IDENTIFICATION OF 6-O-α-D-MANNOPYRANOSYL *MYO*-INOSITOL[1] FROM *SACCHAROMYCES CEREVISIAE*[2]

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SUMMARY: A mannosyl inositol isolated from Baker's yeast was shown to be α-linked from the 1 position of mannose to the 6 position of *myo*-inositol by comparison of the products of permethylation, hydrolysis, and reduction of the disaccharide. The structure was established using chemical and enzymatic methods, gas chromatography, and combined gas chromatography-mass spectrometry.

INTRODUCTION.

Recently, a galactoside of *myo*-inositol from human and rat milk and rat mammary gland was isolated and identified as 6-O-β-D-galactosyl *myo*-inositol (1). Because it is structurally similar to 1L-1-O-α-D-galactopyranosyl *myo*-inositol (1-α-galactinol) of plant origin, it was given the trivial name, 6-β-galactinol (1). A related glycoside of *myo*-inositol synthesized by yeast was identified by Tanner as O-α-D-mannopyranosyl-*myo*-inositol (2). It was proposed that this compound may donate mannosyl groups in certain reactions of yeast in analogy with the role played by 1-α-galactinol in the transfer of galactosyl units in plants (3,4). This attractive hypothesis, however, may not be applicable to the mannosyl *myo*-inositol from *Saccharomyces cerevisiae* since, in this report, we present evidence that the mannosyl group is linked not to the 1 position but to the 6 position. Thus, it is more closely analogous to the galactoside of mammary origin which is β-linked to the 6 position of *myo*-inositol.

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MATERIALS AND METHODS.

Saccharomyces cerevisiae cells (Anheuser-Busch, Inc., St. Louis, Mo.) were grown aerobically in 10 ℓ of sterilized medium (5) for 40 hr at 30°C. The cells (260 g) were harvested by centrifugation at 15,000 rpm for 20 min. The mannosyl *myo*-inositol was isolated essentially as described by Tanner (2). In the present preparation, a column (50 x 4.5 cm) of prewashed charcoal (Darco G-60):Celite (Johns Manville, New York) (1:1, w/w) was employed. The deionized extract was pumped onto the column at a rate of 2-3 ml/min and monosaccharides were eluted with 8.2 ℓ of water. The mannosyl inositol was eluted with 1.6 ℓ of 2% ethanol. Samples of 160 ml of the effluent were reduced to 5 ml and aliquots monitored by gas chromatography of the trimethylsilylated residue (6). The compound emerged in a broad peak prior to the elution of contaminating trehalose from the charcoal column with 10% ethanol (v/v). The combined samples were concentrated to a small volume, streaked onto sheets (25 x 45 cm) of Whatman 3 MM paper, and the chromatogram was developed by an ascending technique using four passes of a solvent system consisting of butanol-pyridine-water-acetic acid (6:4:3:0.3, by volume). Sugars were detected by the silver nitrate procedure of Trevelyan (7). The band moving just ahead of authentic 1-α-galactinol (Calbiochem, La Jolla, CA.) was eluted from the paper with water for analysis. Gas chromatography of the fully trimethylsilylated sugar was carried out on a Hewlett-Packard Model 402 instrument equipped with a hydrogen flame detector, and a 3 mm x 1.8 m glass column packed with 5% SP 2401 (Supelco, Bellefonte, PA.) on GasChrom Z, 80-100 mesh. The column temperature was 240°C. Permethylation, hydrolysis, and reduction of the hydrolysis products were carried out as previously published (8-10). Samples of the purified disaccharide preparation were hydrolyzed in 2 N H_2SO_4 for 1.5 hr in a boiling water bath. After neutralization with 0.3 N $Ba(OH)_2$, the salt-free supernatant solution was dried and trimethylsilylated in the presence of the internal standard, methyl α-D-mannosylpyranoside, as previously reported (6). Combined gas-liquid chromatography-mass spectrometry of the permethylated sugar and its

hydrolysis-reduction products were recorded at an electron energy of 70 eV with a LKB 9000 mass spectrometer. The relative abundance of fragments was displayed as bar graphs by means of an on-line data acquisition and processing program (11). The source temperature was 290°, accelerating voltage 3.5 KV, and the ionizing current 60 μA. Sample introduction was via the GC inlet using a 3 mm x 1.8 m glass coil packed with 3% OV-1 on Supelcoport (80-100 mesh). The column temperature was 240°C.

Enzymatic hydrolysis was carried out by incubating aliquots of the isolated mannoside with α-mannosidase (EC 3.2.1.24) from jack bean (Boehringer Mannheim, New York, N.Y.). The reaction mixture contained 100 mM sodium acetate buffer, pH 4.6, 10 mM $ZnSO_4$, and 50 units of α-mannosidase based on the hydrolysis of p-nitrophenyl β-D-mannopyranoside (Sigma, St. Louis, Mo.), and was incubated for 6 hr at 37°C. The reaction was terminated by placing in a boiling water bath for 3 min, and after cooling, galactitol was added as an internal standard. The mixture was deionized by the addition of 2-3 g of washed MB-3 resin (Rohm & Haas, Philadelphia, Pa.), and after drying, the residue was trimethylsilylated and gas chromatographed as before (10). Similarly, samples of the mannosyl inositol were incubated with 100 mM sodium acetate buffer, pH 4.6, 10 mM $ZnSO_4$, and 10 mg of hemicellulase (grade II from Rhizopus mold, Sigma, St. Louis, Mo.) containing 4 units (μmoles p-nitrophenol produced/min) of β-mannosidase activity for 24 hr at 37°C.

RESULTS AND DISCUSSION.

Gas chromatography of the trimethylsilylated mannosyl *myo*-inositol purified on charcoal and Whatman 3 MM paper revealed a single peak in the disaccharide region [raffinose (Sigma, St. Louis, MO.) was added as an internal standard] (Fig. 1) and the absence of peaks in the monosaccharide region (data not shown). The trimethylsilylated mannosyl *myo*-inositol eluted from the SP 2401 column earlier than either 1-α- or 6-β-galactinol. When the disaccharide was acid hydrolyzed, mannose and *myo*-inositol were identified by gas chromatography of the trimethylsilyl derivatives as the only products and were present in a 1:1

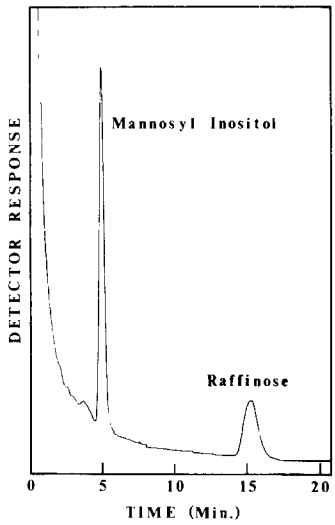

Fig. 1. Gas-liquid chromatography of the fully trimethylsilylated 6-O-α-D-mannopyranosyl *myo*-inositol isolated from yeast on a 3 mm x 1.8 m glass column packed with 5% SP 2401 on GasChrom Z (80-100 mesh). The column temperature was 242°C. The raffinose derivative was an added internal standard.

molar ratio as determined by quantitative gas chromatography. The hydrolysis of the mannosyl inositol by 50 units of α-mannosidase calculated from *myo*-inositol released was 0.7% in 6 hours. With the appropriate p-nitrophenyl α- or β-D-mannopyranoside, the α-mannosidase preparation was found to be contaminated with approximately 2% of β-activity. We, therefore, subjected the inositol mannoside to crude β-mannosidase which contained no activity toward the p-nitrophenyl α-D-mannopyranoside. After incubation for 24 hr, no hydrolysis of the mannoside was detectable. We, therefore, conclude from these experiments that the yeast mannoside is α-oriented but is a very poor substrate for jack bean α-mannosidase compared with the p-nitrophenyl substrate.

These data thus confirmed the findings of Tanner (3) and verified that the disaccharide isolated was suitable for structural analysis by combined gas chromatography-mass spectrometry to determine the position of linkage between mannose and *myo*-inositol. The fragmentation pattern of the permethylated

Table 1. Gas chromatography of derivatives of *myo*-inositol disaccharides

Derivatives	Retention Time (Min)		
	1-α-Galactinol	Glucosylinositol	Mannosylinositol
Permethyl[a]	11.8	7.7	7.6
Trimethylsilyl[b]	6.8	---	6.0

[a]Column was 3% OV-1, 3 mm x 1.8 m on Chromosorb W, 100-120 mesh, and the temperature was 220°C.

[b]Column was 5% SP 2401, 3 mm x 1.8 m on GasChrom Z, 80-100 mesh, and the temperature was 240°C.

derivative of mannopyranosyl *myo*-inositol was found to include ions at *m/e* 293, 233, and 201 in the high mass region, and *m/e* 75, 88 (base), and 101 in the low mass region. This fragmentation pattern has been shown to be characteristic of aldohexose-*myo*-inositol type disaccharides (unpublished data).

The position on *myo*-inositol to which mannose was linked was determined as previously published by Ballou (12), Ueda *et al.* (13), and Naccarato and Wells (1). After permethylation, hydrolysis, and reduction of mannosyl *myo*-inositol, the products were identified as 2,3,4,6-tetramethylmannitol, using authentic methyl α-D-mannopyranoside (General Biochemicals, Chagrin Falls, OH.) as a model, and 1,2,3,4,5-pentamethyl *myo*-inositol by comparison of retention times (Table 1) and mass spectra of the corresponding pentamethyl inositol derived from an authentic sample of 6-O-α-D-glucopyranosyl *myo*-inositol obtained from H. E. Carter, Tucson, AR., by means of a gift to R. S. Bandurski, East Lansing, MI. The pentamethyl *myo*-inositol product was likewise identical to that obtained previously from 6-O-β-D-galactopyranosyl *myo*-inositol from rat mammary gland (1). As in the case of the latter compound, insufficient material has made it impossible to identify whether the linkage at the 6 position is to 1D or 1L *myo*-inositol and thus in both instances, this final structural feature remains to be determined.

Table 2. Gas chromatography of methylated hydrolysis products of *myo*-inositol disaccharides[a]

Compound Methylated	Products	Retention Time (Min)
1-α-Galactinol	2,3,4,6-Tetra-O-methyl galactitol	6.8
	1,2,4,5,6-Penta-O-methyl *myo*-inositol	4.8
Glucosylinositol	2,3,4,6-tetra-O-methyl glucitol	6.6
	1,2,3,4,5-penta-O-methyl *myo*-inositol	6.2
Mannosylinositol	2,3,4,6-tetra-O-methyl mannitol	6.7
	1,2,3,4,5-penta-O-methyl *myo*-inositol	6.2

[a]Column was 3% OV-1, 3 mm x 1.8 m on Chromosorb W, 100-120 mesh, and the temperature was 148°C.

Nevertheless, the discovery that the linkage of the mannopyranosyl group of the yeast disaccharide is on the 6 position of *myo*-inositol, as in the case of 6-β-galactinol, whereas galactinol which acts as a galactosyl transfer agent in plants is linked 1-α- casts some doubt on the universality of a cofactor role for the former two compounds in reactions of glycosylation in yeast and mammary cells. We have not been able to obtain evidence for 6-β-galactinol in rat tissues other than the mammary gland and in its secretory product, milk (unpublished data). Similarly, the mannopyranosyl *myo*-inositol of yeast cells may be an excretory metabolite of the type originally described by Dawson *et al.* (14) found in the medium of growing yeast cells. The biosynthesis of 1-α-galactinol in plants involves transfer of galactose from UDP-galactose to *myo*-inositol (15). However, it is possible that nucleotides do not participate in the biosynthesis of 6-O-α-D-mannosyl *myo*-inositol from yeast or 6-β-galactinol from

mammary gland because of the position of linkage to *myo*-inositol. In regard to the metabolic origin of 6-O-α-D-mannosyl *myo*-inositol, it is of interest that a mannosyl *myo*-inositol moiety is found in glycolipids of yeast (16) and glycerolipids of *Mycobacterium* (17). The position of mannose substitution on *myo*-inositol in glycolipids of yeast is not known, but in *Mycobacterium*, the *myo*-inositol in glycerolipids is substituted on the 2 and 6 positions with mannose (17). In view of these structural similarities, the possibility that the mannosyl *myo*-inositol from yeast is a catabolite of the more complex glycolipid warrants further investigation.

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