Gas chromatographic separation of diastereomeric esters of α-alkyl-α-amino acids on dimethylpolysiloxane[a]

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ABSTRACT

The separation of 28 (R,S)-α-alkyl-α-amino acids (AAAs) with the structure $H_2NC(R^1)(R^2)COOH$ (R^1, R^2 = alkyl, aralkyl, which may be further substituted; R^1, $R^2 \neq H$) was systematically investigated by capillary gas chromatography on the stationary phase dimethylpolysiloxane (CP-Sil 5); the AAAs were used as their N(O)-pentafluoropropionyl (PFP)-AAA $(S)(+)$-2-alkyl esters (alkyl − 2-butyl to 2-octyl and 3-methyl-2-butyl). In selected cases, (R,S)-3,3-dimethyl-2-butyl esters, (R,S)-3-octyl esters, (R,S)-2-dodecyl esters and ( )-menthyl esters were also employed. Baseline resolution was achieved for these AAAs by derivatization with suitable alcohols. By using AAAs of known absolute configuration, it was possible to establish the order of emergence as (S)-AAAs before (R)-AAAs for PFP-(R,S)-AAA $(S)(+)$-2-alkyl esters. This is the opposite elution order to that of α-amino acids having a C^α-hydrogen atom. In spite of their opposite elution orders on achiral stationary phases, conformational analysis of PFP $(S)(+)$-2-alkyl esters of (S)-α-amino acids and (S)-AAAs shows that they have the same bulkiness chirality.

INTRODUCTION

As a consequence of the substitution of the C^α-hydrogen atom of protein amino acids by alkyl or alkaryl groups, the resulting chiral α-alkyl-α-amino acids (AAAs) of the type $H_2NC(R^1)(R^2)COOH$ ($R^1 \neq R^2 \neq H$) show different chemical and chromatographic behaviours to their parent compounds [1,2]. In spite of the increasing interest in the use of chiral and non-chiral AAAs as sterically constrained, non-protein amino acids, *e.g.*, for peptide drug design [3,4], the systematic investigation of their chiral analytical properties is still hampered by the fact that only a few racemic and no optically pure AAAs are currently commercially available (see Experimental), although stereoselective syntheses [5] and enzymic scaled-up resolutions of (R,S)-AAA amides [6] have been described.

The natural microheterogeneity of the 20-mer peptide antibiotic paracelsin served as a possible model for reversed-phase interaction mechanisms in high-per-

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formance liquid chromatography (HPLC) [7,8] and, in the case of synthetic *homo*-Aib oligopeptides, the thin-layer chromatographic recognition of 3_{10}-helical conformations of these peptides [9] has been realized. Further, by using α-aminoisobutyric acid (α-methylalanine, 2-methylalanine, Aib) and isovaline (2-ethylalanine, Iva) as highly specific marker compounds for the detection of a unique group of polypeptide antibiotics of the peptaibol family, it was shown that these AAAs are common metabolites of certain genera of fungi [10,11,12] and that Aib and Iva, therefore, are neither exceedingly rare amino acids in the biosphere nor, if occurring in sediments, necessarily of extraterrestrial origin [13].

Using capillary gas chromatography (GC) and the diastereomeric approach, Pollock [14] demonstrated that (*R,S*)-Iva was partly separated as the N-trifluoroacetyl (TFA)-Iva (*S*)(−)-2-alkyl esters (alkyl = *n*-pentyl to *n*-octyl) but not as the TFA-Iva (*S*)(+)-2-butyl ester. The formation of N-pentafluoropropionyl (PFP)-(*R,S*)-Iva (*S*)(+)-3-methyl-2-butyl esters was used for the revison of the configuration of Iva in the peptide antibiotic antiamoebin [15]. Chang *et al.* [16] demonstrated that a number of (*R,S*)-AAAs are separable as N-TFA isopropyl esters on optically active diamide stationary phases and we have reported the separation of derivatives of selected (*R,S*)-AAAs using the chiral stationary phases Chirasil–L-Val and XE-60 L-Val (*S*)-α-phenylethylamide [17]. Further, it was shown that the resolution of (*R,S*)-AAAs is also possible by chiral ligand-exchange HPLC using either chiral additives, such as N-dialkyl-L-amino acids together with Cu^{II} salts [18,19], to eluents or by employing chiral stationary phases such as Chiral ProCu [19,20–24]. Moreover, HPLC and precolumn derivatization of (*R,S*)-AAAs with *o*-phthaldialdehyde together with N-acyl-L-cysteine (acyl = acetyl, *tert.*-butyloxycarbonyl) [22,23,25], derivatization of (*R,S*)-AAAs using Marfey's reagent [21,24] and thin-layer chromatography using Chiralplate [26] have also made the separation of (*R,S*)-AAAs possible.

EXPERIMENTAL

Gas chromatography

A Hewlett-Packard Model 5880 A gas chromatograph equipped with a flame ionization detector and a 26 m × 0.22 mm I.D. wall-coated open-tubular (WCOT) fused-silica column coated with 0.21-μm dimethylpolysiloxane (CP-Sil 5) (Chrompack, Middelburg, The Netherlands) was used. The carrier gas was helium at 100 kPa (1.0 bar), the splitting ratio was *ca.* 1:30 and the injector and detector temperatures were 250°C.

Abbreviations and sources of α-alkyl-α-amino acids

In order to have simple nomenclature and abbreviations, in this work AAAs are considered as being formally derived by substitution of a C$^{\alpha}$-hydrogen atom in α-amino acids by an alkyl group. Substitution of the C$^{\alpha}$-hydrogen atom of Ala by an *n*-alkyl group (alkyl = ethyl to *n*-octyl) thus leads to the homologous series α-Et-Ala to α-Oct-Ala (for abbreviations of alkyl groups, see the next paragraph) and substitution of the C$^{\alpha}$-hydrogen atom in α-amino-*n*-butyric acid (Abu) by an *n*-alkyl group (alkyl = *n*-propyl to *n*-hexyl) leads to the homologous series α-Prop-Abu to α-Hex-Abu (see also Fig. 1).

Derivatives of the following AAAs were investigated {for racemic AAAs the

prefix DL- is used as given by the manufacturers, for enantiomers (*R,S*)- is used in this paper in order to avoid ambiguities [24]} DL-α-methylaspartic acid (α-Me-Asp), DL-α-methylglutamic acid (α-Me-Glu), DL-α-methylornithine (α-Me-Orn), DL-α-methylserine (α-Me-Ser), DL-α-methyl-*m*-methoxyphenylalanine (α-Me-*m*-PheOMe), DL- and L-α-methyltyrosine (α-Me-Tyr) and DL- and L-α-methyl-(3,4-dihydroxyphenyl)alanine (α-Me-Dopa) were purchased from Sigma (St. Louis, MO, U.S.A.), DL-α-methylleucine (α-Me-Leu) from Bachem (Bubendorf, Switzerland) and DL-α-ethylphenylglycine (α-Et-Phg) from EMKA Chemie (Markgröningen-Talhausen, F.R.G.).

Commercially unavailable AAAs were synthesized in our laboratory according to the Strecker procedure by reaction of the respective ketones (from Fluka, Buchs, Switzerland) with potassium cyanide and ammonium chloride and subsequent saponification of the nitriles with hydrochloric acid. The AAAs were liberated from the hydrochlorides by treatment with tributylamine and purified by crystallization from methanol–water. The following AAAs were synthesized (abbreviations and other names used in the relevant literature are given in parentheses): DL-α-ethylalanine (α-Et-Ala or isovaline, Iva), DL-α-propylalanine (α-Prop-Ala, α-methylnorvaline), DL-α-butylalanine (α-But-Ala, α-methylnorleucine), DL-α-pentylalanine (α-Pent-Ala, 2-methyl-2-aminoheptanoic acid), DL-α-hexylalanine (α-Hex-Ala, 2-methyl-2-aminooctanoic acid), DL-α-heptylalanine (α-Hept-Ala, 2-methyl-2-aminononanoic acid), DL-α-octylalanine (α-Oct-Ala, 2-methyl-2-aminodecanoic acid), DL-α-nonylalanine (α-Non-Ala, 2-methyl-2-aminoundecanoic acid), DL-α-propyl-α-amino-*n*-butyric acid (α-Prop-Abu, α-ethylnorvaline), DL-α-butyl-α-amino-*n*-butyric acid (α-But-Abu, α-ethylnorleucine), DL-α-pentyl-α-amino-*n*-butyric acid (α-Pent-Abu, 2-ethyl-2-aminoheptanoic acid) and DL-α-hexyl-α-amino-*n*-butyric acid (α-Hex-Abu, 2-ethyl-2-aminooctanoic acid). Pure enantiomers of α-Et-Ala and α-Prop-Ala were obtained by digestion of the respective N-chloroacetyl- or N-trifluoroacetyl derivatives with acylase I or carboxypeptidase [21]. L-α-Me-Leu, L-α-Me-Phg, L-α-Me-Tyr-Ome, L-α-Et-Phe, DL-α-Et-Phe, L-α-Me-*homo*-Phe and DL-α-Me-*homo*-Phe were gifts from Dr. J. Kamphuis (DSM Research, Geleen, The Netherlands) (configuration as designated by the supplier). (*S*)(+)-2-Butanol, (*S*)(+)-2-pentanol, (*S*)(+)-2-octanol, (*R,S*)-2-dodecanol, (*R,S*)-3-octanol and (*R,S*)-3,3-dimethyl-2-butanol (pinacolic alcohol) were obtained from Fluka, (*S*)(+)-3-methyl-2-butanol, (*S*)(+)-2-hexanol and (*S*)(+)-2-heptanol from Chemical Dynamics (EHEMALOG) (South Plainfield, NJ, U.S.A.) and (1*R*,2*S*,5*R*)(−)-menthol from Aldrich (Steinheim, F.R.G.). (*S*)(+)-3-Methyl-2-butanol and (*S*)(+)-2-pentanol were also synthesized in our laboratory according to procedures described in the literature [27]. Pentafluoropropionic anhydride (PFPA) was obtained from Pierce (Rockford, IL, U.S.A.), acetyl chloride of pro analysi (p.a.) grade from Fluka and dichloromethane (p.a.) from Merck (Darmstadt, F.R.G.).

Procedures for derivatization of amino acids for GC

Aliphatic and aromatic AAAs. About 0.6 mg of the AAAs in 0.1 ml of a 20% (v/v) solution of acetyl chloride in the respective alcohol was sonicated and heated for 1 h in a 1-ml Reacti-vial (Wheaton, Milleville, NJ, U.S.A.). The mixture was evaporated to dryness in a stream of dry nitrogen, the residue was dissolved in 100 μl of dichloromethane (DCM) and 25 μl of PFPA were added. After 20 min at 100°C the reagents were removed in a stream of nitrogen, the residue was dissolved in 50 μl ml of DCM and 0.8-μl portions were subjected to GC in the split mode.

TABLE I

COMPARISON OF NET RETENTION TIMES, t (min), AND RESOLUTION FACTORS, α, AT THE TEMPER-ATURE T (°C) OF N(O)-PENTAFLUOROPROPIONYL DERIVATIVES OF (R,S)-α-ALKYL-α-AMINO ACID (S) (+)-2-ALKYL ESTERS [(R,S)-AAAs] ON DIMETHYLPOLYSILOXANE (CP-SIL 5)

Carrier gas, helium (100 kPa). Resolution factors in italics are highest factors in the series of alcohols.

(R,S)-AAA	2-Butyl ester			3-Methyl-2-butyl ester			2-Pentyl ester		
	t	T	α	t	T	α	t	T	α
α-Et-Ala	12.20 12.40	80	1.016	7.30 7.45	100	1.021	7.81 8.00	100	*1.024*
α-Prop-Ala	10.97 11.13	90	1.015	8.37 8.53	105	1.019	6.87 7.13	110	*1.038*[+a]
α-But-Ala	7.24 7.40	110	1.022	8.52 8.78	115	1.031[-]	7.06 7.43	120	*1.052*[+]
α-Pent-Ala	7.64 7.83	120	1.025	8.71 9.01	125	1.034[-]	5.90 6.21	135	1.053[+]
α-Hex-Ala	7.94 8.16	130	1.028	8.93 9.24	135	1.035[-]	7.48 7.90	140	1.056[+]
α-Hept-Ala	8.21 8.43	140	1.027	9.08 9.38	145	1.033[-]	7.61 8.02	150	1.054[i]
α-Oct-Ala	6.84 7.01	155	1.025	9.28 9.58	155	1.032[+]	6.39 6.69	165	1.047[+]
α-Non-Ala	6.89 7.09	165	1.029	9.20 9.46	165	1.028[+]	5.12 5.33	180	1.041[i]
α-Prop-Abu	8.23 —	105	n.r.[b]	9.91 —	110	n.r.	10.36 10.54	110	*1.017*
α-But-Abu	8.44 —	115	n.r.	9.93 —	120	n.r.	10.20 10.53	120	1.032[+]
α-Pent-Abu	8.65 8.78	125	1.015	9.94 10.08	130	1.014	10.25 10.67	130	1.041[+]
α-Hex-Abu	8.87 9.02	135	1.017	9.96 10.13	140	1.017	10.27 10.70	140	1.042[+]
α-Me-Leu	7.49 7.66	105	1.023	7.15 7.37	115	1.031[+]	7.44 7.72	115	*1.038*[+]
α-Me-Met	6.58 6.77	135	1.029	7.48 7.79	140	1.041[+]	7.41 7.86	140	1.061[i]
α-Me-Ser	8.53 8.81	95	1.033	7.79 8.06	105	1.035[i]	6.44 6.81	110	1.057[+]
α-Me-Asp	10.50 10.65	130	1.014		—[c]		8.85 9.14	150	*1.033*[+]
α-Me-Glu	8.04 8.17	150	1.016	8.08 8.21	165	1.016	6.79 7.05	170	1.038[i]
α-Me-Orn	6.57 6.75	145	1.027	8.95 9.22	145	1.030[i]	5.78 6.14	155	1.062[+]

2-Hexyl ester			2-Heptyl ester			2-Octyl ester		
t	T	α	t	T	α	t	T	α
8.56 8.73	110	1.020	9.16 9.32	120	1.017	9.72 9.86	130	1.014
7.35 7.59	120	1.033$^+$	6.32 6.51	135	1.030^1	8.07 8.30	140	1.029$^+$
7.32 7.64	130	1.044$^+$	7.41 7.77	140	1.049$^+$	7.48 7.84	150	1.048$^+$
5.91 6.20	145	1.049^1	7.26 7.71	150	*1.062$^+$*	7.15 7.58	160	1.060$^+$
5.86 6.16	155	1.051$^-$	5.73 6.08	165	1.061$^+$	6.90 7.36	170	*1.067$^+$*
5.93 6.25	165	1.054$^-$	5.64 6.00	175	1.064$^+$	6.62 7.09	180	*1.071$^+$*
5.93 6.25	175	1.054$^+$	5.51 5.85	185	1.062$^+$	6.37 6.80	190	*1.068$^-$*
6.98 7.35	180	1.053$^+$	6.46 6.87	190	1.063^1	6.05 6.46	200	*1.068$^+$*
10.98 11.08	120	1.009	11.26 11.45	130	*1.017*	9.15 9.31	145	*1.017*
10.51 10.82	130	1.029^1	10.42 10.85	140	*1.041$^+$*	8.38 8.72	155	*1.041$^+$*
10.28 10.71	140	1.042$^+$	9.89 10.45	150	1.057$^+$	9.68 10.26	160	*1.060$^+$*
9.98 10.44	150	1.046$^+$	9.47 10.08	160	1.064$^+$	9.09 9.72	170	*1.069$^+$*
7.82 8.05	125	1.029$^+$	8.15 8.40	135	1.031$^+$	8.24 8.46	145	1.027$^+$
7.38 7.83	150	1.061$^-$	7.50 8.00	160	*1.067$^+$*	7.16 7.63	170	1.066^1
6.64 7.03	120	1.059$^-$	6.75 7.22	130	*1.070^1*	7.10 7.60	140	*1.070$^+$*
8.01 8.20	170	1.024	7.17 7.30	190	1.018	9.56 9.87	200	1.032
7.01 7.34	185	1.047$^-$	7.44 7.86	200	1.056$^+$	9.26 9.84	210	*1.063$^+$*
5.41 5.81	165	1.074$^-$	5.12 5.57	175	*1.088$^-$*		$-^c$	

TABLE 1 (continued)

(R,S)-AAA	2-Butyl ester			3-Methyl-2-butyl ester			2-Pentyl ester		
	t	T	α	t	T	α	t	T	α
α-Me-Phg	6.37 6.52	140	1.024	7.06 7.25	145	1.027[1]	7.12 7.60	145	1.067[+]
α-Et-Phg	8.75 8.89	140	1.016	9.66 9.78	145	1.012	7.98 8.42	150	1.055[+]
α-Me-Phe	9.60 9.83	140	1.024	8.60 8.93	150	1.038[+]	8.96 9.12	150	1.018
α-Et-Phe	8.72 8.88	150	1.018	7.86 8.05	160	1.024	8.05 8.14	160	1.011
α-Me-homo-Phe	7.53 7.69	160	1.021	8.17 8.38	165	1.026	8.24 8.64	165	1.049[+]
α-Me-p-Tyr	6.79 6.92	160	1.019	8.82 9.06	160	1.027	7.39 7.47	165	1.011
α-Me-m-Tyr	6.21 6.40	160	1.031	8.08 8.46	160	1.047[1]	6.95 7.06	165	1.016
α-Me-p-TyrOMe	7.29 7.43	170	1.019	9.59 9.85	170	1.027[1]	9.90 —	170	n.r.
α-Me-m-PheOMe	6.65 6.77	170	1.018	7.25 7.45	175	1.028[+]	8.90 8.97	170	1.008
α-Me-Dopa	7.97 8.15	160	1.023	8.01 8.25	165	1.030[+]	8.51 —	165	n.r.

[a] Baseline resolution is indicated by a superscript "plus" sign.

[b] n.r. = Not resolved.

[c] No derivatization achieved under the conditions used.

Acidic and basic AAAs. About 0.6 mg of the amino acid in 300 μl of 2.5 M hydrochloric acid in methanol were heated in a 1-ml Reacti-vial at 100°C for 1 h. The reagents were removed in a stream of nitrogen and the resulting methyl ester was transesterified by addition of 100 μl of acetyl chloride in the respective alkanol by heating at 100°C for 1 h. Acylation with PFPA was carried out as described above. Derivatization of sparingly soluble AAAs was performed by stirring with a PTFE-coated, triangular stirring bar. Derivatization procedures and treatment with nitrogen were carried out in a heating/stirring module from Pierce equipped with an aluminium block.

2-Hexyl ester			2-Heptyl ester			2-Octyl ester		
t	T	α	t	T	α	t	T	α
7.09 7.52	155	1.061^+	6.97 7.45	165	1.069^1	5.82 6.20	180	1.065^+
7.95 8.36	160	1.052^+	7.63 8.12	170	1.064^+	7.45 7.95	180	1.067^+
8.96 9.12	160	1.018	8.70 8.87	170	1.020	8.45 8.59	180	1.017
7.79 7.93	170	1.018	9.17 9.38	175	1.023	10.93 11.18	180	1.023
7.89 8.28	175	1.049^+	7.45 7.90	185	1.060^-	8.73 9.29	190	1.064^+
8.45 8.61	170	1.019	9.63 9.83	175	1.021	8.97 9.19	185	1.025
7.78 7.95	170	1.022	7.25 7.42	180	1.023	8.47 8.68	185	1.025
11.38	175	n.r.	12.95	180	n.r.	14.79 —	185	n.r.
10.33 10.42	175	1.009	11.99 12.13	180	1.012	13.68 13.80	185	1.009
7.30 7.40	175	1.014	8.29 8.42	180	1.016	7.63 7.78	190	1.020

RESULTS AND DISCUSSION

Selection of derivatives, stationary phase and GC conditions

In a previous paper [17], it was shown that satisfactory resolution of N(O)-perfluoroacyl-(S)(+)-2-alkyl esters of selected (R,S)-AAAs was achieved by use of capillary columns coated with CP-Sil 19 CB (Chrompack). In order to investigate systematically the influence of the respective alkyl esters on the resolution factors, we used the N(O)-PFP esters exclusively and 100% dimethylpolysiloxane (CP-Sil 5) as a standard stationary phase. Suitable isothermal temperatures (80–190°C) and carrier gas pressures were selected in order to obtain about equal retention times of *ca.* 7–12 min and the highest resolution factors for the various derivatives.

Resolution of N(O)-pentafluoropropionyl-α-alkyl-α-amino acid 2-alkyl esters

Derivatives of 28 AAAs were investigated. Retention times, isothermal temperatures and resolution factors (α) are given in Table I and for selected AAAs in Table II. AAAs are grouped as the α-alkylalanines and the α-alkyl-α-amino-*n*-butyric acids with increasing alkyl chain lengths, and then as neutral, acidic, basic and aromatic side-chain α-methyl amino acids (Table I; for nomenclature of AAAs, see Fig. 1 and Experimental). Derivatives of (R,S)-AAAs for which baseline resolution was achieved are indicated by a superscript "plus" sign on the resolution factors in the tables. Sections of gas chromatograms of PFP-(R,S)-AAA (S)(+)-2-octyl esters are shown in Fig. 2. Gas chromatograms of PFP-(R,S)-AAA (S)(+)-3-methyl-2-butyl esters [19] and selected gas chromatograms of the series (S)(+)-2-pentyl to (S)(+)-2-heptyl esters have been published elsewhere [21,22,24]. The influence of the structures of the 2-alkanols on the α values of the diastereomeric PFP-AAA 2-alkyl esters is discussed below.

(S)(+)-2-Butyl esters [14,27–30]. Most AAAs were separated more or less satisfactorily, but in no case was complete baseline resolution achieved. α-Prop-Abu and α-But-Abu were not resolved. In most instances the use of these esters is less advantageous in comparison with those described below.

(S)(+)-3-Methyl-2-butyl esters [27,29–31]. In comparison with the (S)(+)-2-butyl esters an increase of α was achieved in most instances together with baseline resolution of the enantiomers in many instances. However, α-Prop-Abu and α-But-Abu were again not resolved. α-Me-Phg, α-Me-Phe, α-Et-Phe, α-Me-*m*-PheOMe and Me-Dopa were baseline resolved and the best resolution of α-Me-Tyr and analogs was also obtained by use of this reagent. The significantly increased resolution of AAAs by esterification with branched 2-alkanols is also demonstrated for (R,S)-3,3-dimethyl-2-butanol (pinacolic alcohol) in some selected cases (Table II). This reagent provides the highest resolution factors for α-Et-Ala and α-Me-Phe. However, this alcohol also shows significant kinetic discrimination [30,32].

(S)(+)-2-Pentyl esters [14,27,29,30]. Most aliphatic side-chain AAAs showed baseline resolution with this reagent. α-Me-Phg, α-Et-Phg and α-Me-*homo*-Phe showed baseline resolution, in contrast to α-Me-Phe, α-Et-Phe and hydroxylated aromatic AAAs, which exhibited incomplete separation. This behaviour was also found for the (S)(+)-2-hexyl, (S)(+)-2-heptyl and (S)(+)-2-octyl esters of these aromatic AAAs.
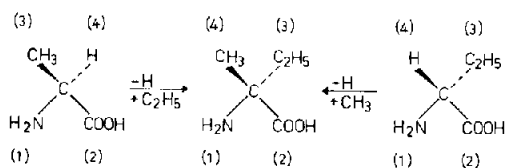

Fig. 1. (R)-α-Ethylalanine (α-Et-Ala, isovaline), as an example of an α-alkyl-a-amino acid, might be *formally* derived from either L-Ala [= (S)-Ala] by C^α-hydrogen substitution by an ethyl group, or from D-Abu [= (R)-Abu] (Abu, α-amino-*n*-butyric acid) by C^α-hydrogen substitution by a methyl group. Numbers indicate the priority of C^α substituents according to the Cahn-Ingold Prelog rule. Formal substitution of the C^α-hydrogen by alkyl groups in Ala and Abu leads to the series α-alkyl-Ala and α-alkyl-Abu, respectively, or, in general, to α-alkyl-α-amino acids.
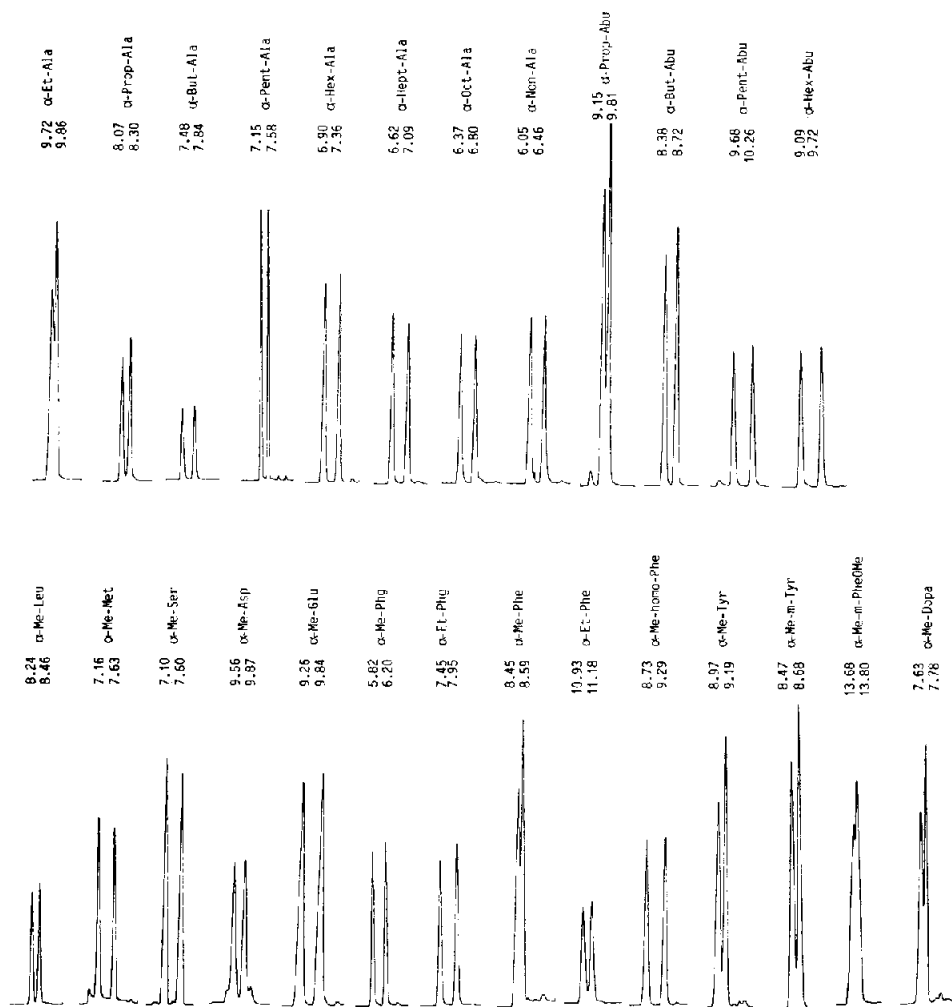
Fig. 2. GC of N(O)-pentafluoropropionyl-(R,S)-α-alkyl-α-amino acid (S)(+)-2-octyl esters on dimethyl-polysiloxane (CP-Sil 5). Net retention times (min) at isothermal temperatures (cf., Table I) are given. Carrier gas, helium at 100 kPa (1.0 bar).

(S)(+)-2-Hexyl [14,29], (S)(+)-2-heptyl [14] and (S)(+)-2-octyl esters [14,28,30]. In comparison with the pentyl esters, higher α were found for these esters for aliphatic AAAs. α-Me-Phg, α-Et-Phg and α-Me-*homo*-Phc were baseline resolved and the other aromatic AAAs were partially resolved by use of these alcohols, with the exception of α-Me-Tyr-OMe, which remained unresolved. In most instances increasing α values were found in the series of pentyl to octyl esters, with the highest α values for (S)(+)-2-octyl esters (Fig. 2). An exception is α-Et-Ala, which exhibited decreasing α values on going from the pentyl to the octyl ester.

(R,S)-2-Dodecyl, (R,S)-3-octyl [30] and (R,S)-3,3-dimethyl-2-butyl esters [27,29,30]. [It is understood that by derivatization of (S)-AAAs with (R,S)-2-alka-

TABLE II

COMPARISON OF NET RETENTION TIMES, t (min), AND RESOLUTION FACTORS, α, AT THE TEMPERATURE T (°C) OF N(O)-PENTAFLUOROPROPIONYL DERIVATIVES OF (S)-α-AL-KYL-α-AMINO ACID (R,S)-2-ALKYL ESTERS [(S)-AAAs] ON CP-SIL 5

Carrier gas, helium (100 kPa).

(S)-AAA	2-Dodecyl ester			3-Octyl ester			3,3-Dimethyl-2-butyl ester		
	t	T	α	t	T	α	t	T	α
α-Et-Ala	10.16 —	170	n.r.[a]	8.75 —	130	n.r.	9.26 9.48	100	1.024[+b]
α-Me-Leu	8.31 8.51	185	1.024[+]	7.84 7.94	145	1.013	7.69 7.86	120	1.022
α-Me-Phe	7.15 7.36	220	1.029[+]	7.90	180	n.r.	10.97 11.59	150	1.057[+]
α-Me-Tyr	8.05 8.41	220	1.045[+]	8.37 —	185	n.r.	7.18 7.56	170	1.053[+]

[a] n.r. = not resolved.

[b] Baseline resolution is indicated by a superscript "plus" sign.

nols actually the 2-alkanols are resolved. As α does not change when (R,S)-AAAs are resolved with (R) or (S)-2-alkanols, respectively, for simplicity the terminology that follows is used]. This tendency for higher α values with increasing chain length of the 2-alkanols is exemplified by (R,S)-2-dodecanol for selected (S)-AAAs, giving baseline resolution for α-Me-Leu, α-Me-Phe and α-Me-Tyr (Table II). α-Et-Ala was not resolvable with (R,S)-2-dodecanol. (R,S)-3-Octanol, in striking contrast to (R,S)-2-octanol, gave no resolution for α-Et-Ala, α-Me-Phe and α-Me-Tyr and low resolution for α-Me-Leu (Table II). Remarkably, (R,S)-3,3-dimethyl-2-butanol gave the highest α values for all derivatives investigated of α-Et-Ala, α-Me-Phe and α-Me-*homo*-Phe, but not for α-Me-Leu (cf., Tables I and II). α-Prop-Abu was partially resolved by 3,3-dimethyl-2-butanol, but the resolution coefficient ($\alpha = 1.014$) was lower than that with 2-pentanol ($\alpha = 1.017$) under the same conditions (cf., Table I). By analogy with protein amino acids, significant diastereomeric fractionation would be expected for AAAs by esterification with this sterically highly constrained alkanol [30,32].

(1R,2S,5R)(−)-Menthyl esters [27,33]. Excellent resolution was achieved for aromatic AAAs by esterification with (−)-menthol, in particular for those which were unsatisfactorily resolved by $(S)(+)$-2-alkanols (Table III). However, no separation was obtained for α-alkylalanines by esterification with (−)-menthol and, as a major disadvantage, low derivatization yields were obtained with this reagent, which did not increase on transesterification of the respective methyl esters.

Relative derivatization yields of AAAs with alcohols

The relative derivatization yields of the isomeric amino acids (assumed to give equal flame ionization detector responses) valine (Val), norvaline (Nva) and isovaline (Iva) were investigated on CP-Sil 5 by derivatization with 1- and 2-propanol. With

(R,S)-AAA	t	T	α	(R,S)-AAA	t	T	α
α-Et-Phg	7.10 7.31	195	1.030^{+a}	α-Me-p-Tyr	7.74 8.11	200	1.048^{+}
α-Me-Phe	9.73 10.11	190	1.039^{+}	α-Me-m-Tyr	7.55 8.03	200	1.064^{+}
α-Et-Phe	8.36 8.70	200	1.041^{+}	α-Me-p-TyrOMe	9.02 9.31	210	1.032^{1}
α-Me-$homo$-Phe	10.23 10.39	200	1.016	α-Me-m-PheOMe	8.32 8.71	210	1.047^{+}

^{a} Baseline resolution is indicated by a superscript "plus" sign.

1-propanol, Nva (unbranched side-chain) and Val (branched side-chain), both having C^{α}-hydrogen atoms, gave equal peak areas in GC, and are therefore assumed to give 100% relative derivatization yields (r.d.y.), whereas Iva (alkyl-substituted C^{α}-hydrogen atoms) gave 80% r.d.y. With 2-propanol, Nva gave 100%, Val 77% and Iva 37% r.d.y. This clearly shows the steric hindrance resulting from the side-chain branching of the amino acids and from the branching of the alcohols. Further, by analogy with protein amino acids [30], for the determination of the ratios of enantiomers of AAAs using the diastereomeric approach a possible kinetic discrimination [30,32] in the esterification process has to be evaluated, in particular for highly branched alcohols such as 3,3-dimethyl-2-butanol.

Elution order and assignment of absolute configuration of AAAs

As pure or enriched enantiomers of AAAs (*cf.*, Experimental) were available, the elution order of enantiomers as their PFP-AAA $(S)(+)$-2-alkyl esters was determined unambiguously for these AAAs. In all instances the (S)-AAAs eluted before the (R)-AAAs when using $(S)(+)$-2-alkanols, and (R)-AAAs before (S)-AAAs after esterification with $(R)(-)$-2-butanol. By analogy this is assumed to be valid for all AAAs investigated and also for AAAs which are structurally related. This elution order of AAAs is the opposite of that of amino acids having a C^{α}-hydrogen atom. The elution order, optical rotation and absolute configuration of AAAs have also been correlated by chiral ligand-exchange chromatography [20].

Configuration and elution order of α-alkyl-α-amino acids as compared with protein amino acids

Feibush and Gil-Av [34] presented a model for the elution order of enantiomeric N-TFA-amino acid esters of the general type $CF_3CONHCHRCOOR'$ on a chiral "ureido" phase. They postulated that the order of emergence is dependent on the relative effective size of the substituents H, R and COOR' at the asymmetric carbon
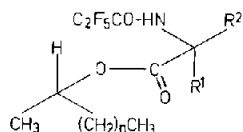
Fig. 3. Scheme of spatial arrangement and bulkiness chirality of N-PFP-(S)-α-amino acid (S)(+)-2-alkyl esters for α-amino acids (R^1 = H, R^2 = alkyl, alkaryl) and for α-alkyl-α-amino acids (R' = Me, Et; R^2 = alkyl, alkaryl, bulkiness of R^2 > R^1).

atom. Feibush [35] applied a similar model to the separation of diastereomeric α-ace-toxypropionic 2-alkyl esters and found for the LD (= S,S) isomers, which showed the longer retention time of the LD–DD pair, that the substituents on both asymmetric carbon atoms are arranged in the same direction with regard to their size in decreas-ing order ("bulkiness chirality") when viewing the molecule along its long axis.

This is also true for diastereomeric esters of proteinogenic amino acids, but the corresponding α-methyl-α-amino acids show the opposite order of elution, although the respective derivatives of amino acids and AAAs do have the same bulkiness chirality (Fig. 3). On chiral stationary phases, however, enantiomeric esters of amino acids and AAAs, such as N-TFA-1-propyl esters, show the same elution order for R- and S-enantiomers, respectively.

CONCLUSIONS

Systematic investigation of the separation of 28 diastereomeric N(O)-PFP-(R,S)-AAA (S)(+)-2-alkyl esters [series (S)(+)-2-butanol to (S)(+)-2-octanol and (S)(+)-3-methyl-2-butanol; with in selected instances the use of 3,3-dimethyl-2-buta-nol, (R,S)-3-octanol, (R,S)-2-dodecanol and (−)-menthol] by GC on dimethylpolysi-loxane showed that no one alcohol made the baseline separation of all AAAs pos-sible, but that baseline resolution could be achieved for most AAAs by use of an appropriate alcohol (see tables and Fig. 2). The most difficult AAA to resolve as diastereomeric esters on CP-Sil 5 are those which have the relatively smallest differ-ences in sizes of the *geminal* alkyl groups, i.e., α-Et-Ala (Iva) and α-Prop-Abu (cf., Tables I and II).

For determinations of the ratios of enantiomers in a certain (R,S)-AAA, not only the optical purity of the alkanol employed but also the kinetic discrimination in the esterification process as a function of the branching of the alcohol and of the side-chains of AAAs have to be determined.

(S)-AAAs are eluted before (R)-AAAs in the cases of diastereomeric PFP-(R,S)-AAA (S)(+)-alkyl esters and the use of achiral stationary phases. This is the opposite elution order to that of α-amino acids having a C$^\alpha$-hydrogen atom, where R-(= D) enantiomers elute before S-(= L) enantiomers. However, with respect to their bulkiness chirality [35] (i.e., arrangement of alkyl and aryl substituents in de-creasing order; cf., Fig. 3), PFP-(S)-AAA (S)(+)-2-alkyl esters show the same elution order as PFP-(R)-AA (S)(+)-2-alkyl esters.

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