carried out on each 10-ml. aliquot, after quenching by addition to a large volume of water plus sodium acetate, by dilution to the proper concentration range and application of the colorimetric method of Lowry and Lopez.[10] A Beckman spectrophotometer was used throughout the colorimetry, with the scheme calibrated at a wave length of 700 mμ.

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[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, UNIVERSITY OF BRISTOL]

Mannose-Containing Polysaccharides. I. The Galactomannans of Lucerne and Clover Seeds[1]

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The galactomannans of lucerne and clover seed are found to be similarly constituted. Both polysaccharides are highly branched and contain D-galactopyranose end-groups combined with chains of 1,4- or 1,6-linked D-mannose residues, which are probably in the pyranose form.

Galactomannans are common constituents of the ungerminated seeds of leguminous plants, amounting in some cases to more than 40% of the total seed. They occur as mucilages in the endosperms of the seeds, from which they may be isolated by extraction with water. It is known that when the seeds germinate both the mucilage and the endosperm disappear, hence it is argued that the galactomannan is a food reserve polysaccharide.[3] An extensive investigation of the seeds of more than 160 species of legumes by Wise and Appling[4] and Anderson[5] has revealed that many of them contain galactomannans. The galactomannan of gum gatto (from *Ceratonia siliqua* seeds), commonly known as locust bean gum, is widely used in the textile, paper, food and other industries, and the purpose of examining so many other seeds was to find substitutes. The amounts of mucilaginous polysaccharide in the various seeds were found to vary greatly from one species to another; the highest yields of mucilage were obtained from carob seed (38% of the weight of the seed) and guar seed (35%). The composition of the galactomannans is also variable, although all those examined by these workers contained less galactose than mannose. For example, the molecular ratio of these sugars was 16:81 in *Sophora japonica*, whilst in guar seed galactomannan ("guaran"[6]) the figures were 38:58.5. Apparently, galactomannans derived from one species only are of variable composition, since the proportion of D-galactose to D-mannose in gum gatto has been variously reported as 16:84 by Hirst and Jones,[7] 20:80 by Smith[8] and Wise and Appling,[4] 27:73 by Spada[9] and 18:82 by Lew and Gortner.[10]

Two other galactomannans are known in which the ratios of the component hexoses are outside the range of those given above. Thus, the galactomannan of Fenugreek seed (*Trigonella foenum-graecum*) was deduced[11] from the optical rotation of the hydrolysis products, to contain D-galactose and D-mannose in the proportions 48:52, and Hirst, Jones and Walder[12] obtained from a sample of lucerne seed (*Medicago sativa*) a galactomannan ($[\alpha]_D$ + 89° in water) which is unique in that it contains more D-galactose than D-mannose, the ratio being 2:1. The latter polysaccharide was extracted from the milled seed with hot 10% sodium hydroxide solution. This paper describes the isolation in similar yield (*ca.* 5.5%) of a galactomannan ($[\alpha]_D$ + 118 ± 11°) from the seeds, *var. Provence*, by extraction with hot water only. (Further extraction of the seeds with alkali gave only another 1% of material, which contained some xylan.) Both this product and that of Hirst, Jones and Walder were purified by copper complex formation, followed by regeneration of the galactomannan with cold dilute mineral acid. The new preparation contains D-galactose and D-mannose in the proportions 4:5; the two polysaccharides are, therefore, differently constituted (see below). By the same method, a galactomannan ($[\alpha]_D$ + 78 ± 11°), containing D-galactose and D-mannose in the proportions 7:9, has been isolated from clover seed (*Trifolium pratense*).

Evidence concerning the structure of several galactomannans had earlier been obtained by studies of the methylated polysaccharides, and also by periodate oxidation techniques. From the fission products of methylated gum gatto, Hirst and Jones[7] identified 2,3,4,6-tetramethyl-D-galactose (1 part), 2,3,6-trimethyl-D-mannose (4 parts) and 2,3-dimethyl-D-mannose (1 part), whilst Smith[8] obtained precisely the same compounds, but in the proportions 1:2–3:1, respectively. Evidently the galactose in the gum is present in the pyranose form, occupying a terminal position, and is attached to a skeleton of mannose residues by 1,4- and/or 1,6-linkages Guaran[4-6] appears to have a similar

(1) A summary of this paper was read in New York at the XII International Congress of Chemistry on September 12, 1951.

(2) Department of Chemistry, The University, Bristol, England.

(3) Schulze, *Landw. Jahrb.*, **23**, 1 (1894); *Ber. deut. bot. Ges.*, **14**, 66 (1896).

(4) L. E. Wise and J. W. Appling, *Ind. Eng. Chem., Anal. Ed.*, **16**, 28 (1944).

(5) E. Anderson, *Ind. Eng. Chem.*, **41**, 2887 (1949).

(6) E. Heyne and R. L. Whistler, THIS JOURNAL, **70**, 2249 (1948).

(7) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1278 (1948).

(8) F. Smith, THIS JOURNAL, **70**, 3249 (1948).

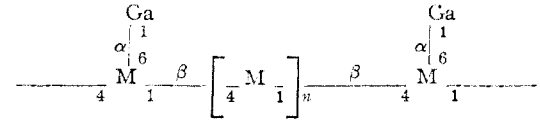
(9) A. Spada, *Atti. soc. natc. mat. Modena*, **70**, 20 (1939).

(10) B. Lew and R. A. Gortner, *Arch. Biochem.*, **1**, 325 (1943).

(11) K. M. Daoud, *Biochem. J.*, **26**, 255 (1932).

(12) E. L. Hirst, J. K. N. Jones and W. O. Walder, *J. Chem. Soc.*, 1143 (1947).

structure, since studies on the methyl derivative[13-15] have shown that again all the galactose exists as end-group, an equivalent number of mannose units are linked through positions 1, 4 and 6, while the remaining mannose units are linked through positions 1 and 4 only. These results are consistent with several possible structures for guaran and gum gatto but the recent isolation,[16-17] from the partial hydrolysis products of guaran, of the two disaccharides 4-(β-D-mannopyranosyl)-β-D-mannopyranose and 6-(α-D-galactopyranosyl)-β-D-mannopyranose is in agreement with a structure in which the terminal galactopyranose units are joined by 1,6-α-glycosidic links to a chain of mannopyranose units, united by links of the 1,4-β type:



(Ga = D-galactopyranose; M = D-mannopyranose)

Fig. 1.

This structure is in agreement with the findings of Palmer and Ballantyne,[18] who investigated guaran using X-ray diffraction methods.

The galactomannans isolated from lucerne and clover seed show a structural resemblance one to another and also to guaran and gum gatto, since the methyl derivatives of both gave, on hydrolysis, 2,3,4,6-tetramethyl-D-galactose, 2,3,6-trimethyl-D-mannose and 2,3-dimethyl-D-mannose. These methyl sugars were identified by separation on a column of hydrocellulose by partition chromatography,[19] followed by conversion into characteristic crystalline derivatives. Quantitative analysis of the methyl sugar mixtures by separation on paper chromatograms and subsequent estimation by oxidation with alkaline hypoiodite[20] gave the molecular proportions of tetra-, tri- and dimethyl sugars as 4:1:4 for the lucerne and 7:2:7 for the clover. Hence, all the galactose was in pyranose form as end-group, with an equivalent number of branching points formed by mannose units linked through positions 1, 4 and 6 with the remaining mannose units linked through positions 1 and 4. Since both polysaccharides contained over 40% of galactose as end-groups, the degree of branching was very great.

The galactomannan obtained from lucerne seed by Hirst, Jones and Walder[12] differs both in composition and structure from the four galactomannans described above, namely, guaran, gum gatto, lucerne and clover. The results of these authors[12] indicated that half the galactose formed all the end-group, the remaining galactose being attached through positions 1 and 3, and that the branching points are at C_2 or C_6 on the mannose residues, whereas in the other galactomannans the branching points are on mannose residues at C_4 or C_6. The reason for the difference between the polysaccharide examined by these authors and the lucerne polysaccharide described in this paper is not understood.

The oxidation of gum gatto[7] and guaran[14] with the periodate ion has been found to give yields of formic acid in close agreement with those expected from the structures suggested by methylation studies. Similarly, the lucerne and clover galactomannans, on oxidation with potassium metaperiodate by the method of Brown, Halsall, Hirst and Jones,[21] yield results in excellent agreement with those obtained by the methylation procedure. After the theoretical yields of formic acid had been liberated, the oxidized polysaccharides were isolated and hydrolyzed. An examination of the hydrolysis products on the paper chromatogram revealed in each case that all the galactose had been oxidized, but that approximately 10% of the original mannose remained unoxidized. This result was unexpected, since the methylation evidence indicated that all the mannose residues possess free hydroxyl groups on adjacent carbon atoms (C_2 and C_3). However, prolonged oxidation of the polysaccharides with an excess of sodium metaperiodate at *p*H 7 resulted in the destruction of both hexoses, thus confirming the methylation results. The considerable difference in the rates of oxidation of the galactose and the mannose is probably attributable to a steric effect, resulting from the highly ramified structure of the galactomannans, in which mannose units form all the branching points. On the other hand, the galactose is more accessible to the periodate reagent, since it is present as terminal units only. Similar findings have been reported for guaran[14] and amylopectin.[22]

Source of galactomannan	% end-group By periodate oxidation	% end-group By methylation
Lucerne seed	43.7	44.2
Clover seed	43.2	43.2

The function of the galactomannans in nature is obscure. The general structure (Fig. 1), in which the galactose residues are attached, possibly at random, to a chain of mannose residues, could be regarded as a method of storing galactose in the seed. If the galactose is metabolized during the life of the seed, this may well account for some, at least, of the variability in the composition of galactomannans. Alternatively, it must be borne in mind that they may consist of a mixture of polysaccharides, such as a mannan and a galactomannan, in varying proportions.

Experimental

Micro-analyses were by Mr. W. Eno, Bristol. Melting points are uncorrected.

Extraction of the Galactomannan from Lucerne Seed (*var. Provence*).—The milled seeds (100 g.) were extracted with water (2 l.) at 70–80° for 2 hours with occasional stirring. The cooled mixture was then centrifuged, and the

(13) J. W. Swanson, THIS JOURNAL, **71**, 1510 (1949).
(14) Z. F. Ahmed and R. L. Whistler, *ibid.*, **72**, 2524 (1950).
(15) C. F. Rañque and F. Smith, *ibid.*, **72**, 4634 (1950).
(16) R. L. Whistler and J. Z. Stein, *ibid.*, **73**, 4187 (1951).
(17) R. L. Whistler and D. F. Durso, *ibid.*, **73**, 4189 (1951).
(18) K. J. Palmer and M. Ballantyne, *ibid.*, **72**, 739 (1950).
(19) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).
(20) S. K. Chanda, E. L. Hirst, J. K. N. Jones and E. G. V Percival, *ibid.*, 1289 (1950); J. K. N. Jones, *ibid.*, 3292 (1950).
(21) F. Brown, T. G. Halsall, E. L. Hirst and J. K. N. Jones, *ibid.*, 27 (1948).
(22) G. C. Gibbons and R. A. Boissonas, *Helv. Chim. Acta*, **33**, 1477 (1950).

solid (A) collected. Fehling solution (200 ml.) was added to the supernatant liquor, and the resultant insoluble copper complex was separated by filtration under gravity on a coarse sintered-glass filter. The complex was washed with water, and the copper removed by the addition of *N* hydrochloric acid (*ca.* 100 ml.) with vigorous stirring to its suspension in ice-cold water (1 l.) until solution was complete. The resultant viscous solution was filtered and the filtrate poured into alcohol (2 l.) with the formation of a white precipitate, which was collected on the filter and washed with alcohol until free from copper; it was further purified by solution in water and reprecipitation with alcohol. The product was washed first with absolute alcohol, then ether, and dried *in vacuo*, to give a white fibrous polysaccharide; yield 5.5 g. Found: N, < 0.3; sulfated ash, 1.3%; OMe, nil; titration with 0.1 *N* sodium hydroxide, negligible; $[\alpha]^{19}D$ +118 ± 11° in water (*c* 0.4; observations made on a centrifuged solution, and concentration determined by evaporation to dryness and weighing of residue). Only 62% of this desiccated material was soluble in water and it was also incompletely soluble in 2 *N* sodium hydroxide. The aqueous solution gave no coloration with iodine. A qualitative examination of the hydrolysis products on the paper chromatogram[23,24] indicated the presence of galactose and mannose only.

The residual seed material from (A) was further extracted with 6% sodium hydroxide solution (1.5 l.) at 70–80°. The insoluble material was removed on the centrifuge, and Fehling solution (100 ml.) added to the supernatant alkaline liquor. Since the copper complex so produced was not completely soluble in dilute hydrochloric acid, the copper was removed by grinding it with acidified alcohol, the product washed with alcohol and ether, and dried *in vacuo* (yield 1 g.). Hydrolysis was found to give galactose, mannose and xylose, the latter sugar forming about half the total. This material was not further examined.

Analysis of the Hydrolysis Products of the Purified Galactomannan.—A dispersion of the galactomannan (0.45 g.) in *N* sulfuric acid (20 ml.) was heated at 95° for 15 hours. The reaction mixture was neutralized with barium carbonate, filtered and evaporated under reduced pressure to a sirup. The sugar mixture was separated by partition chromatography on a column of hydrocellulose[19] (22 × 2 cm.), using *n*-butanol half saturated with water as the mobile phase, to give D-galactose [m.p. and mixed m.p. 165°; $[\alpha]^{18}D$ +79°, equil. value in water (*c* 0.8)] and D-mannose [m.p. and mixed m.p. 132°; $[\alpha]^{18}D$ +14.5°, equil. value in water (*c* 1.2)]; both sugars were crystallized from methyl alcohol.

The galactomannan (61.6 mg.) was hydrolyzed in a sealed tube with *N* sulfuric acid (2 ml.) for 15 hours at 100°. The tube was opened and the contents washed into a small beaker containing ribose (27.4 mg.); the solution was then neutralized with barium carbonate, filtered and concentrated to a thin sirup. The sugars were separated on a paper chromatogram using a mixture of butanol, ethanol and water (40:11:19) as the mobile phase. After separation, the chromatogram paper was dried in a current of air, and the sugars determined by the method of Hirst and Jones.[25] (Found: galactose, 1.79, 2.17; mannose, 2.27, 2.67; ribose, 1.66, 2.00 mg.) Assuming complete recovery of the ribose, these figures correspond to the production, from the polysaccharide, of galactose, 26.6, 26.8, and mannose, 33.8, 32.9 mg. (both calcd. as $C_6H_{10}O_5$). The corresponding galactose:mannose ratios are 1.00:1.27 and 1.00:1.23.

Periodate Oxidation[21] of the Purified Galactomannan.—The galactomannan (280.0 mg.) was dispersed in water (25 ml.) in a steamed-out stoppered bottle, and potassium chloride (3 g.) and sodium metaperiodate solution (0.36 *N*, 25 ml.) added. The reaction mixture was shaken, and as the oxidation proceeded, the polysaccharide passed into solution. At intervals, portions (5 ml.) of the solution were withdrawn, ethylene glycol (2 ml.) was added and, the formic acid content of the solution determined by titration with 0.01 *N* sodium hydroxide. [Found (quoted as polysaccharide yielding 1 g.-mole formic acid): 382 g. (96 hr.), 373 g. (147 hr.), 371 g. (240 and 342 hr.).] The latter value corresponds to the presence of 43.7% of hexose, containing three contiguous hydroxyl groups. Excess ethylene glycol was added after 342 hours, the solution dialyzed, and evaporated to dryness under reduced pressure. Hydrolysis of a portion of the oxidized material with *N* sulfuric acid yielded some mannose, but no galactose. A quantitative determination showed that the oxidized polysaccharide contained 9% of mannose (calcd. as $C_6H_{10}O_5$).

A further sample of the galactomannan (0.3 g.) was suspended in water (20 ml.) and sodium metaperiodate solution (0.5 *N*, 30 ml.) added. The solution was maintained at *p*H 7 with dilute sodium hydroxide, and the reaction allowed to proceed in the dark at room temperature for 10 days. The oxidized polysaccharide was recovered as above, and a portion (*ca.* 10 mg.), when hydrolyzed with *N* sulfuric acid (2 ml.) and examined on the paper chromatogram, was found to contain no galactose and only a trace of mannose.

Methylation.—The galactomannan (6 g.) was dissolved in sodium hydroxide solution (300 ml., 40%) and methyl sulfate (300 ml.) added dropwise with vigorous stirring. After being stirred overnight, the mixture was neutralized with glacial acetic acid and dialyzed against tap water for 24 hours. The solution was then concentrated under reduced pressure to *ca.* 100 ml. and the methylation procedure repeated by the addition of sodium hydroxide (50 g.) and methyl sulfate (100 ml.), followed by more sodium hydroxide (100 g.) and methyl sulfate (200 ml.). The mixture was then neutralized, dialyzed, concentrated and remethylated as described above. The final reaction mixture, after dialysis, was extracted with chloroform (3 portions of 700 ml.); removal of the chloroform under reduced pressure gave the methylated galactomannan (5.4 g., OMe, 44.6%) as a crisp white solid.

The methylated material was fractionated by boiling with mixtures of petroleum ether (b.p. 40–60°) and chloroform (200 ml. for 2 hr. in each case), giving mainly one fraction (4.7 g.), soluble in 80% petroleum ether, but insoluble in 90%. This fraction had OMe, 45.0% and $[\alpha]^{19}D$ +66 ± 3° in chloroform (*c* 1.2).

Fission of the Methylated Polysaccharide.—The methylated material (1.45 g.) was dissolved in methanolic hydrogen chloride (2% by weight of hydrogen chloride, 30 ml.) and the solution boiled under reflux for 16 hours. The solution was then neutralized by the addition of a slurry of silver carbonate in methanol, filtered and concentrated under reduced pressure to a sirup. This sirupy mixture of glycosides was hydrolyzed in *N* hydrochloric acid (50 ml.) at 95°. The optical rotation of the solution was constant after about 12 hours heating. After 14 hours, the solution was neutralized with silver carbonate, filtered, treated with hydrogen sulfide, again filtered and concentrated under reduced pressure to give a nearly colorless sirup (1.42 g.) of reducing methyl sugars.

Analysis of the Mixture of Methyl Sugars.—Examination of the mixture of methyl sugars on the paper chromatogram using as the mobile phase a mixture of benzene, ethanol and water (167:47:15; top layer, clarified by the addition of a little ethanol), indicated the presence of tetramethyl (R_G 0.95),[26] trimethyl (R_G 0.63) and dimethyl (R_G 0.26) hexoses.

(This benzene-containing solvent has proved very efficacious for the separation of methyl sugars on the paper chromatogram; it gives a wide spatial separation of tetra-, tri- and dimethyl hexoses in 5 to 6 hours on Whatman No. 1 filter paper at 20°. It was also found of utility for the separation of methyl sugars on cellulose columns.)

A portion of the above mixture of methyl sugars was analyzed by separation on the paper chromatogram, followed by a quantitative determination of the components by oxidation for 15 hours with alkaline hypoiodite.[20] (Found: in duplicate experiments, expressed as ml. of 0.01 *N* iodine consumed —"tetra," 3.36, 4.34; "tri," 0.86, 1.18; "di," 3.34, 4.38). These results correspond to an average tetra:tri:di ratio of 4.00:1.05:4.02.

Identification of the Methyl Sugars.—A portion of the methyl sugar mixture (0.73 g.) was separated on a column of hydrocellulose (22 × 2 cm.) using *n*-butanol:petroleum ether (b.p. 80–100°) (40:60 v./v.) as the mobile phase.[19] As a result of a qualitative examination of small portions of the effluent on the chromatogram, it was divided into three parts, which, on evaporation, gave:

(23) S. M. Partridge, *Nature*, **158**, 270 (1946); *Biochem. J.*, **42**, 238 (1948).

(24) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

(25) E. L. Hirst and J. K. N. Jones, *ibid.*, 1659 (1949).

(26) Rate of movement relative to tetramethylglucopyranose, see E. L. Hirst, L. Hough and J. K. N. Jones, *ibid.*, 928 (1949).

Fraction 1, (0.29 g.), which sirup contained 2,3,4,6-tetramethyl-D-galactose (R_G 0.95), had $[\alpha]^{18}D$ +110° in water (*c* 2.5).

Anal. Calcd. for $C_{10}H_{20}O_6$: OMe, 52.5. Found: OMe, 50.8. The sirup (0.22 g.) when heated under reflux with aniline (0.10 g.) in ethyl alcohol (5 ml.) gave crystalline 2,3,4,6-tetramethyl-D-galactose anilide (0.26 g.), which had m.p. and mixed m.p. 190° and $[\alpha]^{19}D$ +40°, const. value in acetone (*c* 1.2), after recrystallization from ethyl alcohol.

Fraction 2, (0.09 g.) contained a trimethyl sugar (R_G 0.63) and a small amount of a dimethyl sugar (R_G 0.26).

Anal. Calcd. for trimethyl hexose: OMe, 41.8. Found: OMe, 39.3. The two sugars were separated on a large sheet-paper chromatogram, using the benzene/ethanol/water solvent, giving a sirupy trimethyl sugar (0.045 g. Found: OMe, 41.2) with $[\alpha]^{18}D$ −2° in water (*c* 1.1), and a small amount of dimethyl sugar which was combined with Fraction 3. The trimethyl sugar (*ca.* 5 mg.), after demethylation[24] (with 48% hydrobromic acid (1 ml.) at 100° for 12 min.) and examination on the paper chromatogram gave a small amount of mannose, but no galactose. The sugar was oxidized with bromine water in the usual way, and the product, isolated as the sirupy lactone, did not crystallize; when boiled with alcoholic phenylhydrazine it gave, in 60% yield, 2,3,6-trimethyl-D-mannonic acid phenylhydrazide,[7] m.p. 130° (raised to 131° when mixed with an authentic specimen, m.p. 131°) after recrystallization from ethyl alcohol, and $[\alpha]^{19}D$ −20° in water (*c* 0.9).

Anal. Calcd. for $C_{15}H_{24}O_6N_2$: N, 8.5; OMe, 28.4. Found: N, 8.3; OMe, 28.3.

Fraction 3, (0.25 g.) contained a dimethyl hexose only.

Anal. Calcd. for $C_8H_{16}O_6$: OMe, 29.8. Found: OMe, 28.7. It gave, after oxidation with bromine water, crystalline 2,3-dimethyl-γ-D-mannonolactone, m.p. and mixed m.p. 108° after recrystallization from acetone–ether.

Anal. Calcd. for $C_8H_{14}O_6$: OMe, 30.1. Found: OMe, 28.9. When heated with phenylhydrazine in ethyl alcoholic solution, the lactone gave 2,3-dimethyl-D-mannonic acid phenylhydrazide[5] which, when crystallized from ethyl alcohol, had m.p. 168° and $[\alpha]^{19}D$ −24° in water (*c* 0.8).

Anal. Calcd. for $C_{14}H_{22}O_6N_2$: N, 8.9. Found: N, 8.7.

Extraction of the Galactomannan from Clover Seed (*var. Broad Red*).—The polysaccharide was extracted from the milled seeds (100 g.) with water, and purified as the copper complex, as described above, to give a white fibrous material, yield 3.5 g. [Found: N, 0.9; sulfated ash, 2.2%; OMe, nil; titration with 0.1 *N* sodium hydroxide, negligible; $[\alpha]^{18}D$ +78 ± 11° in water (*c* 0.4; determined as with the lucerne galactomannan)]. This material was only 63% soluble in water, and incompletely soluble in 2 *N* sodium hydroxide.

Hydrolysis of the Galactomannan.—Hydrolysis of a small portion (*ca.* 10 mg.) of the material with *N* sulfuric acid, followed by neutralization with barium carbonate and examination of a concentrate on the chromatogram showed the presence of galactose and mannose only. A larger portion (0.5 g.) was similarly hydrolyzed, and the products separated on a cellulose column, giving D-galactose [m.p. and mixed m.p. 165°, $[\alpha]^{18}D$ +79.6°, equil. value in water (*c* 1.2)] and D-mannose [m.p. and mixed m.p. 132°, $[\alpha]^{18}D$ +14.1°, equil. value in water (*c* 1.4)]; both sugars were crystallized from methyl alcohol.

The galactomannan (46.3 mg.) was hydrolyzed as above, ribose (19.7 mg.) was added to the hydrolysate, which was neutralized with barium carbonate, and the mixture of sugars, after separation, estimated by the periodate oxidation method.[25] (Found: galactose, 2.03, 2.18; mannose, 2.61, 2.73; ribose, 1.89, 1.99 mg.). Assuming complete recovery of the ribose, these figures correspond to the production from the polysaccharide of galactose, 19.0, 19.4 and mannose, 24.5, 24.6 mg. (both calcd. as $C_6H_{10}O_5$). The corresponding galactose:mannose ratios are 1.00:1.29 and 1.00:1.27.

Periodate Oxidation.[21]—The galactomannan (261.0 mg.) was oxidized with potassium metaperiodate as described above; the yield of formic acid was determined by titration with 0.01 *N* sodium hydroxide solution. [Found (quoted as g. of polysaccharide yielding 1 g.-mole of formic acid): 379 (96 hr.), 376 (147 hr.), 375 (240 hr.), 374 (342 hr.)]. The latter value corresponds to 43.2% of the hexose residues possessing three contiguous hydroxyl groups.

After hydrolysis of the oxidized material (recovered as described above), it was found to contain 10.5% of mannose (calcd. as $C_6H_{10}O_5$).

The polysaccharide, after oxidation for 10 days in the dark at *p*H 7 with an excess of sodium metaperiodate, yielded neither galactose nor mannose on hydrolysis with *N* sulfuric acid.

Methylation and Hydrolysis.—The galactomannan (4 g.) when methylated in the manner described above, gave the methyl derivative (3.3 g.; OMe, 43.7%). Extraction of this material (2.5 g.) with mixtures of chloroform and petroleum ether (b.p. 40–60°) gave mainly a fraction (2.3 g.) with OMe, 43.8%, and $[\alpha]^{19}D$ +76 ± 2° in chloroform (*c* 1.3). Methanolysis of the methylated galactomannan (1.50 g.) in the usual way, followed by hydrolysis of the glycosides with *N* hydrochloric acid, gave a mixture of reducing methyl sugars (1.46 g.).

Analysis of the Mixture of Methyl Sugars.—When examined on the paper chromatogram, using the benzene/ethanol/water solvent, the mixture appeared very similar to that obtained from the methylated lucerne galactomannan. The relative amounts of tetra- (R_G 0.95), tri- (R_G 0.63) and dimethyl (R_G 0.26) sugars were determined by the alkaline hypoiodite method.[20] [Found (results quoted as ml. of 0.01 *N* iodine consumed): "tetra," 3.25, 3.94; "tri," 1.02, 1.12; "di," 3.30, 4.05]. These values correspond to an average tetra:tri:di molecular ratio of 3.36:1.00:3.43. A portion (1.07 g.) of the mixture of methyl sugars was separated into its components by partition chromatography on a hydrocellulose column[19] (22 × 2 cm.), using the benzene/ethanol/water solvent. Three fractions were obtained:

Fraction 1, (0.45 g.), $[\alpha]^{19}D$ +112° in water (*c* 5.6) contained 2,3,4,6-tetramethyl-D-galactose.

Anal. Calcd. for tetramethyl hexose: OMe, 52.5. Found: OMe, 50.6. The sirup (0.30 g.) when boiled with aniline (0.11 g.) in absolute ethanol, gave 2,3,4,6-tetramethyl-D-galactose anilide (0.34 g.), m.p. and mixed m.p. 189–190°, $[\alpha]^{18}D$ +39°, const. value in acetone (*c* 0.9).

Fraction 2 (0.12 g.) had $[\alpha]^{18}D$ −4° in water (*c* 1.2).

Anal. Calcd. for trimethyl hexose: OMe, 41.8. Found: OMe, 40.7. After oxidation with bromine water, it gave crystalline 2,3,6-trimethyl-γ-D-mannonolactone, m.p. and mixed m.p. 81° after crystallization from acetone–ether–petroleum ether.

Anal. Calcd. for $C_9H_{16}O_6$: OMe, 42.2. Found: OMe, 41.5. The lactone, when heated with phenylhydrazine in alcohol, gave 2,3,6-trimethyl-D-mannonic acid phenylhydrazide,[7] m.p. and mixed m.p. 131–132°.

Anal. Calcd. for $C_{15}H_{24}O_6N_2$: N, 8.5. Found: N, 8.2.

Fraction 3 (0.43 g.) had $[\alpha]^{19}D$ −16° in water (*c* 5.0).

Anal. Calcd. for dimethyl hexose: OMe, 29.8. Found: OMe, 29.8. After oxidation with bromine water, the sugar gave 2,3-dimethyl-γ-D-mannonolactone, m.p. and mixed m.p. 109°.

Anal. Calcd. for $C_8H_{14}O_6$: OMe, 30.1. Found: OMe, 28.5. The lactone, when boiled with alcoholic phenylhydrazine, gave 2,3-dimethyl-D-mannonic acid phenylhydrazide,[7] m.p. 167° and $[\alpha]^{18}D$ −23° in water (*c* 0.7).

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