THE SEPARATION OF SIMPLE SUGARS BY CELLULOSE THIN-LAYER CHROMATOGRAPHY*

D. W. VOMHOF AND T. C. TUCKER
Department of Agricultural Chemistry and Soils, College of Agriculture, University of Arizona, Tucson, Ariz. (U.S.A.)
(Received June 18th, 1964)

INTRODUCTION

Thin-layer chromatography (TLC) is a useful tool for the rapid separation of compounds of biological interest. Although many different sorbents are available which are suitable to TLC, the majority of the workers have used silica gel with a binder for the sorbent layer[1]. One group of compounds for which silica gel has not been a satisfactory medium is the naturally occurring free sugars. Poor separation of some of the more common sugars and the low capacity of the plates are two disadvantages in this application.

Although the simple sugars have been separated for many years using paper chromatography, a major disadvantage is the long elution time required with the associated problems of temperature fluctuations[2]. When cellulose thin layers are used, the advantages of the partitioning properties of cellulose and the increased loading of sample are coupled with the separations which are characteristic of thin-layer chromatography. RANDERATH[3] has found cellulose thin-layer chromatography superior to paper chromatography in the separation of nucleotides. The authors have found no more than one single paper[4] in the literature which deals with the separation of a few simple sugars using cellulose layers for thin-layer chromatography.

In terms of solute detectability and time, it is advantageous to find a solvent or solvents which will give good separation of sugars in one dimension. Since cellulose thin-layer plates possess the same partition properties as paper, it seemed advisable to evaluate several solvents previously used for the separation of sugars by paper chromatography to determine their applicability for the separation of sugars on cellulose layers.

Nine solvents were evaluated in this study. Special emphasis was placed upon the property of the solvent to separate sucrose, glucose, and fructose in one dimension without a long period of pre-saturation of the plate. These sugars are of principal interest because they are the most commonly occurring free sugars in higher plants.

---

* Technical Paper No. 889 of the Agricultural Experiment Station, University of Arizona, Tucson. The mention of specific sources of equipment or products to the exclusion of others that may be suitable should not be considered as an endorsement by the University of Arizona of a particular manufacturer or product.

EXPERIMENTAL

(*A*) *Solvents*

The following solvents were evaluated:

(1) Formic acid–methyl ethyl ketone–*tert.*-butanol–water (15:30:40:15, v/v)[5].
(2) Ethyl acetate–pyridine–water (2:1:2, v/v)[6].
(3) Ethyl acetate–isopropanol–water (65:23.5:11.5, v/v)[7].
(4) *n*-Butanol–acetic acid–water (6:3:1, v/v)[7].
(5) Methyl ethyl ketone–acetic acid–methanol (3:1:1, v/v)[7].
(6) Ethyl acetate–acetic acid–water (3:2:3, v/v)[8].
(7) *n*-Butanol–pyridine–water (45:25:40, v/v)[8].
(8) *iso*-Propanol–pyridine–acetic acid–water (8:8:1:4, v/v)[9].
(9) Phenol aq. (ca. 90 %)–water (10:1.25, v/v) + 0.002 % 8-hydroxyquinoline[10].

(*B*) *Detection reagent*

The detection reagent used was 2-aminodiphenyl–oxalic acid dissolved in 85 % ethanol[9]. This reagent locates disaccharides as well as hexoses and pentoses. The 2-aminodiphenyl is no longer commercially available because of its suspected carcinogenic properties. Several methods of preparation are given in the literature[11,12].

(*C*) *Preparation of plates*

The plates were washed with a detergent, rinsed well with tap water, followed by distilled water and finally methanol (analytical grade).

The cellulose slurry was prepared as follows: 15 g of cellulose 300 MN (Macherey, Nagel & Company) were mixed with 90 ml of a deionized water–methanol solution (5:1, v/v) by adding small portions of the solution to the powder and stirring well. A homogeneous slurry resulted. This was sufficient to cover five 20 × 20 cm plates and two 5 × 20 cm plates. A 0.37 mm thick layer was applied with an adjustable applicator (Desaga).

The plates were always dried in a hood for 2 h and then stored in a desiccator cabinet overnight before use. (The relative humidity in the laboratory seldom was over 15 %.)

(*D*) *Sample preparation*

The sugar solutions were made by dissolving 1 mg of sugar in 10 ml of 10 % isopropanol. A mixture of glucose, fructose and sucrose was prepared in the same way. The following sugars were studied:

Disaccharides: sucrose, lactose, cellobiose, maltose;
Aldohexoses: β-D-glucose, D-mannose, D-galactose;
Ketohexoses: D-fructose, L-sorbose;
Aldopentoses: D-arabinose, L-arabinose, D-lyxose, L-xylose, D-ribose.

(*E*) *Chromatographic procedure*

The samples were spotted at 1 cm intervals using a micropipette. The pentoses were applied at 30 μg per spot and the others at 40 μg per spot. The origin was 2 cm above the bottom edge of the plate. The film was broken 15 cm above the origin. The film was also broken vertically 0.5 cm from each side to eliminate edge effects[13].

Solvent was placed in the tank 15 min before the plates were added. The laboratory temperature was 23°.

After the plates were developed and dried they were sprayed with the detecting reagent. The sugars were located by heating the plates with an industrial hot air drier. A lighter background resulted by this procedure than when heated for 10 min in an oven at 110°.

RESULTS AND DISCUSSION

These nine solvents ascend more slowly on cellulose layers than on silica gel layers. The development time is still rapid when compared to the usual developing times of 24 h or longer for paper chromatography. A slightly faster development time can be achieved using cellulose with $CaSO_4$ as a binder. No other differences were observed in this laboratory between cellulose with and without a binder.

Table I lists the characteristic colors produced by the spray reagent. The order of appearance and relative intensities are also given. These results were the same for all the solvents tested.

Table II lists the $R_F \times 100$ values for the various solvents. The $R_G \times 100$ values for solvent 1 also are given. In this case, the plate was developed twice in the same direction. Several of the solvents gave severe streaking, especially the more volatile, faster running solvents.

Formic acid–methyl ethyl ketone–*tert.*-butanol–water is the preferred solvent. The spots containing pentoses enlarged to about twice the diameter of the original spot after being developed twice. The higher molecular weight sugars diffused even less. All spots were nearly circular with no bearding or tailing whereas for all of the other solvents tested the spots were elongated and bearded, and there was an occasional double spot. Fig. 1 shows the results of a 15 cm development using solvent 3.

Since the R_F values are low in the formic acid–methyl ethyl ketone–*tert.*-butanol–water solvent, multiple development can be used to advantage[14]. Fig. 2 shows the amount of separation after one 15 cm development in solvent 1.

Fig. 3 shows the increased separation achieved after two developments in the same direction.

The literature to date indicates that the use of thin-layer chromatography for the separation of sugars is inferior to paper chromatography because of the small quantities of sugars that can be used. These results were obtained on silica gel. With the solvent 1–cellulose substrate combination, mixtures containing 100 μg or more of sucrose, glucose, and fructose can be separated using the multiple development technique. The upper limits for the separation of most pentoses appear to be 50 μg.

Galactose can be separated from glucose using this system. After two developments with solvent 1, about 15 μg of galactose can be separated from the same quantity of glucose. If larger amounts of these sugars are present, three or more developments may be required. With the phenol–water system galactose moves farther than glucose, permitting a satisfactory separation of as much as 50 μg of each sugar in one development.

Using solvent 1, the sugars are separated into classes, *i.e.*, trisaccharides remain nearest the origin, disaccharides above them, aldohexoses next, etc.

TABLE I

CHARACTERISTIC COLORS OF SUGARS SPRAYED WITH 2-AMINODIPHENYL

Class	*Color*	*Order of appearance*	*Relative intensity*
Disaccharides	Light tan	3	3
Aldohexoses	Very dark brown	2	2
Ketohexoses	Green, change to green-brown on prolonged heating	4	4
Aldopentoses	Red	1	1

TABLE II

R_F VALUES OF SUGARS IN THE NINE SOLVENTS

	$R_F \times 100$									$R_G \times 100$
Compound/solvent	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*1*
Sucrose	10	20	4	S	0	63	40	67	37	65
Lactose	4	17	1	5	0	56	31	39	37	26
Maltose	6	S	2	7	0	62	34	48	34	38
Cellobiose	5	27	1	4	0	60	33	45	32	32
β-D-Glucose	19	S	S	17	0	63	40	47	35	100
D-Mannose	23	40	S	S	0	65	43	60	40	123
D-Galactose	17	S	S	S	0	60	37	53	40	91
D-Fructose	24	S	11	22	0	62	41	61	47	130
L-Sorbose	23	S	10	20	0	63	41	60	37	123
D-Arabinose	27	40	S	23	0	63	41	61	50	145
D-Lyxose	33	48	S	S	0	67	46	57	46	170
L-Xylose	30	47	S	25	0	67	46	65	41	160
D-Ribose	39	53	S	S	0	69	49	69	57	191
L-Arabinose	29	39	S	23	0	63	40	60	51	151
Mixture: α-D-Glucose–D-Fructose–Sucrose	Y	N	N	N	N	N	N	N	Y	Y
Approximate time (h) for solvent to travel 15 cm at 23°	3	2	3	4	½	3	4	4	6	6 total

N = No separation of the mixture; Y = Yes, mixture separated; S = Badly streaked.

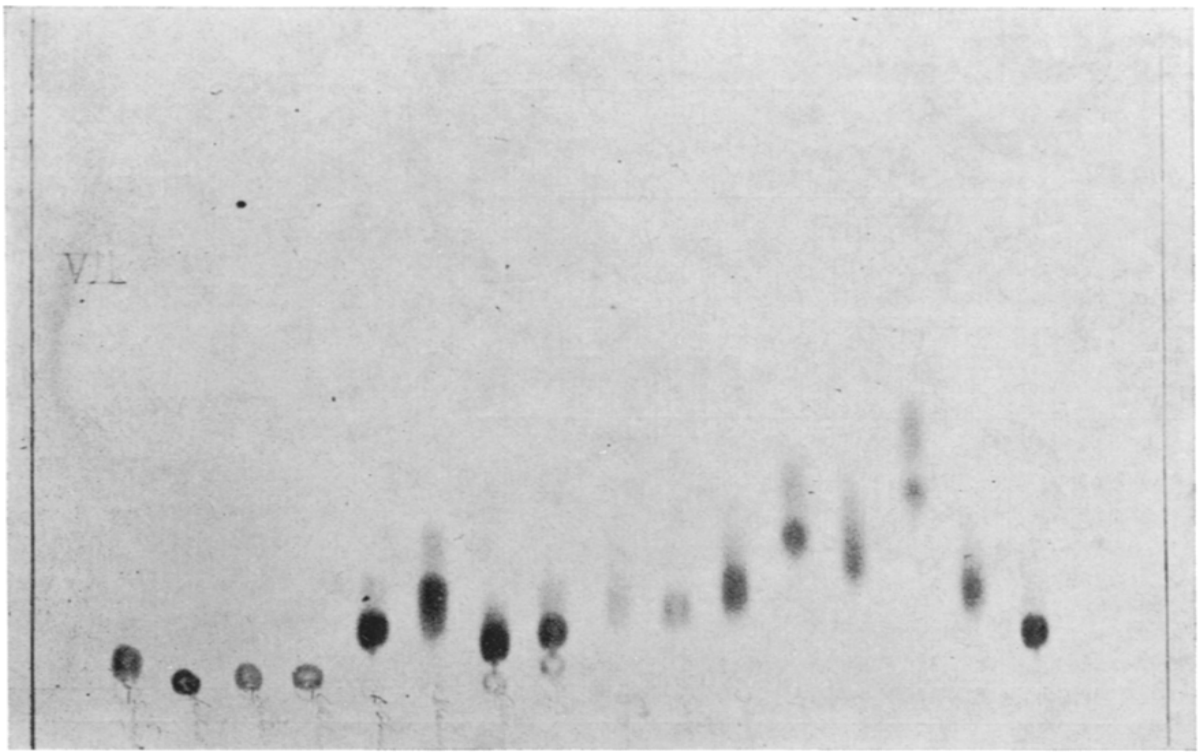

Fig. 1. A 15 cm development in ethyl acetate–isopropanol–water showing typical bearding and poor separation. From left to right the sugars are: sucrose, lactose, maltose, cellobiose, D-glucose, D-mannose, D-galactose, mixture, D-fructose, L-sorbose, D-arabinose, D-lyxose, L-xylose, D-ribose, and L-arabinose.

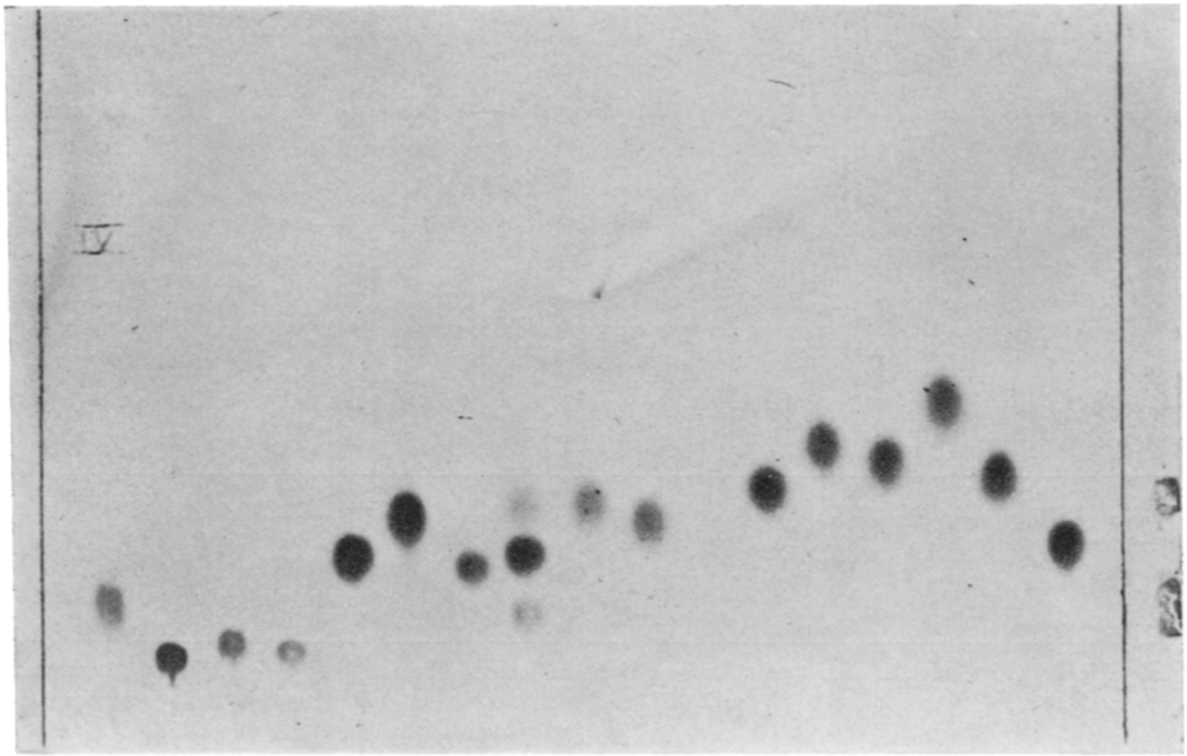

Fig. 2. A single 15 cm development in the formic acid–methyl ethyl ketone–*tert.*-butanol–water solvent. The sugars are in the same sequence as in Fig. 1.

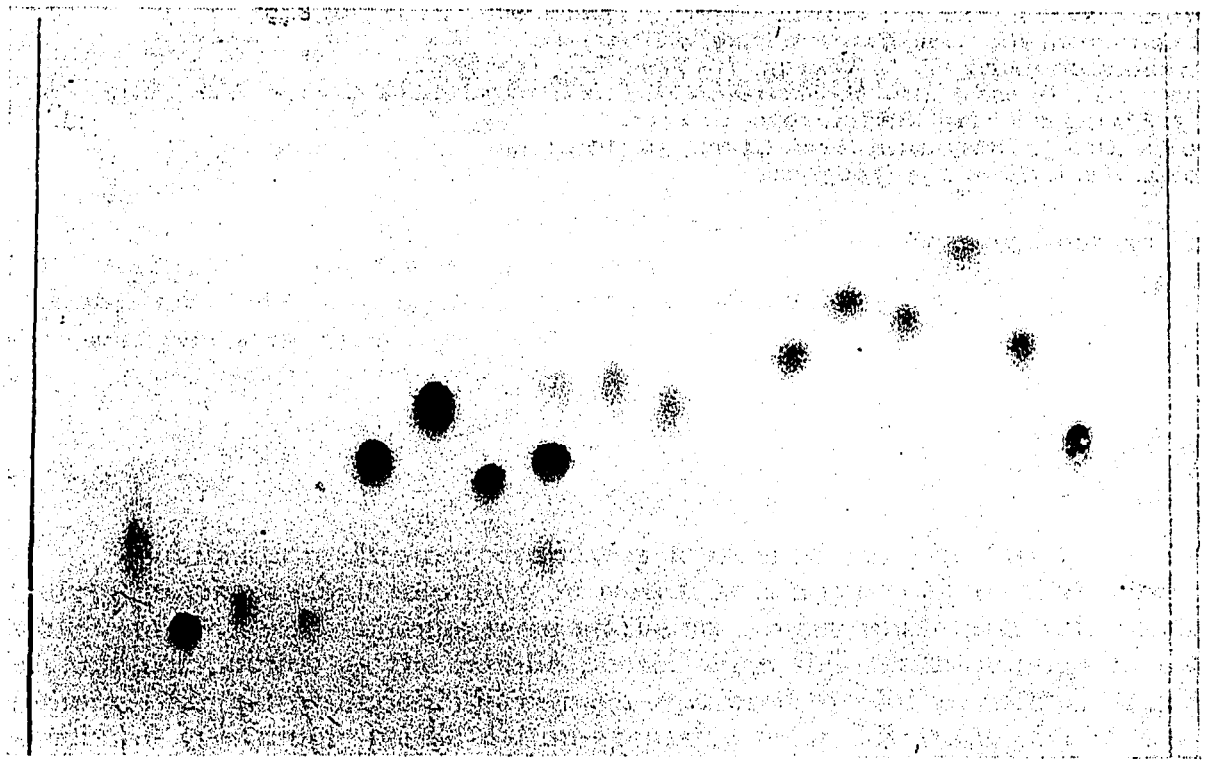

Fig. 3. The separation of sugars achieved after two developments of 15 cm. The sugars are in the same sequence as in Fig. 1.

ACKNOWLEDGEMENTS

This investigation was sponsored in part by Grant CPI 63–38 from the Cotton Producers Institute of the National Cotton Council of America.

The authors also acknowledge the technical assistance of THOMAS C. TUCKER, Jr.

SUMMARY

Several solvents previously used in paper chromatography for the separation of sugars were evaluated for use with cellulose thin-layer plates. Several simple sugars which were difficult to separate by one-dimensional chromatography on either paper or silica gel can be separated on cellulose using a solvent of formic acid–methyl ethyl ketone–*tert.*-butanol–water.

REFERENCES

1 J. M. BOBBIT, *Thin-Layer Chromatography*, Reinhold, New York, 1963, pp. 17, 27.
2 R. J. BLOCK, E. L. DURRUM AND G. ZWEIG, *A Manual of Paper Chromatography and Paper Electrophoresis*, 2nd Ed., Academic Press, New York, 1958, pp. 172–174.
3 K. RANDERATH, *Thin-Layer Chromatography*, Academic Press, New York, 1963, pp. 185–199.
4 A. SCHWEIGER, *J. Chromatog.*, 9 (1962) 374.
5 K. FINK, R. E. CLINE AND R. M. FINK, *Anal. Chem.*, 35 (1963) 389.
6 F. A. ISHERWOOD AND M. A. JERMYN, *Biochem. J.*, 48 (1951) 515.
7 E. STAHL (Editor), *Dünnschicht-Chromatographie*, Springer-Verlag, Berlin, 1962, p. 475.
8 R. J. BLOCK, E. L. DURRUM AND G. ZWEIG, *A Manual of Paper Chromatography and Paper Electrophoresis*, 2nd Ed., Academic Press, New York, 1958, p. 173.

9 H. T. GORDON, W. THORNBURG AND L. WERUM, *Anal. Chem.*, 28 (1956) 849.
10 E. F. WELLINGTON, *Biochim. Biophys. Acta*, 7 (1951) 238.
11 H. A. SCARBOROUGH AND W. A. WATER, *J. Chem. Soc.*, (1927) 91.
12 F. BEILSTEIN, B. PRAGER AND P. JACOBSON (Editors), *Organische Chemie*. Vol. 12. *Systeme 1592–1793*, Springer Verlag, Berlin, 1929, p. 1317.
13 T. L. BROWN AND J. BENJAMIN, *Anal. Chem.*, 36 (1964) 446.
14 J. A. THOMA, *Anal. Chem.*, 35 (1963) 214.