THE URONIC ACID OF HEPARIN*

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The direct isolation of a uronic acid from heparin has not been reported. The lability of uronic acids under conditions necessary for hydrolysis of heparin, requires indirect methods for identification of the uronic acid (Wolfrom and Karabinos, 1945). Following treatment of heparin with bromine in sulfuric acid, Wolfrom and Rice (1946) isolated glucaric acid. Yosizawa (1960) found principally glucitol following reduction of both the carboxyl and aldehyde groups of a mixture of oligosaccharides obtained by the acid hydrolysis of heparin. The presence of glucuronic acid was also indicated by the isolation of glucose (Cifonelli and Dorfman, 1961; Wolfrom and Vercellotti, 1961) in relatively small yields following reduction of the carboxyl groups of desulfated heparin and by the isolation of glucuronic acid following hydrolysis of N-acetylated heparin (Foster, et al., 1961). The presence of a ketouronic acid in heparin has been suggested on the basis of colorimetric data (Brown, et al., 1961). However, a product obtained by reduction of esterified heparin with sodium borohydride (Cifonelli and Dorfman, 1961) was negative by the ketose reaction (Dische and Borenfreund, (1951).

Since hydrolysis of heparin and of the desulfated N-acetylated heparin did not yield free uronic acid other conditions were sought which might

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result in the release of free uronic acids. When oligosaccharides, obtained after partial hydrolysis of N-acetylated heparin, were again acetylated and subjected to further hydrolysis with 1 N HCl from 1 to 3 hours, evidence was obtained of the release of free uronic acids. Figure 1 demonstrates that hydrolysis of acetylated oligosaccharides (AOH) contain compounds which migrated as do glucuronolactone and iduronolactone. No such compounds were observed as a result of direct hydrolysis of heparin (HH). Increased amounts of free uronolactones could be obtained by repeating acetylation and hydrolysis. For further identification, the material migrating as iduronolactone was isolated by paper chromatography using a solvent of ethyl acetate:acetic acid:H_2O (3:1:3).

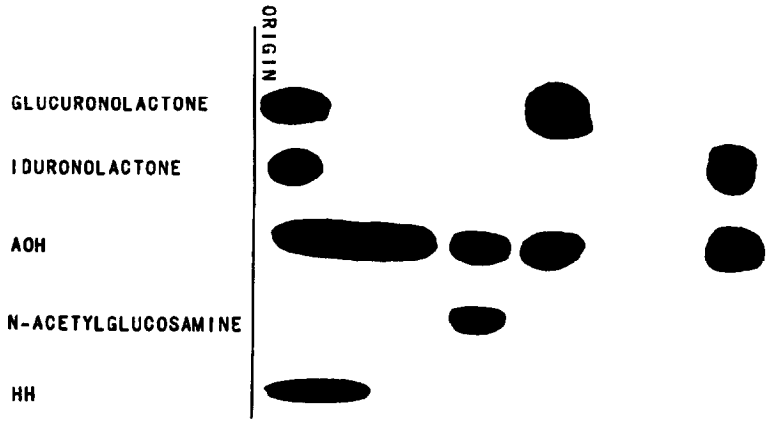

Fig. 1. Paper chromatogram. Solvent is t-amyl alcohol:i-propanol:H_2O (4:1:2). Descending chromatogram, 18 hours, developed with aniline oxalate. AOH = acetylated oligosaccharide hydrolysate; HH = heparin hydrolysate.

The results of paper chromatography of these purified fractions are shown in Figure 2. Clearly the isolated compound migrates as does iduronolactone. After reduction with sodium borohydride, and deionization, the product migrated as idonolactone by paper chromatography. Under these conditions it could be differentiated from gulonolactone, gluconolactone and mannonolactone (Figure 3). Chromatography in 3 other solvent systems yield similar results.

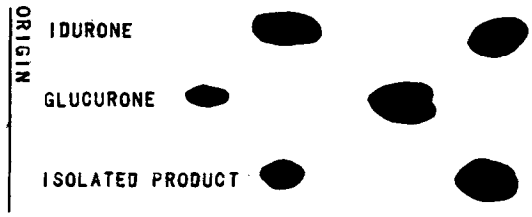

Fig. 2. Paper chromatogram of purified product. Solvent is t-amyl alcohol: 1-propanol:H_2O (4:1:2). Descending chromatogram, 18 hours, developed with aniline oxalate.

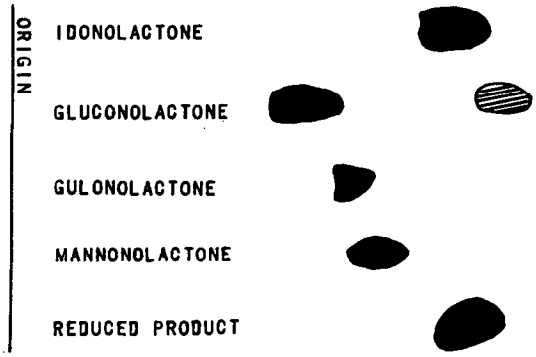

Fig. 3. Paper chromatogram of reduced uronic acid. Solvent is t-amyl alcohol: 1-propanol:H_2O (4:1:2). Descending chromatogram, 18 hours, developed with silver nitrate.

The iduronolactone isolated by paper chromatography and purified with charcoal had an $[\alpha]_D^{24} = +27^\circ$ [H_2O:C,1]. The reported optical rotation of L-iduronic acid $[\alpha]_D^{22} = +33^\circ$ (Shafizadeh and Wolfrom, 1955).

Since chondroitinsulfuric acid-B (CSA-B) is known to contain L-iduronic acid (Hoffman, et al., 1956; Cifonelli, et al., 1957), it was important to ascertain whether the iduronic acid isolated might have originated from contaminating CSA-B. The following facts suggest that this was not the case: (1) free uronic acid could not be demonstrated following hydrolysis of the heparin preparation under conditions which result in the release of iduronic acid from CSA-B, (2) following the addition of 10% CSA-B to the heparin, the release of iduronic acid by hydrolysis was readily demonstrated, (3) following complete hydrolysis of the heparin preparation with 4 N HCl for 14 hours, no

galactosamine could be detected, (4) the heparin used was purified by precipitation with cetylpyridinium chloride in the presence of 1.4 M NaCl. No CSA-B was found in the precipitate under these conditions. The material was further purified by chromatography on Dowex 1, Cl (2X, 200-400 mesh) as described by Schiller, et al., (1961), and (5) no N-acetyl could be detected in the heparin preparation.

Application of similar procedures to a purified fraction of heparin monosulfate resulted in the isolation of products which showed a similar pattern on paper chromatography. These data again point out the similarity in structure between heparin monosulfuric acid and heparin.

The unexpected finding in heparin and heparin monosulfuric acid of a uronic acid behaving as L-iduronic acid is of considerable interest in that CSA-B shares with heparin certain anticoagulant properties (Grossman and Dorfman, 1957), in contrast to the glucuronic acid-containing isomers, chondroitinsulfuric acids A and C. Of further interest is the fact that CSA-B and heparin monosulfuric acid are the two polysaccharides excreted and deposited in abnormal amounts in the Hurler syndrome (Dorfman and Lorincz, 1957). The presence of two different uronic acids in heparin is of interest in view of the recent finding (Cifonelli and Dorfman, 1961) that the amino sugar is substituted in both the 4 and 6 positions.

Acknowledgments

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References

Brown, K. D., Rosenthal, W. A., and Helbert, J. R. A.C.S. Abstracts 8D, Sept., 1961, Chicago.
Cifonelli, J. A., Ludowieg, J. and Dorfman, A., J. Biol. Chem., 233, 541 (1958).
Cifonelli, J. A. and Dorfman, A., Biochem. Biophys. Res. Communs., 4, 328 (1961).
Dische, Z. and Borenfreund, E., J. Biol. Chem., 192, 583 (1951).
Dorfman, A. and Lorincz, A. E., Proc. Natl. Acad. Sci., U.S., 43, 443 (1957).
Foster, A. B., Olavesen, A. H., Stacey, M., and Webber, J. M., Chem. and Ind., 143 (1961)

Grossman, B. J. and Dorfman, A., Pediatrics 20, 506 (1957).
Hoffman, P., Linker, A., and Meyer, K., Science 124, 1252 (1956).
Schiller, S., Slover, G. A. and Dorfman, A., J. Biol. Chem., 236, 983 (1961).
Shafizadeh, F. and Wolfrom, M. L., J. Am. Chem. Soc., 77, 2568 (1955).
Wolfrom, M. L. and Karabinos, J. V., J. Am. Chem. Soc., 67, 679 (1945).
Wolfrom, M. L. and Rice, F. A. H., J. Am. Chem. Soc., 68, 532 (1946).
Wolfrom, M. L., Vercelotti, J. R. and Thomas, G. H. S., J. Org. Chem., 26, 2160 (1961).
Yozizawa, Z., Tohoku J. Exp. Med., 72, 140 (1960).