Methanolysis of Fucoidan. I. Preparation of Methyl α-L-Fucoside and L-Fucose[1]

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Fucoidan from *Macrocystis pyrifera* is subjected to methanolysis. The polysaccharide is depolymerized to its monomer, and the sulfate groups (which previously had been found to be very stable) are removed quantitatively. Methyl α-L-fucoside can be obtained in crystalline form in high yield, so the procedure may be applied as a preparative method for methyl α-L-fucoside and, after subsequent hydrolysis, L-fucose.

Fucoidan is a water-soluble polysaccharide which occurs in brown alga in various proportions depending on the species of alga and, to a lesser extent, on location and season.[2a] It was first isolated and described by Kylin[2b] as a polymer with fucose as the unit. It is now generally accepted that fucoidan is composed mainly of L-fucose units which are sulfated[3] in the 4-position and connected through α-(1→2) linkages.[4] However, there are still doubts about the details of the structure, branching, and the presence of other sugar units.[3,5]

Fucoidan and methylated fucoidan have been depolymerized by hydrolysis and by acetolysis in order to solve structure problems. In other investigations, the main object was the preparation of L-fucose, which is difficult to synthesize.[6] Günther and Tollens[7] were the first who obtained L-fucose from seaweed extracts in preparative quantities. Since the direct crystallization after hydrolysis was difficult, they formed and separated the phenylhydrazone and then decomposed it with benzaldehyde to obtain crystalline L-fucose. Essentially the same method but using different seaweeds was applied later by Clark[8] and by Hudson, *et al.*[9] Black, *et al.*,[10] succeeded in crystallizing L-fucose directly from the hydrolyzate of a sample of fucoidan from *Pelvetia canaliculata*.[11] Trabert[12] separated L-fucose from D-mannitol in crystalline form from the hydrolyzate of an extract of *Fucus serratus* L.

The present article gives information on another method of depolymerization, namely, methanolysis of fucoidan. This produces methyl α-L-fucoside in high yields, so it can be used as a preparative method. Crystalline L-fucose may be obtained also if, before the isolation of the methyl L-fucoside, the solution is refluxed with mineral acid.

Fucoidan, isolated from the exudate of *Macrocystis pyrifera*, was treated with methanolic hydrogen chloride under various conditions. In all experiments, methyl α-L-fucoside was isolated as the principal crystalline compound, the yield of which depended greatly on the concentration of hydrogen chloride, the amount of fucoidan per liter of methanol, and the reaction temperature, but very little on the reaction time. The yield was determined by the weight of methyl α-L-fucoside which actually could be crystallized rather than on the reducing power of the hydrolyzed reaction mixture, the optimum of which need not be identical with optimum yields of crystalline product. Only the fractions of crystals with sufficient purity (correct melting point) were considered in these determinations.

Table I indicates optimum yields at about 60°. A rapid decrease of the yield is noticed at lower temperatures probably because of insufficient depolymerization. The slight decrease at higher temperatures probably is due to degradative impurities (deeper color of the reaction mixture), which make crystallization more difficult.

TABLE I

METHANOLYSIS OF FUCOIDAN AT DIFFERENT TEMPERATURES

Fucoidan, 150 g.; Methanol, 1000 ml.; Hydrogen Chloride, 50–60 g.

Temperature	L-Fucoside, g.	M.p.	Sirup, g.
20–25	3.1	153–156	21
60	14.0[a]	157–158	17.4
	2.5[b]	140–152	
80	10.4[a]	155–157	21
	2.85[b]	147–155	
	2.75[c]	105–120	
110	10.5[a]	156–159	21
	1.55[b]	152–156	

[a] First fraction of crystals. [b] Second fraction of crystals. [c] Third fraction of crystals.

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TABLE II
METHANOLYSIS OF FUCOIDAN USING DIFFERENT AMOUNTS OF HYDROGEN CHLORIDE
Temp., 60°; Methanol, 1000 ml.

HCl, g.	L-Fucoside, g.	M.p.	Sirup, g.
10	0	...	1.4
20	9.8[a]	155–158	9
	5.6[b]	106–119	
40	14.4[a]	148–155	8.6
	5.3[b]	122–143	
84	14.2[a]	147–156	19.4
	1.7[b]	119–144	

[a] First fraction of crystals. [b] Second fraction of crystals.

The results of varying the hydrogen chloride concentration (Table II) show that crystalline L-fucoside is obtained only if more than 10 g. of hydrochloric acid/l. of methanol is present. Optimum yields are produced with 45–70 g./l.

Similarly, L-fucoside can be crystallized only if the amount of fucoidan/l. of methanol (Table III) is below 600 g.

TABLE III
METHANOLYSIS OF VARYING AMOUNTS OF FUCOIDAN
Methanol, 1000 ml.; Hydrogen Chloride, 40–50 g.

Fucoidan, g.	L-Fucoside, g./150 g. fucoidan	M.p.	Sirup, g.
600	0	...	<5
300	11.2[a]	154–158	16
	2.2[b]	143–155	
150	14.4[a]	148–155	8.6
	5.3[b]	122–143	
75	15.4[a]	153–156	15.6
	6.0[b]	103–134	

[a] First fraction of crystals. [b] Second fraction of crystals.

The yield increases steadily with dilution and, if extrapolated to 0 g. of fucoidan, is about 11% of the starting material. However, considering that only 23.9% of the weight of the polymer is present as L-fucose, the yield accounts for over 40% of the total L-fucose content. The second crystallizate contains another 10–15%, and the non-crystallizing sirup (obtained after concentration of the filtrate) about 15–20%, of which nearly 10% can be isolated as crystalline L-fucose after subsequent hydrolysis. When added up, this accounts for 70–80% of the total amount of L-fucose present. A loss of around 20% during all the reaction steps is reasonable and must be expected. An insoluble residue which is formed during methanolysis and which amounts to almost 40% of the weight of the starting material consists mainly of inorganic salts and contains very little L-fucose (usually not more than 1–2%).

While the first fraction of methyl α-L-fucoside is identical with an authentic sample, the second fraction usually has a low, very broad melting range and a much higher optical rotation than expected. This and the absence of larger proportions of other sugars suggest the presence of methyl β-L-fucoside in mixture with the α-L-form. Final evidence was obtained by isolation and characterization of methyl β-L-fucoside as the potassium acetate adduct. The quantity of methyl β-L-fucoside may be roughly estimated by comparing the rotation of the mixture with the rotation of the pure α- and β-forms, −197° and +14°, respectively.[9]

If the fucoidan is purified by reprecipitation with alcohol, the L-fucose content and, correspondingly, the yields after methanolysis increase. This increase, however, does not compensate for the high losses of fucoidan during its purification.

Instead of methyl α-L-fucoside, L-fucose may be obtained if the solution of the fucoside is heated with dilute sulfuric acid. The free sugar then can be readily crystallized in a yield of 50–60%. If hydrochloric acid is used, the crystallization of L-fucose is difficult.

Summarizing all results, methanolysis under the conditions described above produces methyl α-L-fucoside and, to a smaller extent, methyl β-L-fucoside as the only compounds which crystallize directly. The high yields of these two derivatives and the lack of chromatographic evidence for oligosaccharides establish that fucoidan is completely depolymerized by cleavage of the glycosidic linkages and that the sulfate groups (which are unusually stable[4,13]) are split off quantitatively. Methanolysis under milder conditions, as reported recently by McKinnell and Percival,[13] does not attack the glycosidic linkages very intensively and removes the sulfate groups only partially. For the purpose of preparing methyl α-L-fucoside or L-fucose, the most favorable conditions are 50–60 g. of hydrogen chloride gas and 100–150 g. of fucoidan per liter of methanol, a reaction temperature of 60°, and a reaction time of about twenty hours.

Experimental

Isolation and Purification of Fucoidan.—The slimy exudate from recently harvested *Macrocystis pyrifera* (harvested off the coast of southern California) was collected, filtered through a cloth, and slowly poured into one volume of isopropyl alcohol with stirring. The soft, fibrous precipitate was removed and washed (and hardened) with fresh isopropyl alcohol. After being dried at 45° in the presence of an air stream, the light brown material was ground to a fine powder and extracted in a Soxhlet extractor with 90% methanol for 48 hr. This material was dried at 45° and used in the following experiments as unpurified fucoidan.

Anal. L-Fucose[14] 23.9; sulfate, 18.03.

For further purification, unpurified fucoidan was dissolved in distilled water to make a 3–4% solution. After filtration of this solution on a Büchner funnel through cotton, the polymer was precipitated from it by addition of 1.3 volumes of isopropyl alcohol, and washed and hardened with fresh isopropyl alcohol as described above. Dissolving in water and precipitating with isopropyl alcohol was repeated a second time and the material then obtained was powdered and extracted in a Soxhlet extractor with 90%

(13) J. P. McKinnell and E. Percival, *Biochem. J.*, **80**, 43P (1961).
(14) W. A. P. Black, W. J. Cornhill, E. T. Dewar, E. G. V. Percival, and A. G. Ross, *Soc. Chem. Ind.* (London), **69**, 317 (1950).

methanol for 30 hr., and dried at 45° in the presence of an air stream; yield, about one-third of the weight of the starting material.

Anal. L-Fucose, 24.9; sulfate, 18.85.

Methanolysis of Fucoidan.—Unpurified fucoidan (150 g.) was suspended in 1000 ml. of methanol into which 56 g. of hydrogen chloride gas had been introduced. The mixture was placed in a pressure-reaction apparatus and stirred at 110° for 6 hr. Then the heater was turned off and stirring was continued for a further 14 hr. The temperature then had dropped to 46°. An insoluble residue (59 g.) was removed by filtration (fritted-glass) and washed with methanol. An analysis of the residue indicated 1.2% L-fucose, 51.3% ash, and 14.8% sulfate. The dark brown filtrate was stirred with 410 g. of lead carbonate (1 mole/mole of hydrogen chloride), filtered, and concentrated to a sirup. After dilution with water and heating on a steam bath, a solution of ammonium carbonate was added until formation of precipitate was complete. The mixture was filtered and then passed through anion- and cation-exchange resin columns until neutral. Most of the color was removed during treatment with the exchange resins and with activated carbon, but the solution was still slightly yellow. It was concentrated *in vacuo* to a sirup which was diluted with methanol. Ether was added and, after seeding with methyl α-L-fucoside, the solution was kept in the refrigerator for 1 day to complete crystallization. The crystals were removed, washed with a cold mixture of methanol and ether, and dried; m.p. 156–159°,[15] $[\alpha]^{20}D$ −180.7 ± 5° (*c* 0.5, water). After one recrystallization from ethanol–ether the substance had m.p. 159.5° and $[\alpha]^{20}D$ −187.2 ± 5° (*c* 0.5, water). When mixed with methyl α-L-fucoside prepared from authentic L-fucose, the m.p. was undepressed. After concentration of the filtrate, dilution with ethanol and ether, and refrigeration, a second crop of crystals was obtained. The filtrate was concentrated again to a sirup, from which no further crystals could be separated.

In all other experiments the procedure was essentially the same. Variations of the reaction temperature, of the concentration of hydrogen chloride, of the amount of fucoidan/l. of methanol, and all results are given in Tables I, II, and III. The reaction time was 20–24 hr. and the temperature indicated was maintained during this period of time. The solutions obtained after passage through the exchange resin columns were colorless.

Methanolysis of 150 g. of purified fucoidan (60°, 1000 ml. of methanol, 47 g. of hydrogen chloride) yielded 20.8 g. of methyl α-L-fucoside, m.p. 153–157°, 10.1 g. of a second fraction of crystals, m.p. 98–104°, and 9.8 g. of noncrystallizing sirup.

Chromatographic Examinations.—A second fraction of crystals with m.p. 103–130° was chromatographed using ethyl acetate–acetic acid–formic acid–water (18:3:1:4 v./v.) as the irrigant. When sprayed with permanganate–periodate[16] and with silver nitrate,[17] no compounds with low R_f values could be detected.

Part of the crystallizate was hydrolyzed by refluxing in *N* sulfuric acid for 5 hr. After removal of the sulfuric acid as barium sulfate, the solution was chromatographed using the same irrigant as above. When sprayed with aniline hydrogen phthalate[18] and permanganate-periodate, only fucose and a very small proportion of a hexose could be detected.

Identification of Methyl β-L-Fucoside.—A saturated ethanolic solution of an impure fraction of crystals with m.p. 103–130° and $[\alpha]^{20}D$ −77.2 ± 2° (*c* 1.6, water) was prepared and 1.5–2.0 volumes of a saturated ethanolic solution of potassium acetate was added. This mixture was kept at room temperature for 1 day. The crystalline precipitate (silky needles) was filtered off, washed with cold ethanol, and dried in a desiccator over calcium chloride; m.p. 209–212°, $[\alpha]^{20}D$ +6.5 ± 2.2° (*c* 1.6, water). The data for the potassium acetate adduct of methyl β-L-fucoside reported by Hudson, *et al.*,[19] are m.p. 208–212°, $[\alpha]^{20}D$ +8.9°.

The experiment was repeated using a fraction with m.p. 108–116° and $[\alpha]^{20}D$ −41.1 ± 2.8 (*c* 0.6, water). The same crystalline product was isolated.

Hydrolysis of Noncrystallizing Residue.—The noncrystallizing residue (40 g.) obtained from different experiments after removal of crystalline methyl α- and β-L-fucosides was diluted with 200 ml. of *N* sulfuric acid, refluxed for 5 hr., treated with activated carbon, and filtered. The sulfuric acid then was precipitated quantitatively as barium sulfate with a solution of barium hydroxide. After treatment with some Amberlite IR-120-(H^+) cation-exchange resin and activated carbon, the colorless solution was concentrated *in vacuo* to a sirup which was diluted with hot methanol. Ether was added and, after being seeded with L-fucose, the mixture was refrigerated for 10 days. Crystals occasionally were removed from the sides of the flask with a spatula. The crystallizate was filtered off on a fritted-glass funnel, washed with a cold mixture of methanol and ether, and dried; yield, 7.5 g., m.p. 136–138°, $[\alpha]^{20}D$ −75.0° ± 1.5° (*c* 1.3, water). When admixed with an authentic sample of L-fucose, the m.p. was undepressed.

Preparation of L-Fucose.—Fucoidan (75 g.) was subjected to methanolysis at 60° for 24 hr. using 500 ml. of methanol and 25 g. of hydrogen chloride. The procedure from here until after passage through the cation- and anion-exchange resin columns was the same as described above. The neutral, colorless solution, however, then was concentrated to a smaller volume, acidified with sufficient sulfuric acid to make it *N* with respect to the acid, and refluxed for 5 hr. After filtration, precipitation of the sulfuric acid with barium hydroxide, and treatment with activated carbon and some Amberlite IR-120-(H^+) cation-exchange resin, the colorless solution was concentrated *in vacuo* to a sirup. The sirup was diluted with hot methanol to which ether was added. This mixture was left in the refrigerator for crystallization, the crystals being occasionally removed from the sides of the flask. After about 2 weeks, L-fucose was filtered off on a fritted-glass funnel, washed with a cold mixture of methanol and ether, and dried. The yield was 7.6 g., m.p. 136–139°, $[\alpha]^{20}D$ −75.3 ± 1.4° (*c* 1.3, water). When a sample was mixed with authentic L-fucose, the m.p. was undepressed. The noncrystallizing sirup weighed 29.5 g.

(15) All melting points are uncorrected.

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