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Note

Chromatographic separation of alditols and some aldoses on *O*-(carboxymethyl)cellulose paper in the lanthanum, calcium, and barium forms

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The most difficult problem encountered in paper chromatography of carbohydrates is the separation of stereoisomeric alditols. The use of several solvent-systems has been reported in the literature[1–6]. Among them, those relatively the most efficient exploit boric acid as a component of the solvent

TABLE I

RELATIVE PAPER-CHROMATOGRAPHIC MOBILITIES OF ALDITOLS AND SOME ALDOSES[a]

Compound	*CMC-paper in the form*				*Whatman No. 1*
	La	*Ca*	*Ba*	*H*	
Glycerol	8	6	6	4.0	3.9
Erythritol	4.5	3.7	3.6	2.7	2.4
D-Threitol	4.0	3.1	3.3	2.5	2.3
Ribitol	2.8	2.4	2.2	1.70	1.75
D-Arabinitol	2.4	2.1	1.94	1.63	1.73
Xylitol	1.64	1.69	1.64	1.51	1.63
Allitol	2.0	1.56	1.46	1.18	1.16
D-Altritol	1.60	1.31	1.29	1.15	1.11
D-Mannitol	1.58	1.20	1.22	1.07	1.09
Galactitol	1.38	1.05	1.02	0.95	0.92
D-Glucitol	1.00	1.00	1.00	1.00	1.00
L-Iditol	0.81	0.85	0.84	0.96	0.93
D-Ribose	2.9	2.4	2.3	2.1	2.2
D-Lyxose	3.6	2.7	2.5	1.80	1.94
D-Xylose	3.4	2.6	2 3	1.70	1.82
L-Arabinose	2.7	2.1	1.78	1.33	1.46
D-Talose	2.2	1.67	1.60	1.71	1.78
D-Mannose	2.5	2.0	1.62	1.34	1.40
D-Allose	1.62	1.14	1.24	1.02	1.06
D-Glucose	1.90	1.48	1.27	0.99	1.01
D-Galactose	1.55	1.09	0.96	0.83	0.81

[a]Referred to that for D-glucitol.

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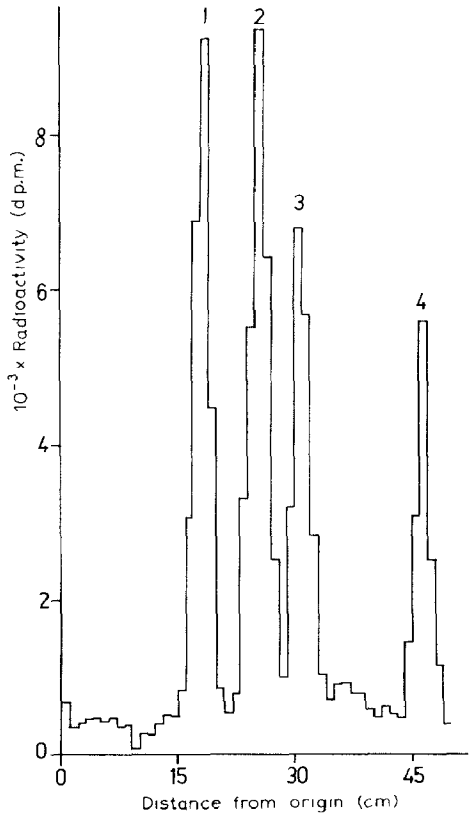

Fig. 1. Separation of some [U-^{14}C]alditols on CMC-paper in the lanthanum form: 1, D-glucitol; 2, galactitol, 3, D-mannitol; and 4, D-arabinitol

mixture[4–6]; complex formation between borate ions and polyols operates in these chromatographic systems. Combination of a boric acid-containing solvent-system and *O*-(2-diethylaminoethyl) modified chromatographic paper[7] has been found to be the most efficient thus far for these purposes. Alkaline-earth and rare-earth cations, among them barium(II), calcium(II), and lanthanum(III) ions, have been known as strictly differentiating complexants for polyols if used, in paper electrophoresis[8] and column chromatography on cation exchangers[9–11]. We now report new chromatography systems utilizing *O*-(carboxymethyl)cellulose (CMC) paper with the bound cations just mentioned.

Mobilities of alditols and aldoses on unmodified CMC-paper were very similar to those on Whatman No. 1 paper, if development was with 10:1:2 (v/v/v) 1-butanol–ethanol–water. When CMC-paper in the lanthanum, calcium, or barium forms was used with the same eluent, significant changes in the mobilities were observed (Table I). In agreement with the known fact that, compared with other cations, lanthanum(III) ions form the most stable complexes with polyols[10,12], the differences in mobilities of the stereoisomeric alditols in a certain group were the

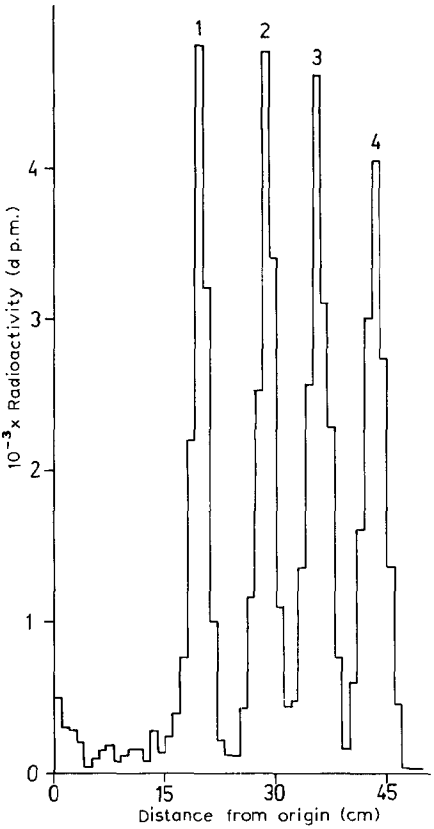

Fig. 2. Separation of D-[U-^{14}C]glucitol (1), [U-^{14}C]galactitol (2), D-[U-^{14}C]galactose (3), and D-[U-^{14}C] glucose (4) on CMC-paper in the lanthanum form.

greatest with the lanthanum form. Thus, we achieved excellent resolution of both tetritols, all pentitols, and all hexitols, except for the pair altritol–mannitol. Because of slow migration, the time necessary for sufficient resolution of alditols utilizing the whole length (57 cm) of the CMC-paper was relatively long (2 days for tetritols and up to 7 days for hexitols), but the spots were sharp. For satisfactory resolution of pentitols, it is possible to choose an eluent in which these compounds move faster, for example a 24-h development with 8:2:1 (v/v/v) ethyl acetate–pyridine–water. In this system, the mobilities referred to that of D-glucitol were for xylitol 1.66, D-arabinitol 2.4, and ribitol 3.4, but the spots were more diffuse. In addition to partition and permeation effects operating in classical paper chromatography, chemisorption also took part in this chromatographic system. Complex formation between lanthanum(III) ions and polyols sometimes caused dramatic changes that resulted in reversal of their mobility sequence (for instance, pentoses). As is apparent from Table I, the procedure permits efficient separation not only most of the alditols from each other, but also most common aldoses from each other and from their corresponding alditols.

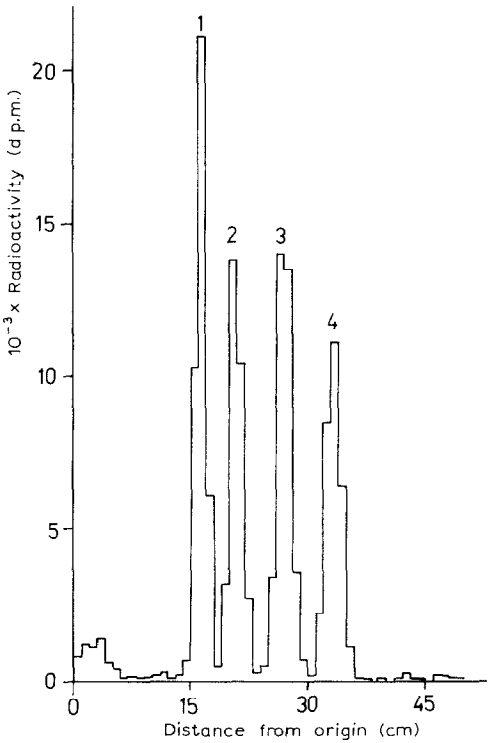

Fig. 3. Separation of some [U-^{14}C]alditols on CMC-paper in the calcium form: 1, D-glucitol; 2, D-mannitol; 3, xylitol; and 4, D-arabinitol

Somewhat less-effective separations of alditols and aldoses were achieved on CMC-paper in the Ca and Ba forms. However, D-altritol and D-mannitol were partially resolved and the separation of xylitol from D-mannitol was excellent.

The efficiency of the chromatographic systems developed is apparent in the resolution of several artificial mixtures of [U-^{14}C]alditols and [U-^{14}C]aldoses (Figs. 1–3). For the resolution of a mixture containing all five [U-^{14}C]alditols used, two successive separations were necessary. After separation on the La-CMC-paper, the mixture of D-[U-^{14}C]mannitol and [U-^{14}C]xylitol remained then to be resolved on the Ca-CMC-sheet. The effective paper-chromatography systems presented here are reproducible unambiguously, in contrast to that reported by Conrad and coworkers[7]. The next advantage of our systems lies in use of an ion-free eluent. It is also important to remove borate ions from an alditol mixture, if prepared by sodium borohydride reduction, before its separation, in order to avoid formation of competitive complexes with the polyols resolved.

Application of these new paper-chromatographic procedures for analysis of oligo- and poly-saccharides is in progress.

EXPERIMENTAL

General. — The radioactivity was measured with a scintillation spectrometer (Packard TriCarb 3390). Paper chromatograms cut into 1 × 4-cm strips were dipped into measuring vessels containing the scintillation mixture (5 mL) prepared from PPO (3.5 g) and POPOP (50 mg) in toluene (1 L).

Preparation of chromatographic papers. — CMC-sheets (Whatman CM 82, nominal capacity 2.6 μequiv/cm^2) were irrigated with 0.1M aqueous lanthanum acetate, 0.15M calcium acetate, or 0.15M barium acetate for 24 h and then with water for 2 days by the descending technique. After being dried at room temperature, the papers were ready for use in chromatographic separations.

Separation of alditols and aldoses. — Aqueous solutions of alditols or aldoses (50–200 μg) were spotted at the starting line of CMC-paper (in the La, Ca, or Ba forms), unmodified CMC-paper, and Whatman No. 1 paper. Chromatograms were developed in 10:1:2 (v/v/v) 1-butanol–ethanol–water at room temperature for 2–7 days. After the chromatograms had been dried, spots were detected by spraying with a freshly prepared, 9:1:1 (v/v/v) mixture of 2% aqueous sodium periodate, 2% potassium permanganate, and 4% sodium carbonate.

Separation of common [U-^{14}C]alditols, D-*[U-^{14}C]glucose, and* D-*[U-^{14}C]galactose.* — From a mixture containing 0.2 mg each of D-[U-^{14}C]glucose (465 Bq), D-[U-^{14}C]galactose (506 Bq), D-[U-^{14}C]mannose (375 Bq), D-[U-^{14}C]xylose (435 Bq), and D-[U-^{14}C]arabinose (270 Bq), a mixture of the corresponding D-[U-^{14}C]alditols was prepared as follows. To the mixture of the D-[U-^{14}C]aldoses in water (5 μL), M carbonate buffer of pH 8.5 (5 μL) and 0.5M sodium borohydride in 0.1M sodium hydroxide (5 μL) were added. The mixture was kept for 40 min at 50°. It was then passed through a column (1 × 5 cm) of Dowex 50W X-8 (H^+ form) and eluted with water at a flow rate of 0.25 mL/min. The total amount of effluent (15 mL) was collected and evaporaed *in vacuo* (35–40°). The evaporation was repeated 5 times after adding 2 mL of water in order to concentrate the residue, and finally 5 times after adding 2 mL of methanol. The residue, dissolved in 0.2 mL of water, was applied to the starting line (spot area 0.5 × 3 cm) of the La-CMC-paper and the chromatogram was developed in the aforementioned solvent system for 7 days at room temperature. Water elution of individual 1-cm segments of the paper chromatogram and evaporation of the fractions afforded the separated D-[U-^{14}C]glucitol (428 Bq, 92%), [U-^{14}C]galactitol (466 Bq, 92%), D-[U-^{14}C]arabinitol (232 Bq, 85%), and a mixture of D-[U-^{14}C]mannitol and [U-^{14}C]xylitol (730 Bq). Successive separation of this two-component mixture on Ca-CMC-paper under the same conditions as already mentioned yieded D-[U-^{14}C]mannitol (330 Bq, 80%) and [U-^{14}C]xylitol (358 Bq, 83%).

The mixture of [U-^{14}C]alditols not containing galactitol was resolved in a single separation on the Ca-CMC-sheet. Similarly, both the mixture not containing xylitol and that of D-[U-^{14}C]glucitol, [U-^{14}C]galactitol, and their parent D-[U-^{14}C]aldoses were resolved on the La-CMC-sheets.

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