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Note

Partial benzoylation of β-gentiobiose

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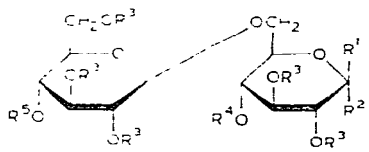
The selective acylations of a series of (1→4)-D-linked oligosaccharides[1–6] and their derivatives[6–10] have been previously investigated. No report has been found, however, on the preferential acylation of oligosaccharides having other glycosidic linkages. This paper describes the partial benzoylation of β-gentiobiose (6-*O*-β-D-glucopyranosyl-β-D-glucopyranose)[11] (**1**) with benzoyl chloride in pyridine.

Reaction of **1** with 8 mol. equiv. of benzoyl chloride in pyridine at −40° gave a mixture of four products (t.l.c.) which was fractionated by column chromatography. The first-eluted component was obtained in crystalline form in 31% yield and identified as β-gentiobiose octabenzoate (**2**) by comparison with an authentic specimen synthesized from **1** with an excess of benzoyl chloride.



1 $R^1 = OH, R^2 = R^3 = R^4 = R^5 = H$
2 $R^1 = OBz, R^2 = H, R^3 = R^4 = R^5 = Bz$
3 $R^1 = OBz, R^2 = R^5 = H, R^3 = R^4 = Bz$
4 $R^1 = R^5 = H, R^2 = OBz, R^3 = R^4 = Bz$
5 $R^1 = OBz, R^2 = H, R^3 = R^4 = Bz, R^5 = Me$
6 $R^1 = H, R^2 = OBz, R^3 = R^4 = Bz, R^5 = Me$
7 $R^1 = OAc, R^2 = H, R^3 = R^4 = Ac, R^5 = Me$
8 $R^1 = OBz, R^2 = R^4 = H, R^3 = R^5 = Bz$
9 $R^1 = OBz, R^2 = H, R^3 = R^5 = Bz, R^4 = Me$
10 $R^1 = OBz, R^2 = H, R^3 = R^5 = Bz, R^4 = Ac$
11 $R^1 = OBz, R^2 = R^4 = R^5 = H, R^3 = Bz$
12 $R^1 = OBz, R^2 = H, R^3 = Bz, R^4 = R^5 = Me$
13 $R^1 = OBz, R^2 = H, R^3 = Bz, R^4 = R^5 = Ac$

The second component eluted from the column moved as a single component on t.l.c. in a variety of solvent systems, and was obtained as an amorphous powder in 26% yield. Elementary analysis showed that it was a hepta-*O*-benzoyl derivative which subsequently was shown to be a 2:1 mixture of 1,2,3,4,2′,3′,6′-hepta-*O*-benzoyl-β- (**3**) and α-gentiobiose (**4**) as follows: The n.m.r. spectrum of the mixture in chloroform-*d* solution exhibited two doublets due to the anomeric protons at τ 3.31 and 3.82 with relative proton intensities of 1:2, thus indicating the presence of a 2:1

mixture of the β and α anomers (**3** and **4**). To determine the position of the free hydroxyl group, the mixture of **3** and **4** was converted into a mixture of the hepta-*O*-benzoyl-mono-*O*-methyl derivatives **5** and **6** by methylation with diazomethane–boron trifluoride etherate[12]. *O*-Debenzoylation of the mixture of **5** and **6**, followed by methanolysis, gave methyl 4-*O*-methyl-α,β-D-glucopyranoside and methyl α,β-D-glucopyranoside, identified by g.l.c. as the *O*-trimethylsilyl derivatives, which indicated that the free hydroxyl group of **3** and **4** was located at C-4 or -4′. *O*-Debenzoylation of the mixture of **5** and **6** and subsequent acetylation gave the known 1,2,3,4,2′,3′,6′-hepta-*O*-acetyl-4′-*O*-methyl-β-gentiobiose[13] (**7**), thus establishing the structure of **3** and **4**.

The third component eluted was obtained in crystalline form in 26% yield. Elementary analysis indicated a heptabenzoyl derivative which proved to be 1,2,3,2′,3′,4′,6′-hepta-*O*-benzoyl-β-gentiobiose (**8**) as follows: On methylation, **8** gave the hepta-*O*-benzoyl-mono-*O*-methyl derivative **9** which was *O*-debenzoylated and methanolyzed to afford methyl 4-*O*-methyl-α,β-D-glucopyranoside and methyl α,β-D-glucopyranoside (g.l.c.). This result, combined with the data for the structural elucidation of the mixture of **3** and **4**, indicated that **8** is the positional isomer of the mixture of **3** and **4** having HO-4′ free. Hence, the free hydroxyl group in **8** was assigned to HO-4. In the n.m.r. spectrum of **8** in chloroform-*d*, the anomeric proton signal appeared at τ 3.90 as a doublet with *J* 8.0 Hz, consistent with the β configuration at C-1. Acetylation of **8** gave the crystalline 4-*O*-acetyl-1,2,3,2′,3′,4′,6′-hepta-*O*-benzoyl derivative **10**.

The fourth component eluted was obtained as an amorphous powder in 12% yield. The structure of 1,2,3,2′,3′,6′-hexa-*O*-benzoyl-β-gentiobiose (**11**) was assigned to this compound on the basis of the results of the elementary analysis, of n.m.r. data, and sequential methylation. This gave the hexa-*O*-benzoyl-di-*O*-methyl derivative **12** which was *O*-debenzoylated and methanolyzed to give methyl 4-*O*-methyl-α,β-D-glucopyranoside as the sole product (g.l.c.). Acetylation of **11** afforded the 4,4′-di-*O*-acetyl-1,2,3,2′,3′,6′-hexa-*O*-benzoyl derivative **13** in crystalline form.

On the basis of the yields of the reaction products, HO-4 and -4′ of **1** have the lowest, similar reactivities. The remarkable inertness of HO-3 towards benzoylation with benzoyl chloride in pyridine in maltose[2], methyl β-lactoside[7], methyl β-cellobioside[6], and β-cyclodextrin[5] is known. It was also shown[6] that HO-3 in β-maltose monohydrate and methyl β-maltoside monohydrate is the least reactive group towards benzoylation, followed by HO-4′. The very slow benzoylation of HO-3 in maltose has been attributed[2] to the high steric hindrance due to the (1→4)-α-D-glycosidic linkage, which causes the close proximity of the two D-glucopyranose rings and a strong intramolecular hydrogen bond between HO-3 and HO-2′. In **1**, which has a (1→6)-D-β-glycosidic linkage, no or at least only little steric interactions exist between the two monosaccharide moieties. The lack of the steric hindrance in **1** may be responsible for the observed reactivity of HO-4 and 4′, lower than that of HO-3, whereas the inverse order was observed for β-maltose monohydrate and methyl β-maltoside monohydrate[6].

EXPERIMENTAL

General methods. — Unless otherwise stated, the general experimental conditions were the same as those described previously[6]. G.l.c. was performed under the same conditions as described previously[14]. N.m.r. spectra were recorded on chloroform-*d* solutions with tetramethylsilane as the internal standard. T.l.c. and column chromatography were performed with 9:1 (v/v) benzene–ethyl acetate as eluent.

1,2,3,4-Tetra-O-benzoyl-6-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-β-D-glucopyranose (**2**). — To a cooled solution of **1** (200 mg) in dry pyridine (8 ml) was added benzoyl chloride (0.8 ml), and the mixture was kept for 2 days at room temperature, and then poured into ice–water. The precipitate formed was filtered off, washed with water, and dried. Crystallization from ethanol–acetone gave **2** (588 mg, 87%), m.p. 189–191°, $[\alpha]_D^{20}$ +22.8° (*c* 1.6, chloroform); n.m.r. τ 3.80 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1).

Anal. Calc. for $C_{68}H_{54}O_{19}$: C, 69.50; H, 4.63. Found: C, 69.72; H, 4.68.

Benzoylation of **1** *with 8 mol. equiv. of benzoyl chloride.* — Benzoyl chloride (10.75 ml, 8 mol. equiv.) was added dropwise over a period of 20 min to a stirred solution of **1** (3.95 g) in anhydrous pyridine (160 ml) at −40°. The reaction mixture was stirred for 1 h at −30°, 2 h at −20°, and 1 h at 0°, and then poured into ice–water. The resulting precipitate was filtered off, washed extensively with water, and dried. T.l.c. showed the presence of four spots having R_F values of 0.82 (**2**), 0.65 (**3** and **4**), 0.52 (**8**), and 0.25 (**11**), respectively. The mixture was fracticnated on a column of Silica gel (400 g). The first fraction gave **2** (4.16 g, 30.7%), m.p. and mixed m.p. 189–191° (ethanol–acetone), $[\alpha]_D^{17}$ +22.0° (*c* 1.0, chloroform).

The second fraction gave a 2:1 mixture of 1,2,3,4-tetra-*O*-benzoyl-6-*O*-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-β- (**3**) and α-D-glucopyranose (**4**) (3.21 g, 26.0%) as an amorphous powder, $[\alpha]_D^{17}$ +54.8° (*c* 1.5, chloroform); n.m.r. τ 3.31 (d, $J_{1,2}$ 3.0 Hz, H-i of **4**) and 3.82 (d, $J_{1,2}$ 8.0 Hz, H-1 of **3**) (ratio of peaks at τ 3.31 and 3.82, 1:2).

Anal. Calc. for $C_{61}H_{50}O_{18}$: C, 68.41; H, 4.71. Found: C, 68.30; H, 4.78.

The third fraction gave 1,2,3-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-β-D-glucopyranose (**8**) (3.22 g, 26.1%), m.p. 223–225° (methanol–acetone), $[\alpha]_D^{17}$ +9.4° (*c* 1.7, chloroform); n.m.r. τ 3.90 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1).

Anal. Calc. for $C_{61}H_{50}O_{18}$: C, 68.41; H, 4.71. Found: C, 68.36; H, 4.62.

The fourth fraction gave 1,2,3-tri-*O*-benzoyl-6-*O*-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-β-D-glucopyranose (**11**) (1.33 g, 11.9%) as an amorphous powder, $[\alpha]_D^{20}$ +22.0° (*c* 1.7, chloroform); n.m.r. τ 3.90 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1).

Anal. Calc. for $C_{54}H_{46}O_{17}$: C, 67.08; H, 4.80. Found: C, 67.20; H, 4.74.

Mixture of 1,2,3,4-tetra-O-benzoyl-6-O-(2,3,6-tri-O-benzoyl-4-O-methyl-β-D-glucopyranosyl)-β- (**5**) *and -α-D-glucopyranose* (**6**). — Diazomethane in dichloromethane was gradually added to a cooled solution of the mixture of **3** and **4** (1.1 g) in dichloromethane (8 ml) containing BF_3 etherate (0.1 ml) until a pale-yellow color persisted, and the mixture was kept for 2 h at room temperature. Polymethylene was

filtered off, and the filtrate was washed successively with aqueous $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated. The residue was fractionated on a column of Silica gel (30 g), and the fraction containing the major product was evaporated to give a 2:1 mixture of **5** and **6** as an amorphous solid (940 mg, 87%), $[\alpha]_D^{20}$ +54.6° (*c* 1.7, chloroform); n.m.r. τ 3.27 (d, $J_{1,2}$ 3.5 Hz, H-1 of **6**), 3.82 (d, $J_{1,2}$ 8.0 Hz, H-1 of **5**) (ratio of peaks at τ 3.27 and 3.82, 1:2), and 6.64 (s, 3 H, OMe).

Anal. Calc. for $C_{62}H_{52}O_{18}$: C, 68.63; H, 4.83. Found: C, 68.80; H, 4.76.

A solution of the mixture of **5** and **6** (100 mg) in dry methanol (5 ml) was treated with methanolic M sodium methoxide (0.1 ml). The solution was kept for 3 h at room temperature, and then neutralized with Amberlite IR-120 (H^+) ion-exchange resin and evaporated to dryness. Methanolysis of the residue (1% methanolic HCl, 5 ml; reflux, 16 h) and g.l.c. of the resulting methyl glycosides as their per-*O*-(trimethylsilyl) derivatives gave peaks corresponding to methyl 4-*O*-methyl-α,β-D-glucopyranoside (8.4 and 9.6 min) and methyl α,β-D-glucopyranoside (13.8 and 15.2 min).

1,2,3,4-Tetra-O-*acetyl*-6-O-(*2,3,6-tri*-O-*acetyl*-4-O-*methyl*-β-D-*glucopyranosyl*)-β-D-*glucopyranose* (**7**). — The mixture of **5** and **6** (300 mg) was *O*-debenzoylated, as just described, and the residue was acetylated with 1:1 (v/v) acetic anhydride–pyridine (1 ml) overnight at room temperature. Isolation in the usual way gave **7** (155 mg, 81%), m.p. 158–159° (ethanol), $[\alpha]_D^{20}$ −10.8° (*c* 0.9, chloroform); lit.[13]: m.p. 159–160°, $[\alpha]_D^{25}$ −10.2°.

1,2,3-Tri-O-*benzoyl*-4-O-*methyl*-6-O-(*2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl*)-β-D-*glucopyranose* (**9**). — Treatment of **8** (1.21 g) with diazomethane–BF_3 etherate in dichloromethane, as just described, afforded **9** (1.06 g, 86%), m.p. 176–177° (ethanol–chloroform), $[\alpha]_D^{20}$ +5.5° (*c* 1.9, chloroform); n.m.r. τ 3.94 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1) and 6.84 (s, 3 H, OMe).

Anal. Calc. for $C_{62}H_{52}O_{18}$: C, 68.63; H, 4.83. Found: C, 68.75; H, 4.76.

O-Debenzoylation of **9** (100 mg), followed by methanolysis as just described, and g.l.c. of the methanolyzate as the per(trimethylsilyl) ethers gave peaks corresponding to methyl 4-*O*-methyl-α,β-D-glucopyranoside and methyl α,β-D-glucopyranoside.

1,2,3-Tri-O-*benzoyl*-4-O-*methyl*-6-O-(*2,3,6-tri*-O-*benzoyl*-4-O-*methyl*-β-D-*glucopyranosyl*)-β-D-*glucopyranose* (**12**). — Methylation of **11** (920 mg), as just described, gave **12** (840 mg, 89%), m.p. 205–206° (ethanol), $[\alpha]_D^{18}$ +13.2° (*c* 1.4, chloroform); n.m.r. τ 3.97 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 6.60, and 6.85 (s, 6 H, 2 OMe).

Anal. Calc. for $C_{56}H_{50}O_{17}$: C, 67.60; H, 5.07. Found: C, 67.48; H, 5.13.

After sequential *O*-debenzoylation of **12**, methanolysis, and trimethylsilylation, g.l.c. examination showed the presence of methyl 4-*O*-methyl-α,β-D-glucopyranoside as the sole product.

4-O-*Acetyl*-*1,2,3-tri*-O-*benzoyl*-6-O-(*2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl*)-β-D-*glucopyranose* (**10**) *and* 4-O-*acetyl*-6-O-(4-O-*acetyl*-*2,3,6-tri*-O-*benzoyl*-β-D-*glucopyranosyl*)-*1,2,3-tri*-O-*benzoyl*-β-D-*glucopyranose* (**13**). — Conventional acetylation of **8** (150 mg) with 1:1 (v/v) acetic anhydride–pyridine (3 ml) gave **10** (134 mg, 86%),

m.p. 191–192° (from ethanol–chloroform), $[\alpha]_D^{20}$ +52.2° (*c* 1.6, chloroform); n.m.r. τ 3.89 (d, 3 H, $J_{1,2}$ 7.5 Hz, H-1) and 8.12 (s, 3 H, OAc).

Anal. Calc. for $C_{63}H_{52}O_{19}$: C, 67.98; H, 4.71. Found: C, 67.80; H, 4.88.

Acetylation of **11** (150 mg) afforded **13** (145 mg, 89%), m.p. 218–219° (ethanol–chloroform); n.m.r. τ 3.81 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 8.08, and 8.13 (s, 6 H, 2 OAc).

Anal. Calc. for $C_{58}H_{50}O_{19}$: C, 66.28; H, 4.80. Found: C, 66.20; H, 4.91.

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