The Reaction of Ammonia with Acylated Disaccharides. VII. The Wohl Reaction with Octa-*O*-acetyllactobiononitrile

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From the reaction of aqueous ammonia with octa-*O*-acetyllactobiononitrile, 3-*O*-(β-D-galactopyranosyl)-D-arabinose, 1,1-diacetamido-1-deoxy-3-*O*-(β-D-galactopyranosyl)-D-arabinitol (I), and *N*-acetyl-3-*O*-(β-D-galactopyranosyl)-D-arabinofuranosylamine (II) were isolated. The acyclic structure of I was established by periodate oxidation and by formation of a hepta-*O*-acetyl derivative. 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.

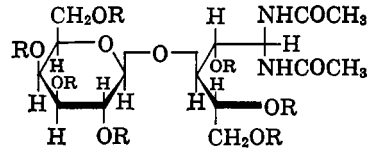
In a previous paper of this series[1] we studied the Wohl degradation applied to octa-*O*-acetylcellobiononitrile. When the Wohl reaction with disaccharides is compared with studies carried out up to the present on the reaction of acylated nitriles of aldonic acids with ammonia,[2] we observe a greater complexity regarding the number of isolated substances. In the monosaccharide field, 1,1-diacylamido-1-deoxyalditols with one carbon atom less than the original nitrile were generally obtained as unique products, although Brigl, Mühlschlegel, and Schinle[3] degraded hexa-*O*-benzoyl-D-*glycero*-D-*galacto*-heptononitrile and obtained not only 1,1-dibenzamido-1-deoxy-D-mannitol, but also an *N*-benzoyl-D-mannopyranosylamine. Another exception was the isolation, by Hockett and Chandler,[4] of an *N*-acetyl-D-glucofuranosylamine from the degradation of hexa-*O*-acetyl-D-*glycero*-D-*gulo*-heptononitrile. The application of column chromatography allowed a more careful study of these complex competitive reactions in the disaccharide field.

Deferrari and Cadenas[5] showed that the reaction of ammonia with acetylated disaccharides with 1,4 glycosidic linkages affords the free sugars as well as 1,1-diacetamido-1-deoxyaldobiitols and *N*-acetylaldobiosylamines; the yields of reducing sugars and nitrogenated compounds depend on the medium in which the reaction is carried out. In this paper we report the reaction of octa-*O*-acetyllactobiononitrile with aqueous ammonia.

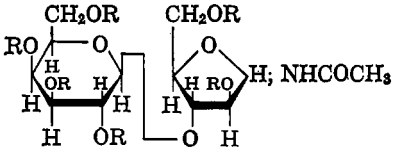
Zemplén[6] synthesized octa-*O*-acetyllactobiononitrile for the first time and obtained a syrup that contained only 65% of the nitrile. We modified Zemplén's original technique and prepared that nitrile with the same properties described by Kuhn and Kirschenlohr.[7]

The reaction of octa-*O*-acetyl-lactobiononitrile with 25% aqueous ammonia gave a syrup from which the acetamide formed in the ammonolysis was removed by repeated extraction with boiling ethyl acetate, and basic substances were eliminated by treatment with Zeokarb 225 sulfonic acid resin. By chromatography on a charcoal–Celite column, 1.9% of a 3-*O*-(β-D-galactopyranosyl)-D-arabinose, m.p. 155–156°, $[\alpha]^{25}D$ +5.3° (final value), 17.6% of 1,1-diacetamido-1-deoxy-3-*O*-(β-D-galactopyranosyl)-D-arabinitol, m.p. 185–186° dec., $[\alpha]^{21}D$ +49.7°, and 4.4% of an *N*-acetyl-3-*O*-(β-D-galactopyranosyl)-D-arabinosylamine, m.p. 174–175° dec., $[\alpha]^{27}D$ +26.0°, were isolated.

The physical constants of our 3-*O*-(β-D-galactopyranosyl)-D-arabinose did not agree with those described by other authors, but the product gave the same osazone.[6-8] We suppose that this substance is an isomer of the already reported sugar. This indicates that the method of synthesis of the free aldobiose governs the stereochemistry of the product. Work to clarify this problem is now in progress. The D-galactosyl-D-arabinose structure of this sugar was established by acid hydrolysis and chromatographic identification on paper of the two monosaccharide units by comparison with pure specimens of D-galactose and D-arabinose employing different solvent systems.



I, R = H
III, R = $COCH_3$



II, R = H
IV, R = $COCH_3$

The acyclic structure in the nitrogenated moiety of 1,1-diacetamido-1-deoxy-3-*O*-(β-D-galactopyranosyl)-D-arabinitol (I) was established by oxidation with sodium metaperiodate[9] in which 3 moles of oxidant was consumed and 1 mole of formaldehyde was produced per mole of sugar. Acetylation of I with acetic anhydride and pyridine gave a hepta-*O*-acetyl derivative, m.p. 71–72°, $[\alpha]^{27}D$ +42.9°.

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Acetylation of the *N*-acetyl-3-*O*-(β-D-galactopyranosyl)-D-arabinosylamine (II) gave a hexa-*O*-acetyl derivative, m.p. 54–56°, $[\alpha]^{20}D$ −11.6°, which suggests a cyclic structure for the D-arabinosylamine moiety. To demonstrate its structure, II was methylated with methyl iodide and barium oxide in dimethylformamide.[10] Subsequent hydrolysis of the methylated product and paper chromatography of the methyl sugars on Whatman 3 MM paper afforded 2,5-di-*O*-methyl-D-arabinose and 2,3,4,6-tetra-*O*-methyl-D-galactose, whose physical constants agree with those described in the literature[11,12]; these facts show that II is an *N*-acetyl-3-*O*-(β-D-galactopyranosyl)-D-arabinofuranosylamine.

Experimental Section

A 25% aqueous solution of ammonia was employed. Paper chromatography was carried out on Whatman No. 1 paper using butan-1-ol–ethanol–water (5:1:4 v./v., top layer) as eluent, by the descending technique. D-Glucose was used as standard. The reagents used were (A) silver nitrate–sodium methoxide[13] and (B) aniline hydrogen phthalate.[14] Evaporations were carried out at reduced pressure and below 60°. Melting points are uncorrected.

Octa-*O*-acetyllactobiononitrile.—Sodium (14 g.) was dissolved in 600 ml. of 96% ethanol and this solution was slowly added with stirring to a solution of 62.5 g. of hydroxylamine hydrochloride in 50 ml. of water previously cooled to −5°. After 0.5 hr. the sodium chloride formed was filtered off and washed with 10 ml. of ethanol.

Lactose monohydrate (100 g.) was dissolved in 100 ml. of warm water, and the ethanolic solution of hydroxylamine was slowly added at 60° to avoid precipitation. After 1 hr. at 65° the reaction mixture was evaporated to a thick syrup. The residue was repeatedly dissolved in methanol, the solution was evaporated in order to eliminate the water present, and the lactose oxime obtained was dried in a vacuum desiccator.

Anhydrous sodium acetate (116 g.) was mixed with the powdered lactose oxime obtained as above and 675 ml. of acetic anhydride was added; the mixture was slowly and cautiously warmed in a water bath and was shaken continually, avoiding a violent reaction by immersing the flask whenever necessary in ice–water. When the reaction had apparently ceased and the syrup had dissolved, the solution was kept at 100° for 1 hr. and then poured into 3 l. of ice–water. After 24 hr. the water was decanted and the gummy product obtained was washed by stirring several times with cold water until a powder was obtained which was filtered and washed with water to neutrality. Crude octa-*O*-acetyllactobiononitrile (167 g., 88.9%) was obtained which by dissolving in 100 ml. of benzene crystallized as hexagonal prisms, m.p. 88–90° (softened at 75°), $[\alpha]^{21}D$ +39.4° (*c* 1.25, chloroform), $[\alpha]^{28}D$ +35.8° (*c* 0.8, methanol). Kuhn and Kirschenlohr[7] give m.p. 90–93, $[\alpha]^{25}D$ +35.5° (methanol). The analytical sample was dried at 111° and 2 mm.

Anal. Calcd. for $C_{28}H_{37}NO_{18}$: C, 49.75; H, 5.52; N, 2.07. Found: C, 49.30; H, 5.35; N, 2.38.

Reaction of Octa-*O*-acetyllactobiononitrile with Aqueous Ammonia.—Octa-*O*-acetyllactobiononitrile (40 g.) was suspended in 1 l. of aqueous ammonia and dissolved by shaking during 13 hr. at room temperature. The solution was allowed to stand 24 hr. and then evaporated to dryness. The syrup obtained was extracted with 50-ml. portions of boiling ethyl acetate to remove the acetamide produced. The residual syrup from these extractions was dissolved in 750 ml. of methanol and the solution was shaken with 200 ml. of Zeokarb 225 sulfonic acid resin during 6 hr. The resin was then separated by filtration, the solution was evaporated to dryness, and the syrup obtained was dried to a powder in a vacuum desiccator. Paper chromatography of this syrup and development of the chromatogram with reagent A showed three spots of R_g 0.92, 0.77, and 0.38. Development with reagent B showed only one reducing spot of R_g 0.92.

Isolation of 3-*O*-(β-D-Galactopyranosyl)-D-arabinose.—The syrup obtained was dissolved in 40 ml. of water and chromatographed on a charcoal–Celite 535 column (5:1 by weight, 550 × 50 mm.). The column was eluted with water (fractions 1–16, total 4 l.) and with increasing concentrations of ethanol in water, as follows: 1.5% (fractions 17–20, total 1 l.), 3% (fractions 21–36, total 4 l.), 5%)fractions 37–48, total 3 l.), 7.5% (fractions 49–56, total 2 l.), 10% (fractions 57–72, total 4 l.), 12% (fractions 73–92, total 5 l.), 15% (fractions 93–104, total 3 l.), 16% (fractions 105–112, total 2 l.), 17.5% (fractions 113–128, total 4 l.), 20% (fractions 129–140, total 3 l.), 22.5% (fractions 141–144, total 1 l.), 25% (fractions 145–152, total 2 l.), 30% (fractions 153–164, total 3 l.), 35% (fractions 165–168, total 1 l.), 60% (fractions 169–172, total 1 l.), and finally 2 l. of 96% ethanol. Fractions of 250 ml. were collected, evaporated separately, taken up with methanol, and combined according to their composition as revealed by paper chromatography.

Fractions 1–33 gave by dissolution in methanol 230 mg. (1.9%) of 3-*O*-(β-D-galactopyranosyl)-D-arabinose as needles of m.p. 155–156°, $[\alpha]^{25}D$ +72° (5 min.) → +5.3° (5 days) (*c* 0.37, water). Paper chromatography of this substance gave only one spot of R_g 0.92 after development with reagents A and B, which pointed out the reducing character of this compound. The analytical sample was dried at 90° and 0.001 mm.

Anal. Calcd. for $C_{11}H_{20}O_{10}\cdot H_2O$: C, 39.97; H, 6.74. Found: C, 39.99; H, 6.80.

Isolation of 1,1-Diacetamido-1-deoxy-3-*O*-(β-D-galactopyranosyl)-D-arabinitol (I).—Fractions 37–88 gave 4.31 g. (17.6%) of I, m.p. 181–184° dec. After two recrystallizations from methanol, needles of m.p. 185–186° dec., $[\alpha]^{21}D$ +49.7° (*c* 0.32, water), were obtained. Paper chromatography of this substance and development of the chromatogram with reagent A showed only one spot of R_g 0.38. Development with reagent B did not show any spot, pointing out the nonreducing character of this substance. The analytical sample was dried at 130° and 2 mm.

Anal. Calcd. for $C_{15}H_{28}N_2O_{11}$: C, 43.66; H, 6.84; N, 6.79. Found: C, 43.31; H, 7.16; N, 6.49.

Isolation of *N*-Acetyl-3-*O*-(β-D-galactopyranosyl)-D-arabinofuranosylamine (II).—Fractions 93–164 gave 930 mg. (4.4%) of II as needles which were recrystallized from methanol, 174–175° dec., $[\alpha]^{27}D$ +26.0° (*c* 0.58, water). Paper chromatography of this substance and spraying the chromatogram with reagent A showed only one spot of R_g 0.77. Spraying with reagent B did not show any spot, pointing out the nonreducing character of III. The analytical sample was dried at 100° and 2 mm.

Anal. Calcd. for $C_{13}H_{23}NO_{10}\cdot 0.5H_2O$: C, 43.06; H, 6.67; N, 3.86. Found: C, 43.05; H, 6.70; N, 4.08.

Characterization of 3-*O*-(β-D-Galactopyranosyl)-D-arabinose. A. Phenylosazone.—A mixture of 50 mg. of the 3-*O*-(β-D-galactopyranosyl)-D-arabinose in 1 ml. of water, 100 mg. of phenylhydrazine hydrochloride, and 150 mg. of sodium acetate was heated in a boiling-water bath. After 30 min. the solution was cooled and the phenylosazone was filtered and recrystallized three times from 80% methanol; it gave m.p. 233–235° dec. Pazur and co-workers[8] gave m.p. 234–236° dec. and Whistler and Yagi[8] gave for the same substance 236° dec. A nitrogen analysis gave 12.01% [calcd. for 3-*O*-(β-D-galactopyranosyl)-D-arabinose phenylosazone, 11.43%].

B. Hydrolysis.—3-*O*-(β-D-Galactopyranosyl)-D-arabinose (10 mg.) was dissolved in 3 ml. of 1 *N* sulfuric acid. The solution was heated 1 hr. in a boiling-water bath, then neutralized with barium carbonate, filtered, and concentrated to a volume of 0.5 ml. Paper chromatography gave two reducing spots which were identifiable with D-arabinose and D-galactose used as standards. These sugars were also compared by paper chromatography employing butan-1-ol–pyridine–water (10:4:3 v./v.) and ethyl acetate–propan-2-ol–water (4:1:2, top layer).

1,1-Diacetamidohepta-*O*-acetyl-1-deoxy-3-*O*-(β-D-galactopyranosyl)-D-arabinitol (III).—I (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 solution was allowed to stand 24 hr. at room temperature and then evaporated to dryness in a vacuum desiccator. The syrup was dissolved in 2 ml. of methanol and, by addition of ethyl acetate to turbidity, 210 mg. (61.2%) of III was obtained as prisms of m.p. 60–62°. After two recrystallizations from methanol–ethyl acetate, it gave m.p. 70–71°,

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TABLE I

Time, min.	$NaIO_4$ consumed, moles/mole of substance	Formaldehyde, moles/mole of substance
5	1.22	0.99
15	1.75	1.01
30	1.91	1.01
60	2.29	1.00
120	2.37	1.01
300	2.94	1.01
1440	2.97	1.02

$[\alpha]^{27}D$ +42.9° (*c* 0.55, chloroform). The analytical sample was dried at 30° and 2 mm.

Anal. Calcd. for $C_{29}H_{42}N_2O_{18}$: C, 49.26; H, 5.98; N, 3.96. Found: C, 49.32; H, 6.20; N, 4.30.

N-Acetylhexa-O-acetyl-3-O-(β-D-galactopyranosyl)-D-arabinofuranosylamine (IV).—II (100 mg.) was dissolved in 3.6 ml. of a 1:1 mixture of pyridine–acetic anhydride by heating 5 min. in a boiling-water bath. The mixture was left 24 hr. at room temperature and then evaporated to dryness in a vacum desiccator. The syrup was dissolved in 1.5 ml. of methanol, giving IV as prisms of m.p. 54–56°, $[\alpha]^{20}D$ −11.6° (*c* 0.6, chloroform). The analytical sample was dried at 80° and 2 mm.

Anal. Calcd. for $C_{25}H_{39}NO_{16}$: C, 49.56; H, 5.83; N, 2.31. Found: C, 49.77; H, 5.81; N, 2.63.

Oxidation of 1,1-Diacetamido-1-deoxy-3-O-(β-D-galactopyranosyl)-D-arabinitol.—This substance (3.68 mg.) was dissolved in 3.42 ml. of a 0.015 *M* solution of sodium metaperiodate. The solution was held at 30°. Samples of 0.1 ml. were taken at intervals and diluted with water to 25 ml.; the periodate consumed and the formaldehyde produced were determined according to spectrophotometric methods.[9] Results are given in the Table I.

Methylation of N-Acetyl-3-O-(β-D-galactopyranosyl)-D-arabinofuranosylamine.—Methyl iodide (2.28 g., 1.6×10^{-2} mole) was added to a solution of 80 mg. of II (2.24×10^{-4} mole) in 3 ml. of dimethylformamide which contained 500 mg. of barium oxide (3.25×10^{-3} mole) in suspension. The suspension was shaken for 10 hr. at room temperature and then poured into 100 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, dried with anhydrous sodium sulfate, and finally evaporated to dryness. The residual syrup weighed 90 mg. and did not show any spot by development of paper chrmatograms with reagent B.

Hydrolysis of the Methylated N-Acetyl-3-O-(β-D-galactopyranosyl)-D-arabinofuranosylamine.—The methyl derivative of II (90 mg.) obtained as above was dissolved in 5 ml. of 1 *N* sulfuric acid and heated in a boiling-water bath during 6 hr. The solution was neutralized with barium carbonate, filtered, and evaporated to dryness. The residue was taken up with ethyl ether, the solution was evaporated, and the residue was dried exhaustively, yield 78.4 mg. Paper chromatography[15] gave two spots of 2,5-di-*O*-methyl-D-arabinose (R_g 0.80) and 2,3,4,6-tetra-*O*-methyl-D-galactose (R_g 0.86).

The mixture was fractionated on Whatman 3 MM paper and pure 2,5-di-*O*-methyl-D-arabinose of $[\alpha]^{35}D$ +21.3° (*c* 0.32, water) was obtained [lit.[11] $[\alpha]^{20}D$ +20.0° (water)]. 2,3,4,6-Tetra-*O*-methyl-D-galactose was also obtained, $[\alpha]^{32}D$ +115° (final value) (*c* 0.29, water) [lit.[12] $[\alpha]^{20}D$ +150° → +114° (water)].

(15) 2,3,4,6-Tetra-*O*-methyl-D-glucose was employed as standard.

Studies on Condensed Aromatic Nitrogenous Compounds. XXV. Product Distribution in Ribosylation of Purines and Deazapurines by the Mercuri Method[1,2]

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Product distribution in ribosylation of several heterobicycles, including purines, by the mercuri method was closely examined by use of alumina, gas, and paper chromatography. Ribosylation of purine (IV) and 3*H*-imidazo[4,5-*b*]pyridine (V) afforded, in addition to already reported major products, a minor amount of isomeric products, XIX and XVII, respectively. The ribosylation of 6-bromo-3*H*-imidazo[4,5-*b*]pyridine (VI) also gave a minor amount of 7*H* isomer as well as a predominant amount of 9*H* isomer. On the other hand, the 3-deaza analogs of the purines examined (compounds VII, VIII, and IX) gave on ribosylation each of two possible isomeric products in almost equal amounts. A common structural feature of bases whose ribosylation gave one of the two possible isomers in predominant amounts is that they have a nitrogen atom adjacent to the imidazole ring. Bases which have no nitrogen adjacent to the imidazole ring gave rise to each of two possible isomers in about equal amounts. In connection with the present investigation, several new ribonucleosides were prepared, such as XIV, XV, XVII, XIX, XXII, and XXIII.

The synthesis of purine nucleosides by the condensation of poly-*O*-acylglycosyl halides with the heavy metal salts of purines has wide application.[4] One of the main features of this reaction is that position 9 of purines undergoes substitution.[4b] Several exceptions have been known: some of them are theophylline (I),[5] 3-benzylhypoxanthine (IIa),[6a] 6-benzoylamido-3-benzyl- (IIb),[6b] and 6-dimethylamino-2,8-dimethylthiopurine (III).[7] It has been reported that ribosylation of I, IIa, and IIb showed a high preference for respective 7*H* isomers,[5,6] whereas ribosylation of III afforded twice as much 9 isomer as 3 isomer.[7] On the other hand, Mizuno and co-workers[8] have shown that ribosylation of 5-nitrobenzimidazole (VIII) gave

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