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Unusual Reducing Sugar from *Coccidioides immitis*

E. SCHEER, T. TERAI, S. KULKARNI, N. F. CONANT, R. W. WHEAT, AND E. P. LOWE

Departments of Microbiology and Biochemistry, Duke University Medical Center, Durham, North Carolina 27706 and Fort Detrick, Frederick, Maryland 21701

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Documentation is offered for the identification of 3-*O*-methyl-mannose as one of several neutral sugars found in defatted arthrospore and mycelial cell walls of *Coccidioides immitis*.

Several monosaccharides have been reported in *Coccidioides immitis* mycelium culture filtrate and autolysate materials. Mannose, glucose, glucosamine, and traces of galactose have been identified (5, 9; E. P. Goldschmidt and G. W. Taylor, Bacteriol. Proc., p. 127, 1958). The identity of an additional fast-moving neutral sugar (5; E. P. Goldschmidt and G. W. Taylor, Bacteriol. Proc., p. 127, 1958), suggested to be a monomethoxy mannose derivative (E. P. Goldschmidt and G. W. Taylor, Bacteriol. Proc., p. 127, 1958), has not been further documented. We recently observed small amounts of similar compounds in hydrolysates of defatted mycelium, arthrospore cells, and cell walls of *C. immitis*. The isolated unknown compound corresponds in chromatographic mobility and staining characteristics to 3-*O*-methyl-mannose and can be differentiated from 3-*O*-methyl-glucose, 3-*O*-methyl-galactose, and 6-*O*-methyl-glucose.

Arthrospores and mycelium were grown, harvested, and killed as previously described (9). Cells were broken by multiple passage of defatted cells through a Ribi-Sorvall refrigerated cell fractionator at 50,000 psi, and cell walls were isolated by fractional centrifugation. Both cells and cell walls were defatted by methanol-chloroform extraction; 10- to 100-mg samples were then hydrolyzed in 2 N HCl at 100 C for 2 hr. Hydrolysates were filtered and evaporated to dryness; water was added and the process was repeated several times to remove acid. The dried residue was dissolved in 1 ml of water and passed through a Dowex 50.H column (1 by 7.0 cm) to remove amino sugars and amino acids. After washing with 10 column volumes of water, the eluted neutral fraction was dried and dissolved in a minimal amount of water. As shown in Table 1, sugars detected on thin-layer chromatograms included galactose (trace), glucose, mannose, and two or more fast-moving unknowns. Slow-running materials, presumably oligosaccharides, were also observed. The fast-moving unknown, spot d in Table 1, has not been identified. The other fast-moving unknown, spot e in Table 1, corresponded in mobility and color reactions to synthetic 3-*O*-methyl-mannose, synthesized by a modification of the route of Aspinall and Zweifel (1). Reference standards of 3-*O*-methyl-D-galactose and 6-*O*-methyl-D-glucose were gifts from E. Gros (2) and C. Ballou (10), respectively, whereas 3-*O*-methyl-D-glucose was purchased from General Biochemicals Corp., Chagrin Falls, Ohio. Unknown spot e and 3-*O*-methyl-mannose exhibited similar color development under a variety of different staining conditions, as described for various methyl-*O*-sugars (4), including a positive reaction of the unknown with 2% triphenyltetrazolium chloride, which ruled out a 2-*O*-methyl-sugar. The isolated unknown compound e also co-chromatographed by gas-liquid chromatography with 3-*O*-methyl-mannose as the peracetylated alcohol derivative (7). This sugar and 3-*O*-methyl-D-mannose were found in all of the above parameters to be identical with a similar compound which could be isolated from mycelium, culture filtrate, cells, and cell walls of *C. immitis*, confirming previous reports (5; Goldschmidt and Taylor, Bacteriol. Proc., p. 127, 1958) of the occurrence in mycelial products of an unidentified, fast-moving neutral sugar. Separation of the individual monosaccharides from either arthrospores or mycelium hydrolysates for analysis and quantification was accomplished by thin-layer cellulose chromatography (Machery and Nagel MN 300, Brinkman and Co., Westbury, N.Y.), and the chromatograms were developed in *n*-butanol-pyridine-water (6:4:3). The separated sugar-containing bands, located by spraying guide strips, were scraped off and eluted from the cellulose; the eluate was dried and redissolved in a minimal amount of water for further assays and chromatography. Relative molar concentrations of the sugars in one arthrospore cell wall sample examined were found to be approximately 0.08:

TABLE 1. *Chromatographic identification of Coccidioides immitis arthrospore reducing sugars*

Compound	$R_{Glucose}$ values[a,c]							
	Paper chromatography in solvent system			Thin-layer chromatography in solvent system[b]				
	A	B	C	A	B	C	D	E
Arthrospore cell wall hydrolysate								
a				0.86	Streak	1.00	Streak	Streak
b	1.00	1.00	1.00	0.99	0.72–1.10	Streak	0.76–1.19	0.66–1.11
c	1.19	1.29	1.08	1.13	1.18	1.07–1.24		
d				1.28	1.42	1.70	1.29	
e	1.92	2.57	1.90	1.59	1.77	2.00	1.51	1.61
3-*O*-methyl-mannose		2.58	1.88	1.53	1.76	1.98	1.50	1.62
3-*O*-methyl-glucose				1.56	1.66	1.87	1.58	1.76
3-*O*-methyl-galactose				1.28	1.47	1.88	1.33	1.57
6-*O*-methyl-glucose				1.41	1.74	1.85	1.43	1.63
Mannose	1.19	1.30	1.09	1.10	1.21	1.15	1.12	0.86
Galactose				0.85	0.99	1.13	0.93	0.80
Ribose				1.37	1.51	1.57	1.37	0.76
Glucose	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[a] Spots were detected in systems A to D with *o*-amino-biphenyl (8), or with aniline hydrogen phthalate (4); detection in system E, 50% chromic-sulfuric acid charring.

[b] Solvent systems A to D, thin-layer cellulose plates; solvent system E, thin-layer silica gel G plates.

[c] Solvent systems: (A) *n*-butanol-pyridine-water (6:4:3); (B) *n*-butanol-glacial acetic acid-water (5:1:2); (C) phenol saturated with water; (D) ethyl acetate-pyridine-glacial acetic acid-water (5:5:1:3); (E) chloroform-methanol (2:1).

2.0:1.3:0.03 for the unknown sugar identified as 3-*O*-methyl-mannose, mannose, glucose, and galactose, respectively, as determined by ferricyanide reduction (6) by using a mannose standard.

Although various monomethyl and dimethyl ethers of fucose, rhamnose, 6-deoxytalose, and glucose have been found as hydrolysis products of certain type-specific glycolipids of mycobacteria (3, 4, 10), the present report is the first identification of 3-*O*-methyl-mannose as a constituent of a fungal polysaccharide.

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