L-Iduronic Acid in Purified Heparin

By M. L. WOLFROM,* S. HONDA, and P. Y. WANG
(*Department of Chemistry, The Ohio State University, Columbus, Ohio* 43210)

COMMERCIAL UPJOHN HEPARIN was purified through the crystalline barium acid salt[1,2] (three times recrystallized, 60% yield†), followed by reconversion into the sodium salt. The purified heparin was hydrolysed by the Klason method[3] using 72% sulphuric acid at 25° for 2 hr. The viscous solution was then diluted to an acid concentration of 3% and refluxed for 2·5 hr. The acid was neutralized by barium carbonate and the solution was filtered. Percolation through a column of cation-exchange resin removed the barium ion and the amino-sugar component from the hydrolysate. Concentration of the percolate gave the uronic acid fraction as a syrup (yield 13% of theory). Paper chromatography‡ resolved this syrup into four distinct components: R_{Glc} 0·70 (D-glucuronic acid), 1·21 (L-iduronic acid), 2·18 (D-glucuronolactone), and 3·23 (major, L-iduronolactone). The last component was isolated by preparative paper chromatography to give a syrup; $[\alpha]_D^{22}$ 0 → +25° (3 days; *c* 1 in water), lit. (free acid)[4] $[\alpha]_D^{22}$ +33° (4·5 hr.; *c* 3 in water). Attempted crystallization of this

† This procedure will remove some sulphuric acid groups from the amino-functions but this is not critical for the hydrolytic degradations reported.

‡ Descending paper chromatography was performed on Whatman No. 1 and No. 3 MM papers using the butan-1-ol–acetic acid–water (4 : 1 : 2) system at 18°. Zones were located by spraying with alkali–silver nitrate, naphthoresorcinol, or aniline hydrogen phthalate. R_{Glc} refers to mobilities relative to D-glucose.

isolated syrupy fraction was unsuccessful, and rechromatography showed the presence of two components: R_{Glc} 1·21 and 3·23, apparently due to rapid acid–lactone equilibration. Part of the syrup was treated with an ethanolic solution of brucine. The crystalline brucinium salt obtained had the formula $C_{29}H_{36}O_{11}N_2$ and exhibited a *X*-ray powder diffraction pattern completely identical with that of the brucinium salt of authentic[4] L-iduronic acid; m.p. and mixed m.p. 160—161° (decomp.).

Since the heparin was exhaustively purified by an established procedure, the L-iduronic acid isolated did not originate from any dermatan sulphate. However, it could be derived from D-glucuronic acid through C-5 epimerization. Fischer and Schmidt[5] have obtained L-iduronic acid by heating a neutral, aqueous solution of sodium D-glucuronate. Consequently, sodium chondroitin 6-sulphate and sodium D-glucuronate were hydrolysed by the Klason method,[3] but no traces of L-iduronic acid were found. These results minimized the possibility that the L-iduronic acid isolated from the hydrolysate of purified heparin was an artifact.

The presence in heparin of L-iduronic acid, as well as D-glucuronic acid, necessitates the reassessment of our previous work on the carboxyl-reduced heparin. Essentially the same result was obtained as reported[6] when 4N- or 1·5 N-hydrochloric acid was used for hydrolysis. When the partially acetylated, desulphated, and carboxyl-reduced heparin used in our previous work was hydrolyzed by the Klason method, L-idose and L-iduronic acid were readily detected among other expected products. The hydrolytic conditions used in most of our previous work[6,7] and some reported by others[8] appear to be too drastic to give all representative acid degradation fragments from heparin.

Some reports which inferred the presence of L-iduronic acid in heparin were, unfortunately, based on commercial preparations of unestablished purity.[9] Cifonelli and Dorfman[10] have found (chromatographically) L-iduronic acid in the hydrolysate derived from a Cetavlon-purified heparin. The evidence reported establishes the presence of L-iduronic acid in heparin, purified through the crystalline barium acid salt. The apparent predominance of L-iduronic acid over D-glucuronic acid in our partial hydrolysates may be due to the greater ease of hydrolysis of the L-iduronic acid linkage over that of the D-glucuronic acid.[10]

This work was supported by the U.S. Public Health Service, National Institutes of Health, and by the Office of Research, The Ohio State University.

(*Received, March 14th,* 1968; *Com.* 314.)

[1] A. F. Charles and D. A. Scott, *Biochem. J.*, 1936, **30**, 1927.
[2] M. L. Wolfrom, D. I. Weisblat, J. V. Karabinos, W. H. McNeely, and J. McLean, *J. Amer. Chem. Soc.*, 1943, **65**, 2077.
[3] Tappi Standard T-13 m-54; G. A. Adams in "Methods in Carbohydrate Chem.," ed. R. L. Whistler, Academic Press, New York, 1965, vol. V, p. 185.
[4] F. Shafizadeh and M. L. Wolfrom, *J. Amer. Chem. Soc.*, 1965, **77**, 2568.
[5] F. G. Fischer and H. Schmidt, *Chem. Ber.*, 1959, **92**, 2184.
[6] M. L. Wolfrom, J. R. Vercellotti, and H. G. S. Thomas, *J. Org. Chem.*, 1961, **26**, 2160, *ibid.*, 1964, **29**, 536; M. L. Wolfrom, J. R. Vercellotti, and D. Horton, *ibid.*, 1962, **27**, 705; 1963, **28**, 278, 279; 1964, **29**, 540, 547.
[7] M. L. Wolfrom and F. A. H. Rice, *J. Amer. Chem. Soc.*, 1946, **68**, 532.
[8] I. Danishevsky, H. B. Eiber, and E. Langholtz, *J. Biol. Chem.*, 1962, **237**, 1413; J. A. Cifonelli and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1961, **4**, 328; A. B. Foster, A. H. Olavesen, M. Stacey, and J. M. Webber, *Chem. and Ind.*, 1961, 143.
[9] Z. Yosizawa, *Tohoku J. Exp. Med.*, 1960, **72**, 140 (*Chem. Abs.*, 1961, **55**, 8519); B. Radhakrishnamurthy and G. S. Berenson, *Arch. Biochem. Biophys.*, 1963, **101**, 360; *Analyt. Chem.*, 1963, **35**, 1316; M. Marini and J. R. Helbert, *Abstracts Papers Amer. Chem. Soc.*, 1966, 151, C1; A. S. Perlin, private communication.
[10] J. A. Cifonelli and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1962, **7**, 41.