ACTA CHEMICA SCANDINAVICA 27 (1973) 1595–1600

1-*O*-β-D-Galactopyranosyl-D-ribitol from *Xanthoria parietina**

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Dedicated to Professor *František Šorm* on his 60th birthday

O-α-D-Galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→1)-D-glyceritol and 1-*O*-β-D-galactopyranosyl-D-ribitol have been isolated from the lichen *Xanthoria parietina*. The latter substance, and the corresponding L-ribitol derivative, were synthesized in order to determine the configuration of the ribitol moiety in the natural product.

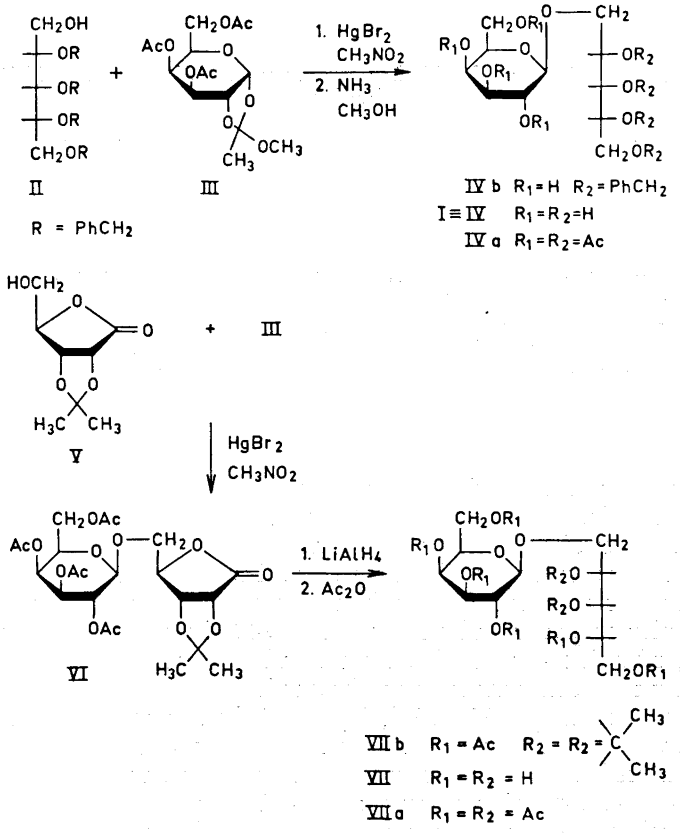
Low molecular weight carbohydrates, such as alditols, alditol glycosides, and disaccharides have been isolated from various lichens.[2a] In the present communication, studies on the low molecular weight carbohydrates in *Xanthoria parietina* are reported. The water soluble part of a methanol extract of the lichen was fractionated by carbon column chromatography followed by chromatography on paper and on Sephadex G-25. In addition to *myo*-inositol, D-arabinitol, ribitol, D-mannitol, sucrose, and α,α-trehalose, two components not previously found in lichens were obtained.

One component, m.p. 190 – 193°, $[\alpha]_{578}$ +89° in water, on acid hydrolysis yielded D-galactose and glyceritol in the molar ratio 2:1. It was indistinguishable (m.p., mixed m.p., IR) from an authentic sample of *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→1)-D-glyceritol, a common component in glycolipids.[2b] The non-acylated compound has also been isolated from a red alga.[3]

The second component (I), $[\alpha]_{578}$ −3° (in water), which did not crystallize, on acid hydrolysis yielded equimolar quantities of D-galactose and ribitol. The rate of acid hydrolysis indicated the presence of a pyranosidic linkage. The low optical rotation indicated that the component was a β-D-galactopyranoside. In accordance with these findings, it was hydrolyzed, although

* Part 29 in *Studies on the Chemistry of Lichens*. Part 28, Ref. 1.

at a low rate, by the action of β-D-galactosidase. In the NMR spectrum (D_2O) the anomeric proton gave a signal at 4.4 ppm (d), $J_{1,2}$ 8 Hz, confirming *trans*-geometry of H-1 and H-2 in the galactopyranosyl residue and thereby the presence of a β-D-galactopyranoside. On periodate oxidation, 5.3 mol reagent were consumed, with the simultaneous release of 1.08 mol formaldehyde and 2.81 mol formic acid. These findings are consistent with I being 1-*O*-β-D-galactopyranosyl-D- or -L-ribitol. In order to distinguish between these alternatives, the two substances were synthesized and compared with the natural product.



2,3,4,5-Tetra-*O*-benzyl-D-ribitol (II) was prepared from D-ribose diethyl dithioacetal[4] by benzylation, hydrolysis in the presence of mercuric chloride and mercuric oxide, and finally reduction with sodium borohydride. Condensation of II with 3,4,6-tri-*O*-acetyl-1,2-methylorthoacetyl-α-D-galactopyranose (III) in nitromethane using mercuric bromide as catalyst and the general conditions devised by Kochetkov and co-workers,[5] followed by deacetylation, yielded the β-D-galactopyranoside IVb. Removal of benzyl groups by catalytic hydrogenation

yielded 1-*O*-*β*-D-galactopyranosyl-D-ribitol (IV), which was purified as its octaacetate (IVa).

A similar orthoester synthesis, using the orthoester III and 2,3-*O*-isopropylidene-1,4-D-ribonolactone, but with a mixture of *p*-toluenesulphonic acid and mercuric bromide as catalyst, yielded crude VI. Reduction of VI with lithium aluminium hydride yielded the ribitol *β*-D-galactopyranoside derivative VIIb, which, however, was contaminated with the corresponding α-galactoside. Another example of low stereoselectivity in the orthoester glycoside synthesis has recently been described.[6] On both occasions recourse had to be taken to more strongly acidic conditions than those generally used, in order to obtain condensation. The isopropylidene group in VIIb was removed by hydrolysis in trifluoroacetic acid. The product was fully acetylated and then separated from the α-anomer VIII by chromatography on dimethyl sulphoxide-impregnated silica gel.[7] The faster-moving component, VIII, had $[\alpha]_D$ +73° in chloroform and the slower-moving one, VIIa, $[\alpha]_D$ +13° indicating that they were the α- and *β*-D-galactopyranosides, respectively. Both glycosides (VII and VIII) were converted into their octamethyl ethers, which could be separated by GLC, but gave identical mass spectra (MS) except for minor variations attributable to stereoisomerism. The MS were also identical to those of the fully methylated IV and the fully methylated natural product I, demonstrating that they were all 1-*O*-D-galactopyranosyl-ribitols. The fully methylated I, IV, and VII could not be separated on GLC. The fully trimethylsilylated I and IV were also inseparable, but had a different retention time to that of the corresponding derivative of VII. The negative rotations of the octaacetates Ia and IVa, $[\alpha]_D$ −7° and −11°, respectively, in contradistinction to the positive value for VIIa, +13°, lends further support to the identity of I and IV. Deacetylation of IVa and VIIa afforded 1-*O*-*β*-D-galactopyranosyl-D- and -L-ribitol, $[\alpha]_D$ −7° and +30°, respectively; $[\alpha]_{578}$ for I was −3°. The natural product therefore is 1-*O*-*β*-D-galactopyranosyl-D-ribitol.

Sugar alcohols have low optical rotations, but high values are observed for their molybdate complexes. Pyranosides, on the other hand, do not complex with molybdate.[8] It is therefore expected that the main contributions to the optical rotations of IV and VII should come from the 1-substituted D- and L-ribitol moieties, respectively, and have different signs, but approximately the same magnitude. In agreement with this, IV and VII, in 0.037 M sodium molybdate buffered to pH 5.5, showed $[\alpha]_{589}$ −78°, +75°, $[\alpha]_{578}$ −81°, +89°, $[\alpha]_{546}$ −95°, +92°, $[\alpha]_{436}$ −202°, +180°, and $[\alpha]_{365}$ −455°, +357°, respectively.

EXPERIMENTAL

General methods. Melting points are corrected. Concentrations were performed at reduced pressure and a bath temperature not exceeding 40°, unless otherwise stated. Optical rotations were recorded at room temperature, at *c* 0.05−0.5, using a Perkin-Elmer 141 instrument. UV and visible spectra were measured using Beckman DK2 and Beckman DB instruments, respectively. NMR spectra were recorded with a Varian A60-A instrument, using tetramethylsilane as internal standard. The NMR spectra, determined for all new compounds, were in agreement with the postulated structures. TLC was performed on silica gel F_{254} (Merck) plates. When necessary, sulphuric acid was used as spray reagent. Silica gel column chromatography was performed using Mallinck-

rodt 100 mesh silicic acid. Whatman No. 1 and 3MM papers were used for analytica and preparative paper chromatography, respectively. Solvent systems: *A*. Ethyl acetate, acetic acid, water, 3:1:1. *B*. Ethyl acetate, pyridine, water, 8:2:1. *C*. Same solvent, but 2:1:1. Silver acetate-sodium hydroxide in ethanol and *p*-anisidine hydrochloride in ethanol were used as spray reagents. GLC and GLC-MS were performed using Perkin-Elmer 900 and Perkin-Elmer 270 instruments, respectively (manifold temperature 200°, ionization potential 70 eV, ionization current 80 μA, temperature at the ion source chamber 80°). A column of XE-60 (3 %) on Gas Chrom Q (100 – 120 mesh) was used for the disaccharide derivatives.

Extraction and fractionations of lichen components. Dry, ground *Xanthoria parietina*, collected at Fiskebäckskil, Bohuslän, Sweden, (400 g), was extracted in a continuous extractor, first with diethyl ether for one week and then with methanol for one week. The methanol extract was concentrated, partitioned between chloroform and water, and the aqueous phase deionized with Dowex 50 (H^+) and Dowex 3 (free base) and then concentrated to a syrup (29 g). D-Mannitol (7 g) was obtained from the syrup by crystallization from ethanol. The remainder of the syrup, in water (40 ml) was added to the top of a carbon-Celite column (5.3 × 47 cm) which was irrigated, first with water (3.5 l) and then with aqueous ethanol (8 l 0→20 %, linear gradient). The fractionation was monitored by paper chromatography. The substances were eluted in the following order: *myo*-inositol, D-arabinitol, ribitol, D-mannitol, α,α-trehalose, sucrose, galactosylribitol and galactosylgalactosylglyceritol. No components were obtained pure, but had to be further purified by preparative paper chromatography, giving the following: *Myo*-inositol, m.p. 216 – 222°. D-Arabinitol, m.p. 102 – 104°, $[\alpha]_{578}$ +11° (saturated aqueous sodium tetraborate). Ribitol, m.p. 102 – 104°. D-Mannitol, m.p. 163 – 164°, $[\alpha]_{578}$ +33° (saturated aqueous sodium tetraborate). α,α-Trehalose dihydrate, m.p. 91 – 93°, $[\alpha]_{578}$ +175° (water). The various compounds were indistinguishable from authentic samples (m.p., IR).

O-α-D-*Galactopyranosyl-(1→6)*-O-β-D-*galactopyranosyl-(1→1)*-D-*glyceritol.* The fraction (300 mg) containing the title compound was purified by paper chromatography (solvent *A*) followed by chromatography on a Sephadex G-25 (superfine) column (1.6 × 175 cm). The pure compound (53 mg) crystallized from aqueous ethanol, m.p. 190 – 193°, $[\alpha]_{578}$ +89° (water). It was indistinguishable from an authentic sample of the title compound [3] (m.p., IR).

1-O-β-D-*Galactopyranosyl*-D-*ribitol (I).* The combined fractions containing the title compound were purified by paper chromatography (solvent *C*), followed by chromatography on the Sephadex G-25 column. The product (I, 50 mg), which gave a single spot on paper chromatography, showed $[\alpha]_{578}$ −3° (water) and did not crystallize. The hydrolysis of I in 0.05 M aqueous sulphuric acid at 80° was followed polarimetrically and reached a constant, positive value (indicating D-galactose) after 35 h. 2-*O*-β-D-Galactofuranosyl-D-arabinitol (umbilicin) was completely hydrolysed in 2 h. Paper chromatography of the hydrolysate showed the presence of galactose and ribitol. Analysis, by GLC, of the product obtained after borohydride reduction and acetylation showed, in addition to galactitol and ribitol acetates, smaller amounts (less than 2 % of each) of the acetylated arabinitol, mannitol, and glucitol. I was oxidized with 0.03 M aqueous sodium metaperiodate. The consumption of oxidant was followed spectrophotometrically,[9] the formation of formaldehyde by the reaction with chromotropic acid,[10] and the formation of formic acid by treatment with ethylene glycol followed by titration with 0.01 M sodium hydroxide.

2,3,4,5-Tetra-O-*benzyl*-D-*ribose diethyl dithioacetal.* D-Ribose diethyl dithioacetal [4] (2.5 g) was benzylated by the procedure devised by Brimacombe and Ching.[11] Part of the product was purified by TLC ($CHCl_3$) $[\alpha]_D$ +20° (chloroform). (Found: C 72.1; H 7.07; O 10.5. $C_{37}H_{44}O_4S_2$ requires: C 72.0; H 7.19; O 10.4.) Most of the product (6.0 g) was used, without purification, in the next step.

2,3,4,5-Tetra-O-*benzyl*-D-*ribitol (II).* The above compound (6.0 g) in acetone (55 ml) and water (3.5 ml) was transformed into the aldehyde by treatment with mercuric chloride (4.0 g) and yellow mercuric oxide, and worked up as described for analogous syntheses.[12] Part of the crude product was purified by TLC (toluene), yielding syrupy 2,3,4,5-tetra-*O*-benzyl-D-ribose, $[\alpha]_D$ +13° (chloroform). The remainder (5.0 g), in methanol (50 ml) containing sodium methoxide (from 50 mg sodium), was treated with sodium borohydride (2.3 g) at room temperature overnight. The product was acidified and partitioned between water and chloroform, and the chloroform phase (II) purified

by chromatography on a silica gel column (5 × 30 cm) (chloroform). Pure II (2.1 g) showed $[\alpha]_D$ −14° (chloroform). (Found: C 77.2; H 7.10; O 15.7. $C_{33}H_{36}O_5$ requires: C 77.3, H 7.08; O 15.6.)

3,4,6-Tri-O-*acetyl-1,2*-O-*methylorthoacetyl*-α-D-*galactopyranose (III)* was prepared from 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide as described by Kochetkov and co-workers for the corresponding ethyl orthoacetate,[5] and purified by chromatography on silica gel (diethyl ether). The yield of pure product, $[\alpha]_D$ +122° (chloroform), was 61 %.

2,3,4,5-Tetra-O-*benzyl-1*-O-(β-D-*galactopyranosyl*)-D-*ribitol* (*IVb*). The orthoester III (1.3 g) and II (1.3 g) were dissolved in nitromethane (15 ml). Solvent was distilled off at constant volume for 4 h, by the continuous addition of nitromethane. Mercuric bromide (50 mg) was added and the distillation at constant volume continued for 2 h. The mixture was filtered, concentrated, and deacetylated in methanol (120 ml) containing 1.67 % ammonia at room temperature overnight. The product was concentrated, dissolved in acetone, insoluble material removed by filtration, and the filtrate concentrated. The product (1.87 g) was separated on a silica gel column (ethyl acetate, methanol, and water 80:15:5). The main component (1.25 g) was almost pure according to TLC. Part of it was further purified by TLC in the above solvent yielding chromatographically homogeneous, amorphous IVb, $[\alpha]_D$ −6° (chloroform).

1-O-β-D-*Galactopyranosyl*-D-*ribitol octaacetate (IVa)*. Catalytic hydrogenation of IVb (1.25 g), using 5 % palladium on carbon, followed by treatment with acetic anhydride in pyridine yielded crude IVa. This was purified by chromatography on silica gel (chloroform, diethyl ether, 8:2). The amorphous product (0.99 g) showed $[\alpha]_D$ −11° (chloroform). (Found: C 50.0; H 5.71; O 44.1. $C_{27}H_{38}O_{18}$ requires: C 49.8; H 5.89; O 44.3.)

5-O-(*2,3,4,6-Tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-*2,3*-O-*isopropylidene-1,4*-D-*ribonolactone* (*VI*). A solution of the orthoester III (6.5 g), *p*-toluenesulphonic acid (2 mg) and 2,3-*O*-isopropylidene-1,4-D-ribonolactone (V, 4.0 g) in nitromethane (50 ml) was distilled at constant volume for 2 h, when according to TLC all of III had reacted. Mercuric bromide (250 mg) was added and the mixture refluxed for 1 h. After dilution with chloroform, the solution was shaken with aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$) and concentrated to a syrup (9.0 g). Fractionation of this syrup on a silica gel column (diethyl ether) afforded VI (3.7 g), contaminated with about 8 % of V. This material was used in the next step.

4,5-Di-O-*acetyl-1*-O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-*2,3*-O-*isopropylidene*-L-*ribitol* (*VIIb*). A solution of VI (3.7 g) and lithium aluminium hydride (1.4 g) in tetrahydrofuran (50 ml) was refluxed overnight. The product was worked up as usual and acetylated with acetic anhydride in pyridine. Purification of the product by silica gel chromatography (chloroform, acetone 9:1) yielded VIIb (1.65 g), $[\alpha]_D$ +33° (chloroform). This material was contaminated with the corresponding α-form (see below). (Found: C 51.6; H 6.34. $C_{26}H_{38}O_{16}$ requires: C 51.5; H 6.31.)

1-O-β-D-*Galactopyranosyl*-L-*ribitol octaacetate* (*VIIa*). The isopropylidene derivative VIIb (1.6 g) was treated with 90 % aqueous trifluoroacetic acid (10 ml) for 10 min at room temperature. The product was concentrated to a syrup, dried by repeated concentrations with toluene, acetylated with acetic anhydride in pyridine and purified by TLC (chloroform, acetone 9:1). Chromatography on dimethyl sulphoxide impregnated paper (diisopropyl ether), as devised by Wickberg,[7] revealed the presence of two components, the slower of which (β-anomer) predominated. Separation on dimethyl sulphoxide impregnated silica gel (diisopropyl ether) yielded the two components. The α-form (180 mg) showed $[\alpha]_D$ +73° (chloroform). (Found: C 50.1; H 5.82. $C_{27}H_{38}O_{18}$ requires: C 49.9; H 5.89.) The β-form (250 mg) showed $[\alpha]_D$ +13° (chloroform). (Found: C 50.0; H 6.01).

The octamethyl ethers prepared from VIIb and its α-anomer, and also those for I and IV, gave indistinguishable mass spectra, except for minor differences in intensities. The following spectrum, from methylated IV, is typical (relative intensities in brackets): 40 (5), 41 (13), 43 (13), 44 (34), 45 (54), 53 (5), 55 (10), 57 (9), 58 (6), 59 (16), 69 (6), 71 (85), 72 (7), 73 (11), 75 (21), 83 (5), 85 (5), 88 (100), 89 (27), 95 (5), 97 (5), 101 (21), 102 (9), 103 (6), 111 (6), 115 (6), 127 (10), 133 (5), 159 (6), 187 (10), 191 (10), 192 (5), 251 (4). On GLC (XE-60)fully methylated I, IV, and VII were indistinguishable, but the methylated α-anomer of VII had a shorter retention time.

I, IV, and VII were also converted into their fully trimethylsilylated derivatives. Those of I and IV were indistinguishable on GLC (XE-60, 170 − 190°) but that of VII had a different retention time ("mixed" chromatography).

Deacetylation of IVa and VIIa afforded 1-*O*-β-D-galactopyranosyl-D- and -L-ribitol, $[\alpha]_D$ −7° and +30° (water), respectively.

Acknowledgements. The authors are indebted to Dr. C. A. Wachtmeister for the lichen material, to Professor B. Wickberg for reference material, and to *Statens Naturvetenskapliga Forskningsråd* for financial support.

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Received December 5, 1972.