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Influence of the kind of the alcoholic modifier on chiral separation on a Chiralpak AD column

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Abstract

The enantioselective separation of substituted 3-phenoxy-1,2-propanediols (PPDs) and aziridinecarboxylic acid derivatives (ACAs) on Chiralpak AD, an amylose-based chiral stationary phase, was investigated. All solutes could be separated using hexane–alcohol mobile phases. The retention times for sixteen out of seventeen substituted PPDs and from all the ACAs increased on changing the alcoholic modifier from 2-propanol to ethanol or ethanol–methanol mixtures. The separation factors of sixteen out of seventeen PPDs and nine out of twelve ACAs were improved using ethanol or ethanol–methanol mixtures instead of 2-propanol.

Keywords: Mobile-phase composition; Enantiomer separation; Chiralpak AD; Alcoholic modifier; 3-Phenoxy-1,2-propanediols; Aziridine carboxylic acid derivatives

1. Introduction

Chiralpak AD is useful amylose-based chiral column for the separation of a wide range of chiral compounds [1]. Usually 2-propanol is used as an alcoholic modifier. Recently, it was found that changing the modifier from 2-propanol to ethanol and methanol leads to an improvement of the chiral separation [2,3]. However, systematic investigations on the influence of polar modifiers in chiral separations using Chiralpak AD have not been carried out, to the best of our knowledge. In this paper, we report on the influence of ethanol and mixtures of ethanol and methanol on the chiral separation of substituted 3-phenoxyl-1,2-propanediols (PPDs) and aziridinecarboxylic acid derivatives (ACAs) (Fig. 1). Stereochemically defined aziridine-2-carboxylic acid derivatives are useful intermediates for the synthesis of modified optically active amino acids. Either substituted α-amino acids or β-amino acids may be prepared by nucleophilic attack on the aziridine ring [4,5]. The 1,2-diol functionality is found in a series of synthetic intermediates [6], pharmaceuticals and pharmaceutical intermediates [7,8]. Therefore, methods for the preparation of enantiomerically pure 1,2-diols are of increasing interest [9].

2. Experimental

2.1. Chemicals and materials

The synthesis of the racemic 3-phenoxy-1,2-propanediols was described previously [9,10].

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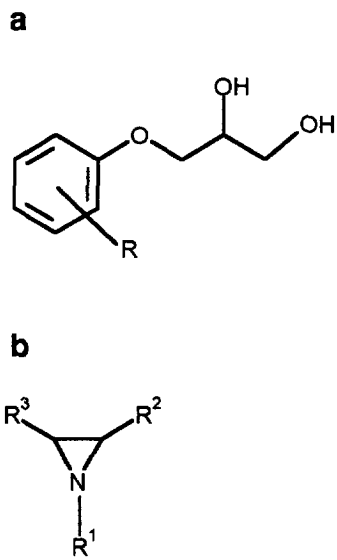

Fig. 1. Structures of the compounds. (a) 3-Phenoxy-1,2-propanediols: R see Table 1; aziridine carboxylic acid derivatives: R^1, R^2 and R^3 see Table 4.

The aziridine derivatives A1 and A6 were synthesized as described in Ref. [11], A3 and A4 as in Ref. [12], A2 as in Ref. [13] and A8 as in Ref. [14]. A7 was prepared by reaction of aziridine-2-carboxylic acid methyl ester with phenyl isocyanate in dichloromethane and A10, A11 and A12 by reaction of A1 in chloroform in the presence of equimolar amounts of triethylamine and *p*-nitrobenzoyl chloride, *p*-toluenesulfonyl chloride and triphenylchloromethane, respectively. A9 was obtained in an analogous manner to A10, using A2 as starting material.

The Chiralcel AD column (250 × 4.6 mm I.D.) was purchased from J.T. Baker (Gross-Gerau, Germany). The mobile phase components 2-propanol, ethanol, methanol and hexane were LiChrosolv quality (Merck, Darmstadt, Germany).

2.2. Apparatus and chromatographic conditions

Two liquid chromatographic systems were used. One consisted of a Merck–Hitachi L 6200 pump, a Rheodyne RH 7125 injection valve (20-μl loop), either a variable-wavelength spectrophotometric (Knauer, Berlin, Germany) on an RI 71 refractive index detector (Merck) and an Auswert 2 data system (ZIOC, Berlin, Germany). The other was a Merck–Hitachi system with a diode-array detector and an autosampler (Merck). The UV detection wavelength was set at 230 nm. The aziridine derivatives without phenyl groups were detected with the RI 71 refractive index detector. The eluents used for the chiral separation were hexane–2-propanol, hexane–ethanol and hexane–(ethanol–methanol) (1:1, v/v) in the proportions indicated in the tables. The flow-rate was set at 1 ml/min. The dead time, determined with 1,3,5-tri-*tert.*-butylbenzene, was 2.85 min for all the eluents used. The column temperature was maintained at 23°C. The sample concentration was about 0.5 mg/ml for UV detection and 2 mg/ml for RI detection. The capacity factors k'_1 and k'_2 were determined as $(t_1 - t_0)/t_0$ and $(t_2 - t_0)/t_0$, respectively, the separation factor as $\alpha = k'_2/k'_1$ and the resolution factor as $Rs = 2(t_2 - t_1)/(W_1 + W_2)$.

3. Results and discussion

The capacity factors of the second-eluted peaks and the separation factors of the substituted 3-phenoxy-1,2-propanediols on a Chiralpak AD column with different modifiers and different modifier concentrations are given in the Tables 1–3. Figs. 2 and 3 show examples of chromatograms of 4-t-C_8H_{17}-PPD and 2-t-Bu-PPD, respectively. The results of the chiral resolution of the aziridinecarboxylic acid derivatives are summarized in the Tables 4 and 5 and in Fig. 4.

3.1. Capacity factors

It can be seen that the capacity factors increase significantly on changing from 2-propanol to ethanol or ethanol–methanol mixtures using the same volume fraction of the modifier, except for 2-t-Bu-PPD (see Tables 1–3). The retention times of the 3-phenoxy-1,2-propanediols with a bulky substituent in the *para*-position of the aromatic ring and of the aziridine derivatives, in which the substituent in the 1-position is an electron-withdrawing group (e.g. $COCH_6H_5$, $CONHC_6H_5$, $COC_6H_4NO_2$, $SO_2C_6H_4CH_3$) are

Table 1
Enantiomeric separation of substituted 3-phenoxy-1,2-propanediols using 10% of modifier in hexane

Compound		Modifier 1		Modifier 2		Modifier 3	
No.	R	k'_2	α	k'_2	α	k'_2	α
P1	H	2.81	1.14	6.18	1.34	5.81	1.33
P2	4-Me	2.76	1.21	6.22	1.27	5.38	1.25
P3	2-Me	1.83	1.12	3.17	1.25	2.71	1.24
P4	3-Me	1.76	1.07	3.55	1.30	2.95	1.28
P5	2,4-Me_2	1.98	1.16	3.36	1.17	2.80	1.11
P6	4-t-Bu	1.70	~1	4.88	1.19	5.20	1.13
P7	2-t-Bu	0.94	~1	0.83	~1	0.74	~1
P8	3-t-Bu	1.01	~1	1.22	1.22	1.18	1.33
P9	2,4-t-Bu_2	0.49	~1	0.62	1.29	0.50	1.25
P10	4-t-C_8H_{17}	0.88	~1	2.02	1.12	2.48	1.29
P11	4-Ph	3.95	~1	12.61	1.18	11.70	1.05
P12	2-Ph	2.03	~1	2.46	1.21	2.05	1.18
P13	4-CH_2Ph	4.39	1.16	9.80	1.10	9.31	1.08
P14	2-CH_2Ph	2.33	1.07	2.88	1.18	2.35	1.16
P15	4-COPh	10.81	~1	27.50	~1	22.86	~1
P16	2-COPh	6.40	~1	9.53	1.19	5.92	1.12
P17	4-OPh	3.56	1.10	8.79	1.09	7.55	1.13

Column, chiralpak AD; flow-rate, 1 ml/min; column temperature, 23°C; UV, detection at 230 nm. Modifiers: 1 = 2-propanol; 2 = ethanol; 3 = ethanol–methanol (1:1, v/v). k'_2 = capacity factor of the second-eluting peak; α = separation factor.

Table 2
Enantiomeric separation of substituted 3-phenoxy-1,2-propanediols using 20% of modifier in hexane

Compound		Modifier 1		Modifier 2		Modifier 3	
No.	R	k'_2	α	k'_2	α	k'_2	α
P1	H	0.90	1.13	1.53	1.33	1.96	1.33
P2	4-Me	1.03	1.18	2.08	1.28	2.07	1.25
P3	2-Me	0.72	~1	1.19	1.31	1.10	1.24
P4	3-Me	0.68	~1	1.25	1.26	1.18	1.28
P5	2,4-Me_2	0.80	~1	1.27	1.23	1.21	1.09
P6	4-t-Bu	0.64	~1	1.87	1.22	2.02	1.12
P7	2-t-Bu	0.38	~1	0.39	~1	0.33	~1
P8	3-t-Bu	0.40	~1	0.51	1.19	0.51	1.30
P9	2,4-t-Bu_2	0.20	~1	0.35	1.52	0.25	1.25
P10	4-t-C_8H_{17}	0.34	~1	0.81	~1	1.06	1.13
P11	4-Ph	1.43	~1	3.63	1.09	4.06	1.05
P12	2-Ph	0.78	~1	0.98	1.18	0.82	1.17
P13	4-CH_2Ph	1.60	1.15	2.97	1.12	3.49	1.08
P14	2-CH_2Ph	0.90	~1	1.20	1.19	0.98	1.17
P15	4-COPh	2.47	~1	5.90	1.10	5.63	~1
P16	2-COPh	1.99	~1	3.23	1.14	2.02	1.11
P17	4-OPh	1.27	1.09	2.36	1.02	2.72	1.13

For chromatographic conditions and abbreviations, see Table 1.

Table 3
Influence of the modifier concentration on the separation of substituted 3-phenoxyl-1,2-propanediols

Substituent R	Modifier	Modifier concentration (%)							
		20		10		5		2	
		k'_2	α	k'_2	α	k'_2	α	k'_2	α
2-t-Bu	1	0.38	~1	0.94	~1	2.39	1.05	8.28	1.07
2,4-t-Bu_2	1	0.20	~1	0.49	~1	1.23	~1	4.41	1.09
2-t-Bu	2	0.39	~1	0.83	~1	2.06	1.08	6.20	1.10
2,4-t-Bu_2	2	0.35	1.52	0.62	1.29	1.58	1.26	4.52	1.22
2-t-Bu	3	0.33	~1	0.74	~1	1.81	1.09	6.14	1.13
2,4-t-Bu_2	3	0.25	~1	0.50	1.25	1.22	1.28	4.22	1.37

For chromatographic conditions and abbreviations, see table 1.

more influenced with the ethanol–methanol mixture than with ethanol. The retention times of the 3-phenoxy-1,2-propanediols with a bulky substituent in the *ortho*-position of the aromatic ring, such as 2-t-Bu, 2-Ph and 2-CH_2Ph, and the aziridine derivatives with H in the 1-position and the trityl-substituted compound are less increased with ethanol–methanol than with ethanol modifier compared with 2-propanol.

3.2. Separation factors

Of the seventeen 3-phenoxy-1,2-propanediols investigated, sixteen show improved separation factors using ethanol or ethanol–methanol modifier instead of 2-propanol. The same behaviour was found for nine out of the twelve aziridine derivatives investigated. Fig. 2 shows chromatograms of the 2-t-C_8H_{17}-PPD influenced by the alcoholic modifier. Fig. 4 demonstrates chromatograms of two aziridine derivatives under the influence of different alcoholic modifiers.

Decreasing the modifier concentration does not improve the separation factors in most cases. On the other hand, the chiral resolution of the 2-t-Bu-PPD (Fig. 3 and Table 3) and the trityl-substituted ACA (Table 4) was achieved only by decreasing the modifier concentration. A further improvement of the resolution of these two compounds was obtained by decreasing the flow-rate (from 1 to 0.5 ml/min) and decreasing the column temperature (7°C instead of 23°C). 2,4-t-Bu_2PPD shows decreasing separation factors with decreasing ethanol volume fraction but increasing α-values with decreasing ethanol–methanol in the eluent. This means that the resolution can be improved with both increasing and decreasing modifier concentration. A slight increase in the separation factors with increasing modifier concentration is also seen in the case of compounds A5 and A10 (Tables 4 and 5) and 2,4-Me_2-PPD (Tables 1 and 2). Tables 4 and 5 show, on the other hand, that the capacity factors of the ACAs are of the same order of magnitude with 20% 2-propanol as with 50% ethanol, hence the separation factors are similar with 20% and 50% ethanol modifier.

4. Discussion

The carbamate structural unit has been considered as the most important adsorbing site of the phenylcarbamate derivatives of polysaccharides which are used for chiral recognition. On the one hand, there are interactions through hydrogen bonding between the carbonyl group of the carbamate and the OH and NH groups of the solutes. On the other hand, there are interactions of the same type between the NH group of the carbamate unit and the carbonyl groups of the solute [1,15]. Furthermore, $\pi-\pi$ interactions between the racemic solutes and the phenyl group of the chiral stationary phase (CSP) have been discussed [16]. A third contribution to chiral recognition is due to a regular higher order

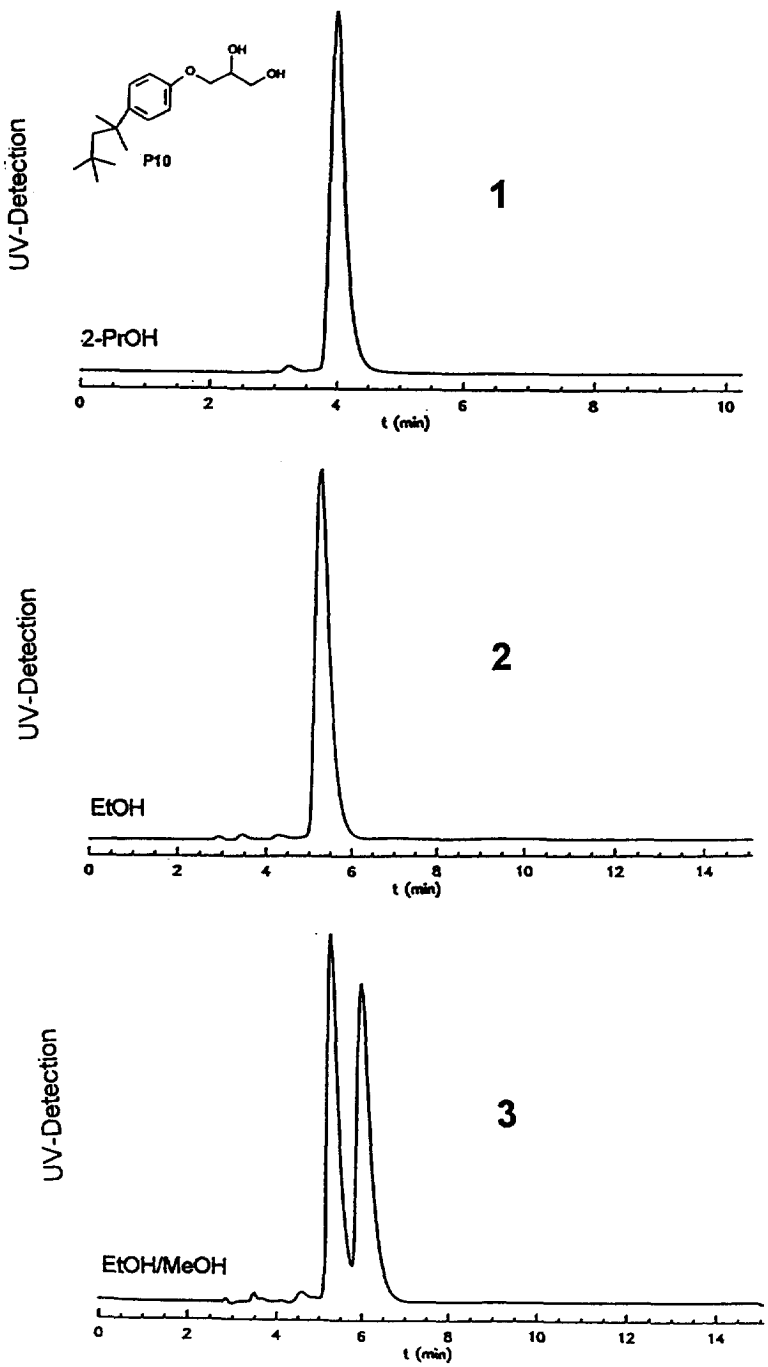

Fig. 2. Influence of the alcoholic modifier on the chiral separation of the 4-t-C_8H_{17}-PPD chromatographic conditions: flow-rate, 1 ml/min; column temperature, 23°C; UV detection at 230 nm; 20% of modifier: (1) 2-propanol, (2) ethanol and (3) methanol–ethanol (1:1, v/v) in hexane.

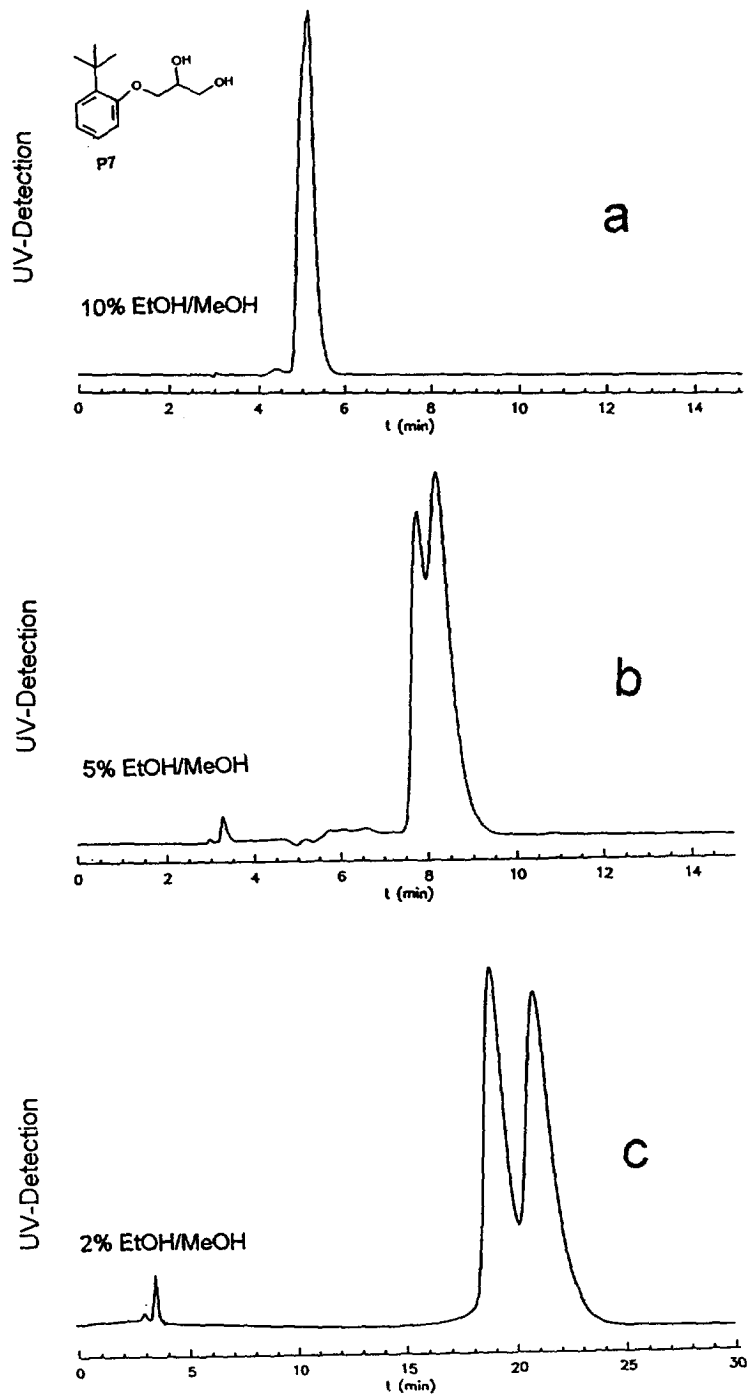

Fig. 3. Resolution of the 2-t-C_4H_9-PPD depending on the modifier concentration. Chromatographic conditions as in Fig. 2; modifier concentration: (a) 10%, (b) 5% and (c) 2% of methanol–ethanol (1:1, v/v) in hexane.

Table 4
Enantiomeric separation of aziridinecarboxylic acid derivatives using 20% of modifier in hexane

Compound				Modifier 1		Modifier 2		Modifier 3	
No.	R_1	R_2	R_3	k'_2	α	k'_2	α	k'_2	α
A1	H	$CONH_2$	H	0.76	~1	1.41	~1	1.62	1.23
A2	H	CN	H	1.52	1.56	2.24	1.59	1.83	1.42
A3	H	CN	*cis*-Me	1.13	1.31	1.37	1.20	1.15	1.08
A4	H	CN	*trans*-Me	3.31	2.11	4.07	1.98	2.85	1.83
A5	CONHPh	CN	H	1.85	1.16	5.54	1.63	9.17	2.97
A6	CONHPh	$CONH_2$	H	1.60	1.36	4.17	1.42	5.36	1.79
A7	CONHPh	COOMe	H	3.28	2.69	10.36	4.68	12.25	5.37
A8	COPh	CN	H	1.35	1.02	4.29	2.02	6.54	3.20
A9	CO(4-NO_2Ph)	CN	H	5.52	1.09	13.36	2.13	14.92	2.62
A10	CO(4-NO_2Ph)	$CONH_2$	H	7.12	2.10	17.21	2.79	15.47	2.89
A11	*p*-Tosyl	$CONH_2$	H	3.23	~1	7.69	1.14	7.77	1.11
A12	Trityl	$CONH_2$	H	0.72	~1	1.06	~1	0.70	~1
A12[a]	Trityl	$CONH_2$	H	5.45	1.23	5.66	~1	3.66	~1

[a] Modifier concentration 5% in hexane.
For chromatographic conditions and abbreviations, see Table 1, except refractive index detection of compounds A1–A4.

structure of the chiral sites of the CSP [1]. Therefore, the resolving abilities of amylose and cellulose derivatives with the same 3,5-dimethylphenylcarbamate moiety are very different. The cellulose derivative has been reported to exist in a conformation of a left-handed threefold (3/2) helix. The amylose derivative can be regarded as a left-handed fourfold (4/1) helix [17,18]. In both substituted chiral biopolymers a helical groove exists with polar carbamate residues and 3,5-dimethylphenyl groups. To achieve chiral discrimination, the racemic solutes have to enter this groove and interact as described above with the CSP. The interaction forces of solutes with the NH and CO groups of the CSP decrease on increasing the polarity of the mobile phase. This means that an increasing modifier volume fraction and a higher polarity of the modifier should cause a decrease in the capacity factors. As can be seen from Tables 1, 2, 4 and 5, higher concentrations of the modifier cause a decrease in the retention times, but the same volume fraction of ethanol instead of 2-propanol leads to increased capacity factors. In addition, the chiral resolution is improved by ethanol and ethanol–methanol mixtures compared with 2-propanol. Although the higher polarity of the alcoholic modifier should lead to weaker interactions by hydrogen bonding between the CSP and the solute, increased capacity and separation factors are found. The main reason for the improved separation properties should be a changed geometry and/or size of the chiral groove, which is caused by the kind of alcoholic modifier.

Table 5
Enantiomeric separation of aziridinecarboxylic acid derivatives using 50% of ethanol in hexane

Compound				k'_2	α
No.	R1	R_2	R_3		
A5	CONHPh	CN	H	1.49	1.67
A6	CONHPh	$CONH_2$	H	0.97	1.35
A7	CONHPh	COOMe	H	3.42	4.68
A8	COPh	CN	H	1.60	1.95
A9	CO(4-NO_2Ph)	CN	H	4.04	2.06
A10	CO(4-NO_2Ph)	$CONH_2$	H	3.97	2.96
A11	*p*-Tosyl	$CONH_2$	H	1.74	1.14

For chromatographic conditions, see Table 1.

So far we have been unable to realize a general relationship between the structure of the solutes and the enantioselectivity of their separation on Chiralpak AD. However, the separation parameters of the *para*-substituted PPDs are more improved by changing the alcoholic modifier from 2-propanol to ethanol or ethanol–

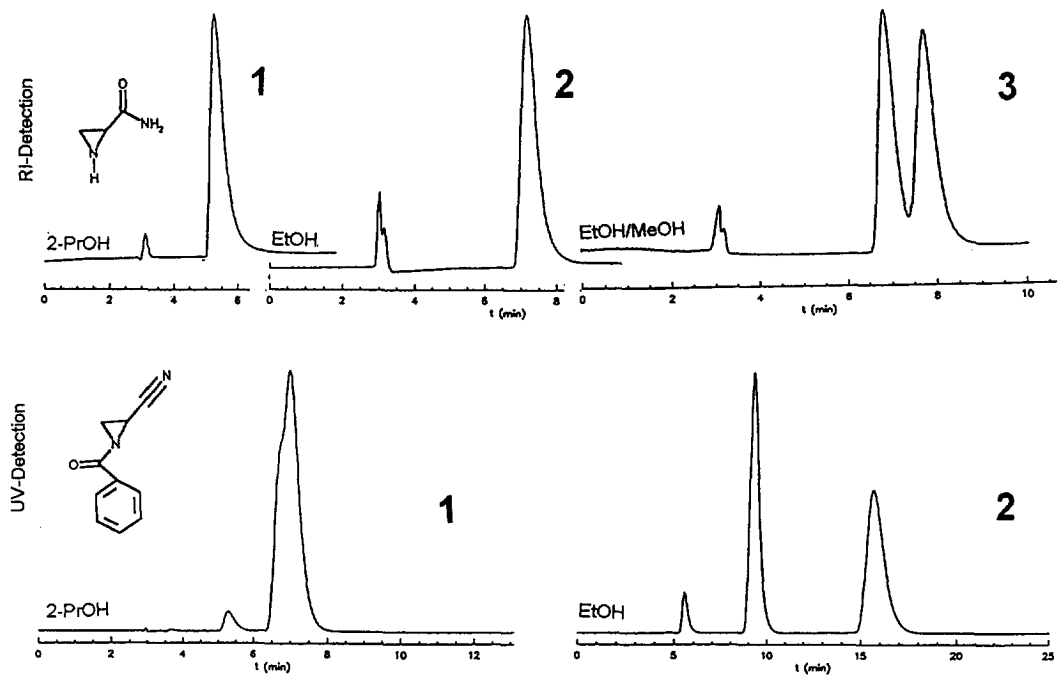

Fig. 4. Influence of the alcoholic modifier on the chiral separation of the aziridinecarboxylic acid derivatives A1 and A5. Chromatographic conditions as in Fig. 2, except for compound A1, RI detection.

methanol mixtures than those of the *ortho*- and *meta*-substituted PDPs. The same behaviour was found for the ACAs with electron-withdrawing substituents at the aziridine nitrogen related to the NH derivatives.

5. Conclusions

Chiralpak AD is suitable for the optical resolution of substituted 3-phenoxy-1,2-propanediols and aziridinecarboxylic acid derivatives, even aziridine derivatives without chromophores, which have been detected by refractive index measurement. The chiral resolution is improved by ethanol or ethanol–methanol mixtures as modifier instead of 2-propanol in most cases. An inverse polarity effect of the eluent is observed: 28 out of 29 compounds show increased capacity factors and 25 out of 29 show improved separation factors. Hence the statement in the instruction manual of the Chiralpak AD column that "Retention time is generally shorter with ethanol than with 2-propanol" cannot be sustained.

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