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Note

Synthesis of raffinose and an isomer

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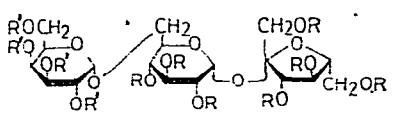
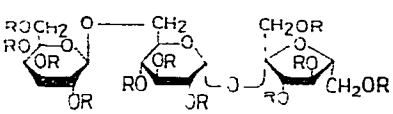
Raffinose occurs widely in the plant world and its structure has been established as *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside[1]. Its enzymic synthesis was described by Hestrin *et al.*[2] in 1955.

In connection with previous papers on sucrose chemistry[3–5], we have attempted to synthesize raffinose (**5**) chemically. Recently, successful attempts to prepare α-D-linked disaccharides by reaction of glycosyl halides having a non-participating group at C-2[6] with alcohols have been described[7–11]. By employing the reaction between 2,3,4,1′,3′,4′,6′-hepta-*O*-acetylsucrose[4,12] (**2**) and tetra-*O*-benzyl-α-D-galactopyranosyl chloride[9] (**1**), we have synthesized **5**. In this reaction, the use of tetra-*O*-acetyl-α-D-galactopyranosyl bromide[13] (**7**) instead of **1** resulted in the formation of an isomeric trisaccharide, *O*-β-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside (**9**).

The condensation of **2** with **1** in dry benzene in the presence of mercuric cyanide and "Drierite" afforded *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-1,3,4,6-tetra-*O*-acetyl-β-D-fructofuranoside (**3**) in 53% yield. Deacetylation of **3** in 0.1M methanolic sodium methoxide yielded *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside (**4**). Hydrogenolysis of **4** with Raney nickel catalyst



3 R = Ac R′ = Bn
4 R = H R′ = Bn
5 R = H R′ = H
6 R = Ac R′ = Ac



8 R = Ac
9 R = H

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afforded **5** pentahydrate in 25% yield, identical with natural raffinose pentahydrate. Acetylation of **5** afforded the undecaacetate (**6**) in 81% yield.

Partial hydrolysis of **5** in 0.1M hydrochloric acid, followed by acetylation and chromatographic fractionation, yielded β-melibiose octaacetate[17,18].

The p.m.r. spectrum of **5** revealed two doublets at δ 5.01 (*J* 3.0 Hz) and 5.44 (*J* 3.5 Hz). The former signal was attributed to the anomeric proton of the α-D-galactopyranosyl group and the latter was ascribed to that of the α-D-glucopyranosyl group, as that of the α-D-galactopyranosyl group in melibiose showed its signal at δ 4.96 (*J* 2.9 Hz)[19] and that of the α-D-glucopyranosyl group in sucrose appeared at δ 5.45 (*J* 3.2 Hz)[5,19].

The isomeric trisaccharide **9** was prepared in 59% yield as the undecaacetate (**8**) by condensation of **2** with **7** in nitromethane in the presence of mercuric cyanide and "Drierite". Deacetylation of **8** in 0.1M methanolic sodium methoxide afforded **9** in 53% yield. Partial hydrolysis of **9** and subsequent acetylation of the hydrolyzate gave β-allolactose octaacetate[14].

The p.m.r. spectrum of **9** showed two doublets at δ 4.44 (*J* 7.5 Hz) and 5.44 (*J* 3.5 Hz), which were attributed to the anomeric proton of the β-D-galactopyranosyl moiety and that of the α-D-glucopyranosyl one, because that of the β-D-galactopyranosyl group in allolactose[14] showed its signal at δ 4.44 (*J* 7.9 Hz).

Therefore, the structure of **9** was established to be the above described trisaccharide.

EXPERIMENTAL

General. — Melibiose and raffinose were purchased from commercial sources. Melting points were determined in capillary tubes, unless otherwise noted, and are corrected. Solutions were evaporated below 40° under diminished pressure. I.r. spectra were determined for potassium bromide discs with a Hitachi EPI-2 spectrophotometer. Optical rotations were measured on a Japan Spectroscopic DIP-SL polarimeter. P.m.r. spectra were determined at 60 MHz with a Varian A-60D spectrometer in chloroform-*d* or deuterium oxide with tetramethylsilane or sodium 4,4-dimethyl-4-silapentane-1-sulfonate as an internal standard. The peak positions are given in δ-values. T.l.c. was performed on silica gel (Wakogel B-10) plates, and silica gel (Wakogel C-200) was used for column chromatography.

Tetra-O-benzyl-α-D-galactopyranosyl chloride (**1**). — This product was prepared by the method of Austin *et al.*[9]; $[\alpha]_D^{20}$ +135° (*c* 4.27, benzene); p.m.r. data ($CDCl_3$): δ 6.18 (d, 1 H, *J* 3.3 Hz, H-1) and 7.2–7.4 (m, 2OH, phenyl); lit.[9] $[\alpha]_D$ +147° (*c* 2.0, benzene).

2,3,4,1',3',4',6'-Hepta-O-acetylsucrose[4,12] (**2**). — This product was prepared by the method of Otake[12].

O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucoyranosyl)-(1→2)-1,3,4,6-tetra-O-acetyl-β-D-fructofuranoside (**3**). — To a solution of **1** (1.73 g, 3.1 mmoles) and **2** (1.60 g, 2.5 mmoles) in dry benzene (30 ml),

mercuric cyanide (2.00 g, 7.9 mmoles) and "Drierite" (2.00 g) were added and the mixture was heated for 24 h under reflux with mechanical agitation and exclusion of moisture. The mixture was filtered, and the filtrate was washed with water and evaporated to give a solid residue (3.24 g). The residue was chromatographed on a silica gel column (100 g, 2.8 × 50 cm) in 1:5 butanone–toluene. Fractions that showed a single spot having R_F 0.38 on t.l.c. in the same solvent mixture were combined and concentrated, giving **3** as an amorphous solid (1.54 g, 53%), $[\alpha]_D^{20}$ +65.40° (*c* 1.87, chloroform); p.m.r. data ($CDCl_3$): δ 1.96 (s, 3H, OAc), 2.02 (s, 6H, 2OAc), 2.08 (s, 6H, 2OAc), 2.10 (s, 3H, OAc), 2.14 (s, 3H, OAc), 5.71 (d, 1H, *J* 3.5 Hz, H-1, α-Glc), and 7.2–7.4 (m, 20H, Phenyl).

Anal. Calc. for $C_{60}H_{70}O_{23}$: C, 62.17; H, 6.09. Found: C, 61.98; H, 5.76.

Fractions having R_F 0.33 by t.l.c. in the same solvent system were combined and evaporated to give a solid product (0.24 g, 8.2%), $[\alpha]_D^{20}$ +47.80° (*c* 1.36, chloroform); p.m.r. data ($CDCl_3$): δ 1.93 (s, 3H, OAc), 1.99 (s, 3H, OAc), 2.03 (s, 9H, 3OAc), 2.08 (s, 3H, OAc), 2.13 (s, 3H, OAc), 5.71 (d, 1H, *J* 3.5 Hz, H-1, α-Glc) and 7.2–7.4 (m, 2OH, phenyl). This product was probably an anomeric isomer of **3**, but was not further examined.

O-(*2,3,4,6-Tetra*-O-*benzyl*-α-D-*galactopyranosyl*)-(*1→6*)-O-α-D-*glucopyranosyl*-(*1→2*)-β-D-*fructofuranoside* (**4**). — A solution of **3** (1.21 g, 1.0 mmole) in 0.1M methanolic sodium methoxide (20 ml) was kept overnight at room temperature. The solution was then deionized with Dowex-50W (H^+) to pH 7 and evaporated, giving a glassy solid (0.78 g, 86%), $[\alpha]_D^{20}$ +53.6° (*c* 1.12, chloroform).

Raffinose (**5**). — To a solution of **4** (0.62 g, 0.7 mmole) in 95% aqueous methanol (35 ml), Raney nickel T-4[15] (~0.5 g) was added. The suspension was shaken under hydrogen at 3.4 atm pressure for 16 h. The catalyst was removed by filtration and the filtrate was evaporated, giving a solid residue (0.30 g, 82%), which showed a single spot at R_F 0.8 on t.l.c. in 1:3:3 chloroform–butanone–methanol. The product was crystallized from a mixture of 90% aqueous ethanol and methanol to give 0.11 g (25%) of needle-like crystals, m.p. 80–82°, $[\alpha]_D^{20}$ +105° (*c* 1.04, water). The product did not reduce Fehling's solution and its i.r. spectrum was superposable on that of natural raffinose pentahydrate. The mixed m.p. with an authentic sample did not show any depression. P.m.r. data (D_2O): δ 5.01 (d, 1H, *J* 3.0 Hz, H-1, α-Gal) and 5.44 (d, 1H, *J* 3.5 Hz, H-1, α-Glc). Lit[16] m.p. 80°, $[\alpha]_D^{20}$ +105.2° (*c* 4, water).

Anal. Calc. for $C_{18}H_{32}O_{16}\cdot 5H_2O$: C, 36.36; H, 7.12. Found: C, 36.66; H, 6.80.

Raffinose undecaacetate (**6**). — After drying over phosphorous pentoxide, **5** (73 mg, 0.15 mmole) was acetylated with acetic anhydride (5 ml) in pyridine (5 ml) overnight. The solution was evaporated and the residue was dissolved in chloroform. The solution was passed through a column of active alumina and evaporated giving a crude product (122 mg, 87%). The product was crystallized from a mixture of ethanol, methanol and water (3:2:5) to give needles (113 mg, 81%), m.p. 99–100°, $[\alpha]_D^{20}$ +92.8° (*c* 5.14, ethanol). The i.r. spectrum was superimposable on that of an authentic sample of raffinose undecaacetate and the mixed m.p. with the sample showed no depression. Lit.[16] m.p. 99–101°, $[\alpha]_D^{20}$ +92° (*c* 8, ethanol). P.m.r. data

($CDCl_3$): δ 1.96 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.04 (s, 6H, 2OAc), 2.09 (s, 6H, 2OAc), 2.10 (s, 6H, 2OAc), 2.11 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.17 (s, 3H, OAc) and 5.68 (d, 1H, *J* 3.5 Hz, H-1, α-Glc).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.68; H, 5.63. Found: C, 49.68; H, 5.44.

Partial hydrolysis of **5**. — A 120-mg portion of **5** was hydrolyzed in 0.1M hydrochloric acid for 10 min at 100° and the solution was neutralized with Amberlite IRA-400 (OH^-). The solution was concentrated and the residue was acetylated with acetic anhydride in pyridine. The product was chromatographed on a silica gel column (45 g, 2.8 × 20 cm) in 1:3 butanone–toluene. Fractions having R_F 0.3 on t.l.c. in the same solvent mixture were combined and evaporated, giving a solid product (70 mg, 43%). The product (65 mg) was deacetylated in 0.1M methanolic sodium methoxide as described for **4**. The product was acetylated again in acetic anhydride with anhydrous sodium acetate for 2.5 h at 100° and the mixture was poured into ice-cold water, which was extracted with chloroform. The chloroform solution was washed with water, dried over sodium sulfate, and evaporated. The residue was recrystallized repeatedly from abs. ethanol to give crystals (31 mg, 23%), m.p. 176–177°, $[\alpha]_D^{20}$ +103° (*c* 2.51, chloroform). The product was identified as β-melibiose octaacetate by i.r. spectral comparison and mixed m.p. with an authentic sample; p.m.r. data ($CDCl_3$): δ 2.00 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.05 (s, 6H, 2OAc), 2.11 (s, 3H, OAc), 2.14 (s, 6H, 2OAc) and 5.76 (d, 1H, *J* 7.5 Hz, H-1, β-Glc).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.73; H, 5.57.

Lit.[17] m.p. 177–178°, $[\alpha]_D^{20}$ +104° (*c* 0.8, chloroform); Lit.[18] m.p. 172–173°, $[\alpha]_D^{20}$ +97.2° (chloroform).

*Tetra-*O*-acetyl-*α*-*D*-galactopyranosyl bromide* (**7**). — This product was prepared by the method of Ohle *et al.*[13].

O-β-D-*Galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside undecaacetate* (**8**). — To a solution of **2** (1.01 g, 1.6 mmole) and **7** (1.01 g, 2.5 mmoles) in dry nitromethane (20 ml), mercuric cyanide (2.00 g, 7.9 mmoles) and "Drierite" (2.00 g) were added. The mixture was stirred for 48 h in the dark with exclusion of moisture at room temperature, and then filtered. The filtrate was evaporated and the residue was dissolved in chloroform. The solution was washed with 5% sodium hydrogen carbonate and water, dried over sodium sulfate, and evaporated. The residue (1.67 g) was acetylated with acetic anhydride in pyridine and the product was chromatographed on a silica gel column (85 g, 2.8 × 30 cm) in 2:3 butanone–toluene. Fractions having R_F 0.56 on t.l.c. in the same solvent mixture were combined and concentrated to give a glassy solid. The product was crystallized from 30% aqueous isopropyl alcohol to give **8** (0.91 g, 59%), m.p. 75–77°, $[\alpha]_D^{20}$ +46.5° (*c* 2.92, chloroform); p.m.r. data ($CDCl_3$): δ 1.97 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.04 (s, 6H, 2OAc), 2.08 (s, 3H, OAc), 2.10 (s, 6H, 2OAc), 2.11 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.17 (s, 3H, OAc), 4.54 (d, 1H, *J* 7.5 Hz, H-1, β-Gal) and 5.77 (d, 1H, *J* 3.5 Hz, H-1, α-Glc).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.68; H, 5.63. Found: C, 49.81; H, 5.46.

O-β-D-Galactopyranosyl-(1→6)-O-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside (**9**). — A 425-mg portion of **8** (0.44 mmole) was deacetylated in 0.1M methanolic sodium methoxide as described for **4**. The crude product (205 mg) was crystallized from ethanol–methanol–water (10:1:1) giving **9** (121 mg, 53%), m.p. 130–134° (hot stage), $[\alpha]_D^{20}$ +55.6° (*c* 0.67, water). Recrystallization from abs. ethanol yielded an analytically pure sample, m.p. 132–135°. The product did not reduce Fehling's solution. P.m.r. data (D_2O): δ 4.44 (d, 1H, *J* 7.5 Hz, H-1, β-Gal) and 5.44 (d, 1H, *J* 3.5 Hz, H-1, α-Glc).

Anal. Calc. for $C_{18}H_{32}O_{16}$: C, 42.86; H, 6.39. Found: C. 42.70; H, 6.54.

Partial hydrolysis of **9**. — A 141-mg portion of **9** was hydrolyzed in 0.1M hydrochloric acid for 10 min at 90° and the solution was neutralized with Amberlite IRA-400 (OH^-). The solution was concentrated and the residue was acetylated with acetic anhydride and anhydrous sodium acetate. The crude product was crystallized from abs. ethanol to give crystals (97 mg, 53%), m.p. 166–167°, $[\alpha]_D^{20}$ +1.3° (*c* 2.71, chloroform). The product was identified as β-allolactose octaacetate[14] by i.r. spectral comparison and mixed m.p. with an authentic sample; p.m.r. data ($CDCl_3$): δ 1.99 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.15 (s, 3H, OAc), 4.55 (d, 1H, *J* 7.4 Hz, H-1, β-Gal) and 5.78 (d, 1H, *J* 7.6 Hz, H-1, β-Glc).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.64; H, 5.54.

β-Allolactose octaacetate. — This product was prepared by the method of Helferich and Sparmberg[14] in a yield of 73%; m.p. 166–167°, $[\alpha]_D^{20}$ +2.1° (*c* 4.10, chloroform). Lit.[14] m.p. 165–166°, $[\alpha]_D$ 0°.

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.54; H, 5.78.

Allolactose. — This product was prepared by the method of Helferich and Sparmberg[14] in 82% yield; m.p. 176–177°, $[\alpha]_D^{20}$ +31.6° (*c* 2.06, water). Lit.[14] m.p. 174–176°, $[\alpha]_D^{18}$ +30.7° (water); lit.[20] $[\alpha]_D$ +25° (c, 2 ,water).

Anal. Calc. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 42.48; H, 6.39.

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