APPENDIX

The Separation of Iodobenzene-*p*-sulphonylamino Acids (Pipsylamino Acids) by Thin-Layer Chromatography

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The use of pipsyl chloride for the identification of the *N*-terminal residues of peptides involves the determination of a single derivative from many samples rather than the determination of several derivatives from the same sample. We have therefore investigated the separation of pipsylamino acids by the rapid and convenient technique of thin-layer chromatography.

EXPERIMENTAL

Plates (20 cm. × 20 cm.) were spread with a layer (250 μ thick) of a suspension (30 g./60 ml. of water) of Kieselgel G (E. Merck A.-G., Darmstadt, Germany). The layer was allowed to set for 15 min. and then the plates were dried in a draught oven at 110° for 30 min. Immediately before use, the adsorbent was activated by heating for 30 min. at 150°. After application of the load in ethanol, ethanol-water (1:1, v/v) or pyridine-acetone-water (1:1:1, by vol.) the plates were developed (in Shandon Chromatanks, lined with filter paper) in chloroform-tetrahydrofuran-acetic acid (18:1:1, by vol.) for 1 hr. The plates were dried at 60° for 30 min., and then developed in the second dimension in ethyl acetate-propan-2-ol-aq. ammonia (sp.gr. 0.88)-water (9:7:1:1, by vol.) for 2 hr., followed by heating at 150° for 30 min. The pipsylamino acids were detected by exposing the plates to chlorine gas (Reindel & Hoppe, 1954), followed by spraying with a saturated solution of *o*-tolidine in acetic acid-water (1:49, v/v), and also by the use of specific reagent sprays. A diagram of the two-dimensional separation attained is shown in Fig. 1.

REFERENCE

Reindel, F. & Hoppe, W. (1954). *Chem. Ber.* **87**, 1103.

Fig. 1. Separation of pipsylamino acids by thin-layer chromatography. Development was first in direction A with chloroform-tetrahydrofuran-acetic acid (18:1:1, by vol.) followed by development in direction B with ethyl acetate-propan-2-ol-aq. ammonia (sp.gr. 0.88)-water (9:7:1:1, by vol.). Detection was by exposing plates to chlorine gas, followed by spraying with a saturated solution of *o*-tolidine in acetic acid-water (1:49, v/v), and also by specific reagent sprays. Spots: 1, pipsylisoleucine; 2, pipsylvaline; 3, pipsyl-leucine; 4, iodobenzene-*p*-sulphonamide; 5, pipsylproline; 6, pipsylphenylalanine; 7, pipsyl-methionine; 8, *NO*-dipipsyltyrosine; 9, pipsylalanine; 10, *N*-pipsyltyrosine; 11, *N* α *N* ϵ -dipipsyl-lysine; 12, pipsyl-glycine; 13, pipsyltryptophan; 14, pipsylglutamic acid; 15, pipsylaspartic acid; 16, pipsylserine; 17, pipsyl-threonine; 18, pipsylmethionine sulphone; 19, pipsylcysteic acid; 20, *O*-pipsyltyrosine; 21, *N* α -pipsyl-lysine; 22, *N* ϵ -pipsyl-lysine; 23, pipsylarginine; 24, *N* α -pipsylhistidine; 25, iodobenzene-*p*-sulphonic acid; 26, *NN'*-dipipsylcystine. The identifications of spots 10 and 21 are only tentative.