Carbohydrate Research, 26 (1973) 351–356

PREPARATION AND STRUCTURE OF 1,2,6,2′,3′,4′,6′-HEPTA-*O*-BENZOYL-β-LACTOSE AND 6-*O*-BENZOYLLACTOSE*

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(Received June 12th, 1972; accepted in revised form, October 13th, 1972)

ABSTRACT

1,2,6,2′,3′,4′,6′-Hepta-*O*-benzoyl-β-lactose (**1**) was prepared and its structure ascertained. Ammonolysis gave 6-*O*-benzoyllactose and lactose. The stability of the 6-*O*-benzoyl group was investigated and is discussed.

INTRODUCTION

The first benzoylations of disaccharides[1–3] were performed with benzoyl chloride in a 20% sodium hydroxide solution. They gave mixtures of partially benzoylated compounds that were difficult to separate by crystallization. Benzoylation with benzoyl chloride in pyridine gave octa-*O*-benzoylated disaccharides[4–6] with the exception of maltose which gave a mixture of octa-*O*-benzoyl-β-maltose and 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-β-maltose[7].

Skraup[1] and Kueney[2] have described a hexa-*O*-benzoyllactose, and Panormow[3] a hepta-*O*-benzoyllactose. As we were interested in obtaining partially blocked disaccharide derivatives, of interest as starting materials, we benzoylated lactose with benzoyl chloride in 20% sodium hydroxide solution. From this reaction, 24% of a crystalline hepta-*O*-benzoyllactose (**1**) was obtained; however, the melting point differs from that of the heptabenzoate previously described[3].

To determine the position of the free hydroxyl group, compound **1** was methylated several times with diazomethane–boron trifluoride etherate[7–9], to give hepta-*O*-benzoyl-*O*-methyllactose (**2**). Debenzoylation and hydrolysis of **2** gave D-galactose and 3-*O*-methyl-D-glucose.

Further benzoylation of **1** with benzoyl chloride–pyridine gave an octa-*O*-benzoyllactose (**3**), to which the β configuration was assigned on the basis of the optical rotation and n.m.r. data, benzoyl derivatives having the α-D-glucopyranosyl structure show an H-1 eq signal at τ 3.00–3.50, whereas those having the β-D-glucopyranosyl structure show no signal in this region and the signal for H-1 ax appears at approximately τ 3.65, superimposed over the signals of the other protons[10]. Therefore, **1** has the β configuration, in agreement with its n.m.r. data, and is 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-β-lactose, and **2** is 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-3-*O*-methyl-β-lactose.

*Dedicated to Professor V. Deulofeu, in honor of his 70th birthday.
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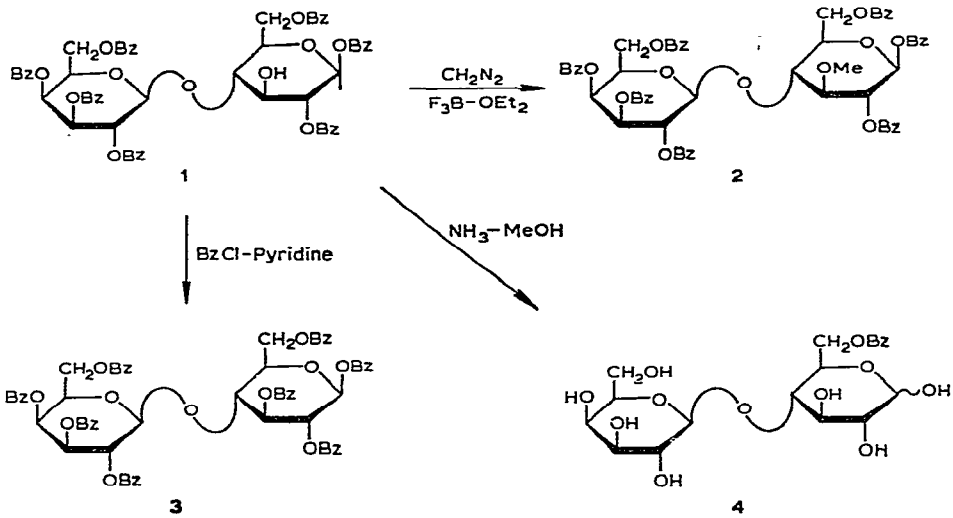
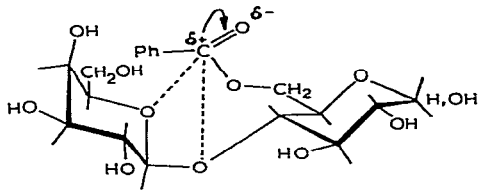
Treatment of **1** with methanolic ammonia gave both lactose (75.1%) and 6-*O*-benzoyl-lactose (**4**) (21.5%), and no nitrogenated compounds could be detected. Similar results had been observed with 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-β-maltose[7],



which is an additional proof that no benzoyl group is present at O-3; a benzoyl group at this position being required for the migration reaction that leads to the formation of nitrogenous compounds. This has been shown[11,12] for the ammonolysis of penta-*O*-benzoylhexoses labeled with benzoyl-*1*-^{14}C in which the substituents at O-3 and O-4 contribute most to the formation of the *N*-benzoyl derivatives at C-1. From the results of the ammonolysis of compound **1** and 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-β-maltose, we can assume that the benzoyl groups at O-2 and O-6 of the reducing moiety and those of the nonreducing moiety are not essential in the formation of *N*-benzoyl derivatives at C-1.

The structure of the mono-*O*-benzoyllactose isolated from the ammonolysis of compound **1** was demonstrated by periodate oxidation, controlled acid hydrolysis, and chromatographic separation of D-galactose and 6-*O*-benzoyl-D-glucose.

A comparison of the yields of 6-*O*-benzoyl disaccharides, prepared by ammonolysis of hepta-*O*-benzoyl disaccharides, showed that maltose gave 40% of 6-*O*-benzoylmaltose, while 21.5% of 6-*O*-benzoyllactose was isolated from lactose. The formation of a 6-benzoyl derivative could be explained for maltose by a sterically more favored position, due to the α glycosidic linkage, which stabilize the 6-*O*-benzoyl group by interactions with the three oxygen atoms[7]. Examination of the molecular model suggests the same interactions for 6-*O*-benzoyllactose (**4**), but the β glycosidic linkage allows only interaction with two oxygen atoms (Scheme 1), and therefore the benzoyl group is less stable and the yield of the 6-*O*-benzoyl disaccharide decreases.



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Scheme 1

The stability of the benzoyl group gives a qualitative idea of these possible interactions. 6-*O*-Benzoyllactose required 4 days in methanolic ammonia for the elimination of the benzoyl group, whereas 6-*O*-benzoyllactitol required only 4 h. This suggests that the disappearance of one of the pyranose rings decreases the stabilization of the benzoyl group and, consequently, a stabilizing effect of the ring oxygen of the reducing moiety; although it is not possible to visualize this effect in molecular models. These results agree qualitatively with those obtained in the same reaction of 6-*O*-benzoylcellobiose (for 7 days) and 6-*O*-benzoylcellobiitol (for 6 h) and show that, in cellobiose and lactose, these interactions are less favored than in maltose, where the benzoyl group is eliminated only after 15 days, and in 6-*O*-benzoylmaltitol, where it is eliminated within 2 days. In 6-*O*-benzoyl-D-glucose, where no such interactions can be postulated, the 6-*O*-benzoyl group is split off within 2 h and the same lenght of time was observed for 6-*O*-benzoyl-D-glucitol[6].

Benzoylation of lactose with benzoyl chloride in pyridine at 60° gave octa-*O*-benzoyllactose in 90% yield[10], whereas maltose, under the same conditions, gave 54% of 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-β-maltose and 46% of octa-*O*-benzoyl-β-maltose[7]. In maltose, this result may be explained by the high steric hindrance due to the α-glycosidic linkage, which causes a close proximity of the two moieties of the molecule and hydrogen bonding between the hydroxyl groups at C-3 and C-2′, thus, the hydroxyl group at C-3 is specially hindered for benzoylation. In lactose, which has a β-glycosidic linkage, no or at least only small interactions exist and the octabenzoate is obtained in good yield. Only after the reaction conditions were changed, the temperature being decreased to 0° and a 20% sodium hydroxide solution being used, was a syrup obtained from which 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-β-lactose crystallized in 24% yield. The mother liquors contain some octa-*O*-benzoyllactose and other products, which were assumed to be partially benzoylated compounds on the basis of the chromatographic behavior. Their study is in progress.

EXPERIMENTAL

General procedures. — Chromatography was performed on Whatman No. 1 and 3MM papers and on cellulose columns with 5:2:2 butyl alcohol–ethanol–water as developing solvent. The spray reagents used were (a) silver nitrate–sodium methox-

ide[13], (b) aniline hydrogen phthalate[14], and (c) permanganate–periodate[15]. Thin-layer chromatography was performed on Silica Gel G (Merck) plates with 19:1 benzene–ethyl acetate as eluent and iodine vapor for detection. Melting points are not corrected. The n.m.r. spectra were measured on a Varian A-60 spectrometer in chloroform-*d* with tetramethylsilane as internal standard.

*1,2,6,2',3',4',6'-Hepta-*O*-benzoyl-β-lactose* (**1**). — α-Lactose hydrate (10 g) was dissolved in an ice-cooled 20% sodium hydroxide solution (480 ml), and benzoyl chloride (55 ml) was added portionwise with good shaking. The mixture was stirred for 1 h and the whole mass solidified. It was kept for 1 h at room temperature, water was added, and the solid was filtered off and washed until neutral. The solid was extracted with boiling methanol (700 ml) and the residual syrup dissolved in 1:1 chloroform–methanol to give crystals. After three crystallizations, 7.52 g (24%) was obtained as needles, m.p. 196–197°, $[\alpha]_D^{20}$ +71.2° (*c* 1.2, chloroform); the n.m.r. spectra show no signals at τ 3.00–3.50.

Anal. Calc. for $C_{61}H_{50}O_{18}$: C, 68.31; H, 4.70. Found: C, 68.10; H, 4.69.

*Octa-*O*-benzoyl-β-lactose* (**2**). — Compound **1** (2 g) was dissolved in dry pyridine (5 ml) and benzoyl chloride (1.5 ml) was added. The reaction mixture was heated for 2 h at 50° and for 30 min at 90°, and then cooled and dissolved in chloroform. The solution was washed with cold 0.5M sulfuric acid, saturated sodium hydrogen carbonate solution, and water, dried over anhyd. sodium sulfate, and evaporated to dryness. From 2:1 acetone–methanol **2** (2.05 g, 93%) was obtained as rectangular prisms, m.p. 140–142°, $[\alpha]_D^{20}$ +38.1° (*c* 0.9, chloroform); the n.m.r. spectra show no signals at τ 3.00–3.50.

Anal. Calc. for $C_{68}H_{54}O_{19}$: C, 69.48; H, 4.64. Found: C, 69.20; H, 4.73.

*1,2,6,2',3',4',6'-Hepta-*O*-benzoyl-3-*O*-methyl-β-lactose* (**3**). — Compound **1** (3 g) was methylated seven times with diazomethane (prepared each time from 16 g of 1-methyl-1-nitrosourea) and boron trifluoride etherate[7–9] in chloroform to give **3** in chromatographically pure form. Crystallization from 4:1 chloroform–methanol gave 2.60 g (86%) of needles, m.p. 203–204°, $[\alpha]_D^{20}$ +52.9° (*c* 0.9, chloroform); the n.m.r. spectra showed the *O*-methyl signal at τ 6.31 and no signals at τ 3.00–3.50.

Anal. Calc. for $C_{62}H_{52}O_{18}$: C, 68.63; H, 4.79; OMe, 2.9. Found: C, 68.69; H, 4.73; OMe, 2.8.

Debenzoylation and hydrolysis of **3**. — Compound **3** (860 mg) was debenzoylated with sodium methoxide in methanol. After 20 h the solution was neutralized with Amberlite IR-120 resin, evaporated to dryness, and hydrolyzed with 0.25M hydrochloric acid (10 ml) during 4 h at 100°. The solution was neutralized with De-Acidite G resin and evaporated. The residue was fractionated by chromatography on Whatman 3MM paper to give D-galactose (133 mg), $[\alpha]_D^{20}$ +79.5°(at equil., *c* 1, water), lit.[16]: $[\alpha]_D$ +80.5° (equil., water); and 3-*O*-methyl-D-glucose (81 mg), $[\alpha]_D^{20}$ +55.6° (equil., *c* 0.9, water), lit.[17]: $[\alpha]_D$ +55.5° (equil., water). 3-*O*-Methyl-D-glucose was characterized by the osazone, m.p. 171–173°, $[\alpha]_D^{20}$ −42.1° (equil., *c* 0.2, ethanol); lit.[17]: $[\alpha]_D$ −41.6 (equil., ethanol).

*6-*O*-Benzoyllactose* (**4**). — Compound **1** (20 g) was dissolved by agitation in

16% methanolic ammonia (500 ml). The solution was kept for 24 h at room temperature, evaporated to dryness, and extracted with ethyl acetate. The residue was dissolved in hot water, and ethanol added until a 80% ethanolic solution was obtained, which was kept at room temperature. The crystallized lactose was filtered off, the mother liquors were evaporated, and the residue was treated as just described. This treatment, repeated several times, gave 3.37 g of lactose, m.p. 219–220°, $[\alpha]_D^{20}$ +55.4° (equil., *c* 1.2, water); lit.[16]: $[\alpha]_D$ +55.3° (equil., water). The remaining solution was evaporated and the residue chromatographed on a column (4 × 70 cm) of Whatman CF 11 cellulose with fractions of 15 ml each. Fractions 1–48 and 81–300 gave, after evaporation, 1.44 g of lactose (total yield 75.1%). Fractions 53–80 gave 1.79 g of **4** (21.5%), which precipitated from 2-propanol as an amorphous solid, and showed no sharp melting point; $[\alpha]_D^{20}$ +55.6° (equil., *c* 0.9, methanol). On paper chromatogram, it showed only one reducing spot, R_F 0.34, $R_{Lactose}$ 8.9.

Anal. Calc. for $C_{19}H_{26}O_{12}$: C, 51.12; H, 5.87. Found: C, 51.17; H, 6.09.

Periodate oxidation of **4**. — Compound **4** (3.0 mg) was dissolved in a 17mM sodium periodate solution (4 ml). Samples were taken at 1-h intervals and the periodate uptake measured with a Beckman DU spectrophotometer at 222.5 μm[18]: 4 moles of periodate were consumed and no formaldehyde was detected[19]. Over-oxidation was observed after 48 h.

Hydrolysis of 6-O-benzoyllactose (**4**). — Compound **4** (182 mg) was hydrolyzed with 0.25M hydrochloric acid (5 ml) during 4 h at 100°. The solution was neutralized with De-acidite G resin, evaporated, and chromatographed on Whatman 3MM paper. D-Galactose (40 mg), $[\alpha]_D^{20}$ +80.2° (equil., *c* 1.1, water) and 6-*O*-benzoyl-D-glucose (32 mg) were obtained. The latter compound was identified by paper chromatography, $R_{2,3,4,6\text{-tetra-}O\text{-methyl-D-glucose}}$ 0.84 in 5:1:4 butyl alcohol–ethanol–water (lit.[20]: R_{TMG} 0.82), and $[\alpha]_D^{21}$ +45.7° (equil., *c* 0.9, ethanol) [lit.[21]: $[\alpha]_D$ +48° (ethanol)].

6-O-Benzoyllactitol. — Compound **4** (204 mg) was dissolved in water (3 ml) and reduced with sodium borohydride (33.6 mg) in water (1.7 ml) for 3 h. Amberlite IR-120 resin was added to destroy the excess of reagent and to remove the sodium ions. The solution was filtered and evaporated. The residue was dried by several additions and evaporations of methanol to give a syrup (194 mg, 97%), $[\alpha]_D^{20}$ +28.7° (*c* 0.9, water).

Anal. Calc. for $C_{19}H_{28}O_{12}$: C, 50.89; H, 6.29. Found: C, 51.10; H, 6.60.

Ammonolysis of 6-O-benzoyllactose (**4**) *and 6-O-benzoyllactitol* (**5**). — A sample of the 6-*O*-benzoyl derivatives (10 mg) was dissolved in methanolic ammonia (3 ml) and the reaction was controlled by paper chromatography. The spot corresponding to **4** disappeared after 4 days, and that corresponding to **5** after 4 h.

ACKNOWLEDGMENTS

We thank the Consejo Nacional de Investigaciones Científicas y Téchnicas of Argentina and the University of Buenos Aires for partial financial support and the Instituto Nacional de Farmacología y Bromatologia for a scholarship (I. M. V.).

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