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Journal of Chromatography A, 786 (1997) 13–21

JOURNAL OF CHROMATOGRAPHY A

Regioselectively carbamoylated polysaccharides for the separation of enantiomers in high-performance liquid chromatography

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Received 14 February 1997; received in revised form 29 April 1997; accepted 5 May 1997

Abstract

The chromatographic behavior of new chiral stationary phases (CSPs) based on mixed substituted cellulose, amylose or amylopectin is reported. Most CSPs have a 3,5-dimethylphenylcarbamate at the 2 and 3 positions of the glucose unit and either an (*R*)-, (*S*)- or (*RS*)-phenylethylcarbamate at the 6 position. This study shows that configuration of the phenylethylcarbamate at the 6 position largely influences the chiral recognition depending upon the polysaccharide studied. Some of the new CSPs exhibit a better enantioselective power for several racemates as compared to the corresponding symmetrical substituted polysaccharides CSPs. The resolution of some benzodiazepines is also reported. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Stationary phases, LC; Polysaccharides; Benzodiazepines

1. Introduction

Over the last ten years, optical resolution by high-performance liquid chromatography (HPLC) has become a practical and useful method not only to determine optical purity but also to obtain pure optical isomers. Many publications have already reported the abilities of chiral stationary phases (CSPs) based upon cellulose, amylose or amylopectin derivatives coated on macroporous silica gel [1–4]. Recently, we made a contribution to the interesting work reported by Kaida and Okamoto, dealing with heterogeneous substituted polysaccharides as CSPs [5]. We discussed the resolution power of cellulose derivatives with functional groups of a different nature on the same glucose unit: a benzoate at the 6 position or the 2,3 position and a phenylcarbamate at the 2,3 or the 6 position, respectively [6]. The best results were obtained when using a chiral phenylcarbamate at the 6 achiral position, the chiral 2 and chiral 3 positions being substituted with an achiral carbamate.

In order to apply this work to other polysaccharides, we have introduced on amylose and amylopectin a chiral (*R*)-, (*S*)- or (*R,S*)-phenylethylcarbamate moiety at the 6 position of the glucose unit and a 3,5-dimethylphenylcarbamate group at the 2,3 position. The chromatographic behavior of these CSPs were studied in detail and compared to the cellulose based ones. Some other interesting mixed cellulose-based CSPs were also prepared. The different types of synthesized polysaccharide are represented in Scheme 1.

The CSPs of the three series (**1–3**), (**4–6**) and (**7–9**), cellulose, amylose and amylopectin based, respectively, has a 3,5-dimethylphenylcarbamate at

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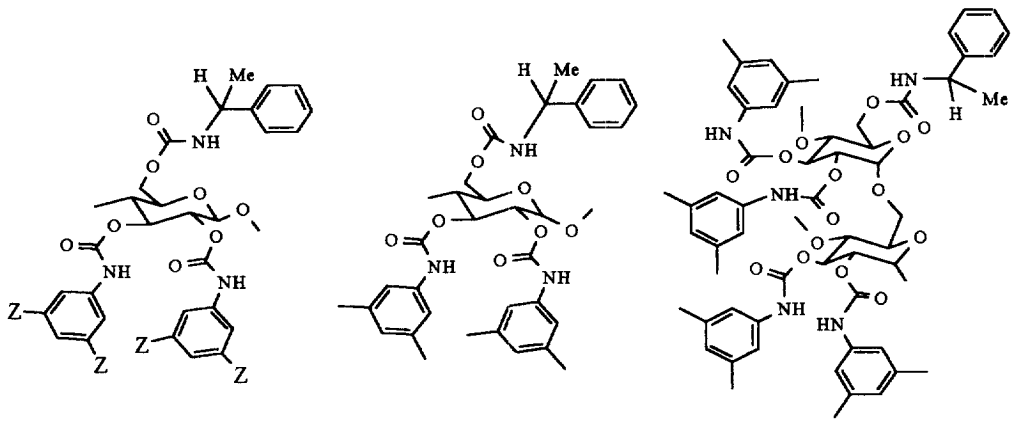
PII S0021-9673(97)00553-0



Cellulose	Amylose	Amylopectin
1: 6-(R); Z=CH_3	**4**: 6-(R)	**7**: 6-(R)
2: 6-(S); Z=CH_3	**5**: 6-(S)	**8**: 6-(S)
3: 6-(R,S); Z=CH_3	**6**: 6-(R,S)	**9**: 6-(R,S)
10: 6-(R); Z = H		
11: 6-(R); Z = Cl		

Scheme 1.

the 2,3 position and a phenylethylcarbamate moiety at the 6 position. The cellulose based series (**10–11**) has a phenylcarbamate or a 3,5-dichlorophenylcarbamate at the 2 and 3 positions and a (*R*)-phenylethylcarbamate at the 6 position respectively.

2. Experimental

The chemical pathway for the synthesis of regioselectively derivatized polysaccharides was described previously [6]. These products were identified by elemental analysis (Table 1), IR spectrometry and 1H NMR at 400 MHz, showing the stereospecificity of the different reactions as already published [6]. The packing chiral materials were prepared as reported elsewhere [7]. Each chiral material was packed in stainless-steel tubes (250× 4.6 I.D. mm) by a slurry method.

Chromatographic resolutions were carried out with an HPLC system comprising of a Jasco UV-975 UV–Vis detector and a Jasco PU-980 HPLC pump.

3. Results and discussion

Fig. 1 shows a chromatogram of the resolution of the Tröger's base on CSP **2**. The dead-time of the column (t_0) was determined by injection of 1,3,5-tri-*tert.*-butylbenzene. The capacity factors (k'_1 and k'_2), defined as $(t_1-t_0)/t_0$ and $(t_2-t_0)/t_0$ where t_1 and t_2 are the retention times of the enantiomers, and were 0.94 and 1.52, respectively. The separation factor

Table 1
Elemental analysis on the different CSPs

		C%	H%	N%
1	Found	66.12	6.00	6.81
	Calculated	65.66	6.18	6.96
2	Found	66.20	6.13	7.13
	Calculated	65.66	6.18	6.96
3	Found	65.95	6.54	6.89
	Calculated	65.66	6.18	6.96
4	Found	65.71	5.92	6.87
	Calculated	65.66	6.18	6.96
5	Found	65.52	6.03	6.82
	Calculated	65.66	6.18	6.96
6	Found	65.51	5.94	6.68
	Calculated	65.66	6.18	6.96
7	Found	65.81	5.96	7.11
	Calculated	65.66	6.18	6.96
8	Found	65.96	5.87	6.86
	Calculated	65.66	6.18	6.96
9	Found	65.72	6.03	6.66
	Calculated	65.66	6.18	6.96
10	Found	63.54	5.38	7.78
	Calculated	63.62	5.30	7.68
11	Found	51.16	3.77	6.11
	Calculated	50.80	3.65	6.13

($\alpha = k'_2/k'_1$) and the resolution factor ($R_s = 2(t_2 - t_1)/(\omega_1 + \omega_2)$) were 1.62 and 3.71, respectively.

The chromatographic results obtained with CSPs **1–11** for the separation of seven classical racemates **a–g** (Scheme 2) used as test compounds on polysaccharide-CSPs are reported in Table 2.

In our recent works on mixed derivatized cellulose CSPs [6], we have shown how the achiral interactions driven by the 6 position of the polysaccharide glucose units were involved in the chiral recognition mechanism. We have obtained an improvement of enantioselectivity when using a chiral substituent at this position only. The achiral and harmful interactions due to the inadequate polysaccharide shape must remain hidden from the racemate. Increasing the whole chirality of the discriminating support thanks to a chiral screen effect reveals itself as a powerful tool.

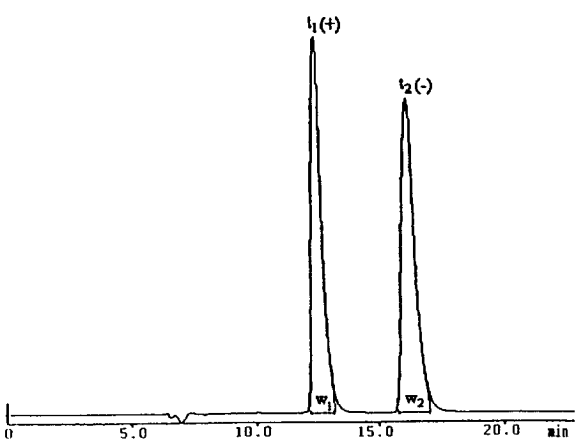


Fig. 1. Optical resolution of Tröger's base on CSP **2**. Mobile phase: hexane–isopropanol (90:10). Flow-rate, 0.5 ml/min.

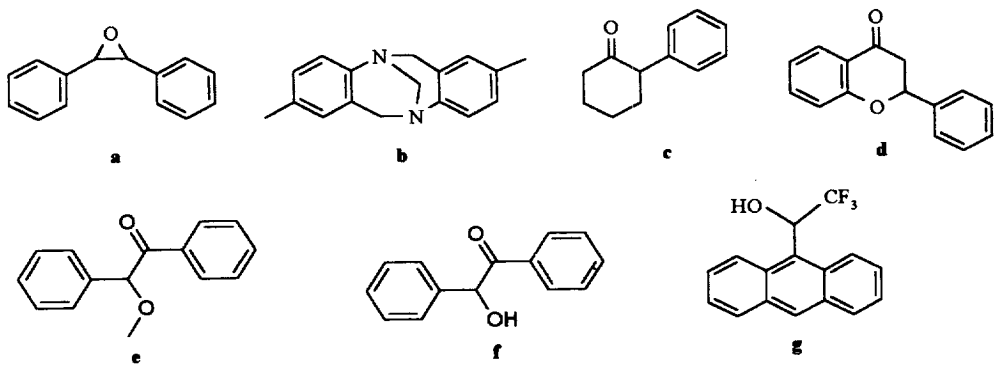
The first part of this report is devoted to examine how the absolute configuration of the chiral moiety at the 6 position may affect the selectivity. Its influence is also discussed according to the kind of polysaccharide used. The second part is a first attempt to explain in which way the substituent at the 2,3 position can modify the recognition mechanism.

The only difference between CSPs **1–9**, except for the nature of the polysaccharide, is the configuration of the phenylethylcarbamate (PEC) at the 6 position of the glucose unit. All those CSPs reveal themselves as very suitable for chiral recognition (Table 2) but their chromatographic behavior is quite different from the corresponding homosubstituted CSPs. It is not unusual to obtain better results with heterosubstituted CSPs. For example, **d** was separated on CSP **5** with an α value of 1.36 although it was either poorly recognized ($\alpha = 1.12$) or not at all on Chiralpak AD and Chiralpak AS, respectively [8] (Table 2). This phenomenon is observed in most cases with **b** and **c** on cellulose, **d** and **g** on amylose, **d**, **e** and **f** on amylopectin-based CSPs (Tables 2 and 3). The widest range of separations was obtained with cellulose and amylose derivatives. Although having a higher specificity, amylopectin-based CSPs give excellent results since **a** and **d** were better separated on CSP **9**. The retention factors obtained with CSP **7** are particularly low. Amylopectin with a *R*-PEC at the 6 position and a 3,5-dimethylphenylcarbamate at the 2,3 position is partially soluble in the eluent. The desorption of some amount of chiral selector leads to fast elution and low resolution in spite of high selectivity. A phenomenon of interest is the complementarity of the three polysaccharides. **b**, **c** and **g** were well recognized on cellulose, **d** and **f** on amylopectin and **e** on amylose. The chromatographic behaviors converge as the polymeric structures be-

Table 2
Optical resolution of racemates **a–g** on CSPs **1–11**, Chiralpak AS and AD [8]

CSPs	**a**			**b**			**c**			**d**			**e**			**f**			**g**		
	k'_1	α	Rs	k'_1	α	Rs	k'_1	α	Rs	k'_1	α	Rs	k'_1	α	Rs	k'_1	α	Rs	k'_1	α	Rs
1	0.70	1.58	3.14	0.80 (+)	1.72	4.07	1.17	1.35	3.60	1.26	1.25	2.26	0.92	1.30	2.14	2.38 (+)	1.31	3.53	2.05 (−)	2.01	7.27
2	0.86	1.39	2.67	0.94 (+)	1.62	3.71	1.33	1.29	2.45	1.67	1.21	1.90	1.15	1.32	2.63	3.09 (+)	1.38	3.92	2.40 (−)	2.19	7.08
3	0.58	1.00	0	0.60 (+)	1.48	2.61	0.88	1.25	1.48	1.33	1.06	0	0.85	1.12	0.86	2.19 (+)	1.23	1.73	1.96 (−)	1.50	3.68
4	0.57	1.75	3.70	0.85 (+)	1.13	0.91	0.95	1.00	0	1.51	1.37	3.26	1.14	1.65	4.80	4.23 (+)	1.21	2.93	3.41 (+)	1.21	1.87
5	0.49	1.60	2.30	0.69 (+)	1.31	1.77	1.02	1.14	1.00	1.90	1.36	2.62	0.95	4.22	10.5	3.10 (+)	1.32	2.65	1.32 (−)	1.33	1.63
6	0.52	1.78	3.82	0.72 (+)	1.22	1.27	0.90	1.00	0	1.78	1.08	0.62	0.96	3.64	10.9	3.99 (+)	1.14	1.21	1.38 (−)	1.31	2.26
7	0.27	1.00	0	0.10 (+)	8.00	0.92	–	–	–	0.60	2.00	~	0.69	1.00	0	1.64	1.00	0	–	–	–
8	0.43	1.44	1.62	1.17	1.00	0	–	–	–	1.81	1.00	0	0.85	2.83	7.09	2.40 (+)	1.46	3.28	–	–	–
9	0.25	1.80	1.50	0.93	1.00	0	–	–	–	1.04	1.79	1.81	0.63	3.33	4.00	2.34 (+)	1.32	1.27	–	–	–
10	0.68	1.35	1.57	0.94 (+)	1.45	1.82	1.83	1.22	2.41	2.21	1.07	0.69	1.72	1.14	1.13	4.36 (+)	1.04	~	1.76 (−)	1.41	1.77
11	0.16	1.60	0.49	0.28 (+)	1.43	0.50	0.68	1.29	0.6	0.74	1.00	0	0.61	1.00	0	1.21	1.00	0	1.05	1.00	0
AS	0.61	1.28	1.52	0.90 (+)	2.38	4.43	1.50	1.21	1.68	3.02	1.00	0	–	–	–	4.29 (+)	1.98	9.10	1.95 (−)	1.88	5.67
AD	0.42	3.04	6.67	0.53 (+)	1.58	2.30	0.61	1.00	0	0.93	1.12	0.77	–	–	–	3.14 (−)	1.21	2.07	1.30 (+)	1.15	0.75

Mobile phase: hexane–isopropanol (90:10); flow-rate: 0.5 ml/min. The sign in parentheses shows optical rotation of the first eluted enantiomer. The configuration of **b**(+), **f**(+) and **g**(+) is, respectively (5*R*,11*R*), *S* and *R*.



Scheme 2.

come closer. The first indication is the good resolution of **a** on amylose and amylopectin (α=1.78 on CSP **6** and α=1.80 on CSP **9**). The second indication is the increase in selectivity obtained for **d** both on amylose and amylopectin heterosubstituted CSPs as compared to the homosubstituted ones. This common behavior could be explained by the similarities between the two polysaccharides shapes, more precisely the presence of α glucosidic linkages in both polymer chains.

It is obvious that the enantioselectivities change as soon as the PEC configuration is modified. A dramatic fall of selectivity is observed with **e** when the configuration goes from *S* (CSP **5**; α=4.22) to *R* (CSP **4**; α=1.65). There seems to be no regular rules for the CSPs **1–9**. On the one hand, **a** is well recognized on CSP **1** (*R*-PEC; α=1.58) and not at all on CSP **3** (*RS*-PEC; α=1). On the other hand, **a** is not separated on CSP **7** (*R*-PEC; α=1) and well separated on CSP **9** (*RS*-PEC; α=1.8). After all, some trends appear within series prepared with the same polysaccharide. Higher separation factors were obtained with cellulose-based CSP **1** and CSP **2**, *R*-PEC and *S*-PEC, respectively. It was most surprising because the cellulose tris(*RS*-PEC) results surpass those of cellulose tris(*S*-PEC) [9,10]. Thus, using chiral substituent at the 6 position only is an effective method to improve selectivity with cellulose CSPs. Within the amylose series, the α values for CSP **5** having a *S*-PEC are elevated, in accordance with the results obtained with amylose tris(PEC) [8]. At last, the astonishing behavior of the CSP **9** must be pointed out. We have reported the good ability of amylopectin tris(*S*-PEC) for the discrimination of racemic mixtures [4]. Amylopectin tris(*RS*-PEC) was not mentioned because it was unsuitable for chiral recognition. In this case, CSP **9**, i.e. amylopectin substituted at the 6 position with a *RS*-PEC, gives the best selectivities. The average results obtained on CSP **7** and **8** having a chiral carbamate at the 6 position reveal that the artificially added chiral center is not responsible for the selec-

Table 3
Selectivities obtained on homosubstituted polysaccharide based CSPs for racemic compounds **b**, **c**, **d**, **e**, **f** and **g** [4,8–10]

Polysaccharide	Cellulose		Amylose		Amylopectin		
Racemate	b	c	d	g	d	e	f
OD	1.32	1.15					
AD			1.12	1.15			
3,5-DMPC					1.32	1.20	1.10
R-PEC	1.22	1.12	1.07	1.05	1.00	1.00	1.00
S-PEC	1.00	1.00			1.04	1.96	1.46
AS			1.00	1.88			
R,S-PEC	1.00	1.09	1.11	1.14	1.00	1.00	1.00

Conditions: hexane–iPrOH (90:10); flow-rate, 0.5 ml/min; 3,5-DMPC=tris(3,5-dimethylphenylcarbamate), PEC=phenylethylcarbamate.

tivity increase. So it would be simply the positive influence of the steric effect induced by the phenylethylcarbamate.

The retention orders are not affected by the PEC absolute configuration. **b**(+/5*R*,11*R*), **f**(+/*S*) and **g**(−/*S*) were eluted first on all the CSPs (except for **g** on CSP **4**). As a result, the 6 position is not responsible for the absolute configuration of the active sites. So it is not one independent function supported by an asymmetric carbon which controls the selectivity but the whole chirality of the polysaccharide macrostructure. The less retained enantiomers were the same on heterosubstituted CSPs **1–3** and on cellulose tris(3,5-dimethylphenylcarbamate) [1]. Conversely, same elution orders were observed between CSPs **4–6** and the corresponding amylose tris(PEC) [9,10] or between CSPs **7–9** and the corresponding amylopectin tris(PEC). As a result, the interactions driven by the 6 position seem to be more involved in the discrimination mechanism as soon as amylose and amylopectin were concerned. This common behavior could be explained, as we have discussed above, by the closer shape of the polysaccharides.

In conclusion, using a chiral phenylcarbamate at the 6 position induces a whole selectivity increase. Yet, the reason for the improvement differs according to the polysaccharide used. Although the chiral screen effect is clearly involved for cellulose and amylose, the recognition mechanism is more complicated for amylopectin, which is a branched polysaccharide. In which case, it would be the steric hindrance of the PEC that creates the desired effect.

The only difference between CSPs **1**, **10** and **11** is the nature of the phenylcarbamate at the 2,3 position of the glucose unit. Cellulose with *R*-PEC at the 6 position and a 3,5-dichlorophenylcarbamate at the 2,3 position is partially soluble in the eluent leading to very low retention and resolution like those observed on CSP **7**. After all, it would be of interest to graft derivatives **11**, and also **7**, on silica gel in order to increase the stability of the CSP and to use the most of their chiral resolution power. Although the seven test racemates were recognized on CSPs **1** and **10**, the a values were always lower on CSP **10**. The phenylcarbamates at the 2 and 3 positions are supported by two neighboring carbons. So the steric repulsion becomes stronger as soon as the substituent volume at the *para* position of the phenylcarbamate increases. As a consequence, the secondary structure of the polysaccharide is presumably disturbed. The crystallinity decrease induces new amorphous parts, commonly called chiral cavities, improving the inclusion of the solutes in the polymer layer. This phenomenon is developed with derivative **1** having a 3,5-dimethylphenylcarbamate at the 2 and 3 positions leading to best separations for CSP **1**.

4. Applications

The separations of four benzodiazepins on CSPs **1–3** and CSPs **4–6** are shown in Tables 4 and 5, respectively. In the same chromatographic conditions, only the temazepam was well recognized on amylopectin based CSPs with α values of 1.59 on CSP **8** and 1.63 on CSP **9**. Although the four racemates were always recognized on CSPs **1–6**, the separation amplitude was influenced by the polysaccharide nature. Within CSPs **1–3**, oxazepam was the best separated racemate (Fig. 2) since lorazepam was poorly recognized. The latter presents a chlorine

Table 4
Separation of benzodiazepins on cellulose CSPs

Compounds	CSPs								
	1			**2**			**3**		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
Oxazepam	3.85	1.34	2.23	4.09	1.41	2.43	5.33	1.46	2.77
Lorazepam	3.86	1.09	0.61	4.40	1.15	0.99	5.29	1.17	1.19
Temazepam	4.78	1.21	2.29	5.03	1.24	2.35	5.68	1.41	3.97
Lormetazepam	5.96	1.08	0.86	6.53	1.19	1.68	6.94	1.34	3.21

Conditions: hexane–iPrOH (80:20), octanoic acid 10 m*M*; flow-rate, 1 ml/min.

Table 5
Separation of benzodiazepins on amylose CSPs

Compounds	CSPs								
	4			**5**			**6**		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
Oxazepam	4.41	1.21	1.76	4.31	1.05	0.80	3.38	1.17	0.88
Lorazepam	4.46	1.17	1.36	4.82	1.15	0.96	3.74	1.18	0.89
Temazepam	4.79	1.82	8.09	6.64	1.77	5.20	6.02	1.82	4.59
Lormetazepam	7.92	1.68	8.42	10.80	1.05	~	1.82	2.27	1.64

Conditions: hexane–iPrOH (80:20), octanoic acid 10 m*M*; flow-rate, 1 ml/min.

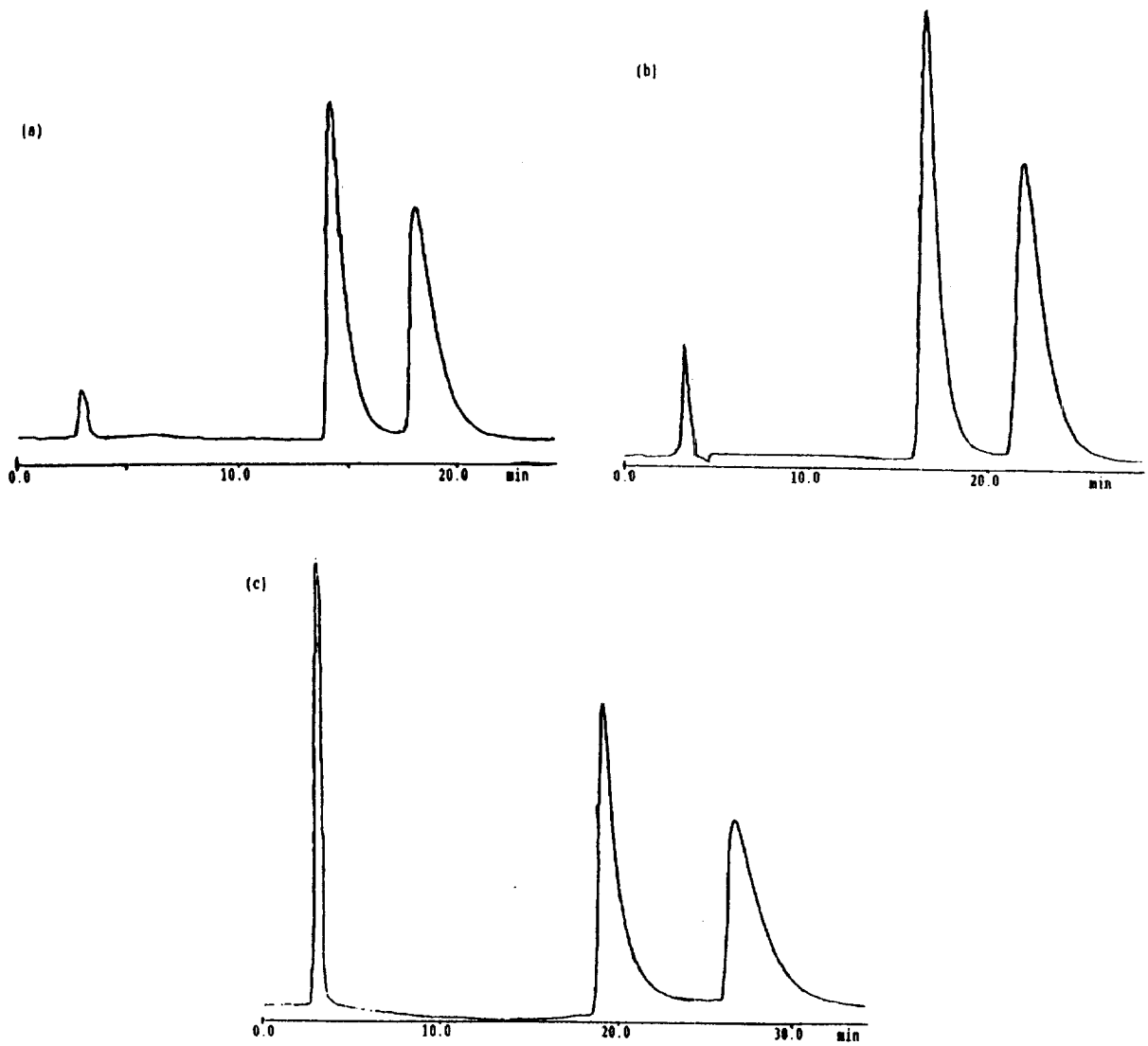
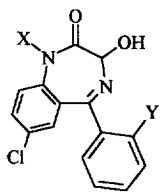


Fig. 2. Optical resolution of oxazepam on CSPs **1** (a), **2** (b) and **3** (c). Mobile phase: hexane–isopropanol (80:20), octanoic acid 10 m*M*. Flow-rate, 1 ml/min.



X=H; Y=H : Oxazepam
X=H, Y=Cl : Lorazepam
X=CH_3; Y=H : Temazepam
X=CH_3; Y=Cl : Lormetazepam

Scheme 3.

substituent at the *ortho* position of the phenyl (Scheme 3). The resulting rotational hindrance prevents the molecule from adopting a planar configuration. As a result, it becomes difficult for lorazepam to penetrate the chiral cavities and leads to low selectivity. Within the CSPs **4–6**, temazepam was the best separated racemate (Fig. 3) since lorazepam and oxazepam were poorly recognized. The two last-mentioned racemates present a secondary amine proton (Scheme 3) with the ability to build hydrogen bonds with the CSP. The interactions not supported by the chiral carbon of the racemate reveal themselves as harmful for discrimination. Lormetazepam which has an *ortho* chloro group is surprisingly well resolved on CSP **4** and CSP **6**. Consequently, the recognition mechanism is quite different between cellulose and amylose depending on the size and the spatial shape of the chiral cavities: steric effects becoming a main parameter with small cavities,

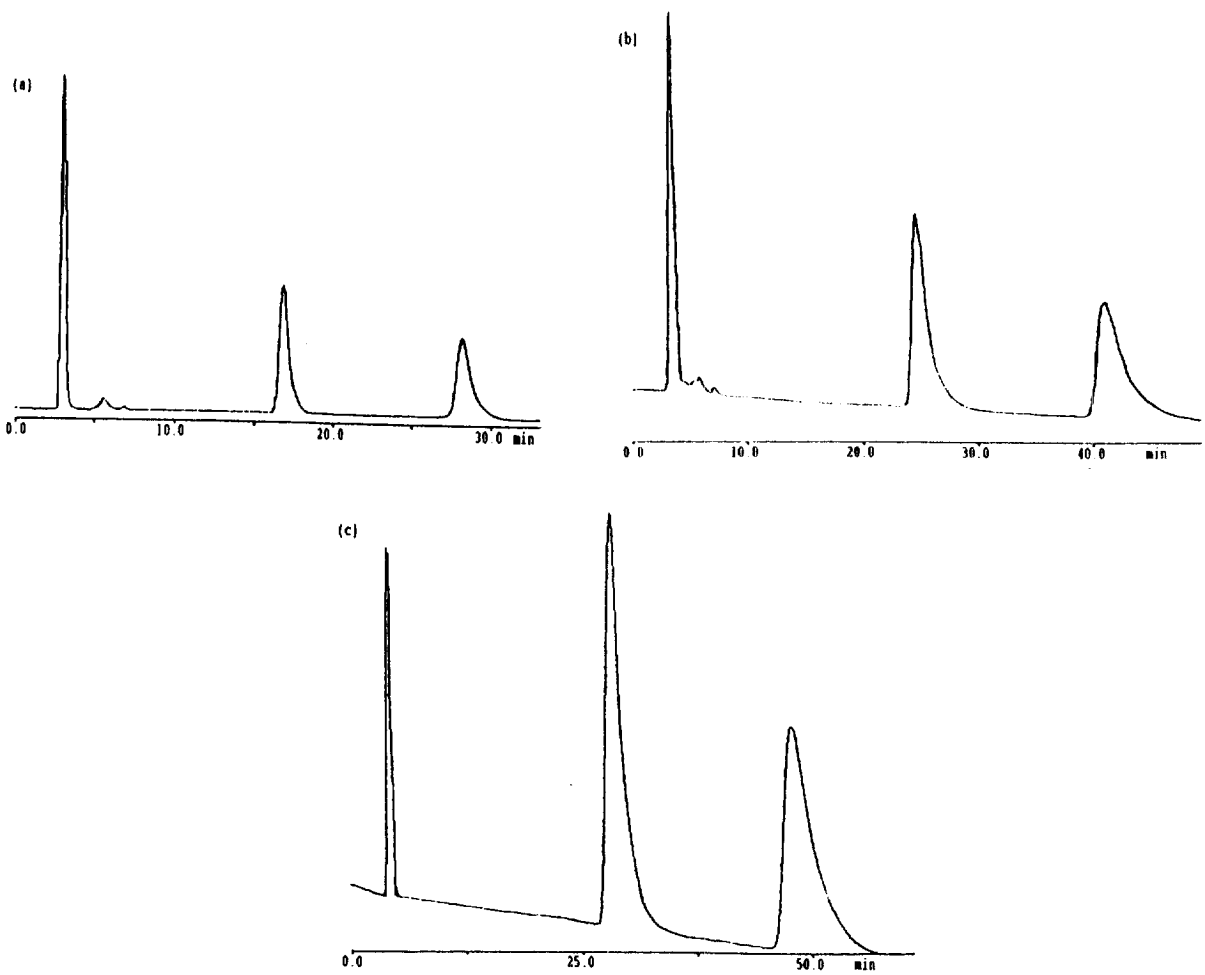


Fig. 3. Optical resolution of temazepam on CSPs **4** (a), **5** (b) and **6** (c). Mobile phase: hexane–isopropanol (80:20), octanoic acid 10 m*M*. Flow-rate, 1 ml/min.

hydrogen bonds going over it with wider ones. Only a few separations of benzodiazepins were reported in normal-phase using commercially available CSPs based on homosubstituted polysaccharides [11,12]. Oxazepam was recognized on Chiralcel OD and chiralpak AS with an α value of 1.56 and 1.48, respectively. Any resolution of lorazepam, temazepam and lormetazepam was reported on homosubstituted carbamate cellulose and amylose CSPs as shown in Chirbase [13]. As a result, the polysaccharide regioselective derivatization does not improve the benzodiazepins discrimination as soon as amylose is involved. However, heterosubstituted cellulose-based CSPs are preferred to the corresponding homosubstituted ones when the separation of the whole four racemics is required.

5. Conclusion

Eleven regioselectively derivatized polysaccharide-coated CSPs were synthesized. They reveal themselves as suitable for chiral discrimination and sometimes give better results than commercial CSPs. Racemic compounds shown in this paper were most effectively resolved with the 6-(*R*-phenylethylcarbamate)-2,3-(3,5-dimethylphenylcarbamate) cellulose and the 6-(*S*-phenylethylcarbamate)-2,3-(3,5-dimethylphenylcarbamate) amylose derivatives. The crucial role of the phenylethylcarbamate configuration at the 6 position has been pointed out according to the kind of polysaccharide used. The influence of the phenylcarbamate substituent at the 2,3 position was also discussed. Finally, using cellulose with a chiral phenylethylcarbamate at the 6 position allows the improvement of benzodiazepins separation.

Acknowledgments

The authors wish to thank the Conseil Régional d'Aquitaine for its financial support.

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