

Studies on the Chemistry of Lichens

VIII*. Investigation of a *Dermatocarpon* and Some *Roccella* Species

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The systematic investigation of low-molecular carbohydrates in lichens¹ has been extended to a *Dermatocarpon* and some *Roccella* species. From *D. miniatum* mannitol, volemitol, α,α -trehalose and sucrose were isolated. From the *Roccella* species erythritol, arabitol, mannitol, galactose and the internal salt choline sulphate, were isolated. The presence of the rare sugar tagatose, combined with galactose in the form of a di- and a tri-saccharide was indicated.

In Part IV¹ of this series the investigation of the low-molecular carbohydrate constituents of a number of lichens, using chromatographic methods, was reported. Most lichens were investigated by paper chromatography only, but for some of them, all belonging to the order *Gymnocarpeae*, the compounds were separated on carbon columns and isolated as crystalline substances. The genus *Roccella* of this order, which is known to contain erythritol and thus differs from the others, was not represented. In the present paper investigations of *Dermatocarpon miniatum*, order *Pyrenocarpeae*, and three *Roccella* species are reported.

From *D. miniatum* D-mannitol (0.4 %), D-volemitol (4 %), α,α -trehalose (1.1 %) and sucrose (0.17 %) were isolated. Mannitol and volemitol were detected in the previous investigation of *Dermatocarpon* species, but only the volemitol was isolated and the present isolation of mannitol is complementary to these results. The disaccharides are the same as those found in lichens of the order *Gymnocarpeae*.

As mentioned above, lichens of the genus *Roccella* are known to contain erythritol and thus differ from other lichens of the order *Gymnocarpeae*. In the previous investigation D-arabitol and D-mannitol were found in all lichens belonging to this order. Now three *Roccella* species, *R. fucoformis*, *R. hypomecha* and *R. linearis*, have been investigated. Besides considerable amounts

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of erythritol, small amounts of arabitol and mannitol were isolated, so in this respect the genus does not differ from the others of this order. An internal salt, choline sulphate, $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{O}\bar{\text{S}}\text{O}_3$, was also isolated from these lichens. Woolley and Peterson² isolated the same substance from the fungus *Aspergillus sydowi*, and this seems to be the only previous report of the isolation of this substance from natural material. As a lichen consists of an alga and a fungus, living in symbiosis, one can expect to find identical or similar substances in lichens and in fungi and choline sulphate is an example of a rather rare substance occurring in both groups. An investigation of isolated algae and fungi components of some lichens, would be of special interest from this point of view.

Two small fractions, containing a di- and a tri-saccharide, respectively, were also isolated from the *Roccella* lichens. These, on hydrolysis, yielded two reducing sugars, an aldose and a ketose which were chromatographically indistinguishable from galactose and tagatose. From the intensity of the spots, the proportions of aldose to ketose could be estimated as 1:1 and 2:1. Chromatographic evidence for the presence of the free sugars in the extracts was also obtained, and the galactose was isolated as its methylphenylhydrazone. Neither the tagatose nor the saccharides have as yet been obtained in a state of purity. The saccharides are very sensitive to hydrolysis and have probably been partly decomposed during the extraction and fractionation procedures. An epimerization, although improbable, is not excluded, and a further investigation of these saccharides is necessary. Tagatose has only been found once before in Nature, by Hirst, Hough and Jones³ in the gum of *Sterculia setigera*. Galactose, glucose and mannose were the only sugars found in the hydrolysates of the extracted *Roccella* lichens.

EXPERIMENTAL

(Melting points uncorrected)

Dermatocarpon miniatum. The lichen (320 g) was extracted and the extract worked up as described in Part III⁴. Of the carbohydrate fraction (25 g) only a part (7 g) was fractionated on a carbon-Celite column, using the gradient elution technics, also described in Part III. The following substances were isolated: Mannitol (0.65 g), M. p. 161–162°; Volemitol (3.6 g), M. p. 149–150°; α,α -Trehalose (0.99 g), M. p. 96–100°; Sucrose (0.16 g), M. p. 178–180°. The melting points of all these compounds were not depressed on admixture with the corresponding authentic materials.

Roccella hypomecha. The lichen (90 g) was extracted and worked up as above. The carbohydrate fraction (5.8 g) was separated on a carbon column and the following substances were isolated:

- Erythritol 2.3 g. M. p. 118–119°.
- Choline sulphate 0.25 g. M. p. 310° (decomp.).
- "Disaccharide" (amorphous) 0.25 g.
- "Trisaccharide" (amorphous) 0.10 g.

The presence for arabitol and mannitol was indicated by means of paper chromatography.

Roccella linearis. The lichen (500 g) was extracted and worked up as above. The extract (59 g), was fractionated on a carbon column, using gradient elution with aqueous ethanol. The first fractions were shown to contain erythritol by paper chromatography but owing to mischance these fractions were lost before the amount of erythritol present

could be determined. Erythritol (2.9 g), m. p. 120–121°, and choline sulphate (0.8 g), m. p. 310° (decomp.), were isolated and in the mother liquor the presence of tagatose, galactose, arabitol and mannitol was indicated by paper chromatography. Crude fractions containing the disaccharide (0.8 g) and the trisaccharide (1.1 g) mentioned above, mixed with several unknown components, were also obtained.

The mother liquors from the erythritol and choline sulphate fractions were concentrated to a sirup (2.0 g) and fractionated on a hydrocellulose column (40 × 4.5 cm), using butanol saturated with water as solvent. Erythritol (0.28 g), m. p. 118–119°, arabitol (0.09 g), m. p. 99–100°, mannitol (0.05 g), m. p. 164–165°, and choline sulphate (0.04 g) were isolated. From one of the mother liquors galactose was isolated as its methylphenylhydrazone (0.05 g), m. p. 185–187°. The melting points of all these compounds were undepressed on admixture with the corresponding authentic materials. The ketose believed to be tagatose was found in the mother liquors from the arabitol but could not be separated from that substance. It gave colour reactions for ketoses, red with resorcinol-hydrochloric acid and yellow with anisidine phosphate. Fructose and tagatose have almost identical R_F -values in many solvent systems. In butanol-pyridine-water (3:1:1.5), however, they are separated, tagatose having the highest R_F -value of the two.

Roccella fuciformis. The lichen (300 g) was extracted as above, the methanol extract concentrated to dryness and treated with water (500 ml). Undissolved material was removed by filtration and the solution was deionized by stirring with a mixture of Amberlite IR 120 and IR 4 B, until the conductivity decreased to a constant value. The solution was then concentrated to a mixture of crystals and sirup and dissolved in boiling 90 % ethanol. On cooling crystals (7.5 g), m. p. 115–117°, separated. From the mother liquors a second crop (1.0 g) of the same m. p. was obtained. The combined crystalline fractions were refluxed with a mixture of acetic anhydride (100 ml) and pyridine (50 ml). Part of the material, which remained undissolved, was filtered off and recrystallised from ethanol, yielding pure choline sulphate (0.8 g), m. p. 308–317° (decomp.). The acetic anhydride-pyridine solution was concentrated to a sirup under reduced pressure and dissolved in hot ethanol. On cooling, the tetraacetate of erythritol (16.0 g), m. p. 85–87°, separated.

The mother liquors from the recrystallisation of the crude carbohydrate fraction were concentrated to a sirup (2.5 g) and fractionated on a hydrocellulose column as above. Erythritol (0.50 g), m. p. 116–118°, arabitol (0.18 g), m. p. 94–98°, mannitol (0.10 g), m. p. 163–164°, and choline sulphate (0.01 g), were isolated. In addition to these substances the presence of small amounts of galactose and tagatose in the mother liquors was indicated by means of paper chromatography. Crude fractions, containing small amounts of the di- and trisaccharide, were also obtained.

Choline sulphate. The "internal salt" isolated from the *Roccella* lichens was purified by recrystallisation from 90 % ethanol. It was first believed to be an ordinary salt, but later it was observed that it could not be removed from an aqueous solution by filtering through the ion exchange resins Amberlite IR 120 and IR 4 B. (Found: C 33.0; H 7.16; N 7.85; S 17.3. Calc. for $C_6H_{13}O_4NS$ (183.2): C 32.7; H 7.16; N 7.65; S 17.5).

Authentic material was prepared according to Schmidt⁵ and was further purified by ion exchange and recrystallisation from 90 % ethanol. M. p. 310° (decomp.). The natural and the synthetic materials behaved identically on heating and a supersaturated solution of one of them immediately crystallised when seeded with the other.

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