CHROM. 3692

Filter paper electrophoresis of purines and pyrimidines: mobility data

In our laboratory, a combination of filter paper electrophoresis and partition chromatography has been applied to the separation and identification of a number of purines, pyrimidines, and nucleosides isolated from urine of normal and leukemic subjects[1]. As a preliminary step, electrophoretic mobilities were obtained for some 94 compounds, and these are reported here.

Experimental

The Durrum Model B paper electrophoresis cell was used with a rack which accommodates eight filter paper strips (Schleicher and Schüll 2043-A, 3 cm × 30.4 cm). Sodium borate buffer, ionic strength of 0.1 at pH 9.2, was used throughout. Aliquots of solutions of the authentic compounds, containing from 2–5 μg, were applied as small spots (2–3 mm) at the center of the paper strips. After the rack of strips was placed in the cell the papers were moistened with buffer to within 1 cm of the initial positions of the applied compounds. The cell was covered, and a potential of 300 V was applied across the strips, generally for 4 h. (Some compounds, especially nucleotides, migrate too rapidly and hence a two-hour period was used for these.) After the filter paper strips had dried at room temperature, the compounds were visualized under ultraviolet light, and the distance of migration, either to the anode or cathode, was measured from the point of origin.

In an attempt to reduce the variation in mobility values obtained with replicate determinations in the same or separate series of runs, the effect of equilibration was studied using the following procedure. A 3 mm hole was punched equidistant from the ends of the paper strips, prior to placing the strips on the rack and positioning it in the cell. The papers were moistened with buffer and allowed to equilibrate in the covered cell for 2 h at room temperature. The standard solutions were applied to individual 3 mm paper discs and allowed to dry. The discs were then inserted with the use of forceps through an aperture in the cover of the Durrum cell and placed in the circular holes previously punched from the paper strips. The aperture was closed, and an additional equilibration period of 30 min was used before the electrophoresis was started, using the same voltage and time as above.

Results and discussion

The mobility data are tabulated in Tables I–IV. In the first three tables, the compounds are arranged according to average mobility, to permit easy recognition of what compounds might overlap and thus be considered in separation and identification procedures. The classification in these three tables was made on the basis of direction of migration (anode or cathode) and the period of time used for electrophoresis (2 or 4 h). In Table IV the compounds, together with average mobilities, are arranged in alphabetical order in order to determine whether a specific compound has been studied and to facilitate location of that compound in the preceding tables.

The fluctuations in mobilities for a given compound are probably inherent in the procedure, with lack of uniformity of filter paper as one variable. The technique with the insertion of the discs to which the standard has been applied permitted

TABLE I

ELECTROPHORETIC DATA FOR PURINES AND PYRIMIDINES AND THEIR DERIVATIVES ARRANGED ACCORDING TO MOBILITIES TOWARD THE ANODE

Compound	*Mobility (cm/4 h)*			*Number of Determinations*
	Average	*Range*		
Xanthosine	11.5	13.1	11.2	3
5-Fluorouracil	11.3	11.5	11.0	3
Xanthine	9.3	9.9	8.3	14
Inosine	8.3	8.8	7.9	4
8-Hydroxypurine	8.0	8.6	7.3	5
1,3-Dimethyluric acid	7.9	8.0	7.8	3
1-Methylxanthine	7.9	8.3	7.3	3
7-Methyluric acid	7.6	7.8	7.4	3
6,8-Dihydroxypurine	7.4	7.8	7.0	6
3-Methyluric acid	7.4	7.7	7.0	4
Uric acid	7.4	7.6	7.4	3
1-Methyluric acid	7.2	7.6	6.9	3
2-Oxypurine	7.1	7.9	6.8	6
Uridine	6.9	7.2	6.3	3
3,7-Dimethyluric acid	6.8	7.2	6.4	3
7-Methylxanthine	6.8	7.2	6.6	4
1,7-Dimethyluric acid	6.6	7.0	6.2	3
5-Fluorodeoxyuridine	6.6	6.9	6.4	3
3-Methylxanthine	6.4	7.0	6.1	5
Guanosine	6.2	6.5	6.1	3
5-Hydroxymethyluridine	6.1	6.4	5.8	5
5-Ribosyluracil	5.7	6.3	5.2	5
1,3-Dimethylxanthine	5.5	5.8	5.3	5
1,7-Dimethylxanthine	5.5	5.8	5.3	5
Thymine ribonucleoside	5.5	5.7	5.3	3
5-Acetylamino-6-amino-3-methyluracil	5.4	5.5	5.4	3
N^2-Dimethylguanosine	5.2	5.6	4.8	22
Inosine-5'-phosphate	5.1	5.7	4.4	3
Hypoxanthine	5.0	5.3	4.7	7
1-Methylinosine	4.8	5.3	4.5	13
4-Amino-5-imidazolecarboxamide ribonucleoside	4.5	4.9	4.2	4
6-Ethylaminopurine	4.4	5.2	4.0	5
Cytidine	4.3	4.6	4.0	3
Adenosine	4.1	4.3	4.0	3
Isoguanine	4.0	4.6	4.0	3
N^6-Methyladenosine	4.0	4.2	3.8	16
1-Methylguanosine	3.7	4.0	3.0	16
2-Amino-8-hydroxypurine	3.2	3.9	2.9	6
7-Methylhypoxanthine	3.1	3.2	3.0	3
8-Hydroxy-7-methylguanine	3.0	3.3	2.7	3
1-Methylhypoxanthine	3.0	3.4	2.8	3
6-Amino-8-hydroxypurine	2.9	3.2	2.6	5
Deoxyinosine	2.9	3.0	2.9	5
9-Methylhypoxanthine	2.6	2.9	2.3	4
Uracil	2.5	2.8	2.2	7
2-Amino-6,8-dihydroxypurine	2.2	2.2	2.2	2
2-Methylhypoxanthine	2.1	2.4	1.9	3
Deoxyguanosine	1.8	1.9	1.7	3
7-Methylguanine	1.8	2.3	1.2	7
3-Methylcytidine	1.8	2.2	1.3	15
Deoxyuridine	1.7	1.8	1.6	3
N^2-Dimethylguanine	1.5	1.8	1.2	3

(*continued on p. 345*)

TABLE I (*continued*)

Compound	*Mobility (cm/4 h)*			*Number of Determinations*
	Average	*Range*		
1-Methyladenosine	1.4	1.7	1.1	14
Guanine	1.3	1.4	1.1	7
5-Hydroxymethyldeoxyuridine	1.3	1.7	1.1	4
N^2-Methylguanine	1.2	1.3	1.0	4
2-Aminopurine	1.1	1.2	0.7	5
6-Methyluracil	0.3	0.7	−0.7	6
Thymidine	0.3	0.6	0.0	5
3-Methyluracil	0.2	0.4	−0.2	5
1-Methyluracil	0.1	0.4	−0.1	5

equilibration of the paper and samples prior to electrophoresis, but with the equilibration times employed, there did not appear to be a significant improvement in the reproducibility.

TABLE II

ELECTROPHORETIC DATA FOR PURINES AND PYRIMIDINES AND THEIR DERIVATIVES ARRANGED ACCORDING TO MOBILITIES TOWARD THE ANODE

Compound	*Mobility (cm/2 h)*			*Number of Determinations*
	Average	*Range*		
6-Succinoaminopurine	8.4	9.0	7.9	4
Orotidine-5′-phosphate	8.1	8.6	7.7	3
Orotidine cyclohexyl ammonium salt	7.3	7.6	7.0	4
Uridine-2′,3′-phosphate	7.3	7.5	6.9	3
Orotic acid	7.2	7.6	6.9	4
Thymidine-5′-phosphate	7.1	7.4	6.9	3
Cytidine-2′,3′-phosphate	6.7	6.9	6.5	3
Guanosine-2′,3′-phosphate	6.5	6.9	6.2	4
Deoxycytidine-5′-phosphate	6.4	7.0	6.2	3
5-Methylorotic acid	6.4	6.7	6.3	3
Adenosine-2′,3′-phosphate	6.3	6.7	5.9	3
Deoxyguanosine-5′-phosphate	6.2	6.4	6.1	3
Deoxyadenosine-5′-phosphate	6.0	6.3	5.8	3

The electrophoretic mobility data included in the tables have been of use in the identification of a variety of purines and pyrimidines found in urine. Because of overlapping mobilities it is often not possible to separate the components of a mixture using electrophoresis for the separations of these compounds in urine. As reported previously[1], we have first used ion-exchange chromatography to obtain a crude fraction. Subsequently filter paper partition chromatography with *n*-butanol, water and concentrated ammonium hydroxide (86:14:1) was employed to provide some separation of the ultraviolet absorbing components, and for the most part move them away from interfering fluorescent substances. Finally, use of electrophoresis in borate buffer provided a second-dimensional resolution, and yielded more readily identifiable spots.

TABLE III

ELECTROPHORETIC DATA FOR PURINES AND PYRIMIDINES AND THEIR DERIVATIVES ARRANGED ACCORDING TO MOBILITIES TOWARD THE CATHODE

Compound	*Mobility (cm/4 h)*			*Number of Determinations*
	Average	*Range*		
3-Methylcytosine	3.4	3.8	3.1	10
Cytosine	2.7	3.1	2.3	7
1,3,7-Trimethylxanthine	2.6	2.8	2.2	4
1,3-Dimethyluracil	2.6	2.8	2.5	3
1-Methyladenine	2.6	3.0	2.1	12
1,3,7,9-Tetramethyluric acid	2.3	2.7	2.1	3
5-Methylcytosine	2.3	2.7	2.0	3
Deoxycytidine	2.3	2.4	2.2	3
4-Amino-5-imidazolecarboxamide	2.1	2.2	1.9	4
7-Methyladenine	1.9	2.1	1.7	4
Deoxyadenosine	1.9	2.0	1.7	4
1,7-Dimethylguanine	1.9	2.2	1.6	5
9-Methyladenine	1.8	2.2	1.5	4
2,6-Diamino-7-methylpurine	1.7	1.8	1.7	3
1-Methylguanine	1.1	1.4	0.8	3
N^6-Methyladenine	0.9	1.2	0.5	3
2-Methyladenine	0.9	1.1	0.7	6
Thymine	0.8	1.0	0.7	7
3,7-Dimethylxanthine	0.5	1.1	0.0	4
Adenine	0.1	0.5	0.1	7

TABLE IV

ALPHABETICAL ARRANGEMENT OF COMPOUNDS, TOGETHER WITH AVERAGE MOBILITY[a]

5-Acetylamino-6-amino-3-methyluracil	5.4	N^2-Dimethylguanosine	5.2
Adenine	0.1[c]	1,3-Dimethyluracil	2.6[c]
Adenosine	4.1	1,3-Dimethyluric acid	7.9
Adenosine-2',3'-phosphate	6.3[2]	1,7-Dimethyluric acid	6.6
2-Amino-6,8-dihydroxypurine	2.2	3,7-Dimethyluric acid	6.8
2-Amino-8-hydroxypurine	3.2	1,3-Dimethylxanthine	5.5
6-Amino-8-hydroxypurine	2.9	1,7-Dimethylxanthine	5.5
4-Amino-5-imidazolecarboxamide	2.1[c]	3,7-Dimethylxanthine	0.5[c]
4-Amino-5-imidazolecarboxamide ribonucleoside	4.5	6-Ethylaminopurine	4.4
2-Aminopurine	1.1	5-Fluorodeoxyuridine	6.6
Cytidine	4.3	5-Fluorouracil	11.3
Cytidine-2',3'-phosphate	6.7[2]	Guanine	1.3
Cytosine	2.7[c]	Guanosine	6.2
Deoxyadenosine	1.9[c]	Guanosine-2',3'-phosphate	6.5[2]
Deoxyadenosine-5'-phosphate	6.0[2]	5-Hydroxymethyldeoxyuridine	1.3
Deoxycytidine	2.3[c]	8-Hydroxy-7-methylguanine	3.0
Deoxycytidine-5'-phosphate	6.4[2]	5-Hydroxymethyluridine	6.1
Deoxyguanosine	1.8	8-Hydroxypurine	8.0
Deoxyguanosine-5'-phosphate	6.2[2]	Hypoxanthine	5.0
Deoxyinosine	2.9	Isoguanine	4.0
Deoxyuridine	1.7	Inosine	8.3
2,6-Diamino-7-methylpurine	1.7[c]	Inosine-5'-phosphate	5.1
6,8-Dihydroxypurine	7.4	1-Methyladenine	2.6[c]
1,7-Dimethylguanine	1.9[c]	2-Methyladenine	0.9[c]
N^2-Dimethylguanine	1.5	7-Methyladenine	1.9[c]
		9-Methyladenine	1.8[c]

(*continued on p. 347*)

TABLE IV (*continued*)

N^6-Methyladenine	0.9[c]	1-Methylxanthine	7.9
1-Methyladenosine	1.4	3-Methylxanthine	6.4
N^6-Methyladenosine	4.0	7-Methylxanthine	6.8
3-Methylcytidine	1.8	Orotidine cyclohexyl ammonium salt	7.3[2]
3-Methylcytosine	3.4[c]	Orotidine-5'-phosphate	8.1[2]
5-Methylcytosine	2.3[c]	Orotic acid	7.2[2]
1-Methylguanine	1.1[c]	2-Oxypurine	7.1
7-Methylguanine	1.8	5-Ribosyluracil	5.7
N^2-Methylguanine	1.2	6-Succinoaminopurine	8.4[2]
1-Methylguanosine	3.7	1,3,7,9-Tetramethyluric acid	2.3[c]
1-Methylhypoxanthine	3.0	Thymidine	0.3
2-Methylhypoxanthine	2.1	Thymidine-5'-phosphate	7.1[2]
7-Methylhypoxanthine	3.1	Thymine	0.8[c]
9-Methylhypoxanthine	2.6	Thymine ribonucleoside	5.5
1-Methylinosine	4.8	1,3,7-Trimethylxanthine	2.6[c]
5-Methylorotic acid	6.4[2]	Uracil	2.5
1-Methyluracil	0.1	Uric acid	7.4
3-Methyluracil	0.2	Uridine	6.9
6-Methyluracil	0.3	Uridine-2',3'-phosphate	7.3[2]
1-Methyluric acid	7.2	Xanthine	9.3
3-Methyluric acid	7.4	Xanthosine	11.5
7-Methyluric acid	7.6		

[a] The superscript 2 designates mobility toward anode in 2 h; c designates mobility toward cathode in 4 h; all other mobilities are toward the anode in 4 h.

Acknowledgements

This research was supported in part by U.S. Public Health Service Grant CA-02433, and by the American Cancer Society Grant P-313.

Department of Medicine, School of Medicine, University of California at Los Angeles, Los Angeles, Calif. (U.S.A.)

WILLIAM S. ADAMS
MISAE NAKATANI

1 K. FINK AND W. S. ADAMS, *Arch. Biochem. Biophys.*, 126 (1968) 27.

Received July 9th, 1968