at 100°. The solution was filtered through charcoal, concentrated to dryness *in vacuo* and the last traces of water were removed by codistillation with absolute ethanol. After standing for one month, the residual sirup crystallized. Recrystallization from a mixture of methanol and acetone gave 140 mg. (53%) of VI, decomposing above 178°. The product showed mutarotation, from $[\alpha]^{24}_{D}$ + 125° (after 10 minutes) to $[\alpha]^{26}_{D}$ +100 ± 1° (after 17 hours, in water, *c*, 1.09). *Anal.* Calcd. for $C_7H_{15}O_5NCl$: C, 36.61; H, 7.02; OCH_3, 13.51. Found: C, 36.93; H, 7.22; OCH_3, 13.23.

2-Deoxy-2-(2'-hydroxynaphthylidenamino)-4-*O*-methyl-α-D-galactopyranose (VII).—A solution of 37 mg. of VI in 1.0 ml. of water was treated as previously described[11] with 70 mg. of 2-hydroxynaphthaldehyde and 50 mg. of sodium acetate. The product was purified by chromatography on silicic acid. Forty-eight milligrams (87%) of crystalline fractions was eluted with pure acetone and mixtures of acetone and methanol. Recrystallization from a mixture of methanol and ether gave 43 mg. (77%) of yellow prismatic needles (VII), m.p. 207–209° (with decomposition). The product showed mutarotation from $[\alpha]^{27}_{5461}$ +187° (after 7 minutes) to $[\alpha]^{27}_{5461}$ +168 ± 2° (after 20 hours, in methanol, *c* 1.40). *Anal.* Calcd. for $C_{18}H_{21}O_6N$: C, 62.24; H, 6.09. Found: C, 62.27; H, 6.18.

Methyl 2-Acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-methyl-α-D-galactopyranoside (IX).—Sixty milligrams of VI was treated with 1.0 ml. of dry pyridine and 0.6 ml. of acetic anhydride for two days at room temperature. After addition of two drops at methanol the solution was evaporated *in vacuo* and dried overnight in a desiccator over sulfuric acid and soda lime to give the crude sirupy 2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-methyl-D-galactopyranose (VIII). The sirup was refluxed for two hours with 5 ml. of 2% hydrochloric acid in methanol. After cooling, the solution was treated with an excess of silver carbonate and the silver salts were filtered. The remaining soluble silver ions were precipitated with hydrogen sulfide, the solution was filtered over a double layer of charcoal and Celite and evaporated *in vacuo* to give 69 mg. of crude crystalline methyl 2-acetamido-2-deoxy-4-*O*-methyl-D-galactopyranoside. The dry crystalline residue was acetylated by standing with a mixture of 0.5 ml. of dry pyridine and 0.3 ml. of acetic anhydride for two days at room temperature. After evaporation *in vacuo* and elimination of the last traces of solvent by codistillation with dry toluene, the sirupy residue was dissolved in benzene and chromatographed on silicic acid. Elution with mixtures of ether and ethyl acetate gave 52 mg. of crystalline fractions. Recrystallization from ether supersaturated with pentane gave 30 mg. of prismatic needles, m.p. 114–115°, $[\alpha]^{25}_{D}$ +82 ± 2° (in chloroform, *c* 1.30). *Anal.* Calcd. for $C_{14}H_{23}O_8N$: C, 50.44; H, 6.95; OCH_3, 18.62. Found: C, 50.42; H, 6.85; OCH_3, 18.76.

The crystallization of IX was difficult and the partially crystalline residues were directly transformed to X (see below).

Methyl 2-Acetamido-2-deoxy-4-*O*-methyl-α-D-galactopyranoside (X).—A solution of 16.0 mg. of IX in methanol was treated with 0.5 ml. of a solution of 0.2 *N* barium methoxide in methanol. After standing overnight at 0°, the solution was filtered through a column of Dowex 50 in the H form. The solution was evaporated *in vacuo* and the crystalline residue was recrystallized from a mixture of methanol and ether to give 11.5 mg. (95%) of needles, m.p. 241–242°, $[\alpha]^{20}_{D}$ +147 ± 6° (in methanol, *c* 0.52). *Anal.* Calcd. for $C_{10}H_{19}O_6N$: C, 48.18; H, 7.68. Found: C, 48.13; H, 7.62.

Twenty-two milligrams of mother liquors from the preparation of IX was similarly transformed to X. The product was purified by chromatography on silicic acid. Mixtures of acetone and methanol eluted crystalline fractions. Recrystallization from a mixture of methanol and ether gave 8.5 mg. of X. The total yield of X, calculated on the basis of VI, was 46%.

Acknowledgments.—The authors are indebted to Miss Ann Foley and to Miss Shirley Phillips for technical assistance.

(11) R. W. Jeanloz, THIS JOURNAL, **74**, 4597 (1952).

BOSTON, MASS.

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

Syntheses of 4-*O*-Methyl-β-D-galactopyranose and 2,4-Di-*O*-methyl-α-D-galactopyranose[1,2]

BY ROGER W. JEANLOZ

RECEIVED JUNE 9, 1954

4-*O*-Methyl-β-D-galactopyranose and 2,4-di-*O*-methyl-α-D-galactopyranose have been prepared from 1,6:2,3-dianhydro-4-*O*-methyl-β-D-talopyranose by the action of aqueous potassium hydroxide and sodium methoxide, respectively, with subsequent hydrolysis in each case.

Although 4-*O*-methyl-β-D-galactopyranose (VI) and more often 2,4-di-*O*-methyl-α-D-galactopyranose (IX) have been isolated many times in the study of the structure of galactose-containing polysaccharides, their syntheses have not yet been reported.[3] The opening of the epoxy ring of 1,6:2,3-dianhydro-β-D-talopyranose by the action of basic reagents has been reported[4] to yield the 2-amino-2-deoxy derivative of galactose on treatment with ammonia, and the 2-*O*-methyl derivative of galactose on treatment with sodium methoxide.

In the preceding paper,[5] the preparation of crystalline 1,6:2,3-dianhydro-4-*O*-methyl-β-D-talopyranose (III) and its reaction with ammonia has been described. The further investigation of the reactions of the readily accessible III with alkaline reagents appeared to provide an attractive route to the synthesis of VI and IX, and was followed through as described below.

Treatment of III with aqueous potassium hydroxide for 40 hours gave 1,6-anhydro-4-*O*-methyl-β-D-galactopyranose (I), which was purified through its 2,3-di-*O*-acetyl derivative II, in 87% yield (calculated on the basis of a theoretical yield from III). No idose derivative was obtained. Hydrolysis of the 1,6-anhydro ring gave crystalline 4-*O*-methyl-

(1) This is publication No. 166 of the Robert W. Lovett Memorial Foundation for the Study of Crippling Diseases, Department of Medicine, Harvard Medical School, Boston, and the Massachusetts General Hospital. 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 (Grant A-148-C1).

(2) Presented before the Division of Carbohydrate Chemistry at the 126th Meeting of the American Chemical Society, New York, N. Y., September 1954.

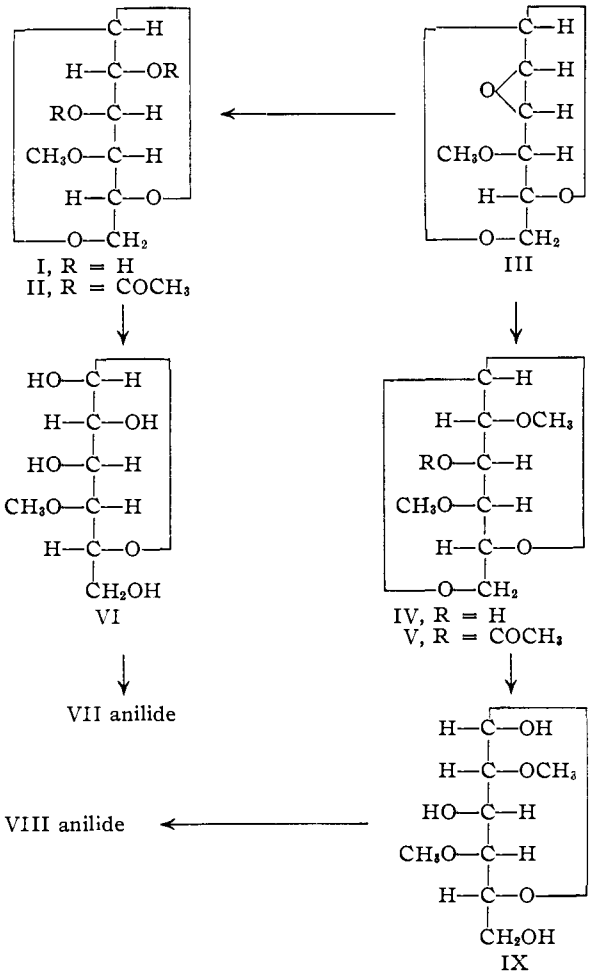
(3) D. J. Bell, *Adv. Carbohydrate Chem.*, **6**, 11 (1951).

(4) S. P. James, F. Smith, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 625 (1946).

(5) R. W. Jeanloz and P. J. Stoffyn, THIS JOURNAL, **76**, 5682 (1954).

β-D-galactose (VI), characterized by its crystalline anilide VII. The properties of VI and of its anilide correspond to those of the 4-O-methyl-β-D-galactopyranose isolated by Hirst and Jones[6] from methylated damson gum. The structure of the latter compound was assigned on the basis of its reaction with phenylhydrazine to give the known 4-O-methylgalactose phenylosazone.

When III was treated with sodium methoxide, it gave principally a sirupy 1,6-anhydro-2,4-di-O-methyl-β-D-galactopyranose (IV). When crude IV was acetylated and chromatographed, the products were a crystalline 3-O-acetyl derivative (V) in a 70% yield and a sirup consisting mainly of 1,6-anhydro-2-O-acetyl-3,4-di-O-methyl-β-D-idopyranose in a 10% yield. (Yields are based on the theoretical yield calculated from III). Hydrolysis of IV gave crystalline 2,4-di-O-methyl-α-D-galactose (IX) from which the crystalline anilide, VIII, was prepared. The properties of VIII and IX are in agreement with those of the 2,4-di-O-methyl-α-D-galactopyranose isolated by Baldwin and Bell[7] from methylated *Helix pomatia* galactogen, and with those of its anilide. The structure of IX was established by Smith,[8] who isolated it from methylated arabic acid.



(6) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 506 (1946).
(7) E. Baldwin and D. J. Bell, *ibid.*, 1461 (1938).
(8) F. Smith, *ibid.*, 1724 (1939).

From the sirupy idose derivative hydrolyzed to the free 3,4-di-O-methyl-D-idose, and transformed to the anilide, no crystalline product could be prepared, but an additional 1% of VIII was isolated.

The preponderant yield of galactose over idose derivatives is in good agreement with the results previously obtained on treating 1,6:2,3-dianhydrotalose with basic reagents.

Experimental[9]

2,3-Di-O-acetyl-1,6-anhydro-4-O-methyl-β-D-galactopyranose (II).—A solution of 620 mg. of 1,6:2,3-dianhydro-4-O-methyl-β-D-talose (III) in 5% aqueous potassium hydroxide was refluxed for 48 hours. After cooling, the solution was neutralized with carbon dioxide and evaporated *in vacuo* to dryness. The residue was exhaustively extracted with chloroform. The solvent was then removed, and the residual sirup was acetylated as follows: it was dissolved in 3 ml. of dry pyridine and 2 ml. of acetic anhydride, and allowed to stand for two days at 20°. The excess acetic anhydride was decomposed by the addition of methanol with cooling, and the solution was evaporated *in vacuo* to dryness. The last traces of pyridine were removed by codistillation *in vacuo* with several portions of dry toluene. The residual sirup was dissolved in benzene and chromatographed on silicic acid. The products were eluted with mixtures of benzene and ether 4:1 and 2:1. The first product was 46 mg. of a sirup, $[\alpha]^{28}D$ −31 ± 2° (in chloroform, *c* 0.87). In the following fractions, 870 mg. of substance II was isolated as a sirup, $[\alpha]^{28}D$ −16 ± 1° (in chloroform, *c* 6.66). *Anal.* Calcd. for $C_{11}H_{16}O_7$: C, 50.77; H, 6.20; OCH_3, 11.85. Found: C, 50.75; H, 6.24; OCH_3, 11.91.

From the first fraction, approximately 20 mg. of 4-O-methylgalactose (VI) was subsequently obtained, making the total yield of II 890 mg. (87%).

1,6-Anhydro-4-O-methyl-β-D-galactopyranose (I).—To a solution of 830 mg. of II in 10 ml. of methanol was added 4.5 ml. of a solution of 1.6 *N* barium methoxide. After standing overnight at 0° the solution was neutralized with sulfuric acid and evaporated *in vacuo* to dryness. The residue was extracted with acetone and after evaporation of the solvent the residual sirup was purified by dissolving it in methanol, adding an equal volume of ether and filtering through a double layer of Darco-G 60 and Celite. Evaporation *in vacuo* gave a colorless sirup (I), weighing 525 mg. (93%), $[\alpha]^{28}D$ −28 ± 1° (in chloroform, *c* 1.67). *Anal.* Calcd. for $C_7H_{12}O_5$: C, 47.72; H, 6.87. Found: C, 47.74; H, 6.96.

Attempts to obtain crystalline di-*p*-tolylsulfonyl or di-(2′,4′-dinitrobenzoyl) derivatives were unsuccessful.

4-O-Methyl-β-D-galactopyranose (VI).—A solution of 235 mg. of I in 5 ml. of 2 *N* sulfuric acid was heated overnight on a steam-bath. After removal of the sulfate ion with barium carbonate and filtration through Darco G-60 and Celite, the solution was evaporated *in vacuo* and the crystalline residue was recrystallized from a mixture of water, ethanol and acetone or from glacial acetic acid, giving 210 mg. (81%) of prisms, m.p. 218–221°. The compound showed mutarotation from $[\alpha]^{24}D$ +61° (after 10 minutes) to $[\alpha]^{22}D$ +83 ± 2° (after 17 hours, in water, *c* 2.17).[10] *Anal.* Calcd. for $C_7H_{14}O_6$: C, 43.30; H, 7.27; OCH_3, 15.98. Found: C, 43.37; H, 7.33; OCH_3, 16.05.

4-O-Methyl-*N*-phenyl-D-glucosylamine (VII).—A solution of 0.5 ml. of aniline in 0.7 ml. of absolute ethanol was added to 67 mg. of VI.[11] After standing at 20° for two days, the mixture was diluted with 5 ml. of absolute ethanol, refluxed for two minutes and then evaporated to dryness *in vacuo*. The last traces of aniline were removed by codistillation with several additions of dry toluene. The crystalline residue was recrystallized from a mixture of methanol and ether, giving 82 mg. (88%) of prismatic needles (VII); the m.p. depended on the rate of heating. When heated slowly from 20°, the product melted at 167–168°[12]; when started at 175°, the product melted at 182–

(9) R. W. Jeanloz, THIS JOURNAL, **76**, 555 (1954).
(10) Hirst and Jones[6] reported m.p. 207° and $[\alpha]^{20}D$ +62° mutarotating to +92° (after 9 hours, in water, *c* 1.5).
(11) See E. J. Bourne, personal communication in the paper by D. J. Bell and D. J. Manners, *J. Chem. Soc.*, 1145 (1954).
(12) Hirst and Jones[6] reported 168°.

183°. The product showed mutarotation, from $[\alpha]^{21}D$ −84° (after 7 minutes) to $[\alpha]^{22}D$ −39 ± 1° (after 17 hours, in methanol, *c* 1.33). *Anal.* Calcd. for $C_{13}H_{19}O_8N$: C, 57.98; H, 7.11. Found: C, 57.96; H, 7.16.

3-*O*-Acetyl-1,6-anhydro-2,4-di-*O*-methyl-β-D-galactopyranose (V).—A solution of 760 mg. of 1,6:2,3-dianhydro-4-*O*-methyl-β-D-talose (III) in 50 ml. of methanol containing 1 g. of sodium was refluxed for 24 hours in a moisture-free system. After cooling, the solution was diluted with water, neutralized with carbon dioxide and evaporated *in vacuo* to dryness. The residue was extracted with hot chloroform. After evaporation of the solvent, 960 mg. of sirup remained, which was acetylated with 4 ml. of dry pyridine and 2 ml. of acetic anhydride, following the procedure described above for II. The residue was dissolved in benzene and chromatographed on silicic acid. Elution was obtained with mixtures of benzene and ether, 4:1 and 2:1. The first product was a sirup which was chromatographed a second time (see next paragraph). Crystalline fractions were obtained on elution with the 2:1 mixture. Recrystallization from a mixture of ether and pentane gave 765 mg. of prismatic needles of the D-galactose derivative V, m.p. 108°, $[\alpha]^{24}D$ −68 ± 2° (in chloroform, *c* 1.62). *Anal.* Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.95; OCH_3, 26.72. Found: C, 51.64; H, 6.98; OCH_3, 26.68.

Chromatography of the non-crystalline fraction described above and of the mother liquors of V gave 15 mg. of V, and 115 mg. (10%) of a sirup (presumed to be mainly 1,6-anhydro-2-*O*-acetyl-3,4-di-*O*-methyl-β-D-idopyranose) with $[\alpha]^{27}D$ −86 ± 2° (in chloroform, *c* 0.91). *Anal.* Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.95; OCH_3, 26.72. Found: C, 51.81; H, 6.94; OCH_3, 26.64. Hydrolysis of this sirup and reaction of the resulting sirup with aniline gave besides 5 mg. of the anilide VIII only sirupy fractions. The total yield of V from III is thus 70%.

1,6-Anhydro-2,4-di-*O*-methyl-β-D-galactopyranose (IV).—To a solution of 400 mg. of V in 5 ml. of methanol was added 5 ml. of a solution of 0.4 *N* barium methoxide. After heating to boiling for two minutes, the solution was cooled, neutralized with sulfuric acid and evaporated *in vacuo* to dryness. The residue was extracted with chloroform, the solvent was evaporated and the residue, redissolved in chloroform, was chromatographed on silicic acid. Mixtures of chloroform and ether eluted fractions which gave by evaporation 315 mg. (96%) of a colorless sirup IV, $[\alpha]^{27}D$ −46 ± 2° (in chloroform, *c* 1.51). *Anal.* Calcd. for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.55; H, 7.51.

2,4-Di-*O*-methyl-α-D-galactopyranose (IX).—A solution of 205 mg. of IV in 5 ml. of 2 *N* sulfuric acid was heated overnight on a steam-bath. After addition of an excess of barium carbonate, the solution was filtered through a double layer of Darco G-60 and Celite. Evaporation *in vacuo* to dryness gave 223 mg. of a sirup which crystallized in the cold after addition of a few drops of 95% ethanol. Recrystallization from a mixture of 95% ethanol, acetone and ether gave 206 mg. (92%) of prisms, m.p. 105–108° (dried over P_2O_5). The compound crystallized as a hydrate and showed mutarotation from $[\alpha]^{27}D$ +113° (after 10 minutes) to $[\alpha]^{27}D$ +85 ± 2° (after 22 hours in water, *c* 1.15 for the dry product), indicating the product was the α-anomer.[13] *Anal.* Calcd. for $C_8H_{16}O_6 \cdot H_2O$: C, 42.47; H, 8.02; OCH_3, 27.44; H_2O, 7.96. Found: C, 42.43; H, 8.10; OCH_3, 27.40; H_2O, 7.98.

2,4-Di-*O*-methyl-*N*-phenyl-D-glucosylamine (VIII).—A solution of 61 mg. of IX in 2 ml. of absolute ethanol was refluxed with 28 mg. of aniline for two hours. The solution was then concentrated *in vacuo* and ether added to complete crystallization. Recrystallization from a mixture of methanol and ether gave 73 mg. (88%) of very small prismatic needles, m.p. 219–220°, $[\alpha]^{26}D$ +30 ± 3° (in methanol, *c* 0.68).[14] *Anal.* Calcd. for $C_{14}H_{21}O_5N$: C, 59.35; H, 7.47. Found: C, 59.29; H, 7.60.

Acknowledgments.—The author is indebted to Miss Ann Foley and to Miss Shirley Phillips for technical assistance.

(13) Baldwin and Bell[7] reported m.p. 100–103° and $[\alpha]^{20}D$ +85.7° (in water at equilibrium). Smith[8] reported m.p. 103° and $[\alpha]^{18}D$ +122.7° (after 5 minutes) mutarotating to +87.5° (in water at equilibrium).

(14) Smith[8] reported m.p. 216°.

BOSTON, MASSACHUSETTS

[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, COLUMBIA UNIVERSITY COLLEGE OF PHYSICIANS AND SURGEONS AND THE EDWARD DANIELS FAULKNER ARTHRITIS CLINIC OF THE PRESBYTERIAN HOSPITAL]

Structural Studies on Chondroitin Sulfuric Acid.[1,2] I. The Nature of Chondrosine

BY EUGENE A. DAVIDSON AND KARL MEYER

RECEIVED MAY 6, 1954

Chondrosine, a disaccharide from chondroitin sulfate of cartilage, has been prepared by an improved method and obtained in crystalline form for the first time. The disaccharide was shown to be 3(?)-(β-D-glucopyranosyluronic acid)-2-deoxy-2-amino-D-galactopyranose by reduction of the crystalline methyl ester hydrochloride with sodium borohydride to a 3(?)-(β-D-glucopyranosyl)-2-deoxy-2-amino-D-galactitol, demonstrated by the isolation of glucose pentaacetate after N-acetylation and acid hydrolysis. The N-acetyl derivative was hydrolyzed by β-glucosidase thus indicating the β-configuration of the disaccharide.

Chondrosine was isolated by Hebting[3] as the crystalline ethyl ester hydrochloride after hydrolysis of chondroitin sulfate with oxalic acid, and shown to be a desulfated and deacetylated disaccharide. Levene[4] isolated a crystalline methyl ester hydrochloride to which he assigned the structure of a chondrosaminidoglucuronic acid. Recently, Wolfrom, Madison and Cron[5] confirmed the results obtained by Levene and assigned to the disaccharide the structure 4-(2-amino-2-deoxy-β(?)-D-galactopyranosyl)-D-glucuronic acid. In contrast to these findings, in 1951 Masamune and co-workers,[6] on the basis of a positive Elson–Morgan reaction and periodate oxidation of N-acetylchondrosine ethyl ester, assigned to the disaccharide the structure 3-(D-glucopyranosyluronic acid)-2-deoxy-2-amino-D-galactopyranose. In previous publications from the same laboratory, various other structures had been reported.[7–9]

On the basis of the similarity of chondroitin sul-

(1) This work was supported by grants from the National Institutes of Health, the Helen Hay Whitney Foundation and the New York Chapter of the Arthritis and Rheumatism Foundation.

(2) Taken in part from a thesis to be submitted by Eugene A. Davidson in partial fulfillment of the requirments for the Ph.D. degree, Faculty of Pure Science, Columbia University.

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(4) P. A. Levene, *J. Biol. Chem.*, **140**, 267 (1941).

(5) M. L. Wolfrom, R. K. Madison and M. J. Cron, THIS JOURNAL, **74**, 1491 (1952).

(6) H. Masamune, Z. Yosizawa and M. Maki, *Tohoku J. Exp. Med.*, **55**, 47 (1951).

(7) H. Masamune and S. Osaki, *ibid.*, **45**, 121 (1943).

(8) T. Kobayashi, *ibid.*, **49**, 84 (1947).

(9) T. Nagaoka, *ibid.*, **53**, 29 (1950).