The Behavior of 2-Acetamido-2-deoxy-D-mannose with Isopropenyl Acetate in the Presence of *p*-Toluenesulfonic Acid. I. Isolation and Identification of Derivatives of 2-Amino-D-glucal (2-Amino-1,2-dideoxy-D-*arabino*-hex-1-enopyranose) and of Other Products

N. PRAVDIĆ[1] AND HEWITT G. FLETCHER, JR.

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland 20014

Received January 3, 1967

Acetylation of 2-acetamido-2-deoxy-D-mannose (**1**) with isopropenyl acetate in the presence of a trace of *p*-toluenesulfonic acid affords a variety of products in contrast to the analogous acetylation of 2-acetamido-2-deoxy-D-glucose reported earlier. In addition to the two anomeric 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannopyranoses (α and β **2**), there is formed 2-(D-*glycero*-1,2-diacetoxyethyl)-4-(*N*-acetylacetamido)furan (**3**), 3,4,6-tri-*O*-acetyl-2-(*N*-acetylacetamido)-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (**4**), and 1,1,3,4,5,6-hexa-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-D-mannose aldehydrol (**5**). 4-Acetamido-2-(D-*glycero*-diacetoxyethyl)furan (**6**) was also isolated; whether it is a primary reaction product or arose from **3** through the loss of one *N*-acetyl group during chromatography, is not known. The unsaturated compound **4** is a derivative of the hitherto unknown 2-acetamido-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (**10**, "2-acetamido-D-glucal") and is readily converted into the latter compound by alkaline deacetylation. The structures of **10** and of its tri-*O*-acetyl derivative (**11**) were determined by catalytic reduction to the corresponding 1,5-anhydroglycitol derivatives (**12** to **15**) which have the D-*gluco* and D-*manno* configurations. Neither of the two anomeric 1,3,4,6-tetra-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-D-mannopyranoses was detected among the products from the acetylation of **1**.

Recent work in this laboratory[2] has demonstrated the utility of isopropenyl acetate (containing a trace of *p*-toluenesulfonic acid) as a reagent for the acetylation of sugars and sugar derivatives. In contrast to other acetylating agents, it not only attacks the hydroxyl group but also the acylamido group,[2,3] converting the latter to a di-*N*-acylamine. Thus, for example, 2-acetamido-2-deoxy-D-glucose gives a mixture of the two anomeric 1,3,4,6-tetra-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-D-glucopyranoses.[2] Further investigation of the behavior of carbohydrates with this reagent has now shown that 2-acetamido-2-deoxy-D-mannose (**1**, *N*-acetyl-D-mannosamine) gives products which stand in sharp contrast to those obtained from its D-glucose analog. In this paper we will describe the isolation and proof of structure of these products; in the following paper[4] we will present evidence bearing upon the mechanism of some of the reactions involved.

Treatment of 2-acetamido-2-deoxy-D-mannose (**1**) with a large excess of boiling isopropenyl acetate, containing a catalytic quantity of *p*-toluenesulfonic acid, for 24 hr afforded a mixture of at least five products which could, in part, be resolved by chromatography on a column of silica gel. The main product (31%) proved to be a mixture of the anomeric 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannopyranoses (**2**); gas–liquid partition chromatography (glpc) showed that the β anomer predominated over the α anomer in this mixture by 2.5 to 1.

Another component of the mixture was obtained in amorphous but chromatographically homogeneous form and proved to be an unsaturated compound[5] with an elementary composition corresponding to $C_{14}H_{17}NO_7$. No amide proton was present but the nmr spectrum of the substance included signals from two nitrogen-attached acetyl groups, two oxygen-attached acetyl groups, and two vinyl protons. Other features of the nmr spectrum were consistent with the presence of a diacetoxyethyl group. On the basis of this evidence, structure **3** was tentatively assigned to this substance. Deacetylation with sodium methoxide led to the isolation of a crystalline product with a melting point of 109–111° and a specific rotation of $[\alpha]^{24}D$ +7.8° (*c* 1.2, H_2O). Kuhn and Krüger[6] have reported that warm, aqueous sodium carbonate solution degrades 2-acetamido-2-deoxy-D-glucose to (*inter alia*) a substance of mp 115–117° and $[\alpha]^{20}D$ +12.1 ± 1° (*c* 1.32, H_2O). They showed that this product, which they called "chromogen III," was 2-(1,2-dihydroxyethyl)-4-acetamidofuran (**7**). The nmr spectral characteristics of our product are in agreement with structure **7**[7] and the infrared absorption spectrum is identical with that reported by Kuhn and Krüger.[6] Our material was further characterized through the preparation of a crystalline di-*O*-*p*-nitrobenzoyl derivative (**8**) and of an amorphous di-*O*-acetyl derivative (**6**); the latter compound proved to be identical with a substance isolated in the column chromatography of the products from the acetylation of 2-acetamido-2-deoxy-D-mannose (**1**). Although Kuhn and Krüger[6] described a "di-*O*-acetylchromogen III" as having mp 62–64°, efforts to obtain our product in crystalline form were unsuccessful. Catalytic reduction of the substance gave an amorphous tetrahydro derivative (**9**) with an nmr spectrum which suggested that it was probably (as might be expected) a mixture of isomers.

Since **3** is optically active and retains in its structure the asymmetric carbon atom which was originally C-5 of a D-aldohexose, it may be designated as 2-(D-*glycero*-diacetoxyethyl)-4-(*N*-acetylacetamido)furan, although the possibility of partial racemization during its forma-

(1) Associate in the Visiting Program of the National Institutes of Health, 1965–1966; on leave from the "Ruder Bošković" Institute, Zagreb, Yugoslavia.

(2) T. D. Inch and H. G. Fletcher, Jr., *J. Org. Chem.*, **31**, 1815 (1966).

(3) H. J. Hagemeyer, Jr., U. S. Patent 2,656,360 (Oct. 20, 1953).

(4) N. Pravdić and H. G. Fletcher, Jr., *J. Org. Chem.*, **32**, 1811 (1967).

(5) In preliminary experiments, Dr. Thomas D. Inch, Visiting Fellow in this laboratory, 1964–1965, isolated this substance, though, apparently, in slightly impure form.

(6) R. Kuhn and G. Krüger, *Ber.*, **90**, 264 (1957).

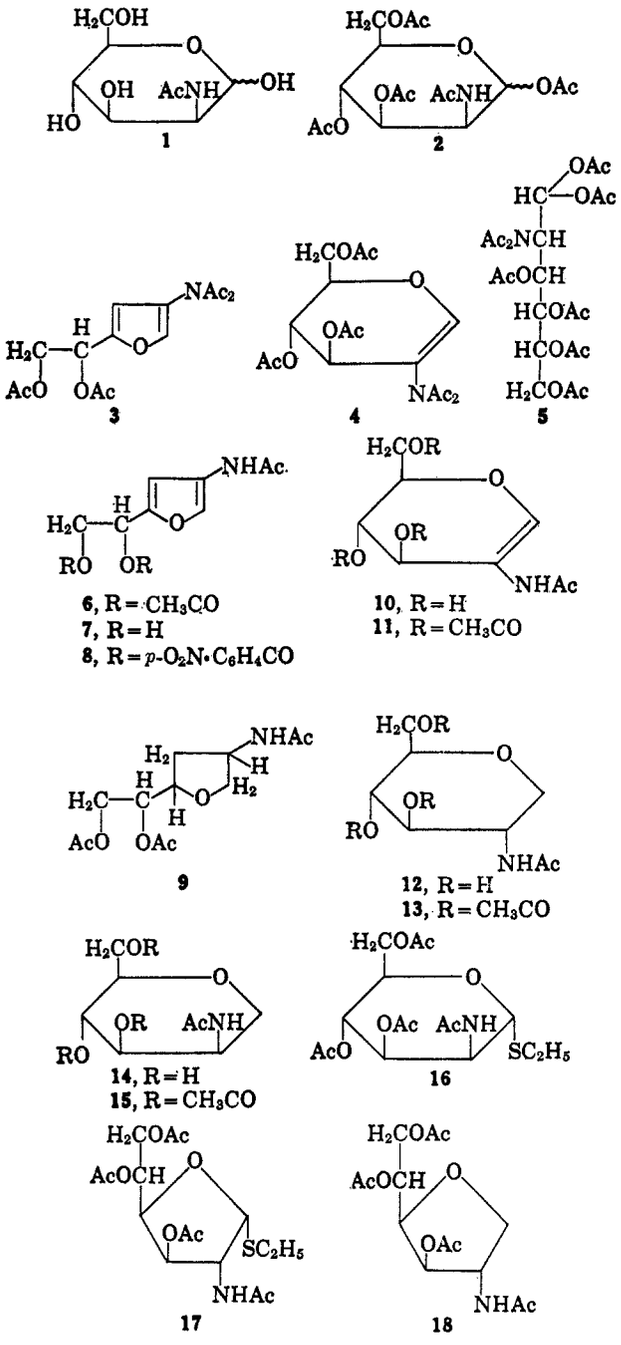
(7) The signal from an amide proton is, of course, readily identified through D_2O exchange. Such a signal is normally split by the adjacent carbon-bound proton to produce a broad doublet, J_{CHNH} being *ca.* 6–9 cps. That the NH signal in **7** is a singlet and at lower field than normal indicates that the nitrogen atom is attached to a vinyl position. The spectra of **6**, **8**, **10**, and **11** similarly show low-field singlets for amide protons.

tion is not, of course, wholly excluded. Like the majority of di-*N*-acylamines,[2] **3** readily loses one of its *N*-acetyl groups to give **6** and it is probable that **3** is a primary product of the acetylation of **1** while **6** is formed from **3** during the chromatography on silica gel.

A minor product from the acetylation of 2-acetamido-2-deoxy-D-mannose (**1**) was a crystalline substance, isolated in 0.36% yield; its physical properties (infrared, nmr) as well as analytical data (elementary and acetyl analyses) clearly defined it as the octaacetyl derivative of an aminodeoxyhexose. After treatment with sodium methoxide and trimethylsilylation, the substance was shown (by glpc) to be a derivative of 2-acetamido-2-deoxy-D-mannose (**1**); it may therefore be regarded as 1,1,3,4,5,6-hexa-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-D-mannose aldehydrol (**5**).[8]

Another product from the acetylation of **1** was also isolated in crystalline form; like **3**, this was an unsaturated compound. Its nmr spectrum clearly showed the presence of two *N*-acetyl and three *O*-acetyl groups while a low-field singlet at τ 3.30 suggested the presence of a vinyl proton. On this evidence, the substance was tentatively presumed to be 3,4,6-tri-*O*-acetyl-2-(*N*-acetylacetamido)-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (**4**) although efforts to reduce the compound with hydrogen in the presence of palladium catalyst were uniformly unsuccessful. Deacetylation with sodium methoxide readily removed one *N*-acetyl and the three *O*-acetyl groups to give a crystalline product (presumably **10**) which was also unsaturated and gave a signal at τ 3.10. Catalytic reduction of the deacetylated compound afforded two products. One, formed in very low yield, was shown by glpc of its trimethylsilyl derivative to be the known 2-acetamido-1,5-anhydro-2-deoxy-D-glucitol (**12**).[9] The major product from the reduction was obtained in crystalline form (79% yield) and, as will be seen below, was shown to be 2-acetamido-1,5-anhydro-2-deoxy-D-mannitol (**14**). Since this anhydride had not hitherto been reported, we undertook its synthesis by an unequivocal route. 2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-mannopyranose (β **2**)[10] was treated with ethanethiol and zinc chloride to give an ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-D-mannopyranoside in amorphous but chromatographically homogeneous form; the dextrorotation of

CHART I



the substance ($[\alpha]^{21}D$ + 82.4° in chloroform), as well as the presumed mechanism involved in its formation,[11] suggests that the substance is most probably the α anomer (**16**). Reductive desulfurization of this thioglycoside with Raney nickel afforded authentic 2-acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-mannitol (**15**, Chart I).

By way of confirming the structure of the compound from the acetylation of 2-acetamido-2-deoxy-D-mannose as **4**, its deacetylation product (**10**) was acetylated with pyridine and acetic anhydride to give a chromatographically homogeneous syrup with an nmr spectrum fully consistent with 2-acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (**11**). Catalytic reduction of this material afforded a mixture of a major and a minor product. These were separated

(8) Such peracetylated derivatives of the acyclic forms of aldoses have been encountered as products of acetolyses; *cf.* E. M. Montgomery, R. M. Hann, and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 1124 (1937). The formation of **5** appears to require the intermediacy of acetic acid or acetic anhydride. Since both **1** and the *p*-toluenesulfonic acid were introduced as monohydrates, acetic acid should have been present at the outset; E. A. Jeffrey and D. P. N. Satchell [*J. Chem. Soc.*, 1876 (1962)] have shown that acetic acid is converted in high yield to acetic anhydride through the action of isopropenyl acetate. One may envisage acetylium ions, derived from isopropenyl acetate, as attacking the ring oxygen of the sugar, breaking its bond with C-1, and forming a carbonium ion which is stabilized as a cyclic cation by an acetyl group attached to the nitrogen atom; subsequent attack by acetic acid or acetic anhydride would give **5**. Isopropenyl acetate is known to convert enolizable keto compounds to enol acetates [H. J. Hagemeyer, Jr., and D. C. Hull, *Ind. Eng. Chem.*, **41**, 2920 (1949); E. A. Jeffrey and D. P. N. Satchell, *J. Chem. Soc.*, 1906 (1962)], and it may be significant that vinyl acetate reacts with acetic acid in the presence of sulfuric acid to give ethylidene diacetate [E. S. Rothman, S. Serota, T. Perlstein, and D. Swern, *J. Org. Chem.*, **27**, 3123 (1962)], but the intermediacy of an enol acetate of **1** in the formation of **5** would have involved loss of asymmetry at C-2 and would have given rise to at least some of the D-glucose analog of **5**.

(9) L. Hough and M. Taha [*J. Chem. Soc.*, 2042 (1956)] described this substance and its 3,4,6-tri-*O*-acetyl derivative. However, we have found the procedure which D. Horton and M. L. Wolfrom [*J. Org. Chem.*, **27**, 1794 (1962)] used for the synthesis of the latter compound to be more convenient.

(10) A. N. O'Neill, *Can. J. Chem.*, **37**, 1747 (1959).

(11) D. Horton and D. H. Hutson, *Advan. Carbohydrate Chem.*, **18**, 135 (1963).

by chromatography and the major (54%) component was obtained in crystalline form; glpc showed it to be identical with authentic 2-acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-mannitol (**15**). The minor (4%) component of the reduction mixture was similarly shown to be 2-acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-glucitol (**13**).[9]

The conversion of **4** into 1,5-anhydro-D-glucitol derivatives (**12** and **13**) and 1,5-anhydro-D-mannitol derivatives (**14** and **15**) appears to establish its structure as 3,4,6-tri-*O*-acetyl-2-(*N*-acetylacetamido)-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (**4**). Structures **4**, **10**, and **11** may be regarded as derivatives of 2-amino-D-glucal (2-amino-1,2-dideoxy-D-*arabino*-hex-1-enopyranose); as far as we are aware, the only related structure which has been reported in the literature is 1,2-dideoxy-2-(*N*-methyl-*p*-toluenesulfonamido)-D-*arabino*-hex-1-enopyranose, a substance which Micheel and Opitz[12] recently found to be a product of the action of sodium methoxide on 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*N*-methyl-*p*-toluenesulfonamido)-α-D-glucopyranosyl fluoride.

That **4** is formed from **1** but not from acetamido-2-deoxy-D-glucose and that this acetylation of **1** has not led to the isolation of either anomer of 1,3,4,6-tetra-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-D-mannopyranose are striking facts which have led to further experiments, designed to throw light upon the mechanism of the formation of **4**; these experiments are described and discussed in the following paper.[4]

It has been noted that the D-*manno* rather than the D-*gluco* configuration predominates when **10** and **11** are reduced catalytically. Hydrogenations which proceed rapidly under mild conditions are recognized as involving *cis* addition to the less hindered side of a molecule;[13] inspection of models of **10** and **11** clearly shows that approach of a catalyst surface to the upper side of the molecules (to give the D-*gluco* configuration) is hindered. It is not surprising, therefore, that the D-*manno* configuration, arising from the less hindered approach of the bottom of the molecules to the catalyst, predominates. With two acetyl groups attached to the nitrogen atom (as in **4**), approach of the catalyst surface to either side of the molecule is hindered and, indeed, as we have noted, attempts to reduce **4** were unsuccessful.

In the course of this work, 2-acetamido-3,5,6-tri-*O*-acetyl-1,4-anhydro-2-deoxy-D-glucitol (**18**) was synthesized through the reductive desulfurization of ethyl 2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-glucofuranoside (**17**).[14] Glpc readily distinguished **18** from **13** and **15** (see Chart I).

Experimental Section[15]

Reaction of 2-Acetamido-2-deoxy-D-mannose (1) with Isopropenyl Acetate.—2-Acetamido-2-deoxy-D-mannose monohydrate[16] (5 g) was treated with isopropenyl acetate (150 ml) containing *p*-toluenesulfonic acid monohydrate (*ca.* 60 mg) and the mixture was boiled under reflux for 24 hr. Chromatography (tlc, ether) of the reaction mixture revealed the presence of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannopyranose (**2**) by the end of the first hour. After 3–4 hr, two other components were detected while, after 24 hr, a further and fourth component was evident. The solvent was evaporated *in vacuo* and the dark syrup, dissolved in ether, was passed through a column of silica gel (60 ml). Elution was made with ether (350 ml) and then with methanol. Concentration of the methanol eluate and extraction of the resulting syrup with hot ether afforded a mixture of the anomeric 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannopyranoses (**2**, 2.5 g, 31%). The ethereal eluate was concentrated *in vacuo* and the light yellow syrup (4.2 g) was chromatographed on a column of silica gel (450 ml) using ether as eluent.

Fractions 37–43 were pooled and concentrated to yield a yellowish oil (870 mg, 13%) which, as described below, was found to be **2-(D-*glycero*-1,2-diacetoxyethyl)-4-(*N*-acetylacetamido)furan (3)**.

Fractions 44–47 contained a mixture of **3** and **3,4,6-tri-*O*-acetyl-2-(*N*-acetylacetamido)-1,2-dideoxy-D-*arabino*-hex-1-enopyranose** (**4**) from which the latter compound could be separated by repeated crystallization from ethanol. Fractions 48–55 yielded **4**, crystallized from ethanol (1.05 g, 14%).

Fractions 56–58 proved to contain a mixture. Concentration of fractions 59 to 123 gave a semicrystalline residue (200 mg) to which ether was added and **1,1,3,4,5,6-hexa-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-D-mannose aldehydrol** (**5**) was removed by filtration (40 mg, 0.36%). Concentration of the ethereal filtrate afforded **4-acetamido-2-(D-*glycero*-1,2-diacetoxyethyl)furan** (**6**) as a yellow oil (160 mg, 3%).

The Anomeric 2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannopyranoses (2).—Infrared and nmr spectra of this partially crystalline mixture showed the presence of an acetamido group. The mixture was effectively resolved by glpc at 210°, giving two peaks which were identical with those of a product obtained through the acetylation of **1** with pyridine–acetic anhydride. The peaks were distinguished by cochromatography with an authentic sample of the crystalline 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-mannopyranose (β **2**);[10] comparison of the peak areas indicated that the ratio of α **2** to β **2** was 1:2.5.

2-(D-*glycero*-Diacetoxyethyl)-4-(*N*-acetylacetamido)furan (3).—This substance partially decomposed to **6** on chromatography on a silica gel column. A once-chromatographed sample was dissolved in ether and the solution was chilled to *ca.* −70°. A yellow oil was deposited and the colorless supernatant solution was decanted, diluted with pentane, and again chilled to *ca.* −70°. The colorless syrup which separated was dried *in vacuo* at 50° and found to be homogeneous by glpc: $[\alpha]^{20}D$ +43° (*c* 0.65, $CHCl_3$); infrared absorption (neat) at 1720 (C=O) and 1615 cm^{-1} (C=C); nmr peaks at τ 2.66 (singlet, α-H), 3.73 singlet, β-H, 4.02 (quartet, ethyl H_1, $J_{1,2}$ = 4.9 cps, $J_{1,2}$ = 7.0 cps), 5.60 (doublet, ethyl H_2, $J_{1,2}$ = 4.9 cps), 5.63 (doublet, ethyl H_2', $J_{1,2}$ = 7.0 cps), 7.72 (6 H, NAc), 7.94 (3 H, OAc), and 8.00 (3 H, OAc). On tlc the substance gives a positive test with permanganate and with fluorescein–bromine.

Anal. Calcd for $C_{14}H_{17}NO_7$ (311.28): C, 54.01; H, 5.50; N, 4.50; Ac, 55.31. Found: C, 53.81; H, 5.39; N, 4.40; Ac, 56; mol wt, 318.[17]

4-Acetamido-2-(D-*glycero*-1,2-hydroxyethyl)furan (7).—A solution of **3** (1.47 g) in methanol (30 ml) and 0.1 *N* sodium methoxide solution (4 ml) was kept at room temperature overnight and then treated with Amberlite IR-120 (H^+) and concentrated *in vacuo*

(12) F. Micheel and W. Opitz, *Ber.*, **96**, 1965 (1963).

(13) H. O. House, "Modern Synthetic Reactions," W. A. Benjamin, Inc., New York, N. Y., 1965, p 22.

(14) M. L. Wolfrom, S. M. Olin, and W. J. Polglase, *J. Am. Chem. Soc.*, **72**, 1724 (1950).

(15) Melting points are corrected. Thin layer chromatography was conducted on silica gel G (E. Merck A.G., Darmstadt) using the solvent system specified, components being detected by spraying with 10% sulfuric acid and heating at 100°. For the specific detection of unsaturated compounds, the plates were sprayed with either aqueous potassium permanganate or, alternatively, with aqueous fluorescein (0.04%, w/v), dried without heating, and then exposed to bromine vapors, the unsaturated compounds appearing as yellow spots on a red background; *cf.* E. Stahl, Ed., "Thin-Layer Chromatography," Springer-Verlag, Berlin, 1965, p 496. Column chromatography was conducted on silica gel (0.05–0.20 mm) of E. Merck A.G., 15-ml fractions being collected. Nmr spectra were obtained in $CDCl_3$ solution (unless otherwise specified) using a Varian A-60 spectrometer and tetramethylsilane as an internal standard. Infrared spectra were recorded for Nujol mulls (or neat in the case of amorphous materials) on a Perkin-Elmer Model 137 spectrometer or in dry chloroform solution using a Perkin-Elmer Model 21 instrument. An F & M Model 500 instrument, equipped with a flame ionization detector, was used for glpc; the column employed (0.25 in. × 6 ft) was filled with 3% SE 52 on Gaschrom A (Applied Science Laboratories, Inc., State College, Pa.). The "Tri-Sil" reagent of the Pierce Chemical Co., Rockford, Ill., was used for the preparation of trimethylsilyl derivatives.

(16) Pfanstiehl Laboratories, Inc., Waukegan, Ill.

(17) Determined in a Model 301A vapor pressure osmometer of Mechrolab, Inc., Mountain View, Calif.

to a yellow syrup. The crude product was chromatographed on silica gel (*ca.* 150 ml) using ether–methanol–dichloromethane (3:1:1, v/v) to give a crystalline material (710 mg, 81%), mp 105–107°. The substance was recrystallized from isopropyl alcohol–ether: mp 109–111°; $[\alpha]^{24}D$ +7.8° (*c* 1.2, H_2O); nmr peaks (dimethyl sulfoxide-d_6) at τ 0.17 (broad singlet, disappearing after D_2O exchange, NH[7]), 2.17 (singlet, H_2), 3.73 (singlet, H_4), 4.77 (doublet, 5.8 cps,[18] disappearing after D_2O exchange, secondary OH), 5.35 (triplet, 5.8 cps, disappearing after D_2O exchange, primary OH), 5.54 (quartet, J = 5.8 and 11.3 cps, collapsing to a triplet after D_2O exchange, ethyl H_1), 6.45 (2 H, triplet, J = 5.8 cps, collapsing to a doublet on D_2O exchange, ethyl H_2), and 8.02 (NAc).

Anal. Calcd for $C_8H_{11}NO_4$ (185.18): C, 51.89; H, 5.99; N, 7.56. Found: C, 52.17; H, 5.96; N, 7.62.

Kuhn and Krüger[6] reported mp 115–117° and $[\alpha]^{20}D$ +12.1 ± 1° (*c* 1.32, H_2O) for their "chromogen III;" the infrared absorption spectrum of the product prepared as described above is identical with that found by Kuhn and Krüger[6] for "chromogen III."

4-Acetamido-2-(D-*glycero*-1,2-diacetoxyethyl)furan (6).—4-Acetamido-2-(D-*glycero*-1,2-dihydroxyethyl)furan (**7**, 180 mg) was acetylated with acetic anhydride (3 ml) and pyridine (5 ml) at room temperature overnight and the reaction mixture was poured onto ice. The crude product was extracted with dichloromethane and the extract was washed successively with 1 *N* sulfuric acid, aqueous sodium bicarbonate solution, and water. Moisture was removed with sodium sulfate and the solution was concentrated *in vacuo* to a syrup which was chromatographed on a column of silica gel (50 ml) using ether–acetone (10:1, v/v). Removal of solvent from the eluate afforded a nearly colorless syrup which, on tlc, gave positive tests for unsaturation with both permanganate and fluorescein–bromine: 200 mg (76%); $[\alpha]^{27}D$ +123° (*c* 1.14, $CHCl_3$); infrared absorption (neat) at 3400 (NH), 1740 (OAc), 1670, and 1560 cm^{-1} (amide); nmr peaks at τ 1.78 (singlet, NH[7]), 2.13 (singlet, α-H), 3.80 (singlet, β-H), 4.06 (quartet, $J_{1,2}$ 5.8 and $J_{1,2'}$ = 7.0 cps, ethyl H_1), 5.62 (doublet, $J_{1,2}$ = 5.8 cps, ethyl H_2), 5.64 (doublet, $J_{1,2'}$ = 7.0 cps, ethyl $H_{2'}$), 7.92 (NAc), 7.95 (OAc), and 7.98 (OAc).

Anal. Calcd for $C_{12}H_{15}NO_6$ (269.26): C, 53.53; H, 5.62; N, 5.20. Found: C, 53.54; H, 5.34; N, 5.10.

Kuhn and Krüger[6] reported their "di-*O*-acetyl-chromogen III" as a crystalline product of mp 62–64°, but gave no specific rotation for the substance. In our hands, the compound proved somewhat unstable, particularly when impure, and attempts to crystallize it were unsuccessful. Indeed, as isolated subsequent to the acetylation of **1**, the substance could not be fully purified, all samples prepared by this route being yellow and contaminated with one or two minor impurities as shown by tlc. However, this material had an infrared absorption spectrum and an nmr spectrum identical with those of the preparation from **7**.

An ether solution of **3** was stirred with chromatographic grade silica gel at room temperature overnight; tlc then showed **6** to be the sole product.

4-Acetamido-2-(D-*glycero*-1,2-di-*p*-nitrobenzoyloxyethyl)furan (8).—4-Acetamido-2-(D-*glycero*-1,2-dihydroxyethyl)furan (**7**, 500 mg) was dissolved in dry pyridine (15 ml) and the cooled solution was treated with *p*-nitrobenzoyl chloride (1.1 g). The reaction mixture was stored at room temperature overnight and the excess reactants were removed in conventional fashion to give a syrup (1.1 g) which was chromatographed on a column of silica gel (250 ml) using ether–acetone (10:1, v/v). The product was obtained in crystalline form: 380 mg (29%); mp 179–180° (recrystallization from ethanol failed to change this melting point); $[\alpha]^{22}D$ +62° (*c* 1.28, $CHCl_3$); infrared absorption at 3350 (NH), 1730 (C=O), 1650 and 1530 (NHAc), and 1350 cm^{-1} (NO_2); nmr peaks (dimethyl sulfoxide-d_6) at τ 0.15 (singlet, NH[7]), 1.6–1.8 (aromatic), 1.92 (α-H), 3.21 (β-H), and 7.96 (NAc).

Anal. Calcd for $C_{22}H_{17}N_3O_{10}$ (483.40): C, 54.66; H, 3.54; N, 8.69. Found: C, 54.62; H, 3.33; N, 9.02.

4-Acetamido-2-(D-*glycero*-1,2-diacetoxyethyl)tetrahydrofuran (9).—A solution of 4-acetamido-2-(D-*glycero*-1,2-diacetoxyethyl)-furan (**6**, 670 mg) in glacial acetic acid (20 ml) was treated with palladium black (700 mg) and shaken with hydrogen until absorption of the gas had ceased (20 hr). The catalyst was removed by filtration and the solution was concentrated *in vacuo* to a syrup which was chromatographed on a column of silica gel (100 ml) using ether–methanol (19:1, v/v). Fractions 72–74, containing a mixture (230 mg) of two substances with very similar migration rates (tlc), were combined and rechromatographed on silica gel, chloroform–methanol–benzene (10:1:1, v/v) being used as eluent. A material which appeared to be chromatographically homogeneous in this solvent system was obtained in the form of a syrup: $[\alpha]^{20}D$ −3.6° (*c* 0.84, $CHCl_3$); infrared absorption at 3350 (NH), 1740 (C=O), and 1650 and 1540 cm^{-1} (NHAc); mass spectrum, highest peak at 273. The nmr spectrum showed four acetyl peaks with a total intensity of 9, suggesting that the material is a mixture of isomers.

Anal. Calcd for $C_{12}H_{19}NO_8$ (273.29): C, 52.74; H, 7.01; N, 5.13. Found: C, 52.44; H, 7.05; N, 4.86; mol wt, 270.5.[17]

1,1,3,4,5,6-Hexa-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-D-mannose Aldehydrol (5).—Crystallized from ethanol–pentane, the substance had mp 155–156°; $[\alpha]^{23}D$ +10° (*c* 0.4, $CHCl_3$); infrared absorption ($CHCl_3$) at 1760 (OAc) and 1685 cm^{-1} (NAc); nmr peaks at τ 2.97 (doublet, $J_{1,2}$ = 6.0 cps, H_1), 3.96 (quartet, H_3, $J_{2,3}$ = 8.3 cps and $J_{3,4}$ = 1.6 cps), 4.76 (quartet, H_4, $J_{3,4}$ = 1.6 cps, $J_{4,5}$ = 8.0 cps), 4.80–4.95 (multiplet, H_5), 5.33 (quartet, H_2, $J_{1,2}$ = 6.0 cps and $J_{2,3}$ = 8.3 cps), 5.85–6.00 (multiplet, H_6), 7.58 (NAc), 7.89 (OAc), 7.92 (OAc), and 7.99 (OAc); ratio of NAc to OAc, 1:3.

Anal. Calcd for $C_{22}H_{31}NO_{14}$ (533.50): C, 49.53; H, 5.86; N, 2.63; Ac, 64.55. Found: C, 49.83; H, 6.03; N, 2.53; Ac, 64.9.

A sample (15 mg) of the compound was treated with sodium methoxide in the usual fashion and the product was converted into its trimethylsilyl derivative. On glpc the product afforded the same pattern of peaks as shown by a trimethylsilylated sample of 2-acetamido-2-deoxy-D-mannose (**1**).

3,4,6-Tri-*O*-acetyl-2-(*N*-acetylacetamido)-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (4).—Crystallized from absolute ethanol and then from benzene, the substance had mp 95–96° and $[\alpha]^{20}D$ −18.3° (*c* 1.02, $CHCl_3$). On tlc the substance decolorized permanganate but failed to give a positive fluorescein–bromine test; attempts to reduce the substance in glacial acetic acid solution in the presence of palladium black were unsuccessful. On storage, samples of this compound were found (by tlc) to have decomposed in part to **11**. Its infrared spectrum ($CHCl_3$) showed absorption at 1860 (OAc), 1722 (NAc), and 1675 cm^{-1} (C=C). The nmr spectrum of the substance showed peaks at τ 3.30 (singlet, H_1, vinyl), 4.33 (doublet, H_3, $J_{3,4}$ = 5.0 cps), 4.66 (triplet, H_4, $J_{3,4}$ = 5.0 cps, $J_{4,5}$ = 6.0 cps), 7.58 (NAc), 7.87 (OAc), and 7.94 (OAc); the ratio of the intensities of the NAc signals to the OAc signals was 2:3.

Anal. Calcd for $C_{16}H_{21}NO_9$ (371.36): C, 51.75; H, 5.70; N, 3.77. Found: C, 51.63; H, 5.63; N, 3.72, mol wt, 359.[17]

2-Acetamido-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (10).—A solution of **4** (660 mg) in absolute methanol (15 ml) containing sodium methoxide (1 ml, 0.1 *N*) was left at room temperature for 1 hr. Amberlite IR-120 (H^+) was then added and the solution was stirred until neutral. After filtration, the solution was concentrated *in vacuo* to a syrup (360 mg, 100%) which crystallized when triturated with chloroform. The product was recrystallized from isopropyl alcohol: mp 124–125°; $[\alpha]^{23}D$ +64.7° (*c* 0.34, H_2O); infrared absorption (KBr) at 3430 (OH), 3280 (NH), 1670 (C=C), and 1640 and 1540 cm^{-1} (NAc); nmr peaks (dimethyl sulfoxide-d_6) at τ 1.50 (NH, singlet disappearing after exchange with D_2O[7]), 3.10 (H_1, vinyl), and 8.09 (NAc). The substance failed to give a color with tetranitromethane but gave a positive test with fluorescein–bromine on tlc. Toward Fehling solution it appeared to be stable even after prolonged boiling.

Anal. Calcd for $C_8H_{13}NO_5$ (203.20): C, 47.29; H, 6.45; N, 6.89. Found: C, 47.55; H, 6.71; N, 6.99; mol wt, 189.[17]

Catalytic Reduction of 2-Acetamido-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (10).—To a solution of **10** (500 mg) in glacial acetic acid (25 ml) was added palladium black (300 mg) and the suspension was agitated with hydrogen until absorption of the gas had ceased (5 hr). The catalyst was removed by filtration and the solution was concentrated *in vacuo* at 40° (bath) to a syrup; tlc (methanol–ether, 1:1 and 1:3, v/v) then showed the presence of a major and a minor component. The syrup was chromatographed on a column of silica gel (120 ml) using ether–methanol (3:1, v/v) as eluent. Fractions 13–16

(18) The vicinal coupling constant between the hydroxyl proton and the adjacent carbon-bound proton (J_{CHOH}) can be observed in dimethyl sulfoxide solution because of the relatively slow rate of hydroxyl proton exchange in this solvent: J. J. Hebel and H. W. Goodwin, *J. Org. Chem.*, **31**, 2040 (1966). Coupling constants for CHOH protons are in the range of 4–7 cps: R. H. Bible, Jr., "Interpretation of NMR Spectra," Plenum Press, New York, N. Y., 1965, p 60.

contained a colorless syrup (60 mg); a portion of this was trimethylsilylated and then subjected to glpc and two compounds were detected. The first was identified by cochromatography with the trimethylsilyl derivative of 2-acetamido-1,5-anhydro-2-deoxy-D-glucitol (12).[9] The second substance was identified by cochromatography with the trimethylsilylated derivative of 2-acetamido-1,5-anhydro-2-deoxy-D-mannitol (14, see below).

Fractions 28–40 contained the major component from the reduction; removal of the solvent afforded a colorless syrup which crystallized on standing (400 mg, 79%). Recrystallization from isopropyl alcohol gave pure 2-acetamido-1,5-anhydro-2-deoxy-D-mannitol (14): mp 197–198°; $[\alpha]^{22}D$ −60.1° (*c* 1.1, H_2O); infrared absorption (KBr) at 3400 (OH), 3300 (NH), and 1650 and 1555 cm^{-1} (NAc); nmr peaks (dimethyl sulfoxide-d_6) at τ 2.47 (broad doublet, NH), 5.23 (2 H, doublet, 4.1 cps H_1), and 8.12 (NAc). At 0° over the course of 2 hr the substance consumed 1.08 molar equiv of periodate.

Anal. Calcd for $C_8H_{15}NO_5$ (205.22): C, 46.82; H, 7.37; N, 6.83. Found: C, 46.95; H, 7.54; N, 6.70.

2-Acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (11).—A sample of **10** (200 mg) was acetylated with acetic anhydride (3 ml) and pyridine (5 ml) in normal fashion to give, after removal of excess reactants, a syrup (280 mg) which was chromatographed on a column of silica gel (40 ml) using ether–methanol (19:1, v/v) as eluent. As thus obtained, 2-acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (**11**) was a colorless syrup which, on tlc, gave a positive test for unsaturation with fluorescein–bromine: 240 mg (74%); $[\alpha]^{23}D$ −24.6° (*c* 0.5, $CHCl_3$); infrared absorption ($CHCl_3$) at 3390 (NH), 1750 OAc), 1685 and 1535 (NAc), and 1670 cm^{-1} (C=C); nmr peaks at τ 2.84 (singlet, H_1, vinyl), 3.20 (singlet, NH[7]), and 7.96 and 8.03 (N and OAc).

Anal. Calcd for $C_{14}H_{19}NO_8$ (329.31): C, 51.06; H, 5.82; N, 4.25. Found: C, 51.51; H, 5.87; N, 4.19.

Catalytic Reduction of 2-Acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (11).—Chromatographically pure **11** (250 mg) was dissolved in glacial acetic acid (20 ml) and palladium black catalyst (200 mg) was added. The suspension was shaken with hydrogen at room temperature until the absorption of the gas had ceased (4 hr) and the catalyst was then removed by filtration. The filtrate was concentrated *in vacuo* (40° bath) to a syrup which was shown by tlc to consist of two components. The syrup was then chromatographed on a column of silica gel (60 ml) using ether–methanol (9:1, v/v) for elution.

Fractions 12–15 were pooled and freed of solvent to give 2-acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-mannitol (**15**) as an apparently amorphous powder (135 mg, 54%). From its solution in isopropyl ether (containing a few drops of isopropyl alcohol), the compound crystallized in pure form: mp 64–65°; $[\alpha]^{22}D$ −9.8° (*c* 0.5, $CHCl_3$); infrared absorption (Nujol) at 3350 (NH), 1750 (OAc), and 1660 and 1540 cm^{-1} (NAc); nmr peaks at τ 4.08 (doublet, NH), 5.02 (2 H, doublet, 5.1 cps, H_1) and at 7.95, 8.01, and 8.08 (N and OAc). A sample dissolved in pyridine was homogeneous on glpc; by both tlc and glpc the substance was indistinguishable from **15** prepared through the reductive desulfurization of ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-mannopyranoside (**16**) as described later in this paper. De-*O*-acetylation of the material, followed by trimethylsilylation, gave a product which was indistinguishable (glpc) from one made by the trimethylsilylation of **14**, derived from **10**.

Anal. Calcd for $C_{14}H_{21}NO_8$ (331.33): C, 50.75; H, 6.39; N, 4.23. Found: C, 50.78; H, 6.65; N, 4.20.

The material contained in fractions 16 and 17 was not homogeneous. Concentration of fractions 18 and 19 afforded nearly pure 2-acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-glucitol (**13**) as a syrup (10 mg, 4%). A small quantity of **15** was shown to be present (glpc); the major component was chromatographically indistinguishable from an authentic sample of **13**.[9]

Ethyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-mannopyranoside (16).—2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-mannopyranose[10] (β **2**, 6.0 g) was added to a cold solution of anhydrous zinc chloride (3.0 g) in ethanethiol (30 ml) and the mixture was stirred at 0° for 24 hr. As there was still a heavy layer of undissolved material, the mixture was heated (40° bath) under a reflux condenser.[19] After 3 hr the reaction mixture became homogeneous. It was then poured into a saturated solution of sodium bicarbonate (400 ml) and the precipitate which formed was removed by filtration. The solid was washed three times with boiling dichloromethane and the aqueous layer was also extracted with this solvent. The combined extracts and washings were washed with water, dried with sodium sulfate, and concentrated *in vacuo* to a syrup. The syrup was dissolved in ether–methanol (9:1, v/v) and the β **2** which crystallized was removed by filtration. The filtrate was then chromatographed on a column of silica gel (450 ml) using ether–methanol (9:1). Pure ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-mannopyranoside (**16**) was obtained as a colorless syrup: 3.75 g (62%), $[\alpha]^{21}D$ +82.4° (*c* 0.8, $CHCl_3$).

Anal. Calcd for $C_{16}H_{25}NO_8S$ (391.45): C, 49.09; H, 6.44; S, 8.19. Found: C, 49.28; H, 6.23; S, 8.48.

Reductive Desulfurization of Ethyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-mannopyranoside (16).—A solution of the thioglycoside (**16**, 340 mg) in ethanol (15 ml) was treated with Raney nickel (*ca.* 8 g) and the suspension was boiled under reflux. The reduction was complete (tlc) after 15 min. After 30 min, the reaction mixture was filtered through a layer of Celite, the nickel being washed with hot ethanol. On concentration, the combined filtrate and washings yielded 2-acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-mannitol (**15**, 210 mg, 73%) as a syrup which was homogeneous and chromatographically indistinguishable (tlc and glpc) from the major product from the reduction of **11**. On prolonged standing, the product crystallized, mp 63–64°.

Reductive Desulfurization of Ethyl 2-Acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-glucofuranoside (17).—A solution of **17**[14] (1.4 g) in ethanol (60 ml) was boiled under reflux with Raney nickel (*ca.* 20 g) for 1 hr. The hot solution was filtered through a bed of Celite and the residue was washed thoroughly with hot ethanol. Concentration *in vacuo* of the combined filtrate and washings afforded a thick syrup which was chromatographed on a column of silica gel (120 ml) using ether–methanol (9:1, v/v) to give a crystalline product (1.05 g, 89%). Recrystallization from ethyl acetate–pentane afforded pure 2-acetamido-3,5,6-tri-*O*-acetyl-1,4-anhydro-2-deoxy-D-glucitol (**18**): mp 110–111°; $[\alpha]^{21}D$ +22.7° (*c* 1.16, $CHCl_3$); infrared absorption (Nujol) at 3320 (NH), 1750 (OAc), and 1640 and 1550 cm^{-1} (NAc); nmr peaks at τ 3.53 (doublet, NH), 4.51 (2 H, doublet, 4.8 cps, H_1), 7.92 and 8.03 (OAc and NAc).

Anal. Calcd for $C_{14}H_{21}NO_8$ (331.33): C, 50.75; H, 6.39; N, 4.23. Found: C, 50.75; H, 6.41; N, 4.37.

Registry No.—**1**, 6730-06-9; α **2**, 4539-83-7; β **2**, 6730-10-5; **3**, 10293-53-5; **4**, 10293-54-6; **5**, 10293-55-7; **6**, 10293-56-8; **7**, 10293-57-9; **8**, 10316-18-4; **9**, 10293-58-0; **10**, 10293-59-1; **11**, 10293-60-4; **14**, 10293-61-5; **15**, 10293-62-6; **16**, 10277-32-4; **18**, 10316-19-5; isopropyl acetate, 108-22-5; *p*-toluenesulfonic acid, 104-15-4.

Acknowledgments.—We wish to express our thanks to Dr. Henry M. Fales of the National Heart Institute, for mass spectra and to the staff of the Section on Microanalytical Services and Instrumentation of this Institute, for spectra and elementary analyses.

(19) Hough and Taha[9] prepared ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside through the action of ethanethiol and anhydrous zinc chloride on 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose at 0° for 24 hr and these reaction conditions may be regarded as typical of those normally employed for the conversion of an acetylated aldose to an acetylated alkyl 1-thioaldoside. In the present case, however, we found such mild conditions to give **16** in but 20% yield.