SYNTHESIS AND MASS SPECTRA OF DIASTEREOISOMERIC CYCLOHEXAPEPTIDES CYCLO(GLY-ALA-LEU)$_2$ AND CYCLO(GLY-VAL-LEU)$_2$*

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Diastereoisomeric cyclo(Gly-L-Ala-L-Leu)$_2$ and cyclo(Gly-L-Ala-D-Leu)$_2$ and an analogous cyclohexapeptide pair with the L-valine residue instead of L-alanine were prepared from the corresponding linear hexapeptides with the use of the azide method in the cyclisation step. As indicated by high resolution mass spectra, the fragmentation of cyclo(Gly-Ala-Leu)$_2$ is limited to that initiating by ring cleavage at the leucine residue while with cyclo(Gly-Val-Leu)$_2$, the fragmentation begins both at the leucine and valine residue. Differences in mass spectra inside the diastereoisomeric pairs are discussed in connection with their stereochemistry.

In connection with investigations on the steric arrangement of cyclic peptides numerous compounds containing the amino-acid residues of glycine, phenylalanine, and leucine[1-3] have been prepared and their properties examined[4,5]. The basic type of these compounds was represented by diastereoisomeric cyclohexapeptides with the Gly-Phe-Leu-Gly-Phe-Leu sequence,** the regularity of which in primary structure and absolute configuration may be reflected in conformational regularity. The effect of the aromatic ring in the side chain of phenylalanine might be elucidated with the use of analogous peptides with modified side chains. The synthesis and CD spectra of cyclohexapeptides with the S-benzylcysteine residue instead of the phenylalanine residue have been described some time ago[6]. In this paper, we wish to report preparation of additional compounds with exclusively aliphatic side chains of a various bulkiness and placed at positions previously occuppied by the benzyl side chain of phenylalanine.

The sequence Gly-Ala-Leu-Gly-Ala-Leu, the methyl groups of which represent a side chain of the lowest steric requirements, and the sequence Gly-Val-Leu-Gly--Val-Leu with a bulky isopropyl side chain were selected. In both sequences, both

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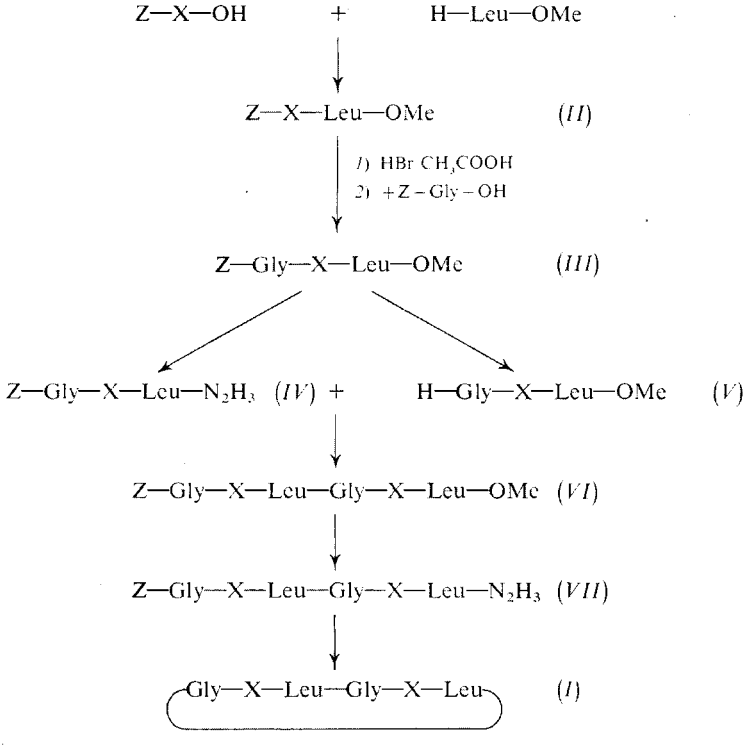
** The nomenclature and symbols follow the published rules[7,8].

possible diastereoisomers with C_2 symmetry were synthesised, namely, cyclo(glycyl--L-alanyl-L-leucyl-glycyl-L-alanyl-L-leucyl) (*Ia*), cyclo(glycyl-L-alanyl-D-leucyl-glycyl--L-alanyl-D-leucyl) (*Ib*), cyclo(glycyl-L-valyl-L-leucyl-glycyl-L-valyl-L-leucyl) (*Ic*), and cyclo(glycyl-L-valyl-D-leucyl-glycyl-L-valyl-D-leucyl) (*Id*). All these four cyclohexapeptides were prepared according to Scheme 1 by an earlier reported procedure[1,2]. The final step consisted in cyclisation of linear hexapeptides by the azide method proceeding in about 50% yields. The isolation of these cyclohexapeptides was somewhat difficult because of the high solubility in aqueous media. For the properties of thus-prepared compounds see Table I.



SCHEME 1

Series *a*: X = L-Ala, Leu = L-Leu; *b*: X = L-Ala, Leu = D-Leu; *c*: X = L-Val, Leu = L-Leu; *d*: X = L-Val, Leu = D-Leu.

Mass Spectrometry

Fig. 1*A* – *D* (p. 3647) shows mass spectra of both diastereoisomeric pairs cyclo(Gly-L-Ala-L-Leu)$_2$ (*Ia*), cyclo(Gly-L-Ala-D-Leu)$_2$ (*Ib*) and cyclo(Gly-L-Val-L-Leu)$_2$ (*Ic*), cyclo (Gly-L-Val-D-Leu)$_2$ (*Id*). Intensive peaks of *m/e* 30, 44 (or 72), and 86 are formed by the

TABLE I

Physical Data and Elemental Analyses

Compound	M.p.[a], °C (yield, %)	M.p.[b], °C solvent[c]	Formula (mol.wt.)	Calculated/Found % C	% H	% N	$[\alpha]_D^{25}$, (*c*, %; solvent[c])
Z-L-Val-D-Leu-OCH_3 (*IId*)	125—131 (73)	131·5—132·5 A	$C_{20}H_{30}N_2O_5$ (378·5)	63·47 63·68	7·99 7·92	7·40 7·20	+10·3° (0·5, B)
Z-Gly-L-Ala-L-Leu-OCH_3[d] (*IIId*)	103—110 (81)	111—112 A	$C_{20}H_{29}N_3O_6$ (407·5)	58·95 58·68	7·17 7·08	10·31 10·51	−20·6° (0·5, C)
Z-Gly-L-Ala-D-Leu-OCH_3[e] (*IIIb*)	106—109·5 (65)	107·5—109·5 A	$C_{20}H_{29}N_3O_6$ (407·5)	58·95 59·01	7·17 7·36	10·31 10·29	+10·1° (0·5, C)
Z-Gly-L-Val-L-Leu-OCH_3[f] (*IIIc*)	113—115 (50)	116—118 A	$C_{22}H_{33}N_3O_6$ (435·5)	60·67 60·73	7·64 7·52	9·65 9·74	−46·5° (0·5, *B*)
Z-Gly-L-Val-D-Leu-OCH_3 (*IIId*)	131—137 (44)	141·5—142·5 A	$C_{22}H_{33}N_3O_6$ (435·5)	60·67 60·84	7·64 7·67	9·65 9·83	+1·9° (0·5, C)
Z-Gly-L-Ala-L-Leu-N_2H_3 (*IVa*)	189—191 (77)	189—181 B	$C_{19}H_{29}N_5O_5$ (407·5)	56·00 55·78	7·17 7·11	17·19 17·45	−20·8° (0·5, C)
Z-Gly-L-Ala-D-Leu-N_2H_3 (*IVb*)	182—185 (72)	182—185 D	$C_{19}H_{29}N_5O_5$ (407·5)	56·00 56·38	7·17 7·31	17·19 17·15	+1·2° (0·5, C)
Z-Gly-L-Val-L-Leu-N_2H_3 (*IVc*)	193—197 (100)	198—201 E	$C_{21}H_{33}N_5O_5$ (435·5)	57·91 57·80	7·64 7·62	16·08 16·05	−37·0° (0·5, B)
Z-Gly-L-Val-D-Leu-N_2H_3 (*IVd*)	193—205 (91)	207·5—209 E	$C_{21}H_{33}N_5O_5$ (435·5)	57·91 57·94	7·64 7·50	16·08 15·85	+10·1 (0·5, B)
Z-Gly-L-Ala-L-Leu-Gly-L-Ala--L-Leu-OCH_3 (*VIa*)[g]	191—194 (62)	192—196 F	$C_{31}H_{48}N_6O_9$ (648·8)	57·39 57·11	7·46 7·42	12·96 13·14	−28·6 (0·5, C)

Z-Gly-L-Ala-D-Leu-Gly-L-Ala--D-Leu-OCH_3	217—221	219—221	$C_{31}H_{48}N_6O_9$	57·39	7·46	12·96	+7·2
(*VIb*)[9]	(61)	G	(648·8)	57·26	7·42	13·04	(0·5, C)
Z-Gly-L-Val-L-Gly-L-Val--L-Leu-OCH_3	220—230	234—236	$C_{35}H_{56}N_6O_9$	59·64	8·01	11·92	—27·7
(*VIc*)	(83)	B	(704·9)	59·60	8·13	11·98	(0·5, B)
Z-Gly-L-Val-D-Leu-Gly-L-Val--D-Leu-OCH_3	208—212	209—212	$C_{35}H_{56}H_6O_9$	59·64	8·01	11·92	—4·5
(*VId*)	(87)	F	(704·9)	59·56	7·87	11·59	(0·5, B)
Z-Gly-L-Ala-L-Leu-Gly-L-Ala--L-Leu-N_2H_3	240—243	240—243	$C_{30}H_{48}N_8O_8$	55·54	7·46	17·27	—34·2
(*VIIa*)	(68)	F	(648·8)	55·79	7·71	17·38	(0·5, C)
Z-Gly-L-Ala-D-Leu-Gly-L-Ala--D-Leu-N_2H_3	214—219	218—219	$C_{30}H_{48}N_8O_8$	55·54	7·46	17·27	+1·8
(*VIIb*)	(88)	H	(648·8)	55·37	7·70	17·36	(0·5, C)
Z-Gly-L-Val-L-Leu-Gly-L-Val--L-Leu-N_2H_3	257—260	262—264	$C_{34}H_{56}N_8O_8$	57·93	8·01	15·90	—55·2
(*VIIc*)	(95)	F	(704·9)	57·70	8·03	15·70	(0·5, B)
Z-Gly-L-Val-D-Leu-Gly-L-Val--D-Leu-N_2H_3	248—253	248—253	$C_{34}H_{56}N_8O_8$	57·93	8·01	15·90	—6·3
(*VIId*)	(91)	J	(704·9)	57·78	8·00	15·68	(0·5, K)
Cyclo(Gly-L-Ala-L-Leu-Gly-L-Ala--L-Leu)	317—323 (dec.)	317—323 (dec.)	$C_{22}H_{38}N_6O_6$	54·75	7·94	17·42	—101·2
(*Ia*)	(52)	L	(482·6)	54·40	7·87	17·34	(0·5, K)
Cyclo(Gly-L-Ala-D-Leu-Gly-L-Ala--D-Leu)	320—330 (dec.)	320—330 (dec.)	$C_{22}H_{38}N_6O_6$	54·75	7·94	17·42	—39·8
(*Ib*)	(27)	H	(482·6)	55·04	7·98	17·52	(0·3, K)

TABLE I
(*Continued*)

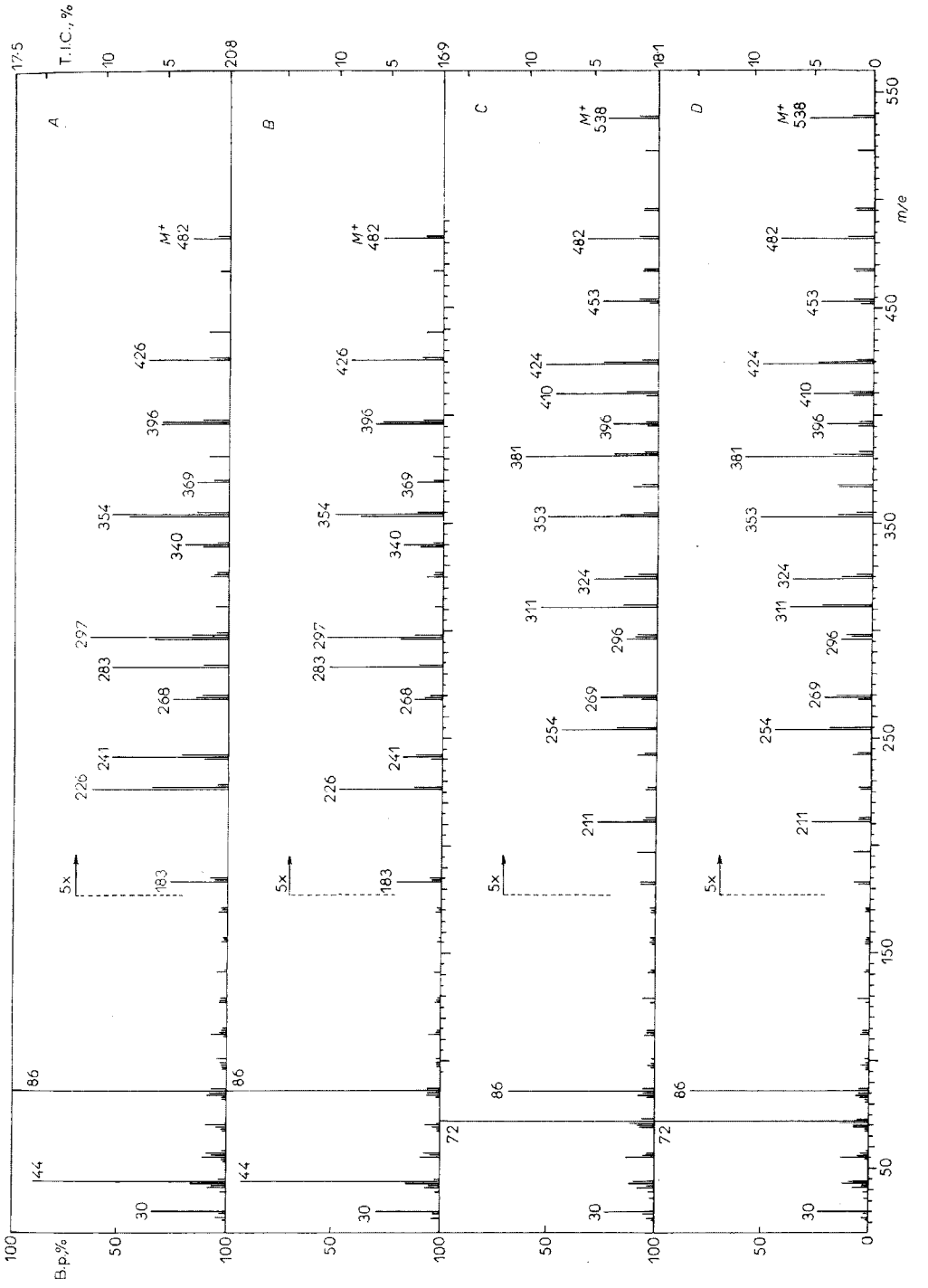
Compound	M.p.[a], °C (yield, %)	M.p.[b], °C solvent[c]	Formula (mol.wt.)	Calculated/Found % C	% H	% N	$[\alpha]_D^{25}$, (*c*, %; solvent[c])
Cyclo(Gly-L-Val-L-Leu-Gly-L-Val--L-Leu) (*Ic*)	>360 (41)	>360 L	$C_{26}H_{46}O_6$ (538·7)	57·97 57·70	8·61 8·58	15·60 15·32	−151·8 (0·3, K)
Cyclo(Gly-L-Val-D-Leu-Gly-L-Val--D-Leu) (*Id*)	>360 (55)	>360 L	$C_{26}H_{46}N_6O_6$ (538·7)	57·97 57·96	8·61 8·63	15·60 15·23	−93·6 (0·3, K)

[a] Melting point of the particular compound obtained in the yield stated. [b] Melting point of the sample for analysis and physical measurements. [c] A, ethyl acetate–light petroleum; B, methanol; C, dimethylformamide; D, methanol–water; E, ethanol; F, methanol–ether; G, ethyl acetate with a few drops of methanol–ether–light petroleum; H, methanol–ether–light petroleum; J, acetic acid–methanol–ether; K, acetic acid; L, acetic acid–water. [d] From Z-L-Ala-L-Leu-OCH_3, lit.[9] (Z-D-Ala-D-Leu-OCH_3). [e] From Z-L-Ala-D-Leu-OCH_3, lit.[9]. [f] From Z-L-Val-L-Leu--OCH_3, lit.[10]. [g] Isolation: The material was dissolved in ethyl acetate, the solution washed with 1M-HCl, 0·5M-$NaHCO_3$, and water, and dried over anhydrous sodium sulfate.

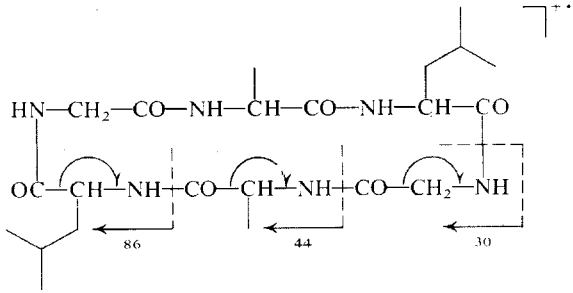
FIG. 1

Electron Impact Mass Spectra (70 eV) of Cyclo(Gly-L-Ala-L-Leu)$_2$ (*Ia*), Cyclo(Gly-L-Ala-D-Leu)$_2$ (*Ib*), Cyclo(Gly-L-Val-L-Leu)$_2$ (*IIa*), and Cyclo(Gly-L-Val-D-Leu)$_2$ (*IIb*), resp.

Direct inlet, ion source temperature 180—190°C, b.p. base peak, T.I.C. total ion current.


T.I.C., %
A
B
C
D
M+ 482
M+ 538
5x
B.p.%
m/e

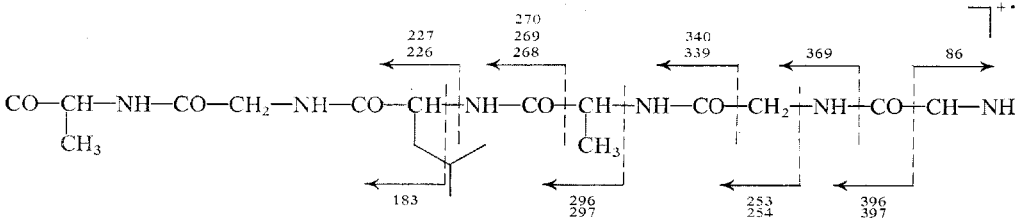
"amine cleavage" (*cf.*[11-13]) and correspond to alkylideneimmonium ions of the $RCH{=}NH_2^{(+)}$ type (Scheme 2). The upper region of mass spectra of cyclohexapeptides contains peaks of ions formed by stepwise fragmentation of the linear molecular ion. Chain opening of the cyclic molecular ion occurs at a position of the N-terminal peptide bond of the amino acid with the longest side chain[12] and the fragmentation is initiated by elimination of this amino acid in the form of an alkylideneimine molecule ("azomethine cleavage", *cf.*[12,13]), sometimes along with elimination of the hydrogen atom. The thus-formed $(M - 85)^{+\cdot}$ or $(M - 86)^{+}$ ions undergo further decompositions by a gradual loss of HNCO and RCHNHCO fragments (Scheme 3). In the case of cyclo(Gly-Ala-Leu), a single sequential series of significant



SCHEME 2

Formation of Alkylideneimmonium Ions $RCH{=}NH_2^{(+)}$ in the Fragmentation of Cyclo(Gly-Ala-Leu)$_2$.

The symmetrical cleavage is not shown.



SCHEME 3

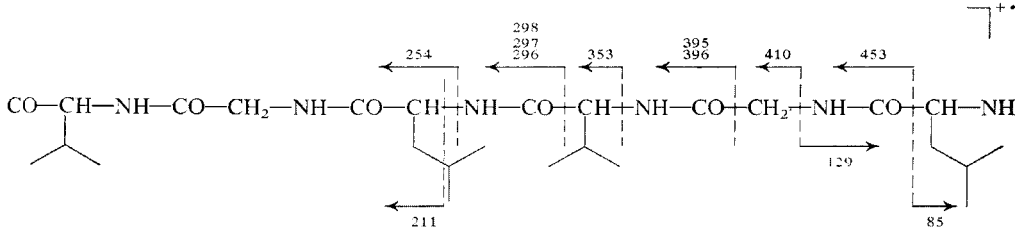
Fragmentation of Cyclo(Gly-Ala-Leu)$_2$ by Decomposition of the Open-Chained Molecular Ion

In the last residue on the right side the side chain (isobutyl) is omitted.

fragments is formed which might be used to obtain important information on the amino acid sequence. In accordance with the assumed formation in fragmentations of the open chain, the ions shown in Scheme 3 did not exhibit any differences in relative intensities in spectra of both diastereoisomers. However, a marked difference may be observed in population of the *m/e* 241 ion in the cleavage of the cyclic $M^{+\cdot}$

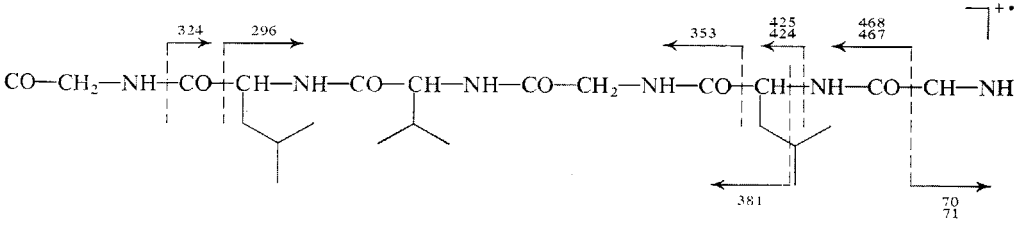
molecular ion into two symmetrical halves by a transannular reaction. In the case of cyclo(Gly-L-Ala-L-Leu)$_2$ (*Ia*), the population of this ion is 2·3 times higher than with the diastereoisomer *Ib*.

The mass spectra of cyclo(Gly-L-Val-L-Leu)$_2$ and cyclo(Gly-L-Val-D-Leu)$_2$ are much more complicated than those of the above discussed pair of isomeric cyclohexapeptides. This observation might be explained as follows. The ring opening of the cyclic molecular ion occurs not only at a position adjacent to the leucine residue but also (though to a lesser extent) at the N-end of the valine residue, the side chain of which is of a similar length as that of leucine. Consequently, the ions of the "first sequential series" (Scheme 4) are accompanied by less abundant ions of the "second sequential series" due to decomposition of the competitive linear molecular ion (Scheme 5).



SCHEME 4

Fragmentation of the Molecular Ion of cyclo(Gly-Val-Leu)$_2$ after Opening at a Position Vicinal to the Leucine Residue (Formation of the "First Sequential Series" Fragments)



SCHEME 5

Fragmentation of the Molecular Ion of cyclo(Gly-Val-Leu)$_2$ after Opening at a Position Vicinal to the Valine Residue (Formation of the "Second Sequential Series" Fragments)
In the last residue on the right side the side chain (isopropyl) is omitted.

It may be inferred from the higher abundancy of the *m/e* 410 and 211 ions (Scheme 4) in the spectrum of cyclo(Gly-L-Val-L-Leu)$_2$ (when compared with the other diastereoisomer) that the ring opening of the cyclic molecular ion at a position adjacent to the leucine residue and the subsequent fragmentation according to Scheme 4 are more preferred processes in the case of the former isomer. Accordingly, the *m/e*

324 ion, product of the other fragmentational path (Scheme 5), is more abundant in the spectrum of cyclo(Gly-L-Val-D-Leu)$_2$. The yield of the symmetrical cleavage of the cyclic molecular ion (with the formation of the *m/e* 269 ion) is almost equal with both isomers in contrast to different intensities of the analogous *m/e* 241 ion in spectra of the isomeric pair of alanine cyclohexapeptides.

The above differences in mass spectra of the two diastereoisomeric pairs must be due to different spatial structures. An explanation is therefore attempted as follows. As inferred from temperature dependence of CD spectra[14], conformation of the L,D-isomers with an unequal absolute configuration of the two vicinal amino-acid residues (*Ib*, *Id*) is thermodynamically more advantageous and more stable than that of L,L-isomers (*Ia*, *Ic*). With the pair *Ib* and *Id*, the steric interactions between side chains are obviously of a lesser importance than in the case of the pair *Ia* and *Ic*. An analogous situation (at least to a certain degree) may also be assumed with the cyclic molecular ion. In accordance with this idea, the molecular ions of L,L-isomers (*Ia*, *Ic*) in both diasteroisomeric pairs exhibit a higher content of energy, are less stable, the probability of their decomposition is higher and the peak of the corresponding molecular ion in the mass spectrum is lower (in relative intensities of $M^{+\cdot}$ ions, 3·3% and 5·5% with alanine derivatives *Ia* and *Ib*, resp., and 4·6% and 6% with valine derivatives *Ic* and *Id*, resp.). Easier decomposition of the molecular ion can affect various fragmentation reactions to a different degree. Thus in the series of alanine derivatives let us mention the transannular symmetrical bisection of the ring into two identical tripeptide fragments (*m/e* 241), the relative intensity of which is 11% with the L,L-isomer and only 3·8% with the L,D-isomer. (There is not any substantial difference in the readiness of a further decomposition of diastereoisomeric acyclic *m/e* 241 tripeptide fragments, only an enhanced population of the more readily formed fragment may be observed). According to the present knowledge[15] the most probable spatial arrangements of cyclohexapeptides are characterized by two antiparallel tripeptide units connected through β-turn-like structures. In conformations of this type the possibility exists for transannular reactions between central amino acid residues of both tripeptide units.

As inferred from ^{1}H-NMR spectra[5] these central positions may be occupied in cyclo(Gly-Ala-Leu)$_2$ not only by glycine residues (as in analogous valine or phenylalanine derivatives) but also by alanine residues owing to the low bulkiness of the alanine side chain. The probability of a transannular reaction is thus greater with alanine derivatives than in the case of valine derivatives; in this situation, a higher steric strain (limited mobility of side chains) could manifest itself with the L,L-isomer *Ia*. With valine derivatives, the preferential opening takes place on the valine-leucine bond with the formation of an acyclic ion, the longer side chains (with a higher conformational freedom) of which are capable of absorbing the energy liberated by opening of the cyclic molecular ion. The relatively rigid attachment of side chains in the cyclic system is replaced by a considerably higher conformational mobility

in the acyclic system; this change necesserily manifests itself by an increased entropy accompanying the fragmentation process (this increase is greater in the case of the L,L-isomer *Ic* since the side chain mobility of vicinal amino-acid residues was more restricted). The probability of this fragmentation should be thus higher with cyclo-(Gly-L-Val-L-Leu)$_2$ (*Ic*) than with the diastereoisomer *Id*, in accordance with experimental observations.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Samples for analysis and physical measurements were dried for one day at 0·5 Torr over phosphorus pentoxide (and potassium hydroxide pellets when crystallisations were performed from acetic acid). Optical rotations were measured on a photoelectric polarimeter. Purity of samples was checked by thin-layer chromatography on silica gel in the solvent systems 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) and 2-butanol–99% formic acid–water (75 : 12·3 : 12·7). Spots were detected by chlorination and the subsequent treatment with the *ortho*-tolidine agent.

Benzyloxycarbonyl-L-valyl-D-leucine Methyl Ester (*IId*)

D-Leucine methyl ester (obtained from 6·0 g of the hydrochloride by the action of chloroform saturated with ammonia) and N,N′-dicyclohexylcarbodiimide (6·2 g) were added at −10°C to a solution of benzyloxycarbonyl-L-valine (7·5 g) in ethyl acetate (80 ml) and the mixture kept at 0°C overnight. Four drops of acetic acid were added, the mixture kept at room temperature for 2 h, and filtered to remove N,N′-dicyclohexylurea. The filtrate was washed with 1M-HCl, 0·5M-$NaHCO_3$, and water, dried over anhydrous sodium sulfate, and evaporated to afford the ester *IId*.

Benzyloxycarbonylglycyl-L-valyl-D-leucine Methyl Ester (*IIId*)

Hydrogen bromide in acetic acid (50 ml of 35% solution) was added to the ester *IId* (8·3 g), the mixture kept at room temperature for 40 min, and evaporated. The residue was repeatedly triturated with ether and subjected to the action of a saturated solution of ammonia in chloroform to liberate the dipeptide ester. The ammonium bromide was filtered off, the filtrate evaporated, and the residue dissolved in ethyl acetate (80 ml). Benzyloxycarbonylglycine (4·6 g) was added to the solution, the mixture cooled to −10°C, treated with N,N′-dicyclohexylcarbodiimide (4·6 g), and kept at 0°C overnight. Acetic acid (4 drops) was added, the mixture kept at room temperature for 2 h, and filtered to remove N,N′-dicyclohexylurea. The filtrate was washed with 1M-HCl, 0·5M-$NaHCO_3$, and water, dried over anhydrous sodium sulfate, and evaporated to afford the protected tripeptide *IIId*.

Benzyloxycarbonylglycyl-L-valyl-D-leucine Hydrazide (*IVd*)

The tripeptide *IIId* (1·7 g) was refluxed in methanol (15 ml) with 98% hydrazine hydrate (1·7 ml) for 3 h, the mixture kept at room temperature overnight, evaporated, and the residue washed with water and ether to afford the hydrazide *IVd*.

Benzyloxycarbonylglycyl-L-valyl-D-leucyl-glycyl-L-valyl-D-leucine Methyl Ester (*VId*)

Hydrogen chloride in tetrahydrofuran (14 ml of 1·6M solution) was added to the hydrazide *IVd* (1·15 g) in dimethylformamide (15 ml) with stirring at −30°C, followed by n-butyl nitrite (0·63 ml; over 1 min) and, after 4 min, a precooled (−30°C) solution of glycyl-L-valyl-D-leucine methyl ester hydrobromide (*Vd*; obtained from *IIId* by the action of hydrogen bromide in acetic acid). The mixture was adjusted to pH 8·5 by means of N-ethylpiperidine, kept at 0°C overnight, and evaporated. The residue was repeatedly triturated with 1M-HCl, water, and ether to afford the protected hexapeptide *VId*.

Benzyloxycarbonylglycyl-L-valyl-D-leucyl-glycyl-L-valyl-D-leucine Hydrazide (*VIId*)

The protected hexapeptide *VId* (500 mg) was refluxed in methanol (25 ml) with 92% hydrazine (0·55 ml) for 10 h and the mixture kept at room temperature overnight. Another portion of hydrazine (0·20 ml) was then added, the mixture refluxed for 10 h, kept at room temperature overnight, and evaporated. Trituration of the residue with water and ether afforded the hydrazide *VIId*.

Cyclo(glycyl-L-valyl-D-leucyl-glycyl-L-valyl-D-leucyl) (*Id*)

Hydrogen bromide in acetic acid (8 ml of 35% solution) was added to the hydrazide *VIId* (185 mg), the mixture kept at room temperature for 30 min, and evaporated. The residue was triturated with ether to afford a powder (195 mg) which was dissolved in 0·5M-HCl (5 ml). A solution of sodium nitrite (19 mg) in water (5 ml) was added dropwise with stirring at 1°C, the mixture kept at 1°C for 10 min, and poured into water (700 ml). The solution was adjusted to pH 6·6 by means of sodium hydrogen carbonate, kept at 0°C for 6 days, stirred with Dowex 50 (H^+) ion exchange resin (25 ml), and filtered. The filtrate was passed through a column of Dowex 50 (H^+) resin (100 ml) and then a column of Amberlite IR-4B (OH^-) resin (100 ml). The effluent was evaporated to afford the cyclohexapeptide *Id*.

Cyclo(glycyl-L-alanyl-L-leucyl-glycyl-L-alanyl-L-leucyl) (*Ia*)

The benzyloxycarbonyl protecting group of the hydrazide *VIIa* (541 mg) was removed by hydrogenolysis in methanol (35 ml) containing 1·7 ml of concentrated hydrochloric acid. The cyclisation was performed as above (reaction time, 14 days), the solution evaporated, the residue triturated with 1M-HCl and water, and recrystallised from a mixture of acetic acid and water.

Mass Spectrometry

Mass spectra were measured on a MS 902 double focussing mass spectrometer (AEI, Manchester) with the use of direct inlet, ion source temperature of 180—190°C and ionisation energy of 70 eV. High resolution measurements were performed at the resolving power of 15000; all exact masses were found in the range of ±3 p.p.m. of the corresponding theoretical value. The following partial high resolution spectra were obtained (*m/e*, formula; with multiplets, percentage is given in brackets from the ionic current of ions of the same *m/e*):

cyclo(Gly-L-Ala-L-Leu)$_2$ (*Ia*): 482(M), $C_{22}H_{38}N_6O_6$; 426, $C_{18}H_{30}N_6O_6$; 397, $C_{17}H_{27}N_5O_6$; 396, $C_{17}H_{26}N_5O_6$; 369, $C_{16}H_{27}N_5O_5$; 354, $C_{16}H_{26}N_4O_5$; 353, $C_{16}H_{25}N_4O_5$; 340, $C_{15}H_{24}N_4O_5$; 339, $C_{15}H_{23}N_4O_5$; 297, $C_{14}H_{23}N_3O_4$; 296, $C_{14}H_{22}N_3O_4$; 283, $C_{13}H_{21}N_3O_4$; 270,

$C_{12}H_{20}N_3O_4$; 269, $C_{12}H_{19}N_3O_4$; 268, $C_{12}H_{18}N_3O_4$; 241, $C_{11}H_{19}N_3O_3$, 227, $C_{11}H_{19}N_2O_3$; 226, $C_{11}H_{18}N_2O_3$; 183, $C_8H_{11}N_2O_3$ (65%); 183, $C_9H_{15}N_2O_2$ (35%); 141, $C_8H_{15}NO$; 112, $C_5H_6NO_2$; 86, $C_5H_{12}N$; 44, C_2H_6N; 30, CH_4N;

cyclo(Gly-L-Val-L-Leu)$_2$ (*Ic*): 538(M), $C_{26}H_{46}N_6O_6$; 496, $C_{23}H_{40}N_6O_6$; 495, $C_{23}H_{39}N_6O_6$ (55%); 495, $C_{25}H_{45}N_5O_5$ (45%); 468, $C_{22}H_{38}N_5O_5$; 467, $C_{22}H_{37}N_5O_6$ (65%); 467, $C_{22}H_{39}N_6O_5$ (35%); 453, $C_{31}H_{35}N_5O_6$; 424, $C_{21}H_{36}N_4O_5$; 410, $C_{20}H_{34}N_4O_5$; 396, $C_{19}H_{32}N_4O_5$; 381, $C_{18}H_{29}N_4O_5$; 353, $C_{18}H_{31}N_3O_4$ (85%); 353, $C_{16}H_{25}N_4O_5$ (15%); 324, $C_{16}H_{25}N_5O_4$; 311, $C_{15}H_{25}N_3O_4$; 298, $C_{14}H_{24}N_3O_4$; 297, $C_{14}H_{23}N_3O_4$; 296, $C_{14}H_{22}N_3O_4$ (65%); 296, $C_{15}H_{26}N_3O_3$ (35%); 269, $C_{13}H_{23}N_3O_3$; 254, $C_{13}H_{22}N_2O_3$; 211, $C_{10}H_{15}N_2O_3$ (80%); 211, $C_{11}H_{19}N_2O_2$ (20%); 129, $C_6H_{13}N_2O$; 86, $C_5H_{12}N$; 72, $C_4H_{10}N$; 30, CH_4N.

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