[CONTRIBUTION FROM THE ROBERT W. LOVETT MEMORIAL FOUNDATION FOR THE STUDY OF CRIPPLING DISEASES, MASSACHUSETTS GENERAL HOSPITAL, AND THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

3-O-Methyl-D-galactosamine Hydrochloride (2-Amino-2-deoxy-3-O-methyl-D-galactose Hydrochloride)[1a,b]

BY PIERRE J. STOFFYN AND ROGER W. JEANLOZ

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3-O-Methyl-D-galactosamine hydrochloride (2-amino-2-deoxy-3-O-methyl-D-galactose hydrochloride) has been prepared and transformed to the crystalline N-(2'-hydroxynaphthylidene) derivative.

Galactosamine (chondrosamine) isolated in 1913 from chondroitin sulfuric acid[2] has been shown recently to be present in an increasing number of biologically important muco- and glycoproteins.[3] Parallel with the work carried on in this Laboratory to establish the structure of glucosamine-containing polysaccharides by methylation procedures, a study of the galactosamine-containing polysaccharides has been undertaken.

Although it is probable some partially methylated galactosamines have been contained in the mixtures resulting from the hydrolysis of methylated chondroitin sulfate[4] no pure substance has been reported as yet. Preparation of pure methylated galactosamines by synthesis has been undertaken and the present work describes the synthesis of 3-O-methyl-D-galactosamine hydrochloride (VI).

Besides its value for identification, 3-O-methyl-D-galactosamine was synthesized to establish evidence for the location of the benzylidene group in the methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (II), because such a compound is a valuable intermediate in the synthesis of the other methylated galactosamines. The sequence of reactions starting from methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (I)[5] is shown in the accompanying diagram and is identical to the one established for the synthesis of 3-O-methyl-D-glucosamine.[6]

Methylation of methyl 2-acetamido-2-deoxy-3-O-methyl-α-D-galactopyranoside (IV) to the known 3,4,6-tri-O-methyl derivative VII[5] showed that position 5 is not involved in the methylation, thus no shifting of the ring had occurred during formation of II or III. Periodate oxidation of IV, which showed a negligible amount of oxidant consumed was proof that the methyl group was not located in position 6, thus the benzylidene group could not be located in position 3 and 4. In the glucosamine sequence, Neuberger[6] had shown by periodate oxidation of the 2-amino-2-deoxy-D-gluconic acid derived from the 3-O-methyl-D-glucosamine that the methyl group was not in position 4, *i.e.*, that the benzylidene ring was located in position 4,6. Such direct evidence could unfortunately not be obtained in the galactosamine series, as we were unable to prepare a pure crystalline 2-amino-2-deoxy-D-galactonic acid by oxidation of VI. However, location of the benzylidene group in positions 3 and 6 would require formation of a seven-membered ring, which has never to our knowledge been observed in benzylidene derivatives of sugars. Consequently the methyl group can be assigned to position 3 with a high degree of probability. 3-O-Methyl-D-galactosamine hydrochloride (VI) was obtained in a sirupy form, which could not be crystallized as yet. Transformation to the known 3-O-methyl-D-galactose phenylosazone[7] was unsuccessful, but reaction with 2-hydroxynaphthaldehyde[8] gave an easily recrystallizable Schiff base.

Experimental[9]

Methyl 2-Acetamido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (II).—A mixture of 1.13 g. of methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (I),[10] m.p. 217–219°, 1.13 g. of zinc chloride and 6 ml. of benzaldehyde was shaken overnight at room temperature. The solution was poured into water and shaken with pentane. The precipitate was filtered, washed with pentane and dried *in vacuo*. Recrystallization from a mixture of methanol and ether gave 1.28 g. (82%) of fine needles. When heated the substance sublimes at about 170° and recrystallizes to form long needles at 190–210°. These melt at 243–245°, $[\alpha]^{29}D$ +149 ± 2° (in chloroform, *c* 1.04). *Anal.* Calcd. for $C_{16}H_{21}O_6N$: C, 59.43; H, 6.55. Found: C, 59.23; H, 6.55.

Methyl 2-Acetamido-4,6-O-benzylidene-2-deoxy-3-O-methyl-α-D-galactopyranoside (III).—The methylation was

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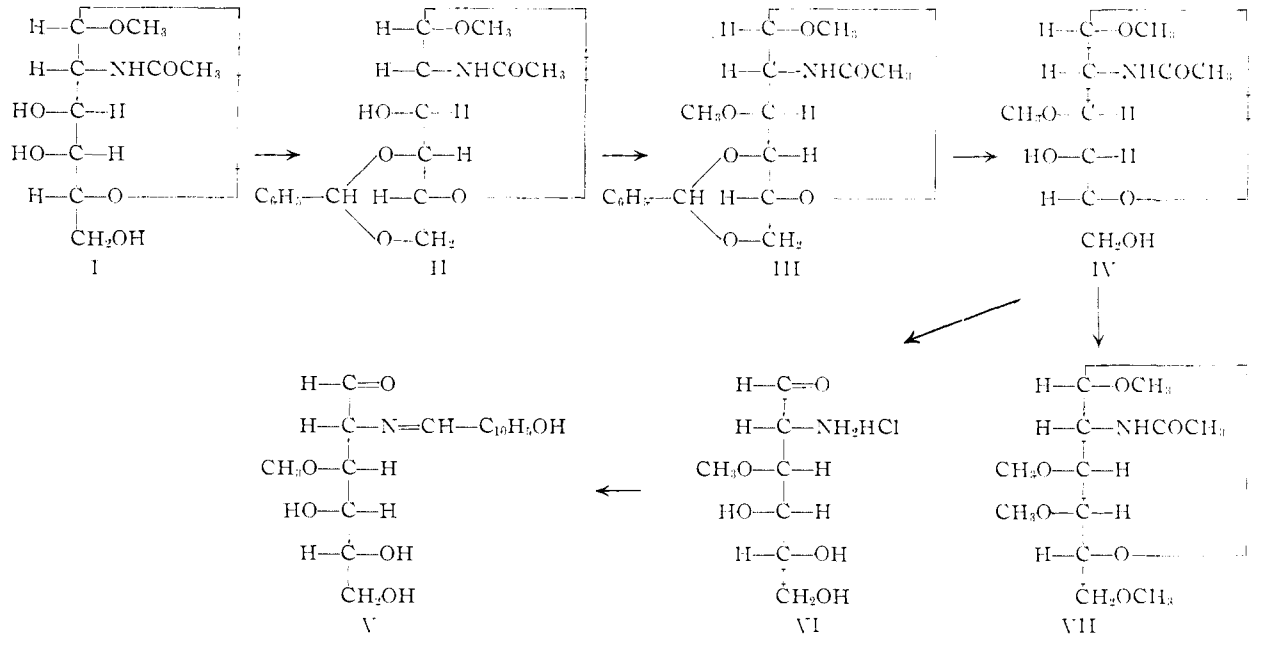
(10) Methyl 2-acetamido-2-deoxy-α-D-galactopyranoside was prepared from β-pentaacetylgalactosamine, in an identical way as followed by Stacey[5] starting from α-pentaacetylgalactosamine. In a total of six acetylations of galactosamine hydrochloride with acetic anhydride and pyridine at room temperature, five times β-pentaacetylgalactosamine (m.p. 245–246°) was the predominating form obtained. The α-form (m.p. 178–181°) was obtained once.



carried out by dissolving 0.70 g. of II in 35 ml. of dioxane, previously distilled over sodium, and adding 8.0 ml. of 30% sodium hydroxide solution and 3.0 ml. of methyl sulfate in 10 portions at 10-minute intervals. The temperature was maintained at 55–60° and the mixture was vigorously stirred. The cooled solution was diluted with 100 ml. of water and neutralized with CO_2. After standing overnight in the ice-box, the crystalline precipitate (tiny needles) was collected and washed with water, affording 0.55 g. of III, m.p. 284–285°. By concentration of the mother liquors an additional crop of 0.15 g. was obtained, with a total yield of 96%. Recrystallization from a mixture of methanol and ether did not change the m.p. With slow heating, a transformation to long needles occurred at 255–270° and the product sublimed below 280°; $[\alpha]^{29}D$ +172 ± 2° (in chloroform *c* 0.92). *Anal.* Calcd. for $C_{17}H_{23}O_6N$: C, 60.52; H, 6.87; OCH_3, 18.40. Found: C, 60.41; H, 6.95; OCH_3, 18.22.

Methyl 2-Acetamido-2-deoxy-3-*O*-methyl-α-D-galactopyranoside (IV).—A solution of 1.15 g. of III in 25 ml. of 60% acetic acid was heated on the water-bath for 30 minutes. The solution was evaporated *in vacuo*, water was added and evaporated again *in vacuo* to eliminate the last traces of acetic acid and benzaldehyde. Traces of benzoic acid were separated by chromatography on a short column (10 g.) of silicic acid. Elution with acetone and methanol gave 790 mg. of crude IV. Recrystallization from a mixture of methanol, acetone and ether gave 575 mg. (68%) of needles; m.p. 190–193°; $[\alpha]^{27}D$ +183 ± 2° (in methanol, *c* 1.0). *Anal.* Calcd. for $C_{10}H_{19}O_6N$: C, 48.18; H, 7.68; OCH_3, 24.90. Found: C, 48.03; H, 7.60; OCH_3, 24.71.

Acetylation of IV with acetic anhyride and pyridine in the usual manner gave the **4,6-di-*O*-acetyl derivative**; m.p. 137–139°, $[\alpha]^{26}D$ +136 ± 2° (in chloroform, *c* 0.97). *Anal.* Calcd. for $C_{14}H_{23}O_8N$: C, 50.44; H, 6.95. Found: C, 50.43; H, 6.89.

Fifty-nine milligrams of IV dissolved in 1 ml. of acetone was refluxed with 10 ml. of methyl iodide and 200 mg. of silver oxide; after 15 hours, 200 mg. of silver oxide was added and the reflux continued for 24 hours. The silver salts were filtered, the solution evaporated and the sirupy residue treated again in a similar way. After filtration through celite and evaporation of the solution to dryness, the residue was crystallized from a mixture of acetone and pentane affording 29 mg. (44%) of methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-α-D-galactopyranoside (VII) as fine needles; m.p. 191–192°, $[\alpha]^{28}D$ +142 ± 3° (in methanol, *c* 0.9). *Anal.* Calcd. for $C_{12}H_{23}O_6N$: C, 51.97; H, 8.36; OCH_3, 44.76. Found: C, 51.80; H, 8.23; OCH_3, 44.49. Admixture with authentic material[5] did not depress the melting point.

Periodate oxidation of IV was carried out under conditions similar to those used for methyl 2-acetamido-2-deoxy-α-D-glucopyranoside[11]; 2.5 moles of sodium periodate was added for each mole of sugar and the oxidation performed at 5° and *p*H 4.5. After 10, 24 and 48 hours 0.1 mole of oxidant was consumed.

3-*O*-Methyl-D-galactosamine Hydrochloride (2-Amino-2-deoxy-3-*O*-methyl-D-galactose Hydrochloride) (VI).—A solution of 200 mg. of IV in 5 ml. of 3 *N* hydrochloric acid was heated for three hours on the water-bath. After concentration *in vacuo*, the sirup was dissolved in absolute ethanol and evaporated to dryness. The residue was left overnight in a desiccator over sulfuric acid and sodium hydroxide, then dissolved in methanol and filtered through a layer of Darco G-60. Evaporation gave a colorless sirup in a quantitative yield; $[\alpha]^{28}D$ +119 ± 2° (in water, *c* 1.38). *Anal.* Calcd. for $C_7H_{16}O_5NCl$: C, 36.61; H, 7.02; OCH_3, 13.51; Cl, 15.44. Found: C, 36.55; H, 7.15; OCH_3, 13.65; Cl, 15.26.

2-Deoxy-2-(2′-hydroxynaphthylidenamino)-3-*O*-methyl-D-galactose (V).—A solution of 48 mg. of VI in 1.0 ml. of water was treated as previously described[8] with 90 mg. of 2-hydroxynaphthaldehyde in 10 ml. of methanol and 60 mg. of sodium acetate. Purification was obtained by chromatography. Elution with a mixture of acetone and methanol 9:1 gave crystalline fractions, which after recrystallization from a mixture of pyridine, methanol and ether gave 28 mg. (39%) of yellow microcrystals, m.p. 198–202°. A second recrystallization raised the m.p. to 205–207°; $[\alpha]^{26}_{5461}$ +132 ± 5° (at the equilibrium in methanol, *c* 0.18). *Anal.* Calcd. for $C_{18}H_{21}O_6N$: C, 62.24; H, 6.09. Found: C, 62.32; H, 6.23.

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