Tetrahedron. Vol. 26, pp. 1361 to 1368. Pergamon Press 1970. Printed in Great Britain

INTERACTION BETWEEN ASYMMETRIC SOLUTES AND SOLVENTS

PEPTIDE DERIVATIVES AS STATIONARY PHASES IN GAS LIQUID PARTITION CHROMATOGRAPHY

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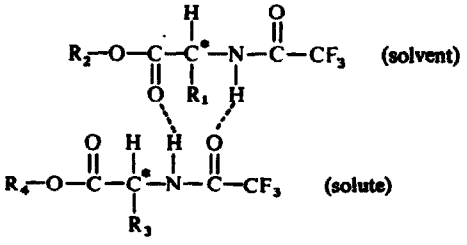
(*Received in the UK* 11 *November* 1969; *Accepted for publication* 20 *November* 1969)

Abstract—Structural considerations leading to the development of efficient optically active stationary phases for the separation of enantiomers by gas chromatography are presented. The phases which are derivatives of the di- and the tripeptide of L-valine give the best separation reported thus far for the antipodes of N-trifluoroacetyl (TFA)-α-amino acid esters. The ratio (r) of the retention times of the enantiomers studied has been found to be influenced by the structure of the esterifying alcohol and the temperature. At 100°, e.g., the r values for the N-TFA-alanine esters of primary alcohols are about 1·1, of secondary alcohols about 1·14, and of t-butanol about 1·22, with the L-isomers having throughout the longer retention time. Differences in the enthalpy and entropy of solution of the enantiomers of N-TFA-alanaine t-butyl ester were found to be 0·63–0·67 kcal./mole and 1·22–1·36 cal./mole°K, respectively. The phases are a new tool for the qualitative and quantitative determination of the enantiomers of α-amino acids.

RECENT studies [1-4] on the resolution of enantiomers by optically active stationary phases have led to the recognition of some of the structural factors involved in the mechanism of separation. These findings were applied to the development of some particularly efficient asymmetric solvents, described in the present paper.

The resolution of enantiomeric amino acid derivatives by solvents of the type $R_1C^*H(NHCOCF_3)COOR_2$ has been explained by the formation of H-bonded "diastereomeric" association complexes, such as:



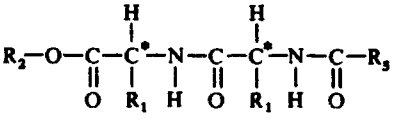
(1)

where R_1, R_2, R_3 and R_4 are hydrocarbon radicals, and the solvent differs from the solute essentially by the fact that R_2 is much larger than R_4. In an association complex of type (1), no more than two H-bonds can be formed, since only one hydrogen donor is available in each of the molecules considered. It was reasoned that by placing a second hydrogen donor in a suitable position of the solvent molecule, the selectivity of the phase could be increased through the formation of three H-bonds in the vicinity of the asymmetric centers. A judicious choice of the substituents R_1 and R_2 could further

optimize the efficiency of the phase, as the ease of resolution[1,3] increases in the following order:

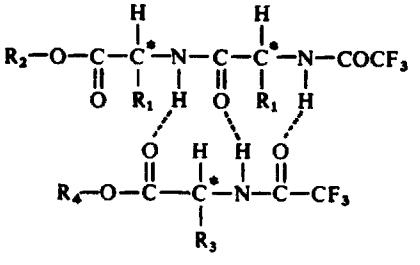
for group R_2: Primary<secondary<tertiary; and
for group R_1: tertiary<primary<secondary.

These desirable structural characteristics are found in the N-acyl derivative of dipeptide esters of formula



Phase I—$R_1 = R_2 = CH(CH_3)_2$; $R_3 = CF_3$
Phase II—$R_1 = CH(CH_3)_2$; R_2 = Cyclohexyl; $R_3 = CF_3$
Phase III—$R_1 = R_2 = CH(CH_3)_2$; $R_3 = CH_3$

Inspection of Courtauld models showed that association through three hydrogen bonds between NH and CO groups bringing the asymmetric centers of the two molecules close together, as in (2), could indeed occur.



(2)

The expectation of high resolution factors for these phases was fully confirmed by the experimental results, which are given in Tables 1 and 2 and Fig. 1.

The efficiency of the dipeptide phases is clearly seen, when the behavior of the t-butyl derivatives on various phases is compared. Thus, at 90° $r_{L/D}$ (see Tables for definition of r) is equal to 1·031 on N-TFA-L-phenylalanine cyclohexyl ester, and 1·039 on N-TFA-L-isoleucine dodecyl ester, whereas on the dipeptide phase I at 110° the resolution factor is as high as 1·21.

The change of the resolution factor with temperature has been measured for the t-butyl ester on phase I. By extrapolating the curve given in Fig. 2 to 90° (i.e., 17–19° below the m.p.), a value of $r_{L/D} = 1·27$ is found. This means that the improvement of efficiency is still higher than apparent from the figures cited above. Since the resolution factors have such large values, separation of enantiomers can be achieved with dipeptide phases not only on capillary, but also on packed columns.[4]

As has been mentioned above, the inspection of models indicated that an association complex of N-TFA-α-amino acid esters with the dipeptide, containing three H-bonds, appears possible. In addition the model shows that, when such association occurs, a particular conformation is imposed on the solute, with the acceptor and donor groups forming part of a spiral turn, the handedness of which is determined by the configuration of the amino acids in the dipeptide. The resulting distortion, which

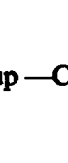
occurs in the same direction for both enantiomers on the L-stationary phases I, II and III, introduces an additional (conformational) element of chirality. In the conformation in which they are hydrogen bonded to the solvent, the enantiomeric solutes thus cease to be mirror images of each other and become "conformational" diastereomers. Interactions of such "diastereomers" with the solvent will in general be different. This aspect of the association of the dipeptide phase with the enantiomeric N-TFA-amino acid esters further helps to understand the very high resolution factors found.

The ease of separation greatly depends on the nature of the alcohol group. As in the case where the stationary phase is a N-TFA-α-amino acid ester, such as the N-TFA-L-phenylalanine cyclohexyl ester, the resolution factors increase in the order primary <secondary <tertiary alchol.

In Table 2 results are given for the resolution on N-*acetyl*-L-valyl-L-valine isopropyl ester (III) of both N-TFA and N-acetyl-α-amino acid esters. The results are of interest for assessing the role played by the trifluoromethyl group in the mechanism of resolution. Phase III melts at 129–131° and had, therefore, to be tested at higher temperature than I. Hence, the behaviour of the N-TFA derivatives on the two phases can be compared directly only for the t-butyl ester, for which $r_{II/I}$ on phase I at 140° (Table 3) is higher by 0·012 only. For the other N-TFA esters, the effect of changing from phase I to III is, by analogy, estimated to be closely similar. Furthermore, when the trifluoromethyl group is absent from both the solute and the solvent, as in N-acetyl-amino acid esters chromatographed on phase III, the resolutions obtained are still very good. Thus, $r_{II/I}$ for N-acetyl-alanine isopropyl ester on phase III at 139° is only lower by 0·023 than that of the corresponding N-TFA derivative on I at 110°.

It has been reported previously, on the other hand that when the trifluoroacetyl group in N-TFA-L-phenylalanine cyclohexyl ester is replaced by isobutyryl, the phase becomes almost incapable of resolving N-TFA-α-amino acid esters. This result has been explained by the increased ability for H-bonding of NH attached to a trifluoroacetyl group. The present data show that this indirect effect of the trifluoromethyl group may be only a minor factor in some systems. It should be recalled, however, that an important effect of the TFA group is the decrease of the melting point of the dipeptide phase, which permits to operate at lower temperatures with correspondingly higher resolution factors.

In addition to the N-TFA esters mentioned in Table 1, the methyl esters of α-amino-n-butyric acid, α-amino-n-valeric acid, valine and leucine were separated on phase I with resolution factors of 1·06–1·07, determined under the same conditions as given in Table 1.

All compounds discussed thus far contain the group —CO—NH—  —CO—. The

resolution obtainable is very sensitive to modifications of this structural feature. Thus, N-TFA-2-aminoheptane showed only a shoulder (3), and the isopropyl esters of N-2, 2, 2-trifluoroethylalanine and N-TFA-β-aminobutyric acid were not resolved (the experiments were carried out on phase I, under the conditions given in Table 1). It is easily seen that the above three derivatives can form less readily, or not at all.

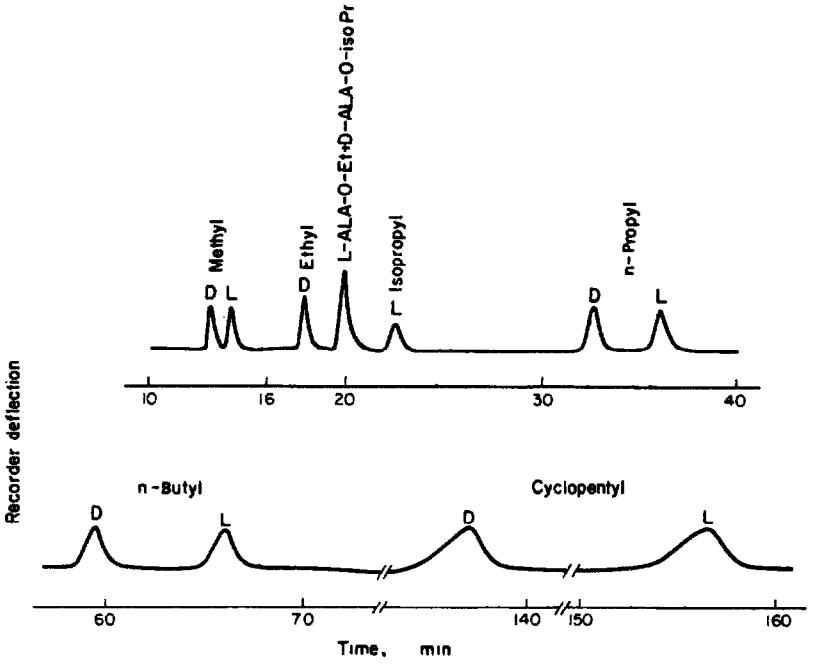


FIG. 1 Chromatogram of N-TFA-(±)- alanine esters on a capillary column coated with N-TFA-L-valyl-L-valine cyclohexyl ester (II); column temperature, 100°; injector, 155°; detector, 125°; carrier gas N_2, pressure 20 p.s.i.

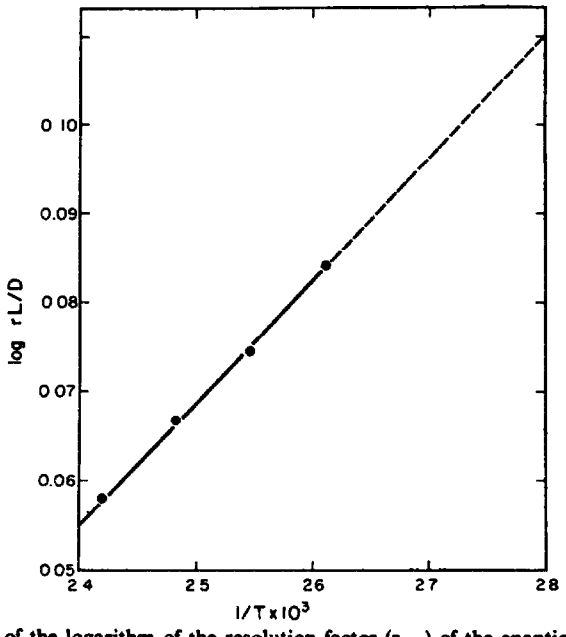


FIG. 2 Plot of the logarithm of the resolution factor ($r_{L/D}$) of the enantiomers of N-TFA-alanine t-butyl ester on N-TFA-L-valyl-L-valine isopropyl ester (I) versus the inverse of the absolute temp.

association complexes with phase I, held together through three H-bonds. It would also appear that in the case of the dipeptides with their numerous possibilities for H-bonding, the formation of *selective* association, through two bridges only, has a relatively low statistical weight (e.g., in the case of N-TFA-2-aminoheptane).

An attempt was made to investigate the effect of extending the peptide chain by synthesizing N-TFA-(L-val)$_3$-O-isopropyl (IV). The compound has a m.p. as high as

TABLE 1. RELATIVE CORRECTED RATENTION TIMES OF N-TFA-(±)—ALANINE ESTERS ON DIPEPTIDE DERIVATIVES AS THE STATIONARY PHASE

N-TFA-L-valyl-L-valine Isopropyl Ester[a] (I)
Temperature 110°

Ester	1st Peak	2nd Peak	$r_{II/I}$[b]
Methyl	0·929	1·000[c]	1·076
Ethyl	1·220	1·346	1·103
Isopropyl	1·327	1·494	1·126
n-Propyl	2·088	2·304	1·103
t-Butyl	1·355	1·644	1·214
n-Butyl	3·576	3·959	1·107
Cyclopentyl	7·455	8·416	1·133

N-TFA-L-valyl-L-valine Cyclohexyl Ester[d] (II)
Temperature 100°

Ester	1st Peak	2nd Peak	$r_{L/D}$
Methyl	0·926	1·000[j]	1·080
Ethyl	1·269	1·404	1·106
Isopropyl	1·395	1·589	1·136
t-Butyl	1·468	1·802	1·227
n-Propyl	2·293	2·536	1·106
n-Butyl	4·175	4·646	1·113
Cyclopentyl	9·674	11·02	1·139

[a] Chromatographic conditions: glass capillary column 70 m × 0·25 mm; injector, 165°; detector, 135°; N_2 pressure, 20 p.s.i.; m.p. of phase I, 107–109°.

[b] Corrected retention time of the second peak over that of the first peak. By extrapolation of the peak assignment made for the esters chromatographed on phase II, it is assumed that $r_{II/I} = r_{L/D}$; see footmote *e*.

[c] Corrected retention time of reference compound, 21·85 min.

[d] Chromatographic conditions: glass capillary column 60 × 0·25 mm; injector, 155°; detector, 125°; N_2 pressure 20 p.s.i.

[e] Peak identifications of enantiomers separated on phase II were made for the methyl, isopropyl and butyl esters; the other esters were assumed to emerge in the same order.

[f] Corrected retention time of reference compound, 14·2 min.

TABLE 2. RELATIVE CORRECTED RETENTION TIMES OF ALANINE DERIVATIVES ON N-ACETYL-L-VALYL-L-VALINE ISOPROPYL ESTER[a] (III)

	Temperature[b] 139°		
N-TFA-alanine esters	1st Peak	2nd Peak	$r_{II/I}$[c]
Methyl	0·932	1·000[d]	1·072
n-Propyl	1·817	1·973	1·086
t-Butyl	1·117	1·263	1·130
n-Butyl	2·936	3·208	1·090
Cyclopentyl	6·056	6·636	1·095
N-Acetyl-alanine esters			
Methyl	0·951	1·000[e]	1·052
Isopropyl	1·190	1·313	1·103

[a] Glass capillary column 70 m × 0·25 mm I.D.; nitrogen pressure, 20 p.s.i.; injector, 195°; detector, 149°.
[b] M.p. of phase II is 129–131·5°.
[c] Retention time of the second peak over that of the first.
[d] Corrected retention time of reference compound, 8.3 min.
[e] Corrected retention time of reference compound, 32·4 min.

TABLE 3. RESOLUTION OF N-TFA—(±)-ALANINE-t-BUTYL ESTER ON MIXTURES OF N-TFA-L-VALYL-L-VALINE ISOPROPYL (I) AND N-TFA-(L-VALYL)$_2$-L-VALINE ISOPROPYL ESTER (IV)

	Relative corrected retention time, $r_{L/D}$			
Temp °C	X[a] = 0	X = 0·28	X = 0·42	X = 0·55
80	—	—	1·288	—
90	—	—	1·269	—
100	—	1·242	1·239	1·216
110	1·214	1·205	1·206	1·203
120	1·187	1·180	1·179	1·176
130	1·165	1·156	1·159	1·154
140	1·142	1·139	—	1·135
Differences in enthalpy and entropy of solution of the enantiomers				
$\Delta\bar{H}_0 = \bar{H}_0(D) - \bar{H}_0(L)$[b] kcal./mole	0·63	0·64	0·67	0·66
$\Delta\bar{S} = \bar{S}_0(D) - \bar{S}_0(L)$[b] cal./mole °K	1·22	1·29	1·37	1·36

[a] $X = n_{IV}/(n_I + n_{IV})$, where n_I and n_{IV} are the number of moles of phase I and phase IV, respectively, in the binary mixture.
[b] The letters in brackets characterize the enantiomers to which the symbols refer.

202°. Therefore, only lower melting binary mixtures of I and IV could be studied. The results are given in Table 3. It is seen that the addition of the tripeptide derivative tends to depress slightly the resolution of N-TFA-alanine t-butyl ester and similar observations were made with the methyl, ethyl, n-propyl, n-butyl and cyclopentyl esters of N-TFA-alanine. Nevertheless, the tripeptide ester is an excellent phase and its further investigation seems of interest. In particular, it should be pointed out that for some binary compositions the m.p. is below 80° (Table 3), and very high resolution factors can be obtained at this lower temperature. For instance, N-TFA-alanine t-butyl ester has a resolution factor as high as 1·29, at 80°, on a binary mixture of the di- and tripeptide, containing 0·42 mole fraction of the latter.

The logarithm of the relative retention times listed in Table 3 was plotted versus the inverse of the absolute temperature and gave straight lines, as illustrated in Fig. 2 (for X = 0). From the slopes of the curves the differences in enthalphy and entropy of solution of the enantiomers were calculated (Table 3). The values for $\Delta\bar{H}_0$ are relatively high (0·63–0·67 kcal.). However, both the differences in enthalphy and entropy play an important role in determining the differences in the free energy of solution of the enantiomers (Table 3). It is further seen that changes in the composition of the binary mixture affect the $\Delta\bar{S}_0$ and $\Delta\bar{H}_0$ values only slightly. In the range of temperatures studied, the entropy was found to vary within a few per cent only.

EXPERIMENTAL

Materials. The stationary phases used had the following properties:

Stationary phase	*m.p.* (°C)	$[\alpha]_D$ (*temp*, % *in* $CHCl_3$)
I	107–109	−14·76 (30°, 4%)
II	96–98	−16·47 (28°, 5%)
III	129–131	−25·25 (29°, 5%)
IV	202	−74·1 (30°, 2·7%)

N-*carbobenzoxy*-L- valyl-L-*valine cyclohexyl ester* (V). Cyclohexyl ester of L-valine (0·05 mole) was dissolved in 130 ml dry $CHCl_3$, cooled to 0°, and 0·055 mole Et_3N added. N-carbobenzoxy-L-valine (0·055 mole; a commercial product from Miles–Yeda Ltd., Rehovoth, Israel) in 110 ml dry $CHCl_3$ equally cooled to 0°, was mixed with the first soln. After the addition of 0·055 mole dicyclohexylcarbodiimide, the mixture was left at room temp overnight, and then washed 3 times with 1% HCl aq, once with water, and 3 times with 5% $NaHCO_3$ aq. After drying over $MgSO_4$, the $CHCl_3$ was removed *in vacuo*. The residue was taken up in ether, filtered, if necessary, and again evaporated *in vacuo*. The residue of the second evaporation was dissolved in a minimum amount of ether and pentane added. After standing overnight in the refrigerator, the ppt of V was filtered off; yield 46%; m.p. 109–110°.

Hydrochloride of L-*valyl*-L-*valine cyclohexyl ester* (VI). V (10 g) was dissolved in 100 ml EtOH, and a few drops AcOH and 0·4 g Pd-C (10%) added. A slow stream of H_2 was passed through the soln for 2 hr, while stirring vigorously. HCl was then added until acid reaction, followed by filtration and evaporation of the alcohol *in vacuo*. On addition of ether a gel formed, which was filtered off and again treated with dry ether. After standing over-night, the ether was decanted and the remaining traces of solvent eliminated *in vacuo*; yield 70%.

N-*TFA*-L-*valyl*-L-*valine cyclohexyl ester* (II). A suspension of 2·7 g of VI in 40 ml CH_2Cl_2 was cooled to −40°, and 3 ml trifluoroacetic anhydride added in small portions, while stirring, in order to avoid raising the temp. The reaction mixture was allowed to heat up to room temp, stirred overnight, washed once with water, 3 times with 1% HCl aq, again with water, and finally 3 times with a 5% $NaHCO_3$ aq. After drying over $MgSO_4$ and filtering, the CH_2Cl_2 was evaporated *in vacuo*. The material was then recrystallized from pentane; yield 80%, m.p. 96–98°.

N-*TFA*-L-*valyl*-L-*valine isopropyl ester* (I) and N-*acetyl*-L-*valyl*-L-*valine-isopropyl ester* (III) were prepared by the same procedure as phase II, using the appropriate reagents.

N-TFA-(L-valyl)$_2$-L-valine isopropyl ester (IV) was a commercial product purchased from Yeda Ltd., Rehovoth, Israel. The compound was recrystallized from pentane before use.

The various N-TFA amino acid derivatives of primary and secondary alcohols used as solutes were prepared by first esterifying the amino acid with the appropriate alcohol, using gaseous HCl as catalyst, followed by trifluoroacetylation, as described above for II, except that stirring at room temp was continued for 1 hr. only. N-TFA-alanine t-butyl ester was synthesized by condenstion of N-TFA-alanine with isobutylene in $CHCl_3$ with H_2SO_4 as catalyst.[6]

Capillaries of 70–100 m × 0·25 I.D. were drawn from Pyrex glass of 1·2 m length, 6 mm O.D. and 2·5 mm I.D. in a machine built according to Desty *el al.*[7] The columns were first coated with a layer of detergent,[8] by passing consecutively 4 ml of a soln of "Tide" (made up by dissolving 58 g of detergent in 135 ml of water and 58 g of EtOH, and filtering), 4 ml of abs alcohol, 4 ml of ether and a flow of dry N_2 overnight. The coating of the phase was then carried out by the plug method with a soln of 20% ether and a plug of 3 m length, moving at a rate of 10 cm/min. After chasing the solvent as above, and conditioning at the temp of operation, the column was ready for work. A typical capillary coated with phase I had, for instance, 70 m length and an efficiency of 140,000 plates with respect to heptyl acetate.

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