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OPTIMIZATION OF THE DIRECT CHIRAL SEPARATION OF POTENTIAL CYTOTOXIC α-METHYLENE-γ-BUTYROLACTONES AND α-METHYLENE-γ-BUTYROLACTAMS BY LIQUID CHROMATOGRAPHY

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SUMMARY

The direct enantiomeric resolution of a series of racemic α-methylene-γ-butyrolactones and α-methylene-γ-butyrolactams was carried out on various commercially available chiral stationary phases (CSPs). Particular interest was paid to compounds which exhibit physiological activity as cytotoxic agents. On a Pirkle-type column packed with (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (DNBPG) covalently bonded to γ-aminopropylsilanized silica gel, only α-methylene-γ-lactams containing two aromatic groups were resolved; a chiral recognition mechanism is proposed. (+)-Poly-(triphenylmethyl methacrylate) (PTrMA) coated on silica gel [Chiralpak OT(+)] with methanol as eluent afforded fairly good selectivities (up to 1.8) especially for α-methylene γ-lactones. Temperature had a great influence on the resolution. Cellulose tribenzoate and triphenylcarbamate (Chiralcel OB and OC, respectively) coated on macroporous silica gel displayed selectivities from 1.1 to 1.3, but, because of the very poor efficiency of these CSPs, no baseline resolution was achieved. Investigations on two protein CSPs are reported, α_1-acid glycoprotein (α_1-AGP) and bovine serum albumin (BSA) immobilized on microparticulate silica gel (Enantiopac and Resolvosil-BSA, respectively). The separations were performed using an aqueous sodium phosphate buffer as eluent and 2-propanol as organic modifier to regulate retention and selectivity. Both CSPs exhibited a good chiral recognition ability, especially towards cytotoxic solutes with resolution factors between 1 and 2.

INTRODUCTION

The synthesis of various α-methylene γ-butyrolactams has been carried out by Alami *et al.*[1,2] in order to develop a new series of therapeutic agents for the treatment of cancer. This study was performed[2,3] by comparison with α-methylene-γ-

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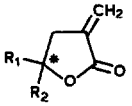
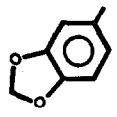
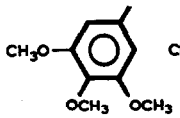
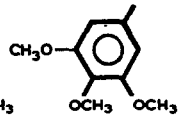
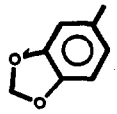
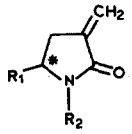
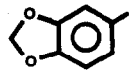
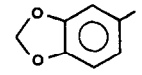
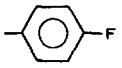
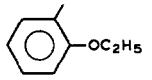
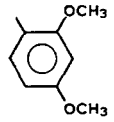
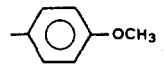
TABLE I

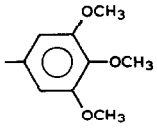
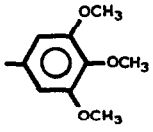
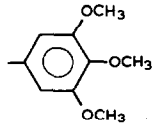
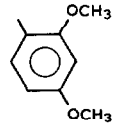
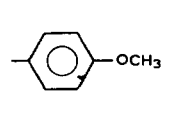
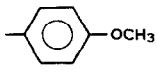
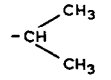
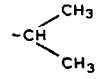
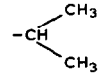
STRUCTURES OF THE α-METHYLENE-γ-BUTYROLACTONES AND α-METHYLENE-γ-BUTYROLACTAMS STUDIED

α-Methylene γ-lactones: 

Substituent	*1*	*2*	*3*	*4*
R_1				
R_2	H	H	H	

α-Methylene γ-lactams: 

Substituent	*5*	*6*	*7*	*8*	*9*	*10*
R_1						
R_2					-CH₃	

	11	*12*	*13*	*14*	*15*
R_1					
R_2		-CH₃			

butyrolactones[4], already known as cytotoxic agents but which often display toxic effects. The major direct factor responsible for the cytotoxicity of these compounds is the introduction of the $O=\overset{|}{C}-\overset{|}{C}=CH_2$ group. However, Alami[2] observed that some other specific structural parameters were required for biological activity, *e.g.*, the presence of an alkyl group on the nitrogen atom was necessary and lipophilic aryl groups R_1 (methoxyphenyl, 3,4-methylenedioxyphenyl) greatly enhanced the cytotoxicity (Table I, cytotoxic compounds in bold type). All these compounds bear an asymmetric carbon.

It has been extensively reported[5] that the two enantiomers of a chiral drug often provide different biological responses. Improvement of the therapeutic activity of a chiral drug molecule needs pharmacological investigations on the relative activities (toxicity/cytotoxicity for the case of our solutes) of its enantiomers. Thus, the development of rapid and accurate methods, such as liquid chromatography on chiral stationary phases (CSPs), for the determination of the enantiomeric purity of chiral solutes constitutes a necessary tool for biologists. This paper deals with the direct separation, without prior derivatization, of the aforementioned lactones and lactams on various commercially available CSPs. We intended to test easily accessible CSPs with very different structures and operating with various mobile phases in order to optimize the separations and make them suitable for further optical purity controls.

THEORETICAL

According to the solute structures, three types of CSPs were chosen after examination of literature data. Our classification of the CSPs studied was based on their chemical structures and on the possible chiral recognition mechanisms that were supposed to be involved. Type I CSPs are obtained by covalently bonding a chiral moiety on an achiral support via an achiral spacer; we used a Pirkle-type column derived from (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (DNBPG) grafted on microparticulate silica gel. The resolution of some lactones[6,7], phthalides[6,7] and β-, γ- and δ-lactams[6,8] have been previously described on such a CSP. However, the chiral recognition mechanism proposed by the authors (on an ionic CSP) for 3-aryl-γ-lactams predicted that substitution of the hydrogen on the nitrogen atom led to a decrease in selectivity[9].

Type II CSPs consists of synthetic or derivatized natural polymers coated (in thin layers) on macroporous silica gel. These polymers possess many centres of asymmetry and/or asymmetric environments called chiral cavities. We report investigations on optically active (+)-poly(triphenylmethyl methacrylate) [(+)-PTrMA]-derived CSP [Chiralpak OT(+)]. Okamoto and Hatada[10] suggested that this CSP is able to resolve various compounds lacking functionalities but bearing aryl groups (suitable for hydrophobic interactions) in the vicinity of the asymmetric centre, and/or having a rigid structure similar to our compounds. The resolution of lactones[11,12] and lactams[12] on cellulose derivative CSPs such as cellulose tribenzoate (Chiralcel OB) and triphenylcarbamate (Chiralcel OC) has been reported. Here also the geometric structure of the solute seems to play an important role in chiral recognition.

Finally, Type III CSPs include two protein CSPs which have been developed

recently and which appear to be very useful for the resolution of chiral pharmaceuticals[13,14]. Enantiopac and Resolvosil BSA columns were synthetized by immobilization of α_1-acid glycoprotein[13] (α_1-AGP) and bovin serum albumin (BSA)[14], respectively, on wide-pore microparticulate silica gel. These proteins have interesting binding properties in biological media which could make them very attractive for the resolution of our potential cytotoxic compounds.

EXPERIMENTAL

Apparatus

Analytical chromatography was performed with a Model 1084B liquid chromatograph (Hewlett-Packard, Waldbronn, F.R.G.) equipped with an automatic sampling system (79842 A) and a variable-wavelength detector (190–540 nm) (79875 A), or with a Model 8100 liquid chromatograph (Spectra-Physics, Santa Clara, CA, U.S.A.) equipped with a variable-wavelength detector (190–600 nm) (SP-8440) and a dual-channel computing integrator (SP 4200).

Experiments on the Chiralpak OT(+) column were performed with a modular liquid chromatograph (Gilson, Villiers-le-Bel, France) equipped with a Model 303 pump, a Model 802C manometric modula, a Gilson 811 (1.5-ml) dynamic mixer and a Holochrome HMD (analytical cell, 11 μl) spectrophotometer. All results were recorded with a Shimadzu CR3A integrator (Touzart et Matignon, Vitry-sur-Seine, France). For the control of the column and solvent temperature we used a Haake Model D8-V circulator bath (0–150°C) (Roucaire, Vélizy-Villacoublay, France) and a water-cooling jacket (purchased with the column). All tubing connections were heat-insulated.

UV detection was carried out at 254 nm with organic mobile phases and at 230 nm with aqueous buffer eluents.

Materials

n-Hexane, ethanol, 2-propanol, methanol and acetonitrile were of LiChrosolv grade, purchased from Merck (Darmstadt, F.R.G.). Chloroform stabilized with 0.6% (w/w) of ethanol was of analytical-reagent grade, purchased from Prolabo (Paris, France). Aqueous buffer solutions used with protein CSPs were prepared either directly[15] from mixtures of 0.2 *M* solutions of sodium dihydrogenphosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and sodium hydrogenphosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$), extra-pure grade, purchased from Merck, or from a commercially available sodium phosphate buffer (25 mmol, pH 6.88).

Deionized water was doubly distilled on a Büchi-Fontavapor 285 apparatus (Roucaire). The pH of the aqueous buffer eluents was controlled with a Model Minisis 8000 pH/millivoltmeter (Tacussel, Villeurbanne, France), Tacussel glass TB/HS and Tacussel C8 calomel reference electrodes.

Aqueous solvents were filtered through 0.65-μm Type DAWP Millipore membrane filters (Touzart et Matignon) before being degassed in an ultrasonic bath, whereas organic mobile phases were filtered through a 0.5-μm Type FHLP Millipore membrane filter and then degassed with helium (with the Spectra-Physics chromatograph) or *in vacuo* (with the Hewlett-Packard chromatograph).

Sodium chloride was of analytical-reagent grade, purchased from Merck.

LiChrosorb-NH_2 γ-aminopropylsilica gel (irregular particles; diameter, d_p = 5 μm) was purchased from Merck.

Pirkle-type column preparation

The chiral moiety, (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (DNBPG), was prepared as described[16]. Covalent grafting of the (*R*)-N-3,5-DNBPG was carried out as previously described[17]. Starting from 100 g of γ-aminopropylsilica gel and 27.6 g of chiral moiety (0.8 mmol g^{-1}) we obtained 114 g of CSP with the following elemental analysis: C 18.7, H 2.5, N 2.2, Si 21.9%, corresponding to 0.43 mmol of chiral sites per gram of support. The CSP was then packed into 250 × 4.6 mm I.D. stainless-steel columns by the usual slurry technique at 400 bar with ethanol as pumping solvent.

Commercially available CSPs

Chiralpak OT(+), Chiralcel OB and Chiralcel OC packings consisted of optically active (+)-PTrMA, cellulose tribenzoate and cellulose triphenylcarbamate, respectively, coated on macroporous silica gel (particle diameter, d_p = 10 μm; mean pore diameter 1000 Å). Packed in 250 × 4.6 mm I.D. stainless-steel columns, they are available from Daicel Chemical Industries (J. T. Baker, Sochibo, Velizy-Villacoublay, France).

The 100 × 4.0 mm I.D. Enantiopac column was purchased from LKB (Les Ulis, France): the plasma protein α_1-AGP is immobilized on a 10 μm diethylaminoethylsilica gel by ionic bonding followed by cross-linking.

BSA-silica of 7 μm particle size was purchased as Resolvosil-BSA-7 from Macherey-Nagel (Düren, F.R.G.) (150 × 4.0 mm I.D. stainless-steel column).

Solutes

Samples of α-methylene-γ-butyrolactones and α-methylene-γ-butyrolactams were given to us by Professor J. Villieras, Laboratory of Selective Organic Synthesis, University of Nantes, France. The syntheses have been described previously by Alami[2]. The solvents used for solubilizing the solutes were chosen as follows: hexane–2-propanol (50:50, v/v) with the Pirkle-type and Chiralcel OB and OC columns, methanol with the Chiralpak OT(+) column and phosphate bufferr (7.5 m*M*, pH 7)–2-propanol (50:50, v/v) with the protein columns. On the protein columns the solubility of the solute was improved by addition of 2-propanol; the peak shape was then improved (less broad and more symmetric). We often noticed that the efficiency and as a consequence, the resolution could be greatly affected by the choice of solubilizing solvents.

RESULTS AND DISCUSSION

Type I CSP: Pirkle-type phase

Enantiomers of the α-methylene-γ-butyrolactones in Table I were not resolved on the Pirkle-type CSP. Only γ-butyrolactams bearing an aryl substituent on the nitrogen atom (*i.e.*, R_2, Table I) were resolved (Table II). These results suggest the mechanism for chiral recognition depicted in Fig. 1. This model involves the formation of a charge-transfer complex between the π-electron acceptor 3,5-dinitroben-

TABLE II

RESOLUTION OF α-METHYLENE γ-LACTAMS ON A PIRKLE-TYPE CSP

Solute	*2-Propanol in mobile phase** (%, v/v)	k'_2**	α***	R_s§
5	5	4.4	1.16	1.3
6	5	3.7	1.12	0.9
7	12	4.5	1.55	3.5
8	12	11.2	1.58	4.4
10	12	10.1	1.21	3.0
11	12	16.0	–	–

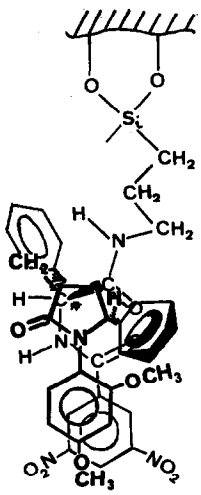
* Mobile phase: *n*-hexane–2-propanol.

** k'_2 is the capacity factor of the second eluted enantiomer, $k'_2 = (t_{r_2}/t_0) - 1$, where t_{r2} is the retention time of the second eluted enantiomer and t_0 the retention time of a non-retained solute.

*** The selectivity between two enantiomers is the ratio of their respective capacity factors (k'_2/k'_1).

§ R_s (resolution factor) = 2(distance of the two enantiomer peak positions/sum of the band width of the two peaks at their bases): $R_s = 2(t_{r_2} - t_{r_1})/(w_2 + w_1)$.

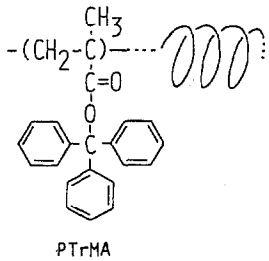
zoyl group of the CSP and the π-electron donor aryl substituent R_2 of the lactam, and hydrogen bonding of the 3,5-dinitrobenzamide hydrogen of the CSP and the carboxy oxygen of the lactam. The difference in size between the hydrogen atom and the phenyl group R_1 on the chiral carbon of the solute induces a steric discrimination of the *R* and *S* configurations. In the case of the *R* configuration our model shows that the bulky phenyl group (R_1) would be inserted between the CSP and the lactam ring, which may hinder both π–π interaction and hydrogen bonding, thus leading to a less stable diastereomeric complex. In Fig. 1 the conformation of the lactam 8 was



(S)-enantiomer

Fig. 1. Proposed mechanism for chiral recognition of racemate 8 on a Pirkle-type CSP.

chosen according to the examination of the CPK* molecular models in order to give the preferred one (where the mean plane of the lactam ring and the aromatic nucleus R_2 are coplanar). It also illustrates more obviously the chiral discrimination. The (*R*)-N-(3,5 DNB) phenylglycine CSP was drawn according to the conformation proposed by Lipkowitz *et al.*[18] from the conformational analysis computed with the Allinger MM_2 force field method. The observed α values are in good agreement with our model, which shows the importance of the π–π interaction between the nitrogen substituent R_2 and the CSP: α increases regularly with the π-electron donor character of the aryl group R_2 (Table II); compound 10 was resolved but compound 11 was not. From this we can argue that the stronger π-electron donor trimethoxyphenyl group (R_1) competes with the less strong methoxyphenyl group (R_2) for the π-acceptor site of the CSP, leading to a lack of selectivity. In addition, we failed to resolve the lactam containing the phenyl group at R_1 and a hydrogen atom as R_2. This is in accordance with our model of discrimination: a π–π interaction between R_1 and the 3,5-DNB group of the CSP and simultaneous hydrogen bonding or a dipolar interaction between the amide group of the lactam and the 3,5-DNB amide group of the CSP cannot occur because of the rigid quasi-planar conformation of the lactam ring. This quasi-planar system provides an additional steric restraint. The magnitude of the resolution factor R_s shows that this CSP is suitable for chiral preparative chromatography of compounds 7, 8 and 10.



Scheme 1. Structure of the PTrMA polymer chains.

Type II CSPs

(+)-PTrMA coated silica gel [Chiralpak OT(+) column]. As we failed to resolve the cytotoxic lactones and some lactams on the DNBPG column, we investigated the chiral recognition ability of optically active PTrMA coated on silica gel. The polymer possesses a rigid helical conformation which arises from the asymmetric polymerization of triphenylmethyl methacrylate (Scheme 1). With methanol as the eluent most of the compounds were resolved. Data obtained at 5 and 25°C are presented in Table III (compounds that have been omitted were not separated). It was puzzling that compounds 13 and 14 were not resolved whatever the temperature, whereas enantiomers of lactams 12 and 15 were separated with selectivity values of *ca.* 1.2 at 5°C ($R_s > 1.1$).

For all compounds, methanol appeared to be the most suitable eluent; ex-

* CPK precision molecular models are improved versions of the Corey–Pauling models designed at the California Institute of Technology in the late 1940s, with new connectors by Dr. W. Koltun.

TABLE III

INFLUENCE OF TEMPERATURE ON THE RESOLUTION OF SOME α-METHYLENE-γ-BUTYROLACTONES AND -γ-BUTYROLACTAMS

Stationary phase, (+)-PTrMA coated on silica gel [Chiralpak OT(+) column]; mobile phase, methanol; flow-rate, 0.5 ml min^{-1}; column, 250 × 4.6 mm I.D.

Compounds	*Solute*	*5°C*			*25°C*		
		k'_2	α	R_s	k'_2	α	R_s
α-Methylene-γ-butyrolactones	1	0.76	1.40	1.8	0.54	1.30	1.2
	2	0.61	1.13	0.6	0.46	1.07	0.5
	3	0.61	1.24	1.1	0.44	1.15	0.7
	4	2.00	1.32	1.5	1.49	1.45	2.7
α-Methylene-γ-butyrolactams	5	1.09	1.09	0.5	0.81	–	–
	6	0.59	1.05	0.4	0.46	–	–
	7	1.32	1.43	0.85	1.16	1.32	1.05
	8	1.17	1.30	1.2	0.99	1.31	1.25
	10	1.56	1.29	1.2	1.11	1.13	0.8
	11	1.30	–	–	1.05	1.19	0.9
	12	1.07	1.19	1.1	0.68	1.13	0.8
	15	0.65	1.23	1.15	0.49	1.23	0.95

periments carried out with hexane–ethanol or hexane–2-propanol mixtures did not result in chiral recognition. This suggests a contribution of hydrophobic interactions between the numerous non-polar sites of our compounds (R_1 and R_2 are often aryl substituents) and the triphenylmethyl groups of the polymer chains; these hydrophobic interactions occur in polar media such as methanol and are thus disfavoured with apolar hexane–alcohol mobile phases, leading to a decrease in enantioselectivity. The chiral recognition mechanism may thus result from a combination of these hydrophobic interactions and an inclusion phenomenon of the solute in the intrinsically chiral (+)-PTrMA.

The importance of temperature on the resolution of the racemates is evident from the data at 25 and 5°C given in Table III. However, this dependence on tem-

TABLE IV

EFFECT OF TEMPERATURE ON THE EFFICIENCY OF THE (+)-PTrMA COLUMN FOR RACEMATE 4

T (°C)	k'_2	N_2*	h_2**
5	2.00	780	32
10	1.81	940	27
15	1.73	1240	20
20	1.61	1420	18
25	1.49	1870	13

* The efficiency N was calculated according to the equation given by Foley and Dorsey[20] for non-Gaussian peaks.

** $h = L/(Nd_p)$, where L is the column length and d_p the mean diameter of silical gel particles. Other operating conditions as in Table III.

perature varied greatly according to the compound and we sometimes observed opposite behaviours. For all compounds the efficiency of the column increased with increasing temperature (higher solute diffusion coefficients in the mobile phase). The theoretical plate number, N, and the reduced plate height, h, for the best resolved compound, the lactam 4, are listed in Table IV. N more than doubled when the temperature increased from 5 to 25°C.

A parallel study of the dependence of the selectivity, α, on temperature revealed unusual trends such as an increase in α with increasing temperature for compounds 4 and 11 (Fig. 2). This abnormal behaviour has already been observed by Okamoto *et al.*[19] on (+)-PTrMA columns. They also studied the chiral discrimination ability of (+)-PTrMA as a function of the density of polymer coated on the silica gel. They suggested that heavily (+)-PTrMA-coated silica gel could show an ordered structure of associated chains leading to the formation of chiral spaces between the chains responsible for the chiral recognition process. With lightly coated silica gel, the polymer chains remained isolated from each other and could act as independent chiral

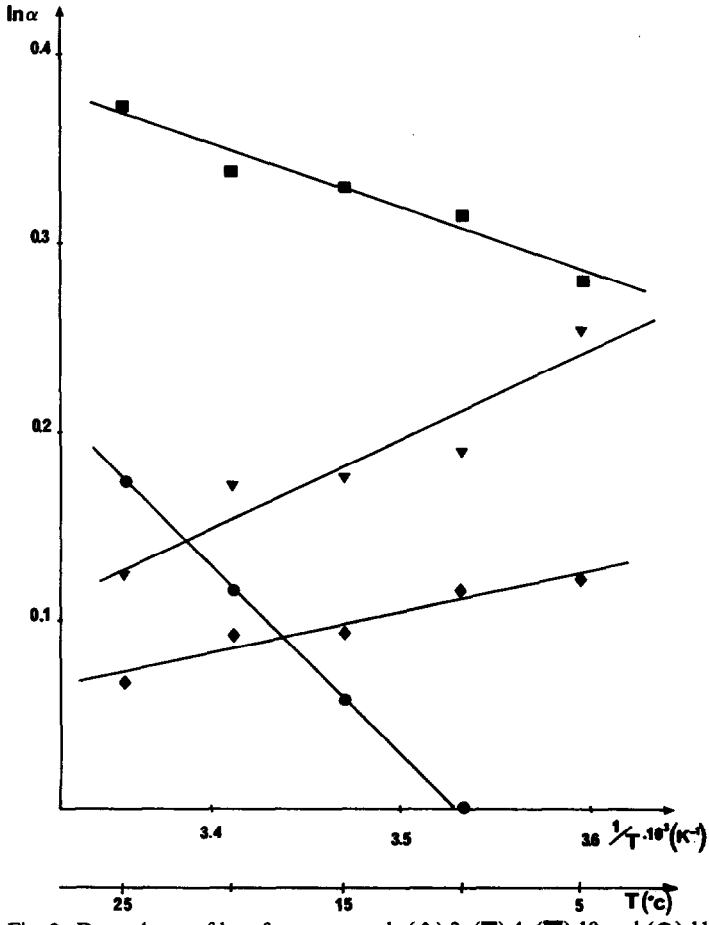


Fig. 2. Dependence of ln α for compounds (◆) 2, (■) 4, (▼) 10 and (●) 11 on temperature. Operating conditions as in Table III.

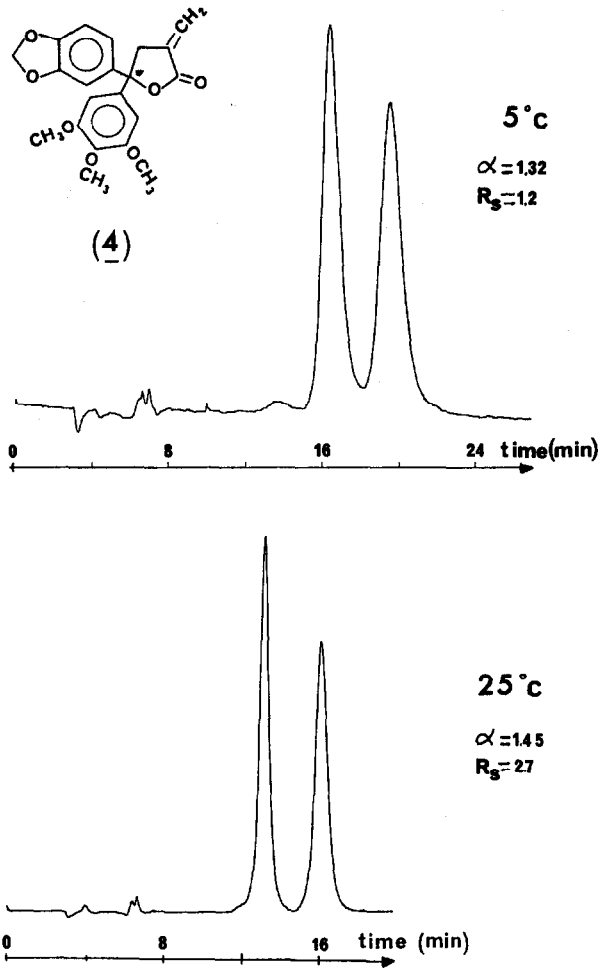


Fig. 3. Optimization of the resolution of solute 4 on the (+)-PTrMA column by increasing the temperature. Operating conditions as in Table III; UV detection at 254 nm.

sites. For a given compound one of these two chiral recognition mechanisms may be more suitable.

However, we do not know the (+)-PTrMA to silica gel weight ratio of the commercially available columns we used. We can assume that even small variations in temperature may alter the conformation of the polymer chains, modifying either the helical structure or the shape of the chiral spaces. This may then entail changes in the chiral recognition mechanism whether the chains act isolatedly or cooperatively; for some solutes an increase in temperature may thus happen to be more suitable for chiral discrimination. Also, a change in temperature may induce an alteration of the conformation of the solute itself that may be either favourable or unfavourable with respect to stereoselectivity. These comments emphasize the role of inclusion with such polymers; the solute has to fit to the chiral macromolecular structure.

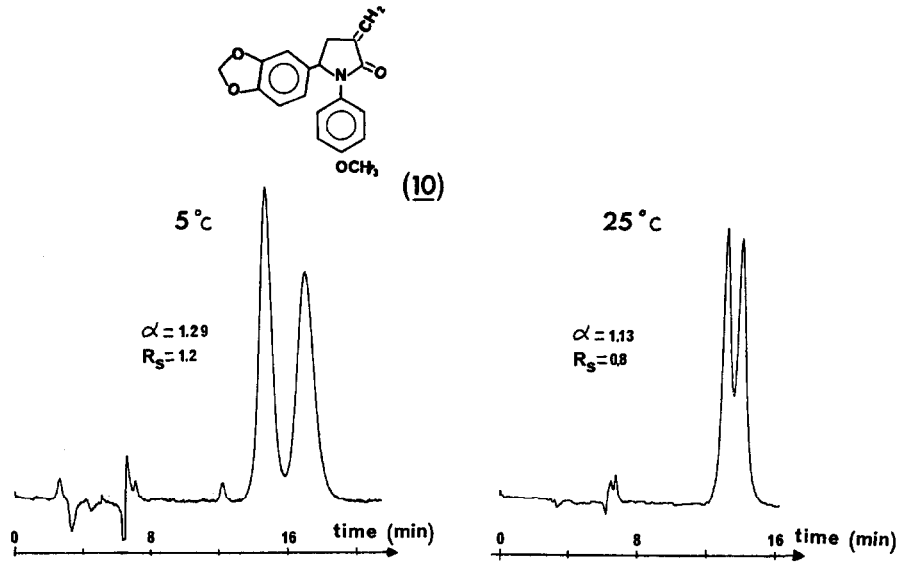
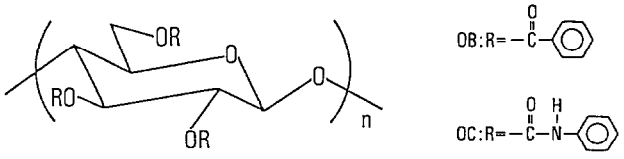


Fig. 4. Optimization of the resolution of solute 10 on the (+)-PTrMA column by decreasing the temperature. Operating conditions as in Table III; UV detection at 254 nm.

In Fig. 3 the chromatograms obtained at 5 and 25°C for the lactone 4 show that both the efficiency and selectivity increase with increasing temperature. However, for lactam 10 the resolution was improved at low temperatures (Fig. 4) because the selectivity gain, with a decrease in temperature from 25 to 5°C, was important enough to compensate for the small decrease in efficiency.

In conclusion, we can say that 10–15°C is a suitable temperature range for the use of a (+)-PTrMA column, noting that Okamoto *et al.*[19] have shown that the solvolysis of the triphenylmethyl ester groups of the polymer was reduced below 15°C. With regard to the magnitude of the resolution factor, R_s, optical purity determinations can be carried out on a (+)-PTrMA column for compounds 1, 4, 8 and 10.

Cellulose derivative CSPs. The structures of the two commercially available cellulose derivative CSPs that we used are depicted in Scheme 2. Mobile phases consisting of hexane–ethanol, hexane–2-propanol and hexane–2-propanol–acetonitrile were studied. OB- and OC-CSPs display good ability for chiral recognition towards our compounds (Tables V and VI). The selectivity values varied from 1.1 to 1.3; OC-CSP was more efficient than OB-CSP for the separation of γ-lactams. However, because of the low efficiency of the columns (a reduced plate height of 40–50, *i.e.*,



Scheme 2. Structures of the cellulose derivative CSP Chiralcel OB and Chiralcel OC.

TABLE V

RESOLUTION OF SOME α-METHYLENE-γ-LACTONES AND -γ-LACTAMS ON A CELLULOSE TRIPHENYLBENZOATE DERIVATIVE COATED ON SILICA GEL (CHIRALCEL OB COLUMN)

Mobile phase, *n*-hexane–polar solvent mixture; flow-rate, 1 ml min^{-1}, temperature, 26°C; column, 250 × 4.6 mm I.D.

Solute	*Polar solvent in mobile phase (%, v/v)*	k'_2	α	R_s
1	Ethanol (8)	6.9	1.27	1.2
2	Acetonitrile–2-propanol (75:25, v/v) (8)	5.4	1.22	1.0
6	2-Propanol (15)	4.8	1.24	0.6
13	2-Propanol (15)	7.9	1.70	0.8
15	Acetonitrile–2-propanol (75:25, v/v) (1)	9.5	1.64	1.0

600 theoretical plates for the first peak of 1 on the OB-CSP), baseline resolution was not achieved.

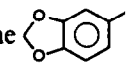
Figs. 5 and 6 show the chromatograms of the lactone 1 on OB-CSP and of the lactams 9 and 13 on OC-CSP. These two CSPs are sensitive to the geometric structure of the solutes. For example, we noticed that the compounds containing the  unit on the asymmetric carbon were partly resolved on OC-CSP (compounds 2, 4, 9 and 10). Unfortunately, our investigations on γ-lactams and γ-lactones did not allow us to formulate simple rules concerning chiral recognition on such CSPs. Nevertheless, some general assumptions have been proposed previously by other workers[21] which can suit our experiments: the chiral recognition process may involve the formation of π–π interactions, occurring between the phenyl group of the CSPs and the aryl groups of the solutes; hydrogen bonding between the carboxy group of

TABLE VI

RESOLUTION OF SOME α-METHYLENE-γ-LACTONES AND -γ-LACTAMS ON A CELLULOSE TRIPHENYLCARBAMATE DERIVATIVE COATED ON SILICA GEL (CHIRACEL OC COLUMN)

Mobile phase, *n*-hexane–ethanol; flow-rate, 1 ml min^{-1}; temperature, 26°C; column, 250 × 4.6 mm I.D.

Solute	*Polar solvent in mobile phase (%, v/v)*	k'_2	α	R_s
2	15	9.2	1.14	0.7
4	25	10.8	1.08	0.6
8	15	10.8	1.29	1.1
9	15	8.9	1.17	0.9
10	20	15.4	1.22	1.0
12	20	10.7	1.08	0.7
13	15	7.6	1.28	1.0
15	10*	5.3	1.10	0.6

* Mobile phase, *n*-hexane–2-propanol.

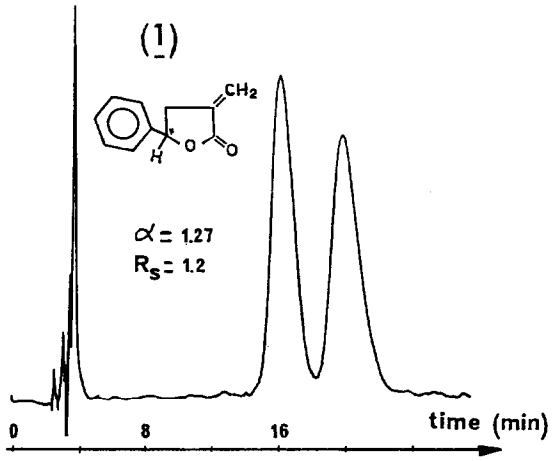


Fig. 5. Separation of the enantiomers of the lactone 1 on the Chiralcel OB column. Column, 250 × 4.6 mm I.D.; mobile phase, *n*-hexane–ethanol (92:8, v/v); flow-rate, 1 ml min^{-1}; temperature, 26°C; UV detection at 254 nm.

the lactones and lactams, and the carbamate hydrogen for the case of OC-CSP, may also contribute to the chiral recognition. A steric chiral discrimination based on the inclusion of the solute in the chiral cavities of the CSPs is probably essential and involves the structure and "flexibility" of the molecule.

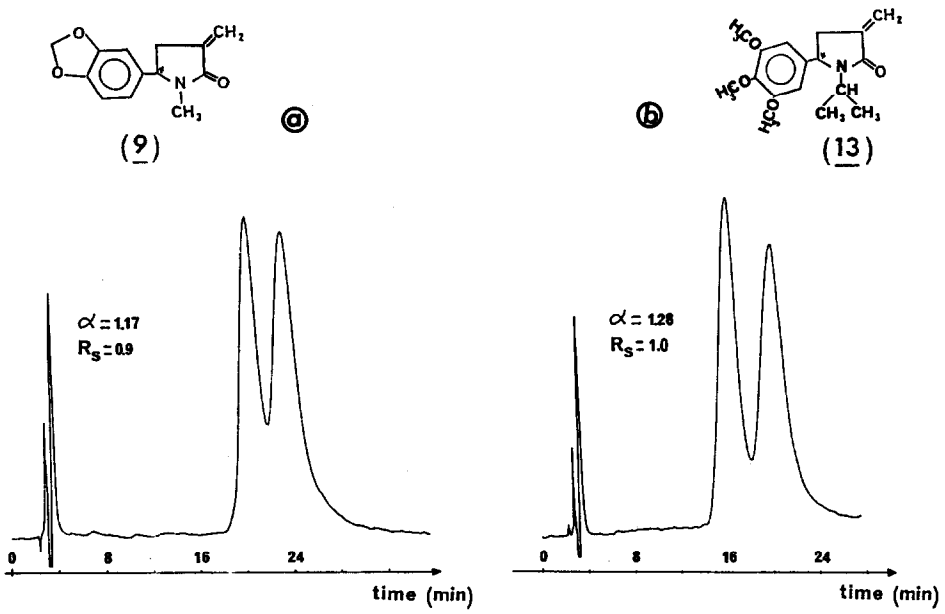
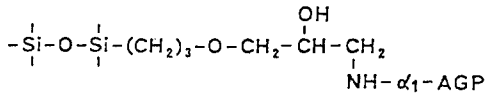


Fig. 6. Separation of the enantiomers of the lactams 9 and 13 on the Chiralcel OC column. (a) Racemate 9; (b) racemate 13. Column, 250 × 4.6 mm I.D.; mobile phase, *n*-hexane–ethanol (85:15, v/v); flow-rate, 1 ml min^{-1}; temperature, 26°C; UV detection at 254 nm.



Scheme 3. Structure of the α_1-AGP CSP.

Type III CSPs: protein CSPs

Investigations carried out on the BSA-CSP (Resolvosil-BSA) led to surprising results: most of the compounds were not (or poorly) resolved ($R_s \approx 0.5$–0.6), except for lactone 2, for which baseline resolution was achieved (selectivity $\alpha = 2.1$) within a short analysis time (*ca.* 15 min). On the other hand, the α_1-AGP-CSP (Enantiopac; see Scheme 3) exhibited a noticeable chiral recognition ability towards our compounds, and especially towards cytotoxic ones, with selectivity values varying from 1.3 to 4.7. Table VII gives the selectivities and resolution factors for compounds 1–15 on the α_1-AGP-CSP. Because of the very low efficiency of the α_1-AGP columns a selectivity higher than 1.5 is generally required to achieve a baseline resolution. Furthermore, the columns are sensitive to an increase in pressure; a bed compression was then observed together with a decrease in efficiency which affected the resolution.

The 2-propanol content in the mobile phase was responsible for the regulation of the retention and selectivity factors and was adjusted for each solute. From Table VII, α-methylene-γ-butyrolactams, 5–8, are eluted with similar magnitudes of α, R_s and k', indicating that the phenyl group R_1 may play an important part in the chiral recognition (through hydrophobic and steric interactions). Compounds 14 and 15,

TABLE VII

RESOLUTION OF α-METHYLENE-γ-BUTYROLACTONES AND -γ-BUTYROLACTAMS ON α_1-AGP IMMOBILIIZED ON SILICA GEL (ENANTIOPAC COLUMN)

Mobile phase, 8 m*M* sodium phosphate buffer (pH 7)–0.1 *M* NaCl–2-propanol; flow-rate, 0.3 ml min^{-1}; temperature, 25°C; column, 100 × 4 mm I.D.

Solute	*2-Propanol in mobile phase (%, v/v)*	k'_2	α	R_s
1	2	7.7	2.02	3.0
2	2	5.5	1.23	0.9
3	2	5.8	1.74	1.2
4	2	34	1.29	1.2
5	6	9.0	1.09	<0.5
6	6	10.3	1.17	0.6–0.7
7	6	10.8	1.17	0.5–0.6
8	6	10.0	1.15	0.5–0.6
9	2	4.5	1.50	1.2
10	6	8.4	1.26	0.8
11	2	17.0	1.10	0.5
12	4	4.1	4.7	1.7
13	2	9.0	1.8	1.0
14	6	5.5	1.34	0.9
15	4	6.6	1.39	1.0

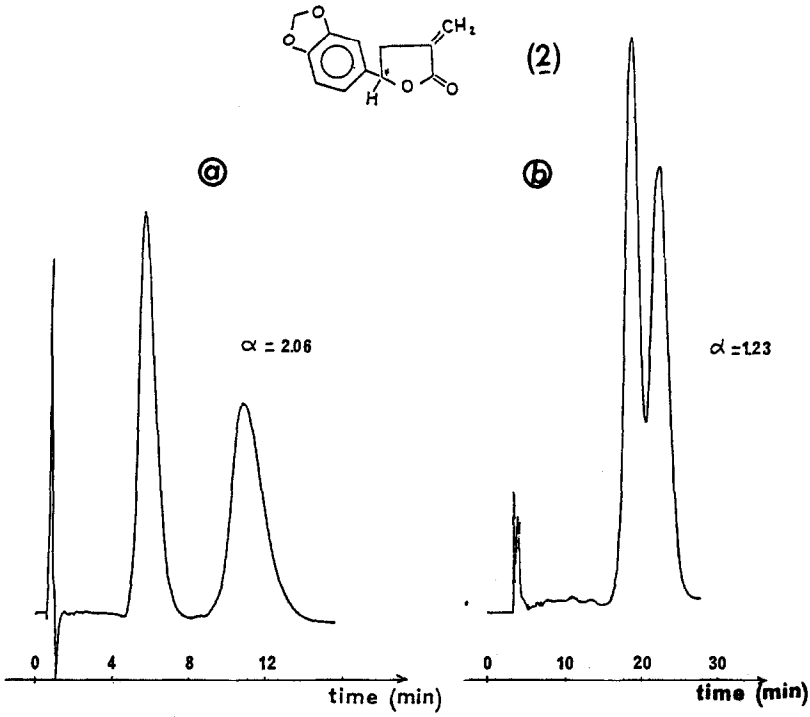


Fig. 7. Comparison of the resolution of racemate 2 on two protein-derived CSPs. (a) BSA immobilized on silica gel (Resolvosil-BSA-7 column), d_p = 7 μm. Column, 150 × 4.6 mm I.D.; mobile phase, 7.5 m*M* phosphate buffer (pH 6.9)–1.5% (v/v) 2-propanol; D = 2 ml min^{-1}. (b) α_1-AGP immobilized on silica gel (Enantiopac column), d_p = 10 μm. Column, 100 × 4 mm I.D.; mobile phase, 8 m*M* sodium phosphate buffer (pH 7)–0.1 *M* NaCl–2% (v/v) 2-propanol, D = 0.3 ml min^{-1}. Temperature, 25°C; UV detection at 230 nm.

which have similar structures, also exhibit similar chromatographic behaviour. The presence of an alkyl substituent R_2 on the nitrogen atom favours contribution to the chiral discrimination, as shown by comparing compounds 9 and 10 or 11 and 12. The chiral recognition process associated with these macromolecular proteins (BSA and α_1-AGP) is complex. Hydrogen bonding and hydrophobic interactions are the largest contributors. However, these proteins consists of hundreds of amino acids all possessing asymmetric carbon atoms available for chiral discrimination. Also, the secondary structure of the proteins takes part in the chiral recognition mechanism by means of helical parts of the protein, which are similar to the helical polymer chains of (+)-PTrMA.

Figs. 7–9 are typical chromatograms obtained on the BSA and α_1-AGP columns for the present series of lactams and lactones.

CONCLUSION

Each racemic α-methylene-γ-butyrolactone and -γ-butyrolactam was resolved on at least one of the CSPs studied in this work. The control of the optical purity for

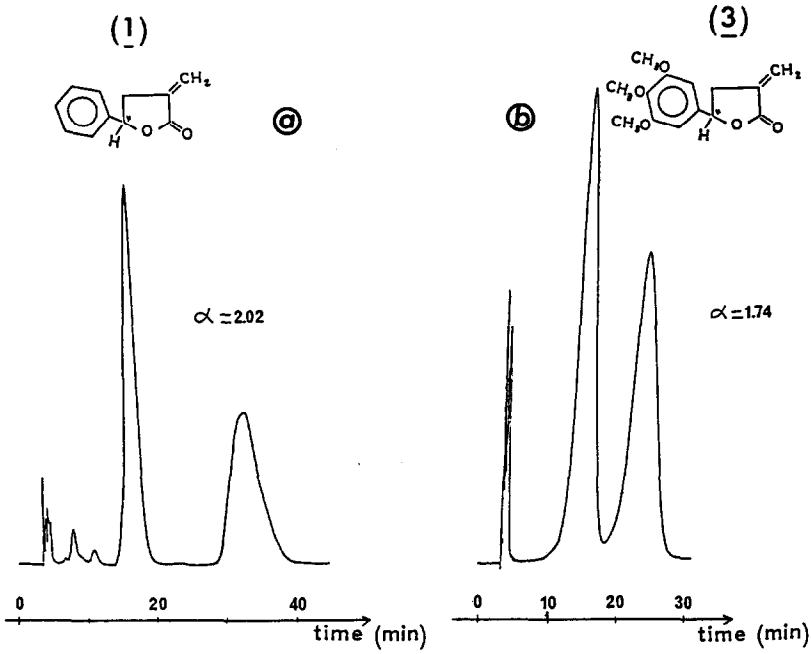


Fig. 8. Resolution of cytotoxic γ-methylene-γ-lactones 1 and 3 on the Enantiopac column. (a) Racemate 1; (b) racemate 3. Mobile phase, 8 m*M* sodium phosphate buffer (pH 7)–0.1 *M* NaCl–2% (v/v) 2-propanol; other operating conditions as for racemate 2 in Fig. 7.

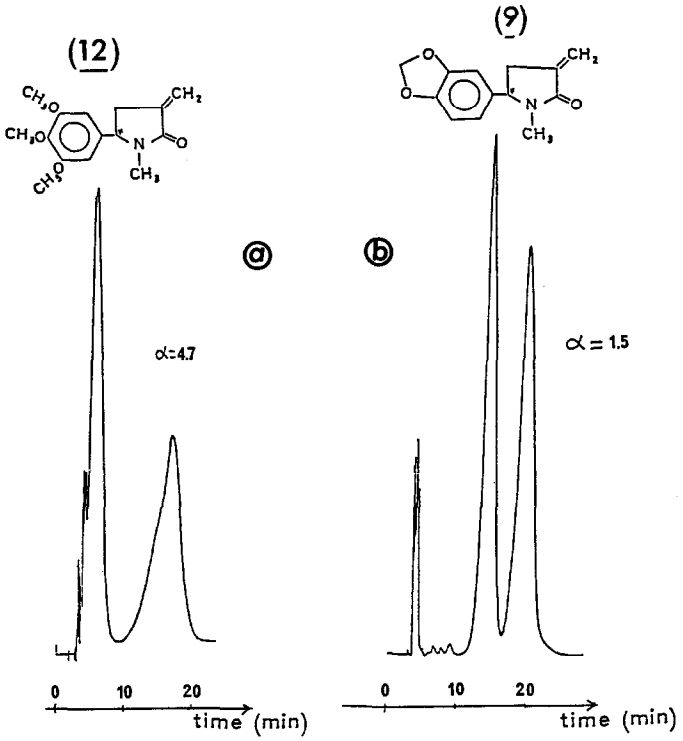


Fig. 9. Resolution of cytotoxic γ-methylene-γ-lactams 9 and 12 on the Enantiopac column. (a) Racemate 12; (b) racemate 9. Mobile phase, 8 m*M* sodium phosphate buffer (pH 7)–0.1 *M* NaCl–2-propanol [2 and 4% (v/v), respectively, for 9 and 12]; other operating conditions as for racemate 2 in Fig. 7.

TABLE VIII

CHOICE OF THE CSP FOR THE RESOLUTION OF CYTOTOXIC α-METHYLENE-γ-LACTONES AND -γ-LACTAMS

The most suitable CSPs are given in bold type.

Solute	*CSP*
1	**Enantiopac (α_1-AGP)**
	Chiralpak OT(+)
	Chiralcel OB
2	**Resolvosil-BSA-7**
	Enantiopac (α_1-AGP)
	Chiralcel OB and OC
3	**Enantiopac (α_1-AGP)**
	Chiralpak OT(+)
4	**Chiralpak OT(+)**
9	**Enantiopac (α_1-AGP)**
	Chiralcel OC
12	**Enantiopac (α_1-AGP)**
	Chiralpak OT(+)

such compounds, in particular for the cytotoxic agents, can be easily carried out. However, it should be noted that none of these CSPs was able to resolve the complete series of lactones and lactams (with R_s values higher than 0.8). There is an antagonism for one given CSP to exhibit both high enantioselectivities and a large scope of application. In Table VIII are reported the different CSPs suitable for the resolution of α-methylene-γ-lactones and -γ-lactams of our series which can act as cytotoxic agents; for each compound the CSP which gives the best resolution is in bold type, (+)-PTrMA [Chiralpak OT(+)] and α_1-AGP (Enantiopac) display the widest applicability. The chiral recognition ability of these polymers is related to the presence of numerous chiral sites of interaction different in nature and available for various types of interactions (asymmetric centres, chiral spaces arising from helical structures, etc.). However, the kinetics of the chromatographic process are slow, probably owing to the polymer structure of the CSPs, which results in low efficiencies of the columns. Baseline resolution often requires a selectivity higher than 1.5. It should also be mentioned that these CSPs are fragile and there can be problems concerning reproducibility.

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