# CHROMATOGRAPHY OF URONIC ACIDS, SUGAR ACID LACTONES, AND GLUCURONOSIDES ON ANION-EXCHANGE PAPER OR DEAE-CELLULOSE

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For the adsorption chromatography of sugar acids and their lactones on regular paper various solvent systems have been proposed[1-8], most of them containing organic acids. After column chromatography on ion-exchange resins had become a standard method in analytical biochemistry[9,10], the introduction of ion-exchange paper offered new possibilities to the chromatographic analysis of such polar compounds[11], since it combined the well known advantages of paper chromatography with those of ion-exchange procedures. In the present communication the application of paper chromatography on anion-exchange paper to the separation of uronic acids, lactones and glucuronosides is described.

## MATERIALS AND METHODS

50 or 100 $\mu$g of the following compounds were submitted to descending or ascending paper chromatography:

1. D-Glucuronic acid, sodium salt (Serva Entwicklungslabor, Heidelberg)
2. D-Galacturonic acid (Deutsche Hoffmann-La Roche, AG, Grenzach)
3. D-Glucuronic acid $\gamma$-lactone (Deutsche Hoffmann-La Roche, AG, Grenzach)
4. D-Gluconic acid $\gamma$-lactone (Serva Entwicklungslabor, Heidelberg)
5. D-Galactonic acid $\gamma$-lactone (Deutsche Hoffmann-La Roche, AG, Grenzach)
6. L-Gulonic acid $\gamma$-lactone (Serva Entwicklungslabor, Heidelberg)
7. $\alpha$-D-Glucoheptonic acid $\gamma$-lactone (Serva Entwicklungslabor, Heidelberg)
8. Phenolphthalein glucuronoside (Schering AG, Berlin)
9. Isonicotinic acid hydrazide glucuronoside (Hormonchemie, München).

The following commercially available anion-exchange papers were used:

Whatman DE-20, ion-exchange capacity: 0.4 mequiv./g

Whatman ET-20, ion-exchange capacity: 0.3 mequiv./g

Schleicher & Schüll Anionen-Austauscher Papier, with approx. 5 % Dowex 2 X8.

For the application of the substances to paper strips of 35 × 3 cm, a mixture of pyridine–methanol (1:1 v/v) was employed. The paper strips were cut as suggested in a previous publication[12] in order to produce compact spots. Of the various solvent systems tried the following mobile phases were selected for use with the appropriate anion-exchange paper:

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A. Methanol–water–acetic acid (80:15:5 v/v)
B. Ethanol–water–acetic acid (85:10:5 v/v)
C. Isopropanol–water–formic acid (80:18:2 v/v)
D. Isopropanol–water–formic acid (85:14:1 v/v)
E. Ethanol–0.13 *M* acetate buffer of pH 4.7 (80:20 v/v)
F. Ethanol–0.5 *M* acetate buffer of pH 4.8 (75:25 v/v)
G. Ethanol–0.5 *M* acetate buffer of pH 4.8 (85:15 v/v).

After the solvent front had reached a line approximately 30 cm from the origin, the time of development depending on the type of paper used as well as the solvent system, the chromatogram was removed and thoroughly dried for 2 hours at 60°. For detection of the different compounds three spot tests were performed:

1. Spraying with benzidine reagent (0.5 g benzidine in 80 ml ethanol and 200 ml acetic acid) and drying for 10 min at 100°[13].

2. Dipping into silver nitrate reagent (0.1 ml saturated silver nitrate, added to 20 ml acetone, and diluted with enough water to dissolve the precipitate), spraying with 0.5 *N* sodium hydroxide in 80% ethanol, drying at room temperature, and washing with 10% sodium thiosulfate[14–16].

3. Spraying with 0.1% naphthoresorcinol in 20% trichloroacetic acid in *n*-butanol and heating for 5 min at 100°[17].

Finally, the $R_F$ values of each substance were determined from three individual runs.

In order to separate glucuronosides from glucuronic acid or glucuronic acid $\gamma$-lactone by column chromatography, a mixture of such compounds in 1.0 ml methanol was applied to a column (height: 2.5 cm; diameter: 0.9 cm) of 400 mg Whatman DEAE-cellulose (Whatman DE-50 floc; ion-exchange capacity: 1.0 mequiv./g), prepared with methanol. Elution was carried out with 20 ml of the following solvents:

methanol
methanol–water (80:20 v/v)
methanol– 0.5% acetic acid (80:20 v/v)
methanol– 1.0% acetic acid (80:20 v/v)
methanol– 2.5% acetic acid (80:20 v/v)
methanol– 5.0% acetic acid (80:20 v/v)
methanol–10.0% acetic acid (80:20 v/v)
methanol–15.0% acetic acid (80:20 v/v)
methanol–20.0% acetic acid (80:20 v/v)
methanol–40.0% acetic acid (80:20 v/v)

at a flow rate of 1 ml/min. Each fraction was evaporated in vacuo at 50° and the residue redissolved in 1 ml methanol. In one aliquot of each fraction the amount of glucuronic acid present was determined by the naphthoresorcinol method[18]. The remaining aliquot of all fractions containing naphthoresorcinol-positive material was subjected to chromatography on Whatman ET-20 in solvent system A in order to establish the identity of compounds eluted from the column.

## RESULTS

The $R_F$ values of the various compounds, obtained after chromatography on different anion-exchange papers are recorded in Tables I-III. As indicated, in addition to the

TABLE I

DESCENDING PAPER CHROMATOGRAPHY ON WHATMAN ET-20

| Compound | $R_F$ value in solvent system: | | | |
|---|---|---|---|---|
| | *A* | *C* | *E* | *F* |
| Glucuronic acid | 0.33 | 0.32 | 0.05 | 0.14 |
| Galacturonic acid | 0.38 | 0.35 | 0.07 | 0.16 |
| Glucuronic acid $\gamma$-lactone | 0.68 | 0.53 | (0.05) 0.56 | (0.15) 0.58 |
| Gluconic acid $\gamma$-lactone | 0.76 | 0.60 | 0.74 | (0.21) 0.73 |
| Galactonic acid $\gamma$-lactone | 0.70 | 0.58 | 0.65 | (0.22) 0.64 |
| Gulonic acid $\gamma$-lactone | 0.66 | 0.51 | (0.07) 0.55 | (0.16) 0.61 |
| Glucoheptonic acid $\gamma$-lactone | 0.64 | 0.47 | (0.08) 0.51 | (0.20) 0.59 |
| Phenolphthalein glucuronoside | 0.78 | 0.63 | 0.88 | 0.75 |
| Isonicotinic acid hydrazide glucuronoside | 0.76 | 0.62 | 0.73 | 0.71 |
| Development at 22° | 3–4 h | 5–6 h | 3 h | 4–5 h |

Parentheses indicate a minor spot.

TABLE II

DESCENDING PAPER CHROMATOGRAPHY ON WHATMAN DE-20

| Compound | $R_F$ value in solvent system: | | |
|---|---|---|---|
| | *A* | *C* | *F* |
| Glucuronic acid | 0.16 | 0.14 | 0.21 (0.56) |
| Galacturonic acid | 0.23 | 0.21 | 0.25 |
| Glucuronic acid $\gamma$-lactone | (0.16) 0.71 | (0.14) 0.44 | (0.21) 0.56 |
| Gluconic acid $\gamma$-lactone | (0.29) 0.76 | (0.30) 0.50 | (0.38) 0.70 |
| Galactonic acid $\gamma$-lactone | (0.30) 0.74 | (0.31) 0.49 | (0.38) 0.69 |
| Gulonic acid $\gamma$-lactone | (0.27) 0.75 | (0.26) 0.47 | (0.36) 0.65 |
| Glucoheptonic acid $\gamma$-lactone | (0.25) 0.70 | (0.22) 0.41 | (0.28) 0.55 |
| Phenolphthalein glucuronoside | 0.82 | 0.64 | 0.74 |
| Isonicotinic acid hydrazide glucuronoside | 0.76 | 0.52 | 0.69 |
| Development at 22° | 5–6 h | 8–10 h | 5–6 h |

Parentheses indicate a minor spot.

TABLE III

DESCENDING PAPER CHROMATOGRAPHY ON SCHLEICHER & SCHÜLL ANIONEN-AUSTAUSCHER PAPIER

| Compound | $R_F$ values in solvent system: | | |
|---|---|---|---|
| | *B* | *D* | *G* |
| Glucuronic acid | 0.19 | 0.37 | 0.11 |
| Galacturonic acid | 0.28 | 0.39 | 0.15 |
| Glucuronic acid $\gamma$-lactone | 0.58 | 0.60 | 0.57 |
| Gluconic acid $\gamma$-lactone | 0.70 | 0.66 | (0.25) 0.72 |
| Galactonic acid $\gamma$-lactone | 0.61 | 0.63 | 0.64 |
| Gulonic acid $\gamma$-lactone | 0.54 | 0.52 | 0.16 0.58 |
| Glucoheptonic acid $\gamma$-lactone | 0.51 | 0.48 | 0.16 (0.55) |
| Phenolphthalein glucuronoside | 0.77 | 0.81 | 0.80 |
| Isonicotinic acid hydrazide glucuronoside | 0.69 | 0.65 | 0.69 |
| Development at 22° | 4–5 h | 8–9 h | 3–4 h |

Parentheses indicate a minor spot.

TABLE IV

ASCENDING PAPER CHROMATOGRAPHY ON WHATMAN ET-20

| *Compound* | *$R_F$ value in solvent system A* |
|---|---|
| Glucuronic acid | 0.23 |
| Galacturonic acid | 0.31 |
| Glucuronic acid $\gamma$-lactone | 0.57 |
| Gluconic acid $\gamma$-lactone | 0.62 |
| Galactonic acid $\gamma$-lactone | 0.68 |
| Gulonic acid $\gamma$-lactone | 0.53 |
| Isonicotinic acid hydrazide glucuronoside | 0.69 |
| Development at 20° | 4–5 h |

spots of assumedly pure material frequently other minor spots could be detected, especially after chromatography on DEAE-cellulose in the form of Whatman DE-20 anion-exchange paper. As compared to descending paper chromatography the more time-consuming ascending technique led to similar results. However, the corresponding $R_F$ values were found to be slightly lower (Table IV). Fig. 1 represents a typical elution diagram obtained by column chromatography of phenolphthalein glucuronoside and glucuronic acid $\gamma$-lactone on DEAE-cellulose (Whatman DE-50 floc). In contrast to phenolphthalein glucuronoside, which is eluted with 0.5% acetic acid–methanol (20:80 v/v), practically all the glucuronosides occurring in normal human urine can be removed from the column by methanol and 80% methanol.

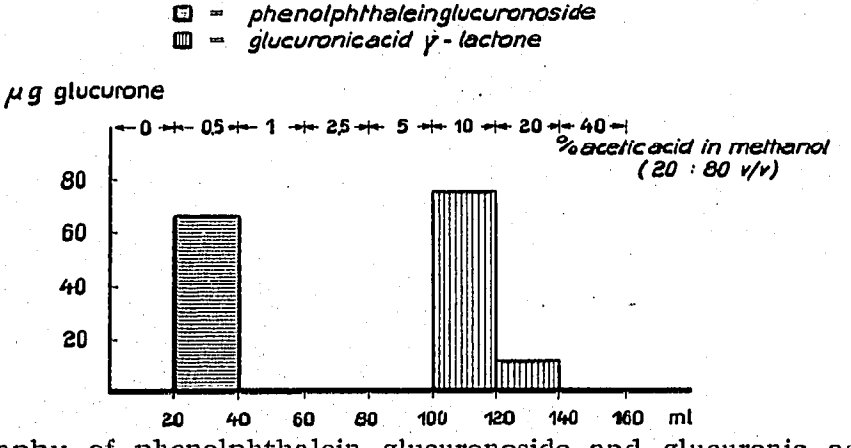


Fig. 1. Chromatography of phenolphthalein glucuronoside and glucuronic acid $\gamma$-lactone on DEAE-cellulose (Whatman DE-50 floc).

## DISCUSSION

From the data presented it becomes evident, that anion-exchange paper of the type DEAE-cellulose (Whatman DE-20), ECTEOLA cellulose (Whatman ET-20) and resin-impregnated paper (Schleicher & Schüll Anionen-Austauscher Papier, with Dowex 2 X8) may be successfully used for chromatography of uronic acids, sugar acid lactones, and glucuronosides. The results obtained with certain anion-exchange papers compare favorably with those reported for procedures involving ordinary paper. For instance, by using ECTEOLA paper ET-20 and solvent system A adequate

separation of various compounds seems possible within a rather short time.

A disadvantage of the anion-exchange papers, especially DEAE paper DE-20 obviously consists in an apparent shift of the equilibrium between sugar acid and its lactone. The hydrolysis of lactones becomes more pronounced upon chromatography on paper with a higher anion-exchange capacity and application of less acidic solvent systems.

Column chromatography of uronic acids, sugar acid lactones, and glucuronosides may be recommended for the separation of larger amounts of such compounds, which cannot be handled by paper chromatography.

Based on the experimental data presented in this communication a paper chromatographic method for the estimation of glucuronosides in urine and plasma has been developed. It involves chromatography of urinary extracts on ECTEOLA ET-20, in order to improve the specificity of such a determination, usually performed without any purification. Details of this procedure will be given in another periodical. Likewise, a rapid and quantitative separation of steroid sulfates and glucuronosides can be achieved by paper or column chromatography on anion-exchange cellulose, which will be described in a subsequent paper.

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SUMMARY

For the separation of uronic acids, sugar acid lactones, and glucuronosides paper chromatography on anion-exchange paper, such as Whatman ET-20, Whatman DE-20, and Schleicher & Schüll Anionenaustauscher Papier with Dowex 2 X8 may be employed. Mixtures of alcohols, water, and organic acids or buffers serve as mobile phases. In a similar manner, glucuronosides and glucuronic acid or glucuronic acid $\gamma$-lactone can be successfully chromatographed on columns of anion-exchange cellulose (Whatman DE-50 floc).

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