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**Abstract**

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# THIN-LAYER CHROMATOGRAPHY OF POLYHYDROXY COMPOUNDS ON CELLULOSE

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In a previous paper (^1), undertaken by us in connection with the study of the products of oxidative cleavage of certain macrolide antibiotics, it was shown that polyhydroxy compounds can be separated by thin-layer chromatography on silica gel and detected with specific reagents for the $\alpha$-glycol grouping. However, the sensitivity of these reagents proved to be 2-5 times lower than in paper chromatography, and development of the chromatograms required a rather long time (up to 4 hr). In order to avoid these drawbacks and achieve better separation of polyhydroxy compounds, we tested cellulose powder as the adsorbent. Cellulose has recently been used for thin-layer chromatography of alkaloids (^2) and nucleotides (^3). In the first case the cellulose was applied to the plates as an aqueous suspension, in the second as an acetone suspension, after which the plates were dried with warm air. After development, the substances were detected by their absorption in ultraviolet light.

When, in our attempts, we tried to use plates prepared in this way for the separation of polyhydroxy compounds, it turned out that the adsorbent layer was not strong and crumbled when sprayed with the reagents used for detection. Therefore we mixed cellulose powder* (150-200 mesh) with gypsum and water (5 g cellulose, 0.3 g gypsum, 15 ml water), applied the resulting paste to plates (13 × 18 cm), and dried them for 5-10 hr in air at room temperature and for 45 min at 104-106°. The cellulose layer fixed in this way did not crumble when sprayed with detecting agents, and the plates obtained were sufficiently standard (deviations in the values of $R_f$ did not exceed 5%).

In the separation of carbohydrates on such plates, effective developing systems proved to be mixtures of tert-butanol–*n*-propanol–water (8 : 2 : 3) (I), tert-butanol–ethyl acetate–water (6 : 13 : 3) (II), sec-butanol–ethyl acetate–water (8 : 12 : 3) (III), isopropanol–ethyl acetate–water (25 : 65 : 11) (IV), *n*-butanol –pyridine–water (10 : 3 : 3) (V), acetone–butanol–water (7 : 2 : 1) (VI), *n*-propanol–ethyl acetate–water (15 : 2 : 3) (VII). Mixtures of polyhydric alcohols were well separated in systems I-VII and in mixtures of *n*-butanol–25% $NH_4OH$ –water (16 : 1 : 2) (VIII), *n*-butanol–pyridine–water (6 : 4 : 3) (IX), and *n*-

butanol—ethanol—25% $NH_4OH$—water (8 : 3 : 1 : 8) (X). Development of the chromatograms continued for 40-45 min (the exceptions are systems I and X, in which development takes 1.5 hr). For detection of aldoses the following reagents were used: aniline—phthalic acid (^4) and aniline—diphenylamine—phosphoric acid (^5), whose sensitivity on cellulose plates proved to be appreciably higher (0.5-2 $\gamma$) than in paper chromatography (2-5 $\gamma$). For detec-

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* Cellulose powder was obtained by boiling 800 g of cotton cellulose with 5 l of a 10% solution of hydrogen chloride in absolute ethyl alcohol for 20-25 min, followed by washing with water and methanol and drying in air.

Table 1

| Compound | I | II | III | IV | V | VI | VII | VIII | IX | X |
|---|---|---|---|---|---|---|---|---|---|---|
| **Developer systems** | I | II | III | IV | V | VI | VII | VIII | IX | X |
| $R_f$ **of polyhydric alcohols** | | | | | | | | | | |
| Inositol | — | — | 0.02 | 0.02 | 0.08 | — | — | 0.03 | — | — |
| Mannitol | 0.50 | 0.15 | 0.12 | 0.12 | 0.27 | 0.20 | 0.31 | 0.18 | 0.44 | 0.52 |
| Sorbitol | 0.51 | 0.15 | 0.12 | 0.10 | 0.26 | 0.20 | 0.28 | 0.17 | 0.42 | 0.51 |
| Dulcitol | 0.51 | 0.15 | 0.14 | 0.10 | 0.28 | 0.20 | 0.28 | 0.19 | 0.43 | 0.53 |
| Glycerol | 0.71 | 0.45 | 0.37 | 0.41 | 0.55 | 0.57 | 0.60 | 0.46 | 0.64 | 0.65 |
| 2-Methylpentanetriol-1,2,3 | 0.88 | 0.90 | 0.90 | 0.92 | 0.85 | 0.92 | 0.84 | 0.84 | 0.89 | 0.85 |
| 2,3-Dihydroxy-2-methylpentanoic acid* | | | | | 0.41 | | | 0.29 | 0.51 | 0.62 |
| $R_f$ **of carbohydrates** | | | | | | | | | | |
| Lactose | 0.19 | 0.03 | 0.01 | 0.01 | 0.12 | 0.03 | 0.07 | — | — | — |

| Compound | I | II | III | IV | V | VI | VII | VIII | IX | X |
|---|---|---|---|---|---|---|---|---|---|---|
| Galactose | 0.37 | 0.11 | 0.06 | 0.07 | 0.27 | 0.12 | 0.21 | – | – | – |
| Glucose | 0.40 | 0.13 | 0.08 | 0.09 | 0.32 | 0.14 | 0.23 | – | – | – |
| Mannose | 0.44 | 0.19 | 0.12 | 0.14 | 0.37 | 0.22 | 0.32 | – | – | – |
| Lyxose | 0.48 | 0.24 | 0.17 | 0.19 | 0.44 | 0.38 | 0.42 | – | – | – |
| Ribose | 0.52 | 0.32 | 0.21 | 0.23 | 0.46 | 0.44 | 0.40 | – | – | – |

* For I-IV and VI, VII gives a long diffuse spot.

Fig. 1. Separation of mixtures of polyhydroxy compounds. A and B—tert-butanol-ethyl acetate-water system (6 : 13 : 3). 1—lactose, 2—galactose, 3—mannose, 4—ribose, 5—glucose, 6—lyxose. C—n-butanol-25% $NH_4OH$-water system (16 : 1 : 2). 7—inositol, 8—mannitol, 9—2,3-dihydroxy-2-methylpentanoic acid, 10—glycerol, 11—2-methylpentanetriol-1,2,3.

For the detection of polyhydric alcohols we used the usual reagent for the $\alpha$-glycol grouping, potassium periodate-benzidine (6). The sensitivity of this reagent ranged from 0.5 to 6 $\gamma$, depending on the structure of the chromatographed substances (0.5 $\gamma$ for mannitol, sorbitol, dulcitol; 6 $\gamma$ for 2,3-dihydroxy-2-methylpentanoic acid and 2-methylpentanetriol-1,2,3; inositol does not give a distinct white spot even at 30 $\gamma$, but is detected as a diffuse spot in an amount of 3 $\gamma$).

In all developer systems containing branched alcohols (I-IV) or bases (V, VIII-X), the spots after detection had sharply outlined boundaries ($d$ = 3-5 mm); in systems VI and VII the spots were somewhat diffuse.

The $R_f$ values found (averages from 3-5 experiments) are given in Table 1. Chromatograms obtained in the separation of several mixtures of polyhydroxy compounds are presented in Fig. 1.

Separation of carbohydrate mixtures proceeds better when small amounts of substances are applied (5-120 $\gamma$). In the case of polyhydric alcohols, good separation can be achieved both when small amounts (4-5 $\gamma$) and relatively large amounts (up to 2000 $\gamma$) are applied; however, the $R_f$ values decrease with increasing concentration.

The described procedure for separating polyhydroxy compounds on a fixed layer of cellulose powder has a number of advantages over paper chromatography (a considerable reduction in the time for developing chromatograms, the pos-

···the possibility of chromatographing smaller amounts of substances, smaller spot size) and can be successfully used for the chromatography of complex mixtures.

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*Note: Figure translations are in progress. See original paper for figures.*