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RESOLUTION OF α-METHYL-α-AMINO ACID DERIVATIVES BY GAS CHROMATOGRAPHY ON OPTICALLY ACTIVE DIAMIDE STATIONARY PHASES

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SUMMARY

The resolution of α-methyl-α-amino acids derivatized as either N-trifluoroacetyl (TFA) isopropyl esters or N-TFA-*tert.*-butylamides was investigated on chiral diamide stationary phases R'''CONHCH(R'')CONHR', where R'' = methyl, isobutyl, phenyl, benzyl. The alanine phase (I) is the only solvent which shows chiral recognition for a relatively wide range of the N-TFA isopropyl esters. On the other hand, the N-TFA-*tert.*-butylamides, including that of isovaline, are well resolved on all phases studied. In particular, the phenylalanine phase (IV) shows high resolution factors and is recommended for enantiomeric analysis of the α-methyl-α-amino acids via their N-TFA-*tert.*-butylamides. Similarities and differences between the behaviour of α-methyl and α-H-amino acid derivatives are discussed.

INTRODUCTION

In recent years more and more instances of the presence of α-methyl-α-amino acids in nature have been reported. In particular, α-aminoisobutyric acid and isovaline have been found in a series of lipophilic polypeptidic antibiotics[1–4], and α-methylserine has been identified in amecitin[5]. The replacement of the α-hydrogen in α-amino acids by a methyl group may significantly change various properties, such as hydrophobicity and reactivity towards chemical reagents and enzymes. These effects may evidently fulfill certain purposes in nature.

Investigation of the pharmacological properties of α-methyl-α-amino acids has resulted in the observation that these compounds can act as inhibitors of enzymes. For instance, α-methyl-L-tyrosine methyl ester[6] and α-methyl-3,4-dihydroxy-L-phenylalanine (α-methyl-L-DOPA)[7] inhibit the action of monoamino oxidase and aromatic amino acid decarboxylase, respectively. The introduction of L-DOPA as an antihypertensive drug[8] has resulted from this work.

Other areas in which α-methylamino acids have become important are research on the origin of life and the study of meteorites. In fact, these compounds are formed in experiments simulating primitive Earth conditions[9], and have further been identified in chondritic meteorites, *e.g.*, the Murchison meteorite[10].

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For the study of the above topics, a knowledge of the configuration and/or optical purity of the pertinent α-methyl-α-amino acids is often essential. The present research had as its objective the development of relevant, improved methods of analytical resolution by gas chromatography.

For the enantiomeric analysis of isovaline in meteorite extracts, some authors developed procedures of gas chromatographic resolution based on the use of diastereomers, such as N-pentafluoropropionyl esters of 2-*n*-pentyl[11], 2-*n*-hexyl[11] or 3-methyl-2-butyl alcohol[12], on symmetric stationary phases. Better results were obtained with N-trifluoroacetyl (TFA)-isovaline-L-leucine isopropyl ester on chiral N-docosanoyl-L-valine *tert.*-butylamide[13].

In our laboratory the separation of enantiomeric α-methyl-α-amino acid derivatives was studied. Several years ago, the resolution and the order of emergence of a series of α-methyl-α-amino acid esters on carbonylbis-(N-L-valine isopropyl ester) were reported[14]. Subsequently[15], it was found that the monoamide, N-lauroyl-(*S*)-α-(1-naphthyl)ethylamide, shows chiral recognition for certain of the α-methyl-α-amino acid esters resolved on the former phase (r = 1.008–1.026, at 100°C), but not for isovaline or α-methylleucine.

The present work is concerned with the behaviour of both N-TFA esters and N-TFA *tert.*-butylamides of α-methyl-α-amino acids on different diamide phases, of a type introduced previously[16–18]. The purpose of this research was both to improve the available procedures for analytical resolution, as well as to study the effect of the α-methyl group on chiral recognition by diamides.

MATERIALS

The racemic α-methyl derivatives of α-aminobutyric acid, valine, norvaline, leucine, norleucine and phenylalanine were synthesized in our laboratory[14,19] from the corresponding ketones[20] by reaction with ammonium carbonate and sodium cyanide, followed by hydrolysis of the hydantoins formed. α-Methylaspartic acid was a gift from Dr. P. E. Hare, Geophysical Laboratory, Carnegie Institution of Washington, Washington, DC, U.S.A.

Samples enriched in L-isovaline and D-α-methylvaline were also available in our laboratory[14,19] from the enzymatic hydrolysis[21] with Acylase I of the corresponding racemic N-TFA-α-methyl-α-amino acids.

For the preparation of the N-TFA isopropyl esters[14] and the N-TFA *tert.*-butylamides[22], previously described procedures[15,21,22] were used. It should, however, be mentioned that, since the α-methylamino acids are resistant to racemization, it is not necessary to maintain carefully the mild conditions recommended for derivatization of α-H-amino acids.

The phases examined were N-lauroyl *tert.*-butyl amide derivatives of L-alanine (I), L-leucine (II), D-phenylglycine (III) and L-phenylalanine (IV), the properties of which have been described previously[23], as well as the novel chiral solvent N-docosanoyl-L-leucine *tert.*-butylamide (V)[24].

Chromatographic conditions

The solvents were coated by the plug method on stainless-steel capillary columns [100 ft. × 0.02 in. I.D. (for I, II and V) or 150 ft. × 0.02 in. I.D. (for III and

IV)]. Chromatography was carried out with a helium flow of 3 ml/min and a flame-ionization detector. Temperatures employed are given in the tables and figures.

RESULTS AND DISCUSSION

Previously, we had attempted to separate enantiomeric N-TFA isopropyl esters of α-methyl-α-amino acids on several diamide phases derived from L-valine. However, only in one case, *viz.* on N-docosanoyl-L-valine-2-(2-methyl)heptadecylamide (VI)[25], success was achieved. Chiral recognition was, indeed, found, but was limited to the N-TFA isopropyl ester of α-methylvaline, and did not extend to either isovaline, or the α-methyl derivatives of norvaline, leucine and norleucine. On the other hand, on the polymeric phase Chirasil–Val, the N-TFA *n*-propyl ester of isovaline was well resolved[12].

These sporadic results prompted us to study the topic more systematically, and in this article we report the behaviour of the N-TFA isopropyl esters and the N-TFA *tert.*-butyl amides of α-methyl-α-amino acids on a number of diamide stationary phases derived from amino acids other than valine[23,24].

N-TFA isopropyl esters of α-methyl-α-amino acids

N-Lauroyl-α-amino acid *tert.*-butylamides with a relatively bulky R″ group, such as isobutyl (II), phenyl (III) and benzyl (IV), did not behave differently from the valine phase VI: only the α-methylvaline ester could be separated into its enantiomers

TABLE I

SEPARATION OF ENANTIOMERS OF N-TFA ISOPROPYL ESTERS OF α-METHYLAMINO ACIDS ON N-LAUROYL-L-ALANINE *tert.*-BUTYLAMIDE (I) AND N-DOCOSANOYL-L-LEUCINE *tert.*-BUTYLAMIDE (V)

For chromatographic conditions see Experimental section.

Phase		*T* (°C)	*Configuration*	*Amino acid N-TFA isopropyl ester*							
				α-Me-valine		*α-Me-norvaline*		*α-Me-leucine*		*α-Me-norleucine*	
				*r**	$r_{L\,D}$**	*r**	$r_{L\,D}$**	*r**	$r_{L\,D}$**	*r**	$r_{L\,D}$**
I	N-Lauroyl L-alanine *tert.*-butylamide	80	D	35.28	1.063	28.22	1.034	37.14	1.018	54.02	1.033
			L	37.66		29.40		37.82		55.78	
		90	D	22.54	1.052	18.86	1.021	24.00	1.014	33.96	1.030
			L	23.72		19.26		24.36		34.96	
		100	D	15.14	1.046	13.04	1.020	16.44	1.000	22.46	1.018
			L	15.84		13.30		16.44		22.86	
		120	D	7.64	1.026	4.64	1.000	8.24	1.000	11.16	1.000
			L	7.84		4.64		8.24		11.16	
V	N-Docosanoyl L-leucine *tert.*-butylamide	100	D	12.40	1.087	9.26	1.000				
			L	13.48		9.26					

* 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 place.

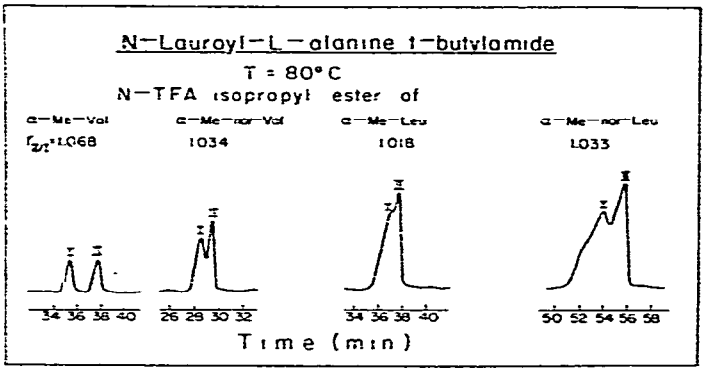


Fig. 1. Chromatogram of N-TFA isopropyl esters of some D,L-α-methylamino acids on phase I.

with resolution factors similar as on VI [r = 1.066 (IV), 1.056 (III) and 1.046 (II) at 130°C, while for VI at 80°C r = 1.086 had been reported[25]].

On the other hand, when the α-alkyl substituent of the solvent is methyl (phase derived from alanine, I), the range of chiral recognition is widened. As can be seen in Table I, all the amino acids listed were resolved at 90°C and below. However, when the temperature was raised to 120°C, only the enantiomers of α-methylvaline were separated. The chromatograms (at 80°C, Fig. I) show baseline separation for α-methylvaline, while for the other amino acids under the experimental conditions only partial peak resolution could be obtained. Another drawback of phase I is that no chiral recognition for the isovaline ester was found.

Attempts to separate the enantiomers of α-methylaspartic acid and of α-methylphenylalanine, as their N-TFA isopropyl esters, on phases I, II and IV were unsuccessful.

The order of emergence has not as yet been established experimentally. The result for the isovaline ester on Chirasil–Val, reported in the literature[12], indicates that the L-isomer in analogy should appear after the D-isomer, *i.e.* as in the corresponding α-H-α-amino acids.

N-TFA tert.-butylamides of α-methyl-α-amino acids

Recently, it was found that N-TFA *tert.*-butylamides[22] and N-TFA isopropylamides[26] of the α-H-α-amino acids are in certain cases more effectively resolved on diamide phases than the corresponding N-TFA isopropyl esters. Application of this approach to α-methyl-α-amino acids is described in the present section.

The chromatographic data listed in Table II (see also Figs. 2 and 3), show that this strategy was effective. In Table III, the resolution factors of the N-TFA isopropyl esters and the N-TFA *tert.*-butylamides on I are compared. When the difference in temperature is taken into account, it is apparent that chiral recognition for the diamide derivatives is definitely better. Also, α-methylphenylalanine, and even isovaline, were resolved on all the phases tried (Table II). However, in general, resolution factors, although sufficient for analytical purposes, remain markedly below those of the corresponding α-H-α-amino acid derivatives[22].

The close similarity of α-methyl- and α-H-α-amino acids permits the assumption that corresponding interactions with solvents are similar. Diastereomeric solute–

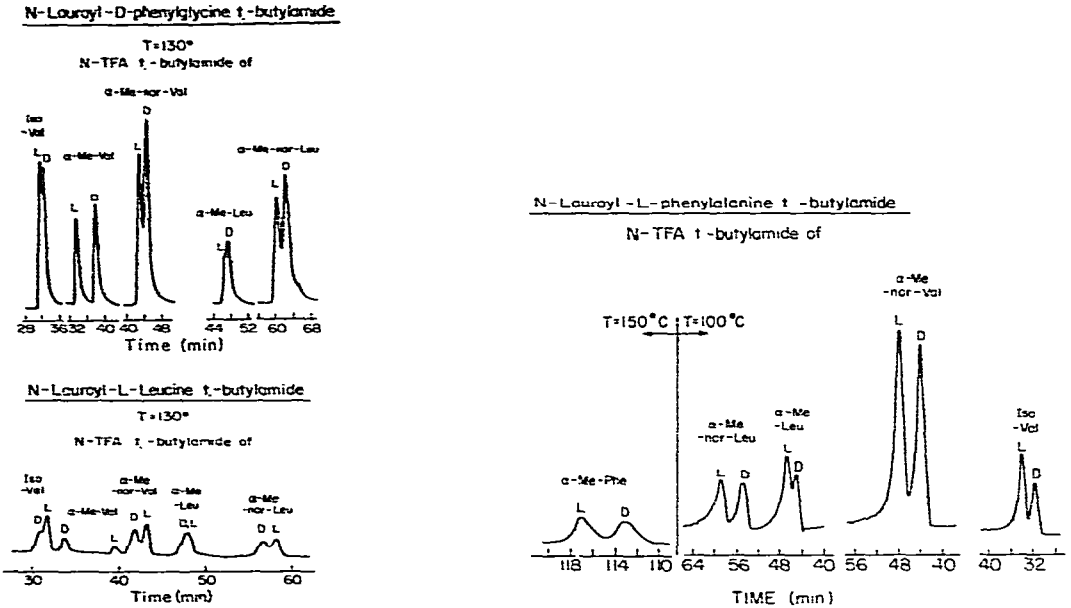


Fig. 2. Chromatogram of N-TFA *tert.*-butylamides of L-enriched isovaline, D-enriched α-Me-Val and D,L-α-Me-nor-Val, α-Me-leu and α-Me-nor-Leu, on phases II and III.

Fig. 3. Chromatogram of N-TFA *tert.*-butylamides of L-enriched isovaline and D,L-α-Me-nor-Val, α-Me-Leu, α-Me-Nor-Leu and α-Me-Phe, on phase IV.

solvent complexes, considered to be responsible for resolution, have been discussed previously[16–18,22–25]. Models of such associations involving, respectively, N-TFA esters and N-TFA *tert.*-butyl amides of α-methyl-α-amino acids, are represented in Fig. 4.

For steric reasons, it might be expected that the α-methyl group will weaken hydrogen bonding with the stationary phases. Indeed, considerably lower retention is found throguhout for the derivatives of the α-methyl- as compared with those of the α-H-α-amino acids. Thus, on phase I, the retention time of the N-TFA *tert.*-butylamide of D-valine was 75 min (140°C), as against 22 min (130°C) for the D-α-methylvaline (Table II), and that of the N-TFA isopropyl ester of D-leucine was 15.7 min (130°C), as against 8.24 min (120°C) for D-α-methylleucine (Table I). Looser association should result in a reduction of the concentration of the selective complexes formed in the stationary phase, as well as in larger distances between the associating molecules. Both of these circumstances will reduce the capacity for chiral recognition, in agreement with the findings for the two classes of derivatives investigated.

Chiral differentiation of the enantiomeric solutes in the stereoselective association complexes should be more difficult in the case of the α-methyl-α-amino acid derivatives, because they have a lesser degree of asymmetry, *i.e.*, a relatively smaller difference in the size of the substituent (R *vs.* methyl, as compared with R *vs.* H, for the α-H-α-amino acids). Isovaline, in which the alkyl substituents at the asymmetric carbon differ only by one methylene group, illustrates this effect particularly clearly,

TABLE II

SEPARATION OF ENANTIOMERS OF N-TFA *tert.*-BUTYLAMIDES OF α-METHYL-α-AMINO ACIDS ON N-LAUROYL-L-ALANINE *tert.*-BUTYLAMIDE (I), N-LAUROYL-L-LEUCINE *tert.*-BUTYLAMIDE (II), N-LAUROYL-D-PHENYLGLYCINE *tert.*-BUTYLAMIDE (III), N-LAUROYL-L-PHENYLALINE *tert.*-BUTYLAMIDE (IV) AND N-DOCOSANOYL-L-LEUCINE *tert.*-BUTYLAMIDE (V) AS STATIONARY PHASES

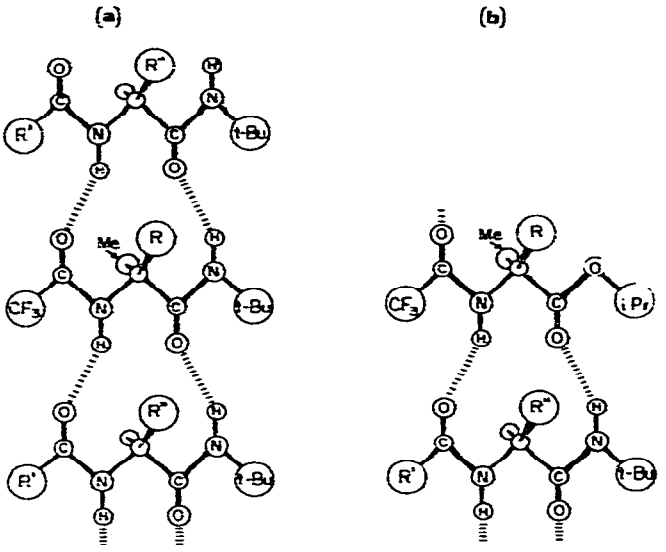
For chromatographic conditions, see Experimental section, for definition of r and $r_{L/D}$, see Table I.

Phase	*T*(°C)	*Configuration*	*N-TFA tert.-butylamide*													
			Isovaline		*α-Methylvaline*		*α-Methylnorvaline*		*α-Methylleucine*		*α-Methylnorleucine*		*α-Methylphenylalanine*			
			r	$r_{L/D}$*	r	$r_{L/D}$*	r	$r_{L/D}$*	r	$r_{L/D}$*	r	$r_{L/D}$*	*T*(°C)	*Configuration*	r	$r_{L/D}$*
I	110	D	37.10	1.016	37.60	1.122	49.90	1.033	58.30	1.022	71.80	1.032				
		L	37.70		42.20		51.54		59.60		74.10					
	120	D	24.60	1.000	25.40	1.098	32.30	1.018	39.00	1.000	47.00	1.017				
		L	24.60		27.90		32.90		39.00		47.80					
	130	D	21.80	1.000	22.30	1.078	28.30	1.014	33.80	1.000	40.00	1.010	140	D	122.00	1.026 (shoulder)
		L	21.80		24.04		28.70		33.80		40.40			L	125.20	
II	130	D	30.90	1.029	33.60	1.176	41.60	1.034	47.80	1.000	56.60	1.027				
		L	31.80		39.50		43.00		47.80		58.10					
	140	D	22.20	1.018	26.20	1.137	29.14	1.026	37.20	1.000	43.80	1.018	170	D	98.40	1.024
		L	22.60		29.80		29.90		37.20		44.60			L	100.80	

III**	110	L D	82.28 85.20	1.036	–	–	–	–	121.40 125.00	1.030	–	–		–	
	130	L D	31.40 32.30	1.026	35.36 38.00	1.075	42.80 44.40	1.037	46.60 47.40	1.017	60.20 62.28	1.035			
IV	100	D L	30.32 32.48	1.071	39.00 53.00	1.359	42.00 46.00	1.095	45.00 46.60	1.036	54.80 58.88	1.074			
	110	D L	19.80 21.00	1.061	25.00 31.80	1.272	27.40 29.20	1.066	35.00 36.12	1.032	42.20 44.80	1.062			
	120	D L	16.00 16.80	1.050	21.20 25.60	1.208	21.80 22.90	1.054	30.60 31.00	1.013	24.60 26.00	1.057			
	130	D L	13.00 13.60	1.046	13.40 15.60	1.164	17.80 18.60	1.045	19.20 19.20	1.000	22.60 23.20	1.027	150	D L	113.00 117.00	1.035
V	100	D L	19.20 20.70	1.078											
	110	D L	15.10 15.95	1.056											
	120	D L	10.87 11.13	1.024											

* The order of emergence ($r_{L/D} > 1$) determined with optically enriched mixutres for isovaline (L > D) and for α-Me-valine (D > L) was by extrapolation assumed to be valid also for the other α-Me-amino acids.

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



R' : $C_{11}H_{23}$ -; $C_{21}H_{43}$

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

Fig. 4. Schmatic representation of hydrogen-bonded associates assumed to be responsible for resolution by diamide phases. (a) Solvent–solute–solvent complex with parallel orientation of the solvent molecules and an intercalated N-TFA *tert.*-butylamide of an L-α-methyl-α-amino acid[22]. To represent the corresponding diastereomeric complexes with D-solutes, the relative position of Me and R have to be inverted. (b) Solvent–solute complex with an N-TFA isopropyl ester of an α-methyl-α-amino acid; in diastereomeric association complexes with D-solutes the relative positions of the Me and R groups are inverted.

TABLE III

COMPARISON OF THE RESOLUTION FACTORS OF N-TFA ISOPROPYL ESTERS AND N-TFA-*tert.*-BUTYLAMIDES OF α-METHYL-α-AMINO ACIDS ON N-LAUROYL-L-ALANINE *tert.*-BUTYLAMIDE (I)

Column: 100 ft. × 0.02 in.

Amino acid	*N-TFA tert.-Butylamide*	*N-TFA Isopropyl ester*
	$r_{L,D}$* *at 110°C*	$r_{L,D}$* *at 80°C*
α-Methylvaline	1.122	1.068
α-Methylnorvaline	1.033	1.034
α-Methylleucine	1.022	1.018
α-Methylnorleucine	1.032	1.033

* For $r_{L,D}$, see Table I.

and it is, indeed, the most difficult compound to resolve in the series studied (also its N-TFA ester is not separated on I, see above). It should be mentioned that the nature of the R substituent of the solute also has more subtle effects, which cannot be understood without a more detailed knowledge of the conformation of the solute–solvent association complexes. Thus, α-methylvaline, in which R is branched at its β-position, gives the highest $r_{II/I}$ value observed in this research (1.359, Table II). On the other hand, α-methylleucine, with the R group branched in the γ-position, is as difficult to resolve as isovaline. The same influence of substitution is indicated by the behaviour of the N-TFA esters (Table I)*.

The increase in the $r_{L,D}$ values for the diamide derivatives of the α-methyl-α-amino acids, as compared with the N-TFA isopropyl esters, is ascribed to the difference in the mode of interaction with the stationary phases. With reference to Fig. 4, it is seen that the N-TFA *tert.*-butylamide derivatives, which are capable of forming four hydrogen bonds, can intercalate between two solvent molecules (complex a). On the other hand, association of N-TFA esters with a second solvent molecule would be rather loose, as only one hydrogen bond is available for that purpose (complex b). The resulting lesser restriction of the solutes would appear to be related to the relatively lower chiral differentiation in the latter case.

The order of emergence (L after the D isomer on the L-phases) has been experimentally determined on only two enriched mixtures (isovaline and α-methylvaline, Table II). By extrapolation, and by analogy with the same consistent elution sequence found for the α-H-α-amino acid derivatives, it is provisionally assumed that, for the α-methyl-α-amino acids derivatives, also, $r_{L/D}$ on the L-phases is larger than unity, throughout. These results are in agreement with the above assumption of analogous stereoselective solute–solvent association for both types of amino acids.

As was seen for the N-TFA esters, discussed in the preceding sections, the nature of the substituent R″ in the diamide solvent is observed to play (Table II) an important role in determining the magnitude of the resolution factors. In the series I–IV of the diamides R‴CONHCH(R″)CONHR′ with the same R‴ and R′ groups, chiral recognition improves as R″ increases, except in the case of the D-phenylglycine derivative III, where the proximity of the phenyl to the chiral centre creates a special situation. For R″ = benzyl, the highest $r_{L,D}$ values were obtained throughout, and the corresponding diamide IV is the best phase found, thus far, for the enantiomeric analysis of the α-methyl-α-amino acids. It is recalled that for the N-TFA isopropyl esters, the best results were, however, obtained on the diamide with the smallest R″ group (I).

As was found for the α-H-α-amino acids, lengthening of the R‴ chain reduces to some extent the resolution factors (V, as compared with II), but has the advantage of permitting the range of column working temperatures to be widened.

APPLICATIONS

The determination of the configuration of isovaline in natural substances is of topical interest. Recently, Brueckner and co-workers[12] definitely established the D-

* In contrast, for the N-TFA esters of the α-H-α-amino acid derivatives, the effects of branching of R in the β- and γ-positions are reversed[23].

configuration of isovaline in polypeptidic antibiotics, using gas chromatographic procedures expecially developed for that purpose. Previously the opposite assignment had been made for this α-methyl-α-amino acid in antiamoebins[27] and emericidins[28]. Apparently, the erreneous conclusion was reached by the assumption that chiral recognition of α-methyl-α-amino acids is as easy as that of the α-H analogues. The new derivatives and chiral phases introduced in the present research will supplement and improve the enantiomeric analysis of isovaline, as well as of other α-methyl-α-amino acids. Furthermore, the data discussed above show clearly that, besides certain similarities, there are also marked differences between the α-methyl and α-H-α-amino acids in their interaction with chiral diamide stationary phases.

Brueckner and co-workers[12] have already pointed out the special diffuclties involved in the determination of the configuration of α-methyl-α-amino acids, such as the inapplicability[29] of the Clough–Lutz–Jirgenson rule. Also, it has been found[14] that, in this series, assignment through enzymatic hydrolysis of the N-acyl esters cannot be effected in all cases, because of resistance to attack by Acylase I. Experience has shown that the order of emergence of solutes on the chiral phases studied so far can be correlated in a consistent way with the configuration. In particular, on the diamides[16–18,22–25] no exception has yet been found to the rule that derivatives of α-H-α-amino acids elute in the order L-after the D-isomer on L-phases. Furthermore, it should be added that measurements[14,19] for five optically enriched N-TFA esters of α-methyl-α-amino acids gave throughout the same sequence of elution ($r_{L/D} > 1$) on carbonylbis-(N-L-valine isopropyl ester). There are, therefore, good reasons to conclude that the order of emergence of derivatives of α-methyl-α-amino acids on diamide stationary phases promises to become a useful tool for the determination of the configuration in this series.

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REFERENCES

1 T. Ooka, Y. Shimojima, T. Akimoto, I. Takeda, S. Senoh and J. Abe, *Agr. Biol. Chem.*, 30 (1966) 700.
2 R. C. Pandey, H. Meng, J. C. Cook and K. L. Rinehart, *J. Amer. Chem. Soc.*, 99 (1977) 5203.
3 G. Jung, N. Dubischar and D. Leibfritz, *Eur. J. Biochem.*, 54 (1975) 395.
4 G. Irmscher and G. Jung, *Eur. J. Biochem.*, 80 (1977) 165.
5 E. H. Flynn, J. W. Hinman, E. L. Caron and D. O. Woolf, Jr., *J. Amer. Chem. Soc.*, 75 (1953) 5867.
6 T. H. Svensson and B. Waldeck, *Psychopharmacologia*, 18 (1970) 357.
7 J. A. Oates, L. Gillespie, S. Udenfriend and A. Sjoerdsma, *Science*, 131 (1960) 1890.
8 L. Gillespie, Jr., *Ann. N.Y. Acad. Sci.*, 88 (1960) 1011.
9 M. S. Chadha, J. Lawless, J. Flores and C. Ponnamperuma, in R. Buvet and C. Ponnamperuma (Editors), *Molecular Evolution I, Proceedings of the International Conference on the Origin of Life, Pont-a-Mousson, France, April 20–25, 1970*, North-Holland, Amsterdam, 1971, p. 143.
10 K. A. Kvenvolden, J. G. Lawless and C. Ponnamperuma, *Proc. Nat. Acad. Sci. U.S.*, 68 (1971) 486.
11 G. E. Pollock, *Anal. Chem.*, 44 (1972) 2368.
12 H. Brueckner, G. J. Nicholson, G. Jung, K. Kruse and W. A. Koenig, *Chromatographia*, 13 (1980) 209.
13 J. J. Flores, W. A. Bonner and M. A. Van Dort, *J. Chromatogr.*, 132 (1977) 152.
14 N. Frydman, T. Tamari, B. Feibush and E. Gil-Av, *Proc. Isr. Chem. Soc. 42nd Meeting, Dec. 1972*, p. 11.

15 S. Weinstein, B. Feibush and E. Gil-Av, *J. Chromatogr.*, 126 (1976) 97.
16 B. Feibush, *Chem. Commun.*, (1971) 544.
17 R. Charles, U. Beitler, B. Feibush and E. Gil-Av, *J. Chromatogr.*, 112 (1975) 121.
18. U. Beitler and B. Feibush, *J. Chromatogr.*, 123 (1976) 149.
19 A. Schwartz, *Ph. D. Thesis*, Weizmann Institute of Science, Rehovot, Israel, 1977.
20 H. T. Bucherer and V. A. Lieb, *J. Prakt. Chem.*, 141 (1934) 5.
21 J. P. Greenstein, M. Winitz, *Chemistry of the Amino Acids*, Wiley, New York, 1961, p. 2572.
22 S.-C. Chang, R. Charles and E. Gil-Av, *J. Chromatogr.*, 202 (1980) 247.
23 S.-C. Chang, R. Charles and E. Gil-Av, *J. Chromatogr.*, 235 (1982) 87.
24 R. Charles and E. Gil-Av, unpublished data.
25 R. Charles and E. Gil-Av, *J. Chromatogr.*, 195 (1980) 317.
26 N. Ōi, M. Horiba and H. Kitahara, *J. Chromatogr.*, 202 (1980) 299.
27 R. C. Pandey, J. C. Cook and K. L. Rinehart, *J. Antibiot.*, 31 (1978) 241.
28 R. C. Pandey, J. C. Cook and K. L. Rinehart, *J. Amer. Chem. Soc.*, 99 (1977) 5205.
29 M. Winitz, S. M. Birnbaum and J. P. Greenstein, *J. Amer. Chem. Soc.*, 77 (1955) 716.