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Studies on the Chemistry of Lichens

19 *. Mannitol Glycosides in *Peltigera* species

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The mannitol galactoside in *Peltigera horizontalis* has been shown to be 3-*O*-β-D-galactofuranosyl-D-mannitol. This substance was absent in nine other *Peltigera* species, which all contained the 3-*O*-β-D-glucopyranosyl-D-mannitol, previously isolated from *Peltigera aphthosa*.

The isolation of a mannitol galactoside from the lichen *Peltigera horizontalis* (Huds.) Baumg. was reported by Pueyo.[1] During an investigation of *Peltigera aphthosa* (L). Willd. we recently isolated another glycoside, 3-*O*-β-D-glucopyranosyl-D-mannitol.[2] In the present paper structural studies on the mannitol galactoside and a search for glycosides in other *Peltigera* species are reported.

The mannitol galactoside, m.p. 161—163°, $[\alpha]_D$ −64°, was isolated from *Peltigera horizontalis*. Assuming that the contribution from the mannitol residue is insignificant, the value for the optical rotation is lower than expected for a β-D-galactopyranoside. β-D-Galactofuranosides have optical rotations of this order of magnitude. The value for umbilicin,[3] 2-*O*-β-D-galactofuranosyl-D-arabinitol,[4] is −80°. A sample of the mannitol galactoside was hydrolysed, almost to completion, by treatment with 0.01 M aqueous hydrochloric acid at 100° for 3 h, which also indicates a furanosidic structure. In agreement with this assumption, periodate oxidation of the galactoside with a limited amount of periodate, followed by borohydride reduction and acid hydrolysis, yielded arabinose. This sugar is evidently formed by fission of the C-5 — C-6 linkage in the galactofuranose residue.

A decision between the three possible structures for a D-mannitol-β-D-galactofuranoside could be made by a comparison of the paper electrophoretic mobilities of the natural product and some other substances in germanate [5] and sulphonated phenyl boronic acid [6] buffers. (Table 1). Acyclic polyols,

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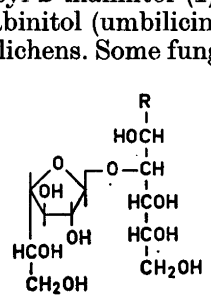
Table 1. Mobilities of the mannitol galactofuranoside and some other substances on paper electrophoresis.

Substance	M_M* germanate	M_M* sulphonated phenyl boronic acid
Mannitol galactofuranoside	0.4	0.3
1-*O*-*β*-D-Glucopyranosyl-D-mannitol	0.7	—
1,6-Di-*O*-acetyl-D-mannitol	—	0.8
2-*O*-Methyl-D-glucitol	0.8	1.2
4-*O*-Methyl-D-arabinitol	0.5	0.5
3-*O*-*β*-D-Glucopyranosyl-D-mannitol	0.3	0.3
2-*O*-Methyl-D-arabinitol	0.1	0.1
Methyl-*β*-D-galactofuranoside	0.1	0.05
Methyl-*β*-D-glucopyranoside	0.0	0.0

* Mobility, relative to that of D-mannitol.

containing one or several pairs of *α-trans* glycol groupings, have high mobilities in these buffers. Thus, D-mannitol has a high mobility and substitution in the 1- or 1,6-position does not reduce this mobility very much. No 2-*O*-substituted mannitol was available, but from the high mobilities of the related 2-*O*-methyl-D-glucitol and 4-*O*-methyl-D-arabinitol, it was expected that 2-*O*-substituted mannitols should also have high mobilities.* In a 3-substituted mannitol (*e.g.* 3-*O*-*β*-D-glucopyranosyl-D-mannitol) and also in 2-*O*-methyl-D-arabinitol, one of the *α-trans* glycol hydroxyls is blocked and the mobility is considerably reduced. The low mobility of the mannitol *β*-D-galactofuranoside from *P. horizontalis* thus shows that it is 3-*O*-*β*-D-galactofuranosyl-D-mannitol. The latter has a somewhat higher mobility in germanate buffer than 3-*O*-*β*-D-glucopyranosyl-D-mannitol. A galactofuranoside generally shows a higher mobility in germanate than a glucopyranoside.

The 3-*O*-*β*-D-galactofuranosyl-D-mannitol (I) is thus closely related to the 2-*O*-*β*-D-galactofuranosyl-D-arabinitol (umbilicin, II)[4] which occurs in *Umbilicaria pustulata* and some other lichens. Some fungal polysaccharides, as galacto-



I R = CH_2OH
II R = H

* *Added in proof:* 2-*O*-Benzyl-D-mannitol has recently been prepared. Its electrophoretic mobility in sulphonated phenyl boronic acid buffer was almost as high as that of D-mannitol, in agreement with the expectation (P.J. Garegg, unpublished results).

carolose,[7] which contain D-galactofuranose residues are known but umbilicin and the mannitol galactoside from *P. horizontalis* are the only known natural galactofuranosides of low molecular weight.

The occurrence of glycosides in some *Peltigera* species, *P. horizontalis* (Huds.) Baumg., *P. canina* L. (Willd.), *P. leucophlebia* (Nyl.) Gyeln., *P. spuria* (Ach.) DC., *P. praetextata* (Flk.) Vain., *P. scutata* (Dicks.) Duby, *P. rufescens* (Weis.) Humb., *P. scabrosa* Th. Fr. and *P. polydactyla* (Neck.) Hoffm., has been studied. From *P. horizontalis* the crystalline mannitol galactoside [1] was isolated in a good yield. In addition small amounts of an amorphous material was obtained, which was chromatographically indistinguishable from the 3-*O*-β-D-glucopyranosyl-D-mannitol and on hydrolysis yielded mannitol and glucose. The presence of the glucoside, albeit in small amounts, in this lichen is indicated.

Crystalline 3-*O*-β-D-glucopyranosyl-D-mannitol, identical with the substance previously isolated from *P. aphthosa*,[2] was isolated from all the eight remaining *Peltigera* species. Its presence in *P. venosa* (L.) Hoffm., of which only a small quantity was available, was indicated by paper chromatography.

The mannitol galactoside was not found in any other species than *P. horizontalis*. The galactoside has about the same chromatographic mobility as sucrose, and the faint spot below the glucoside, observed on paper chromatograms of some of the extracts, could be developed with a sucrose reagent. The mannitol galactoside found in *P. horizontalis* thus seems to be virtually absent in all the other *Peltigera* species investigated. The name suggested for that substance, peltigeroside,[1] is therefore unfortunate, as *P. horizontalis* seems to hold a unique position amongst the lichens belonging to the genus *Peltigera*.

EXPERIMENTAL

Isolation of the glycosides. The lichen (2 – 12 g) was extracted in a continuous extractor with ether, acetone and methanol. Almost all the glycosidic material was found in the methanol extract, which was concentrated and partitioned between water and chloroform. The aqueous phase was deionised and concentrated. All the extracts revealed on paper chromatography the presence of arabinitol (for some in traces only), mannitol and a component in the disaccharide region. The latter was isolated by chromatography on thick filter paper (ethyl acetate, acetic acid, water, 3:1:1) and crystallised from aqueous ethanol. From *P. horizontalis* (12.6 g) the galactoside was obtained in a yield of 0.30 g, part of which crystallised directly from the deionised methanol extract. Further some amorphous material (11 mg) was obtained, which showed the same chromatographic mobility as the glucoside and on hydrolysis yielded glucose and mannitol.

The glucoside, m.p. 97 – 100°, $[\alpha]^{20}_D$ – 6°, (water, *c* 2.0) was isolated from the other lichens in yields between 0.5 – 1.5 %. Its identity with the 3-*O*-β-D-glucopyranosyl-D-mannitol isolated from *P. aphthosa* [2] was demonstrated by mixed m.p. determinations, hydrolysis and chromatographic investigations of the hydrolysate and, for some samples, by IR.

The mannitol galactoside. The mannitol galactoside, after crystallisation from 96 % ethanol, melted at 161 – 163° and showed $[\alpha]^{20}_D$ – 61° (water, *c* 2.0). Some samples melted partially below 100°, resolidified and melted again at the higher temperature. On drying a sample *in vacuo* at 60° over phosphorus pentoxide it lost 5.3 % in weight, corresponding to one mole of water of crystallisation. The optical rotation increased to – 64°.

A solution of the galactoside in 0.01 M hydrochloric acid was kept at 100° for 3 h and then neutralised. A paper chromatographic investigation showed that almost all the material had been hydrolysed into galactose and mannitol.

The galactoside (25 mg) was dissolved in water (1 ml) and 0.4 M periodic acid (1 ml) was added. After 1 h at room temperature, sodium borohydride (25 mg) was added, the solution kept for 1 h and then concentrated to dryness and extracted with pyridine. The extract was hydrolysed with 0.01 M hydrochloric acid as above. Paper chromatographic examination of the hydrolysate revealed the presence of arabinose.

Paper electrophoresis in 0.05 M germanate buffer at pH 10.7 and in 0.05 M sulphonated phenylboronic acid buffer of pH 6.5 was performed as previously described.[5,6]

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