The chromatographic separation of diastereoisomeric dipeptides

Diastereoisomeric dipeptides and their derivatives have variable physical properties and therefore can be sometimes separated by crystallisation[1–4], countercurrent distribution[5], ion-exchange[6], or gas chromatography[7]. Some authors report a difference of the paper chromatographic R_F values for diastereoisomeric peptides[2,8].

The present work reports a satisfactory separation of some diastereoisomeric dipeptides by ascending paper chromatography on Whatman paper No. 1.

The simplest dipeptide containing two asymmetric carbon atoms can exist in four isomeric modifications: L-L, D-D, L-D, D-L. Several dipeptides of known configuration were obtained from L- and/or D-amino acids (see Table I). The values of R_F for the L-L peptides were identical with those observed for the D-D compounds (since L-L is the mirror image of D-D) and differed markedly from those of the L-D and D-L isomers.

The best separation of diastereoisomers was obtained with the following basic solvent systems:

TABLE I

THE R_F VALUES OF DIASTEREOISOMERS OF DIPEPTIDES IN RELATION TO THEIR CONFIGURATION

Solvents: S_1 = Ethyl acetate–pyridine–acetic acid–water (5:5:1:3).
S_2 = Pyridine–water (4:1).
Paper: Whatman No. 1 (ascending), length 30 cm.
Detection: Ninhydrin.

Dipeptides	*Configuration of component amino acids*									
	R_F *in* S_1					R_F *in* S_2				
	DL-DL	L-L	D-D	L-D	D-L	DL-DL	L-L	D-D	L-D	D-L
Ala-Ala	do not separate					0.44 0.37	0.44			
Ala-But	0.53		0.53			0.48		0.48		
	0.47				0.47	0.43				0.43
Ala-Leu	0.79	0.79				0.60	0.60			
	0.73					0.54				
Ala-Phe	0.69	0.69				0.72	0.71			
	0.62				0.62	0.55				0.56
Ala-Val	0.60	0.60				0.73	0.73			
	0.54				0.54	0.50				0.51
But-Ala	0.58					0.68				
	0.51				0.50	0.51				0.51
But-But	0.68					0.73				
	0.59				0.59	0.51				0.51
Glu-Asp	0.29	0.28								
	0.23									
Glu-Glu	0.41	0.40								
	0.34									
Phe-Ala	0.70	0.70				0.73				
	0.58			0.58		0.60			0.60	
Phe-Val	0.80	0.80				0.83	0.82			
	0.70					0.69				
Val-Leu	0.80	0.80				0.83	0.82			
	0.70					0.71				
Val-Phe	0.86	0.86				0.80	0.80			
	0.78					0.71				
Val-Val	0.83	0.83				0.80	0.80			
	0.72					0.71				

S_1: Pyridine–ethyl acetate–acetic acid–water (5:5:1:3)
S_2: Pyridine–water (4:1)

The differences of the R_F coefficients (ΔR_F) in these systems had the values 0.05–0.23.

The dipeptides synthesised from two racemic amino acids (a mixture of four diastereoisomers: L–L, L-D, D-D, D-L) were separated in these systems and gave two non-overlapping spots which corresponded to two racemates (L-L/D-D and L-D/D-L). The upper spot of the mixture of these racemates had the R_F value corresponding to the L-L and D-D isomers, the lower spot to the L-D and D-L isomers.

Relative amounts of diastereoisomers can be quantitatively determined according to BOISSONNAS[9] with an accuracy of 2%.

There is a regular dependence of the R_F value of the dipeptides upon the relative configuration of the amino acids in all experiments:

$$R_F \text{ (L-L/D-D)} > R_F \text{ (L-D/D-L)}$$

Similar regularities are observed in the paper chromatography of hydroxy-amino acids with two asymmetric centres; *erythro*-isomers of phenylserine[10] or threonine[11] travel more quickly than the *threo*-isomers.

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