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Low-molecular Carbohydrates in Algae

V *. Investigation of *Laminaria cloustoni*

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The brown alga *Laminaria cloustoni* has been investigated with respect to its low-molecular carbohydrates. Mannitol, 1-mannitol β-glucoside, 1,6-mannitol di-(β-glucoside) and 1-mannitol acetate were isolated. All of these substances have been found previously in another brown alga, *Fucus vesiculosus*. In addition to these substances, the disaccharide laminarobiose was isolated in a low yield and the presence of laminarotriose demonstrated by paper chromatography. Finally, a great number of spots, mostly due to unidentified substances, was observed on the chromatograms of the different fractions obtained after separation on carbon and cellulose columns.

In previous papers in this series the investigations of the brown algae *Fucus vesiculosus* (Part I[1]) and *Pelvetia canaliculata* (Part IV) were reported. Both these algae contain D-mannitol, 1-mannitol β-glucoside and 1,6 mannitol di-(β-glucoside), and *P. canaliculata* also D-volemitol and the corresponding volemitol glucosides. 1-Mannitol acetate was isolated from *F. vesiculosus* but not from *P. canaliculata*. In the present paper a similar investigation of a third brown alga, *Laminaria cloustoni*, is reported.

By fractionation of the carbohydrate fraction on carbon and cellulose columns mannitol (7 %), its monoglucoside (0.5 %), diglucoside (0.08 %) and monoacetate (0.01 %) were isolated, *i.e.* all the substances previously found in *F. vesiculosus*. Laminarobiose (0.02 %) was further isolated and characterised as its crystalline β-octaacetate, and the presence of laminarotriose demonstrated by paper chromatography. The carbohydrate fraction was very complex, and the presence of at least twenty unidentified substances was demonstrated by means of paper chromatography. Most of these substances seem to occur in rather small quantities, and attempts to isolate some of them in a state of purity by further separations were not successful. Some of the substances were reducing, others nonreducing carbohydrates, while some after hydrolysis gave a

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positive ninhydrin reaction. It seems not improbable that some of the substances are artefacts, formed by condensation of sugars with amino compounds.

In Table 1 the relative amounts of sugar alcohol derivatives, calculated as percentage of free sugar alcohol found in the algae, are given. The yields obtained by the fractionation are of course not of analytical accuracy, but the figures obtained for the mono- and di-glucosides are surprisingly similar for the different plants, and it seems possible that these substances are characteristic for at least a group of brown algae.

For the monoacetate, however, the figures differ very much, from 2 % in *Fucus* to nil in *Pelvetia*. The *Laminaria* was richer in mannitol than the other algae and a greater amount of material was extracted, so if the *Pelvetia* contained monoacetates in a relative percentage of the same magnitude as that of the *Laminaria*, this would correspond to rather small amounts (10—20 mg), which might easily be overlooked. It is therefore not improbable that the mannitol monoacetate occurs in the same group of brown algae. It is safer, however, to await generalisations until several further species of brown algae from different families have been investigated.

Table 1. Sugar alcohol derivatives, in percentage of free sugar alcohol isolated.

Alga	Monoglucoside	Diglucoside	Monoacetate
Fucus vesiculosus	10.0	2.4	2.0
Laminaria cloustoni	7.1	1.1	0.1
Pelvetia canaliculata (mannitol)	9.2	1.5	—
» » (volemitol)	10.0	2.2	—

Free reducing sugars, except glucose and fructose, which are probably formed by hydrolysis of the sucrose, have not been found in algae before. Those found here, laminarobiose and laminarotriose, have not previously been found in Nature but are obtained by partial hydrolysis of laminaran, a glucan with 1,3-β-bonds occurring in brown algae. Their occurrence in *L. cloustoni* may be due to either of two causes. Either they are present in the living plant as precedents in the biosynthesis of laminaran or they are formed from this polysaccharide by a post-mortal, enzymatic hydrolysis. As no special precautions were taken to destroy the enzyme systems when the plant was collected, it is impossible to distinguish between these possibilities.

A substance, m.p. 250° (slight decomp.), which travels very fast on the carbon column but slow on paper was also isolated in a yield of 0.03 %. It might be a cyclitol and is now subject to further investigations.

EXPERIMENTAL

(Melting points uncorrected, all evaporations under reduced pressure, 40°).

The air-dried, ground alga (1 045 g), kindly supplied by Dr. E. T. Dewar, Inst. of Seaweed Research, Inveresk, Scotland, was extracted with ether for 3 days and with methanol for 9 + 7 days. The methanol extracts were concentrated to dryness, the residues treated with water and undissolved material removed by filtration. After treatments

Table 2. Fractionation of the low-molecular carbohydrates from L. cloustoni on a Carbon-Celite column.

(Fractions listed in the order of elution from the carbon column. Chromatograms run on Whatman 1 filter paper, ethyl acetate-acetic acid-water, 3:1:1, used as solvent and, unless otherwise stated, silver nitrate-sodium ethoxide [2] as reagent.)

Fraction	Yield, g		Contents, by paper chromatography
	1:st extr.	2:nd extr.	
a	0	0.2	Salts.
b	24.0	23.5	Mannitol, glucose, fructose, strong spot just faster than sucrose, strong spot between mannose and xylose, which gives positive reaction with anisidine phosphate.
c_1, c_2	6.0	7.0	Mannitol, glucose, fructose and faint, unidentified spots. In c_2 only mannitol.
d	1.33	0.66	Mannitol, four intense spots between those of mannose and rhamnose, all with positive anisidin ephosphate reactions and one with positive resorcinol-hydrochloric acid reaction.
e	1.29	—	Eight unidentified spots, one with positive anisidine phosphate reaction, same R_F as glucose.
f	4.28	1.37	Mannitol monoglucoside, trace mannitol monoacetate.
g	0.52	0.31	Laminarobiose, trace mannitol monoglucoside.
h	0.40	0.41	Mannitol diglucoside, two weak, unidentified spots.
i	0.34	0.39	Traces of mannitol diglucoside and of unknown substances.
j_2	—	0.20	Intense spot between mannitol mono- and di-glucosides.
k	0.31	0.28	Trace of laminarotriose, large number of unidentified spots.
l	0.28	0.27	Laminarotriose.
m	0.20	0.44	Unidentified spots
50 %	0.77	0.60	» » , an intense spot of low R_F, strongly reducing with anisidine phosphate reagent.

with lead acetate and hydrogen sulphide the aqueous solutions were concentrated to dryness. Yields, 179 g and 42 g. Ionic material in the first extract was removed by ion exchange (Amberlite resins IR 120 and IR 4B) and the residue (69 g) was dissolved in aqueous ethanol, when mannitol (20 g), m. p. 161—163°, separated. The mother liquors were concentrated and fractionated on a carbon-Celite column (50 × 5 cm), using the gradient elution techniques. Eluants: 12 l aqueous ethanol, 1—45 %, followed by 4 l 50 % ethanol. The eluate was divided into fractions, which were investigated by paper chromatography and similar fractions combined and concentrated to dryness. The second extract, without previous ion exchange and removal of mannitol, was subjected to the same fractionation procedure. The results of these fractionations, which gave very similar results, are given in Table 2.

Fraction b. The fraction was deionised, most of the mannitol removed by crystallisation and the residue concentrated to an orange syrup, which on standing deposited colourless crystals of high m. p. (1.34 g). Part of the syrup was fractionated on a hydrocellulose column, using aqueous butanol as eluant. Mannitol, glucose and a slow moving substance, identical to that which crystallised from the syrup, were isolated. These high melting fractions were combined and recrystallised five times from ethanol. Yield, 0.28 g. M. p.

250° (Slight decomp.). The substance is optically inactive, contains only C, H and O, reduces the silver nitrate-sodium ethoxide reagent and has an R_F-value slightly higher than that of D-inositol.

Fraction c. On recrystallisation pure D-mannitol was obtained, m. p. 164—165°, undepressed on admixture with authentic material.

Fraction e_1. By fractionation of this fraction on thick filter paper (Whatman 3 MM), a chromatographically pure, amorphous substance (100 mg) was obtained. The non-reducing substance had an R_F-value slightly higher than that of 1-mannitol β-glucoside and on hydrolysis yielded a mixture of mannitol and glucose, why the substance might be an isomer to 1-mannitol β-glucoside.

Fractions f_1 *and* f_2. Pure 1-mannitol β-glucoside (1.06 g) was obtained by crystallisation from f_2. M. p. 137—138°, undepressed on admixture with authentic material.

Fraction f_1 was fractionated on a hydrocellulose column (50 × 7 cm) using *iso*propanol-butanol-water (7:1:2) as eluant. 1-Mannitol acetate (180 g), m. p. 119—120, and 1-mannitol β-glucoside (3.4 g), m. p. 136—138°, identical to authentic materials, were obtained. In addition, a compound with colour reactions and R_F-values in different solvent systems identical to those of gentiobiose, was obtained in a low yield (5 mg).

Fraction g. Chromatographically pure laminarobiose was isolated from this fraction by separation on thick filter paper. By acetylation with sodium acetate-acetic anhydride at 130—140°, it was converted to β-laminarobiose octaacetate, m. p. 158—159°, undepressed on admixture with authentic material.

Fraction h. The main spot in this fraction was chromatographically indistinguishable from that of authentic 1,6-mannitol di-(β-glucoside). Partial hydrolysis yielded glucose, mannitol and 1-mannitol β-glucoside.

Fraction l. This fraction, in colour reactions and R_F-values in different solvents, was indistinguishable from laminarotriose. Glucose was the only substance detected after hydrolysis.

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