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Thin-layer chromatographic separation of enantiomeric dansylamino acids using a macrocyclic antibiotic as a chiral selector

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Abstract

The enantiomeric resolution of ten dansyl-DL-amino acids was achieved on thin silica gel plates impregnated with a macrocyclic antibiotic, erythromycin, as the chiral selector. Different combinations of 0.5 *M* aqueous NaCl–MeCN–MeOH were found to be successful in resolving various dansyl-DL-amino acids. Spots were detected using a fixed dual-wavelength (254 nm) ultraviolet chamber.

Keywords: Chiral stationary phases, TLC; Enantiomer separation; Amino acids; Antibiotics; Erythromycin

1. Introduction

Thin-layer chromatography (TLC) continues to be used for the resolution of enantiomers of a variety of compounds because it is simple and inexpensive and has several other advantages. Methods for the resolution of enantiomers of amino acids, their derivatives, etc., have been discussed and reviewed in detail [1–3]. Impregnation of TLC plates with a suitable chiral selector for enantiomeric resolution of amino acids [4,5] and their phenylthiohydantoin derivatives [6] has been reported from this laboratory. Bovine serum albumin has been used as an impregnating agent for the resolution of dansyl-DL-amino acids [7], dinitropyridyl, dinitrophenyl and dinitrobenzoyl derivatives of amino acids [8], tryptophan [9], fluorenylmethoxycarbonylamino acids [10], miscellaneous compounds including substituted amino acids [11] and several enantiomeric compounds [12] by reversed-phase (RP) planar chromatography. Recently vancomycin, a macrocylic antibiotic, has been used as a chiral mobile phase additive for the resolution of dansyl-DL-amino acids by RP-TLC [13]. Rifamycin-B, thiostrepton and vancomycin have been used as bonded stationary phase chiral selectors to resolve a number of enantiomeric compounds including dansylamino acids by RP-HPLC [14].

These antibiotics seem to have many of the useful enantioselectivity properties of proteins and other polymeric selectors [14]. There is no report on the resolution of dansyl-DL-amino acids using erythromycin by normal-phase TLC. This paper reports the use of erythromycin as a chiral

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impregnating reagent while making thin silica gel plates to resolve dansyl-DL-amino acids by TLC.

2. Experimental

All dansylamino acids were purchased from Sigma (St. Louis, MO, USA). The chiral antibiotic erythromycin was obtained from Ranbaxy (India). Silica gel G (Merck India, Bombay, India), with calcium sulphate (13%), iron and chloride (0.03% each) and giving pH 7 in an aqueous suspension, was used. Reagents were of analytical-reagent grade from Merck, India. The standard solutions of dansyl-DL-amino acids and dansyl-L-amino acid (10^{-3} *M*) were prepared in 70% ethanol.

Erythromycin (1 g) was dissolved in a minimum volume of chloroform by warming to 40°C. After filtration, the solution was kept at −15°C. The crystals were removed by filtration and dried under vacuum; these were nearly colourless (0.9 g), m.p. 135–140°C, resolidifying and melting at 190–193°C.

Impregnated TLC plates (20 cm × 20 cm × 0.5 mm) were prepared by spreading a slurry of silica gel (50 g) in distilled water (100 ml, containing 0.05 g of erythromycin) with a Stahl-type applicator. The plates were dried overnight at 60°C. The samples of dansyl-DL- and -L-amino acids were applied side by side at the 500-ng level using a 25-μl Hamilton syringe. An aqueous solution of NaCl (0.5 *M*) was added to all mobile phases to stabilize the plate binder. Mixtures of acetonitrile, *n*-butanol, acetic acid, formic acid, *n*-propanol and methanol were tried as developers in varying proportions; only the finally successful combinations are reported. The chromatograms were dried, cooled to room temperature and the spots were revealed under UV radiation at 254 nm.

3. Results and discussion

Erythromycin is a complex (fourteen membered ring) antibiotic, characterized by a molecular structure containing a large lactone ring linked with amino sugars through glycosidic bonds [15]. It is a therapeutically useful wide-range antibiotic produced by a strain of *Streptomyces erythreus* and contains one methoxyl, two N-methyl and eight or more (18%) C-methyl groups [16]. Of the erythromycins, only erythromycin-A is certified by the US Food and Drug Administration; it is basic in nature ($pK_a = 8.6$) and has a specific optical rotation $[\alpha]_D^{25} = -78°$ ($c = 1.9$ in ethanol) [17].

The hR_F values for resolved enantiomers (D and L) of dansylamino acids and pure form (L) are given in Table 1. The results are the averages of at least five identical runs. The successfully resolved dansly-DL-amino acids were phenylalanine, valine, leucine, serine, glutamic acid, aspartic acid, norleucine, α-amino-*n*-butyric acid, methionine and tryptophan. Spots of these amino acids were identified at 254 nm as fluorescent green to greenish yellow spots. A photograph of the actual chromatograms showing resolution of dansyl-DL-Phe, -Val and -Leu as typical results is given in Fig. 1.

It was interesting that the addition of MeOH up to 0.5 ml for the resolution of Dns-DL-Glu in the solvent system 0.5 *M* aqueous NaCl–MeCN (22:1) did not have any effect on the enantioselectivity except that R_F for both the D and L resolved components decreased relatively slightly; further addition of methanol resulted in poor resolution and enantioselectivity. Similarly, several other combinations of the solvent system 0.5 *M* aqueous NaCl − MeCN were successful without MeOH for the resolution of Dns-DL-Glu, -Phe, -Val, -Leu and -*n*-butyric acid. Only dansyl-DL-norleucine requires acetic acid instead of MeOH as one of the components of the solvent system. On the other hand, a small volume of MeOH in the solvent system was required for the resolution of Dns-DL-Ser, -Try, -Met and -Asp. Variation of the concentration of acetonitrile in the mobile phase also affected the resolution of dansyl-DL-amino acids, i.e., tailing appeared with increasing concentration of MeCN.

The effect of the concentration of erythromycin as impregnating reagent with silica gel was also studied. It was found that the best resolution, as reported, was with 0.05% of the impre-

Table 1
hR_F values of enantiomers of dansylamino acids resolved on plates impregnated with erythromycin

Dansyl-DL-amino acid	hR_F			Solvent system 0.5 *M* aq. NaCl–MeCN–MeOH
	Pure L	From DL-mixture		
		D	L	
Serine	64	68	64	10:4:1
	30	36	30	15:1:1
Glutamic acid	45	56	45	22:1:0.5
	56	65	56	22:1:0
	52	59	52	26:1:0
Phenylalanine	50	65	50	15:2:0
	20	27	20	15:1:0
Valine	22	30	22	15:1:0
Leucine	24	32	24	15:1:0
Tryptophan	38	47	38	18:1:0.25
Methionine	56	63	56	25:2:0.5
	50	57	50	25:1:0.5
Aspartic acid	50	63	50	28:1.5:0.5
γ-Amino-*n*-butyric acid	42	51	42	12:1:0
Norleucine	63	71	63	16:1:0:0.4 HOAc

Temperature, 25 ± 2°C (34°C for methionine); time, 20–25 min; solvent front, 10 cm; detection, UV at 254 nm.

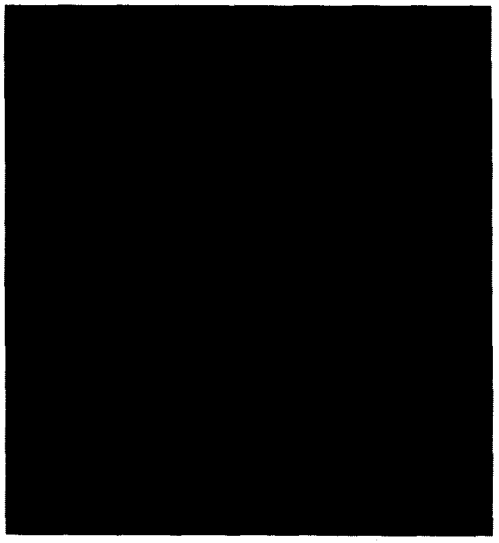

Fig. 1. Photograph of the actual chromatograms showing resolution of dansyl-DL-Phe, -Val and -Leu as typical results. Left to right: 1 = Dns-DL-Phe; 2 = Dns-L-Phe; 3 = Dns-DL-Val; 4 = Dns-L-Val; 5 = Dns-DL-Leu; 6 = Dns-L-Leu; 1, 3 and 5 show resolution of L and D forms, the former having lower R_F). Temperature, 25 + 2°C (34°C for methionine); time, 20–25 min; solvent front, 10 cm; detection, UV at 254 nm; solvent system, 0.5 *M* aq. NaCl–MeCN (15:1).

gnating reagent; as the concentration was increased to 0.1% or 0.5% the resolution became poor, and a further increase to 0.75% or 1.0% resulted in long tailing.

An increase in temperature from 20 to 27°C improved the resolution with an increase in the distance of the two spots of the enantiomers of the danslyamino acids resolving from the DL-mixture; dansyl-DL-methionine was resolved at 34°C whereas at 25°C there was either no resolution or it was very poor, showing a weak "eight"-shaped spot.

Enantioseparation may be possible via $\pi-\pi$ complexation, hydrogen bonding, inclusion in a hydrophobic pocket, dipole stacking, steric interactions or combinations thereof. Accordingly the resolutions are significantly affected by variations in the solution environment, which in turn tend to affect the chiral antibiotic which is ionizable, contains hydrophobic and hydrophilic moieties and is somewhat flexible. Armstrong et al. [14] considered association with a hydrophobic pocket rather than a true hydrophobic inclusion

complex as the possible reason for the enantioselectivity of vancomycin.

The above TLC systems can be considered to be rapid, reliable, simple and inexpensive for the resolution of enantiomers of dansylamino acids in comparison with the reported methods involving RP-TLC [13] or RP-HPLC [14]. Various macrocyclic antibiotics will continue to play a major role in future enantiomeric separations.

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