Preparation of Galactinol and Myoinositol From Sugar Beet Sirup by Chromatography on a Cation Exchange Resin

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Galactinol (O-α-D-galactopyranosyl-myoinositol dihydrate) was first isolated by Brown and Serro from sugar beet sirup in 1953 (2)[2]. By methylation studies, Kabat *et al.* showed the structure of this new glycoside is D-1-O-α-D-galactopyranosyl-myoinositol (5). Frydman and Neufeld in 1963 synthesized galactinol by catalyzing with an enzyme preparation from unripe peas the transfer of D-galactosyl from uridine diphospho-D-galactose (UDP-D-gal) to myoinositol (4). They also showed that this enzyme, which they designated as UDP-D-galactose:inositol galactosyl transferase, catalyzes the transfer of D-galactopyranosyl to scyllo-, dextro-, and levoinositol to yield galactosides different from sugar beet galactinol. No mechanism for synthesis of galactinol from sugar beets has been demonstrated, but there is little doubt that beet galactinol is synthesized by a similar transferase mechanism. Because of their vital roles in microbiology and biochemistry, there has been renewed interest in the cyclitols and their derivatives (1).

Galactinol is now commercially available in milligram quantities[3], but for some syntheses it is more practical to use gram quantities. The carbon column chromatographic method used by Brown and Serro is somewhat laborious so it seemed worthwhile to describe a new procedure for isolating galactinol and myoinositol from a beet molasses sirup.

Experimental

B-Molasses Saccharate.—The starting material was B-molasses saccharate sirup (kindly furnished by The Great Western Sugar Company[4]), a concentrate prepared by lime treatment of their feedstuff (Concentrated Johnstown Filtrate). We decomposed the lime precipitate with carbon dioxide, filtered off calcium carbonate, concentrated the solution at pH 8.9 to 70% refractometric dry solids (RDS). B-Molasses saccharate is a thick dark sirup

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[4] The Great Western Sugar Company, Sugar Building, Denver Colorado.

and contains sucrose, raffinose, galactinol, myoinositol and many other unidentified substances. Quantitative bioassay of this sample of saccharate sirup, with *Saccharomyces carlsbergensis*[5] showed 1.23% free myoinositol and 3.04% myoinositol after acid hydrolysis. By difference bound myoinositol was 1.81%, which was calculated to be 3.62% as anhydrous galactinol. The assay on galactinol was negative.

Fermentation of B-Molasses Saccharate.—Two kilos of saccharate sirup (pH 8.9, 70% RDS) were diluted with about 7 liters of water at 30° C to 15% RDS. The pH was adjusted to 6.0 with about 5 ml of 85% phosphoric acid, and three pounds of baker's yeast was added. After five hours the Clinistix test for glucose was negative indicating that fermentation was complete. The RDS dropped to 9%. Fermentation removes glucose, fructose, and sucrose, and converts raffinose to melibiose. Galactinol, melibiose, myoinositol, yeast metabolites, salts and other compounds remain. Yeast was removed by centrifugation and melibiose was destroyed by adding 150 g of calcium hydroxide and boiling the solution for 20 minutes at pH 12.3. Carbon dioxide was bubbled in to about pH 8 to precipitate calcium carbonate and the solution was boiled 3 minutes to free it from excess carbon dioxide. After the calcium carbonate was filtered off, the solution had a pH of 7.8, 10% RDS, and contained 2.1 equivalents of unidentified anions in 7 liters.

Ion Exchange.—The solution was passed through 5 liters of a cation exchange resin, Dowex 50 X8 (H). The effluent at pH 3.1 was passed immediately through 5 liters of a weak anion exchange resin, Doulite A-4 (OH). The pH rose to 7 but dropped to 3.6 before all of the solution was through the column, and, after washing was complete, the 28 liters of effluent and wash water were at pH 3.6 and 1% RDS. It was neutralized with ammonium hydroxide and concentrated *in vacuo* at 40-50° C to 2 liters and 12% RDS. The pH was adjusted to 8 with potassium hydroxide. The pH of the solution dropped to 5.7 overnight.

Separation on a Cation Exchange Resin.—Fractionation according to molecular size of sugars was described by Jones *et al.* (6) and Carruthers *et al.* (3) with water on columns of Dowex 50W × 2 (2% DVB 200-400 mesh, Li^+ form). Our ion exclusion and molecular species separations in the experiment reported here were conducted at 80° C in a steam-jacketed column[6] of

[5] Analyses were run by Wisconsin Alumni Research Foundation.

[6] This ion exclusion column was designed and its construction was supervised by William G. Schultz of the Engineering and Development Laboratory, Western Regional Research Laboratory, Albany, California.

175 cm² in cross section containing 54 liters of Dowex 50W X4 (4% DVB, 50-100 mesh, K^+ form) operating at a rate of 175 ml of effluent per minute. The first one-third of the column effluent (18 liters) was discarded. The RDS reached its maximum due to salts and polymers, and, when the RDS decreased below 1%, thirty fractions of 600 ml each and 6 fractions of 900 ml each were collected for the remainder of the run. Figure 1 shows variations of RDS *vs.* effluent volume during the run.

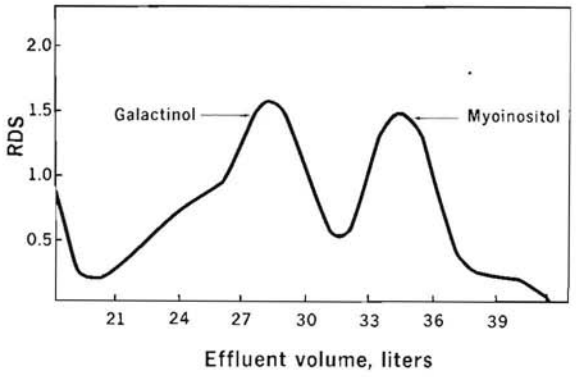

Figure 1.—Plot of RDS *vs.* volume of column effluent for separation of galactinol and inositol.

Paper Chromatography of Fractions.—Each fraction was analyzed by paper chromatography. The solvent system was the organic phase of a mixture of 1-butanol, glacial acetic acid and deionized water (4:1:5) v/v. One microliter of juice (about 10% solids) was spotted on sheets of Schleicher and Schuell No. 2043-B paper and developed by descent for 20 hours. Air-dried papers were dipped in an indicator containing 1 ml of saturated silver nitrate in 200 ml of acetone, dried and dipped in 0.5% sodium hydroxide in ethanol. The sheets were air dried for about one hour, then dipped first in saturated sodium thiosulfate in 60% ethanol and rinsed in 60% ethanol. The ethanol-washed chromatograms were dried in air. R (sucrose) values for galactinol and myoinositol were about 0.36 and 0.76, respectively. Fractions 5 to 24 were combined to recover galactinol, and fractions 25 to 32 were combined to crystallize myoinositol.

Crystallization.—The combined fractions mentioned above were concentrated to 35-40% RDS, ethanol added to turbidity (2 to 3 volumes for galactinol and more for myoinositol), and the solutions were heated on the steam bath to 50° C, and more ethanol added. Galactinol dihydrate (30 g) and anhydrous myoinositol (12 g) crystallized during cooling overnight at 25°

C. Samples were recrystallized as described. Gas chromatography of the trimethyl silyl ethers of galactinol showed only one component (7).

Properties of Galactinol Dihydrate.—The $[\alpha]_D^{25}$, + 135.8 (c, 2% in water) agrees with that reported earlier by Brown and Serro (2). Water loss *in vacuo* at 80° C overnight was 9.29% (calculated 9.52). Freshly crystallized galactinol and the oven-dried sample were examined by proton magnetic resonance (PMR). If the sample is first dissolved in deuterium oxide, PMR can determine the number of hydrogens attributable to the water of crystallization. These results indicate the fomula $C_{12}H_{22}0_{11} \cdot 2H_20$ for the dihydrate and $C_{12}H_{22}0_{11}$ for the oven-dried anhydrous galactinol.

The melting point of the dihydrate in a closed tube on a Kofler hot stage was 114° C and in an open tube 232-235° C. The melting point depended somewhat upon the method of heating. For example, crystals placed on the stage at 190° C melted immediately, then partially recrystallized. Crystals heated from below 160° C partially melted then resolidified without crystallization. Crystals melted in an open tube at 190° C and subsequently heated to 235° C showed by paper chromatography, myoinositol and galactinol, but no galactose. This shows that during open tube melting some of the galactinol is decomposed.

Crystal Properties.—Galactinol dihydrate crystallizes from aqueous ethanol solutions in the form of colorless, splintery needles in loose clusters. If a slush of crystal fragments is covered on a microscope slide with a cover glass and then warmed cautiously to dissolve about half the solid, on cooling the undissolved remnants will grow in the form of prisms belonging to the orthorhombic crystal system. When viewed in polarized light, the crystals show symmetrical extinction.

The refractive indices determined by immersion methods in white light at 26° C are: $\alpha = 1.540$; $\beta = 1.562$; $\gamma = 1.578$. The refractive index for longitudinal vibrations is always α and the minimum and maximum refractive indices for crosswise vibrations are β and γ, respectively. The optical character is (—) with optical axial angle (2F), being too large to measure on any view seen.

Although the crystals of galactinol dihydrate will lose their water of crystallization when vacuum dried, attempts to grow crystals of the anhydrous compound from numerous organic solvents failed. Crystals of the dihydrate left exposed under ambient conditions lost part of their water of crystallization.

Partially oven-dried crystals showed many cracks, and the refractive index for longitudinal vibrations became greater than 1.540.

X-Ray Powder Data.—X-ray powder photographs were taken of these preparations of galactinol dihydrate and anhydrous myoinositol with Ni-filtered copper Kα radiation, $\lambda = 1.5418$ Å. Values are reported in Table 1.

Table 1.—X-ray powder data.

Galactinol dihydrate[1]		Myoinositol	
d-Values	I/I_o	d-Values	I/I_o
9.73	2	6.03	6
7.36	46	5.76	17
6.16	4	5.09	31
5.99	4	4.73	100
5.03	3	4.39	62
4.85	3	3.70	22
4.73	100	3.19	9
4.35	31	3.08	20
4.20	6	2.879	17
4.09	3		
3.90	23		
3.69	27		
3.59	9		
3.51	4		
3.255	12		
3.203	9		
3.090	9		
3.037	14		
2.987	11		

[1] Intensities visually estimated by comparison with a calibrated scale.

Summary

Galactinol and myoinositol were isolated and crystallized from an enriched source of beet molasses by a process including fermentation, alkaline oxidation of melibiose, ion exchange, separation of molecular species on a cation exchange column and crystallization. Some crystal and X-ray powder data are presented.

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Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

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