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Journal of Chromatography A, 725 (1996) 219–224

JOURNAL OF CHROMATOGRAPHY A

Comparison of chiral separations on polysaccharide chiral stationary phases to an improved Pirkle phase

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Received 16 May 1995; revised 18 August 1995; accepted 4 September 1995

Abstract

Polysaccharide chiral stationary phases (CSPs) Chiralcel OD, OJ, Chiralpak AD, AS, and a chemically derived Whelk-O 1 were evaluated for separation of different types of racemic compounds. When possible, small differences in a structure such as conversion of ketone to alcohol were compared to investigate differences in chiral separation capabilities of a given CSP. Comparison of separations on the Whelk-O 1 and polysaccharide CSPs are presented. Correlations between aromatic bulk of a molecule and chiral recognition as well as functional groups on molecules which enhance chiral recognition are discussed.

Keywords: Chiral stationary phases; Enantiomer separation

1. Introduction

Chiral chromatography continues to grow much the same way as high-performance liquid chromatography several years ago where numerous phases were being developed for specific classes of compounds. These chiral stationary phases (CSPs) are of basically four types including protein, ligand exchange, inclusion, or brush type. Whelk-O 1 is a new brush or Pirkle CSP initially developed to separate naproxen enantiomers [1,2]. Brush-type CSPs have always been useful for preparative separations due to their high chiral selectivity. However, the high chiral recognition ability of these type phases has limited their flexibility. Brush or Pirkle-type CSPs contain a small chiral selector, covalently bonded to the silica surface. Separations on these CSPs are based on a three-point interaction model where enantiomers will have three possible interaction points with the CSP. One enantiomer will interact more strongly than the other and thus it will be retained longer. Best separations occur when the analyte has structural features similar to the CSP. The separation mechanism can be predicted by evaluating the effect of incremental changes in the CSP on enantioselectivity [3]. The (*S,S*)-Whelk-O 1 CSP is illustrated in Fig. 1.

This CSP has both π-acid (*p*-nitrobenzyl) and π-base (naphthyl) functionality. This dual functionality allows separation of many more types of compounds on a single phase. The elution order of enantiomers is predictable since the stereochemistry of the CSP is known. Exceptions in elution order may result from inversion in priority based upon the Cahn–Ingold–Perlog stereochemical nomenclature system. Primary points of interaction on the Whelk-O 1 are: (1) hydrogen bonding of the amide (CSP) and a carboxyl group on the analyte; (2) $\pi-\pi$ face-to-face interaction between the dinitrobenzamide (CSP) and naphthyl (or other aromatic) on the analyte; and (3) $\pi-\pi$ face-to-face interaction

0021-9673/96/$15.00
SSDI 0021-9673(95)01001-7

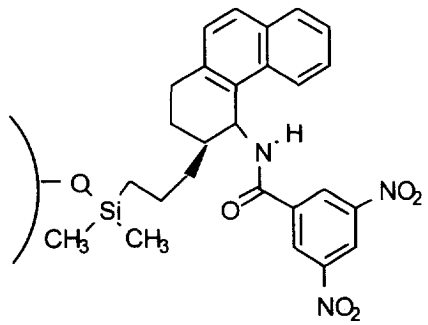

Fig. 1. Structure of (*S,S*)-Whelk-O 1 CSP.

with the tetrahydrophenenthrene system (CSP) and an aromatic function on the analyte [4]. Studies indicate that the length of the tether (carbon chain) used to bond the phase to the silica has an effect on enantioselectivity [5]. This suggests that more variations are possible to make this type CSP more versatile.

Polysaccharide phases based on cellulose and amylose have been used extensively to solve chiral separation problems [6–8]. These CSPs are very versatile, utilizing multiple mechanisms to achieve separations. Cellulose and amylose differ in configuration, cellulose being linear and amylose being helical. Manufacturing processes for these phases involve isolation of the amylose or cellulose from the natural carbohydrate. Several commercially available polysaccharide CSPs are shown in Fig. 2.

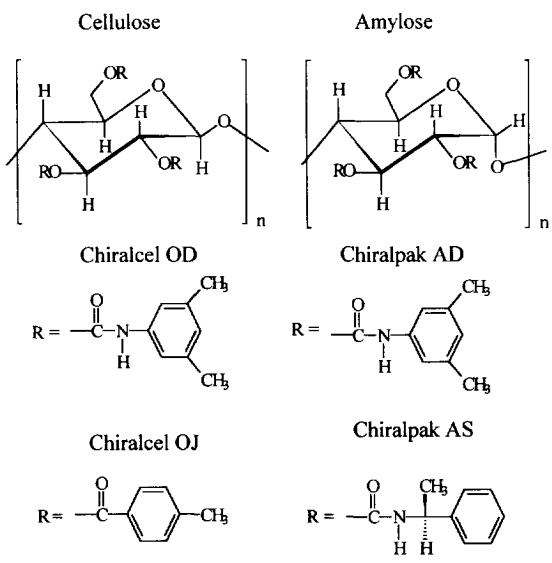

Fig. 2. Structures of carbohydrate CSP.

2. Experimental

The Whelk-O 1 columns used in these studies were purchased from Regis Chemical. Chiralcel OD and OJ (cellulose), Chiralpak AD and AS (amylose) columns were purchased from Chiral Technologies. Normal-phase chromatographic eluents were isopropanol or ethanol in hexane. Reversed-phase eluents consisted of acetonitrile and water with pH adjusted to 2.5 using phosphoric acid. Other mobile-phase modifiers investigated included acetic acid, trifluoroacetic acid, and octyl amine. All columns were 250×4.6 mm I.D. and column temperatures were controlled at 40 or 45°C. Flow-rates were 1.0 ml/min and wavelengths for the various compounds were chosen based on λ_{max}. The separations were characterized by resolution (R_s) and separation factor (α) of enantiomers.

3. Results and discussion

In order to evaluate the versatility of the selected CSPs, numerous types of compounds were chosen as candidates for evaluation. These are presented in Fig. 3. Compounds were modified by reduction of the carbonyl function (**3**,**4**) or removal of the aromatic group (**4**,**5**) to assess the relationship of a functional group on selectivity of a particular CSP.

The enantiomers of compounds **2** and **3** were separated on several of the polysaccharide phases using isopropanol–hexane (10:90). This data is presented in Table 1. Under these operating conditions, separation of these compounds on the cellulose-based OD and OJ was not as good as on the amylose-based AS and AD. Attempts to optiminize separation conditions with other alcohols did not improve the separation of **2** or **3** on the OD or OJ CSPs. This data suggests that the bulky aromatic character of these molecules and the inability to fit into the cavity of the planar cellulose phases may be

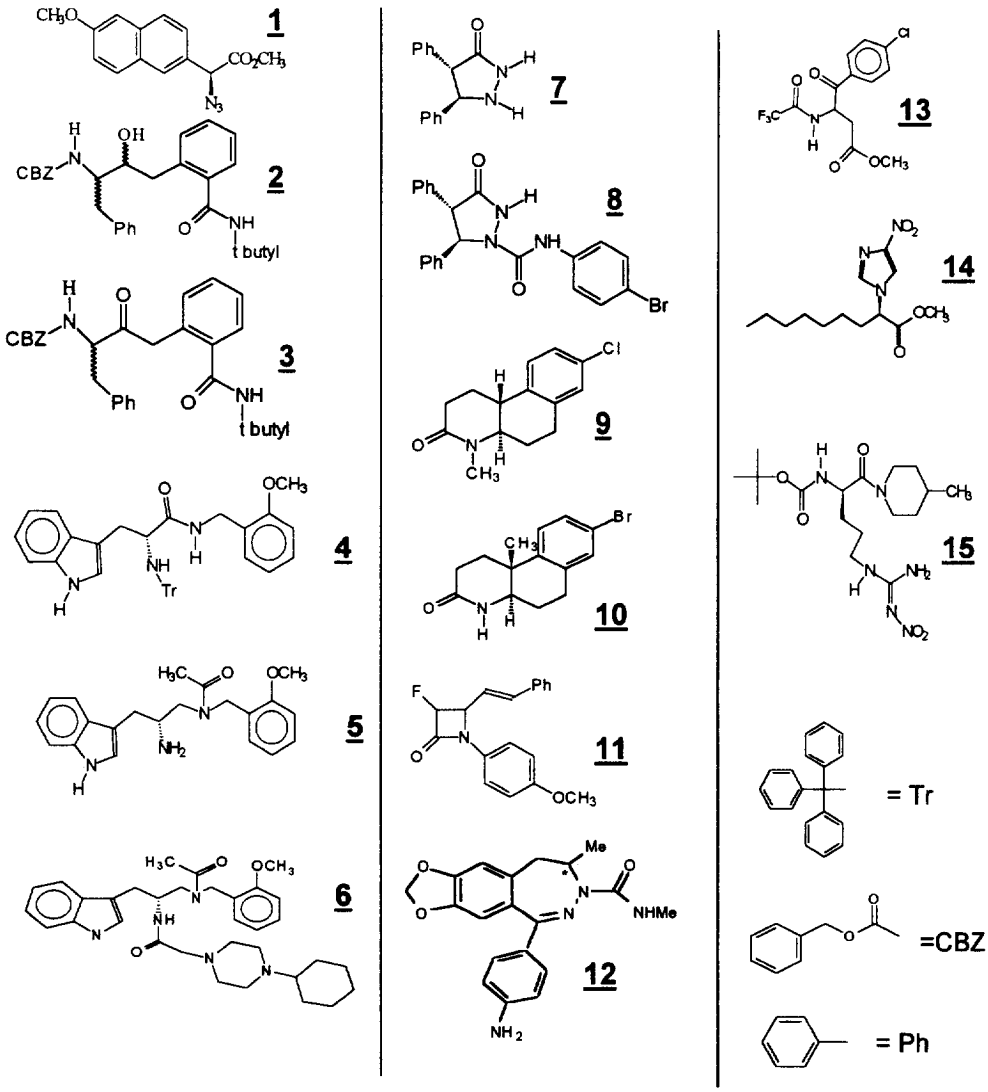

Fig. 3. Compounds separated on carbohydrate and Whelk-O 1 CSP.

Table 1
Comparison of separations on carbohydrate CSP

Compound	Stationary phase							
	Chiralcel OD		Chiralcel OJ		Chiralpak AS		Chiralpak AD	
	α	R_s	α	R_s	α	R_s	α	R_s
2	1.07	0.70	1.29	1.25	1.00	0	1.86	2.10
3	1.06	0.60	1.00	0	1.00	0.80	1.12	1.55

important in understanding the chiral recognition mechanism on polysaccharide CSPs. Evidence of steric fit of the aromatic functionality being the cause of poor chiral recognition was gained by study of phenylalanine portion on the molecules. Phenylalanine enantiomers with CBZ blocked amine functions are readily separated on the cellulose phases under the same conditions. Cellulose CSPs tend to be more suitable for linear molecules. Higher α values for separations are achieved when molecules are *para* substituted versus *ortho* or *meta* [9]. The degree of aromatic character did not appear to be as significant on the amylose phases as evidenced by R_s values greater than 1.5 for both molecules. Spatial differences between the three phenylcarbamate functions on the cellulose and amylose CSPs have been reported and may be responsible for the differences in chiral recognition mechanism of the two CSPs [10]. The pseudo helical nature of amylose allows more inclusion into the phase and better chiral recognition possibilities than the planar cellulose surface.

Work on polysaccharide CSPs suggested that selectivity could be changed by substitution of different alcohols in the eluent. Hydrogen bonding through the carbonyl oxygen on the dimethoxybenzyl group is a major interaction point on these phases and alcohols in the eluent are known to compete with analyte for these interaction sites resulting in differences in selectivity [11]. Table 2 is a summary of results obtained for **2** and **3** when ethanol is used rather than isopropanol in the eluent on the Chiralpak AD. Separation factor and resolution were improved in both cases. The Chiralpak AD was also able to resolve diastereomers of **2** from the enantiomers. Compounds **2** and **3** were separated on the Whelk-O 1 CSP as illustrated in Fig. 4. Separation factor and resolution for **3** were better on Whelk-O 1 than on the Chiralcel OD under these operating conditions.

Table 2
Comparison of alcohol on Chiralpak AD

Compound	Alcohol used in eluent			
	Ethanol		Isopropanol	
	α	R_s	α	R_s
2	2.05	2.55	1.86	2.10
3	1.67	2.55	1.15	1.55

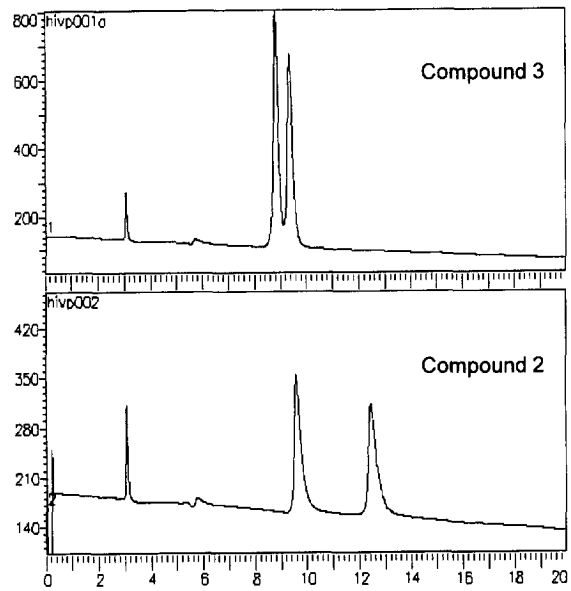

Fig. 4. Chiral HPLC chromatograms of racemates on Whelk-O 1. The chromatographic conditions are: eluent isopropanol–hexane (10:90), flow-rate 1.0 ml/min, 40°C, and UV at 230 nm.

The diastereomers of **2** were not evaluated on Whelk-O 1.

Tryptophan derivatives (**4–6**) were examined on both type phases. Compound **4** was separated on the Whelk-O 1 but not on the cellulose phases. Aromatic nature of the trityl function was suspected as being the primary reason for lack of chiral recognition. When the carbonyl function and trityl group are removed (**5,6**), separation occurs on the Chiralcel OD but not on the Whelk-O 1. Carbonyl or oxygen function and adjacent aromatic system are necessary components for chiral recognition on the Whelk-O 1 CSP. Pyrazolidinone type compounds (**7,8**) were resolved on both polysaccharide and Whelk-O 1 CSP. The α values from optimized separations were higher on the Whelk-O 1 possibly due to better steric fit of the enantiomers into the chiral selector. Separation data from these compounds indicate that increasing aromatic bulk may lead to decreasing chiral recognition ability on cellulose type CSPs. This effect is not as significant on amylose type Chiralpak AD where good separation of enantiomers **7** and **8** occurred.

Since the Whelk-O 1 CSP is chemically bonded to the silica, the column may be used in either reversed-

phase or normal mode to separate compounds. Fig. 5 is a comparison of normal- and reversed-phase separation of **8** on the Whelk-O 1. Cellulose phases such as Chiralcel OD can be used in both modes as well with proper care being taken to convert from one to another [12]. Data in this report on the polysaccharide CSPs was generated using non-aqueous systems.

A summary of comparative separation data for all compounds examined on the Whelk-O 1 and two polysaccharide CSPs is presented in Table 3. It is notable that most molecules successfully separated on the Whelk-O 1 contained a carbonyl or hydroxyl function α to the asymmetric center as well as a conjugate aromatic system. When the carbonyl function is not present (**5**) or is not adjacent to the asymmetric center (**6**,**9**,**10**), chiral recognition is poor on Whelk-O 1 CSP. The polysaccharide CSPs tend to be very sensitive to molecular size adjacent to the asymmetric center. Results from the compounds studied suggest that a high degree of aromatic character or non-linear structure (**2**,**3**,**4**,**12**) prevents interaction between the CSP and chiral center resulting in low chiral recognition on planar cellulose phases such as Chiralcel OD. Molecular bulk appears to be less important on a helical amylose phase such as the Chiralpak AD where more inclusion may occur and result in better chiral recognition.

Molecular size does not appear to be an important factor on the Whelk-O 1 as illustrated by the trityl blocked tryptophan compounds (**4**), pyrazolidinone compounds (**7**,**8**), or benzdiazepine (**12**) where a high degree of aromatic character is present. The chiral selector on the Whelk-O 1 CSP is relatively small thus allowing a good steric fit of the analyte into the CSP. Both polysaccharide and Whelk-O 1 CSP require some π character in the analyte for chiral recognition. The nitro quanidine type compound (**15**) separated on Chiralcel OD but not on Whelk-O 1. The π functionality in the form of the two carbonyl groups was sufficient for chiral recognition on Chiralcel OD.

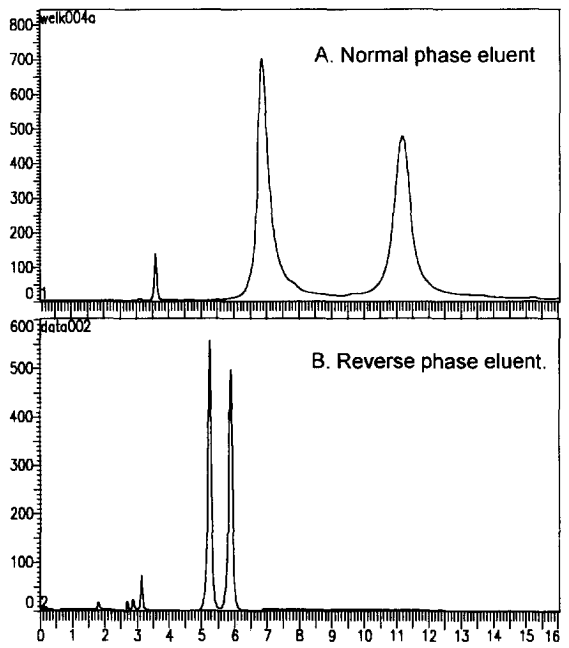

Fig. 5. Comparison of normal-phase to reversed-phase elution for compound **8**. Conditions are as follows: (A) eluent, isopropanol–hexane (45:55); flow-rate, 1.0 ml/min; temperature, 45°C; (B) eluent, acetonitrile–water (50:50), pH 2.5 with H_3PO_4; flow-rate, 1.5 ml/min; temperature, 40°C. UV detection at 230 nm for both systems.

4. Conclusions

All chiral and achiral interactions of the analyte and CSP are important considerations when designing a chiral separation. Since hydrogen bonding is a major source of chiral recognition for both types of CSPs, derivatization of carboxylic acids or eluent modifiers such as acetic acid or diethyl amine may be necessary. The polysaccharide and Whelk-O 1 CSP have been used to separate a variety of different types of compounds. Experiences with separation of many types of different compounds suggest that the polysaccharide CSPs tend to be more versatile than the Whelk-O 1. The position of molecular substituents present on the analyte is not as critical for chiral recognition on a polysaccharide CSP as on the Whelk-O 1. The Whelk-O 1 CSP is more predictable and capable of separating both π-acid and π-basic type compounds. The Whelk-O 1 is available in either *R*,*R* or *S*,*S* configuration, thus allowing some control of elution order of enantiomers as well as identification of the stereochemistry of the analyte. This is an advantage for both trace analytical and preparative applications. Steric hindrance adjacent to the asymmetric carbon does not affect stereoselec-

Table 3
Summary of compounds investigated

Compound	Whelk-O 1	Chiralcel OD	Chiralpak AD
1	α=1.34 R_s=2.10 IPA–hexane (60:40)	No evaluation	No evaluation
2	α=1.29 R_s=2.10 IPA–hexane (10:90)	α=1.07 R_s=0.70 IPA–hexane (10:90)	α=1.86 R_s=2.10 IPA–hexane (10:90)
3	α=1.10 R_s=0.95 IPA–hexane (10:90)	α=1.06 R_s=0.60 IPA–hexane (10:90)	α=1.15 R_s=1.55 IPA–hexane (10:90)
4	α=1.32 R_s=2.10 IPA–hexane (50:50)	No separation	No evaluation
5	No separation	α=1.10 R_s=0.95 IPA–hexane (10:90)	No evaluation
6	No separation	α=1.58 R_s=1.60 IPA–hexane (10:90)	No evaluation
7	α=2.17 R_s=2.20 IPA–hexane (55:45)	α=1.28 R_s=1.0 NPA–hexane (20:80)	α=1.21 R_s=1.0 EtOH–hexane (20:80)
8	α=1.57 R_s=2.20 IPA–hexane (55:45)	No separation	α=1.44 R_s=2.10 EtOH–hexane (20:80)
9	α=1.04 R_s=0.6 IPA–hexane (10:90)	α=1.14 R_s=1.5 IPA–hexane (10:90)	No evaluation
10	α=1.04 R_s=0.6 IPA–hexane (10:90)	α=1.09 R_s=1.5 IPA–hexane (10:90)	No evaluation
11	α=1.13 R_s=1.50 IPA–hexane (15:85)	α=1.18 R_s=1.5 IPA–hexane (15:85)	No evaluation
12	α=1.22 R_s=1.50 IPA–hexane (40:60)	No separation	No evaluation
13	α=1.11 R_s=1.50 IPA–hexane (10:90)	No separation	No evaluation
14	No separation	α=1.50 R_s=2.10 IPA–hexane (30:70)	No evaluation
15	No separation	α=1.69 R_s=2.00 IPA–hexane (30:70)	No evaluation

IPA=isopropanol; NPA=propanol; EtOH=ethanol.

tivity of the Whelk-O 1 to the extent that it affects selectivity on a polysaccharide CSP.

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