the product was as just described, and a pale yellow sirup resulted in 92% yield. The specific rotation in chloroform was −43.6°.

Anal. Calcd. for a monotosyl triacetyl derivative, $C_{28}H_{33}O_{12}S$: S, 5.4; Cl, 0.0; CH_3CO, 21.7; OCH_2CH_2, 7.4. Found: S, 4.9, 5.0, 5.1; Cl, 1.53, 1.58, 1.54; CH_3CO, 21.8, 22.1; OCH_2CH_2, 7.7, 7.9. A preparation made at 21° instead of 0° had S, 4.7, 4.8; Cl, 2.09, 2.30.

Benzyl 2,4,6-Tri-*O*-acetyl-3-*O*-iodoethyl-β-D-glucoside (IId).—A 1-g. sample of the tosyloxyethyl triacetate and 1 g. of sodium iodide were heated in 20 cc. of dry acetonylacetone at 110–115° for 2 hr. The solution was poured into 300 ml. of water and the mixture was extracted with ether; the extract was washed with dilute aqueous sodium thiosulfate to remove free iodine, was dried and evaporated. The residual amber sirup (yield 90%) crystallized, and after three recrystallizations from ethanol the white needles melted sharply at 114–115° and had a specific rotation in chloroform of −26.8°.

Anal. Calcd. for a benzyl iodoethyl glucose triacetate, $C_{21}H_{27}O_9I$: I, 23.1. Found: I, 22.7, 22.8.

When the tosyloxy derivative was iodinated for five hours in boiling acetone (56°), the uncrystallized product had only 10.9% of iodine.

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MONTREAL, CANADA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Derivatives of D-Glucose Containing the Sulfoamino Group[1]

BY M. L. WOLFROM, R. A. GIBBONS[2] AND A. J. HUGGARD[3]

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Methyl 2-deoxy-2-sulfoamino-α-D-glucopyranoside (sodium salt monohydrate, XIII) was prepared from methyl 2-amino-*N*-(benzoxycarbonyl)-2-deoxy-α-D-glucopyranoside (I) by acetylation followed by hydrogenolysis of the *N*-blocking group, sulfation (without deacetylation) and final deacetylation. Analogous reactions were carried out with the corresponding trimethyl ether of I. Sulfation and subsequent deacetylation of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose (III) yielded 2-sulfoamino-2-deoxy-D-glucose (IX).

The sulfoamino group is present in the 2-amino-2-deoxy-D-glucose (D-glucosamine, chitosamine) unit of heparin.[4] The only derivatives of this sulfoamino sugar hitherto described are the amorphous 2-deoxy-2-sulfoamino-D-glucose (ammonium salt)[5] and the amorphous barium salt of methyl 2-deoxy-2-sulfoamino-β-D-glucopyranoside trisulfate dihydrate.[6] The first compound was obtained by the direct sulfation of 2-amino-2-deoxy-D-glucose base and was believed to be a mixture of sulfate and sulfoamino derivatives having some 80% of the sulfur function on the nitrogen atom. We describe herein the synthesis of the crystalline sodium salt (monohydrate) of methyl 2-deoxy-2-sulfoamino-α-D-glucopyranoside (XIII), its triacetate XI, and the crystalline sodium salt of tetra-*O*-acetyl-2-deoxy-2-sulfoamino-β-D-glucose (VI). Our preparation of 2-deoxy-2-sulfoamino-D-glucose (IX) was an amorphous anomeric mixture but was analytically pure and was obtained through crystalline intermediates. Our methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-sulfoamino-α-D-glucopyranoside (XII), although obtained through crystalline intermediates, was amorphous and contained extraneous ash (not sulfate).

Methyl 2-amino-*N*-(benzoxycarbonyl)-2-deoxy-α-D-glucopyranoside (I), prepared by the glycosidation of 2-amino-*N*-(benzoxycarbonyl)-2-deoxy-D-glucose according to Neuberger and Rivers,[7] was acetylated, the amino blocking group was removed by catalytic hydrogenation and the resultant amine was isolated as the hydrochloride V. When the reaction contained the equivalent amount of hydrogen chloride to exactly neutralize the amine, the product V was obtained in good yield without deacetylation. In one experiment wherein slightly more than one equivalent of acid was present, a crystalline monoacetyl derivative of methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride was obtained. The amino-blocked glycoside I was also methylated by treatment with methyl iodide and thallous hydroxide followed by reaction with methyl iodide and silver oxide. The crystalline trimethyl ether IV then was converted to the hydrochloride VII by catalytic hydrogenation. Utilizing a different synthetic route, Cutler, Haworth and Peat[8] have recorded the same substance (VII).

For the purposes of *N*-sulfation, a study was first made of the conditions required to sulfate the 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose (III) of Bergmann and Zervas.[9] This free base was sulfated with sulfur trioxide in pyridine. The product VI was isolated as the crystalline sodium salt which contained acid-hydrolyzable sulfate and exhibited negative tests for the amino

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(2) Postdoctoral Fellow of the Foreign Research Scientists Program of the Foreign Operations Administration (Project TAOI-101-3006 EPA 151).

(3) Recipient of a Postdoctoral Fellowship from The Ohio State University Advisory Committee on Research Grants (Project 557).

(4) J. E. Jorpes, H. Boström and V. Mutt, *J. Biol. Chem.*, **183**, 607 (1950); A. B. Foster, E. F. Martlew and M. Stacey, *Abstracts Papers Am. Chem. Soc.*, **126**, 6D (1954); A. B. Foster and A. J. Huggard, *Advances in Carbohydrate Chem.*, **10**, 352 (1955).

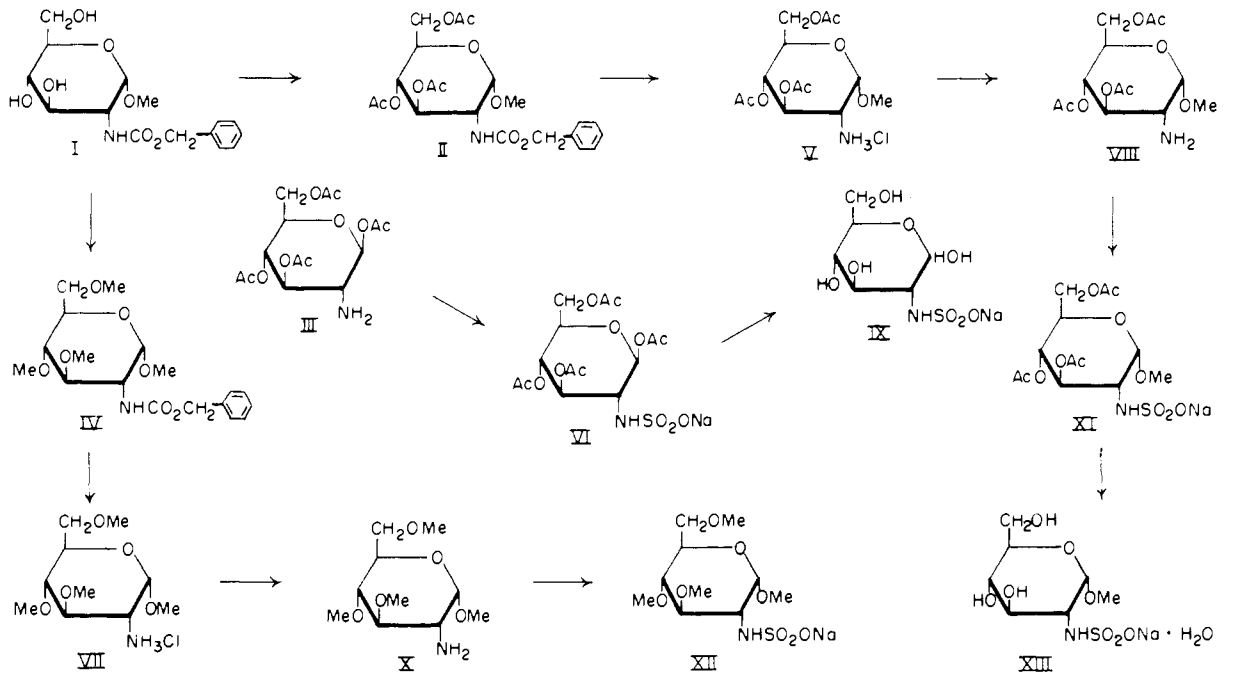
(5) K. H. Meyer and D. E. Schwartz, *Helv. Chim. Acta*, **33**, 1651 (1950).

(6) M. L. Wolfrom, T. M. Shen and C. G. Summers, THIS JOURNAL, **75**, 1519 (1953); T. M. Shen Han, Ph.D. Dissertation, The Ohio State University, 1954.

(7) A. Neuberger and Rosalind Pitt Rivers, *J. Chem. Soc.*, 122 (1939).

(8) W. O. Cutler, W. N. Haworth and S. Peat, *ibid.*, 1979 (1937).

(9) M. Bergmann and L. Zervas, *Ber.*, **64**, 975 (1931).



group (by ninhydrin) and the acetamido (by the Morgan–Elson assay[10]) function. No significant O → N acetyl migration had therefore occurred and the isolated material was *N*-sulfated and was not deacetylated. Removal of the acetate groups from VI by alkaline ester exchange yielded 2-deoxy-2-sulfoamino-D-glucose (IX) as an amorphous but analytically pure substance. The hydrochloride salts of the acetylated (V) and methylated (VII) derivatives of the methyl glycoside were converted to the free bases (VIII and X) with an ion exchange resin. The bases were not herein characterized but were sulfated in the same manner to yield the sodium salts XI and XII. Methyl 2-deoxy-tri-*O*-methyl-2-sulfoamino-α-D-glucopyranoside (sodium salt, XII) was not isolated in pure form but the corresponding triacetate derivative XI was and this on deacetylation yielded the crystalline methyl 2-deoxy-2-sulfoamino-α-D-glucopyranoside (sodium salt monohydrate, XIII).

The stability toward acidity and alkalinity of the sulfoamino group in these derivatives of 2-amino-2-deoxy-D-glucose will be the subject of a future communication.

Experimental

Methyl 3,4,6-Tri-*O*-acetyl-2-amino-*N*-(benzoxycarbonyl)-2-deoxy-α-D-glucoside (II).—An amount of 4 g. of methyl 2-amino-*N*-(benzoxycarbonyl)-2-deoxy-α-D-glucoside (I),[7] $[\alpha]^{24}D$ +94° (*c* 2, pyridine), m.p. 156°, was dissolved in 20 ml. of dry pyridine and 16 ml. of acetic anhydride was added. The mixture was left at room temperature for 14 hr. and then poured onto 300 g. of crushed ice. The resultant precipitate was washed with water and was crystallized from ethanol; yield 4.1 g. (74%), m.p. 105–107°, $[\alpha]^{25}D$ +95° (*c* 2.5, pyridine).

Anal. Calcd. for $C_{15}H_{18}O_7N(CH_3CO)_3$: C, 55.6; H, 6.0; N, 3.1; CH_3CO, 28.45. Found: C, 55.2; H, 6.1; N, 3.1; CH_3CO, 28.4.

Methyl 2-Amino-*N*-(benzoxycarbonyl)-2-deoxy-3,4,6-tri-*O*-methyl-α-D-glucoside (IV).—A solution of thallous acetate (100 g.) in 300 ml. of water was passed down a column (40 × 3 cm., diam.) of Amberlite IR-400[11] (OH^- form). The basic eluate was concentrated under reduced pressure to 350 ml. and 12 g. of compound I (see above), dissolved in the minimum amount of hot water, was added to it. The solution was well mixed and freeze-dried. To the dry solids was added 250 ml. of methyl iodide, and the mixture was shaken at 0° for 72 hr. At the end of this time, the methyl iodide was removed under reduced pressure and the solid residue was extracted with five 40-ml. portions of chloroform. Solvent removal, under reduced pressure, from the dried extract yielded a pale yellow sirup which formed waxy crystals from aqueous methanol on standing for 24 hr. at −5°; yield 2.8 g. A considerable quantity of oil which resisted crystallization could be obtained from the mother liquor by dilution with water. The oil was dissolved in methyl iodide (150 ml.), 10 g. of freshly prepared silver oxide was added and the whole was refluxed for 24 hr. The reaction mixture was processed as described above and the crystalline product was combined with the first crop of crystals and recrystallized twice from light petroleum (b.p. 60–110°); yield 10.9 g. (80%) of waxy crystals, m.p. 119–121°, $[\alpha]^{25}D$ +98.5° (*c* 2.5, $CHCl_3$).

Anal. Calcd. for $C_{14}H_{15}O_3N(OCH_3)_4$: C, 58.5; H, 7.4; N, 3.8; OCH_3, 33.6. Found: C, 58.7; H, 7.4; N, 3.9; OCH_3, 34.3.

Methyl 3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucoside Hydrochloride (V).—An amount of 5 g. of compound II above was dissolved at 0° in 75 ml. of dry methanol containing exactly 0.01104 mole of hydrogen chloride. One gram of a palladium-on-charcoal catalyst (10%)[12] was added (*CAUTION*). A rapid stream of hydrogen was then passed into the methanolic solution through a sintered glass diffuser. The use of a small vessel (100 ml.) and a large (1 × 2 cm.) gas diffuser appears to be essential if the reaction is to be completed in a reasonable time and without loss of acetyl groups. The emergent gases were passed through lime water periodically and when carbon dioxide was no longer detectable, the reaction was complete (165 min.). After filtration, the neutral methanolic solution was concentrated under reduced pressure to 2–3 ml. from which crystals separated on cooling to −5°. Recrystallization was effected from ethanol(warm)–ether by maintaining at −5° overnight; yield 2.5 g. (65%), long, well formed needles, m.p. 230–238° dec., $[\alpha]^{26}D$ +154° (*c* 1.8, water).

(10) W. T. J. Morgan and L. A. Elson, *Biochem. J.* **28**, 988 (1934).

(11) A product of the Rohm and Haas Co., Resinous Products Division, Philadelphia 5, Pa.

(12) A product of the Matheson, Coleman and Bell Division of the Matheson Chemical Co., Inc., Norwood, O.

Anal. Calcd. for $C_6H_{10}O_4ClN(OCH_3)(CH_3CO)_3$: C, 43.9; H, 6.21; N, 3.94; CH_3CO, 36.2; OCH_3, 8.7. Found: C, 43.9; H, 6.21; N, 4.01; CH_3CO, 35.8; OCH_3, 8.7.

Methyl 2-Amino-2-deoxy-3,4,6-tri-*O*-methyl-α-D-glucoside Hydrochloride (VII).—With minor differences, the reductive cleavage of the benzoxycarbonyl group in IV was accomplished in the same manner as that in II. Thus, 3.69 g. of IV, in 55 ml. of methanol, was reduced at room temperature in 100 min. with 0.75 g. of 10% palladium-on-charcoal catalyst[12] in the presence of 0.01 equivalent of hydrogen chloride. The methanolic filtrate and washings were taken to dryness under reduced pressure and crystallized from ethyl acetate containing 5% chloroform; yield 0.7 g. A further crop (1.4 g.) was obtained by the addition of 10 vol. of ether to the mother liquor; total yield 2.1 g. (77%), small prisms, m.p. 225–238° dec., $[\alpha]^{27}D$ +147° (*c* 2.6, water). Cutler, Haworth and Peat[8] cite dec. 237° with softening at 210° and $[\alpha]^{20}D$ +129.6° for this substance prepared by a different synthetic route.

Anal. Calcd. for $C_6H_{10}OClN(OCH_3)_4$: C, 44.2; H, 8.1; N, 5.2; OCH_3, 45.9. Found: C, 44.5; H, 7.7; N, 5.3; OCH_3, 46.1.

Tetra-*O*-acetyl-2-deoxy-2-sulfoamino-β-D-glucose, Sodium Salt (VI).—1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose[9] (III, 10 g.) was dissolved in 80 ml. of dry pyridine and this was added to a reagent prepared by the slow addition of 5.5 ml. of sulfuric anhydride[13] to 100 ml. of dry pyridine, previously cooled to near its freezing point (−42°). The flask was rinsed with 20 ml. of dry pyridine and this was added to the reaction mixture. The reaction vessel was closed with a glass stopper, and the mixture was stirred with a magnetic stirrer for 24 hr. at room temperature. A small aliquot of the reaction mixture was withdrawn, diluted with 10 vol. of water, neutralized, and tested for (1) free amino groups (ninhydrin), (2) *N*-acetyl groups,[10] and (3) acid-hydrolyzable sulfate. Tests 1 and 2 were negative. Test 3 was performed by adding an aqueous solution of barium chloride to the aliquot, removing the precipitate by centrifugation, acidifying the supernatant with hydrochloric acid and heating in a boiling water-bath. A copious precipitate formed almost immediately, indicating the presence of acid-labile sulfate ion.

When 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose (III) hydrochloride (2 g.) was subjected to the same sulfation conditions, tests 1 and 2 were found to be positive to the extent of some 60–70% of that of the original starting material.

The above sulfation reaction mixture was divided into three parts; the first portion (20 ml.) was added to the 100 ml. of ethanol containing 2.2 g. of benzidine. The second portion (60 ml.) was added to 750 ml. of water (near 0°) containing 7 g. of sodium bicarbonate and the resultant solution was freeze-dried. The third portion (120 ml.) was added to 1.5 liters of water (near 0°) containing 22.5 g. of anhydrous barium acetate and the solution was freeze-dried.

The dried preparation of the sodium salt was extracted with one 50-ml., and two 25-ml. portions of dry methanol. The first extract was colorless and neutral, whereas the second and third extracts were slightly alkaline and had a pale yellow tint. Accordingly, the first extract was concentrated, under reduced pressure and at room temperature, to 10 ml. whereupon fine rosettes of crystals separated. These were removed and recrystallized from (ethyl acetate)–ether; yield 0.29 g., m.p. 146–148°, $[\alpha]^{27}D$ +10.5° (*c* 3.0, water). The material appears to be a monohydrate.

Anal. Calcd. for $C_6H_8O_8NNaS(CH_3CO)_4 \cdot H_2O$: C, 35.8; H, 4.73; N, 3.0; S, 6.9; Na, 5.0; CH_3CO, 36.8. Found: C, 36.0; H, 4.74; N, 3.02; S, 7.0; Na, 5.2; CH_3CO,[14] 37.0.

The second and third methanolic extracts, together with the filtrate from the first extract, were combined and 10 vol. of ether was added. A further amount of material (apparently amorphous) was obtained; yield 1.0 g., $[\alpha]^{26}D$ +10.1° (*c* 1.4, water), m.p. 145–146°.

Anal. Found: C, 35.5; H, 4.35; N, 2.81; S, 7.3.

(13) Sulfan B, stabilized sulfuric anhydride, General Chemical Division, Allied Chemical and Dye Corp., New York, N. Y.

(14) Method of A. Chaney and M. L. Wolfrom, *Anal. Chem.*, **28**, 1614 (1956).

The above material was employed for deacetylation to obtain compound IX (see below). No satisfactory preparations were obtained from the benzidine or barium salts.

Methyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-sulfoamino-α-D-glucoside, Sodium Salt (XI).—Ten grams of V was dissolved in 25 ml. of water and passed down a column (15 × 2 cm., diam.) of Amberlite IR-400[11] (bicarbonate form) and the eluate was freeze-dried; yield 8 g. of a white hygroscopic solid exhibiting a positive ninhydrin test, a negative chloride ion test and yielding a basic solution in water. This solid was not further characterized but was sulfated under the same conditions as described above for 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose (III), employing 4.5 ml. of sulfuric anhydride. The reaction mixture was poured into 1.75 liters of water (near 0°) containing 25 g. of sodium bicarbonate and the resultant solution was freeze-dried. The dry solids were extracted with three 100-ml. portions of dry ethyl acetate and the solvent was removed under reduced pressure at room temperature; yield 10 g. of a pale yellow solid, ninhydrin (−), acid-hydrolyzable sulfate (+). A 2-g. portion of this material was dissolved in 10 ml. of warm ethanol and ether added to opalescence. After storage at 0° for 2 weeks, a crystalline precipitate was removed; yield 1.3 g. (55%), m.p. 159°, $[\alpha]^{27}D$ +70.4° (*c* 3.5, water).

Anal. Calcd. for $C_7H_{11}O_8NNaS(CH_3CO)_3$: C, 37.04; H, 4.76; N, 3.32; S, 7.60; Na, 5.46; CH_3CO, 30.68. Found: C, 37.16; H, 4.81; N, 3.29; S, 7.46; Na, 5.62; CH_3CO,[14] 30.35.

Methyl 2-Deoxy-3,4,6-tri-*O*-methyl-2-sulfoamino-α-D-glucoside, Sodium Salt (XII).—An amount of 5 g. of VII was dissolved in 20 ml. of methanol and passed down a column (6 × 1.5 cm., diam.) of Amberlite IR-400[11] (bicarbonate form) which had been well washed with methanol. The column was eluted slowly with methanol until the washings gave a negative ninhydrin test. The eluate was evaporated on a boiling water-bath at 145 mm. There remained a viscous oil which could be distilled at 100° at 0.01 mm. The oil solidified in the refrigerator (2–3°) but was not further characterized; it has been characterized as a distilled sirup by Cutler, Haworth and Peat.[8] This material was sulfated with 2.5 ml. of sulfuric anhydride in 75 ml. of pyridine, as described above. About half of the reaction mixture was accidentally lost. The remainder was poured into 300 ml. of water (near 0°) containing 3.5 g. of barium acetate (anhydrous) and the resultant solution was freeze-dried.

The dry solids were extracted with four 25-ml. portions of dry methanol, and the methanolic extracts were taken to dryness at room temperature and reduced pressure. The dry solid contained acid-hydrolyzable sulfate but gave a positive ninhydrin color. Accordingly, its solution in water (20 ml.), made just alkaline with barium hydroxide, was extracted with three 20-ml. portions of chloroform, after which the aqueous layer no longer gave a positive ninhydrin test. The aqueous layer was neutralized by the dropwise addition of 2 *N* sulfuric acid, the barium sulfate was removed by centrifugation and the supernatant was freeze-dried. The residual solid was dissolved in methanol and precipitated by the addition of ether; yield 1.4 g. Analysis showed that this preparation contained about 50% of extraneous ash. The crude barium salt (600 mg.) was dissolved in 5 ml. of water and to this was added 25 ml. of acetone. The precipitate which formed was washed with acetone/water (5:1) and contained only a trace of acid-hydrolyzable sulfate. The filtrate and washings were taken to dryness under diminished pressure and dissolved in 10 ml. of water. A saturated solution of sodium sulfate was added dropwise until no further barium ion could be detected in the solution. The solution was filtered and the filtrate was freeze-dried. The residue was extracted with four 10-ml. portions of ethyl acetate, the extracts were condensed under diminished pressure to 5 ml., and 5 ml. of light petroleum (b.p. 65–110°) was added. A white amorphous precipitate formed; yield 187 mg., m.p. 77° with preliminary softening, $[\alpha]^{27}D$ +98.2° (*c* 1, water). The product was not pure and contained extraneous ash.

Anal. Calcd. for $C_6H_8O_4Na(OCH_3)_4$: C, 35.6; H, 5.9; N, 4.1; S, 9.5; ash, 21.1; OCH_3, 36.8. Found: C, 32.4; H, 5.7; N, 3.6; S, 8.1; ash (sulfate), 29.3; OCH_3, 32.1.

2-Deoxy-2-sulfoamino-D-glucose (IX).—1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-sulfoamino-β-D-glucose, sodium salt (VI,

1 g.), was dissolved in 30 ml. of dry methanol, cooled to 0°, a small piece of sodium added, and the whole left in the ice-box overnight. A yellow amorphous precipitate formed which was separated by centrifugation, washed twice with dry methanol and dried; yield 0.7 g. This material was dissolved in 50 ml. of hot methanol and decolorized with carbon. The material in the filtrate was precipitated by the addition of acetone. The precipitate was redissolved in methanol and reprecipitated with acetone; yield 0.31 g. (49.5%) of a white powder, m.p. 148–150°, $[\alpha]^{27}D$ +52.1° (*c* 2, water).

Anal. Calcd. for $C_8H_{12}O_8NNaS$: C, 25.6; H, 4.3; N, 4.9; S, 11.4; Na, 8.2. Found: C, 25.9; H, 4.4; N, 4.8; S, 11.2; Na, 8.4.

Methyl 2-Deoxy-2-sulfoamino-α-D-glucopyranoside, Sodium Salt Monohydrate (XIII).—The crude reaction product (5 g.) from the above-described sulfation of VIII, was dissolved in 50 ml. of dry ethanol and deacetylated overnight in the cold (0–4°) in the presence of a small amount of sodium ethoxide. An amorphous precipitate formed which was separated by centrifugation, washed with ethanol until the washings were neutral, taken up in 50 ml. of dry methanol and an equal volume of dry ethanol added. A precipitate formed which was removed by centrifugation, and the supernatant was evaporated at low pressure and at room temperature to crystallization. The material was recrystallized from aqueous ethanol; yield 1.02 g. (30%), m.p. 159–161°, $[\alpha]^{27}D$ +103.1° (*c* 2.1, water). The material appears to be a monohydrate.

Anal. Calcd. for $C_7H_{14}O_8NNaS \cdot H_2O$: C, 26.8; H, 5.1; N, 4.5; S, 10.0; Na, 7.3. Found: C, 26.75; H, 4.9; N, 4.5; S, 9.8; Na, 7.6.

Methyl Mono-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranoside Hydrochloride.—Methyl tri-*O*-acetyl-2-amino-*N*-(benzoxycarbonyl)-2-deoxy-α-D-glucopyranoside (II, 8.3 g.) was mixed with 1 g. of a palladium-on-charcoal catalyst and added to 175 ml. of dry methanol containing 0.025 equivalent (1.2 molar ratio) of dry hydrogen chloride. Hydrogen was bubbled slowly through the solution for 12 hr. The solution was filtered and concentrated to dryness under reduced pressure to give a white solid (4.9 g.) which was dissolved in 20 ml. of ethanol and precipitated with light petroleum. The amorphous solid (4.3 g.) was filtered and was crystallized with difficulty from ethanol–acetone mixtures; m.p. 205–215° dec., $[\alpha]^{25}D$ +113.5° (*c* 2.6, water).

Anal. Calcd. for $C_7H_{15}O_5ClN(CH_3CO)$: C, 39.68; H, 6.68; Cl, 13.05; N, 5.16; CH_3CO, 15.8. Found: C, 39.75; H, 6.66; Cl, 13.17; N, 5.17; CH_3CO, 16.0.

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE RACKHAM ARTHRITIS RESEARCH UNIT, AND THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND BACTERIOLOGY, UNIVERSITY OF MICHIGAN]

The Preparation of Glucosamine Oligosaccharides. I. Separation[1,2]

BY SYLVIA T. HOROWITZ, SAUL ROSEMAN[3] AND HAROLD J. BLUMENTHAL

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Chitosan was partially degraded by the use of hydrochloric acid. The hydrolysate was fractionated by means of ion-exchange chromatography and yielded five discrete peaks. Analysis indicated that the materials in the peaks were glucosamine oligosaccharides which were eluted in order of increasing molecular weight. Procedures are presented for the analysis of glucosamine oligo- or polysaccharides and for large scale gradient chromatography.

In the course of studies in this Laboratory concerned with the biochemistry of chitin, it was desirable to prepare oligosaccharides of glucosamine (2-amino-2-deoxy-D-glucose) and N-acetylglucosamine (2-acetamido-2-deoxy-D-glucose). The preparation of glucosamine oligosaccharides has not been reported previously. The preparation of N-acetylglucosamine oligosaccharides has been attempted by subjecting chitin to acetolysis, or to partial hydrolysis, followed by fractionation of the resulting mixtures by conventional techniques. Acetolysis of chitin[4] was reported to yield the fully acetylated disaccharide, octaacetylchitobiose (1,3,6-triacetyl-2-acetamido-2-deoxy-4-(3,4,6-triacetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-glucose) in 16.2% yield as the crude product. Subsequent partial hydrolysis of chitin[5] followed by fractionation of the chitodextrin mixture and acetylation gave low yields of the fully acetylated disaccharide and trisaccharide. It has been reported recently[6] that saponification of octaacetyl chitobiose with methanolic ammonia yields five products from which crystalline N,N¹-diacetylchitobiose was obtained by chromatography.

This report presents a method for the preparation of glucosamine oligosaccharides. Subsequent studies will deal with the isolation, properties and characterization of certain of these compounds. Although chitin is a polymer of N-acetylglucosamine, the amino groups can be liberated by deacetylation of chitin to chitosan. Partial hydrolysis of the resulting polyhexosamine yields the glucosamine oligosaccharides which are separated by ion-exchange chromatography. The separations achieved in this manner offer the following advantages over the older methods: (1) the free amino sugars can be isolated, (2) the separation appears to be much more efficient, (3) fractions of higher molecular weight than the trisaccharide can be obtained. For present purposes, effort was directed primarily toward the separation of the lower molecular weight materials.

The literature indicates the difficulty in obtaining purified chitin. The usual technique involves repeated treatment of the crude material with hot acid, hot alkali and alkaline permanganate at room temperature to remove colored material. It was found that preparations exhibiting satisfactory analyses could be obtained by treatment of

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(2) A preliminary report has appeared, *Federation Proc.*, **14**, 740 (1955).

(3) To whom requests for reprints and additional information should be addressed.

(4) M. Bergmann and E. Silberkweit, *Naturwiss.*, **19**, 20 (1931); *Ber.*, **64**, 2436 (1931).

(5) L. Zechmeister and G. Tóth, *ibid.*, **64**, 2028 (1931).

(6) F. Zilliken, C. A. Braun, C. S. Rose and P. György, THIS JOURNAL, **77**, 1296 (1955).