-phosphate.

Location of O-acetyl Substituent—Most of the previous assignments made on the serogroup A polysaccharide were conveniently made on the de-O-acetylated product. However, the spectrum of the native polysaccharide (Fig. 3a), although more complex, does yield some remarkable information on closer analysis. It has previously been established by integration of the CH3 signals of the O-acetyl and acetamido groups that the polysaccharide is only O-acetylated to the extent of 0.72 mole per acetamido-mannosyl phosphate unit. This is also apparent in other resonances of the spectrum of the native polysaccharide. Some of the resonances of the de-O-acetylated polysaccharide are readily discernible and are indicated without a prime in Fig. 3a (C-3, C-4, C-2, and the CH3 signal of —NHCOCH3). This suggests that the native polysaccharide is composed partially of unacylated units, and of monoacylated units (resonances indicated as primed in Fig. 3a). It also suggests that the introduction of an O-acetyl group has an effect on many of the chemical shifts of the carbon atoms of the 2-acetamido-2-deoxymannosyl phosphate residues. This has been previously studied in detail on a model compound, 2-acetamido-3-O-acetyl-2-deoxy-d-glucose (4), and it was demonstrated that the O-acetyl group on C-3 caused widespread effects on its chemical shifts in comparison with its unacetylated precursor. The major effects were found in the α and β carbon atoms to the O-acetyl group; a downfield shift of 3.0 p.p.m. of the C-3 signal, and an upfield shift of the adjacent β carbons (C-2 and C-4) of approximately 2.0 p.p.m. This large shielding effect of the β carbons by the O-acetyl group is in direct contrast to the minimal shielding effect found on the β carbons of all the polysaccharides by the phosphodiester group (Table I). A similar lack of shielding of the β carbons by the phosphodiester group has also been found in polyuridylic acid (16). On the basis of the shielding effects in the model compound a complete analysis of the spectrum of the native serogroup A polysaccharide can be made which is consistent only with the O-acetyl groups on C-3 of individual 2-acetamido-2-deoxymannosyl phosphate residues. The resonances due to the 3 O-acetyl-2-acetamido-2-deoxymannopyranose moieties are indicated by primes in the assignments of Fig. 3a. One can thus assign the resonance at 73.2 p.p.m. to the C-3′ resonance of the acetylated moieties. This resonance is approximately 2.6 p.p.m. in both cases and is of the same order as that found in 2-acetamido-3-O-acetyl-2-deoxy-d-glucose (4) (2.0 p.p.m.). The alternative assignment of the O-acetyl group to C-4′ would necessitate interpretation of the spectrum in terms of a large downfield shift of C-4 (5.0 p.p.m.) and an even larger upfield shift for C-3 (5.0 p.p.m.), with a negligible shift for the C-5 resonance. The two further shifts are much larger than expected from the model compound. Although this latter interpretation is the least plausible one cannot discount the fact that a model compound in the gluco configuration was used, whereas the polysaccharide is in the manno configuration. However, to avoid any ambiguity in this assignment the fact that C-2 shifts to this large extent (2.6 p.p.m.) can only be interpreted as being due to the O-acetyl group being located at C-3′ as the studies on the model compound in the gluco configuration did establish that the substituent effect of the O-acetyl group did not extend to any appreciable degree to the γ carbon atoms (4). To finally corroborate the assignment one should be able to estimate the O-acetyl content by comparison of the integrated areas of any of the analogous signals of the O-acetylated and unacyetylated residues. Integration of the well separated signals (C-2, C-2′, and the acetamido CH3 and CH′3) gave an identical result with
that found by a comparison of the integrals of the O-acetyl and acetamido groups, discussed under "Composition."

Conformation—Couplings between $^3$P and $^{13}$C are listed in Table III and yield significant information on the conformation of the polysaccharides. In the nucleotides the couplings through 2 bonds ($\psi_{3,14}$) were found to be 5 ± 1 Hz (11, 12) and the 3-bond couplings in the anomers of 2-acetamido-2-deoxyglucopyranosyl phosphates also fell within this range (4). A similar range is noted in the polysaccharides, although the larger line-widths sometimes made accurate measurement difficult (serogroup A).

The 3-bond couplings between $^3$P and $^{13}$C ($\psi_{3,14}$) have been shown previously to depend on the dihedral angle between the $^3$POC and OOC planes, allowing an estimation of the populations of the possible rotational isomers (11, 12, 16, 17). The coupling for the trans conformation (dihedral angle of 180°) was found to be 8 to 10 Hz, whereas that for the gauche conformation was approximately 2 Hz (11, 12, 17). The data in Table III show that for rotamers about O-C1 (Fig. 4), in all the polysaccharides the phosphorus atom preferentially adopts the PIII position, trans to C-2 and gauche to the ring oxygen, avoiding the acetamido group at C-2. This can be interpreted as indicating the P-III position, 2-trans to C-2 and gauche to the ring oxygen, the polysaccharides the phosphorus atom preferentially adopts the PIII position, trans to C-2 and gauche to the ring oxygen, avoiding the acetamido group at C-2. This can be interpreted as indicating the P-III position, 2-trans to C-2 and gauche to the ring oxygen, the polysaccharides the phosphorus atom preferentially adopts the PIII position, trans to C-2 and gauche to the ring oxygen.

TABLE III

<table>
<thead>
<tr>
<th>Compound</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
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<td>α-D-GlcNac 4-phosphate, NH₄⁺</td>
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</table>

a Estimated accuracy ±0.7 Hz.
b Poorly resolved, ±1.5 Hz.
c Unresolved.

CONCLUSION

This work on the phosphorylated homopolymers of 2-acetamido-2-deoxy-hexopyranoses augments previous studies (3, 6, 7) in demonstrating the remarkable resolution and scope of $^{13}$C NMR for the studies of polysaccharides. It has been shown that this nondestructive technique provides information on the composition, sequence, anomeric configuration, position of linkages, location of substituents, and conformation of these phosphorylated polysaccharides. This basic work indicates the feasibility of the use of this technique in solving structural and conformational problems associated with a wide range of phosphorylated bacterial polysaccharides (18, 19) of biological importance.

The limiting factor in this technique, which is pertinent to all the conclusions reached on the structure of these polysaccharides, is the signal to noise ratio obtained in the spectra. In all the spectra (Figs. 1, 2, and 3) it was estimated that it would not be possible to detect fine structural detail represented by individual sugar units or carbon-containing substituents which were present in the polysaccharide to the extent of less than 5%.

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