[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Carbohydrates of Gramineae. V. The Constitution of a Hemicellulose of the Endosperm of Wheat (*Triticum Vulgare*)[1,2]

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The constitution of a hemicellulose from the "squeegee" fraction of wheat flour has been investigated by methylation studies. The methylated hemicellulose gives upon hydrolysis 2,3,5-tri-*O*-methyl-L-arabofuranose (14 moles), 2,3-di-*O*-methyl-D-xylose (24 moles), 2-*O*-methyl-D-xylose (7 moles) and D-xylose (4 moles) together with small amounts of two unknown products. The highly branched structure of this hemicellulose conforms to the general type of structure found for the hemicelluloses of the endosperm of the *Gramineae*.

When wheat flour, freed from gluten, is suspended in water and centrifuged, it separates into a tightly packed lower layer of wheat starch above which is a mucilaginous material. The latter, which has been variously called the "amylodextrin"[3] or "squeegee"[4] or "tailings"[5] of wheat flour, plays an important role in the starch–gluten separation process. The chemical composition of the squeegee fraction of wheat starch has not been extensively studied but it is known to contain starch granules and a considerable amount of pentosan and proteinaceous material.

This paper is concerned with the composition and structure of a hemicellulose of the "squeegee" fraction of wheat flour. By the prolonged hydrolysis of the "squeegee" material with pancreatin until it no longer gives a color with iodine, Simpson[6] has obtained a water-insoluble product amounting to 0.5–1% of the whole wheat flour. This insoluble material was used in the present study. It was fractionated by forming the acetate and separating it into an acetone soluble fraction, showing $[\alpha]^{27}D$ $+17°$ in pyridine, and an acetone-insoluble fraction which could not be dissolved in any of the common solvents. Deacetylation of the acetone-insoluble fraction, which amounted to about 70% of the crude acetate, was achieved by heating a pyridine dispersion of it with 25% potassium hydroxide. The resulting material was purified by extraction with *N* sodium hydroxide to give a white amorphous product which dissolved in hot water and showed $[\alpha]^{22}D$ $-108°$ in 0.5 *N* sodium hydroxide, a value in close agreement with that ($[\alpha]D$ $-103°$ in *N* sodium hydroxide) shown by wheat straw hemicellulose.[7]

Upon hydrolysis with acid, the purified hemicellulose gave D-xylose (59%), L-arabinose (39%) and D-glucose (2%), the quantitative analysis being carried out by the phenol–sulfuric acid procedure.[8] In periodate oxidation studies, 0.8 mole of periodate was consumed per anhydropentose residue. The resulting hemicellulose polyaldehyde after reduction with hydrogen using a Raney nickel catalyst followed by acid hydrolysis of the polyalcohol gave a hydrolysate which was shown by chromatographic analysis to contain D-xylose.[9] The hemicellulose thus appeared from the periodate studies to have a branched structure.

In order to determine the mode of union of the component sugars, the hemicellulose was methylated with 45% potassium hydroxide and methyl sulfate. Fractional precipitation of the methylated hemicellulose from an acetone–ether solution with petroleum ether gave a series of fractions, the properties of which were constant ($[\alpha]^{22}D$ $-165°$ in acetone and $-OCH_3$, 38.8) indicating that the methylated hemicellulose was essentially homogeneous. One striking property of the methylated hemicellulose was the highly viscous nature of its solutions in acetone.

Methanolysis of the methylated hemicellulose with 2% methanolic hydrogen chloride gave a mixture of glycosides which was hydrolyzed with dilute hydrochloric acid to give the corresponding methylated reducing sugars. Chromatography on a cellulose–hydrocellulose column[10] using methyl ethyl ketone–water azeotrope as the developing solvent, coupled with the isolation of crystalline derivatives, showed that this mixture of methylated sugars consisted of 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose and D-xylose; by means of paper chromatography and the phenol–sulfuric acid analytical method[8] for determining sugars, the proportions of these four cleavage products of the methylated hemicellulose were found to be 14, 24, 7 and 4 moles, respectively. Although these data do not allow a definite structure to be assigned to the polysaccharide, it is seen that a framework of D-xylopyranose units linked through positions 1 and 4 is present. The presence of 2-*O*-methyl-D-xylose indicates that the carbohydrate polymer has a branched structure in which the side chains, terminated by arabofuranose residues, are attached to xylose units of the framework through position 3.

The isolation of 7 moles of 2-*O*-methyl-D-xylose

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requires the presence of 7 side chains while the 4 moles of D-xylose require 8, making a total of 15, each of which must be terminated by an arabofuranose residue. This requirement for the average repeating unit is in good agreement with the number (14) found. The "squeegee" pentosan is therefore highly branched and appears to be similar to that found in the water-soluble extracts of wheat flour[11,12] and barley flour.[13]

In one column chromatographic separation, small amounts of two unknown methyl sugars (components A and B) were isolated; they appeared to be derived from the same parent sugar. The significance of this is not yet understood but it may mean either that the structure of the hemicellulose under discussion is more complicated than depicted above or it may be that the methylated polysaccharide subjected to hydrolysis is a mixture.

Experimental

Isolation of Pentosan from the "Squeegee" Fraction of Wheat Flour.—The pentosan fraction was isolated by Dr. F. J. Simpson[6] of the Prairie Regional Laboratory, Saskatoon, by prolonged hydrolysis of the squeegee starch protein complex with pancreatin until the material no longer gave a color with iodine. The yield of water-insoluble material was 0.5 to 1% of the whole flour. It was not completely soluble in 0.5 *N* sodium hydroxide. Purification of the product by extraction with 0.5 *N* sodium hydroxide followed by acidification (acetic acid) and precipitation with ethanol gave a white amorphous powder which showed $[\alpha]^{22}D$ −80° in 0.5 *N* sodium hydroxide.

Acetylation of the Wheat Flour Hemicellulose.—The material (10 g.) was suspended in formamide (130 ml.) and after two days the thick jelly-like mass was warmed to 68°. The gel was vigorously stirred with pyridine (390 ml.) and treated at room temperature with acetic anhydride (200 ml.) in portions with shaking. Heat was generated but no external cooling was applied. After keeping the viscous reaction mixture for three days at room temperature, it was poured into water with stirring. The acetate was filtered, washed with water and dried (yield 15 g.). The acetate did not dissolve completely in acetone, chloroform or pyridine.

Separation of the Crude Pentosan Acetate into an Acetone-soluble and an Acetone-insoluble Fraction.—The acetate (15 g. approx.) was refluxed with acetone (500 ml.) for three hours and the mixture allowed to stand overnight. The insoluble fraction of the acetate was removed (centrifuge), washed three times with acetone and dried (yield 10.5 g.).

Deacetylation of the Acetone-insoluble Fraction of the Acetate.—One-sixth of the total amount of the acetone-insoluble acetate obtained above was dispersed in pyridine (50 ml.); it did not dissolve but formed a jelly-like material which could be separated by centrifugation. Other solvents such as chloroform, formamide, dimethylformamide, acetic acid and *m*-cresol likewise had no solvent action on it. To the pyridine dispersion, 25% potassium hydroxide (35 ml.) was added and the mixture was heated on a boiling water-bath. The upper pyridine layer became less viscous and finally became watery in appearance as deacetylation proceeded. Two-thirds of the pyridine was allowed to distil as an azeotrope with water, after which the mixture was cooled, acidified with glacial acetic acid and treated with methanol (4 volumes). The amorphous white precipitate was removed, dissolved in 1 *N* sodium hydroxide (30 ml.) and the solution centrifuged to remove a small amount of insoluble matter. The solution was acidified with glacial acetic acid and treated with methanol (4 volumes). The precipitate of the pentosan was purified by two precipitations from aqueous solution with ethanol. It was finally washed with absolute ethanol, petroleum ether and dried *in vacuo*.

Deacetylation of the rest of the crude hemicellulose acetate in the same way afforded 4.2 g. of the purified polysaccharide. In the purification by dissolution in 1 *N* sodium hydroxide 0.6 g. of insoluble material was removed.

The white stringy amorphous product dissolved in hot water to give a clear solution. It dissolved in formic acid and in dilute sodium hydroxide and showed $[\alpha]^{22}D$ −108° in 0.5 *N* sodium hydroxide.

Paper chromatographic separation followed by quantitative analysis using the phenol–sulfuric acid method[8] showed that the hemicellulose gave upon hydrolysis with dilute mineral acid D-glucose (2%), D-xylose (59%) and L-arabinose (39%).

In periodate oxidation studies, 0.8 mole of sodium periodate was consumed per anhydropentose residue. The polyaldehyde so formed, without previous isolation, was reduced with a pressure (750 lb. per sq. inch) of hydrogen at 80–90° for 7 hours using a Raney nickel catalyst.[9] The resulting solution was made 1 *N* with respect to sulfuric acid and heated under reflux for 10 hours. The acid was neutralized with "Duolite A-4" anion-exchange resin and the aqueous solution evaporated to dryness *in vacuo*. Partition chromatography of the residue using butanol–ethanol–water (4:1:5) as the developing liquid indicated the presence of xylose; no glucose or arabinose was detected.

Examination of the Acetone-soluble Portion of the Hemicellulose Acetate.—The acetone extract of 15 g. of crude acetate (see above) was concentrated to 200 ml. and poured into light petroleum ether. The precipitate was filtered, washed with petroleum ether and dried *in vacuo* (yield 4.0 g.). This product showed $[\alpha]^{22}D$ +17° in pyridine (*c* 0.5). Deacetylation of 1 g. of this acetate in the usual way gave 0.45 g. of a polysaccharide which had $[\alpha]^{23}D$ −14° (*c* 0.5) in 0.5 *N* sodium hydroxide. This material was not examined further.

Methylation of the Hemicellulose Regenerated from the Acetone-insoluble Acetate.—The polysaccharide (4.0 g.) was dissolved in 45% potassium hydroxide (700 ml.) and methylated by the dropwise addition of methyl sulfate (150 ml.) during 3.5 hours at room temperature. Heat was generated during the reaction; no external cooling was applied. Frothing was brought under control by adding butanol (10 ml.). When the addition of the methyl sulfate had been completed, stirring was continued for three hours after which the reaction mixture was heated on the boiling water-bath. The solution was cooled, neutralized with 20% sulfuric acid and dialyzed to remove salts. Concentration of the solution *in vacuo* gave a pale yellow residue and while still containing some solvent (20–30 ml.) the product was remethylated with methyl sulfate (85 ml.) and 45% potassium hydroxide (250 ml.) at 55°; the reagents were added in one-tenth portions every 10 minutes and acetone was added when required to keep the methylated polysaccharide in solution. The reaction was completed by heating for 30 minutes on the boiling water-bath whereupon the methylated polysaccharide separated as granular particles which readily settled. The hot supernatant liquid was decanted and after dissolving the product in acetone (80 ml.) the methylation was repeated as before. Three more methylations were applied in the same way.

After the final methylation the product was washed with boiling water to remove salts, dissolved in acetone and evaporated *in vacuo* to dryness to remove moisture.

Fractional Precipitation of the Methylated Polysaccharide.—The residue from the previous experiment was dissolved in acetone (500 ml.), centrifuged to remove a small amount

TABLE I

FRACTIONAL PRECIPITATION OF METHYLATED "SQUEEGEE" HEMICELLULOSE

Fraction	Wt., g.	$[\alpha]^{22}D$ in acetone (*c* 0.4)	OCH_3, %
IA[a]	0.28	....	..
I	.24	−165°	38.2
II	.31	−162°	38.4
III	1.07	−165°	38.8
IV	0.70	−165°	38.3
V	0.53	−169°	38.4
VI (from mother liquors)	0.89	....	..

[a] This fraction did not give a clear solution in acetone presumably because salts were present and was not examined further.

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TABLE II

SEPARATION OF THE CLEAVAGE PRODUCTS OF METHYLATED "SQUEEGEE" HEMICELLULOSE ON THE HYDROCELLULOSE–CELLULOSE COLUMN[10] WITH METHYL ETHYL KETONE–WATER AZEOTROPE

Tube no. (inclusive)[a]	Component	Wt., g.	OMe, %	R_f[b]	$[\alpha]^{23}D$
10–14	Sudan IV (marking solvent front)	....	..		...
17–27	2,3,5-Tri-*O*-methyl-L-arabinose	0.2305	46.7	0.80	−35° (MeOH)
28–35	Unknown A	.0202	..	.74	−31.5° (MeOH)
38–73	2,3-Di-*O*-methyl-D-xylose	.2596	34.4	.58	+16° (MeOH)
80–105	Unknown B	.0466	..	.53	−27° (H_2O)
138–148	2-*O*-Methyl-D-xylose	.1079	..	.16	+32° (H_2O)

[a] Collection time for tubes 10 to 98 was 10 minutes and thereafter it was 30 minutes per tube. [b] The R_f values for the standard methyl sugars determined on Whatman No. 1 filter paper at the same time with methyl ethyl ketone–water azeotrope were as follows: 2,3,5-tri-*O*-methyl-L-arabinose (0.80), 2,3-di-*O*-methyl-D-xylose (0.58), 2-*O*-methyl-D-xylose (0.16).

of insoluble material and fractionally precipitated by adding ether (100 ml.) followed by increasing amounts of petroleum ether. Each precipitated fraction was dissolved in acetone (highly viscous solutions were formed) and precipitated by pouring the solution into an excess of petroleum ether. The fractions obtained are shown in Table I.

The methylated product appeared from these results to be essentially homogeneous.

Hydrolysis of the Methylated Hemicellulose.—When a portion (0.966 g.) of fraction III was boiled for 8 hours with 2.9% methyl alcoholic hydrogen chloride (50 ml.) the solution showed $[\alpha]^{21}D$ +18° (final value). Heating for an additional four hours effected no further change in rotation.

The solution was concentrated *in vacuo* (no heat applied) to a sirup which was dissolved in 0.9 *N* hydrochloric acid (100 ml.) and the solution heated on the boiling water-bath for 19 hours when the final constant observed rotation was αD (1 dm.) +0.03°. The solution was neutralized ("Duolite A_4" anion-exchange resin) and concentrated *in vacuo* to dryness. Extraction with methanol afforded a sirup (0.897 g.).

Separation of the Cleavage Products Obtained from the Methylated Hemicellulose.—Paper chromatographic analysis of the mixture of sugars, obtained in the previous experiment using methyl ethyl ketone–water azeotrope as the developing solvent, revealed the presence of four components, the R_f values of which corresponded to 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose and D-xylose.

Analysis of the Mixture of Methylated Sugars. (a) By Paper Chromatography.—The mixture of methylated sugars (101 mg.) derived from the methylated polysaccharide (fraction II, 106 mg.) was dissolved in 20% aqueous methanol (10 ml.). An aliquot of this solution (0.1 ml.) containing approximately 1 mg. of the mixture of sugars was accurately transferred with a micropipet to a sheet (8″ × 22″) of Whatman No. 1 filter paper. A drop of the solution of the mixture of sugars was also put on the starting line in marginal strips ruled 1.25″ from either side of the chromatogram. The chromatogram was developed (descending technique) with methyl ethyl ketone–water azeotrope until the solvent front had almost reached the bottom of the paper. The chromatogram was dried in air and the marginal strips cut off and the four methylated sugars located by spraying in the usual way. The chromatogram was reassembled and those areas of the central unsprayed portion of the chromatogram containing the component methyl sugars were cut out and extracted separately with water. The amount of water used for extracting each methylated sugar was adjusted so that a 2-ml. aliquot contained approximately 10 μg. The extract was filtered through glass wool to remove cellulose fibers and an aliquot (2 ml.) treated with 80% aqueous phenol (0.1 ml.) followed by concd. sulfuric acid (5 ml.).[8,14] The mixture was shaken and the absorbance of the orange yellow color was determined by a Coleman Junior colorimeter when the solution had cooled to room temperature.

The absorbance was determined at λ 415 mμ for 2,3,5-tri-*O*-methyl-L-arabinose, at λ 480 mμ for 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose and D-xylose.

A blank experiment was carried out on a sheet of filter paper irrigated in the same way as the chromatogram in order to correct for the trace amounts of carbohydrates extracted from the filter paper that otherwise would affect the results.

By reference to standard curves, the relative amounts of the methyl sugars were found, in duplicate experiments, to be as follows: 2,3,5-tri-*O*-methyl-L-arabinose (13.8 moles), 2,3-di-*O*-methyl-D-xylose (24.1 moles), 2-*O*-methyl-D-xylose (7.0 moles) and D-xylose (4 moles). In additional experiments, the mole ratio of 2-*O*-methyl-D-xylose and D-xylose was checked further by developing the chromatogram until the monomethyl sugar was near to the bottom of the paper. This provided better separation of the two sugar bands and consequently more reliable results.

(b) By Column Chromatography.—The sirupy mixture of sugars (0.897 g. from fraction III) was extracted with acetone to give a sirup (0.709 g.). The acetone-insoluble material (0.19 g.) contained D-xylose. The acetone-soluble material was dissolved in methyl ethyl ketone–water azeotrope (1 ml.) and placed on the cellulose–hydrocellulose column for separation as described previously, using a mechanical fractionator and methyl ethyl ketone–water azeotrope as the developing solvent.[10] Sudan IV dye was used as the marker for the solvent front. Examination of the fractions furnished the results in Table II.

After all the 2-*O*-methyl-D-xylose had emerged from the column, the D-xylose was displaced by elution with a mixture of methyl ethyl ketone–water azeotrope (4 parts) and methanol (1 part).

Examination of the Cleavage Products of Methylated "Squeegee" Hemicellulose. (1) Identification of 2,3,5-Tri-*O*-methyl-L-arabinose.—The tri-*o*-methyl-L-arabinose (0.19 g.) was dissolved in water (10 ml.) and treated with bromine (0.5 ml.) at room temperature for 7 days when the solution was non-reducing to Fehling solution. The solution was freed from bromine by aeration, neutralized (Ag_2O), filtered and passed through a column of "Amberlite IR 120" cation-exchange resin. The eluate was concentrated *in vacuo* and the sirupy 2,3,5-tri-*O*-methyl-L-arabono-γ-lactone, after purification by extraction with ether, distilled giving 0.124 g., b.p. (bath temp.) 120°, 0.01 mm., $[\alpha]^{23}D$ −38°, initial value in water (*c* 1.0).

When treated with methanolic ammonia in the usual way the lactone gave 2,3,5-tri-*O*-methyl-L-arabonamide, m.p. and mixed m.p. 137°, $[\alpha]^{23}D$ +14° in water (*c* 1.0) (after crystallization from ethanol and from ethyl acetate–light petroleum ether).

(2) Identification of 2,3-Di-*O*-methyl-D-xylose.—Treatment of the di-*O*-methyl-D-xylose (0.2 g.) with aniline (0.15 ml.) in boiling ethanol (5 ml.) for 3 hours, followed by removal of solvent *in vacuo* gave a crystalline residue. Recrystallization from ethanol and from ethyl acetate–petroleum ether gave 2,3-di-*O*-methyl-D-xylose anilide, m.p. and mixed m.p. 126°, $[\alpha]^{24}D$ +183° in ethyl acetate (*c* 0.8), (no mutarotation).[15]

(3) Identification of 2-*O*-Methyl-β-D-xylose.—The crystalline material obtained from the column (tubes 138–148) was purified by several crystallizations from ethanol, m.p. 136°, $[\alpha]^{22}D$ −8° changing to +32.4°, equilibrium value in water (*c* 2.5). It gave no depression of the melting point when mixed with an authentic specimen of 2-*O*-methyl-D-xylose (m.p. 133°).

(4) Identification of D-Xylose.—Evaporation of the methyl ethyl ketone–water–methanol eluate furnished crystalline D-xylose (40 mg.) which was identified by conversion into its crystalline benzylidene dimethylacetal derivative, m.p. and mixed m.p. 207°, $[\alpha]^{20}D$ −10° in chloroform (*c* 0.8).[16]

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Examination of Two Unknown Components Obtained from the Methylated Polysaccharide.—During the column chromatographic analysis of the sugars from fraction III of the methylated polysaccharide described above two components, A and B, were isolated (see Table II). Component A (20.2 mg.) collected in tubes 28–35 had R_f 0.74, and showed $[\alpha]^{23}D$ −13.5° in methanol (*c* 0.6). Chromatographically, using methyl ethyl ketone–water azeotrope as the developing solvent, it differed from 2,3,4-tri-*O*-methyl-D-xylose (R_f 0.82), 2,3,4-tri-*O*-methyl-L-arabinose (0.58), 2,3,5-tri-*O*-methyl-L-arabinose (0.85), 2,3,4-tri-*O*-methyl-L-rhamnose (0.87), and from tetra-*O*-methyl derivatives of D-mannopyranose (0.79), D-galactofuranose (0.88), D-galactopyranose (0.68) and D-glucopyranose (0.83). This unknown compound was recovered unchanged after treatment with 2 *N* sulfuric acid for 13 hours on a boiling water-bath. A was therefore not a methylated oligosaccharide.

The unknown component B (46.6 mg.) (R_f 0.53 on methyl ethyl ketone–water azeotrope) was purified by paper chromatography to remove a small amount (5 mg.) of 2,3-di-*O*-methyl-D-xylose. The residue (41 mg.) obtained on removal of solvent was heated for 18 hr. with *N* sulfuric acid on a boiling water-bath. The solution was neutralized ($BaCO_3$), filtered and evaporated to dryness *in vacuo* giving a sirupy residue (40 mg.), $[\alpha]^{26}D$ −27° in water (*c* 1.1), which crystallized spontaneously m.p. 115°, $[\alpha]^{26}D$ −83° changing to −55° equilibrium value in water (*c* 1.0). The crystalline material (10 mg.) had the same R_f value (0.53) as the original sirup and it reduced boiling Fehling solution. It was not a ketose (modified anthrone test)[17] and from its melting point, rotation and chromatographic properties, it was readily distinguished from the 2,3-, 2,4-, 3,4- and 3,5-di-*O*-methyl derivatives of D-xylose. This crystalline component B failed to give a crystalline anilide and complete methylation of this sirupy anilide with silver oxide and methyl iodide also yielded a sirupy anilide.[14] Treatment of the latter with dilute acid furnished the fully methylated reducing sugar as a sirup which had R_f 0.74, using methyl ethyl ketone–water azeotrope as the developing solvent. This appeared from chromatographic analysis to be the same as the unknown component A. When this fully methylated sugar was demethylated with 48% hydrogen bromide[18] it underwent complete decomposition and the parent sugar was not recognized.

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[CONTRIBUTION FROM THE NATIONAL RESEARCH COUNCIL, MARITIME REGIONAL LABORATORY]

3,6-Anhydro-D-galactose as a Constituent of κ-Carrageenin[1]

BY A. N. O'NEILL

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Mercaptolysis of the κ-fraction of carrageenin has led to the isolation of the diethyl mercaptals of D-galactose and 3,6-anhydro-D-galactose. The latter was characterized by conversion to 3,6-anhydro-D-galactose phenylosazone and to 2,4,5-tri-*O*-*p*-nitrobenzoyl-3,6-anhydro-D-galactose dimethyl acetal. Further confirmation was obtained by comparison with authentic material synthesized from methyl α-D-galactopyranoside through the crystalline intermediates methyl 6-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside and methyl 3,6-anhydro-α-D-galactopyranoside. Spectrophotometric evidence has indicated that the 3,6-anhydro-D-galactose residues constitute about 24% of the κ-fraction of carrageenin.

Carrageenin[2,3] is the chief polysaccharide of the red alga *Chondrus crispus*, where it occurs as a structural material, and from which it can be isolated by hot water extraction. Polysaccharides with similar compositions and physical properties have been isolated from the closely related seaweeds *Gigartina stellata*[4] and *Chondrus ocellatus*.[5]

The heterogeneous nature of carrageenin preparations has long been suspected,[6–12] but only recently the polysaccharide has been separated into two definite components designated κ-carrageenin (40%) and λ-carrageenin (60%).[13]

The composition and constitution of this polysaccharide have been the subject of several investigations.[4–9,14–21] The present conception of structure is that of a highly branched backbone of D- and L-galactose residues joined through C_1 and C_3 with branching at C_6, to which are attached long chains of D-galactopyranose units joined in α-1,3-glycosidic linkages, with each galactose residue carrying a half-ester sulfate on C_4. This interpretation, however, does not take into account that, although the chief carbohydrate constituent of carrageenin is D-galactose, this sugar represents only 35–40% of the polysaccharide or about 60% of the organic matter. Fructose repeatedly has been reported to be present but neither fructose nor any of its derivatives ever has been isolated from carrageenin. Small quantities of D-glucose and D-xylose have been reported but these are considered impurities arising from contamination by floridean starch and a xylan. Young and Rice[19] isolated a crystalline derivative of 2-ketogluconic acid in about 3% yield, but this is now believed to be an artifact.

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