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THE REACTION OF AMMONIA WITH ACYLATED DISACCHARIDES

PART X. OCTA-*O*-BENZOYL-LACTOSE AND OTHER BENZOYL DERIVATIVES OF LACTOSE

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ABSTRACT

From the reaction of octa-*O*-benzoyl-lactose (**1**) with methanolic ammonia, lactose, 1,1-bis(benzamido)-1-deoxy-4-*O*-β-D-galactopyranosyl-D-glucitol (**2**), and *N*-benzoyl-4-*O*-β-D-galactopyranosyl-D-glucopyranosylamine (**3**) were obtained. The behavior of some other octabenzoylated disaccharides in the ammonolysis reaction is discussed.

INTRODUCTION

Benzoylation of lactose under conditions that give octabenzoylated disaccharides[1–3] led to the formation of a mixture (**1**) of 66% of octa-*O*-benzoyl-α-lactose and 34% of octa-*O*-benzoyl-β-lactose.

Mixture **1** was treated with methanolic ammonia, and gave lactose (82%), 1,1-bis(benzamido)-1-deoxy-4-*O*-β-D-galactopyranosyl-D-glucitol (**2**, 6.7%), and a mixture that, by paper chromatography, showed two overlapping zones that suggested the probable presence of a mono-*O*-benzoyl-lactose and *N*-benzoyl-4-*O*-β-D-galactopyranosyl-D-glucopyranosylamine (**3**)**. Isolation of **3** (0.7%) was only possible after treatment of the mixture with sodium methoxide, followed by chromatographic separation.

The formation of 6-*O*-benzoylated disaccharides had been observed in the ammonolysis of octa-*O*-benzoyl-β-cellobiose[2], octa-*O*-benzoyl-β-maltose[3], 1,2,6,-2′,3′,4′,6′-hepta-*O*-benzoyl-β-maltose[4], and 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-β-lactose[5].

The formation of compounds nitrogenated on C-1 by migration of benzoyl groups is a reaction competitive with that of elimination of these groups by ammonolysis or by transesterification[6] with the methanol used as the solvent. Table I shows

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Compound **2 was characterized by acetylation to its octaacetate (**4**), and **3** to its heptaacetate (**5**).

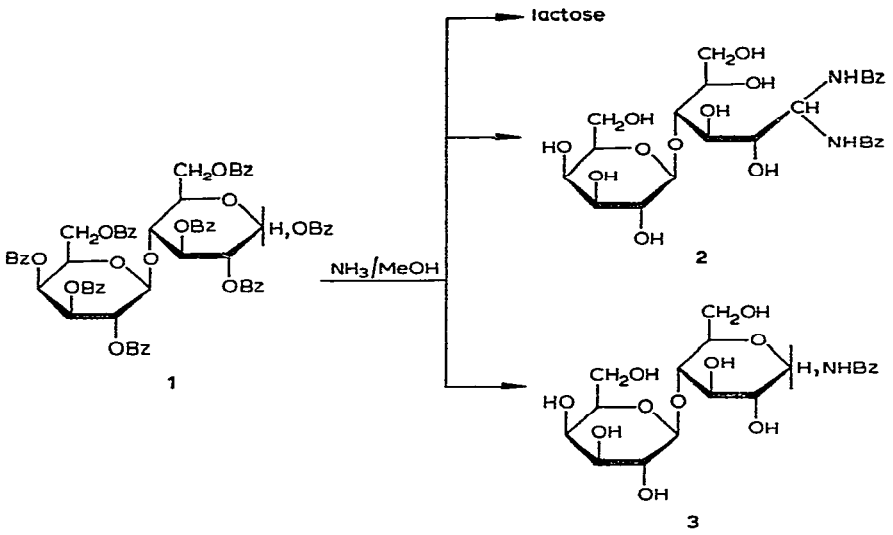


TABLE I

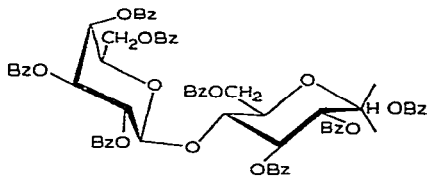
YIELDS (%) OF COMPOUNDS OBTAINED BY AMMONOLYSIS OF OCTA-*O*-BENZOYLATED DISACCHARIDES

Compound ammonolyzed	*Product*[a]		
	A	*B*	*C*
	Yield (%)		
Octa-*O*-benzoyl-α,β-lactose (**1**)	6.7	0.74	[b]
Octa-*O*-benzoyl-β-cellobiose (**6**)	7.8	0.92	6.6
Octa-*O*-benzoyl-β-maltose (**7**)	20.8	0 1	31.6

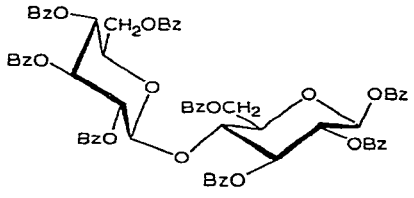
[a]A. 1,1-bis(benzamido)-1-deoxy-4-*O*-β-D-glycopyranosyl-D-glucitol; B. *N*-benzoyl-4-*O*-β-D-glycopyranosyl-D-glucopyranosylamine; and C. 6-*O*-benzoyl disaccharide. [b]Not isolated.

the yields of nitrogenated compounds formed by ammonolysis of two octa-*O*-benzoylated disaccharides having a β-D-(1→4) glycosidic linkage, and of octa-*O*-benzoyl-β-maltose (**7**) [which has an α-D-(1→4) glycosidic linkage]. The similar yields of nitrogenated products in the ammonolysis of octa-*O*-benzoyl-lactose (**1**) and octa-*O*-benzoylcellobiose[2] (**6**) may be attributable to a similar steric situation arising from the β-D-glycosidic linkage for these two compounds, as shown in **1a** and **6**.

The relative contribution of substituents on each carbon atom to the formation of 1,1-bis(benzamido)-1-deoxyalditols has been determined for penta-*O*-benzoyl-hexoses[6,7] by means of benzoyl groups labeled with carbonyl-^{14}C groups; these results were discussed for acylated monosaccharides[8]. Based on them, it may be assumed that the benzoyl group on O-3 should participate strongly in the formation of products having nitrogen on C-1. The 3-hydroxyl group, liberated by the migration,



1a



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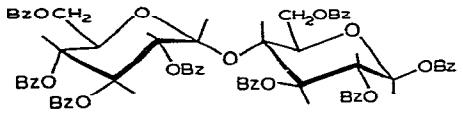
may be an intermediate step in the intramolecular migration of a second benzoyl group, giving rise to the bis(benzamido) derivatives. Previous studies on benzoylated hexoses[6,7] demonstrated that the substituents on O-2 and O-6 make little contribution to this migration. For the disaccharides having (1→4)-glycosidic linkage, these considerations give great relevance to the C-3 position, both for the migration of the 3-*O*-benzoyl group to the nitrogen atom on C-1 and as regards the possible participation of the free hydroxyl group as an intermediate step in the migration of other benzoyl groups. The importance of the 3-*O*-benzoyl group is confirmed by the fact that, in the ammonolysis of 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-β-maltose[4] and 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-β-lactose[5], compounds nitrogenated on C-1 could not be detected. In both heptabenzoates, the 3-OH group has been shown to be relatively resistant to further benzoylation and to methylation[4,5]; this may be attributable to steric hindrance caused by crowding of the benzoyl groups, or to some other interaction (*e.g.*, hydrogen bonding) that makes this group less available for these reactions. The same kind of situation could make this hydroxyl group unavailable as an intermediate step in the migration reaction.

In the three disaccharides studied, the reducing moiety is the same, and the orthoester intermediate postulated for the migration between C-3 and the nitrogen atom on C-1 should also be the same. The difference in yields arises from steric factors, in the molecules, that could hinder the ammonolysis of the benzoyl groups to give benzamide and, in contrast, favor their migration. These steric factors also stabilize the 6-*O*-benzoyl group, which, especially in 6-*O*-benzoyl-maltose, is relatively resistant to attack by ammonia[3,5].

Inspection of molecular models suggested that the reaction of ammonia with the benzoyl groups to give benzamide is less favored in octa-*O*-benzoyl-β-maltose (**7**) owing to the *endo* situation of these groups that hinders the approach of ammonia to the reaction centers. Octa-*O*-benzoyl-lactose (**1a**) and octa-*O*-benzoyl-β-cellobiose (**6**)



7

have some of their benzoyl groups more peripheral, and these are open to an *exo* attack by ammonia. In the three cases, the benzoyl group on O-1 must be eliminated first, giving a free hydroxyl group on C-1. In **7**, after liberation of the 1-hydroxyl group, the crowding of the benzoyl groups could favor the opening of the pyranose ring of the reducing moiety, and its C-1 carbonyl group would then be attacked fast by ammonia, allowing facile migration of the other benzoyl groups. In **1a** and **6**, ammonia could readily eliminate most of the benzoyl groups as benzamide, by an *exo* attack. At the stage in which C-1 has the amino group attached and ready to combine with the migrating groups, only few would be available, and so the yields of nitrogenated sugar derivatives would be low, compared to those from compound **7**. As previously postulated[3,5], the 6-*O*-benzoyl groups would be more resistant to attack by ammonia, owing to the stabilizing interactions of this group with the ring and with the glycosidic oxygen atoms.

EXPERIMENTAL

General procedures. — A methanolic solution of ammonia (16%) was used. Chromatography, on Whatman No. 1 and 3MM papers, and on cellulose columns, was conducted with 5:2:2 butyl alcohol–ethanol–water as the developing solvent. The spray reagents used were (*a*) silver nitrate–sodium methoxide[9] and (*b*) aniline hydrogen phthalate[10]. Thin-layer chromatography was performed on plates of Silica Gel (Merck, Germany), with 19:1 benzene–ethyl acetate as the eluant, and iodine vapor for detection. Melting points are not corrected. The optical rotations were determined at 22°. N.m.r. spectra were recorded with a Varian A-60 spectrometer, with tetramethylsilane as the internal standard.

The octa-O-benzoyl-lactoses. — Lactose (50 g) was suspended in dry pyridine (500 ml), and benzoyl chloride (170 ml) was added portionwise, the mixture being shaken vigorously and kept in a water bath at 15°. After 1 h at room temperature, it was heated for 4 h at 60° and for 15 min at 100°. The solution was cooled and poured into ice–water, and the syrup obtained was washed by decantation until a powdery solid was obtained. The yield was 145.5 g (90%) of an amorphous substance, m.p. 110–120°, that, by reprecipitation from 1:3 acetone–methanol gave 136 g of octa-*O*-benzoyl-α,β-lactose (**1**); m.p. 119–120°; $[\alpha]_D$ +88.0° (*c* 1.0, chloroform); it showed two spots on t.l.c. It could not be further purified by reprecipitation or by column chromatography.

Anal. Calc. for $C_{68}H_{54}O_{19}$: C, 69.48; H, 4.64. Found: C, 69.50; H, 5.05.

The anomeric mixture was resolved by preparative t.l.c. and gave the following. (*a*) Octa-*O*-benzoyl-α-lactose (66%) was obtained as an amorphous substance having m.p. 124–126°, $[\alpha]_D$ +113° (*c* 0.8, chloroform). N m.r. data (chloroform-*d*): a doublet at τ 3.22, $J_{1,2}$ 3.5 Hz; this signal corresponds to H-1 of the α-anomeric benzoates[11].

Anal. Calc. for $C_{68}H_{54}O_{19}$: C, 69.48; H, 4.64. Found: C, 69.66; H, 5.00.

(*b*) Octa-*O*-benzoyl-β-lactose (34%) crystallized as needles, m.p. 140–142°, $[\alpha]_D$ +36.7° (*c* 0.8, chloroform) (lit.[5] m.p. 140–142°). N.m.r. data (chloroform-*d*) showed no signal between τ 3.00 and 3.50; this corresponds to the β-anomeric benzoate[11].

Reaction of **1** *with methanolic ammonia.* — (*A*) *Isolation of lactose.* Mixture **1** (40 g) was suspended in methanolic ammonia (1 liter), and dissolved by shaking during 5 h. After being kept for 24 h at room temperature, the solution was evaporated to dryness, and the residue was extracted with ethyl acetate (10 × 50 ml); then it was dissolved in methanol, and, from this solution, lactose crystallized. It was filtered off, the filtrate was evaporated to dryness, the solid was extracted several times with ethyl acetate, and the residue was dissolved in methanol, from which a further amount of lactose crystallized (8.4 g). Recrystallization from methanol gave needles having m.p. 220–222°, $[\alpha]_D$ +58.9° (equil.; *c* 1.9, water), (lit.[12] $[\alpha]_D$ +55.3°).

(*B*) *Isolation of 1,1-bis(benzamido)-1-deoxy-4-*O*-β-*D*-galactopyranosyl-*D*-glucitol* (**2**). The residual syrup was chromatographed on a column (97 × 4.8 cm) of Whatman CF-11 cellulose, and 400 fractions (15 ml each) were collected. Fractions 1–20 gave benzamide on evaporation; fractions 31–105 gave 0.83 g of **2**; and fractions 110–200 gave a mixture of nitrogenated compounds which were separated on Whatman 3MM paper, to afford 0.46 g of **2** (total yield 6.7%). By recrystallization from 1:1 methanol–ethyl acetate, needles were obtained; m.p. 196–198°, $[\alpha]_D$ −20.9° (*c* 0.9, water); R_F 0.53.

Anal. Calc. for $C_{26}H_{34}N_2O_{12}$: C, 55.12; H, 6.05; N, 4.94. Found: C, 55.34; H, 5.88; N, 4.56.

Fractions 210–400 gave 1.0 g. of lactose (total yield 82%).

(*C*) *Isolation of* N*-benzoyl-4-*O*-β-*D*-galactopyranosyl-*D*-glucopyranosylamine* (**3**). From the chromatography (on 3MM paper) of fractions 110–200, another nitrogenated fraction was obtained (0.38 g) that was treated with sodium methoxide in methanol. After 24 h, the base was neutralized with Amberlite IR-120 (H^+) ion-exchange resin, the solution was evaporated to dryness, and the residue was chromatographed on Whatman 3MM paper. Lactose (0.19 g) and compound **3** (0.12 g; 0.74%) were obtained. Compound **3** gave a hygroscopic, amorphous powder from 1:1 methanol–ethyl acetate, $[\alpha]_D$ +63.3° (*c* 0.9, water); R_F 0.37.

Anal. Calc. for $C_{19}H_{27}NO_{11}$: N, 3.14. Found: N, 3.43.

Periodate oxidation of compounds **2** *and* **3**. — Periodate oxidation by the micromethod described[13] gave, for (*a*) compound **2**: uptake of 4 moles of periodate per mole and formation of 0.9 mole of formaldehyde; oxidation was complete after

3 h, and overoxidation began after 10 h. (*b*) Compound 3: uptake of 3 moles of periodate per mole; no formaldehyde could be detected; oxidation was complete after 24 h, and, after 50 h, overoxidation was observed.

Octa-O-acetyl-1,1-bis(benzamido)-1-deoxy-4-O-β-D-galactopyranosyl-D-glucitol (**4**). — Compound **2** (180 mg) was acetylated with 1:1 acetic anhydride–pyridine (6 ml); the mixture was kept for 24 h at room temperature, and then heated for 30 min at 60°, and evaporated to dryness in a vacuum desiccator (sulfuric acid and sodium hydroxide). Recrystallization from ethanol gave 0.26 g (96%) of **4** as needles, which, after three recrystallizations from benzene, had m.p. 178–180°, $[\alpha]_D$ −12.5° (*c* 0.8, chloroform).

Anal. Calc. for $C_{42}H_{50}N_2O_{20}$: C, 55.87; H, 5.58; N, 3.10. Found: C, 55.98; H, 5.56; N, 3.40.

Hepta-O-acetyl-N-benzoyl-4-O-β-D-galactopyranosyl-D-glucopyranosylamine (**5**). — Compound **3** (100 mg) was dissolved in 1:1 acetic anhydride–pyridine (10 ml), and kept for 10 h at room temperature. The solution was heated for 30 min at 60°, and evaporated to dryness. After purification from methanol–water, compound **5** was obtained in 40% yield as an amorphous material; m.p. 108–110°, $[\alpha]_D$ +47° (*c* 0.8, chloroform).

Anal. Calc. for $C_{33}H_{41}NO_{18}$: C, 53.58; H, 5.58; N, 1.89. Found: C, 53.23; H, 5.72; N, 2.13.

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