Constituents of the Mucilage of Gloiopeltis Furcata

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Certain species of the *Gloiopeltis* family of *Rhodophyceae* contain a water-soluble mucilage, which on dissolution in water forms a highly viscous solution. It is widely used in this country as a sizing agent for silk textiles and as a thickening agent for mortar and plaster. The mucilage is a metallic salt of the sulfate ester of a polysaccharide, which on acid hydrolysis produces chiefly D-galactose together with a small amount of L-arabinose and L-fucose[1,2]. But there has also been reported the presence of a considerable amount of an unidentified constituent showing a Seliwanoff's ketose reaction[1,2]. On the other hand, it is established that agar-agar contains 3, 6-anhydro-L-galactose as well as D-galactose as the chief constituent[3], and that carrageenin contains an enanthiomorph of the anhydro-sugar present in agar in addition to D-galactose[4,5]. Both agar and carrageenin also contain a small amount of L-galactose[5,6]. Since 3, 6-anhydro-galactose shows a distinct Seliwanoff's reaction, it is most probable that the unidentified constituent of the *Gloiopeltis* mucilage is 3, 6-anhydro-galactose. This has been proved to be the case as reported herein, and the anhydro-sugar has been shown to be of the L-series. In addition, the presence of a small amount of L-galactose has also been confirmed.

The mucilage used in this study was extracted from commercial "sheet Funori", which is prepared from *Gloiopeltis furcata* by conglutinating the plant bodies with each other by means of their own mucilage. The mucilage extracted has a specific rotation, $[\alpha]_D^{15} = -20.6°$, in water and contains 18.5% of sulfate. It was then subjected to complete methanolysis. After an appropriate treatment the methanolysate was separated by column chromatography into 3, 6-anhydro-L-galactose dimethylacetal and a mixture of methyl D- and L-galactoside. The former product was hydrolysed to give a reducing sugar, which was then identified as its phenylosazone. The latter mixture was further separated into crystalline methyl α-D-galactoside and a residual syrup, which on hydrolysis gave rise to a mixture of D- and L-galactose. Then D-galactose was removed therefrom as much as possible by crystallization, and the residue was treated with ethylmercaptan and concentrated hydrochloric acid. L-Galactose was isolated in the form of crystalline DL-galactose diethylmercaptal, which was further identified as its penta-acetate. The yields of the products have indicated that the *Gloiopeltis* mucilage used contains 3, 6-anhydro-L-galactose, D-galactose and L-galactose in the approximate ratio of 8 : 12 : 1.

Experimental

Evaporation and concentration were carried out under reduced pressure below 40°. Specific rotations were measured in aqueous solutions unless otherwise stated. The melting points are uncorrected.

Material.—Twenty grams of commercial sheet of *Gloiopeltis furcata* was extracted with water (2 l.) in a boiling water bath for two hours. After filtration through a bed of Celite-active carbon, the extract was concentrated and precipitated with 95% ethanol. The product, collected and pressed in a cotton sack, was dehydrated with absolute ethanol and then ether, air-dried and finally ground to powder; yield 6.5 g. (moisture 9.33%); $[\alpha]_D^{15} = -20.6°$ (c 0.63); found: ash, 13.14%; SO_4 total, 18.48%; SO_4 in ash, 6.84%, of the mucilage, respectively, on the moisture-free basis.

1) K. Kawakami, *J. Soc. Chem. Ind. Japan*, 13, 341 (1910).
2) E. Takahashi, *J. Coll. Agr. Hokkaido Imp. Univ.*, 8, 183 (1920).
3) f. i. see C. Araki, This Bulletin, 29, 543 (1956).
4) C. Araki and S. Hirase, ibid, 29, 991 (1956); A. N. O'Neil, *J. Am. Chem. Soc.*, 77, 2837, 6324 (1955).
5) E. E. Percival, *Chemistry & Industry*, 1954, 1487.
6) C. Araki and K. Arai, Collection of Treatises in Commemoration of 45th Anniversary of Kyoto Technical College, p. 80 (1948).

Methanolysis.—The mucilage (1.50 g., moisture 9.33%) in 3% methanolic hydrogen chloride (20 cc.) was heated under reflux for twenty-five hours. The resulting solution was neutralized with silver carbonate, filtered and concentrated to a syrup, which was then treated with 0.3N-barium hydroxide solution (20 cc.) at 60° for two hours. Excess barium hydroxide was removed by neutralization with carbon dioxide and filtration, and the filtrate was concentrated to a syrup. It was dissolved in water and deionized by passing the solution through columns of Amberlite IR-120 and IR-4B resins in succession, the resins being subsequently washed with water. The effluent and washings were combined and concentrated to a syrup; yield 0.779 g. (57.3% of the mucilage); $[\alpha]_D^{24}=+46.9°$ (*c* 0.64). Paper chromatographic examination, using *n*-butanol-ethanol-water (4:1:2 by volume) as a solvent and *o*-aminophenol reagent[7] as a spraying reagent, indicated the presence of 3,6-anhydrogalactose dimethylacetal (R_f 0.67[8]).

Chromatographic Separation.—The deionized methanolysate (0.754 g.) obtained above was chromatographed on a column (2.5×30 cm.) of powdered filter paper, *n*-butanol-water (6:1) being used as a developer until the effluent showed no longer a spot of 3,6-anhydro-galactose dimethylacetal on a paper chromatogram. Then the column was developed with 80% aqueous methanol (300 cc.). Butanolic and methanolic effluents were separately evaporated to dryness, giving 3,6-anhydro-L-galactose dimethylacetal and a mixture of methyl D- and L-galactoside, respectively, in yields of 0.290 g. and 0.456 g. Since L-galactose amounts to 7.9% of total galactose as described later, 3,6-anhydro-L-galactose, D-galactose and L-galactose are in the molar ratio 7.6:11.6:1.

3,6-Anhydro-L-galactose Dimethylacetal.—3,6-Anhydro-L-galactose dimethylacetal above obtained was a colorless syrup, which was further purified by dissolution in dry acetone followed by filtration and subsequent evaporation; $[\alpha]_D^{27}=-25.9°$ (*c* 0.80); OCH_3, found: 27.73% (calcd. for $C_8H_{16}O_6$: 29.81%). It showed a distinct Seliwanoff's ketose reaction.

3,6-Anhydro-L-galactose.—The above dimethylacetal (0.3 g.) was hydrolysed with 0.02N-sulfuric acid (10 cc.) in a boiling water bath for two hours. The solution was neutralized with barium carbonate, filtered and concentrated to dryness, when 3,6-anhydro-L-galactose was obtained as a syrup (0.2 g.). It reduced a Fehling's solution at room temperature.

Phenylosazone: The sugar above obtained was converted to its phenylosazone by reaction with phenylhydrazine in the presence of dilute acetic acid in the usual manner. The osazone was twice recrystallized from ethanol-water (2:1), forming yellow needles; m.p. 217°; $[\alpha]_D^{30}=-65.9°$ (an initial value, pyridine-methanol (2:3), *c* 0.44) → −52.3° (an equilibrium value). Reported values[9] are m.p. 217° and $[\alpha]_D=-52.8°$ (an equilibrium value, pyridine-methanol). Admixture with an authentic sample showed no depression of the melting point.

Methyl α-D-Galactoside.—When the mixture of methyl D- and L-galactoside, obtained from the methanolic effluent of chromatography, was dissolved in ethanol and kept in a refrigerator, methyl α-D-galactoside was deposited as its monohydrate; yield 0.191 g. It was recrystallized from ethanol, giving prisms; m.p. 94–98°, not depressed on admixture with an authentic sample; $[\alpha]_D^{32}=+175°$ (*c* 1.00); OCH_3, found: 14.63% (calcd. for $C_7H_{14}O_6\cdot H_2O$: 14.61%).

D-Galactose.—The mother liquor, separated from crystals of methyl α-D-galactoside monohydrate, was concentrated to a syrup (0.262 g.), which was then subjected to hydrolysis with N-sulfuric acid (5 cc.) in a boiling water bath for four hours. The product (0.239 g.), isolated in the usual manner, was crystallized from methanol, affording D-galactose; yield 0.107 g. The recrystallized sample melted at 164–165° and had a specific rotation $[\alpha]_D^{27}=+80.8°$ (an equilibrium value, *c* 0.73). Mixed melting point determination showed no depression.

DL-Galactose Diethylmercaptal.—The mother liquor, separated from crystals of D-galactose, was concentrated to a syrup (0.123 g.), which was then combined with two other batches. The resulting mixture was extracted with boiling absolute ethanol. On cooling the extract gave crystals (0.10 g.) melting at 140–144° and having a specific rotation, $[\alpha]_D^{28}=+32.5°$ (an equilibrium value, *c* 0.80). The value of the specific rotation indicates that the crystals are a mixture of D-galactose and L-galactose, the former predominating. The filtered mother liquor was concentrated to a syrup (0.20 g.), $[\alpha]_D^{27}=+4.6°$ (an equilibrium value, *c* 0.65). It showed on a paper chromatogram a single spot corresponding to galactose. This observation and the low value of a specific rotation may show that the above-mentioned syrup is nearly pure DL-galactose. Then, on being calculated for a single batch, the yield (0.067 g.) of DL-galactose amounts to 15.8% of the whole galactose, and hence that of L-galactose is 7.9%.

For conclusive identification, the syrupy DL-galactose above obtained was converted to its diethylmercaptal as follows. The syrup (0.20 g.) was dissolved in concentrated hydrochloric acid (*d*= 1.20, 2 cc.), and ethylmercaptan (2 cc.) was added. After being vigorously shaken for fifteen minutes under ice-cooling, the reaction mixture was diluted with ice-water, immediately neutralized with excess lead carbonate, filtered and concentrated to dryness. The residue was extracted with boiling acetone. On evaporation the extract afforded DL-galactose diethylmercaptal melting at 121-124°; yield 0.18 g. A pure compound was obtained on recrystallization from acetone; m.p. 126-127°; $[\alpha]_D^{28}=\pm0°$ (*c* 0.80); SC_2H_5[10], found: 42.80% (calcd. for

7) S. Hirase, C. Araki and S. Nakanishi, This Bulletin, **26**, 183 (1953).
8) C. Araki and S. Hirase, ibid., **27**, 109 (1954).
9) C. Araki, *J. Chem. Soc. Japan*, **65**, 725 (1944).
10) S. Hirase and C. Araki, This Bulletin, **28**, 481 (1955).

$C_{10}H_{22}O_5S_2$: 42.68%). The values previously reported by two of the present authors[11] are m.p. 126-127° and $[\alpha]_D = \pm 0°$. The admixture of an authentic sample showed no depression of the melting point.

For further identification, the diethylmercaptal was converted to its penta-acetate by acetylation with acetic anhydride and pyridine. The acetate obtained was recrystallized from aqueous methanol; m.p. 112–113°; $[\alpha]_D^{28} = \pm 0°$ (chloroform, *c* 0.75). Reported values are m.p. 112–113° and $[\alpha]_D = \pm 0°$ (chloroform). Admixture with an authentic sample showed no depression of the melting point.

Summary

1. The mucilage of *Gloiopeltis furcata* has been investigated by the complete methanolysis method.

2. 3,6-Anhydro-L-galactose dimethylacetal and methyl D-galactoside have been isolated from the methanolysate. In addition, L-galactose has also been isolated in the form of the crystalline DL-galactose diethylmercaptal.

3. The yields of the isolates have indicated that the mucilage used contains 3,6-anhydro-L-galactose, D-galactose and L-galactose in the approximate molar ratio of 8:12:1.

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11) C. Araki and S. Hirase, This Bulletin,, **26**, 463 (1953).