Evidence for the Nature of the Link between the Arabinogalactan and Peptidoglycan of Mycobacterial Cell Walls*

(Received for publication, April 2, 1990)

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The long-posed question of the nature of the link between the mycolylarabinogalactan and the underlying peptidoglycan of the cell walls of Mycobacterium sp. has been addressed. The insoluble cell wall matrix of Mycobacterium leprae, Mycobacterium tuberculosis, and Mycobacterium bovis was partially hydrolyzed with acid either before or after per-O-methylation and the resulting oligosaccharides further derivatized and analyzed by gas chromatography/mass spectrometry. The structures of fragments arising from the reducing end of arabinogalactan demonstrated the existence of the terminal sequence \(-\text{Gal}-(1\rightarrow4)-\text{Rhap}-(1\rightarrow3)-\text{D-GlcNAc}\). Other analyses confirmed the presence of muramyl-6-P within the peptidoglycan of these mycobacteria. Based on the acid lability of the 3-linked GlcNAc unit, the presence of about equimolar amounts of Rhap-(1\rightarrow3)-D-GlcNAc and muramyl-6-P in an isolated cell wall fragment, and \(^{31}\text{P}\) NMR analysis, it was concluded that the GlcNAc residue of the terminal triglosyl unit of arabinogalactan is joined by 1-O-phosphoryl linkage to the 6-position of some muramyl residues within the peptidoglycan. Thus, it is reasoned that the massive mycolylarabinogalactan of mycobacteria, responsible for aspects of disease pathogenesis and much of the antibody response in infections, is attached to the peptidoglycan framework by the actinomycete-specific diglycosyolphosphoryl bridge, L-Rhap-(1\rightarrow3)-D-GlcNAc-(1\rightarrowP), perhaps thereby providing a unique target for site-directed chemotherapy of mycobacterial infections.

Research extending back over 50 years has implicated the components of the insoluble cell wall matrix of mycobacteria in many of the host responses associated with tuberculosis, leprosy, and other mycobacterioses (1, 2). The mycobacterial cell wall may be regarded as consisting of two massive entities, mycolyl-D-arabino-D-galactan and peptidoglycan (1, 3, 4). It has long been held that the mycolylarabinogalactan polymer is connected at intervals by a phosphodiester bridge, possibly extending from an arabinosyl unit (5) to the glycan chain of peptidoglycan, which consists of repeating units of GlcNAc (1\rightarrow4)-\(N\)-glycolylmuramic acid (4). The evidence for a phosphodiester link stems from the recognition of muramic acid-6-P, initially in Mycobacterium butyrium (6) and subsequently in cell wall preparations of several other mycobacteria (7). However, Kanetsuna and San Blas (3) had also implied the presence of a phosphate-free direct glycosidic link between mycolylarabinogalactan and peptidoglycan. In view of renewed interest in the immunogenicity and pathogenesis of mycobacterial cell walls (2, 8) and the apparent crucial singular role of a linker arm in maintaining the structural integrity of a unit with a molecular mass of perhaps \(2 \times 10^6\) kDa, we have returned, after a hiatus of 20 years (3), to the fundamental question of the nature of this bond.

EXPERIMENTAL PROCEDURES

Mycobacteria—Mycobacterium leprae was isolated from the livers and spleens of experimentally infected armadillos (9). A virulent strain of Mycobacterium tuberculosis (Erdman; Trudeau mycobacterial collection no. 107) was grown and harvested as described (10). Mycobacterium bovis BCG (Danish strain Lilly) was obtained in lyophilized form from S. D. Chaparas (Bureau of Biologics, Food and Drug Administration, Bethesda, MD).

Isolation of the mAGPP Complex—Mycobacteria were disrupted by sonication and impure cell walls obtained by centrifugation at 27,000 \(\times\) g for 30 min (11, 12). These were subsequently extracted repeatedly with 2% sodium dodecyl sulfate at 56 °C to obtain the crude insoluble mAGPP complex (10-12) of the respective species.

Preparation of Per-O-alkyl-(oligo)glycosyl Alditol Fragments—The mAGPP complex from M. leprae was per-O-methylated, partially hydrolyzed for 40 min at 75 °C with 2 M CF\(_3\)COOH, reduced, and per O-pentadeuterioethylated to arrive at a mixture of partially O-methylated, partially O-pentadeuterioethylated oligoglycosyl alditols, as described (10). Alternatively, the mAGPP complex from M. leprae, M. tuberculosi, and M. bovis BCG was partially hydrolyzed with 0.2 M H\(_2\)SO\(_4\) at 100 °C for 30 min and centrifuged at 2400 \(\times\) g for 35 min to obtain a solubil fraction, which was neutralized with BaCO\(_3\). The resulting mixture of oligosaccharides was reduced with NaBH\(_4\), per-O-methylated, and purified in bulk on Sep-Pak cartridges (Waters Associates, Milford, MA) as described (10).

Preparation of Soluble AGP—The mAGPP complex from M. bovis BCG (1 g) was digested with Pronase (100 mg; Type IV, Sigma) in 50 ml of phosphate-buffered saline containing 0.5% Triton X-100 at 25 °C for 18 h. The digest was centrifuged at 27,000 \(\times\) g, washed thoroughly with water, extracted with 90% phenol, and washed repeatedly with water and ethanol (7) to yield 0.55 g of the protein-free complex (i.e. mAGP). Mycic acid were removed by saponification of mAGP in refluxing 2% KOH in methanol/benzene (1:1) for 48 h (7). The insoluble residue was extracted with methanol to yield 288 mg of the resulting AGP complex. In order to partially cleave the constituent peptidoglycan, mAGP was re-N-acetylated with acetic anhydride in Na\(_2\)CO\(_3\) at 7 °C for 18 h (7), recovered by centrifugation.
Arabinogalactan-Peptidoglycan Linkage

at 27,000 x g, washed with water, and digested with lysozyme (72 mg; Worthington) in 0.05 M ammonium acetate buffer (pH 6.3) in the presence of toluene at 25 °C for 18 h, followed by centrifugation at 27,000 x g.

Preparation of Partially Depolymerized AGP—The lysozyme-solubilized AGP was concentrated and applied directly to a column (15 x 1 cm) of Bio-Gel P-60 (Bio-Rad) in 100 mM ammonium bicarbonate buffer (pH 7.8). The partially depolymerized carbohydrate-reactive material was collected and lyophilized. In order to eliminate the peptide side chains attached to the reducing-end muramy1 residues (13, 14), the product was treated with 1 M NaOH (pH 11.0) at 37 °C for 2 h, neutralized, and resolated as the voided product from the same sizing column. This material, in turn, was applied to a column (1 x 12.5 cm) of DEAE-Sephadex (A25; Sigma) in 100 mM ammonium bicarbonate (pH 7.8) followed by a gradient of 10 mM to 1 M ammonium bicarbonate (total volume, 150 ml). Fractions (4.5 ml) were collected, and those containing carbohydrate (no. 9-25) were combined. About 35 mg of partially depolymerized AGP were recovered.

GC/MS Analysis—GC/MS was performed on a HP-1 (Hewlett-Packard, Avondale, PA) 12-m capillary column (10). The temperature program for resolution of per-O-alkylated oligosaccharides involved a 150 °C setting for 1 min, followed by a 30 °C/min rise to 200 °C and an 8 °C/min rise to 320 °C, a temperature which was held for 8 min. The temperature program which was applied to all (CH3)2Si derivatives (i.e. of methyl glycosides, alditols, and muramyl-6-P) involved a 50 °C hold for 1 min followed by a 30 °C/min rise to 140 °C, a 6 °C/min rise to 260 °C, a 30 °C/min rise to 280 °C, and a final 2-min hold at this final temperature. A stringently clean injector port was critical to the successful analysis of the (CH3)2Si derivatives of muramyl-6-P.

Other Analytics—Neutral sugars were analyzed as alditol acetates (15). Amino sugars, including muramic acid, were also analyzed as the alditol acetates after hydrolysis with 4 M HCl at 100 °C for 4 h; the published protocol for the formation of volatile derivatives of muramic acid by alditol acetate chemistry was followed (16). Amino acids were analyzed as the N-(O)-heptfluorobutnyl isobutyl esters (17). Phosphate was assayed colorimetrically (18). The precise conditions for quantitation of muramyl-6-P were apparently critical for reproducibility. Samples (1-2 mg) were hydrolyzed with 6 M HCl for 1 h at 105 °C. Acid was evaporated from the sample with a stream of filtered air which was then dissolved in 0.5 ml of water, filtered through an Acro LC3a 0.45-m filter (Gelman Sciences, Ann Arbor, MI), and again dried with filtered air. Pyridine (50 µl), N,O-bis(trimethylsilyl)trifluoroacetamide (100 µl; Supelco, Bellefonte, PA), and trimethylchlorosilane (1 µl; Sigma) were added to the sample which was heated at 150 °C for 15 min and then held at room temperature for 2 days. Aliquots were injected directly onto the HP-1 capillary GC column.

Another critical protocol concerned the quantitation of reducing sugars generated under conditions of partial acid hydrolysis. The mAGP complex (2 mg) from several mycobacteria was hydrolyzed with 0.2 M H2SO4 (0.5 ml) at 85 °C for 20 min and centrifuged at 2400 x g. The supernatant was neutralized with BaCO3 and dried under a stream of filtered air at 60 °C. The product was reduced with NaB[3H], (1 mg in 100 µl of 1 M NH2OH) as described (19) and methanolyzed (1 M HCl in CH3OH, 500 µl) at 85 °C for 18 h to produce a mixture of methyl glycosides and free alditols. Amino groups were N-acetylated in methanol/pyridine/acetic anhydride (5:1:1) for 15 min at 25 °C, recovered after evaporation of reagents, trimethylsilylated (15), and the resulting mixture of (CH3)3Si methy1glycosides and (CH3)2Si alditols analyzed by GC/MS with concomitant elimination of minimal standards. Selectively, mAGP (2 mg) was partially hydrolyzed with 0.2 M CF3COOH (500 µl) for 20 min at 85 °C, centrifuged, the supernatant evaporated to dryness, reduced with NaB[3H],, hydrolyzed with 4 M HCl for 4 h, reduced with NaB[3H],, acetylated, and analyzed by GC/MS (15, 19). The ratio of [3H]/[H] at C-1 for the alditol acetates of GlcNac and GalNAc was determined by the relative amount of the m/e 85 and m/ 84 ions (19).

Samples for 3P NMR analysis were dissolved in 1 ml of [H]2O and the pH titrated to about 10.0 with Na2CO3; the reference was to external 85% H3PO4 at 0.0. NMR was performed on a Bruker 500-MHz NMR at the Colorado State University Regional NMR Center.

RESULTS

The Presence of the Reducing End Group, →4)-D-Rhap(1→3)-D-GlcNAc, on Arabinogalactan—Previously, we had ob-
mAGPP of *M. tuberculosis* was subjected to partial acid hydrolysis, its proceeds reduced with NaB\[^{1-2}\text{H}\]_3, methylated, the methylated oligoglycosyl alditols separated by high performance liquid chromatography, and the peak containing product \(\text{4}\) collected. The methylated oligoglycosyl alditol \(\text{4}\) was hydrolyzed with 2 \(\text{M}^{+}\text{CF}_2\text{COOH}\), reduced, and the resulting partially methylated alditol acetates analyzed by GC/MS. The expected alditol acetates, 1,5-di-O-Ac-1-C\[^{1-2}\text{H}\]_1,3,4-tri-O-CH\_3-rhamnitol (m/z 115, 118, 131, 162, 175) and 3-O-Ac-2-deoxy-1-C\[^{1-2}\text{H}\]_1-(N-methylacetamido)-1,4,5,6-tetra-O-CH\_3-glucitol (m/z 89, 131, 247, 290), were identified in approximately equimolar amounts. In order to confirm that the N-acetamido sugar is GlcNAc, rather than GalNAc, which is also present in mycobacterial cell walls (21), the mixture containing products \(\text{4}\) and \(\text{5}\) was reduced with NaB\[^{1-2}\text{H}\]_3, methanolyzed, N-acetylated, and trimethylsilylated. The \(R\), value and mass spectrum of the glycosylaminitol on the HP-1 capillary column was identical to that of 2-N-acetamido-2-deoxy-1-C\[^{1-2}\text{H}\]_1,3,4,5,6-penta-O-(CH\_3)\_2Si-glucitol (m/z 14.00) and different from that of the 2-(N-acetamido)-2-deoxygalactitol derivative (\(R\), 14.20).

Further samples of products \(\text{4}\) and \(\text{5}\) were butanoylated with optically pure S\((+)\)-2-butanol by the method of Gerwig et al. (22). GC/MS of the (CH\_3)\_2Si derivatives as described (22) demonstrated that the GlcNAc is D and the Rha is L.

The structures of these two key products, \(\text{4}\) and \(\text{5}\), clearly imply that L-Rha is universally attached to C-3 of the D-GlcNAc residue, and the sequence of sugars within the oligosaccharide fragment, Gal-(1→4)Rha-(1→3)GlcNAc, demonstrates that the reducing end of a arabinogalactan proper is occupied by the unit, L-Rha-(1→3)d-GlcNAc.

**Recognition of L-Rhap-(1→3)-d-GlcNAc-(1→P)—** The recovery of the partial acid hydrolysis products \(\text{4}\) and \(\text{5}\), in which the relatively acid-stable GlcNAc rather than the acid-labile Rhap is the reducing-end sugar, suggested that these fragments are not part of the greater glycan chain of peptidoglycan. Rather, the composition of these products implies that the terminal GlcNAc in situ is linked to peptidoglycan proper via an acid-labile link, possibly a phosphodiester (23). To test this possibility, the mAGF complex from *M. tuberculosis* was treated with mild acid under conditions known to dephosphorylate GlcNAc-(1→P) (23, 24). The solubilized material was further reacted with NaB\[^{1-2}\text{H}\]_3, in order to determine the extent of the generation of reducing sugar and to identify the newly formed alditol. The results (not shown) demonstrated that the conditions selected (0.2 \(\text{M}\,\text{H}_2\text{SO}_4\), 20 min, 85 °C) were sufficiently mild such that only 36 and 41% of the acid-labile Gal and Ara residues, respectively, were cleaved; only about 10% of the Rha residues were hydrolyzed. On the other hand, under these conditions, all of the solubilized GlcNAc was hydrolyzed and converted to the 1-C\[^{1-2}\text{H}\]-derivative, and the molar ratio of the reduced GlcNAc to Rha was about 1:1, as expected. In control experiments, it was demonstrated that the commercially available disaccharide (d-GlcNAc-(1→4)-L-Rhap-(1→4)-d-GlcNAc (Sigma), and its \(\alpha\)-methylglycoside were not appreciably cleaved under these conditions. Thus, the generation of products \(\text{4}\) and \(\text{5}\) can be readily explained if considered as part of a larger phosphodiester-linked unit. Incidentally, under similar mild acid hydrolysis conditions (0.2 \(\text{M}\) trifluoroacetic acid, 20 min, 85 °C), the GalNAc, which persists in
cell wall preparations, was solubilized but not reduced, demonstrating that it does not occupy a reducing-end position on arabinogalactan and is not involved in a 1-O-phosphoryl linkage.

Seeking more direct evidence for a phosphodiester link, a partially degraded water-soluble segment of mAGP of M. bouis BCG was generated by the process of demycolylation, partial lysozyme digestion, and chromatographic fractionation, as described under "Experimental Procedures." Analysis of this material (Table III) demonstrates the presence of sizable quantities of the arabinogalactan polymer proper, 2–3 mol of the muramyl tetra- (or penta-) peptide unit, about 1 mol of Rha, and about 1.5 mol of P; 1 mol of P was shown by 31P NMR to exist as a phosphodiester (see below). The amount of GlcNAc was larger than expected even considering that these quantities represent a combination of that found in both the residual peptidoglycan and the proposed linker unit. Nevertheless, the overall data in Table III are supportive of the case for a \( (\rightarrow 4)\)-L-Rhap-(1\( \rightarrow 3\))-D-GlcNAc-(1\( \rightarrow P \)) unit joining arabinogalactan and peptidoglycan proper. The presence of substantial quantities of GalNAc within the soluble cell wall fragment is discussed below.

31P NMR analysis of the partially degraded soluble AGP fragment (Fig. 4A) showed the predominance (about 75%) of a signal with a chemical shift (\( \delta -1.71 \)) indicative of a phosphodiester (25–27); this signal is significantly upfield from the phosphomonoester region (\( \delta -4 \)) at pH 10. In addition, subsequent treatment of the soluble AGP fragment with \( H_2SO_4 \), under the conditions (0.2 \( \text{N} \) \( H_2SO_4 \), 20 min, 85 °C) used to generate reducing GlcNAc, resulted in a shift of this \( 31 \text{P} \) NMR signal to the phosphomonoester region (\( \delta 3.7 \)) (Fig. 4B). Clearly, conversion of phosphodiester to phosphomonoester parallels cleavage of the glucosaminyl linkage.

Demonstration That the \( (\rightarrow 4)\)-L-Rha-(1\( \rightarrow 3\))-D-GlcNAc-(1\( \rightarrow P \)) Unit Is Linked to the 6-Position of Some Muramyl Residues within Peptidoglycan—The mAGP complexes from M. leprae, M. tuberculosis, and M. bouis BCG were hydrolyzed with 6 \( \text{N} \) HCl at 105 °C for 1 h, trimethylsilylated, and the (CH8)Si products analyzed by GC/MS. Under the stringent GC conditions described under "Experimental Procedures," the (CH8)Si derivatives of muramyl-6-P were readily identified, and positive proof of identification was provided by a comparison of mass spectra (m/z 73, 147, 185, 217, 259, 299, 315, 341, 387, 583, 601, and 658, indicative of 2-N-(CH8)Si-1,4,9-tri-O-(CH3)3Si-6-[di-O-(CH3)3Si-phosphate]-muramic acid) and retention times (R, 21.51 and 21.71) to those of the

![Figure 2](http://www.fc.org)  
**Figure 2.** GC/MS analysis of the per-O-alkylated oligoglycosyl alditols prepared from the cell walls of M. leprae. The elution of products 2 and 3 (Table I) is indicated. A, the total ion chromatogram. B, the use of the selected ion chromatogram of m/z 238 in the region defined by the brackets in A to locate products 2 or 3. C, the electron intact mass spectrum of product 2.

### Table 1

**Structures of those partially O-methylated partially O-pentadeuterioethylated oligoglycosyl fragments derived from the mAGP complex of M. leprae and M. tuberculosis and containing a rhamnogalactose reducing end group**

<table>
<thead>
<tr>
<th>Product number</th>
<th>GC R*</th>
<th>Mass spectral ions</th>
<th>Structure of product</th>
<th>Abbreviated structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1'</td>
<td>23.3</td>
<td>101, 155 (aA, 206 (aA), 238 (aA)), 244 (aldJ, 304 (aldJ), 442 (bJ, 448 (caldJ), 508 (caldJ))</td>
<td>2-O-C[14]H, 2,3,6-tri-O-CH3-Galf- -+B-Galf-(1( \rightarrow 6))-Galf-(1( \rightarrow 5))-Galf-</td>
<td>((\rightarrow 5))-Galf-(1( \rightarrow 6))-Galf-(1( \rightarrow 5))-Galf-</td>
</tr>
<tr>
<td>2</td>
<td>11.45</td>
<td>101, 155 (aA, 206 (aA), 238 (aA)), 244 (aldJ, 304 (aldJ), 442 (bJ, 448 (caldJ), 508 (caldJ))</td>
<td>2,3,6-tri-O-CH3-5-O-C[14]H, Galf-</td>
<td>((\rightarrow 5))-Galf-(1( \rightarrow 4))-Rhap</td>
</tr>
<tr>
<td>3</td>
<td>12.60</td>
<td>88, 155 (aA, 206 (aA), 238 (aA)), 157 (bJ, methanol), 189 (bJ), 249 (bJ)</td>
<td>2,3,6-tri-O-CH3-5-O-C[14]H, Galf-</td>
<td>((\rightarrow 5))-Galf-(1( \rightarrow 4))-Rhap-(1( \rightarrow OMe)</td>
</tr>
</tbody>
</table>

*GC/MS was performed on a HP-1 capillary column under the temperature gradient conditions described in the text.
*See earlier publications (15, 20) for explanation of ion designation.
*Obtained from the M. tuberculosis mAGP only.
(CH₃)₂Si derivatives of authentic muramyl-6-P, prepared from crude M. bovis BCG cell walls as described (6). In the same fashion, the soluble purified AGP fragment (Table III) was shown to contain muramyl-6-P. Quantitation by the external standard method demonstrated that about 95% of the phosphate present in the partially degraded purified AGP segment was in the form of muramyl-6-P (Table III). It should also be noted that the quantities of phosphate and muramyl-6-P in this AGP fragment are similar to those in whole cell walls from Mycobacterium smegmatis (28).

**DISCUSSION**

In a broader context, we have devoted considerable effort to defining the major structural features of the cell walls of human and avian tubercle and leprosy bacilli, especially those entities responsible for cell-mediated and humoral immunity in infections (29, 30). In the present context, we have dwelled solely on the purely chemical question of the nature of the link between arabinogalactan proper and peptidoglycan, a linkage which is responsible for the structural integrity of the mycolylarabinogalactan-peptidoglycan-protein complex of Mycobacterium sp., perhaps one of the largest multifunctional polymers within Prokaryotae. This particular question had not been addressed since the early demonstration of the presence of muramyl-6-P among the hydrolysis products of mycobacterial cell walls (3, 6) and with it the conclusion that the appropriate linkage is, in part, phosphodiester, spanning arabinogalactan proper and the occasional muramyl residue within the basal peptidoglycan (1, 4, 31, 32). Draper (21) had previously noted the presence of GalNAc in the cell wall of Mycobacterium lepraemurium and in amounts consistent with a role in a linkage between arabinogalactan and peptidoglycan; indeed, the quantities of GalNAc in the processed cell walls of M. bovis BCG (Table III) tended to support such a conclusion. However, the cell walls of M. smegmatis and M. tuberculosis H37Ra do not contain GalNAc although still containing Rha. Moreover, the GalNAc released from other mycobacterial cell walls by various degradations cannot be reduced with NaBH₄, and is not present in any of the relevant isolated linkage fragments. Thus, this combined evidence suggests that GalNAc does not have a direct role in the linkage between arabinogalactan and peptidoglycan. Contrarily, the presence of GalNAc in the partially degraded AGP complex, which is largely devoid of peptidoglycan (Table III), indicates that GalNAc is part of the arabinogalactan polymer proper. In addition, the data of Table III show an excess of GlcNAc over that attributable to peptidoglycan and the linker region, suggesting that GlcNAc may also be part of the arabinogalactan polymer. Arabinosyl-1-P, which had been isolated from mycobacterial cell walls after base treatment (5), had also been proposed as a constituent of the putative link between arabinogalactan and peptidoglycan. However, it now seems more likely that it occurs as simple arabinosyl-1-P side chains attached to the arabinogalactan proper; we have recently indicated that inositol-1-P may appear in a similar format on the arabino segments of lipoarabinomannan (33).

Dissection of the massive cell wall armament of mycobacteria has been hindered by the paucity of appropriate hydrolytic enzymes; for instance, mycobacterial cell walls are resistant to conventional muramidases. Accordingly, the approach developed herein is based entirely on the practice of chemical cleavage, relying on the lessons derived from several earlier key publications (3, 7, 23, 24, 34) and our own recent applications (10) of the strategy of partial depolymerization of per-O-alkylated polysaccharides (19) to analysis of the cell wall arabinogalactan. A summary of the experimental strategy based on the susceptibility of various key linkages to different sources is given in Fig. 3.

![GC/MS analysis of per-O-methylated oligosaccharide alditols prepared by partial acid hydrolysis, reduction, and O-methylation of the cell walls of M. leprae. The time of elution of products 4 and 5 (Table II) is indicated by the arrows. A, the total ion chromatogram. B, the selected ion profile of the J₃ ion (m/z 277) produced by the 2-deoxy-1-C-[¹H]₂-(N-methylacetamido) 1,4,5,6-tetra-0-CH₃-glucitol which was used to locate products 4 and 5 within the mass of other materials. C, the mass spectrum of 4.](http://www.jbc.org/)

**TABLE II**

**Structures of the per-O-methylated oligoglycosyl-N-acetyl-glucosaminitol isolated from the mAGPP complexes of M. leprae, M. tuberculosis, and M. bovis (BCG)**

<table>
<thead>
<tr>
<th>Product number</th>
<th>GC R₀</th>
<th>Mass spectral ions</th>
<th>Structure of product</th>
<th>Abbreviated structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>13.50</td>
<td>88, 189 (aArs), 157 (aArs), 277 (aAlld₃) and the alditol cleavage ions 89, 131, 349, 393, 436, and 437</td>
<td>2,3,4-tri-O-CH₃ Rha-[1→3]-2-deoxy-1-C-[¹H]₂-(N-methylacetamido)-1,4,5,6-tetra-0-CH₃-glucitol</td>
<td>Rha-(1→3)-GlcNAc</td>
</tr>
<tr>
<td>5</td>
<td>21.40</td>
<td>219 (aArs), 155 (aArs), 277 (aAlld₃), 451 (bAlld₃), 383 (bArs), and 533, 597, 640, and 641 (alditol cleavage ions)</td>
<td>2,3,5,6-tetra-0-CH₃ Gal-[1→4]-2,3-di-O-CH₃ Gal-[1→4]-Rha-(1→3)-GlcNAc</td>
<td>Rha-[1→3]-2-deoxy-1-C-[¹H]₂-(N-methylacetamido)-1,4,5,6-tetra-0-CH₃-glucitol</td>
</tr>
</tbody>
</table>

* GC/MS conditions were those mentioned in Table I.
The origin of the partially degraded segment of AGP is described under "Experimental Procedures." The Table III compositional analysis of the partially degraded water-soluble segment of AGP from M. bovis BCG. The origin of the partially degraded segment of AGP is described under "Experimental Procedures." The Table III compositional analysis of the partially degraded water-soluble segment of AGP from M. bovis BCG. The Table III compositional analysis of the partially degraded water-soluble segment of AGP from M. bovis BCG. The origin of the partially degraded segment of AGP is described under "Experimental Procedures." The origin of the partially degraded segment of AGP is described under "Experimental Procedures."


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