THE ISOLATION AND SYNTHESIS OF THE METHYL ESTER-METHYL α-GLYCOSIDE OF 3-O-β-D-GLUCURONOSYL-N-ACETYL-D-GLUCOSAMINE (HYALOBIURONIC ACID)[1]

Sir:

Polysaccharide components of animal connective tissue, such as hyaluronic acid, chondroitin sulfate and dermatan sulfate, are built of alternate units of uronic acid and hexosamine linked at positions 4 and 3, respectively. Isolation of a 3-*O*-β-D-glucuronosyl-hexosamine disaccharide, chondrosin, from chondroitin sulfate was reported already in 1914,[2] whereas a similar disaccharide, 3-*O*-(β-D-glucopyranosyluronic acid)-2-amino-2-deoxy-D-glucose (hyalobiuronic acid) (I) recently has been isolated from hyaluronic acid.[3,4] We wish to report the first synthesis of this type of disaccharide, isolated as the fully acetylated methyl ester-methyl-α-glycoside of I, namely, methyl 3-*O*-(methyl tri-*O*-acetyl-β-D-glucopyranosyluronate)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (II). The same compound II was obtained directly by degradative methanolysis of hyaluronic acid, then by acetylation, and from hyalobiuronic acid (I), by glycoside formation and acetylation.

Dried hyaluronic acid (1.10 g.) from human umbilical cord,[5] was refluxed with 6% methanolic hydrochloric acid for 24 hr. The resulting sirup, treated with pyridine and acetic anhydride gave, after purification by chromatography on silicic acid, 0.76 g. of crystalline material. Recrystallization afforded 0.22 g. of II, m.p. 236–238°, $[\alpha]^{26}D$ +30° (*c* 0.68, $CHCl_3$). *Anal.* Calcd. for $C_{26}H_{37}O_{17}N$: C, 49.13; H, 5.87; N, 2.20; OCH_3, 9.77. Found: C, 49.19; H, 5.95; N, 2.35; OCH_3, 10.36. Hydrolysis of *II* with barium methylate in methanol at 0° gave methyl 3-*O*-(β-D-glucopyranosyluronic acid)-2-acetamido-2-deoxy-α-D-glucopyranoside (III) in 95% yield, m.p. 207–210°, $[\alpha]D$ +31° (*c* 0.74, CH_3OH). *Anal.* Calcd. for $C_{15}H_{15}O_{12}N \cdot H_2O$: C, 41.96; H, 6.34. Found: C, 41.94, H, 6.40. Esterification of III with diazomethane or methanolic hydrochloric acid afforded IV, identical with the compound described below. 3-*O*-(β-D-glucopyranosyluronic acid)-2-acetamido-2-deoxy-D-glucose,[6] obtained by *N*-acetylation of I, was refluxed with 1.5 *N* methanolic hydrochloric acid and gave, after purification by chromatography on silicic acid, methyl 3-*O*-(methyl β-D-glucopyranosyluronate)-2-acetamido-2-deoxy-α-D-glucopyranoside (IV) in 50% yield, m.p. 223–225°, $[\alpha]^{23}D$ +16° (*c* 1.09, CH_3OH). Acetylation of IV gave II in 80% yield.

Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[7] (V) was allowed to react with an excess of (methyl tri-*O*-acetyl-α-D-glucopyranosyluronate) bromide and mercuric cyanide in a mixture of nitromethane and benzene for 2 days. The resulting product was purified by chromatography on silicic acid, heated for 15 min. with 60% acetic acid, and acetylated with acetic anhydride in pyridine solution, to give II in 42% over-all yield, m.p. 237–238°, $[\alpha]^{23}D$ +30° (*c* 0.98, $CHCl_3$), identical with the product described above. *Anal.* Calcd. for $C_{26}H_{37}O_{17}N$: C, 49.13; H, 5.87; OCH_3, 9.77. Found: C, 49.27; H, 5.96; OCH_3, 9.91. Reduction of II with lithium borohydride in tetrahydrofuran, followed by acetylation, gave a product identical with VI, described below.

The product resulting from the reaction of V with equimolecular quantities of tetra-*O*-acetyl-α-D-glucopyranosyl bromide and mercuric cyanide in benzene–nitromethane at 30° for 3 days was deacetylated catalytically with sodium methoxide, and heated with 60% acetic acid. After purification through a charcoal–Celite (1:1) column, methyl 3-*O*-(β-D-glucopyranosyl)-2-acetamido-2-deoxy-α-D-glucopyranoside (VI) was obtained in 35% yield, m.p. 252–254°; $[\alpha]^{27}D$ +44° (*c* 1.00, H_2O). *Anal.* Calcd. for $C_{15}H_{27}O_{11}N$: C, 45.33; H, 6.85. Found: C, 45.88; H, 7.13. Acetylation of VI with acetic anhydride in pyridine solution gave the hexa-*O*-acetyl derivative in 75% yield, m.p. 236–237°, $[\alpha]^{28}D$ +24° (*c* 1.11, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{39}O_{17}N$: C, 49.92; H, 6.05. Found: C, 50.02; H, 6.08.

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THE CYCLOADDITION OF "SULFENE" TO KETENE DIETHYLACETAL

Sir:

Sulfenes as reactive intermediates have been proposed by various workers. Diphenylsulfene was suggested as an intermediate in the reaction of diphenyldiazomethane with sulfur dioxide which decomposed to form tetraphenylethylene.[1] Likewise a similar structure was postulated by Kloosterziel and Backer.[2] "Methylenesulfene" ($CH_2=SO_2$) was proposed by Hesse and Reichold[3] as formed by the interaction of diazomethane with sulfur

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