The Reaction of Ammonia with Acylated Disaccharides. V. The Wohl Reaction with Octa-O-acetylcellobionic Acid Nitrile

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From the reaction of aqueous ammonia with octa-O-acetylcellobionic acid nitrile, 3-O-β-D-glucopyranosyl-1,1-bis(acetamido)-1-deoxy-D-arabitol (I), 3-O-β-D-glucopyranosyl-N-acetyl-D-arabofuranosylamine (II), and 3-O-β-D-glucopyranosyl-D-arabinose (III) were isolated. The hepta-O-acetyl derivative of I was prepared and the acyclic structure of the nitrogenated moiety was established by oxidation with sodium metaperiodate. By methylation of II, subsequent hydrolysis, and isolation of the methyl sugars produced, the presence of a furanose ring in its D-arabinose moiety was demonstrated.

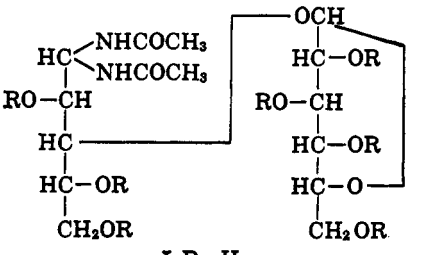
The reaction of acylated nitriles of aldonic acids with ammonia has been extensively studied in the monosaccharide field. The principal product obtained is a monosaccharide with two N-acyl groups on C-1 and one carbon atom less than the original nitrile. These substances have been called "aldose amides," N,N'-diacylaldosylidendiamines, or otherwise 1,1-bis-(acylamido)-1-deoxyglycitols, if they are considered as derivatives of the corresponding polyols.

This reaction was studied at first only with acetylated nitriles of aldonic acids,[1-8] but later Brigl and co-workers[9] and Restelli de Labriola and Deulofeu[10] degraded benzoylated nitriles of monosaccharides, and Deulofeu and Gimenez[11] carried out similar experiments with propionylated nitriles of aldonic acids.

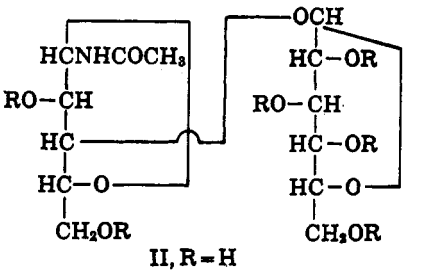
Some systematic studies[12,13] demonstrated that the yields of "aldose amides" depends on the medium in which the reaction is carried out; they are higher in aqueous ammonia than in alcoholic ammonia. Studies developed mainly by Brigl and co-workers[9] and Deulofeu and Deferrari[14] pointed out that the ammonolysis of acylated aldose esters also gave this type of nitrogenated substances. Only one short reference has been published about the reaction of ammonia with acylated nitriles of disaccharides by Zemplén in 1926[15]; he applied the Wohl reaction to octa-O-acetylcellobionic acid nitrile and obtained a nitrogenated sirup that was not studied.

The Wohl degradation of acetylated aldobionic acid nitriles could lead to the obtention of mono- and diamide derivatives of disaccharides with one carbon atom less than the original compound and the infrequent hexosylpentose structure.

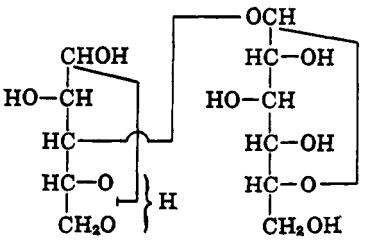
Octa-O-acetylcellobionic acid nitrile treated with 25% aqueous ammonia gave a sirup from which the acetamide formed in the ammonolysis was removed by extraction with ethyl acetate; by treatment with Zeokarb 225 sulfonic resin some basic substances produced were eliminated. By chromatography of the sirup that was obtained on charcoal–Celite,[16] 3-O-β-D-glucopyranosyl-1,1-bis(acetamido)-1-deoxy-D-arabitol (I), m.p. 214–215° dec., $[\alpha]^{20}D$ +25.2° (water), was isolated in 24.2% yield, as well as 3-O-β-D-glucopyranosyl-N-acetyl-D-arabofuranosylamine (II), m.p. 130–131°, $[\alpha]^{23}D$ +72.7° (water), 4.2% yield, and a reducing sugar, 3-O-β-D-glucopyranosyl-D-arabinose (III), m.p. 143–145°, $[\alpha]^{20}D$ +37.4° (water), 0.38%



I, R = H
IV, R = CH_3CO



II, R = H
V, R = CH_3CO



III

yield, whose physical constants did not agree with those described by Zemplén for 3-O-β-D-glucopyranosyl-D-arabinose in his study on the degradation of octa-O-acetylcellobionic acid nitrile with sodium methoxide.[15] The glucosyl-D-arabinose structure of this sugar was established by hydrolysis and identification of the D-glucose and D-arabinose obtained by paper

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(10) E. Restelli de Labriola and V. Deulofeu, *J. Org. Chem.*, **12**, 726 (1947).
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(12) E. Gros, A. Lezerovich, E. F. Recondo, V. Deulofeu, and J. O. Deferrari, *Anales asoc. quím. arg.*, **50**, 185 (1962).
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(15) G. Zemplén, *Ber.*, **59**, 1254 (1926).
(16) R. L. Whistler and D. F. Durso, *J. Am. Chem. Soc.*, **72**, 677 (1950).

chromatography. This substance undoubtedly is an isomer of Zemplén's sugar. This poses the question of stereospecificity in the synthesis of the free aldobiose in the ammonolysis reaction, which would depend on the solvent used. Work to clarify this problem is now in progress.

By acetylation of I with acetic anhydride and pyridine, a crystalline hepta-O-acetyl derivative of m.p. 74–75°, $[\alpha]^{17}D$ +55.3° (chloroform), was obtained which points out an acyclic structure in the nitrogenated moiety. This structure was confirmed by oxidation with sodium metaperiodate[17]; 3 moles of metaperiodate was consumed and 1 mole of formaldehyde was produced by each mole of substance.

The acetylation of II gave a hexa-O-acetyl derivative of m.p. 124–125°, $[\alpha]^{20}D$ −3.95° (chloroform). The furanose structure of the D-arabinose moiety in II was demonstrated by methylation and subsequent hydrolysis of the hexa-O-methyl derivative. By methylation of II with methyl iodide a compound was obtained which, after hydrolysis with 1 *N* sulfuric acid at 100° during 20 hr. and subsequent separation by chromatography on Whatman 3 MM paper, gave 2,5-di-O-methyl-D-arabinose and 2,3,4,6-tetra-O-methyl-D-glucose whose optical rotations agree with those described in the literature.[18,19]

Experimental

A 25% aqueous solution of ammonia was employed. Paper chromatography was carried out on Whatman No. 1 paper, using 1-butanol–ethanol–water (50:10:40 v./v., top layer) as eluent, by the descending technique. D-Glucose was used as standard. The sprays used were (A) silver nitrate–sodium methoxide,[20] and (B) aniline hydrogen phthalate.[21] Evaporations were carried out at reduced pressure and below 60°. Melting points are not corrected.

Reaction of Octa-O-acetylcellobionic Acid Nitrile with Aqueous Ammonia.—Twenty grams of octa-O-acetylcellobionic acid nitrile[15] was suspended in 500 ml. of aqueous ammonia and dissolved by shaking for 3 hr. at room temperature. The solution was allowed to stand at room temperature for 24 hr. and evaporated to dryness, and the sirup was extracted with five 50-ml. portions of warm ethyl acetate to remove acetamide. The sirup was dried, then dissolved in 50 ml. of water, and passed through a column containing 500 ml. of Zeokarb 225 sulfonic resin. To elute the neutral sugars the column was washed with 6 l. of water and, after evaporation of the eluate to dryness, a sirup was obtained which was dried to a powder in a vacuum desiccator. Paper chromatography of this sirup and development of the chromatogram with reagent A showed three spots of R_g 1.16, 0.98, and 0.57. Development with reagent B, to detect reducing sugars, showed only one spot of R_g 0.96.

Isolation of 3-O-β-D-Glucopyranosyl-1,1-bis(acetamido)-1-deoxy-D-arabitol (I).—The sirup obtained was fractionated into its components by chromatography on a column (600 × 50 mm.) of a mixture of Darco G-60–Celite 535 (5:1 by weight). A solution of the sirup in 25 ml. of water was added to the column. To elute the material, the following solvents or mixtures were used: 0.5% ethanol (fractions 1–8, total 1 l.), 1% ethanol (fractions 9–18, total 1 l.), 5% ethanol (fractions 19–22, total 1 l.), 10% ethanol (fractions 23–26, total 1 l.), 20% ethanol (fractions 27–42, total 5 l.), 50% ethanol (fractions 43–47, total 1.25 l.), and finally 1.5 l. of 96% ethanol. Fractions of 250 ml. were collected.

Fractions 1–4 gave only acetamide. Fractions 5–26 gave an amorphous hygroscopic powder which did not crystallize. Fractions 27–31 gave 3.01 g. (24.2%) of I. After three recrystallizations from methanol, prismatic crystals, m.p. 214–215° dec., were obtained, $[\alpha]^{20}D$ +25.2° (*c* 0.99, water). Paper chromatography of this substance and development of the chromatogram with reagent A showed only one spot of R_g 0.57. Development with reagent B, for reducing sugars, did not show any spot, pointing out the nonreducing character of this substance. The analytical sample was dried at 138° and 2 mm.

Anal. Calcd. for $C_{15}H_{28}N_2O_{11}$: C, 43.60; H, 6.84; N, 6.79. Found: C, 43.55; H, 6.86; N, 6.62.

Isolation of 3-O-β-D-Glucopyranosyl-N-acetyl-D-arabofuranosylamine (II).—Fractions 32–33 gave 350 mg. (3.27% yield) of II. After three recrystallizations from methanol, needles, m.p. 130–131°, were obtained, $[\alpha]^{23}D$ +72.7° (*c* 0.50, water). Paper chromatography of this substance and development of the chromatogram with reagent A showed only one spot of R_g 1.10. Development with reagent B did not show any spot. The analytical sample was dried at 111° and 2 mm.

Anal. Calcd. for $C_{13}H_{23}NO_{10}\cdot H_2O$: C, 42.02; H, 6.79; N, 3.72. Found: C, 41.82; H, 6.82; N, 3.76.

Isolation of 3-O-β-D-Glucopyranosyl-D-arabinose (III).—Fractions 34 and following, together with fractions 5–26, gave 2.07 g. of a sirup which was rechromatographed on a column of Darco G-60–Celite 535 (5:1 by weight, 450 × 35 mm.). The column was eluted with water (1.2 l.) and with increasing concentrations of ethanol in water, as follows: 1% (500 ml.), 2% (500 ml.), 4% (700 ml.), 6% (700 ml.), 7% (1 l.), 10% (400 ml.), 12% (300 ml.), 14% (400 ml.), 16% (300 ml.), 18% (500 ml.), and 96% (1 l.). The 1% ethanol fractions gave 30 mg. (0.38% yield) of needles of III, m.p. 143–145°, $[\alpha]^{20}D$ +37.4° (*c* 0.55, water). This substance did not mutarotate after 7 days. The analytical sample was dried at 100° and 2 mm.

Paper chromatography gave only one spot of R_g 0.96 after development with reagents A and B. This pointed out the reducing character of this substance.

Anal. Calcd. for $C_{11}H_{20}O_{10}\cdot H_2O$: C, 39.97; H, 6.74. Found: C, 39.90; H, 6.72.

Fractions containing 2–16% ethanol gave a sirup. The 18% ethanol fractions gave 100 mg. of II, m.p. 130–131°. The total amount of II was 450 mg., yield 4.20%.

Hydrolysis of 3-O-β-D-Glucopyranosyl-D-arabinose (III).—III (10 mg.) was dissolved in 3 ml. of 1 *N* sulfuric acid. The solution was left 1 hr. in a boiling water bath, and then neutralized with barium carbonate, filtered, and concentrated to a volume of 0.5 ml. Paper chromatography gave two reducing spots identifiable with D-arabinose and D-glucose standards.

Hepta-O-acetyl-3-O-β-D-glucopyranosyl-1,1-bis(acetamido)-1-deoxy-D-arabitol (IV).—I (200 mg.) was dissolved in 7 ml. of a 1:1 pyridine–acetic anhydride mixture by heating 3 min. in a boiling water bath. The mixture was allowed to stand for 24 hr. at room temperature and the liquid was evaporated to dryness in a vacuum desiccator. By dissolution of the sirup in 5 ml. of benzene, 260 mg. (76.4% yield), of IV was obtained. Recrystallization from benzene gave needles, m.p. 74–75° (sintering from 60°), $[\alpha]^{17}D$ +55.3° (chloroform). The analytical sample was dried at 100° and 2 mm.

Anal. Calcd. for $C_{29}H_{42}N_2O_{18}$: C, 49.26; H, 5.98; N, 3.96. Found: C, 49.16; H, 5.88; N, 4.19.

Hexa-O-acetyl-3-O-β-D-glucopyranosyl-N-acetyl-D-arabofuranosylamine (V).—II (200 mg.) was dissolved in 7 ml. of a 1:1 mixture of pyridine–acetic anhydride by heating 5 min. in a boiling water bath. The mixture was allowed to stand for 24 hr. at room temperature and then evaporated to dryness in a vacuum desiccator. The sirup was dissolved in 2 ml. of benzene and 250 mg. (73.5% yield) of needles of V was obtained, m.p. 106–107°, which, after three recrystallizations from benzene, had m.p. 124–125°, $[\alpha]^{20}D$ −3.95° (*c* 0.75, chloroform). The analytical sample was dried at 100° and 2 mm.

Anal. Calcd. for $C_{25}H_{35}NO_{16}$: C, 49.56; H, 5.83; N, 2.31. Found: C, 49.30; H, 5.78; N, 2.43.

Oxidation of 3-O-β-D-Glucopyranosyl-1,1-bis(acetamido)-1-deoxy-D-arabitol (I).—This substance (3.48 mg.) was dissolved in 3.14 ml. of a 0.015 *M* solution of sodium metaperiodate. The solution was kept in a thermostat at 30°. Samples of 0.1 ml. were taken at intervals and diluted with water to 25 ml.;

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

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the periodate consumed and the formaldehyde produced were determined according to spectrophotometric methods.[17] Results are given in Table I.

TABLE I

Time, min.	$NaIO_4$ consumed, moles/mole of substance	Formaldehyde, moles/mole of substance
5	1.03	1.02
20	2.27	1.01
50	2.45	1.01
80	2.49	1.00
140	2.52	1.02
240	2.60	1.03
300	2.64	1.01
720	2.79	1.02
900	2.97	1.02
1020	3.00	1.01

Methylation of 3-O-β-D-Glucopyranosyl-N-acetyl-D-arabofuranosylamine (II).—Methyl iodide (1.14 g., 8 × 10^{-3} mole) was added to a solution of 0.030 g. of II (8.4 × 10^{-5} mole) in 3 ml. of dimethylformamide which contained 140 mg. of barium oxide (9.1 × 10^{-4} mole) in suspension. The suspension was shaken for 6 hr. at room temperature and then poured into 20 ml. of chloroform and filtered. The chloroform solution was washed with cold 1 *N* sulfuric acid until no more barium sulfate appeared in the interphase; it was then washed with water, a saturated solution of sodium hydrogen carbonate, and water, and dried with anhydrous sodium sulfate and finally evaporated to dryness. The residual sirup obtained weighed 19.1 mg. and did not show any spot by development of paper chromatograms with aniline hydrogen phthalate.[21] The yield of hexa-O-methyl-3-O-β-D-glucopyranosyl-N-acetyl-D-arabofuranosylamine (VI) was 51.3%.

Hydrolysis of Hexa-O-methyl-3-O-β-D-glucopyranosyl-N-acetyl-D-arabofuranosylamine (VI).—VI (19 mg.) was dissolved in 2 ml. of 1 *N* sulfuric acid and heated in a boiling-water bath for 20 hr. The solution was neutralized with barium carbonate, filtered, and evaporated to dryness. The residue was dissolved with ethyl ether and dried exhaustively; yield, 12 mg. Paper chromatography gave two distinct spots of 2,5-di-O-methyl-D-arabinose (R_g 0.80)[22] and 2,3,4,6-tetra-O-methyl-D-glucose (R_g 1).

The mixture was fractionated on Whatman 3 MM paper and pure 2,5-di-O-methyl-D-arabinose of $[\alpha]^{22}D$ +20.5° (*c* 0.12, water) was obtained; the literature[18] gives $[\alpha]^{20}D$ +20.0° (water). The 2,3,4,6-tetra-O-methyl-D-glucose was also obtained, $[\alpha]^{25}D$ +86.0° (*c* 0.11, water); the literature[19] gives $[\alpha]^{20}D$ +92° → +84.0° (water).

(22) 2,3,4,6-Tetra-O-methyl-D-glucose was employed as standard. We acknowledge Dr. F. Smith's gift of a sample of 2,4-di-O-methyl-D-arabinose for chromatographic comparison.

Three Chemically Related Metabolites of *Streptomyces*. II. Structural Studies[1]

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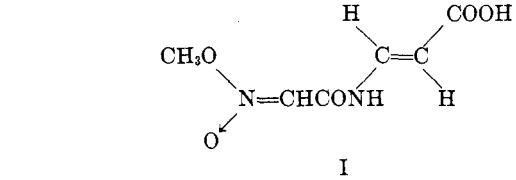
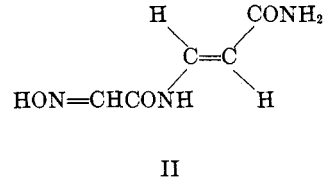
Two new metabolites of *Streptomyces* have been shown to be 3-(oximinoacetamido)acrylamide (II) and 4-(O-methyl-*aci*-nitro)crotonic acid (III). The relationship of these compounds to enteromycin (I, seligocidin) is discussed.

The isolation of three low molecular weight, chemically related compounds from *Streptomyces* fermentation broths has been reported recently.[2] One of these compounds (I) has been found to be identical with the antibiotic enteromycin (seligocidin), reported by Nakamura, Maeda, and Umezawa,[3] and whose structure has been established by Mizuno.[4] The other two compounds, U-15,774 (II) and U-22,956 (III), are new. Enteromycin and U-15,774 are produced by *Streptomyces achromogenes*, which also produces streptozotocin,[5] while U-22,956 is produced by *Streptomyces fervens var. melrosporus*. In spite of the close chemical relationship of these compounds, U-15,774 (II) does not have antibacterial activity, while the other two are quite active against various bacteria.

This paper discusses studies which establish that the structures of U-15,774 and U-22,596 are represented by the expressions II and III, respectively, and which more firmly identify the third compound as being enteromycin (I).



I



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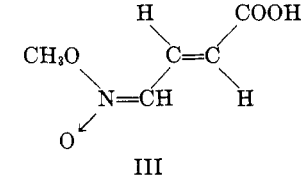

III

The previously reported[2] analytical values obtained from I and the molecular weight, determined by titration of an acidic group, established a molecular formula of $C_6H_8N_2O_5$, as was found by Mizuno[4] for enteromycin. The ultraviolet spectra of the two compounds were identical. The infrared spectrum of I differed from that reported for enteromycin in the 1400–1100-cm.$^{-1}$ region, and there was considerable difference in the reported melting points. However, the melting point reported by Mizuno[4] is very close to that of the acid V obtained by thermal degradation of enteromycin, and it seems likely that the reported value of 172° resulted from formation of V in the process of taking the melting point. A study of the melting point exhibited by I showed that the value found depended a great deal on

(1) A preliminary report of this work has been presented orally: see R. R. Herr, P. F. Wiley, F. A. MacKellar, and A. D. Argoudelis, 4th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, N. Y., Oct. 26–28, 1964.

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