

Characterization of 3-*O*-Methylmannose from *Coccidioides immitis*

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3-*O*-methylmannose isolated from *Coccidioides immitis* was characterized by gas chromatographic comparison of derivatives with those of synthetic 3-*O*-methylmannose. Gas chromatographic behavior of three derivatives of the natural and synthetic sugars was identical.

3-*O*-methylmannose has been identified in *Streptomyces griseus* (2), *Escherichia coli* 08 (3), *Klebsiella* type K 57:05 (3), and *Klebsiella* types 2 and 6 (Gormus and Wheat, unpublished data) and was tentatively identified in *Coccidioides immitis* (5). The present work documents characterization of the *C. immitis* sugar as 3-*O*-methylmannose.

The naturally occurring methylated sugar was obtained from pooled, dialyzed culture filtrates of *C. immitis* Silveira strain. The fungus was grown in glucose-yeast dialysate medium in a shaking broth culture for 3 days at 37 C. Culture filtrate (spent medium) was separated from the mycelial mat by filtration through Whatman no. 1 paper on a Büchner funnel. Culture filtrate solubles were passed through a Selas candle (porosity 03) to insure sterility, dialyzed against deionized water, and lyophilized. The lyophilized retentate (150 mg) was hydrolyzed with 15 ml of 2 N hydrochloric acid for 2 hr at 100 C, and acid was removed by distillation under reduced pressure. The hydrolysate was deionized with Dowex 50-H⁺ and then with Dowex 1-HCO₃⁻, concentrated, and then streaked on six thin-layer plates coated with a 0.5-mm layer of microcrystalline cellulose (Machery, Nagel & Co.). Glucose and synthetic (1) 3-*O*-methyl-D-mannose were run on either side of the hydrolysate streak. The plates were developed twice in *n*-butanol-acetic acid-water (5:1:2) and then sprayed with alkaline silver nitrate or Timell's reagent as described previously (5) to locate the standard sugars and the edges of the hydrolysate bands. Bands with R_{Glc} values corresponding to synthetic 3-*O*-methylmannose were scraped from the plates and eluted with water. The slurry was filtered on Whatman no. 1 paper, and the filtrate was passed through a 0.45- μ m filter disc (Milli-

pore Corp.) before being lyophilized (yield, 4.1 mg, 2.7%).

In an earlier report (5), tentative identification of the natural methylated sugar was made by thin-layer chromatography. Gas chromatography has now been used to compare several derivatives of the natural and synthetic sugars. The major peaks observed on gas-liquid chromatograms of the natural and synthetic preparations were found to be additive in all cases. The derivatives were prepared by (i) conversion of the sugars to their alditol acetates, (ii) preparation and acetylation of the methyl glycosides, and (iii) acetylation of the sugars without prior reduction.

The alditol acetates were prepared by the method of Sawardeker et al. (4). For preparation of the acetylated methylglycosides, a 20- μ g sample of the sugar was heated under reflux for 2 hr with 1 ml of 2% methanolic hydrogen chloride. The solvent was removed in a rotary evaporator, and the residue was then heated at 138 C with 0.25 ml of an acetic anhydride-pyridine mixture (2:1, v/v) for 2.5 hr. Water was added to destroy excess acetylation reagent, and the solvents were removed at reduced pressure. The peracetylated sugars were prepared by heating 10 μ g of the sugar with 0.25 ml of the same acetylation reagent for 2 hr at 138 C. Excess reagent again was destroyed, and the solvents were removed.

Chromatography was performed with ECNSS-M columns (3% on 100 to 200 mesh GasChrom-Q) in a gas chromatograph (F & M model 400) equipped with a flame ionization detector. Helium carrier gas flow was 110 ml/min. The results (Table 1) indicate correspondence of the three derivatives of both the natural and synthetic sugars. The natural material did not appear to be completely pure since one or more small addi-

TABLE 1. Gas chromatographic data for derivatives of natural and synthetic 3-O-methylmannose

Derivative	Column size (inches)	Column temp (C)	Retention time of major peaks ^a (min)	Retention time of additional smaller peak ^b (min)
Alditol acetates.....	72 by 3/16	183	13.1	5.3
Peracetylated methyl glycosides.....	54 by 3/16	153	20.1	7.3, 9.8, 43.6
Peracetates.....	54 by 3/16	178.6	18.5, 21.5	8.2

^a In chromatograms from both natural and synthetic sugars.

^b From the natural material.

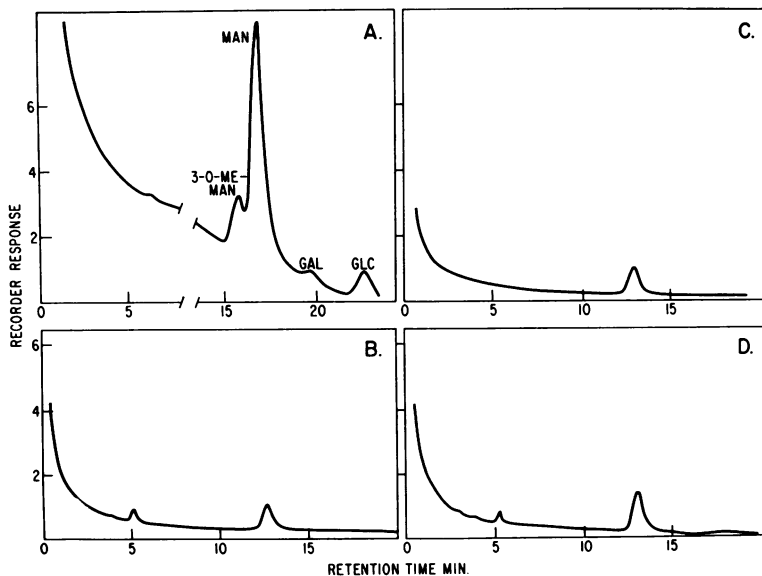


FIG. 1. Gas-liquid chromatograms of monosaccharide alditol acetate derivatives prepared from (A) *Coccidioides immitis* culture filtrate polysaccharide hydrolysate, (B) 3-O-methylmannose fraction eluted from thin-layer cellulose chromatograms, (C) synthetic 3-O-methyl-D-mannose, and (D) co-chromatography of a mixture of synthetic and natural 3-O-methylmannoses.

tional peaks were observed in chromatograms of derivatives of it. However, the major peaks were identical to those of the synthetic sugar in each case (Fig. 1). Finally, it should be mentioned that 3-O-methylmannose has been repeatedly demonstrated by these procedures in culture filtrate and mycelial polysaccharides obtained from different subcultures of *C. immitis* strain Silveira and also from subcultures of strains M-11, Cash, and 3384.

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