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Structural Characterization of the Lipopolysaccharide O Antigens of *Burkholderia pseudomallei*

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A serologically typical strain of *Burkholderia pseudomallei* (strain 304b) was found to produce two S-type lipopolysaccharides (LPS) differing in the chemical structures of their O-polysaccharide (O-PS) components. Structural analysis revealed that one O-antigenic polysaccharide (O-PS I) is an unbranched high-molecular-weight polymer of 1,3-linked 2-*O*-acetyl-6-deoxy-β-D-*manno*-heptopyranose residues. The other LPS O antigen (O-PS II) is an unbranched polymer of repeating disaccharide units having the structure -3)-β-D-glucopyranose-(1-3)-6-deoxy-α-L-talopyranose-(1- in which ca. 33% of the L-6dTal*p* residues bear 2-*O*-methyl and 4-*O*-acetyl substituents while the other L-6dTal*p* residues carry only 2-*O*-acetyl substituents. Analysis of a serologically atypical strain of *B. pseudomallei* (strain 824a) produced a single LPS O-PS which was chemically identical to the 6-deoxy-D-*manno*-heptan O-PS I. The production of two distinct LPS raises the interesting question of their relative immunogenicities and consequently their relative importance for diagnostic serology and for the possible development of conjugate vaccines.

Melioidosis, or *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei* [28]) infection, is an important cause of mortality in Thailand and other parts of Southeast Asia and northern Australia (12). The infection occurs in three clinical forms, asymptomatic, localized, and septicemic. In northeastern Thailand alone, a population of 7 million people are at risk, and septicemic melioidosis accounted for 19% of cases of, and 40% of deaths from, community-acquired septicemia in a 12-month period from 1986 to 1987 (10). Although melioidosis does occur in previously healthy individuals, diabetes mellitus appears to be a particular risk factor (23). In addition to its virulence, *B. pseudomallei* is also intrinsically resistant to penicillin and gentamicin, the usual empirical treatment for suspected septicemia in many parts of the developing world. Even under the best circumstances, prolonged treatment with expensive antibiotics is required for eradication of the organism.

No effective method of prevention of melioidosis currently exists. It is reasonable to predict that a vaccine inducing protective antibodies would be efficacious because of the septicemic nature of the severe disease. During the course of studies aimed at determining the protective potential of antibodies directed against O-polysaccharide (O-PS) of *B. pseudomallei*, a rabbit polyclonal antibody and a monoclonal antibody raised against a tetanus toxoid conjugate of *B. pseudomallei* O-PS and excised sodium dodecyl sulfate-polyacrylamide gel bands, respectively, were found to be protective against challenge in an animal model of infection (9). These same antibodies recognized more than 40 strains of *B. pseudomallei* isolated from infected patients in northeast Thailand, indicating the presence of a common antigen. Only one clinical strain of *B. pseudomallei* (i.e., strain 824a) was nonreactive both by enzyme-linked immunosorbent assay (ELISA) and by immunoblot. Furthermore, the antisera gave negative ELISA and agglutination reactions with a wide range of other gram-negative and gram-positive bacteria, with the exception of several strains of *Burkholderia cepacia* serogroup A, which showed weak reactivity against the rabbit O-PS antiserum.

As a first step in defining the antigenic determinants of the lipopolysaccharide (LPS) cell wall components produced by *B. pseudomallei* which can be incorporated into a conjugate vaccine, the fine structure of their O-PS was determined. This investigation records the analysis of the O-PS of the LPS produced by a prototype strain, *B. pseudomallei* 304b, and also that of an LPS from an atypical strain, *B. pseudomallei* 824a.

Through the application of chemical and physical analytical methods, the *B. pseudomallei* 304b strain was demonstrated to produce two distinct LPSs. One LPS had an O-PS component of a 1,3-linked homopolymer of 2-*O*-acetylated 6-deoxy-β-D-*manno*-heptopyranosyl residues. The other LPS had an O-PS component composed of repeating disaccharide units having the structure -3)-β-D-glucopyranose-(1-3)-6-deoxy-α-L-talopyranose-(1-, in which the 6-deoxy-L-talopyranosyl residues were partially (ca. 33%) methylated at the *O*-2 position and which were also variably substituted by *O*-acetyl groups. The atypical *B. pseudomallei* strain 824a produced one LPS in which the O-PS component had the same structure as that of the 6-deoxy-D-*manno*-heptan produced by the typical *B. pseudomallei* strains.

(Results of this investigation were presented at the Canadian Bacterial Diseases Centres of Excellence Symposium, Vancouver, Canada, June 1993.)

MATERIALS AND METHODS

Preparation of *B. pseudomallei* LPS and O-PSs. Strains of *B. pseudomallei* (304b and 824a), clinical isolates from patients in northeastern Thailand, were gifts of M. D. Smith, Wellcome-Mahidol University, Oxford Tropical Medicine Research Programme, Bangkok, Thailand. They were grown in 3.7% brain heart infusion broth (Difco) at 37°C in a Microferm fermentor (New Brunswick; 28 liters) and, at 18 h, were chilled to 4°C and killed by the addition of phenol to a final concentration of 2%. Cells were collected by Sharples continuous centrifugation and extracted by a modified enzyme hot aqueous-phenol method (21). The separated phenol and aqueous phases from the extraction were collected, dialyzed against tap water until free from phenol, and then lyophilized. Aqueous solutions of the two products were treated sequentially at 37°C with RNase, DNase, and finally with protease K. The solutions were cleared by low-speed

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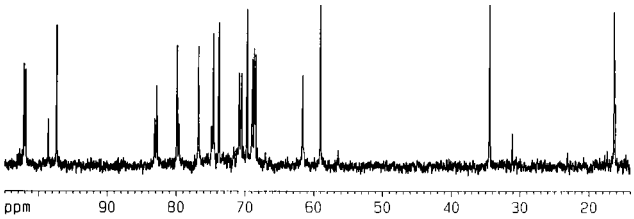

FIG. 1. ^{13}C NMR spectrum of the *O*-deacetylated total LPS O-PS fraction from *B. pseudomallei* 304b.

centrifugation and subjected to ultracentrifugation (105,000 × *g*, 12 h, 4°C) to yield precipitated gels of LPS which were taken up in distilled water and lyophilized.

Fission of the LPS (2%) in 5% acetic acid (100°C, 2 h) released lipid A, and the lyophilized water-soluble product was fractionated by Sephadex G-50 gel filtration column chromatography (column size, 2 by 90 cm) with 0.05 M pyridinium acetate (pH 4.6) as the mobile phase. Collected fractions (10 ml) were analyzed for neutral glycose by the phenol-sulfuric acid method (15), for aminodeoxyglycose by a modified Elson-Morgan method (16), and for 3-deoxy-D-manno-octulosonic acid (Kdo) (1) and phosphate (11). Appropriate fractions were collected and lyophilized.

NMR spectroscopy. Measurements were made on samples dissolved in D_2O at 318 K and containing a trace of acetone. ^1H nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz, and ^{13}C NMR spectra were recorded at 125 MHz with a Bruker AM 500 spectrometer equipped with an Aspect 3000 computer with standard Bruker software. Chemical shifts are recorded in parts per million relative to internal acetone (2.225 ppm [^1H] and 31.07 ppm [^{13}C]). Two-dimensional homonuclear chemical shift correlated spectroscopy (COSY [2] and relayed COSY [27]) were carried out with spectral widths of 2,400 or 900 Hz. Heteronuclear ^{13}C-^1H chemical shift correlations were measured in the ^1H-detected mode via multiple quantum coherence ($^1H\{^{13}C\}$HMQC) (3) with a Bruker 5-mm inverse broad-band probe by use of reverse electronics as described previously (7). The two-dimensional heteronuclear multiple-bond-correlated ^1H-^{13}C ($^1H\{^{13}C\}$HMBC) experiments were made with the pulse sequence described by Bax and Summer (4). $^1J_{C,H}$ values were determined by gated decoupling.

Analytical methods. Analytical gas-liquid chromatography (GLC) and GLC-mass spectrometry (GLC-MS) were done with a Hewlett-Packard 5890 Series II GLC-MS system operating in the electron impact (EI) mode and with a DB-17 fused silica capillary column (30 m by 0.25 mm) with an ionization potential of 70 eV. The following temperature program was used: 180°C (delay, 2 min) and 2°C/min to 240°C. The retention times of glycose derivatives were recorded relative to that of 1,2,3,4,5,6-hexa-*O*-acetyl-D-glucitol (T_{GA}) or 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (T_{GM}), and they were compared, as were the corresponding mass spectra of authentic samples.

Samples (~1 mg) were hydrolyzed with 4 M trifluoroacetic acid (1 ml, 120°C, 2 h), constituent liberated glycoses were reduced (sodium borodeuteride [$NaBD_4$]) and acetylated, and the resulting products were subjected to GLC analysis (17). Methylation of samples (~1 mg) was made by the Hakomori method (18). Methylated samples were dialyzed to remove low-molecular-weight impurities, and the products were hydrolyzed (4 M trifluoroacetic acid, 1 h, 125°C) and analyzed by GLC-EIMS of the reduced ($NaBD_4$) and acetylated alditol derivatives of the released methylated glycoses.

Preparative paper chromatography was done on water-washed 3MM filter paper with pyridine-ethyl acetate-water (2:5:5 [vol/vol/vol], top layer) as the mobile phase. Glycose mobilities are quoted relative to D-galactose (R_{Gal}). Specific optical rotations were determined at 20°C in 10-cm microtubes with a Perkin-Elmer model 243 polarimeter. D-Glucose was determined by the D-glucose oxidase method (19), and *O*-acetyl was determined by the colorimetric Hestrin method (14).

RESULTS

Fermentor-grown cells of *B. pseudomallei* 304b were extracted by a modified hot aqueous-phenol method (21), and the LPSs were obtained by ultracentrifugation of the separated phenol and water phases, yielding 3.5% phenol-phase LPS and 0.7% aqueous-phase LPS on the basis of dry cell weight. Chemical analyses indicated that the aqueous-phase product was essentially a lipooligosaccharide (R-type LPS). The phenol-phase product was essentially S-type LPS and was used in all subsequent analyses.

B. pseudomallei 304b LPS was hydrolyzed with hot, dilute acetic acid, and after removal of semisolid oily precipitated lipid A, the concentrated water-soluble products were fractionated by Sephadex G-50 gel filtration column chromatography (2 by 90 cm) to give an O-PS fraction (K_{av}, 0.01 to 0.42; 84%) which had a $[\alpha]_D$ of −55° (c 0.2, water), a core oligosaccharide (K_{av}, 0.75; 7%) having a $[\alpha]_D$ of +75.0° (c 0.1, water), and a low-molecular-weight product (K_{av}, 0.98; 4%) containing phosphate and Kdo.

Preparative paper chromatographic separation of the hydrolysis products of the total O-PS fraction (140 mg) afforded four glycoses which were characterized as follows. The glycose 6-deoxy-talose (24 mg) had a $[\alpha]_D$ of −20.1° (c 0.12, water), gave a single spot on paper chromatography (R_{Gal}, 1.44), and upon reduction ($NaBD_4$) and acetylation gave 1,2,3,4,5-penta-*O*-acetyl-6-deoxy-L-talitol-1-d (25) having a GLC retention time (T_{GA}, 0.59) and MS identical to that of a reference sample. Treatment with phenylhydrazine gave L-fucosazone having a melting point and a mixture melting point of 180 to 181°C and a $[\alpha]_D$ of −59° (c 0.1; pyridine/ethyl alcohol, 2:3) (24).

The glycose 6-deoxy-2-*O*-methyl-L-talose (9 mg) had a $[\alpha]_D$ of −17° (c 0.1, water), gave a single spot on paper chromatography (R_{Gal}, 1.57), and upon reduction ($NaBD_4$) and acetylation gave 1,3,4,5-tetra-*O*-acetyl-6-deoxy-2-*O*-methyl-L-talitol-1-d having a GLC retention time (T_{GA}, 0.50) and MS fragmentation pattern identical to that of an authentic sample. Upon hydrolysis, reduction ($NaBD_4$), and acetylation, the methylated glycose gave 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methyl-L-talitol-1-d identified by GLC-MS.

The glycose D-glucose (30 mg) had a $[\alpha]_D$ of +52.2° (c 0.22, water), gave a single spot on paper chromatography (R_{Gal}, 1.08), and upon reduction ($NaBD_4$) and acetylation gave hexa-*O*-acetyl-D-glucitol-1-d characterized by GLC-MS (T_{GA}, 1.00). The glycose was completely oxidized by D-glucose oxidase (19).

The glycose 6-deoxy-D-*manno*-heptose (23 mg) had a $[\alpha]_D$ of +24.2° (c 0.14, water), gave a single spot on paper chromatography (R_{Gal}, 1.27), and upon reduction ($NaBD_4$) and acetylation gave 1,2,3,4,5,7-hexa-*O*-acetyl-6-deoxy-D-*manno*-heptitol-1-d having a GLC retention time (T_{GA}, 1.15) and MS identical to that of an authentic sample. Upon methanolysis (methanol-HCl), the glycose gave methyl 6-deoxy-α-D-*manno*-heptopyranoside (94%), which upon acetylation (pyridine-acetic anhydride [Ac_2O]) afforded crystalline methyl 2,3,4,7-tetra-*O*-acetyl-6-deoxy-α-D-*manno*-heptopyranoside, which had a melting point and a mixture melting point of 78°C, a $[\alpha]_D$ of +60° (c 0.11, chloroform), and gave a single peak on GLC (T_{GA}, 0.86), corresponding in properties with those of authentic derivatives.

TABLE 1. Proton and carbon-13 chemical shifts for *B. pseudomallei* O-PS I

6-Deoxy-D-*manno*-heptose	Chemical shift (ppm)[a]														
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	H-7	C-1	C-2	C-3	C-4	C-5	C-6	C-7
Native O-PS I	4.91 (<2)	5.30	3.97	3.49	3.51	2.17	1.73	3.78	96.9 (163)	69.9	79.6	69.4	73.5	34.3	58.7
O-Deacetylated O-PS I	4.83 (<2)	4.26	3.87	3.62	3.47	2.11	1.74	3.74	97.5 (163)	68.8	80.0	69.8	73.9	34.5	59.2

[a] Coupling constants in hertz are given in parentheses.

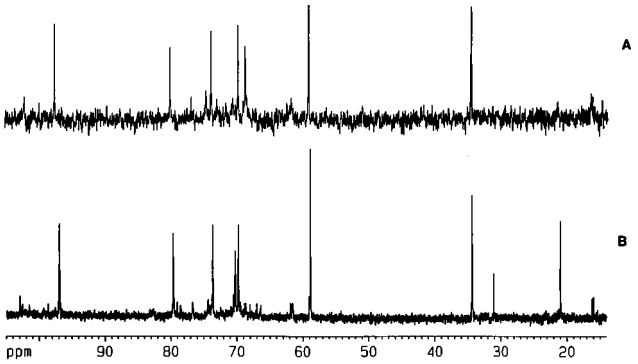

FIG. 2. ^{13}C NMR spectrum of *O*-deacetylated O-PS I (A) and native O-PS I (B) from *B. pseudomallei* 304b.

Analysis of the hydrolyzed O-PS by the GLC alditol acetate method indicated a glycose composition of D-glucose, 6-deoxy-D-*manno*-heptose, 6-deoxy-L-talose, and 2-*O*-methyl-6-deoxy-L-talose in the approximate molar ratio 1:2:0.7:0.3.

The ^{1}H and ^{13}C NMR spectra of the total O-PS fraction (Fig. 1) were complex as a result of *O*-acetyl substitution (ca. 15%). The *O*-deacetylated (dil. NH_4OH) O-PS gave a simplified ^{13}C NMR spectrum (Fig. 1) consistent with the presence of anomeric carbons, OCH_3, CH_3, and CH_2 signals of the chemically identified component glycoses. The nonstoichiometric glycose composition of the O-PS fraction indicated by the ^{1}H NMR spectrum and by chemical analyses first suggested that the O-PS fraction was composed possibly of two distinct O-PSs, a fact confirmed by the results of two-dimensional nuclear Overhauser effect (NOE) and COSY experiments.

Fractions collected from the eluate of the Sephadex G-50 gel filtration chromatography of the O-PS afforded an essentially homogeneous high-molecular-weight polymer (O-PS I; K_{av}, 0.01 to 0.08; 19%), a heterogeneous fraction (K_{av}, 0.18 to 0.25; 63%), and a fraction containing a lower-molecular-weight, essentially homogeneous polymer (O-PS II; K_{av}, 0.25 to 0.42; 17%).

O-PS I had a $[\alpha]_D$ of −62.0° (c 0.12, water) containing *O*-acetyl (ca. 18%), and upon hydrolysis was found to be composed of 6-deoxy-D-*manno*-heptose (76%). Upon methylation analysis, O-PS I gave a major product (94%) identified as 1,3,5,-tri-*O*-acetyl-6-deoxy-2,4,7-tri-*O*-methyl-D-*manno*-heptitol-1-d, having a GLC retention time (T_{GM}, 1.56) and MS identical to that of an authentic derivative, thus indicating that O-PS I was a polymer of 1,3-linked 6-deoxy-D-*manno*-heptopyranoside residues. The ^{1}H NMR spectrum of the native O-PS I showed that each heptose unit was stoichiometrically substituted by a single *O*-acetyl group. Two-dimensional homonuclear shift-correlated COSY spectroscopic analysis (2) of the native and *O*-deacetylated O-PS I allowed the assignment of the anomeric resonances and all of the ring proton resonances (Table 1). The ^{13}C NMR spectrum of the *O*-deacetylated O-PS I (Fig. 2A) showed, as expected, seven carbon signals, and their assignments were made from a heteronuclear multiple quantum coherence experiment (Table 1). The ^{13}C NMR spectrum of the native O-PS I polysaccharide (Fig. 1B) showed signals at 21.0 ($\underline{C}H_3CO$) and 174.0 ($CH_3\underline{C}O$) ppm attributed to the *O*-acetyl substituent. Consideration of the negative specific optical rotation of both the native and *O*-deacetylated O-PS I and the $J_{C1,H1}$ values (165 Hz) determined from gated-decoupling experiments together with the high-field H-1 signals at 4.91 and 4.83 ppm seen in the respective native and *O*-deacetylated O-PS I ^{1}H NMR spectra established a β-D- assignment for the glycosidic linkages. The carbon chemical shift for the C-2 signal from 68.8 in the *O*-deacetylated O-PS I to 69.9 ppm in the native O-PS I is characteristic of an α-effect (20) of an acetyl substitution at the O-2 position of the heptose residue. The O-2 location of the acetyl group is also consistent with the H-2 down-field shift to 5.30 ppm seen in the ^{1}H NMR spectrum of the native O-PS I from 4.26 ppm in the *O*-deacetylated O-PS I (Table 1). The accumulated evidence leads to the characterization of the O-PS I as an unbranched linear homopolymer of 1,3-linked 2-*O*-acetyl-6-deoxy-β-D-*manno*-heptopyranosyl residues.

O-PS II had a $[\alpha]_D$ of −75.0° (c 0.21, water), contained *O*-acetyl (ca. 14%), and by GLC analysis was indicated to contain the glycoses (80%) D-glucose, 6-deoxy-L-talose, and 6-deoxy-2-*O*-methyl-L-talose in the molar ratio 1.0:0.70:0.30. A small amount (ca. 6%) of 6-deoxy-D-*manno*-heptose was present from contamination with O-PS I.

Methylation analysis of O-PS II by GLS-MS gave two products identified as 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-glucitol-1-d (T_{GM}, 1.30) and 1,3,5-tri-*O*-acetyl-6-deoxy-2,4-di-*O*-methyl-L-talitol-1-d (T_{GM}, 0.87) (1:1), showing that the component glycoses were involved in an unbranched linear polymer of 1,3-linked hexopyranosyl residues.

The ^{1}H NMR spectrum of the native O-PS II showed characteristic signals for two major anomeric peaks at 5.24 ($J_{1,2}$, ~2 Hz) and 4.62 ppm ($J_{1,2}$, 8.1 Hz) together with a minor anomeric signal at 5.42 ppm from 6-deoxy-2-*O*-methyl-L-talose residues, CH_3 of 6-deoxyhexoses at 1.11 to 1.28 ppm, *O*-acetyl at 2.17 to 2.14 ppm, and OMe at 3.44 ppm (Table 2). The ^{13}C NMR spectrum of native O-PS II (Fig. 3B) showed two major anomeric carbon signals at 102.4 ($J_{C1\text{-}H1}$, 161 Hz) and 99.3 ppm ($J_{C1\text{-}H1}$, 174 Hz) together with a minor C-1 signal at 98.6 ppm arising from the 6-deoxy-2-*O*-methyl-L-talose residues, 6-deoxyhexose CH_3 signals at 16.0 and 16.2 ppm, *O*-methyl at 58.9

TABLE 2. Proton NMR chemical shifts for *B. pseudomallei* O-PS II

Glycose residue	Chemical shift (ppm)[a]						
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′
Native O-PS II							
-3)-α-L-6dTal*p*-(1-	5.24 (~2)	5.24	4.25	3.96	4.36	1.25	
-3)-2-*O*-Me-α-L-6dTal*p*-(1-	5.42 (~2)	3.74	4.36	5.34	4.46	1.11	
-3)-β-D-Glc*p*-(1-	4.62 (8.1)	3.47	3.62	3.49	3.45	3.75	3.86
O-Deacetylated O-PS II							
-3)-α-L-6dTal*p*-(1-	5.29 (~2)	4.15	4.10	3.95	4.33	1.28	1.28
-3)-2-*O*-Me-α-L-6dTal*p*-(1-	5.37 (~2)	3.80	4.14	3.90	4.31	1.28	1.28
-3)-β-D-Glc*p*-(1-	4.68 (8.1)	3.55	3.64	3.49	3.49	3.78	3.94

[a] Coupling constants in hertz are given in parentheses.

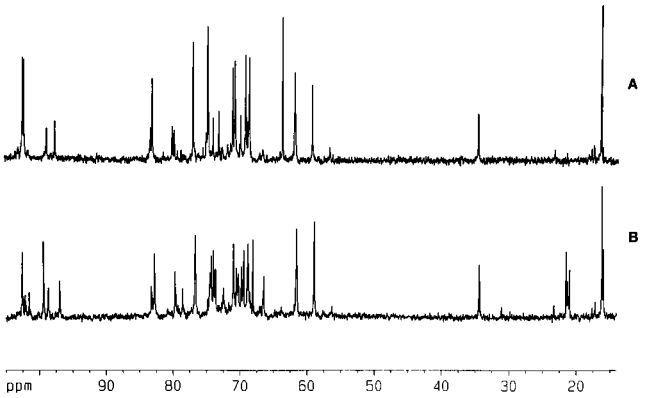

FIG. 3. ^{13}C NMR spectrum of *O*-deacetylated O-PS II (A) and native O-PS II (B) from *B. pseudomallei* 304b (ca. 6% contamination with O-PS I).

ppm, and *O*-acetyl signals at 174.2 and 174.5 ppm ($CH_3\underline{C}O$) and 21.2 and 21.4 ppm ($\underline{C}H_3CO$) (Table 2).

COSY NMR analysis (2) of both the native and *O*-deacetylated O-PS II allowed unambiguous assignment of all of the proton signals in the NMR spectrum of each polymer (Table 2). The chemical shifts of the anomeric protons and their $J_{1,2}$ coupling constants were consistent with the D-glucopyranosyl residues having the β-configuration and the 6-deoxy-L-talopyranosyl and 6-deoxy-2-*O*-methyl-L-talopyranosyl residues having the α-configuration. Assignment of signals in the ^{13}C NMR spectra of native and *O*-deacetylated O-PS II (Table 3), made from heteronuclear multiple quantum coherence experiments, were consistent with the structural deductions made from the ^1H NMR spectra as were the anomeric configurations determined from the $J_{C1,H1}$ coupling constants for the D-glucopyranosyl residues at 102.4 ppm (161 Hz) and for the 6-deoxy-L-talopyranoside residues at 98.6 and 99.3 ppm (173 to 174 Hz) (Table 3).

Location of the *O*-acetyl substituents determined from two-dimensional COSY analysis indicated essentially stoichiometric *O*-acetyl substitution at O-2 of the 6-deoxy-α-L-talopyranosyl residues and at O-4 of the 6-deoxy-2-*O*-methyl-α-L-talopyranosyl residues. The combined experimental evidence leads to the O-PS II being characterized as an unbranched polymer of disaccharide repeating units having the basic structure -3-)-β-D-glucopyranose-(1-3)-α-L-6-deoxy-talopyranose-(1- in which ca. one-third of the 6-deoxy-L-talose residues are substituted by 2-*O*-methyl and 4-*O*-acetyl groups and the residual two-third 6-deoxy-L-talose residues bear 2-*O*-acetyl substituents.

The unusual serological properties of *B. pseudomallei* 824a prompted a study of its LPS product. The LPS (4% yield) was isolated in equal yields from both the phenol- and water-phase cell extracts. Mild acetic acid hydrolysis of the LPS from both phases afforded a single homogeneous O-PS, which by chemical and NMR analyses as described above, was found to be identical to the 2-*O*-acetyl-6-deoxy-D-*manno*-heptan O-PS I. No trace of O-PS II was detected in the LPS preparations.

DISCUSSION

The structure of O-PS I and O-PS II described in this paper are similar to those reported by Knirel et al. (22) for several strains of *B. pseudomallei* isolated in Vietnam, although *O*-methyl substitutions in those isolates were not reported. Simultaneous production of two or more LPS O-PSs by a gram-negative bacterium is not unusual. *Salmonella boecker* (group H, O:16,14) produces two distinct O chains (8) while *Salmonella madelia* (O:1,6,14,25) has been demonstrated to produce three structurally different LPS O antigens (13).

The similarity between the structures of the O-PS of organisms from diverse geographical locations suggests that there may be only one serotype of *B. pseudomallei*. Indeed, homogeneity of the LPS of *B. pseudomallei* has been demonstrated previously serologically and by immunoblots with our collection of 44 clinical isolates from Thailand (9) and with a panel of 12 human and animal strains collected from different parts of Asia and Australia between 1923 and 1990 (26). The LPS of *B. pseudomallei* thus appears to be both highly conserved and constant, so that an anti-LPS vaccine against *B. pseudomallei* may be widely applicable. In a related study, we have shown that the previously reported protective monoclonal antibody is directed against O-PS I (18a). Since antibodies against the flagellin protein of *B. pseudomallei* have also been shown to be protective (5), a conjugate vaccine using these two components of the bacterium is currently being developed (27a). Such a vaccine will consist of defined antigenic components which can be monitored for purity and efficiency of conjugation. Furthermore, knowledge of the structure of the components will allow for modifications to enhance immunogenicity.

Both *B. pseudomallei* LPS O antigens are recognized by sera from patients with localized or septicemic *B. pseudomallei* infections (18a). Anti-LPS or anti-O-PS antibodies are not detected in controls. These findings suggest that antibodies against O-PS I and II would be of diagnostic value for an acute infection with *B. pseudomallei* in a setting where such infections are not endemic. However, since a number of asymptomatic individuals from an area of endemicity are seropositive, a positive result would be less indicative of an active infection under those circumstances.

The observed serological reaction of the *B. pseudomallei*

TABLE 3. Carbon-13 NMR chemical shifts for *B. pseudomallei* O-PS II

Glycose residue	Chemical shift (ppm)[a]					
	C-1	C-2	C-3	C-4	C-5	C-6
Native O-PS II						
-3)-α-L-6dTal*p*-(1-	99.3 (174)	70.9	74.0	69.4	68.0	16.2
-3)-2-*O*-Me-α-L-6dTal*p*-(1-	98.6 (173)	78.8	72.5	70.5	66.7	16.0
-3)-β-D-Glc*p*-(1-	102.4 (161)	74.8	82.7	68.7	76.6	61.5
O-Deacetylated O-PS II						
-3)-α-L-6dTal*p*-(1-	102.2 (174)	70.4	74.5	70.8	68.4	16.4
-3)-2-*O*-Me-α-L-6dTal*p*-(1-	98.6 (173)	79.6	74.6	70.7	68.5	16.3
-3)-β-D-Glc*p*-(1-	102.0 (161)	74.5	82.7	68.8	76.7	61.5

[a] Coupling constants in hertz are given in parentheses.

polyclonal antiserum with strains of *B. cepacia* serotype A is surprising since the LPS O chain of this bacterium was found to be a linear polymer of repeating trisaccharide units having the structure [-3)-α-D-Gal*p*NAc-(1-3)-β-D-Gal*p*NAc-(1-4)-α-L-Rha*p*-(1-] (6), unrelated to the O chains of *B. pseudomallei* and having no immediately obvious common epitopic features. Since this serological cross-reaction was observed only with polyclonal rabbit antiserum, it is possible that the positive reaction could have arisen from antibody directed against contaminating cross-reactive protein or against common epitopes in LPS core oligosaccharide moieties.

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