[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, HARVARD MEDICAL SCHOOL, AND THE MASSACHUSETTS GENERAL HOSPITAL]

3-Amino-3-deoxy-D-idose and 3-Amino-3-deoxy-D-gulose[1]

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Methyl 3-acetamido-3-deoxy-α-D-idopyranoside, obtained by ammonolysis of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-talopyranoside, was transformed into 3-acetamido-1,6-anhydro-3-deoxy-β-D-idopyranose, which was identical with the product obtained in a side reaction of the ammonolysis of 1,6:2,3-dianhydro-β-D-talopyranose. 3-Amino-3-deoxy-D-gulose hydrochloride and 3-amino-1,6-anhydro-3-deoxy-β-D-gulose hydrochloride were obtained from methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-idopyranoside.

The recent isolation of 3-amino-3-deoxy-D-glucose as a constituent of the antibiotic kanamycin,[4] and of 3,6-diamino-3,6-dideoxy-D-glucose from the antibiotic neomycin,[5] after the isolation of 3-amino-3-deoxy-D-ribose from puromycin,[6] has brought interest to the 3-amino-3-deoxy-D-hexoses. Whereas all the 2-amino-2-deoxy-D-hexoses have now been synthesized, only the 3-amino-3-deoxy derivatives of D-allose,[7,8] D-altrose,[9] D-glucose,[10] and D-idose[11,12] are known. In the present publication the synthesis of a new 3-amino-3-deoxy-D-hexose, 3-amino-3-deoxy-D-gulose is described, as well as new derivatives of 3-amino-3-deoxy-D-idose.

In the preceding publication,[13] the synthesis of methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-idopyranoside (III) has been described, using the ammonolysis of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-talopyranoside or of methyl 4,6-*O*-benzylidene-2,3-di-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside. Removal of the benzylidene group of III gave the crystalline methyl 3-acetamido-3-deoxy-α-D-idopyranoside (I). A better yield of I was obtained by purification through the crystalline 2,4,6-tri-*O*-acetyl derivative II. It is possible that the conditions used to remove the benzylidene group, 60% acetic acid, may produce some acetylation[14] of the primary hydroxyl group, resulting in the contamination of the final product, I, with its 6-*O*-acetyl derivative. Action of hydrochloric acid on I resulted in the formation in high yield of the 1,6-anhydro derivative, which was characterized through the formation of its *N*-acetyl derivative IX possessing a low solubility and excellent properties of crystallization. Very little of the 3-amino-3-deoxy-D-idose (as its *N*-acetyl derivative) could be detected in the mother liquors of IX by paper chromatography. Thus, 3-amino-3-deoxy-D-idose forms an anhydro derivative as easily as the 2-amino-2-deoxy derivative[13,15] or the parent sugar, D-idose.[16] The same 3-acetamido-1,6-anhydro-3-deoxy-β-D-idopyranose (IX) was obtained by alkaline hydrolysis of 3-acetamido-2,4-di-*O*-acetyl-1,6-anhydro-3-deoxy-β-D-idopyranose (X), a side product of the ammonolysis of 1,6:2,3-dianhydro-β-D-talopyranose (XI).[12] The ease of formation, as well as the properties of crystallization of the 1,6-anhydro derivative IX, makes it an ideal compound for the characterization of 3-amino-3-deoxy-D-idose.

The synthesis of 3-amino-3-deoxy-D-gulose hydrochloride XII was achieved using the method of inversion introduced by Baker, *et al.*[17] in the carbohydrate field and already used in the synthesis of a 3-amino-3-deoxy-D-hexose derivative, methyl 3-acetamido-2,4,6-tri-*O*-acetyl-3-deoxy-α-D-allopyranoside.[18] The crystalline 2-mesylate IV

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(3) Special Investigator of the Arthritis and Rheumatism Foundation.

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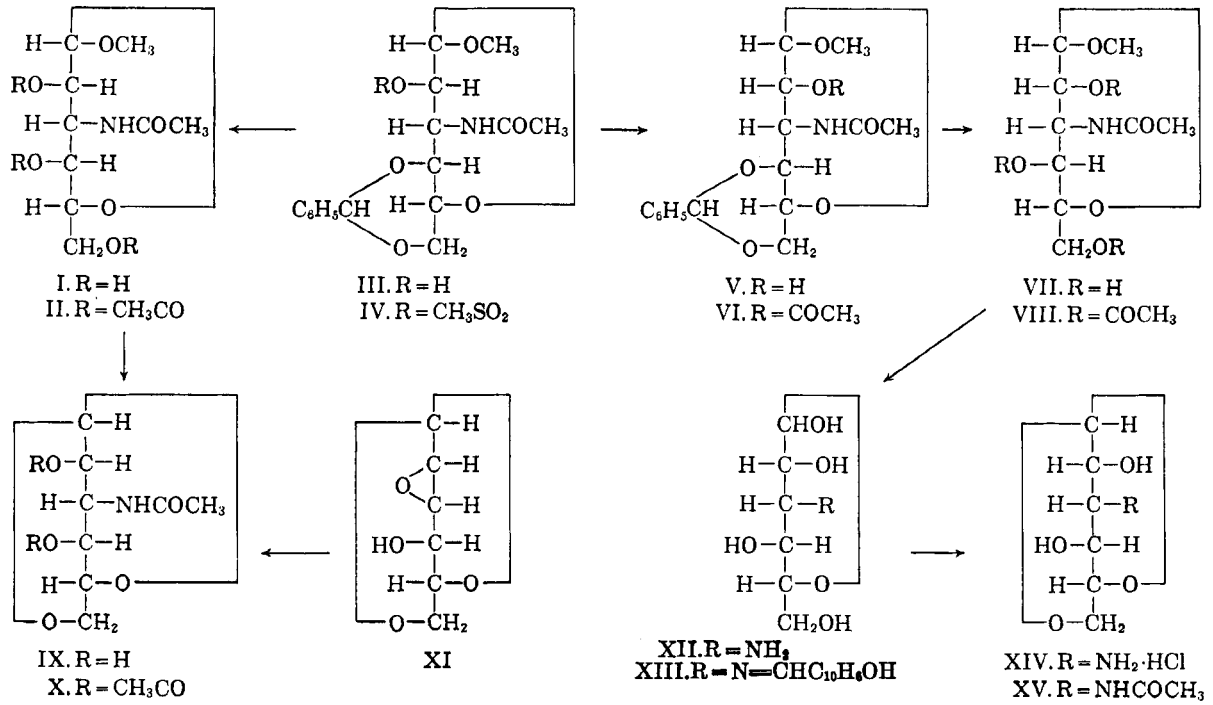

reacted very easily with sodium acetate in Methyl Cellosolve solution to give methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-gulopyranoside (V) with the best yield so far obtained in this type of transformation (97%). It is noteworthy that the spatial configuration of the carbons at positions 2 and 3 involved in the inversion are in opposite conformation (axial) to those of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methylsulfonyl-α-D-galactopyranoside, the only 4,6-*O*-benzylidene derivative of a 2 or 3-aminodeoxy sugar which has resisted inversion under the standard conditions used.[19] Removal of the benzylidene group of V gave methyl 3-acetamido-3-deoxy-α-D-gulopyranoside (VII). As described above for the idoside derivative, a better yield was obtained by purification through the 2,4,6-tri-*O*-acetyl derivative VIII. The effect of secondary acetylation was, however, less important.

When the glycoside VII was hydrolyzed with 2*N* hydrochloric acid at 100°, three different compounds were formed, as detected by paper chromatography (Fischer-Nebel solvent system[20]; Whatman No. 1 and No. 54 papers). The compound moving most slowly had a $R_{Glucosamine}$ of 1.06, reacted strongly with the aniline phthalate and alkaline silver reagents, and gave a purple color with ninhydrin. This compound was formed when hydrolysis was carried out for thirty minutes; but when hydrolysis lasted two or six hours, it could be detected in small quantities only. On the basis of its properties, it was assumed to be 3-amino-3-deoxy-D-gulose (XII). The second substance formed had a $R_{Glucosamine}$ of 1.28 to 1.33; it gave a purple spot with ninhydrin, reacted very slowly with the alkaline silver reagent, and not at all with aniline phthalate. The identity of this substance has not been established. The fastest moving compound had a $R_{Glucosamine}$ of 1.57 (R_f 0.45); it did not react with aniline phthalate, and only slowly with the alkaline silver reagent; with ninhydrin it gave a brown color. Judging from the intensity of the ninhydrin reaction on samples hydrolyzed thirty minutes, two hours and six hours, it was inferred that formation of this third compound was favored by duration of hydrolysis.

These various observations indicated this fast moving substance to be 3-amino-1,6-anhydro-3-deoxy-β-D-gulose. It has been isolated as a crystalline hydrochloride (XIV), obtained in 58% yield after a ten-hour hydrolysis of VII. The tendency to form a 1,6-anhydro ring seems, at least qualitatively, to be much greater for the 3-amino-3-deoxy derivative than for the 2-amino-2-deoxy derivative,[21,22] and in the same order of magnitude as for the parent sugar.[23] The ease of formation of the anhydro derivative XIV as well as its 3-acetamido derivative XV makes them ideal compounds for the identification of 3-amino-3-deoxy-D-gulose. Characterization of 3-amino-3-deoxy-D-gulose (XII) was obtained by reaction of 2-hydroxynaphthaldehyde[24] with the product of

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hydrolysis of VII, after most of the anhydro derivative XIV had been removed by crystallization. This gave the Schiff's base (XIII) with two characteristically colored forms of crystals.

EXPERIMENTAL

Melting points were taken on a hot stage, equipped with a microscope, and correspond to "corrected melting point." Rotations were determined in semimicro or micro (for amounts smaller than 3 mg.) tubes with lengths of 100 or 200 mm., using a Rudolph Photoelectric Polarimeter Attachment, Model 200; the chloroform used was A. R. grade and contained approximately 0.75% of ethanol. Chromatograms were made with the flowing method using silicic acid; "Silica Gel Davison," from the Davison Co., Baltimore 3, Md. (grade 950; 60–200 mesh) was used without pretreatment. When deactivation by contact with moist air occurred, reactivation was obtained by heating to 170–200° (manufacturer's instructions). The sequence of eluants was hexane, benzene or chloroform, ether, ethyl acetate, acetone, and methanol individually or in binary mixtures. The proportion of weight of substance to be adsorbed to weight of adsorbent was 1 to 50–100. The proportion of weight of substance in g. to volume of fraction of eluant in ml. was 1 to 100. The ratio of diameter to length of the column was 1 to 20. Evaporations were carried out *in vacuo*, with an outside bath temperature kept below 45°. Amounts of volatile solvent smaller than 20 ml. were evaporated by blowing dry nitrogen. The microanalyses were done by Dr. K. Ritter, Basel, and Dr. M. Manser, Zurich, Switzerland.

Methyl 3-acetamido-2,4,6-tri-O-acetyl-3-deoxy-α-D-idopyranoside (II). A solution of 200 mg. of methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-idopyranoside (III)[13] in 6 ml. of glacial acetic acid was heated on the water bath and 4 ml. of water was added slowly. After 1 hr. the solution was cooled and evaporated to dryness. To the crystalline residue was added 0.5 ml. of anhydrous pyridine and 0.3 ml. of acetic anhydride. After standing at room temperature overnight absolute ethanol was added to decompose the excess of anhydride. The solution was evaporated to dryness, the last traces of pyridine being removed by codistillation with toluene. The crystalline residue was recrystallized from a mixture of methanol and ether and from a mixture of acetone, ether, and pentane to give 203 mg. (93%) of prismatic needles, m.p. 134–135°, $[\alpha]_D^{25}$ +48 ± 1° (in chloroform, *c* 1.60).

Anal. Calcd. for $C_{15}H_{23}O_9N$: C, 49.86; H, 6.42. Found: C, 49.80; H, 6.55.

Methyl 3-acetamido-3-deoxy-α-D-idopyranoside (I). To a precooled solution at 0° of 150 mg. of II in 1 ml. of methanol was added 0.1 ml. of 1*N* barium methoxide. After standing overnight at 0°, the solution was evaporated to dryness. The residue was dissolved in water, and the solution was passed through Dowex 50 in the acid form. After evaporation, the residue was dissolved in methanol, filtered through Celite and Darco G-60 and evaporated. The crystalline residue was recrystallized from a mixture of acetone and ether to give 80 mg. (82%) of elongated prisms, m.p. 146–148°. From a mixture of methanol and ether, a second crystalline form, needles, was obtained, m.p. 157–158°, $[\alpha]_D^{29}$ +66 ± 1° (in methanol, *c* 0.67).

Anal. Calcd. for $C_9H_{17}O_6N$: C, 45.95; H, 7.28. Found: C, 45.80; H, 7.26.

Direct preparation of I from III, without purification through the intermediate 2,4,6-triacetate II, gave low yields (about 20%).

3-Acetamido-1,6-anhydro-3-deoxy-β-D-idopyranose (IX). *a*) *From* X. A solution of 180 mg. of 3-acetamido-2,4-di-*O*-acetyl-1,6-anhydro-3-deoxy-β-D-idopyranose (X)[12] in 2.5 ml. of 0.2*N* sodium methoxide was refluxed for a few minutes. After removal of the cations and purification as described above, the crystalline residue was recrystallized from methanol to give 128 mg. (99%) of elongated prisms, m.p. 245–246°, $[\alpha]_D^{26}$ −96 ± 1° (in methanol, *c* 1.54).

Anal. Calcd. for $C_8H_{13}O_5N$: C, 47.29; H, 6.45. Found: C, 47.30; H, 6.59.

b) *From* I. A solution of 60 mg. of I in 0.5 ml of 2*N* hydrochloric acid was heated in a sealed tube for 6 hr. at 100°. After evaporation to dryness the last traces of hydrochloric acid were removed by codistillation with toluene. The product failed to crystallize and it was *N*-acetylated by dissolving it in 2 ml. of methanol and adding 70 mg. of silver acetate and 0.1 ml. of acetic anhydride. After standing overnight at room temperature the mixture was refluxed for a few minutes, then filtered through Celite and Darco G-60 and evaporated. The crystalline residue was recrystallized from a mixture of methanol and ether to give 37 mg. (70%) of prismatic needles, m.p. 243–244°, $[\alpha]_D^{26}$ −95 ± 1° (in methanol, *c* 0.61). In admixture with the product described under *a*) the melting point was not depressed.

Examination of the residual mother liquors by descending paper chromatography on Whatman No. 54 paper showed only traces of material reacting with silver nitrate. It had a $R_{Glucosamine}$ of 1.60 in the mixture pyridine, ethyl acetate, acetic acid, water in the proportions 5:5:1:3 (at the bottom of the tank: pyridine, ethyl acetate, and water in proportions 11:40:6)[20] ($R_{Glucosamine}$ of *N*-acetyl-D-glucosamine 1.52), and a $R_{Glucosamine}$ of 1.13 in the mixture propanol-water-ammonia 70:29:1 ($R_{Glucosamine}$ of *N*-acetyl-D-glucosamine 1.10). This material is probably 3-acetamido-3-deoxy-D-idose.

Examination by descending paper chromatography of the sirupy 3-amino-1,6-anhydro-3-deoxy-β-D-idopyranose hydrochloride gave the following results: In the Fischer-Nebel mixture (see above)[20] on Whatman No. 1 paper, the substance traveled with a R_f of 0.46, compared to D-glucosamine hydrochloride 0.29, D-galactosamine hydrochloride 0.25, 2-amino-1,6-anhydro-2-deoxy-β-D-galactopyranose hydrochloride[12] 0.38, 2-amino-1,6-anhydro-2-deoxy-β-D-gulopyranose hydrochloride[21] 0.41. In the mixture propanol, water, and ammonia in the proportions 70:29:1, on Whatman No. 1 paper, the above substances traveled with the following respective R_f: 0.67, 0.52; 0.47; 0.58; 0.65.

Methyl 3-acetamido-4,6-O-benzylidene-3-deoxy-2-O-methylsulfonyl-α-D-idopyranoside (IV). To a solution of 500 mg. of III in 10 ml. of anhydrous pyridine cooled at −20° was added 0.3 ml. (2.5 moles) of methanesulfonyl chloride. After standing 48 hr. at 0° the excess chloride was decomposed by ice and the solution diluted with 200 ml. of water. After a few hours at 0° the crude product was filtered and the mother liquors were evaporated to dryness (472 mg.). The residue was extracted with chloroform, the solution washed two times with a saturated sodium bicarbonate solution, then with water, dried over sodium sulfate, and evaporated to dryness (123 mg.). Both residues were combined, dissolved in methanol, and the solution filtered through Darco G-60 and Celite. The residue left after evaporation was recrystallized from mixtures of chloroform, methanol, and pentane or acetone, ether, and pentane to give 570 mg. (92%) of hexagonal prisms or iridescent hexagonal plates, m.p. 198–200°, with slight decomposition, $[\alpha]_D^{24}$ +78° ± 1° (in chloroform, *c* 1.56).

Anal. Calcd. for $C_{17}H_{23}O_8NS$: C, 50.86; H, 5.77; S, 7.99. Found: C, 50.83; H, 5.91; S, 8.13.

Methyl 3-acetamido-4,6-O-benzylidene-3-deoxy-α-D-gulopyranoside (V). A solution of 500 mg. of IV and 500 mg. of sodium acetate trihydrate in 50 ml. of 95% Methyl Cellosolve was heated under reflux for 2 days. After concentration to dryness, the residue was suspended in water and extracted with chloroform. The chloroform layer was washed two times with water, dried with sodium sulfate, and

(24) Z. E. Jolles and W. T. J. Morgan, *Biochem. J.*, 43, 1183 (1940).

evaporated. The crystalline residue was recrystallized from acetone or from a mixture of methanol, ether, and pentane to give 390 mg. (97%) of needles, m.p. 181–182°, $[\alpha]_D^{26}$ +91 ± 2° (in chloroform, *c* 1.13).

Anal. Calcd. for $C_{16}H_{21}O_8N$: C, 59.43; H, 6.55. Found: C, 59.36; H, 6.63.

Acetylation of 30 mg. of V with acetic anhydride and pyridine in the usual way gave the *2-O-acetyl* derivative (VI). Crystallization from a mixture of acetone, ether, and pentane gave 32 mg. (95%) of rectangular plates, m.p. 149–150°, $[\alpha]_D^{26}$ +89 ± 2° (in chloroform, *c* 0.52).

Anal. Calcd. for $C_{18}H_{23}O_7N$: C, 59.17; H, 6.34. Found: C, 59.23; H, 6.41.

Methyl 3-acetamido-2,4,6-tri-O-acetyl-3-deoxy-α-D-gulopyranoside (VIII). A solution of 200 mg. of V in 6 ml. of glacial acetic acid was heated on the water bath and 4 ml. of water was added slowly. After 1 hr. the solution was cooled and evaporated to dryness. To the crystalline residue was added 0.5 ml. of anhydrous pyridine and 0.3 ml. of acetic anhydride. After standing at room temperature overnight absolute ethanol was added to decompose the excess anhydride. The solution was evaporated to dryness, the last traces of pyridine being removed by codistillation with toluene. The sirupy residue was dissolved in methanol and the solution was filtered through Celite and Darco G-60 to give, after evaporation, a crystalline residue. Recrystallization from a mixture of acetone and ether afforded 233 mg. (97%) of prismatic needles, m.p. 158–159°, $[\alpha]_D^{24}$ +86 ± 1° (in chloroform, *c* 1.52).

Anal. Calcd. for $C_{15}H_{23}O_9N$: C, 49.86; H, 6.42. Found: C, 49.98; H, 6.49.

Methyl 3-acetamido-3-deoxy-α-D-gulopyranoside (VII). To a cold solution of 130 mg. of VIII in 2 ml. of methanol was added 0.5 ml. of 1*N* sodium methoxide. After standing overnight at 0° the solution was evaporated to dryness. The residue was dissolved in water and the solution was passed through a column of Dowex 50 in the acid form and evaporated to dryness. The residue was dissolved in methanol and the solution filtered through Celite and Darco G-60 and evaporated. The crystalline residue was recrystallized from methanol to give 84 mg. (98%) of prismatic needles, m.p. 175–176°, $[\alpha]_D^{26}$ +87 ± 1° (in methanol, *c* 1.90).

Anal. Calcd. for $C_9H_{17}O_6N$: C, 45.95; H, 7.28. Found: C, 45.97; H, 7.28.

When VII was obtained directly from V without purification through the 2,4,6-tri-*O*-acetyl derivative VIII, the yield was only 69%.

Hydrolysis of methyl 3-acetamido-3-deoxy-α-D-gulopyranoside (VII).[25] Samples of 0.5 mg. of VII were hydrolyzed in sealed tubes with 0.02 ml. of 2*N* hydrochloric acid at 100° for 30 min., 2 hr., and 6 hr. The hydrolyzates were diluted with 2 to 3 drops of absolute ethanol, and the solvents evaporated in a vacuum desiccator over calcium chloride and sodium hydroxide. After 15 min. the dry residues were dissolved in 0.05 ml. of water and separated into five portions of 0.1 mg. each of which were chromatographed on Whatman No. 1 and No. 54 papers. The chromatography was done by the descending method in the solvent system of Fischer and Nebel.[20] The reference products were D-glucosamine hydrochloride, D-galactosamine hydrochloride, 2-amino-1,6-anhydro-2-deoxy-β-D-gulopyranose hydrochloride,[21] 3-amino-1,6-anhydro-3-deoxy-β-D-gulopyranose hydrochloride (XIV) (see below) and the mother liquor of the preparation of XIV. The spots were detected on Whatman No. 54 paper with the alkaline silver reagent, and on Whatman No. 1 paper with ninhydrin and with aniline phthalate.

Glucosamine and galactosamine ($R_{Glucosamine}$ 0.85) gave spots with the three reagents (purple with ninhydrin, brown with aniline phthalate). The 2-amino-1,6-anhydro-2-deoxy-β-D-gulopyranose ($R_{Glucosamine}$ 1.46) gave a purple spot with ninhydrin, reacted slowly with the alkaline silver reagent, but gave no color with aniline phthalate. The 3-amino-1,6-anhydro-3-deoxy-β-D-gulopyranose ($R_{Glucosamine}$ 1.59) gave a brown spot with ninhydrin, reacted slowly with the alkaline silver reagent, but gave no color with aniline phthalate. The 30-min. hydrolyzate was observed to contain three different substances: 1) a product of $R_{Glucosamine}$ 1.05, reacting strongly with the three reagents used; 2) a product of $R_{Glucosamine}$ 1.33, detected only with ninhydrin (pale pink spot); and 3) a product of $R_{Glucosamine}$ 1.57, giving a brown spot with ninhydrin, and not detected by the other two reagents. The 2-hr. hydrolyzate gave only two spots: $R_{Glucosamine}$ 1.06 (weak) and $R_{Glucosamine}$ 1.57 (stronger). The 6-hr. hydrolyzate gave weak spots of $R_{Glucosamine}$ 1.05 and 1.29, and a strong spot of $R_{Glucosamine}$ 1.57 (brown color with ninhydrin). The identity of the substance having a $R_{Glucosamine}$ 1.29–1.33 could not be ascertained, but the spot of $R_{Glucosamine}$ 1.05–1.06 was seen to represent 3-amino-3-deoxy-D-gulose (XII), and that of $R_{Glucosamine}$ 1.57 the 1,6-anhydro derivative of XII.

3-Amino-1,6-anhydro-3-deoxy-β-D-gulose hydrochloride (XIV). A solution of 80 mg. of VII in 10 ml. of 2*N* hydrochloric acid was heated in a sealed tube at 100° for 10 hr. After evaporation to dryness the residue was dissolved in methanol and the solution was filtered through Celite and Darco G-60. After evaporation the crystalline residue was recrystallized from a mixture of methanol and absolute ethanol to give 42 mg. (58%) of very small white prisms. At 140–150° the compound became yellow; above 200° it sublimed into prismatic needles, melting with decomposition at 245–248°. The product showed no mutarotation from 5 min. until 22 hr., $[\alpha]_D^{26}$ +44 ± 2° (in water, *c* 0.51). Dried at 60° for 4 hr. in high vacuum or in a desiccator at room temperature, the product gave identical analytical values.

Anal. Calcd. for $C_6H_{12}O_4NCl$: C, 36.47; H, 6.12; Cl, 17.94. Found: C, 36.06; H, 6.31; Cl, 17.61.

3-Acetamido-1,6-anhydro-3-deoxy-β-D-gulopyranose (XV).[25] The mother liquors of the preparation of XIV (40 mg.) were equilibrated by heating in a sealed tube with 0.5 ml. of 2*N* hydrochloric acid at 100° for 6 hr. After concentration and drying by repeated additions of absolute ethanol and toluene, the residue was dissolved in 2 ml. of methanol and 70 mg. of silver acetate and 0.2 ml. of acetic anhydride were added. After standing overnight at room temperature, the mixture was refluxed for a few minutes, then filtered over Celite and 2 drops of 2*N* hydrochloric acid were added to the filtrate. After filtration over Celite and Darco G-60, the filtrate was concentrated to dryness by repeated additions of absolute ethanol and toluene to give 40 mg. of a yellow sirup. It was chromatographed on silicic acid. A mixture of ethyl acetate and acetone 1:1 eluted 16 mg. of crystalline fractions. Recrystallization from a mixture of methanol, acetone and ether gave 11 mg. of small prisms, m.p. 203–204°, $[\alpha]_D^{26}$ + 81 ± 3° (in methanol, *c* 1.80).

Anal. Calcd. for $C_8H_{13}O_5N$: C, 47.29; H, 6.45. Found: C, 47.17; H, 6.70.

The sirupy fractions eluted with pure acetone were not further investigated.

3-Deoxy-3-(2'-hydroxynaphthylideneamino)-D-gulose (XIII). To a solution of 34 mg. of the mother liquors of XIV in 1 ml. of methanol was added 27 mg. of sodium acetate and 60 mg. of 2-hydroxynaphthaldehyde. The solution was treated as previously described.[26] In the purification by chromatography on silicic acid, the mixture ethyl acetate and acetone 9:1 eluted crystalline fractions. Recrystallization from a mixture of acetone and ether gave 35 mg. (68%) of two interchangeable forms of crystals. In a very concentrated solution elongated red-orange prisms deposited rapidly, m.p. 160–165°. In a dilute solution small bushes of

(25) This experiment was carried out by Dr. A. Rapin.

(26) R. W. Jeanloz, *J. Am. Chem. Soc.*, **74**, 4597 (1952).

yellow prisms deposited slowly, m.p. 229–230°. The fused red form recrystallized into the yellow form. The product showed no mutarotation from 4 min. to 16 hr., $[\alpha]_{5461}^{24}$ +204 ± 3° (in methanol, *c* 0.37).

Anal. Calcd. for $C_{17}H_{19}O_6N$: C, 61.26; H, 5.75. Found C, 61.21; H, 5.82.

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[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[2]]

Alkali Sensitivity of Polysaccharides: Periodate Starches, Periodate Dextran and a Polygalacturonide[1]

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Reactions in alkali of periodate dextran, periodate cornstarches of three degrees of periodation, and a polygalacturonide were studied with the chlorite method, specific for aldehyde groups. All were found to be alkali-sensitive in various ways. The periodated substances underwent reactions which, at *p*H 10.2, first increased and then decreased the aldehyde content, corresponding to hydrolysis and dialdehyde intra-action, respectively. At lower *p*H and lower dialdehyde content both reactions were slower but dialdehyde intra-action relatively more, so that hydrolysis dominated aldehyde content for a longer period. At higher *p*H, dialdehyde intra-action dominated. The polygalacturonide showed limited depolymerization, approximately five scissions per chain molecule, indicating that only one de-esterification out of eighty led to scission. These findings permit conclusions regarding the reactions occurring in alkaline reagents.

In a forthcoming article by the writers[3] it will be shown that polysaccharides such as starches, dextrans, and their periodated forms, an araban and a polygalacturonide, usually give misleading aldehyde results by alkaline methods. A typical method using iodine in a carbonate-bicarbonate buffer at *p*H 10.2 gives values from 16 to 76-fold greater than those of the *pH* 3 chlorite method. The errors are due to side-reactions in the presence of alkali.

Not all of the alkali-sensitive substances give completely erroneous results with alkaline methods: Periodate starches and periodate dextran give aldehyde values with a carbonate-iodine method employing carbonate-bicarbonate buffer at *p*H 10.2 which are only 1 to 21% higher than chlorite results. With the Willstätter-Schudel method[4] employing unbuffered sodium hydroxide at *p*H 11.9 the results are 8 to 31% higher. Although these percentages represent large absolute increases in aldehyde groups, as the initial values are high, the agreement appears fair by comparison.

Such agreement is particularly noteworthy in view of the fact that these materials, by analogy to periodate cellulose,[5–16] could be expected to be extremely sensitive to degradation by alkali by virtue of the presence of dialdehyde groups. Furthermore, Hofreiter, *et al.*[17] have shown that periodate starches of all degrees of periodation react with sodium hydroxide, and devised a method for estimating dialdehyde group content by measuring the alkali consumed.

An explanation for such agreement between iodine and chlorite results on periodated starch and dextran, aside from assuming no alkali-sensitivity, is that it is fortuitous and due to compensating reactions. Such reactions might be hydrolysis, which would create aldehyde groups, and intra-action of dialdehyde groups, which would destroy them. The dialdehyde groups would undergo mutual oxidation and reduction of a Cannizzaro-type, giving rise to a hydroxyl and a carboxyl group at the site of the former dialdehyde.

In view of this, alkaline reagents could be expected to give low results for aldehyde content because of dialdehyde intra-action, and the copper reagent (*p*H 10.2) at 100° does so, but both the carbonate-iodine (*p*H 10.2) and the hydroxide-

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