Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE STRUCTURE OF A GALACTOMANNAN FROM THE SEED OF *Gleditsia triacanthos*

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(Received May 18th, 1970)

ABSTRACT

A homogeneous galactomannan composed of D-mannose and D-galactose in a molar ratio of 3.2–3.5:1 was isolated in 15–20% yield from the seeds of *Gleditsia triacanthos*. On periodate oxidation, all of the D-mannose and D-galactose residues were attacked, as hydrolysis of the reduced, oxidized polysaccharide gave only glycerol and erythritol. The periodate consumption was 4.7 molecules per four hexose residues, and 1 molecule of formic acid was produced. Hydrolysis of the methylated polysaccharide (1 mole) yielded 2,3,4,6-tetra-*O*-methyl-D-galactose (1 mole), 2,3,6-tri-*O*-methyl-D-mannose (2.5 moles), and 2,3-di-*O*-methyl-D-mannose (1 mole). Traces of two other compounds (which, according to their chromatographic behavior, could be 2,3,4- and 2,3,6-tri-*O*-methyl-galactose) were also found.

INTRODUCTION

Gleditsia triacanthos is a leguminous tree, native to the central United States, that grows to be 20–30 m in height and 0.50–0.75 m in diameter; it was introduced into the Argentine Republic 50–60 years ago under the name of "Acacia negra", and is used for ornamental purposes in the Provincia de Buenos Aires.

As early as 1900, Goret[1] studied the mucilage contained in its seed and recognized that it consists mainly of a galactomannan; its proportion was later[2] determined as ~27%. This polysaccharide, usually known as "honey-locust galactomannan", was studied by periodate oxidation[3,4], with contradictory results; it is now realized that the conditions used were inadequate. Coffee-bean α-D-galactosidase splits α-D-galactosyl groups from this polymer, as with other galactomannans[5].

Because of our interest in the galactomannan from Gleditsia trees, especially as use of this substance has been advised for industrial applications[6], it was considered useful to conduct an investigation of its structure, to determine whether it is similar to that of the galactomannans whose structure is already known.

RESULTS AND DISCUSSION

The polysaccharide was extracted from the dry, ground seed with warm water. Ethanol was added to the aqueous extract until a 10% concentration thereof was

obtained, and the insoluble matter was removed by centrifuging; addition of alcohol to the supernatant liquor to give a 30% concentration of alcohol caused precipitation of the polysaccharide as long, white fibers; yield 15–20%; $[\alpha]_D$ +23.0° (water); proteins 1.8%.

This crude product was purified by two methods. Method *1* consisted in fractional precipitation from its aqueous solution by increasing the concentration of ethanol stepwise; almost 80% of the initial weight was precipitated at an ethanol concentration lying between 30 and 35%. Only small proportions were obtained at lower or higher concentrations (upper limit 60%); $[\alpha]_D$ +25.0° (water); proteins 1.9%. In method *2*, precipitation of the copper complex by addition of freshly prepared Fehling solution to an aqueous solution (0.2%) of the crude polysaccharide gave a precipitate that was separated by centrifuging; it was redissolved in water at pH 5.0. Ethanol was added to the upper limit (30%) of solubility of the galactomannan, and the suspension was centrifuged. Precipitation of the pure product was obtained by increasing the concentration of ethanol to 35%; yield 70–75%; $[\alpha]_D$ +25.0° (water); proteins 1.5%.

Evidence of the homogeneity of the polysaccharide was provided by the following observations: (*a*) its precipitation from aqueous solution over a narrow range of concentration of ethanol; (*b*) the fact that the values of the optical rotation and limiting-viscosity number (see Table I), and its infrared (i.r.) spectrum remain unchanged through the purification processes; (*c*) the precipitation of its acetate, from solution in acetone, by ethanol occurs over a narrow range of concentration of ethanol, and the product obtained after purification through this acetate[7] displays the same physical properties as those of material obtained by other purification procedures; and (*d*) a single peak was found in the sedimentation patterns obtained with an ultracentrifuge.

The i.r. spectrum was identical with that of the galactomannan[8] from *Gleditsia amorphoides*; it showed absorption bands at 810–815 and 870–875 cm^{-1}, indicating the presence of α-linked galactopyranose residues and β-linked mannopyranose residues, respectively[8]. The same bands were observed in the i.r. spectrum of the galactomannan from *Ipomoea muricata*[9].

The ultracentrifugations were performed at three different concentrations, giving sedimentation constants ($S_{20,w}$) that decreased with increasing concentration of the solute; this dependence is usual in polymers of asymmetrical form[10]. As the values obtained lay on a straight line, they were extrapolated to zero concentration, giving a value of 2.03×10^{-13} for the sedimentation constant at infinite dilution.

A quantitative relationship has been obtained between the rotatory power and the molar ratio of D-mannose: D-galactose for water-soluble galactomannans having a structure consisting of a backbone of β-D-(1→4)-linked D-mannopyranose residues with side chains of α-D-(1→6)-linked D-galactopyranose residues[11]. The specific rotation of the *Gleditsia triacanthos* galactomannan suggests a D-mannose:D-galactose ratio of ~3.5. Acid hydrolysis of the purified polysaccharide, and quantitative determination of sugar components in the hydrolyzate showed a molar ratio of 3.2:1

(methylation analysis indicated a ratio of 3.5:1), a value in close agreement with that suggested by the aforementioned relationship. This agreement also corroborates the hypothesis mentioned that the deviations from the idealized structure that are suggested by the methylation analysis are not quantitatively important.

The degree of polymerization (D.P.) was determined chemically[12], and a value of 110–120 was obtained; this is almost identical to that of the *Gleditsia amorphoides* galactomannan (D.P. 116). This identity was confirmed by the similar values of the limiting-viscosity numbers for both products (see Table I), which, according to the equations of Staudinger and Mark, indicate similar molecular weights[13].

Variable results were obtained in periodate-oxidation studies. When 0.01M periodate was used, four molecules of oxidant were consumed for every four hexose residues, and reduction of the oxidized polysaccharide followed by hydrolysis indicated that a considerable proportion of the D-mannose residues survived the oxidation. However, when the reaction was conducted with 0.1M periodate, 4.7 molecules of it were consumed for every four hexose residues, and reduction followed by hydrolysis gave glycerol and erythritol (as in the former case), with only traces of D-galactose and D-mannose. This resistance of some D-mannose residues has been observed in several instances[8], and has been attributed to cyclic acetal formation[8]. The fact that only traces of hexoses survived the periodate treatment indicates that significant proportions of (1→3)-linkage are not present. The molecular proportion of periodate consumed and of formic acid produced (1 molecule per every four hexose residues, in both experiments), as well as the erythritol and glycerol released on acid hydrolysis of the polyalcohol, are the results to be expected from the well-known structure consisting of a backbone of (1→4)-linked D-mannopyranose residues with side chains of (1→6)-linked D-galactopyranose residues.

Hydrolysis of the methylated galactomannan yielded 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose, and 2,3-di-*O*-methyl-D-mannose in the molar ratios of 2:5:2 (by weight); these were separated by column chromatography, and identified in the usual way. Some evidence was obtained of the presence of trace amounts of two compounds that, from their chromatographic behavior, could be 2,3,4- and 2,3,6-tri-*O*-methyl-D-galactose. That the proportions of these compounds was very small was proved by further column chromatography of the fraction containing the tri-*O*-methylated sugars (in which, both compounds appear); no resolution of the mixture was found possible, and the specific rotation of each of the three subfractions separated was that of 2,3,6-tri-*O*-methyl-D-mannose (−8.0°, −13.0°, and −8.8°; lit. value, −10.0°) which, in view of the rotations of the methylated D-galactoses (+119.0° and +87.0° for 2,3,4- and 2,3,6-tri-*O*-methyl-D-galactose, respectively), indicated very little contamination by the latter compounds.

The presence of small proportions of 2,3,4-tri-*O*-methyl-D-galactose had previously been detected in the products of hydrolysis of the methylated galactomannans of *Gleditsia ferox*[14] and *Gleditsia amorphoides*[8]. Methylation and hydrolysis of the galactomannan of *Trifolium repens*, and of the insoluble fraction obtained by the action of coffee-bean α-D-galactosidase on the *Gleditsia ferox* galactomannan,

showed the presence[5] of this D-galactose derivative in proportions of ~1.0–1.5%. That the tri-*O*-methylated galactoses are not artifacts produced by partial demethylation of the 2,3,4,6-tetra-*O*-methyl-D-galactose has been demonstrated by Courtois and Le Dizet[5]. To the best of our knowledge, the presence of 2,3,6-tri-*O*-methyl-D-galactose in methylated, water-soluble galactomannans has not previously been suggested.

The preceding results constitute good evidence that the basic structure of the galactomannan of *Gleditsia triacanthos*, like that of the other water-soluble galactomannans known, consists of a backbone of β-D-(1→4)-linked D-mannopyranose residues with side chains (one of every 3.2–3.5 D-mannose residues, on the average) formed by an α-D-(1→6)-linked D-galactopyranose residue. In a small number of these side chains, another D-galactose residue could be connected through a (1→6) linkage[14]. It is also possible that some (1→4)-linked D-galactose residues form part of these side chains, or of the backbone of the molecule.

EXPERIMENTAL

General. — Chromatographic separations were performed on Whatman No. 1 paper and on microcrystalline cellulose (Avicel) with the following solvents: (*A*) 4:1:5 butyl alcohol–ethanol–water, upper layer; (*B*) butanone–water azeotrope, and (*C*) 5:5:3:1 pyridine–ethyl acetate–water–acetic acid. The spray reagents used were (*a*) aniline phthalate in butyl alcohol saturated with water, and (*b*) benzidine–periodate reagent. The concentrations of ethanol in the purification steps are given in wt. of ethanol/wt. of solution. All evaporations were conducted in a rotary evaporator under diminished pressure at 35–40° (bath temperature). The optical rotations given are equilibrium rotations. Melting points are uncorrected.

Viscosities. — Viscosities of aqueous solutions of the crude polysaccharide, and of samples purified by methods *1* and *2*, were measured at 20° in a Hoeppler, type C viscosimeter at different concentrations between 0.05 and 0.25%; the results were extrapolated to zero concentration, to obtain the limiting-viscosity numbers (see Table I). Parallel determinations were conducted with similar solutions of crude and ethanol–purified *Gleditsia amorphoides* galactomannan (see Table I). Determinations on solutions of both polysaccharides (purified by method *1*) in 0.1M sodium

TABLE I

LIMITING-VISCOSITY NUMBERS [η] OF AQUEOUS SOLUTIONS [a] OF GALACTOMANNANS OF *Gleditsia triacanthos* (A) AND *Gleditsia amorphoides* (B)

Type	*Crude polysaccharide*	*Purified*[b]	
		1	2
A	4.4	4.0	4.8
B	4.4	3.9	—

[a] 100 ml/g. [b] *1*, by alcohol precipitation; *2*, through the copper complex.

chloride gave results identical to those given in Table I; the results have a standard deviation of ±12%.

Infrared spectra. — The spectra were recorded with a Perkin–Elmer Model 137 Infracord spectrophotometer for films of the polysaccharides or of their methylated or acetylated derivatives. The films were prepared by evaporating a solution (0.5%) of the material in a suitable solvent (water for the polysaccharide, and chloroform for its derivatives) to dryness on a mercury surface in a vacuum desiccator at room temperature.

Ultracentrifugation. — The sedimentation patterns were obtained with a Spinco Model E ultracentrifuge, at 20°, by use of 1.0, 0.7, and 0.3% solutions of the ethanol-purified polysaccharide in 0.1M sodium chloride. The sedimentation coefficients ($S/_{20,w}$), calculated in the usual way, were 1.06×10^{-13}, 1.29×10^{-13}, and 1.78×10^{-13}, respectively. When these were plotted against the concentrations, a straight line was obtained which, extrapolated to zero concentration, gave the sedimentation constant (2.03×10^{-13}) at infinite dilution.

Isolation of the galactomannan. — Seeds of *Gleditsia triacanthos* were fed through a mill fitted with a 20-mesh screen. The powdered seeds (100.0 g) were extracted for 24 h with water (5 liters) at 50°, with constant mechanical stirring; this procedure was repeated until no more precipitate was obtained when the extract was added to two volumes of ethanol. The extracts were combined, and cooled to room temperature, and ethanol was slowly added, with vigorous stirring, until the concentration of alcohol was 10% (previous experiments had shown that, at this concentration, the polysaccharide remains in solution). The precipitate was removed by centrifuging, and alcohol was added to the supernatant liquor until the concentration of alcohol was 30%; all of the galactomannan is precipitated under these conditions as long, white fibers. The liquor was decanted, and the product was squeezed between sheets of filter paper and kept under ethanol. It was washed by solvent exchange with absolute ethanol and then ether, and dried in a vacuum desiccator at room temperature; yield 15–20%, $[\alpha]_D$ +23.0° (*c* 0.6, water), limiting-viscosity number 4.4, proteins 1.8%.

Purification of the galactomannan. — Method 1. Precipitation with ethanol. The crude product (8.0 g) was dissolved in distilled water (4 liters) at room temperature. Ethanol (1.7 liters) was added in 100-ml portions, without formation of any precipitate after stirring for 24 h. Further addition of a total of 450 ml of ethanol precipitated the galactomannan; the mixture was kept overnight, and centrifuged, and the precipitate was dried as before; yield 6.3 g (78.3%), $[\alpha]_D$ +25.0° (*c* 0.4, water), limiting-viscosity number 4.0, proteins 1.9%. The product was precipitated at a concentration of ethanol of 30–35%. Increase in the concentration of ethanol to 60% produced only a small precipitate that was discarded.

Method 2. Precipitation of the copper complex. Freshly prepared Fehling solution (200 ml) was slowly added to a 0.2% solution of the crude galactomannan in water (4 liters). The white precipitate formed was kept overnight, and removed by centrifuging. It was suspended in water (4 liters), and cold 0.5M hydrochloric acid

was added until dissolution was complete; ethanol was added to the upper limit of solubility of the polysaccharide (30%), and the suspension was centrifuged. Precipitation of the pure galactomannan was obtained by increasing the concentration of ethanol to 35%. The product was isolated, and dried as before; yield 70–75%; $[\alpha]_D$ +25.0° (*c* 0.5, water), limiting-viscosity number 4.8, proteins 1.5%.

Acetylation of the galactomannan. — The purified galactomannan (4.0 g) was dispersed in formamide (200 ml), and anhydrous pyridine (40 ml) was slowly added, followed by acetic anhydride (40 ml) added dropwise during 2 h. After being shaken overnight, the brown, viscous solution was poured, with stirring, into water. The grayish white precipitate was centrifuged out, washed with water until it became white, and dried by solvent exchange (absolute ethanol, petroleum ether) and in a vacuum desiccator at room temperature; yield 5.93 g (81.0%); acetyl 38.95%; $[\alpha]_D^{20}$ +8.8° (*c* 0.5, chloroform), $[\alpha]_D^{21}$ +23.3° (*c* 0.5, acetone).

The acetate (3.0 g) was dissolved in acetone (160 ml) and ethanol was added in portions until a concentration of 60% was obtained. (Further addition did not produce any precipitate.) A total of 2.60 g (87.5%) was recovered (range of precipitation, 49.5–56.0% of ethanol), and, from it, 2.42 g (93.4% of the recovered material) was precipitated in the range of 49.5–52.5% concentration of ethanol; $[\alpha]_D^{22}$ +12.3° (*c* 0.5, chloroform); $[\alpha]_D^{22}$ +25.5° (*c* 0.4, acetone).

Regeneration of the galactomannan from its acetate. — A solution of the purified acetate (1.0 g) in acetone (30 ml) was added to 30 ml of aqueous potassium hydroxide solution (45%). Nitrogen was bubbled through the solution, which was boiled for 3 h under reflux. When the reaction was complete, the aqueous lower layer (which had become a gel) was separated from the acetone layer, diluted with water, dialyzed against running tap-water for 48 h, and freeze-dried; yield 0.58 g (95%); $[\alpha]_D^{22}$ +23.9° (*c* 0.4, water).

Hydrolysis of the galactomannan. — A solution of the purified polysaccharide (0.2 g) in 20 ml of 0.5M sulfuric acid was heated in a sealed tube for 12 h at 95°, neutralized (barium carbonate), filtered, the filtrate evaporated to dryness, and the residue extracted with absolute ethanol. The mixture of sugars thus obtained was analyzed by paper chromatography and t.l.c, with solvents *A* and *C*. Chromatograms sprayed with reagent *a* showed spots of galactose and mannose, only. Quantitative determination of these sugars was performed by densitometry[15]; a molar ratio of mannose: galactose of 3.2:1 was obtained.

Determination of the degree of polymerization of the galactomannan. — The galactomannan (~40 mg) was dissolved in 10 ml of distilled water, sodium borohydride (45 mg) was added, and the mixture was kept for 96 h at room temperature. It was acidified with 50% aqueous acetic acid, and treated with sodium periodate (53 mg). The pH was then adjusted[16] to 7.5 by addition of sodium hydrogen carbonate, and the mixture was kept for at least 96 h (which previous experiments had indicated as sufficient) in the dark at room temperature. The sodium iodate formed and the excess of periodate were removed by addition of lead formate (70 mg) and the volume of the solution was increased to 25 ml. An aliquot (5 ml) was withdrawn,

and dialyzed in a closed system against distilled water (5 ml) for 48 h. The external solution (1 ml) was used for the determination of formaldehyde by the chromotropic acid method[12,17,18]; the results indicated a degree of polymerization of 110–120 for the alcohol-purified galactomannan.

Periodate oxidation of the galactomannan. — The polysaccharide (50 mg) was dissolved in water, sodium periodate (1.070 g) was added, and the solution was diluted to 50 ml, to give a 0.1M concentration of the oxidant. The reaction was conducted in the dark, at room temperature. The formic acid produced was determined by titration with 0.01M sodium hydroxide, and the periodate consumption by the Fleury–Lange method[16]. After 240 h, the liberation of formic acid and the uptake of periodate were constant, corresponding to 0.98 molecule of formic acid and 4.70 molecules of sodium periodate for every four hexose residues.

When the reaction was performed under the same conditions, but with 0.01M sodium periodate, the uptake of periodate after 240 h was 3.99 molecules and the liberation of formic acid was 0.99 molecule for every four hexose residues.

Periodate degradation of the galactomannan. — The purified galactomannan (0.2 g) was dissolved in water (10 ml), and a solution of 535 mg of sodium periodate in 10 ml of water was added. The volume was increased to 25 ml, so as to obtain a 0.1M concentration of the oxidant. The oxidation was conducted in the dark, at 5°, and was monitored polarimetrically; 5 days after the rotation had become constant, the solution was dialyzed for 72 h against running tap-water (to eliminate the excess of periodate and other salts), and sodium borohydride (0.1 g) was added, to reduce the oxopolysaccharide. After 24 h at room temperature, the solution was redialyzed, and concentrated sulfuric acid was cautiously added, to give a 0.5M solution; this was heated for 8 h at 95°, cooled to room temperature, and neutralized (barium carbonate), centrifuged, and the supernatant liquor evaporated to dryness. Chromatography of the residue (solvents A and C, spray reagent *b*) showed spots corresponding to erythritol and glycerol. Traces of a mannose were also detected.

When the polysaccharide that had been oxidized with 0.01M sodium periodate was reduced and the product hydrolyzed as just described, chromatography showed not only glycerol and erythritol but also a considerable proportion of unattacked mannose.

Methylation of the galactomannan. — The polysaccharide (4.0 g) was dissolved in water (100 ml), sodium borohydride (0.1 g) was slowly added with mechanical stirring, and the mixture was kept for 48 h at room temperature. Sodium hydroxide (100 ml of 3:2, w/v), which had been pretreated by bubbling nitrogen through it, was added, to give a final concentration of 3:7 (w/v), and the polysaccharide was subjected to five methylations by the Haworth method, conducted at 30°. The partially methylated product thus produced formed an emulsion with, but was not soluble in, chloroform. Accordingly, it was subjected to six such treatments, at 50°. The final mixture was concentrated, centrifuged, and extracted with chloroform, and the methylated galactomannan was isolated in the usual way as a yellow, glassy solid (3.5 g).

This product was dissolved in anhydrous *N,N*-dimethylformamide and methylated (Kuhn method[19]) with methyl iodide–barium oxide. Isolation in the usual way yielded 2.8 g of a product having MeO 41.9%. A further methylation by the same method gave a product that had MeO 42.1%; $[\alpha]_D^{23}$ +16.5° (*c* 0.4, acetone); $[\alpha]_D^{23}$ +30.1° (*c* 0.5, chloroform).

Hydrolysis of the methylated galactomannan. — A solution of the methylated galactomannan (1.0 g) in formic acid (90%; 20 ml) was heated for 1 h at 95°. The formic acid was evaporated off, and three 30-ml portions of methanol were added and evaporated. The syrupy residue was dissolved in 0.5M sulfuric acid (60 ml) and the solution was heated for 7 h at 95°, cooled, neutralized (barium carbonate), filtered, and the filtrate evaporated to dryness. The residue was dissolved in methanol (10 ml), the suspension was filtered, and the filtrate was evaporated to a light-yellow syrup. The mixture of methylated sugars was separated by cellulose-column chromatography (solvent *B*), the eluate being collected in 10-ml fractions with an automatic fraction-collector. After paper-chromatographic examination (solvent *B*) of every third tube, it was possible to combine the appropriate fractions into four large fractions.

*Fraction 1. Identification of 2,3,4,6-tetra-*O*-methyl-*D*-galactose.* This component [160 mg; $[\alpha]_D^{22}$ +107.0° (*c* 0.6, water); lit.[20] $[\alpha]_D^{22}$ +109.5° (water)], recovered from tubes 1–10, showed R_G 0.86 (solvent *A*) and R_F 0.69 (solvent *B*); its properties corresponded to those of 2,3,4,6-tetra-*O*-methyl-D-galactose. Treatment of the methylated sugar with aniline, in the usual way, yielded 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine; m.p. 186–187°, $[\alpha]_D^{22}$ +38.7° (*c* 0.4, acetone); lit. m.p. 186–188° (Ref. 21), $[\alpha]_D^{22}$ +39.0° (acetone) (Ref. 22).

Fraction 2. From tubes 11–17, a mixture of two compounds was obtained [110 mg; $[\alpha]_D^{22}$ +70.9° (*c* 1.8, water)] having R_G 0.86 and 0.80 (solvent *A*), and R_F 0.69 and 0.48 (solvent *B*). They were chromatographically identical to 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-mannose (see fraction 3), respectively; the optical rotation of the mixture indicated that the two sugars were present in approximately the same proportions.

*Fraction 3. Identification of 2,3,6-tri-*O*-methyl-*D*-mannose.* Evaporation of the fractions collected in tubes 18–42 gave a syrupy material (440 mg) which showed $[\alpha]_D^{21}$ −11.5° (*c* 0.8, water); lit. value[23] for 2,3,6-tri-*O*-methyl-D-mannose, $[\alpha]_D$ −10.0° (water). Paper chromatography indicated that the syrup contained a major component corresponding to this sugar (solvent *A*, R_G 0.79; solvent *B*, R_F 0.50), and traces of two other components whose chromatographic behavior suggested that they were a 2,3,4-tri-*O*-methyl-galactose (solvent *A*, R_G 0.63; solvent *B*, R_F 0.38) and a 2,3,6-tri-*O*-methyl-galactose (solvent *A*, R_G 0.73; solvent *B*, R_F 0.44). Attempts to separate the three components by further column-chromatography failed; the mixture was then fractionated into three fractions (80, 330, and 30 mg, respectively) in an attempt to concentrate the trace components; the rotations of these fractions were −8.0, −13.0, and −8.8°, which, considering the rotations of the possible components of the mixture (2,3,4-tri-*O*-methyl-D-galactose[24], +119.0°; 2,3,6-tri-*O*-methyl-D-galactose[24], +87.0°; and 2,3,6-tri-*O*-methyl-D-mannose[23], −10.0°), indicated very

little contamination. Treatment of the syrup with aniline, in the usual way, gave 2,3,6-tri-*O*-methyl-*N*-phenyl-D-mannosylamine, m.p. 126–128°, $[\alpha]_D^{22}$ −135.0 → −39° (*c* 0.4, methanol); lit. m.p. 127–128° (Ref. 25), $[\alpha]_D$ −155.0 → 39.0° (methanol) (Ref. 26).

*Fraction 4. Identification of 2,3-di-O-methyl-*D*-mannose.* This component (190 mg) recovered from tubes 68–110, showed R_G 0.54 (solvent A) and R_F 0.23 (solvent B); $[\alpha]_D^{21}$ −16.5° (*c* 1.8, water), $[\alpha]_D^{21}$ +6.0° (*c* 0.5, methanol), corresponding to 2,3-di-*O*-methyl-D-mannose; lit.[27] $[\alpha]_D$ −15.8° (water) and +6.0° (methanol). 2,3-Di-*O*-methyl-D-mannose 1,4,6-tris-*p*-nitrobenzoate was obtained on treatment of the syrup with *p*-nitrobenzoyl chloride[26]; m.p. 192–193°, $[\alpha]_D^{21}$ +64.3° (*c* 0.4, chloroform); lit.[26] m.p. 194°, $[\alpha]_D$ +65.0° (chloroform).

ACKNOWLEDGMENT

This work was supported by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas.

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