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Note

On the structure of mammalian-lung galactan

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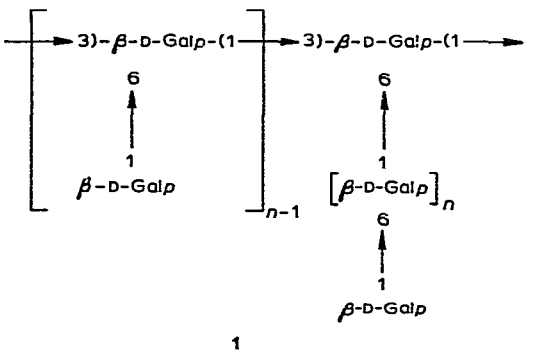
Wolfrom and co-workers showed, in 1952, that beef-lung galactan consists mainly of (1→3)- and (1→6)-linked D-galactopyranosyl residues[1]. Some years later, Heidelberger *et al.* examined this polysaccharide by immunochemical methods[2], and this laboratory extended these investigations in 1973 and proposed structure **1** for the lung galactan[3]. We now present additional data to support this general structure.



Crude lung-galactan was chromatographed on DEAE-cellulose to yield a neutral and an acidic fraction. The neutral fraction was free from protein, and, when hydrolyzed, yielded arabinose (8.3%), galactose (83%), and glucose (8.7%). Chromatography on Sephadex (G-25, G-50, G-100, and G-200) failed to give significant resolution: only on G-200 was there a more-or-less extended elution-pattern (without any separated fractions). The middle portion (7.2% arabinose, 84% galactose, and 9% glucose) was taken for the work reported here, but hydrolysis of all portions of the pattern yielded the three sugars mentioned, in about the same ratios.

In order to verify our earlier proposal[3] that the backbone of the lung galactan is (1→3)-linked and the side branches are composed of (1→6)-linked galactopyranosyl residues, the following experiment was performed. Purified polysaccharide was oxidized with sodium metaperiodate, the excess of periodate was decomposed, and the product was dialyzed. The retained material was reduced with sodium boro-

TABLE I

ALDITOL ACETATES DERIVED FROM METHYLATED LUNG-GALACTAN

Alditol acetate of	*Mole percent*	*Molar ratio*
2,3,5-Tri-*O*-methylarabinitol	7.1	1.0
2,3,4,6-Tetra-*O*-methylgalactitol	26.3	3.7
2,3,6-Tri-*O*-methylglucitol	7.1	1.0
2,3,4-Tri-*O*-methylgalactitol	29.2	4.1
2,4-Di-*O*-methylgalactitol	26.3	3.7
2,4,6-Tri-*O*-methylgalactitol	3.0	0.4
2,3-Di-*O*-methylgalactitol	0.9	0.1

TABLE II

CHROMIUM TRIOXIDE OXIDATION OF ACETYLATED LUNG-GALACTAN

Time of oxidation (h)	*Arabinose*	*Galactose*	*Glucose*	myo-*Inositol*
0	1.10	5.80	2.2	10
1	0.41	0.32	1.2	10
2	0.08	0.08	0.7	10

hydride, and the product hydrolyzed at room temperature with 0.5M sulfuric acid. After neutralization of the acid, the material was methylated, the permethylated polysaccharide was methanolyzed, and the products were analyzed by gas–liquid chromatography (g.l.c.). Only methyl 2,4,6-tri-*O*-methylgalactoside was detected; this shows the polymeric backbone to be a (1→3)-galactopyranan.

Methylation[4] of the purified, original polysaccharide, followed by acid hydrolysis, gave a series of methylated sugars that were converted into their alditol acetates, and these were analyzed by g.l.c. (see Table I).

It may be recalled that the polysaccharide itself shows the following molar ratios: arabinose:galactose:glucose 1:10:1. From Table I, it may be seen that the methylated polysaccharide shows ratios of arabinose:galactose:glucose of 1:12:1; thus, some arabinose and some glucose were lost in the methylation. However, the general outline of the structure, as indicated by the methylated fragments and the periodate oxidation, is fairly straightforward: the proportions of tetra-*O*-methylgalactitol and 2,4-di-*O*-methylgalactitol are identical; the molar amount of 2,3,4-tri-*O*-methylgalactitol is also equal to that. As Smith degradation yielded a (1→3)-linked galactan, these data are precisely compatible with the occurrence of such a structural element as **1**, and this accounts for ~82% of the total structure. In addition, there appear to be occasional backbone units consisting of unsubstituted →3)-Gal*p*-(1→.

The →4)-Glc*p*-(1→ and terminal arabinose units apparently present cannot be placed until more information is acquired.

Finally, in our earlier work, we showed immunochemically the likelihood of the presence of the galactan residues as β-linked units[3]. We have confirmed this chemically by oxidation of the acetylated lung-galactan with chromium trioxide[5,6]. In this method, only an axially oriented 1-proton is abstracted[7], to yield a 5-hexulosonic acid, thus leading to disappearance of the sugar residues having such an α proton. It is clear from the results given in Table II that the galactan is linked by β-glycosidic linkages.

EXPERIMENTAL

General. — G.l.c. was performed with a Hewlett–Packard Model 5730A chromatograph. The columns used were stainless steel (1.83 m × 3.17 mm) packed with 15% of DEGS on Chromosorb W (column *A*), and glass (1.83 m × 6.35 mm) packed with 3% of ECNSS-M on Gas-Chrom Q (column *B*).

Purification of the lung galactan. — Crude pneumogalactan from Hoffmann–La Roche was added to the top of a column (25 × 3 cm) of DEAE-cellulose in 0.05M 2-(hydroxymethyl)-1,3-propanediol (Tris) hydrochloride buffer, pH 8.0. The column was first washed with the same buffer (500 mL). The first 60 mL contained material giving a positive phenol–sulfuric acid[8] reaction; it was dialyzed, and lyophilized (85 mg, fraction A). The column was next eluted with a citric acid–disodium phosphate buffer, pH 4.0, and this removed another material giving a positive phenol–sulfuric acid reaction. Dialysis and lyophilization gave fraction B (250 mg). Fractions A and B both precipitated with murine myeloma immunoglobulins having specificity for β-(1→6)-linked D-galactosyl units, but fraction B did so only weakly. Fraction B precipitated very strongly with a solution of 1% DEAE-dextran in phosphate-buffered saline, pH 7.4, thus indicating its acidic nature[9]. Fraction A did not precipitate

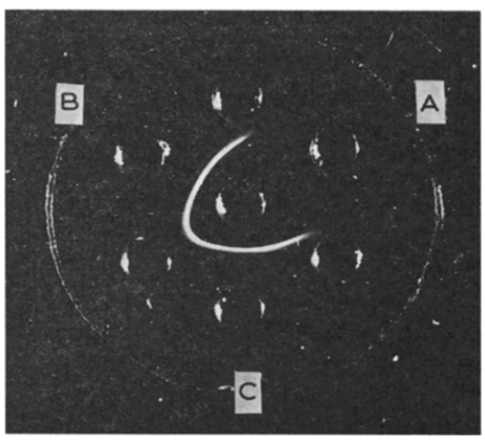


Fig. 1. Agar-gel double-diffusion of lung-galactan fractions *versus* DEAE-dextran. (Outer wells: A, 1% neutral fraction of lung galactan; B, 1% acid fraction of lung galactan; C, 1% sample of lung galactan before purification. Center well: 1% DEAE-dextran.)

with this basic polysaccharide (see Fig. 1). An amino acid analyzer revealed the absence, from fraction A, of amino acids, and the presence of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, histidine, lysine, and large proportions of tyrosine and phenylalanine in fraction B.

Fraction A was passed through a column of Sephadex G-200, and the middle portion of the eluted material was taken. The purified lung-galactan (2 mg) was hydrolyzed with 0.5M sulfuric acid on a steam-bath for 12 h. The mixture was made neutral (barium carbonate), the suspension filtered, and the filtrate de-ionized, and concentrated to 2 mL. The material was reduced with sodium borohydride (50 mg) for 4 h, and the resulting alditols were acetylated with acetic anhydride–pyridine. Analyses were performed by g.l.c. on column *B* at 195°, and showed arabinitol acetate (7.2%), galactitol acetate (84%), and glucitol acetate (8.8%).

Periodate oxidation of lung galactan. — The purified polysaccharide (21 mg) was dissolved in water (5 mL), and sodium metaperiodate (86 mg) in water (5 mL) was added. The oxidation was allowed to proceed in the dark for 112 h. Ethylene glycol (30 mg) was added, and, after 2 h, the solution was dialyzed against distilled water for 48 h. The contents of the dialysis bag were concentrated to 2 mL and treated with sodium borohydride (50 mg) for 3 h. The hydride was decomposed by the addition of Dowex 50-W X-8 resin. An equal volume of 0.5M aqueous sulfuric was added, and the solution was kept for 8 h at room temperature, and the acid neutralized by the addition of Rexyn 203 anion-exchange resin. Residual boric acid (if any) was removed by evaporation with methanol. The lyophilized residue (2 mg) was dissolved in *N*,*N*-dimethylformamide and methylated with methyl iodide and silver oxide[10], and the permethylated polysaccharide was methanolyzed with 4% hydrogen chloride in methanol. Processed as usual, the material was examined by g.l.c. (column *A*, 190°), which revealed the presence of methyl 2,4,6-tri-*O*-methylgalactoside (compared with an authentic sample).

Methylation analysis of the lung galactan. — The polysaccharide was methylated first according to the Hakomori method[4], and then according to the method of Kuhn[10], and the methylated polysaccharide (5 mg) was passed through a column (20 × 1.5 cm) of Sephadex LH-20 in 2:1 chloroform–acetone. Hydrolysis with 90% formic acid for 1 h, followed by 0.5M sulfuric acid for 20 h at 100°, gave the component sugars, which were converted into their alditol acetates in the usual way; these were analyzed by g.l.c. (column *B*, 175°), and the results are listed in Table I.

Determination of the anomeric configuration. — Lung galactan (2 mg), mixed with *myo*-inositol (2 mg) was dissolved in formamide (0.4 mL) and acetylated with acetic anhydride (0.5 mL) and pyridine (1 mL) overnight. The reagents were removed *in vacuo*, the residue was taken up in glacial acetic acid (3 mL), and powdered chromium trioxide (300 mg) was added. The stirred suspension was kept at 50°, and aliquots were removed, deacetylated, hydrolyzed, and analyzed for their sugar content (as their alditol acetates) by g.l.c. with column *B* at 190°. The results are given in Table II.

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