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Amino acids and peptides

XV. Separation of N-benzyloxycarbonyl amino acids and esters*

The recent success of polyamide thin-layer chromatography in separating various derivatives of amino acids, *e.g.*, dansyl[2], phenylthiohydantoin[3] and dinitrophenyl[4], suggested an additional application to N-benzyloxycarbonyl amino acids and esters. The purity of these compounds is important in peptide synthesis and identification had been achieved previously on such supports as Kieselgel G[5–8] and potassium silicate–glass fiber[9]. We wish to describe here a convenient chromatographic method using a solvent resistant polyester film polyamide layer[10], which is now commercially available**. The new technique is faster and more highly sensitive than existing procedures.

Methods and materials

The N-benzyloxycarbonyl amino acids were purchased from various vendors; the corresponding esters were prepared by standard methods. All solvents were

TABLE I

R_F VALUES OF EIGHTEEN N-BENZYLOXYCARBONYL AMINO ACIDS AND THREE N-BENZYLOXYCARBONYL AMINO ACID ESTERS

Polyamide layer was prepared on poly(ethyleneterephthalate) film using Amilan CM 1011 of Toyo Rayon Co., Tokyo, Japan. Solvents: I = 90% formic acid–water (1:1); II = chlorobenzene–glacial acetic acid (4:1); III = chlorobenzene–90% formic acid–dimethylformamide–water (90:12:5:10); IV = benzene–chloroform–90% formic acid–dimethylformamide–*n*-hexane (5:22:1:2:2); V = benzene–glacial acetic acid (4:1). Detection: visible under a U.V. lamp (Mitamura Riken Kogyo Inc., Tokyo, Japan) after drying (80°). Distance: 10 cm. An abbreviated designation of amino acid derivatives is applied to these compounds[12].

	Solvents	*I*	*II*	*III*	*IV*	*V*
	Time (min)	*120*	*90*	*56*	*88*	*49*
Z-ala		0.51	0.65	0.26	0.86	0.59
Z-arg		0.00	0.02	0.00	0.00	0.05
Z-ω-nitro-arg		0.53	0.10	0.00	0.16	0.08
Z-asn		0.67	0.15	0.00	tails	0.23
Z-asp		0.55	0.15	0.02	0.32	0.23
Z-gln		0.68	0.21	0.00	0.26	0.30
Z-gly		0.57	0.38	0.14	0.65	0.45
Z-ilu		0.24	0.73	0.25	front	0.70
Z-leu		0.28	0.69	0.43	0.87	0.71
Z-lys		0.86	0.17	0.00	0.06	0.22
Z-met		0.37	0.60	0.35	0.85	0.35
Z-phe		0.21	0.65	0.42	0.85	0.67
Z-pro		0.44	0.77	0.29	front	0.72
Z-ser		0.63	0.17	0.03	0.36	0.26
Z-thr		0.60	0.26	0.05	0.46	0.36
Z-trp		0.12	0.35	0.11	0.55	0.37
Z-tyr		0.07	0.73	0.48	0.96	0.74
Z-val		0.37	0.67	0.42	0.87	0.70
Z-ala-gly-OEt		0.63	0.80	0.52	front	0.79
Z-gly-gly-OEt		0.70	0.75	0.41	front	0.75
Z-val-gly-OEt		tails	0.86	0.58	front	0.85

* For the previous paper in this series, see ref. 1.

** Chen Chin Trading Co., Ltd., Taipei, Taiwan, Republic of China and Gallard-Schlesinger Chemical Mfg. Corp., Long Island, N. Y., U.S.A.

purified to meet chromatographic standards. The polyamide film was made by following the earlier literature directions[10]. Visualization was achieved by irradiation of the chromatograms with ultraviolet light (2538 Å), after spraying with Rhodamine B solution[11].

Results and discussion

Table I summarizes the R_F values of eighteen N-benzyloxycarbonyl amino acids and three similar N-benzyloxycarbonyl amino acid ester derivatives in five different solvent systems. The spread of R_F values is sufficient for most purposes. It is planned to extend these results to other amino acid and peptide derivatives in the near future.

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