Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 7, pp. 665–673, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00
Pergamon Journals Ltd.

Enantiomeric separation of chiral carboxylic acids, as their diastereomeric carboxamides, by thin-layer chromatography

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Abstract: A thin-layer chromatographic (TLC) method is described for the enantiomeric separation of chiral carboxylic acids using chiral derivatization and non-chiral TLC conditions (ordinary plates and mobile phases) to separate the diastereomeric carboxamides obtained.

New chiral derivatizing agents, "levobase" (1R, 2R)-(−)-1-(4-nitrophenyl)-2-amino-1,3-propanediol, and "dextrobase" (the enantiomer of levobase) are used for carboxamide formation in the presence of dicyclohexylcabodiimide as coupling agent. The procedure is very simple and convenient to carry out. Good resolution is obtained for a wide range of carboxylic acid enantiomeric pairs containing one to two chiral centres.

Keywords: *Chiral carboxylic acids; enantiomeric TLC separation; chiral derivatization; new chiral derivatizing agents.*

Introduction

Due to the rapidly increasing interest in asymmetric synthesis and the resolution of chiral compounds, the use of chromatographic methods (mainly HPLC and TLC) for monitoring enantiomeric purity has grown considerably in recent years [1–3].

Despite the fact that TLC presents a great number of advantages (simplicity, rapidity, reproducibility and low cost), few examples of enantiomeric separations by TLC have been reported [4–17].

As in HPLC, three general techniques can in principle be used, namely:

(i) Separation on chiral stationary phases (including ordinary reversed-phase plates impregnated with copper(II)-complexes of chiral *N*-alkyl-α-amino acid derivatives) using ordinary ("non-chiral") mobile phases [4–14];

(ii) Separation on ordinary stationary phases by means of chiral additives in the mobile phases which form diastereomeric complexes with the substrate. As far as the authors are aware, this principle has not been exploited in TLC;

(iii) Derivatization of the sample with chiral reagents to produce covalently bonded diastereomeric molecules which can be separated using ordinary chromatographic systems [15–17].

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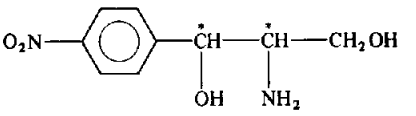
As a result of the present study a new and widely applicable TLC method for enantiomeric separation of chiral carboxylic acids as their diastereomeric amides has been developed. For chiral derivatization, "levobase"(I) and "dextrobase"(II), two new derivatizing agents are used in the presence of dicyclohexylcarbodiimide (DCC) as coupling agents.



I,II

I: (1R, 2R)-(−)-1-(4-nitrophenyl)-2-amino-1,3-propanediol;
II: (1S, 2S)-(+)-1-(4-nitrophenyl)-2-amino-1,3-propanediol.

Experimental

Chemicals and reagents

All solvents used were of analytical grade and purchased from Reanal (Budapest, Hungary). Levobase and dextrobase, produced by EGIS Pharmaceuticals (Budapest, Hungary) were available in high enantiomeric purity (m.p.: 163–165°C for both enantiomers; $[\alpha]_D^{20}$ = −29.5 and +29.4 (c = 1, 6 M HCl), respectively). *Cis*-permethrinic acid, fenoprofen and naproxene were made available by Chinoin Pharmacochemical Works Ltd. (Budapest, Hungary). Other compounds obtained from commercial sources were: dicyclohexylcarbodiimide, lactic acid, mandelic acid and *N*-α-acetylphenylalanine, Aldrich (Beerse, Belgium); racemic 3-bromo-2-methylpropionic acid, Toyosoda (Tokyo, Japan); (2R, 3R)-di-*O*-benzoyl-tartaric acid 1-dimethylamide, Industria (Budapest, Hungary).

(S)-Proline-*N*-tosylamide [18], racemic 2-methoxy-3-benzoylpropionic acid [19] and 3-bromo-2-methyl-propionic acid enantiomeric mixture enriched in the (2S) form [20] were synthesized as described in the literature.

Derivatization procedure

From freshly prepared stock solutions of the substrate, that of levobase (or dextrobase) and DCC (all 0.1 mM in dry methanol or tetrahydrofuran), the following volume ratios were mixed and homogenized (DCC was added last):

substrate–levobase (or dextrobase)–DCC (1:1.5:1.5, v/v/v).

After a 10-min derivatization period at ambient temperature, 5–10 μl portions of the reaction mixture were directly applied onto the TLC plate.

Chromatography

TLC plates (Silica gel F_{254}; 100 × 200 mm; layer thickness: 0.25 mm) and a LiChroprep Si 60 prepacked column (240 × 10 mm i.d.) were obtained from E. Merck (Darmstadt, FRG). Solutions were applied manually onto the TLC plates. Chambers were pre-saturated for 2 h. Developments were carried out over 150 mm, the plates were

then dried by a hot air stream and evaluated under UV radiation at 254 nm. The mobile phase consisted of chloroform–ethanol–acetic acid (9:1:0.5, v/v/v).

Instruments

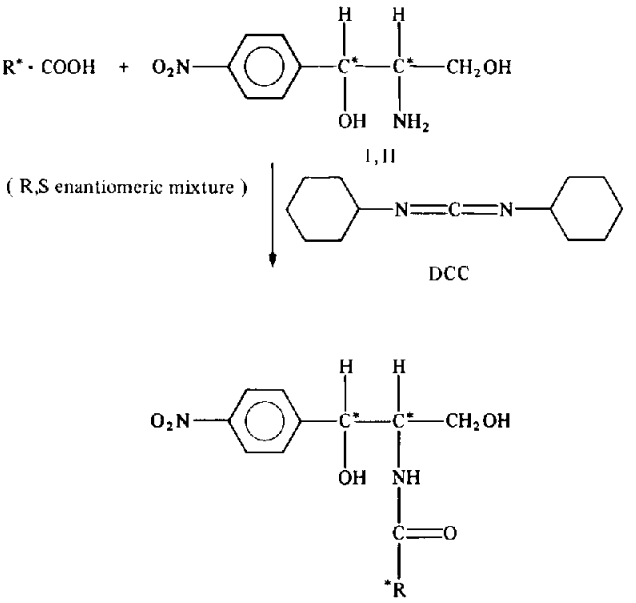
Infrared spectra were recorded as potassium bromide discs using a Pye Unicam SP-3-200 IR spectrophotometer (Pye Unicam Ltd., Cambridge, UK). Mass spectra were recorded using a Jeol JMS 01SG-2 type mass spectrometer (Jeol Ltd., Tokyo, Japan). For column chromatography, a Labor MIM IS-232 solvent delivery pump (Labor MIM, Budapest, Hungary) was used.

Results and Discussion

The carboxylic acids **1–10** (Fig. 1) were reacted with either of the chiral derivatizing agents in the presence of DCC and the resulting diastereomeric carboxamides (for structure, see Fig. 3) resolved by conventional silica plate thin-layer chromatography.

A typical example, namely the resolution of 3-bromo-2-methylpropionic acid, is shown in Fig. 2.

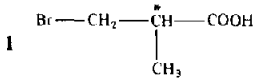
The derivatization reaction scheme may be written as:



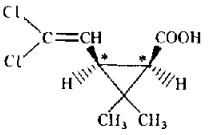
Mixture of diastereomeric Carboxamides

In principle, if dextrobase (1S, 2S configuration) is used instead of levobase (1R, 2R configuration) for derivatization of a given individual enantiomer (of "R" configuration for example), the resulting carboxamide will migrate with the same R_f-value as the respective "S" enantiomer derivatized with levobase (and vice versa) under the same "non-chiral" chromatographic conditions (Table 1). R_f-values obtained for carboxylic acids (listed in Fig. 1) are presented in Table 2.

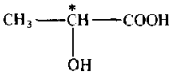
From Tables 1 and 2, it may be concluded that R_f-values for enantiomer pairs can be determined with any individual enantiomer, by performing two separate derivatization



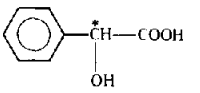
1 3-bromo-2-methylpropionic acid



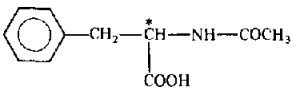
2 *Cis*-permethrinic acid [2,2-dimethyl-3-(2′-dichlorovinyl)-cyclopropane carboxylic acid]



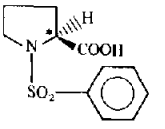
3 Lactic acid



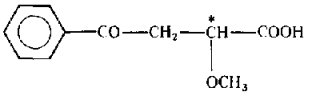
4 Mandelic acid



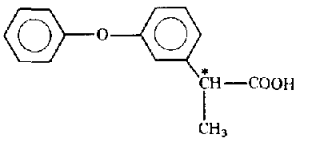
5 *N*-α-acetylphenylalanine



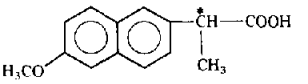
6 (S)-Proline-*N*-tosylamide



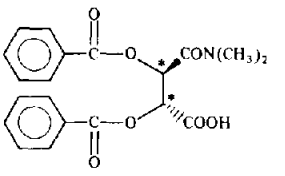
7 2-methoxy-3-benzoylpropionic acid



8 2-(3-phenoxyphenyl)-propionic acid (Fenoprofen)



9 2-(6-methoxy-2-naphtyl)-propionic acid (Naproxen)



10 (2R,3R)-(-)-di-O-benzoyltartaric acid 1-dimethylamide

Figure 1
Chemical structure of the investigated carboxylic acids.

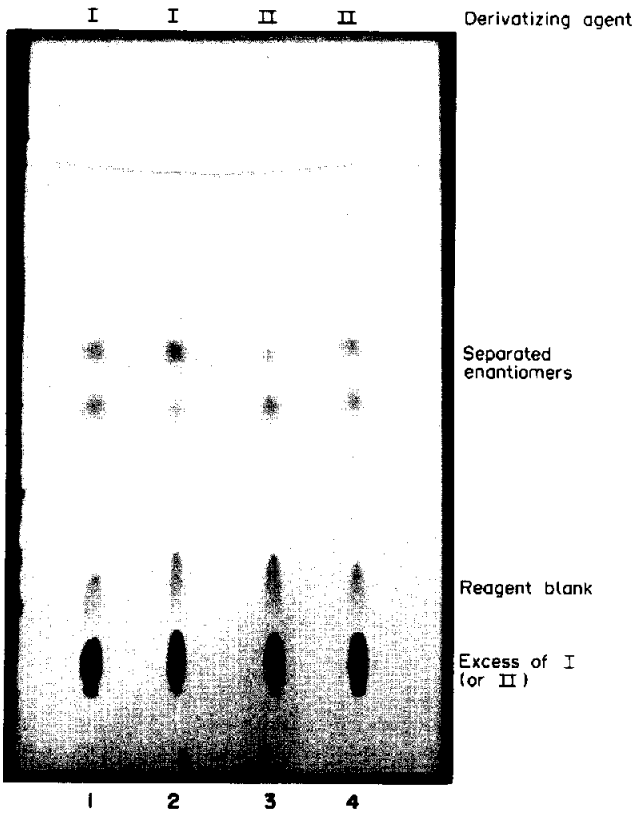


Figure 2
Optical resolution of 3-bromo-2-methylpropionic acid by TLC. **2** and **3**: enantiomeric mixture enriched in "S" form (ee: about 50%). **1** and **4**: racemic mixture. Reagent blank: DCC + I(or II). Chromatographic conditions: see Experimental. R_f-values: as in Table 2.

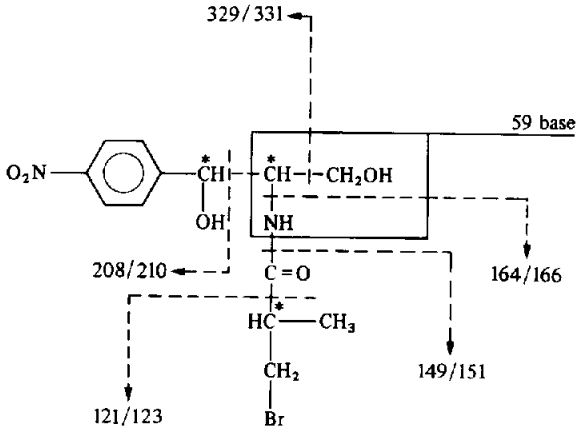


Figure 3
Structure and mass spectrometric fragmentation of the first eluted diastereomer obtained by chiral derivatization of racemic 3-bromo-2-methylpropionic acid with levobase. Numbers at the end of the arrows indicate *m/z* values for fragments detected.

Table 1
Chiral derivatization of carboxylic acid enantiomers with "levobase" and "dextrobase"

Absolute configuration of the enantiomer*	Derivatizing agent	Abbreviation for absolute configuration of the diastereomer formed	R_f-values†
R	Levobase	RRR } enantiomer	R_f (R)
S	Dextrobase	SSS } pairs	R_f (R)
R	Dextrobase	RSS } enantiomer	R_f (S)
S	Levobase	SRR } pairs	R_f (S)

*Enantiomers with one asymmetric carbon atom.
†Enantiomeric carboxamides (RRR/SSS and RSS/SRR in the Table) give the same R_f-value (indicated as R_f (R) and R_f (S), respectively) under ordinary chromatographic conditions.

Table 2
Enantiomeric separation of chiral carboxylic acids as their diastereomeric carboxamides by TLC

Carboxylic acid (as in Fig. 1)	Absolute configuration	R_f-values* with Dextrobase	R_f-values* with Levobase	ΔR_f	Remarks
1	(R)	0.34	0.45	0.11	Enantiomer mixture enriched in S-form
	(S)	0.45	0.34		
2	(1S, 2S)	0.61	0.57	0.04	
	(1R, 2R)	0.57	0.61		
3	(S)	0.48	0.43	0.05	R-enantiomer and racemate were investigated
	(R)	0.43	0.48		
4	(S)	0.33	0.26	0.07	
	(R)	0.26	0.33		
5	(R)	0.48	0.35	0.13	Racemization was found for both enantiomers
		0.35	0.48		
		0.48	0.35		
	(S)	0.35	0.48		
6	(S)	0.72	0.62	0.09	Only S-enantiomer was investigated
7		0.59	0.52	0.07	Only racemate was investigated
		0.52	0.59		
8		0.65	0.54	0.11	Only racemate was investigated
		0.54	0.65		
9	(R)	0.63	0.53	0.1	Racemate and S-enantiomer were investigated
	(S)	0.53	0.63		
10	(2R, 3R)	0.44	0.38	0.06	Only (2R, 3R) enantiomer was investigated

*Corresponding R_f-values have been obtained in the same TLC run.

reactions as described above, one with levobase and the other with dextrobase, followed by a TLC separation of the resulting diastereomers.

In addition the carboxamides obtained by derivatization of racemic 3-bromo-2-methylpropionic acid with levobase have been separated by semipreparative column chromatography. The presumed carboxamide structure for the resolved fractions was proved conclusively by mass spectrometry (Fig. 3) and IR spectroscopy.

The mass spectrum (obtained by electron impact ionization) does not exhibit a parent peak. The base peak is at $m/z = 59$ and can be attributed to the fragment $[HN{=}CH{-}CH_2{-}OH]^+$. The second-eluted diastereomer gave a very similar mass spectrum.

Characteristic IR absorption bands (1645 cm^{-1}: Amide I band; 1510 cm^{-1}: Amide II band; 1350 cm^{-1}: Amide III band) in the spectra of the separated diastereomers, as well as the absence of frequencies characteristic of aliphatic esters (which can theoretically be formed in the derivatization procedure), tend to confirm the proposed carboxamide structure.

The use of DCC as coupling agent to obtain diastereomeric carboxamides from *N*-protected amino acids by means of O-(4-nitrophenyl)-tyrosine methylesters (R and S forms) as chiral derivatizing agents has been presented in the excellent paper of Görög *et al.* [21]. They found that a side reaction can take place to a small extent between the free carboxylic group of the amino acid and DCC to give an *N*-acylurea derivative. However, it was shown that this side-reaction does not affect the enantiomeric peak area ratio (because the ratio of the rates of the main and side-reactions does not show any enantioselectivity). Therefore it is likely that the appearance of products from this type of side reaction (which could, in principle, occur with the present derivatization procedure) may be ignored.

Significantly different reaction rates for the diastereomeric carboxamide formation can, in principle, change the enantiomer spot area (or spot intensity) ratio, if the derivatization process is incomplete ("kinetic resolution") [22]. Preliminary HPLC results obtained for racemic 3-bromo-2-methylpropionic acid and *cis*-permethrinic acid enantiomers revealed that the measured enantiomer peak area ratios are in very good agreement with the true enantiomeric composition of the mixture studied, even when the derivatization is incomplete. Moreover, TLC spots of underivatized carboxylic acid (found after a 10-min derivatization period) generally can be ignored with respect to the main spot. Kinetic resolution (if any), therefore, has not to be taken into consideration.

As a result of the very low R_f-values (0.05–0.1) of the reagent spots (that of DCC, I and II) under the present experimental conditions, the evaluation of the main spots is found to be easy.

An accidental racemization (partial or complete) could be a serious source of error (in the case of *N*-acetylphenylalanine). Accordingly, when elaborating a new enantiomeric separation as described above, a preliminary study of that possibility is necessary. This can be done by testing any individual enantiomer of the mixture to be separated.

The new chiral derivatizing agents (I and II) can be obtained as the chiral key intermediate and the side product, respectively, in the most commonly used method for the manufacture of chloramphenicol [23]. They have previously been used as resolving agents [24] (giving diastereomeric salts of different solubility with carboxylic acid enantiomer pairs) but not as chiral derivatizing agents. They are non-hazardous, low-cost and commercially available chemicals.

Recrystallized from water as the chlorhydrate salt, their enantiomeric purity can be

higher than 99.8% (measured by differential scanning calorimetry). Stored under anhydrous conditions at ambient temperature in a well-sealed, dark bottle, no significant change has been found in their physicochemical properties (melting point, optical rotatory power, and enantiomeric purity) even after a period of three years. Due to their strong UV absorption, detection limits in TLC for the carboxamides obtained are relatively low (~1 μg in the case of (2S)-3-bromo-2-methyl-propionic acid on the ordinary silica plate used).

The potential analytical applications of the described procedure may be designated as follows:

(i) Controlling the progress of preparative optical resolutions in which enantiomers of unknown optical activity are involved;

(ii) Detecting or determining enantiomeric impurities in pharmaceuticals, synthesis intermediates, etc. For this purpose it is advisable to use that form of the chiral derivatizing agents (I or II), for which the enantiomeric impurity gives a lower R_f-value than the more abundant enantiomer.

Our preliminary investigations show the utility of this derivatization technique in enantiomeric separation of chiral carboxylic acids by HPLC [23].

Conclusions

Enantiomeric separation by TLC of a wide range of chiral carboxylic acids as their diastereomeric carboxamides, formed by the use of levobase and dextrobase as new, stable, low-cost and commercially available chiral derivatizing agents, has been found to be a useful and efficient technique. Simple reaction conditions, reproducibility, low detection limits, flexibility and little chance of racemization during the course of the analyses are general characteristics of the method.

Acknowledgements: The authors wish to thank Miss E. Szabó and Miss É. Furdyga for their helpful contribution in recording and interpreting the mass and infrared spectra. Also they are grateful to Mrs I. Kaminszki for her skilful assistance in TLC and for Miss I. Sztruhár for valuable discussions.

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[*Received for review 7 January 1987*]