Assignment of the Configuration of Optical Isomers by Gas Chromatography with Asymmetric Phases. The Order of Emergence of Amino-alkanes, and α-, β-, and γ-Amino-acids on Carbonylbis-(*N*-L-valine isopropyl ester)

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The interaction of the enantiomers of *N*-trifluoroacetyl derivatives of amines and α-, β-, and γ-amino-acid esters with the optically active stationary phase carbonylbis-(*N*-L-valine isopropyl ester) has been studied systematically by g.l.c. The general formula of the solutes is $CF_3{\cdot}CO{\cdot}NH{\cdot}C^*HR^1R^2$, in which R^1 = alkyl and R^2 is either a different alkyl group or an ester-bearing substituent. When the solute molecules are viewed in the direction from the asymmetric carbon to the nitrogen atom, the other three substituents are seen to be arranged, according to decreasing size, in either a clockwise or an anticlockwise direction, depending on the enantiomer considered. The enantiomers with the clockwise arrangement have been found to have throughout the larger retention time on the L-phase studied. The magnitude of the resolution factor increases with the difference in size between R^1 and R^2.

REGULAR relationships between the configuration and the order of emergence of diastereoisomers have been observed in series of related compounds chromatographed on optically inactive phases.[1-3] Consistent dependency of the order of emergence on configuration has equally been found for enantiomers separated by gas chromatography on optically active phases.[4-6] The value of $\Delta(\Delta F^0)$ of solution of the optical isomers can be ascribed essentially to the difference in interaction of those parts of the chiral solute and solvent molecules which adjoin the asymmetric centres. The difference of the retention volumes should, therefore, be the result of a less complex sum total of interactions than in diastereoisomers. It seemed, hence, that in chiral solute–solvent systems a better understanding of the nature of the interactions could be gained, increasing the reliability of the assignment of configuration by g.l.c.

A mechanism of separation based on the formation of hydrogen-bonded 'diastereoisomeric' association compounds between the chiral solutes and solvents was suggested before.[4-6] In the present study attention was focused on the effect of the structure of the solutes on the strength of association with the solvent.

Two general types of chiral solvent have been used: (1) derivatives of α-amino-acids, and di- and tri-peptides;[4,6] (2) *NN'*-disubstituted urea derivatives,[5] particularly carbonylbis-(*N*-L-valine isopropyl ester) (hereafter called the L-ureide).

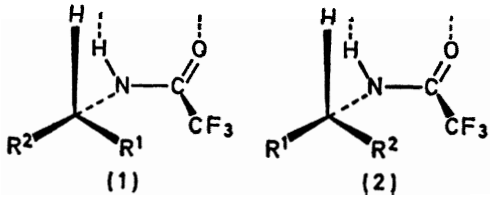
The relative position and/or number of donor and acceptor groups required in the solute for achieving resolution are different in the two cases. For solvents of the first category, efficient separation is dependent on the presence of the group –CO–NH · · · C* · · · CO– with the acceptor and donor groups flanking the asymmetric carbon (C*) on both sides. For the ureide, the presence of $-CO-NH-\underset{|}{\overset{|}{C^*}}-$ in the solute molecule suffices, permitting, in particular, the ready resolution of amino-alkanes in addition to that of amino-acids.

The influence of substituents on resolution differs significantly for the two types of phase. With solvents of the first class, all α-amino-acid derivatives studied, $RC^*H(NH{\cdot}CO{\cdot}CF_3)CO_2R'$, emerge in the order D before L on the L phases, with little effect on resolution shown by lengthening of the chain of either the α-alkyl or the ester groups. On the other hand, for the L-ureide phase the order of emergence varies with the relative size of R and CO_2R', and whereas for all *N*-trifluoroacetyl esters of alanine the D-isomer is eluted before the L-isomer, the reverse is true for the methyl *N*-trifluoroacetyl leucinate. Further, the enantiomers of *N*-trifluoroacetylalaninate and the 2-amino-*N*-trifluoroacetylalkanes were found to emerge in a consistent manner, when account was taken of the arrangement in space according to size of the substituents of the asymmetric carbons in both classes of compound. Finally, on the L-ureide phase the *N*-trifluoroacetyl derivatives of both alanine esters and 2-amino-alkanes showed resolution factors which increase with the disparity in chain length of R and CO_2R', respectively, the methyl and alkyl groups.

These observations led us to suggest a mechanism of resolution of solutes on the ureide based only on the effect of the bulk of the substituents, even if they are not all apolar groups. It is suggested that the ureide is linked through two hydrogen bonds to the amide group of the solute, and that thus the two molecules are oriented in a particular manner towards each other. Viewing the asymmetric carbon of the associated solute molecule from the side remote from its nitrogen atom, one obtains the projection formulae for the two enantiomers, having respectively, a clockwise (1) and an anticlockwise (2) arrangement of R^1, R^2, and H. The order of emergence and the magnitude of the resolution factors will depend on the relative stability of these

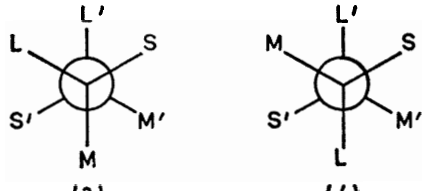
[1] E. Gil-Av and D. Nurok, *Proc. Chem. Soc.*, 1962, 146.
[2] J. W. Westley and B. Halpern, 'Gas Chromatography, 1968,' ed. S. L. A. Harbourn, Institute of Petroleum, London, 1969, p. 119.
[3] E. Gil-Av and D. Nurok, in 'Resolution of Optical Isomers by Gas Chromatography of Diastereomers,' in 'Advances in Chromatography,' eds. G. C. Giddings and R. A. Keller, Marcel Dekker, New York, in the press.
[4] E. Gil-Av, B. Feibush, and R. Charles-Sigler, in 'Gas Chromatography, 1966,' ed. A. B. Littlewood, The Institute of Petroleum, 1967, p. 227; p. 235 (authors' additional comments).
[5] B. Feibush and E. Gil-Av, *J. Gas Chromatog.*, 1967, **5**, 257.
[6] B. Feibush and E. Gil-Av, *Tetrahedron*, 1970, **26**, 1361.

'diastereoisomeric' association complexes. It is assumed that the difference in stability can be interpreted in terms of preferred conformations, and that the



(1) (2)

non-bonded interactions between the chiral solvent and the enantiomers can be described as if the two asymmetric centres were on vicinal carbons [(3) and (4)].



(3) (4)

With this simplified approach, it appears reasonable that one of the combinations of the enantiomers with the chiral solvent [see (1) and (2)], must be less stable than the other by analogy with the pair (3) and (4),* where (3) should be less stable because of the interaction between the bulkiest substituents L and L'. The larger the difference in size between M and L, the larger should be the effect on the relative stability and hence on the resolution factor. Further, for a given configuration of the solvent (*i.e.*, by analogy, a given handedness of L'M'S) the order or emergence will depend on the clockwise L→M→S † or anticlockwise L←S←M arrangement according to size of the substituents R^1, R^2, and H. Thus the configuration of an enantiomer could be determined from its order of emergence on the L-ureide phase if the relative size of the substituents R^1 and R^2 is known. Also for *N*-trifluoroacetyl-α-amino-acid derivatives a reversal of the order of emergence for a given amino-acid could occur depending on the relative size of R^1 and R^2, which are in this case, respectively, alkyl and alkoxycarbonyl groups.

Admittedly the working hypothesis suggested involves a greatly simplified representation of the interaction between the solutes and the solvent. Its use appears justified by the success with which it permits the accommodation of experimental data and its utility in making predictions.

RESULTS AND DISCUSSION

In order to test the validity of the suggested mechanism and its consequences, a systematic study of the influence of the size of substituents on resolution of *N*-trifluoroacetyl amino-acid esters on the L-ureide phase was undertaken. Experimentally these solutes are convenient for the purpose since many of them are readily available optically pure.

α-Amino-acids.—When considering the experimental results given in Table 1, it should be recalled with reference to projection formulae (1) and (2) that R^1 = alkyl and R^2 = alkoxycarbonyl, so that the D-enantiomer corresponds to (1) and the L-enantiomer to (2). In the horizontal rows of Table 1 the relative size of the alkoxycarbonyl (CO_2R') group increases as compared with the α-alkyl group on going from left to right, and the numerical values of $r_{L/D}$ ‡ increase in the same sense (see also Figures 1 and 2). On the other hand, in vertical rows, for amino-acids with normal chains, the relative size of the α-alkyl group (R) increases with respect to the alkoxycarbonyl group on going from the top to the bottom. Correspondingly, there is a decrease of the values of $r_{L/D}$ in this direction, *e.g.*, for the methyl

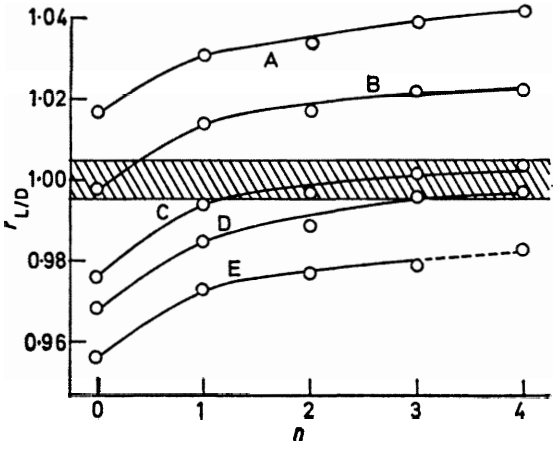


FIGURE 1 Influence of the alcohol chain (R' = $[CH_2]_nMe$) on the resolution factor of normal *N*-trifluoroacetyl-α-amino-acid esters $RCH(NH{\cdot}CO{\cdot}CF_3)CO_2R'$. The shaded area, corresponding to $r = 1{\cdot}000 \pm 0{\cdot}005$, divides the graph into two regions. In the upper part the D- precedes the L-enantiomer, whereas the reverse is true for the lower part. Within the shaded area no resolution of enantiomers takes place under the experimental conditions; A, alanine; B, α-aminobutyric acid; C, α-aminovaleric acid; D, α-aminohexanoic acid; E, α-amino-octanoic acid

esters the *r* values become first equal to 1·00 (no resolution) and then drop below 1·00, which means inversion of the order of emergence (see also Figures 3 and 4). Further, the opposing effects of CO_2R' and R are seen to result in cancellation of resolution ($r = 1{\cdot}00$) for compounds situated along an approximately diagonal band across the upper (framed) part of Table 1; above

* (3) and (4) should be preferred conformations, as in both the small groups S and S' are placed between the larger ones.

† L, M, and S stand for large, medium, and small groups, respectively.

‡ $r_{L/D}$ = Ratio of the corrected retention volume of the L-isomer over that of the D-compound. $r_{II/I}$ = Ratio of the corrected retention volume of the second peak over that of the first peak. The relationship $\Delta F = -RT \ln r_{L/D}$ shows that $\ln r_{L/D}$ would be a better measure of the difference of the interaction of the enantiomers with the ureide phase. However, the numerical values can be used instead, since the resolution factors measured are throughout near unity, and, hence, $r - 1 \simeq \ln r$.

TABLE 1

Resolution factors [a] of *N*-trifluoracetyl-α-amino-acid esters, $RCH(NH{\cdot}CO{\cdot}CF_3)CO_2R'$, with carbonyl-bis-(*N*-L-valine isopropyl ester) as the stationary phase [b] at 120 °C

R′ = Alcohol residue

		Me		Et		Pr^n		Bu^n		$n\text{-}C_5H_{11}$		Pr^i		Et_2CH	
		r	$r_{L/D}$	r	$r_{L/D}$	r	$r_{L/D}$	r	$r_{L/D}$	r	$r_{L/D}$	r	$r_{L/D}$	r	$r_{L/D}$
Alanine	D [c]	0·72	1·017	0·86	1·031	1·45	1·034	2·45	1·039	4·18	1·042	0·82	1·046	2·23	1·069
	L [c]	0·73		0·89		1·49		2·55		4·36		0·86		2·38	
α-Amino-butyric acid	D [c]	0·85	1·000	1·02	1·014	1·69	1·017	2·85	1·022	4·84	1·023	0·97	1·027	2·61	1·051
	L [c]			1·04		1·72		2·92		4·95		1·00		2·75	
α-Amino-valeric acid	D [c]	1·51	0·976	1·75	0·994	2·85	(0·997) [d]	4·64	(1·002) [d]	7·66	(1·004) [d]	1·69	1·005	4·36	1·034
	L [c]	1·47		1·74								1·70		4·47	
		$r_{II/I}$ = 1·024		$r_{II/I}$ = 1·006		$r_{II/I}$ = (1·003) [d]									
α-Amino-hexanoic acid	D [c]	2·46	0·968	2·83	0·985	4·58	0·989	7·54	0·994	12·32	(0·997) [d]	2·74	(1·002) [d]	4·14	1·027
	L [c]	2·38		2·78		4·53		7·49						4·25	
		$r_{II/I}$ = 1·032		$r_{II/I}$ = 1·015		$r_{II/I}$ = 1·011		$r_{II/I}$ = 1·006		$r_{II/I}$ = (1·003) [d]					
α-Amino-octanoic acid	D [c]	7·19	0·956	8·16	0·973	13·03	0·977	19·62	0·979			7·69	0·990	12·42	1·016
	L [c]	6·88		7·94		12·75		19·21				7·62		12·72	
		$r_{II/I}$ = 1·045		$r_{II/I}$ = 1·027		$r_{II/I}$ = 1·023		$r_{II/I}$ = 1·021				$r_{II/I}$ = 1·010			
Valine	D [c]	0·68	1·012	0·83	1·030	1·32	1·033	2·23	1·034	3·77	1·036	0·83	1·043	2·23	1·058
	L [c]	0·69		0·86		1·37		2·30		3·90		0·87		2·35	
Leucine	D [c]	2·27	0·957	2·59	0·977	4·23	0·981	6·66	0·983	11·61	0·987	6·48	1·000	7·24	1·025
	L [c]	2·17		2·53		4·15		6·55		11·46				7·43	
		$r_{II/I}$ = 1·043		$r_{II/I}$ = 1·023		$r_{II/I}$ = 1·019		$r_{II/I}$ = 1·017		$r_{II/I}$ = 1·013					
t-Leucine	D [c]	0·54	1·000	0·66	1·015							0·66	1·019		
	L [c]			0·67								0·68			

[a] For the definition of $r_{L/D}$ and $r_{II/I}$ see text; r = relative corrected retention volume with respect to decyl acetate as reference compound. [b] On a glass capillary column of 80 m × 0·25 mm; other chromatographic conditions, see text. [c] Peak identification was made with mixtures enriched in one of the enantiomers. [d] Estimated by interpolation. [e] Peak identification was based on the rules correlating configuration with order of emergence.

this band the order of emergence is D before L, while the reverse is true for the region below it, with the resolution improving as the distance from the diagonal increases. As predicted, reversal of the order of emergence occurs also in some cases for the same amino-acid on changing the chain length of the esterifying alcohol (see, *e.g.*, the α-amino-valerates and -hexanoates).

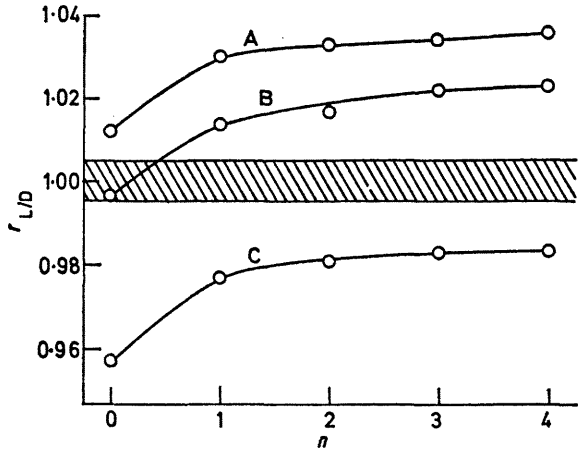


FIGURE 2 Influence of the alcohol chain (R′ = $[CH_2]_nMe$) on the resolution factor of *N*-trifluoroacetyl esters of A, valine, B, α-aminobutyric acid, and C, leucine; see legend to Figure 1

It is readily recognized from the structure of the alanine esters that R^1 = methyl is the medium-sized substituent as compared with H and $R^2 = CO_2R'$. The experimental data show that the L-alaninates with the clockwise arrangement of L→M→S emerge last and have a stronger interaction with the L-ureide, than the D-isomers with the anticlockwise arrangement L←S←M. The reversals in both horizontal and vertical rows agree with the relationship found for the alanine esters; the correspondence between the

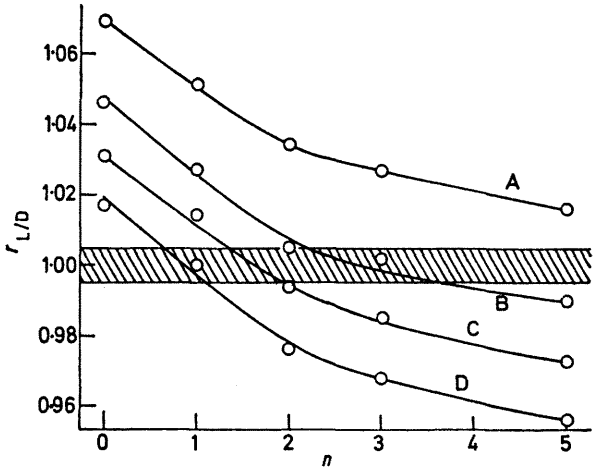


FIGURE 3 Influence of the α-alkyl group (R = $[CH_2]_nMe$) on the resolution factor of α-amino-acid esters $RCH(NH{\cdot}CO{\cdot}CF_3)$-$CO_2R'$. For legend see Figure 1; A, R′ = Et_2CH; B, R′ = Pr^i; C, R′ = Et; D, R′ = Me

order of emergence and the handedness of the spatial arrangement of the substituents according to size is seen thus to have general validity.

The influence of the relative size of the substituents on resolution expresses itself not only in a predictable qualitative behaviour but also in quantitative relationships. As can be seen by the parallel plots in the Figures, and the data in Table 2, identical changes in

TABLE 2

Change of the resolution factor for equivalent changes in structure of *N*-trifluoroacetyl-α-amino-acid esters, $RCH(NH{\cdot}CO{\cdot}CF_3)CO_2R'$

Effect of the α-alkyl substituent R

	Alanine (R = Me)	α-Aminobutyric acid (R = Et)		α-Aminohexanoic acid (R = Bu^n)	
R′	$r_{L/D}$	$r_{L/D}$	Δr_1 [a]	$r_{L/D}$	Δr_2 [a]
Me	1·017	1·000	−0·017	0·968	−0·049
Et	1·031	1·014	−0·017	0·985	−0·046
Pr^n	1·034	1·017	−0·017	0·989	−0·045
Bu^n	1·039	1·022	−0·017	0·994	−0·045
n-C_5H_{11}	1·042	1·023	−0·019		

Effect of the ester group CO_2R'

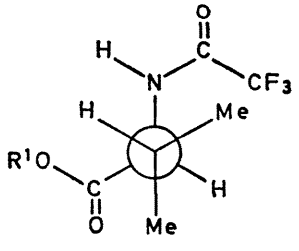
	R′ = Me	R′ = Et		R′ = Pr^n	
Amino-acid	$r_{L/D}$	$r_{L/D}$	Δr_3 [a]	$r_{L/D}$	Δr_4 [a]
Alanine	1·017	1·031	0·014	1·034	0·017
α-Aminobutyric acid	1·000	1·014	0·014	1·017	0·017
α-Aminovaleric acid	0·976	0·994	0·018	(0·997) [b]	
α-Aminohexanoic acid	0·968	0·985	0·017	0·989	0·021
α-Amino-octanoic acid	0·956	0·973	0·017	0·977	0·021

[a] $\Delta r_1 = r_{L/D}$ (R = Et) − $r_{L/D}$ (R = Me); $\Delta r_2 = r_{L/D}$ (R = Bu^n) − $r_{L/D}$ (R = Me); $\Delta r_3 = r_{L/D}$ (R′ = Et) − $r_{L/D}$ (R′ = Me); $\Delta r_4 = r_{L/D}$ (R′ = Pr^n) − $r_{L/D}$ (R′ = Me). [b] Estimated by interpolation, see Table 3, footnote *b*.

R or R′ lead to about equal increments of the resolution factor. The effect of a methylene group in R is about twice that in R′.

Branching of R′ leads to larger increments of the resolution factor than mere lengthening of the chain. The same phenomenon is observed for an R group branched in the γ-position as in the leucine esters. On the other hand, in the β-branched valine and t-leucine

* In its preferred conformation the *N*-trifluoroacetyl valine ester has a hydrogen atom in a staggered position between the ester and the amide substituents. On the other hand, for



α-aminobutyric acid, for instance, the conformer with a methyl group in this same position will be relatively more stable. If the interaction of R with the solvent occurs from the side defined by the amide and the ester groups, it is understood why higher and not lower values are found for valine compared with α-aminobutyric acid.

(2-amino-3,3-trimethylbutyric acid), it is found that the alkyl groups, R, which are normally considered to have high steric hindrance, show a lesser effective size than the corresponding normal alkyls of the same carbon number. This behaviour ascribed to conformational effects * does not contradict the working hypothesis.

Isosteric Group.—Since the effect of the size of R and CO_2R' on resolution are of opposite sign, cases occur, as already mentioned, in which the compounds are unresolvable on the L-ureide phase ($r_{L/D}$ = 1·00). These compounds serve to define isosteric [7] substituents R and CO_2R', *i.e.*, groups having the same (effective) size as appraised by the phenomenon of resolution.

Table 3 compares pairs of compounds of the general formula $X{-}C^*H(NHCOCF_3){-}Y$ belonging to the classes of the aminoalkane and α-amino-acid ester having isosteric groups Y and common alkyl substituents X. The resolution factors of equivalent derivatives agree remarkably well. The results confirm the parallelism between the mechanism of resolution of the derivatives

TABLE 3

Comparison of resolution factors of enantiomeric A, *N*-trifluoroacetylaminoalkanes; and B, *N*-trifluoroacetylamino-acid esters, $X{-}CH(NH{\cdot}CO{\cdot}CF_3){-}Y$, where Y is an isosteric group and X an alkyl substituent

X	A $r_{D/L}$ [b]	B [a] $r_{L/D}$ [b]
	$X{-}CH(NH{\cdot}CO{\cdot}CF_3){-}Bu^n$	$X{-}CH(NH{\cdot}CO{\cdot}CF_3){-}CO_2Pr^i$
Me	1·049 [c]	1·046
Et	1·027 [d]	1·027
Pr^n	Shoulder [d]	1·005
Bu^n	1·000 (symmetric)	(1·002) [b]
	$X{-}CH(NH{\cdot}CO{\cdot}CF_3){-}Bu^i$ [e]	$X{-}CH(NH{\cdot}CO{\cdot}CF_3){-}CO_2Pr^i$
Me	1·047 [e]	1·046
	$X{-}CH(NH{\cdot}CO{\cdot}CF_3){-}Pr^n$	$X{-}CH(NH{\cdot}CO{\cdot}CF_3){-}CO_2Bu^n$
Me	1·040 [e]	1·039
Et	*f*	1·022
Pr^n	1·000 (symmetric)	(1·002) [b]
Bu^n	Shoulder [d]	0·994
	$X{-}CH(NH{\cdot}CO{\cdot}CF_3){-}Et$	$X{-}CH(NH{\cdot}CO{\cdot}CF_3){-}CO_2Me$
Me	1·027 [e,g]	1·017
Et	1·000 (symmetric)	1·000
Pr^n	*f*	0·976
Bu^n	1·027 (0·973) [h]	0·968

[a] See Table 1. [b] Differences of ±0·002—0·005 are to be expected, because of the experimental error on r = 1·00. Where several esters of a certain amino-acid could not be resolved, the corrected data (values in parentheses, see Table 1) were used to choose the derivative with resolution factor nearest to unity. [c] Ref. 5. [d] E. Gil-Av and B. Feibush, unpublished results. [e] Isobutyl and isopropoxycarbonyl are isosteric, as the *N*-trifluoroacetyl-leucine isopropyl ester is not resolved (Table 1). [f] Not determined. [g] The experimental error on r is larger than usual for this compound, because of its relatively short retention time. [h] Since peak reversal occurred in the corresponding α-amino-acid ester, the value in parentheses must be used for comparison.

of aminoalkanes and α-amino-acid esters, and thus further support the working hypothesis. The above comparison of isosteric groups in the two series also demonstrates that the substituents at the asymmetric

[7] R. L. Stern, B. L. Karger, W. J. Keane, and H. C. Rose, *J. Chromatog.*, 1969, **39**, 17.

carbon exert their influence on resolution independently of each other in the derivatives examined.

As in the preceding section, a comparison involving a β-substituted α-amino-acid derivative gave an unexpected result, in that methoxycarbonyl and t-butyl groups were found to be isosteric. However, when X = methyl is opposed to these two groups, different values are found for the resolution factor, namely, 1·017 for alanine methyl ester and 1·059 for 2-amino-3,3-dimethylbutane. The small effective size of the β-substituted groups in α-amino-acid derivatives seems thus to be linked with the simultaneous presence of an ester group in the molecule.

Absolute Configuration and Order of Emergence.—Determination of the configuration based on the above empirical relationships requires a knowledge of the effective size of R (= R^1) and CO_2R' (= R^2). The $CO_2R' < R$. In order to correlate configuration with the sign of rotation, it is of course necessary that an optically enriched mixture be chromatographed.

An interesting case is t-leucine. According to Table 1, $r_{II/I}$ increases with the size of the esterifying alcohol. Hence, the effective size of ethoxycarbonyl is already larger than that of the t-butyl group and the first peak should correspond to the D-isomer. By chromatographing a mixture of the *N*-trifluoroacetyl isopropyl ester enriched in one of the isomers [8] it was confirmed that the L-enantiomer has the longer retention time. This finding is further evidence for the now accepted configurational assignment of the t-leucine enantiomers. As recently as 1964 [8] it was deemed necessary to reconfirm by a series of physical methods the pertinent conclusions of Izuma and his co-workers,[9] based on enzymic tests.

TABLE 4

Resolution factors [a] of *N*-trifluoroacetyl-β- and γ-amino-acid esters, $RCH(NH{\cdot}CO{\cdot}CF_3){\cdot}[CH_2]_n{\cdot}CO_2R'$, with carbonylbis-(*N*-L-valine isopropyl ester) as the stationary phase [b] at 120 °C

R′ = Alcohol residue

		Me		Et		Pr^n		Bu^n		Pr^i		Et_2CH	
Ester		r	$r_{L/D}$	r	$r_{L/D}$	r	$r_{L/D}$	r	$r_{L/D}$	r	$r_{L/D}$	r	$r_{L/D}$
β-Aminobutyrate	D [c]	1·85	1·016	2·40	1·023	2·53	1·026	6·76	1·028	2·68	1·030	6·62	1·037
	L [c]	1·88		2·46		2·61		6·95		2·76		6·88	
β-Aminovalerate	D [d]	2·77	1·000	3·53	1·006	5·63	1·012	9·50	1·014	6·12	1·016	9·10	1·023
	L [d]			3·55		5·69		9·63		6·23		9·31	
β-Aminohexanoate	D [c]	4·45	0·985	5·64	0·991	8·16	0·994			5·82	1·000		
	L [c]	4·38		5·59		8·11							
		$r_{II/I}$ 1·015		$r_{II/I}$ 1·009		$r_{II/I}$ 1·006							
γ-Aminovalerate	D [d]	5·47	1·053	9·36	1·060	14·90	1·061	19·98	1·064	9·06	1·064	20·36	1·065
	L [d]	5·76		9·93		15·81		21·27		9·64		21·70	

[a] For the definition of r, $r_{L/D}$, and $r_{II/I}$ see text. [b] Chromatographic conditions as in Table 2, footnote *b*. [c] Peak identification was made with mixtures enriched in one of the enantiomers. [d] Peak identification was based on the rules correlating configuration with order of emergence.

bulk relationship between these two substituents may be evaluated from the formulae, particularly with reference to the magnitude of the $r_{L/D}$ values of appropriate compounds in Table 1 and to the groups recognized to be isosteric (Table 3). It can also be determined experimentally by following the change of the ratio of the retention volumes of a series of esters, $r_{II/I}$ (not $r_{L/D}$, Table 1), on lengthening the alcohol chain, R′. If with increasing length of *R′* $r_{II/I}$ increases the CO_2R' groups considered are of larger effective size than R; the reverse is true, if $r_{II/I}$ decreases.

Let us view the molecule of the solute in the direction from the asymmetric carbon to the nitrogen as in (1) and (2). The experimental results show that the enantiomer in which the order of the above substituents according to their effective size is anticlockwise (L←S←M), will be the one to emerge first; if the order is clockwise, it will emerge last. Thus, for the α-amino-acid derivatives the rule is: *the first peak will have the* D-*configuration if* $CO_2R' > R$, *and the* L-*configuration if*

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β- *and* γ-*Amino-acids.*—Let us now assume that the validity of the model can be extended to amino-acid derivatives of formula $RC^*H(NH{\cdot}CO{\cdot}CF_3){\cdot}[CH_2]_n{\cdot}CO_2R'$, where $n = 1$ or 2, and examine the experimental results accordingly. Chromatographic data for β-amino-acid derivatives are given in Tables 4 and 5. In parallel with

TABLE 5

Change of the resolution factor for equivalent changes in structure of *N*-trifluoroacetyl-β-amino-acid esters,[a] $RCH(NH{\cdot}CO{\cdot}CF_3)CH_2{\cdot}CO_2R'$

N-Trifluoroacetyl ester of	R′ = Me	R′ = Et		R′ = Pr^n	
	$r_{L/D}$	$r_{L/D}$	Δr_1 [b]	$r_{L/D}$	Δr_2 [b]
β-Aminobutyric acid (R = Me)	1·016	1·023	0·007	1·026	0·010
β-Aminohexanoic acid (R = Pr^n)	0·985	0·991	0·006	0·994	0·009
Δr_3 [b]	0·031	0·032		0·032	

[a] See Table 4. [b] $\Delta r_1 = r_{L/D}$ (R′ = Et) − $r_{L/D}$ (R′ = Me); $\Delta r_2 = r_{L/D}$ (R′ = Pr^n) − $r_{L/D}$ (R′ = Me); $\Delta r_3 = r_{L/D}$ (R = Me) − $r_{L/D}$ (R = Pr^n).

the results for α-amino-acid derivatives, equivalent changes in structure have been found to lead to approximately the same change in the resolution factors (Table 5). Further (Table 4), depending on the relative size of the

substituents, certain derivatives are not resolvable (*e.g.*, methyl *N*-trifluoroacetyl-β-aminovalerate), or a reversal of the order of emergence appears to occur (see β-aminohexanoic acid). Since the influence of substituents follows a similar pattern as for α-amino-acids, application of the above rules for the determination of the configuration of β-amino-acids seems to be justified.

Accordingly, it was concluded that the esters of β-aminobutyric and of β-aminovaleric acids emerge with the D- preceding the L-enantiomers, whereas for the β-aminohexanoic acid, the methyl, ethyl, and n-propyl esters emerge in the order L before D. These conclusions were confirmed experimentally, with optically enriched mixtures for β-amino-butyric and -hexanoic acids.

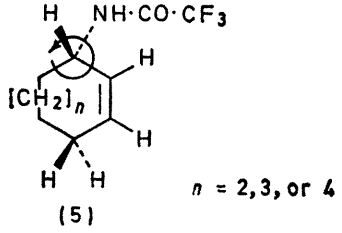
Compared with α-amino-acids having the same R group substituting the asymmetric carbon, the β-amino-acid derivatives show smaller resolution factors.

On introduction of a dimethylene group between the asymmetric carbon and the ester group ($n = 2$) as in γ-aminovaleric acid, a relatively larger increase of the resolution factor $r_{L/D}$ is observed with respect to the corresponding alanine and β-aminobutyric acid derivatives. This is in keeping with the expected influence of increase in size of the substituent carrying the ester group. The effect of lengthening of the chain of normal alkoxy-groups is similar to that observed for the β-amino-acids (Table 4). Though only one example of this class has been studied it is felt that, in view of the evidence accumulated in this study, γ-amino-acids can be considered as coming under the above rule.

Amines.—The suggested mechanism of resolution is based on the assumption that the size of the substituents, rather than their chemical nature, determines the order of energence. An important argument presented for this view was the observation that the L-2-amino-n-alkanes emerge before their D-enantiomers.[5] Generalizations on the order of elution of other types of amine can be made by applying the conclusions of the present work.

By reference to the above rule it can be stated that amino-n-alkanes having the amino-function at any position of the chain emerge in the same order as the 2-amino-derivatives. No such general statement can, however, be made for branched amines, since in this case the nomenclature is not necessarily linked to the spatial arrangement of the substituents according to their effective size. A number of specific cases will be discussed in a forthcoming publication.

The cyclic amines have not yet been investigated thoroughly. However, the *N*-trifluoroacetyl-*cis*- and *trans*-3-methylcyclohexylamines emerge[5] in the order 1-*R* before 1-*S*,* as expected. As to the *N*-trifluoroacetyl-3-aminocyclenes, experimental identification of the peaks has not been made in any of the examples studied.[5] In order to apply the rule to this case, it is necessary to determine whether the double bond is to be included formally in the 'medium' or the 'large' substituent. When 3-aminocyclo-octene is dissected by a plane passing through the asymmetric carbon and the hydrogen and nitrogen atoms attached to it, two unequal ring sections are clearly formed, with the double bond situated in the smaller one. The unsaturated part is thus taken to be the medium-sized substituent. The decrease of the resolution factor with diminishing ring size demonstrates that this conclusion also holds for the other cyclo-olefinic amines studied (see the assessment of relative effective size of substituents from $r_{II/I}$ for α-amino-acids). Accordingly it is predicted that the enantiomer (5) which has the anticlockwise arrangement of substituents L←S←M will emerge first.



(5)

The Selective Association Complex.—The following remarks refer essentially to the interaction between the ureide phase and *N*-trifluoroacetyl-α-amino-acid esters. According to the working hypothesis, the solute in the selective association complex is hydrogen-bonded to the solvent molecule through its $NH{\cdot}CO{\cdot}CF_3$ group. In the alternative arrangement, in which the ester carbonyl serves as the acceptor group of the solute, the difference between the antipodes would express itself in the relative positions of the hydrogen and the R substituent linked to the asymmetric carbon. Such a complex could not account for the observed experimental facts. The same argument applies to triply bonded forms. Incidentally, association through only

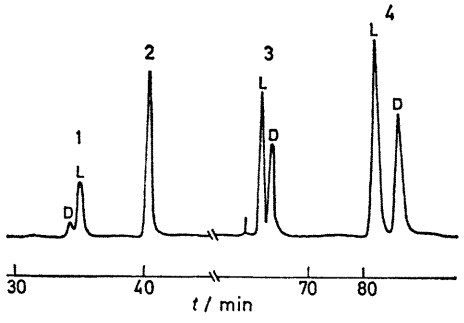


FIGURE 4 Peak reversal of *N*-trifluoroacetyl-α-amino-acid methyl esters $RCH(NH{\cdot}CO{\cdot}CF_3)CO_2Me$ with change in size of R. Chromatographed on a glass capillary column 80 m × 0·25 mm, coated with the L-ureide phase; other conditions as in text. The groups of peaks represent 1, alanine; 2 α-amino-butyric acid; 3, α-aminovaleric acid; 4, α-aminohexanoic acid

* The DL nomenclature is unsatisfactory in this case; in the *RS* nomenclature L-2-amino-n-alkanes are assigned *R* configuration.

one hydrogen bond would exert too little constraint to force the asymmetric centres together.

Since the two *N*-substituents of the ureide are identical, bonding to either of its NH groups is equivalent. On the other hand, the NH of the solute has two different options for hydrogen bonding to the solvent. It has been found[10] that for sulphonylbis-(*N*-L-valine isopropyl ester) resolution is extremely weak and this seems to argue for an essential role played by the central carbonyl group. However, further work is needed to establish this. In addition to the number and nature of the groups involved in hydrogen bonding, the conformation of the solute–solvent complex will have to be elucidated in order to gain a better understanding of the nature of the selective association.

EXPERIMENTAL

Materials.—The *N*-trifluoroacetylamino-acid esters were prepared as described.[11] The racemic and optically pure amino-acids used were mostly commercial reagents. Racemic β-amino-valeric and -hexanoic acids were synthesized by addition of ammonia to the corresponding αβ-unsaturated acids.[12] Racemic β-aminobutyric acid was prepared by reduction of the acetylhydrazone of ethyl acetoacetate with aluminium amalgam,[13] and hydrolysis of the ester obtained. γ-Aminovaleric acid was obtained by reductive amination of levulinic acid.[14]

L-*t-Leucine.* t-Leucine was converted into its methyl ester, and resolved by crystallization of the salt formed with dibenzoyl-L-tartaric acid.[8] A crop of the L-t-leucine methyl ester dibenzoyl-L-hydrogen tartrate had $[\alpha]_D^{23}$ −84·5° (*c* 2 in MeOH) [lit.,[8] −83·7° (*c* 1·0 in MeOH)]. The salt (200 mg) was refluxed with 10% KOH, neutralized, the aqueous solution evaporated to dryness, and the residue submitted to esterification with propan-2-ol containing 1·2N-HCl and then *N*-trifluoroacetylated in the usual way.[11] G.l.c. on the L-ureide showed that the L-t-leucine was over 95% optically pure and coincided with the second peak of the racemic mixture. The hydrolysis products of dibenzoyltartaric acid did not interfere with the g.l.c.

L-β-*Amino-butyric and -hexanoic acids.* These optically active amino-acids were synthesized by the Arndt–Eistert homologation reaction from L-alanine and L-α-amino-valeric acid, respectively, by the procedure of Balenovic and Stimac.[15] The structures of the intermediates and the end products were determined by n.m.r. Details will be given elsewhere.

The L-β-aminobutyric acid isolated was not quite pure and had $[\alpha]_D$ +13·5° (*c* 4·0 in 6N-HCl) {lit.,[16] $[\alpha]_D^{19}$ +18·7° (*c* 5·0 in 6N-HCl)}. The *N*-trifluoroacetyl-*O*-isopropyl derivatives prepared from the hydrochloride showed, however, that no racemization had taken place, and that the impurities did not interfere with the g.l.c. identification. Only a single peak coinciding with the second one of the racemic isopropyl *N*-trifluoroacetyl-β-aminobutyrate was observed in the relevant part of the chromatogram.

The L-β-aminohexanoic acid isolated had $[\alpha]_D^{19}$ +24·9° (*c* 4·0 in 6N-HCl) [lit.,[15] +36° (*c* 0·29 in 5N-HCl)]. By g.l.c. on the L-ureide only a single peak was observed, and further, no impurity could be detected in the n.m.r. spectrum. The discrepancy between the $[\alpha]_D$ values might be due to the errors attached to the measurement of optical rotation in dilute solution. The *N*-trifluoroacetyl derivative of the racemic and the optically active methyl ester of β-aminohexanoic acid were chromatographed by injection at short intervals from each other. Measurement of the retention volume showed that L- preceded the D-isomer.

Chromatographic Conditions.—The chromatographic columns were capillaries of 0·25 mm i.d. and 70—100 m length drawn from Pyrex glass (Sivorel, France). Before coating they were washed successively with 4 ml of a solution of 15 g of Tide in 135 ml of water and 58 ml of ethanol; 4 ml of ethanol; 4 ml of dry ether; and then dried in a stream of nitrogen overnight. The coating was carried out by the plug method with a 20% solution of the phase in ether, enough liquid being used to fill *ca.* 3 m of the capillary. After being left again in a stream of dry nitrogen overnight, the column was brought gradually to the operating temperature of 120 °C. This temperature is most suitable for operation on this phase, since the m.p. is 114 °C and above 130 °C the noise level is too high. The efficiency of the columns, as measured with n-decyl acetate, was in the range of 25,000—50,000 plates. Subsequently it was found that satisfactory columns having longer life and better reproducibility on coating could be obtained with stainless steel (0·5 mm i.d. × 50—200 m). The carrier gas was helium at a pressure of 20 lb in^{-2}, corresponding to a flow of 1—2 ml min^{-1}. Samples of *ca.* 1 μl (1—5% solution) were injected with a split ratio of *ca.* 50 : 1. The injector temperature was 170 °C, and that of the flame ionization detector 150 °C.

With a few exceptions, the values of $r_{L/D}$ were found to be reproducible within *ca.* ±0·001. The data are less accurate in the range of 1·000 ± 0·010, because of the high number of theoretical plates required for resolution in such cases. It should be pointed out that the efficiency of the columns was in general *ca.* 30—50% less for the *N*-trifluoroacetylamino-acid esters than for the reference substance.

By use of the relationships shown in Table 2 between compounds for which an equivalent change in structure is made, values of resolution factors can be predicted. This is done by interpolation from data of properly chosen higher and lower homologues in both R and R′. Interpolations were made mainly in cases where the experimental values of *r* were near unity, in order to have a better estimate of isosteric groups (Table 3). Such values appear in parentheses in Table 1; they have also been used in the Figures. A check of the reliability of these estimations was made for ethyl *N*-trifluoroacetyl-α-aminobutyrate and isopropyl *N*-trifluoroacetyl-α-amino-octanoate. The values predicted before experiment were, respectively, $r_{L/D}$ 1·014 (found, 1·014) and 0·987—0·990 (found, 0·990).

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