On Sulphate Placement in Heparin

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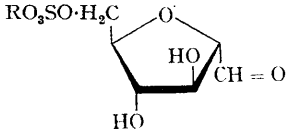
PREVIOUS publications from this laboratory have reported the isolation and characterization of crystalline degradation products from heparin which established the alternating α-D-(1→4) stereochemical relationship between the D-glucuronic acid and 2-amino-2-deoxy-D-glucose units in this heteropolymer.[1–3] The remaining structural problem on this natural blood anticoagulant is the exact placement of the five sulphate groups known to be present per tetrasaccharide repeating unit.[4,5] Two of these sulphates have been shown to be sulphoamino in nature.[6] There remains the placement of three sulphate ester groups on oxygen.

Nominé and co-workers[7] have presented evidence

that the C-3 hydroxyl of the 2-amino-2-deoxy-D-glucose unit is not sulphated through the isolation and characterization of 2-amino-2-deoxy-3-*O*-methyl-D-glucose on hydrolysis of a partially methylated heparin. Foster, Stacey, and co-workers[8] have obtained, presumably, an equimolar mixture of mono- and di-sulphated disaccharides by the nitrous acid deamination of *N*-desulphated heparin, and their results on the mixture (periodate treatment) suggest the presence of 2- and 6-sulphate groups in heparin.

Purified heparin[1] was oxidized[9] with periodate until exactly one mole of the oxidant per tetrasaccharide unit was consumed.[10] The oxidation apparently occurred at the glycol group of the unsubstituted D-glucuronic acid unit.[3] The oxidized product was reduced with borohydride and then hydrolyzed with 0·08N-sulphuric acid for 3 hr., which effected *N*-desulphation and cleaved the acetal linkage of the oxidized unit. Subsequent deamination with nitrous acid (generated from barium nitrite) followed by paper electrophoresis in borate solution revealed the presence of four distinct components: M_{Gl} 1·27, 1·02, 0·84, and 0·67. The zone with M_{Gl} 0·84 was identical in mobility with that from 2,5-anhydro-D-mannose[11] 6-(sodium sulphate) (I) prepared by the direct sulphation[12] of 2-amino-2-deoxy-D-glucose hydrochloride followed by nitrous acid deamination. The infrared spectrum of (I) has a clearly defined band at 816 cm.$^{-1}$, characteristic of a sulphated equatorial primary hydroxyl group, and thus of a 6-sulphate.[13] The material corresponding to M_{Gl} 0·84 was then isolated by preparative paper chromatography and converted[14] into a crystalline brucinium salt whose *X*-ray powder diffraction pattern was completely identical with that of the brucinium salt from the synthetic product (II); m.p. and mixed m.p. 200—201° (decomp.). Compound (II) exhibited satisfactory elemental analyses and was homogeneous on paper chromatography or paper electrophoresis after conversion into (I).

The evidence reported herein establishes, on a crystalline, isolative basis, that the C-6 hydroxyl group of the 2-amino-2-deoxy-D-glucose unit of heparin is sulphated. The characterization of the materials with M_{Gl} 1·27, 1·02, and 0·67 is in progress.



(I; R = Na)
(II; R = H·brucine)

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