The sirup from zone R_f 0.76 was chromatographically homogeneous on Whatman No. 1 paper in solvent A (R_f 0.94) and solvent B (R_f 0.84) with mobility corresponding to the R_f values of 2,3,6-tri-*O*-methyl-D-glucose in each solvent. The sirup was taken up in a small amount of ethanol, and ether and petroleum ether added until incipient turbidity. After the sirup was cooled overnight, crystals (small needles) formed which were isolated; yield 3 mg. (7.5% of sirup applied to plates; 37% of sirup isolated); m.p. 120–124° (lit.[57] m.p. 118–119°); X-ray powder diffraction data, identical with that of an authentic sample of 2,3,6-tri-*O*-methyl-α-D-glucose[45]: 11.65 (s) (2), 10.10 (s) (2,2), 7.61 (m), 5.76 (m), 5.09 (w), 4.61 (m) (3), 4.25 (vs) (1), 4.05 (m), 3.86 (m), 3.67 (w), 3.41 (w), 3.26 (w), 2.97 (w), 2.81 (w), 2.73 (w), 2.47 (w), 2.38 (w), 2.33 (w), 2.24 (w).

The sirup from the zone of R_f 0.61 was chromatographically homogeneous on Whatman No. 1 paper in solvent A (R_f 0.707) and solvent B (R_f 0.67) with mobility corresponding to the R_f values of 2-amino-2-deoxy-3,4,6-tri-*O*-methyl-D-glucose hydrochloride in each solvent. The sirup was taken up in a small amount of ethanol, and ether and petroleum ether were added until incipient turbidity. After the solution was cooled overnight, small crystalline granules formed which were isolated; yield 2 mg. (5% of sirup applied to plates; 28% of sirup isolated); m.p. 211–214° dec.[58]; X-ray powder diffraction data identical with that of authentic sample of 2-amino-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucose hydrochloride[45]: 9.88 (s) (3), 7.04 (m), 6.11 (s) (2), 5.20 (w), 4.45 (w), 4.14 (w), 3.85 (m), 3.63 (w), 3.38 (vs) (1), 2.96 (w), 2.63 (m).

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(57) W. Charlton, W. N. Haworth, and S. Peat, *J. Chem. Soc.*, 89 (1926).

(58) The literature gives 210° for 2-amino-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucose hydrochloride, W. O. Cutler, W. N. Haworth, and S. Peat, *ibid.*, 1979 (1937). The authentic sample available melted at 208–211°. From the reference cited and R. W. Jeanloz, *Advan. Carbohydrate Chem.*, **13**, 196 (1958), the β-D form should predominate under these crystallizing conditions.

Methylation Studies on Carboxyl-Reduced Heparin. 2-Amino-2-deoxy-3,6-di-*O*-methyl-α-D-glucopyranose from the Methylation of Chitosan[1]

M. L. WOLFROM, J. R. VERCELLOTTI, AND D. HORTON

Department of Chemistry, The Ohio State University, Columbus 10, Ohio

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Permethylation and acid hydrolysis of partially acetylated, completely desulfated, carboxyl-reduced heparin gave, by thin layer chromatographic resolution, crystalline 2,3,6-tri-*O*-methyl-β-D-glucose and 2-amino-2-deoxy-3,6-di-*O*-methyl-D-glucose (isolated as the crystalline α-D, *N*-acetyl derivative); this provides further evidence for regular (1 → 4) linkages in heparin. *O*-Methylation of chitosan to a high degree of substitution has been achieved, and acid hydrolysis of the product provides a convenient source of 2-amino-2-deoxy-3,6-di-*O*-methyl-D-glucose and its crystalline *N*-acetyl derivative, key reference compounds in the structural work on heparin.

Structural studies on heparin by the methylation procedure are beset with difficulties arising from limited solubility, and hindrance to substitution.[2] In the present study, these difficulties were avoided by performing the methylation procedure on a heparin modification which was completely desulfated, carboxyl-reduced, and partially acetylated.[3] It was possible, with this material, to achieve almost complete *O*-methylation, and hydrolysis of the product provided crystalline, partially methylated monosaccharide fragments, whose structures provided further definitive evidence for the presence of (1 → 4) linkages in the heparin macromolecule.

The completely desulfated, carboxyl-reduced, partially acetylated heparin,[3] which was soluble in *N*,*N*-dimethylformamide, was twice methylated in this medium with dimethyl sulfate in the presence of barium oxide–barium hydroxide, by the procedure of Kuhn and Trischmann,[4] to give a nondialyzable product which contained 78% of the theoretical methoxyl content. Dialysis stages were employed during each methylation to remove low-molecular-weight degradation products; it was considered important to retain only nondialyzable, methylated high polymer, at the expense of yield, in the procedure. Further methylation, in *N*,*N*-dimethylformamide, with methyl iodide in the presence of barium oxide–barium hydroxide,[4] gave a product with methoxyl content 97% of the theoretical value for complete *O*-methylation when determined after *N*-acetylation.[5] Acid hydrolysis of the methylated polymer was initiated in 90% aqueous formic acid, in which the material was soluble, and the partially hydrolyzed, water-soluble product was then hydrolyzed completely in 1 *N* hydrochloric acid. The hydrolysate was N-acetylated,[5] and was then found by thin layer chromatography to consist of two principal components, R_f 0.41 and 0.59 in the system employed. Isolative thin layer chromatography gave the material in the two zones as chromatographically homogeneous sirups in good yield. The sirup from the slower moving zone crystallized, to give 2-acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucose, identical by X-ray powder diffraction pattern, melting point, and thin layer chromatographic techniques with an authentic sample.[6,7] The sirup from the faster moving zone crystallized from the anomeric mixture to give 2,3,6-tri-*O*-methyl-β-D-glucose, identical by X-ray powder diffraction pattern, melting

(1) Preliminary communication: M. L. Wolfrom, J. R. Vercellotti, and D. Horton, *J. Org. Chem.*, **28**, 278 (1963).

(2) A. B. Foster and A. J. Huggard, *Advan. Carbohydrate Chem.*, **10**, 356 (1955).

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

(4) R. Kuhn and H. Trischmann, *Ber.*, **96**, 284 (1963).

(5) S. Roseman and J. Ludowieg, *J. Am. Chem. Soc.*, **76**, 301 (1954).

(6) R. W. Jeanloz, *J. Org. Chem.*, **26**, 905 (1961).

(7) R. Kuhn and A. Gauhe, *Ber.*, **95**, 518 (1962).

point, and thin layer chromatographic techniques with an authentic sample.[8]

The isolation and definitive characterization of the prior two hydrolytic components from the permethylated, carboxyl-reduced heparin derivative provides further firm evidence for the presence of (1 → 4) linkages in heparin and supports the evidence in the preceding paper[9] based on isolation of disaccharide fragments. Rotatory data[9] would strongly indicate that all these linkages are of the α-D form.

A methylation linkage analysis study on a partially carboxyl-reduced, desulfated, *N*-acetylated heparin preparation has been reported very briefly, in a paper[10a] describing the observation of a paper chromatographic zone with the anticipated properties of 2-amino-2-deoxy-3,6-di-*O*-methyl-D-glucose and a component considered to be 2,3,6-tri-*O*-methyl-D-glucose. No yields or physical constants were given, but the data as presented are in agreement with our results.[10b] Methylation studies on unmodified heparin have been described[11,12]; homogeneous methylation of the Hyamine[13] quaternary ammonium[11] salt of heparin, with methyl iodide and silver oxide, gave a product containing one methyl ester and one methyl ether group per disaccharide unit. Isolation of 2-amino-2-deoxy-3-*O*-methyl-D-glucose following hydrolysis of this product furnished definitive evidence that the 3-position of the amino sugar unit is not a linkage point in the heparin molecule. This is in agreement with our present findings.

The 3,6-dimethyl ether of 2-amino-2-deoxy-D-glucose was required as a reference compound in connection with the degradative studies[9] on carboxyl-reduced heparin. Direct synthesis[6] of this derivative from 2-amino-2-deoxy-D-glucose involves many stages, and a shorter route was sought. Permethylation followed by hydrolysis of chitin, a readily available linear β-D-(1 → 4)-linked 2-acetamido-2-deoxy-D-glucan, presented, in theory, a simple direct preparative route for the dimethyl ether. However, reports in the literature on the attempted methylation of chitin were discouraging; for example, Schorigin and Makarowa-Semljanskaya[14] stated that, after forty-five successive methylations of chitin with dimethyl sulfate–sodium hydroxide (Haworth procedure), the product still contained less than one methoxyl group per monosaccharide unit. The great resistance of chitin toward solubilizing reagents and chemical attack[15] led us to examine the feasibility of methylating *N*-deacetylated chitin (chitosan), which can be dispersed in dilute aqueous acids.

A dilute hydrochloric acid solution of a commercial chitosan preparation,[16] which was probably approximately 70% *N*-deacetylated,[15] was treated with an excess of sodium hydroxide to precipitate the polysaccharide in a highly hydrated form suitable for chemical attack. Methylation of this material with dimethyl sulfate, according to the usual Haworth procedure, gave a product with 29% of the methoxyl content calculated for complete methylation. A limited degree of *N*-methylation[17,18] of exposed free amino groups may have occurred during this stage. The dialyzed product was then amenable to acetylation in formamide–acetic anhydride[19] to a product soluble in organic media. Methylation in *N*,*N*-dimethylformamide solution with methyl iodide in the presence of barium oxide–barium hydroxide[7] raised the methoxyl content to 66%. Repetition of the acetylation and methylation stages twice more, followed by reacetylation, gave methylated chitin with 92% of the theoretical maximum methoxyl content. Dialysis operations were performed at each stage in the methylation procedure, to ensure that low-molecular-weight degradation products were not present in the product. Degradation was particularly apparent during the methylations in *N*,*N*-dimethylformamide solution. Preparation of 92% methylated chitin represents a twofold increase in the highest degree of methylation previously reported[14] for this polysaccharide.

Hydrolysis of the 92% methylated chitin required vigorous conditions, which undoubtedly caused some *O*-demethylation and destruction of the sugars; some 2-amino-2-deoxy-D-glucose hydrochloride was isolated from the hydrolysate. The remainder of the hydrolyzate was *N*-acetylated[6] and resolved by preparative thin-layer chromatography into one minor and three major zones. From one zone crystalline 2-acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucose was obtained in 14% yield. One of the other major zones corresponded to 2-acetamido-2-deoxy-D-glucose; the third major zone exhibited the mobility anticipated for a monomethyl derivative.

In addition to furnishing the required reference derivative, this work demonstrates, for the first time by the methylation procedure, the presence of the (1 → 4) linkage in chitin.

Experimental[20]

Completely Desulfated, Partially Acetylated, Carboxyl-Reduced Heparin.—Partially desulfated, partially acetylated, carboxyl-reduced heparin was prepared according to the method of Wolfrom and co-workers.[3]

Anal. Found: S, 0.9.

This modified heparin (4.1 g.) was dissolved in 0.12 *N* methanolic hydrogen chloride (250 ml.) and shaken for 2 days.[21] The solution was dialyzed against running distilled water for 2 days, and the concentrated dialysate was freeze-dried; yield 3.2 g.

Anal. Found: S, trace.

Permethylated, *N*-Acetylated, Completely Desulfated, Carboxyl-Reduced Heparin.—Completely desulfated, partially acet-

(8) W. Charlton, W. N. Haworth, and S. Peat, *J. Chem. Soc.*, 69 (1926).

(9) M. L. Wolfrom, J. R. Vercellotti, and D. Horton, *J. Org. Chem.*, **29**, 540 (1964).

(10) (a) I. Danishefsky, H. B. Eiber, and A. Williams, *Federation Proc.*, **22**, 539(1963). (b) NOTE ADDED IN PROOF.—A subsequent full paper [I. Danishefsky, H. B. Eiber, and A. Williams, *J. Biol. Chem.*, **238**, 2895 (1963)], which appeared after our paper was submitted, gives yield data for sirupy 2-acetamido-2-deoxy-3,6-di-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-glucose; the latter was identified as the crystalline 1,4-di-*p*-nitrobenzoate.

(11) L. Velluz, G. Nominé, and D. Bertin, *Compt. rend.*, **248**, 2354 (1959); L. Velluz, G. Nominé, and J. Mathieu, *Bull. Soc. Chim. Biol.*, **41**, 415 (1959); G. Nominé, R. Bucourt, and D. Bertin, *Bull. Soc. Chim. France*, 561 (1961).

(12) K. Onodera and S. Hirano, *Agr. Biol. Chem.* (Tokyo), **27**, 143 (1963).

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

(14) P. Schorigin and N. N. Makarowa-Semljanskaya, *Ber.*, **68**, 969 (1935).

(15) A. B. Foster and J. M. Webber, *Advan. Carbohydrate Chem.*, **15**, 389 (1960).

(16) A product of the Kelco Co., Chicago, Ill.

(17) S. A. Barker, M. Stacey, and D. J. Tipper, *Nature* (London), **184**, 1718 (1959).

(18) N. J. Wojciechowski, R. Daniels, and B. Ecanow, *J. Pharm. Sci.*, **50**, 888 (1961).

(19) M. L. Wolfrom and J. W. Spoors, *J. Org. Chem.*, **25**, 308 (1960).

ylated, carboxyl-reduced heparin (3.2 g.) was dissolved in *N,N*-dimethylformamide (75 ml.). Following the procedure of Kuhn and Trischmann,[4] barium oxide (10 g.) and barium hydroxide octahydrate (10 g.) were placed in the stirred mixture maintained at 0°. Dimethyl sulfate (20 ml.) was added, and the mixture was stirred under nitrogen and allowed to warm to room temperature. After 18 hr., the reaction mixture was again cooled to 0°, the addition of methylating reagents repeated, and the reaction mixture left for 12 hr. under nitrogen with stirring. The reaction mixture was diluted with chloroform (500 ml.), cooled to 0°, and the barium salts filtered. The chloroform was evaporated, and the residual solution dialyzed against running, distilled water for 2 days. During dialysis, insoluble inorganic salts formed which were filtered from the dialysate. The filtrate was evaporated to a sirup from which inorganic crystals separated. The dried residue was extracted with tetrahydrofuran, the insoluble inorganic material filtered, and the tetrahydrofuran evaporated to give a sirup. The above methylation procedure was repeated; yield 210 mg. (7%).

Anal. Calcd. for $C_{12}H_{15}O_4(NHCOCH_3)(OCH_3)_6$: OCH_3, 35.64. Found: OCH_3, 27.81 (78% of theory for the fully methylated polymer).

The partially methylated sirup (181 mg.) was dissolved in *N,N*-dimethylformamide (10 ml.), to which was added barium oxide (2 g.) and barium hydroxide octahydrate (1 g.). After cooling to 0°, methyl iodide (5 ml.) was added to the reaction mixture, and the slurry was stirred under nitrogen for 48 hr. at room temperature.[4] The reaction mixture was diluted with chloroform (200 ml.), the diluted solution cooled to 0°, and the salts removed by centrifugation. The supernatant was passed through a glass cloth filter pad, and the precipitate of salt washed several times with chloroform. The combined chloroform filtrates were twice extracted with water (50-ml. portions), aqueous 0.1 *N* sodium thiosulfate (50 ml.), and again with water. The aqueous washings were extracted once with chloroform and the combined extracts dried over anhydrous sodium sulfate for 2 hr. The chloroform was evaporated, and the resulting sirup codistilled several times with 1-propanol to remove the last traces of *N,N*-dimethylformamide. The dried sirup was extracted with chloroform, and the insoluble salts filtered from the solution through a glass cloth filter pad with Celite.[22] The sirup was dried under reduced pressure at 100° and was then *N*-acetylated by the technique of Roseman and Ludowieg.[5] The water-insoluble sirup was solubilized with a 2:1 water–tetrahydrofuran mixture (15 ml.). Methanol (2 ml.) and Dowex-1 ion-exchange resin (CO_3^{-2} form, 10 ml.) were added; the mixture was cooled to 0°, and acetic anhydride (2 ml.) added to the stirred mixture. After 1 hr. at 0°, the resin was filtered and the solution evaporated to a sirup; yield 124 mg.; $[\alpha]^{27}D$ +66° (*c* 0.45, $CHCl_3$); λ_{max}^{KBr} (μ) 6.08, 6.53 (NHAc), OH absent.

Anal. Calcd. for $C_{12}H_{15}O_4(NHCOCH_3)(OCH_3)_6$: OCH_3, 35.64. Found: OCH_3, 34.59 (97.2% of theory for the fully methylated polymer).

Hydrolysis of Permethylated, Desulfated, Carboxyl-Reduced Heparin.—The methylated sirup (94 mg.) was heated for 6 hr. at 100° in 10 ml. of 90% formic acid, the solution was evaporated, 1 *N* hydrochloric acid (10 ml.) was added, and the solution was heated at 90° for 5 hr. Removal of the acid by codistillation with 1-propanol gave a sirup, which was *N*-acetylated by dissolving in methanol (2 ml.), adding water (10 ml.), Dowex-1 ion-exchange resin (CO_3^{-2} form) (5 ml.), and acetic anhydride (0.2 ml.). After stirring for 1 hr., the resin was filtered, the filtrate evaporated to a sirup, and the sirup codistilled several times with 1-propanol. The sirup crystallized on standing at room temperature; yield 50.2 mg. (54.6%).

Chromatography of the sirup on silica gel G[20] in benzene–methanol (7:3) revealed eight zones. The two principal zones had R_f 0.41 (2-acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucose) and R_f 0.59 (2,3,6-tri-*O*-methyl-β-D-glucose).

2-Acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucose.—Isolative thin layer chromatography was used to separate the components of the hydrolysate. The hydrolysate (50.2 mg.) was applied to two preparative thin layer plates[20] and developed with benzene–methanol (7:3). Zones were located on the edges of the plate with alkaline permanganate spray reagent. The zone with R_f 0.41 was excised and eluted with methanol. Removal of the solvent gave a chromatographically homogeneous, sirupy mixture of anomers; yield 15.8 mg. (31.5%). This product crystallized from ethanol–ether–petroleum ether to give 2-acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucose; yield 11 mg. (70% of the sirup), m.p. 212–216° dec.

For this compound Jeanloz[6] reports m.p. 232–233°. An authentic sample kindly furnished by Professor R. Kuhn melted at 221–224°.

The compound gave 65.6% of the color observed with an equivalent amount of 2-acetamido-2-deoxy-D-glucose in the Morgan–Elson determination.[23]

The X-ray powder diffraction pattern of this compound[1,9] was identical with that of the authentic 2-acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucose obtained from Professor Kuhn.

2,3,6-Tri-*O*-methyl-β-D-glucose.—On thin layer chromatography of the hydrolysate described above, the zones from R_f 0.59 to the solvent front separated poorly. The material in these zones was eluted from the silica gel with methanol, the solution evaporated to a sirup (12 mg.) and the sirup rechromatographed using the previously described conditions.[20] Elution of the zone of R_f 0.59 gave a chromatographically homogeneous, sirupy, anomeric mixture; yield 7.5 mg. (62.5% of sirup chromatographed). This mixture crystallized from ethanol–ether–petroleum ether; yield 2.5 mg. (33%), m.p. 112–115°. Charlton, Haworth, and Peat[8] reported m.p. 117–118°.

The sample was identical by X-ray powder diffraction pattern with an authentic sample of 2,3,6-tri-*O*-methyl-β-D-glucose.[1,9]

Methylation of Chitosan.—Chitosan[16] (*N*-deacetylated chitin, 38 g.) was dissolved with stirring in 1 *N* hydrochloric acid (1 l.). Sodium hydroxide pellets (500 g.) were dropped into the solution with vigorous mechanical agitation to form a thick pasty suspension, to which water (500 ml.) was then added. Dimethyl sulfate (200 ml., Sp. Gr. 1.33) was introduced during 1 hr. with external cooling, and the mixture was left to stir for 8 hr., whereupon sodium hydroxide (40 g.) and dimethyl sulfate (40 ml.) were carefully added. Additions of like proportions of sodium hydroxide and dimethyl sulfate were repeated twice more at 8-hr. intervals, and the mixture was stirred for 48 hr. after the final addition. The mixture was neutralized with concentrated hydrochloric acid, dialyzed against running water for 4 days, concentrated to a small volume, and freeze-dried; yield 36 g., positive ninhydrin reaction.

Anal. Calcd. $C_6H_{10}O_2ClN(OCH_3)_2$: OCH_3, 28.8. Found: OCH_3, 8.25 (29% of theoretical OCH_3 for fully methylated chitosan).

A suspension of this material (10 g.) in formamide (400 ml.) was acetylated by shaking 1 day with acetic anhydride (50 ml.) and pyridine (25 ml.) with addition of further acetic anhydride (25 ml.) and pyridine (12 ml.) after 24 hr. After shaking for another 24 hr., the practically homogeneous reaction mixture was dialyzed against running water for 3 days. The solution was concentrated and the partially methylated and acetylated chitin was freeze-dried. The product was ninhydrin negative.

The partially acetylated, partially methylated chitin (3 g.) was further methylated in *N,N*-dimethylformamide solution (200 ml.) by the addition of barium oxide (18 g.), barium hydroxide (1.8 g.), and methyl iodide (21 ml.). The reaction temperature

(20) Paper chromatography was carried out by the descending technique with the upper layer of a 4:1:5 1-butanol–ethanol–water system (solvent A), 5:5:3:1 pyridine–ethyl acetate–water–acetic acid [solvent B according to F. G. Fischer and H. J. Nebel, *Z. Physiol. Chem.*, **302**, 10 (1955)], and 9:2:2 ethyl acetate–acetic acid–water (solvent C). Zones were located by the silver nitrate–sodium hydroxide procedure [W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature* (London), **166**, 444 (1950)] as well as by ninhydrin (0.2% in ethanol). R_g refers to mobility relative to that of glucose. Thin layer chromatography was performed by the ascending technique, with benzene–methanol developing mixtures, on silica gel G (E. Merck, Darmstadt, Germany) activated at 100°. Zones were located by spraying the plates with 0.1% potassium permanganate in 10% sodium hydroxide or by heating the plates at 100° after spraying with concentrated sulfuric acid. For isolative purposes, 19.5 × 19.5 cm. plates were used with 0.5-mm. thickness of silica gel G activated for 12 hr. at 110°. Melting points were determined on a Hershberg-type apparatus (A. Thompson and M. L. Wolfrom, in "Methods in Carbohydrate Chemistry," Vol. I, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press Inc., New York, N. Y., 1961, p. 517). Infrared spectra were measured on a Perkin-Elmer Infracord spectrometer. The finely powdered sample was pressed into a pellet with dried, reagent grade potassium bromide. Methoxyl values were determined by the method of D. O. Hoffman and M. L. Wolfrom, *Anal. Chem.*, **19**, 225 (1947).

(21) M. L. Wolfrom, J. R. Vercellotti, H. Tomomatsu, and D. Horton, *Biochem. Biophys. Res. Commun.*, **12**, 8 (1963).

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

(23) W. T. J. Morgan and L. A. Elson, *Biochem. J.*, **28**, 988 (1934); D. Aminoff, W. T. J. Morgan, and W. M. Watkins, *ibid.*, **51**, 379 (1952).

was controlled by external cooling. After the initial exothermic reaction (1 hr.), the mixture was left shaking for another 24 hr. The homogeneous solution was cooled, methanol added (200 ml.), and the barium salts filtered. The filtrate was dialyzed for 3 days, the solid materials collected by concentrating the dialysate, and the product freeze-dried; yield, 3.2 g.

Anal. Calcd. for $C_6H_7O_2(NHCOCH_3)(OCH_3)_2$: OCH_3, 26.8. Found[20]: OCH_3, 17.2 (66% of theory for fully methylated chitin).

The acetylation and methylation procedures were repeated twice more, followed by reacetylation.

Anal. Calcd. for $C_6H_7O_2(NHCOCH_3)(OCH_3)_2$: OCH_3, 26.8. Found[20]: OCH_3, 24.17 (92% of theory for fully methylated chitin).

Hydrolysis of Permethylated Chitin and Isolation of 2-Acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucopyranose.—The above-methylated product (1.5 g.) was hydrolyzed in 6 *N* hydrochloric acid (200 ml.) at 100° for 12 hr. After removal of acid by concentration and codistillation with 1-propanol, the resulting sirup was dissolved in methanol, treated with carbon, the carbon removed by filtering through a sintered glass Büchner funnel covered with a 5-mm. bed of Celite,[22] and the filtrate concentrated to a yellow sirup. The sirup was dissolved in water (5 ml.) and the solution placed on a column (20 × 200 mm.) of Amberlite IR-120 ion-exchange resin (H^+ form). After washing the column to neutrality, the amino sugar derivative was eluted with 1 *N* hydrochloric acid (25 ml.) and sufficient water to give a neutral eluate. The combined eluate and washings were then concentrated to a small volume and the final traces of acid removed by codistillation with 1-propanol; yield 0.6 g. of sirup. Upon concentrating the sirup several times with ethanol, crystals formed, which were filtered and identified by X-ray powder diffraction pattern[24] as 2-amino-2-deoxy-α-D-glucose hydrochloride; yield 68 mg. (12.5%).

The remaining sirup (0.35 g.) was *N*-acetylated by the Roseman and Ludowieg method[6] described above in the preparation of 2-acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucopyranose from methylated, carboxyl-reduced heparin. The *N*-acetylated, ninhydrin-negative sirup (148 mg.) was chromatographed by the thin layer technique with 7:3 benzene–methanol.[20] Zones, located with alkaline potassium permanganate spray reagent,[20] corresponded to 2-acetamido-2-deoxyglucose (1.00), $R_{2\text{-acetamido-2-deoxyglucose}}$ 1.8 (strong intensity), 2.62 (medium intensity), and 3.10 (weak intensity). Isolative thin layer chromatography on 19.5 × 19.5 cm. plates with a 0.5-mm. thickness of Silica Gel G yielded on elution of the zone with $R_{2\text{-acetamido-2-deoxyglucose}}$ 2.62 from the silica gel with methanol and concentration, a sirup which crystallized from ethanol–ether–petroleum ether in long needles; yield 21 mg. (14.2% of the sirup chromatographed), m.p. 229–232°, $[\alpha]^{20}D$ +38 ± 5° (final, *c* 0.18, water). For this compound Jeanloz reports[6] $[\alpha]^{25}D$ +90 (15 min.) → +35 ± 5° (24 hr., *c* 0.29, water). This compound was chromatographically homogeneous in solvents A, B, and C and possessed in these solvents the same chromatographic mobility as a sample of 2-acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucose obtained from Professor R. Kuhn. The preparation gave an X-ray powder diffraction pattern identical with that of the authentic compound obtained from Professor Kuhn as well as samples of the same compound isolated on hydrolysis of both a methylated disaccharide from carboxyl-reduced heparin[9] and of the permethylated, carboxyl-reduced heparin described above.

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(24) I. Werner, *Mikrochim. Acta*, **39**, 133 (1952).

Stereochemical Effects in the Nucleophilic Displacement Reactions of Primary Carbohydrate Benzenesulfonate Esters with Sodium Iodide[1]

JAMES M. SUGIHARA AND WILFORD J. TEERLINK

Department of Chemistry, University of Utah, Salt Lake City, Utah

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Reactivities of seven primary benzenesulfonate esters, 1,2,3,4-tetra-*O*-acetyl-6-*O*-(phenylsulfonyl)-β-D-glucopyranose (I), 1,2:3,4-di-*O*-isopropylidene-6-*O*-(phenylsulfonyl)-D-galactopyranose (II), 2,4:3,5-di-*O*-methylene-1-*O*-(phenylsulfonyl)-DL-ribitol (III), 2,4:3,5-di-*O*-methylene-1-*O*-(phenylsulfonyl)-DL-xylitol (IV), 2,4:3,5-di-*O*-benzylidene-1-*O*-(phenylsulfonyl)-L-xylitol (V), 2,4:3,5-di-*O*-methylene-1,6-di-*O*-(phenylsulfonyl)-D-mannitol (VI), and 2,3,4,5-di-*O*-benzylidene-1,6-di-*O*-(phenylsulfonyl)-D-mannitol (VII), toward nucleophilic displacement by sodium iodide in acetonylacetone were determined. Rate constants and activation parameters were established. The data demonstrated that the compounds may be placed into two classes on the basis of ease of reaction. An explanation is proposed based upon the differences in the extent of field interaction of the nucleophilic reagent with electronegative atoms in these as well as other substrates.

In reactions involving displacement of primary iodides with cyanide ion to effect synthesis of deoxynitrile derivatives of carbohydrates, differences in reactivity were observed for the several substrates applied.[2] A rationalization was proposed based upon the degree of interaction of the nucleophilic reagent with the electronegative atmosphere found on the backside of the carbon atom undergoing displacement. An inspection of a summary[3] of data, describing the general reaction applied in carbohydrate chemistry of displacing primary sulfonate esters with iodide ion, suggested the possibility of making the same interpretation for this reaction. Accordingly, a study was made to confirm this observation.

Seven substrates were selected for the kinetic studies described herein. All compounds contained primary benzenesulfonate ester groups so that the departing group could be the same in the displacement reactions studied. 1,2,3,4-Tetra-*O*-acetyl-6-*O*-(phenylsulfonyl)-β-D-glucopyranose (I),[4] 1,2:3,4-di-*O*-isopropylidene-6-*O*-(phenylsulfonyl)-D-galactopyranose (II),[5] 2,4:3,5-di-*O*-methylene-1-*O*-(phenylsulfonyl)-DL-ribitol (III),[6] and 2,4:3,5-di-*O*-methylene-1-*O*-(phenylsulfonyl)-DL-xylitol (IV)[7] were prepared by methods

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