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Note

A new, crystalline glucotetraose (6^2-β-D-glucopyranosyl-laminaratriose)

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A previous publication[1] from this laboratory described some of the gluco-oligosaccharides obtained from sclerotia of *Sclerotinia sclerotiorum* (Lib.) by acid hydrolysis. Of these oligosaccharides, laminaratriose was isolated as crystals[2]. In addition, a crystalline glucotetraose (**1**), eluted from the carbon column with 35% ethanol, has now been identified as 6^2-β-D-glucopyranosyl-laminaratriose.

Complete hydrolysis of **1** with dilute sulfuric acid gave D-glucose only. The degree of polymerization (d.p.) of **1** was found to be **4**. Partial hydrolysis of **1** with acid gave laminarabiose and gentiobiose. The hydrolyzate of fully methylated **1** contained 2,3,4,6-tetra-*O*-methyl-D-glucose (2 parts), 2,4,6-tri-*O*-methyl-D-glucose (1 part), and 2,4-di-*O*-methyl-D-glucose (1 part), showing that **1** is a branched tetrasaccharide consisting of D-glucose residues only, in (1→3)- and (1→6)-D-glucosidic linkages. From the results (shown in Table I) of methylation and fragmentation analysis, 6^2-β-D-glucopyranosyl-laminaratriose (I), 6^1-β-laminarabiosyl-laminarabiose (II), and 6^1-β-D-glucopyranosyl-laminaratriose (III) could be envisaged as possible structures for **1** by a consideration of the three methylated D-glucoses, and/or the fragments laminarabiose and gentiobiose.

Table I shows the theoretical results for the periodate oxidation of the three possible glucotetraoses and the three glucotetraitols (I′, II′, and III′) derivable therefrom. Periodate oxidation of **1** gave, per mol of **1**, 4.94 mol of periodate consumed and 1.98 mol of formic acid produced. On the other hand, the periodate oxidation of the alditol of **1** gave, per mol thereof, 7.12 mol of periodate consumed, and 3.12 mol of formic acid and 2.11 mol of formaldehyde produced. These results are in agreement with the theoretical values for I and I′.

The alditol of **1**, formed by reduction with sodium borohydride, was fully methylated, hydrolyzed, and acetylated in the usual way. The alditol acetates thus obtained were analyzed[3] by combined g.l.c.–m.s., and 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methyl-D-glucitol (**4**) was identified; this compound must be derived from the 3-*O*-substituted D-glucose constituting the reducing, terminal residue. If the structure of **1** were II or III, 3,6-di-*O*-acetyl-1,2,4,5-tetra-*O*-methyl-D-glucitol would be produced

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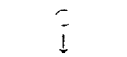
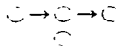
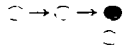
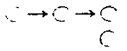
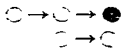
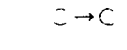
TABLE I

THEORETICAL VALUES FOR PERIODATE OXIDATION OF GLUCOTETRAOSES[a] AND THEIR ALDITOLS

Sugar	*Type*	*Periodate consumption (mol/mol)*	*Formic acid production (mol/mol)*	*Formaldehyde production (mol/mol)*
	I	5	2	0
	I′	7	3	2
	II	5	2	0
	II′	6	2	1
	III	5	2	0
	III′	6	2	1

[a]Key: ○, D-glucopyranose; ●, D-glucitol; →, (1→3)-β-glucosidic linkage; and ↓, (1→6)-β-glucosidic linkage.

from the reducing, terminal residue of either compound. Consequently, the structure of **1** was found to be 6^2-β-D-glucopyranosyl-laminaratriose.

β-D-Glc*p*
1
↓
6
β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-D-Glc*p*
1

In addition, partial hydrolysis of the alditol of **1** with acid gave gentiobiose, showing that the reducing-terminal, D-glucose residue of **1** does not have a branched structure with a (1→6)-β-linkage as in structures II and III.

The i.r. spectrum of crystalline **1** showed two characteristic absorption-peaks[4] at 896 (type 2b) and 862 cm^{-1} (type 2a). However, the type-2a absorption was very weak for amorphous **1**. The possibility of the presence of some of the α anomer in crystalline **1** is thus suggested by the i.r. spectrum (and 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 4:1:2 (v/v) 1-butanol–acetic acid–water. Aniline hydrogenphthalate was used as the spray reagent. G.l.c. was performed with a Shimadzu gas chromatograph, Model GC-4APF, fitted with a flame-ionization detector, under the following conditions: glass column (200 × 0.3 cm), 3% by weight of ECNSS-N on Neopak 1A (80–100 mesh); temp. 170°; carrier-gas flow, 100 mL of N_2/min. For quantitative evaluation of the results, a Shimadzu digital integrator ITG-4A was used. G.l.c.–m.s. was performed with a combined gas chromatograph–mass spectrometer, Hitachi M-52. The compounds were separated on a glass column (100 × 0.3 cm) containing 2% of OV-1 on Chromosorb W (80–100 mesh) at 160° with He as the carrier gas at 0.8 kg/cm^2, and identified by the spectrum at 20 eV (ionizing potential).

Isolation of **1**. — In accordance with the procedure already described[1], 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 35% ethanol as the eluant. Syrupy **1** (4.5 g) was dissolved in 80% methanol, the insoluble material was filtered off, and the filtrate was evaporated to a syrup. This treatment was repeated 3 or 4 times, and, finally, a solution of the syrup in 3:2 ethanol–water was kept at room temperature. Globular crystals separated after 30 days; recrystallized from the same solvent, compound **1** had m.p. 199–200°, and $[\alpha]_D^{20}$ +5.7° (2 min) → +1.8° (final; *c* 1, water). The d.p. of **1**, as determined by the method of Peat *et al.*[5], was found to be 4. The water content of crystalline **1**, measured by the usual method, was 5.14%, indicating a dihydrate (calc. for 2 H_2O, 5.13%).

Anal. Calc. for $C_{24}H_{42}O_{21} \cdot 2\ H_2O$: C, 41.03; H, 6.60. Found: C, 40.60; H, 6.91.

The i.r. spectrum of crystalline **1** in the region 970–730 cm^{-1} showed absorption peaks at 930 (type 1), 970, 896 (type 2b), 862 (type 2a), and 777 cm^{-1} (type 3). On the other hand, the type-2a absorption-band of amorphous **1** [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**.

Acetylation of **1**. — A mixture of **1** (50 mg), acetic anhydride (1 mL), and anhydrous sodium acetate (50 mg) was heated for 30 min at 120° and then for 2 h at 100°. The product was isolated in the usual way, and crystallized from ethanol–petroleum ether. Recrystallization from the same solvent gave the peracetate, m.p. 149–150°, $[\alpha]_D^{20}$ −27.7° (*c* 1, chloroform).

Anal. Calc. for $C_{52}H_{72}O_{35}$: C, 49.77; H, 5.69. Found: C, 49.85; H, 5.69.

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

Partial hydrolysis of **1** *with acid.* — A portion (10 mg) of **1** was reduced with sodium borohydride in the usual way. The alditol was hydrolyzed with 0.1M hydrochloric acid (1 mL) in a sealed tube for 3 h at 100°. D-Glucose (R_{Glc} 1.00), laminarabiose (R_{Glc} 0.78), gentiobiose (R_{Glc} 0.64), and 3,6-di-*O*-β-glucosyl-D-glucose (R_{Glc} 0.53) were detected on the chromatogram, triply developed with the solvent, of the hydrolyzate. The R_{Glc} values refer to the chromatographic mobility relative to that of D-glucose as unity.

Periodate oxidation of **1**. — A portion of **1** (33 mg) was oxidized with 0.01M sodium periodate (50 mL) in the dark for 48 h at 2–5°. At intervals, the periodate uptake was determined by the arsenite procedure[6], and the formic acid production was determined by titration with 0.01M sodium hydroxide; the results are shown in Table II. Periodate oxidation of **1** was complete within 24 h, 4.94 mol of periodate being consumed, and 1.98 mol of formic acid liberated, per mol of **1**.

Periodate oxidation of the alditol of **1**. — Compound **1** (33 mg) in water (3 mL) was reduced with 1% sodium borohydride (30 mL) for 5 h at room temperature. The solution was made neutral with acetic acid, 0.01M sodium periodate (70 mL) was added, and oxidization conducted for 48 h as already described. The data are given in Table III. After oxidation was complete, consumption of 7.12 mol of periodate per mol of **1** was determined. At the same time, production of 3.12 mol of formic acid and of 2.11 mol of formaldehyde, per mol of **1**, was found (by the chromotropic acid method[7]).

Methylation analysis of **1**. — A portion of **1** (20 mg) was methylated with methyl iodide and methylsulfinyl anion in dimethyl sulfoxide (Hakomori method[8]). The

TABLE II

PERIODATE OXIDATION OF **1**

Time (h)	0.25	0.50	1	2	4	10	24	48
Periodate[a] consumption	1.24	1.48	1.75	2.08	2.52	3.22	4.94	4.94

[a]In mol per mol of **1** (glucotetraose).

TABLE III

PERIODATE OXIDATION OF THE ALDITOL OF **1**

Time (h)	0.25	0.50	1	3	6	10	24	48
Periodate[a] consumption	1.65	2.12	2.38	3.64	4.97	5.71	7.12	7.12

[a]In mol per mol of the alditol of **1** (glucotetraose).

permethylated compound was dissolved in one drop of ethanol, and hydrolyzed with M hydrochloric acid (0.5 mL) for 5 h at 100° in a sealed tube. The hydrolyzate was made neutral with barium carbonate, the suspension filtered, and the filtrate concentrated. A portion of the concentrate was treated with sodium borohydride, and the product acetylated with acetic anhydride–pyridine in the usual way. G.l.c. analysis of the partially methylated, alditol acetates showed the presence of 2,3,4,6-tetra-, 2,4,6-tri-, and 2,4-di-*O*-methyl-D-glucose (*T* 1.00, 1.88, and 4.66, respectively) in the molar ratios of 2:1:1.

Methylation analysis of the alditol of **1**. — The alditol of **1** (20 mg), prepared by reduction with sodium borohydride, was methylated, hydrolyzed, reduced, and acetylated as just described. Analysis by g.l.c.–m.s. of the partially methylated, alditol acetates showed 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (**2**), 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methyl-D-glucitol (**3**), and 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methyl-D-glucitol (**4**). Mass spectra: **2**, *m/e* 205 (18%), 161 (54), 145 (54), 129 (53), 117 (59), 101 (100), 87 (18), 71 (12), and 45 (12); **3**: *m/e* 233 (9%), 201 (4), 189 (20), 173 (5), 129 (58), 117 (100), and 87 (23); and **4**: *m/e* 249 (9%), 205 (41), 189 (4), 173 (18), 145 (5), 133 (19), 101 (74), 89 (100), 59 (23), and 45 (23).

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