Preparation and Properties of Dialditylamines[1]

J. E. HODGE AND B. F. MOY

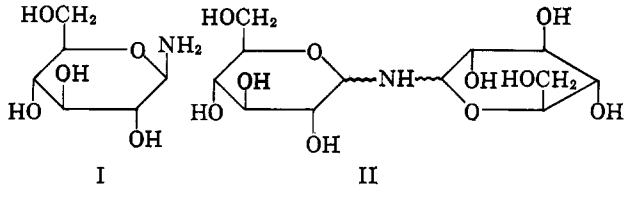
Northern Regional Research Laboratory,[2] Peoria, Illinois

Received April 22, 1963

D-Glucose, ammonia, and ammonium chloride reacted to produce a mixture of the hydrochloride salts of mono- and, mainly, di-D-glucosylamine. Catalytic hydrogenation of the mixed salts in aqueous methanol gave di-1,1′-D-glucitylamine hydrochloride as the only spontaneously crystallizing compound in 66% yield. D-Mannose, D-galactose, and D-arabinose gave the corresponding dialditylamine salts in comparable yields. In addition to hydrochloride salts, the free bases, *N*-acetyl derivatives, and some copper chelate salts were crystallized. The neutral salts of all known mono- and di-1-alditylalkylamines follow the optical rotation rule that has been established for *N*-1-alditylarylamines by Weygand. Because two basic monoalditylalkylamines do not comply, a modification of the rule is suggested. Improved methods for making mono- and di-D-glucosylamine and their *N*-acetyl derivatives are given.

Investigation of the sequestration of metal ions by glucose–ammonia derivatives showed that, although di-D-glucosylamine excelled mono-D-glucosylamine, both are generally poor sequestering agents.[3] Because hydrolysis and cyclic structures probably were limiting factors, more stable derivatives of di-D-glucosylamine were sought by isomerization and hydrogenation. This paper reports the preparation and properties of the hydrogenated derivative, di-1,1′-D-glucitylamine (iminobis-1-deoxy-D-glucitol), and congeners in this little-investigated class of carbohydrate derivatives.

Muskat[4] has claimed that an evaporated solution of D-glucose in anhydrous liquid ammonia yields a residue, perhaps the aldehyde–ammonia addition compound, which, when taken up in alcohol, deposits D-glucosylamine (I) in quantitative yield. Subsequent reports indicate that yields of I do not approach quantitative either by Muskat's procedure or in closely allied variations.[5–7]



Our analyses of reaction mixtures of D-glucose and liquid ammonia, prepared according to Muskat,[4] showed 14% of the theoretical amount of di-D-glucosylamine (II) in the ammonia-evaporated residues, and at least 17% of II in the alcohol-precipitated solids. Reaction of D-glucose with liquid ammonia at 60° under pressure (according to Klug's example 1)[6] gave 8% of II mixed with I in the reaction products. Products were analyzed by acetylation in pyridine–acetic anhydride at 25°, followed by fractional crystallization of the highly insoluble α- and β-octaacetates of II.[8] The octaacetates were judged not to be artifacts generated during the acetylation because a control acetylation of pure β-D-glucopyranosylamine (I) gave barely a trace of the octaacetates of II.

A survey of the previously reported analyses for nitrogen in various preparations of I showed that all values are somewhat low; *e.g.*, 7.51,[4] 7.2,[6] 5.8,[7] 7.6,[8] 6.77,[9] 7.5,[10] as against the calculated 7.82% N. Various lots of I prepared in this laboratory by the Muskat[4] and Lobry de Bruyn[11] methods likewise were low in nitrogen and gave noticeable amounts of the octaacetates of II upon acetylation. Analytically pure I was finally made by the Lobry de Bruyn method,[11] but only by starting with more than 30% by weight of ammonia in the methanol. The high ammonia concentrations hastened crystallization of I and repressed coprecipitation of II. Recrystallization of the first precipitate from ammoniacal alcohol, with storage of the pure compound in an atmosphere of ammonia near 0° (to inhibit spontaneous conversion to II by loss of ammonia), gave I with the theoretical nitrogen content.

The presence of II in the various reaction mixtures and products was expected because it had been shown that I and II form in an equilibrated mixture whenever D-glucose is chromatographed in alcoholic aqueous ammonia.[12–14] These same reports indicated that ammonium salts catalyze diglucosylamine formation.

In experiments designed to increase the yield of II at the expense of I by heating the evaporated residue in alcohols and other solvents to drive out ammonia,[8,14] decomposition of the glucosylamines with browning was a serious drawback. At first browning was minimized by conducting the entire reaction, starting in liquid ammonia, in the presence of powdered anhydrous calcium sulfate. Then, with the addition of ammonium chloride as a condensation catalyst, yields of II were boosted to about 65%.

Di-D-glucosylamine was not isolated directly from the reaction mixture, but was obtained from the residue of mixed hydrochloride salts by acetylation, fractionation of the acetates, and deacetylation of the octaacetate(s) of II. The "α-diglucosylamine" of Brigl and Keppler[8] (presumably the α,α-form because of its most highly positive optical rotation) crystallized. The pentaacetate of I was isolated from the first mother liquor in 27% yield. Therefore, about 92% of the D-

(1) Presented at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., September 9–14, 1962.

(2) One of the laboratories of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(3) J. E. Hodge, E. C. Nelson, and B. F. Moy, *J. Agr. Food Chem.*, **11**, 126 (1963).

(4) I. E. Muskat, *J. Am. Chem. Soc.*, **56**, 693 (1934).

(5) W. Wayne and H. Adkins, *ibid.*, **62**, 3314 (1940).

(6) E. D. Klug, U. S. Patent 2,197,540 (April 16, 1940).

(7) E. J. Lorand, U. S. Patent 2,235,938 (March 25, 1941).

(8) P. Brigl and H. Keppler, *Z. Physiol. Chem.*, **180**, 38 (1929).

(9) W. Pigman, E. A. Cleveland, D. H. Crouch, and J. H. Cleveland, *J. Am. Chem. Soc.*, **73**, 1976 (1951).

(10) A. R. Ling and D. R. Nanji, *J. Chem. Soc.*, **121**, 1682 (1922).

(11) C. A. Lobry de Bruyn, *Ber.*, **28**, 3082 (1895).

(12) R. J. Bayly, E. J. Bourne, and M. Stacey, *Nature*, **169**, 876 (1952).

(13) I. D. Raacke-Fels, *Arch. Biochem. Biophys.*, **43**, 289 (1953).

(14) H. S. Isbell and H. L. Frush, *J. Org. Chem.*, **23**, 1309 (1958).

TABLE I

OPTICAL ROTATIONS OF SOME ALDITOLS, ADITYLAMINES, AND THEIR SALTS

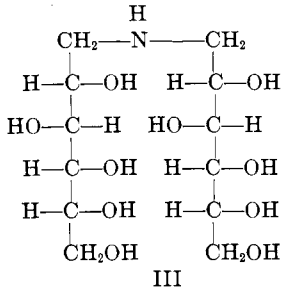
([α]D in water)

Alditol radical Orientation of C-2 hydroxyl[a]	D-Glucosyl right	D-Mannityl left	D-Galactityl right	D-Arabinityl left	D-Ribityl right
Alditol	− 2[b]	− 0.2[b]	*meso*	*ca.* 0[c]	*meso*[c]
Monoalditylamine	− 8[d]	− 2[e], +1[f]	− 3[d]	(+ 4)[g]	+ 5[f]
Hydrochloride salt		+ 5[f]		+15[f]	− 8[f]
Neutral oxalate salt	−15[d]	+ 4[e]	−11[d]	(+13)[g]	
N-Acetyl derivative		+13[f]		+23[f]	−24[f]
Dialditylamine	−22	+ 6	−11	+13	
Hydrochloride salt	−27	+14	−22	+22	
N-Acetyl derivative	−27	+34	−16	+19	
N-Alditylpiperidine	−18[h]	− 7[h,i]	− 8	+ 9	
Hydrochloride salt	−31[h]	+17[h,i]	−21	+22	
N-Alditylmethylamine[j]	−17[i]				
Hydrochloride salt	−25[i]				

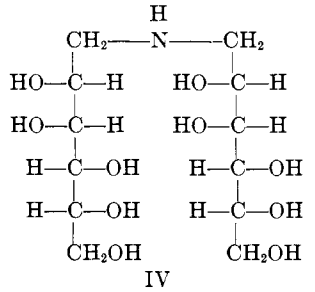
[a] In the conventional Fischer projection formula. [b] R. L. Lohmar and R. M. Goepp, Jr., *Advan. Carbohydrate Chem.*, **4**, 211 (1949). [c] E. Fischer, *Ber.*, **26**, 635 (1893). [d] E. Roux, *Ann. chim. phys.*, [8] **1**, 72 (1904). [e] E. Roux, *Compt. rend.*, **138**, 503 (1904). [f] J. K. N. Jones, M. B. Perry, and J. C. Turner, *Can. J. Chem.*, **40**, 503 (1962). [g] L-Arabinitylamine values reversed in sign, E. Roux, *Compt. rend.*, **136**, 1079 (1903). [h] J. E. Hodge and C. E. Rist, *J. Am. Chem. Soc.*, **74**, 1494 (1952). [i] Optical rotations determined by B. F. Moy. [j] Sample of "*N*-methylglucamine" supplied by Commercial Solvents Corp.

glucose was converted to the mixture of II (65%) and I (27%).

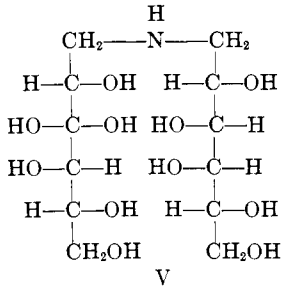
That the yield of II in the mixture of hydrochloride salts was at least 60% was confirmed after a high-pressure hydrogenation of the mixture. Di-1,1′-D-glucitylamine hydrochloride crystallized cleanly from the hydrogenation filtrate in 60% yield. Recrystallization of the salt from ammoniacal aqueous alcohol gave the same base (III) that was formed by hydrogenation of the isolated "α-diglucosylamine." Di-1,1′-D-mannitylamine (IV), di-1,1′-D-galactitylamine (V), and di-1,1′-D-arabinitylamine (VI) were prepared similarly; but the preparation was more convenient



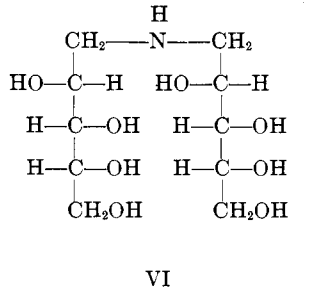
III



IV



V



VI

starting with the sugar in methanol–ammonium hydroxide–ammonium chloride (procedure B). Some evidence for the existence of III was given in a patent[15]; however, isolation of III has not been reported. Compounds IV and VI are new. Di-1,1′-D-galactitylamine (V) has been prepared indirectly by hydrogenolysis of the *N*-benzyl derivative.[16]

(15) J. T. Massengale and R. H. Elliott, U. S. Patent 2,872,301 (February 3, 1959).

(16) F. Kagan, M. A. Rebenstorf, and R. V. Heinzelman, *J. Am. Chem. Soc.*, **79**, 3541 (1957).

Identity of the aldityl radicals in III and IV was made certain by oxidizing them back to the corresponding hexoses with bromine.[17,18] D-Glucose was the only aldose produced from III, and only D-mannose was formed from IV, as was shown by clear chromatographic separations on paper in two different solvent systems.

Acetylation of II with acetic anhydride in pyridine at 25° gave mixtures of α- and β-octa-*O*-acetyl derivatives; none of the *N*-acetyl-octa-*O*-acetyl derivative could be isolated. The latter was prepared under more drastic reaction conditions with zinc chloride as the catalyst. On the other hand, acyclic di-1,1′-D-glucitylamine (III) was readily acetylated in pyridine–acetic anhydride at 25° to form the N-acetyl-deca-*O*-acetyl derivative, illustrating a significant degree of steric hindrance of the nitrogen atom in the bicyclic structure II.

The selective *N*-acetylation of amino sugars has been accomplished nicely by using acetic anhydride in water.[19,20] Our application of this method to I gave the *N*-acetyl derivative quickly and in purer form than by a recently published method that prescribes either methanol or dimethylformamide solvents.[21] The aqueous method did not work on II because of hydrolysis; however, the *N*-acetyl derivative of II was produced by the method of Brigl and Keppler,[8] and it was crystallized for the first time. *N*-Acetyl derivatives of the dialditylamines were readily obtained by the aqueous method.

The specific optical rotations of the dialditylamines, their hydrochloride salts, and *N*-acetyl derivatives (for the sodium D-line in water) are shown in Table I, together with rotations of the corresponding alditols and some monoalditylamines. The 1-amino-1-deoxyalditols show increased rotations over the very low values of the corresponding alditols, and the dialditylamines show uniformly increased rotations over the corresponding monoalditylamines. Reduction in the basicity of the nitrogen atom, either by salt or amide formation, results in further increased numerical values

(17) F. Weygand and G. Schaefer, *Chem. Ber.*, **84**, 603 (1951).

(18) E. Roux, *Ann. chim. phys.*, [8] **1**, 72 (1904).

(19) S. Roseman and J. Ludowieg, *J. Am. Chem. Soc.*, **76**, 301 (1954).

(20) G. A. Levvy and A. McAllan, *Biochem. J.*, **73**, 127 (1959).

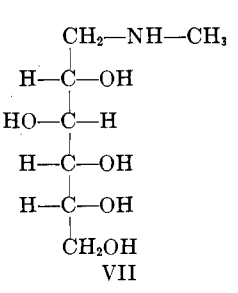
(21) K. Onodera and S. Kitaoka, *J. Org. Chem.*, **25**, 1322 (1060).

(disregarding the sign) and, also, differences in the direction of the increase in rotation depending on the steric orientation of the hydroxyl group on C-2. The increased rotations of the neutral salts follow the rule that Weygand has established for *N*-alditylarylamines,[22] although the low rotations of the strongly basic D-ribitylamine and *N*-D-mannitylpiperidine do not follow the rule. Weygand states that when the C-2 hydroxyl of an *N*-polyhydroxyalkylaminobenzene is on the right in the Fischer projection formula, the $[\alpha]_D$ in pyridine is negative—and the converse. From the examples of Table I, wherein the salts follow Weygand's rule but the basic monoalditylamines do not, it appears that this modification of the rule should now be considered. When the C-2 hydroxyl of an *N*-1-alditylamine is on the right in the conventional Fischer projection formula, the $[\alpha]_D$ of the base in water will become more levorotatory upon conversion of the base to the neutral salt; conversely, when the C-2 hydroxyl is on the left, the $[\alpha]_D$ will become more dextrorotatory upon conversion of the base to its neutral salt.

Kuhn and Weygand showed previously with flavins that the contribution of the asymmetric carbon atom nearest the light-absorptive center is far greater than the sum of the contributions of the more remote asymmetric carbon atoms.[22,23] The same effect had been demonstrated in several other sugar derivatives, notably amides and *N*-substituted amides, which also contain a chromophoric group that absorbs in the ultraviolet.[24,25] This nearest asymmetric-carbon effect is now shown in the *salts* of *N*-1-alditylalkylamines, which show no absorption band in the near (>2200 Å.) ultraviolet. Protonated amino groups therefore can be guessed to absorb at longer wave lengths in the far ultraviolet than basic amino or hydroxyl groups.

The varying compositions of some crystalline copper chelate salts of the dialditylamines are reported below. These salts are more soluble in aqueous alcohol than the corresponding dialditylamines, and they show surprisingly sharp melting (decomposition) points. In aqueous solutions they give no precipitate with hydroxide, carbonate, or ferrocyanide ions, showing sequestration of the cupric ion.

Greater sequestration of cupric ion by di-1,1′-D-glucitylamine (III) over *N*-1-D-glucitylmethylamine (VII) at pH 8.5 and at pH 12 was demonstrated in



VII

previous work.[3] Confirmation and an explanation of the stronger sequestration came after isolation and analysis of the crystalline copper chelate salts that were formed under identical conditions. The copper complex from VII gave a precipitate of cupric ferrocyanide upon addition of the ferrocyanide ion to a dilute aqueous solution, whereas the copper complexes from III, IV, and V gave none under the same conditions. Analyses for C, H, N, Cu, and acetyl showed that the complex from the monoalditylamine VII contained one mole of basic cupric acetate that probably was bound without proton elimination from the glucityl radical; whereas, the complexes from the dialditylamines III, IV, V contained at least one atom of copper that was bound salt-like with the elimination of protons, plus varying amounts of basic cupric acetate apparently bound without proton elimination. For example, when equimolar amounts of cupric acetate and IV in separate aqueous alcohol solutions were combined, the mixture was neutral, and all cupric ion was removed after crystallization of the chelate salt. Analyses of the dried, recrystallized salt, $C_{14}H_{29}NO_{13}Cu_2$, indicated that one mole of basic cupric acetate, $CH_3COO(OH)Cu$, had merely added and that the second atom of copper had combined to form an alcoholate salt with loss of two protons from the carbohydrate derivative. Although the chelate salts from III, IV, and V were of different compositions, each contained fewer protons than the calculated number for simple addition compounds. Two quite different salts containing 5:2 and 3:2 ratios of copper to nitrogen were produced from III under the same conditions that gave a single compound with 2:1 ratio from IV. The compositions of these and other chelate salts of alditylamines are being investigated further, possibly to correlate the stoichiometry with stereochemical configurations.

Experimental[26]

β-D-Glucopyranosylamine (I).—A solution of 27 g. of anhydrous D-glucose in 80 ml. of 31% (wt./wt.) anhydrous ammonia in methanol was allowed to stand at 25° protected from the entry of atmospheric moisture. Seed crystals of I were added after 2 days, and crystallization proceeded over a 5-day period. Filtration and thorough washing of the filter cake with methanol, followed by drying over calcium chloride at atmospheric pressure, gave 10.8 g. (40%) of crude I, m.p. 125–126°. Recrystallization from 550 ml. of 4% ammonia in methanol gave 5.5 g. (21%), m.p. 128–129°, $[\alpha]^{25}_D$ +20° (*c* 2.0 in water); lit. m.p. 128–129°,[9] 127–128°,[10] 125–127°,[14] 126–128°[21]; $[\alpha]^{20}_D$ +20.3° (water),[10] +20.8°,[14] +22°.[21]

Anal. Calcd. for $C_6H_{13}NO_5$: C, 40.2; H, 7.31; N, 7.82. Found: C, 40.2; H, 7.54; N, 7.87.

The recrystallized I (1 g.) was shown to be free of II by acetylation in pyridine with excess acetic anhydride at 25°, whereupon less than 1 mg. of the highly water-insoluble octaacetate of II was found. Upon paper chromatography of pure I in 1-butanol–pyridine–water (6:4:3), a distinct spot of II as always seen because an equilibrium exists between I and II in solution.[12–14] The spot for I usually streaked into the faster moving D-glucose formed by hydrolysis.

***N*-Acetyl-β-D-glucopyranosylamine**.—The method that Levvy and McAllan[20] used on 2-amino-2-deoxy-D-glucopyranose was adapted as follows. β-D-Glucopyranosylamine (2.5 g., 14 mmoles) was dissolved in 18 ml. of water, and 2.4 g. (23.5 mmoles) of acetic anhydride was immediately added. The mixture was shaken as the anhydride dissolved, then the solution was allowed to stand at 25° for 20 min. Evaporation under 20-mm. pressure and at 50° bath temperature gave a crystalline residue which was separated, dissolved in 100 ml. of hot methanol, and recrystallized after concentration under vacuum to one-half volume. The white crystals, which separated on cooling to 2°, were filtered off, washed with methanol, and dried to a weight of 2.45 g.

(22) F. Weygand, *Ber.*, **73B**, 1278 (1940).

(23) R. Kuhn and F. Weygand, *ibid.*, **68B**, 166, 1001 (1935).

(24) C. S. Hudson, *J. Am. Chem. Soc.*, **39**, 462 (1917); **40**, 813 (1918); C. S. Hudson and K. P. Monroe, *ibid.*, **41**, 1140 (1919); C. S. Hudson and S. Komatsu, *ibid.*, **41**, 1141 (1919); N. K. Richtmeyer and C. S. Hudson, *ibid.*, **64**, 1612 (1942).

(25) K. Freudenberg and W. Kuhn, *Ber.*, **64B**, 703 (1931).

(26) Melting points were determined in capillary tubes and are corrected.

(80%), m.p. 257° dec., $[\alpha]^{25}D$ −21° (*c* 2.0 in water). A second recrystallization from 90 ml. of 3:1 methanol–water gave 2.15 g. (70%), m.p. 258° dec., $[\alpha]^{25}D$ −22.8° (*c* 3.0 in water); lit.[21] m.p. 256°, $[\alpha]^{16}D$ −20° (water); lit.[14,27] m.p. 260°, $[\alpha]^{25}D$ −22.8° (water).

Anal. Calcd. for $C_6H_{15}NO_6$: C, 43.4; H, 6.84; N, 6.33. Found: C, 43.7; H, 6.88; N, 6.27.

Identity was proved by *O*-acetylation of the product at 0° in pyridine–acetic anhydride to give *N*-acetyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine, m.p. 163°, $[\alpha]^{25}D$ +17.5° (*c* 3 in chloroform); lit.[14,27] m.p. 163°, $[\alpha]^{25}D$ +17° (*c* 1 in chloroform).

Mixture of Di- (Mainly) and Mono-D-glucosylamines.—In a 100-ml. cylindrical reaction flask fitted with a stirrer and inlet and outlet tubes connected to calcium sulfate drying columns, 2.7 g. (0.05 mole) of dry ammonium chloride was placed. After cooling the flask in a solid carbon dioxide–acetone bath, column-dried gaseous ammonia was led in until 50 ml. was condensed. Anhydrous D-glucose (18.0 g., 0.10 mole) and anhydrous calcium sulfate (20 g., powdered soluble anhydrite) were then stirred into the liquid ammonia solution. With the cooling bath removed, the mixture was stirred vigorously for 2 hr. to evaporate most of the ammonia. Absolute methanol (30 ml.) was added to the sirupy residue, then the mixture constantly stirred was heated by a water bath held at 40° for 3 hr. and at 70° for 1 hr. Ammonia was still being emitted slowly at the end of the heating period; nevertheless, heating was stopped because the browning decomposition had noticeably increased. The reaction mixture was first diluted with methanol (50 ml.), then filtered, and the filtrate was concentrated under vacuum (at 40° bath temperature in a rotating evaporator) to a dry residue.

The residue was shaken continuously for 24 hr. at 25° with 160 ml. of pyridine and 108 ml. of acetic anhydride. The crystalline product that separated during the last hours of the acetylation was filtered and identified as the α-octaacetate of di-D-glucosylamine,[8] yield, 9.7 g., m.p. 210–211°, $[\beta]^{25}D$ +89.3° (*c* 2.6 in chloroform). The filtrate was poured into 1400 ml. of ice–water to produce more of the α- and some β-octaacetate; yield, 12.7 g., m.p. 200–204°, making the total crude yield 66%. The crop of mixed α- and β-anomers (12.7 g.) was recrystallized from a mixture of 250 ml. of absolute ethanol and 145 ml. of acetone; yield, 11.4 g., m.p. 207–208°, $[\alpha]^{25}D$ +88.6° (*c* 2.6 in chloroform). The combined 11.4- and 9.7-g. crops (62%) were recrystallized three times from ethanol–acetone to give pure α-octaacetate; m.p. 211–212°, $[\alpha]^{25}D$ +89.4° (*c* 2.5 in chloroform).[8]

Anal. Calcd. for $C_{28}H_{39}NO_{18}$: C, 49.6; H, 5.80; N, 2.07. Found: C, 49.9; H, 5.84; N, 2.05.

The aqueous filtrate from the 12.7-g. crop was extracted with chloroform, and the aqueous pyridine-soluble acetates were isolated in the usual way. Recrystallizations from chloroform–petroleum ether gave 27% of the theoretical amount of *N*-acetyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine, m.p. 159–160°, $[\alpha]^{25}D$ +17° (*c* 1 in chloroform).[47]

Two other preparations conducted and analyzed in this way gave 60% yields of octa-*O*-acetyl-di-D-glucosylamine(s) with m.p. *ca.* 205° and $[\alpha]^{25}D$ +86°. When the preparative method was repeated, starting with 21% (wt./wt.) ammonia in methanol at 25°, the yeld of octaacetates was lowered to 47%. Magnesium sulfate as the drying agent gave less octaacetates than did calcium sulfate. When calcium sulfate was omitted, yields were only 37%.

Di-α-D-glucosylamine.—Octa-*O*-acetyl-di-α-glucosylamine (20 g.), m.p. 211–212°, was held in methanol–ammonia solution (24% ammonia, wt./wt.) at 2° for 2 days. After filtration and evaporation of the filtrate under vacuum at 25°, crystallization occurred. The first crop was filtered, then addition of ethanol to the filtrate gave an additional crop which, when combined with the first, washed with methanol and ethanol, and dried in a vacuum desiccator over sulfuric acid, weighed 8.4 g. (84%), m.p. 157–159°. After extraction with 260 ml. of boiling methanol, the yield was 6.6 g. (66%), m.p. 165–166° dec., $[\alpha]^{25}D$ +90° (3–5 min., *c* 2.0 in water, pH 7.35), decreasing very slowly thereafter; lit.[8] m.p. 167–168°, $[\alpha]D$ +85.1 (water), for substance of m.p. 164–165°.

Anal. Calcd. for $C_{12}H_{23}NO_{10}$: C, 42.2; H, 6.79; N, 4.10. Found: C, 42.1; H, 6.78; N, 4.06.

Di-α-D-glucosylamine is only slightly soluble in hot methanol, and is practically insoluble in absolute ethanol or other cold organic solvents, except dimethyl sulfoxide. It is soluble in warm pyrrolidones. When recrystallized from a hot methanol solution, with or without added ammonia, its melting point is lowered. It does not reduce 2,6-dichloroindophenol in 0.1 *N* sodium hydroxide solution at 25° until 5 min. or more have elapsed. Paper chromatography in aqueous solvent systems shows an isolated spot near the origin for II, also a streak running from the position of mono-D-glucosylamine to that of D-glucose.

***N*-Acetyl-octa-*O*-acetyl-di-α-D-glucosylamine.**—Prepared in 70% yield[8] the recrystallized nonaacetate melted at 195°, $[\alpha]^{25}D$ −7.1° (*c* 4.3 in chloroform).

Anal. Calcd. for $C_{30}H_{41}NO_{19}$: C, 50.1; H, 5.74; N, 1.95. Found: C, 50.1; H, 5.85; N, 1.99.

***N*-Acetyl-di-D-glucosylamine.**—Seven grams of the recrystallized nonaacetate of II was held in 200 ml. of 17% (wt./wt.) ammonia in methanol at 2° for 2 days and at 25° for 4 hr. Concentration under reduced pressure gave a sirup which, when dissolved in 20 ml. of ethanol, 0.2 ml. of water, and with ether added to incipient turbidity, gave 3.7 g. (91%) of the crystalline dihydrate. Recrystallization from methanol, with drying over calcium chloride in a desiccator for a day, gave 3.1 g. containing 1.6 moles of water of hydration. Drying at 110° under high vacuum for 5 hr. gave the anhydrous compound, m.p. *ca.* 165°, $[\alpha]^{26}D$ +21° (*c* 1.0 in water).

Anal. Calcd. for the anhydrous $C_{14}H_{24}NO_{11}$: C, 43.9; H, 6.57; N, 3.65. Found: C, 44.0; H, 6.99; N, 3.61.

The anhydrous compound regained 1.6 moles of water per mole at 25°, 45% relative humidity, before spontaneously drying to 0.33 mole of water (constant), with a change in crystalline form, but with no change in specific rotation of the anhydrous compound.

Attempted acetylation of II in water by the method used on I, and also at pH 8–10, gave chiefly D-glucose and *N*-acetylated mono-D-glucosylamine (proved by reacetylation).

Di-1,1′-D-glucitylamine.—Di-α-D-glucosylamine, m.p. 165–166° (5.0 g.), in 150 ml. of aqueous methanol (85%) was hydrogenated with a Raney nickel catalyst[28] for 5.5 hr. at 100°, 2470 p.s.i. The filtered solution gave no reduction of hot Fehling solution. After storage at 2° for 2 days, 4.0 g. (80%) of the crude product, m.p. 145–150°, crystallized. Three recrystallizations from 80% methanol gave 2.0 g. (40%), m.p. 162–164°, $[\alpha]^{20}D$ −22.1° (*c* 5.0 in water, pH 10.2).

Anal. Calcd. for $C_{12}H_{27}NO_{10}$: C, 41.7; H, 7.88; N, 4.06; neut. equiv., 345. Found: C, 41.5; H, 7.77; N, 4.07; neut. equiv., 342.

Di-1,1′-D-glucitylamine Hydrochloride. A.—The mixture of di- and mono-D-glucosylamines, prepared as described to the point of analysis by acetylation, was hydrogenated in 150 ml. of 4:1 methanol–water with 10 g. of a 64% nickel-on-kieselguhr catalyst (Girdler G-49A,[29] prehydrogenated just before use). The theoretical drop in pressure was obtained at 100°, 2500 p.s.i., within 2 hr. After 2.5 hr. the hydrogenation was stopped, the bomb was opened while still warm (50°), and the contents were immediately filtered. The hydrochloride salt crystallized promptly from the colorless filtrate. Neutralization of the liberated ammonia with hydrochloric acid increased the yield to 11.4 g. (60%). Recrystallization of the crude product from 210 ml. of 4:1 methanol–water gave 9.3 g. (48%) of the pure hydrochloride salt, m.p. 164–165°, $[\alpha]^{25}D$ −26.7° (*c* 5.0 in water, pH 5.2).

Anal. Calcd. for $C_{12}H_{28}NO_{10}Cl$: C, 37.8; H, 7.39; N, 3.67; Cl, 9.29. Found: C, 37.9; H, 7.37; N, 3.78; Cl, 9.20.

Basic di-1,1′-D-glucitylamine, prepared as described, gave this same hydrochloride salt when mixed with hydrochloric acid in aqueous methanol.

Recrystallization of 4.5 g. of the hydrochloride salt from 120 ml. of 0.1 *M* ammonium hydroxide in 4:1 methanol–water gave the free base in 86% yield. The base absorbed only 1% moisture upon standing 3 days at 21°, 50% constant relative humidity.

B. One-half of 18 g. (100 mmoles) of anhydrous D-glucose was stirred with 1.33 g. (25 mmoles) of ammonium chloride and 59 mmoles of ammonia (4.0 ml. of 14.8 *N* ammonium hydroxide) in 250 ml. of absolute methanol at 50–60° in a closed (mineral oil trapped) reflux system at atmospheric pressure. When the sugar dissolved (15 min.), the remaining half of the D-glucose was

(27) C. Niemann and J. T Hays, *J. Am. Chem. Soc.*, **62**, 2960 (1940).

(28) A. A. Pavlic and H. Adkins, *ibid.*, **68**, 1471 (1946).

(29) Mention of manufacturer or trade-names does not constitute endorsement by the U. S. Department of Agriculture.

added, and heating was continued at 60–70° for 0.5 hr. until the second batch of sugar dissolved. The bath temperature was increased to 70° and refluxing was continued for 2 hr. in the closed system. After the solvent was removed under reduced pressure (water aspirator, rotating evaporator), the residue was thoroughly dried at 50° (20 mm.). The residue was then dissolved in 100 ml. of boiling methanol, and the solution was added to 10 g. of a nickel-on-kieselguhr catalyst (Girdler G-49A,[29] prehydrogenated for 1 hr. at 200°, 2500 p.s.i. in 50 ml. 90% methanol–water) in a high-pressure hydrogenation bomb. After hydrogenation at 100°, 2500 p.s.i., for 2.5 hr. (constant pressure after 1.5 hr.), cooling to 25°, and filtering, most of the reaction product had crystallized and remained with the catalyst in the filter cake. Extraction of the filter cake with hot 50% methanol–water, and then with 400 ml. of hot water—along with neutralization of the filtrate with hydrochloric acid—produced a total of 12.6 g. (66%) of recrystallized hydrochloride salt, m.p. 164–165°, identical with the di-1,1′-D-glucitylamine hydrochloride prepared as described in A.

The filtrate contained sorbitol (10%) and reducing sugar (1%, as glucose by copper reduction) which were separated by passage of the total filtrate through strongly acidic cation-exchange resin in hydrogen form. Sorbitol crystallized from the effluent after concentration and was identified as the hexa-*o*-acetyl derivative. A mixture of mono- and di-D-glucitylamine hydrochlorides was eluted from the resin with 1 *N* hydrochloric acid; then passage of the acid solution through a strongly basic anion-exchange column gave a solution of the mixed bases (identified by paper chromatography). Concentration of the basic effluent under vacuum, with the addition of methanol, gave additional di-D-glucitylamine as the crystalline free base (1%).

Procedure B was repeated with one-half the specified quantity of ammonium hydroxide with a crude yield of 66%. When 150 ml. of 94% methanol–water was used as the solvent medium in place of 250 ml. of absolute methanol, the crude yield also was 66%.

***N*-Acetyl-deca-*O*-acetyl-di-1,1′-D-glucitylamine.**—One gram of III was acetylated in 10 ml. of pyridine with 6 ml. of acetic anhydride by shaking the mixture continuously for 5 hr. at 25°. After decomposition of the excess acetic anhydride in ice–water, the first-formed precipitate dissolved. Extraction with chloroform, with elimination of pyridine acetate in the usual manner, and evaporation under vacuum gave the fully acetylated product as a solid residue, 1.5 g. (66%), m.p. 59–64°. Reprecipitation by solution in ether, addition of petroleum ether (b.p. 33–57°) and cooling to 0° gave 1.0 g. (44%) of analytically pure compound, m.p. 64–66°, $[\alpha]^{19}D$ +0.6° (*c* 1.0 in chloroform, *l* 2).

Anal. Calcd. for $C_{34}H_{49}NO_{21}$: C, 50.5; H, 6.11; N, 1.73; $COCH_3$, 58.5. Found: C, 50.7; H, 6.20; N, 1.62; $COCH_3$ (Elek),[30] 58.2.

***N*-Acetyl-di-1,1′-D-glucitylamine.**—One gram (2.9 mmoles) of di-1,1′-D-glucitylamine was dissolved in 7 ml. of water and 0.8 ml. (7.8 mmoles) of acetic anhydride was added. After standing at 25° with occasional shaking for 1 hr., the clear solution was concentrated under vacuum to a sirupy residue. A solution of the residue in 100 ml. of water was passed through an ion-exchange column (Amberlite IRC-50 H^+-form[29]) to remove the unchanged diglucitylamine; then the acidic effluent from the column was concentrated under vacuum to a neutral residue. The residue was dissolved in 25 ml. of hot 98% methanol; 0.50 g. (44%) of colorless crystals separated on cooling. Recrystallization in the same way with air-drying gave 0.44 g. (39%) of a neutral, hydrated compound which shrank in a capillary tube and partially melted from 85 to 92°, swelled at 93°, and did not completely melt until 130°. A desiccator-dried sample was further dried to constant weight at 65° under high vacuum (with loss of 1.0% water) before analysis, $[\alpha]^{25}D$ −27° (*c* 1.0 in water).

Anal. Calcd. for $C_{14}H_{29}NO_{11}$: C, 43.4; H, 7.55; N, 3.62. Found: C, 43.5; H, 7.73; N, 3.49.

The dry sample was exposed at 21°, 50% relative humidity, for 2 days. The gain in weight became constant at 2.2%, representing 0.5 mole of hydrate water.

Di-1,1′-D-mannitylamine Hydrochloride.—When procedure A was applied to D-mannose, the yield of dimannitylamine hydrochloride salt was 33%, m.p. 199–200°, $[\alpha]^{20}D$ +13.5° (*c* 5.0 in water, pH 5.9).

Anal. Calcd. for $C_{12}H_{28}NO_{10}Cl$: C, 37.8; H, 7.39; N, 3.67; Cl, 9.29. Found: C, 37.8; H, 7.47; N, 3.52; Cl, 9.19.

By procedure B, using only 145 ml. of absolute methanol in the first mixture (because of the greater solubility of D-mannose over D-glucose), the crude yield was 15.2 g. (80%), m.p. 198–200°. Recrystallization from 370 ml. of 70% methanol gave the pure hydrochloride salt (80% recovery) described.

Di-1,1′-D-mannitylamine.—Di-1,1′-D-mannitylamine hydrochloride (2.50 g.) was recrystallized from 70 ml. of 70% (v./v.) methanol–water that contained 1 ml. of 14.8 *N* ammonium hydroxide, yielding 2.24 g. (99%) of chloride-free crystals, m.p. 186–187°, $[\alpha]^{20}D$ +6.4° (*c* 5.0 in water, pH 11.0). Recrystallization did not raise the melting point. The base was slightly hygroscopic; it took up 0.58% of water upon standing at 20°, 50% relative humidity, for 1 day. It was dried at 64° under high vacuum before analysis.

Anal. Calcd. for $C_{12}H_{27}NO_{10}$: C, 41.7; H, 7.88; N, 4.06; neut. equiv., 345. Found: C, 41.8; H, 8.04; N, 4.05; neut. equiv., 350.

***N*-Acetyl-di-1,1′-D-mannitylamine.**—One gram of dimannitylamine, 0.8 ml. of acetic anhydride, and 9 ml. of water were mixed and held at 25° for 1 hr. Vacuum distillation of the solvent and recrystallization of the residue gave 0.72 g. (65%), m.p. 155–157°. Ion-exchange column purification as described, recrystallization from 85% methanol–water, and drying for 4 hr. at 110° under high vacuum gave 0.46 g. of anhydrous *N*-acetyl-di-1,1′-D-mannitylamine (41%), m.p. 165–166°, $[\alpha]^{20}D$ +34° (*c* 1.0 in water, pH 6.5).

Anal. Calcd. for $C_{14}H_{29}NO_{11}$: C, 43.4; H, 7.55; N, 3.62. Found: C, 43.7; H, 7.69; N, 3.50.

Di-1,1′-D-galactitylamine Hydrochloride.—Application of procedure B, but with more water added to dissolve the D-galactose (74% methanol), produced 9.9 g. (51%) of nearly pure hydrochloride salt, m.p. 239–241°. Recrystallization from 11 parts of water gave 9.3 g. (49%) of the pure compound, m.p. 240–241°, in agreement with Kagan.[16] The optical rotation, previously unreported, is $[\alpha]^{20}D$ −22° (*c* 1.4 in water, pH 5.6). Crude D-galactitol (dulcitol), m.p. 184–185°, was crystallized from the mother liquor in 4.5% yield.

Di-1,1′-D-galactitylamine.—Recrystallization of the hydrochloride salt from aqueous ammoniacal alcohol, as described, gave the free amine in 95% yield, m.p. 199–201°. Recrystallization of the free base from water raised the melting point to 202–204°, $[\alpha]^{20}D$ −9.4° (*c* 1.0 in water, supersaturated, pH 10.6); lit.[16] $[\alpha]^{20}D$ −11° (*c* 0.50 in water, *l* 4).

***N*-Acetyl-di-1,1′-D-galactitylamine.**—Prepared as described for *N*-acetyl-di-D-mannitylamine, this neutral hygroscopic compound (35%, after two recrystallizations from 4:1 methanol–water) melted at 168–169°. The dry compound absorbed 1.3 moles of water at 25°, 50% relative humidity, and was much more soluble in water (*ca.* 5%) than the free base (<1%). A desiccated sample that was not further dried before analysis gave C, H, and N percentages in agreement with the hemihydrate. Samples that were dried to constant weight at 110° under high vacuum immediately before analysis gave $[\alpha]^{20}D$ −16° (*c* 1.0 in water, pH 6.8).

Anal. Calcd. for $C_{14}H_{29}NO_{11}$: C, 43.4; H, 7.55; N, 3.62. Found: C, 43.3; H, 7.58; N, 3.53.

Di-1,1′-D-arabinitylamine Hydrochloride.—Application of procedure B produced a total of 12.1 g. (76%) of nearly pure hydrochloride salt, m.p. 204–205°, Recrystallization from 370 ml. of 60% methanol–water gave 10.7 g. (67%), m.p. 206°, $[\alpha]^{20}D$ +22.2° (*c* 5.0 in water, pH 5.5).

Anal. Calcd. for $C_{10}H_{24}NO_8Cl$: C, 37.3; H, 7.52; N, 4.35; Cl, 11.0. Found: C, 37.7; H, 7.71; N, 4.36; Cl, 11.0.

Di-1,1′-D-arabinitylamine.—Two grams of the hydrochloride salt, prepared as described, was dissolved in 100 ml. of warm 60% methanol–water that was made alkaline with ammonium hydroxide. After no crystallization occurred on cooling to 2°, 10 ml. of absolute ethanol was added. Crystallization then occurred after storage at 2° yielding 1.7 g. (96%). To remove a trace of chloride, the 1.7 g. was recrystallized from 85 ml. of 75% methanol–water, yielding 1.4 g. (79%), m.p. 177–178°, $[\alpha]^{20}D$ +13° (*c* 2.0 in water, pH 10.6).

Anal. Calcd. for $C_{10}H_{23}NO_8$: C, 42.1; H, 8.13; N, 4.91. Found: C, 42.2; H, 8.11; N, 4.79.

***N*-Acetyl-di-1,1′-D-arabinitylamine.**—By the procedure described for *N*-acetylation of dimannitylamine, 1.0 g. of diarabinitylamine, 0.8 ml. of acetic anhydride, and 8 ml. of water gave the tertiary acetamide in 70% yield, m.p. 166–169°. Two recrystallizations from 90% methanol gave the pure compound, m.p. 168–169°, $[\alpha]^{20}D$ +19° (*c* 1.0 in water, pH 6.6).

(30) A. Elek and R. A. Harte, *Ind. Eng. Chem., Anal. Ed.*, **8**, 267 (1936).

Anal. Calcd. for $C_{12}H_{25}NO_8$: C, 44.0; H, 7.70; N, 4.28. Found: C, 44.4; H, 7.57; N, 4.12.

Paper Chromatography.—The compounds were separated by the descending method on Whatman[29] no. 1 filter paper at 20° in 1-butanol–pyridine–water (6:4:3) for 18 hr., and in ethyl acetate–acetic acid–water (3:1:3, upper phase) for 44 hr. Ammoniacal silver nitrate spray and heating located all compounds, whereas the amines were indicated separately by either ninhydrin or bromcresol green spray reagents. The glycosylamines were nearly completely hydrolyzed by the acidic solvent (Table II).

TABLE II

THE CHROMATOGRAPHIC MOBILITIES RELATIVE TO D-GLUCOSE

Compound	*n*-BuOH: C_5H_5N: H_2O 6:4:3	EtOAc: AcOH:H_2O 3:1:3, upper phase
D-Glucose	1.00[a]	1.00
D-Mannose	1.21	1.29
D-Galactose	0.87	0.90
D-Arabinose	1.12	1.53
D-Glucitol	0.94	1.02
D-Mannitol	0.98	1.07
D-Galactitol	0.93	1.19
Mono-D-glucosylamine	0.58[b]	...
Di-D-glucosylamine	0.43[b]	...
Mono-D-mannosylamine	0.59[b]	...
Di-D-mannosylamine	0.28[b]	...
Di-1,1′-D-glucitylamine	0.23	0.17
Di-1,1′-D-mannitylamine	0.23	0.17
Di-1,1′-D-galactitylamine	0.18	0.14
Di-1,1′-D-arabinitylamine	0.36	0.32
N-Acetyl-β-D-glucopyranosylamine	1.23	1.39
N-Acetyl-di-D-glucosylamine	0.75	0.50
N-Acetyl-di-D-glucitylamine	0.38	0.31
N-Acetyl-di-D-mannitylamine	0.44	0.31
N-Acetyl-di-D-galactitylamine	0.38	0.31
N-Acetyl-di-D-arabinitylamine	0.70	0.57

[a] $R_F = 0.31 \pm 0.02$ at 20°. [b] Partially hydrolyzed, with spots showing for the parent sugar and the secondary (or primary) amine.

Copper Chelate Salts.—The dialditylamine (1.2 mmoles) was dissolved in about 15 ml. of 80% methanol–water by heating. To the warm solution was added a warm solution of 1.2 mmoles of cupric acetate monohydrate dissolved in 8 ml. of 80% methanol–water. The combined solution was neutral or weakly acidic. Nothing crystallized on cooling; but when 20 ml. of absolute ethanol was added to the warm mixed solution, blue crystals formed during storage at 2°. The alcohol-washed, desiccated crystals contained alcohol of crystallization, as was shown by alkoxyl determinations. Drying at 110° (0.1 mm.) with intervening humidifications, was necessary to remove all the alcohol. The melting points were sharp, and all melts turned red with reduced copper. Dilute solutions of each copper salt gave no precipitate upon adding hydroxide, carbonate, or ferrocyanide ion; however, precipitation did occur with hydrogen sulfide or disodium hydrogen phosphate additions. *N*-1-D-Glucitylmethylamine and *N*-1,1′-di-D-arabinitylamine gave no crystalline precipitate by this procedure, the salts being much more soluble.

A. **Di-1,1′-D-glucitylamine** (0.40 g.) gave a mixture of two different compounds (0.26 g. less soluble and 0.20 g. more soluble) that were fractionally recrystallized from 80% methanol–water. The 110° vacuum-dried, less-soluble fraction contained more copper and melted sharply at a lower temperature (162–163°) than the more soluble fraction (179–181.5°). Both dried fractions were hygroscopic.

Anal. Calcd. for $C_{26}H_{48}N_2O_{22}Cu_5$: C, 29.5; H, 4.57; N, 2.65; Cu, 30.0; 1 $COCH_3$, 4.07. Found, for the less-soluble fraction: C, 29.4; H, 4.42; N, 2.68; Cu, 29.8; $COCH_3$ (Elek),[30] 3.50.

Anal. Calcd. for $C_{26}H_{52}N_2O_{22}Cu_3$: C, 33.4; H, 5.61; N, 3.00; Cu, 20.4; 1 $COCH_3$, 4.60. Found for the more-soluble fraction: C, 32.7; H, 5.41; N, 2.92; Cu, 19.2; $COCH_3$, 0.78.

B. **Di-1,1′-D-mannitylamine** removed all traces of cupric ion from the mixed solution. A single compound crystallized; when dried over calcium chloride and sulfuric acid at room temperature, atmospheric pressure, 0.4 g., m.p. 186–188°, was recovered. Recrystallization from aqueous alcohol with drying at 60° (0.1 mm.) for 4 hr. gave material of m.p. 195–196°.

Anal. Calcd. for $C_{16}H_{34}NO_{14}Cu_2$: C, 32.5; H, 5.63; N, 2.37; Cu, 21.5; 1 $COCH_3$, 7.29; 1 C_2H_5OH, 7.79. Found: C, 33.0; H, 5.72; N, 2.46; Cu, 21.4; $COCH_3$ (Elek),[30] 7.34.

The loss on further drying at 110° (0.1 mm.) to constant weight was 8.06% leaving a dried hygroscopic material, m.p. 188–189°.

Anal. Calcd. for $C_{14}H_{29}NO_{13}Cu_2$: C, 30.8; H, 5.35; N, 2.56; Cu, 23.3; 1 $COCH_3$, 7.88. Found: C, 30.2; H, 5.21; N, 2.51; Cu, 24.3; $COCH_3$, 6.82.

C. **Di-1,1′-D-galactitylamine** (1.2 mmoles) was dissolved in 6 ml. of warm water and 1.2 mmoles of cupric acetate in 4 ml. of water was added. The dark blue, neutral solution was warmed and diluted with 25 ml. of absolute ethanol. On storage at 2° crystallization occurred; 0.3 g., m.p. 158–162°, was isolated. Recrystallization from 4 ml. of water plus 15 ml. of ethanol did not change the melting point. A sample dried to constant weight at 110° (0.1 mm.) gave:

Anal. Calcd. for $C_{12}H_{25}NO_{10}Cu + {}^1/_3Cu(OH)(OCOCH_3)$: C, 33.6; H, 5.85; N, 3.09; Cu, 18.7. Found: C, 33.6; H, 5.85; N, 3.05; Cu, 18.1.

D. ***N*-1-D-Glucitylmethylamine** (***N*-methylglucamine, VII**) (0.40 g., 2.0 mmoles) was dissolved in 7 ml. of 80% methanol–water, and 0.40 g. (2.0 mmoles) of hydrated cupric acetate in 4 ml. of 80% methanol–water was added. No precipitation occurred after extensive vacuum concentration and cooling. Further vacuum concentration gave 0.7 g. of hygroscopic, blue, solvated crystals, m.p. 92–96°. When recrystallized from absolute ethanol plus ether, these gave 0.18 g. (27%) of hygroscopic salt, m.p. 164–167° dec., after drying at 64° (0.1 mm.) to constant weight.

Anal. Calcd. for $C_9H_{21}NO_8Cu$: C, 32.3; H, 6.32; N, 4.18; Cu, 19.0; $COCH_3$, 12.8. Found: C, 32.6; H, 6.39; N, 4.48; Cu, 18.6; $COCH_3$ (Elek),[30] 12.7.

***N*-1′-D-Alditylpiperidines.**—The D-galactityl- and D-arabinitylpiperidines were prepared by the method previously described for D-mannitylpiperidine (Table I, footnote *h*). The products were contaminated with the alditol, and three recrystallizations from aqueous alcohol were required to reach an analytically pure sample.

***N*-1-D-Galactitylpiperidine** melted at 139–140°, $[\alpha]^{20}D$ −8.3 (*c* 2.0 in water, pH 11.2). After neutralzation of the optical rotation solution to pH 6.5 with hydrochloric acid, the rotation became $[\alpha]^{20}D$ −21°. Evaporation of the neutral solution gave the hydrochloride salt, m.p. 155–156°, when recrystallized from absolute ethanol.

Anal. Calcd. for $C_{11}H_{23}NO_6$: C, 53.0; H, 9.30; N, 5.62. Found: C, 52.8; H, 9.23; N, 5.35.

***N*-1-D-Arabinitylpiperidine** melted at 133–134°, $[\alpha]^{20}D$ +8.5 (*c* 2.0 in water, pH 11.3). After neutralization to pH 6.5 with hydrochloric acid, the rotation became $[\alpha]^{20}D$ +22°. The hydrochloride salt obtained by evaporation of the neutral solution and recrystallization from absolute ethanol melted at 136–137°.

Anal. Calcd. for $C_{10}H_{21}NO_4$: C, 54.8; H, 9.65. Found: C, 54.7; H, 9.64.

Acknowledgment.—The authors thank Mrs. Clara McGrew, Mrs. Bonita Heaton, and Miss Kay Horton for the microanalyses, R. L. Reichert for assistance with the hydrogenations, and E. C. Nelson for preparations of D-glucosylamine by Klug's procedure. The work was done under the general supervision of R. J. Dimler.