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Journal of Chromatography A, 684 (1994) 162–167

JOURNAL OF CHROMATOGRAPHY A

Short communication

Diastereomeric and enantiomeric separation of monoesters prepared from *meso*-cyclopentanediols and racemic carboxylic acids on a silica phase and on amylose and cellulose chiral stationary phases

Annamarie Kunath[a,*], Fritz Theil[a], Jürgen Wagner[b]

[a]*Institute of Applied Chemistry, Rudower Chaussee 5, D-12484 Berlin-Adlershof, Germany*

[b]*Humboldt University, Institute of Organic and Bioorganic Chemistry, Invalidenstrasse 42, D-10115 Berlin, Germany*

First received 26 May 1994; revised manuscript received 15 July 1994

Abstract

The diastereomeric and enantiomeric separation of cyclopent-2-ene-1,4-diol and bicyclo[3.1.0]hexane-1,4-diol derivatives was carried out by HPLC on chiral stationary phases consisting of 3,5-dimethylphenylcarbamates of cellulose (Chiracel OD) and amylose (Chiralpak AD) coated on macroporous silica. The separation on the AD column is dramatically influenced by the kind of alcohol in the mobile phase. The retention times and separation factors increase on changing the mobile phase modifier from 2-propanol to ethanol and to a mixture of methanol and ethanol. On the OD column solutes elute faster with ethanol than with 2-propanol modifier, as expected from the higher polarity of ethanol.

1. Introduction

The lipase-catalysed transesterfication of *meso*-diols with racemic carboxylic esters [1,2] leads to four stereoisomers with at least three asymmetric centers (Fig. 1). The isomers 1/1 and 1/2 and also 2/1 and 2/2 are enantiomeric. The pairs 1/1 + 1/2 and 2/1 + 2/2 are diastereomeric. Therefore, a complete analytical treatment includes first the separation of the diastereomeric pairs, which are subjected to chiral chromatography in a second step.

* Corresponding author.

2. Experimental

2.1. Chemicals and materials

The synthesis of the racemic monoesters was described previously [2]. Chiracel OD and Chiralpak AD analytical columns (250 × 4.6 mm I.D.) were purchased from J.T. Baker (Gross-Gerau, Germany). Silica gel Si 60 (7 μm) (Merck, Darmstadt, Germany) was slurry packed in a stainless-steel column (150 × 4 mm I.D.) and used for diastereomeric separation. Mobile phase components were carefully dried analytical-reagent grade 2-propanol, ethanol, methanol, diethyl ether and *n*-hexane (Merck).

0021-9673/94/$07.00
SSDI 0021-9673(94)00684-9

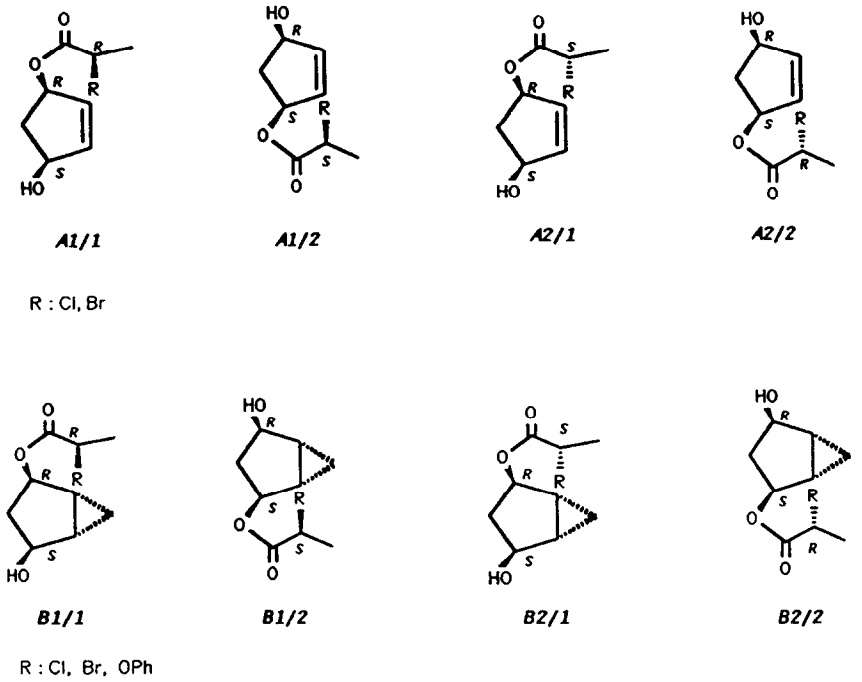

Fig. 1. Structures of compounds. A = cyclopent-2-ene-1,4-diol derivatives; B = bicyclo[3.1.0]hexane-1,4-diol derivatives.

2.2. Apparatus and chromatographic conditions

The liquid chromatographic systems consisted of a Merck–Hitachi L 6200 pump, a Rheodyne RH 7125 injection valve (20-μl loop) a variable-wavelength spectrophotometer (Knauer, Berlin, Germany) and an Auswert 2 data system (ZIOC, Berlin, Germany). Optical rotation was followed with a Chiralyser (IBZ Messtechnik, Hannover, Germany) in series with the UV detector. The detection wavelength of the spectrophotometer and the flow-rate were set at 215 nm (except for the OPh compounds, 254 nm) and 1 ml/min, respectively. The column temperature was maintained at 23°C. The eluents used for the chiral separation were hexane–2-propanol (80:20, v/v), hexane–ethanol (80:20, v/v) and hexane–ethanol–methanol (80:10:10, v/v/v). Diastereomeric separations were carried out using hexane–2-propanol (97:3, v/v) or hexane–diethyl ether (1:1, v/v) as mobile phases. The sample concentration was about 10 mg/ml for the preparative mode and about 1 mg/ml for the analytical mode. Preparative separation of the diastereomers was achieved on a silica gel column. Compounds with index 1 elute first (fraction F_1); index 2 represents the corresponding diastereomers with longer retention (fraction F_2) (see Fig. 2). After the evaporation of the mobile phase using a stream of nitrogen, the separated diastereomers were dissolved in 100 μl of the mobile phase and subjected to chiral separation.

Circular dichroism (CD) spectra were recorded at room temperature on a JASCO J-710 spectropolarimeter.

3. Results and discussion

3.1. Diastereomeric separation

The capacity factors and the α-values of the diastereomeric separation are given in Table 1. Fig. 2 shows as an example the chromatogram of the preparative separation of compound B (R = OPh). The results clearly indicate that the bulkier the substituent R, the better is the separation factor and the shorter the retention time. In all

Table 1
Separation of the diastereomers on a silica gel column

Solute	Substituent	Eluent[a]	k'_1	k'_2	α
A	Cl	1	5.85	6.48	1.11
A	Br	1	5.40	6.24	1.16
B	Cl	2	5.03	5.56	1.10
B	Br	2	4.61	5.53	1.20
B	OPh	2	3.95	5.08	1.29

[a] Eluent 1 = hexane–diethyl-ether (1:1, v/v); 2 = hexane–2-propanol (97:3, v/v).

instances the resolutions was sufficient enough for the preparative separation.

3.2. Enantiomeric separation

Enantiomeric separation of the solutes A1, A2, B1 and B2 was achieved on chiral stationary phases (CSP) consisting of 3,5-dimethylphenylcarbamates of cellulose (Chiracel OD) and amylose (Chiralpak AD) coated on silica. The AD column gave better results in all instances. The chromatographic parameters obtained are summarized in Tables 2 and 3. Examples of chromatograms are shown in Figs. 3 and 4.

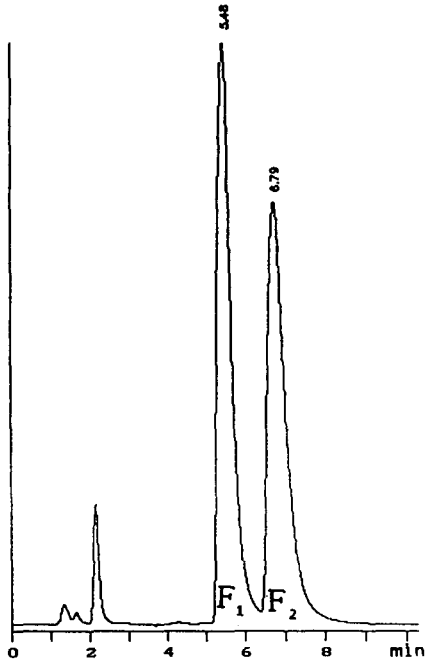

Fig. 2. Separation of the diastereomers of bicyclo[3.1.0]-hexane-1,4-diol derivatives (R = OPh) on a silica gel column. Eluent: hexane–2-propanol (97:3, v/v). F_1 = B1/1 and B1/2; F_2 = B2/1 and B2/2.

Most striking is the influence of the kind of polar modifier in the mobile phase on the capacity factors k' and also the chiral recognition. With the CSP OD, as expected, the solutes elute faster with ethanol than with 2-propanol as polar modifier [3] (Table 3).

The chromatographic parameters obtained on CSP AD showed an unexpected behaviour. Both the capacity factors and the separation factors increase on changing from 2-propanol to ethanol and to ethanol–methanol (see Table 2 and Fig. 3). It is seen that with respect to retention, the second-eluting enantiomers (ester group in an *S*-configuration) are much more retained than the corresponding first-eluting *R*-enantiomers. Type B solutes are especially influenced. The retention also depends on the substituent R of the ester group. The strongest retention is obtained for solute B with the chloropropionate residue in an *S*-configuration and hexane–ethanol–methanol as eluent. The weakest retention is shown by solute A with the chloropropionate residue in an *R*-configuration and hexane–2-propanol as eluent. There seems to be no simple relationship between the type of substituent in the propionate side-chain and retention. In all instances the hexane–ethanol–methanol mobile phase system leads to an increase in optical resolution. For example, the α-value of B1 solute (R = OPh) increases by a factor of nine.

The chiral recognition process reflects the sum of all stereospecific interactions between the solute and the CSP [4,5]. Possible modes of the interaction arise between the free OH group and the ester carbonyl of the solutes and the CO group and the NH group of the polysaccharide carbamate as dipole–dipole interactions and/or hydrogen bonding. In addition, the chiral cavity of the CSP plays an important role. As the chemical derivatization of both cellulose and amylose is the same, the difference in chiral recognition of cellulose and amylose derivatives must be due to the different configuration of the glucose residue and higher order structure [5–7].

Table 2
Enantiomeric separation of the cyclopent-2-ene-1,4-diol and bicyclo[3.1.0]hexane-1,4-diol derivatives on a Chiralpak (AD) column

Substituent	Solute	Eluent[a]	$k'_{1/1}$	$k'_{1/2}$	α	$k'_{2/1}$	$k'_{2/2}$	α
Cl	A	1	0.65	0.78	1.20	0.67	0.77	1.14
Cl	A	2	0.95	2.10	2.21	1.01	1.66	1.64
Cl	A	3	0.94	2.30	2.45	1.05	1.78	1.69
Br	A	1	0.75	0.76	1.02	0.73	0.84	1.15
Br	A	2	1.18	1.63	1.38	0.94	2.03	2.16
Br	A	3	1.15	1.80	1.56	0.96	2.12	2.20
Cl	B	1	0.97	2.15	2.22	0.93	2.03	2.18
Cl	B	2	1.90	13.29	6.99	2.01	10.82	5.38
Cl	B	3	1.98	22.34	11.28	3.01	16.23	5.39
Br	B	1	1.17	1.81	1.55	1.02	1.67	1.64
Br	B	2	3.00	13.42	4.47	2.94	12.59	4.28
Br	B	3	3.11	18.22	5.86	3.11	18.00	5.79
OPh	B	1	0.79	1.22	1.54	0.80	1.26	1.57
OPh	B	2	0.97	9.71	10.01	2.38	11.09	4.66
OPh	B	3	0.85	12.09	14.22	2.43	16.60	6.83

[a] Eluent 1 = hexane–2-propanol (80:20, v/v); 2 = hexane–ethanol (80:20, v/v); 3 = hexane–ethanol-methanol (80:10:10, v/v/v).

In order to explain the observed effect of polar organic modifiers on optical resolution, two possibilities must be taken into account: (i) the solvation or the conformation of either the solute or the CSP is affected by changes in the nature of the modifier; (ii) an alternation in the steric environment of the chiral cavity on the stationary phase is induced by changing the modifier. For example, CD experiments for solutes B1/2 (R = Cl) and B2/2 (R = Cl, Br) were performed to confirm whether a conformational change of the solute occurred depending on the alcohol used. In each instance identical CD spectra were obtained in 2-propanol and methanol (Cotton effect λ_{max}/sign: B1/2 233.5 nm/+; B2/2 (R = Cl) 233/5 nm/−; B2/2 (R = Br) 239.0 nm/−). These results show that the conformation of the solutes should not alter and therefore the modifier effect is probably related to changes in the CSP. To the best of our knowledge, there is no

Table 3
Enantiomeric separation of the cyclopent-2-ene-1,4-diol and bicyclo[3.1.0]hexane-1,4-diol derivatives on a Chiralcel (OD) column

Substituent	Solute	Eluent[a]	$k'_{1/1}$	$k'_{1/2}$	α	$k'_{2/1}$	$k'_{2/2}$	α
Cl	A	1	0.52	0.66	1.27	0.54	0.61	1.12
Cl	A	2	0.42	0.48	1.14	0.45	0.45	ca.1
Br	A	1	0.56	0.71	1.27	0.56	0.68	1.21
Br	A	2	0.45	0.49	1.09	0.45	0.49	1.09
Cl	B	1	0.47	0.48	1.02	0.46	0.50	1.09
Cl	B	2	0.37[b]	0.34	1.09	0.33	0.38	1.15
Br	B	1	0.53	0.53	ca.1	0.52	0.51	ca.1
Br	B	2	0.45	0.49	1.13	0.45	0.49	1.21
OPh	B	1	0.89[b]	0.65	1.37	0.67	1.19	1.78
OPh	B	2	0.50	0.50	ca.1	0.46	0.68	1.48

[a] See Table 2.
[b] Elution order has changed.

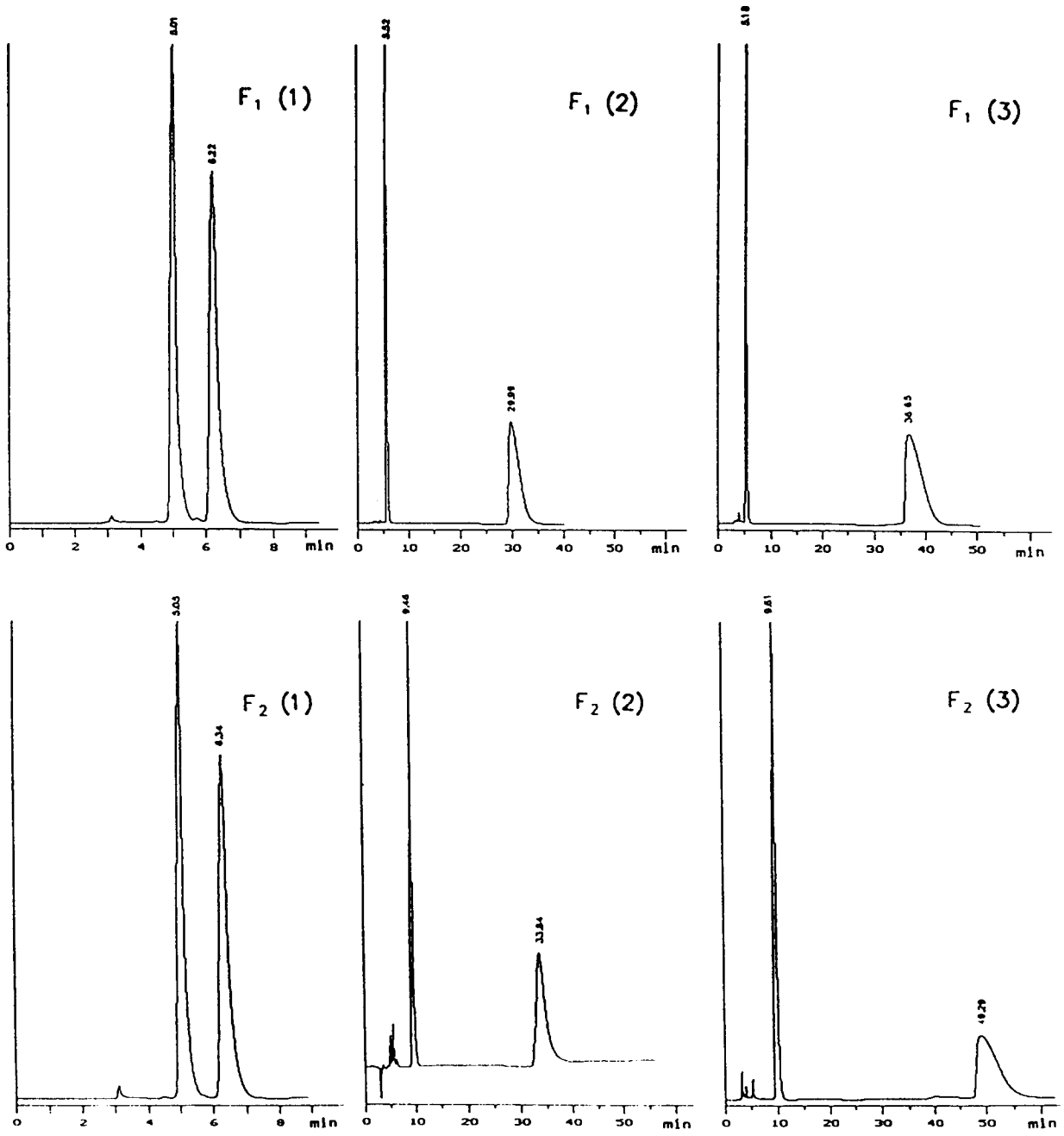

Fig. 3. Optical resolution of the silica gel fractions F_1 and F_2 on a Chiralpak AD column. The first-eluting compounds are B1/1 (F_1) and B2/1 (F_2) and the second B1/2 (F_1) and B2/2 (F_2). For eluents (1), (2) and (3), see Table 2.

information about the influence of solvents on the helix and chiral cavity of amylose carbamate derivatives. Solvents may affect the groove of the helix and the steric fit of a solute can become better or worse during the interaction with carbamate groups of two or more glucose units.

A similar solvent effect was found by Balmer et al. [8] in the optical resolution of timopraxole

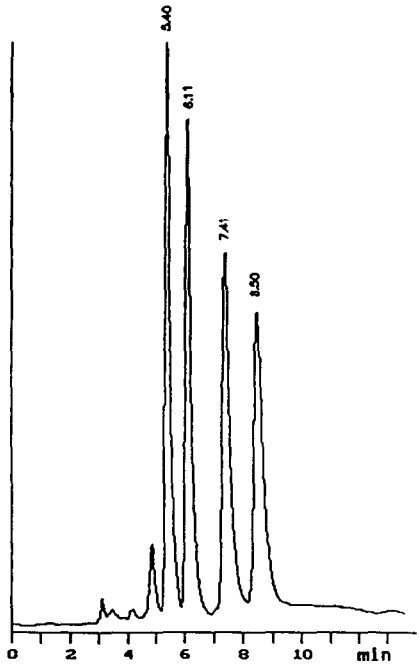

Fig. 4. Optical resolution of cyclopent-2-ene-1,4-diol derivatives (R = Br) on a Chiralpak AD column. Eluent: hexane–ethanol (80:20, v/v). Retention times: 540 min = A2/1, 611 min = A1/1; 7.41 min = A1/2; 8.50 min = A2/2.

on an AD column. They used hexane–2-propanol (80/20, v/v) as eluent and to this were added 1–20% of methanol. From 1% to about 6% of methanol the capacity factors decreased, as expected from the higher polarity of the eluent. On increasing the methanol content from 6 to 8%, the capacity factor of the second-eluting (+)-enantiomer increased by a factor of 2 whereas the retention of the (−)-enantiomer was only slightly influenced. This means that an alteration of the chiral forces of the AD CSP probably occurs when the concentration of methanol in the eluent is about 5–10%.

4. Conclusion

OD and AD CSPs are suitable for the optical resolution of cyclopent-2-ene-1,4-diol and bicyclo[3.1.0]hexane-1,4-diol derivatives. Using the AD column an inverse polarity effect of the eluent is observed. Both retention and separation factor increase on changing the polar organic modifier from 2-propanol to ethanol and to a mixture of ethanol and methanol.

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