PEPTIDES OF NUCLEOAMINO ACIDS

PEPTIDES OF β-(1-URACILYL)-α-ALANINE (DL-WILLARDIINE)

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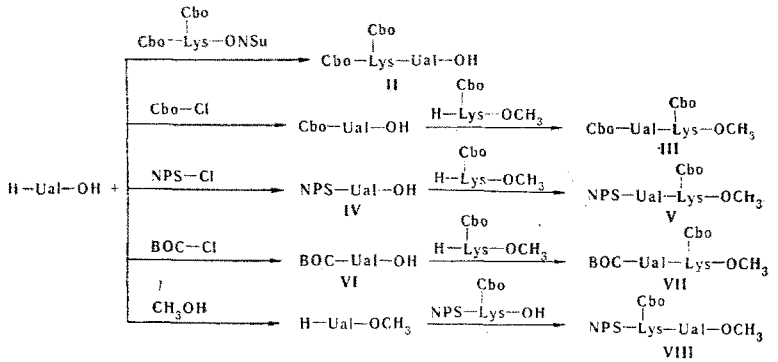
Dipeptides of β-(1-uracilyl)-α-alanine with lysine were obtained by the method of activated esters and by the dicyclohexylcarbodiimide method.

We have previously described [1] the preparation of pentides of β-(1-uracilyl)-α-alanine (DL-willardiine) (I) with several protein amino acids. Compounds I were also subjected to polycondensation, and the corresponding polyamino acids were obtained [2]. Polypeptides of regular structure obtained by polycondensation of di- and oligopeptides of willardiine and protein amino acids are, in our opinion, of interest. Polypeptides containing L-lysine may have particularly interesting properties, inasmuch as lysine molecules may endow the resulting polymers with polycationic properties and increase their solubility in water.

In the present research we set out to synthesize dipeptides of willardiine with lysine containing willardiine as the N-terminal or C-terminal fragment.

The method in [3], which is based on the use of N-hydroxysuccinimide esters for the creation of a peptide bond, was used for the synthesis of L-lysyl-DL-willardiine. N^{α}, N^{ε}-Dicarbobenzoxy-L-lysyl-DL-willardiine (II) is formed in the reaction of N^{α}, N^{ε}-dicarbobenzoxylysine N-hydroxysuccinimide ester (IX) with willardiine in dimethylformamide (DMFA).



N-Carbobenzoxy-DL-willardiyl-N^{ε}-carbobenzoxy-L-lysine methyl ester (III) was obtained by the carbodiimide method by condensation of N-carbobenzoxy-DL-willardiine [2] with N^{ε}-carbobenzoxy-L-lysine methyl ester.

o-Nitrophenylsulfenyl (NPS) and tert-butoxycarbonyl (BOC) groups, which are selectively cleaved in the presence of a carbobenzoxy (Cbo) group, were used as the blocking groups for the amine function of willardiine in order to obtain dipeptides with different protecting groups. A Cbo group was used in all cases to protect the N^{ε} group of lysine.

N-(o-Nitrophenylsulfenyl)-DL-willardiine (IV) was obtained by direct addition of o-nitrobenzenesulfenyl chloride to an alkaline solution of willardiine at room temperature. The purification of IV is com-

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plicated by its low solubility in nonpolar solvents, and this eliminates the possibility of its isolation in the form of the dicyclohexylammonium salt.

Condensation of protected willardiine IV with N^{ε}-Cbo-L-lysine methyl ester in the presence of dicyclohexylcarbodiimide gives N-NPS-DL-willardiyl-N^{ε}-Cbo-L-lysine methyl ester (V), whereas condensation with N^{ε}-Cbo-L-lysine pentachlorophenyl ester [4] in the presence of dicyclohexylcarbodiimide by the method in [5, 6] was unsuccessful. An acylurea of N-NPS-DL-willardiine was isolated from the reaction mixture. This is apparently explained by the low reactivity of N-(o-nitrophenylsulfenyl)-DL-willardiine (IV), owing to which the competitive reaction of the activated lysine ester with the free amino group of a second molecule of lysine proceeds more rapidly.

Because of the complexity of the preparation of N-NPS derivatives of willardiine, we checked the possibility of the use of a tert-butoxycarbonyl group for the protection of the α-amino group of willardiine. N-BOC-DL-willardiine (VI) was synthesized by the method in [7] with certain changes. Reaction of VI with N^{ε}-Cbo-L-lysine methyl ester in the presence of dicyclohexylcarbodiimide gives N-BOC-DL-willardiyl-N^{ε}-Cbo-L-lysine methyl ester (VII) in good yield.

The carbodiimide method was selected to prepare L-lysyl-DL-willardiine with various protecting groups. Condensation of willardiine methyl ester [2] with N^{α}-NPS-N^{ε}-Cbo-L-lysine gave N^{α}-NPS-N^{ε}-Cbo-L-lysyl-DL-willardiine methyl ester (VIII).

The structures of all of the compounds obtained were confirmed by the results of hydrolysis to the corresponding free amino acids.

EXPERIMENTAL

Filtrak FN-11 paper was used for chromatography. The following solvent systems were used: butyl alcohol–acetic acid–water (9:1:2) (A) and isopropyl alcohol–ammonium hydroxide–water (7:1:2) (B). The substances were detected in UV light (compounds containing an amino group were detected by means of ninhydrin). The R_f values presented below pertain to ascending chromatograms.

N^{α},N^{ε}-Dicarbobenzoxy-L-lysyl-DL-willardiine (II). A 1.65-g (3 mmole) sample of N^{α},N^{ε}-dicarbobenzoxylysine N-hydroxysuccinimide ester (IX) was added to 0.7 g (3 mmole) of willardiine in 30 ml of DMFA and 1.4 ml of triethylamine, after which the mixture was stirred at room temperature for 24 h. It was then diluted with 100 ml of ethyl acetate and washed with 30 ml of a 0.5% solution of citric acid. The ethyl acetate solution was washed with water and dried over anhydrous sodium sulfate. The solvent was removed in vacuo, and the residual oil was crystallized from 50% ethanol to give 0.42 g (25%) of a product with mp 129–130° (from ethyl acetate) and R_f 0.45 (A). Found: C 56.7; H 5.6; N 11.5%. $C_{29}H_{33}N_5O_9$. Calculated: C 56.8; H 5.8; N 11.8%.

N-Carbobenzoxy-DL-willardiyl-N^{ε}-carbobenzoxy-L-lysine Methyl Ester (III). A 2.06-g (10 mmole) sample of dicyclohexylcarbodiimide was added at 0° with stirring to a solution of 2.66 g (10 mmole) of N^{ε}-Cbo-L-lysine methyl ester in 20 ml of tetrahydrofuran (THF). After 15 min at the same temperature, a solution of 3.3 g (10 mmole) of N-Cbo-DL-willardiine [1] in 75 ml of DMFA was added. The mixture was then stirred at room temperature for 20 h, after which it was filtered. The filtrate was vacuum evaporated, and the residual oil was crystallized by trituration with ether to give 4.5 g (74%) of a product with mp 134–135° (from ethyl acetate) and R_f 0.93 (A) and 0.96 (B). Found: C 58.9; H 5.6; N 11.5%. $C_{30}H_{35}N_5O_9$. Calculated: C 59.1; H 5.7; N 11.5%.

N-(o-Nitrophenylsulfenyl)-DL-willardiine (IV). A 1.9-g (10 mmole) sample of o-nitrobenzenesulfenyl chloride and 5.7 ml of 2 N sodium hydroxide solution were added in small portions with stirring to a solution of 2.17 g (10 mmole) of I in 15 ml of dioxane and 5.2 ml of 2 N sodium hydroxide solution (the pH of the mixture was maintained rigorously at 7.5–8). The mixture was stirred at room temperature for 3 h, after which it was diluted with 30 ml of water, and the precipitated disulfide was removed by filtration. The filtrate was acidified to pH 4–5 with 2 N sulfuric acid, and the resulting light-yellow precipitate was crystallized twice from ethanol to give 2.7 g (77%) of a product with mp 202–203° and R_f 0.82 (A) and 0.88 (B). Found: C 44.4; H 3.0; N 15.3%. $C_{13}H_{12}N_4O_6$. Calculated: C 44.3; H 3.4; N 16.0%.

N-(o-Nitrophenylsulfenyl)-DL-willardiyl-N^{ε}-carbobenzoxy-L-lysine Methyl Ester (V). A solution of 2.76 g (10 mmole) of N^{ε}-Cbo-4-lysine methyl ester in 20 ml of DMFA was cooled to 0°, and 1.94 g (10 mmole) of dicyclohexylcarbodiimide was added to it with stirring. The mixture was stirred for 30 min, after which a solution of 3.5 g (10 mmole) of IV in 30 ml of DMFA was added, and the mixture was allowed

to stand for 48 h. It was then vacuum evaporated to dryness, and the residue was extracted with ethyl acetate solution gave a yellow oil, which was crystallized by trituration with petroleum ether to give 3.5 g (55%) of a product with mp 82-83° (from ethyl acetate) and R_f 0.90 (A) and 0.92 (B). Found: C 54.0; H 5.2; N 13.6%. $C_{28}H_{32}N_6O_9$. Calculated: C 53.5; H 5.1; N 13.4%.

N-tert-Butoxycarbonyl-DL-willardiine (VI). A 4-ml sample of a 50% solution of tert-butoxycarbonyl azide in dioxane was added with stirring to a solution of 2.17 g (10 mmole) of I in 15 ml of water, 15 ml of dioxane, and 2.5 ml of triethylamine, and the mixture was stirred at room temperature for 48 h. It was then filtered, and the filtrate was evaporated to half its original volume in vacuo. Water (15 ml) was added, and the mixture was acidified to pH 3-4 with 2 N hydrochloric acid. The resulting precipitate was removed by filtration to give 1.7 g (57%) of a product with mp 188-189° (from 50% ethanol) (mp 198° [7]) and R_f 0.87 (A) and 0.89 (B). Found: C 45.9; H 5.9; N 13.0%. $C_{12}H_{17}N_3O_6 \cdot H_2O$. Calculated: C 45.4; H 6.0; N 13.2%.

N-tert-Butoxycarbonyl-DL-willardiyl-N^{ε}-carbobenzoxy-L-lysine Methyl Ester (VII). A solution of 2.66 g (10 mmole of N^{ε}-carbobenzoxy-L-lysine methyl ester in 30 ml of THF was cooled to 0°, 2.06 g (10 mmole) of dicyclohexylcarbodiimide was added, and the mixture was stirred for 30 min. A 2.99-g (10 mmole) sample of VI was added to the reaction mixture, and stirring was continued for 12 h. The precipitated dicyclohexylurea was removed by filtration, and the filtrate was evaporated to dryness. The residual oil was crystallized by trituration with ether to give 4.1 g (71%) of a compound with mp 111-112° (from ethyl acetate) and R_f 0.96 (A) and 0.97 (B). Found: C 56.0; H 6.7; N 11.9%. $C_{27}H_{37}N_5O_9$. Calculated: C 56.3; H 6.5; N 12.2%.

N^{α}-(o-Nitrophenylsulfenyl)-N^{ε}-carbobenzoxy-L-lysyl-DL-willardiine Methyl Ester (VIII). A mixture of solution of 7.7 g (12.5 mmole) of the dicyclohexyl ammonium salt of N^{ε}-carbobenzoxy-N^{α}-(o-nitrophenylsulfenyl)-L-lysine and 3.0 g (12.5 mmole) of willardiine methyl ester in a mixture of 250 ml of dioxane and 250 ml of DMFA was cooled to 0°. A 2.6-g (12.5 mmole) sample of dicyclohexylcarbodiimide was added to the mixture, and it was then stirred for 48 h. The precipitated dicyclohexylurea was removed by filtration, and the filtrate was evaporated to dryness. The residual oil was crystallized by treatment with petroleum ether to give 5.8 g (74%) of a product with mp 127-128° (from ethyl acetate) and R_f 0.95 (B). Found: C 52.9; H 5.1; N 13.5%. $C_{28}H_{32}N_6O_9$. Calculated: C 53.5; H 5.1; N 13.4%.

N^{α},N^{ε}-Dicarbobenzoxy-L-lysine N-hydroxysuccinimide Ester (IX). A 4.53-g (22 mmole) sample of dicyclohexylcarbodiimide was added with stirring at 0° to 9.18 g (22 mmole) of N^{α},N^{ε}-dicarbobenzoxy-L-lysine and 2.53 g (22 mmole) of N-hydroxysuccinimide in 30 ml of DMFA, after which the mixture was allowed to stand overnight in a refrigerator. The solution was then diluted with 400 ml of ethyl acetate and washed with a saturated solution of sodium bicarbonate and water. The solution was then dried with anhydrous sodium sulfate, and the solvent was removed by vacuum distillation. The residual oil began to crystallize on treatment with isopropyl alcohol. Workup gave 3.6 g (32%) of a product with mp 102-103° (from ethyl acetate). Found: C 61.8; H 7.4; N 8.0%. $C_{26}H_{29}N_3O_8$. Calculated: C 61.1; H 7.6; N 8.2%.

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