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Structural features affecting chiral discrimination of terpene derivatives on a carbamated amylose stationary phase

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Abstract

The chiral discrimination of enantiomeric derivatives of α-pinene was studied using amylose tris(3,5-dimethylphenylcarbamate) as a chromatographic stationary phase. The effect of structural features of these enantiomeric pairs on their chromatographic resolution was systematically studied to understand further the correlation between these features and chiral discrimination by the carbamated amylose. Structural analysis by molecular mechanics indicated that the conformation of the α-pinene skeleton was preserved with substitution in all its derivatives. However, in spite of the rigidity of the molecular backbone of these molecules, their resolution capabilities were different. Apparently, the site of the hydrogen-bonding substituents affected chiral discrimination by the stationary phase rather than conformational changes. Separation was achieved in spite of the fact that none of the members in the series had an aromatic moiety, and therefore π–π interactions with the stationary phase were insignificant. Hence it was concluded that the most important interaction of the terpene derivatives with the carbamated amylose was hydrogen bonding.

1. Introduction

Terpenes are well known natural products in plant leaves, flowers and fruits [1]. One of the most widespread bicyclic monoterpenes is α-pinene, whose (+)- and (−)-enantiomers are both found in nature. It is used industrially as a solvent (turpentine) and as starting material in the manufacture of α-terpinol and camphor [1]. The alcoholic derivative, *cis*-verbenol is found in the oleo-resin of the East African tree *Boswellia carterii*, and its corresponding ketone, verbenone, is a major constituent of Spanish verbena oil [1]. The (−)- and (+)-verbenone (via verbenol) were the starting materials for the first synthesis of the (−)- and (+)-enantiomers of Δ^1-tetrahydrocannabinol (Δ^1-THC). The (−)-enantiomer is the major psychoactive constituent of *Cannabis sativa* and its preparations (marijuana, hashish, etc.) [2].

The enantiomeric purity of the terpenic derivatives dictates the degree of purity of the final product of the chiral synthesis of cannabinoids. Therefore, it is essential to ensure that optically pure terpenoids are used in the syntheses. An example is the synthetic (3*S*,4*S*)-1,1-dimethylheptyl-Δ^6-THC (HU-211), which is potentially an antiemetic agent [3] and acts as a

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functional N-methyl-D-aspartate (NMDA) receptor blocker [4] without producing psychotropic effects. It also has been found that HU-211 has cerebroprotective effects after head trauma in rats [5]. The corresponding (3*R*, 4*R*)-enantiomer (HU-210) is one of the most potent psychotropic cannabinoids known [6]. The total synthetic route of these two enantiomers, using commercial (+)- and (−)-α-pinene as a starting material, involves 4-oxomyrtenyl pivalate and its corresponding alcohol as intermediates [7] (see Fig. 1). The minimum requirement for optical purity of HU-211 (and its precursors) should be greater than 99.8 enantiomeric excess (e.e.), in order to prevent psychotropic effects.

Stereoselective analysis of enantiomeric drugs using liquid chromatography has been the focus of intensive research in recent years [8]. Separations of enantiomers and diastereomers of terpenes and their alcohols by liquid chromatography, using cyclodextrin inclusion complexes in the mobile or in the stationary phase, have been reported [9,10]. The inclusion capability of cyclodextrin is frequently compared with that of amylose, which can also form inclusion complexes, although more flexible ones [11]. Therefore, the resolution of enantiomeric terpenes has been attempted on an amylose-based stationary phase as well. A successful preliminary resolution of two derivatives of α-pinene was reported in a previous paper [12]. A comparative study of their chromatographic behaviour was made to shed more light on the mechanism of chiral discrimination in the amylose stationary phase. The understanding of chiral discrimination by the stationary phase is essential in order to use a more rational approach in the optimization of the separation.

Polysaccharides such as amylose consist of linked D-(+)-glucose units with α(1→4)-glycosidic linkages that form a helical structure. Derivatives of amylose, particularly carbamates, have exhibited a relatively high degree of chiral discrimination [13]. It has been proposed that the mechanism of chiral discrimination by amylose involves stereoselective inclusion of enantiomers based on hydrogen bonding and dipole–dipole interactions with the CO and NH portions, in addition to π–π interactions with the phenylcarbamate [13,14]. Most of the studies of chiral discrimination by the carbamated polysaccharides have involved solutes with aromatic fragments [15]. We demonstrate in this work that the efficient resolution of non-aromatic mole-

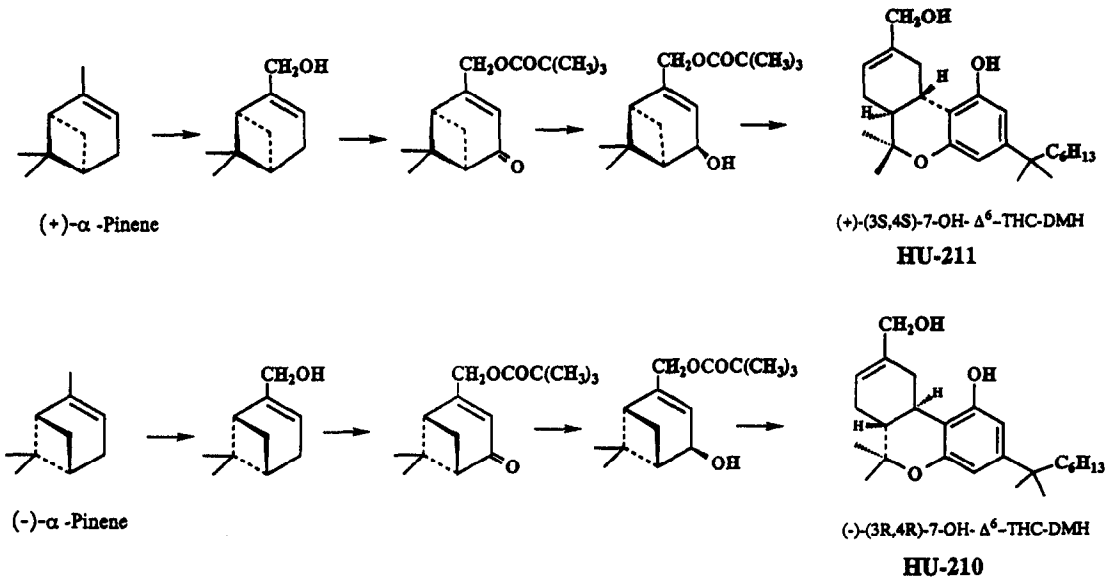


Fig. 1. Total step-synthesis of HU-210 and HU-211.

cules, such as terpenoids, can also be obtained using a carbamated polysaccharide stationary phase. Such a resolution indicates that in some instances the contribution of $\pi-\pi$ interactions to chiral discrimination might not be as significant as perceived generally. Another example of the resolution of aliphatic enantiomers by a substituted polysaccharide stationary phase, benzoylcellulose, was reported by Francotte and Wolf [16].

In addition to the chromatographic studies, conformations of the terpenic enantiomers were analysed by molecular mechanics to confirm that the rigid skeleton of α-pinene is preserved throughout the entire series.

2. Experimental

2.1. Instrumentation and materials

The HPLC analysis was performed using an HP1050 instrument (Hewlett-Packard, Palo Alto, CA, USA) equipped with a diode-array UV detector, an HPCHEM data station, and a ThinkJet printer. A Rheodyne (Cotati, CA, USA) injection valve was used, equipped with a 20-μl loop. A ChiralPak AD chiral column (250 mm × 4.6 mm I.D.; 10 μm film thickness (Daicel Chemical Industries, Tokyo, Japan) was used.

HPLC-grade solvents (ethanol or 2-propanol) were purchased from Lab-Scan (Dublin, Ireland) and α-pinene and myrtenol from Aldrich (Milwaukee, WI, USA). The other terpenic pairs were prepared as described previously [7].

2.2. Procedure of analysis

A flow-rate of 1 ml/min at room temperature was used in all the experiments. Each run was monitored at two wavelengths simultaneously, 254 and 240 nm. At the end of a session the column was washed with ethanol–hexane (20:80). When regeneration was needed, the column was washed with pure ethanol. When not in use, the column was stored in 2-propanol–hexane (5:95).

2.3. Computational method

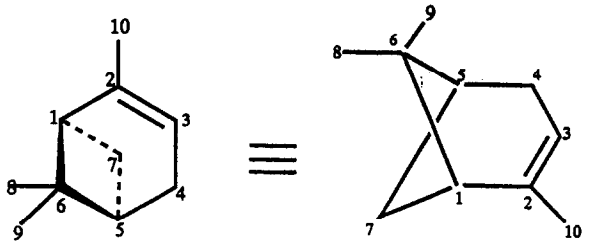
Construction and treatment of the terpene structures were performed with the InsightII/Discover 2.0.0 software package from BIOSYM Technologies (San Diego, CA, USA). All calculations were carried out on a Silicon Graphics 4D/310VGX workstation. The following molecular mechanics (MM) potential energy function was used:

$$E_{\text{Tot.}} = E_s + E_q + E_f + E_{\text{VWD}} + E_{\text{elec}} \quad (1)$$

where E_s is the stretching energy, E_q is the bending energy, E_f is the dihedral (torsion) energy, E_{VWD} is the Van der Waals energy and E_{elec} is the electrostatic energy. The force field used in the calculations was CVFF (consistent valence force field). All parameters defining the geometry of the molecule were modified by small increments until the overall structural energy reached a local minimum. First, 1000 iterations were made in the steepest descent algorithm, then it was transferred to the conjugate gradient minimizer until the convergence criterion had been achieved.

3. Results and discussion

Nine pairs of bicyclic monoterpenes that belong to the α-pinene series were studied. The structural skeleton of the α-pinene moiety according to the terpenic numbering system is shown in Fig. 2. The structures of the nine bicyclic monoterpenes are presented in Fig. 3. A



(+)-α -Pinene

Fig. 2. Structure of α-pinene according to the terpenic numbering system.

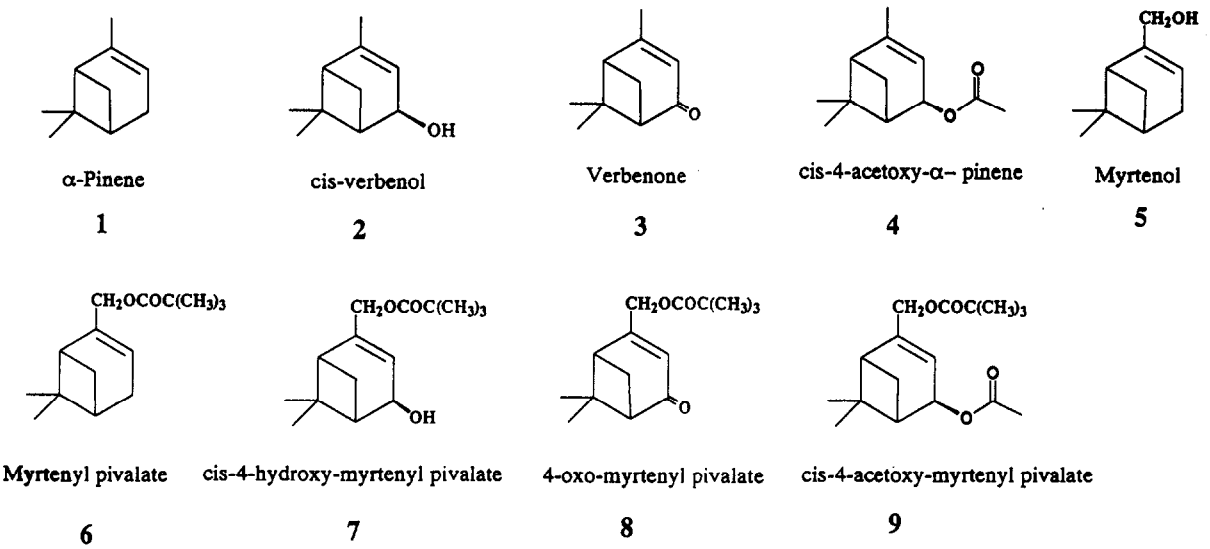


Fig. 3. Structures of the nine enantiomeric bicyclic monoterpenes used.

major question in this study was what structural features are essential to the chiral discrimination of these solutes by the amylose tris(3,5-dimethylphenylcarbamate). Conformational analysis by molecular mechanics was performed parallel to the chromatographic studies to answer this question.

3.1. Structural analysis

Systematic and comparative studies of the structural features of solutes that give rise to chiral discrimination can be greatly advanced by using molecular modelling of the enantiomers. The feature common to the monoterpenes in this study is the α-pinene skeleton, and therefore the first step was to simulate the most stable conformation of α-pinene. The structure obtained was used as a starting point for the construction of the other terpenes. All the energy-minimized structures of the substituted pinenes were superimposed on α-pinene, which was used as a reference structure, to examine whether there was a change in the conformation of the skeleton as a result of substitution. A quantitative criterion of the deviation between two different structures or their portions, when superimposed on each other, is given by the RMS values. These values are the least-squares fit between the two sets of *xyz* coordinates (Å units) of the two superimposed structures, and are calculated as follows:

$$\mathrm{RMS} = \sqrt{\sum_{i=1}^{N} \frac{(x-x_0)^2 + (y-y_0)^2 + (z-z_0)^2}{N}} \quad (2)$$

where N is the number of atoms compared. Each structure in the terpenoid series was superimposed on the α-pinene skeleton, and the RMS deviations of the common ten heavy atoms were calculated. The results are given in Table 1. The very small RMS values (≤ 0.023) that were obtained for all the terpenes indicated that the conformation of the α-pinene skeleton was preserved. Therefore, differences in the chromatographic behaviour of the terpenic pairs can be correlated with the type and position of their substituents rather than with conformational changes.

3.2. Chromatographic analysis

Chiral discrimination by amylose tris(3,5-dimethylphenylcarbamate) was studied from the chromatographic behaviour of the nine pairs,

Table 1
Values of the root mean square (RMS) of the differences in *xyz* coordinates of the ten heavy atoms common to all the structures compared with (+)-α-pinene

No.	Solute	RMS (Å)
1	(+)-α-Pinene	000
2	(+)-*cis*-Verbenol	0.021
3	(+)-Verbenone	0.017
4	(+)-*cis*-4-Acetoxy-α-pinene	0.010
5	(+)-Myrtenol	0.017
6	(+)-Myrtenyl pivalate	0.020
7	(+)-*cis*-4-Hydroxymyrtenyl pivalate	0.022
8	(+)-4-Oxomyrtenyl pivalate	0.023
9	(+)-*cis*-4-Acetoxymyrtenyl pivalate	0.021

using ethanol or 2-propanol as the mobile phase additives to hexane. The parameters studied were the retention factor, k', selectivity factor, α, resolution, R_s, and elution order. Tables 2 and 3 summarize these results using 1–10% of ethanol and 1–10% of 2-propanol in hexane, respectively. The results show the following general behaviour: the alcoholic and ketonic derivatives (pairs 2, 3, 7 and 8) could be easily separated, whereas compounds without these groups, α-pinene (pair 1), myrtenyl pivalate (pair 6) and *cis*-4-acetoxy-α-pinene (pair 4), were not separated at all. Myrtenol (pair 5) was partially separated and so was 4-acetoxymyrtenyl pivalate (pair 9), but the latter also exhibited an inversion of the elution order from +, − to −, +.

Structural features required for chiral discrimination

Role of hydroxyl and carbonyl groups. On the basis of previous studies on amylose-based stationary phases [12–14], it is reasonable to assume that hydrogen bonding and dipole–dipole interactions between the solutes and the carbamate moieties are responsible for the chiral fit of the separated terpenes. Therefore, it was not surprising that the presence of hydroxyl or ketone groups permitted the resolution of the enantiomeric pairs 2, 3 and 7, 8, whereas α-pinene (pair 1) was not separated at all. The alcoholic derivatives were better resolved than the ketonic derivatives, as can be seen in Figs. 4 and 5. The difference between the ketones and the alcohols can be attributed to inductive effects of the methyl groups on the phenyl carbamate. As a result, the hydrogen bonding between the hydroxylic solute and CO is probably stronger than that between the ketonic solute with NH of the carbamate in the stationary phase. Although the enantiomeric terpenes had no aromatic portions, separation took place, *i.e.*, the contribution of $\pi-\pi$ interactions in the chiral discrimination process was insignificant. This observation highlights the domination of hydrogen bonding in the mechanism of chiral recognition.

Substitution of position 4 vs. position 10. The presence of hydroxyl group did not necessarily ensure separation. It was the combination of hydrogen bonding at the appropriate position that allowed their chiral discrimination. A comparison between the derivative with a hydroxyl group in the 4-position, *cis*-verbenol (pair 2), and that with a hydroxyl group in the 10-position, myrtenol (pair 5), reveals that the two solutes behaved differently. Myrtenol was only partially separated, whereas *cis*-verbenol was easily separated in both solvents. This difference indicates that the hydroxyl group in position 4 had an anchoring function in the chiral fit into the stationary phase. Further, esterification of position 10 did not affect the resolution, as shown by myrtenyl pivalate (pair 6), which was not resolved. On the other hand, the combination of pivalate ester in the 10-position and hydroxyl in the 4-position (*cis*-4-hydroxymyrtenyl pivalate, pair 7) yielded a high selectivity factor, relative to the other enantiomeric pairs of terpenes. This enantiomeric pair was resolved under all conditions studied here, even with a relatively high percentage of the alcoholic modifiers (10%) in the mobile phase, as shown in Fig. 4.

Blocking the hydroxyl group by acetylation. The hydroxyl group in the 4-position of *cis*-verbenol (pair 2) and in *cis*-4-hydroxymyrtenyl pivalate (pair 7) was blocked with an acetyl ester group.

Table 2
Chromatographic parameters of the nine pairs of bicyclic monoterpenes in Fig. 3 using various ethanol concentrations (1–10%, v/v) in the mobile phase

Terpenic pair No.	1%				2%				4%				6%				8%				10%			
	k'_+ [a]	k'_- [a]	α [b]	R_s [c]	k'_+	k'_-	α	R_s	k'_+	k'_-	α	R_s	k'_+	k'_-	α	R_s	k'_+	k'_-	α	R_s	k'_+	k'_-	α	R_s
1	Eluted as void peak																							
2	2.26	2.55	1.12	1.19	1.65	1.84	1.12	1.09	0.88	0.99	1.13	0.95	0.78	0.85	1.09	0.94	0.63	0.68	1.08	0.75	0.53	0.57	1.07	0.66
3	2.82	3.01	1.07	0.88	2.03	2.17	1.07	0.91	1.16	1.23	1.06	0.52	1.02	1.06	1.04	0.43	0.87	0.89	1.02	0.22	0.75	0.77	1.02	0.26
4	0.42	0.42	1.00	0.00	0.30	0.31	1.00	0.00																
5	2.46	2.61	1.06	0.61	1.61	1.71	1.06	0.72	0.84	0.90	1.06	0.41												
6	Eluted as void peak																							
7	3.79	6.84	1.81	4.56	2.42	4.38	1.81	8.28	1.15	1.97	1.72	5.69	0.78	1.30	1.66	4.77	0.58	0.96	1.63	3.13	0.48	0.76	1.58	3.24
8	2.93	4.25	1.45	5.07	2.07	2.85	1.37	4.04	1.15	1.51	1.31	2.78	1.10	1.27	1.17	2.11	0.91	1.05	1.15	2.97	0.8	0.89	1.12	1.80
9	1.64	1.44	1.14	0.5	0.32	0.27	1.18	0.35																

[a] $k' = (t_R - t_0)/t_0$, where t_R is the retention time and t_0 is the void time.
[b] $\alpha = k'_-/k'_+$, whenever the (+)-isomer eluted first.
[c] $R_s = 2[t_{R(-)} - t_{R(+)}]/[w_{(-)} + w_{(+)}]$, where w is the peak width at the base.

Table 3
Chromatographic parameters of the nine pairs of bicyclic monoterpenes in Fig. 3 using various 2-propanol concentrations (1–10%, v/v) in the mobile phase

Terpenic pair No.	1%				2%				4%				6%				8%				10%			
	k'_+ [a]	k'_- [a]	α [b]	R_s [c]	k'_+	k'_-	α	R_s	k'_+	k'_-	α	R_s	k'_+	k'_-	α	R_s	k'_+	k'_-	α	R_s	k'_+	k'_-	α	R_s
1	Eluted as void peak																							
2	3.22	3.37	1.05	0.65	2.19	2.32	1.06	0.70	1.18	1.26	1.07	0.67												
3	3.43	3.86	1.13	1.59	2.07	2.22	1.07	0.80	1.23	1.36	1.05	0.48	0.84	0.87	1.03	0.21	0.73	0.75	1.03	0.17	0.64	0.66	1.03	0.16
4	0.99	0.97	1.02	0.08	0.63	0.62	1.00	0.03																
5					2.45	2.53	1.03	0.42	1.4	1.45	1.04	0.40	0.95	0.96	1.00	0.00								
6	Eluted as void peak																							
7	6.91	9.69	1.40	4.11	4.00	5.43	1.36	4.26	1.73	2.28	1.32	1.38	1.09	1.43	1.30	2.75	0.78	1.00	1.28	1.89	0.61	0.79	1.29	1.62
8	4.05	5.04	1.24	2.9	2.56	2.84	1.1	1.31	1.5	1.62	1.08	0.98	1.08	1.12	1.04	0.57	0.88	0.91	1.04	0.59	0.75	0.75	1.00	0.00
9	0.84	0.63	1.34	0.85	0.62	0.51	1.21	0.78																

[a–c] See Table 2.

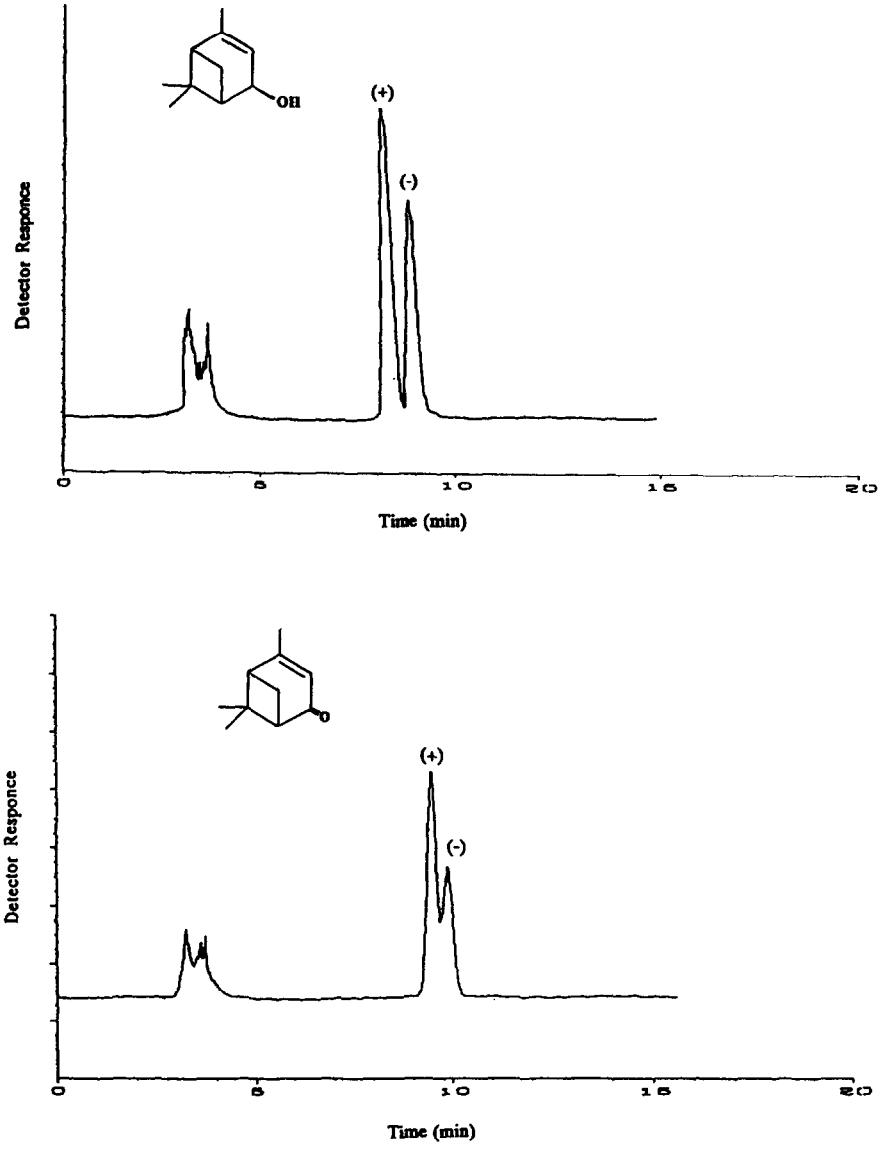


Fig. 4. Chromatograms showing the separation of the (−)- and (+)-enantiomers of *cis*-verbenol (pair 2) and verbenone (pair 3). Mobile phase, hexane–ethanol (98:2, v/v); wavelength of detection, 240 nm.

As a hydrogen-bonding group in the 4-position is essential for chiral discrimination, blocking this sensitive position with an acetoxy group was expected to diminish the resolution. Indeed, the separation of *cis*-4-acetoxy-α-pinene (pair 4) was lost. On the other hand, *cis*-4-acetoxymyrtenyl pivalate (pair 9) behaved unexpectedly. An unusual reversal of elution order of the (+)- and (−)-enantiomers was observed using 1 and 2% 2-propanol in the mobile phase with partial resolution. This change indicates a possible change in the recognition process by the station-

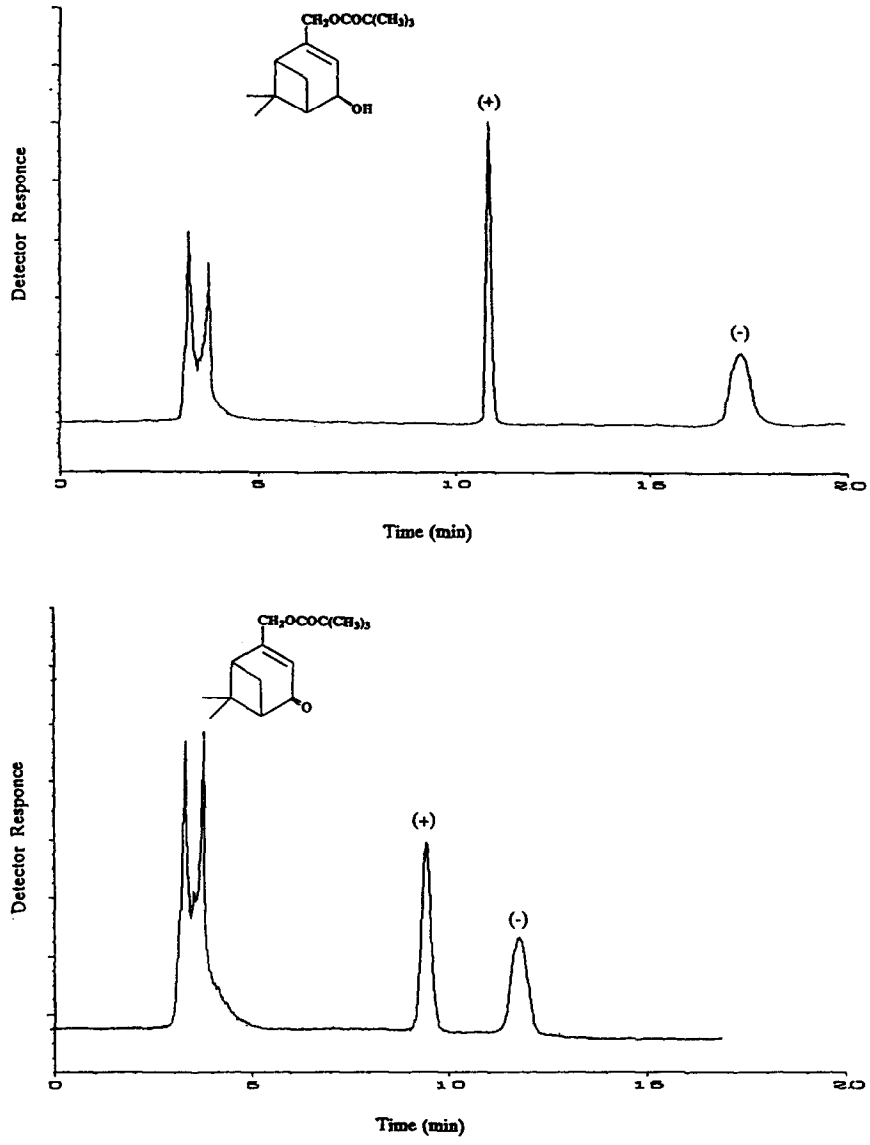


Fig. 5. Chromatograms showing the separation of the (−)- and (+)-enantiomers of *cis*-4-hydroxymyrtenyl pivalate (pair 7) and 4-oxomyrtenyl pivalate (pair 8). Conditions as in Fig. 4.

ary phase. A similar observation was reported by Wainer *et al.* [14] using an aryl-substituted alcohol in a cellulose tribenzoate.

Effects of solvent additives

Generally, normal-phase behaviour was observed for all the solutes in this study. Shorter retention times were obtained with ethanol as the modifier, and the retention decreased with the increase in the percentage of modifier in the mobile phase. The chromatographic parameters obtained using 1–10% of the two modifiers are given in Tables 2 and 3.

Solvent effects were observed in two instances. First, with 4-hydroxymyrtenyl pivalate (pair 7) and 4-oxomyrtenyl pivalate (pair 8), the selec-

tivity and resolution were better using ethanol as the mobile phase modifier. Second, 4-acetoxymyrtenyl pivalate (pair 9) was separated only in 2-propanol, displaying a reversal of elution order. It has already been reported in some instances that the two alcoholic modifiers were not always interchangeable [12,14]. It is also known that the degree of helicity of the amylose and its conformation may be solvent dependent [11]. In some instances, therefore, modification of the mobile phase composition may change the mechanism of chiral recognition and cause a reversal of elution order, especially when the hydroxyl functionality is lost.

4. Conclusions

This systematic comparative study of a series of α-pinene derivatives indicated that substitution of the 4-position on the α-pinene skeleton with hydrogen-bonding functional groups (OH and CO) facilitated chiral discrimination by the carbamated amylose. Molecular modelling of all the solutes under study indicated that substitution did not significantly change the most stable conformation of the α-pinene skeleton, hence the position of the functional groups rather than their effect on the overall conformation was more important. The role of hydrogen bonding as the major interaction was highlighted by the fact that the carbamated stationary phase was capable of resolving the enantiomers although they had no aromatic moiety.

5. Acknowledgements

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