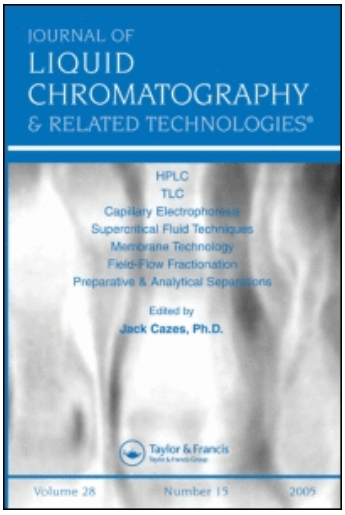

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M. M. Warnke[a]; C. R. Mitchell[a]; R. V. Rozhkov[a]; D. E. Emrich[a]; R. C. Larock[a]; D. W. Armstrong[a]
[a] Department of Chemistry, Iowa State University, Ames, Iowa, USA

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M. M. Warnke, C. R. Mitchell, R. V. Rozhkov, D. E. Emrich, R. C. Larock, and D. W. Armstrong
Department of Chemistry, Iowa State University, Ames, Iowa, USA

Abstract: The enantioselectivity of native and derivatized cyclodextrin stationary phases and macrocyclic glycopeptides for chiral pterocarpans was evaluated using high performance liquid chromatography (HPLC). All enantiomers could be baseline resolved in the reverse phase mode on cyclodextrin-based Cyclobond chiral stationary phases (CSPs). The hydroxypropyl-β-cyclodextrin, acetyl-β-cyclodextrin, and gamma-cyclodextrin CSPs show the broadest enantioselectivity in the reverse phase mode. Some compounds were baseline separated on the ristocetin A and vancomycin macrocyclic glycopeptide chiral stationary phases in the reverse phase mode. Separations on the ristocetin A columns produced the highest resolutions (up to $\sim$7.1) in this study. The 3,5-dimethylphenyl carbamate derivatized cyclodextrin column showed the broadest enantioselectivity in normal phase LC. Of the macrocyclic glycopeptide CSPs, ristocetin A and teicoplanin aglycone (Chirobiotic R and TAG, respectively) showed enantioselectivity for the most compounds in the normal phase mode. However, baseline separations were only achieved with the teicoplanin and teicoplanin aglycone.

Keywords: Column liquid chromatography, Cyclodextrin, Macrocyclic glycopeptides, Pterocarpans

Address correspondence to D. W. Armstrong, Department of Chemistry, Iowa State University, Gilman Hall, Ames, IA 50011-3111, USA. E-mail: sec4dwa@iastate.edu

INTRODUCTION

Pterocarpans are *cis*-fused benzopyran benzofuranyl structures and one of the largest groups of natural isoflavanoids, second only in prevalence to the isoflavones.[1] Isoflavanoids have been isolated mainly from fodder crops, beans, peas, and some shrubs. Many pterocarpans are phytoalexins, or antifungal compounds formed in a plant after it has been infected by fungal organisms.[2,3] Some pterocarpans have shown anti-microbial activity[4] and activity against snake and spider venom.[5] The structure and numbering system for pterocarpans is shown in Figure 1.

Although there are two stereogenic centers at carbons 6a and 11a, pterocarpans exist as only one set of enantiomers because of the *cis*-fused ring system. In most cases, the (−)-(6a*R*, 11a*R*)-isomer is the biologically produced pterocarpan enantiomer.[3−5] Synthetic pterocarpans are often produced as racemates.[6−8] Thus, enantioseparation is important in isolating the active enantiomer. Previously, enantiomeric separations have been achieved for both natural and synthetic pterocarpan compounds using both HPLC and capillary electrophoresis.[9,10]

Native α, β, and γ cyclodextrins are macrocyclic compounds formed from 6, 7, or 8 gluco-pyranose units, respectively. Chiral stationary phases (CSP) based on cyclodextrins are able to separate enantiomers by forming inclusion complexes in the reverse phase mode of operation.[11,12] The hydrophobic part of the analyte molecule complexes with the hydrophobic interior cavity of the cyclodextrin. Interactions with the hydroxyl groups or derivative groups on the rim complete the necessary three points of interaction required to achieve enantioselective complexation.[11] The cavity size increases as the number of gluco-pyranose units increases. The native β-cyclodextrin CSP (Cyclobond I 2000) was shown to effectively separate enantiomers of polycyclic aromatic hydrocarbons.[13,14]

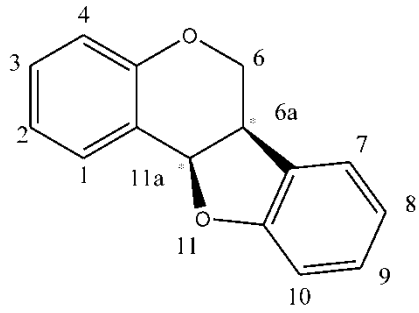


Figure 1. Numbering system of an unsubstituted pterocarpan. The stereocenters are denoted with asterisks.

Derivatized cyclodextrin based CSPs offer additional sites for interactions leading to chiral recognition. The hydroxypropyl-β-cyclodextrin (Cyclobond I 2000 RSP) has been shown to separate compounds not separated on the native β-cyclodextrin CSP.[15] Cyclodextrins derivatized with aromatic groups are effective for separations in the normal phase mode.[16,17] Nonpolar solvent molecules occupy the cyclodextrin cavity in the normal phase mode, therefore, π-π interactions, dipole stacking, and hydrogen bonding interactions are important for chiral recognition.[16]

Macrocyclic glycopeptide based CSPs have also been shown to separate a wide variety of chiral compounds.[18] The commercially available CSPs are those based on the macrocyclic glycopeptide antibiotics vancomycin, ristocetin A, teicoplanin, and teicoplanin aglycone. All of these chiral selectors have a similar peptide backbone, multiple stereogenic centers, and functionalities such as carboxylic acids, amines, sugar moieties, and aromatic rings.[19] The teicoplanin aglycone is the only chiral selector without saccharide groups attached. Along with a variety of functional groups, these chiral selectors have a secondary structure in the form of a twisted "C" shaped basket that is relatively non-polar.[20,21] Interactions between an analyte molecule and the functional groups or hydrophobic cavity of the macrocyclic glycopeptide based CSP can lead to enantioselectivity.

EXPERIMENTAL

Materials

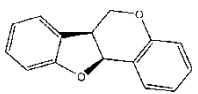
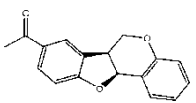
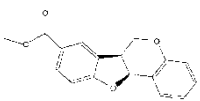
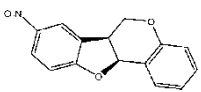
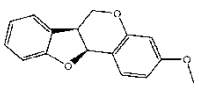
The pterocarpan compounds studied are shown in Table 1. The compounds were synthesized by reacting 1 equivalent of a substituted 2-iodophenol and 2 equivalents of benzopyran or a substituted benzopyran with 5% palladium acetate catalyst, 1 equivalent of Na_2CO_3, and 15% n-Bu_4NCl in DMF at 100°C for 1 day.[22]

The methanol (MeOH), acetonitrile (ACN), and heptane used in the mobile phases are all HPLC grade and were purchased from Fisher Scientific (Fair Lawn, New Jersey). The ethyl alcohol (EtOH) was purchased from Aaper Alcohols (Shelbyville, Kentucky) and is of punctilious grade. Water used in the separations was filtered and deionized using activated charcoal and a 5 μm filter. Mobile phases were degassed through ultrasonication and vacuum for 5 minutes.

Equipment

The cyclodextrin based chiral stationary phases used were Cyclobond I 2000, Cyclobond I 2000 DM (consisting of dimethylated β-cyclodextrin),

Table 1. Retention factor (k_1), enantioselectivity (α) and enantioresolution (Rs) of pterocarpans on columns listed

Cyclodextrin based chiral stationary phases	1 			2 			3 			4 			5 		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
Reverse phase mode															
Cyclobond I 2000 (a)	7.12	1.01	0.32	7.78	—	—	10.01	1.11	1.96	8.40	—	—	8.52	1.06	1.21
Cyclobond II(b)	3.23	1.08	1.33	3.43	1.08	1.39	4.26	1.13	1.03	2.68	1.06	0.86	3.98	—	—
Cyclobond III (c)	3.48	—	—	4.25	—	—	7.96	—	—	5.99	—	—	5.84	—	—
Cyclobond I 2000 RSP(d)	4.33	1.12	1.93	3.29	1.07	1.17	4.56	1.11	1.75	3.30	—	—	5.73	1.13	2.09
Cyclobond I 2000 AC(e)	8.01	1.22	2.78	8.23	1.15	1.69	10.00	1.18	2.27	8.33	1.17	2.00	11.90	1.11	1.44
Cyclobond I 2000 DM(f)	6.92	1.09	0.73	6.15	1.06	0.76	8.82	—	—	5.99	1.08	0.89	7.55	1.03	0.56
Cyclobond I 2000 DMP(d)	7.76	—	—	11.98	1.05	0.68	14.02	—	—	9.88	—	—	15.39	1.04	0.60
Cyclobond I 2000 RN (g)	15.31	—	—	14.00	1.03	0.63	20.40	1.05	1.11	8.96	—	—	17.40	1.06	1.33

Normal phase mode															
Cyclobond I 2000 DMP (h)	0.22	—	—	2.85	1.10	1.40	1.09	1.06	1.21	3.17	1.12	2.03	0.58	—	—
Cyclobond I 2000 RN (h)	0.15	—	—	2.31	—	—	0.90	—	—	2.13	—	—	0.37	—	—
Macrocyclic glycopeptide based chiral stationary phases	$k_{1'}$	α	R_s	$k_{1'}$	α	R_s	$k_{1'}$	α	R_s	$k_{1'}$	α	R_s	$k_{1'}$	α	R_s
Reverse phase mode															
Chirobiotic R(e)	1.82	—	—	2.52	1.29	4.19	3.52	1.65	7.07	4.36	1.25	3.75	2.47	—	—
Chirobiotic V (g)	4.04	—	—	6.10	1.10	1.71	12.20	1.06	1.12	6.61	—	—	5.69	—	—
Chirobiotic TAG(i)	20.12	—	—	23.80	—	—	30.00	—	—	10.72	—	—	22.62	—	—
Chirobiotic T(g)	9.06	—	—	17.89	1.03	0.54	19.25	—	—	10.13	—	—	12.20	—	—
Normal phase mode															
Chirobiotic R(j)	0.28	—	—	2.35	1.02	0.65	0.85	1.04	0.90	2.19	1.08	1.38	0.30	—	—
Chirobiotic V(j)	0.21	—	—	2.46	1.05	1.26	0.85	—	—	1.89	—	—	0.27	—	—
Chirobiotic TAG(h)	0.29	—	—	6.00	1.07	0.98	2.19	1.02	0.44	4.65	1.36	3.24	0.51	—	—
Chirobiotic T(j)	0.15	—	—	2.14	—	—	0.66	—	—	1.79	1.20	3.39	0.48	—	—

Mobile Phase Conditions: (a) 80/20 water/acetonitrile; (b) 90/10 water/acetonitrile; (c) 80/20 water/methanol; (d) 60/40 methanol/water; (e) 60/40 water/methanol; (f) 80/20 water/acetonitrile; (g) 70/30 water/methanol; (h) 95/5 heptane/ethanol; (i) 75/25 water/acetonitrile; (j) 90/10 heptane/ethanol.

Cyclobond I 2000 RSP (consisting of hydroxypropyl derivatized β-cyclodextrin), Cyclobond I 2000 AC (consisting of acetylated β-cyclodextrin), Cyclobond I 2000 DMP (consisting of 3,5-dimethylphenylcarbamate derivatized β-cyclodextrin), Cyclobond I 2000 RN (consisting of naphthylethyl carbamate derivatized β-cyclodextrin), Cyclobond II (γ-cyclodextrin), and Cyclobond III (α-cyclodextrin). The macrocyclic glycopeptide based chiral stationary phases used in this study were the Chirobiotic R (based on ristocetin A), Chirobiotic V (based on vancomycin), Chirobiotic T (based on teicoplanin), and Chirobiotic TAG (based on teicoplanin aglycone). All stationary phases were obtained from Astec (Whippany, New Jersey, USA).

The chromatographic experiments were performed on an HP1050 HPLC, equipped with an autosampler, quaternary pump, and VWD UV detector. All experiments were performed at ambient temperature and a flow rate of 1.0 mL/min.

Calculations

The retention factor for the first enantiomeric peak, k_1, was calculated by the equation: $t_{r1} - t_m/t_m$, where t_{r1} is the retention time of the first peak and t_m is the retention time of the void volume. The selectivity, α, is determined as follows: k_2/k_1, where k_2 is the retention factor of the second enantiomeric peak. The resolution is calculated as follows: $2(t_{r2} - t_{r1})/(w_1 + w_2)$, where w_1 and w_2 are the widths at the peak's base.

RESULTS AND DISCUSSION

The β-cyclodextrin and γ-cyclodextrin CSPs were the most useful native cyclodextrin CSPs, separating all five compounds. The difference between the two cyclodextrins is one gluco-pyranose unit, which creates a larger cavity for the gamma cyclodextrin. Figure 2 shows separations on both the beta and gamma cyclodextrin CSPs. On the former column, there is a definite substituent effect on enantioselectivity. The unsubstituted pterocarpan (compound 1) has very low enantioselectivity, while the substituted pterocarpans (compounds 5 and 3) have much higher enantioselectivities and resolutions. The substituent can be on the benzofuran or benzopyran portion of the molecule, and either improves enantioseparation on the β-cyclodextrin column. Enantiomers of the unsubstituted pterocarpan (compound 1) are almost completely resolved on the γ-cyclodextrin CSP, which is a vast improvement from the results on the β-cyclodextrin CSP. This can only be due to the difference in how well the analyte fits into the cyclodextrin cavity versus the larger γ-cyclodextrin cavity. However, it should be noted that the γ-cyclodextrin CSP was ineffective in separating pterocarpans with substituted benzopyran groups.

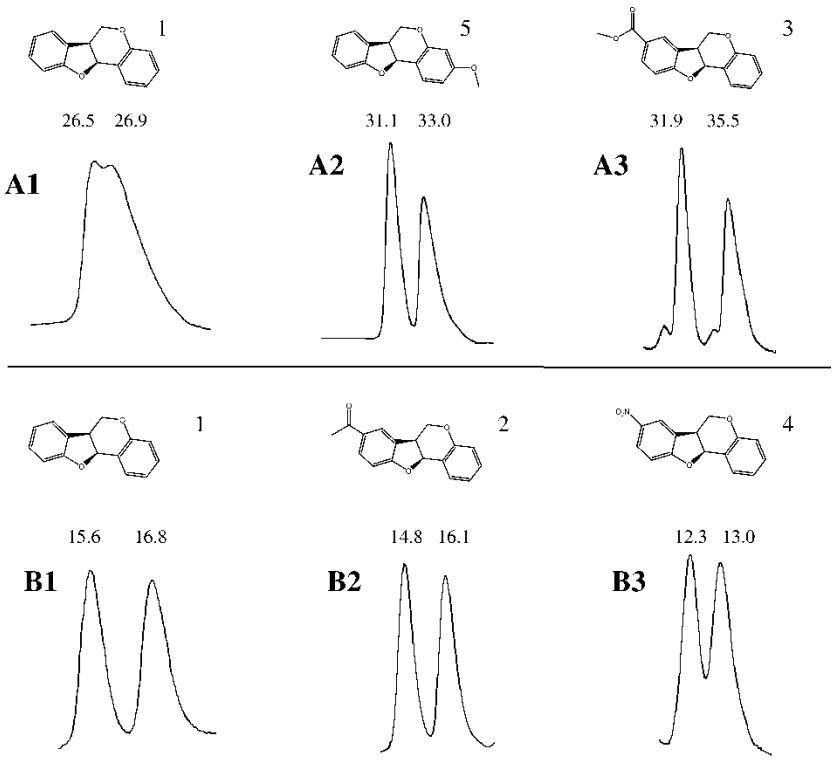


Figure 2. Comparison of separations on beta and gamma cyclodextrin based CSPs. A1, A2, and A3 are the enantioseparations of compounds 1, 5, and 3 respectively on the beta cyclodextrin. A1-A3 mobile phase conditions are 20/80 ACN/H_2O. B1, B2, and B3 are the enantioseparations of compounds 1, 2, and 4 respectively on the gamma cyclodextrin. B1-B3 mobile phase conditions are 10/90 ACN/H_20.

The nonaromatic derivatized cyclodextrin based CSPs produced the best separations of all the cyclodextrin based CSPs. All five pterocarpan compounds were baseline or near baseline separated on the Cyclobond I 2000 AC CSP. The Cyclobond I 2000 RSP CSP and Cyclobond I 2000 DM CSP each separated four of the five compounds. However, separations on the Cyclobond I 2000 RSP CSP had higher resolutions than those on the Cyclobond I 2000 DMP CSP (see Table 1).

Compound 1 had the highest resolution and enantioselectivity of all of the compounds on the Cyclobond I 2000 AC CSP. The enantioselectivity, resolution, and retention factors are similar for compounds 2, 3, and 4, which are pterocarpans with substituents on the benzene ring of the benzofuran moiety. Because of these similarities, it appears that the type of substituent plays only a minor role in the enantiomeric separation on the acetylated

β-cyclodextrin CSP. Compound 5, with a methyl ether substituent on the aromatic ring of the benzopyran moiety, has a lower enantioselectivity and resolution and a higher retention factor than the other pterocarpans.

The Cyclobond I 2000 DM CSP showed similar trends to the AC CSP. On the DM CSP, pterocarpans without a substituent or with a substituent on the benzofuran moiety (compounds 1, 2, and 4), had similar resolutions and enantioselectivities. The benzopyran substituted compound 5 had lower resolution and enantioselectivity.

Conversely, compound 5 had higher enantioselectivity and resolution on the Cyclobond I 2000 RSP CSP (Figure 3A) than on the Cyclobond I 2000 DM CSP (Figure 3B). On the other derivatized cyclodextrin CSPs, compounds 1-4 showed similar separations and were always better than those for compound 5. The enantioseparation of compound 5 was the best on the RSP CSP (highest α and Rs). The nature of the benzofuran substituent appears to affect the enantioseparation on the RSP CSP. Compound 2, with a methyl ketone substituent, has a smaller resolution and enantioselectivity than compound 3, with its methyl ether substituent. The two enantiomers of the nitro-substituted pterocarpan, compound 4, were not separated.

The aromatic derivatized cyclodextrin CSPs, the Cyclobond I 2000 DMP and Cyclobond I 2000 RN, were not highly effective in the reverse phase mode, with no baseline separations despite long retention times. However, the normal phase mode was much more successful. The Cyclobond I 2000 DMP had higher resolutions and selectivities and lower retention factors. A comparison of the performance of the Cyclobond I 2000 DMP column in the reverse phase mode and the normal phase mode is shown in Figure 4. In the reverse phase mode, compound 2 shows a slight shoulder. In the normal phase mode, a much better separation of the two enantiomers is achieved.

The macrocyclic glycopeptide CSPs are also effective for the enantioseparation of these pterocarpans. From the data in Table 1, it is clear that only the pterocarpans with substituents on the benzofuran side can be separated

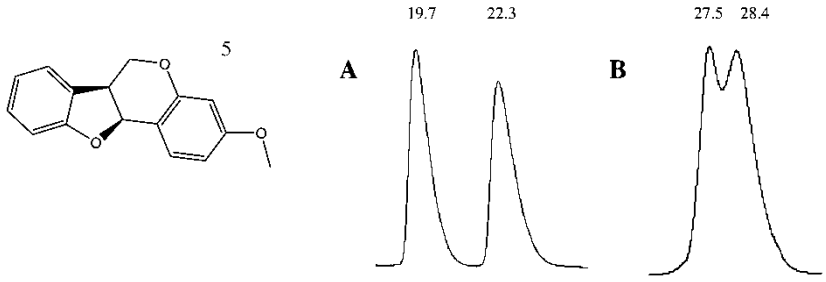


Figure 3. Enantioseparation of methyl ether substituted pterocarpan (compound 5). A) Separation on Cyclobond I 2000 RSP using 40/60 MeOH/H_20. B) Separation on Cyclobond I 2000 DM using 20/80 ACN/H_20.

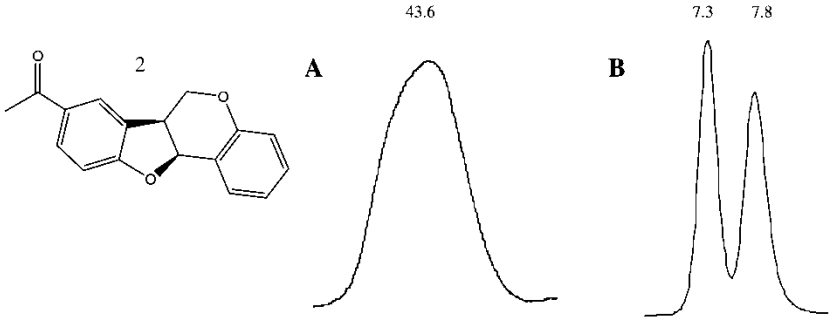


Figure 4. Comparison of separations in normal and reverse phase modes. Compound 2 was separated on the Cyclobond I 2000 DMP column using the following mobile phase conditions: A) 40/60 MeOH/H_20 and B) 95/5 Heptane/EtOH.

on the Chirobiotic columns in either the reverse phase or normal phase mode. The location of the substituent on the pterocarpan along with interactions between the substituent and chiral selector appear to be necessary for chiral recognition with the glycopeptide chiral selectors.

The principle of complementary separations for the macrocyclic glycopeptide based CSPs states, that if a compound partially separates on one of the Chirobiotic columns, it is likely it will baseline separate on one of the other columns using the same or similar mobile phase conditions.[18] Figure 5 illustrates this concept. Compound 2 separated with an α value of 1.10 and baseline resolution on the Chirobiotic V CSP. The separation on the Chirobiotic R CSP has an α value of 1.29 and over twice the resolution of the Chirobiotic V CSP. The difference in performance of the Chirobiotic V and R CSPs is much more dramatic for the separation of compound 3. In this case, a non baseline resolved set of peaks with a selectivity of 1.06 on the Chirobiotic V column, improves to a selectivity of 1.65 and a resolution of 7.07 on the Chirobiotic R column.

In the normal phase mode separations with the macrocyclic glycopeptide columns, the Chirobiotic TAG and R columns separated the most enantiomers. Again, only the pterocarpans with benzofuranyl substituents separated. Compound 4 was baseline separated on the Chirobiotic T, and TAG columns in the normal phase mode. The electron- withdrawing nitro substituent makes the aromatic systems of the pterocarpan somewhat π electron deficient, so there can be stronger π-π interactions with the aromatic systems in the stationary phase.

CONCLUSIONS

The native cyclodextrin CSPs were effective in the enantioseparation of all five pterocarpan compounds in the reverse phase mode. The Cyclobond I 2000 RSP

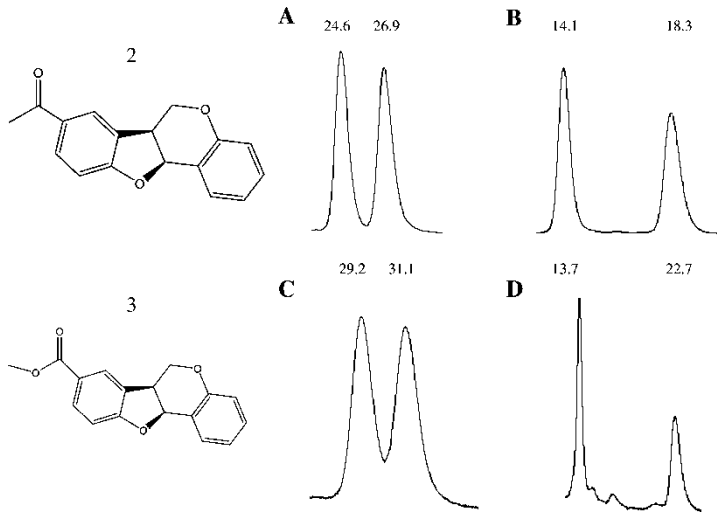


Figure 5. Enantioseparations on macrocyclic glycopeptide CSP. The methyl ketone substituted pterocarpan (compound 2) was separated on A) Chirobiotic V at 30/70 MeOH/H_2O and B) Chirobiotic R at 40/60 MeOH/H_2O. The methyl ester substituted pterocarpan (compound 3) was separated on C) Chirobiotic V at 30/70 MeOH/H_2O and D) Chirobiotic R at 40/60 MeOH/H_2O.

and Cyclobond I 2000 AC columns were the most effective of this class of CSPs. In the reverse phase mode, baseline separations were achieved for all compounds using these two CSPs. The Chirobiotic R CSP separated the most compounds in the reverse phase mode and had the best resolution and enantioselectivities found in this study. In the normal phase mode, the Cyclobond I 2000 DMP was the only cyclodextrin based CSP to show enantioselectivity. Also, in normal phase mode, some of the pterocarpans were partially separated on the Chirobiotic R and Chirobiotic V CSPs, and baseline separations were achieved for one compound on the Chirobiotic T and Chirobiotic TAG CSPs.

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