PREPARATION OF FRAGMENTS OF BRADYKININ ANALOGUES CONTAINING OPTICALLY ACTIVE PIPECOLIC ACID

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Some peptides containing optically active L- and D-pipecolic acid, as fragments of pipecolic acid-bradykinin analogues, were prepared and their physical constants were determined.

For investigating the connections between chemical structure and biological activity of known peptide hormones, preparation and study of the biological properties of analogues containing one or several "non-proteinogenic" amino acids is of great importance [1]. The well-known tissue hormone bradykinin (arginyl-prolyl-prolyl-glicyl-phenylalanyl-seryl-prolyl-phenylalanyl-arginine) with complex effects contains three prolines. In order to clear up the biological role of the latter, it seems theoretically interesting to substitute one or more prolines by "non-proteinogenic" amino acids of only slightly different structure.

Among the numerous possibilities, our research group used optically active pipecolic acid* and α-L-homoproline for the substitution of proline.

α-L-homoproline had not been applied earlier in peptide chemistry. Publication of some results concerning rational synthesis of α-L-homoproline, preparation of several protected and active derivatives and of α-L-homoproline-bradykinin elaborated in our group as well as of the results of biological investigations is in course [2].

Numerous papers on substitution of proline by pipecolic acid can be found in the literature of peptide chemistry. The first report on application of L- and D-pipecolic acid is found in connection with the synthesis of collagen models [3]. 7-L-Pipecolic acid-oxytocin [4] and 7-L-pipecolic acid-angiotensine II [5] were also prepared, the latter by the method of solid phase peptide synthesis [6]. L-Pipecolic acid was applied for preparing a sequence-polypeptide [7], too.

The synthesis of further analogues with conventional [9] and solid phase peptide synthesis methods [10] followed that of the first pipecolic acid-bradykinin [8] only about ten years later.

* Nomenclature and abbreviations are those accepted by IUPAC-IUB for peptide chemistry: Pip = pipecolic acid (piperidine-2-carboxylic acid); HPro = α-homoproline (pyrrolidine-2-acetic acid). Z = benzyloxycarbonyl; BOC = *t*-butyloxycarbonyl; ONb = *p*-nitrobenzyl; OMe = methyl.

In order to fill up gaps in literature, special attention was paid to peptide chemical application of optically active pipecolic acids. Therefore the preparation of different protected and active derivatives of both L- and D-pipecolic acid [11, 12] and possibilities of their applications [13] were published in detail.

In our preceding paper [14] we reported on preparation of some di- and tripeptides containing L- and D-pipecolic acids by the stepwise method. In this paper we describe the preparation of greater fragments, namely tetra-, penta-, hexa-, hepta- and octapeptides. A publication on the synthesis of pipecolic acid-bradykinin analogues from these fragments, on their purification and biological assays is under preparation [15].

The synthesis of each fragment described (Table I) started from the C-terminal tripeptides [14] and proceeded by stepwise condensation (**1—6**) and (**9—13**), acylating with an active derivative of the respective protected amino acid. For hexapeptides (**7** and **8**), fragment condensation starting from tripeptides was applied. For preparation of tetrapeptides (**3** and **4**) azide coupling without isolation, for the penta- and hexapeptides (**3—6**) the N-hydroxysuccinimid esters of the protected acyl components were used. Coupling of the tripeptide fragments to hexapeptides (**7** and **8**) was performed by the azide method. Acylation of heptapeptides (**9** and **10**) to octapeptides could be succesfully performed only by the dicyclohexylcarbodiimid method. In the latter case the active ester methods did not prove suitable; an exact explanation for this fact cannot be given up to now.

Experimental

Melting points were determined with a Kofler block, optical rotations with a Zeiss polarimeter. The values given are uncorrected. TLC on Kiesel G (Merck) was used for purity control with the following systems:

1. *n*-butanol—acetic acid—water 4:1:1
2. ethyl acetate—pyridine—acetic acid—water 60:20:6:11
3. ethyl acetate—pyridine—acetic acid—water 30:20:6:11
4. ethyl acetate—pyridine—acetic acid—water 120:20:6:11
5. chloroform—methanol 8:2
6. chloroform—methanol—acetic acid 85:10:5

p-Nitrobenzyl t-butyloxycarbonyl-L-seryl-D-pipecolyl-L-phenylalanyl--L-nitroargininate (**1**)

5.34 g (7.5 mmole) protected tripeptide ester [14] was treated with slight excess of trifluoroacetic acid at room temperature for 1 hour. The excess acid was evaporated in vacuum, and the trifluoroacetate of the tripeptide ester was precipitated with ether, then filtered, washed with ether several times and dried in vacuum. 1.64 g (7.5 mmole) *t*-butyloxycarbonyl-L-serine hydrazide was dissolved in 10 ml dimethylformamide and the solution cooled to -15°C. 3.75 ml 6*N* hydrochloric acid and concentrated aqueous solution of 0.54 g sodium nitrite were added dropwise to the solution under stirring, and stirred for further 5 minutes. The trifluoroacetate of the tripeptide ester was dissolved similarly in 10 ml dimethylformamide, cooled to -15°C, then

0.83 ml (7.5 mmole) N-methylmorpholine was added dropwise to the solution and stirred for 3 minutes. The azide solution was added to the latter solution and the reaction mixture was stirred under $-5°C$ for 2 hrs, then left to stand in a refrigerator overnight. After pouring the reaction mixture into water, the organic phase was extracted with ethyl acetate, the extract washed with diluted solutions of citric acid and sodium hydrocarbonate, then with 1*N* ammonium hydroxide and water. After drying, the extract was evaporated in vacuum and the residue crystallized.

Methyl t-butyloxycarbonyl-L-phenylalanyl-L-seryl-L-pipecolyl- -L-phenylalanyl-L-nitroargininate (**4**)

2.41 g (4 mmole) protected tetrapeptide ester (**2**) was treated with slight excess of trifluoroacetic acid at room temperature for 1 hour. The excess acid was evaporated in vacuum, and the trifluoroacetate of the tetrapeptide ester precipitated with ether, then filtered, washed with ether and dried in vacuum. The dried product was dissolved in 20 ml dimethylformamide, the solution cooled to 0°C, then equivalent quantity of N-methylmorpholine and finally 1.45 g (4 mmole) N-hydroxysuccinimide *t*-butyloxycarbonyl-L-phenylalaninate were added. The reaction mixture was stirred at the same temperature for 2 hrs, then left to stand at room temperature for 24 hrs. After pouring the reaction mixture into water, the organic phase was extracted with ethyl acetate, the extract washed with diluted solutions of citric acid and sodium hydrocarbonate, then with 1*N* ammonium hydroxide and water. The extract was dried, evaporated in vacum, and the residue was crystallized.

p-Nitrobenzyl t-butyloxycarbonyl-glycyl-L-phenylalanyl-L-seryl-D-pipecolyl- -L-phenylalanyl-L-nitroargininate (**5**)

3.79 g (4 mmole) protected pentapeptide ester (**3**) was treated in slight excess of trifluoroacetic acid at room temperature for 1 hour. The excess acid was evaporated in vacuum, and the trifluoroacetate of the pentapeptide ester precipitated with ether, then filtered, washed with ether and dried in vacuum. The dried product was dissolved in 15 ml dimethylformamide, the equivalent quantity of N-methylmorpholine was added and the solution cooled to 0°C. Finally 1.18 g (4 mmole) N-hydroxysuccinimide *t*-butyloxycarbonyl-glycynate was given to the reaction mixture and worked up as above (**4**).

Methyl benzyloxycarbonyl-glycyl-L-phenylalanyl-L-seryl-L-pipecolyl- -L-phenylalanyl-L-nitroargininate (**7**)

1.87 g (3 mmole) methyl benzyloxycarbonyl-L-pipecolyl-L-phenylalanyl-L-nitroargininate [14] was dissolved in excess 4*N* hydrogen bromide—acetic acid, stirred at 0°C for 45 minutes and the hydrobromide of the tripeptide ester was precipitated with ether, filtered, washed with ether and dried in vacuum over P_2O_5 and potassium hydroxide. The dried product was dissolved in 10 ml dimethylformamide, the solution cooled to $-15°C$ and neutralized with N-methylmorpholine. At the same time, 1.37 g (3 mmole) benzyloxycarbonyl-glycyl-L-phenylalanyl-L-serine hydrazide was dissolved in 6 ml dimethylformamide, the solution cooled to $-15°C$, then 1.5 ml 6*N* hydrochloric acid and 0.22 g cc. aqueous sodium nitrite

Table I

Physical properties and analytical data of the synthesized peptides[1]

Peptide	Method[2]	Crystallized from[3]	Yield %	M. p. °C	$[\alpha]_D^{25}$ c = 1 DMF	Formula	Analysis, % Calc. N	Found N	
BOC-Ser-D-Pip-Phe-Arg(NO_2)-ONb (**1**)	A	a	57	102—105	−32°	$C_{36}H_{49}O_{12}N_9$	15.7	15.6	
BOC-Ser-Pip-Phe-Arg(NO_2)OMe (**2**)	A	a	64	92—97	−30°	$C_{30}H_{46}O_{10}N_8$	16.5	16.3	
BOC-Phe-Ser-D-Pip-Phe-Arg(NO_2)-ONb (**3**)	A	a	88	113—116	−20°	$C_{45}H_{58}O_{13}N_{10}$	14.8	14.7	
BOC-Phe-Ser-Pip-Phe-Arg(NO_2)-OMe (**4**)	A	a	77	95—98	−38°	$C_{39}H_{56}O_{11}N_9$	15.3	15.3	
BOC-Gly-Phe-Ser-D-Pip-Phe-Arg(NO_2)-ONb (**5**)	A	b	81	114—118	−18°	$C_{47}H_{61}O_{14}N_{11}$	15.4	15.2	
BOC-Gly-Phe-Ser-Pip-Phe-Arg(NO_2)-OMe (**6**)	A	b	80	108—113	−33°	$C_{41}H_{58}O_{12}N_{11}$	15.9	15.7	
Z-Gly-Phe-Ser-Pip-Phe-Arg(NO_2)-OMe (**7**)	B	c	67	163—168	−35°	$C_{44}H_{56}O_{12}N_{10}$	15.3	15.2	
Z-Gly-Phe-Ser-Pip-Phe-Arg-(NO_2)-ONb (**8**)	B	c	73	164—170	−38°	$C_{50}H_{59}O_{14}N_{11}$	14.9	14.8	
BOC-Pro-Gly-Phe-Ser-D-Pip-Phe-Arg(NO_2)-ONb (**9**)	A	c	80	122—124	−30°	$C_{52}H_{68}O_{15}N_{12}$	[4] Arg 0.96, Phe 1.88,	Pro 0.85, Ser 0.85,	Pip 0.88 Gly 1.0
BOC-Pip-Gly-Phe-Ser-Pip-Phe-Arg(NO_2)-OMe (**10**)	A	c	78	121—124	−40°	$C_{47}H_{67}O_{13}N_{11}$	Arg 0.95, Phe 1.88,	Ser 0.8,	Pip 1.8 Gly 1.0
BOC-Pro-Pro-Gly-Phe-Ser-D-Pip-Phe-Arg(NO_2)-ONb (**11**)	A	d	56	128—132	−31°	$C_{57}H_{75}O_{16}N_{13}$	Arg 0.96, Phe 1.87,	Pro 1.92, Ser 0.85,	Pip 0.93 Gly 1.0
BOC-Pro-Pip-Gly-Phe-Ser-Pip-Phe-Arg(NO_2)-OMe (**12**)	A	d	55	126—130	−48°	$C_{52}H_{74}O_{14}N_{12}$	Arg 0.98, Phe 1.87,	Pro 0.97, Ser 0.86,	Pip 1.84 Gly 1.0
BOC-D-Pip-Pro-Gly-Phe-Ser-Pro-Phe-Arg(NO_2)-ONb (**13**)	A	d	58	127—131	−32°	$C_{57}H_{75}O_{16}N_{13}$	Arg 0.96, Phe 1.86,	Pro 1.9, Ser 0.84,	Pip 0.9 Gly 1.0

[1] EtOAc = ethyl acetate, EtOH = ethanol, MeOH = methanol, Et_2O = ether, P. E. = petroleum ether
[2] A = stepwise condensation, B = fragment condensation
[3] a = EtOAc-P. E., b = EtOH/Et_2O, c = MeOH/Et_2O, d = acetone/Et_2O
[4] amino-acid analysis was made instead of elemental analysis

solution was added dropwise. After further stirring for 5 minutes the azide solution was added to the solution of the amino-component and the reaction mixture was stirred under −5°C for 3 hrs. After 24 hrs standing in a refrigerator, the solution was eveporated in vacuum and the residue suspended in 1:1 ethyl acetate—water. The organic phase was washed with diluted hydrochloric acid, diluted sodium carbonate solution and water. After drying and evaporating the ethyl acetate solution in vacuum, the residue was crystallized.

p-Nitrobenzyl t-butyloxycarbonyl-L-prolyl-glycyl-L-phenylalanyl-L-seryl--D-pipecolyl-L-phenylalanyl-L-nitroargininate (**9**)

2.5 g (2.5 mmole) protected hexapeptide ester (**5**) was treated in slight excess of trifluoroacetic acid at room temperature for 1 hour. The excess acid was evaporated in vacuum, the trifluoroacetate of the hexapeptide ester precipitated with ether, then filtered and washed with ether. The product was dissolved in 8 ml dimethylformamide, the solution cooled under 0°C and neutralized with N-methylmorpholine added dropwise. After adding 0.78 g (2.5 mmole) N-hydroxysuccinimide *t*-butyloxycarbonyl-L-prolinate the reaction mixture was worked up as described for **1**.

Methyl t-butyloxycarbonyl-L-prolyl-L-pipecolyl-glycyl-L-phenylalanyl--L-seryl-L-pipecolyl-L-phenylalanyl-L-nitroargininate (**12**)

0.91 g (1 mmole) protected heptapeptide ester (**10**) was treated with slight excess of trifluoroacetic acid at room temperature for 1 hour. The excess acid was evaporated in vacuum, the remaining trifluoroacetate of the heptapeptide ester precipitated with ether, filtered, washed with ether and dissolved in 5 ml dimethylformamide. The solution was cooled to 0°C and, after adding 0.14 ml triethylamine, the precipitated triethylamine hydrochloride was filtered. 0.12 g (1.0 mmole) *t*-butyloxycarbonyl-L-proline and 0.2 g (10 mmole) dicyclohexylcarbodiimide were added to the filtrate and the reaction mixture was stirred at the same temperature for 6 hrs, then left to stand in a refrigerator for 24 hrs. After filtering off the dicyclohexylurea, the filtrate was evaporated in vacuum, the residue dissolved in acetone and the protected octapeptide ester was crystallized by addition of ether.

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СИНТЕЗ ФРАГМЕНТОВ АНАЛОГОВ БРАДИКИНИНА, СОДЕРЖАЩИХ ОПТИЧЕСКИ АКТИВНУЮ ПИПЕКОЛИНОВУЮ КИСЛОТУ

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Авторами синтетизированы и определены физические константы нескольких пептидов, содержащих «L» и «D» пипеколиновую кислоту, которые соответствуют фрагментам аналогов брадикинина с пипеколиновой кислотой.