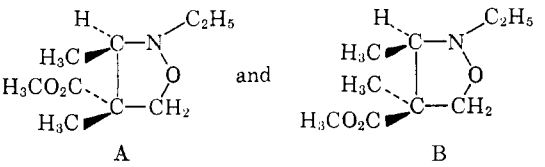
water as formed in the reaction.] The disappearance of the peroxide band at ~11.8 μ indicated that at least 90% of the peroxide had reacted in *72 hr.* After removal of 2 ml. for g.l.c. analyses, methyl methacrylate (10.2 g.; 0.1 mole) was added and the reaction mixture heated at ~60° for 17 hr. At the end of this time the 6.2-μ band had essentially disappeared, being replaced by the 6.1-μ carbon–carbon double bond stretch of methyl methacrylate. The mixture was concentrated by distillation and the concentrate fractionated through a micro-Vigreux column (see Table I).

TABLE I

Fraction	Wt., g.	B.p., °C. (mm.)	$n^{22.5}D$	Isomer distribution (B:A) % of mixture
1	1.48	99–101/28	1.4378	80:20
2	5.57	101–102.5/28	1.4383	77:23
3	1.42	102/28	1.4392	63:36
4	0.29	60↓/5	1.4414	37:58
Residue	.7			

Fractions 1–4 were analyzed by g.l.c. (85°, 10 p.s.i.g. N_2, 10-ft. Silicone SE-30) and found to consist of principally two isomers differing in retention time by ~3 min. under the stated conditions. We *presume* A and B to have the structures shown.[4]

The nuclear magnetic resonance spectra were determined for fraction 2 and for individual isomers A and B collected at the exit end of a g.l.c. column. The n.m.r. analysis[12] was consistent with geometrical isomers having the proposed structure (O attachment to —CH_2—) and distribution; however, it was not possible to establish the identity of the individual isomers and the assignment of A to the higher boiling isomer is not certain.



A B

An aliquot of fraction 2 was taken for analysis.

Anal. Calcd. for $C_9H_{17}NO_3$: C, 57.6; H, 9.15; N, 7.52. Found: C, 57.6; H, 9.2; N, 7.8, 7.9 (Dumas).

The infrared spectrum of III shows a characteristic ester band at 5.72, strong absorption in the 8–9-μ region, and a characteristic set of moderately strong, sharp bands at 10.08, 10.57, 11.36, 12.10, and 13.18 μ.

An aliquot of another reaction mixture was converted to a 2,4-dinitrophenylhydrazone (m.p. 145–147°; ~85% yield).

Anal. Calcd. for $C_8H_8N_4O_4$: C, 42.9; H, 3.6; N, 25.0. Found: C, 43.0; H, 3.8; N, 24.6.

Mixture melting point with the higher melting modification of acetaldehyde 2,4-dinitrophenyl hydrazone (m.p. 167–168°) showed no depression.

(12) We are indebted to our colleagues J. L. Jungnickel and C. A. Reilly for the determination and resolution of the n.m.r. spectra.

The Reaction of Ammonia with Acylated Disaccharides. I. Acetyl Derivatives of Cellobiose

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Received July 2, 1962

The reaction of methanolic ammonia with α-octa-O-acetylcellobiose is described. From the reaction were isolated cellobiose, N,N′-diacetylcellobiosylidenediamine, and a hepta-O-acetyl-N-acetylcellobiosylamine which is considered the alpha anomer of the product already described as hepta-O-acetyl-N-acetylcellobiosylamine. The acetyl derivative of N,N′-diacetylcellobiosylidenediamine was prepared and the influence of solvent upon the reaction studied is discussed.

Wohl's study[1] on ammonia degradation of acetylated D-gluconononitrile, introduced for the first time the so-called "aldose–diacetamides," derivatives which possessed two acetyl groups on carbon atom 1.

Later work on this subject, developed mainly by Brigl, Mühlschlegel, and Schinle[2] and Deulofeu and Deferrari[3–5] pointed out that these types of compounds could be obtained not only by degradation of acetylated nitriles, but by ammonolysis of partially or fully acetylated aldoses. In all cases, the principal product of the reaction was the corresponding "aldose–diamide," obtained in yields varying between 20 and 80%, depending both on the nature of the sugar and the substituents. An exception was found by Hockett and Chandler[6] and by Nieman and Hays[7] in the case of glucose acetates, which gave as a principal product N-acetyl-D-glucofuranosylamine.

In this paper we extended the knowledge of this reaction to the field of disaccharides. The nitrogenated products obtained through this reaction could be named, in a general manner, "aldobiose–amides" (or more correctly, N,N′-diacylaldobiosylidenediamines and N-acylaldobiosylamines), and this study would allow us to know the influence upon the course of reaction by the carbohydrate moiety glycosidically linked to carbon atom 4 of the reducing moiety of the disaccharide.

The first known attempt at degradation of acetylated disaccharides by ammonia was Zemplen's, who attempted to apply Wohl's reaction to octa-O-acetylcellobionic acid nitrile and obtained amorphous substances.[8]

Zemplen supposed he had obtained acetamido derivatives on carbon atom 1 which were not useful for his proposed studies and therefore did not continue his investigation.

Zechmeister and Toth[9] dissolved octa-O-acetylcellobiose in liquid ammonia and maintained the solution at 55° for forty-eight hours. From the products of the reaction they isolated an N-acetylcellobiosylamine (m.p. 246°; $[\alpha]^{20}D$ −20.3°, water) whose acetate had m.p. 196°; $[\alpha]^{20}D$ −8.4° (chloroform). Acetylation of the residual sirup led to the isolation of a fully acety-

(1) A. Wohl, *Ber.*, **26**, 730 (1893).
(2) P. Brigl, H. Mühlschlegel, and R. Schinle, *ibid.*, **64**, 2921 (1931).
(3) V. Deulofeu and J. O. Deferrari, *J. Org. Chem.*, **17**, 1087 (1952).
(4) J. O. Deferrari and V. Deulofeu, *ibid.*, **17**, 1093 (1952); **17**, 1097 (1952).
(5) J. O. Deferrari and V. Deulofeu, *ibid.*, **22**, 802 (1957).
(6) R. C. Hockett and L. R. Chandler, *J. Am. Chem. Soc.*, **66**, 957 (1944).
(7) C. Nieman and J. T. Hays, *ibid.*, **67**, 1302 (1945).
(8) G. Zemplen, *Ber.*, **59**, 1254 (1926).
(9) L. Zechmeister and G. Toth, *Ann.*, **525**, 14 (1936).

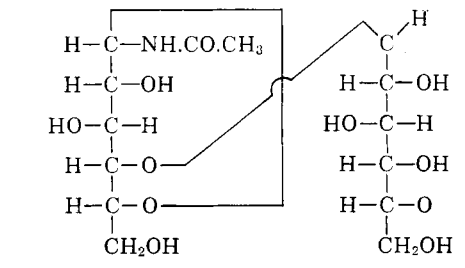
lated N,N′-diacetylcellobiosylidenediamine (m.p. 196°; some samples softened at 140°; $[\alpha]^{20}D$ −3.3°, chloroform).

Elimination of O-acetyl groups with barium hydroxide afforded N,N′-diacetylcellobiosylidenediamine ($[\alpha]^{20}D$ −20°, water) which they did not obtain in crystalline form.

Micheel, *et al.*,[10] submitted octa-O-acetylcellobiose to the action of 40% methanolic ammonia at 50° during 120 hours. Under these conditions, they did not isolate the N,N′-diacetylcellobiosylidenediamine but cellobiosylamine, dicellobiosylamine and the same N-acetylcellobiosylamine of Zechmeister and Toth, as the acetate.

We carried out this reaction under the same conditions that were used before by Deulofeu and Deferrari[3-5] and other authors in studies with acylated monosaccharides, employing 16% methanolic ammonia at room temperature.

The principal product of this reaction was cellobiose, which was obtained in high yield. The direct crystallization of N,N′-diacetylcellobiosylidenediamine from the mother liquors was hindered by the presence of substances of basic character, of unknown structure. The elimination of these substances was accomplished in two stages, the first being a partial elimination by passage through a carboxylic acid resin, after which practically all the cellobiose still present, crystallized. The second stage was passage through a sulfonic acid resin which eliminates all remanent basic substances. From the eluate, crystalline N,N′-diacetylcellobiosylidenediamine (m.p. 113–115°; $[\alpha]^{26.5}D$ −23.5° water) was isolated. Acetylation of the residue afforded a crystalline acetate m.p. 230°, which on further purification by column chromatography gave hepta-O-acetyl-N-acetylcellobiosylamine (acetyl derivative of I) (m.p. 242°; $[\alpha]^{27}D$ +54.09° chloroform). We consider this acetate as the alpha anomeric form of the acetate, m.p. 196°, $[\alpha]^{20}D$ −8.4°, obtained by Zechmeister and Toth and by Micheel, *et al.* Elimination of the O-acetyl groups gave a sirup which, because of its nonreducing character, confirms the position of the N-acetyl group on carbon atom 1.



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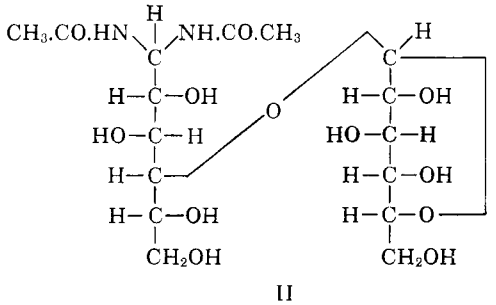

II

The N,N′-diacetylcellobiosylidenediamine presents high stability in ammoniacal media; acetylation of this substance affords a crystalline acetate which could be deacetylated, by means of methanolic ammonia, without elimination of the N-acetyl groups, recovering the original sugar. The specific rotation for this acetate is different from that reported by Zechmeister and Toth.

For this substance we postulate structure II, instead of the structures proposed by Zechmeister and Toth.[9] This problem will be clarified by a structural study, which is in progress.

The reaction of ammonia upon acetylated carbohydrates can be considered as the result of a balance among several simultaneous and competitive reactions. A comparison of the results of this investigation with those of Zechmeister and Toth and Micheel, *et al.*, illustrates clearly that temperature and solvent are not critical factors in determining the yield of N,N′-diacetylcellobiosylidenediamine. The unusually low yield of N,N′-diacetylcellobiosylidenediamine compared with the yields obtained in the field of monosaccharides can in all probability be attributed to the steric hindrance exerted by the nonreducing moiety of the disaccharide upon the ortho ester mechanism already proposed for the reaction.[11-13]

Experimental

A 16% methanolic solution of ammonia was employed. Paper chromatography was performed on Whatman no. 1 paper, employing the following systems: (A) ethyl acetate–pyridine–water (10:4:3 v./v.); (B) *n*-butyl alcohol–ethanol–water (50:10:40 v./v., top layer). Glucose was used as standard. Evaporations were carried out at reduced pressure and below 60°. Melting points are not corrected.

Reaction of α-Octa-O-acetylcellobiose with Methanolic Ammonia. (a) **Isolation of Cellobiose.**—Twenty grams of α-octa-O-acetylcellobiose[14] was suspended in 500 ml. of methanolic ammonia and dissolved by shaking for 20 min. at room temperature. The solution was allowed to stand at room temperature for 24 hr. and concentrated to 200 ml. After 3 days 6.950 g. of cellobiose, m.p. 234–236°, crystallized (lit., m.p. 225°); $[\alpha]^{25}D$ +26.7° (*c* 2.15 water, at 30 min.); phenylosazone, m.p. 200°, Paper chromatography in system B gave only one spot of R_g 0.49 at 25°, detected with the silver nitrate–sodium methoxide reagent.[15] The filtrate was evaporated to dryness and extracted with five 50-ml. portions of warm ethyl acetate to remove the acetamide formed in the ammonolysis. The residual sirup dissolved in methanol gave, by evaporation at room temperature, a further gram of cellobiose.

The filtrate was diluted with water to 50 ml. and passed through a column of Amberlite IR C-50 (250 ml.); it was eluted with 4 l. of water. The residue obtained after evaporation was dried, then dissolved in methanol from which 990 mg. of cellobiose crystallized. The over-all yield of cellobiose was 89.3%. Repeated extractions with ethyl acetate and drying to a powder is of special importance to obtain crystallization from the sirup.

(b) **Isolation of N,N′-Diacetylcellobiosylidenediamine (II).**—The sirup obtained on solvent removal after separation of cellobiose, was dissolved in 100 ml. of water and passed through 750 ml. of an Amberlite IR-120 resin. Six liters of water was employed for the elution and was then evaporated to dryness; the residue, dissolved in methanol, was allowed to stand at room temperature until needles separated. Thus was obtained 500 mg.

(10) F. Micheel, R. Frier, E. Plate, and A. Hiller, *Chem. Ber.*, **85**, 1092 (1952).

(11) H. S. Isbell and H. L. Frush, *J. Am. Chem. Soc.*, **71**, 1579 (1949).

(12) R. C. Hockett, V. Deulofeu, and J. O. Deferrari, *ibid.*, **72**, 1840 (1950).

(13) V. Deulofeu and J. O. Deferrari, *Anales Asoc. Quim. Arg.*, **38**, 241 (1950).

(14) G. Braun, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 124.

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

of II, m.p. 105–112°, which dissolved in 12 ml. of ethanol and left during 24 hr., without stirring or scratching, gave crystals, m.p. 113–115°, $[\alpha]^{26.5}D$ −23.3° (*c* 0.27, water); yield 3.7%.

Anal. (for a sample dried at 100° and 2 mm.). Calcd. for $C_{16}H_{30}O_{12}N_2 \cdot H_2O$: C, 41.73; H, 6.95; N, 6.08. Found: C, 41.91; H, 6.88; N, 6.02.

Anal. (for a sample dried at 120° and 2 mm.). Calcd. for $C_{16}H_{30}O_{12}N_2$: C, 43.43; H, 6.78; N, 6.33. Found: C, 43.35; H, 6.79; N, 6.02.

Octa-O-acetyl-N,N′-diacetylcellobiosylidenediamine (III).—Fifty milligrams of II was dissolved in a boiling suspension of 1 ml. of acetic anhydride and 40 mg. of anhydrous sodium acetate. The mixture was warmed for 0.5 hr. on a boiling water bath. It was then cooled and poured into a mixture of ice and water. After 24 hr. the solution was extracted with five 20-ml. portions of chloroform. The chloroform extracts were washed with a saturated solution of hydrogen sodium carbonate and then with water, dried over anhydrous sodium sulfate, and evaporated. Thus was obtained 90 mg. of crude material (71%) which when recrystallized from water gave 60 mg., m.p. 195–196° (softens at 140°); $[\alpha]^{21}D$ +6.6° (*c* 0.18, chloroform). Recrystallizations from ethanol gave a product which melts at 140°, affording a sirup which shrinks at 195°.

Anal. Calcd. for $C_{32}H_{46}O_2N_2$: C, 49.35; H, 5.91; N, 3.59. Found: C, 48.70; H, 6.29; N, 3.69.

Ammonolysis of Octa-O-acetyl-N,N′-diacetylcellobisylidenediamine.—Three hundred milligrams of III was dissolved in 9 ml. of methanolic ammonia. After 24 hr. at room temperature and subsequent evaporation, the residue was extracted with five 3-ml. portions of ethyl acetate and then dried. By dissolution in 10 ml. of absolute ethanol and spontaneous evaporation at room temperature, 130 mg. of needles, m.p. 111–115°, were obtained, which after two recrystallizations from absolute ethanol gave m.p. and m.m.p. with II 113–115°, $[\alpha]^{25}D$ −23.0° (*c* 0.26, water).

(c) **Acetylation of the Residual Sirup. Isolation of Hepta-O-acetyl-N-acetyl-α-cellobiosylamine.**—The solution obtained after isolation of II, was evaporated to dryness and the residual powder (450 mg.) was dissolved in 12 ml. of a 1:1 mixture of pyridine and acetic anhydride, at room temperature. The solution was left to stand for 24 hr. and then left 40 min. in a boiling water bath. The cooled mixture was poured into ice–water and a solid (285 mg.) was filtered; from the filtrate, by extraction with chloroform, washing the chloroform extracts in the usual way, and evaporating, 90 mg. of III was obtained, which recrystallized from water gave m.p. 195–196° (softens at 140°) $[\alpha]^{27}D$ +6.1° (*c* 0.2, chloroform).

The solid was recrystallized from ethanol giving 250 mg. of needles, m.p. 230–233°, which were dissolved in a mixture of chloroform (7 ml.) and benzene (5 ml.). This solution was chromatographed in a column of talc–Celite 503 (5:1 by wt., 30 mm. by 220 mm.). Elution with 110 ml. of a mixture of benzene–absolute ethanol (100:0.25 v./v.) afforded 115 mg. of α-octa-O-acetylcellobiose, m.p. and m.m.p. 224–225°, $[\alpha]^{24}D$ +42.6° (*c* 0.51, chloroform). Then a benzene–absolute ethanol solution (100:1 v./v., 100 ml.) was used, which on solvent removal, did not give any residue. Finally, 300 ml. of benzene–absolute ethanol (100:3 v./v.) were used, which by evaporation and recrystallization of the residue from ethanol gave 100 mg. of hepta-O-acetyl-N-acetyl-α-cellobiosylamine (IV), m.p. 242–243°, $[\alpha]^{27}D$ +54.09 (*c* 0.28, chloroform).

Anal. Calcd. for $C_{28}H_{39}O_{18}N$: C, 49.61; H, 5.80; N, 2.07. Found: C, 49.58; H, 5.80; N, 2.37.

Ammonolysis of Hepta-O-acetyl-N-acetyl-α-cellobiosylamine.—One hundred and seventy five milligrams of IV was dissolved in 5 ml. of methanolic ammonia and left at room temperature 24 hr. The solution was evaporated to dryness and extracted with four 3-ml. portions of ethyl acetate. The residual sirup did not crystallize from ethanol, absolute ethanol or isopropyl alcohol and from mixtures of these solvents with ethyl ether. Chromatography in solvent (A) and spraying with silver nitrate–sodium methoxide reagent[15] showed only one spot R_x 0.95 at 25°. With the picric acid–sodium methoxide reagent[15] no spots were detected. This result points out the nonreducing character of the N-acetylcellobiosylamine (I) formed by the elimination of O-acetyls.

The Reaction of Ammonia with Acylated Disaccharides. II. Acetyl Derivatives of Lactose

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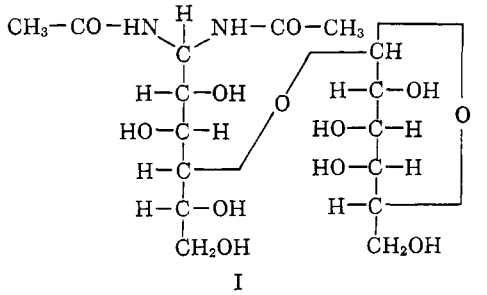
Received August 20, 1962

The reaction between ammonia and β-octa-O-acetyllactose was studied. As reaction products, N,N′-diacetyllactosylidenediamine and N-acetyl-α-lactosylamine were isolated and characterized *via* the corresponding acetyl derivatives.

In the first paper of this series[1] we have reported on the products obtained by the reaction of methanolic ammonia on octa-O-acetylcellobiose. This reaction follows the pattern already shown for acetylated and benzoylated derivatives of monosaccharides, by Brigl, Mühlschlegel, and Schinle,[2] Deulofeu, and Deferrari,[3] and other authors,[4] who have isolated "aldosediamides" and "aldosemonoamides" (N,N′-diacylaldosylidenediamines and N-acylaldosylamines, respectively).

However, certain differences appear in the case of disaccharides, the most evident being the lowering of the yields on the "aldobiose-diamides" (N,N′-diacylaldobiosylidenediamines) (I). Whereas the usual yields in the case of monosaccharides vary between 20 and 80%, in the disaccharides these values diminish to about 4%.



I

In the first instance, this variation can be attributed to the bulky moiety attached glycosidically to the monosaccharide unit which undergoes the transformation leading to the N,N′-diacyl derivatives. The mechanism for the formation of these derivatives was postulated by Isbell and Frush[5] for acylated monosaccharides. The intramolecularity of this mechanism

(1) J. O. Deferrari and R. A. Cadenas, *J. Org. Chem.*, **28**, 1070 (1963).
(2) P. Brigl, H. Mühlschlegel, and R. Schinle, *Ber.*, **64**, 2921 (1931).
(3) V. Deulofeu and J. O. Deferrari, *J. Org. Chem.*, **17**, 1087 (1952); **17**, 1093 (1952); **17**, 1097 (1952); **22**, 802 (1957).
(4) R. C. Hockett and L. R. Chandler, *J. Am. Chem. Soc.*, **66**, 957 (1944).
(5) H. S. Isbell and H. L. Frush, *ibid.*, **71**, 1579 (1949).