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

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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—$C_6H_4$—$COOC_2H_5$ wherein X designates Ala, Ile, Pro, Abu of the L and D series, Gly, and Sar, were structurally related to terpenoid juvenile hormone analogues[4] 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.

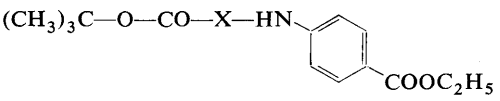
Protected esters of dipeptides *I* – *V* were prepared by condensation of tert-butyloxycarbonyl derivatives[5] of the appropriate amino acids with ethyl *p*-aminobenzoate by the action of phosphorus trichloride in pyridine[6] (compounds *I* and *II*) or N,N'-dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole[7] (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*-aminobenzoate[2] (*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.

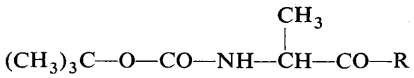
The acid *VII* was prepared by saponification of the ester *VI* with sodium hydroxide in acetone. By condensation of the acid *VII* with ethylamine, diethylamine or glycine

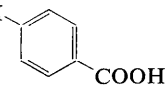
* BOC, butyloxycarbonyl; abbreviations of amino acids correspond to the IUPAC-IUB nomenclatural rules[3].

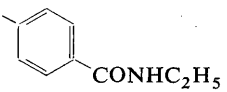
ethyl ester in the presence of the phosphorus trichloride-pyridine reagent, there were obtained compounds *VIII*, *IX*, and *X*. The same reagent was used in the preparation of compound *XI* from tert-butyloxycarbonyl-L-alanine and 3,4-methylenedioxyaniline[8]. N,N′-Dicyclohexylcarbodiimide and 1-hydroxybenzotriazole 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-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-D-alanyl-L-alanyl-*p*-aminobenzoate (*XV*) was prepared by a similar procedure.



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



*VII*, R = 

*VIII*, R = 

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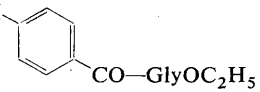
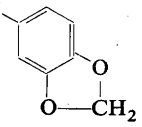
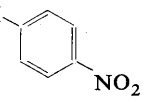
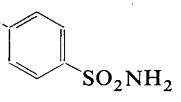
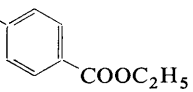
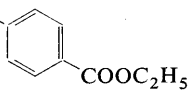
*X*, R = 

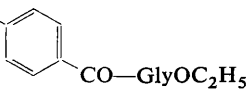
*XI*, R = 

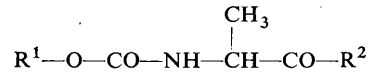
*XII*, R = 

*XIII*, R = 

*XIV*, *XV*, R = Ala-NH 

*XVI*, R = Ala-Ala-NH 

*XVII*, R = Ala-NH 

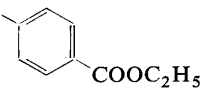


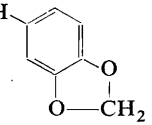
*XVIII*, $R^1 = CH_3—CH_2$

*XX*, $R^1 = CH_3—CH_2—CH(CH_3)$

*XIX*, $R^1 = CH_3$

*XXI*, *XXII*, $R^1 = CH_3—CH_2—C(CH_3)_2$

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

*XXII*, $R^2 =$ 

In the alanine derivatives *XVIII*–*XXII*, the tert-butyloxycarbonyl group was replaced by other residues such as the methyloxycarbonyl, ethyloxycarbonyl, sec-butyloxycarbonyl, and tert-pentyloxycarbonyl group. Compounds *XVIII* to *XXII* were 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 media[9]; 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 toluene[10].

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 derivatives[2].

## 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

TABLE I

Peptidic Analogues of Insect Juvenile Hormones

| Compound (method) yield, % | Starting compounds: acyl compound / amino compound | M.p., °C solvent |
|---|---|---|
| *I* (*A*) 34 | BOC-L-Val[d] / PABE | 127–128[a] ether–light petroleum |
| *II* (*A*) 46 | $N^{\alpha}$-BOC-$N^{\varepsilon}$-Z-L-Lys / PABE | foam[b] |
| *III* (*B*) 51 | BOC-L-Leu / PABE | 73–75[c] benzene–light petroleum |
| *IV* (*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*) 33 | BOC-L-Ala-PABH / $GlyOC_2H_5$ | 153–155 aqueous ethanol |
| *XI* (*A*) 78 | BOC-L-Ala / MDA | 147–149 ethyl acetate |
| *XII* (*B*) 55 | BOC-L-Ala / $H_2N-C_6H_4-NO_2$ | 172–173 aqueous ethanol |
| *XIII* (*B*) 89 | BOC-L-Ala / $H_2N-C_6H_4-SO_2NH_2$ | 200–202 methanol |
| *XVIII* (*A*) 67 | EOC-L-Ala / PABE | 110–112 ethyl acetate–light petroleum |
| *XIX* (*A*) 70 | MOC-L-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 $N^{\varepsilon}$-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

TABLE I
(*Continued*)

| Formula (m.wt.) | Calculated/Found | | | $[\alpha]_D^{22}$ (*c*)[e] |
|---|---|---|---|---|
| | % C | % H | % N | |
| $C_{19}H_{28}N_2O_5$ | 62·62 | 7·74 | 7·69 | +15·7° (0·5) |
| (364·5) | 62·61 | 7·80 | 7·69 | |
| $C_{20}H_{31}N_3O_5$ | 61·04 | 7·94 | 10·68 | 0·0° (0·24) |
| (393·5) | 61·18 | 7·96 | 10·54 | |
| $C_{20}H_{30}N_2O_5$ | 63·47 | 7·99 | 7·40 | +3·1° (0·5) |
| (378·5) | 63·51 | 7·94 | 7·22 | |
| $C_{18}H_{26}N_2O_6$ | 59·00 | 7·15 | 7·65 | +3·0° (0·5) |
| (366·4) | 59·21 | 7·17 | 7·42 | |
| $C_{18}H_{25}N_3O_6$ | 56·98 | 6·64 | 11·07 | −13·7° (0·5) |
| (379·4) | 56·88 | 6·82 | 11·30 | |
| $C_{17}H_{25}N_3O_4$ | 60·88 | 7·51 | 12·53 | −2·1° (0·21) |
| (335·4) | 61·08 | 7·68 | 12·29 | |
| $C_{19}H_{29}N_3O_4$ | 62·79 | 8·04 | 11·56 | −2·4° (0·25) |
| (363·5) | 63·14 | 8·26 | 11·15 | |
| $C_{19}H_{27}N_3O_6$ | 58·00 | 6·92 | 10·68 | −11·8° (0·26) |
| (393·4) | 58·28 | 7·06 | 10·49 | |
| $C_{15}H_{20}N_2O_5$ | 58·42 | 6·53 | 9·09 | −44·4° (0·5)[f] |
| (308·3) | 58·53 | 6·51 | 9·01 | |
| $C_{14}H_{19}N_3O_5$ | 54·36 | 6·20 | 13·60 | −18·7° (0·5) |
| (309·3) | 54·62 | 6·41 | 13·58 | |
| $C_{14}H_{21}N_3O_5S$ | 48·96 | 6·16 | 12·24 | −15·1° (0·28) |
| (343·4) | 49·31 | 6·48 | 12·45 | |
| $C_{15}H_{20}N_2O_5$ | 58·42 | 6·53 | 9·09 | −15·2° (0·49) |
| (308·3) | 58·41 | 6·48 | 9·15 | |
| $C_{14}H_{18}N_2O_5$ | 57·13 | 6·16 | 9·52 | −14·4° (0·5) |
| (294·3) | 57·11 | 6·20 | 9·62 | |
| $C_{17}H_{24}N_2O_5$ | 60·70 | 7·19 | 8·33 | −15·2° (0·49) |
| (336·4) | 60·81 | 7·02 | 8·38 | |
| $C_{18}H_{26}N_2O_5$ | 61·69 | 7·48 | 7·99 | −12·7° (0·12) |
| (350·4) | 61·48 | 7·38 | 8·22 | |
| $C_{16}H_{22}N_2O_5$ | 59·61 | 6·88 | 8·69 | −20·5° (0·17) |
| (322·4) | 59·71 | 6·76 | 8·95 | |

(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 1M acetic acid (pH 2·4) and pyridine acetate (pH 5·7); detection with ninhydrin.

Preparation of Compounds *I*—*XXII*

*Method* A (*cf.*[6]). 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 crude residue was either 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 Schnabel[5].

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: $[\alpha]_D^{20}$ −25·1° (*c* 0·4, ethanol). For $C_{15}H_{20}N_2O_5$ (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: $[\alpha]_D^{25}$ −13·9° (*c* 0·5, dimethylformamide). For $C_{20}H_{29}N_3O_6$ (407·5) calculated: 58·95% C, 7·17% H, 10·31% N; found: 58·96% C, 7·48% H, 10·31% N.

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°C) which was recrystallised from ethyl acetate to afford 1·25 g (61%) of the ester *XV*, m.p. 152—154°C. Optical rotation: $[\alpha]_D^{25}$ −35·8° (*c* 0·5, dimethylformamide). For $C_{20}H_{29}N_3O_6$ (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°C) was recrystallised from 2-methoxyethanol to afford 2·08 g (62%) of the ester *XVI*, m.p. 241—243°C. Optical rotation: $[\alpha]_D^{25}$ −12·9° (*c* 0·51, dimethylformamide). For $C_{23}H_{34}N_4O_7$ (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: $[\alpha]_D^{23}$ −5·64° (*c* 0·5, dimethylformamide). For $C_{22}H_{32}N_4O_7$ (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°C/15 Torr. For $C_5H_7NO_3$ (129·1) calculated: 46·51% C, 5·47% H, 10·85% N; found: 46·19% C, 5·28% H, 11·12% 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).

TABLE II

Insect Juvenile Hormone Activity of Some Efficient Peptidic Analogues

| Insect | *I* | *III* | *IV* | *V* | *VII* | *VIII* | *X* | *XI* | *XIV* | *XV* | *XVIII* | *XIX* | *XX* | *XXI* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Dysdercus cingulatus* | 0·0007 | 500 | 30 | 1 | 300 | — | [a] | [a] | 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 μl 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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