Journal of Chromatography, 395 (1987) 569–590
Elsevier Science Publishers B V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1096

DETERMINATION OF α-ALKYL-α-AMINO ACIDS AND α-AMINO ALCOHOLS BY CHIRAL-PHASE CAPILLARY GAS CHROMATOGRAPHY AND REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

HANS BRÜCKNER* and INGRID BOSCH
Institute of Food Technology, University of Hohenheim, D-7000 Stuttgart 70 (F.R.G.)
and
THEODOR GRASER and PETER FÜRST
Institute of Biological Chemistry and Nutrition, University of Hohenheim, D-7000 Stuttgart 70 (F.R.G.)

SUMMARY

The enantiomeric resolution by fused-silica capillary gas–liquid chromatography (GLC) of non-protein DL-α-alkyl-α-amino acids of the structure $H_2NCR^1R^2COOH$ (R^1 = alkyl, R^2 = alkyl, alkaryl) was investigated by using chiral [L-valine-*tert.*-butylamide, linked to a statistical polymer of dimethylsiloxane and (2-carboxypropyl)methylsiloxane, Chirasil-L-Val, and XE-60–*S*-Val-*S*-α-phenylethylamide] and non-chiral (methylphenylcyanopropylvinylpolysiloxane, CP-Sil-19 CB) stationary phases. To evaluate the resolution coefficients, N-acylamino acid *n*-propyl esters (acyl = acetyl, propionyl, trifluoroacetyl, pentafluoropropionyl, heptafluorobutyryl) and diastereomeric esters with *S*(−)-2-methyl-1-butanol, *S*(+)-2-butanol and *S*(+)-2-octanol were used. Although α-alkyl-α-amino acids in general gave lower resolution coefficients than the enantiomers of protein amino acids, most α-alkyl-α-amino acids could be resolved by using suitable derivatization procedures and, preferably, isothermal conditions. In addition, a number of DL-α-alkyl-α-amino acids could be separated by ligand-exchange chromatography (L-hydroxyproline/Cu^{2+}) by both thin-layer chromatography (Chiralplate) and high-performance liquid chromatography (HPLC) (Nucleosil Chiral-1). Further, a standard mixture composed of fifteen α-amino acids and eleven α-amino alcohols could be completely separated by C_{18} HPLC after derivatization with *o*-phthaldialdehyde–2-mercaptoethanol (OPA–2-ME). The time and temperature dependences of the relative fluorescence of the adducts were investigated kinetically.

INTRODUCTION

As pointed out in a previous paper[1], the formal substitution of the C_α hydrogen atoms of α-amino acids by alkyl or alkaryl groups yields non-protein α-alkyl (alkar-

* Dedicated to Professor E. Bayer on the occasion of his 60th birthday.

0021-9673/87/$03.50

yl)-α-amino acids. These products may also be considered as $C_{\alpha,\alpha}$-disubstituted glycines of the general formula $H_2NCR^1R^2COOH$, where R^1, R^2 = alkyl, alkaryl, both of which may bear additional functional groups[2]. In some instances, these compounds show strongly deviating chemical and biological behaviour when compared with protein amino acids and have, therefore, attracted considerable interest as, for example, potential antagonists of proteinogenic amino acids or, in the case of α-methyltryptophan (α-Me-Trp) for its ability to increase the activity of hepatic tryptophan pyrrolase[3]. α-Methyldihydroxyphenylalanine (α-Me-Dopa) is used therapeutically as an anti-hypertensive agent[4–7].

Further, a particular group of fungal metabolites, the "peptaibols*", is characterized and defined as N-acylated peptides with a high content of the simplest α-alkyl-α-amino acid, *viz.*, α-aminoisobutyric acid (Aib) and the presence of an amino alcohol, linked by a peptide bond to the C-terminal amino acid[8]. Substitution of protein amino acids in suitable positions in synthetic peptide hormones will increase their lifetime in the plasma or may lock biologically active peptides in more rigid secondary structures. This is a most important device for evaluating quantitative structure–activity relationships (QSAR). By analogy with protein amino acids, α-alkyl-α-amino acids having an asymmetric C_α atom show optical activity. The simplest chiral α-alkyl-α-amino acid, therefore, is α-ethylalanine (usually designated as isovaline, Iva). Methods routinely applicable to the enzymatic separation of protein amino acids generally proceed with markly diminished reaction rates in the case of α-alkyl-α-amino acids, or even fail completely[9–12]. Therefore, methods for the enantioselective synthesis of α-alkyl-α-amino acids are of great interest[13,14]. Work with optically active α-alkyl-α-amino acids requires analytical methods for the accurate and definite proof of optical purity. In the past, stereospecific enzymatic digestion or the formation of diastereomeric salts and measurement of the optical rotation and chemical correlation were almost the only methods suitable for the preparative separation and assignment of the optical configuration of α-alkyl-α-amino acids[9,15–17]. However, more recently, X-ray analysis has provided definite proof, for example, in the case of D-Iva[18]. Gas–liquid chromatography (GLC) on chiral and non-chiral stationary phases has been shown to be the most sensitive and rapid technique for the analytical separation of enantiomers[19–22]. Our interest in the sequence analysis[23] and chemical synthesis[24] of the above-mentioned peptaibol mycotoxins prompted us to investigate the analytical behaviour of α-alkyl-α-amino acids in more detail.

We recently demonstrated that all α-alkyl-α-amino acids show drastically decreased reactivity with respect to the α-amino group, as manifested in very low colour yields in the quantitative ninhydrin reaction, a low relative fluorescence of their adducts with *o*-phthaldialdehyde–2-mercaptoethanol (OPA–2-ME) and partially incomplete reactions in the derivatization procedures for GLC. These phenomena could be attributed to steric constraints of geminal C_α-alkyl(alkaryl) groups, but could be partly overcome by applying more drastic reaction conditions[1].

As pointed out above, peptaibols are also characterized by the presence of a C-terminally bonded α-amino alcohol such as phenylalaninol (Pheol, peptaibophol

---

* Peptaibol antibiotics (mycotoxins) are available from Bachem Feinchemicalien AG, Hauptstrasse 144, CH-4416 Bubendorf, Switzerland.

antibiotics[8]), valinol[25] (Valol), leucinol[26] (Leuol) or tryptophanol[27] (Trpol). We have demonstrated that the difficulties encountered in the determination of Valol[25] (which gives about 7.4% of the colour yield of Leu) and Pheol, which is not eluted from the column of an automated ion-exchange amino acid analyser under standard conditions[8], can be overcome by pre-column derivatization and reversed-phase high-performance liquid chromatography (RP-HPLC) of the OPA–2-ME adducts[1], which in general is a powerful method[28–30]. In this paper, we illustrate the general applicability of the method for the determination of α-amino alcohols and fifteen α-amino acids. Further, we compare the separation of a number of α-methyl-α-amino acids into enantiomers by capillary GLC on chiral and non-chiral stationary phases and demonstrate that optically active α-alkyl-α-amino acids can be resolved by thin-layer chromatography (TLC) and HPLC by using recently developed techniques of ligand-exchange chromatography.

EXPERIMENTAL

Sources and abbreviations of α-alkyl-α-amino acids and α-amino alcohols

DL-Isovaline and D-isovaline (α-ethylalanine, α-methyl-α-amino-*n*-butyric acid, Iva) were prepared according to the literature[16]. DL-α-Methylleucine was obtained from Calbiochem-Behring (La Jolla, CA, U.S.A.) and DL-α-methylaspartic acid (α-Me-Asp), DL-α-methyl-3-(3,4-dihydroxyphenyl)alanine (α-Me-Dopa), DL-α-methylglutamic acid · $0.5H_2O$ (α-Me-Glu), DL-α-methylhistidine · 2HCl (α-Me-His), DL-α-methylmethionine (α-Me-Met), DL-α-methylornithine (α-Me-Orn), DL-α-methyl-*m*-methoxyphenylalanine (α-Me-PheOCH_3), DL-α-methylphenylalanine (α-Me-Phe), DL-α-methylserine (α-Me-Ser), DL-α-methyltryptophan (α-Me-Trp), DL-α-methyltyrosine and L-α-methyltyrosine (α-Me-Tyr) from Sigma (St. Louis, MO, U.S.A.). DL-α-Methylvaline (α-Me-Val), DL-α-methylnorvaline (α-Me-Nva) and DL-α-methylnorleucine (α-Me-Nle) were donated by E. Gil-Av (Weizman Institute of Science, Rehovot, Israel). α-Aminoisobutyric acid (α-methylalanine, 2-methylalanine, Aib) was purchased from Fluka (Buchs, Switzerland), L-isoleucinol (L-Ileol), DL-valinol (DL-Valol), DL-alaninol (DL-Alaol), DL-methioninol (DL-Metol), L-tyrosinol (L-Tyrol), L-leucinol (L-Leuol), DL-serinol (DL-Serol), cycloleucinol (Cleol) and L(+)-phenylglycinol (L(+)-Phgol) from Sigma and L-leucinol (L-Leuol), L-histidinol (L-Hisol), L-phenylalaninol (L-Pheol) and 2-amino-2-methyl-1-propanol (Aibol) from Serva (Heidelberg, F.R.G.).

Gas–liquid chromatography

GLC was carried out on Model 4100 (Fractovap series) and Model HRGC 5160 (Mega series) chromatograph, both from Carlo Erba (Rodano, Italy). They were equipped with a Model 410 LT programmer, an EL 440 electrometer (Carlo Erba) and a Chromatopac C-R3A integrator–printer–plotter (Shimadzu, Kyoto, Japan).

Columns. The following capillary columns were used: (I) a 25 m × 0.22 mm I.D. wall-coated open-tubular (WCOT) fused-silica column, coated with chemically bonded CP-Sil-19 CB [methyl(85%)–phenyl(7%)–cyanopropyl(7%)–vinyl(1%)-polysiloxane (equal to OV-1701)], with film thickness 0.21 μm, phase ratio 260, capacity factor (k') (decanoic acid methyl ester, carrier gas hydrogen, 115°C) 6.4, num-

ber of theoretical plates 82 000, height equivalent to a theoretical plate (HETP) 0.233 mm and coating efficiency 85%; (II) a 25 m × 0.22 mm I.D. WCOT fused-silica column, coated with Chirasil-L-Val, with film thickness 0.13 μm, phase ratio 412; k' (L-methionine, carrier gas hydrogen, 140°C) 4.6, number of theoretical plates 82 000, HETP 0.306 mm and coating efficiency 60%; and (III) a 50 m × 0.22 mm I.D. WCOT fused-silica column, coated with XE-60–*S*-Val-*S*-α-phenylethylamide, with film thickness 0.13 μm, phase ratio 430, k' (L-methionine, carrier gas helium, 165°C) 4.5, number of theoretical plates 216 000, HETP 0.23 mm and coating efficiency 79%.

All columns were obtained from Chrompack (Middelburg, The Netherlands). The specifications were certified by the manufacturer.

Derivatization procedure in GLC. Samples of 2 mg of α-methyl-α-amino acids were transferred into 1-ml heavy-walled Reacti-vials (Wheaton, Millville, NJ, U.S.A.), and each was derivatized with 200 μl of 2.5 *M* HCl in 1-propanol (Merck, Darmstadt, F.R.G.), or *S*(−)-2-methyl-1-butanol (Fluka), *S*(+)-2-butanol (Fluka) or *S*(+)-2-octanol (Fluka), containing 20% of acetyl chloride. The vials were sealed tightly with a Teflon-lined screw-cap, heated for 1 h at 100°C in an aluminium heating block, and the contents were evaporated to dryness at 40°C in a stream of dry nitrogen. Each residue was dissolved in 200 μl of dichloromethane and then 50 μl of pentafluoropropionic anhydride (Pierce, Rockford, IL, U.S.A.), heptafluorobutyric anhydride (Fluka), trifluoroacetic anhydride (Merck), acetic anhydride (Merck) or propionic anhydride (Merck) were added. After being heated for 1 h at 100°C, the reagents were removed in a stream of dry nitrogen at ambient temperature and the residue was dissolved in 200 μl of dichloromethane. Samples of 0.8–1.0 μl were applied to the injector of the gas chromatograph, using a 10-μl syringe (Hamilton, Bonaduz, Switzerland) and operating in the split mode (splitting ratio *ca.* 1:30).

High-performance liquid chromatography

Apparatus. The HPLC system (LKB, Bromma, Sweden) consisted of two Model 2150 pumps, a Model 2152 controller for gradient programming and a modified Model 2153 autoinjector with a 20-μl filling loop for automated on-line derivatization. A Rheodyne (Cotati, CA, U.S.A.) No. 7125 injection valve with a 20-μl filling loop was used for manual injections. Fluorescence was routinely monitored with a Model RF-530 fluorescence spectromonitor (Shimadzu) at an excitation wavelength of 330 nm and an emission wavelength of 450 nm with a 12-μl flow cell. UV absorption was measured with a Model BT 3030 UV detector (Biotronik, Maintal, F.R.G.).

Automated sample injector. An LKB 2153 autoinjector was modified so as to permit automated pre-column derivatization and subsequent injection. Thus, the normal digitally pre-set functions of the autoinjector for flush cycles and the injection–fill cycles were changed into digitally pre-set functions for pump time (0–90 s) and derivatization time (0–990 s), respectively. The on-line derivatization was facilitated by attaching a peristaltic pump to the autoleader. This permitted mixing of the sample with the OPA–2-ME reagent in an external mixing T-piece (reaction vessel) in the pumping mode. The prepared samples and the reagent bottle were placed in a Thermobox on the top of the autoinjector, which was held at ambient temperature (25°C) or cooled to 4°C. As a result of the warming of the reaction vessel (which was not thermostated), the temperature of the reactants was 7°C after a 1.5-min reaction time.

In the complete HPLC system, the autoinjector was connected to the controller by an interface cable. Full control of the analysis time and gradient profile for each sample injection and of the number of repetitive injections from each vial was then achieved. A ready-to-use OPA–2-ME reagent solution (Pierce 26025) and a 1.0 *M* potassium borate buffer (pH 10.4) (Pierce 27035) were obtained from Pierce.

Separation of α-alkyl-α-amino acids and α-amino alcohols

Column. For the separation of α-alkyl-α-amino acids and α-amino alcohols, a Hyperchrome HPLC column (125 × 4.6 mm I.D.) (Bischoff-Analysentechnik, Leonberg, F.R.G.) and a guard column (10 × 4.6 mm I.D.) were used, both packed with Spherisorb ODS II, 3 μm (Phase Separations, Queensferry, U.K.). The columns were kept in a Model 4000 Thermobox (Bischoff) and maintained at 25°C by a Model 2761 thermostat (Eppendorf Gerätebau, Netheler & Hinz, Hamburg, F.R.G.).

Eluent system. Gradients were formed between two helium-conditioned solvents; 12.5 m*M* Na_2HPO_4–NaH_2PO_4 at pH 7.2 was used to buffer the organic solvent mixtures (acetonitrile and tetrahydrofuran). Degassing was carried out by using a Model 2156 solvent conditioner (LKB). Further details of the analytical conditions are given in the figure legends.

Preparations of standard solutions. For the determination of α-alkyl-α-amino acids and α-amino alcohols, 10-μl aliquots of a 2.5 m*M* solution of each component were diluted with 10 ml of water and 2 ml of potassium borate buffer (1.0 *M*, pH 10.4). Each standard vial was filled with 1 ml of this solution and placed in the top of the thermostated autoinjector.

Reagents. Ultra-pure water, which was used exclusively in the preparation of buffers, was generated with an Elgastat Spectrum water purification system that includes reverse osmosis, activated carbon and a nuclear-grade ionization cartridge (Elga, Lane End, U.K). All chemicals used were of analytical-reagent grade and the solvents were of HPLC grade. $NaH_2PO_4 \cdot H_2O$, $Na_2HPO_4 \cdot 2H_2O$, copper(II) acetate hydrate, methanol and acetonitrile were purchased from Merck.

Chiral separation by TLC and HPLC. Ligand-exchange TLC was performed on Chiralplate (Macherey, Nagel & Co., Düren, F.R.G.) after activation for 15 min at 100°C. Aliquots of 3 μl of 1% solutions of DL-α-alkyl-α-amino acids in 50% aqueous methanol were used, and ascending chromatography was carried out over a distance of 7–8 cm. The plates were dried in an oven at 100°C and then sprayed with 0.2% ninhydrin in ethanol. The solvent systems are specified in Table VII.

For ligand-exchange HPLC, a Nucleosil Chiral-1 column (250 mm × 4 mm I.D.) (Macherey, Nagel & Co.) was used and maintained at 60°C in a Model 2155 column oven (LKB). Of each component, 2.5 m*M* solutions were prepared in 1 m*M* copper acetate buffer (pH 5.6), and 50 nmol of each sample were injected manually by using a Model 7125 injection valve (Rheodyne). The eluent was 5% acetonitrile in 1 m*M* copper acetate buffer (pH 5.6). The UV absorption was monitored at 245 nm at a range of 0.04 a.u.f.s.

The compounds Z-Gly-Aib-L-Pheol, Z-L-Leu-Aib-Aib-Gln-Valol, Z-L-Glu-Aibol, Z-L-Glu-Leuol and Z-L-Gln-Gln-Pheol (Z = benzyloxycarbonyl protecting group) were synthesized according to methods described elsewhere[24,25].

TABLE I

COMPARISON OF NET RETENTION TIMES, t (min), TEMPERATURES, T (°C) AND RESOLUTION FACTORS, α, OF N-ACETYL (Ac), N-PROPIONYL (Prop), N-TRIFLUOROACETYL (TFA), N-PENTAFLUOROPROPIONYL (PFP) AND N-HEPTAFLUOROBUTYRYL (HFB) DERIVATIVES OF DL-α-ALKYL-α-AMINO ACID *n*-PROPYL ESTERS ON THE CHIRAL PHASE CHIRASIL-VAL

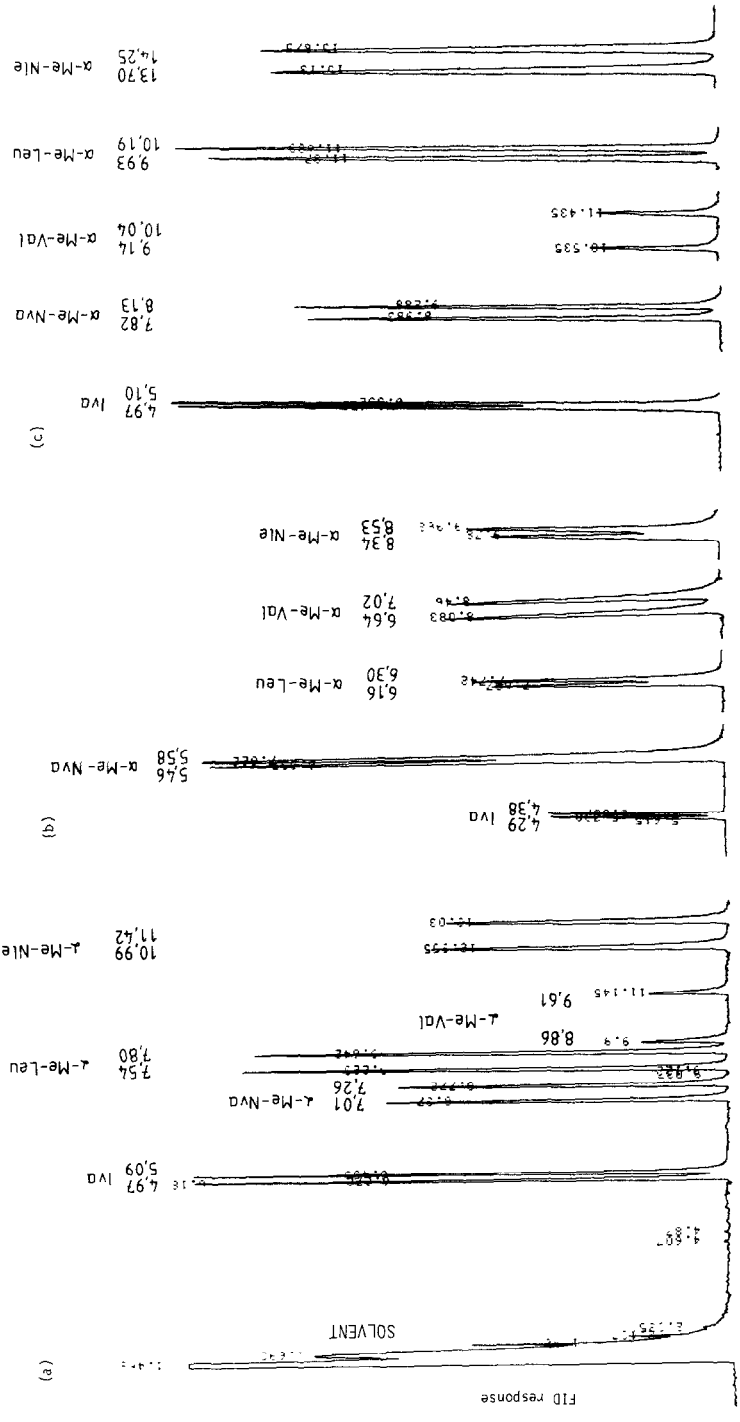
Temperature programme, 5 min at 85°C, then increased at 3°C/min to 185°C; carrier gas, hydrogen, 0.55 bar.

Amino acid	*Ac*			*Prop*			*TFA*			*PFP*			*HFB*		
	t	T	α	t	T	α	t	T	α	t	T	α	t	T	α
DL-Iva	14.54 14.75	113.6 114.3	1.014	16.51 16.65	119.5 120.0	1.009	5.61 5.69	86.8 87.1	1.014	4.81	85.0	1.000	6.81 6.90	90.4 90.7	1.013
DL-α-Me-Nva	17.12 17.38	121.4 122.1	1.015	18.49 18.68	125.5 126.0	1.010	6.31 6.41	88.9 89.2	1.016	6.41 6.52	89.2 89.6	1.017	6.91 6.99	90.7 91.0	1.012
DL-α-Me-Leu	17.51 17.77	122.5 123.3	1.015	19.34 19.55	128.0 128.7	1.011	7.13 7.22	91.4 91.7	1.013	7.64 7.73	92.9 93.2	1.012	7.93 8.00	93.8 94.0	1.009
DL-α-Me-Val	18.98 19.52	126.9 128.6	1.029	19.90 20.96	129.7 132.9	1.053	7.26 7.53	91.8 92.6	1.037	7.08 7.36	91.2 92.1	1.040	7.67 7.88	93.0 93.6	1.027
DL-α-Me-Nle	20.11 20.38	130.3 131.1	1.013	21.58 21.76	134.7 135.3	1.008	9.10 9.22	97.3 97.7	1.013	9.35 9.46	98.1 98.4	1.012	9.70 9.79	99.1 99.4	1.009

RESULTS AND DISCUSSION

For the resolution of optically active α-amino acids[31] by GLC two major approaches have been found to be successful. The first uses the difference in the free energies of diastereomers formed by esterification and acylation with chiral reagents and has the disadvantage of needing derivatizing reagents of the highest possible purity for the quantitative determination of enantiomers. This is very difficult to achieve and these reagents are relatively expensive. Therefore, it was a great breakthrough when Gil-Av *et al.*[32] succeeded in the second approach, *i.e.*, the optical resolution of amino acid enantiomers on glass capillary columns coated with optically active N-trifluoroacetyl-L-Ile dodecyl esters or N-trifluoroacetyl-L-Phe cyclohexyl esters. They also included selected α-alkyl-α-amino acids in their investigations for the evaluation of separation mechanisms of chiral phases[10,11]. With the design of a novel chiral phase in which L-valine-*tert.*-butylamide is linked covalently to polysiloxane, Bayer and co-workers designed a very thermally stable chiral phase, named Chirasil-Val[33–35], which also covers the need for an industrially manufactured chiral capillary column. The very reproducible resolution allows the separation of all protein amino acids in a single run and gives comparable analyses in different laboratories. A useful and versatile variant, developed by König[36], is XE-60–*S*-valine-*S*-α-phenylethylamide. We use Chirasil-Val routinely for the analysis of racemization in processed food proteins[37], synthetic peptides and for the determination of the configuration of Iva in peptaibol mycotoxins[38]. This method recently also revealed the presence of D-Iva in minor components (sequence analogues) of alamethicin[39]. However, the detection of L-Iva in the polypeptide antibiotic efrapeptin[40] (which we have confirmed, using the methods described below) shows the need for an assignment of the configuration in each particular instance.

The resolutions of N-acyl *n*-propyl esters of DL-α-alkyl-α-amino acids (acyl = acetyl, Ac; propionyl, Prop; trifluoroacetyl, TFA; pentafluoropropionyl, PFP; and heptafluorobutyryl, HFB) were compared on Chirasil-Val, using a standard temperature programme of 85°C for 5 min, then increased at 3°C/min to 185°C (Table I). Enantiomeric resolution was achieved for DL-Iva, DL-α-Me-Nva, DL-α-Me-Leu, DL-α-Me-Val and DL-α-Me-Nle (with the exception of the PFP derivative of DL-Iva, which could be resolved with isothermal programming, see below). Remarkably, the acidic, basic and aromatic α-alkyl-α-amino acids could not be eluted as either acetyl or propionyl derivatives at column temperatures below 190°C, which is close to the recommended maximal operating temperature (200°C). α-Me-Met and α-Me-Ser could not be resolved. α-Me-Phe and α-Me-Glu were separated as their PFP *n*-propyl derivatives with low resolution factors of 1.006 for both derivatives and 1.005 for the TFA *n*-propyl derivative of α-Me-Phe by means of a temperature programme of 70°C for 3 min, then increased at 3°C/min to 200°C. However, these last two separations were achieved with a laboratory-prepared glass capillary column (G. J. Nicholson, University of Tübingen, F.R.G.), which had been selected from a series of Chirasil-Val-coated capillary columns on the basis of extremely high resolution factors for α-amino acids. The TFA, PFP and HFP derivatives of aromatic side-chain, basic and acidic α-alkyl-α-amino acids and α-Me-Met and α-Me-Ser could not be resolved by means of the above temperature programme. Therefore, no or very low resolution was expected under isothermal conditions.


FID response
Time (min)
SOLVENT
4,97
5,09
Iva
7,01
7,26
4-Me-Nva
7,54
7,80
4-Me-Leu
8,86
4-Me-Val
9,61
10,99
11,42
4-Me-Nle
(a)
4,29
4,38
Iva
5,46
5,58
α-Me-Nva
6,16
6,30
α-Me-Leu
6,64
7,02
α-Me-Val
8,34
8,53
α-Me-Nle
(b)
4,97
5,10
Iva
7,82
8,13
α-Me-Nva
9,14
10,04
α-Me-Val
9,93
10,19
α-Me-Leu
13,70
14,25
α-Me-Nle
(c)

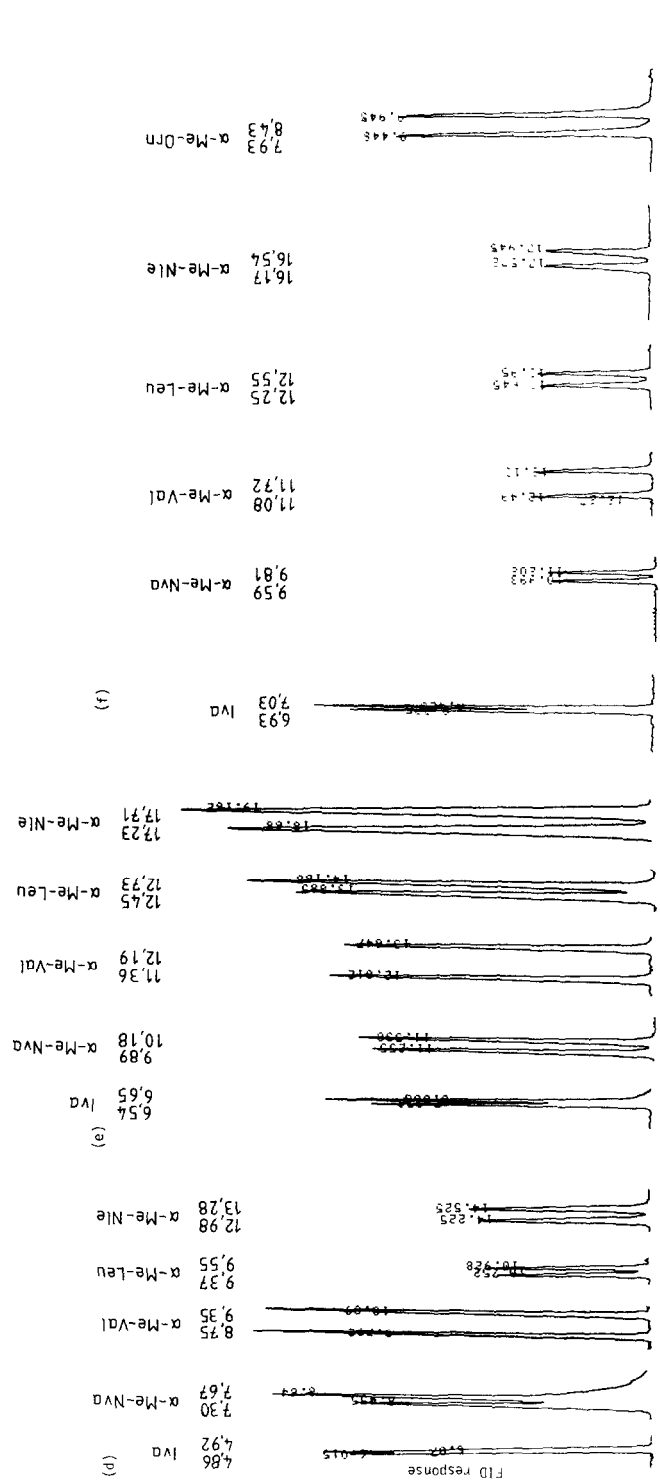


Fig. 1. GC of (a)–(e) N-acyl DL-α-alkyl-α-amino acid *n*-propyl esters, and (f) diastereomeric N-pentafluoropropionyl DL-α-alkyl-α-amino acid S(−)-2-methyl-1-butyl esters on the chiral phase Chirasil-Val. Carrier gas, hydrogen; $p = 0.55$ bar (55 kPa). Isothermal temperature for each N-acyl group: (a) acetyl, 120°C; (b) propionyl, 130°C; (c) trifluoroacetyl, 80°C; (d) pentafluoropropionyl, 80°C; (e) heptafluorobutyryl, 80°C; and (f) pentafluoropropionyl, 95°C.

TABLE II

COMPARISON OF NET RETENTION TIMES, t (min), AND RESOLUTION FACTORS, α, AT THE TEMPERATURE T (°C) OF N-ACYLATED DL-α-ALKYL α-AMINO ACID n-PROPYL ESTERS ON THE CHIRAL PHASE CHIRASIL-VAL

Carrier gas, hydrogen, 0.55 bar; for abbreviations of acyl groups, see Table I.

Amino acid	*Ac*			*Prop*			*TFA*			*PFP*			*HFB*		
	t	T	α	t	T	α	t	T	α	t	T	α	t	T	α
DL-Iva	4.97 5.09	120	1.024	4.29 4.38	130	1.023	4.97 5.10	80	1.026	4.86 4.92	80	1.012	6.54 6.65	80	1.017
DL-α-Me-Nva	7.01 7.26	120	1.036	5.46 5.58	130	1.022	7.82 8.13	80	1.040	7.30 7.67	80	1.051	9.89 10.18	80	1.029
DL-α-Me-Leu	7.54 7.80	120	1.035	6.16 6.30	130	1.023	9.93 10.19	80	1.023	9.37 9.55	80	1.019	12.45 12.73	80	1.023
DL-α-Me-Val	8.86 9.61	120	1.085	6.64 7.02	130	1.057	9.14 10.04	80	1.099	8.75 9.35	80	1.069	11.36 12.19	80	1.073
DL-α-Me-Nle	10.99 11.42	120	1.039	8.34 8.53	130	1.023	13.70 14.25	80	1.040	12.98 13.28	80	1.023	17.23 17.71	80	1.028

TABLE III

COMPARISON OF NET RETENTION TIMES, t (min) AND RESOLUTION FACTORS, α, AT 95°C OF N-PENTAFLUOROPROPIONYL L-α-ALKYL-α-AMINO ACID $S(-)$-2-METHYL-1-BUTYL ESTERS ON THE CHIRAL PHASE CHIRASIL-VAL

Carrier gas, hydrogen, 0.55 bar.

Amino acid	t	α	*Amino acid*	t	α
DL-Iva	7.03 6.96	1.010	DL-α-Me-Leu	12.55 12.25	1.024
DL-α-Me-Nva	9.81 9.59	1.023	DL-α-Me-Nle	16.54 16.17	1.023
DL-α-Me-Val	11.72 11.08	1.058	DL-α-Me-Orn*	8.43 7.93	1.063

* 150°C.

For comparison of the influence of N-acyl groups on the respective resolution factors, the *n*-propyl esters were investigated with isothermal programming (Fig. 1a–e and Table II). The perfluoroacyl esters were resolved at 80°C, the N-acetyl esters at 120°C and the N-propionyl esters at 130°C. DL-Iva and DL-α-Me-Nle showed the best resolution as TFA derivatives, DL-α-Me-Nva as the PFP derivatives and DL-α-Me-Leu and DL-α-Me-Val as acetyl derivatives. The highest separation factor among all α-alkyl-α-amino acids investigated was shown by the TFA derivative of α-Me-Val (α = 1.099); this is attributable to the optimal interaction of the chiral selector and selectand. α-Me-Met and α-Me-Ser were not resolved. For DL-Iva and DL-α-Me-Tyr, for which the pure enantiomers were available, the order of emergence of all derivatives could be established as D before L by spiking of the racemate.

The diastereomeric N-PFP DL-α-alkyl-α-amino acid $S(-)$-2-methyl-1-butyl esters were also investigated by GC on Chirasil-Val (Fig. 1f and Table III). DL-Iva, DL-α-Me-Nva, DL-α-Me-Val, DL-α-Me-Leu and DL-α-Me-Nle were well resolved at 95°C and DL-α-Me-Orn at 150°C. However, this derivatization offers no essential advantage over the use of *n*-propyl esters.

By analogy with Chirasil-Val, the chiral phase XE-60–*S*-valine-*S*-α-phenylethylamide has been shown to exhibit chiral recognition towards protein amino acids and many other groups of chiral compounds[41–43]. This column (having a length of 50 m, compared with the 25 m of the Chirasil-Val column) did resolve the PFP and HFB *n*-propyl esters of the aromatic side-chain α-alkyl-α-amino acids, DL-α-Me-Phe, DL-α-Me-Tyr and DL-α-Me-PheOCH$_3$, and the diastereomeric PFP esters with $S(-)$-2-methyl-1-butanol. The application of the latter combined with the chiral column therefore does not offer any advantage (Fig. 2a–c and Table IV).

α-Me-Dopa and α-Me-Trp were not eluted as the above derivatives below 190°C, and none of the other α-alkyl-α-amino acids could be separated as their TFA *n*-propyl esters, despite the fact that this column showed a very high resolution factor for the TFA isopropyl ester of DL-Met (α = 1.094). This selectivity toward α-alkyl (α-alkaryl) α-amino acids is attributable to the chiral selector phenylethylamide of the stationary phase giving rise to π–π interactions of aromatic ring systems. How-

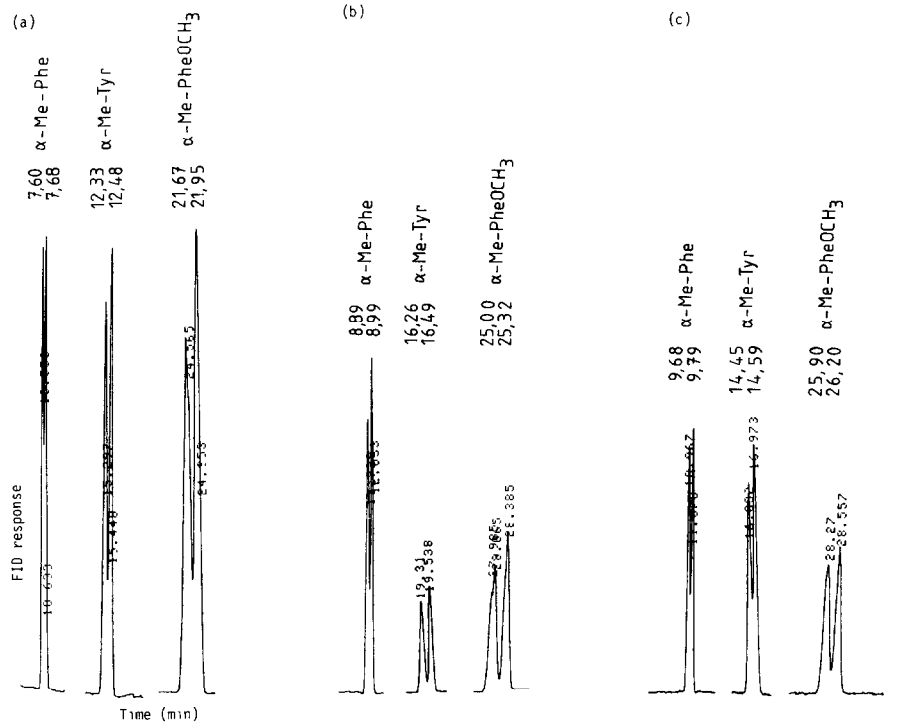


Fig. 2. GC of (a) N-pentafluoropropionyl DL-α-alkyl-α-amino acid *n*-propyl esters, (b) N-heptafluorobutyryl DL-α-alkyl-α-amino acid *n*-propyl esters and (c) N-pentafluoropropionyl DL-α-alkyl-α-amino acid *S*(−)-2-methyl-1-butyl esters on the chiral phase XE-60–*S*-Val-*S*-α-phenylethylamide. Conditions: (a) and (b) p = 1.4 bar (140 kPa), 165°C; (c) p = 1.8 bar (180 kPa), 170°C.

TABLE IV

COMPARISON OF NET RETENTION TIMES, t (min) OF N-PENTAFLUOROPROPIONYL DL-α-ALKYL-α-AMINO ACID *S*(−)-2-METHYL-1-BUTYL ESTERS (PFP_1), N-PENTAFLUOROPROPIONYL DL-α-ALKYL-α-AMINO ACID *n*-PROPYL ESTERS (PFP_2) AND N-HEPTAFLUOROBUTYRYL DL-α-ALKYL-α-AMINO ACID *n*-PROPYL ESTERS (HFB) AT THE TEMPERATURE T (°C) ON THE CHIRAL PHASE XE-60-*S*-VALINE-*S*-α-PHENYLETHYLAMIDE

Carrier gas, hydrogen, 1.8 bar (PFP_1) and 1.4 bar (PFP_2 and HFB).

Amino acid	*PFP_1*			*PFP_2*			*HFB*		
	t	T	α	t	T	α	t	T	α
DL-α-Me-Phe	9.68 9.79	170	1.011	7.60 7.68	165	1.011	8.89 8.99	165	1.011
DL-α-Me-Tyr	14.45 14.59	170	1.010	12.33 12.48	165	1.012	16.26 16.49	165	1.014
DL-α-Me-$PheOCH_3$	25.90 26.20	170	1.012	21.67 21.95	165	1.013	25.00 25.32	165	1.014

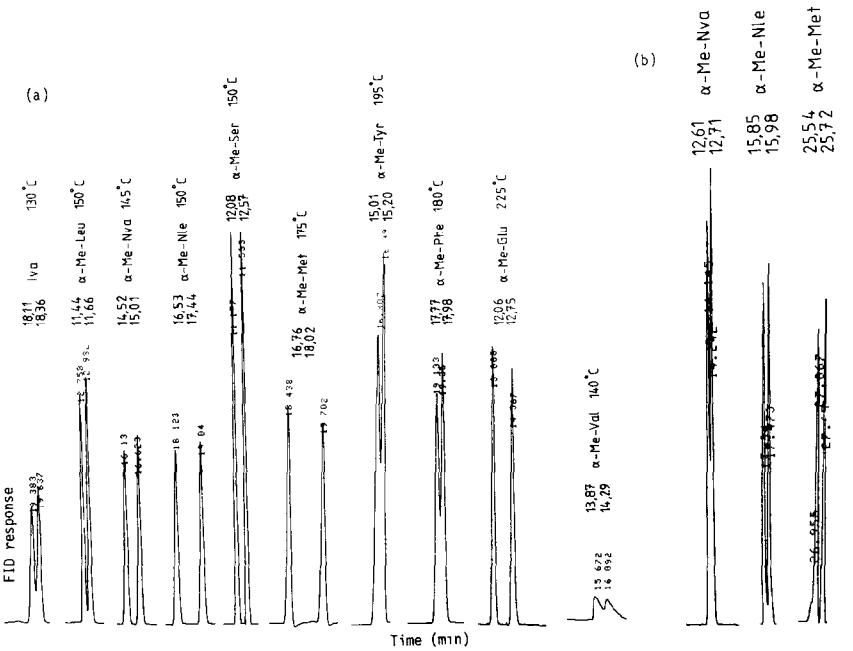


Fig. 3. GC of (a) N-pentafluoropropionyl DL-α-alkyl-α-amino acid *S*(+)-2-octyl esters and (b) N-pentafluoropropionyl DL-α-alkyl-α-amino acid *S*(+)-2-butyl esters on achiral methylphenylcyanopropylvinylpolysiloxane (CP-Sil-19 CB). Carrier gas, hydrogen. (a) p = 0.5 bar (50 kPa), isothermal temperature; (b) p = 0 5 bar (50 kPa), temperature programme, 5 min at 95°C, then increased at 3°C/min to 165°C.

TABLE V

COMPARISON OF NET RETENTION TIMES, t (min), TEMPERATURE, T (°C), AND RESOLUTION FACTORS, α, OF N-PENTAFLUOROPROPIONYL DERIVATIVES OF DL-α-ALKYL α-AMINO ACID *S*(+)-2-OCTYL ESTERS ON METHYLPHENYLCYANOPROPYLVINYL-POLYSILOXANE (CP-SIL-19 CB)

Amino acid	t	T	α	*Amino acid*	t	T	α
DL-Iva	18.11 18.36	130	1.014	DL-α-Me-Ser	12.08 12.57	150	1.041
DL-α-Me-Leu	11.44 11.66	150	1.019	DL-α-Me-Met	16.76 18.02	175	1.075
DL-α-Me-Nle	16.53 17.44	150	1.055	DL-α-Me-Glu	12.06 12.75	225	1.057
DL-α-Me-Val	13.87 14.29	140	1.030	DL-α-Me-Phe	17.77 17.98	180	1.012
DL-α-Me-Nva	14.52 15.01	145	1.034	DL-α-Me-Tyr	15.01 15.20	195	1.013

ever, N-Ac and N-Prop *n*-propyl esters of the aromatic α-methyl-α-amino acids were not eluted below 190°C (the maximal allowable temperature of the column is 200°C).

Owing to the differences in the free energies of the diastereomeric amino acid esters, which are readily available by esterification with optically active alcohols, α-alkyl-α-amino acids can be resolved without the need for a chiral stationary phase. Esterification with *S*(+)-2-octanol[44] proved to be the most universally applicable method for all the α-alkyl-α-amino acids and gave, in part, excellent resolution factors under isothermal conditions when a capillary column coated with methylphenylcyanopropylvinylpolysiloxane (CP-Sil-19 CB) was used (Fig. 3a and Table V). However, α-Me-PheOCH$_3$ showed a very low resolution factor ($\alpha = 1.005$), and α-Me-Orn, α-Me-His and α-Me-Asp gave rise to several peaks in the chromatogram, which are not unequivocally assignable to the respective diastereomers. α-Me-Val gave a very low flame ionization detection (FID) response and showed peak broadening, probably owing to incomplete derivatization of this sterically highly hindered α-methyl-α-amino acid[1].

The behaviour of diastereomeric N-PFP α-alkyl-α-amino acid *S*(+)-2-butyl esters on CP-Sil-19 CB was also investigated (Fig. 3b and Table VI). All derivatives were eluted from the column when a temperature programme was used, but they could not be resolved, with the exception of DL-α-Me-Nva, DL-α-Me-Nle and DL-Me-Met, which gave relatively low resolution factors.

Finally, in agreement with the behaviour of α-amino acids[45] diastereomeric esters of N-acetyl, N-trifluoroacetyl and N-pentafluoropropionyl α-alkyl-α-amino acid *S*(−)-2-methyl-1-butyl esters could not be resolved on achiral phases (OV-17, SE-30, CP-Sil-19 CB and CP-Sil-5), in contrast to diastereomers formed with *S*(+)-3-methyl-2-butanol[38,46].

Enantiomeric resolution of α-alkyl-α-amino acids by ligand-exchange chromatography

Among the numerous methods for resolving enantiomeric amino acids by liquid chromatographic procedures, ligand-exchange chromatography is one of the

TABLE VI

COMPARISON OF NET RETENTION TIMES, *t* (min), TEMPERATURES, *T* (°C), AND RESOLUTION FACTORS, α, OF N-PENTAFLUOROPROPIONYL DL-α-ALKYL-α-AMINO ACID *S*(+)-2-BUTYL ESTERS ON METHYLPHENYLCYANOPROPYLVINYLPOLYSILOXANE (CP-SIL-19 CB)

Temperature programme, 5 min at 95°C, then increased at 3°C/min to 165°C; carrier gas, hydrogen, 0.5 bar.

Amino acid	*t*	*T*	α
DL-α-Me-Nva	12.61 12.72	107.8 108.1	1.008
DL-α-Me-Nle	15.85 15.98	117.6 117.9	1.008
DL-α-Me-Met	25.54 25.72	146.6 147.2	1.007

most promising approaches[47,48]. This method takes advantage of the stereoselectivity of the Cu^{2+} complex of, *e.g.*, hydroxyproline, which is covalently linked through derivatization of the imino group to an alkylsilica stationary phase. Because it is applicable to underivatized amino acids and has a relatively high degree of stereoselectivity, this technique should offer advantages on a preparative scale. However, until recently, the method suffered from a lack of commercially available stationary phases, manufactured under standardized procedures.

This situation has changed with the introduction of TLC plates pre-coated with alkylsilica and the chiral selector L-hydroxyproline–Cu^{2+} (Chiralplate)[49,50] and of a chiral HPLC column, based on a similar principle (Nucleosil Chiral-1). Chiralplate has also been found to be very valuable in the optical resolution of N-substituted or halogenated amino acids, thiazolinone derivatives and dipeptides. This method is also capable of separating α-alkyl-α-amino acids (Fig. 4). To investigate the influence of the composition of the mobile phase on the resolution factors of DL-α-alkyl-α-amino acids when Chiralplate is used, aqueous acetonitrile and aqueous acetone with and without admixture of alcohols (methanol, ethanol, propanol) were investigated (Table VII). The best resolution factors [*i.e.*, $R_{F(II)}/R_{F(I)}$] are obtained for α-methyl-α-amino acids having aromatic side-chains, *i.e.*, DL-α-Me-Phe, DL-α-Me-PheOCH$_3$, DL-α-Me-Dopa and DL-α-Me-Trp, in solvent system VII (see Table VII). DL-α-Me-Val, DL-α-Me-Nva and DL-α-Me-Nle show the best resolution factors in system II and DL-α-Me-Met in system III. DL-α-Me-Leu showed the same resolution in systems II and VII. The best resolution factor among all DL-α-methyl-α-amino acids in this series was shown by DL-α-Me-Nle in system II and DL-α-Me-PheOCH$_3$ in system VII, both giving $\alpha = 1.35$. Replacement of methanol in system I by ethanol

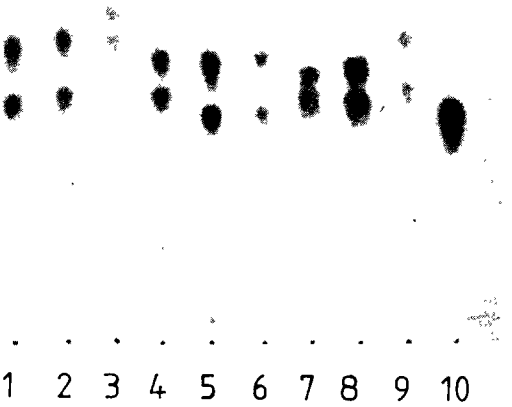


Fig. 4. Ligand-exchange TLC of DL-α-alkyl-α-amino acids on Chiralplate. (1) DL-α-Me-Phe; (2) DL-α-Me-PheOCH$_3$, (3) DL-α-Me-Dopa; (4) DL-α-Me-Met; (5) DL-α-Me-Leu; (6) DL-α-Me-Trp; (7) DL-α-Me-Nva; (8) DL-α-Me-Val; (9) DL-α-Me-Nle; (10) Aib, non-chiral standard; the bold letter refers to the enantiomer having the higher R_F value as established by comparison with optically pure enantiomers. Application of 3-μl aliquots of 1% solutions of amino acids in 50% aqueous methanol, detection with ninhydrin. Solvent system, acetonitrile–methanol–water (4:1:1).

TABLE VII

COMPARISON OF R_F VALUES AND RESOLUTION FACTORS, α, OF DL-α-ALKYL-α-AMINO ACIDS BY TLC ON CHIRALPLATE

Solvent systems: I, acetonitrile–methanol–water (80:20:20); II, acetonitrile–ethanol–water (80:20:20); III, acetonitrile–1-propanol–water (80:20:20); IV, acetone–methanol–water (80:20:20); V, acetone–ethanol–water (80:20:20); VI, acetone–1-propanol–water (80:20:20); VII, acetonitrile–water (80:40); VIII, acetonitrile–water (60:40); acetone–water (80:20).

Amino acid	*Solvent system*																	
	I		*II*		*III*		*IV*		*V*		*VI*		*VII*		*VIII*		*IX*	
	R_F	α	R_F	α	R_F	α	R_F	α	R_F	α	R_F	α	R_F	α	R_F	α	R_F	α
DL-α-Me-Val	0.62 0.56	1.11	0.63 0.55	1.14	0.65 0.58	1.12	0.80 0.75	1.07	0.76 0.73	1.05	0.83 0.78	1.06	0.59 0.54	1.09	0.69 0.69	1.00	0.79 0.75	1.05
DL-α-me-Nva	0.63 0.55	1.15	0.64 0.54	1.19	0.68 0.60	1.13	0.79 0.73	1.08	0.78 0.73	1.07	0.83 0.78	1.06	0.59 0.54	1.09	0.72 0.72	1.00	0.79 0.74	1.07
DL-α-Me-Leu	0.64 0.54	1.19	0.68 0.54	1.25	0.65 0.50	1.30	0.74 0.67	1.10	0.79 0.71	1.11	0.84 0.77	1.09	0.57 0.44	1.30	0.73 0.67	1.09	0.73 0.66	1.00
DL-α-Me-Nle	0.65 0.52	1.25	0.66 0.49	1.35	0.68 0.52	1.31	0.82 0.73	1.12	0.77 0.71	1.08	0.86 0.76	1.13	0.62 0.49	1.27	0.65 0.57	1.14	0.80 0.72	1.11
DL-α-Me-Met	0.64 0.58	1.10	0.68 0.59	1.14	0.66 0.55	1.20	0.74 0.68	1.09	0.81 0.74	1.09	0.84 0.78	1.08	0.57 0.47	1.21	0.74 0.70	1.06	0.73 0.67	1.09
DL-α-Me-Phe	0.70 0.59	1.19	0.73 0.58	1.26	0.72 0.56	1.28	0.74 0.68	1.09	0.82 0.82	1.00	0.89 0.78	1.14	0.64 0.48	1.33	0.66 0.61	1.08	0.75 0.69	1.09
DL-α-Me-PheOCH$_3$	0.72 0.59	1.22	0.74 0.60	1.23	0.74 0.58	1.28	0.77 0.70	1.10	0.82 0.75	1.09	0.90 0.80	1.13	0.66 0.49	1.35	0.66 0.62	1.06	0.77 0.70	1.10
DL-α-Me-Dopa	0.77 0.71	1.08	0.78 0.71	1.09	0.80 0.68	1.18	0.83 0.79	1.05	0.82 0.82	1.00	0.93 0.93	1.00	0.73 0.60	1.22	0.83 0.76	1.09	0.78 0.78	1.00
DL-α-Me-Trp	0.71 0.66	1.08	0.74 0.62	1.19	0.73 0.60	1.22	0.78 0.72	1.08	0.82 0.76	1.08	0.88 0.81	1.09	0.68 0.51	1.33	0.63 0.58	1.09	0.75 0.70	1.07
Aib*	0.53		0.58		0.53		0.69		0.72		0.73		0.46		0.77		0.70	

* Internal, non-chiral standard.

and 1-propanol increased the resolution in every instance, but the replacement of acetonitrile by acetone gave relatively lower resolution factors in all instances. Under the conditions used, we could not resolve DL-Iva, and this technique was not applicable to the basic and acidic DL-α-methyl-α-amino acids DL-α-Me-His, DL-α-Me-Orn, DL-α-Me-Asp and DL-α-Me-Glu. Activation of the TLC plates for 15 min at 100°C did not increase the resolution factors. Saturation of the TLC chambers by coating with filter-paper gave rise to less intense spots with ninhydrin owing to enhanced diffusion.

Three selected α-alkyl-α-amino acids were investigated by ligand-exchange HPLC on Nucleosil Chiral-1. They separated with excellent resolution factors: DL-Iva (L/D = 1.17), DL-α-Me-Tyr (L/D = 1.45), and DL-α-Me-Leu (II/I = 1.37) (Fig. 5a–c). The order of emergence was determined by spiking a sample of DL-amino acids with the pure enantiomer. The almost complete separation is of great importance, because this method should permit the preparative isolation of enantiomers of DL-α-alkyl-α-amino acids after removal of the contaminating Cu^{2+} ions in the effluent by H_2S (thioacetamide) or stronger complex-forming agents.

Separation of α-amino alcohols and α-amino acids by RP-HPLC

We recently demonstrated that the difficulties encountered in the detection and quantification of two amino alcohols (Valol and Pheol) in the above-mentioned peptaibol myctoxins could be overcome by derivatization of hydrolysates with OPA–2-ME and subsequent RP-HPLC[1]. This method allowed the separation and quantification of the peptaibol constituent α-amino acids, α-alkyl-α-amino acids and α-amino alcohols in a single chromatogram. To evaluate and extend the scope of the method, we now show that a standard mixture of fifteen α-amino acids and eleven α-amino alcohols can be completely separated as their OPA–2-ME adducts on octadecylsilyl-bonded silica with Na_2HPO_4 buffer–acetonitrile gradient (Fig. 6). This method offers the advantage that, with few exceptions, α-amino alcohols show a fluorescence response that is about equal to that of the standards Leu or Leuol, in contrast to their colour yield in the quatitative ninhydrin reaction. Valol, for example, gives a colour ratio of about 7.3% of that of Leu (= 100%) with ninhydrin, and Pheol is not eluted from ion-exchange columns of automated amino acid analysers under standard conditions[8]. Serol, Alaol, Metol, Valol, Pheol and Ileol show a relative fluorescence that is about equal to that of Leuol (= 100%), depending more or less on the derivatization temperature T_1 (7°C) and T_2 (25°C) (Table VIII). Hisol shows a relatively low fluorescence of 22.5% (F_{T_1}) and 24.7% (F_{T_2}), but Cleol 16.8% (F_{T_1}) and 15.5% (F_{T_2}), Phgol 12.4% (F_{T_1}) and 11.6% (F_{T_2}), and Aibol 5.2% (F_{T_1}) and 7.7% (F_{T_2}) show a much lower relative fluorescence, the last compound having the lowest relative fluorescence of all α-amino alcohols investigated. It is obvious that the spatial arrangement of the substituents at the C_α atom is responsible for this decrease in fluorescence. From the time dependence of the relative fluorescence (Fig. 7) with OPA–2-ME at a derivatization temperature of 25°C, it is obvious that the plateau of the relative fluorescence of Pheol, Valol and Leuol is reached after *ca.* 3 min and remains stable for a further *ca.* 12 min (longer reaction times have not yet been investigated). This corresponds to the relative fluorescence of Glu and Leu, although the latter shows a very slight decrease in the relative fluorescence after 3 min. In contrast, Gly exhibits a very strong decrease of the relative fluorescence

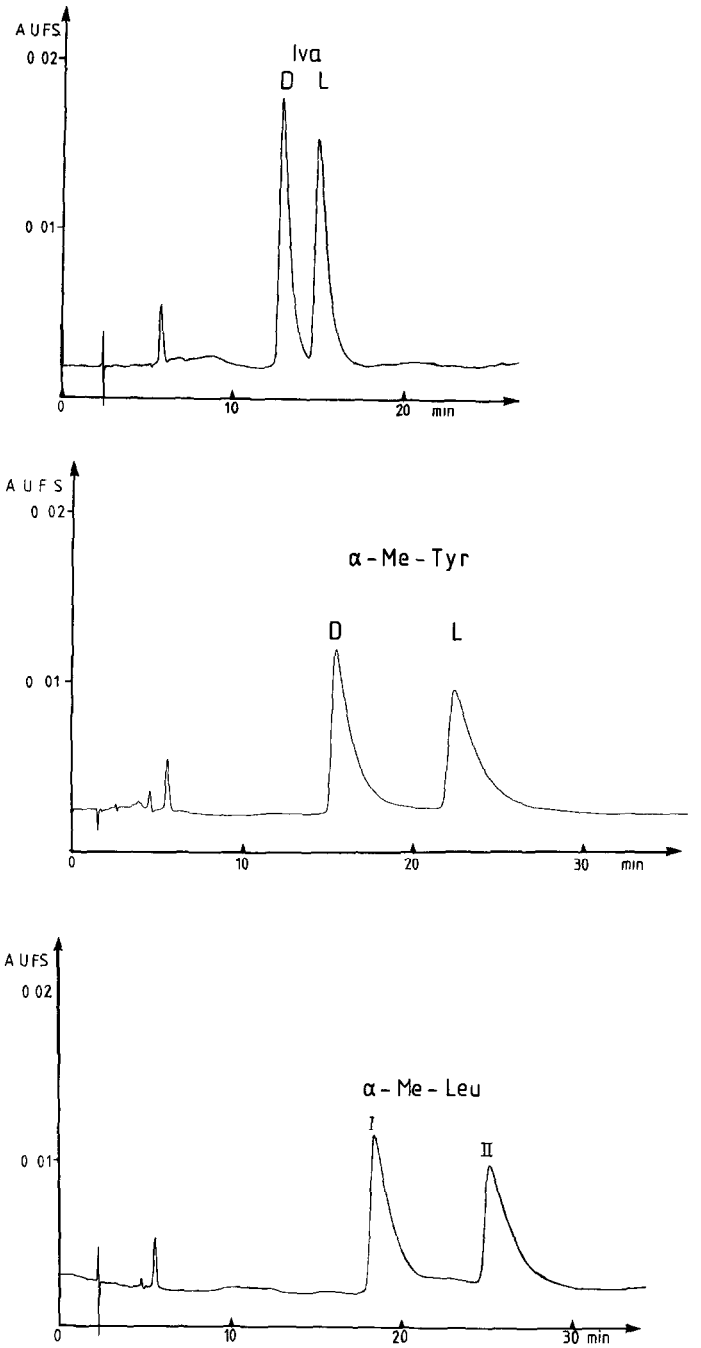


Fig. 5. Separation of selected DL-α-alkyl-α-amino acids [(a) DL-Iva; (b) DL-α-Me-Tyr; (c) DL-α-Me-Leu] by ligand-exchange HPLC. Nucleosil Chiral-1 column (250 mm × 4 mm I.D.); eluent, 5% acetonitrile in 1.0 m*M* copper acetate buffer adjusted with HCl to pH 5.6; temperature, 60°C; flow-rate, 1.0 ml/min; pressure, 7 MPa (70 bar); injection, 50 nmol in 20 μl; absorption, 245 nm.

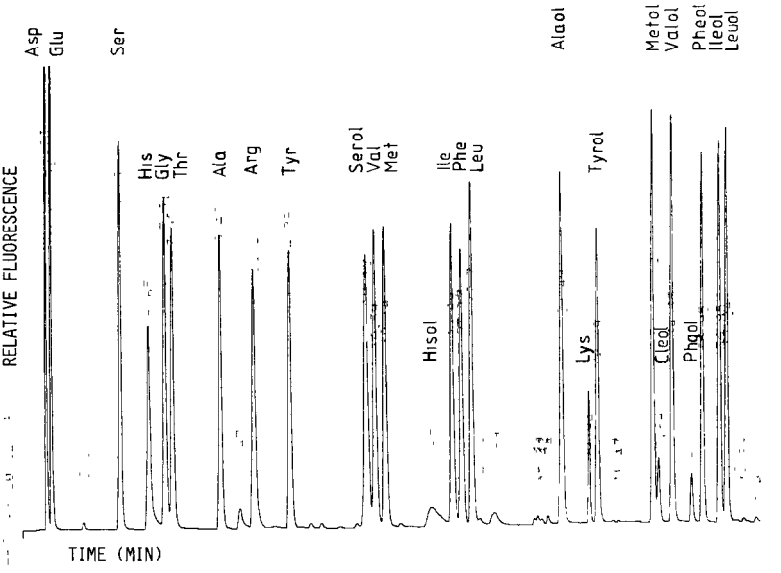


Fig. 6. RP-HPLC of an OPA–2-ME-derivatized standard, composed of α-amino acids and α-amino alcohols. Conditions: Spherisorb ODS II column (125 mm × 4 mm I.D.) with a 10 mm × 4.6 mm I.D. guard column; particle size, 3 μm; eluent A, 1% acetonitrile–1.5% tetrahydrofuran; eluent B, 50% acetonitrile in 12.5 m*M* Na_2HPO_4–NaH_2PO_4 buffer (pH 7.2); elution, 1 min with 10% B, linear gradient of 23 min to 45% B and 14 min to 100% B; flow-rate, 1.5 ml/min; pressure, 18 MPa (180 bar); injection of 28.5 pmol of each component in 20 μl after automated derivatization for 90 s at 25°C; temperature, 20°C.

TABLE VIII

RETENTION TIMES, *t* (min), OF α-AMINO ACIDS AND α-AMINO ALCOHOLS AND TEMPERATURE DEPENDENCE OF THEIR RELATIVE FLUORESCENCE, F_{T_1} AND F_{T_2} COMPARED WITH LEUOL (= 100%), AFTER DERIVATIZATION WITH OPA–2-ME FOR 1.5 min

Compound	*t* *(min)*	F_{T_1} *(7°C)*	F_{T_2} *(25°C)*	*Compound*	*t* *(min)*	F_{T_1} *(7°C)*	F_{T_2} *(25°C)*
Asp	1.23	87.9	69.6	Phe	23.17	87.2	77.3
Glu	1.51	92.3	79.0	Leu	23.70	104.8	86.9
Ser	5.15	93.8	78.5	Alaol	28.49	107.4	95.5
His	6.65	71.3	60.6	Lys	29.94	23.2	27.7
Gly	7.51	93.9	83.3	Tyrol	30.39	77.7	98.9
Thr	7.92	86.6	72.2	Aibol	31.19	5.2	7.7
Ala	10.43	87.3	71.7	Metol	33.32	105.8	78.9
Arg	12.20	88.2	83.0	Cleol	33.64	16.8	15.5
Tyr	14.09	86.1	72.6	Valol	34.35	104.3	96.9
Serol	18.12	108.7	109.4	Phgol	35.39	12.4	11.6
Val	18.59	95.4	83.0	Pheol	35.95	89.9	128.6
Met	19.14	95.9	83.0	Ileol	36.85	96.5	96.5
Hisol	21.55	22.5	24.7	Leuol	37.24	100.0	100.0
Ile	22.69	95.6	87.0				

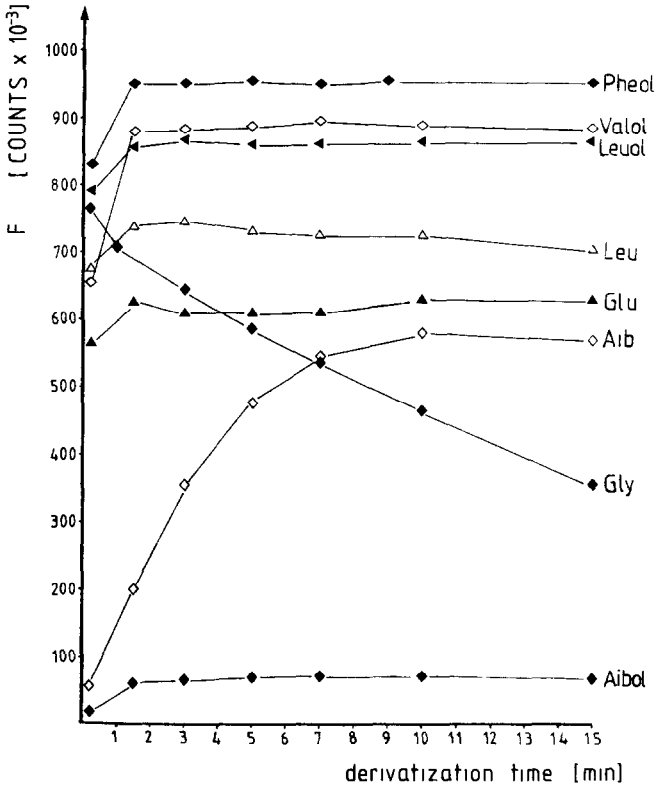


Fig. 7. Time dependence of the relative fluorescence (F) of selected α-amino acids and α-amino alcohols after derivatization with OPA–2-ME at 25°C. Conditions as in Fig. 6.

directly after mixing with derivatizing reagent, giving half of the relative fluorescence after a 15-min reaction time, and the relative fluorescence of Aib reaches the plateau of the reaction curve after a *ca.* 10-min reaction time. The corresponding amino alcohol of Aib, Aibol, shows a unique behaviour, giving a very low relatively fluorescence after a 3-min reaction time with an almost negligibly low slope to a 15-min reaction time. As a suitable compromise, a derivatization temperature of 25°C and a reaction time of 10 min were selected for the simultaneous determination of α-alkyl-α-amino acids, α-amino alcohols and α-amino acids.

The applicability of the method to the quantitative determination of the constituents of model peptides containing α-amino alcohols is demonstrated below (hydrolysis with 6 *M* HCl at 110°C for 24 h; Z = benzyloxycarbonyl protecting group). Derivatization with OPA–2-ME reagent was carried out for 90 s (first value) and 600 s (second value), with the exception of the last two peptides, where only the values for 90 s are given. The amino acid on which the ratio is based is marked with an asterisk: for Z-Gly-Aib-L-Pheol, Gly* (1.00, 1.00), Aib (1.12, 0.99), Pheol (0.91, 0.96); for Z-L-Leu-Aib-Aib-Gln-Valol, Leu* (1.00, 1.00), Aib (2.20, 2.12), Glu (0.97, 1.06). Valol (0.96, 0.90); for Z-L-Glu-Aibol, Glu* (1.00, 1.00), Aibol (1.20, 1.15); for Z-L-Gln-Leuol, Glu* (1.00), Leuol (0.93); and for Z-L-Gln-Gln-Pheol, Glu* (2.00), Pheol (1.17); Gln is hydrolyzed to Glu.

CONCLUSIONS

As a result of the space-filling properties of the disubstituted C_α atoms, the GC separation of DL-α-alkyl-α-amino acids into enantiomers is in principle more difficult to achieve than that of the respective protein amino acids. However, in contrast to the latter, a large number of which usually must be resolved simultaneously in hydrolysates for the determination of the degree of racemization, the optical configuration of the relatively few α-alkyl-α-amino acids found in natural products must be assigned unambigously. Alternatively, the enatiomeric purity of α-alkyl-α-amino acids, obtained by enantioselective syntheses or by enzymatic or chemical resolution of racemates, must be determined. From this point of view, it is not necessary to separate a very large number of DL-α-alkyl-α-amino acids in a single chromatogram.

The applications given show that GC is a rapid and convenient method which permits the resolution of most DL-α-alkyl-α-amino acids, preferably under isothermal conditions. The chiral phase Chirasil-Val provides a good resolution of those α-alkyl-α-amino acids having exclusively C_α-alkyl side-chains. The resolution factor depends to a certain extent on the different derivatization procedures. The α-alkyl-α-amino acids having aromatic side-chains are not resolved on Chirasil-Val under the experimental conditions used; in contrast, the chiral phase XE-60–*S*-Val-*S*-α-phenylethylamide shows good enantioselectivity towards DL-α-Me-Phe, DL-α-Me-PheOCH$_3$ and DL-α-Me-Tyr. It should be emphasized that the results presented have been obtained with commercially manufactured capillary columns. Batch modifications of the stationary phase, different column material and various deactivation and coating procedures will influence the resolution to some extent.

An excellent resolution of most DL-α-alkyl-α-amino acids was shown by the diastereomeric N-pentafluoropropionyl *S*(+)-2-octyl esters on an achiral column (CP-Sil-19 CB). However, for the quantification of enantiomers, the optical purity of the alcohol used for derivatization and the completeness of the derivatization procedures[1] must be carefully tested.

Very recently the enantiomeric resolution by ligand-exchange chromatography was extended to the analytical and preparative separation and determination of the optical rotations of most α-alkyl-α-amino acids involved in this study[51]. This method, therefore, should also be advantageous for the correlation of chiroptical properties of these compounds and their configurations.

ACKNOWLEDGEMENTS

We are deeply obliged to E. Gil-Av, Weizman Institute of Science, Rehovot, Israel, for samples of commercially unavailable DL-α-alkyl-α-amino acids and are grateful to D. Seebach, Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, Zürich, Switzerland, for several standards of L-α-methyl-α-amino acids, obtained by enantioselective syntheses[52]. We also thank Macherey, Nagel & Co. for providing the Nucleosil Chiral-1 column. This work was supported by grants from the Deutsche Forschungsgemeinschaft (Br 880/3-1), the Ministerium für Wissenschaft und Kunst Baden-Württemberg (Schwerpunkt 33) and the Bundesministerium für Wirtschaft (Industrielle Gemeinschaftsforschung), AIF Nr. 6668.

REFERENCES

1 H. Brückner, I. Bosch, T. Graser and P. Fürst, *J. Chromatogr.*, 386 (1987) 251.
2 K. Kociolek, U. Slomaczynska and M. T. Leplawy, in D. Theodoropoulos (Editor), *Peptides 1986, Proceedings of the 19th European Peptide Symposium*, Walter de Gruyter, Berlin, 1987, p. 251.

3 T. L. Sourkes and E. E. Townsend, *Can. J. Biochem.*, 33 (1955) 735.
4 T. L. Sourkes, *Arch. Biochem. Biophys.*, 51 (1954) 444.
5 J. A. Oates, L. Gillespie, S. Udenfriend and A. Szoerdsma, *Science*, 131 (1960) 1890.
6 H. Smirk, *Br. Med. J.*, 146 (1963) 146.
7 M. Hamilton and H. Kopelman, *Br. Med. J.*, 146 (1963) 151.
8 H. Brückner and M. Przybylski, *Chromatographia*, 19 (1984) 188.
9 J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, Vol. 1, Wiley, New York, 1961, p. 743.
10 T. Frydman, B. Tamari, B. Feibush and E. Gil-Av, *Proc. Isr. Chem. Soc. 42nd Meet.*, (1972) 11.
11 S.-C. Chang, R. Charles and E. Gil-Av, *J. Chromatogr.*, 238 (1982) 29.
12 J. Turk, G. T. Panse and G. R. Marshall, *J. Org. Chem.*, 40 (1975) 953.
13 U. Schöllkopf, U. Groth, K.-O. Westphalen and C. Deng, *Synthesis*, 1981, 969.
14 B. Kübel, P. Gruber, R. Hurnaus and W. Steglich, *Chem. Ber.*, 112 (1979) 128.
15 E. M. Wilson and E. E. Snell, *J. Biol. Chem.*, 237 (1962) 3180.
16 C. G. Baker, S.-C. J. Fu, S. M. Birnbaum, H. A. Sober and J. P. Greenstein, *J. Am. Chem. Soc.*, 74 (1952) 4701.
17 S. Terashima, K. Achiva and S. Yamada, *Chem. Pharm. Bull.*, 13 (1965) 1399.
18 R. Bosch, H. Brückner, G. Jung and W. Winter, *Tetrahedron*, 38 (1982) 3579.
19 E. Gil-Av, *J. Mol. Biol.*, 6 (1975) 131.
20 R. H. Liu and W. W. Ku, *J. Chromatogr.*, 271 (1983) 309.
21 V. Schurig, *Angew. Chem.*, 96 (1984) 733; *Angew. Chem., Int. Ed. Engl.*, 23 (1984) 747.
22 H. Frank, in W. Fresenius, H. Günzler, W. Huber, I. Lüderwald and G. Tölg (Editors), *Gas-Chromatographie von Aminosäuren*, Springer Verlag, Berlin, 1984, p. 337.
23 M. Przybylski, I. Dietrich, I. Manz and H. Brückner, *Biomed. Mass Spectrom.*, 11 (1984) 569.
24 H. Brückner, in D. Theodoropoulos (Editor), *Peptides 1986, Proceedings of the 19th European Peptide Symposium*, Walter de Gruyter, Berlin, 1987, p. 231.
25 H. Brückner and G. Jung, *Justus Liebigs Ann. Chem.*, (1982) 1677.
26 T. Fujita, Y. Takaishi, K. Matsuura, Y. Takeda, Y. Yoshioka and H. Brückner, *Chem. Pharm. Bull.*, 32 (1984) 2870.
27 D. Davoust, B. Bodo, S. Rebuffat and N. Platzer, *Biochem. Biophys. Res. Commun.*, 116 (1983) 1.
28 H. Godel, T. Graser, P. Földi, P. Pfaender and P. Fürst, *J. Chromatogr.*, 297 (1984) 49.
29 K. P. Kohse, T. A. Graser, H. G. Godel, C. Rössle, H. E. Franz and P. Fürst, *J. Chromatogr.*, 344 (1985) 319.
30 T. A. Graser, H. G. Godel, S. Albers, P. Földi and P. Fürst, *Anal. Biochem.*, 151 (1985) 142.
31 V. Schurig, *Kontakte (Darmstadt)*, (1986) 3.
32 E. Gil-Av, B. Feibush and R. Charles-Sigler, in A. B. Littlewood (Editor), *Gas Chromatography 1966*, Institute of Petroleum, London, 1967, p. 227.
33 H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr. Sci.*, 15 (1977) 174.
34 H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr.*, 167 (1978) 187.
35 B. Koppenhoefer and E. Bayer, *Chromatographia*, 19 (1984) 123.
36 W. A. König, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 588.
37 H. Brückner, S. Schäfer, D. Bahnmüller and M. Hausch, *Fresenius Z. Anal. Chem.*, 327 (1987) 30.
38 H. Brückner, G. J. Nicholson, G. Jung, K. Kruste and W. A. König, *Chromatographia*, 13 (1980) 209 and 516.
39 H. Brückner, M. Bokel and M. Przybylski, in C. M. Deber, V. J. Hruby and K. D. Kopple (Editors), *Peptides, Structure and Function*, Pierce, Rockford, IL, 1985, p. 109.
40 D. A. Bullough, C. G. Jackson, P. J. F. Henderson, F. H. Cottee, R. B. Beechey and P. E. Linnett. *Biochem. Int., 4 (1982) 543.*
41 W. A. König, I. Benecke and H. Bretting, Angew. Chem., 93 (1981) 688; *Angew. Chem., Int. Ed. Engl.*, 20 (1981) 693.
42 W. A. König, I. Benecke and S. Sievers, *J. Chromatogr.*, 217 (1981) 71.
43 W. A. König and I. Benecke, *J. Chromatogr.*, 209 (1981) 91.
44 E. Gil-Av, R. Charles and G. Fischer, *J. Chromatogr.*, 17 (1965) 408.
45 W. A. König, *Chromatographia*, 9 (1976) 72.
46 W. A. König, W. Rahn and J. Eyem, *J. Chromatogr.*, 133 (1977) 141.
47 V. A. Davankov and A. V. Semechkin, *J. Chromatogr.*, 141 (1977) 313.
48 V. A. Davankov and A. A. Kurganov, *Chromatographia*, 17 (1983) 686.
49 K. Günther, M. Schickedanz and J. Martens, *Naturwissenschaften*, 72 (1985) 149.
50 U. A. Th. Brinkman and D. Kamminga, *J. Chromatogr.*, 330 (1985) 375.
51 H. Brückner, *Fresenius Z. Anal. Chem.*, 327 (1987) 32.
52 D. Seebach, J. D. Aebi, R. Naef and T. Weber, *Helv. Chim. Acta*, 68 (1985) 144, and references cited therein.