Determination of the D and L Configuration of Phenolic Glycolipids of *M. leprae* and *M. bovis*

TO THE EDITOR:

It has been reported by Hunter, *et al.* that the structure of the sugar part of the species-specific phenolic glycolipid-I (PGL-I) of *Mycobacterium leprae* is *O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-α-L-rhamnopyranose ([15, 16]). This structure has generally been accepted and used elsewhere. However, the absolute structure of each sugar has only been assumed and has not been determined in that report ([16]). Several laboratories have shown by synthetic study that the disaccharide and trisaccharide of PGL-I synthesized from D-glucose and L-rhamnose have almost the same activity as that of PGL-I ([1–4, 6–10]). This suggests that the assumption of Hunter, *et al.* is correct, but there has been no direct evidence concerning the absolute configurations of the sugar residues. On the other hand, Demarteau-Ginsberg and Lederer have reported that the structure of the sugar part of the PGL of *M. bovis* (mycoside B) is 2-*O*-methyl-D-rhamnose, which was determined by an optical rotation study ([5]). However, D-rhamnose is a very rare sugar, and the reports which appeared after that paper did not treat the absolute configuration of the sugar residue ([11, 12]). Therefore, it is necessary to make sure of this determination by a more direct method. This paper provides the gas chromatographic determination of the absolute structure of the sugar residues of the PGLs of *M. leprae* and *M. bovis*.

Analytical procedures of the absolute configuration were based on the glycosidation with optically active alcohol, (+)-2-butanol ([13]). Five hundred μg of PGL-I from human-*M. leprae*-infected armadillo liver, synthesized trisaccharide, *p*-(2-methoxycarbonylethyl)phenyl *O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-α-L-rhamnopyranoside ([9]), or synthesized 2-*O*-methyl-L-rhamnopyranose was heated for 15 hr in 0.5 ml of (±)-2-butanol or (+)-2-butanol in the presence of 25 mg of powdered Amberlite IR 120 (H^+). The mixture was filtered, evaporated with the repeated addition of methanol, and then dissolved in 1 ml of methanol. Insoluble materials were filtered out with the aid of Celite. It was evaporated and dried. The residue was trimethylsilylated with 0.1 ml of TMS-PZ (Tokyo Kasei Co.) by heating the mixture at 40°C for 30 min, and an aliquot was analyzed by a capillary gas chromatograph (Hitachi G3000). Gas-liquid chromatography (GLC) conditions were as follows: Column; chemical bonded OV-1; d.f. 0.5 μm; length 5 m (0.25 mm i.d.); temp. program = 150°C for 3 min then →200°C at 4°C/min; carrier gas = 34 cm/min; split ratio = 25:1; injection temp. = 220°C.

Figure 1 shows the results of GLC of the

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FIG. 1. GLC of the sugar derivatives of the PGL-I of *M. leprae*: (+)-butanol-treated PGL-I (a), (±)-butanol-treated PGL-I (b), and (+)-butanol-treated synthetic trisaccharide (c) were trimethylsilylated and subjected to capillary GLC. (GLC conditions are given in the text.)

FIG. 2. GLC of the sugar derivatives of the phenolic glycolipid of *M. bovis*: (+)-butanol-treated PGL (a), (±)-butanol-treated PGL (b), and (+)-butanol-treated 2-*O*-methyl-L-rhamnopyranose (c) were trimethylsilylated and subjected to capillary GLC.

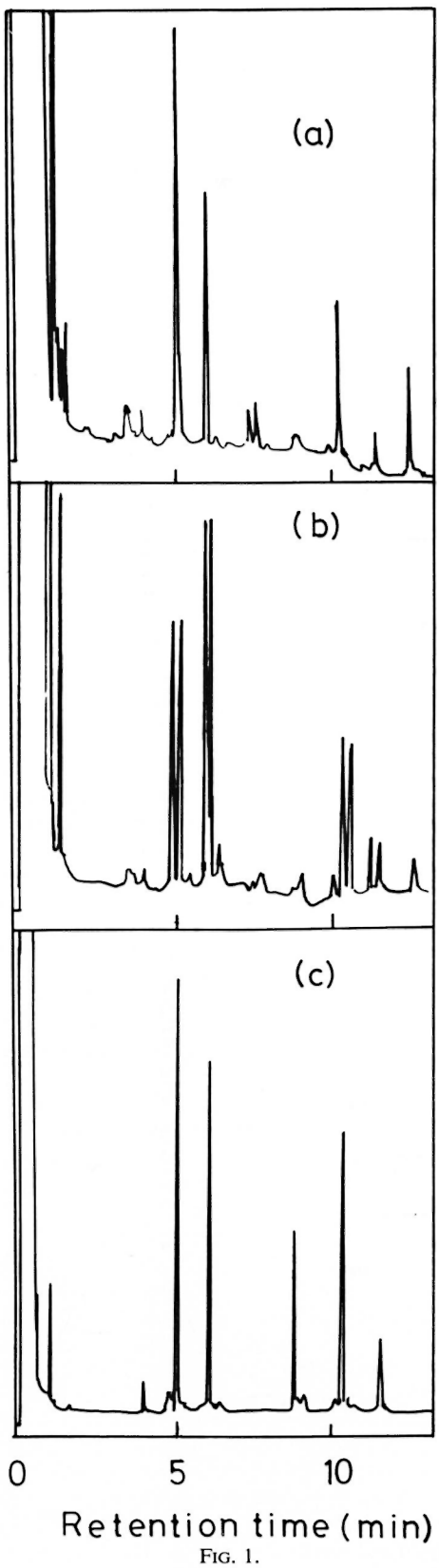

FIG. 1.

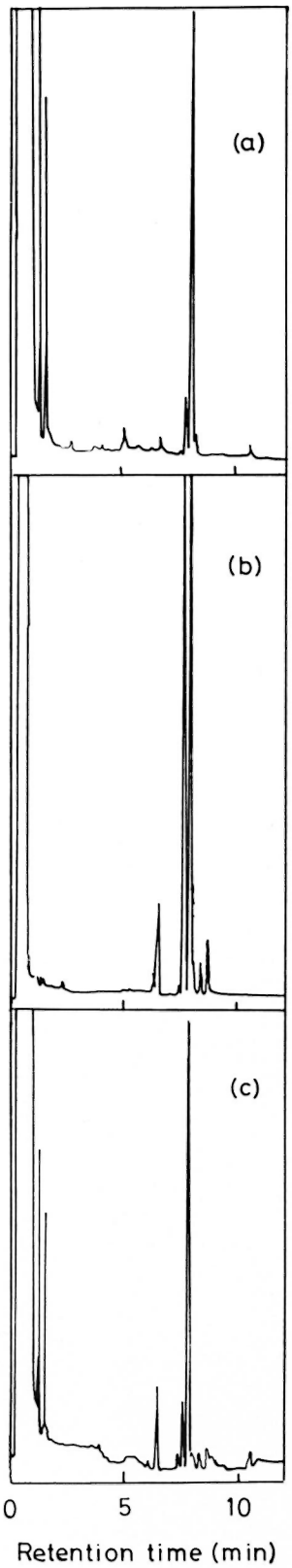

FIG. 2.

sugar derivatives of the PGL-I of *M. leprae*. GLC of the sugar derivatives of PGL-I treated with (±)-2-butanol showed four 1:1 doublet-peaks corresponding to the derivatives of 2,3-di-*O*-methyl-D- and L-rhamnose (5.10 min and 5.30 min, (+)-2-butyl 2,3-di-*O*-methyl-D-rhamnoside should have the same retention time as (−)-2-butyl 2,3-di-*O*-methyl-L-rhamnoside); 3-*O*-methyl-D- and L-rhamnose (6.10 and 6.25 min); 3,6-di-*O*-methyl-α-D- and β-L-glucose (10.60 and 10.80 min); and 3,6-di-*O*-methyl-α-L- and β-D-glucose (11.45 and 11.75 min). In the case of PGL-I treated with (+)-2-butanol, four singlet-peaks were observed at 5.30, 6.27, 10.60, and 11.80 min. Synthesized trisaccharide gave four singlet-peaks at 5.30, 6.25, 10.58, and 11.75 min and one additional singlet-peak at 8.95 min, which corresponded to *p*-hydroxyphenylpropionate. Cochromatography of the (+)-2-butanol-treated PGL and the (+)-2-butanol-treated synthesized trisaccharide gave the same pattern as that of (+)-2-butanol-treated PGL, except that the peak was at 8.95 min (*p*-hydroxyphenylpropionate). Therefore, the absolute configurations of the three sugars of *M. leprae* PGL-I were D for 3,6-di-*O*-methylglucose and L for both 2,3-di-*O*-methylrhamnose and 3-*O*-methylrhamnose.

Figure 2 shows the results of GLC of the sugar derivatives of the PGL of *M. bovis*. GLC of the (±)-2-butanol-treated PGL shows only one doublet-peak at 7.60 and 7.80 min, which corresponds to the derivatives of 2,3-di-*O*-methyl-D- and L-rhamnose, respectively. In the case of the PGL treated with (+)-2-butanol, only one singlet-peak was observed at 7.80 min, which was completely in accord with that of (+)-2-butanol-treated 2-*O*-methyl-L-rhamnose. Cochromatography of (+)-2-butanol-treated PGL and (+)-2-butanol-treated 2-*O*-methyl-L-rhamnose gave the same pattern as that of (+)-2-butanol-treated PGL, showing that 2-*O*-methylrhamnose of *M. bovis* was in the L-configuration.

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