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Separation of the four pairs of enantiomers of vincamine alkaloids by enantioselective high-performance liquid chromatography

Salvatore Caccamese*, Grazia Principato

Dipartimento di Scienze Chimiche, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy

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Abstract

The four enantiomeric pairs of vincamine group alkaloids were separated by HPLC using Chiralpak AD as chiral stationary phase (CSP) and various *n*-hexane–2-propanol and *n*-hexane–ethanol mobile phases. (+)-*cis*-Vincamine, which is used in pharmaceutical preparations, is eluted much faster than its optical isomer, with separation factors of 2.4 and 3.5, respectively in these mobile phases. Other CSPs gave negative results. A chiral recognition mechanism is proposed and circular dichroism spectra of the individual enantiomers are presented. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Vincamine alkaloids; Alkaloids

1. Introduction

Eburnamine-vincamine alkaloids comprise a large group of pharmacologically active bases [1]. Among these bases (+)-vincamine exhibits a valuable therapeutic activity in cerebral insufficiencies [2]. Vincamine was first isolated from periwinkle (*Vinca minor* L. *Apocynaceae*) [3] and several strategies were developed for the stereoselective synthesis of the *cis* and *trans* series at the C-3 and C-16 positions and related epimers at the C-14 position [4] (for a review), [5] as well as for the synthesis of racemic [6,7] and enantiopure forms [8,9] of vincamine itself. Due to the presence of three stereogenic centers, eight stereoisomers (four enantiomeric pairs) are in fact possible as shown in Fig. 1. Considering that great attention has to be given to enantiomeric purity during the development of chiral drugs [10,11] we wanted to develop a suitable chromatographic method that affords the determination of the enantiomeric purity of vincamine group alkaloids. In addition, we also observed the effect of local stereochemical differences in the various compounds on the recognition by a chiral stationary phase (CSP).

To obtain optically pure vincamines, treatments of racemic precursors of vincamine with (−)-dibenzoyltartaric acid and subsequent reactions are reported [12–14]. However, so far only two reports have dealt with the high-performance liquid chromatographic enantioseparation of vincamine alkaloids. The first one used a chiral ion exchanger in the normal phase; the separation factor α of the enantiomeric pairs never exceeded 1.10 [15]. The second report used α-cyclodextrin as stationary phase and phosphate buffer as mobile phase to separate only (±)-*cis*-vincamine obtaining a separation factor α of 1.19; experiments were done on a

*Corresponding author. Tel.: +39-095-738-5029; fax: +39-95-580-138.

E-mail address: scaccamese@dipchi.unict.it (S. Caccamese).

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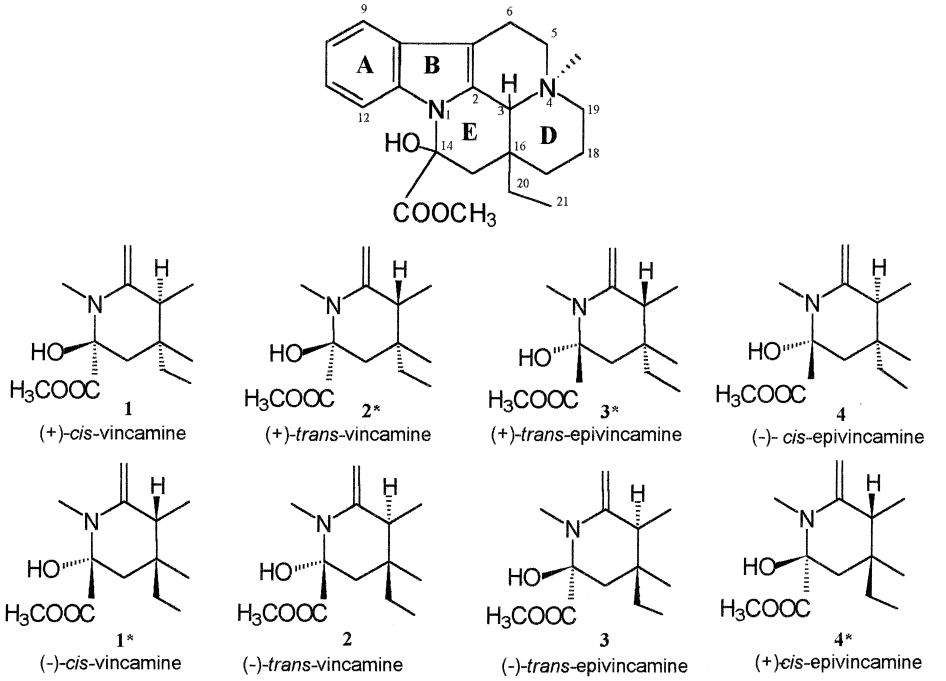

Fig. 1. Structure of the vincamine group, and of E ring of the enantiomers. The asterisk * denotes enantiomeric pairs (e.g., **1**/**1***).

large preparative scale with elution times of 8–12 h [16].

In this paper we report the direct separation of the four pairs of enantiomers by enantioselective high-performance liquid chromatography (HPLC) on a polysaccharide derived CSP (Chiralpak AD). Good α values are obtained for all enantiomeric pairs, as well as for the epimeric pairs. Resolution factors (R_s) are satisfactory for (±)-*cis*-vincamine, (±)-*cis*-epivincamine and (±)-*trans*-epivincamine. Moreover, a study of the energy minimized conformations of some stereoisomers affords a hypothesis on the chiral discrimination mechanism between the CSP and the analytes.

2. Experimental

2.1. Instrumentation

The HPLC system consisted of a Varian 5060 liquid chromatograph with Valco 10-μl sample loop, a Jasco Uvidec 100-III UV spectrophotometric detector operating at 280 nm and a Hewlett-Packard 3395 integrator. Circular dichroism (CD) spectra were recorded on a Jasco 600 spectropolarimeter. Molecular modeling conformations were obtained using MacroModel Version 4.5 with a Silicon Graphics Indigo XZ 4000 Workstation. The column (25 cm× 4.6 mm I.D.) used for the experiments reported in Table 1 was packed with Chiralpak AD (amylose tris-3,5-dimethylphenylcarbamate) coated on 10-μm silica gel from Daicel (Tokyo, Japan). Two columns (25 cm×4.6 mm I.D.) used for experiments not reported in Table 1 were packed with Chiralcel OD (cellulose tris-3,5-dimethylphenylcarbamate) coated on 10-μm silica gel from Daicel and with (3*S*,4*R*)-Whelk O-1 [4-(3,5-dinitrobenzamido)-tetrahydrophenanthrene] covalently bonded to 5 μm 3-propyl silica, from Regis (Morton Grove, IL, USA).

2.2. HPLC procedure

Sample solutions (0.2 mg/ml) were prepared by dissolving the individual enantiomers in 2-propanol. Volumes (5–10 μl) of these solutions or their mixtures were injected into the chromatographic column. Mobile phases were HPLC-grade *n*-hexane–

Table 1
HPLC resolution of the enantiomeric pairs of the vincamine groups on Chiralpak AD

No.	Compound[a]	A[b]	%[c]	t	k'[d]	α[e]	R_s
1	(+)-*cis*-Vincamine	2-PrOH	30	11.28	2.52	2.23	2.7
1*	(−)-*cis*-Vincamine	2-PrOH	30	21.21	5.63		
1	(+)-*cis*-Vincamine	2-PrOH	20	16.80	4.25	2.43	3.4
1*	(−)-*cis*-Vincamine	2-PrOH	20	36.30	10.34		
1	(+)-*cis*-Vincamine	EtOH	20	8.42	1.63	3.03	2.2
1*	(−)-*cis*-Vincamine	EtOH	20	18.98[f]	4.93		
1	(+)-*cis*-Vincamine	EtOH	10	13.25[f]	3.14	3.52	3.4
1*	(−)-*cis*-Vincamine	EtOH	10	38.55[f]	11.05		
2*	(+)-*trans*-Vincamine	2-PrOH	10	10.94	2.42	NS[g]	
2	(−)-*trans*-Vincamine	2-PrOH	10	10.94	2.42		
2*	(+)-*trans*-Vincamine	2-PrOH	8	10.84	2.39	1.17	<0.5
2	(−)-*trans*-Vincamine	2-PrOH	8	12.10	2.78		
2*	(+)-*trans*-Vincamine	2-PrOH	5	15.79	3.93	1.24	0.5
2	(−)-*trans*-Vincamine	2-PrOH	5	18.77	4.86		
2*	(+)-*trans*-Vincamine	2-PrOH	10[h]	13.23	1.76	1.12	<0.5
2	(−)-*trans*-Vincamine	2-PrOH	10[h]	14.32	1.98		
2*	(+)-*trans*-Vincamine	EtOH	10	9.17	1.87	1.68	0.6
2	(−)-*trans*-Vincamine	EtOH	10	13.24	3.14		
3*	(+)-*trans*-Epivincamine	2-PrOH	20	8.21	1.56	1.38	1.1
3	(−)-*trans*-Epivincamine	2-PrOH	20	10.12	2.16		
3*	(+)-*trans*-Epivincamine	2-PrOH	8	10.57	2.30	1.59	1.4
3	(−)-*trans*-Epivincamine	2-PrOH	8	14.95	3.67		
3*	(+)-*trans*-Epivincamine	2-PrOH	5	13.02	3.07	1.66	1.5
3	(−)-*trans*-Epivincamine	2-PrOH	5	19.52	5.10		
3*	(+)-*trans*-Epivincamine	2-PrOH	2	26.42[i]	7.26	1.81	1.7
3	(−)-*trans*-Epivincamine	2-PrOH	2	45.19[i]	13.12		
3*	(+)-*trans*-Epivincamine	EtOH	10	6.60	1.06	2.11	1.2
3	(−)-*trans*-Epivincamine	EtOH	10	10.37	2.24		
4	(−)-*cis*-Epivincamine	2-PrOH	30	4.52	0.41	3.31	1.6
4*	(+)-*cis*-Epivincamine	2-PrOH	30	7.57	1.37		
4	(−)-*cis*-Epivincamine	2-PrOH	20	5.67	0.77	3.34	2.5
4*	(+)-*cis*-Epivincamine	2-PrOH	20	11.48	2.58		
4	(−)-*cis*-Epivincamine	2-PrOH	10	8.54[j]	1.67	3.64	2.6
4*	(+)-*cis*-Epivincamine	2-PrOH	10	22.65[j]	2.65		
4	(−)-*cis*-Epivincamine	EtOH	10	6.87[j]	1.15	2.86	2.0
4*	(+)-*cis*-Epivincamine	EtOH	10	13.69	3.28		

[a] See the formulas in Fig. 1.
[b] Polar component in *n*-hexane. Flow-rate of 1 ml/min (t_0=3.2 min), unless specified otherwise.
[c] Percentage of A in the mobile phase.
[d] Capacity factor.
[e] Separation factor=k'_2/k'_1.
[f] Broad peak with large tail.
[g] Not separated, shoulder in the rising edge of the peak.
[h] Flow-rate of 0.7 ml/min, t_0=4.8 min.
[i] Broad peak with large tail, unstable baseline.
[j] Impurity in the descending edge of the peak.

2-propanol or *n*-hexane–ethanol mixtures. The column void time (t_0) was measured by injection of 1,3,5-tri-*tert*.-butylbenzene as a non retained sample [17]. Retention times (t_R) were mean values of two replicate determinations. All separation were carried out at room temperature.

2.3. Chemicals

The pure enantiomers [(**1**–**4**) and (**1***–**4***)] of the vincamine group alkaloids used in this study were a kind gift from Gedeon Richter (Budapest, Hungary) and they were synthesized in their laboratory some years ago [5].

3. Results and discussion

The chromatographic results for the eight stereoisomers (formulas shown in Fig. 1) are presented in Table 1. Considering the data obtained using 2-propanol as the polar constituent of the mobile phase, enantioselectivity (α) ranges from 3.64 for the pair **4**/**4*** to 1.24 for the pair **2**/**2*** in the optimal mobile phase composition. This parameter is in fact important to obtain good separation factors α. Indeed, using a flow-rate of 1 ml/min, a decrease in the polarity of the mobile phase from 30 to 20% and 30 to 10% of 2-propanol in hexane for the two enantiomeric pairs **1**/**1*** and **4**/**4***, respectively, markedly increases the separation factor as well as the resolution (R_s). The decrease in the percentage of 2-propanol in hexane is crucial for a successful separation of the pair **2**/**2***, which remains unresolved using *n*-hexane–2-propanol (9:1) and is instead resolved using a (95:5) mixture of the same solvents, although the R_s value remains low. A decrease in the flow-rate of the same mobile phase (from 1.0 to 0.7 ml/min, *n*-hexane–2-propanol, 9:1) also results in the separation of **2**/**2***.

The enantioresolution of the pair **3**/**3*** is also affected by the polarity of the mobile phase, although less markedly, the separation factor α ranging from 1.38 to 1.66 in *n*-hexane–2-propanol (8:2 to 95:5), respectively. Moreover, the resolution (R_s) also improves from 1.1 to 1.5 for the same variation of propanol concentration. In Table 1 the enantioseparation of **3**/**3*** using a very low polarity mixture (2% of 2-propanol in hexane) is reported. Although a slight increase in the α and R_s values is obtained, the enantiomeric peaks exhibit too high elution times and retention factor k' resulting in broad and tailing shapes caused by a drop in the efficiency of the chromatographic system. It is noteworthy that the polarity of the mobile phase useful to obtain good separation factor and reasonable elution times for both enantiomers is higher for **1**/**1*** and **4**/**4*** than for the remaining enantiomeric pairs, indicating a stronger interaction with the CSP; and this interaction results also in a better enantiomeric discrimination, as shown by the comparison of the α values of these pairs with respect to the other pairs. Indeed, the **1**/**1*** and **4**/**4*** are both *cis* stereoisomers at the C-3 and C-16 positions, and remarkably the CSP is much more effective in their enantiodiscrimination with respect to the *trans* stereoisomers. This behavior can be explained by a recognition mechanism proposed in a following paragraph. Fig. 2 shows typical chromatograms obtained for all the pairs in *n*-hexane–2-propanol mobile phase.

Table 1 also reports chromatographic results obtained using ethanol as the polar constituent of the mobile phase. The separation factors for the pairs **1**/**1***, **2**/**2*** and **3**/**3*** are better than those obtained in *n*-hexane–2-propanol. In detail, the enantioseparation of the **2**/**2*** pair is much better (α=1.68), although resolution still remains insufficient. A reverse behavior is obtained instead for the **4**/**4*** pair. As expected by the lower viscosity of the mobile phase, the speed of the separation is also improved and small impurity peaks are observed in the chromatogram of the **4**/**4*** pair. Fig. 3 shows typical chromatograms obtained for all the pairs in *n*-hexane–ethanol mobile phase. However a more marked tailing of the peaks with respect to the experiments in *n*-hexane–2-propanol can be noted, although the analysis time is strongly reduced.

The need for different concentrations of 2-propanol in *n*-hexane for optimal separation of different enantiomeric pairs precludes of course the isocratic separation of a mixture of all enantiomeric and diastereomeric pairs. However (+)-*cis*-vincamine (**1**) and (+)-*trans*-vincamine (**2***), starting and final products, respectively in an epimerization reaction at the D/E ring junction [13], are easily separated together with their corresponding enantiomers **1*** and

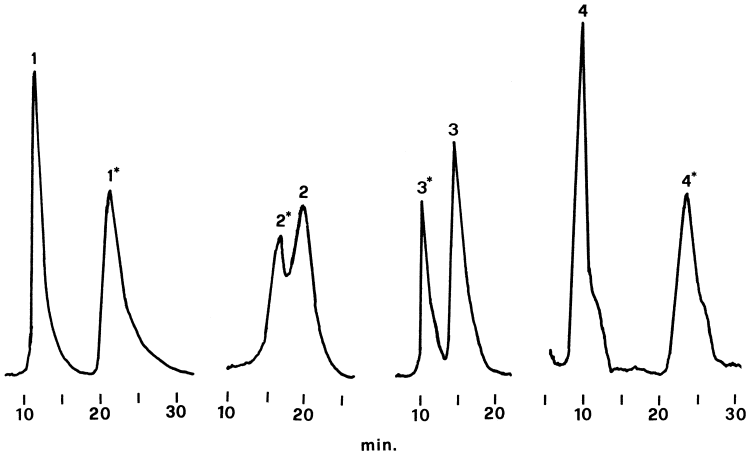

Fig. 2. Typical HPLC separation of the four enantiomeric pairs. Conditions: stationary phase Chiralpak AD; mobile phase *n*-hexane–2-propanol at 1 ml/min: (**1**/**1***) 7:3, (**2**/**2***) 95:5, (**3**/**3***) 92:8, (**4**/**4***) 9:1.

2 using a gradient elution profile as shown in the left trace of Fig. 4. The separation of six of the eight stereoisomers is achieved using another gradient elution profile, as shown in the right trace of Fig. 4. The pair **2**/**2*** unluckily overlap with the peak of **3***, using this and other gradient profiles and thus was not added in the mixture. Analogously, (+)-*cis*-epivincamine (**4***) and (+)-*trans*-epivincamine (**3***) (C-3 epimers) are well separated in isocratic mode (10% 2-propanol in *n*-hexane) together with their corresponding enantiomers **4** and **3**, as shown in Table 1 and from unreported experiments performed on their mixtures.

Thus the versatility of the proposed method can be useful in testing the enantiomeric purity of (+)-*cis*-vincamine used in the medical practice, as well as the stereoisomeric purity of other compounds obtained by epimerization procedures.

At an early stage of the work, trials with all the enantiomeric pairs using a cellulose-based CSP (Chiralcel OD) did not yield enantiomeric resolution although several experimental conditions, such as various concentrations of 2-propanol or ethanol in *n*-hexane, were evaluated. It is well known that the difference in the size of the helical cavity of the cellulose and amylose-derived phases (Chiralcel OD

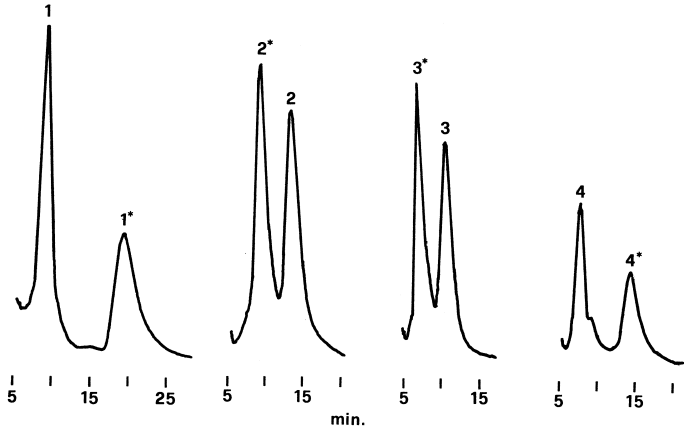

Fig. 3. Typical HPLC separation of the four enantiomeric pairs. Conditions: stationary phase Chiralpak AD; mobile phase *n*-hexane–ethanol at 1 ml/min: (**1**/**1***) 8:2, (**2**/**2***), (**3**/**3***) and (**4**/**4***) 9:1.

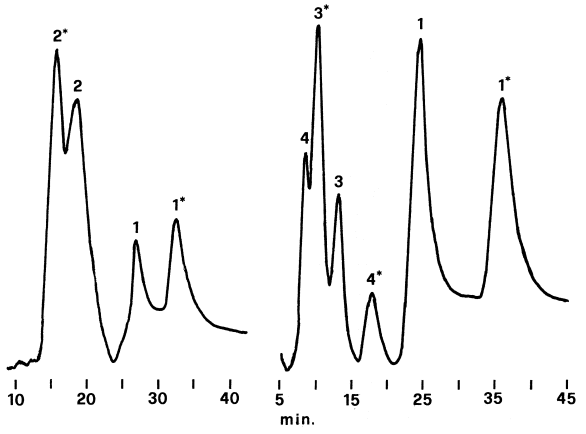

Fig. 4. Left trace: HPLC separation of C-3 epimers of vincamine and related enantiomers (**1**/**1***) and (**2**/**2***). Conditions: stationary phase Chiralpak AD; mobile phase *n*-hexane–2-propanol (95:5) steady for 18 min. Gradient to 8:2 from 18 to 20 min, steady until 40 min. Flow, 1 ml/min. Right trace: HPLC separation of a mixture of six stereoisomers of vincamine group (**1**/**1***), (**3**/**3***), (**4**/**4***). Conditions: stationary phase Chiralpak AD; mobile phase *n*-hexane–2-propanol (9:1) steady for 15 min. Gradient to 7:3 from 15 to 20 min, steady until 45 min. Flow, 1 ml/min.

and Chiralpak AD, respectively) [18,19] plays a significant role in the chiral recognition of many classes of compounds and this can explain the observed behavior for the vincamine alkaloids. Analogously, the Pirkle-type CSP Whelk O-1, which exhibits a general ability to resolve enantiomers and which acts through face-to-face and face-to-edge π–π interactions [20], was ineffective resulting in a unique broad peak eluting at long time. This was probably due to the large dimension of the selectand with respect to the chiral moiety of the selector, to the presence of three stereogenic centers in the selectand and to too many complementary selectand–selector interactions.

Besides the above analytical observations, which are consistent with the normal-phase behavior of the Chiralpak AD phase, some interesting observations are made about the influence of the stereochemistry of the analyte on the chiral recognition of the CSP. According to the general chiral recognition model proposed for amylose tris (3,5-dimethylphenylcarbamate) and other polysaccharide CSPs [18,19], the vincamine alkaloids interact with the CSP via a π–π interaction between the indole moiety and the phenyl group of the CSP and via hydrogen bondings of the lone pair of the N-4 and the OH group in ring E with the carbamate moiety of the CSP.

Fig. 5 shows the energy-minimized conformations for compounds possessing the *cis* or *trans* ring fusion at D/E, **1** and **3***, respectively. Compound **1** in its preferred conformation exhibits the lone pair of N-4 directed markedly toward the indole moiety and the D ring is curved toward the ''interior'' of the molecule. Instead, compound **3*** in its preferred conformation exhibits that nitrogen lone pair pointing perpendicular to the indole plane and the D ring is prolonging far away from the indole moiety. This fact causes, in our opinion, a different recognition process of these pairs from the CSP, resulting in a better α value for **1**/**1***. Moreover, due to the helical cavity of the CSP, considering the same orientation (towards the observer) of the indole moiety and of

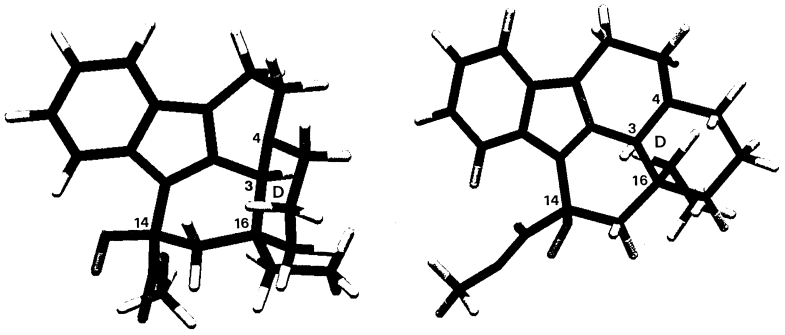

Fig. 5. Polytube computer models of the minimum energy conformations of stereoisomers **1** (left) and **3*** (right).

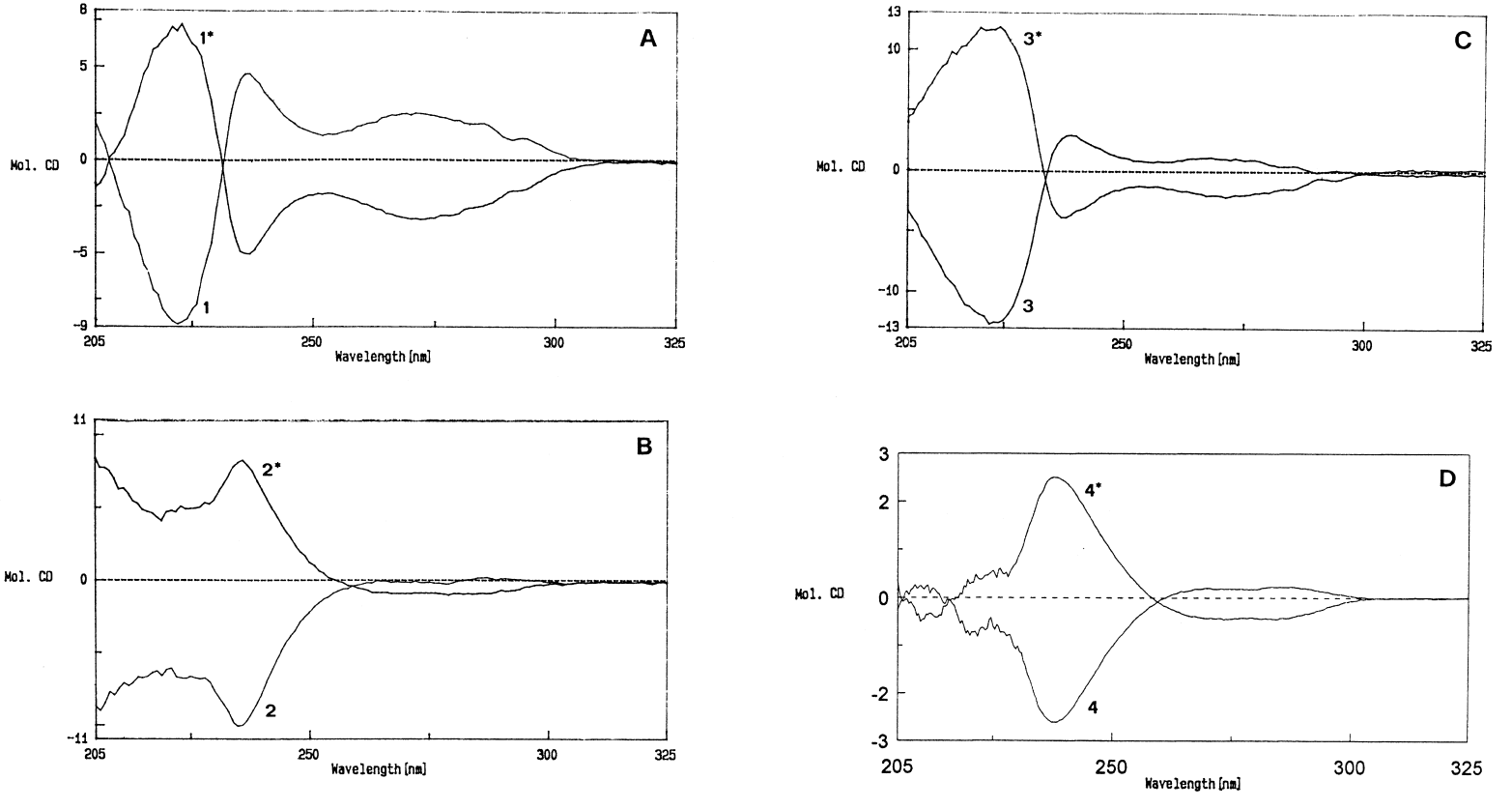

Fig. 6. CD spectra of the enantiomeric pairs of vincamine group, in ethanol at 22°C. A: (**1**/**1***), B: (**2**/**2***), C: (**3**/**3***), D: (**4**/**4***).

the ring C for compounds **1** and **3***, the intercalation of them in the groove of the CSP has to be the same and this can explain why **1** and **3*** are the first eluted enantiomers in the **1**/**1*** and **3**/**3*** pairs, respectively. The same observation perfectly applies to the other pair possessing the *cis* ring fusion at D/E, **4**/**4***. The enantiodiscrimination operated by the CSP is also very efficient for this pair ($\alpha > 3$) and the intercalation of the enantiomer in the groove of the CSP is the same as proposed for **1**/**1***, with **4** being the first eluted enantiomer in the **4**/**4*** pair.

The CD spectra of the eight stereoisomers are presented in Fig. 6. The spectra are grouped according to the enantiomeric nature of the compounds. Remarkably, compounds **2*** and **4*** both with 3*R*,14*S* absolute configurations exhibit diagnostic positive Cotton effect (CE) at 235 nm, while compounds **1*** and **3***, both with 3*R*,14*R* absolute configurations exhibit instead positive CE at 220 nm. In the literature [21] the CD spectra of these compounds are reported in other solvents and in several truncated scales of molecular CD ($\Delta\epsilon$) and do not cover the entire wavelength range (205–325 nm) in a single run. Thus, our data offer more understandable spectra with respect to the previous ones. The usefulness of these CD spectra is evident, as a sensitive identification method of the chromatographic peaks resulting from an epimerization process, or to confirm the enantiomeric nature of chromatographic peaks in a mixture.

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