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Note

High-resolution ^{1}H- and ^{13}C-n.m.r. spectra of the group A-variant streptococcal polysaccharide*

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L-Rhamnose is a major component of many bacterial polysaccharides including the streptococcal, group-specific polysaccharides. The sugar is believed to be the primary antigenic determinant of the groups A-variant[1], B, and G (ref. 2), streptococcal polysaccharides, of several capsular polysaccharides of *Pneumococci* and *Klebsiella*[3], and of an antigenic polysaccharide of *Mycoplasma pneumoniae*[4], among others. In order to use effectively n.m.r. methods to study the structures of these polysaccharides, several workers have synthesized various rhamnose oligosaccharides and studied their ^{1}H-n.m.r.[5–7] and ^{13}C-n.m.r.[8–10] spectra.

We report here the results of both ^{1}H- and ^{13}C-n.m.r. studies of the streptococcal group A-variant polysaccharide. This polysaccharide is of particular interest since it has been proposed that it constitutes the backbone of several streptococcal, group-specific polysaccharides including the groups A and C polysaccharides[11].

Methylation analysis and periodate oxidation studies indicated that this polysaccharide is a linear homopolymer of alternating (1→2)- and (1→3)-linked L-rhamnose units[1].

The ^{1}H-n.m.r. spectrum of the A-variant polysaccharide was recorded, and computer simulation was employed to determine chemical shifts and coupling constants (Table I). The experimental and computer-simulated spectra are shown in

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TABLE I

CHEMICAL SHIFTS (δ) FOR THE ^{1}H-N.M.R. SPECTRUM OF THE A-VARIANT STREPTOCOCCAL POLYSACCHARIDE[a]

Linkage position	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*
2	5.21	4.09	3.97	3.53	3.85	1.34
	$J_{1,2}$ 1.5	$J_{2,3}$ 3.09	$J_{3,4}$ 9.6	$J_{4,5}$ 9.6	$J_{5,6}$ 6.17	
3	4.97	4.18	3.87	3.57	3.78	1.29
	$J_{1,2}$ 1.5	$J_{2,3}$ 3.09	$J_{3,4}$ 9.6	$J_{4,5}$ 9.6	$J_{5,6}$ 6.17	

[a]Relative to the signal of internal sodium 4,4-dimethyl-4-sila-[2,2.3,3-$^2H_4$]pentanoate. *J* values in Hz.

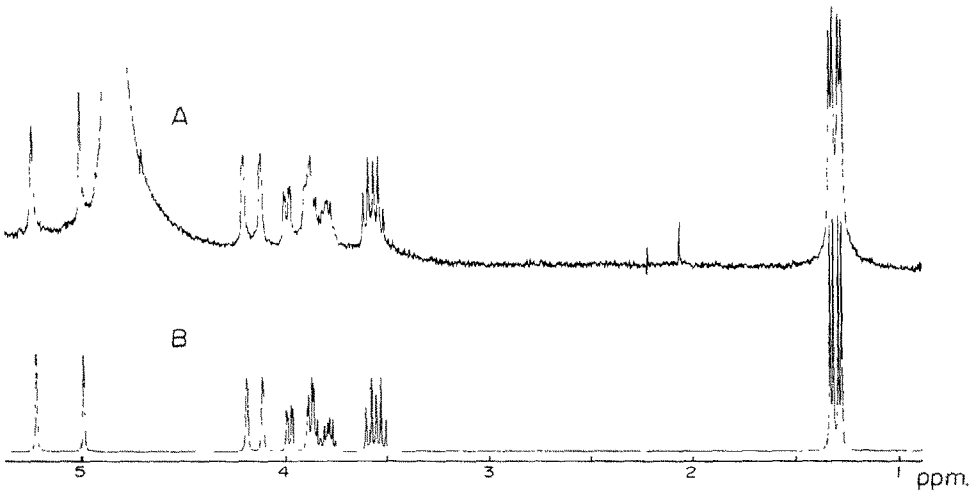

Fig. 1. ^{1}H-N.m.r. spectrum of the streptococcal group A-variant polysaccharide (A) recorded relative to internal sodium 4,4-dimethyl-4-sila-[2,2.3,3-$^2H_4$]pentanoate, and the computer-simulated ^{1}H-n.m.r. spectrum (B).

Fig. 1. The proton resonances of each of the two L-rhamnose residues in the polysaccharide were assigned by sequential, selective-decoupling experiments starting at the well-resolved H-1 resonances. The two sets of proton resonances thus obtained were assigned to either a 2- or 3-linked L-rhamnose residue, based upon the results of ^{13}C-heteronuclear-decoupling experiments. Selective heteronuclear-decoupling of the downfield, anomeric-proton resonance (δ 5.21), in the proton-coupled ^{13}C spectrum, caused the upfield C-1 doublet (δ 101.61) to collapse. Similarly, decoupling of the upfield, anomeric-proton resonance (δ 4.97) caused the downfield C-1 (δ 102.75) doublet to collapse. The upfield, ^{13}C-anomeric carbon resonance (δ 101.61) was assigned to a 2-linked L-rhamnose unit, based upon model-compound data[10]. This

TABLE II

CHEMICAL SHIFTS (δ) FOR THE ^{13}C-N.M.R. SPECTRUM OF THE A-VARIANT STREPTOCOCCAL POLYSACCHARIDE[a]

Linkage position	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
2	101.61	78.75	70.67	72.99	70.11	17.49
3	102.75	70.67	78.29	72.38	70.11	17.37

[a]Relative to the signal of an internal standard of dimethyl sulfoxide, at δ 39.55 relative to tetramethylsilane.

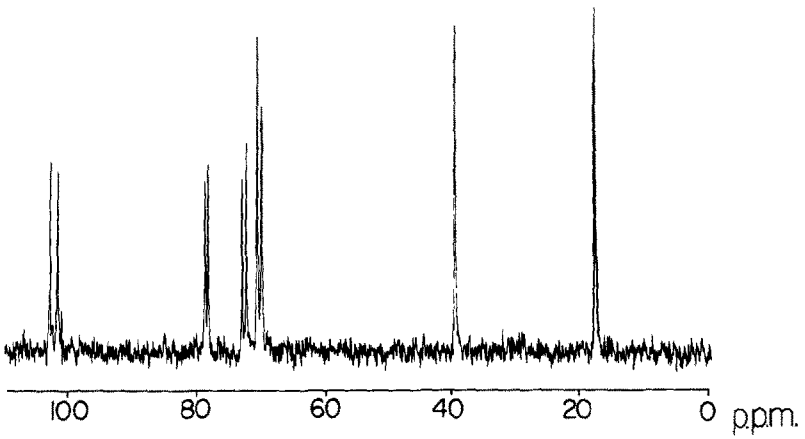

Fig. 2. ^{13}C-N.m.r. spectrum of the streptococcal group A-variant polysaccharide recorded relative to internal dimethyl sulfoxide (δ 39.55).

assignment is consistent with the expected upfield shift (β-effect) for C-1 of a 2-linked L-rhamnose unit. Fig. 2 shows the ^{13}C-n.m.r. spectrum of the A-variant streptococcal polysaccharide, and the chemical shifts are listed in Table II. The nonglycosidically linked C-2 and -3 resonances were not resolved from each other, nor were the C-5 resonances. The C-4 resonance assignments were identified by off resonance heteronuclear-decoupling, and differentiated on the basis of model-compound data[10]. The assignments are consistent with an expected, upfield shift for a C-4 of a 3-linked L-rhamnose unit. The assignment of the methyl carbon resonances was based upon the observation that the upfield methyl peak was also present in the spectrum of the group A streptococcal polysaccharide. Since both the A and A-variant polysaccharide have in common a 3-linked L-rhamnose unit, the upfield methyl resonance was assigned to this unit[1].

Laffite *et al.*[6] pointed out that the anomeric configuration of L-rhamnose saccharides could be deduced from the chemical shifts of the H-5 protons. A large

deshielding of the H-5 proton was observed in all compounds having the β-L configuration, as compared to the compounds having the α-L configuration. The H-5 resonances observed, in the spectrum of the A-variant streptococcal polysaccharide, at δ 3.85 and 3.78, are both consistent with α-L-glycosidic linkages. In the proton-coupled, ^{13}C-n.m.r. spectrum, both C-1 signals appeared as doublets with a spacing of 170.9 Hz, further confirming the presence of α-L-glycosidic linkages.

These results support the previously proposed structure of the group A-variant polysaccharide that was based upon methylation analysis data[1]. In addition, the aforementioned results establish for the first time that the glycosidic linkages in the polysaccharide have the α-L configuration.

EXPERIMENTAL

General. — ^{1}H- and ^{13}C-spectra were recorded with a Bruker WH-400 spectrometer operating in the Fourier-transform mode. The ^{13}C-spectra were obtained by use of broadband decoupling and a 3.3-s recycle delay. A minimum of 1500 free-induction decays were collected. Proton chemical-shifts were measured relative to internal sodium 4,4-dimethyl-4-sila[2,2,3,3-$^{2}H_4$]pentanoate (TSP). Carbon-13 chemical-shifts were measured with an internal standard of dimethyl sulfoxide, whose chemical shift was set to δ 39.55 relative to tetramethylsilane.

Preparation of the group-A variant streptococcal polysaccharide. — The group A-variant carbohydrate was extracted with formamide[1] at 180° from acetone-dried cells of strain A486 streptococci. The extract was mixed with acidified ethanol (2 vol., 19:1, v/v, ethanol–M hydrochloric acid), and the precipitate was discarded. The polysaccharide was precipitated with acetone (5 vol.). This material was dissolved in water and charged molecules were removed by sequentially passing the extract through columns of anion- and cation-exchange resins. The solution was lyophilized.

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