

Low-molecular Carbohydrates in Algae

IV*. Investigation of *Pelvetia canaliculata*

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The investigation of the brown alga *Fucus vesiculosus*, in which mannitol, 1-mannitol β -glucoside, 1,6-mannitol di-(β -glucoside) and 1-mannitol acetate were found, was reported in Part I¹ of this series. It was of interest to study other brown algae and in the present paper a similar investigation of *Pelvetia canaliculata* is reported.

This alga contained, in addition to D-mannitol, another sugar alcohol, D-volemitol, in yields of about 1.3 and 0.85 % respectively. Mannitol is quite a common constituent of algae and although volemitol has been isolated from some cryptogams, the mushroom *Lactarius volemus*² and lichens of the genera *Dermatocarpon*^{3,4} and *Endocarpon*⁴, it has not previously been found in algae.

A mixture of monoglucosides of mannitol and volemitol, representing about 0.2 % of the alga, was also isolated. From this mixture, 1-mannitol β -glucopyranoside, identical to the specimen from *Fucus vesiculosus*, was obtained in a pure state. Another substance, m.p. 182—184° and specific rotation -17° (Water) on hydrolysis yielded equimolecular amounts of volemitol and glucose. The analysis was consistent with the formula of a volemitol glucoside monohydrate. The substance consumed 7 moles of periodic acid with the formation of 5 moles of formic acid, indicating that the substance is (1 or 7)-D-volemitol- β -D-glucopyranoside. Crystalline preparations of volemitol glucoside, melting gradually between wide limits (e.g. 140—160°) but chromatographically indistinguishable from the specimen above, were also obtained. Similar results with periodate oxidation suggest a mixture of 1- and 7-volemitol glucosides. A further reason for this assumption is advanced below.

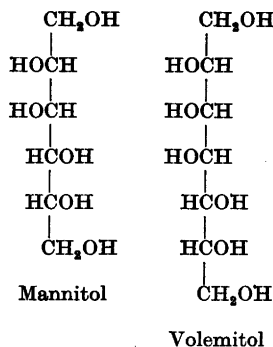
Finally, diglucosides of mannitol and volemitol were obtained. The mannitol diglucoside could not be distinguished chromatographically from that isolated from *Fucus vesiculosus*. The volemitol diglucoside, amorphous, was chromatographically but not chemically pure. On acid hydrolysis, it yielded two moles of glucose and one of volemitol. The values obtained for the consumption of periodic acid and formation of formic acid were inconsistent

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with any formula but their ratio (1.56) is rather close to that calculated for the 1,7-volemitol diglucoside (1.6). On periodate oxidation no formaldehyde was formed, and these facts, together with the specific rotation, -13° (Water) are consistent with the formula of 1,7-volemitol di-(β -glucopyranoside).

In all the isolated glucosides, primary hydroxyl groups are glucosidically linked. In mannitol and volemitol the primary hydroxyl groups are very similar, having identical configurations on two adjacent carbon atoms. It is therefore reasonable to assume that they should be almost equivalent in their reactions with enzymes, and that the glucose should be almost statistically distributed between these groups. According to this theory, which is consistent with the yields obtained, both 1- and 7-volemitol monoglucoside should occur in the alga. Although only one of them has been isolated in a state of purity, this is a further argument, in addition to the experimental, for the occurrence of both substances.

In addition to these substances, the presence of sucrose and a small amount of glucose and fructose was demonstrated chromatographically.



EXPERIMENTAL

The alga was collected at Lofoten, Norway, in July and August 1952. The coarsely ground alga (630 g) was extracted in a continuous extractor with ether for 2 days and with methanol for 16 days. The methanol extract was concentrated to dryness under reduced pressure, the residue 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 sulphide. Salts were removed by treatment with the Amberlite resins IR 120 and IR 4B and the solution on concentration to dryness gave a light yellow syrup (19.1 g).

Half of the carbohydrate fraction was dissolved in 1 % aqueous ethanol (100 ml) and added to the top of a carbon-Celite column (35 \times 4.5 cm). The column was eluted with the same solvent (3 000 ml) followed by aqueous ethanol (4 000 ml), the concentration of which was increased continuously from 1 to 25 %. The eluate, collected in fractions, was investigated by paper chromatography (Solvents: butanol-ethanol-water, 4 : 1 : 5 and ethyl acetate-acetic acid-water, 3 : 1 : 1), and similar fractions combined and concentrated. The main fractions in the order of their elution were

Mannitol, 3.4 g,
Mannitol and volemitol, about 50 % of each, 1.7 g,
Volemitol, 2.2 g,

Mannitol monoglucoside, 0.1 g,

Mannitol and volemitol monoglucosides, 0.5 g,

Volemitol monoglucoside 0.1 g.

Mixture of several substances, sucrose recognized by R_F-value and colour reactions.

Mannitol diglucoside, 0.03 g,

Mannitol and volemitol diglucosides 0.05 g,

Volemitol diglucoside, 0.03 g.

Sugar alcohols. The mannitol and volemitol, both recrystallised from methanol, melted at 163–164° and 152–153°, undepressed on admixture with authentic samples. They showed positive rotations in borax solutions and thus both belonged to the D-series.

Mannitol monoglucoside. The mannitol monoglucoside was crystallised from aqueous methanol, m.p. 137–139°, undepressed on admixture with the 1-mannitol β-glucoside obtained from *Fucus vesiculosus*. The same substance was obtained from the mixture of monoglucosides by chromatography on thick filter paper.

Volemitol monoglucoside. Chromatographically pure preparations of volemitol monoglucoside were obtained by the separation on the carbon column, by separation of the mixed monoglucosides on thick filter paper (Whatman 3 MM, three successive 24 hour developments with ethyl acetate-acetic acid-water solvent) or by spontaneous crystallisation from a solution of mixed glucosides in aqueous ethanol. Some fractions were amorphous, others crystalline but with a varying range in melting points, e. g. 140–153°, 155–168° and 182–184°. On the paper chromatograms, the preparations behaved identically. The specimen melting at 182–184° was obtained from the mixed glucosides by spontaneous crystallisation and subsequent recrystallisation from aqueous ethanol. The m. p. was unchanged on further recrystallization so it was considered to be a pure substance. $[\alpha]_D^{20} -17^\circ$ (water, c = 2). On hydrolysis it yielded glucose and volemitol. The glucose was characterised by paper chromatography and its ready fermentation with baker's yeast. The volemitol was isolated by chromatography on thick filter paper and recrystallised from ethanol. M. p. 151.5–152.5°, undepressed on admixture with authentic volemitol. The ratio of glucose to volemitol, 1 : 1.2 was determined by quantitative paper chromatography⁵, and the analysis was consistent with the formula of a volemitol monoglucoside monohydrate. (Found: C 39.5; H 7.24. Calc. for C₁₃H₂₈O₁₂ · H₂O (392.3): C 39.8; H 7.19.) On periodate oxidation with 0.1 M solution at pH 3.9 and room temperature overnight, the substance consumed 7.1 ± 0.2 mole periodate. By oxidation with sodium metaperiodate under similar conditions, 5.1 ± 0.1 mole of formic acid were formed. A sample, m.p. 140–153°, obtained by separation on thick filter paper, gave similar values.

Mannitol diglucoside. The sample obtained from the separation on the carbon column yielded mannitol and glucose on hydrolysis. On paper chromatography in different solvents (e. g. ethyl acetate-acetic acid-water, three successive 24 hours developments) it could not be distinguished from the 1,6-mannitol di-(β-glucoside) from *Fucus vesiculosus*. It was not investigated further.

Volemitol diglucoside. The hydrolysis of the chromatographically pure sample from the carbon column yielded glucose and volemitol in the proportion 2 : 1.1. On periodate oxidation, with conditions as for the monoglucoside, 5.87 mole of periodate were consumed and 3.76 mole of formic acid (but no formaldehyde) were formed. These figures indicate that the substance is not pure, and the specific rotation, -13° (water, c = 2) is significant only by its sign and relative magnitude.

SUMMARY

The carbohydrates in the brown alga *Pelvetia canaliculata* have been investigated. The sugar alcohols mannitol and volemitol and mono- and di-β-glucosides of these substances in which glucose is linked to a primary hydroxyl group, have been isolated.

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REFERENCES

1. Lindberg, B. *Acta Chem. Scand.* **7** (1953) 1119.
2. Bougault, E. *J. pharm. chim.* [6] **2** (1895) 385.
3. Asahima, Y. and Kasitani, M. *Ber.* **67** (1934) 804.
4. Lindberg, B., Wachtmeister, C. A. and Wickberg, B. *Acta Chem. Scand.* **7** (1953) 591.
5. Hirst, E. L. and Jones, J. K. N. *J. Chem. Soc.* **1949** 1659.

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