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Note

Direct liquid chromatographic separation of enantiomeric and diastereomeric terpenic alcohols as β-cyclodextrin inclusion complexes

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Sobrerol[1], a fluidificant mucoregulatory drug widely used for the treatment of respiratory diseases as the *trans* racemate[2,3] and a new more potent mucofluidifying terpenic alcohol, CO/1408[4], exhibit two asymmetric centres on the cyclohexene ring. Therefore, each compound produces four isomers: two enantiomeric pairs derived from the two *cis* and *trans* diastereoisomers. Their absolute configurations are shown in Figs. 1 and 2.

The ability of cyclodextrins (CDs) to form inclusion complexes with many molecules and ions has been known for a long time. However, their utilization, especially for analytical purposes, started only a few years ago when CDs became available in larger amounts and at lower cost. The highly selective inclusion properties of CDs have been applied successfully in high-performance liquid chromatography (HPLC) in two different approaches, CDs being used either as a specific modifier of the mobile phase[5–8] or as a chemically bonded stationary phase[9–12].

This paper describes the separation of the enantiomeric and diastereomeric pairs of sobrerol and CO/1408 by reversed-phase (RP) using β-CDs in the above two approachers.

EXPERIMENTAL

A Varian 2010 liquid chromatograph, equipped with a Model 2050 variable-wavelength UV detector, a Rheodyne 7125 sample injector with a 10-μl loop and a Varian Model 9176 recorder, was used at a detection wavelength of 205 nm. Experiments were carried out with prepacked LiChrosorb RP-18 (10 μm) and LiChrospher RP-18 (5 μm) columns (250 × 4.0 mm) (Merck, Darmstadt, F.R.G.) and with a Cyclobond I column (250 × 4.6 mm I.D.) packed with 5-μm silica gel with chemically bonded β-CD (ASTEC, Whippony, NJ, U.S.A.).

Chemicals used for buffer preparation were of analytical-reagent grade and

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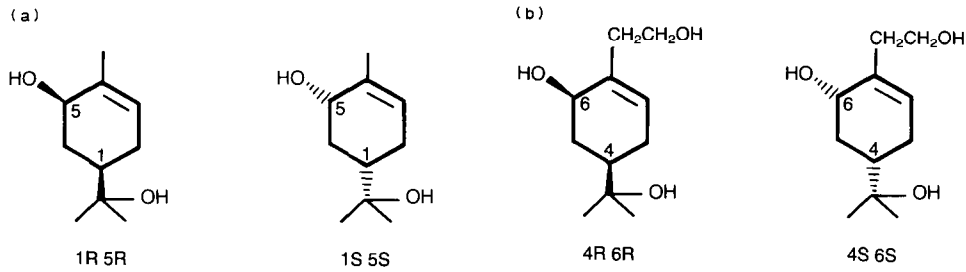

Fig. 1. (a) Enantiomeric pair of *cis*-sobrerol: (1*R*,5*R*)- and (1*S*,5*S*)-5-hydroxy-α,α,4-trimethyl-3-cyclohexene-1-methanol. (b) Enantiomeric pair of *cis*-CO/11408: (4*R*,6*R*)- and (4*S*,6*S*)-6-hydroxy-4(1-hydroxy-1-methylethyl)-1-cyclohexene-1-ethanol.

obtained from Merck. The eluents were prepared with Merck HPLC-grade solvents and were degassed prior to use. β-Cyclodextrin was of analytical-reagent grade and supplied by Fluka (Buchs, Switzerland). Racemates and enantiomers of *cis*- and *trans*-sobrerol and CO/1408 were synthesized in our laboratories; their purity was checked by polarimetry and differential scanning calorimetry.

RESULTS AND DISCUSSION

HPLC allowed the partial separation of the diastereomeric *cis* and *trans* terpenic alcohol pairs using the conventional Rp mode phase but failed in the resolution of their enantiomers. The addition of β-CD to the mobile phase decreased the retention times of the diastereoisomers and improved their separation (see Table I and Fig. 3).

The use of α-CD instead of β-CD did not improve the two diastereomeric separations compared with those obtained with the conventional RP analysis, as shown in Table I. The results suggest that the α-CD cavity is too small to include these terpenic compounds whereas the cavity of β-CD is appropriate for the formation of inclusion complexes with these alcohols.

Another aim of this work was to determine whether β-CD might be a selective mobile phase modifier for the resolution of the two terpenic alcohol racemates by HPLC. From the structures of the two terpenic compounds, able to form inclusion complexes with the β-CD cavity (probably via the cyclohexene ring), enantiomeric separation could be expected according to the three-point interaction model originally proposed by Dalgliesh[13] and later used by Hinze *et al.*[14] to explain enantiomeric

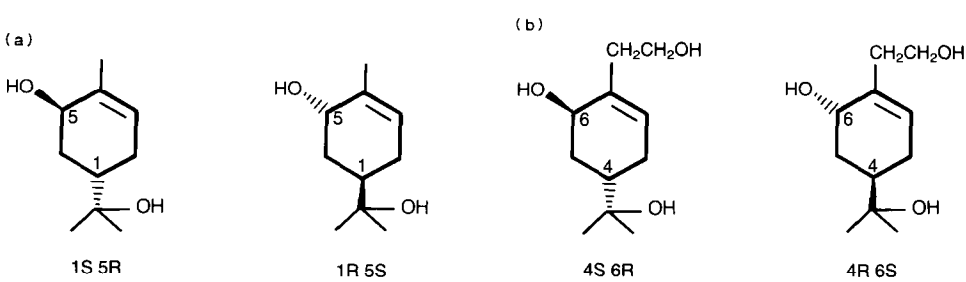

Fig. 2. (a) Enantiomeric pair of *trans*-sobrerol. (b) Enantiomeric pair of *trans*-CO/1408.

TABLE I

DIASTEREOMERIC SEPARATION OF *cis/trans*-TERPENIC ALCOHOLS USING DIFFERENT CD MOBILE PHASES

Mobile phase: potassium phosphate buffer solution (0.025 *M*; pH 7.25) with ethanol as organic modifier (20% for sobrerol and 10% for CO/1408). A flow-rate of 1 ml/min was used for the 250 × 4.0 mm I.D. (10 μm) LiChrosorb RP-18 column and the mobile phase was saturated with different CDs. Parameters: k' = capacity factor; α = separation factor; R_s = resolution

Diastereomeric pair	k'^a	α	R	*Mobile phase*
cis/trans-Sobrerol	6.00	1.07	0.89	Without CD
	6.00	1.07	0.89	α-CD
	3.44	1.36	2.44	β-CD
cis/trans-CO/1408	6.00	1.11	0.86	Without CD
	6.00	1.11	0.86	α-CD
	2.33	1.81	3.40	β-CD

[a] Value for the first-eluting enantiomer (*cis*-sobrerol and *cis*-CO/1408).

separations with cyclodextrins. The possibility of this chiral recognition and subsequent chromatographic optical resolution are probably due, after inclusion with β-CD, to steric interactions with the outer rim of β-CD. These interactions may involve hydrogen bonding between the terpenic alcohols and β-CD hydroxyl groups and potential steric interactions between alkyl substituents of terpenic compounds and the outer rim of β-CD.

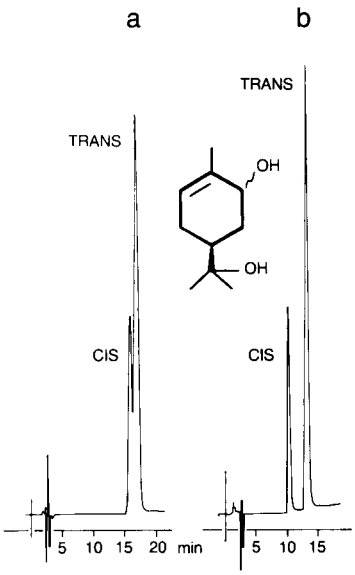

Fig. 3. Separation of *cis*- and *trans*-sobrerol on a LiChrosorb RP-18 (10 μm) column (250 × 4.0 mm I.D.). Mobile phase: 20% ethanol–potassium phosphate buffer solution (0.025 *M*; pH 7.25), (a) without β-CD and (b) saturated with β-CD. Flow-rate: 1 ml/min.

TABLE II

ENANTIOMERIC SEPARATION OF TERPENIC ALCOHOLS USING A β-CD MOBILE PHASE

Mobile phase: potassium phosphate buffer solution (0.025 *M*; pH 7.25) with ethanol as organic modifier. A flow-rate of 0.3 ml/min was used for the 250 × 4.0 mm I.D. (5 μm) LiChrospher 100 RP-18 column and the mobile phase was saturated with β-CD.

Enantiomeric pair	k'^a	α	R_s	*Mobile phase*[b]
(±)-*trans*-sobrerol	24.4	1.08	1.45	7.5 : 92.5
(±)-*cis*-sobrerol	Not resolved			
(±)-*trans*-CO/1408	20.6	1.08	1.46	0.8 : 99.2
(±)-*cis*-CO/1408	Not resolved			

[a] Value for the first-eluting enantiomer [(−)-*trans*-sobrerol and (−)-*trans*-CO/1408].
[b] Numbers represent the volume percentage of ethanol added to buffer solution.

Table II gives separation data for *trans*-sobrerol and *trans*-CO/1408 racemates resolved on the LiChrospher 100 RP-18 column with aqueous ethanol–potassium phosphate buffer as the mobile phase, saturated with β-CD. The order of elution of enantiomers determined by injecting single antipodes and the chromatogram of CO/1408 is shown as an example in Fig. 4. Enantiomeric resolutions were obtained with a more efficient column, a smaller percentage of cosolvent (ethanol) and a lower flow-rate compared with the parameters used for *cis–trans* separations (see Tables I and II) according to the general concept that these factor changes can improve the resolution of inclusion complexes[14].

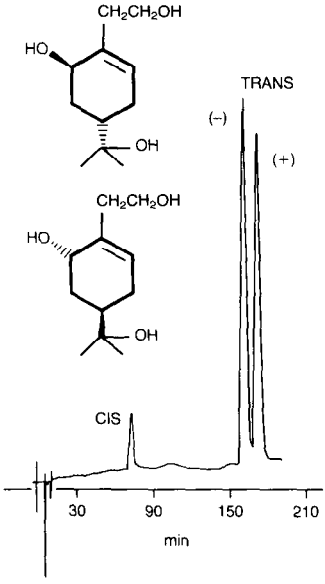

Fig. 4. Enantiomeric resolution of (±)-*trans*-CO/1408 on a LiChrospher 100 RP-18 (5 μm column (250 × 4.0 mm I.D.). Mobile phase: 0.8% ethanol–potassium phosphate buffer solution (0.025 *M*; pH 7.25) saturated with β-CD. Flow-rate: 0.3 ml/min.

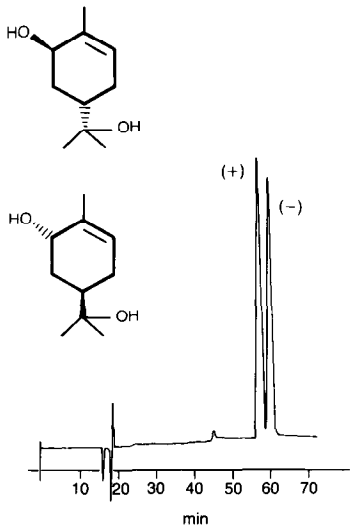

Fig. 5. Enantiomeric resolution of (±)-*trans*-sobrerol on a chiral β-CD Cyclobond I (5 μm) column (250 × 4.6 mm I.D. Mobile phase: 7% acetonitrile–aqueous triethylammonium acetate (0.005 *M*; pH 6.3). Flow-rate: 0.2 ml/min.

The enantiomeric separations of *cis*- and *trans*-sobrerol and CO/1408 were repeated with the Cyclobond I column. In this instance we were able not only to separate *trans*-sobrerol (Fig. 5) and *trans*-CO/1408 racemates faster than when using β-CD saturated eluents (compare Table III with Table II), but also to resolve the *cis*-sobrerol enantiomers (Table III). To obtain this separation within a reasonable elution time it was necessary to modify the chromatographic conditions used for the

TABLE III

ENANTIOMERIC SEPARATION OF TERPENIC ALCOHOLS USING A CHIRAL 250 × 4.6 mm I.D. (5 μm) β-CD CYCLOBOND I COLUMN AND DIFFERENT MOBILE PHASES

Enantiomeric pair	k'^a	α	R_s	*Mobile phase*[b]	*pH*
(±)-*trans*-sobrerol	2.67	1.08	1.55	Acetonitrile–buffer	4.40
	2.55	1.07	1.33	(7 : 93)[c]	5.25
	2.48	1.08	1.20		6.32
	2.43	1.08	1.09		7.30
(±)-*cis*-sobrerol	4.45	1.06	0.75	Acetonitrile–isopropanol–buffer (7 : 0.25 : 92.75)[d]	6.32
(±)-*trans*-CO/1408	2.06	1.04	0.75	Acetonitrile–buffer (7 : 93)[c]	6.32
(±)-*cis*-CO/1408	Not resolved				

[a] Value for the first-eluted enantiomer [(+)-*trans*-sobrerol, (+)-*trans*-CO/1408 and (−)-*cis*-sobrerol].

[b] Buffer: 0.005 *M* triethylammonium acetate of different pH.

[c] Flow-rate: 0.2 ml/min.

[d] Flow-rate: 0.3 ml/min.

separations of *trans*-sobrerol enantiomers by increasing the flow-rate and adding 0.25% of 2-propanol to the eluent. In fact, using the Cyclobond I column, *cis*-sobrerol and *cis*-CO/1408 are retained longer than their *trans* forms. Hence the elution order of the diastereoisomers is opposite to that observed with β-CD dissolved in the mobile phase. Further, an inversion of the elution order was also noted for the enantiomers. This HPLC retention behaviour has also been observed by other workers[15] and it is in agreement with the inclusion mechanism generally proposed to rationalize chromatographic separations with cyclodextrins[15].

The effect of mobile phase pH on the resolution was studied for sobrerol and the results are summarized in Table III. It is apparent that there was no significant change in the separation factor but an appreciable improvement in resolution was observed as the pH was reduced.

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