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# Chromatographic resolution on methylbenzoylcellulose beads

# Modulation of the chiral recognition by variation of the position of the methyl group on the aromatic ring

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ABSTRACT

The preparation of cellulose-based chiral stationary phases (CSPs) in the pure polymer form (*i.e.*, without an achiral support such as silica gel), according to a process already reported for benzoyl cellulose, was extended to substituted benzoylcellulose derivatives. The chiral recognition abilities of benzoylcellulose and *o*-, *m*- and *p*-methylbenzoylcellulose were compared. The four CSPs are shown to exhibit different selectivities and in numerous instances an inversion of the elution order was observed on the different methylbenzoylcellulose CSPs, indicating that the CSPs undergo different interactions with the same racemic solute. The broad variety of racemic structures that can be resolved on the four CSPs indicate the great application potential of the new cellulose-based sorbents. This should be the case especially for preparative separations, as the materials are not deposited on an achiral supporting matrix and thus have a higher loading capacity than coated CSPs.

## INTRODUCTION

There is an increasing demand for the isolation of the enantiomerically pure forms of new chiral biologically active compounds owing to the necessity to investigate the relationships between the stereochemistry of these compounds and their biological activity. Therefore, the individual enantiomers should be isolated easily and at low cost with high optical purity, in amounts adequate for performing biological tests, toxicological studies and even, at a later stage, clinical or field tests. Chromatography on chiral stationary phases has been recognized as a useful tool in respect of these requirements and is now established as a simple, efficient and generally applicable method for this purpose.

Among the numerous chiral stationary phases (CSPs) that have been investigated and developed during the last decade, cellulose-based phases have been identified as very versatile and useful chiral sorbents for the separation of enantiomers [1]. Numerous applications have been reported on microcrystalline cellulose triacetate (CTA I) used in the pure polymeric form [1] and a variety of other cellulose derivatives have been introduced for the same purpose by Okamoto *et al.* [1] as a coating of *ca.* 20% polymer weight on silica. Most of these silica-coated materials are now commercially available and they show very different selectivities depending on the derivatizing groups on cellulose.

We recently developed a process for the preparation of benzoylcellulose beads in the pure cellulosic form, avoiding the use of the expensive macroporous silica support, and producing a material that consists of 100% chiral sorbent, resulting in a high loading capacity [2,3]. Owing to the excellent chro-

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matographic properties and its high loading capacity, this new chiral sorbent is useful for both analytical applications and preparative purposes. Using the same procedure we prepared further benzoylcellulose derivatives in the pure polymer form [2] and examined their chiral recognition abilities. In this study, we investigated the influence of the position of the methyl group of methylbenzoylcellulose on the chiral recognition abilities of the CSP. For this purpose a series of racemic compounds were chromatographed on benzoylcellulose and on *o*-, *m*- and *p*-methylbenzoylcellulose.

## EXPERIMENTAL

### *Preparation of the chiral phases*

*General procedure for the preparation of methylbenzoylcellulose (ortho-, meta- or para).* To a suspension of 10 g of microcrystalline cellulose in a mixture of 100 ml of pyridine and 45 ml of triethylamine containing 0.2 g of dimethylaminopyridine are slowly added at room temperature 40 g of toluoyl chloride (*ortho-, meta-* or *para*). The temperature rises to 40–50°C. After the addition, the mixture is stirred for 20 h at 120°C under nitrogen. After cooling to room temperature, the solid mass obtained is treated with 4 l of methanol. The insoluble part is filtered and washed twice with methanol. The product is dissolved in dichloromethane and precipitated twice in ethanol. The light brown powder is dried for 2 days at 100°C under vacuum. Yield: 95–98%. Elemental analysis: methylbenzoylcellulose (*ortho-, meta-* or *para-*) $(C_{30}H_{28}O_8)_n$, calculated, C 69.8, H 5.5, O 24.8%; *o*-methylbenzoylcellulose (OMBC), found C 69.2, H 5.5, O 25.3%; *m*-methylbenzoylcellulose (MMBC), found C 69.3, H 5.5, O 25.2%; *p*-methylbenzoylcellulose (PMBC), found C 69.5, H 5.6, O 24.9%.

*p-Methylbenzoylcellulose beads.* A solution of 10 g of *p*-methylbenzoylcellulose dissolved in a mixture of 300 ml of dichloromethane and 50 ml of heptanol is added dropwise to an aqueous solution (240 ml) of sodium lauryl sulphate (0.7%) stirred mechanically at 400 rpm in a 1-l flask. After the addition, stirring is maintained and the emulsion is heated at 40–42°C (bath temperature) to remove the dichloromethane slowly by distillation. After complete removal of dichloromethane, the residual suspension is filtered and the solid is washed first with water and then with ethanol. The powder is dried under vacuum at 80°C for 20 h (yield 9.5 g, 95%). The material consists of more or less spherical particles of cellulose benzoate as determined by electron microscopy [3] (size 5–10 μm) and can be used directly for chromatographic separations. The specific surface are determined by the BET method was 69 $m^2/g$. Differential scanning calorimetric (DSC) analysis of the material showed a melting point at 267°C.

*m-Methylbenzoylcellulose beads.* Analogously, 10 g of *m*-methylbenzoylcellulose in 300 ml of dichloromethane and 50 ml of heptanol are added to an aqueous solution (240 ml) of sodium lauryl sulphate (0.7%). After removal of the dichloromethane, the solid is washed and dried (yield, 9.4 g, 94%). The particle size was 10–20 μm, specific surface area 58 $m^2/g$ and melting point by DSC 246°C.

*o-Methylbenzoylcellulose beads.* A solution of 5 g of *o*-methylbenzoylcellulose dissolved in a mixture of 300 ml of tetrahydrofuran (THF) and 12 ml of heptanol is added dropwise to an aqueous solution (300 ml) of sodium lauryl sulphate (0.7%) stirred mechanically at 400 rpm in a 1-l flask. After the addition, stirring is maintained and the emulsion is heated at 70–75°C (bath temperature) to remove the THF slowly by distillation. After complete removal of THF the residual suspension is filtered and the solid is washed first with water and then with ethanol. The powder is dried under vacuum at 80°C for 20 h (yield 4.9 g, 98%). The material consists of more or less spherical particles of cellulose methylbenzoate (size 5–20 μm) and can be used directly for chromatographic separations. The specific surface area determined by the BET method was 36 $m^2/g$. DSC analysis of the material showed a melting point at 246°C.

### *Chromatographic conditions*

The methylbenzoylcellulose beads were slurry packed as a suspension in hexane–isopropanol (9:1) or methanol in stainless-steel high-performance liquid chromatographic (HPLC) columns (25 × 0.46 cm I.D.). The columns were washed with the same mobile phase until UV absorption was no longer detected at a wavelength of 254 nm. Chromatography was performed using the same mobile phase composition (hexane–isopropanol or metha-

nol) at a constant flow-rate (usually 1 ml/min). The dead time of the columns was determined by injection of 1,3,5-tri-*tert.*-butylbenzene used as a non-retained compound. Typically, 10 $\mu$l of a 1% solution of racemate dissolved in the mobile phase used for chromatography were injected.

*General*

Solvents were of analytical-reagent grade. All compounds synthesized in this work were characterized spectroscopically ($^1$H NMR, IR) and gave satisfactory elemental analyses. NMR measurements were recorded on a Bruker VM 250-MHz spectrometer. X-ray measurements were performed on a Philips powder diffraction instrument as described previously [3]. Melting points were determined by DSC on a Mettler TA3000 system.

*Apparatus*

The HPLC experiments were performed with a modular liquid chromatograph composed of an Altex Model 110A pump and a Shimadzu Model UV-120-02 multi-wavelength UV detector in series with a Perkin-Elmer Model 241 LC polarimeter equipped with an 80-$\mu$l cell (length 10 cm). Both signals (UV absorption and optical rotation) were recorded and processed by an IBM PC-AT3 microcomputer, via a DYSC WD 24 analogue interface module using MAXIMA 820 chromatographic software (Carlo Erba, Milan, Italy).

## RESULTS AND DISCUSSION

*Preparation of stationary phases*

Analogously to the preparation of benzoylcellulose beads reported recently [3], *p*- and *m*-methylbenzoylcellulose beads were prepared from a suspension of the corresponding cellulose derivative dissolved in dichloromethane, in water containing sodium lauryl sulphate and in the presence of a long aliphatic chain alcohol (Fig. 1). In the case of *o*-methylbenzoylcellulose, when using dichloromethane as a solvent, the specific surface area of the beads produced is very low, resulting in very bad chromatographic properties. In contrast, when *o*-methylbenzoylcellulose (OMBC) is dissolved in THF, a good specific surface area is obtained, yielding an efficient chromatographic support material. X-ray investigations (Fig. 2) and DSC measure-

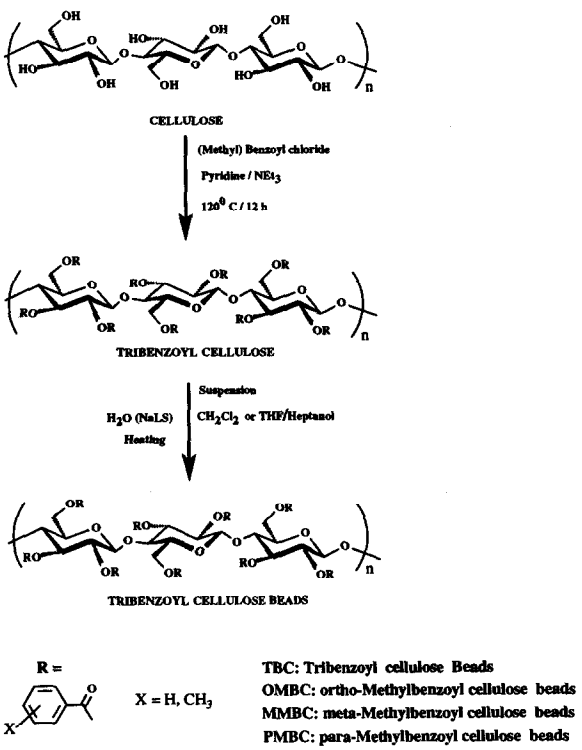



Fig. 1. Preparation of methylbenzoylcellulose beads.

ments (see Experimental) indicate that the different cellulose derivatives are at least partially crystalline, confirming the necessity to have a certain supramolecular structure in the polymer in order to achieve good chiral recognition.

*Chromatographic resolutions*

In order to investigate the influence of the position of the methyl group attached to the benzoyl moiety on the chiral recognition ability, various classes of racemates were chromatographed on benzoylcellulose and on the different methylbenzoylcellulose derivatives. The cellulose-based chiral stationary phases were found to exhibit very different selectivities, behaving like completely different sorbents.

The acetate derivatives of various racemic aliphatic alcohols or diols were chromatographed on the different benzoylcellulose esters reported in this work and the results are summarized in Table I. The best resolutions of the enantiomers are clearly obtained with unsubstituted tribenzoylcellulose

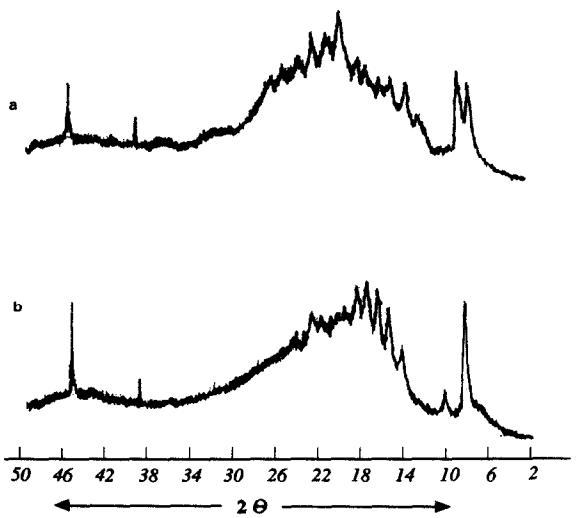



Fig. 2. X-ray powder diffractograms of (a) MMBC and (b) PMBC beads (after annealing of the samples for 45 min at 200°C).

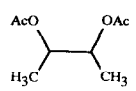
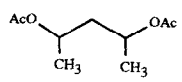
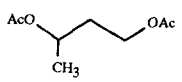
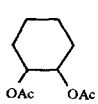
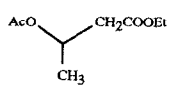
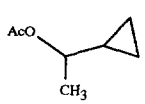
(TBC), whereas the substituted methylbenzoyl derivatives exhibit only poor chiral recognition, independently of the position of the methyl group. *p*-Methylbenzoylcellulose (PMBC) shows virtually no selectivity. These results indicate that TBC is the most appropriate CSP for relatively small molecules, especially for aliphatic compounds (Fig. 3), as we have illustrated more widely in a previous paper [3].

We have reported previously [3] that numerous racemic aryl alkanols can be well resolved on TBC beads. The comparative results presented in Table II confirm that TBC is generally the most appropriate stationary phase for the resolution of this class of compounds, except for **12**, which is not resolved on TBC but very well on MMBC. Only partial resolutions could be achieved for phenylethanol (**7**) and phenylpropanol (**8**) on MMBC and PMBC. Moreover, in the series of phenylalkanol derivatives **7**–

TABLE I

CHROMATOGRAPHIC RESULTS FOR ACETATE DERIVATIVES OF ALIPHATIC ALCOHOLS

$k'_2$ = Capacity factor of the more strongly retained enantiomer and, in parentheses, its absolute configuration or sign of the optical rotation at 365 nm; $\alpha$ = separation factor; HPLC column, 25 × 0.46 cm I.D.; mobile phase, hexane–2-propanol (90:10). Ac = Acetyl; Et = ethyl.

| Compound | Structure | Parameter | Chiral stationary phase | | | |
|---|---|---|---|---|---|---|
| | | | TBC | OMBC | MMBC | PMBC |
| **1** |  | $k'_2$ | 6.98 (+) | 1.82 | 10.66 (−) | 2.35 |
| | | $\alpha$ | 3.13 | 1.00 | 1.21 | 1.00 |
| **2** |  | $k'_2$ | 1.80 (−) | 0.59 (−) | 2.28 | 2.18 |
| | | $\alpha$ | 1.32 | 1.00 | 1.00 | 1.00 |
| **3** |  | $k'_2$ | 10.24 (−) | 1.78 | 4.75 | 3.54 |
| | | $\alpha$ | 2.14 | 1.00 | 1.11 | 1.00 |
| **4** |  | $k'_2$ | 3.62 (−) | 0.57 | 2.28 (+) | 2.14 |
| | | $\alpha$ | 1.48 | 1.00 | 1.22 | 1.00 |
| **5** |  | $k'_2$ | 5.04 (+) | 1.39 (+) | 2.60 | 2.72 |
| | | $\alpha$ | 1.40 | 1.0 | 1.00 | 1.00 |
| **6** |  | $k'_2$ | 2.67 (−) | 1.86 | 0.90 (−) | 0.10 (−) |
| | | $\alpha$ | 1.19 | 1.00 | 1.0 | 1.0 |

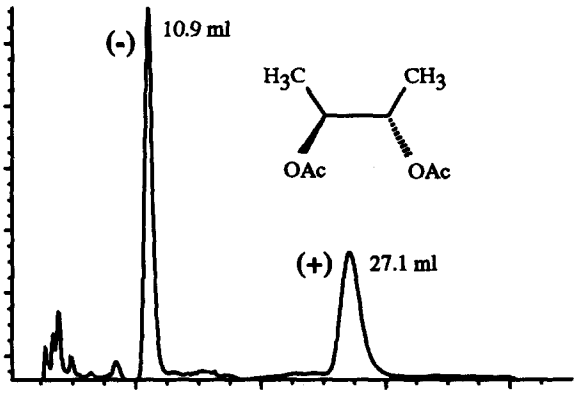



Fig. 3. Chromatographic separation of the enantiomers of racemic 2,3-butanediol diacetate (**1**) on TBC beads. HPLC column, 25 × 0.46 mm I.D.; mobile phase, hexane–2-propanol (9:1); flow-rate, 1 ml/min.

**11**, the separation factors and the capacity factors decrease on TBC as the chiral centre carrying the alcohol function is further away from the phenyl ring. The inversion of the elution order observed for **12** as the position of the methyl group on the CSP is changed from the *ortho* to the *meta* position and again from the *meta* to the *para* position clearly demonstrates that small alterations of the chemical structure of the cellulose-based CSP (even far away from the chiral carbon centres located on the sugar moieties) can totally modify the chiral recognition ability of the CSPs.

The chromatographic results summarized in Table III for a series of 3-phenylcyclopentanone and cylohexanone derivatives show that the chiral recognition is strongly dependent on the substituent attached to the phenyl ring of the racemic solute. The enantiomers of the 3-phenylcyclopentanone derivatives **17** and **18** are only resolved on MMBC, but the elution order is reversed for both compounds (*S* more retained for **17** and *R* for **18**), indicating that the interaction mechanisms are different. Both compounds have been preparatively re-

TABLE II

CHROMATOGRAPHIC RESULTS FOR ARYLAKANOLS

For definitions and chromatographic conditions, see Table I.

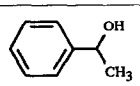
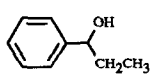
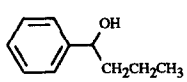
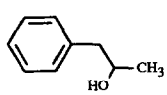
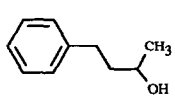
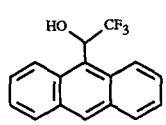
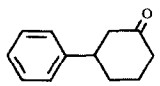
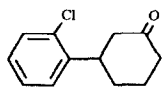
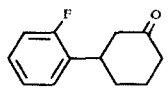
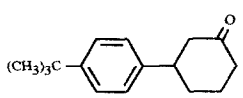
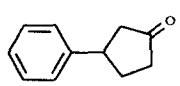
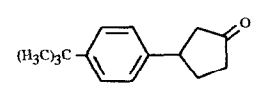
| Compound | Structure | Parameter | Chiral stationary phase | | | |
|---|---|---|---|---|---|---|
| | | | TBC | OMBC | MMBC | PMBC |
| **7** |  | $k'_2$<br>$\alpha$ | 5.06 (*R*)<br>1.76 | 0.63 (*R*)<br>1.0 | 3.81 (*R*)<br>1.15 | 3.95 (*R*)<br>1.13 |
| **8** |  | $k'_2$<br>$\alpha$ | 2.72 (*R*)<br>1.43 | 0.54 (*R*)<br>1.0 | 3.02 (*R*)<br>1.12 | 3.35 (*R*)<br>1.05 |
| **9** |  | $k'_2$<br>$\alpha$ | 2.62 (+)<br>1.48 | 0.38 (+)<br>1.0 | 2.38<br>1.00 | 2.62<br>1.00 |
| **10** |  | $k'_2$<br>$\alpha$ | 2.03 (+)<br>1.10 | 0.57 (+)<br>1.0 | 2.41 (−)<br>1.0 | 3.08<br>1.00 |
| **11** |  | $k'_2$<br>$\alpha$ | 1.47 (+)<br>1.0 | 0.46<br>1.0 | 2.26<br>1.00 | 2.62<br>1.00 |
| **12** |  | $k'_2$<br>$\alpha$ | 5.92<br>1.00 | 3.97 (*S*)<br>1.25 | 38.14 (*R*)<br>1.84 | 22.80 (*S*)<br>1.18 |

TABLE III

CHROMATOGRAPHIC RESULTS FOR PHENYLCYCLOALKANONE DERIVATIVES

For definitions and chromatographic conditions, see Table I.

| Compound | Structure | Parameter | Chiral stationary phase | | | |
|---|---|---|---|---|---|---|
| | | | TBC | OMBC | MMBC | PMBC |
| **13** |  | $k'_2$<br>$\alpha$ | 3.80<br>1.00 | 5.90 (+)<br>1.51 | 7.36 (+)<br>1.0 | 6.32 (−)<br>1.0 |
| **14** |  | $k'_2$<br>$\alpha$ | 4.27 (−)<br>1.0 | 5.83 (+)<br>1.40 | 7.45 (+)<br>1.0 | 5.61 (−)<br>1.0 |
| **15** |  | $k'_2$<br>$\alpha$ | 3.60 (−)<br>1.0 | 4.38 (+)<br>1.36 | 4.96 (−)<br>1.0 | 4.78 (−)<br>1.0 |
| **16** |  | $k'_2$<br>$\alpha$ | 1.46 (−)<br>1.0 | 0.96 (−)<br>1.0 | 8.41 (+)<br>1.96 | 2.83 (+)<br>1.0 |
| **17** |  | $k'_2$<br>$\alpha$ | 7.09 (*R*)<br>1.0 | 5.49 (*R*)<br>1.0 | 12.75 (*S*)<br>1.12 | 9.36 (*S*)<br>1.0 |
| **18** |  | $k'_2$<br>$\alpha$ | 2.39 (*S*)<br>1.0 | 1.48 (*R*)<br>1.0 | 6.00 (*R*)<br>1.32 | 4.60 (*R*)<br>1.0 |

solved as intermediates for the synthesis of new fungicides [4]. The enantiomers of 3-phenylcyclohexanone and of both *o*-halo-3-phenylcyclohexanone derivatives **14** and **15** (Fig. 4) are only separated on OMBC, whereas the *tert.*-butyl derivative **16** (Fig. 4) is only resolved on MMBC, as in the case of the corresponding cyclopentanone derivative **18**. Moreover, for most of the cycloalkanone derivatives we observed variations of the elution order depending on the position of the methyl group on the methylbenzoylcellulose CSP.

Various alkyl and phenyl lactone derivatives were

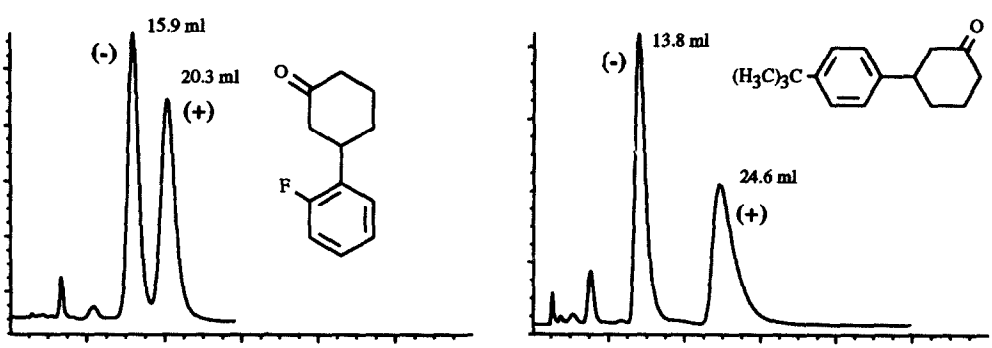
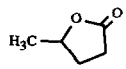
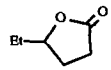
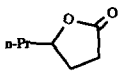
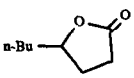
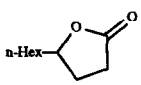
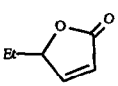
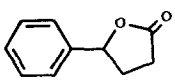
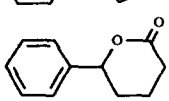
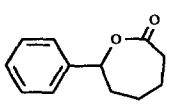
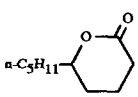
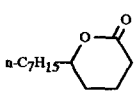



Fig. 4. Chromatographic resolution of the racemic cyclohexanone derivatives **15** on OMBC (left) and **16** on MMBC (right). For chromatographic conditions, see Fig. 3.

TABLE IV

CHROMATOGRAPHIC RESULTS FOR LACTONE DERIVATIVES 19–25

For definitions and chromatographic conditions, see Table I. Et = ethyl; Pr = propyl; Bu = butyl; Hex = hexyl;

| Compound | Structure | Parameter | Chiral stationary phase | | | |
|---|---|---|---|---|---|---|
| | | | TBC | OMBC | MMBC | PMBC |
| **19a** |  | $k'_2$<br>α | 17.29 (*S*)<br>1.15 | 5.80 (*R*)<br>1.0 | 10.94 (*S*)<br>1.05 | 10.90<br>1.00 |
| **19b** |  | $k'_2$<br>α | 11.83 (*R*)<br>1.06 | 4.49 (*R*)<br>1.0 | 7.28 (*S*)<br>1.0 | 8.45<br>1.00 |
| **19c** |  | $k'_2$<br>α | 9.47 (*R*)<br>1.04 | 3.69 (*R*)<br>1.0 | 6.17 (*S*)<br>1.04 | 7.30<br>1.0 |
| **19d** |  | $k'_2$<br>α | 6.14<br>1.00 | 3.06<br>1.00 | 5.20<br>1.00 | 5.66<br>1.00 |
| **19e** |  | $k'_2$<br>α | 4.00<br>1.00 | 2.23<br>1.0 | 3.85<br>1.00 | 4.14<br>1.00 |
| **20** |  | $k'_2$<br>α | 17.15 (*R*)<br>1.17 | 5.39 (*R*)<br>1.0 | 10.00<br>1.00 | 11.71<br>1.00 |
| **21** |  | $k'_2$<br>α | 36.90 (*R*)<br>1.39 | 13.18<br>1.00 | 27.22 (*R*)<br>1.24 | 27.90<br>1.00 |
| **22** |  | $k'_2$<br>α | 37.76 (+)<br>1.17 | 16.32<br>1.00 | 23.70<br>1.00 | 33.64 (−)<br>1.08 |
| **23** |  | $k'_2$<br>α | 13.53<br>1.66 | 8.98<br>3.19 | 7.51<br>1.00 | 11.48<br>1.00 |
| **24** |  | $k'_2$<br>α | 4.95 (+)<br>1.13 | 2.29 (−)<br>1.0 | 3.77<br>1.00 | 4.53<br>1.00 |
| **25** |  | $k'_2$<br>α | 3.67 (+)<br>1.08 | 1.79<br>1.00 | 2.45<br>1.00 | 3.55<br>1.00 |

chromatographed on TBC, OMBC, MMBC and PMBC (Table IV). In the series of five- and six-membered ring lactones, the best enantiomeric resolution is always observed on the TBC beads CSP. For the alkyl-substituted lactones, the separation factors decrease as the length of the alkyl substituent increases, contrasting with the results obtained with cellulose triacetate [5], where a maximum α value was reached for the butyl derivative **19d**. The inversion of the elution order on TBC between the methyl **19a** (*S* more strongly retained) and the ethyl **19b** (*R* more retained) derivative cannot be explained.

The size of the lactone ring also has a strong in-

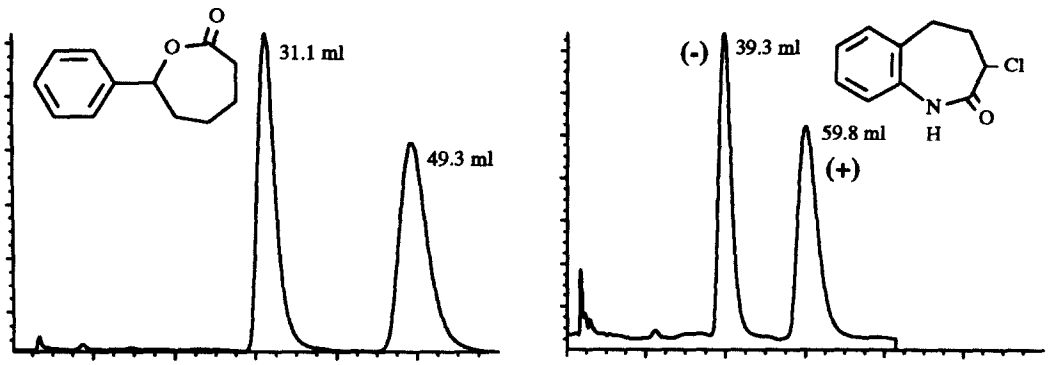



Fig. 5. Chromatographic resolution of both seven-membered-ring compounds **23** (left) and **28** (right) on TBC beads. For chromatographic conditions, see Fig. 3.

fluence on the separation, as indicated by the chromatographic results of the phenyl lactone derivatives **21–23** on the four different CSPs. While the enantiomers of phenylbutyrolactone (**21**) and phenylvalerolactone (**22**) are better separated on TBC beads, the seven-membered ring lactone derivative **23** is better resolved on OMBC (Fig. 5).

Racemic acylated nitrogen compounds or weakly basic nitrogen derivatives can also be resolved on CSPs consisting of cellulose esters. This is illustrated by the chromatographic resolution of **28** (Fig. 5) and **29** on TBC, of **30** on OMBC and of **31** on MMBC (Table V). For the resolution of racemic molecules with a $C_2$ axis of symmetry, PMBC seems to be the most appropriate CSP, as observed for binaphthol (**26**) and the biphenyl derivative **27** (Table V). For these compounds, which exhibit very high capacity factors using hexane–2-propanol as a mobile phase, pure methanol as the eluent strongly reduces the retention time, without a decrease in selectivity, however. The results obtained with both glycidyl ether derivatives **32** and **33** show that the CSP is very sensitive to the steric hindrance of the solute. For the methyl-substituted compound **32**, OMBC exhibits the best chiral recognition, whereas for the *tert.*-butyl derivative **33**, PMBC gives the best selectivity.

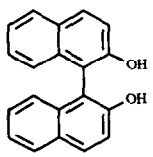
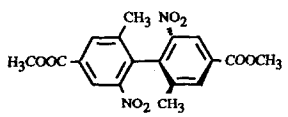
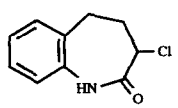
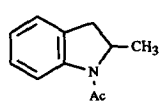
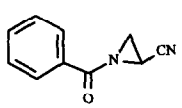
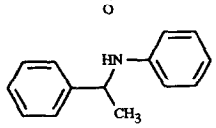
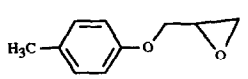
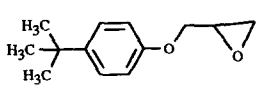
In addition to the racemic compounds presented before, a variety of racemic drugs and biocides were investigated on the four cellulose-based CSPs. Although a prediction of the separation was not possible, we could identify in most instances at least one among the four different investigated CSPs which was able to separate the enantiomers efficiently (Table VI). Even for analogous structures we found that the best selectivity was not necessarily achieved on the same CSP. For example, for the hypnotic drugs mephobarbital (**40**) and hexobarbital (**41**), the former is better resolved on MMBC and the latter on TBC. Racemic thalidomide (**42**), which is not soluble in hexane–2-propanol (9:1), was chromatographed with methanol as the mobile phase and a baseline resolution was achieved on PMBC (Fig. 6). The enantiomers of glutethimide (**39**) are well resolved either on OMBC or on PMBC (Fig. 6), whereas the metabolite **43** of methsuximide, a five-membered ring imide analogue of glutethimide, is better resolved on TBC. The resolution of the methyl ester of the non-steroidal anti-inflammatory drug (NSAID) oxindazac (**34**) was easily achieved on MMBC, similarly to other propionic acid methyl ester derivatives. The four isomers of the fungicide CGA 80000 (**38**) [6] can be baseline separated on MMBC (Fig. 8). This example illustrates the ability of MMBC to recognize simultaneously the chirality at the carbon centre of the lactone ring and the atrope isomerism induced by the hindered rotation of the trisubstituted phenyl ring around the nitrogen atom. Although the selectivity exhibited by TBC for the enantiomeric separation of the anti-cancer agent **35** [7] is higher with hexane–2-propanol (60:40) as the mobile phase ($\alpha$ = 3.55), the resolution can also be performed with methanol. Moreover, the selectivity can be considerably enhanced by addition of water to the methanol mobile phase (Fig. 8).

The various examples reported in Tables I–VI show that the cellulose-based CSPs are very versatile and in many instances also complementary in their selectivities. The dependence of the chiral recognition ability of methylbenzoylcellulose on the position of the methyl substituent on the phenyl group has already been observed for the corresponding silica-coated CSPs [8], but no inversion of the elution order for the same solute has so far been reported. The inversion of the elution order observed for many racemates (compounds **10**, **12–17**, **19a–c**, **26**, **28–32**), as illustrated in Fig. 9 for com-

TABLE V

CHROMATOGRAPHIC RESULTS FOR MISCELLANEOUS RACEMIC COMPOUNDS

For definitions and chromatographic conditions, see Table I.

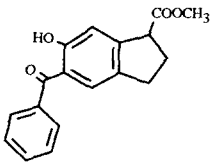
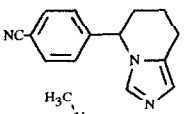
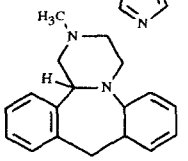
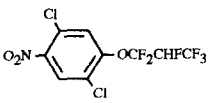
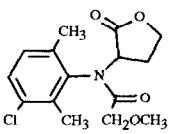
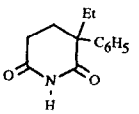
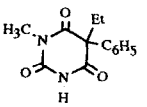
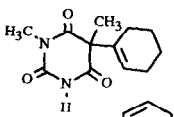
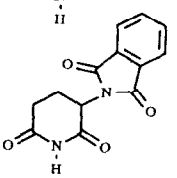
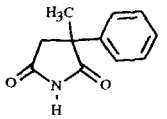
| Compound | Structure | Parameter | Chiral stationary phase | | | |
|---|---|---|---|---|---|---|
| | | | TBC | OMBC | MMBC | PMBC |
| **26** |  | $k'_2$ | 1.43 | 7.89 | 32.45 (*R*) | 63.11 (*S*) |
| | | $\alpha$ | 1.00 | 1.00 | 1.17 | 1.45 |
| | | $k'_2$ | 0.62 (*S*)[a] | – | – | 2.45 (*S*)[a] |
| | | $\alpha$ | 1.0 | – | – | 1.42[a] |
| **27** |  | $k'_2$ | 72.75 | 31.10 | 22.82 | 129.51 |
| | | $\alpha$ | 1.00 | 1.00 | 1.00 | 1.25 |
| | | $k'_2$ | 5.33[a] | – | – | 9.09 (–)[a] |
| | | $\alpha$ | 1.00 | – | – | 1.24[a] |
| **28** |  | $k'_2$ | 16.63 (+) | 8.05 (–) | 15.86 (+) | 13.50 (+) |
| | | $\alpha$ | 1.57 | 1.0 | 1.22 | 1.10 |
| **29** |  | $k'_2$ | 19.20 (–) | 3.58 (+) | 8.70 (–) | 9.01 (–) |
| | | $\alpha$ | 2.53 | 1.0 | 1.22 | 1.0 |
| **30** |  | $k'_2$ | 37.05 (*R*) | 16.21 (*R*) | 33.02 (*S*) | 24.16 (*S*) |
| | | $\alpha$ | 1.16 | 1.19 | 1.16 | 1.10 |
| **31** |  | $k'_2$ | 11.16 (*R*) | 2.56 (*R*) | 18.85 (*R*) | 13.27 (*S*) |
| | | $\alpha$ | 1.13 | 1.16 | 1.43 | 1.10 |
| **32** |  | $k'_2$ | 7.21 (*R*) | 3.69 (*S*) | 10.09 (*R*) | 11.23 (*R*) |
| | | $\alpha$ | 1.15 | 1.31 | 1.15 | 1.12 |
| **33** |  | $k'_2$ | 2.51 | 1.28 | 6.04 | 6.95 |
| | | $\alpha$ | 1.00 | 1.00 | 1.11 | 1.19 |

[a] Mobile phase: methanol.

TABLE VI

CHROMATOGRAPHIC RESULTS FOR VARIOUS DRUGS AND BIOCIDES

For definitions and chromatographic conditions, see Table I.

| Compound | Structure | Parameter | Chiral stationary phase | | | |
|---|---|---|---|---|---|---|
| | | | TBC | OMBC | MMBC | PMBC |
| **34** |  | $k'_2$ | 15.83 | 5.95 | 25.11 | 27.81 |
| | | $\alpha$ | 1.28 | 1.00 | 2.29 | 1.05 |
| **35** |  | $k'_2$ | 2.26 (+)[a] | – | – | 1.93 (+)[a] |
| | | $\alpha$ | 1.62[a] | – | – | 1.10[a] |
| **36** |  | $k'_2$ | 1.17 (−)[a] | – | – | 2.92 (+)[a] |
| | | $\alpha$ | 1.0[a] | – | – | 1.31[a] |
| **37** |  | $k'_2$ | 2.96 (−) | 1.63 | 5.33 | 4.62 |
| | | $\alpha$ | 1.24 | 1.28 | 1.00 | 1.00 |
| **38** |  | $k'_2$ | – | – | 14.40 (+)[b] | 14.16 (+)[b] |
| | | $\alpha$ | – | – | 2.11[b] | 1.29[b] |
| | | $k'_2$ | – | – | 23.53 (+)[b] | 14.65 (+)[b] |
| | | $\alpha$ | – | – | 2.30[b] | 1.34[b] |
| **39** |  | $k'_2$ | 17.74 (−) | 17.31 (−) | 32.26 | 28.14 (−) |
| | | $\alpha$ | 1.23 | 1.76 | 1.31 | 1.65 |
| **40** |  | $k'_2$ | 16.33 | 11.92 | 38.27 | 53.44 |
| | | $\alpha$ | 1.00 | 1.29 | 1.30 | 1.0 |
| **41** |  | $k'_2$ | 19.87 | 8.21 | 21.49 | 37.32 |
| | | $\alpha$ | 1.46 | 1.0 | 1.0 | 1.28 |
| **42** |  | $k'_2$ | 2.59 (−)[a] | – | – | 4.26 (−)[a] |
| | | $\alpha$ | 1.26 | – | – | 1.39 |
| **43** |  | $k'_2$ | 20.86 | 12.96 | 19.95 | 42.04 |
| | | $\alpha$ | 1.00 | 1.00 | 1.20 | 1.56 |

[a] Mobile phase: methanol.

[b] Mobile phase: hexane–2-propanol (60:40).

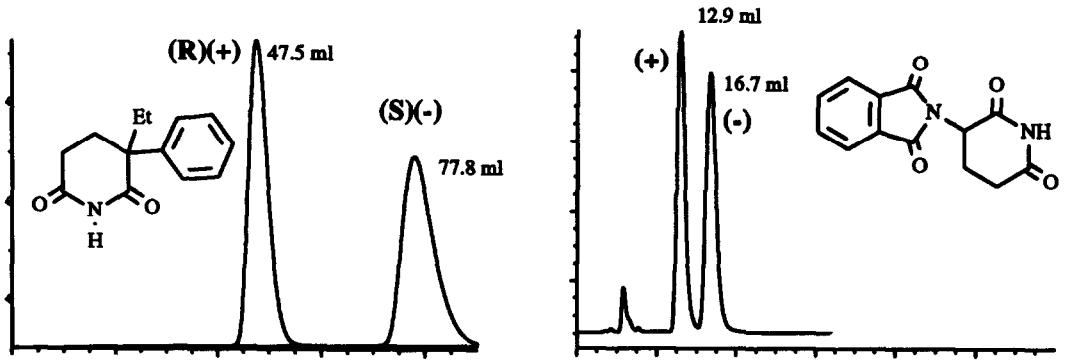



Fig. 6. Chromatographic resolution on PMBC of racemic glutethimide (**39**) (left) (for chromatographic conditions, see Fig. 3) and thalidomide (**42**) (right) (chromatographic conditions as in Fig. 3, but with methanol as the mobile phase).

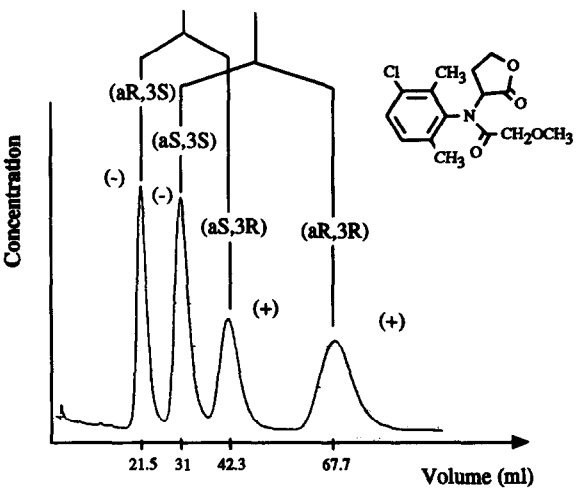



Fig. 7. Chromatographic resolution of the four optical isomers of CGA 80 000 on MMBC. HPLC column 25 × 0.46 cm I.D.; mobile phase, hexane–2-propanol (6:4); flow-rate, 1 ml/min.

pounds **12, 30** and **31**, demonstrates the dramatic influence of the position of the methyl radical attached to the benzoyl group on the chiral recognition, showing that the three methylbenzoylcellulose derivatives behave like totaly different sorbents. The preparative separation of the enantiomers of **30** on MMBC and the absolute configuration have been reported previously [9]. The elution order of the enantiomers of **31** has been determined by injection of the individual enantiomers obtained by condensation of the corresponding enantiomers of phenylethylamine with bromobenzene [10].

The great differences in selectivities exhibited by TBC, OMBC, MMBC and PMBC indicate that very slight modifications of the substituents or its position on the benzoyl group can lead to important alterations of the shape of the chiral receptors. This shape is defined by the arrangement of the polymer

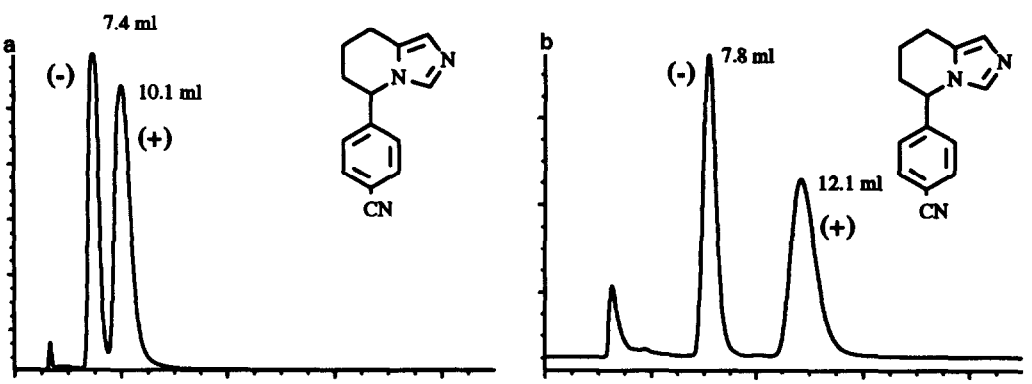



Fig. 8. Chromatographic resolution of **35** on TBC beads. HPLC column, 25 × 0.46 mm I.D.; mobile phase, (a) methanol at a flow-rate of 1 ml/min and (b) methanol–water (95:5) at a flow-rate of 0.5 ml/min.

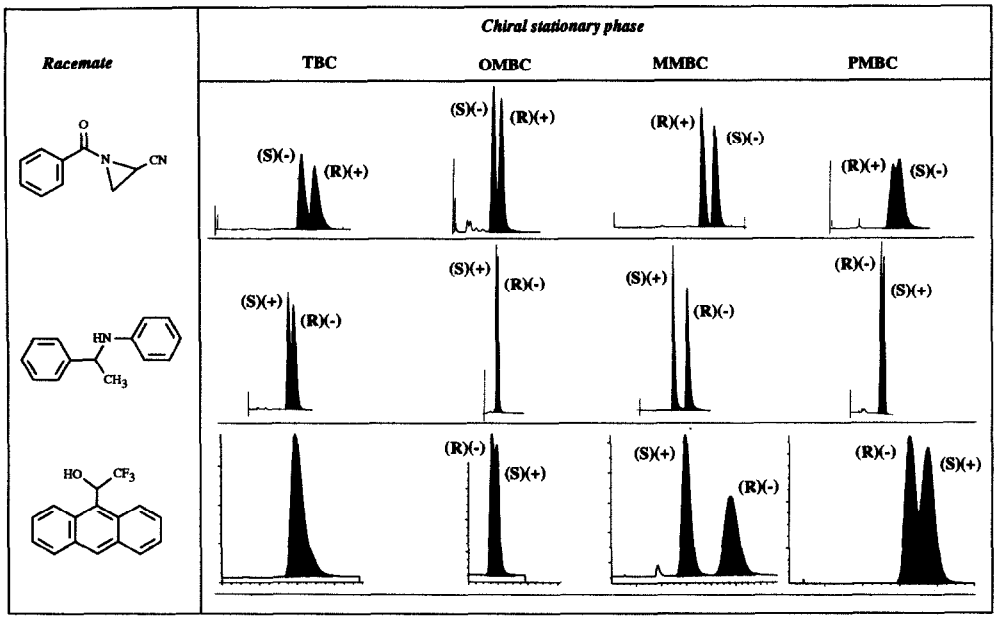



Fig. 9. Analytical resolutions of racemic compounds **30** (top), **31** (middle) and **12** (bottom) on TBC, OMBC, MMBC and PMBC beads. For chromatographic conditions, see Fig. 3.

chains and/or by the actual conformation of the benzoyl groups. Although achiral *per se*, the benzoyl groups can obviously transfer the chiral information of the sugar moiety to which they are attached, further into the space around the polymer chains, thus creating chiral cavities.

Whereas the main-chain conformation of the polymer is presumably not dramatically affected by slight variations on the phenyl groups, the conformation of the phenyl groups themselves is certainly altered. Also, the packing of the polymer chains in ordered (crystalline or at least semi-crystalline) arrays must be affected. The influence of the supramolecular structure of cellulose derivatives on their chiral recognition has been revealed by studies carried out on cellulose triacetate [11]. In this instance, a change in the actual crystalline packing scheme, without any chemical changes, was found to reverse the elution order of some enantiomers. It is therefore not surprising that the presence and position of a methyl group on the phenyl group of methyl benzoylcellulose can induce similar effects. However, these effects are a clear indication that the chiral recognition process on cellulose derivatives is based on a large number of weak, well balanced, interactions and not, or only rarely, on a few, but very well defined, strong interactions between distinct atom groups (*e.g.*, hydrogen bonds or $\pi$-$\pi$ interactions). This may explain the surprisingly broad range of different racemic compounds that can be resolved on derivatized cellulose CSPs.

These results also illustrate the difficulty of making predictions concerning a separation. Although some empirical rules can be applied, it is very difficult to decide by a rational method which CSP has to be used for a given compound.

The new CSPs described in this work are not only suitable for analytical purposes but also, because of their high loading capacity, they are particularly useful for preparative separations as we have already demonstrated for benzoylcellulose beads (TBC) [3]. Such preparative applications will be reported later.

## CONCLUSIONS

Various cellulose-based CSPs have been prepared in the pure polymeric form (without supporting material) according to a new process. By simple variation of the position of the methyl substituent on

the phenyl group of the methylbenzoylcellulose CSPs, completely different chiral recognition abilities have been observed. In some instances, even an inversion of the elution order of the enantiomers has been observed, depending only on the position of the methyl group.

In most instances, a good resolution of the racemates could be achieved on at least one of the benzoylcellulose CSPs, indicating that the four different CSPs have complementary selectivities. These results demonstrate that a large number of racemates can probably be resolved with a limited number of cellulose derivatives, starting from the same inexpensive material, cellulose. Although a rationalization of the influence of the derivatizing group of cellulose is still impossible, the versatility of the cellulose derivatives offers a large application potential. Mechanistically, the results suggest that supramolecular effects (crystal packing) play an important role in the chiral recognition, in accordance with our previous investigations on cellulose triacetate [11]. A better understanding of these effects could lead to a controlled modulation of the chiral recognition by variations of the substituent and its position on the phenyl group of the respective benzoylcellulose derivatives.

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