Presence of 3-*O*-Methyl-L-xylose in the Lipopolysaccharide of *Pseudomonas maltophilia* N.C.T.C. 10257

By FRANKLAND BROWN, DAVID J. NEAL and STEPHEN G. WILKINSON
Department of Chemistry, University of Hull, Kingston-upon-Hull HU6 7RX, U.K.

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3-*O*-Methyl-L-xylose was isolated from whole cells of *Pseudomonas maltophilia* N.C.T.C. 10257. The sugar is a component of lipopolysaccharide from which a polysaccharide also containing L-rhamnose and L-xylose was released by mild acid hydrolysis. 3-*O*-Methyl-L-xylose was absent from five other strains of *Ps. maltophilia* and one strain of *Pseudomonas geniculata*.

Neutral monosaccharides detected in isolated envelopes from *Pseudomonas maltophilia* N.C.T.C. 10257 were rhamnose, xylose, mannose, glucose, galactose and an unidentified sugar (Wilkinson, 1968). The high paper-chromatographic mobilities and the colour reactions of this sugar ('Compound X') suggested that it was an *O*-methyl derivative of an aldopentose. The characterization of Compound X and its location in lipopolysaccharide are now reported.

Materials and Methods

Cells of *Ps. maltophilia* N.C.T.C. 10257 were grown for 24 h (or 16 h for the preparation of envelopes) at 37°C in nutrient broth no. 2 (Oxoid Ltd., London E.C.4, U.K.). Envelopes were prepared and used for the extraction of lipopolysaccharide as described by Key *et al.* (1970). Degraded polysaccharide, obtained by hydrolysis of lipopolysaccharide with 1% (v/v) acetic acid at 100°C for 2 h, was fractionated by chromatography on Sephadex G-50 (Pharmacia, Uppsala, Sweden) as described by Drewry *et al.* (1971).

Monosaccharides were released by hydrolysis with 0.5M-H₂SO₄ at 100°C for 1 h (whole cells) or with 2M-HCl at 105°C for 2 h (polysaccharide). After neutralization with BaCO₃ and deionization, hydrolysates from whole cells were fractionated on a column [(34.5 cm × 4.8 cm) (14 in × 1.5 in)] of Whatman CC31 cellulose at 4°C. The samples (about 1.5 g) were applied in and eluted with solvent system A [butan-1-ol/ethanol/water (4:1:1, by vol.)]. Fractions (10 ml) were examined by paper chromatography with solvent system B [ethyl acetate/pyridine/water (5:2:5, by vol., upper phase)]. Fractions 40–55, containing Compound X, were combined, and the product was purified by preparative chromatography on Whatman no. 3MM paper with solvent

systems A (to remove rhamnose) and B (to remove glycerol). Further samples of Compound X, rhamnose and xylose were obtained by direct paper chromatography of deionized hydrolysates of (lipo)-polysaccharide. Compounds were detected by using alkaline AgNO₃ (Trevelyan *et al.*, 1950), aniline hydrogen phosphate (Bryson & Mitchell, 1951) and periodate/Schiff reagents (Baddiley *et al.*, 1956).

Methods given by Drewry *et al.* (1975) were used for the following: preparation of alditols and methyl glycosides; acetylation of aldoses and alditols; paper electrophoresis of aldoses and aldonic acids; determination of rhamnose by using cysteine/H₂SO₄. Trimethylsilyl derivatives of aldoses and alditols were prepared by the method of Sweeley *et al.* (1963). Pentoses were determined by the orcinol reaction (Umbreit *et al.*, 1972) and the aniline reaction (Goodwin, 1972). Demethylation of Compound X was done by the methods of Saier & Ballou (1968) or McOmie *et al.* (1968). After purification by paper chromatography, samples of the product (alone, and mixed with either D- or L-xylose) were converted into osazones by the method of Clarke & Humphreys (1966). Other samples were treated with glucose oxidase (EC 1.1.3.4) and peroxidase (EC 1.11.1.7) as described by Weckesser *et al.* (1971).

N.m.r. (nuclear-magnetic-resonance) spectra were recorded by using a JEOL 4H-100 spectrometer, and optical rotations were determined by using a Bendix 143A polarimeter. Mass spectra of aldose peracetates were obtained by using an AEI MS902 spectrometer, and the spectrum of the alditol acetate from Compound X was obtained by combined g.l.c.-mass spectrometry with an LKB model 2091 spectrometer. G.l.c. separations were carried out with a Pye 104 chromatograph and packed columns containing the following stationary phases: I, 10% (w/w) poly-(2,2-dimethylpropane-1,3-diol sebacate); II, 3% (w/w) ECNSS-M; III, 3% (w/w) SE-52.

Results and Discussion

The characterization of Compound X was largely achieved with material isolated from whole cells. Its chromatographic isolation was facilitated by its high mobilities, e.g. $R_{\text{Rhamnose}} = 1.2$ with solvent system B. Colorimetric determinations with orcinol or aniline confirmed its reactivity as a pentose. Evidence for the suspected *O*-methyl substituent was provided by the n.m.r. spectrum of Compound X in $[\text{D}_5]\text{pyridine}$, which contained two prominent singlets near 6.1τ , consistent with an anomeric mixture. The parent pentose, obtained by demethylation of Compound X with BBr_3 , was identified as xylose by the following criteria: (a) paper chromatography of the aldose and alditol (solvent systems A and B); (b) high-voltage electrophoresis in sodium borate buffer, pH 9.2; (c) g.l.c. of the peracetates and the pertrimethylsilyl ethers of the aldose (columns I at 200°C and III at 150°C respectively), and of the alditol acetate (column I at 200°C).

Although the amount of xylose obtained was insufficient for the determination of its specific rotation, the product (like Compound X) was clearly laevorotatory. The *L*-configuration was confirmed by comparing the osazones prepared from the demethylation product alone and mixed with comparable amounts of *D*- and *L*-xylose. The osazones from individual isomers and the mixture of demethylation product and *L*-xylose formed long needles, whereas that from the mixture with *D*-xylose formed irregular plates characteristic of the racemate. No xylonic acid was formed when the demethylation product was incubated with glucose oxidase and peroxidase under conditions that led to complete oxidation of *D*-xylose.

Initial evidence for the location of the *O*-methyl substituent at C-3 came from studies of the alditol derived from Compound X. On a paper chromatogram sprayed with the periodate/Schiff reagents, this derivative ($R_{\text{Glycerol}} = 0.87$ with solvent system B) gave a purple spot which developed a yellow halo then turned green. These colour changes, indicating the formation of both formaldehyde and malondialdehyde (Hardy & Buchanan, 1963), could only arise with 3-*O*-methyl-*L*-xylitol. This inference was confirmed by further studies of the alditol acetate. On g.l.c. (column II at 160°C) this derivative had a retention time relative to that for 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol of 2.93, close to the value (2.92) quoted for 1,2,4,5-tetra-*O*-acetyl-3-*O*-methylxylitol (Björndal *et al.*, 1970). The *O*-methyl signal at 6.48τ in the n.m.r. spectrum of the alditol acetate in C^2HCl_3 matched that for the authentic compound, and differed slightly from that (6.53τ) determined for the 2-*O*-methyl isomer. The mass spectrum of the derivative contained the expected peaks at m/e 275, 189, 129, 87 and 43 (Björndal *et al.*, 1970). Additional peaks at m/e 276, 190, 130 and 88

appeared when NaB^2H_4 in $^2\text{H}_2\text{O}$ was used for the reduction of Compound X. The identification of Compound X as 3-*O*-methyl-*L*-xylose was completed by comparison with authentic 3-*O*-methylxylose as follows: (a) paper chromatography with seven solvent systems; (b) high-voltage electrophoresis in borate buffer, pH 9.2; (c) g.l.c. of the peracetates (column I at 200°C), the pertrimethylsilyl derivatives (column III at 140°C) and the methyl glycosides (column II at 150°C); (d) mass spectrometry of the peracetates (De Jongh & Biemann, 1963).

Partially methylated sugars (including 2-, 3- and 4-*O*-methylxylose) have been increasingly reported as components of bacterial polysaccharides in recent years, and 3-*O*-methyl-*L*-xylose has been found in lipopolysaccharides from *Rhodopseudomonas viridis* (Weckesser *et al.*, 1971). In addition to this sugar, the lipopolysaccharide from *Ps. maltophilia* N.C.T.C. 10257 contained rhamnose (the major component), xylose, glucose and mannose. Fractionation on Sephadex G-50 of the water-soluble products obtained by mild hydrolysis of the lipopolysaccharide with 1% (v/v) acetic acid showed that the aldohexoses were mainly present in a phosphorylated oligosaccharide provisionally designated the core unit. Rhamnose, 3-*O*-methylxylose and xylose (molar proportions about 15:4:1) were confined to a polysaccharide fraction excluded by Sephadex G-50. Both the rhamnose ($[\alpha]_{\text{D}}^{20} + 8^\circ$, c 3 in water) and the xylose (resistant to glucose oxidase) were the *L*-isomers.

3-*O*-Methyl-*L*-xylose is consistently present in lipopolysaccharides from *Rh. viridis* (Weckesser *et al.*, 1974), and different methylated sugars are found in polysaccharides from other phototrophic bacteria (Weckesser *et al.*, 1970, 1973, 1975; Hurlbert *et al.*, 1976). Whole cells from other strains of *Ps. maltophilia* (N.C.T.C. 10258 and 10259; N.C.I.B. 9201, 9202 and 9204) and a non-fluorescent strain (N.C.I.B. 9428), listed as *Ps. geniculata* but more likely to be *Ps. maltophilia* (Mandel, 1966; Wilkinson, 1967), were examined for neutral sugar composition by paper chromatography (solvent system B). No 3-*O*-methylxylose was detected, although xylose was probably present in all strains except strains N.C.I.B. 9204 and 9428. Neutral sugars in strain N.C.I.B. 9204 included arabinose and a sugar with the chromatographic mobility of Compound X but the reactions of a hexose.

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