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Preliminary communication

Non-destructive determination of the monosaccharide composition and the structure of the O-specific polysaccharide of *Pseudomonas cepacia*

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In the preceding communication[1], we demonstrated the advantage of homonuclear ^1H and selective heteronuclear $^{13}C\{^1H\}$ double-resonance for structural analysis of carbohydrates. We now report on an application of this approach exclusively to determine the monosaccharide composition and structure of the O-specific polysaccharide from *P. cepacia*, strain IMV 4137.

The polysaccharide was obtained by acid hydrolysis (1% CH_3CO_2H, 100°, 1.5 h) of the lipopolysaccharide isolated from dry bacterial cells by the Westphal procedure[2]. Its ^1H-n.m.r. spectrum was well-resolved (Fig. 1) and contained signals for two anomeric protons at δ 5.15 (s, $J_{1,2}$ <0.5 Hz) and 5.03 (d, $J_{1,2}$ 3.5 Hz), one *C*-methyl group of a 6-deoxyhexose at δ 1.26. (d, 3H, $J_{5,6}$ 6 Hz), and ten protons in the region δ 3.4–4.3. The ^{13}C-n.m.r. spectrum of the polysaccharide (62.89 MHz, D_2O, 50°) contained signals for two anomeric carbon atoms (100.6 and 98.9 p.p.m.), one *C*-methyl group of a 6-deoxyhexose (17.8 p.p.m.), and one *C*-hydroxymethyl group of a hexose (62.0 p.p.m.), as well as for eight secondary carbon atoms bearing oxygen in the region 68–79 p.p.m. Therefore, it was proposed that the polysaccharide was made up of disaccharide repeating-units containing one hexosyl and one 6-deoxyhexosyl residue.

The absence of ^{13}C signals in the regions 103–110 and 82–86 p.p.m., which are characteristic of C-1 and C-4 of aldofuranosides, respectively[3], showed that both sugar residues are pyranoid. The relatively large $^1J_{CH}$ values of 171.1 and 169.2 Hz, determined from the gated-decoupling spectrum of the polysaccharide, indicated[4] both residues to be α.

All the signals in the ^1H-n.m.r. spectrum were assigned by homonuclear double-resonance, and the coupling constants were determined. From the data obtained, it followed that the hexose has the *galacto* configuration ($J_{2,3}$ 10.5, $J_{3,4}$ 2.5, $J_{4,5}$ <0.5, $J_{5,6}$ 6.5 Hz) and the 6-deoxyhexose has the *manno* configuration (rhamnose, $J_{2,3}$ 4, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5, $J_{5,6}$ 6 Hz). Further, the ^{13}C-n.m.r. spectrum was fully interpreted by means of selective heteronuclear $^{13}C\{^1H\}$ double-resonance (Table I). The relatively low-field signals at 78.5 and 77.2 p.p.m., belonging to non-anomeric carbon atoms involved in the glycosidic linkages, were unambiguously assigned to C-2 of rhamnose and C-4 of glactose (cf. refs. 5 and 6).

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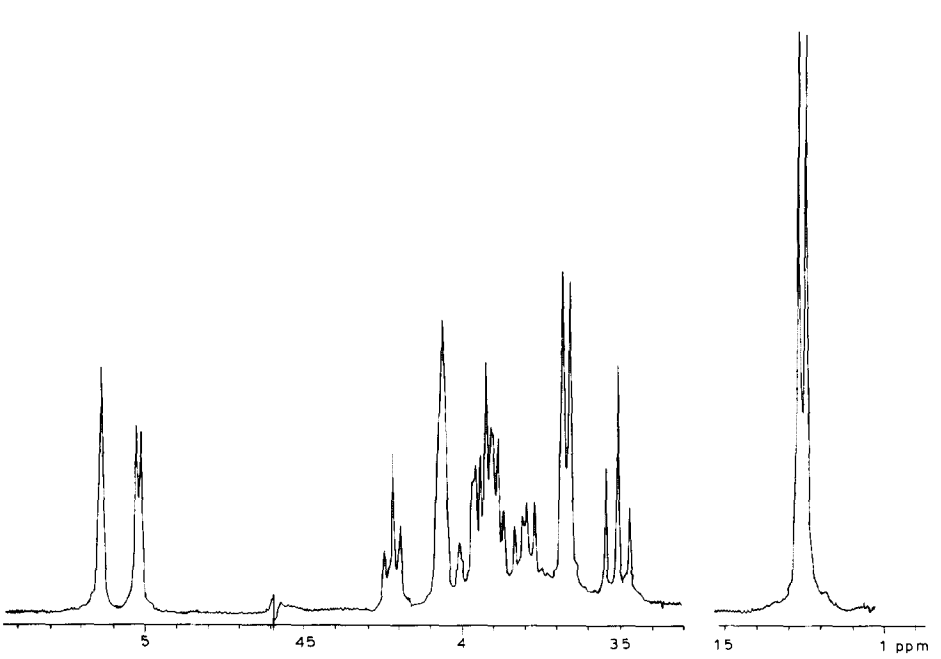

Fig. 1. The ^{1}H-n.m.r. spectrum of *P. cepacia* O-specific polysaccharide (250 MHz, D_2O, 40°).

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS (p.p.m.) FOR THE POLYSACCHARIDE

Unit	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Galactose	98.9	70.6	68.7	77.2	72.3	62.0
Rhamnose	100.6	78.5	70.9	73.3	70.3	17.8

TABLE II

OPTICAL ROTATION DATA [a]

Compound	$[\alpha]_D$ *(degrees)*	*Ref.*	$[M]_D$ *(degrees)*
Methyl α-D-galactopyranoside	+178.8	8	+347
Methyl α-L-rhamnopyranoside	−67.2	9	−120
Polysaccharide, observed	+48.3		+149
Polysaccharide [b]			
α-D-Galactopyranose and α-L-rhamnopyranose	+74		+227
α-D-Galactopyranose and α-D-rhamnopyranose	+152		+467
α-L-Galactopyranose and α-D-rhamnopyranose	−74		−227
α-L-Galactopyranose and α-L-rhamnopyranose	−152		−467

[a] For aqueous solutions. [b] Values calculated for a repeating unit containing the residues indicated.

The absolute configurations of the monosaccharides were determined by calculation of the molecular rotation of the polysaccharide, according to Klyne's rule[7]. The results in Table II revealed the similarity in the observed $[\alpha]_D$ value and that calculated for a repeating unit containing D-galactosyl and L-rhamnosyl residues.

Thus, the O-specific polysaccharide of *P. cepacia* has the structure →4)-α-D-Gal*p*-(1→2)-α-L-Rha*p*-(1→.

The results of acid hydrolysis and methylation analysis accorded with the structure assigned.

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