Arginine-Vasotocin, a Synthetic Analogue of the Posterior Pituitary Hormones Containing the Ring of Oxytocin and the Side Chain of Vasopressin*

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Several analogues of the posterior pituitary hormones, oxytocin I and the vasopressins II and III, have been synthesized in this laboratory (1–5) in an attempt to detect possible correlations between chemical structures and biological properties in this group of compounds. Among these analogues is oxypressin IV (1), a cyclic octapeptide amide which contains a cyclic pentapeptide amide portion identical with the one existing in the vasopressins, linked to the tripeptide amide side chain that is present in oxytocin. It may be recalled that oxypressin possesses very low pressor activity, although it contains the ring structure that is present in the vasopressins. This may be attributable (2) to the absence of a strongly basic amino acid in the side chain, such as is present in the vasopressins.

It was therefore of interest to synthesize and determine the biological properties of a compound which, like oxypressin, would also be a combination of the two moieties existing in oxytocin and vasopressin but, unlike oxypressin and histidine-vasopressin, would have a strongly basic amino acid in the side chain.

**Scheme I**

\[
\begin{align*}
\text{CyS} & \text{-Tyr. Ileu. Glu-NH}_2 \text{, Asp-NH}_2, \text{CyS} & \text{- Pro. Leu. Gly-NH}_2 \\
\text{Oxytocin} \quad & \\
\text{CyS} & \text{-Tyr. Phe. Glu-NH}_2 \text{, Asp-NH}_2, \text{CyS} & \text{- Pro. Arg. Gly-NH}_2 \\
\text{Arginine-vasopressin} \quad & \\
\text{CyS} & \text{-Tyr. Phe. Glu-NH}_2 \text{, Asp-NH}_2, \text{CyS} & \text{- Pro. Lys. Gly-NH}_2 \\
\text{Lysine-vasopressin} \quad & \\
\text{CyS} & \text{-Tyr. Phe. Glu-NH}_2 \text{, Asp-NH}_2, \text{CyS} & \text{- Pro. Leu. Gly-NH}_2 \\
\text{Oxypressin} \quad & \\
\text{CyS} & \text{-Tyr. Ileu. Glu-NH}_2 \text{, Asp-NH}_2, \text{CyS} & \text{- Pro. Arg. Gly-NH}_2 \\
\text{Arginine-vasotocin} \quad & \\
\end{align*}
\]

This paper describes the synthesis and purification of such a compound. We have tentatively named the compound “arginine-vasotocin,” since it is conceivable that lysine-vasotocin might sometime be synthesized. The arginine-vasotocin would be the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-arginylglycinamide. In other words, it would be the cyclic octapeptide amide that contains a cyclic pentapeptide amide portion identical with the one existing in oxytocin I, linked to the tripeptide amide side chain that is present in arginine-vasopressin II. It will be noted that the combination of “hormone moieties” existing in arginine-vasotocin is the reverse of the one existing in oxytocin.

The synthesis of this octapeptide amide followed the pattern used in this laboratory for the synthesis of the posterior pituitary hormones (6–9) in that it involved the preparation of a protected nonapeptide amide, in this case S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-arginylglycinamide hydrobromide, cleavage of the protecting groups with sodium in liquid ammonia, and oxidation of the resulting sulphydryl nonapeptide to the cyclic octapeptide amide.

The preparation of the protected nonapeptide intermediate was accomplished by an approach similar to that used for the synthesis of arginine-vasopressin (9). For arginine-vasotocin the protected hexapeptide amide (10), carbobenzyoxyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-arginylglycinamide hydrobromide, was deprotected with HBr in acetic acid and the resulting hexapeptide amide dihydrobromide was converted to the monohydrobromide with triethylamine and oxidation of the resulting sulphydryl nonapeptide to the cyclic octapeptide amide.

This paper describes the synthesis and purification of such a compound. We have tentatively named the compound

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1 The vasopressins isolated from beef and hog posterior pituitary glands have been found to differ in amino acid composition and have been named arginine-vasopressin and lysine-vasopressin, respectively.
treated with sodium in liquid ammonia to remove the protecting groups. The reduced material obtained after evaporation of the ammonia was oxidized by aeration in dilute aqueous solution at pH 6.5 and then tested for biological activity. The solution was assayed for pressor activity in the rat (13), for avian depressor activity by the Coon method (14), and for oxytocic activity by the rat uterine strip method (15, 16), and it was found to possess all three activities.

The treatment with sodium in liquid ammonia and subsequent oxidation of a total of 1.45 gm. of crude nonapeptide in several batches gave a solution which possessed approximately 70,000 pressor units. This solution, upon concentration and lyophilization, yielded a solid which weighed 3.5 gm. and consisted of a mixture of peptide material and inorganic salts. Upon assay, this solid was found to possess a total of 25,000 pressor units which indicated that during concentration and lyophilization approximately 60 per cent of the activity was lost. A similar situation was encountered, although not to as great an extent, during synthetic studies on arginine-vasopressin (9, 17).

For purification, the crude active material was subjected to countercurrent distribution in the system 2-butanol-0.06 m-p-toluenesulfonic acid. Assay for pressor activity was used to locate the active material during the distribution. After 1440 transfers, the activity was concentrated in a single peak having a partition coefficient (K) of 0.58. The contents of the tubes containing the bulk of the material in the peak were combined, concentrated, and lyophilized to give 155 mg. of a product with approximately 150 avian depressor units per mg.

Determination of the amino acid content of this product gave the expected results. This purified material was also assayed for pressor and oxytocic activity and found to possess approximately 125 pressor units per mg. and 75 oxytocic units per mg. in relation to the U.S.P. Standard Posterior Pituitary Powder.

The high pressor activity of the compound substantiates the assumption (2) that a strongly basic amino acid in the side chain is one requirement for high pressor activity. On the other hand, the high avian depressor and oxytocic activities of arginine-vasotocin indicate the importance of an oxytocin-like ring structure in the molecule for possession of these activities in a high degree.

**EXPERIMENTAL**


The reaction mixture was stirred at 0° for 4 hours and allowed to stand overnight in the refrigerator after the addition of a few drops of acetic acid. The precipitated N,N'-dicyclohexylurea (2.3 gm., m.p. 232°) was filtered off and the filtrate was concentrated to dryness in a vacuum. The semisolid residue was triturated with 30 ml. of warm ethyl acetate and the crystallized product (4 gm.) was separated by filtration. The ethyl acetate filtrate was washed successively with water, aqueous potassium bicarbonate, water, dilute acetic acid, and water, and then it was dried over MgSO₄ and concentrated to dryness in a vacuum. The residue, after trituration with ether, amounted to 0.9 gm.

The combined crude product (4.9 gm.) was recrystallized from hot methanol to give 4.5 gm. (78 per cent) of material as long needles, m.p. 193°-195° for this compound.


To a solution of 0.5 gm. (0.75 mmole) of S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-isoleucine hydrazide (11) in a mixture of 35 ml. of acetic acid and 10 ml. of 2 N hydrochloric acid, cooled at 0°, were added 57 mg. (0.82 mmole) of sodium nitrite dissolved in 1 ml. of water. The mixture was stirred for 7 minutes at 0° and the azide was precipitated with the addition of 200 ml. of cold water. It was then filtered off and washed successively with ice-cold water, aqueous potassium bicarbonate, and water, and dried in a vacuum at 0° over P₂O₅. The product, m.p. 185°-187°, weighed 0.48 gm.

To a solution of 0.54 gm. (0.58 mmole) of the hydrazide in 7 ml. of dimethylformamide and 0.11 mmole of triethylamine was added 0.48 gm. (0.72 mmole) of the azide. The reaction mixture was stirred overnight at 5° and for 2 hours at room temperature. The product was precipitated with the addition of ethyl acetate, washed with ethyl acetate and ether, and dried; 0.64 gm. A total of 1.45 gm. of crude protected nonapeptide amide dihydrobromide were precipitated from methanol with each other; 0.7 gm.


The crude protected nonapeptide amide hydrobromide were dissolved in 250 ml. of liquid ammonia in a 250-ml. round bottom flask. The solution before concentration and lyophilization was assayed for pressor activity in the rat uterine strip method (15, 16), and it was found to possess approximately 150 avian depressor units per mg. Determination of the amino acid content of this product gave the expected results.

**EXPERIMENTAL**

**S-Benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-isoleucine Methyl Ester—A solution of 2 gm. (0.111 mole) of L-isoleucine methyl ester (19) in 30 ml. of tetrahydrofuran and 1.5 ml. of triethylamine was stirred for 15 minutes and the precipitated triethylamine hydrochloride was filtered off. The filtrate was cooled to 0° and 3.3 gm. (0.011 mole) of S-benzyl-N-tosyl-L-cysteinyl-L-tyrosine (8, 11) dissolved in 20 ml. of tetrahydrofuran were added along with 2.3 gm. of N,N'-dicyclohexylcarbodiimide.

2 The United States Pharmacopeia has assigned a value of 0.40 unit per mg. to the Posterior Pituitary Standard Powder.

1 The treatment with sodium in liquid ammonia to remove the protecting groups.

2 The United States Pharmacopeia has assigned a value of 0.40 unit per mg. to the Posterior Pituitary Standard Powder.
for pressor activity in the rat (13) and found to possess approximately 70,000 pressor units. However, after concentration and lyophilization of this solution, 3.5 gm. of solid were obtained which upon assay were found to possess a total of 28,000 pressor units.

Purification and Isolation of Active Product—The lyophilized crude product (3.5 gm.) was placed in the first 14 tubes of the all glass countercurrent distribution apparatus (21) and distributed in the system 2-butanol-0.06 M-p-toluenesulfonic acid. The progress of the purification was followed by determining the Folin color (22) and the pressor activity (13) of selected tubes. After 1440 transfers, the solvent from the tubes containing the most active segments were combined and lyophilized to give 25 mg. of white, fluffy solid. The biological assays were performed on this solid with the results already presented.

Amino acid analysis by the starch column chromatographic method (23) of the synthetic material after acid hydrolysis showed the expected composition, expressed as follows in molar ratios with the ratio for isoleucine arbitrarily taken as 1: isoleucine 1.0, tyrosine 0.9, proline 0.8, glutamic acid 1.0, aspartic acid 1.1, glycine 1.1, arginine 1.0, cystine 0.8, and ammonia 3.5.

**REFERENCES**

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