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

Derivatives of 4-*O*-β-D-Galactopyranosyl-3,6-anhydro-D-galactose from κ-Carrageenin[1]

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Partial mercaptolysis of κ-carrageenin yielded the crystalline diethyl mercaptals of D-galactose and 3,6-anhydro-D-galactose. Acetylation of the sirup obtained after removal of the monosaccharide components and chromatographic resolution of the mixture of acetates on Magnesol has led to the isolation of a crystalline acetylated diethyl mercaptal of a disaccharide. Deacetylation of this disaccharide derivative followed by reductive desulfurization yielded 4-*O*-β-D-galactopyranosyl-1-deoxy-3,6-anhydro-D-galactitol which was characterized by acid hydrolysis and periodate oxidation. The significance of these results is discussed with respect to the molecular constitution of κ-carrageenin.

The polysaccharide, carrageenin, as usually obtained by extraction of the marine alga *Chondrus crispus* with hot water recently has been shown to be a complex mixture of at least five different polysaccharides.[2-4] The two main components separable by potassium chloride have been designated κ-carrageenin and λ-carrageenin. These differ in that the κ-component is composed of sulfated D-galactose and 3,6-anhydro-D-galactose residues[5] in the approximate ratio of 1.2:1, while λ-carrageenin is composed almost entirely of sulfated D-galactose.[4]

By methylation of unfractionated carrageenin, Percival[6-8] had previously established that C_3 of each D-galactose residue was involved in a linkage and that C_4 contained the half-ester sulfate group. Some of the D-galactose residues were joined through both C_3 and C_6 creating a branch. With the identification of 3,6-anhydro-D-galactose as a component of κ-carrageenin[5] it became of interest to establish the manner in which this residue is incorporated in the polymer.

The κ-carrageenin used in the present investigation contained D-galactose 38.1%, 3,6-anhydro-D-galactose 28.1%, sulfate (as SO_3Na) 28.0%, in the molecular proportions of 6:5:7, respectively. This material was subjected to partial mercaptolysis and, after removal of the diethyl mercaptals of D-galactose and 3,6-anhydro-D-galactose, the residue was acetylated. A crystalline compound I, m.p. 118–119° and $[\alpha]^{25}D$ −4.0° was obtained by chromatographic resolution of the mixture of acetates on Magnesol. Elementary analysis and the molecular weight indicated the formula $C_{28}H_{42}O_{15}S_2$. Deacetylation yielded a crystalline compound II with m.p. 116.5–117.5° and $[\alpha]^{25}D$ +4.0° which on acid hydrolysis gave D-galactose, ethyl mercaptan, 5-hydroxymethyl-2-furaldehyde and other degradation products of 3,6-anhydro-D-galactose. This, together with the analyses and molecular weight, establishes I as the hexaacetate diethyl mercaptal of a disaccharide composed of D-galactose and 3,6-anhydro-D-galactose.

The deacetylated compound II was subjected to reductive desulfurization and a crystalline compound, $C_{12}H_{22}O_9$ (III), with m.p. 135–136° and $[\alpha]^{25}D$ +12.5° was isolated. On hydrolysis this compound gave D-galactose and 1-deoxy-3,6-anhydro-D-galactitol, thereby establishing the reducing end of the parent disaccharide as 3,6-anhydro-D-galactose and the non-reducing end as D-galactose.

The linkage between the residues was established by periodate oxidation. A 1→5-glycosidic linkage almost certainly can be eliminated since this would require the 3,6-anhydro-D-galactose units in the polysaccharide in the furanose form, but in the galactose series the presence of both a furanose and hydrofuranol ring is sterically impossible. Periodate oxidation of mercaptals is not satisfactory. This was evident when D-galactose diethyl mercaptal was tried as a model compound. It consumed 7.2 moles of sodium metaperiodate in 6 hr. without any apparent break which would indicate that sulfur was being oxidized through the sulfoxide to the sulfone. Methyl 3,6-anhydro-α-D-galactopyranoside consumed no periodate as was to be expected if the anhydro ring is stable under the conditions of oxidation. The compound obtained by reductive desulfurization III was subjected to periodate oxidation and the results (Table I) show that this compound consumed two moles of sodium metaperiodate with the production of one mole of formic acid. Neither formaldehyde nor acetaldehyde was formed. This compound then must be 4-*O*-β-D-galactopyranosyl-1-deoxy-3,6-anhydro-D-galactitol. A similar disaccharide with a 1→2-glycosidic linkage would consume three moles of periodate. The configuration of the linkage is assumed to be β because of the low positive rotations of II and III and the change to a negative rotation in the acetate I.

TABLE I

OXIDATION OF COMPOUND III IN 0.0025 *M* SOLUTION WITH 0.04 *M* SODIUM METAPERIODATE AT 25°

Time, hr.	Moles per mole of substance: Oxidant consumed[a]	Formic acid produced
0.17	1.75	
.25	1.84	0.72
.5	1.92	.77
.75	1.94	
1.0	1.98	
1.25	1.98	
1.5		.90
2.0	1.98	
4.0	1.98	
5.0		.99
24.0		.99

[a] In the dark.

The molecular proportions of D-galactose and

(1) Issued as N.R.C. 3780.

(2) D. B. Smith and W. H. Cook, *Arch. Biochem. Biophys.*, **45**, 232 (1953).

(3) D. B. Smith, W. H. Cook and J. L. Neal, *ibid.*, **53**, 192 (1954).

(4) D. B. Smith, A. N. O'Neill and A. S. Perlin, *Can. J. Chem.*, **33**, 1352 (1955).

(5) A. N. O'Neill, THIS JOURNAL, **77**, 2837 (1955).

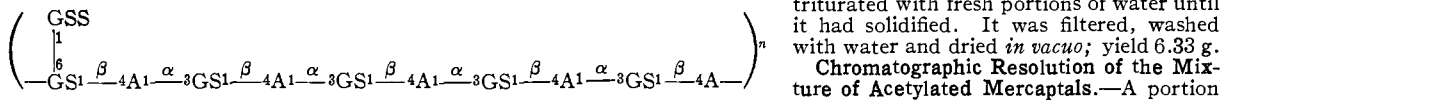
(6) J. Buchanan, E. E. Percival and E. G. V. Percival, *J. Chem. Soc.*, 51 (1943).

(7) E. T. Dewar and E. G. V. Percival, *ibid.*, 1622 (1947).

(8) R. Johnston and E. G. V. Percival, *ibid.*, 1994 (1950).

3,6-anhydro-D-galactose calculated on the basis of the weights of the isolated mercaptals is 1.1:1 in close agreement to the value 1.2:1 obtained directly by the analysis of the polysaccharide.

The high yield of the diethylmercaptal of 4-*O*-β-D-galactopyranosyl-3,6-anhydro-D-galactose suggests that it is unlikely that any of the 3,6-anhydro-D-galactose residues occur together in the chain. The simplest structure for κ-carrageenin might be



GS = D-galactopyranose-4-sulfate
GSS = D-galactopyranose-3,4-disulfate or D-galactopyranose-3,6-disulfate
A = 3,6-anhydro-D-galactopyranose

Previous methylation studies[8] on unfractionated carrageenin indicated that the polysaccharide possessed a branched structure. The present analytical data suggest, if we assume a uniform chain, that every tenth unit in the chain should be a point of branching. This agrees with the prediction of Dillon[9] from studies of methylation and periodate oxidation on a desulfated and degraded sample of unfractionated carrageenin. Analysis for sulfate suggests that some of the D-galactose residues are disubstituted. Since κ-carrageenin consumes no periodate, these are considered to be end groups.

Experimental

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

Mercaptolysis of κ-Carrageenin.[10]—Purified κ-carrageenin (10 g.) 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 (70 ml.) at 0° was added slowly with stirring until a thin slurry was obtained. After 40 minutes ethyl mercaptan (30 ml.) was added dropwise and the mixture stirred at 0° for 3 hr. when the temperature was increased to 12–14°. The material was stirred rapidly at this temperature for 48 hr.

The brown solution so obtained was poured with stirring into a suspension of 175 g. of lead carbonate in 250 ml. of ice and water. The mixture was filtered and the precipitate washed well with cold water. The colorless filtrate and washings were combined and saturated with hydrogen sulfide and the lead sulfide removed by filtration. The resulting acid solution was deionized by shaking with exchange resins Amberlites IR-100 and IR-4B.[11]

D-Galactose Diethyl Mercaptal.—The above neutral solution was concentrated under reduced pressure to about 200 ml. when crystallization began. After several hours at 5° the crystals were filtered and washed with cold water. Concentration of the solution was repeated until no further crystals were obtained after standing overnight at 5°, yield 1.78 g., m.p. 141–142° unchanged on admixture with D-galactose diethyl mercaptal, $[\alpha]^{25}D$ −4.7° (*c* 1.0, water).

3,6-Anhydro-D-galactose Diethyl Mercaptal.—The final filtrate obtained after removal of D-galactose diethyl mercaptal was continuously extracted with ether for 24 hr. On cooling to 25° the ether solution deposited an additional 350 mg. of D-galactose diethyl mercaptal bringing the total yield to 2.13 g.

The resulting ether solution was concentrated and on cooling to 5° deposited 0.71 g. of crystalline 3,6-anhydro-D-galactose diethyl mercaptal, m.p. 112–113°, $[\alpha]^{25}D$ −10° (*c* 1.0, water). An additional 1.12 g. was obtained from the mother liquors bringing the total yield of this material to 1.83 g.

Acetylation of the Residual Mercaptolysate.—The aqueous solution remaining after the above ether extraction was concentrated under reduced pressure to a thick sirup which was repeatedly dried by the distillation of added ethanol. It was finally dried in a vacuum desiccator over phosphorus pentoxide; yield 4.1 g.

The dried amorphous solid was dissolved in 40 ml. of pyridine and cooled to 0°. Acetic anhydride (30 ml.) was added with cooling and the whole allowed to remain at 5° for 4 days. The acetylation mixture was poured with stirring into 125 ml. of ice and water and the resulting sirup triturated with fresh portions of water until it had solidified. It was filtered, washed with water and dried *in vacuo;* yield 6.33 g.

Chromatographic Resolution of the Mixture of Acetylated Mercaptals.—A portion (200 mg.) of the above acetate mixture was chromatographed on a 190 mm. × 35 mm. (diam.) column of Magnesol–Celite (5/1 by wt.) by development with 1200 ml. of a mixture of benzene–*t*-butyl alcohol (100/1 by vol.). An alkaline permanganate streak on the extruded column indicated that most of the material was in a zone in the center of the column. This section was cut out and eluted with acetone. The sirupy material obtained by distilling off the acetone under reduced pressure was rechromatographed in the same manner until only a single zone was obtained. The compound thus purified crystallized from aqueous ethyl alcohol on seeding with a small amount of activated carbon. This crystalline material was used to seed a solution of the residual impure mixture of acetylated mercaptals in aqueous ethyl alcohol. Several recrystallizations from the same solvent produced pure material; yield 4.8 g., m.p. 118–119°, $[\alpha]^{25}D$ −4.0° (*c* 1.2, chloroform). An additional 400 mg. was obtained by chromatographing the residue on Magnesol–Celite.

Anal. Calcd. for $C_{28}H_{42}O_{15}S_2$: C, 49.3; H, 6.15; S, 9.38; mol. wt., 682. Found: C, 49.78; H, 6.09; S, 9.33; mol. wt., 678 (ebullioscopic in butanone).

Deacetylation of the Mixture of Acetylated Mercaptals.—The above crystalline material (1.5 g.) was dissolved in 15 ml. of absolute methyl alcohol and 4.5 ml. of a solution of sodium methoxide (0.5 g. of sodium in 100 ml. of absolute methyl alcohol) was added. The solution was allowed to stand at 5° for 18 hr. when it was neutralized with dilute acetic acid and deionized by shaking with exchange resins Amberlites IR-100 and IR-4B. It was concentrated under reduced pressure to a sirup which was dried in a vacuum desiccator over phosphorus pentoxide. The material dried in this manner crystallized from an ethanol–ether mixture and was recrystallized from the same solvent; yield 774 mg., m.p. 116.5–117.5°, $[\alpha]^{25}D$ +4.0° (*c* 1.8, water). Hydrolysis with 0.2 *N* sulfuric acid for 18 hr. at 100° yielded D-galactose, ethyl mercaptan, 5-hydroxymethyl-2-furaldehyde and other degradation products of 3,6-anhydro-D-galactose. The H.M.F. was identified by paper chromatography and by its ultraviolet absorption spectrum, and the D-galactose by isolation of the crystalline material after removal of the other compounds by adsorption on carbon.

Reductive Desulfurization.—An amount of 400 mg. of the above deacetylated compound was dissolved in 10 ml. of 70% ethanol and 4 to 6 g. of freshly prepared W-4 Raney nickel catalyst[12] added. The mixture was refluxed for 3.5 hr. after which the nickel was filtered off and thoroughly washed. The filtrate and washings were concentrated under reduced pressure to a sirup. This was dissolved in a small amount of water, deionized by shaking with exchange resins and decolorized with charcoal. After filtration the solution was again concentrated under reduced pressure to a sirup which was further dried *in vacuo*. This material was crystallized from absolute ethanol and recrystallized from the same solvent; yield 166 mg., m.p. 135–136°, $[\alpha]^{25}D$ +12.5° (*c* 1.0, water).

Anal. Calcd. for $C_{12}H_{22}O_9$: C, 46.4; H, 7.1. Found: C, 46.6; H, 6.9.

Hydrolysis of this material with 0.5 *N* sulfuric acid for 20 hr. at 100° yielded D-galactose and 1-deoxy-3,6-anhydro-D-galactitol. The latter compound was identified by comparison on paper chromatograms with authentic material synthesized by reductive desulfurization of 3,6-anhydro-D-galactose diethyl mercaptal. This material was obtained as a sirup and has not yet been crystallized.

(9) T. Dillon and P. O'Colla, *Proc. Roy. Irish Acad.*, **54B**, 51 (1951).

(10) For the preparation of this material see reference 5. It was further purified by dialysis and several reprecipitations from aqueous solution with ethyl alcohol.

(11) Products of the Rohm and Haas Co., Philadelphia, Penna.

(12) A. A. Pavlic and H. Adkins, THIS JOURNAL, **68**, 1471 (1946).

Periodate Oxidation.—The above compound, obtained by reductive desulfurization, in a concentration of 0.0025 *M* was oxidized with 0.04 *M* sodium metaperiodate at 25° in the dark. Samples were taken at intervals and the periodate consumed was determined by the arsenite method. Formic acid was estimated, after destruction of excess periodate with ethylene glycol, by titration with 0.01 *M* sodium hydroxide with phenolphthalein as indicator. The absence of formaldehyde and acetaldehyde was shown by no reaction with dimedone.

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[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

D-*glycero*-D-*allo*-Heptose, L-*allo*-Heptulose, D-*talo*-Heptulose and Related Substances Derived from the Addition of Cyanide to D-Allose[1]

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The addition of cyanide to D-allose (I) has led to the production of D-*glycero*-D-*allo*-heptose (VI) and its non-crystalline epimer V. Hydrogenation of these aldoheptoses and oxidation of the heptitols thus obtained by *Acetobacter suboxydans* yielded L-*allo*-heptulose (X) and D-*talo*-heptulose (IX). D-*allo*-Heptulose (IV) was prepared by the rearrangement in alkali of the epimeric aldoheptoses.

Oxidation by *Acetobacter suboxydans* of heptitols which have the favorable D-*erythro* configuration for the terminal triol group has been utilized in this Laboratory to prepare L-*galacto*-heptulose (perseulose), L-*gluco*-heptulose, D-*manno*-heptulose, D-*altro*-heptulose (sedoheptulose), L-*gulo*-heptulose and D-*ido*-heptulose.[2] We now wish to report that the procedure has been successfully employed to obtain the last remaining heptuloses which can be prepared in this manner, *viz.*, crystalline L-*allo*-heptulose (X) from *meso*-*glycero*-*allo*-heptitol (VIII) and crystalline D-*talo*-heptulose (IX) from the epimeric D-*glycero*-D-*altro*-heptitol (synonym, D-*glycero*-L-*allo*-heptitol) (VII). Neither these ketoses nor their enantiomorphs have been obtained previously. We have thus completed the catalog of heptulose configurations[3] and have reconfirmed the *Acetobacter* specificity rule of Bertrand as extended to *A. suboxydans* by Hann, Tilden and Hudson. Our co-equal interest in these sugars—their behavior in hot, aqueous acid—will be considered in a subsequent publication.[4]

The addition of hydrogen cyanide to D-allose followed by the usual hydrolysis yielded a pair of epimeric acids which were conveniently separated as their lactones when one of the epimers was found to crystallize readily. The phenylhydrazide of the temporarily non-crystalline lactone was prepared from the mother liquor, and this compound likewise was obtained epimerically pure after one or two recrystallizations.

A phenylhydrazide was prepared from the crystalline (*"allo"*) lactone III in the usual manner in order that the "phenylhydrazide rule" might be applied. The (*"altro"*) phenylhydrazide which was obtained originally was decomposed to yield the corresponding lactone II. This compound likewise crystallized. Thus it appears that the fortuitous separation of epimers is not necessarily to be expected on repetition of this procedure.

In order to assign the correct configuration to the lactones we adduce the evidence and arguments which follow. The lactone which first crystallized—D-*glycero*-D-*allo*-heptonolactone—showed $[\alpha]^{20}D$ +3.9°, while its epimer showed $[\alpha]^{20}D$ +26.3°. Hudson[5] calculated the values −10 and +26°, respectively, for these lactones. More significantly, the phenylhydrazides showed $[\alpha]^{20}D$ (*"allo"*) +16.7° and (*"altro"*) −22°; these values justify our assignment of configuration at C2 in accordance with the "phenylhydrazide rule."[6] Finally, sodium amalgam reduction of the *"allo"* lactone yielded a crystalline sugar (VI) which was further reduced to an optically inactive (and therefore *meso*-*glycero*-*allo*-) heptitol (VIII).

The preparation of D-*allo*-heptulose (IV) is announced because of some interest in the compound. A fuller report is planned.

Experimental

D-*glycero*-D-*allo*-Heptono-γ-lactone (III).—D-Allose (I)[7] (45 g.) was treated with sodium cyanide (27 g.) using Hudson's modification of the cyanohydrin synthesis.[8,7] After standing at 5° for 20 hours, the solution was boiled to hydrolyze the nitriles and expel ammonia. Sodium ions were removed by passage of the solution through a cation-exchange resin (Amberlite IR-120-H), and the eluate was concentrated *in vacuo* to a thick sirup and then heated *in vacuo* for one hour at 70–80° to effect lactonization. The sirup was dissolved in 120 ml. of absolute ethanol and placed in the refrigerator. After several days 26.6 g. (51%) of crystalline material was obtained. After recrystallization from 8 parts of 95% ethanol, the small, water-white prisms melted at 147.5–148.5° and showed $[\alpha]^{20}D$ +3.94° in water (*c* 8.5) with no evidence of mutarotation.

Anal. Calcd. for $C_7H_{12}O_7$: C, 40.38; H, 5.81. Found: C, 40.47; H, 5.85.

D-*glycero*-D-allo-Heptonic Phenylhydrazide.—A solution of 2 g. of the heptonolactone III and 2 ml. of phenylhydra-

(1) Presented in part before the Division of Carbohydrate Chemistry at the New York Meeting of the American Chemical Society, September 17, 1954.

(2) For a short review of this work, as well as pertinent references, see J. W. Pratt N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **74**, 2210 (1952).

(3) With the preparation of D-*allo*-heptulose also announced in this paper, only D-*gulo*, L-*ido*- and L-*talo*-heptuloses remain unknown.

(4) In a preliminary experiment (see Abstracts of Papers, New York Meeting of the American Chemical Society, Sept. 12–17, 1954, p. 22D) we have found that L-*allo*-heptulose is transformed to the extent of about 50% to a non-reducing anhydride which has been characterized as its crystalline tetraacetate (m.p. 116°, $[\alpha]^{20}D$ +46° in chloroform). D-*talo*-Heptulose appears to produce about 10% of an anhydride under the same conditions.

(5) C. S. Hudson, THIS JOURNAL, **61**, 1525 (1939).

(6) P. A. Levene, *J. Biol. Chem.*, **23**, 145 (1915); P. A. Levene and G. M. Meyer, *ibid.*, **31**, 623 (1917); C. S. Hudson, THIS JOURNAL, **39**, 462 (1917).

(7) J. W. Pratt and N. K. Richtmyer, *ibid.*, **77**, 1906 (1955).

(8) C. S. Hudson, *ibid.*, **73**, 4498 (1951).