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Abstract

Full Text

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CHEMISTRY

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DESCENDING THIN-LAYER CHROMATOGRAPHY OF POLYHYDROXY COMPOUNDS

(Presented by Academician M. M. Shemyakin, 13 XI 1962)

In connection with work in the field of macrolide antibiotics, we developed a method of ascending chromatography of polyhydroxy compounds on plates with a fixed layer of cellulose, which has a number of advantages over chromatography on paper (a considerable reduction in development time, the possibility of chromatographing smaller amounts of substances, smaller spot size) (^{1}). However, this method did not make it possible to separate certain mixtures of substances with very close R_f values (glucose–galactose, lyxose–ribose, arabinose–mannose, etc.). For the separation of such "critical pairs" we have now developed a method of descending flow chromatography.

Fig. 1. Plate holder

Chromatography was carried out on plates coated with a fixed layer of cellulose (^{1}), in a glass chamber with a support made of glass or stainless steel, from which was suspended the trough customarily used in paper chromatography. A duralumin vertical holder for the plates was placed beside it (Fig. 1). The eluent was fed onto the plate through a strip of filter paper (previously wetted with the same eluent), one edge of which was placed in the trough, while the other was in close contact with the adsorbent layer. The plates were coated with a paste of cellulose powder, gypsum, and water, as described earlier (^{1}). After drying to an air-dry state (10–12 hr), the side edges of the plates were cleaned of adsorbent (5 mm on each side) so that the adsorbent layer did not come into contact with the metal surface of the holder.

The shape of the adsorption layer has a great influence on the resolving power

Fig. 2

Figure 2: Fig. 2

of the plate. In the chromatography of carbohydrates, optimum results were obtained when the adsorption layer was given the form shown in Fig. 2. The substance was applied at a distance of 15 mm from the upper edge of the plate and at a distance of 10 mm from the edge of the strip of filter paper transferring the eluent to the plate*.

Fig. 2. Separation of carbohydrate mixtures. **1** –system: *n*-butanol–25% N H_4OH–water (16:1:2); time 7 h, arabinose–xylose–ribose–rhamnose; **2** –system: *n*-butanol–pyridine–water (10:3:3); time 10 h; galactose–glucose–mannose–xylose–ribose–rhamnose. **3** –system: phenol–*n*-butanol–acetic acid –water (5:5:2:10) (upper layer, amount of phenol in grams) time 10 h, **a** –mannose–arabinose; **b** –lyxose–ribose. The separated substances are listed in the order of their distance from the starting line.

Optimum separation of carbohydrate mixtures with close R_f values was achieved in systems containing bases: 1) *n*-butanol–pyridine–water (10:3:3) (I) and *n*-butanol–25% NH_4OH–water (16:1:2) (II). Only in the case of the mixtures lyxose–ribose and arabinose–mannose was separation carried out in the acidic system phenol–butanol–acetic acid–water (5:5:2:10) (upper layer; the amount of phenol is given in grams) (IV). The time for developing the chromatograms took (depending from the system) from 7 to 18 hours (in paper chromatography, development in the same systems takes from 3 to 9 days (2)). For the detection of carbohydrates, as in the preceding work (1), the reagent aniline–phthalic acid was used. The described method for separating carbohydrates proved to be approximately 10-20 times more sensitive than paper chromatography in a stream (2), and makes it possible to detect 0.5-1 γ of substance when the mixture is applied as a spot and 5-10 γ when the mixture is applied as a band.

Using the indicated method, we separated a number of carbohydrate mixtures that do not separate in ascending chromatography on cellulose plates (Fig. 2). The values of R_g and R_a are presented in Table 1.

Table 1

Values of R_g and R_a for polyoxy compounds*

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* To obtain reproducible data it is necessary to observe all the indicated dimensions.

Compound	R_g I a	R_g I b	R_g II a	R_g II b	R_g III a	R_a IV b
Galactose	0.82	0.88	0.88	0.86		—
Glucose	1	1	1	1		—
Mannose	1.27	1.15	1.50	1.23		0.85
Xylose		1.27	1.87	1.45		—
Ribose		1.38	2.29	1.65		1.27
Rhamnose		1.56	3.09	2.06		—
Arabinose	1.27					1
Lyxose						1.03
Erythro-2,3-dioxy-2-methylpentanoic acid					1.93	
Threo-2,3-dioxy-2-methylpentanoic acid					2.23	

* Roman numerals denote the developer systems;
a —application as a spot, *b* —as a band.

The method of descending thin-layer chromatography on cellulose also proved very effective for the separation of stereoisomeric polyoxy acids. The most suitable system in this case was a mixture of *n*-butanol —8% NH_4OH —water (saturated with borax) (8 : 1 : 2) (III); detection was carried out with a solution of periodate—potassium cuprate ([3]). Using this method, threo- and erythro-2,3-dioxy-2-methylpentanoic acids ([4]) were separated (see Table 1), which cannot be achieved by any of the previously used thin-layer chromatography methods ([1,5]).

Thus, the method of descending chromatography in a thin layer of cellulose can be recommended for the separation of complex mixtures of polyoxy compounds (including carbohydrates) with very close R_f values.

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

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