The synthetic caviunin was characterized as its diacetate, colorless crystals from ethanol, m.p. and mixed m.p. 197.5°.

Anal. Calcd. for $C_{19}H_{16}O_6$ $(OCH_3)_4$; C, 60.26; H, 4.84; OCH_3, 27.1. Found: C, 59.91; H, 5.18; OCH_3, 28.7.

The natural and synthetic caviunin gave identical infrared (Nujol mull) and ultraviolet spectra. The infrared spectra (Nujol mull) of the diacetates were also identical.

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[CONTRIBUTION FROM THE ARTHRITIS RESEARCH LABORATORY, DEPARTMENTS OF MEDICINE AND BIOCHEMISTRY, UNIVERSITY OF ALABAMA MEDICAL CENTER]

Methyl Derivatives of D-Mannosamine

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By replacement of a *p*-tolylsulfonyloxy group with hydrazine and subsequent reductions, 2-amino-2-deoxy-3-*O*-methyl-D-mannose hydrochloride and crystalline 2-amino-2-deoxy-3,5,6-tri-*O*-methyl-D-mannose hydrochloride and their crystalline methyl β-glycosides were prepared. Other new amorphous intermediates are reported.

The interest in the preparation of methylated derivatives of 2-amino-2-deoxy-D-mannose arises from the finding of D-mannosamine as a structural entity of the biochemically important neuraminic acid.[1] The methods for the preparation of 2-amino-2-deoxy-D-mannose[2–6] require the separation of this sugar from its epimeric isomer in one step of the procedure. We are reporting the preparation of methyl ethers of D-mannosamine by a method which avoids such a separation and leads unambiguously only to compounds with a 2-amino-2-deoxy-D-mannose configuration.

It has been shown that the replacement of a *p*-tolylsulfonyloxy group with hydrazine[7] in appropriately substituted sugars proceeds with Walden inversion.[8–10] The application of this reaction to 2-*O*-*p*-tolysulfonyl-D-glucose derivatives should therefore yield compounds with a 2-hydrazino-2-deoxy-D-mannose configuration in which the hydrazino group should be reducible to an amino group.[8,11]

In an effort to get the unsubstituted D-mannosamine, we prepared the methyl 2-*O*-*p*-tolylsulfonyl-3,5,6-tri-*O*-benzyl-α,β-D-glucofuranoside by tosylation of methyl 3,5,6-tri-*O*-benzyl-α,β-D-glucofuranoside[12] in pyridine. The replacement of the *p*-tolylsulfonyloxy group with hydrazine, however, was not achieved even after a prolonged period of refluxing (four days). The steric effect of a large benzyl group in the 3- position or even in the 5- and 6- positions seems the probable basis for this lack of reactivity.

In order to test this supposition, the reaction was then carried out on the corresponding 3-*O*-methyl-5,6-di-*O*-benzyl derivative. This compound was prepared by benzylation of 1,2-*O*-isopropylidene-3-*O*-methyl-D-glucofuranose (I)[14] with benzyl chloride and potassium hydroxide, yielding 1,2-*O*-isopropylidene-3-*O*-methyl-5,6-di-*O*-benzyl-D-glucofuranose (II). With methanolic hydrogen chloride the isopropylidene group was split off, and a mixture of the α- and β-glycosides (III) was formed. Tosylation of III in pyridine yielded methyl 2-*O*-*p*-tolylsulfonyl-3-*O*-methyl-5,6-di-*O*-benzyl-α,β-D-glucofuranoside (XV). For this compound, also, a replacement of the *p*-tolysulfonyloxy group with hydrazine could not be achieved.

To show whether benzyl groups in the 5- or 6-positions would prevent a back-side displacement by hydrazine of the *p*-tolylsulfonyloxy group, the methyl 2-*O*-*p*-tolylsulfonyl-3,5,6-tri-*O*-methyl-β-D-glucofuranoside was prepared by tosylation of the known methyl 3,5,6-tri-*O*-methyl-β-D-glucofuranoside.[13] This compound was found to react with hydrazine. On subsequent hydrogenation with Raney nickel catalyst the methyl 2-amino-2-deoxy-3,5,6-tri-*O*-methyl-β-D-mannofuranoside was isolated as a crystalline hydrochloride. Hydrolysis with hydrochloric acid yielded the crystalline 2-amino-2-deoxy-3,5,6-tri-*O*-methyl-D-mannose hydrochloride.

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(2) P. A. Levene, *J. Biol. Chem.*, **36**, 73 (1918); **39**, 69 (1919).
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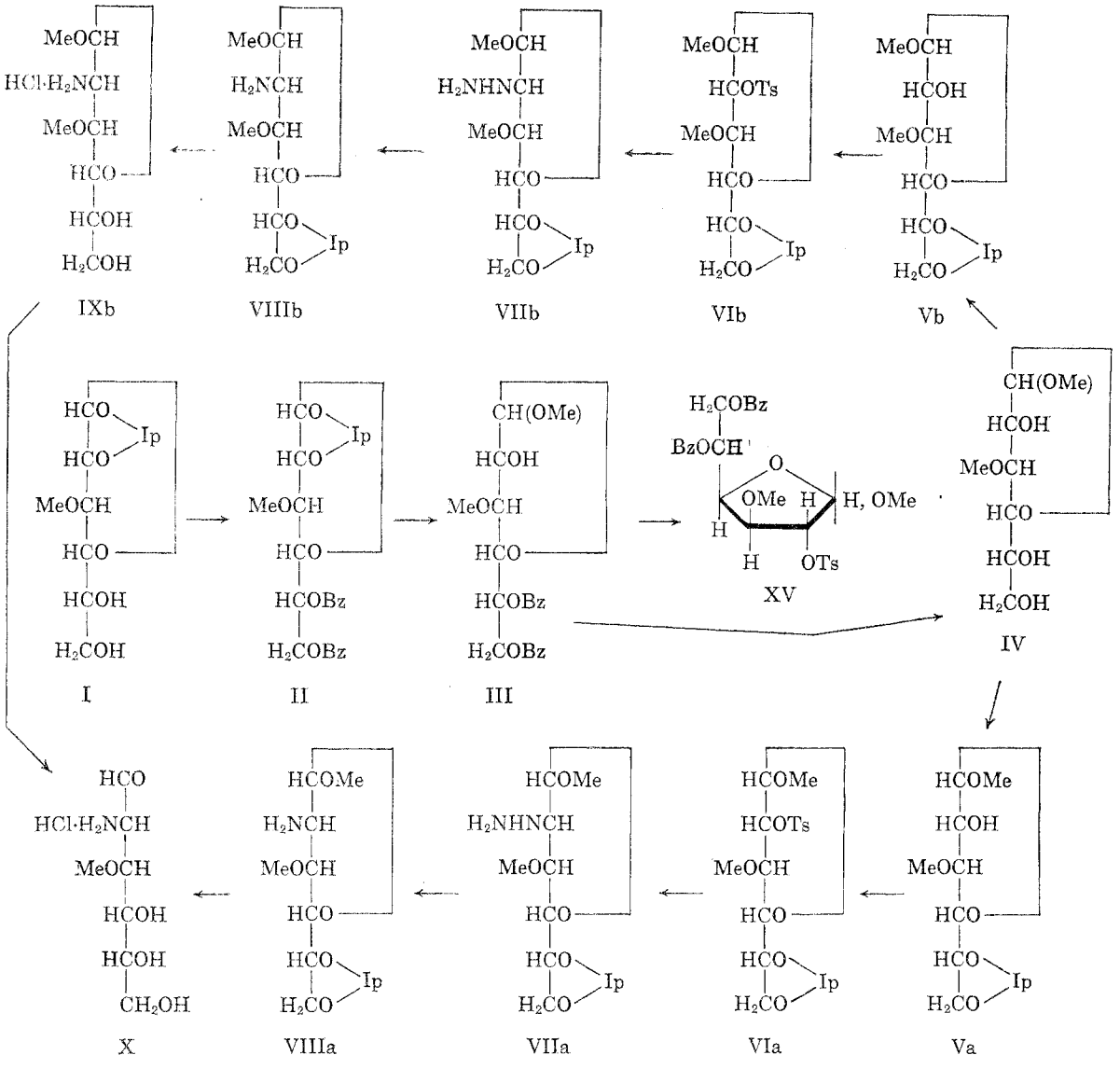

To prepare the corresponding 2-amino-2-deoxy-3-*O*-methyl-D-mannose, the methyl 3-*O*-methyl-5,6-di-*O*-benzyl-α,β-D-glucofuranoside (III) was hydrogenated with palladium catalyst to eliminate the benzyl groups (IV). By treatment with acetone and cupric sulfate, an isopropylidene group was introduced, and high vacuum distillation of the resulting compound (V) allowed the separation of the α- and β-glycosides (Va, Vb) which subsequently were tosylated in pyridine to yield VIa and VIb.

These compounds were subjected to hydrazine treatment and the resulting products (VIIa and VIIb) were hydrogenated with Raney nickel catalyst to yield the amino compounds VIIIa and VIIIb. Attempts to isolate VIIIb as a hydrochloride failed, caused the elimination of the isopropylidene group and produced the crystalline methyl 2-amino-2-deoxy-3-*O*-methyl-β-D-mannofuranoside hydrochloride (IXb). Acid hydrolysis of VIIIa and IXb yielded the same compound (X) which could only be isolated as an amorphous hydrochloride. *N*-Acetylation[15] of X did not lead to a crystalline compound.

The different optical rotations of the hydrochlorides of X and 2-amino-2-deoxy-3-*O*-methyl-D-glucose ($[\alpha]_D^{20}$ − 23.3°, final, compared with +91.0° final,[16] and their different chromatographic properties (Rg value 1.09 compared with 1.19 in 1-butanol–pyridine–water, 6:4:3) seems reasonable evidence that the replacement of the *p*-tolysulfonyloxy group had proceeded with Walden inversion. In the presence of the blocking groups, the reaction of VIa and VIb could only yield these two compounds.

EXPERIMENTAL

Methyl 2-O-p-tolylsulfonyl-3,5,6-tri-O-benzyl-α,β-D-glucofuranoside (XI). To a solution of 12 g. of methyl 3,5,6-tri-*O*-benzyl-α,β-D-glucofuranoside[12] in 60 ml. of dry pyridine, a

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(16) A. Neuberger, *J. Chem. Soc.*, 50 (1941).

solution of 7.5 g. of *p*-tolylsulfonyl chloride in 15 ml. of chloroform was added at 0°. After the reaction mixture had been kept at room temperature for 12 hr., 1.5 ml. of water was added and the solution was vigorously stirred for 1 hr. Subsequently, chloroform (150 ml.) was added, and the solution was poured in 1 l. of water. The chloroform layer was separated, washed twice with 10% sulfuric acid, twice with saturated sodium bicarbonate solution, and finally with water. The chloroform layer was then dried with calcium chloride and evaporated. The colorless sirup could not be distilled in high vacuum without decomposition.

Anal. Calcd. for $C_{35}H_{38}O_8S$: C, 67.94; H, 6.19; S, 5.18. Found: C, 68.13; H, 6.35; S, 5.11.

*Methyl 2-O-p-tolylsulfonyl-3,5,6-tri-O-methyl-β-*D*-glucofuranoside* (XII). Methyl 3,5,6-tri-*O*-methyl-β-D-glucofuranoside[13] was treated with tolylsulfonyl chloride as described for the benzyl derivative (XI). The product could not be distilled in high vacuum without decomposition.

Anal. Calcd. for $C_{17}H_{26}O_8S$: C, 52.29; H, 6.71; S, 8.21. Found: C, 52.03; H, 6.66; S, 7.96.

*Methyl 2-amino-2-deoxy-3,5,6-tri-O-methyl-β-*D*-mannofuranoside hydrochloride* (XIII). A mixture of 25 g. of XII and 50 g. of anhydrous hydrazine (95 + %) was heated under reflux (bath temperature 140°) for 36 hr. After cooling, the final homogeneous solution was extracted four times with 100-ml. portions of ether. The combined ether fractions were then extracted with 200 ml. of water. Subsequently the water phase was treated with Raney nickel catalyst (*ca.* 3–4 g.) for 6 hr. at room temperature and then hydrogenated for 20 hr. at 3 atm. pressure, using Raney nickel catalyst added before. The catalyst was filtered off, and the solution evaporated *in vacuo*. The remaining sirup was dissolved in ether. When methanolic hydrogen chloride was added, a crystalline compound precipitated, which was recrystallized from alcohol. Yield 15 g.; m.p. 227–232° dec. $[\alpha]_D^{20}$ −57.2° (*c*, 1, water).

Anal. Calcd. for $C_{10}H_{22}O_5NCl$: C, 44.30; H, 8.16; N, 5.16. Found: C, 44.31; H, 8.29; N, 5.24.

*2-Amino-2-deoxy-3,5,6-tri-O-methyl-*D*-mannose hydrochloride* (XIV). A solution of 12 g. of XIII in 50 ml. of 2.5*N* hydrochloric acid was heated on a steam bath for 2 hr. and then evaporated *in vacuo*. The residue was recrystallized from alcohol, yield 10 g.; m.p. > 300°, turns dark at 190°; $[\alpha]_D^{20}$ −13.0° final (*c*, 1, water); +16.7° (extrap.) → −87° (*c* 1, pyridine).

Anal. Calcd. for $C_9H_{20}O_5NCl$: C, 41.94; H, 7.82; N, 5.44. Found: C, 41.74; H, 7.76; N, 5.50.

*Methyl 2-O-p-tolylsulfonyl-3-O-methyl-5,6-di-O-benzyl-α,β-*D*-glucofuranoside* (XV). Compound III was treated with tolylsulfonyl chloride as described for the preparation of XI. The resulting sirup could not be distilled without decomposition, even in high vacuum (10^{-3} mm.).

Anal. Calcd. for $C_{29}H_{34}O_8S$: C, 64.19; H, 6.32; S, 5.91. Found: C, 63.70; H, 6.10; S, 5.61.

*1,2-O-Isopropylidene-3-O-methyl-5,6-di-O-benzyl-*D*-glucofuranose* (II). In a three-necked flask provided with a mechanical stirrer and a condenser, 90 g. of 1,2-*O*-isopropylidene-3-*O*-methyl-D-glucofuranose (I) was dissolved in 1 l. of dry, freshly distilled benzyl chloride, and 250 g. of powdered potassium hydroxide was added with vigorous stirring. The reaction mixture was heated to 100° while the stirring was continued. After 30 min., another portion of 250 g. powdered potassium hydroxide was added, and the heating and stirring was extended 4 more hours. After cooling, water was added, and the water phase when separated was extracted twice with ether. The extracts were combined with the benzyl chloride phase, dried with potassium hydroxide and evaporated *in vacuo*, finally at 5×10^{-3} mm. and 100°. The residue, free of benzyl chloride and benzyl alcohol, was then distilled in a molecular still at 3×10^{-3} mm. at 140°, yield 85%; $[\alpha]_D^{20}$ −32.4° (*c*, 1, chloroform).

Anal. Calcd. for $C_{24}H_{30}O_6$: C, 69.54; H, 7.30. Found: C, 69.88; H, 7.49.

*Methyl 3-O-methyl-5,6-di-O-benzyl-α,β-*D*-glucofuranoside* (III). A solution of 132 g. of II in 1 l. of methanol containing 0.5% hydrogen chloride was heated for 6 hr. under reflux. After cooling the solution was neutralized with lead carbonate, filtered, and evaporated *in vacuo;* yield of the remaining sirup, 95%.

Anal. Calcd. for $C_{22}H_{28}O_6$: C, 68.02; H, 7.26. Found: C, 67.89; H, 7.31.

*Methyl 3-O-methyl-α,β-*D*-glucofuranoside* (IV). A solution of 118 g. of III in 500 ml. tetrahydrofuran was hydrogenated with hydrogen using palladium black catalyst (*ca.* 1 g.) at 3 atm. pressure for 12 hr., and 2 moles of hydrogen was absorbed. The catalyst was removed by filtration, the solution evaporated *in vacuo*, the residue dissolved in acetone and the solution again evaporated; yield of the remaining sirup, 95%.

Anal. Calcd. for $C_8H_{16}O_6$: C, 46.15; H, 7.75. Found: C, 45.90; H, 7.67.

*Methyl 3-O-methyl-5,6-O-isopropylidene-α and β-*D*-glucofuranosides* (V). To a solution of 69 g. of IV in 2 l. of acetone, 30 g. of anhydrous cupric sulfate was added, and the reaction mixture was stirred for 10 days. The precipitate was removed by filtration, and the yellow solution evaporated *in vacuo*. Subsequently, the residue was fractionated by high vacuum distillation. At 90–92° and 5×10^{-3} mm. a fraction (Va) was collected as a colorless sirup, yield 39%, $[\alpha]_D^{20}$ +67.2° (*c*, 2, chloroform).

Anal. Calcd. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C 53.12; H, 8.07.

At 114–116° and 5×10^{-3} mm., another compound (Vb) was isolated as a light yellow sirup; yield 42%, $[\alpha]_D^{20}$ −54.1° (*c*, 1, chloroform).

Anal. Calcd. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 53.43; H, 8.01.

*Methyl 2-O-p-tolylsulfonyl-3-O-methyl-5,6-O-isopropylidene-α-*D*-glucofuranoside* (VIa). Compound Va was treated with *p*-tolylsulfonyl chloride as described for the preparation of XI, yield 85% of light yellow sirup, $[\alpha]_D^{20}$ + 75.8 (*c*, 1, chloroform).

Anal. Calcd. for $C_{18}H_{26}O_8S$: S, 7.97. Found: S, 7.73.

*Methyl 2-O-p-tolylsulfonyl-3-O-methyl-5,6-O-isopropylidene-β-*D*-glucofuranoside* (VIb). Compound Vb was treated with *p*-tolylsulfonyl chloride as described for the preparation of XI; yield 90% of light yellow sirup, $[\alpha]_D^{20}$ −28.5 (*c*, 1, chloroform).

Anal. Calcd. for $C_{18}H_{26}O_8S$: S, 7.97. Found: S, 8.07.

*Methyl 2-amino-2-deoxy-3-O-methyl-β-*D*-mannofuranoside hydrochloride* (IXb). Compound VIb (20 g.) was treated with hydrazine and afterwards hydrogenated, as described for the preparation of XIII. The solvent was evaporated and the residue dissolved in ether, then methanolic hydrogen chloride was added and after 15 min. petroleum ether. The precipitated sirup was separated and dissolved in alcohol. On the addition of ether, crystallization occurred. The reaction product was recrystallized from alcohol; yield 65%, m.p. 180–185° dec., $[\alpha]_D^{20}$ −90.7° (*c*, 2, water).

Anal. Calcd. for $C_8H_{18}O_5NCl$: C, 39.43; H, 7.45; N, 5.75. Found: C, 39.80; H, 7.57; N, 5.77.

*2-Amino-2-deoxy-3-O-methyl-*D*-mannose hydrochloride* (X) (A) A solution of 2 g. of IXb in 2*N* hydrochloric acid was heated on a steam bath for 1 hr. and then evaporated *in vacuo*. The residue was dissolved in methanol, treated with activated carbon, and precipitated with acetone. The resulting sirup was dissolved two more times in methanol and precipitated with acetone $[\alpha]_D^{20}$ −23.3° final (*c*, 5, water).

Anal. Calcd. for $C_7H_{16}O_5NCl$: C, 36.60; H, 7.02; N, 6.10. Found: C, 36.41; H, 6.81; N, 6.39.

(B) Compound VIa was treated with hydrazine and subsequently hydrogenated as described for the preparation of XIII. The reduction product was dissolved in 2*N* hydrochloric acid and heated on the steam bath for 1 hr. and then

worked up as described under (A). The resulting product showed the same properties as listed under (A).

Chromatography on Whatman No. 1 paper with 1-butanol–pyridine–water, 6:4:3 resulted in a single spot, Rg value 1.09. The substance is ninhydrin positive.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Reaction of Amylose with 1-Acrylamido-1-deoxy-D-glucitol to Introduce Extended Branches[1]

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Amylose reacts with 1-acrylamido-1-deoxy-D-glucitol in aqueous base. Ten molar lithium chloride solution as solvent permits a completely homogeneous reaction and suppresses amide hydrolysis. The product of the reaction is given the trivial name "glucamidoethylamylose." Fractionation of glucamidoethylamylose by ethanol precipitation yields fractions having degrees of molar substitution ranging from 0.20 to 0.82. Solubility in water increases with the amount of substitution. The solutions give a blue color with iodine, but do not show complex formation on titration with iodine. These derivatives of amylose are hydrolyzed by acid and by α-amylase, but are much less susceptible to the action of β-amylase than is amylose. They show no tendency toward retrogradation, nor do they complex with butanol.

A structure consisting of an amylose main chain with grafted poly(1-deoxy-1-β-oxypropionamido-D-glucitol) branches is consistent with these findings and is supported by chromatographic analysis of an acid hydrolyzate.

Introduction of a small amount of neutral substituent into a linear polysaccharide tends to increase its water solubility and its stability in solution. Solubility is further enhanced if the substituent group is hydrophilic. Hydroxyethylation and carbamylethylation are familiar examples of substitution by neutral, hydrophilic groups. The introduction of a sugar or an open-chain polyol as a substituent might provide a still greater enhancement of solubility and solution stability.

Sugar-substituted polysaccharides have recently been prepared by Husemann and Reinhardt,[2] who used the modified Koenigs-Knorr method of Bredereck and co-workers[3] wherein a carbohydrate trityl ether is treated with an acetobromo sugar in the presence of silver perchlorate. The synthesis in this laboratory[4] of 1-acrylamido-1-deoxy-D-glucitol (*N*-acryloyl-D-glucamine) has provided a sugar-substituted acrylamide which has now been shown to undergo reaction, through its activated double bond, with the hydroxyl groups in the linear polysaccharide, corn amylose. The product of the reaction is an *N*-substituted carbamylethylamylose to which the trivial name "glucamidoethylamylose" is given.

In preliminary investigations *N*-acryloyl-D-glucamine reacted with a dispersion of amylose in dilute sodium hydroxide solution. Base concentrations between 0.1 and 1.0*M*, temperatures between 50° and 100° and reaction periods between one half and twelve hours were investigated for mixtures containing one mole of *N*-acryloyl-D-glucamine per D-glucose residue of the amylose. Maximum incorporation of nitrogen to 0.85% *N*, or a molar substitution[5] of 0.12, occurs without the appearance of carboxyl groups in 0.3*M* base at 70° for two hours. An increase in either base strength, temperature, or time brings about partial hydrolysis of amide linkages. Since amylose is not readily soluble in 0.3*M* sodium hydroxide solution, the reaction mixture is not always completely homogeneous. Dissolution of the amylose in 10*M* lithium chloride solution provides a homogeneous reaction mixture, and at the same time reduces the amount of water available for hydrolysis of amide bonds. Consequently, base concentrations up to 0.6*M* in 10*M* lithium chloride solution may be used without the appearance of carboxyl groups in the product. Nitrogen contents up to 2.15%, corresponding to an M.S.[5] of 0.39, are obtained when four moles of *N*-acryloyl-D-glucamine are treated per D-glucose residue of the amylose in this manner.

Fractionation of glucamidoethylamylose on the basis of its solubility in water-ethanol mixtures gives rise to fractions having different nitrogen contents (Table I). As the concentration of ethanol is increased, fractions containing an increasing amount of nitrogen are obtained. The fractions are soluble in water, and their solubility increases with increasing amounts of substitution.

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(1) Presented before the Division of Cellulose Chemistry at the 138th Meeting of the American Chemical Society, New York, September 1960; Journal Paper No. 1689 of the Purdue Agricultural Experiment Station.

(2) E. Husemann and M. Reinhardt, *Angew. Chem.*, **71**, 429 (1959); Abstracts of Papers, 138th Meeting, American Chemical Society, September 1960, 8D.

(3) H. Bredereck, A. Wagner, G. Faber, H. Ott, and J. Rauther, *Chem. Ber.*, **92**, 1135 (1959).

(4) R. L. Whistler, H. P. Panzer, and H. J. Roberts, *J. Org. Chem.*, **26**, 1583 (1961).

(5) Molar substitution (M.S.) is defined as the number of moles of substituent introduced per D-glucose residue.