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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.

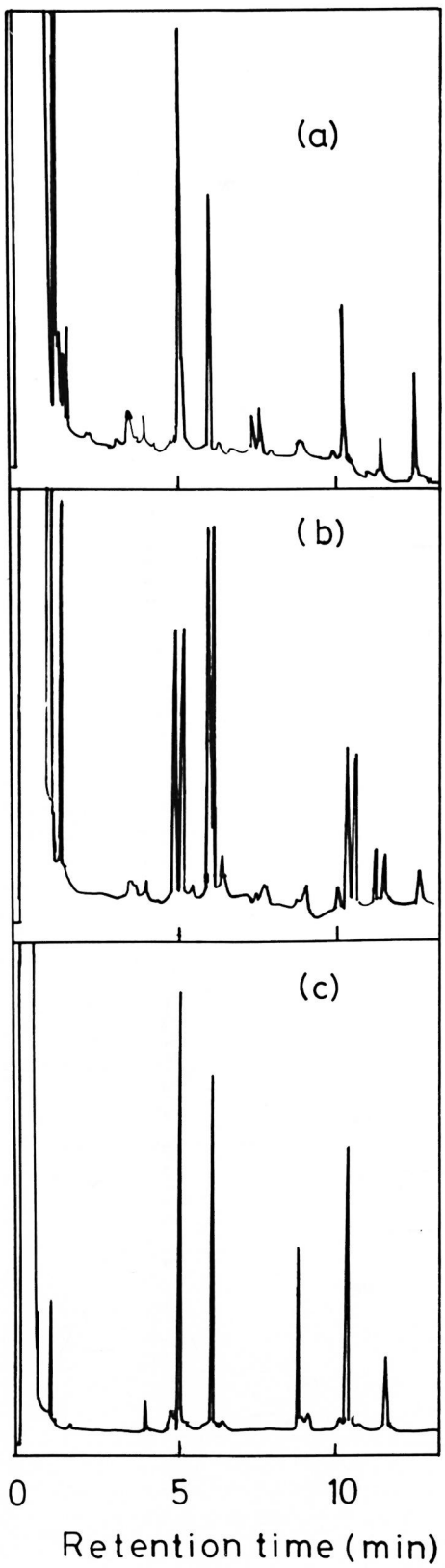

FIG. 1.

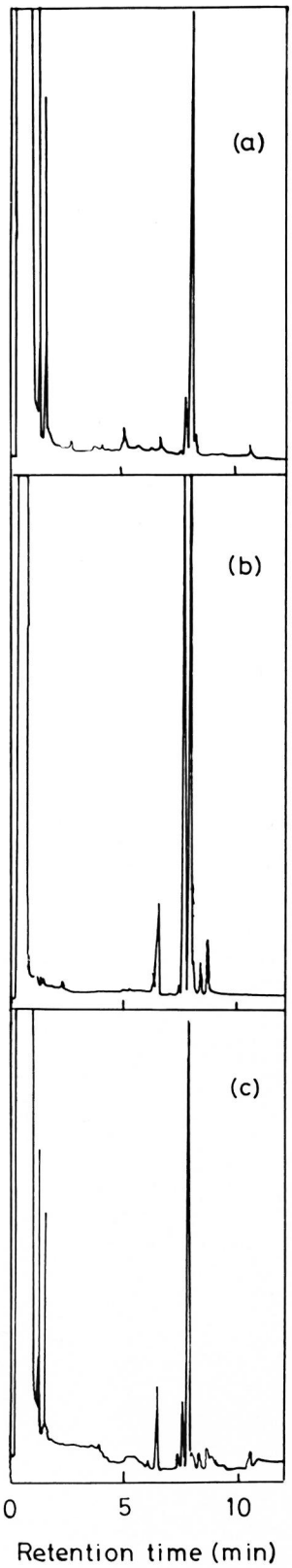

FIG. 2.

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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Effect of Shipment of Skin-Biopsy Specimens to a Distant Laboratory on Viability of *Mycobacterium leprae*

TO THE EDITOR:

In some clinical trials of antileprosy chemotherapy, measurement of the response to treatment requires shipment of biopsy specimens to a distant laboratory where *Mycobacterium leprae* are recovered from the specimens and inoculated into mice. Earlier work ([4]) had demonstrated that *M. leprae* survived periods of storage at 0–4°C, justifying shipment of specimens to distant laboratories for inoculation into mice, but the number of specimens studied and the method employed were insufficient to exclude a small but systematic loss of viable organisms in the course of shipment.

In a series of clinical trials among patients with lepromatous leprosy at the Leonard Wood Memorial Leprosy Research Laboratory, Cebu, The Philippines ([1, 2]), conducted between mid-1969 and early 1974, patients were subjected to skin biopsy at intervals, and 409 biopsy specimens were divided, one portion of each specimen being processed for inoculation of mice in Cebu and the second portion air-shipped on wet ice to the U.S. Public Health Service Hospital, San Francisco, California, where mice were also inoculated. Specimens obtained during the morning in Cebu were usually put aboard an afternoon flight to Manila, and transferred to an international flight that left Manila that evening, arriving in San Francisco on the same evening (because of the west-to-east crossing of the International Date Line). In San Francisco, the specimens were usually picked up during the evening of arrival and, when possible, processed for mouse inoculation the following day. Thus, the elapsed time between biopsy in Cebu and inoculation of mice in San Francisco was frequently no more than 48 hr. Occasionally, a longer period of storage intervened between biopsy and inoculation of mice in San Francisco.

In both Cebu and San Francisco, the technique of Shepard ([3, 5]) was employed for recovery of *M. leprae* from the biopsy specimens, counting the organisms, inoculating mice, and harvesting the organisms from mice. The generation time (G), defined as the number of days per doubling of *M. leprae,* was calculated according to the relationship:

$$G = \frac{\text{number of days between inoculation and harvest}}{\text{logarithm}_2 \left(\text{number AFB harvested} / \text{number of AFB inoculated}\right)}.$$

This calculation assumes that all of the inoculated organisms were capable of multiplication, began multiplying on the day of inoculation, and multiplied at a constant rate from the day of inoculation to the day of harvest. Although these assumptions are untenable, this measurement has provided a useful means of evaluating the effects of treatment in a number of clinical trials among patients with lepromatous leprosy. In addition to the values for G, the difference between the dates of inoculation in the two laboratories (D) and the year of biopsy (Y) were recorded for each specimen.