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Comparative HPLC enantioseparation of new chiral hydantoin derivatives on three different polysaccharide type chiral stationary phases

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Dedicated to Professor Dr Gottfried Blaschke on the occasion of his 65th birthday.

Abstract

The enantioseparation of eighteen new chiral hydantoin derivatives was studied on three different polysaccharide type chiral stationary phases (CSP) Chiralpak AD, Chiralcel OD and Chiralcel OJ in the normal-phase HPLC mode. Chiralpak AD material exhibited the most universal chiral resolving ability and allowed the enantioseparation of 17 out of 18 compounds followed by Chiralcel OD (10 enantioseparations of 12 tested compounds) and Chiralcel OJ (eight enantioseparation from 13 tested analytes). Some complementary separations were observed and all of 18 compounds could be resolved at least with one of the three chiral CSP under the conditions of this study. With regard to the structure of the analytes, bulky electron rich substituents at C5 of the hydantoin nucleus appear to favor stereoselective interactions.

Keywords: Chiral hydantoins; Enantioseparations; HPLC; Polysaccharide-type chiral; Stationary phases; Chiralcel OD; Chiralpak AD; Chiralcel OJ

1. Introduction

The imidazolidinedione ring, better known under the name hydantoin, is one of the key heterocycles in medicinal chemistry and extensively used in combinatorial chemistry libraries [1,2]. The hy-

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dantoin moiety is part of numerous structures of drugs or drug candidates. The best known example is 5,5′-diphenylhydantoin or phenytoin, a drug launched in the 1950s and still used worldwide as one of the major antiepileptic drugs [3,4]. Despite the synthesis and evaluation of numerous analogues no compound proved to be superior to the parent drug with the exception of the phenytoin prodrug fosphenytoin as an injectable drug for the treatment of the status epilepticus [5]. The hydantoin heterocycle is also part of ligands of the serotonine [6] and somatostastin [7] receptors as well as the antiviral drugs, 5-(3,4-dichlorophenyl) -methylhydantoin [8].

Based on the structures of 1-alkyl-3-(1-naphthoyl)pyrroles [9] and of diarylpyrazoles [10] as ligands of the cannabinoid receptor, we investigated the potential of the 5,5′-diphenylhydantoin moiety as a new template for cannabinoid receptor recognition. Thus, a series of 3-alkyl-(5,5′-diphenyl)imidazolidinediones was synthesized [11] and three compounds exhibited significant affinity for the human cannabinoid receptor ($Ki < 100$ nM). In the course of continuing structures–activity relationships studies, new chiral hydantoin derivatives, the chiral center being C5 of the heterocycle, have been synthesized and evaluated as racemic mixtures for competitive displacement of radioactive ligands from the CB_1 cannabinoid receptor [12]. Derivatives with indole substituents in position 5 displayed the highest activity.

Based on the known difference of pharmacological and toxic properties of enantiomers [13,14] including chiral ligand-receptor recognition, the pharmacological and toxicological properties of the individual enantiomers of new chiral hydantoin derivatives [15] summarized in Fig. 1 have to be evaluated. For this purpose assays are required to assess the stereochemical purity of the compounds following stereoselective synthesis or preparative chiral chromatography. In addition, stereoselective bioanalytical assays may be required for further in vitro and in vivo studies.

The aim of the present study was to evaluate the potential of the three most widely used polysaccharide type chiral stationary phases (CSPs) cellulose tris-(4-methylbenzoate) (Chiralcel OJ), cellulose tris-(3,5-dimethylphenylcarbamate) (Chiralcel OD) and amylose tris-(3,5-dimethylphenalcarbamate) (Chiralpak AD) (Fig. 2) for analytical scale enantioseparations of the new chiral hydantoin derivatives. Based on the enantioseparations the most suitable CSP may be selected for preparative scale enantioseparations of some of the hydantoin analytes. In addition, preliminary structure–enantioseparation relationships may be derived from results.

2. Experimental

2.1. Materials and reagents

Chemicals and solvents for the synthesis of the compounds were obtained from Acros (Geel, Belgium). HPLC grade *n*-hexane and 2-propanol were from Merck (Darmstadt, Germany). The hydantoin derivatives were synthesized in two steps according to standard procedures described for the preparation and alkylation of phenytoin [16]. Briefly, the first step included the formation of the hydantoin ring starting from the corresponding amino acid phenylalanine or tryptophan, respectively, and potassium cyanate. In a second reaction, the compounds were alkylated in position 3 of the hydantoin ring at room temperature using bromo- or chloroalkanes in dimethylformaide in the presence of potassium carbonate. 5-(4-Chlorophenyl)-5′-phenylhydantoin (**18**) was synthesized from 4-chlorophenylphenylketone, ammonium carbonate and potassium cyanide in water-dimethylformamide under pressure and vigorous heating. All compounds were recrystallized and characterized by NMR, mass spectroscopy and elemental analysis.

2.2. High performance liquid chromatography

The HPLC system consisted of an isocratic Knauer HPLC pump 64 (Knauer, Berlin, Germany), a Merck Hitachi 655A variable wavelength UV detector and a Merck Hitachi D-2500 Chromato-Integrator (Merck, Darmstadt, Germany). The separations were performed using 250×4.6 mm HPLC columns of Chiralcel OJ, Chiralcel OD and Chiralpak AD obtained from Daicel Chemical

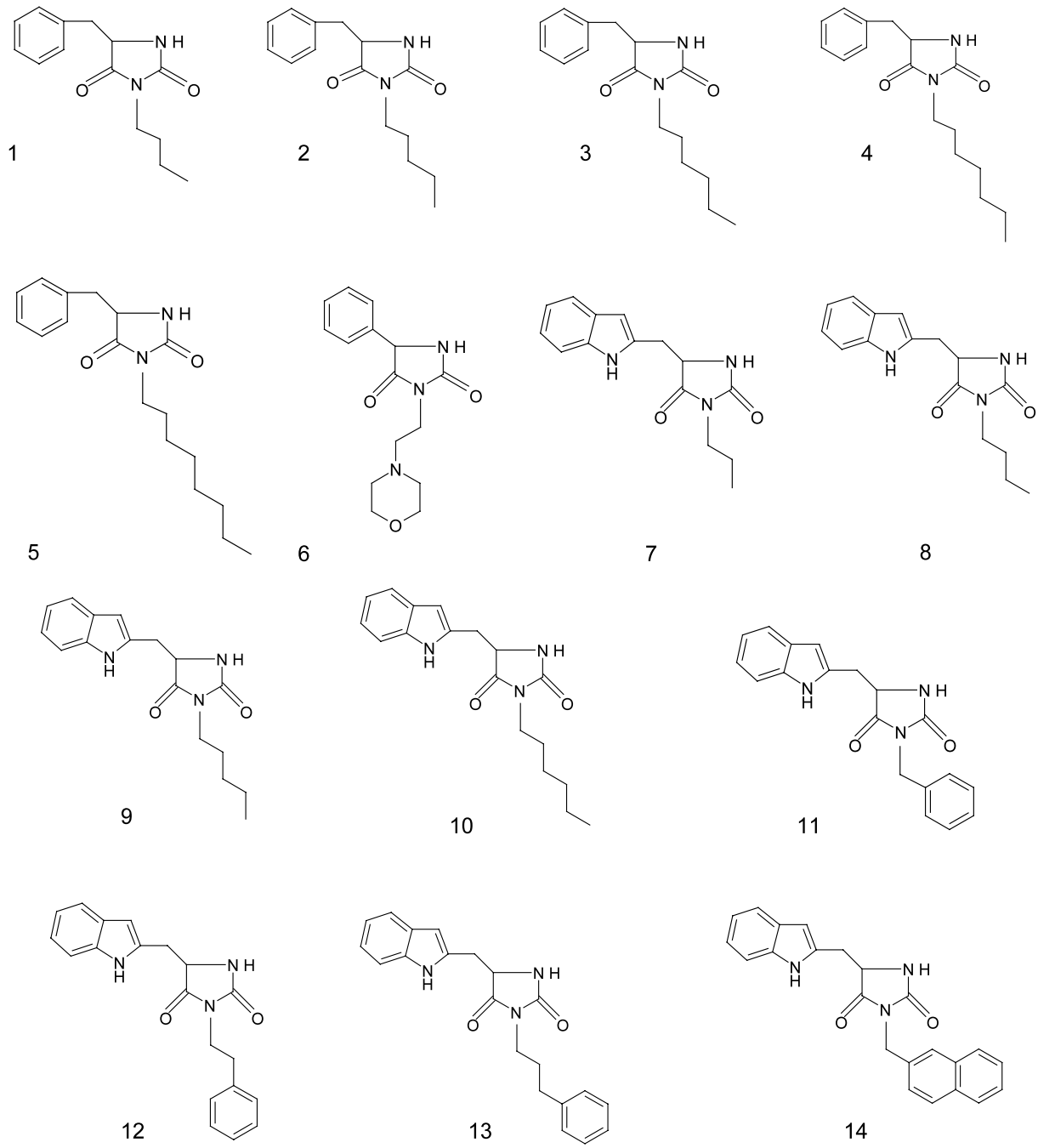

Fig. 1. Structure of chiral hydantoins.

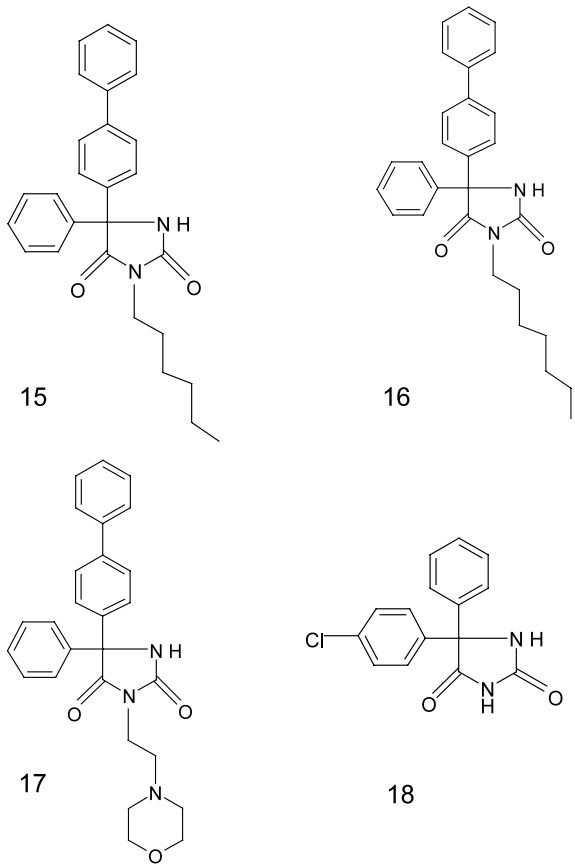

Fig. 1. (*Continued*)

Ind. (Tokyo, Japan). The mobile phases were *n*-hexane/2-propanol mixtures of various ratios (v/v) delivered at a flow rate 1 ml/min. UV detection was carried out at 254 nm.

3. Results and discussion

The structures of the chiral hydantoin derivatives are summarized in Fig. 1 while Fig. 2 gives a schematic representation of the CSPs used in the present study. The role of the substituent on the carbohydrate backbone of the CSP, i.e. methylbenzyl versus dimethylphenylcarbamoyl, on the separation of the phenytoin enantiomers may be estimated by comparing Chiralcel OJ and Chiralcel OD. The influence of the carbohydrate backbone may be derived from a comparison of Chiralcel OD containing cellulose and the amylose-based CSP Chiralpak AD. For this purpose the hydantoins were divided into three groups. The first group included compounds **1**–**6** which contain a benzyl substituent and a proton at the chiral C5. With the exception of the ethylmorpholino substituent in compound **6** they differ in the chain length of the N3 alkyl substituent. Group 2 comprises analytes **7**–**14** with the 2-(indoyl)methyl moiety instead of the benzyl group at C5. Compounds **15**–**18** containing two aromatic substituents form group 3. Compound **18** contains a phenyl and a 4-chlorophenyl substituent while compounds **15**–**17** are substituted with a phenyl and a diphenyl moiety.

3.1. Enantioseparations on Chiralcel OJ

The results of the separations using Chiralcel OJ are summarized in Table 1. The CSP did not

Table 1
Enantioseperations of chiral hydantoins

Compound	Chiralcel OJ					Chrialcel OD					Chrialpak AD				
	k'_1	k'_2	α	R_S	Condition[a]	k'_1	k'_2	α	R_S	Condition[a]	k'_1	k'_2	α	R_S	Condition[a]
1	0.63	0.63	1.00	–	A	1.22	1.60	1.31	1.4	A	2.69	3.98	1.48	1.2	B
2	0.40	0.40	1.00	–	A	1.12	1.44	1.29	1.3	A	2.38	3.52	1.48	1.2	B
3	0.38	0.38	1.00	–	A	–	–	–	–	A	2.42	2.97	1.23	0.7	B
4	0.30	0.30	1.00	–	A	1.01	1.26	1.25	1.3	A	2.11	3.06	1.45	1.2	B
5	–	–	–	–	–	0.94	1.18	1.25	1.0	A	1.86	2.71	1.45	1.3	B
6	–	–	–	–	–	9.00	15.00	1.66	5.0	A	5.38	6.47	1.20	0.6	B
7	4.63	4.63	1.00	–	B	7.96	12.30	1.55	2.5	A	4.75	8.80	1.85	1.7	B
8	3.22	4.00	1.24	0.7	B	7.01	13.20	1.88	4.0	A	4.17	7.55	1.81	1.7	
9	2.18	2.56	1.18	0.5	B	6.37	14.80	2.33	6.0	A	3.86	7.24	1.87	2.0	
10	2.71	3.27	1.20	0.5	B	5.68	10.80	1.90	3.6	A	3.46	6.18	1.79	1.8	
11	–	–	–	–	–	–	–	–	–	–	9.32	14.52	1.56	1.5	
12	15.50	18.90	1.22	0.8	B	–	–	–	–	–	10.30	10.30	1.00	–	
13	8.16	11.20	1.37	1.0	B	–	–	–	–	–	7.24	11.50	1.59	1.6	B
14	–	–	–	–	–						14.50	21.90	1.52	1.6	B
15	2.40	4.40	1.80	1.5	C	1.33	1.33	1.00	–	A	7.01	13.20	1.88	4.0	B
16	2.40	6.20	2.60	2.0	C	1.22	1.22	1.00	–	A	11.00	12.00	1.09	–	B
17	11.8	13.2	1.11	–	C	2.34	2.94	1.26	1.1	A	2.74	4.33	1.58	1.2	B
18	3.36	6.92	2.06	1.32	C	13.00	13.00	1.00	–	–	4.78	7.17	1.49	1.1	D

[a] Experimental conditions.

A, *n*-hexane/2-propanol 90/10 (v/v), flow rate 1.0 ml/min; B, *n*-hexane/2-propanol 95/5 (v/v), flow rate 1.0 ml/min; C, *n*-hexane/2-propanol 90/10 (v/v), flow rate 0.5 ml/min; D, *n*-hexane/2-propanol 98/2 (v/v), flow rate 1.0 ml/min.

exhibit chiral recognition abilities towards the hydantoins of group 1. In fact they were not strongly retained on this material as can be derived from the k' values and eluted as unresolved peaks. Increasing the chain length of the N3 alkyl substituent led to a decrease of the retention. Substituting the phenyl group by the larger indole moiety improved the interaction between the CSP and the analytes. Thus, partial resolution of the enantiomers of the compounds of group 2, i.e. hydantoins **8**–**10**, **12** and **13** were observed. As within group 1 a decrease of the retention factor k' upon increasing the N3 alkyl chain occurred with the exception of compound **10**. In contrast, the bulkier arylalkyl substituents in compounds **12** and **13** improved the interaction between the CSP and the analyte enantiomers resulting in a better although not complete separation.

Replacing the hydrogen at C5 with aromatic substituent significantly improved the chiral recognition ability of Chiralcel OJ material towards the hydantoins. Thus, the biphenyl derivative **15** was baseline resolved on Chiralcel OJ column with *n*-hexane/2-propanol 90/10 (v/v) as a mobile phase. The cyclic 1,4-oxazan moiety seems to contribute significantly to the nonenantioselective selector-an-

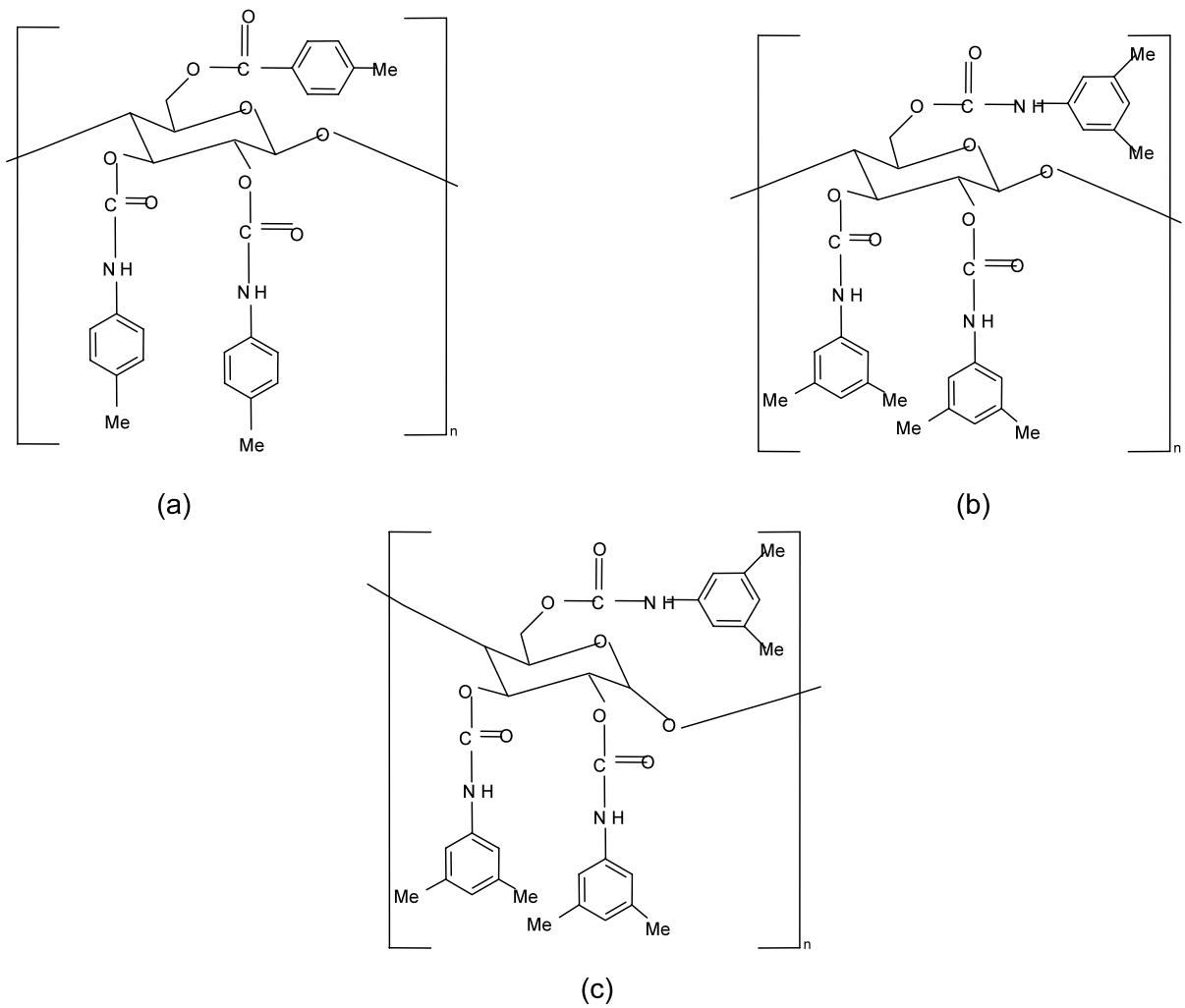

Fig. 2. Schematic representation of polysaccharide derivatives contained in CSPs: (a) cellulose tris(4-methylbenzoate) (Chiralcel OJ); (b) cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD); and (c) amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD).

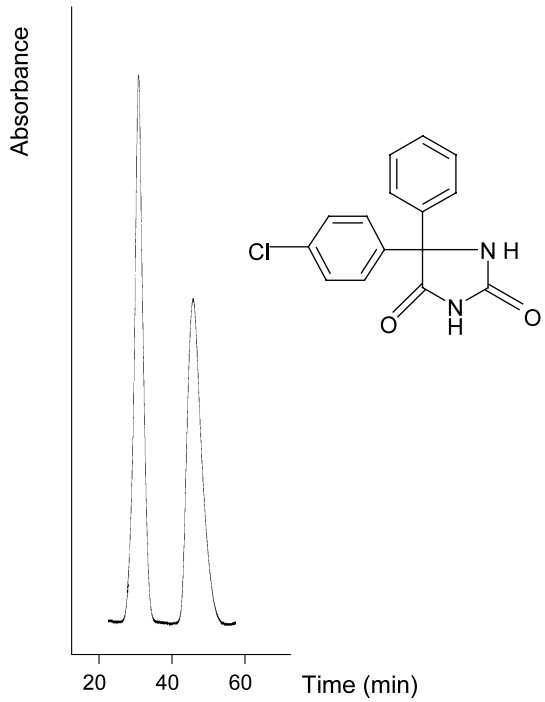

Fig. 3. Enantioseparation of 5-phenyl-5′-(4-chlorophenyl)-hydantoin (**18**) on Chiralcel OJ column using *n*-hexane/2-propanol (90/10, v/v) as a mobile phase with a flow rate 0.5 ml/min.

alyte interactions as the highest retention factors but the lowest enantioselectivity was observed for compound **17**. Interestingly, in contrast to group 1 and 2 of the compounds, the retention factor appears to increase with increasing length of the alkyl substituent at N3 within group 3. The enantiomers of 5-(4′-chlorophenyl)-5-phenylhydantoin (**18**) could be effectively separated using a Chiralcel OJ column (Fig. 3). Overall, bulky and electron rich substituents at C5 improve the chiral recognition ability of the CSP towards the hydantoins and thus the enantioseparation.

3.2. Enantioseparations on Chiralcel OD

This CSP contains cellulose tris(3,5-dimethylphenylcarbamate) as chiral selector offering additional hydrogen-bonding sites for selector-analyte interactions compared to the 4-methylbenzoate substituents on Chiralcel OJ. Therefore, the Chiralcel OD column exhibits in general higher chiral resolving ability compared to Chiralcel OJ column for numerous compounds [17,18]. This trend was also confirmed in the present study (Table 1). Thus, from the analytes of group 1 which were not resolved on the Chiralcel OJ column, four were almost baseline separated on the Chiralcel OD material. The general trend of decreasing retention factors k' with increasing alkyl chain length of the substituent at N3 was also observed for the Chiralcel OD column. The analytes of group 2 which contained an indole moiety in the position 5 and alkyl substituents of different lengths in the position N3 were the best resolved compounds for the Chiralcel OD column. Typical chromatograms of the resolution of the enantiomers of hydantoins **9** and **10** are shown in Fig. 4. Surprisingly, the peak ratio deviated significantly from 1:1 in the case of analytes **7** and **10**. This may be attributed to incomplete racemization during the synthesis. The synthetic procedure starts from amino acids which are enantiomerically pure. The second alkylating step involves the use of a strong base leading to racemization of the compounds.

The compounds of the third group containing biphenyl substituents at the center of chirality **15**–**17** were less retained and not as well resolved by Chiralcel OD as by Chiralcel OJ material except for compound **17** which was almost baseline resolved on Chiralcel OD with a rather short retention time.

The enantioseparation abilities of Chiralcel OJ and Chiralcel OD appear to be somewhat complementary with respect to the present set of analytes. Thus, although Chiralcel OD allowed the resolution of more analytes, some of the compounds which were effectively resolved on Chiralcel OJ column were not resolved on Chiralcel OD material. For example, compound **18** eluted from Chiralcel OD column as an unresolved wide peak with a relatively high retention factor ($k' = 13.0$) while it was baseline resolved on Chiralcel OJ column using the same mobile phase (Fig. 3). Chiralcel OD column allowed the enantioseparation of compound **6** with rather high enantioselectively but a plateau was observed between two resolved peaks. This may be indication of configurational instability of this compound (Fig. 5).

3.3. Enantioseparations on Chiralpak AD column

As described in numerous previous studies the enantiomer resolving ability of polysaccharide derivatives depends not only on the substituents on the hydroxyl groups but also on the structure of polysaccharide backbone [17,18]. Thus, the most widely used cellulose and amylose phenylcarbamate derivatives, Chiralcel OD and Chiralpak AD, not only exhibit complementary chiral recognition ability but may also reverse the elution order of the enantiomers of many chiral compounds [18]. Towards the chiral hydantoin derivatives of this study Chiralcel OD and Chiralpak AD materials exhibited comparable chiral recognition ability (Table 1). All of the chiral analytes of the first structural group were almost baseline resolved on Chiralpak AD column although the eluent contained only 5% 2-propanol in this case. The aforementioned trend of a decreasing retention factor k' with increasing chain length of the alkyl substituent in position N3 for compounds **1**–**5** was also observed for this CSP. The group of indole compounds was also resolved well on Chiralpak AD although the resolution was not as good as found for Chiralcel OD. Compound **17** containing the 1,4-oxazane moiety on N3 together with a biphenyl moiety at C5 was almost baseline resolved on both phenylcarbamate derivative but not as well resolved on the phenylester derivative of the polysaccharides. Thus, not only the substituents immediately linked to the center of chirality but also to the rather remote nitrogen atom appears to be important for chiral recognition.

As shown by the data summarized in Table 1, Chiralpak AD material was successful for the enantioseparation of most analytes compared to other two polysaccharide derivatives. Although both phenylcarbamate derivatives appear to be more universal than the phenylester derivative, the superiority of Chiralpak AD over Chiralcel OD is somewhat apparent because mobile phase optimization has been performed in some cases for the former but not for the Chiralcel OD column.

4. Conclusions

Polysaccharide phenylester and phenylcarbamate derivatives represent useful chiral selectors for the HPLC enantioseparation of chiral hydantoin derivatives. The phenylcarbamate derivatives

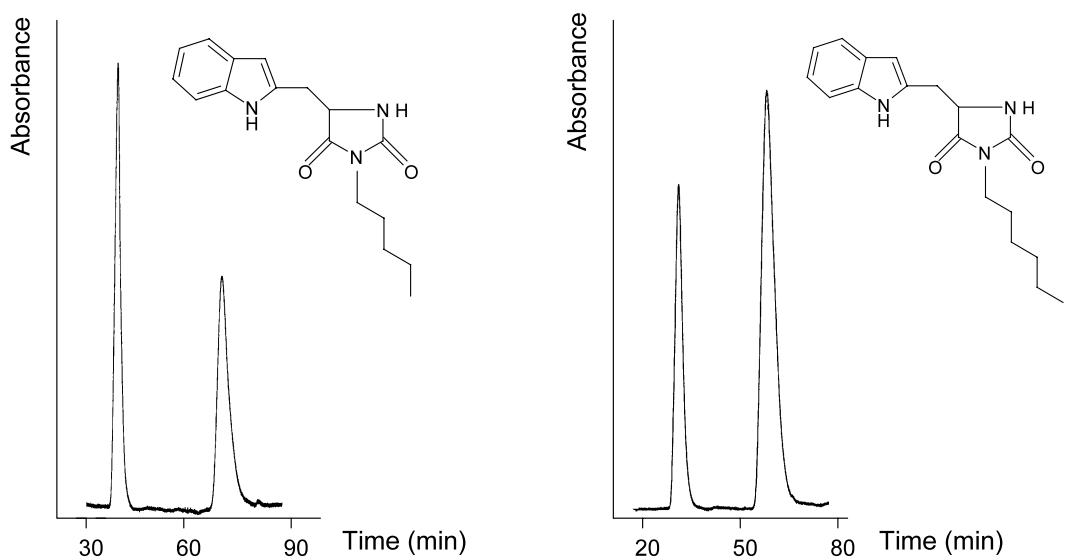

Fig. 4. Enantioseparation of analytes 9 and 10 on Chiralcel OD column. Mobile phase *n*-hexane/2-propanol 90/10 (v/v). Flow rate 1 ml/min.

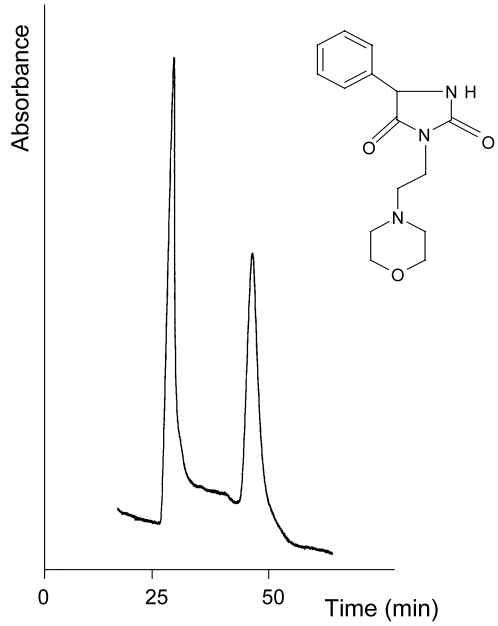

Fig. 5. Enantioseparation of analytes 6 on Chiralcel OD column. Mobile phase *n*-hexane/2-propanol 90/10 (v/v). Flow rate 1 ml/min.

of cellulose and amylose exhibited superior chiral recognition ability compared to cellulose phenylester derivative. A combination of one of the phenylcarbamate CSPs and a Chiralcel OJ column allowed to resolve the enantiomers of most of the compounds under study with only minor optimizations of the mobile phase. High enantioseparation factors observed for several analytes may allow to apply the polysaccharide derivatives for the isolation of pure enantiomers on a (micro) preparative scale using SMB chromatography.

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