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RESOLUTION OF ENANTIOMERIC AMIDES ON A CELLULOSE TRIBENZOATE CHIRAL STATIONARY PHASE

MOBILE PHASE MODIFIER EFFECTS ON RETENTION AND STEREOSELECTIVITY

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SUMMARY

The effect of the steric structure and concentration of the mobile phase modifier on the retention (k') and stereoselectivity (α) of a series of enantiomeric amides has been investigated. The amides were chromatographed on a commercially available cellulose tribenzoate chiral stationary phase (CSP) using mobile phases composed of hexane and two homologous series of alcohols: methanol, ethanol, 1-propanol and 2-propanol, 2-butanol, 2-pentanol, 2-hexanol. The results of the study indicate that the alcoholic mobile phase modifiers compete with the solutes for achiral and chiral binding sites and that the steric bulk around the hydroxyl moiety of the modifier plays a role in this competition. Increased steric bulk tends to result in increased k' and α. However, the results also suggest that the effect of the alcoholic mobile phase modifiers on stereoselectivity may also be due to binding to achiral sites near or at the chiral cavities of the CSP which alters the steric environment of these cavities.

INTRODUCTION

A number of cellulose-based high-performance liquid chromatography (HPLC) chiral stationary phases (CSPs) have been developed and are now commercially available[1–2]. We have recently reported our initial work on the mechanism of chiral resolution of enantiomeric amides on one of these phases, a cellulose tribenzoate chiral stationary phase (OB-CSP)[4]. The results of this previous study indicated that the formation of solute–CSP diastereomeric complexes between enantiomeric solutes and the OB-CSP are based on a combination of hydrogen bonding, π–π and amide dipole interactions. In addition, the results suggested that chiral recognition is a function of the fit of the assymetric portion of the solute in a chiral cavity (or

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channel) of the CSP and that this fit has rigid steric requirements. We have continued this study with an investigation of the effect of varying the steric structure and concentration of the mobile phase modifier (MPM) on retention (k') and stereoselectivity (α).

The effect of the steric structure of alcoholic MPMs on k' and α in the Pirkle-type CSP has been investigated by a number of groups[5–7]. In the systems studied, hydrogen bonding interactions were important for the formation and stabilization of the solute–CSP complexes. The alcoholic MPM, compete with the solute for hydrogen bonding sites on the CSP and the result of this competition effects both k' and α. In the work reported by Zief *et al.*[5], 2,2,2-trifluoro-1-(9-anthryl(ethanol was resolved using mobile phases of hexane and either ethanol, 2-propanol or *tert.*-butanol as the polar modifier. The polarity (p') of the mobile phases were kept relatively constant, 0.48, 0.48 and 0.50, respectively, and thus, the only difference lay in the steric structure of the alcohols. Of the three alcohols tested the use of *tert.*-butanol as the MPM gave the best stereoselectivity, $\alpha = 1.62$ *versus* $\alpha = 1.56$ for 2-propanol and $\alpha = 1.33$ for ethanol. The increase in α was due primarily to an increase in the k' of the more tightly bound enantiomer while the k' of the first eluted enantiomer was essentially unchanged. The authors concluded that an increase in the bulk of the alcohol increases the ability of both of the solute enantiomers to displace the modifier from the CSP.

Pescher *et al.*[6] have reported similar results in the resolution of enantiomeric phosphine oxides. Pirkle-type CSPs were used with hexane–alcohol mobile phases. The stereoselectivity of the system increased with the increasing steric hindrance of the alcohol.

Macaudiere *et al.*[7] reported the resolution of a series of enantiomeric amides using a Pirkle-type CSP under supercritical fluid chromatographic conditions. The amides were chromatographed using subcritical carbon dioxide modified with an alcohol. The results of the study indicated that at constant k', the stereoselectivities are greater for alcohols with large steric hinderance close to the hydroxyl moiety.

Since the Pirkle-type CSP and OB-CSP both utilize attractive interactions such as hydrogen bonding in the formation of the solute–CSP complex, a change in the steric structure of an alcoholic MPM should have a similar effect in both CSPs. However, the Pirkle–type CSP and OB-CSP differ in a number of important aspects. One of the key differences is the fact that in the Pirkle-type CSP the solute–CSP interactions take place on the surface of the CSP while in the OB-CSP these interactions involve chiral cavities (or channels) within the CSP. In this respect, the OB-CSP reflects some of the properties of the microcrystalline cellulose triacetate CSP (CTA-CSP).

Mobile phase effects on k' and α on the CTA-CSP have been investigated by Koller *et al.*[8]. In this study, the eluent was changed from methanol to ethanol–water (96:4) to 2-propanol. Three enantiomeric solutes were chromatographed. The k'values of each enantiomer increased throught the series while the effect on α varied according to the structure of the solute. The authors concluded that the polarity of the eluents may not be the key to their elution power. If the solutes are included (*i.e.*, enter into) cavities or channels of the CTA-CSP, the elution power may be determined instead by the steric size of the eluent.

The work of Koller *et al.*[8] suggests that the steric bulk of the eluent affects the

steric environment of the chiral cavities or channels of the CTA-CSP. This possibility also exists in the OB-CSP.

In this study, we have investigated the effect of the steric bulk of an alcoholic MPM on k' and α on the OB-CSP. Two homologous series: methanol, ethanol, 1-propanol and 2-propanol, 2-butanol, 2-pentanol, 2-hexanol, were used as polar modifiers. The results of the study indicated that as in the Pirkle-type CSP the solute and MPM compete for both chiral and achiral binding sites on the CSP. The results also suggest that like the CTA-CSP the MPM may bind to sites near or at the chiral cavities of the CSP changing the steric environment at these cavities and, thus, the stereoselectivity of the CSP.

EXPERIMENTAL

Apparatus

The chromatography was performed with a Perkin-Elmer (Norwalk, CT, U.S.A.) Series 400 liquid chromatograph equipped with a Perkin-Elmer LC auto-control, a Perkin-Elmer LC-85B variable-wavelength spectrophotometric detector and a Perkin-Elmer LCI-100 laboratory computing integrator. The column used with this system was stainless-steel (25 cm × 4.6 mm I.D.) packed with a tribenzoate cellulose adsorbed on microporous silica (OB column, Daicel Chemical Industries, New York, U.S.A.) (Fig. 1).

Materials

The preparation of the amides used in this study has been previously described[1]. HPLC-grade *n*-hexane, 2-propanol and acetonitrile were purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.) and HPLC-grade 1-propanol was purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). 2-Butanol, 2-Pentanol and 2-hexanol were purchased from Aldrich (Milwaukee, WI, U.S.A.) and *tert.*-butanol was purchased from Fisher Scientific (Springfield, NJ, U.S.A.).

Sample preparation

The solutes (0.1 mg) were dissolved in 10 ml of methylene chloride.

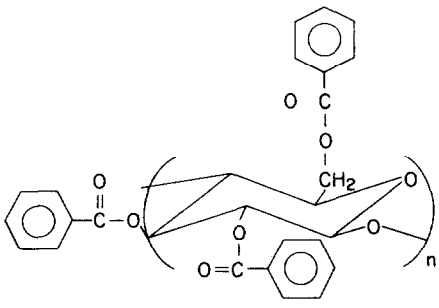

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Fig. 1. Structure of the chiral stationary phase used in this study.

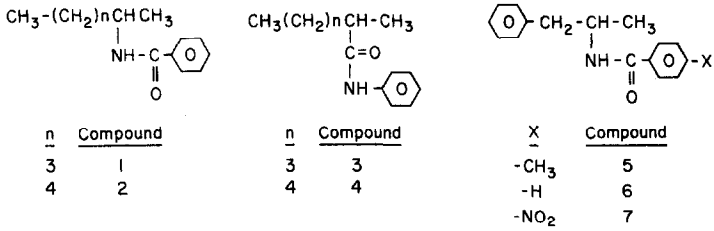

Fig. 2. Structure of the solutes used in this study.

Chromatographic conditions

A temperature of 20°C and a flow-rate of 1 ml/min were maintained throughout the study. The mobile phases were composed of various mixtures of hexane and alcohol. The solutes were monitored at 254 nm.

RESULTS AND DISCUSSION

The structures of the solutes used in this study are presented in Fig. 2. The chromatographic results obtained with solutes 1–4 and the various alcoholic mobile phase modifiers employed in this study are presented in Table I. The chromatographic results obtained using 1- and 2-propanol as modifiers and solutes 5–7 are presented in Table II.

The effect of the structure of the mobile phase modifier on retention and stereoselectivity

The change in the mobile phase modifier from methanol to ethanol to 1-propanol at constant molar concentration results in a steady increase in k' and α for solutes 1–4. The increases in k' average around 30% overall for solutes 1 and 2 and 10% overall for solutes 3 and 4. The change in mobile phase modifier from methanol to ethanol also results in an increase in α of about 20% for 1–4 and when 1-propanol is substituted for ethanol, the increase in α averages 5%.

TABLE I

CHROMATOGRAPHIC RESULTS FOR COMPOUNDS 1–4

Mobile phase: 0.67 *M* alcohol in hexane.

Alcohol	*Solute 1*		*Solute 2*		*Solute 3*		*Solute 4*	
	k'*	α	k'*	α	k'*	α	k'*	α
Methanol	1.66	1.22	1.39	1.30	1.85	1.24	1.39	1.25
Ethanol	2.00	1.48	1.67	1.53	2.10	1.50	1.65	1.52
1-Propanol	2.16	1.61	1.83	1.61	2.15	1.56	1.71	1.62
2-Propanol	3.70	1.62	3.57	1.81	3.62	1.66	2.91	1.93
2-Butanol	2.70	2.19	2.48	2.56	3.49	1.83	2.76	1.97
2-Pentanol	3.91	3.21	3.56	3.46	4.80	2.11	4.03	2.01
2-Hexanol	3.62	2.28	3.02	2.23	4.01	1.66	3.19	1.60
tert.-Butanol	5.70	4.59	6.34	4.88	9.74	1.93	9.12	1.96

* Capacity factor first eluted enantiomer.

TABLE II

CHROMATOGRAPHIC RESULTS FOR COMPOUNDS 5–7

Mobile phase: 1.34 *M* alcohol in hexane

Compound	*1-Propanol*		*2-Propanol*	
	k'^{*}	α	k'^{*}	α
5	1.58	4.99	3.19	5.85
6	1.63	3.40	3.20	3.95
7	7.17	1.41	14.45	1.69

* Capacity factor first eluted enantiomer.

These results are consistent with a decreasing ability of the MPM to displace the solutes from the CSP due to a decrease in solvent polarity. The solvent polarity parameters (p') of methanol, ethanol and propanol are 5.1, 4.3 and 4.0 respectively[9].

When 2-propanol is substituted for 1-propanol, there is a dramatic increase in the retention of solutes 1–4 with an average increase of 76% in the observed capacity factors, Table III and Fig. 3. For solutes 5–7, the effect is even larger where change from 1- to 2-propanol results in a two-fold increase in the observed capacity factors, Tables II and III.

The effect of the change of MPM from 1- to 2-propanol on stereoselectivity is less dramatic. The chiral resolution of solute 1 is virtually unchanged (a 0.6% increase in α) and there is only a 6% increase in α for solute 3. However, for solutes 2, 4 and 5–7, the increase in α averages 17%, Table III.

Since the p' values for 1- and 2-propanol are virtually the same, 4.0 and 3.9 respectively, the observed increases in retention and stereoselectivity are more than likely due to the steric difference between the two molecules which can be expressed at both the achiral and chiral binding sites. The increases in k' and α are consistent with a decreasing ability of the MPM to displace the solutes from these sites. The greater effect on retention *versus* stereoselectivity could be simply due to a greater number of achiral binding sites and/or an easier access to these sites.

TABLE III

PERCENT CHANGE IN RETENTION AND STEREOSELECTIVITY WHEN 1-PROPANOL IS REPLACED BY 2-PROPANOL AS THE MOBILE PHASE MODIFIER

Solute	$\%k'^{*}$	$\%\alpha$
1	71.3	0.6
2	95.1	12.4
3	68.4	6.4
4	70.2	19.1
5	101.9	17.2
6	96.3	16.2
7	101.5	19.9

* Capacity factor of first eluted enantiomer.

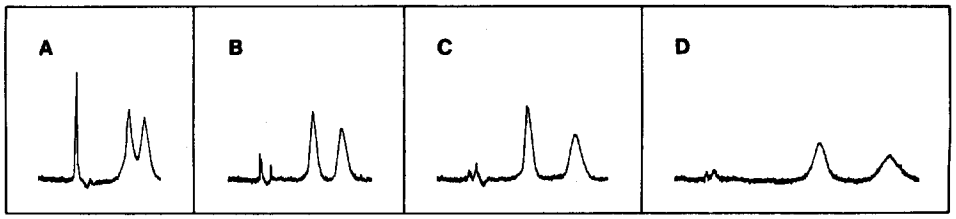

Fig. 3. The effect of the structure of the mobile phase modifier on the chromatography of solute 4 on the OB-CSP. Mobile phases: A = 0.67 *M* methanol in hexane; B = 0.67 *M* ethanol in hexane; C = 0.67 *M* 1-propanol in hexane; D = 0.67 *M* 2-propanol in hexane.

It is interesting to note the lesser affect on the stereoselectivity of the smaller solutes 1 and 3, *versus* the larger ones, 2 and 4–7. Previous work in this laboratory has suggested that the magnitude of chiral recognition is due to the positioning of the solute relative to the CSP and the steric fit of the solute into a chiral cavity on the CSP[4]. A possible explanation is that the MPM not only competes for chiral binding sites with the solute but can also alter the steric nature of the chiral cavities on the CSP by binding to achiral sites at or near the chiral cavity. In this system, the increase in steric bulk at the chiral cavities caused by the replacement of 1-propanol by the bulkier 2-propanol does not appear to be great enough to significantly affect the fit (and thus α) of the smaller solutes.

The effect on k' through the homologous series 2-propanol to 2-hexanol is presented in Fig. 4. The change from 2-propanol to 2-butanol results in an average

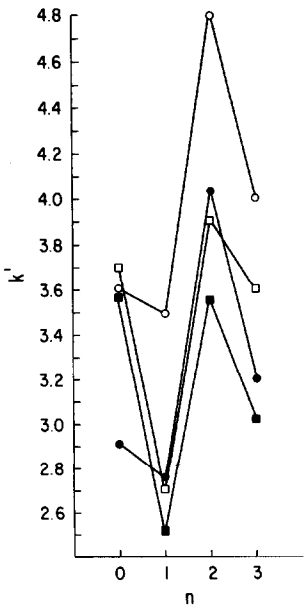

Fig. 4. The effect of increasing steric size of the mobile phase modifier [$CH_3(CH_2)nCHOHCH_3$] on the capacity factor mobile phase 0.67 *M* alcohol in hexane. □ = Solute 1; ■ = solute 2; ○ = solute 3; ● = solute 4.

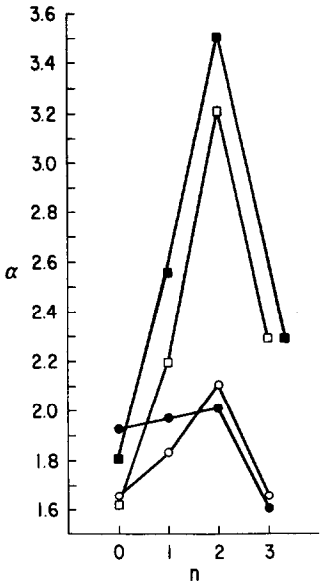

Fig. 5. The effect of increasing steric size of the mobile phase modifier [$CH_3(CH_2)n$CHOHCHCH_3] on stereoselectivity mobile phase 0.67 *M* alcohol in hexane. □ = Solute 1; ■ = solute 2; ○ = solute 3; ● = solute 4.

30% decrease in k' for solutes 1 and 2 and 5% decrease for solutes 3 and 4. When 2-pentanol is substituted for 2-butanol the k' values for solutes 1–4 increase by about 43% while the use of 2-hexanol results in an average 15% decrease in k' relative to the results obtained with 2-pentanol.

The change in the structure of the mobile phase modifier from 2-propanol to 2-butanol to 2-pentanol increases the chiral resolution of solutes 1–4, Fig. 5. However, solutes 1 and 2 are affected to a greater degree than solutes 3 and 4. For solutes 1 and 2, the substitution of 2-butanol for 2-propanol and the substitution of 2-pentanol for 2-butanol each result in an increase in α of approximately 40%. The same changes result in smaller increases in α for solute 3, 10 and 15%, respectively, and have almost no affect on the chiral resolution of solute 4 where α increases by about 2% with each substitution.

The substitution of 2-hexanol for 2-pentanol results in a decrease in the stereoselectivity. The chiral resolution of solutes 1 and 2 falls by an average 32% and there is an average 20% decrease in α for solutes 3 and 4.

The basis behind the varying effect on k' through the homologous series 2-propanol to 2-hexanol is not immediately evident. However, the difference in the magnitude of the effect on solutes 1 and 2 *versus* 3 and 4 when 2-butanol in the MPM suggests that the observed decrease in k' could be the results of secondary steric effects (see discussion below). The decrease in k' (and α) when 2-hexanol is the MPM may be due to inclusion of the alkyl side chain of the molecule in the hydrophopic cavities of the cellulose-based CSP decreasing both chiral and achiral binding of the solute.

The increases in stereoselectivity with the changes from 2-propanol to 2-bu-

tanol to 2-pentanol is consistent with the view that the MPMs are altering the steric environment of the chiral cavities on the CSP. This approach is supported by the fact that solutes 1 and 2 are affected to a greater extent than solutes 3 and 4. This effect does not appear to be affected by the chirality of the MPM. Solute 2 was chromatographed using (*S*)-(+)-2-butanol as the MPM and the observed k' and α were identical to those obtained using the racemic alcohol.

Previous work in this laboratory[4] has demonstrated a reversal in the enantiomeric elution order between amides derived from enantiomeric amines (solutes 1 and 2) and amides derived from the corresponding enantiomeric carboxylic acids (solutes 3 and 4). This reversal was attributed to a different positioning of the solute on the CSP most probably due to the opposite directions of the amide dipoles.

If the observed effects of 2-propanol, 2-butanol and 2-pentanol on stereoselectivity are due to an alteration in the steric environment of the chiral cavity, then solutes which orient themselves differently relative to this cavity should be affected to different extents.

The results obtained using *tert.*-butanol as the MPMs are consistent with this approach. For solutes 1 and 2, there is a dramatic increase in the observed α relative to α obtained with 1-propanol as the MPM (185 and 203%, respectively). The relative increases for solutes 3 and 4, however, are only 24 and 21%, respectively.

As discussed above, the decrease in the observed α when 2-hexanol is the MPM relative to the α obtained with 2-pentanol may be due to an inclusion of the C_4 side chain in the chiral cavity of the CSP. This could be confirmed by the use of 2-heptanol and 2-octanol as mobile modifiers.

Effect of 1-propanol concentration on retention and stereoselectivity

The effects on the retention and stereoselectivity of solutes 1–4 resulting from increases in the molar concentration of the MPM 1-propanol are presented in Table IV and the resulting chromatograms for solute 4 are presented in Fig. 6.

From this data it is evident that consecutive increases in the molar concentration of 1-propanol result in corresponding decreases in retention as would be normally expected. The magnitude of this affect steadily diminishes from an average 50% decrease in k' for the increase in 1-propanol molar concentration from 0.40 to

TABLE IV

THE EFFECT OF THE CONCENTRATION OF 1-PROPANOL ON RETENTION AND STEREOSELECTIVITY

Mobile phase: 1-propanol in hexane.

Molar conc.	*Solute 1*		*Solute 2*		*Solute 3*		*Solute 4*	
1-propanol	k'^*	α	k'^*	α	k'^*	α	k'^*	α
0.40	3.20	1.64	2.95	1.86	3.99	1.58	3.04	1.60
0.67	1.87	1.59	1.58	1.77	1.83	1.55	1.44	1.62
0.94	1.19	1.57	0.98	1.75	1.12	1.54	1.00	1.60
1.21	0.96	1.55	0.69	1.76	0.84	1.54	0.60	1.61

* Capacity factor first eluted enantiomer.

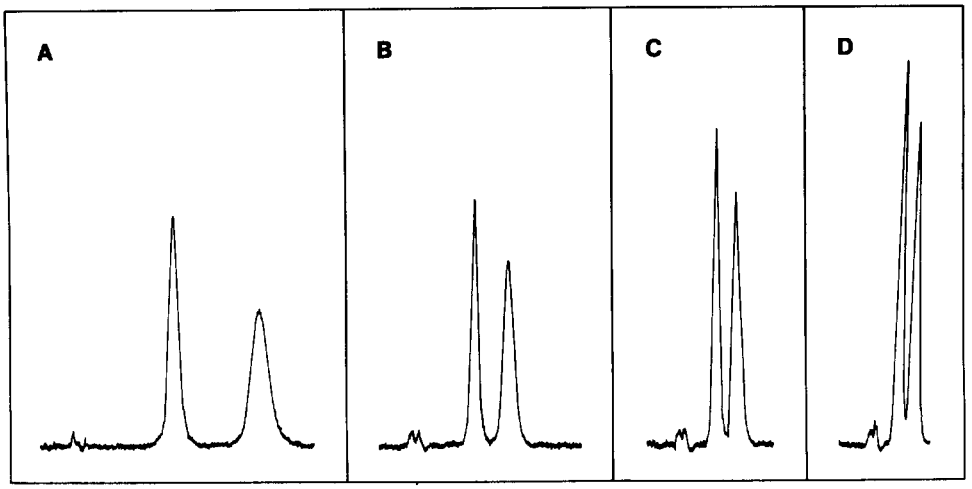

Fig. 6. The effect of the concentration of 1-propanol on the chromatography of solute 4 on the OB-CSP. Mobile phases: A = 0.40 *M* 1-propanol in hexane; B = 0.67 *M* 1-propanol in hexcane; C 0.94 *M* 1-propanol in hexane; D = 1.21 *M* 1-propanol in hexane.

0.67 *M* to an average 25% decrease in k' when the 1-propanol molar concentration is increased from 0.94 to 1.21 *M*.

While the increases in 1-propanol molar concentration decrease k', they seem to have very little effect on the stereoselectivity. The increase in 1-propanol molar concentration from 0.40 to 1.21 *M* results in a 5% decrease in α for solutes 1 and 2, a 3% decrease for solute 3 and no change at all for solute 4.

Two conclusions are possible from these results. First, there are more achiral binding sites on the CSP than chiral sites. This was also suggested by the increase in k' when 2-propanol was substituted for 1-propanol. Secondly, at a MPM concentration of 0.40 *M* the competition for the chiral binding sites between the solute and the MPM has reached saturation, *i.e.* the maximum effect of the MPM has been reached, and an increase in MPM concentrations has no further effect on α. The decreasing effect on k' following serial increases in the MPM concentration indicates that the competition for the achiral binding sites is also a saturable process and a maximum effect on k' will be reached at some 1-propanol concentration greater than 1.21 *M*.

CONCLUSION

The results of this study indicate that the retention and chiral recognition mechanisms operating on the OB-CSP are complex and interrelated. The indication that the binding to achiral sites can alter the steric environment at the chiral cavities of this CSP and thus the stereoselectivity opens up a number of possibilities including the manipulation of the composition of the mobile phase to accomplish desired chiral resolutions.

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