AMINO ACIDS AND PEPTIDES. CVII.*

STUDIES ON ISOMERIC CYCLOHEXAPEPTIDES CONTAINING GLYCINE, PHENYLALANINE AND LEUCINE: SYNTHESIS AND CHARACTERISATION OF FURTHER DIASTEREOISOMERIC AND CYCLOENANTIOMERIC COMPOUNDS

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For physical measurements 9 isomeric cyclohexapeptides, belonging to three series, were synthesized: one with the sequence c(Gly-Phe-Leu-Gly-Phe-Leu), four with c(Gly-Leu-Phe-Gly-Leu-Phe), and four with c(Gly-Phe-Leu-Gly-Leu-Phe). Two of the cyclopeptides from the last series belong in the category of cycloenantiomers. Structures were characterised by mass spectrometry.

The synthesis of cyclohexapeptides, started in this laboratory some years ago[1], was motivated by an attempt to use such substances as models for studies of the interactions in peptide molecules and for studies of the properties of peptide bonds[2]. Cyclopeptides appeared to be versatile models in which relatively simple structural alterations could markedly change the conformation of the entire molecule or parts of it. The relation of this change to selected interactions could be studied. This concept received some justification over succeeding years and has been used in a number of instances in this laboratory[3-8] as well as elsewhere[9-13]. In the basic sequence glycyl-phenylalanyl-leucyl-glycyl-phenylalanyl-leucyl the first structural parameter chosen for alteration was the absolute configuration of amino acid residues where changes resulted in a series of diastereoisomers[1]. The second parameter chosen was the sequence of amino acid residues, carried out by preparing two retro-isomers[14].

The present communication deals with the preparation and properties of further cyclohexapeptide isomers with altered structural parameters in order to complement the total series. From 2 residues of glycine, 2 of phenylalanine and 2 of leucine we can construct a series of 16 positional isomers of cyclohexapeptides, two of which will consist of enantiomeric pairs and *meso*-forms, four of cycloenantiomers[15], two of cyclodiastereoisomers[15] and eight of enantiomers only; in all there are 120 enantiomeric pairs and 4 *meso*-forms. Since measurements of chiroptical properties[4,6] and NMR spectra[8] have shown that data interpretation is more fruitful in compounds with some degree of structural regularity, we have centred our interests on a series of positional isomers in which there is some degree of symmetry. Thus in the series with the se-

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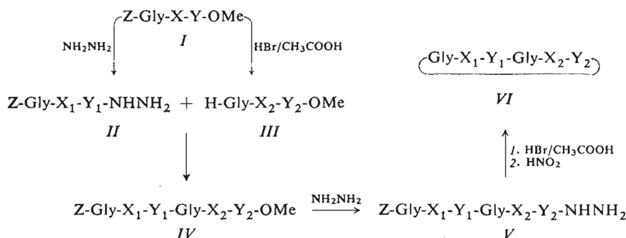
quence c(Gly-Phe-Leu-Gly-Phe-Leu), isomers* -,L,L,-,L,L and -,D,L,-,D,L have a two-fold rotational axis C_2, and both *meso*-forms -,D,L,-,L,D and -,L,L,-,D,D have a centre of symmetry C_i. There is an analogous situation in the retro-series c(Gly-Leu-Phe-Gly-Leu-Phe) where the -,L,L,-,L,L and -,L,D,-,L,D isomers possess a two-fold rotational axis C_2, and the *meso*-forms -,D,L,-,L,D and -,L,L,-,D,D have a center of symmetry C_i. In none of the members of the other positional isomeric series can an element of symmetry occur. Instead of *meso*-forms with a centre of symmetry, substances which can be categorised as cycloenantiomers can exist in some positional isomeric series. These are particularly interesting because of their chiroptic properties[9,10]. Cycloenantiomers can occur in 4 sequences (two pairs in each): c(Gly-Phe-Leu-Gly-Leu-Phe), c(Gly-Phe-Phe-Gly-Leu-Leu), c(Gly-Gly-Leu-Phe-Phe-Leu) and c(Gly-Gly-Phe-Leu-Leu-Phe). We decided to synthesize two compounds with the first sequence (isomers -,D,D,-,L,L and -,D,L,-,L,D) because this would make possible the maximal utilisation of intermediates from previous synthetic work[14].

All of the cyclohexapeptides cited below were prepared by a uniform synthetic scheme[1,14]. First we synthesized two tripeptide fragments derived from the benzyloxycarbonyltripeptide ester *I* with a glycine residue at the amino end; one as a benzyloxycarbonyltripeptide hydrazide *II*, the other as a tripeptide ester *III*. Azide coupling yielded the benzyloxycarbonylhexapeptide ester *IV* from which the benzyloxycarbonylhexapeptide hydrazide *V* (Table I) was obtained. The final cyclopeptides *VI* were prepared by the decarbobenzoxylation of the protected hydrazides *V* and subsequent cyclisation by the azide method. In the case of compounds with a twofold rotational axis of symmetry we also used an alternative synthesis, cyclisation-dimerisation of the corresponding hydrazide, again by the azide method.



In a number of cases it was possible to use tripeptide derivatives prepared by previously reported procedures[14]. In the remaining cases the dipeptide or tripeptide derivatives were syn-

* The absolute configuration of the residue in the sequence is given. For greater clarity the positions of the glycine residues are denoted by dashes.

thesized which are enantiomeric with compounds previously prepared. With all of these enantiomeric pairs a good agreement in physical properties was attained; in particular the optical rotation $[\alpha]_D$ of the enantiomers were of equal magnitude but opposite sign. For the preparation of tripeptide derivatives with a sequence Gly-Leu-Phe, but with various configurations we used not only the benzyloxycarbonyl group but also *o*-nitrobenzenesulphenyl group, split off with hydrogen chloride in methanol[6,16] for the synthesis of the intermediary dipeptides. This considerably simplified the work.

In all, we prepared 8 new isomers. The series of compounds with the sequence c(Gly-Phe-Leu-Gly-Phe-Leu) was completed by the synthesis of the last heretofore unknown stereoisomer – the *meso*-form -,L,L,-,D,D – obtained by cyclisation of both enantiomers of the corresponding linear hexapeptide hydrazide (glycyl-L-phenylalanyl-L-leucyl-glycyl-D-phenylalanyl-D-leucine hydrazide and glycyl-D-phenylalanyl-D-leucyl-glycyl-L-phenylalanyl-L-leucine hydrazide). Of the series of retro-isomers with the sequence c(Gly-Leu-Phe-Gly-Leu-Phe) two have been previously described[14]: one asymmetric -,L,D,-,L,L and one symmetric -,L,D,-,L,D. The last listed was originally prepared by dimerisation[14]; in the present work it was prepared by the alternative route using the linear hexapeptide. In addition, a second symmetric, optically active diastereoisomer -,L,L,-,L,L was prepared, again by cyclisation of the linear hexapeptide as well as by cyclisation-dimerisation of tripeptide fragments. Cyclisation of the corresponding hexapeptides (glycyl-L-leucyl-L-phenylalanyl-glycyl-D-leucyl-D-phenylalanine hydrazide and glycyl-L-leucyl-D-phenylalanyl-glycyl-D-leucyl-L-phenylalanine hydrazide resp.) yielded both possible *meso*-forms: -,L,L,-,D,D and -,L,D,-,D,L. In the series of compounds with sequence c(Gly-Phe-Leu-Gly-Leu-Phe) we prepared compounds formally corresponding to four symmetrical isomers of both series mentioned above. Substances with the same absolute configuration in terms of amino acid residues of the same type, *i.e.* -,L,L,-,L,L and -,D,L,-,L,D, do not show symmetry in this case. Both were prepared by cyclisation of the corresponding linear hexapeptide hydrazides. In the same manner we also prepared the last two isomers c(glycyl-D-phenylalanyl-L-leucyl-glycyl-D-leucyl-L-phenylalanyl) and c(glycyl-D-phenylalanyl-D-leucyl-glycyl-L-leucyl-L-phenylalanyl) which represent possible cycloenantiomeric pairs in this series.

The properties and yields of the prepared cyclohexapeptides are presented in Table II. As observed[1,6,17,18], the yield was related to the relative configuration of chiral atoms in the chain. Higher yields were obtained in all cases in which both L and D forms were present in the same molecule. The homogeneity of the cyclohexapeptides was controlled by thin layer chromatography among other methods. In the system diisopropyl ether–chloroform–formic acid there was some degree of separation of diastereoisomers. Somewhat larger R_F values were shown by isomers containing both L and D residues. The structure of all the cyclohexapeptides were studied by mass spectrometry. The molecular weight in all cases corresponded to cyclohexapeptides of the given structure, even if data from elemental analysis corresponded to variously hydrated forms which are usual in the cyclohexapeptides[19]. Fragmentation determined was in agreement with observations on substances prepared previously.

EXPERIMENTAL

Melting points were determined on a Kofler block. Optical rotation was measured with a photoelectric polarimeter or the Jasco ORD-UV/5 spectropolarimeter. Samples for analysis were dried at 70°C/1 Torr over phosphorus pentoxide for 8—16 h, unless otherwise stated. Intermediate products, i.e. derivatives of di-, tri-, and hexapeptides, were analysed for purity both by thin layer chromatography on silica gel (Kieselgel G, Merck) in the solvent system 1-butanol—acetic acid—water (4 : 1 : 1) and 2-butanol—3% aqueous ammonia (3 : 1), and by paper electrophoresis on Whatman 3 MM in a moist chamber in 6% acetic acid at a potential gradient of about 20 V/cm. Compounds with a free amino groups were detected with ninhydrin and the protected peptides with tolidine reagent after chlorination[20]. Substances used in further stages were pure by these criteria. All solutions were evaporated on a rotating evaporator at 40°C/15 Torr.

o-Nitrobenzenesulphenyl-D-leucyl-L-phenylalanine Methyl Ester

To a solution of the dicyclohexylammonium salt of *o*-nitrobenzenesulphenyl-D-leucine (6·0 g) and the hydrochloride of L-phenylalanine methyl ester (3·0 g) in chloroform (100 ml) dicyclohexylcarbodiimide (3·2 g) was added. The mixture was left standing overnight in the refrigerator, the solution was filtered and evaporated, the residue was dissolved in ethyl acetate, the solution shaken with 0·5M-H_2SO_4, water, 5% $NaHCO_3$, and water, dried and evaporated. The residue was crystallised from a mixture of ethyl acetate and light petroleum, giving a yield of 4·7 g (72%), m.p. 127—129°C, $[\alpha]_D$ −12·1° (*c* 0·50, methanol). For $C_{22}H_{27}N_3O_5S$ (445·5) calculated: 59·31% C, 6·11% H, 9·43% N; found: 59·29% C, 5·86% H, 9·48% N. The analogously prepared enantiomeric ester had m.p. 128—129°C, $[\alpha]_D$ +11·8° (*c* 0·50, methanol).

o-Nitrobenzenesulphenyl-D-leucyl-D-phenylalanine Methyl Ester

A mixture of the dicyclohexylammonium salt of *o*-nitrobenzenesulphenyl-D-leucine (12·6 g), the hydrochloride of D-phenylalanine methyl ester (6·0 g) and dicyclohexylcarbodiimide (6·3 g) in chloroform (200 ml) was worked up as in the preceding preparation. The yield was 8·4 g (70%) of a substance with m.p. 107—108°C, $[\alpha]_D$ +30·3° (*c* 0·50, methanol). For $C_{22}H_{27}N_3O_5S$ (445·5) calculated: 59·31% C, 6·11% H, 9·43% N; found: 59·64% C, 6·03% H, 9·11% N. An analogously prepared enantiometric ester had a m.p. of 108—109°C, $[\alpha]_D$ −30·1 (*c* 0·50, methanol).

D-Leucyl-L-phenylalanine Methyl Ester Hydrochloride

To a solution of *o*-nitrobenzenesulphenyl-D-leucyl-L-phenylalanine methyl ester (1·5 g) in methanol (10 ml) 9 ml of 0·5M-HCl in methanol were added. The mixture was left standing at room temperature (the course of the reaction was followed by thin layer chromatography on silica gel in benzene[21]) for 10 min, evaporated, the residue was ground with ether and let stand for 30 min at room temperature. The substance was filtered and crystallised from methanol–ether with a yield of 0·93 g (84%), m.p. 215—220°C, $[\alpha]_D$ −30·7° (*c* 0·50, methanol). For $C_{16}H_{25}ClN_2O_3$ (328·8) calculated: 58·44% C, 7·66% H, 8·25% N; found: 58·70% C, 7·78% H, 8·59% N. The analogously prepared enantiomer had a m.p. of 215—220°C, $[\alpha]_D$ +30·9° (*c* 0·50, methanol).

D-Leucyl-D-phenylalanine Methyl Ester Hydrochloride

This compound was obtained in the same manner as above from 1 g of *o*-nitrobenzenesulphenyl-D-leucyl-D-phenylalanine methyl ester in a yield of 0·65 g (89%), m.p. 199—201°C, $[\alpha]_D$ −10·6° (*c* 0·50, methanol). For $C_{16}H_{25}ClN_2O_3$ (328·8) calculated: 58·44% C, 7·66% H, 8·52% N; found: 58·59% C, 7·77% H, 8·63% N. The analogously prepared enantiomer had a m.p. of 204—205°C, $[\alpha]_D$ +10·6° (*c* 0·50, methanol).

Benzyloxycarbonyl-D-phenylalanyl-D-leucine Methyl Ester

To a solution of benzyloxycarbonyl-D-phenylalanine (8 g) and D-leucine methyl ester (4 g) in ethyl acetate (150 ml) 6·3 g of dicyclohexylcarbodiimide was added. The mixture was left standing overnight in the refrigerator and then 0·5 ml of dilute acetic acid was added. After 1 h at 0°C N,N'-dicyclohexylurea separated out and was filtered off, the filtrate was washed with 1M-HCl, 5% $NaHCO_3$, and water, dried over sodium sulphate and evaporated. The residue was taken up in ethyl acetate and light petroleum and the product crystallised out at a yield of 9·4 g (82%), m.p. 112–113°C, $[\alpha]_D$ +25·7° (*c* 0·50, methanol). The literature[22] reports for the enantiomer m.p. 110–111°C, $[\alpha]_D$ −25·0° (*c* 2·8, methanol).

o-Nitrobenzenesulphenylglycyl-D-leucyl-L-phenylalanine Methyl Ester

A mixture of the dicyclohexylammonium salt of *o*-nitrobenzenesulphenylglycine (1·0 g), the hydrochloride of D-leucyl-L-phenylalanine methyl ester (0·8 g) and dicyclohexylcarbodiimide (0·6 g) in chloroform (20 ml) was worked up as in preceding preparations; the yield 0·8 g (66%) m.p. 128–130°C (ethyl acetate–light petroleum). For $C_{24}H_{30}N_4O_6S$ (502·6) calculated: 57·35% C, 6·02% H, 11·15% N; found: 57·78% C, 6·18% H, 11·11% N.

Benzyloxycarbonylglycyl-D-phenylalanyl-D-leucine Methyl Ester (*I*, X = D-Phe, *Y* = D-Leu)

The above procedure was used to obtain 4·2 g (88%) of the title compound from benzyloxycarbonylglycine (7 g), D-phenylalanyl-D-leucine methyl ester (10 g) in ethyl acetate (200 ml) and dicyclohexylcarbodiimide (7·0 g); m.p. 90–92°C, $[\alpha]_D$ +21·2° (*c* 0·50, dimethylformamide). After decarbobenzoxylation with hydrogen bromide in acetic acid and decomposition of the crude hydrobromide with ammonia in chloroform the free tripeptide methyl ester was obtained, m.p. 111–112°C (methanol–ether). The literature gives m.p. 84–86°C, $[\alpha]_D$ −17·4° (*c* 0·40, 98% acetic acid) for the enantiomer of the protected tripeptide methyl ester and m.p. 108–109°C for the enantiomeric unprotected tripeptide ester.

Benzyloxycarbonylglycyl-L-leucyl-L-phenylalanine Hydrazide (*II*, X_1 = L-Leu, Y_1 = L-Phe)

To a solution of benzyloxycarbonylglycyl-L-leucyl-L-phenylalanine methyl ester[14] (1·5 g) in methanol (5 ml) 82% hydrazine hydrate (0·5 ml) was added. The mixture was refluxed for 2 h, left overnight at 0°C and then diluted with water. The crystals which separated out were recrystallised from aqueous methanol, giving a yield of 1·32 g (88%) of the hydrazide, m.p. 191–192°C, $[\alpha]_D$ −28·3° (*c* 0·50, dimethylformamide). For $C_{25}H_{33}N_5O_5$ (483·5) calculated: 62·10% C, 6·80% H, 14·50% N; found: 62·03% C, 7·02% H, 14·49% N.

Benzyloxycarbonylhexapeptide Methyl Esters *IV*

As an example we present the synthesis of substance *IVi*. Isomers *IVa*–*IVh* were prepared in the same manner. Yields and analytical data are presented in Table I. To a solution of benzyloxycarbonylglycyl-D-phenylalanyl-L-leucine hydrazide[1] (1·0 g) in dimethylformamide (20 ml) 5·5 ml 3·5M-HCl in tetrahydrofurane was added, followed by the dropwise addition of 0·45 ml of butyl nitrite at −30°C with stirring. After 4 min a solution of hydrobromide of glycyl-L-leucyl-D-phenylalanine methyl ester[14] (2·6 g) in dimethylformamide (5 ml) was added and the mixture was neutralised with N-ethylpiperidine to pH 8·5. After 12 h standing at 0°C the solvents were removed and the residue was taken up in 30 ml 1M-HCl. After 1 h at 0°C the solid substance which formed

TABLE 1

Yields, Specific Rotations in Dimethylformamide and Analyses of Benzyloxycarbonyl Hexapeptide Esters *IV* and Hydrazides *V*

Compound	X_1 X_2	Y_1 Y_2	Yield, % M.p., °C	$[\alpha]_D$ *c*	Found % C	Found % H	Found % N
			Esters *IV*[a]				
IVa[b]	D-Phe	D-Leu	47	+ 7·2°	64·49	7·04	10·53
	L-Phe	L-Leu	239—240	(0·21)			
IVb	L-Leu	L-Phe	67	—43·8°	64·81	7·06	10·36
	L-Leu	L-Phe	210—211	(0·28)			
IVc[c]	L-Leu	D-Phe	67	+ 3·7°	64·24	7·05	10·22
	L-Leu	D-Phe	217—218	(0·50)			
IV[d]	L-Leu	L-Phe	45	—15·0°	64·28	7·32	10·25
	D-Leu	D-Phe	230—232	(0·20)			
IVe[e]	L-Leu	D-Phe	87	+ 8·9°	64·48	7·08	10·58
	D-Leu	L-Phe	212—213	(0·50)			
IVf	L-Phe	L-Leu	44	—25·7°	64·70	7·01	10·61
	L-Leu	L-Phe	240—242	(0·50)			
IVg	D-Phe	L-Leu	76	+ 5·0°	64·82	7·28	10·81
	D-Leu	L-Phe	189—190	(0·50)			
IVh	D-Phe	D-Leu	60	—19·5°	64·29	6·96	10·62
	L-Leu	L-Phe	230—232	(0·50)			
IVi	D-Phe	L-Leu	70	+ 7·8°	64·34	7·12	10·49
	L-Leu	D-Phe	205—207	(0·50)			
			Hydrazides *V*[f]				
Va[g]	L-Phe	L-Leu	80	— 3·5°[h]	63·18	7·01	13·79
	D-Phe	D-Leu	260—265	(0·50)			
Vb[i]	L-Leu	L-Phe	92	—54·4°	62·36	7·75	13·95
	L-Leu	L-Phe	246—248	(0·50)			
Vc	L-Leu	D-Phe	82	+ 2·8°	62·68	7·11	14·06
	L-Leu	D-Phe	180—182[k]	(0·12)			
Vd	L-Leu	L-Phe	75	— 8·6°	62·66	6·79	14·00
	D-Leu	D-Phe	245—247	(0·50)			
Ve	L-Leu	D-Phe	86	+ 5·6°	62·63	6·96	14·00
	D-Leu	L-Phe	226—227[l]	(0·50)			
Vf	L-Phe	L-Leu	84	—31·5°	63·14	7·15	14·26
	L-Leu	L-Phe	246—248[l]	(0·50)			
Vg[i]	D-Phe	L-Leu	77	— 4·6°	62·32	7·11	13·93
	D-Leu	L-Phe	160—162[m]	(0·50)			
Vh	D-Phe	D-Leu	80	— 5·6°	62·93	7·01	13·92
	L-Leu	L-Phe	254—256	(0·19)			
Vi	D-Phe	L-Leu	80	+27·4°	63·00	7·14	13·76
	L-Leu	D-Phe	228—230[l]	(0·50)			

TABLE II

Yields and Analyses of Cyclohexapeptides *VI*

Compound	X_1 / X_2	Y_1 / Y_2	Yield, % / m.p., °C	Formula (M.v.)	Calculated/Found % C	% H	% N
VIa[a]	L-Phe	L-Leu	30	$C_{34}H_{46}N_6O_6.2\ H_2O$	60·88	7·51	12·53
	D-Phe	D-Leu	—	(670·8)	60·89	7·24	12·29
VIb	L-Leu	L-Phe	23[b]	$C_{34}H_{46}N_6O_6$	64·32	7·30	13·24
	L-Leu	L-Phe	314—316	(634·8)	64·05	7·67	13·14
VIc	L-Leu	D-Phe	43[c]	$C_{34}H_{46}N_6O_6.2\ H_2O$	60·88	7·51	12·53
	L-Leu	D-Phe	314—316	(670·8)	61·06	7·53	12·57
Vd	L-Leu	L-Phe	35	$C_{34}H_{46}N_6O_6.2\ H_2O$	60·88	7·51	12·53
	D-Leu	D-Phe	[d]	(670·8)	61·03	7·54	12·59
VIe	L-Leu	D-Phe	43	$C_{34}H_{46}N_6O_6.H_2O$	62·55	7·41	12·88
	D-Leu	L-Phe	[e]	(652·8)	62·68	7·33	12·53
VIf	L-Phe	L-Leu	31	$C_{34}H_{46}N_6O_6.\frac{1}{2}\ H_2O$	63·37	7·30	13·05
	L-Leu	L-Phe	295—298	(643·8)	63·27	7·71	12·72
VIg	D-Phe	L-Leu	37	$C_{36}H_{46}N_6O_6.2\ H_2O$	60·88	7·51	12·53
	D-Leu	L-Phe	307—309	(670·8)	61·19	7·52	12·66
VIh	D-Phe	D-Leu	58	$C_{34}H_{46}N_6O_6.\frac{1}{2}\ H_2O$	63·37	7·30	13·05
	L-Leu	L-Phe	315—317	(643·8)	63·09	7·32	12·98
VIi	D-Phe	L-Leu	34	$C_{34}H_{46}N_6O_6.2\ H_2O$	60·88	7·51	12·53
	L-Leu	D-Phe	307—310	(670·8)	60·89	7·14	12·55

[a] Substance synthesized from enantiomeric -,L,L,-,D,D hydrazide *Va* decomposes over 330°C; substance synthesized from -,D,D,-,L,L enantiomer with a yield of 27% did not melt to 360°C. Changes in crystalline form in both cases at 305—310°C. [b] Substance identical with cyclopeptide obtained by cyclisation-dimerisation with a yield of 19%, m. p. 312—315°C. [c] Substance identical with product of cyclisation-dimerisation[14]. [d] Did not melt at 350°C, change in crystaline form at 320°C. [e] Decomposition at about 360°C, change in crystalline shape at about 350°C.

Continuation of Table I:

[a] For $C_{43}H_{56}N_6O_9$ (800·9) calculated: 64·50% C, 7·00% H, 10·50% N; [b] enantiomer *IVa* -,L,L,-,D,D was prepared with the same yield, m.p. 237—240°C; [c] crystallised from 2-propanol–ethyl acetate; [d] crystallised from aqueous dimethylformamide; [e] crystallised from 2-propanol, the same compound was synthesized starting with non-crystalline glycyl-D-leucyl-L-phenylalanine methyl ester (prepared by deblocking of the *o*-nitrobenzenesulphenyl derivative; [f] for $C_{42}H_{56}N_8O_8$ (800·9) calculated: 62·98% C, 7·05% H, 13·99% N; [g] enantiomer *Va* -,D,D,-,L,L gave the same yield, m.p. 262—264°C, $[\alpha]_D$ +2·4° (*c* 0·50, pyridine); [h] measured in pyridine; [i] for $C_{42}H_{56}N_8O_8.{}^1/_2\ H_2O$ (809·9) calculated: 62·36% C, 7·25% H, 13·95% N; [k] solidifies and melts at 212°C; [l] crystallised from aqueous methanol; [m] crystallised from methanol–ether.

was filtered off, washed with 1M-HCl, 5% $NaHCO_3$, and water and dried *in vacuo*. The yield was 1·5 g, m.p. 185—195°C, after crystallisation from 2-propanol–ether–light petroleum the yield was 1·16 g (70%), m.p. 204—207°C.

Benzyloxycarbonylhexapeptide Hydrazides *V*

As an example we present the synthesis of substance *Vi*. Isomers *Va*—*Vh* were prepared in the same manner, *cf.* Table I. To a solution of hexapeptide ester *IVi* (0·5 g) in methanol (5 ml) 0·5 ml 92% hydrazine was added. The mixture was refluxed for 6 h, left standing 20 h, diluted with water and left standing 1 h at 0°C. The precipitate which separated out was sucked off and dried *in vacuo*; the yield was 0·45 g, m.p. 226—228°C; after crystallisation from aqueous methanol the yield was 0·40 g (80%), m.p. 228—230°C.

Cyclohexapeptides *VI*

As an example the synthesis of peptide *VIi* is presented. Isomers *VIa*—*VIh* were prepared in the same manner (Table II). Hydrazide *Vi* (0·5 g) was left standing in a 33% solution of hydrogen bromide in acetic acid for 30 min at room temp. The mixture was diluted with ether, the hydrobromide which separated out was ground with ether and dried *in vacuo*. A solution of sodium nitrite (40 mg) in water (5 ml) was added dropwise to a solution of purified hydrobromide hydrazide (0·45 g) in acetic acid (10 ml) and 1M-HCl (30 ml) with constant stirring at 0°C. After a further 15 min mixing at 0°C the mixture was added to 1 000 ml of ice water and the pH was adjusted to 6·7 with a solution of $NaHCO_3$ and Na_2CO_3. The reaction mixture was left standing at 3°C for 6 days. The solid substance which separated out was filtered off and dried in an exsiccator, giving a yield of 160 mg, m.p. 270—290°C. After crystallisation from acetic acid–water the yield was 121 mg (34%); m.p. 307—310°C. With isomer *VIf* the reaction time was 28 days, *VIb* 9 days, and the remaining isomers were left standing for 3—6 days. In the case of isomer *IVa* with the given dilution a product was isolated containing a small admixture of a high molecular weight substance, perhaps an oligomer according to mass spectrometry. In repeated syntheses, therefore, the dilution was increased threefold. The yields presented in Table II refer to product crystallised once from acetic acid and values given are the highest from repeated measurements.

The synthesis of isomer VIb by cyclisation dimerisation: Hydrazide *II* (X_1 = L-Leu, Y_1 = = L-Phe) (0·8 g) was decarbobenzoxylated as described for cyclisation of hexapeptide hydrazide *Vi*. To a solution of the impure hydrobromide (0·70 g) in 1M-HCl (26 ml) was added dropwise a solution of sodium nitrite (101 mg) in water (8 ml) at −5°C with stirring. The mixture was stirred 15 min at 0°C, added to 300 ml ice water, left standing 4 days and worked up as above.

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