Anal. Calcd. for $C_{26}H_{36}O_7Br_2$: C, 50.34; H, 5.85; O, 18.05. Found: C, 50.12; H, 5.62; O, 18.39.

12β-Hydroxyprednisolone 11β,12β-Acetonide 21-Acetate (Xa). (A) From the Dibromo Compound IXg.—To 2 ml. of collidine was added 250 mg. of IXg and the resulting solution was allowed to reflux for 1.25 hours under an atmosphere of nitrogen. At the end of this time the total mixture was taken up in 50 ml. of ethyl acetate and the solution was then washed several times with 2% hydrochloric acid. After washing with aqueous sodium bicarbonate solution followed by water, the solvent was dried and evaporated to leave 200 mg. of gum. Following adsorption on 4 g. of silica gel and elution with benzene–acetone (19:1) there was obtained 75 mg. of crystals, m.p. 238–250°. Repeated recrystallization from acetone–ether then provided 25 mg. of Xa, m.p. 273–276°, identical in all respects with that obtained below in B.

(B) By Selenium Dioxide Oxidation of IXe.—Three hundred mg. of IXe was allowed to reflux for 48 hours in 20 ml. of *t*-butyl alcohol containing 0.05 ml. of pyridine and 0.2 g. of selenium dioxide. At the end of this time an additional 0.2 g. of selenium dioxide was added and the heating was continued for another 48 hours. Following evaporation, the residue was triturated with water and then dissolved in benzene. After being chromatographed twice over silica gel, elution with benzene–acetone (19:1) provided 70 mg. of oily crystals. Three recrystallizations from acetone–ether then led to 50 mg. of crystals, m.p. 274–276°, $[\alpha]_D$ +131°, λ_{max} 242 mμ, log ε 4.24; λ_{max}^{KBr} 3.02(s), 5.71(vs), 5.79(vs), 6.01(vs), 6.17(vs), 11.38(s) μ.[26]

Anal. Calcd. for $C_{26}H_{34}O_7$: C, 68.10; H, 7.47; O, 24.43. Found: C, 67.75; H, 7.55; O, 24.52.

12β-Hydroxyprednisolone 21-Acetate (Xb).—A solution of 60% aqueous formic acid (9.2 ml.) and 370 mg. of Xa was allowed to reflux for 20 min., whereafter it was evaporated almost to dryness under reduced pressure and the residue was dissolved in 50 ml. of methylene chloride. The resulting solution was washed several times with aqueous sodium bicarbonate solution and finally with water. After drying and evaporation there remained 270 mg. of a colorless froth which could not be obtained crystalline from any of the ordinary solvent systems even after careful chromatography. Using methylene chloride–ether the material could be obtained as a gel which upon drying exhibited melting points varying from m.p. 150–159° to m.p. 170–177°, $[\alpha]_D$ +78°, λ_{max} 242 mμ, log ε 4.14; λ_{max}^{KBr} 2.92(vs, broad), 5.80(vs, broad), 6.02(vs), 6.20(s) and 8.00(s) μ. The characteristic band at 11.4 μ associated with the 11β,12β-acetonide moiety[26] was no longer present. Paper chromatography indicated that the substance was considerably more polar than a standard of prednisolone acetate and that it contained a small amount of still more polar material, presumably the 21-acetate free tetrol.

Proof that no major structural change had occurred was forthcoming when it was observed that 50 mg. of the amorphous material upon treatment with acetone (0.5 ml.) containing 70% perchloric acid (2 drops) for 2 hours at room temperature provided after chromatography over silica gel *ca.* 10 mg. of Xa, m.p. 250–258°. A single recrystallization from acetone–ether gave m.p. 271–274°. The infrared spectrum of this compound was identical with that of authentic Xa and a mixture melting point was not depressed.

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[CONTRIBUTION FROM THE OLYMPIC RESEARCH DIVISION, RAYONIER, INC.]

The Nature of a Galactoglucomannan Associated with Wood Cellulose from Southern Pine[1a,b]

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A mixture of galactoglucomannans and araboxylans was extracted with 5% sodium hydroxide from wood cellulose produced from a 1:1 combination of slash pine (*Pinus elliottii* var. elliottii) and longleaf pine (*Pinus palustris* Mill) by the conventional kraft process. These polysaccharides were acetylated and readily separated into an acetone-insoluble araboxylan acetate and an acetone-soluble galactoglucomannan acetate in which the ratio of D-galactose:D-glucose:D-mannose was approximately 1:1:3. Methylation of the galactoglucomannan acetate yielded a product (44.3% OMe, $[\alpha]^{25}_D$ +9.5°, *c* 3, $CHCl_3$) that was essentially homogeneous as shown by fractional precipitation studies. Hydrolysis of the methylated polymer gave 2,3-di-*O*-methyl-D-mannose (10.0%), 2,3-di-*O*-methyl-D-glucose (3.2%), 2,3,6-tri-*O*-methyl-D-mannose (55.0%), 2,3,6-tri-*O*-methyl-D-glucose (13.2%), 2,3,4,6-tetra-*O*-methyl-D-mannose (3.2%) and 2,3,4,6-tetra-*O*-methyl-D-galactose (15.3%). Periodate oxidation and formic acid production studies were in good agreement with the branched structure indicated by methylation. Qualitative graded acid hydrolysis studies are also presented. The structural significance of these findings and the relationship of this family of polysaccharides to the coniferous wood cellulose system in general is discussed.

Coniferous and decidous woods have both been shown to contain true glucomannans.[2] In the case of the deciduous woods, the glucomannan, although representing what is probably the predominant mannose-containing polymer, constitutes only a minor portion of the total hemicellulosic material.[3] The mannose-containing polysaccharides of conifers, however, represent the major hemicellulosic component of these woods. About half of the anhydromannose units of a number of conifers has been shown to occur as an essentially linear polymer composed of glucose and mannose in which the ratio of these sugars is about 1 to 3.[2–6] Investigations of other conifers have shown that glucomannan polymers may be isolated which contain a very high percentage of anhydromannose units joined to only a few glucose units.[2,6,7] However, glucomannans are not the only mannose-containing polysaccharides present in coniferous woods since mannose-containing polymers also have been shown to be associated with galactose.[2,6]

(1) (a) Contribution No. 47 from the Olympic Research Division, Rayonier Incorporated, Shelton, Wash. (b) Presented at the Northwest Regional Meeting of the American Chemical Society, Seattle, Wash., June, 1959.

(2) J. K. Hamilton and N. S. Thompson, *Pulp and Paper Mag. Can.*, **59**, (10) 233 (1958). [A review of the hemicelluloses of hardwoods and softwoods is included in this paper.]

(3) The alder glucomannan described in ref. 2 was erroneously given a specific rotation of −51.5°; however, it has been found that this polymer from both alder holocellulose and semichemical wood cellulose has a rotation very close to −32°.

(4) J. K. Hamilton, H. W. Kircher and N. S. Thompson, THIS JOURNAL, **78**, 2508 (1956).

(5) J. K. Hamilton and H. W. Kircher, *ibid.*, **80**, 4703 (1958).

(6) J. K. Hamilton and E. V. Partlow, *ibid.*, **80**, 4880 (1958).

(7) G. G. S. Dutton and K. Hunt, *ibid.*, **80**, 5697 (1958).

Galactose-containing polysaccharides have been isolated in small quantities from the water-soluble extracts of a number of coniferous and deciduous woods.[2] In the case of western larch, which differs from other coniferous woods in that it contains a much higher proportion of galactose-containing polysaccharides, the water-soluble polysaccharides appear to be arabogalactans. Lesser amounts of similar polysaccharides also appear to be present in the cold water extracts of Douglas fir, black spruce and western red cedar, although their structural formulas have yet to be established. Recently, it has become apparent that some of the galactose units of coniferous woods are associated with a mannose-containing polymer. Adams[8] was able to separate the water-soluble extract of white spruce woodmeal into what appeared to be an arabogalactan and a galactoglucomannan. In this Laboratory, it was found that several holocelluloses and kraft wood celluloses also contained what appeared to be galactoglucomannans.[9] Other workers likewise have reported the existence of this type of polymer.[10]

In work described in a previous communication,[9] the conventional kraft wood celluloses of western hemlock (*Tsuga heterophylla*), redwood (*Sequoia sempirvirens*) and southern pine [a mixture of equal parts of slash pine (*Pinus elliottii* var. elliottii) and longleaf pine (*Pinus palustris* Mill)] were extracted with 5% sodium hydroxide. Following acidification of the sodium hydroxide extracts, the acid-insoluble fractions of these extracts appeared to consist of similar polymers composed of arabinose and xylose and which, in the case of the southern pine extract, was shown by methylation and periodate oxidation studies to be an araboxylan.[11] It was concluded that this polymer was an "artifact" hemicellulose and was produced by the selective alkaline hydrolysis of 4-*O*-methylglucuronic acid units from the original 4-*O*-methylglucuronoaraboxylan of the wood.[9,11] The marked similarity of the corresponding extracts from the other two coniferous conventional kraft wood celluloses (hemlock and redwood) suggested that they, too, were araboxylans. The fraction from the southern pine kraft wood cellulose which was extracted with 5% sodium hydroxide but which was soluble upon acidification with acetic acid was also isolated.[11] Following acetylation, this polysaccharide acetate was separated, on the basis of solubility in acetone, into two fractions. Upon hydrolysis, the acetone-insoluble acetate gave rise to arabinose and xylose, while the acetone-soluble acetate gave rise to galactose, glucose and mannose. Deacetylation of the latter polysaccharide, accompanied by a mild form of fractionation brought about by trituration with water, gave rise to a polymer containing galactose, glucose and mannose in an approximate 1:1:3 molar ratio.

This paper is specifically concerned with the structure of the above galactoglucomannan. At the same time, a brief comparison also is made of the sugar ratios and specific rotations of heteropolymers containing galactose, glucose and mannose which have been isolated from other conifers both in this Laboratory and elsewhere.

Numerous techniques were employed in an attempt to fractionate this galactoglucomannan into simpler hetero- and homopolymers. Methods used included (a) treatment with sodium borate (test for galactomannan), (b) precipitation using Fehling solution, (c) fractional precipitation using complexing agents such as barium hydroxide or sodium borate in conjunction with cetyltrimethylammonium bromide, and (e) fractional precipitation of the acetate from acetone solution. An alternative method of isolating the galactoglucomannan from the crude 5% sodium hydroxide extracts was also employed based on work described by Adams.[8] The crude hemicellulose was extracted with water, and the galactoglucomannan was precipitated with Fehling solution. The use of these fractionation techniques, in some cases, separated small quantities of glucan and xylan; but in no case was it demonstrated that the galactoglucomannan was a mixture of polymers. Similar galactoglucomannan polymers from western hemlock and redwood wood conventional kraft celluloses were also isolated. They were subjected to the above-mentioned fractionation techniques with the same result; *i.e.*, no substantial separation was achieved. However, the galactoglucomannans from the western hemlock conventional kraft wood cellulose and chlorine dioxide holocellulose were found to have an approximate ratio of galactose to glucose to mannose of 1:1.5:5 which differed from the ratio of approximately 1:1:3 found for both the pine and redwood galactoglucomannans.

The previously mentioned galactoglucomannan acetate (from the southern pine conventional kraft wood cellulose) was methylated, first with methyl sulfate and powdered sodium hydroxide in dry tetrahydrofuran and then with methyl iodide and silver oxide.[5,12] It was eventually possible to isolate a fully methylated polymer which, upon fractionation, appeared to concentrate the trace quantities of the methyl ethers of glucan and xylan in the most soluble fraction. This fraction was discarded. After hydrolysis of the purified methylated galactoglucomannan, the mixture of methylated sugars was separated by paper partition chromatography. The components of the hydrolyzate, which were identified by comparison of their melting points and optical rotations with those of authentic specimens and/or authentic derivatives, were found to consist of 2,3,4,6-tetra-*O*-methyl-D-mannose (3.2%), 2,3,6-tri-*O*-methyl-D-mannose (55.0%) and 2,3-di-*O*-methyl-D-mannose (10.0%), 2,3,6-tri-*O*-methyl-D-glucose (13.2%) and 2,3-di-*O*-methyl-D-glucose (3.2%) and 2,3,4,6-tetra-*O*-methyl-D-galactose (15.3%). These results indicated that the galactoglucomannan is a branched polymer joined through positions 1, 4 and occasionally 6 of the glucose and mannose repeating units. Periodate oxidation results confirmed this picture by giving periodate consumption and formic acid production values

(8) G. A. Adams, *Tappi*, **40**, 721 (1957).

(9) J. K. Hamilton, E. V. Partlow and N. S. Thompson, *ibid.*, **41**, 803 (1958).

(10) J. K. N. Jones and T. J. Painter, *J. Chem. Soc.*, 573 (1959); see also D. H. Ball, J. K. N. Jones, W. H. Nicholson and T. J. Painter, *Tappi*, **39**, 438 (1956).

(11) J. K. Hamilton, E. V. Partlow and N. S. Thompson, *ibid.*, **41**, 811 (1958).

(12) E. L. Falconer and G. A. Adams, *Can. J. Chem.*, **34**, 338 (1956).

close to those required by the conjectured structure. The periodate consumption curve was of the same general shape as that obtained from the periodate oxidation of a highly branched cherry gum[13] but was not the same as the periodate consumption curves from either the less highly branched 4-*O*-methylglucurono-xylan or the straight-chain glucan controls. The relatively slow oxidation of the galactoglucomannan may be explained by the bulky constituent attached at the sixth carbon atom of some of the glucose and mannose units. The occurrence of this phenomenon has been established in the case of glucose derivatives by Smith, *et al.*[14]

From the preceding data, the m[illegible]uctural features of the galactoglucomannar[illegible] be deduced. The fractionation experim[illegible] indicate that it is a reasonably homogeneous polymer. It is evident from the methylation data that both the 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,4,6-tetra-*O*-methyl-D-galactose are representative of terminal D-mannopyranose and D-galactopyranose units in the galactoglucomannan polymer. From the large amounts of 2,3,6-tri-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-glucose, it is probable that the main body of the polymer consists of D-mannose and D-glucose pyranose units linked through positions 1 and 4. It is also probable that the 2,3-di-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-glucose are representative of branch points through position 6 along the main body of the polymer. The low positive optical rotation of the polymer indicates a predominance of β-bonds, but it does not preclude the possibility that some of the bonds may be of the α-configuration. The behavior of the polymer during graded acid hydrolysis indicates that most of the galactose residues are joined to the polymer by a labile linkage (see Experimental). Further graded hydrolysis studies, in which crystalline di- and trisaccharides are isolated and identified, are essential before a more definite structure can be assigned.

Although a unique solution to the structure of this polymer is not possible on the basis of the data presented, a simplified structure consistent with the facts so far ascertained is presented

$$M_{p1}\text{—}{}_4\overset{\overset{Gal_p}{\vdots\,1\atop\vdots\,6}}{G_{p1}}\text{—}({}_4M_{p1})_4\text{—}{}_4G_{p1}\text{—}{}_4\overset{\overset{Gal_p}{\vdots\,1\atop\vdots\,6}}{M_{p1}}$$

Additional variations of this structure are possible in which glucose and/or mannose units which give rise to 2,3,6-tri-*O*-methyl-*D*-glucose or 2,3,6-tri-O-methyl-D-mannose are interposed between the side-chain terminal galactose units and the branch point. Alternatively, the polymer may have an irregularly branched structure.

As mentioned previously, galactoglucomannans were also isolated from the hemlock and redwood conventional kraft wood celluloses. The galactoglucomannan from redwood resembled that from southern pine. The galactoglucomannans obtained from western hemlock kraft wood cellulose and chlorine dioxide holocellulose however, had ratios of sugars and optical rotations which differed from those of the polymers obtained from the other two wood celluloses. This galactoglucomannan (from the kraft pulp) was fractionated in a manner identical to that used upon the southern pine galactoglucomannan, but no separation could be achieved. These results, together with the properties of galactoglucomannans from different sources, as determined by other workers, are summarized in Table I.

TABLE I

A COMPARISON OF GALACTOGLUCOMANNANS

Wood source	Method of isolation	$[\alpha]^{25}D$	Molar ratio of Galactose	Glucose	Mannose
So. pine conv.	A[a]	+ 5.2°	1.0	1.1	3.2
kraft	B[a]	+12.5	1.2	1.0	3.0
Hemlock conv.	A[a]	+ 2	1.0	1.3	4.8
kraft	B[a]	− 2	1.0	1.5	5.0
Hemlock ClO_2 holocellulose	B[d]	− 8	0.8	1.5	5.0
Redwood conv. kraft	A[a]	+17.5	1.0	1.0	3.0
White spruce wood	[b]	+11	1.0	1.0	3.7
Loblolly pine wood	Dil. alk.	−20[c]	1	7	19

[a] See Experimental section for details. [b] The method employed by Adams[8] for the isolation of this polysaccharide material was a water extraction followed by Fehling precipitation. [c] Jones and Painter[10] did not consider this fraction homogeneous. [d] Isolated by method B and Fehling precipitation of the 10% KOH extract of the holocellulose.

These data in Table I suggest that galactoglucomannans may be present in many coniferous woods. Whether or not these polymers have the same general type of structure as the galactoglucomannan isolated from southern pine remains to be seen. It is apparent that differences among the carbohydrate polymers from various wood sources do occur not only in the same species[7,15] but probably also in the same tree.[15a]

Galactose-containing compounds have been detected in many coniferous wood celluloses prepared by the conventional kraft, neutral sulfite semichemical and various holocellulose procedures. However, neither acid sulfite nor prehydrolyzed kraft wood celluloses from the same wood sources contain galactose to any significant extent. Therefore, it is concluded that the galactoglucomannans are relatively unstable to acid hydrolysis. This conclusion was corroborated when 50% of the southern pine galactoglucomannan was converted to monosaccharides by refluxing in 0.05 *N* oxalic acid solution for 6 hours. Galactose was the first sugar and mannose was the next sugar to appear during the hydrolysis. Glucose units were still more resistant to hydrolysis and were the last to appear. It is interesting to note that although galactose was the first sugar to appear during the hydrolysis, it was not completely removed from the

(13) J. K. N. Jones, *J. Chem. Soc.*, 558 (1939); 1055 (1947); 3141 (1949).

(14) E. F. Garner, I. J. Goldstein, R. Montgomery and F. Smith, THIS JOURNAL, **80**, 1206 (1958).

(15) G. O. Aspinall, R. A. Laidlaw and R. B. Rashbrook, *J. Chem. Soc.*, 4444 (1957).

(15a) It has been reported[7] that sitka spruce (*Piceo sitchensis*) contains a glucomannan in which the ratio of glucose to mannose is 1 to 9. Independent studies in this Laboratory and by Aspinall[15] also indicate the presence of a glucomannan in this wood which contains glu cose and mannose in the more common ratio for coniferous woods of 1 to 3.

residual unhydrolyzed polymer even after 6 hours reflux with the oxalic acid solution.

A number of oligosaccharides were produced as a result of this mild hydrolysis. A reducing substance having the same chromatographic mobility as mannobiose (and giving mannose only upon hydrolysis) was produced in seemingly greatest yield. Other reducing substances with chromatographic mobilities close to those of glucosidomannose and mannosidoglucose were produced in lesser amounts. Many substances with chromatographic mobilities in the region of the higher oligosaccharides also were detected.

Experimental

Chromatographic Techniques.—The chromatographic solvents employed in this investigation were: (A) ethyl acetate–pyridine–water (8:2:1, v./v.); (B) ethyl acetate–acetic acid–water (3:1:3, v./v. upper phase); (C) ethyl acetate–acetic acid–formic acid–water (18:3:1:4, v./v.); and (D) 2-butanone–water (10:1, v./v). The spray reagents used for development of color was *p*-anisidine trichloroacetic acid or a modification of this reagent in which a small amount of pyridine had been added.[16] Chromatographic papers included Whatman No. 1 for qualitative and quantitative purposes and Whatman No. 3 and Whatman No. 17 for the separation and isolation of larger amounts of pure sugars.

Quantitative paper chromatographic analyses were by a method similar to that used by McCready and McComb.[17,18] The indicator employed was aniline phthalate, and spot densities were determined with a Beckman spectrophotometer.

Isolation of the Crude Hemicellulose.—The wood cellulose used in this study was prepared from a mixture of equal parts of longleaf pine (*Pinus palustris* Mill) and slash pine (*Pinus elliottii* var. elliottii) wood chips by digestion in a conventional kraft cooking liquor under conditions similar to those employed commercially. The raw wood cellulose was then bleached by conventional techniques giving a wood cellulose containing 91.4% α-cellulose, 7.7% xylose, 9.2% mannose and no 4-*O*-methyl-D-glucuronic acid.

The wood cellulose was extracted with ten times its weight of 5% sodium hydroxide (20° for 20 minutes), was filtered, and the extract was made slightly acidic with acetic acid. The resulting insoluble araboxylan[11] was allowed to settle for one week prior to its removal by centrifugation.

The centrifugate was dialyzed against running water for 10 days and then was concentrated to one-twentieth of its original volume by evaporation at diminished pressure at approximately 40°. The concentrate was poured into four volumes of methanol, and the precipitate was isolated by centrifugation. This precipitate was dissolved in water, bleached with chlorine dioxide,[6] reprecipitated with methanol and solvent exchanged through methanol, acetone and ether and was finally isolated as a white powder; found: yield 1.1% (based on oven dry wood); molar ratio of galactose, glucose, mannose, arabinose, xylose and 4-*O*-methyl-D-glucuronic acid after hydrolysis 1.2:1.8:5.2:1:12:0; intrinsic viscosity in *M* cupriethylenediamine hydroxide, 0.49 (expressed as dl./g.).

Five grams of the crude hemicellulose was dissolved in 72% sulfuric acid, adjusted to 1 *N* with water and hydrolyzed for 8 hours at 100°. Following removal of sulfate ions (with barium carbonate) and cations (with Amberlite IR-120(H) resin), the hydrolyzate was evaporated to a convenient volume and spotted on Whatman No. 3 chromatographic paper. After chromatographing for 24 hours on solvent A, the individual sugars were isolated by elution of the appropriate zones of the paper with water. All five sugars (galactose, glucose, mannose, xylose and arabinose) were isolated as light yellow sirups which crystallized readily upon nucleation with the appropriate seed; found: for D-glucose, m.p. and mixed m.p. 164–167°, $[\alpha]^{25}D$ +53° (*c* 1, H_2O); for D-galactose, m.p. and mixed m.p. 144–146°, $[\alpha]^{25}D$ +79.4° (*c* 1, H_2O); for D-mannose, m.p. and mixed m.p. 130–132°, $[\alpha]^{25}D$ +14.3° (*c* 1, H_2O); for arabinose, m.p. and mixed m.p. 160°; for D-xylose, m.p. and mixed m.p. 148°, $[\alpha]^{25}D$ +18.5° (*c* 1, H_2O).

Isolation of a Crude Galactoglucomannan (Method A).—A portion of the crude hemicellulose referred to previously (43 g.) was swollen for 70 hours in 300 ml. of formamide. Pyridine (800 ml.) was added followed by three 200-ml. aliquots of acetic anhydride after 0, 1 and 8 hours reaction time. After standing for 48 hours (with occasional shaking), the reaction mixture was poured into dilute aqueous hydrochloric acid. Two hours later the precipitated hemicellulose acetate was recovered by filtration, was washed first with water and secondly with methanol and then dried.

The dry hemicellulose acetate (59 g.) was triturated for 4 hours with 800 ml. of acetone. Following removal of the supernatant solution, the insoluble residue was re-extracted with acetone as before. The residue remaining after the second extraction with acetone was hydrolyzed and examined by means of paper partition chromatography on solvent A which showed the presence of arabinose and xylose only.

The combined acetone extracts were concentrated to 100 ml. and poured into 800 ml. of diethyl ether. The precipitate was isolated by centrifugation, washed with ether and dried. This galactoglucomannan acetate was employed for subsequent fractionation and methylation experiments; found: yield 0.44% (based on oven dry wood); molar ratio of galactose, glucose, mannose, arabinose, xylose and 4-*O*-methyl-D-glucuronic acid in the hydrolyzed extract, 1:1.6:4:0:0.2:0.

The galactoglucomannan acetate (10 g.) was dissolved in 400 ml. of acetone and was refluxed for 1 hour with an equal volume of 45% aqueous potassium hydroxide. The slight color in the acetone layer was gradually transferred to the aqueous layer. After standing overnight at room temperature, the aqueous alkaline layer was separated from the turbid acetone layer and was added to four volumes of methanolic acetic acid. After washing the precipitate with methanol, the sample was triturated with water, and the water-soluble fraction was precipitated again with methanol, was solvent exchanged as before, dried and labeled GGM-2. The water-insoluble residue was resuspended in the original turbid acetone suspension and fresh 45% potassium hydroxide was added. After shaking for 24 hours, the alkali-soluble fractions were isolated separately as before. Once again, the alkali-soluble fraction was triturated with water, and the water-insoluble fraction was combined with the acetylated acetone-soluble material. This water-soluble fraction was called GGM-3. Fractions GGM-2 and GGM-3 had identical compositions and were considered identical; found: combined yield from the deacetylation of fractions GGM-2 and GGM-3, 82%; molar ratio of galactose, glucose, mannose and xylose after hydrolysis, 1.0:1.1:3.2:0.1; intrinsic viscosity in *M* cupriethylenediamine hydroxide, 0.29 dl./g.; $[\alpha]^{25}D$ +5.2° (*c* 1, H_2O).

The combined yield of acetone-soluble and water-insoluble fractions from the deacetylation amounted to 18% having a molar ratio of galactose, glucose, mannose, xylose of 0.4:1.0:2.2:0.8 (after acid hydrolysis) and an intrinsic viscosity of 0.34 dl./g. in *M* cupriethylenediamine hydroxide.

Isolation of a Crude Galactoglucomannan (Method B).—The crude hemicellulose mixture of araboxylan and galactoglucomannan may be separated into its components by a method based upon a procedure devised by Adams for the separation of a galactoglucomannan from an arabogalactan. The crude hemicellulose was shaken with twenty times its weight of water for 48 hours. Because of the formation of a very fine suspension of hemicellulose in water, it was necessary to centrifuge at 18000 r.p.m. in order to get a clear centrifuge. An equal volume of freshly prepared Fehling solution was added to the water-soluble (chlorine dioxide bleached[6]) hemicellulose centrifugate. The precipitate so formed was recovered by centrifugation, suspended in water, the copper complex was destroyed with acetic acid and the clear solution was dialyzed against running water until free of cupric ion. The water-insoluble hemicellulose fraction produced xylose and arabinose upon hydrolysis, while the water-soluble, Fehling solution-insoluble hemicellulose fraction appeared to be a galactoglucomannan and was called GGM-4; found: molar ratio of galactose, glucose, mannose, arabinose, xylose, in the hydrolyzed extract was 1.2:1.0:3.0:0.1; intrinsic viscosity

(16) J. K. Hamilton and N. S. Thompson, THIS JOURNAL, **79**, 6464 (1957).

(17) R. M. McCready and E. A. McComb, *Anal. Chem.*, **26**, 1645 (1954).

(18) J. E. Jeffery, E. V. Partlow and J. W. Polglase to be published.

in *M* cupriethylenediamine, 0.31 dl./g.; $[\alpha]^{25}D$ +12.5° (*c* 1, H_2O).

Qualitative Graded Acid Hydrolysis Studies.—Galactoglucomannan (1 g.) was dissolved in 100 cc. of 0.05 *N* oxalic acid and was heated under reflux. At hourly intervals, 1-ml. aliquots were removed and the unhydrolyzed material precipitated with methanol and isolated by centrifugation. One-half of the hemicellulose was methanol insoluble after 2 hours and only 8% after 6 hours hydrolysis. The soluble residues were extracted with ether, evaporated to dryness and examined by qualitative paper chromatography. Galactose was the first sugar to appear as a result of this hydrolysis, followed by mannose, while glucose was the last sugar to appear.

Fractionation of the Galactoglucomannan.—Galactoglucomannan acetate (0.5 g.) was dissolved in acetone (100 ml.), and water was added to the point of incipient precipitation. The hemicellulose acetate was fractionally precipitated into four fractions by bubbling air through the acetone–water solution (to remove acetone) and with periodic removal of the precipitated hemicellulose acetate by centrifugation. Qualitative paper chromatographic examination (solvent A) of the acid hydrolyzate of these four fractions showed no difference in the apparent ratios of galactose to glucose to mannose.

Galactoglucomannan fraction GGM-2 (100 mg.) was dissolved in 10 ml. of water, and an equal volume of freshly prepared Fehling solution was added. Virtually all the sample (90%) was recovered by this technique. Quantitative analyses indicated this hemicellulose had a molar ratio of galactose to glucose to mannose of 1:1:3 and $[\alpha]^{25}D$ +7.4° (*c* 1, H_2O).

Galactoglucomannan fraction GGM-2 also failed either to precipitate or to form a gel with borax under the conditions described by Smith[19] which indicated that no galactomannan was present.

Galactoglucomannan fraction GGM-4 (0.400 g.) was dissolved in 50 ml. of water and was fractionally precipitated by the addition of aliquots of saturated aqueous barium hydroxide.[2,5,20] The precipitated fractions were suspended in a little water, neutralized with acetic acid and reprecipitated by adding methanol. After thorough methanol and ether washings, the fractions were dried and analyzed; the results are given in Table II.

TABLE II

FRACTIONATION OF A GALACTOGLUCOMANNAN WITH SATURATED AQUEOUS BARIUM HYDROXIDE

Cumulative vol. of $Ba(OH)_2$	Recovered, %	Molar ratio of sugars: Galactose	Glucose	Mannose	$[\alpha]^{25}D$
9	22.5	1	1	3	+10°
19	54.4	1	1	3	+16°
49	6.5	1	1	3	...
Soluble	4.6	1	2	4	...

Galactoglucomannan fraction GGM-4 (465 mg.) was dissolved in 50 ml. of water and 5 ml. of a 20% aqueous solution of cetyltrimethylammoniun bromide was added. No precipitate appeared and addition of 20 ml. of 5% boric acid also caused no precipitate. The *p*H of the solution was then adjusted from 5 to 7, using dilute sodium hydroxide, and a precipitate appeared which was isolated by centrifugation. One ml. of sodium hydroxide (10%) was added, and the precipitate so formed was isolated. An additional 3 ml. of 10% sodium hydroxide was added, and the soluble and insoluble fractions so formed were isolated separately (see Table III below). This fractionation technique was a modification of the cetavlon–sodium borate complexing method of Barker, Stacey and Zweifel.[21]

Periodate Oxidations.—A sample of the galactoglucomannan (0.5 g.), prepared by the copper complex isolation technique, was dissolved in 100 ml. of water. Samples of 4-*O*-methylglucurono-xylan, a short-chain β-1–4-glucan and cherry gum (analyzing for galactose, mannose, arabinose, xylose, rhamnose and glucuronic acid in a 14.3:1.0:35.4:2.2:1.0:7.5 molar ratio), were also suspended in water and served as controls. The samples and a blank were all adjusted to a *p*H of 4.6 and were placed in 250-ml. volumetric flasks. Then, 50 ml. of 0.74 molar sodium periodate was added, and the solutions were diluted to volume and stored in the dark at room temperature. The consumption of periodate ion was measured by the method of Fleury and Lange.[22] The "overstrength" oxidation was employed since preliminary experiments indicated the oxidation using less concentrated sodium periodate required an inconveniently long period of time to achieve a constant oxidation rate. The control experiments on the glucan and 4-*O*-methylglucurono-xylans went rapidly to their maximum theoretical consumption and virtually stopped. The samples of cherry gum and galactoglucomannan did not reach a maximum consumption of periodate but (after 60 hours for the former and 40 hours for the latter) achieved a constant rate of oxidation. Extrapolation to zero time showed that the cherry gum consumed 6.1 moles periodate per kilogram, and the galactoglucomannan consumed 7.5 moles periodate per kilogram (required 7.7 moles periodate per kilogram for the conjectured structure).

TABLE III

FRACTIONATION OF THE BORATE COMPLEX OF THE GALACTOGLUCOMANNAN WITH CETAVLON

Fraction	Recovered, %	Molar ratio of sugars: Galactose	Glucose	Mannose
Insol. (*p*H 7)	24	1.0	1.0	3.3
Insol. (1 ml. NaOH)	69	0.9	1.0	3.7
Insol. (4 ml. NaOH)	7	.9	1.0	3.7
Soluble residue	8	.5	1.0	2.3

Formic acid production was followed by the sodium thiosulfate method.[23] The theoretical formic acid production was 1.23 moles per kilogram of sample which corresponded closely with the observed maximum value of 1.25 moles of formic acid produced per kilogram of galactoglucomannan.

Upon completion of the oxidation, the excess periodate ion was destroyed with ethylene glycol, and the sample was dialyzed free of iodate and iodide ions. The dialyzate was evaporated to dryness, dissolved in 20 ml. of 0.1 *N* sodium hydroxide and was reduced using a large excess of sodium borohydride. The excess borohydride was destroyed, and the sodium ions were removed by Amberlite IR120(H) resin. The solution was evaporated to dryness and was concentrated from methanolic acetic acid until a negative test for boron was achieved. Hydrolysis of this reduced material, followed by paper chromatographic analysis, showed that no glucose, mannose nor galactose was present.

In another similar experiment, the oxidation was terminated when about half complete. Chromatographic examination of the acid hydrolyzate showed that, while glucose and mannose were not completely oxidized, no galactose whatsoever could be detected.

Methylation of the Acetone-soluble Hemicellulose Acetate.—Ten grams of the acetone-soluble galactoglucomannan acetate was dissolved in 400 ml. of tetrahydrofuran and methylated by the addition of 7 aliquots of powdered sodium hydroxide (10 g.) and 7 aliquots of dimethyl sulfate (12 ml.) over a period of two days.[12] The mixture was heated to reflux after the second addition to remove residual acetyl groups. Following the addition of the seventh aliquot (final), the mixture was shaken for two days and then heated to boiling bath temperature for one hour to decompose any residual dimethyl sulfate. After cooling and centrifuging, the solid residue was washed twice with tetrahydrofuran (100 ml.), and the combined tetrahydrofuran solutions evaporated to one-tenth of the original volume. This solution was dissolved in chloroform (300 ml.), washed with water (300 ml.), and the chloroform layer evaporated to dryness. A small portion of this sirupy material was dissolved in acetone and precipitated by pouring into petroleum ether (50 ml.) to give a near-white solid (OMe, 32.3%).

The partially methylated galactoglucomannan was subjected to five methylations with Purdie reagents[24] using methyl iodide (40 ml.) and silver oxide (10 g.), the reaction mixture being refluxed each time for 48 hours. After removal of the methyl iodide, the product was dissolved in

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(20) H. Meier, *Acta Chem. Scand.*, **12**, 144 (1958).

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(22) P. Fleury and P. Lange, *J. Pharm. Chem.*, [8] **17**, 107 (1933).

(23) K. H. Meyer, *Adv. in Enzymol.*, **3**, 109 (1943).

(24) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).

acetone and the insoluble material removed by centrifugation. Evaporation gave the partially methylated galactoglucomannan (OMe, 39.4%).

The methylated galactoglucomannan was dissolved in acetone (150 ml.) and methylated with dimethyl sulfate (90 ml.) and 30% sodium hydroxide (260 ml.) according to the method of Haworth.[25] The reaction was completed by heating at boiling bath temperature for 0.5 hours. After cooling, the reaction mixture was acidified with sulfuric acid and extracted three times with chloroform, and the combined chloroform solutions washed with water, dried (Na_2SO_4), and evaporated to dryness (OMe, 43.2%). The product was subjected to two additional methylations with Purdie[24] reagent as before (OMe, 43.5%).

Fractional Precipitation of Methylated Galactoglucomannan.—The methylated galactoglucomannan was dissolved in chloroform (60 ml.) and diethyl ether (60 ml.) and was fractionally precipitated with petroleum ether in the usual manner. The results are given in Table IV.

TABLE IV

FRACTIONAL PRECIPITATION OF METHYLATED GALACTOGLUCOMANNAN

Fraction no.	1	2	3	4	5	6
Total petroleum ether added, ml.	250	380	520	850	[b]	.....
Weight, g.	0.17[a]	1.51	1.68	0.83	0.79	0.86[c]
$[\alpha]^{28}D$ ($CHCl_3$, c 3)		+9.5°	+10.6°	+6.5°	+1.9°	.....
Methoxyl, %	.....	43.7	44.2	44.8	44.4	30.0

[a] Contaminated with inorganic material, not further used. [b] Solvent mixture remaining after removal of fraction 4 was evaporated and the residue dissolved in 10 ml. of chloroform and poured into 800 ml. of petroleum ether. [c] Material soluble in petroleum ether after precipitation of fraction 5.

All of the fractions shown in Table IV precipitated as oils. Fractions 2, 3, 4 and 5, after being dissolved in chloroform (10 ml.), were poured, with stirring, into petroleum ether (400 ml.) and formed tan-colored flocculent precipitates.

Hydrolysis of the Methylated Galactoglucomannan and Separation of the Methylated Sugars.—Preliminary experiments involving the hydrolysis and paper chromatographic examination of fractions 2, 3, 4 and 5 indicated that these fractions contained the same number of components, namely six, with identical R_{tmg} values (relative to 2,3,4,6-tetra-*O*-methyl-D-glucose) on solvent D. These fractions were therefore combined.

The combined fractions of the methylated galactoglucomannan (3.80 g.) were dissolved in acetone (150 ml.), and aqueous 1 *N* sulfuric acid (150 ml.) was slowly added. This clear solution was refluxed for 21 hours, removed from reflux and placed in a boiling water-bath, and the acetone was allowed to evaporate. The solution was further hydrolyzed under reflux for 15 hours.

The solution was neutralized ($BaCO_3$), filtered, and the cations were removed with an ion exchange resin (Amberlite IR-120). Evaporation gave the methylated sugars as a light yellow sirup (3.43 g.).

The methylated sirup (3.13 g.) was subjected to paper partition chromatography (solvent D) for 40 hours using Whatman No. 17 chromatographic paper to which a Whatman No. 50 wick was attached.[6] The areas corresponding to the various methylated sugars were excised and eluted. The two di-*O*-methyl sugars were not completely separated from each other; similarly, the two tri-*O*-methyl sugars were not completely separated. These mixtures were purified by further chromatography (solvent D). Results of the paper partition chromatography are shown in Table V.

Identification of the Cleavage Products of Methylated Galactoglucomannan. (1) **2,3-Di-*O*-methyl-D-mannose** was isolated as a sirup (calcd. OCH_3: 29.8%; found: 28.7% OCH_3). Upon demethylation in a sealed tube (1 ml. of 48% hydrobromic acid at boiling bath temperature for 20 minutes), this component was shown chromatographically (solvent A) to consist of mannose only.

The 2,3-di-*O*-methyl-D-mannose (75 mg.) was dissolved in water (6 ml.) containing bromine (0.25 ml.), and the reaction mixture was stored in the dark for four days. The reaction mixture was worked up in the usual manner, and the corresponding γ-lactone was obtained. After crystallization from acetone–petroleum ether, a m.p. of 109–110° was obtained (lit.[26] m.p. 109–110°).

The lactone gave the phenylhydrazide of 2,3-di-*O*-methyl-D-mannonic acid, m.p. and mixed m.p. 160–161° (lit.[27] m.p. 158°).

(2) **2,3-Di-*O*-methyl-D-glucose.**—This component, a sirup, had the following analysis: 28.9% OCH_3 (calcd. OCH_3, 29.8%). Upon demethylating a portion of this sirup, using the technique described, chromatographic examination (solvent A) showed that only glucose was present.

The sirup, when nucleated with an authentic seed crystal, yielded crystalline 2,3-di-*O*-methyl-D-glucose. The crude crystalline material was placed on a porous tile to remove adhering sirup, and the crystals were washed twice with diethyl ether, m.p. and mixed m.p. 116–118° (lit.[28] m.p. 117–119°).

(3) **2,3,6-Tri-*O*-methyl-D-mannose.**—This component (1.37 g.) was isolated as a sirup, $[\alpha]^{22}D$ −10.5° (*c* 3.9 in water). A portion of this sirup (400 mg.) was oxidized with bromine water in the manner previously described. The reaction mixture was worked up in the usual manner, and the corresponding γ-lactone was obtained. The lactone gave the phenylhydrazide of 2,3,6-tri-*O*-methyl-D-mannonic acid which crystallized spontaneously. Following recrystallization from ethanol (m.p. 136–140°), the crystals were melted (*in vacuo*) and recrystallized from absolute ethanol to give the anhydrous phenyl hydrazide of 2,3,6-tri-*O*-methyl-D-mannonic acid, m.p. and mixed m.p. 146–148°, $[\alpha]^{22}D$ −17.5° (*c* 1.0 in water) (lit.[29] m.p. 144°, $[\alpha]^{20}D$ −16.5° (*c* 0.9 in water)).

(4) **2,3,6-Tri-*O*-methyl-D-glucose.**—This component (333 mg.) crystallized when nucleated with an authentic specimen of 2,3,6-tri-*O*-methyl-D-glucose. The crystals were placed on a porous tile for two days to remove adhering sirup and then recrystallized from ether–petroleum ether mixture, m.p. and mixed m.p. 120–121°, $[\alpha]^{22}D$ +66.5° equilibrium value in water (*c* 1.0) (lit. m.p.[30] 121–123°, $[\alpha]D$ +70° (water)).

(5) **2,3,4,6-Tetra-*O*-methyl-D-galactose.**—This component (382 mg.) was isolated as a sirup, $[\alpha]^{22}D$ +101.0° (*c* 1.3 in water). Treatment of the sirup with boiling alcoholic aniline in the usual way afforded 2,3,4,6-tetra-*O*-methyl-D-galactose-N-phenylglucosylamine, m.p. and mixed m.p. 192–193°, $[\alpha]^{22}D$ −142° in pyridine (*c* 1.0) (lit.[29] m.p. 192°, $[\alpha]^{22}D$ −141° in pyridine (*c* 0.8)).

TABLE V

PAPER PARTITION CHROMATOGRAPHY OF THE HYDROLYZATES OF METHYLATED GALACTOGLUCOMANNAN

Component	R_{tmg}[a]	Weight, g.	Molar ratio (approx.)	%
2,3-Di-*O*-methyl-D-mannose	0.16	0.25	4	10.0
2,3-Di-*O*-methyl-D-glucose	.20	0.08	1	3.2
2,3,6-Tri-*O*-methyl-D-mannose	.45	1.37	19	55.0
2,3,6-Tri-*O*-methyl-D-glucose	.56	0.33	5	13.2
2,3,4,6-Tetra-*O*-methyl-D-galactose	.73	.38	5	15.3
2,3,4,6-Tetra-*O*-methyl-D-mannose	1.00	.08	1	3.2
Total		2.49[b]		

[a] Relative to distance traveled by 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent D. [b] Allowing 10% for loss incurred in locating the areas containing methylated sugars following chromatographic separation, this value corresponds to a 90% recovery.

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(6) **2,3,4,6-Tetra-*O*-methyl-D-mannose.**—This chromatographically pure component (75 mg.) was obtained as a sirup $[\alpha]^{22}D$ +29.2° in water (*c* 3.9). Although chromatographically pure, the possibility existed that this component could be a mixture of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-mannose since it has been shown that a variety of chromatographic solvents failed to separate these two compounds.[6]

The aniline derivative of this component was prepared and the crude crystals were separated from the adhering sirup by use of a porous tile. These crystals were fractionally crystallized from ether–petroleum ether mixture into four fractions, all of which had melting points and mixed melting points of 143–146°. The four fractions were combined and had $[\alpha]^{22}D$ −8.4° in methanol (*c* 1.5) (lit.[31] m.p. 144–145°, $[\alpha]^{15}D$ −7.5° (in methanol)). From the preceding data, and from the values given in the literature for the aniline derivative of 2,3,4,6-tetra-*O*-methyl-D-glucose, m.p. 137–138°,[32] it was concluded that only 2,3,4,6-tetra-*O*-methyl-D-mannose was originally present in this component.

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Alkaline Degradation of Alginates[1]

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Alginates are readily degraded by alkaline solutions at 80–120° with the formation of new 6-carbon, dicarboxylic saccharinates, 3-deoxy-2-C-hydroxymethyl-pentarates.

Vollmert reported that methyl alginate is depolymerized in alkaline solutions while sodium alginate is relatively more stable.[2] However, both esterified and ionic alginates are degraded in alkaline solutions and form salts of saccharinic-type acids by the ordinary process of sequential elimination of the reducing end unit and its subsequent transformation into a saccharinate (Fig. 1).[3] The alkaline degradation reaction is more pronounced in high concentrations of alkalies and at high temperatures.

Alginic acids are heteroglycuronoglycans of D-mannopyranosyluronic acid units and L-guluronic acid units.[4] Alginic acid from *Macrocystis pyrifera* contains mainly D-mannopyranosyluronic acid units and 20–40% of L-guluronic acid units.[4c] The D-mannopyranosyluronic acid units are connected with β-(1→4)-linkages.[5–7] The position on the L-guluronic acid units to which the glycosidic linkages are attached is not known, but periodate oxidation[8] gives no indication of linkages at either C2 or C3. If all uronic acid units are connected with (1→4)-linkages, the saccharinates formed would be the isosaccharinate type[9] with a carboxylate group at the C5 position (C6 of the original uronic acid unit). Because of the presence of both D- and L-hexuronic acid units, this new acid, 3-deoxy-2-C-hydroxymethyl-pentaric acid, would be produced in both D- and L-isomeric forms. Experience has shown that the process of alkaline degradation forms a saccharinic-type acid with predominantly one configuration of C2.

The isomeric, 6-carbon, dicarboxylic saccharinic acids have been isolated in the course of the present work. Structures are assigned to these acids on the basis of the currently accepted mechanism for the formation of products from (1→4)-linked D-hexo- and D-pentoglycans.[2] Supporting evidence for the proposed structures is afforded by the equivalent weight (detd. 100, calcd. 97), the production of formaldehyde upon periodate oxidation (0.5 mole per acid equivalent), and by comparison of the nitric acid oxidation products with those of α-D-isosaccharinic acid. Nitric acid oxidation of α-D-isosaccharinic acid (I) produces a carboxyl group from the primary alcohol group (C2′) so that the asymmetry of C2 is destroyed. A tricarboxylic acid, 2-C-carboxy-3-deoxy-D-*glycero*-pentaric acid (III), is formed in which only the penultimate carbon atom is asymmetric. Nitric acid oxidation of the two 3-deoxy-2-C-hydroxymethyl-pentaric acids produces two tricarboxylic acids, one which has the same rotation as that of the known 2-C-carboxy-3-deoxy-D-*glycero*-pentaric acid and one which has an opposite rotation. The isolated 3-deoxy-2-C-hydroxymethyl-pentarate whose nitric acid oxidation product has the same rotation as that of the known 2-C-carboxy-3-

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