Communications TO THE EDITOR

Carboxyl-Reduced Heparin

Sir:

Heparin (sodium salt), purified through the cetyltrimethylammonium salt, was treated with methanolic hydrogen chloride to yield a product containing one sulfate acid ester group per disaccharide unit, in agreement with Wolfrom and co-workers[1] and with Danishevsky and associates.[2] *N*-Acetylation allowed some further desulfation[2] to give I: $[\alpha]_D^{25}$ + 66° (*c* 0.8, water). *Anal.* S, 3.8. Repetition of this stepwise procedure with 0.15*N* methanolic hydrogen chloride for 2 days at 25° followed by treatment with ion exchange resins gave a nearly desulfated product (II); $[\alpha]_D^{25}$ + 66° (*c* 0.5, water). *Anal.* C. 39.55; H, 5.39; N, 3.21; S, 0.2; Na, 0.1.

Product I was freeze-dried to a solid which dissolved in formamide or *N*,*N*-dimethylformamide (as does heparin, sodium salt, in formamide) and was acetylated homogeneously according to the procedure utilized by Wolfrom and Spoors[3] in the peracetylation of sodium chondroitin sulfate A. The product (III) was isolated as the sodium salt of an incompletely *O*-acetylated polymer (reacetylation did not increase the acetyl value) by pouring the reaction mixture into ether-ethanol (2:1), centrifuging, dissolving in very dilute (*ca.* 0.001*N*) sodium carbonate, dialyzing, and freeze-drying; $[\alpha]_D^{24}$ + 15° (*c* 1, water). *Anal.* Calcd. for $C_{22}H_{24}O_8(CO_2Na)_2(NHCOCH_3)_2(OCOCH_3)_{4.3}(OH)_{2.6}(OSO_2Na)_{1.1}$: C, 39.06; H, 4.33; $COCH_3$, 24.3; Na, 6.3. Found: C, 39.28; H, 4.55; $COCH_3$, 24.8; Na, 5.7; OCH_3, 0.0. An aqueous solution of III was passed through Amberlite IR-120 (H^+) ion exchange resin and the product (IV), isolated by freeze-drying, was dissolved in bis(2-methoxyethyl) ether and *N*,*N*-dimethylformamide (4:1) and reduced with diborane,[4] following the adaptation of Smith and Stephen.[5]

The reduced product (V), isolated by dialysis and freeze-drying, was partially de-*O*-acetylated with methanolic sodium methoxide; $[\alpha]_D^{25}$ + 52.5° (*c* 1, water). *Anal.* Calcd. for $C_{24}H_{28}O_8(NHCOCH_3)_2(OCOCH_3)_{2.5}(OH)_{6.6}(OSO_2Na)_{0.9}$: C, 43.4; H, 5.5; N, 3.1; S, 3.2; $COCH_3$, 21.15; Na, 2.26. Found: C, 43.7; H, 5.7; N, 3.6; S, 3.5; $COCH_3$, 20.8; Na, 1.6; uronic acid,[6] trace. The water-soluble product was hydrolyzed in 4*N* hydrochloric acid (*c* 1.0) at reflux for 8.5 hr. (rotation constant $[\alpha]_D^{25}$ + 40°, at 7 hr.) and the hydrolyzate was deionized. Paper chromatography on the hydrolyzate solution showed three spots with $R_{glucose}$ values: 0.32, 0.72 (2-amino-2-deoxyglucose), and 1.0. Concentration of the hydrolyzate to a sirup and addition of ethanol yielded crystals identified as 2-amino-2-deoxy-α-D-glucose hydrochloride. Isolative paper chromatography yielded sirups from the spots of $R_{glucose}$ 0.32 and 1.00. The latter sirup was converted to the acetylated diethyl dithioacetal according to the procedure of Wolfrom and Karabinos,[7] and the crystalline product obtained, in very low yield (*ca.* 2%), was identified as glucose diethyl dithioacetal; m.p. 46–47° unchanged on admixture with authentic material, x-ray powder diffraction pattern identical with that of authentic material.

The previous isolation of potassium hydrogen D-glucarate by oxidative hydrolysis[8] and its identification by optical rotation and analysis limit the uronic acid present in the heparin to D-glucuronic acid or L-guluronic acid. The above isolation of the D-glucose derivative eliminates the latter and confirms the recent notice of Foster, Stacey, and coworkers.[9] The sirup of $R_{glucose}$ 0.32 is under further investigation.

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