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Note

Isolation of 3-*O*-β-gentiobiosyl-D-glucose from the sclerotia of *Sclerotinia libertiana*

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In a previous report[1], the sclerotia of *Sclerotinia libertiana* were partially hydrolyzed by acid and the oligosaccharide fragments resulting were fractionated by carbon-column chromatography. The present work involves determination of the structure of a crystalline oligosaccharide (**1**) obtained from the hydrolyzate by elution with 25% ethyl alcohol.

The fraction, which gave a single spot on a paper chromatogram, crystallized slowly from methanol. On hydrolysis, the oligosaccharide (**1**) gave D-glucose only. The degree of polymerization of **1** was 3 and therefore, it was a glucotriose.

Hydrolysis of fully methylated **1** gave 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose, and 2,4,6-tri-*O*-methyl-D-glucose, as detected by paper chromatography. This result shows that **1** has (1→3) and (1→6) glucosidic linkages in the molecule. Partial hydrolysis of reduced **1** gave (papergram) gentiobiose and laminaribiitol, indicating that **1** is 3-*O*-β-gentiobiosyl-D-glucose.

Acetylation of **1** with hot acetic anhydride and anhydrous sodium acetate gave 5 parts of a hendecaacetate as prisms (**2**), m.p. 169–170°, and 1 part of a hendecaacetate as needles (**3**), m.p. 179–180°. Acetylation of α- or β-D-glucose with acetic anhydride and pyridine at 0° occurs without any anomeric change[2], whereas the β-acetate is favored at high temperature when sodium acetate is used as a catalyst. Acetylation of **1** with acetic anhydride and pyridine at low temperature gave mostly the prisms (**2**) and a small amount of the needles (**3**), suggesting that **2** is the β-acetate, and **3** is the α-anomer. If this assumption is correct, the original sugar must have been a mixture of mainly the β-anomer and a little of the α-anomer. The assumption is also supported by the fact that crystalline **1** showed upward mutarotation, and that the characteristic absorption peak[3] at 850 cm^{-1} (type 2a) for α-anomer was present in the i.r. spectrum, although it was very weak. Crystallization of the free sugars was attempted with various solvents at various temperatures, but only one product (m.p. 223–224°), having the same m.p. and i.r. spectra, was obtained, indicating that the β-anomer preponderates in crystalline **1**.

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3-*O*-β-Gentiobiosyl-D-glucose has been isolated from laminaran[4] and yeast glucan[5], and has been synthesized[6] by the Königs-Knorr reaction, but it was not previously obtained as crystals.

EXPERIMENTAL

General. — Evaporations were conducted under diminished pressure at 40° in a rotary evaporator. All melting points are uncorrected. Optical rotations were obtained with an automatic polarimeter MP-1T (Applied Electric Lab. Co., Ltd., Tokyo). I.r. spectra were recorded with a JASCO-DS 391 spectrometer (Japan Spectroscopic Co., Ltd.). Paper chromatography was performed on Toyo filter paper No. 2 with following solvent systems (*v/v*): (*A*) 4:1:2 butyl alcohol–acetic acid–water, and (*B*) 40:10:49:1 butyl alcohol–ethyl alcohol–water–ammonia. The upper layer was used. Reducing sugars were located by aniline hydrogen phthalate (*a*). Reducing and non-reducing sugars were located by silver nitrate–sodium hydroxide (*b*).

Isolation of **1**. — Following the previous procedure[1], the defatted powder of sclerotia was hydrolyzed with 10mM sulfuric acid in an autoclave for 30 min at 150°. The hydrolyzate was neutralized and fractionated on a charcoal–Celite column. An oligosaccharide[1] (**1**, a glucotriose) was isolated from the eluate obtained with 25% ethyl alcohol. Compound **1** (1 g) was dissolved in a little water, methanol (10 ml) was added, and the solution was refluxed. Insoluble material was filtered off and the filtrate was evaporated to a syrup. This treatment was repeated several times, and the 80% methanolic solution was kept for 6 months at room temperature until a globular substance crystallized out; yield 800 mg. This product was dissolved in water (0.7 ml) and methanol (6 ml) was added. After refluxing the solution, the oligosaccharide crystallized. Recrystallization gave prisms, m.p. 223–224° (decomp.), $[\alpha]_D^{15}$ −4.35° (2 min) → −1.58° (final) (*c* 1, water).

The water content of crystalline **1**, as determined by the method of Takiura *et al.*[7], was 6.81%, indicating a dihydrate structure (H_2O, 6.66%).

Anal. Calc. for $C_{18}H_{32}O_{16}\cdot 2H_2O$: C, 40.00; H, 6.73. Found: C, 39.84; H, 6.73.

The i.r. spectrum of crystalline **1** in the region 970–730 cm^{-1} showed absorption peaks[3] at 908 (type 1 or 2b, s), 850 (type 2a, w), and 777 cm^{-1} (type 3, w).

Complete acid hydrolysis of **1**. — Fifty mg of **1** was hydrolyzed with 0.25M sulfuric acid (2.5 ml) in a sealed tube for 5 h at 100°, and neutralized with barium carbonate. The hydrolyzate showed a single spot for a glucose on a paper chromatogram. This hydrolyzate was treated with acidified, methanolic *p*-nitroaniline[8] to give *N*-phenyl-D-glucosylamine, m.p. and mixed m.p. 180–181°.

Degree of polymerization of **1**. — The d.p. of **1**, as determined by the method of Peat *et al.*[9], was 2.9.

Reducing power of **1**. — The reducing power of **1**, expressed as D-glucose equivalent, was determined by the methods of Somogyi[10] and Willstätter–Schudel[11]. The total reducing power was measured after hydrolysis of the sugar in 0.25M sulfuric acid for 4 h at 100°. The reducing power was 33.8 units by the Somogyi method and

by the Willstätter–Schudel method. [Calc. $(C_6H_{12}O_6/C_{18}H_{32}O_{16}) \times 100 = 35.7$]. The total reducing power was 106.4 and 106.8, respectively. [Calc. $(3C_6H_{12}O_6/C_{18}H_{32}O_{16}) \times 100 = 107.1$].

Hydrolysis of methylated **1**. — Methylation was effected by the method of Hakomori[12] as follows: a mixture of sodium hydride (40 mg) and dimethyl sulfoxide (4 ml) was stirred for 45 min and added to **1** (36 mg) dissolved in dimethyl sulfoxide (2 ml). The mixture was then stirred with a magnetic stirrer under a nitrogen stream for 2.5 h at room temperature. Methyl iodide (0.3 ml) was added to the mixture, which was stirred for an additional 3 h. The reaction mixture was diluted with water, and the methylated product was extracted with chloroform. The chloroform solution was washed with water and evaporated to a syrup. The residue was dissolved in a diethyl ether–petroleum ether mixture, washed with water, and evaporated. The hydrolyzate of methylated **1**, obtained by hydrolysis with M hydrochloric acid for 5 h at 100°, gave three spots corresponding to 2,3,4,6-tetra-*O*-methyl-D-glucose (R_F 0.84), 2,3,4-tri-*O*-methyl-D-glucose (R_F 0.75), and 2,4,6-tri-*O*-methyl-D-glucose (R_F 0.71) on the chromatogram, doubly developed in solvent (*B*), with spray (*a*).

Acetylation of **1** *at high temperature*. — A mixture of **1** (150 mg), acetic anhydride (3 ml), and anhydrous sodium acetate (300 mg) was kept for 30 min at 120°. Water was added to the reaction mixture, and the acetate was extracted with chloroform (30 ml × 3). The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The crude acetate was dissolved in ethyl alcohol (2 ml) and kept overnight at 5° to give a globular, crystalline product; yield 210 mg, m.p. 165–166°. The product was recrystallized from 80% ethyl alcohol to afford a mixture of needles (**2**) and prisms (**3**). When the crystals were added to 80% ethyl alcohol kept at 60°, the needles dissolved, but some of the prisms did not. The insoluble prisms (28 mg) were filtered off quickly, and recrystallized from ethyl alcohol to give pure **3**, m.p. 179–180°, $[\alpha]_D^{15}$ −11.0° (chloroform).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.55; H, 5.63.

The mother liquors were kept at 5° to give **2** (155 mg), which was recrystallized from ethyl alcohol giving pure **2**, m.p. 169–170°, $[\alpha]_D^{15}$ −27.9 (chloroform).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.52; H, 5.66.

Acetylation of **1** *at low temperature*. — A mixture of **1** (100 mg), pyridine (670 mg), and acetic anhydride (500 mg) was kept for 48 h at 2°. Water (3 ml) was added to the reaction mixture, and the precipitate was filtered off and washed with water; yield 220 mg. The acetate of **1** crystallized from ethyl alcohol (2.5 ml) at 5°. The crystals were dissolved with warm 80% ethyl alcohol and filtered as already described to give **3** (20 mg, m.p. 179–180°). Compound **2** (100 mg, m.p. 169–170°) was obtained from the mother liquors.

Partial hydrolysis of **1** *and its alditol*. — Thirty mg of **1** was hydrolyzed with 0.2M sulfuric acid (1 ml) in a sealed tube for 1 h at 100° and the mixture was neutralized with barium carbonate. The hydrolyzate showed the presence of D-glucose ($R_{Glucose}$ 1.0), laminarabiose ($R_{Glucose}$ 0.81), gentiobiose ($R_{Glucose}$ 0.67), and unchanged **1** ($R_{Glucose}$ 0.55), on the chromatogram triply developed in solvent (*A*) with spray (*a*).

Fifty mg of **1** was dissolved in water (5 ml), sodium borohydride (50 mg) was added, and the solution was kept for 4 h at 30°; it was then treated with Amberlite IR-120 and evaporated to a syrup. After the addition of methyl alcohol, the evaporation was repeated. The resulting amorphous product (**4**) was hydrolyzed in the same way as for **1**. On the paper chromatograms, D-glucose ($R_{Glucose}$ 1.0), laminaribiitol ($R_{Glucose}$ 0.80), gentiobiose ($R_{Glucose}$ 0.67), and **4** ($R_{Glucose}$ 0.55) were detected (solvent *A*, threefold development, sprays *a* and *b*).

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