Carbohydrate Research, 45 (1975) 237–243

DISTRIBUTION OF D-GALACTOSYL GROUPS IN GUARAN AND LOCUST-BEAN GUM*

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(Received April 14th, 1975; accepted for publication, May 1st, 1975)

ABSTRACT

Distribution of α-D-galactopyranosyl side-chain groups in two galactomannans, guaran and locust-bean gum, was determined by measurement of the *O*-acetyl-*O*-methyl-D-mannitol derivatives obtained from the corresponding primary *C*-*p*-tolylsulfonyl polysaccharide derivatives. The *O*-acetyl-*O*-methyl-D-mannitol derivatives were produced by β-elimination and methylation, with sodium (methylsulfinyl)methide and methyl iodide, of the primary *C*-*p*-toluenesulfinylated galactomannans, followed by sequential acid hydrolysis, reduction, and acetylation of the partially degraded *p*-tolyl sulfones. The results indicated that side-chain units of guaran are alternately disposed along the D-mannan backbone, whereas those of locust-bean gum are disposed in uniform blocks along the backbone.

INTRODUCTION

Endosperms of seeds of leguminous plants contain D-galacto-D-mannans. Two of these, guaran and locust-bean gum, are important gums of commerce. They are used in a wide variety of food products and in numerous industrial applications because of their compatibility with other components, but especially because of their useful rheological characteristics[1]; one of these is their ability to produce stable solutions having high viscosities even at low concentrations of polysaccharide. Although similar in many ways, the polysaccharides differ in their ability to improve viscosity and gel strength of carrageenan dispersions, wherein guaran shows no interaction, whereas locust-bean gum effects a synergistic increase in viscosity and gel strength.

Both polysaccharides are galactomannans that consist of (1→4)-β-D-mannopyranosyl residues, some of which bear an α-D-galactopyranosyl group[2] at O-6. A plausible, primary structure for guaran was proposed on the basis of (*a*) its gross composition[3], (*b*) the structure and yield of oligosaccharides resulting from acid[4] and enzymic[5] hydrolysis, and (*c*) general, physical characteristics of the polymer[3,6]. From analogous investigations[7], locust-bean gum was considered to have a similar

*Dedicated to the memory of Dr. Hewitt G. Fletcher, Jr.

primary structure in which the α-D-galactopyranosyl groups are more widely, but randomly, spaced.

Recently, examination of the products resulting from enzymic hydrolysis[8] of locust-bean gum, and the ability of the gum to interact with some polysaccharide systems possessing a high order of molecular association[9], have led to the belief that its α-D-galactopyranosyl substituents are not randomly disposed, but occur in blocks. Thus, consecutively substituted backbone units were theorized to be separated by numerous, contiguous, β-D-mannopyranosyl residues bearing no substituents.

To determine the primary structures of guaran and locust-bean gum chemically, a method that permits selective depolymerization either by way of glycosidic stabilization or activation would be useful. In the present work, we elected to use the observation that 6-deoxy-6-*p*-tolylsulfonylhexopyranosides readily undergo alkali-catalyzed glycosidic hydrolysis[10]. This alkali-lability of such derivatized hexopyranosyl linkages has been used to remove nonreducing D-galactopyranosyl groups from branches in a (1→6)-linked chain[11] and the D-glucopyranosyl groups from

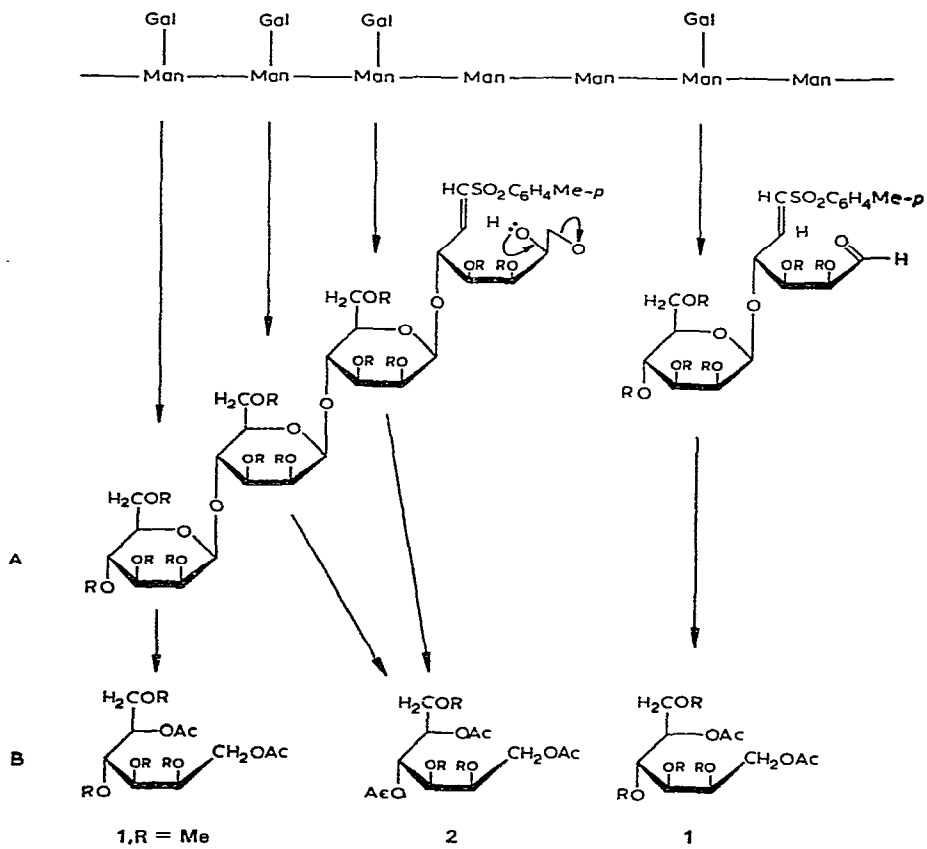

Fig. 1. Alkaline degradation by (A) β-elimination and methylation, followed by (B) acid hydrolysis, reduction, and acetylation.

branches in a (1→6)-linked glucan (dextran)[12]. Application of this principle should make possible the depolymerization of a galactomannan chain in such a way as to provide D-mannopyranosyl units glycosidically bound either to a neighboring, sulfonylated unit or to a neighboring, underivatized D-mannopyranosyl residue. Simple methylation would, therefore, provide a means for distinguishing between those isolated β-D-mannopyranosyl residues in the chain which bear a D-galactopyranosyl group and those adjacent β-D-mannopyranosyl residues that bear a D-galactopyranosyl group.

Alkaline degradation by β-elimination[10] and methylation (see Fig. 1A) with sodium (methylsulfinyl)methide and methyl iodide[13], followed by sequential acid hydrolysis[14], reduction[15], and acetylation (see Fig. 1B) of the partially degraded material, would produce *O*-acetyl-*O*-methyl-D-mannitol derivatives characteristic of the distribution pattern of the α-D-galactopyranosyl side-chains in the parent galactomannan. From the sequence of the β-elimination and methylation, and of the acid hydrolysis, reduction, and acetylation, it is evident that D-mannopyranosyl units substituted at O-6 with α-D-galactopyranosyl groups and at O-4 by a D-mannopyranosyl group without substitution at O-6 will produce 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol (**1**), whereas, if the D-mannopyranosyl residue attached at O-4 bears a D-galactopyranosyl group at O-6, this D-mannopyranosyl residue will give rise to 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol (**2**).

If the α-D-galactopyranosyl groups are isolated from each other along the D-mannan backbone, compound **1** would result from the D-mannopyranosyl residues to which they are attached. However application of the reaction sequence to a block type of pattern of substitution would yield compounds **1** and **2** derived from all D-mannopyranosyl units in the block save the one on the end nearest the nonreducing end of the chain. Each initial, nonreducing β-D-mannopyranosyl group of consecutively substituted portions of the D-mannan chain would give rise to **1**, whereas the remaining β-D-mannopyranosyl residues of this block would yield **2**. The α-D-galactopyranosyl groups and the β-D-mannopyranosyl residues originally possessing unsubstituted hydroxymethyl groups would yield unsaturated *p*-tolyl sulfones that would undergo alkali-induced rearrangements[10].

EXPERIMENTAL*

General methods. — Guaran, isolated from guar flour** by ethanol precipitation[16], and locust-bean gum*** were analyzed by sequential permethylation[13], acid hydrolysis[14], reduction[15], and acetylation[17] (see Table I). Iodide content was determined by combustion and iodometric titration[18]. Sulfur content was determined after wet combustion, using thorin as the titrimetric indicator[19]. All analyses (see

*Abbreviations used: DMF, *N*,*N*-dimethylformamide; Me_2SO, dimethyl sulfoxide; HMPA, hexamethylphosphoramide; NIS, *N*-iodosuccinimide; and Ph_3P, triphenylphosphine.

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TABLE I

ANALYSIS OF GUARAN AND LOCUST-BEAN GUM BY PERMETHYLATION, ACID HYDROLYSIS, REDUCTION, AND ACETYLATION[17]

Sample	*Derived* D-*alditol*[a]	*Molar ratio of alditols from each polymer*
Guaran	A	0.99
	B	1.00
	C	1.00
Locust-bean gum	A	4.75
	B	1.02
	C	1.00

[a]Key: A = 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol produced from β-D-mannopyranosyl residues bearing no 6-*O*-mono-α-D-galactopyranosyl groups; B = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol, produced from α-D-galactopyranosyl groups; and C = 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-mannitol produced from β-D-mannopyranosyl residues bearing 6-*O*-mono-α-D-galactopyranosyl groups.

TABLE II

ELEMENTAL ANALYSIS OF SYNTHESIZED SAMPLES

Sample	*Element*	*Element*	
		Expected (%)	*Found* (%)
3	I	35.94	34.84
4	I	41.68	42.10
3a	I	35.94	35.10
4a	I	41.68	42.00
5	S	8.41	8.17
6	S	9.64	9.78

Table II) gave results lying within the experimental error when the exact composition of each galactomannan (see Table I) was considered. Infrared (i.r.) spectra, determined for KBr disks, were obtained with a Perkin–Elmer Model 337 spectrophotometer. Gas–liquid chromatographic (g.l.c.) analysis[20] was performed with a Varian Aerograph, series 1200, instrument. Evaporations were performed under diminished pressure in a Büchi evaporator at 45°. Samples were lyophilized in a Virtis freeze-dryer. Glassware for anhydrous reactions was dried for 16 h at 110° prior to use.

Primary p-*tolyl sulfone* (**5**) *of guaran.* — Dry guaran (1.215 g) and freshly recrystallized, dry NIS[21] (5.60 g, 24.8 mmoles) were magnetically stirred for 30 min at 0° in dry HMPA (75 ml). Freshly recrystallized, dry Ph_3P[22] (6.56 g, 25 mmoles) was added by aliquots (1 g/15 min) to the stirred suspension, and the mixture was gradually warmed to 95° during 24 h. An aliquot was withdrawn, dialyzed, freeze-dried, and analyzed (see Table II). The temperature was raised to 110°, and dry

sodium *p*-toluenesulfinate (17.8 g, 0.10 mole) was added. The solution was stirred for 24 h at 110°, cooled, purified by dialysis for 30 h against de-ionized water, filtered, and concentrated to 150 ml; a tan product (1.950 g) was isolated by freeze-drying.

Primary p-*tolyl sulfone* (**6**) *of locust-bean gum.* — Dry locust-bean gum (1.216 g), freshly recrystallized, dry NIS (7.00 g, 31 mmoles), and dry Ph_3P (6.56 g, 25 mmoles) were allowed to react in dry HMPA (100 ml) in exactly the same way as for **5**. An aliquot (**4a**) was analyzed (see Table II). The temperature was raised to 110°, and dry sodium *p*-toluenesulfinate (22.2 g, 124 mmoles) was added. The solution was stirred for 24 h at 110°, and a brown product (2.109 g) was isolated by the same procedure as was used for **5**.

β-Elimination and methylation of guaran primary p-*tolyl sulfone* (**7**). — A solution of dry **5** (381 mg) in dry Me_2SO[23] (40 ml) was treated[13] with 2M sodium (methylsulfinyl)methide (10 ml), magnetically stirred for 8 h at 22°, and then cooled to 15°; methyl iodide (0.15 ml) was added, and the solution was warmed, and stirred for 2 h at 22°. This alternating procedure of addition was repeated (2 × 10 ml, and 2 × 0.15 ml, respectively). After the final addition of methyl iodide with stirring, an excess of methyl iodide (3 ml) was added, the solution was stirred for 2 h at 22°, and dry methanol (5 ml) was slowly added. The solution was poured into water (100 ml), and the mixture extracted with chloroform (4 × 10 ml). The extracts were combined, washed with 10% (w/v) aqueous sodium chloride (5 × 250 ml), dried (anhydrous sodium sulfate), filtered, and the filtrate evaporated to a syrup (400 mg).

β-Elimination and methylation of locust-bean gum primary p-*tolyl sulfone* (**8**). — A solution of dry **6** (415 mg) in dry Me_2SO (50 ml) was treated and isolated in the same way as for **7**. The chloroform filtrate was evaporated to a syrup (435 mg).

Production of D-*mannitol derivatives.* — Each dry syrup (**7** and **8**) was separately hydrolyzed in 50 ml of hydrochloric acid solution (sp. gr. 1.125) for 2.5 h. The suspension was filtered, and the filtrate was made neutral and concentrated under diminished pressure to 10 ml; this was reduced for 16 h at 22° with sodium borohydride (400 mg)[15], the alkali neutralized with IR-120 (H^+) cation-exchange resin, the suspension filtered, and the filtrate evaporated to dryness. The residue was co-

TABLE III

GAS–LIQUID CHROMATOGRAPHY OF *O*-ACETYL-*O*-METHYL-D-MANNITOL PRODUCTS

Sample	D-*Mannitol derivative*	*Retention time*[a] *(min)*	*Molar ratio*[b]
Guaran (**7**)	**1**[c]	12.5[c]	0.99
Locust-bean gum (**8**)	**1**[c]	12.5[c]	0.04
	2[d]	26.8[d]	1.00

[a]A 2.4-m column of 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 180°, with nitrogen at a flow rate of 40 ml.min^{-1}. [b]Relative ratio of product obtained from degradation sequence in Fig. 1. [c]Identical to authentic 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol[17]. [d]Identical to authentic 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol[28].

evaporated with neat methanol[24] (5 × 150 ml), and the dry syrup was acetylated with acetic anhydride–pyridine. This acetylated mixture was prepared for g.l.c. analysis by successive co-evaporation with water (5 × 150 ml) and ethanol (5 × 150 ml).

G.l.c. analysis. — A portion (20–25 mg) of the dry, acetylated syrup was dissolved in chloroform (5 ml). A sample (1.0 μl) of this solution was analyzed by standard g.l.c.[20]. The retention time and peak area of the *O*-acetyl-*O*-methyl-D-mannitol derivatives were compared to those of authentic samples, as shown in Tables I and III.

RESULTS AND DISCUSSION

Replacement of the primary hydroxyl groups of guaran and of locust-bean gum by *C*-*p*-tolylsulfinyl groups was effected by bimolecular displacement at the corresponding iodomethyl groups. The excellent reaction of Trippett[25] and its extension to carbohydrates by Hanessian and co-workers[26,27] was utilized to introduce the deoxy-iodo functions. The reaction is highly specific for primary hydroxyl groups[25].

When the galactomannans were dissolved in HMPA and treated with NIS and Ph_3P for 24 h at 95°, the primary deoxy-iodo derivatives were obtained having the appropriate degree of substitution (d.s.) of 1.0. The groups in compounds **3** and **4** were incompletely displaced by *p*-toluenesulfinate ion, even after repeated reaction[17]. However, if the deoxy-iodo derivatives were not isolated, but were treated with an excess of *p*-toluenesulfinate ion immediately after their formation[26] (see **3a** and **4a** in Table II), the corresponding, primary *C*-*p*-tolyl sulfones, **5** (d.s. 1.0) and **6** (d.s. 1.0), were produced[17].

Compounds **5** and **6** were degraded by β-elimination, and methylated with sodium (methylsulfinyl)methide and methyl iodide[13] to a partially degraded, methylated guaran (**7**) or locust-bean gum (**8**). Products **7** and **8** were sequentially hydrolyzed with acid[14], reduced with sodium borohydride[15], and acetylated with acetic anhydride–pyridine. The *O*-acetyl-*O*-methyl-D-mannitol derivatives generated were quantitatively measured by g.l.c.[20], and identified by comparison with authentic compounds (see Table III).

The results confirmed the previously suggested regularity in the primary structure of guaran[3]. This polymer has a primary structure consisting of regular repetitions of the trisaccharide 4-*O*-(6-*O*-α-D-galactopyranosyl-β-D-mannopyranosyl)-β-D-mannopyranose. Locust-bean gum, on the other hand, does not contain singularly disposed α-D-galactopyranosyl side-chains, but has these side groups located on D-mannopyranosyl residues in blocks.

As the tetramethyl ether **1** is produced from the first nonreducing β-D-mannopyranosyl group in the block of consecutively substituted β-D-mannopyranosyl units, its relative ratio indicates that each substituted block is composed of 25 β-D-mannopyranosyl residues. Assuming (*a*) a molecular weight of 210,000 for locust-bean gum[1], and (*b*) that these substituted blocks are separated by nonsubstituted β-D-mannopyranosyl units of equal length, the average length of each unsubstituted block is 85

linearly linked β-D-mannopyranosyl residues. This long length of unsubstituted chain agrees well with the observed association of locust-bean gum with some segmented, non-gelling carrageenans, and with agaran[9].

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