

## Studies on the Chemistry of Lichens

III\*. Disaccharides from *Umbilicaria pustulata* (L.) Hoffm.

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In Part II of this series the isolation of an arabitol galactoside from the lichen *Umbilicaria pustulata* (L.) Hoffm. was reported. The name umbilicin was proposed for the substance, and as the result of periodate oxidation experiments it was concluded that it might be either 3-D-arabitol  $\beta$ -D-galactopyranoside or, less likely, a galactofuranoside. Further investigations have shown, however, that the first suggestion is incorrect, and there is evidence in favour of the furanoside structure. In order to continue these investigations we required larger amounts of umbilicin, and as the method for its isolation previously described is rather tedious an alternative was sought. Fractionation of the carbohydrate mixture from the acetone extract on a carbon column, as described by Whistler and Durso<sup>1</sup> proved to be very satisfactory and further amounts of umbilicin could be obtained by continuing the extraction with methanol and fractionating the carbohydrates on a carbon column, using the gradient elution method<sup>2</sup>. In this way arabitol, mannitol and umbilicin were isolated in a pure state, together with smaller amounts of  $\alpha$ ,  $\alpha$ -trehalose and sucrose. The last two substances have not been isolated from lichens previously, but as sucrose occurs almost universally in the plant kingdom and  $\alpha$ , $\alpha$ -trehalose is a common constituent of fungi, these results are not particularly surprising. From the amounts of carbohydrates isolated the following approximative percentages could be estimated:

Arabitol 0.6 %, mannitol 0.8 %,  $\alpha$ , $\alpha$ -trehalose 0.2 %,  
umbilicin 2.5 %, sucrose 0.2 %.

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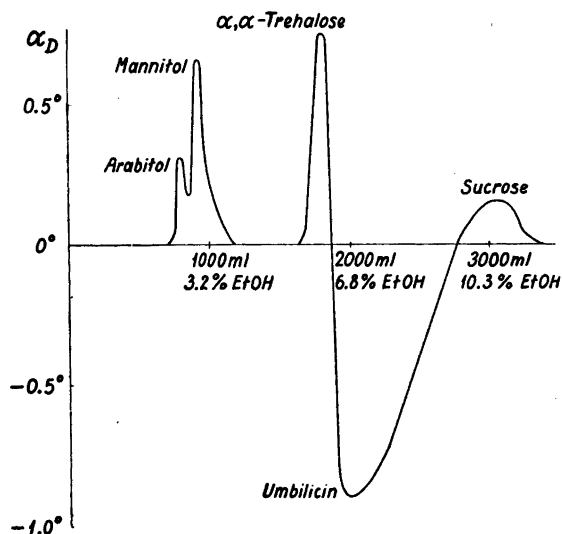


Fig. 1. Fractionation of the methanol extract on a carbon-Celite column.

#### EXPERIMENTAL

Air-dried, ground *Umbilicaria pustulata* (550 g) was extracted in a continuous extractor with ether for one week, then with acetone for one week and finally twice with methanol for the same time. The acetone and methanol extracts were worked up similarly. The solvent was removed under reduced pressure, the residue treated with water and undissolved material was removed by filtration. An excess of basic lead acetate was added to the aqueous solution, the precipitate formed was filtered off, and the excess of lead was precipitated with hydrogen sulfide. Coloured impurities were partly removed by passing the solution through a short column of aluminium oxide, and the eluate was evaporated to dryness. From the acetone extract and the two methanol extracts the residues 7 g, 21 g and 0.5 g respectively were obtained.

The carbohydrate fraction (7 g) from the acetone extraction was dissolved in 1 % ethanol (70 ml) and absorbed on to the top of a column (23 cm  $\times$  3.3 cm) of equal parts (by weight) of "carbo animalis pro analysi" (J. P. Riedel & E. de Haën) and Celite. The absorbent had been filled into the tube as a thick slurry in 1 % ethanol. The column was washed successively with 1 % ethanol (1 000 ml), 10 % ethanol (500 ml) and 50 % ethanol (1 000 ml). The 1 % ethanol eluted a crude mixture of arabitol and mannitol (2.5 g) and the 10 % alcohol crude umbilicin (2.8 g), which, however, did not crystallize. When this material was acetylated with acetic anhydride in pyridine, pure umbilicin octaacetate, corresponding to 1.8 g of umbilicin, was obtained.

The methanol extract was worked up in batches and in a typical run 6 g in 60 ml of 1 % ethanol was put on a carbon-Celite column (35 cm  $\times$  4.5 cm). The carbohydrates were eluted with aqueous ethanol (4 000 ml) the concentration of which was continuously increased from 1 % to 15 %. The elute was divided into fractions by means of an auto-

matic fraction collector, and the optical rotation of each fraction was determined (2 dm tube). The fractions with zero rotation were examined for arabitol and mannitol by investigating their rotation in acidified molybdate solution<sup>3</sup>. (5 ml solution, 5 ml 10 % aqueous ammonium molybdate, 2.5 ml *N* sulfuric acid, 2 dm tube). The results obtained are given in Fig. 1. The fractions were also investigated paper chromatographically and the intermediate fractions, containing two substances, were discarded. When the pure fractions were concentrated, five crystalline substances, corresponding to the peaks in Fig. 1, were obtained. These were further purified by recrystallization from ethanol or aqueous ethanol and identified as the following.

*Arabitol*: 0.2 g, m.p. 97–99°. (All melting points are uncorrected.)

*Mannitol*: 0.8 g, m.p. 162–163°.

*α,α-Trehalose*: 0.3 g, m.p. 94–95°. Non-reducing, yielded only glucose on hydrolysis.

Acetate, m.p. 75–78°.

*Umbilicin*: 3.0 g, m.p. 136–137°.  $[\alpha]_D^{20} - 80^\circ$  (water,  $C = 2$ ). Acetate, m.p. 84–85°.

$[\alpha]_D^{20} - 22^\circ$  (chloroform,  $C = 2$ ).

(As larger amounts of umbilicin and its acetate have been available, we consider these values more accurate than those previously recorded.)

*Sucrose*: 0.35 g, m.p. 178–180°. Non-reducing, yielded glucose and fructose on hydrolysis. Acetate, m.p. 87–88°.

All the substances were compared with authentic specimens and no melting point depressions were obtained.

The fractions prior to those containing arabitol, contained some unidentified material (0.4 g) which gave a strong colour reaction with ninhydrin, and when the column was washed with 50 % ethanol, a further small amount of material (0.75 g) was obtained.

#### SUMMARY

In addition to the previously known constituents arabitol, mannitol and umbilicin, *α,α*-trehalose and sucrose have been isolated from the lichen *Umbilicaria pustulata* (L.) Hoffm.

An improved method for the isolation of umbilicin from this lichen has been developed.

#### REFERENCES

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