STRUCTURES OF THE GALACTOMANNANS FROM SEEDS OF *Annona muricata*, *Arenga saccharifera*, *Cocos nucifera*, *Convolvulus tricolor*, AND *Sophora japonica*

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(Received March 12th, 1971; accepted for publication in revised form, May 22nd, 1971)

ABSTRACT

Galactomannans were isolated from ripe seeds of *Annona muricata* (*Annonaceae*), *Convolvulus tricolor* (*Convolvulaceae*), *Sophora japonica* (*Leguminosae*), and from immature seeds of *Arenga saccharifera* and *Cocos nucifera* (both *Palmae*). Their sugar compositions were determined and their structures studied by the methylation and periodate-oxidation techniques. All the galactomannans studied are of the leguminous type, the molecules having main chains consisting of (1→4)-linked β-D-mannose residues, with differing proportions of side chains consisting of single α-D-galactose residues linked to the main chains by (1→6)-bonds. The molecular weights were found to vary from 6,000 (*Sophora*) to 17,000 (*Arenga*). The isolation of the galactomannan of *Annona* is the first recorded occurrence of this type of polysaccharide in the family *Annonaceae*, whereas there has been a previous report of the occurrence of a galactomannan in the *Convolvulaceae*; the study of the structure of the *Sophora* galactomannan is the first one in the tribe *Sophoreae* of the *Leguminosae*.

INTRODUCTION

Galactomannans occur in the endosperm of many *Leguminosae*[1]; they are prevalent in several tribes of the three subfamilies[2]. The chemical structures of the galactomannans of many species which have been investigated are remarkably uniform and comprise main chains of β-(1→4)-linked D-mannopyranose residues and side chains of single D-galactopyranose residues attached by α-(1→6)-bonds. However, exceptions to this general type of structure have been found[3]. The galactomannans isolated from different species differ mainly in the mannose–galactose ratio and in molecular weight. In some cases, a marked molecular heterogeneity within one endosperm sample was encountered[4].

Galactomannans have been found in other plant families. Among the Dicotyledons, the only galactomannan outside the *Leguminosae* is one from *Ipomoea muricata* (*Convolvulaceae*); its structure is similar to the leguminous-type[5]. Among the Monocotyledons, galactomannans occur in unripe endosperms of some *Palmae*. Thus, *Borassus flabellifer* contains a leguminous-type galactomannan[6], whereas

that from *Cocos nucifera* not only has an unusually high, negative value for its specific optical rotation, but is also reported to have side chains of both mannose and galactose residues, and a considerable proportion of non-terminal galactose residues, some of which are branch points[7]. The cell walls of the endosperms of all ripe palm-seeds studied so far consist, for a considerable part, of mannans containing only a few percent of galactose residues. However, a galactomannan containing as much as 9% of galactose residues has been extracted from ripe *Phoenix dactylifera* endosperm. The latter polysaccharide is reported to have mainly mannose end-groups and mainly non-terminal galactose residues[8].

We now report on several galactomannans obtained from the following sources. During a study of the amyloid isolated from the seeds of *Annona muricata*, the seeds were found to contain a galactomannan as a second polysaccharide component[9]. In order to obtain more information on the polysaccharides of unripe palm-seeds, the gelatinous endosperm of *Arenga saccharifera* (commercially available as "kulang kaleng", a sugar syrup imported from Indonesia) was investigated. The main water-soluble polysaccharide component proved to be a galactomannan. Since the reported structure of *Cocos* galactomannan[7] is, in certain details, rather different from those of the *Borassus*[6] and *Arenga* galactomannans, the former polysaccharide was reinvestigated. For the purpose of comparative phytochemistry, a study of a relative of *Ipomoea muricata* was thought to be important. The seeds of *Convolvulus tricolor* were available, and they contain a galactomannan. Finally, a galactomannan from the seeds of a *Leguminosae* (*Sophora japonica*) belonging to the tribe *Sophoreae*, of which no representative had been investigated previously, was studied.

RESULTS

Annona muricata L.

The decorticated seeds were ground and defatted and then extracted at room temperature successively with water, and 0.2M and M sodium hydroxide. After acidification of the alkaline extracts with acetic acid, precipitates were formed which contained the crude amyloid; the filtrates contained the crude galactomannan which was precipitated with ethanol. The dried product was dissolved in water and incubated with heated cellulase from *Myrothecium verrucaria*[10] which hydrolyzed the contaminating amyloid to give D-glucose and oligosaccharides; on precipitation of the galactomannan with ethanol, a purified preparation was obtained which was used for structural investigation. Part of the crude galactomannan was insoluble in water and had a somewhat higher mannose-to-galactose ratio (5.1:1) than the soluble material and was not studied further.

The purified, water-soluble galactomannan (2.2% of the defatted starting material) had $[\alpha]_D$ +3.4° (*c* 1, water), and a mannose-to-galactose ratio of 4.46:1.

The methylated polysaccharide had $[\alpha]_D$ +4.7° (*c* 3.5, chloroform), and on hydrolysis it yielded (paper chromatography) 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 proportions 1:3.55:1.02.

On oxidation, the polysaccharide consumed 1.07 moles of periodate per mole of hexosyl residue, yielding 0.17 mole of formic acid (expected values 1.09 and 0.18, respectively). The molecular weight of the polysaccharide, estimated at 8,700, indicates a content of 44 mannose and 10 galactose residues.

Arenga saccharifera Labill.

The galactomannan was separated from the dilute sodium hydroxide extract of the gelatinous endosperm of unripe seeds by precipitation with ethanol after acidification. The crude, water-soluble polysaccharide was purified via the copper complex to give a galactomannan (~5% of the wet endosperm) having $[\alpha]_D$ +50° (*c* 1, water) and +35° (*c* 1, M sodium hydroxide). The mannose-to-galactose ratio, which remained unchanged during purification, was 2.26:1.

The methylated polysaccharide had $[\alpha]_D$ +33.5° (*c* 3.3, chloroform), and on hydrolysis 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 proportions 1:1.30:1.05.

On periodate oxidation, the polysaccharide consumed 1.07 moles of oxidant, and 0.26 mole of formic acid was formed (expected values 1.15 and 0.31, respectively). Estimation of the molecular weight gave a value of 17,000 which indicates a content of 73 mannose and 32 galactose residues.

Cocos nucifera L.

Unripe, gelatinous endosperm was extracted with dilute alkali, and the galactomannan was precipitated from the acidified extract with ethanol. The crude polysaccharide, purified via the copper complex, gave a galactomannan (1% of the wet endosperm) having $[\alpha]_D$ +27° (*c* 1.5, 0.1M sodium hydroxide) which did not change on repeated conversion into the copper complex. The mannose-to-galactose ratio was 2.57:1. The methylated polysaccharide had $[\alpha]_D$ +14° (*c* 1.5, chloroform), and on hydrolysis it gave 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 proportions 1:1.44:1.15.

On oxidation, the polysaccharide consumed 1.09 moles of periodate and gave 0.27 mole of formic acid (expected values 1.14 and 0.28, respectively). The molecular weight was 7,200, indicating a content of 32 mannose and 12 galactose residues.

Convolvulus tricolor L.

Ground, defatted seeds were extracted with boiling water, and the crude polysaccharide was precipitated with ethanol. The galactomannan, after purification via the copper complex, had $[\alpha]_D$ +44° (*c* 1.49, water) and a mannose-to-galactose ratio of 1.75:1.

The methylated polysaccharide had $[\alpha]_D$ +41° (*c* 1.67, chloroform), and on hydrolysis 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 proportions 1:1.15:1.14.

On oxidation, the polysaccharide consumed 1.09 moles of periodate, yielding 0.30 mole of formic acid (expected values 1.18 and 0.36, respectively). The molecular weight was 11,000, corresponding to a content of 43 mannose and 25 galactose residues.

Sophora japonica L.

The ground, defatted seeds were extracted with cold water, and the crude polysaccharide was precipitated with ethanol. The galactomannan was purified via the copper complex. Subsequent treatment of the powdered seeds with hot water and with dilute alkali at room temperature yielded highly viscous extracts from which galactomannans were precipitated with ethanol. These preparations were almost insoluble in water or alkali; they were not studied further.

The purified polysaccharide had $[\alpha]_D$ −9° (*c* 1.33, water) and a mannose-to-galactose ratio of 5.26:1. The methylated galactomannan had $[\alpha]_D$ −25° (*c* 7.8, benzene), and on hydrolysis gave 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 proportions 1:4.26:1.05. The polysaccharide consumed 1.12 moles of periodate and yielded 0.13 mole of formic acid (expected values 1.08 and 0.16, respectively). The molecular weight was 6,000, indicating a content of 31 mannose and 6 galactose residues.

DISCUSSION

The foregoing results indicate that the galactomannans have similar, basic structures. The molecules consist of (1→4)-linked D-mannose residues having branches each of which is a D-galactose residue attached by a (1→6)-link. These structures are similar to most of those published for leguminous galactomannans and to that of the galactomannan of unripe *Borassus* endosperm. The previously reported structure of the galactomannan from unripe endosperm of *Cocos nucifera*[7] was not confirmed. Terminal mannose residues and non-terminal galactose residues have not been found in any of the galactomannans studied. Tetra-*O*-methyl-D-mannose, tri-*O*-methyl-D-galactose, and di-*O*-methyl-D-galactose were absent from the hydrolysates of the methylated galactomannans. The proportions of the isolated methyl sugars are in close agreement with the mannose-to-galactose ratios of the polysaccharides. The specific optical rotations of the polysaccharides and of the methylated polysaccharides have values comparable to those of other galactomannans and indicate that the types of linkage are similar. Thus, most, or all, of the mannose residues in the main chain are β-D-linked, whereas most or all of the galactose residues are α-D-linked. The results of the periodate-oxidation experiments agree well with the results of the methylation studies, although the values found for periodate consumption and formic acid production are somewhat low in comparison with those expected for galactomannans having (1→4)-linked mannose residues and (1→6)-linked galactose side-groups. Incomplete oxidation of galactomannans and mannans has been reported[11–14] repeatedly and may be caused by cyclic acetal

formation[13]. Comparison of the estimated values for the molecular weights of the galactomannans shows that the *Arenga* polysaccharide has the largest, and the polysaccharide extracted from *Sophora* with cold water the smallest molecule. The latter polysaccharide is probably the low-molecular part of a family of similar galactomannans, most of which have higher molecular weights and can be extracted from *Sophora* seeds with hot water and with dilute alkali.

It is remarkable that, whereas galactomannans are predominant in the unripe endosperms of the palm seeds so far investigated, the cell walls of ripe palm-seeds consist chiefly of mannans containing at most a few percent of galactose residues[14]. Probably, most of the galactose groups are removed during maturation of the seeds, resulting in cell walls which are much less liable to swelling and dissolution in water. This process occurs concomitantly with the transition of the endosperm from the hydrated, gelatinous phase to the dehydrated, solid mature-state.

In some plant taxa belonging to other plant families, cell-wall mannans occur in the seeds as reserve polysaccharides (*e.g. Coffea*[2,15], *Diospyros*[2,16], *Melampyrum*[17], *Phacelia*[2], *Strychnos*[2,18], *Tamus*[2]). In no instance has the nature of the polysaccharides present in the immature seeds been investigated. It would be of interest to study the mannose-to-galactose ratio during the ripening of leguminous seeds, since there might be a gradual diminishing of the proportion of galactose residues as maturation occurs. In the *Leguminosae* and in other taxa having galactomannan-containing seeds (so far only *Annona*, *Convolvulus*, and *Ipomoea*[5]), this would not proceed to the mannan end-point as in the *Palmae*, but would be arrested at an earlier stage.

EXPERIMENTAL

Melting points are corrected. Specific optical rotations are equilibrium rotations. Evaporations were done at $<30°$. Paper chromatography was performed by the descending method on either Whatman No. 1 or 3MM papers, with (*A*) butyl alcohol–pyridine–water (6:4:3), (*B*) butyl alcohol–ethanol–water (40:11:19), (*C*) butanone–water (2:1). *p*-Anisidine phosphate[19] was used for the detection of sugars.

Preparation of galactomannans. — (*a*) Seeds of *Annona muricata* were obtained from Indonesia. The decorticated seeds were ground and then defatted in a Soxhlet apparatus with ethanol–benzene (1:1). The dried powder (90 g) was extracted with water (1 litre) for two days at room temperature, and the filtrate was discarded. Subsequently, the residue was thrice extracted with 0.2M sodium hydroxide (1 litre) and then thrice with 2M sodium hydroxide (1 litre), each treatment lasting 24 h at room temperature. The combined extracts were acidified with acetic acid, and the precipitates were removed by centrifugation. The clear supernatant liquids were treated with 2 vol. of ethanol, and the precipitates were collected, washed successively with 70% and 96% ethanol, and ether, and dried. The 0.2M and the 2M sodium hydroxide extracts yielded 1.66 g and 9.07 g of crude galactomannan, respectively.

Samples of the products were hydrolyzed conventionally with 0.5M sulphuric acid and examined by paper chromatography (solvent *A*); apart from mannose and galactose, they contained xylose, glucose, some uronic acid, and a probable trace of rhamnose. The combined preparations of crude galactomannan were dissolved in 0.2M sodium hydroxide (300 ml), the solution was neutralized to pH 5.5, and heated *Myrothecium* cellulase (10 ml) was added. The solution was incubated at 37° with toluene (1 ml) added to prevent microbial contamination. After 1 week, the mixture was centrifuged, the sediment was washed successively with water, 50% and 96% ethanol, and ether, and then dried to give "water-insoluble" galactomannan (1.66 g). The clear liquid was treated with 2 vol. of ethanol, and the precipitated galactomannan was washed with 70% ethanol and dehydrated by solvent exchange. The yield of dried product was 1.94 g.

(*b*) Unripe endosperms of *Arenga saccharifera* (~170 g, preserved in sugar syrup) were obtained commercially and homogenized. The viscous mass was stirred for 24 h with 0.1M sodium hydroxide (1 litre) at room temperature and then centrifuged. The clear, supernatant liquid was acidified with acetic acid, and the galactomannan was precipitated with an equal volume of ethanol. After washing of the precipitate with 70% ethanol, the polysaccharide was dried by solvent exchange and then *in vacuo* (yield 8.48 g). The galactomannan (1 g) was dissolved in water (200 ml), and the solution was filtered through diatomaceous earth. Fehling's solution (2.5 ml diluted to 25 ml) was added, and the precipitated copper complex was separated and washed with water. The precipitate was suspended in water (100 ml) and 2M hydrochloric acid (1 ml) was added to destroy the copper complex. The regenerated galactomannan was precipitated with ethanol, washed, and dried (yield 0.78 g).

(*c*) Unripe endosperms of *Cocos nucifera* (~200 g) were obtained from Surinam. After homogenization, the material was twice extracted with boiling water (500 ml) and then with 0.2M potassium hydroxide at room temperature for 24 h. The combined filtrates were neutralized with acetic acid and treated with 2 vol. of ethanol. The precipitate was washed with 70% ethanol, and dried by solvent exchange and then *in vacuo* (yield 2.55 g). The crude galactomannan was purified *via* the copper complex as in (*b*).

(*d*) Seeds of *Convolvulus tricolor* (50 g) were harvested from plants grown in the Botanical Garden of this Laboratory. They were ground and defatted with ethanol–benzene (1:1) in a Soxhlet apparatus. The powder was extracted with boiling water (1 litre), and the filtrate was treated with 2 vol. of ethanol. The crude galactomannan was purified as described in (*b*) (yield 4.50 g).

(*e*) *Sophora japonica* seeds (100 g) were ground and defatted. The powder was extracted with cold water (2.5 l), and the filtrate was treated with 2 vol. of ethanol (yield 4.40 g) .The precipitate was purified as described in (*b*) (yield 0.98 g).

Analysis of the galactomannans. — The galactomannans were hydrolyzed, and the amounts of the liberated sugars were determined by using a modified hypoiodite method[20]. The molecular weights of the galactomannans were determined by the hypoiodite method[21], and the periodate consumption and formic acid production

were determined by the method of Chanda *et al.*[22]. The data in Table I were extrapolated to zero time to obtain net values.

TABLE I

PERIODATE OXIDATION OF GALACTOMANNANS

Periodate consumption (moles/mole)

Time (days)	*1*	*2*	*3*	*4*	*6*	*7*	*10*	*11*	*14*	*17*	*18*	*21*	*25*
Annona	0.93		1.05		1.16		1.18		1.23	1.30		1.35	
Arenga	0.99		1.08		1.14		1.12		1.15	1.22		1.26	
Cocos	0.995	1.10		1.12		1.27		1.23			1.36		1.45
Convolvulus	1.15					1.31	1.46		1.53			1.63	
Sophora	1.15		1.13			1.36			1.52			1.64	

Formic acid production (mole/mole)

Time (days)	*1*	*3*	*6*	*7*	*10*	*14*	*17*	*18*	*21*	*36*
Annona	0.18	0.22	0.23		0.29	0.31	0.34		0.37	
Arenga	0.24	0.28	0.29		0.31	0.33	0.34		0.37	
Cocos					0.30			0.33		0.38
Convolvulus	0.27	0.32		0.36	0.39	0.42			0.49	
Sophora	0.14	0.18		0.22	0.26	0.31			0.41	

Methylation of the galactomannans. — The polysaccharides were methylated in the usual way by the Haworth procedure, followed by treatments by the Purdie method until the i.r. hydroxyl-absorption was negligible. The methylated polysaccharide was dissolved in benzene (5 ml/g), an equal volume of light petroleum was added, and the solution was centrifuged to remove a small amount of impurity. The methylated polysaccharide was precipitated by adding 10 vol. of light petroleum (b.p. 40–60°). The precipitate was washed with light petroleum and finally dried.

Hydrolysis of the methylated polysaccharides. — The methylated galactomannans were hydrolyzed as described previously[23], and the methyl sugars were separated by paper chromatography (solvent *C*). In each case, the following fractions were obtained.

Fraction *A* had the R_F of 2,3,4,6-tetra-*O*-methyl-D-galactose (solvents *B* and *C*) and was identified by its specific optical rotation {$[\alpha]_D$ +100° (water) (lit.[25] +117°)} and by the melting point (195–196°) of its aniline derivative (lit.[26] 197°). Fraction *B* was chromatographically indistinguishable from 2,3,6-tri-*O*-methyl-D-mannose (solvents *B* and *C*) and, on demethylation[24], yielded mannose. It was identified by its specific optical rotation {$[\alpha]_D$ −8° (water); lit. [27,28] +6°, −10°}, and by the melting point (121–122°, lit.[29] 131°) and specific optical rotation {$[\alpha]_D$ −140→−40° (methanol); lit.[29] −155→−39°} of its aniline derivative. Fraction *C* was identical

with 2,3-di-*O*-methyl-D-mannose on paper chromatograms (solvents *B* and *C*) and, on demethylation, yielded mannose. It was identified by its specific optical rotation $\{[\alpha]_D -16°$ (water); lit.[30] $-16°\}$, its M_G value (0.25) on paper electrophoresis in 0.12M borate solution at pH 10, and by the melting point (110°; lit.[31] 109–110°) of the lactone following oxidation with bromine.

The parts of the Whatman No. 3 MM sheets in between the zones of the fractions *A*, *B*, and *C* contained traces of some unidentified methyl sugars, together accounting for ~2% of the methylated polysaccharide. In addition, the material moving between the origin line and fraction *C*, which probably contained products of either undermethylation or demethylation, amounted to ~3% of the methylated polysaccharide. These products were not identified and were considered to have no structural significance.

ACKNOWLEDGMENTS

Expert technical assistance by Miss D. C. Reuvers is gratefully acknowledged. A gift of 2,3-di-*O*-methyl-D-mannono-1,4-lactone by Professor J. K. N. Jones is gratefully acknowledged. The infrared spectra were determined by Dr. A. van Veen (Delft). Many thanks are due to Mr. T. Babock for a generous gift of *Annona muricata* seeds, to Mr. and Mrs. W. Dijkland- Salomon (Paramaribo, Surinam) for providing unripe coconuts, and to Mr. L. W. J. C. Caron for growing *Convolvulus tricolor* and harvesting the seeds.

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