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Direct TLC Enantioseparations of Four Neutral Aliphatic DL-Amino Acids Using Teicoplanin as Chiral Selector

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TLC experiments were performed to resolve the enantiomers for four neutral aliphatic DL-α-amino acids, DL-alanine, DL-valine, DL-leucine and DL-isoleucine on silica gel plates using teicoplanin as chiral selector with same developing solvent *n*-butanol-methanol-water (5:0.5:1.2, v/v). The effects of constitute of developing solvents, the ratio of mobile phase, concentration of chiral selector and temperature on the enantioseparation had been studied. To our best of knowledge, this is the first report on thin layer chromatographic chiral resolution using teicoplanin as the chiral selector.

Keywords: Amino acid, Teicoplanin, Chiral selector, Enantioseparation, Thin layer chromatography.

INTRODUCTION

Chirality is a phenomenon which is of great importance to some biological and chemical processes. Many pharmaceutical and bioactive compounds are racemic mixtures with chiral isomers having nearly identical physical and chemical properties. The racemates have been resolved into their enantiomers either directly or indirectly using different chromatographic techniques. TLC can provide a direct method for resolution of enantiomers and has many advantages over other chromatographic techniques such as simple and inexpensive. Different chiral selectors have been used for TLC resolution of a variety of compounds into their enantiomers. Some reviews for TLC enantioseparations have been published[1-5].

Naturally occurring amino acids, which are the building blocks and language of all kinds of proteins, differ only in the nature of side chain residue R. Search of the literature reveals that impregnation of TLC plates with (-)-brucine[6], (1R,3R,5R)-2-azabicyclo [3,3,0] octan-3-carboxylic acid[7], L-proline-Cu(II)-complex[8] and (2S,4R,2'RS)-4-hydroxy-l-(2'-hydroxydodecyl)-proline-Cu-(II)-complex[9] have been used for resolution of DL-amino acids. Impregnation of TLC plates has also been used to resolve dansyl-DL-amino acids using a macrocyclic antibiotic erythromycin[10], vancomycin[11] and bovine serum albumin[12] *etc*. Further, enantiomeric RP-TLC separations of amino acids and their derivatives are achieved with cyclodextrins[13], bovine serum[14] and vancomycin[15] in chiral mobile phase.

Currently, the antibiotics of last resort are glycopeptides of the vancomycin family. The vancomycin-related antibiotics bind to the bacterial cell-wall d-alanyl-d-alanine terminal group, blocking the process of wall building. It turned out that chiral stationary phases (CSPs) based on these macrocyclic antibiotics were extremely useful in the chiral separation of native and unusual amino acids[16-20].

The objective of the present study is to present results on the resolution of four neutral aliphatic DL-α-amino acids into their enantiomers on thin layer silica gel plates impregnated teicoplanin as chiral selector with the same developing solvent. To the best of our knowledge, this is the first report on thin layer chromatographic chiral resolution using teicoplanin as the chiral selector (Fig. 1).

EXPERIMENTAL

All the four L-, D- and DL-amino acids *viz*., alanine, valine, leucine and isoleucine were obtained from Fluka. Silica gel G, with 14 % calcium sulphate as binder, having chloride, iron impurities up to 0.02 % and with a pH 7 in a 10 % aqueous suspension, was supplied by Qingdao Ocean Chemical Factory (China). Teicoplanin was obtained from Xiaogan Shenyuan Chemical Industry Ltd. (China). All other organic solvents and chemical reagents were of at least analytical-reagent grade (Beijing Chemical Factory, China).

Impregnated thin-layer plates (10 cm × 10 cm × 0.5 mm) were prepared by spreading a slurry of silica gel G (25 g) in methanol-H_2O (1:1, v/v, 50 mL) containing recrystallized teicoplanin (1.8 g). The plates were dried overnight at 60 °C.

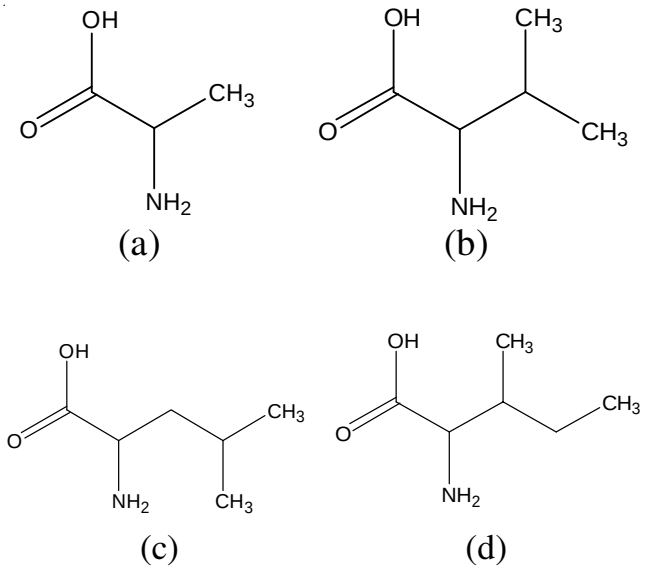

Fig. 1. Structures of four neutral aliphatic DL-α-amino acids. (a) Alanine; (b) Valine; (C) Leucine; (d) Isoleucine

Solutions of both DL-amino acids and their L- or D- forms (10^{-2} M) were prepared in 50 % ethanol and were applied side-by-side to the plates with a syringe.

Chromatograms were developed in solvent systems comprising of *n*-butanol-methanol-water 5:0.5:1.2 (v/v) in a paper-lined rectangular glass chamber at 13 °C. These were pre-equilibrated with developers for 15 min. The chromatograms were developed 8.5 cm. The plates were then sprayed with freshly prepared ninhydrin solution (0.2 % in ethanol) and heated between 100 and 110 °C for 10 min to reveal the characteristic spots of the amino acids.

RESULTS AND DISCUSSION

In order to investigate the effect of concentration of the impregnating reagent on resolution, 0.095, 0.19, 0.36, 0.72, 1.08, 1.44 to 1.80 g of teicoplanin were added to 25 g of silica gel to prepare thin layer, respectively. The experiments observe that the best resolution was at 1.80 g of chiral selector teicoplanin for the four racemic amino acids. However, more contents of teicoplanin weren't studied due to enough chiral resolution obtained for four animo acids.

For the resolution of DL-amino acids into their enantiomers on plates impregnated with teicoplanin, different solvent compositions of mixtures of water, butanol, chloroform, acetic acid, acetonitrile, methanol, ethyl acetate, carbon tetrachloride and propionic acid were systematically tried. The best solvent system was *n*-butanol-methanol-water 5:0.5:1.2 (v/v) for the four DL-amino acid, whereas, the other compositions of mixtures resulted in poor or even no resolutions. The photograph of the chromatograms showing resolution of DL-alanine, valine, leucine and isoleucine on teicoplanin impregnated plates is presented in Fig. 2.

In the solvent system *n*-butanol-methanol-water 5:0.5:1.2 (v/v), the effect of methanol and water content in the mobile phase were studied by using *n*-butanol-methanol-water 5:0.3:1.2 (v/v), *n*-butanol-methanol-water 5:0.8:1.2 (v/v), *n*-butanol-methanol-water 5:0.5:0.8 (v/v) and *n*-butanol-methanol-water 5:0.5:1.6 (v/v). The effect of butanol content

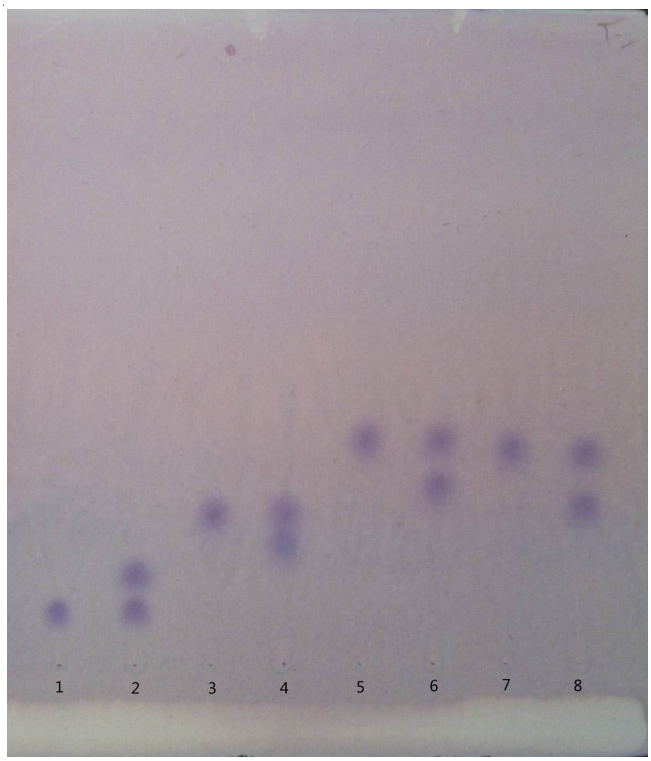

Fig. 2. Photograph of a chromatogram showing resolution of DL-amino acids. From left to right: spot 1, pure D-alanine; spot 2, lower spot for D-alanine and upper spot for L-alanine resolved from the racemic mixture; spot 3, pure L-valine; spot 4, lower spot for D-valine and upper spot for L-valine resolved from the racemic mixture; spot 5 pure L-leucine; spot 6, lower spot for D-leucine and upper spot for L-leucine resolved from the racemic mixture; spot 7, pure L-isoleucine; spot 8, lower spot for D-isoleucine and upper spot for L-isoleucine resolved from the racemic mixture. Solvent front, 8.5 cm; temperature, 13 °C; solvent system, *n*-butanol-methanol-water 5:0.5:1.2 (v/v)

was also studied by using *n*-butanol-methanol-water 4:0.5:1.2 (v/v) and *n*-butanol-methanol-water 6:0.5:1.2 (v/v) as mobile phase. The experimental results showed that the best composition of mobile phase was *n*-butanol-methanol-water 5:0.5:1.2 (v/v) for all the four amino acids. The different composition of solvent system also influenced the separation time. The data of additional experiments and the distance of the two spots center are presented in Figs. 3-5, respectively.

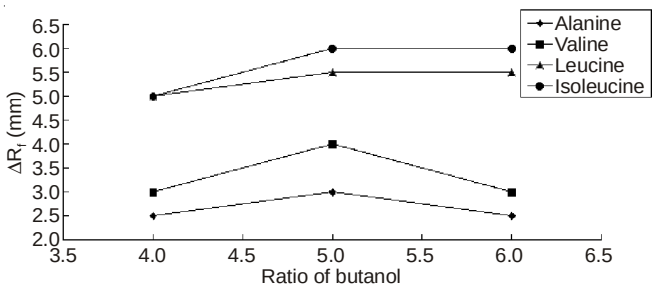

Fig. 3. Relationship between the ratio of butanol and ΔR_f of the four amino acids. Solvent front, 8.5 cm; temperature, 13 °C; solvent system, *n*-butanol-methanol-water 4:0.5:1.2 (v/v), *n*-butanol-methanol-water 5:0.5:1.2 (v/v) and *n*-butanol-methanol-water 6:0.5:1.2 (v/v)

Since chiral interaction between the chiral selector and the analyte are known to be affected by temperature, additional

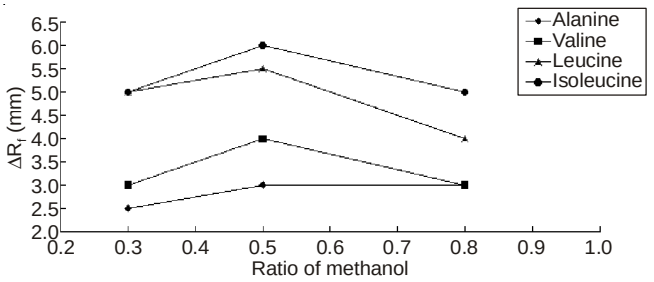

Fig. 4. Relationship between the ratio of methanol and ΔR_f of the four amino acids. Solvent front, 8.5 cm; temperature, 13 °C; solvent system, *n*-butanol-methanol-water 5:0.3:1.2 (v/v), *n*-butanol-methanol-water 5:0.5:1.2 (v/v) and *n*-butanol-methanol-water 5:0.8:1.2 (v/v)

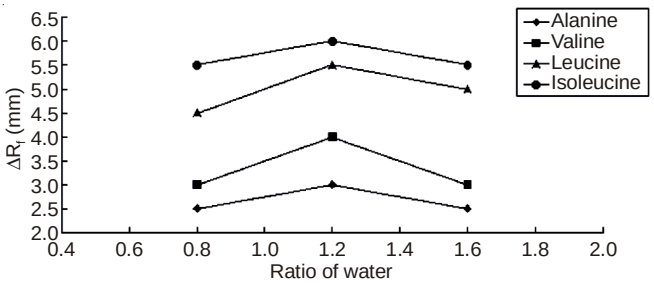

Fig. 5. Relationship between the ratio of water and ΔR_f of the four amino acids. Solvent front, 8.5 cm; temperature, 13 °C; solvent system, *n*-butanol-methanol-water 5:0.5:0.8 (v/v), *n*-butanol-methanol-water 5:0.5:1.2 (v/v) and *n*-butanol-methanol-water 5:0.5:1.6 (v/v)

experiments with successful mobile phases were performed at 4 °C and 35 °C. Each temperature was maintained inside an incubator and the chamber was pre-equilibrated for 20 min at each temperature. Studies on the effect of temperature on enantioseparation of racemic amino acid in TLC with teicoplanin as chiral selector revealed that the best resolution was obtained at 4 °C. The higher temperature got poor resolution. But the lower temperature increased the resolution time. Therefore, the 13 °C was a better resolution temperature due to enough chiral resolution and less time spend for four animo acids. The relationship between temperature (°C) and ΔR_f of four amino acids is presented in Fig. 6.

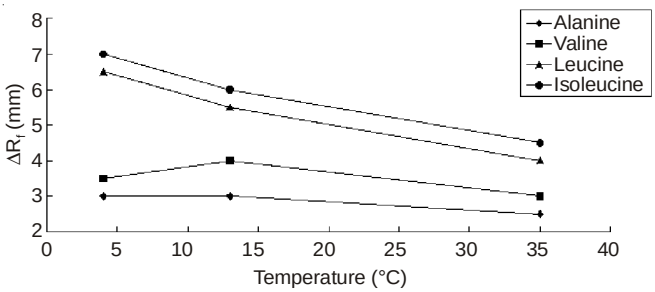

Fig. 6. Relationship between the temperature and ΔR_f of the four amino acids. Solvent front, 8.5 cm; temperature, 4, 13 and 35 °C; solvent system, *n*-butanol-methanol-water 5:0.5:1.2 (v/v)

Chiral recognition requires a minimum of three-points of interaction[20]. Earlier studies[16] showed that the teicoplanin ammonium group is the most available and logical site for initial docking and enantio selective retention. The secondary and tertiary structure of the teicoplanin molecule play an additional important role in chiral recognition by supplying appropriate hydrogen bonding, hydrophobic and steric interaction sites[16]. The shape of the chiral selector, the three-dimensional structure of the molecule and the different spatial arrangements of the functional groups are responsible for the different stereoselective capability of the macrocyclic teicoplanin.

Conclusion

During present studies on enantiomeric resolution of DL-amino acids using teicoplanin, it was found that teicoplanin is sufficiently stable, had good enantioselective property. This method can be considered as an improvement compared with earlier reports. The technique is versatile, flexible, simple, direct and economical and may become the method of choice compared with other chromatographic techniques for fast routine analysis.

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