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Note

Crystalline α-laminaratriose

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Laminaratriose has been obtained by partial hydrolysis of such polysaccharides as laminaran[1], pachyman[2], and yeast β-glucan[3]. In addition, the trisaccharide has been synthesized chemically by the Koenigs–Knorr reaction[4]. However, it has not been isolated and characterized in the crystalline state. We now describe the isolation of crystalline laminaratriose from the partial hydrolyzate of sclerotia of *Sclerotinia sclerotiorum* (Lib.).

A previous publication[5] from this laboratory described some of the gluco-oligosaccharides obtained from the sclerotia by acid hydrolysis. Of these oligosaccharides, a noncrystalline trisaccharide (**1**) eluted from a carbon column with 25% ethanol was tentatively identified as laminaratriose on the basis of its behavior in paper and carbon-column chromatography. Compound **1** has now been isolated as crystals from methanol solution.

The hydrolysis of fully methylated **1** gave 2,3,4,6-tetra-*O*-methyl-D-glucose (1 part) and 2,4,6-tri-*O*-methyl-D-glucose (2 parts), showing that **1** is a trisaccharide composed only of D-glucose residues in (1→3)-glucosidic linkage. Moreover, D-glucose and laminarabiose were detected in the partial, acid hydrolyzate of **1**. The acetyl derivative, prepared by treatment with acetic anhydride and sodium acetate in the usual way, had m.p. 121–122°, $[\alpha]_D^{20}$ −33° (*c* 1, chloroform). Peat *et al.*[1] reported m.p. 120–121°, $[\alpha]_D^{17}$ −40° (chloroform) for the β-acetate of laminaratriose.

Crystalline **1** showed a mutarotation of +7.1→+3.3° in water. The specific optical rotations of α- and β-laminaratriose in water, calculated from the values for α- and β-laminarabiose ($[\alpha]_D$ +24.9° and +7°, respectively[6]) by using an equation proposed by Yamauchi and Matsuda[7], were +7.2° and −5.0°, respectively. The equilibrium value for laminaratriose in water was calculated to be +2.9°, and so the observed value is in good agreement with the calculated one.

The i.r. spectrum of crystalline **1** showed two characteristic absorption peaks[8] at 896 (type 2b) and 848 cm^{-1} (type 2a). However, the absorption of type 2a was very weak for amorphous **1**. The possibility of the presence of some of the α anomer in crystalline **1** is thus enhanced by the i.r. spectrum, as well as by the downward mutarotation.

EXPERIMENTAL

General. — Evaporations were conducted under diminished pressure at 40° in a rotary evaporator. Optical rotations were measured at 20° with an Automatic Polarimeter MT-IT (Applied Electric Lab. Co., Ltd., Tokyo). I.r. spectra were recorded with a Japan Spectroscopic spectrometer model IRA-1. Paper chromatography was performed on Toyo filter paper No. 2 with the following solvents: (*1*) 6:4:3 (v/v) 1-butanol–pyridine–water and (*2*) 4:1:2 (v/v) 1-butanol–acetic acid–water. Aniline hydrogenphthalate was used as the spray reagent. Thin-layer chromatography (t.l.c.) was performed on 0.25-mm layers of silica gel G with the upper phase of 10:5:3 (v/v) 2-butanol–water–ammonium hydroxide as the solvent. The positions of the compounds on the chromatogram were located by spraying with 50% sulfuric acid. Gas–liquid chromatography (g.l.c.) was performed with a Shimadzu gas chromatograph GC-4APF, fitted with a flame-ionization detector, under the following conditions: column (300 × 0.3 cm), containing 15% of 1,4-butanediol succinate polyester on Celite 545 (80–100 mesh); temp. 210°; carrier-gas flow, 82 mL of N_2/min. For quantitative evaluation of the results, a Shimadzu digital integrator ITG-4A was used.

Isolation of **1**. — In accordance with the procedure described in the previous paper[5], a defatted powder of the sclerotia was hydrolyzed with 0.01M sulfuric acid in an autoclave for 30 min at 150°. The acid was neutralized with barium carbonate, and the hydrolyzate was fractionated on a column of 1:1 carbon–Celite. An oligosaccharide was isolated from the fraction obtained with 25% ethanol as the eluant. Syrupy **1** (0.8 g), contaminated with a trace of laminarabiose, was dissolved in methanol containing a small proportion of water, and the solution refluxed. The amorphous precipitate was filtered off, and the filtrate was evaporated to a syrup. This treatment was repeated several times, and finally, a solution of the syrup in 4:1 methanol–water was kept at room temperature. Globular crystals separated after 30 days; recrystallized from methanol, compound **1** had m.p. 164–165°, and $[\alpha]_D^{20}$ +7.1 (2 min)→+3.3° (final; *c* 1, water). The water content of crystalline **1**, as determined by the usual method, was 6.80%, indicating a dihydrate (calc. for 2 H_2O, 6.66%).

Anal. Calc. for $C_{18}H_{32}O_{16} \cdot 2\,H_2O$: C, 40.00; H, 6.73. Found: C, 39.82; H, 6.84.

The i.r. spectrum of crystalline **1** in the region of 970 to 730 cm^{-1} showed absorption peaks at 920 (type 1, w), 896 (type 2b, s), 848 (type 2a, m), and 777 cm^{-1} (type 3, m). On the other hand, the type-2a absorption-band of amorphous **1**, which was obtained by dissolving crystalline **1** in water followed by evaporation (repeated several times), was very weak, although the rest of the spectrum was very similar to that of crystalline **1**.

Complete hydrolysis with acid. — A portion (10 mg) of **1** was hydrolyzed with 0.2M sulfuric acid (0.5 mL) in a sealed tube for 5 h at 100°. The hydrolyzate showed a single spot of D-glucose on the paper chromatogram.

Partial hydrolysis with acid. — A portion (20 mg) of **1** was hydrolyzed with 0.2M sulfuric acid in a sealed tube for 1 h at 100°. D-Glucose (R_F 0.69), laminarabiose

TABLE I

GAS–LIQUID CHROMATOGRAPHY OF REDUCTION–ACETYLATION PRODUCTS OF THE HYDROLYZATE OF METHYLATED **1**

Corresponding alditol acetate from	*Relative retention time*	*Molar ratio*
2,3,4,6-Tetra-*O*-methyl-D-glucose	**1.00**	1
2,4,6-Tri-*O*-methyl-D-glucose	1.84	1.9

(R_F 0.60), and unchanged **1** (R_F 0.49) were detected on the chromatogram of the hydrolyzate, triply developed with solvent *2*.

Methylation analysis. — Compound **1** (30 mg) was methylated by the Hakomori method[9], and the methylation product was hydrolyzed with M hydrochloric acid for 5 h at 100°. The hydrolyzate was analyzed by t.l.c., and the following two methyl ethers of D-glucose were identified: 2,3,4,6-tetra- and 2,4,6-tri-*O*-methyl-D-glucose. For g.l.c. analysis, the hydrolyzate was reduced with sodium borohydride, and the product acetylated with acetic anhydride–pyridine in the usual way. Compounds corresponding to the individual peaks on the chromatogram were identified by comparison of their retention times with those of authentic samples under the same conditions. The mole ratio of these compounds was determined to be ~1:2, from the areas under the peaks. The results are shown in Table I.

Acetylation. — A mixture of **1** (50 mg), acetic anhydride (0.5 mL), and anhydrous sodium acetate (25 mg) was heated for 1 h at 120°. The product was isolated in the usual way, and crystallized from methanol–ethanol. Recrystallization from the same solvent gave the peracetate, m.p. 121–122°, $[\alpha]_D^{20}$ −33° (*c* 2, chloroform).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.64; H, 5.63. Found: C, 49.62; H, 5.66.

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