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Studies on the Chemistry of Lichens

IV.* Investigation of the Low-molecular Carbohydrate Constituents of Different Lichens

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Much work has been devoted to the study of the lichen acids, but the carbohydrate constituents of lichens, however, have been rather neglected. The occurrence of erythritol in *Rocella* species, of D-arabitol and D-mannitol in different lichens and of D-volemitol in *Dermatocarpon miniatum* is mentioned in Asahina's surveys [1]. Following the discovery of an arabitol galactoside, umbilicin, in *Umbilicaria pustulata* [2], we became interested in this field and in the present paper an investigation of the low molecular carbohydrate constituents of some 60 different lichens is reported.

Most lichens have been investigated by paper chromatography, by which method the sugar alcohols arabitol, mannitol and volemitol are easily detected. In several cases these substances were also isolated and characterized. Paper chromatography is not suitable for the detection of umbilicin, which gives rather weak spots with the silver nitrate-sodium ethoxide reagent [3], nor for the disaccharides α,α-trehalose and sucrose, previously found in Umbilicaria pustulata (Part III), as they generally occur in rather low concentrations and the usual reagents are not very sensitive to these sugars. Hydrolysis of umbilicin yields galactose, and the disaccharides yield glucose, both sugars being easily detected even in low concentrations, and by this indirect method the presence of these substances has been indicated in several lichens. This method is of course unsatisfactory, and for some lichens the carbohydrate components have been fractionated on a column of animal charcoal, as described in Part III. The results of the investigation are summarized in Tables 1 and 2.

* Part III. *Acta Chem. Scand.* **7** (1953) 140.

Table 1. Carbohydrate constituents of lichens, found by paper chromatography.

Species	Arabitol	Mannitol	Volemitol
PYRENOCARPEAE			
Dermatocarpon lachneum (Ach.) A.L.Sm.	−	+	++
» *miniatum* (L.) Mann	−	+	++
» » var. *complicatum* (Lightf.) Th. Fr.	−	+	++
» *fluviatile* (Web.) Th.Fr.	−	(+)	++
Endocarpon adscendens (Anzi) Müll. Arg.	−	+	+
GYMNOCARPEAE			
Sphaerophorus fragilis (L.) Pers.	+	+	−
Crocynia spec.	+	+	−
Lobaria pulmonaria (L.) Hoffm.	+	+	−
Nephroma arcticum (L.) Torss	+	++	−
Peltigera aphthosa (L.) Willd.	+	++	−
Rhizocarpon spec.	+	++	−
Cladonia alpestris (L.) Rabh.	+	+	−
» *uncialis* (L.) Web.	+	+	−
» *rangiferina* (L.) Web.	+	+	−
Stereocaulon spec.	+	+	−
Umbilicaria pustulata Hoffm.	++	++	−
» *papulosa* (Ach.) Nyl.	+	+	−
» *pennsylvanica* Hoffm.	+	+	−
» *polyrrhiza* (L.) Ach.	+	+	−
» *cylindrica* (L.) Del.	+	+	−
» *arctica* (Ach.) Nyl.	+	+	−
» *vellea* (L.) Ach.	+	+	−
» *hyperborea* (Ach.) Hoffm.	+	+	−
» *spodochroa* (Ach.) Frey	+	++	−
» *crustulosa* (Ach.) Frey	+	+	−
» *deusta* (L.) Baumg.	+	+	−
» *proboscidea* (L.) Schrad.	+	+	−
» *hirsuta* (Sw.) Ach.	+	+	−
» *torrefacta* (Lightf.) Schrad.	+	+	−
» *polyphylla* (L.) Hoffm.	+	+	−
» *virginis* Schaer	+	+	−
» *decussata* (Vill.) Frey	+	+	−
» *rigida* (DR.) Frey	+	+	−
Pertusaria amara (Ach.) Nyl.	+	++	−
» *corallina* (L.) Arn	+	+	−
Lecanora spec.	++	+	−
Ochrolechia tartarea (L.) Mass.	+	+	−
Haematomma ventosum (L.) Mass.	+	(+)	−

Parmelia furfuracea (L.) Ach.	++	+	−
» *caperata* (L.) Ach.	+	(+)	−
» *physodes* (L.) Ach.	+	(+)	−
» *conspersa* (Ehrh.) Ach.	++	+	−
» *omphalodes* (L.) Ach.	+	+	−
Cetraria glauca (L.) Ach.	+	(+)	−
» *islandica* (L.) Ach.	+	+	−
Letharia vulpina (L.) Vain	+	+	−
Alectoria sarmentosa Ach.	+	+	−
» *implexa* (Hoffm.) Nyl.	+	+	−
Ramalina polymorpha Ach.	+	++	−
» *farinacea* (L.) Ach.	+	+	−
Thamnolia vermicularis (Sur.) Ach.	++	+	−
Xanthoria spec.	+	+	−
Anaptychia ciliaris (L.) Kbr	+	+	−
» *fusca* (Huds.) Vain	+	+	−
Lepraria chlorina Ach.	+	+	−

++ Isolated.
\+ Identified by paper chromatography.
(+) » » » » , but only as traces.
− Not found by paper chromatography.

Table 2. Carbohydrate constituents of lichens, isolated by separation on a charcoal column. (The numbers are given in per cent of dry lichen and are very approximative.)

Species	Trehalose	Sucrose	Umbilicin
Cladonia rangiferina (L.) Web.	0.025	0.06	0
Umbilicaria pustulata Hoffm.	0.2	0.2	2.5
Umbilicaria rigida (DR) Frey	0.1	0.05	0.2
Haematomma ventosum (L.) Mass.	0.015	0.2	0.15
Cetraria islandica (L.) Ach.	0.025	0.07	0.05
Lecanora atra (Huds.) Ach.	0.04	Identified but not isolated	0

DISCUSSION

Arabitol occurs in all lichens of the order *Gymnocarpeae* which have been investigated, but not in the lichens of the genera *Dermatocarpon* and *Endocarpon*. These, on the other hand, contain *volemitol*, which was not, however, detected in *Gymnocarpeae*. Thus there seems to be a sharp chemical difference between the order *Gymnocarpeae* and the genera of the order *Pyrenocarpeae*. Because the classification of the lichens is not natural, and the order *Pyreno-*

carpeae is rather heterogenous, no generalizations can be drawn from these observations.

Mannitol has been found in all the lichens investigated and appears to be a generally occurring constituent.

α,α,-Trehalose and *sucrose* have been found in all six lichens investigated by separation of the carbohydrate fraction on a charcoal column. The presence of glucose in the hydrolysate of the acetone extract from most lichens, is evidence of the general occurrence of *α,α*-trehalose and sucrose.

Umbilicin has been isolated from four of the six more carefully investigated lichens. The percentage, however, varies between wide limits, and the occurrence of umbilicin in concentrations of 0.01 % or less might have escaped detection. The presence of galactose in many hydrolysates of carbohydrate fractions from lichens of the order *Gymnocarpeae*, indicates that umbilicin is a substance frequently occurring in lichens belonging to this order.

Spots due to unidentified substances have been found frequently. Some of these spots could be developed with ninhydrin and thus contain amines, whereas others gave phenol reactions and were probably due to lichen acids. Some spots, however, could only be developed with the silver nitrate-sodium ethoxide reagent, but nothing is known of the nature of the substances giving such spots. For example the presence of erythritol in very small concentrations in some of the lichens is not definitely excluded.

An interesting question is whether the substances discussed above are formed by the algae or by the fungi of the lichen or are products of the symbiosis. The solution of that problem necessitates the separate investigation of the isolated fungi and algae.

EXPERIMENTAL

The air-dried, ground lichen (2—25 g) was extracted continuously with ether for three days. Small amounts of arabitol were sometimes found in the ether extract of lichens rich in this substance. The extraction was then continued for 3 days with acetone. Volemitol and mannitol have low solubilities in acetone and often separated as crystals and it was found that almost pure arabitol separated from the extract of lichens very rich in arabitol. These sugar alcohols were purified by recrystallization and characterized. The acetone extract was concentrated to dryness, extracted with water and the water extract investigated by paper chromatography.

The chromatograms were prepared on Whatman No. 1 paper, using butanol-ethanol-water (4 : 1 : 5). The chromatograms were run for about 20 hours and developed with silver nitrate-sodium ethoxide and with aniline hydrogen phtalate or *p*-anisidine phosphate [4]. Generally no reducing sugars were found with the latter reagents, but in some cases small amounts of hexoses were found, probably indicating that partial hydrolysis of disaccharides and of umbilicin had occurred. Part of the extract was hydrolysed

with 0.1 *N* hydrochloric acid at 100° over night and then investigated for reducing sugars by paper chromatography as described above. The results are summarized in Table 1.

For the more carefully investigated lichens larger amounts (100—200 g) were extracted and the extracts worked up as described in Part III. The results are summarized in Table 2.

SUMMARY

The low-molecular carbohydrate constituents of a number of lichens have been investigated. All the substances found, arabitol, mannitol, volemitol, α,α-trehalose, sucrose and umbilicin, previously have been occasionally observed, but they have now been shown to be either lichen constituents of general occurrence or to be characteristic for specific taxonomic groups.

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