PREPARATION OF SOME DI- AND TRIPEPTIDES CONTAINING OPTICALLY ACTIVE PIPECOLIC ACID AS FRAGMENTS OF PIPECOLIC ACID-BRADYKININ ANALOGUES

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Some di- and tripeptides of L- and D-pipecolic acid, fragments of the corresponding pipecolic acid-bradykinin analogues, are described.

Changes in the structure of known peptide hormones by substituting amino acids of similar character for the constituting amino acids are very important for the investigation of the connections between hormone structure and biological activity [1]. Among the amino acids substituted several "non-proteinogenic" amino acids [2] are found, mainly as corresponding homologues of "proteinogenic" amino acids.

Proline is an important constituent of several biologically active peptide hormones. Three of the nine amino acids contained in the well-known tissue hormone Bradykinin (I) are prolines:

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
(I)

Thus it is easy to see that the use of proline homologues in peptide chemistry may yield much valuable information. The most important homologues to be considered in their optically active form are pipecolic acid*, azetidine-2-carboxylic acid, oxazolidine-4-carboxylic acid, thiazolidine-4-carboxylic acid and α-homoproline.

According to literature, oxazolidine-4-carboxylic acid has not been used in peptide chemistry owing to various circumstances, and only in three instances [3, 4, 17c] the use of azetidine-2-carboxylic acid is found; three papers [3—5] were published on the substitution of thiazolidine-4-carboxylic acid, while α-homoproline was applied only most recently, after a suitable way of synthesis had been elaborated [6].

* The abbrevations are those accepted by IUPAC—IUB for peptide chemistry: Pip = pipecolic acid (piperidine-2-carboxylic acid); Aze = azetidine-2-carboxylic acid; Thz = thiazolidine-4-carboxylic acid; Oxz = oxazolidine-4-carboxylic acid; HPro = α-homoprolin (pyrrolidine-2-acetic acid). Z = benzyloxycarbonyl; Boc = *t*-butyloxycarbonyl; ONb = *p*-nitrobenzyl.

Pipecolic acid was applied more frequently than the above amino acids, mainly in connection with the preparation of the corresponding analogues. The first papers on this topic were concerned with collagen models [7] and 3-L-pipecolic acid-bradykinin [8]. Then the synthesis of 7-L-pipecolic acid-oxytocin [9] followed. All these publications give very little information on the synthetic work, therefore it seemed justified to elaborate a suitable resolution method for preparing optically active pipecolic acids and derivatives suitable for peptide chemical purposes [10]. At the same time with our paper [10a] the first use of pipecolic acid in solid phase peptide synthesis for preparing 7-L-pipecolic acid-angiotensine II was described [11]. In the last years, several papers concerning the use of pipecolic acid for preparing sequence-polypeptides [3] as well as pipecolic acid-bradykinin analogues, both with solid-phase and conventional peptide synthesis [12, 13], are found in literature. In the total synthesis of an analogue of the antibiotic Penicillin-Cephalosporin, too [14], an optically active pipecolic acid derivative was used. Most recently, isolation and structure determination of Actinomycin [15] and Amphomycin [16] also points to the presence of optically active pipecolic acids. The structure supporting syntheses are to be expected in the next future.

During the synthetic work aiming at the preparation of pipecolic acid-bradykinin analogues, numerous di- and tripeptides containing pipecolic acids were prepared in our research group. The present paper wishes to review the latter and to compare them with the results other authors of, published after our work had been finished [17a—c].

The dipeptides containing L- and D-pipecolic acids were prepared (Table I) with the mixed anhydride method [18] to obtain protected dipeptide esters (**1**—**4**), or by acylating the salt of the amino component with the N-hydroxysuccinimid esters of the protected acyl component [19] to form protected dipeptides (**5**—**7**). For comparison, in the case of two protected dipeptide esters (**1** and **2**) the corresponding protected dipeptides were prepared by alkaline hydrolysis (**6** and **7**), too. From the corresponding dipeptide derivatives the C-terminal (**8**—**11**) and N-terminal (**12**—**13**) protected tripeptide esters or protected tripeptides (Table II) used for the synthesis of the pipecolic acid-bradykinin analogues were also prepared with the mixed anhydride method. The preparation of greater fragments obtained from the latter or with other methods will be described in a subsequent paper [20]

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. chloroform—methanol 8:2
4. chloroform—methanol—acetic acid 85:10:5.

Methyl benzyloxycarbonyl-L-pipecolyl-L-pipecolate (**1**). 14.48 g (55 mmole) benzyloxycarbonyl-L-pipecolic acid, solved in 75 ml chloroform, was cooled to −15°C. 6.05 ml (55 mmole) N-methylmorpholine and 5.28 ml (55 mmole) ethyl chloroformate was added dropwise to the cooled solution. After three minutes stirring 9.98 g (50 mmole) methyl L-pipecolinate hydrochloride and 5.55 ml (50 mmole)

N-methylmorpholine, both solved in 75 ml chloroform and cooled, were added to the reaction mixture and the stirring continued at −5°C for 3 hrs, then at room temperature for 2 hrs. After standing in a refrigerator overnight, the small amount of precipitated N-methylmorpholine hydrochloride was filtered off, the solution evaporated and the rest solved in ethyl acetate. The solution was washed with diluted sodium hydrocarbonate, diluted hydrochloric acid, then with water. After drying, the ethyl acetate solution was evaporated to obtain an oil, which was crystallized.

Benzyloxycarbonyl-L-pipecolyl-D-pipecolic acid (**7**). 7.2 g (20 mmole) N-hydroxysuccinimide benzyloxycarbonyl-L-pipecolate and 3.25 g (25 mmole) D-pipecolic acid were solved in a mixture of 15 ml water and 15 ml pyridine. The reaction mixture was brought to pH 8.8 by adding 4*N* sodium hydroxide under continuous stirring and held at the same pH for 3 hrs. Then the solution was adjusted to pH 8.0 by adding 4*N* hydrochloric acid, saturated with solid sodium hydrocarbonate and extracted with ethyl acetate. The aqueous solution was acidified to pH 2.0 with 4*N* hydrochloric acid and the precipitate solved in ethyl acetate, washed with water and dried, the ethyl acetate evaporated in vacuum and the oil obtained was crystallized.

p-Nitrobenzyl t-butyloxycarbonyl-D-pipecolyl-L-phenylalanyl-L-nitroargininate (**10**). Trifluoroacetate of *p*-nitrobenzyl-L-phenylalanyl-L-nitroargininate, obtained from the protected compound with trifluoroacetic acid, was solved in 30 ml dimethylformamide and 1.33 ml (12 mmole) N-methylmorpholine was added to the solution. Then, after 1 minute, 3.26 g (10 mmole) N-hydroxysuccinimide *t*-butyloxycarbonyl-D-pipecolinate was added under stirring. After 24 hrs stirring at room temperature the reaction mixture was poured in water and the suspension obtained solved in ethyl acetate, the cooled ethyl acetate solution rapidly washed with diluted hydrochloric acid, diluted sodium hydrocarbonate and water. After drying and evaporating, an oil was obtained, which was crystallized.

Benzyloxycarbonyl-L-nitroarginyl-L-pipecolyl-L-pipecolic acid (**13**). 4.2 g (12 mmole) benzyloxycarbonyl-L-nitroarginine was solved in 15 ml dimethylformamide, the solution cooled under −15°C, then 1.33 ml (12 mmole) N-methylmorpholine and 1.15 ml ethyl chloroformate were added dropwise. After 3 minutes, the cooled solution of dipeptide hydrochloride ($R_f(1)=0.5$, $R_f(3)=0.35$) was added to the reaction mixture. The dipeptide hydrochloride solution was prepared in advance from 3.74 g (10 mmole) benzyloxycarbonyl-L-pipecolyl-L-pipecolic acid (**6**), solved in hydrochloric acid—metanol, by hydrogenation in the presence of Pd-C catalyst, then solving in 15 ml dimethylformamide and adding 1.11 ml (10 mmole) N-methylmorpholine. The reaction mixture was stirred at a temperature lower than −5°C for 3 hrs and held in a refrigerator overnight. The solution was evaporated in vacuum and the remaining oil digerated with diluted sodium hydrocarbonate and 0.1*N* hydrochloric acid, filtered, washed several times with diluted sodium hydrocarbonate solution, diluted hydrochloric acid, water and finally with ether, then crystallized.

* * *

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Table I

*Physical properties of dipeptides**

Peptide		Method	Crystallized from	Yield %	M. p. °C	$[\alpha]_D^{25}$ c=1, DMF	Analysis (%) Calculated / Found C	H	N
Z-L-Pip-L-Pip-OMe	(1)	MA	EtOH—petroleum ether	69	67—70	−62	64.9 64.9	7.3 7.3	7.2 7.1
Z-L-Pip-D-Pip-OMe**	(2)	MA	EtOH—petroleum ether	78	56—59	+16	64.9 64.8	7.3 7.3	7.2 7.0
Z-D-Pip-L-Pip-OMe	(3)	MA	EtOH—petroleum ether	75	66—68	+18	64.9 64.8	7.3 7.3	7.2 7.1
Z-D-Pip-D-Pip-OMe***	(4)	MA	EtOH—petroleum ether	81	64—66	+64	64.9 64.8	7.3 7.3	7.2 7.0
Z-L-Pip-L-Pro-OH	(5)	SuOH	MeOH	68	170—173	−72	63.3 63.1	6.7 6.7	7.8 7.6
Z-L-Pip-L-Pip-OH	(6)	SuOH hydrolysis	MeOH MeOH	60 93	172—174 171—174	−58 −57	64.1 64.0 63.8	7.0 6.9 6.9	7.5 7.5 7.4
Z-L-Pip-D-Pip-OH	(7)	SuOH hydrolysis	MeOH MeOH	65 95	170—173 170—173	+16 +16	64.1 64.0 64.1	7.0 6.9 6.9	7.5 7.3 7.4

* EtOH = ethanol; MeOH = methanol; SuOH = N-hydroxysuccinimide activated ester; MA = mixed anhydride; hydrolysis-ester hydrolysis

** [17] DCCl method, oil, $[\alpha]_D^{25} = +22.6°$C (c=0.15, in methanol). Found: C: 64.70 H: 6.82, N: 7.47%

*** [17] DCCl method, m.p. 61—62.5°C, $[\alpha]_D^{25} = +76.6°$ (c=0.4, in methanol). Found: C: 64.8, N: 7.34, N: 7.02%

Table II

*Physical properties of tripeptides**

Peptide		Method	Crystallized from	Yield %	M. p. °C	$[\alpha]_D^{25}$ c=1, DMF	Analysis (%) Calculated / Found C	H	N
Boc-L-Pip-Phe-Arg(NO_2)-OMe	**(8)**	SuOH	EtOAc—petroleum ether	83	77—83	−23	54.9 55.2	6.9 6.9	16.5 16.3
Boc-D-Pip-Phe-Arg(NO_2)-ONb	**(9)**	SuOH	EtOAc—petroleum ether	82	76—84	−5	55.9 55.6	6.2 6.1	15.7 15.5
Z-L-Pip-Phe-Arg(NO_2)-OMe	**(10)**	SuOH	EtOH—petroleum ether	78	130—137	−26	57.6 57.5	6.3 6.2	15.7 15.4
Z-L-Pip-Phe-Arg(NO_2)-ONb	**(11)**	SuOH MA	EtOAc—ether EtOAc—ether	70 72	114—117 113—117	−14 −14	58.0 58.0 57.8	5.7 5.7 5.6	15.1 15.0 15.0
Z-Arg(NO_2)-L-Pip-Pro-OH	**(12)**	MA	MeOH—ether	61	72—77	−20			16.6 16.5
Z-Arg(NO_2)-L-Pip-L-Pip-OH	**(13)**	MA	MeOH—ether	55	74—86	−16			16.2 16.0

*EtOAc=ethyl acetate

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СИНТЕЗ ДИ- И ТРИПЕПТИДОВ В СОСТАВЕ НЕСКОЛЬКИХ МОЛЕКУЛ ОПТИЧЕСКИ АКТИВНОЙ ПИПЕКОЛИНОВОЙ КИСЛОТЫ, КОТОРЫЕ СООТВЕТСТВУЮТ ФРАГМЕНТАМ АНАЛОГОВ БРАДИКИНИНА С ПИПЕКОЛИНОВОЙ КИСЛОТОЙ

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Авторы сообщают синтез и физические константы ди- и трипептидов, в составе нескольких молекул пипеколиновой кислоты „L" и „D", которые соответствуют фрагментам аналогов брадикинина с пипеколиновой кислотой.