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Separation of acidic amino acids by high-voltage paper electrophoresis and chromatography

A method has been described previously for the separation of acidic amino acids likely to be encountered in various fern genera, based on high-voltage electrophoresis at pH 3.4 for the first dimension followed by chromatography in *n*-butanol–acetic acid–water for the second dimension[1]. If the concentrations of acidic amino acids were low and those of interfering substances high, it was convenient to carry out a preliminary electrophoretic separation at pH 5.3 to isolate an acidic amino acid fraction before proceeding to the two-dimensional separation.

As published, the two-dimensional method of separating acidic amino acids was limited in its application, for other types of plants contain several acidic amino acids[2–6] unknown in ferns. So that the separation method could be applied more generally in phytochemical surveys, the positions of these additional acidic amino acids were determined and now are related to the data previously published.

γ-Glutamyl peptides, which behave as acidic amino acids in this separation system, also occur in some plant species[7]. Their relative positions have not been mapped, but their presence can be checked routinely by comparing the acidic amino acid pattern before and after weak acid hydrolysis (2 *N* HCl, 100°, 2 h).

TABLE I

ELECTROPHORETIC MIGRATION DISTANCES (cm)[a], CHROMATOGRAPHIC VALUES (R_{GLU}) AND NINHYDRIN COLOURS FOR VARIOUS AMINO ACIDS

Compound	*Electrophoresis*		*Chromatography* R_{GLU}	*Colour*	*Reference*
	pH 5.3	*pH 3.4*			
Aspartic acid	22.7	9.4	0.81	grey-blue	1
Glutamic acid	19.6	3.9	1.0	blue	1
erythro-γ-Methyl-γ-hydroxyglutamic acid	19.7	13.8	0.99	grey-blue	1
α-Aminopimelic acid	14.7	1.0	1.42	blue	1
Cysteic acid	25.5	32.4	0.39	blue	1
S-Carboxymethylcysteine	20.4	14.4	0.89	blue	2
S-Carboxyethylcysteine	17.0	4.1	1.18	blue	2
S-Carboxyisopropylcysteine	16.5	4.6	1.45	blue	2
Dichrostachinic acid	16.2	16.3	0.54	blue	3
m-Carboxyphenylglycine	16.2	7.0	1.49	yellow[b]	4
m-Carboxy-*p*-hydroxyphenylglycine	15.4	17.9	1.17	yellow[c]	4
m-Carboxyphenylalanine	14.8	5.3	1.56	blue	4
m-Carboxy-*p*-hydroxyphenylalanine	14.1	15.1	1.36	blue	4
cis-α-(Carboxycyclopropyl)glycine	18.6	3.9	1.10	blue	5
trans-α-(Carboxycyclopropyl)glycine	18.3	5.2	1.10	blue	5
Nicotianine	0.0	0.0	0.42	brown-blue	6
Asparagine	0.0	0.0	0.63	brown	1

[a] As electro-osmotic flow takes place during electrophoresis, the migration distances recorded have been measured from the position of the common amide asparagine.
[b] Rapid colour change, yellow through brown to blue (2 h).
[c] Slow colour change, yellow through brown to blue (24 h).

Experimental

The additional acidic amino acids, separated by the experimental techniques previously described, are listed in Table I together with several used for comparison. A complete separation of all compounds in Table I is possible by electrophoresis at pH 3.4 followed by chromatography. If the rapidly migrating amino acids of the cysteic acid family are absent, it is advantageous to increase the electrophoresis period to achieve a better resolution of the remaining components.

Several structural correlations are apparent. Increasing chain length within the group of S-substituted cysteines results in decreased electrophoretic mobility at pH 5.3, but chromatographic values (R_{GLU}) increase. A similar relationship was noted for *m*-carboxyphenylglycine and *m*-carboxyphenylalanine. The addition of a hydroxyl group to *m*-carboxyphenylglycine or *m*-carboxyphenylalanine gave compounds having lower R_{GLU} values after chromatography, but higher electrophoretic mobilities at pH 3.4 when compared with the respective parent substances. The amino acid nicotianine [*i.e.*, 2-amino-4-(3'-carboxypyridin-1'-yl)butyric acid] behaved as a neutral amino acid and therefore would not be recorded by this method in phytochemical surveys.

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