Eur. J. Biochem. *141*, 513–519 (1984)

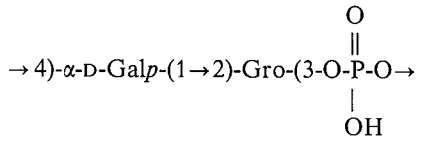
Structure of the capsular antigen of *Neisseria meningitidis* serogroup H

Arie VAN DER KAADEN, Judith I. M. VAN DOORN-VAN WAKEREN, Johannis P. KAMERLING, Johannes F. G. VLIEGENTHART, and Rudy H. TIESJEMA

Department of Bio-Organic Chemistry, State University of Utrecht, Utrecht; and National Institute for Public Health, Bilthoven

(Received January 4, 1984) – EJB 84 0008

The capsular polysaccharide of *Neisseria meningitidis* serogroup H is composed of the following repeating unit, Gro = glycerol;



Partial *O*-acetylation of the D-Gal*p* moieties is found for C-2 (21 %) and C-3 (57 %). The structural elucidation of the biopolymer is based on sugar analysis, methylation analysis, partial acid hydrolysis, using gas-liquid chromatography/mass spectrometry studies, and NMR spectroscopy with ^{1}H, ^{13}C and ^{31}P.

Recently, the structures of the capsular polysaccharides of *Neisseria meningitidis* serogroups A, B, C, 29E, W135, X, Y and Z have been reviewed [1]. The structural elucidation of the capsular polysaccharide isolated from *N. meningitidis* serogroup L has also been published [2]. In 1981 Shao-Qing et al. [3] reported on the detection of three new meningococcal serogroups, which were classified as H(1890), I(1486) and K(1811), respectively.

Here we describe the isolation and structural elucidation of the group H capsular antigen.

MATERIALS AND METHODS

Bacterial growth and isolation of the capsular polysaccharide

The *Neisseria meningitidis* strain H(1890) organism was grown in a modified Frantz' medium, under control of pH (7.0) and pO_2 (10 % air saturation, or 20 mbar), at a temperature of 35 °C. After 16 h the polysaccharide was isolated by Cetavlon precipitation and purified as described earlier [4].

General analytical methods

GLC analyses were performed on a Varian 3700 gas chromatograph equipped with a flame-ionization detector, in combination with a Varian CDS-101 electronic integrator. Separations were achieved on a CP-Sil 5 WCOT fused silica capillary column (25 m × 0.32 mm internal diameter) (column A) and a SP-1000 WCOT glass capillary column (25 m × 0.26 mm internal diameter) (column B), both with nitrogen as carrier gas (1.5 ml/min).

GLC-MS analyses were carried out with a Carlo Erba GC/Kratos MS80/Kratos DS55 system, operating in the electron impact mode at a potential of 70 eV. For GLC separations column A and a glass column (2 m × 2 mm internal diameter) packed with 3 % OV-225 on Chromosorb WHP, 100–200 mesh (column C) were applied.

Infrared spectra (KBr discs) were recorded with a Perkin Elmer spectrophotometer (model 621). Specific rotations were measured at ambient temperature with a Perkin Elmer 241 polarimeter, using a 10-cm micro-cell.

Phosphorus was determined according to the method of Chen et al. [5]. The *O*-acetyl content of the polysaccharide was assayed according to Hestrin [6]. The protein and nucleic acid contents were established as described [7, 8].

Sugar analyses

Gas chromatographic sugar analyses on column A were performed essentially as described previously [9, 10]. For the separation of the sugar derivatives obtained after methanolysis and trimethylsilylation, an oven temperature program of 70 → 220 °C at a rate of 2 °C/min was used. Identifications were confirmed by GLC-MS using the same gas chromatographic parameters as for GLC. The absolute configuration of the monosaccharide unit was determined by GLC of the corresponding trimethylsilylated (–)-2-butyl glycosides [11] using column A, with an oven temperature program of 130 → 220 °C at a rate of 2 °C/min.

Methylation analysis

The polysaccharide (10 mg), dissolved in dimethylsulfoxide (1 ml), was methylated with methyl iodide (1 ml) in the presence of 2 M sodium methylsulfinylmethanide in dimethylsulfoxide

Abbreviations. p, pyranose; *f*, furanose; Gro, glycerol; GLC, gas-liquid chromatography; GLC-MS, gas-liquid chromatography/mass spectrometry.

(1 ml) [12, 13]. After dialysis and lyophilization, solvolysis of the methylated material was accomplished with 90% formic acid for 1 h at 100 °C. Then the solution was concentrated to dryness and treated with 0.13 M H_2SO_4 for 16 h at 100 °C. Partially methylated sugars were analyzed as their [1-^{2}H]alditol acetates by GLC, using column B, at an oven temperature of 180 °C, and by GLC-MS, using column C, with an oven temperature program of 70 → 200 °C at a rate of 2 °C/min [13].

Solvolysis with hydrogen fluoride

The polysaccharide (50 mg) was treated with 48% hydrogen fluoride (2 ml) for 48 h at 4 °C. The solution was then frozen in and evaporated in a vacuum desiccator over solid sodium hydroxide. The residue was fractionated on a Bio-Gel P-2 (100 – 200 mesh) column (130 × 1.8 cm), using water as eluent at a flow rate of 27.5 ml/h. The effluent was monitored for the presence of carbohydrates using orcinol/H_2SO_4. The major carbohydrate-containing fraction was investigated in its underivatized form by ^{1}H-NMR and ^{13}C-NMR spectroscopy, after pertrimethylsilylation by GLC and GLC-MS using column A with an oven temperature program of 70 → 220 °C at a rate of 2 °C/min and further by sugar analysis.

O-*Deacetylation*

The polysaccharide (100 mg) was treated with aqueous ammonia (pH 11) for 6 h at room temperature. Then the solution was concentrated to dryness, the residue was dissolved in water, dialyzed and recovered by lyophilization.

Nuclear magnetic resonance spectroscopy

500-MHz ^{1}H-NMR spectra were recorded on a Bruker WM-500 spectrometer (SON hf-NMR Facility, Department of Biophysical Chemistry, University of Nijmegen, The Netherlands). Prior to analysis the saccharides were repeatedly treated with ^2H_2O at room temperature, with intermediate lyophilization. Finally, the saccharides were dissolved in ^2H_2O (99.96 mol% ^{2}H). The spectra were recorded at probe temperatures of 70 °C or 27 °C. Resolution enhancement was achieved by Lorentzian-to-Gaussian transformation [14]. Chemical shifts (δ) were expressed in ppm downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, but were actually measured by reference to internal acetone (δ = 2.225 ppm at 27 °C and δ = 2.215 ppm at 70 °C).

Proton-noise-decoupled ^{13}C-NMR spectra were recorded at 20 MHz on a Varian CFT-20 instrument, or at 50.76 MHz on a Bruker WM-200 spectrometer, in ^2H_2O at a probe temperature of 27 °C. Chemical shifts (δ) were given in ppm downfield from external tetramethylsilane, but were actually measured by reference to internal acetone (δ = 31.4 ppm).

36.4-MHz ^{31}P-NMR spectra were recorded on a Bruker WH-90 instrument, in ^2H_2O at a probe temperature of 20 °C. Chemical shifts (δ) were reported in ppm relative to external 50% (w/v) aqueous orthophosphoric acid.

RESULTS AND DISCUSSION

General characteristics of the polysaccharide

The H polysaccharide has been obtained by isolation from the culture liquid of a *Neisseria meningitidis* H(1890) fermentation in a yield of 34 mg/l. Sugar analysis [9, 10] reveals the presence of galactose and glycerol only. The absolute configuration [11] of galactose has been identified as D. The polysaccharide has $[\alpha]_D^{20}$ + 145° (*c* 0.3, water). Chemical analyses indicate the presence of phosphorus (7.5%), calcium (4.9%) and *O*-acetyl groups (2.05 mmol/g polysaccharide). The material turns out to be slightly contaminated with protein (0.6%) and nucleic acids (0.2%). The infrared spectrum of the Ca-salt of the polysaccharide shows a strong P = O absorption band at 1230 cm^{-1}, suggesting the occurrence of a phosphoester moiety; *O*-acetylation is evident from a band at 1720 cm^{-1}. No indications for *N*-acetyl (1650 cm^{-1} and 1550 cm^{-1}) or carboxylate (1620 cm^{-1}) groups are observed.

Solvolysis with hydrogen fluoride

Treatment of the polysaccharide with 48% HF and subsequent fractionation on Bio-Gel P-2 yields a major cleavage product containing equimolar amounts of galactose and glycerol as has been determined by sugar analysis. GLC-MS of the pertrimethylsilylated compound, together with ^{1}H-NMR and ^{13}C-NMR spectroscopy of the underivatized cleavage product point to structure 1, as will be discussed below.

α-D-Gal*p*-(1 → 2)-Gro

1

The mass spectrum of the main gas chromatographic peak of the pertrimethylsilylated material shows, *inter alia*, peaks at *m/z* 581 (*M*-CH_3-Me_3SiOH), *m/z* 539 [15], *m/z* 493 (*M*-Me_3SiOH-CH_2OSiMe_3), *m/z* 491 (581-Me_3SiOH), *m/z* 464 (*M*-Me_3SiOH-Me_3SiOCH_2CHO), *m/z* 451 (aA_1), *m/z* 361 (aA_2), *m/z* 337 (abJ_1), *m/z* 271 (aA_3) and *m/z* 219 (bA_1); see [16] for the explanation of the symbols used. These data together with those from the sugar analysis indicate a Gal → Gro sequence. Evidence for Gal in the pyranose form is obtained from the ratio of the intensities of the peaks at *m/z* 204 ($Me_3SiOCHCHOSiMe_3$) and 217 ($Me_3SiOCHCHCHOSiMe_3$), being 204/217 ≫ 1 [15]. In accordance with this observation, the peak at *m/z* 205 consists only of the isotopic peak of *m/z* 204. Information on the type of glycosidic linkage is not deductable from the mass spectrum, as is evident from comparison of the mass spectra of the trimethylsilylated authentic samples of β-Gal*p*-(1 → 1)-Gro [17], β-Gal*f*-(1 → 1)-Gro [17] and α-Gal*p*-(1 → 2)-Gro. According to the GLC-MS analysis the fraction also contains a minor quantity (5%) of a compound which shows essentially the same mass spectrum as the main GLC peak, but which has not yet been identified.

The 500-MHz ^{1}H-NMR spectrum of compound 1 is presented in Fig. 1. The Gal H-1 signal at δ = 5.145 ppm ($J_{1,2}$ = 4.0 Hz) indicates an α-glycosidic linkage between D-Gal*p* and glycerol. The Gal H-2, H-3, H-4 and H-5 resonances have been assigned on the basis of selective decoupling experiments. The spectrum is identical with that of authentic α-Gal*p*-(1 → 2)-Gro.

The ^{13}C-NMR spectral data of compound 1 are presented in Table 1. Comparison of these data with those of reference α-D-Gal*p*, Me-α-D-Gal*p* and glycerol enables complete assignment of the resonances. The α-configuration of the D-Gal*p* unit is evident from the resonance at δ = 99.2 ppm. The signals at δ = 61.5, 62.3 and 62.5 ppm are characteristic for non-substituted primary carbon atoms, which implies that α-D-Gal*p* is coupled to glycerol C-2′. This is confirmed by the downfield shift for the glycerol C-2′ resonance ($\Delta\delta$ = 6.6 ppm) and the

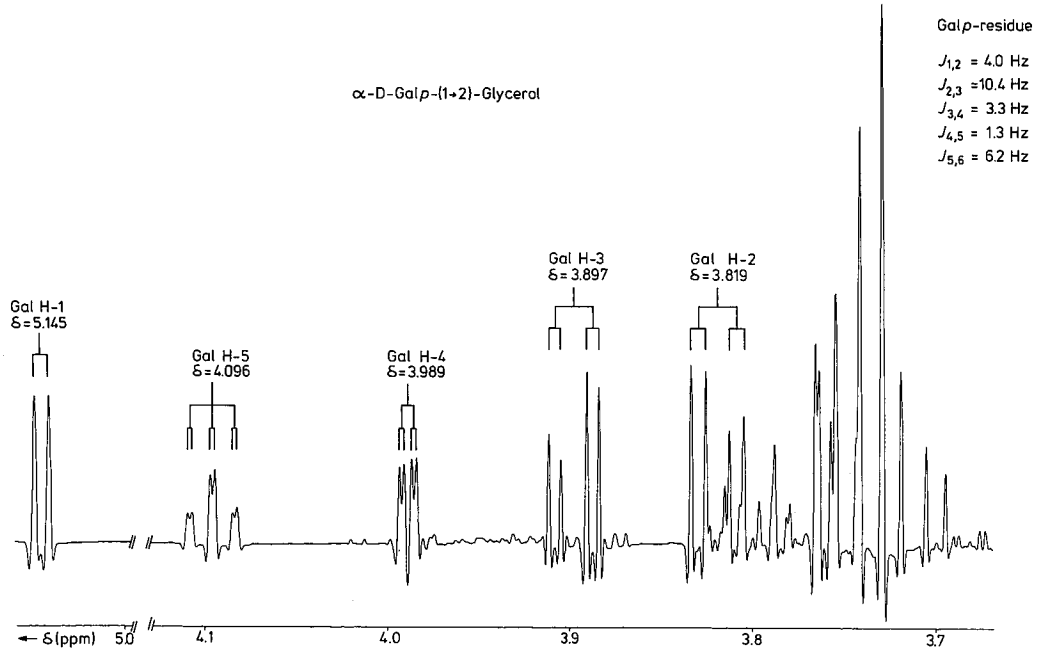


Fig. 1. *Resolution-enhanced 500-MHz ^1H-NMR spectrum of* α-D-Gal*p*-*(1 → 2)-Gro (residue 1), recorded in* ^2H_2O, *at 27 °C.* Chemical shift values are presented relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, using internal acetone ($\delta = 2.225$ ppm)

Table 1. ^{13}C *Chemical shifts and* ^{31}P-^{13}C *coupling constants (in parenthesis) in the* ^{13}C-*NMR spectra of* α-D-Gal*p*-*(1 → 2)-Gro,* Neisseria meningitidis *group H polysaccharide (native and* O*-deacetylated) and some reference substances*
Chemical shift values are given at 27 °C, relative to external tetramethylsilane in ^2H_2O, but were actually measured relative to internal acetone ($\delta = 31.4$ ppm)

Compounds	Chemical shifts (couplings)										
	Gal*p* moiety								glycerol moiety		
	C-1	C-2	C-3	C-4	C-5	C-6	*O*-acetyl		C-1′	C-2′	C-3′
							−Me	−C=0			
α-D-Gal*p*[a]	93.2	69.4	70.2	70.3	71.4	62.2					
Me-α-D-Gal*p*[a]	100.1	69.2	70.5	70.2	71.6	62.2					
α-D-Gal*p*-(1 → 2)-Gro	99.2	69.6	70.5[c]	70.4[c]	72.2	62.3[d]			62.5[d]	79.9	61.5[d]
Glycerol[b]									63.8	73.3	63.8
Glycerol-3-*P*[b]									62.8	71.6 (6.4)[f]	65.2 (4.8)[e]
H polysaccharide (*O*-deAc)	99.3	69.6	70.0	75.8 (5.8)[e]	71.9 (2.8)[f]	62.0			62.5	78.3 (7.6)[f]	65.8 (4.8)[e]
H polysaccharide (native)	99.3	69.7	70.1	75.8 (5.8)[e]	71.7 (3.5)[f]	62.1			62.5	78.5 (6.2)[f]	65.6 (n.d.)[e]
	96.7[g]	72.1[g]	67.9[g]				21.7[g]	175.0[g]			
		67.2[h]	72.8[h]	73.2 (5.8)[e, h]			22.0[h]	175.0[h]			

[a] Taken from [18].
[b] Taken from [19].
[c] Assignments may be reversed.
[d] Assignments may be interchanged.
[e] $^2J(^{31}P$-$^{13}C)$ in Hz.
[f] $^3J(^{31}P$-$^{13}C)$ in Hz.
[g] 2-*O*-acetyl-D-Gal*p* residues.
[h] 3-*O*-acetyl-D-Gal*p* residues.

small upfield shifts found for glycerol C-1′ ($\Delta\delta = -1.3$ ppm) and glycerol C-3′ ($\Delta\delta = -2.3$ ppm), as compared to glycerol [19].

Methylation analysis

Permethylated H polysaccharide, subjected to alditol acetate analysis, gives rise mainly to the formation of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-[1-^{2}H]galactitol. This result provides evidence for a 4-substituted D-Gal*p* residue 2:

$$\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{-}(1 \rightarrow$$
$$\quad\quad 2$$

It should be noted that a small, but reproducible, degree of undermethylation of the galactose unit has been observed.

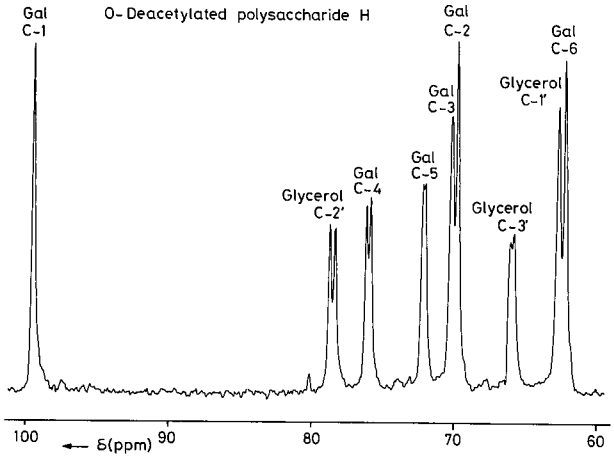


Fig. 2. *^{13}C-NMR Spectrum of the O-deacetylated H polysaccharide (ammonium salt form, pH = 6) recorded in ^2H_2O, at a temperature of 27 °C and 20 MHz.* Chemical shifts are given relative to external tetramethylsilane, using internal acetone (δ = 31.4 ppm)

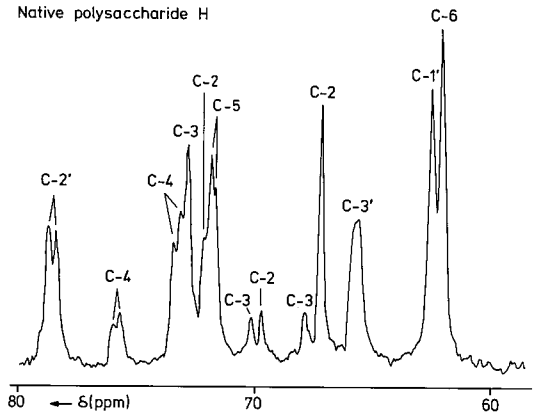


Fig. 3. *Partial ^{13}C-NMR spectrum of the native group H polysaccharide (calcium salt form, pH = 6), recorded in ^2H_2O at a temperature of 27 °C and 20 MHz*

NMR spectroscopy of the O-*deacetylated polysaccharide*

The nine-resonance ^{13}C-NMR spectrum of the *O*-deacetylated polysaccharide is presented in Fig. 2. The chemical shifts and ^{31}P-^{13}C coupling constants are included in Table 1.

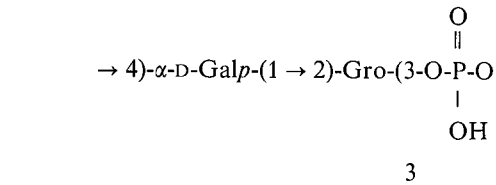
In the anomeric region the resonance at δ = 99.3 ppm (Gal C-1) points to α-configuration of the D-Gal*p* unit. The Gal C-4 resonance (δ = 75.8 ppm; $^2J(^{31}P\text{-}^{13}C)$ = 5.8 Hz) is found $\Delta\delta$ = 5.4 ppm downfield from the Gal C-4 position in compound 1. This shift effect together with the ^{31}P-^{13}C coupling demonstrate that in the polysaccharide a phosphate ester group is attached to Gal C-4 [20, 21]. Owing to this substitution, the resonances of the adjacent carbon atoms C-3 (δ = 70.0 ppm) and C-5 (δ = 71.9 ppm) show small upfield shifts of $\Delta\delta$ = −0.5 ppm and $\Delta\delta$ = −0.3 ppm, respectively. Moreover, the Gal C-5 resonance gives rise to a doublet due to three-bond ^{31}P-^{13}C coupling (2.8 Hz).

In comparison to product 1, the glycerol C-3′ resonance (δ = 65.8 ppm; $^2J(^{31}P\text{-}^{13}C)$ = 4.8 Hz) shows a downfield shift increment of $\Delta\delta$ = 4.3 ppm. This shift effect in conjunction with the ^{31}P-^{13}C coupling evidences the location of a phosphate ester at glycerol C-3′. This conclusion is supported by the upfield shift for C-2′ ($\Delta\delta$ = −1.6 ppm). The glycerol C-2′ resonance is a doublet due to three-bond ^{31}P-^{13}C coupling (7.6 Hz). A similar $\Delta\delta$-value (−1.7 ppm) is observed when the positions of the C-2′ resonances of glycerol and glycerol 3-phosphate are compared.

The 500-MHz ^{1}H-NMR spectrum of the *O*-deacetylated material shows two structural reporter group resonances (Fig. 4a). The resonance of Gal H-1 at δ = 5.200 ppm ($J_{1,2}$ = 3.9 Hz) is in agreement with α-glycosidic linkage. The downfield shift $\Delta\delta$ = 0.56 ppm of the Gal H-4 resonance (δ = 4.554 ppm; $J_{3,4}$ = 2.8 Hz, $J_{4,5}$ < 1.0 Hz and $^3J_{H-4,P}$ = 9.0 Hz) as compared with that of compound 1, confirms the 4-substitution of α-D-Gal*p*. The large $^3J_{H-4,P}$ = 9.0 Hz identifies this substitution as a phosphate ester [22].

The one-resonance ^{31}P-NMR spectrum (δ = 2.98 ppm) of the *O*-deacetylated material demonstrates one type of phosphate moiety. Consequently, the phosphate feature contributes to the backbone as its monophosphate diester. It should be noted that a pyrophosphate unit would imply non-equivalent phosphorus nuclei, yielding resonances with different chemical shifts.

On the basis of these NMR results and the structures 1 and 2, the repeating unit for the primary structure of the *O*-deacetylated group H polysaccharide is proposed to be:



3

Location of the O-*acetyl substituents*

The *O*-acetyl substitution pattern of the native polysaccharide is derived on the basis of the ^{13}C-NMR and ^{1}H-NMR spectral data.

The ^{13}C-NMR spectrum of the native polymer is given in Fig. 3. The chemical shifts and ^{31}P-^{13}C coupling constants are compiled in Table 1. Comparison of these data with those of the *O*-deacetylated material (Fig. 2, Table 1) specifies that Gal C-2 as well as Gal C-3 are partially *O*-acetylated. For C-2 this is evident from the α effect of *O*-acetylation on the resonance of Gal C-2 ($\Delta\delta$ = 2.4 ppm) and the β effects on the resonances of Gal C-1 ($\Delta\delta$ = −2.6 ppm) and Gal C-3 ($\Delta\delta$ = −2.2 ppm). Partial *O*-acetylation of Gal C-3 is derived from the β effect of *O*-acetylation on the Gal C-2 and the Gal C-4 resonances which corresponds with upfield shifts of $\Delta\delta$ = −2.5 and $\Delta\delta$ = −2.6 ppm, respectively, whereas a downfield α effect is observed for the Gal C-3 resonance ($\Delta\delta$ = 2.7 ppm). The values of the individual shift increments correspond to those generally found for *O*-acetyl substituents [23–25]. From the peak areas of the signals it is concluded that the degree of substitution is considerably higher for Gal C-3, than for Gal C-2. There are no indications for *O*-acetyl groups at Gal C-6 and glycerol C-1′, if it is assumed that, as a consequence of substitution, α effects of $\Delta\delta \approx$ 2.5 ppm would be observed.

The ^{1}H-NMR spectrum of the native polysaccharide is depicted in Fig. 4b. Comparison of Fig. 4a and 4b learns that as a consequence of *O*-acetylation additional groups of resonances are found in the structural reporter group region. For the native polysaccharide (Fig. 4b) the resonance at

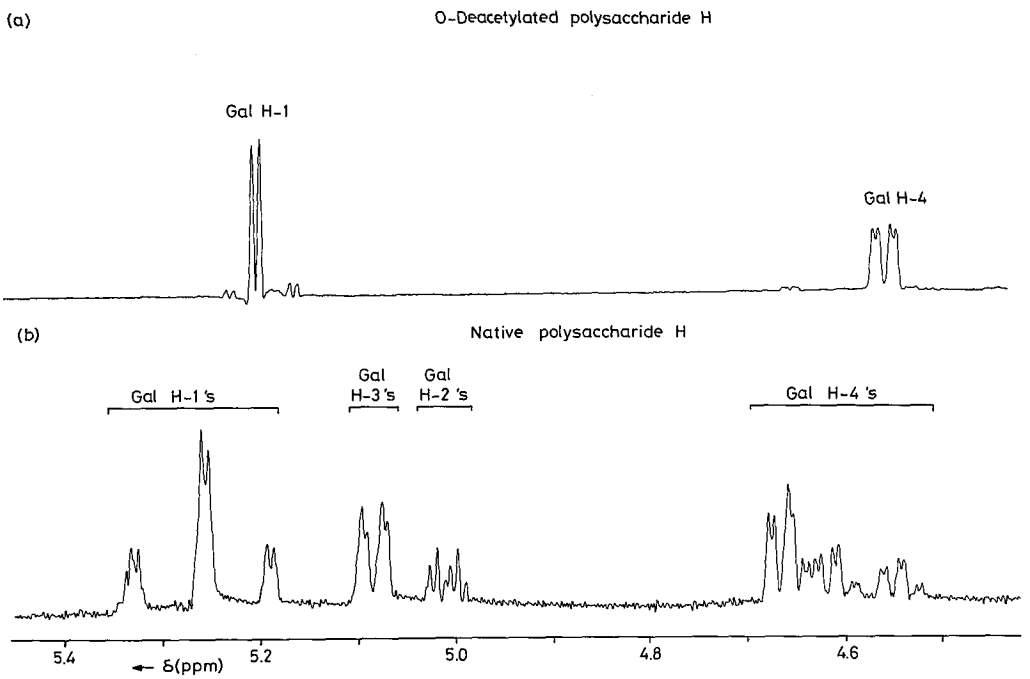
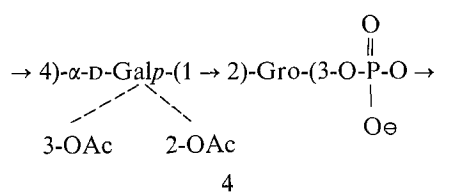


Fig. 4. *Structural reporter group regions of the resolution-enhanced 500-MHz ^1H-NMR spectra of the* O-*deacetylated (a) and the native (b) group H polysaccharide, recorded in ^2H_2O, at a probe temperature of 70 °C.* The *O*-deacetylated material has been analysed in its ammonium form, the native polysaccharide has been measured as its calcium salt. Chemical shift values are presented relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate using internal acetone ($\delta = 2.215$ ppm)

$\delta = 5.188$ ppm corresponds with the Gal H-1 resonance of the *O*-deacetylated material (Fig. 4a). The groups of resonances at $\delta \approx 5.08$ ppm and $\delta \approx 5.01$ ppm are associated with the presence of *O*-acetyl substituents at C-3 and C-2, respectively. Based on the characteristic coupling patterns the group of resonances at $\delta \approx 5.08$ ppm is assigned to Gal H-3 and that at $\delta \approx 5.01$ ppm is attributed to Gal H-2. For these resonances shift increments are found of $\Delta\delta \approx 1.1$ ppm as compared to the *O*-deacetylated material. These values agree with α effects of $\Delta\delta = 1.2-1.5$ ppm found for *O*-acetylation of secondary hydroxyl functions in sialic acids [26]. The presence of *O*-acetyl substituents at C-2 and C-3 of the same galactose moiety is excluded, as no concomitant β effects ($\Delta\delta \approx 0.2$ ppm [26]) are observed for the structural reporter group resonances of H-2 and H-3.

As a consequence of *O*-acetylation at Gal C-2 or Gal C-3 β and γ effects are observed for the Gal H-1 and Gal H-4 resonances. The shifted resonances of Gal H-1 ($\delta \approx 5.33$ ppm and $\delta \approx 5.26$ ppm) can be assigned on the basis of their intensities, as compared with those of the Gal H-2 and Gal H-3 resonances at $\delta \approx 5.01$ ppm and $\delta \approx 5.08$ ppm, respectively. Therefore the resonances at $\delta \approx 5.26$ ppm are attributed to Gal H-1 of 3-*O*-acetylated galactose moieties, whereas the signals at $\delta \approx 5.33$ ppm stem from Gal H-1 of 2-*O*-acetylated galactose units. The same approach can be used for the interpretation of the Gal H-4 signals. It should be noted that the resonances of the native material (Fig. 4b) exhibit multiple peak patterns, which will be discussed in a subsequent section.

According to the intensities of the H-1 resonances, the degree of *O*-acetylation at the D-Gal*p* residues is estimated as: 22% non-acetylated, 21% 2-*O*-acetylated and 57% 3-*O*-acetylated. Therefore the total degree of *O*-acetylation of the native polymer is approximately 78%. Based on structural element 3 and taking into account the calcium salt form of the native material this value is in good agreement with the *O*-acetyl content mentioned above (2.05 mmol/g polysaccharide ≙ 78% total degree of *O*-acetylation). In conclusion the repeating unit of the primary structure of the native polysaccharide has been established to be structure 4. The absolute configuration of the glycerol moiety remains to be established.



4

Conformational aspects of the polysaccharide

Based on NMR spectroscopic data, some remarks can be made with respect to the conformation of the polysaccharide.

In the ^{13}C-NMR spectra the values for the three-bond $^3J(^{31}P\text{-}^{13}C)$ coupling constants provide information about the conformation around the phosphate diester. These values depend on the dihedral angle between the P-O and the C-C bonds involved, according to a Karplus-like relation [27] (see Table 2). The small angle of 56° for the rotamer about O-(C-4) would imply that the phosphodiester group is situated closely above the Gal*p* ring, which results in unfavourable steric interactions. Therefore the alternative disposition is preferred, whereby the phosphodiester extends from the Gal*p* ring. Some flattening of the Gal*p* ring occurs, as for the angle between (C-3)-(C-4)-(C-5) values in the range of 150−160° are deduced. The three-bond phosphate coupling at C-2′ indicates a *trans*-like disposition (154°) of the glycerol (C-2′)-(C-3′) bond and the P-O bond. The alternative angle of 37° would imply an unfavourable *gauche*-like disposition.

Table 2. *Three-bond ^{31}P-^{13}C coupling constants and dihedral angles for the O-(C-4) and O-(C-3′) rotamers in the native and O-deacetylated polysaccharide*

Poly-saccharide	Rotamer	Coupling	Dihedral angles
		Hz	
O-Deacetyl-ated	O-(C-4)	$^3J_{C-3,P} < 0.5$	80–90°
		$^3J_{C-5,P} = 2.8$	56/116°
	O-(C-3′)	$^3J_{C-2',P} = 7.6$	37/154°
Native	O-(C-4)	$^3J_{C-3,P} < 0.5$	80–90°
		$^3J_{C-5,P} = 3.5$	52/120°
	O-(C-3′)	$^3J_{C-2',P} = 6.2$	42/142°

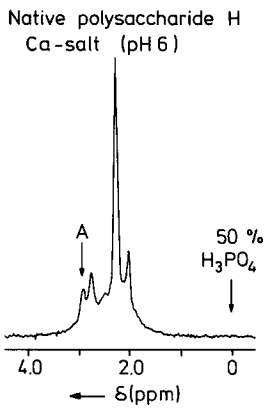


Fig. 5. *36.4-MHz ^{31}P-NMR Spectrum of the native group H polysaccharide, recorded in ^2H_2O at 20 °C.* A indicates the position of the resonance of the phosphorus nuclei from the *O*-deacetylated material. Orthophosphoric acid 50% (w/v) is used as external reference

The three-bond phosphate couplings in the native polysaccharide can be measured less accurately, as the heterogeneity in the *O*-acetylation pattern of the Gal*p* moieties broadens the resonance peaks and overlapping signals are observed. On the basis of the coupling constants estimated (see Table 2), it can be suggested that for the rotamers about O-(C-4) and O-(C-3′) only small changes in the dihedral angles occur as compared to the *O*-deacetylated polysaccharide. Therefore it is concluded that both the *O*-deacetylated and the native polysaccharide possess a rather rigid, linear structure around the phosphodiester moiety.

The multiple peak patterns in the 500-MHz ^{1}H-NMR spectrum of the native polysaccharide (Fig. 4b) probably are constituted of pairs of multiplets slightly shifted from each other. This suggests diversity in the physico-chemical environments of the individual resonating nuclei, intrinsic to a certain conformation of the polysaccharide. Specific sequences of *O*-acetylated Gal moieties may induce such a conformation, but effects of the calcium salt form of the polymer can not be excluded.

The ^{31}P-NMR spectrum of the native material (Fig. 5) shows at least five resonances with chemical shifts displaced to higher field, as compared to the *O*-deacetylated material. This demonstrates a non-equivalency of the phosphate groups, which might be caused by different *O*-acetyl substitution patterns of the galactose moieties. The occurrence of a dominating resonance ($\delta = 2.29$ ppm) is compatible with the major *O*-acetyl substitution at Gal C-3 of the polymer.

Concluding remarks

A polysaccharide structure composed of partially *O*-acetylated → 4)-α-D-Gal*p*-(1 → 2)-Gro-3-P → repeating units underlies the antigenic serogroup H specificity of *N. meningitidis*. Within the class of *N. meningitidis* capsular polysaccharides its structure is closest related with the group Z polysaccharide [21]: → 3)-α-D-GalNAc*p*-(1 → 1)-Gro-3-*P* →. On the other hand it shows a similarity with the teichoic acid polymer, consisting of a → 6)-β-D-Gal*p*-(1 → 1)-Gro-3-*P* → repeating unit, which has been reported as a component of the cell wall of *Bacillus licheniformis* ATCC 9945 [28]. For this reason the group H polysaccharide may as well be classified as a teichoic acid. Interestingly, this capsular polymer is produced by a gram-negative organism, a class of bacteria which does not produce cell-wall-incorporated teichoic acids.

The authors are indebted to Dr J. Kistemaker (FOM-Institute for Atomic and Molecular Physics, Amsterdam) for this continuous interest. They wish to thank Drs H. van Halbeek, B. de Kruijf and J. C. Roos-Venekamp for running the NMR spectra and Ms A.C. van der Kerk-van Hoof for running the GLC-MS analyses. This investigation was supported by the Netherlands Organization for the Advancement or Pure Research (ZWO) and the Netherlands Foundation for Chemical Research (SON). A. van der K. is on leave from FOM Institute for Atomic and Molecular Physics, Amsterdam, Netherlands.

REFERENCES

1. Jennings, H. J. (1983) *Adv. Carbohydr. Chem. Biochem. 41*, 155–208.
2. Jennings, H. J., Lugowski, C. W., Ashton, F. E. & Ryan, J. A. (1983) *Carbohydr. Res. 112*, 105–111.
3. Shao-Qing, D., Ren-Bang, Y. & Huan-Chun, Z. (1981) *J. Biol. Stand. 9*, 307–315.
4. Bundle, D. R., Jennings, H. J. & Kenny, C. P. (1974) *J. Biol. Chem. 249*, 4797–4801.
5. Chen, P. S., Toriba, J. U. & Warner, H. (1965) *Anal. Chem. 28*, 1756.
6. Hestrin, S. (1949) *J. Biol. Chem. 180*, 249–261.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. J. & Rondall, R. J. (1951) *J. Biol. Chem. 193*, 265.
8. Wetmur, J. G. & Davidson, N. (1968) *J. Mol. Biol. 31*, 349–370.
9. Kamerling, J. P., Gerwig, G. J., Vliegenthart, J. F. G. & Clamp, J. R. (1975) *Biochem. J. 151*, 491–495.
10. Kamerling, J. P. & Vliegenthart, J. F. G. (1982) *Cell Biol. Monogr. 10*, 95–125.
11. Gerwig, G. J., Kamerling, J. P. & Vliegenthart, J. F. G. (1978) *Carbohydr. Res. 62*, 349–357.
12. Hakomori, S. (1964) *J. Biochem. (Tokyo) 55*, 205.
13. Jansson, P. E., Kenne, L., Liedgren, H., Lindberg, B. & Lönngren, J. (1976) *Chem. Commun. Univ. Stockholm 8*, 1–75.
14. Ernst, R. R. (1966) *Adv. Magn. Res. 2*, 1–135.
15. Kamerling, J. P., Vliegenthart, J. F. G., Vink, J. & De Ridder, J. J. (1971) *Tetrahedron 27*, 4275–4288.
16. Kovacik, V., Bauer, S., Rosik, J. & Kovac, P. (1968) *Carbohydr. Res. 8*, 282–290.
17. Veerkamp, J. H. & Van Schaik, F. W. (1974) *Biochim. Biophys. Acta 348*, 370–387.
18. Bock, K. & Thøgersen, H. (1982) *Ann. Reports on NMR Spectroscopy 13*, 1–57.
19. De Boer, W. R., Kruyssen, F. J., Wouters, J. T. M. & Kruk, C. (1976) *Eur. J. Biochem. 62*, 1–6.
20. Branefors-Helander, P., Kenne, L. & Lindqvist, B. (1980) *Carbohydr. Res. 79*, 308–312.

21. Jennings, H. J., Rosell, K.-G. & Kenny, C. P. (1979) *Can. J. Chem. 57*, 2902–2907.
22. Kenne, L., Lindberg, B. & Madden, J. K. (1979) *Carbohydr. Res. 73*, 175–182.
23. Bundle, D. R., Jennings, H. J. & Smith, I. C. P. (1973) *Can. J. Chem. 51*, 3812–3819.
24. Bundle, D. R., Smith, I. C. P. & Jennings, H. J. (1974) *J. Biol. Chem. 249*, 2275–2281.
25. Bhattacharjee, A. K., Jennings, H. J., Kenny, C. P., Martin, A. & Smith, C. P. (1975) *J. Biol. Chem. 250*, 1926–1932.
26. Haverkamp, J., Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Pfeil, R. & Schauer, R. (1982) *Eur. J. Biochem. 122*, 305–311.
27. Kochetkov, N. K., Nifant'ev, E. E., Koroteev, M. P., Zhane, Z. K. & Borisenko, A. A. (1976) *Carbohydr. Res. 47*, 221–231.
28. Burger, M. M. & Glaser, L. (1966) *J. Biol. Chem. 241*, 494–506.

A. van der Kaaden, Fundamenteel Onderzoek der Materie Instituut voor Atoom- en Molecuulfysica, Kruislaan 407, NL-1098-SJ Amsterdam, The Netherlands

J. I. M. van Doorn-van Wakeren, J. P. Kamerling, and J. F. G. Vliegenthart, Afdeling Bio-Organische Chemie, Organisch-Chemisch Laboratorium, Rijksuniversiteit Utrecht, Croesestraat 79, NL-3522-AD Utrecht, The Netherlands

R. H. Tiesjema, Rijks Instituut voor Volksgezondheid, Postbus 1, NL-3720-BA Bilthoven, The Netherlands