water was added dropwise with stirring during 15 minutes. Heating was continued for 15 minutes after which the solution was brought to *p*H 7 with sodium hydroxide and evaporated on the steam-bath under reduced pressure to dryness. The residue was extracted with 300 ml. of hot acetone, and this extract was evaporated to dryness. Again the residue was taken up in 400 ml. of hot acetone. The solution was filtered, evaporated to 50 ml. and then allowed to evaporate slowly to dryness. A white crystalline residue was left mixed with a little gum. It was triturated with a very little ice-cold, absolute ethanol, collected on a filter and air-dried. The yield was 0.9 g. It was recrystallized from a mixture of acetone, methanol and petroleum ether; m.p. 141–142°.

Anal. Calcd. for $C_9H_{13}NO_4$: N, 7.03. Found: N, 6.92.

4-Amino-6-methyl-2,5-dihydroxymethylpyridine (V).—One gram of dimethyl 4-amino-6-methyl-2,5-pyridinedicarboxylate[1c] was allowed to react with 1 g. of lithium aluminum hydride in 250 ml. of anhydrous ether, and the mixture was worked up in a manner previously described.[1a] There was obtained 0.62 g. (83% yield) of 4-amino-6-methyl-2,5-dihydroxymethylpyridine, m.p. 143–145°. A sample for analysis was recrystallized from absolute ethanol–ethyl acetate–petroleum ether mixture; m.p. 147–148°.

Anal. Calcd. for $C_8H_{12}N_2O_2$: C, 57.12; H, 7.19; N, 16.66. Found: C, 56.69; H, 7.17; N, 16.52.

Ethyl 5-Cyano-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate.—Ethyl ethoxymethylenetrifluoroacetoacetate[6] and β-aminocrotononitrile were allowed to condense, and the mixture was worked up in a manner previously described for similar reactions.[2] The resulting impure ethyl 5-cyano-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate was obtained in 63% yield as a colorless liquid; b.p. 130–140° (4 mm.); 97–102° (0.7 mm.).

Anal. Calcd. for $C_{11}H_9F_3N_2O_2$: C, 51.17; H, 3.51; N, 10.85. Found: C, 51.72; H, 3.82; N, 11.43.

Ethyl 5-Carbamyl-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate.—Ethyl 5-cyano-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate, was treated with concentrated sulfuric acid and the mixture was worked up as described above to give ethyl 5-carbamyl-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate in 60–70% yield. It was a white crystalline solid which melted at 167–168° after recrystallization from ether–petroleum ether.

Anal. Calcd. for $C_{11}H_{11}F_3N_2O_3$: C, 47.83; H, 3.98; N, 10.14. Found: C, 48.01; H, 3.22; N, 10.07.

5-Amino-6-methyl-2-trifluoromethyl-3-pyridinecarboxylic Acid.—This was prepared from ethyl 5-carbamyl-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate by reaction with hypochlorite in the same way as described above for the preparation of 5-amino-6-methyl-2,3-pyridinedicarboxylic acid. The compound was obtained in 88% yield. A sample was recrystallized from water; m.p. 215–216° dec. (uncor.).

Anal. Calcd. for $C_8H_7F_3N_2O_2$: C, 43.64; H, 3.21; N, 12.73. Found: C, 43.87; H, 3.36; N, 12.81.

Attempted Preparation of 3-Amino-5-hydroxymethyl-2-methyl-6-trifluoromethylpyridine.—The above noted 3-amino-2-methyl-6-trifluoromethyl-5-pyridinecarboxylic acid was esterified by heating under reflux for 20 hours in methanol saturated with hydrogen chloride. Isolation of the ester in the usual way gave a viscous sirup which did not crystallize. The crude ester was reduced in ether solution with lithium aluminum hydride and the product isolated as described previously for similar reactions.[1a] It was a white solid, soluble in water, alcohol, or ether, but it appeared to be unstable. All attempts to purify the material resulted in its decomposition, and no analytical sample was obtained.

(6) R. G. Jones, THIS JOURNAL, **73**, 3684 (1951).

INDIANAPOLIS, INDIANA RECEIVED SEPTEMBER 29, 1951

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

The Structure of Chondrosine and of Chondroitinsulfuric Acid[1]

BY M. L. WOLFROM, R. K. MADISON AND M. J. CRON

Chondroitinsulfuric acid from cartilage is electrophoretically homogeneous and consumes 1 mole of periodate per disaccharide unit to cleave a glycol in the D-glucuronic acid portion. The component disaccharide, chondrosine (further characterized as the crystalline heptabenzoyl methyl ester derivative), was converted to the crystalline diamide glycitol IV which on periodate oxidation underwent formaldehyde and formic acid scission in the reduced portion to yield crystalline V with the N-acetamido-2-desoxy-D-galactopyranosyl radical intact; further periodate oxidation cleaved a glycol in this hexosamine moiety. Chondrosine is therefore 4-(2-amino-2-desoxy-β(?)-D-galactopyranosyl)-D-glucuronic acid and in the heteropolymer one sulfate acid ester group and the glycosidic attachment of the adjacent D-glucuronic acid group are yet to be partitioned between positions 3, 4 and 6 of each chondrosamine unit. The high yield of chondrosine obtainable by acid hydrolysis characterizes chondroitinsulfuric acid as very probably a linear type polymer.

Chondroitinsulfuric acid is the heteropolysaccharide present in combination with protein in cartilage. It was adequately described by Krukenberg[2] and by Mörner[3] and has been extensively investigated by Levene and co-workers.[4] Its component monosaccharide units[5] are D-glucuronic acid[6,7] and N-acetylchondrosamine[8] (N-acetyl-2-amino-2-desoxy-D-galactose[9]). To these two units is attached one sulfuric acid group as an acid ester. The component disaccharide, designated chondrosine, of this polysaccharide has long been known as its crystalline ethyl ester hydrochloride.[10] It is readily obtainable in high yield by differential acid hydrolysis dependent upon the resistance toward hydrolysis exhibited by the 2-amino-2-desoxyglycosidic linkage. The uronic acid is therefore the reducing component. The high yield of this disaccharide obtained in our work indicates that the polysaccharide is very probably of the linear type with this component as the repeating unit. In his last and posthumously published article,[11] the late Dr. P. A. Levene described a number of crystalline derivatives of this disaccharide but did not succeed in establishing its structure.

Levene reduced the crystalline methyl ester hydrochloride of chondrosine (II, Fig. 1) to the gly-

(1) A preliminary report of the work establishing the structure of chondrosine appeared in *Abstracts Papers Am. Chem. Soc.*, **118**, 7R (1950).

(2) C. F. W. Krukenberg, *Z. Biol.*, **20**, 307 (1884).

(3) C. T. Mörner, *Skand. Arch. Physiol.*, **1**, 210 (1889).

(4) P. A. Levene, "Hexosamines and Mucoproteins," Longmans, Green and Co., London, 1925.

(5) M. L. Wolfrom, D. I. Weisblat, J. V. Karabinos, W. H. McNeely and J. McLean, THIS JOURNAL, **65**, 2077 (1943).

(6) P. A. Levene and W. A. Jacobs, *J. Exptl. Med.*, **10**, 557 (1908).

(7) H. G. Bray, J. E. Gregory and M. Stacey, *Biochem. J.*, **38**, 142 (1944).

(8) P. A. Levene, *J. Biol. Chem.*, **31**, 609 (1917).

(9) Sybil P. James, F. Smith, M. Stacey and L. F. Wiggins, *Nature*, **156**, 308 (1945); *J. Chem. Soc.*, 625 (1946).

(10) J. Hebting, *Biochem. Z.*, **63**, 353 (1914).

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

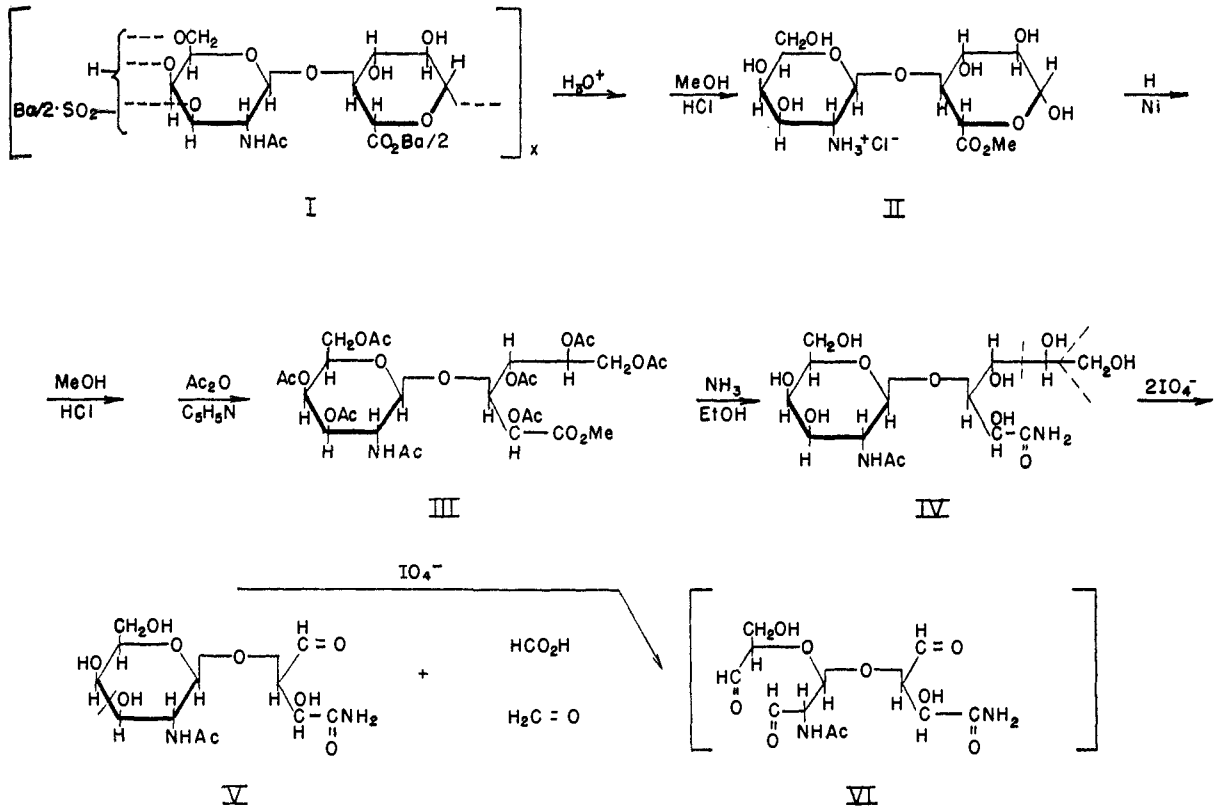

Fig. 1.—Degradation of barium chondroitinsulfate.

citol, characterized as its crystalline octaacetyl derivative III. Chondrosine was herein further characterized as its crystalline heptabenzoyl methyl ester derivative. It is noteworthy that II exhibited no mutarotation and did not undergo glycosidation. In the present work, III was converted to the crystalline diamide IV with alcoholic ammonia. Periodate oxidation showed the rapid consumption (Fig. 2) of two moles of oxidant (per mole of IV) with the concomitant formation of one mole each of formic acid and formaldehyde. At this point substance V could be isolated as a crystalline product. Prolonged action of the oxidant led to the consumption of one further mole with the formation of VI (not isolated). It is of interest to note that this last glycol undergoing scission is configurationally *cis*. The resistance toward glycol splitting thus noted previously[12] in the hexosamine portion of N-acetylhexosamine glycosides is therefore due to the presence of the acetamido group on the adjacent carbon. These periodate oxidation results uniquely establish IV as the structure for the diamide and in chondrosine, therefore, the disaccharide union is from C-1 of the hexosamine portion to C-4 of the hexuronic acid. The high levorotations exhibited by IV (−100°) and V (−77°) make the β-D disaccharide linkage probable but not definitive. Chondrosine may therefore be designated as 4-(2-amino-2-desoxy-β-(?)-D-galactopyranosyl)-D-glucuronic acid.

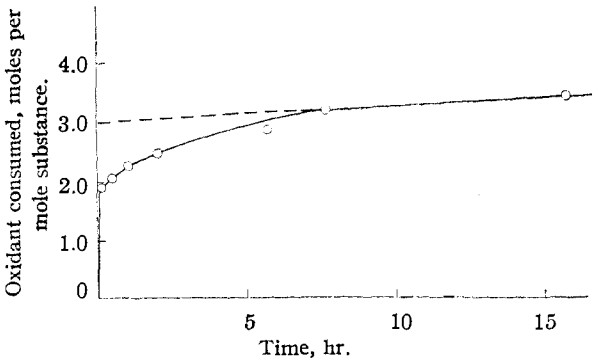

Fig. 2.—Oxidation of 0.005 *M* 4-(N-acetyl-2-amino-2-desoxy-D-galactopyranosyl)-L-gulonamide (IV) with 0.025 *M* sodium metaperiodate at 12°, initial *p*H 6.0. Formic acid (moles per mole substance): 3 min., 1.0: 5 hr., 1.1. Formaldehyde (dimedon method): 45 min., 0.8 mole per mole substance.

We find that our preparation of sodium chondroitinsulfate is electrophoretically homogeneous[13] and shows by periodate consumption the presence in it of one glycol grouping per disaccharide unit. The oxidized product was isolated and it was demonstrated that this contained acid-hydrolyzable sulfate and hexosamine but that the glycuronic acid moiety had been destroyed. The glycol grouping in the disaccharide unit was therefore in the D-glucuronic acid portion, thus limiting the position of esterification with sulfuric acid to the hexosamine portion. Two of the positions 3, 4 and 6 of the chondrosamine portion are accordingly concerned in sulfate acid ester formation and in the glycosidic polysaccharide union but definite alloca-

(12) R. U. Lemieux, C. W. DeWalt and M. L. Wolfrom, THIS JOURNAL, **69**, 1838 (1947); M. L. Wolfrom and C. W. DeWalt, *ibid.*, **70**, 3148 (1948).

(13) See footnote 20 in Experimental portion.

ion of these two points must await further data. Finally, it may be stated that these results are not in harmony with previously postulated[14,15] structures for chondroitinsulfuric acid based upon the application of other methods of structural proof. It is hoped that future work may reconcile these differences and it is also not excluded that various types of chondroitinsulfuric may occur in nature.

Experimental[16]

Preparation of Salts of Chondroitinsulfuric Acid.—Commercial crude sodium chondroitinsulfate (50 g.) from cartilage[17] was dissolved in 500 ml. of water and filtered through diatomaceous earth (Filter-Cel) to give a clear amber solution. Addition of 3000 ml. of glacial acetic acid precipitated a crude sodium acid salt which was filtered after 4 hr. and washed with glacial acetic acid. The precipitate was immediately dissolved in 400 ml. of water and precipitated by pouring into 1500 ml. of 95% ethanol. The filtered product was washed with 95% ethanol (800 ml.) until the filtrate was neutral to litmus and was then dissolved in 400 ml. of water and precipitated by pouring into a mixture of 1500 ml. of 95% ethanol and 800 ml. of ether. The precipitate was removed by centrifugation and washed successively with 95% ethanol, abs. ethanol and ether. The white, amorphous, crude sodium acid chondroitinsulfate gave a negative biuret test and was free of inorganic sulfate; yield 40 g.

An amount of 50 g. of the above product was precipitated as described above from aqueous solution by glacial acetic acid and then from aqueous solution by 95% ethanol and the filtered product was washed with 95% ethanol until the washings were neutral to litmus. The precipitate was then immediately dissolved in 400 ml. of water and 10% NaOH (*ca.* 20 ml.) was added slowly with cooling to neutralization. This solution was poured into 1500 ml. of 95% ethanol and the resultant fibrous precipitate of amorphous sodium chondroitinsulfate was removed by filtration and washed successively with 95% ethanol, abs. ethanol and ether; yield 41 g.

An amount of 50 g. of the above sodium salt was dissolved in 1000 ml. of water and the solution passed successively through two columns (43 × 4.3 cm., diam.) holding Amberlite IR-100 cation exchange resin.[18] The effluent was collected in a flask containing the calculated amount of barium acetate. The columns were washed with water (2500 ml.) until the effluent was neutral to litmus. The combined effluent and washings were concentrated under reduced pressure to 800 ml. and poured with stirring into 2500 ml. of 95% ethanol. The resulting precipitate of neutral barium chondroitinsulfate was removed by filtration, washed with abs. ethanol and with ether and dried over P_2O_5 under reduced pressure; yield 56–58 g. of a white, amorphous powder, $[\alpha]^{30}D$ −24° (*c* 2.1, water).

Anal. Calcd. for $C_{14}H_{19}O_{14}NSBa$: N, 2.36; S, 5.39; Ba, 23.10. Found: N, 2.45; S, 5.01; Ba, 23.60.

Electrophoretic Behavior of Sodium Chondroitinsulfate.—A 1% solution of the above described sodium chondroitinsulfate in acetate buffer was dialyzed against 2.5 l. of buffer solution for 4 days at 2–6°. A modified Tiselius apparatus[19] was used and the boundaries were photographed by the Schlieren method. Only one component was present[20]; its mobility was evaluated as -12×10^{-5} cm.^{2} v.$^{-1}$ sec.$^{-1}$ at *p*H 5.6, ionic strength 2 and 0.6°.

Preparation of Methyl O-Heptaacetyl-4-(N-acetyl-2-amino-2-desoxy-D-galactopyranosyl)-L-gulonate (III).—Following Levene,[11] barium chondroitinsulfate (50 g.) was partially hydrolyzed to the amorphous disaccharide chondrosine hydrochloride; yield 26.7–29.3 g. (81–89%), $[\alpha]^{27}D$ +39° (*c* 3, water). An amount of 10 g. of this product was converted to the crystalline methyl ester hydrochloride (II) according to Levene[11]; yield 7.3–9.9 g. (70–95%) of material of good purity; on one recrystallization from abs. ethanol; m.p. 155–156°, $[\alpha]^{25}D$ +39° (*c* 4, methanol, no mutarotation in 5 hr. observation), reducing to Fehling solution.

Anal. Calcd. for $C_{12}H_{21}O_{10}NCl(OCH_3)$: C, 38.48; H, 5.96; N, 3.45; OCH_3, 7.65; equiv. wt., 406. Found: C, 38.06; H, 5.90; N, 3.49; OCH_3, 8.27; equiv. wt., 418.

An amount of 10 g. of the above methyl ester hydrochloride was reduced as described by Levene[11] except that an aqueous solution was employed and reduction was effected at 60° for 20 hr. at 1100 p.s.i. The sirup obtained on catalyst and solvent removal was dissolved in 75 ml. of a 1% solution of hydrogen chloride in abs. methanol and refluxed for 15 hr. The sirup obtained on solvent removal was twice precipitated from a small amount of methanol by the successive addition of abs. ethanol and ether; yield 7.3 g., $[\alpha]^{21}D$ −35° (*c* 2.8, methanol). This amorphous product was suspended in 50 ml. of pyridine and 35 ml. of acetic anhydride was added with cooling. After maintaining the mixture at 10° for 24 hr. and then at room temperature for 24 hr., it was poured into an excess of ice and water and extracted (after 4 hr. standing) with chloroform. Pyridine was removed from the extract by washing with an excess of a saturated aqueous solution of cadmium chloride. The filtered insoluble cadmium chloride–pyridine complex was washed with chloroform and the combined washings and filtrate were concentrated under reduced pressure to a sirup from which several portions of abs. ethanol were distilled under reduced pressure. The residual sirup was crystallized from methanol–ether (dry solvents); yield 4.7 g., m.p. 118–119°. Pure material was obtained on recrystallization from abs. ethanol by the addition of petroleum ether (b.p. 30–60°); yield 4.0 g., m.p. 121–123°, $[\alpha]^{28}D$ −23° (*c* 1.8, ethanol). For this substance Levene[11] cites the constants: m.p. 122°, $[\alpha]^{25}D$ −21° (*c* 3.2, abs. ethanol).

Anal. Calcd. for $C_{14}H_{17}O_{11}N(CH_3CO)_7(OCH_3)$: C, 49.22; H, 5.84; N, 1.98; OCH_3, 4.39; CH_3CO, 48.7. Found: C, 49.22; H, 5.70; N, 2.10; OCH_3, 4.85; CH_3CO, 48.6.

O-Hexabenzoyl-N-benzoylchondrosine Methyl Ester.[21]—To a solution of 300 ml. of dry, alcohol-free chloroform and 80 ml. of pyridine, 8.9 g. of crystalline chondrosine hydrochloride methyl ester was added followed by the slow addition at 0° of 25 ml. of benzoyl chloride. The mixture was protected from moisture and stirred vigorously for 24 hr. at 0°. The clear solution which resulted was then allowed to stand at 15° for 2 days. After distillation under reduced pressure until the appearance of a white, crystalline precipitate, the solution was poured into 700 ml. of ether. The viscous sirup which precipitated was removed and triturated first with 200 ml. of water and then, after removal of moisture on a clay plate, with 200 ml. of ether. After solution in 200 ml. of chloroform, treatment with activated charcoal, and drying over anhydrous sodium sulfate, the residue was again precipitated by pouring into excess ether. After decanting the mother liquor, the sirup which remained was allowed to stand for 30 min. and to it was then added 200 ml. of methanol. The sirup dissolved with the simultaneous formation of a white, crystalline precipitate; yield 6.4 g. (26%). Pure material was obtained on recrystallization from acetone–methanol; m.p. 126–129°, $[\alpha]^{22}D$ +83° (*c* 2, chloroform).

Anal. Calcd. for $C_{62}H_{51}O_{18}N$: C, 67.82; H, 4.68; N, 1.27. Found: C, 67.63; H, 4.27; N, 1.36.

4-(N-Acetyl-2-amino-2-desoxy-D-galactopyranosyl)-L-gulonamide (IV).—A solution of 3.46 g. of methyl 4-(O-heptaacetyl-N-acetyl-2-amino-2-desoxy-D-galactopyranosyl)-L-gulonate in 50 ml. of abs. methanol was cooled in an ice–salt-bath and a stream of anhydrous ammonia gas was passed in for 15 min. After standing at room temperature for 3 hr., the solvent was removed by distillation under re-

(14) K. H. Meyer, M. E. Odier and A. E. Siegrist, *Helv. Chim. Acta*, **31**, 1400 (1948).

(15) H. Masamune, *Chem. Researches (Japan)*, **4**, *Biochem.*, 1 (1949); *C. A.*, **44**, 5818 (1950).

(16) Unless otherwise noted, all experimental work was performed by Mr. R. K. Madison.

(17) A product of The Wilson Laboratories, Chicago, Illinois.

(18) A product of The Resinous Products Division of the Rohm and Haas Co., Philadelphia, Pennsylvania.

(19) L. G. Longsworth and D. A. MacInnes, *Chem. Revs.*, **24**, 271 (1939).

(20) All of the "non-moving" components of heparin, chondroitinsulfuric acid and mucoitinsulfuric acid mentioned in our previous publication with F. A. H. Rice, THIS JOURNAL, **69**, 2918 (1947), are artifacts produced by an unusually large boundary anomaly. Isolation and analytical experiments showed that no organic material was present in these areas. See also K. Meyer, Eleanor Chaffee (and D. H. Moore), *J. Biol. Chem.*, **138**, 491 (1941).

(21) Experimental work by Mr. M. J. Cron.

duced pressure and the resultant sirup was precipitated from a small volume of methanol by the addition of abs. ether; yield 1.82 g. This amorphous product was crystallized from methanol–chloroform; yield 1.50 g., m.p. 132–134°, $[\alpha]^{27}D$ −100° (*c* 1.9, water).

Anal. Calcd. for $C_{12}H_{23}O_{10}N_2(CH_3CO)$: C, 42.21; H, 6.58; N, 7.03; CH_3CO, 10.8. Found: C, 42.21; H, 6.91; N, 7.09; CH_3CO, 11.0.

Oxidation of 4-(N-Acetyl-2-amino-2-desoxy-D-galactopyranosyl)-L-gulonamide to 2-(N-Acetyl-2-amino-2-desoxy-D-galactopyranosyl)-D-erythruronamide (V).—An amount of 0.9987 g. (2.51 millimoles) of crystalline 4-(N-acetyl-2-amino-2-desoxy-D-galactopyranosyl)-L-gulonamide (IV) was oxidized at 12° in 100 ml. of solution with 5.28 millimoles of sodium metaperiodate. After 15 hr., titration of an aliquot showed that all of the periodate had been reduced. Thereupon ionic material was removed by successive passage of the solution over Amberlite[18] exchange resins (200 × 40 mm., diam.) IR-4 and IR-100 and the sirup obtained on solvent removal was crystallized from methanol by the addition of abs. ethanol. The crystals were dried under reduced pressure at 78°; yield 0.313 g., m.p. 177–179°, $[\alpha]^{27}D$ −77° (*c* 1.5, water).

Anal. Calcd. for $C_{10}H_{17}O_8N_2(CH_3CO)$: C, 42.86; H, 5.99; N, 8.33; CH_3CO, 12.8. Found: C, 42.87; H, 5.29; N, 8.08; CH_3CO, 13.1.

Periodate Oxidation of Sodium Chondroitinsulfate (Neutral Salt).—An amount of 2.01 g. (4.0 millimoles per disaccharide unit of calcd. 503 mol. wt.) of neutral sodium chondroitinsulfate (S, 6.01; $[\alpha]^{28}D$ −24°, *c* 2 in water) was oxidized at 26° in 100 ml. of solution with 7.92 millimoles of sodium metaperiodate. The initial *p*H was 5.5. Aliquots showed that slightly more than 1 mole (per disaccharide unit) of oxidant was consumed with the formation of small amounts (*ca.* 0.1 mole) of formic acid or 1.0 mole oxidant consumed at 60 hr. when corrected (decreased) for formic acid produced. At this point the excess periodate was destroyed by the addition of ethylene glycol, the solution was reduced in volume and treated with excess barium chloride. The precipitated barium iodate was removed by filtration and the organic material was precipitated in the filtrate by the successive addition of ethanol and ether. The filtered precipitate was dissolved in water and deionized by successive passage through Amberlite[18] IR-100 and IR-4 exchange resin columns (17.5 × 2.5 cm., diam.). The effluent was concentrated under reduced pressure to a sirup which was precipitated from a small amount of water by three volumes of ethanol. The precipitate was removed by filtration, washed with alcohol and ether and dried at room temperature in a vacuum desiccator. This product exhibited a negative Dische hexuronic acid color test[22] and a positive hexosamine color test.[23] It gave a negative sulfate test which became positive after acid hydrolysis. It is to be noted that iodate ion interferes with both of the color tests cited above by producing a green color in the Dische test when hexuronic acid is present and by preventing the formation of the pink color in the hexosamine test.

(22) Z. Dische, *J. Biol. Chem.*, **167**, 189 (1947).

(23) J. W. Palmer, Elizabeth M. Smyth and K. Meyer, *ibid.*, **119**, 491 (1937).

COLUMBUS 10, OHIO RECEIVED OCTOBER 8, 1951

[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

N-Glycosyl Derivatives of Secondary Amines

BY JOHN E. HODGE AND CARL E. RIST

New data on the optical rotations of glycosyl derivatives of piperidine, diethanolamine and dibenzylamine are presented. The D-glucosyl derivatives of piperidine and diethanolamine do not show mutarotation in dry pyridine, whereas the D-galactosyl and D-mannosyl derivatives of piperidine do. Acid catalysts and alcoholic reaction media were found to be unnecessary in the preparation of N-glycosyl derivatives of strongly basic amines. Evidence is given to show that the compound heretofore known as N-D-glucosyldibenzylamine is actually a 1-desoxy-1-aminofructose (isoglucosamine) derivative.

Investigations of glycosylamines and amino-sugar reactions have increased in recent years because of results of research on nucleic acids, vitamins of the B-complex, coenzymes and intermediates in the non-enzymatic browning of foods. The lack of knowledge on the structural configuration of N-substituted glycosylamines, and particularly on their transformations in solution, has recently been discussed.[2a,3,4] Recent studies on the mutarotations of glycosylamines have been reported by Isbell and Frush[5] and by Pigman, Cleveland, Couch and Cleveland.[6] We have new data to report on the optical rotations of tertiary glycosylamines.

Kuhn and Birkofer,[7] because of their observations on the unexpected mutarotation of N-D-glucosylpiperidine and N-D-glucosyldibenzylamine in pyridine, formulated a theory of mutarotation,[2b,7b] in which they postulated quaternary base or salt formation as a requisite for the assumed Schiff base intermediate between anomeric α- and β-glycosidic forms. Our data show that N-D-glucosylpiperidine does not mutarotate in pyridine,[8] and the compound considered as N-D-glucosyldibenzylamine[7a] is actually a 1-desoxy-1-amino-D-fructose (isoglucosamine) derivative. These results indicate that more information on the optical rotations of tertiary glycosylamines is needed to confirm the theory.

In dry pyridine, a pure glycosyl derivative of a secondary amine should not show mutarotation through a Schiff base form, because protons would not be available for formation of the necessary cation. We have observed no mutarotation in dried pyridine for either N-D-glucosylpiperidine or N-D-glucosyldiethanolamine. On the other hand, we found that N-D-mannosylpiperidine and N-D-galactosylpiperidine do show mutarotation in dried pyridine. These mutarotations may not occur through the Schiff base form, however; they may occur by other intramolecular rearrangement. Evidence for a rearrangement of N-D-mannosylpiperidine in pyridine was noted when we failed to obtain an acetyl

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) W. W. Pigman and R. M. Goepp, Jr., "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948, (a) p. 376; (b) p. 386.

(3) J. Honeyman and A. R. Tatchell, *J. Chem. Soc.*, 967 (1950).

(4) G. P. Ellis and J. Honeyman, *Nature*, **167**, 239 (1951).

(5) H. S. Isbell and H. L. Frush, *J. Research Natl. Bur. Standards*, **46**, 132 (1951).

(6) W. Pigman, E. A. Cleveland, D. H. Couch and J. H. Cleveland, THIS JOURNAL, **73**, 1976 (1951).

(7) R. Kuhn and L. Birkofer, *Ber.*, **71**, (a) 621, (b) 1535 (1938).

(8) Two attempts to prepare Kuhn and Birkofer's mutarotating N-D-glucosylpiperidine of m.p. 115° resulted only in the initial formation of an impure product. After several recrystallizations the compound melted at 128–129° (dec.) and showed no mutarotation in pyridine.