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Note

Structural studies of the *Eubacterium saburreum* strain O 2 antigen

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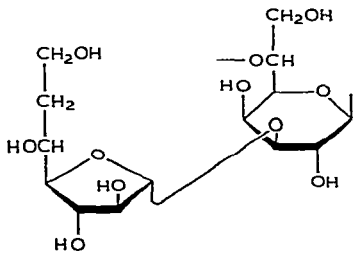
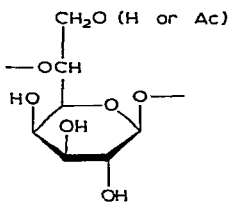
The recent publication by Kondo *et al.*[1] on the structure of the *Eubacterium saburreum* strain O 2 antigen prompts us to report on our studies of the same antigen.

The antigens from *E. saburreum* strains L 44[2] and L 49[3] both contain a chain of β-D-*glycero*-D-*galacto*-heptopyranosyl residues. In the latter, every second heptosyl residue is substituted with a 6-deoxy-α-D-*altro*-heptofuranosyl group. The identification of the 6-deoxyheptose as the D-*altro* isomer, which was tentative in the original publication[3], has been confirmed by an unambigous synthesis[4].

The antigen from *E. saburreum* strain O 2 was found to contain the same two sugars in equimolecular proportions. Structural studies demonstrated that the polysaccharide antigen has a comb-like structure and is composed of the disaccharide repeating-unit **1**, in complete agreement with the results of Kondo *et al.*[1]. The methods used were also the same as those used by these authors and the details are therefore not reported here.



1



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In agreement with this structure, the ^{1}H-n.m.r. spectrum of the antigen showed, *inter alia*, signals for anomeric protons at δ 5.20 ($J_{1,2}$ low, 1 H) and 4.62 ($J_{1,2}$ 7 Hz, 1 H), and for the H-6 protons of the 6-deoxyheptose at δ 1.8 (m, 2 H). In addition,

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signals at δ 2.17 (s, 1.4 H) were observed, indicating a non-stoichiometric amount of *O*-acetyl groups (~0.4 per repeating unit).

In the ^{13}C-n.m.r. spectrum, signals at 22.3 and 175.8 p.p.m. confirmed the presence of *O*-acetyl groups. The other signals could be divided into two groups, one of which contained 7 sharp signals. Terminal groups have a higher mobility than chain residues, and the signals given by the carbon atoms of the former are often stronger than those given by the carbon atoms of the latter. Comparison with the chemical shifts of the configurationally related methyl β-D-galactofuranoside[5] (Table I) also indicated that these signals were given by the 6-deoxy-α-D-*altro*-heptofuranosyl groups, and tentative assignments of the signals could be made.

The other group of 7 signals (Table II) were broader, as expected for signals given by chain residues. Some were especially broad or appeared as pairs, which is most probably due to the effect of the *O*-acetyl groups. The ^{13}C-n.m.r. spectrum of methyl β-D-*glycero*-D-*galacto*-heptopyranoside was also determined, and assignment of the signals could be made by comparison with the spectrum of the configurationally related methyl β-D-galactopyranoside[5]. Because of the effects of the substituents at O-3 and O-6, the assignments of some of the signals given by the β-D-*glycero*-D-*galacto*-heptopyranosyl residues in the spectrum of the antigen should be considered

TABLE I

^{13}C-N.M.R. SHIFTS FOR TERMINAL GROUPS AND METHYL β-D-GALACTOFURANOSIDE

Substance[a]	*Chemical shifts (p.p.m.)*						
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-7*
A	109.2	81.9	77.8	84.0	72.0	63.9	—
B	106.5	83.3	78.6	88.7	70.1	36.5	60.5

[a] *A*, Methyl β-D-galactofuranoside; *B*, the 6-deoxy-α-D-*altro*-heptofuranosyl groups of the *E.s.* strain O 2 antigen.

TABLE II

^{13}C-N.M.R. SHIFTS FOR CHAIN RESIDUES AND RELATED COMPOUNDS

Substance[a]	*Chemical shifts (p.p.m.)*						
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-7*
A	104.9	71.8	73.9	69.8	76.8	62.1	—
B	104.9	71.9	74.2	69.9	75.6	68.8	64.0
C	103.8, 103.3[b]	71.4	79.2	66.2	74.7	78[c]	65.3, 62.4[b]

[a] *A*, Methyl β-D-galactopyranoside; *B*, methyl β-D-*glycero*-D-*galacto*-heptopyranoside; and *C*, the β-D-*glycero*-D-*galacto*-heptopyranosyl residues in the *E.s.* strain O 2 antigen. [b] These two signals were of comparable intensities and about half as strong as the others. [c] Broad signal, overlapping with the C-3 signals of the heptosyl and 6-deoxyheptosyl residues.

as uncertain. However, the signals at 65.3 and 62.4 p.p.m. could be assigned to C-7, with or without acetyl on O-7, respectively. In the *E. saburreum* strain L 44 antigen, having the structure **2**, some 65% of the 7-positions are *O*-acetylated, and the signals for C-7 appear at 65.1 and 62.3 p.p.m. with the relative intensities 2:1. The signal for C-1 also appeared as a pair, at 103.8 and 103.3 p.p.m. for both the L 44 and the O 2 antigens.

Our studies of the *E. saburreum* strain O 2 antigen therefore confirm that it is composed of the disaccharide repeating-unit **1**, as demonstrated by Kondo and Sato[1], and further demonstrate that some 40% of the β-D-*glycero*-D-*galacto*-heptosyl residues carry an *O*-acetyl group at O-7. Such a structure agrees with the demonstration of serological identity between the O 2 antigen and the de-acylated polysaccharide antigen[6] of *E. saburreum* strain L 49.

The observations by Kondo and Sato[1] that the 6-deoxy-α-D-*altro*-heptofuranosyl groups are immunodominant and that the serological properties of the antigen are unaffected by treatment with alkali are in agreement with the location of the *O*-acetyl groups on the heptopyranosyl residues.

EXPERIMENTAL

The antigen, which was isolated and purified as previously described for other *E. saburreum* antigens[7], had $[\alpha]_{578}$ +61° (*c* 0.1, water). N.m.r. spectra were recorded with a JEOL FX-100 instrument for solutions in D_2O, using external tetramethylsilane (^{13}C) and internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate (^1H) as references.

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