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Note

Sophorose produced in the fusion and Koenigs–Knorr reactions

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Sophorose (**1**, 2-*O*-β-D-glucopyranosyl-D-glucose) has been produced *via* the fusion reaction of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (**2**) in the presence of toluene-*p*-sulphonic acid[1], and by the Koenigs–Knorr reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**3**) and methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[2,3]. In contrast, kojibiose (**4**, 2-*O*-α-D-glucopyranosyl-D-glucose) is produced in the fusion reaction of 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose (**5**) in the absence of catalyst[4], and in the Koenigs–Knorr reaction of **2** and **3**[5,6]. A mixture of **1** and **4** was produced in the fusion reaction of **2** with $ZnCl_2$ as catalyst[1,7].

We now report the formation of sophorose (**1**) *via* the fusion reaction of **5**, or of a mixture of **2** and **5**, in the presence of toluene-*p*-sulphonic acid, and in the Koenigs–Knorr reaction of **2** and **3**.

Compound **2**, **5**, or a mixture of **2** and **5** melted at 65–130° in the presence of toluene-*p*-sulphonic acid or $ZnCl_2$ (Table I). The reaction products were deacetylated, and separated into three fractions by chromatography on Bio-Gel P-2. A series of oligo- and poly-saccharides was detected by p.c. (R_{Glc} 0.54, 0.33, 0.26, 0.20, and slowly moving spots; solvent *A*). Essentially the same products were formed in these reactions, but the fusion of **5** in the presence of $ZnCl_2$ produced only a trace of oligo- and poly-saccharides (p.c.), and **2** did not produce **5**. The oligo- and poly-saccharide fraction was obtained in up to 38.6% yield; reaction D gave a slightly higher yield than reactions A–C (Table I).

The product having R_{Glc} 0.54, isolated by preparative p.c. as a syrup from reactions A–D in yields of 19, 16, 13, and 20%, respectively, was a mixture of **1** and **4**. It had a reducing value of 45–55% compared with D-glucose, and exhaustive digestion with β-D-glucosidase gave a mixture of glucose and disaccharide **4** (R_{Glc} 0.54; reducing value, 52%). The product from reaction C[1,7] contained a significant amount of **4**, but only traces were present in the corresponding products from reactions A, B[1], and D.

The product (R_{Glc} 0.54) from reaction D gave a crystalline octa-acetate monohydrate with R_F values [t.l.c.; 0.63 (solvent *C*), 0.59 (solvent *D*)] indistinguishable from those of the octa-acetates of **1** and **4**. The octa-acetate monohydrate had $[\alpha]_D^{20}$

TABLE I

OLIGO- AND POLY-SACCHARIDES PRODUCED IN THE FUSION REACTION

Reaction	*Monosaccharide derivative*[a]	*Catalyst*[b]	*Reaction*		*Oligo- and poly-saccharides*				
			Temperature (degrees)	*Time (min)*	*Fraction*	*Yield (%)*	*D.p.*[c]	*Anthrone value*[d] *(%)*	$[\alpha]_D^{17}$ *(c 1, water) (degrees)*
A	5	TsOH	65 ± 3	10	a	trace	n.d.	n.d.	n.d.
					b	32	3.0	96	+13
B[e]	2	TsOH	105 ± 3	30	a	5	9.2	95	− 7
					b	26	3.5	96	+ 7.4
C[e]	2	$ZnCl_2$	105 ± 3	60	a	7	11	98	− 7.5
					b	22	4.0	90	+35[f]
D	2 + 5	TsOH	130 ± 3	40	a	7.3	10	93	− 3
					b	31	3.0	98	+15

[a]2, 1,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranose; 5, 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose. [b]TsOH, *p*-Toluenesulfonic acid. [c]Degree of polymerization. [d]Calc. as D-glucose. [e]Similar reactions were reported in Refs. 1 and 7. [f]α-D-Glucoside was present (Refs. 1 and 7).

+42° (*c* 0.55, chloroform) (*cf.* +45° for the anhydrous compound[13]), showed eight signals for acetate groups in the n.m.r. spectrum ($CDCl_3$), and had ν_{max}^{KBr} at 890 cm^{-1} characteristic of a β-D-glucopyranoside. The deacetylated product gave only D-glucose (p.c.) on complete hydrolysis (2M HCl, 100°, 3 h), had a reducing value of 48% compared with D-glucose, and was completely hydrolysed by β-D-glucosidase.

The other oligo- and poly-saccharides were not further fractionated. Fraction **a** consumed 0.9–1.1 mol of periodate per D-glucose residue, and formic acid could not be detected by n.m.r. spectroscopy[12]. The $[\alpha]_D$ value (−3° to −7.5°) accorded with that (−8°) of (1→2)-β-D-glucan[8].

The products which were obtained by the Koenigs–Knorr reaction of **2** and **3** in nitromethane[5,6] in the presence of $Hg(CN)_2$, after deacetylation, gave 52% of a syrupy disaccharide fraction which had chromatographic behaviour identical with that of the disaccharide fraction obtained from reaction C above. Exhaustive digestion with β-D-glucosidase gave D-glucose and **4** (detected by t.l.c.), and indicated that **1** and **4** (~3:2) were present in the disaccharide fraction. Compound **4** was previously isolated in 21% yield from the same reaction[5,6].

EXPERIMENTAL

Compounds **2**[5], **3**[9], and **5**[10] were prepared by the conventional methods. Almond β-D-glucosidase (EC 3.2.1.21) was obtained from Sigma (Lot. 29B-1280); the enzyme (1 mg) liberated ~2.5 μmol of D-glucose per min from salicin at pH 5.2 and 37°.

P.c. was performed by the descending method on Whatman No. 1 or 3MM filter papers with *A*, acetic acid–1-butanol–ethanol–water (12:50:5:25), *B*, 1-butanol–pyridine–water (3:2:1:5), and detection with alkaline silver nitrate. T.l.c. was performed on Silica Gel G (Merck, Type 60) with *C*, benzene–ethyl acetate (4:3), *D*, benzene–acetone (4:1), and detection by charring with sulphuric acid. The reducing-sugar values were obtained by the Schales method[11], and the degree of polymerisation (d.p.) was calculated therefrom. The other methods were described previously[12].

Fusion reactions. — The reactions A–D were performed by using 1–3 g of **2** or **5** (Table I). The products were deacetylated with methanolic ammonia and eluted from a column (1.7 × 213 cm) of Bio-Gel P-2 with 10% ethanol (7-ml fractions). Fractions were monitored by the anthrone reaction[12] and by p.c., and grouped as follows: **a** (fractions 20–30) contained products having low mobility, and R_{Glc} 0.20 and 0.26 (solvent *A*); **b** (fractions 31–43), R_{Glc} 0.26, 0.33, 0.54. and 1.00 (trace); and **c** (fractions 44–58), R_{Glc} 0.54 (trace) and 1.00. Fractions **b** and **c** were rechromatographed to remove monosaccharide, concentrated *in vacuo* at 40° to a small volume, and lyophilized. All the products were amorphous and soluble in water.

Fraction **b** (277 mg) from reaction D was subjected to preparative p.c. on three sheets (46 × 57 cm) of Whatman 3MM paper, and the compound having R_{Glc} 0.54 was eluted from the appropriate areas with hot water. The extract was concen-

trated *in vacuo*, and the resulting syrup (184 mg), $[\alpha]_D^{15}$ +20° (*c* 1, water), was treated with acetic anhydride and pyridine. Crystallisation of the product from ether–light petroleum (b.p. 40–60°) gave the octa-acetate (78%), m.p. 74–76°, $[\alpha]_D^{21}$ +42° (*c* 0.55, chloroform); ν_{max}^{KBr} 1760 (acetyl C=O) and 1240 (acetyl C-O), 1090–1040 (C–O), 890 cm^{-1} (β-D-Glc).

Anal. Calc. for $C_{28}H_{38}O_{19} \cdot H_2O$: C, 48.27; H, 5.79. Found: C, 48.61; H, 5.60.

The octa-acetate (1 mg) was treated with 0.2M NaOH in ethanol (0.2 ml) at room temperature overnight. Ethanol was evaporated *in vacuo*, and the residue was made up to 5 ml with 0.2M acetate buffer (pH 5.2). The reducing-sugar value was 44% against D-glucose. β-D-Glucosidase (0.1 mg) was added to the solution, and the mixture was incubated at 37°. Aliquots (0.5 ml) were withdrawn at intervals, and the reducing-sugar value was determined. The value reached 98% against D-glucose after incubation for 2 h.

The Koenigs–Knorr reaction. — To a solution of **2** (1.88 g) in nitromethane (40 ml) was added $Hg(CN)_2$ (2 g) with stirring. To the mixture was added a solution of **3** (2.23 g) in anhydrous chloroform (10 ml), and stirring was continued for 24 h in the dark. More **3** (1.6 g × 3) was added, and **2** disappeared (t.l.c.) after 72 h. The mixture was filtered, and concentrated to dryness. The residue was deacetylated with methanolic sodium methoxide, and the products were eluted from a column (3 × 60 cm) of Silica Gel 60 (Merck) with 1-butanol–ethyl acetate–2-propanol water (2:1:1:1) to remove monosaccharide. The disaccharide fraction was isolated as syrup (0.97 g), R_{Glc} 0.57 (p.c., solvent *B*). The octa-acetate had R_F 0.62 (solvent *C*) and 0.59 (solvent *D*) in t.l.c.

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