ACTA CHEMICA SCANDINAVICA 17 (1963) 1348–1350

Studies on the Chemistry of Lichens

18 *. 3-*O*-β-D-Glucopyranosyl-D-Mannitol from *Peltigera aphthosa* (L) Willd.

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The low molecular weight carbohydrates in *Peltigera aphthosa* (L) Willd. have been investigated. In addition to *myo*-inositol, D-arabinitol, D-mannitol and sucrose, a new glycoside, 3-*O*-β-D-glucopyranosyl-D-mannitol was isolated. The structure of the latter was proved by its synthesis from 4-*O*-β-D-glucopyranosyl-D-mannose.

Sugar alcohols and the non-reducing disaccharides trehalose and sucrose are the low molecular weight carbohydrates generally found in lichens. Some lichens also contain glycosides of sugar alcohols. For example, umbilicin, 2-*O*-β-D-galactofuranosyl-D-arabinitol [1] was isolated from *Umbilicaria pustulata* [2] and some other lichens.[3] A D-mannitol-D-galactoside, peltigeroside, possibly related to umbilicin, has recently been isolated from *Peltigera horizontalis* by Pueyo [4]. Smith [5] reports the isolation of a glycoside, probably identical with peltigeroside, from *Peltigera polydactyla*.

In the present communication, a study of the low molecular weight carbohydrates in *Peltigera aphthosa* is reported. The lichen was extracted successively with ethyl ether, acetone and methanol. The water soluble parts of the acetone and methanol extracts were worked up and fractionated by carbon column chromatography as described in the experimental part. *Myo*-inositol, D-arabinitol and D-mannitol were isolated and characterised. *Myo*-inositol has not been isolated from lichens before and it seems most probable that it has been overlooked in earlier investigations. In the disaccharide region, sucrose and a non-reducing substanse, which had a paper chromatographic mobility very close to that of α,α-trehalose, were observed. The sucrose was not isolated in a crystalline state but was chromatographically indistinguishable from an authentic sample and was readily hydrolysed by invertase.

* Part 17. *Acta Chem. Scand.* **16** (1962) 2240.

The other substance crystallised from aqueous ethanol. The product, which contained water of crystallisation, melted at 97—100° and showed $[\alpha]_D$ −6° in water. On hydrolysis it yielded equal parts of D-glucose and D-mannitol. These observations, and the fact that it was more resistant to acid hydrolysis than expected of a furanoside, indicated that it was a β-D-glucopyranosyl-D-mannitol. It was different from the 1-*O*-β-D-glucopyranosyl-D-mannitol isolated from *Fucus vesiculosus* [6] and other brown algae.

Of the three isomeric β-D-glucopyranosides of D-mannitol, the 1- and 2-derivatives, in which the *a-trans* hydroxyl groups at C-3 and C-4 in the mannitol residue are free, should have high electrophoretic mobilities in germanate buffer.[7] In the 3-derivative, one of these positions is substituted and the mobility should be low. The mobility of the glucoside from brown algae was slightly less than that of mannitol, but that of the glucoside from *P. aphthosa* was low, indicating the 3-derivative. 3-*O*-β-D-Glucopyranosyl-D-mannitol was consequently prepared by borohydride reduction of 4-*O*-β-D-glucopyranosyl-D-mannose. This disaccharide has been synthesised by several routes (summarised in Ref.[8]) and, like the sample [9] used in this investigation, it has also been obtained from soft wood glucomannans by partial hydrolysis. The synthetic 3-*O*-β-D-glucopyranosyl-D-mannitol and the natural product proved to be indistinguishable, and by this means the structure of the latter was proved.

The glucoside crystallises with two moles of water of crystallisation. Neither the anhydrous glucoside nor its nona-acetate could be induced to crystallise.

EXPERIMENTAL

Air dried, ground *Peltigera aphthosa* (430 g), collected at Abisko, Lappland, Sweden, was continuously extracted with ether for 3 days, with acetone for 3 days and with methanol for 12 days. The acetone and methanol extracts were concentrated to dryness, yielding residues of 17.5 g and 60.8 g, respectively. These were partitioned between chloroform and water, the aqueous phases treated with basic lead acetate, filtered, excess lead precipitated by hydrogen sulphide and deionised. A chromatographic examination of the solution from the acetone extract revealed that it contained essentially arabinitol and mannitol, and it was not further investigated. The solution from the methanol extract was filtered through a short column of charcoal-Celite (1:1) which was then washed with 50 % aqueous ethanol. The filtrate and washings were combined and concentrated to a syrup (25 g) which was dissolved in water (80 ml) and added to the top of a charcoal-Celite column (71 × 8 cm) which was irrigated with 0→20 % aqueous ethanol (18 l, linear gradient) and then with 50 % aqueous ethanol (8 l). Fractions (50 ml) were collected and examined by paper chromatography. Similar fractions were combined and concentrated. The following main components, listed in the order in which they were eluted, were obtained. The identities of the first three were established by comparison with authentic samples.

Myo-inositol (50 mg), melting at 220—222° after crystallisation from aqueous ethanol and vacuum sublimation.

D-*Arabinitol* (600 mg), m.p. 99—102°, $[\alpha]_D^{20}$ +12° (*c*, 2.0, saturated sodium tetraborate in water), after crystallisation from aqueous acetone.

D-*Mannitol* (4.6 g), m.p. 162—164°, $[\alpha]_D^{20}$ +28° (*c*, 2.0, saturated sodium tetraborate in water), after crystallisation from aqueous ethanol.

Mannitol glucoside (2.9 g), m.p. 97—100°, $[\alpha]_D^{20}$ −6° (*c*, 2.0, water) after crystallisation from aqueous ethanol. The glucoside (50 mg) was hydrolysed in 1 M hydrochloric acid (2.5 ml) at 100° overnight. After removal of the acid by ion exchange, the product, which on paper chromatography in ethyl acetate-acetic acid-water (3:1:1) revealed the presence of glucose and mannitol, was fractionated on thick filter paper, using the same solvent

system. D-Mannitol (17 mg), m.p. 164—166° and D-glucose (15 mg), m.p. 143—146°, indistinguishable from authentic samples, were obtained.

On drying in a vacuum over phosphorus pentoxide at 60° the glucoside lost 9.75 % in weight. Two moles of water of crystallisation correspond to 9.47 %. (Found (for the dried substance): C 41.8; H 7.18; O 51.3. Calc. for $C_{12}H_{24}O_{11}$: C 41.9; H 7.03; O 51.1.)

Synthesis of 3-O-β-D-glucopyranosyl-D-mannitol. 4-O-β-D-glucopyranosyl-D-mannose (20 mg) and sodium borohydride (20 mg) were dissolved in water (1 ml). After 3 h at room temperature the solution was diluted with water (5 ml) and treated with 2 ml of Dowex 50 (H^+). The ion exchange resin was removed by filtration and the solution was concentrated under reduced pressure. Boric acid was removed by repeated distillation with methanol. The residue was crystallised from aqueous ethanol, yielding 3-*O*-β-D-glucopyranosyl-D-mannitol (9 mg), m.p. 97—100°. The substance proved to be identical with the natural product (paper chromatography and electrophoresis, mixed m.p., optical rotation, IR).

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Received January 30, 1963.