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Constitution of Mucilage from *Ulva Conglobata*. I. Separation of Rhamnobiose Derivative by Partial Methanolysis

Kiyo MITA

Home Economics, Hiroshima Women's University, Hiroshima

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Partial methanolysis of the mucilage from *Ulva conglobata* gave methyl α-L-rhamnoside (29.07%), methyl α-D-xyloside (7.04%) and methyl α-D-glucoside (5.13%). Separation of a new disaccharide (methyl α-L-rhamnobioside, 3.4%) from methanolysate was achieved chromatographically. The biose was attacked by maltase but not by emulsin. The results and infrared spectrum show that the rhamnobiose derivative consists of rhamnose units linked by α-link.

It was shown[1] that an acid hydrolysis of the mucilage from *Ulva conglobata* collected in Saga prefecture gave L-rhamnose, D-xylose and D-glucose as neutral constituents and D-glucuronic acid and sulfuric acid as acidic constituents.

The present investigation was to determine the manner of the linkages of these constituents in the polysaccharide molecule.

Percival *et al.*[2-7] have studied water-soluble sulfated polysaccharides which contain glucose, galactose, mannose, arabinose, xylose, rhamnose and uronic acid residues, from *Caulerpa filiformis*, *Acrosiphonia centralis*, *Cladophora rupestvis*, *Ulva lactuca*, *Enteromorpha compressa*, *Codium fragile*, *Chaetomorpha capillaris*. Furthermore, partial hydrolysis of the above mucilages led to the isolation of 3-*O*-β-D-xylopyranosyl-D-xylose, 4-*O*-α-D-glucopyranosyl-D-glucose, 4-*O*-D-xylopyranosyl-L-rhamnose, 4-*O*-D-glucopyranosyl-L-rhamnose and 4-*O*-glucuronosyl-L-rhamnose-2-sulfate.

Partial methanolysis was conducted by refluxing the suspension of the mucilage in a methanolic hydrogen chloride (1%) for 2 hr. The methanolysate was treated with a barium hydroxide solution (0.3 N) to effect saponification. The neutral methanolysate passed through a cation- and anion exchange resins was subjected to charcoal chromatography, which led to the isolation of methyl α-L-rhamnoside (29.07%), methyl α-D-xyloside (7.04%), methyl α-D-glucoside (5.13%), a mixture of methyl glycosides of disaccharides (5.5%) and methyl glycosides of oligosaccharides (32.5%).

The mixture of methyl glycosides of disaccharides was further resolved into its components by chromatography on a powdered filter paper column (Table 2). Fraction III-a in Table 2 gave only L-rhamnose by acidic hydrolysis. The fraction was not attacked at all by emulsin but by maltase. The results are very interesting because methyl α-L-rhamnobioside has not yet been isolated from natural substance and its synthesis has not been reported.[8]

Barker *et al.*[9,10] have studied structures of carbohydrates by means of IR spectra and reported that α-anomers give absorption at 844±8 cm^{-1} and β-anomers at 891±7 cm^{-1}. This seems to be one of the C–H deformation modes. The absorption bands are observed in all saccharides. Judging from the absorption band at 850 cm^{-1}, the isolated rhamnobioside appears to be α-anomer.

The IR spectrum of xyloside fraction indicated the presence of hydroxy groups from the absorption bands at 3400 and 1100—1000 cm^{-1} and α-anomer from the absorption band at 740 cm^{-1} (type 3 of pyranose ring). The IR spectrum of glucoside fraction also identified as α-anomer from the absorption bands: 3400, 1100—1000 (OH), 840 (type 2a of pyranose ring) and 745 cm^{-1} (type 3 of pyranose ring). Furthermore, these α-anomers were identified by direct comparison of the melting

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point, the specific rotation and the IR spectrum with authentic samples.

Experimental

Materials. The mucilage was obtained from *Ulva conglobata* (collected in Saga prefecture) as described in the previous paper.[11] All reagents were of analytical or practical grade. Reagents of practical grade were purified by recrystallization and distillation. All melting points are uncorrected.

Apparatus. The IR spectra were obtained with KBr disk on a Hitachi EPI-S2 infrared spectrophotometer.

Partial Methanolysis of the Mucilage. Powdered mucilage (20 g, moisture 12.2 %, ash 17.6 %) was swelled in a 1 % methanolic hydrogen chloride solution (300 m*l*) at room temperature for 12 hr and then refluxed for 2 hr. After filtration, the insoluble substance (7.0 g) was dried *in vacuo* at 40°C and refluxed again with a 1 % methanolic hydrogen chloride solution (200 m*l*) for 2 hr and then filtered. Finally, the insoluble substance (1.85 g) was washed with methanol and dried *in vacuo*.

All filtrates and washings were combined and neutralized with silver carbonate. After filtration, the filtrate was evaporated to a syrup (22.3 g) *in vacuo* at 40°C. This was saponified at 60°C for 2 hr with a 0.3 N barium hydroxide solution (350 m*l*), and excess barium hydroxide was neutralized with carbon dioxide. After filtration, the filtrate was evaporated to a syrup (18.05 g).

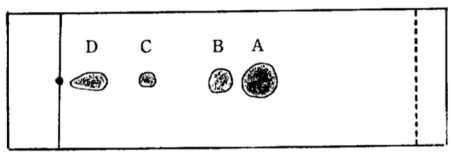

Fig. 1. Paper chromatogram of the neutral fraction of methanolysates.
Solvent system is *n*-butanol : ethanol : water : ammonia = 40 : 10 : 49 : 1 (v/v).[13]
A: methyl α-L-rhamnoside
B: methyl α-D-xyloside
C: methyl α-D-glucoside
D: unknown

Separation of the Neutral Methanolysate. The saponified syrup was dissolved in water (10 %) and the aqueous solution was allowed to pass through columns (3×40 cm) of Amberlite IR 120 (H form; 210 m*l*) and then Amberlite IR 45 (OH form; 210 m*l*). Both columns were washed with water (5 *l*) at a flow rate of 200 m*l*/hr. The combined eluates and washings were evaporated in order to obtain the neutral syrup (8.45 g); $[\alpha]_D^{30}$ −21.4° (*c* 1.5, water), OCH_3 15.31 %. The neutral fraction from the methanolysates gave four distinct colored spots with R_f values 0.10 (D: orange brown), 0.43 (C: orange brown), 0.55 (B: violet blue) and 0.64 (A: orange, main) on a paper chromatogram with *o*-aminophenol reagent,[12] as shown in Fig. 1.

Adsorption Chromatography for the Neutral Methanolysate. The neutral methanolysate (8.0 g) was dissolved in water (72 m*l*), placed on a charcoal-Celite 545 (1 : 1 w/w) column (5×34 cm) and eluted successively with water and then aqueous ethanol (2, 5, 7.5, 15 and 30 %) at room temperature according to the method of Whistler.[14]

The eluates were measured for specific rotation and chromatographed on papers. The chromatograms were sprayed with *o*-aminophenol[12] and potassium periodate-potassium permanganate reagents.[15] The results are summarized in Table 1.

Separation and Identification of Methyl α-D-Xyloside, Methyl α-L-Rhamnoside and Methyl α-D-Glucoside. Fraction I in Table 1 gave three distinct spots on a paper chromatogram: R_f 0.64 (orange; corresponding to methyl α-L-rhamnoside), 0.55 (violet-blue; corresponding to methyl α-D-xyloside) and 0.43 (brown-orange; corresponding to methyl α-D-glucoside).

Fraction I (2.7 g) was further separated through a charcoal-Celite 545 column (2×35 cm), water being used as a mobile phase. Each eluate was examined by specific rotation and then chromatographed on paper. The eluates gave the same specific rotation and R_f values were combined and then evaporated to dryness. They were identified as follows.

Methyl α-D-Xyloside: The xyloside fraction (white solid, 1.05 g) was dissolved in methanol-acetone (1 : 3

Table 1. Chromatographic separation of neutral methanolysate

Fraction	I	II	III	IV	V	VI
Solvent	W*1	2% E*1	5% E*1	7.5% E*1	15% E*1	30% E*1
Vol. (*l*)	20	12	20	21	11	17
Yield (g)	2.7	0.55	0.5	0.5	1.1	1.0
$[\alpha]_D^{30}(H_2O)$	—	−87.31°	−89.77°	−84.17°	−57.25°	−53.17°
OCH_3 (%)	—	17.5	6.43	3.58	3.70	4.22
R_f value	0.64 0.55 0.43	0.64	0.30 0.24	0.14*2 0.10	0.10	0.10

*1 W: water, E: ethanol, *2 trace

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v/v) on heating and then filtered. The filtrate gave white crystals (1.0 g), $[\alpha]_D^{22.8}$ +143.96° (*c* 2.7, water), mp 103.5°C and identified as methyl α-D-xyloside by means of mixed melting point and comparison of the IR spectrum (KBr): 3400, 1100—1000 (OH), 740 cm^{-1} (type 3 of pyranose ring) with that of the authentic sample.

Found: OCH_3, 18.55 %. Calcd for $C_5H_9O_4(OCH_3)$: OCH_3, 18.9 %.

Methyl 2,3,4-Tri-O-acetyl-α-L-rhamnopyranoside: After dissolving the methyl α-L-rhamnoside fraction (1.1 g) in pyridine (10 m*l*), acetic anhydride (20 m*l*) was added. After 24 hr the solution was dropped into ice water and the white solid (0.88 g) separated was filtered. Recrystallization from aqueous-methanol (1 : 2 v/v) gave white crystals, $[\alpha]_D^{14.4}$ −8.10° (*c* 0.29, methanol), mp 84—85°C (lit 86—87°C),[16] identified as methyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside by means of mixed melting point and comparison of the IR spectrum (KBr): 1745, 1375 and 1225 cm^{-1} (OAc), 850 cm^{-1} (type 2a of pyranose ring) with the authentic sample.

Found: OCH_3, 18.00%. Calcd for $C_6H_{11}O_4(OCH_3)$: OCH_3, 17.4%.

Methyl α-D-Glucoside: The glucoside fraction (white solid: 0.58 g) was dissolved in methanol-acetone (1 : 2 v/v) on heating and then filtered. The filtrate gave white crystals (0.55 g) mp 163—164°C, $[\alpha]_D^{28.0}$ +150.30° (*c* 0.24, water) which recrystallized from the same mixed solvent. The crystals were identified as methyl α-D-glucoside by means of mixed melting point and comparison of the IR spectrum (KBr): 840 cm^{-1} (type 2a of pyranose ring), 745 cm^{-1} (type 3 of pyranose ring) with that of the authentic sample.

Found: OCH_3, 15.40%. Calcd for $C_6H_{11}O_5(OCH_3)$: OCH_3, 15.97%.

Separation and Identification of Methyl α-L-Rhamnobioside. A solution (3 m*l*) of *n*-butanol-water (6 : 1 v/v) containing fraction III (0.5 g) was chromatographed on a column (2×50 cm) of cellulose powder with *n*-butanol saturated with water according to the method of Hough[17] and the eluates (5 m*l* each) were collected automatically and analyzed by means of specific rotation and paper chromatography. The eluates containing the same components were combined, and then *n*-butanol was distilled off *in vacuo*. The residues dried on pottassium hydroxide in a dessiccator gave white crystals.

TABLE 2. CHROMATOGRAPHIC SEPARATION OF FRACTION III ON CELLULOSE POWDER

Fraction	Eluate No.	Yield (g)	R_f value
III-a	51— 58	0.11	0.31
III-b	59—100	0.33	0.31, 0.24
III-c	101—110	0.04	0.24

Fraction III-a in Table 2 recrystallized from methanol-water (4 : 1 v/v) gave needles, mp 123—125°C, $[\alpha]_D^{22.5}$ −16.0° (*c* 2.0, water), R_f 0.31 (orange brown by *o*-aminophenol reagent).

Found: C, 48.11; H, 7.39 %. Calcd for $C_{13}H_{24}O_9$: C, 48.14; H, 7.40 %.

Found: OCH_3, 8.89%. Calcd for $C_{12}H_{21}O_8(OCH_3)$: OCH_3, 9.56%.

Fraction III-a was hydrolyzed with 0.1 N sulfuric acid solution (5 m*l*) in a boiling water bath for 1 hr, neutralized with barium carbonate and then filtered. The filtrate was vacuum evaporated and chromatographed. It gave only rhamnose spot (R_f 0.37).

Enzymic Hydrolysis of Fraction III-a. Fraction III-a (20 mg) was hydrolyzed with β-glucosidase (emulsin, 0.1 g) in an acetic acid-sodium acetate buffer solution (pH 4.1) at 30°C in a water bath for 1 hr. After filtration, the filtrate was chromatographed on paper. The R_f value did not change at all before treatment with β-glucosidase. However, Fraction III-a gave rhamnose by hydrolysis with maltase in a phosphoric buffer solution (pH 6.4) at 37°C for 1.5 hr.

Fractions III-b and III-c in Table 2 were hydrolyzed with 0.1 N sulfuric acid solution as Fraction III-a and then chromatographed. The hydrolysates from both fractions gave two spots corresponding to xylose and rhamnose by paper chromatography.

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