Journal of Chromatography, 363 (1986) 81–93
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 661

PREPARATIVE SEPARATION OF RACEMIC TERTIARY PHOSPHINE OXIDES BY CHIRAL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

It is shown that preparative chromatography on a chiral stationary phase (CSP) may constitute a useful alternative to chemical methods for the separation of enantiomers of some tertiary phosphine oxides starting from racemic mixtures. Optimization of the mobile phase, carried out by addition of a third solvent (chloroform) to the classical binary mixture (hexane–alcohol), results in a higher selectivity per unit time. A column packed with 500 g of CSP prepared by bonding (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine to an aminopropylsilica gel (7 μm) enabled the injection of 1 g of methyl(4-methoxy-1-naphthyl)phenylphosphine oxide in a single experiment. In this manner the two enantiomers were obtained with an optical purity greater than 99% and a good yield (96%).

INTRODUCTION

The preparation of enantiomers is usually achieved by fractional recrystallization of diastereoisomeric salts; another method is enantioselective synthesis. However, it is not always possible to use those techniques; for instance, the first method cannot be used in the case of compounds lacking reactive functional groups[1].

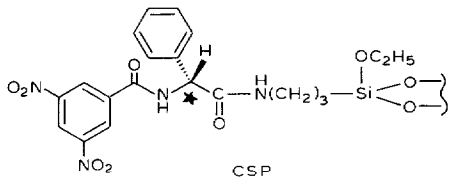
Pirkle and co-workers[2,3] recently showed that liquid chromatography on a preparative scale with a chiral stationary phase (CSP) is an interesting method for obtaining enantiomers in high optical purities. These authors described the resolution of a number of compounds belonging to various chemical classes and reported the preparation of enantiomers with optical purities higher than 99%. Quantities of $\geqslant 1$ g were obtained in a single experiment in favourable cases.

In a previous paper[4] we have shown that some tertiary phosphine oxides including an aromatic substituent derived from naphthalene were resolved on various CSPs, especially on that derived from (*R*)-phenylglycine using hexane–2-propanol as a classic mobile phase. In order to determine the absolute configuration of the dif-

0021-9673/86/$03.50

ferent enantiomers and possibly to specify the reaction mechanisms occurring during the liquid chromatographic resolution, we had to prepare enantiomers with a high optical purity.

In this work we describe the experiments done on the preparative separation of three tertiary phosphine oxides using 500 g of CSP derived from (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine covalently bonded to an aminopropyl silica gel of fine particle diameter (7 μm):



CSP

The mobile phase was chosen according to a previous analytical study[5] which revealed that a ternary mixture hexane–chloroform–ethanol exhibits a good selectivity within a short analysis time.

EXPERIMENTAL

Apparatus

Analytical chromatography was performed with a Model 1084 B liquid chromatograph (Hewlett-Packard, Waldbronn, 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 liquid chromatograph (Spectra-Physics, Santa Clara, CA, U.S.A.) equipped with a variable-wavelength detector (190–600 nm) (SP-8440) operating at 280 nm and a dual-channel computing integrator (SP 4200). The column temperature was 40°C.

Preparative chromatography was performed with a Modulprep apparatus (Jobin-Yvon, Longjumeau, France). The chiral stationary phase (500 g) was packed into the column (22 cm × 80 mm I.D.) by axial compression under 15 bar. UV detection was carried out at 325 nm with a variable-wavelength detector (195–370 nm) (Model SM-25, Jobin-Yvon). The preparative chromatograph was operated at room temperature. The eluent inlet pressure was about 9–10 bar, which gave a flow-rate of *ca.* 110 ml min^{-1}.

Melting points were measured on a Buchi-Tottli hot-stage apparatus and are given without correction. Optical rotations were measured on a Perkin-Elmer 141 micropolarimeter with thermostatted 1-dm quartz cells and using high purity solvents (usually from Merck). NMR spectra were recorded on a Brucker WP-200 (200 MHz) using tetramethylsilane (TMS) as an internal standard and [^{2}H]chloroform as a solvent. Chemical shifts (δ) values are given in ppm and coupling constants in Hz.

The compounds followed by "analysis" had elemental analyses consistent with their formulae within ± 0.30% (Service Central de Microanalyse du Centre National de Recherche Scientifique.

Materials

The solvents [*n*-hexane, ethanol, chloroform stabilized with 0.6% (w/w) etha-

nol] were analytical grade and were purchased from Prolabo (Paris, France). Classical chromatographic purifications were carried out on Merck silica gel 60; analytical thin-layer chromatography (TLC) was performed on Merck F 254 silica gel plates.

Synthesis of chiral stationary phase

The chiral moiety, (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine, $[\alpha]_D^{25} = -86.9°$ [C = 0.92% (w/w); tetrahydrofuran, THF], m.p. 209–211°C, was prepared as described[6].

Solid (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (43 g, 124 mmol) and N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (39.4 g, 150 mmol) were successively added with swirling to 500 g of LiChrosorb NH_2 (7 μm), suspended in 2.5 l of dichloromethane. The reaction mixture turned dark as the reagents dissolved, and was periodically swirled over 2 h, then the supernatant was removed by suction. The solid was washed three times with portions of 500 ml of methanol. The last washing was done with 500 ml of diethyl ether. After drying under vacuum, 576 g of CSP were obtained with the following elemental analysis: 24.59% C, 3.47% H, 2.10% N, 24% Si, corresponding to 0.30 mmol of chiral sites per gram of support. This means that only 34% of the aminopropyl sites were bonded.

Synthesis of racemic phosphine oxide

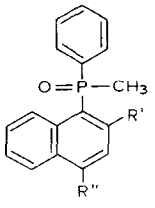
Three racemic compounds (±)-I–III were studied (Table I), of which I and II have been described previously[4]. Compound III was prepared in a similar manner. Experiments showed that a good yield was obtained reproducibly when the methylphenylphosphonyl chloride was freshly prepared.

(±)-Methyl(4-methoxy-1-naphthyl)phenylphosphine oxide (III), m.p. 199–201°C (toluene), yield 62%. ^{1}H NMR ([^{2}H]chloroform): 2.12 (d, J_{P-CH_3} = 13, P–$\underline{CH}_3$), 4.02 (s, O–$\underline{CH}_3$), 6.84 (d, J_{P-H3} = 1.7, $\underline{H}^3$), 7.87 (d, J_{P-H2} = 15, $\underline{H}^2$), 7.71 (d, J_{P-H9} = 12.1, $\underline{H}^9$), 7.4–8.36 (m, 7H). Analysis (C, H, P): $C_{18}H_{17}O_2P$.

1-Bromo-4-methoxynaphthalene, used as an intermediate for the preparation of III, was prepared as follows.

TABLE I

PHOSPHINE OXIDES STUDIED



Phosphine oxide	*R'*	*R''*
I	H	H
II	OCH_3	H
III	H	OCH_3

1-Bromo-4-hydroxynaphthalene

To a solution of iodine (1390 g, 5.47 mol) in 3.2 l of acetic acid, was added, at 20°C, bromine (837 g, 5.24 mol) within 2 h, then the mixture was stirred at 50°C for 3 h and at room temperature for 16 h.

To a solution of 1-hydroxynaphthalene (750 g, 5.2 mol) in 2.4 l of acetic acid was added at 10–15°C for 4 h the solution prepared as above. Stirring was maintained for 16 h. The reaction mixture was added in 15 min to a solution of sodium bisulphite (857 g) in 28 l of water. After stirring for 0.5 h the mixture was neutralized by adding 8.6 kg of sodium bicarbonate (pH 5). The organic material was then extracted successively with 2 l of diisopropyl ether and with 500 ml (twice) of the same solvent. The diisopropyl ether fractions were pooled and then washed (twice) with 500 ml of water then brine. The insoluble part was discarded by filtration through Celite. After evaporation of the solvent, the crude product was dissolved in 600 ml of dichloromethane and then cooled. The resulting crystalline precipitate was collected by filtration, washed with 200 ml of heptane–dichloromethane (9:1) and dried under vacuum at room temperature. A 264-g amount (22%) of 1-bromo-4-hydroxynaphthalene was obtained, m.p. 122–123°C (lit.[7] 121–122°C). ^{1}H NMR([^{2}H]chloroform): 5.34 (s, OH).

1-Bromo-4-methoxynaphthalene

To a solution of 1-bromo-4-hydroxynaphthalene (33.45 g, 150 mmol) in 450 ml acetonitrile was added potassium carbonate (22.8 g, 165 mmol) and the resulting suspension was stirred for 1 h under nitrogen. Then methyl iodide (42.6 g, 300 mmol) was added and the mixture was heated under reflux for 3 h. The precipitate was removed by filtration and washed twice with 100 ml acetonitrile. The filtrate was then evaporated and the organic material extracted with 200 ml benzene, and washed successively with water (150 ml, four times) until neutral and with brine. After drying by filtration through an hydrophobic filter and stripping off the solvent 34.6 g of crude product were obtained. The compound was purified by chromatography on 1200 g of silica gel with hexane as eluent, and 25.2 g (70%) of 1-bromo-4-methoxynaphthalene as were obtained a pale yellow viscous oil and used directly for the preparation of the Grignard reagent. ^{1}H NMR([^{2}H]chloroform): 3.81 (s, $OC\underline{H}_3$).

RESULTS AND DISCUSSION

Analytical optimization

Recently we have shown[5] that the ternary mixture hexane–chloroform–alcohol, obtained by mixing two isoeluotropic binary mixtures hexane–alcohol and hexane–chloroform, always gives a higher selectivity per unit time than each isoeluotropic binary mixture. This interesting phenomenon is observed whatever the nature of the alcohol. Fig. 1 shows the results obtained with ethanol. A large decrease in the capacity factor is observed when the two isoeluotropic binary mixtures are mixed in about equal volumes. The selectivity increases linearly with increasing chloroform content. So a compromise between a short separation time and the highest possible selectivity led us to choose a ratio of 20:80 between the binary mixtures A (hexane–ethanol, 93:7) and B (hexane–chloroform, 60:40) corresponding to the following composition: hexane–chloroform–ethanol (66.6:32:1.4).

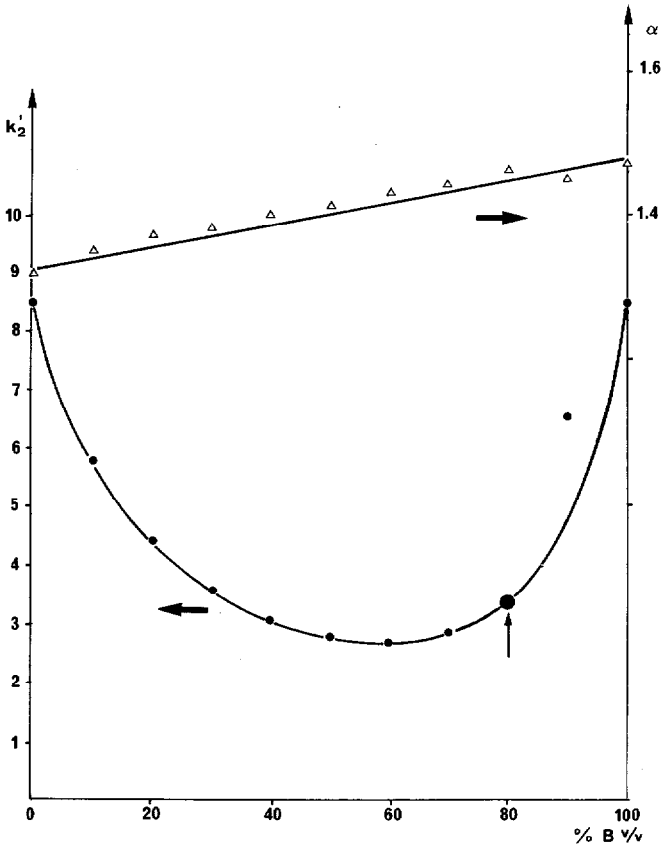


Fig. 1. Influence of the mobile phase composition on the capacity factor (●–●) and selectivity (△–△). The mobile phase is obtained by mixing binary mixture A (hexane–ethanol, 93:7) with binary mixture B (hexane–chloroform, 60:40) in different proportions. Solute: racemate (±)-methyl(4-methoxy-1-naphthyl)phenylphosphine oxide (III).

The chromatographic data are given in Table II for both analytical and preparative columns. The quantity injected on the preparative column is small compared to the amount of CSP and can be considered as an analytical one. The length of the stationary phase bed in the preparative column cannot be measured directly, but can be calculated from measurements of the dead volume, V_m, for the analytical and preparative columns. Assuming that the total porosities, ε, are not very different for the two columns ($\varepsilon = 0.77$ for the analytical one), one can derive a length of *ca* 22 cm for the preparative column. The only difference in operating conditions between the columns is the temperature. It is noted that the capacity factors of the less strongly retained isomers (isomers 1) are very close to each other on both columns, while those of the more strongly retained isomers (isomers 2) are higher on the preparative column. This behaviour is in agreement with a normal temperature dependence and results in a higher selectivity with the preparative column. The plate number is also higher for isomers 1 on both columns and is systematically higher on the analytical than on the preparative column. This can be explained by differences in the temper-

TABLE II

ANALYTICAL CHROMATOGRAPHIC DATA FOR THE OPTICAL ISOMER PAIRS STUDIED

Stationary phase: LiChrosorb NH_2 (7 μm) bonded with (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine. Mobile phase: hexane–chloroform–ethanol (66.6:32.0:1.4). UV detection at 280 nm. Anal. = analytical column (25 cm × 0.46 cm I.D.) containing 2 g of CSP. Temperature: 40°C. Flow-rate: 2 ml min^{-1}. Dead volume: 3.2 ml. Injection: 10 μl of a 10 mg ml^{-1} solution (0.1 mg of the racemate III). Prep. = preparative column (22 cm × 8 cm I.D.) containing 500 g of CSP. Temperature: ambient. Flow-rate: 112 ml min^{-1}. Dead volume: 860 ml. Injection: 5 ml of a 2 mg ml^{-1} solution (10 mg of the racemate III).

Compound	*Capacity ratio, k'*		*Plate number, N*		*Selectivity*		*Resolution, R_s*	
	Anal.	*Prep.*	*Anal.*	*Prep.*	*Anal.*	*Prep.*	*Anal.*	*Prep.*
Naphthyl derivative (I)	3.3 4.3	3.3 4.9	4400 3800	1700 1300	1.30	1.48	3.0	2.4
2-Methoxynaphthyl derivative (II)	4.2 5.6	4.2 6.4	3700 3500	1400 1100	1.33	1.52	3.3	2.4
4-Methoxynaphthyl derivative (III)	5.5 8.0	5.6 9.4	4800 4100	1600 1100	1.45	1.68	4.5	3.0

ature, length and packing quality. In addition, the flow-rate (112 ml min^{-1}) on the preparative column might be a little too low. If we assume a diffusion constant in the mobile phase of about $2.6 \cdot 10^{-9}$ m^2 s^{-1} (ref. 8), the reduced velocity would be about 1.3, which indicates that there might be a slight band broadening due to axial diffusion.So, in spite of its higher selectivity, the preparative column produces a lower resolution than the analytical one, but the resolution remains quite satisfactory for preparative purposes.

For the sake of simplicity, results concerning only the (±)-methyl(4-methoxy-1-naphthyl)phenylphosphine oxide (III) will be presented and discussed.

Preparative chromatography

Fig. 2 shows three preparative chromatograms obtained from injections of three different amounts of the racemic compound III. One can see a strong decrease in the overall retention of each enantiomer, compared to the analytical chromatogram. The analytical retention volumes, V_R, and standard deviations, σ, for each isomer, calculated from the data in Table II, on the preparative column are V_{R_1} = 5676 ml, σ_1 = 142 ml and V_{R_2} = 8944 ml, σ_2 = 270 ml. For the three injections of Fig. 2, the injected volume, V_0, remains lower than σ_1. The injected concentration profile is "seen" by the column as a sharp impulse. There is no band broadening

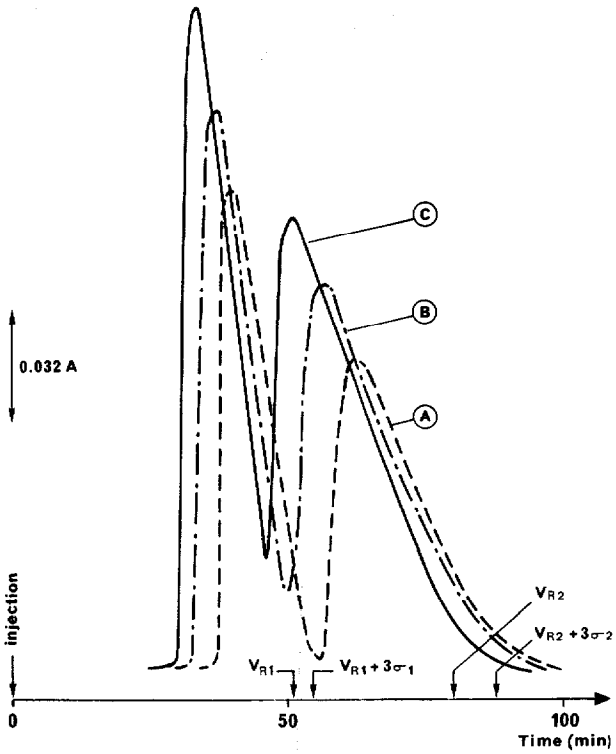


Fig. 2. Preparative chromatograms of the racemate III. Operating conditions as in Table II except injected amount: (A) 500 mg (10 ml of a 50 g l^{-1} solution in chloroform; (B) 750 mg (100 ml of a 7.5 g l^{-1} solution in the mobile phase) (C) 1 g (100 ml of a 10 g l^{-1} solution in the mobile phase). UV detection at 325 nm.

TABLE III

QUANTITATIVE ANALYSIS OF FRACTIONS RECOVERED DURING THE SEPARATION OF 1 g OF RACEMATE III (100 ml OF A 10 g l^{-1} SOLUTION IN THE MOBILE PHASE)

Preparative chromatogram: see Fig. 3. Operating conditions for analytical chromatography: see Table II, except injected volume 20 µl. Tr = cumulative recovery ratio; Ti = cumulative impurity ratio.

Fraction No.	*Volume (ml)*	*Total concentration · 10^3 (M)*	*Isomer 1 (%)*	*Isomer 2 (%)*	*Tr(1) (%)*	*Ti(1) (%)*	*Tr(2) (%)*	*Ti(2) (%)*
1	900	1.441	>99.9	<0.1	72.9	<0.1		
2	340	0.671	99.5	0.5	85.7	<0.1		
3	230	0.433	99.0	1.0	91.2	<0.2		
4	200	0.262	98.7	1.3	94.1	<0.2		
5	220	0.180	91.3	8.7	96.1	<0.4		
6	230	0.513	19.3	80.7	97.4	5.9	99.7	3.9
7	1120	0.724	5.1	94.9	99.7	49.2	94.4	2.7
8	1120	0.386	0.8	99.2			51.1	0.5
9	2350	0.205	0.3	99.7			27	0.3

arising from the injected volume, or no volume overload as it is sometimes referred to[9–11].

We also note from Fig. 2 that the elution profiles exhibit similar peak tails, resembling an envelope curve. These tails join the baseline at an elution volume of approximately $V_R + 3\sigma$. All these properties were described previously[11–12] and are in accord with a strongly non-linear behaviour of the preparative column. This behaviour, arising from a strongly non-linear solute distribution isotherm, is surprising in so far as the injected amount (500 mg to 1 g) lies in the range of 1–2 mg per g of stationary phase, within which the column behaviour is usually only slightly non-linear. It could be related to the fact that the silica gel support has undergone two consecutive bondings, with aminopropyl and (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine moieties, which result in a low number of chiral sites and thereafter in a low stationary phase capacity. This explanation is supported by the elemental analysis data given in the Experimental.

Because of this strong non-linearity, the maximum injected amount leading to a total recovery of pure enantiomers is lower than expected according to a non-linear model for injection optimization[11]. It seems that the elution profiles begin to overlap on the chromatogram when the injected amount is 1 g (Fig. 2C). However it is often difficult to assess the actual purity of a compound from the on-line detection signal only. Fig. 3 shows the fractions collected manually during the separation and quantitatively analyzed on the analytical column. The results are given in Table III. The volumes, V_i, total racemate concentrations, C_i, and percentage enantiomeric compositions were determined for each fraction *i*. From these data one can calculate the cumulative recovery and impurity ratios[13] as follows: the recovery ratio of the first enantiomer eluted, obtained when the first *j* fractions are pooled, is

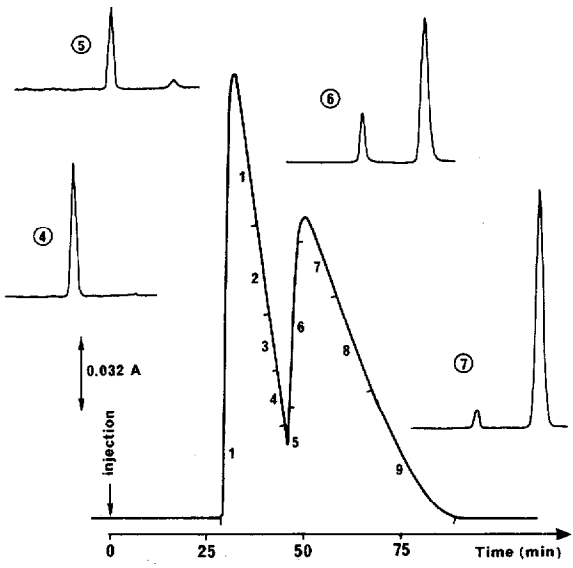


Fig. 3. Fraction collection during the preparative separation of 1 g of racemate III (100 ml of a 10 g l^{-1} solution in the mobile phase). Operating conditions: preparative chromatography, see Fig. 2; analytical chromatography, see Table III.

$$\mathrm{Tr}(1)_j = \frac{\sum_{i=1}^{j} \%(1)_i \cdot C_i V_i}{\frac{1}{2}\sum_{i=1}^{9} C_i V_i}$$

but, according to the mass balance, we have:

$$\frac{1}{2}\sum_{i=1}^{9} C_i V_i = 0.5\ \mathrm{g} = 1.779\ \mathrm{m}M$$

So, $\mathrm{Tr}(1)_j = [\sum_{i=1}^{j} \%(1)_i \cdot C_i V_i]/1.779$. The impurity ratio in these first j fractions is:

$$\mathrm{Ti}(1)_j = \frac{\sum_{i=1}^{j} \%(2)_i \cdot C_i V_i}{\sum_{i=1}^{j} \%(1)_i \cdot C_i V_i} = \frac{\sum_{i=1}^{j} \%(2)_i \cdot C_i V_i}{1.779\ \mathrm{Tr}(1)_j}$$

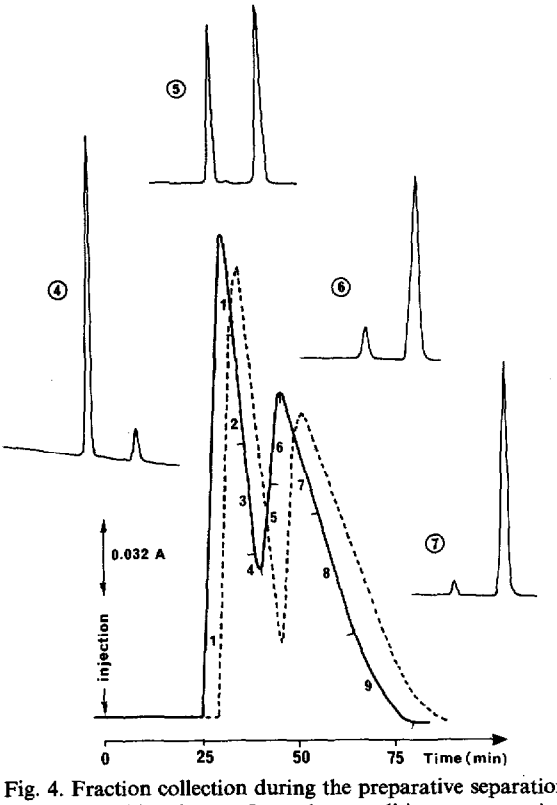


Fig. 4. Fraction collection during the preparative separation of 1 g of racemate III (10 ml of a 100 g l^{-1} solution in chloroform). Operating conditions: preparative chromatography, see Fig. 2 and Table II; analytical chromatography, see Table IV. The preparative chromatogram of Fig. 3 is shown as a dashed line.

The recovery and impurity ratios of the last isomer eluted, obtained when the last j fractions are pooled, are calculated in a similar manner (Table III). By pooling the first five fractions, about 96% of the first enantiomer eluted can be recovered with less than 0.5% impurity. Likewise, about 70–75% of the last enantiomer eluted can be recovered with less than 1% impurity, if fractions 9, 8 and a part of fraction 7 are pooled. Fraction 6 and the other part of fraction 7 should be discarded or recycled.

Fig. 4 allows one to compare two preparative chromatograms obtained by injection of the same amount, 1 g, but different concentration–volume (C_0, V_0) pairs. The dashed line corresponds to the chromatogram discussed above (V_0 = 100 ml, C_0 = 10 g l^{-1}; see Fig. 2). The full line was obtained from a ten-fold more concentrated racemic solution in chloroform. The effluent was fractionated as in the previous case and quantitative results from the analytical chromatography are given in Table IV. A decrease in the overall retention of both peaks and an apparently poorer resolution are observed. This is in agreement with the analytical chromatographic pattern for fractions 4–7 in Figs. 3 and 4, and indicates that the behaviour of the preparative column is, as expected, more non-linear with a more concentrated solution. However, if we calculate the cumulative recovery and impurity ratios (Table IV), we can see that about 92–93% of the first enantiomer eluted can be recovered with approximately 0.5% impurity, while 65–60% of the second enantiomer eluted is recovered with about 1% impurity. These figures are only slightly lower than those derived from Table III.

The optical purity of each fraction was also measured in order to confirm the purity attainable by preparative chromatography. The results are in good agreement with the values calculated from the difference between the percentages of each isomer (Table IV).

After each preparative chromatography the various fractions corresponding to an enantiomeric purity higher than 99% were pooled, evaporated and the final product was crystallized from benzene–diisopropyl ether (1:1). The following enantiomers were obtained. (*S*)-(+)-Methyl(1-naphthyl)phenylphosphine oxide, I-*S*(+), m.p. = 140–144°C, $[\alpha]_D^{22}$ = + 21°(C = 2, methanol); lit.[14], m.p. = 142°C, $[\alpha]_D^{22}$ = + 18.6° (C = 2, methanol). Enantiomer I-*R*(−) has m.p. = 143–145°C, $[\alpha]_D^{22}$ = −17.3° (C = 2, methanol). For the enantiomers corresponding to the racemic compounds II and III the absolute configurations are unknown. (+)-Methyl(2-methoxy-1-naphthyl)phenylphosphine oxide, II-(+), m.p. = 131–132°C, $[\alpha]_D^{22}$ = +124° (C = 2, methanol). II-(−), m.p. = 130–132°C, $[\alpha]_D^{22}$ = −120.5° (C = 2, methanol). (+)-Methyl(4-methoxy-1-naphthyl)phenylphosphine oxide III-(+), m.p. = 145–150°C, $[\alpha]_D^{22}$ = + 29° (C = 2, methanol); III-(−), m.p. = 149–151°C, $[\alpha]_D^{22}$ = − 25.3° (C = 2, methanol).

It must also be borne in mind that chloroform, in spite of its high solubilizing power with regard to the racemic mixture (300 g l^{-1} at room temperature), cannot be advocated as a sample solvent owing to its too high eluent strength: with chloroform as mobile phase, the capacity factors of both enantiomers are 3.2 and 4.1 and the selectivity falls to 1.29. The ternary mobile phase is preferred (solubility of racemic mixture 35 g l^{-1} at room temperature). However, small volumes of chloroform, as here, can be injected without any detrimental effect on the separation.

Finally, it can be concluded that the recovery and impurity ratios are very satisfactory for injections of about 1 g of racemic mixture. it seems possible to in-

TABLE IV

QUANTITATIVE ANALYSIS OF FRACTIONS RECOVERED DURING THE SEPARATION OF 1g OF RACEMATE III (10 ml OF A 100 g l^{-1} SOLUTION IN CHLOROFORM)

Preparative chromatogram: see Fig. 4. Analytical chromatography as in Table III.

Fraction No.	*Volume (ml)*	*Total concentration · 10^3 (M)*	*Isomer 1 (%)*	*Isomer 2 (%)*	*Tr(1) (%)*	*Ti(1) (%)*	*Tr(2) (%)*	*Ti(2) (%)*	*Optical purity (%)*	
									Calculated	*Measured*
1	780									
2	340	1.245	>99.9	<0.1	78.4	<0.1				
3	340	0.566	>99.9	<0.1	89.2	<0.1			100	100
4	225	0.427	89.3	10.7	94.0	0.7			78.6	73
5	225	0.480	38.7	61.3	96.4	4.5	99.4	6.0	22.6	23
6	240	0.941	11.8	88.2	97.9	12.1	95.7	3.8	76.4	74
7	1100	0.840	4.0	96.0	99.9	50.7	84.5	2.5	92	92
8	1100	0.327	0.1	99.9			34.6	<0.1	100	100
9	1400	0.183	<0.1	>99.9			14.4	<0.1		

crease the purified amount recovered per injection and thereafter the time yield by increasing the injected amount to 2 g, but at the slight expense of the recovery ratio. We also have shown that it is better to inject a rather diluted racemic solution in the mobile phase, in order to reduce the effects of the non-linear isotherm and to improve the preparative resolution. Based on the analytical data taken from Table II, the linear theory for injection volume optimization[11,15] predicts injections of up to 2000 ml. Unfortunately, the injection volume is presently limited to about 100 ml for technological reasons. The maximum pressure the apparatus can withstand imposes a severe compromise on the choices of column length, stationary phase particle size and mobile phase flow-rate.

ACKNOWLEDGEMENT

We are grateful to Arlette Begos (Centre d'Études du Bouchet) for technical assistance in the experimental part of this work.

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