Polish J. Chem., **76**, 1425–1431 (2002)

New Proctolin Analogues Modified in Position 2 or 3 of the Peptide Chain and Their Myotropic Effects in Insects*

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(Received May 13th, 2002; revised manuscript June 17th, 2002)

New analogues of insect neuromodulator proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) modified in position 2 and 3 of the peptide chain by natural or non-natural amino acid residues were synthesized. For modification of proctolin at position 2 a series of novel L-homophenylalanine derivatives H-Hpa(4-NO_2)-OH (**1**), Boc-Hpa(4-NO_2)-OH (**2**), Boc-Hpa(4-NH_2)-OH (**3**), Boc-Hpa(4-NH-Z)-OH (**4**), Boc-Phg(4-Me_2N)-OH (**5**) were obtained. The following two groups of proctolin analogues modified at positions 2 and 3such as: 1/ H-Arg-X^2-Leu-Pro-Thr-OH, where X^2 = Hpa (**6**), Hpa(4-NO_2) (**7**), Hpa(4-NH_2) (**8**), and Hpa(4-N-Me_2) (**9**), and 2/ H-Arg-Tyr-X^3-Pro-Thr-OH, where X^3 = Ile (**10**), Phe (**11**), Arg (**12**), Sar (**13**), Nva (**14**), Nle (**15**), Asn (**16**), Asp (**17**), Gln (**18**), Glu (**19**), Lys (**20**), and γ-Abu (**21**), were synthesized. Myotropic activity of proctolin analogues (**6**–**21**) was assayed *in vitro* on the semi-isolated heart of the yellow mealworm *Tenebrio molitor*. Analogues **10–12** and **20** retained more that 60% of proctolin activity. Other analogues showed a weak or none activity.

Key words: proctolin, proctolin analogues, insect peptide proctolin, new L-homophenylalanine derivatives

Continuing our studies [1,2] on the structure-function relationship of the insect neuropeptide proctolin, Arg-Tyr-Leu-Pro-Thr, we became interested in its analogues modified in position 2 and 3 of the peptide chain by natural or non-natural amino acid residues. The subject of these investigation was synthesis of the following two groups of proctolin analogues modified in positions 2 and 3 by such residues as: 1/ H-Arg-X^2-Leu-Pro-Thr-OH, where X^2 = Hpa (**6**), Hpa(4-NO_2) (**7**), Hpa(4-NH_2) (**8**) and Hpa(4-N,N-Me_2) (**9**), 2/ H-Arg-Tyr-X^3-Pro-Thr-OH, where X^3 = Ile (**10**), Phe (**11**), Arg (**12**) Sar (**13**), Nva (**14**), Nle (**15**), Asn (**16**), Asp (**17**), Gln (**18**), Glu (**19**), Lys (**20**), and γ-Abu (**21**). The aim of the synthesis of the mentioned proctolin analogues (**6**–**21**) was to explain the role of amino acid residues in position 2 and 3 for myotropic activity in insect.

Earlier structure-myotropic function studies on insects have prompted the syntheses of proctolin and many of its analogues [1,2]. The role of the aromatic acid resi-

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* The symbols of the amino acids, peptides, and their derivatives are in accordance with the Recommendation of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (1984) [*Eur. J. Biochem*., **138**, 9 (1984)] and *J. Pept. Sci*., **5**, 465 (1999).

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due in position 2 has been thoroughly discussed in the literature [1–7], whereas the role of Leu-3 in proctolin was not too amply discussed yet [1,2].

Among proctolin analogues modified at position 2, the Tyr residue was replaced by Phe [1,2,6] and Phg derivatives [7] modified in position 2 of the aromatic ring. Analogues modified in position 2 by 4′-substituted Phe-derivatives (in which the benzene ring was linked to the C-α atom through the methylene group) showed higher cardiostimulatory effects in insects than proctolin itself [1,2], whereas analogues containing L- or D-Phg or their 4′-substituted derivatives (amino acids which lack the methylene group between the C-α atom and the benzene ring) had weak or none myotropic activity in the heart of insects [7]. These results testified that the presence of the methylene group next to the benzene ring of the amino acid residue in position 2 of proctolin is essential for myotropic activity.

To explain the role of the methylene group in the side chain of the aromatic residues in position 2 for proctolin myotropic properties in insects we performed the synthesis of further analogues, where native Tyr-2 was replaced by Hpa and its derivatives (**6**–**9**). The L-homophenylalanine derivatives were chosen for modification of proctolin in position 2 of the peptide chain because they have two methylene groups between the C-α atom and the benzene ring.

Studies on the significance of Leu in position 3 of the proctolin skeleton for its myotropic function in insects may shed some light on the hydrophobic interaction of the proctolin molecule with its receptor site. In earlier studies a series of modified proctolin analogues was synthesized by replacement of Leu-3 by the natural or non natural amino acids [1,2,5]. Among tested analogues only [N-Me-Leu^3]-proctolin preserved almost full proctolin activity, whereas [Val^3]- and [Gly^3]-proctolin showed a weak activity [8]. Basing on these results a hypothesis was formulated that Leu-3 plays an essential role in the hydrophobic interaction of proctolin with its receptor. Moreover, it should be pointed out, that the Leu residue in proctolin is situated between the Tyr residue, which plays an important role in creating cardiotropic activity in insects [1,2], and the Pro residue, which is responsible for stabilization of the biologically active proctolin conformation [9].

These suppositions inspired us to perform further studies on the role of the Leu^3 residue in myotropic effect in insects. The subject of these studies was synthesis of further proctolin analogues modified at position 3 (**10**–**21**) by hydrophobic and hydrophilic amino acid residues.

EXPERIMENTAL

Chemical part: General procedures: Amino acid compositions were determined on an amino acid analyzer Mikrotechna T339 (Czechoslovakia). The optical activity of the chiral compounds was measured with a Jasco DIP-1000 polarimeter (±1.2°) (Jasco, Japan). The molecular weights of the peptides were determined using a Finigan Mat TSQ 700 (USA) mass spectrometer. The purity and homogeneity of all final products were checked by HPLC (Beckman Peptide Gold System) and TLC on silica gel plates, amino acid analysis, and molecular weight determinations. All peptides purity was about 100%.

Proctolin was obtained from Sigma Chemical Co. Ltd. All peptides (**6**–**21**) were synthesized by the classical solid-phase Boc procedure [6]. Dicyclohexylcarbodiimide (DCC) in the presence of HOBt (hydroxybenzotriazole) as a coupling reagent was used. The C-terminal amino acid derivative Boc-Thr (OBzl)-OH was connected to the chloromethylated classical Merrifield resin containing 0.8 mmol of Cl/g resin by standard caesium salt procedure. The protected amino acids were coupled using DCC method. The following amino acid derivatives were used: Boc-Arg(Tos)-OH, Boc-Leu-OH, Boc-Pro-OH, Boc-Thr(OBzl)-OH, Boc-Ile-OH, Boc-Phe-OH, Boc-Sar-OH, Boc-Nva-OH, Boc-Nle-OH, Boc-Asn-OH, Boc-Asp(OBzl)-OH, Boc-Gln-OH, Boc-Glu(OBzl)-OH, Boc-Lys(Z)-OH, and Boc-γ-Abu-OH (Bachem). A series of new Hpa derivatives: H-Hpa(4-NO_2)-OH (**1**), Boc-Hpa(4-NO_2)-OH (**2**), Boc-Hpa(4-NH_2)-OH (**3**), Boc-Hpa(4-NH-Z)-OH (**4**), and Boc-Hpa(4-N,N-Me_2)-OH (**5**) was synthesized (Table 1). The N-protected amino acids and DCC were used at the three fold excess. The N^{α}-Boc group was subsequently removed with 30% TFA in dichloromethane (DCM) according to standard methods. The neutralization was performed with 10% triethylamine (TEA) in DCM. Final peptides were obtained by deprotection and cleavage from the support resin with trifluoromethanesulfonic acid (TFMSA) in anisole. All free peptides were desalted with Amberlite CG-4B and then purified on a Sephadex G-10 column, with 5% acetic acid as eluent. Analytical RP-HPLC was conducted on a Beckman Peptide Gold System chromatograph with C-18, 5 μm Beckman column (ODS 4.6×250 mm), ultrasphere plus 4.6×4.5 mm precolumn. Solvent systems: S1 – 0.1% aqueous TFA, S2 – 80% acetonitrile; linear gradient from 0–100% of S2 for 60 min., flow rate 1.0 ml/min., determined at 223 nm. An isocratic system (18% acetonitrile) was also applied to check the purity.

Final purification was carried out by semi-preparative HPLC on an Alltech Econsil C-18, 10 μm column (ODS 22×250 mm), linear gradient 23–39% S2 for 15 min., flow rate 7 ml/min., determined at 223 nm.

Purity and homogeneity of the free peptides was established by amino acid analysis and determination of molecular weights and optical activity. The physico-chemical data of Hpa derivatives (**1**–**5**) are summarized in Table 1 and of free peptides (**6**–**21**) in Table 2.

Biological part: Peptides were bioassayed *in vitro* on the semi-isolated heart preparations of *T. molitor* according to the Rosiński and Gäde method [4] on adult males (7-day-old). The dose response relationship was established for each proctolin analogue (of separate determinations for 8 insects) (8.0 ± SEM) (Fig. 1).

L-4-Nitro-homophenylalanine (H-Hpa(4-NO_2)-OH) (1). The product was obtained in the manner presented for H-Phe(4-NO_2)-OH [11]. Hpa (40.0 g, 223.2 mmol) was dissolved in concentrated sulfuric acid (60 ml) and then concentrated nitric acid (14 ml) was added slowly dropwise with stirring at 0°C. The solution was mixed for 30 min. at 0–5°C, dropped to 400 ml of cold water and pH was adjusted to 6.5 with aqueous ammonia. The yellow product was filtered and crystallized from water. 29.7 g of the product were obtained. Physico-chemical data are presented in Table 1.

L-N^{α}-(*tert*-Butoxycarbonyl)-4-nitro-homophenylalanine (Boc-Hpa(4-NO_2)-OH) (2). Compound **1** (29.0 g, 129.3 mmol) was dissolved in 1 M NaOH (150 ml) and then water (100 ml) was added. 32.0 g (146.6 mmol) of Boc_2O were dissolved in 100 ml of dioxane and poured into the solution of compound **1**. The reaction was carried out according to [12]. After crystallization from diethyl ether-pentane (1:3), 33.6 g of the product were obtained. Physico-chemical data are presented in Table 1.

L-N^{α}-(*tert*-Butoxycarbonyl)-4-amino-homophenylalanine (Boc-Hpa(4-NH_2)-OH) (3). Compound **2** (15.0 g, 46.2 mmol) was dissolved in methanol (50 ml) and the nitro group was reduced by catalytic hydrogenation in the presence of 10% Pd/C (0.1 g) for 20 h. The reaction was carried out according to [12]. After crystallization from methanol-pentane (1:3), 11.0 g of the product were obtained. Physico-chemical data are presented in Table 1.

L-N^{α}-(*tert*-Butoxycarbonyl)-4-(N,N-dimethylamino)-homophenylalanine (Boc-Hpa(4-N,N-Me_2)-OH) (4). 7.0 g (21.6 mmol) of compound **2** were dissolved in 40 ml of methanol, 8 ml of 30% formaldehyde were added and the solution was hydrogenated in the presence of 10% Pd/C (0.1 g) for 40 h. The reaction was carried out according to [13]. Crystallization from diethyl ether-pentane (1:3) gave 5.0 g of the title product. Physico-chemical data are presented in Table 1.

L-N^{α}-(*tert*-Butoxycarbonyl)-4-(N-benzyloxycarbonyl)-homophenylalanine (Boc-Hpa(4-N-Z)-OH) (5). 5.0 g (17 mmol) of compound **3** were dissolved in 4 N NaOH (15 ml). Benzyl chlorocarbonate (2.5 ml, 17.4 mmol) and 4 N NaOH (10 ml) were added slowly dropwise. The reaction was carried out according to standard method. The yellow product was filtered and crystallized from acetone-water (1:1). 2.0 g of the product were obtained. Physico-chemical data are presented in Table 1.

Table 1. Physicochemical data of L-Hpa derivatives.

Compound		Yield (%)	M.p. [°C]	$[\alpha]_D^{20}$ c = 1% CH_3OH	Rt[a] (HPLC)	Analysis %C Calc.	%C Found	%H Calc.	%H Found	%N Calc.	%N Found	Mw Calc.	Mw Found	T.L.C.[b] Rf X	Y	W
H-Hpa(4-NO_2)-OH	(**1**)	59	228–230	+15.5*	20.5	53.57	53.7	5.36	5.4	12.5	12.5	224.2	225.0	0.27	0.62	0.59
Boc-Hpa(4-NO_2)-OH	(**2**)	78	98	+6.5	26.3	55.38	55.4	6.46	6.5	8.61	8.8	324.3	323.0	0.26	0.34	0.24
Boc-Hpa(4-NH_2)-OH	(**3**)	80	127–130	+16.3	24.1	61.2	61.4	7.5	7.5	9.5	9.8	294.2	293.4	0.76	0.83	0.72
Boc-Hpa-(4-N-Z)-OH	(**4**)	27	112–114	–5.6	34.8	64.5	64.5	6.5	6.5	6.5	6.5	428.2	427.2	0.85	0.82	0.16
Boc-Hpa-(4-N,N-Me_2)-OH	(**5**)	72	82–85	+8.1	26	63.3	63.5	8.1	8.0	8.7	9.9	322.2	321.0	0.41	0.85	0.43

[a]HPLC on Ultrasphere ODS column (Beckman) 4.5×250 mm S1 – 0.1% aqueous TFA, S2 – 80% acetonitrile in water; linear gradient: 0–100% of S2 in 60 min.
[b]T.L.C. on silica gel plates (Merck), eluents: X = n-butanol:Ac-OH:water (4:1:5), Y = n-butanol:pyridine:Ac-OH:water (30:20:6:24),
W = n-butanol:Ac-OH:ethyl acetate:water (1:1:1:1), *c = 1% in 50% in CH_3COOH.

Table 2. Physicochemical data of proctolin analogues modified in position 2 and 3 of the peptide chain.

Peptide		Yield (%)	$[\alpha]_D^{20}$ CH_3OH	Rt[a] (HPLC)	Amino acids analysis	Mw Calc.	Mw Found	T.L.C.[b] Rf X	Y	W	Z
H-Arg-Hpa-Leu-Pro-Thr-OH	(**6**)	45	–83.93 c = 0.3	27.30	Arg 1.12 Leu 0.9 Pro 0.98 Thr 1.0	646.8	647.0	0.10	0.76	0.54	0.13
H-Arg-Hpa(4-NO_2)-Leu-Pro-Thr-OH	(**7**)	56	–24.10 c = 1	20.42	Arg 1.1 Leu 0.9 Pro 0.9 Thr 1.1	691.8	692.0	0.04	0.63	0.48	–
H-Arg-Hpa(4-NH_2)-Leu-Pro-Thr-OH	(**8**)	34	–32.80 c = 0.5	17.15	Arg 0.9 Leu 1.0 Pro 1.1 Thr 1.0	661.8	662.7	–	0.53	0.41	0.16
H-Arg-Hpa-(4-N,N-Me_2)-Leu-Pro-Thr-OH	(**9**)	35	–15.60 c = 0.3	19.10	Arg 1.18 Leu 0.92 Pro 1.0 Thr 1.0	689.8	692.1	–	0.42	0.13	–
H-Arg-Tyr-Ile-Pro-Thr-OH	(**10**)	82	–12.87 c = 2	16.79	Arg 1.2 Tyr 0.9 Ile 0.9 Pro 1.0 Thr 1.0	648.8	649.3	0.1	0.48	0.43	0.12
H-Arg-Tyr-Phe-Pro-Thr-OH	(**11**)	83	+13.38 c = 0.6	19.20	Arg 1.1 Tyr 1.0 Phe 1.0 Pro 0.9 Thr 1.0	682.8	683.1	0.47	0.5	0.45	–
H-Arg-Tyr-Arg-Pro-Thr-OH	(**12**)	32	+21.62 c = 1.5	18.92	Arg 0.9 Tyr 1.0 Arg 0.9 Pro 1.1 Thr 1.1	691.8	690.4	0.39	0.45	0.43	0.2
H-Arg-Tyr-Sar-Pro-Thr-OH	(**13**)	84	–17.23 c = 0.1	16.53	Arg 0.9 Tyr 1.0 Sar 0.9 Pro 1.1 Thr 1.1	606.7	607.2	0.1	0.59	0.15	0.11
H-Arg-Tyr-Nva-Pro-Thr-OH	(**14**)	55	–14.58 c = 0.6	14.75	Arg 1.12 Tyr 0.98 Pro 0.9 Thr 1.0	634.7	635.0	0.45	0.54	0.39	0.21
H-Arg-Tyr-Nle-Pro-Thr-OH	(**15**)	47	–23.20 c = 0.4	16.71	Arg 0.9 Tyr 1.1 Pro 0.9 Thr 1.1	648.8	649.6	0.78	0.74	0.48	0.81
H-Arg-Tyr-Asn-Pro-Thr-OH	(**16**)	67	–41.18 c = 0.4	18.85	Arg 1.21 Tyr 0.89 Asn 0.9 Pro 1.1 Thr 1.0	649.7	650.0	0.1	0.59	0.15	0.11
H-Arg-Tyr-Asp-Pro-Thr-OH	(**17**)	76	–29.77 c = 5	11.80	Arg 1.1 Tyr 1.0 Asp 1.0 Pro 0.9 Thr 1.0	650.7	651.0	–	0.61	0.24	–
H-Arg-Tyr-Gln-Pro-Thr-OH	(**18**)	95	–39.50 c = 1	15.02	Arg 0.81 Tyr 0.81 Gln 1.0 Pro 1.09 Thr 1.09	663.7	664.0	–	0.58	0.36	0.71
H-Arg-Tyr-Glu-Pro-Thr-OH	(**19**)	78	–21.12 c = 1	13.47	Arg 1.13 Tyr 0.8 Glu 0.9 Pro 1.0 Thr 1.17	664.7	665.4	–	0.51	0.43	0.27
H-Arg-Tyr-Lys-Pro-Thr-OH	(**20**)	35	–24.60 c = 1	17.18	Arg 1.2 Tyr 0.9 Lys 0.9 Pro 1.0 Thr 1.0	663.8	664.8	0.27	0.54	0.40	0.15
H-Arg-Tyr-Abu-Pro-Thr-OH	(**21**)	87	–36.30 c = 1	13.05	Arg 0.8 Tyr 1.0 Abu 0.9 Pro 1.2 Thr 1.1	619.6	621.1	0.44	0.57	0.51	0.21

[a]HPLC on Ultrasphere ODS column (Beckman) 4.5×250 mm; solvent system: S1 – 0.1% aqueous TFA, S2 – 80% acetonitrile in water ; linear gradient: 0–100% of S2 in 60 min.
[b]T.L.C. on silica gel plates (Merck), eluents: X = n-butanol:Ac-OH:water (4:1:5), Y = n-butanol:pyridine:Ac-OH:water (30:20:6:24),
W = n-butanol:Ac-OH:ethyl acetate:water (1:1:1:1), Z = n-butanol:Ac-OH:water (4:1:1).

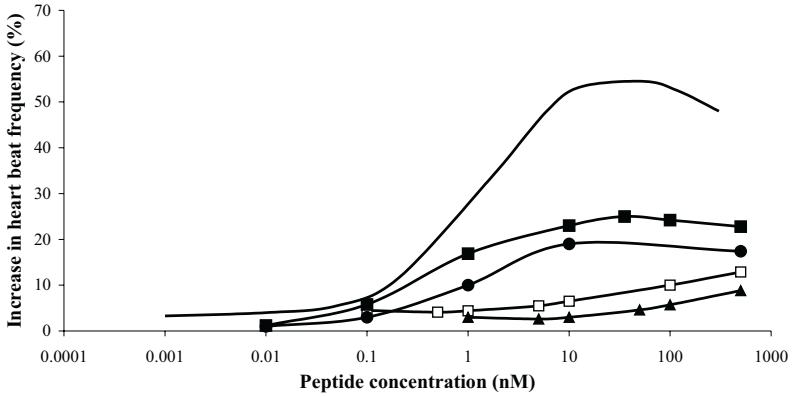

Figure 1. Cardioexitatory effect of proctolin and its analogues on *Tenebrio molitor* heart beat frequency (8.0±SEM): —— – proctolin, **A** – H-Arg-Hpa-Leu-Pro-Thr-OH (**6**), ▲ – H-Arg-Hpa(4-NO_2)-Leu-Pro-Thr-OH (**7**), ● – H-Arg-Hpa(4-NH_2)-Leu-Pro-Thr-OH (**8**), ■ – H-Arg-Hpa(4-N,N-Me_2)-Leu-Pro-Thr-OH (**9**).

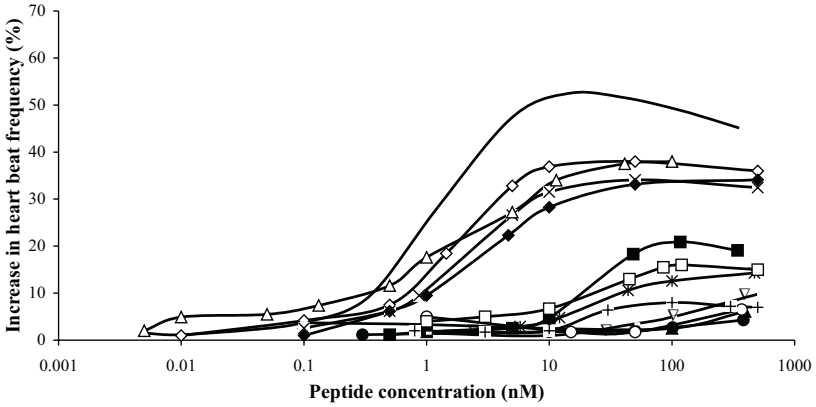

Figure 2. Cardioexitatory effect of proctolin and its analogues on *Tenebrio molitor* heart beat frequency (8.0 ±SEM): —— – proctolin, ◊ – H-Arg-Tyr-Ile-Pro-Thr-OH (**10**), ✻ – H-Arg-Tyr-Phe-Pro-Thr-OH (**11**), ◆ – H-Arg-Tyr-Arg-Pro-Thr-OH (**12**), ❍ – H-Arg-Tyr-Sar-Pro-Thr-OH (**13**), ▲ – H-Arg-Tyr-Nva-Pro-Thr-OH (**14**), ∇ – H-Arg-Tyr-Nle-Pro-Thr-OH (**15**), ■ – H-Arg-Tyr-Asn-Pro-Thr-OH (**16**), Δ – H-Arg-Tyr-Asp-Pro-Thr-OH (**17**), + – H-Arg-Tyr-Gln-Pro-Thr-OH (**18**), – H-Arg-Tyr-Glu-Pro-Thr-OH (**19**), × – H-Arg-Tyr-Lys-Pro-Thr- OH (**20**), ● – H-Arg-Tyr-γ-Abu-Pro-Thr-OH (**21**).

H-Arg-Hpa-Leu-Pro-Thr-OH (6). The peptide was obtained by a stepwise elongation of the peptide chain by the method outlined above. 1.5 g of Boc-Thr(OBzl)-resin (substitution level 1.08 mmol/g) was suspended in solution of 30% TFA in DCM. The mixture was stirred for 30 min. at room temp. Then it was filtered and washed for 10 min. with DCM and chloroform, 3 times each. The solution was neutralized with 10% TEA in DCM for 10 min. and washed as above with chloroform and then DCM. The next amino acid, Boc-Pro-OH (0.88 g, 4.1 mmol), was dissolved in DCM and coupled to the resin in the presence of one equivalent of DCC/HOBt for 2 h. The end of the reaction was determined by the Kaiser test. The further Boc-amino acids, Boc-Leu-OH, Boc-Hpa-OH, and Boc-Arg(Tos)-OH, were connected to the resin in the same way. The protected pentapeptide resin was dried overnight over KOH under reduced pressure.

The free peptide was obtained according to the following procedure: The peptide resin was mixed with 0.9 ml of anisole, 0.45 ml of ethane-1,2-ditiol, 7.5 ml of TFA, and 1.2 ml of TFMSA. The mixture was kept at room temperature for 2 hours. The resin was filtered off and the filtrate was triturated with diethyl ether (200 ml). The above reaction mixture gave a precipitate, which was washed with diethyl ether, dried *in vacuo* over KOH and then dissolved in water. The aqueous solution was subsequently stirred with Amberlite CG-4B (acetate form) for 30 min., filtered, and lyophilized. The peptide was desalted on a Sephadex G-10 column eluted with 5% acetic acid. The peptide was then purified by preparative HPLC. The main fractions were pooled and lyophilized. The data are presented in Table 2.

H-Arg-Hpa(4-NH_2)-Leu-Pro-Thr-OH (8). This peptide was obtained in the same manner as peptide **6**. The Hpa was introduced to the peptide chain as Boc-Hpa(4-NH-Z)-OH (**4**). Peptides **7**, **9**–**21** were obtained and purified in the same manner as peptide **6**. Their data are presented in Table 2.

RESULTS AND DISCUSSION

The bioassay data reported here reveal that structural modification of proctolin in position 2 or 3 results in analogues of which some are biologically active. Thus, peptides (**6**–**9**) retained a weak (10–20%) proctolin activity (Fig. 1) when applied to the heart of *T. molitor* at physiological concentration ranging from 10^{-9} to 10^{-7} M. The myotropic effects, observed in insects, depend probably on the structure requirements of the amino acid residue in position 2 of the proctolin molecule. In the homophenylalanine analogues of proctolin, there is a longer distance between the side chain aromatic ring at position 2 and the peptide chain. It seems that this change resulting from the presence of additional methylene group between the C-α atom and the aromatic ring in these analogues is so drastic that binding of the homophenylalanine analogues to the proctolin receptor is not possible. Thus, the presence of only one methylene group next to the benzene ring of the amino acid residue at position 2 is essential for myotropic activity in insects.

The biological activity of proctolin analogues, modified in the position 3 of the peptide chain tested (Fig. 2) on the *T. molitor* heart, showed that the presence of hydrophobic residues in position 3 of the proctolin peptide chain is not so important for myotropic properties in insects as we postulated in our earlier paper [8]. Only described earlier [8] [Val^3]-proctolin, and [Ile^3]-proctolin (**10**) presented here with a branched side chain retain a high cardiostimulatory activity. Proctolin analogues containing hydrophobic amino acids without branched side chain, such as: Phe (**11**), Sar (**13**), Nva (**14**), Nle (**15**), and γ-Abu (**21**) were practically inactive. On the other hand, proctolin analogues modified in position 3 by basic amino acids, such as (**12**) and (**20**) preserved more than 60% of proctolin activity. Analogues containing acidic amino acid residues in position 3, like Asp (**17**) or Glu (**19**) showed 60% and 30% of the native peptide activity, respectively.

CONCLUSIONS

From analysis of the myotropic effects of proctolin analogues **6**–**21** on *T. molitor* the following conclusions can be drawn:

1) The biological results pointed out that the presence of the methylene group between the C-α atom and the aromatic ring of the side chain of an amino acid residue in position 2 of the peptide chain is important for the myotropic activity in insects.
2) The elongation of the side chain in position 2 and bringing the aromatic ring in longer distance to the peptide chain.
3) A lower or none myotropic activity of the mentioned analogues in insects, as compared to proctolin, is probably the result of the change in the shape of the proctolin molecule.
4) The presence of a hydrophobic amino acid residue with a branched side chain in position 3 is important for cardiotropic activity in yellow mealworm.
5) The basic or acidic character of residues in position 3 of the peptide chain of proctolin does not destroy the myotropic activity on the insect heart.

Acknowledgments

This work was supported by grants from the Wrocław University Scientific Foundation.

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