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Structure of simusan, a new acidic exopolysaccharide from *Arthrobacter* sp.

Soph'ya N. Senchenkova [a,*], Yuriy A. Knirel [a],
Leonid M. Likhosherstov [a], Aleksander S. Shashkov [a],
Vladimir N. Shibaev [a], Lyubov' A. Starukhina [b],
Vladimir V. Deryabin [b]

[a] *N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russian Federation*
[b] *A.O. Biotechnology, Moscow, Russian Federation*

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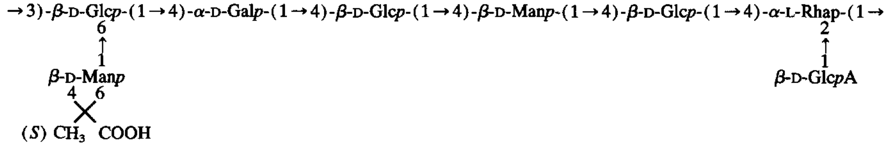
Abstract

Simusan, a major exopolysaccharide produced by an ethanol-utilizing *Arthrobacter* sp. strain CE-17, contains D-glucose, D-mannose, D-galactose, L-rhamnose, D-glucuronic acid, and pyruvic acid in the ratios ~3:2:1:1:1:1 as well as *O*-acyl groups (presumably residues of acetic and palmitic acid). On the basis of chemical modifications of the polysaccharide, solvolysis with anhydrous hydrogen fluoride resulting in a penta- and an octa-saccharide fragment, Smith degradation, and ^{1}H and ^{13}C NMR analysis, the following structure of the repeating unit was established:



$$\begin{array}{lr}
\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow & \\
\qquad 6 & 2\qquad \\
\qquad \uparrow & \uparrow\qquad \\
\qquad 1 & 1\qquad \\
\beta\text{-D-Man}p & \beta\text{-D-Glc}p\text{A} \\
\quad 4\ \ 6 & \\
\qquad \times & \\
(S)\ \text{CH}_3\ \ \text{COOH} &
\end{array}$$

It is suggested that at least one of the glucose residues and the galactose residue are *O*-acetylated.

Keywords: Bacterial polysaccharides; Industrial carbohydrates; NMR spectroscopy; Solvolysis

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1. Introduction

Bacteria of genus *Arthrobacter* are known as producers of exopolysaccharides having potential importance for industry. Some of the polysaccharides contain mainly glucose with

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[*] Corresponding author.

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β-(1 → 4) linkages in the backbone; acidic components such as glucuronic, mannuronic, 2,3-diacetamido-2,3-dideoxymannuronic, succinic, and acetal-linked pyruvic acid are present in polysaccharides from various *Arthrobacter* strains [1–6]. We are interested in a new exopolysaccharide, called simusan [7–9], which is produced by an ethanol-utilizing *Arthrobacter* sp. strain CE-17 isolated from soil and has useful physico-chemical properties. It may be used for food and for technical purposes as a highly effective thickener, a stabilizer for emulsions and foams, a strong flocculant, and a water retention and suspension agent. We report now the results of our structural study of simusan, aiming at correlation between its structure and physico-chemical properties.

2. Results and discussion

The polysaccharide simusan (PS) was isolated from the cultural fluid by precipitation with 2-propanol.

Attempts to obtain a ^{13}C NMR spectrum of PS failed owing to its low solubility. Treatment of PS with aq 12% ammonia followed by ultrasonication and purification by ion-exchange chromatography on DEAE-Trisacryl M resulted in the *O*-deacetylated polysaccharide (PS_{NH_4OH}). As judged by the ^{13}C NMR spectrum (Fig. 1), a similar polysaccharide was obtained by treatment of PS with 0.1 M sodium hydroxide in the presence of 1 M sodium borohydride.

Sugar analysis of PS_{NH_4OH} revealed the presence of glucose, mannose, galactose, rhamnose, and glucuronic acid in the ratios ~3:2:1:1:1. The D configuration of glucose and glucuronic acid (after carboxyl reduction [10]) was determined by oxidation with D-glucose oxidase and the D configuration of mannose and galactose and the L configuration of rhamnose by GLC of acetylated (+)-2-octyl glycosides [11]. Pyruvic acid was identified by the colorimetric method [12,13] and found to be present in the same molar amount as rhamnose on the basis of comparison of the integral intensities of the signals for their methyl groups in the ^{1}H and ^{13}C NMR spectra of PS_{NH_4OH} (δ_H 1.31 and 1.47 ppm, δ_C 18.0 and 26.1 ppm for rhamnose and pyruvic acid, respectively).

Treatment of PS with aq 2% acetic acid and ultrasonication led to another modified polysaccharide (PS_{HOAc}). According to the ^{13}C NMR spectrum, PS_{HOAc} lacked pyruvic acid but contained *O*-acetyl groups (a signal at 21.6 ppm with an integral intensity com-

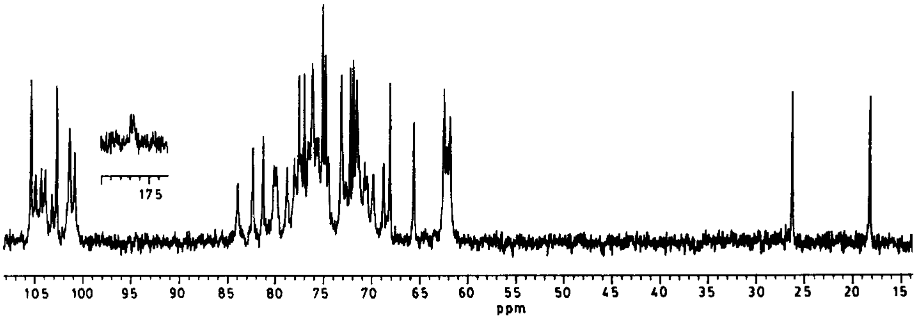


Fig. 1. ^{13}C NMR spectrum of the *O*-deacetylated polysaccharide (PS_{NH_4OH}).

Table 1
^{1}H NMR chemical shifts (δ in ppm)

Unit	H-1	H-2	H-3	H-4	H-5	H-6
Oligosaccharide **1**						
β-D-Glc*p*A-(1→	4.66	3.38	3.55	3.55	3.87	
β-D-Glc*p*-(1→(II)	4.52	3.32	3.53	3.41	3.52	[a]
→4)-β-D-Man*p*-(1→	4.79	4.13	3.78	3.82	3.59	[a]
→4)-β-D-Glc*p*-(1→(I)	4.69	3.44	3.72	3.72	3.60	[a]
→4)-L-Rha-ol 2 ↑	[a]	3.97	4.01	4.03	4.15	1.29
Oligosaccharide **2**						
β-D-Glc*p*A-(1→	4.66	3.37	3.54	3.54	3.65	
β-D-Man*p*-(1→	4.72	4.05	3.67	3.60	3.40	[a]
→6)-β-D-Glc*p*-(1→(III)	4.68	3.38	3.54	3.42	3.62	3.82, 4.24
→4)-α-D-Gal*p*(1→	5.46	3.96	3.96	4.26	4.05	[a]
→4)-β-D-Glc*p*-(1→(II)	4.53	3.37	3.81	3.66	3.66	[a]
→4)-β-D-Man*p*-(1→	4.80	4.14	3.79	3.83	3.59	[a]
→4)-β-D-Glc*p*-(1→(I)	4.70	3.45	3.73	3.73	3.60	[a]
→4)-L-Rha-ol 2 ↑	[a]	3.94	4.02	4.03	4.15	1.39

[a] Not assigned.

parable with that of the signal for Me of rhamnose). Fatty acid analysis showed the presence of palmitic acid (up to 1.5%) in the preparations of PS_{HOAc} from all batches of bacterial cells studied; other fatty acids varied from batch to batch and were thus derived from contaminating lipids and/or lipopolysaccharide.

Solvolysis of PS_{NH_4OH} with anhydrous hydrogen fluoride at −78°C resulted in an oligosaccharide which was isolated by gel chromatography on Sephadex G-15. It contained glucose, mannose, rhamnose, and glucuronic acid in the ratios 2:1:1:1. Reduction of this oligosaccharide with sodium borohydride resulted in conversion of rhamnose into rhamnitol (Rha-ol) showing that this sugar occupied the reducing end.

The ^{1}H and ^{13}C NMR spectra of the reduced compound (**1**) confirmed its structure as a tetraglycosylrhamnitol.

The ^{1}H NMR spectrum of **1** (Table 1) contained four signals for anomeric protons at 4.44, 4.60, 4.62 (all doublets, $J_{1,2}$ 7.6–7.9 Hz), and 4.72 ppm (d, $J_{1,2}$ 0.8 Hz), the signal for a methyl group (H-6 of rhamnitol) at 1.20 ppm (d, $J_{1,2}$ 6.5 Hz), and signals for other protons in the region 3.2–4.1 ppm.

The ^{13}C NMR spectrum of **1** (Table 2) contained the signals for four anomeric carbons at 101.1, 103.7, 103.8, and 104.3 ppm, a methyl group (C-6) of the rhamnitol residue at 19.2 ppm, four CH_2OH groups (C-6 of three hexoses and C-1 of rhamnitol) at 61.2, 61.4, 61.7, and 61.9 ppm, and 20 other sugar carbons in the region 69–83 ppm. The signal for the COOH group (C-6 of GlcA) was not clearly observed probably because of an insufficient amount of the material.

Methylation analysis [14] of **1** led to the identification of 1,3,5-tri-*O*-methylrhamnitol, 2,3,4,6-tetra-*O*-methylglucose, 2,3,6-tri-*O*-methylglucose, and 2,3,6-tri-*O*-methylmannose

Table 2
^{13}C NMR chemical shifts (δ in ppm). The spectra were assigned using 2D H-detected multiple-quantum ^{1}H,^{13}C correlation (for **2**), tentatively by the computer-assisted method [16,17] (for **3**–**5**), and by comparison with the spectra of **2**–**5** (for **1**). The values calculated by the published method [16,17] are given in parentheses

Sugar unit	C-1	C-2	C-3	C-4	C-5	C-6
Oligosaccharide **1**						
β-D-Glc*p*A-(1→	103.6	73.9	76.5	76.1	[a]	
β-D-Glc*p*-(1→(II)	103.7	74.1	76.5	70.5	77.0	61.6[b]
→4)-β-D-Man*p*-(1→	101.0	71.1	72.5	77.5	76.1	61.3[b]
→4)-β-D-Glc*p*-(1→(I)	104.2	74.1	75.1	79.6	75.1	61.1[b]
→4)-L-Rha-ol 2 ↑	61.8[b]	81.4	69.4	82.5	69.6	19.1
Oligosaccharide **2**						
β-D-Glc*p*A-(1→	103.4	74.0	76.6	72.7	76.8	[a]
β-D-Man*p*-(1→	101.5	71.4	73.8	67.7	77.2	61.9[b]
→6)-β-D-Glc*p*-(1→(III)	104.7	74.6	76.6	70.7	75.8	69.8
→4)-α-D-Gal*p*-(1→	100.7	70.6	69.9	79.5	72.1	61.9[b]
→4)-β-D-Glc*p*(1→(II)	103.4	74.0	76.8	77.7	75.5	61.5[b]
→4)-β-D-Man*p*-(1→	100.9	71.0	72.5	77.4	76.1	61.7[b]
→4)-β-D-Glc*p*-(1→(I)	104.2	74.1	75.0	79.5	75.2	61.2[b]
→4)-L-Rha-ol 2 ↑	61.9[b]	81.4	69.4	82.4	69.7	19.0
Oligosaccharide **3**						
α-L-Rha*p*-(1→	102.5	71.8	71.6	73.4	70.3	17.9
	(102.3)	(71.6)	(71.3)	(73.5)	(70.5)	(18.0)
→3)-β-D-Glc*p*-(1→(III)	103.2	75.3	83.5	69.5	77.2	61.9
		(75.3)	(83.4)	(69.6)	(77.2)	(62.1)
→2)-D-Thr-ol	61.9	81.0	72.0	63.6		
Oligosaccharide **4**						
β-D-Glc*p*-(1→(I)	104.6	74.4	77.1	70.8	77.3	62.0
	(104.3)	(75.0)	(77.0)	(70.9)	(77.2)	(62.1)
→4)-α-L-Rha*p*-(1→	102.1	71.7	71.5	82.5	68.6	18.0
	(102.3)	(71.6)	(71.3)	(82.4)	(68.7)	(18.0)
→3)-β-D-Glc*p*-(1→(III)	103.0	75.2	83.4	69.4	77.1	62.0
		(75.3)	(83.4)	(69.6)	(77.2)	(62.1)
→2)-D-Thr-ol	62.0	80.9	72.1	63.5		
Oligosaccharide **5**						
α-L-Rha*p*-(1→	102.2	71.7	71.7	73.4	70.3	17.8
	(102.3)	(71.6)	(71.3)	(73.5)	(70.5)	(18.0)
→3)-β-D-Glc*p*-(1→(III)	104.8	75.5	83.9	69.7	77.2	62.2[b]
	(104.9)	(75.3)	(83.4)	(69.6)	(77.2)	(62.1)
→4)-α-D-Gal*p*-(1→	99.4	7.02	71.3	79.6	73.0	62.3[b]
		(69.6)	(71.1)	(79.9)	(72.9)	(62.4)
→2)-D-Ery-ol	61.3	80.2	72.2	63.9		

[a] Not found.

[b] Assignment could be interchanged.

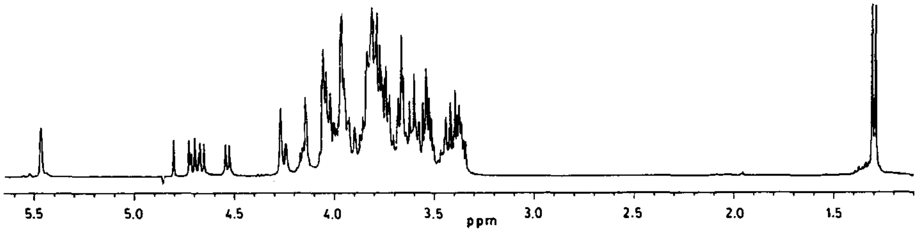


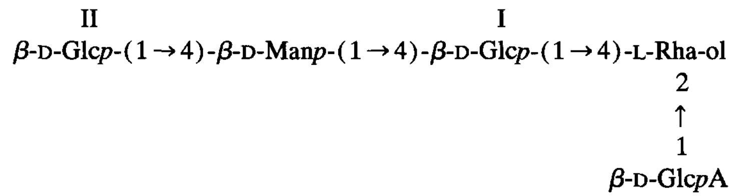
Fig. 2. ^{1}H NMR spectrum of the octasaccharide-alditol **2**.

as alditol acetates. When methylated **1** was carboxyl-reduced with lithium borohydride prior to hydrolysis, in addition to the above-mentioned sugars, 2,3,4-tri-*O*-methyl glucose derived from the GlcA residue was identified. Therefore, **1** is branched at the rhamnitol residue, substituted at positions 2 and 4. The GlcA residue and one of the glucose residues are terminal, while the second glucose residue and the mannose residue are 4-substituted.

The ^{1}H NMR spectrum of **1** was assigned using 1D sequential, selective spin-decoupling, 2D shift-correlated spectroscopy (COSY), and COSY with relayed coherence transfer (COSYRCT), and the results are given in Table 1. The relatively large values of the coupling constants $J_{1,2}$ 7.6–7.9 Hz for the residues of glucose (GlcI and GlcII) and GlcA were indicative [15] of the β configuration of these sugar units.

The following interresidue NOEs between transglycosidic protons were observed on sequential, selective pre-irradiation of H-1 of each sugar residue: H-1 GlcII at δ 4.52/H-4 Man at 3.82; H-1 Man at δ 4.79/H-3 or H-4 GlcI at δ 3.72; H-1 GlcI at δ 4.69/H-4 Rha-ol at δ 4.03; H-1 GlcA at δ 4.66/H-2 Rha-ol at δ 3.97. These data suggest the sequence of the sugar residues in **1** shown below. The appearance of NOEs on H-4 Man and H-2 and H-4 Rha are in agreement with the methylation data (see above). The NOEs on H-3,5 Man observed on pre-irradiation of H-1 of the same residue proved the β configuration of the mannose residue.

Therefore, the oligosaccharide **1** has the following structure:



$$\begin{array}{l}
\text{II} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \text{I} \\
\beta\text{-D-Glc}p\text{-}(1\rightarrow4)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow4)\text{-L-Rha-ol} \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 2 \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \uparrow \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 1 \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \beta\text{-D-Glc}p\text{A}
\end{array}$$

1

Solvolysis of PS with anhydrous hydrogen fluoride at −78°C resulted in a larger oligosaccharide isolated by gel chromatography on TSK HW-50. Its borohydride reduction led to an oligosylalditol (**2**) which was isolated by gel filtration on Sephadex G-15 and proved to be homogeneous by anion-exchange chromatography in borate buffer. It contained the same sugar components (except that rhamnitol was present instead of rhamnose) in the same ratios as PS and thus represented a modified octasaccharide repeating unit.

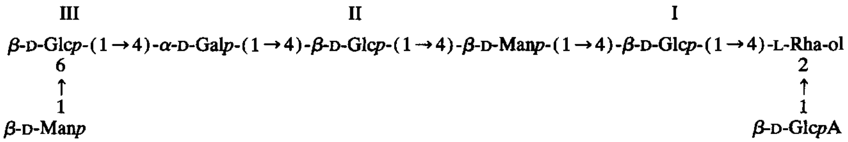
The ^{1}H NMR spectrum of **2** (Fig. 2) was assigned using 2D total correlation spectroscopy (TOCSY, Table 1). The values of the coupling constants $J_{1,2}$ 7–8 Hz showed [15] that all three glucose residues and the GlcA residue are β, while the value $J_{1,2} \leq 4$ Hz indicated the

Table 3
ROESY data for oligosaccharide **2**

Sugar unit	Proton	Cross-correlation peaks with H-1 of						
		β-GlcA	β-Man	β-Glc (III)	α-Gal	β-Glc (II)	β-Man	β-Glc (I)
β-GlcA	H-2	+						
	H-3	+						
	H-5	+						
β-Man	H-2		+					
	H-3		+					
	H-5		+					
β-Glc (III)	H-2			+				
	H-3			+				
	H-5			+				
	H-6		+					
α-Gal	H-2				+			
	H-4			+				
	H-5			+				
β-Glc (II)	H-3					+		
	H-4				+			
	H-5					+		
β-Man	H-2						+	
	H-3						+	
	H-4					+		
	H-5						+	
β-Glc (I)	H-3							+
	H-4						+	
	H-5							+
Rha-ol	H-2	+						
	H-3	+						
	H-4							+
	H-5							+

galactose residue to be α. On the basis of relatively upfield positions of the signals for H-1 at 4.72 and 4.80 ppm and the appearance of the correlation peaks H-1/H-3 and H-1/H-5 in the 2D NOE spectrum (Table 3), it is suggested that both mannose residues are β.

Linkage and sequence analysis of the oligosaccharide **2** was performed using 2D rotating-frame NOE spectroscopy (ROESY, Table 3). The interresidue NOE contacts listed in Table 3 allowed determination of the following structure for the octasaccharide **2** which is in agreement with the structure of the pentasaccharide-alditol **1**.



$$\begin{array}{llllll}
\text{III} & & \text{II} & & \text{I} & \\
\beta\text{-D-Glc}p\text{-}(1\rightarrow4)\text{-} & \alpha\text{-D-Gal}p\text{-}(1\rightarrow4)\text{-} & \beta\text{-D-Glc}p\text{-}(1\rightarrow4)\text{-} & \beta\text{-D-Man}p\text{-}(1\rightarrow4)\text{-} & \beta\text{-D-Glc}p\text{-}(1\rightarrow4)\text{-} & \text{L-Rha-ol} \\
6 & & & & & 2 \\
\uparrow & & & & & \uparrow \\
1 & & & & & 1 \\
\beta\text{-D-Man}p & & & & & \beta\text{-D-Glc}p\text{A}
\end{array}$$

2

This structure and, in particular, the positions of substitution of the constituent monosaccharides, including rhamnitol, were confirmed by assignment of the ^{13}C NMR spectrum of

2 (Table 2), which was performed using 2D H-detected multiple-quantum $^{1}H,^{13}C$ correlation (HMQC).

It is evident that the octasaccharide-alditol **2** was obtained as a result of selective cleavage of the rhamnosidic linkage, while the pentasaccharide-alditol **1** was formed upon cleavage of the rhamnopyranosidic and α-galactopyranosidic linkages; a lower stability of α-hexopyranosides towards anhydrous hydrogen fluoride as compared with β-hexopyranosides has been described [16].

Smith degradation of PS_{NH_4OH} resulted in the destruction of both mannose units, galactose, and GlcA, and the partial destruction of glucose, with rhamnose remaining intact (the ratio Glc/Rha is 1.3:1 after oxidation). Mild acid hydrolysis of the degraded polysaccharide gave an oligosaccharide-alditol isolated by gel chromatography on TSK HW-40. According to GLC of alditol acetates, it contains equal amounts of glucose, rhamnose, and threitol (Thr-ol).

Computer-assisted ^{13}C NMR-based analysis [17,18] of the Smith degradation product revealed only one structure **3** conforming to the experimental spectrum (Table 2). It was characterized by the smallest sum of the squared deviations of the chemical shifts in the calculated and experimental spectra per one sugar unit (S = 0.2), while all other structures had the value S > 1. This structure was confirmed by methylation analysis which resulted in identification of 2,3,4-tri-*O*-methylrhamnose and 2,4,6-tri-*O*-methylglucose.

$$\alpha\text{-L-Rha}p\text{-}(1\rightarrow3)\text{-}\overset{\text{III}}{\beta\text{-D-Glc}p}\text{-}(1\rightarrow2)\text{-Thr-ol}$$

3 (S = 0.2)

Smith degradation of PS itself led to two additional oligosaccharide-alditol products **4** and **5** isolated by HPLC on reversed-phase C18; **4** contained glucose, rhamnose, and threitol in the ratios ~ 2:1:1, while **5** contained almost equal amounts of glucose, galactose, rhamnose, and erythritol (Ery-ol). As in the case of **3**, the structures of these oligosaccharide alditols were elucidated using computer-assisted ^{13}C NMR analysis (Table 2). Two structures with the values S 0.1 and 0.4 conforming to the experimental spectrum were revealed for **4** and four structures with the values S 0.4 (two structures) and 0.6 for **5**. The alternative structures differed in the position of substitution of one of the sugar residues (rhamnose in **4** or galactose in **5**), and the correct structures were easily chosen by methylation analysis.

$$\overset{\text{I}}{\beta\text{-D-Glc}p}\text{-}(1\rightarrow4)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow3)\text{-}\overset{\text{III}}{\beta\text{-D-Glc}p}\text{-}(1\rightarrow2)\text{-Thr-ol}$$

4 (S = 0.1)

$$\alpha\text{-L-Rha}p\text{-}(1\rightarrow3)\text{-}\overset{\text{III}}{\beta\text{-D-Glc}p}\text{-}(1\rightarrow4)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow2)\text{-Ery-ol}$$

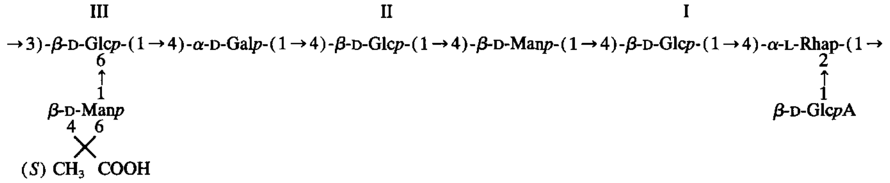
5 (S = 0.4)

The structures **3–5**, and in particular the attachment of the Rha → Glc disaccharide to threitol, derived from galactose in **3** and **4**, and to galactose in **5**, suggest the sequence

Rha → Glc → Gal and thus show that Rha is attached to GlcIII. The complete sequence of the sugar residues in PS is thus elucidated.

The ^{13}C NMR spectrum of $\text{PS}_{\text{NH}_4\text{OH}}$ (Fig. 1) contained a signal for a CH_2 group at 65.5 ppm (attached-proton test data [19]), which was absent from the spectrum of PS_{HOAc}. Evidently, this signal belonged to C-6 of a hexose residue carrying the pyruvic acid residue, which caused the downfield shift of this signal by >3 ppm (cf. the published data [20]). The only sugar which is able to carry the pyruvic acid acetal at position 4 and 6 is the lateral mannose residue. The chemical shift of 26.1 ppm for the methyl group of the pyruvic acid residue showed [20] that it has the (*S*) configuration.

On the basis of these data, it was concluded that $\text{PS}_{\text{NH}_4\text{OH}}$ has the following structure:



$$\rightarrow 3)\text{-}\underset{\substack{6\\ \uparrow\\ 1\\ \beta\text{-D-Man}p\\ 4\ \ 6\\ \times\\ (S)\ \text{CH}_3\ \ \text{COOH}}}{\overset{\text{III}}{\beta\text{-D-Glc}p}}\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 4)\text{-}\overset{\text{II}}{\beta\text{-D-Glc}p}\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow 4)\text{-}\overset{\text{I}}{\beta\text{-D-Glc}p}\text{-}(1\rightarrow 4)\text{-}\underset{\substack{2\\ \uparrow\\ 1\\ \beta\text{-D-Glc}p\text{A}}}{\alpha\text{-L-Rha}p}\text{-}(1\rightarrow$$

The exact sites of *O*-acetylation in simusan remain unknown. Formation of oligosaccharides **4** and **5**, i.e., survival of GlcI and Gal during Smith oxidation of PS suggests that at least these two sugar residues are *O*-acetylated. The presence of fatty acids has been reported, e.g., for emulsan, an industrially important exopolysaccharide of *Acinetobacter calcoaceticus* RAG-1 [21]. In simusan we found palmitic acid; however, its covalent attachment to the polysaccharide was not proved.

Our structural study showed that simusan has a unique structure among the known exopolysaccharides of *Arthrobacter*. It is interesting that simusan and an exopolysaccharide from *Arthrobacter stabilis* NRRL-3225 show similar rheological properties. In particular, the viscosity of aqueous solutions increases under similar conditions (a decrease of pH to 3.0, the presence of K^+, heat treatment) [2,7,22]. Structural elucidation of the latter polysaccharide would reveal whether it has any structural element in common with simusan.

3. Experimental

General methods.—The ^{1}H and ^{13}C NMR spectra were recorded with Bruker WM-250 and Bruker AM-300 spectrometers, respectively, for solutions in D_2O at 30°C for oligosaccharides and 60°C for polysaccharides (internal standard acetone, δ_H 2.225, δ_C 31.45). The standard Bruker software was used to obtain 2D COSY and COSYRCT spectra. 2D TOCSY, ROESY, and HMQC spectra were recorded with a Bruker AMX-400 spectrometer at 30°C.

GLC was performed with a Hewlett–Packard 5890 instrument equipped with a glass capillary column (0.2 mm × 25 m) coated with cross-linked methyl silicone gum. GLC–MS was carried out on a Varian MAT 311 instrument.

GPC was carried out on a column (70 × 2.2 cm) of Sephadex G-15 in water and monitored by a 2138 Uvicord S at 206 nm and on a column (80 × 1.6 cm) of TSK HW-40 (S) in water; monitoring was performed by a Knauer differential refractometer. HPLC was carried out on a column (30 × 0.8 cm) of Silasorb SPH C18 eluted with water and monitored by a Knauer differential refractometer. Anion-exchange chromatography of polysaccharides was performed on a column (10 × 0.3 cm) of DEAE-Trisacryl M with stepwise gradients of 0.01 M → 0.1 M Na_2HPO_4 (pH 6.3) containing 0 → 0.5 M NaCl and monitored by the orcinol–H_2SO_4 reaction. Anion-exchange chromatography of monosaccharides was performed on a column (20 × 0.6 cm) of Durrum DAx4 in 0.5 M sodium borate buffer (pH 9.0) at 55°C and monitored as above.

Cultivation of bacterium and isolation of exopolysaccharide.—*Arthrobacter* sp. strain CE-17 was grown in 0.75-L flasks with 100 mL of a medium containing (g/L): EtOH, 15; $Na_2HPO_4 \cdot 12H_2O$, 3.62; KH_2PO_4, 1.77; NH_4NO_3, 0.6; $MgSO_4 \cdot 7H_2O$, 0.4; $CaCl_2 \cdot 2H_2O$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.01; yeast extract, 0.2; pH 7.0, shaken at 180 rpm at 29°C during 48 h. The culture broth (1 L) was diluted with distilled water (9 L) and centrifuged (12 000*g*, 30 min). The supernatant solution was dialyzed against distilled water for 3 days and centrifuged as described above. PS was precipitated from the supernatant solution with 2-propanol (2 vol), washed several times with EtOH, then with acetone, and dried in vacuum. The yield of PS was 8.5 g.

Modifications of exopolysaccharide.—For *O*-deacetylation, PS (100 mg) was treated with aq 12.5% ammonia (100 mL, 16 h, 37°C), and the solution was concentrated in vacuum to minimal volume (2–3 mL) and passed through a Sephadex G-15 column. The viscous eluate was ultrasonicated [23] with a USDN-2T instrument at 44 kHz (0°C), and the major acidic polysaccharide (PS_{NH_4OH}, 43 mg) and a minor neutral polysaccharide (4 mg) were isolated by anion-exchange chromatography on DEAE-Trisacryl M. The latter was not studied further.

Alternatively, PS (100 mg) was treated with 1 M $NaBH_4$ in 0.1 M NaOH (100 mL, 16 h, 50°C); after neutralization with glacial AcOH, PS_{NH_4OH} (90 mg) was isolated by gel filtration on Sephadex G-15.

For removal of pyruvic acid, PS (100 mg) in aq 2% AcOH was heated for 2 h at 100°C and PS_{HOAc} (70 mg) was isolated by gel filtration on Sephadex G-15 with subsequent ultrasonication [23].

Analytical methods.—For sugar analysis, PS_{NH_4OH} (0.2 mg) was hydrolyzed in sealed tubes with 2 M CF_3CO_2H for 2 h at 121°C, and the hydrolysates were evaporated in vacuum and analyzed using a Biotronik LC-2000 analyzer equipped with a column (15 × 3.7 cm) of Chromex DA-X8-11 in 0.5 M sodium borate buffer (pH 8.0) at 65°C for neutral sugars and 0.04 M KH_2PO_4 buffer (pH 2.4) at 70°C for uronic acids; detection was effected with 2,2′-bicinchoninate using a sugar analyzer.

For determination of absolute configurations, the hydrolysate (from 8 mg of PS_{NH_4OH}) was fractionated by anion-exchange chromatography on Durrum DAx4; rhamnose, mannose, and galactose were converted into acetylated (+)-2-octyl glycosides [11] and analyzed by GLC. The absolute configurations of glucose and glucuronic acid (after carboxyl reduction) were determined using D-glucose oxidase. For carboxyl reduction [10], PS_{NH_4OH} (1 mg) was methanolyzed with 1 M HCl in MeOH at 85°C for 4 h, reduced with $LiBH_4$ in aq 50% 2-propanol (20°C, 16 h), and hydrolyzed as described above.

Pyruvic acid was determined colorimetrically with 4-dimethylaminobenzaldehyde [12]; before measurement, the pyruvic acid derivative was purified [13] on a column of neutral Al_2O_3.

Fatty acids were determined by GLC after methanolysis (0.5 M HCl in MeOH, 16 h, 80°C).

Methylation was performed by the published method [14], the methylated products were hydrolyzed with 2 M CF_3CO_2H for 1 h at 121°C, and sugars were conventionally converted into alditol acetates and analyzed by GLC–MS. Reduction of the methylated products with $LiBH_4$ was performed as described [24].

Solvolysis with anhydrous HF.—PS_{NH_4OH} (100 mg) was treated with anhyd HF (~10 mL, 10 min, −78°C), the cold solution was poured into cold 1:1 ether–hexane, the precipitate was collected on a steel filter, washed with cold ether, and dissolved in water, the insoluble portion was removed by centrifugation, and the supernatant solution was separated on gel TSK HW-40. The pentasaccharide obtained was conventionally reduced with $NaBH_4$ to give oligosaccharide **1** (12 mg). Similarly, oligosaccharide **2** (8.5 mg) was obtained from PS (150 mg).

Smith degradation.—PS_{NH_4OH} (75 mg) was oxidized with 0.05 M $NaIO_4$ with stirring in the dark (75 mL, 72 h, room temperature). After addition of ethylene glycol (0.2 mL), the solution was dialyzed and a precipitate was removed by centrifugation. The supernatant solution was reduced with 1 M $NaBH_4$ in 0.1 M NaOH (16 h), neutralized with concd AcOH, desalted by gel filtration on Sephadex G-15, and freeze-dried, and the product was hydrolyzed with aq 2% AcOH (2 h, 100°C) to give oligosaccharide **3** (10 mg) isolated by gel chromatography on TSK HW-40.

PS (200 mg) was degraded under similar conditions and the resulting oligosaccharides **4** (4.5 mg) and **5** (5 mg) were isolated by HPLC on reversed-phase C18.

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