[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]

4,6-Di-*O*-methyl-D-galactosamine Hydrochloride (2-Amino-2-deoxy-4,6-di-*O*-methyl-D-galactose Hydrochloride)[1a,b]

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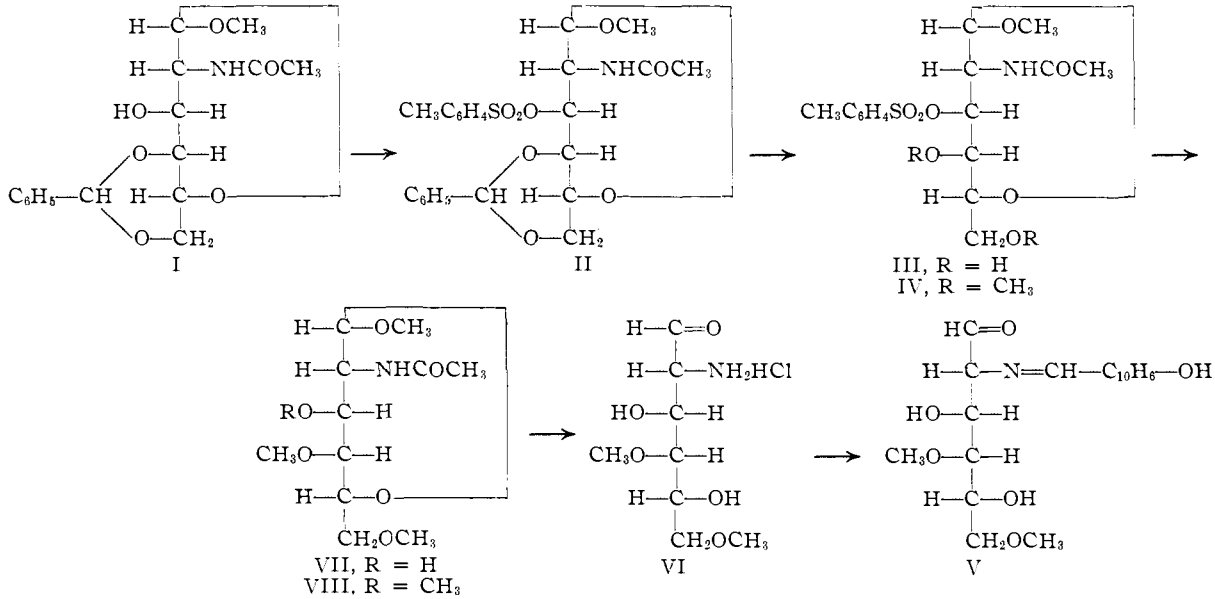
RECEIVED MAY 7, 1953

4,6-Di-*O*-methyl-D-galactosamine hydrochloride (2-amino-2-deoxy-4,6-di-*O*-methyl-D-galactose hydrochloride) has been prepared in crystalline form and characterized by its crystalline N-(2'-hydroxynaphthylidene) derivative.

Synthesis of the various methylated 2-amino-2-deoxygalactopyranoses has been undertaken[2] with the purpose of using them as reference compounds. Synthesis of the 4,6-di-*O*-methyl-D-galactosamine hydrochloride (2-amino-2-deoxy-4,6-di-*O*-methyl-D-galactose hydrochloride(VI) has been accomplished, starting from methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside,[2] through a route already described for the synthesis of 4,6-di-*O*-methyl-D-glucosamine hydrochloride[3] and shown in the accompanying diagram. The protection of the group in position 3 was obtained by tosylation; no side reactions were observed as was the case in the glucosamine series,[3] and the over-all yield from I to the methyl 2-acetamido-2-deoxy-4,6-di-*O*-methyl-α-D-galactopyranoside (VII) (48%) was double the one obtained in the glucosamine series (23%).



Preparation of the known 3,4,6-tri-*O*-methyl derivative VIII[4] constitutes proof that no shifting of the ring occurred during methylation.

4,6-Di-*O*-methyl-D-galactosamine hydrochloride (VI) was obtained in crystalline form and, as it showed a mutarotation in water from +107° to +91°, it was assumed to be in the α-form.

Like D-glucosamine hydrochloride and its crystalline *O*-methyl derivatives, VI decomposes without showing a definite melting point; characterization was obtained by transformation to the Schiff base[5] with 2-hydroxynaphthaldehyde.

Experimental[3]

Methyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (II).—Dry methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (I)[2] (1.0 g.) was treated with *p*-toluenesulfonyl chloride as previously described.[3] The crude resulting sirup was chromatographed on silicic acid. Elution with mixtures of ether and ethyl acetate afforded crystalline fractions which after two recrystallizations from a mixture of methanol, ether and pentane gave 0.971 g. (66%) of large prisms II; m.p. 190–192°, $[\alpha]^{29}D$ +152 ± 2° (in chloroform, *c* 1.11). *Anal.* Calcd. for $C_{23}H_{27}O_8NS$: C, 57.85; H, 5.70; S, 6.72. Found: C, 57.86; H, 5.84; S, 6.81.

Methyl 2-Acetamido-2-deoxy-3-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (III).—Seven hundred and forty mg. of II was hydrolyzed with acetic acid as previously described.[3] Crystallization from a mixture of acetone and ether gave 522 mg. (86%) of prisms III, m.p. 146–148°. Several recrystallizations raised the m.p. to 154–157°; $[\alpha]^{27}D$ +100 ± 2° (in chloroform, *c* 1.02). *Anal.* Calcd. for $C_{16}H_{23}O_8NS$: C, 49.34; H, 5.95; S, 8.23. Found: C, 49.47; H, 5.93; S, 8.41.

Acetylation of 30 mg. of III with acetic anhydride and pyridine in the usual manner gave the **4,6-di-*O*-acetyl de-**

(1) (a) Studies on hyaluronic acid and related substances XI. This is publication No. 150 of the Robert W. Lovett Memorial Foundation for the Study of Crippling Diseases, Harvard Medical School, Boston, Massachusetts. This investigation has been supported by research grants from Eli Lilly and Company and from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service: (b) presented before the Division of Carbohydrate Chemistry at the 124th Meeting of the American Chemical Society, Chicago, Illinois, September, 1953.

(2) P. J. Stoffyn and R. W. Jeanloz, THIS JOURNAL, **76**, 561 (1954).

(3) R. W. Jeanloz, *ibid.*, **76**, 555 (1954).

(4) M. Stacey, *J. Chem. Soc.*, 272 (1944).

(5) Z. E. Jolles and W. T. J. Morgan, *Biochem. J.*, **34**, 1183 (1940); R. W. Jeanloz, THIS JOURNAL, **74**, 4597 (1952).

rivative. After adsorption on silicic acid, the product was eluted with mixtures of ether and ethyl acetate and mixtures of ethyl acetate and acetone. Crystallization from a mixture of acetone, ether and pentane gave 25 mg. (68%); m.p. 152–153°, $[\alpha]^{27}D$ +90 ± 2° (in chloroform, *c* 0.90). *Anal.* Calcd. for $C_{20}H_{27}O_{10}NS$: C, 50.73; H, 5.75. Found: C, 50.60; H, 5.84.

Methyl 2-Acetamido-2-deoxy-4,6-di-*O*-methyl-3-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (IV).—Four hundred and eighty mg. of III was methylated with methyl iodide and silver oxide as previously described.[3] Purification was carried out by chromatography on silicic acid. Mixtures of ethyl acetate and acetone eluted 490 mg. (95%) of sirup (IV); $[\alpha]^{27}D$ +89 ± 2° (in chloroform, *c* 1.17). *Anal.* Calcd. for $C_{18}H_{27}O_8NS$: C, 51.78; H, 6.52; OCH_3, 22.30. Found: C, 51.76; H, 6.58; OCH_3, 22.49.

Methyl 2-Acetamido-2-deoxy-4,6-di-*O*-methyl-α-D-galactopyranoside (VII).—To a solution of 610 mg. of sirupy IV in 30 ml. of 90% methanol was added 9 g. of 2.5% sodium amalgam. After shaking overnight, the mixture was diluted with 20 ml. of water, neutralized with CO_2, filtered and evaporated to dryness *in vacuo*. The residue was extracted with chloroform and filtered. After concentration to dryness, crystallization from a mixture of methanol and ether afforded 341 mg. (89%) of fine needles; m.p. 227–229°, $[\alpha]^{29}D$ +141 ± 2° (in methanol, *c* 0.79). *Anal.* Calcd. for $C_{11}H_{21}O_6N$: C, 50.18; H, 8.04; OCH_3, 35.36. Found: C, 49.84; H, 8.65; OCH_3, 35.13.

Thirty-six mg. of VII was methylated with methyl iodide and silver oxide as previously described. After crystallization from a mixture of acetone and pentane, the theoretical yield of methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-α-D-galactopyranoside (VIII) was obtained. The product melted at 191–192° and did not depress the m.p. in admixture with authentic material[4]; $[\alpha]^{28}D$ +147 ± 2° (in methanol, *c* 1.00).

Acetylation of 38 mg. of VII with acetic anhydride and pyridine in the usual way gave the **3-*O*-acetyl derivative**. Crystallization from a mixture of acetone, ether and pentane gave 26 mg. (60%); m.p. 111–112°, $[\alpha]^{28}D$ +106 ± 2° (in chloroform, *c* 0.94). *Anal.* Calcd. for $C_{13}H_{23}O_7N$: C, 51.14; H, 7.59. Found: C, 51.32; H, 7.69.

4,6-Di-*O*-methyl-α-D-galactosamine Hydrochloride (2-Amino-2-deoxy-4,6-di-*O*-methyl-α-D-galactose Hydrochloride) (VI).—A solution of 145 mg. of VII in 3 *N* hydrochloric acid was treated as previously described.[3] The residual sirup was crystallized from a mixture of acetone and methanol, affording 112 mg. (84%) of small square platelets, decomposing at 190°. The compound showed mutarotation from $[\alpha]^{26}D$ +107° (after 5 minutes) to $[\alpha]^{26}D$ +91 ± 2° (after 48 hours in water, *c* 1.02). *Anal.* Calcd. for $C_9H_{18}O_5NCl$: C, 39.43; H, 7.44; OCH_3, 25.47; Cl, 14.55. Found: C, 39.56; H, 7.57; OCH_3, 25.51; Cl, 14.65.

2-Deoxy-2-(2′-hydroxynaphthylideneamino)-4,6-di-*O*-methyl-D-galactose (V).—The preparation was carried out on 59 mg. of VI as previously described.[3] Purification was obtained by chromatography. Elution with mixtures of ethyl acetate and acetone gave 32 mg. (39%) of yellow microcrystals, m.p. 183–186°, $[\alpha]^{26}_{5461}$ +223 ± 3° (at the equilibrium in methanol, *c* 0.62). *Anal.* Calcd. for $C_{19}H_{23}O_6N$: C, 63.14; H, 6.41. Found: C, 63.01; H, 6.37.

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[CONTRIBUTION FROM THE DEPARTMENTS OF NEUROLOGY, MICROBIOLOGY AND BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, THE NEUROLOGICAL INSTITUTE, PRESBYTERIAN HOSPITAL AND THE BIOPHYSICS SECTION, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Precipitation of C^{14}-Labeled Dextran by Human Anti-dextran[1a]

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RECEIVED MAY 28, 1953

A large proportion of C^{14}-labeled clinical dextran was specifically precipitated by an excess of anti-dextran produced by the injection of 1 mg. of dextran into human beings. An unrelated specific precipitate of blood group A substance with human anti-A formed in the presence of C^{14}-labeled dextran did not carry down significant quantities of radioisotope.

Recent studies from this Laboratory[1b] and confirmed by Maurer,[1c] have demonstrated that purified dextrans are antigenic in man; injection of small quantities of dextran stimulates the production of precipitins and of skin sensitivity. In addition to other lines of evidence indicating that the precipitins formed were indeed antibodies to dextran and not to some contaminating substance, it was considered desirable to show that dextran itself was contained in the precipitate formed by the antidextran. The recent preparation[2] of highly purified dextran randomly labeled with C^{14} by biosynthesis from C^{14}-sucrose made it possible to establish that the antibody formed in humans injected with various dextrans[1b] specifically precipitated a substantial portion of the C^{14}-labeled dextran.

Materials and Methods

Human Antidextran.—Serum samples 20_{D-2} and 30_{D-2} from individuals who had been injected with 1 mg. of clinical Swedish dextrans OP155 and OP163, respectively, were available. The quantitative precipitin data on the reaction of these antisera with various native and clinical dextrans as well as with the C^{14} clinical dextran and C^{14}-dextran fractions have already been published.[1b]

C^{14}-Labeled Dextrans.—The C^{14}-labeled clinical dextran[2] Lot 21-2-Ci-L-D was obtained through the National Research Council. Fractions 1 and 9 of this C^{14}-dextran obtained by fractional precipitation with methanol were prepared at the National Bureau of Standards[3] and provided through the kindness of Drs. S. G. Weissberg and H. S. Isbell; fraction 1 had a number average molecular weight of 60,800, while fraction 9 had a number average molecular weight of 13,230.

Control Materials.—A sample of anti-A prepared by injection of hog blood group A substance into humans of blood group B[4] and a sample of purified hog blood group A substance (Hog 14)[5] were employed.

Procedure.—From the quantitative precipitin curves for the reactions of the C^{14}-dextran and dextran fractions with

(1) (a) This investigation was carried out in part under a grant (RG 34) from the National Institutes of Health, U. S. Public Health Service and under the William J. Matheson Commission.

(1) (b) E. A. Kabat and D. Berg, *Ann. N. Y. Acad. Sci.*, **55**, 471 (1952); *J. Immunol.* **70**, 514(1953); (c) P. H. Maurer, *Proc. Soc. Exp. Biol. and Med.*, **83**, 879 (1953).

(2) N. J. Scully, H. E. Stavely, J. Skok, A. R. Stanley, J. K. Dale, J. T. Craig, E. B. Hodge, W. Chorney, R. Watanabe and R. Baldwin, *Science*, **116**, 87 (1952).

(3) S. G. Weissberg and H. S. Isbell, National Bureau of Standards Reports, 1160 (1951) and 1713 (1952).

(4) Kindly donated by the Knickerbocker Foundation, New York City.

(5) A. Bendich, E. A. Kabat and A. E. Bezer, THIS JOURNAL, **69**, 2163 (1947).