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Uronic Acid of Chondroitin Sulfate B

Chondroitin sulfate B has been isolated as the major mucopolysaccharide component of skin, ligamentum nuchae, tendon, and heart valves, and it has also been found in other tissues (*1*). It was differentiated from the chondroitin sulfates A and C by its rotation, its solubility in aqueous ethanol, and its inertness to testicular hyaluronidase (*2*). Desulfated B was differentiated from desulfated A by its resistance to the bacterial hyaluronidases. All the chondroitin sulfates show molar ratios of unity of uronic acid-to-N-acetylgalactosamine-to-sulfate when the uronic acid is determined by the orcinol colorimetric (*3*) or the Tracey CO_2 (*4*) method. However, the carbazole colorimetric value (*5*) of B is markedly lower. The low value is retained in the desulfated product and also in fractions of low molecular weight obtained after acid hydrolysis.

The five available uronic acids or their lactones were investigated, and three yielded low carbazole values (Table 1), indicating that a uronic acid other than glucuronic may be present in B.

Chondroitin sulfate B (carbazole-to-orcinol ratio = 0.41) was hydrolyzed on the steam bath in the presence of Dowex 50 (H^+). The products of hydrolysis were fractionated on a Dowex 1 × 10 (acetate) column with increasing concentrations of acetic acid. The first acidic fraction removed was a mixed disaccharide fraction that was separated

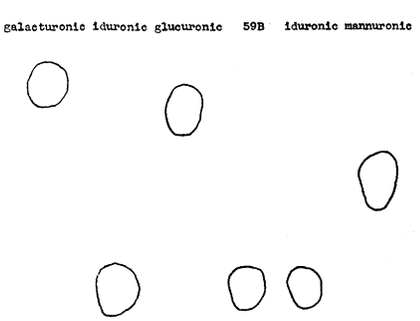

Fig. 1. Trace of a paper chromatogram of the uronic acids and of sample 59B isolated from chondroitin sulfate B. Guluronic acid (not shown) has a mobility similar to that of glucuronic acid in this solvent system (*7*).

Table 1. Colorimetric uronic acid values of the uronic acids or their lactones and fractions isolated from the Dowex-1 column. D-Glucurone was used as a standard.

Compound	Carbazole	Orcinol	Carbazole/orcinol ratio
D-Glucurone	100	100	1.0
L-Gulurone	32	106	.30
L-Iduronic acid	29	130	.22
D-Mannurone	17	128	.13
D-Galacturonic acid	120	127	.95
57B	21	66	.32
59B	41	92	.45
Disaccharide I	12	53	.23
Disaccharide II	33	29	1.2

preparatively by paper chromatography (butanol, acetic acid, and water; 50:12:25) into disaccharides I and II. Disaccharide I, the major disaccharide product, yielded a carbazole-to-orcinol ratio similar to that obtained from iduronic acid (Table 1), and a hydrolyzed sample produced a spot on a paper chromatogram with an R_f value identical to that of iduronic acid. The disaccharide has recently been obtained in crystalline form and is under investigation. It was similarly shown that disaccharide II contains glucuronic acid as its uronic acid moiety. Both disaccharides contained galactosamine as the only demonstrable hexosamine present under the conditions of the ninhydrin oxidation method (*6*).

The next acidic fraction from the Dowex-1 column was a sirup. It was passed through an Amberlite IRC 50 (Na^+) column and freeze dried as the crude sodium salt. Samples 57B and 59B (Table 1) were obtained in two different runs. The reducing values indicated that these were monosaccharides. Paper chromatography (Fig. 1) revealed a spot with an R_f value equal to that of crystalline L-iduronic acid. A trace spot with an R_f value equal to that of glucuronic acid was also present, and this accounts for the difference in carbazole-to-orcinol ratios of the isolated acid and synthetic iduronic acid.

Aqueous solutions of the free uronic acids or their lactones, with the exception of galacturonic acid, which does not form a lactone, each showed two spots on paper chromatograms due to acid-lactone equilibria. The isolated uronic acid and the synthetic iduronic acid had identical R_f values for both acid and lactone in four different solvent systems. Identical mobilities of the isolated and synthetic acids were also obtained on paper electrophoresis in 0.1*M* borate buffer (*p*H 9.4) and in 0.1*N* acetic acid.

This appears to be the first isolation of iduronic acid from a biological source and of a uronic acid other than glucuronic acid from an acid mucopolysaccharide. However, there is an interesting parallel in the isolation of L-guluronic acid from alginic acid (*8*), for L-guluronic acid is the C-5 epimer of D-mannuronic acid, the same relationship that L-iduronic acid bears to D-glucuronic acid (*9*).

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Anomalous Carbon-14 Content of Carbon Dioxide from Sewer Gas

During a study of the carbon-14 content of atmospheric carbon dioxide, an attempt was made to use sewer gas as a source of modern carbon. In the course of this work, an anomalous, high content of C^{14} in the CO_2 fraction of this gas has been found.

Sewer gas was piped directly into our laboratory from the adjacent sewage-treatment plant that processes sewage from the District of Columbia. The plant employs primary treatment followed by anaerobic digestion. The gas from the digestion process consists principally of methane (65 to 70 percent) and carbon dioxide (30 to 35 percent) with low percentages of nitrogen, oxygen, carbon monoxide, and other gases. A similar material was used in the first identification of C^{14} in nature (*1*).

Carbon dioxide samples were obtained by passing the sewer gas through a sodium hydroxide solution. The remainder of the CO_2 was removed by suitable absorbers, the CO_2-free methane was converted to CO_2 by passage over hot copper oxide, and the CO_2 was absorbed