Journal of Chromatography A, 654 (1993) 53–64
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 373

Resolution of chiral cannabinoids on amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase: effects of structural features and mobile phase additives

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(First received April 6th, 1993; revised manuscript received June 17th, 1993)

ABSTRACT

The separation of six pairs of chiral cannabinoids was achieved using a dimethylphenylcarbamate derivative of amylose, immobilized on silica gel (ChiralPak AD, Daicel), using 2-propanol and ethanol as the modifiers of *n*-hexane in the mobile phase. Good separation was achieved for most of the solutes in both solvent systems under various conditions. The chromatographic parameters of various cannabinoids in the two solvent systems were determined. The pairs differ from each other in small structural features such as the degree of saturation, position of a double bond and closure of a pyran ring. Therefore, a comparative study could give some clues regarding the mechanism of discrimination between the enantiomeric pairs on the chiral stationary phase. Preliminary measurements of limit of determination showed that it was possible to assess 99.9% enantiomeric purity of the cannabinoids, owing to the high efficiency of the separation. Enantiomers of two monoterpenes, used as intermediates or as starting materials in the chiral synthesis of cannabinoids, were also separated, hence the described procedure is capable of assessing whether the chiral centres in the molecules were sustained throughout the synthetic procedures.

INTRODUCTION

The increased interest in and importance of problems related to the stereoselectivity of drug action [1] have made the development of procedures of enantioselective analysis by chromatographic methods a focus of intensive research [2,3]. Chiral stationary phases, which serve as chiral discriminators during the chromatographic process, are of central importance. The stationary phase is prepared by derivatization and immobilization of chiral compounds on the surface of the support material, generally silica gel.

An effective group of chiral stationary phases are derivatized polysaccharides immobilized on silica [4–9]. Polysaccharides such as cellulose and amylose consist of linked D-glucose units, forming natural polymers with a highly ordered structure. Differential access to the helical backbone or to the glucose chiral cavities can affect discrimination between enantiomers. While resolution can sometimes be achieved using the natural cellulose as the stationary phase, the immobilized version with ester or phenylcarbamate derivatives has shown far better performance. Numerous compounds of pharmaceutical

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0021-9673/93/$06.00

importance have been separated using polysaccharide-based stationary phases [2–9].

Understanding the mechanism of discrimination of enantiomers in a chiral stationary phase can facilitate a rational approach to the optimization of their resolution. Studies on the separation of enantiomeric amides, using cellulose tribenzoate stationary phases [7–9], suggested that the formation of transient diastereomeric complexes between enantiomeric solutes and the chiral binding sites on the stationary phase is based on a combination of hydrogen bonding, $\pi-\pi$ and dipole interactions with the aromatic amide. Once the solutes have been bound by these interactions, chiral recognition is based on the fit of the asymmetric portion of the solute into a chiral cavity of the chiral discrimination site. This fit has rigid steric requirements.

Optimization of the resolution is based on the type and proportion of the mobile phase modifier. Solvent effects on the chromatographic parameters in several stationary phases have been systematically studied. Zief *et al.* [10] examined the effect of a series of alcoholic mobile phase modifiers on the chromatographic parameters of 2,2,2-trifluoro-1-(9-anthryl)ethanol using a Pirkle-type stationary phase. It was concluded that an increase in the bulk of alcohol increased the ability of the enantiomers to displace it from the stationary phase. Another study of solvent effects on the resolution of enantiomers in cellulose triacetate [11], using methanol, ethanol and 2-propanol as mobile phase modifiers, suggested that the polarity of the modifier may not be the key to its elution performance. If the elution involves access into a chiral cavity on the stationary phase, the effectiveness of the resolution may be determined by the steric size of the alcoholic modifier, rather than its polarity. A study of the effect of the steric bulk of an alcoholic mobile phase modifier on k' and α, using cellulose tribenzoate as the stationary phase, similarly indicated that the alcoholic modifier competes for both chiral and achiral binding sites on the stationary phase [12]. It was suggested that, as in cellulose triacetate described above, the mobile phase modifier may bind to sites near (or at) the chiral cavities of the stationary phase, changing their steric environment and, presumably, their stereoselectivity.

Numerous cannabinoids [13,14], including Δ^1-tetrahydrocannabinol (Δ^1-THC), the major psychoactive constituent of *Cannabis sativa* and its preparations (marijuana, hashish, etc.), have shown therapeutic activities [15] in addition to their psychotropic properties. This group of compounds has regained attention recently with the discovery of a cannabinoid receptor in the brain [16], its cloning [17] and the isolation from the brain of the endogenous substrate [18] that binds to the receptor. A new field of research can evolve from these findings and open the way for new therapeutic compounds.

The plant-derived cannabinoids are usually optically pure; Δ^1-THC has a 3*R*,4*R* stereochemistry. Synthetic procedures for the preparation of both enantiomers of most cannabinoids have been developed [13,19]. Most of the procedures are based on commercial chiral starting materials of various enantiomeric purity. Unless chiral purification has been done at some stage of the synthesis, the end products may not be of very high optical purity, owing to the variability of the commercial products.

The therapeutic properties of the cannabinoids have led numerous groups to investigate the possibility of the separation of their undesirable psychotropic effects from the desirable effects by chemical modification. A major advancement in this field was the establishment that some of the unnatural (3*S*,4*S*)-cannabinoid enantiomers are antiemetic [20] and exhibit functional NMDA antagonism [21] without producing THC-like psychotropic effects. The most thoroughly investigated compound in this series is the (3*S*,4*S*)-7-hydroxy Δ^6-THC-dimethylheptyl homologue (HU-211). Its 3*R*,4*R* enantiomer (HU-210) is one of the most potent psychotropically active cannabinoids [22]. Hence any future development of (3*S*,4*S*)-cannabinoids as therapeutic agents will depend to a large extent on the stereochemical efficiency of the enantiomeric synthesis. The products of such syntheses will have to be monitored by analytical procedures capable of separating enantiomeric mixtures, in which the undesirable enantiomer may be present in minute amounts. In the case of HU-211 the optical purity [enantiomeric excess (e.e.)] required, in order to prevent psychotropic effects, would be >99.8 e.e.

This paper describes the resolution of enantiomeric pairs of cannabinoids, using a commercial Daicel ChiralPak-AD column, which is based on amylose tris(3,5-dimethylphenylcarbamate) (ADMPC) supported on macroporous silica gel (Fig. 1). This chromatographic system fulfils the above requirements for enantiomeric excess of the cannabinoids. Understanding of the mechanism of chiral recognition by this polymeric chiral stationary phase is a complex task in the absence of the exact structural features of the immobilized polymeric backbone and the chiral sites. Nevertheless, some suggestions, made by Okamoto and co-workers [4–6] and Wainer and co-workers [7,12], may give preliminary guidance for the design of the appropriate chromatographic separation system. As a rule, chirality is a property of the molecule as a whole, hence all the possible chiral and achiral interactions between the solute and the chiral stationary phase should be accounted for. It has been proposed that the main chiral adsorbing sites are the carbamate polar functional groups, which interact with the solute via hydrogen bonding (through NH and CO groups) and dipole–dipole interactions on CO. The $\pi-\pi$ interactions of the dimethylphenyl groups, with the aromatic groups of the solute, are also important. The presence of the dimethyl groups on the phenylcarbamate moiety probably increases the electron density at the carbonyl oxygen group, which in turn intensifies its hydrogen bonding with hydroxyl groups in the solutes. The two methyl groups on the phenylcarbamate can also play a role in controlling the steric fit of the solute into the chiral cavity on the stationary phase. A comparative study is presented here, aimed at further understanding the mechanism of chiral discrimination in polysaccharide-based stationary phases.

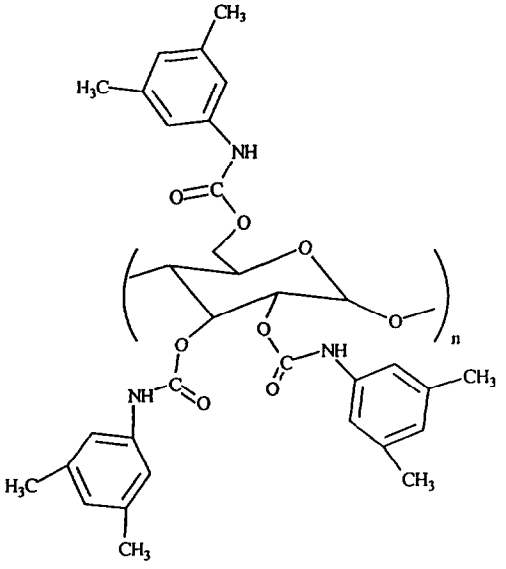

Fig. 1. An adsorption site in the chiral stationary phase used in this study.

EXPERIMENTAL

Instrumentation

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

Materials

HPLC-grade solvents were all purchased from Merck (Darmstadt, Germany). Six pairs of enantiomeric cannabinoids and two pairs of enantiomeric monoterpenes were subjected to the enantiomeric analysis.

Pair 1. (−)-(3*R*,4*R*)-Δ^1-THC, $[\alpha]_D$ −175° ($CHCl_3$), was isolated from hashish or prepared by partial synthesis from natural cannabidiol (CBD) as described previously [23]. (+)-(3*S*,4*S*)-Δ^1-THC, $[\alpha]_D$ +176° ($CHCl_3$), was prepared according to a published procedure [24]. In order to obtain a high enantiomeric excess, an intermediate in its synthesis, (+)-verbenol, was recrystallized to a constant m.p. 72°C and a constant rotation $[\alpha]_D$ +9.9° ($CHCl_3$).

Pair 2. (−)-(3*R*,4*R*)-Δ^6-THC, $[\alpha]_D$ −252° ($CHCl_3$), was prepared from CBD [23]. (+)-(3*S*,4*S*)-Δ^6-THC, $[\alpha]_D$ +252° ($CHCl_3$), was prepared according to a published procedure [24]. The intermediate (+)-verbenol was prepared as described for pair 1.

Pair 3. (−)-(3*S*,4*R*)-CBD, m.p. 66°C, $[\alpha]_D$ −79° ($CHCl_3$) and −129° (EtOH), was isolated

from hashish [23,25]. (+)-(3*R*,4*S*)-CBD, m.p. 65°C, $[\alpha]_D$ +79° ($CHCl_3$) and +128° (EtOH), was prepared according to a published procedure [26].

Pair 4. (−)-(3*R*,4*R*)-7-Hydroxy-Δ^6-THC, dimethylheptyl homologue (HU-210), m.p. 141–142°C, $[\alpha]_D$ −277° ($CHCl_3$), and (+)-(3*S*,4*S*)-7-hydroxy-Δ^6-THC, dimethylheptyl homologue (HU-211), m.p. 141–142°C, $[\alpha]_D$ +227° ($CHCl_3$), were prepared as described previously [19].

Pair 5. (−)-(1*R*,3*R*,4*R*)-7-Hydroxyhexahydrocannabinol, dimethylheptyl homologue, (HU-243), m.p. 80–82°C, $[\alpha]_D$ −92° ($CHCl_3$), and (+)-(1*S*,3*S*,4*S*)-7-hydroxyhexahydrocannabinol ($CHCl_3$), dimethylheptyl homologue (HU-251), m.p. 80–82°C, $[\alpha]_D$ +92° ($CHCl_3$), were prepared by reduction of HU-210 and HU-211, respectively, as derived previously [27].

Pair 6. The tetracyclic HU-249, m.p. 156–158°C, $[\alpha]_D$ +178° ($CHCl_3$), and HU-250, m.p. 156–158°C, $[\alpha]_D$ −178° ($CHCl_3$), were prepared as described previously [28].

Pair 7. (−)-4-Oxomyrtenyl pivalate, m.p. 42–43°C, $[\alpha]_D$ −165° ($CHCl_3$), and (+)-4-oxomyrtenyl pivalate, m.p. 42–43°C, $[\alpha]_D$ +165° ($CHCl_3$), were prepared as described previously [19].

Pair 8. (−)-*cis*-Verbenol and (+)-*cis*-verbenol were purchased from Aldrich (Milwaukee, WI, USA).

Procedure

The mobile phase consisted of various mixtures of *n*-hexane with ethanol or 2-propanol (1–20%, v/v). A flow-rate of 1 ml/min was used in all the experiments at room temperature. Each run was monitored at two wavelengths simultaneously; one of them was either 260 or 270 nm, depending on the cannabinoid, and the other was 220 nm. In each instance, *ca.* 0.1 mg of analyte was dissolved in 1 ml of the appropriate solvent (mixture of 2-propanol or ethanol with *n*-hexane, according to the composition of the mobile phase) and injected both individually and as a racemic mixture. The day-to-day reproducibility was high; the R.S.D. was <1% for capacity factors and <2% for selectivity factors.

RESULTS AND DISCUSSION

Six pairs of cannabinoids were studied, using various proportions of either ethanol or 2-propanol as alcoholic additives to *n*-hexane in the mobile phase. The six pairs are shown in Fig. 2. The group consisted of members that differed from each other in small structural features. The parameters studied were the retention factor, k', which combines the extent of selective and non-selective retention of the enantiomers, the selectivity factor, α, which expresses the degree of discrimination between the two enantiomers, the resolution, R_s, which indicates the efficiency of the separation, and elution order, which indicates the type of stereoselective fit into the binding site.

Optimization of the separation of the six enantiomeric pairs of cannabinoids was performed, using various compositions of *n*-hexane with a modifier, either ethanol or 2-propanol, in the mobile phase. Results for the k', α and R_s using 1–20% ethanol are summarized in Table I and using 2–20% 2-propanol in Table II.

The chromatographic system operated very well in terms of discrimination and efficiency of the enantiomeric resolution, judging from the α and R_s values in Tables I and II. Apart from the two enantiomers of Δ^6-THC, all the enantiomeric pairs could be easily separated using various percentages of 2-propanol or ethanol in the mobile phase. Values of $\alpha \geq 1.2$ were easily obtained at relatively high resolution values. The system operated in the normal-phase mode in terms of average retention of the enantiomeric pairs. Fig. 3 shows two chromatograms of the (+)- and (−)-enantiomers of Δ^6-THC, obtained using *n*-hexane–2-propanol (98:2, v/v) and *n*-hexane–ethanol (98:2, v/v). Shorter retention times were obtained when ethanol was the modifier. Also typical was the decrease in retention parameters with increase in the percentage of modifier in the mobile phase.

Detection limits and enantiomeric purity

The stereoselectivity of the pharmacological activity of chiral medicinal compounds cannot be established quantitatively unless a sensitive method for the determination of enantiomeric

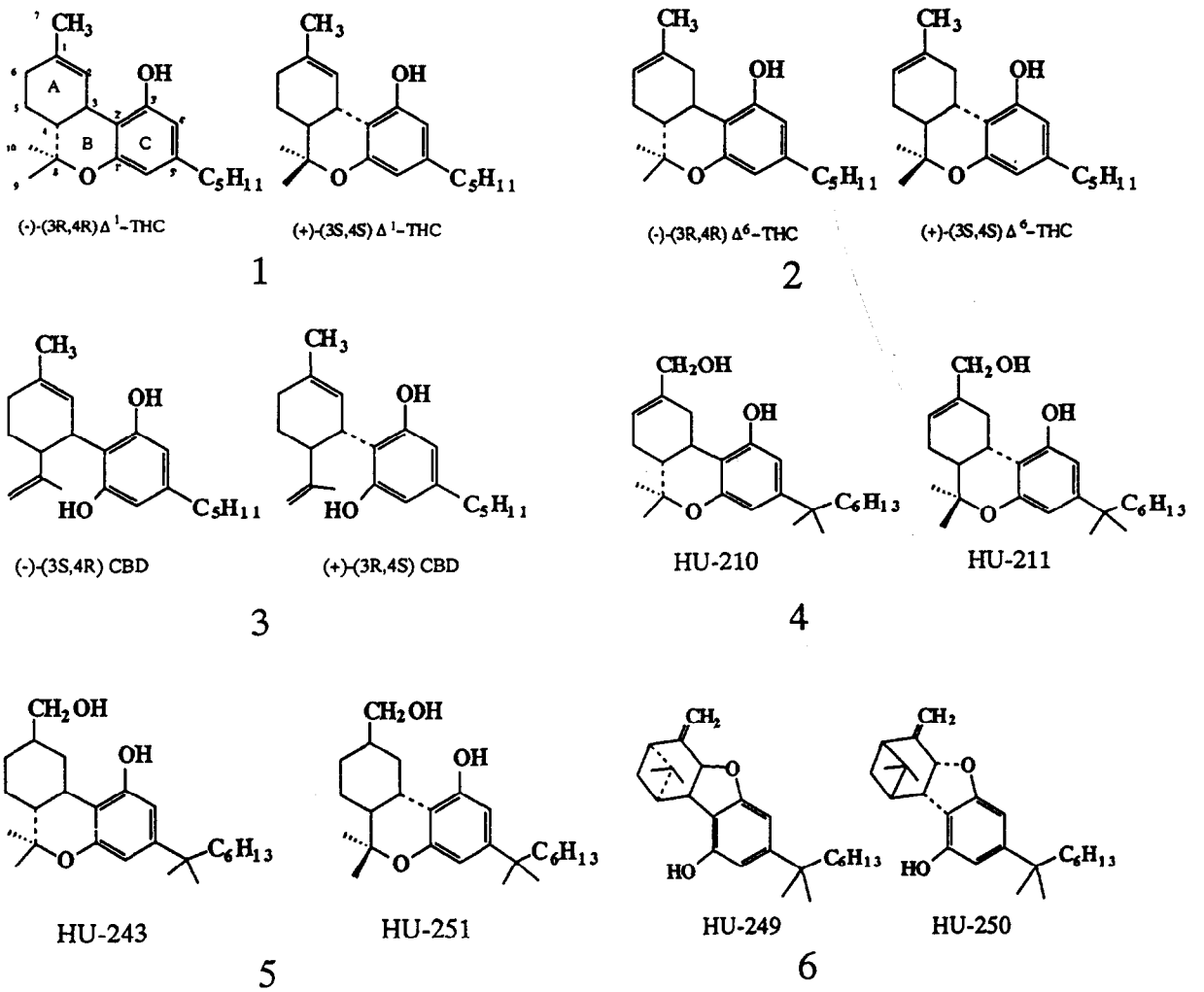

Fig. 2. Structures of the six pairs of cannabinoids studied.

purity is available. Research aimed at the development of therapeutic derivatives of cannabinoids devoid of psychotropic side-effects should include the determination of optical purity. The quantitative criterion of the minimum degree of optical purity of the therapeutic enantiomer is dictated by the pharmacological potency of the contamination. The higher the psychotropic activity of the enantiomer, the stricter is the requirement for optical purity. An extreme example is the enantiomeric pair HU-211 and HU-210, in which the very high undesirable psychotropic effects of HU-210 require that HU-211 should be at least 99.8% optically pure.

A full quantitative study of the limits of detection and determination that constrain the maximum optical purity that can be measured with the ChiralPak column for cannabinoids is currently under study. Preliminary studies involved calibration graphs for the (+)- and (−)-enantiomers of 4-oxomyrtenyl pivalate, the precursors of HU-210 and HU-211. Typical correlation coefficients were above 0.999 and the limits of determination were *ca.* $1 \cdot 10^{-5}$ mol/l (*ca.* 60

TABLE I

CHROMATOGRAPHIC PARAMETERS OF THE SIX PAIRS OF CANNABINOIDS IN FIG. 2 USING VARIOUS ETHANOL CONCENTRATIONS (1–20%, v/v) IN THE MOBILE PHASE

Pair No.	1%				2%				5%				10%				15%				20%			
	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					3.21	5.93	1.85	9.58	1.13	2.14	1.89	5.08	0.56	1.19	2.10	3.18	0.38	0.86	2.26	2.41				
2	4.01	3.79	1.06	0.85	3.15	2.96	1.06	0.57	1.04	1.04	1.00	0												
3					6.12	8.59	1.40	14.7	1.99	2.84	1.42	2.00	0.83	1.15	1.38	0.42	0.51	0.67	1.30	0.15	0.40	0.49	1.23	0.09
4					4.50	6.85	1.52	44.0	1.07	1.61	1.50	1.05	0.39	0.59	1.51	0.27	0.27	0.42	1.57	0.13	0.17	0.29	1.63	0.11
5					4.52	8.78	1.94	32.6	1.08	1.95	1.79	1.92	0.54	0.80	1.48	0.27	0.26	0.50	1.89	0.19	0.20	0.32	1.63	0.16
6					2.00	7.95	3.96	44.0	0.70	2.68	3.80	6.30	0.38	1.39	3.60	1.75	0.22	0.91	4.00	1.01	0.16	0.71	4.29	0.71

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

TABLE II

CHROMATOGRAPHIC PARAMETERS OF THE SIX PAIRS OF CANNABINOIDS IN FIG. 2 USING VARIOUS 2-PROPANOL CONCENTRATIONS (2–20%, v/v) IN THE MOBILE PHASE

Pair No.	2%				5%				10%				15%				20%			
	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	7.32	11.3	15.4	18.4	2.34	6.73	2.87	14.5	1.06	2.78	2.62	7.92	0.65	1.70	2.60	4.80	0.47	1.24	2.63	3.01
2	6.19	7.79	1.26	9.07	2.01	2.62	1.30	1.40	0.93	1.21	1.30	0.38	0.61	0.78	1.29	0.38	0.38	0.50	1.31	0.11
3	12.9	13.3	1.08	7.77	3.35	3.64	1.09	0.83	1.36	1.48	1.09	0.18	0.76	0.82	1.08	0.07				
4	11.6	21.5	1.84	11.3	3.34	5.14	1.54	7.08	1.07	1.56	1.46	0.86	0.54	0.77	1.42	0.26	0.32	0.45	1.43	0.13
5	9.63	13.5	1.40	9.64	5.29	6.60	1.25	7.64	1.87	2.03	1.08	0.35	0.94	0.95	1.01	0.08				
6	4.93	25.4	5.15	226	1.37	6.45	4.72	19.7	0.62	2.73	4.42	3.8	0.40	1.70	4.22	1.74	0.30	1.23	4.0	1.02

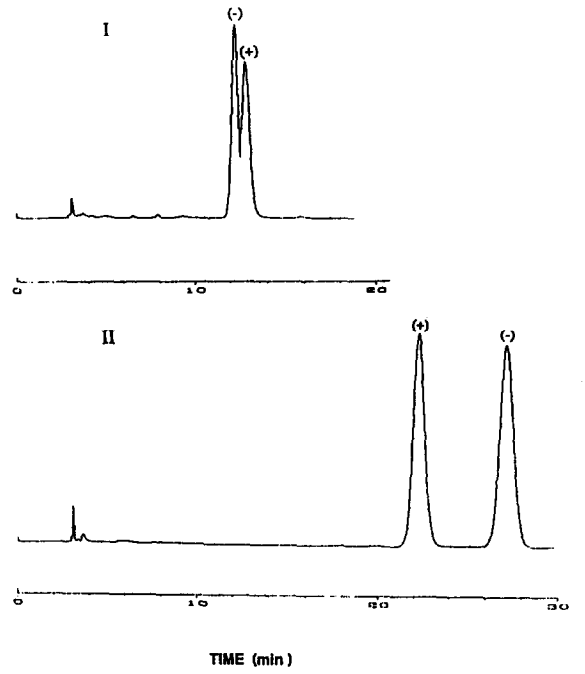

Fig. 3. Chromatograms showing the separation of (+)- and (−)-Δ^6-THC in ethanol and 2-propanol as mobile phase modifiers. Mobile phase: (I) *n*-hexane–ethanol (98:2, v/v); (II) *n*-hexane–2-propanol (98:2, v/v).

ng). Identical calibration graphs were obtained for the individual and the mixed enantiomers owing to the high selectivity and resolution values obtainable in this system. With such limits of determination an enantiomeric purity of 99.9% could be easily assessed. Preliminary studies of Δ^6-THC and Δ^1-THC have shown similar limits of determination. Enantiomeric purities ≥99.9% of all the enantiomers used in the present study could be easily determined.

Sustaining the chiral centre during synthesis

Optically pure cannabinoids can be prepared from chiral starting materials if the chirality is sustained throughout the entire synthesis. It is essential to begin the synthesis with optically pure compounds, and a sensitive enantioselective analysis is required for every stage of the synthesis. For example, (+)-Δ^6-THC can be prepared from (+)-verbenol whereas HU-211 and HU-210 can be prepared from the corresponding (+)- or (−) 4-oxomyrtenyl pivalate. The starting material should be optically pure, otherwise it may yield products of poor enantiomeric purity. Therefore, enantioselective analysis is currently being developed for the complete set of starting materials, intermediates and final products in the synthesis of cannabinoids. Two examples are presented here, the separation of enantiomers of *cis*-verbenol and 4-oxomyrtenyl pivalate in Figs. 4 and 5, respectively. Good separation and sensitivity were observed in both instances.

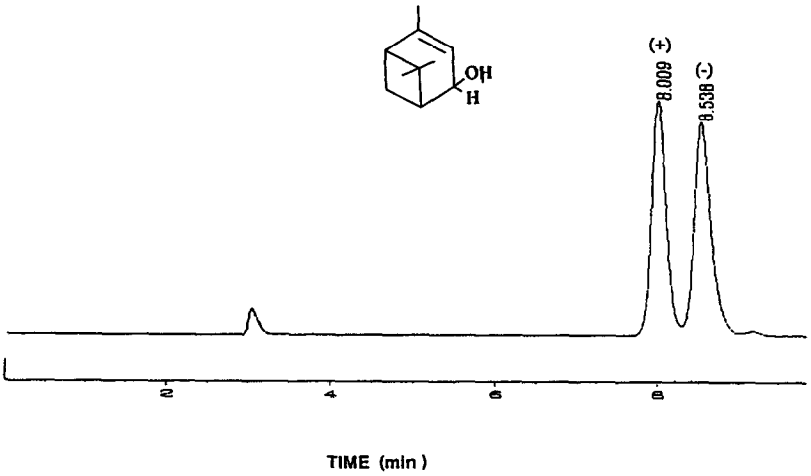

Fig. 4. Chromatogram showing the separation of (+)- and (−)-*cis*-verbenol. Mobile phase, *n*-hexane–ethanol (98:2, v/v); wavelength of detection, 210 nm.

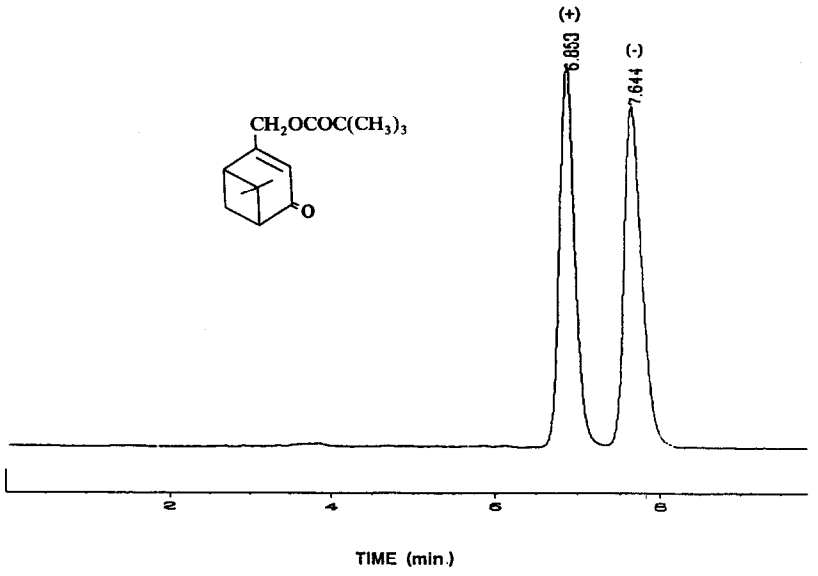

Fig. 5. Chromatogram showing the separation of (+)- and (−)-4-oxomyrtenyl pivalate. Mobile phase, *n*-hexane–2-propanol (95:5, v/v); wavelength of detection, 220 nm.

Selective solvent effects and structural features

The influence of the type and composition of the modifier in the mobile phase on solute retention, elution order, selectivity and resolution was studied with reference to previous observations on other types of polysaccharide-based stationary phases. Values of α and R_s obtained using 2% and 5% of the two modifiers (see Tables I and II) are presented in Figs. 6 and 7. This type of illustration serves as a quick reference to the chromatographic parameters during examination of the structural effects.

The availability of several enantiomeric pairs of cannabinoid compounds made possible preliminary comparative studies of chiral recognition by the chiral sites of the dimethylphenylcarbamate derivatives of amylose. The features, common to all these solutes, are the aromatic moiety and at least one hydroxyl group near the chiral centres. The pairs, shown in Fig. 2, vary from each other in small structural features: position of a double bond on ring A (pairs 1 and 2); open and closed ring B (pairs 1 and 3); and non-saturated and saturated ring A (pairs 4 and 5). These aspects are considered below.

Position of the double bond: Δ^1-THC vs. Δ^6-

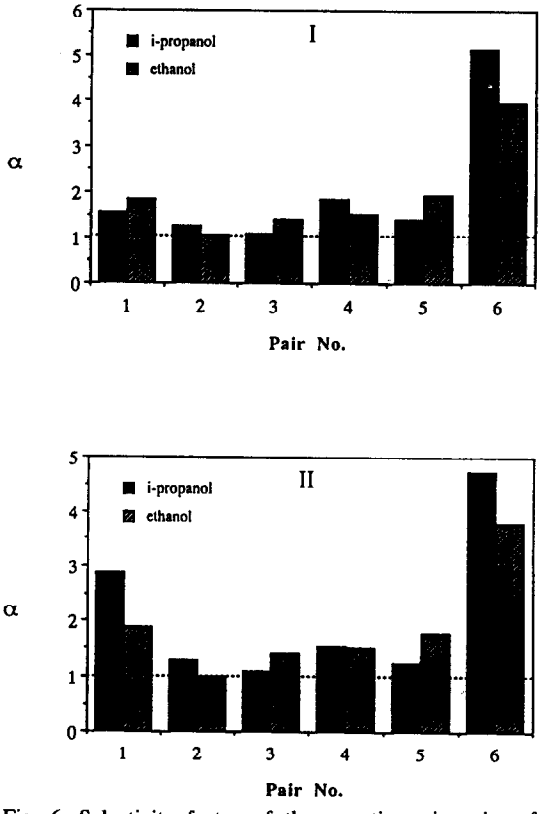

Fig. 6. Selectivity factor of the enantiomeric pairs of the cannabinoids using (I) 2% and (II) 5% (v/v) 2-propanol and ethanol in the mobile phase.

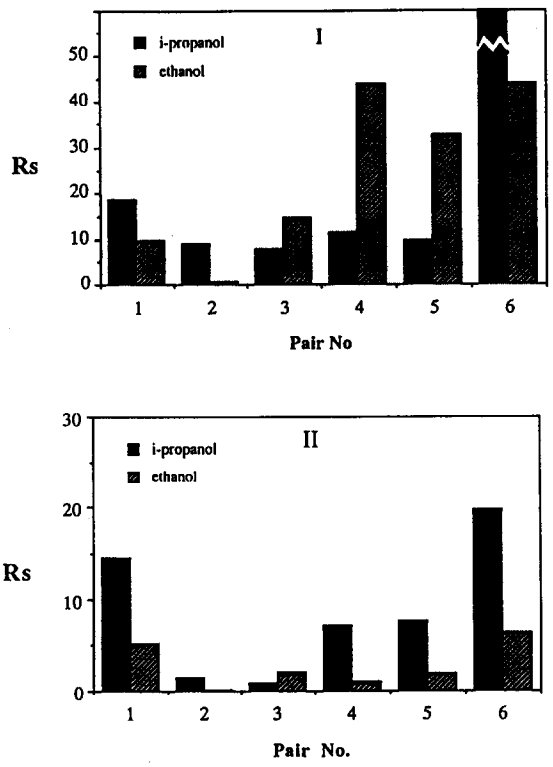

Fig. 7. Resolution between the enantiomeric pairs of the cannabinoids using (I) 2% and (II) 5% (v/v) 2-propanol and ethanol in the mobile phase.

THC. Pair 1 are the natural active cannabinoid (−)-(3*R*,4*R*)-Δ^1-THC and the synthetic (+)-(3*S*,4*S*)-Δ^1-THC enantiomer; pair 2 are the natural (−)-(3*R*,4*R*)-Δ^6-THC and the synthetic (+)-(3*S*,4*S*)-Δ^6-THC enantiomer. The structural difference between the two pairs is the position of the double bond on ring A. The different position of the double bond affected the chromatographic behaviour of the two pairs of enantiomers considerably, the following effects being observed.

The two enantiomers of Δ^1-THC were generally retained longer than the two Δ^6-THC enantiomers, using either modifier, in spite of the presumably subtle difference in polarity between them (see Tables I and II). Apparently, the position of the double bond in ring A dictates the differences in capacity factors of the two enantiomeric pairs of THC.

The selectivity factors and resolution values were generally better for the enantiomers of Δ^1-THC in both solvent systems. Therefore, better selectivity and efficiency, combined with the higher capacity factor of the Δ^1-THC enantiomers compared with Δ^6-THC, indicate a better steric fit with the chiral adsorption sites on the stationary phase.

According to the α and R_s values in Figs. 6 and 7, 2-propanol was the preferred solvent for both the Δ^6-THC and Δ^1-THC pairs. Moreover, the Δ^6-THC enantiomers could not be separated at all using ethanol at concentrations above 2% in the mobile phase. Examination of the efficiency of the resolution revealed that although selectivity factors observed for the (+)- and (−)-Δ^1-THC enantiomers using 2-propanol were comparable to those using ethanol, the resolution was significantly better with 2-propanol. The different efficiency of the separation between ethanol and 2-propanol suggests that the kinetics of the chromatographic process (efficiency of the distribution) in the chiral column are also affected by the solvent.

The elution order was (+)- and then (−)-enantiomers for all the pairs except (+)- and (−)-Δ^6-THC. An unusual reversal of the elution order of (+)- and (−)-Δ^6-THC was observed when the solvent was changed from 2-propanol to ethanol, as shown in Fig. 3. Although the average k' behaved as expected for an achiral normal-phase mode of retention, the elution order of the two enantiomers was changed. The k' values of the two (+)- and (−)-Δ^6-THC enantiomers decreased as the percentage of modifier in the mobile phase increased, keeping the reversed elution order, the (+)-enantiomer being first to elute when 2-propanol was used whereas the (−)-enantiomer was first to elute when ethanol was used.

In contrast to Δ^6-THC, the closely related Δ^1-THC enantiomers showed regular normal-phase retention behaviour with an elution order similar to those for the other enantiomeric pairs in this study.

The reversal of the elution order of the (+)- and (−)-enantiomers of Δ^6-THC indicates that it is sensitive to the steric environment at the chiral binding site. Wainer and co-workers [7,12] suggested that the mobile phase modifier, which is

constantly present at the binding site, plays a role in the chiral discrimination at that site. Either it may alter the steric environment or it has to be displaced from the binding site for a better fit of the solute to the chiral site. The displacement of the modifier molecules from the chiral site can be non-selective, and is common to all the other enantiomers. However, the reversal of the elution order of the (+)- and (−)-enantiomers of Δ^6-THC indicates that there was also an alteration of the shape of the chiral site by ethanol. This suggestion seems to explain the considerable difference between the elution properties of the two alcohol modifiers in the carbamate amylose stationary phase.

In conclusion, the two pairs behaved very differently in the given chromatographic system in spite of the subtle conformational differences between them. Preliminary molecular mechanics calculations on the two isomers (−)-Δ^6-THC and (−)-Δ^1-THC were carried out using the Insight II/Discover 2.0.0 software package of BIOSYM Technologies (San Diego, CA, USA). Superimposition of the two structures gave rise to a very small root mean square difference in the x, y, z coordinates of the heavy atoms, indicating that they have very similar structures. Therefore, the unequal chiral discrimination of the two THC enantiomeric pairs in the chiral binding site obviously cannot be explained by the structural differences between them alone. The position of the double bond in terms of intramolecular distances rather than total conformational changes seems to have a considerable effect on the enantioselective fit of these analytes to the chiral sites on the stationary phase. This assumption is currently being studied, using molecular modelling techniques, to provide new insights into the mechanism of chiral recognition in the amylose-based stationary phase and to explain the differences in the chromatographic behaviour.

Open and closed ring B. Δ^1-THC has a completely different conformation from that of CBD, hence the differences in their chromatographic behaviour are understandable. Δ^1-THC has three rings, A, B and C, with one free phenolic group; CBD has no ring B, and two phenolic groups, with the two A and C rings being almost perpendicular to each other.

The capacity factors observed for the two pairs (see Tables I and II) showed that CBD is retained longer than Δ^1-THC over the entire range of percentages of either ethanol or 2-propanol modifier in the mobile phase. This is a typical behaviour in the normal-phase mode of retention, where an additional hydroxyl group enhances the interaction with the stationary phase. In spite of the longer retention times of CBD, the selectivity factor and resolution were both better for the two enantiomers of Δ^1-THC using both ethanol and 2-propanol. Apparently, the opening of ring B reduced the extent of discrimination between the two CBD enantiomers by the chiral stationary phase.

The selectivity factors and resolution between the two pairs showed that the preferable solvent for the CBD enantiomers was ethanol, in contrast to Δ^1-THC (and Δ^6-THC). Both the selectivity and efficiency of the separation between the CBD enantiomers were better using ethanol. Apparently, the modification of the chiral site by ethanol in the mobile phase (indicated in the previous section) improved the steric fit of CBD enantiomers into the chiral cavity, in contrast to the two THC pairs.

Saturated and non-saturated ring A. Pair 4 (7-OH-DMH-Δ^6-THC) and pair 5 (7-OH-DMH-HHC) have a hydroxyl group on atom 7 attached to ring A. The difference between them is the degree of saturation of ring A. Compounds with unsaturated rings are expected to be retained longer than homologous compounds with saturated rings in the achiral normal-phase retention mode. According to the retention data in Tables I and II, the two enantiomers of HHC (saturated ring A) were retained longer in all instances but one, *viz.*, with *n*-hexane–2-propanol (98:2, v/v). Under these conditions selectivity between the two 7-OH-DMH-Δ^6-THC enantiomers (non saturated ring A) sharply increased, indicating domination of selective interactions.

Examination of the chromatographic parameters of the two pairs in the two solvent systems (Tables I and II, Figs. 6 and 7) reveals that selectivity factors in both solvent systems were comparable, with a slight better discrimination between (+)- and (−)-7-OH-DMH-Δ^6-THC enantiomers. Also, an increase in the percentage of

modifier affected both solutes similarly in all their chromatographic parameters.

Fig. 7 highlights the observation that the two alcoholic modifiers were interchangeable at low percentages for both enantiomeric pairs. 2-Propanol was the preferred modifier at *n*-hexane–modifier (95:5, v/v), whereas ethanol was preferred at *n*-hexane–modifier (98:2, v/v).

It was surprising that the position of the double bond of ring A contributed more to the chromatographic resolution than the saturation of the same ring. It is interesting also that the chromatographic parameters of all three pairs 1, 4 and 5 (Δ^1-THC, 7-OH-DMH-Δ^6-THC and 7-OH-DMH-HHC) were similar in terms of selectivity, efficiency and elution order. Apparently, neither the addition of a bulky alkyl group on ring C nor the hydroxyl attached to atom 7 had a dramatic effect on the capability of the stationary phase to discriminate between the enantiomers.

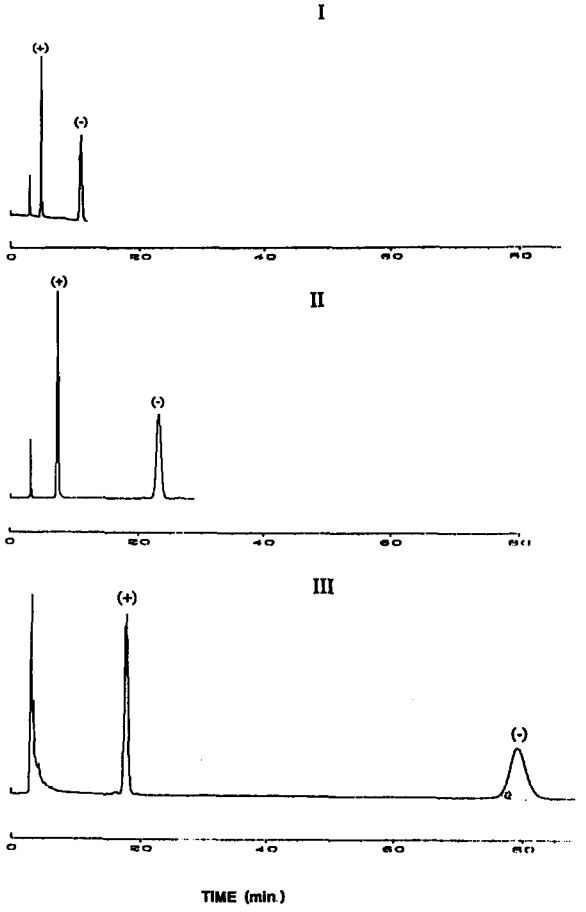

Fig. 8. Chromatograms showing the separation of the tetracyclic HU-249 and HU-250 using the following mixtures of *n*-hexane–2-propanol in the mobile phase: (I) 90:10; (II) 95:5; (III) 98:2 (v/v). Wavelength of detection, 260 nm.

Change of both ring A and ring B. Pair 6, the tetracyclic HU-249 and HU-250, are different from the other cannabinoids in rings A and B; ring C is the same as in pairs 4 and 5. The carbamate derivative of amylose showed an extraordinary capability to discriminate between this two enantiomers under all conditions, even at relatively high percentages of the alcoholic modifiers in the mobile phase, as shown in Fig. 8, where three chromatograms of the two enantiomers, using 2%, 5% and 10% 2-propanol in the mobile phase, are presented. This unusually high degree of discrimination and efficiency of the separation, relative to the other enantiomeric pairs of the cannabinoids studied, supports the suggestion that the conformations of rings A and B, next to the chiral centres, play a key role in the steric fit with the chiral adsorption site.

CONCLUSIONS

The resolution of six enantiomeric pairs of cannabinoids and two pairs of monoterpenes was achieved using an amylose tris(3,5-dimethylphenylcarbamate) stationary phase. The chromatographic system described is capable of assessment of enantiomeric excesses of the cannabinoids ≥99.9%. A comparative study of the various pairs indicated that the conformations of rings A and B next to the chiral centres in the cannabinoids are features of major importance in the chiral discrimination by the stationary phase. 2-Propanol and ethanol were not interchangeable in the separation of some of the enantiomeric pairs studied, and their polarity was not the determining factor in their elution properties. These findings supported indications from previous studies on polysaccharide stationary phases that the solvent modifiers participate in the process of chiral discrimination. Molecular modelling of all the solutes that were studied and their steric fit into the chiral sites on the stationary phase is currently being explored in order to understand better the mechanism of chiral discrimination in the present chromatographic system.

ACKNOWLEDGEMENTS

Financial support of this work by the Israel Ministry of Health is gratefully acknowledged. We thank Noa Sobe for her technical help.

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