Carbohydrate Research, 86 (1980) 247–257
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

STRUCTURE OF THE D-GALACTAN ISOLATED FROM *Aloe barbadensis* MILLER*

GAURHARI MANDAL** AND AMALENDU DAS†
Department of Chemistry, Jadavpur University, Calcutta-700032 (India)
(Received May 8th, 1980; accepted for publication, June 3rd, 1980)

ABSTRACT

Hot-water extraction of the pulp obtained by dehydrating the jelly of the fleshy leaves of *Aloe barbadensis* furnished a mixture of polysaccharides containing mainly pectic acid, along with a D-galactan, a glucomannan, and an arabinan. The pectic acid was partly removed by treatment with calcium chloride, and the resulting, hexose-enriched, polysaccharide mixture was fractionated through a column of DEAE-cellulose to yield a D-galactan containing D-galactose (92.9%) and D-galacturonic acid (3.8%). Hydrolysis of the permethylated D-galactan furnished 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methylgalactose in the molar ratios of 1:26:1. On periodate oxidation, the D-galactan reduced 0.95 molar equivalent of the oxidant per hexosyl residue, and liberated one molar equivalent of formic acid per 26 galactosyl residues. Smith degradation of the D-galactan afforded mainly threitol. From these results, a structure has been assigned to the repeating unit of the D-galactan. The number-average, molecular weight of the peracetylated galactan has been found to be 3.74×10^4.

INTRODUCTION

The polysaccharide composition of the mucilaginous jelly isolated from the fleshy leaves of the genus *Aloe* (Liliaceae) seems to vary widely with the species. The jelly from *Aloe vera*[1] has been reported to contain equimolar proportions of glucose and mannose, in addition to 2.4% of uronic acid, and that from *Aloe plicatilis* Miller[2] was found to contain only one acetylated glucomannan having glucose and mannose in the molar ratio of 1:2.8. A South Indian species of *A. vera*[3] mainly consists of several acetylated glucomannans, along with traces of galacturonic acid, galactose, xylose, and arabinose. It is apparent from these reports that the principal polysaccharide material present in these sources is essentially acetylated glucomannan(s).

In the course of our investigation on *Aloe barbadensis* Miller, we found that

*Characterization of the Polysaccharides of *Aloe barbadensis*, Part I.
**Present address: Department of Chemistry, Mahishadal Raj College, P.O. Mahishadal, Dist. Midnapur, West Bengal, India.
†To whom enquiries should be directed.

the constituents of the polysaccharide (Ps) from this source differ significantly from the previous ones, in that, not only does it contain a very high percentage of pectic acid, but also, at least three more polysaccharides, *viz.*, an arabinan, a galactan, and a glucomannan, and this glucomannan differs from the previous one in not being acetylated. Also, except for the presence of D-glucose and D-mannose, the composition reported[4] (without any structural information) for an Oklahoma (U.S.A.) species of *A. barbadensis* seems to have very little in common with our species of *A. barbadensis*. It is to be noted that, although *A. vera* and *A. barbadensis* are often mentioned[5,6] as being synonyms, there are still two to three varieties[5,6] whose exact delimitations are not yet clear. Hence, the difference in polysaccharide composition of our species may be attributable to this circumstance and to local variations.

Furthermore, the polysaccharide composition of our species has been found to vary with the season, because the material collected during April of 1977 contained ~85% of galacturonic acid and the neutral sugars were essentially present in traces, whereas that collected (from the same kind of plants in the same locality) during October of the same year contained only ~70% of galacturonic acid. However, despite the differences in composition, the medicinal importance[5-7] of *A. barbadensis* in India is the same as that of the other species of the genus *Aloe*.

We now report the isolation and characterization of a galactan from *A. barbadensis* Miller, collected from a field in West Bengal.

RESULTS AND DISCUSSION

The fleshy leaves of *A. barbadensis** used in this investigation were collected in April, 1977. The green covering of the leaves was carefully removed, and the colorless, thick jelly was homogenized with 95% ethanol. The mixture was filtered, and the residue was dried by solvent exchange, to yield the pulp. The pulp was extracted with water on a boiling-water bath, and the extract was cooled, and filtered through a Nylon cloth. The filtrate was centrifuged, and the clear, supernatant liquor was precipitated with ethanol at pH 4.5, giving crude polysaccharide A in ~0.01% yield with respect to the weight of the green leaves (a fact that reflects the remarkable, gel-forming capacity of the constituent polysaccharides).

It may be mentioned that a similar extraction with aqueous ammonium oxalate (0.5%), instead of water, did not cause any significant change in the composition of the resulting polysaccharide B. Hence, subsequent lots were isolated by extraction with water only.

Polysaccharide A contained mainly galacturonic acid (85%) and the other sugars, present in very small proportions, were galactose, rhamnose, arabinose, xylose, mannose, and glucose (see Table I). Because of the presence of such a high proportion of uronic acid, the hexose content of A could not be determined by the L-cysteine method. Such a high percentage of galacturonic acid, along with the neutral sugars

*Identified at the Herbarium, Botanical Survey of India, Calcutta.

mentioned, indicated that this material was another, typical, pectic substance[8], and, as such, it was different from the polysaccharides (*viz.*, glucomannans[2,3]) reported to be present in the same genus. The i.r. spectrum of A showed bands at 1740 (*m*), 1600 (*s*), and 1420 (*s*) cm^{-1}. As these could have been due to the presence[9(a),(b)] of pectin or acetyl groups, or both, no conclusion could be drawn at this stage regarding the presence or absence of acetyl groups.

To obtain a hexose-enriched fraction, an aqueous solution of A was treated with a solution of calcium chloride at pH 8.5. The precipitated calcium pectate was removed, and the supernatant liquor was processed to afford a solid. The optimal conditions for ensuring the highest hexose content were determined by pilot experiments with 0.20, 0.15, and 0.10% solutions of A, and the respective polysaccharides (C_1, C_2, and C_3) were isolated. Of these, C_3 had the highest (52.2%) hexose content (see Table I), and hence, subsequent lots were isolated under these conditions. Polysaccharide C_3 was again treated with calcium chloride, to yield C_4, which had $[\alpha]_{589.5}$ +61.2° and contained 60.2% of hexose (of which the galactose content was ~40%, as calculated from the molar ratio) and 26.3% of uronic acid. No further enrichment in hexose content could be achieved by repeating the treatment with calcium chloride.

Further fractionation of C_4 was effected by chromatography through a column of DEAE-cellulose (OH^-), using, first, water, and then sodium chloride solution of increasing molarity, as the irrigant. The elution profile is given in Fig. 1. Water eluted fractions 4–10 (which responded positively to 1-naphthol[10], but negatively to the carbazole[11] reaction) were pooled, and the resulting polysaccharide C_5 (yield 7.5%, with respect to C_4) was isolated in the usual way. This polysaccharide C_5 had $[\alpha]_{589.5}$ +84.0°, and contained galactose (92.9%) and galacturonic acid (3.8%). Polysaccharide C_5 was recycled through a fresh column of DEAE-cellulose, but the composition remained practically unchanged.

Elution with sodium chloride furnished polysaccharide fractions C_6, C_7, C_8,

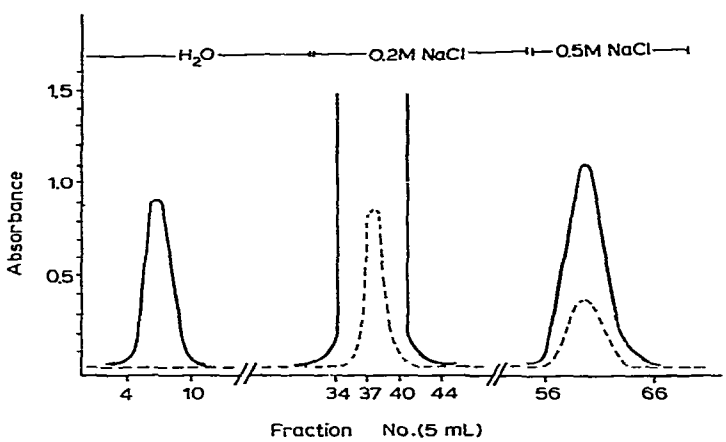


Fig. 1. Chromatography of Ps-C_4 on a column of DEAE-cellulose (——, 1-naphthol, and – – – –, carbazole reaction).

TABLE I

COMPOSITION OF POLYSACCHARIDES A, B, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, AND C_9

Method of isolation	*Ps fraction*	$[\alpha]_{580.5}$ *(degrees)*[a]	*Composition*		*Mole ratio*[b]					
			GalA (%)	*Total hexose (%)*	*Gal*	*Glc*	*Man*	*Ara*	*Xyl*	*Rha*
Hot-water extraction	A	+185.02 (0.45)	85.20	—	9.20	trace	trace	1.00	trace	7.20
Hot ammonium oxalate extraction	B	+181.82 (0.40)	84.00	—	8.10	trace	trace	1.00	trace	5.70
$CaCl_2$ treatment of Ps-A solution										
0.2%	C_1	+186.66 (0.40)	75.50	—	4.70	trace	trace	1.00		3.70
0.15%	C_2	+49.79 (0.50)	41.74	40.00	44.78	1.00	7.90	4.12		4.42
0.1%	C_3	+54.86 (0.40)	40.48	52.22	10.56	1.24	2.80	2.35		1.00
$CaCl_2$ treatment of Ps-C_3 (0.25% soln.)	C_4	+61.22 (0.30)	26.31	60.20	19.92	1.06	1.00	3.63		5.63
Column chromatography with Ps-C_4										
water	C_5	+84.0 (0.50)	3.80	92.90	not done[c]					
0.2M NaCl	C_6	+46.06 (0.90)	15.00	54.23	13.78			1.58		1.00
	C_7	+54.26 (0.50)	27.67	21.15	3.48			1.00		1.49
	C_8	+31.25 (0.50)	26.31	24.30	2.80			1.00		1.06
0.5M NaCl	C_9	+89.28 (0.50)	55.34	—				1.00		5.06

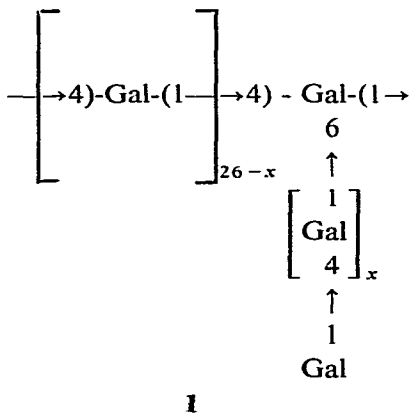
[a]In water; concentration in parentheses. [b]From g.l.c.; —, could not be determined by the L-cysteine method; blank spaces indicate absence. [c]Question of mole ratio does not arise.

and C_9, having increasing proportions of uronic acid (see Table I). Of these, C_6 was recycled, to yield some more of C_5, but the others were not investigated further.

Although complete removal of the uronic acid was not possible, gradual elimination of the uronic acid during purification processes, and the absence of any aldobiouronic acid in the hydrolyzates of the different polysaccharide fractions, indicated that the galactose residues and, hence, the galactan, was not chemically bound to the glycuronan.

Permethylated C_5, prepared by a one-step, Hakomori treatment, had $[\alpha]_{589.5}$ −46.0°, and contained OCH_3, 43.8%. There was no OH band in its i.r. spectrum, and, on attempting fractionation by adding petroleum ether to its chloroform solution, it yielded only one fraction, showing that the permethylated polysaccharide C_5 was reasonably homogeneous. It was hydrolyzed in two steps by treatment with formic acid, and the partially methylated sugars were examined by p.c. and g.l.c., which (see Table II) revealed the presence of 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methylgalactose in the molar ratios of 1:26:1.1.

From these results, it is apparent that the galactosyl residues are linked through O-1 and O-4 in the main chain of the galactan, and the branched unit is linked through O-1, O-4, and O-6. The presence of one tetra-*O*-methyl sugar per 27 residues of the other two methylated sugars indicates that the galactan is branched, and that the average length of the repeating unit consists of 28 hexosyl residues. Hence, structure **I** may be assigned for the average repeating-unit of the galactan, in which, however, the length of the side chain remains uncertain.



I

where Gal represents a D-galactopyranosyl group or residue.

The alternative possibility, of branching from O-4 of the branched unit while that is itself linked through O-1 and O-6 in the main chain, would be very unusual.

On periodate oxidation, the galactan (C_5) reduced 0.95 mol of oxidant per hexosyl residue, and liberated one molar equivalent of formic acid per 25.6 hexosyl units. Smith degradation of the galactan furnished mainly threitol, with a trace of glycerol. These findings are also consistent with the structure proposed for the galactan.

On treatment with β-D-galactosidase, the galactan slowly released D-galactose.

TABLE II

IDENTIFICATION OF THE METHYLATED GALACTOSES OBTAINED FROM THE GALACTAN OF *A. barbadensis*

Components (alditol acetates from)	*RRT*[a]		*Mole ratio*
	Col. I	*Col. II*	
2,3,4,6-Tetra-*O*-methylgalactose	1.26 (1.25)	1.20 (1.19)	1
2,3,6-Tri-*O*-methylgalactose	2.46 (2.42)	2.25 (2.22)	26
2,3-Di-*O*-methylgalactose	5.73 (5.68)	4.79 (4.70)	1.1

[a]Relative retention times with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity. Literature values[26] are given in parentheses.

This fact, and the low specific rotation of the galactan and negative specific rotation of its permethylated derivative, indicate the presence of β-D-linkages in the D-galactan, as has been found elsewhere[12].

The galactan was peracetylated, and the number-average molecular weight ($\overline{M}_n$) of this product was determined by osmometry, using the Van't Hoff equation $\overline{M}_n = RT/(\pi/C)_{C\to 0}$, in a solution of chloroform containing 4% (v/v) of ethanol. It was expected that, under these conditions, there would be minimum association amongst the polymer molecules. The negative slope of the curve (see Fig. 2) probably indicates the poor character[13] of the solvent. The value of $\overline{M}_n$ was found to be 3.74×10^4.

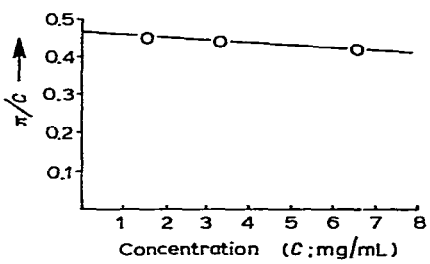


Fig. 2. Plot of π/C against C for peracetylated Ps-C_5 (Galactan fraction). (From the intercept, the value of $(\pi/C)_{C\to 0}$ was found to be 0.47.)

EXPERIMENTAL

Materials and methods. — Paper partition-chromatography (p.c.) was conducted on Whatman No. 1 paper, using the following solvent systems (v/v): (*A*) 8:2:1 ethyl acetate–pyridine–water, (*B*) 5:5:1:3 ethyl acetate–pyridine–acetic acid–water, (*C*) 4:1:1 1-butanol–ethanol–water, (*D*) 4:1:5 1-butanol–ethanol–water (upper layer), and (*E*) 40:11:19 1-butanol–ethanol–water. The staining reagents used were (*a*) alkaline silver nitrate, (*b*) benzidine periodate, and (*c*) aniline hydrogenoxalate.

Neutral monosaccharides were estimated by the L-cysteine–sulfuric acid method[14], taking dichromatic readings at 414 and 380 nm for total hexose, and also by g.l.c. of their alditol acetates using *myo*-inositol as the internal standard. Uronic acid was estimated by the carbazole method[11] at 535 nm. Methoxyl content was estimated by the semimicro method[15].

All specific rotations were measured at equilibrium. Spectral data were obtained, and ultracentrifugation and g.l.c. were performed, with the instruments mentioned earlier[16]. For g.l.c., glass columns (1.83 m × 6 mm) containing (*I*) 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 190° (for alditol acetates of sugars) and at 170° (for partially methylated alditol acetates), and (*II*) 5% of OV-225 on SIL RUB (80–100 mesh) at 170° (for partially methylated sugars) were used. A Hewlett–Packard, high-speed, Standard Membrane Osmometer, model 501, was used for osmometric measurements.

Unless otherwise stated, all evaporations were performed at 35–40° under diminished pressure.

Isolation of the crude polysaccharide. — The chlorophyll-containing, outer layer of the leaves (5.5 kg, average length 40–50 cm, weight 200–250 g per piece) was carefully skinned off, and the almost colorless, jelly-like mass was taken up in alcohol (95% v/v, 2.5 L). This mixture was thoroughly homogenized in a blender for 5 min, and then filtered through a Nylon cloth. The residue (pulp) was thoroughly squeezed, and air-dried, to yield a white, fibrous material (10 g). The opaque filtrate, which yielded very little residue upon concentration and precipitation with acetone, was discarded.

The dry pulp (5 g) was heated with water (500 mL), with stirring, on a boiling-water bath for 5 h, and the suspension was cooled, filtered through a Nylon cloth, and the filtrate centrifuged at 6000 r.p.m. during 45 min at 15°. The clear, supernatant liquor was acidified (pH ~4.5) with cold acetic acid, and the polysaccharide material was precipitated with 95% (v/v) ethanol (3.5 vol.), affording a transparent jelly which was collected by centrifugation, and dried by solvent exchange with 95% ethanol, absolute ethanol, and dry ether, to give a white, amorphous powder (polysaccharide A, yield 625 mg).

A similar extraction with 0.5% ammonium oxalate (500 mL) of another 5-g lot of the pulp yielded polysaccharide B (600 mg), having practically the same composition as A (see Table I), so polysaccharides A and B were combined.

Separation and identification of the monosaccharide components. — Polysaccharide A (20 mg) was hydrolyzed with 0.5M H_2SO_4 (4 mL) during 14 h on a boiling-water bath, and the solution made neutral with prewashed $BaCO_3$, and decationized with Dowex 50W-X8 (H^+) resin in the usual way. The resulting solution was evaporated to a syrup, and this was examined by p.c. (solvents *A*, *B*, and *C*, and spray reagent *a*), which showed it to contain GalA, Gal, Glc, Man, Ara, Xyl, and Rha, but no aldobiouronic acid could be detected.

The syrup was diluted to ~1.5 mL, and the solution applied to a column

(0.4 cm × 30 cm) of Dowex 1-X4 (formate). This was eluted with water, and the effluent (200 mL) was lyophilized.

(*a*) *Neutral sugars.* The aqueous effluent from the column responded positively to the 1-naphthol[10], but negatively to the carbazole[11] test. P.c. (solvents *A* and *C*, and staining agents *a* and *b*) corroborated the earlier findings. A portion of the solution was lyophilized, and the resulting solid (~0.5 mg) in 1 mL of H_2O was reduced with potassium borohydride (~50 mg). The mixture was then treated with Dowex 50 (H^+) resin, generating boric acid, which was removed as methyl borate. The material was dried over P_2O_5, and converted[17] into the peracetates by treatment with acetic anhydride and pyridine (1 mL each) for 30 min at 90°. G.l.c. (column *I*, 190°) of the resulting material indicated the presence of the same monosaccharides, *viz.*, Gal, Glc, Man, Ara, Xyl, and Rha.

(*b*) *Hexuronic acid.* The acidic component was eluted from the column of Dowex 1-X4 (formate) with 0.3M formic acid (25 mL), and the formic acid removed; the residue gave a single spot (p.c., solvents *A* and *B*, stain *a*) corresponding to galacturonic acid. This acid was reduced[18], and the neutral sugar resulting was identified (p.c., solvent *A* and stain *a*; and g.l.c. of the corresponding alditol acetate[17], col. *I*, 190°) as galactose. A part of this galactose was treated[19] with D-galactose oxidase, whereupon it developed the typical, yellow color.

Fractionation of crude polysaccharide. — Polysaccharide A (10 g) was thoroughly dispersed in water (1.5 L) by stirring and warming. The suspension was centrifuged at 19,000 r.p.m. during 45 min at 10°, and the clear, supernatant liquor was treated with 95% (v/v) ethanol (4 vol.) at room temperature. The precipitate (polysaccharide C, yield 9.8 g) was dried by solvent exchange.

(*a*) *Treatment of polysaccharide (C) with calcium chloride.* — A clear, aqueous solution (0.2%) of the foregoing polysaccharide (3 g) was made ammoniacal (pH ~8.5), and the carbohydrate precipitated by dropwise addition of 5% aqueous calcium chloride solution (60 mL) at room temperature, with stirring. Stirring was continued for a further 2 h, and then the precipitate was allowed to settle at 4°. The mixture was centrifuged at 6,000 r.p.m. for 40 min at 10°, to remove calcium pectate, and the supernatant liquor was extensively dialyzed against distilled water. The dialyzate was concentrated, and a small amount of precipitate appearing at this stage was removed by centrifugation. From the clear, supernatant liquor, polysaccharide C_1 (770 mg, yield 25.7%) was precipitated with ethanol (4 vol.). Similar calcium chloride treatment of 0.15 and 0.10% solutions of polysaccharide C furnished polysaccharides C_2 (54 mg, yield 2.25%) and C_3 (23 mg, yield 1.76%). The compositions of these fractions are given in Table I. Polysaccharide C_3, obtained by removing calcium pectate from the 0.10% solution of polysaccharide C, was comparatively more enriched in hexose (52.2%); hence, subsequent lots were isolated under these conditions.

For further enrichment in the hexose content, a solution of C_3 (200 mg) in water (80 mL) was precipitated with 5% calcium chloride (20 mL) at pH 8.5, and the resulting polysaccharide C_4 (135 mg) was isolated in the usual way. It had $[\alpha]_{589.5}$

+61.2° (*c* 0.3, water), and contained uronic acid (26.3%) and hexose (60.2%) (see Table I). No further enrichment in hexose content could be achieved by repeating the treatment.

(*b*) *Chromatography of polysaccharide* C_4 *on a column of DEAE-cellulose* (OH^-)[20]. — Polysaccharide C_4 (120 mg, containing total hexose 60.2%, and uronic acid 26.3%; see Table I) was dissolved in water (25 mL), and decationized with Dowex 50 (H^+) resin. The filtrate was concentrated (~2 mL), adsorbed on a column (22 × 1 cm) of DEAE-cellulose (OH^-), and the material eluted with water (5-mL fractions) at the rate of 60 mL/h, the fractionation (see Fig. 1) being monitored by both the 1-naphthol[10] and the carbazole[11] reaction. Fractions 4–10 (35 mL), which responded positively to the 1-naphthol but negatively to the carbazole reaction, were pooled, filtered through glass wool, and lyophilized, to give polysaccharide C_5 (9 mg), containing galactose (92.9%) and galacturonic acid (3.8%).

The column was next eluted with 0.2M sodium chloride solution, and three fractions (*viz.*, 34–37, 38–40, and 41–44; each responding positively to both tests) were dialyzed and lyophilized, to yield polysaccharide fractions C_6 (25.9 mg). C_7 (25.69 mg), and C_8 (9.5 mg). Finally, the column was eluted with 0.5M sodium chloride solution, and fractions 56–66, responding positively to both color reactions, were processed, to give polysaccharide C_9 (11.26 mg). An M sodium chloride eluate failed to give any color reaction in the color tests. Ps-C_6 was recycled with water, to yield some more Ps-C_5 (~7 mg), and the combined C_5 fraction (~16 mg) was recycled through a fresh column of DEAE-cellulose (OH^-), using water as the irrigant. The resulting polysaccharide (14 mg) had the same composition as C_5. Subsequent lots of C_5 were isolated in the same way. The 0.5M sodium chloride eluate furnished only a trace of polysaccharide. The compositions of C_5, C_6, C_7, C_8, and C_9 are given in Table I. There was no ester band in the i.r. spectrum of C_5.

Methylation[21] *studies on polysaccharide* C_5. — Polysaccharide C_5 (12 mg, dried over P_2O_5) in dimethyl sulfoxide (12 mL; dried over molecular sieve 4A) was treated with 2M methylsulfinyl sodium[22] (12 mL) under nitrogen. The resulting solution was agitated in an ultrasonic bath for 1 h and kept for 2 h at room temperature. Methyl iodide (14 mL) was added dropwise, with external cooling in ice–water. The resulting, turbid solution was agitated ultrasonically for 1 h, when a clear solution was obtained. The excess of methyl iodide was removed with a jet of nitrogen, and the mixture was dialyzed at 4°. The permethylated polysaccharide was extracted into chloroform, and attempted fractionation with petroleum ether (b.p. 40–60°; 5 vol.) gave a single fraction (10 mg), $[\alpha]_{589.5}$ −46.0° (*c* 0.4, chloroform), that had no OH band in its i.r. spectrum. The permethylated polysaccharide (2 mg) was heated with 90% formic acid (1 mL) in a sealed tube for 6 h at 100°. Then, water (3 mL) was added, and the material was hydrolyzed[2] for 2 h at 100°. The formic acid was completely removed by repeated evaporation with water (3 × 2 mL), and the hydrolyzate (containing partially methylated monosaccharides) was examined by p.c. (using authentic samples for comparison, and solvents *D* and *E*; stain *c*); it furnished three spots, corresponding to 2,3-di-, 2,3,6-tri-, and 2,3,4,6-tetra-*O*-methyl-D-galactose. The rest of the hydrolyzate

(~2 mL) was reduced with potassium borohydride (50 mg), and the products were converted into the alditol acetates[23] in the usual way, and subjected to g.l.c. on columns *I* and *II* (both at 170°). The results are given in Table II.

Periodate-oxidation studies. — Polysaccharide C_5 (4.0 mg, in water; in duplicate) was oxidized with 8 mM sodium metaperiodate at 4° in the dark. The uptake of periodate was monitored spectrophotometrically[24] at intervals. The uptake became constant in 40 h, and 0.95 mol of the oxidant was reduced per hexosyl residue. In another experiment, C_5 (20 mg, in water; in duplicate) was oxidized with periodate under the same conditions, and the amount of formic acid formed was determined by titration with 0.01M sodium hydroxide in the usual way. The liberation of the formic acid became constant in ~36 h, and formation of one molar equivalent of formic acid corresponded to the oxidation of 25.6 (average value) hexosyl residues. After determination of the formic acid, the duplicate oxidation mixtures were pooled, dialyzed at 4°, and concentrated by lyophilization. The material in the resulting solution (2 mL) was reduced with potassium borohydride (100 mg), the excess of borohydride was decomposed with ice-cold, 6M acetic acid, and the solution was dialyzed, and lyophilized, to afford the reduced polysaccharide (~6 mg). A portion of the reduced, oxidized product (~2 mg) was hydrolyzed with 0.5M H_2SO_4 for 10 h on a boiling-water bath, and, after the usual treatment, p.c. (solvent *A*, stain *b*) of the hydrolyzate revealed the presence of threitol (major, R_{Gal} 1.45) and a trace of glycerol (R_{Gal} 1.80). No hexose survived the oxidation.

Treatment with β-D-galactosidase. — A solution of polysaccharide C_5 (5 mg) in 0.01M citrate buffer (0.5 mL; pH 4.0) containing 1 unit (0.5 mL) of β-D-galactosidase (Sigma; β-D-galactoside galactohydrolase *from Aspergillus niger*, EC 3.2.1.23) was incubated at 30° under a drop of toluene. Aliquots (0.1 mL) of the reaction mixture were deactivated at intervals by heating for 15 min at 60°, de-ionized (Amberlite monobed MB-3), and examined by p.c. (solvents *A* and *B*; stain *a*). Control experiments respectively having a polysaccharide blank and an enzyme blank were examined simultaneously. Polysaccharide C_5 released D-galactose in ~40 h.

Acetylation[25] of polysaccharide C_5. — Polysaccharide C_5 (19.4 mg, dried over P_2O_5) was dispersed in dry *N*,*N*-dimethylformamide (4 mL) by stirring for 4 h at room temperature. To the suspension were added pyridine and acetic anhydride (4 mL of each), and stirring was continued for 3 days at room temperature. The mixture was cooled, diluted with cold acetic acid (10 mL), and poured into ice-cold water (100 mL) with stirring. The residue was separated by centrifugation, washed with water until free from acid, and dried over P_2O_5. The acetylation process was repeated twice more, to give peracetylated C_5 (14 mg) which had no OH bands in its i.r. spectrum.

Determination of molecular weight. — The number-average molecular weight ($\overline{M}_n$) of peracetylated C_5 (galactan fraction) was determined by osmometry, using a type S&S 08 (permeability limit 10,000) membrane and a mixed solvent (density 1.462) consisting of 24:1 (v/v) chloroform–ethanol. Sample pressures (*P*) of solutions having concentrations (*C*) 6.50, 3.25, and 1.625 (in mg/mL; in duplicate) and solvent

pressure (P_0) were measured at 30°; from these the osmotic pressure π ($= P - P_0$) for each concentration was calculated. The plot of π/C *vs.* C was extrapolated to zero concentration, and, from the value (0.47) of the intercept at $(\pi/C)_{C\to 0}$ (see Fig. 2), $\overline{M}_n$ was calculated to be 3.74×10^4.

ACKNOWLEDGMENTS

The authors are grateful to the U.G.C. (New Delhi) and to Mahishadal Raj College for providing an F.I.P. Fellowship and study leave to G.M.

REFERENCES

1 E. ROBOZ AND A. J. HAAGEN-SMIT, *J. Am. Chem. Soc.*, 70 (1948) 3248–3249.
2 B. S. PAULSEN, E. FAGERHEIM, AND E. ØVERBYE, *Carbohydr. Res.*, 60 (1978) 345–351.
3 D. C. GOWDA, B. NEELISIDDAIAH, AND Y. V. ANJANEYALU, *Carbohydr. Res.*, 72 (1979) 201–205.
4 G. R. WALLER, S. MANGIAFICO, AND C. R. RITCHEY, *Proc. Okla. Acad. Sci.*, 58 (1978) 69–76.
5 R. N. CHOPRA, S. L. NAYAR, AND I. C. CHOPRA, *Glossary of Indian Medicinal Plants*, C.S.I.R., New Delhi (India), 1956, p. 13.
6 *The Wealth of India*, A Dictionary of Indian Raw Materials and Industrial Products, Vol. I, C.S.I.R., New Delhi (India), 1948, pp. 60–62.
7 K. R. KIRTIKAR AND B. D. BASU, *Indian Medicinal Plants*, 2nd edn., Vol. IV, Basu, Allahabad (India), reprinted 1975, pp. 2504–2506.
8 D. A. REES, *Adv. Carbohydr. Chem. Biochem.*, 24 (1969) 298–303.
9(a) L. J. BELLAMY, *The Infra-red Spectra of Complex Molecules*, 2nd edn., Wiley, New York, 1958, p. 179. (b) R. M. SILVERSTEIN AND G. C. BASSLER, *Spectrometric Identification of Organic Compounds*, 2nd edn., Wiley, New York, 1967, p. 92.
10 D. GLICK, *Methods Biochem. Anal.*, 2 (1955) 320–321.
11 Z. DISCHE, *J. Biol. Chem.*, 167 (1947) 189–198.
12 N. N. DAS AND A. DAS, *Carbohydr. Res.*, 56 (1977) 337–349.
13 C. TANFORD, *Physical Chemistry of Macromolecules*, 4th printing, Wiley, New York, 1966, pp. 210–221.
14 Z. DISCHE, L. B. SHETTLES, AND M. OSNOS, *Arch. Biochem.*, 22 (1949) 169–184.
15 E. P. CLARK, *Semimicro Quantitative Organic Analysis*, Academic Press, New York, 1943, pp. 68–72.
16 S. K. SIKDER AND A. DAS, *Carbohydr. Res.*, 71 (1979) 273–285.
17 U. LINDAHL, *Biochem. J.*, 116 (1970) 27–34.
18 G. O. ASPINALL, K. HUNT, AND I. M. MORRISON, *J. Chem. Soc., C*, (1967) 1080–1086.
19 A. DAS, M. HEIDELBERGER, AND R. BROWN, *Carbohydr. Res.*, 48 (1976) 304–305.
20 H. NEUKOM AND W. KUENDIG, *Methods Carbohydr. Chem.*, 5 (1965) 14–17.
21 S.-I. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
22 P. A. SANDFORD AND H. E. CONRAD, *Biochemistry*, 5 (1966) 1508–1517.
23 H. BJÖRNDAL, B. LINDBERG, AND S. SVENSSON, *Acta Chem. Scand.*, 21 (1967) 1801–1804.
24 J. S. DIXON AND D. LIPKIN, *Anal. Chem.*, 26 (1954) 1992–1993.
25 N. N. DAS AND A. DAS, *Carbohydr. Res.*, 64 (1978) 155–167.
26 P. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LÖNNGREN, *Chem. Commun.* (Univ. Stockholm), 8 (1976) 22–23.