

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.*, dansyl², phenylthiohydantoin³ and dinitrophenyl⁴, 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 G⁵⁻⁸ and potassium silicate-glass fiber⁹. We wish to describe here a convenient chromatographic method using a solvent resistant polyester film polyamide layer¹⁰, 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 compounds¹².

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 directions¹⁰. Visualization was achieved by irradiation of the chromatograms with ultraviolet light (2538 Å), after spraying with Rhodamine B solution¹¹.

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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Department of Chemistry, National Taiwan University,
Taipei, Taiwan (Republic of China)

KUNG-TSUNG WANG
KUANG-YU CHEN

Department of Chemistry, Stanford University,
Stanford, Calif. 94305 (U.S.A.)

BORIS WEINSTEIN

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Separation of simple indole derivatives by thin layer chromatography*

In recent years a number of adsorbent systems for thin layer chromatography (TLC) have been described for the successful separation of several compounds. The work reported here is an effort to compare the chromatographic behaviour of 23 simple indole derivatives, their R_F values and color reactions on Eastman Chromagram and Gelman Instant thin layer chromatography (ITLC) sheets with the standard chromatoplates.

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