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PREPARATION AND EVALUATION OF NEW CHIRAL STATIONARY PHASES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS

COMPARISON OF ENANTIOMERIC AND DIASTEREOMERIC PHASES BASED ON (α)-1-NAPHTHYLETHYLAMINE FOR THE RESOLUTION OF DERIVATIVES OF AMINO ACID ENANTIOMERS

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SUMMARY

Three new chiral stationary phases [two diastereomeric phases based on *R*-phenylglycyl-*R*/*S*-(α)-1-naphthylethylamide and one enantiomeric phase based on glycyl-*S*-(α)-1-naphthylethylamide] have been prepared and evaluated.

There were marginal differences between the phases for the separation of enantiomers of 3,5-dinitrobenzoyl amino acid esters when used in conjunction with isopropanol–hexane mobile phases. However, there were marked differences when dichloromethane–hexane mobile phases were used. With the latter mobile phase the *R*/*R* chiral support did not separate any of the derivatives into their enantiomers and the *R*/*S* chiral support gave larger separation factors than the enantiomeric (*S*) phase. The *R*/*S* chiral support gave comparable separation factors with either dichloromethane or isopropanol containing mobile phases.

The *R*/*R* chiral phase gave the largest separation factor for the enantiomers of the N-α-3,5-dinitro benzoyl derivative of ethyl-α-amino-2-imidazolepropionate (isohistidine ethyl ester).

INTRODUCTION

The use of high-performance liquid chromatography (HPLC) for the separation of enantiomers has increased rapidly in recent years[1] with a number of chiral stationary phases becoming commercially available. [Pirkle type columns are available from J. T. Baker and Phase Separations, β-cyclodextrin-bonded columns from Advanced Separation Technologies and bovine serum albumin bonded columns (Resolvosil) from Macherey-Nagel.] As a result the range of racemic mixtures of chiral compounds including many drugs capable of being separated into their enantiomers has also increased[2].

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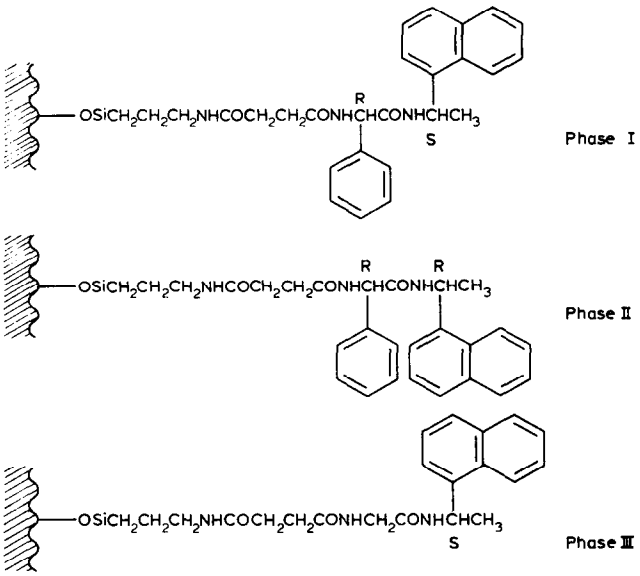
However, these commercial phases have some disadvantages, notably less efficient columns and small separation factors resulting in reduced resolution which makes the determination of small quantities ($<0.5\%$) of one enantiomer in the other difficult. Our requirements for a chromatographic method with a chiral phase for the separation of enantiomers and determination of optical purity are as follows: (1) selectivity over a wide range of compounds; (2) resolution sufficient to determine levels of $\leqslant 0.1\%$ of one enantiomer in the other; (3) good compound sensitivity so that the methods may be used for trace analysis work, *i.e.* drug metabolism studies; (4) readily prepared from easily available starting materials; (5) can be used on standard HPLC equipment.

It is clear from previous investigations[3,4] that chiral amide bonded phases have a wide range of applications. In particular those based on *R*- or *S*-(α)-1-naphthylethylamine[5,6] showed a suitable range of separation factors when associated with compounds derivatised with 3,5-dinitrobenzoyl chloride. The use of the 3,5-dinitrobenzoyl group is particularly attractive as it has a large molar extinction coefficient in the UV spectrum thus enabling small amounts of compound to be detected.

It is less clear from the literature[7,8] how chiral phases with a single asymmetric centre compare with those with more than one asymmetric centre and whether the separation can be improved by adding additional asymmetric centres to the chiral phase.

For gas chromatography the most widely used commercially available stationary phase (Chirasil-Val) is diastereomeric as is another phase XE-60-S-Valine-*S*-(α)-Phenylethylamide developed by König *et al.*[9].

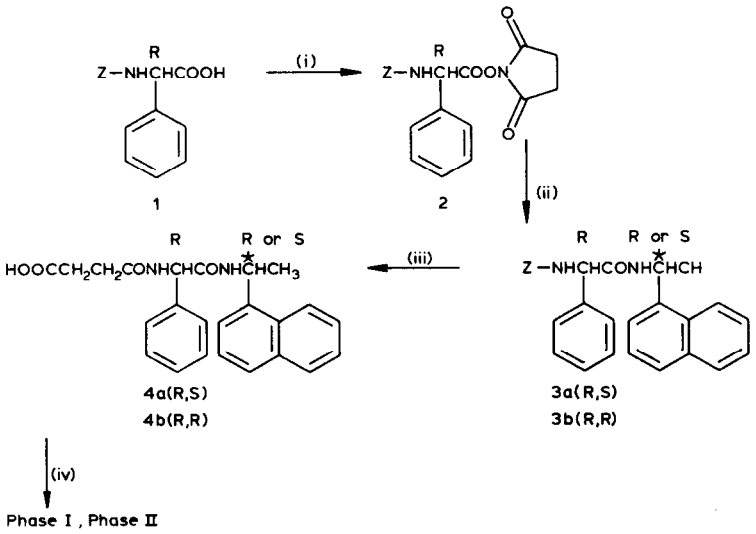
This paper describes the preparation and evaluation of three new chiral phases based on (α)-1-naphthylethylamine as shown in Scheme 1.



Scheme 1. Three new chiral phases based on (α)-1-naphthylethylamine.

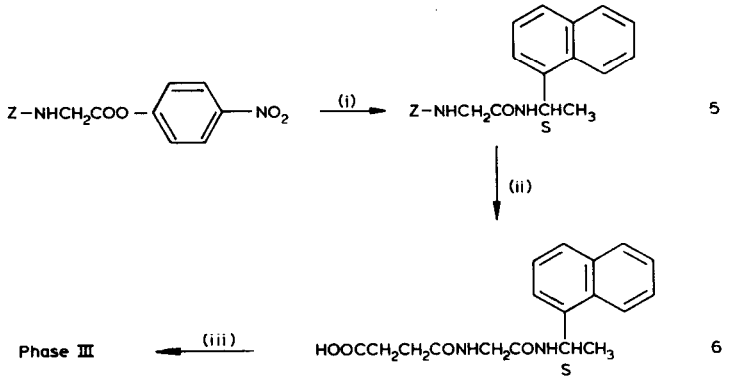
Phase III, a single enantiomer phase, provided a reference from which the performance of the diastereomeric phases I and II could be compared.

EXPERIMENTAL



Scheme 2. Synthetic scheme for the preparation of chiral phases I and II. z = Benzyloxycarbonyl; (i) N-hydroxysuccimide–dicyclohexylcarbodiimide; (ii) *R*- or *S*-(α)-1-naphthylethylamine; (iii)Hydrogen bromide in acetic acid followed by succinic anhydride in pyridine; (iv) 2-ethoxy-1-ethoxy carbonyl-1,2-dihydroquinoline (EEDQ)–Apex 5 amino silica.

The synthetic scheme for the preparation of chiral phases I and II is shown in Scheme 2. The synthetic scheme for the preparation of chiral phase III is shown in Scheme 3.



Scheme 3. Synthetic scheme for the preparation of chiral phase I. Z = Benzyloxycarbonyl; (i) *S*-(α)-1-naphthylethylamine–triethylamine; (ii) hydrogen bromide in acetic acid followed by succinic anhydride in pyridine; (iii) EEDQ–Apex 5 amino silica.

Synthetic methods

R-Phenyl glycine, *R*- and *S*-(α)-naphthylethylamine were all supplied by Aldrich. Benzyloxycarbonylglycine-*p*-nitro phenyl ester was supplied by Sigma. Apex 5 amino was supplied by Jones Chromatography.

R-Benzyloxycarbonyl-phenylglycine (1). *R*-Phenylglycine (15.1 g, 0.1 mol) was dissolved in 2 *M* sodium hydroxide (50 ml) and cooled in an ice water bath. Benzylchloroformate (16 ml, 0.11 mol) and 2 *M* sodium hydroxide (50 ml) were added in five portions over 30 min with stirring. After addition a further 20 ml 2 *M* sodium hydroxide was added. The mixture was stirred and allowed to rise to ambient temperature over 45 min. The mixture was extracted with diethyl ether (2 × 100 ml) and acidified with 6 *M* hydrochloric acid (*ca.* 20 ml). The precipitate was collected, washed with water and dissolved in ethylacetate (500 ml). The organic solution was washed with water (2 × 100 ml) and the aqueous layer back-extracted with ethyl acetate (100 ml). The combined organic layer was dried ($MgSO_4$). The solution was filtered and evaporated. The solid residue was dissolved in ethanol–water (1:1, 140 ml) with heating; the solution was filtered and allowed to cool. The oil which separated slowly crystallised. A further 70 ml of water was added and the mixture cooled at 0°C. The crystals were collected, washed with water and dried. Yield 22 g, m.p. 128–131°C, $[\alpha]_D^{21}$ − 116.5° (*c* = 4% in ethanol) (lit.[10] 130–130.5°C, $[\alpha]_d^{21}$ − 119°).

R-Benzyloxycarbonyl-phenylglycine-N-hydroxysuccinimide ester (2). *R*-Benzyloxycarbonylphenylglycine (13.42 g, 0.047 mol) and N-hydroxy succinimide (5.42 g, 0.047 mol) were dissolved in dimethoxyethane (80 ml) and stirred at 0°C. Dicyclohexylcarbodiimide (10.70 g, 0.052 mol) was added and the mixture stirred for 1 h at 0°C and kept at 0°C for 72 h. The precipitate was collected, washed with dichloromethane (2 × 100 ml). The combined filtrate was diluted with diethyl ether (300 ml) and the precipitate collected. The filtrate was evaporated *in vacuo* and the residue triturated with diethyl ether. The solid was collected. The combined solids (16.5 g) were recrystallised from dichloromethane (130 ml) diluted with diethylether (300 ml). The solid was collected, washed with diethylether (100 ml) and dried. Yield 14.9 g, m.p. 158–161°C, $[\alpha]_{365}^{20}$ − 209.6° (*c* = 0.5% in dichloromethane).

R-Benzyloxycarbonylphenylglycyl-S-(α)-naphthylethylamide (3a). *R*-Benzyloxycarbonyl-phenylglycine-N-hydroxysuccimide ester (2.25 g, 6 mmol) was dissolved in dichloromethane (50 ml) and cooled in an ice bath. *S*-(α)-Naphthylethylamine (1 g, 6 mmol) in dichloromethane (20 ml) was added over 5 min with stirring. The solution was stirred at 0°C for 1 h and left to stand at ambient temperature for 72 h. Some solid separated. The mixture was diluted with dichloromethane (100 ml), and washed with 1% orthophosphoric acid, 0.2 *M* potassium hydroxide (100 ml), deionised water (100 ml) and dried ($MgSO_4$). The solution was filtered, evaporated and the residual solid suspended in cyclohexane, filtered and dried. The filtrate was evaporated, the residual solid suspended in cyclohexane, and collected. Combined solids weight 2.25 g. Recrystallisation from toluene (50 ml) gave 2.05 g, m.p. 193.5°C. (Found: C, 76.87; H, 5.94; N, 6.25; $C_{28}H_{26}O_3N_2$ requires C, 76.69; H, 5.98; N, 6.39) ^1H NMR (300 MHz) δ (C^2HCl_3) 1.50 (doublet, $-CH_3$), 5.0, (multiplet, $-CH_2O-$), 5.1, (broad doublet, –NH–), 5.9, (multiplet, 2 × –CH–), 6.12 (broad doublet, –NH–), 7.2–8.05, (multiplet, aromatic –CH–), $[\alpha]_{365}^{20}$ − 169.6° (*c* = 1% in dichloromethane).

R-Benzyloxycarbonylphenylglycyl-R-(α)-naphthylethylamide (3b). *R*-Benzyl-

oxycarbonyl-phenylglycine-N-hydroxysuccimide ester (11.25 g, 0.03 mol) was suspended in dichloromethane (150 ml) and 0°C and *R*-(α)-naphthylethylamine (5 g, 0.03 mol) in dichloromethane (40 ml) added over 10 min. The mixture stood at 0°C for 1 h and then 24 h at ambient temperature. The mixture was cooled in an icebath. The solid was collected, washed with dichloromethane (100 ml) and dried. Yield 9.7 g, m.p. 225–226°C. (Found: C, 76.51; H, 5.97; N, 6.39; $C_{28}H_{26}O_3N_2$ requires C, 76.69; H, 5.98; N, 6.39) ^{1}H NMR (300 MHz) δ (C^2HCl_3) 1.66, (doublet, $-CH_3$), 5.0, (multiplet, $-CH_2O-$), 5.26, (broad doublet, –NH–), 5.8, (multiplet, –CH–), 6.1 (broad doublet, –CH–), 6.2, (broad doublet, –NH–), 7.1–7.8 (multiplet, aromatic –CH–), 244, $[\alpha]_{365}^{20}$ − 370.4° (*c* = 1% in trichloromethane).

Benzyloxycarbonyl-glycyl-S-(α)-naphthylethylamide (5). Benzyloxycarbonyl-glycine-*p*-nitrophenylester (10 g, 0.03 mol), *S*-(α)-naphthylethylamine (5 g, 0.03 mol) and triethylamine (2 ml) were dissolved in dichloromethane (100 ml) and left to stand at ambient temperature for 24 h. The solution was washed with water (3 × 100 ml), 0.3 *M* sodium hydroxide (3 × 75 ml) and water (3 × 100 ml) and evaporated *in vacuo*. The solid residue was suspended in diethyl ether (100 ml), filtered, washed with diethyl ether and dried. Yield 7.5 g. The filtrate was evaporated; the residue suspended in diethyl ether, filtered and dried. Yield 1 g (second crop). The aqueous washings were filtered, washed with water, diethylether to give a further 1 g. This was combined with second crop material and recrystallised from toluene to give 1.5 g; combined with first crop material and recrystallised from toluene (75 ml) to give 8.5 g. m.p. 133°C (Found: C, 72.94; H, 6.15; N, 7.77; $C_{22}H_{22}O_3N_2$ requires C, 72.91; H, 6.12; N, 7.73). ^{1}H NMR (300 MHz) δ (C^2HCl_3), 1.64, (doublet, $-CH_3$), 3.82, (doublet, $-CH_2CO$), 5.04, (multiplet, $-CH_2O-$), 5.46, (broad singlet, –NH–), 5.88, (multiplet, –CH–), 6.44, (broad doublet, –NH–), 7.2–8.1 (multiplet, aromatic –CH), $[\alpha]_{365}^{20}$ − 105.8° (*c* = 1% in trichloromethane).

Succinyl-R-phenylglycyl-S-(α)-naphthylethylamide (4a). *R*-Benzyloxycarbonyl-phenylglycyl-*S*-(α)-naphthylethylamide (7.4 g, 0.017 mol) was suspended in glacial acetic acid (75 ml) and hydrogen bromide–acetic acid (45:55) (25 ml) added. The solution which formed was kept at ambient temperature for 5 h. The solution was concentrated *in vacuo* and the residue diluted with diethylether (200 ml). The solid was collected, washed with diethyl ether and dried. The solid was suspended in pyridine (20 ml) and succinic anhydride added. The mixture was left to stand at ambient temperature for 24 h. The mixture was evaporated and the residue treated with 5% orthophosphoric acid (200 ml). The solid was collected, washed with water and dried. Recrystallisation from ethanol–water (80:20) (300 ml) gave 4.5 g. m.p. 215–216°C (dec.). (Found: C, 71.18; H, 5.96; N, 6.90; $C_{24}H_{24}O_4N_2$ requires C, 71.27; H, 5.98; N, 6.93). ^{1}H NMR (300 MHz) δ [d_6DMSO (dimethyl sulphoxide] 1.44, (doublet, $-CH_3$), (multiplet, $-CH_2CH_2$), 5.56, (multiplet, –CH–), 5.68 (multiplet, –CH–), 7.2–8.9, (multiplet, aromatic –CH), $[\alpha]_{365}^{20}$ − 138.0° (*c* = 1% in DMSO).

Succinyl-R-phenylglycyl-R-(α)-naphthylethylamide (4b). *R*-Benzyloxycarbonyl-phenylglycyl-*R*-(α)-naphthylethylamide (9.6 g, 0.022 mol) was suspended in glacial acetic acid (50 ml) and hydrogen bromide–acetic acid (45:55) (35 ml) added. The solution which formed was kept at ambient temperature for 5 h. The solution was concentrated *in vacuo* and the residue diluted with diethyl ether (250 ml). The solid was collected, suspended in pyridine (25 ml) and succinic anhydride (1.92 g) added. The mixture stood at ambient temperature for 36 h. The mixture was evaporated and

the residue treated with 10% orthophosphoric acid (200 ml). The solid was collected, washed with water and recrystallised from ethanol–water (80:20) (250 ml) to give a gelatinous solid. Yield 5.5 g, m.p. 225.7°C (dec.). (Found: C, 71.06; H, 5.85; N, 6.99; $C_{24}H_{24}O_4N_2$ requires C, 71.27; H, 5.98; N, 6.93). ^{1}H NMR (300 MHz) δ (d_6DMSO) 1.52, (doublet, $-CH_3$), 2.44, (multiplet, $-CH_2CH_2-$), 5.58, (multiplet, –CH–), 5.66, (multiplet, –CH–), 7.2–9.0, (multiplet, aromatic –CH), $[\alpha]_{365}^{20}$ – 293.8° (c = 1% in DMSO).

Succinyl-glycyl-S-(α)-naphthylethylamide (6). Benzyloxycarbonyl-glycyl-*S*-(α)-naphthylethylamide (8.2 g, 0.023 mol) was suspended in glacial acetic acid (60 ml) and hydrogen bromide–acetic acid (45:55) added. The solution was kept at ambient temperature for 5 h. The solution was concentrated *in vacuo* and the residue suspended in diethyl ether (200 ml). The precipitate was collected and dissolved in pyridine (25 ml). Succinic anhydride (1.75 g) was added and the mixture kept at ambient temperature for 36 h. The mixture was evaporated, water added to the residue and re-evaporated. The residue slowly solidified. The residue was collected, washed with 10% orthophosphoric acid and water. The solid was recrystallised from ethanol–water (50:50) to give 4.2 g. m.p. 188–190°C. (Found: C, 65.69; H, 6.13; N, 8.60; $C_{18}H_{20}O_4N_2$ requires C, 65.84; H, 6.14; N, 8.53). ^{1}H NMR (300 MHz) δ (d_6DMSO), 1.50, (doublet, $-CH_3$), 2.42 (multiplet, $-CH_2CH_2-$), 3.76, (multiplet, $-CH_2CO-$), 5.76, (multiplet, –CH–), 7.4–8.4, (multiplet, aromatic –CH), $[\alpha]_{365}^{20}$ – 60.1° (c = 1% in DMSO).

Chiral phases I, II, III (4)

Apex 5 Amino (5 g), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (2 g) and chiral agent 4a, 4b or 6 (2.5 g) were suspended in tetrahydrofuran (THF) (100 ml) and sonicated for 5 min. The mixture was left to stand at ambient temperature with occasional swirling for 24 h. The solid was collected, washed with THF (100 ml), methanol (2 × 100 ml), and diethyl ether (50 ml) and dried. Yield *ca.* 5.3 g. Phase I; found: C, 9.28; H, 1.23; N, 1.45. Phase II; found: C, 8.22; H, 1.02; N, 1.38. Phase III; found: C, 7.36; H, 0.88; N, 1.27.

Chromatographic methods

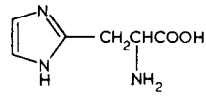
The three phases were packed into 15 × 0.4 cm stainless-steel columns on a Shandon column packer set in the upward mode. Chromatography solvents were Rathburn HPLC grade. 3,5-Dinitrobenzoyl chloride was supplied by Fluka and L and DL amino acid methyl ester hydrochlorides were supplied by Sigma.

The liquid chromatograph consisted of a Constammetric II pump, Rheodyne valve (7120), ACS UV detector fitted with 254-nm filter, chart recorder and Spectra-Physics Autolab System I integrator or LDC Cl-10 integrator.

Derivatisation conditions: A mixture of L and DL amino acid ester hydrochloride (*ca.* 3 mg) and 3,5-dinitrobenzoyl chloride (*ca.* 3 mg) were suspended in dichloromethane (0.5 ml) and triethylamine (1 drop from Pasteur pipette) added. The solution stood at ambient temperature. Water (0.5 ml) was added and the mixture thoroughly shaken. The organic layer was separated and an aliquot (5 μl) injected onto the column.

The following derivatives were prepared (DNB = dinitrobenzoyl): 3,5-DNB-Val-OCH_3 (Val); 3,5-DNB-Asp-OCH_3 (Asp); 3,5-DNB-Ala-OCH_3 (Ala); 3,5-

DNB-Phe-OCH_3 (Phe); 3,5-DNB-Met-OCH_3 (Met); 3,5-DNB-Ser-OCH_3 (Ser), mono-derivatives as determined by mass spectrometry (MS); 3,5-DNB-Threo-OCH_3 (Threo) analysis of HPLC fractions (off-line LC–MS); 3,5-DNB-Tyr-OCH_3 (Tyr); 3,5-DNB-Trp-OCH_3 (Trp); 3,5-DNB-Isohis-OC_2H_5 (Isohis, see structure below), mono-derivative determined by NMR spectroscopy; 3,5-DNB-Pro-OCH_3 (Pro), proline converted to methyl ester with methanol–thionyl chloride prior to derivatisation.

Isohis = Isohistidine 

A sample of D-isohistidine ethyl ester monohydrochloride was also derivatised for optical purity determination.

The void volumes of the columns were determined by injecting dichloromethane (5 μl) and measuring the first recorder pen deflection.

RESULTS AND DISCUSSION

Overall the results show that there are significant differences in selectivity between the various mobile phases and hence comparisons between the chiral phases must take this into account (Tables I–III).

The consistency for the separation factors for phase III and the chiral phase described by Ôi[5] (Table I) show that bearing in mind that only three compounds were examined some viable inferences can be drawn. These two phases differ in that in phase III a glycyl group has been inserted between the succinyl group and the chiral centre. The base silica is Apex Amino (5 μm, spherical) in one instance and Lichrosorb NH_2 (10 μm, irregular) in the other. The separation factors observed would appear to be entirely due to the chemical structure of the phase and not influenced by the base silica and bonding chemistry. Chiral phase II gives separation factors less than and chiral phase I gives separation factors greater than (except 3,5-DNB-Phe-OCH_3) phase III and the phase described by Ôi[5]. Recently Ôi has reported[8] improved separation factors in some instances for diastereomeric phases

TABLE I

COMPARISON WITH THE CHIRAL PHASE, *S*-(α)-NAPHTHYLETHYLAMINO-SUCCINYL-AMINO-PROPYLSILYL BONDED SILICA, PREPARED BY N. ÔI *et al.*[5]

α = Separation factor, k' = capacity factor.

	Phase I			*Phase II*			*Phase III*			*Phase II (Ôi)**		
	k'**	α	*R/S***	k'	α	*R/S*	k'	α	R/S	k'	α	*R/S*
Val	2.92	1.81	*R*	2.43	1.32	*R*	2.55	1.56	*R*	1.60	1.56	*R*
Ala	5.86	1.48	*R*	5.56	1.26	*R*	5.62	1.39	*R*	4.40	1.40	*R*
Phe	6.25	1.24	*R*	4.67	1.28	*R*	5.0	1.30	*R*	3.26	1.24	*R*

* Values as reported in the literature.

** Mobile phase hexane–dichloromethane–ethanol (48:15:1) at 2 ml/min.

*** Configuration of first eluted enantiomer, k' of first eluted enantiomer.

TABLE II

COMPARISON OF CHIRAL PHASES, I, II AND III USING DICHLOROMETHANE–HEXANE MOBILE PHASES

	Phase I				*Phase II**				*Phase III*			
	k'	α	*MP***	*R/S****	*k'*	α	*MP*	*R/S*	*k'*	α	*MP*	*R/S*
Val	0.67	1.85	A	*R*	0.71	1.0	A	–	0.59	1.35	A	*R*
Asp	1.04	1.27	A	*R*	1.12	1.0	A	–	1.15	1.14	A	*R*
Ala	1.57	1.32	A	*R*	1.69	1.0	A	–	1.57	1.20	A	*R*
Phe	1.11	1.38	A	*R*	1.05	1.0	A	–	0.95	1.20	A	*R*
Met	1.22	1.60	A	*R*	1.39	1.0	A	–	1.23	1.43	A	*R*
Pro	1.60	1.0	B	–	18.34	1.0	C	–				

* Phase II did not give any separation with dichloromethane–hexane mobile phases.

** Mobile phase (MP) A = dichloromethane–hexane (8:20) at 1 ml/min, B = dichloromethane–hexane (40:60) at 1 ml/min, C = dichloromethane–hexane (20:80) at 2 ml/min. (See ref. 11 for a discussion on mobile phase selectivity.)

*** Configuration of first eluted enantiomer, *k'* of first eluted enantiomer.

from *S*-valine-*S*-(α)-naphthylethylamide and *S*-valine-*R*-(α)-naphthylethylamide and suggested that the second chiral centre (*S*-valine) improved the enantioselectivity.

Differences in phases I, II and III are most pronounced when using dichloromethane–hexane mobile phases (Table II). Only the non-polar or totally

TABLE III

COMPARISON OF CHIRAL PHASES I, II AND III USING ISOPROPANOL–HEXANE MOBILE PHASES

	Phase I				*Phase II*				*Phase III*			
	*k'**	α	*MP***	*R/S****	*k'*	α	*MP*	*R/S*	*k'*	α	*MP*	*R/S*
Val§	5.49	2.03	A	*R*	4.26	1.79	A	*S*	4.05	1.89	A	*R*
Asp§§	14.77	1.20	A	–	13.61	1.19	A	–	13.09	1.22	A	–
Ala	8.35	1.54	A	*R*	7.23	1.54	A	*S*	6.73	1.52	A	*R*
Phe	13.4	1.26	A	*R*	9.5	1.54	A	*S*	9.24	1.40	A	*R*
Met	4.55	1.58	B	*R*	3.75	1.59	B	*S*	3.55	1.70	B	*R*
Ser	11.41	1.31	B	*R*	11.46	1.28	B	*S*	9.89	1.24	B	*R*
Threo	6.07	1.40	B	*R*	6.15	1.24	B	*S*	5.58	1.33	B	*R*
Tyr	12.11	1.47	C	*R*	11.77	1.25	C	*S*	9.39	1.39	C	*R*
Trp	10.46	1.19	C	*R*	8.6	1.17	C	*S*	7.26	1.33	C	*R*
Isohis	9.21	1.33	A	*R*	9.72	1.42	A	*S*	8.31	1.32	A	*R*
Pro§§§	4.01	1.00	A	–	4.08	1.00	A	–	3.60	1.00	A	–

* *k'* for first eluted enantiomer.

** Mobile phase A = isopropanol–hexane (10:90), B = isopropanol–hexane (20:80), C = isopropanol–hexane (30:70), all at 2 ml/min.

*** Configuration of first eluted enantiomer as determined by using known mixtures of L and DL amino acid.

§ 3,5-DNB derivatives of methyl–ethyl esters.

§§ Racemate only run, configuration of first eluted enantiomer not determined.

§§§ Enantiomers of proline not resolved.

protected amino acids eluted with this system. Phase II did not separate any of the amino acids and phase I showed better enantioselectivity than phase III indicating that the second chiral centre (*R*-phenylglycine) has a significant effect in phase I with dichloromethane–hexane mobile phase.

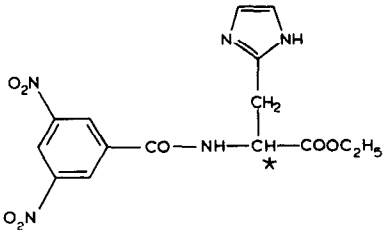
In contrast only slight differences were observed between the phases when isopropanol–hexane mobile phases were used. These mobile phases eluted both the polar and non-polar amino acid derivatives and no single phase exhibited greater enantioselectivity. For the non-polar amino acid derivatives, (Val, Asp, Ala, Phe, Met, Pro) phase I showed increased enantioselectivity for 3,5-DNB-Val-OCH_3 and the least enantioselectivity for 3,5-DNB-Phe-OCH_3 and *vice versa* for phase II. Phase III gave increased enantioselectivity for 3,5-DNB-Met-OCH_3 compared with phases I and II (Table III).

For the polar amino acid derivatives (Ser, Threo, Tyr, Trp, Isohis) phase I showed greater enantioselectivity than the other two phases except for 3,5-DNB-Isohis-OC_2H_5 where phase II showed increased enantioselectivity and for 3,5-DNB-Trp-OCH_3 where phase III showed increased enantioselectivity.

Pirkle and Hyun[12] have recently prepared two new chiral phases based on *R*-(α)-naphthylethylamine and *R*-(α)-(6,7-dimethyl-1-naphthyl)isobutylamine with a C_{10} hydrocarbon chain linking the chiral amide to the silica support. These phases demonstrate larger separation factors for 3,5-DNB-Ala-OCH_3, 3,5-DNB-Val-OCH_3 and 3,5-DNB-Phe-OCH_3 with isopropanol–hexane mobile phases compared with the phases described here. However, the order of elution for the enantiomers was reversed *i.e.* the *R*-enantiomer eluted first instead of the *S*-enantiomer. It was suggested that the mode of attachment may affect the chiral recognition process. From the results reported in this paper, phases I–III are comparable to that described by Ôi[5].

All attempts to separate the enantiomers of 3,5-DNB-Pro-OCH_3 were unsuccessful. Similarly a method described by Hara and Dobashi[13], which used a chiral agent (N-acetyl-L-valine-*tert.*-butylamide) in the mobile phase (chloroform–hexane, 40:60) and a silica gel column, did not separate the enantiomers of 4-nitrobenzoyl-proline isopropyl ester, although twelve other amino acids were separated into their enantiomers. The mechanism proposed was that of a chelate diastereomeric solvate formed with the chiral agent in the mobile phase by intermolecular NH $\cdots$ O=C hydrogen bonds. Since proline is a secondary amino acid such a diastereomeric solvate is less likely to be formed. Should a similar mechanism occur with chiral phases I, II and III then it may account for their inability to separate the enantiomers of 3,5-DNB-Pro-OCH_3. Further investigations with chiral secondary amines would be necessary in order to determine whether such a phenomenon is generally applicable. It should be noted that amide derivatives of β-blockers (secondary amino alcohols) have been successfully separated into their enantiomers on Pirkle columns[14,15].

Chiral phase II (based on *R*-phenylglycyl-*R*-(α)-naphthylethylamide) gave the largest separation factor (1.42) for the enantiomers (see structure below) of 3,5-dinitrobenzoyl-isohistidine ethyl ester, a derivative of Ro 31-2502/002, using isopropanol–hexane (10:90) as mobile phase. By modifying the mobile phase with acetonitrile it was possible to reduce the band tailing and maintain the separation



factor (1.37) (Fig. 1). This allowed the determination of 0.06% of the L-isomer:

In this instance, the amount of L-isomer arising from the derivatisation reaction has not been determined. Moreover, the use of triethylamine as the base may be far from ideal. Nevertheless, the results demonstrate that it is possible to determine small amounts of one enantiomer in the presence of the other.

CONCLUSIONS

Three new chiral stationery phases based on (α)-naphthylethylamine have been prepared and evaluated.

The diastereomeric phase I [based on *R*-phenylglycyl-*S*-(α)-naphthylethylamide] demonstrated the best overall performance in terms of resolution and selectivity when used with differing mobile phases for a number of 3,5-dinitrobenzoyl amino acid methyl esters.

The diastereomeric phase II [based on *R*-phenylglycyl-*R*-(α)-naphthylethylam-

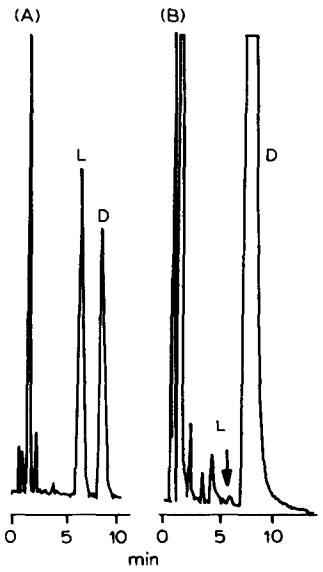


Fig. 1. Resolution of DL-3,5-dinitrobenzoyl-isohistidine ethyl ester. (A) Separation of the racemate, (B) Determination of the optical purity of D-3,5-dinitrobenzoyl-isohistidine ethyl ester; L-isomer content 0.06%. Column: chiral phase II, mobile phase: 10% isopropanol–1% acetonitrile–hexane at 2 ml/min.

ide] gave the largest separation factor for the enantiomers of 3,5-dinitrobenzoyl-isohistidine ethyl ester. This led to the development of a method for the determination of the optical purity of Ro 31-2502/002, D-isohistidine ethyl ester measuring < 0.1% of the L-enantiomer (see Fig. 1).

In general the performance of the better diastereomeric phase I was not a marked improvement on the enantiomeric phase III [based on glycyl-*S*-(α)-naphthylethylamide]. This suggests that the second chiral centre, in this instance, *R*-phenylglycine, plays a lesser role in chiral selectivity which nevertheless for particular compounds can result in better resolution compared to enantiomeric phases.

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