Carbohydrate Research, 203 (1990) 79–90
Elsevier Science Publishers B.V., Amsterdam

Isolation and analysis by the reductive-cleavage method of linkage positions and ring forms in the *Mycobacterium smegmatis* cell-wall arabinogalactan*

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(Received September 13th, 1989; accepted, in revised form, December 14th, 1989.)

ABSTRACT

The *Mycobacterium smegmatis* arabinogalactan polysaccharide has been isolated from the cell wall by saponification and extraction to remove lipids and subsequent solubilization by treatment with lysozyme. Analysis for neutral sugars demonstrated the presence of D-arabinose and D-galactose in a ratio of 3:1, respectively. Reductive cleavage of the fully methylated polysaccharide in the presence of triethylsilane and trimethylsilyl trifluoromethanesulfonate and subsequent acetylation *in situ* gave six partially methylated 1,4-anhydroalditol acetates as the major products and three partially methylated 1,5-anhydroalditol acetates as minor products. Partially methylated 1,5-anhydroalditol acetates were not formed when reductive cleavage was accomplished with triethylsilane and a mixture of trimethylsilyl methanesulfonate and boron trifluoride etherate as the catalyst, demonstrating that the polysaccharide is exclusively comprised of furanosyl residues. The partially methylated anhydroalditols so produced were identified by comparison to authentic standards. Their identities are consistent with the presence in the *M. smegmatis* arabinogalactan of an octasaccharide repeating unit comprised of a nonreducing terminal D-arabinofuranosyl group, a 2-*O*-linked D-arabinofuranosyl residue, three 5-*O*-linked D-arabinofuranosyl residues, a 3,5-di-*O*-linked D-arabinofuranosyl residue, a 5-*O*-linked D-galactofuranosyl residue, and a 6-*O*-linked D-galactofuranosyl residue.

INTRODUCTION

An arabinogalactan polysaccharide is known to be a major component of the cell walls of such notable pathogens as *Mycobacterium tuberculosis, M. leprae,* and *Corynebacterium diphtheriae*[1] and, in fact, is one of the major serological antigens of *Mycobacteria, Corynebacteria, Nocardia,* and *Rhodococcus* species[2]. Because of their biological significance, arabinogalactans have been the subject of extensive investigation[3–7], but in spite of these efforts, not even one such polymer has been fully characterized. For those that have been examined, differences have been reported in their glycosyl compositions and the position(s) of linkage and ring form of individual glycosyl residues. Although these differences may arise as a result of the use of different bacterial strains, two more

* This investigation was supported by PHS grant number CA15325, awarded by the National Cancer Institute, DHHS.
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0008-6215/90/$ 03.50

fundamental problems are likely to also contribute. The first such problem is the lack of a uniform isolation procedure. Various workers have utilized a *soluble* polysaccharide derived by alkali extraction of cell walls[4,8], Wax D[6] (which is thought to be a product of partial cell-wall autolysis), of defatted cell walls[7]. Another such problem is the inability of standard methylation analysis to distinguish between 4-*O*-linked aldopyranosyl and 5-*O*-linked aldofuranosyl residues. Using a procedure developed by Darvill *et al.*[9], McNeil *et al.*[7] addressed this problem and determined that all D-galactosyl and D-arabinosyl residues[3] in the *M. tuberculosis* and *M. leprae* arabinogalactan polysaccharides were furanoid. The latter workers pointed out, however, that small proportions of pyranose residues might be missed by their analyses.

The procedure for distinguishing between 4-*O*-linked aldopyranosyl and 5-*O*-linked aldofuranosyl residues that was developed by Darvill *et al.*[9] is laborious and quite complex, as it involves sequential methylation, partial acid hydrolysis, reduction with sodium borodeuteride, ethylation, total acid hydrolysis, reduction with sodium borodeuteride, acetylation, and subsequent gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.) of the derived alditol acetates. In contrast, the reductive-cleavage method[10] is a much simpler procedure for distinguishing between 4-*O*-linked aldopyranosyl and 5-*O*-linked aldofuranosyl residues and, in addition, the method can be used to simultaneously establish the identities[11] of constituent monomers and their positions of linkage[12–18]. However, most previous studies have utilized polysaccharides that exclusively contain pyranosyl residues, and no studies have been carried out on polysaccharides comprised of either arabinofuranosyl or galactofuranosyl residues.

Our continuing interest in the structure[19,20] and immunology[21] of mycobacterial cell-wall components therefore led us to examine the *M. smegmatis* arabinogalactan by the reductive-cleavage technique. Because of conflicting past reports as to its composition and structure, these studies were begun by developing an isolation protocol designed to yield the covalently attached cell-wall polysaccharide in highly pure form.

RESULTS

Isolation of the M. smegmatis *arabinogalactan.* — The goal of the isolation protocol was to isolate, in soluble form, that form of the arabinogalactan covalently linked to peptidoglycan of the bacterial cell wall. The cell walls of *M. smegmatis* were isolated as previously described[22] and carefully washed by centrifugation to remove water-soluble contaminants. Analysis of the crude cell-wall preparation for amino acids and amino sugars (Table I) revealed the usual constituents of peptidoglycan, *i.e.*, D-muramic acid (MurN), D-glucosamine (GlcN), alanine (Ala), glutamic acid (Glu), and diaminopimelic acid (Dap), as well as other amino acids. Analysis of the total sugar content by the method of Dubois *et al.*[23] gave a value of 29% by weight, and analysis for neutral sugars[24] indicated the presence of D-arabinose, D-galactose, D-mannose, and D-glucose in relative molar ratios of 3.4:1.0:1.4:0.4, respectively. After saponification and extraction to remove mycolic acids[25], the cell walls were reanalyzed (Table I) and

found to be depleted in amino acids of non-peptidoglycan origin, to have a higher carbohydrate content (52% by weight), and to contain only D-arabinose and D-galactose (molar ratio 3:1, respectively). Finally, the saponified cell wall was solubilized by treatment[26] with lysozyme, and the arabinogalactan was purified by gel-permeation chromatography on Bio-Gel A-1.5m, where it eluted at the void volume. Amino acid and amino sugar analysis (Table I) demonstrated that non-peptidoglycan amino acids, except for glycine, were absent. Analysis for total carbohydrate gave a value of 83% (by weight), and neutral sugar analysis demonstrated that the D-arabinose:D-galactose ratio (3:1, respectively) was unchanged from that present in the saponified cell wall.

TABLE I

Amino acid and carbohydrate analysis of *M. smegmatis* cell-wall preparations and the lysozyme-solubilized arabinogalactan

	Crude Cell Wall	*Saponified Cell Wall*	*Arabinogalactan*
Peptidoglycan components (Molar Ratios)[a]			
MurN	0.95	1.2	0.92
GlcN	0.70	1.6	1.3
Ala	5.1	3.4	3.1
Glu	2.3	0.76	0.71
Dap	1.0	1.0	1.0
Other amino acids[a]			
Asp	1.8	—	—
Thr	1.6	0.07	—
Ser	1.1	0.05	—
Pro	1.3	—	—
Gly	2.2	0.10	0.08
Ile	1.0	0.06	—
Leu	2.3	0.13	—
Tyr	0.50	—	—
Phe	0.74	—	—
His	0.49	—	—
Lys	1.3	—	—
Neutral carbohydrates (Molar Ratios)[b]			
Ara	3.4	3.0	3.0
Gal	1.0	1.0	1.0
Man	1.4	—	—
Glc	0.4	—	—
Total carbohydrate (Weight-%, dry solid)[c]			
	29	52	83

[a] Determined by acid hydrolysis and amino acid analysis. Values are normalized to Dap. [b] Determined by acid hydrolysis and analysis of alditol acetates by g.l.c.[24]. Values are normalized to Gal. [c] Determined by the phenol–sulfuric acid assay[23] with a mixture of D-arabinose and D-galactose (3:1, respectively) as standard.

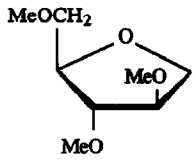
Standard methylation analysis. — The arabinogalactan was subjected to standard methylation analysis[27] in order to identify those residues for which the position(s) of linkage and ring form were ambiguous. The fully methylated polysaccharide was hydrolyzed, and the resulting monomers were subjected to reduction ($NaBH_4$) and acetylation. Analysis of the resulting mixture of partially methylated alditol acetates by g.l.c. combined with chemical-ionization (c.i.) and electron-impact (e.i.) mass spectrometry revealed the presence of six components which were integrated and corrected for molar response[29]. The results (Table II) were suggestive of an octasaccharide repeating unit, but it was not possible to determine whether three of the components were derived from 4-*O*-linked pyranosyl or 5-*O*-linked furanosyl residues. The positions of linkage and ring form in the remaining residues were clearly indicated, however. The results could therefore be interpreted in terms of an octasaccharide repeating unit comprised of a terminal (nonreducing) D-arabinofuranosyl group, a 2-*O*-linked D-arabinofuranosyl residue, three 5-*O*-linked D-arabinofuranosyl or 4-*O*-linked D-arabinopyranosyl residues or the possible permutations thereof, a 3,5-di-*O*-linked D-arabinofuranosyl or 3,4-di-*O*-linked D-arabinopyranosyl residue, a 5-*O*-linked D-galactofuranosyl or 4-*O*-linked D-galactopyranosyl residue, and a 6-*O*-linked D-galactofuranosyl residue.

TABLE II

Identities and molar ratios of the partially methylated alditol acetates derived by standard methylation analysis of the *M. smegmatis* arabinogalactan

Methylated alditol acetate	*Molar ratio*	*Linkage indicated*
1,4-Di-*O*-acetyl-2,3,5-tri-*O*-methyl-D-arabinitol	1.0	terminal Ara*f*
1,2,4-Tri-*O*-acetyl-3,5-di-*O*-methyl-D-arabinitol	1.0	2-*O*-linked Ara*f*
1,4,5-Tri-*O*-acetyl-2,3-di-*O*-methyl-D-arabinitol	2.9	5-*O*-linked Ara*f* or 4-*O*-linked Ara*p*
1,3,4,5-Tetra-*O*-acetyl-2-*O*-methyl-D-arabinitol	0.9	3,5-di-*O*-linked Ara*f* or 3,4-di-*O*-linked Ara*p*
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-galactitol	1.0	5-*O*-linked Gal*f* or 4-*O*-linked Gal*p*
1,4,6-Tri-*O*-acetyl-2,3,5-tri-*O*-methyl-D-galactitol	0.9	6-*O*-linked Gal*f*

Reductive-cleavage analysis. — Based upon the results of standard methylation analysis, it was apparent that total reductive cleavage of the arabinogalactan could give rise to as few as six, or as many as nine, partially methylated anhydroalditols. The nine possible anhydroalditol derivatives (**1–9**) were therefore prepared by independent synthesis[30], and the g.l.c. retention times and c.i. (NH_3)- and e.i.-mass spectra of their acetates (**1**, **2a–9a**) were compared to those of the products derived by reductive cleavage of the fully methylated arabinogalactan. Reductive cleavages were performed with triethylsilane (Et_3SiH) as the reducing agent and, separately, with the two catalysts previously shown to effect total reductive cleavage, *i.e.*, trimethylsilyl trifluoromethanesulfonate[14] ($Me_3SiOSO_2CF_3$) or a mixture[31] of trimethylsilyl methanesulfonate (Me_3SiOSO_2Me) and boron trifluoride etherate ($BF_3{\cdot}Et_2O$).



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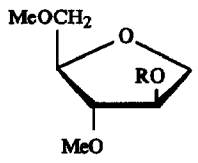
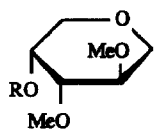
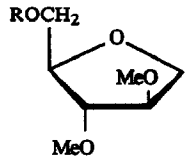


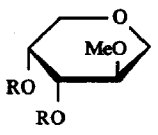
2a
b



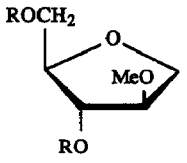
3a
b



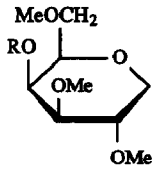
4a
b



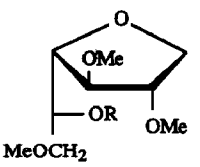
5a
b



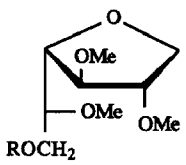
6a
b



7a
b



8a
b



9a
b

a: R = Ac
b: R = Bz

Reductive-cleavage with Et_3SiH and $Me_3SiOSO_2CF_3$. — Shown in Fig. 1 is the gas–liquid chromatogram obtained when the reductive cleavage of permethylated arabinogalactan was performed in the presence of Et_3SiH and $Me_3SiOSO_2CF_3$, with subsequent acetylation *in situ*[14]. The numbered peaks were identified as indicated, integrated, and corrected for molar response[12,29] to give the molar ratios listed in Table III. As is evident (Fig. 1, Table III), all nine possible anhydroalditol derivatives were observed in this reaction, but three products (**3a, 5a** and **7a**) were observed in molar proportions substantially less than unity. The three minor products (**3a, 5a, 7a**) were all pyranoid isomers, and, in each case, these were derived from residues (4-*O*-linked pyranoses or 5-*O*-linked furanoses) sensitive[32] to rearrangement during Me_3SiO-SO_2CF_3-catalyzed reductive cleavage. The major products (**1, 2a, 4a, 6a, 8a, 9a**) of reductive cleavage were furanoid isomers, however. So it was assumed that the polysaccharide was exclusively comprised of furanosyl residues and that the three 5-*O*-linked furanosyl residues were subject to ring isomerization during reductive cleavage. If this assumption were valid, then the combined molar ratios of **3a** + **4a** (3.0), **5a** + **6a** (0.97) and **7a** + **8a** (1.03) would be whole numbers, within experimental error, and the molar ratios of all glycosyl residues would be those expected based upon compositional analysis and standard methylation analysis. These results are therefore consistent with the presence in the polysaccharide of an octasaccharide repeating unit comprised of one non-reducing terminal D-arabinofuranosyl group, one 2-*O*-linked D-arabinofuranosyl

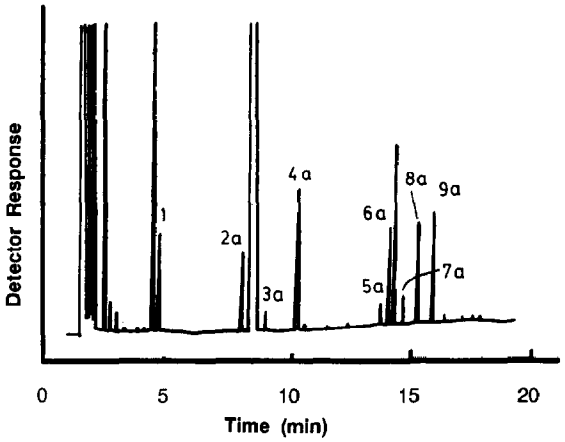


Fig. 1. Gas–liquid chromatogram of the partially methylated anhydroalditol acetates derived by Me_3SiO-SO_2CF_3-catalyzed reductive-cleavage of the per-*O*-methylated *M. smegmatis* arabinogalactan. The peaks were numbered with the compound numbers. Unnumbered peaks were present in a reagent control.

TABLE III

Molar ratios of products derived by reductive cleavage of the fully methylated *M. smegmatis* arabinogalactan with either $Me_3SiOSO_2CF_3$ or $Me_3SiOSO_2Me/BF_3{\cdot}Et_2O$ as the catalyst

Compound	*Molar Ratio*[a]	
	$Me_3SiOSO_2CF_3$	*$Me_3SiOSO_2Me/BF_3{\cdot}Et_2O$*
1	1.2	1.1
2a	1.2	1.2
3a	0.31	—
4a	2.7	3.0
5a	0.26	—
6a	0.71	0.75
7a	0.25	—
8a	0.78	0.65
9a	0.88	0.43

[a] Normalized to the proportion of **4a** or the combined proportions of **3a** and **4a** set at 3.0.

residue, three 5-*O*-linked D-arabinofuranosyl residues, one 3,5-di-*O*-linked D-arabinofuranosyl residue, one 5-*O*-linked D-galactofuranosyl residue, and one 6-*O*-linked D-galactofuranosyl residue.

Reductive-cleavage with Et_3SiH and $Me_3SiOSO_2Me/BF_3{\cdot}Et_2O$. — In our previous work[31], a combination of Me_3SiOSO_2Me and $BF_3{\cdot}Et_2O$ as the catalyst has been found to give the best results for glycosyl residues that are prone to ring isomerization during reductive cleavage. Indeed, reductive cleavage of permethylated arabinogalactan with this catalyst (Fig. 2, Table III) failed to give rise to any of the pyranoid isomers (**3a, 5a, 7a**) observed upon reductive cleavage with $Me_3SiOSO_2CF_3$. Instead, only furanoid isomers (**1, 2a, 4a, 6a, 8a,** and **9a**) were formed, demonstrating that the arabinogalactan

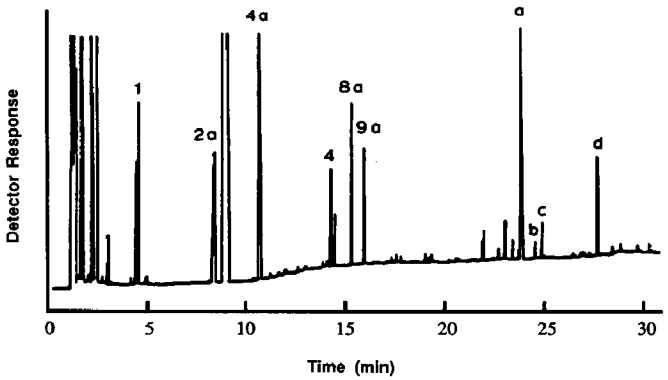


Fig. 2. Gas–liquid chromatogram of the partially methylated anhydroalditol acetates derived by Me_3SiO-$SO_2Me/BF_3{\cdot}Et_2O$-catalyzed reductive cleavage of the per-*O*-methylated *M. smegmatis* arabinogalactan. The peaks were numbered with the compound numbers except for a, docosane (added as a reference); b, a di-*O*-acetyl-tri-*O*-methyl-D-arabinosyl–anhydro-D-arabinitol; c, a mono-*O*-acetyl-penta-*O*-methyl-D-galactosyl–anhydro-D-arabinitol or mono-*O*-acetyl-penta-*O*-methyl-D-arabinosyl–anhydro-D-galactitol, and d, a di-*O*-acetyl-hexa-*O*-methyl-D-galactitolyl–anhydro-D-galactitol. Unnumbered peaks were present in a reagent control.

is comprised exclusively of furanosyl residues. The reaction was not very satisfactory in a quantitative sense (Table III), however, as incomplete reductive cleavage was observed (Fig. 2). For example, peak b (Fig. 2) was found by c.i.m.s. (NH_3) to have a molecular weight of 392 daltons [$(M + NH_4)^+ = m/z$ 410], which could only correspond to that of a di-*O*-acetyl-tri-*O*-methyl-D-arabinosyl–anhydro-D-arabinitol dimer. Similarly, peak c (Fig. 2) was found to have a molecular weight of 408 daltons [$(M + H)^+ = m/z$ 409; $(M + NH_4)^+ = m/z$ 426], which could only have arisen from a mono-*O*-acetyl-penta-*O*-methyl-D-galactosyl–anhydro-D-arabinitol or a mono-*O*-acetyl-penta-*O*-methyl-D-arabinosyl–anhydro-D-galactitol dimer. Surprisingly, peak d (Fig. 2) was found to have a molecular weight of 496 daltons [$(M + H)^+ = m/z$ 497; $(M + NH_4)^+ = m/z$ 514], which could only correspond to that of a di-*O*-acetyl-hexa-*O*-methyl-D-galactitolyl–anhydro-D-galactitol dimer (bearing an *acyclic* D-galactitol group). The latter product had to have arisen by *endocyclic* reductive cleavage of a D-galactofuranosyl residue. These products were not further characterized, but clearly their presence reduces the proportions of products (**6a, 8a, 9a**) derived from the 3,5-di-*O*-linked D-arabinofuranosyl, 5-*O*-linked D-galactofuranosyl, and 6-*O*-linked D-galactofuranosyl residues (Table III).

Analysis of benzoylated reductive-cleavage products by ^{1}H-n.m.r. spectroscopy. — In order to confirm the structures of the reductive cleavage products, these were isolated as their benzoyl esters by high-performance liquid chromatography (h.p.l.c.), and their ^{1}H-n.m.r. spectra were obtained. Permethylated arabinogalactan (7 mg) was subjected to reductive cleavage for 4 h in the presence of Et_3SiH and $Me_3SiOSO_2CF_3$, followed by benzoylation *in situ* with benzoic anhydride, and the products were chromatographed by normal-phase h.p.l.c. (Fig. 3A). The large peaks near the solvent front, which were present in a reagent control, interfered with the isolation of compound **6b**, so they were

removed by applying the reductive-cleavage mixture to a small column of silica gel and eluting with chloroform (with a small loss of compound **6b**). The reductive-cleavage products were subsequently eluted with ethyl acetate and then rechromatographed by h.p.l.c. (Fig. 3B). The indicated peaks in Fig. 3B were collected, analyzed by ^{1}H n.m.r. spectroscopy, and found to be identical to authentic standards[30] as indicated. The purity of each peak was checked by g.l.c. and, in each case, was found to be >98% pure. Noticeably different in this experiment, versus the separate experiment (Fig. 1) in which acetate esters were analyzed, was the virtual absence of the pyranoid isomers (**3b, 5b, 7b**) arising as a result of ring isomerization. In fact, only a very small proportion of compound **5b** (Fig. 3) was detected. This variability is quite common in $Me_3SiOSO_2CF_3$-catalyzed reactions, as the amount of ring isomerization is known[32] to be a function of the amount of water present during reductive cleavage. Also different in this experiment was the absence of compound **1**, which was not detected due to its lack of ultraviolet absorbance.

The experiment just described was also performed using a mixture of Me_3SiO-SO_2Me and $BF_3{\cdot}Et_2O$ as the catalyst, and the products were benzoylated in a separate experiment. Separation of the products by h.p.l.c. gave chromatograms (not shown) virtually identical to those in Fig. 3. However, the pyranoid isomers (**3b, 5b, 7b**) were not observed in this experiment. Nor were the dimers that were detected in the separate experiment (Fig. 2) in which the products were analyzed as their acetates. Such results suggest that, in this experiment, reductive cleavage was complete.

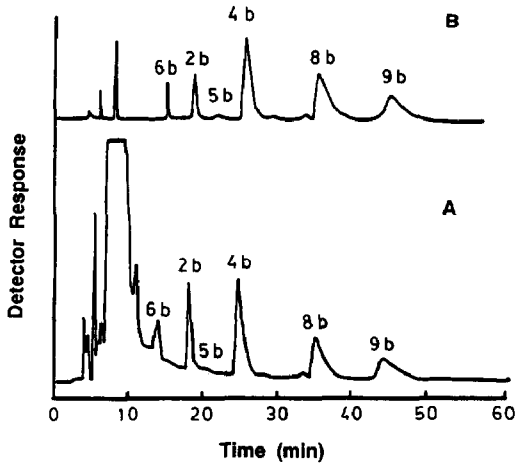


Fig. 3. High-performance liquid chromatograms of the partially methylated anhydroalditol benzoates derived by reductive-cleavage of permethylated arabinogalactan both before (A) and after (B) removal of benzoate side products. The peaks were numbered with the compound numbers.

DISCUSSION

The simple, but effective, procedure for isolation of the *M. smegmatis* arabinogalactan that is described herein takes advantage of its *insolubility* when it is attached to a peptidoglycan, thus facilitating the removal of soluble polysaccharides, proteins, nucleic acids, etc. Subsequent treatment of the cell wall with lysozyme resulted in its complete solubilization and gave a polysaccharide preparation that was very soluble in water and easy to fully methylate. When lysozyme treatment was omitted, however, the particulate peptidoglycan–arabinogalactan complex was difficult to methylate and, as a consequence, reductive-cleavage mixtures were complex due to the presence of additional, partially methylated anhydroalditol acetates. The molecular weight of the polysaccharide preparation was obviously large, based upon the fact that it was excluded from Bio-Gel A-1.5m. This result probably does not reflect the true molecular size of the polysaccharide itself, since lysozyme digestion of the cell wall probably gives rise to a mixture of peptidoglycan fragments which individually contain more than one polysaccharide chain.

Analysis of the polysaccharide for neutral sugars established the presence of D-arabinose and D-galactose (in a ratio of 3:1, respectively), suggestive of the presence of some multiple of a tetrasaccharide repeating unit. Importantly, the polysaccharide preparation did not contain any of the wide variety of sugars commonly found in other mycobacterial polysaccharides. Standard methylation analysis of the polysaccharide, as expected, gave ambiguous results as to the positions of linkage and ring forms of its constituent monomers, but this experiment did establish the positions of linkage and ring form of three of the eight residues in the presumed octasaccharide repeating unit.

Analysis of the polysaccharide by the reductive-cleavage method confirmed the results of standard methylation analysis for the three residues for which ambiguities of ring form and position(s) of linkage were not present and, moreover, established the ring form and position(s) of linkage of the five remaining residues of the repeating unit. Reductive cleavage in the presence of Et_3SiH and $Me_3SiOSO_2CF_3$ gave six methylated 1,4-anhydroalditols as major products and three methylated 1,5-anhydroalditols as minor products. The latter were interpreted as arising *via* ring isomerization of the 5-*O*-linked furanosyl residues during reductive cleavage. Reductive cleavage with Et_3SiH and $Me_3SiOSO_2Me/BF_3{\cdot}Et_2O$ supported this interpretation as the partially methylated 1,5-anhydroalditol products were not observed. We have since confirmed this interpretation through extensive model studies[33] which demonstrate that 5-*O*-linked-D-arabinofuranosyl and -D-galactofuranosyl residues are subject to ring isomerization in $Me_3SiOSO_2CF_3$-catalyzed reactions but are only slightly, if at all, subject to isomerization in $Me_3SiOSO_2Me/BF_3{\cdot}Et_2O$-catalyzed reactions. The combined results of these experiments demonstrate that the *M. smegmatis* arabinogalactan is comprised exclusively of furanosyl residues, and they are consistent with the presence of an octasaccharide repeating unit containing one terminal D-arabinofuranosyl group, one 2-*O*-linked D-arabinofuranosyl residue, three 5-*O*-linked D-arabinofuranosyl residues, one 3,5-di-*O*-linked D-arabinofuranosyl residue, one 5-*O*-linked D-galactofuranosyl

residue, and one 6-*O*-linked D-galactofuranosyl residue. The sequence of these residues was not determined in the present study, but it is obvious that isolation and characterization of the dimers arising from incomplete reductive cleavage (peaks b–d, Fig. 2) would be useful in this regard[11].

EXPERIMENTAL

General. — Triethylsilane, trimethylsilyl trifluoromethanesulfonate, and boron trifluoride etherate were obtained from Aldrich Chemical Co. Trimethylsilyl methanesulfonate was prepared as previously described[31]. Trimethylsilyl trifluoromethanesulfonate was redistilled under vacuum and stored in sealed ampoules under dry nitrogen or argon. Dimethyl sulfoxide was distilled over barium oxide at reduced pressure, and dichloromethane was distilled from calcium hydride. Both were stored over 4-Å molecular sieves. Methylation was carried out by a modification[34] of the Hakomori[35] procedure.

Analytical g.l.c. was performed using a Hewlett–Packard model 5890A gas–liquid chromatograph equipped with a Hewlett–Packard 3392A integrator, a flame ionization detector, and a J. & W. Scientific DB-5 fused silica capillary column (0.25 mm × 30 m). The temperature of the column was held for 10 min at 110° then programmed at 10.min^{-1} to 300°. High-performance liquid chromatography was performed using a Waters Associates model ALC/GPC-244 liquid chromatograph equipped with a Waters model 440 UV detector and an Alltech preparative silica column (10 mm × 25 cm, 10 μm particle size). The column was eluted with 4:1 (v/v) hexane–ethyl acetate at a flow rate of 3.0 mL·min^{-1}. G.l.c.–m.s. analyses were performed using either a Finnigan 4000 mass spectrometer equipped with a VG Multispec data system or a VG Analytical LTD model VG 7070E-HF high-resolution, double-focusing mass spectrometer. Column effluents were analyzed by c.i.-mass spectrometry with ammonia as the reagent gas and by e.i.-mass spectrometry. ^{1}H-N.m.r. spectra were recorded on an IBM NR-300 n.m.r. spectrometer in $CDCl_3$ and were referenced to internal tetramethylsilane.

Isolation of the M. smegmatis *arabinogalactan.* — Cell walls of *M. smegmatis*, isolated from acetone-defatted cells as previously described[22,25], were washed three times by centrifugation from 0.05M $NaHPO_4$, pH 7. The pelleted cell walls were suspended in water and lyophilized. The product, termed "crude cell wall", was analyzed for amino acids and amino sugars (Microchemical Facility, University of Minnesota), neutral sugars[24], and total carbohydrate[23] (Table I). Saponification of the crude cell walls was carried out in 2N NaOH under nitrogen atmosphere for 16 h at room temperature, and the insoluble product was isolated by centrifugation from water until neutral. The saponified cells were lyophilized, then analyzed as before (Table I). Solubilization of the saponified cell walls (500 mg) was accomplished by treatment with lysozyme (30 mg) in 0.05M phosphate buffer, pH 6.3 (150 mL), with a drop of toluene added, for 16 h at 37° (ref. 26). The reaction mixture was then dialyzed against running water, and the nondialyzable fraction was reduced in volume and chromatographed on a 4 × 100-cm

column of Bio-Gel A-1.5m in water. The arabinogalactan, which eluted at the void volume, was collected and lyophilized, then analyzed for composition (Table I). The yield was 310 mg of arabinogalactan from 500 mg of saponified cell walls.

Reductive cleavages. — Reductive cleavage with $Me_3SiOSO_2CF_3$ as the catalyst and subsequent acetylation *in situ* was carried out as previously described[14], except that reductive cleavage was allowed to proceed for 4 h. The analysis was performed on 2 mg of sample. Benzoylation *in situ* was performed as described for acetylation except that benzoic anhydride was used. Reductive cleavage with a mixture of Me_3SiOSO_2Me and $BF_3{\cdot}Et_2O$ as the catalyst was performed by a modification of the previously published procedure[31]. A solution of Et_3SiH (1.0M), Me_3SiOSO_2Me (1.0M), and $BF_3{\cdot}Et_2O$ (0.2M) in dry CH_2Cl_2 was prepared immediately before use in a 3-mL Wheaton V-vial whose inner surface had previously been silylated[14]. A volume of the solution equal to 10 equiv. each of Et_3SiH and Me_3SiOSO_2Me and 2 equiv. of $BF_3{\cdot}Et_2O$ per equiv. of acetal was then added to the dry, methylated sample (2 mg), and reductive cleavage was allowed to proceed for 4 h at room temperature. The reaction was processed, and the products were acetylated as previously described[31]. Separate benzoylations were carried out using 2 equiv. of benzoic anhydride per equiv. of hydroxyl group in a 10-fold excess of pyridine for 8 h at room temperature or for 1 h at 100°. The reaction was processed in the usual way[12].

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