OBSERVED AND CALCULATED R_F VALUES OF SEVERAL SYNTHETIC PEPTIDES IN PAPER CHROMATOGRAPHY*

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The widespread use of paper chromatography in studies on peptides, particularly in protein-structure investigations, and the availability of large numbers of peptides in this Laboratory made it desirable to institute a systematic inquiry into the behavior of peptides in paper chromatography. In addition, data collected in such a study would supplement the extensive information on the properties of amino acids and peptides already obtained in this Laboratory[1-7].

The chromatographic behavior of 88 synthetic peptides and 29 amino acids was investigated in 15 solvent systems. For 9 of these solvent systems, comparisons were made between observed and calculated R_F values, the latter obtained by use of a method developed by PARDEE[8]. From considerations of free energy relationships of the peptides and amino acids in respective solvent systems, PARDEE derived the following equation:

$$RT \ln (1/R_F - 1)_P = (n - 1) A + B + \Sigma RT \ln (1/R_F - 1)_{AA} \quad (1)$$

where R is the molar gas constant (cal. per degree), T, the absolute temperature, n, the number of amino acid residues in the peptide, and A and B are constants. The subscripts P and AA refer to peptide and amino acid, respectively. The constants A and B are evaluated from a plot of $RT \ln (1/R_F - 1)_P - \Sigma RT \ln (1/R_F - 1)_{AA}$ *versus* the number of peptide bonds, $(n - 1)$, where A is the slope and B the intercept. For the determination of these constants, R_F values obtained in the 9 solvent systems for 33 dipeptides and 11 tripeptides were utilized.

EXPERIMENTAL

Compounds

The 88 peptides and 29 amino acids employed had theoretical elemental analyses, and the amino acids had optical rotation values in agreement with those in the literature[1,2,5-7].

Paper

Whatman No. 4 chromatographic paper was used for all determinations.

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TABLE I

COMPOSITION OF SOLVENT SYSTEMS[a]

Solvent (composition see below)	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*
Distilled water	15	50	25	25		d	e	23	30	40	20		20	30	10
88% Formic acid	15														
Glacial acetic acid		10	10	5											
Methyl alcohol (abs.)													80		
Ethyl alcohol (abs.)								77		10					
n-Propyl alcohol									70						60
n-Butyl alcohol		40	65	12.5						50					
tert.-Butyl alcohol	70										40				
tert.-Amyl alcohol														35	
Methyl ethyl ketone											40				
Acetone												60			
Liquefied phenol[b]				25	c	d									
m-Cresol							e								
Pyridine													4	35	
0.5% Aqueous urea												40			
10% Trisodium citrate					c										
Conc. ammonium hydroxide															30
Reference	14	14	14	15	14	14	14	14	16	17	14	14	14	14	17
Phase[g]	M	U	M	U	U	U	U	M	M	M	M	M	M	M	M

A Formix
B 4:1:5 Butanol–acetic acid
C BuOH–acetic acid
D PBA
E Phenol (citrate)
F Phenol (H_2O) (NaCN and NH_3 atmos.)
G *m*-Cresol
H 77% Ethanol
I 70% Propanol
J BuOH–EtOH
K TBK[f]
L Acetone–urea
M Pyridine mix
N Pyridine–amyl alcohol
O Propanol–NH_3

[a] All reagent grade except *m*-cresol, which was practical grade.
[b] Mallinckrodt U.S.P. Gilt Label.
[c] Liquefied phenol saturated aqueous 10% trisodium citrate.
[d] Liquefied phenol saturated with water with addition of 4 to 6 crystals of sodium cyanide; a beaker of 0.3% NH_4OH present in the tank during each run.
[e] *m*-Cresol saturated with water; a beaker of 0.03% NH_4OH present in the tank during each run.
[f] A beaker of 5% aqueous diethylamine (Eastman No. 92) present in the tank during each run.
[g] M:miscible; U:upper layer used.

Solvent mixtures

Compositions of the 15 solvent mixtures employed are given in Table I.

The peptides and amino acids were dissolved in water at a 5 mmolar concentration, except where low solubility did not permit this concentration, in which case a saturated solution was used. The quantity of each solution applied to the paper was 0.01 ml, except for the sarcosyl peptide solutions where 0.05 ml was applied in 5 portions, with drying between each application. The solutions were spaced at 1-inch intervals along a line $1^1/_2$ inches from the bottom of 16 by 21 inch sheets of paper. Leucine and alanine were run on each sheet as standards.

Ascending chromatograms were run overnight in museum jars at room temperature[9]. The sheets were dried 20 to 30 min in warm air, dipped in a 0.25% solution of ninhydrin in acetone[10], and again dried in warm air to speed color development. After treatment with ninhydrin, most of the glycyl peptides first appeared as yellow spots which gradually turned to a blue-purple color within 10 to 20 min. The R_F values were determined at the middle of each spot with the aid of an elastic rule[11]. Each compound was run at least twice in each solvent mixture, and about 1/3 of them were run in triplicate.

RESULTS AND DISCUSSION

The R_F values of the peptides and amino acids are shown in Table II.

The R_F values of the glycyl and diglycyl peptides of the aliphatic amino acids increase with increasing amino acid chain length in all of the solvent mixtures, as do those of the free amino acids. The alanyl peptides of this series show a similar increase.

The addition of one or more glycyl residues to a particular aliphatic amino acid has a variable effect on the R_F values. In solvent systems (B), (C) and (D), there is little change in the R_F values, but in solvents (A), (H) and (J) there is a decrease in the R_F values. In *phenol* (*citrate*) (E) the R_F value decreases as one glycyl residue is added, but increases when two glycyl residues are added. In the *phenol* solvent system (F), the R_F values generally increase when one or two glycyl residues are added to aliphatic amino acids.

Examination of groups of aliphatic compounds with equal numbers of carbon atoms reveals a decrease in R_F values for all solvent mixtures as the number of peptide bonds increases, but the location of the bond within the molecule has no effect on the migration rate. For example, the six-carbon dipeptides, glycyl-L-amino-*n*-butyric acid and alanylalanine, move slower than norleucine and faster than glycylglycylglycine, but there is no difference in R_F values for the two different dipeptides.

There is no significant difference in the R_F values for DL, L, and D stereoisomers except for the cystinylcystine peptides. L-Cystinyl-D-cystine has the same R_F value as D-cystinyl-L-cystine, but L-cystinyl-L-cystine moves much faster than the other two isomers in all 15 systems. Earlier work[12] indicates that the L-L isomer employed was the monomeric cyclo-L-cystinyl (monopeptide) and the L-D and D-L isomers the dimeric cystinyl-cystine (dipeptide).

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	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
	Formix	Butanol-Acetic 4:1:5	BuOH:Acetic 6.7:1:2.5	PBA	Phenol (citrate)	Phenol (H_2O) (NaCN & NH_3 atmos.)	*m*-Cresol (NH_3 atmos.)	77% Ethanol	70% Propanol	BuOH: EtOH	TBK	Acetone-Urea	Pyridine Mix	Pyridine-Amyl	Propanol-NH_3
Glycylglycine	0.32	0.22	0.12	0.27	0.37	0.61	0.15	0.18	0.21	0.05	0.19	0.59	0.46	0.20	0.41
Cobalt complex of glycylglycine	0.04	0.04	0.02	0.06	0.23	0.32	0.02	0.21	0.21	0.00	0.06	0.68	0.52	0.19	0.26
Glycylsarcosine	0.42	0.17	0.11	0.41	0.68	0.80	0.44	0.33	0.30	0.05	0.18	0.73	0.49	0.21	0.43
DL-Alanylglycine	0.47	0.23	0.16	0.41	0.53	0.69	0.25	0.32	0.28	0.06	0.29	0.68	0.63	0.29	0.49
D-Alanylglycine	0.46	0.23	0.17	0.34	0.57	0.70	0.30	0.33	0.35	0.06	0.25	0.68	0.53	0.27	0.52
DL- Leucylglycine	0.75	0.54	0.53	0.66	0.68	0.86	0.56	0.61	0.63	0.23	0.60	0.83	0.75	0.59	0.74
L-γ-Glutamylglycine	0.35	0.15	0.12	0.23	0.18	0.32	0.04	0.16	0.18	0.02	0.05	0.63	0.52	0.16	0.25
Glycyl-DL-alanine	0.46	0.25	0.19	0.36	0.54	0.72	0.30	0.28	0.35	0.06	0.26	0.70	0.51	0.26	0.51
Glycyl-L-alanine	0.47	0.30	0.21	0.44	0.50	0.71	0.25	0.27	0.28	0.07	0.28	0.67	0.60	0.27	0.48
Glycyldehydroalanine	0.50	0.29	0.20	0.32	0.41	0.67	0.22	0.32	0.30	0.08	0.39	0.79	0.53	0.30	0.52
Cobalt complex of glycyldehydroalanine	0.00	0.00	0.00	0.00	0.24	0.42	0.00	0.36	0.27	0.04	0.04	0.78	0.63	0.31	0.39
Sarcosyl-DL-alanine	0.53	0.25	0.13	0.55	0.83	0.82	–	0.41	0.35	0.05	0.30	0.68	0.59	0.21	0.54
Sarcosyldehydroalanine	0.63	0.35	0.27	0.55	0.81	0.82	0.50	0.41	0.40	0.12	0.48	0.86	0.64	0.36	0.68
DL-Alanyl-L-alanine	0.61	0.39	0.31	0.55	0.68	0.78	0.31	0.42	0.34	0.10	0.36	0.66	0.71	0.34	0.57
L-Alanyl-D-alanine	0.57	0.38	0.27	0.50	0.66	0.79	0.42	0.41	0.46	0.10	0.32	0.74	0.63	0.33	0.59
D-Alanyl-L-alanine	0.56	0.38	0.26	0.48	0.64	0.76	0.43	0.38	0.46	0.10	0.31	0.72	0.59	0.33	0.57
D-Alanyl-D-alanine	0.57	0.36	0.28	0.50	0.65	0.76	0.41	0.40	0.43	0.09	0.31	0.74	0.63	0.32	0.57
L-Alanyldehydroalanine	0.64	0.39	0.28	0.45	0.61	0.78	0.35	0.47	0.41	0.13	0.50	0.87	0.66	0.49	0.66
α-L-Glutamyl-L-alanine	0.53	0.25	0.25	0.39	0.32	0.48	0.03	0.24	0.10	0.04	0.08	0.63	0.54	0.16	0.28
γ-L-Glutamyl-β-alanine	0.40	0.20	0.19	0.38	0.33	0.42	0.04	0.22	0.21	0.03	0.05	0.66	0.58	0.20	0.28
Glycyl-L-serine	0.27	0.11	0.08	0.15	0.29	0.52	0.10	0.18	0.26	0.03	0.17	0.66	0.45	0.21	0.41
Glycyl-D-serine	0.27	0.18	0.08	0.16	0.29	0.54	0.10	0.19	0.25	0.03	0.17	0.61	0.43	0.22	0.41
Sarcosyl-α-amino-β-methylaminopropionic acid·HCl	0.21	0.10	0.05	0.40	0.74	0.89	0.52	0.22	0.21	0.04	0.18	0.29	0.27	0.22	0.48
Glycyl-α-aminoisobutyric acid	0.54	0.40	0.31	0.56	0.61	0.80	0.41	0.37	0.42	0.09	0.35	0.69	0.61	0.30	0.56
Glycyl-DL-α-amino-*n*-butyric acid	0.61	0.32	0.26	0.43	0.64	0.74	0.40	0.35	0.42	0.08	0.28	0.75	0.58	0.31	0.57
Glycyl-L-α-amino-*n*-butyric acid	0.56	0.39	0.30	0.51	0.62	0.82	0.38	0.41	0.44	0.10	0.34	0.74	0.65	0.32	0.58
L-Alanyl-L-α-amino-*n*-butyric acid	0.71	0.50	0.45	0.61	0.78	0.86	0.53	0.55	0.48	0.12	0.37	0.80	0.72	0.44	0.65
D-Alanyl-L-α-amino-*n*-butyric acid	0.69	0.45	0.42	0.56	0.72	0.82	0.52	0.51	0.50	0.15	0.37	0.74	0.65	0.41	0.64
Glycyl-L-threonine	0.35	0.24	0.12	0.20	0.41	0.66	0.16	0.24	0.32	0.04	0.21	0.71	0.50	0.26	0.48
Glycyl-D-valine	0.63	0.43	0.41	0.54	0.64	0.82	0.45	0.46	0.48	0.13	0.43	0.74	0.68	0.43	0.58
Glycyldehdrovaline	0.53	0.40	0.33	0.54	0.65	0.85	0.49	0.29	0.35	0.08	0.32	0.83	0.49	0.29	0.58
N-Dimethylglycyl-α-amino-β-dimethylaminopropionic acid	0.26	0.10	0.71	0.75	0.89	0.20	0.09	0.41	0.29	0.09	0.47	0.79	0.67	0.38	0.70
Glycyl-L-isovaline	0.61	0.49	0.41	0.57	0.68	0.84	0.50	0.47	0.48	0.13	0.45	0.78	0.64	0.38	0.64
Glycyl-D-isovaline	0.63	0.50	0.44	0.58	0.64	0.86	0.48	0.46	0.49	0.13	0.44	0.77	0.63	0.41	0.63
Glycyl-α,α-diethylglycine	0.72	0.61	0.50	0.63	0.76	0.85	0.59	0.50	0.53	0.18	0.52	0.87	0.70	0.54	0.70
Glycyl-D-norvaline	0.64	0.49	0.45	0.54	0.65	0.84	0.51	0.47	0.51	0.13	0.45	0.74	0.65	0.44	0.64
Glycyldehydronorvaline	0.58	0.46	0.43	0.54	0.65	0.84	0.49	0.36	0.39	0.10	0.40	0.85	0.57	0.36	0.60
L-Alanyl-L-norvaline	0.79	0.60	0.58	0.69	0.84	0.88	0.64	0.62	0.58	0.21	0.49	0.80	0.74	0.53	0.71
D-Alanyl-L-norvaline	0.77	0.57	0.52	0.63	0.76	0.86	0.61	0.56	0.60	0.21	0.48	0.84	0.69	0.50	0.71
Glycyl-DL-leucine	0.72	0.61	0.56	0.66	0.73	0.85	0.58	0.57	0.63	0.19	0.53	0.82	0.70	0.55	0.72
Glycyl-L-leucine	0.73	0.61	0.57	0.66	0.70	0.86	0.57	0.52	0.59	0.23	0.54	0.77	0.70	0.55	0.71
Glycyldehydroleucine	0.65	0.58	0.55	0.63	0.70	0.87	0.55	0.43	0.52	0.23	0.54	0.87	0.61	0.48	0.65
α-Glutamyl-L-leucine	0.70	0.64	0.60	0.65	0.52	0.63	0.20	0.68	0.66	0.19	0.20	0.80	0.73	0.35	0.50
Glycyl-DL-isoleucine	0.74	0.60	0.53	0.69	0.69	0.84	0.52	–	0.51	0.23	0.52	0.71	0.69	0.49	0.70
Glycyl-L-isoleucine	0.74	0.63	0.55	0.64	0.71	0.86	0.57	0.52	0.58	0.20	0.53	0.80	0.71	0.54	0.70
Glycyl-D-isoleucine	0.73	0.62	0.54	0.63	0.71	0.87	0.55	0.53	0.58	0.20	0.52	0.81	0.69	0.54	0.69
Glycyl-L-alloisoleucine	0.71	0.59	0.54	0.63	0.72	0.83	0.56	0.53	0.57	0.20	0.54	0.79	0.70	0.52	0.71
Glycyldehydroisoleucine	0.64	0.51	0.49	0.62	0.69	0.88	0.60	0.34	0.44	0.14	0.42	0.84	0.51	0.37	0.59
Glycyl-L-norleucine	0.72	0.65	0.58	0.65	0.73	0.86	0.60	0.57	0.63	0.21	0.58	0.83	0.69	0.57	0.74
Glycyl-D-norleucine	0.72	0.61	0.54	0.64	0.73	0.84	0.58	0.57	0.63	0.20	0.57	0.63	0.70	0.57	0.74
Glycyl-DL-α-amino-*n*-caprylic acid	0.86	0.71	0.63	0.70	0.77	0.89	0.65	0.59	0.74	0.40	0.66	0.90	0.71	0.65	0.81
Glycyldehydrophenylalanine	0.59	0.52	0.43	0.58	0.67	0.85	0.54	0.36	0.52	0.24	0.56	0.89	0.55	0.55	0.70
Sarcosyl-DL-phenylalanine	0.70	0.50	0.42	0.74	0.80	0.81	0.77	0.52	0.57	0.22	0.53	0.80	0.67	0.60	0.64
Sarcosyldehydrophenylalanine	0.66	0.55	0.47	0.75	0.85	0.85	0.49	0.50	0.52	0.42	0.62	0.81	0.63	0.49	0.70
Sarcosyl-N-methylglycyldehydrophenylalanine	0.76	0.59	0.39	0.78	0.90	0.92	0.75	0.48	0.57	0.31	0.59	0.88	0.67	0.64	0.78
DL-Alanyl-DL-phenylalanine	0.76	0.62	0.55	0.72	0.76	0.87	0.62	0.52	0.59	0.30	0.56	0.83	0.72	0.60	0.75
α-L-Glutamyl-L-phenylalanine	0.73	0.62	0.56	0.62	0.51	0.64	0.18	0.35	0.31	0.09	0.20	0.84	0.68	0.37	0.43

Table II. Experimental R_f Values of Peptides and Amino Acids (continued)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
	Formix	Butanol-Acetic 4:1:5	BuOH:Acetic 6.7:1:2.5	PBA	Phenol (citrate)	Phenol (H_2O) (NaCN & NH_3 atmos.)	*m*-Cresol (NH_3 atmos.)	77% Ethanol	70% Propanol	BuOH: EtOH	TBK	Acetone-Urea	Pyridine Mix	Pyridine-Amyl	Propanol-NH_3
L-Cystinyl-L-cystine	0.38	0.32	0.21	0.35	0.46	0.68	0.20	0.34	0.38	0.18	0.42	0.75	0.53	0.43	0.56
L-Cystinyl-D-cystine	0.08	0.11	0.03	0.17	0.08	0.37	0.02	0.02	0.12	0.02	0.08	0.30	0.18	0.09	0.30
D-Cystinyl-L-cystine	0.08	0.10	0.03	0.15	0.16	0.34	0.02	0.00	0.11	0.01	0.15	0.41	0.22	0.09	0.31
Glycyl-D-methionine	0.64	0.41	0.31	0.49	0.71	0.81	0.45	0.38	0.47	0.12	0.41	0.80	0.58	0.46	0.64
Glycyl-DL-tryptophan	0.60	0.53	0.41	0.52	0.73	0.86	0.49	0.35	0.51	0.14	0.50	0.82	0.56	0.56	0.64
Glycyl-L-tryptophan	0.57	0.52	0.42	0.62	0.68	0.83	0.47	0.34	0.43	0.19	0.51	0.73	0.59	0.57	0.65
Glycyl-D-tryptophan	0.60	0.49	0.41	–	0.73	0.82	0.51	0.35	0.50	0.15	0.50	0.81	0.55	0.54	0.64
α-L-Aspartyl-D-histidine	0.12	0.07	0.04	0.24	0.38	0.45	0.05	0.04	0.08	0.01	0.06	0.41	0.27	0.12	0.23
Glycyl-L-lysine · H_2SO_4	0.12	0.05	0.07	0.13	0.35	0.83	0.51	0.07	0.08	0.02	0.13	0.39	0.14	0.09	0.41
Glycyl-L-aspartic acid	0.16	0.12	0.05	0.14	0.35	0.56	0.11	0.12	0.23	0.05	0.12	0.60	0.32	0.18	0.33
Glycyl-L-glutamic acid	0.34	0.31	0.11	0.16	0.17	0.29	0.03	0.14	0.20	0.03	0.07	0.62	0.43	0.14	0.24
α-L-Glutamyl-L-glutamic acid	0.41	0.25	0.21	0.24	0.07	0.10	0.00	0.15	0.17	0.02	0.03	0.63	0.50	0.08	0.18
Glycyl-D-tyrosine	0.50	0.41	0.32	0.41	0.56	0.74	0.27	0.37	0.44	0.09	0.36	0.81	0.59	0.53	0.52
Glycylglycylglycine	0.24	0.15	0.09	0.22	0.44	0.72	0.22	0.15	0.28	0.03	0.21	0.69	0.40	0.21	0.34
D-Alanylglycylglycine	0.39	0.20	0.13	0.33	0.62	0.79	0.34	0.25	0.35	0.03	0.27	0.76	0.54	0.30	0.45
DL-Leucylglycylglycine	0.65	0.50	0.44	0.67	0.82	0.85	0.60	0.59	0.59	0.16	0.54	0.87	0.74	0.57	0.73
D-Leucylglycylglycine	0.66	0.49	0.42	0.66	0.79	0.88	0.57	0.59	0.58	0.18	0.53	0.87	0.74	0.58	0.71
Glycyl-DL-alanylglycine	0.35	0.23	0.13	0.36	0.59	0.79	0.33	0.28	0.36	0.04	0.23	0.73	0.52	0.30	0.47
Glycyl-D-alanylglycine	0.35	0.24	0.13	0.36	0.58	0.77	0.33	0.26	0.36	0.04	0.22	0.74	0.52	0.29	0.48
Glycyl-DL-leucylglycine	0.65	0.58	0.47	0.63	0.75	0.88	0.61	0.57	0.60	0.17	0.55	0.85	0.72	0.56	0.73
Glycyl-DL-phenylalanylglycine	0.63	0.47	0.38	0.61	0.76	0.84	0.58	0.44	0.54	0.14	0.49	0.85	0.65	0.59	0.68
Glycylglycyl-DL-alanine	0.37	0.25	0.15	0.38	0.61	0.81	0.34	0.29	0.34	0.04	0.26	0.74	0.52	0.29	0.47
Glycylglycyl-L-alanine	0.38	0.22	0.15	0.38	0.61	0.80	0.37	0.28	0.31	0.05	0.27	0.76	0.52	0.28	0.46
Glycylsarcosyldehydroalanine	0.51	0.32	0.23	0.58	0.74	0.85	0.50	0.38	0.34	0.09	0.32	0.80	0.56	0.38	0.55
Glycylglycyl-DL-leucine	0.65	0.54	0.47	0.66	0.82	0.86	0.67	0.55	0.58	0.16	0.51	0.86	0.72	0.51	0.68
Glycylglycyl-DL-phenylalanine	0.65	0.49	0.41	0.63	0.82	0.88	0.58	0.42	0.51	0.11	0.50	0.85	0.65	0.54	0.64
Glycylglycyldehydrophenylalanine	0.51	0.46	0.35	0.58	0.73	0.91	0.36	0.35	0.42	0.09	0.36	0.86	0.54	0.44	0.60
Glycylglycyl-L-cystine	0.07	0.08	0.07	0.09	0.19	0.52	0.05	0.04	0.15	0.01	0.05	0.51	0.15	0.10	0.22
DL-Alanyl-DL-alanyl-L-cystine	0.16	0.08	0.08	0.16	0.33	0.65	0.09	0.09	0.13	0.01	0.09	0.57	0.29	0.16	0.34
Glycylglycylglycylglycine	0.18	0.12	0.06	0.22	0.58	0.75	0.24	0.17	0.24	0.02	0.16	0.64	0.37	0.21	0.32
Glycylglycylglycylglycylglycylglycine	0.12	0.08	0.05	0.25	0.66	0.71	0.22	0.16	0.24	0.02	0.12	–	0.35	0.21	0.30
Glycine	0.41	0.17	0.13	0.22	0.40	0.50	0.14	0.30	0.29	0.08	0.17	0.54	0.41	0.24	0.38
Sarcosine	0.47	0.18	0.09	0.52	0.77	0.77	0.52	0.42	0.35	0.15	0.25	0.69	0.56	0.36	0.56
Alanine	0.51	0.28	0.20	0.39	0.56	0.60	0.28	0.40	0.36	0.13	0.24	0.66	0.59	0.36	0.50
α-Amino-n-butyric acid	0.69	0.34	0.30	0.46	0.71	0.75	0.47	0.51	0.46	0.18	0.29	0.88	0.65	0.41	0.54
Norvaline	0.80	0.49	0.44	0.62	0.79	0.81	0.60	0.81	0.60	0.29	0.39	0.74	0.71	0.51	0.64
δ-Aminovaleric acid	0.65	0.46	0.35	0.62	0.81	0.81	0.59	0.48	0.38	0.11	0.20	0.62	0.63	0.28	0.47
Norleucine	0.87	0.63	0.56	0.70	0.83	0.85	0.71	0.67	0.74	0.43	0.52	0.80	0.75	0.61	0.72
ε-Amino-n-heptylic acid	0.90	0.71	0.68	0.77	0.85	0.88	0.77	0.70	0.83	0.54	0.62	0.85	0.77	0.68	0.83
ε-Amino-n-caprylic acid	0.94	0.77	0.76	0.80	0.87	0.88	0.84	0.75	0.87	0.59	0.71	0.91	0.80	0.73	0.87
Valine	0.73	0.45	0.37	0.56	0.75	0.78	0.57	0.58	0.60	0.24	0.38	0.74	0.73	0.46	0.67
Leucine	0.78	0.61	0.58	0.70	0.78	0.82	0.67	0.61	0.61	0.41	0.51	0.78	0.75	0.61	0.74
Isoleucine	0.82	0.58	0.52	0.68	0.81	0.85	0.68	0.64	0.68	0.37	0.47	0.80	0.77	0.56	0.73
Serine	0.37	0.16	0.12	0.19	0.32	0.45	0.10	0.32	0.29	0.08	0.28	0.60	0.44	0.28	0.39
Threonine	0.44	0.17	0.15	0.26	0.48	0.64	0.21	0.42	0.38	0.13	0.48	0.71	0.58	0.38	0.47
Phenylalanine	0.71	0.53	0.49	0.68	0.84	0.85	0.72	0.57	0.61	0.34	0.54	0.77	0.69	0.63	0.73
Tyrosine	0.59	0.24	0.30	0.35	0.59	0.84	0.31	0.45	0.44	0.24	0.34	0.79	0.61	0.59	0.56
Diiodotyrosine	0.68	0.62	0.59	0.67	0.76	0.83	0.86	0.51	0.50	0.84	0.81	0.77	0.84	0.66	0.42
Tryptophan	0.59	0.43	0.35	0.50	0.70	0.75	0.53	0.39	0.46	0.17	0.51	0.65	0.47	0.55	0.64
Proline	0.58	0.27	0.23	0.65	0.88	0.84	0.75	0.49	0.42	0.13	0.25	0.69	0.62	0.37	0.53
Hydroxyproline	0.39	0.13	0.13	0.36	0.66	0.66	0.42	0.40	0.33	0.08	0.18	0.61	0.52	0.32	0.42
Histidine	0.16	0.10	0.08	0.37	0.61	0.72	0.42	0.17	0.23	0.13	0.22	0.51	0.38	0.27	0.43
Arginine	0.25	0.10	0.08	0.34	0.59	0.93	0.61	0.12	0.19	0.05	0.05	0.18	0.17	0.17	0.36
Ornithine	0.16	0.09	0.07	0.21	0.37	0.76	0.40	0.14	0.13	0.04	0.09	0.18	0.13	0.15	0.32
Lysine	0.22	0.08	0.08	0.25	0.54	0.87	0.46	0.12	0.16	0.03	0.10	0.19	0.20	0.14	0.36
Aspartic acid	0.31	0.16	0.09	0.10	0.08	0.19	0.00	0.20	0.13	0.02	0.05	0.53	0.44	0.13	0.19
Glutamic acid	0.42	0.17	0.17	0.24	0.14	0.26	0.03	0.22	0.20	0.03	0.05	0.64	0.54	0.17	0.25
Methionine	0.69	0.44	0.36	0.57	0.77	0.82	0.59	0.56	0.51	0.25	0.39	0.73	0.68	0.52	0.65
Cysteine · HCl	0.07	0.08	0.03	0.08	0.25	0.32	0.06	0.12	0.13	0.02	0.05	0.31	0.22	0.12	0.25
Cystine	0.07	0.05	0.03	0.06	0.15	0.39	0.05	0.11	0.11	0.00	0.05	0.30	0.30	0.11	0.19

Table III. Comparison of Observed and Calculated R_f Values of Peptides

Peptide	A*			B*			E*			F*			G*			H*			K*			M*			O*		
	Obs.	Theory	Δ	Obs.	Theory	Δ	Obs.	Theory	Δ	Obs.	Theory	Δ	Obs.	Theory	Δ	Obs.	Theory	Δ	Obs.	Theory	Δ	Obs.	Theory	Δ	Obs.	Theory	Δ
Glycylglycine	0.32	0.37	0.05	0.22	0.20	-0.02	0.37	0.37	0.00	0.61	0.56	-0.05	0.15	0.11	-0.04	0.18	0.22	0.04	0.19	0.20	0.01	0.46	0.34	-0.12	0.41	0.38	-0.03
Glycylsarcosine	0.42	0.43	0.01	0.17	0.21	0.04	0.68	0.75	0.07	0.80	0.81	0.01	0.44	0.44	0.00	0.33	0.33	0.00	0.18	0.29	0.11	0.52	0.49	-0.03	0.43	0.56	0.13
Alanylglycine	0.47	0.47	0.00	0.23	0.32	0.09	0.55	0.53	-0.02	0.70	0.66	-0.04	0.28	0.22	-0.06	0.35	0.31	-0.02	0.27	0.28	0.01	0.58	0.48	-0.10	0.51	0.50	0.01
Leucylglycine	0.75	0.75	0.00	0.54	0.65	0.11	0.68	0.76	0.08	0.86	0.85	-0.01	0.56	0.59	0.03	0.61	0.51	-0.10	0.60	0.56	-0.04	0.75	0.69	-0.06	0.74	0.74	0.00
Glycylalanine	0.47	0.47	0.00	0.28	0.32	0.04	0.52	0.53	0.01	0.72	0.66	-0.06	0.28	0.22	-0.06	0.28	0.31	0.03	0.27	0.28	0.01	0.56	0.48	-0.08	0.50	0.50	0.00
Sarcosylalanine	0.53	0.53	0.00	0.25	0.33	0.08	0.83	0.85	0.02	0.82	0.87	0.05	–	–	–	0.41	0.43	0.02	0.30	0.39	0.09	0.59	0.67	0.08	0.54	0.68	0.14
Alanylalanine	0.58	0.57	-0.01	0.38	0.47	0.09	0.66	0.68	0.02	0.77	0.74	-0.03	0.39	0.40	0.01	0.40	0.41	0.01	0.33	0.37	0.04	0.64	0.69	0.05	0.58	0.62	0.04
α-Glutamylalanine	0.53	0.48	-0.05	0.25	0.32	0.07	0.32	0.22	0.10	0.48	0.40	-0.08	0.03	0.05	0.02	0.24	0.23	-0.01	0.08	0.09	0.01	0.54	0.65	0.09	0.28	0.35	0.07
Glycylserine	0.27	0.34	0.07	0.15	0.19	0.04	0.29	0.29	0.00	0.53	0.51	-0.02	0.10	0.07	-0.03	0.19	0.24	0.05	0.17	0.32	0.15	0.44	0.37	-0.07	0.41	0.39	-0.02
Glycyl-α-amino-n-butyric acid	0.59	0.66	0.07	0.36	0.38	0.02	0.63	0.69	0.06	0.78	0.79	0.01	0.39	0.39	0.00	0.53	0.41	-0.11	0.31	0.33	0.02	0.62	0.58	-0.04	0.58	0.54	-0.04
Alanyl-α-amino-n-butyric acid	0.70	0.74	0.04	0.48	0.54	0.06	0.75	0.81	0.06	0.84	0.85	0.01	0.53	0.60	0.07	0.53	0.52	-0.01	0.37	0.44	0.07	0.69	0.74	0.05	0.65	0.66	0.01
Glycylthreonine	0.35	0.40	0.05	0.24	0.20	-0.04	0.41	0.45	0.04	0.66	0.60	-0.06	0.16	0.16	0.00	0.24	0.33	0.09	0.21	0.53	0.32	0.50	0.51	0.01	0.48	0.47	-0.01
Glycylvaline	0.63	0.70	0.07	0.43	0.49	0.06	0.64	0.73	0.09	0.82	0.82	0.00	0.45	0.49	0.04	0.46	0.46	0.02	0.43	0.43	0.00	0.68	0.67	-0.01	0.58	0.67	0.09
Glycylnorvaline	0.64	0.77	0.13	0.49	0.53	0.04	0.65	0.77	0.12	0.84	0.84	0.00	0.51	0.52	0.01	0.47	0.51	0.04	0.45	0.44	-0.01	0.65	0.65	0.00	0.64	0.64	0.00
Alanylnorvaline	0.78	0.84	0.06	0.59	0.69	0.10	0.80	0.80	0.00	0.87	0.89	0.02	0.63	0.72	0.09	0.59	0.62	0.03	0.49	0.55	0.06	0.72	0.79	0.07	0.71	0.74	0.03
Glycylleucine	0.73	0.75	0.02	0.61	0.65	0.04	0.72	0.76	0.04	0.86	0.85	-0.01	0.58	0.59	0.01	0.55	0.51	-0.04	0.54	0.56	0.02	0.70	0.69	-0.01	0.72	0.74	0.02
α-Glutamylleucine	0.70	0.76	0.06	0.64	0.65	0.01	0.52	0.43	-0.09	0.63	0.67	0.04	0.20	0.22	0.02	0.68	0.41	-0.27	0.20	0.25	0.05	0.73	0.79	0.06	0.50	0.61	0.11
Glycylisoleucine	0.74	0.80	0.06	0.62	0.63	0.01	0.70	0.79	0.09	0.86	0.86	0.00	0.55	0.61	0.06	0.50	0.55	0.05	0.52	0.52	0.00	0.70	0.72	0.02	0.70	0.73	0.03
Glycylnorleucine	0.72	0.85	0.13	0.63	0.67	0.04	0.73	0.81	0.08	0.85	0.88	0.03	0.59	0.64	0.05	0.57	0.58	0.01	0.58	0.57	-0.01	0.70	0.69	-0.01	0.74	0.72	-0.02
Glycyl-α-amino-n-caprylic acid	0.86	0.93	0.07	0.71	0.80	0.09	0.77	0.86	0.09	0.89	0.90	0.01	0.65	0.79	0.14	0.59	0.67	0.08	0.66	0.75	0.09	0.71	0.75	0.04	0.81	0.87	0.06
Sarcosylphenylalanine	0.66	0.78	0.12	0.50	0.59	0.09	0.80	0.96	0.16	0.81	0.96	0.15	0.77	0.93	0.16	0.52	0.60	0.08	0.53	0.70	0.17	0.67	0.75	0.08	0.64	0.85	0.21
Alanylphenylalanine	0.76	0.80	0.04	0.62	0.72	0.10	0.76	0.90	0.14	0.87	0.92	0.05	0.62	0.82	0.20	0.52	0.58	0.06	0.56	0.69	0.13	0.72	0.78	0.06	0.75	0.81	0.06
α-Glutamylphenylalanine	0.73	0.74	0.01	0.62	0.57	-0.05	0.51	0.53	0.02	0.64	0.72	0.08	0.18	0.26	0.06	0.35	0.37	0.02	0.20	0.27	0.07	0.68	0.74	0.06	0.43	0.59	0.16
L-Cystinyl-L-cystine	0.38	0.01	-0.37	0.32	0.02	-0.30	0.46	0.06	-0.40	0.68	0.34	-0.32	0.20	0.01	-0.19	0.34	0.02	-0.32	0.42	0.14	-0.28	0.53	0.17	-0.36	0.56	0.08	-0.48
L-Cystinyl-D-cystine	0.08	0.01	-0.07	0.11	0.02	-0.09	0.12	0.06	-0.06	0.36	0.34	-0.02	0.02	0.01	-0.01	0.01	0.02	0.01	0.12	0.14	0.02	0.20	0.17	-0.03	0.31	0.08	-0.23
Glycylmethionine	0.64	0.66	0.02	0.41	0.48	0.07	0.71	0.75	0.04	0.81	0.85	0.04	0.45	0.51	0.06	0.38	0.46	0.08	0.41	0.44	0.03	0.59	0.62	0.03	0.64	0.65	0.01
Glycyltryptophan	0.59	0.55	-0.04	0.51	0.47	-0.04	0.71	0.67	-0.04	0.84	0.79	-0.05	0.49	0.45	-0.04	0.35	0.30	-0.05	0.50	0.56	0.06	0.57	0.40	-0.17	0.64	0.64	0.00
α-Aspartylhistidine	0.12	0.10	-0.02	0.07	0.11	0.04	0.38	0.15	-0.13	0.45	0.44	-0.01	0.05	–	.–	0.04	0.08	0.04	0.06	0.08	0.02	0.27	0.34	0.07	0.23	0.23	0.00
Glycyllysine · H_2SO_4	0.12	0.20	0.08	0.05	0.09	0.04	0.35	0.51	0.16	0.83	0.90	0.07	0.51	0.38	-0.13	0.07	0.08	0.01	0.13	0.12	-0.01	0.14	0.16	0.02	0.41	0.36	-0.05
Glycylaspartic acid	0.16	0.28	0.12	0.12	0.19	0.07	0.35	0.07	-0.28	0.56	0.23	-0.33	0.11	–	–	0.12	0.14	0.02	0.12	0.06	-0.06	0.32	0.37	0.05	0.33	0.19	-0.14
Glycylglutamic acid	0.34	0.38	0.04	0.31	0.20	-0.11	0.17	0.13	-0.04	0.29	0.31	0.02	0.03	0.02	-0.01	0.14	0.16	0.02	0.07	0.06	-0.01	0.43	0.47	0.04	0.24	0.25	0.01
α-Glutamylglutamic acid	0.41	0.39	-0.02	0.25	0.20	-0.05	0.07	0.03	-0.04	0.10	0.14	0.04	0.00	–	–	0.15	0.11	-0.04	0.03	0.14	0.11	0.50	0.60	0.10	0.18	0.15	-0.03
Glycyltyrosine	0.50	0.55	0.05	0.41	0.27	-0.14	0.56	0.56	0.00	0.74	0.69	-0.05	0.27	0.25	-0.02	0.37	0.36	-0.01	0.36	0.39	0.03	0.59	0.54	-0.05	0.52	0.56	0.04
Glycylglycylglycine	0.24	0.30	0.06	0.15	0.14	-0.01	0.44	0.40	-0.04	0.72	0.63	-0.09	0.22	0.12	-0.10	0.18	0.21	0.03	0.21	0.17	-0.04	0.40	0.25	-0.15	0.34	0.34	0.00
Alanylglycylglycine	0.39	0.39	0.00	0.20	0.24	0.04	0.62	0.56	-0.06	0.79	0.72	-0.07	0.34	0.25	-0.09	0.28	0.29	0.01	0.27	0.24	-0.03	0.54	0.50	-0.04	0.45	0.46	0.01
Leucylglycylglycine	0.66	0.69	0.03	0.50	0.55	0.05	0.81	0.78	-0.03	0.87	0.89	0.02	0.59	0.64	0.05	0.59	0.49	-0.10	0.54	0.51	-0.03	0.74	0.68	-0.06	0.72	0.71	-0.01
Glycylalanylglycine	0.35	0.39	0.04	0.24	0.24	0.00	0.58	0.56	-0.02	0.78	0.72	-0.06	0.33	0.25	-0.08	0.28	0.29	0.01	0.23	0.24	0.01	0.52	0.50	-0.02	0.48	0.46	-0.02
Glycylleucylglycine	0.65	0.69	0.04	0.58	0.55	-0.03	0.75	0.78	0.03	0.88	0.89	0.01	0.61	0.64	0.03	0.57	0.49	-0.08	0.55	0.51	-0.04	0.72	0.68	-0.04	0.73	0.71	-0.02
Glycylphenylalanylglycine	0.63	0.66	0.03	0.47	0.47	0.00	0.76	0.84	0.08	0.84	0.91	0.07	0.59	0.69	0.10	0.44	0.45	0.01	0.49	0.54	0.05	0.65	0.61	-0.04	0.66	0.70	0.04
Glycylglycylalanine	0.38	0.39	0.01	0.24	0.24	0.00	0.61	0.56	-0.05	0.81	0.72	-0.09	0.36	0.25	-0.11	0.29	0.29	0.00	0.27	0.24	-0.03	0.52	0.50	-0.02	0.47	0.46	-0.01
Glycylglycylleucine	0.65	0.69	0.04	0.54	0.55	0.01	0.82	0.78	-0.04	0.86	0.89	0.03	0.67	0.64	-0.03	0.55	0.49	-0.06	0.51	0.51	0.00	0.72	0.68	-0.04	0.68	0.71	0.03
Glycylglycylphenylalanine	0.65	0.66	0.01	0.49	0.47	-0.02	0.82	0.84	0.02	0.88	0.91	0.03	0.58	0.69	0.11	0.42	0.45	0.03	0.50	0.54	0.04	0.65	0.61	-0.04	0.64	0.70	0.06
Glycylglycylcystine	0.07	0.04	-0.03	0.08	0.04	-0.04	0.19	0.18	-0.01	0.52	0.53	0.01	0.05	0.04	-0.01	0.04	0.07	0.03	0.05	0.05	0.00	0.15	0.23	0.08	0.22	0.16	-0.06
Alanylalanylcystine	0.16	0.09	-0.07	0.08	0.13	0.05	0.33	0.45	0.12	0.65	0.71	0.06	0.09	0.21	0.12	0.09	0.16	0.07	0.09	0.11	0.02	0.29	0.56	0.27	0.34	0.35	0.01
Glycylglycylglycylglycine	0.18	0.23	0.05	0.12	0.10	-0.02	0.58	0.44	-0.14	0.75	0.70	-0.05	0.24	0.15	-0.09	0.17	0.20	0.03	0.16	0.15	-0.01	0.37	0.31	0.06	0.32	0.31	-0.01
Glycylglycylglycylglycylglycine	0.12	0.18	0.06	0.08	0.07	-0.01	0.66	0.47	-0.19	0.71	0.75	0.04	0.22	0.17	-0.05	0.16	0.19	0.03	0.12	0.13	0.01	0.35	0.23	-0.12	0.30	0.27	-0.03

*See Table I. for solvent mixtures designated in table by capital letters

No difference in R_F values was noted for groups of peptides in which the sequence of amino acids was altered. For example, alanylglycine migrates at the same rate as glycylalanine, and leucylglycine the same as glycylleucine. The tripeptides, alanylglycylglycine, glycylalanylglycine, and glycylglycylalanine, all have identical R_F values.

Comparison of the R_F values for a peptide with those for the constituent amino acids shows no regular pattern. In some systems a dipeptide has R_F values intermediate between those of the amino acids, but in other solvents the peptide migrates faster or slower than either amino acid. If a peptide is composed of a small amino acid such as glycine and a much larger one, such as leucine, phenylalanine, methionine, or glutamic acid, the R_F values of the peptide correspond more closely to those of the larger amino acid in most of the solvents.

TABLE IV

VALUES OF CONSTANTS A AND B IN EQUATION (1)

Solvent mixture	A *Cal/mole*	B *Cal/mole*
A. Formix	—14.0	—112.6
B. Butanol–acetic acid (4:1:5)	—695.9	—349.7
E. Phenol (citrate)	—322.8	157.4
F. Phenol–(H_2O) (NaCN and NH_3 atmos.)	—172.5	27.8
G. *m*-Cresol	—1187.2	303.6
H. 77% Ethanol	—456.2	187.2
K. TBK	—828.4	—232.9
M. Pyridine mix	—172.3	125.0
O. Propanol–NH_3	—190.5	—103.4

In Table III the observed R_F values in 9 solvents are listed with the theoretical values (Eqn. 1) and the differences between the two for each of the 46 peptides included in the theoretical calculations. For the great majority of these compounds, the difference between the two R_F values is less than $\pm$ 0.05. The only peptide which consistently varies by a wide margin from the calculated value is L-cystinyl-L-cystine. Calculated values for L-cystinyl-L-cystine are useless in this study since the compound employed was a cyclic monopeptide with only a single cystine residue[12] and PARDEE's formula[8] is inapplicable. Other peptides that occasionally vary by more than 0.10 from the calculated values usually contain sarcosine, glutamic acid, aspartic acid, lysine, or phenylalanine.

The values employed for the constants A and B in Eqn. (1) are listed in Table IV. Even though the tetra- and pentapeptides were not included in the evaluation of the constants, their theoretical R_F values calculated with constants A and B are in close agreement with the observed values except in one solvent system, *phenol (citrate)* (E), where the differences exceeded 0.10. More data on the behavior of peptides of greater chain length are needed to test the usefulness of Eqn. (1) for predicting R_F values of longer peptides.

The standard errors of the mean of 10 to 15 determinations of the R_F values for leucine and for alanine were calculated for each of the 15 solvent systems. The *acetone–*

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urea (L) solvent mixture had the highest standard error of 0.016, and *77% ethanol* (H), *Formix* (A), *TBK* (K), and *BuOH–acetic* (C) had standard errors from 0.011 to 0.014. The other systems had standard errors of 0.006 to 0.010.

The solvent mixtures which appear to be better ones on the basis of compactness and color intensity of the spots, rapidity of solvent migration, and separation of the various amino acids are: *Formix* (A), *phenol (citrate)* (E), *77% ethanol* (H), *70% propanol* (I), *TBK* (K), *pyridine mix* (M), *pyridine–amyl alcohol* (N), and *propanol–NH_3* (O).

The R_F values on which PARDEE based his calculations[8] were taken from KNIGHT[13]. The solvents used by KNIGHT are not the same as used here, although his phenol–water system is similar to the *phenol (citrate)* (E) used here. Eleven of the peptides reported by KNIGHT are included in this study, and when results with KNIGHT's phenol–water system are compared with those obtained with solvent system (E), the R_F values for nine of these are within 0.05 of those reported in this paper. General review of the literature revealed few instances where the conditions employed were the same as those in this study, but in the few cases in which they were, agreement in results was noted.

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SUMMARY

To supplement extensive information on peptides and amino acids, data were obtained on the paper-chromatographic behavior of 88 synthetic peptides and 29 amino acids in 15 solvent systems. Theoretical R_F values were calculated for 46 peptides in 9 solvent systems and were found to be in good agreement with the observed values.

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