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INTERACTION BETWEEN ASYMMETRIC SOLUTES AND SOLVENTS

DIAMIDES DERIVED FROM L-VALINE AS STATIONARY PHASES IN GAS–LIQUID PARTITION CHROMATOGRAPHY

UZI BEITLER and BINYAMIN FEIBUSH*

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot (Israel)

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SUMMARY

The resolution of enantiomers of (±)-α-amino acid derivatives on optically active diamides derived from L-valine, $R_1CONHCH[CH(CH_3)_2]CONHR_2$, as gas chromatographic stationary phases was studied. The effects of the structure of R_1 and R_2 in the stationary phases, the structure of the racemic α-amino acid derivatives, temperature, etc., on the separation factors are reported. In the light of these and related results, a more probable mechanism of resolution is presented.

INTRODUCTION

The gas chromatographic (GC) resolution of N-TFA**-α-amino acid esters on optically active N-acyl-dipeptide O-alkyl esters was first reported in the period 1967–1970[1–4]. Later workers tried mainly to achieve the separation of all naturally occurring amino acids[5–8], but none used chemical variations of the stationary phases other than to use different analogous amino acids as components of the dipeptide moiety.

It was found that the selectivity of the dipeptide stationary phases is due essentially to the N-terminal amino acid, while the C-terminal group has only a minor effect on resolution[9,10]. In agreement with this observation, it was shown[11] that N-lauroyl-L-valine *tert.*-butylamide, a stationary phase that contains only one amino acid, but includes the necessary two amide functional groups and no other polar substituents, is superior to all previously reported dipeptide stationary phases. These findings were also deducible from the "1:1 three-hydrogen-bonded solute–solvent associate", regarded as the model for resolutions[4,6], which will be discussed later and modified to give a more appropriate model.

The GC resolution of enantiomers, beside its obvious practical value, elucidates stereochemical structural aspects. The resolution factors are directly related to the difference in the stereospecific interaction of the corresponding antipodic solutes

* Present adress: Intergen Research Ltd., 31 Bayit-Vegan Str., Jerusalem, Israel.
** TFA = trifluoroacetyl.

with the chiral stationary phases. Their dependence on structural changes may lead to a better understanding of the mechanism of resolution, the conformation of the interacting molecules and the nature of the aggregates in the melt. These aspects, in the particular system studied, involve the fit of antipodic α-amido ester solutes into the network of chiralic diamide solvents, *i.e.*, the spatial correlation of the different polar and apolar groups —interacting through attraction or repulsion— of the solute and solvent molecules.

EXPERIMENTAL

Instruments

The IR spectra were measured with a Perkin-Elmer Infracord instrument, the NMR data with a Varian A/60 instrument and the optical rotation with a Perkin-Elmer Model 141 polarimeter and a Schmidt & Haensch, Berlin S. GC was carried out on a Perkin-Elmer 801 chromatograph and a Varian Aerograph 1200 chromatograph adjusted to operate with capillary columns.

The chromatographic columns were stainless-steel capillaries, 150 ft. × 0.02 in. I.D., coated with the appropriate stationary phases by the plug method with 5% dichloromethane solutions. The temperatures used were: columns, 130° (except in a few instances specified in the text); injector and detector, 200°. The carrier gas was helium at a pressure of 10 p.s.i.

Materials

Compounds I–XIX (Table I) were commercial products. The melting and boiling points given below are not corrected.

Cyclooctanecarboxylic acid. 1-(N-Pyrrolidyl)-1-cyclohexene was prepared according to Szmuszkovicz[12] in 75% yield, b.p. 95–97° (3.5 mm). This compound was treated with acrolein to form 2-(N-pyrrolidyl)bicyclo-(3.3.1)-nonan-9-one in 55% yield, b.p. 125–127° (0.6 mm), which was converted by reaction with methyl iodide into 2-(N-pyrrolidyl)bicyclo-(3.3.1)-nonan-9-one methiodide[13], crystallized from light petroleum (b.p. 40-60°) in 80% yield, m.p. 216–217°. Under reflux in 20% sodium hydroxide solution, a 40% yield of 4-cyclooctenecarboxylic acid, b.p. 118–120°(0.5 mm), was obtained. This compound was hydrogenated in ethanol in the presence of 10% Pd/C to the corresponding cyclooctanecarboxylic acid, b.p. 98–100° (0.1 mm).

Dipentylacetic acid. Diethyl malonate, under reflux in methanol in the presence of 2 equiv. of sodium methoxide and 2 equiv. of 1-bromopentane, gave dimethyl dipentylmalonate, b.p. 104–105° (0.15 mm), which, after saponification and acidification, gave dipentylmalonic acid, m.p. 111.5–113°. This acid was decarboxylated to yield dipentylacetic acid, b.p. 117–119° (0.5 mm).

6-Undecylamine. This compound was prepared by the Leuckart method[14] from 6-undecanone (Fluka, Buchs, Switzerland).

N-Succinimido alkanoates (XX). A solution of 0.1 mole of the appropriate carboxylic acid and 0.1 mole of N-hydroxysuccinimide in 200 ml of dry chloroform was cooled to 10° and 0.1 mole of dicyclohexyl carbodiimide was added. The mixture was stirred for 12 h, filtered and the filtrate was concentrated under reduced pressure and diluted with dry diethyl ether. The mixture was filtered, the solvent removed under reduced pressure and the residue dispersed in light petroleum (b.p. 40–60°) for 3 h.

The precipitate was filtered and dried in a desiccator. The following N-succinimido compounds were prepared in 70–90% yield: isobutyrate, m.p. <35°; pivaloate, m.p. 69–70°; *tert.*-butylacetate, m.p. 111–112°; laurate, m.p. 73–74°; dipentylacetate, oil; cyclohexanecarboxylate, m.p. 83.5–85.5°; cycloheptanecarboxylate, m.p. 98–99°; cyclooctanecarboxylate, m.p. 87–89°; and 1-adamantanecarboxylate, m.p. 172–174°.

*N-Cbz**-L-*valine N-succinimido ester (XXI).* Was prepared in a similar manner from N-Cbz-L-valine (Miles-Yeda, Rehovot, Israel) and crystallized from diethyl ether, m.p. 113–114°, $[\alpha]_D^{18}$ −17.9° (c8 in $CHCl_3$)**.

*N-Cbz-*L-*valine alkylamide (XXII).* A solution of 0.1 mole of XXI in 500 ml of dry chloroform was cooled in an ice-bath and 0.15 mole of triethylamine and 0.1 mole of the appropriate alkylamine were added. The mixture was stirred for 36 h at 5° and the solvent removed under reduced pressure. The residue was dissolved in 300 ml of hot ethanol and 150 ml of water were slowly added. The mixture was stirred at room temperature for 3 h, filtered and the precipitate was washed with ethanol–water (2:1), dispersed in water for 2 h and air dried. When necessary, the product was further purified by chromatography on silica gel (Merck, Darmstadt, G.F.R.) containing an additional 6% of water, starting with *n*-hexane as eluent and increasing the polarity with ethyl acetate. The following N-Cbz-L-valine compounds were obtained in 75–85% yield: isopropylamide, m.p. 180–181°, $[\alpha]_D^{25}$ −12.2° (c8 in $CHCl_3$)**; *tert.*-butylamide, m.p. 108.5–109.5°, $[\alpha]_D^{25}$ −8.5° (c10 in $CHCl_3$)**; neopentylamide, m.p. 116.5–117.5°, $[\alpha]_D^{25}$ −21.6° (c10 in $CHCl_3$)**; *n*-dodecylamide, m.p. 126.5–127.5°, $[\alpha]_D^{23}$ −13.1° (c10 in $CHCl_3$)**; 6-undecylamide, m.p. 142–143°, $[\alpha]_D^{24}$ −5.8° (c8 in $CHCl_3$)**; cyclohexylamide, m.p. 202–204°, $[\alpha]_D^{24}$ −6.5° (c8 in $CHCl_3$)**; cycloheptylamide, m.p. 184–185°, $[\alpha]_D^{24}$ −11.6° (c8 in $CHCl_3$)**; and cyclooctylamide, m.p. 152.5–153.5°, $]\alpha]_D^{25}$ −15.2° (c10 in $CHCl_3$)**.

L-*Valine alkylamide (XXIII).* Hydrogen gas was bubbled at a low flow-rate for 4 h through an alcoholic solution of 0.2 mole of XXII containing 1.6 g of 10% Pd/C. The catalyst was filtered off and the solvent removed from the filtrate under reduced pressure to give XXIII quantitatively. It should be noted that before and after the use of hydrogen, the reaction mixture should be flushed with nitrogen.

*N-Acyl-*L-*valine alkylamide.* A solution of 0.02 mole of XXIII in 300 ml of dry chloroform was cooled in an ice-bath, stirred and 0.025 mole of triethylamine and 0.025 mole of the appropriate XX were added. The mixture was stirred at 5° for 36 h. The solvent was removed under reduced pressure, the residue dissolved in 200 ml of ethanol and 12 ml of concentrated ammonia solution were added to destroy the excess of XX. After stirring for 1/2 h, the precipitate was filtered, washed with water, dissolved in chloroform, extracted with 2% hydrochloric acid and 5% sodium hydrogen carbonate solution and dried on anhydrous magnesium sulphate. The compound that was recovered from an ether filtrate was crystallized from light petroleum (b.p. 40–60°). When it showed impurities, it was chromatographed under the conditions described previously. The following compounds were prepared (see Table I).

*N-Lauroyl-*L-*valine (XXIV).* This compound was prepared by the following

* Cbz = carbobenzoxy.

** Optical purity was not determined.

TABLE I

PHYSICAL PROPERTIES OF SOME OF THE STATIONARY PHASES STUDIED

Stationary phase	*Compound*	*M.p. (°C)*	*Optical purity (%)***	*$[\alpha]_D$ obs. in $CHCl_3$*	*Elemental analysis*					
					Found			*Calculated*		
					C(%)	*H(%)*	*N(%)*	*C(%)*	*H(%)*	*N(%)*
I	N-Lauroyl-L-valine amide	165–167 (d)*	70.1	$t = 26°C$ + 3.6° (c5)***	68.01	11.21	9.48	68.41	11.48	9.39
II	N-Lauroyl-L-valine isopropylamide	126.5–128	81.1	$t = 25°C$ −23.8° (c10)	69.99	11.57	7.89	70.54	11.84	8.23
III	N-Lauroyl-L-valine *tert.*-butylamide	<57	75.6	$t = 24°C$ −21.5° (c4)	71.32	11.91	7.83	71.13	11.94	7.90
IV	N-Lauroyl-L-valine neopentylamide	71.5–73.5	73.2	$t = 26°C$ −38.1° (c10)	71.53	12.25	7.87	71.68	12.03	7.60
V	N-Lauroyl-L-valine 6-undecylamide	81– 83	67.4	$t = 24°C$ −28.4° (c4)	74.31	12.25	6.10	74.28	12.47	6.19
VI	N-Lauroyl-L-valine cyclohexylamide	161.5–162.5	73.5	$t = 24°C$ −32° (c4)	72.58	11.85	7.38	72.58	11.65	7.36
VII	N-Lauroyl-L-valine cycloheptylamide	143–144	77.1	$t = 24°C$ −28.5° (c4)	72.80	11.60	6.90	73.04	11.75	7.10
VIII	N-Lauroyl-L-valine cyclooctylamide	113–114.5	80.2	$t = 25°C$ −26.1° (c9)	73.33	11.90	6.89	73.47	11.84	6.86
IX	N-Lauroyl-L-valine 1-adamantylamide	130–144	85.0	$t = 25°C$ −21.1° (c10)	74.75	11.15	6.31	74.95	11.18	6.48

X	N-Lauroyl-L-valine *n*-dodecylamide	113–114.5	90.1	$t = 22°C$ $-20.7°$ (c5)	74.78	12.33	5.82	74.62	12.52	6.00
XII	N-Isobutyryl-L-valine *n*-dodecylamide	124–125	95.8	$t = 24°C$ $-29.7°$ (c10)	70.96	11.96	7.86	71.13	11.94	7.90
XIII	N-Pivaloyl-L-valine *n*-dodecylamide	77.5–78.5	94.5	$t = 22°C$ $-23°$ (c10)	71.71	11.83	7.40	71.68	12.03	7.60
XIV	N-*tert.*-Butylacetyl-L-valine *n*-dodecylamide	72.5–73.5	74.3	$t = 26°C$ $-38.9°$ (c10)	72.02	11.95	7.68	72.20	12.12	7.32
XV	N-Dipentylacetyl-L-valine *n*-dodecylamide	100–104	68.2	$t = 26°C$ $-28.9°$ (c10)	74.57	12.32	6.27	74.62	12.53	6.00
XVI	N-Cyclohexanecarbonyl-L-valine *n*-dodecylamide	165.5–167	94.1	$t = 22°C$ $-24°$ (c3)	73.28	11.50	7.29	73.04	11.75	7.10
XVII	N-Cycloheptanecarbonyl-L-valine *n*-dodecylamide	142–144	91.0	$t = 22°C$ $-22.2°$ (c10)	73.56	11.60	6.75	73.47	11.84	6.86
XVIII	N-Cyclooctanecarbonyl-L-valine *n*-dodecylamide	88–89.5	85.4	$t = 22°C$ $-18.9°$ (c10)	73.64	11.75	6.40	73.88	11.92	6.63
XIX	N-Adamantane-1-carbonyl-L-valine *n*-dodecylamide	120–121.5	85.3	$t = 22°C$ $-20.4°$ (c6)	75.33	11.12	6.18	75.28	11.28	6.27

* (d) = decomposition.

** The optical purity was measured by GC (see Experimental).

*** Solvent = CF_3CO_2H

method[15]. Sodium hydroxide (0.2 mole) and L-valine (0.1 mole) were dissolved in 60 ml of water, cooled to *ca.* 5°, stirred vigorously and 0.11 mole of lauroyl chloride added dropwise, with further stirring for 1/2 h. The solution was diluted with 900 ml of water and acidified to congo red with 6 *N* hydrochloric acid. The precipitate was filtered, washed with water, air dried and recrystallized by diluting a concentrated chloroform solution with 300 ml of light petroleum (b.p. 40–60°), m.p. 103–104°, $[\alpha]_D^{25}$ + 22.4° (c10 in $CHCl_3$)*.

N-Lauroyl-L-valine N-succinimido ester (XXV). This compound was obtained from XXIV as described previously; m.p. 94–95.5°, $[\alpha]_D^{25}$ −21.4° (c10 in $CHCl_3$)*. Stationary phases I and IX were obtained by treatment of XXV with ammonia and adamantylamine, respectively.

N-Lauroyl-L-β-amino-α-piperidone (XXVI). L-β-Amino-α-piperidone was prepared according to Fischer and Zemplen[16] from L-ornithine, and without further purification was treated with N-succinimido laurate according to the previous procedure to give XXVI, m.p. 114–115°, $[\alpha]_D^{18}$ + 55.6° (c5 in $CHCl_3$)*; elemental analysis required for $C_{17}H_{32}N_2O_2$, C 68.87%, H 10.88% and N 9.45%; found, C 68.88%, H 11.00% and N 9.44%.

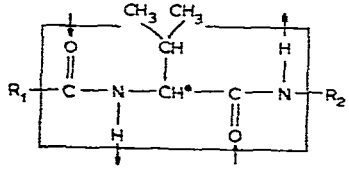
N-TFA-(±)-amino acid esters. These esters were prepared according to Gil-Av *et al.*[17].

Determination of the optical purity of the diamide products

Hydrolysis of 50 mg of N-acylvaline alkylamide was carried out by refluxing with 20 ml of 6 *N* hydrochloric acid for 4 h. The hydrochloric acid was removed under reduced pressure and the residue converted into its N-TFA isopropyl ester[18]. This derivative was gas chromatographed on stationary phase III and the optical purity was calculated from the areas of the peaks corresponding to the derivatives of D- and L-valine.

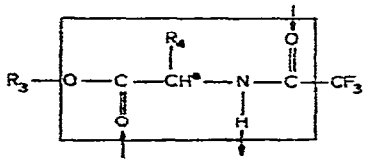
RESULTS AND DISCUSSION

The chiral diamide compounds



contain a strongly polar asymmetric centre surrounded by three alkyl substituents: an isopropyl radical and two alkyl groups, R_1 and R_2 (their shape and size can be readily varied). These compounds, when used as gas chromatographic stationary phases, resolve racemic α-amino acid esters

* Optical purity was not determined.

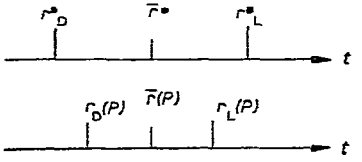


which have suitably disposed functional groups that are capable of interacting with the diamide by hydrogen bonding.

In this study, separation factors of the above solutes were measured on diamides derived from L-valine. This amino acid was chosen because of the high resolution factors achieved with L-valyl-L-valine derivatives, which are the analogous dipeptide stationary phases[6]. In order to compare the steric effect of the substituent of the N-acyl groups (R_1) and the substituent at the N-amide side (R_2) on resolution factors, two series of diamide stationary phases were studied (Table I): (i) N-lauroyl-L-valine alkylamides and (ii) N-acyl-L-valine dodecylamides. In the first series, R_2 = H (I), isopropyl (II), *tert.*-butyl (III), neopentyl (IV), 6-undecyl (V), cyclohexyl (VI), cycloheptyl (VII), cyclooctyl (VIII), 1-adamantyl (IX) and *n*-dodecyl (X); in the second series, R_1 = isopropyl (XII), *tert.*-butyl (XIII), neopentyl (XIV), 6-undecyl (XV), cyclohexyl (XVI), cycloheptyl (XVII), cyclooctyl (XVIII) and 1-adamantyl (XIX).

Calculation of separation factors on optically pure stationary phases from separation factors obtained with partially active stationary phases

The separation factors of racemic solutes on a given stationary phase, at a certain temperature, depend on its optical purity. The conditions used to prepare the diamide stationary phases (see experimental) were such that different degrees of racemization occurred. A comparison of the efficiencies of these stationary phases in resolving racemic solutes could not be made unless the separation factors were measured or calculated for a certain optical purity.



Let $r_D(P)$ and $r_L(P)$ be the corrected retention times of enantiomers D and L on a stationary phase of optical purity* P, respectively, let r^*_D and r^*_L be the respective values at 100% optical purity of the stationary phase under the same chromatographic conditions and let $\Delta r(P)$ and Δr^* be the differences in the corrected retention times of the enantiomers on a stationary phase with optical purity P% and 100%, respectively.

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* $P = \frac{100\,(X_L - X_D)}{X_L + X_D}$, where X_L and X_D are the appropriate molar fractions of enantiomers L and D, respectively.

If the resolution of the enantiomers on the chiral stationary phase satisfies the following two assumptions: (a) $\Delta r(P)$ is linearly dependent on P, and (b) the value $\bar{r}(P) = 1/2\ [r_L(P) + r_D(P)]$ is independent of P, then the resolution factors at 100% optical purity, $r^*_{L/D}$, can be calculated by means of eqn. 1 (see below). These two assumptions are reasonable approximations in instances when the difference in the total interactions of the D and L enantiomers with the solvent molecules results of all 1:1 solute–solvent bimolecular interactions in an additive way; where the free energy of solvation of the L-solute results from all possible L–L′ and L–D′ associates* and that of the D-solute from the D–L′ and D–D′ pairs, while usually, interaction of L–D′ equals that of D–L′ associate and that of L–L′ equals that of D–D′ associate.

From (a):

$$\Delta r(P) = \Delta r^* P/100$$

Therefore,

$$\Delta r^* = [r_L(P) - r_D(P)]\ 100/P$$

From (b):

$$\bar{r}^* = \bar{r}(P) = \frac{1}{2}[r_L(P) + r_D(P)]$$

Rearranging these equations into

$$r^*_L = \bar{r}^* + \frac{\Delta r^*}{2} = \bar{r}(P) + \frac{\Delta r(P)}{2}\ 100/P = 1/2\ [r_L(P) + r_D(P)] + \\ + 1/2\ [r_L(P) - r_D(P)]\frac{100}{P}$$

and

$$r^*_D = \bar{r}^* - \frac{\Delta r^*}{2} = \bar{r}(P) - \frac{\Delta r(P)}{2}\ 100/P = 1/2\ [r_L(P) + r_D(P)] - \\ - 1/2\ [r_L(P) - r_D(P)]\frac{100}{P}$$

and substituting $r_{L/D}$ for $r_L(P)/r_D(P)$ and $r^*_{L/D}$ for r^*_L/r^*_D, eqn. 1 is derived:

$$r^*_{L/D} = r^*_L/r^*_D = \frac{(r_{L/D} + 1)P + (r_{L/D} - 1)\ 100}{(r_{L/D} + 1)P - (r_{L/D} - 1)\ 100} \qquad (1)$$

Two columns with stationary phase III of optical purities 59.3% and 75.6%, respectively, were prepared. Separation factors of N-TFA-α-amino acid methyl and

* L′ and D′ are each a solvent molecule of L-or D-configuration, respectively.

TABLE II

MEASURED AND CALCULATED CORRECTED SEPARATION FACTORS ($r_{L/D}$ AND $r^*_{L/D}$, RESPECTIVELY) OF N-TFA-α-AMINO ACID ESTERS ON STATIONARY PHASE III AT 130°

α-Amino acid	*Methyl ester*				*Isopropyl ester*			
	$r_{L/D}$ *for* $P = 59.3\%$	$r^*_{L/D}$ *calculated*	$r_{L/D}$ $P = 75.6\%$	$r^*_{L/D}$ *calculated*	$r_{L/D}$ *for* $P = 59.3\%$	$r^*_{L/D}$ *calculated*	$r_{L/D}$ *for* $P = 75.6\%$	$r^*_{L/D}$ *calculated*
Alanine	1.175	1.313	1.235	1.323	1.194	1.351	1.255	1.351
Aspartic acid	1.044	1.076	1.062	1.080	1.046	1.079	1.062	1.083
Leucine	1.228	1.420	1.301	1.419	1.265	1.490	1.356	1.490
Methionine	1.183	1.329	1.230	1.319	1.205	1.372	1.276	1.382
Phenylalanine	1.170	1.304	1.222	1.304	1.193	1.349	1.265	1.347
Valine	1.129	1.233	1.171	1.232	1.172	1.309	1.225	1.308

isopropyl esters were measured on these columns and corrected for 100% optical purity of the phase according to eqn. 1. The results, which are summarized in Table II, are in good agreement and prove the usefulness of eqn. 1.

Dependence of the separation factors, $r^*_{L/D}$, on the alkyl substituents R_1 and R_2 of the chiral diamide solvent

The nature of the alkyl substituents R_1 and R_2 has an influence on the separation factors, through their bulkiness and shape, which also affect the configuration of the two amide groups, as will be discussed later. The correlation of the last aspect can elucidate the mechanism of resolution.

Two series of diamides were studied: (i) stationary phases I–X, where R_1 is a straight-chain $C_{11}H_{23}$ group and R_2 has various shapes, and (ii) stationary phases XII–XIX, where R_2 is a straight-chain group and R_1 has the structures of R_2 in series (i). Using these stationary phases, resolution of N-TFA-α-amino acid esters and other compounds[19] was achieved. When L stationary phases were used, the L enantiomers of all the α-amino acid derivatives emerged last from the column.

Tables III and IV summarize the corrected resolution factors, $r^*_{L/D}$, of the above methyl and isopropyl ester derivatives. In general, it is observed that series (i), where the branching is on the N-amide radical, leads to higher resolution factors than those obtained on series (ii); *e.g.*, the $r^*_{L/D}$ values of N-TFA-leucine isopropyl ester on different phases (given in parentheses) at 130° are: 1.499 (III) > 1.376 (VIII) >

TABLE III

RESOLUTION FACTORS ($r^*_{L/D}$) OF N-TFA-α-AMINO ACID METHYL ESTERS CALCULATED FOR 100% OPTICAL PURITY OF THE STATIONARY PHASES AT 130°

α-Amino acid	Stationary phase											
	III	*IV*	*V*	*VIII*	*IX**	*X*	*XII*	*XIII*	*XIV*	*XV*	*XVIII*	*XIX*
Alanine	1.323	1.221	1.123	1.221	1.168	1.136	1.125	1.130	1.262	1.098	1.166	1.121
Aspartic acid	1.080	1.057	1**	1.051	1**	1.036	1**	1.044	1.033	1**	1.057	1**
α-Aminobutyric acid	1.302	1.223	1.064	1.218	1.158	1.112	1.114	1.119	1.202	1**	1.152	1.084
Glutamic acid	1.264	1.141	1.109	1.182	1.142	1.123	1.090	1.108	1.160	1.094	1.149	1.098
α-Aminohexanoic acid	1.344	1.236	1.132	1.248	1.175	1.138	1.121	1.129	1.232	1.072	1.176	1.087
Leucine	1.419	1.287	1.167	1.320	1.223	1.167	1.163	1.155	1.270	1.098	1.200	1.121
tert.-Leucine	1.116	1.086	1**	1.089	1**	1**	1**	1.049	1.081	1**	1**	1**
Methionine	1.319	1.227	1.145	1.233	1.176	1.132	1.123	1.135	1.206	1.076	1.173	1.096
α-Aminooctanoic acid	1.333	1.231	1.113	1.249	1.174	1.134	1.128	1.124	1.229	1.063	1.131	1.084
Phenylalanine	1.304	1.225	1.137	1.249	1.178	1.138	1.122	1.142	1.232	1.058	1.190	1.107
Proline	1.071	1.056	1**	1***	1**	1**	1**	1.034	1.061	1**	1.043	1**
Serine	1.167	1.133	1.078	1.156	1.077	1.080	1.057	1.089	1.098	1.047	1.096	1.087
Threonine	1.175	1.146	1.135	1.156	1***	1.086	1**	1.107	1.121	1.058	1.120	1.093
α-Aminovaleric acid	1.345	1.247	1.141	1.241	1.180	1.156	1.145	1.129	1.240	1.068	1.162	1.093
Valine	1.232	1.176	1**	1.171	1.122	1.086	1**	1.082	1.139	1**	1.119	1**

* At 150°.

** No resolution.

*** Shoulder.

TABLE IV

RESOLUTION FACTORS ($r^*_{L/D}$) OF N-TFA-α-AMINO ACID ISOPROPYL ESTERS CALCULATED FOR 100% OPTICAL PURITY OF THE STATIONARY PHASES AT 130°

α-Amino acid	*Stationary phase*											
	III	*IV*	*V*	*VIII*	*IX**	*X*	*XII*	*XIII*	*XIV*	*XV*	*XVIII*	*XIX*
Alanine	1.323	1.270	1.174	1.282	1.206	1.162	1.174	1.150	1.270	1.142	1.228	1.145
Aspartic acid	1.083	1.070	1.036	1.097	1.044	1.048	1.045	1.051	1.051	1***	1.063	1.043
α-Aminobutyric acid	1.350	1.259	1.105	1.268	1.179	1.143	1.144	1.126	1.212	1.068	1.181	1.119
Glutamic acid	1.285	1.234	1.153	1.231	1.168	1.137	1.136	1.122	1.169	1.106	1.181	1.109
α-Aminohexanoic acid	1.395	1.299	1.180	1.307	1.206	1.154	1.167	1.144	1.237	1.102	1.186	1.127
Leucine	1.499	1.375	1.246	1.376	1.251	1.216	1.223	1.190	1.329	1.172	1.280	1.166
tert.-Leucine	1.136	1.109	1**	1.110	1.079	1.050	1**	1.064	1.110	1**	1.054	1.058
Methionine	1.382	1.301	1.189	1.282	1.199	1.148	1.178	1.146	1.218	1.137	1.187	1.181
α-Aminooctanoic acid	1.405	1.282	1.180	1.307	1.203	1.153	1.171	1.135	1.246	1.110	1.181	1.118
Phenylalanine	1.347	1.293	1.170	1.304	1.201	1.149	1.172	1.143	1.222	1.165	1.205	1.125
Phenylglycine	1.210	1.118	1.078	1.121	1.084	1.060	1.064	1.068	1.093	1.062	1.076	1.039
Proline	1.055	1***	1**	1**	1**	1***	1**	1.031	1.038	1**	1***	1***
Serine	1.198	1.183	1.107	1.183	1.122	1.098	1.102	1.112	1.117	1.122	1.140	1.110
Threonine	1.239	1.203	1.162	1.218	1.126	1.119	1.141	1.150	1.170	1.134	1.147	1.143
α-Aminovaleric acid	1.391	1.295	1.175	1.294	1.204	1.173	1.161	1.145	1.234	1.099	1.201	1.127
Valine	1.308	1.223	1.067	1.252	1.163	1.125	1.114	1.127	1.175	1**	1.152	1.113

* At 150°.
** No separation.
*** Shoulder.

1.375 (IV) > 1.329 (XIV) > 1.280 (XVIII) > 1.246 (V) > 1.233 (XII) > 1.216 (X) > 1.190 (XIII) > 1.172 (XV) > 1.166 (XIX); and 1.251 (IX) at 150°. For the same derivative of methionine at 130°, the values are: 1.382 (III) > 1.301 (IV) > 1.282 (VIII) > 1.218 (XIV) > 1.189 (V) = 1.187 (XVIII) > 1.181 (XIX) > 1.178 (XII) > 1.148 (X) > 1.146 (XIII) > 1.137 (XV); and 1.199 (IX) at 150°.

The best resolution of the above and similar racemic solutes (see Tables III–V), was given by stationary phase III, N-lauroyl-L-valine *tert.*-butylamide, which has a combination of R_1 = *n*-undecyl for lowering its vapour pressure and melting point and R_2 = *tert.*-butyl for improved stereoselectivity.

The correlation between the selectivity and the type of R_1 and R_2 substituents can be used in preparing GC stationary phases that are capable of operating over a wider range of temperature without losing their ability to resolve the appropriate antipodes. Moreover, a combination of diamide stationary phases at adjusted optical purities should be considered for the complete separation of all natural and "unnatural" enantiomers of the protein amino acids.

Dependence of separation factors, $r^*_{L/D}$, on the substituents R_3 and R_4 in the racemic solutes

In general, the α-amino acid solutes behave on the diamide stationary phases in a similar manner to that on the dipeptide stationary phases: (a) the order of emer-

TABLE V

RESOLUTION FACTORS ($r^*_{L/D}$) OF N-TFA-ALANINE AND -LEUCINE ESTERS CALCULATED FOR 100% OPTICAL PURITY AT 130°

α-Amino acid	*Ester*	*Stationary phase*											
		III	*IV*	*V*	*VIII*	*IX**	*X*	*XII*	*XIII*	*XIV*	*XV*	*XVIII*	*XIX*
Alanine	Methyl	1.323	1.221	1.123	1.221	1.168	1.136	1.125	1.130	1.262	1.098	1.165	1.121
	Ethyl	1.331	1.233	1.141	1.258	1.181	1.151	1.129	1.137	1.268	1.106	1.200	1.127
	n-Propyl	1.314	1.254	1.150	1.261	1.186	1.134	1.154	1.137	1.210	1.131	1.201	1.116
	n-Butyl	1.313	1.258	1.148	1.268	1.181	1.153	1.160	1.141	1.230	1.106	1.207	1.120
	Isopropyl	1.351	1.270	1.174	1.282	1.206	1.162	1.174	1.150	1.278	1.142	1.228	1.145
	3-Pentyl	1.402	1.313	1.192	1.327	1.216	1.197	1.184	1.177	1.293	1.155	1.252	1.166
	Cyclopentyl	1.321	1.249	1.128	1.257	1.185	1.141	1.138	1.121	1.218	1.089	1.188	1.117
Leucine	Methyl	1.419	1.287	1.167	1.320	1.223	1.167	1.163	1.155	1.270	1.098	1.200	1.121
	Ethyl	1.431	1.323	1.198	1.337	1.235	1.181	1.175	1.174	1.293	1.104	1.231	1.140
	n-Propyl	1.433	1.329	1.206	1.323	1.217	1.193	1.164	1.176	1.286	1.091	1.233	1.126
	n-Butyl	1.433	1.308	1.206	1.339	1.231	1.183	1.174	1.174	1.296	1.090	1.220	1.121
	Isopropyl	1.499	1.375	1.246	1.376	1.251	1.216	1.223	1.190	1.329	1.172	1.280	1.166
	3-Pentyl	1.576	1.422	1.264	1.435	1.298	1.246	1.235	1.217	1.393	1.184	1.336	1.200
	Cyclopentyl	1.467	1.341	1.200	1.337	1.223	1.195	1.189	1.141	1.283	1.115	1.233	1.139

* At 150°.

gence on the L stationary phases is L after the D enantiomers; (b) branching of the alkyl ester group, R_3, improves the separation factors (isopropyl and 3-pentyl esters in comparison with *n*-alkyl derivatives; see Table V); the best separation factors are obtained for 3-pentyl, poorer for isopropyl (shortening of the side chains) and worst for cyclopentyl (rigidity); (c) branching of the alkyl substituent, R_4, on the β-carbon lowers the separation factors; but (d) branching of the γ-carbon improves the separation factors, *i.e.*, leucine derivatives have the highest $r^*_{L/D}$ values, phenylalanine is one of the best resolved compounds, whereas phenylglycine has one of the poorest resolution factors, etc.; (e) a decrease in $r^*_{L/D}$ is observed when R_4 includes a polar carbonyl group, in particular at the β-position, which competes for the hydrogen bonding with the α-carbonyl ester radical participating in the "selective associate" discussed later.

From a study on the behavior of β- and γ-amino acids[19] on stationary phase V, it was found that the D enantiomers emerge after the L enantiomers, which is the reverse order of emergence of α-amino acid derivatives. The separation factors of γ-amino acids on stationary phase V are *ca.* 1.1, whereas those of β-amino acids are only *ca.* 1.04.

Association of L-aspartic and L-glutamic acids with the molecules of the stationary phase through the α-carbonyl is analogous to that of regular apolar L-α-amino acids (see Fig. 1, left) and association through the β- or γ-carbonyl is analogous to that of D-β- or D-γ-amino acids, respectively, as shown in Fig. 1 (right). From the relative disposition of the groups, it happens that the L-diamide stationary phases interact more strongly with the L-diacids in both associative forms than with those of their D-antipodes. However, as reported above, the selectivity of the diamide stationary phase towards the β-carbonyl is small and this additional associative form strongly decreases the separation factors of aspartic acid, while the γ-carbonyl competing as-

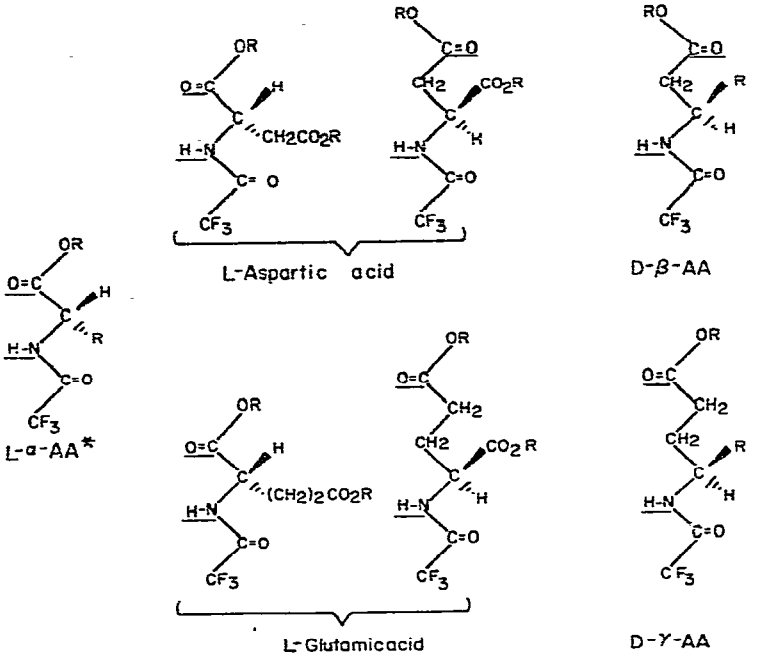


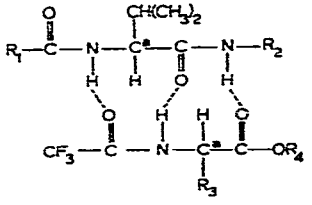
Fig. 1. Schematical spatial disposition of L-aspartic and L-glutamic acid derivatives in comparison with those of L-α-AA and D-β- or D-γ-AA, respectively. The groups participating in a particular bimolecular 1:1 solute–solvent association are underlined. AA = amino acid.

sociative form, being of high stereoselectivity, does not lower the resolution factors of glutamic acid (or, similarly, the separation factors of serine and threonine derivatives).

It is of interest that the proline derivative, which lacks the amide hydrogen atom that participates in the "selective associate" (see the following section), is resolvable. This is also the only amino acid whose methyl ester is better resolved than its isopropyl ester derivative.

Mechanism of resolution

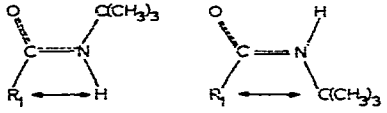
The high stereoselectivity of the active diamides towards the resolution of racemic amino acid derivatives, in comparison with the less effective stationary phases, N-TFA-L-α-amino acid esters[1], was presumed to result from the spatial requirements of the "three-hydrogen-bonded associate"[4], represented schematically as



and its similar opposite oriented associate[6].

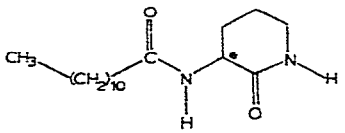
The C–N amide bond in $R_1CONR_2R'_2$ has π-bond character, leading to separate *cis*- and *trans*-isomers. The activation energy of their interconversion is 16–20

kcal/mole for compounds where $R_1 \neq H$[20]. It was found by NMR measurements that the *trans*-isomer is the exclusive form (>99%) for compounds in which R_1, R_2 = alkyl and R'_2 = H. On the other hand, similar compounds in which R_2 is an aromatic substituent have a great proportion of the *cis*-isomer, which in certain instances becomes the predominant form[20]. Another example is the N-alkylformamides (R_1, R'_2 = H and R_2 = alkyl), in which the *cis*-form is present in increasing percentages in compounds that have bulky R_2 alkyl groups, *e.g.*, it reaches 20% for R_2 = *tert.*-butyl[21]. This last case is unique for the homologous series, where a hydrogen replaces the R_1 group and the steric factors work in an opposite direction, in favour of the less hindered *cis*-isomer.



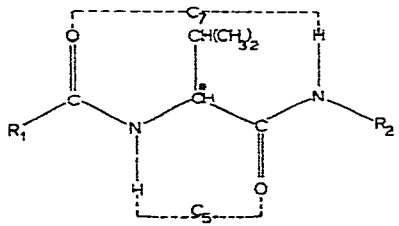
trans *cis*

The stationary phase N-lauroyl-L-β-amino-α-piperidone



which has the corresponding amide group only in the *cis*-conformation, was synthesized, and was found to be incapable of resolving racemic α-amino acids. Moreover, as mentioned previously, the order of increasing selectivity of the phases in series (i) is: X (R_2 = *n*-dodecyl) < V (R_2 = 6-undecyl) < IV (R_2 = neopentyl) < VIII (R_2 = cyclooctyl) < III (R_2 = *tert.*-butyl); where at least III is exclusively in the *trans*-amide conformation.

The difficulties in building the above "three-hydrogen-bonded selective associate" with C.P.K. (Corey, Pauling, Koltun) models confirms the experimental results that the corresponding amide bond of the solvent in the selective associate is in the *trans*-conformation:



Diamides of this type, when dissolved in carbon tetrachloride at high dilution (*ca.* 10^{-4} *M*), form mixtures of two possible intramolecular hydrogen-bonded rings, the planar "C_5" ring[22–29] and the folded "C_7" ring, which has two adjacent planes intersecting at 115° (refs. 26, 27) as well as the unassociated open structure[22–29]. The

last form becomes predominant at higher temperatures[27–29]. The "C_5" and "C_7" forms disappear at higher concentrations, where intermolecular hydrogen-bonded associative oligomers of the extended structure are formed[29,30].

Mizushima and co-workers[30,31] studied the IR spectra of the crystalline structure of different diamides with polarized light and concluded that, for example, N-acetyl-L-leucine methylamide (see Fig. 2a) possesses the crystalline structure of poly-L-alanine[32], keratin[33], etc. Ichikawa and Iitaka[34] analyzed the X-ray pattern of the above racemic modification, N-acetyl-D,L-leucine methylamide (see Fig. 2b) and found that the "C_7" site was not associated as suggested above, but involved bonding of three molecules. This structure is not possible for diamides that have large alkyl substituents, *e.g.*, with the N-*tert.*-butylamide derivative. For such derivatives, the structure represented in Fig. 2a is more probable.

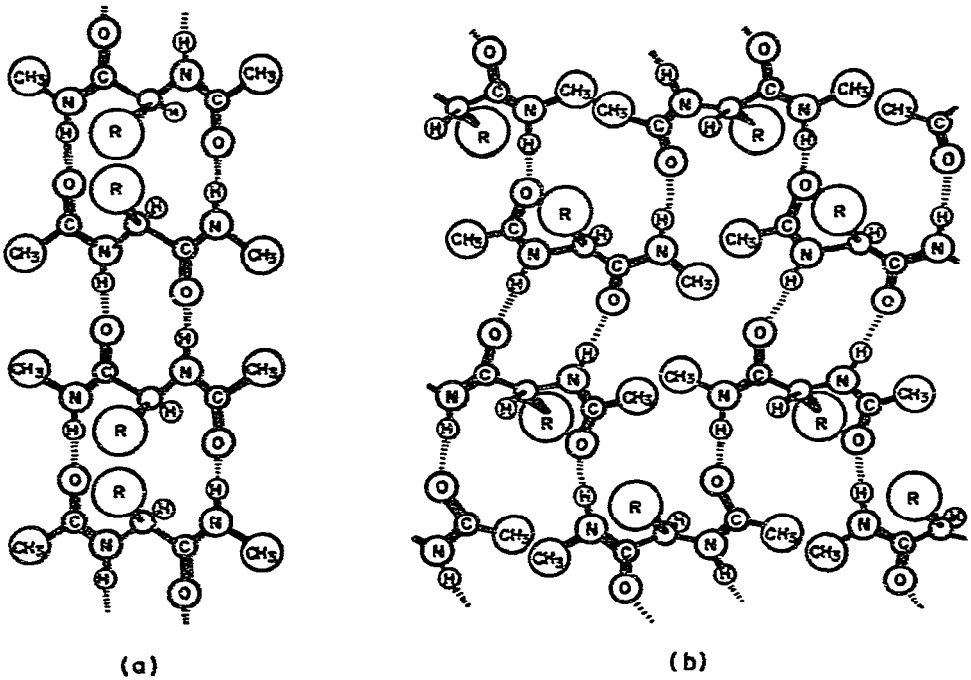


Fig. 2. (a) The structure of N-acetyl-L-leucine methylamide derived from IR measurements[30,31] (R = isobutyl). (b) The structure of N-acetyl-D,L-leucine methylamide derived from X-ray measurements[34] (R = isobutyl).

The assumption that over a short range (a distance of a few molecules) an associative oligomeric structure, similar to that in Fig. 2a, exists to a considerable extent in the melt at 130° is reasonable and agrees with all known experimental results. This structure gives a good fit with the solutes studied, as can be seen on C.P.K. models, and explains the stereoselectivity via association, represented in Fig. 3. The substituent R_3 of the solute is above the plane of the paper and *syn* to the isopropyl group of the L solvent for the L′ enantiomer and below the plane and *anti* for the D′ antipode.

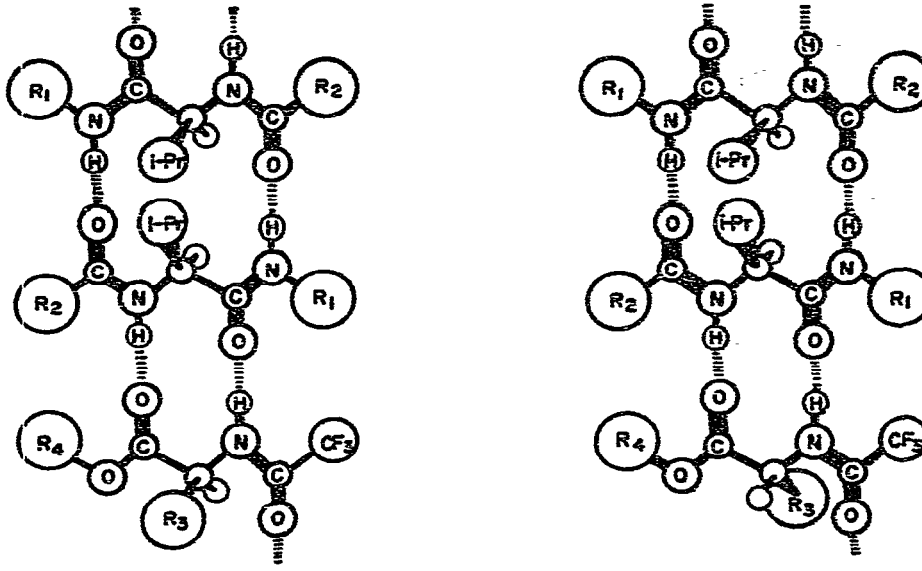


Fig. 3. The stereoselective hydrogen bonds association of the L- and D-amino acid derivatives solvated in the active diamide phase.

The experimental results indicate that the L–L′ combination, in which the isopropyl and R_3 groups are in close proximity, have a larger difference in free energy of solvation than the L–D′ system.

The difference in $\bar{G}_0$, $\bar{H}_0$ and $\bar{S}_0$ of the enantiomers in the chiral diamide solvent

The resolution factors are a direct measure of the difference in the free energy of solution:

$$\Delta\bar{G}_0 = \bar{G}_0(\text{L}) - \bar{G}_0(\text{D}) = -RT\ln r_{\text{L/D}} = \Delta\bar{H}_0 - T\Delta\bar{S}_0$$

and from its temperature dependence the other quantities are obtainable:

$$\ln r_{\text{L/D}} = -\frac{\Delta\bar{H}_0}{R}\cdot\frac{1}{T} + \frac{\Delta\bar{S}_0}{R}$$

$$\frac{\partial(\ln r_{\text{L/D}})}{\partial\left(\frac{1}{T}\right)} = -\frac{\Delta\bar{H}_0}{R}$$

From the experimental results summarized in Table VI, the slopes $[\partial(\ln r^*_{\text{L/D}})/\partial(1/T)]$ for some compounds were calculated, taking into account the least-square deviations. It is noticeable that $\Delta\bar{S}_0$ is independent of temperature (See Table VII) which

TABLE VI

CORRECTED RESOLUTION FACTORS ($r^*_{L/D}$) OF N-TFA ESTERS OF ALANINE AND LEUCINE AS A FUNCTION OF TEMPERATURE ON STATIONARY PHASE IV

Temperature (°C)	*Alanine ester*				*Leucine ester*			
	Methyl	*Ethyl*	*Isopropyl*	*3-Pentyl*	*Methyl*	*Ethyl*	*Isopropyl*	*3-Pentyl*
100	1.339	1.364	1.413	1.525	1.463	1.510	1.572	
110	1.293	1.316	1.362	1.445	1.417	1.446	1.506	1.560
120	1.254	1.274	1.315	1.391	1.355	1.391	1.441	1.504
130	1.221	1.233	1.270	1.316	1.287	1.318	1.375	1.422
140	1.179	1.198	1.223	1.270	1.236	1.265	1.302	1.343
150	1.139	1.157	1.179	1.217	1.190	1.208	1.245	1.278

TABLE VII

DIFFERENCE IN ENTHALPY ($\Delta\bar{H}_0$ kcal/mole), ENTROPY ($\Delta\bar{S}_0$ cal/mole·deg) AND FREE ENERGY OF SOLVATION ($\Delta\bar{G}_0$ kcal/mole) OF N-TFA ESTERS OF ALANINE AND LEUCINE ON STATIONARY PHASE IV

$\Delta\bar{H}_0 = \bar{H}_0(\text{L}) - \bar{H}_0(\text{D})$, $\Delta\bar{S}_0 = \bar{S}_0(\text{L}) - \bar{S}_0(\text{D})$ and $\Delta\bar{G}_0 = \bar{G}_0(\text{L}) - \bar{G}_0(\text{D})$.

α-Amino acid	*Ester*	$\Delta\bar{H}_0$	*110°*		*130°*		*150°*	
			$\Delta\bar{G}_0$	$\Delta\bar{S}_0$	$\Delta\bar{G}_0$	$\Delta\bar{S}_0$	$\Delta\bar{G}_0$	$\Delta\bar{S}_0$
Alanine	Methyl	−0.995	−0.196	−2.09	−0.160	−2.07	−0.109	−2.09
	Ethyl	−1.016	−0.209	−2.11	−0.168	−2.10	−0.123	−2.11
	Isopropyl	−1.130	−0.235	−2.33	−0.192	−2.33	−0.138	−2.34
	3-Pentyl	−1.363	−0.280	−2.83	−0.220	−2.83	−0.165	−2.83
Leucine	Methyl	−1.331	−0.265	−2.80	−0.202	−2.80	−0.146	−2.80
	Ethyl	−1.404	−0.281	−2.93	−0.221	−2.93	−0.160	−2.94
	Isopropyl	−1.474	−0.312	−3.03	−0.255	−3.02	−0.184	−3.05
	3-Pentyl	−1.611	−0.338	−3.32	−0.282	−3.30	−0.206	−3.32

strengthens the model of the selective association discussed previously, in which no steric hindrance or strain in forming the associate is observed, but is mainly due to the difference between the disposition of the alkyl group, R_3, of the antipodes relative to the isopropyl radical of the stationary phase (see Fig. 3).

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