Isolation of Agarobiose Derivative from the Mucilage of Gloiopeltis Furcata

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The mucilage of a red seaweed *Gloiopeltis furcata** resembles carrageenin (the mucilage of *Chondrus* algae) in the respect that it has a high content of sulfate residues as well as that it forms on dissolution in water a highly viscous solution. But our recent report has pointed out the chemical difference that the *Gloiopeltis* mucilage is chiefly composed of D-galactose and 3,6-anhydro-L-galactose as in the case of agar[1], while that carrageenin is composed of D-galactose and the anhydro-D-sugar, an enantiomorph of the above one[2]. Thus the complete methanolysis of the former mucilage afforded methyl D-galactoside and 3,6-anhydro-L-galactose dimethylacetal together with a small amount of a L-galactose derivative[1]. On the other hand, partial methanolysis of agar led to the isolation of crystalline agarobiose dimethylacetal (4-*O*-β-D-galactopyranosyl-3,6-anhydro-L-galactose dimethylacetal) in a good yield[3] and also of crystalline 4-*O*-(4,6-*O*-1′-carboxyethylidene-β-D-galactopyranosyl)-3,6-anhydro-L-galactose dimethylacetal as an acidic product in a slight yield[4]. With this in mind, the *Gloiopeltis* mucilage has been subjected to partial methanolysis, the results being reported herein.

The partial methanolysis has been conducted by refluxing the suspension of the mucilage in 0.5% methanolic hydrogen chloride for two hours as in the case of agar[3]. The methanolysate was treated with a barium hydroxide solution to effect saponification and then with cation- and anion-exchange resin in succession. An acidic substance, which was adsorbed by the anion-exchange resin, was eluted therefrom by displacement with excess of sulfuric acid and isolated as a barium salt. The salt has proved to be entirely barium methylsulfate, derived from sulfate residues present in the mucilage molecule. No evidence was obtained to indicate the presence of pyruvic acid[5] or any other carboxylic acid.

The neutral methanolysate, which passed through the resins without being retained, was subjected to charcoal chromatography[6], which led to the isolation of methyl D-galactoside (6%), a mixture of 3,6-anhydro-L-galactose dimethylacetal (4%) and methyl D-xyloside (2%), and agarobiose dimethylacetal (55%), the yields being based on the neutral methanolysate by weight.

The mixture of 3,6-anhydro-L-galactose dimethylacetal and methyl D-xyloside was further resolved into the components by chromatography on a powdered filter paper column. The methyl D-xyloside, obtained as a syrup, was hydrolyzed to give the free sugar, which was then identified as crystalline di-*O*-benzylidene-D-xylose dimethylacetal after Breddy and Jones[7]. This has been the first report as to the identification of D-xylose as far as the *Gloiopeltis* mucilage is concerned. But the yield was so low that the sugar might be possibly derived from a contaminating xylan.

Agarobiose dimethylacetal has been isolated as crystals, the physical constants of which are identical with those of the sample isolated previously from agar by two of the present writers[3]. The dimethylacetal was hydrolyzed and subsequently converted into agarobiose phenylosazone, which again agreed well with an authentic sample. On acetylation the dimethylacetal yielded hexa-acetate melting at 136~137°C, the value being in disagreement with the melting point 87~88°C reported previously by two of the writers[3], but in agreement with the melting point 137.5~138.5°C reported recently by Clingman, Nunn and Stephen[8]. The experiment

* Japanese name is "Fukuro-funori".

1) S. Hirase, C. Araki and T. Itô, This Bulletin, **29**, 985 (1956).
2) C. Araki and S. Hirase, ibid., **29**, 770 (1956); A. N. O'Neill, *J. Am. Chem. Soc.*, **77**, 2837, 6324 (1955).
3) C. Araki and S. Hirase, This Bulletin, **27**, 109 (1954).
4) S. Hirase, ibid., **30**, 70, 75 (1957).
5) S. Hirase, ibid., **30**, 68 (1957).
6) R. L. Whistler and D. F. Durso, *J. Am. Chem. Soc.*, **72**, 677 (1950).
7) L. J. Breddy and J. K. N. Jones, *J. Chem. Soc.*, **1945**, 738.
8) A. L. Clingman, J. R. Nunn and A. M. Stephen, ibid., **1957**, 197.

was repeated, but the acetate melting at 87~88°C could be prepared neither from the dimethylacetal of a *Gloiopeltis* origin nor from that of an agar origin.

It necessarily follows from the isolation of the agarobiose derivative that D-galactopyranose residues are connected through β-galactoside links with the 4-positions of 3,6-anhydro-L-galactose residues to form agarobiose residues in the mucilage molecule, and that if sulfate residues are left out of consideration, the disaccharide represents the repeating unit in the molecule of the mucilage as in that of agarose, a principal polysaccharide of agar[9].

Experimental

Evaporation and concentration of solutions were carried out under reduced pressure below 40°C. The melting points are uncorrected.

Partial Methanolysis of the Mucilage. — The mucilage preparation used in this study was the same one as that used in the previous work[1]. The mucilage (10 g., moisture 9.33%), after being soaked in 0.5% methanolic hydrogen chloride (100 cc.) overnight at room temperature, was heated under reflux for two hours, and then undissolved material (1.8 g.) was filtered off, washed with methanol and dried in vacuo. The filtrate and washings were combined, neutralized with silver carbonate, refiltered and evaporated to a syrup (9.0 g.). It was then heated with 0.3 N barium hydroxide solution (80 cc.) at 60°C for two hours to effect saponification, excess of the barium hydroxide removed by neutralization with carbon dioxide and subsequent filtration, and the filtrate was concentrated to a syrup, which was dried by repeated dissolution in methanol followed by evaporation; yield 7.3 g.; $[\alpha]_D^{13}$ −9.6° (*c* 0.83 in water).

Barium Methylsulfate.—The syrup obtained above was dissolved in water, the resulting solution allowed to pass through columns of the cation-exchange resin Amberlite IR-120 (100 cc) and the anion-exchange resin Amberlite IR-4B (100 cc.) in succession, and the columns were washed with water (1.3 *l.*). The combined effluents and washings were evaporated to recover the neutral methanolysate, which formed a syrup; yield 4.3 g. (59% of the consumed mucilage on the moisture-free basis); $[\alpha]_D^{13}$ −10.7° (*c* 0.75 in water).

The IR-4B resin, which adsorbed an acidic substance, was transferred to a beaker and stirred with 2 N sulfuric acid (100 cc.) under ice-cooling for a brief period of time. The resin was retransferred to a glass tube to form a column, excess of the liquid allowed to drain, and the column was washed with additional 100 cc. of 2 N sulfuric acid and water in succession. All the effluents were combined, neutralized immediately with a hot saturated barium hydroxide solution, filtered and evaporated, the residue being obtained as a colorless solid; yield 3.5 g.; $[\alpha]_D^{13} \pm 0°$ (*c* 1.0 in water). Paper chromatographic examination showed the absence of any carbohydrate compound. Determination of the ash content proved that the solid obtained above was barium methylsulfate;

Anal. Found: Ash (as barium sulfate), 83.2. Calcd. for $(CH_3SO_4)_2Ba \cdot H_2O$: $BaSO_4$, 83.3%.

Chromatography of the Neutral Methanolysate. — The neutral methanolysate (4.3 g.), obtained above from the effluent which passed through the ion exchangers, was dissolved in water (100 cc.), and the solution was soaked into the top of a charcoal*-Celite column (5.0×20 cm.), which was then developed successively with water, 2%, 5%, 7.5%, 15% and 30% ethanol in water. The effluents were separately evaporated to dryness, and the resultant residues were weighed, measured for specific rotation, and examined on paper chromatograms[6], which were irrigated with *n*-butanol-ethanol-water (4 : 1 : 2 v/v) and which were sprayed with *o*-aminophenol reagent[10]. The results are given in Table I.

Experiments described below enabled the following identification: fraction I as methyl D-galactoside, fraction II as a mixture of 3,6-anhydro-L-galactose dimethylacetal and methyl D-xyloside, and fraction IV as agarobiose dimethylacetal. From the paper chromatographic examination, there was also obtained an indicative evidence to show that fraction III was a mixture of 3,6-anhydro-L-galactose dimethylacetal and agarobiose dimethylacetal, and that fractions V, VI and VII were mixtures of higher oligosaccharide derivatives. But these fractions were not studied.

Identification of Methyl D-Galactoside. — Fraction I was dissolved in 95% ethanol and the solution left in a refrigerator, when methyl D-galactoside was obtained as crude crystals of monohydrate; yield 0.06 g.; m. p. and mixed m. p. 95~98°; $[\alpha]_D^{12}$ +172° (*c* 0.57 in water); OCH_3, found 14.69% (calculated for $C_6H_{11}O_5(OCH_3) \cdot H_2O$: 14.62%).

The mother liquor was concentrated to a syrup (0.18 g.), which was then subjected to hydrolysis with 1N sulfuric acid in the usual manner, affording D-galactose; yield 0.15 g.; m. p. and mixed m. p. 160°C; $[\alpha]_D^{12}$ +81.3° (an equilibrium value, *c* 0.64 in water).

Separation and Identification of 3,6-Anhydro-L-galactose Dimethylacetal and Methyl D-Xyloside. — Fraction II showed two distinct spots on a paper chromatogram; one was yellowish brown in color, corresponding to 3,6-anhydro-L-galactose dimethylacetal, and the other blue in color, corresponding to methyl D-xyloside. The mixture (0.38 g.) was separated on a column of powdered filter paper, *n*-butanol-water (6 : 1 v/v)

9) C. Araki, This Bulletin, **29**, 543 (1956); *Mem. Fac. Ind. Arts, Kyoto Tech. Univ.*, **5**, 21 (1956).

10) S. Hirase, C. Araki and S. Nakanishi, This Bulletin, **26**, 183 (1953).

* Charcoal used was "Shirasagi", manufactured by Takeda Pharmaceutical Industries, Ltd., Osaka.

TABLE I

CHROMATOGRAPHIC SEPARATION OF THE NEUTRAL METHANOLYSATE

Fraction	I	II	III	IV	V	VI	VII
Developer	W	2% E	5% E	5% E	7.5% E	15% E	30% E
〃 (*l.*)	3.0	3.6	0.7	9.3	3.0	4.0	3.0
Yield (g.)	0.26	0.38	0.10	2.37	0.13	0.62	0.57
$[\alpha]_D$ (° in W)	+98	+17	−22	−27	−10	−4	−19

W: Water E: Ethanol

being used as a mobile phase. The effluents (7 cc. per fraction) were examined on paper chromatograms and the fractions showing the same contents were combined and evaporated to dryness, two main components being recovered as colorless syrups, respectively.

The faster-moving component was proved to be 3,6-anhydro-L-galactose dimethylacetal; yield 0.19 g.; $[\alpha]_D^{12}$ −26.0° (*c* 0.50 in water); OCH_3, found: 28.72% (calculated for $C_6H_{10}O_4(OCH_3)_2$: 29.81%). When hydrolyzed with 0.02 N sulfuric acid in a boiling water bath for two hours, it afforded 3,6-anhydro-L-galactose (0.12 g.) in a syrupy form. The sugar was identified by its conversion into the phenylosazone in the usual manner[11]; m. p. and mixed m. p. 217°C; $[\alpha]_D^{18}$ −52.0° (*c* 0.50 in pyridine-methanol (2 : 3)).

The slower moving component was proved to be methyl D-xyloside; yield 0.10 g.; $[\alpha]_D^{18}$ +28.0° (*c* 0.50 in water). On hydrolysis with N sulfuric acid in the usual manner, it afforded D-xylose in a sirupy form; yield 0.06 g.; $[\alpha]_D^{18}$ +18.8° (*c* 1.28 in water). The sugar was dissolved in one cc. of Breddy and Jones' reagent[7] which consisted of benzaldehyde (4 cc.) and 0.36 N methanolic hydrogen chloride (14 cc.), and the resulting solution was let stand for a week at room temperature. The di-*O*-benzylidene-D-xylose dimethylacetal which formed was filtered off, washed with water and methanol in succession, and dried at 100°C; yield 0.05 g.; m. p. 207～208°C. Recrystallization from chloroform-ligroin afforded needles; m. p. 208～209°C; $[\alpha]_D^{18}$ −10° (*c* 1.0 in chloroform). Admixture with an authentic sample showed no depression of the melting point.

Anal. Found: C, 66.98; H, 6.51. Calcd. for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50%.

Identification of Agarobiose Dimethylacetal.—Fraction IV of the charcoal chromatography was dissolved in ethanol (5 cc.), acetone (5 cc.) added, and the resulting solution was left in a refrigerator. Crystals of agarobiose dimethylacetal were filtered off, washed with ethanol and dried; yield 1.91 g.; m. p. 162～163°C. Recrystallization was best effected by dissolving the crude crystals in hot methanol, adding a double volumes of acetone, filtering the resulting solution while it was hot, and allowing the filtrate to cool. Pure agarobiose dimethylacetal formed colorless prisms; m. p. 165～166°C; $[\alpha]_D^{10}$ −29.3° (*c* 1.50 in water); $[\alpha]_D^{10}$ −38.7° (*c* 1.50 in methanol). Two of the writers report m. p. 162～164°C, $[\alpha]_D$ −29.1° (in water) and $[\alpha]_D$ −37.4° (in methanol)[3], and Clingman, Nunn and Stephen report m. p. 163～166°C and $[\alpha]_D$ −36° (in methanol)[8]. Admixture with an authentic sample showed no depression of the melting point.

Anal. Found: C, 45.20; H, 7.23; OCH_3, 16.74. Calcd. for $C_{12}H_{20}O_9(OCH_3)_2$: C, 45.40; H, 7.07; OCH_3, 16.76%.

Agarobiose Phenylosazone.—Agarobiose dimethylacetal obtained above was heated in 0.02 N sulfuric acid in a boiling water bath for two hours to effect hydrolysis, and the resulting agarobiose was converted into its phenylosazone in the usual manner. The crude osazone obtained was purified by recrystallization from ethanol, forming yellow crystals; m. p. 219～220°C; $[\alpha]_D^{10}$ −136.6° (an initial value, *c* 0.41 in pyridine-ethanol (2 : 3)) → −107.3° (after forty-eight hours). The values reported from this laboratory are m. p. 218～219°C[12], 220～221°C[13] and 221～222°C[13], and $[\alpha]_D$ −108.8°[3] and −110.8°[13] in pyridine-ethanol (2 : 3). Clingman and others report m. p. 222～223.5°C and $[\alpha]_D$ −115° in the same solvent mixture[8]. Admixture with an authentic sample showed no depression of the melting point.

Hexa-*O*-acetylagarobiose Dimethylacetal.—Agarobiose dimethylacetal (0.5 g.) obtained above was acethylated with acetic anhydride (5 cc.) and pyridine (5 cc.) for two days at room temperature. When the reaction solution was poured into a mixture of ice and water with stirring, the hexaacetate was precipitated as fine powders, which were then filtered off, washed with water and dried in vacuo; yield 0.7 g.; m. p. 136～137°C. The crude crystals were recrystallized from methanol-water (1 : 2), affording the pure acetate in stout needles; m. p. 136～137°C; $[\alpha]_D^{10}$ −5.8° (*c* 1.20 in chloroform) and $[\alpha]_D^{10}$ −12.5° (*c* 1.20 in benzene). Two of the writers reported m. p. 87～88°C, $[\alpha]_D$ −5.8° (in chloroform) and −12.5° (in benzene)[3], and Clingman and others report m. p. 137.5～138.5°C and $[\alpha]_D$ −13.5° (in benzene)[8].

Anal. Found: C, 50.11; H, 6.28; OCH_3, 9.66; $COCH_3$, 41.57. Calcd. for $C_{12}H_{14}O_9(COCH_3)_6(OCH_3)_2$: C, 50.16; H, 6.15; OCH_3, 9.97; $COCH_3$, 41.57%.

Since the observed melting point was in disagreement with that reported by two of the writers, acetylation was re-examined for agarobiose dimethylacetal isolated from agar, but the result was exactly the same as that described above. The reason for the discrepancy is obscure.

11) C. Araki, *J. Chem. Soc. Japan*, (*Nippon Kagaku Kaishi*), **65**, 725 (1944).

12) C. Araki, ibid., **65**, 533 (1944).

13) S. Hirase and C. Araki, This Bulletin, **27**, 105 (1954).

Summary

1. The mucilage of *Gloiopeltis furcata* has been subjected to partial methanolysis, which resulted in the isolation of methyl D-galactoside, 3, 6-anhydro-L-galactose dimethylacetal, methyl D-xyloside and agarobiose dimethylacetal.

2. Since the agarobiose derivative was obtained in a good yield, it has been concluded that the disaccharide represents the chief repeating unit of the mucilage molecule, if the sulfate residues present in the mucilage are left out of consideration.

3. Xylose has been identified for the first place so far as the *Gloiopeltis* mucilage is concerned. But the yield was so low that the sugar might be possibly derived from a contaminating xylan.

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