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Studies on the Chemistry of Lichens

V *. The Furanoside Structure of Umbilicin

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The D-arabitol D-galactoside, umbilicin, isolated from *Umbilicaria pustulata* as described in part II[1] of this series, was later shown to be a constituent of several lichens of the order Gymnocarpeae (Part IV). The substance has the specific rotation —80° in water, indicating a β-glycosidic structure. The results of the periodate oxidation of umbilicin during which 10 moles of oxidant were consumed, indicate that umbilicin is either 3-D-arabitol β-D-galactopyranoside (I) or a galactofuranoside (II). Furanosides of aldohexoses are rather rare in Nature so we considered the former structure more probable. In order to elucidate the structure, I was synthesised from lactose by degradation to 3-D-arabinose β-D-galactopyranoside according to Zemplén [2] and finally reduction with sodium borohydride. The amorphous substance could be hydrolysed to galactose and arabitol, but the R_F-value was less than that of umbilicin and a furanosidic structure must now be considered. The rate of acid hydrolysis of umbilicin was rapid which is characteristic of furanosides [3]. In the hydrolysate of fully methylated umbilicin the presence of a substance having the same R_F-value and colour reactions as 2,3,5,6-tetra-*O*-methyl-galactose but different from those of 2,3,4,6-tetra-*O*-methyl-galactose was demonstrated.

The former substance was prepared by Haworth, Ruell and Westgarth [4] from the liquid methyl galactoside obtained by cold treatment of galactose with methanolic hydrogen chloride. We used crystalline ethyl β-galactofuranoside [5] as starting material and obtained the galactose tetramethyl ether as a liquid with the specific rotation —32° in water. The somewhat higher value obtained by Haworth *et al.* indicates that their substance contained some galactopyranose tetramethyl ether. Humphreys, Pryde and Waters [6] characterised the galactofuranose tetramethyl ether by transformation into the corresponding galactonic amide. The yield in this series of reactions is rather low however, and adopting the method of Boissonnas [7] for the identification of methylated glucoses, we reduced the furanoside to 2,3,4,6-tetra-*O*-methyl-D-galactitol, and transformed the latter to the diazoate, m.p. 99—100°.

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Finally, a larger amount of methylated umbilicin was hydrolysed and the galactose tetramethyl ether and the arabitol tetramethyl ether separated by distillation. The galactose tetramethyl ether was reduced to the D-galactitol derivative and azoylated, yielding derivatives identical with authentic specimens. The arabitol tetramethyl ether was obtained as an oil, the azoate of which has not crystallized.

The periodate oxidation of umbilicin, at 50° and pH 3.5, was studied as a function of time in an endeavour to learn more about the structure. The periodate consumption increased continously however, and no turning point was observed. A similar result was obtained when ethyl β-D-galactofuranoside was subjected to periodate oxidation under the same conditions.

EXPERIMENTAL

(All melting points uncorrected.)

3-D-Arabitol β-D-galactopyranoside. A solution of 3-D-arabinose β-D-galactopyranoside [2] (2.5 g) in water (5 ml) was dropped into a solution of sodium borohydride (0.2 g) in water (3 ml). The addition was made over five minutes and after a further fifteen minutes' excess borohydride was destroyed with acetic acid. The reaction mixture was concentrated to dryness and the residue acetylated with acetic anhydride (30 ml) and pyridine (15 ml), poured into water and extracted with chloroform. The chloroform solution was washed with water, dried over calcium chloride and concentrated. The yield of syrupy acetate was almost quantitative. The substance did not crystallise even after innoculation with umbilicin acetate. The product obtained after deacetylation with sodium ethoxide in ethanol was still amorphous and had a lower R_F-value than umbilicin, using butanol-ethanol-water (4 : 1 : 5) as solvent. The presence of arabitol and galactose in the hydrolysate of the substance was demonstrated by paper chromatography.

Hydrolysis of umbilicin. The hydrolysis of umbilicin at 100° in 0.01 *N* hydrochloric acid was followed polarimetrically. The value of $10^5 \times k$ expressed in minutes and Brigg's logarithms was determined to 2 200, a value of the magnitude characteristic for furanosides [3] and about one hundred times greater than expected for a pyranoside.

Ethyl β-galactofuranoside tetramethyl ether. Ethyl β-galactofuranoside (5.0 g) and powdered sodium hydroxide (23 g) were added to anhydrous peroxide-free dioxan (210 ml). Methyl sulphate (27.2 ml in total) was added with vigorous stirring, as the temperature was raised to 70°. The intention was to carry out the methylation as described previously[3], but the temperature was raised too quickly for the present mixture and the solid material melted and formed hard lumps. Water (15 ml) was added and the methylation continued in the liquid two-phase system. The dioxan phase was concentrated and the residue, combined with small amounts of methyl ethers isolated from the aqueous phase, was subjected to a second methylation with methyl sulphate (14 ml) and sodium hydroxide (11.5 g) in dioxan (150 ml). Following methylation, the dioxan solution was filtered off and the filter cake washed with dioxan. The combined solutions were concentrated, the residue dissolved in chloroform (50 ml) and washed with water (10 ml). The chloroform solution was dried over anhydrous magnesium sulphate, filtered and concentrated. The residual oil amounted to 6.21 g, part of which was distilled at 2—3 mm and 120—122°. (Found: C 54.3; H 9.13. Calc. for $C_{12}H_{24}O_6$ (264.3): C 54.3; H 9.17.)

2,3,5,6-Tetra-O-methyl-D-galactose. The crude, methylated ethyl galactoside (4.00 g) was dissolved in 0.2 *N* sulphuric acid (120 ml) and kept at 100° for 22 hours. The solution was neutralised with sodium hydrogen carbonate, concentrated to 30 ml and extracted with chloroform (2 × 10 + 8 × 5 ml). The extract was dried over magnesium sulphate, the solvent distilled off and the residual sirup distilled under reduced pressure. Yield 2.96 g, b.p. 113—114° at 0.35 mm. $[\alpha]_D^{20}$ −32° (Water, c = 2.3). The overall yield from the ethyl galactoside was 81 %. (Found: OCH_3 51.9. Calc. for $C_{10}H_{20}O_6$ (236.3): OCH_3 52.3. Molecular weight, by hypoiodite oxidation [9], 231.)

2,3,5,6-Tetra-O-methyl-D-galactitol. Raney nickel (100 mg) was added to a solution of 2,3,5,6-tetra-*O*-methyl-D-galactose (1.037 g) in water (10 ml) and the pH of the mixture

adjusted to 7.2 with sodium hydroxide. After hydrogenation for 10 hours at 120° and 155 atm. the mixture was filtered. Hypoiodite titration [9] showed that the reduction had reached 96 % of completion. The filtrate was concentrated to a syrup, dissolved in chloroform, filtered through a layer of aluminium oxide and reconcentrated. The residue, a colourless oil (1.00 g) was distilled. Yield, 0.87 g, b.p. 122—124° at 0.25 mm. The distillate crystallised, and recrystallisation from benzene-light petroleum (b.p. 40—50°) yielded hygroscopic needles, m.p. 41—41.5°, $[\alpha]_D^{20}$ —24° (Water, $c = 2$). Tipson and Levene [10] prepared the same substance by similar methods. Their preparation melted at 83—84° and had the specific rotation —26.8° (water). However, not until the conclusion of this work were we able to obtain the substance in this higher melting modification.

*2,3,5,6-Tetra-*O*-methyl-*D*-galactitol 1.4 diazoate.* To the galactitol tetramethyl ether (121 mg) in purified, dry pyridine (4 ml), azoyl chloride (300 mg) was added and the mixture kept at 40° for 12 hours and then at 100° for 24 hours. The reaction mixture was allowed to cool, water (0.5 ml) slowly added and most of the pyridine distilled off under reduced pressure. The residue was dissolved in chloroform, and the azoic acid which separated was filtered off. The chloroform solution was washed twice with 0.5 *N* sulphuric acid, precipitating further amounts of azoic acid which were filtered off, and finally washed with sodium hydrogen carbonate solution. The chloroform was distilled off and the crude azoate (310 mg), dissolved in dry benzene, was added to the top of a column (2.5 × 13 cm) of acid treated aluminium oxide. The column was developed with dry, alcohol-free chloroform (300 ml) giving a wide zone in the middle of the column and two narrow zones near the top. The main zone was extracted with methanol, yielding the crude azoate (255 mg), which was recrystallised from ethanol. M. p. 99—100°. (Found: N 8.30. Calc. for $C_{36}H_{38}O_8N_4$ (654.7): N 8.57).

Methylation of umbilicin. Umbilicin octaacetate [11] (2.49 g) was methylated twice with methyl sulphate (8.8 ml) and sodium hydroxide (8.6 g) in pure dioxan (85 ml), as previously described [8]. The oil obtained on concentrating the dioxan-benzene solution was dissolved in chloroform and washed with a small amount of water, yielding on concentration under reduced pressure a pale yellow oil (1.58 g). A small amount of this substance decomposed on attempted distillation, 160° and 0.01 mm.

*Hydrolysis of octa-*O*-methyl-umbilicin.* Methylated umbilicin (530 mg) was hydrolysed in 0.5 *N* sulphuric acid (10 ml) for 16 hours at 100°. The hydrolysate was neutralised with barium carbonate and filtered through a short column of aluminium oxide. The filtrate was concentrated and the residual syrup partitioned between chloroform (10 ml) and a few drops of water. The syrup obtained on concentrating the chloroform phase (442 mg) on paper chromatograms (Solvent: benzene-light petroleum, 1 : 1, saturated with water. Reagent: aniline hydrogen phtalate) gave only one spot, indistinguishable from that of authentic 2,3,5,6-tetra-*O*-methyl-D-galactose. On distillation of this syrup two fractions were obtained. The first (212 mg) b.p. 80—100° at 0.25 mm was a thin syrup, containing 13 % of galactose tetramethyl ether, determined by hypiodite titration. The main constituent of this fraction must be an arabitol tetramethyl ether. The second fraction (195 mg), b.p. 106° at 0.25 mm, was more viscous and consisted of galactose tetramethyl ether of 99 % purity, determined as above, $[\alpha]_D^{20}$ —32° (Water, $c = 3.8$). The main part of this fraction (189 mg) was hydrogenated as described above, yielding 175 mg of distilled product, b. p. 116—118 at 0.2 mm. Recrystallisation from benzene-light petroleum afforded crystals melting at 40.5—41.5°, but owing to the poor reproducibility of this value, a mixed m.p. determination with authentic material was considered insignificant. Instead, a sample (46 mg) was azoylated as described above, yielding a product (30 mg) melting at 97—99°, undepressed on admixture with 2,3,5,6-tetra-*O*-methyl-D-galactitol 1,4-diazoate.

SUMMARY

The furanoside configuration of umbilicin has been shown by the isolation of 2,3,5,6-tetra-*O*-methyl-D-galactose from the hydrolysate of the fully methylated product. The galactose tetramethyl ether was characterised by reduction to the corresponding D-galactitol derivative and subsequent azoylation.

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