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Direct resolution of optically active isomers on chiral packings containing ergoline skeletons

II. Enantioseparation of carboxylic acids

Massimo Sinibaldi*

Istituto di Cromatografia del CNR, Area della Ricerca di Roma, C.P. 10, I-00016 Monterotondo Stazione, Rome, (Italy)

Miroslav Flieger

Institute of Microbiology, Academy of Sciences of the Czech Republic, Videnskà 1083, C-142 20 Prague 4, (Czech Republic)

Ladislav Cvak

Galena Opava, Opava, (Czech Republic)

Antonella Messina and Alessandro Pichini

Dipartimento di Chimica, Università di Roma "La Sapienza", Pl. Aldo Moro 5, I-00185, Rome (Italy)

ABSTRACT

Structurally different carboxylic acids and dansyl derivatives of amino acids were examined on a chiral stationary phase for high-performance liquid chromatography. The packing was prepared by bonding to silica gel an aminopropyl derivative of the ergot alkaloid (+)-terguride. Optimization of the enantioseparations was attained through the study of the influence of the organic modifier content and the pH and ionic strength of the buffer in the eluent. Electrostatic and hydrophobic interactions between the ergot alkaloid and the analyte contribute to a large extent to the retention and chiral discrimination. The selector was shown to be effective for the resolution of dicarboxylic acids, 2-arylcarboxylic acids and amino acid derivatives.

INTRODUCTION

Small-molecule naturally occurring products are an attractive source of resolving agents for the development of highly efficient and selective chiral stationary phases (CSPs) with a low cost of production [1]. Several papers have dealt with the use of cinchona alkaloids [2,3], D-penicillamine [4], penicillin derivatives [5], riboflavin [6] and berberine [7].

In this connection, a large group of natural compounds with high potential applicability is the ergot alkaloids, owing to the presence in the molecules of (a) two or three asymmetric carbons, (b) a π-acceptor represented by the indole ring, (c) basic nitrogens as electrostatic interaction sites and (d) steric hindering groups, all being distributed along the rigid ergoline skeleton. We recently reported the preparation of a (+)-terguride-based stationary phase for the separation of structurally analogous lisuride and terguride enantiomers [8]. In this paper, the

* Corresponding author.

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enantioselectivity of this packing for a series of organic acids, including arylpropionic, hydroxycarboxylic and phenylcarboxylic acids and dansylamino acid derivatives, is reported.

EXPERIMENTAL

Instrumentation

The liquid chromatographic system consisted of a Perkin-Elmer (Norwalk, CT, USA) Series 410 solvent-delivery pump equipped with a Rheodyne Model 7125 injection valve and connected to a Varian (Walnut Creek, CA, USA) Model 2550 variable-wavelength detector. Chromatograms were recorded by a Shimadzu (Kyoto, Japan) Chromatopac CR3A integrator.

Materials and methods

The chiral packing was prepared from silica gel Nucleosil 100-S (particle size 5 μm, average pore diameter 100 Å) (Macherey–Nagel, Düren, Germany), 3-glycidoxypropyltrimethoxysilane (Serva, Heidelberg, Germany) and (+)-1-(3-aminopropyl)-(5*R*,8*S*,10*R*)-terguride according to the procedure described previously [8].

The t_0 times of the column at different flow-rates were obtained by injection of chloroform and using methanol as the eluent.

The elution sequence of the isomers was determined by using D- and L- or *R*- and *S*-forms of appropriate standards. Dansylamino acid derivatives were purchased from Sigma (St. Louis, MO, USA) and α-hydroxycarboxylic acids, tropic, citronellic, 1,2-*trans*-cyclohexanedicarboxylic, 2-phenylpropionic, 2-phenylbutyric, 3-phenylbutyric and phenylsuccinic acid from Fluka (Buchs, Switzerland). (*R*,*S*)-*m*-Phenoxyhydratropic acid (fenoprofen), (*R*,*S*)-*m*-benzoylhydratropic acid (ketoprofen), (*R*,*S*)- and (*S*)-2-(6-methoxy-2-naphthyl)propionic acid (naproxen) ethyl ester, (±)-*trans*-chrysanthemic acid ethyl ester and (+)-(1*R*,3*R*)-*trans*-chrysanthemic acid ethyl ester were kindly provided by Professor E. Cernia, University of Rome, Rome, Italy.

Hydrolysis of naproxen and chrysanthemic acid esters was carried out by refluxing 1 g of compound with 10 ml of 20% sodium hydroxide solution for 1–2 h until the ester layer of suspension disappeared. The cooled alkaline mixture was acidified with dilute sulphuric acid and the carboxylic acid was extracted with chloroform.

All solvents and other reagents were of HPLC or analytical-reagent grade and obtained from Carlo Erba (Milan, Italy).

RESULTS AND DISCUSSION

(+)-(5*R*,8*S*,10*R*)-Terguride was derivatized to the 1-(3-aminopropyl) derivative (AMP-TER) in order to provide the ergot alkaloid with a suitable reactive group to bond it to the silica gel surface. The structure of the chiral selector is shown in Fig. 1.

Several classes of carboxylic acids and a series of dansylamino acid derivatives (Fig. 2) were examined on the chiral packing. The corresponding capacity factors (k') and enantioselectivity factors (α) are reported in Tables I and II. For each resolved species the elution sequence was the (−)- before the (+)-enantiomer.

The data obtained for the class of compounds **I** (hydroxycarboxylic acids) show that dipole, hydrophobic content and pK_a of the acid are determining factors for the k' and α values. Mandelic acid *p*- (**Ia**) and *m*-OH (**Ib**) isomers showed different values of retention and selec-

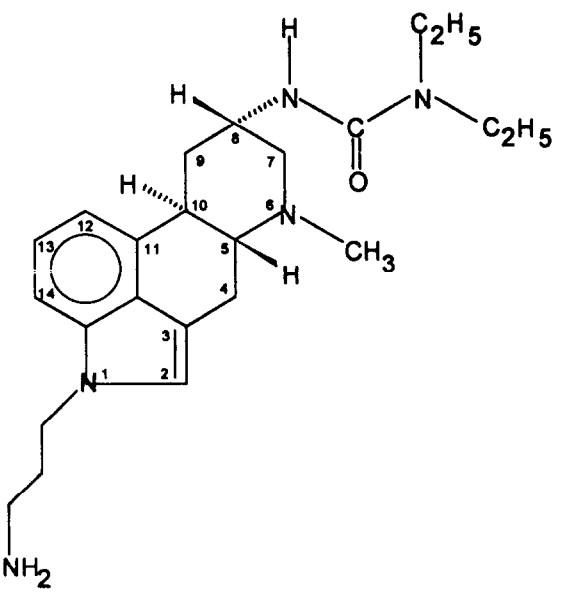

Fig. 1. Structure of the chiral selector.

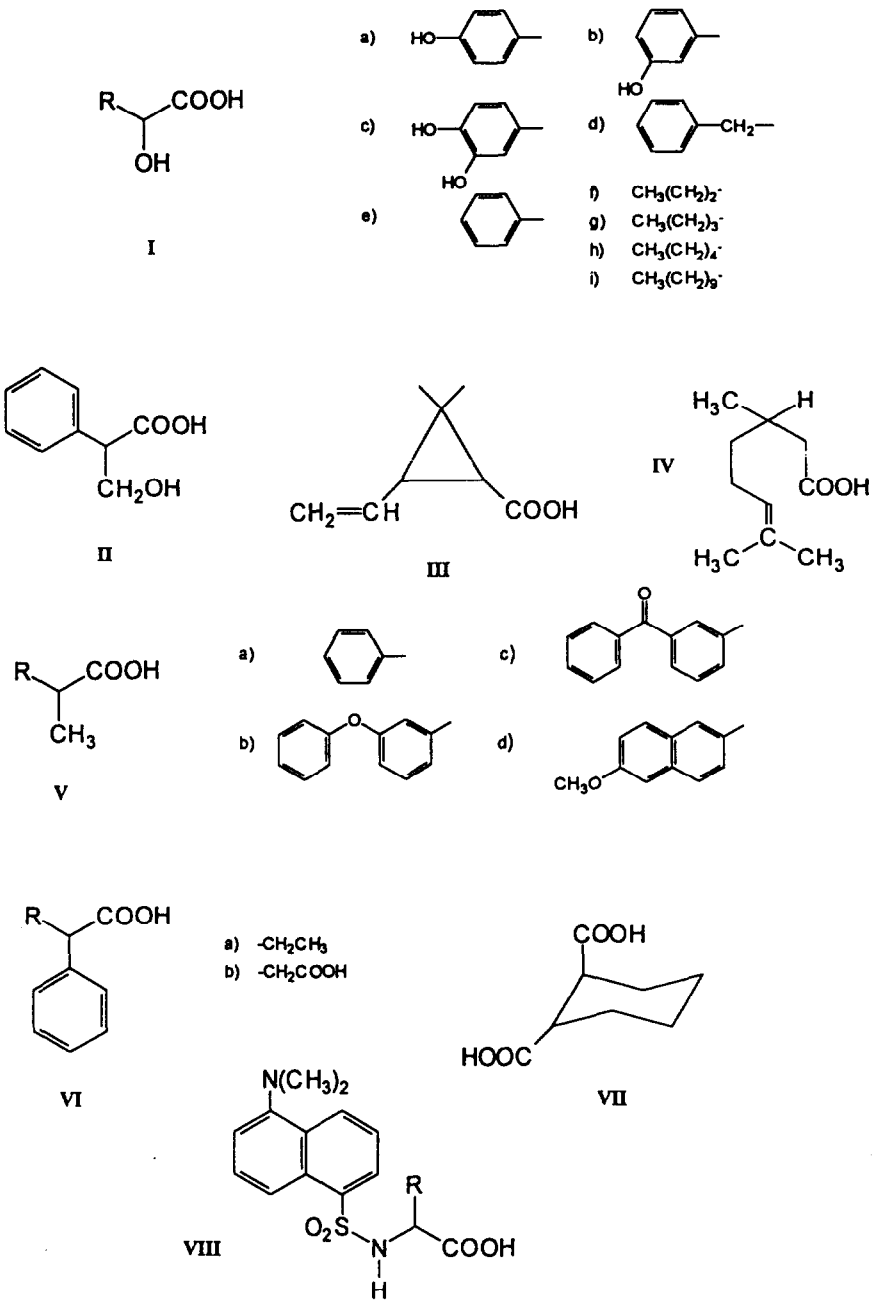

Fig. 2. Structures of the compounds examined.

tivity, owing to the different pK_a ionization constants [9] and position of the hydroxy group on the aromatic ring, while 3,4-dihydroxy-mandelic acid (**Ic**), to be resolved, required a lower concentration of organic modifier in the eluent.

The dependence of the α values on the polarity of the molecules is well reflected by the behaviour of **If**, **Ig**, **Ih** and **Ii**. The longer the alkyl chain, the better was the separation of the acids. No resolution was observed for phenyllactic acid

TABLE I

OPTICAL RESOLUTION DATA OBTAINED FOR A SERIES OF CARBOXYLIC ACIDS ON A (+)-(5*R*,8*S*,10*R*) TERGURIDE-BASED PACKING

Column, 15 × 0.46 cm I.D.; flow-rate, 0.7 ml/min; amount injected, 0.6 nmol; detection, UV at 254 or 230 nm.

No.	Compound	Mobile phase[a]	$k'_{(-)}$	$k'_{(+)}$	α[b]
Ia	*p*-Hydroxymandelic acid	A	3.71	3.93	1.06
Ib	*m*-Hydroxymandelic acid	A	2.79	2.91	1.04
	m-Hydroxymandelic acid	A′	1.85	1.90	1.02
Ic	3,4-Hydroxymandelic acid	A	1.65	1.65	1.00
		A″	2.27	2.32	1.02
Id	β-Phenylllactic acid	A	4.50	4.50	1.00
Ie	Mandelic acid	A	4.15	4.40	1.06
If	α-Hydroxybutyrric acid	A	6.28	6.28	1.00
Ig	α-Hydroxyvaleric acid	A	8.00	8.00	1.00
Ih	α-Hydroxycaproic acid	A′	3.40	3.45	1.02
Ii	α-Hydroxydecanoic acid	B	11.5	12.5	1.08
II	Tropic acid	A′	4.13	4.20	1.02
III	*trans*-chrysanthemic acid	A‴	4.83	5.33	1.10
IV	Citronellic acid	A′	8.53	8.53	1.00
Va	2-Phenylpropionic acid	A‴	8.90	8.90	1.00
Vb	*m*-Phenoxyhydratropic acid	A‴	10.4	11.3	1.09
Vc	*m*-Benzoylhydratropic acid	A‴	11.7	12.3	1.06
Vd	2-(6-Methoxy-2-naphthyl)propionic acid	A‴	4.30	4.65	1.08
VIa	2-Phenylbutyric acid	B	7.22	7.22	1.00
VIb	2-Phenylsuccinic acid	B	22.6	23.3	1.03
VIc	3-Phenylbutyric acid	B	9.12	9.12	1.00
VII	*trans*-1,2-Cyclohexanedicarboxylic acid	B′	1.37	1.46	1.06

[a] Mobile phases: (A) 0.05 *M* acetate buffer (pH 4.6)–methanol (80:20, v/v); (A′) 0.05 *M* acetate buffer (pH 5.5)–methanol (80:20 v/v); (A″) 0.05 *M* acetate buffer (pH 4.6)–methanol (90:10, v/v); (A‴) 0.05 *M* acetate buffer (pH 4.6)–acetonitrile (70:30 v/v); (B) 0.02 *M* phosphate buffer (pH 3.8)–acetonitrile (50:50, v/v); (B′) 0.02 *M* phosphate buffer (pH 4.5)–methanol (60:40 v/v).

[b] Selectivity factor $\alpha = k'_{(+)}/k'_{(-)}$.

(**Id**) and topic acid (**II**), which have the carboxylic group in a β-position with respect to the asymmetric centre.

Chrysanthemic acid (**III**) exists as *cis* and *trans* isomers, each in two enantiomeric forms. The strongest insecticidal activity is ascribed to the (+)-(1*R*,3*R*)-*trans*-enantiomer [10]. The resolution of the two *trans* stereoisomers, (+)-(1*R*,3*R*) and (−)-(1*S*,3*S*), is shown in Fig. 3.

Citronellic acid (**IV**) did not show any resolution under several eluent conditions.

Compounds **Vb**, **Vc** and **Vd** are 2-arylpropionic acid non-steroidal anti-inflammatory drugs (NSAIDs), marketed as fenoprofen, ketoprofen and naproxen, respectively. There is great interest in NSAIDs as most of these therapeutic agents undergo a unidirectional stereoselective metabolic pathway associated with the chiral inversion of the inactive *R*-enantiomer to the pharmacologically active *S*-form [11]. Several direct resolutions of derivatized or underivatized NSAIDs on HPLC CSPs have been published. These methods utilized a variety of CSPs based on (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine [12], tris(3,5-dimethylphenylcarbamate) derivatives of cellulose and amylose [13], α_1-acid glycoprotein [14], human serum albumin [15] and, more recently, a 3,5-dinitrobenzoyl derivative of

TABLE II

CAPACITY FACTORS (K') AND RESOLUTION FACTORS (α) OBTAINED FOR A SERIES OF DANSYLAMINO ACID DERIVATIVES ON A (+)-(5*R*,8*S*,10*R*)-TERGURIDE-BASED PACKING

Chromatographic conditions: column, 15 × 0.46 cm I.D.; mobile phase, 0.02 *M* phosphate buffer (pH 4.6)–methanol (60:40 v/v); flow-rate, 1.0 ml/min; amount injected, 1 μg; detection, UV at 254 nm; room temperature.

Compound	k'_L	k'_D	α
Dns-valine	11.4	12.8	1.12
Dns-norvaline	12.3	13.3	1.08
Dns-leucine	13.5	14.1	1.05
Dns-norleucine	15.3	16.2	1.06
Dns-methionine	15.2	16.4	1.08
Dns-phenylalanine	23.2	26.1	1.13
Dns-tryptophan	35.9	45.6	1.27
Dns-threonine	9.23	11.2	1.22
Dns-serine	10.7	12.8	1.19
Dns-α-aminobutyric acid	11.9	13.3	1.11
Dns-glutamic acid[a]	38.8	41.2	1.06
Dns-aspartic acid[a]	55.9	63.1	1.13

[a] Mobile phase: 0.05 *M* phosphate buffer (pH 4.6)–methanol (60:40, v/v).

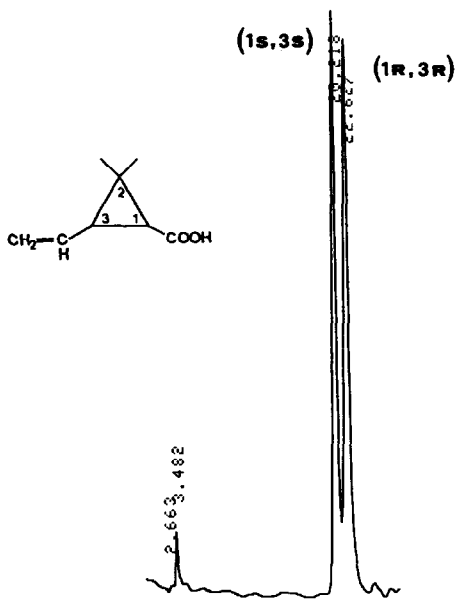

Fig. 3. Separation of (−)-(1*S*,3*S*)- and (+)-(1*R*,3*R*)-*trans*-chrysanthemic acid enantiomers on a 15 × 0.46 cm I.D. column. Eluent, 0.02 *M* acetate buffer (pH 4.6)–acetonitrile (75:25 v/v); flow-rate, 0.8 ml/min; detection, UV at 230 nm. Numbers at peaks indicate retention times in min.

TABLE III

CAPACITY FACTORS (k') AND SELECTIVITY FACTORS ($\alpha = k'_S/k'_R$) FOR FENOPROFEN ENANTIOMERS AS A FUNCTION OF THE ORGANIC MODIFIER (ACETONITRILE) COMPOSITION, pH AND IONIC STRENGTH OF THE BUFFER

Chromatographic conditions: column, 15 × 0.46 cm I.D., buffer potassium acetate; flow-rate, 1.0 ml/min; detection, UV at 254 nm; room temperature.

Variable	Value	k'_R	k'_S	α
pH[a]	2.9	11.3	11.8	1.04
	3.5	13.5	14.3	1.06
	4.0	14.6	15.6	1.07
	4.6	15.8	16.9	1.07
	5.0	12.6	13.4	1.06
	5.5	9.3	9.8	1.05
	6.0	6.9	6.9	1.00
Acetonitrile concentration (%)[b]	60	14.0	14.8	1.06
	50	14.4	15.6	1.08
	40	17.1	18.3	1.07
	30	25.5	27.5	1.08
Ionic strength (*M*)[c]	0.01	24.6	26.2	1.07
	0.02	14.4	15.6	1.08
	0.04	9.6	10.3	1.07

[a] Mobile phase: acetonitrile–0.02 *M* sodium acetate (60:40, v/v).
[b] Mobile phase: 0.02 *M* sodium acetate (pH 3.8).
[c] Mobile phase: acetonitrile–sodium acetate (50:50, v/v) (pH 3.8).

1,2,3,4-tetrahydrophenanthrene [16]. Here, the resolution of all three compounds was achieved, corresponding to $\alpha = 1.07$. In order to optimize the separation of the fenoprofen enantiomers, the effect of changes in organic modifier was also studied. Plots of k' *vs.* acetonitrile content at pH 4.6 and ionic strength 0.02 *M* of the buffer are shown in Fig. 4. As the amount of the organic modifier was increased, a decrease in retention was observed, according to a reversed-phase mode [17]. However, no substantial change in the α values occurred in a k' range between 3 and 1. The dependence of the eluent parameters is summarized in Table III. These data indicate that an essential part of the overall interaction of the analyte with the stationary phase is caused by

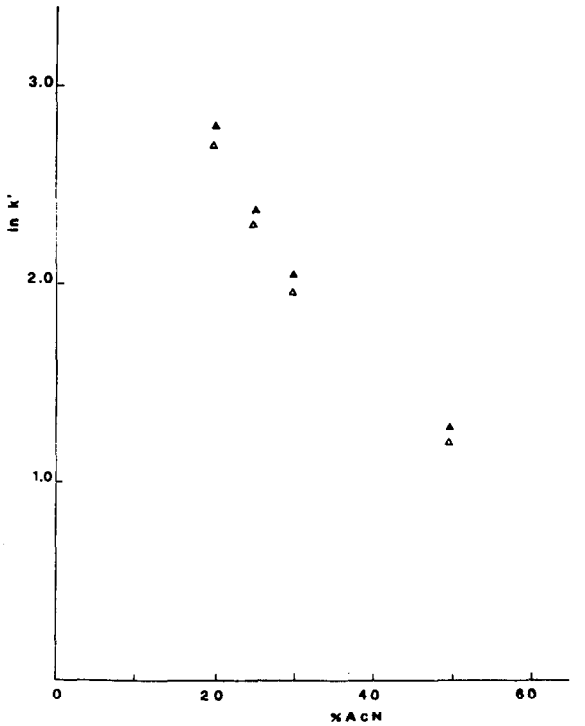

Fig. 4. Plots of ln k' *vs.* acetonitrile (AcN) content of the mobile phase for (▲) (*S*)- and (△) (*R*)-fenoprofen enantiomers. Chromatographic conditions: column, 15 × 0.46 cm I.D.; buffer, 0.02 *M* potassium acetate (pH 4.6); flow-rate, 0.7 ml/min; detection, UV at 254 nm; room temperature.

hydrophobic and ion-exchange effects. This last interaction appears as the effect on the retention of increases in the ionic strength and buffer pH, corresponding to a higher concentration of acetate counter ions. At pH >6, fenoprofen was not resolved. The enantioseparations of fenoprofen, ketoprofen and naproxen are shown in Fig. 5.

Of the three phenylcarboxylic acids (**VIa**, **VIb** and 3-phenylbutyric acid), only 2-phenylsuccinic acid (**VIb**) was resolved.

Interesting data were found for **VII**. *trans*-1,2-Cyclohexanedicarboxylic acid has a hydrophobic content (cyclohexane ring) in its molecule not higher than those of **Va** and **VIa**. However it was well resolved ($\alpha = 1.06$) in a short elution time.

Table II summarizes the chromatographic data obtained for a series of twelve dansylamino acid derivatives. The high enantioselectivity of (+)-terguride for α-amino acids (AA) is shown by the α values ranging between 1.05 (leucine) and 1.27 (tryptophan) for non-polar AA, 1.19 (serine) and 1.22 (threonine) for polar AA and 1.06 (glutamic acid) and 1.13 (aspartic acid) for acidic AA, which are strongly retained. A peculiarity of the chiral selector is that it resolves most of the pairs of enantiomers each separated from the others, making this packing suitable for

Fig. 5. Chromatograms of arylcarboxylic acids. Compounds (from left to right): 2-phenylpropionic acid; fenoprofen; ketoprofen; naproxen. Column, 15 × 0.46 cm I.D.; eluent, 0.02 *M* acetate buffer (pH 4.6)–acetonitrile (70:30, v/v); flow-rate, 1.0 ml/min; detection, UV at 254 nm; room temperature. Numbers at peaks indicate retention times in min.

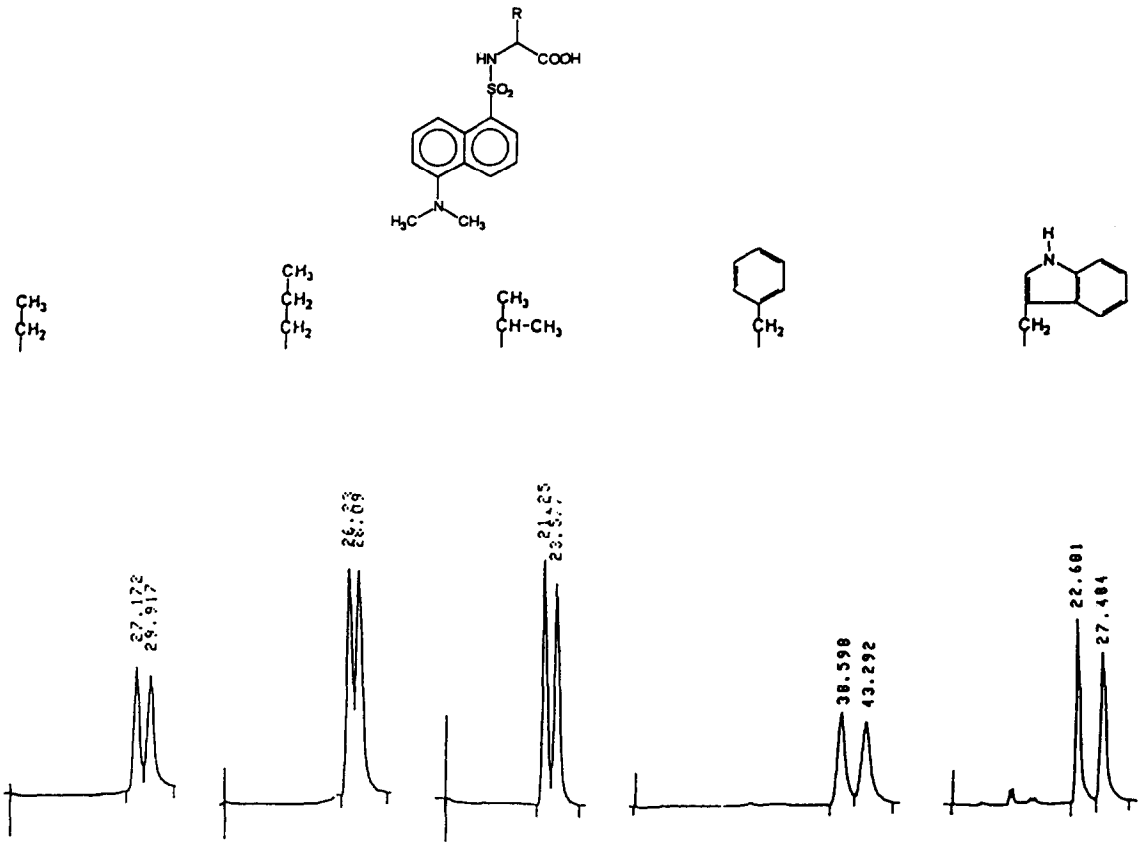

Fig. 6. Separation of dansylamino acid enantiomers. Eluent, 0.02 *M* phosphate buffer (pH 4.5)–methanol (50:50, v/v). Other conditions as in Fig. 3.

the analysis of complex mixtures. The resolution of some dansylamino acid derivatives is shown in Fig. 6.

The AMP-TER CSP is a useful additive to the growing number of stationary phases available for the resolution of NSAIDs and amino acids by HPLC. This packing also allows the direct enantioseparation of underivatized hydroxyalkylcarboxylic and *trans*-chrysanthemic acids, which otherwise are resolved as diastereomeric derivatives [18] or as derivatized compounds [19]. To our knowledge, this is the first report of the separation of *trans*-1,2-cyclohexanedicarboxylic acid enantiomers.

In order to interpret the chiral discrimination mechanism operating on the AMP-TER packing, a detailed NMR study of the adducts formed between the selector and 2-arylpropionic acids is in progress.

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REFERENCES

1 W.J. Lough (Editor), *Chiral Liquid Chromatography*, Blackie, Glasgow, 1989.
2 P. Salvadori, D. Pini, C. Rosini, C. Bertucci and G. Uccello-Baretta, *Chirality*, 4 (1992) 43.
3 C. Rosini, P. Altemura, D. Pini, C. Bertucci, G. Zullino and P. Salvadori, *J. Chromatogr.*, 348 (1985) 79.
4 N. Ôi, H. Kitahara and R. Kira, *J. Chromatogr.*, 592 (1992) 291.
5 Y. Saotome, T. Miyazawa and T. Endo, *Chromatographia*, 28 (1989) 509.
6 Y.H. Kim, A. Tishbee and E. Gil Av, *J. Am. Chem. Soc.*, 102 (1980) 5915.

7 R. Bhushan and I. Ali, *Fresenius' Z. Anal. Chem.*, 329 (1988) 793.
8 M. Flieger, M. Sinibaldi, L. Cvak and L. Castellani, *Chirality*, in press.
9 R.C. Weast (Editor), *Handbook of Chemistry and Physics*, Chemical Rubber, Cleveland, OH, 70th ed., 1900, p. D161.
10 R. Staudinger, *Helv. Chim. Acta*, 7 (1924) 177.
11 J. Caldwell, A.J. Hutt and S. Fournel-Gigleux, *Biochem. Pharmacol.*, 37 (1988) 105.
12 J.R. Kern, *J. Chromatogr.*, 543 (1991) 355.
13 Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, N. Inotsume and M. Nakano, *Chirality*, 1 (1989) 239.
14 J. Hermansson and M. Eriksson, *J. Liq. Chromatogr.*, 9 (1986) 621.
15 S.G. Allenmark and S. Anderson, *Chirality*, 1 (1989) 154.
16 W.H. Pirkle, C.J. Welch, J. Liq. Chromatogr., 15 (1992) 1947.
17 W.R. Melander and Cs. Horváth, in Cs. Horváth (Editor), *High Performance Liquid Chromatography*, Vol. 2, Academic Press, New York, 1980, p. 113.
18 S.G. Allenmark, *Chromatographic Enantioseparation —Methods and Applications*, Ellis Horwood, Chichester, 1988, p. 54.
19 N. Oi, M. Nagase, Y. Inda and T. Doi, *J. Chromatogr.*, 259 (1983) 487.