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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ST. PAUL, MINNESOTA

[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.

(1) Issued as N.R.C. No. 3585. Presented in part before the Division of Carbohydrate Chemistry of the American Chemical Society, September, 1954.

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It is thus apparent that there is a considerable portion of the polysaccharide as yet unidentified, and this communication reports the isolation from the κ or gel fraction of carrageenin of a heretofore unknown component which has been identified as 3,6-anhydro-D-galactose.

κ-Carrageenin has been shown by hydrolysis to contain 38.5% D-galactose. No L-galactose was present since all the galactose was fermented with yeast. The only other component in the hydrolysate was 5-hydroxymethyl-2-furaldehyde as shown by its characteristic ultraviolet absorption spectrum, and by comparison with authentic material on paper chromatograms. Control experiments showed that the H.M.F. did not arise from galactose. The presence of H.M.F. in carrageenin hydrolysates explains the ketose reactions formerly reported and the presence of formic acid which presumably arises from the further decomposition of the H.M.F.

From our previous studies it was known that κ-carrageenin consumed no periodate and therefore contained no contiguous hydroxyl groups. Unfractionated carrageenin was hydrolyzed by acid and degraded by heat[22] much more readily than would be expected of a normal galactan with galactose units joined through C_1 and C_3. When the polysaccharide was hydrolyzed with N hydrochloric acid at 30° two distinct first-order reactions were distinguished, an initial and very rapid hydrolysis followed by one that was relatively much slower. Almost 30% of the total reducing sugar was formed in the first 50 hours while the complete hydrolysis required over 500 hours.

The high sensitivity toward acid of both the unknown component and the polysaccharide is consistent with the presence of pyranose residues of 3,6-anhydro-D-galactose which are known to be sensitive toward acid.[23] The highly strained glycosidic bonds involving C_1 of these residues would be expected to undergo rapid hydrolysis under relatively mild conditions to give 3,6-anhydro-*aldehydo*-D-galactose which in turn would undergo a series of dehydrations with ring rearrangement to give H.M.F. Araki[24] has shown that methyl 3,6-anhydro-α-D-galactopyranoside is converted into H.M.F. by heating at 140° in 3% oxalic acid.

This hypothesis was verified by the observation that treatment of κ-carrageenin with ethyl mercaptan and hydrochloric acid afforded crystalline 3,6-anhydro-D-galactose diethyl mercaptal,[25] which in turn was transformed into 3,6-anhydro-*aldehydo*-D-galactose. The latter was characterizied as its osazone[23,26] and by conversion to the 2,4,5-tri-*O*-*p*-nitrobenzoyl dimethyl acetal derivative.[23] The strongly reducing 3,6-anhydro-*aldehydo*-D-galactose gave a positive Seliwanoff test. The presence of D-galactose in the mercaptolysis products of κ-carrageenin was demonstrated by the isolation of its crystalline diethyl mercaptal.

The amount of 3,6-anhydro-D-galactose could not be determined by mercaptolysis because of incomplete degradation, but it was accomplished by making use of the fact that the H.M.F. produced by the action of acid upon the 3,6-anhydro-D-galactose residues displays a strong selective absorption band the intensity of which could be measured spectrophotometrically. The results of these measurements indicated that κ-carrageenin contains about 24% of 3,6-anhydro-D-galactose, or approximately two of these units for every three sulfated D-galactose residues.

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Experimental

All melting points were taken on a Kofler micro melting point apparatus and are corrected.

Preparation of the Polysaccharide.—Carrageenin was isolated from washed *C. crispus* by hot water extraction at 80°, clarification and alcohol precipitation. Fractionation was accomplished by the slow addition of 1.0 M potassium chloride to a rapidly stirred solution of carrageenin at 25°. The initial concentration of the carrageenin solution was 0.1% and the final concentration of potassium chloride 0.15 M. The precipitated κ-fraction was centrifuged down, dialyzed and precipitated by alcohol. It was refractionated in the same manner to eliminate most of the occluded λ-material and finally was converted to the sodium salt; galactose, 38.5%; sulfate, 26.3%; $[\alpha]^{24}D$ +60.3° (c 0.3, water). The ultraviolet absorption spectrum of a neutralized acid hydrolyzate of this preparation had maxima at 2280 and 2850 Å. and was identical to that of 5-hydroxymethyl-2-furaldehyde.

Mercaptolysis of κ-Carrageenin.—A portion (10 g.) of the κ-fraction was weighed into a three-neck flask, fitted with a stirrer through a precision ground glass fitting. The flask was placed in an ice and water bath at 0° and concentrated hydrochloric acid (60 ml.) at 0° was added slowly. Rapid stirring was maintained throughout the addition and for 30 min. after when most of the lumps had disappeared and a consistent slurry was obtained. Ethyl mercaptan (25 ml.) was added dropwise. After the addition the mixture was stirred at 0° for 3 hr. when the temperature was increased to 12–14°. The material was stirred rapidly at this temperature for 96 hr., and 6 ml. of ethyl mercaptan were added after 24 hr.

The brown solution so obtained was poured with stirring into a suspension of 150 g. of lead carbonate in 250 ml. of ice and water. Frothing was reduced by the addition of a few drops of capryl alcohol. The precipitate was filtered and washed thoroughly with cold water. The colorless filtrate and washings were combined and saturated with hydrogen sulfide, and the lead sulfide was removed by filtration. The resulting acidic solution was deionized by shaking with exchange resins IR-100 and IR-4 B.[27]

Isolation of D-Galactose Diethyl Mercaptal.—The neutral solution was concentrated under reduced pressure to about 300 ml. when crystallization began. After several hours at 5° the crystals were filtered off and washed with cold water; yield 400 mg. This material was recrystallized from ethyl alcohol, m.p. 141–142°, not depressed by admixture with an authentic specimen of D-galactose diethyl mercaptal synthesized according to Wolfrom's modification of Fischer's method,[28] $[\alpha]^{25}D$ −4.8° (c 2.0, water). Concentration of the solution was repeated until no further crystals were obtained after standing overnight at 5°. The total yield of D-galactose diethyl mercaptal at this point was 2.28 g.

Anal. Calcd. for $C_{10}H_{22}O_5S_2$: C, 41.93; H, 7.74; S, 22.4. Found: C, 41.82; H, 7.55; S, 22.6.

Isolation of 3,6-Anhydro-D-galactose Diethyl Mercaptal.—The final filtrate from the above was continuously extracted

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with 250 ml. of ether for 18 hr. On cooling the ether solution deposited an additional 330 mg. of D-galactose diethyl mercaptal (m.p. 140–142°) bringing the total yield of this material to 2.61 g.

The resulting ether solution was concentrated and deposited crystalline material on cooling; yield 520 mg., m.p. 110–112°. This material was recrystallized from ethyl acetate containing a little petroleum ether; m.p. 112–113°, undepressed on admixture with an authentic specimen of 3,6-anhydro-D-galactose diethyl mercaptal, synthesized as described below, $[\alpha]^{25}D$ −10° (*c* 1.0, water), +26.8° (*c* 1.0, pyridine); final yield 1.48 g.

Anal. Calcd. for $C_{10}H_{20}O_4S_2$: C, 44.75; H, 7.51; S, 23.9. Found: C, 44.93; H, 7.36; S, 24.1.

3,6-Anhydro-*aldehydo*-D-galactose.—One hundred mg. of 3,6-anhydro-D-galactose diethyl mercaptal, dissolved in 5 ml. of water, was treated with 175 mg. of mercuric chloride and 300 mg. of cadmium carbonate for 6 hr. at 50°. The resulting mixture was filtered and the excess mercuric chloride was removed by extraction with ether. Concentration of the solution by distillation under reduced pressure yielded a sirup which reduced Fehling solution at 25°, gave the Seliwanoff reaction and restored the color to Schiff solution. On treatment in aqueous solution with phenylhydrazine hydrochloride and sodium acetate for 2 hr. at 80° it yielded a phenylosazone, which on recrystallization from methyl alcohol had m.p. 216–217°, $[\alpha]^{24}D$ + 71° (*c* 0.3, methyl alcohol). The melting point was undepressed on admixture with authentic 3,6-anhydro-D-galactose phenylosazone prepared from methyl 3,6-anhydro-α-D-galactopyranoside. Percival[26] reports m.p. 215° and $[\alpha]^{16}D$ +71° for this compound.

Anal. Calcd. for $C_{18}H_{20}O_3N_4$: C, 63.5; H, 5.9; N, 16.5. Found: C, 63.35; H, 5.66; N, 16.41.

2,4,5-Tri-*O*-*p*-nitrobenzoyl-3,6-anhydro-D-galactose Dimethyl Acetal.—3,6-Anhydro-*aldehydo*-D-galactose was converted through the sirupy dimethyl acetal into the crystalline 2,4,5-tri-*O*-*p*-nitrobenzoyl derivative according to the method of Haworth.[23] The compound melted at 111–112° in agreement with the value previously reported, and was undepressed on admixture with authentic material prepared in the same manner from methyl 3,6-anhydro-α-D-galactopyranoside.

Methyl 6-*O*-*p*-Tolylsulfonyl-α-D-galactopyranoside.—By the method described in reference 23, anhydrous methyl α-D-galactopyranoside (20 g.) was treated with 22 g. (1.1 moles) of *p*-toluenesulfonyl chloride. The yield of desired product was 5.6 g., m.p. 172–174° dec., $[\alpha]^{27}D$ +106° (*c* 1.2, pyridine).

Anal. Calcd. for $C_{14}H_{20}O_8S$: C, 48.3; H, 5.8; S, 9.2. Found: C, 48.48; H, 5.68; S, 8.9.

Methyl 3,6-Anhydro-α-D-galactopyranoside.—The above 6-tosyl derivative (3.0 g.) was converted into this compound by treatment in ethanol solution with sodium hydroxide,[23] to give 1.34 g. of crystalline methyl 3,6-anhydro-α-D-galactopyranoside, m.p. 139–140°, $[\alpha]^{26}D$ +80° (*c* 1.0, water).

Anal. Calcd. for $C_7H_{12}O_5$: C, 47.7; H, 6.8. Found: C, 47.93; H, 6.80.

3,6-Anhydro-D-galactose Diethyl Mercaptal.—Methyl 3,6-anhydro-α-D-galactopyranoside (500 mg.) was dissolved in 0.75 ml. of concentrated hydrochloric acid precooled to 0°. Ethyl mercaptan (0.5 ml.) was added and the mixture shaken at 0° for 90 min. The solution was diluted with ice and water and the crystalline 3,6-anhydro-D-galactose diethyl mercaptal filtered and washed with cold water. It was dried *in vacuo* over potassium hydroxide and recrystallized from ethyl acetate–petroleum ether; yield 485 mg., m.p. 112–113°, $[\alpha]^{26}D$ −9.1° (*c* 1.15, water), +27.0° (*c* 1.1, pyridine).

Anal. Calcd. for $C_{10}H_{20}O_4S_2$: C, 44.75; H, 7.51; S, 23.9. Found: C, 44.87; H, 7.55; S, 23.8.

Quantitative Determination of 3,6-Anhydro-D-galactose.—Ten 25-mg. samples of the polysaccharide were dissolved in 2 ml. of 0.15 *N* hydrochloric acid and hydrolyzed in sealed tubes at 100°. Tubes were removed at intervals during a 24-hr. period, the contents neutralized with barium carbonate and filtered quantitatively into 10-ml. volumetric flasks. The solutions were made up to volume and diluted 1 → 50. Measurements of optical density were made on these dilutions at 2850 Å. in a Beckman model DU spectrophotometer. Maximum production of H.M.F. was found at 8 hr. To account for the first-order decomposition[29] of H.M.F. to formic and levulinic acids during the hydrolysis, the log of the optical density was plotted against time and the straight line portion of the curve concerned with this decomposition was extrapolated to zero time. From the value of the intercept (0.09—corresponding to an optical density of 1.23) and the molar extinction coefficient (16,500)[30] it was calculated that the H.M.F. formed represented 18.7% of the κ-carrageenin which corresponds to 24% of 3,6-anhydro-D-galactose.

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HALIFAX, N. S., CANADA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, GENERAL MILLS, INC.]

Reactions of Long Chain Amines. V. Reactions with Sugars[1,2]

BY JOHN G. ERICKSON

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Aldohexoses react with primary long chain alkylamines at room temperature, giving N-alkylglycosylamines in good yields. Observations are offered regarding the stability and ease of hydrolysis of these compounds. Ketohexoses react with primary long chain amines at room temperature to form the corresponding N-alkylketosylamines, as well as substantial amounts of products formed from one mole of sugar and two moles of amine. At higher temperatures several moles of amine may react with one mole of either aldohexose or ketohexose. Reducing disaccharides may also react with more than one mole of primary amine. Possible structures for these products are discussed.

Hodge's[3] comprehensive survey of the browning reaction discusses many types of products formed by reactions of amines with sugars. The present paper deals with the reactions between primary long chain aliphatic amines and various kinds of sugars. Some of the products are of types not generally heretofore recognized.

Sorokin[4] prepared N-arylglycosylamines by heating amines and sugars in ethanol. When Mitts and Hixon[5] used this method to prepare glucosyl derivatives of primary long chain amines yields fell noticeably short of theoretical and there was much decomposition, as shown by the very dark color developed. Pigman, Cleveland, Couch and Cleveland[6] modified the procedure by using hot methanol

(1) Paper No. 173, Journal Series, General Mills, Inc., Research Dept.

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