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Note

Synthesis of a new disaccharide: *O*-β-D-glucopyranosyl-(1→3)-D-fructose (laminarabiulose)

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INTRODUCTION

During our studies on the structure of a polysaccharide from the fungus *Cyttaria johowii*[1,2], a glucosyl-fructose was detected among the products of partial acid-hydrolysis. The retention time in gas–liquid chromatography of the trimethylsilyl derivative was very similar to that of turanose [*O*-α-D-glucopyranosyl-(1→3)-D-fructose], although the compounds could be separated by cochromatography. This result suggested that the compound could be the β-glucosidic disaccharide, laminarabiulose.

Glucosylfructoses are usually obtained by alkaline isomerization of the corresponding aldobiose[3]. Only the (1→1)-linked glucosylfructoses have been synthesized by the Koenigs–Knorr reaction. We now report the first synthesis of β-D-glucopyranosyl-(1→3)-D-fructose, useful for the identification of this component in the polysaccharide.

The disaccharide obtained by mild acid-degradation of the polysaccharide was indistinguishable from the synthetic compound by paper chromatography and g.l.c. of the trimethylsilyl ethers (unpublished results).

RESULTS AND DISCUSSION

Condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**1**) with 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**2**) in 1:1 nitromethane–benzene in the presence of mercuric cyanide during 5 h at 45° gave 1,2:4,5-di-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-fructopyranose (**3**) in 26% yield.

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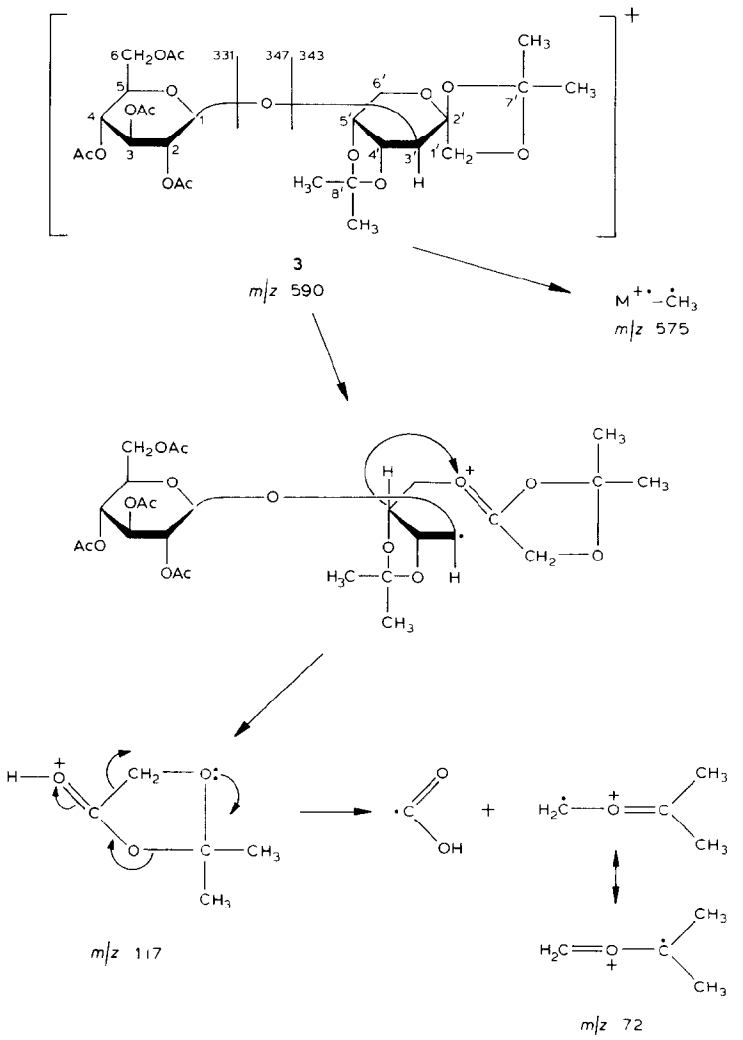
Gas–liquid chromatographic examination of the mixture showed unreacted **2**, 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose formed by decomposition of **1**, and two disaccharide derivatives. Both compounds gave very similar fragmentations by g.l.c.–m.s., indicating they were the anomeric (1→3)-α- and β-D-glucosyl-fructose derivatives. The peak observed by g.l.c. of pure **3** was coincident with that of the main compound in the mixture. Its mass spectrum showed the M − 15 ion at *m/z* 575. The fragments at *m/z* 117 and 72 provided evidence for 3-*O*-substitution of the fructopyranose ring[6] (Scheme I). The other ions corresponded to primary and secondary fragmentations of the individual rings.



Scheme I. Mass-spectral fragmentations of 1,2:4,5-di-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-fructopyranose (**3**).

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS (δ_c) OF 1,2:4,5-DI-*O*-ISOPROPYLIDENE-3-*O*-(2,3,4,6-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSYL)-β-D-FRUCTOPYRANOSE AND RELATED COMPOUNDS

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	*C-7'*	*C-8'*	*Ref.*
2							72.4	104.7	70.3	77.4	73.5	60.9	111.9	109.5	8
6							65.5	102.9	70.9[a]	70.7[a]	70.0[a]	61.2	108.9	108.4	
3	101.5	72.9	71.5[a]	68.5	71.9[a]	61.9	72.3[a]	103.5	78.5	75.3	71.4[a]	61.0	111.6	108.8	
7	99.4	72.9	73.1	68.6	71.6	62.0	105.1	83.0	81.3	80.6	72.3	66.3	112.0	108.6	9
8	101.3	72.1	73.1	68.9	71.8	62.2	106.5	84.2	75.2	79.2	71.6	70.2	112.3	101.0	9
9	91.8	70.5	72.9	68.0	72.9	61.6									10
4	103.9	73.6[a]	76.6[b]	70.1	76.2[b]	62.0	71.9	104.1	79.5	76.1[b]	73.4[a]	60.7	112.0	108.9	
12	103.9	74.8	77.1	71.2	77.1	62.4	97.2	74.8	86.7	69.6	77.1	62.4			12

[a,b]Assignments may be interchanged.

TABLE II

EFFECT OF SUBSTITUTION OF THE 1-*O*-ACETYL GROUP IN 1,2,3,4,6-PENTA-*O*-ACETYL-β-D-GLUCOPYRANOSE BY DI-*O*-ISOPROPYLIDENEHEXOSE GROUPS

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*Ref.*
3	+9.7	+2.4	−1.4	+0.48	−1.0	+0.3	
7	+7.6	+2.4	+0.2	+0.6	−1.3	+0.4	9
8	+9.5	+1.6	+0.2	+0.9	−1.1	+0.6	9
10	+7.0	+1.0	0	+0.2	−0.5	+0.3	11
11	+2.2	+0.8	+0.1	+0.5	−1.8	+0.2	11

The i.r. spectrum showed absorptions at 1760 (carbonyl of acetate), 859–833 (dioxolane), and 890 cm^{-1} (β-glucoside).

The ^{1}H-n.m.r. spectrum revealed, besides the acetate and the isopropylidene protons, the H-1 doublet at δ 5.15, $J_{1,2}$ 8 Hz, indicating a β-glucosidic bond, and a doublet at δ 3.7 for H-3′ ($J_{3',4'}$ 7 Hz). A value of 3.49 was reported for H-3 of 1,2:4,5-di-*O*-isopropylidene-3-*O*-methyl-β-D-psicopyranose[7].

For the assignment of the ^{13}C-n.m.r. spectrum of **3**, chemical shifts of compound[8] **2**; 2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**6**); 1,2:5,6-di-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucofuranose[9] (**7**); 1,2:3,5-di-*O*-isopropylidene-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucofuranose[9] (**8**); and 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose[10] (**9**) were used as model compounds (Table I). Four acetate and two isopropylidene groups were evident. Comparison of the chemical shifts for the acetal carbons of **2**, **3**, and **6** shows that this quaternary carbon signal appears at a lower field when the dioxolane ring involves an exocyclic primary alcohol. The values for compound **3** prove that no acetal migration occurred under the reaction conditions[9]. Chemical shifts for the anomeric carbon atoms were readily assigned at 103.5 and 101.4. The last chemical shift is consistent with the presence of a β-glucopyranoside structure. Glucosylation at position 3 of **2** resulted in a downfield shift of +8.2 p.p.m., which is in agreement with the value reported[9] for compound **7**.

The shifts observed when the 1-*O*-acetyl group of **9** is replaced by a 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranosyl group are shown in Table II. The shifts for the laminarabiulose and gentiobiose derivatives were calculated, for comparison, from the ^{13}C-n.m.r. data[9]. The shifts observed for 2,3:5,6-di-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-mannofuranose[11] (**10**) and 2,3:5.6-di-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-mannofuranose[11] (**11**), the last one with respect to α-D-glucopyranose pentaacetate, are also included in Table I. Compound **11** was the only α-linked disaccharide derivative having the same protective groups, found in the literature. A significant downfield shift is observed for C-1 on β-glycosidation (7–9.7 p.p.m.) and a similar shift (+2.4) is observed on the β-carbon atom of **3** and of the laminarabiulose derivative.

Treatment of **3** with sodium methoxide gave crystalline 3-*O*-(β-D-glucopyranosyl)-1,2:4,5-di-*O*-isopropylidene-β-D-fructose (**4**). The ^{13}C-n.m.r. spectrum (Table I) showed two signals at δ 112.0 and 108.9 for the dioxolane quaternary carbon atoms. Typical downfield shifts caused by deacetylation were observed for the ^{13}C-signals of the glucopyranosyl moiety. The signals were assigned by comparison with the data for laminarabiose[12] (**12**, Table I).

By removal of the isopropylidene groups, β-D-glucopyranosyl-(1→3)-D-fructose (**5**) was obtained as a homogeneous syrup having $[\alpha]_D^{20}$ −43° (equil., *c* 0.5, water). The disaccharide may be named "laminarabiulose" by taking into account the common name for the isomeric aldobiose. The disaccharide was characterized as *O*-β-D-glucopyranosyl-(1→3)-D-*arabino*-hexose phenylosazone, whose constants were similar to those reported in the literature for the phenylosazone obtained from laminarabiose.

Laminarabiulose can potentially exist in solution in five tautomeric forms. As with maltulose, cellobiulose[15], and turanose[16], compound **5** exhibits only three detectable tautomeric forms at equilibrium; the two furanoses and the β-pyranose, as shown by six anomeric-carbon signals in the ^{13}C pulse-Fourier-transform spectrum. Thirty five resonances were listed, but it was not possible to assign all of them unambiguously because of the complexity introduced by the presence of the glucopyranosyl ring.

EXPERIMENTAL

General methods. — Melting points were determined with a Kofler apparatus and are uncorrected. Optical rotations were recorded with a Perkin–Elmer 141 polarimeter. N.m.r. spectra were obtained at 25.2 MHz with a Varian XL-100-15 spectrometer operating in the f.t. mode by using a 620 L-100 computer interfaced to a Sykes 7000 dual disk-drive. Samples were spun in 12-mm tubes at ~30°. Substituted sugars were dissolved in chloroform-*d* with Me_4Si as the internal standard. The free disaccharide was dissolved in D_2O and 1,4-dioxane was used as the external standard (δ_c 67.4 p.p.m. downfield from Me_4Si). Mass spectra were performed with a Varian MAT Ch-7A mass spectrometer at 70 eV coupled to a Varian MAT data-system 166. G.l.c. was effected with a Hewlett–Packard 5840 A gas chromatograph equipped with glass columns (120 × 0.2 cm) packed with 2% of OV-101 on Chromosorb W AW-DMCS (60–80 mesh): *A*, with nitrogen at a flow rate of 20 mL.min^{-1}, T_i 280°, T_d 290°, T_c 190–280°, rate 1°/min; *B*, T_i 250°, T_d 225°, T_c 160–210°, rate 1°/min, nitrogen: 32 mL.min^{-1}.

T.l.c. was performed on Silica Gel G (Merck) with (*a*) 4:1 (v/v) dichloromethane–ethyl acetate, or (*b*) 9:1 (v/v) chloroform–methanol. For the detection of ketoses, the resorcinol reagent[17] was modified as follows. The plates were first sprayed with 1% resorcinol in 1-butanol and then with 50% sulfuric acid in acetic acid. By heating for 5 min at 80°, only ketoses gave visible spots; further heating at higher temperatures was used to detect all sugars. Column chromatog-

raphy was performed on Silica Gel H (Merck) with 4:1 dichloromethane–ethyl acetate as solvent.

Descending paper-chromatography was performed on Whatman No. 1 paper with (*A*) 5:2:2 (v/v) 1-butanol–ethanol–water; (*B*) 6:4:3 (v/v) 1-butanol–pyridine–water, and (*C*) 10:4:3 (v/v) ethyl acetate–pyridine–water.

1,2:4,5-Di-O-isopropylidene-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-fructopyranose (**3**). — A solution of 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose[18] (**2**, 3.24 g) in 1:1 (v/v) benzene–nitromethane (110 mL) was concentrated at atmospheric pressure to one-half volume, cooled to 45°, and stirred with mercuric cyanide (3.5 g) and tetra-*O*-acetyl-α-D-glucopyranosyl bromide[19] (**1**, 5.7 g) for 5 h under anhydrous conditions. The mixture was filtered and the filtrate evaporated under diminished pressure. A solution of the residue in dichloromethane (200 mL) was successively washed with 5% aqueous potassium iodide (50 mL), water (2 × 50 mL), saturated aqueous sodium hydrogencarbonate (50 mL), and water (2 × 50 mL), dried (magnesium sulfate) and evaporated to yield 7.02 g of a syrup, t.l.c. of which (solvent *a*) showed three spots having R_F 0.72 (major) and R_F 0.64 and 0.47 (minor). G.l.c. of the mixture under conditions (*A*) showed peaks having R_T (min, compound): 0.54, **2**; 2.25, 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose; 3.51, unidentified; 21.03, 1,2:4,5-di-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-fructopyranose; 22.88, **3**; and 26.73, unidentified. The relative peak-area ratio was: 6.6:3.8:1:2.6:15.7:1.3.

Half of the mixture was fractionated by column chromatography (15 × 5 cm) and elution was monitored by t.l.c. Fractions 12–22, showing one spot (R_F 0.72), were evaporated and compound **3** (0.53 g) crystallized upon addition of 2-propanol. It was recrystallized from the same solvent; m.p. 149–151°, $[\alpha]_D^{20}$ −81.5° (*c* 0.8, dichloromethane), ν_{max}^{Nujol} 1760 (acetate, C=O), 859–833 (dioxolane), and 890 cm^{-1} (β-glucoside). G.l.c. under conditions (*A*) showed a single peak, having *T* 22.88 min. ^{1}H-N.m.r. data: δ (p.p.m.) 1.33 (s, 3 H, CH_3), 1.38 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 1.98 (s, 3 H, Ac), 2.0 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), 2.1 (s, 3 H, Ac), 3.7 (d, $J_{3',4'}$ 7 Hz, H-3'), 4.0–4.5 (m, 8 H), 4.68 (dd, $J_{1,2}$ and $J_{2,3}$ 8 Hz, H-2), 4.95–5.15 (m, H-3 and H-4), and 5.15 (d, $J_{1,2}$ 8 Hz, H-1); ^{13}C-n.m.r. data see Table I; *m/z* 575 (M − 15, 3.4%) 474 (1.1); 473 (1.5), 347 (1.4), 331 (26), 271 (8.6), 243 (5.8), 185 (18.9), 170 (13.4), 169 (92.1), 157 (9.1), 145 (11.9), 143 (66), 127 (32), 117 (3.2), 115 (22.6), 109 (74), 100 (29.5), 85 (23.2), 72 (11.6), 69 (23.2), 59 (22.2), and 43 (100).

Anal. Calc. for $C_{26}H_{38}O_{15}$: C, 52.80; H, 6.44. Found: C, 53.03; H, 6.70.

Fractions 23–30 (0.4 g) contained (t.l.c.) compound **3** and starting material (**2**, R_F 0.64), which was recovered by recrystallization. Fractions 35–40 (0.45 g) contained (t.l.c.) a main component, R_F 0.42, characterized as 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose; m.p. 132–134° lit.[20] m.p. 132–134°. By seeding, compound **3** crystallized from the mixture in 26% yield without previous chromatographic purification.

1,2:4,5-Di-O-isopropylidene-3-O-β-D-glucopyranosyl-β-D-fructopyranose

(**4**). — Deacetylation of **3** (0.36 g) was effected with 0.1M sodium methoxide in methanol (10 mL) at 5°. The reaction was monitored by t.l.c. (solvent *a*) until no starting material was detected (2 h). The solution was made neutral with Dowex 50 (H^+ resin), and evaporated to give a crystalline mass which, on recrystallization from benzene, afforded **4** (0.174 g); m.p. 103–104°, $[\alpha]_D^{20}$ −112° (*c* 0.7, chloroform); R_F 0.60 (solvent *b*); ^{1}H-n.m.r. data: δ (p.p.m.) 1.36, 1.46, 1.48, 1.54 (4 s, 12 H, 4 CH_3), 3.3–4.5 (br. m, 14 H); ^{13}C-n.m.r. data see Table I; ν_{max}^{Nujol} 830–850 (dioxolane) and 890 cm^{-1} (β-glucoside).

Anal. Calc. for $C_{18}H_{30}O_{11}$: C, 51.18; H, 7.1. Found: C, 50.97; H, 7.2.

O-β-D-*Glucopyranosyl-(1→3)*-D-*fructose* (**5**). — A solution of **4** (70 mg) in water (10 mL) was heated for 4 h at 60° with Bio-Rad 50 (H^+) ion-exchange resin. The mixture was filtered, the filtrate made neutral with barium carbonate, and evaporated. The syrup (30 mg) gave (p.c.) only one spot detected with the reagent for ketoses; R_F 0.73 (solvent *A*); R_F 0.90 (solvent *B*); R_F 0.60 (solvent *C*); $[\alpha]_D^{20}$ −43° (*c* 0.5, water). The ^{13}C-n.m.r. spectrum showed 35 signals, among them those of the anomeric carbon atoms at δ_c 105.55, 103.98, 103.83, 103.35 102.62, and 98.62. G.l.c. of the trimethylsilyl derivative gave one peak having retention time 20.99 min under conditions (*B*).

A solution of **5** (30 mg) in water (0.5 mL) was heated with phenylhydrazine hydrochloride (50 mg) and sodium acetate (100 mg) during 30 min in a boiling-water bath. After cooling, *O*-β-D-glucopyranosyl-(1→3)-D-*arabino*-hexulose phenylosazone crystallized as prismatic yellow needles; m.p. 196–198°, $[\alpha]_D^{20}$ −70.9° (*c* 0.5, ethanol), lit.[21] m.p. 198°, $[\alpha]_D^{20}$ −75° (ethanol); lit.[22] m.p. 199–201°, $[\alpha]_D^{20}$ −76° (ethanol).

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