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Note

Enantiomeric separation of N-carbamyltryptophan by thin-layer chromatography on a chiral stationary phase

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The development of a reversed-phase silica gel thin-layer chromatographic (TLC) plate containing a chiral stationary phase (2*S*,4*R*,2'*RS*)-4-hydroxy-1-(2-hydroxydodecyl)proline and copper(II) ions (Chiralplate™)[1], allowed the separation of amino acids, N-methylamino acids, N-formylamino acids and dipeptides[2–5] using a solvent consisting of methanol, acetonitrile and water. The separation of enantiomers on Chiralplates is now extended to another amino acid derivative, N-carbamyltryptophan using copper (II) acetate in a water–methanol solvent system at reduced temperature.

EXPERIMENTAL

A Chiralplate, 10 × 20 cm (Macherey-Nagel, Düren F.R.G.), was activated at 105°C for 20 min and allowed to cool; 1 cm from the bottom of the plate 2-μl samples were applied using 2-μl glass micropipettes. After the spots had dried, the plate was immersed *ca.* 3 mm in a solution of 1 m*M* copper (II) acetate (Sigma), 5% methanol (Aldrich)(pH 5.8) at 16°C and developed until the solvent front reached the 14-cm mark (about 4 h). The TLC tank had been equilibrated with the solvent at 16°C overnight. The plate was dried, sprayed with Ehrlich's reagent (1 g Ehrlich's reagent in 100 ml hydrochloric acid–methanol, 1:3) and heated at 105°C until the color developed (about 5 min). Spots of N-carbamyltryptophan were blue against a yellow background. The sensitivity of detection of N-carbamyltryptophan is 100 μg/ml or less.

RESULTS AND DISCUSSION

Attempts to separate enantiomers of N-carbamyltryptophan on Chiralplates with solvent systems designed to separate amino acids and their derivatives[2–5] proved unsuccessful. However, a system described for chiral HPLC[6] was moderately effective for this separation, and modifications improved its effectiveness significantly. A solution of 1 m*M* copper (II) acetate, 5% methanol (pH 5.8) used at 16°C achieved the separation of the optical isomers of N-carbamyl-D,L-tryptophan (Fig. 1). The temperature at which the development takes place is important for good resolution. At 23°C, the enantiomers were observed to overlap, but as the temperature was reduced,

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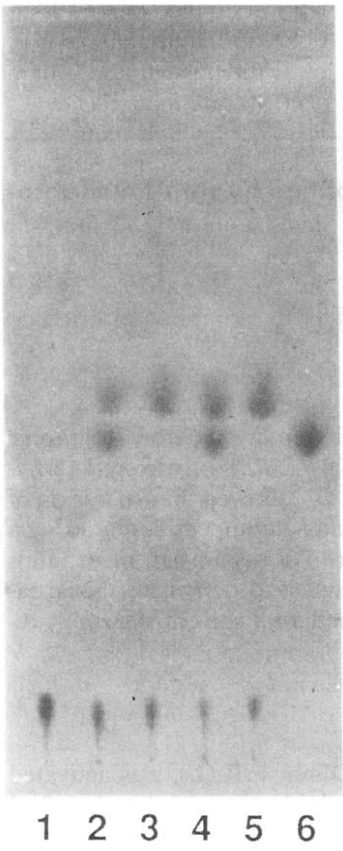

Fig. 1. Separation of N-carbamyl-D,L-tryptophan. Samples of N-carbamyl-D-tryptophan and N-carbamyl-L-tryptophan were spotted on a Chiralplate, developed, and visualized as described in Experimental. Lane 1: blank; lane 2: 1 mg/ml N-carbamyl-D,L-tryptophan; lane 3: 1 mg/ml N-carbamyl-D-tryptophan; lane 4: 1 mg/ml N-carbamyl-D,L-tryptophan; lane 5: 1 mg/ml N-carbamyl-D-tryptophan; lane 6: 1 mg/ml N-carbamyl-L-tryptophan.

the separation improved. At 16°C, the R_F of the D-isomer was 0.55 and that of the L-isomer was 0.44 with no overlap of the two enantiomers. Increased solvent front regularity was achieved by the addition of 5% methanol. When less or no methanol was added, excellent separation was achieved, but the jagged solvent front made lane-to-lane comparisons difficult.

So far, attempts to resolve other enantiomeric carbamylamino acids have been unsuccessful. However, further modifications of this system may make it generally useful for these compounds.

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