Identification of guanidine derivatives by coupled electrophoresis–paper chromatography

For some time now, we have adopted a technique for the identification of the nitrogen components in biological material, fundamentally based on the ideas of BISERTE *et al.*[1], into which we have introduced some modifications[2].

Although bibliographical references to electrophoretic and chromatographic methods of investigation are very numerous when dealing with amine compounds, they are quite small in the field of guanidine derivatives, and even then the number of compounds studied is very small[3–5]. Recently new natural guanidine derivatives have been isolated, especially in different invertebrates[6–9], about which there are very few details which can lead to their identification in other biological materials by their electrophoretic and chromatographic characteristics. Furthermore, the study of the intermediary metabolism of guanidine derivatives raises difficult problems in connection with the identification of compounds, which are sometimes formed in minimum quantities.

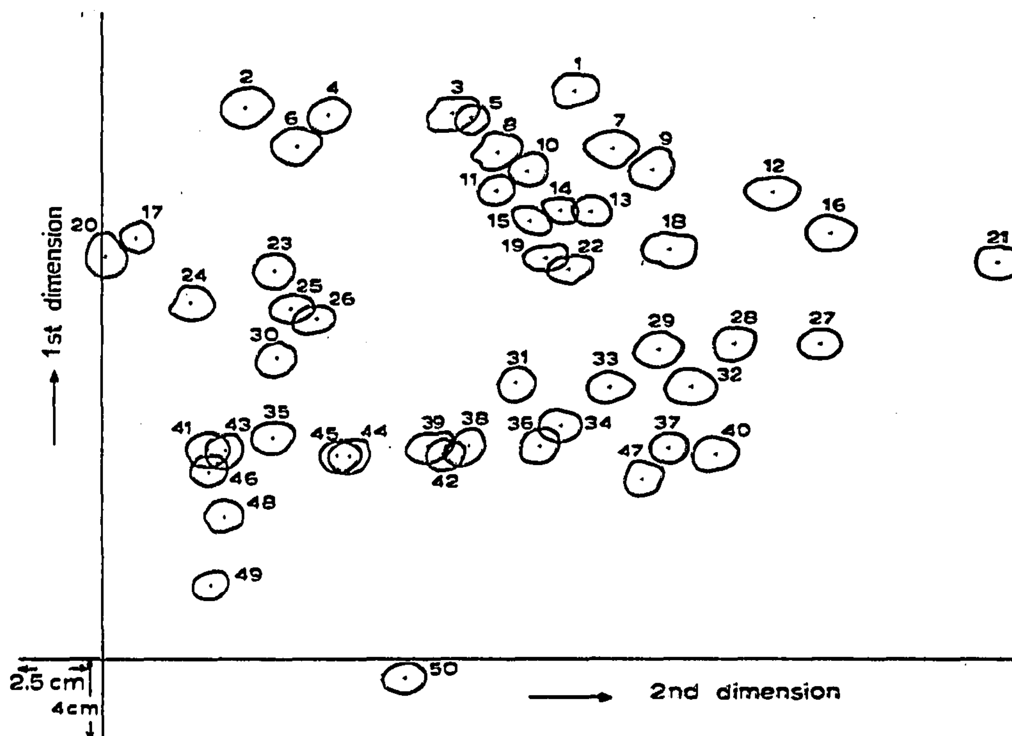

Fig. 1. Two-dimensional electrophoresis–chromatography. Paper: Schleicher & Schüll 2043b (30 × 29 cm). First dimension: electrophoresis; buffer, pyridine–acetic acid–water (3:10:487), pH 3.9; electrical potential: 350 V; time: 1.5 h. Second dimension: ascending chromatography; solvent system, *n*-butanol–acetic acid–water (12:3:5); time: 25 h.

We have considered it interesting to collect the data we obtained during several years of work on guanidine bases, during which we have frequently been confronted with problems of identification. In addition, we think that it would be useful to complete these data by the inclusion of the guanidine derivatives recently isolated, which we have synthesized because they are not commercially available.

Schematically, the technique of fractionation consists of two-dimensional

coupled electrophoresis–chromatography, carried out on sheets of Schleicher & Schüll 2043b paper 30 cm × 29 cm. Electrophoretic development is carried out in a tank in an inverted "V" with a pyridine–acetic acid–water buffer (3:10:487) of pH 3.9, at 350 V for 1 h 30 min, with subsequent chromatographic development in a *n*-butanol–acetic acid–water system (12:3:5), by the ascending method, for 25 h.

The reagent of Sakaguchi as modified by JEPSON *et al.*[10] was employed as the

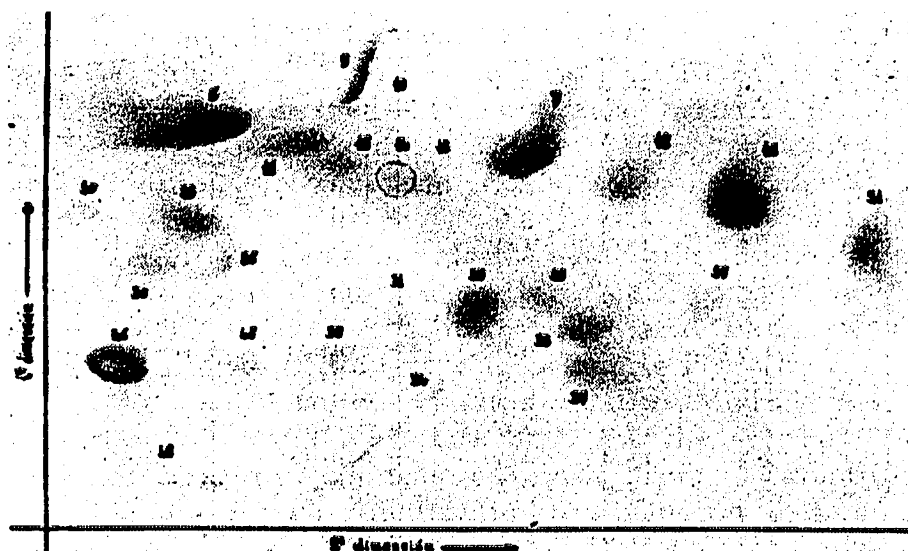

Fig. 2. Separation of 26 guanidine derivatives by two-dimensional electrophoresis–chromatography.

basic developer Other visualisation methods used were the ninhydrin reaction[11], the diacetyl-α-naphthol system[12], the Jaffé reaction modified by AMES AND RISLEY[13], the reaction of carbonyl derivatives with 2,4-dinitrophenylhydrazine[14], and the Ehrlich reaction for urea and derivatives[15].

TABLE I

ABSOLUTE AND RELATIVE MOBILITIES OF GUANIDINE COMPOUNDS

R_G = mobility relative to that of glycine. Paper: Schleicher & Schüll 2043b (30 × 29 cm). Electrophoresis: pyridine–acetic acid–water (3:10:487) buffer, pH 3.9; 350 V for 1 h 30 min. Chromatography: *n*-butanol–acetic acid–water (12:3:5), ascending technique for 25 h. Colour of guanidine compounds with various specific reagents. D = Diacetyl-α-naphthol; N = ninhydrin; J = Jaffé; P = 2,4-dinitrophenylhydrazine; E = Ehrlich. c = reaction + only with previous heating at 100° for 2 h.

Substance	*Electrophoresis*		*Chromatography*		*Sakaguchi*	D	N	J	P	E
	cm	R_G	*cm*	R_G						
1 Guanidine	19.5	2.8	13.3	1.9		+				
2 N-Aminopropylagmatine	18.9	2.7	4.1	0.6	+ red	+	+			
3 Agmatine	18.8	2.7	9.9	1.4	+ red	+	+			
4 Histaguanidine	18.6	2.7	6.4	0.9	+ purple	+				
5 Homoagmatine	18.5	2.6	10.4	1.5	+ red	+	+			
6 Hirudonine	17.6	2.5	5.5	0.8	+ red	+				
7 Methylguanidine	17.6	2.5	14.4	2.1	+ orange	+				
8 Arcain	17.4	2.5	11.2	1.6	+ red	+				
9 N,N-Dimethylguanidine	16.9	2.4	15.7	2.2		+				
10 Audouine	16.7	2.4	12.0	1.7	+ red	+				

(continued on p. 302)

TABLE I *(continued)*

Substance	*Electrophoresis*		*Chromatography*		*Sakaguchi*	*D*	*N*	*J*	*P*	*E*
	cm	R_G	*cm*	R_G						
11 Mercaptoethylguanidine	16.1	2.3	11.1	1.6	+ red	+				
12 Ethylguanidine	16.0	2.3	19.0	2.7	+ orange	+				
13 Guanidinoethanol	15.5	2.2	13.7	2.0	+ pink	+				
14 Hydroxyguanidine	15.3	2.2	13.0	1.9	+ yellow					
15 γ-Guanidinobutanol	15.0	2.1	12.0	1.7	+ orange	+				
16 Propylguanidine	14.8	2.1	20.6	2.9	+ orange	+				
17 Streptidine	14.4	2.1	0.9	0.1	+ pink	+				
18 γ-Guanidinobutyraldehyde	14.0	2.0	16.1	2.3	+ orange	+			+	
19 γ-Guanidinobutyramide	13.7	2.0	12.6	1.8	+ orange	+				
20 Streptomycin	13.7	2.0	0.0	0.0	+ pink	+				
21 Isoleucaguanidine	13.6	1.9	25.5	3.6	+ red	+				
22 Creatinine	13.4	1.9	13.2	1.9				+		
23 α-Amino-β-guanidinopropionic acid	13.2	1.9	4.9	0.7	+ red	+	+			
24 Canavanine	12.2	1.7	2.6	0.4	+ brown	+	+			
25 Arginine	12.0	1.7	5.5	0.8	+ orange	+	+			
26 Homoarginine	11.6	1.7	6.0	0.9	+ orange	+	+			
27 ε-Guanidinocaproic acid	10.8	1.5	20.4	2.9	+ orange	+				
28 δ-Guanidinovaleric acid	10.8	1.5	17.9	2.6	+ orange	+				
29 γ-Guanidinobutyric acid	10.7	1.5	15.8	2.3	+ orange	+				
30 Hydroxyhomoarginine	10.3	1.5	4.9	0.7	+ pink	+	+			
31 β-Hydroxy-γ-guanidinobutyric acid	9.4	1.3	11.8	1.7	+ pink	+				
32 β-Guanidinoisobutyric acid	9.3	1.3	16.8	2.4	+ orange	+				
33 β-Guanidinopropionic acid	9.3	1.3	14.5	2.1	+ orange	+				
34 Argininic acid	7.9	1.1	13.0	1.9	+ orange	+				
35 α-Keto-γ-guanidoxybutyric acid	7.6	1.1	4.9	0.7	+ brown	+			+	
36 α-Guanidinopropionic acid	7.3	1.0	12.5	1.8	+ red	+				
37 α-Guanidinobutyric acid	7.2	1.0	16.2	2.3	+ orange	+				
38 Creatine	7.2	1.0	10.4	1.5		+		+ c		
39 Guanidinoacetic acid	7.2	1.0	9.4	1.3	+ orange	+		+ c		
40 α-Chloro-δ-guanidinovaleric acid	7.1	1.0	17.4	2.5	+ orange	+				
41 Lombricine	7.0	1.0	3.1	0.4	+ orange	+	+			
42 α-Keto-δ-guanidinovaleric acid	7.0	1.0	9.7	1.4	+ orange	+			+	
43 Octopine	7.0	1.0	3.4	0.5	+ red	+				
Glycine	7.0	1.0	7.0	1.0						
44 Citrulline	6.9	1.0	6.9	1.0			+			+
45 Taurocyamine	6.9	1.0	6.8	1.0	+ orange	+				
46 Desmethyloctopine	6.4	0.9	3.1	0.4	+ red	+				
47 Urea	6.3	0.9	15.4	2.2						+
48 Arginosuccinic acid	4.9	0.7	3.6	0.5		+	+			
49 Arginine-Nα,Nα-diacetic acid	2.5	0.4	3.1	0.4	+ orange	+				
50 Creatine phosphate	—0.7	—0.1	8.7	1.2		+		+ c		

The origin of the different compounds studied was as follows: creatinine, canavanine, arginine, creatine, citrulline and guanidinoacetic acid, were obtained from The Nutritional Biochemical Corporation, creatine phosphate, α-amino-β-guanidinopropionic acid, α-guanidinopropionic acid and arginosuccinic acid from Calbiochem, guanidine from Doesder, urea from Merck, and streptomycin from Antibioticos S.A.

Agmatine, methylguanidine, arcain, ethylguanidine, guanidinoethanol, N,N-

dimethylguanidine, N-aminopropylagmatine, hirudonine, propylguanidine, mercaptoethylguanidine, isoleucaguanidine, histaguanidine, hydroxyguanidine, hydroxyhomoarginine, audouine, homoagmatine, taurocyamine, homoarginine, and α-guanidinobutyric acid, ε-guanidinocaproic acid, β-hydroxy-γ-guanidinobutyric acid, δ-guanidinovaleric acid, γ-guanidinobutyric acid, β-guanidinoisobutyric acid and β-guanidinopropionic acid, were synthetized by us starting from the corresponding amino derivative and S-ethylthiourea, according to the method described for the synthesis of guanidinoacetic acid by BRAND AND BRAND[16].

Argininic acid and α-chloro-δ-guanidinovaleric acid were synthetized in our laboratory according to HAMILTON AND ORTIZ[17]. Octopine was prepared according to the technique of HERBST AND SWART[18] with α-bromopropionic acid; desmethyloctopine and arginine-N^{α},N^{α}-diacetic acid were synthetized in the same way with monochloroacetic acid.

α-Keto-δ-guanidinovaleric acid and α-keto-γ-guanidoxybutyric acid were obtained enzymatically, according to LACOMBE *et al.*[19], starting from arginine and canavanine, respectively, and L-amino acid oxidase of viper poison. Streptidine was prepared from streptomycin, according to HUNTER *et al.*[20].

We synthetized γ-guanidinobutyraldehyde by adaptation of the method described by WITT AND HOLZER[21] for the synthesis of succinic semialdehyde. γ-Guanidinobutyramide was obtained from γ-guanidinobutyric acid with thionyl chloride, and γ-guanidinobutanol by the sulphydric reduction of γ-guanidinobutyraldehyde.

Finally, lombricine was kindly supplied to us by Prof. A. H. ENNOR (Department of Biochemistry, John Curtin School of Medical Research, Canberra, Australia).

In Table I a summary is made of all the compounds tested, with their mobilities after electrophoretic and chromatographic development expressed in cm (absolute), and relative to those of glycine (R_G). An indication is also given of their behaviour with the detecting reagents tested, with special reference to the reagent of Sakaguchi.

Fig. 1 shows the spacial disposition of all the substances tested in the two-dimensional development, while Fig. 2 represents a real separation of 26 of the guanidine derivatives which reacted positively with the Sakaguchi reagent.

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The effect of solvent purity on the chromatography of indole-3-acetic acid

In the course of an investigation on the growth substance relationships of diploid and tetraploid races of *Ranunculus ficaria* L. certain anomalies were found with regard to the R_F values quoted for indole-3-acetic acid in various chromatographic solvents.

NITSCH[1] stated that the R_F of indole-3-acetic acid (IAA) using a solvent system of isobutanol, methanol and water (16:1:3, by volume) was 0.24, whilst we have consistently obtained values of 0.80–0.90 using BDH Analar materials. Certain other observations on the effects of solvents on bio-assays prompted us to investigate the possibility of there being present an impurity in one or more of the solvent components causing the differences in R_F quoted. Purification of the isobutanol was achieved by refluxing the alcohol over potassium hydroxide for 2 h, and then fractionating the liquid. The fraction distilling over between 106° and 108° was collected. Methanol was purified by the method of GORDON AND PALEG[2], whilst the water used was doubly glass distilled and passed through a deionizer.

The R_F values obtained using combinations of repurified and un-repurified components of the solvent mixture are shown in Table I. In all cases purified water was used. Chromatography was carried out using strips of Whatman No. 3 paper, spotted with 2 μl of a methanolic solution of IAA (10^3 p.p.m.). The strips were spotted

TABLE I

THE EFFECT OF DISTILLATION OF ALCOHOL COMPONENTS OF THE SOLVENT SYSTEM ON THE R_F VALUE FOR PURE IAA

Distilled, deionized water used.

Solvent composition	*R_F value of IAA*
Distilled isobutanol Distilled methanol	0.20–0.30
Distilled isobutanol Undistilled methanol	0.20–0.30
Undistilled isobutanol Distilled methanol	0.80–0.90
Both undistilled	0.80–0.90