Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

STRUCTURAL STUDIES OF THE COMMON-CORE POLYSACCHARIDE OF THE CELL-WALL LIPOPOLYSACCHARIDE FROM *Salmonella typhimurium*

CARL GUSTAF HELLERQVIST
Institutionen för Organisk Kemi, Stockholm Universitet, Stockholm (Sweden)

AND ALF A. LINDBERG
Department of Bacteriology, Statens Bakteriologiska Laboratorium, Stockholm (Sweden)

(Received March 23rd, 1970)

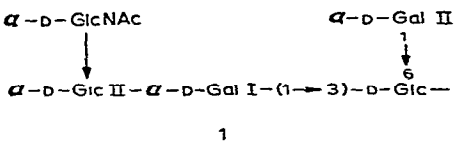
ABSTRACT

The structure of the common-core polysaccharide connecting the O-antigenic side-chains to the lipid part in the *Salmonella typhimurium* lipopolysaccharides (LPS) has been investigated. The LPS produced by one semi-rough and four rough mutants of *S. typhimurium* were used in these studies. The LPS were first converted into lipid-free polysaccharides and then subjected to methylation analysis. As a result of these studies, the sequence of the sugar residues and the positions to which they are linked have been determined, assuming a sequential addition of the sugar residues in the biosynthesis. The presence of *O*-acetyl groups and/or other alkali-labile groups in some positions in the common-core polysaccharide has been demonstrated.

INTRODUCTION

The lipopolysaccharides (LPS) of *Salmonella* bacteria are composed of three different parts: the lipid part (Lipid A), the basal core, and the O-antigenic side-chains[1]. The O-antigenic side-chains consist of repeating oligosaccharide units and determine the serological specificity of the different serotypes. The basal core is an oligosaccharide chain believed to have the same structure in most, if not all, *Salmonella* LPS. The outer part of the core, the common-core polysaccharide, is a pentasaccharide residue, containing D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-glucose residues in the ratio 2:2:1, and is synthesised by sequential addition of the individual sugar residues[2]. Any defect in the synthesis of the core results in R (rough) mutants termed[3] *rfa*. These mutants generally produce and polymerise the O-antigenic oligosaccharides, but cannot attach the O side-chain as the core is incomplete. A defect in the synthesis of the O-antigenic oligosaccharides or in the production of the translocase, which links them to the core, results in R-mutants termed *rfb*, the LPS of which have a complete core but no O-antigenic side-chains. The repeating oligosaccharide units are linked to each other by the action of a polymerase; mutants which are deficient in the production of this enzyme (SR = semirough or *rfc* mutants) synthesise a LPS in which only a single repeating unit is attached to the core.

In LPS from S (smooth) forms, only a minor part of the sugar residues belong to the core. Structural studies of the core region are therefore preferably performed with LPS from R or SR mutants. As a result of such studies, the partial structure **1** was proposed for the outer region of the core[4]. Some of the linkages were determined by graded hydrolysis followed by the isolation and identification of oligosaccharides. In a recent paper, Nikaido[5] demonstrated that the O-antigenic side-chain is linked to Glc II and not to GlcNAc as had been assumed.



In structural studies of different smooth LPS by methylation analysis[6–11], several methyl ethers of D-glucose and D-galactose were obtained as minor components. It was inferred that these were not derived from the O-antigenic side-chains but from the core. Because of the low percentage of these components, and, further, because the expected 2-acetamido-2-deoxy-D-glucose derivatives were missing in these studies, we made no attempt to draw any conclusions concerning the structure of the core. Methylation analyses of LPS from R and SR mutants should give more-reliable information. Such studies, performed on LPS from four R mutants and a SR mutant, are reported in the present communication.

METHODS AND RESULTS

Bacterial strains. — The bacterial strains were derived from *S. typhimurium.* LT2. The SH strains came from the collection of Dr. P. H. Mäkelä, State Serum Institute, Helsinki, Finland and the SL and TV strains from the collection of Dr. B. A. D. Stocker, Department of Microbiology, Stanford University, Stanford, Calif., U. S. A. The characteristics of the strains are given in Table I. Each strain was re-isolated from a single colony and tested for nutritional characteristics and phage sensitivity before and after cultivation. The bacteria were grown as described earlier[6].

Isolation of the LPS. — The phenol–water method[8] is applicable for the extraction of LPS from S as well as from R forms. It was shown, however, that the enzymic defect leading to roughness in three of the four R mutants used (TV 148, TV 160, and SL 733) was incomplete ("leaky" mutants), which resulted in the finding of significant amounts of O-specific material in the LPS preparations[19], in addition to the core. A new method[20], based upon the solubility of the lipophilic LPS from R-mutants in chloroform–light petroleum, was therefore applied to the R-mutants. The LPS preparations obtained contained exclusively core sugars.

The LPS preparations were investigated by passive hemagglutination inhibition[19] and phage inactivation[21]. The results of these studies will be published elsewhere.

Preparation and analysis of the lipid-free polysaccharides. — Treatment of the LPS with aqueous acetic acid at pH 3.4 for 1 h at 100° cleaves the acid-labile 3-deoxy-

TABLE I

CLASSIFICATION OF *Salmonella typhimurium* MUTANTS

	Genotype[3]	*Chemotype*[12]	*Phage pattern*[13,14]	*Further description and reference to the strain*
S. typhimurium SH 777	*rfc*	XIV	Smooth	Derived from a cross *S. montevideo* × *S. typhimurium* LT2
S. typhimurium SH 180	*rfb*, non-leaky mutation[a]	Ra	Rough sensitive	*rfb* deletion mutant his-658[15]
S. typhimurium SL 733	*rfa*, leaky mutation	Rb	Rough resistant-1 but 6SR sensitive	Ref. 6
S. typhimurium TV 160	*rfa*, leaky mutation	Rb	Rough resistant-1	Ref. 17
S. typhimurium TV 148	*rfa*, leaky mutation	Rb	Rough resistant-2	Ref 17

[a]The designations non-leaky and leaky mutations are based on (*1*) behaviour towards phage P22, (*2*) sugar analyses of phenol–water-extracted LPS purified by repeated cycles of ultracentrifugation, and (*3*) serological specificity in passive hemagglutination inhibition.

octulosonic acid (KDO) linkages[22], yielding a polysaccharide with a diminished content of lipid.

The polysaccharide from each mutant was hydrolysed to give a mixture of monosaccharides which were analysed as alditol acetates by g.l.c.[23]. Special precautions (see Experimental) were taken to account for the 2-acetamido-2-deoxy-D-glucose. The different sugars were identified solely by their retention times (*T* values) and mass spectra[24] (m.s.). The sugars have, however, been fully characterised in previous investigations[25]. The results are summarised in Table II. The molar percentage of abequose in the SR polysaccharide (SH 777) should have been equivalent to those of D-mannose or L-rhamnose; the low value found is due to the fact that no special precautions were taken to avoid destruction of abequose during the acid hydrolysis, as done previously[6].

TABLE II

SUGAR COMPOSITION IN THE DIFFERENT R MUTANTS AS ANALYSED AS THEIR CORRESPONDING ALDITOL ACETATES

Mutants	*Sugars*						
	Abe	L-*Rha*	D-*Man*	D-*Gal*	D-*Glc*	*Heptose*[a]	*GlcNAc*[a]
SH 777	7	10	10	29	19	20	9
SH 180	—	—	—	41	50	53	25
SL 733	—	—	—	28	32	29	—
TV 160	—	—	—	38	28	51	—
TV 148	—	—	—	32	41	87	—

[a]These values have been adjusted by using response factors (heptose 1.60, GlcNAc 1.98) found for model compounds.

As will be described elsewhere, in passive hemagglutination tests, the LPS from the mutants SH 777 and SL 733 inhibited the *S. typhimurium*/anti 05 factor serum in concentrations less than 8 μg/ml. This finding demonstrated the presence of structures cross-reacting with 2-*O*-acetyl-α-abequosyl groups in this LPS[6]. Accordingly, on treatment with methanolic hydrogen chloride, both LPS samples yielded methyl acetate, identified by g.l.c.–m.s. A more-detailed study of alkali-labile groups in these and the other mutant polysaccharides is in progress.

Methylation analysis. — The lipid-free polysaccharides were subjected to methylation analysis as previously described[6]; the mixtures of methylated sugars

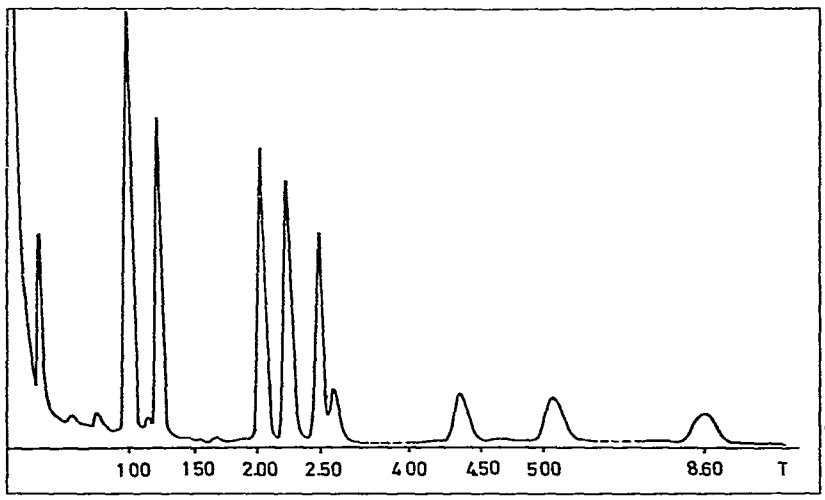


Fig 1. G.l c. separation of methylated sugars, as their alditol acetates, obtained from the hydrolysate of the fully methylated SH 777 polysaccharide.

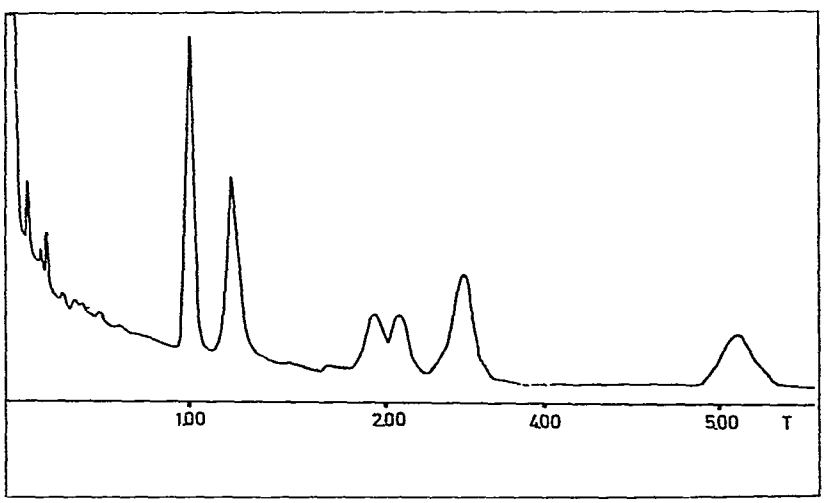


Fig. 2. G.l c. separation of methylated sugars, as their alditol acetates, obtained from the hydrolysate of the fully methylated SL 733 polysaccharide.

obtained were transformed into their alditol acetates and analysed by g.l.c.[26]–m.s.[27]. The procedure was slightly modified (see Experimental) to release all 2-acetamido-2-deoxy-D-glucose residues. In order to facilitate identifications by m.s., the reduction of the methylated sugars was performed with borodeuteride. Typical g.l.c. separations for the SH 777 and SL 733 polysaccharides are shown in Figs. 1 and 2. The results of the analyses are summarised in Table III.

TABLE III

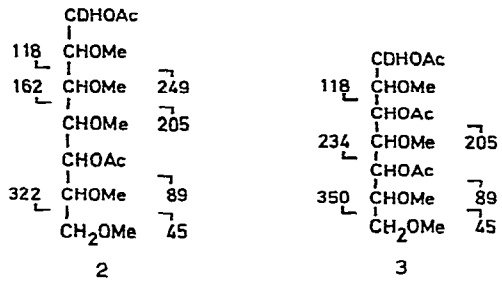
METHYL ETHERS FROM THE HYDROLYSATE OF METHYLATED POLYSACCHARIDES

Sugars	T[a]	*Molar proportions*				
		SH 777	*SH 180*	*SL 733*	*TV 160*	*TV 148*
2,4-Di-*O*-methylabequose	0.32	7.2	—	—	—	—
2,3-Di-*O*-methyl-L-rhamnose	0.98	12.4	—	—	—	—
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.00	—	—	22.0	9.0	9.5
2,3,4,6-Tetra-*O*-methyl-D-galactose	1 25	9.1	15.3	15.2	57.5	35.0
2,4,6-Tri-*O*-methyl-D-glucose	1.95	1.0	3.1	8.8	3 3	—
3,4,6-Tri-*O*-methyl-D-glucose	1.98	—	20.9	—	—	—
2,4,6-Tri-*O*-methyl-D-mannose	2.09	12.2	—	—	—	—
2,3,4,6,7-Penta-*O*-methylheptose	2.15	—	10.6	9.8	—	15.0
2,4,6-Tri-*O*-methyl-D-galactose	2 28	12.2	—	—	—	—
2,3,4-Tri-*O*-methyl-D-glucose	2.48	—	—	—	—	37.5
3,4,6-Tri-*O*-methyl-D-galactose	2.50	11.9	20.4	21.8	—	—
2,3,6-Tri-*O*-methyl-D-glucose	2.60	2.9	—	—	—	—
2,4,6,7-Tetra-*O*-methylheptose	4 15	—	—	—	—	10.5
3,6-Di-*O*-methyl-D-glucose	4.30	8 8	—	—	—	—
2,4-Di-*O*-methyl-D-glucose	5.10	9.6	15.3	14.0	30.0	—
2-Deoxy-3,4,6-tri-*O*-methyl-2(*N*-methyl-acetamido)-D-glucose	8.60	8.8	16.5	—	—	—

[a]Retention times of the corresponding alditol acetates on the ECNSS-M S.C.O.T-Column relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

Most of the methylated sugars were also obtained in our previous studies of *Salmonella* LPS, and their identification will not be discussed again. One peak had the same *T* value (8.60) and m.s.[28] as the alditol acetate from 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucose. Since 2-acetamido-2-deoxy-D-glucose is the only amino sugar found in the polysaccharides, no further characterization was needed. A minor component (*T* 2.15) was obtained from some polysaccharides. Its m.s. contained, *inter alia*, major fragments at *m/e* 45, 89, 162, 205, 249, and 322. These are the primary fragments expected for the alditol acetate derived from a 2,3,4,6,7-penta-*O*-methylheptose (**2**), monodeuterated at C-1. Another component (*T* 4.15), obtained from two polysaccharides, showed the primary fragments (45, 89, 118, 205, and 234) expected for the alditol acetate derived from a 2,4,6,7-tetra-*O*-methylheptose (**3**).

There is good agreement between the original sugar analysis and the methylation analysis for the five polysaccharides, except for the content of abequose and



L-*glycero*-D-*manno*-heptose. Part of the former was probably lost as volatile ethers during the analysis[6]. The heptose residues are esterified with phosphoric acid[29], and little is known about the reactions of these residues and their fate under the conditions used for methylation analysis.

Determination of the positions of alkali-labile groups. — Alkali-labile groups were located in the SH 777 and SL 733 polysaccharides, by methylation analysis, after protection of all free hydroxyl groups by acetalation with methyl vinyl ether[30]. The alditol acetate from 2-*O*-methylabequose, a sugar also present in the LPS from two *S. typhimurium* strains, 395 MS[6] and LT2[7], was obtained from the SH 777 polysaccharide, together with abequitol acetate. The relative proportion of these two derivatives indicated that ~60% of the abequose residues in the SH 777 polysaccharide carry an alkali-labile group, known[6] to be *O*-acetyl, in the 2-position.

Another component (*T* 7.90) having the m.s. of a 2-*O*-methylhexose derivative was also observed in the analyses of the SH 777 polysaccharide. This *T*-value corresponds to either 2-*O*-methyl-D-glucose (*T* 7.90) or 2-*O*-methyl-D-mannose (*T* unknown), but not to 2-*O*-methyl-D-galactose (*T* 8.30). A 2-*O*-methylhexose (*T* 7.90) was also observed in the analysis of the SL 733 polysaccharides. This must be a D-glucose derivative, since D-mannose residues are absent.

In the methylation analysis of the acetalised SL 733 polysaccharide, the D-galactose content was only 70% of that found in the sugar analysis. A new component (*T* 10.2), identified as the 4-*O*-methyl-D-galactose derivative, accounted for this difference.

DISCUSSION

The partial structure **1** of the common-core polysaccharide is based upon results from biosynthetic studies[2], linkage analysis[4], and periodate oxidations[5]. The present results afford an independent means of deducing a partial structure for the common-core polysaccharide, assuming that it is synthesised by sequential addition of sugar residues and that it has the regular structure discussed below.

The polysaccharide from TV 148 contains one mole of D-glucose and somewhat less than one mole of D-galactose per two moles of heptose. Methylation analysis shows that all D-galactose units are terminal, and are connected to the 6-position of D-glucose residues. The remaining D-glucose units are all terminal (structures **4** and **5**).

D-Glc*p*-(1→3)-Hep D-Gal*p*-(1→6)-D-Glc*p*-(1→3)-Hep

4 5

The polysaccharide from TV 160 contains *ca.* one mole of D-glucose and more than one mole of D-galactose per two moles of heptose. Methylation analysis shows that most of the D-glucose residues are substituted in the 3-position and most of them also in the 6-position, giving partial structure **6**. The remaining D-glucose residues are all terminal, indicating that not all D-glucose units (Glc I) in the TV 160 polysaccharide have been substituted with D-galactose in the 3- and 6-positions. In the postulated structure **6**, as in following structures, a dotted line indicates that not all molecules carry the indicated substituent.

D-Gal*p* D-Gal*p* Gal II

1 1

⋮ ⋮

6 6

D-Gal*p*-(1→3)-D-Glc*p*-(1→3)-Hep D-Glc*p*-(1→2)-D-Gal*p*-(1→3)-D-Glc*p*-(1→3)-Hep

Glc II Gal I Glc I

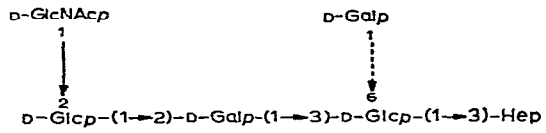
6 7

The polysaccharide from SL 733 contains approximately equal proportions of D-glucose, D-galactose, and heptose. As is evident from the methylation analyses, part of the D-glucose residues (50%) are terminal and are linked to the 2-position of D-galactose. The remainder of the D-glucose residues are substituted in the 3- and 6-positions (70%) or in the 3-position (30%). The amount of terminal D-galactose residues is equivalent to that of branched D-glucose. This demonstrates that D-glucose (Glc II) is linked to D-galactose (Gal I) as in partial structure **7**, and that the terminal D-galactose (Gal II) is linked to the 6-position of D-glucose (Glc I).

The finding that not all Glc I residues in the polysaccharides from TV 160 and SL 733 carried Gal II can be interpreted in two ways. Either, there is full substitution during the biosynthesis, but part of the Gal II has been removed after the biosynthesis by the action of galactosidase; or, the presence of Gal II is not an absolute requirement for the further addition of sugar residues in the biosynthesis of the core.

The SH 180 polysaccharide contains one mole of 2-acetamido-2-deoxy-D-glucose (GlcNAc), two moles of D-glucose, and somewhat less than two moles of D-galactose per two moles of heptose. The methylation analysis shows that all of the GlcNAc is terminal. The 2,3,4,6-tetra-*O*-methyl-D-glucose obtained from the SL 733 polysaccharide is replaced by 3,4,6-tri-*O*-methyl-D-glucose, demonstrating that GlcNAc is linked to the 2-position of G II. In the polysaccharide from the SL 733 mutant, as was also found in the TV 160 polysaccharide, not all D-glucose residues (Glc I) have a terminal D-galactose residue (Gal II) linked to their 6-position. The partial structure **8** is therefore proposed for this polysaccharide.

By deducting from the total sugar values of the SH 777 polysaccharide the contributions from the O-antigenic repeating unit (1 mole each of abequose, L-rhamnose, D-mannose, and D-galactose), the core sugars GlcNAc, D-galactose,

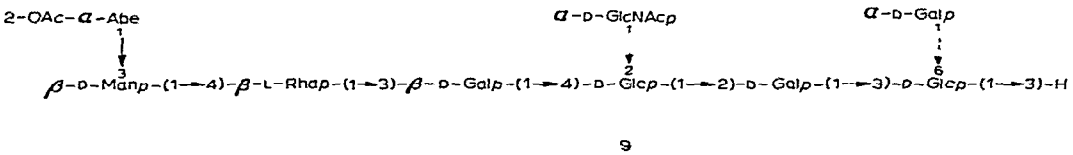


8

D-glucose, and heptose were calculated to be in the approximate relative proportions 1:2:2:2. The methylation analysis indicates that the O-antigenic oligosaccharide has the same structure as the biological repeating-unit in the complete *S. typhimurium*-LPS[6,7], and is thus terminated by a 3-*O*-α-abequosyl-D-mannose residue. In the LPS from S strains of *S. typhimurium* (12_2 positive forms), some D-galactose residues carry a terminal D-glucose unit on the 4-position. This structural feature is not present in the SR polysaccharide, as neither 2,3,4,6-tetra-*O*-methyl-D-glucose nor 2,6-di-*O*-methyl-D-galactose was found in the methylation analysis. In smooth LPS, all[6] or most[7] of the abequose residues are acetylated in the 2-position, a feature also observed in the SR polysaccharide. The methylation analysis shows that Glc II is now substituted also in the 4-position, and thus the O-antigenic oligosaccharide is linked to that position. The reducing, terminal, sugar residue of the biological repeating-unit is D-galactose; consequently, this is the residue that is linked to Glc II. A small but significant proportion of 2,3,6-tri-*O*-methyl-D-glucose is probably derived from D-glucose (Glc II), indicating that some of these residues do not carry a GlcNAc residue in their 2-position. The percentage of 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucose also corresponds to that of 3,6-di-*O*-methyl-D-glucose, but this is less significant as the quantitative analysis of the GlcNAc derivatives is not as accurate as those of the neutral sugar derivatives. The partial deficit in terminal GlcNAc residues could be explained in the same way as was discussed for the lack of stoichiometry between the Gal II and Glc I residues in the TV 160 and SL 733 polysaccharides.

In the partial structure **9** proposed for the SH 777 polysaccharide, previous results concerning the anomeric nature of some sugar residues are included. Two heptose residues, which are believed to be linked to their 3-positions and to be esterified by phosphoric acid, precede D-glucose I in the core structure[29]. The methylation analyses of some of the polysaccharides indicated the presence of terminal heptose (SH 180, SL 733, and TV 148) and of heptose substituted in the 3-position (TV 148). Since so little is known about methylation analyses of phosphor-esterified sugars, these values cannot be given any structural significance. In addition to the *O*-acetyl groups on the abequose residues in the SH 777 polysaccharide, the presence of alkali-labile groups in the common-core polysaccharide was also demonstrated. A low percentage of such groups, linked to C-2 in D-glucose residues, was demonstrated in the SH 777 and SL 733 polysaccharides. A high percentage of alkali-labile groups, linked to C-4 in D-galactose residues, was also demonstrated in the SL 733 polysaccharide. It is questionable if the alkali-labile group linked to the latter position could be an *O*-acetyl, as a facile migration to other positions would be expected, and the analysis did not indicate the presence of alkali-labile groups in

other positions. Alkali-labile groups have not been observed before in the common-core polysaccharide. The significance of this finding remains to be explained.



9

EXPERIMENTAL

The methods used were essentially those described in the investigations of *Salmonella typhimurium* 395 MS[6] and *S. newport* and *S. kentucky*[11]. Additional procedures were as follows.

Sugar analysis. — In the sugar analysis, it was essential to avoid *N*-deacetylation during hydrolyses, since 2-amino-2-deoxy-D-glucosides are hydrolysed only with difficulty. Experiments with methyl 2-acetamido-2-deoxy-α-D-glucopyranoside demonstrated that a complete hydrolysis of the glycosidic linkages was obtained by treatment with 90% aqueous acetic acid for 8 h at 100°. The polysaccharides were treated with 90% aqueous acetic acid for 8 h at 100°, and after evaporation to dryness, 0.25M sulphuric acid was added and the material was hydrolysed for 12 h at 100°. After conversion of the free sugars into their alditols, the material was acetylated with acetic anhydride–pyridine for 2 h at 100°.

Methylation analysis. — Experiments with methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucoside demonstrated that complete hydrolyses of the glycosidic linkage was obtained by treatment with a mixture of 4 ml of 90% aqueous acetic acid and 0.35 ml of 2M sulphuric acid. The polysaccharides were treated with a mixture of 4 ml of 90% aqueous acetic acid and 0.35 ml of 2M sulphuric acid for 36 h at 100°, and after removal of the acetic acid, water (4 ml) was added, and the solutions were heated for 4 h at 100°. The sugar derivatives were converted into the alditols and acetylated as described above.

ACKNOWLEDGMENT

We are indebted to Professor Bengt Lindberg for his interest. We thank Drs. G. Hämmerling, O. Lüderitz, O. Westphal, and H. Nikaido for allowing us to read their manuscripts before they were submitted for publication. The skilled technical assistance of Miss Gunnel Ljunggren and Mrs. Jana Cederstrand is acknowledged.

This work has been supported by grants from the Swedish Medical Research Council (to A. A. L. No B 69-40x-656-04A to CGH No B 69-13x-2522-01) from the Swedish Natural Research Council, from Harald Jeanssons Stiftelse, from Emil and Wera Cornells Stiftelse, and from Stiftelsen Sigurd och Elsa Goljes minne.

REFERENCES

1 O. LÜDERITZ, K. JANN, AND R. WHEAT, *Comprehensive Biochem.*, 26A (1968) 105.
2 M. J. OSBORN, S. M. ROSEN, L. ROTHFIELD, L. D. ZELEZNICK, AND B. L HORECKER, *Science*, 145 (1964) 783.
3 P. H. MÄKELÄ AND B A. D. STOCKER, *Am. Rev. Genet.*, 3 (1969) 291.
4 I. W. SUTHERLAND, O. LUDERITZ, AND O. WESTPHAL, *Biochem. J.*, 96 (1965) 439.
5 H. NIKAIDO, *J. Biol. Chem.*, 244 (1969) 2835
6 C. G. HELLERQVIST, B. LINDBERG, S. SVENSSON, T. HOLME, AND A. A. LINDBERG, *Carbohyd. Res.*, 8 (1968) 43.
7 C G. HELLERQVIST, B. LINDBERG, S SVENSSON, T. HOLME, AND A. A. LINDBERG, *Carbohyd. Res.*, 9 (1969) 237.
8 C. G. HELLERQVIST, B. LINDBERG, S SVENSSON, T. HOLME, AND A. A. LINDBERG, *Acta Chem. Scand*, 23 (1969) 1588.
9 C. G. HELLERQVIST, O. LARM, B. LINDBERG, T. HOLME. AND A. A. LINDBERG, *Acta Chem. Scand*, 23 (1969) 2217
10 C. G. HELLERQVIST, B. LINDBERG, A. PILOTTI, AND A. A. LINDBERG, *Acta Chem. Scand*, jn press.
11 C. G. HELLERQVIST, B LINDBERG, S. SVENSSON, AND A. A. LINDBERG, *Carbohyd. Res.*, in press.
12 O. LUDERITZ, A. M. STAUB, AND O. WESTPHAL, *Bacteriol. Rev.*, 30 (1966) 192.
13 R. G. WILKINSON, Ph. D. THESIS, University of London, 1966.
14 B. A. D. STOCKER, *Colloq. Intern. Centre Nat. Rech Sci*, Paris, 174 (1969) 189.
15 H. NIKAIDO, M. LEVINTHAL, K. NIKAIDO, AND K. NAKANE, *Proc. Nat. Acad. Sci. U S.*, 37 (1967) 1825.
16 Y. NAIDE, H. NIKAIDO, P. H. MAKELA, R. G. WILKINSON, AND D. A. B. STOCKER, *Proc. Nat. Acad. Sci. U.S.*, 53 (1965) 147.
17 T. V. SUBBAIAH AND B. A. D. STOCKER, *Nature*, 201 (1964) 1298.
18 O. WESTPHAL, O. LUDERITZ, AND F. BISTER, *Z. Naturforsch*, 7b (1952) 148.
19 A. A. LINDBERG AND T. HOLME, *J. Gen. Microbiol.*, 52 (1968) 55.
20 C. GALANOS, O. LUDERITZ, AND O. WESTPHAL, *Europ. J. Biochem.*, 9 (1969) 245.
21 A. A. LINDBERG, *J. Gen. Microbiol*, 48 (1967) 225.
22 W. DROGE, O. LUDERITZ, AND O. WESTPHAL, *Europ. J. Biochem.*, 4 (1968) 126.
23 J. S. SAWARDEKER, J. SLONEKER, AND A. R. JEANES, *Anal. Chem.*, 12 (1965) 1602.
24 O. S. CHIZHOV, L. S. GOLOVKINA, AND N. S. WULFSON, *Izv. Akad. Nauk SSSR, Otd Khim. Nauk*, (1966) 1915.
25 L. KRUGER, O. LUDERITZ, J L. STROMINGER, AND O. WESTPHAL, *Biochem. Z.*, 335 (1962) 548.
26 H. BJORNDAL, B. LINDBERG, AND S. SVENSSON, *Acta Chem. Scand*, 21 (1967) 1801.
27 H. BJORNDAL, B. LINDBERG, AND S. SVENSSON, *Carbohyd. Res*, 5 (1967) 433.
28 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem.*, in press.
29 W. DROGE, E. RUSCHMANN, O. LUDERITZ, AND O. WESTPHAL, *Europ. J. Biochem.*, 4 (1968) 134.
30 A. N. de BELDER AND B. NORRMAN, *Carbohyd. Res*, 8 (1968) 1.