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Note

Structure of the O-antigen polysaccharide from *Escherichia coli* O18ac: a revision using computer-assisted structural analysis with the program CASPER

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We have recently shown that structural investigations of regular polysaccharides can be performed rapidly by computer-assisted analysis using the computer program CASPER[1,2]. This uses data from sugar and methylation analysis together with the unassigned ^{1}H- or ^{13}C-n.m.r. spectrum or the two-dimensional C,H-correlation n.m.r. spectrum. The program generates all possible structures and simulates their n.m.r. spectra. The latter are ranked according to their fit to the experimental spectrum and, for some of these spectra, a high degree of similarity is observed. For several linear and branched polysaccharides of known structure, with repeating units of three to five sugar residues, an exclusive structure can be selected[1,2].

Among the polysaccharides of known structure used for testing CASPER was the O-antigen polysaccharide from *Escherichia coli* O18ac, the structure of which was investigated a few years ago[3]. A pentasaccharide repeating-unit was proposed from composition, fragmentation procedures, methylation analysis, and n.m.r. spectroscopy.

In the computer-assisted analysis, information on components and linkages together with the ^{13}C-n.m.r. data were used as input to CASPER. That the material was identical to that used earlier was evident from a comparison with the published ^{13}C-n.m.r. spectrum as well as by comparison of methylation analysis data. The result of the analysis with CASPER is shown in Scheme 1. Structures with spectra in which the C-1–H-1 and H-1–H-2 coupling constants were not compatible with the observed values[2] were excluded from the analysis. For the suggested structure 1, a spectrum with a considerably lower deviation from the experimental spectrum than that for structure 2 was obtained, the difference being so large that it and the following structures could be considered as being incompatible with the experimental spectrum. The deviation termed deltasum is the difference between the experimental spectrum and the simulated spectrum obtained by a peak-by-peak comparison. The structure proposed earlier appears as suggested structure 11, with a spectrum

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ECO18

No. Polysaccharide.

1 —4ADGAL —3ADGLCN—2ALRHA —6ADGLC —
3
BDGLCN

2 —4ADGAL —2ALRHA —6ADGLC —3ADGLCN—
3
BDGLCN

3 —3ADGAL —6ADGLC —3ADGLCN—2ALRHA —
4
BDGLCN

11 —4ADGAL —6ADGLC —3ADGLCN—2ALRHA —
3
BDGLCN

No.	13C Deltasum	13C Sum /sig	13C Check#	C-Branch Check#
1	9.8	0.29	0.41	0.01
2	13.0	0.38	0.50	0.01
3	13.3	0.39	0.50	0.01
11	15.0	0.44	0.50	0.01

J12 = 131 and JCH = 41 used to eliminate structures

13C Experimental spectrum.

175.2	174.7	104.2	99.9	99.7	98.8	96.8	79.3	77.7	76.6
76.6	76.6	75.1	73.7	73.1	72.8	72.8	72.6	71.4	71.4
71.1	70.4	70.0	69.7	68.9	67.4	62.0	61.2	60.6	56.7
52.8	23.1	23.1	17.9						

Spectrum number 1.

175.7	175.1	103.9	100.2	100.2	98.8	97.6	79.3	78.2	77.6
77.5	76.7	74.8	73.6	73.0	73.0	72.8	72.4	71.7	71.4
71.1	70.7	70.3	69.8	69.2	67.2	61.8	61.4	60.7	56.9
53.5	23.1	22.9	17.0						

Spectrum number 2.

175.7	175.1	103.9	100.1	98.9	98.9	98.8	79.0	78.2	77.6
77.6	76.7	74.8	73.8	73.0	72.7	72.6	72.3	72.1	71.1
71.0	70.7	70.5	69.8	68.8	67.4	61.8	61.1	60.8	56.9
54.0	23.1	22.9	17.0						

Spectrum number 11.

175.7	175.1	103.7	100.0	99.0	99.0	97.6	80.1	79.3	77.9
76.8	76.7	74.6	74.1	73.0	72.9	72.6	71.9	71.4	71.3
70.8	70.8	70.5	70.3	69.4	66.7	62.0	61.7	61.4	56.8
53.5	23.1	22.9	17.1						

Spectrum number 1.

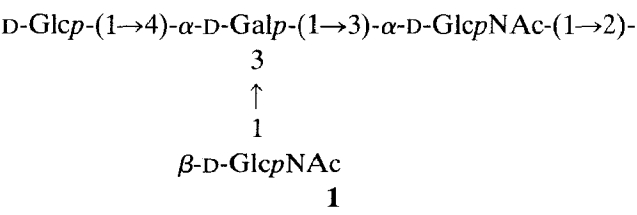
103.9	56.9	74.8	71.1	76.7	61.8	23.1	175.7
100.2	69.2	78.2	77.5	72.4	60.7		
97.6	53.5	79.3	71.4	73.0	61.4	22.9	175.1
98.8	77.6	70.7	73.0	69.8	17.0		
100.2	72.8	73.6	70.3	71.7	67.2		

Scheme 1. CASPER run on *E. coli* O18ac.

deltasum of 15.4 p.p.m., compared to 9.8 p.p.m. for that of structure 1. The low check numbers[1,2] indicate that the quality of the simulated spectra is good. The difference between structures 1 and 11 is in the order of sugar residues in the main chain. Thus, the branch point α-D-galactopyranosyl residue and the 1,6-linked α-D-glucopyranosyl residue are transposed.

Experimental evidence indicating that structure 1 is correct was obtained from a NOESY-spectrum (Table I) in which connectivities between neighbouring residues were observed as cross-peaks between the signals from anomeric protons and the protons on the linkage carbons. The signal from the anomeric proton of the 1,6-linked α-D-glucopyranosyl residues has cross-peaks to the H-3 and H-4 signals of the branched α-D-galactopyranosyl residue. For structure 11, cross-peaks between the latter signals and the signal from the anomeric proton of the 1,2-linked α-L-rhamnopyranosyl residue would have been observed instead.

From the above results, it can be concluded that the repeating unit of the *E. coli* O18ac O-polysaccharide has structure **1**.



1

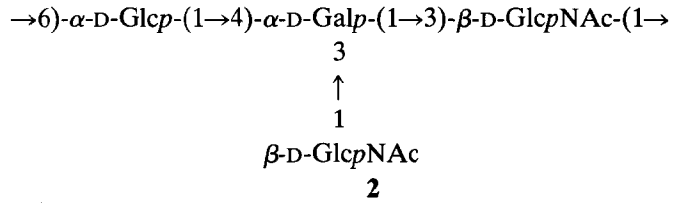
The O-antigen polysaccharide from *Serratia marcescens* O8, which is composed of tetrasaccharide repeating-units with the structure **2**, cross-reacts strongly[4] with the O-polysaccharide from *E. coli* O18ac. Most of the structure of the repeating unit from *Serratia marcescens* O8 is present in the revised structure of the repeating unit of *E. coli* O18ac, thus explaining the strong cross-reactivity.

TABLE I

CHEMICAL SHIFTS OF SIGNALS FOR H-1 TO H-3 AND OBSERVED INTERGLYCOSIDIC N.O.E CONTACTS IN THE NATIVE O-POLYSACCHARIDE FROM *E. coli* O18ac

Sugar residue	*H-1*	*H-2*	*H-3*[a]	*Interglycosidic n.O.e. contacts between H-1 and*
→4)-α-D-Galp-(1→ 3 ↑	5.47	3.95	3.81	4.01 (H-3, α-D-GlcpNAc)
→3)-α-D-GlcpNAc-(1→	5.02	4.09	4.01	4.02 (H-2, α-L-Rhap)
→6)-α-D-Glcp-(1→	4.93	3.49	3.70	3.81 (H-3, α-D-Galp) 4.27 (H-4, α-D-Galp)
→2)-α-L-Rhap-(1→	4.85	4.02	3.92	3.91 (H-6, α-D-Glcp)
β-D-GlcpNAc-(1→	4.64	3.60	3.58	3.81 (H-3, α-D-Galp)

[a]Chemical shifts were obtained from cross-peaks in the 2D relayed COSY-spectra.



2

EXPERIMENTAL

N.m.r. spectroscopy. — N.m.r. spectra of solutions in deuterium oxide were recorded with a JEOL GX-270 instrument, using acetone δ_C 31.00 (70°) or sodium 3-trimethylsilylpropanoate-d_4 δ_H 0.00 (60°) as internal reference. For the NOESY experiment, a mixing time of 350 ms was used. CASPER version 2.0[2] was used for the computer analysis.

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