Partial Methanolysis of the Mucilage of Chondrus Ocellatus Holmes[1]

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(Received April 26, 1956)

The mucilage of *Chondrus ocellatus Holmes* closely resembles carrageenin, the mucilage of *Chondrus crispus*, in properties and chemical structure[2]. Both mucilages are the metallic salts of sulfate esters of polysaccharides containing D-galactose residues, which are connected through their positions 1 and 3[3]. The galactoside linkages were inferred to be of the α-type[3b,4]. Small amounts of L-galactose[5], D-glucose[6] and 2-keto-D-gluconic acid[7] were also reported to be present in one of the mucilages. Recently, O'Neil found the presence of an appreciable amount of 3, 6-anhydro-D-galactose in the mucilage of *C. crispus*[8], using the mercaptolysis method which had been developed by the present authors in the investigation of agar[9]. The anhydro-sugar found is the enanthiomorph of that present in agar, and the result obtained by O'Neil was confirmed by Percival[10]. On the other hand, the present authors succeeded in the isolation of agarobiose dimethylacetal(4-*O*-β-D-galactopyranosyl-3, 6-anhydro-L-galactose dimethylacetal) in a high yield by the partial methanolysis method from agar[11]. With this in mind, the method has been applied to the mucilage of *C. ocellatus*, and the results obtained are reported herein.

The mucilage has been prepared by extraction of *C. ocellatus Holmes* (*f. canaliculatus* Okam.) with hot water and precipitation of the extract with ethanol. The product showed a specific rotation $[\alpha]_D+54°$ in water, and contained 24% sulfate. The partial methanolysis has been carried out in a manner similar to that used for agar[11]. After saponification the methanolysate was deionized with ion exchange resins. The acidic product, absorbed by the anion resin, was eluted from the resin and was isolated as a barium salt. The sole product obtained was barium monomethylsulfate arising from the sulfate residue present in the mucilage. The deionized methanolysate was separated by column chromatography, which led to the isolation of the following products in the yields indicated by weight percentages based on the deionized material: methyl D-galactoside(IV, 6%), 3,6-anhydro-D-galactose dimethylacetal (V, 5%), 4-*O*-β-D-galactopyranosyl-3, 6-anhydro-D-galactose dimethylacetal (III, 68%) and methyl 4-*O*-β-D-galactopyranosyl-3, 6-anhydro-β-D-galactoside (II, 5%).

4-*O*-β-D-Galactopyranosyl-3, 6-anhydro-D-galactose dimethylacetal (III) could not be crystallized, but it was characterized as its crystalline hexa-acetate (VI) having the formula $C_{12}H_{14}O_9(COCH_3)_6(OCH_3)_2$. Saponification of the acetate regenerated the non-crystallizable dimethylacetal (III), which was then hydrolysed with a 0.02 N oxalic acid solution to give a reducing disaccharide (I). Its phenylosazone was prepared in a crystalline condi-

1) After this article had been submitted to be read at the 9th Annual Meeting (1956) of the Chemical Society of Japan, the authors saw O'Neil's report of the isolation of 4-O-β-D-galactopyranosyl-3, 6-anhydro-D-galactose diethylmercaptal from the mercaptolysis product of carrageenin; A.N. O'Neil, *J. Am. Chem. Soc.*, 77. 6324 (1955).

2) T. Mori, *Advances in Carbohydrate Chemistry*, 8, 315 (1953).

3) a) T. Mori and T. Tsuchiya, *J. Agr. Chem. Soc. Japan*, 14. 585 (1941). b) R. Johnston and E.G.V. Percival, *J. Chem. Soc.*, 1950, 1994.

4) T. Mori and S. Fumoto, *J. Agr. Chem. Soc. Japan*, 23, 81 (1949).

5) C. Araki and K. Arai, Collected Papers for the Celebration of the 45th Anniversary of the Founding of Kyoto Technical College, 80 (1948).

6) A. Müther and B. Tollens, *Ber.*, 37, 298 (1904); P. Haas and B. Russell-Wells, *Biochem. J.*, 23, 425 (1929).

7) E.G. Yong and F.A.H. Rice, *J. Biol. Chem.*, 164, 35 (1946).

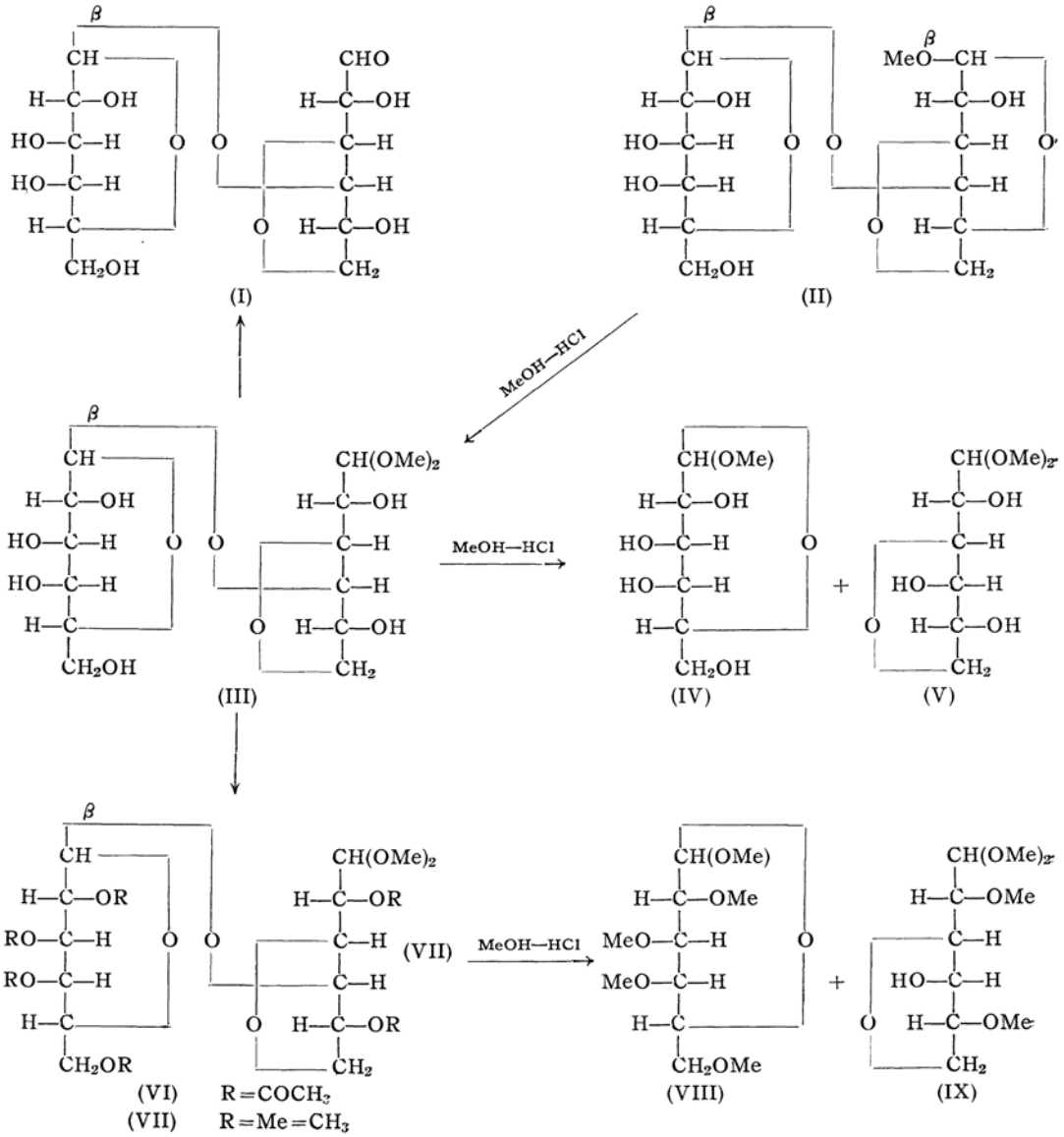
8) A.N. O'Neil, *J. Am. Chem. Soc.*, 77, 2837 (1955).

9) a) C. Araki and S. Hirase, This Bulletin, 26, 463 (1953). b) S. Hirase and C. Araki, ibid., 27, 105 (1954).

10) E.E. Percival, *Chem. & Ind.*, 1954, 1487.

11) C. Araki and S. Hirase, This Bulletin, 27, 109 (1954).

tion. The structure assignment to III has arisen for the following reasons. Methanolysis afforded methyl D-galactoside (IV) and 3,6-anhydro-D-galactose dimethylacetal (V), indicating that III is composed of D-galactose and 3,6-anhydro-D-galactose residues. On the other hand, the hexa-acetate (VI) was treated with dimethylsulfate and potassium hydroxide, and was further methylated with Purdie's reagents. When the resulting methylated dimethylacetal (VII) was subjected to methanolysis, there was obtained a mixture of methyl 2,3,4,6-tetra-*O*-methyl-D-galactoside (VIII) and 2,5-di-*O*-methyl-3,6-anhydro-D-galactose dimethylacetal (IX). The controlled hydrolysis converted only the latter product into the corresponding reducing sugar, from which the former product was separated by extraction with petroleum ether. Methyl 2,3,4,6-tetra-*O*-methyl-D-galactoside obtained was hydrolysed to give the corresponding reducing sugar, which was identified as its well known anilide. 2,5-Di-*O*-methyl-3,6-anhydro-D-galactose was oxidized with bromine water to give crystalline 2,5-di-*O*-methyl-3,6-anhydro-D-galactonic acid, the identity of which was established by comparison with its enanthiomorph previously obtained from methylated agarobiose derivatives[9b,12]. The amide was also prepared in a crystalline

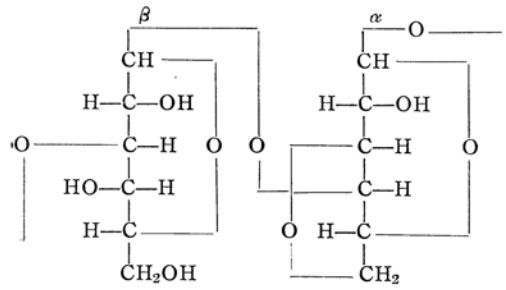


12) C. Araki, *J. Chem. Soc., Japan*, 65 533, 627 (1944).

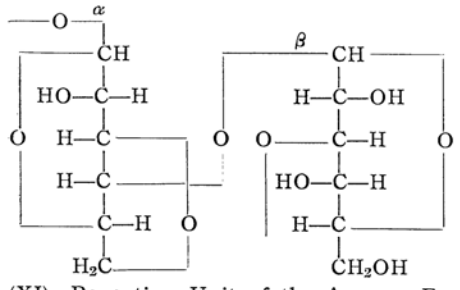
condition. This result has indicated that the D-galactose residue is glycosidically joined with the position 4 of the 3,6-anhydro-D-galactose residue. Moreover, from the low positive value of the specific rotation of III, it has been suggested that the galactoside linkage has the β-configuration, in disagreement with the inference so far drawn[3b,4].

Methyl 4-*O*-β-D-galactopyranosyl-3,6-anhydro-β-D-galactoside (II) was isolated as crystalline monohydrate, having the formula $C_{12}H_{19}O_9(OCH_3)$. On treatment with 0.5% methanolic hydrogen chloride for a short period, it afforded the foregoing dimethylacetal (III), in agreement with the structure (II) assigned. In addition, on the basis that the methyl glycoside (II) has a highly negative value of the specific rotation ($[\alpha]_D$ −78.6° in water), the β-configuration has been assigned to the methoxyl group.

From the high yield, in which the disaccharide (I) has been isolated as its dimethylacetal (III) and methyl glycoside (II), and also from the fact that D-galactose residues are connected by 1,3-linkages[3], it necessarily follows that the most part of the carbohydrate of the *Chondrus* mucilage employed is composed of such repeating units as are illustrated in the formula (X), the α-configuration being assigned to the 3,6-anhydro-D-galactoside linkages because of the positive value of the specific rotation of the mucilage.



(X) Repeating Unit of the Carbohydrate of the *Chondrus* Mucilage.



(XI) Repeating Unit of the Agarose Fraction of Agar[13]

The repeating unit (X) suggested above forms a novel contrast with that (XI) of the agarose fraction of agar[13], because they are of "half-antipode" with each other. Since the parent disaccharide of XI was isolated from agar and termed agarobiose[12], it is now suggested that the parent disaccharide (I) of X is conveniently named carrabiose. Accordingly, the dimethylacetal (III) and methyl glycoside (II) isolated in this work are respectively called carrabiose dimethylacetal and methyl β-carrabioside.

Last, it should be emphasized that further investigation is needed to establish the end group and the molecular shape as well as the disposition of sulfate groups of the mucilage.

Experimental

General Procedure.—Evaporation and concentration were carried out under reduced pressure below 40°. The Melting points are uncorrected. Specific rotations were measured in aqueous solutions unless otherwise stated. The paper chromatograms were irrigated with *n*-butanol-ethanol-water (4 : 1 : 2 by volume) in the ascending manner, and were sprayed with *o*-aminophenol reagent[14] unless otherwise stated.

Material.—The mucilage was extracted from sun-dried *C. ocellatus Holmes* (*f. canaliculatus* Okam.) (30 g.) with hot water (3 *l.*). After filtration through a Celite-bed, the extract was concentrated and precipitated by 95% ethanol. The product was obtained as fibres, which were then ground to powder; yield 15.8 g.; $[\alpha]_D^{15}$ +53.7° (*c*, 0.54); SO_4, 24.14; Ash, 17.34; SO_4 in ash, 7.25%, of the mucilage on the moisture-free basis, respectively.

Partial Methanolysis.—The mucilage (20.0 g., moisture 11.32%) was suspended in 0.5% methanolic hydrogen chloride (200 cc.). After being left overnight at room temperature, the suspension was heated under reflux for two hours and filtered. The filtrate was neutralized with silver carbonate, refiltered, and concentrated to a sirup (A). The insoluble material (5.2 g.), which had escaped the first methanolysis, was again subjected to methanolysis in a similar manner. An insoluble material (2.5 g.) and a sirup (B) were obtained, respectively.

The sirups A and B were combined, dissolved in a 0.3 N barium hydroxide solution (300 cc.), and saponified at 60° for two hours. Excess barium hydroxide was removed by neutralization with carbon dioxide and filtration, and the filtrate was concentrated to a sirup; yield 17.0 g.; $[\alpha]_D^7$ +17.7° (*c* 1.13). It was dissolved in water (200 cc.) and passed through a column of Amberlite IR-120 (200 cc.) to remove cations and then through a column of Amberlite IR-4B (250 cc.), where anions were absorbed. The resins were thoroughly washed with water, and the neutral effluent and

13) C. Araki, This Bulletin, **29**, 543 (1956).
14) S. Hirase, C. Araki and S. Nakanishi, This Bulletin, **26**, 183 (1953).

washings were combined and concentrated to a sirup; yield 9.0 g., $[\alpha]_D^9$+29.3°. Paper chromatographic examination revealed two distinct spots having respective R_f values 0.67 and 0.34.

Meanwhile, anions, which were retained by the IR-4B resin, were displaced from the resin by stirring it for fifteen minutes with excess 2 N sulfuric acid under ice-cooling. After filtration the resin was washed with water (1 *l.*). The combined filtrate and washings were immediately neutralized with saturated barium hydroxide solution and filtered. Evaporation gave crystals of barium monomethylsulfate dihydrates; yield 7.8 g. No evidence was obtained to suggest the presence of uronic acid derivatives and other organic acids.

Chromatography of the Deionized Methanolysate.—The deionized methanolysate (9.0 g.), dissolved in water (200 cc.), was placed on a column (5.0×26 cm.) of active carbon Shirasagi[15]-Celite (4:3 by weight), and developed successively with water, 5%, 10%, 15% and 30% ethanol at room temperature (10–12°), according to Whistler and Durso[16]. The effluent was grouped into six fractions containing polarimetrically and chromatographically identical materials. Table I shows the yields and properties of the fractions obtained.

Fraction I was rechromatographed on a column (2.5×30 cm.) of powdered filter paper, *n*-butanol-water (6:1 by volume) being used as a mobile phase until the components showing R_f values 0.67 and 0.34 were successively removed from the column. The column was then washed with 80% methanol (300 cc.). The eluates were separately evaporated to dryness, and three fractions Ia, Ib and Ic were obtained (Table II).

TABLE I

CHARCOAL CHROMATOGRAPHY OF THE DEIONIZED METHANOLYSATE OF THE CHONDRUS MUCILAGE

Fraction	Eluted by	Yield, g.	$[\alpha]_D$	R_f value
I	W (6 *l.*)	1.3	+64.9	0.34, 0.67
II	5%-E (0.7 *l.*)	0.7	+19.3	0.34, 0.67
III	5%-E (12 *l.*)	5.1	+18.1	0.34
IV	10%-E (6 *l.*)	0.6	−36.2	0.34
V	15%-E (5 *l.*)	0.4	+55.0	0.26
VI	30%-E (5 *l.*)	0.7	+60.0	0.18

W: water; E: ethanol.

TABLE II

RECHROMATOGRAPHY OF FRACTIONS I AND II

Fraction	Yield g.	$[\alpha]_D$	OCH_3%	R_f value
Ia	0.37	+26.0	28.2	0.67
Ib	0.36	+22.0	16.9	0.34
Ic	0.54	+105.0	15.8	0.31*
IIa	0.09	+27.0	28.6	0.67
IIb	0.61	+21.0	16.9	0.34

* The spot was located by a lead tetraacetate reagent[17].

15) Product of Takeda Pharmaceutical Industries, Ltd., Osaka.

16) R.L. Whistler and D.F. Durso, *J. Am. Chem. Soc.*, 72, 677 (1950).

17) J.G. Buchanan, C.A. Dekker and A.G. Long, *J. Chem. Soc.*, 1950, 3162.

Fraction II was also rechromatographed in the same way as above, giving two fractions IIa and IIb. In this case, the 80% methanolic eluate left no material on evaporation.

Fractions III and IV were chromatographically pure, and respectively treated in the manner described later. Fractions V and VI are not treated in this communication.

3, 6-Anhydro-D-galactose Dimethylacetal.—Data listed in Table II indicate that fractions Ia and IIa, obtained as colorless sirups, are 3, 6-anhydro-D-galactose dimethylacetal. It was hydrolysed with 0.02 N sulfuric acid in a boiling water bath for two hours, producing 3, 6-anhydro-D-galactose, $[\alpha]_D^{10}$+21.3° (*c*, 0.75), which was then converted into the diphenylhydrazone in the usual manner[18]; m.p. 153–155°; $[\alpha]_D^{14}$ + 34.5° (initial) ⟶ + 23.6° (after twenty-four hours, methanol, *c*, 0.55). Accepted values[18] for 3, 6-anhydro-D-galactose diphenylhydrazone are m.p. 154° and $[\alpha]_D$+23.9° (after twenty-four hours, methanol).

Anal. Found: C, 65.68; H, 6.25; N, 8.11. Calcd. for $C_{18}H_{20}O_4N_2$: C, 65.82; H, 6.14; N, 8.34%.

Methyl D-Galactoside.—Fraction Ic, a sirup, was a mixture of α- and β-forms of methyl D-galactoside, from which the α-form was crystallized as monohydrates from ethanol-ethyl acetate; yield 0.21 g., m.p. and mixed m.p. 109°; $[\alpha]_D^{10}$+175° (*c*, 1.00). The mother liquor was concentrated and subsequently hydrolysed with N sulfuric acid, affording D-galactose only; m.p. and mixed m.p. 165°; $[\alpha]_D^{10}$+80.4° (*c*, 0.51).

Methyl β-Carrabioside(II).—Fraction IV crystallized spontaneously. Recrystallization twice from ethanol-acetone afforded monohydrates of II; needles; m.p. 136-138°; $[\alpha]_D^9$−78.6° (*c*, 0.70).

Anal. Found: C, 43.56; H, 6.60; OCH_3, 8.76. Calcd. for $C_{12}H_{19}O_9(OCH_3)\cdot H_2O$: C, 43.81; H, 6.79; OCH_3, 8.70%.

It migrated at the same rate as carrabiose-dimethylacetal on a paper chromatogram. Since the fraction IV produced a single spot on a paper chromatogram, it has been considered that the fraction is a mixture composed of the dimethylacetal (0.18 g.) and the methyl glycoside (0.42 g.), the proportion being calculated from the values of a specific rotation.

Conversion of Methyl β-Carrabioside into the Dimethylacetal(III).—Methyl β-carrabioside monohydrate (0.15 g.) in 0.5% methanolic hydrogen chloride (15 cc.) was heated under reflux. The specific rotation of the solution was rapidly changed from the initial value −76° to a constant value +30° after thirty minutes. The solution was neutralized with silver carbonate, filtered and evaporated to dryness. Carrabiose dimethylacetal was obtained as an amorphous powder; yield 0.15 g., $[\alpha]_D^{12}$+26.4° (*c*, 0.34); OCH_3, found: 17.02%, (calcd. for $C_{12}H_{20}O_9\cdot(OCH_3)_2$: 16.74%).

18) C. Araki and K. Arai, Collected Papers for the Celebration of the 45th Anniversary of the Founding of Kyoto Technical College, 84 (1948).

For identification, the dimethylacetal was converted into its hexa-acetate, m. p. 147–149°, $[\alpha]_D^{16}$ −16.0° (benzene, *c*, 1.00). Its m. p. was not depressed on admixture with the sample described below.

Hexa-*O*-acetyl-carrabiose Dimethylacetal(VI). —Fractions Ib, IIb and III, having the same properties, were combined and purified by dissolution in absolute ethanol and filtration. Evaporation afforded carrabiose dimethylacetal in an amorphous condition; yield 6.0 g.; $[\alpha]_D^{10}$+19.0° (*c*, 1.00); OCH_3, found: 16.58 (calcd. for $C_{12}H_{20}O_9(OCH_3)_2$: 16.74%).

It was then dissolved in pyridine (30 cc.) and acetylated with acetic anhydride (60 cc.) at room temperature for two days. The reaction mixture was poured into ice-water with stirring, and the crystals deposited were filtered, washed with water, and dried in vacuo; yield 9.0 g. Two recrystallizations from ethanol-water gave pure needles of VI; m. p. 147–149°; $[\alpha]_D^{16}$ −16.3° (benzene, *c*, 1.23); ±0° (chloroform, *c*, 1.22).

Anal. Found: C, 50.42; H, 6.19; CH_3CO, 41.41; OCH_3, 9.92%; M. W. (Rast), 620. Calcd. for $C_{12}H_{14}O_9(CH_3CO)_6(OCH_3)_2$: C, 50.16; H, 6.15; CH_3CO, 41.49; OCH_3, 9.96%; M.W., 622.

Saponification of Hexa-*O*-acetyl-carrabiose Dimethylacetal.—The hexa-acetate (VI) (5.0 g.) was suspended in absolute methanol (20 cc.), and a sodium methoxide solution (5 cc.), prepared by dissolving metallic sodium (0.2 g.) in absolute methanol (25 cc.), was added. The crystals of the acetate were soon dissolved. After being left overnight, the solution was carefully neutralized with dilute acetic acid, deionized with ion exchange resins, and evaporated to dryness. Carrabiose dimethylacetal (III) was obtained as a hygroscopic amorphous powder, which could not be crystallized; yield 3.0 g.; $[\alpha]_D^{11}$ +25.0° (*c*, 1.32), +28.0° (methanol, *c*, 1.42); OCH_3, found: 16.69% (calcd. for $C_{12}H_{20}O_9(OCH_3)_2$: 16.74%). It migrated on a paper chromatogram at the same rate as agarobiose dimethylacetal (Rf, 0.34).

Carrabiose(I).—The dimethylacetal (1.0 g.), obtained by saponification of the acetate, was hydrolysed with a 0.02 N oxalic acid solution (50 cc.) in a boiling water bath until the optical rotation of the solution reached a constant value: $[\alpha]_D$ +25.0° (initial); +11.0° (0.5 and 1.0 hour); +12.5° (1.5 hour); +14.0° (2.0 and 2.5 hours). The solution was neutralized with calcium carbonate, filtered, and concentrated to a sirup, which was purified by dissolution in absolute methanol and filtration. Evaporation gave carrabiose as an amorphous material; yield 0.8 g., $[\alpha]_D^{11}$ +15.6° (an equilibrium value, *c*, 1.28). It slowly reduced a Fehling's solution at room temperature, showed a strong Seliwanoff's reaction, and restored the color to a Schiff's reagent. The last observation suggests that the sugar seems to exist in an aldehyde form.

Phenylosazone.—The above disaccharide (0.5 g.) was dissolved in water (10 cc.) and phenylhydrazine (0.5 g.) and 50% aqueous acetic acid (1 cc.) were added. The mixture was heated in a boiling water bath for thirty minutes, at which time oily precipitates were removed by decantation. Additional phenylhydrazine (0.3 g.) was added to the supernatant, and the solution was again heated for one and a half hours. On cooling it deposited yellow precipitates, which were filtered, washed with cold ethanol, and recrystallized three times from aqueous ethanol. The osazone was obtained as yellow needles in a poor yield; m. p. 216°; $[\alpha]_D^{10}$ +46.0° (no mutarotation) in pyridine-ethanol (2:3) (*c*, 0.50).

Anal. Found: N, 11.24. Calcd. for $C_{24}H_{30}O_8N_4$: N, 11.15%.

Methanolysis of Carrabiose Dimethylacetal. Carrabiose dimethylacetal (1.0 g.), obtained by saponification of the acetate, was dissolved in 3% methanolic hydrogen chloride (30 cc.) and the solution was boiled until the optical rotation of the solution reached a constant value after fifteen hours ($[\alpha]_D$ +30° ⟶ +70°). It was neutralized with silver carbonate, filtered and concentrated. The residual sirup (0.9 g.) was chromatographed on a column of powdered filter paper in a manner similar to the separation of the fraction I of the charcoal chromatography. The following two products were obtained and identified in the same manner as already described: 3,6-anhydro-D-galactose dimethylacetal (0.45 g.), $[\alpha]_D^{10}$ +29.7° (*c*, 0.64) and methyl α,β-D-galactoside (0.45 g.), $[\alpha]_D^{10}$ +100° (*c*, 0.50).

Hexa-*O*-methyl-carrabiose Dimethylacetal (VII).—The hexa-acetate (VI) (2.1 g.) in acetone-water (3:1, 20 cc.) was treated with dimethylsulfate (50 cc.) and a 30% potassium hydroxide solution (90 cc.) at 30° over two days. The product (1.5 g., OCH_3 47.2%), which was isolated by extraction with chloroform and subsequent evaporation of the extract, was further methylated with methyl iodide (20 g.) and silver oxide (10 g.) twice in the usual manner. VII was obtained as a slightly colored sirup; yield 1.4 g.; $[\alpha]_D^{11}$ +25.0° (*c*, 0.80), +6.9° (chloroform, *c*, 0.87); OCH_3, found: 53.67% (calcd. for $C_{12}H_{14}O_3(OCH_3)_8$: 54.63%).

Methanolysis of Hexa-*O*-methyl-carrabiose Dimethylacetal. — Hexa-*O*-methylcarrabiose dimethylacetal (1.3 g.) in 3% methanolic hydrogen chloride (30 cc.) was boiled for fifteen hours, when the optical rotation of the solution became constant ($[\alpha]_D$+17.5°→+75.0°). The solution was neutralized with silver carbonate, filtered and concentrated to a sirup; yield 1.3 g.; $[\alpha]_D^{10}$ +73.7° (*c*, 0.95). This sirup proved to be a mixture of methyl 2,3,4,6-tetra-*O*-methyl-D-galactoside (VIII) and 2,5-di-*O*-methyl-3,6-anhydro-D-galactose dimethylacetal(IX). The separation was effected by the selective hydrolysis and subsequent solvent fractionation as described below.

Methyl 2, 3, 4, 6-Tetra-*O*-methyl-D-galactoside (VIII). — The above mixture (1.3 g.) in a 0.02 N oxalic acid solution (20 cc.) was heated in a boiling water bath for two hours. With this

treatment, only the anhydro-sugar derivative(IX) was hydrolysed to give the corresponding reducing sugar. Then the solution was neutralized with calcium carbonate, filtered and evaporated to dryness. Extraction with warm petroleum ether followed by evaporation afforded VIII as a mobile sirup; yield 0.60 g.; n_D^{25} 1.4481; $[\alpha]_D^{10}$ +111.8° (*c*,1.10); OCH_3, found: 60.17% (calcd. for $C_6H_7O(OCH_3)_5$: 62.05%). The petroleum ether-insoluble residue was kept aside.

2, 3, 4, 6-Tetra-*O*-methyl-D-galactose. — The methylated galactoside (0.50 g.), obtained above, was hydrolysed with N-sulfuric acid for four hours in the usual manner. 2, 3, 4, 6-Tetra-*O*-methyl-D-galactose was obtained as a colorless sirup; yield 0.40 g.; n_D^{25} 1.4613; $[\alpha]_D^{10}$ +108.2° (an equilibrium value, *c*, 0.98); OCH_3, found: 52.28% (calcd. for $C_6H_8O_2(OCH_3)_4$: 52.56%.).

For identification, it was converted into an anilide in the usual manner; m.p. and mixed m.p. 193°; $[\alpha]_D^{10}$ −85.4° (acetone, an initial value, *c*, 0.41).

2, 5-Di-*O*-methyl-3, 6-anhydro-D-galactose. —The petroleum ether-insoluble residue, separated from VIII, was shown to be 2, 5-di-*O*-methyl-3, 6-anhydro-D-galactose on solvent removal; a colorless sirup; yield 0.55 g.: n_D^{25} 1.4758; $[\alpha]_D^{10}$ + 28.8° (an equilibrium value, *c*, 0.59); OCH_3, found: 33.08% (calcd. for $C_6H_8O_3(OCH_3)_2$: 32.60%). It reduced a cold Fehling's solution, showed a strong Seliwanoff's reaction, and restored the color to a Schiff's reagent.

2, 5-Di-*O*-methyl-3, 6-anhydro-D-galactonic Acid.—2, 5-Di-*O*-methyl-3, 6-anhydro-D-galactose (0.45 g.) in water (15 cc.) was oxidized with bromine (0.5 cc.) in the usual manner, affording 2, 5-di-*O*-methyl-3, 6-anhydro-D-galactonic acid (0.35g.), which was twice recrystallized from ethyl acetate; m.p. 161°; $[\alpha]_D^{10}$ +62.5° (*c*, 0.64). Recorded values for its enanthiomorph are m. p. 161° and $[\alpha]_D$ −54.3°[12], −58.6°[9b] and −65°[19].

Anal. Found: C, 46.23; H, 6.67; OCH_3, 29.91. Calcd. for $C_6H_8O_4(OCH_3)_2$: C, 46.61; H, 6.84; OCH_3, 30.07%.

Amide: The acid obtained above was converted into the amide through its methyl ester in the usual manner; m. p. 172° (from methanol-ether); $[\alpha]_D^{10}$ +72.0° (*c*, 1.00). Recorded values for its enanthiomorh are m. p. 172°[12,9b] and 173°[20], and $[\alpha]_D$ −64.8°[12], −67.0°[20] and −75.7°[22].

Anal. Found: N, 6.55; OCH_3, 30.12. Calcd. for $C_8H_{15}O_5N$: N, 6.82; OCH_3, 30.22%.

Summary

1. The mucilage of *Chondrus ocellatus Holmes* has been investigated by the partial methanolysis method.

2. A new disaccharide, the structure of which is shown to be 4-*O*-β-D-galactopyranosyl-3, 6-anhydro-D-galactose, has been isolated in the form of its dimethylacetal and methyl β-glycoside in a good yield.

3. The disaccharide has been named carrabiose.

4. It has been suggested that 1→3′ linked carrabiose represents the repeating unit for the most part of the carbohydrate of the Chondrus mucilage.

The authours wish to express their hearty thanks to Mr. T. Ito for his assistance in this study and to Mr. K. Arai for the microanalysis.

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20) W.G.M. Jones and S. Peat, *J. Chem. Soc.*, **1942**, 225.