SYNTHESIS OF DIPEPTIDES CONTAINING 1-AMINOCYCLOALKYLCARBOXYLIC ACIDS

PART II[1]

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ABSTRACT

Six new dipeptides containing either 1-aminocyclopentanecarboxylic acid or 1-aminocyclohexanecarboxylic acid placed at either the N- or the C-terminal of L-phenylalanine or L-leucine have been synthesized. Two molecules of 1-aminocyclopentanecarboxylic acid were also linked with a peptide bond. These seven dipeptides were cyclized into the corresponding substituted 2,5-piperazinediones.

INTRODUCTION

The synthetic amino acid 1-aminocyclopentanecarboxylic acid has been shown to be an interesting amino acid. It is active against certain types of tumors (1–3). The biochemical studies done so far have not yet elucidated its mechanism of action (4), nor explained its toxicity. It is known that the alkylation of the cyclopentane ring destroys the antitumor activity of the compound. However, the incorporation of this synthetic amino acid into a short peptide chain preserves the antitumor effect (2), while lowering the toxicity.

In order to enhance the antitumor index, Connors and Ross (5) and Tailleur and Berlinguet (6) have recently reported the synthesis of a few short-chain peptides having glycine and DL-phenylalanine attached to 1-aminocyclopentanecarboxylic acid or to 1-aminocyclohexanecarboxylic acid. Shankman *et al.* (7) have just reported the synthesis of other peptides containing 1-aminocyclopentanecarboxylic acid.

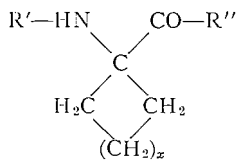
Since these peptides were intended for biological assays, it was important to incorporate the L-form of the natural amino acids. We have synthesized six new dipeptides containing either 1-aminocyclopentanecarboxylic acid or 1-aminocyclohexanecarboxylic acid attached to L-leucine or L-phenylalanine. We have also prepared the dipeptide containing two molecules of 1-aminocyclopentanecarboxylic acid. The list of these peptides is given in Table I. For these syntheses the classical method of Sheehan and Hess (8) was used in which the N-carbobenzoxy derivative of one amino acid was condensed with the benzyl ester of the other amino acid in the presence of N,N′-dicyclohexylcarbodiimide. The protected dipeptides were then hydrogenolized in the presence of 10% palladium on carbon, and the free dipeptides isolated by two different procedures according to their solubilities.

During these manipulations, the free peptides had a strong tendency to cyclize into the corresponding diketopiperazines, especially when heated in anhydrous conditions. Therefore, one has to be careful when purifying the free peptide because in some cases the solubilities of the peptide and of the cyclized diketopiperazine are quite similar, the analytical values being also very close. We found that the separation by chromatography and the analysis by titration are the most reliable methods to detect traces of contamination by diketopiperazines.

In order to prevent this cyclization three methods can be used: (1) water can be added

[1]*For part I, see reference 6.*

TABLE I



R′	R″	x	Name	
L-Leucyl-	-H	2	1-(L-Leucylamino)cyclopentanecarboxylic acid	(XI)
L-Leucyl-	-H	3	1-(L-Leucylamino)cyclohexanecarboxylic acid	(XII)
L-Phenylalanyl-	-H	2	1-(L-Phenylalanylamino)cyclopentanecarboxylic acid	(XIII)
L-Phenylalanyl-	-H	3	1-(L-Phenylalanylamino)cyclohexanecarboxylic acid	(XIV)
H-	-L-Phenylalanine	2	1-Aminocyclopentanecarbonyl-L-phenylalanine	(VIII)
H-	-L-Phenylalanine	3	1-Aminocyclohexanecarbonyl-L-phenylalanine	(IX)
H-	-1-Aminocyclopentane carboxylic acid	2	1-Aminocyclopentanecarbonyl-1-aminocyclopentanecarboxylic acid	(X)

to the ethanolic solution of the protected dipeptide before hydrogenolysis, (2) the hydrogenolysis can be done in acid medium where the liberated peptide would be ionized, thus preventing cyclization, and (3) if these conditions are not realized, care must be taken to avoid too much heating of the solutions especially if the content of water is low.

The spatial configuration of 1-aminocyclopentanecarboxylic acid and 1-aminocyclohexanecarboxylic acid seems to be responsible for the tendency to cyclize of the peptides containing these two amino acids attached to L-phenylalanine or L-leucine either by the C- or N-terminals. In fact, when those dipeptides are heated, they give a sublimate between 175° and 200° which melts between 175° and 195° depending on the peptide. The corresponding diketopiperazines obtained by different syntheses also give a sublimate between 175° and 200°. When these peptides are heated separately at 200° for a few minutes and then studied chromatographically they give two spots, one corresponding to the unchanged dipeptide and the other spot corresponding to the diketopiperazine. The dipeptide containing two molecules of 1-aminocyclopentanecarboxylic acid also cyclizes very easily by heating.

Preliminary testing of these peptides on tumors are now in progress and will be reported elsewhere.

EXPERIMENTAL

All melting points are uncorrected. The optical rotations were measured on a Rudolph polarimeter with a photoelectric cell alignment. The carbobenzoxy derivatives of L-leucine (9), L-phenylalanine (10), 1-aminocyclopentanecarboxylic acid (6), 1-aminocyclohexanecarboxylic acid (6), and the benzyl esters of L-phenylalanine (11), 1-aminocyclopentanecarboxylic acid (6), and 1-aminocyclohexanecarboxylic acid (6) were prepared according to published procedures.

N-Carbobenzoxydipeptide Benzyl Esters

The protected dipeptides were prepared as follows: to 0.01 mole of the N-carbobenzoxyamino acid dissolved in methylene chloride were added 0.011 mole of the appropriate amino acid benzyl ester and 0.011 mole of N,N′-dicyclohexylcarbodiimide. After 4 hours at room temperature, the N,N′-dicyclohexylurea formed was removed by filtration and the filtrate evaporated *in vacuo* on a water bath, after the addition of 2 ml of glacial acetic, which decomposes the excess of carbodiimide.

The oily residue was dissolved in 25 ml of ethyl acetate, and the solution filtered after 20 minutes to remove

the excess of N,N′-dicyclohexylurea formed after the addition of acetic acid. The filtrate was successively washed with 1 *N* hydrochloric acid, 1 *M* sodium carbonate, and water, and dried over anhydrous sodium sulphate. The solution was evaporated to dryness, and the N-carbobenzoxydipeptide benzyl ester was crystallized with the appropriate solvent system. The optical rotations were taken at 25° at a concentration of 2% in ethanol.

(I) Benzyl N-carbobenzoxy-1-(L-leucylamino)cyclopentanecarboxylate.—Yield: 3.7 g (78%), m.p. 116°, from ether–*n*-hexane, $[\alpha]_D$ −28°. Anal. Calc. for $C_{27}H_{34}N_2O_5$: N, 6.00%. Found: N, 6.08%.

(II) Benzyl N-carbobenzoxy-1-(L-leucylamino)cyclohexanecarboxylate.—Yield: 3.9 g (81%), m.p. 124–126°, from ether–*n*-hexane, $[\alpha]_D$ −31°. Anal. Calc. for $C_{28}H_{36}N_2O_5$: N, 5.85%. Found: 5.91%.

(III) Benzyl N-carbobenzoxy-1-(L-phenylalanylamino)cyclopentanecarboxylate.—Crude yield of the product which was isolated as an oil: 4.1 g (82%).

(IV) Benzyl N-carbobenzoxy-1-(L-phenylalanylamino)cyclohexanecarboxylate.—Crude yield of the product which was isolated as an oil: 4.0 g (78%).

(V) N-Carbobenzoxy-1-aminocyclopentanecarbonyl-L-phenylalanine benzyl ester.—Yield: 4.0 g (80%), m.p. 117°, from ethanol – petroleum ether, $[\alpha]_D$ −13°. Anal. Calc. for $C_{30}H_{32}N_2O_5$: N, 5.59%. Found: N, 5.58%.

(VI) N-Carbobenzoxy-1-aminocyclohexanecarbonyl-L-phenylalanine benzyl ester.—Yield: 4.2 g (82%), m.p. 98°, from ethanol – petroleum ether, $[\alpha]_D$ −9°. Anal. Calc. for $C_{31}H_{34}N_2O_5$: N, 5.44%. Found: 5.56%.

(VII) Benzyl N-carbobenzoxy-1-aminocyclopentanecarbonyl-1-aminocyclopentanecarboxylate.—Yield: 3.7 g (80%), m.p. 138°, from ethanol – petroleum ether. Anal. Calc. for $C_{27}H_{32}N_2O_5$: N, 6.03%. Found: N, 5.92%.

Free Dipeptides

The neutral equivalent values of the peptides were determined by titration with perchloric acid in glacial acetic acid, using crystal violet as indicator, according to Toennies and Callan (12). It was assumed that the molecular weight and the neutral equivalent are identical. The R_f values are for ascending chromatography in collidine–lutidine–water (1:1:2), the dipeptides and substituted diketopiperazines being detected by the Cl_2–KI–starch method of Rydon and Smith (13). The optical rotations were taken at 25° at a concentration of 1% in the given solvent.

(a) Peptides Soluble in Water

The appropriate N-carbobenzoxydipeptide benzyl ester (0.01 mole) was dissolved in 30 ml of hot ethanol containing 1 ml of glacial acetic acid. Then a suspension of 0.3 g of palladium (10%) on carbon in 6 ml of water was added. This hot solution was hydrogenolized at low pressure during 6 hours in a Parr apparatus, after which time the solution was heated if necessary to entirely dissolve the peptide, and the catalyst was removed by filtration. The clear filtrate was evaporated to a few milliliters, and then was added a mixture of water and ether to dissolve respectively the free dipeptide and the unreacted protected dipeptide. If at this stage there remains an insoluble product it is the substituted diketopiperazine. Being insoluble in both these solvents it is easily removed by filtration and can be identified by melting point and chromatography. The ethereal extract was decanted and discarded, and the aqueous fraction concentrated to allow the peptide to crystallize in the cold after the addition of acetone.

(VIII) 1-Aminocyclopentanecarbonyl-L-phenylalalanine.—Yield: 2.0 g (72%), m.p. 278–280° (subl. 180°), from water–acetone, $[\alpha]_D$ −2.0° (in water), R_f 0.64. Anal. Calc. for $C_{15}H_{20}N_2O_3$: N, 10.13%; mol. wt., 276. Found: N, 10.20%; mol. wt., 277.

(XIX) 1-Aminocyclohexanecarbonyl-L-phenylalanine.—Yield: 2.0 g (69%), m.p. 278–280° (subl. 180°), from water–acetone, $[\alpha]_D$ −1.3° (in water), R_f 0.67. Anal. Calc. for $C_{16}H_{22}N_2O_3$; N, 9.63%; mol. wt., 290. Found: N, 9.60%; mol. wt., 296.

(X) 1-Aminocyclopentanecarbonyl-1-aminocyclopentanecarboxylic acid.—Yield: 1.6 g (66%). The compound sublimates at 185° and does not decompose below 300°. It was recrystallized from water–acetone and had a R_f value of 0.54. Anal. Calc. for $C_{12}H_{20}N_2O_3$: N, 11.65%; mol. wt., 240. Found: N, 11.60%; mol. wt., 243.

(b) Peptides Insoluble in Water

The N-carbobenzoxydipeptide benzyl ester (0.01 mole) was dissolved in 20 ml of hot ethanol, and then 3.0 ml of 3.5 *N* hydrochloric acid and 0.3 g of palladium (10%) on carbon were added. This solution was hydrogenolized as described in the preceding part. After the water–ether treatment, there was no insoluble compound. The ethereal fraction was discarded and the water fraction neutralized with 3.5 *N* sodium hydroxide. The precipitated peptide was then filtered and washed with a little cold water, and recrystallized with the appropriate solvent system.

(XI) 1-(L-Leucylamino)cyclopentanecarboxylic acid.—Yield: 1.9 g (78%), m.p. 291–292° (subl.), from methanol–water, $[\alpha]_D$ +10.0° (in methanol), R_f 0.50. Anal. Calc. for $C_{12}H_{22}N_2O_3$: N, 11.54%; mol. wt., 242. Found: N, 11.45%; mol. wt., 241.

(XII) 1-(L-Leucylamino)cyclohexanecarboxylic acid.—Yield: 2.1 g (82%), m.p. 295–297° (subl.), from methanol–water, $[\alpha]_D$ +22.5° (in methanol), R_f 0.60. Anal. Calc. for $C_{13}H_{24}N_2O_3$: N, 10.92%; mol. wt., 256. Found: N, 10.87%; mol. wt., 260.

(XIII) 1-(L-Phenylalanylamino)cyclopentanecarboxylic acid.—Yield: 2.0 g (72%), m.p. 284–285° (subl. 180°), from aqueous ethanol 60%, $[\alpha]_D$ +57° (in glacial acetic acid), R_f 0.61. Anal. Calc. for $C_{15}H_{20}N_2O_3$: N, 10.13%; mol. wt., 276. Found: N, 9.98%; mol. wt., 279.

(XIV) 1-(L-Phenylalanylamino)cyclohexanecarboxylic acid.—Yield: 2.2 g (75%), m.p. 278° (subl. 180°),

from aqueous ethanol 60%, $[\alpha]_D$ +46° (in glacial acetic acid), R_f 0.63. Anal. Calc. for $C_{16}H_{22}N_2O_3$: N, 9.63%; mol. wt., 290. Found: N, 9.59%; mol. wt., 294.

Cyclization of Dipeptides into Substituted Diketopiperazines

The dipeptides (0.002 mole) were heated during 3 hours at 145° in 4 g of β-naphthol according to Lichtenstein (14).

The yellowish solutions so obtained were cooled and then thoroughly washed with four portions of 15 ml of ether to remove the β-naphthol. The insoluble 2,5-piperazinediones were crystallized with the appropriate solvent. The optical rotations were taken at 25° at a concentration of 0.2% in glacial acetic acid.

(XV) 1,4-Diazaspiro[4.5]decane-2,5-dione-3-benzyl.—(*a*) From 1-aminocyclopentanecarbonyl-L-phenylalanine: Yield: 0.41 g (79%), m.p. 287–289° (subl. 180°), from *n*-butanol, $[\alpha]_D$ +58°, R_f 0.90. Anal. Calc. for $C_{15}H_{18}N_2O_2$: N, 10.83%. Found: N, 10.78%. (*b*) From 1-(L-phenylalanylamino)cyclopentanecarboxylic acid: Yield: 0.42 g (80%), m.p. 289° (subl. 180°), from *n*-butanol, $[\alpha]_D$ +60°, R_f 0.91. Anal. Calc. for $C_{15}H_{18}N_2O_2$: N, 10.83%. Found: N, 10.87%.

(XVI) 1,4-Diazaspiro[5.5]undecane-2,5-dione-3-benzyl.—(*a*) From 1-aminocyclohexanecarbonyl-L-phenylalanine: Yield: 0.43 g (80%), m.p. 289–291° (subl. 180°), from *n*-butanol, $[\alpha]_D$ +59°, R_f 0.91. Anal. Calc. for $C_{16}H_{20}N_2O_2$: N, 10.28%. Found: N, 10.40%. (*b*) From 1-(L-phenylalanylamino)cyclohexanecarboxylic acid: Yield: 0.43 g (80%), m.p. 290–291° (subl. 180°), from *n*-butanol, $[\alpha]_D$ +56°, R_f 0.92. Anal. Calc. for $C_{16}H_{20}N_2O_2$: N, 10.28%. Found: N, 10.38%.

(XVII) 6,13-Diazaspiro[4.2.4.2]tetradecane-7,14-dione.—Yield: 0.36 g (82%), from 1-aminocyclopentanecarbonyl-1-aminocyclopentanecarboxylic acid. The compound, recrystallized from 75% acetic acid, began to sublime at 185°, and decomposed above 300°, R_f 0.92. Anal. Calc. for $C_{12}H_{18}N_2O_2$: N, 12.62%. Found: N, 12.70%.

(XVIII) 1,4-Diazaspiro[4.5]decane-2,5-dione-3-isobutyl.—Yield: 0.35 g (77%), from 1-(L-leucylamino)-cyclopentanecarboxylic acid, m.p. 289–293° (subl.), from *n*-butanol, $[\alpha]_D$ −10°, R_f 0.92. Anal. Calc. for $C_{12}H_{20}N_2O_2$: N, 12.51%. Found: N, 12.56%.

(XIX) 1,4-Diazaspiro[5.5]undecane-2,5-dione-3-isobutyl.—Yield: 0.38 g (80%), from 1-(L-leucylamino)-cyclohexanecarboxylic acid, $[\alpha]_D$ −20°, R_f 0.92. Anal. Calc. for $C_{13}H_{22}N_2O_2$: N, 11.76%. Found: N, 11.85%.

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RÉSUMÉ

On décrit la synthèse de six nouveaux dipeptides non-naturels. Ils contiennent d'une part un acide aminé de synthèse, soit l'acide amino-1 cyclopentane carboxylique, soit l'acide amino-1 cyclohexane carboxylique et, d'autre part, un acide aminé naturel, soit la L-phénylalanine, soit la L-leucine. On rapporte aussi la synthèse d'un dipeptide contenant deux molécules d'acide amino-1 cyclopentane carboxylique. Ces sept peptides furent cyclisés en pipérazinedione-2,5 substituées.

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