Journal of Chromatography, 202 (1980) 247–254
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,185

CHIRAL RECOGNITION IN GAS CHROMATOGRAPHY BY DIAMIDE–DIAMIDE SOLUTE–SOLVENT INTERACTION

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(Received July 28th, 1980)

SUMMARY

The resolution of N-trifluoroacetyl (N-TFA) *tert.*-butylamides of Ala, Val, Leu and Pro by gas chromatography on the N-lauroyl *tert.*-butylamide derivatives of L-alanine (I), L-leucine (II), D-phenylglycine (III) and L-phenylalanine (IV) as stationary phases was investigated, and the results compared with those obtained for the corresponding N-TFA isopropyl esters.

Efficiency of resolution of the two classes of compounds and the differences between them depend greatly on the nature of the phase. N-Lauroyl-L-Phe-*tert.*-butylamide (IV), which is highly efficient for the resolution of the N-TFA esters of α-amino acids, shows even more selectivity for the N-TFA *tert.*-butylamides.

Proline, which is resolved only with great difficulty as its N-TFA ester, behaves distinctly differently from the amino acids having a primary amine group, and on all phases shows high resolution factors, ranging from 1.173 to 1.384.

The chromatography of α-amino acids as N-TFA *tert.*-butylamides would appear to have considerable interest from the analytical point of view. Some ideas on the possible mechanism of chiral recognition, involving, in particular, a diamide–diamide intercalation model, are discussed.

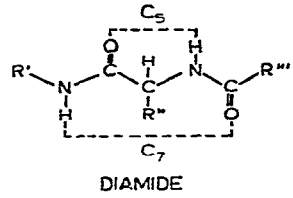
INTRODUCTION

Much of the research carried out in our laboratory in recent years on the resolution of optical isomers has centred around systems in which the solute–solvent association necessary for chiral recognition is effected through hydrogen bonding. In particular, we have studied extensively the highly selective diamide phases derived from α-amino acids, R'''CONHCH(R'')CONHR', where R'' = isopropyl. These investigations have dealt with the effects[1–3] of R' and R''' on selectivity and performance of these phases, and have recently been extended to the study of the influence of the R'' group[4,5]. These diamides permit the resolution of different classes of compounds. However, their most important application lies in the area of α-amino acids, which were chromatographed hitherto exclusively as N-perfluoroacyl ester derivatives.

The mechanism of resolution of N-acyl-α-amino acid esters on diamide phases

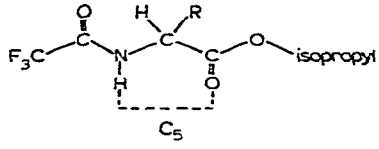
0021-9673/80/0000–0000/$02.25

has been discussed before[2,3,6–8]. It suffices here to recall that the interaction between pertinent solute and solvent molecules is assumed to occur through hydrogen-bonded association of the "C_5–C_5" or "C_5–C_7" type. Recently[8], arguments have been produced for the possible larger contribution of the "C_5–C_7" solute–solvent association to chiral recognition:

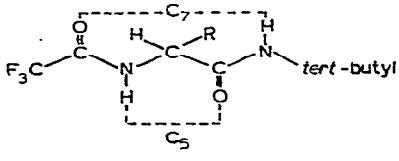


DIAMIDE

N-Acyl-α-amino acid esters have only a C_5 conformation:



It seemed of great interest to study solutes derivatized in such a way as to have both a C_5 and a C_7 side, for instance, the N-trifluoroacetyl (N-TFA) α-amino acid *tert.*-butylamides:



In the present paper we report on the stereoselectivity observed in the interaction of N-TFA-*tert.*-butylamide derivatives of a number of α-amino acids with several diamide phases, and compare the results with those obtained for the corresponding N-TFA-isopropyl esters.

EXPERIMENTAL

Materials

Chromatographic resolution was carried out on the following stationary phases: N-lauroyl-L-alanine *tert.*-butylamide (I)[4]; N-lauroyl-L-leucine *tert.*-butylamide (II)[5]; N-lauroyl-D-phenylglycine *tert.*-butylamide (III)[5] and N-lauroyl-L-phenylalanine *tert.*-butylamide (IV)[5]. These compounds were synthesized according to the general procedure described previously[3]; full details will be reported elsewhere[5].

The N-TFA *tert.*-butylamides of Ala, Val, Leu and Pro were prepared from the N-TFA-amino acids, synthesized according to Weygand and Geiger[9], by one of the following procedures.

(1) To a solution of N-TFA-α-amino acid in chloroform or ethyl acetate, kept at −5 to −10°C, N-hydroxysuccinimide (1.1 equiv.) and dicyclohexylcarbodiimide (1.0 equiv.) were added. After 24 h the dicyclohexylurea formed was filtered off, and into the stirred solution, cooled as above, was added dropwise a mixture of *tert.*-butylamine and N-methylmorpholine (1.0 equiv. each); stirring was then continued in the cold (48 h). The reaction mixture was washed successively with 2% HCl, water, 5% $NaHCO_3$, water and then dried over $MgSO_4$. The residue left on evaporation of the solvent was the desired compound, as checked by NMR spectroscopy. During this reaction no racemization was observed to occur in analogous cases[5].

(2) First, the acid chloride of the N-TFA-α-amino acid in dry dichloromethane was formed by reaction with a slight excess of thionyl chloride at room temperature for 1 h. The solvent was removed by evaporation with a nitrogen stream. The residue was redissolved in dry dichloromethane, cooled in a water–ice bath and *tert.*-butylamine (2 equiv.) was added dropwise. The solution was then stirred for another hour. The reaction mixture was washed as above, and the desired compound obtained on evaporation of the solvent.

The second procedure is more rapid and convenient than the first. However, some racemization of the α-amino acids with a primary amine group (but not of proline) occurs under the above conditions. For the purpose of the present study, where non-racemic mixtures were prepared only for peak identification, both methods can be used.

Chromatographic conditions

Stainless-steel capillary columns (100 ft. × 0.02 in.) were coated by the plug method with I and II (mounted in a Varian Series 2700 chromatograph) and (150 ft. × 0.02 in.) with III and IV (mounted in a Varian Series 1200 chromatograph). Both instruments were provided with a splitter and a flame ionization detector. The temperatures of the injector and detector were 240°C; column temperatures used are given in Table I. The helium flow-rate was 3 ml/min for all columns. The order of elution of the peaks was established for Ala and Pro by operating with mixtures enriched in the D and the L enantiomer, respectively. The result was extrapolated to Val and Leu.

DISCUSSION

The results are listed in Table I and some typical chromatograms are given in Figs. 1–4.

The order of elution for both classes of compounds is the same on I–IV. As expected, on the D-phenylglycine phase (III), the order is reversed with respect to I, II and IV, which are derived from L-α-amino acids.

As to the magnitude of the resolution factors, it is advantageous to discuss the data for the α-amino acids having a primary group separately from those for proline. Although the temperature at which the isopropyl esters and the corresponding diamide derivatives were chromatographed is not the same, there is no difficulty in judging the differences in selectivity for the two classes of compounds.

As can be seen in Table I, Ala, Val and Leu show different behaviour on the four phases. Thus, on I (derived from alanine) the two classes of derivatives show

TABLE I

RESOLUTION OF N-TFA ISOPROPYL ESTERS AND OF N-TFA *tert.*-BUTYLAMIDES OF α-AMINO ACIDS ON DIAMIDES, R'''CONHCH(R'')CONHR', AS STATIONARY PHASES

Optical purity: 98% (I), 99% (II), 81% (III) and 99% (IV). For chromatographic conditions, see Experimental.
r = Corrected retention time (min). $r_{L/D}$ = resolution factor = ratio of the corrected retention time of the enantiomer eluting last over that of the enantiomer eluting first, calculated with r values expressed to the second decimal.

Phase	*N-TFA-alanine*						*N-TFA-valine*		
	Isopropyl ester			*tert.-Butylamide*			*Isopropyl ester*		
	r	$r_{L/D}$	*T*(°C)	*r*	$r_{L/D}$	*T*(°C)	*r*	$r_{L/D}$	*T*(°C)
I. N-Lauroyl-L-alanine *tert.*-butylamide	D 5.00	1.080	130	46.6	1.083	140	6.60	1.061	130
	L 5.40			50.5			7.00		
II. N-Lauroyl-L-leucine *tert.*-butylamide	D 8.46	1.163	150	48.2	1.143	160	12.50	1.138	150
	L 9.84			55.1			14.22		
III. N-Lauroyl-D-phenylglycine *tert.*-butylamide*	L 8.60	(1.140)	130	65.20	(1.055)	150	12.00	(1.117)	130
	D 9.80			68.80			13.40		
IV. N-Lauroyl-L-phenylalanine *tert.*-butylamide	D 4.82	1.212	130	30.24	1.223	150	7.42	1.207	130
	L 5.84			37.00			8.96		

* III being a D phase, the order of elution is reversed. Resolution factors listed are $r_{D/L}$.

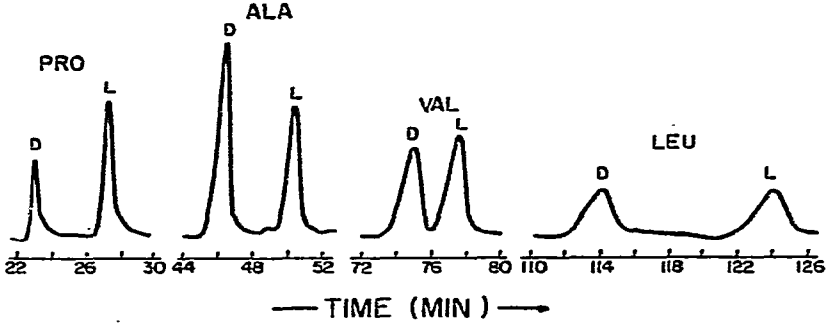


Fig. 1. Chromatogram of N-TFA *tert.*-butylamides of L-enriched Pro, D-enriched Ala, D,L-Val and D,L-Leu on a stainless-steel capillary column (100 ft. × 0.02 in.) coated with N-lauroyl-L-alanine *tert.*-butylamide. For chromatographic conditions, see Experimental. Temperature, 140°C.

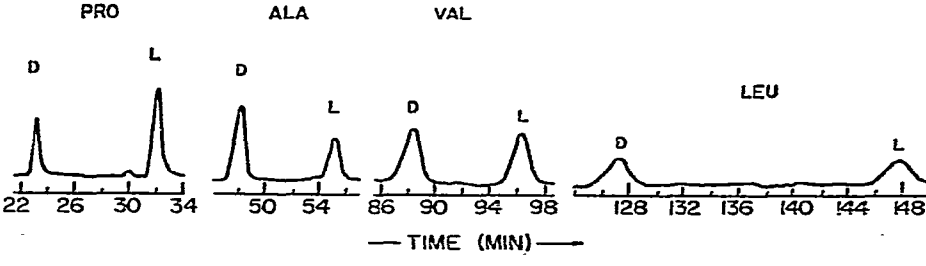


Fig. 2. Chromatogram of N-TFA *tert.*-butylamides of L-enriched Pro, D-enriched Ala, D,L,Val and D,L-Leu on a stainless-steel capillary column (100 ft. × 0.02 in.) coated with N-lauroyl-L-leucine *tert.*-butylamide. For chromatographic conditions, see Experimental. Temperature, 160°C.

N-TFA-leucine						N-TFA-proline					
tert.-Butylamide			*Isopropyl ester*			*tert.*-Butylamide			*Isopropyl ester*			*tert.*-butylamide		
r	$r_{L/D}$	*T*(°C)	*r*	$r_{L/D}$	*T*(°C)	*r*	$r_{L/D}$	*T*(°C)	*r*	$r_{L/D}$	*T*(°C)	*r*	$r_{L/D}$	*T*(C°)
75.20	1.034	140	15.70	1.095	130	114.30	1.087	140	17.00	1.000	130	23.10	1.186	140
77.80			17.20			124.20			17.00			27.40		
88.40	1.089	160	24.30	1.216	150	127.20	1.162	160	29.02	1.036	150	23.20	1.384	160
96.30			29.54			147.80			30.06			32.10		
90.80	(1.066)	150	11.00	(1.145)	150	140.80	(1.020)	150	40.40	(1.000)	130	43.80	(1.173)	150
96.80			12.60			143.80			40.40			51.40		
53.00	1.216	150	6.50	1.215	150	78.32	1.343	150	22.42	1.000	130	17.18	1.314	150
64.48			7.90			105.20			22.42			23.40		

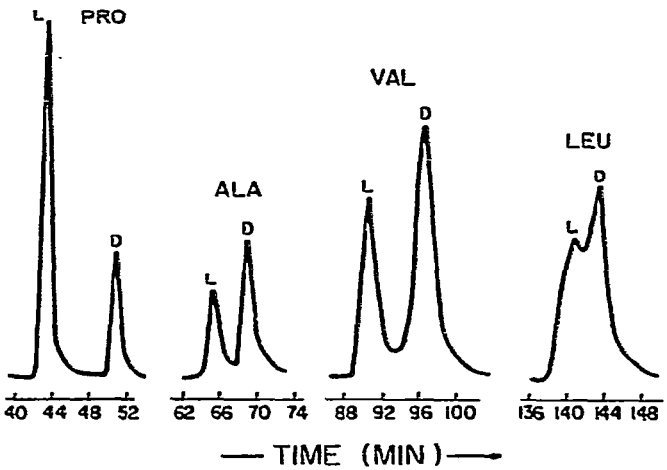


Fig. 3. Chromatogram of N-TFA *tert.*-butylamides of L-enriched Pro, D-enriched Ala, D,L-Val and D,L-Leu on a stainless-steel capillary column (150 ft. × 0.02 in.) coated with N-lauroyl-D-phenylglycine *tert.*-butylamide. For chromatographic conditions, see Experimental. Temperature, 150°C.

approximately the same *r* values. On the leucine phase (II) the resolution for the *tert.*-butylamines is approximately equal for the alanine derivative and lower for valine and leucine, as compared with the corresponding N-TFA isopropyl esters. With the D-phenylglycine phase (III) the diamide solutes have considerably lower resolution factors. On the other hand, on the highly selective phase IV (derived from Phe), the diamide solutes have even higher resolution factors than the isopropyl esters.

Diamides can interact through hydrogen bonding in a number of ways to form structures of the pleated sheet type[10,11]. The diamide molecules can, for instance, be aligned parallel or antiparallel to each other as illustrated in Fig. 5. As in the chro-

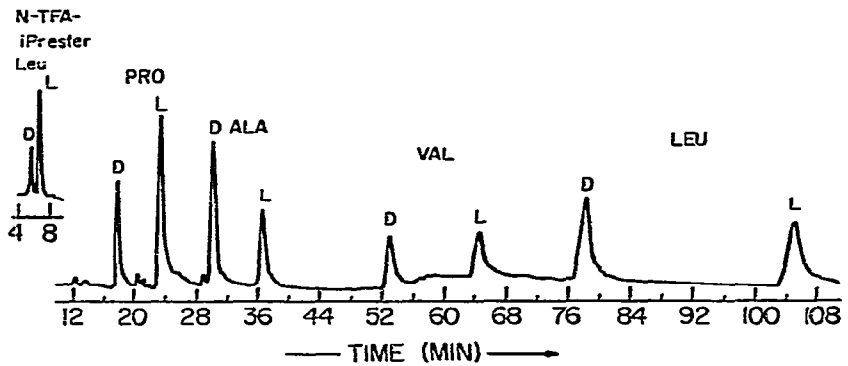
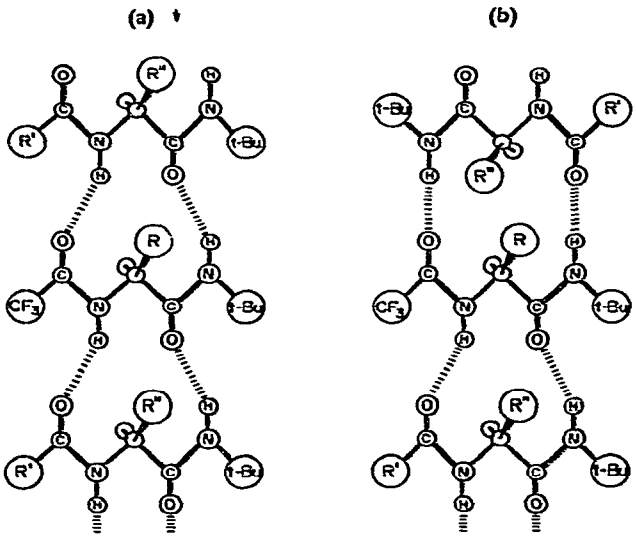


Fig. 4. Chromatogram of N-TFA *tert.*-butylamides of L-enriched Pro, D-enriched Ala, D,L-Val and D,L-Leu and of the N-TFA isopropyl esters of L-enriched Leu on a stainless-steel capillary column (150 ft. × 0.02 in.) coated with N-lauroyl-L-phenylalanine *tert.*-butylamide. For chromatographic conditions, see Experimental. Temperature, 150°C.

matographic process the solute is always present in a very low concentration in the liquid phase, its potential for hydrogen bonding will be fully implemented by interaction with surrounding solvent molecules, resulting in intercalation (Fig. 5). The stereoselectivity observed should result from the differences in interaction between



R: CH_3; $CH(CH_3)_2$; $CH_2CH(CH_3)_2$

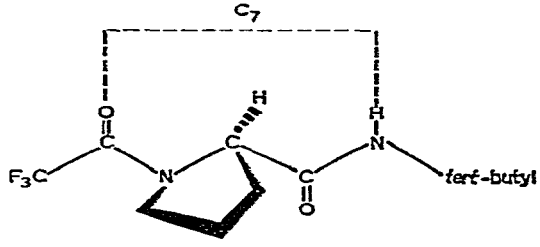
R': $C_{11}H_{23}$

R": CH_3; $CH_2CH(CH_3)_2$; ; CH_2

Fig. 5. Schematic representation of the intercalation of a diamide solute molecule in the diamide solvent aligned parallel (a) or antiparallel (b).

the substituents at the asymmetric centres in the diastereomeric intercalation complexes.

For proline the picture is different than for Ala, Val and Leu. Indeed, N-TFA-proline isopropyl ester does not possess a C_5 side. Its *r* values are small on all diamide phases, and the mechanism of chiral recognition differs from that of the other N-TFA-amino acids which have a primary amine group. In contrast, for the diamide of proline a C_7 conformation is available:



Association with the solvent through a C_7–C_5 or a C_7–C_7 hydrogen-bonded ring is possible. The relatively large resolution factors are ascribed to these associations, with the rigidity imparted to the solute molecule by the ring structure of proline presumably also playing a role. It should also be pointed out that the diamides of proline have lower retentions than those of Ala, Val and Leu. For the isopropyl esters, on the other hand, the inverse is true: their retention is approximately the same as that of the leucine derivatives. This behaviour is ascribed to the fact that, in contrast to the other α-amino acid derivatives, the N-TFA-proline *tert.*-butylamide can interact with neighbouring solvent molecules only by three and not by four hydrogen bonds. The proline derivatives cannot form an intercalation complex of the type shown in Fig. 5, and hence their total interaction with the phase is smaller.

From an analytical point of view the use of diamide solutes has great interest for the determination of the enantiomeric composition of proline and its analogues. The data for phase IV further indicate that the chromatography of N-TFA *tert.*-butylamides of α-amino acids having a primary amine group may lead to more efficient resolution than that of the corresponding N-TFA isopropyl esters. The methods used for the formation of the N-TFA *tert.*-butylamides are described in the Experimental. Further work is required to develop procedures which are rapid and which also completely avoid racemization during derivatization.

It has been shown[12,13] that an intercalation mechanism can explain satisfactorily the chiral recognition observed for the interaction of aromatic monoamides of the type $ArCH(CH_3)NHCOR$. In the present paper we suggest that also in the case of chiral diamides derived from α-amino acids, intercalation of N-TFA *tert.*-butylamides of α-amino acids between the solvent molecules leads to the observed stereoselective effects. It will be the objective of future studies to ascertain by spectroscopic methods, as well as by calculations, the modes of alignment between solvent molecules in the different phases in the liquid state. Subsequently, it would be necessary to estimate the differences in energy of the diastereomeric intercalation products in an attempt to interpret the experimental data.

ACKNOWLEDGEMENT

S.-C. Chang is indebted to the Chung-Shan Institute of Science and Technology, Taiwan, R.O.C., for the grant of a scholarship.

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