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OLIGO-OXAALKANOYL TETRAAMIDES DERIVED FROM L-PHENYLALANINE AS STATIONARY PHASES IN CAPILLARY GAS CHROMATOGRAPHIC RESOLUTION OF D,L-AMINO ACIDS

GERARDO PALLA*, ARNALDO DOSSENA and ROSANGELA MARCHELLI
Istituto di Chimica Organica dell'Università, Via Massimo D'Azeglio 85, 43100 Parma (Italy)

SUMMARY

The resolution of enantiomers of (±)-α-amino acid derivatives by capillary gas chromatography was studied using a series of optically active tetraamide stationary phases derived from L-phenylalanine, $[(CH_3)_3\ CNHC\ (O)\ CH\ (CH_2C_6H_5)\ NHC\ (O)\ CH_2]_2\ X$ with X = O, $O(CH_2CH_2O)_n$ and n = 1–3. The effects of the structure of X on the separation factors are reported; the tetraamide phases were stable at high temperature (200°C) and allowed the separation of amino acid derivatives of high and low volatility in one run.

INTRODUCTION

The use of chiral amide stationary phases for the gas chromatographic resolution of (±)-α-amino acid derivatives is a well known method and has recently been reviewed.[1] The technique is successful in many instances also for the separation of chiral hydroxy acid derivatives[2], amines[3], amino alcohols and sugars[4]. The mechanism of resolution has been studied, and stereoselective hydrogen bonding associations have been suggested to be responsible for the separation[5,6]. However, the known non-bonded amide phases could not be used at the high temperatures required for the resolution of amino acids of low volatility as they lack thermal stability; to solve the problem, binding processes of chiral amides to silicone phases have been developed, resulting in stable derivatives, such as the commercially available Chirasil-Val[7].

As a contribution to the understanding of the phenomenon of chiral resolution via hydrogen bonding, we utilized a series of dicarboxylic chiral ligands, containing L-phenylalanine, which had been previously synthesized to study the selective complexation and extraction of ions from aqueous into organic solutions[8]. We derivatized these ligands with *tert.*-butylamine and tested the corresponding tetraamides (Fig. 1) as a new chiral stationary phase for the resolution of D,L-amino acids by capillary gas chromatography.

We also studied the influence of the bridge binding the two amide chains and determined the dimensions corresponding to optimal resolution. Tetraamides with L-valine and diamides with an oxaalkane chain were also synthesized and compared with the corresponding L-phenylalanine derivatives.

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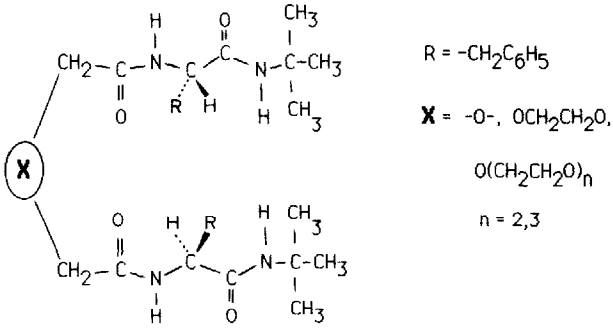

Fig. 1. Structure of the oligo-oxaalkanoyl tetraamides used as stationary phases.

EXPERIMENTAL

Instruments

IR spectra were recorded with a Perkin-Elmer 298 instrument in liquid films (KBr for ligand 5). ^{1}H NMR spectra were recorded on a Varian 360 instrument at a frequency of 60 MHz. $[\alpha]_D$ values were measured on a Rudolph Research Polarimeter III at 20°C in 95% ethanol. Melting points are uncorrected (Büchi apparatus). Preparative HPLC was carried out with a Waters Model 440 liquid chromatograph, equipped with a UK-6 septumless injector, a 6000 A pump and a dual-wavelength (254 and 280 nm) UV detector, using a Perkin-Elmer C_{18} reversed-phase column (25 × 2.5 cm I.D.).

Gas–liquid chromatography (GLC)

Capillary GLC was carried out with a Dani 3900 instrument, equipped with a flame-ionization detector, using glass columns (25 m × 0.3 mm I.D.) with injector and detector temperatures of 250°C and helium at a pressure of 0.6 atm. The glass columns were leached with 20% HCl at 180°C, deactivated with barium carbonate[9] and statically wall-coated with a dichloromethane solution of the chiral amide phase (1–8) (0.15%) and of a commercial silicone gum (OV-101) (0.15%). The columns were conditioned for 10 h at 180°C (phases 1–6) or at 160°C (phases 7 and 8).

Materials

The starting compounds N,N′-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), amino acids, trifluoroacetic anhydride (TFAA), heptafluorobutyric anhydride (HFBA), oxalyl chloride, glycols and suberic acid were commercial products.

Synthesis of the tetraamide ligands 1–4 and 6. Ligands were synthesized by allowing L-phenylalanine (ligands 1–4) or L-valine (ligand 6) sodium salts to react with the bis-acid chlorides $X(CH_2COCl)_2$, obtained from the corresponding $X(CH_2CH_2OH)_2$ glycols by reaction with nitric acid and then with oxalyl chloride, as previously described[8]. The dicarboxylic chiral ligands were purified by preparative reversed-phase high-performance liquid chromatography and then dissolved (10 mmol) in dry dioxane (50 ml) and treated at 20°C with DCC (22 mmol) and NHS (20 mmol). The solution was stirred overnight. Dicyclohexylurea was filtered off and

the solution containing the active bis-succinimidic esters was added directly to a cooled solution of *tert.*-butylamine (20 mmol) dissolved in dioxane (20 ml). After 10 h the solution was concentrated under vacuum, poured into ice water (300 ml) at pH 2.0 and extracted twice with 50-ml volumes of dichloromethane. The organic solution was dried, concentrated and flash-chromatographed on a silica gel column (20–40 μm; 20 × 3.5 cm I.D.), with *n*-hexane–ethyl acetate (7:3, v/v) as the eluent. The pure tetraamides thus obtained (total yield 50–60%) were characterized by IR and NMR spectroscopy and microanalysis.

Synthesis of the tetraamide 5. Tetraamide 5 was synthesized by allowing L-phenylalanine sodium salt to react with suberic acid bis-chloride, and proceeding as described for the synthesis of ligands 1–4.

Synthesis of the diamides 7 and 8. The ligands were synthesized by condensing the L-phenylalanine or L-valine sodium salt with $CH_3OCH_2CH_2OCH_2CH_2OCH_2COCl$ obtained by oxidation of the triethyleneglycol monomethyl ether with nitric acid and subsequent treatment with thionyl chloride. The chiral carboxylic acid so obtained was transformed into the active ester by reaction with equimolecular amounts of NHS and DCC, proceeding as reported above for the synthesis of tetramides.

Properties. The main properties of the chiral amides 1–8 obtained are reported in Table I. The ^1H NMR chemical shifts ($CDCl_3$, δ ppm down field from TMS, 60 MHz) are as follows: (1) 1.15 [18H, s, $C(CH_3)_3$], 3.0 (4H, d, $CH_2C_6H_5$), 4.10 (4H, s, CH_2CO), 4.8 (2H, m, CH), 6.0 (2H, s, broad, NH), 7.1–7.2 (10H, s, broad, arom.), 7.5 (2H ,d, broad, NH); (2) 1.15 [18H, s, $C(CH_3)_3$], 3.0 (4H, d, $CH_2C_6H_5$), 3.5 (4H, s, $-CH_2CH_2-$), 3.9 (4H, s, CH_2CO); 4.5 (2H, m, CH); 5.5 (2H, s, broad, NH), 7.1–7.2 (10H, s, broad, arom.), 7.3 (2H, d, NHJ); (3) 1.20 [18H, s, $C(CH_3)_3$], 3.0 (4H, d, $CH_2C_6H_5$), 3.4 (8H, s, $-CH_2CH_2-$), 3.8 (4H, s, CH_2CO), 4.8 (2H, m, CH), 6.4 (2H, s, broad, NH), 7.1–7.2 (10H, s, broad, arom.), 7.5 (2H, d, NH); (4) 1.15 [18H, s, $C(CH_3)_3$], 3.0 (4H, d, $CH_2C_6H_5$), 3.65 (12H, s, broad, $-CH_2CH_2-$), 4.6 (2H, m, CH), 5.90 (2H, s, broad, NH), 7.1–7.2 (10H, s, broad, arom.), 7.4 (2H, d, NH); (5) 1.2 [18H, s, $C(CH_3)_3$], 1.0–1.7 [8H, m, $-(CH_2)_4-$], 1.9–2.1 (4H, m, CH_2CO), 3.2 (4H, d, $CH_2C_6H_5$), 4.7 (2H, m, CH), 5.5 (2H, m, NH), 7.2–7.3 (2H, d, broad, NH), 7.3 (10H, s, broad, arom.); (6) 0.6–0.9 [12H, d, broad, $C(CH_3)_2$]; 1.35 [18H, s, $C(CH_3)_3$], 1.6–2.1 [2H, m, $CH(CH_3)_2$], 3.75 (8H, s, $-CH_2CH_2-$); 4.2 (4H, s, CH_2CO), 5.8 (2H, s, broad, NH), 7.6 (2H, d, broad, NH); (7) 1.25 [9H, s, $C(CH_3)_3$], 3.1 (2H, d, $CH_2C_6H_5$), 3.45 (3H, s, OCH_3), 3.7 (8H, s, $-CH_2CH_2-$), 4.07 (2H, s, CH_2CO), 4.65 (1H, m, CH), 5.75 (1H, s, broad, NH), 6.3 (1H, s, broad, NH); (8) 0.6–0.9 [6H, d, broad, $C(CH_3)_2$], 1.33 [9H, s, $C(CH_3)_3$], 1.6–2.1 [1H, m, $CH(CH_3)_2$], 3.45 (3H, s, OCH_3); 3.65–3.75 (8H, s, broad, $-CH_2CH_2-$), 4.10 (2H, s, $-CH_2CO$), 4.35 (1H, m, CH), 5.85 (1H, s, broad, NH), 6.8 (1H, d, broad, NH).

Synthesis of solutes. N-TFA- and N-HFB-amino acid isopropyl and *n*-butylesters were synthesized as described previously[10].

RESULTS AND DISCUSSION

The characteristics of the capillary columns and the resolution factors r(L/D) obtained for N-TFA-amino acid *n*-butyl esters on stationary phases 1–4 are given in Table II. Fig. 2 shows a chromatogram of several amino acid derivatives, recorded in 30 min by programming the temperature from 100 tot 200°C. The best resolution

TABLE I

PHYSICO-CHEMICAL PROPERTIES OF THE STATIONARY PHASES STUDIED (1–8)

No.	*Name*	*X*	*R*	*Mol. wt.*	*M.p. (°C)*	$[\alpha]_D^{20}$ *(ethanol)*	*Main IR bands (cm^{-1})*
1	Phe-1-O	–O–	$CH_2C_6H_5$	538.6	Wax	+7.6 ($c=5$)	3280, 2960–2900, 1645, 1540, 1220, 740,
2	Phe-2-O	OCH_2CH_2O	$CH_2C_6H_5$	582.7	Wax	+15.7 ($c=7$)	3280, 2960–2900, 1660–1640, 1540, 1220, 1110, 740–730, 695
3	Phe-3-O	$O(CH_2CH_2O)_2$	$CH_2C_6H_5$	626.7	Wax	+24.3 ($c=8$)	3300–3290, 2980–2880, 1645, 1545–1520, 1220, 1110, 700
4	Phe-4-O	$O(CH_2CH_2O)_3$	$CH_2C_6H_5$	670.8	Wax	+20.8 ($c=3$)	3300–3280, 2960–2860, 1660–1640, 1540–1520, 1220, 1110, 740, 700
5	Phe-2-C	$(CH_2)_4$	$CH_2C_6H_5$	578.7	218–219	−2.4 ($c=5$)	3240, 2900, 1730, 1630, 1540
6	Val-3-O	$O(CH_2CH_2O)_2$	$CH(CH_3)_2$	530.7	Wax	−10.9 ($c=3$)	3300, 2960–2860, 1740, 1650–1520, 1450, 1360, 1210
7*	Phe-diamide	–	$CH_2C_6H_5$	370.4	Wax	+3.9 ($c=4$)**	3280, 2920–2860, 1660–1640, 1520, 740, 700
8*	Val-diamide	–	$CH(CH_3)_2$	332.4	Wax	−3.6 ($c=2$)	3300, 2960–2860, 1740, 1650, 1520

* 7 = $CH_3OCH_2CH_2OCH_2CH_2OCH_2CONHCH(CH_2C_6H_5)CONHC(CH_3)_3$;
8 = $CH_3OCH_2CH_2OCH_2CH_2OCH_2CONHCH[CH(CH_3)_2]CONHC(CH_3)_3$.

** Ethyl acetate.

TABLE II

COLUMN CHARACTERISTICS AND RESOLUTION FACTORS FOR TFA-AMINO ACID *n*-BUTYL ESTERS

Column	*Length (m)*	*I.D. (mm)*	*HEPT × 10^3*	*Film thickness (μm)*	*Resolving power, r(L/D)**										
					150°C							*190°C*			
					Ala	*Thr*	*Val*	*Ile*	*Nva*	*Leu*	*Nleu*	*Met*	*Asp*	*Phe*	*Glu*
Phe-1-O	24	0.33	17	0.2	1.038	1.027	1.038	1.036	1.044	1.054	1.050	1.029	1.00	1.023	1.026
Phe-2-O	25	0.30	24	0.2	1.036	1.022	1.035	1.029	1.034	1.040	1.037	1.024	1.00	1.020	1.022
Phe-3-O	25	0.30	18	0.23	1.047	1.036	1.046	1.040	1.048	1.057	1.049	1.034	1.01	1.027	1.031
Phe-4-O	25	0.30	24	0.2	1.035	1.030	1.035	1.029	1.039	1.040	1.037	1.024	1.00	1.018	1.024
Phe-2-C	24	0.29	15	0.2	1.000	–	–	–	–	1.00	–	–	–	–	–

* Ratio of the corrected retention times of the L-enantiomer over that of the D-isomer.

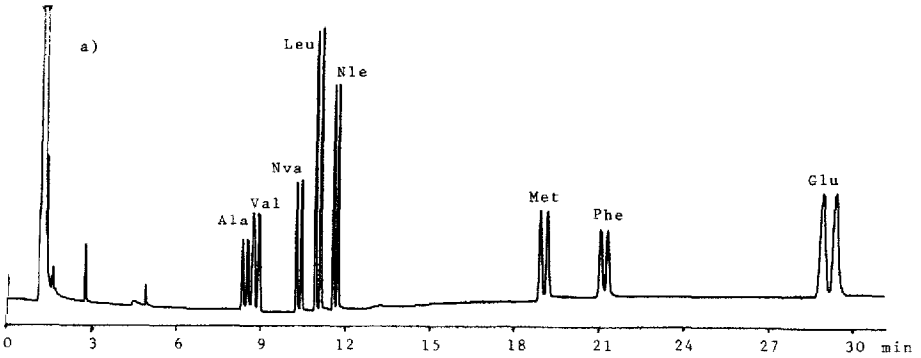

Fig. 2. Enantiomeric separation of N-TFA-amino acid *n*-butyl esters on a 25 m × 0.30 mm I.D. glass column wall-coated with the Phe-3-O (3) phase.

factors were obtained with the Phe-3-O ligand (3); the phases with a shorter (1,2) or longer bridge X (4) gave inferior results.

The resolving powers of stationary phases 1–5 were compared with those of the well established N-lauroyl-L-valyl-*tert.*-butylamide[11], which showed better resolution factors (R = 1.09–1.10 for Ala, Thr, Val, Ile, Nva, Leu, Nleu at 130°C). However, R factors for Met, Asp, Phe and Glu could not be measured because above 140°C the N-lauroyl phase bled to much.

By substituting the oxaalkane bridge X with the less polar $-(CH_2)_4-$ group (5), the resolution factors decreased dramatically. We think that this phenomenon is connected with the lower solubility of this ligand in the solvent used to fill the column, which is probably due to the formation of strong intramolecular hydrogen bonds between the two diamide chains. This reduces the chiral interactions and favours the formation of crystals along the column, thus decreasing the ability to cover the glass surface.

The oxaalkane bridge X seems to play an important role in chiral resolution, as it confers to the phase the waxy character necessary for a good dispersion during the column coating. The lower efficiency in chiral resolution shown by stationary

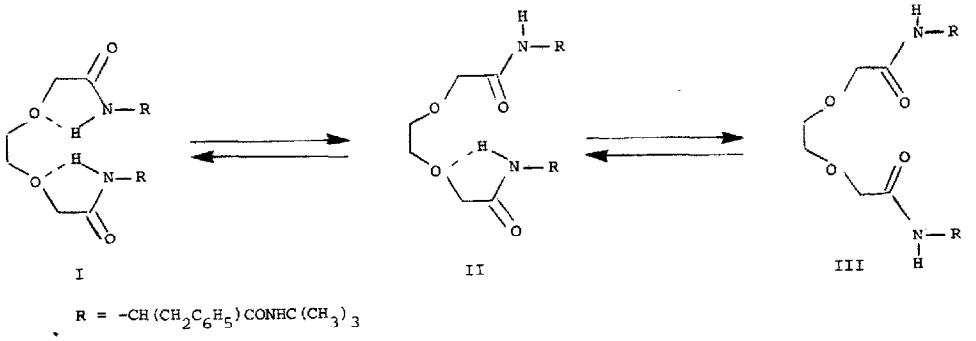

Fig. 3. Conformational behaviour expected for the Phe-2-O tetraamide ligand.

phases 1, 2 and 4 compared with 3 could be connected with the conformational equilibria that ligands undergo in solution. Intramolecular hydrogen bonding between the amide NH and the ethereal oxygens of the bridge X can maintain the two chains in a rigid conformation and at a certain distance from one another, thus influencing the formation of the chiral D–L and L–L associates between the stationary phase and the amino acid derivatives. Conformational studies by NMR of the dicarboxylic acid Phe-2-O provided evidence for the presence of an equilibrium between "open" and "closed" forms determined by intramolecular hydrogen bonding[8]. The tetraamide Phe-2-O, which gives the lowest resolution factors, could show similar equilibria (Fig. 3), with a favoured closed form I in less polar solvents, less available for chiral D–L or L–L associates, an intermediate form II with only one intramolecular hydrogen bond and an open form III, in which both the NH groups are well exposed to give chiral association with the solute.

Glass capillary wall-coated columns carrying the chiral stationary phase Phe-3-O (3) have been used at high temperatures (190–200°C) for long periods (100 h) without evidence of bleeding or reduction in the resolving power. Aspartic acid was only partially resolved and proline not at all; the detection of tryptophan required derivatization with 2-propanol and pentafluoropropionic anhydride, as the retention time of the N-TFA *n*-butyl ester derivative was too high, even at 200°C. GLC experiments performed with a maximum temperature of 220°C for several hours produced only a slow loss of resolution and a slight increase of the baseline.

The characteristics of the Phe-3-O phase were compared with those of the corresponding tetraamide ligand Val-3-O (6), synthesized from L-valine, usually considered to give more effective chiral ligands in amino acid resolution. However, Val-3-O gave resolutions of the same order as Phe-3-O and showed a lower resistance to temperature; the maximum operating temperature had to be reduced to 180°C. Diamide phases with L-Phe, L-Val and an oxadecanoyl chain were synthesized (ligands 7 and 8, Table I) to study the influence of the number of amide chains on the resolution factors. Although diamides 7 and 8 and tetraamides 3 and 6 gave similar resolution factors, the former required lower temperature limits, 180°C for ligand 7 and 160°C for ligand 8.

We also tried to prepare a bonded stationary phase starting from ligand 8 by deactivating the glass surface with Carbowax 1500, adding dicumyl peroxide (5%) to the stationary phase and slowly conditioning from 100 tot 160°C. The stability of the chiral phase towards higher temperatures actually increased, but the resolution slowly decreased to coalescence, and bleeding gradually became significant.

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