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Journal of Chromatography A, 1011 (2003) 37–47
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Cyclodextrin-based liquid chromatographic enantiomeric separation of chiral dihydrofurocoumarins, an emerging class of medicinal compounds

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Received 21 February 2003; received in revised form 10 June 2003; accepted 25 June 2003

Abstract

A set of 28 racemic dihydrofurocoumarins in which the stereogenic center is located in the furan ring have been synthesized. Currently no effective asymmetric synthesis of this class of compounds exists, although their enantiomers are produced biologically by certain plants. Their diverse medicinal properties are being investigated in several laboratories. The enantioselective separation of these dihydrofurocoumarins by three native and six derivatized cyclodextrins has been evaluated in the reversed-phase mode, the polar organic mode, and normal-phase mode. The hydroxypropyl-β-cyclodextrin is the most effective chiral stationary phase (CSP) at separating the dihydrofurocoumarins into enantiomers, showing some enantioselectivity for 22 dihydrofurocoumarins, and baseline resolving 16 of the 28 compounds in the reversed-phase mode. The acetyl-β-cyclodextrin and 2,3-dimethyl-β-cyclodextrin also showed enantioselectivity for a large number (18 and 17, respectively) of dihydrofurocoumarins in the reversed-phase mode. The native cyclodextrins are ineffective and the aromatic derivatized β-cyclodextrins are only marginally effective at separating the furocoumarin enantiomers in the reversed-phase mode. The polar organic mode and the normal-phase mode have also been evaluated with these CSPs, but no enantioseparations were observed.

Keywords: Enantiomer separation; Chiral stationary phase, LC; Cyclodextrins; Dihydrofurocoumarins

1. Introduction

Over the last several years, furocoumarins have received considerable attention from chemists, biologists, and pharmacologists. Two general classes of furocoumarins are the psoralens and angelicins (Fig. 1). Both of these classes of compounds contain the parent coumarin structure fused to a dihydrofuran ring. Furocoumarins are found many places in nature, most often in plants. Different substituted furocoumarins have been found in celery [1], bark extracts [2], citrus oil [3], and culinary herbs (parsley [4], dill, fennel, and cumin).

Furocoumarins are known to exhibit a variety of biological effects. Most significant are their photosensitizing and DNA intercollating properties [5–8]. Ancient Egyptians used psoralens in the form of plant extracts for the treatment of skin disorders [9].

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0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)01128-2

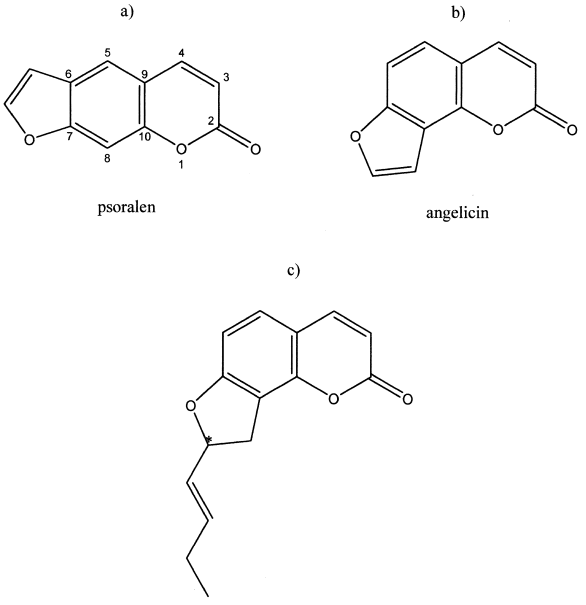


Fig. 1. (a) Psoralen type compounds are ‘‘linear’’ derivatives of coumarin where the furan ring is fixed to the 6,7-segment of the coumarin. The structure is numbered as the parent coumarin would be for consistency of comparisons in the discussion. (b) Angelicin type compounds are derivatives of coumarin where the furan ring is fused to the 7,8-segment of the coumarin. (c) A chiral, substituted angelicin analogue where the stereogenic center is denoted by an asterisk.

In more recent times, furocoumarins have been used for the treatment of psoriasis and vitiligo (skin depigmentation). Naturally occurring psoralen, bergapten, and xanthotoxin were found to be most active against skin diseases and were used in PUVA therapy (Psoralen-UltraViolet A). Upon exposure to long wavelength UV light (320–380 nm) furocoumarins form adducts with DNA nucleotides [10,11]. These adducts prevent the proliferation of cells from damaged or diseased tissues by halting DNA replication, which disrupts cellular division. While mono-adducts have therapeutic effects, inter-strand cross-linked di-adducts are primarily responsible for un-repairable DNA damage and undesired mutagenic effects [6]. More recently, furocoumarins have been investigated for their inhibition of acetylcholinesterase [12], their cytotoxicity against KB cells [2] (a line of cancerous cells), and for distinguishing between active and inactive rRNA [13].

It is well known that the biological activity of enantiomeric compounds can vary greatly. Consequently, it has become standard practice to assess the biological activity of each enantiomer of a chiral molecule and to produce drugs and food products mainly as single enantiomers [14]. Only recently has there been any investigation into the biological activity of chiral dihydrofurocoumarins [2,15]. To date, the syntheses of chiral dihydrofurocoumarins has been limited [16,17] and there have been no methods published in the literature pertaining to the enantioseparation of chiral dihydrofurocoumarins. As such, methods must be developed to obtain both enantiomers in their pure form and to determine the activity of each.

Cyclodextrin-based chiral stationary phases (CSPs) have been shown to be broadly applicable in their ability to separate enantiomers of a wide variety of compounds [18,19]. They are quite successful at resolving the enantiomers of chiral molecules with aromatic substituents [20–24]. Furthermore, it has been shown that cyclodextrins are useful in the analysis of various coumarin derivatives, as a CSP for the enantioseparation of warfarin, coumachlor, coumafuryl, phenprocoumon [23,25], and as post column fluorescence enhancing reagents for psoralen and phenprocoumon [25,26]. Consequently, cyclodextrin-based CSPs are a natural choice as CSPs for addressing the liquid chromatographic chiral separation of these compounds.

Recent efforts by Rozhkov et al. [27] have generated chiral dihydrofurocoumarins by the palladium-catalyzed annulation of 1,3-dienes by *o*-iodo-hydroxycoumarins. Substituents on the dihydrofuran portion of the heterocycle create a stereogenic center (Fig. 1c). The aim of this work is to evaluate the enantioselectivity of native and derivatized cyclodextrin based CSPs for these chiral dihydrofurocoumarins. Both substituted psoralens and substituted angelicins are examined in different chromatographic modes.

2. Experimental

2.1. Materials

The CSPs were obtained from Advanced Separation Technologies (Whippany, NJ, USA). All stationary phases used consisted of the chiral selector

bonded to 5 μm spherical silica gel. The chiral selectors used are the underivitized cyclodextrins and the derivatized β-cyclodextrins, which are illustrated in Fig. 2. The dimensions of the columns are 250× 4.6 mm. The triethylamine, methanol, acetonitrile, 2-propanol, and hexane used were HPLC grade from Fisher (Fairlawn, NJ, USA). Sodium chloride and acetic acid were ACS certified grade from Fisher. All substituted dihydrofurocoumarin were prepared as outlined previously [27].

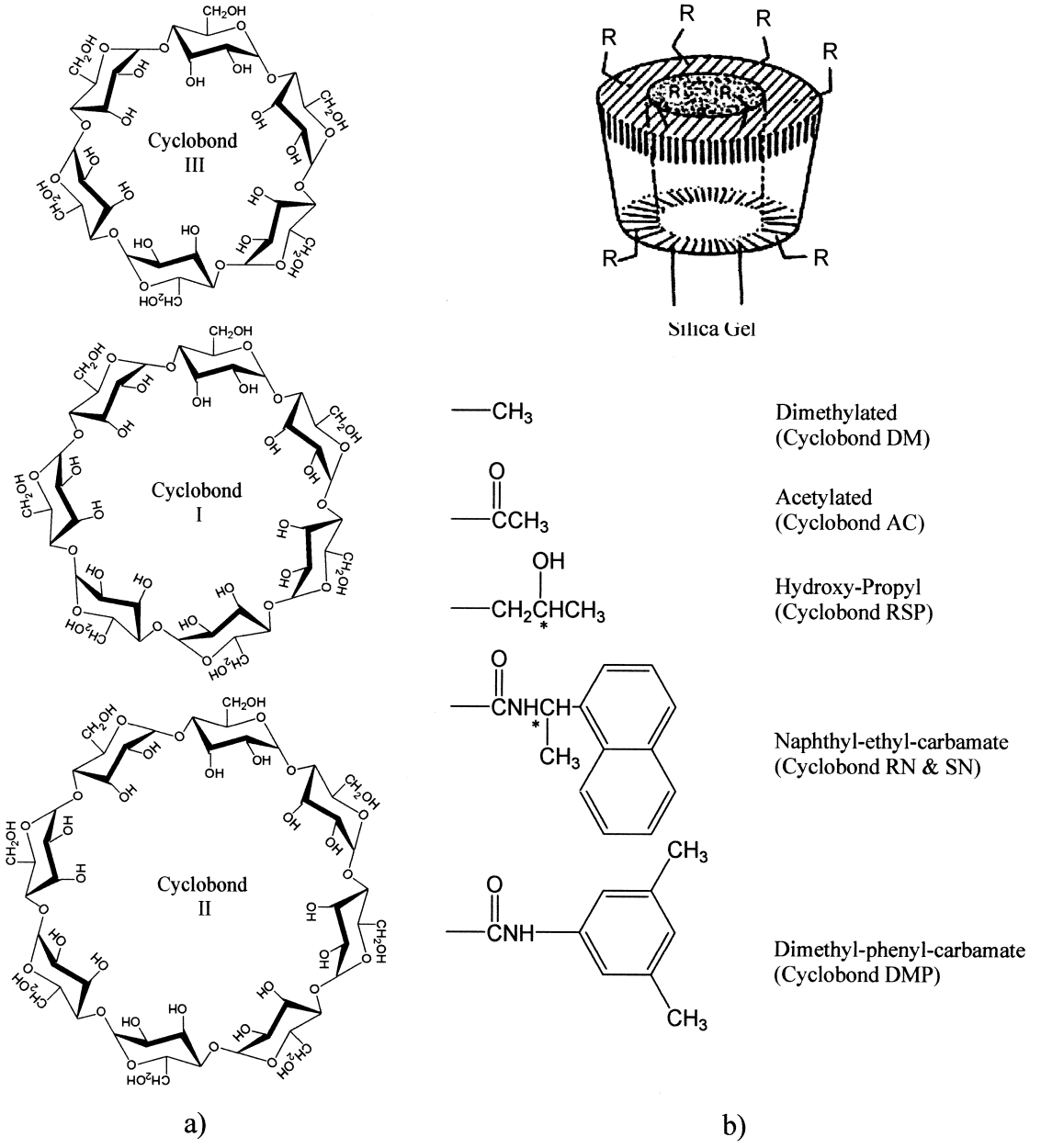


Fig. 2. (a) Native α, β, and γ cyclodextrins (i.e., Cyclobond III, I and II, respectively). (b) Types of derivatized cyclodextrins. An asterisk denotes the stereogenic center.

2.2. Equipment

The HPLC system used consisted of a quaternary pump, an auto sampler, a UV VWD detector (1050, Hewlett-Packard, Palo Alto, CA, USA), and an integrator (3395, Hewlett-Packard). Mobile phases were degassed by ultra-sonication under vacuum for 10 min. UV detection was carried out at 220 nm. All separations were carried out at room temperature (~23 °C).

2.3. Column evaluation

The performance of each stationary phase was evaluated in the reversed-phase mode using acetonitrile–water and methanol–water mobile phases. The aromatic derivatized CSPs, Cyclobond DMP, RN, and SN, were also evaluated in the normal-phase mode (isopropanol–hexane) and in the polar organic mode (100% acetonitrile). The composition of the mobile phase was optimized for resolving the enantiomers of each compound at a flow-rate of 1.0 ml min^{-1}.

2.4. Calculations

Dead times (t_M) were estimated using the refractive index solvent peak on each CSP. Retention factors (k) were calculated using the equation $k = (t_r - t_M)/t_M$. Enantioselectivity (α) was calculated using the equation $\alpha = k_2/k_1$. Resolution factors (R_s) was calculated using the equation $R_s = 2 \times (t_{r2-}t_{r1})/(w_1 + w_2)$, where t_{r2} and t_{r1} are the retention times of the second and first enantiomers, respectively, and w_1 and w_2 are the base peak widths of the corresponding peaks.

3. Results and discussion

A series of 28 racemates, including seven substituted psoralen derivatives, 14 substituted angelicin derivatives, five substituted dihydrofurocoumarins, and two substituted coumarins were evaluated on nine different cyclodextrin based CSPs in the reversed-phase mode (see Table 1 for structures and separation data). Fig. 3 is a summary of the performance of each CSP in the reversed-phase mode. Clearly the best CSP for these chiral dihydrofurocoumarins utilizes hydroxypropyl-β-cyclodextrin as the chiral selector (Cyclobond I RSP). The acetyl-β-cyclodextrin (Cyclobond I AC) and 2,3-dimethyl-β-cyclodextrin (Cyclobond I DM) based CSPs were also able to resolve a large number of dihydrofurocoumarins. The remaining CSPs, native cyclodextrins and aromatic derivatized β-cyclodextrin, were either ineffective or showed enantioselectivity for a small number of the examined dihydrofurocoumarin compounds in the reversed-phase mode. A partial separation of enantiomers is reported in Fig. 3 if there is an observable enantioselectivity ($\alpha > 1.02$) and a baseline separation of enantiomers is reported if the peak-to-peak resolution (R_s) exceeds 1.5.

The effect of mobile phase composition was also investigated. All 28 compounds were analyzed in the reversed-phase mode with both acetonitrile–water and methanol–water mobile phases on all CSPs. Generally, comparable results for enantioselectivity and resolution were obtained with each solvent system; however, there were several cases where an acetonitrile–water mobile phase successfully separated enantiomers where the methanol–water mixture failed. This is thought to be due to hydrogen bonding of the methanol molecules to the hydroxyl groups on the cyclodextrin, which may interfere with the enantioselective complexation process. The effect of pH (4.00, 5.00, 6.00, 7.00, and unbuffered [pH 6.20], 0.1% (v/v) triethylamine–acetic acid) and ionic strength (0, 0.10, 0.20, 0.30, 0.40, and 0.50 *M* NaCl) were also investigated. However, neither appreciably affected selectivity or resolution (data not shown). This is due to the fact that the dihydrofurocoumarins are neutral, hydrophobic compounds with no ionizable groups (see Fig. 1 and Table 1).

3.1. Cyclobond I RSP, AC, and DM chiral stationary phases

Table 1 summarizes the separation data for the most effective Cyclobond AC, DM, and RSP columns in the reversed-phase mode of operation. The structure of each dihydrofurocoumarin and the optimal mobile phase compositions are given, as well as the values for k, R_s, and α.

It is well known that cyclodextrin CSPs excel at

Table 1
Retention factor (k'), enantioselectivity (α) and enantioresolution (R_s) of all dihydrofurocoumarins on Cyclobond I 2000AC, DM, and RSP CSPs

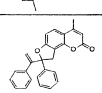
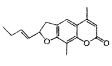
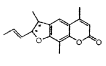
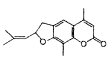
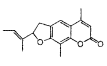
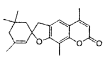
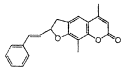
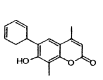
		Cyclobond AC				Cyclobond DM				Cyclobond RSP			
Compound #	Structure	k	α	Rs	Mobile Phase [a]	k	α	Rs	Mobile Phase [a]	k	α	Rs	Mobile Phase [a]
1		3.32	1.18	1.93	A	5.54			D	3.83	1.21	1.31	K
2		3.11	1.10	0.77	A	6.23	1.14	1.92	D	6.49	1.21	1.58	K
3		5.28	1.24	2.24	A	4.78	1.39	4.02	A	12.46	1.21	1.80	K
4		5.02	1.07	0.55	A	4.20			A	6.57	1.13	1.76	J
5		9.65	1.18	1.73	B	3.29			A	23.07	1.13	1.03	D
6		2.24			A	5.03	1.04	0.27	A	11.99			I
7 [b]		5.54			C	5.14	1.40	3.31	D	6.70	1.57	5.67	A
8		4.33	1.17	1.63	B	4.13			A	6.99	1.08	0.66	I
9		6.24	1.23	2.10	C	5.77			A	6.16	1.09	0.64	K
10		10.20	1.25	2.62	D	8.38	1.19	1.43	D	7.26	1.41	3.20	E
11		6.50	1.16	1.65	D	6.82	1.17	1.56	D	11.34	1.14	1.75	A
12		4.46			A	15.16	1.05	0.68	A	20.75	1.04	0.30	A
13		10.41	1.15	2.47	A	6.49	1.24	1.64	A	2.25	1.61	6.25	F
14		6.19	1.14	0.82	I	6.07			J	4.67	1.44	3.57	G
15		2.80	1.28	2.14	B	2.78	1.07	0.37	C	6.44	1.14	1.74	C
16		4.49	1.06	0.68	G	6.66	1.04	0.48	D	4.53	1.28	2.73	J
17		2.78	1.10	0.93	G	2.54	1.05	0.44	D	3.88	1.17	2.17	I

Table 1. Continued

18		3.20	1.09	0.67	G		5.33	1.16	1.70	D		5.01	1.31	3.27	A	
19		3.04	1.05	0.47	I		3.26			A		8.75	1.10	1.78	A	
20		2.78			A		7.42			D		2.25			I	
21 [b]		2.44			A		5.81	1.12	1.37	D		1.82			I	
22		2.75			D		8.77	1.07	0.74	D		17.40	1.08	1.26	D	
23		4.61	1.07	0.51	D		6.35	1.24	2.42	D		19.38	1.11	1.69	D	
24		2.75			A		8.49	1.06	0.76	D		4.74	1.07	0.85	A	
25		6.80			A		10.25	1.14	1.16	A		9.63	1.24	2.08	E	
26		1.74			J		2.79			G		19.21			A	
27		2.12			D		4.51			I		5.03	1.18	1.70	C	
28		2.09			D		1.62			I		2.34			J	

[a] Mobile phase composition: (A) 20:80 ACN–water, (B) 30:70 MeOH–water, (C) 35:65 MeOH–water, (D) 15:85 ACN–water, (E) 50:50 MeOH–water, (F) 50:50 ACN–water, (G) 30:70 ACN–water, (H) 55:45 MeOH–water, (I) 40:60 MeOH–water, (J) 25:75 ACN–water, (K) 45:55 MeOH–water.

[b] Separation of diastereomers.

enantioseparations where the analytes contain large aliphatic groups or multiple aromatic rings [20–24]. For example, the separation of angelicin derivatives 1, 2, and 3 clearly show that an increase in steric bulk about the stereogenic center improves the separation on all three of the non-aromatic derivatized β-cyclodextrin CSPs (Table 1). On the hydroxypropyl-β-cyclodextrin CSP, the resolution of these compounds is enhanced (see Fig. 4). Compound 14 also shows that an excess of steric bulk can hinder a separation on some CSPs (Cyclobond I AC and DM) and enhance selectivity on others (Cyclobond I RSP).

Other examples of the importance of steric interactions near the chiral center are shown in the separation of compounds 9, 10, and 11 on these CSPs. While these molecules are structurally similar, the addition or removal of one methyl group α to or β to the stereogenic center can greatly affect the observed enantioselectivity (see Fig. 5). The methyl groups create additional steric bulk near the chiral center, which enhances chiral recognition. Conversely, compounds 6, 8, and 12 have little steric bulk near the chiral center, leading to diminished enantioselectivity. Therefore, steric bulk must play a significant role in the selectivity of these types of compounds.

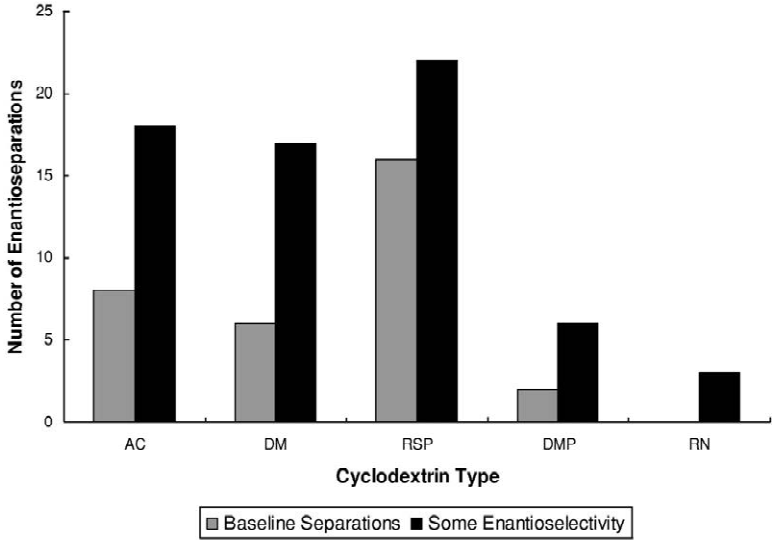


Fig. 3. Number of separations in the reversed-phase mode using cyclodextrin-based CSPs. The various types of cyclodextrins and their designated abbreviations are illustrated in Fig. 1. Black bars: number of observable enantioselective separations, enantioselectivity, $\alpha > 1.02$. Grey bars: number of baseline separations, enantioresolution, $R_s > 1.5$.

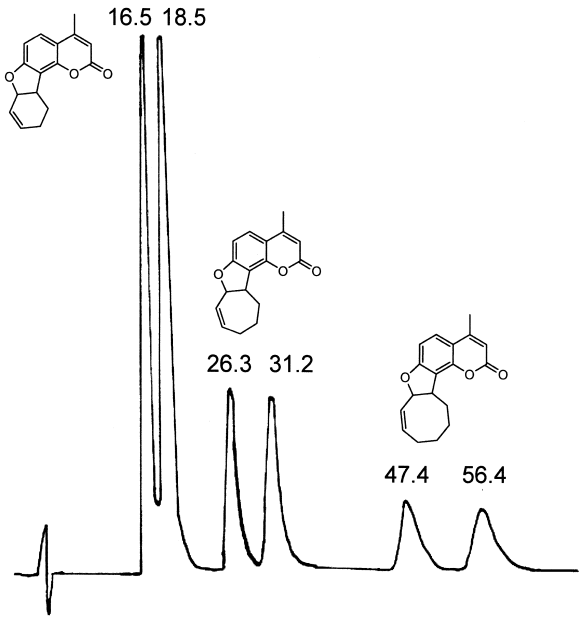


Fig. 4. Enantioseparation of dihydrofurocoumarins 1, 2, and 3 (in order of elution) on Cyclobond RSP. Mobile phase: 45:55 methanol–water.

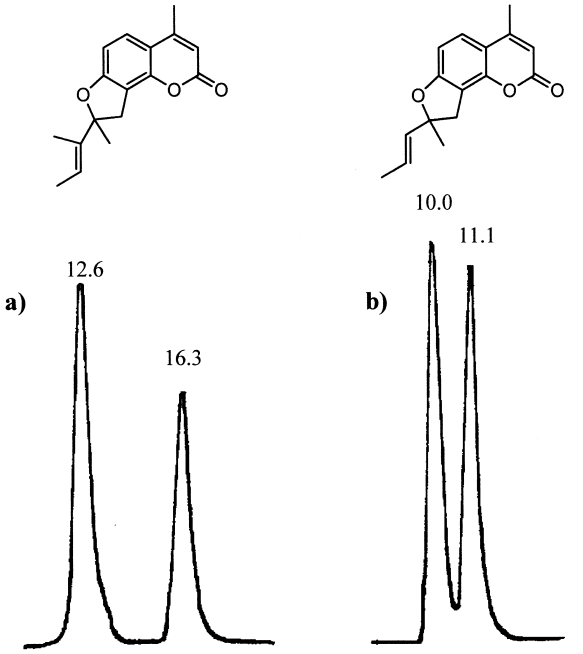


Fig. 5. The effect of steric bulk on the enantioseparations. Separations performed on the Cyclobond RSP CSP with a 30:70 ACN–water mobile phase. (a) Dihydrofurocoumarin 10; (b) Dihydrofurocoumarin 11.

The enantioseparations of the dihydrofuroangelicin derivatives and their corresponding structural isomers (the dihydrofuropsoralen derivatives) is also of interest. While these pairs of analytes are quite similar, the more-linear psoralen derivatives are generally less well resolved than their angelicin derivative counterparts. This is the case for compounds 8 and 20, 9 and 24, and 13 and 25. The results for compounds 8 and 20, and 13 and 25 are shown in Fig. 6. The difference in enantioselectivity between dihydrofuroangelicin derivatives and dihydrofuropsoralen derivatives must be due to the spatial orientation of the dihydrofuran group, which limits the rotational or reorientational ability of the analyte in the inclusion complex. It is also of interest to note that, when comparing dihydrofuroangelicin derivatives with the dihydrofuropsoralen derivative structural analogs, a separation of enantiomers is not achieved in the case of the dihydrofuropsoralen analytes. For example, compare compounds 7, 8, and 12, which are dihydrofuroangelicin derivatives, with compounds 21, 20, and 26 which are the corresponding dihydrofuropsoralen derivatives (which are not separated into enantiomers). There is only one case where a dihydropsoralen analogue is better resolved than its dihydroangelicin counterpart: compounds 6 vs. 22.

It was also observed that the orientation of the dihydrofuran oxygen in relation to the coumarin affects the enantiomeric separation. For example, compounds 17 and 18 are very similar in structure, as are compounds 15 and 16. Fig. 7 is a comparison of the enantiomeric separation of compounds 17 and 18 (which differ only in the location of the oxygen heteroatom in the furan ring) on the Cyclobond I RSP. The best chiral selector for this class of compounds is the hydroxypropyl-β-cyclodextrin (Cyclobond I RSP), as all four of these analytes are baseline resolved ($R_s \geq 1.5$). Greater selectivity was observed when the dihydrofuran oxygen was α to position 5 on the coumarin for the Cyclobond I RSP (compounds 16 and 18) and α to position 6 for the Cyclobond I AC column (compounds 15 and 17).

Obviously the exact location of the fused dihydrofuran ring (on the parent coumarin) has a

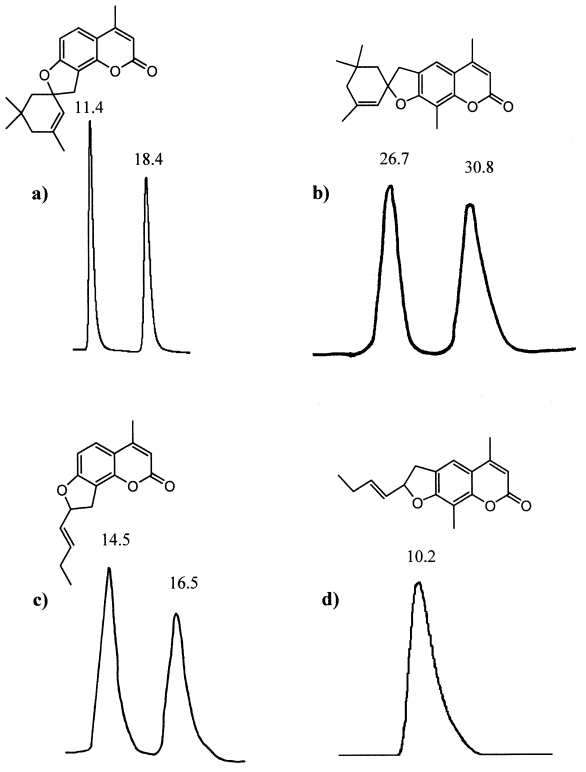


Fig. 6. Angelicin–psoralen analogue enantioseparations. Separations of dihydrofurocoumarins 13 and 25 performed on the Cyclobond RSP CSP. Separations of dihydrofurocoumarins 8 and 20 performed on the Cyclobond AC CSP. (a) Angelicin analogue 13. (b) Psoralen analogue 25. (c) Angelicin analogue 8. (d) Psoralen analogue 20.

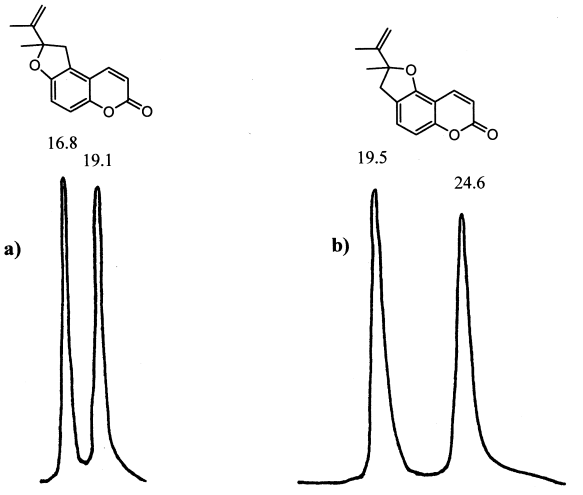


Fig. 7. The effect of the dihydrofuran orientation on the separation of enantiomers on the Cyclobond RSP CSP. (a) Dihydrofurocoumarin 17. (b) Dihydrofurocoumarin 18.

significant impact on the separation. This is further shown by comparing the results from compounds 1, 15, 16, and 19 on the Cyclobond I AC and RSP CSPs. The best orientation for enantioresolution on these CSPs is when the dihydrofuran moiety is fused to the 5 and 6 positions on the coumarin, as is the case for compounds 15 and 16.

3.2. Other CSPs: native and aromatic derivatized cyclodextrins

Other cyclodextrin-based CSPs were much less effective in separating enantiomers of these types of compounds in the reversed-phase mode. These remaining CSPs can be divided into two categories: aromatic derivatized cyclodextrin CSPs (Cyclobond I RN, SN and DMP) and native cyclodextrin CSPs (Cyclobond I, II, and III). The results of these analyses are presented in Table 2. The aromatically derivatized cyclodextrins (Cyclobond I DMP, RN, and SN) are not as successful for this class of compounds. As only a limited number of separations were observed with the aromatically derivatized cyclodextrins, it is reasonable to conclude that an excess of aromatic steric bulk on the chiral selector is detrimental to the enantioseparation of most chiral dihydrofurocoumarins. The native cyclodextrins did not show any selectivity for any of the analytes investigated.

Table 2
Retention factor (k'), enantioselectivity (α), and enantioresolution (R_s) for chiral dihydrofurocoumarins separated on Cyclobond RN, and DMP CSPs

Compound no.	k	α	R_s	Mobile Phase[a]
Cyclobond DMP CD				
10	8.70	1.11	1.34	C
11	4.45	1.05	0.33	B
19	3.84	1.04	0.76	E
20	7.11	1.04	0.32	B
26	4.71	1.32	2.38	A
Cyclobond RN CD				
15	4.55	1.05	0.60	D
19	3.41	1.05	0.53	E

[a] Mobile phase composition: (A) 75:25 MeOH–water, (C) 60:40 MeOH–water, (B) 55:45 MeOH–water, (D) 50:50 MeOH–water, (E) 40:60 ACN–water.

3.3. Normal phase and polar organic modes

The normal-phase mode was investigated on all of the aromatically derivatized CSPs. The Cyclobond I RN, SN, and DMP columns were each evaluated with a 5:95 isopropanol–hexane mobile phase. All analytes were appreciably retained, but no enantioselectivity was observed. The polar organic mode was also investigated under the weakest condition (100% acetonitrile) where all compounds eluting at the dead time of the column.

3.4. Mechanistic observations

The binding of a dihydrofurocoumarin analyte to a cyclodextrin CSP is a dynamic process. Both the furan portion and the lactone portion of a dihydrofurocoumarin molecule can enter the cyclodextrin cavity to form an inclusion complex in the reversed-phase mode, but only one of the two inclusion complex orientations will produce the enantioselectivity which leads to the observed chiral separation. It is well established that, for a cyclodextrin to form an enantioselective diastereomeric complex, the substituents off of the stereogenic center of the analyte must be in close proximity to the secondary hydroxyls at the mouth of the cyclodextrin in order to achieve the necessary three-points of interaction [19,24,28]. If the furan portion of the molecule resides in the cavity of the cyclodextrin upon inclusion, the stereogenic center will be buried inside the cyclodextrin torus, not in close proximity to the secondary hydroxyl groups (or the derivative groups on these hydroxyls) on the larger rim of the molecule. In this case, the substituents on or near the analyte's stereogenic center will be unable to interact with the portion of the chiral selector that is most responsible for chiral recognition. It is then reasonable to conclude that, for chiral recognition to occur, the lactone portion of the analyte molecule must occupy the cyclodextrin cavity and the furan portion is in close proximity to the mouth of the cyclodextrin cavity where the secondary hydroxyls and their substituents are located.

The size of these analytes (Tables 1 and 2) supports the contention that the same portion of these polycyclic analytes must protrude from the torus of the cyclodextrin cavity when an inclusion complex is

formed. The hydroxylpropyl-β-cyclodextrin CSP and acetyl-β-cyclodextrin CSP (Cyclobond I RSP and AC) are very successful at resolving larger analytes where significant portions of the included molecule protrude from the cyclodextrin (23, 29), whereas native cyclodextrins are not. The hydroxylpropyl and acetyl groups of the derivatized cyclodextrins are also known to extend beyond the mouth of the cyclodextrin cavity (28) and are in a position to interact with both the dihydrofuran moiety and any substituents attached to the stereogenic center. This has previously been shown to be the most prominent interaction that leads to enantioselectivity in the cases when the hydroxylpropyl-β-cyclodextrin CSP is superior to the native β-cyclodextrin CSP [29]. Therefore, the additional interactions produced by these derivative groups are essential for chiral recognition. Taking into consideration the fact that native cyclodextrins CSPs are completely ineffective in separating these compounds, one must conclude that when the dihydrofurocoumarins form an enantioselective inclusion complex with a derivatized cyclodextrin in the reversed-phase mode, their stereogenic center is located near the mouth of the cyclodextrin selector.

4. Conclusions

The Cyclobond I AC, DM and RSP are the most effective cyclodextrin-based CSPs for resolving the enantiomers of chiral dihydrofurocoumarins in the reversed-phase mode. This is due to the analyte complexing with these chiral selectors in such a way that the substituents off of the dihydrofurocoumarin stereogenic center interact with the derivative moieties on the cyclodextrin molecule. The presence of steric bulk about the analytes chiral center greatly enhances the chiral recognition of these enantiomers. The orientation of the furan oxygen as well as the spatial placement of the dihydrofuran moiety on the parent coumarin molecule both play a major role in the selectivity of the separation. Generally, the angelicin-type coumarins are better resolved than their psoralen analogues. The aromatic derivatized and native cyclodextrins are mostly ineffective at resolving these types of analytes. The normal-phase and polar organic modes could not be used to separate any of these compounds into their enantiomers with these CSPs.

Acknowledgements

Support of this work by the National Institutes of Health, NIH RO1 GM53825-06 is gratefully acknowledged. R.V. Rozhkov and R.C. Larock also acknowledge the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research and Johnson Matthey Inc. and Kawaken Fine Chemicals Co., for donations of palladium acetate.

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