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STRUCTURE OF THE D-GALACTAN ISOLATED FROM GARLIC (*Allium sativum*) BULBS*

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ABSTRACT

Hot-water extraction of defatted garlic-bulbs yielded a mixture of polysaccharides containing a D-galactan, a D-galacturonan, an L-arabinan, a D-glucan, and a D-fructan. A trace of L-rhamnose was also detected in the polysaccharide hydrolyzate. The pectic acid was partially removed by precipitation with aqueous calcium chloride; from the remaining polysaccharide mixture, a pure D-galactan containing 97.3% of D-galactose was isolated by fractional precipitation and repeated chromatography through a column of DEAE-cellulose. Methanolysis and hydrolysis of the permethylated D-galactan yielded 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methyl-D-galactose in the molar proportions of 1:2:1. On periodate oxidation, the D-galactan reduced 1.18 molar equivalents of the oxidant per D-galactosyl residue, and liberated one molar equivalent of formic acid per 4.13 D-galactosyl residues. Smith degradation of the D-galactan was also conducted. From these results, a structure has been assigned to the repeating unit of the D-galactan.

INTRODUCTION

Garlic (*Allium sativum*) is widely cultivated in India, and comes onto the market in two forms: having (*a*) a single bulb per piece, and (*b*) a bunch of bulbs per piece. The work reported here was done with form *b*. Numerous medicinal applications of garlic are known[1], particularly in the treatment of heart diseases, respiratory troubles, digestive disorders, rheumatic pains, and nerve diseases. Diverse observations as to the monosaccharide constituents of garlic have been reported[2–6], but none of the authors seem to have conducted any systematic investigations on the structure of the polysaccharides present. Abdel-Fattah and co-workers[7] reported the presence of pectic acid in garlic skins; it was also found in the bulbs, along with a galactan, an

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*Characterization of the Polysaccharides of Garlic (*Allium sativum*) Bulbs, Part I.
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arabinan, a glucan, and a fructan. In this paper, isolation of the galactan in the pure state, and elucidation of its structure, are reported.

Defatted garlic-bulbs were extracted with ammonium oxalate (0.5%) on a boiling-water bath; on precipitation with ethanol, the extract yielded a polysaccharide material (polysaccharide A) containing ash 4.06, galacturonic acid 22.8, galactose 24.1, arabinose 7.2, rhamnose 3.1, and glucose plus fructose 37.1% (total hexose, including galactose, 61.2%). A similar extraction with water, instead of ammonium oxalate, yielded polysaccharide B, which contained all of these monosaccharides, but the proportion of total hexoses (76.6%) was considerably higher. Obviously, ammonium oxalate solubilized more of the acidic constituents. On concentration, and precipitation with cold acetone, the supernatant liquor from B furnished polysaccharide C, containing mainly glucose and fructose (total hexose, 92.8%), and small proportions of arabinose (1.3) and galacturonic acid (2.8%). Characterization of the monosaccharides isolated after hydrolysis is described in Table I.

The presence in our preparations of mannose, reported[2] by earlier workers, could not be substantiated for the following reasons: (*a*) the monosaccharide portion corresponding to fructose and mannose (which are chromatographically inseparable using the listed solvent systems) was isolated from the paper chromatogram. G.l.c. of this portion as the per(trimethylsilyl) derivative did not show the characteristic mannose peaks [RRT*, 0.70(α) and 1.08(β)], whereas the fructose peak (RRT*, 0.69) could be well identified. However, g.l.c. of this portion (as the alditol acetates) furnished two peaks, corresponding to glucose and mannose; this was obviously due to formation from fructose during borohydride reduction. (*b*) Oxidation of a part of the aforementioned monosaccharide portion with bromine water, followed by paper chromatography, did not show the presence of mannonic acid, whereas, on similar treatment, standard mannose was oxidized to the aldonic acid. Furthermore, on similar oxidation, a mixture of mannose and fructose (in the molar ratio of 1:5) furnished mannonic acid. D-Fructose (standard, as well as that obtained from garlic hydrolyzates) remained unoxidized under identical conditions.

To obtain a galactan-enriched fraction, polysaccharide B was dispersed in water, and treated with aqueous calcium chloride (5%) at pH 8.5. The precipitated calcium pectate was removed, and, on concentration and fractional precipitation with ethanol, the supernatant liquor yielded polysaccharide B_1, containing all of the monosaccharide residues except rhamnose, and containing a higher proportion of galactose. On concentration and precipitation, the supernatant liquor from B_1 finally yielded polysaccharide B_2, which was found to contain mainly glucose and fructose (total hexose 94.2%). The other sugars, *viz.*, galacturonic acid, galactose, and arabinose were present in traces; there was no rhamnose. The disappearance of rhamnose indicates that, in all probability, it is not chemically bound to the rest of the

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*RRT refers to relative retention times with respect to the per(trimethylsilyl) derivative of α-D-glucose as unity.

TABLE I

CHARACTERIZATION OF THE MONOSACCHARIDES FROM GARLIC POLYSACCHARIDE HYDROLYZATES

Sugars	$[\alpha]_{589.5}$ *(degrees)*[a]	*Lit.* $[\alpha]_D$		*Crystalline derivative*	*M.p. and mixed m.p.* (°)	*Lit. m.p.*		*RRT value*[b] *of the sugars as*	
		(degrees)	*Ref.*			(°)	*Ref.*	*Alditol acetate* (*1*)	*Silyl derivatives* (*2*)
D-Galacturonic acid	+51	+53.4	21	galactaric acid	211–212	212–213	20	—	—
D-Galactose	+80	+83.3	18(a)	*N*-*p*-nitrophenyl-D-galactosylamine	215–216	217–218	17	0.88	—
D-Glucose	+54	+52.5	18(c)	*N*-*p*-nitrophenyl-D-glucosylamine	183–184	184	17	1.00	1.00(*α*) 1.57(*β*)
D-Fructose	−89	−92.0	19(b)	1,3,4,6-tetra-*O*-benzoyl-D-fructose	122–123	124–125 113	19(b) 18(b)	1.00 0.76	0.69, 1.04 (very weak)
L-Arabinose	+107	+104.5	19(a)	—	—	—	—	0.30	0.28(*β*), 0.33(*α*) 0.38(small)
L-Rhamnose	+7	+8.9	18(d)	—	—	—	—	0.18	0.30

[a] In water. [b] RRT values refer to the relative retention times with respect to (1) D-glucitol hexaacetate, and (2) the per(trimethylsilyl) derivatives of D-glucose.

TABLE II

COMPOSITION OF POLYSACCHARIDES A, B, C, B_2, B_3, B_4, B_5, B_6, AND B_7

Poly-saccharide fraction	$[\alpha]_{589.5}$ *(degrees)*[a]	*Ash* (%)	*Composition*					*Other sugars present*[b]	
			Galact-uronic acid (%)	*Total hexose* (%)	*Galactose* (%)	*Arabinose* (%)	*Rhamnose* (%)	*Glucose*	*Fructose*
A	−11.99 (1.0)	4.06	22.8	61.2	24.1	7.2	3.1	+ +	+ +
B	−5.94 (1.0)	3.79	12 5	76.6	21.1	3.6	1.1	+ +	+ +
C	−35.92 (1.0)	0.24	2.8	92.8	—	1.3	—	+ + +	+ + +
B_2	−43.77 (1.0)	0.16	1.6	94.2	1.1	2.7	—	+ + +	+ + +
B_3	+43.59 (1.0)	2.64	28.9	51.4	49.3	10.2	6.3	+	+
B_4	+59.94 (1.0)	8.17	54.0	28.5	25.8	4.6	3.0	+	+
B_5	+66.5 (1.0)	2.68	9.3	80.9	81.4	5.7	—	—	—
B_6	+70.0 (1.0)	0.12	—	97.3	97.1	1.6	—	—	—
B_7	+45.23 (1.0)	6.32	27.1	50.4	46.1	7.1	7.8	+	—

[a] In water; concentration in parentheses. [b] + + + major, + + medium, and + very small peaks in the g.l.c. of the alditol acetates.

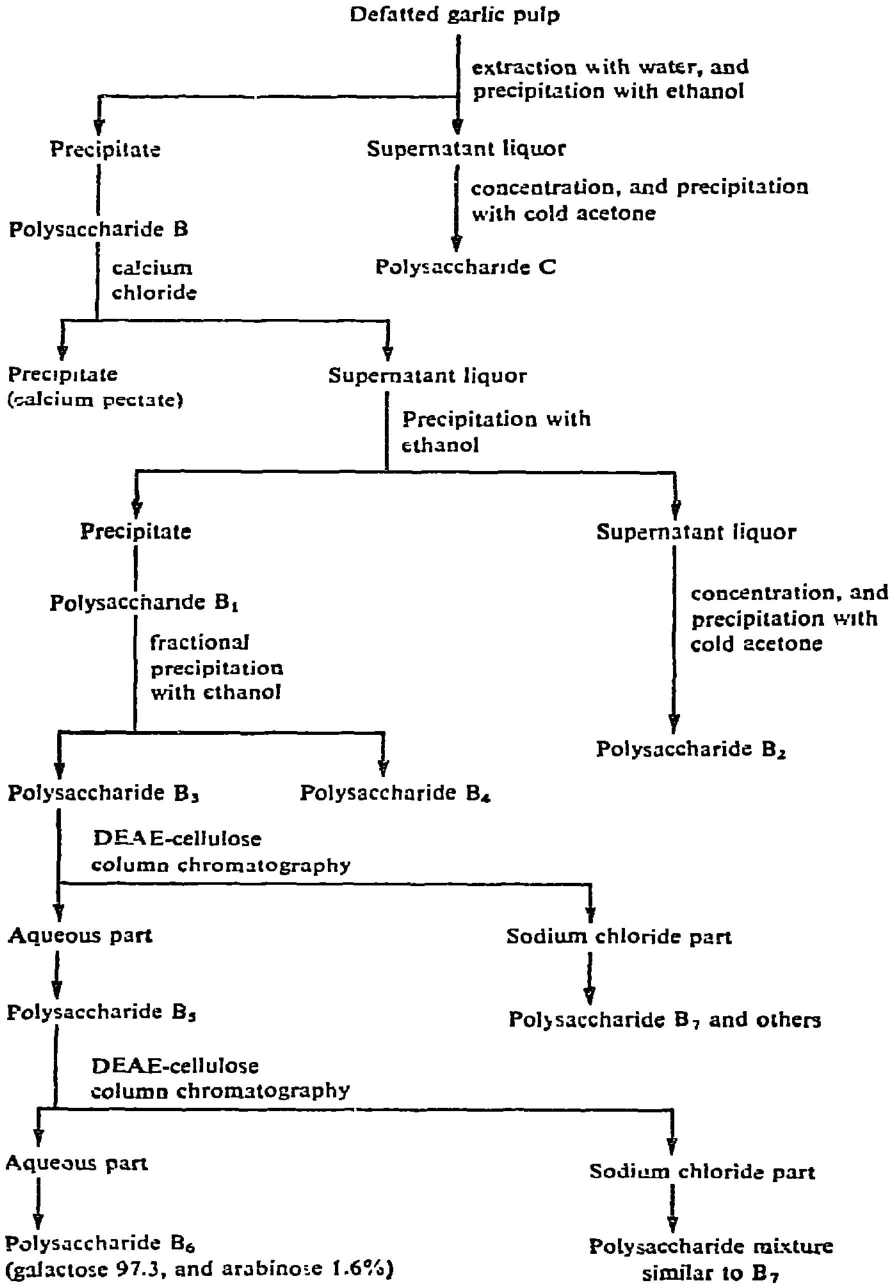
polysaccharides. Polysaccharide B_1 was further separated into polysaccharides B_3 and B_4 by fractional precipitation with ethanol. Of these, B_3 had the highest galactose (49.3%) to uronic acid (28.9%) ratio (see Table II).

To achieve further fractionation, polysaccharide B_3 was applied to a column of DEAE-cellulose, and eluted first with water and then with sodium chloride gradients of different molarity. The water-eluted fractions, which responded positively in the 1-naphthol[15] and carbazole[12] reactions, were pooled and lyophilized, to yield a white, amorphous material, polysaccharide B_5, containing ash 2.68, galacturonic acid 9.3, galactose 81.4, and arabinose 5.7%. The fractions eluted with sodium chloride (polysaccharide B_7 and others) contained most or all of the sugars. Polysaccharide B_7 could not be recycled, because of its extreme insolubility in water or in electrolyte. On recycling through the DEAE-cellulose column, polysaccharide B_5 furnished polysaccharide B_6, which was found to be an almost pure galactan (galactose 97.3, and arabinose 1.6%). The fractions eluted from this column with sodium chloride had compositions similar to that of B_7. The compositions of all these fractions are given in Table II, and the isolation procedure is summarized in Scheme 1.

Although presence of the galactan as a separate entity in pectic substances has been variously reported[8,9], this contention is now well verified by the isolation of a galactan of high purity. Furthermore, none of the polysaccharides A, B, C, B_3, B_4, and B_7 furnished any aldobiouronic acid under conditions suitable for their liberation; this indicates that the D-galacturonic acid residues are not glycosidically bound to any of the neutral sugars, and that they constitute the D-galacturonan component reported[7a,b] to be present in the polysaccharide mixture.

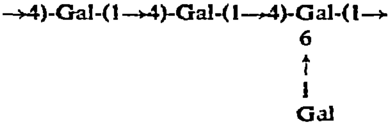
Polysaccharide B_6 was methylated, first with dimethyl sulfate and sodium hydroxide, and then with methyl iodide and silver oxide. The permethylated product, having $[\alpha]_{589.5}$ $-53.7°$ and OMe 43.2%, had no band in the hydroxyl region of its i.r. spectrum. The methylated product was resolved into four fractions by precipitation from its chloroform solution with increasing proportions of petroleum ether. The fact that these fractions had very close values of specific rotation and methoxyl content indicated that the starting material was practically homogeneous. The major fraction was methanolyzed and hydrolyzed; on demethylation[22], the hydrolyzate furnished only galactose. On paper chromatography, the hydrolyzate was resolved into three components, corresponding to 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methyl-D-galactose. The mixture of methylated sugars was chromatographically separated on thick filter-papers, and the components were identified by preparing appropriate derivatives, and by g.l.c. The mole ratio of tetra-:tri-:di-*O*-methyl sugars was found to be 1:2:1 by quantitative separation of the mixture on a paper chromatogram, and estimation of the components by the alkaline hypoiodite method[23].

From these results, some conclusions can be drawn regarding the structure of the repeating unit of the D-galactan. In the main chain, the D-galactopyranose residues are linked through O-1 and O-4, and the branched unit is linked through O-1, O-4, and O-6. The fact that, in the methylated polysaccharide, there is one tetra-*O*-methyl sugar for every three of the other methylated hexose residues indicates that the



Scheme 1. The fractionation of the polysaccharides of defatted garlic-bulbs.

galactan is highly branched, and that the average length of the repeating unit consists of four hexose residues. From these results, structure I may be assigned for the average repeating-unit of the galactan. The low specific rotation of the D-galactan and the negative specific rotation of its permethylated derivative indicate the preponderance of β-D-linkages in the galactan. However, with the data available, it is not possible to ascertain the length of the side chain.



$$\rightarrow 4)\text{-Gal-}(1\rightarrow 4)\text{-Gal-}(1\rightarrow 4)\text{-}\underset{\substack{6\\ \uparrow\\ 1\\ \text{Gal}}}{\text{Gal}}\text{-}(1\rightarrow$$

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where Gal represents D-galactopyranosyl groups or residues.

On periodate oxidation, polysaccharide B_6 reduced 1.18 molar equivalents of the oxidant per mole of hexosyl residue and liberated 1 molar equivalent of formic acid per 4.13 hexosyl residues. These values are in good agreement with the structure suggested for the repeating unit of the polysaccharide. Hydrolysis of the periodate-oxidized and then reduced polysaccharide furnished glycol aldehyde, glycerol, and a sugar derivative (R_{Gal} 1.08) having a chromatographic mobility corresponding to that of a 4-carbon segment (*e.g.*, erythritol, R_{Gal} 1.10) which might be the expected threitol, but this could not be verified due to nonavailability of an authentic sample. The oxidized-reduced polysaccharide contained no galactose or galactitol residues. These results further support the conclusion regarding the position of linkages in the main chain and at the branch points.

EXPERIMENTAL

Materials and methods. — The following solvent systems (A.R. grade; v/v) were used for irrigation in the paper partition chromatography: (*A*) 8:2:1 ethyl acetate–pyridine–water, (*B*) 5:5:1:3 ethyl acetate–pyridine–acetic acid–water, (*C*) 10:1:2 1-butanol–ethanol–water, (*D*) 4:1:5 1-butanol–ethanol–water, (*E*) 40:11:19 1-butanol–ethanol–water, and (*F*) 5:1:4 1-butanol–ethanol–water. The spray reagents used were (*a*) alkaline silver nitrate, (*b*) aniline hydrogenoxalate, and (*c*) benzidine periodate. Small quantities of sugar mixtures were separated on Whatman No. 1 chromatographic paper, the solvent being allowed to flow against the direction of the machine lines; larger quantities (up to 200 mg) were separated on Whatman No. 3MM papers. All specific rotations were recorded at equilibrium. Unless otherwise stated, all evaporations were conducted at 40°, under diminished pressure.

The glycuronic acid was identified by reducing[10] its methyl ester methyl glycoside with potassium borohydride to the corresponding neutral sugar, and chromatographing this with solvents *A* and *C*. The uronic acid was further characterized by oxidation[20] to its aldaric acid. Glucose and galactose were characterized through the corresponding *N*-*p*-nitrophenylglycosylamines[17], prepared by refluxing a methanolic solution of the sugar with a solution of *p*-nitroaniline in methanol

(4.5%) containing a trace of hydrochloric acid. Fructose was converted into its tetra-*O*-benzoyl derivative[19(b)] by adding benzoyl chloride to its solution in pyridine. Arabinose and rhamnose were identified by g.l.c. of the corresponding alditol acetates and per(trimethylsilyl) derivatives.

Determination of monosaccharides was performed spectrophotometrically. The neutral sugars were determined by the L-cysteine-sulfuric acid method[11], by taking dichromatic readings at 414 and 380 nm for total hexose[11], at 390 and 420 nm for arabinose[13], and at 396 and 432 nm for rhamnose[14]. Galactose was estimated with galactostat (Worthington Biochemical Corp., Freehold, N.J.), the yellow color being measured[16] at 425 nm. Uronic acid was determined by the carbazole method[12] at 535 nm.

G.l.c. of the neutral sugars as (*a*) their alditol acetates was conducted isothermally at 195° on a column of 3% of ECNSS-M supported on Gaschrom Q, and (*b*) as their per(trimethylsilyl) derivatives at 170° on a column of 3% of SE-52 on a stationary phase of Chromosorb WAW. For methyl glycosides of, and alditol acetates from, methylated sugars, a column of 15% of diethylene glycol succinate on Chromosorb WAW at 200° and an OV-225 column at 190°, respectively, were used. The glycuronic acid in a polysaccharide hydrolyzate was removed by treatment with Amberlite IR-45 (OH^-) resin, and the resulting mixture of the neutral sugars was detected by g.l.c. of the corresponding alditol acetate mixture. All g.l.c. experiments were performed in a Hewlett-Packard Gas Chromatograph, Model 5730A, equipped with flame-ionization detection and a recorder, model 7127A, nitrogen being used as the carrier gas. Spectrophotometric studies were performed with a Pye-Unicam spectrophotometer, model SP500 (for the u.v. and visible regions) and a Perkin-Elmer spectrophotometer model 337 (for the i.r. region). All specific rotations were measured in a Perkin-Elmer Polarimeter, model 241.

Isolation of crude polysaccharides, and identification of the components. — The skins were peeled off garlic-bulbs (1 kg), and the bulbs were thoroughly crushed under ethanol in a blender. The blended material was filtered through a nylon cloth, and the residue was defatted by treating it with boiling 1:1 ether-petroleum ether (b.p. 60–80°) (500 ml) for 2 h under reflux; the suspension was cooled, and filtered through a nylon cloth. The process of defatting was repeated five times, and the residue was air-dried, to yield a brownish, pasty material (183 g). To this was added aqueous ammonium oxalate (0.5%, 1.2 liters), and the suspension was heated, with stirring, on a boiling-water bath for 4 h, cooled, and filtered through a nylon cloth. The filtrate was centrifuged at 6,000 r.p.m. for 40 min at 15°, the clear, supernatant liquor was acidified to pH 4.5 with glacial acetic acid, and the polysaccharide was precipitated with 95% ethanol (4 vol.). The precipitate (polysaccharide A) was collected by centrifugation, and successively triturated with 95% ethanol, absolute ethanol, and dry ether, to afford a white, amorphous powder (30.5 g). This had $[\alpha]_{589.5}$ −11.99° (*c* 1.0, water), and contained ash 4.06, galacturonic acid 22.8, total hexose 61.2, galactose 24.1, arabinose 7.2, and rhamnose 3.1%; glucose and fructose were also present.

A similar extraction with water, instead of ammonium oxalate solution, of another lot of defatted pasty material (200 g) furnished polysaccharide B (31.1 g, $[\alpha]_{589.5}$ $-5.94°$ (*c* 1.0, water) having all the constituents present in A (see Table II). The supernatant liquor from B was concentrated to 500 ml, and the polysaccharide was precipitated as a gummy material with cold acetone (3 vol.). A pale-yellow powder (polysaccharide C) was obtained on trituration of the gum with absolute ethanol and dry ether; yield 68.8 g; $[\alpha]_{589.5}$ $-35.92°$ (*c* 1.0, water).

The constituent monosaccharides in the hydrolyzates of polysaccharides A, B, and C were isolated in the pure state by repeated paper chromatography with solvent *C*. The pure monosaccharides were identified chromatographically by using solvents *A*, *C*, and *D* for neutral sugars, and *A* and *B* for uronic acid, and spray reagents *a* and *b*. A portion of each neutral sugar, as its alditol acetate and as its per(trimethylsilyl) derivative, was examined by g.l.c. The per(trimethylsilyl) derivative of the monosaccharide corresponding to fructose, which always co-chromatographed with mannose on paper, gave a strong peak for fructose. A very weak peak appearing in the mannose position (which is also the position for one of the peaks for glucose) did not establish the presence of mannose conclusively. The corresponding alditol acetates, however, gave strong peaks for mannose and glucose. An aqueous solution of the fructose portion (~20 mg in 5 ml of water) from the polysaccharide hydrolyzate was oxidized with bromine in the presence of $SrCO_3$ (100 mg) for 4 days at room temperature. The excess of bromine was flushed out with nitrogen, the suspension was filtered, and the filtrate was treated with silver acetate, and de-ionized with Dowex-50 X-8 (H^+) resin. Paper chromatography (solvent *A*) then showed the presence of fructose only. Similar oxidation was conducted separately on mannose (~5 mg), fructose (~5 mg), and a 1:5 mixture of the two. After the usual processing, chromatography in solvent *A* showed that, both individually and in the mixture, mannose was oxidized to mannonic acid, but fructose remained unaffected in both cases. Finally, the monosaccharides were characterized by preparing appropriate derivatives, as described earlier (see Table I). Polysaccharides A and B were found to contain galacturonic acid, galactose, glucose, fructose, arabinose, and rhamnose, whereas polysaccharide C contained mainly glucose and fructose with small proportions of galacturonic acid and arabinose (see Table II).

Isolation of the pure galactan from polysaccharide B. — A homogeneous suspension of polysaccharide B (15.0 g) in water (1.5 liters) was prepared by stirring for 4 h at room temperature. To this suspension, made ammoniacal (pH 8.5), was added aqueous calcium chloride (5%; 200 ml). The mixture was stirred for 4 h at room temperature, and the resulting calcium salt of pectic acid was removed by centrifugation. The supernatant liquor was dialyzed extensively, and then concentrated to 900 ml. A small amount of precipitate appearing at this stage was removed by centrifugation. On treatment with ethanol (4 vol.), the clear, supernatant liquor furnished a galactan-enriched fraction (polysaccharide B_1, 3.2 g). The supernatant liquor from this was concentrated to 100 ml, and treated with cold acetone (4 vol.), to yield a gummy material (polysaccharide B_2, 4.0 g). Polysaccharide B_1 (3.0 g) was dispersed in water (450 ml) and fractionally precipitated with ethanol, to yield polysaccharides B_3 (1.15 g) and B_4 (0.12 g). The fractions were collected in the usual way, and their compositions determined (see Table II).

Chromatography of polysaccharide B_3 on a column of DEAE-cellulose. — Polysaccharide B_3 (2 g), containing galacturonic acid 28.9, total hexose 51.4, galactose 49.3, arabinose 10.2, and rhamnose 6.3%, was dispersed in water (150 ml), and de-ionized with Amberlite IR-120 (H^+) resin. The solution was concentrated to ~2 ml, and adsorbed on a column (20 × 3 cm) of DEAE-cellulose. The column was eluted with water at the rate of 1 ml per min, and the eluate was collected in 10-ml fractions. Fractions 11–38, which responded positively in both the 1-naphthol[15] and carbazole reactions[12], were pooled (300 ml), and lyophilized, to yield polysaccharide B_5 (625 mg); this contained galacturonic acid 9.3, galactose 81.4, and arabinose 5.7%; other sugars were absent. After being washed with water (600 ml), the column was successively eluted with 0.1–1M, 1–2M, and 2–4M sodium chloride gradients. All of the polysaccharide-containing fractions, whose pooling was guided by the aforementioned color reactions, were separately dialyzed, and the products isolated in the usual way. The 0.1–1M gradient yielded polysaccharide B_7 (1.1 g), containing galacturonic acid 27.1, total hexose 50.4, galactose 46.1, arabinose 7.1, and rhamnose 7.8%. Colorimetric analysis and g.l.c. showed that other fractions eluted with sodium chloride had approximately the same composition as that of B_7. Polysaccharide B_7 could not be rechromatographed, as it was sparingly soluble in water or in electrolyte solution.

The aqueous fraction B_5 (600 mg) was recycled through a fresh column of DEAE-cellulose, using water as the irrigant. The effluent (fractions 12–24) responded positively to the 1-naphthol, but negatively to the carbazole, reaction. These fractions were pooled and lyophilized, to afford a white, polysaccharide material (polysaccharide B_6, 82 mg). At this stage, gradient elution with sodium chloride (0.1–1M) again furnished a material having a composition similar to that of B_7. The experiments were repeated several times, in order to obtain sufficient B_6, which contained galactose 97.3 and arabinose 1.6%.

Methylation studies on polysaccharide B_6. — Polysaccharide B_6 (350 mg), containing galactose 97.3 and arabinose 1.6%, was methylated, first with dimethyl

TABLE III

FRACTIONATION OF METHYLATED POLYSACCHARIDE B_6

Fraction no.	*Vol. of pet. ether (ml)*	*Ratio of chloroform: pet. ether (v/v)*	*Yield (mg)*	$[\alpha]_{589.5}$ *(degrees)*[a]	*OMe (%)*
b_1	16	1:1.6	180.5	−54.6	43.4
b_2	24	1.2.4	15.3	−53.8	43.0
b_3	37	1:3.7	8.3	−54.2	43.6
b_4	47	1:4.7	11.1	−51.8	42.7

[a] In chloroform.

sulfate and sodium hydroxide (4 times), and then with methyl iodide and silver oxide (5 times); yield 252 mg, $[\alpha]_{589.5}$ −53.7° (*c* 1.0, chloroform), OMe 43.2%. The final, methylated product, which had no OH band in its i.r. spectrum, was dissolved in chloroform (10 ml), and fractionally precipitated with petroleum ether (b.p. 40–60°), to afford fractions b_1, b_2, b_3, and b_4, as shown in Table III.

Hydrolysis and identification of methylated sugars. — The methylated fraction b_1 (160 mg) was completely methanolyzed (monitored polarimetrically) by refluxing with dry, methanolic hydrogen chloride (5%; 25 ml) on a hot-water bath for 16 h. The solvent was removed, and the resulting syrup was hydrolyzed with aqueous hydrochloric acid (M; 20 ml) on a boiling-water bath for 14 h. The hydrolyzate was made neutral (Ag_2CO_3), and filtered, and the filtrate was deionized with Amberlite IR-120 (H^+) and Amberlite IR-45 (OH^-) resins, and evaporated to a syrup (145 mg).

On demethylation[22] with constant-boiling hydrobromic acid, and chromatography (solvents *A* and *F*), the syrup (~5 mg) gave mainly galactose, along with some partially demethylated sugars. On chromatography (solvents *E* and *F*), the mixture of methylated sugars was resolved into three fractions, f_1, f_2, and f_3, corresponding to 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methyl-D-galactose. Fractions f_1, f_2, and f_3 were isolated by chromatography on thick paper (solvent *F*). The methylated sugars in these chromatographically pure fractions were identified by preparing crystalline derivatives, and by comparative g.l.c.

In another experiment, a small amount of the mixture of methylated sugars was quantitatively separated on paper (solvent *F*); with the guide of reference spots, the individual components were eluted with water, and estimated by the alkaline hypoiodite method[23]. Tetra-, tri-, and di-*O*-methyl-D-galactose were found to be present in the mole ratios of 1.02:1.99:1.00.

Fraction f_1. — This fraction, $[\alpha]_{589.5}$ +108° (*c* 0.6, water) (lit.[18(e)] +114.5°, lit.[8] +109°), OMe 51.4%, was identified as 2,3,4,6-tetra-*O*-methyl-D-galactose by preparing its aniline derivative (m.p. and mixed m.p. 186–187°; lit.[8] 187°, lit.[24] 188–190°, lit.[25] 186–188°). The methyl glycosides of a portion (~2 mg) of f_1 gave g.l.c. peaks identical to those given by an authentic sample of the methyl 2,3,4,6-tetra-*O*-methyl-D-galactopyranosides.

Fraction f_2. — The syrup had $[\alpha]_{589.5}$ +89° (*c* 1.0, water) (lit.[26] +79.4°, lit.[18(f)] +87°), and OMe 40.7%. G.l.c. data for the methyl glycosides of this fraction corresponded to those of an authentic sample of 2,3,6-tri-*O*-methyl-D-galactose. This material (~20 mg) was oxidized with bromine–water, and the resulting lactone, crystallized from ether–petroleum ether (b.p. 60–80°), was identified as 2,3,6-tri-*O*-methyl-D-galactonolactone (m.p. and mixed m.p. 96–97°, lit.[8] 96–97°, lit.[27] 97–98°, lit.[28] 101°).

Fraction f_3. — The syrupy material, having $[\alpha]_{589.5}$ +78° (lit.[26] +80°) and OMe 29.2%, was identified as 2,3-di-*O*-methyl-D-galactose through the preparation of its crystalline aniline derivative (m.p. and mixed m.p. 151–153°, lit.[26] 152–154°). Comparative g.l.c. of the alditol acetate of this fraction and g.l.c. of a mixture with an authentic sample confirmed the identification.

Periodate oxidation studies. — Polysaccharide fraction B_6 (4.02 mg; in duplicate), containing galactose 97.3 and arabinose 1.6%, was subjected to oxidation with sodium metaperiodate in the dark at 4°. The periodate uptake, which was monitored spectrophotometrically[29], became constant in 24 h, when 1.18 moles of the oxidant were reduced per mole of hexosyl residue. In another experiment, the same polysaccharide (18.6 mg in 9 ml of water) was oxidized, in duplicate, under the same conditions as before, and the liberation of formic acid was determined at intervals by titration with 0.01M sodium hydroxide solution. The liberation of formic acid became constant in 22 h; the liberation of one molar equivalent of formic acid corresponded to the oxidation of 4.13 molar equivalents of hexosyl residues. After 30 h, the duplicate oxidation mixtures were pooled, and the excess of periodate was decomposed with ethylene glycol (1 ml). After extensive dialysis, the solution was concentrated by lyophilization to ~5 ml, and the solute reduced with potassium borohydride (100 mg) for 24 h at 10°. The excess of borohydride was decomposed with ice-cold acetic acid (6M), and the solution was dialyzed and then lyophilized. After hydrolysis of the resulting material (2.1 mg) with 0.5M H_2SO_4 for 6 h, paper chromatography (solvent *A*) revealed the presence of glycol aldehyde (R_{Gal} 1.56), glycerol (R_{Gal} 1.24) and another spot (R_{Gal} 1.08). No galactose survived the oxidation.

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