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Note

Structure of the oligosaccharide component of a serologically active phenolic glycolipid isolated from *Mycobacterium leprae**

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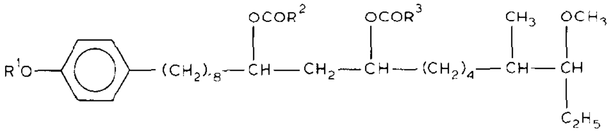
A phenolic glycolipid can be extracted[1] in relatively high amounts (~2% of the dry weight) from *Mycobacterium leprae* isolated from infected armadillo liver. This glycolipid or mycoside[2] (**1**) is a *p*-substituted phenolic glycoside and possesses a branched glycol chain, the hydroxyl functions of which are esterified with branched-chain fatty acids. A recent communication[2] suggested that the carbohydrate moiety of this glycolipid is a partially methylated glucosyl-rhamnosyl trisaccharide, but the anomeric configurations of the components were not determined. We now confirm the composition of, and the linkage positions within, the trisaccharide and present evidence that the three components are β-D-glucopyranosyl, α-L-rhamnopyranosyl, and α-L-rhamnopyranosyl (the absolute configurations are assumed).

Methanolysis of the purified mycoside (see Experimental) gave three glycosides (g.l.c. after trimethylsilylation) before, and two glycosides (which corres-



1 R^1 = oligosaccharide ; R^2,R^3 = branched-chain fatty acids

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*Preliminary communication, P. Draper, S. N. Payne, and E. Tarelli, Abstr. Roy. Soc. Chem. Carbohydrate Group Meeting, University of Birmingham, April 5–7th, 1982.

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ponded to rhamnose and glucose; molar ratio of 2:1) after, *O*-dealkylation (BCl_3–CH_2Cl_2). Acid hydrolysis of the *O*-dealkylated mycoside gave (t.l.c.) only rhamnose and glucose. Using standard procedures[3], g.l.c.–m.s. of the alditol acetates derived from the mycoside revealed 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methylhexitol, 1,4,5-tri-*O*-acetyl-6-deoxy-2,3-di-*O*-methylhexitol, 1,2,4,5-tetra-*O*-acetyl-6-deoxy-3-*O*-methylhexitol, and from the methylated mycoside, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol, 1,4,5-tri-*O*-acetyl-6-deoxy-2,3-di-*O*-methylhexitol, and 1,2,5-tri-*O*-acetyl-6-deoxy-3,4-di-*O*-methylhexitol. Thus, the oligosaccharide is a trisaccharide comprising a terminal 3,6-di-*O*-methylglucopyranosyl group together with a 4-linked 2,3-di-*O*-methylrhamnopyranosyl residue and a 2-linked 3-*O*-methylrhamnopyranosyl residue. Identical results have been reported[2] using similar methods. Furthermore, partial hydrolysis studies[4] with acid have shown that the 2,3-di-*O*-methylrhamnose is the central residue and therefore the oligosaccharide chain is *O*-(3,6-di-*O*-methyl-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-L-rhamnose.

The anomeric configurations within this trisaccharide were assigned on the basis of 270-MHz ^{1}H-n.m.r. data ($CDCl_3$) for the mycoside. The resonances in the

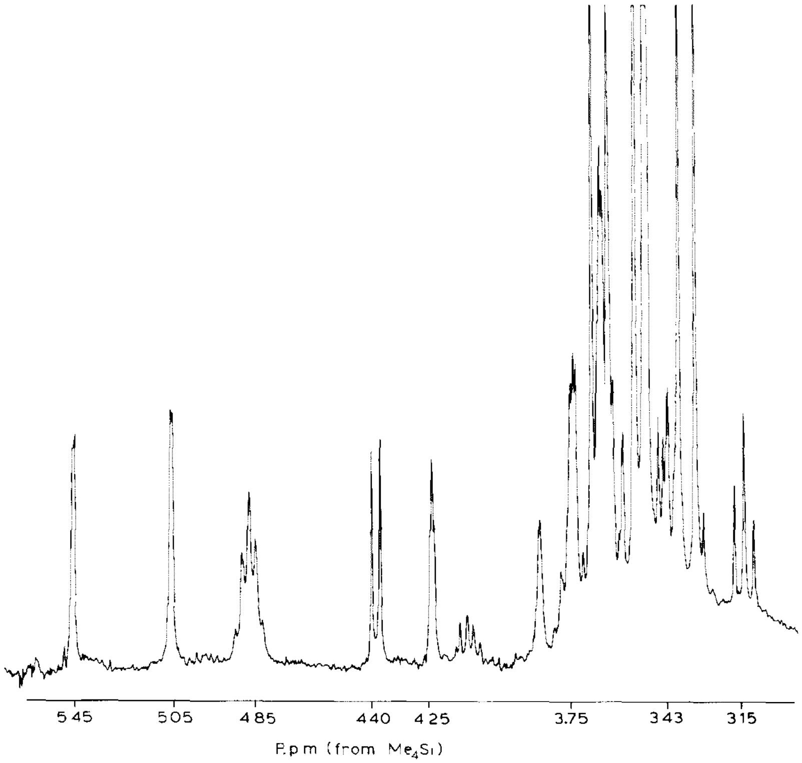


Fig. 1. ^{1}H-N.m.r. spectrum (3–5.5 p.p.m.) of a 5% solution of the mycoside in $CDCl_3$ at 270 MHz and 25°.

region for anomeric and other deshielded protons (δ 4–5.5) are shown in Fig. 1. The 2-proton quintet centred at δ 4.85 can be attributed to the two methine protons located at the ester linkages in the lipid chain. The remaining four 1-proton multiplets were assigned as follows. The doublet at δ 4.40 (J 7.5 Hz) is assigned to H-1 of a β-D-glucopyranosyl residue since[5] these protons should resonate at δ <4.8 (*cf.* δ 5–5.5 and $J_{1,2}$ ~3 Hz for α anomers) and have $J_{1,2}$ values of 7–8 Hz. Furthermore, this doublet cannot be associated with either an α- or a β-L-rhamnopyranosyl residue because the J value is too large[6]. Irradiation of the doublet at δ 4.40 caused partial collapse of a multiplet centred at δ 3.43 which, in turn, when irradiated, caused the doublet to collapse to a sharp singlet and, in addition, the triplet at δ 3.15 to collapse to a doublet (J 9.5 Hz). These observations accord with these resonances being due to H-1, H-2, and H-3 of a β-D-glucopyranosyl residue.

Two of the remaining low-field signals must be due to H-1 of L-rhamnopyranosyl residues. The narrow doublets (J ~1.5 Hz) at δ 5.45 and 5.05 suggest[6–8] the presence of α-L-rhamnopyranosides. These doublets collapsed to sharp singlets on irradiation at δ 4.25 and 3.75, respectively. The signals at δ 4.25 and 3.75 can therefore be assigned to H-2 of rhamnose residues. Furthermore, it was possible to differentiate the anomeric protons of the two rhamnosyl residues by observing the effect of irradiation at the aromatic proton resonances. When the higher-field doublet at δ 6.89 (aromatic protons *ortho* to the glycosidic linkage[9]) was irradiated, there was a small, but significant, n.O.e. to the resonance centred at δ 5.45. Therefore[10], this doublet may be assigned to H-1 of the *p*-substituted-phenyl rhamnopyranoside residue and consequently that at δ 4.25 to H-2 of the same residue. The relative deshielding of H-1 and H-2 of this rhamnoside residue is probably due to the proximity of the aromatic nucleus.

The foregoing conclusions were essentially confirmed by the 50-MHz ^{13}C-n.m.r. data (Fig. 2), particularly from the resonances for anomeric carbons observed at 104.8, 98 3, and 97.0 p.p.m. downfield from that of Me_4Si. The resonance at 104.8 p.p.m. is to higher field than those for C-1 of an α- or a β-L-rhamnopyranoside[7,11–14], but is in good agreement with chemical shift values reported[15–17] for β-D-glucosides, β-D-gluco-oligosaccharides, and β-D-glucans. In contrast to D-glucosides, for which there is a large chemical shift difference between the resonance for C-1α and C-1β, the values observed for α- and β-L-rhamnosides are similar and it is not usually possible to distinguish between them on this basis. Consideration of the resonances at 67–73 p.p.m., however, suggests the presence of two α-L-rhamnosyl residues. This region contains seven separate resonances (*i.e.*, 7 non-identical carbons). Apart from the two carbons involved in the ester links, it is not expected that any other carbons of the lipid moiety would give rise to signals within this range of chemical shifts. Consequently, between 67 and 73 p.p.m., there are five resonances due to carbons of the trisaccharide moiety, and these may be assigned to C-4,6 of the β-D-glucosyl residue together with the two C-5 atoms and the unsubstituted C-4 of α-L-rhamnosyl residues. There is good agreement (Table I) between the calculated and the observed values of the chemical

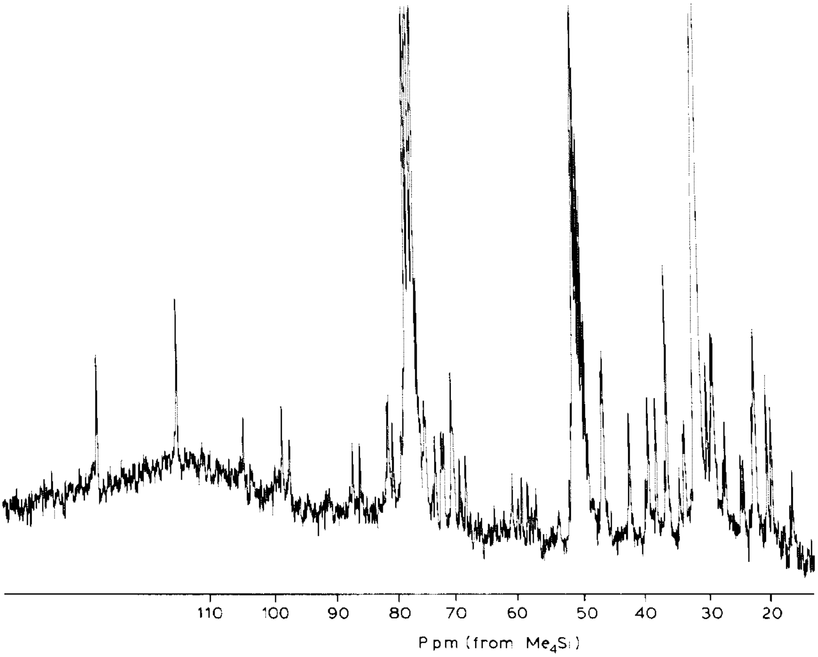


Fig. 2. ^{13}C-N.m.r. spectrum of a 5% solution of the mycoside in $CDCl_3$ at 50 MHz and 25°.

TABLE I

CALCULATED ^{13}C-CHEMICAL SHIFT VALUES (67–73 P P M FROM THE SIGNAL FOR Me_4Si) FOR THE TRISACCHARIDE MOIETY **2**, AND THE OBSERVED VALUES

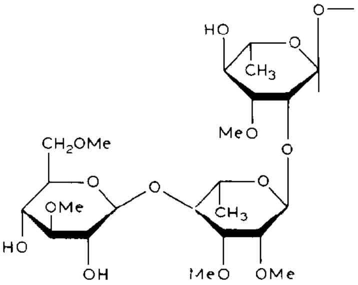
Residue	*Carbon*	*Shift in unsubstituted hexose*[16]	*Increment*[a]	*Calculated shift*	*Observed shift*[b]
3,6-Di-*O*-methyl-β-D-glucopyranosyl	6	61.8	+10	71.8	72.9
	4	70.7	−1	69.7	68.9
3-*O*-Methyl-α-L-rhamnopyranosyl	4	73.2	−1	72.2	71.9
	5	69.5	0	69.5	71.5
2,3-Di-*O*-methyl-α-L-rhamnopyranosyl	5	69.5	−1	68.5	67.9

[a] The values for increments are from Gorin[17]; for *O*-methylation (or *O*-glycosylation), +10 p.p.m. for the α carbon, −1 p.p.m. for the β carbon. No account is taken of solvent effects. [b] Arranged to give the best fit, and are therefore not necessarily in this order.

shifts for these carbons, and poor agreement for any other permutation of anomeric configurations. The assignment of anomeric configuration to L-rhamnopyranosides based on chemical shift values, particularly of C-3 and C-5, is well documented[13,14] and, for the (unsubstituted) α-L anomers, these carbons resonate at fields significantly higher than for the β-L anomers.

As might be expected, much of the ^{13}C-n.m.r. spectrum is not amenable to detailed analysis. However, the five or six resonances observed at 80–87 p.p.m. may be accommodated by the structure proposed for the glycolipid, which contains six *O*-glycosyl/*O*-methyl secondary, non-anomeric, carbons. The signal at 86.6 p.p.m. has a chemical shift which accords with that observed[18] for C-3 of tetra-*O*-methyl-β-D-glucopyranosides.

Thus, it is concluded that the oligosaccharide component of the mycoside has the structure **2**, and this is supported by the finding that synthetic[19] propyl 4-*O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-2,3-di-*O*-methyl-α-L-rhamnopyranoside is a competitive inhibitor of the natural mycoside in a number of bioassay systems[20]. Since the completion of the work described here, a publication has appeared[21] which essentially confirms our findings, and, recently, serological activity of the purified mycoside has been demonstrated[22,23], thus making the mycoside a potentially useful diagnostic agent for leprosy.



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EXPERIMENTAL

The mycoside was extracted[1] from *Mycobacterium leprae* (isolated from infected armadillo liver) and purified[22] by column and thick-layer chromatography on silica gel.

Methanolysis. — The mycoside (5 mg) was treated with methanolic M HCl (1 mL) under nitrogen at 85° for 6 h. The solution was neutralised (Ag_2CO_3), centrifuged, and concentrated to dryness, and the residue was extracted with hexane (3 × 2 mL) to remove lipid material. The residue was then trimethylsilylated and analysed by g.l.c. on a column (3 m × 2 mm) of SE-30 with a temperature programme 120→210° at 2°/min, using a Hewlett–Packard gas chromatograph Model 5750B.

O-*Dealkylation.* — The mycoside (5 mg) was treated with boron trichloride (1 mL) in dichloromethane (5 mL) at 20° for 60 h and the solution was then concentrated to dryness. Half of the residue was methanolysed, and the products were trimethylsilylated and analysed by g.l.c. Two products were observed with retention times identical to those of trimethylsilylated, methanolysed D-glucose and L-rhamnose. The remaining residue was hydrolysed in M sulphuric acid (0.5 mL) at 100° under nitrogen for 16 h, and then neutralised (Ag_2CO_3). T.l.c. (silica gel; ethyl acetate–pyridine–water, 20:7:5) of the hydrolysate showed, after spraying with aqueous 20% H_2SO_4–1% 1-naphthol and heating at 140°, the presence of two saccharides corresponding to glucose and rhamnose.

Methylation analysis. — Essentially the method of Jansson *et al.*[3] was used. The mycoside (5 mg) was methylated (Hakomori), and the alditol acetates derived from this and the native material (5 mg) were analysed by g.l.c.–m.s. Separations were effected on OV-225, using a Perkin–Elmer Sigma 3 gas chromatograph interfaced with a Kratos MS25 mass spectrometer, and the data were acquired on a DS505 computer data system. The methylated alditol acetates noted in the Discussion were identified by comparison with standard spectra[3].

N.m.r. spectra. — ^{1}H-N.m.r. spectra were recorded at ambient temperature for 5% solutions in $CDCl_3$ (internal Me_4Si) with a Bruker WH-270 spectrometer operating at 270 MHz. The ^{13}C-n.m.r. spectrum was obtained with proton broadband noise-decoupling at ambient temperature for a 5% solution in $CDCl_3$ (internal 1,4-dioxane) with a Bruker WH-200 spectrometer operating at 50 MHz.

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