The Reaction of Ammonia with Acylated Disaccharides. VI. Octa-O-benzoylcellobiose and Benzoyl Derivatives of Cellobiose

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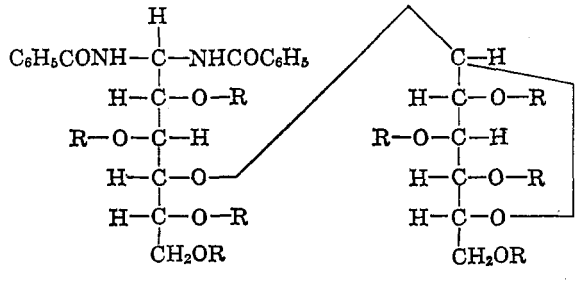
Received March 23, 1965

β-Octa-O-benzoylcellobiose (I) was prepared and, from its reaction with methanolic ammonia, cellobiose (II), 4-O-β-D-glucopyranosyl-1,1-bis(benzamido)-1-deoxy-D-glucitol (IIIa), N-benzoylcellobiosylamine (IVa), and 6-O-benzoylcellobiose (V) were obtained. The structure of compounds IIIa and V were established.

The reaction of methanolic ammonia with acetylated disaccharides was described in previous papers of this series.[2,3] We have now extended this reaction to disaccharide benzoates assuming that the yields of 1,1-bis(benzamido)-1-deoxyaldobiitols would be higher than the yields of 1,1-bis(acetamido)-1-deoxyaldobiitols obtained from the disaccharide acetates, because the greater resistance of benzoyl groups to ammonolysis should allow them to participate more extensively in the ortho ester mechanism which leads to this type of compound.[3a]

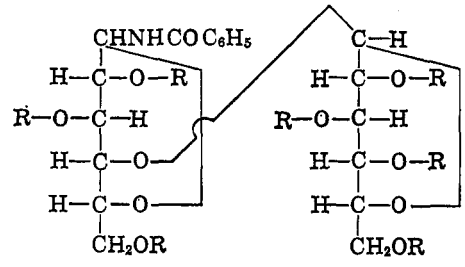
In the present paper we describe the results of the reaction of methanolic ammonia with β-octa-O-benzoylcellobiose. The benzoates of cellobiose reported in the literature are a hepta-O-benzoyl and a penta-O-benzoylcellobiose, obtained by Hintikka[4] by benzoylation of cellobiose with benzoyl chloride and sodium hydroxide. By benzoylation of cellobiose with benzoyl chloride in pyridine, we obtained β-octa-O-benzoylcellobiose (I) of m.p. 188–191°, $[\alpha]^{26}D$ +37°, which was found to be homogeneous by thin layer chromatography on silicic acid–starch plates. This octabenzoate was ammonolyzed with 16% methanolic ammonia under the same experimental conditions described for disaccharide acetates.[2,3] By repeated chromatography on cellulose columns we obtained 49.6% of cellobiose (II), 7.8% of 4-O-β-D-glucopyranosyl-1,1-bis(benzamido)-1-deoxy-D-glucitol (IIIa), 0.92% of N-benzoylcellobiosylamine (IVa), and 6.6% of 6-O-benzoylcellobiose (V).

Octa-O-acetyl-4-O-β-D-glucopyranosyl-1,1-bis(benzamido)-1-deoxy-D-glucitol (IIIb) was obtained by the acetylation of 4-O-β-D-glucopyranosyl-1,1-bis(benzamido)-1-deoxy-D-glucitol (IIIa). The periodate oxidation of the latter required 3.9 moles of sodium metaperiodate and yielded 1 mole of formaldehyde. These results agree with an open-chain structure of the nitrogenated moiety, as represented by IIIa. This was also supported by the production of an octa-O-acetyl derivative and by the analogous structures for the N-acetyl derivatives demonstrated by Deferrari and Cadenas.[5]



IIIa, R = H
b, R = CH_3CO

The N-benzoylcellobiosylamine must have the pyranose structure IVa, since the hydroxyl group at carbon 4 is blocked by the glycosidic linkage and acetylation of this substance affords a hepta-O-acetyl-N-benzoylcellobiosylamine (IVb).



IVa, R = H
b, R = CH_3CO

The structure of 6-O-benzoylcellobiose (V) was demonstrated in the following ways: by benzoylation it produced α-octa-O-benzoylcellobiose; by methylation using the method of Kuhn, Baer, and Seeliger[6] with methyl iodide in dimethylformamide and barium oxide, followed by elimination of the O-benzoyl group with sodium methoxide and hydrolysis of the glycosidic linkage with 1 N sulfuric acid, it afforded 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose, and 2,3-di-O-methyl-D-glucose. The nearly quantitative yield of 2,3,4,6-tetra-O-methyl-D-glucose showed that the O-benzoyl group must have been present in the reducing moiety of the methylated disaccharide and therefore the original monobenzoate must have been the 6-O-benzoylcellobiose and not the 6′-O-benzoylcellobiose. The production of 2,3,6-tri-O-methyl-D-glucose is attributed to partial debenzoylation of the 6-O-benzoylcellobiose during the methylation reaction. Our dimethylglucose was identified as 2,3-di-O-methyl-D-glucose by its specific rotation and also by oxidation with sodium metaperiodate at pH 1–2; it gave 0.9 mole of formaldehyde with an uptake of 1.8 moles of sodium metaperiodate. Further proof of the structure of this methylated glucose was obtained by reduction to 2,3-di-O-methylglucitol and oxidation of this substance with sodium meta-

(1) Part of this work has been presented at the Internationales Symposium über die Chemie der Kohlenhydrate, Münster, Germany, July 1964.
(2) J. O. Deferrari and R. A. Cadenas, *J. Org. Chem.*, **28**, 1070 (1963).
(3) (a) R. A. Cadenas and J. O. Deferrari, *ibid.*, **28**, 1072 (1963); (b) *ibid.*, **28**, 2613 (1963).
(4) S. V. Hintikka, *Ann. Acad. Sci. Fennicae*, 9 (1923); *Chem. Zentr.*, III, 1603 (1923).
(5) J. O. Deferrari and R. A. Cadenas, *J. Org. Chem.*, **30**, 2007 (1965).
(6) R. Kuhn, H. H. Baer, and A. Seeliger, *Ann.*, **611**, 236 (1958).

periodate at pH 1–2, which gave 0.9 mole of formaldehyde and took up 1.8 moles of metaperiodate.

We also oxidized the 6-*O*-benzoylcellobiose (V) with sodium metaperiodate to establish that the benzoyl group had not migrated during methylation; the uptake of 4 moles of oxidant and the nonproduction of formaldehyde agrees with the structure pointed out for this substance. Further proof of this structure was obtained by the oxidation of the 6-*O*-benzoylcellobiitol (VI) obtained by reduction of 6-*O*-benzoylcellobiose with sodium borohydride, which gave 1.1 moles of formaldehyde with an uptake of 4.1 moles of metaperiodate.

The 6-*O*-benzoyl group showed great resistance to ammonolysis. 6-*O*-Benzoylcellobiose was dissolved in methanolic ammonia and after 3 days the solution gave by paper chromatography a faint spot of the starting material and two strong spots of cellobiose and benzamide.

Benzoylation of 6-*O*-benzoylcellobiose with benzoyl chloride in pyridine gave crystalline α-octa-*O*-benzoylcellobiose (VII), m.p. 194–196°, $[\alpha]^{27}D$ +77.7°, which was indistinguishable by thin layer chromatography from β-octa-*O*-benzoylcellobiose (I) obtained by direct benzoylation of cellobiose. The reason by which the direct benzoylation of cellobiose gave the β-octabenzoate and the benzoylation of the 6-*O*-benzoylcellobiose gave the α-octabenzoate could be attributed to the influence of the 6-*O*-benzoyl group which would probably produce an important steric effect, leading benzoylation to the formation of the α anomer. Anomerization of β-octa-*O*-benzoylcellobiose (I) of $[\alpha]D$ +37° with zinc chloride and benzoic acid, adapting the method of Ness, Fletcher, and Hudson,[7] gave α-octa-*O*-benzoylcellobiose (VII) of $[\alpha]D$ +76.5°. The anomerization of the last product under the same experimental conditions, produced the octabenzoate of $[\alpha]D$ +38.2°, which establishes the anomeric character of both substances.

We observed that the presence of benzoyl groups attached to the disaccharide molecule, being more resistant to nucleophilic attack by ammonia, increases the yield of nitrogenated products when compared with similar experiments with octa-*O*-acetylcellobiose. Mono-*O*-acyl derivatives have not been isolated from experiments carried out under the same conditions starting from acetyl and benzoyl derivatives of monosaccharides or acetyl derivatives of disaccharides. This would suggest a differential behavior of the benzoyl derivatives of disaccharides, that we attribute especially to the reciprocal steric effect of both moieties of the disaccharide, which renders nucleophilic attack by ammonia difficult.

Experimental

A 16% methanolic solution of ammonia was used. Paper chromatography was carried out on Whatman No. 1 paper, using butan-1-ol–ethanol–water (5:1:4 v./v., top layer) as developing solvent; the sprays used were (a) silver nitrate–sodium methoxide[8] and (b) aniline hydrogen phthalate.[9] Thin layer chromatography was performed on silicic acid–starch plates,[10] developing with benzene–ethyl acetate (95:5 v./v.); the plates were revealed with spray a by heating at 110° during 15–30 min. Melting points are not corrected.

β-Octa-O-benzoylcellobiose (I) by Benzoylation of Cellobiose.—Cellobiose (25 g., 0.073 mole), m.p. 232–234°, was suspended in 250 ml. of pyridine and 85 ml. (0.72 mole) of benzoyl chloride was added portionwise, shaking the mixture vigorously and keeping it in a water bath at 15°. After standing 1 hr. at room temperature, it was heated 4 hr. at 60° and 15 min. at 100°. The solution was poured into ice–water and the sirup obtained was washed until it gave a pulverized solid. The yield was 83.3 g. (98%) of I, m.p. 185–190°, which precipitated from methanol–acetone (5:1) solution as an amorphous substance, m.p. 188–191°, $[\alpha]^{26}D$ +37° (*c* 1.14, chloroform); the substance was homogeneous by thin layer chromatography and, after aluminum oxide column chromatography, maintained its properties.

Anal. Calcd. for $C_{68}H_{54}O_{19}$: C, 69.48; H, 4.64. Found (for a sample dried at 120° and 1 mm.): C, 69.45; H, 4.89.

Reaction of β-Octa-*O*-benzoylcellobiose (I) with Methanolic Ammonia. A. Isolation of Cellobiose (II).—Forty grams of I was suspended in 1000 ml. of methanolic ammonia and dissolved by shaking during 10 hr. After standing 24 hr. at room temperature the solution was evaporated to dryness and the residue was extracted with seven 50-ml. portions of ethyl acetate. By dissolving in 200 ml. of methanol and spontaneous evaporation at room temperature, 4.22 g. of II, m.p. 230–233°, $[\alpha]^{23}D$ +46.5° (6 min.) → +32.0° (24 hr.) (*c* 0.96, water), was obtained. This substance was identified as cellobiose by paper chromatography, spraying the chromatogram with reagents a and b. The mother liquors from the cellobiose crystallization were evaporated to dryness and the sirupy residue was extracted with four 50-ml. portions of ethyl acetate. The residue was dissolved in 150 ml. of methanol and shaken during 2 hr. with 100 ml. of Zeo-Karb 225 sulfonic resin to eliminate basic substances. The resin was separated by filtration and 1.1 g. of II crystallized from the methanolic solution on evaporation of the solvent. Subsequent column chromatographies carried out to isolate the other components of the mixture gave an additional 0.35 g. of II (total yield 5.69 g., 49.6%).

B. Isolation of 6-*O*-Benzoylcellobiose (V).—The mother liquors from the crystallization of II were dried, extracted with ethyl acetate, and chromatographed on a cellulose column of 59 × 2.8 cm. which was loaded with water saturated with butanol and developed with water-saturated butanol. Sixty-one fractions of 10 ml. each were collected. By evaporation of fractions 35–39 0.24 g. (1.5%) of V was obtained as a sirup, which was dissolved in methanol and obtained in a more purified form from this solution by cooling and decantation of the supernatant methanol; dried at 80° and 1 mm. it had $[\alpha]^{22}D$ +34° (10 min.) → +44° (120 hr.) (*c* 1, water); by paper chromatography, and spraying with reagents a and b it gave a reducing spot of R_f 0.30.

Anal. Calcd. for $C_{19}H_{26}O_{12}$: C, 51.12; H, 5.87. Found (for a sample dried at 60° and 0.005 mm.): C, 51.17; H, 6.08.

C. Isolation of *N*-Benzoylcellobiosylamine (IV).—From fractions 51–59 0.14 g. (0.92%) of IV was obtained as a sirup which was purified by repeated precipitation from methanol (as was described for the compound V); dried at 80° and 1 mm. it gave $[\alpha]^{23}D$ +33.8° (*c* 0.85, pyridine); by paper chromatography and spraying with reagents a and b it showed only one nonreducing spot of R_f 0.19.

Anal. Calcd. for $C_{19}H_{27}NO_{11}\cdot H_2O$: C, 49.23; H, 6.28; N, 3.02. Found (for a sample dried at 80° and 1 mm.): C, 49.32; H, 6.66; N, 2.76.

D. Isolation of 4-*O*-β-D-Glucopyranosyl-1,1-bis(benzamido)-1-deoxy-D-glucitol (IIIa).—The fractions from the column above described which did not crystallize were again chromatographed and 43 fractions of 25 ml. each were collected. Fractions 19–23 afforded 1.5 g. (7.8%) of IIIa, which crystallized from methanol–ethyl acetate (1:1) as needles of m.p. 148–150° and $[\alpha]^{26}D$ −33.2° (*c* 0.97, water); by paper chromatography and spraying with reagents a and b it gave only one nonreducing spot of R_f 0.42.

Anal. Calcd. for $C_{26}H_{34}N_2O_{12}$: C, 55.12; H, 6.05; N, 4.94. Found (for a sample dried at 120° and 0.0001 mm.): C, 55.46; H, 6.23; N, 4.80.

(7) R. K. Ness, H. G. Fletcher, and C. S. Hudson, *J. Am. Chem. Soc.*, **72**, 2200 (1950).

(8) R. A. Cadenas and J. O. Deferrari, *Analyst*, **86**, 132 (1961).

(9) M. Patridge, *Nature*, **164**, 443 (1949).

(10) J. O. Deferrari, R. M. de Lederkremer, B. Matsuhiro, and J. F. Sproviero, *J. Chromatog.*, 283 (1962).

Fractions 25–33 gave 0.77 g. of the previously described 6-*O*-benzoylcellobiose (V).

α-Octa-*O*-benzoylcellobiose (VII) from 6-*O*-Benzoylcellobiose (V).—V (170 mg.) was dissolved in 5 ml. of pyridine and 2.5 ml. of benzoyl chloride was added. The mixture was kept 1 hr. at room temperature and heated 4 hr. at 60° and 15 min. at 100°; it was then cooled, poured into 150 ml. of ice–water, and the sirup obtained was washed and macerated with water until it gave a pulverized solid. The yield was 0.41 g. (93%) of VII, m.p. 180–195°; crystallized from acetone–methanol (1:1) needles, m.p. 194–196°, $[\alpha]^{26}D$ +77.7° (*c* 1.2, chloroform), were obtained. Thin layer chromatography showed only one spot of the same R_f as I, which was obtained by direct benzoylation of cellobiose.

Anal. Calcd. for $C_{68}H_{54}O_{19}$: C, 69.48; H, 4.64. Found (for a sample dried at 110° and 1 mm.): C, 69.34; H, 4.51.

Hepta-*O*-acetyl-*N*-benzoylcellobiosylamine (IVb).— IVa (35 mg.) was dissolved in 3 ml. of a mixture (1:1) of acetic anhydride and pyridine; the solution was kept 24 hr. at room temperature, then heated 30 min. at 60°, and evaporated to dryness in a vacuum desiccator. The yield was 0.050 g. (85%) of IVb, which was purified by precipitation from methanol. A sirup was obtained which by drying at 80° and 1 mm. was transformed into an amorphous solid of m.p. 104–107°, $[\alpha]^{28}D$ +37.2° (*c* 0.73, chloroform).

Anal. Calcd. for $C_{33}H_{41}NO_{18}$: C, 53.58; H, 5.58. Found (for a sample dried at 80° and 1 mm.): C, 53.50; H, 5.79.

Octa-*O*-acetyl-4-*O*-β-D-glucopyranosyl-1,1-bis(benzamido)-1-deoxy-D-glucitol (IIIb).—IIIa (220 mg.) was acetylated with 6 ml. of a mixture (1:1) of acetic anhydride and pyridine, according to the technique described above. The yield was 0.30 g. (85%) of IIIb obtained as a sirup, which was precipitated three times from methanol and dried at 80° and 1 mm. to yield an amorphous substance, m.p. 116–117° (softened at 98°), $[\alpha]^{28}D$ −21.1° (*c* 0.83, chloroform).

Anal. Calcd. for $C_{42}H_{50}N_2O_{20}$: C, 55.87; H, 5.58; N, 3.10. Found (for a sample dried at 80° and 1 mm.): C, 55.72; H, 5.88; N, 3.4.

Anomerization of β-Octa-*O*-benzoylcellobiose.—Anhydrous zinc chloride (500 mg.) and 1 g. of benzoic acid were melted at 140° in a stoppered test tube; 1 g. of β-octa-*O*-benzoylcellobiose, $[\alpha]^{26}D$ +37°, was added and the mixture was heated 1 hr. at 140°, then cooled, and partially dissolved in 10 ml. of pyridine. The dark solution was poured into 100 ml. of water and extracted with three 50-ml. fractions of chloroform; the chloroform solution was washed with 1 *N* sulfuric acid, a saturated sodium hydrogen carbonate solution, and finally with water. The solution was dried with anhydrous magnesium sulfate, evaporated to dryness, and dissolved in methanol–acetone (1:1); 0.46 g. of α-octa-*O*-benzoylcellobiose, m.p. 185–192°, $[\alpha]^{28}D$ +76.5° (*c* 0.83, chloroform), was obtained.

Anomerization of α-Octa-*O*-benzoylcellobiose.—α-Octa-*O*-benzoylcellobiose (200 mg.), $[\alpha]^{29}D$ +77°, was anomerized under the same conditions as described above and yielded 0.046 g. of β-octa-*O*-benzoylcellobiose, $[\alpha]^{22}D$ +38.2° (*c* 1.1, chloroform).

Methylation of 6-*O*-Benzoylcellobiose (V).—V (100 mg.) was dissolved in 5 ml. of dimethylformamide and shaken with 4 ml. of methyl iodide and 0.7 g. of barium oxide during 24 hr. The mixture was filtered, then 25 ml. of chloroform was added, and the chloroform solution was washed with 1 *N* sulfuric acid, a saturated sodium hydrogen carbonate solution, and water; it was dried with anhydrous magnesium sulfate and evaporated to dryness. The yield was 0.09 g. of methylated products which were dissolved in 3 ml. of a 0.07% solution of sodium methoxide in methanol, which was neutralized after 4 days and hydrolyzed with 3 ml. of 1 *N* sulfuric acid during 6 hr. at 100°. The sulfuric acid was neutralized with barium carbonate, filtered, and evaporated. The sirup obtained was chromatographed on Whatman No. 3 MM paper with water-saturated butanol. The yield was 0.042 g. of 2,3,4,6-tetra-*O*-methyl-D-glucose of $[\alpha]^{23}D$ +90° (final value) (*c* 1.1, water), lit.[11] $[\alpha]D$ +92°; 0.0175 g. of 2,3,6-tri-*O*-methyl-D-glucose of $[\alpha]^{21}D$ +63° (final value) (*c* 0.64, water), lit.[12] $[\alpha]D$ +70°; and 0.0205 g. of 2,3-di-*O*-methyl-D-glucose of $[\alpha]^{19}D$ +46.4° (*c* 0.5, acetone), lit.[13] $[\alpha]D$ +48.3°.

6-*O*-Benzoylcellobiitol (VIII).—This substance was prepared applying the technique of Thompson[14] for the preparation of D-galactitol. V (180 mg.) dissolved in 3 ml. of water was reduced by a solution of 29 mg. of sodium borohydride in 1.5 ml. of water during 3.5 hr. Zeo-Karb 225 sulfonic resin was added to the solution to destroy the excess reducing substance; the solution was then shaken with 2 ml. of resin during 15 min., filtered, evaporated several times with methanol, and precipitated from absolute ethyl alcohol. The yield was 175 mg. (96%) of VIII which was a sirup at room temperature and when dried at 60° and 1 mm. gave $[\alpha]^{27}D$ −6.1° (*c* 1, water).

Anal. Calcd. for $C_{19}H_{28}O_{12}$: C, 50.89; H, 6.29. Found (for a sample dried at 60° and 0.0001 mm.): C, 50.82; H, 6.28.

Sodium Metaperiodate Oxidations.—The spectrophotometric technique was applied, measuring the sodium metaperiodate uptake with a Beckmann DU spectrophotometer at 222.5 mμ,[15] and at 570 mμ[16] the formaldehyde produced. Oxidations were carried out on samples of 2–6 mg.; the reacting mixtures were kept at 26° during the oxidation. The results are shown in Table I.

TABLE I

SODIUM METAPERIODATE OXIDATIONS

Time, hr.	$NaIO_4$, moles	Formaldehyde, moles
A. 6-*O*-Benzoylcellobiose (V)		
0.5	1.5	0
3.5	2.6	0
10	3.7	0
24	4.0	0
B. 6-*O*-Benzoylcellobiitol (VIII)		
0.15	1.9	1.1
1.5	1.9	1.1
7	3.8	1.1
10.5	4.1	1.1
C. 2,3-Di-*O*-methyl-D-glucose[a]		
0.5	0.7	0.5
3.5	1.6	0.8
7	1.6	0.9
12	1.8	0.9
D. 2,3-Di-*O*-methylglucitol[a,b]		
0.5	1.2	0.8
12	1.6	0.9
24	2.1	0.9
E. 4-*O*-β-D-Glucopyranosyl-1,1-bis(benzamido)-1-deoxy-D-glucitol (IIIa)		
0.15	0.5	0.7
1.5	1.0	1.0
2.5	1.6	1.0
6	3.9	1.0

[a] pH 1–2. [b] 2,3-Di-*O*-methyl-D-glucose (6.4 mg.) was reduced with sodium borohydride (5.3 mg.); the excess of reducing agent was destroyed with formic acid and the pH was adjusted to 1; the oxidation was carried out as described (see Experimental).

Ammonolysis of 6-*O*-Benzoylcellobiose (V).—V (15 mg.) was dissolved in 1.5 ml. of methanolic ammonia; samples were chromatographed on paper every 24 hr. and the spots were detected with reagents a and b. Only after 3 days a diminution of the concentration of V was observed by the low intensity of the spot and after 7 days the last traces of V had disappeared.

(11) J. C. Irvine and J. W. H. Oldham, *J. Chem. Soc.*, **119**, 1744 (1921).

(12) J. C. Irvine and E. L. Hirst, *ibid.*, **121**, 1231 (1922); J. C. Irvine and R. P. McGlynn, *J. Am. Chem. Soc.*, **54**, 356 (1932).

(13) J. C. Irvine and J. P. Scott, *J. Chem. Soc.*, **103**, 575 (1913).

(14) A. Thompson, *Methods Carbohydrate Chem.*, **2**, 67 (1963).

(15) C. E. Crouthamel, H. V. Meek, D. S. Martin, and C. V. Banks, *J. Am. Chem. Soc.*, **71**, 3031 (1949).

(16) D. A. Mac Fadyen, *J. Biol. Chem.*, **158**, 107 (1945).