mixture of methylated neutral sugars was applied to strips of filter paper, pretreated with water, and was resolved with solvent system F. Appropriate sections were located with guide strips, eluted with water and diluted to a suitable volume. The concentration of the solutions was determined by the *o*-aminodiphenyl method,[10] the relation between concentration and absorbance for each sugar having been determined previously.[16] The average value of three determinations was used.

Hypoiodite Oxidation of the Hemicellulose.—The oxidation was carried out with a 0.1 *N* iodine solution buffered to a *p*H of 10.6.[19] The amount of iodine consumed after 2.5 hr. in the dark corresponded to one reducing end-group per 62 anhydroxylose units.

Determination of the Molecular Weight of the Methylated Hemicellulose.—Osmotic pressure measurements were carried out with the osmometer of Zimm and Myerson[62] as improved by Stabin and Immergut.[36] Gel cellophane membranes[63] which had never been allowed to dry, were used, the solvent was chloroform–ethanol (9:1 v./v.) and the temperature was 30 ± 0.01°. The osmotic pressure was determined at six different concentrations by the static method and the values of the reduced osmotic pressure, h/w, as plotted against w, were extrapolated to zero concentration (Table III). The number-average molecular weight, $\bar{M}_n$, was calculated from the relationship $\bar{M}_n = 25{,}700/(h/w)_0$ and the corresponding degree of polymerization, $\bar{P}_n$, from the equation $\bar{P}_n = \bar{M}_n \times n/M_R$, where n was the number of xylose residues present per acid side group and M_R was the molecular weight of the repeating unit of the fully methylated polysaccharide (2457).

TABLE III

OSMOMETRY DATA OBTAINED FOR THE METHYLATED HEMICELLULOSE

w[a]	h[b]	h/w
4.815	9.371	1.946
4.415	7.897	1.789
3.814	6.718	1.761
3.511	6.061	1.726
2.760	4.835	1.752
1.904	3.168	1.664
0	...	1.5 1

[a] Concentration in g./kg. solution. [b] Osmotic height in cm. solvent.

Determination of the Intrinsic Viscosity of the Hemicellulose.—Reduced viscosities (η_{sp}/C) of the potassium salt of the hemicellulose were determined at seven different concentrations with a Craig–Henderson[40] viscometer in *M* cupriethylenediamine. Extrapolation to zero concentration according to Huggins[64] gave an intrinsic viscosity, $[\eta]$, of 0.812 dl./g. and a value of k' of 0.428. Kinetic energy corrections were negligible. According to the relationship $\bar{P}_n = 212[\eta]$, developed earlier for a similar polysaccharide[39] this would correspond to a number-average degree of polymerization of 172.

Estimation of the Polymolecularity of the Hemicellulose.—Hemicellulose (4 g.) was dissolved in 5% aqueous potassium hydroxide (200 ml.) and the solution was diluted to 700 ml. with water and ethanol, until the polysaccharide barely remained in solution. Fractionation was carried out at 25° by gradual addition of ethanol until the solution became cloudy, after which stirring was continued for 10 min. Fourteen fractions were collected on the centrifuge and purified in the same way as the original hemicellulose. Final drying was from petroleum ether (b.p. 30–60°) *in vacuo*. The fractions were weighed and their intrinsic viscosity was determined. The frequency distribution had one maximum located at a $\bar{P}_n$ value of 170 and exhibited a slight negative skewness.

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MONTREAL, QUEBEC

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Acetohalogeno Derivatives and Glycosides of D-Galactosamine[1]

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2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride and bromide have been synthesized from D-galactosamine α- and β-pentaacetates. Under various conditions, they afforded methyl and ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosides, which were further hydrolyzed into methyl and ethyl 2-acetamido-2-deoxy-β-D-galactopyranosides. The 1-chloro derivative was shown to transpose into 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-galactopyranose hydrochloride. Opening of the 1,6-anhydro ring of 2-acetamido-3,4-di-*O*-acetyl-1,6-anhydro-2-deoxy-β-D-galactopyranose with concomitant acetylation was successfully achieved.

Investigations on the metabolism of aminosugars have demonstrated the need for the availability of phosphate esters of D-galactosamine possessing known chemical structures.[3] The prerequisite intermediates in the synthesis of the 1-phosphate esters of D-galactosamine are the acetohalogeno derivatives. Whereas synthesis of this type of derivative in the *N*-acetyl-D-glucosamine series has been the subject of recent numerous publications,[4–13] the analogous derivatives of

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N-acetyl-D-galactosamine have not yet been reported and are the subject of the present paper.

The lack of success of Moggridge and Neuberger[14] in their attempt to isolate the 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl bromide has been shown to be due to the instability of the 1-bromo derivative which isomerizes during the process of recrystallization into 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrobromide.[6,11,12] In order to obtain the β-glycosides, the 1-bromo derivative was not isolated but was treated directly with an alcohol in the presence of silver oxide,[12] silver carbonate[11] or mercuric cyanide[4,13] as a condensing agent. Use of the more stable crystalline 1-chloro derivative[5] leads also to the successful synthesis of β-glycosides.[10,11,13] However, the chloro compound is also somewhat unstable, resulting in its partial transformation into the hydrochloride during crystallization.[11]

The present studies were conducted with D-galactosamine, and led to results identical with those observed in the D-glucosamine series. Action of hydrobromic acid in glacial acetic acid on either of the anomers of D-galactosamine pentaacetate produced 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-galactopyranosyl bromide (I). Because of the known instability of the analogous glucosamine derivative, no attempt was made to isolate I. Its solution was treated with methanol in the presence of mercuric cyanide or silver oxide to give methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (II). Both catalysts gave similar yields, but removal of the silver salts was easier. The total yield of II from D-galactosamine pentaacetate was 76%. Since β-glycosides of D-glucosamine also have been obtained starting from the chloro derivative, which is more convenient to manipulate than the bromo derivative, 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride (III) was synthesized from either α- or β-anomers of D-galactosamine pentaacetate. Two methods were tested, hydrogen chloride in ether[5] and titanium tetrachloride[5]; the latter was found to be simpler. When the chloride III was treated with methanol, in the presence of silver oxide, the β-glycoside II was isolated in a yield of 86%.

Like the glucosamine derivative, III was found to be unstable on recrystallization and to transform into 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-galactopyranose hydrochloride (IV). The structure of IV was ascertained by *N*-acetylation to give the known 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose.[15]

Alkaline hydrolysis of II gave the crystalline methyl 2-acetamido-2-deoxy-β-D-galactopyranoside (V). Similarly reaction of the 1-chloro derivative III with absolute ethanol afforded ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (VI), subsequently hydrolyzed to ethyl 2-acetamido-2-deoxy-β-D-galactoside (VII). The same glycoside VII had been obtained previously during the recrystallization of 2-acetamido-2-deoxy-D-galactose[16] from absolute ethanol and it was identified by transformation into VI.

The preparation of the α-anomer of the ethyl glycoside was carried out by treating D-galactosamine pentaacetate with ethanol in the presence of hydrochloric acid according to the classical procedure.[15]

Since derivatives of 2-acetamido-1,6-anhydro-2-deoxy-β-D-galactopyranose are valuable intermediates in the synthesis of substituted derivatives of D-galactosamine, the 1,6-anhydro ring was opened by acetolysis with the purpose of transforming the resulting acetate into the reactive 1-halogenogalactosamine. Despite the previous report of an unsuccessful attempt to acetolyze 2-amino-1,6-anhydro-2-deoxy-β-D-galactopyranose hydrochloride,[17] its *N*-acetyl-3,4-di-*O*-acetyl derivative VIII showed a change of rotation from −37 to +102°, when treated with acetic anhydride, acetic acid and a catalytic amount of sulfuric acid.[18] Isolation of the resulting product gave the crystalline α- and β-anomers of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-galactopyranose[15] in yields of 61 and 5%, respectively. Reaction of the sirupy mixture of the pentaacetate with hydrobromic acid in glacial acetic acid, followed by direct treatment with methanol and silver oxide, gave the previously described methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (II).

Experimental[19]

2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α,β-D-galactopyranose.—The method described by Stacey[15] was modified.[20] A solution of 2.0 g. of D-galactosamine hydrochloride in a mixture of 15 ml. of anhydrous pyridine and 10 ml. of acetic anhydride was left overnight at room temperature. The mixture was diluted with 300 ml. of chloroform and washed at 0° successively with 15 ml. of water, two times with 10 ml. of saturated sodium bicarbonate solution and then with 10-ml. portions of a 10% solution of cupric sulfate until disappearance of the deep blue pyridine–copper complex, and finally with water. After drying over sodium sulfate, the chloroform was removed *in vacuo* and the sirupy residue was crystallized by addition of a small amount of absolute ethanol. When the β-anomer is present, it crystallizes first. However, in some preparations, only the α-form was isolated. The total yield was improved to 62% by chromatography on silicic acid[19] of the mother liquors, the α-anomer being eluted first with pure ethyl acetate.

Methyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (II) *via* I.—A solution of 500 mg. of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose in 4 ml. of glacial acetic acid saturated at 0° with hydrobromic acid was left overnight at room temperature in the dark, according to Inouye, *et al.*[12] After dilution with 40 ml. of chloroform, the solution was washed rapidly with small portions of ice-cold water, then two times with an ice-cold saturated solution of sodium bicarbonate, and finally with ice-cold water and dried over sodium sulfate. To this solution were added 3 g. of anhydrous sodium sulfate,

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1 g. of silver oxide, a trace of iodine and 5 ml. of methanol. The mixture was shaken in the dark for two days, and filtered over a double layer of Celite and Darco G-60. The filtrate was washed with water, dried over sodium sulfate and the chloroform removed by distillation *in vacuo*. Recrystallization from mixtures of methanol and ether or acetone and ether gave 352 mg. (76%) of elongated prisms, m.p. 216–217°, $[\alpha]^{23}D$ −17 ± 1° (in chloroform, *c* 1.84), $[\alpha]^{20}D$ −17 ± 1° (in methanol, *c* 1.67). *Anal.* Calcd. for $C_{15}H_{23}O_9N$: C, 49.86; H, 6.42; OCH_3, 8.59. Found: C, 49.75; H, 6.32; OCH_3, 8.43.

To avoid complete removal of solvent in the reaction using mercuric cyanide as catalyst,[4] additions of benzene were made during the removal of chloroform by distillation. The yield of II was 60% after purification by chromatography on silicic acid,[19] a mixture of ethyl acetate–acetone 9:1 being the eluent.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl Chloride (III) and 1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-α-D-galactopyranose Hydrochloride (IV).—A solution of 500 mg. of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactopyranose in 20 ml. of alcohol-free chloroform was treated with 0.25 ml. of titanium tetrachloride as described by Baker, *et al.*[5] The residual sirup crystallized from a mixture of ethyl acetate and ether to give approximately 200 mg. of III as rectangular stout prisms, m.p. 131–136° (dec.), $[\alpha]^{23}D$ +134 ± 2° (in chloroform, *c* 2.09). *Anal.* Calcd. for $C_{14}H_{20}O_8NCl$: C, 45.97; H, 5.51; N, 3.83; Cl, 9.69. Found: C, 45.84, 46.11; H, 5.67, 5.65; N, 3.96; Cl, 9.80.

From the mother liquors, 150 mg. of IV crystallized as fine prismatic needles. Successive recrystallizations of III from ethyl acetate–ether mixtures resulted in the formation of more IV, and the addition of a trace of moisture led to a complete transformation. The product recrystallized from a mixture of methanol and ether as a hydrate, m.p. 185–186° dec. $[\alpha]^{20}D$ +129 ± 2° (in methanol, *c* 0.66). *Anal.* Calcd. for $C_{14}H_{20}O_8NCl \cdot H_2O$: C, 43.81; H, 5.78. Found: C, 44.05; H, 5.71.

N-Acetylation of 80 mg. of IV with acetic anhydride in methanol in the presence of silver acetate in the usual manner gave 30 mg. of crystalline 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose, showing no depression of the m.p. in admixture with authentic material.[15]

Methyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (II) from III.—A solution of 33 mg. of III in 1.5 ml. of ethanol-free chloroform was treated with 30 mg. of anhydrous sodium sulfate, 50 mg. of silver oxide, 1 ml. of methanol and a trace of iodine as described for the bromide derivative. After recrystallization, 28 mg. (85%) of II was obtained, m.p. 216–217°, showing no depression of m.p. on admixture with the product described above.

Methyl 2-Acetamido-2-deoxy-β-D-galactopyranoside (V).—To a solution of 269 mg. of II in 4 ml. of methanol was added 0.3 ml. of 1 *N* barium methoxide. After standing overnight at 0°, the solution was neutralized with carbon dioxide and filtered. The solvent was removed and the residue dissolved in water. After treatment with Dowex 50 in the acid form, the water was evaporated *in vacuo* and the residue crystallized from a mixture of methanol and ether to give 158 mg. (90%) of stout needles, m.p. 191–193°, $[\alpha]^{23}D$ −12 ± 1° (in methanol, *c* 1.05). *Anal.* Calcd. for $C_9H_{17}O_6N$: C, 45.95; H, 7.28. Found: C, 45.84; H, 7.31.

Ethyl 2-Acetamido-2-deoxy-β-D-galactopyranoside (VII) and 3,4,6-Tri-*O*-acetyl Derivative (VI).—A solution of 525 mg. of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose in 20 ml. of chloroform was treated as previously described with 0.3 ml. of titanium tetrachloride. The resulting chloride, in sirupy state and without further purification, was dissolved in 7 ml. of chloroform and treated with 2.5 g. of anhydrous sodium sulfate, 0.8 g. of silver oxide, a trace of iodine and 5 ml. of absolute ethanol as previously described. The crystalline product was dissolved in chloroform and purified by chromatography on silicic acid.[19] Elution with ethyl acetate gave 373 mg. of crystalline fractions. Recrystallization from a mixture of acetone and ether afforded 296 mg. (59%) of VI as hexagonal prisms, m.p. 225–226°, $[\alpha]^{24}D$ −19 ± 1° (in methanol, *c* 1.23). *Anal.* Calcd. for $C_{16}H_{25}O_9N$: C, 51.19; H, 6.71. Found: C, 51.23; H, 6.83.

Alkaline hydrolysis with barium methoxide in the usual way of 50 mg. of VI gave, after recrystallization from a mixture of ethanol and ether, 28 mg. (80%) of VII as needles, m.p. 220–222°, $[\alpha]^{24}D$ −9 ± 1° (in methanol, *c* 0.59). *Anal.* Calcd. for $C_{10}H_{19}O_6N$: C, 48.18; H, 7.68. Found: C, 48.08; H, 7.70.

The residual sirup obtained by the *N*-acetylation of 2.0 g. of D-galactosamine according to Roseman and Ludowieg[16] was dissolved in absolute ethanol, and the solvent was removed by boiling. After a few repetitions of this process, the solution was left in the ice-box, and deposited 330 mg. of impure VII, m.p. 180–190°, $[\alpha]^{-5}D$ 0°, in methanol. *Anal.* Calcd. for $C_{10}H_{19}O_6N$: C, 48.18; H, 7.68; N, 5.62; $COCH_3$, 17.27. Found: C, 48.06; H, 7.70; N, 5.49; $COCH_3$, 17.54.

Acetylation of 122 mg. of this material in pyridine solution with acetic anhydride in the usual way gave, after recrystallization, 129 mg. (70%) of VI, m.p. 222–224°, showing no depression of m.p. on admixture with the product described above.

Ethyl 2-Acetamido-2-deoxy-α-D-galactopyranoside.[21]—A solution of 0.7 g. of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactopyranose in 70 ml. of absolute ethanol containing 2% of hydrogen chloride was refluxed for 4 hours. After cooling, the solution was neutralized with basic lead carbonate, filtered over Celite and concentrated *in vacuo*. The crystalline residue was recrystallized from ethanol to give 125 mg. (28%), m.p. 190–192°, $[\alpha]^{27}D$ +191 ± 2° (in methanol, *c* 0.96). *Anal.* Calcd. for $C_{10}H_{19}O_6N$: C, 48.18; H, 7.68. Found: C, 48.29; H, 7.79.

Acetolysis of 2-Acetamido-3,4-di-*O*-acetyl-1,6-anhydro-2-β-deoxy-D-galactopyranose (VIII).—A solution of 400 mg. of VIII in a mixture of 6 ml. of acetic anhydride, 4 ml. of glacial acetic acid and 0.07 ml. of concentrated sulfuric acid[18] was left at room temperature for 60 hours. The optical rotation changed from $[\alpha]D$ −37 to +100° after 30 hours and finally was +102°. After dilution with chloroform and washing with small amounts of ice-cold water, saturated sodium bicarbonate solution and water, and drying over sodium sulfate, the solvent was removed *in vacuo*. The residue was crystallized from ethyl acetate to give a first crop of 29 mg. (5%) of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactopyranose, m.p. 230–240°, $[\alpha]^{24}D$ +2 ± 2° (in chloroform, *c* 0.62), showing no depression of the m.p. in admixture with authentic material.[15] From the mother liquors, 326 mg. (61%) of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose was isolated, m.p. 180–185°, $[\alpha]^{26}D$ +98 ± 2° (in chloroform, *c* 0.93). The product showed no depression of the m.p. in admixture with authentic material.[15] In another experiment under identical conditions the largest part of the isolated material was the β-anomer.

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(21) This compound was prepared by Dr. P. J. Stoffyn.