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ENANTIOMERIC SEPARATION OF TERTIARY PHOSPHINE OXIDES ON PIRKLE'S CHIRAL STATIONARY PHASE

MOBILE PHASE AND TEMPERATURE OPTIMIZATION

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SUMMARY

The enantiomeric resolution of tertiary phosphine oxides on chiral stationary phases (CSP), derived from N-(3,5-dinitrobenzoyl)amino acids (Pirkle's CSP) has been studied by varying the nature of the mobile phase and the temperature. The influence on the separation of the relative ability of the solvent to act as proton acceptor, proton donor or dipole was considered. Alcohols were chosen as proton acceptors, chloroform as a proton donor and dioxane, tetrahydrofuran, ethyl acetate and 1,2-dichloroethane as dipole interactors. The selectivity varies in accordance with the steric hindrance of the alcohol. While the resolution remains constant, a significant decrease in the capacity factor, k', is observed from on going from either of the isoeluotropic binary mixtures (hexane–alcohol or hexane–chloroform) to a ternary mixture of them (hexane–alcohol–chloroform). An explanation of this phenomenon is given. The influence of temperature was also studied and, as expected, a decrease in temperature leads to higher resolution values.

INTRODUCTION

Hitherto, investigations in chiral liquid chromatography have essentially dealt with the development of chiral stationary phases (CSPs)[1]. The most important class of CSPs is derived from a chiral group containing two amide functions. In particular, CSP I (Fig. 1), described by Pirkle and co-workers[2,3], is applicable to a broad spectrum of compounds because it has many sites of possible interactions. In addition, restricted rotation around an amide bond may afford a preferred face for selective interaction with one of a pair of enantiomeric solutes.

Many models of chiral recognition have been proposed for this CSP, and it is generally accepted that selectivity results from various interactions involved in the

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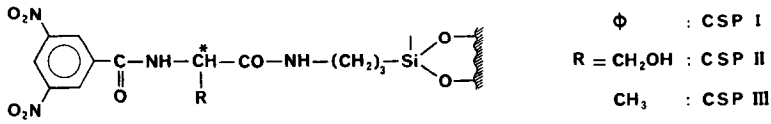

Fig. 1. Structure of CSP I, II and III.

chiral discrimination: hydrogen bonding, electrostatic and/or dipolar interactions, charge-transfer complexes and steric hindrance between the CSP and each enantiomer. Nevertheless, it is clear that any competing interaction involving the mobile phase may alter the enantiomeric resolution. However, most investigators do not take account of the potential solvent–CSP interactions and generally use hexane–2-propanol binary mixtures as the mobile phases. Only Zief *et al.*[4] have shown the importance of the influence of the dipolar and hydrogen donor or acceptor characters (as defined by Snyder[5]) of each component of the mobile phase on the separation quality. Some authors have used ternary[6,7] and even quaternary[8] mixtures, but very few explanations of the elution mechanisms have been given.

In a previous paper[9] we showed that the enantiomers of some tertiary phosphine oxides are separated on CSPs, derived from N-(3,5-dinitrobenzoyl)amino acids (DNB-amino acids) by using hexane–2-propanol binary mixtures as the mobile phase. Here, we deal with the influence of the nature of the solvent on the various chromatographic factors, k', α and R_s, and try to optimize the enantiomeric resolution by using ethanol, 2-propanol, *n*-butanol or *tert.*-butanol as proton acceptors, chloroform as proton donor and tetrahydrofuran, dioxane, ethyl acetate and 1,2-dichloroethane as dipolar compounds. This study has been performed with CSPs I, II, III (Fig. 1), obtained from *R*(−)-DNB-phenylglycine, *S*(+)-DNB-serine and *S*(+)-DNB-alanine, respectively, as chiral precursors. Finally, the influence of the temperature was also studied.

EXPERIMENTAL

Apparatus

Analytical experiments were performed with a Model 1084 B liquid chromatograph (Hewlett-Packard, Walbronn, F.R.G.) equipped with an automatic sampling system (79842 A) and a variable-wavelength detector (190–540 nm) (79875 A) or with a Model 8100 chromatograph (Spectra-Physics, Santa Clara, CA, U.S.A.) equipped with a variable-wavelength detector (190–600 nm) (SP 8440) and a dual-channel computing integrator (SP 4200). Except for the temperature study, all experiments were conducted at 40°C. For thin-layer chromatography (TLC), Merck Si 60 F-254 silica gel plates were used, and column chromatographic separations were carried out over H-60 silica gel (35 g of silica per gram of raw product for purification) (Merck, Darmstadt, F.R.G.).

NMR spectra were recorded on a Bruker WP 200 SY (200 MHz) spectrometer, using tetramethylsilane (TMS) as internal standard and [^{2}H]chloroform as solvent. Chemical shifts (δ) are given in ppm, and coupling constants (J) in Hz. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. The compounds studied had elemental analysis consistent with their formula within ±0.3% (Service Central de Microanalyses du C.N.R.S., Vernaison, France).

The melting points were measured on a Büchi-Tottoli hot-stage apparatus and are uncorrected.

Chiral stationary phase synthesis

The syntheses of CSP I and II have been described[9]. *S*(+)-N-(3,5-Dinitrobenzoyl)alanine was prepared according to the literature method[10]: $(\alpha)_D^{25} = +20.3°$ [C3, tetrahydrofuran (THF)]; m.p. 168–171°C, lit. (ref. 7) 181–192°C.

The coupling procedure for CSP III was the same as that used for CSP I and II[9]: 0.50 mmol of amide per g of CSP (based on N).

Solvents

n-Hexane, 2-propanol and THF were Lichrosolv grade, methanol, ethyl acetate, *tert.*- and *n*-butanol were analytical grade (Merck). Ethanol, propanol, dioxane, chloroform and 1,2-dichloroethane were of analytical grade (Prolabo, Paris, France).

Phosphine oxide synthesis

Racemic tertiary phosphine oxides were prepared according to the reaction shown in Fig. 2. The following compounds have been studied: 1, R = 1-naphthyl; 2, R = 2-naphthyl; 3, R = 1-(2-methylnaphthyl); 4, R = 1-(4-methylnaphthyl); 5, R = 1-(2-methoxynaphthyl); 6, R = 1-(4-methoxynaphthyl); 7, R = 1-(4-benzyloxynaphthyl); 8, R = 1-[4-(ethyl oxyacetate)naphthyl]; 9, R = 1-(4-hydroxynaphthyl); 10, R = 9-phenanthryl. The syntheses of compounds 1, 2, 4–6 and 10 have been described[9–12]. Compounds 3 and 7 were synthesized similarly, the latter from 1-bromo-4-benzyloxynaphthalene.

1-Bromo-4-benzyloxynaphthalene. To a solution of 1-bromo-4-hydroxynaphthalene[9] (19.6 g, 87.9 mmol) in 300 ml of acetonitrile was added potassium carbonate (13.8 g, 100 mmol), and the resulting suspension was stirred for 1 h under a nitrogen atmosphere. Then, benzyl bromide (17.1 g, 100 mmol) was added, and the mixture was heated to reflux for 6 h. The precipitate was removed by filtration and washed with acetonitrile. The filtrate was evaporated and the organic material was extracted with benzene, washed with water until neutral and then with brine. After drying by filtration on an hydrophobic filter and removal of the solvent, 28.5 g of crude product were obtained. Crystallization from heptane–2-propanol (50:1, v/v) yielded 21.6 g (78%) of 1-bromo-4-benzyloxynaphthalene, m.p. 80–82°C. ^{1}H NMR (C^2HCl_3): 5.20 (s, 2H, CH_2), 6.72 [d, ^3J(H, H) = 8.1 Hz, 1 H, 3-naphthyl], 7.32–7.67 (m, 8H), 8.16 [d, ^3J(H, H) = 8.2 Hz, 1 H, 8-naphthyl], 8.35 [d, ^3J(H, H) = 8.4 Hz, 1 H, 5-naphthyl).

(±)Methyl[1-(4-benzyloxynaphthyl)]phenylphosphine oxide, 7. M.p. 178–179°C (diisopropyl ether–2-propanol), yield 45%. ^{1}H NMR (C^2HCl_3): 2.12 [d, ^2J(H, P) = 13 Hz, 3 H, P–CH_3], 5.28 (s, 2 H, CH_2), 6.91 [d-d, ^3J(H, H) = 8.2 Hz, ^4J(H, P) = 1.6 Hz, 1 H, 3-naphthyl], 7.36–7.53 (m, 10 H), 7.66–7.90 (m, 3 H), 8.27–8.43 (m, 2 H).

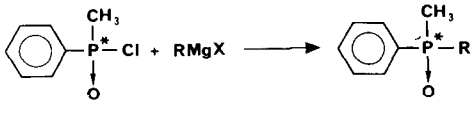

Fig. 2. General reaction for the synthesis of tertiary phosphine oxides. X = halide.

(±)*Methyl[1-(2-methylnaphthyl)]phenylphosphine oxide*, 3. M.p. 119–121°C (diisopropyl ether–hexane), yield 22%. ^{1}H NMR (C^2HCl_3): 2.26 [d, ^2J(H, P) = 13 Hz, 3 H, P–CH_3], 2.74 [d, ^4J(H, P) = 1.8 Hz, 3 H, CH_3], 7.30–7.50 (m, 6 H), 7.61–7.92 (m, 4 H), 8.48 (d, J = 8.5 Hz, 1 H, 8-naphthyl).

(±)*Methyl[1-(4-hydroxynaphthyl)]phenylphosphine oxide*, 9. A solution of (±)methyl[1-(4-methoxynaphthyl)]phenylphosphine oxide (1.184 g, 4 mmol) in 40 ml of dichloromethane was treated dropwise with 10 ml of 1 *M* boron tribromide in dichloromethane (Aldrich) at −15°C under a nitrogen atmosphere. The resulting mixture was stirred at −15°C for 1 h, then at 20°C overnight. The reaction mixture was hydrolyzed by adding water, then neutralized with an aqueous solution of sodium bicarbonate. The solid collected by filtration under suction was washed successively with water, water–ethanol (50:50) and diisopropyl ether, and finally dried under vacuum at 50°C. In this way, 1 g (88%) of pure (±)methyl[1-(4-hydroxynaphthyl)]phenylphosphine oxide was obtained (m.p. 221–223°C). The analytical sample (m.p. 222–225°C) was obtained by crystallization from ethanol. ^{1}H NMR [$(C^2H_3)_2SO$]: 2.10 [d, ^2J(H, P) = 13.4 Hz, 3 H, P–CH_3], 7.02 [d, ^4J(H, P) = 1.8 Hz, 1 H, 3-naphthyl], 7.40–8.42 (m, 10 H), 11.09 (s, 1 H, OH).

(±)*Ethyl[(4-(1-methyl phenyl phosphinyl)naphthalenyl)-oxy]acetate*, 8. To a solution of (±)methyl[1-(4-hydroxynaphthyl)]phenylphosphine oxide, prepared as above (1.1 g, 3.9 mmol, in 50 ml of acetonitrile), were added 0.8 g of potassium carbonate and the resulting suspension was stirred magnetically for 45 min under a nitrogen atmosphere. Then, 0.78 g (4.6 mmol) of ethyl bromoacetate were added, and the mixture was heated under reflux for 4 h. The precipitate was removed by filtration and washed with acetonitrile. The filtrate was then evaporated. The organic material was extracted with dichloromethane, washed with water until neutral and then with brine. The solvent was removed and the raw product was crystallized from diisopropyl ether, yielding 1.3 g (90%) of compound 8, m.p. 127–129°C. ^{1}H NMR (C^2HCl_3): 1.32 [t, ^3J(H, H) = 7.1 Hz, 3 H, CH_2–CH_3], 2.13 [d, ^2J(H, P) = 13 Hz, 3 H, P–CH_3], 4.31 [q, ^3J(H, H) = 7.1 Hz, 2 H, CH_2–CH_3], 4.86 (s, 2 H, O–CH_2), 6.71–8.46 (m, 11 H).

RESULTS AND DISCUSSION

Choice of solutes

The overall optimization study of the mobile phase was carried out with methyl(9-phenanthryl)phenylphosphine oxide, compound 10, as test solute, and methyl[1-(4-methoxynapthyl)]phenylphosphine oxide, compound 6, was used for the study of the temperature influence. The other tertiary phosphine oxides were resolved by using the optimized mobile phase.

Solvent properties

The properties of the various polar solvents studied are summarized in Table I. The polarity index, P', according to Rohrschneider is an indication of the ability of the solvent to take part in strong intermolecular interactions with other, like molecules. The selectivity parameters, x_e, x_d and x_n, can be considered as reflecting the relative ability of the solvent to function as a proton acceptor, proton donor or strong dipole, respectively. Thus, alcohols are considered as essentially proton acceptors,

TABLE I

POLARITY, P', AND SELECTIVITY PARAMETERS, x, AS DEFINED AND CALCULATED BY SNYDER[5] FROM SOLUBILITY DATA REPORTED BY ROHRSCHNEIDER

Underlining indicates the dominant character of the solvent with regard to the values of x_e, x_d and x_n.

Polar solvent	P'	x_e	x_d	x_n
Ethanol	4.3	0.52	0.19	0.29
2-Propanol	3.9	0.55	0.19	0.27
tert.-Butanol	4.1	0.56	0.20	0.24
n-Butanol	3.9	0.59	0.19	0.25
Chloroform	4.1	0.25	0.41	0.33
Dioxane	4.8	0.36	0.24	0.40
Tetrahydrofuran	4.0	0.38	0.20	0.42
Ethyl acetate	4.4	0.34	0.23	0.43
1,2-Dichloroethane	3.5	0.30	0.21	0.49

chloroform as a proton donor and 1,2-dichloroethane, tetrahydrofuran, dioxane and ethyl acetate as dipoles.

The effect of these various solvents on the selectivity was studied at constant analysis time (in all cases hexane was used as the apolar solvent).

TABLE II

RESOLUTION OF METHYL(9-PHENANTHRYL)PHENYLPHOSPHINE OXIDE WITH HEXANE–ALCOHOL BINARY MIXTURES

Alcohol	x_e*	*CSP*	*Alcohol (%, v/v)***	*Mobile phase polarity, P'*	k'_2	α	R_s
Ethanol	0.52	Phenylglycine	10	0.52	10.6	1.37	3.8
		Serine	10	0.52	19.1	1.18	1.7
		Alanine	9	0.48	9.7	1.12	1.2
n-Butanol	0.59	Phenylglycine	18	0.78	9.3	1.39	2.7
		Serine	20	0.86	20.6	1.19	1.1
		Alanine	14	0.63	11.6	1.12	0.8
2-Propanol	0.55	Phenylglycine	20	0.86	10.6	1.45	3.6
		Serine	25	1.05	17.5	1.20	1.3
		Alanine	19	0.82	11.3	1.14	1.1
tert.-Butanol	0.56	Phenylglycine	50	2.10	9.9	1.47	2.2
		Serine	50	2.10	21.0	1.18	0.7
		Alanine	40	1.70	9.0	1.13	#0.4

* Selectivity parameter, reflecting the relative ability of the alcohol to act as a proton acceptor.
** Alcohol content in the mobile phase.

Binary mixtures

Hexane–alcohol. This mixture may give a clue to the influence of proton acceptors.

The results are summarized in Table II. It seems that there is no correlation between the selectivity, α, and x_e for a given CSP. On the contrary, large variations in retention are observed depending on the structure of the alcohol. For a constant analysis time, whatever the CSP, the greater the steric hindrance, the higher must be the alcohol concentration. One explanation is that alcohols may interact at two points with the amide group of the chiral moiety, generating two hydrogen bonds as shown in Fig. 3a. It is reasonable that the more hindered the alcohol, the lower will be the association energy between the alcohol and the CSP. For example, ethanol is much more strongly bonded to CSP than *tert.*-butanol and displaces, more easily, any solute from the CSP. This assumption explains why it is necessary to increase alcohol concentrations when the steric hindrance of the alcohol increases, in order to keep the retention time constant. In addition, we note that, with CSP I, the greater the steric hindrance of the alcohol, the higher is the selectivity; whereas it remains unchanged with CSP II and III.

Finally, with CSP II, the measured selectivities for various alcohols are always lower than those determined with other solvents. This phenomenon is not observed

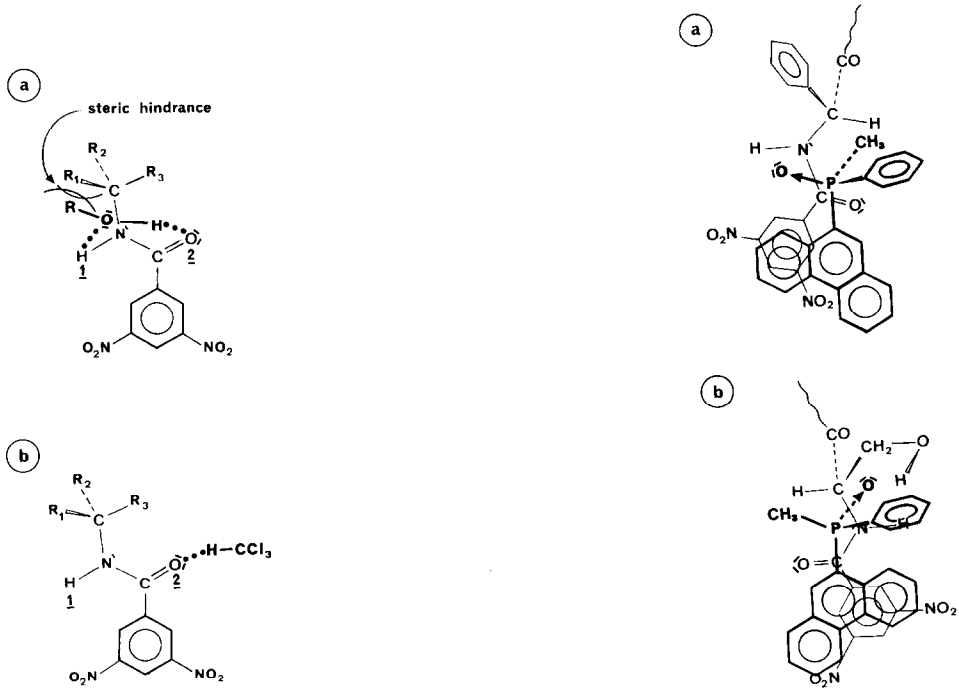

Fig. 3. Models of the interactions between CSP and (a) an alcohol molecule, (b) a chloroform molecule.

Fig. 4. Chiral recognition model, showing the relative arrangement for three simultaneous interactions between (a) (*R*) CSP I and (*S*)-methyl(9-phenanthryl)phenylphosphine oxide, (b) (*S*) CSP II and (*R*)-methyl(9-phenanthryl)phenylphosphine oxide.

TABLE III

RESOLUTION OF METHYL(9-PHENANTHRYL)PHENYLPHOSPHINE OXIDE USING HEXANE–CHLOROFORM BINARY MIXTURES

x_d*	*CSP*	*Polar solvent (%, v/v)***	*Mobile phase polarity, P'*	k'_2	α	R_s
0.41	Phenylglycine	55	2.30	10.6	1.50	3.7
	Serine	75	3.10	21.6	1.41	2.9
	Alanine	65	2.67	10.0	1.18	1.2

* Selectivity parameter reflecting the relative ability of chloroform to act as a proton donor.
** Chloroform content in the mobile phase.

with CSP I and III (Tables II–IV). We can explain this by considering that the primary alcohol function of CSP II interacts with alcohols to develop an hydrogen bond. We have assumed[9] that the primary alcohol group takes part in the chiral recognition mechanism of tertiary phosphine oxides with CSP II, and, as mentioned above, this competing interaction due to the hydroxy group decreases the selectivity. These observations strengthen the assumptions concerning the chiral recognition models proposed in ref. 9:

(a) Hydrogen bonding between the oxygen atom of the solute and an acidic proton of CSP.

TABLE IV

RESOLUTION OF METHYL(9-PHENANTHRYL)PHENYLPHOSPHINE OXIDE USING HEXANE–STRONG DIPOLE SOLVENT BINARY MIXTURES

Polar solvent	x_n*	*CSP*	*Polar solvent (%, v/v)***	*Mobile phase polarity, P'*	k'_2	α	R_s
Dioxane	0.40	Phenylglycine	55	2.69	10.4	1.40	2.9
		Serine	85	4.10	21.5	1.42	2.5
		Alanine	66	3.20	11.0	1.14	1.1
Tetrahydrofuran	0.42	Phenylglycine	50	2.05	9.4	1.24	2.3
		Serine	58	2.36	19.9	1.23	1.8
		Alanine	58	2.26	10.2	1.06	≈0.4
Ethyl acetate	0.43	Phenylglycine	78	3.45	10.5	1.28	2.4
		Serine	90	3.97	22.0	1.22	1.6
		Alanine	92	4.06	9.2	1.06	≈0.4
1,2-Dichloroethane	0.49	Phenylglycine	85	2.99	9.9	1.33	1.9
		Serine	100	3.50	34.0	1.22	1.3
		Alanine	100	3.50	7.9	1.00	0

* Selectivity parameter reflecting the relative ability of the polar solvent to act as a strong dipole.
** Polar solvent content in the mobile phase.

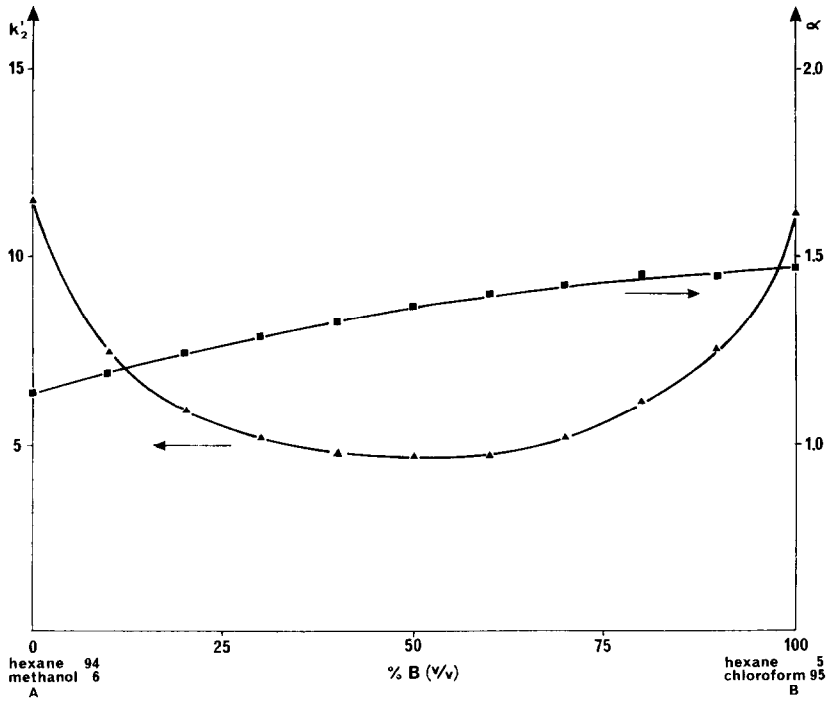

Fig. 5. Dependences of k'_2 (▲) and α (■) for methyl(9-phenanthryl)phenylphosphine oxide on the concentration of binary mixture B [hexane–chloroform (5:95, v/v)] in the ternary mixture A–B [A = hexane–methanol (94:6, v/v)] for CSP II. Column: 250 mm × 4 mm I.D. Flow-rate: 2 ml/min. Temperature: 40°C. Detection: 256 nm.

(b) Electrostatic interaction between the phosphorus atom of the solute and the oxygen atom of the carbonyl group of the amide.

(c) Charge-transfer complex (π–π) formation between the π-donor substituent (naphthyl group) of the solute and the π-acceptor, 3,5-dinitrobenzoyl group, of the chiral support.

(d) Negative interaction, steric in nature, due to the difference in steric hindrance between the methyl and phenyl groups. This implies a difference in stability between the two diastereomeric complexes, occurring during the chromatographic process.

Whereas the last three interactions are common to the three CSPs, the first one depends on the nature of the acidic proton involved. In the cases of CSP I and III, we consider the hydrogen on the amide nitrogen atom (Fig. 4a), whereas we consider the hydrogen of the primary alcohol function in the case of CSP II (Fig. 4b).

Hexane–chloroform (Table III). Chloroform and *tert.*-butanol have the same polarity, P' (Table I). However a chloroform concentration higher than that of *tert.*-butanol is necessary to obtain the same retention time (Tables II and III). In addition, the selectivities are greater with chloroform (α = 1.50) than those measured with alcohols (α = 1.47, maximum value with *tert.*-butanol).

The high chloroform concentration in binary mixtures indicates strong inter-

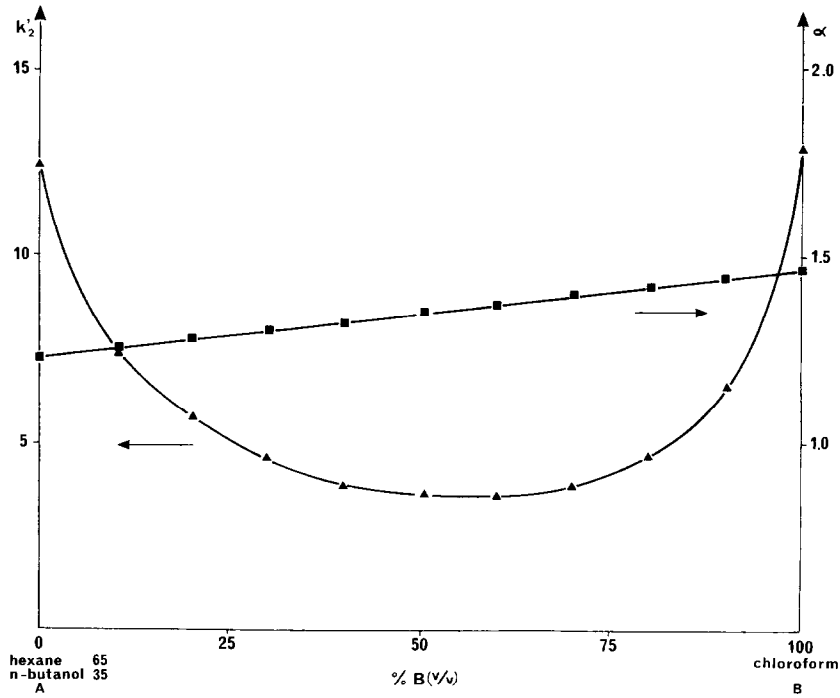

Fig. 6. Dependences of k'_2 (▲) and α (■) for methyl(9-phenanthryl)phenylphosphine oxide on the concentration of solvent B (chloroform) in the ternary mixture A–B [A = hexane–butanol (65:35, v/v)] for CSP II. Other details as in Fig. 5.

actions between the solutes and CSP or weak interactions between the solutes and the mobile phase (or a combination of both). As we shall see, tertiary phosphine oxides are more soluble in chloroform than in an isoeluotropic hexane–ethanol binary mixture. From this last result, only the first assumption can be taken into account. This can be explained by considering that chloroform molecules interact with only one active site instead of two in the case of an alcohol (Fig. 3a and b). Furthermore, Hildebrand partial solubility parameters related to hydrogen bonding[13] have higher values with alcohols, δ_h = 7–8 $cal^{1/2}$ $cm^{-3/2}$, than with chloroform, δ_h = 2.8 $cal^{1/2}$ $cm^{-3/2}$, and, consequently, alcohol molecules are more hard to displace from CSP by solute molecules. Fig. 3b shows that the amide hydrogen remains free to interact with the oxygen atom of the phosphine oxide when the polar solvent in the mobile phase is chloroform, resulting in an enhancement of the solute retention.

Hexane–dipolar solvent. Except for dioxane, the selectivities measured were identical or lower than those obtained with alcohols (Table IV). With regard to the detection, alcohols are preferable owing to their lower UV cut-off.

Ternary mixtures

Taking account of the previous results, hexane–chloroform–alcohol ternary mixtures were studied. With CSP II (derived from serine), methanol, ethanol, 1-propanol, butanol and 2-propanol were chosen. Each ternary mixture was obtained by

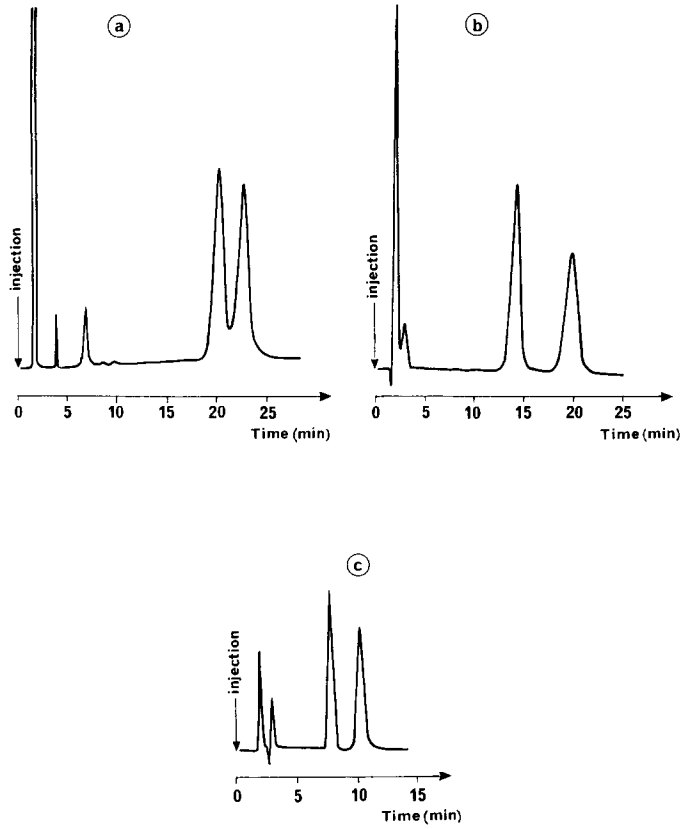

Fig. 7. Separation on CSP II of enantiomers of racemic methyl(9-phenanthryl)phenylphosphine oxide. Mobile phases: (a) hexane–methanol (94:6, v/v); (b) hexane–chloroform (5:95, v/v); (c) hexane–chloroform–methanol (40.6:57.0:2.4, v/v). Other details as in Fig. 5.

TABLE V

INFLUENCE OF THE ALCOHOL NATURE ON $\Delta k'_2/k'_{2bin}$

Alcohol	$\frac{\Delta k'_2}{k'_{2bin}}$	x_e	*Radical of the alcohol*	*No. of carbon atoms in β position to the hydroxyl group*
Methanol	0.60	0.48	CH_3–	0
Ethanol	0.70	0.52	CH_3–CH_2–	1
n-Propanol	0.72	0.54	CH_3–CH_2–CH_2–	1
n-Butanol	0.72	0.59	$CH_3(CH_2)_3$–	1
2-Propanol	0.79	0.55	$(CH_3)_2CH$–	2

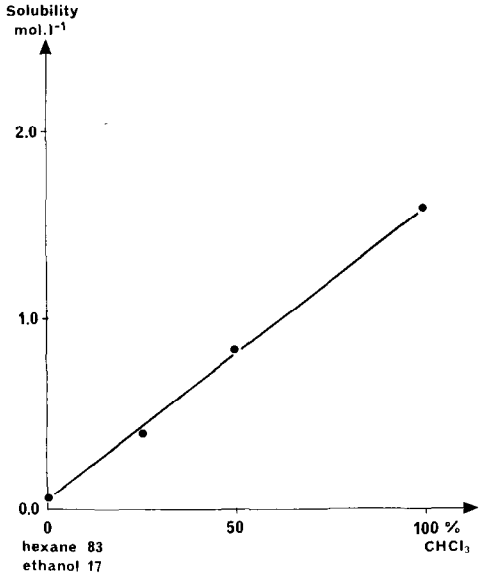

Fig. 8. Solubility of methyl(9-phenanthryl)phenylphosphine oxide at 40°C *versus* the chloroform concentration in A–chloroform ternary mixtures, where A = hexane–ethanol (83:17, v/v).

mixing two isoeluotropic binary mixtures: hexane–alcohol and hexane–chloroform. Figs. 5 and 6, respectively, show the results obtained with methanol and *n*-butanol.

A large decrease in the retention time of the second eluted enantiomer (see k'_2 in Figs. 5 and 6) is observed with a minimum value when the two initial isoeluotropic binary mixtures (hexane–alcohol and hexane–chloroform) are mixed in a ratio close to 50:50 (v/v). Both the selectivity and the resolution increase regularly from a hexane–alcohol mixture to a hexane–chloroform binary mixture (Fig. 7a and b), but the selectivity per unit of time is always greater for the ternary mixtures than for binary mixtures (Fig. 7c). These observations are valid, whatever the nature of the alcohol.

Influence of the alcohol. Let $\Delta k'_2$ be the difference in capacity factors, $k'_{2\,\mathrm{bin}}$ and $k'_{2\,\mathrm{ter}}$, measured for one of the binary mixtures and for the ternary mixture corresponding to the minimum of the curve, respectively (subscript 2 refers to the second eluted enantiomer). For each alcohol, the ratio $\Delta k'_2/k'_{2\,\mathrm{bin}}$, the proton acceptor parameter, x_e, and the number of carbon atoms located in β-position with respect to the hydroxyl group are given Table V. The ratio $\Delta k'_2/k'_{2\,\mathrm{bin}}$ increases with the number of β-carbon atoms. On the other hand, a simple relationship cannot be drawn between x_e and $\Delta k'_2/k'_{2\,\mathrm{bin}}$. The increase in the β-carbon atom number, which enhances the steric hindrance of the alcohol, may account for the decrease in capacity factors: bulkier alcohols may indeed hinder solute molecules from approaching the CSP. However, this decrease in retention has no effect on the selectivity values.

Influence of the nature of the stationary phase. The phenomenon described above is observed for the three chiral stationary phases and also for an aminopropyl-bonded silica without chirality; nevertheless, the ratio $\Delta k'_2/k'_{2\,\mathrm{bin}}$ is lower (0.341) with the latter phase than with CPS.

The capacity factor, k', may be defined as:

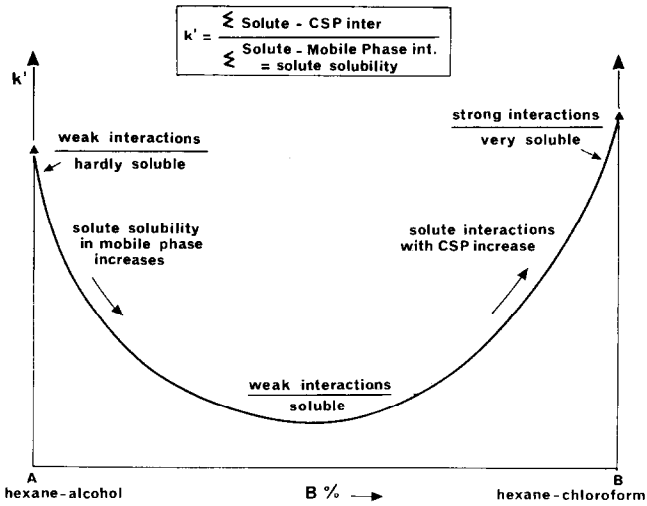

Fig. 9. Concave profile of the measured phosphine oxide capacity factors *versus* chloroform concentration in the hexane–alcohol–chloroform ternary mixture.

$$k' = \frac{\Sigma \text{ solute–stationary phase interactions}}{\Sigma \text{ solute–mobile phase interactions}}$$

To a first approximation, the solubility can be considered as a measure of the solute–mobile phase interactions. Thus when the solubility of methyl(9-phenanthryl)-phenylphosphine oxide is plotted *versus* the chloroform concentration in hexane–chloroform–ethanol ternary mixtures (Fig. 8) a linear increase is observed. Consequently, in ternary mixtures, two phenomena may be assumed: (a) a higher solubility of solute molecules in the mobile phase when chloroform is added; (b) the substitu-

TABLE VI

RESOLUTION OF RACEMIC PHOSPHINE OXIDES ON CSP I

Column: 250 mm × 4 mm I.D. Mobile phase: hexane–chloroform–ethanol (70:26.8:3.2, v/v) except for solutes 1 and 8 (65:29.6:5.4, v/v) and solute 9 (25:66.9:8.1, v/v); flow-rate, 2 ml/min. Detection: 282 nm, except for solute 10, 256 nm. Temperature: 40°C.

Solute	*R*	k'_2	α	h	R_s
1	1-Naphthyl	2.0	1.42	14.1	2.3
2	2-Naphthyl	2.6	1.00	—	—
3	1-(2-Methylnaphthyl)	2.3	1.25	19.3	1.4
4	1-(4-Methylnaphthyl)	4.7	1.57	23.2	3.0
5	1-(2-Methoxynaphthyl)	5.0	1.47	18.2	2.9
6	1-(4-Methoxynaphthyl)	7.5	1.61	15.4	4.0
7	1-(4-Benzyloxynaphthyl)	7.9	1.60	14.5	4.2
8	1-[4-(Ethyloxyacetate)naphthyl]	4.8	1.49	11.4	3.7
9	1-(4-Hydroxynaphthyl)	3.5	1.35	45.7	1.3
10	9-Phenanthryl	6.3	1.58	18.4	3.2

TABLE VII

RESOLUTION OF RACEMIC PHOSPHINE OXIDES ON CSP II

Mobile phase: hexane–chloroform–ethanol (50:44.6:5.4, v/v) except for solutes 6 (25:89:11, v/v) and 9 (15:75.8:9.2, v/v). Other details as in Table VI.

Solute	*R*	k'_2	α	h	R_s
1	1-Naphthyl	8.5	1.24	31.8	1.2
2	2-Naphthyl	7.2	1.00	–	–
3	1-(2-Methylnaphthyl)	2.3	1.19	37.9	0.9
4	1-(4-Methylnaphthyl)	5.3	1.34	39.4	1.5
5	1-(2-Methoxynaphthyl)	6.3	1.39	32.0	1.8
6	1-(4-Methoxynaphthyl)	2.6	1.40	23.6	1.8
7	1-(4-Benzyloxynaphthyl)	7.9	1.34	35.2	1.6
8	1-[4-(Ethyloxyacetate)naphthyl]	8.3	1.27	41.7	1.3
9	1-(4-Hydroxynaphthyl)	5.0	1.27	49.3	1.1
10	9-Phenanthryl	6.3	1.34	30.7	1.8

tion of some alcohol molecules (which are strongly adsorbed on CSP) by chloroform molecules (which are easily displaced from CSP by solute molecules).

Let us examine the curves in Figs. 5 and 6 and the general profile in Fig. 9 which reflects the influence of the addition of chloroform to a hexane–alcohol binary mixture. First, the solute–stationary phase interactions can be considered as weak with hexane–alcohol mobile phases because of the strong adsorption of alcohol molecules onto the CPS. When a small amount of chloroform is added to the mobile phase, the solubility of the solute increases according to (a), whereas, with regard to (b), the chloroform molecules can displace few alcohol molecules adsorbed on the CSP; the increase in solubility overshadows the phenomenon (b) leading to a decrease in k' values. This accounts for the descending left part of the curve. On the contrary, at higher chloroform concentrations the phenomenon (b) becomes predominant,

TABLE VIII

RESOLUTION OF RACEMIC PHOSPHINE OXIDES ON CSP III

Mobile phase: hexane–chloroform–ethanol (75:21.2:3.8, v/v) except for solutes 8 (65:29.6:5.4, v/v), 9 (40:50.8:9.2, v/v) and 10 (67.5:27.5:5, v/v). Other details as in Table VI.

Solute	*R*	k'_2	α	h	R_s
1	1-Naphthyl	5.0	1.18	13.6	1.4
2	2-Naphthyl	4.0	1.00	–	–
3	1-(2-Methylnaphthyl)	3.1	1.07	10.5	0.6
4	1-(4-Methylnaphthyl)	6.3	1.24	18.0	1.8
5	1-(2-Methoxynaphthyl)	7.3	1.17	14.1	1.4
6	1-(4-Methoxynaphthyl)	9.6	1.25	13.9	2.2
7	1-(4-Benzyloxynaphthyl)	10.2	1.25	15.9	2.1
8	1-[4-(Ethyloxyacetate)naphthyl]	7.5	1.17	15.4	1.3
9	1-(4-Hydroxynaphthyl)	6.5	1.09	30.4	0.6
10	9-Phenanthryl	4.9	1.22	26.8	1.3

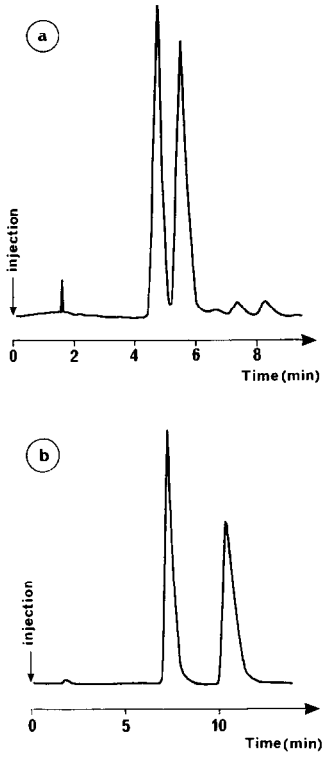

Fig. 10. Separation on CSP I of enantiomers of racemic methyl[1-(2-methylnaphthyl)]phenylphosphine oxide (a) and methyl[1-(4-methylnaphthyl)]phenylphosphine oxide (b). Column: 250 mm × 4 mm I.D. Mobile phase: hexane–chloroform–ethanol (70:26.7:3.3, v/v); flow-rate, 2 ml/min. Temperature: 40°C. Detection: 282 nm.

which causes an increase in the solute–stationary phase interactions and consequently in k', yielding the ascending right part of the curve.

Applications

We have taken advantage of these results to resolve a series of tertiary phosphine oxide racemates by using the optimized ternary mixture hexane–chloroform–methanol as the mobile phase. The capacity factors, selectivities and resolutions measured for these solutes on the three CSPs are summarized in Tables VI–VIII.

It is interesting to compare compounds 3 and 5 (with a methyl or methoxy group in position 2) and compounds 4 and 6 (with a methyl or a methoxy group in position 4): methyl or methoxy substituents induce an higher steric hindrance towards stationary phases when they are located at position 2 of the naphthyl group rather than at position 4. For example, with CSP I and for a given mobile phase, the selectivity for solute 4 is 1.57 for an analysis time of 10 min, while for solute 3 it is 1.25 for an analysis time of 6 min (Fig. 10a and b).

Influence of temperature

It is well known that a decrease in temperature generally leads to higher re-

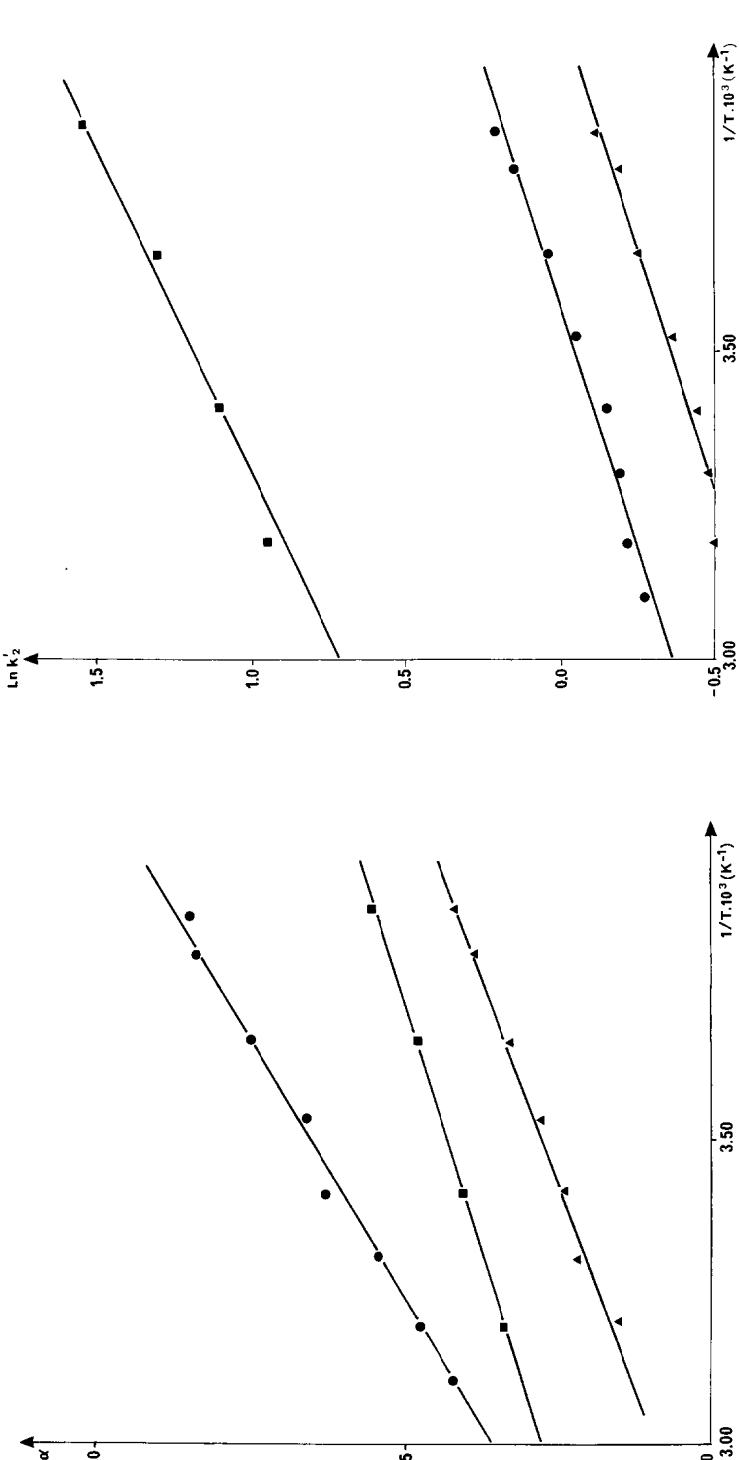

Fig. 11. Dependence of ln α for methyl[1-(4-methoxynaphthyl)]phenylphosphine oxide on temperature for CSP I (●), II (■) and III (▲). Mobile phases: hexane–chloroform–ethanol (25:66.9:8.1, v/v) (CSP I and II); (50:44.6:5.4, v/v) (CSP III). Other details as in Fig. 10.

Fig. 12. Dependence of ln k'_2 for methyl[1-(4-methoxynaphthyl)]phenylphosphine oxide on temperature for CSP I (●), II (■) and III (▲). Operating conditions as in Fig. 11.

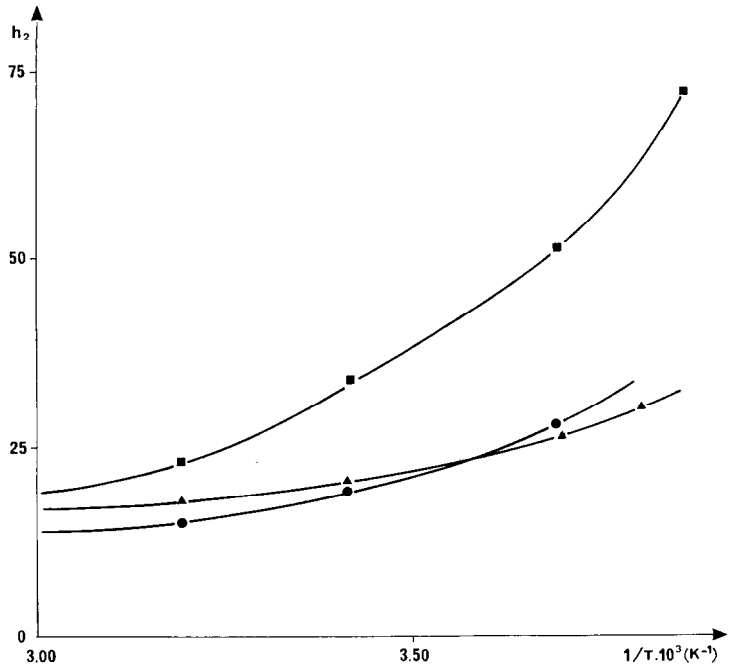

Fig. 13. Dependence of the reduced plate height, h_2, for methyl[1-(4-methoxynaphthyl)]phenylphosphine oxide on temperature for CSP I (●), II (■) and III (▲). Operating conditions as in Fig. 11.

tention times and selectivities[14,15], but few experiments have been described in which enantiomers were separated on CSPs at temperatures lower than 0°C. Here, the resolution of methyl[1-(4-methoxynaphthyl)]phenylphosphine oxide racemate was studied at temperatures between 50 and −15°C.

We observed that a decrease in temperature from 50 to −15°C improves the selectivity which compensates for the loss in efficiency due to the increase in the

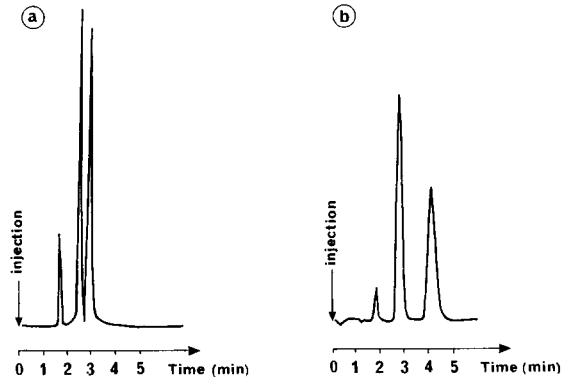

Fig. 14. Effect of the temperature on the separation of racemic methyl[1-(4-methoxynaphthyl)]phenylphosphine oxide on CSP I. Mobile phase: hexane–chloroform–ethanol (25:66.9:8.1, v/v); flow-rate, 2 ml/min. Temperatures: (a) 50°C, (b) −15°C. Other details as in Fig. 10.

mobile phase viscosity. The curves displaying the selectivities, α, the capacity factors, k'_2, and the reduced plate height, h_2, relative to the second eluted enantiomer *versus* $1/T$ are shown in Figs. 11–13. We note that for CSP I the selectivity varies from 1.52 to 2.33, k'_2 from 0.8 to 1.3 and h_2 from 16.4 to 32.9, when the temperature is decreased from 50 to -15°C. At the same time, the resolution factor increases from 1.5 to 2.7 (Fig. 14). These operating conditions give a high resolution per unit of time, 0.55, which is exceptional for chiral separations. In addition, low-temperature high-performance liquid chromatography (HPLC) represents another major advantage in HPLC methodology in that it has the potential for the separation of thermally labile species.

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