CHROM. 5195

Thin-layer chromatography of fructo-oligosaccharides

The technique of paper chromatography (PC) of fructo-oligosaccharides (inulin type) has been used for many years associated with the work on their metabolism in the storage organs of a number of species of Compositae[1,2]. Chicory rootstock is known to contain these fructosans[3] but they are not generally found in the leaves. However, we have observed *de novo* synthesis of fructosans (degree of polymerization (D.P.) up to 20–21) in leaf disks of Chicory, which were incubated for 3 days on a medium consisting of simple sugars and phosphate buffer. A rapid chromatographic procedure was needed for the separation of these polymers since PC of fructo-oligosaccharides of D.P. greater than 14 requires up to 21 days[1]. KARLSSON[4] has reported unidimensional multiple chromatography on phosphate-buffered cellulose thin-layer chromatography (TLC) which requires 8 hours and resolves fructo-oligosaccharides up to D.P. 8. This report describes a TLC procedure utilizing Kieselguhr G plates which requires approximately 90 min. Included are two solvent systems, one for the separation of polymers of D.P. up to 7–8, and another for polymers of D.P. from 2 to 20.

Materials and methods

Equipment. A sandwich-type chamber was obtained from Brinkmann Instruments Inc., Westbury, N.Y. Carbohydrate solutions were applied to the plates with a Hamilton microsyringe. The chromatoplates were prepared using a Desaga adjustable spreader obtained from Desaga, Heidelberg, G.F.R.

Materials. Kieselguhr G was a product of E. Merck, Darmstadt, G.F.R. and solvents were reagent grade and used without further purification. The fructo-oligosaccharides were obtained as follows:

(1) An homologous series of fructo-oligosaccharides was prepared from the rootstock of Chicory (*Cichorium intybus* L). The rootstock was cleaned, diced, and homogenized in an equal volume of distilled water in a Waring blender. The homogenate was boiled for 5 min, filtered through Whatman No. 1 filter paper, and the filtrate centrifuged at 1000 × *g* for 15 min. The supernatant was passed through a mixed-bed ion-exchange column of Dowex 50 (H^+) and Dowex 1 (OH^-) forms. The eluate was treated with decolorizing charcoal filtered through millipore filter (0.45 μ) and the filtrate was then evaporated to dryness *in vacuo*. The residue was dissolved in 50% ethanol to give a final concentration of 25 μg fructose equivalents per μl as determined by the phenolsulfuric acid method of MONTGOMERY[5].

(2) Individual oligofructosans were separated and purified by PC, using the above oligofructosan mixture, according to the procedure of EDELMAN AND DICKERSON[1]. The purified polymers were then eluted, using an elution apparatus described elsewhere[6], and the eluates made up to a final concentration of 2.0 μg fructose equivalents per μl in 50% ethanol.

Procedure. Chromatoplates (20 × 20 × 0.5 cm) were coated with a 250 μ layer of Kieselguhr G according to STAHL[7]. After air-drying, the adsorbent layers were broken horizontally 12 cm above the origin so that the migration distance would be the same on all plates. Also, a 1 cm band of the layer was scraped off from the 2

TABLE I

hR_F VALUES OF FRUCTO-OLIGOSACCHARIDES SEPARATED BY TLC

Glass plates (20 × 20 × 0.5 cm) were coated with a 250 μ thin layer of Kieselguhr G. The fructo-oligosaccharides were applied both individually and in a mixture. All solvent compositions are given in the order: 1-propanol–ethyl acetate–water.

No.	*Solvent ratios*	*Degree of polymerization*																		
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
1	40:50:10	84.7	78.5	52.0	37.7	23.9	14.8	10.1	7.2												
2	40:45:15	89.2	79.8	61.5	46.9	34.3	25.8	18.3	14.0	10.3	6.3										
3	42.5:40:17.5	91.6	84.2	69.3	51.5	41.0	29.7	20.8	15.8	12.4	7.4	5.4									
4	32.5:50:17.5	84.3	51.2	17.4	14.0	8.3															
5	30:50:20	89.5	76.7	52.5	37.6	23.8	14.9	9.9	7.4	5.0											
6	35:45:20	91.8	80.3	54.3	41.6	28.8	18.8	16.7	12.3	10.6	9.0	7.4	6.2	5.0							
7	40:40:20	93.4	89.3	77.3	67.8	55.4	42.6	34.7	30.6	25.6	19.4	16.1	12.4	8.7							
8	50:30:20	96.0	93.0	82.5	76.2	62.9	52.4	44.0	38.6	34.7	30.7	25.7	20.8	15.8	13.4	10.9	7.9	6.0			
9	60:20:20	95.9	94.2	87.3	82.8	76.6	69.7	61.5	54.9	48.4	43.0	38.5	33.6	28.7	23.4	19.3	15.2	12.3	10.0	8.2	7.1

vertical edges and the top edge in order to accomodate the plates in the sandwich-type chamber. One μl (2.0 μg) of each of the individual fructo-oligosaccharides from D.P. 1 (fructose) to D.P. 8, and 1 μl (25 μg) of the fructo-oligosaccharide mixture were spotted along a line 2.5 cm from the lower edge. The plates were developed in solvents containing various proportions of 1-propanol, ethyl acetate, and water (see *Results and discussion*) until the solvent reached the 12 cm line. The plates were then removed, air-dried, and the carbohydrates located by means of urea–metaphosphoric acid spray reagent[8]. Tracings were made using Albanene drawing paper.

Results and discussion

TLC of fructo-oligosaccharides on Kieselguhr G chromatoplates can be achieved by using various proportions of 1-propanol, ethyl acetate, and water and specific ranges of fructosans can be resolved.

The hR_F values of various fructo-oligosaccharide homologs up to D.P. 20 are shown in Table I.

Solvents No. 1 and No. 5 showed almost identical results and were capable of resolving polymers up to D.P. 7–8. A tracing of the separation achieved using solvent No. 1 is shown in Fig. 1. Using solvents Nos. 2 and 3, D.P. up to 10–11 were well separated into distinct spots with little tailing or streaking. Solvent No. 4 was found suitable for separation of fructose, sucrose, and 3 homologs up to D.P. 5. Solvents Nos. 6 and 7 were found to resolve homologs up to D.P. 13. Fig. 2 illustrates the results obtained using solvent No. 6. Fructose and sucrose have high R_F values in these two solvents and are not clearly resolved. Homologs of D.P. 2 to 16–17 could be resolved using solvent No. 8, while solvent No. 9, as shown in Fig. 3 could separate D.P. 2 to 20. The solvent systems described in Table I are arranged in order of in-

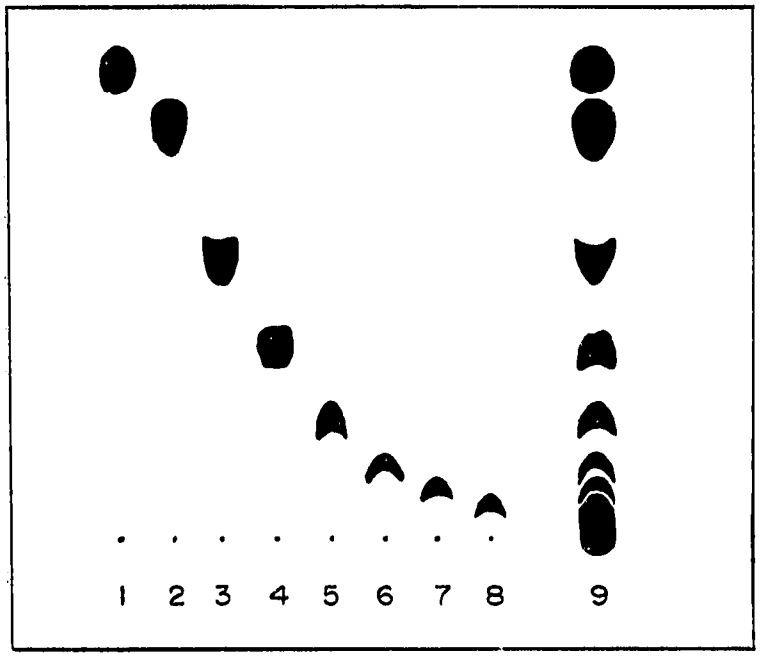

Fig. 1. Separation of fructo-oligosaccharides and fructo-oligosaccharide mixture by TLC in solvent No. 1. 1 = D.P. 1 (F) fructose; 2 = D.P. 2 (GF) sucrose; 3 = D.P. 3 (GF_2); 4 = D.P. 4 (GF_3); 5 = D.P. 5 (GF_4); 6 = D.P. 6 (GF_5); 7 = D.P. 7 (GF_6); 8 = D.P. 8 (GF_7); 9 = Fructo-oligosaccharide mixture.

creasing water content. Ternary solvents containing less than 10 % H_2O by volume were found to produce severe streaking and R_F values too low to resolve fructo-oligosaccharides of D.P. greater than 3. With a water content above 20 % by volume, the R_F values of D.P. less than 20 were too high and poorly resolved into extremely narrow zones. The ethyl acetate component could be replaced by methyl acetate,

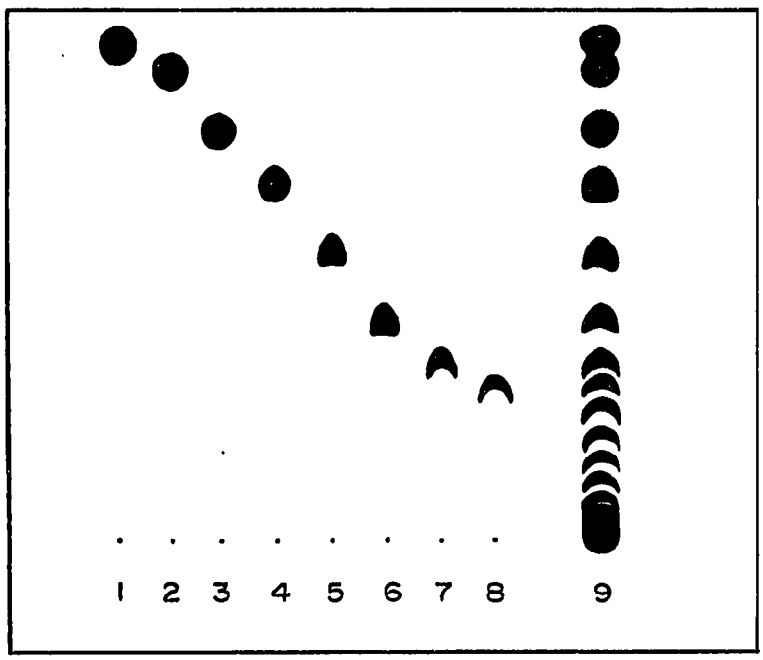

Fig. 2. Separation of fructo-oligosaccharides and fructo-oligosaccharide mixture by TLC in solvent No. 6. For key see Fig. 1.

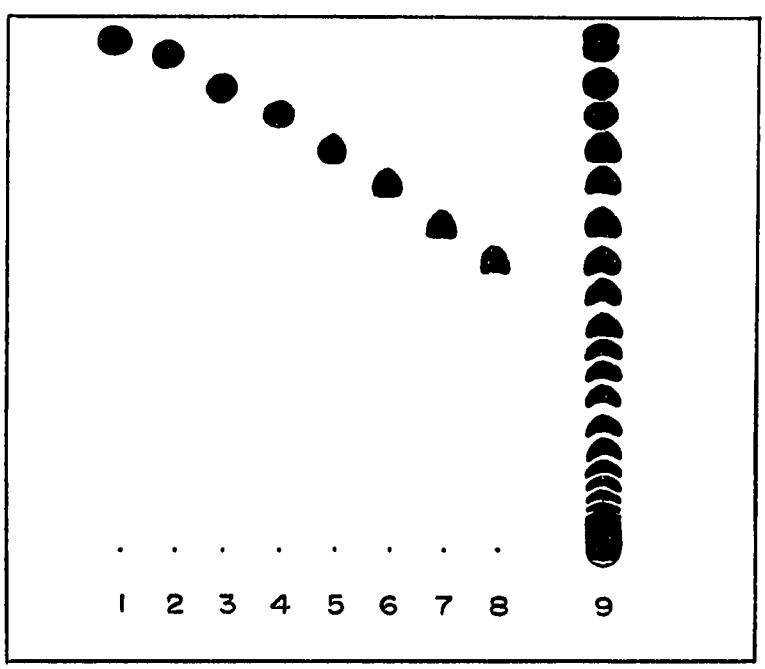

Fig. 3. Separation of fructo-oligosaccharides and fructo-oligosaccharide mixture by TLC in solvent No. 9. For key see Fig. 1.

methyl formate, or ethyl formate without qualitatively changing the separations. Attempts to substitute 1-propanol with 1-butanol, 2-propanol or ethanol, resulted in inferior separations.

TLC, using the practically inactive retentative layers produced with kieselguhr has been advocated for use in partition chromatography of hydrophilic compounds[9]. However, due to the relatively low capacity of the plates, it has found only limited application in separation of oligosaccharides[10]. WEILL AND HANKE[11] using Kieselguhr G TLC and ternary solvent systems of 1-butanol, pyridine, water separated the malto-oligosaccharide homologous series up to D.P. 9. SHANNON AND CREECH[12] have extended this solvent system to resolve D.P. up to 20–25. However, application of these solvent systems to the fructo-oligosaccharide series resulted in poor resolution of individual polymers and R_F values too low to separate higher homologs. Using 1-propanol, ethyl acetate, and water, comparable results were obtained. By varying the proportions of the components in the solvent, the mobilities of various fructo-oligosaccharides could be altered, similar to the observations of SHANNON AND CREECH[12] for malto-oligosaccharides.

In our work, solvent No. 1 composed of 1-propanol–ethyl acetate–water (40:50:10) was used for the best separation of fructose, sucrose and fructo-oligosaccharides up to D.P. 7–8 and solvent No. 9, 1-propanol–ethyl acetate–water (60:20:20) for homologs of D.P. 2 to 20.

The maximum time required for development using these solvent systems described was found to be about 90 min (12 cm run at 25° with solvent No. 9) and represents a considerable improvement over comparable separations by PC or TLC. The procedure also offers increased sensitivity for rapid microgram quantitative analysis of fructo-oligosaccharide mixtures. The flexibility of the solvent systems developed, enables one to scan all or part of the fructo-oligosaccharide series up to D.P. 20 with only one solvent pass without the use of buffered or modified support media.

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Department of Botany, University of Toronto,
Toronto 5, Ontario (Canada)

F. W. COLLINS
K. R. CHANDORKAR

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