gave 258 mg of 1-(3-deoxy-β-D-ribofuranosyl)-4-methoxy-5-methyl-2(1H)-pyrimidinone (**19**), mp 196–198°. Tlc on cellulose in water showed one zone at R_f 0.88: $[\alpha]_D$ +25°, $[\alpha]_{578}$ +27° (*c* 0.77, water); $\lambda_{max}^{H_2O}$, mμ (ε × 10^{-3}), 280 (6.6), 203 (18.6), 215 (infl) (12.0).

Anal. Calcd for $C_{11}H_{16}N_2O_5$: C, 51.56; H, 6.29; N, 10.93. Found: C, 51.26; H, 6.33; N, 10.96.

By reworking the filtrates from the recrystallizations another 186 mg of product, mp 196–198°, was obtained. The total yield was 444 mg (62%).

1-(3-Deoxy-α-D-ribofuranosyl)-4-methoxy-5-methyl-2(1H)-pyrimidinone (21).—By the method described above for the synthesis of **19**, 960 mg (1.73 mmoles) of 2,5-di-O-*p*-nitrobenzoyl-3-deoxy-α-D-ribofuranosyl)-4-methoxy-5-methyl-2(1H)-pyrimidinone (**14**) gave a total of 292 mg (66%) of 1-(3-deoxy-α-D-ribofuranosyl)-4-methoxy-5-methyl-2(1H)-pyrimidinone (**21**), mp 185–187°. Tlc on cellulose in water showed one zone at R_f 0.9: $[\alpha]_D$ −157°, $[\alpha]_{578}$ −166° (*c* 0.22, water), $\lambda_{max}^{H_2O}$, mμ (ε × 10^{-3}), 281 (6.6), 204 (18.9).

Anal. Found: C, 51.42; H, 6.07; N, 11.04.

1-(3-Deoxy-β-D-ribofuranosyl)thymine (23).—A suspension of 395 mg (1.54 mmoles) of 1-(3-deoxy-β-D-ribofuranosyl)-4-methoxy-5-methyl-2(1H)-pyrimidinone (**19**) in 15 ml of methanol was treated with 1.5 ml of 30.6% (w/w) hydrogen chloride in methanol and the solution was kept at 25°. After 6 days no further change in the ultraviolet absorption spectrum could be observed. The solution was concentrated to dryness and one portion of methanol and three successive portions of benzene were distilled from the residue. The residue when crystallized from 1 ml of methanol and 3 ml of ether gave 300 mg (81%) of 1-(3-deoxy-β-D-ribofuranosyl)thymine (**23**) which melted at 96–100°, resolidified, and remelted at 155–157°. For analysis, a sample was twice recrystallized from methanol–ether and dried to constant weight at 56°.

Anal. Calcd for $C_{10}H_{14}N_2O_5$: C, 49.58; H, 5.83; N, 11.57. Found: C, 49.49; H, 5.84; N, 11.22.

1-(3-Deoxy-α-D-ribofuranosyl)thymine (24).—In the manner described above for the synthesis of **23**, 279 mg (1.09 mmoles) of 1-(3-deoxy-α-D-ribofuranosyl)-4-methoxy-5-methyl-2(1H)-pyrimidinone (**21**) gave 200 mg (76%) of 1-(3-deoxy-α-D-ribofuranosyl)-5-methyluracil (**24**), mp 188–191°. Tlc on cellulose in water showed one zone at R_f 0.86.

Anal. Found: C, 49.72; H, 6.07; N, 11.69.

1-(3-Deoxy-β-D-ribofuranosyl)-5-methylcytosine (16).—A mixture of 400 mg (0.72 mmole) of 1-(2,5-di-O-*p*-nitrobenzoyl-3-deoxy-β-D-ribofuranosyl)-4-methoxy-5-methyl-2(1H)-pyrimidinone (**12**) and 5 ml of methanol, saturated with ammonia at 0°, was heated at 100° in a sealed tube for 16 hr. The reaction mixture was worked up as in the synthesis of **15**. Crystallization of the crude product three times from methanol–ether gave a total of 90 mg (52%) of 1-(3-deoxy-β-D-ribofuranosyl)-5-methylcytosine (**16**), mp 223–226°. Tlc on cellulose in water showed one zone at R_f 0.76.

Anal. Calcd for $C_{10}H_{15}N_3O_4$: C, 49.78; H, 6.27; N, 17.42. Found: C, 49.56; H, 6.06; N, 17.76.

1-(3-Deoxy-α-D-ribofuranosyl)-5-methylcytosine (18).—As described above for the preparation of **16**, 300 mg (0.55 mmole) of 1-(2,5-di-O-*p*-nitrobenzoyl-3-deoxy-α-D-ribofuranosyl)-4-methoxy-5-methyl-2(1H)-pyrimidinone (**14**) gave 81 mg (50%) of 1-(3-deoxy-α-D-ribofuranosyl)-5-methylcytosine (**18**), mp 191–192° with a transition at 173°. Tlc on cellulose in water showed a single zone at R_f 0.78.

Anal. Found: C, 49.49; H, 6.23; N, 17.31.

Acknowledgment. —The authors wish to thank the following members of the staff of the Merck Sharp and Dohme Research Laboratories for their contributions to this work: Drs. D. E. Williams, J. Wittick, and K. Hoogsteen for the optical rotatory dispersion measurements, Dr. N. L. Trenner and Mr. B. Arison for determining the nmr spectra, Mr. E. A. MacMullin and his associates for the ultraviolet spectral measurements, and Mr. R. N. Boos and his associates for performing the microanalyses.

2-Amino-2-deoxy-D-xylose and 2-Amino-2-deoxy-D-ribose and Their 1-Thioglycofuranosides[1]

M. L. WOLFROM AND M. W. WINKLEY

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received October 22, 1965

Glycol cleavage of 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-D-glucose diethyl dithioacetal (I) with subsequent aldehyde reduction and acid hydrolysis resulted in a new synthesis of 2-amino-2-deoxy-D-xylose hydrochloride (IV). Improved preparative directions are cited for ethyl 2-acetamido-2-deoxy-1-thio-α-D-xylofuranoside (VII), which on methylsulfonylation and inversion on C-3 by sodium acetate, with subsequent acid hydrolysis, led to a new synthesis of 2-amino-2-deoxy-D-ribose hydrochloride (X). Two crystalline derivatives of a 1-thiofuranoside of X are described which, together with VII, will be used in nucleoside syntheses.

Syntheses of all of the 2-amino-2-deoxypentoses were first reported from this laboratory[2-7] except for the D-*arabino* isomer which was reported by Kuhn and Baschang.[8] Having need of relatively large quantities of 2-amino-2-deoxy-D-xylose[2] and 2-amino-2-deoxy-D-ribose,[5] we have devised new syntheses for these substances. For the former, 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal[9] was converted into its 3,4-di-*O*-isopropylidene cyclic acetal[10] (I) (see Scheme I) and this was oxidized with lead tetraacetate to a syrupy mixture whose main component was undoubtedly the aldehyde II produced by cleavage between C-5 and C-6. Restricted glycol cleavage of a dithioacetal presents difficulties owing to the oxidizability of the sulfur atoms herein present in their most reduced state.[11] II was characterized as its crystalline thiosemicarbazone IIa. Reduction of syrupy, impure II with sodium borohydride produced largely 3,4-di-*O*-isopropylidene-D-xylose diethyl dithioacetal (III) which was charac-

(1) Supported by Grant No. CA 03232-09 from the Department of Health, Education, and Welfare, U. S. Public Health Service, National Institutes of Health, Bethesda, Md. (The Ohio State University Research Foundation Project 759H).

(2) M. L. Wolfrom and K. Anno, *J. Am. Chem. Soc.*, **75**, 1038 (1953).

(3) M. L. Wolfrom, F. Shafizadeh, and R. K. Armstrong, *ibid.*, **80**, 4885 (1958).

(4) M. L. Wolfrom and Z. Yosizawa, *ibid.*, **81**, 3477 (1959).

(5) M. L. Wolfrom, F. Shafizadeh, R. K. Armstrong, and T. M. Shen Han, *ibid.*, **81**, 3716 (1959).

(6) D. Horton, M. L. Wolfrom, and A. Thompson, *J. Org. Chem.*, **26**, 5069 (1961).

(7) M. L. Wolfrom, D. Horton, and A. Böckmann, *ibid.*, **29**, 1479 (1964).

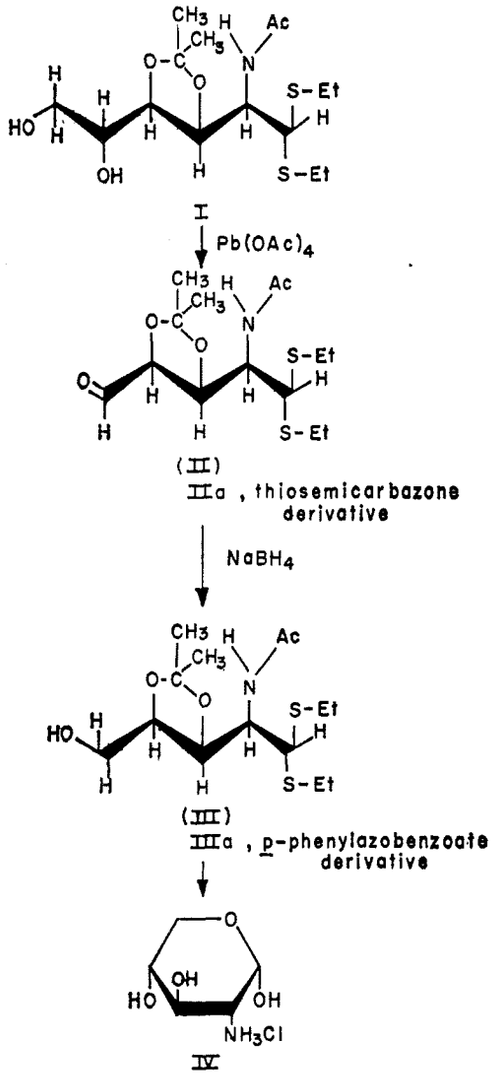
(8) R. Kuhn and G. Baschang, *Ann.*, **628**, 193 (1959).

(9) M. L. Wolfrom and K. Anno, *J. Am. Chem. Soc.*, **74**, 6150 (1952); D. Horton, *Biochem. Prepn.*, in press.

(10) J. Yoshimura and T. Sato, *Nippon Kagaku Zasshi*, **80**, 1479 (1959); *Chem. Abstr.*, **55**, 5355 (1961); A. E. El Ashmaway and D. Horton, *Carbohydrate Res.*, **1**, 164 (1965).

(11) For a discussion of this point, see D. Horton and D. H. Hutson, *Advan. Carbohydrate Chem.*, **18**, 144 (1963).

SCHEME I



terized as its crystalline *p*-phenylazobenzoate IIIa. Hydrolysis of impure, syrupy III gave the crystalline 2-amino-2-deoxy-α-D-xylose hydrochloride (IV). In addition to our previous work,[2] another synthesis of IV has been reported by Gigg and Warren,[12] while its enantiomorph has been recorded by Wolfrom and co-workers.[7] Gigg and Warren utilized as their starting compound an oxazoline derivative of 2-amino-2-deoxy-D-glucose.

While the above procedure makes more available the unsubstituted 2-amino-2-deoxy-D-xylose, our original synthesis[2] produced a 1-thiofuranoside of this same sugar. Such a derivative should be utilizable in nucleoside synthesis and at the same time should be useful in the synthesis of 2-amino-2-deoxy-D-ribose and its 1-thiofuranoside. Accordingly, the original procedure of Wolfrom and Anno[2] was repeated using larger amounts of material with very considerable improvements in the yields at the various steps of the synthesis.

Following Wolfrom and Anno,[2] 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal (V) was converted into ethyl 2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-glucofuranoside (VIa) by the method of Wolfrom, Olin, and Polglase[13] in improved yield (see Scheme II). *O*-Deacetylation of VIa with sodium methoxide followed by oxidative cleavage between C-5 and C-6 with sodium periodate and reduction with sodium borohydride in a similar manner to that reported by Wolfrom and Anno[2] resulted in ethyl 2-acetamido-2-deoxy-1-thio-α-D-xylofuranoside (VII) in improved yield. It was observed that the intermediate aldehyde was partially adsorbed by monobed resin and this was avoided in our present procedure by removing iodate as insoluble barium iodate prior to reduction with sodium borohydride and subsequent deionization with monobed resin.

The next step was to convert this 1-thiofuranoside of 2-amino-2-deoxy-D-xylose into a like derivative of 2-amino-2-deoxy-D-ribose. Methylsufonylation of VII gave a crystalline derivative, ethyl 2-acetamido-2-deoxy-3,5-di-*O*-methylsulfonyl-1-thio-α-D-xylofuranoside (VIII). This was treated with sodium acetate and 95% aqueous 2-methoxyethanol by the general method of Baker and co-workers[14] to give, after acetylation, a syrupy 3,5-diacetate, IX. Hydrolysis of IX with hydrochloric acid yielded crystalline 2-amino-2-deoxy-α-D-ribose hydrochloride (X). In addition to our previous work,[5] other syntheses of X have been reported by Gigg and Warren[12] and by Kuhn and Baschang.[8] The former workers utilized again the oxazoline derivative of 2-amino-2-deoxy-D-glucose as their starting point while the latter employed a Strecker-type synthesis on 2,4-*O*-ethylidene-D-erythrose. Coxon and Hough[15] have recorded a synthesis of 2-acetamido-2-deoxy-D-ribose through the peroxypropionic acid degradation of 3-amino-3-deoxy-D-allose diethyl dithioacetal and its epimeric derivative. Syntheses of the enantiomorphous 2-amino-2-deoxy-L-ribose have been reported by Wolfrom and co-workers[3,5] and by Collins and Overend.[16] The latter investigators utilized reduction of the oxime of methyl 2,3-*O*-isopropylidene-β-L-*erythro*-pentopyranosidulose.

O-Deacetylation of IX yielded syrupy XI, characterized as its crystalline bis(3,5-dinitrobenzoate) XIa, and complete deacetylation of IX with subsequent reaction with 1-fluoro-2,4-dinitrobenzene yielded the crystalline ethyl 2-deoxy-2-(2,4-dinitroanilino)-1-thio-α-D-ribofuranoside (XII).

The utilization of VII and XII in nucleoside synthesis will be the subject of a subsequent communication.

Experimental Section[17]

Lead Tetraacetate Oxidation of 2-Acetamido-2-deoxy-3,4-*O*-isopropylidene-D-glucose Diethyl Dithioacetal (I).—I was prepared

(12) R. Gigg and C. D. Warren, *J. Chem. Soc.*, 1351 (1965).

(13) M. L. Wolfrom, S. M. Olin, and W. J. Polglase, *J. Am. Chem. Soc.*, **72**, 1724 (1950).

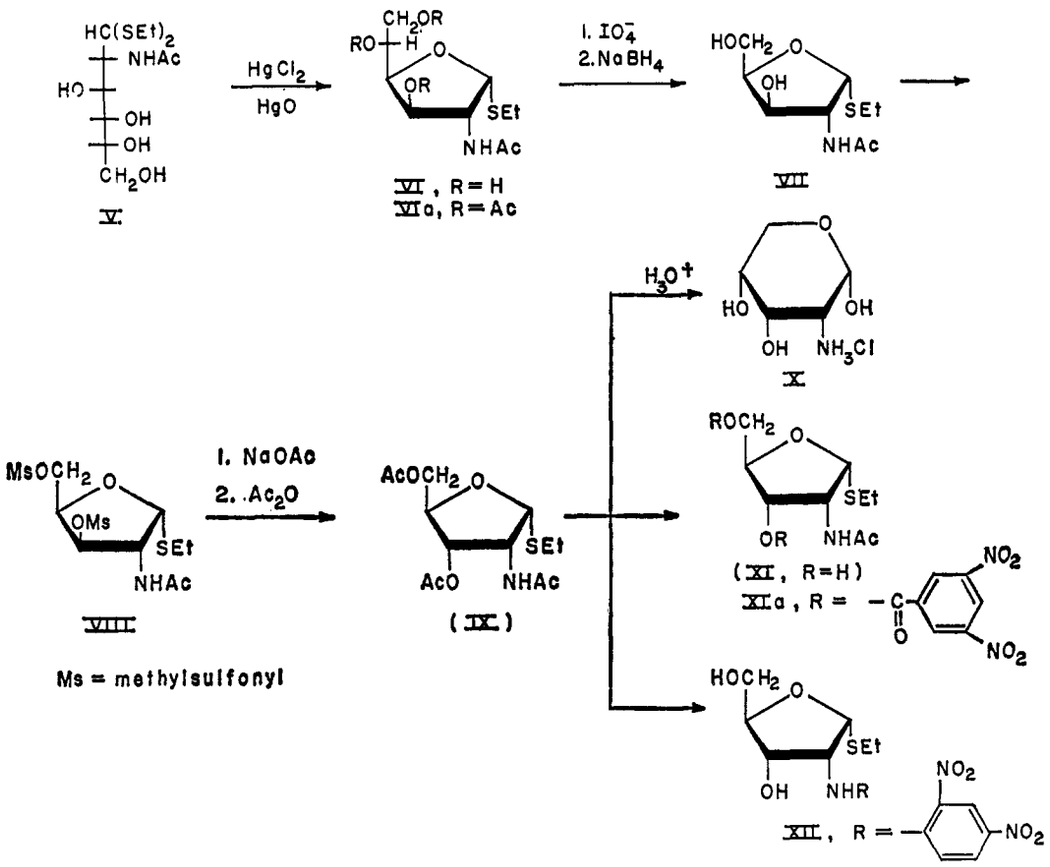
(14) B. R. Baker, R. E. Schaub, and J. H. Williams, *ibid.*, **77**, 7 (1955).

(15) B. Coxon and L. Hough, *J. Chem. Soc.*, 1463, 1643 (1961).

(16) P. M. Collins and W. G. Overend, *ibid.*, 3448 (1965).

(17) Melting points were determined with a Hershberg-type apparatus: A. Thompson and M. L. Wolfrom, *Methods Carbohydrate Chem.*, **1**, 517 (1962). Specific rotations were measured in a 2-dm polarimeter tube. Infrared spectra were measured with a Perkin-Elmer Infracord infrared spectrometer. Microanalytical determinations were made by W. N. Rond. X-Ray powder diffraction data give interplanar spacings, in angstroms, for Cu Kα radiation. Relative intensities were estimated visually: s, strong; m, moderate; w, weak; v, very. The strongest lines are numbered (1, strongest); multiple numbers indicate approximately equal intensities. Tlc was effected with Desaga equipment using silica gel G (E. Merck, Darmstadt, Germany) activated at 110°, with indication by sulfuric acid. Paper chromatography was done on Whatman No. 1 paper using the descending technique. All crystalline compounds described in this work were shown to be homogeneous by either thin layer or paper chromatography. All concentrations (evaporations) were performed at 40° under reduced pressure. Chloroform extracts were washed successively with water, saturated aqueous sodium bicarbonate solution, and again with water, and dried over magnesium sulfate.

SCHEME II



by the method of Yoshimura and Sato,[10] mp 138–139°, $[\alpha]^{21}D$ −28 ± 2° (*c* 2.06, methanol). To a solution of I (2.96 g) in dry, ethanol-free chloroform (40 ml) and dry benzene (200 ml) was added lead tetraacetate (3.96 g, 1:1 molar ratio, commercial brand, dried under reduced pressure over sodium hydroxide for 48 hr at room temperature in the dark). After shaking vigorously at room temperature for 1 hr, the mixture was filtered, chloroform was added to the filtrate, and the syrup (crude II) obtained on solvent removal was examined by tlc with benzene–methanol (9:1 v/v) as developer. Five spots were observed at R_f 0.11, 0.16, 0.21, 0.31, and 0.45. The spot with R_f 0.16 corresponded with a marker of I. The spot with R_f 0.31 was by far the most intense spot. The infrared spectrum of the original syrup indicated an aldehydic absorption at 5.87 μ.

2-Acetamido-2-deoxy-3,4-*O*-isopropylidene-D-*xylo*-pentodialdose 1-(Diethyl Dithioacetal) 5-Thiosemicarbazone (IIa).—To the above syrup in ethanol (5 ml) was added thiosemicarbazide (0.7 g, commercial brand, recrystallized from water), followed by a solution (neutralized with dilute acetic acid to pH 6.0) of sodium acetate trihydrate (1.48 g) in water (3 ml). The mixture was heated under reflux, and water and ethanol were added to achieve solution. The heating was continued for 1 hr. The separated gum, obtained on cooling, was removed by decantation and dissolved in chloroform. The residue obtained on solvent removal from the washed and dried chloroform solution was crystallized from ethyl acetate–hexane, yield 1.66 g (50%), mp 121–123°. This material was extracted with chloroform, the chloroform was removed by concentration, and the syrup was crystallized twice from ethyl acetate–hexane: mp 124–125°; $[\alpha]^{28}D$ +44.5 ± 1° (*c* 2.20, chloroform); λ_{max}^{KBr} 3.10–3.50 (NH), 6.05, 6.62 (NHAc), 6.25 (C=N), and 7.40 μ (CMe_2); X-ray powder diffraction data, 14.98 (w), 11.48 (s, 3.3), 9.72 (vw), 8.59 (s, 2), 7.76 (vs, 1), 6.19 (m), 5.37 (vw), 4.85 (s, 3, 3), 4.42 (w), 4.29 (w), 4.13 (m), 3.97 (vw), 3.85 (m), 3.74 (w), and 3.47 (m).

Anal. Calcd for $C_{15}H_{28}N_4O_3S_3$: C, 44.11; H, 6.90; N, 13.70; S, 23.55. Found: C, 44.18; H, 7.12; N, 13.97; S, 22.86.

2-Acetamido-2-deoxy-3,4-*O*-isopropylidene-5-*O*-(*p*-phenylazobenzoyl)-D-xylose Diethyl Dithioacetal (IIIa).—I (3.66 g) was oxidized with lead tetraacetate as described above. The syrup produced was dissolved in methanol (100 ml) and cooled in ice and water. Sodium borohydride (1.1 g) in methanol (70 ml) was added dropwise to this solution with stirring. After 1 hr at room temperature, the solution was neutralized to pH 7 with Amberlite IR-120 (H^+) cation-exchange resin. The resin was removed by filtration and the solution was concentrated to near dryness. The residue was extracted with chloroform and the washed and dried extract was evaporated to a syrup (III). Thin layer chromatography, using a mixture of benzene and methanol (9:1, v/v) revealed one major component with R_f 0.3 with several other very minor components. The infrared spectrum of the syrup no longer showed an aldehyde absorption at 5.87 μ.

To this reduced, syrupy material (2.43 g), in dry pyridine (40 ml), was added *p*-phenylazobenzoyl chloride (1.91 g) and the mixture was shaken overnight. The reaction mixture was poured into ice and water and extracted with chloroform. The red syrup obtained on solvent removal from the washed and dried extract was crystallized from benzene–hexane, yield 2.31 g (42% from I), mp 50–54°. Recrystallization from benzene–hexane afforded red crystals: mp 55–57°; $[\alpha]^{21}D$ +13 ± 2° (*c* 1.15, chloroform); λ_{max}^{KBr} 3.10 (NH) and 5.82 μ ($-CO_2R$); X-ray powder diffraction data, 11.48 (w), 10.16 (m, 2), 7.63 (m), 6.86 (m), 5.72 (s, 1,1,1), 4.57 (s, 1,1,1), 4.25 (s, 1,1,1), 4.04 (m, 3), 3.79 (m), and 3.04 (w).

Anal. Calcd for $C_{27}H_{35}N_3O_5S_2$: C, 59.41; H, 6.47; N, 7.70; S, 11.75. Found: C, 59.53; H, 6.47; N, 8.04; S, 12.18.

2-Amino-2-deoxy-α-D-xylose Hydrochloride (IV).—I (3.66 g) was oxidized with lead tetraacetate and reduced with sodium borohydride as described above. To the resultant syrup was added 6 *N* hydrochloric acid (60 ml) and the mixture was heated at 95° for 1 hr. The acid was removed by repeated evaporation with 1-propanol and the residue was dissolved in methanol and decolorized with carbon. The residue obtained on solvent removal crystallized slowly from aqueous 1-propanol, yield 0.37 g (20% from I), dec pt 154–158°. Recrystallization from aqueous 1-propanol afforded pure material: dec pt 164–165°; $[\alpha]^{20}D$ +82 (initial, extrapolated) → +46 ± 2° (*c* 1.01, water, final); λ_{max}^{KBr} 3.0–3.5 (OH, NH_3^+), 6.20, and 6.67 μ (NH_3^+); X-ray powder diffraction data, 8.12 (w), 6.03 (s), 4.67 (s, 2), 4.37 (s), 4.06 (s, 3), 3.41 (vs, 1), 3.19 (s), 3.07 (w), 2.96 (vw), 2.77 (vw), 2.69 (vw), 2.61 (m), 2.50 (w), 2.37 (w), 2.33 (w), 2.25 (w), and 2.20 (m).

Anal. Calcd for $C_5H_{12}ClNO_4$: C, 32.36; H, 6.52; Cl, 19.10; N, 7.55. Found: C, 32.69; H, 6.37; Cl, 19.00; N, 8.05.

2-Amino-2-deoxy-α-D-xylose hydrochloride was obtained by hydrolysis of ethyl 2-acetamido-2-deoxy-1-thio-α-D-xylofuranoside according to the method of Wolfrom and Anno.[2] The X-ray powder diffraction pattern of this material was identical with that obtained above. Wolfrom and Anno reported dec pt 165–167°, $[\alpha]^{31}D$ +80 → +40° (water, final) for this compound. Gigg and Warren[12] reported dec pt 168–170°, $[\alpha]D$ +79 → +40° (water, final).

Preparation of Ethyl 2-Acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-glucofuranoside[13] (VIa).—To a suspension of mercuric oxide freshly prepared from mercuric chloride (107 g) by the method of Pacsu and Wilson[18] in water (1 l.) was added 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal (V, 100 g). Mercuric chloride (41.7 g) in water (2 l.) was added dropwise to this suspension under rapid stirring over a period of 3 hr. Stirring was continued for 1 hr after the addition. Pyridine (50 ml) was added and the reaction mixture was allowed to stand at 5° overnight, then filtered through a pad of Celite,[19] and the filtrate was concentrated to small volume. After filtering several times, the solution was finally concentrated to a syrup. The syrup (crude VI) was dried by repeated evaporation with absolute ethanol.

The dried syrup was dissolved in pyridine (250 ml), acetic anhydride (250 ml) was added, with cooling, and the solution was maintained at room temperature overnight. The acetylated mixture was poured into ice and water and extracted with chloroform. The syrup obtained on solvent removal from the washed and dried extract was crystallized from ethanol–water: yield 62 g (51%); mp 122–123°; $[\alpha]^{20}D$ +139 ±3° (*c* 3.81, chloroform); λ_{max}^{KBr} 3.10 (NH), 5.75 (OAc), 6.10, and 6.45 μ (NHAc); X-ray powder diffraction data, 6.92 (vs, 1), 4.80 (s, 2,2,2), 4.77 (m), 4.31 (s, 2,2,2), 3.92 (s, 2,2,2), 3.77 (w), 3.65 (vw), 3.48 (vw), 3.30 (s, 3), 3.13 (s), 3.04 (w), 2.87 (vw), 2.73 (vw), 2.60 (vw).

Wolfrom, Olin, and Polglase[13] reported a yield of crude material of 21%, mp 119–122°, $[\alpha]^{23}D$ +123° (chloroform). Their purified product showed mp 124.5–125.5°, $[\alpha]^{23}D$ +140° (chloroform).

Preparation of Ethyl 2-Acetamido-2-deoxy-1-thio-α-D-xylofuranoside (VII).[2]—VIa (19.55 g) was dissolved in anhydrous methanol (100 ml) and sodium (0.5 g) was added. The solution was allowed to stand for 2 hr at room temperature. The sodium methoxide was neutralized to pH 7 with Amberlite IR-120 (H^+) cation-exchange resin. The resin was removed by filtration and washed with methanol, and the methanolic solution was concentrated to a syrup. The syrup crystallized on removal of the last traces of methanol under reduced pressure.

The O-deacetylated solid (VI) was dissolved in water (200 ml) and the solution was cooled to 10°. To this was added a solution of sodium periodate (11.25 g, 1:1.05 molar ratio) in water (200 ml) at 10°. The reaction was left in the dark at 10° for 30 min. A solution of barium chloride (6.45 g, dihydrate) in water (50 ml) was added and the resulting precipitate of barium iodate was removed by filtration.

To the resultant, stirred solution was added dropwise a solution of sodium borohydride (2.5 g) in water (50 ml) over a period of 10 min. After stirring for an additional 40 min, the solution was neutralized to pH 7 by the dropwise addition of 1 *N* sulfuric acid followed by passage down a column of Amberlite[20] MB-3 (40 × 3.5 cm). The effluent (2 l.) was concentrated to a syrup which crystallized spontaneously. This material was dried by repeated evaporation with absolute ethanol and recrystallized from absolute ethanol: yield 6.08 g (52%); mp 153–155°; $[\alpha]^{22}D$ +212 ± 3° (*c* 6.45, water); λ_{max}^{KBr} 3.05 (NH), 6.05 and 6.50 μ (NHAc); X-ray powder diffraction data, 14.03 (m), 8.27 (s, 2), 7.25 (m), 6.46 (w), 4.85 (m), 4.65 (m), 4.40 (w), 4.23 (vs, 1), 4.13 (vw), 3.93 (s, 3), 3.71 (w), 3.45 (w), 3.19 (m), 3.01 (vw), 2.86 (m), 2.76 (w), 2.68 (w), 2.59 (w), 2.47 (w), and 2.42 (w).

A second crop was obtained: yield 1.65 g (14%), mp 138–145°. Wolfrom and Anno[2] reported a yield of 18% for fairly pure material of mp 154–155° and $[\alpha]^{27}D$ +212° (water) and for pure material they cited mp 157–158° and $[\alpha]^{21}D$ +222° (water).

Ethyl 2-Acetamido-2-deoxy-3,5-di-*O*-methylsulfonyl-1-thio-α-D-xylofuranoside (VIII).—VII (4.85 g) in dry pyridine (100 ml) was cooled to 0° under moisture protection. To the stirred solution was added methanesulfonyl chloride (4.2 ml, 1:2.5 molar ratio) dropwise. The reaction mixture was stored at 0° overnight and was then poured into ice and water and extracted with chloroform. The washed and dried chloroform extract was concentrated to a syrup. Crystallization from ethanol–ether afforded a slightly colored product which was not homogeneous as shown by tlc using ethyl acetate as developer. Recrystallization from ethanol–ether gave a homogeneous, white, crystalline product: yield 5.13 g (64%); mp 99–103°; $[\alpha]^{28}D$ +107 ± 2° (*c* 2.31, chloroform); λ_{max}^{KBr} 3.10 (NH), 6.07, 6.43 (NHAc), 7.50, and 8.54 μ (OSO_2Me); X-ray powder diffraction data, 10.04 (s), 9.61 (w), 8.51 (s, 3), 5.47 (s), 4.85 (m), 4.48 (vs, 1), 4.23 (s, 2,2), 4.10 (s, 2,2), 3.90 (s), 3.68 (vw), 3.35 (vw), 3.19 (s), 3.02 (w), 2.81 (w), 2.68 (w), 2.57 (w), and 2.47 (m).

Anal. Calcd for $C_{11}H_{21}NO_8S_3$: C, 33.75; H, 5.41; N, 3.58; S, 24.58. Found: C, 33.93; H, 5.63; N, 3.91; S, 24.05.

2-Amino-2-deoxy-α-D-ribose Hydrochloride (X).—A mixture of VIII (4.90 g), anhydrous sodium acetate (10.30 g), and calcium carbonate (10.30 g) in 95% aqueous 2-methoxyethanol (Methyl Cellosolve, 120 ml) was heated for 24 hr under reflux with stirring. The cooled mixture was filtered, the filter cake was washed with 2-methoxyethanol, and the filtrate was evaporated to dryness. To the residue was added pyridine (50 ml) and acetic anhydride (50 ml) and the mixture was shaken overnight, then poured into ice and water, and extracted with chloroform. Solvent removal from the washed and dried chlorform extract yielded a syrup (IX) which by tlc using ethyl acetate as developer, was shown to be homogeneous. The infrared spectrum of the syrup indicated complete removal of methylsulfonyl groups by disappearance of the absorption band at 8.54 μ. An ester band at 5.75 μ indicated the presence of acetyl groups.

To the syrup (IX) was added 6 *N* hydrochloric acid (60 ml) and the mixture was heated for 1 hr at 95°. The acid was removed by repeated evaporation at 40° with 1-propanol. The residue was dissolved in aqueous methanol, decolorized with carbon, filtered, and concentrated to dryness. The residue was crystallized from aqueous 1-propanol, yield 1.53 g (66% from VIII), mp 149–153°. Recrystallization from methanol–acetone or concentrated hydrochloric acid–1-propanol afforded pure material: mp 153–155°; $[\alpha]^{29}D$ +13 (initial, extrapolated) → −6 ± 1° (*c* 1.1, water, final); λ_{max}^{KBr} 2.90–3.5 (OH, NH), 6.15, and 6.68 μ (NH_3^+); X-ray powder diffraction data, 7.56 (m) 6.37 (m), 5.87 (w), 5.61 (m), 5.07 (vw), 4.82 (w), 4.19 (vs, 1), 3.95 (m), 3.79 (s, 2), 3.66 (w), 3.49 (w), 3.41 (w), 3.22 (vw), 3.18 (w), 3.03 (s, 3,3), 2.95 (vw), 2.88 (vw), 2.76 (s, 3,3), 2.63 (w), and 2.57 (m).

Anal. Calcd for $C_5H_{12}ClNO_4$: C, 32.36; H, 6.52; Cl, 19.10; N, 7.55. Found: C, 32.72; H, 6.52; Cl, 19.17; N, 7.77.

Wolfrom and co-workers[5] reported mp 144–149° and $[\alpha]^{23}D$ +14.1 → −2.75° (water) for this compound. Kuhn and Baschang[8] reported mp 153–155° and $[\alpha]^{24}D$ +11.6 (2 min) → −5.8° (20 min, water). Gigg and Warren[12] reported mp 147–148° and $[\alpha]D$ +14 → 3° (water). An X-ray diffraction film of material prepared by Kuhn and Baschang was identical with that from our product.

Ethyl 2-Acetamido-2-deoxy-3,5-di-*O*-(3,5-dinitrobenzoyl)-1-thio-α-D-ribofuranoside (XIa).—Dried syrupy IX (0.89 g) was dissolved in anhydrous methanol (10 ml) and sodium (0.1 g) was added. After standing for 2 hr at room temperature, the sodium methoxide was neutralized to pH 7 with Amberlite IR-120 (H^+) cation-exchange resin. The resin was removed by filtration and the solution was evaporated to dryness at 40°. The dried, syrupy product (crude XI, 0.57 g) was dissolved in dry pyridine and cooled in ice and water. 3,5-Dinitrobenzoyl chloride (1.22 g) was added; the mixture was shaken rapidly for a few minutes and allowed to stand at 5° overnight. The reaction solution was then poured into ice and water and extracted with chloroform. The washed and dried extract yielded a cream-colored glass on concentration. Crystallization from ethyl acetate–hexane afforded a poorly crystalline solid. The supernatant was removed by decantation and the residue was dissolved in chloroform, decolorized with carbon, and crystallized from chloroform–ethanol, yield 0.64 g (35% from IX), mp 127–130°. This material was decolorized with carbon and was

(18) E. Pacsu and E. J. Wilson, Jr., *J. Am. Chem. Soc.*, **61**, 1450 (1939).

(19) No. 225, a siliceous filter aid, Johns-Manville, Inc., New York, N. Y.

(20) Amberlite MB-3, a mixture of cation- and anion-exchange resins produced by the Rohm and Haas Co., Resinous Products Division, Philadelphia 5, Pa.

recrystallized twice more to give crystals with a slightly yellow color: mp 131–132°; $[\alpha]^{22}D$ +30 ± 2° (*c* 2.88, pyridine); λ_{max}^{KBr} 3.05 (NH), 5.75 ($-CO_2R$), and 6.02 μ (NH); X-ray powder diffraction data, 13.39 (m), 9.72 (s, 2), 7.03 (m), 5.87 (w), 5.25 (vw), 4.74 (m), 4.25 (s, 1), 3.99 (s, 3), 3.66 (m), 3.47 (m), 3.06 (w), and 2.93 (w).

Anal. Calcd for $C_{23}H_{21}N_5O_{14}S$: C, 44.30; H, 3.39; N, 11.28; S, 5.15. Found: C, 44.27; H, 3.85; N, 11.53; S, 5.20.

Ethyl 2-Deoxy-2-(2,4-dinitroanilino)-1-thio-α-D-ribofuranoside (XII).—To a syrupy XI prepared from VIII (9.20 g) by the above procedure was added saturated barium hydroxide solution (100 ml) and the mixture was heated under reflux for 24 hr.[21] Solid carbon dioxide was added and the bulk of the resulting barium carbonate was removed by filtration. The filtrate was concentrated to small volume and the remaining water was removed by repeated evaporation with absolute ethanol. The residue was extracted with ethanol and the extract was concentrated to a syrup. Examination of this syrup by paper chromatography using 5:5:3:1 pyridine–ethyl acetate–water–acetic acid as solvent[22] revealed a single spot with R_f 0.68 which gave a positive indication with silver nitrate–sodium hydroxide and ninhydrin but not with aniline hydrogen phthalate. To the dried syrup (4.73 g) in water (20 ml) was added sodium bicarbonate (1.87 g) and the mixture was stirred. 1-Fluoro-2,4-dinitrobenzene (4.15 g) was added and the transfer was effected with ethanol (20 ml). The mixture was cooled in water and stirred vigorously for 1 hr. The yellow crystalline solid which separated was removed by filtration, washed with water, and dried under reduced pressure over phosphorus pentoxide: yield 6.73 g (80% from VIII), mp 151–155°. Recrystallization from aqueous methanol afforded pure material: yield 6.18 g (73% from VIII); mp 159–160°; $[\alpha]^{22}D$ −64 ± 1° (*c* 1.63 ethanol); λ_{max}^{KBr} 2.80–3.00 (OH), 3.10 (NH), 6.20, 6.30, 6.70 (aryl C=C), 6.58 (NH, NO_2), 7.46 (NO_2), 12.00, and 13.40 μ (substituted phenyl); X-ray powder diffraction data, 13.60 (w), 11.33 (w), 8.42 (s), 6.03 (w), 5.64 (w), 5.22 (s, 3), 4.82 (s, 1,1,1), 4.40 (m), 4.15 (s, 2), 3.82 (m), 3.59 (s, 1,1,1), 3.44 (vw), 3.11 (s, 1,1,1), and 3.02 (vw).

Anal. Calcd for $C_{13}H_{17}N_3O_7S$: C, 43.43; H, 4.88; N, 11.69; S, 8.92. Found: C, 43.53; H, 5.24; N, 12.13; S, 8.89.

(21) F. J. McEvoy, B. R. Baker, and M. J. Weiss, *J. Am. Chem. Soc.*, **82**, 209 (1960).

(22) F. G. Fischer and H. J. Nebel, *Z. Physiol. Chem.*, **302**, 10 (1955).

Configuration of the Glycosidic Linkage of 2-Amino-2-deoxy-D-glucopyranose to D-Glucuronic Acid in Heparin[1]

M. L. WOLFROM, H. TOMOMATSU, AND W. A. SZAREK

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received October 13, 1965

Partially acetylated, partially desulfated, carboxyl-reduced heparin was completely desulfated by a second treatment with methanolic hydrogen chloride. The desulfated product was *O*-deacetylated and subjected to periodate oxidation. The glycol units in the D-glucose units were oxidized and from the product 2-amino-2-deoxy-D-glucose hydrochloride was isolated on acid hydrolysis. The periodate-oxidized product was reduced with sodium borohydride and subjected to partial acid hydrolysis. Further oxidation with lead tetraacetate and subsequent sodium borohydride reduction and acetylation led to a crystalline compound which was shown by synthesis to be 2-*O*-(2-acetamido-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-1,3-di-*O*-acetylglycerol. This α-D anomer was separated from the anomeric mixture synthesized by a Koenigs–Knorr reaction between tri-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-α-D-glucopyranosyl bromide and *cis*-1,3-*O*-benzylideneglycerol with subsequent acid removal of the benzylidene and *p*-methoxybenzylideneamino groups and acetylation. The pure β-D anomer was synthesized from 2-acetamidotri-*O*-acetyl-2-deoxy-α-glucopyranosyl chloride and *cis*-1,3-*O*-benzylideneglycerol (the product being isolated in two forms shown to be *cis* and *trans* in nature), followed by benzylidene removal and acetylation. These results offer confirmatory evidence for the (1→4) nature of all linkages present in the heparin molecule and demonstrate conclusively that the configuration of the 2-amino-2-deoxy-D-glucose to D-glucuronic acid linkage is α-D.

In previous publications[1,2] from this laboratory it was reported that acid hydrolysis of partially *O*-acetylated, partially desulfated, completely carboxyl-reduced, and *N*-acetylated heparin yielded two crystalline amino sugar containing disaccharides. Methylation studies established that the disaccharides were *O*-α-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-α-D-glucopyranose hydrochloride and *O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→4)-α-D-glucopyranose hydrochloride. Thus, it was demonstrated that the repeating unit sequence of heparin very probably consists of alternating D-glucuronic acid and 2-amino-2-deoxy-D-glucose units linked glycosidically in an α-D-(1→4) manner.

The α-D stereochemical nature of the interglycosidic linkages was based originally upon indirect data, mainly polarimetric in nature. It was desirable to obtain more direct evidence on this point.

One of the disaccharides, *O*-α-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-α-D-glucopyranose hydrochloride, was synthesized in this laboratory[3] from maltose by reduction of its phenylosazone. This work confirmed the α-D-(1→4)-glycosidic linkage of D-glucuronic acid to 2-amino-2-deoxy-D-glucose in heparin.

In the present publication we wish to report degradative and synthetic evidence which establishes the α-D stereochemical nature of the glycosidic linkage of 2-amino-2-deoxy-D-glucopyranose to D-glucuronic acid. Supporting evidence is also presented for the (1→4) nature of all linkages present in the heparin polymer.

Treatment of partially acetylated, partially desulfated, carboxyl-reduced heparin[4] with methanolic hydrogen chloride gave, after dialysis and freeze drying, a completely desulfated product. *O*-Deacetylation in an ethylene glycol–methanol solution of ammonia yielded a completely desulfated, *O*-deacetylated,

(1) Preliminary communications: (a) M. L. Wolfrom, J. R. Vercellotti, H. Tomomatsu, and D. Horton, *Biochem. Biophys. Res. Commun.*, **12**, 8 (1963); (b) W. A. Szarek, M. L. Wolfrom, and H. Tomomatsu, *Chem. Commun.*, 326 (1965).

(2) M. L. Wolfrom, J. R. Vercellotti, and D. Horton, *J. Org. Chem.*, **27**, 705 (1962); **28**, 278, 279 (1963); **29**, 540 (1964).

(3) M. L. Wolfrom, H. El Khadem, and J. R. Vercellotti, *Chem. Ind.* (London), 545 (1964); *J. Org. Chem.*, **29**, 3284 (1964).

(4) M. L. Wolfrom, J. R. Vercellotti, and G. H. S. Thomas, *ibid.*, **26**, 2160 (1961); **29**, 536 (1964).