CHROM. 4625

Dragendorff reactions for visualization of amino acids and amino acid derivatives separated by paper or thin-layer chromatography

Modifications of the Dragendorff reagent are being widely used for detection of alkaloids separated by paper[1] and thin-layer[2] chromatography. One of these containing ethyl acetate is of particular importance, since it has been shown that the specificity, and especially the sensitivity of the reactions of this reagent may be considerably increased in both PC and TLC[3,4] by spraying the chromatogram with dilute sulfuric acid. The advantages of this reagent and the method of sensitization have been exploited for the specific detection of certain other basic compounds which are not alkaloids, such as adenine[5], choline and betaine[6]. With betaine, similar results could also be obtained if the chromatogram was sensitized by spraying with dilute perchloric acid instead of dilute sulfuric acid.

Furthermore, in an earlier communication[7] we described a characteristic reaction of ε-N-trimethyllysine [Me_3-Lys] on thin layers with Dragendorff reagent. The results obtained with this lysine compound with its dual characteristics of an α-amino acid and a quaternary amine, have led us to study the Dragendorff reactions of amino acids and their derivatives under PC and TLC conditions in general.

Materials and methods

The samples of amino acids used and their derivatives, were commercially available, the ε-N-methylated lysines were synthesized[8,9] and several samples were donated. The chromatographic paper used was Schleicher & Schüll 2043b; the sorbents were cellulose powder MN-300 (Macherey, Nagel & Co., Düren, G.F.R.) and Silica Gel G (Merck AG, Darmstadt, G.F.R.). Solvent 1 consisted of chloroform–methanol–25% ammonia (4:4:1) and solvent 2 of *n*-butanol–glacial acetic acid–water (4:1:5).

The polychromatic ninhydrin reagent was made by dissolving 0.5 g of ninhydrin in acetone and then adding 0.05% cadmium chloride. The Dragendorff reagent containing ethyl acetate was prepared by the method described in earlier communications[3–5]. For PC, the reagent was diluted to twice its initial volume, and for TLC it was diluted 4–6 times.

After spraying with the polychromatic ninhydrin reagent, the thin-layer plates were heated at 90° for 10 min and allowed to stand at room temperature for 12 h. After spraying with the Dragendorff reagent, the papers or the cellulose layers were dried and then sprayed with 0.1 *N* H_2SO_4 or $HClO_4$. When using silica gel 1.0 *N* H_2SO_4 or $HClO_4$ was used.

Results and discussion

The R_F values of the amino acids and their derivatives studied under various TLC conditions are given in Table I together with their reaction to the ninhydrin and modified Dragendorff reagent. It appears from this table that only a few of the amino acids or their derivatives give a positive Dragendorff reaction and that many of these are uncertain and short-lived. Those amino acids and their derivatives which give lasting and even specific reactions with the ethyl acetate-containing Dragendorff

TABLE I

COLOR REACTIONS AND $R_F \times 100$ VALUES OF AMINO ACIDS AND THEIR DERIVATIVES

Sorbents: KG = Silica Gel G (Merck); MN-300 = MN-cellulose powder 300 (Macherey, Nagel & Co). Solvent: chloroform–methanol–25% ammonia (4:4·1).

Amino acids and derivatives	*Color reaction*		$R_F \times 100$ *value*[a]	
	Ninhydrin	*Dragendorff + dil. acid*	*KG*	*MN-300*
Aliphatic monoamino acids				
Glycine[c]	+	—	47	33
Sarcosine[i]	+	—	40	38
L(+)-Alanine[c]	+	—	51	51
β-Alanine[c]	+	—	41	31
L-Serine[i]	+	—	42	32
DL-Phosphoserine[c]	+	—		0
DL-α-Amino-n-butyric acid[c]	+	—	82	51
α-Amino-isobutyric acid[c]	+	—		57
L-Threonine[i]	+	—	57	47
DL-β-Amino-isobutyric acid[c]	+	—	44	39
γ-Aminobutyric acid[g]	+	—	42	36
L(+)-Norvaline[i]	+	—		
L(+)-Valine[i]	+	—	68	76
L(+)-Norleucine[i]	+	—		85
L(−)-Leucine[i]	+	—	72	84
L(+)-Isoleucine[i]	+	—	70	85
L-Aspartic acid[b]	+	—	20	6
L-Asparagine hydrate[c]	+	—	43	13
L-Glutamic acid[b]	+	—	35	8
L-N-Methyl glutamic acid[k]	+	—		10
L-Glutamine[i]	+	—	50	38
Aliphatic diamino acids				
β,γ-Diaminopropionic acid[d]	+	—	16	11
L-α,γ-Diaminobutyric acid[d]	+	—	5	9
L(+)-Ornithine[i]	+	—	15	45
Citrulline[i]	+	—	48	37
L(+)-Lysine[k]	+	(+)	16	48
DL(+)-*allo*-δ-Hydroxylysine[c]	+	—	5	12
L-Nα-Methyllysine[i]	+	(+)	13	45
DL-Nε-Methyllysine	+	(+)	12	69
DL-Nε-Dimethyllysine	+	+	32	89
DL-Nε-Trimethyllysine	+	+	7	49
DL-α,α-Diaminopimelic acid[d]	+	—		4
S-Containing mono- and diamino acids				
L(+)-Cysteine[i]	+	—		13
L-Cysteic acid[c]	+	—	24	7
DL-*meso*-Homocysteine[c]	+	—	17	5
L-Methionine[i]	+	—	69	73
DL-Ethionine[g]	+	—	77	50
L(+)-*meso*-Lanthionine[c]	+	—	82	
L(−)-Cystine[i]	+	—	2	10
DL-*allo*-Cystathionine[c]	+	—	4	?
L-Djenkolic acid[c]	+	—	25	5
Aromatic monoamino acids				
DL-Phenylglycine[d]	+	—	70	38
DL-Phenylalanine[i]	+	—	72	83
L-N-Methylphenylalanine[i]	+	—	96	83
DL-o-Tyrosine[i]	+	—	76	34

TABLE I *(continued)*

Amino acids and derivatives	*Color reaction*		$R_F \times 100$ *value*[a]	
	Ninhydrin	*Dragendorff + dil acid*	*KG*	*MN-300*
m-Tyrosine[k]	+	—	64	20
L-Tyrosine[l]	+	—	63	53
L-N-Methyltyrosine[b]	+	—		50
DL-3,4-Dihydroxyphenylalanine[c]	+	—	4	10
β-Phenylserine[h]	+	—	63	27
N-Hetero amino and imino acids				
L-2-Azetidine-carboxylic acid[e]	+	—	30	30
L(−)-Proline[f]	+	—	43	64
L-4-Hydroxyproline[e]	+	—	49	43
DL-Pipecolic acid[e]	+	—	33	49
L-Tryptophan[f]	+	—	69	64
DL-5-Methyltryptophan[d]	+	—	90	54
DL-6-Methyltryptophan[d]	+	—	90	52
L(−)-Histidine[f]	+	(+)	56	54
L-1-Methylhistidine[e]	+	+	80	66
L-3-Methylhistidine[e]	+	+	66	70
L-2-Thiohistidine[e]	+	+	73	13
Ergothioneine[j]	—	+		46
L-Carnosine[e]	+	+	26	15
Guanidine derivatives of α-amino acids				
Glycocyanine[d]	—	—		
Creatine[k]	—	—		
L-Arginine[f]	+	(+)	2	22
L-Nα-Methylarginine[f]	+	(+)	16	34
L-Homoarginine[e]	+	(+)	14	19
L-Canavanine sulphate[e]	+	—	36	9
Creatinine[k]	—	+	92	72

[a] R_F values are estimated
[b] Ajinomoto Co., Inc., Tokyo.
[c] Calbiochem, Los Angeles, U.S.A.
[d] Fluka AG, Buchs SG, Switzerland.
[e] Koch–Light Labs. Ltd., Colnbrook, Bucks., Great Britain.
[f] Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan
[g] Mann Research Labs., New York, U.S.A.
[h] Nutritional Biochemicals Co., Cleveland, Ohio, U.S.A.
[i] Reanal Finomvegyszergyár, Budapest, Hungary
[j] Carl Roth OHG, Karlsruhe, G.F.R.
[k] Sigma Chemical Co., St. Louis, Mo., U.S.A.
[l] Gift of Dr. MITSUO EBATA, Shionogi and Co., Ltd., Osaka, Japan.

reagent when treated with dilute sulfuric acid will be discussed in detail below. It is worth noting that, for most of the compounds, the sensitivity of the Dragendorff reagent, like that of the ninhydrin reagent, is greater on Silica Gel G than on the cellulose layer. In turn, the sensitivity on the cellulose layer is greater than that on the chromatographic paper (see Table II). The compactness of the spot is also greatest on the silica gel layer.

Lysine and its α-N and ε-N-methylated derivatives. Lysine, the three ε-N-methylated lysines and Me$^{\alpha}$-Lys all give a ninhydrin-positive reaction. While the intensity

TABLE II

THIN-LAYER CHROMATOGRAPHY OF AMINO ACIDS

Amino acid	*Sensitivity (μg)*			
	Ninhydrin		*Dragendorff + dil. acid*	
	MN-300	*Silica Gel G*	*MN-300*	*Silica Gel G*
L(+)-Lysine	0.4	0.03	(1 5)	(1.0)
L-*Nα*-Methyllysine	0 5	0.04	(1.0)	(0.7)
DL-*Nε*-Methyllysine	0.4	0.05	(0.8)	(0.7)
DL-*Nε*-Dimethyllysine	0.8	0.09	0.5	0.4
DL-*Nε*-Trimethyllysine	1.0	0.5	0.2	0.2
L(—)-Histidine	0.5	0 5	(1.5)	(0.8)
L-1-Methylhistidine	0.7	0.6	0.7	0.6
L-3-Methylhistidine	0.4	0.6	0.3	0.4
L-2-Thiohistidine	0.5	0.1	0.3	0.3
Ergothioneine	—	—	0.5	0.1
L-Carnosine	4.0	2.0	1.0	0.6
L-Arginine	0.4	0.08	(1.5)	(1 0)
L-*Nα*-Methylarginine	1.0	0.6	(1.2)	(0.7)
L-Homoarginine	0.4	0.2	(0.8)	(0.1)
Creatinine	—	—	0.5	0.1

of the reaction of Me^{ε}-Lys is approximately the same as that of Lys, the reaction of $Me^{\varepsilon}{}_2$-Lys, and especially of $Me^{\varepsilon}{}_3$-Lys, is significantly lower. On the other hand, with the Dragendorff reagent $Me^{\varepsilon}{}_3$-Lys gave the most characteristic and most intensive reaction, and the order of sensitivity is as follows:

$$Me^{\varepsilon}{}_3\text{-Lys} > Me^{\varepsilon}{}_2\text{-Lys} > Me^{\varepsilon}\text{-Lys} > Me^{\alpha}\text{-Lys} > \text{Lys}.$$

While $Me^{\varepsilon}{}_3$-Lys gives a characteristic betaine reaction (orange-red) with the Dragendorff reagent in visible light, the reaction of the Dragendorff reagent with $Me^{\varepsilon}{}_2$-Lys and Me^{ε}-Lys is less marked and only gives a pale yellow color. The same may be said about Me^{α}-Lys. The reaction of Lys in visible light is quite weak and hardly perceptible. After spraying with dilute sulfuric acid or perchloric acid, all three ε-N-methylated lysine derivatives give a characteristic purple color, provided that, before spraying with the Dragendorff reagent, the solvents and any traces of salts have been thoroughly removed from the layers. Sensitivities are of the order: $Me^{\varepsilon}{}_3$-Lys > $Me^{\varepsilon}{}_2$-Lys > Me^{ε}-Lys. After spraying with the dilute acid, the reaction of Me^{ε}-Lys, and especially of Lys, fades within a few minutes.

The positions of Lys and its ε-N-methylated derivatives together with other proteinogenic amino acids on a two-dimensional chromatogram are shown in Fig. 1. The modified Dragendorff reagent (together with dilute acid) may be advantageously used here for the identification of ε-N-methylated lysines on thin layers, since the other proteinogenic amino acids, with the exception of the methylhistidines, give no permanent reactions.

Methyl histidines. Both 1-Me-His and 3-Me-His give marked Dragendorff reactions which are more pronounced and lasting after spraying with acid. The color of these reactions is easily distinguishable from the colors of the ε-N-methylated Lys reactions. The R_F data in Table III show that the methylated histidines can be separated quite clearly from the methylated lysines on thin layers.

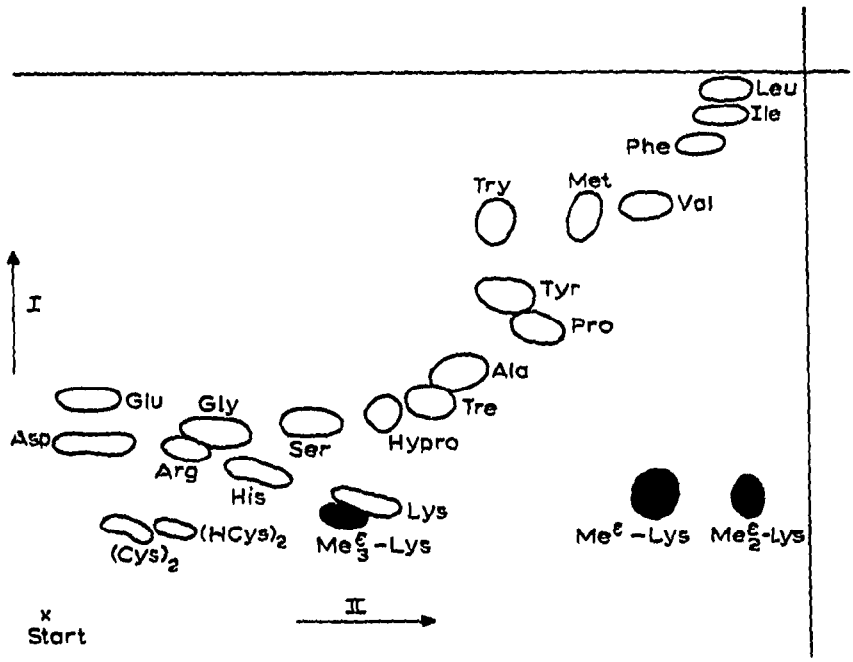

Fig. 1. Two-dimensional thin-layer chromatogram of proteinogenic amino acids. Sorbent: cellulose powder MN-300. Solvent systems. 1 = *n*-butanol–glacial acetic acid–water (4 1·5) (double development); 2 = chloroform–methanol–25% ammonia (4 4·1). White spots are ninhydrin positive; black spots are ninhydrin- and Dragendorff (plus dil. H_2SO_4)-positive.

Ergothioneine, as an α-N fully methylated histidine-like compound, cannot participate in protein formation and thus does not interfere with the examination of the components of protein hydrolysates. It is also shown in Table I that under TLC conditions this compound is easily separated from both methylated histidines and methylated lysines.

Creatine and creatinine and other α-amino acid guanidine derivatives. Me-Arg, and to a lesser degree Homo-Arg, are considered to give positive Dragendorff and Sakaguchi reactions. The Dragendorff reactions, however, are fairly weak and fade within a short time after treatment with sulfuric acid.

TABLE III

R_F × 100 VALUES OF AMINO ACIDS

Amino acid	*MN-300*		*Silica Gel G*	
	Solvent 1[a]	*Solvent 2*[b]	*Solvent 1*	*Solvent 2*
L-Lysine	57	10	14	6
DL-Nε-Methyllysine	69	15	12	5
DL-Nε-Dimethyllysine	89	13	32	4
DL-Nε-Trimethyllysine	49	6	7	3
L-Histidine	54	15	56	8
L-1-Methylhistidine	66	16	80	5
L-3-Methylhistidine	70	18	66	3

[a] Chloroform–methanol–25% ammonia (4 4 1).
[b] *n*-Butanol–glacial acetic acid–water (4:1 5).

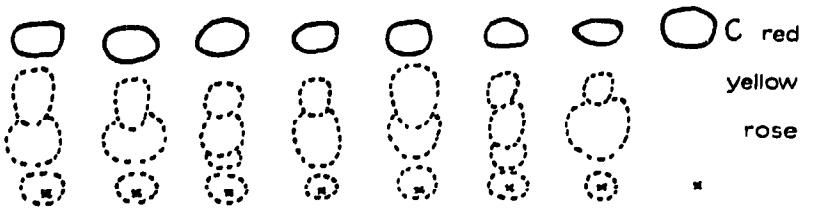

Fig. 2. Thin-layer chromatogram of several urine samples Sorbent: Silica Gel G Solvent: *n*-butanol–glacial acetic acid–water (4:1 5). Reagent ethyl acetate-containing Dragendorff reagent (plus dil. H_2SO_4). C = creatinine (5 μg), 1–7 = urine samples (10 μl)

The behavior of the two guanidine derivatives, namely of creatine and creatinine, deserves special attention. Creatinine gives a characteristic reaction with the Dragendorff reagent which remains and even deepens after treatment with dilute sulfuric acid. Creatine gives no such reaction. Fig. 2 shows the thin-layer chromatograms of human urine when treated with the ethyl acetate-containing Dragendorff reagent followed by spraying with dilute sulfuric acid. 10–20 μl samples were applied. The test can be carried out without preliminary desalting, since none of the other components give this reaction. The Joffe reagent, on the other hand, reacts with the other components of urine as well[10].

Summing up, it seems that the positive Dragendorff reaction with certain amino acids and their derivatives in PC and TLC could be developed into an identification method for these substances.

The authors are grateful to Dr. MITSUO EBATA (Shionogi and Co. Ltd., Japan), Ajinomoto Co. (Tokyo, Japan), Calbiochem (Los Angeles, Calif., U.S.A.), Fluka AG (Buchs, Switzerland), Kyowa Hakko Kogyo (Tokyo, Japan), and Sigma Chemical Co. (St. Louis, Mo., U.S.A.) for several amino acid samples. We also thank Mrs. MARIA MÜLLER and Mrs. EDIT EPER for their technical assistance.

Research Institute for Medicinal Plants,
Budapest (Hungary)

E. TYIHÁK
D. VÁGUJFALVI

1 I. M. HAIS AND K. MACEK, *Handbuch der Papierchromatographie*, Bd. I, VEB Gustav Fischer Verlag, Jena, 1963.
2 E. STAHL, *Dünnschichtchromatographie*, Springer Verlag, Berlin, 1962.
3 D. VÁGUJFALVI, *Planta Med.*, 8 (1960) 34
4 D. VÁGUJFALVI, *Planta Med.*, 13 (1965) 79.
5 E. TYIHÁK, *J. Chromatog.*, 14 (1964) 125
6 E. TYIHÁK, *Naturwiss.*, 51 (1964) 315.
7 E. TYIHÁK, *Herba Hung.*, 8 (1969) 113.
8 J PUSKÁS AND E. TYIHÁK, *Periodica Polytech*, 13 (1969) 261
9 J. PUSKÁS AND E TYIHÁK, unpublished data
10 G. PATAKI, *Die Dünnschichtchromatographie in der Aminosäure und Peptid-Chemie*, Walter De Gruyter, Berlin, 1966.

First received November 26th, 1969; revised manuscript received January 21st, 1970