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Note

Application of n.m.r. spectroscopy, including n.O.e. studies, to the O-specific polysaccharide from *Pseudomonas cepacia* strain IMV 3181

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Using mainly chemical methods, the structure **1** was established[1] for the repeating unit of the O-specific polysaccharide from *P. cepacia*, strain IMV 3181. We now report confirmation of the presence of rarely occurring D-rhamnose as well as the unusual (1→2)-linked β-D-rhamnopyranosyl residue[2] by using n.O.e. and selective double-resonance n.m.r. techniques[3].

$$\underset{A}{\rightarrow 3)\text{-}\alpha\text{-D-Rha}p\text{-}(1} \rightarrow \underset{B}{3)\text{-}\alpha\text{-D-Rha}p\text{-}(1} \rightarrow \underset{C}{2)\text{-}\beta\text{-D-Rha}p\text{-}(1} \rightarrow$$

1

The 300-MHz ^{1}H-n.m.r. spectrum (Fig. 1) of the polysaccharide was fully interpreted by using homonuclear double-resonance, and the data are shown in Table I. The $^3J_{H,H}$ values indicated that *A*–*C* in **1** were 6-deoxymannopyranosyl residues. Irradiation of H-1 of unit *C* caused marked n.O.e.'s for the H-2,3,5 resonances of this unit (Table II), thus indicating the β configuration. In addition, considerable n.O.e.'s for the H-2,3 signals of unit *A* were observed, and thus the sequence *C*–*A* is present. Moreover, these results indicated that unit *A* was substituted at position 3 since n.O.e. for H-3 would not be observed in the case of substitution at position 2.

Irradiation of H-1 of unit *B* caused a considerable n.O.e. for the H-2 resonance of unit *C* which showed the *B*–*C* linkage to be (1→2). The large n.O.e. for H-3 and the relatively small n.O.e. for H-4 of unit *B* observed on irradiation of H-1 of unit *A* indicated that the *A*–*B* linkage was (1→3). The absence of a large n.O.e. for the H-3,5 resonances of unit *A* on irradiation of H-1 and likewise for unit *B* indicated that these units were α.

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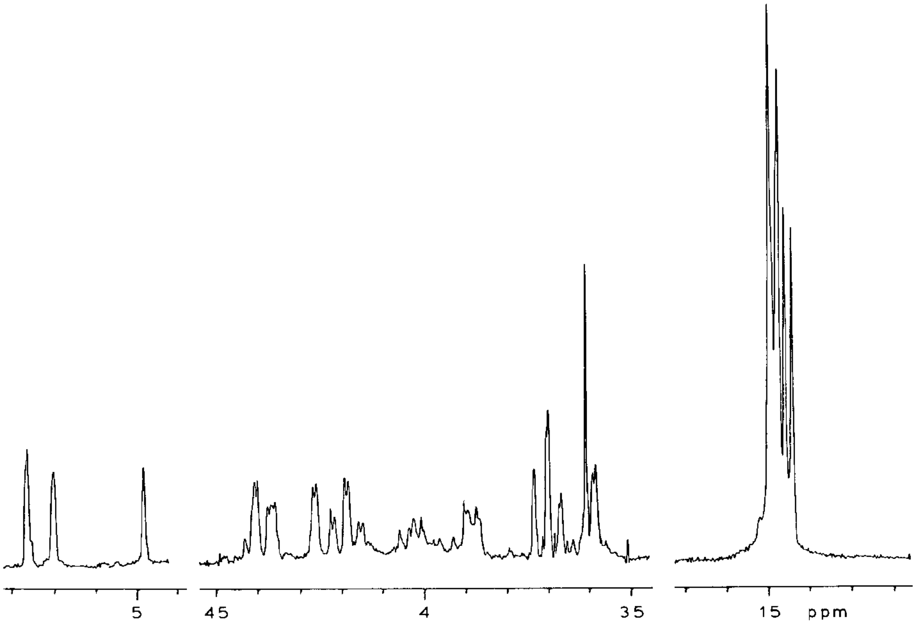

Fig. 1. ^{1}H-N.m.r. spectrum of *P. cepacia* O-specific polysaccharide.

TABLE I

^{1}H-N M.R PARAMETERS FOR THE *P. cepacia* POLYSACCHARIDE

Proton	*Chemical shifts (p.p.m.)*			*Coupling constants (Hz)*		
	Unit A	*Unit* B	*Unit* C	*Unit* A	*Unit* B	*Unit* C
H-1	5.27	5.20	4.98	$J_{1,2}$ 1.6	$J_{1,2}$ 1.6	$J_{1,2}$ 1
H-2	4.40	4.36	4.26	$J_{2,3}$ 2.5	$J_{2,3}$ 2.5	$J_{2,3}$ 2.4
H-3	4.20	4.16	3.88	$J_{3,4}$ 9.8	$J_{3,4}$ 9.8	$J_{3,4}$ 9.6
H-4	3.70	3.70	3.59	$J_{4,5}$ 9.8	n.d.[a]	n.d.
H-5	4.40	4.02	3.59	$J_{5,6}$ 6.1	$J_{5,6}$ 6.1	$J_{5,6}$ 6.1
H-6	1.44	1.48	1.48			

[a] Not determined.

An almost complete interpretation of the ^{13}C-n.m.r. spectrum of the polysaccharide, using selective heteronuclear $^{13}C\{^1H\}$ double-resonance (Table III), confirmed the structure assigned and analysis of the effects of glycosidation indicated that units *A*–*C* had the same absolute configurations. The relatively low-field resonances for C-2 of unit *C* and C-3 of units *A* and *B* (78.8, 78.5, and 79.0 p.p.m., respectively) accord with their positions of substitution (*e.g.*, *cf.* the data in ref. 4). The relatively large $^1J_{C,H}$ values (169.2 and 170.2 Hz, respectively) for the

TABLE II

N.O.e. DATA FOR THE *P. cepacia* POLYSACCHARIDE

Irradiated proton	*Observed proton and n.O.e. values*[a]						
	Unit A		*Unit* B		*Unit* C		
H-1 of unit *A*	H-2 0.12		H-3 0.16	H-4 0.06			
H-1 of unit *B*			H-2 0.21		H-2 0.27		
H-1 of unit *C*	H-2 0.16	H-3 0.09			H-2 0.08	H-3 0.09	H-5 0.18

[a]Values >0.05 are listed. Appearance of n.O.e.'s smaller than 0.05 was due to large correlation times and spin diffusion which are characteristic of polymers. They will be discussed elsewhere.

TABLE III

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

Unit	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
A	103.2	68.7	78.5	71 7	70.3	18.0[a]
B	102.6	71.3	79.0	72.7	70.2	17.9[a]
C	98.1	78.8	74.5	73.5[b]	73.8[b]	17.85[a]

[a,b]Assignments may be interchanged.

anomeric carbons of units *A* and *B*, determined from the gated-decoupling spectrum, showed these units to be α, whereas the small value (159.1 Hz) for C-1 of unit *C* proved it to be β (ref. 5).

The relatively small α-effect (+3.5 p.p.m.) for C-1 of unit *C* and the relatively large (−3.25 p.p.m.) β-effect for C-2 of unit *A*, determined by comparison with published data[6], indicated that these (1→3)-β-linked units had identical absolute configurations[7]. Further, the large α-effect (+8.2 p.p.m.) for C-1 of unit *A* and the small β-effect (−0.65 p.p.m.) for C-2 of unit *B* proved that these (1→3)-α-linked units also had identical configurations[7]. In accordance with Klyne's rule[8], the $[\alpha]_D$ value of +22° (water)[1] of the polysaccharide showed that rhamnose was D.

Thus, the structure assigned[1] to the *P. cepacia* IMV 3181 polysaccharide has been confirmed and it was shown that n.O.e.'s arising from irradiation of anomeric protons are helpful for determining the sites and configurations of glycosidic linkages as well as sequences of monosaccharides in polysaccharides. It is noteworthy that all n.O.e.'s observed for the polysaccharide of *P. cepacia*, as well as those[9] for the polysaccharide of *Shigella flexneri* O-serogroup Y, had negative values.

EXPERIMENTAL

N.m.r. spectra were recorded with an AM-300 (Bruker) instrument for solutions in D_2O at 50° [internal acetone (δ_H 2.23) and methanol (δ_C 50.15)]. N.O.e. data were obtained by the TOE method[10] and performed in the difference mode; the time constants used were D1 = 4 s, relaxation delay; D2 = 0.5 s, build-up n.O.e.

The O-specific polysaccharide of *P. cepacia* IMV 3181 was isolated as described earlier[1].

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