Amino Components of the Lipopolysaccharide from *Pseudomonas aeruginosa* N.C.T.C. 8505

PRESENCE OF 2,4-DIAMINO-2,4,6-TRIDEOXY-D-GLUCOSE

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Configurations were determined for previously identified amino components of the lipopolysaccharide from *Pseudomonas aeruginosa* N.C.T.C. 8505. Glucosamine and galactosamine belong to the D-series, and alanine and aminogalacturonic acid to the L-series. An additional amino component was identified as 2,4-diamino-2,4,6-trideoxy-D-glucose. This compound may be a characteristic component of the O-specific chain in lipopolysaccharides of strains of *Pseudomonas aeruginosa* belonging to Habs sero-group 3.

Recent studies have indicated that lipopolysaccharides from *Pseudomonas aeruginosa* have the same overall architecture as those from enterobacterial species. The respective lipid A and inner-core regions are closely analogous, being derived from phosphorylated oligosaccharides of glucosamine (lipid A) and 3-deoxy-2-octulonic acid with heptose (inner core). The outer-core region for *P. aeruginosa* is more distinctive, being composed of glucose, rhamnose, galactosamine and alanine residues (Chester *et al.*, 1973; Wilkinson & Galbraith, 1975; Drewry *et al.*, 1975). The O-specific fractions seem to be heterogeneous (Wilkinson & Galbraith, 1975; Chester & Meadow, 1975), but are characteristically rich in amino sugars. These include the common hexosamines (glucosamine and galactosamine) and 6-deoxyhexosamines (quinovosamine and fucosamine), as well as various unidentified compounds (Suzuki, 1974; Wilkinson & Galbraith, 1975; Drewry *et al.*, 1975; Koval & Meadow, 1975).

In the lipopolysaccharide from *P. aeruginosa* N.C.T.C. 8505, the known amino components of the O-specific fraction are glucosamine and 2-amino-2-deoxygalacturonic acid (Wilkinson & Welbourn, 1975). The main purpose of the present paper is to report the identification of a further component (Compound Y) as 2,4-diamino-2,4,6-trideoxy-D-glucose (D-bacillosamine). The opportunity to determine the configurations of other amino components of the lipopolysaccharide has also been taken. Most studies of lipopolysaccharides from *P. aeruginosa* have omitted this determination, the importance of which is evident from reports that some lipopolysaccharides contain both D- and L-fucosamine (Suzuki, 1969; Horton & Liav, 1976).

Materials and Methods

Materials

Alanine dehydrogenase (EC 1.4.1.1) and NAD⁺ were obtained from Boehringer Mannheim G.m.b.H., Mannheim, Germany. Galactose oxidase (EC 1.1.3.9) was obtained from Worthington Biochemical Corp., Freehold, NJ, U.S.A. in the form of the Galactostat reagent. The following materials were received as gifts: 2,4-diacetamido-2,4,6-trideoxy-D-glucose, from Professor N. Sharon; lipopolysaccharide from *Rhodopseudomonas viridis* (containing 2,3-diamino-2,3-dideoxy-D-glucose), from Dr. H. Mayer; lipopolysaccharide from *P. aeruginosa* PA1, from Dr. P. M. Meadow.

Methods

Preparation and hydrolysis of lipopolysaccharide. Lipopolysaccharide was obtained from isolated walls of *P. aeruginosa* N.C.T.C. 8505 as described by Wilkinson & Galbraith (1975). Amino compounds were released by hydrolysis with 6.1 M-HCl at 105°C for 4 h under N₂.

Paper chromatography and electrophoresis. Chromatography and high-voltage electrophoresis were carried out with Whatman papers (no. 1 for analytical separations, and water-washed no. 1 or 3MM for preparative work). Solvent systems used were: A, ethyl acetate/pyridine/water/acetic acid (5:5:3:1, by vol.); B, ethyl acetate/pyridine/water (5:2:5, by vol., upper phase); C, propan-1-ol/aq. 1% NH₃ (7:3, v/v). Buffer systems used were: D, pyridine/acetic acid/water (5:2:43, by vol.; pH 5.3); E, pyridine/formic acid/acetic acid/water (2:3:20:180,

by vol.; pH 2.8). Glucose was used to correct m_{GlcN} values for endosmosis. Compounds were detected by heating dry chromatograms, or by using ninhydrin (Cramer, 1955), alkaline AgNO_3 (Trevelyan *et al.*, 1950), aniline hydrogen oxalate (Charalampous & Mueller, 1953), periodate-Schiff reagents (Baddiley *et al.*, 1956), the Ehrlich and Elson-Morgan reagents (Partridge, 1948).

Thin-layer chromatography. Separations were carried out on layers of silica gel G (E. Merck A.-G., Darmstadt, Germany) with the following solvent systems: F, diethyl ether/toluene (2:1, v/v); G, ethyl acetate/acetone (2:1, v/v); H, acetone/ethanol (9:1, v/v). Spots were detected by charring (200°C) with 5% (v/v) H_2SO_4 in ethanol, or by exposure to I_2 vapour.

Gas-liquid chromatography. Aminoalditol acetates were examined by using a Pye 104 gas chromatograph with packed columns containing the following stationary phases: I, 3% (w/w) ECNSS-M at 200°C; II, 1% (w/w) OV-225 at 220°C; III, 2% (w/w) OV-17 at 220°C. Flow rates (N_2) were 30 ml/min in all cases.

Quantitative analyses. Amino compounds were determined by using an amino acid analyser (Locarte Co., London E.C.3, U.K.) with standard sodium citrate buffers (pH 3.25, pH 4.25, pH 5.28, pH 6.65). For Compound Y, determinations were also carried out by the ninhydrin method of Rosen (1957) and by the reaction with 2,4,6-trinitrobenzenesulphonic acid (Snyder & Sobocinski, 1975). Elson-Morgan reactions were carried out by the modifications of Randle & Morgan (1955) and Ludowieg & Benmaman (1968). Morgan-Elson reactions were carried out as described by Strominger *et al.* (1959), Brownlee & Wheat (1966), and (as applied to the determination of 3-amino-3-deoxyhexoses) Ashwell *et al.* (1965). Reducing-sugar values were determined by the method of Park & Johnson (1949). D-Galactosamine was determined by the method of Sempere *et al.* (1965), and L-alanine by the method of Yoshida (1965). Acetaldehyde was determined by the method of Kabat & Mayer (1961) and confirmed by a micro-distillation adaptation of the method (Simmons, 1969), after oxidation of Compound Y with 10 mM- NaIO_4 at 25°C.

Miscellaneous methods. The preparative separation of glucosamine and galactosamine was carried out with a column (0.9 cm $\times$ 53 cm) of Dowex 50 (200–400 mesh, H^+ form) and elution with 0.33 M-HCl at 4 ml/h (Crompton, 1959). N-Acetylation of amino sugars was carried out by the methods of Strominger *et al.* (1959) and Levvy *et al.* (1970). Reduction of Compound Y was carried out with NaBH_4 in 0.05 M- H_3BO_3 at 4°C. The excess of reducing agent was destroyed by the addition of dilute acetic acid, and H_3BO_3 was removed by distillation as trimethyl borate. The reduced Compound Y was peracetylated either by treatment with acetic anhydride in the

presence of residual sodium acetate (Metz *et al.*, 1971), or by treatment with acetic anhydride/pyridine (Niedermeier, 1971) after removal of the salt by preparative paper electrophoresis. Optical rotations were determined with a Bendix model 143A polarimeter and a 1 cm cell.

Results and Discussion

Isolation procedures

Lipopolysaccharide (about 300 mg) was hydrolysed under N_2 with 6.1 M-HCl at 105°C for 4 h. The dark-brown hydrolysate was dried repeatedly *in vacuo* over P_2O_5 and KOH. After filtration (glass sinter, no. 3 porosity), the soluble products were initially fractionated by preparative paper electrophoresis at pH 5.3. Three ninhydrin-positive fractions were collected: a 'neutral' fraction (mainly alanine and 2-amino-2-deoxygalacturonic acid), a 'hexosamine' fraction (essentially glucosamine and galactosamine), and a more mobile cationic fraction (Compound Y). The two hexosamines were separated by chromatography on a column of cation-exchange resin previously calibrated with reference compounds, the eluate being monitored by the method of Randle & Morgan (1955). Much of the brown material in the 'neutral' fraction was removed by further preparative electrophoresis at pH 2.8. Preparative paper chromatography (solvent system A) then gave alanine and aminogalacturonic acid chromatographically pure. Control eluates from appropriate blank areas of paper were prepared for all compounds isolated from chromatograms or electrophoretograms. Eluates of amino sugars were acidified (with HCl) before being dried.

Alanine

The compound had the properties of authentic alanine on paper chromatography, electrophoresis and autoanalysis. About 22 μmol was obtained (measured by autoanalysis), all of which was measured enzymically as the L isomer. The value for the specific rotation ($[\alpha]_D^{20} + 13 \pm 4^\circ$, c 0.15 in 1 M-HCl) supported this assignment. The alanine in the lipopolysaccharide of *P. aeruginosa* N.C.T.C. 1999 is also the L isomer (S. G. Wilkinson & L. Galbraith, unpublished work). L-Alanine has also been found in a lipopolysaccharide from *Proteus mirabilis* (Gmeiner, 1975). These lipopolysaccharides therefore differ from those bacterial wall polymers (e.g. peptidoglycan and teichoic acid) into which D-alanine can be incorporated.

Glucosamine

The compound had the expected mobilities and reactions on paper chromatography (solvent system

A), electrophoresis (buffer system D), preparative ion-exchange chromatography and autoanalysis (continuous elution with citrate buffer, pH 5.28). The identification of glucosamine was confirmed by paper chromatography of the *N*-acetyl derivative (solvent system A), and by g.l.c. of the aminoalditol acetate (stationary phase I). Further confirmation was provided by the insignificant Elson–Morgan reactivity after treatment with alkaline acetylacetone in the cold (Ludowieg & Benmaman, 1968). The specific rotation of the hydrochloride, based on a yield of 90 μ mol (autoanalysis), was $[\alpha]_D^{20} + 71.4^\circ$ (*c* 1.0 in water), in close agreement with literature values for the D isomer. As the glucosamine was derived partly from lipid A and partly from the O-specific fraction, it is apparently the D isomer in both regions of the lipopolysaccharide.

Galactosamine

The compound was identified as galactosamine by applying the tests listed above for glucosamine. The R_{GlcN} value on ion-exchange chromatography was 1.17, in accord with published values (Wheat, 1966). As expected, the compound gave the same absorbance in both the 'hot' and 'cold' versions of the Elson–Morgan reaction described by Ludowieg & Benmaman (1968). The specific rotation of the hydrochloride, based on a yield of 21 μ mol (autoanalysis), was $[\alpha]_D^{20} + 95.7^\circ$ (*c* 0.23 in water), correct for D-galactosamine hydrochloride. The assignment was confirmed by the complete reaction of the amino sugar on treatment with galactose oxidase (Sempere *et al.*, 1965).

Aminogalacturonic acid

Detailed evidence for the identity of this component has already been given (Wilkinson & Welbourn, 1975). The inactivity of galactose oxidase against its reduction product (galactosamine) indicated that the sugar belonged to the L series. The product obtained during the present studies was clearly and strongly laevorotatory, but the small amount available (about 15 μ mol) and the lack of authentic material for the calibration of analytical methods precluded the precise determination of its specific rotation. However, aminogalacturonic acid is stated to give the same molar extinction as galactosamine in one version of the Morgan–Elson reaction (Brownlee & Wheat, 1966). On this basis, $[\alpha]_D^{20}$ for the hydrochloride (*c* about 0.3 in water) was calculated as -113° . The corresponding value based on the assumption of equal extinctions on reaction with ninhydrin during autoanalysis was -80° . Values recorded for 2-amino-2-deoxy-D-galacturonic acid are $+84.5^\circ$ (*c* 1 in dilute HCl, pH 2; Heyns & Beck, 1957) and $+90^\circ$ (*c* 0.06 in water;

Liau *et al.*, 1974). The compound from *P. aeruginosa* can therefore be identified as the previously unknown L isomer.

Compound Y

This basic component was readily isolated by means of paper electrophoresis, in which its mobility was intermediate between the mobilities of hexosamines and basic amino acids, on the one hand, and ethanolamine and polyamines, on the other. Values for m_{GlcN} were about 1.8 with both buffer systems D and E. The compound reduced alkaline $AgNO_3$, and on heating with ninhydrin it gave a brown spot which gradually turned pink–purple. Although the compound was periodate-sensitive (see below), it was not detected with the periodate–Schiff reagents, at least in the amount readily detected by other methods. On prolonged heating with aniline hydrogen oxalate, it gave a light-brown spot with blue fluorescence under u.v. light (glucosamine gave a light-brown spot with yellow fluorescence). The compound reacted with the Elson–Morgan reagents in the same manner and at about the same rate as glucosamine, but did not give a pink colour on treatment with the Ehrlich reagent alone. During heating of a dry chromatogram, Compound Y gave a yellow–brown spot with white fluorescence: this reaction presumably complicated the results of other tests in which treated chromatograms were heated.

On paper chromatography (solvent system A), Compound Y was marginally less mobile than glucosamine (R_{GlcN} about 0.94). When solvent system C was used on an ascending chromatogram, two spots (R_{GlcN} 1.13 and 1.71, possibly linked by a streak) were noted, indicating decomposition in this basic system. On autoanalysis with an elution programme used for the full range of amino acids (final pH 6.65), Compound Y was detected as a broad low peak about 15 min after arginine, with the ratio A_{570}/A_{440} about 1.8:1. With a short column (0.9 cm $\times$ 12 cm), from which glucosamine was eluted in less than 1 h with citrate buffer, pH 5.28, Compound Y was not eluted after 6 h (or was eluted too gradually to be detected).

The major product obtained by treating Compound Y with $NaBH_4$ had unchanged electrophoretic mobility (pH 5.3), and also reduced alkaline $AgNO_3$. However, the reaction with ninhydrin produced a stable yellow colour. A trace product with similar reactions but slightly greater mobility was detected but not studied further. Peracetylation of the reduction products gave material which remained at the origin on t.l.c. with solvent system F, in which glucitol hexa-acetate and glucosaminitol hexa-acetate had R_f values of 0.47 and 0.04 respectively. (T.l.c. in the more polar solvent system

G revealed a more complex mixture, but the major product had R_{GlcN} about 0.27.) Elution of the immobile material, followed by acid hydrolysis (6.1M-HCl at 105°C for 4h) and electrophoresis, confirmed that it was derived from the major ninhydrin-positive product. G.l.c. of the peracetate(s) showed a single component with retention times relative to those of glucosaminitol hexa-acetate as follows: stationary phase I, 1.96; II, 1.48; III, 0.88. The significance of the reversed order of elution with stationary phase III (OV-17) is not clear. As this phase is the least polar of the three, a possible inference is that the product from Compound Y is more polar and more volatile than glucosaminitol hexa-acetate. However, it should be noted that the formation of decomposition products during g.l.c. can be a particular problem with aminoalditol acetates (e.g. Perry & Webb, 1968; Griggs *et al.*, 1971).

The results so far described indicated that Compound Y was a 2-amino sugar (positive Elson-Morgan reaction) with an additional basic function. Supporting evidence came from *N*-acetylation studies. When this was done by the method of Strominger *et al.* (1959), the yield of reducing products (assessed by electrophoresis of equivalent amounts of untreated and acetylated materials) seemed rather low. To avoid the possible loss of basic products during the cation-exchange step used to remove the NaHCO_3 catalyst, the method of Levvy *et al.* (1970) was adopted. In this method, *N*-acetylation is catalysed by the use of an anion-exchange resin (CO_3^{2-} form). Similar amounts of neutral and basic products reducing alkaline AgNO_3 were usually obtained thereby from Compound Y in a single treatment: two treatments gave almost entirely the neutral product. Compound Y-A1, the major basic product, had m_{GlcN} 0.90 at pH 5.3, gave a yellow-brown colour with ninhydrin, a strong violet colour (similar to that from *N*-acetylglucosamine) with the Elson-Morgan reagents, and a similar but weaker colour with the Ehrlich reagent alone (in contrast with the yellow given by Compound Y and glucosamine). When the isolated product was re-examined by electrophoresis after prolonged storage at -20°C, an additional spot (m_{GlcN} about 0.5) linked to compound Y-A1 by a streak was found. The new product (and the streak) also reduced alkaline AgNO_3 , gave a violet colour with the Elson-Morgan reagents and a blue-violet colour in the direct Ehrlich test, but was not detected with ninhydrin. A minor basic product of the original *N*-acetylation was fractionally less mobile than Compound Y-A1, reduced alkaline AgNO_3 and gave a purple colour with ninhydrin. Compound Y-A2, the neutral *N*-acetylation product, was ninhydrin-negative and gave only a faint violet colour with the Ehrlich reagent (alone or as part of the Elson-Morgan treatment). On acid hydrolysis (4M-HCl at 105°C for 2h), it gave Compound Y,

Compound Y-A3, a product (m_{GlcN} 1.13 at pH 5.3) detected only by the yellow colour given with the Ehrlich reagent, and red-brown decomposition products. Compound Y-A3 had the same electrophoretic mobility as Y-A1, reduced alkaline AgNO_3 , gave the typical pink colour in the Elson-Morgan reaction, a yellow spot in the direct Ehrlich test, and a purple one with ninhydrin. These combined results indicated that Compound Y contained two basic functions amenable to *N*-acetylation, giving Compound Y-A2. Monoacetylation gave preferentially a 2-acetamido derivative (Compound Y-A1), in which the less reactive basic function was implicated in the unusual colour reactions with ninhydrin and the Ehrlich and Elson-Morgan reagents, and in the formation of a ninhydrin-negative product on storage. Monodeacetylation of Compound Y-A2 by hydrolysis gave preferentially a 2-amino derivative (Compound Y-A3).

When Compound Y was subjected to *N*-acetylation followed by application of the Morgan-Elson reaction for 2-acetamido sugars (Strominger *et al.*, 1959; Brownlee & Wheat, 1966) or, suitably extended, for 3-acetamido sugars (Ashwell *et al.*, 1965), atypical results were obtained. Whereas the reaction mixtures for authentic reference compounds slowly gave the expected purple-violet colour (A_{max} , 585nm) when warmed with the Ehrlich reagent, reaction mixtures from Compound Y gave a pink colour (A_{max} , 550nm) immediately on addition of the reagent. The colour tended to fade during subsequent warming. When a control reaction was carried out without prior *N*-acetylation, a yellow solution (A_{max} , 415nm) was obtained. It is likely that the pink colour observed in the test mixture for Compound Y corresponds to that produced on electrophoretograms (notably for Compound Y-A1) sprayed with the Ehrlich reagent, either directly or after alkaline treatment of the paper, rather than to a true Morgan-Elson reaction.

Although these results have yet to be explained fully, a negative Morgan-Elson reaction is indicative of substitution in the 4-position of a hexosamine, and is characteristic of, for example, 2,4-diacetamidohexoses (Sharon & Jeanloz, 1960). Also, it should be noted that *N*-acetylated prumycin (4-D-alanyl amino-2-amino-2,4-dideoxy-L-arabinose) gives an atypical red colour in the Morgan-Elson reaction (Omura *et al.*, 1972, 1974), and that at least some 4-amino sugars give a direct Ehrlich reaction (e.g. Lee & Schaffner, 1966; Redmond, 1975). With an unidentified 2-acetamido-4-amino-2,4,6-trideoxyhexose, the coloured product had A_{max} , 550nm and had low heat-stability (Distler *et al.*, 1966). The formation of yellow-brown products on heating alone and during the reaction with ninhydrin is also characteristic of 4-amino sugars (e.g. Wheat *et al.*, 1962; Jann & Jann, 1967; Volk *et al.*, 1970). Another characteristic

Table 1. *Quantitative determinations of Compound Y*
The total amount of Compound Y isolated, determined by various methods, is expressed in terms of glucosamine. Rhamnose was used as the standard in the determination of acetaldehyde liberated during periodate oxidation.

Determination	Method	Amount (μmol)
Reducing sugar	Park & Johnson (1949)	7.75
Amino groups (ninhydrin)	Rosen (1957)	7.61
Amino groups (2,4,6-trinitrobenzene-sulphonic acid)	Snyder & Sobocinski (1975)	15.37
Hexosamine	Rondle & Morgan (1955)	5.41
Acetaldehyde	Kabat & Mayer (1961)	8.28

property of these compounds is the pH-dependent formation of equilibrium mixtures containing *N*-heterocycles (including pyrrolidine, pyrroline and bis-pyrrolidine structures), because of the ready participation of the 4-amino group in cyclization reactions (e.g. Paulsen, 1966; Paulsen *et al.*, 1970). Such reactions clearly complicate attempts to form derivatives of the free amino sugars, and could also explain the behaviour of Compound Y during paper chromatography (solvent system C) and of Compound Y-A1 during storage. Further evidence of reversible isomerization of Compound Y was obtained when the hydrochloride was treated with anion-exchange resin (CO_3^{2-} form) as a control for *N*-acetylation. On electrophoresis, the product moved as a streak from the origin to the position of Compound Y. After treatment with acid (4M-HCl at 105°C for 2h), the original compact spot was obtained. Compound Y is therefore considered to be a 2,4-diamino sugar. Apart from the negative result in the test for a 3-amino group (which would be invalidated by the additional presence of a 2-amino group), the possibility of a 2,3-diamino sugar seemed unlikely from an electrophoretic comparison of Compound Y with 2,3-diamino-2,3-dideoxy-D-glucose. The latter compound had an m_{GlcN} value of only 1.2 at pH 5.3.

To extend the characterization of Compound Y, various quantitative determinations were made with glucosamine hydrochloride as reference compound. The results (Table 1) show good agreement for the determinations of reducing power and ninhydrin-reactive amino groups. In the reaction with ninhydrin, A_{max} was close to 570nm for both Compound Y and glucosamine, but the former gave much greater absorbance at 440nm (values of A_{570}/A_{440} were 2.1 and 16.2 respectively). The presence of a second amino group was clearly

indicated by the reaction with 2,4,6-trinitrobenzene-sulphonic acid. The reaction occurred more slowly than reported for amino acids, but was largely complete by 3h when final readings were taken. As expected (Kelleher & Smith, 1968), A_{max} was close to 480nm for both amino sugars. In the Rondle-Morgan (1955) version of the Elson-Morgan reaction, Compound Y gave A_{max} 530nm, similar to the value for glucosamine, but apparently a lower value of s . In other versions of the reaction (Ludowieg & Benmaman, 1968), colour yields were even lower (36% of the Rondle-Morgan value in the 'hot' reaction, and even less in the 'cold').

The formation of acetaldehyde (molar proportion 1.07, based on the determination of Compound Y as reducing sugar) during periodate oxidation suggested that Compound Y was a 2,4-diamino-2,4,6-trideoxyhexose. The periodate resistance of Compound Y-A2 (based on the electrophoretic comparison of a hydrolysate of material treated with 10mM-NaIO₄ at 4°C for 3 days with a control hydrolysate) is consistent with this suggestion. The only confirmed natural product of this class is 2,4-diamino-2,4,6-trideoxy-D-glucose, isolated as the 4-*N*-acetyl derivative from a polysaccharide of *Bacillus licheniformis* (Sharon & Jeanloz, 1960; Zehavi & Sharon, 1973), although similar material has been isolated from a nucleotide (Distler *et al.*, 1966) and the C-substance (Brundish & Baddiley, 1968) of *Streptococcus pneumoniae*. Compound Y-A2 had the same mobility as 2,4-diacetamido-2,4,6-trideoxy-D-glucose on t.l.c. in solvent system H (which resolves it from the *altro*-, *ido*- and *tal*-compounds; Liav & Sharon, 1973), and on paper chromatography in solvent system B. The components of an acid hydrolysate of the reference compound were identical in their mobilities and reactions with those from Compound Y-A2, on electrophoresis at pH 5.3. Compound Y was compared with the corresponding product isolated from the hydrolysate by the following methods; (a) paper electrophoresis (buffer system E); (b) paper chromatography (solvent systems A and C); (c) autoanalysis; (d) t.l.c. (solvent system G) of the peracetylated reduction product; (e) g.l.c. (stationary phases II and III) of the peracetylated reduction product. No separation was found in any instance. On the basis of these results, Compound Y is identified as 2,4-diamino-2,4,6-trideoxyglucose, Compound Y-A1 as the 2-*N*-acetyl derivative, Compound Y-A3 as the 4-*N*-acetyl derivative, and Compound Y-A2 as the 2,4-di-*N*-acetyl derivative. Previous attempts to isolate the free amino sugar after hydrolysis of its derivatives were unsuccessful (Zehavi & Sharon, 1973), and its physical constants are therefore unknown. However, Compound Y (as the hydrochloride in water) was strongly dextrorotatory, as are the 4-*N*-acetyl and 2,4-di-*N*-acetyl derivatives

of D-bacillosamine (Sharon & Jeanloz, 1960; Zehavi & Sharon, 1973; Liav *et al.*, 1974), and is probably the D isomer. In solution as the hydrochloride, Compound Y probably has the diamino-pyranose structure, rather than a bis-pyrrolidine structure (such as predominates for free 4-amino-4-deoxyglucose; Paulsen *et al.*, 1970) or a pyrrolidine structure (such as predominates for free 2,4-diamino-2,4-dideoxygalactose; Paulsen & Grage, 1974). This was indicated by the measurement, even under the mildly alkaline reaction conditions, of two amino groups by the 2,4,6-trinitrobenzenesulphonic acid method (which will not measure the pyrrolidine imino group of proline; Okuyama & Satake, 1960), and by the formation of a di-N-acetyl derivative (Compound Y-A2) apparently identical with the synthetic compound of known pyranose structure (Liav *et al.*, 1974).

The isolated yield of Compound Y [less than 1% (w/w) of the lipopolysaccharide] is probably much less than the actual content. Despite its successful isolation from a conventional hydrolysate, the acid lability of 4-amino sugars often calls for exceptional conditions of hydrolysis (e.g. Volk *et al.*, 1970; Redmond, 1975), and considerable decomposition was noted during acid hydrolysis of 4-acetamido-2-amino-2,4,6-trideoxy-D-glucose (Zehavi & Sharon, 1973). The acid resistance of the glycosidic bond from residues containing a free 2- or 4-amino group (Paulsen, 1966) is a further obstacle to obtaining good yields of the free amino sugar.

To determine the location of Compound Y in the lipopolysaccharide, hydrolysates were prepared from fractions corresponding to O-specific and core regions (Wilkinson & Galbraith, 1975). Like aminogalacturonic acid, Compound Y was a component of the O-specific fraction. The inference that it may contribute to the serological specificity is supported by the finding of a compound with the same properties (paper chromatography, electrophoresis and autoanalysis) in the lipopolysaccharide from another strain (PAC1) of *P. aeruginosa* belonging to Habs sero-group 3. The identification of Compound Y adds to the range of novel 4-amino sugars (4-amino-4-deoxy-L-arabinose and 4-amino-4,6-dideoxy-D-hexoses with the *gluco*-, *manno*- and *galacto*-configurations) found in bacterial lipopolysaccharides.

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