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# Enantiomeric separation of chiral sulphoxides

## Screening of cellulose-based sorbents with particular reference to cellulose tribenzoate

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ABSTRACT

The possibility of using cellulose tribenzoate as a chiral selector for the resolution of chiral sulphoxides was investigated on analytical and preparative scales. Ten racemic sulphoxides were separated on eight different cellulose-derivatized chiral stationary phases. As a result from this screening it turned out that Chiralcel-OB using cellulose tribenzoate (CTB) as chiral selector was the most suitable for resolving the enantiomers of sulphoxides. To obtain a better understanding of the separation mechanism, the temperature dependence of enantioselectivity ($\alpha$) was measured for Chiralcel-OB and Chiralcel-OD to determine the Gibbs–Helmholtz parameters $\Delta_{(R,S)}\Delta H^0$ and $\Delta_{(R,S)}\Delta S^0$. In addition, a partial least-squares analysis was performed to find a correlation between molecule-independent parameters and $\alpha$. Both approaches indicated that steric hindrance seems to be the main reason for chiral discrimination. A surprising result was observed for the enantiomeric separation of *p*-hydroxyphenyl methyl sulphoxide, which was significantly improved at higher temperatures. In a further study, the developed column materials and strategies, using CTB as chiral selector, were compared. It turned out that microcrystalline CTB I and CTB beads exhibit enantioselectivity equal to that of Chiralcel-OB.

## INTRODUCTION

The occurrence of optically active sulphoxides in nature was described in 1948 by both Stoll and Seebeck [1] and Schmid and Karrer [2]. Chiral sulphoxides are also formed by the metabolism of thioesters [3]. Optically pure sulphoxides have received much attention because of their stereospecific advantages in organic synthesis [4,5] and, as a consequence, the enantiomeric separation of racemic sulphoxides is of analytical and preparative interest.

The first (partial) liquid chromatographic resolution of an optically active sulphoxide was reported in 1959 by Farina *et al.* [6] on $\alpha$-lactose. Other stationary phases followed and a large number of racemic sulphoxides have been separated on "Pirkle-type" columns [7–12] and on protein-bonded stationary phases [13]. The first gas chromatographic separation of various racemic sulphoxides using Chirasil-Val as stationary phase was reported in 1985 [14]. With the introduction of chemically modified cellulose, Ichida *et al.* [15] demonstrated a new type of stationary phase particularly suitable for the separation of chiral sulphoxides. These stationary phases are today well known as Chiralcel and

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have been applied in various sulphoxide separations [16,17].

According to Ichida *et al.*'s study [15], cellulose tribenzoate seems to have the best chiral selection properties of all stationary phases investigated. Another advantage of cellulose tribenzoate is that it can be used in different ways as a stationary phase: in its microcrystalline form (CTB I) (described by Rimböck *et al.* [18]); as a coating material for phenylsilanized silica gel with a pore size of 1000 Å (described by Ichida *et al.* [15]); as a coating material for aminopropylsilanized silica gel with a pore size of 1000 Å (available as Chiralcel-OB from Daicel Chemical Industries, Tokyo, Japan); as a coating material for aminopropylsilanized silica gel with a pore size of 4000 Å (described by Okamoto *et al.* [19]; and as benzoyl cellulose beads (CTB beads) (described by Francotte and Wolf [20]). Nevertheless, in the past few years, the number of new derivatized cellulose stationary phases has increased. The first goal of this study was to screen a series of chiral sulphoxides on eight different cellulose stationary phases (Chiralcel-OA, -OB, -OC, -OD, -OF, -OG, -OJ and -OK) to establish the stationary phase with the best chiral discrimination properties.

To obtain a better insight into the separation mechanism, it was planned to measure enantioselectivity as a function of temperature and to perform a partial least-squares (PLS) analysis with the best stationary phase from the screening mentioned above.

Independently of the results from the first part of the study, it was decided in the second part to compare silica-based CTB (*e.g.*, Chiralcel-OB) with CTB I and CTB beads to investigate their further use in preparative chromatography.

## EXPERIMENTAL

### *Materials*

The structures of the sulphoxides investigated are given in Fig. 1. Racemates **1** and **5** were supplied by EGA, **2** by Fluka and **4** by Aldrich. Racemate **6** was kindly donated by D. Wasmuth and **3** and **7–10** by C.P. Mak (both of Sandoz Pharma, Basel, Switzerland).

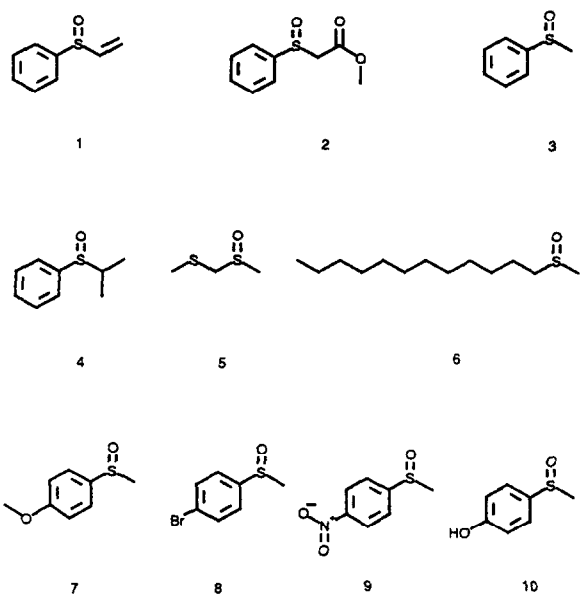



Fig. 1. Structures of investigated sulphoxides.

### *Liquid chromatography: screening with Daicel columns*

A Kontron HPLC pump (Model 420) was used in conjunction with a Kontron variable-wavelength UV detector (Model 430). The following chiral stationary phases (column: 25 cm × 0.46 cm I.D.) were purchased from Daicel Chemical Industries (Tokyo, Japan) and the cellulose derivatives were coated on silica gel with particle size 10 $\mu$m: Chiralcel-OA = cellulose triacetate; Chiralcel-OB = cellulose tribenzoate; Chiralcel-OC = cellulose tris-(phenylcarbamate); Chiralcel-OD = cellulose tris(3,5-dimethylphenylcarbamate); Chiralcel-OF = cellulose tris(4-chlorophenylcarbamate); Chiralcel-OG = cellulose tris(4-methylphenylcarbamate); Chiralcel-OJ = cellulose tris(4-methylbenzoate); and Chiralcel-OK = cellulose tricinnamate.

The mobile phase and chromatographic parameters are given in Table I.

### *Liquid chromatography: screening with CTB-containing columns*

A Kontron HPLC pump (Model 420) was used in conjunction with a Kontron variable-

wavelength UV detector (Model 430). The columns prepared and investigated are summarized in Table VII. CTB I was prepared according to ref. 18 and CTB beads according to ref. 20. CTB 3 was prepared according to ref. 19 whereas CTB 1 and CTB2 were obtained by variations of the cited approach. Tetrahydrofuran was used instead of methylene chloride to study the effect of the solvent used to dissolve CTB (in the case of CTB 2), and silica gel with a pore size of 1000 Å was chosen for CTB 1. CTB 1, having the same silica gel basis as Chiralcel-OB can therefore be used as a standard to rule out differences arising from different packing procedures.

All stationary phases were slurry packed using a suspension in hexane–2-propanol (9:1) and all columns (25 cm × 0.46 cm I.D.) were washed for 40 min with the mobile phase at a flow-rate of 4.5 ml/min. In all instances a stable baseline was achieved at 220 nm. The mobile phase and flow conditions for the above-mentioned columns are given in Table VIII.

## PLS ANALYSIS

PLS analysis was carried out according to the procedure described in detail for computer implementation by Geladi and Kowalski [21].

## RESULTS AND DISCUSSION

### *Sulphoxide screening on derivatized cellulose coated on silica gel (Daicel columns)*

To compare the retentions and enantioselectivities of all the racemates, the whole screening was done under identical conditions. The results obtained are given in Table I, showing significant differences with respect to retention time and enantioselectivity factor $\alpha$. The usefulness of all columns for the separation of sulphoxide enantiomers is obvious (an example is given for the separation of racemate 3 on Chiralcel-OG in Fig. 2). Nevertheless, it must be pointed out that Chiralcel-OB (and thus cellulose tribenzoate) has the highest resolving power of all the columns investigated.

The molecular recognition mechanism is assumed to involve the formation of $\pi-\pi$ interactions between the benzoyl group of the selector and the aromatic ring of the selectand, hydrogen bonding between the sulphoxide group and the stationary phase and steric interactions. It is obvious from our results that the individual contributions differ significantly for the investigated columns.

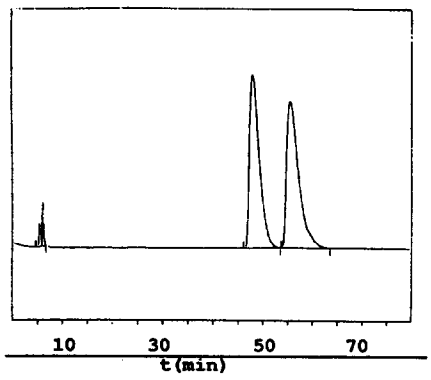



Fig. 2. Enantiomeric separation of racemate 3 on Chiralcel-OG.

Comparing the data obtained with Chiralcel-OA and Chiralcel-OB (triacetate *vs.* tribenzoate) illustrates the importance of $\pi-\pi$ interactions. Chiralcel-OA with no aromatic groups has the poorest resolution capability of all the columns investigated.

Comparing Chiralcel-OC and Chiralcel-OB (phenylcarbamate *vs.* phenyl ester) leads to suggestions about the contribution of the binding group and about the distance of the phenyl group from the stereogenic centre. With Chiralcel-OC significantly higher $k'$ and lower $\alpha$ values are obtained than with Chiralcel-OB. This may be due to strong hydrogen bonding between the NH group of the amide bond and the sulphoxide group. In addition to the $\pi-\pi$ interaction of the aromatic groups, the decrease in enantioselectivity for Chiralcel-OC can be understood in the light of the increased distance between the asymmetric centre of the stationary phase and that of the sulphoxide. This assumption is generally supported by higher $k'$ values for the investigated "carbamate-type" stationary phases.

On comparing the enantioselectivity of various sulphoxides on Chiralcel-OB, the importance of steric interactions becomes evident. The sterically more hindered $i$-$C_3H_7$ group in racemate **4**

TABLE I

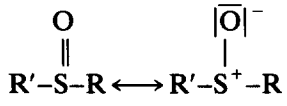
RETENTION AND ENANTIOSELECTIVITY OF CHIRAL SULPHOXIDES **1–10** ON CHIRALCEL COLUMNS

Mobile phase, *n*-hexane–2-propanol (9:1); flow-rate, 0.5 ml/min; temperature, 25°C; injection volume, 20 μl (1–2 mg/ml); UV detection at 220 nm.

| Chiralcel | **1** | | | **2** | | | **3** | | |
|---|---|---|---|---|---|---|---|---|---|
| | $k'_2$ | $\alpha$ | $R_s$ | $k'_2$ | $\alpha$ | $R_s$ | $k'_2$ | $\alpha$ | $R_s$ |
| OA | 2.06 | 1.04 | 0.32 | 10.15 | 1.00 | 0.00 | 2.55 | 1.13 | 1.62 |
| OB | 5.36 | 1.58 | 3.11 | 10.05 | 1.13 | 0.87 | 6.79 | 1.72 | 3.58 |
| OC | 9.78 | 1.18 | 2.40 | | n.e.[a] | | 13.47 | 1.08 | 0.85 |
| OD | 3.45 | 1.24 | 2.78 | 9.74 | 1.19 | 2.27 | 4.26 | 1.22 | 2.05 |
| OF | 15.19 | 1.04 | 0.45 | | n.e. | | | n.e. | |
| OG | 7.07 | 1.31 | 2.43 | 19.72 | 1.10 | 1.00 | 9.23 | 1.18 | 1.73 |
| OJ | 3.82 | 1.18 | 2.25 | 16.36 | 1.34 | 3.64 | 3.44 | 1.03 | 0.27 |
| OK | 3.04 | 1.13 | 1.24 | 12.00 | 1.00 | 0.00 | 3.97 | 1.00 | 0.00 |
| | **4** | | | **5** | | | **6** | | |
| | $k'_2$ | $\alpha$ | $R_s$ | $k'_2$ | $\alpha$ | $R_s$ | $k'_2$ | $\alpha$ | $R_s$ |
| OA | 1.06 | 1.10 | 0.94 | 3.15 | 1.00 | 0.00 | | n.e. | |
| OB | 2.84 | 1.86 | 2.63 | 7.79 | 1.53 | 2.88 | | n.e. | |
| OC | 6.93 | 1.17 | 2.11 | 15.00 | 1.06 | 0.79 | | n.e. | |
| OD | 2.46 | 1.27 | 2.79 | 5.79 | 1.11 | 1.53 | 1.74 | 1.07 | 0.63 |
| OF | 15.75 | 1.00 | 0.00 | 19.00 | 1.05 | 0.51 | 6.29 | 1.05 | 0.36 |
| OG | 4.74 | 1.20 | 1.71 | 9.79 | 1.06 | 0.46 | 2.70 | 1.00 | 0.00 |
| OJ | 1.91 | 1.11 | 1.19 | 4.64 | 1.00 | 0.00 | | n.e. | |
| OK | 1.77 | 1.00 | 0.00 | 5.98 | 1.00 | 0.00 | 1.86 | 1.00 | 0.00 |
| | **7** | | | **8** | | | **9** | | |
| | $k'_2$ | $\alpha$ | $R_s$ | $k'_2$ | $\alpha$ | $R_s$ | $k'_2$ | $\alpha$ | $R_s$ |
| OA | 5.01 | 1.22 | 1.54 | 3.41 | 1.32 | 2.08 | | n.e. | |
| OB | 1.44 | 2.12 | 1.68 | 5.44 | 1.43 | 2.50 | 3.84 | 1.00 | 0.00 |
| OC | | n.e. | | 11.81 | 1.04 | 0.43 | | n.e. | |
| OD | 5.99 | 1.10 | 1.36 | 4.22 | 1.05 | 0.77 | 12.98 | 1.00 | 0.00 |
| OF | | n.e. | | 16.65 | 1.09 | 1.09 | | n.e. | |
| OG | | n.e. | | 10.23 | 1.12 | 1.27 | | n.e. | |
| OJ | 6.83 | 1.06 | 0.68 | 4.64 | 1.00 | 0.00 | | n.e. | |
| OK | 7.12 | 1.03 | 0.29 | 4.43 | 1.00 | 0.00 | | n.e. | |
| | **10** | | | | | | | | |
| | $k'_2$ | $\alpha$ | $R_s$ | | | | | | |
| OA | 5.40 | 1.13 | 1.61 | | | | | | |
| OB | 3.57 | 1.06 | 0.29 | | | | | | |
| OC | 19.02 | 1.01 | 0.10 | | | | | | |
| OD | 17.44 | 1.00 | 0.00 | | | | | | |
| OF | | n.e. | | | | | | | |
| OG | 15.32 | 1.00 | 0.00 | | | | | | |
| OJ | | n.e. | | | | | | | |
| OK | 5.26 | 1.00 | 0.00 | | | | | | |

[a] n.e. = Not eluted within 120 min.

leads to a higher $\alpha$ value compared with the methyl group in racemate **3**, whereas the vinyl group of racemate **1** decreases the enantioselectivity slightly, owing to the more competitive character of the vinyl group to the phenyl group. Steric reasons should be more dominant if a "better" (*i.e.*, larger) sulphoxide tetrahedron is realized. The influence of phenyl substituents can be studied in this respect. Having in mind the possible resonance forms of a sulphoxide:



it is evident that the four sulphur ligands (including the electron pair) of the right-hand form can be better placed in a tetrahedral environment. Hence the capability of electron delocalization seems to improve chiral discrimination. Substituents with electron-accepting properties (such as $NO_2$) will favour a more conjugated electron system which is realized in the left-hand resonance form. Consequently, no separation was obtained for racemate **9**, whereas in the case of racemate **7**, containing an electron-donating methoxy group (thus preferring the right-hand resonance form), the highest $\alpha$ value of 2.12 was found.

### *Thermodynamic parameters of enantiomer resolution*

To obtain more information about the chiral discrimination process, it is worth examining the Gibbs–Helmholtz parameters $\Delta_{(R,S)}\Delta H^0$ and $\Delta_{(R,S)}\Delta S^0$. The difference between the free energies of association can be calculated from the difference in retention via enantioselectivity $\alpha$ according to the equation [22]:

$$-\Delta_{(R,S)}\Delta G^0 = RT \ln K_R/K_S = RT \ln \alpha \qquad (1)$$

where the subscript $R$ refers arbitrarily to the later- and $S$ to the earlier-eluting enantiomer.

The temperature dependence of the enantioselectivity $\alpha$ can be employed to calculate the Gibbs–Helmholtz parameters $\Delta_{(R,S)}\Delta H^0$ and $\Delta_{(R,S)}\Delta S^0$ of chiral recognition according to the equation

$$\ln \alpha = -\frac{\Delta_{(R,S)}\Delta H^0}{R} \cdot \frac{1}{T} + \frac{\Delta_{(R,S)}\Delta S^0}{R} \qquad (2)$$

According to eqn. 2, a plot of ln $\alpha$ against $1/T$ is linear, the slope being the difference between the enthalpy of association of the enantiomers with the stationary phase.

Temperature dependence was studied for nearly all the racemates on Chiralcel-OB and Chiralcel-OD. The results are summarized in Table II and the $\Delta_{(R,S)}\Delta H^0$ values obtained from individual curve fitting can be divided into three groups representing different types of interactions responsible for the chiral discrimination.

In the first group, where $\Delta_{(R,S)}\Delta H^0$ values between −0.05 and −0.1 kcal/mol are observed for racemates **2**, **3** and **8** on Chiralcel-OB and **2** and **8** on Chiralcel-OD, the theory of inclusion chromatography, where chiral discrimination is due only to steric hindrance, is supported because of the low $\Delta_{(R,S)}\Delta H^0$ value, excluding all other types of interaction.

In the second group, where $\Delta_{(R,S)}\Delta H^0$ values between −0.5 and −1.0 kcal/mol are observed, *e.g.*, for racemates **1**, **5** and **7** on Chiralcel-OB and **1**, **3**, **4**, **5** and **7** on Chiralcel-OD, the contribution of steric hindrance is amplified by a second type of interaction, *e.g.*, weak $\pi$–$\pi$ interactions or weak hydrogen bonding.

In the third group, with $\Delta_{(R,S)}\Delta H^0$ values of >1.0 kcal/mol, only one example was found with racemate **4** on Chiralcel-OB [$\Delta_{(R,S)}\Delta H^0$ = 1.51 kcal/mol], where chiral discrimination seems to be the result of an additional strong $\pi$–$\pi$ interaction or hydrogen bond for the most retarded enantiomer.

Most of the racemates investigated showed similar $\Delta_{(R,S)}\Delta H^0$ values for Chiralcel-OD compared with Chiralcel-OB. Only one strong deviation was observed for racemate **4** where the difference of $\Delta_{(R,S)}\Delta H^0$ is >1.0 kcal/mol, clearly demonstrating that the chiral recognition mechanism is different for the two stationary phases.

Another interesting result was obtained for racemate **10** where the sign changed (from − to +) for $\Delta_{(R,S)}\Delta H^0$, now giving a positive $\Delta_{(R,S)}\Delta H^0$ of +1.22 kcal/mol. In other words, chiral discrimination is improved with increasing

TABLE II

EFFECT OF TEMPERATURE ON ENANTIOSELECTIVITY USING CHIRALCEL-OD AND CHIRALCEL-OB

| $1/T \times 10^{-3}$ | Ln $\alpha$ | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | | **2** | | **3** | | **4** | | **5** | | **7** | | **8** | | **10** | |
| | OB | OD | OB | OD | OB | OD | OB | OD | OB | OD | OB | OD | OB | OD | OB | OD |
| 3.4111 | 0.46 | 0.26 | 0.12 | 0.20 | 0.54 | 0.26 | 0.59 | 0.25 | 0.46 | 0.09 | 0.77 | 0.15 | 0.40 | 0.04 | 0.00 | – |
| 3.3539 | 0.45 | 0.25 | 0.11 | 0.20 | 0.52 | 0.25 | 0.52 | 0.25 | 0.44 | 0.08 | 0.75 | 0.14 | 0.40 | 0.04 | 0.00 | – |
| 3.2986 | 0.44 | 0.23 | 0.11 | 0.20 | 0.51 | 0.23 | 0.48 | 0.24 | 0.42 | 0.07 | 0.74 | 0.13 | 0.41 | 0.04 | 0.06 | – |
| 3.2451 | – | 0.23 | – | 0.20 | – | 0.23 | – | 0.23 | – | 0.05 | – | 0.13 | – | 0.03 | – | – |
| 3.1932 | 0.41 | 0.21 | 0.11 | 0.20 | 0.51 | 0.22 | 0.38 | 0.21 | 0.40 | 0.04 | 0.68 | 0.13 | 0.41 | 0.03 | 0.12 | – |
| 3.1431 | – | 0.20 | – | 0.20 | – | 0.20 | – | 0.21 | – | 0.03 | – | 0.12 | – | 0.03 | – | – |
| 3.0944 | 0.40 | 0.18 | 0.13 | 0.19 | 0.52 | – | 0.35 | 0.19 | 0.35 | – | 0.63 | 0.10 | 0.39 | 0.03 | 0.18 | – |
| 3.0473 | – | 0.14 | – | 0.17 | – | 0.14 | – | 0.16 | – | – | – | 0.09 | – | – | – | – |
| $\Delta_{(R,S)}\Delta H^0$ (kcal/mol) | −0.39 | −0.58 | +0.04 | −0.12 | −0.10 | −0.59 | −1.51 | −0.47 | −0.68 | −0.44 | −0.90 | −0.30 | −0.05 | −0.08 | +1.22 | |
| $\Delta_{(R,S)}\Delta S^0$ (cal/mol · K) | −0.42 | −1.40 | +0.34 | +0.02 | +0.70 | −1.48 | −4.06 | −1.10 | −1.42 | −1.34 | −1.44 | −0.74 | +0.62 | −0.74 | +4.18 | |

temperature. The enantiomeric separation is thus entropy controlled. The effect of temperature on the enantiomeric separation of racemate **10** is demonstrated in Fig. 3. No separation occurs at room temperature whereas baseline separation is obtained at 50°C. Since the separation factor is linked to changes in the free energy of association, and the latter depends on the interplay between enthalpy change, entropy change and temperature, according to the equation

$$-\Delta_{(R,S)}\Delta G^0 = -\Delta_{(R,S)}\Delta H^0 + T\Delta_{(R,S)}\Delta S^0 \qquad (3)$$

at lower temperature the separation factor may, in principle, increase again with simultaneous reversal of the order of elution. Several runs with racemate **10** at lower temperatures down to 7°C showed no change in the order of elution. In all instances no separation was obtained. It is therefore likely that with respect to the observed non-linear behaviour, different "separation mechanisms" have to be discussed.

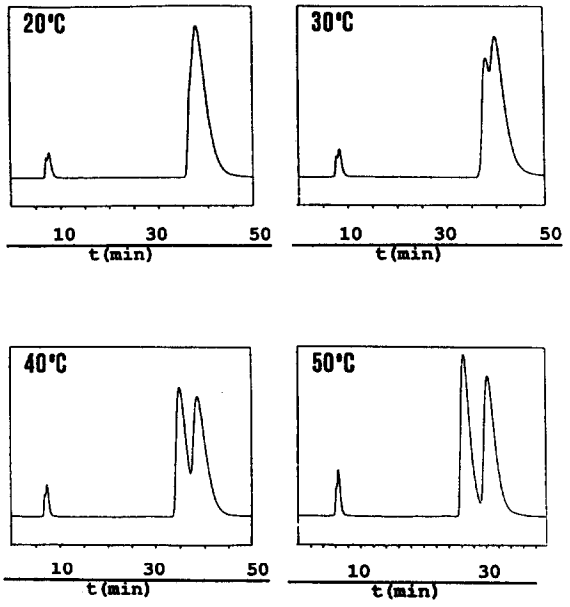



Fig. 3. Effect of temperature on enantiomeric separation of racemate **10** on Chiralcel-OB. Chromatographic conditions as in Table I except for temperature.

*Resolution of chiral sulphoxides on Chiralcel-OB: quantitative structure–retention relationship (QSRR) study*

The successful application of a QSRR study for the prediction of chiral discrimination of aryl alkyl sulphoxides on a $\pi$-acid chiral stationary phase (DACH-DNB) [23] by Altomare *et al.* [24] encouraged us to correlate their set of indepen-

TABLE III

RETENTION, SPECTROSCOPIC AND LIPOPHILICITY PARAMETERS AND QUANTUM CHEMICAL DESCRIPTORS USED IN THE QSRR OF CHIRAL SULPHOXIDES

Log $k_1$ and log $k_2$ are the logarithms of the capacity factors of the first- and second-eluted enantiomer, respectively; $S(Ph)^{HOMO}$ is the sum of the electrophilic superdelocalizabilities calculated for the aromatic carbons [(kcal/mol)$^{-1}$]; $S(S)^{LUMO}$ is the nucleophilic superdelocalizability of the chiral sulphur atom [(kcal/mol)$^{-1}$]; $q_O$ and $q_S$ are the atomic charges of oxygen and sulphur atoms, respectively; $\nu_{SO}$ is the sulphoxide stretching vibration (cm$^{-1}$) measured in 0.025 *M* $CHCl_3$ solution; and log *P* is the partition coefficient between water and *n*-octanol.

| Sulphoxide | Log $k_1$ | Log $k_2$ | Log $\alpha$ | $S(Ph)^{HOMO}$ | $S(S)^{LUMO}$ | $q_O$ | $q_S$ | $\nu_{SO}$ | Log *P* |
|---|---|---|---|---|---|---|---|---|---|
| **3** | 0.595 | 0.832 | 0.236 | 21.880 | 39.060 | −0.729 | 1.003 | 1042 | 0.55 |
| **4** | 0.185 | 0.453 | 0.270 | 22.920 | 72.460 | −0.728 | 1.013 | 1023 | 1.39 |
| **7** | 0.833 | 1.158 | 0.326 | 59.620 | 38.710 | −0.732 | 1.006 | 1035 | 0.79 |
| **8** | 0.581 | 0.736 | 0.155 | 22.870 | 15.190 | −0.724 | 1.005 | 1047 | 1.41 |
| **9** | 0.584 | 0.584 | 0.000 | 10.310 | 2.930 | −0.715 | 1.008 | 1054 | 0.29 |

dent sulphoxide parameters (quantum chemical descriptors, spectroscopic and lipophilicity parameters) with our data for retention and enantioselectivity. The independent variables, implicitly describing steric parameters of our investigated sulphoxides, were taken from Altomare *et al.*'s study [24] and PLS regression analysis [21] was applied to these data together with the dependent variable log $\alpha$ which was determined on a Chiralcel-OB column (see Table III).

PLS analysis was performed with cross-validation, which was carried out in two steps; (1) an analysis using all the explanatory variables (autoscaled) and a number of cross-validated groups equal to the number of compounds provided a cross-validated $r^2$ ($r^2_{cv}$) and the optimum number of components; (2) using this number of components, a final run of PLS without cross-validation gave the PLS model. The results of the PLS analysis are given in Table IV.

By retransformation of the PLS coefficients the linear regression coefficients given in Table V were obtained. These values, after addition of the mean value of the experimental log $\alpha$ (for all compounds), are given in Table VI. The result from PLS analysis, where an optimum correlation ($r^2 = 1.00$) was found, supports the assumption that both steric hindrance and non-bonding interactions are responsible for the chiral discrimination of sulphoxide enantiomers on Chiralcel-OB. In other words, again the ability of electron delocalization seems to be an important factor for enantioseparation.

TABLE IV

RESULTS OF PLS ANALYSIS

| Dependent variable[a] | Independent variables[a] | Cross-validated[b] $r^2_{cv}$ | Conventional $r^2$ |
|---|---|---|---|
| Log $\alpha$ | $S(Ph)^{HOMO}$, $S(S)^{LUMO}$, $q_O$, $q_S$, $\nu_{SO}$, Log *P* | 0.66 (2) | 1.00 (2) |

[a] Autoscaled.

[b] A cross-validated $r^2$ is defined as $r^2_{cv} = 1 - PRESS/SSY$, where SSY is the initial sum of *y* squares of deviations of the observed values from their mean and PRESS is the prediction error sum of squares (*i.e.*, the squared differences between the actual and "predicted" values). The optimum number of components is given in parentheses.

TABLE V

LINEAR REGRESSION COEFFICIENTS OBTAINED BY RETRANSFORMATION OF PLS COEFFICIENTS

| Descriptor | Autoscaled | Ranking of autoscaled coefficients |
|---|---|---|
| $S(Ph)^{HOMO}$ | +0.32 | 2 |
| $S(S)^{LUMO}$ | +0.22 | 3 |
| $q_O$ | −0.39 | 1 |
| $q_S$ | −0.14 | 5 |
| $\nu_{SO}$ | −0.22 | 4 |
| Log $P$ | +0.07 | 6 |

*Comparison of CTB-containing stationary phases*

The results indicated that cellulose tribenzoate (CTB) has the best selection properties for the enantiomeric separation of sulphoxides. Several approaches with different designs for stationary phases containing CTB have been described in order to improve further this chiral selector and to widen its applicability in preparative chromatography. It was therefore decided to check these previous strategies first on an analytical scale. The prepared and investigated columns are given in Table VII. Microcrystalline cellulose tribenzoate (CTB I) was prepared as described by Rimböck *et al.* [18] and cellulose tribenzoate beads (CTB beads), where dissolved CTB was precipitated in the presence of sodium lauryl sulphate, as described by Francotte and Wolf [20]. CTB 3, where CTB was used as a coating material for aminopropylsilanized silica gel, was prepared according to Okamoto *et al.* [19], whereas CTB 1 and CTB 2 are variations of the cited approach (details of all investigated columns are given in Table VII, in the Experimental section and in the cited references). The approach introduced by Ichida *et al.* [15], which is a version of CTB 1, where the silica surface was phenylsilanized (according to Okamoto *et al.* [25]), was not taken into consideration because of too low $\alpha$ values (*e.g.*, $\alpha = 1.0$ for racemate **3** and 1.23 for racemate **1** [15]).

TABLE VI

COMPARISON OF OBSERVED AND PREDICTED ENANTIOSELECTIVITIES

| Sulphoxide | Log $\alpha$ (observed) | Log $\alpha$ (predicted) |
|---|---|---|
| **3** | 0.236 | 0.244 |
| **4** | 0.270 | 0.268 |
| **7** | 0.326 | 0.334 |
| **8** | 0.155 | 0.158 |
| **9** | 0.000 | 0.002 |

The results of the investigation are given in Table VIII, and can be summarized as follows (it should be mentioned that $k'$ values obtained with Chiralcel-OB can not directly be compared with the other investigated columns, because of possible differences during the packing procedure).

*Comparison of CTB 1, CTB 2 and CTB 3.* It is obvious from our results that tetrahydrofuran should be used instead of methylene chloride for the coating procedure of CTB. A significant improvement in enantioselectivity is observed, which supports the assumption of differences in inclusion as the mechanism responsible for chiral discrimination. It is speculated that the con-

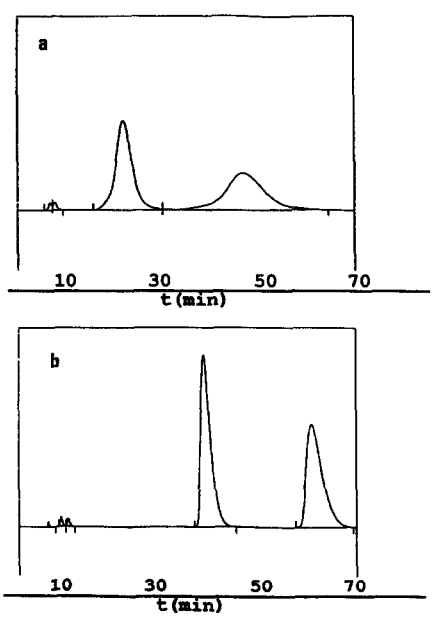



Fig. 4. Enantiomeric separation of racemate **3** on (a) CTB 1 and (b) Chiralcel-OB. Chromatographic conditions as in Table VIII.

TABLE VII

CHARACTERIZATION OF INVESTIGATED COLUMNS USING CELLULOSE TRIBENZOATE AS CHIRAL SELECTOR

*Silica gel-based stationary phases*

| Column | Basis: silica gel | | | Chemical modification | Solvent used for coating procedure | Content of N (%)[b] | Content of CTB (%) |
|---|---|---|---|---|---|---|---|
| | Particle size (μm)[a] | Pore size (Å)[a] | Supplier | | | | |
| Chiralcel-OB | 10 | 1000 | Daicel | Aminopropyl[a] | Not known | Not known | Not known |
| CTB 1 | 7 | 1000 | MN[c] | Aminopropyl[d] | THF[e] | 0.14 | 13.9 |
| CTB 2 | 10 | 4000 | MN[c] | Aminopropyl[d] | THF[e] | 0.15 | 10.6 |
| CTB 3 | 10 | 4000 | MN[c] | Aminopropyl[d] | MEC[e] | 0.15 | 4.8 |

*Cellulose tribenzoate beads*

| Column | Basis: sodium lauryl sulphate | | Solvent used for preparation | Content of CTB (%) |
|---|---|---|---|---|
| | Particle size (μm) | Preparation | | |
| CTB beads | 30[f] | Literature[f] | MEC–heptanol[e] | 100 |

*Microcrystalline cellulose tribenzoate*

| Column | Basis: cellulose microcrystalline | | Chemical modification | In | Content of CTB (%) |
|---|---|---|---|---|---|
| | Particle size (μm) | Supplier | | | |
| CTB (I) | <20[a] | Merck[g] | Benzoylated | Pyridine | 100 |

[a] Data from supplier.
[b] Silica gel after treatment with 3-aminopropyltriethoxysilane.
[c] Macherey–Nagel.
[d] Modification of silica gel according to ref. 19.
[e] THF = tetrahydrofuran; MEC = methylene-chloride.
[f] Data from ref. 20.
[g] Avicel (Merck No. 2331).

formation of CTB is different in methylene chloride and tetrahydrofuran, and that the different conformation is retained after each solvent is removed. The same conclusion was made by Ichida *et al.* [15] for cellulose triacetate. With the exception of racemates **3** and **7**, no enantiomeric separation could be achieved with CTB 3, whereas with CTB 2 nearly all the racemates were separated. An additional improvement in enantioselectivity was obtained by using silica gel with a pore size of 1000 Å instead 4000 Å, which seems to be simply the result of a higher CTB content in CTB 1 compared with CTB 2 (see Table VII).

TABLE VIII

RETENTION AND ENANTIOSELECTIVITY OF CHIRAL SULPHOXIDES ON VARIOUS CHIRAL COLUMNS USING CELLULOSE TRIBENZOATE AS CHIRAL SELECTOR

| Column | Conditions[a] | 1 | | | 2 | | | 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $k_2'$ | $\alpha$ | $R_s$ | $k_2'$ | $\alpha$ | $R_s$ | $k_2'$ | $\alpha$ | $R_s$ |
| OB | 1 | 5.36 | 1.58 | 3.11 | 10.05 | 1.13 | 0.87 | 6.79 | 1.72 | 3.58 |
| CTB 1 | 1 | 2.98 | 1.29 | 1.65 | 6.24 | 1.00 | 0.00 | 5.82 | 2.59 | 1.69 |
| CTB 2 | 1 | 2.17 | 1.00 | 0.00 | 6.18 | 1.00 | 0.00 | 5.81 | 2.75 | 1.82 |
| CTB 3 | 1 | 3.99 | 1.00 | 0.00 | 13.42 | 1.00 | 0.00 | 7.01 | 1.73 | 0.62 |
| CTB beads | 2 | 24.50 | 1.69 | 1.71 | 42.28 | 1.13 | 0.33 | 30.91 | 2.09 | 2.12 |
| CTB beads | 3 | 1.58 | 1.57 | 1.11 | 1.26 | 1.00 | 0.00 | 1.61 | 1.76 | 1.24 |
| CTB I | 3 | 2.00 | 1.25 | 0.37 | 2.32 | 1.00 | 0.00 | 2.82 | 1.87 | 1.26 |
| | | **4** | | | **5** | | | **7** | | |
| | | $k_2'$ | $\alpha$ | $R_s$ | $k_2'$ | $\alpha$ | $R_s$ | $k_2'$ | $\alpha$ | $R_s$ |
| OB | 1 | 2.84 | 1.86 | 2.63 | 7.79 | 1.53 | 2.88 | 1.44 | 2.12 | 1.68 |
| CTB 1 | 1 | 1.32 | 1.62 | 0.79 | 3.66 | 1.34 | 0.63 | 6.51 | 1.85 | 1.27 |
| CTB 2 | 1 | 1.08 | 1.47 | 0.57 | 3.23 | 1.25 | 0.50 | 5.69 | 1.67 | 0.83 |
| CTB 3 | 1 | 1.79 | 1.00 | 0.00 | 5.96 | 1.00 | 0.00 | 10.92 | 1.38 | 0.45 |
| CTB beads | 2 | 13.20 | 2.06 | 1.73 | 28.73 | 1.80 | 1.80 | | n.e.[b] | |
| CTB beads | 3 | 0.88 | 1.26 | 0.26 | 1.05 | 1.24 | 0.35 | 2.54 | 2.07 | 1.68 |
| CTB I | 3 | 1.28 | 1.00 | 0.00 | 1.57 | 1.00 | 0.00 | 3.50 | 1.77 | 1.09 |
| | | **8** | | | **9** | | | **10** | | |
| | | $k_2'$ | $\alpha$ | $R_s$ | $k_2'$ | $\alpha$ | $R_s$ | $k_2'$ | $\alpha$ | $R_s$ |
| OB | 1 | 5.44 | 1.43 | 2.50 | 3.84 | 1.00 | 0.00 | 3.57 | 1.06 | 0.29 |
| CTB 1 | 1 | 3.09 | 1.35 | 0.61 | | n.e. | | 8.13 | 1.07 | 0.18 |
| CTB 2 | 1 | 2.90 | 1.28 | 0.54 | 21.32 | 1.28 | 0.45 | 5.99 | 1.15 | 0.35 |
| CTB 3 | 1 | 5.72 | 1.00 | 0.00 | | n.e. | | 8.65 | 1.00 | 0.00 |
| CTB beads | 2 | 20.57 | 1.55 | 1.36 | | n.e. | | 12.13 | 1.09 | 0.22 |
| CTB beads | 3 | 1.79 | 1.54 | 1.03 | 2.98 | 1.31 | 0.74 | 0.49 | 1.00 | 0.00 |
| CTB I | 3 | 2.76 | 1.34 | 0.50 | 4.85 | 1.13 | 0.26 | 0.78 | 1.00 | 0.00 |

[a] Chromatographic conditions as in Table I except (1) mobile phase = hexane–2-propanol (9:1) and flow-rate = 0.5 ml/min; (2) mobile phase = hexane–2-propanol (9:1) and flow-rate = 1.0 ml/min; and (3) mobile phase = methanol and flow-rate = 0.5 ml/min.

[b] n.e. = Not eluted within 150 min.

*Comparison of CTB 1 and Chiralcel-OB.* CTB 1 exhibits enantioselectivity with the same order of magnitude as Chiralcel-OB. The differences obtained for some $\alpha$ values [*e.g.*, $\alpha$ (rac. 1, OB) > $\alpha$ (rac. 1, CTB 1) and $\alpha$ (rac. 3, OB) < $\alpha$ (rac. 3, CTB 1)] may indicate that Daicel used a solvent other than tetrahydrofuran for the coating procedure. The separations of racemate **3** on both columns are shown in Fig. 4.

*Comparison of CTB beads with Chiralcel-OB and CTB I.* CTB beads offer the possibility of using either hexane–2-propanol (9:1) or methanol as the mobile phase, thus allowing a comparison with Chiralcel-OB and CTB I. It is

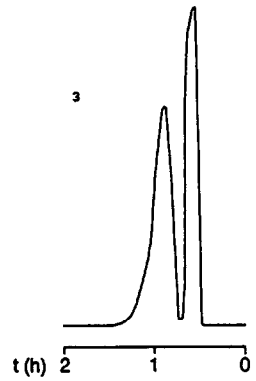



Fig. 5. Enantiomeric separation of 1.2 g of racemate **3** on 120 g of microcrystalline cellulose tribenzoate. Column, 4 cm I.D.; mobile phase, methanol; flow-rate, 5 ml/min; temperature, 25°C; UV detection at 220 nm.

evident from our results that for both mobile phases with CTB beads similar $\alpha$ values were obtained as with Chiralcel-OB. The different polarity of the mobile phase only influenced $k'$. This result additionally supports the assumption that steric hindrance is the major mechanism of chiral discrimination. The comparison of CTB beads with CTB I is of considerable interest because both stationary phases have advantages in preparative liquid chromatography owing to their high loadabilities (an example of a successful preparative separation is given in Fig. 5, where 1.2 g of racemate **3** were baseline separated on 120 g of CTB I within 90 min). As can be seen from Table VIII, CTB beads exhibit some higher $\alpha$ values, combined with lower retention times, compared with CTB I. A higher productivity can therefore be expected with CTB beads. The separation for racemate **5** on the above-mentioned columns is illustrated in Fig. 6.

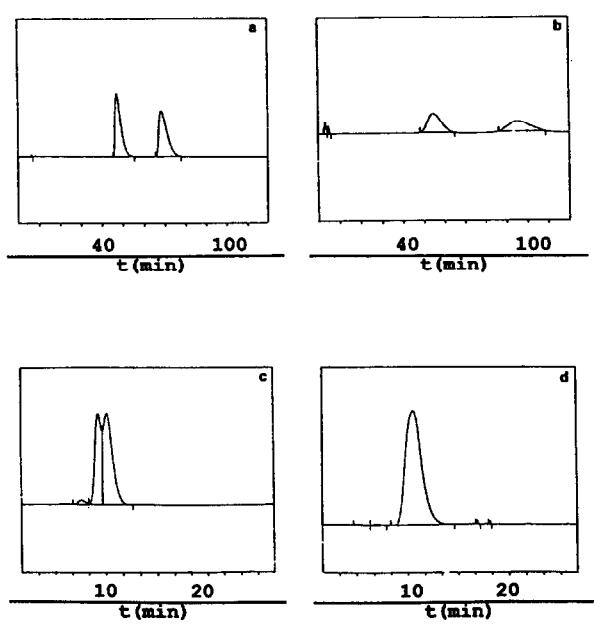



Fig. 6. Enantiomeric separation of racemate **5** on: (a) Chiralcel-OB, chromatographic conditions 1 in Table VIII; (b) CTB beads, chromatographic conditions 2 in Table VIII; (c) CTB beads, chromatographic conditions in Table VIII; (d) CTB I, chromatographic conditions 3 in Table VIII.

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