SYNTHESIS OF PEPTIDES BASED ON β-(N_1-URACILYL)-α-ALANINE

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The synthesis of peptides which include a DL-β-(N_1-uracilyl)-α-alanine (DL-willardiine) residue as an N-terminal or C-terminal group was investigated, dipeptides of DL-willardiine with glycine and L-tyrosine were obtained, and DL-willardiyl-DL-willardiine was synthesized; the latter is the simplest representative of the family of monotonic homopeptides which contain, as a repeating side substituent, a natural nucleic base capable of participation in intermolecular interactions of the complementary type.

Since protein and nucleic acid metabolism plays an important role in life processes, an extremely timely task is the creation of preparations which might actively intervene in these types of metabolism. One of the promising paths for the creation of such preparations is that based on systematic chemical modification of the structure of the key metabolites of protein and nucleic metabolism. Of special interest as potential antimetabolites of these indicated types are such structures in which nucleic acid fragments, on the one hand, and protein fragments, on the other, would be fused. From this point of view, pyrimidyl- and purinyl-α-amino acids are of undoubted interest. The results of investigations of such structures have already been reported in [1-5]. Interest in these compounds has recently undergone an even greater growth due to the observation of several of them in nature, particularly β-(N_1-uracilyl)-α-alanine (I), which was found in a number of plants and is known as "willardiine" [6-14].

Of undoubted interest also are peptides constructed from pyrimidyl- and purinyl-α-amino acid residues [3-5], and nucleopeptides, whose amino acid residues contain nucleic bases are, in our opinion, deserving of special attention.

In this paper we set out to accomplish the synthesis of a number of the simplest peptides which are included as a necessary component of I (DL-willardiine), which is included in the five α-amino acids necessary for the synthesis of diverse nucleopeptides [15].

In the course of the study we decided to work out a method for obtaining the corresponding derivatives of I with respect to the α-amino group and the carboxyl group, to study the behavior of the uracil fragment in peptide synthesis reactions, to compare the different methods for the formation of the peptide bond with participation of I, and also to investigate several methods for the removal of the protective groupings from the peptides obtained, which contain residue I as an N-terminal or C-terminal group.

The protein α-amino acids glycine and L-tyrosine, whose behavior in such reactions has already been studied quite satisfactorily, were used as the second component of the peptide synthesis.

To create the peptide bond we used the method of mixed anhydrides, the method of activated esters, and the dicyclohexylcarbodiimide method. The α-amino groups were protected in all cases by carbobenzoxylation.

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The method of activated esters was used to synthesize the dipeptide DL-willardiylglycine. The reaction of I with carbobenzoxy chloride yielded α-N-carbobenzoxy-DL-willardiine (II). Activation of the carboxyl group in II can be accomplished through the formation of a p-nitrophenyl ester, which is smoothly obtained by the reaction of II with p-nitrophenol in the presence of N,N'-dicyclohexylcarbodiimide. The resulting p-nitrophenyl ester of α-N-carbobenzoxy-DL-willardiine (III) was further subjected to reaction with glycine methyl ester, which gave the methyl ester of α-N-carbobenzoxy-DL-willardiylglycine (IV). It was subsequently established that α-N-carbobenzoxy-DL-willardiylglycine (IX) can be obtained in still higher yields when the triethylammonium salt of glycine rather than glycine methyl ester is used as the amino component in this reaction.

The methyl ester of N-carbobenzoxyglycyl-DL-willardiine (VI) is similarly formed by the reaction of the p-nitrophenyl ester of carbobenzoxyglycine with the methyl ester of DL-willardiine (V).

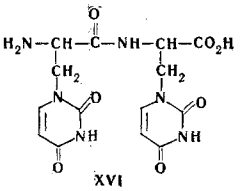
We used the carbodiimide method for the synthesis of the dipeptide DL-willardiyl-L-tyrosine. The methyl ester of α-N-carbobenzoxy-DL-willardiyl-L-tyrosine (VII) was obtained by the condensation of II with L-tyrosine methyl ester in the presence of dicyclohexylcarbodiimide.

We synthesized a derivative of the dipeptide DL-willardiyl-DL-willardiine by the mixed anhydride and activated ester methods. Compound II with ethyl chlorocarbonate readily forms a mixed anhydride which, without isolation, was subjected to reaction with V, as a result of which the methyl ester of α-N-carbobenzoxy-DL-willardiyl-DL-willardiine (VIII) was obtained. However, in connection with side reactions, the yield of this dipeptide via this method was not high enough. Better results were obtained using the method of activated esters. The reaction of III and V proceeds smoothly and leads to formation of VIII in higher yields. Saponification of the ester grouping in the esters of the dipeptides can be accomplished by alkaline hydrolysis.

The corresponding peptides with a free amino group (XV, XIV, and XIII, respectively) were obtained from the thus prepared α-N-carbobenzoxy-DL-willardiylglycine (IX), N-carbomethoxyglycyl-DL-willardiine (X), and α-N-carbobenzoxy-DL-willardiyl-L-tyrosine (XI) by the action of hydrogen bromide in glacial acetic acid.

DL-Willardiyl-DL-willardiine (XVI), i.e., a compound which is the simplest representative of the family of poly(N-pyrimidyl)-α-amino acids and the first member of a series of monotonic homopeptides which contain a uracil ring as a repeating side substituent, was similarly obtained from carbobenzoxydipeptide XIII.

It should be emphasized that the uracil rings in XVI are bonded to the base peptide chain at the same position as in natural polynucleotides. The possibility of participation of the uracil rings in the formation of a specific system of complementary hydrogen bonds with corresponding adenine-containing structures is thereby provided.



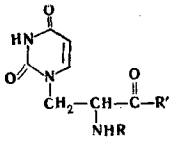
XVI

The structures of all of the compounds obtained were confirmed by IR and UV absorption spectra and by the results of control hydrolysis experiments in which the formation of the corresponding free amino acids was established.

All of the compounds obtained are crystalline substances. Their purity was proved by appropriate analyses and confirmed by means of paper chromatography and electrophoresis (Table 1).

We are continuing our investigations of the synthesis and study of the properties of the nucleopeptides.

TABLE 1.



Comp.	R	R′	Mp, °C	R_f system A	R_f system B	Empirical formula	Found, % C	Found, % H	Found, % N	Calc., % C	Calc., % H	Calc., % N	Yield, %
II	$C_6H_5CH_2OCO$	OH	142—143	0,87	0,71	$C_{15}H_{15}N_3O_6$	54,09	4,44	12,69	54,05	4,50	12,61	51,9
III	$C_6H_5CH_2OCO$	$OC_6H_4NO_2$-p	199—200	0,09	0,19	$C_{21}H_{18}N_4O_8$	55,46	4,66	12,16	55,51	4,18	12,33	59,3
V	H	OCH_3	216	—	—	$C_8H_{11}N_3O_4$	45,49	5,75	18,76	45,57	5,68	19,06	78
VI	$C_6H_5CH_2OCONHCH_2CO$	OCH_3	100—101	0,48	0,33	$C_{18}H_{20}N_4O_7$	53,78	4,84	13,88	53,46	4,95	13,86	58,1
VII	$C_6H_5CH_2OCO$	$NHCH(CH_2C_6H_4OH\text{-}n)CO_2CH_3$	130—131	0,17	0,11	$C_{25}H_{26}N_4O_8$	58,45	5,48	10,63	58,80	5,14	10,97	65,9
VIII	$C_6H_5CH_2OCO$	$NHCH(CO_2CH_3)CH_2U$*	210—211	0,69	0,48	$C_{23}H_{24}N_6O_9$	51,94	4,83	16,29	52,24	4,40	15,90	69,3
IX	$C_6H_5CH_2OCO$	$NHCH_2CO_2H$	135—136	0,31	0,35	$C_{17}H_{18}N_4O_7$	51,92	4,43	14,17	52,29	4,65	14,35	73,8
X	$COCH_2NHCO_2CH_2C_6H_5$	OH	191—193	0,55	0,30	$C_{17}H_{18}N_4O_7$	52,05	4,87	14,07	52,29	4,65	14,35	71,2
XI	$C_6H_5CH_2OCO$	$NHCH(CO_2H)CH_2C_6H_4OH$	132—133	0,11	0,32	$C_{24}H_{24}N_4O_8$	57,66	5,16	10,99	58,05	4,87	11,27	72
XII	H	$NHCH(CH_2C_6H_4OH\text{-}n)CO_2H$	—	0,89	0,73	$C_{16}H_{18}N_4O_6 \cdot HBr \cdot H_2O$	41,16	4,71	11,95	41,65	4,59	12,14	61,3
XIII	$C_6H_5CH_2OCO$	$NHCH(CO_2H)CH_2U$*	330 (decomp)	—	0,1	$C_{22}H_{22}N_6O_9$	51,06	4,65	16,68	51,35	4,31	16,33	58
XIV	$COCH_2NH_2$	OH	260 (decomp)	0,99	0,69	$C_9H_{12}N_4O_5 \cdot H_2O$	39,09	5,38	20,09	39,44	5,15	20,43	59
XV	H	$NHCH_2CO_2H$	—	0,96	0,72	$C_9H_{12}N_4O_5 \cdot HBr$	31,78	4,15	16,26	32,05	3,89	16,62	59,2
XVI	H	$NHCH(CO_2H)CH_2U$*	—	0,94	0,76	$C_{14}H_{16}N_6O_7 \cdot HBr$	36,86	4,35	18,51	36,44	3,72	18,22	88,1

*Uracil ring substituted in the N_1 position.

EXPERIMENTAL

"Chromatographic S" paper from the Volodarsk Leningrad Plant was used for the chromatography. The following solvent systems were used: n-butanol–acetic acid–water (9:1:2) (system A); isopropyl alcohol–28% ammonium hydroxide–water (7:1:2) (system B). The compounds were detected on the chromatograms from the UV absorption, while compounds containing amino groups were developed with ninhydrin. The R_f values presented below pertain to ascending chromatograms.

The electrophoresis was carried out on "chromatographic S" paper. The following systems were used as electrolytes: 85% formic acid–acetic acid–water (7:5:13, pH 0.7) (system 1); 0.1 N KOH (pH 13) (system 2). A voltage of 350 V was supplied with a rectifier during the work with system 1, and the potential gradient on the paper was 9.2 V/cm. The voltage at the output from the rectifier was 600 V in the work with system 2, and the potential gradient on the paper was 13.6 V/cm. The electrophoresis time varied from 2 to 5 h. The compounds were detected on the phoregrams by means of the same methods as used for the chromatograms. The electrophoretic mobilities were calculated from the ratio of the rate of motion of the compound to the electrical field voltage.

α-N-Carbenzoxy-DL-willardiine (II). Carbobenzoxy chloride [3.5 g (0.019 mole)] and 25 ml of 0.1 N sodium hydroxide were added dropwise simultaneously with stirring to a solution of 3.4 g (0.017 mole) of I in 30 ml of 0.1 N sodium hydroxide cooled to 0°. The suspension was stirred for 3 h at 0° and allowed to stand for 12 h at room temperature. At the end of the reaction the solution was extracted with ether, and the aqueous layer was cooled and acidified with concentrated hydrochloric acid to pH 3. The resulting oil was crystallized at low temperature.

p-Nitrophenyl Ester of α-N-Carbenzoxy-DL-willardiine (III). p-Nitrophenol [1.0 g (0.007 mole)] and 1.35 g (0.007 mole) of dicyclohexylcarbodiimide were added with vigorous stirring to 2.0 g (0.007 mole) of II and 225 ml of dry dioxane at 12°. The reaction mass was stirred for 3 h at 12° and allowed to stand for 24 h at room temperature. The resulting precipitate of dicyclohexylurea was separated, and the filtrate was evaporated in vacuo. The oil that was obtained crystallized on trituration with dry methanol.

α-N-Carbobenzoxy-DL-willardiylglycine (IX). A solution of 0.18 g (0.0025 mole) of glycine in 50% ethanol and 0.7 ml of triethylamine were added to a solution of 1.1 g (0.0024 mole) of III in 200 ml of ethanol. The mixture was heated at 50° for 20 h. The solvent was removed by vacuum distillation, and the residue was extracted with 30 ml of ethyl acetate. The aqueous layer was acidified with concentrated hydrochloric acid to pH 3, and the precipitate was filtered.

DL-Willardiine Methyl Ester (V). An intense stream of dry hydrogen chloride was passed without cooling into a suspension of 4.0 g (0.02 mole) of I in 110 ml of absolute methanol. After the amino acid dissolved, the solution was cooled to 2°, the introduction of gas was continued until the mixture was saturated, and the mixture was then allowed to stand in a refrigerator for 48 h. The resulting crystals of the hydrochloride of I were filtered to give 4.1 g (82%) of a product with mp 183°, R_f 0.24 (system A), and R_f 0.38 (system B). This product was washed with ether, dissolved in 50 ml of methanol, and the solution was neutralized with triethylamine to pH 7 and allowed to stand for 24 h at 0°. The resulting precipitate was separated, washed with ether, and dried.

N-Carbobenzoxyglycyl-DL-willardiine Methyl Ester (VI). The p-nitrophenyl ester of N-carbobenzoxyglycine [3.0 g (0.01 mole)] was added to a solution of 2.4 g (0.01 mole) of V in 125 ml of dry dimethylformamide, and the mixture was allowed to stand at room temperature for 7 days. The solution was then evaporated in vacuo, and the oily residue was dissolved in ethyl acetate and extracted with water. The organic layer was dried over $MgSO_4$, vacuum evaporated, and the residue was washed with acetone and dried.

α-N-Carbobenzoxy-DL-Willardiyl-L-tyrosine Methyl Ester (VII). L-Tyrosine methyl ester [0.5 g (0.0025 mole)] was dissolved in 30 ml of tetrahydrofuran, and 0.52 g (0.002 mole) of dicyclohexylcarbodiimide was added in small portions with stirring. The mixture was stirred for another 30 min, a solution of 0.83 g (0.003 mole) of II and dimethylformamide was added, and the mixture was allowed to stand for 24 h. Acetic acid (0.2 ml) was then added, and the precipitate of dicyclohexylurea was filtered. The filtrate was evaporated in vacuo, and the residue was dissolved in ethyl acetate and extracted with water. The organic layer was dried over $MgSO_4$ and vacuum evaporated to dryness.

α-N-Carbobenzoxy-DL-willardiyl-DL-willardiine Methyl Ester (VIII). A solution of 0.7 g (0.0032 mole) of V and 1.45 g (0.0032 mole) of III in 50 ml of dry dimethylformamide was held at room temperature

for 14 days and then vacuum evaporated to give a yellow oil which crystallized on trituration with ethyl acetate. The crystals were filtered and washed with acetone and absolute ethanol.

N-Carbobenzoxyglycyl-DL-willardiine (X). A solution of 0.3 g (0.0007 mole) of N-carbobenzoxyglycyl-DL-willardiine methyl ester (VI) in 20 ml of methanol was cooled to 10°, 1.8 ml of 1 N sodium hydroxide was added in small portions, and the mixture was held for 24 h at room temperature. The methanol was removed by vacuum distillation, and the residual aqueous solution was acidified with 1 N hydrochloric acid to pH 3-4. The resulting white precipitate was filtered, washed with water, and dried.

α-N-Carbobenzoxy-DL-willardiyl-L-tyrosine (XI). This was similarly obtained from VII.

α-N-Carbobenzoxywillardiyl-DL-willardiine (XIII). This was similarly obtained from VIII.

DL-Willardiylglycine Hydrobromide (XV). Compound IX [3.0 g (0.008 mole)] was suspended in 6 ml of glacial acetic acid, and 40 ml of 33% hydrobromic acid in glacial acetic acid was added. The solid gradually dissolved, and after 1 h 200 ml of absolute ether was added to the solution. The resulting precipitate was filtered, washed with ether, and dried. The electrophoretic mobilities* were $-1.6 \cdot 10^{-4}$ cm$^2 \cdot V^{-1} \cdot$ sec^{-1} (system 1) and $+1.4 \cdot 10^{-4}$ cm$^2 \cdot V^{-1} \cdot$ sec^{-1} (system 2). Electrophoretic mobilities: $-1.2 \cdot 10^{-4}$ cm$^2 \cdot V^{-1} \cdot$ sec^{-1} (system 1) and $+1.3 \cdot 10^{-4}$ cm$^2 \cdot V^{-1} \cdot$ sec^{-1} (system 2).

DL-Willardiyl-L-tyrosine Hydrobromide (XII). This was similarly obtained from XI. The electrophoretic mobilities were $-1.1 \cdot 10^{-4}$ cm$^2 \cdot V^{-1} \cdot$ sec^{-1} (system 1) and $+1.4 \cdot 10^{-4}$ cm$^2 \cdot V^{-1} \cdot$ sec^{-1} (system 2).

DL-Willardiyl-DL-willardiine Hydrobromide (XVI). This was similarly obtained from XIII. The electrophoretic mobilities were $-1.1 \cdot 10^{-4}$ (system 1) and $+1.3 \cdot 10^{-4}$ cm$^2 \cdot V^{-1} \cdot$ sec^{-1} (system 2).

Glycyl-DL-willardiine (XIV). This was similarly obtained from X.

Hydrolysis of VI-XVI. A total of 10 mg of the compound was heated for 24 h on a boiling-water bath with 1 ml of 6 N hydrochloric acid, and the appearance of the corresponding amino acids in the solution obtained was proved by means of paper chromatography and electrophoresis.

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*The minus and plus signs indicate migration of the compound to the cathode or anode, respectively.