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Note

A di-D-galactosyl-*myo*-inositol from rapeseed (*Brassica campestris*) meal*

I. R. SIDDIQUI, P. J. WOOD, AND G. KHANZADA
Food Research Institute, Canada Department of Agriculture, Ottawa (Canada)
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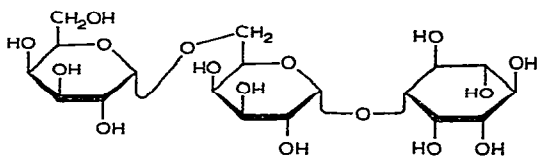
The commonly known galactosyl derivative of *myo*-inositol is galactinol (1L-1-*O*-α-D-galactopyranosyl-*myo*-inositol) which was first isolated from sugar beet[1] (*Beta vulgaris*). During the fractionation of an oligosaccharide preparation (obtained from dehulled, oil-free rapeseed meal) by column chromatography on charcoal–Celite, elution with 5% ethanol gave a disaccharide fraction *B* which contained, *inter alia*, a non-reducing component B_1 now identified as *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→1L)-*myo*-inositol (**1**). The disaccharide derivative **1** appears to be identical with a component previously isolated[2] from vetch seeds.



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Purification of fraction B_1 by acetylation, followed by t.l.c., yielded a crystalline dodeca-acetate, $[\alpha]_D^{25}$ +116° (chloroform), deacetylation of which gave the di-D-galactosyl-*myo*-inositol **1**, $[\alpha]_D^{25}$ +126° (water). Paper chromatography of a total hydrolysate of the purified product yielded only galactose and *myo*-inositol, and oxidation with a D-galactose oxidase preparation (Galactostat) showed that galactose was present in the D form. G.l.c. analysis of a trimethylsilylated portion of the hydrolysate showed that galactose and *myo*-inositol were present in a molar ratio of 2.1:1.0.

Methylation of the di-D-galactosyl-*myo*-inositol **1**, followed by hydrolysis, reduction with sodium borohydride, and acetylation, gave products identical (g.l.c.) with 2,3,4,6-tetra-*O*-methylgalactitol diacetate, 2,3,4-tri-*O*-methylgalactitol triacetate, and 2,3,4,5,6-penta-*O*-methyl-*myo*-inositol monoacetate, in molar ratios of 1.2:1:0.9. The identities of the methylated alditol acetates were confirmed by combined g.l.c.–mass spectrometry[4,5], and 2,3,4,5,6-penta-*O*-methyl-*myo*-inositol monoacetate was

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similarly identified by comparison with an authentic sample prepared from galactinol. The highly positive values for the specific optical rotations of the di-D-galactosyl-*myo*-inositol and its dodeca-acetate strongly suggest that the galactosidic bonds are of the α-D type. Based on the foregoing evidence, the compound is assigned structure **1**.

Biosynthetic studies, using rapeseed-meal enzymes, are needed to elaborate the pathway for the biosynthesis of the di-D-galactosyl-*myo*-inositol **1**. It is conceivable that **1** is a minor by-product of the biosynthetic pathway leading to the synthesis of stachyose, a major oligosaccharide constituent of the rapeseed meal It has been shown[6] that a crude extract from ripening seeds of broad beans (*Phaseolus vulgaris*) efficiently transfers D-galactose from galactinol to raffinose, giving stachyose and *myo*-inositol. The crude extract from the ripening bean-seeds also contains an enzyme which transfers D-galactose-^{14}C from UDP-D-galactose-^{14}C to *myo*-inositol, giving rise to labelled galactinol and uridine 5′-pyrophosphate (UDP). Since galactinol and *myo*-inositol are also constituents of rapeseed meal, it is reasonable to speculate that the same enzyme which transfers D-galactose to raffinose could also transfer D-galactose to galactinol, giving rise to **1** as a minor by-product.

EXPERIMENTAL

General methods. — Paper chromatography was performed by the descending method on Whatman No. 1 and 3MM papers with the organic phases of (*A*) butyl alcohol–pyridine–water (10:3:3), or (*B*) ethyl acetate–pyridine–water (8:2:1). Paper electrophoresis was performed on Whatman No. 3MM paper with 0.2M borate buffer (pH 10), at 800 volts for 2–3 h. Detection was effected with (*A*) aniline hydrogen phthalate[7], (*B*) naphthoresorcinol[8], and (*C*) alkaline silver nitrate[9]. T.l.c.[10] was performed on Silica Gel G (Merck), with a freshly made mixture of 5% methanol in benzene. Sugar acetates were located in t.l.c. by spraying with water. Evaporations were carried out at 35° with a rotary evaporator. Rotations were measured on a Perkin–Elmer 141 polarimeter and are equilibrium values. Melting points are corrected.

*Isolation of di-*D*-galactosyl-*myo*-inositol* **1**. — Material extracted from a large batch of dehulled, oil-free rapeseed meal (3178 g) by boiling 80% aqueous ethanol was freed from bound lipids (168.7 g), yielding an oligosaccharide fraction (454 g). A portion (200 g) of the oligosaccharide fraction was treated with Rexyn 101(H^+) and AG-5(HO^-) resins to yield the deionized oligosaccharide fraction (140 g). The deionized fraction (130 g) was fractionated on a charcoal–Celite column (1:1 v/v; 10 × 60 in.); **1** was found as a minor constituent (~0.03% of the deionized oligosaccharide fraction, on the basis of isolated acetate, see below). It was separated from other components (to be reported elsewhere) by preparative paper chromatography.

Purification and characterization of **1** *as the dodeca-acetate.* — The crude product mixed with an equal weight of freshly fused sodium acetate was heated in acetic anhydride for 3 h at 120°; the dodeca-acetate (63 mg), recovered by chloroform extraction, was purified by t.l.c. The acetylated product (25 mg) was crystallized and

recrystallized from ethanol, giving the dodeca-acetate (19 mg), m.p. 232–233°, $[\alpha]_D^{25}$ +116° (*c* 1, chloroform).

Anal. Calc. for $C_{42}H_{56}O_{28}$: C, 50.00; H, 5.55. Found: C, 50.08; H, 5.13.

*Characterization of di-*D*-galactosyl-*myo*-inositol* **1**. — (*a*) The crystalline dodeca-acetate (15.5 mg) was deacetylated (MeONa), yielding a product (7.7 mg) which showed $[\alpha]_D^{25}$ +126° (*c* 0.47, water); lit.[2] $[\alpha]_D^{29}$ +145° (water). Paper chromatography showed a single component having R_{SUC} 0.023 in solvent *A* and R_{GAL} 0.013 in solvent *B*. Paper electrophoresis also revealed a single component (M_{GLC} 0.44). G.l.c. (Me_3Si derivative) with temperature programming from 100→270°, as indicated below, showed a single, symmetrical peak having a retention time, with respect to trimethylsilylated sucrose, of 1.64.

(*b*) Complete hydrolysis of **1** (4 mg) in M sulphuric acid (0.25 ml) for 4 h at 100° gave galactose and *myo*-inositol as the only detectable components on paper chromatography (solvent *B*). A portion of the hydrolysate (100 μl) containing galactose (~100 μg) was mixed with Galactostat* (2 mg). After incubation for 2 h at 37°, no galactose was detected by paper chromatography (solvent *B*, spray *A*). The remaining hydrolysate, after drying, was trimethylsilylated in pyridine (0.5 ml) with hexamethyldisilazane (0.2 ml) and chlorotrimethylsilane (0.1 ml). After 30 min at room temperature, the reagents were removed by distillation *in vacuo* and the residue was taken up in hexane for g.l.c. analysis. G.l.c. was performed on a Pye 104 Chromatograph, using 5-ft. dual columns of 3% OV-225 on Gas Chrom Q (80–100 mesh). The chromatograms were developed with temperature programming at a rate of 5°/min from 90→240°, using a nitrogen flow-rate of 45 ml/min. The molar ratio of galactose to *myo*-inositol was 2.1:1.0.

(*c*) A solution of **1** (3 mg) in freshly distilled *N*,*N*-dimethylformamide[11] (0.5 ml) was shaken in the dark with silver oxide (40 mg) and methyl iodide (0.25 ml) for 24 h. Methylation was continued by shaking overnight after adding a further batch of methylating agents to the reaction mixture. The methylated product, recovered by chloroform partition, was methylated twice with the Purdie reagents, yielding a syrup (1.5 mg).

G.l.c.–mass spectrometry of methylated components. — The methylated di-D-galactosyl-*myo*-inositol **1** (1.5 mg) was hydrolysed with 0.5M sulphuric acid (0.3 ml) at 100° for 20 h. The hydrolyzate was neutralized with barium carbonate and filtered, and sodium borohydride (5 mg) was added to the filtrate (1 ml). After 18 h, the excess of borohydride was destroyed with glacial acetic acid and, after removal of sodium ions with Rexyn-101(H^+) resin, the solution was repeatedly evaporated with methanol to remove borate. The residue was acetylated with acetic anhydride (0.5 ml) and pyridine (0.25 ml) at 100° for 10 min and left at room temperature for 1 h. Water was added to the reaction mixture and, after removal of pyridine by co-distillation with water, the reaction mixture was evaporated to dryness. A solution of the residue in chloroform was analysed by g.l.c. (as described above), with a nitrogen flow-rate

*A mixture of D-galactose oxidase, peroxidase, and phosphate (Worthington Biochemical Corp.).

of 60 ml/min and temperature programming from 150→250° at a rate of 5°/min. Three components were detected in the ratios 1.2:1.0.0.9 and identified, on the basis of retention times (T) relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucitol diacetate, as 2,3,4,6-tetra-*O*-methylgalactitol diacetate (T 1.09), 2,3,4-tri-*O*-methylgalactitol triacetate (T 1.66), and 2,3,4,5,6-penta-*O* methyl-*myo*-inositol monoacetate (T 0.45).

The identities of the three products were confirmed by direct comparison with authentic specimens by combined g.l.c.–mass spectrometry, using a 5-ft. column of ECNSS-M on Gas Chrom Q (100–120 mesh) coupled to a Bell and Howell 21-490 Mass Spectrometer. The mass spectra were recorded at an inlet temperature of 250°, an ionising potential of 70 eV, and an ion-source temperature of 260°.

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