CHROM. 4549

Electrophoresis of hexuronic acids

The problems involved in the isolation and identification of uronic acids from natural sources have been well documented. These compounds are extraordinarily labile to acid[1,2] and to alkali[3-5], forming a variety of degradation products which interfere in subsequent colorimetric measurements and in separations. To complicate matters further, hexuronic acids lactonize under conditions used for hydrolysis of the polysaccharides[4].

Methods for separation and identification of uronic acids have employed ion-exchange resins[1,6,7], paper chromatography[8], gas–liquid chromatography of the corresponding aldonolactones[9], thin-layer chromatography[10] and electrophoresis[11,12]. HAUG AND LARSEN[11] obtained excellent separation of a number of hexuronic acids using 0.01 *M* borate buffer containing calcium chloride. They noted the anticipated enhanced separation upon addition of calcium ion due to selective cation binding by uronic acids. However, with the apparatus available in this laboratory, their buffer did not give adequate separation and other buffers were sought.

This report describes the electrophoretic separation of hexuronic acids using the acetates of a number of cations which were selected on the basis of reported cation binding by various glycuronans and glycosaminoglycuronans[13,14].

Materials and methods

L-Iduronic acid was synthesized by a modification of the method described by WOLFROM AND THOMAS[15]. L-Guluronic acid was obtained by partial hydrolysis of alginic acid *(Macrocystis pyrifera)*. D-Mannuronolactone was the gift of Dr. B. A. LEWIS.

A Shandon electrophoresis chamber was used with the following modifications: on the support bars were placed in succession: a glass plate (20 × 20 × 0.3 cm), foam rubber (20 × 20 × 1.2 cm), Parafilm (20 × 23 cm), electropherogram, Parafilm (20 × 23 cm), a glass plate (20 × 20 × 0.3 cm) and a Pyrex dish containing ice (22 × 22 × 5 cm)[16]. The chamber cover was not used. By reversal of polarity, two runs could be made with each solution and the electrodes were cleaned with hydrochloric acid between each set of two runs. Except where noted, 0.1 *M* acetate solutions were used. An unregulated Buchler power supply (0–1000 V, 0–200 mA) was used. Runs were started at 600 V and continued for 90 min. During the runs with zinc acetate (0.1 *M*), the voltage dropped to about 580 V while the current rose from 55 to approximately 100 mA. Some cations (barium, calcium, potassium, magnesium) produced current which was prohibitively high for this power supply.

Hexuronic acids were applied at 2 cm intervals along the center of a piece of Whatman No. 1 paper (18 × 31 cm). The paper was wet just to the line of application in the appropriate solution, blotted, immediately placed in the electrophoresis apparatus and current applied. After the run the papers were dried at room temperature, sprayed with ammoniacal silver nitrate and heated at 100° until the spots were moderately brown, avoiding excessive darkening of the background. The hexuronic acid spots darkened considerably upon standing at room temperature, whereas the background darkened only slowly; electropherograms might be best visualized the

TABLE I

ELECTROPHORETIC MOBILITIES OF HEXURONIC ACIDS

0.1 M acetate solution	*pH*	*Migration distance of glucuronic acid*[a]	$M_{glucuronic\ acid}$[b]			
			Mannuronic acid	*Galacturonic acid*	*Iduronic acid*	*Guluronic acid*
Zinc	6.6	5.8	0.75	0.30	0.32	0.44
Cadmium	7.1	5.1	1.01	0.64	0.79	0.55
Calcium	7.3	10.0	0.77	0.50	0.46	0.40
Magnesium	6.8	10.5	0.99	0.93	0.96	0.93
Cobalt	7.2	8.0	0.84	0.51	0.51	0.54
Barium	7.8	8.5	0.79	0.42	0.21	0.26
Potassium	7.6	10.2	0.95	0.89	0.95	0.93
Copper[c]	5.5	−0.4				

[a] Distance in cm measured from *meso*-inositol in 90 min, except for potassium acetate which was 60 min.

[b] Mobility relative to glucuronic acid.

[c] All hexuronic acids moved toward the cathode in cupric acetate. Movement was so slight, however, that measurements of $M_{glucuronic\ acid}$ were misleading.

day after spraying. One microgram of glucuronic acid was easily visualized by this procedure. Although *p*-anisidine trichloroacetate containing 2% phosphoric acid was also used for spraying, it was less sensitive than the silver nitrate reagent. With *meso*-inositol taken as zero, mobilities were measured and expressed relative to the mobility of glucuronic acid.

Results

The mobilities of hexuronic acids in eight acetate solutions are shown in Table I. Zinc acetate (0.01 *M*) and 0.01 *M* borate with 0.005 *M* calcium chloride produced very diffuse spots with virtually no separation. The hexuronic acids migrated toward the anode in all solutions except copper acetate where there was a slight movement toward the cathode. A typical electropherogram run in zinc acetate is shown in Fig. 1.

There were spots in the inositol area in all hexuronic acid solutions except galacturonic acid. The lactones of some of the hexuronic acids, for example, mannuronolactone, are extremely stable in aqueous solution and should be carefully neutralized[4] before electrophoresis.

Glucose, which was included in every run, exhibited no mobility. Thus it was possible to separate neutral from acidic sugars by neutralization of the mixture followed by electrophoresis in an appropriate solution.

Of the eight solutions tested, zinc and barium acetates afforded the best overall resolutions. The separation of those hexuronic acids which occur mutually in nature was excellent, *i.e.*, mannuronic from guluronic acid and glucuronic from iduronic acid. Although galacturonic, iduronic and guluronic acids exhibited similar mobilities, the presence of each in a mixture was readily seen upon electrophoresis in several solutions, for example, zinc and barium acetates.

Although one hesitates to speak of selective cation binding on the basis of experiments with this relatively crude apparatus, it is difficult to explain the results on any other basis. The acid which binds a particular cation most strongly exhibits

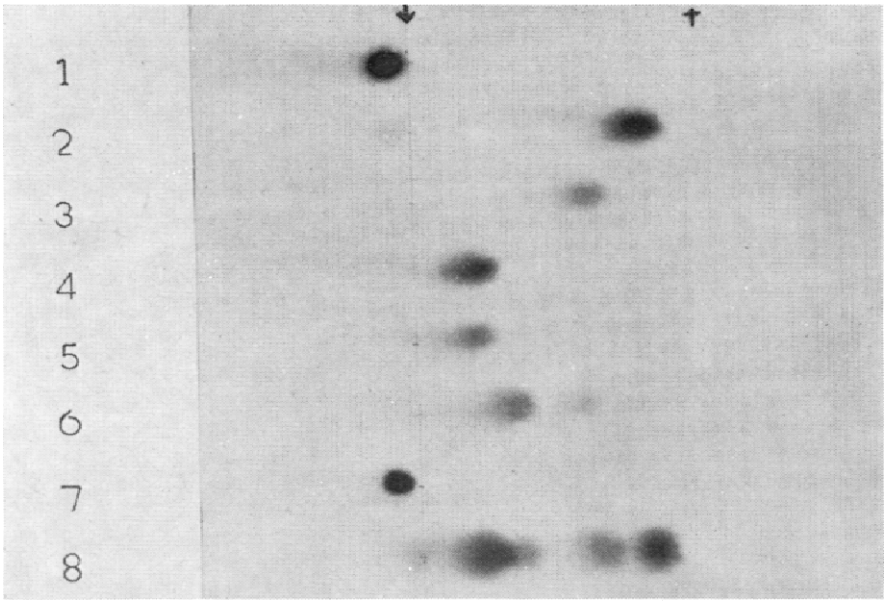

Fig. 1. Electropherogram, 0.1 *M* zinc acetate, 600 V, 90 min. 1 = D-glucose; 2 = D-glucuronic acid (10 μg); 3 = D-mannuronic acid, sodium salt; 4 = D-galacturonic acid; 5 = L-iduronic acid; 6 = L-guluronic acid; 7 = *meso*-inositol; 8 = mixture of 2, 3, 4, 5 and 6. An ammoniacal silver nitrate spray was used.

the lowest mobility in a solution of that cation. Thus this method gives a qualitative comparison of the preferred cation binding of the hexuronic acids. Glucuronic acid does not appear to bind these cations as strongly as the other hexuronic acids tested. Galacturonic, iduronic and guluronic acids have strikingly similar binding patterns and all three bind the tested divalent cations more strongly than either glucuronic or mannuronic acids.

This method affords a simple, rapid, reproducible means of separation of hexuronic acids. It is useful not only for identification but also for preparative purposes. In addition, it is a simple means of screening the cation binding properties of the various hexuronic acids.

This study from the Section of Endocrinology of the Evans Memorial Department of Clinical Research, Boston University School of Medicine, was supported by the grant from the National Institute of Arthritis and Metabolic Diseases, National Institute of Health (AM 12027-03).

Department of Medicine, University Hospital,
Boston University School of Medicine,
750 Harrison Avenue, Boston, Mass. 02118 (U.S.A.)

MARLYS J. ST. CYR

1 A. HAUG AND B. LARSEN, *Acta Chem. Scand.*, 16 (1962) 1908.
2 R. L. WHISTLER AND K. W. KIRBY, *Z. Physiol. Chem.*, 314 (1959) 46.
3 F. SMITH, *J. Chem. Soc.*, (1944) 584.
4 J. D. BLAKE AND G. N. RICHARDS, *Carbohydrate Res.*, 8 (1968) 275.
5 B. CARLSSON, O. SAMUELSON, T. POPOFF AND O. THEANDER, *Acta Chem. Scand.*, 23 (1969) 261.
6 U. LARSSON, T. ISAKSSON AND O. SAMUELSON, *Acta Chem. Scand.*, 20 (1966) 1965.
7 L. A. FRANSSON, L. RODEN AND M. L. SPACH, *Anal. Biochem.*, 23 (1968) 317.
8 F. G. FISHER AND H. DÖRFEL, *Z. Physiol. Chem.*, 302 (1955) 186.

9 M. B. PERRY AND R. K. HULYALKAR, *Can. J. Biochem.*, 43 (1965) 573.
10 W. ERNST, *Anal. Chim. Acta*, 40 (1968) 161.
11 A. HAUG AND B. LARSEN, *Acta Chem. Scand.*, 15 (1961) 1395.
12 H. MAYER AND O. WESTPHAL, *J. Chromatog.*, 33 (1968) 514.
13 J. E. SCOTT, in G. QUINTARELLI (Editor), *The Chemical Physiology of the Mucopolysaccharides*, Little, Brown, Boston, 1968, Ch. 12.
14 R. E. S. PROUT, *Biochim. Biophys. Acta*, 177 (1969) 157.
15 M. L. WOLFROM AND G. H. S. THOMAS, in R. L. WHISTLER AND M. L. WOLFROM (Editors), *Methods in Carbohydrate Chemistry*, Vol. II, Academic Press, New York, 1963, p. 32.
16 D. R. BRIGGS, E. F. GARNER, R. MONTGOMERY AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.

Received December 17th, 1969

CHROM. 4541

An artefact in the chromatography of sugar nucleotides using solvents containing ammonium acetate

The solvents (A) 95% ethanol–1 *M* ammonium acetate pH 7.5 (5:2) and (B) 95% ethanol–1 *M* ammonium acetate pH 3.8 (5:2) are widely used in the analysis and preparation of sugar nucleotides. These solvents were originally described by PALADINI AND LELOIR[1] and were used to assist in studies on the properties of uridine diphosphoglucose (UDPG). In one respect solvent A is the more useful of the two because it allows an easier separation of sugar nucleotide from the related nucleoside 5'-phosphates which often accompany it in biochemical preparations. Some $R_{adenosine}$ values in this solvent are given in Table I. It is the purpose of this communication, however, to show that unreliable analytical results are obtained with solvent A unless certain precautions are taken.

Experimental

Analytical standards were obtained from Sigma, London Ltd. Chromatographic

TABLE I

SOME $R_{adenosine}$ VALUES AT 22° IN SOLVENT A

Compound	$R_{adenosine}$
Adenosine 5'-triphosphate	0.05
Adenosine 5'-diphosphate	0.09
Adenosine 5'-monophosphate	0.24
Adenosine 5'-diphosphoglucose	0.32
Adenosine	1.00
Uridine 5'-triphosphate	0.09
Uridine 5'-diphosphate	0.14
Uridine 5'-monophosphate	0.32
Uridine 5'-diphosphoglucose	0.44
Glucose-1-phosphate	0.39
Glucose-1:2-cyclic phosphate	0.86