by Huque, Jaworzyn and Goring.[48] The solvent was *n*-butyl acetate and the liquid surrounding the cell was a mixture of 2 parts of ethylene glycol and 1 part of water. The light-scattering cells were designed to achieve efficient removal of micellar debris, which, if present, caused vertical striations, making accurate measurements impossible. All solutions were clarified twice by ultracentrifugation, first, at 40,000 r.pm. and subsequently in the light-scattering cells at 20,000 r.p.m. The wave length of the light used was 5460 Å. The refractive index increment was measured with a Brice–Phoenix differential refractometer at a wave length of 4358 Å. The value obtained was 0.085 ml./g. No correction was applied for dissymetries which averaged 1.1 to 1.2 and which did not vary with the concentration. The following values were obtained for the ratio $C/I_{90°}$ at the respective concentrations C (mg./ml.): 2.084(1.98), 2.933(3.99), 3.423(6.06), 3.912(8.16) and 4.420(9.90). Extrapolation to zero concentration gave a value of 1.75 for $(C/I_{90°})_{c=0}$. The molecular weight was calculated from the relationship $1/M_w = H(C/\tau)_0$ where the symbols have their usual significance.

Determination of Intrinsic Viscosity.—The viscometer used was a Craig–Henderson instrument.[49] Reduced viscosities were estimated at 25° at seven different concentrations and extrapolated to zero concentration in the usual way. The solvent was *M* cupriethylenediamine.

Acknowledgment.—The authors wish to express their gratitude to Mr. W. L. Steyn for carrying out the osmotic pressure and light-scattering measurements. A. T. is also thankful to the American Viscose Corporation, Marcus Hook, Pa., for a Fellowship.

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[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY, AND THE WOOD CHEMISTRY DIVISION, PULP AND PAPER RESEARCH INSTITUTE OF CANADA]

Synthesis and Characterization of 2-O-(β-D-Glucopyranosyluronic Acid)-D-xylopyranose[1]

BY W. D. S. BOWERING AND T. E. TIMELL

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2-O-(β-D-Glucopyranosyluronic acid)-D-xylopyranose has been synthesized by condensation of methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucuronate with methyl 3,5-O-isopropylidene-D-xyloside in a Koenigs–Knorr reaction. The aldobiouronic acid was characterized by reduction, methylation and hydrolysis when 2,3,4,6-tetra-O-methyl-D-glucose and 3,4-di-O-methyl-D-xylose were obtained.

In connection with an attempted synthesis of the β-anomer of the ubiquitous 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose[2] the unmethylated aldobiouronic acid 2-O-(β-D-glucopyranosyluronic acid)-D-xylopyranose was also synthesized. The α-anomer of this compound was first obtained by partial hydrolysis of hemicellulose B of corn cob[3,4] and has since been isolated from hemicelluloses in wheat bran,[5] oat hulls,[6] chagual gum,[7] corn hull,[8,9] groundnut shell,[10] wheat straw[11] and the wood of maritime pine.[12] It has been fully characterized through the crystalline methyl ester-pentaacetate of its methyl α- and β-D-glycosides.[9]

The 2-O-(β-D-glucopyranosyluronic acid)-D-xylopyranose was synthesized from D-glucurono-(3→6)-lactone and D-xylose. D-Glucurone was converted by three different methods[13–15] to sirupy methyl (D - glucopyranosyl) - uronate. Acetylation[15,16] yielded the α- and β-anomers of methyl 1,2,3,4 - tetra - O - acetyl - D - glucuronate which both crystallized. The compound was treated with titanium tetrachloride in chloroform[13] when the crystalline methyl 2,3,4-tri-O-acetyl-1-chloro-1-deoxy-α-D-glucuronate was obtained. Treatment with hydrogen bromide in acetic acid converted the tetraacetate to the corresponding bromo compound which was also crystalline, albeit rather unstable.[13,17,18]

D-Xylose was converted to methyl α,β-D-xylofuranoside, either directly by treatment with methanolic hydrogen chloride[19,20] or *via* the 3,5-O-isopropylidene derivative.[21,22] The α- and β-anomers of 3,5-O-isopropylidene D-xyloside were obtained by subsequent condensation with acetone under carefully controlled conditions.[21]

Condensation of methyl 2,3,4-tri-O-acetyl-1-chloro-1-deoxy-α-D-glucuronate with methyl 3,5-

(1) Paper presented at the Symposium on Wood Hemicelluloses before the Division of Cellulose Chemistry at the 136th Meeting of the American Chemical Society in Atlantic City, N. J., September, 1959.

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O-isopropylidene-D-xyloside gave a sirup which, after deacetylation and hydrolysis, contained only glucuronic acid, glucurone and xylose, thus indicating the insufficient reactivity of the acetochloro compound. When methyl 3,5-*O*-isopropylidene-α-(or β)-D-xyloside was treated under similar conditions with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate, the reaction mixture contained an aldobiouronic acid (R_x 0.26) in addition to the above compounds. Resolution by paper chromatography gave the pure aldobiouronic acid and its lactone in a yield of 4.7%, $[\alpha]^{20}D$ +5.7° (*c* 3.0 in water). Recovery of the lactone in the original sugar mixture would probably have increased the total yield to approximately 10%.

The aldobiouronic acid was characterized by reduction of its methyl ester–methyl glycoside and methylation, followed by hydrolysis of the fully methylated disaccharide, when 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose were obtained. The former compound crystallized and both were identified through their aniline derivatives. The methyl 2-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucosyl)-uronate]-3,4-di-*O*-acetyl-α,-β-D-xyloside could not be induced to crystallize.

The ease of lactonization of the 2-*O*-(β-D-glucopyranosyluronic acid)-D-xylopyranose is noteworthy in view of the fact that neither of the α- or β-anomers of its 4-*O*-methyl derivative exhibit this property.[2] The low rotation of the present aldobiouronic acid shows that it contains a β-glycosidic linkage, as could also be expected from its mode of synthesis, the specific rotation of the α-anomer being reported as + 37°,[12] +59°,[3] +84°,[5] +88°,[6] +101°[11] and +108°,[7] respectively. The presence of an α-glycosidic bond in the 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylopyranose previously isolated from corn hull hemicellulose[9] is thus further corroborated.

Experimental

All specific rotations are equilibrium values and melting points are corrected. Evaporations were carried out *in vacuo* at 40–50°.

Paper Chromatography.—Solvents (v./v.) used were (A) ethyl acetate–acetic acid–water (9:2:2), (B) butan-1-ol–pyridine–water (10:3:3) and (C) butan-2-one–ethanol–water (20:5:2). Separations were carried out by the descending technique on Whatman No. 1 and No. 3MM filter paper. *o*-Aminobiphenyl was used as a spray reagent.[23] R_x denotes the distance that the substance has moved on the paper chromatogram relative to D-xylose.

Solvents.—All solvents were of reagent grade quality and made anhydrous in the usual way. Chloroform for the Koenigs–Knorr reaction was specially purified.[24] Reagent grade chloroform (1000 ml.) was shaken with 12% sulfuric acid (25 ml.) for 1 hr. After separation from the acid, the chloroform was washed with saturated sodium bicarbonate solution and then very thoroughly with water. The chloroform was dried over anhydrous calcium chloride, filtered and distilled. The distillate was dried over phosphorus pentoxide, redistilled and stored over Drierite[25] in the dark at +4°. The purified solvent was used within the next few hours.

Methyl (D-Glucopyranosyl)-uronate (A).—D-Glucofuranosyl-(3 → 6)-lactone (D-glucurone, 33.0 g., m.p. 176–177°, $[\alpha]^{19}D$ +19.6° (*c* 3.2 in water)) was boiled under reflux with anhydrous methanol for 72 hr.[13] The methanol was evaporated to yield a yellow sirup which was dissolved in ethanol (200 ml.) and allowed to stand at +3° for 48 hr. A crystalline material was recovered (17.6 g.), $[\alpha]^{20}D$ +19.1° (*c* 3.8 in water), m.p. 176–177°, undepressed on admixture with an authentic sample of D-glucurone. The remaining sirup was concentrated to yield a yellow sirup (18.2 g., 100%).

(B).—D-Glucurone (33.0 g.) was boiled under reflux with anhydrous methanol (600 ml.) containing metallic sodium (0.5 g.) for 10 min. after which the solution was allowed to stand for 2 hr. at room temperature.[14] The sirup obtained was dissolved in ethanol and allowed to stand at 3° for several days. Since no solid material separated, the solvent was evaporated, leaving an orange-colored sirup (31.5 g., 81%).

(C).—D-Glucurone (40.0 g.) was added with stirring in small portions to methanol (300 ml.) containing sodium hydroxide (0.11 g.).[15] The yellow solution (*p*H 8) was allowed to stand for 1 hr. Evaporation of the methanol gave a sirup (47.8 g., 101%).

Methyl 1,2,3,4-Tetra-*O*-acetyl-α-D-glucuronate.—Methyl (D-glucopyranosyl)-uronate (18.2 g.) was dissolved in a mixture of acetic anhydride (100 ml.) and anhydrous pyridine (100 ml.), freshly distilled over barium oxide. The reaction mixture was first cooled at −16° and then allowed to stand at room temperature with occasional shaking for 60 hr. The solution was poured into 1000 ml. of ice-water and the resulting mixture was extracted three times with chloroform. The chloroform extract was washed once with water, twice with 10% aqueous hydrochloric acid, twice with saturated sodium bicarbonate solution and once with water. After drying over anhydrous sodium sulfate and filtration, the chloroform was removed to yield a yellow sirup. The α,β-acetate was dissolved in an isomerizing solution consisting of acetic anhydride (140 ml.), acetic acid (60 ml.) and concentrated sulfuric acid (4.6 ml.).[16] After 24 hr. at +21°, the solution was poured into ice-water, resulting in the separation of an oil. The mixture was extracted three times with chloroform and the extract was washed twice with 10% aqueous sodium bicarbonate, followed by one washing with water. After drying over sodium sulfate and filtration, concentration gave a sirup which was dissolved in ethanol. After several days at room temperature, needle-shaped, white crystals were obtained, 29.8 g., 76%, m.p. 111.5–112.5°, $[\alpha]^{18}D$ +91.4° (*c* 1.3 in chloroform).[26]

Methyl 1,2,3,4-Tetra-*O*-acetyl-α-(and β)-D-glucuronate.—Methyl (D-glucopyranosyl)-uronate (47.8 g.) was dissolved in a mixture of acetic anhydride (150 ml.) and pyridine (100 ml.), the temperature not being allowed to exceed 40°.[15] After standing overnight at +3°, a crystalline material separated which was removed by filtration and dissolved in ethanol containing a little acetone. The solution was treated with Darco G-60 charcoal,[27] filtered and allowed to stand at room temperature. The white, crystalline material, constituting the β-anomer, was recovered by filtration, m.p. 177–178°, $[\alpha]^{20}D$ +8.6° (*c* 2.0 in chloroform).[26] The acetylation mixture, after concentration, yielded additional crystalline material which was recrystallized as above to give a product (10.3 g., 12%) with the same constants as the first product. The mother liquor was further concentrated and dissolved in chloroform. After the usual purification, the chloroform solution was evaporated to give a sirup which was dissolved in ethanol. After treatment with charcoal, the solution on standing deposited small, white, needle-shaped crystals which were recrystallized three times from ethanol (24.8 g., 29%), m.p. 109–110°, $[\alpha]^{20}D$ +85.5° (*c* 2.0 in chloroform).[26]

Methyl 2,3,4-Tri-*O*-acetyl-1-chloro-1-deoxy-α-D-glucuronate.—Methyl 1,2,3,4-tetra-*O*-acetyl-α-D-glucuronate (3.0 g.) was dissolved in anhydrous, alcohol-free chloroform (30 ml.). Titanium tetrachloride (1 ml.)[13] was added and the solution was heated at 40–45° for 3 hr., after which it was poured into ice-water. The chloroform layer was extracted three times with ice-water and dried over anhydrous calcium chloride. The sirupy residue, after filtration and evaporation, was dissolved in ethyl ether and allowed to stand. The white, crystalline material formed (1.0 g., 36%) had m.p. 98.5–100°, $[\alpha]^{20}D$ +162° (*c* 1.0 in chloroform).[13] This procedure was repeated with the β-acetate and the

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(26) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **106**, 63 (1934).

(27) A product of Darco Corp., New York, N. Y.

crystalline α-chloro-compound was obtained in a yield of 81%.

Methyl 2,3,4-Tri-O-acetyl-1-bromo-1-deoxy-α-D-glucuronate.—Methyl 1,2,3,4-tetra-O-acetyl-α-D-glucuronate (5.0 g.) was shaken at room temperature with glacial acetic acid (25 ml.), saturated with anhydrous hydrogen bromide. The solution formed was concentrated to a sirup which was dissolved in chloroform (100 ml.). This solution was cooled to −10° and extracted once with ice-water after which it was dried over anhydrous sodium sulfate at +3°. The sirup obtained after filtration and concentration was dissolved in ethanol (10 ml.). Crystallization occurred instantaneously and after 2 hr. at +3°, the crystals were recovered by filtration, washed with petroleum ether and dried *in vacuo* at +3°. Concentration of the motor liquor gave more crystalline material. The crystals (4.3 g., 82%) had m.p. 104–105.5° and $[\alpha]^{24}D$ +198° (*c* 0.5 in chloroform).[13] The procedure was repeated with the β-anomer of the acetate with identical results. The α-bromo compound decomposed at room temperature within a day but was stable for at least a week at +3°.[17]

Methyl α,β-D-Xylofuranoside. (A).—D-Xylose (m.p. 145–146°, $[\alpha]^{23}D$ +18.6° (*c* 4.2 in water), 100 g.) was added to anhydrous methanol (2.5 l.) containing hydrogen chloride[19,20] (40 g.). The mixture was allowed to stand with occasional shaking at room temperature for 7 days. The clear solution was neutralized with silver carbonate, filtered through Celite[28] and concentrated to a sirup which partly crystallized. Recrystallization from acetone (85 ml.) yielded methyl β-D-xylopyranoside (35.6 g., 33%), m.p. 156.5–157°, $[\alpha]^{20}D$ −65.1° (*c* 4.4 in water). The acetone solution was concentrated to yield a sirup (80.5 g., 74%), a portion of which was subjected to distillation *in vacuo*, giving sirupy methyl α,β-D-xylofuranoside, 24.6 g.), $[\alpha]^{19}D$ +63.2° (*c* 3.5 in ethanol).[19]

(B).—D-Xylose (200 g.) was added to anhydrous methanol (2.5 l.) containing 0.5% hydrogen chloride (w./w.) and the mixture was allowed to stand at room temperature for 5 hr.[29] After neutralization with lead carbonate, filtration through Celite, treatment with hydrogen sulfide and renewed filtration, concentration yielded a crude sirup (220 g.). The sirup was shaken with acetone (2.3 l.), *N* sulfuric acid (4.6 ml.) and anhydrous copper sulfate (460 g.) at room temperature for 60 hr.[21] After filtration, concentrated ammonium hydroxide was added to the filtrate, which was refiltered and concentrated to a sirup. The latter was dissolved in water (220 ml.) and the resulting solution was extracted with chloroform (440 ml.), followed by a second extraction (220 ml.). After drying and concentration, the sirup obtained was dissolved in 70% acetic acid (680 ml.), which was heated at 50° for 2 hr. Concentration yielded sirupy methyl α,β-D-xylofuranoside (71.8 g., 33%).

Methyl 3,5-O-Isopropylidene-α,β-D-xyloside.—Methyl α,β-D-xylofuranoside (55.8 g.) was dissolved in acetone (750 ml.). Anhydrous copper sulfate (150 g.) and *N* sulfuric acid (1.5 ml.) were added and the mixture was shaken at room temperature for 60 hr.[21] The solid material was recovered by filtration and the filtrate was neutralized by addition of concentrated ammonium hydroxide (1.2 ml.). Concentration yielded a sirup (49.8 g.) which was dissolved in water (72 ml.). This solution was extracted twice with chloroform (144 and 72 ml., respectively). The extracts were combined, dried over sodium sulfate and evaporated to yield a sirup (16.1 g.) which was distilled *in vacuo* to give two fractions. The first of these was a fluid, colorless sirup (8.7 g., 12.5%), $[\alpha]^{20}D$ +29.4° (*c* 4.1 in water), and the second was a viscous, yellow sirup (5.7 g., 8.2%), $[\alpha]^{20}D$ −74.1° (*c* 4.0 in water).[30]

2-O-(β-D-Glucopyranosyluronic acid)-D-xylopyranose. (A).—Sirupy methyl 3,5-O-isopropylidene-D-xyloside (5.0 g.) was dissolved in purified chloroform (50 ml.). Drierite (35 g.), freshly prepared silver oxide (17.5 g.) and glass beads (28 g.) were added and the mixture was shaken in the dark at room temperature for 1 hr. Crystalline methyl 2,3,4-tri-O-acetyl-1-chloro-1-deoxy-α-D-glucuronate (8.64 g.), dissolved in chloroform (35 ml.), and iodine (1.75 g.) were added and shaking was continued. After 12 days no ionizable chlorine remained and an additional quantity of the acetochloro derivative (8.7 g. in 35 ml. of chloroform) was added together with iodine (1.75 g.). The reaction was allowed to proceed for 11 days, when the solid material was removed by centrifuging and washed with chloroform. The combined solutions were filtered through Celite and concentrated to a yellow sirup. After treatment with sodium methoxide (2.7 g.) in methanol (75 ml.), followed by hydrolysis with boiling 0.5 *N* sulfuric acid (200 ml.) for 3 hr., concentration yielded a sirup which was examined by paper chromatography (solvent A). Only glucuronic acid, glucurone and xylose were present.

(B).—Methyl 3,5-O-isopropylidene-D-xyloside (12.9 g.) was dissolved in anhydrous, alcohol-free chloroform (135 ml.). After addition of Drierite (90 g.), freshly prepared silver oxide (45 g.) and glass beads (72 g.), the mixture was shaken in the dark at room temperature for 1 hr. Methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucuronate (25.9 g.), dissolved in chloroform (90 ml.) and iodine (4.5 g.) were added. Shaking was continued in the dark until no ionizable bromine was detected (7 days) after which the solids were removed on the centrifuge and washed with chloroform. The combined solutions were filtered through Celite and evaporated to a sirup which was dissolved in methanol (200 ml.) containing sodium methoxide (7 g.) and boiled under reflux for 20 min. The dark sirup obtained on evaporation was dissolved in 0.5 *N* sulfuric acid (500 ml.) and the solution was boiled under reflux for 3 hr. After neutralization (barium carbonate) and filtration through Celite, the filtrate was passed through a column containing Amberlite IR-120 exchange resin (H)[31] and the eluate was concentrated to a sirup (28.4 g.). Paper chromatography (solvent A) indicated the presence of glucuronic acid, glucurone, xylose and an aldobiouronic acid with an R_x value of 0.26.

The sirup was dissolved in water (28 ml.) and the mixture was resolved on 116 sheets of Whatman No. 3MM filter paper (system A, 96 hr.). The sections containing the aldobiouronic acid were excised and extracted five times with water. The extract was concentrated to a small volume, treated with Amberlite IR-120 (H) resin, filtered and evaporated to yield an amorphous solid (3.93 g.). Paper chromatography (system A) suggested the presence of three compounds with R_x values of 0.14, 0.46 and 0.89, respectively. The sirup was redissolved in water (3.5 ml.) and resolved as before on 14 sheets of filter paper. After the usual purification treatment, a lactone (0.603 g., 2.9%) and an acid (0.381 g., 1.8%) were obtained. Each of these substances, when examined chromatographically, gave two spots (system A), corresponding to the acid and lactone forms of the aldobiouronic acid. The specific rotation, $[\alpha]^{20}D$ of the 2-O-(β-D-glucopyranosyluronic acid)-D-xylopyranose was 5.7° (*c* 3.0 in water).

Preparation and Reduction of the Ester Glycoside of the Aldobiouronic Acid.—2-O-(β-D-Glucopyranosyluronic acid)-D-xylopyranose (700 mg.) was dissolved in anhydrous methanol (100 ml.) containing 2% hydrogen chloride (w./w.). Drierite (5 g.) was added and the solution was boiled under reflux for 8 hr. After neutralization with silver carbonate and filtration through Celite, evaporation yielded an amorphous powder of methyl 2-O-[methyl(β-D-glucopyranosyl)-uronate]-D-xylopyranoside (753 mg.).

Anal. Calcd. for $C_{13}H_{22}O_{11}$: OMe, 17.5. Found: OMe, 17.8.

A portion of the methyl ester–methyl glycoside (540 mg.) was dissolved in anhydrous tetrahydrofuran (50 ml.) and lithium aluminum hydride (1 g.) was added, dispersed in the same solvent (15 ml.). After boiling under reflux for 4 hr., excess lithium aluminum hydride was destroyed with ethyl acetate, followed by water (100 ml.). Aluminum hydroxide was removed by filtration and lithium hydroxide and remaining salts by treatment with Amberlite IR-120 (H) and Dowex 1-X4[32] (acetate) exchange resins. Evaporation gave a yellow sirup (369 mg.), $[\alpha]^{20}D$ +47° (*c* 0.4 in water).

Methylation and Hydrolysis of the Reduced Aldobiouronic Acid.—The disaccharide obtained above was dissolved in water (30 ml.) at 0° and aqueous potassium hydroxide (40%, w./w.) and dimethyl sulfate (10 ml.) were added dropwise with vigorous stirring. After further treatment with alkali (310 ml.) and dimethyl sulfate (100 ml.), the reaction mixture was heated to 90° for 1 hr., diluted with water (200 ml.) and continuously extracted with chloroform

(28) A product of Johns-Manville Co., New York, N. Y.

(29) P. A. Levene, A. L. Raymond and R. T. Dillon, *J. Biol. Chem.*, **95**, 699 (1932).

(30) J. M. Anderson and E. Percival, *J. Chem. Soc.*, 819 (1956).

(31) A product of Rohm and Haas Co., Philadelphia, Pa.

(32) A product of Dow Chemical Co., Midland, Mich.

for 60 hr. Evaporation of the extract yielded a partially methylated product (410 mg.) which was dissolved in methyl iodide (50 ml.) containing silver oxide (5 g.) and Drierite. After boiling under reflux for 10 hr., the solution was recovered by filtration and evaporated to dryness. This treatment was repeated four times when an infrared spectrum of the sirup (250 mg.) indicated the absence of any hydroxyl groups.

Anal. Calcd. for $C_{18}H_{34}O_{10}$: OMe, 52.9. Found: OMe, 51.9.

Sirupy methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucosyl)-3,4-di-*O*-methyl-α,β-D-xyloside was boiled under reflux with 0.5 *N* sulfuric acid (50 ml.) for 10 hr. After recovery of the sirupy mixture in the usual way, paper chromatography (solvent C) indicated the presence of a tetra-*O*-methylglucose and a di-*O*-methylxylose in approximately equal amounts.

Identification of 2,3,4,6-Tetra-*O*-methyl-D-glucose.—The sugar mixture obtained after hydrolysis was resolved on strips of Whatman No. 1 filter paper (system C) and the excised sections were eluted five times with ethanol. After treatment with Darco G-60 charcoal and Amberlite IR-120 (H) exchange resin, evaporation yielded chromatographically pure tetra-*O*-methylglucose (68 mg.) and di-*O*-methylxylose (15 mg.).

The 2,3,4,6-tetra-*O*-methyl-D-glucose was crystallized from petroleum ether (b.p. 60–80°), m.p. 87.5–90.5°, $[\alpha]^{21}D$ +82° (*c* 1.7 in water).

Anal. Calcd. for $C_{10}H_{20}O_6$: OMe, 52.5. Found: OMe, 51.3.

The 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine, on recrystallization from petroleum ether, had m.p. and mixed m.p. 135–136.5°, $[\alpha]^{22}D$ +225° (*c* 1.1 in acetone).[33]

(33) J. C. Irvine and A. M. Moodie, *J. Chem. Soc.*, 95 (1908).

Characterization of 3,4-Di-*O*-methyl-D-xylose.—The 3,4-*O*-methyl-D-xylose had an electrophoretic mobility in 0.05 *M* borate solution which was identical to that of an authentic specimen.[34] The remaining sirup was converted to the aniline derivative[35] which, however, failed to crystallize. The infrared spectrum of the 3,4-di-*O*-methyl-*N*-phenyl-D-xylosylamine, $[\alpha]^{22}D$ +137° (*c* 0.5 in ethyl acetate), was indistinguishable from that of an authentic specimen.

Preparation of Methyl 2-*O*-[Methyl (2,3,4-tri-*O*-acetyl-β-D-glucosyl)-uronate]-3,4-di-*O*-acetyl-α,β-D-xyloside.—Methyl 2-*O*-[methyl (β-D-glucopyranosyl)-uronate]-α,β-D-xylopyranoside (200 mg.) was dissolved in anhydrous pyridine (15 ml.) to which freshly distilled acetic anhydride (5 ml.) was added. After 24 hr. at room temperature, the reaction mixture was poured into ice-water to form a solution which was extracted three times with chloroform. The extract was purified in the usual way and concentrated to yield a yellow sirup (238 mg.), $[\alpha]^{21}D$ +28.4° (*c* 1.6 in chloroform), which could not be induced to crystallize in ethanol, ethyl ether or aqueous mixtures of these two solvents.

Anal. Calcd. for $C_{23}H_{32}O_{16}$: OMe, 11.0; ester (as acetyl), 45.8. Found: OMe, 10.3; ester, 42.1.

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MONTREAL, QUE., CANADA

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY, SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION]

Synthesis of 2β-Hydroxytestosterone[1]

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The synthesis of 2β-hydroxytestosterone (III) has been described. It has been shown that 2β-hydroxytestosterone 2,17-diacetate (I) could be selectively hydrolyzed at the C-2 position to give 2β-hydroxytestosterone 17-acetate (V) without isomerization of the 2β-hydroxyl group. Hydrolysis of I and VI by refluxing with potassium bicarbonate or potassium carbonate in aqueous methanol yielded 2,17β-dihydroxyandrosta-1,4-dien-3-one (IV).

2β-Hydroxy steroids have thus far been prepared only by biological hydroxylation.[2] By the usual synthetic methods the 2β-hydroxy group in a Δ^4-3-keto steroid is rather an inaccessible function because it tends to isomerize to the more stable (equatorial) α-form. However, it is of great interest to develop a synthetic route to 2β-hydroxy steroids, and the present paper is concerned with the successful synthesis of 2β-hydroxytestosterone (III).

Steroids having an α- and β-acetoxy group at the C-2 position have been previously synthesized by two methods. The first method consists of acetoxylation of a Δ^4-3-keto steroid with lead tetraacetate[3] and the second one consists of acetolysis of a 6-bromo-Δ^4-3-ketone with potassium acetate[3d,3e,3f,4]

It was claimed previously[3d,3e] that both the 2α- and 2β-acetoxy compounds when hydrolyzed under mild conditions gave the more stable 2α-hydroxy compound. That is, even under seemingly mild hydrolytic conditions the 2β-hydroxy group was isomerized to the 2α-form. However, by the methods worked out in our laboratories the free compound 2β-hydroxytestosterone (III) was definitively synthesized.

Compound I was prepared by acetolysis of 6-bromotestosterone 17-acetate with potassium acetate as described in the literature[3d,3f] with one ex-

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