Biochemical Studies on Carbohydrates

CLIV. Mucosin

Fourth Report

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Jeanloz and Forchielli¹⁾ claimed that, in mucoitin (hyaluronic acid) glucuronic acid combines 3-C of the adjacent acetylglucosamine glucosidically, standing on the basis that mucoitin had used 0.2 to 0.3 mole of periodate per mole, releasing 0.1 mole of formic acid, and a scission product, partially deacetylated by alkali, of the polysaccharide 0.8 to 1.0 mole of the oxidant without evolution of ammonia. Those quantities of periodate reduced pointed to the fact that oxidation had occurred in the chain extremities alone of the native polysaccharide and the disintegration product, and it was reasoned therefrom that the monosaccharide components both possess a pyranose structure.

On the other hand, Kurt H. Meyer and Fellig²⁾ insisted the linkage in question to be 1-4, because they obtained, by permethylation of mucoitin followed by methanolysis, a dimethylmethylglucosaminide which did not react with periodic acid. To draw the conclusion, they hypothesized the pyranose structure of the hexose units.

Both of the two groups of investigators assumed β -glucuronide- and β -glucosaminide-union from the negative optical rotation of mucoitin.

In order to settle the dispute the writer took N-acetylmucosin methyl-ester³⁾ as a material and subjected to periodic acid oxidation under the conditions preferred by Yosizawa⁴⁾ and also estimated the amount of formaldehyde produced according to Masamune and Yosizawa.⁵⁾ The substance reduced 2.96 moles (99% of theor.) of the oxidant per mole and released 0.93 mole (93% of theor.) of the aldehyde. The result indicates that either glucosamine-4- β -glucuronide (1,5) (I) or glucosamine-3- β -glucuronide (1,4) (II) is the configuration to be assigned to mucosin. The enzymological evidence that mucosin is a β -glucuronide has been recorded already in the Third Report. An experiment as to whether

glucuronic acid exists in a pyranose structure or in a furanose structure is now being in progress.

EXPERIMENTAL

N-acetylmucosin methylester was prepared as described in the Second Report.³⁾ 37.54 mg. of it were dissolved in 7 cc. of water and after addition of 3.5 cc. of 0.2595 *M* HIO_4 , maintained at 20°C, until the change of optical rotation ceased (About 6 hours required). Thereupon 4.5 cc. portions of the mixture were pipetted out for estimation of HPO_4 according to Yosizawa⁴⁾ and of formaldehyde according to Masamune and Yosizawa⁵⁾. 0.278 $\times 10^{-3}$ mole of HIO_4 remained unreduced and 10.2 mg. of formaldehyde-dimedon compound [m. p. (cor.) 189–190°C] were separated. Calculated from the values, the substance consumed 2.96 $\left[= (0.2595 \times 3.5 \times 4.5/10.5 - 0.278) \times 10^{-3} \times \frac{429}{37.54 \times 4.5/10.5} \times 10^3 \right]$ moles of HIO_4 per mole and gave 0.93 $\left[= \left(10.2 \times 10^{-3} \times \frac{429}{37.54 \times 4.5/10.5} \times 10^3 \right) / 292 \right]$ mole of the aldehyde.

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References

- 1) Jeanloz, *Experientia*, 1950, **6**, 52; Jeanloz and Forchielli, *J. Biol. Chem.*, 1951, **190**, 537.
- 2) Meyer, Kurt H. and Fellig, *Experientia*, 1950, **6**, 186.
- 3) Isikawa, *Tohoku J. Exp. Med.*, 1951, **54**, 197.
- 4) Yosizawa, *ibid.*, 1950, **52**, 111.
- 5) Masamune and Yosizawa, *ibid.*, 1951, **55**, 43.
- 6) Isikawa, *ibid.*, 1951, **55**, 23.