PEPTIDIC ANALOGUES OF THE INSECT JUVENILE HORMONE CONTAINING URETHAN-TYPE PROTECTING GROUPS

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Received June 6th, 1975

Urethan-like derivatives of amino acids have been synthesized, substituted on the N-end by alkoxycarbonyl (mainly tert-butyloxycarbonyl) groups and on the carboxylic end by derivatives of *p*-aminobenzoic acid or by aromatic amines. Some of the thus-prepared substances exhibit specific juvenile hormone activity on the insect of the family *Pyrrhocoridae*.

Some time ago, synthesis and biological properties of *p*-aminobenzoic-acid-containing peptides have been reported^{1,2}. Several compounds of the general formula* tert-BOC—X—NH— C_6H_4 —COOC₂H₅ wherein X designates Ala, Ile, Pro, Abu of the L and D series, Gly, and Sar, were structurally related to terpenoid juvenile hormone analogues⁴ and exhibited a specific activity on the development of insects. In the present paper, we wish to report synthesis and juvenile activity of some further structural analogues obtained by modifications of particular portions of the molecule.

Protected esters of dipeptides I - V were prepared by condensation of tert-butyloxycarbonyl derivatives⁵ of the appropriate amino acids with ethyl *p*-aminobenzoate by the action of phosphorus trichloride in pyridine⁶ (compounds *I* and *II*) or N,N'dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole⁷ (compounds III - V). With compound *II*, the benzyloxycarbonyl group was split off by hydrogenation on a Pd/C catalyst.

Ethyl tert-butyloxycarbonyl-L-alanyl-p-aminobenzoate² (VI) proved as one of the most active insect juvenile hormone peptidic analogues so far reported. It was therefore of interest to prepare some additional alanine peptides VII - XVII protected on the N-end by the tert-butyloxycarbonyl group and carrying on the C-end derivatives of p-aminobenzoic acid or substituted aromatic amines.

The acid VII was prepared by saponification of the ester VI with sodium hydroxide in acetone. By condensation of the acid VII with ethylamine, diethylamine or glycine

^{*} BOC, butyloxycarbonyl; abbreviations of amino acids correspond to the IUPAC-IUB nomenclatural rules³.

ethyl ester in the presence of the phosphorus trichloride-pyridine reagent, there were obtained compounds VIII, IX, and X. The same reagent was used in the preparation of compound XI from tert-butyloxycarbonyl-L-alanine and 3,4-methylenedioxyaniline⁸. N,N'-Dicyclohexylcarbodiimide and 1-hydroxybenzotriazole were used in condensation of tert-butyloxycarbonyl-L-alanine with the corresponding amino components such as *p*-nitroaniline (XII), *p*-aminobenzenesulfonylamide (XIII), ethyl L-alanyl-*p*-aminobenzoate (XIV; obtained from compound VI), ethyl L-alanyl-*p*-aminobenzoate (XVI; obtained from compound XIV), and L-alanyl-*p*-aminobenzoyl-glycine ethyl ester (XVII) which was prepared from compound X by removal of the tert-butyloxycarbonyl group on treatment with trifluoroacetic acid and liberation of the base by the action of N-ethylpiperidine analogously to the two preceding amino compounds. The D-isomer of compound XIV, namely, ethyl tert-butyloxycarbonyl-L-alanyl-*p*-aminobenzoate (XV) was prepared by a similar procedure.

I, X = Val	III, X = Leu	V, X = Asn
II, X = Lys	IV, X = Thr	VI, X = Ala

$$(CH_3)_3C - O - CO - NH - CH - CO - R$$

$$XII, R = NH$$

VIII,
$$R = NH$$

 $IX, R = NH$

VII, R = NH

$$CON(C_2H_5)_2$$

XI, R = NH00 $-CH_2$

$$XIII, R = NH$$

COOC₂H₅

$$(IV, XV, R = Ala-NH)$$

$$XVI$$
, R = Ala-Ala-NH

$$XVII, R = Ala-NH$$

CO-GlyOC₂H

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$$R^{1} - O - CO - NH - CH - CO - R^{2}$$

XVIII,
$$R^1 = CH_3 - CH_2$$

XX, $R^1 = CH_3 - CH_2 - CH_1$
 $|$
 CH_3

COOC₂H₅

$$XIX, R^{1} = CH_{3} \qquad XXI, XXII, R^{1} = CH_{3} - CH_{2} - C - CH_{3}$$

XXII, $R^2 = NH_{\sim}$

$$XVIII, XIX, XX, XXI, R^2 = NH$$

In the alanine derivatives XVIII - XXII, the tert-butyloxycarbonyl group was replaced by other residues such as the methyloxycarbonyl, ethyloxycarbonyl, secbutyloxycarbonyl, and tert-pentyloxycarbonyl group. Compounds XVIII to XXIIwere prepared by condensation of the corresponding L-alanine alkyloxycarbonyl derivatives with ethyl *p*-aminobenzoate or 3,4-methylenedioxyaniline in the presence of phosphorus trichloride in pyridine. Ethyloxycarbonyl-L-alanine was obtained by reaction of ethyl chloroformate with L-alanine in alkaline media⁹; the remaining alkoxycarbonyl derivatives of L-alanine were prepared by reaction of methanol, sec-butyl alcohol or tert-pentyl alcohol with the isocyanate of L-alanine methyl ester and the subsequent hydrolysis by the action of sodium hydroxide in acetone. The appropriate isocyanate was obtained by reaction of L-alanine methyl ester hydrochloride with phosgene in toluene¹⁰.

The thus-prepared peptidic analogues were tested with respect to their juvenile hormone activity on freshly molted last instar larvae of the hemipterans *Pyrrhocoris apterus* and *Dysdercus cingulatus* (*Pyrrhocoridae*), *Graphosoma italicum* (*Pentatomidae*) and on the freshly molted pupae of *Tenebrio molitor* (*Tenebrionidae*) and were found to be active on the hemipterans of the family *Pyrrhocoridae* only (Table II), similarly to the earlier reported derivatives².

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Samples for elemental analysis were dried over phosphorus pentoxide at 1 Torr and room temperature for several hours. Solutions were taken down on a rotatory evaporator (water pump, bath temperature of about 35°C). The dimethylformamide-containing mixtures were evaporated at 1 Torr. Optical rotations were determined on a photoelectric polarimeter at 25°C. Electrophoresis of the present peptidic analogues was performed (after removal of the protecting alkyloxycarbonyl groups by the action

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TABLE I

Peptidic Analogues of Insect Juvenile Hormones

	Compound	Starting compounds	M.p., °C			
	(method) yield, %	acyl compound amino compound	solvent			
	I(A)	BOC-L-Val ^d	$127 - 128^{a}$			
	34	PABE	ether-light petroleum			
	II (A) 46	N ^α -BOC-N ^ε -Z-l-Lys PABE	foam ^b			
	<i>III (B)</i> 51	BOC-L-Leu PABE	73-75 ^c benzene-light petroleum			
	1V (B) 87	BOC-L-Thr PABE	136–138 ethyl acetate–light petroleum			
	V (B) 67	BOC-L-Asn PABE	162–165 aqueous ethanol			
	VIII (A) 45	BOC-L-Ala-PABH ethylamine.	foam			
	IX (A) 33	BOC-L-Ala-PABH diethylamine	foam			
	X(A)	BOC-L-Ala-PABH	153-155			
	33	GlyOC ₂ H ₅	aqueous ethanol			
	XI (A)	BOC-L-Ala	147—149			
	78	MDA	ethyl acetate			
	XII (B)	BOC-L-Ala	172-173			
	55	$H_2N-C_6H_4-NO_2$	aqueous ethanol			
	XIII (B)	BOC-L-Ala	200-202			
	89	$H_2N-C_6H_4-SO_2NH_2$	methanol			
	XVIII (A)	EOC-L-Ala	110-112			
	67	PABE	ethyl acetate-light petroleum			
	XIX (A) 70	MOC-1-Ala PABE	132–134 aqueous ethanol			
	XX (A) 60	sec-BOC-L-Ala PABE	127—128 aqueous ethanol			
•	XXI (A) 55	tert-POC-L-Ala PABE	104–107 ethyl acetate-light petroleum			
	XXII (A) 57	tert-POC-L-Ala MDA	140-142 ethyl acetate-light petroleum			

^a Chromatographed on silica gel (particle size, 30-60 micron) in benzene; ^b the N^e-Z-derivative was chromatographed on silica gel (60-120 micron) in benzene-ether (1:1) and the Z group was removed by hydrogenation (15 h) over 5% Pd/C in ethanol; ^c chromatographed on silica gel

Peptidic Analogues of the Insect Juvenile Hormone

TABLE I

(Continued)

	Formula	Cale	culated/Fo	ound	$[\alpha]_{D}^{22}(c)^{e}$		
9	(m.wt.)	% C	% H	% N			
	C ₁₉ H ₂₈ N ₂ O ₅ (364·5)	62·62 62·61	7·74 7·80	7·69 7·69	+15·7° (0·5)		
	C ₂₀ H ₃₁ N ₃ O ₅ (393·5)	61·04 61·18	7·94 7·96	10·68 10·54	0·0° (0·24)		
	$C_{20}H_{30}N_2O_5$ (378.5)	63·47 63·51	7·99 7·94	7·40 7·22	+3·1° (0·5)		
	C ₁₈ H ₂₆ N ₂ O ₆ (366·4)	59·00 59·21	7·15 7·17	7·65 7·42	$+3.0^{\circ}$ (0.5)		
	C ₁₈ H ₂₅ N ₃ O ₆ (379·4)	56·98 56·88	6·64 6·82	11·07 11·30	-13.7° (0.5)		
	C ₁₇ H ₂₅ N ₃ O ₄ (335·4)	60·88 61·08	7·51 7·68	12·53 12·29	-2.1° (0.21)		
	C ₁₉ H ₂₉ N ₃ O ₄ (363·5)	62·79 63·14	8·04 8·26	11·56 11·15	-2·4° (0·25)		
	C ₁₉ H ₂₇ N ₃ O ₆ (393·4)	58.00 58.28	6·92 7·06	10·68 10·49	-11·8° (0·26)		
	$C_{15}H_{20}N_2O_5$ (308.3)	58·42 58·53	6·53 6·51	9·09 9·01	$-44\cdot4^{\circ}(0\cdot5)^{f}$		
	$C_{14}H_{19}N_{3}O_{5}$ (309·3)	54·36 54·62	6·20 6·41	13·60 13·58	-18·7° (0·5)		
	$C_{14}H_{21}N_{3}O_{5}S_{(343\cdot4)}$	48·96 49·31	6·16 6·48	12·24 12·45	-15.1° (0.28)		
	$C_{15}H_{20}N_2O_5$ (308.3)	58·42 58·41	6·53 6·48	9·09 9·15	-15.2° (0.49)		
	$C_{14}H_{18}N_2O_5$ (294·3)	57·13 57·11	6·16 6·20	9·52 9·62	-14.4° (0.5)		
	C ₁₇ H ₂₄ N ₂ O ₅ (336·4)	60·70 60·81	7·19 7·02	8·33 8·38	-15.2° (0.49)		
	$C_{18}H_{26}N_2O_5$ (350.4)	61·69 61·48	7·48 7·38	7·99 8·22	-12·7° (0·12)		
	$C_{16}H_{22}N_2O_5$ (322.4)	59·61 59·71	6·88 6·76	8·69 8·95	- 20·5° (0·17)		
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(60-120 micron) in benzene; ^d abbreviations: PABE, ethyl *p*-aminobenzoate; Z, benzyloxycarbonyl; PABH, *p*-aminobenzoic acid; MDA, 3,4-methylenedioxyaniline; EOC, ethyloxycarbonyl; MOC, methyloxycarbonyl; and POC, pentyloxycarbonyl. ^e In dimethylformamide; ^f in ethanol.

of trifluoroacetic acid) on paper Whatman No 3MM (20 V/cm; 45 min) in the buffer solutions 1_{M} acetic acid (pH 2·4) and pyridine acetate (pH 5·7); detection with ninhydrin.

Preparation of Compounds I - XXII

Method A (cf.^b). Into a solution of the amino component (10 mM) in pyridine (25 ml), there was added at -20° C phosphorus trichloride (0.45 ml) and the mixture kept at -20° C for 30 min and then at room temperature for additional 30 min. The acyl component (10 mM) was then added, the whole mixture refluxed for 3 h, cooled down, filtered with active charcoal, and the filtrate evaporated under diminished pressure. The residue was dissolved in ethyl acetate and the solution washed successively with 10% aqueous citric acid, water, 5% aqueous sodium hydrogen carbonate, and water again, dried over anhydrous sodium sulfate, and evaporated under diminished pressure. The crystalline or sirupous.

Method B (cf.⁷). 1-Hydroxybenzotriazole (11 mM) and N,N'-dicyclohexylcarbodiimide (11 mM) were added at -7° C to a stirred solution of the acyl component (11 mM) in dimethylformamide (10 ml) and the mixture kept at the same temperature for 30 min. The amino component (10 mM) was then added at -7° C, the whole mixture kept at 0°C for 24 h, and the N,N'-dicyclohexylurea filtered off. The filtrate was evaporated under diminished pressure and the residual oil dissolved in ethyl acetate (50 ml). The solution was successively washed with 10% aqueous citric acid, water, 5% aqueous sodium hydrogen carbonate, and water again, dried over anhydrous sodium sulfate, and evaporated under diminished pressure. In some cases, the residual oil solidified on addition of light petroleum.

The tert-butyloxycarbonyl derivatives of amino acids were prepared by reaction of tert-butyloxycarbonyl azide with amino acids according to Schnabel⁵.

Tert-butyloxycarbonyl-L-alanyl-p-aminobenzoic Acid (VII)

The ethyl ester (0.67 g) of compound *VII* was dissolved in acetone (9 ml) and the solution stirred with 1M sodium hydroxide (2 ml) at room temperature for 60 h. The reaction mixture was then washed with ethyl acetate and the aqueous layer acidified with 10% aqueous citric acid. The precipitate was collected with suction and washed with water (2 ml). Yield, 0.58 g of the acid *VII*, m.p. 190–195°C; recrystallisation from aqueous methanol afforded 0.48 g (78%), m.p. 198–200°C. Optical rotation: $[\alpha]_D^{20} - 25 \cdot 1^\circ$ (*c* 0.4, ethanol). For C₁₅H₂₀N₂O₅ (308·3) calculated: 58·43% C, 6·53% H, 9·09% N; found: 58·39% C, 6·68% H, 9·29% N.

Ethyl Tert-butyloxycarbonyl-L-alanyl-L-alanyl-p-aminobenzoate (XIV)

Method *B* was used to condense tert-butyloxycarbonyl-L-alanine (0.95 g) with ethyl L-alanyl*p*-aminobenzoate which was obtained from the corresponding tert-butyloxycarbonyl derivative (1.68 g) by treating with trifluoroacetic acid (5 ml) for 30 min, evaporating under diminished pressure, washing the oily trifluoroacetate with three 30 ml portions of ether, dissolving in dimethylformamide (10 ml), and liberating the base by the action of N-ethylpiperidine (0.7 ml). The crude product (1.49 g; m.p. 187–191°C) was recrystallised from ethyl acetate to afford 1.32 g (65%) of the ester *XIV*, m.p. 189–193°C. Optical rotation: $[\alpha]_D^{2.5} - 13.9^\circ$ (*c* 0.5, dimethylformamide). For C₂₀H₂₉N₃O₆ (407.5) calculated: 58.95% C, 7.17% H, 10.31% N; found: 58.96% C, 7.48% H, 10.31% N.

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Ethyl Tert-butyloxycarbonyl-D-alanyl-L-alanyl-p-aminobenzoate (XV)

Tert-butyloxycarbonyl-D-alanine (0.95 g) was processed analogously to the preparation of compound XIV to yield 1.44 g of a crude product (m.p. $150-153^{\circ}$ C) which was recrystallised from ethyl acetate to afford 1.25 g (61%) of the ester XV, m.p. $152-154^{\circ}$ C. Optical rotation: $[\alpha]_{D}^{25} - 35.8^{\circ}$ (c 0.5, dimethylformamide). For $C_{20}H_{29}N_{3}O_{6}$ (407.5) calculated: 58.95% C, 7.17% H, 10.31% N; found: 58.94% C, 7.19% H, 10.04% N.

Ethyl Tert-butyloxycarbonyl-L-alanyl-L-alanyl-L-alanyl-p-aminobenzoate (XVI)

The title protected tetrapeptide ester XVI was prepared according to the condensation method B from tert-butyloxycarbonyl-L-alanine (1·32 g) and the tripeptide ester obtained by removal of the tert-butyloxycarbonyl protecting group from compound XIV (2·85 g). The crude product (2·3 g; m.p. $236-241^{\circ}$ C) was recrystallised from 2-methoxyethanol to afford 2·08 g (62%) of the ester XVI, m.p. $241-243^{\circ}$ C. Optical rotation: $[\alpha]_{D}^{25} - 12\cdot9^{\circ}$ (c 0·51, dimethylformamide). For C₂₃H₃₄N₄O₇ (478·6) calculated: 57·73% C, 7·16% H, 11·71% N; found: 57·53% C, 7·15% H, 11·91% N.

Tert-butyloxycarbonyl-L-alanyl-L-alanyl-p-aminobenzoylglycine Ethyl Ester (XVII)

Condensation (method *B*) of tert-butyloxycarbonyl-L-alanine (0·19 g) with L-alanyl-*p*-aminobenzoyl-glycine ethyl ester (obtained on removal of the tert-butyloxycarbonyl protecting group from 0·39 g of compound X by a procedure similar to that reported in the case of compound XIV) afforded 0·33 g of a crude product (m.p. 235–237°C) which was recrystallised from aqueous ethanol to yield 0·29 g (62%) of the ester XVII, m.p. 237–240°C. Optical rotation: $[\alpha]_D^{23} - 5\cdot64^\circ$ (*c* 0·5, dimethylformamide). For C₂₂H₃₂N₄O₇ (464·5) calculated: 56·89% C, 6·95% H, 12·06% N; found: 57·01% C, 7·04% H, 11·92% N.

N-Isocyanato-L-alanine Methyl Ester

Phosgene was introduced into a stirred solution of L-alanine methyl ester hydrochloride (15 g) in toluene (50 ml) at 150°C for 90 min. Excess phosgene was removed by introduction of air. The toluene was evaporated under diminished pressure and the residual liquid was distilled to afford 17.5 g of the title isocyanate, b.p. $60-61^{\circ}C/15$ Torr. For $C_5H_7NO_3$ (129.1) calculated: $46\cdot51\%$ C, $5\cdot47\%$ H, $10\cdot85\%$ N; found: $46\cdot19\%$ C, $5\cdot28\%$ H, $11\cdot12\%$ N.

Methyloxycarbonyl-L-alanine

A mixture of N-isocyanato-L-alanine methyl ester (1.45 g), methanol (8.5 ml), and pyridine (1.72 g) was refluxed for 2 h and evaporated under diminished pressure. The residual oily methyloxycarbonyl-L-alanine methyl ester (1.12 g) was dissolved in acetone (20 ml) and saponified (90 min)with 2M sodium hydroxide (3 ml). The acetone was then evaporated, the residual alkaline aqueous solution acidified with 10% aqueous citric acid, the oil taken up into ethyl acetate (80 ml), the ethyl acetate extract washed with three 50 ml portions of water, dried over anhydrous sodium sulfate, and evaporated under diminished pressure to afford 0.61 g of methyloxycarbonyl-L-alanine in the form of an oil.

Sec-butyloxycarbonyl-L-alanine

The preceding procedure was used to prepare the title sec-butyloxycarbonyl-L-alanine (1.29 g) from sec-butyl alcohol (10 ml) and N-isocyanato-L-alanine methyl ester (2.48 g).

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TABLE II

Insect Juvenile Hormone Activity of Some Efficient Peptidic Analogues

Insect	Ι	III	IV	V	VII	VIII	X	XI	XIV	XV	XVIII	XIX	XX	XXI
Dysdercus cingulatus	0.0007	500	30	1	300		a	а	300	300	0.01	500	0.06	0.01
Pyrrhocoris apterus	-	-		1	500	80	80	300	_	-	-	•	0.1	·

^a Inactive.

Tert-pentyloxycarbonyl-L-alanine

From tert-pentyl alcohol (6 ml) and N-isocyanato-L-alanine methyl ester (0.72 g) there was prepared the title tert-pentyloxycarbonyl-L-alanine (0.13 g) analogously to the synthesis of methyloxycarbonyl-L-alanine.

Biological Activity

The test substances were applied topically in 1 μ l of acetone or by injection (with pupae) in olive oil. The activity was evaluated on the basis of metamorphosis inhibition of the external morphological insect characters and expressed in activity units which designate such an amount of the test substance in microgrammes which caused half morphological effect under the application conditions stated. In Table II only those analogues of peptides are summarised which were to some extent active. These substances were exclusively active on the hemipterans of the family *Pyrrhocoridae* while they were inactive on other insect species.

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Translated by J. Pliml.