Pergamon

Tetrahedron: *Asymmetry* 13 (2002) 69–75

TETRAHEDRON: *ASYMMETRY*

Enantioselective semi-preparative HPLC of two 2-arylpropionic acids on glycopeptides containing chiral stationary phases

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Received 10 January 2002; accepted 25 January 2002

Abstract—We describe a new enantioselective HPLC procedure for the direct semi-preparative resolution of two unmodified 2-arylpropionic acids. The method is based on the use of novel laboratory-made chiral stationary phases (CSPs) containing macrocyclic glycopeptide antibiotics, such as teicoplanin and A-40,926, covalently bonded to silica gel microparticles. The new CSPs showed high enantioselectivities and broad applicabilities for separations on a semi-preparative scale.

1. Introduction

Enantiomeric resolutions of underivatized carboxylic acid compounds were first performed using the ligand exchange chromatographic (LEC) technique,[1,2] where the samples were separated as Cu^{2+}-coordinating compounds. Pre- or post-column derivatization with UV absorbing[3] or fluorescent reagents[4] solved detection problems, but analysis was limited to compounds containing one or more functional groups able to form coordination complexes with transition metal ions. Another method for rapid and selective separation of charged or ionizable molecules is ion-exchange and ion-pair chromatography,[5–7] amenable to both micro-analytical and preparative studies. However, care needs to be taken to ensure that the solutes are predominantly in a charged form by appropriate selection of the mobile phase pH. Moreover, variations in the enantioselectivity factor (α) values often occurred with mixed ion-pairing conditions, or with different types of ion-pairing reagents, e.g. alkylsulfates versus alkylphosphates.[8]

The 2-arylpropionic acids (2-APAs, the 'profens') are an important sub-group within the class of non-steroidal anti-inflammatory drugs (NSAIDs). These compounds are chiral and the majority are marketed as racemates. The main pharmacological activity (inhibition of cyclooxygenase, which mediates the conversion of arachidonic acid to prostaglandins) resides in the (*S*)-(+)-enantiomer, with the (*R*)-enantiomer being either inactive or weakly active in vitro.[9] Underivatized anti-inflammatory drugs were separated on chiral stationary phases (CSPs) containing either α_1-acid glycoprotein,[10] immobilized human serum albumin,[11] adsorbed or covalently bonded α-chymotrypsin,[12] and quinine and quinidine derivatives as chiral anion exchangers.[13]

Recently, glycopeptide antibiotics have been successfully used as chiral selectors to resolve the enantiomers of a variety of racemic compounds by means of chromatographic (TLC and HPLC) and electrophoretic (HPCE) techniques.[14] These natural compounds contain multiple stereogenic centers and a variety of functional groups. In particular, teicoplanin (TE)[15,16] has a macrocyclic heptapeptide aglycone with three attached sugar units. The aglycone consists of four fused rings forming a 'semi-rigid basket' which incorporates biaryl and biaryl ether units, two of which are chloro-substi-

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0957-4166/02/$ - see front matter
PII: S0957-4166(02)00046-0

tuted. The three sugar units are: α-D-mannose, β-D-*N*-acetyl-glucosamine, β-D-*N*-acyl-glucosamine (acyl = 8-methyl-nonanoyl). Five main components of TE have been identified, differing from each other only in the nature of the hydrocarbon chain of the *N*-acyl-glucosamine moiety. Fig. 1 (left) shows the chemical structure of the prevalent component of TE glycopeptide complex (A_2-2, >85%).

A-40,926 is a natural complex of glycopeptides produced by the *Actinomadura* strain ATCC 39726, from which factors A and B are the major recoverable species.[17–20] The chemical structure of the prevalent component of A-40,926 glycopeptide complex (factor B, >70%) is reported in Fig. 1 (right). It differs from TE for the presence of an additional carboxylic group on the β-D-*N*-acyl-glucosamine, in the lack of the β-D-*N*-acetyl-glucosamine, for the different position of the chlorine atom on the phenyl rings, the presence of an *N*-methyl group on the primary amine in the aglycone portion and finally it has a different acyl group on the β-D-*N*-acyl-glucosamine residue (acyl = 10-methyl-undecanoyl).

Herein we describe an easy, fast and convenient procedure for the direct semi-preparative resolution of two unmodified racemic 2-arylpropionic acids[21] (Fig. 2), by enantioselective HPLC on novel laboratory-made chiral stationary phases, containing covalently bonded teicoplanin (TE-CSP)[22] and A-40,926 (A-40,926-CSP)[23,24] as selectors.

2. Results and discussion

The potential for semi-preparative separations on the A-40,926-CSP was demonstrated in a recent study:[23] large separation factors, very short analysis times and easily removable mobile phases were the attracting features of a chromatographic method developed for racemic *cis*-2-aminocyclohexanecarboxylic acid. We have now successfully extended the range of applications at semi-preparative levels of the teicoplanin-based CSPs to acyclic carboxylic acids (compounds **1** and **2**, see Fig. 2), structurally related to the 2-arylpropionic acid family.

Racemic **1** and **2** were baseline resolved on the analytical scale using A-40,926-CSP and TE-CSP, respectively.

Increasing amounts of racemic **1** or **2** (from 5 to 20 mg) were processed on the corresponding semi-preparative column (Figs. 3 and 4, respectively), using methanol–water (66:34) +0.025 mol/l ammonium acetate as eluent, at a flow-rate of 3.0 mL/min and 2.0 mL/min, respectively.

The enantiomeric excesses (e.e.) of the two collected fractions were measured by HPLC using an analytical column containing the same CSP and using the same mobile phases as the semi-preparative separations. At different loadings, we always obtained the first eluting enantiomer of **1** with e.e. >99.9%, and the second eluting enantiomer with e.e. = 98.3%, with a recovery of

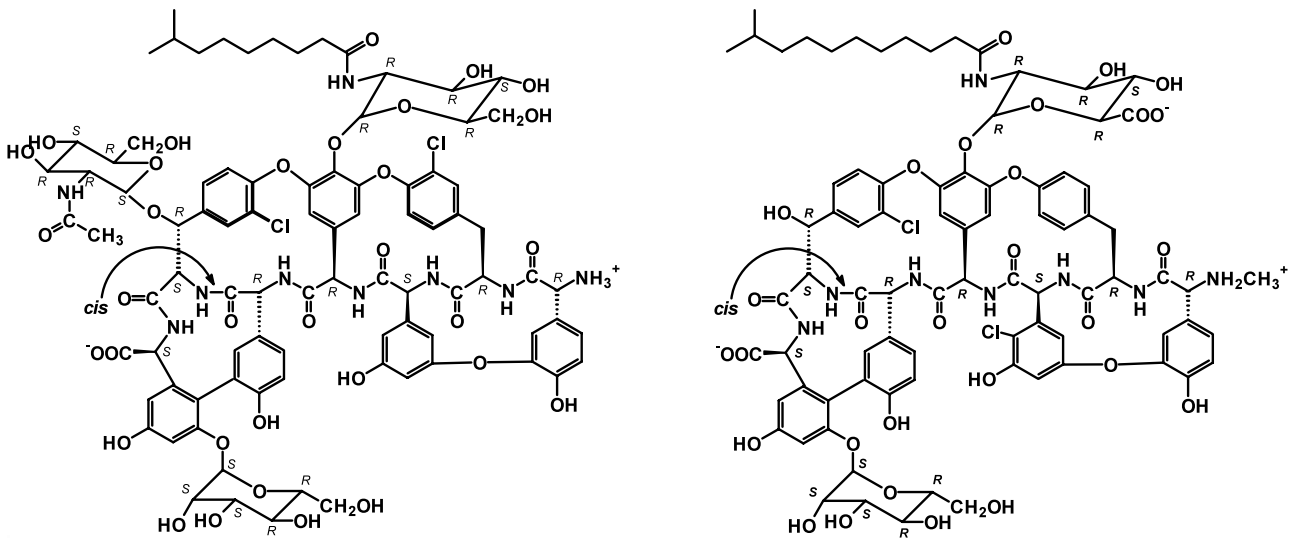

Figure 1. Chemical structures of the prevalent teicoplanin (TE) component (A_2-2, left) and of the prevalent A-40,926 component (factor B, right).

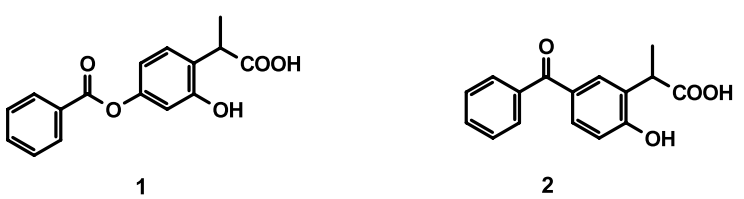

Figure 2. Structures of the two carboxylic acid compounds resolved on our glycopeptides containing CSPs.

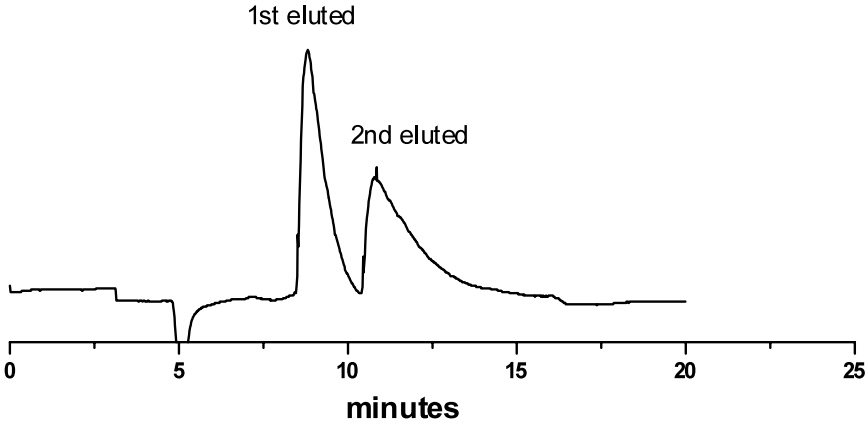

Figure 3. Semi-preparative resolutions of racemic **1** on a 250×10 mm ID column packed with A-40,926. Eluent: MeOH–H_2O (66:34) +0.025 mol/l NH_4OAc. Flow-rate: 3.0 mL/min. Temperature: 20°C. Detection: RI, at room temperature. Injected amount: 15 mg, dissolved in methanol.

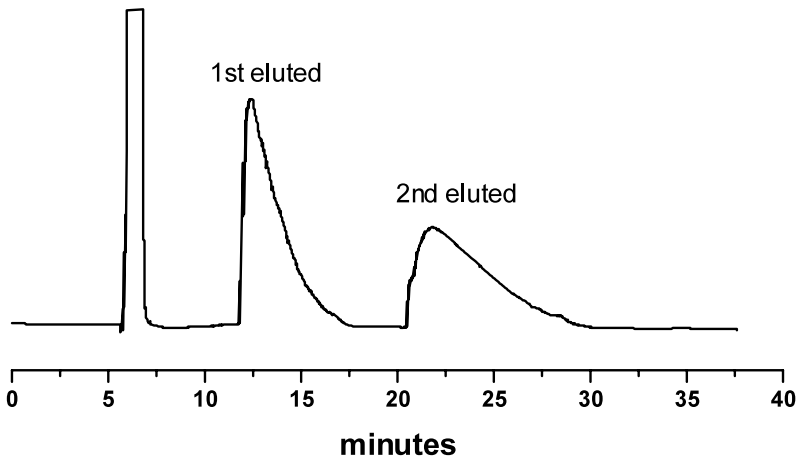

Figure 4. Semi-preparative resolutions of racemic **2** on a 250×10 mm ID column packed with TE. Eluent: MeOH–H_2O (66:34) +0.025 mol/l NH_4OAc. Flow-rate: 2 mL/min. Temperature: 20°C. Detection: RI, at room temperature. Injected amount: 10 mg, dissolved in methanol.

about 90% (Fig. 5). The enantiomers of **2** were obtained with e.e. = 98.2% (first eluted) and e.e. = 97.8% (second eluted) at 95% recovery (Fig. 6). Production rates were about 20 mg/h of each enantiomer for **1** on the A-40,926-CSP and 30 mg/h of each enantiomer for **2** on the TE-CSP. The chiroptical data obtained for the two enantiomers of **1** and **2** are collected in Table 1.

The absolute configurations of the two enantiomers of **1** and **2** were determined by circular dichroism (CD) spectroscopy. The CD spectra of the individual enantiomers (Figs. 7 and 8) were compared with those of enantiomerically pure samples of the structurally related (*R*)-(−)-ketoprofen (Fig. 9). The first eluting enantiomers of **1** and **2** have intense positive bands at 229 and 235 nm, respectively. These bands are primarily due to the n→π* transitions of the carboxyl groups, and secondarily to the ^1L_a (π→π*) transition of the phenyl ring. Additional weak positive bands, observed over the wavelength range 250–300 nm, are due to the ^1L_b transition in the phenyl ring. The weak negative band at 304 nm observed for the first eluting enantiomer of **2** can be associated with the n→π* transition of the carbonyl group, and this band is absent in the CD spectrum of the enantiomers of **1**. The CD spectrum of (*R*)-(−)-ketoprofen shows only a negative band centred around 224 nm. For both **1** and **2**, the first eluting enantiomers were dextrorotatory at the sodium D line, while (*R*)-ketoprofen and other profens of the same configuration are levorotatory.[25] Thus, (*S*)-configuration can be assigned to the first eluting enantiomers of **1** and **2**.

The mechanism of molecular recognition of the enantiomers of carboxylic acid compounds **1** and **2** is consistent with the recently reported binding mode of the acetate anion[26] to vancomycin and ristocetin A, two antibiotics structurally related to teicoplanin. The presence of the hydroxyl group *ortho* to the propionic acid substituent of **1** and **2** plays a key role in the enantioselective recognition process: racemic ketoprofen, in fact, which differs from compound **2** only in the absence of the hydroxyl function, was retained ($k' = 1.02$), under the same chromatographic conditions, but very poorly resolved ($\alpha < 1.1$) on our glycopeptide-containing CSPs.

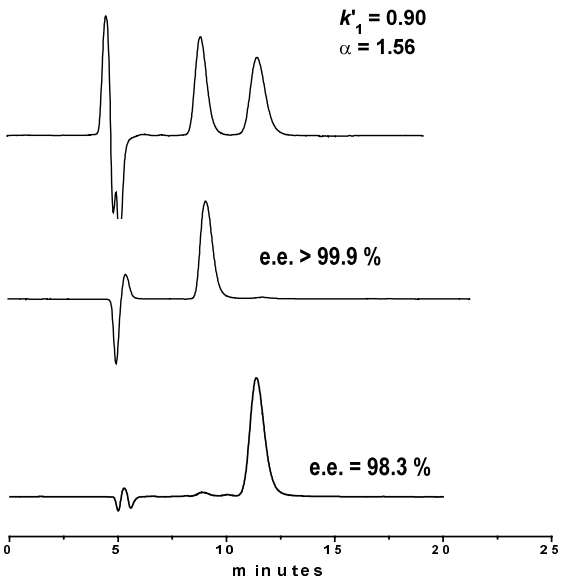

Figure 5. Analytical resolutions on A-40,926-CSP of racemic **1** (top) and control of the fractions collected in the semi-preparative separations (bottom). Eluent: MeOH–H_2O (66:34) +0.025 mol/l NH_4OAc. Flow-rate: 0.5 mL/min. Temperature: 20°C. Detection: UV at 254 nm.

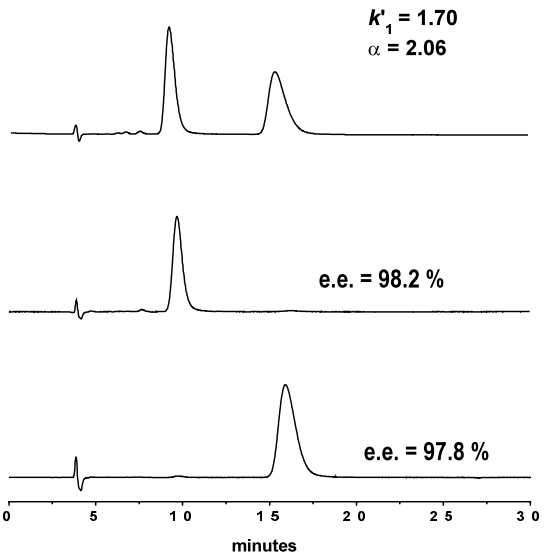

Figure 6. Analytical resolutions on TE-CSP of racemic **2** (top) and control of the fractions collected in the semi-preparative separations (bottom). Eluent: MeOH–H_2O (66:34) +0.025 mol/l NH_4OAc. Flow-rate: 1.0 mL/min. Temperature: 20°C. Detection: UV at 254 nm.

3. Conclusions

Semi-preparative separations of 2-aryl propionic acids in the 5–20 mg range are feasible on 10 mm ID columns packed with novel glycopeptides containing CSPs (TE and A-40,926). The columns used gave easy access to both enantiomers in almost enantiopure form in very short times. Moreover, the absolute configurations and chiroptical data of the two profens, determined by circular dichroism (CD), were consistent with those of other structurally related profens: (*S*) for the dextrorotatory and (*R*) for the levorotatory enantiomers, respectively.

Further studies in this direction are currently in progress in our research group, in order to evaluate the range of applicability and the detailed mechanism of enantioselective binding of the new selectors to 'profen' enantiomers.

4. Experimental

4.1. Apparatus

Analytical liquid chromatography was performed as previously described.[22] Semi-preparative separations were carried out on a Waters Delta Prep 3000 chromatographic system (Waters Chromatography, Milford, MA, USA), equipped with a Rheodyne Model 7010 5 mL loop injector, and a Knauer differential refractometer (RI) detector (Berlin, Germany).

The two collected fractions were then processed on a Hypersil ODS semi-preparative column (250×10 mm ID), in order to remove excess ammonium acetate and convert the enantiomers of **1** and **2** into the undissociated form. Wet fractions were loaded onto the ODS column and recovered using a gradient elution [mobile phase, A: water–acetonitrile (90:10)+0.5% acetic acid; B: acetonitrile+0.5% acetic acid; linear gradient from 30% B to 70% B in 25 min. Flow-rate 5.0 mL/min, $T=25$°C, UV detection at 254 nm].

Optical rotation values were determined on a Perkin–Elmer 341 polarimeter, at 20°C (concentrations expressed in g/100 mL; solvent: methanol). CD spectra were recorded on a Jasco J-710 spectropolarimeter (solvent: methanol).

4.2. Chemicals and reagents

LiChrospher Si 100 silica gel (5 μm particle size, 400 m^2/g) and HPLC-grade solvents were purchased from Merck (Darmstadt, Germany); (3-aminopropyl)-triethoxysilane, dry toluene, dry pyridine, 1,6-diisocyanatohexane and water for HPLC were from Fluka (Sigma-Aldrich Company, Buchs, Switzerland); ammonium acetate and acetic acid were purchased from J. T. Baker (Division of Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA); teicoplanin and A-40,926 were provided by the Lepetit Research Centre (Gerenzano, Italy).

Racemic benzoic acid 4-(1-carboxy-ethyl)-3-hydroxyphenyl ester **1**, 2-(5-benzoyl-2-hydroxyphenyl)-propionic acid **2** and (*R*)-(−)-ketoprofen were supplied from Dompè S.p.A. (L'Aquila, Italy) and used as received, without further purification.

Table 1. Chiroptical data for the resolved enantiomers of compounds **1** and **2**

Sample	Configuration	$[\alpha]_D^{20}$ (methanol)	$\Delta\varepsilon$ (methanol)
First eluting enantiomer of **1**	2*S*	+39 (c=0.26)	+3.678 (λ=229 nm) +0.436 (λ=275 nm)
Second eluting enantiomer of **1**	2*R*	−37 (c=0.31)	–
First eluting enantiomer of **2**	2*S*	+8 (c=0.25)	+2.080 (λ=235 nm) +0.279 (λ=273 nm) −0.156 (λ=304 nm)
Second eluting enantiomer of **2**	2*R*	−8 (c=0.26)	–
(*R*)-(−)-Ketoprofen	2*R*	−43 (c=0.26)	−2.197 (λ=224 nm)

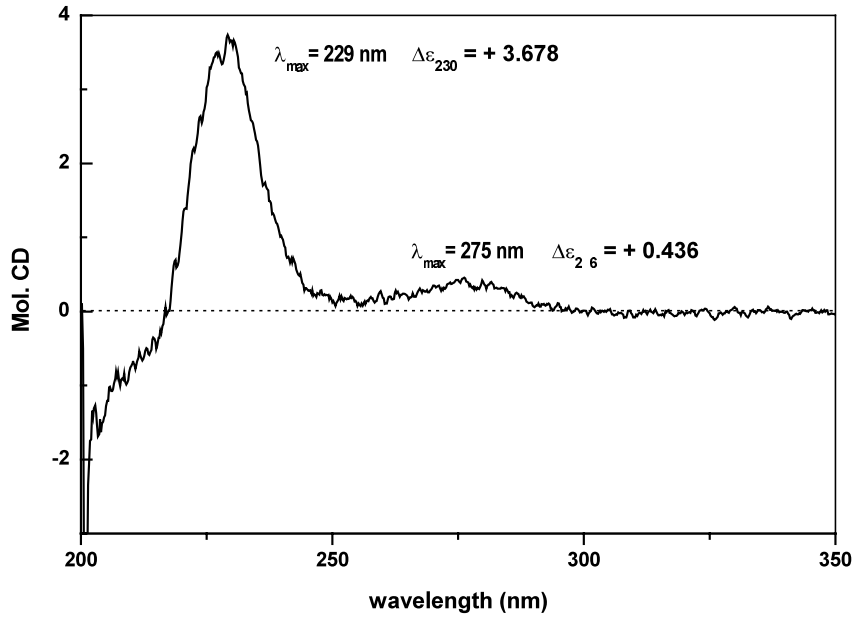

Figure 7. Circular dichroism (CD) spectrum of (*S*)-(+)-**1**. Concentration: 0.20 g/100 mL in methanol. Temperature: 20°C.

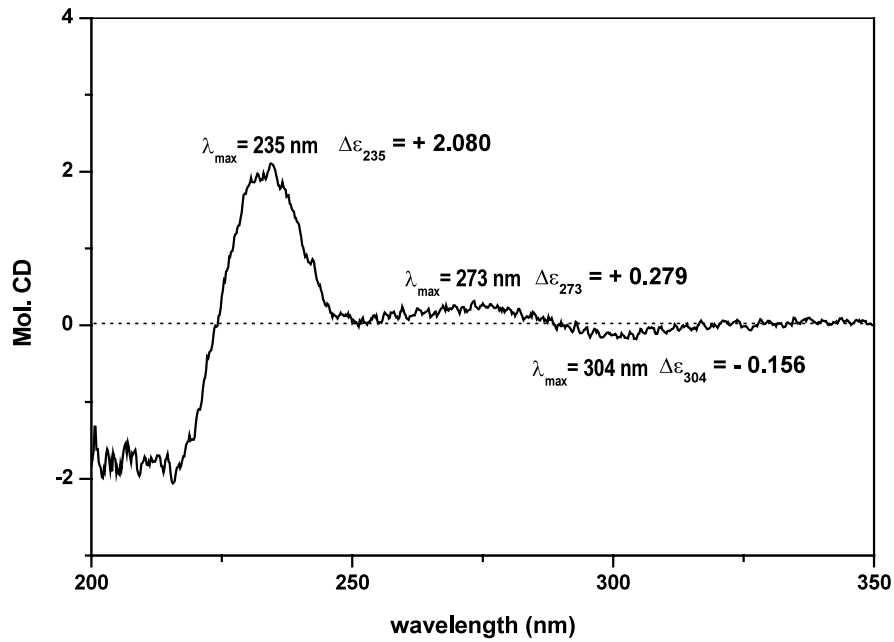

Figure 8. Circular dichroism (CD) spectrum of (*S*)-(+)-**2**. Concentration: 0.20 g/100 mL in methanol. Temperature: 20°C.

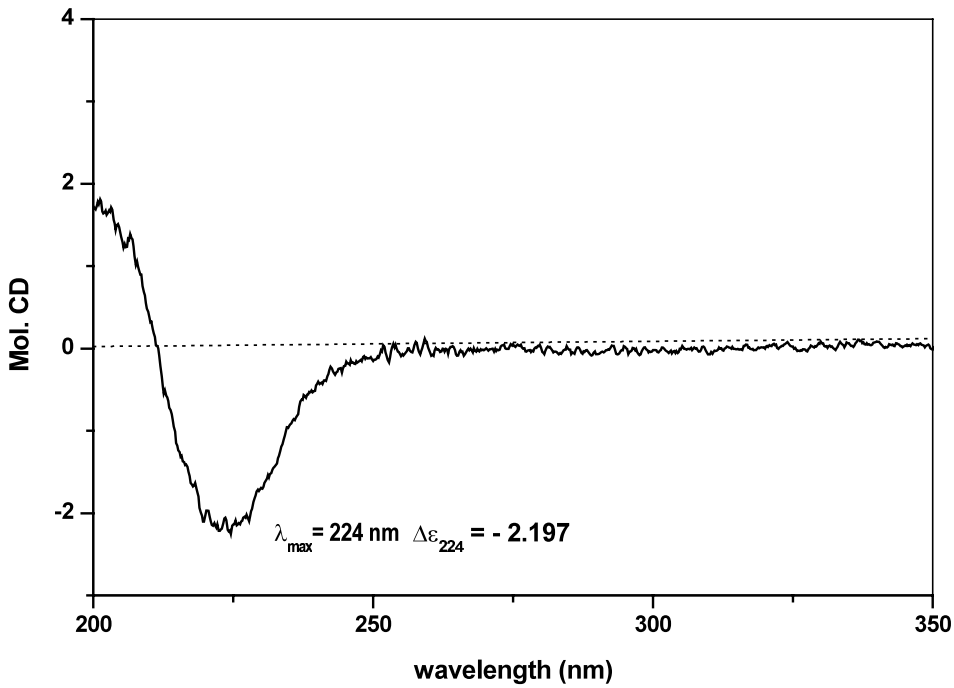

Figure 9. Circular dichroism (CD) spectrum of (*R*)-(−)-ketoprofen. Concentration: 0.20 g/100 mL in methanol. Temperature: 20°C.

4.3. Preparation of the chiral stationary phases

The preparation of the teicoplanin containing chiral stationary phase (TE-CSP) used in the present work was realized according to a synthetic procedure already described,[22] starting from LiChrospher Si 100 silica gel. Elemental analysis for the final TE-CSP gave: C, 16.0; H, 2.70; N, 4.10%, corresponding to ∼120 μmol of teicoplanin per gram of silica (0.30 μmol/m^2), based on the carbon analysis.

The preparation of the A-40,926 containing chiral stationary phase (A-40,926-CSP) used in the present work was completed according to a synthetic procedure already described,[23] starting from LiChrospher Si 100 silica gel. Elemental analysis for the final A-40,926-CSP gave: C, 15.0; H, 2.40; N, 3.70%, corresponding to ∼128 μmol of A-40,926 per gram of silica (0.32 μmol/m^2), based on the carbon analysis.

Stainless steel analytical (250×4.5 mm ID) and semi-preparative (250×10 mm ID) columns were packed with TE-CSP and A-40,926-CSP using a slurry packing procedure.[22]

Acknowledgements

This work was supported by grants from MIUR ('Ministero dell'Istruzione, dell'Università e della Ricerca', Italy) and from CNR ('Consiglio Nazionale delle Ricerche', Project Legge 95/95, Linea 3A, Italy). The authors are greatly indebted to the Lepetit Research Centre (Gerenzano, Italy) for the generous gift of teicoplanin and A-40,926 and to Professor Giovanni Gottarelli (Dip. Chimica Organica 'A. Mangini', Università di Bologna, Italy) for providing circular dichroism (CD) spectra of the first eluting enantiomers isolated from compounds **1** and **2** and of (*R*)-(−)-ketoprofen.

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