Preparation of Alkyl α- and β-L-Fucopyranosides

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Fucoidan from Macrocystis pyrifera is subjected to alcoholysis using primary, secondary, and tertiary alcohols. Ethyl, isopropyl, cyclohexyl, and benzyl α-L-fucopyranosides are obtained in their crystalline forms and in preparative yields. The yields apparently decrease with increasing size of substituent. Under the conditions applied, both primary and secondary alcohols are similarly suitable. Tertiary alcohols, however, do not produce any isolable material. Other crystalline products obtained are methyl and ethyl β-L-fucopyranosides and their potassium acetate adducts.

FUCOIDAN IS A SEAWEED polysaccharide consisting mainly of L-fucose units which presumably are sulfated in the 4-position and connected through α-(1→2)-linkages (*8*). Evidence for the presence of linkages other than (1→2) was obtained recently by Côté (*2*). In a recent article the methanolysis of fucoidan from Macrocystis pyrifera has been described (*9, 10*). The polymer was completely depolymerized forming methyl α- and β-L-fucopyranosides and small quantities of methyl glycosides giving D-galactose and D-xylose on hydrolysis. According to the results it was concluded that the principal constituent of fucoidan from Macrocystis pyrifera is a sulfated galactofucan containing L-fucose and D-galactose in a ratio of about 18:1 (*10*).

This article reports three new fucosides, namely isopropyl, cyclohexyl and benzyl α-L-fucopyranosides, as well as a general method for the preparation of alkyl α- and β-L-fucopyranosides by alcoholysis of fucoidan. Under reaction conditions similar to those applied in the methanolysis, alcohols other than methanol, primary as well as secondary, may be used successfully as demonstrated with ethyl, isopropyl, cyclohexyl, and benzyl alcohols. The product from ethanolysis is identical (m.p. and rotation) with ethyl α-L-fucopyranoside reported by Kuhn, Baer, and Gauhe (*4*). Since the other three fucosides are not reported in the literature they were recrystallized until m.p. and rotation remained constant. Further evidence to support the purity of these compounds was obtained by paper chromatography. The data found are given in Table I. The highly negative specific rotations are in agreement with reported values for α-L-fucopyranosides. The β-anomers have low, usually positive rotations (*3, 4*). The rotations of the corresponding furanosides are considerably higher (*11*). Additionally, the ring size was established by quantitative periodate oxidation studies. So it can be assumed that all three glycosides in Table I are α-L-fucopyranosides.

The approximate yields of crystalline methyl, ethyl, isopropyl, benzyl, and cyclohexyl α-L-fucopyranosides were 52, 46, 39, 32, and 28%, respectively. They appear to decrease with increasing molecular weight of the alcohol. Only relatively small differences in the yields are found when alcoholyses with primary and secondary alcohols of similar molecular weight are compared. A reaction with tertiary alcohols, however, is not possible under these conditions as was demonstrated with *tert*-butyl alcohol.

Of the β-anomers only the methyl and ethyl β-L-fucopyranosides were obtained by isolation of the corresponding potassium acetate adducts and subsequent treatment with ion exchange resins. The adduct of isopropyl β-L-fucoside could not be isolated.

When fucoidan is subjected to alcoholysis the corresponding monoalkyl sulfate, besides alkyl L-fucosides, should be expected. Such a compound, namely methyl potassium sulfate, which could not be isolated in the preceeding work (*9*) is isolated now in a yield of over 50%. After removal of this substance, the yield of crystalline methyl α-L-fucoside can be highly improved.

EXPERIMENTAL

Methyl Potassium Sulfate and Methyl α-L-Fucopyranoside. The preparation of fucoidan and the methanolysis were carried out as described previously (*9*). Fucoidan from Macrocystis pyrifera (100 grams, 26.2% of L-fucose) was suspended in one liter of methanol. Hydrogen chloride gas (51 grams) was introduced and the mixture stirred for 22 hours at 60°. An insoluble residue was removed, the solution stirred with lead carbonate, filtered, and concentrated in vacuo to a small volume. Since it started to solidify to a crystalline mass some methanol was added and the mixture refrigerated to complete crystallization. The crystals were filtered off, washed with methanol, and dried; yield 12.2 grams, m.p. 196–201°, optically inactive. Recrystallized from 90–95% methanol; m.p. 203–206° (dec., no darkening), non-combustible. The substance formed a precipitate of barium sulfate (with barium chloride) only after being heated with alkali or mineral acid.

Anal. Calcd. for CH_3OSO_3K: C 8.05; H 2.01; K 26.02; OCH_3 20.64; SO_4 63.92. Found: C 8.42; H 1.90; K 29.75; OCH_3 20.25; SO_4 62.08.

The filtrate was concentrated to a syrup, the syrup diluted with hot water, and enough of a solution of ammonium carbonate was added to complete the formation of a white precipitate. After cooling to room temperature, the precipitate was filtered off, the filtrate passed through columns with cation and anion exchange resins, Amberlite IR-120(H^+) and Dowex 1-X8(OH^-), until neutral, and the effluent, after further treatment with activated carbon, concentrated to a syrup. The syrup was diluted with methanol, ether was added, then it was refrigerated to complete crystallization. The yield of methyl α-L-fucopyranoside was 14.9 grams with m.p. 155–158° and $[\alpha]_D^{20} - 187.3 \pm 2°$ (*c* 1.5, water). A second crystallizate weighed 2.15 grams, m.p. 116–146°, $[\alpha]_D^{20} - 101.8 \pm 2.5°$ (*c* 1.5 water). After concentration of the filtrate 15.5 grams of a noncrystallizing syrup was obtained.

Other α-L-Fucopyranosides. Alcoholyses with ethanol (800 ml.), isopropyl alcohol (800 ml.), cyclohexyl alcohol (700 ml.), and benzyl alcohol (100 ml.) were carried out under similar conditions as described above for the methanolysis. The reaction temperature was 70°, the reaction time 22–24 hr., the amount of hydrogen chloride 45–55 grams/liter of alcohol, the weight of fucoidan 100 grams. The fucoidan used contained 26.5%, 28%, 28%, and 23.2% of L-fucose in each of the four cases.

The reaction mixture from the alcoholysis with isopropyl-alcohol, after concentration, was diluted with 60% aqueous

methanol. After treatment with exchange resins and concentration to a small volume, some residual cyclohexyl alcohol was removed by steam distillation. The final syrupy material started to produce crystals after about three weeks. The mixture then was diluted with benzene and refrigerated to complete crystallization.

The reaction mixture from the alcoholysis with benzyl alcohol was treated with lead carbonate, filtered, concentrated in vacuo to a syrup, and the residual benzyl alcohol removed by steam distillation. After cooling, the water-insoluble fraction which crystallized was filtered off, washed with water, and recrystallized from ethanol.

An alcoholysis with *tert*-butyl alcohol at 70° did not produce any crystalline material. Only a small amount of syrupy, unidentified end product was obtained. In all other cases, two or three fractions of crystalline α-L-fucosides were obtained. The results are shown in Table II. All four products were recrystallized (and treated with activated carbon) until m.p. and rotation were constant. The solvents used were (a) ethanol-ether, (b) isopropyl alcohol, (c) butanol, and (d) ethanol. The data for ethyl α-L-fucopyranoside then were: m.p. 147°, $[\alpha]_D^{20}$ −190.0 ±1.6° (*c* 1.5, water). Reported data are: m.p. 146°, $[\alpha]_D^{20}$ −191.0° (*c* 1, water) (*4*). The data for the other α-L-fucopyranosides are shown in Table I.

The following analyses were obtained: (b) Calcd. for $C_9H_{18}O_5$: C 52.41; H 8.80; O 38.79. Found: C 52.36; H 8.75; O 38.75; (c) Calcd. for $C_{12}H_{22}O_5$: C 58.52; H 9.00; O 32.48. Found: C 58.41; H 8.94; O 32.33; (d) Calcd. for $C_{13}H_{18}O_5$: C 61.40; H 7.14; O 31.41. Found: C 61.33; H 7.13; O 31.51.

Ethyl and Methyl β-L-Fucopyranosides. (a) The noncrystallizing syrup from the methanolysis above was diluted with 50 ml. of ethanol and 70 ml. of a saturated ethanolic solution of potassium acetate was added. After refrigeration the crystalline precipitate was filtered off, washed with cold ethanol, and dried; yield 4.4 grams, m.p. 209–212.5°. After recrystallization from ethanol: 3.15 grams, m.p. 211–214°, $[\alpha]_D^{20}$ +9.2 ±1.7° (*c* 2.1, water). Reported data are: m.p. 208–212°, $[\alpha]_D^{20}$ +8.9° (water) (*12*).

A portion of the potassium acetate adduct (two grams) was dissolved in water, treated with anion and cation exchange resins until neutral, then with activated carbon, filtered, and concentrated in vacuo to a syrup which crystallized spontaneously. After dissolution in methanol, addition of ether, and refrigeration, 0.4 grams of a crystalline precipitate (thin, long prisms) was collected, m.p. 123–124°, $[\alpha]_D^{20}$ +13.1 ±1.2° (*c* 1.1, water). Recrystallized from methanol-ether: 0.25 grams, m.p. 123–123.5°, $[\alpha]_D^{20}$ +15.5 ±1.4° (*c* 1.2, water). Reported data are: m.p. 121–123°, $[\alpha]_D^{20}$ +14.2° (*c* 3.9, water) (*3*), and m.p. 118.4–20.4 (corr.), $[\alpha]_D^{20}$ +16.04° (*7*). A second crop of crystals (0.3 grams) with m.p. 122.5–123.5° was obtained. The substance is very soluble in ethanol, and slightly soluble in ether.

(b) The potassium acetate adduct of ethyl β-L-fucopyranoside was obtained by the same procedure as described under (a) in a yield of 10.8 grams, m.p. 213–217°. After recrystallization from ethanol: 7.9 grams, m.p. 215–218°, $[\alpha]_D^{20}$ + 12.5 ±1.7° (*c* 1.9, water). Reported data: m.p. 220–221°, $[\alpha]_D^{20}$ + 13.9° (*c* 2.5, water) (*4*).

A portion of the potassium acetate adduct (three grams) was treated as described above. The β-anomer was obtained in its crystalline form (silky needles): yield 1.6 grams. It was recrystallized twice from ethanol-ether and once from ethyl acetate-petroleum ether and dried in vacuo over calcium chloride and paraffin; m.p. 93°, $[\alpha]_D^{20}$ + 23.5° (*c* 1.2, water). Reported data are: m.p. 94–95° and $[\alpha]_D^{20}$ + 21.2° (*5*). If the substance was not entirely dried over paraffin, its melting point appeared to be 66–67°.

An attempt to obtain the potassium acetate adduct of isopropyl β-L-fucopyranoside in its crystalline form was unsuccessful.

Chromatography and Periodate Oxidation. For the paper chromatographic examinations Whatman No. 4 filter paper was used. Irrigant A was a volumetric mixture of butanol-methyl ethyl ketone-water (1:1:1); irrigant B, methyl ethyl ketone saturated with water (*1*); irrigant C, ethyl acetate-acetic acid-formic acid-water (18:3:1:4, v./v.); irrigant D, ethyl acetate-pyridine-water (10:4:3, v./v.). The spray reagent applied was permanganate-periodate (*6*). Each compound tested produced only one spot. Their R_F-values are given in Table III.

For the oxidation with periodate 0.1–0.15 grams of the fucoside was dissolved in water, four ml. of 0.52*M* sodium periodate solution was added, and the volume was adjusted to 10 ml. with water. Both the periodate consumption and the formic acid formation were determined every 20–30 min., using 1 ml. aliquots. The periodate consumption was determined by reducing the residual periodate with 0.1*N* solution and titrating the excess arsenite with 0.1*N* iodine, and the formic acid formation by direct titration with 0.1*N* sodium hydroxide after decomposition of excess periodate with propylene glycol. The values reported in Table II were obtained by extrapolation to zero time. The oxidation of benzyl α-L-fucopyranoside was carried out in

Table I. Melting Point and Specific Rotation of Pure α-L-Fucosides

α-L-Fucoside	M.P.	$[\alpha]_D^{20}$
Isopropyl	156.5[a]	−191(water)
Cyclohexyl	148–148.5	−181(water)
Benzyl	166–167	−184(methanol)

[a] All melting points are uncorrected.

Table II. Data for the Crude α-L-Fucopyranosides

α-L-Fucoside	Yield (G.)	M.P.	$[\alpha]_D^{20}$
Ethyl	14.2[a]	141.5–144	−179.2 ± 1.6[b]
	1.7[c]	141–144	−174 ± 1.0[b]
Isopropyl	13.2[a]	154–156	−188 ± 2.2[b]
	0.8[c]	155–156	−186 ± 2.8[b]
Cyclohexyl	8.0[a]	146–147.5	−173.3 ± 1.6[b]
	2.65[c]	145–147	
Benzyl	7.6[a,e]	166–167	
	1.09[c]	166	
	0.42[d]	165–166.5	−173 ± 2[f]

[a] First fraction of crystals. [b] *c* 1.5, water. [c] Second fraction of crystals. [d] Third fraction of crystals. [e] Recrystallized once from ethanol. [f] *c* 0.6, methanol.

Table III. Chromatographic Examination and Oxidation

	Periodate Oxidation		R_F Values			
L-Fucoside	Periodate Cons. Moles/Mole	Formic Acid Formed Moles/Mole	Solvent A	Solvent B	Solvent C	Solvent D
Ethyl α	2.00(1.94[a])	0.84(0.26[a])	0.68	0.50	0.74	0.84
Ethyl β	2.00	0.94	0.65	0.42	0.70	0.83
Isopropyl	1.80	0.75	0.77	0.68	0.79	0.87
Cyclohexyl	1.81	0.79	0.86	0.83	0.87	0.92
Benzyl[a]	1.99	0.32	0.84	0.82	0.86	0.91

[a] The oxidation with periodate was carried out in aqueous methanol.

aqueous methanol (40–50%). The presence of methanol apparently is responsible for the low amount of formic acid produced. The same observation was made when the oxidation of ethyl α-L-fucopyranoside was repeated in 40–50% methanol.

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