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NEW SYNTHESIS OF [8-ARGININE] VASOPRESSIN AND ITS DE-9-GLYCINE ANALOGS

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A unified scheme is proposed for the synthesis of [8-arginine]vasopressin (I), de-9-glycine[8-arginine]vasopressin (II), and de-9-glycinamide[8-arginine]vasopressin (III) which has been developed on the basis of the tetrapeptide Gln-Asn-Cys(Bzl)-Pro and other homologous fragments of neurohypophyseal hormones as common initial and intermediate compounds. The free dithiols obtained by the reduction of protected derivatives of (I)-(III) by sodium in liquid ammonia have been oxidized to the corresponding cyclic disulfides (I)-(III) with the aid of 1 M H_2O_2 at 0-5°C and pH 8.5. The vasopressor activities of (I)-(III) are, respectively 470 ± 20, 1.7, and 0.5 IU/mg (rat, in vivo).

In view of the high degree of homology of the primary structures of the neurohypophyseal hormones, great interest is presented by unified schemes for their synthesis using a whole series of common initial and intermediate compounds. For the synthesis of arginine- and lysine-vasopressins, oxytocin, and vasotocin, and also their analogs modified in the C- and N-ends of the peptide chain, one such intermediate compound may be the tetrapeptide of sequence 4—7, which is the longest common structural fragment of these hormones. A scheme (in two variants) using Gln-Asn-Cys(Bzl)-Pro for the synthesis of [Phe^2, Orn^8]vasopressin has been described previously [1]. The aim of the present work was the synthesis from this tetrapeptide of arginine-vasopressin (I) and two of its analogs — de-9-glycine[8-arginine]vasopressin (II) [2, 3] and de-9-glycinamide[8-arginine]vasopressin (III) [4, 5].

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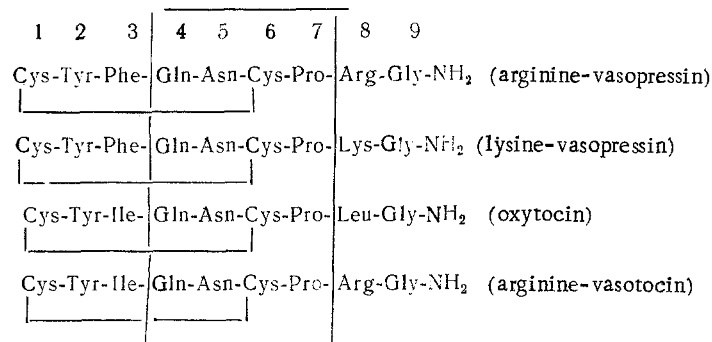
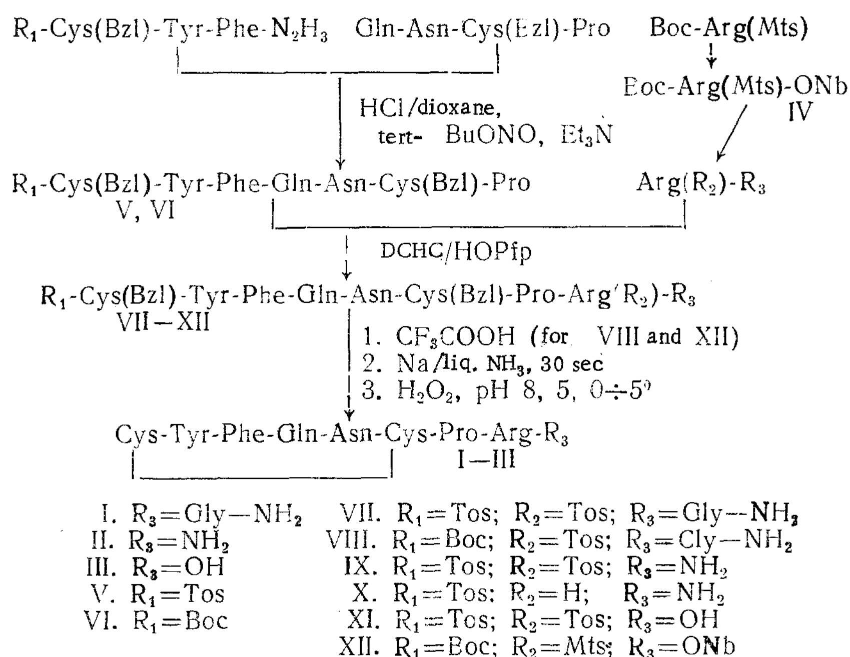


To protect the mercapto group of cysteine we used the benzyl group, which has now become the traditional SH protection in the synthesis of the neurohypophyseal hormones (see scheme). The guanidine function of arginine was protected (with approximately the same results) by tosyl and mesitylenesulfonyl groups, and also by protonation. To block the amino group of the N-terminal cysteine we used the tosyl and tert-butoxycarbonyl groups; however, the former is preferable since the N^{α}-tosyl derivatives of peptides, including the octa- and nonapeptides (VII, IX–XI), are formed in the crystalline state and, in addition to this, their detosylation is effected simultaneously with the splitting out of the S-benzyl groups by means of sodium in liquid ammonia.



According to the scheme of synthesis given, the protected derivatives (VII–XII) of the desired compounds (I–III) were obtained by condensing three blocks – the N-terminal tripeptide R_1-Cys(Bzl)-Tyr-Phe-N_2H_3 with the tetrapeptide Gln-Asn-Cys(Bzl)-Pro, and then with, respectively, the C-terminal dipeptide Arg(Tos)-Gly-NH_2 and with arginine derivatives. Since the N-therminal peptide was added by the azide method and the proline residue (at the C-end of each of the pentapeptides (V) and (VI) is not racemized under the usual conditions of peptide synthesis, the formation of racemates in the condensation of these blocks is practically excluded.

In the second variant of the synthesis of arginine-vasopressin (not described in the present paper), the Boc derivative of the tetrapeptide Gln-Asn-Cys(Bzl)-Pro [1] was condensed with the dipeptide Arg(Tos)-Gly-NH_2 and then to the resulting hexaptide Boc-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)Gly-NH_2 (after the deblocking of the amino group) was added the N-terminal tripeptide R_1-Cys(Bzl)-Tyr-Phe-N_2H_3. However, the overall yields of the nonapeptides (VII and VIII) were lower than in the first variant, described herein.

TABLE 1. Yields and Properties of the Compounds Synthesized

Compound	Empirical formula	Yield, % (method)	mp, °C	$[\alpha]_D^{20}$, deg (c; solvent)	R_f		E	
					A	Б	His	Gly
I	$C_{46}H_{65}N_{15}O_{12}S_2 \cdot 2AcOH \cdot 5H_2O$	42 (A) 40 (B)	Amorph.	—22,0 (0,30; 1 н. AcOH)*	0,04	0,46	0,72	0,96
II	$C_{44}H_{62}N_{14}O_{11}S_2 \cdot 2AcOH \cdot 3H_2O$	40 (A) 41 (B)	Amorph.	—17,2 (0,25; 1 н. AcOH)	0,06	0,50	0,68[2*]	0,91[3*]
III	$C_{44}H_{61}N_{13}O_{12}S_2 \cdot 2AcOH \cdot 2H_2O$	41 (A) 22 (B)	Amorph.	—19,5 (0,20; 1 н. AcOH)	0,08	0,41	0,66	0,82
IV	$C_{27}H_{37}N_5O_8S$	90	66 — 68	—5,6 (1,0; ДМФА)	0,92	0,87		
V	$C_{59}H_{69}N_9O_{13}S_3 \cdot H_2O$	86	204 — 206	—28,9 (1,0; ДМФА)	0,65	0,75		
VI	$C_{57}H_{71}N_9O_{13}S_2 \cdot 0.5\ H_2O$	84,5	190 (decomp.)	—69,4 (1,0; ДМФА)	0,84	0,77		
VII	$C_{74}H_{91}N_{15}O_{16}S_4$	71	213 — 215	—22,0 (1,0; ДМФА)	0,62	0,81		
VIII	$C_{72}H_{93}N_{15}O_{16}S_3$	70	200 (decomp.)	—33,5 (1,0; ДМФА)	0,65	0,82		
IX	$C_{72}H_{88}N_{14}O_{15}S_4$	67	205 — 208[4*]	—19,4 (1,0; ДМФА)	0,66	0,83		
X	$C_{65}H_{82}N_{14}O_{13}S_3 \cdot HCl \cdot 2H_2O$	72	184 — 186	—17,8 (0,73; ДМФА)	0,30	0,75		
XI	$C_{72}H_{87}N_{13}O_{16}S_4 \cdot H_2O$	54	187 — 189	—19,6 (0,50; ДМФА)	0,61	0,72		
XII	$C_{79}H_{98}N_{14}O_{18}S_3 \cdot 2H_2O$	71	199 (decomp.)	—38,2 (1,0; ДМФА)	0,69	0,85		

*22(c0.22; 1 N AcOH) [7]. [2]*0.67 [2]. [3]*0.97 [2]. [4]*205-208° [2].

The splitting out of the protective groups from the protected peptides (VII—XII) was done by reduction with sodium in liquid ammonia in 20-30 sec. In the case of compounds (VIII) and (XII) the Boc group was eliminated beforehand. The free dithiols obtained were oxidized to the corresponding cyclic disulfides (I—III) with the aid of 1 M solution of hydrogen peroxide [6]. The performance of this reaction in highly dilute solutions (0.6-1.0 mM) at a low temperature (0-5°C) and a comparatively high pH (8.3-8.6) prevents the formation of polymeric products. The oxidative closure of the disulfide bond by hydrogen peroxide is complete in 20-30 min under these conditions. At pH values close to 7, and also on oxidation by atmospheric oxygen (pH 6.5-8.0), the reaction takes place considerably more slowly — over 5-12 h. The method of oxidation with the aid of $K_3Fe(CN)_6$ that is in wide use at the present time is distinguished by high efficiency, but in this case the subsequent purification of the peptide becomes more complicated.

The peptides (I-III) obtained were first purified (desalted) with the aid of Amberlite IRC-50 (H^+) and were then subjected to gel filtration through Sephadex G-15 in 0.2 N acetic acid. According to the results of TLC and HPLC and amino acid and elementary analyses, the compounds were obtained in analytically pure form. The yields and physicochemical properties of the compounds synthesized are given in Table 1. The low yield of analog (III) on the use of the p-nitrobenzyl ester of octapeptide (XII) (method B) is explained by a side ammonolysis reaction taking place in the liquid ammonia before the beginning of reduction by metallic sodium.

As biological tests showed, the vasopressor activity of the [8-arginine]vasopressin synthesized was 470 ± 20 IU/mg (487 ± 15 IU/mg [7]), and those of the deglycine analogs (II) and (III) were, respectively, 1.7 and 0.5 IU/mg (rat, in vivo) (see [3]).

EXPERIMENTAL

The melting points of the substances were determined in capillaries (without correction). The homogeneity of the compounds was checked by TLC on plates of silica gel 60 F_{254} (Merck) in the systems: tert-BuOH—n-BuOH—AcOH—water (2:2:1:1) (A); and n-BuOH—AcOH—pyridine—water (15:3:10:6) (B). Ninhydrin, chlorin/benzidine, and the Pauly and Sakaguchi reagents were used for the visualization of the chromatograms. Electrophoretic mobilities (E_{His}, E_{Gly}) were determined on Filtrak FN 17 paper in 5 N AcOH (pH 1.9) at a potential gradient of 30 $V \cdot cm^{-1}$ for 2 h. Analytical reversed-phase HPLC was conducted on Du Pont 830 instrument with a Zorbax C_8 column and the mobile phase acetonitrile—0.2 M ammonium acetate.

The specific optical rotations of the compounds were measured on Perkin-Elmer model 141 M polarimeter.

The peptides were hydrolyzed with 6 N HCl at 105-110°C for 20 h; the amino acid compositions of the hydrolysates were determined on a Liquimat III analyzer, no correction being made for the decomposition of the amino acids. All the optically active amino acids were of the L-configuration.

The compounds Arg(Tos) [8], Arg-$NH_2 \cdot 2HCl \cdot H_2O$ [9], Arg(Tos)-$NH_2 \cdot HBr$ [10], Boc-Arg(Mts) [11], Arg(Tos)-Gly-$NH_2 \cdot CF_3COOH$ [12], Tos-Cys(Bzl)-Tyr-Phe-N_2H_3[13], Boc-Cys(Bzl)-Tyr-Phe-N_2H_3, and Gln-Asn-Cys(Bzl)-Pro$\cdot HCl \cdot 3H_2O$ [1] were obtained by known methods.

Boc-Arg(Mts)-ONb (IV). A solution of 24.0 g (52.7 mmole) of Boc-Arg(Mts) in 100 ml of DMFA was treated with 10.2 ml (73.6 mmole) of Et_3N and 15.90 g (73.6 mmole) of p-nitrobenzyl bromide, and the mixture was heated at 60°C for 2 h and was then left at 20°C for 4 h. The precipitate was separated off by filtration, the filtrate was diluted with water, the oil was extracted with a mixture of ethyl acetate and ether (500 ml, 1:1), and the organic layer was washed with 5% $NaHCO_3$ and with water and was dried over anhydrous Na_2SO_4; the solvent was distilled off, the oil was triturated with hexane, and the resulting precipitate was filtered off, washed with hexane, and dried. Yield 27.95 g.

Tos-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro (V). A solution of 10.70 g (15.5 mmole) of Tos-Cys(Bzl)-Tyr-Phe-N_2H_3 in a mixture of 95 ml of DMFA and 6.2 ml of 5 N HCl in dioxane was cooled to —40°C, and, with vigorous stirring, 1.8 ml (15.5 mmole) of tert-butyl nitrite was added. The reaction mixture was stirred at—30°C for 25 min and was neutralized with Et_3N and then 9.94 g (15.5 mmole) of Gln-Asn-Cys(Bzl)-Pro$\cdot HCl \cdot 3H_2O$ and 2 ml of Et_3N in 50 ml of dimethyl sulfoxide were added, and, with a rise in the temperature to 0°C, the mixture was stirred for another 30 min. Then it was kept at 5°C for 48 h and, after the addition of

1 ml of $Me_2NCH_2CH_2NH_2$, it was stirred for 1 h and was diluted with 0.5 N HCl (300 ml). The precipitate was filtered off, washed with water, dried, and recrystallized from a mixture of DMFA and ethyl acetate. Yield 16.34 g.

Boc-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro (VI). This was synthesized in a similar manner to (V). From 5.42 g (8.5 mmole) of Boc-Cys(Bzl)-Tyr-Phe-N_2H_3 and 5.45 g (8.5 mmole) of Gln-Asn-Cys(Bzl)-Pro·HCl·$3H_2O$ 8.31 g of (VI) was obtained.

Tos-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (VII). At 0°C, 0.45 g (2.2 mmole) of DCHC in 5 ml of DMFA and then (after 1 H) 1.25 g (2.5 mmole) of Arg(Tos)-Gly-NH_2·CF_3COOH and 0.3 ml (2.5 mmole) of N-methylmorpholine were added to a solution of 2.45 g (2.0 mmole) of compound (V) and 0.40 g (2.2 mmole) of pentafluorophenol in 20 ml of DMFA. The mixture was stirred at 20°C for 20 h and was cooled to 0°C, the precipitate was filtered off, and the filtrate was diluted with ethanol. After 20 h (at 5°C) the new precipitate was filtered off, washed with ethanol, dried, and recrystallized from a mixture of DMFA and ethanol. Yield 2.24 g.

Boc-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (VIII). This was synthesized in a similar manner to (VII). From 3.49 g (3.0 mmole) of (VI) and 1.80 g (3.6 mmole) of Arg(Tos)-Gly-NH_2·CF_3COOH 3.17 g of (VII) was obtained.

Tos-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-NH_2 (IX). This was synthesized in a similar manner to (VII). From 1.23 g (1.0 mmole) of the heptapeptide (V) and 0.25 g (1.2 mmole) of Arg(Tos)-NH_2·HBr 1.00 g of (IX) was obtained.

Tos-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg-NH_2 (X). This was synthesized in a similar manner to (VII). From 4.9 g (4.0 mmole) of the heptapeptide (V) and 1.26 g (4.77 mmole) of Arg-NH_2·2HCl·H_2O 4.15 g of (X) was obtained (after recrystallization from a mixture of DMFA and ethyl acetate).

Tos-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos) (XI). This was synthesized in a similar manner to (VII). From 3.07 g (2.5 mmole) of the heptapeptide (V) and 0.99 g (3.0 mmole) of Arg(Tos) 2.08 g of (XI) was obtained.

Boc-Cys-(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Mts)-ONb (XII). A solution of 1.42 g (2.4 mmole) of compound (IV) in 30 ml of 3.2 N HCl in dioxane was prepared, and after 30 min it was diluted with ether (200 ml), and the resulting precipitate was filtered off, washed with ether, and dried in a vacuum desiccator over P_2O_5/KOH. From the Arg(Mts)-ONb·HCl so obtained (1.17 g) and 1.80 g (1.6 mmole) of the heptapeptide (VI) 1.89 g of compound (XII) was synthesized (in a similar manner to (VII)).

[8-Arginine]vasopressin (I). A. Compound (VII) (1.12 g; 0.76 mmole) was dissolved in liquid ammonia (200 ml) and was reduced with sodium until the appearance of a deep blue color persisting for 30 sec. Then the solution was decolorized by the addition of NH_4Cl and the ammonia was distilled off. The dry residue was dissolved in 1 liter of 0.01 N AcOH cooled to 0°C, the solution was made alkaline to pH 8.5 with aqueous ammonia, and a 1 M solution of H_2O_2 (about 5 ml) was added, the oxidation of the mercapto groups being monitored with the aid of the Ellman reagent. After the completion of the reaction (about 30 min) the solution was acidified to pH 4.5 and was desalted by deposition on a column of Amberlite IRC-50 (H^+) followed by the washing of the resin with 0.25% AcOH (800 ml). The peptide was eluted from the column with 50% AcOH (500 ml), the solution was evaporated in vacuum (at a bath temperature not above 30°C) to 20-30 ml, and it was lyophilized. The lyophilizate (600 mg) was dissolved in 5 ml of 0.2 N AcOH, deposited on a column (5.9 × 100 cm) of Sephadex G-15 fine, and eluted with 0.2 n AcOH at the rate of 120 ml/h with detection of the substance at 275 nm. The peptide was isolated by the lyophilization of the appropriate fractions, with additional drying of the lyophilizate in a vacuum desiccator over P_2O_5/KOH. Yield 0.41 g.

B. A solution of 1.52 g (1.0 mmole) of compound (VIII) in 10 ml of CF_3COOH was prepared, and after 30 min it was diluted with ether and the resulting precipitate was filtered off, washed with ether, and dried in a vacuum desiccator over P_2O_5/KOH. Reduction with sodium, oxidation, and purification were carried out as in paragraph A. Yield 0.52 g.

De-9-glycine[8-arginine]vasopressin (II). A. Under the conditions A of the synthesis of (I), 1.52 g (1.0 mmole) of compound (IX) yielded 0.48 g of (II).

B. As in method B for (I), 1.44 g (1.0 mmole) of compound (X) yielded 0.49 g of (II).

De-9-glycinamide[8-arginine]vasopressin (III). A. Under the conditions A of the synthesis of (I), 1.00 g (0.6 mmole) of the octapeptide (XI) yielded 0.29 g of (III).

B. As in method B for (I), 0.83 g (0.5 mmole) of the octapeptide (XII) yielded 0.13 g of (III).

SUMMARY

A new synthesis of [8-arginine]vasopressin and its 9-deglycine analogs has been performed and it has been shown that a unified scheme of synthesis based on the use of derivatives of homologous fragments of the neurohypophyseal hormones as common initial compounds and intermediates is effective and expedient for the preparative synthesis of these hormones.

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