Two-dimensional thin-layer chromatography of amino acids on microcrystalline cellulose

Protein hydrolyzates may be resolved, and the constituent amino acids detected, identified and determined, by ion-exchange column chromatography with an amino acid autoanalyzer[1]. For qualitative work, two-dimensional chromatography on a support of paper[2,3], moisture-equilibrated silica gel[4,5], or cellulose[6], offers the advantage of low cost. The thin-layer techniques[4–6] have the added advantage of greater speed than separations on paper[2,3]. In our hands the silica gel technique[4,5] suffers from disadvantages due to difficulty in standardizing the plates, fragility of the layer, and poor reproducibility in the R_F values obtained. Cellulose supports[6,7] give improved separations, but layers showing good adhesion and physical strength may be difficult to obtain. A form of microcrystalline cellulose known as "Avicel"-Technical Grade (formerly termed "Avirin"), produced commercially by acid treatment of cellulose, gives tenacious layers on glass plates, with excellent physical characteristics for thin-layer chromatography[7]. This support has been used for qualitative separations of sugars and amino acids[8], and for quantitative separations of sugars[9].

We report herein a two-dimensional thin-layer chromatographic procedure on an "Avicel"-Technical Grade support for the separation, detection and identification of amino acids in mixtures containing up to twenty different amino acids.

Experimental

Chromatoplates. "Avicel", Technical Grade (formerly termed "Avirin"), obtained from the Avicel Sales Division of American Viscose Division, FMC Corp., Marcus Hook, Pennsylvania, is blended with water, and applied to clean glass plates (20 × 20 × 0.4 cm) by the method of WOLFROM, PATIN AND DE LEDERKREMER[8], except that a layer thickness of 0.5 mm is used. The plates are allowed to dry without heating, are kept for at least 24 h at room temperature before use, and are not kept in a desiccator. Different commercial lots may require different amounts of water to obtain the same consistency.

Developers. The solvent system for development in the first direction is 1-butanol–acetic acid–water (3:1:1, w/w). For development in the second direction, phenol–water (3:1, w/w) is used.

Separation procedure. Light pencil lines are ruled parallel to, and 1.5 cm from two adjacent edges of the plate. Two more lines are ruled parallel to, and 12 cm from, each of the first two lines. The intersection of the first two lines is the starting point for the two-dimensional chromatogram. A solution containing the mixture to be resolved is applied to the starting point with a fine capillary as a compact zone <0.5 cm in diameter. The solution contains 0.1–0.5 μg of each amino acid standard, or 10–50 μg of a protein hydrolyzate.

A glass jar (29.5 × 27 × 10 cm) containing the first solvent to a depth of 0.5 cm is allowed to equilibrate for at least 6 h. The plate is introduced, with the starting point in the lower left corner, and the solvent is allowed to ascend to the line 12 cm above the starting line (about 1.5 h). The plate is then removed, and is allowed to dry in the air for at least 6 h at room temperature. It is then introduced, with the starting point in the lower right corner, into a second, pre-equilibrated jar containing the second solvent to a depth of 0.5 cm. The plate is removed when the solvent has

ascended to the line 12 cm above the starting line (about 2 h), and is allowed to dry in a hood for at least 12 h, without heating.

For control experiments by one-dimensional chromatography, a row of starting points 1.5 cm from one edge of the plate are marked out with a pencil. Amounts of 0.01–0.05 μg of each amino acid standard, or 1–5 μg of protein hydrolyzate, are used.

Hydrochloric acid hydrolyzates of protein or peptide preparations are freed from acid before chromatography. Hydrolyzates are evaporated in a desiccator over sodium hydroxide, the residues are redissolved in distilled water, and the solutions are re-evaporated over sodium hydroxide. The procedure is repeated at least once more.

Indication of the zones. The dried plates are sprayed lightly with a 0.2 % solution of ninhydrin in 95 % ethanol, and they are kept in the dark for 24 h at room temperature. The amino acids are revealed as purple-blue zones (proline gives a yellow zone), and background color is negligible.

A slight increase in sensitivity is attainable if, directly after spraying, the plates are heated for 5 min at 80°, but it is difficult to avoid the development of colored background, and artifacts resulting from finger contact with the plate.

Results and discussion

Fig. 1 shows the appearance of a chromatoplate on which a mixture of 20 amino acids had been applied, and resolved by the two-dimensional technique. Table I lists the R_F values for 20 amino acids in each of the solvent directions, and gives the limit of visual detection for each amino acid, in the first solvent system with indication at room temperature. Also listed are the detection limits recorded[4,5]

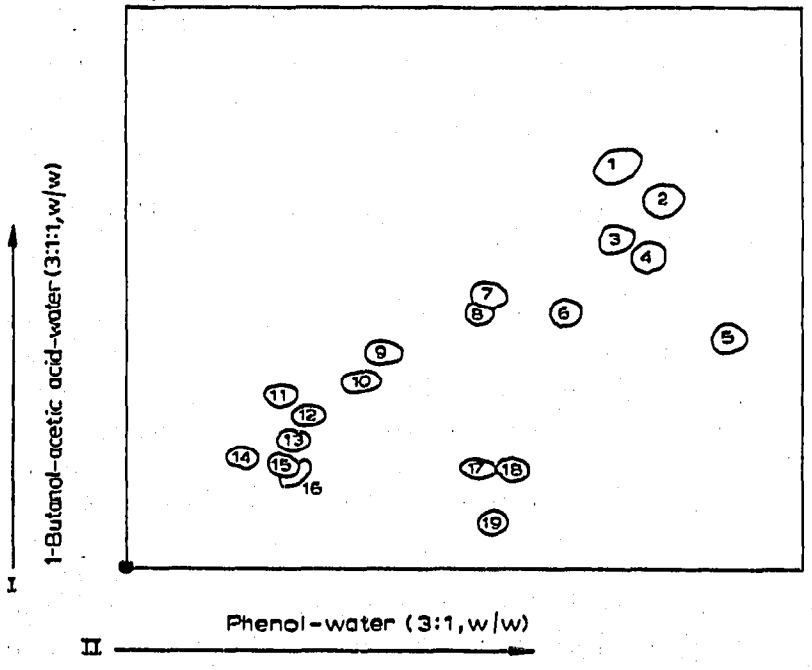

Fig. 1. Two-dimensional thin-layer chromatogram on "Avicel"-Technical Grade of a mixture of amino acids; 1 = isoleucine and leucine; 2 = phenylalanine; 3 = valine; 4 = methionine; 5 = proline; 6 = 4-aminobutyric acid; 7 = tryptophan; 8 = tyrosine; 9 = alanine; 10 = threonine; 11 = glutamic acid; 12 = glycine; 13 = serine; 14 = aspartic acid; 15 = taurine; 16 = lysine; 17 = arginine; 18 = histidine; 19 = cysteine.

TABLE I

R_F VALUES OF AMINO ACIDS AND LIMITS OF DETECTION

Amino acid	*BuOH–AcOH–H_2O (3:1:1, w/w)*		*PhOH–H_2O (3:1, w/w)*	*Limit of detection on silica gel G (μg)**
	R_F	*Limit of detection (μg)*	R_F	
Alanine	0.37	0.02	0.38	0.009
4-Aminobutyric acid	0.44	0.01	0.64	
Arginine	0.17	0.005	0.49	0.01
Aspartic acid	0.19	0.03	0.18	0.1
Cysteine	0.08	0.03	0.56	
Glutamic acid	0.28	0.01	0.23	0.04
Glycine	0.25	0.01	0.32	0.001
Histidine	0.17	0.01	0.60	
Isoleucine	0.70	0.02	0.71	
Leucine	0.71	0.02	0.71	0.01
Lysine	0.17	0.005	0.24	0.005
Methionine	0.55	0.01	0.76	0.01
Phenylalanine	0.64	0.02	0.77	0.05
Proline	0.41	0.05	0.89	0.1
Serine	0.22	0.003	0.24	0.008
Taurine	0.18	0.01	0.22	
Threonine	0.32	0.02	0.34	0.05
Tryptophan	0.47	0.03	0.52	0.05
Tyrosine	0.45	0.02	0.52	0.03
Valine	0.58	0.01	0.73	0.01

* Data from refs. 4 and 5.

for some of these amino acids by one-dimensional chromatography on moisture-equilibrated silica gel G (E. Merck, Darmstadt, Germany). It is noteworthy that the sensitivity of the present method is superior to the silica gel method, except for alanine, glycine, and leucine. The R_F values recorded in Table I for 3:1:1 1-butanol–acetic acid–water, in a weight rather than in a volume ratio, are somewhat larger than those few which were previously reported[8]. We do not find that leucine and isoleucine are separable in admixture.

The R_F values are influenced by the moisture content of the plate, the thickness of the layer, and the amount of sample applied. Plates having 0.5 mm coating, dried as indicated, and with sample amounts in the given ranges, gave R_F values reproducible within $\pm$ 0.02. The one-dimensional R_F values are not noticeably changed by development perpendicular to, rather than along, the direction in which the plates are spread.

Support layers 0.25 mm or 1.0 mm thick gave somewhat less satisfactory results than 0.5 mm layers. "Avicel", the pharmaceutical grade produced by the same manufacturer, gave inferior results.

The technique is in use in this laboratory for identification of amino acids present in biological materials and in hydrolyzates of protein and peptide materials[10]. Compared with thin-layer techniques on moisture-equilibrated silica gel, and other commercial grades of cellulose, it offers the advantage of greater reproducibility, and a tenacious coating which can be marked with a pencil and which does not disintegrate at the point where the sample is applied. On samples of protein hydrolyzates,

the technique gives excellent qualitative agreement with compositions determined with an amino acid autoanalyzer.

The simplicity and low cost of this technique commend its use for instructional, as well as research purposes.

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Purification of polycyclic hydrocarbons by chromatographic decomposition of their picrates, trinitrobenzolates and styphnates

The secondary reactions caused in adsorption chromatography on alumina consisting, for example, in the saponification of glycerides[1] and deacetylation of acetylated sugars[2,3] and other types of decomposition have been reported in the literature.

LEDERER AND LEDERER[4] have referred to the decomposition of the picrates of aromatic hydrocarbons as a practical method for their purification and indicated its application to styphnates and trinitrobenzolates[5,6].

However, LEDERER *et al.* in their publication[5] on ambergris have mentioned the decomposition of the picrate of a hydrocarbon obtained from ambreinolide by passing its solution through alumina and describe the styphnate but not its decomposition.