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The Reaction of Ammonia with Acylated Disaccharides. IV. The Structure of the 1,1-Bis(acetamido)-1-deoxyaldobiitols

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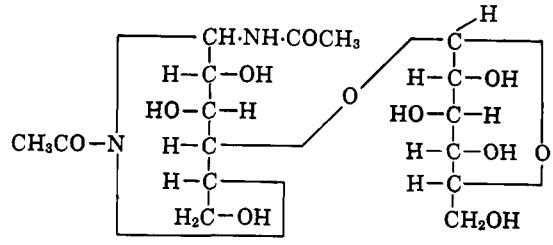
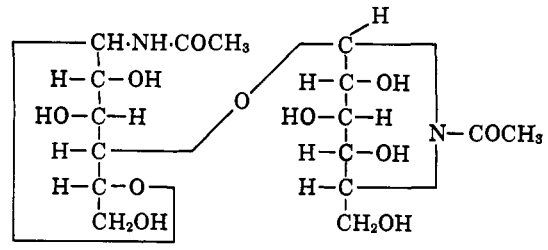
By means of methylation and hydrolysis techniques it was demonstrated that the 1,1-bis(acetamido)-1-deoxycellobiitol, the 1,1-bis(acetamido)-1-deoxylactitol, and the 1,1-bis(acetamido)-1-deoxymaltitol obtained by the reaction of ammonia upon the corresponding acetylated aldobioses have an acyclic structure in the nitrogenated moiety. The separation of a mixture of 2,3,5,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose by gas–liquid and by anion-exchange resin chromatographies is described.

The reaction of ammonia with acetylated disaccharides[1,2] afforded substances which possessed the structure of a disaccharide with two acetamide groups on C-1 and which can be designated as 1,1-bis(acetamide)-1-deoxyaldobiitols.[3]

Zechmeister and Tóth[4] described the reaction of liquid ammonia with octa-*O*-acetylcellobiose and isolated a 1,1-bis(acetamido)-1-deoxycellobiitol, for which they proposed the two possible structures I or II.

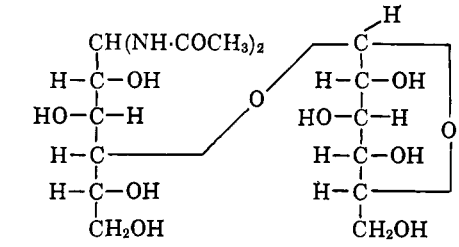


I



II

According to studies performed on the mechanism of this reaction,[5] which is intramolecular, the structure II would not be probable; the analytical data for structure I do not permit a clear distinction between such a structure and the acyclic one (III) which we proposed[1] for 1,1-bis(acetamido)-1-deoxycellobiitol.



III

This structure agrees with that already postulated for analogous monosaccharide derivatives.[6]

Similar open-chain structures were proposed for 1,1-bis(acetamido)-1-deoxymaltitol[2] and for 1,1-bis(acetamido)-1-deoxylactitol.[2] These substances can crystallize with solvent, and the analytical data depend on the drying conditions.

However, the possibility of a ring as shown in I cannot be excluded and recently a group of sugars has been described which bear a nitrogen atom on the hemiacetal ring, such as 5-acetamido-5-deoxy-D-ribopyranose,[7] 5-acetamido-5-deoxy-D-xylopyranose,[7,9] 5-acetamido-5-deoxy-L-arabinopyranose,[7,8] and 5-acetamido-5-deoxy-L-xylopyranose.[10] Although these substances have been obtained by a different path, a similar heterocyclic ring could be possible in I.

We clarified the structure of 1,1-bis(acetamido)-1-deoxycellobiitol, 1,1-bis(acetamido)-1-deoxylactitol, and 1,1-bis(acetamido)-1-deoxymaltitol through a methylation technique which confirmed the acyclic structure in the nitrogenated moiety of these disaccharides, represented by III for the former compound. The usual technique of methylation with dimethyl sulfate in alkaline solution[11] was inadequate for these substances because of their sensitivity to strong alkali. The technique of Kuhn and Baer[12] with methyl iodide and barium oxide in dimethylformamide does not present any difficulty, provided that certain soluble barium

(1) J. O. Deferrari and R. A. Cadenas, *J. Org. Chem.*, **28**, 1070 (1963).

(2) R. A. Cadenas and J. O. Deferrari, *ibid.*, **28**, 1072 (1963); **28**, 2613 (1963).

(3) These substances have been already designated as "aldobiosediamides" or as N,N′-diacylaldobiosylidenediamines, but the name given throughout the text as derivatives of the parent disaccharide alditol seems preferable, considering the acyclic structure of these substances.

(4) L. Zechmeister and G. Tóth, *Ann.*, **526**, 14 (1936).

(5) H. S. Isbell and H. L. Frush, *J. Am. Chem. Soc.*, **71**, 1579 (1949); R. C. Hockett, V. Deulofeu, and J. O. Deferrari, *ibid.*, **72**, 1840 (1950); V. Deulofeu and J. O. Deferrari, *Anales Asoc. Quím. Arg.*, **38**, 241 (1950).

(6) J. O. Deferrari and V. Deulofeu, *J. Org. Chem.*, **17**, 1093 (1952); **17**, 1097 (1952).

(7) S. Hanessian and T. H. Haskell, *J. Org. Chem.*, **28**, 2604 (1963).

(8) J. K. N. Jones and J. C. Turner, *J. Chem. Soc.*, 4699 (1962).

(9) H. Paulsen, *Angew. Chem.*, **74**, 901 (1962); *Ann.*, **670**, 121 (1964); J. K. N. Jones and W. A. Szarek, *Can. J. Chem.*, **41**, 636 (1963).

(10) T. H. Haskell and S. Hanessian, *J. Org. Chem.*, **28**, 2598 (1963).

(11) W. N. Haworth, *J. Chem. Soc.*, **107**, 13 (1915).

(12) R. Kuhn and H. Baer, *Ann.*, **611**, 236 (1958).

compounds formed in the reaction are eliminated from the chloroform solution of the methylation product by washing with sulfuric acid. These barium compounds deacetylate the nitrogens on C-1, when the chloroform solution is concentrated under vacuum.

The methylated disaccharides obtained were very sensitive to the usual conditions of hydrolysis and this made the application of controlled conditions necessary. Hydrolysis of octa-*O*-methyl-1,1-bis(acetamido)-1-deoxylactitol (IV) during 6 hr. with methanolic hydrogen chloride and then for another 4 hr. with aqueous acid led to the isolation of 2,3,4,6-tetra-*O*-methyl-D-galactose but destroyed the other moiety of the methylated disaccharide. When the heating was reduced to 25 min. at 100° with 1 *N* sulfuric acid, it was possible to isolate a mixture of 2,3,5,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-galactose, which were separated using a cellulose column and identified by their optical rotations and preparation of derivatives.

Methylation of 1,1-bis(acetamido)-1-deoxycellobiitol and of 1,1-bis(acetamido)-1-deoxymaltitol, which differ only in their β and α, respectively, glycosidic configurations, led to octa-*O*-methyl-1,1-bis(acetamido)-1-deoxycellobiitol (VI) and octa-*O*-methyl-1,1-bis(acetamido)-1-deoxymaltitol (VII) which, when hydrolyzed under the conditions described, gave the same hydrolytic fragments, *i.e.*, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,5,6-tetra-*O*-methyl-D-glucose. Both methylated monosaccharides could not be separated by means of cellulose or alumina column chromatography, but their presence was demonstrated by a gas–liquid chromatography[13] of a sample of each hydrolysis. Practically both hydrolysis mixtures gave the same pattern, in which the percentage of 2,3,5,6-tetra-*O*-methyl-D-glucose was about 20%, pointing out that, in spite of the mild hydrolysis conditions employed, appreciable destruction of the furanose form had occurred.

The presence of two tetramethyl monosaccharide units in each of the hydrolysis mixtures of the three methylated disaccharides shows that methylation was complete. Infrared spectra of these methylated disaccharides did not show free hydroxyl group bands at 3640–3680 cm.$^{-1}$ and showed amide bands[14] at 1625–1700 and at 3400 cm.$^{-1}$, pointing out that the substituents at C-1 had not been altered by the methylation technique.

The mixture of 2,3,5,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose could be fractionated in a preparative way using the strongly basic ion-exchange resin, Deacidite FF, as was described for isomeric glycosides by Baddiley, *et al.*[15] The separation was sharp and the elution from the resin depended on the handling of the resin, as is described in the Experimental part.

Experimental

Paper chromatography was performed on Whatman No. 1, using 1-butanol–ethanol–water (5:1:4 v./v., top layer). Spray reagent was aniline hydrogen phthalate,[16] and 2,3,4,6-O-methyl-D-glucose was employed as standard. Evaporations were performed at reduced pressure at 50°.

A. Methylation of 1,1-Bis(acetamido)-1-deoxylactitol.—Eight grams (0.056 mole) of methyl iodide was added to a solution of 1 g. of 1,1-bis(acetamido)-1-deoxylactitol (0.0023 mole) in 20 ml. of dimethylformamide which contained 4.35 g. of barium oxide (0.028 mole). The addition was completed in 30 min. and the suspension was shaken for 6 hr. at room temperature. It was then poured into 150 ml. of chloroform, and the barium oxide was filtered off. The chloroform solution was washed with cold 1 *N* sulfuric acid until no more barium sulfate appeared in the interphase, then with water, a saturated solution of sodium hydrogen carbonate, and water, and finally dried with anhydrous sodium sulfate. The aqueous solutions from the different washings were extracted with 100 ml. of chloroform and dried, added to the former chloroform extract, and evaporated to dryness. The residual sirup obtained weighed 1 g. and did not show any spot on paper chromatography; upon strong heating a weak spot of R_g 1.06 appeared. The yield of octa-*O*-methyl-1,1-bis(acetamido)-1-deoxylactitol (IV) was 80%.

This crude product was purified by chromatography on a cellulose column of 580 × 30 mm. using water-saturated 1-butanol as eluent. Eight 50-ml. fractions were collected; in fractions 4–7 practically all the product was eluted as a colorless sirup, which was repeatedly purified by dissolution in water, filtration through activated charcoal, and evaporation to dryness; the same operation was performed many times using ethyl ether as solvent until total elimination of turbidity of the methyl sugar solution was achieved; $[\alpha]^{21}D$ −19° (*c* 0.7, water).

Anal. (for a sample dried at 65° and 0.0001 mm.). Calcd. for $C_{24}H_{46}N_2O_{12}$: C, 51.98; H, 8.30; N, 5.05. Found: C, 52.42; H, 8.24; N, 4.86.

B. Hydrolysis of Octa-*O*-methyl-1,1-bis(acetamido)-1-deoxylactitol (IV).—Compound IV (230 mg.) was dissolved in 10 ml. of 1 *N* sulfuric acid and was heated at 100° in a water bath for 25 min. The solution was neutralized with barium carbonate and evaporated to dryness. The residue was dissolved with ethyl ether and dried exhaustively; yield 140 mg.; paper chromatography gave two distinct spots of 2,3,5,6-tetra-*O*-methyl-D-glucose (R_g 1) and 2,3,4,6-tetra-*O*-methyl-D-galactose (R_g 0.88). The mixture was fractionated on a cellulose column of 360 × 20 mm. and fourteen 5-ml. fractions were collected. Fractions 1–5 gave 20 mg. of starting material; fractions 6–8 gave 50 mg. of 2,3,5,6-tetra-*O*-methyl-D-glucose; fractions 9–10 did not leave any residue; and finally fractions 11–14 gave 70 mg. of 2,3,4,6-tetra-*O*-methyl-D-galactose.

C. Identification of Hydrolytic Fragments.—2,3,5,6-Tetra-*O*-methyl-D-glucose was purified by repeated dissolution in water and in ethyl ether as was described under A for the methylated disaccharide; $[\alpha]^{20}D$ −7.0° (*c* 0.678, water). Irvine, Fyfe, and Hogg[17] gave $[\alpha]^{20}D$ −7.2° (*c* 2.08, water), and Haworth, Porter, and Wayne[18] gave $[\alpha]^{20}D$ −7.6° (*c* 0.9, water). The identification of this sugar was completed by preparation of its aldonic acid lactone and amide, as follows.

2,3,5,6-Tetra-*O*-methyl-D-gluconolactone (V).—2,3,5,6-Tetra-*O*-methyl-D-glucose (30 mg.) was dissolved in 7 ml. of water, and 0.5 ml. of bromine was added. The solution was shaken 24 hr. at room temperature, and the excess bromine was eliminated by aeration. After precipitation of bromide ion with silver oxide, the filtered solution was treated with hydrogen sulfide to eliminate silver ions. The solution was filtered and evaporated to dryness, and the residue was taken up with ethyl ether; $[\alpha]^{21}D$ +63.2° → +31.7° (*c* 0.134, water, 506 hr.). This substance was obtained as a sirup by Humphreys, Pryde, and Waters[19] with $[\alpha]D$ +63.2° → +40.85° (water, 24 days). Haworth, *et al.*,[20] obtained this lactone as a crystalline solid of m.p. 26–27° and $[\alpha]^{21}D$ +62.5° → +32.9° (*c* 1.415, water, 501 hr.).

2,3,5,6-Tetra-*O*-methyl-D-gluconamide.—The lactone V obtained as above (10 mg.) was dissolved in 3 ml. of methanolic ammonia, then left 3 days at 0°, and evaporated to dryness. The residual sirup (8 mg.) had $[\alpha]^{28}D$ +40.4° (*c* 0.136, water). Humphreys, *et al.*,[19] gave $[\alpha]D$ +39.2° (water).

(13) We acknowledge Dr. J. K. N. Jones for carrying out this chromatographic analysis.

(14) R. E. Richards and H. W. Thompson, *J. Chem. Soc.*, 1248 (1947); R. B. Barnes, U. Liddel, and V. Z. Williams, *Ind. Eng. Chem., Anal. Ed.*, **15**, 659 (1943).

(15) P. W. Austin, F. E. Hardy, J. G. Buchanan, and J. Baddiley, *J. Chem. Soc.*, 5350 (1963).

(16) S. M. Partridge, *Nature*, **164**, 443 (1949).

(17) J. C. Irvine, A. W. Fyfe, and T. P. Hogg, *J. Chem. Soc.*, **107**, 524 (1915).

(18) W. N. Haworth, C. R. Porter, and A. C. Wayne, *ibid.*, 2254 (1932).

(19) R. W. Humphreys, J. Pryde, and E. T. Waters, *ibid.*, 1298 (1931).

(20) W. N. Haworth, E. L. Hirst, and E. J. Miller, *ibid.*, 2436 (1927).

The 2,3,4,6-tetra-*O*-methyl-D-galactose obtained in the previously described hydrolysis had $[\alpha]^{21}D$ +107.8° (*c* 2.94, water); the literature[21] gives $[\alpha]D$ +109.5° (water). Its identity was confirmed by preparation of the anilide as follows. The sugar (100 mg.) was dissolved in 1.2 ml. of methanol and 0.5 ml. of freshly distilled aniline was added. The solution was refluxed 2 hr., then was left 24 hr. at +5°, and after dilution with a little ethanol the crystalline solid obtained was centrifuged and washed with cold methanol and with ethyl ether; yield 50 mg. of m.p. and m.m.p. 192°, $[\alpha]^{21}D$ −76.0° (*c* 0.092, acetone); lit.[22] m.p. 192°, $[\alpha]D$ −77.0° (acetone).

D. Methylation of 1,1-Bis(acetamido)-1-deoxycellobiitol.—This substance (500 mg.) was dissolved in 10 ml. of dimethylformamide, then 2.2 g. of barium oxide and 2 ml. of methyl iodide were added, and the suspension was shaken for 6 hr. The technique described under A for 1,1-bis(acetamido)-1-deoxylactitol was followed, and 610 mg. of octa-*O*-methyl-1,1-bis(acetamido)-1-deoxy-cellobiitol (VI) as a sirup finally was obtained in 97.4% yield. This sirup was purified first by chromatography on a cellulose column of 550 × 28 mm. and then by means of repeated dissolutions and evaporations from water and ethyl ether and filtering through activated charcoal until a colorless sirup was obtained. This sirrup had $[\alpha]^{21}D$ +27.0° (*c* 0.37, water).

Anal. (for a sample dried at 65° and 0.0001 mm.). Calcd. for $C_{24}H_{46}N_2O_{12}$: C, 51.98; H, 8.30; N, 5.05. Found: C, 52.00; H, 8.51; N, 4.91.

E. Methylation of 1,1-Bis(acetamido)-1-deoxymaltitol.—This substance (500 mg.) was methylated as described under D for the cellobiitol derivative. Octa-*O*-methyl-1,1-bis(acetamido)-1-deoxymaltitol (VII) (500 mg.) was obtained in 80% yield. The colored sirup was purified through a cellulose column employing water-saturated 1-butanol as eluent. The colorless sirup obtained was treated with water and filtered, and the solution was then evaporated and treated with ethyl ether to obtain a transparent solution. Evaporation of ether gave a sirup of $[\alpha]^{21}D$ +89.9° (*c* 1.35, water).

Anal. (for a sample dried at 65° and 0.0001 mm.). Calcd. for $C_{24}H_{46}N_2O_{12}$: C, 51.98; H, 8.30; N, 5.05. Found: C, 52.31; H, 8.39; N, 5.09.

F. Hydrolysis of VI and VII.—The separate hydrolysis of both substances employing the technique described under B gave the same pair of methylated monosaccharides, *i.e.*, 2,3,5,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose. Both monosaccharides have practically the same R_f and it was impossible to separate them by cellulose and alumina column chromatography. However, a cellulose column of 400 × 20 mm. was used to free the hydrolyzed mixture (100 mg.) from nonhydrolyzed material. The mixture obtained from the column, purified as usual, was submitted to a gas–liquid chromatography at 135° using a column packed with 1 part of Craig polyester (20% on Chromosorb W), 1 part of Apiezon M (20% on Chromosorb W), and 1 part of Apiezon M, 0.1% on glass beads (60–80 mesh). The carrier was argon at 140–150 cc./min. Both hydrolysates gave practically the same pattern. For the hydrolyzed compound VI, the proportion of 2,3,5,6-tetra-*O*-methyl-D-glucose in the mixture was 20.1 and 79.9% for the 2,3,4,6-tetra-*O*-methyl-D-glucose. For hydrolyzed compound VII, the percentages were 20.2 and 79.8%, respectively. The retention time for 2,3,5,6-tetra-*O*-methyl-D-glucose was 0.79, taking 2,3,4,6-tetra-*O*-methyl-D-glucose as reference compound.

G. Separation of 2,3,4,6,-Tetra-*O*-methyl-D-glucose from 2,3,5,6-Tetra-*O*-methyl-D-glucose on Anion-Exchange Resin.[15]—The resin was Permutit Deacidite FF, which was washed with 1 *N* sodium hydroxide and then with water free from carbon dioxide. The mixture of methylated sugars (120 mg.), obtained by hydrolysis of VII, was dissolved in 3 ml. of water and applied to the column (20 × 2 cm.). The elution was carried out with water and 16-ml. fractions were collected at a rate of 5 ml./hr. Evaporation of fractions gave 2,3,4,6-tetra-*O*-methyl-D-glucose (fractions 1–4, 50 mg.), $[\alpha]^{22}D$ +81.3° (*c* 0.92, water, final value); then a mixture of both methyl sugars was obtained (fraction 5, 15 mg.); and finally 2,3,5,6-tetra-*O*-methyl-D-glucose was obtained (fractions 6–10, 30 mg.), $[\alpha]^{22}D$ −11° (*c* 1.05, water). Both sugars gave on paper chromatography a single spot of R_g 1.

When the resin was prepared and allowed to stand 2 weeks under water free from carbon dioxide, the elution of the methyl sugars could not be carried out with water, and the use of pure methanol was necessary to elute the sugars from the column, but no separation was achieved.

(21) J. C. Irvine and A. Cameron, *J. Chem. Soc.*, **85**, 1071 (1904).

(22) R. L. Whistler and H. E. Conrad, *J. Am. Chem. Soc.*, **76**, 1673 (1954).

The Addition of Ethanethiolic Acid to 3β-Acetoxy-5,16-pregnadien-20-one

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On irradiation with ultraviolet light ethanethiolic acid adds *trans* and stereospecifically to the double bonds of 3β-acetoxy-5,16-pregnadien-20-one. The initial attack of the acetylthio radical occurs from the most hindered side (β face) of the molecule. The structures of the adducts are proved by their n.m.r. spectra, rotatory dispersion curves, and chemical reduction products.

Because of the ease[2] with which the Δ^{16}-20-oxo system of steroids adds nucleophilic reagents, a number of small molecules have been added in attempts to enhance certain desirable physiological properties of steroids. Acetone,[3] alcohols,[2,4] amines,[5] diethyl malonate,[6] haloforms,[7] hydrogen cyanide,[8] nitromethane,[9a] and mercaptans[10] have all been added in the presence of base. Ethanethiolic acid,[5b] hydrogen chloride,[9b,5b] mercaptans,[5b] nitromethane,[5b] and vinyl ethers[11] have been added under conditions of acid catalysis. Furthermore, diazomethane,[12] ethyl diazoacetate,[13] meth-

(1) Taken in part from the Ph.D. Thesis of J. R. D., June 1964. This research was partially supported by fellowships from the Sun Oil Co. and The National Science Foundation.

(2) D. F. Fukushima and T. F. Gallagher [*J. Am. Chem. Soc.*, **73**, 196 (1951)] have attributed the ready addition of alcohols to the relief of strain present in the *trans*-fused D-ring of Δ^{16}-20-oxo steroids.

(3) M. E. Wall, S. Serota, H. E. Kenney, and G. S. Abernethy, *ibid.*, **85**, 1844 (1963).

(4) H. Hirschmann, F. B. Hirschmann, and M. A. Daus, *ibid.*, **74**, 539 (1952).

(5) (a) D. Gould, E. L. Shapiro, L. E. Finckenor, F. Gruen, and E. B. Herschberg, *ibid.*, **78**, 3158 (1956); (b) A. S. Hoffman, H. M. Kissman, and M. J. Weiss, *J. Med. Pharm. Chem.*, **5**, 962 (1962).

(6) P. Bladon, *J. Chem. Soc.*, 3723 (1958).

(7) E. Kaspar and R. Wiechert, *Ber.*, **91**, 2664 (1958).

(8) (a) R. H. Mazur and J. A. Cella, *Tetrahedron*, **7**, 130 (1959); (b) J. Romo, *ibid.*, **3**, 37 (1958).

(9) (a) R. M. Dodson, U. S. Patent 2,697,109 (Dec. 14, 1954); (b) R. M. Dodson and P. B. Sollman, U. S. Patent 2,708,201 (May 10, 1955).

(10) (a) J. Romo and G. Conteras, *Bol. Inst. Quim. Univ. Na. Auton. Mex.*, **4**, 101 (1952); (b) J. Romo, M. Romero, C. Djerassi, and G. Rosenkranz, *J. Am. Chem. Soc.*, **73**, 1528 (1951); (c) J. Romo, G. Rosenkranz, and C. Djerassi, *ibid.*, **73**, 4961 (1951).

(11) S. Julia and H. Linares, *Compt. rend.*, **252**, 2560 (1961).

(12) A. Wettstein, *Helv. Chim. Acta*, **27**, 1803 (1944).

(13) G. P. Mueller and B. Riegel, *J. Am. Chem. Soc.*, **76**, 3686 (1954).