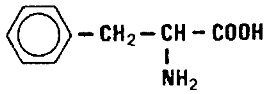
α-Hydroxycarboxylic acids. During investigation of the enantioselective degradation of the biogenic *R*-structured catecholamines norephinephrine (noradrenaline) and epinephrine (adrenaline), Jork and Kany (222) for the first time succeeded in the enantiomeric separation of the resulting 3,4-dihydroxymandelic acid and vanillylmandelic acid, respectively, using the lipophilic eluent mixture dichloromethane–methanol (45:5) and postchromatographic detection with 2,6-dichloroquinone-4-chloroimide (Merck, Cat. No. 3037).

Table 15 shows comparative results for the separation of some α-hydroxycarboxylic acids on Chiralplate and HPTLC-CHIR. The remission-location curves in Figs. 16–18 were recorded with the HPTLC-CHIR plates. Vanadium pentoxide was especially useful for postchromatographic derivatization (259) of the aromatic and aliphatic α-hydroxycarboxylic acids. For aromatic α-hydroxycarboxylic acids, manganese chloride–sulfuric acid (30 min, 120°C) was also suitable (229).

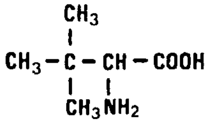
D. Quantitative Evaluation of TLC-Separated Enantiomers

1. General

Phenylalanine (**1**), *tert*-leucine (**2**), 5,5-dimethylthiazolidine-4-carboxylic acid (**3**), and α-hydroxyphenylalanine (**4**) have been chosen as models for the direct quantitative evaluation of thin-layer chromatograms. Emphasis has been placed on the evaluation of detection limits for the TLC-separated enantiomers, because exact determination of trace levels of a D- or L-enantiomer in an excess of the other is increasingly important (220,258,260–262).

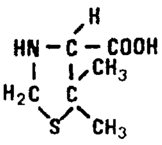


1

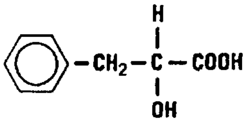


$CH_3-\overset{CH_3}{\underset{CH_3}{C}}-\underset{NH_2}{CH}-COOH$

2



3



4

To enhance specificity and sensitivity, postchromatographic derivatization with ninhydrin or vanadium pentoxide was used. Dipping the plates into the reagent solution proved most useful because it could be automated (263). Quantification of the minor enantiomer was achieved by in situ remission measurement with the CS-930 double-beam scanner (Shimadzu) or the densitometer CD 60 (Desaga) and comparison with external standard solutions. Additionally, possible proportional systemic deviations were excluded by the standard addition method (264).

For every substance investigated, the absorption maximum was determined independently prior to the quantification experiments.

2. Preparation of Test Solutions and Standard Solutions

Successful separation of amino acids on the TLC plate depends inherently on the concentration and often on the hydrochloric acid content of the applied solution. Addition of hydrochloric acid generally improves the solubility of the amino acids and often considerably enhances the enantiomeric resolution.

Phenylalanine test solution (U_{Ph}). Weigh 200 mg of D-phenylalanine into a 10 mL measuring flask and fill to the mark with 50% methanolic hydrochloric acid solution (10 g of acid per liter of solution).

Phenylalanine standard solution (V_{Ph}). Weight 100 mg of L-phenylalanine into a 100 mL measuring flask and fill to the mark with methanol–0.1 M hydrochloric acid (1:1). From this stock solution the standard solutions are prepared for the working range required. Dilute 200 μL, 400 μL, 600 μL, etc., of the stock solution to 10 mL with hydrochloric acid (10 g of acid per liter of solution)–methanol (1:1). Thus 0.1–0.3% solutions of the L-enantiomer relative to the 200 mg of D-phenylalanine are obtained.