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Low-molecular Carbohydrates in Algae

I. Investigation of *Fucus vesiculosus*

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Studies on the carbohydrate constituents of various lichens have been reported in previous communications [1] from this institute. Lichens are composed of algae and fungi and similar studies on algae have now been commenced. The present paper records an investigation of the brown alga *Fucus vesiculosus*.

The only low-molecular carbohydrate previously observed in brown algae is D-mannitol, which is a major constituent. Three additional substances of carbohydrate nature have now been isolated by fractionation of the carbohydrate fraction from *Fucus vesiculosus* on carbon and hydrocellulose columns.

The first substance, m.p. 124—125°, $[\alpha]_D^{20}$ + 4°, had a R_F-value rather close to that of glycerol, but on the carbon column it moved slower than sucrose. It gave the hydroxamate test for esters and on hydrolysis yielded mannitol and acetic acid. Analyses were consistent with the formula of a mannitol monoacetate. The substance consumed 4 moles of periodate with formation of 3 moles of formic acid and is consequently 1-D-mannitol monoacetate. The carbohydrate fraction, after successive treatments with lead acetate and hydrogen sulfide, contained acetic acid which was removed when the solution was concentrated to dryness. The acetate, consequently, might possibly be an artefact, but when, however, a solution of mannitol in 50 % acetic acid was concentrated to dryness, the material was recovered unchanged and not even traces of mannitol acetates could be detected.

The second substance, m.p. 140—141°, $[\alpha]_D^{20}$—18°, moved as a disaccharide on the paper chromatogram and on the carbon column. It was nonreducing and on acid hydrolysis yielded equimolecular amounts of mannitol and glucose. It consumed 6 moles of periodic acid with formation of 4 moles of formic acid, which together with the optical rotation indicates that the substance is 1-D-mannitol β-D-glucoside.

The third substance was amorphous, $[\alpha]_D^{20}$-14°, and moved as a trisaccharide on the paper chromatogram and on the carbon column. It was nonreducing and on acid hydrolysis yielded 1 mole of mannitol and 2 moles of glucose. It consumed 6.5 moles of periodate with formation of 3.4 moles of formic acid. No formaldehyde was formed. The expected values for 1,6-D-mannitol di(β-D-glucopyranoside) are 7 moles of periodate and 4 moles of formic acid,

and bearing in mind that the substance was amorphous and certainly not perfectly pure, the agreement is rather satisfactory.

In addition to these substances the presence of several other minor constituents was demonstrated by paper chromatography. Sucrose and its products of hydrolysis, glucose and fructose were recognized, but the other spots were due to unidentified substances. One of them, however, might be due to α,α-trehalose.

Acetates of glycitols have not previously been observed in Nature. During the investigation of carbohydrates in lichens, however, spots with R_F-values corresponding to that of mannitol monoacetate were frequently observed, the spots were not identified and the amounts of substance were too small to permit isolation. It now appears not impossible that these spots were due to glycitol acetates.

Some glycitol glycosides have been isolated from natural sources. A glycerol α-D-galactoside, floridoside [2], and a D-glyceric acid α-D-mannoside [3] have been isolated from red algae, and a D-arabitol β-D-galactoside, umbilicin[1], from several lichens. Clavicepsin [4], isolated from ergot *(Claviceps purpurea)* is of special interest in this connection. It is a diglucoside of mannitol, and judging from its high specific rotation and α-glucoside, and is thus isomeric with the substance described above.

When the alga was collected no special precautions were taken immediately to inactivate the enzymes. Therefore the possibility that the substances isolated are products of an enzymatic transformation is not excluded.

EXPERIMENTAL

The alga (kindly supplied by Dr. E. Vasseur) was collected on the Swedish west coast and immediately dried in the sun. The crushed alga (975 g) was extracted in a continuous extractor with ether for 3 days and then with methanol for 9 days. The methanol extract was concentrated to dryness under reduced pressure, the residue was treated with water and undissolved material removed by filtration. Lead acetate was added to the aqueous solution, the precipitate filtered and the excess of lead precipitated with hydrogen sulfide. The solution was then concentrated to dryness. Another 9 days extraction with methanol yielded more material, and the two fractions (30 g and 6 g respectively) were combined.

In a preliminary experiment part of the carbohydrate fraction (7 g) was dissolved in 1 % ethanol and absorbed on a carbon-Celite column [5] (35 × 4.5 cm). The column was eluted with aqueous ethanol, the concentration of the ethanol being continuously increased from 1 % to 15 %. The eluate was divided into fractions, which were investigated by paper chromatography. (Solvents: butanol-ethanol-water, 4 : 1 : 5 and ethyl acetate-acetic acid-water, 3 : 1 : 1) Similar fractions were combined and concentrated to dryness under reduced pressure. The results of the separation are summarized below.

0— 750 ml	1.6 g	Salts.
750—1 200 ml	3.5 g	Mannitol, identical with an authentic specimen.
1 200—1 220 ml	0.08 g	Traces of mannitol, glucose, fructose, and several unidentified substances.
1 220—2 870 ml	0.13 g	Traces of unidentified substances.
2 870—3 080 ml	0.06 g	Mannitol monoacetate, traces of sucrose, of a non-reducing substance with the same R_F-value as α,α-trehalose and of unidentified substances.
3 080—3 210 ml	0.19 g	Mannitol monoacetate and monoglucoside.
3 210—3 590 ml	0.23 g	Mannitol monoglucoside, small amounts of mannitol monoacetate.
3 590—4 000 ml	0.03 g	Mannitol monoglucoside.

When the column was eluted with 50 % ethanol, a further amount of material (0.42 g) consisting of the mannitol diglucoside and unidentified substances with lower R_F-values was obtained.

In a new run a larger amount of material (25 g) was absorbed on the same column, and eluted with aqueous ethanol (4 000 ml), the concentration of which was continuously increased from 1 % to 25 %. A fraction (2.5 g) containing a mixture of mannitol monoacetate and mannitol monoglucoside was obtained together with a fraction of chromatographically pure mannitol diglucoside (0.6 g).

The first fraction was combined with the corresponding fractions from the previous run, and the two components separated on a hydrocellulose column (60 × 3 cm), using a mixture of *iso*propanol-butanol-water (7 : 1 : 2) as solvent. The separation was followed by paper chromatography and on concentration of the fractions mannitol monoacetate (0.4 g) and mannitol monoglucoside (2.1 g) were obtained.

Mannitol monoacetate

The mannitol monoacetate, m.p. 100—120, crystallized when the solvent was vaporated. After two recrystallizations the melting point was constant, 124—125°. $[\alpha]_D^{20}$ + 4° (water, C = 2).

A sample (10 mg) was hydrolysed with 0.1 *N* hydrochloric acid (0.5 ml) at 100° overnight, neutralized with sodium hydrogen carbonate and concentrated to dryness. The residue was acetylated with acetic anhydride in pyridine and from the reaction mixture mannitol hexaacetate (11 mg), m.p. 120—121, $[\alpha]_D^{20}$ + 23° (chloroform, C = 2), identical to an authentic specimen, was isolated. Mannitol hexaacetate was also obtained by direct acetylation of the product with acetic anhydride in pyridine.

The volatile acid was identified as acetic acid by Dyer's method [6]. The salt from the acyl group determination was transformed to the free acid and steam distilled at constant volume (15 ml). The distillate was divided into 10 ml-fractions which were titrated with 0.01 *N* sodium hydroxide. A comparable amount of authentic acetic acid (0.05 mmole) was distilled under the same conditions, and the course of distillation for the two samples was found to be identical.

Distillate, ml	Unknown acid %	Acetic acid %
10	27	27
20	44	46
30	58	59
40	70	70
50	77	78
60	84	84
70	88	88
80	91	91

$C_6H_{16}O_7$ (224.2)	Calc.	C 42.9	H 7.19	$COCH_3$ 19.2
	Found	C 42.8	H 7.05	$COCH_3$ 18.9

On periodate oxidation with 0.1 *M* solution at room temperature and pH 3.5 overnight the substance consumed 4.0 ± 0.1 mole periodate. By oxidation with sodium metaperiodate under similar conditions 3.0 ± 0.1 mole of formic acid were formed.

Mannitol monoglucoside

The amorphous product was dissolved in methanol and kept at 0°. After some days the substance (1.55 g) crystallized, m.p. 137—138°. After one further crystallization from methanol the m.p. increased to 140—141°, $[\alpha]_D^{20}$ —18° (water, C = 2). The substance (100 mg) was hydrolyzed with 1 *N* sulphuric acid at 100° overnight. (With 0.1 *N* acid the hydrolysis was not complete.) The sulphuric acid was neutralized with barium carbonate and the barium sulphate removed by filtration. The presence of two substances

in the hydrolysate was demonstrated by paper chromatography. These had the same R_F-values and colour reactions with different developing agents as glucose and mannitol, respectively. As the reducing component was readily fermented with baker's yeast, its identity with glucose is demonstrated. After the fermentation of glucose, the mixture was purified by successive treatments with lead acetate, hydrogen sulfide and the Amberlite resins IR 120 and IR 4B. Mannitol (45 mg) was obtained when the solution was concentrated, and after one recrystallization from methanol melted at 161—163°, undepressed when mixed with an authentic sample. A quantitative analysis of the hydrolysate (0.07 ml about 2 % solution) was made by paper chromatography (the method of Hirst and Jones [7]). Solvent: Ethyl acetate-acetic acid-water, 3 : 1 : 1. The amounts of glucose and mannitol found were 0.782 mg and 0.758 mg, respectively, the proportion 1.04 : 1 being very close the theoretical value.

By periodate oxidation, under the same conditions as the mannitol monoacetate, the substance consumed 6.3 ± 0.1 mole of periodic acid and yielded 4.0 ± 0.1 moles of formic acid.]

$C_{12}H_{24}O_{11}$ (344.3)	Calc.	C	41.9	H	7.03
	Found	»	41.7	»	7.31

Mannitol diglucoside

The material eluted from the carbon column was chromatographically pure but did not crystallize. $[\alpha]_D^{20} -14°$ (water, C = 2). The substance was subjected to the same investigations as the monoglucoside. Mannitol (30 mg) was isolated from the hydrolyzed product (100 mg) after fermentation of the glucose. The proportion of glucose to mannitol was found to be 1.95 : 1 by quantitative paper chromatography. A partially hydrolysed product gave a spot on the chromatogram, identical to that of the monoglucoside. The substance consumed 6.5 moles of periodic acid and yielded 3.4 moles of formic acid.

SUMMARY

Three new carbohydrate components, 1-D-mannitol monoacetate, 1-D-mannitol β-D-glucopyranoside and 1.6-D-mannitol di(β-D-glucopyranoside) have been isolated from the brown alga *Fucus vesiculosus*.

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