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Note

Use of DEAE-cellulose paper in the paper chromatographic separation of uronic acids

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In the course of our studies on polysaccharides we have had occasion to identify by paper chromatography the uronic acids present in polysaccharide hydrolysates. While using the solvent system ethyl acetate–acetic acid–water, (3:1:1), it was found that glucuronic acid could be clearly separated from galacturonic acid and most of the common reducing sugars on DEAE-cellulose paper (Whatman DE-81), whereas these separations could not be achieved by us with Whatman No. 1 paper, a most commonly used cellulose paper, or with Whatman No. 20, a very close grained cellulose paper.

EXPERIMENTAL AND RESULTS

Aliquots (5 μl) of aqueous solutions containing uronic acids and reducing sugars, 5 mg/ml, were applied to the line of origin of the paper chromatograms and eluted descendingly for 24–48 h or over the weekend with the solvent system described above. This system was convenient to use because it was monophasic, did not contain pyridine or other solvents with more objectionable odors, or cause the paper to deteriorate as systems containing strong acids tend to do. The uronic acids and reducing sugars were then located by dipping the DEAE paper in 4% ethanolic aniline malonate, and subsequent air drying and heating as described in Caldes and Prescott[1]. It was necessary to use a higher concentration of aniline malonate than the 2% used for ordinary cellulose papers, otherwise the heating had to be greatly prolonged. The color of the galacturonic acid spot was a deeper color than that of the glucuronic spot, although they were both of orange-brown hue.

Figs. 1 and 2 show typical chromatograms obtained with Whatman No. 1 and Whatman DE-81 paper, respectively. The uronic acids migrated faster on the Whatman No. 1 paper, but glucuronic acid did not clearly separate from galacturonic acid,

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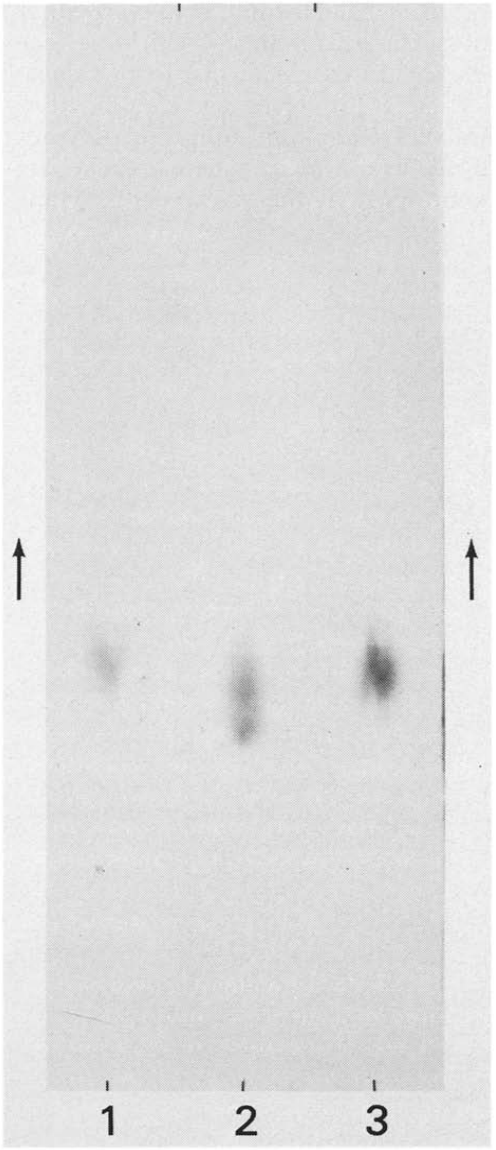

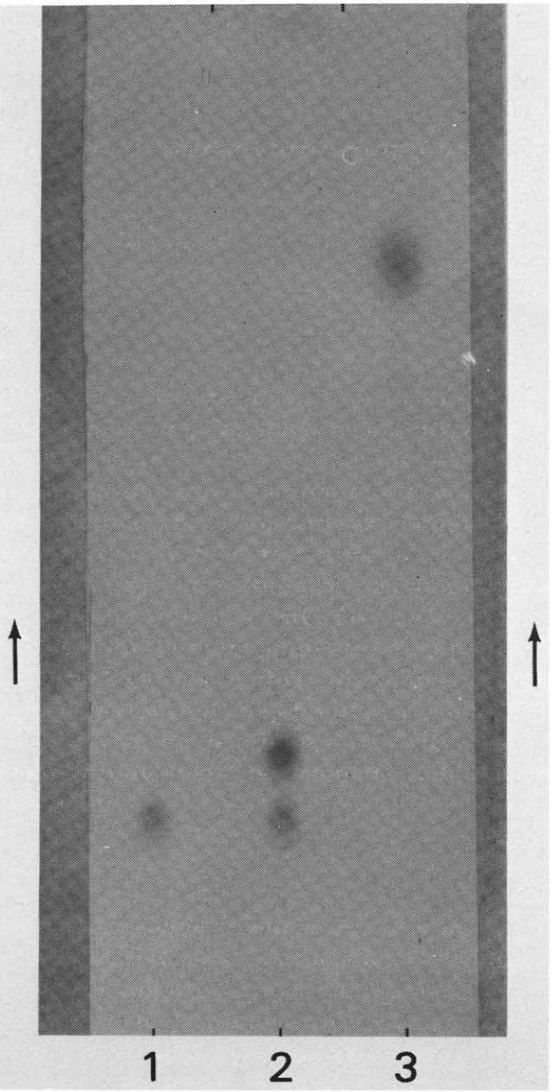

Fig. 1. Descending chromatography of uronic acids and glucose, 50 μg each, on Whatman No. 1 paper for 24 h, with the solvent ethyl acetate–acetic acid–water (3:1:1). Glucuronic acid was applied to spot 1 on the line of origin at the left. A mixture of glucuronic and galacturonic acids was applied to the center spot (2), and glucose was applied to the spot at the right (3).

Fig. 2. As Fig. 1, except that Whatman DE-81 paper was substituted for the Whatman No. 1 paper, and the paper was eluted for 30 h.

although these uronic acids could be distinguished by the difference in color and the separation of the spot nuclei. Increasing the time of elution beyond 24 h did not improve the separations on Whatman No. 1 paper. Other solvent systems tried with Whatman

No. 1 paper in an effort to separate glucuronic from galacturonic acid were ethyl acetate–acetic acid–water, (3:1:3)[2], and butanol–acetic acid–water (4:1:5)[3]. Neither of these solvent systems in our hands completely separated glucuronic from galacturonic acid. Another advantage of using the DE-81 paper for uronic acid identification was that glucose and galactose, reducing sugars very commonly found in polysaccharide hydrolysates, migrated much faster than the uronic acids, whereas on Whatman No. 1 paper they migrated at about the same speed as the uronic acids. Man-

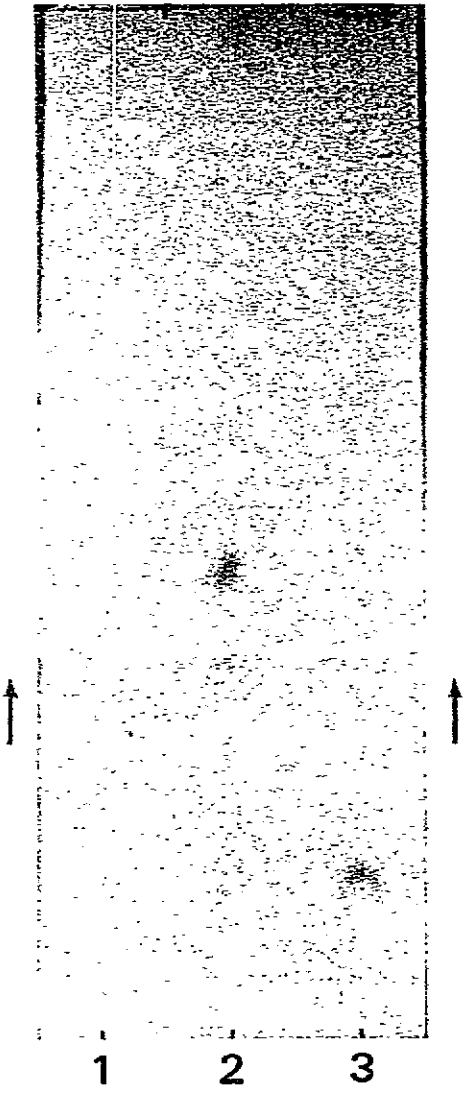

Fig. 3. Descending chromatography of a dilute acid hydrolysate of Type III pneumococcal polysaccharide and standards on Whatman DE-81 paper for 48 h. An amount of hydrolysate equivalent to 125 μg of polysaccharide was applied to spot 1 at the left, 250 μg at the right (spot 3), and 50 μg each of glucuronic, galacturonic, and synthetically prepared cellobiouronic acid at the center spot (2).

nose, arabinose, xylose, and rhamnose also were separated from the uronic acids on DEAE paper, but they could also be separated on Whatman No. 1 paper.

Cellobiuronic acid could be clearly separated from glucuronic and galacturonic acid on DEAE paper, and this property was used to help identify cellobiuronic acid in a hydrolysate of Type III pneumococcal polysaccharide (Fig. 3). Prolonged heating, 3 h at 100°C, was necessary to bring out the color of the cellobiuronic acid spots, even with 4% aniline malonate solution. Increasing the concentration of malonate to 10% speeded the color development.

Mannuronic acid could not be completely separated from galacturonic acid, using the 3:1:1 solvent system, although it did migrate a little faster. It could, however, be easily separated from glucuronic acid. There was one other difference between Whatman DE-81 and Whatman No. 1 paper: on DEAE paper, galacturonic acid migrated faster, whereas on cellulose paper glucuronic acid migrated faster.

Aminoethanol paper (Whatman AE-81) and silica gel paper (Whatman SG-81) were also tried but did not separate the uronic acids with the 3:1:1 solvent system.

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