Carbohydrate Research, 93 (1981) 269–278
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

STRUCTURAL STUDIES OF AN ACETYLATED MANNAN FROM *Pseudomonas diminuta* N.C.T.C. 8545

STEPHEN G. WILKINSON
Department of Chemistry, The University, Hull HU6 7RX (Great Britain)
(Received January 7th, 1981; accepted for publication, January 27th, 1981)

ABSTRACT

An acetylated mannan was isolated from the lipopolysaccharide of *Pseudomonas diminuta* N.C.T.C. 8545 after mild hydrolysis with acid and chromatography of the water-soluble products on Sephadex G-50. Although the presence of a terminal core-oligosaccharide in the mannan could not be demonstrated, chromatographic and electrophoretic studies indicated that the mannan was a real component of the lipopolysaccharide. Degradative and n.m.r. (^{1}H and ^{13}C) studies showed that the mannan had a disaccharide repeating-unit with the following structure, in which about two thirds of the 4-substituted mannopyranosyl residues were acetylated.

→4)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→
6
⋮
OAc

INTRODUCTION

In the last two decades, dissatisfaction with the classification of the aerobic pseudomonads (as well as concern over the etiological importance of some of these organisms) has led to major advances in their phenotypic and genotypic characterisation, and to the recognition of various taxonomic subgroups and relationships. One of the most isolated subgroups is that formed by the closely related species *Pseudomonas diminuta* and *Pseudomonas vesicularis*. Indeed, the view that the distinctive properties[1–5] of these species warrant their removal from the genus *Pseudomonas* is finding increasing favour[6]. This view is supported by the results of studies of the composition of the cell envelopes from the type strains of both species[7–12]. The polar lipids of both strains are distinguished by the absence of phosphatidylethanolamine and by the presence of a unique range of glycolipids, while the lipid A moiety of both lipopolysaccharides is based on 2,3-diamino-2,3-dideoxy-D-glucose rather than 2-amino-2-deoxy-D-glucose. All of these features differentiate *P. diminuta* and *P. vesicularis* from other pseudomonads so far examined.

Other unusual features of the lipopolysaccharide from *P. diminuta* N.C.T.C.

8545 are[10] the absence of phosphate and hexosamine from the core oligosaccharide (components identified were D-glucose, an aldoheptose, and a 3-deoxy-2-octulosonic acid), and the presence of a single sugar (D-mannose) in the presumed O-specific polysaccharide. Structural studies of the latter material are now reported.

RESULTS AND DISCUSSION

During the course of this work, walls were prepared from five batches of *P. diminuta* grown in liquid culture. Analyses for a typical preparation were: P, 0.74; total carbohydrate, 7.6; protein, 49; extractable lipid, 26.7; and lipopolysaccharide, 8.5%. Each batch of lipopolysaccharide gave analyses (P, 0.72; total carbohydrate, 36%) closely resembling those reported previously[10]. Mild hydrolysis of the lipopolysaccharide with acid, followed by chromatography of the water-soluble products on Sephadex G-50, gave the polymeric fraction previously[10] coded DI.

Mannose was the only neutral-sugar component of fraction DI detected by paper chromatography, and essentially the same result was obtained by g.l.c. of the alditol acetates (no heptose derivative was detected, and a very small peak for glucitol hexa-acetate was found only at very high loading of the column). Colorimetric assays for heptose, 3-deoxy-2-octulosonic acid, and phosphorus, and an enzymic assay for D-glucose also gave negative results. Three different assays for the mannose content of fraction DI (expressed as "anhydrohexose") gave results in good agreement: phenol–sulphuric acid method, 91; g.l.c. of the alditol acetate, 92; and enzymic assay, 90% (as the D isomer). Thus, fraction DI is essentially a mannan. However, the i.r. spectrum contained a peak at 1740 cm^{-1}, indicative of an *O*-acyl substituent. This was shown to be an *O*-acetyl group by g.l.c. of a hydrolysate[13], by ^1H-n.m.r. spectroscopy (sharp singlet at δ 2.12 relative to external DSS), and by ^{13}C-n.m.r. spectroscopy (sharp signals at δ 174.4 and 20.7 relative to external Me_4Si). The relevant n.m.r. signals were absent from the spectra of alkali-treated DI. The *O*-acetyl content of DI was determined as 1.87 μmol.mg^{-1} by the ferric hydroxamate method[14]

TABLE I

METHYLATION ANALYSIS OF FRACTION DI

Methylated sugar[a]	T[b]			*Detector response*	*Primary m.s. fragments (m/z)*
	Column a	*Column* b	*Column* c		
2,3,4,6-Man	1.00	1.00	1.00	1.0	117[c], 161[d], 205
3,4,6-Man	1.93	1.62	2.07	15.7	161, 189[c]
2,3,6-Man	2.17	1.76	2.39	16.0	117[c], 233

[a]2,3,4,6-Man = 2,3,4,6-tetra-*O*-methyl-D-mannose, *etc.* [b]Retention time for the corresponding alditol acetate, relative to that for 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol. [c]The *m/z* value was increased by one when $NaBD_4$ was used for reduction of the methylated sugar. [d]About half of the fragments had *m/z* 162 when $NaBD_4$ was used for reduction of the methylated sugar.

and as 2.06 μmol.mg^{-1} by a g.l.c. method[13]. A molar ratio of D-mannose to *O*-acetyl of 3:1 was confirmed by ^{1}H-n.m.r. spectroscopy (equal peak areas for anomeric protons and methyl protons) and by interpretation of the ^{13}C-n.m.r. spectrum (see below).

On periodate oxidation of both native and deacetylated DI, all of the mannose was destroyed. The consumption of periodate per "anhydrohexose" residue was 1.01 mol for native DI and 1.04 mol for deacetylated DI. These results suggested that DI was a linear mannan containing 2- and/or 4-substituted pyranosyl residues, with acetyl substitution at O-6. This inference was confirmed and amplified by the results of methylation analysis (Table I). The results point to a disaccharide repeating-unit with alternate linkages through O-2 and O-4, and to a chain length of ~32 mannosyl residues for the polymer.

Further evidence for a regular polymer having a disaccharide repeating-unit was provided by the ^{1}H- and ^{13}C-n.m.r. spectra. The ^{1}H-n.m.r. spectrum of deacetylated DI contained two signals of equal intensity for anomeric protons (exact chemical shifts were not determined, but at 78° the signals were 0.82 and 1.20 p.p.m. downfield from the HOD signal). The corresponding spectrum for native DI contained one sharp signal at δ 5.37 and partially resolved signals at δ 5.01 and 4.95. The

TABLE II

ASSIGNMENT OF SIGNALS IN THE ^{13}C-N.M.R. SPECTRA OF NATIVE AND DEACETYLATED FRACTION DI[a]

Carbon atom	*Native DI*[b]		*Deacetylated DI*	
	OAc ⋮ *6* →4-α-*Man*-1→	→2-α-*Man*-1→	→4-α-*Man*-1→	→2-α-*Man*-1→
C-1	102.06	100.33	102.06	100.05
	101.92	*100.11*		
		99.94		
C-2	*71.05*	*79.06*	70.98[c]	78.69
	70.91	78.77		
C-3	70.17	70.49	70.18[c]	70.61[c]
C-4	74.84	66.73	74.61	66.90
	74.54			
C-5	71.87	*73.87*	71.86	73.83
	69.34	73.38		
C-6	*64.06*	61.04	61.26[d]	61.03[d]
	61.30	*60.81*		
-OC(O)CH_3	174.43			
-OC(O)CH_3	20.69			

[a]Chemical shifts are given in p.p.m. downfield from external Me_4Si. [b]Italicised numbers indicate the major signals. [c]Assignments of these signals, and of the corresponding signals for native DI, may be interchanged. [d]Assignments of these signals, and of the corresponding signals for native DI, may be interchanged.

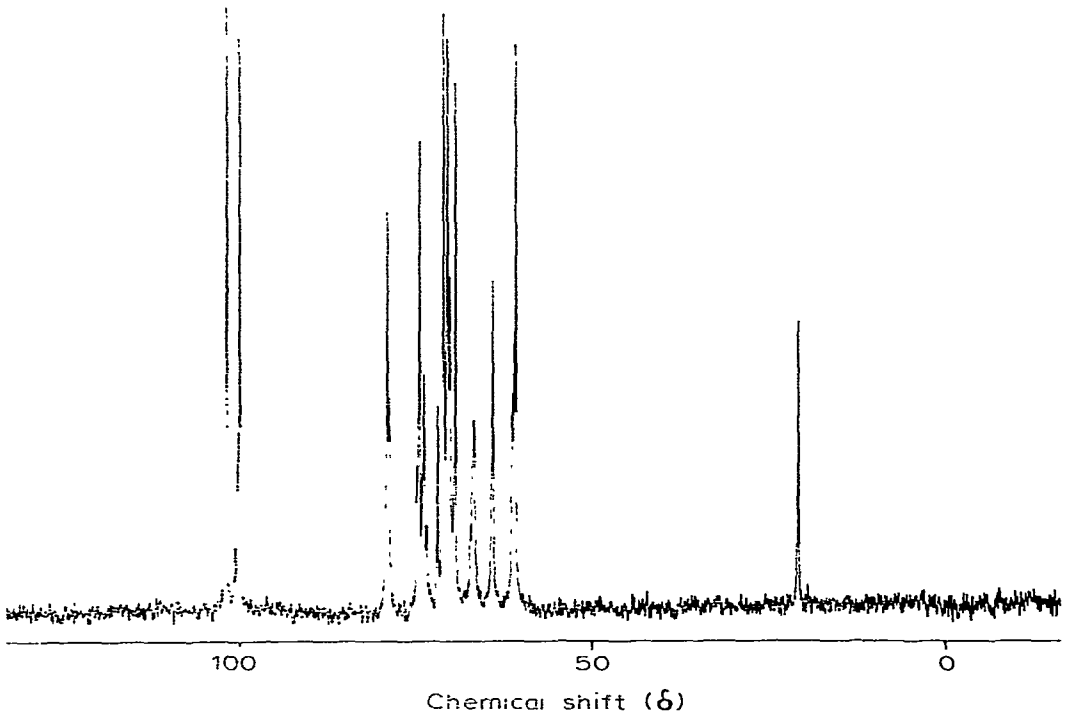

Fig. 1. ^{13}C-N.m.r. spectrum of native fraction D1. The spectrum for the sample in D_2O was obtained at 100.62 MHz and 40° with complete proton-decoupling. In addition to the signals shown, the spectrum contained a signal for carbonyl carbon at δ 174.43 with reference to external Me_4Si.

latter pair (relative areas, ~1:2; total area equal to that for the signal at δ 5.37) presumably arise as a result of incomplete *O*-acetylation of one of the mannosyl residues of the repeating unit. The ^{13}C-n.m.r. spectrum of deacetylated DI (Table II) contained twelve discrete signals, including two for anomeric carbon atoms at δ 102.06 and 100.05. As expected, the corresponding spectrum for native DI (Fig. 1 and Table II) was rather more complex, with fine structure for both anomeric signals. The downfield signal was recorded as two peaks at δ 102.06 and 101.92 (relative areas, ~1:2), whereas the upfield signal gave a major peak at δ 100.11 (about two-thirds of the total peak area) with minor peaks at 100.33 and 99.94. It seems likely that the peak at δ 101.92 corresponds to C-1 of a 6-*O*-acetylated mannopyranosyl residue, and the peak at δ 100.11 to C-1 of the second mannopyranosyl residue flanked on both sides by the acetylated residue, as this sequence should preponderate in the polymer.

Native fraction DI had $[\alpha]_D$ +107° (*c* 1.2, water), suggesting that all residues were α-D-linked. After oxidation of the peracetylated polymer for 2 h at 50° with chromium trioxide, 43% of the mannose residues were apparently destroyed (calculated on the assumption that *myo*-inositol hexa-acetate, the internal standard, was unaffected). Although this result could be taken to indicate the presence of a β

linkage[15], the partial oxidation of acetylated α-mannopyranosyl residues under such conditions is not unknown (*e.g.*, ref. 16). ^{1}H-N.m.r. spectra have been used extensively for the characterisation and classification of mannans from yeasts[17,18] and other micro-organisms. The anomeric signal at δ 5.37 for DI can be assigned to a 2-substituted α-mannopyranosyl residue, as such residues typically give signals at δ ~5.3 (*e.g.*, refs. 19–21). The configuration of the mannopyranosyl residue responsible for the anomeric signal at δ ~5.0 is less obvious. However, the anomeric signal for a 4-substituted mannopyranosyl residue is expected to be at higher field than that for a 2-substituted residue, and for a β linkage the signal should be at higher field than δ 5.0 (ref. 17).

Further evidence about the configurations of the glycosidic bonds was obtained from the ^{13}C-n.m.r. spectra. Although shift data for the anomeric carbon atoms of mannopyranosides are unreliable for the assignment of configuration, the value of the one-bond, anomeric, ^{13}C–^{1}H coupling-constant can be used for this purpose[22,23]. The $^1J_{CH}$ value was 172.6 Hz for both anomeric signals in the proton-coupled spectrum for deacetylated DI, and similar values were obtained for native DI. Thus, both mannopyranosyl residues in the repeating unit have the α configuration. As a final check, polymeric and oligomeric sub-fractions obtained by partial hydrolysis of deacetylated DI with acid were treated with α-D-mannosidase. Mannose was released from both sub-fractions, and the hydrolysis of the oligomeric material at least seemed to be complete.

→4)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→
1

Confirmation of structure **1** for the disaccharide repeating-unit and evidence for the location of the *O*-acetyl substituent were provided by the ^{13}C-n.m.r. spectra. The assignments of signals in the spectrum for deacetylated DI (Table II) were made with the aid of literature data[20,23,24–27] for α-linked mannopyranosides and mannans, and *O*-methyl derivatives thereof. Apart from having a complex fine-structure, the spectrum for native DI contained two distinct, additional, major signals at δ 69.34 and 64.06. In the proton-coupled spectrum, the latter signal was replaced by a triplet, which confirmed its attribution to a hydroxymethyl carbon. The downfield shift of ~3 p.p.m. is the expected consequence of *O*-acetylation. An upfield shift of similar magnitude would also be expected for the adjacent C-5 signal, and a comparison of the two proton-decoupled spectra shows that the disappearance of the signal at δ 69.34 on deacetylation is matched by a major increase in the signal at δ 71.86 (this signal was at δ 71.87 for native DI). As the signal at δ 71.86 can be assigned to C-5 of the 4-substituted mannopyranosyl residue, it follows that about two-thirds of these residues in native DI carry a 6-*O*-acetyl substituent. This conclusion is supported by the inference (*vide supra*) that the anomeric signal at δ ~102, which should be derived from the 4-substituted residue[25,26], corresponds to C-1 of the *O*-acetylated sugar.

Because there was little or no evidence for the presence of a core oligosaccharide

in fraction D1, its identification as an O-specific side-chain of the lipopolysaccharide was dubious. Attempts were therefore made to determine whether D1 was an actual component or a contaminant of the lipopolysaccharide. Initially, the lipopolysaccharide and the water-soluble products of mild hydrolysis with acid [fractions D1 and D2 (the partially degraded, core oligosaccharide)] were examined by chromatography on Sephadex G-100 in the presence of sodium dodecyl sulphate. Fraction D1 was eluted as a rather broad, tailing peak (tubes 21–30, maximum in tubes 23 and 24), and D2 as a sharper, symmetrical peak (tubes 62–68, maximum in tube 65). In contrast, the parent lipopolysaccharide gave two sharp peaks for carbohydrate. The major peak (tubes 21–25) had a maximum (tube 22) which corresponded to the void volume of the column. The material in the much smaller carbohydrate peak (tubes 27–31, maximum in tube 29) contained most or all of the phosphorus applied to the column. Considered in isolation, the results for the lipopolysaccharide could be interpreted either as evidence for a mixture of S- and R-form molecules with the latter preponderating (indicated by the phosphorus distribution), or as evidence for an R-form lipopolysaccharide contaminated by a polysaccharide of higher molecular weight. In turn, D1 (which is clearly not present as such in the lipopolysaccharide) could be regarded either as a degradation product of an S-form lipopolysaccharide or as a result of partial depolymerisation of a polysaccharide contaminant.

In an effort to differentiate between these two possibilities, the water-soluble products from hydrazinolysis of the lipopolysaccharide were also examined by gel-permeation chromatography (without sodium dodecyl sulphate). The major carbohydrate fraction, which was excluded by Sephadex G-50, still contained phosphorus and NH groups, both of which appear to be markers for lipid A[10,11]. After mild hydrolysis of this material with acid, followed by re-chromatography on Sephadex G-50, the product of high molecular weight lacked phosphorus, and was apparently equivalent to fraction D1.

As a final attempt to determine the origin of fraction D1, samples were examined by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulphate. On discontinuous gel-electrophoresis, D1 remained at the top of the stacking gel. No band in this position was found for the parent lipopolysaccharide, but a major band of periodate–Schiff-reactive material was present in the upper part of the separating gel, as expected for an S-form lipopolysaccharide. Thus, it appears that, in the lipopolysaccharide, D1 is attached to a hydrophobic residue capable of binding sodium dodecyl sulphate. Although lipid A is the obvious candidate for such a residue, the use of isolated "cell walls" for the extraction of the lipopolysaccharide does not completely eliminate the possibility of a membrane-associated, hydrophobic carrier-molecule[28]. If D1 is indeed of membrane origin, this would explain the absence of a core oligosaccharide. Whatever its precise significance, the size heterogeneity of D1 indicated by its elution profile on Sephadex G-100 would be comparable with that found for the O-specific fractions of other bacterial lipopolysaccharides[29–31].

→4)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→
6
⋮
OAc

2

Although mannans are relatively common in micro-organisms, structure **2**, proposed for the repeating unit of D1, appears to be unique. Linear α-linked mannans with different repeating-units occur as the O-specific side-chains in lipopolysaccharides from *Klebsiella* O groups 3 (ref. 32) and 5 (ref. 33), *Escherichia coli* O groups 8 (ref. 34) and 9 (ref. 35), and a *Synechococcus* strain[36]. Uncharacterised mannans are also present in lipopolysaccharides from *Acetobacter xylinum*[37] and *Pseudomonas cepacia* (S. G. Wilkinson, unpublished results), while mannose-rich repeating-units are found in the O-specific side-chains of lipopolysaccharides from *Salmonella* group C_1 (ref. 38), a *Citrobacter* strain[39], and *Thiocapsa roseopersicina*[40]. α-Linked mannans are also present in the architecturally diverse "lipopolysaccharides" from *Micrococcus luteus*[41], *Thermoplasma acidophilum*[42], *Mycobacterium smegmatis*[27], and *Streptomyces griseus*[43].

EXPERIMENTAL

General methods. — Concentrations were performed under reduced pressure at bath temperatures below 40°. G.l.c. was performed with a Pye Series 104 chromatograph coupled to a Supergrator-2 integrator (Columbia Scientific Instruments). Glass columns packed with the following stationary phases were used: *a*, 3% of ECNSS-M on Gas Chrom Q (1.6 m × 2 mm); *b*, 10% of OS-138 and 1% of Adpet 80 on Chromosorb W (1.5 m × 3 mm); and *c*, 3% of Silar 10c on Gas Chrom Q (1.6 m × 2 mm). For g.l.c.–m.s. of partially methylated alditol acetates, column *c* was used at 160° in conjunction with an LKB mass spectrometer (model 2091). Paper chromatography was performed on Whatman No. 1 paper with the upper phase of ethyl acetate–pyridine–water (5:2:5). Detection reagents used were alkaline silver nitrate and aniline hydrogenoxalate. Gel-permeation chromatography of the water-soluble products from the mild, acid hydrolysis or the hydrazinolysis of lipopolysaccharide was performed on a column (40 × 2.5 cm) of Sephadex G-50 by elution with pyridine–acetic acid–water (10:4:986; pH 5.4). Fractions (~4 ml) were collected at a flow rate of 10–20 $ml.h^{-1}$, and were analysed for carbohydrate[44], phosphorus[45], or NH groups[46], as appropriate. The same buffer was used for the purification of fraction D2 on a column (82 × 1.5 cm) of Sephadex G-10 (flow rate, 5 $ml.h^{-1}$; fraction size, ~2 ml), and for the separation of products in a partial, acid hydrolysate of fraction D1 on a column (79 × 1.5 cm) of Sephadex G-15 (flow rate, 10 $ml.h^{-1}$; fraction size, ~2 ml).

The lipopolysaccharide and the derived fractions D1 and D2 were also subjected to chromatography on a column (87 × 1.5 cm) of Sephadex G-100 by elution with

0.12M Tris hydrochloride buffer (pH 8.1) containing sodium dodecyl sulphate (1%). Fractions (~2 ml) were collected at a flow rate of 5 ml.h^{-1}. Electrophoresis of the lipopolysaccharide and D1 in the presence of sodium dodecyl sulphate (0.1%) was carried out on separating rod gels of polyacrylamide (10%), as described by Laemmli[47]; bands were detected by using the periodate–Schiff reagents.

The optical rotation of fraction D1 was determined with a Bendix polarimeter (model 143A), and its i.r. spectrum was recorded with a Unicam SP200 spectrophotometer. N.m.r. spectra (^{13}C and ^{1}H) for deacetylated D1 were obtained (by Dr. I. H. Sadler, Department of Chemistry, University of Edinburgh) with a Bruker WH-360 spectrometer. The sample was freeze-dried three times from D_2O and spectra were recorded for a solution in D_2O. The ^{1}H spectrum was obtained at 78°, and chemical shifts for anomeric protons were referenced to the HOD signal. ^{13}C Spectra were obtained at 40° with complete proton-decoupling and with gated decoupling, and chemical shifts were referenced to external tetramethylsilane. N.m.r. spectra for native D1 were similarly obtained (by Dr. B. E. Mann, Department of Chemistry, University of Sheffield), but with a Bruker WH-400 spectrometer. The ^{1}H spectrum was recorded at 85° with sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) as the external standard.

Methods of quantitative analysis. — Standard colorimetric methods were used for the determination of phosphorus[45], total carbohydrate[44], 3-deoxy-2-octulosonic acid[48], aldoheptose[49], and *O*-acetyl groups[14] (α-D-glucopyranose penta-acetate was used as the standard). Total acetyl groups were determined by using g.l.c.[13]. Protein was estimated by autoanalysis of amino acids (Locarte bench analyser) after hydrolysis of the sample with 6.1M hydrochloric acid at 105° for 16 h under nitrogen. D-Glucose was determined by the use of hexokinase (EC 2.7.1.1) and D-glucose 6-phosphate dehydrogenase (EC 1.1.1.49), after hydrolysis of the samples with 2M hydrochloric acid at 105° for 2 h and neutralisation of the hydrolysates with Dowex 1 (HCO_3^-) resin. D-Mannose was determined by using the above enzymes with D-glucose phosphate isomerase (EC 5.3.1.9) and D-mannose phosphate isomerase (EC 5.3.1.8). All enzymes were obtained from Boehringer und Soehne G.m.b.H. Monosaccharides were also determined by g.l.c. of the alditol acetates on column *c* at 190° with *myo*-inositol hexa-acetate as the internal standard.

Growth of bacteria and isolation of lipopolysaccharide. — Cells of *P. diminuta* N.C.T.C. 8545 were grown at 30° for 24 h in Nutrient Broth No. 2 (Oxoid) with aeration at 20 l.min^{-1}. Cell walls were prepared, and the readily extractable lipids and the lipopolysaccharide were isolated, as described previously[10].

Mild hydrolysis of lipopolysaccharide with acid. — Lipopolysaccharide (~10 mg.ml^{-1}) was heated with 1% acetic acid at 100° for 2 h. As lipid A was slow to aggregate, the total hydrolysate was freeze-dried and then dispersed in deionised water. A clear, supernatant solution was obtained by centrifugation of the suspension at 10,000 r.p.m. for 15 min. The pellet was washed twice, and the combined solutions were freeze-dried. The fraction (D1) of high molecular weight was obtained by

chromatography of the products on Sephadex G-50. The oligosaccharide fraction (D2) was purified by re-chromatography on Sephadex G-10.

Hydrazinolysis of lipopolysaccharide. — Lipopolysaccharide (50 mg) was heated with anhydrous hydrazine (2 ml) at 100° for 10 h. After being dried *in vacuo* over P_2O_5 and conc. H_2SO_4, the products were distributed between chloroform and water. The aqueous phase was adjusted to pH ~7 with 0.1M hydrochloric acid and freeze-dried. The residue was fractionated by chromatography on Sephadex G-50. The product of high molecular weight was subsequently subjected to mild hydrolysis with acid and further chromatography on Sephadex G-50, as described for the lipopolysaccharide.

Deacetylation of fraction D1. — The sample (50 mg) was treated overnight at room temperature with 0.1M sodium hydroxide. The hydrolysate was passed down a column of Dowex 50 (H^+) resin, and the eluate was freeze-dried.

Degradative studies of fraction D1. — Methylation analysis was performed by standard procedures[50]. Partially methylated alditol acetates (with or without deuterium-labelling at C-1) were analysed by g.l.c. on column *a* at 150°, column *b* at 180°, and column *c* at 160°, and by means of g.l.c.–m.s. Peracetylation of D1 (10 mg) and oxidation of the product with chromium trioxide (at 50° for 2 h) were performed as described by Lindberg and Lönngren[51]. The oxidation of samples (3–5 mg) of D1 (native and deacetylated) with 50mM sodium periodate was carried out at 4° for 48 h. The consumption of periodate, monitored by the method of Avigad[52], was complete within 24 h. Partial hydrolysis of deacetylated D1 was effected with 0.5M hydrochloric acid at 100° for 20 min. After neutralisation, deionisation, and freeze-drying, the hydrolysate was fractionated on Sephadex G-15. Two separate fractions, corresponding to (*a*) di- to tetra-saccharides and (*b*) higher oligosaccharides, were collected and treated with α-D-mannosidase (EC 3.2.1.24) from jack bean (Boehringer) at 37° for 24 h. The contents of the fractions and the effects of the enzyme treatment were determined by paper chromatography.

ACKNOWLEDGMENTS

The author thanks the Science Research Council for providing access to the high-field n.m.r. services at the Universities of Edinburgh and Sheffield, Drs. I. H. Sadler and B. E. Mann for obtaining the n.m.r. spectra, Dr. S. Binns (Reckitt and Sons Ltd., Hull) for g.l.c.–m.s., Mr. G. Collier for polarimetry and i.r. spectroscopy, Mr. I. A. Pickering for g.l.c., Miss L. Galbraith for technical assistance, and Mrs. D. P. Marchant for carrying out preliminary experiments.

REFERENCES

1 R. W. Ballard, M. Doudoroff, R. Y. Stanier, and M. Mandel, *J. Gen. Microbiol.*, 53 (1968) 349–361.
2 N. J. Palleroni, R. Kunisawa, R. Contopoulou, and M. Doudoroff, *Int. J. Syst. Bacteriol.*, 23 (1973) 333–339.
3 C. M. Kaltenbach, C. W. Moss, and R. E. Weaver, *J. Clin. Microbiol.*, 1 (1975) 339–344.

4 M. S. DUBOW AND T. RYAN, *J. Gen. Microbiol.*, 102 (1977) 263–268.
5 G. L. GILARDI, in G. L. GILARDI (Ed.), *Glucose Nonfermenting Gram-negative Bacteria in Clinical Microbiology*, CRC Press, West Palm Beach, 1978, pp. 15–44.
6 N. J. PALLERONI, *The Pseudomonas Group*, Meadowfield Press, Shildon, Durham, 1978, 80 pp.
7 S. G. WILKINSON, *J. Gen. Microbiol.*, 54 (1968) 195–213.
8 S. G. WILKINSON, *Biochim. Biophys. Acta*, 187 (1969) 492–500.
9 S. G. WILKINSON AND M. E. BELL, *Biochim. Biophys. Acta*, 248 (1971) 293–299.
10 S. G. WILKINSON, L. GALBRAITH, AND G. A. LIGHTFOOT, *Eur. J. Biochem.*, 33 (1973) 158–174.
11 S. G. WILKINSON AND D. P. TAYLOR, *J. Gen. Microbiol.*, 109 (1978) 367–370.
12 S. G. WILKINSON AND L. GALBRAITH, *Biochim. Biophys. Acta*, 575 (1979) 244–254.
13 I. FROMME AND H. BEILHARZ, *Anal. Biochem.*, 84 (1978) 347–353.
14 F. DOWNS AND W. PIGMAN, *Methods Carbohydr. Chem.*, 7 (1976) 241–243.
15 J. HOFFMAN, *Chem. Commun., Univ. Stockholm*, 3 (1977) 1–30.
16 A. K. SEN, A. K. MUKHERJEE, B. GUHATHAKURTA, A. DUTTA, AND D. SASMAL, *Carbohydr. Res.*, 86 (1980) 113–121.
17 P. A. J. GORIN AND J. F. T. SPENCER, *Adv. Appl. Microbiol.*, 13 (1970) 25–89.
18 C. E. BALLOU, *Adv. Microb. Physiol.*, 14 (1976) 93–158.
19 T. HIRATA AND T. ISHITANI, *Agric. Biol. Chem.*, 42 (1978) 775–780.
20 H. YAMAGUCHI AND C. SCHUERCH, *Biopolymers*, 19 (1980) 297–309.
21 H. VAN HALBEEK, L. DORLAND, G. A. VELDINK, J. F. G. VLIEGENTHART, G. STRECKER, J.-C. MICHALSKI, J. MONTREUIL, AND W. E. HULL, *FEBS Lett.*, 121 (1980) 71–77.
22 K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
23 R. KASAI, M. OKIHARA, J. ASAKAWA, K. MIZUTANI, AND O. TANAKA, *Tetrahedron*, 35 (1979) 1427–1432.
24 P. A. J. GORIN, *Can. J. Chem.*, 51 (1973) 2375–2383.
25 P. A. J. GORIN AND M. MAZUREK, *Carbohydr. Res.*, 27 (1973) 325–339.
26 P. A. J. GORIN, R. H. HASKINS, L. R. TRAVASSOS, AND L. MENDONÇA-PREVIATO, *Carbohydr. Res.*, 55 (1977) 21–33.
27 J. E. MAGGIO, *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 2582–2586.
28 K. JANN, B. JANN, V. WINTER, AND C. WOLF-ULLISCH, *J. Gen. Microbiol.*, 110 (1979) 203–210.
29 E. T. PALVA AND P. H. MÄKELÄ, *Eur. J. Biochem.*, 107 (1980) 137–143.
30 R. C. GOLDMAN AND L. LEIVE, *Eur. J. Biochem.*, 107 (1980) 145–153.
31 R. S. MUNFORD, C. L. HALL, AND P. D. RICK, *J. Bacteriol.*, 144 (1980) 630–640.
32 M. CURVALL, B. LINDBERG, J. LÖNNGREN, AND W. NIMMICH, *Acta Chem. Scand.*, 27 (1973) 2645–2649.
33 B. LINDBERG, J. LÖNNGREN, AND W. NIMMICH, *Acta Chem. Scand.*, 26 (1972) 2231–2236.
34 K. RESKE AND K. JANN, *Eur. J. Biochem.*, 31 (1972) 320–328.
35 P. PREHM, B. JANN, AND K. JANN, *Eur. J. Biochem.*, 67 (1976) 53–56.
36 A. KATZ, J. WECKESSER, G. DREWS, AND H. MAYER, *Arch. Microbiol.*, 113 (1977) 247–256.
37 R. F. H. DEKKER, E. T. RIETSCHEL, AND H. SANDERMANN, *Arch. Microbiol.*, 115 (1977) 353–357.
38 O. LÜDERITZ, O. WESTPHAL, A. M. STAUB, AND H. NIKAIDO, in G. WEINBAUM, S. KADIS, AND S. J. AJL (Eds.), *Microbial Toxins*, Academic Press, New York, 1971, Vol. 4, pp. 145–233.
39 B. JANN, P. PREHM, AND K. JANN, *J. Bacteriol.*, 134 (1978) 462–469.
40 R. E. HURLBERT, J. WECKESSER, R. N. THARANATHAN, AND H. MAYER, *Eur. J. Biochem.*, 90 (1978) 241–246.
41 D. D. PRESS, A. S. SCHMIT, AND W. J. LENNARZ, *J. Biol. Chem.*, 250 (1975) 1319–1327.
42 P. F. SMITH, *Biochim. Biophys. Acta*, 619 (1980) 367–373.
43 B. E. KARI AND G. R. GRAY, *J. Biol. Chem.*, 254 (1979) 3354–3357.
44 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
45 G. R. BARTLETT, *J. Biol. Chem.*, 234 (1959) 466–468.
46 P. A. SANDFORD, A. J. NAFZIGER, AND A. JEANES, *Anal. Biochem.*, 44 (1971) 111–121.
47 U. K. LAEMMLI, *Nature (London)*, 227 (1970) 680–685.
48 M. J. OSBORN, *Proc. Natl. Acad. Sci. U.S.A.*, 50 (1963) 499–506.
49 B. G. WRIGHT AND P. A. REBERS, *Anal. Biochem.*, 49 (1972) 307–319.
50 P.-E. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LÖNNGREN, *Chem. Commun., Univ. Stockholm*, 8 (1976) 1–75.
51 B. LINDBERG AND J. LÖNNGREN, *Methods Enzymol.*, 50c (1978) 3–33.
52 G. AVIGAD, *Carbohydr. Res.*, 11 (1969) 119–123.