performed on Sephadex G-25 (200–270 mesh) with 0.2 M acetic acid (elution volume ~340 mL): yield following lyophilization, 50.0 mg; TLC R_f (A) 0.26, R_f (D) 0.63, R_f (F) 0.89; $[\alpha]^{20}_D$ +3° (c 1.2, 50% acetic acid). Amino acid analysis: Cys(O_3H), 1.92; Asp, 1.01; Glu, 1.00; Pro, 1.02; Gly, 1.00; Ile, 0.96; Leu, 1.02; Tyr, 0.85; NH_3, 2.08.

[4-(N^5,N^5-Dimethylglutamine),8-lysine]vasopressin (19). An aliquot of **16** (143 mg, 87 μmol) was deprotected and oxidized to the disulfide and desalted as described for **17**. The product was further purified by gel filtration on the same Sephadex G-15 column with 0.2 M acetic acid, by partition chromatography on Sephadex G-25 using the system 1-butanol–ethanol–pyridine–acetic acid–water (4:1:1:0.4:6.4): R_f 0.22; yield following lyophilization, 69.2 mg. A final gel filtration on Sephadex G-25 (block polymerizate, 200–270 mesh) using 0.2 M acetic acid (~338 mL elution volume) yielded following lyophilization 67.7 mg (76%); TLC R_f (D) 0.25, R_f (F) 0.40; $[\alpha]^{23}_D$ −30° (c 0.6, 1 N acetic acid). Amino acid analysis: Cys(O_3H), 1.91; Asp, 1.02; Glu, 1.04; Pro, 1.06; Gly, 1.00; Tyr, 0.92; Phe, 0.98; Lys, 0.99; NH_3, 1.90.

Biological Assays. Rat uterotonic assays were performed on isolated horns from virgin rats in natural estrus according to the method of Holton as modified by Munsick with the use of Mg^{2+}-free van Dyke–Hastings solution as bathing fluid.[25] For dose–response determinations on the rat uterus in vitro, the conditions were those of above. The individual injection method[26] was used with doses being increased geometrically according to a 0.5 log 10 procedure until a maximal response was reached. Details of the experimental procedure have been previously published.[27] Avian vasodepressor assays were performed on conscious White Leghorn roosters by the method of Coon as described with modifications.[28] Antidiuretic[29] and pressor[30] assays were performed on anesthetized Sprague–Dawley male rats. Whenever measurable activity was detected, the four-point design of Schild[31] was used with at least ten determinations on three animals. Compounds without measurable activity were tested in at least two animals in which posterior pituitary reference standard gave normal responses.

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[5-(N^4,N^4-Dimethylasparagine),8-lysine]vasopressin: The First 5-Position Neurohypophyseal Hormone Analogue to Retain Significant Antidiuretic Potency[1]

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In the proposed biologically active conformation of vasopressin at the antidiuretic receptor, the side-chain carboxamide group of the 5-position asparaginyl residue has been previously suggested to be the key active element in the hormone for its initiation of the antidiuretic response. [5-(N^4,N^4-Dimethylasparagine),8-lysine]vasopressin, the analogue in which the hydrogen atoms of the $-NH_2$ portion of the primary carboxamide have been replaced by methyl groups, has been synthesized and found to retain about 3% of the antidiuretic potency of lysine-vasopressin (i.e., 5.5 ± 0.3 units/mg). This result suggests that the hydrogen atoms of the carboxamide moiety are not essential for antidiuretic activity. In addition, the analogue possesses rat pressor, avian vasodepressor, and rat uterotonic potencies of 2.55 ± 0.05, 0.39 ± 0.03, and less than 0.05 units/mg, respectively.

The 5-position asparaginyl residue plays a crucial role in the proposed "biologically active" conformation of vasopressin (Figure 1) at the antidiuretic receptor.[2] The side-chain carboxamide group acting cooperatively with the basic moiety of the 8-position residue have been suggested to be the "active elements"[3] of the hormone. That the side-chain carboxamide of the asparaginyl residue may be the key active element is demonstrated by the lack of antidiuretic activity of [5-alanine]lysine-vasopressin[4] and

(1) All optically active amino acids are of the L configuration. Abbreviations used follow the recommendations of IUPAC–IUB as found in *Biochemistry* **1975**, *14*, 449, and *Biochem. J.* **1972**, 126, 773. Other abbreviations used are: Asn[$N(CH_3)_2$], N^4,N^4-dimethylasparagine; Boc, *tert*-butyloxycarbonyl; OT, oxytocin; LVP, lysine-vasopressin; DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DMF, dimethylformamide; Z, benzyloxycarbonyl; –OSu, *N*-hydroxysuccinimide ester; –ONp, *p*-nitrophenol ester; HBT, 1-hydroxybenzotriazole.

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Table I. Intermediates in the Stepwise Synthesis of [5-(N^4,N^4-Dimethylasparagine),8-lysine]vasopressin

no.	compd	coupling agent	yield, %	mp, °C	$[\alpha]^{20}_D$, deg (c 1, DMF)	TLC R_f syst A	TLC R_f syst B	anal. calcd[b]
1	Boc-Asp[$N(CH_3)_2$]-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	DCC-HBT	70	115–116	−49.5	0.68	0.81	$C_{41}H_{60}N_8O_{10}S_2$
2	Boc-Gln-Asp[$N(CH_3)_2$]-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	Boc-Gln-ONp	86	165–166	−27.5	0.51	0.75	$C_{46}H_{68}N_{10}O_{13}S_2$
3	Boc-Phe-Gln-Asp[$N(CH_3)_2$]-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	Boc-Phe-OSu	71	125–126	−32.8	0.64	0.80	$C_{55}H_{77}N_{11}O_{13}S_2$
4	Boc-Tyr(Bzl)-Phe-Gln-Asp[$N(CH_3)_2$]-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	Boc-Tyr(Bzl)-OSu	75	188–189	−35.9	0.64	0.86	$C_{71}H_{92}N_{12}O_{15}S_2$
5[c]	Boc-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asp[$N(CH_3)_2$]-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	Boc-Cys(Bzl)-OSu	88	195–196	−45.9	0.76	0.90	$C_{84}H_{101}N_{13}O_{16}S_3$

[a] Only a single spot was detected for loads of at least 50 μg. [b] C, H, and N were within ±0.4% of theory. [c] Amino acid analysis gave the following molar ratios: Asp, 1.00; Glu, 1.00; Pro, 1.07; Gly, 1.07; Tyr, 0.87; Phe, 0.98; Cys(Bzl), 1.99; Lys, 0.29; NH_3, 2.04; Lys(Tos), 0.70.

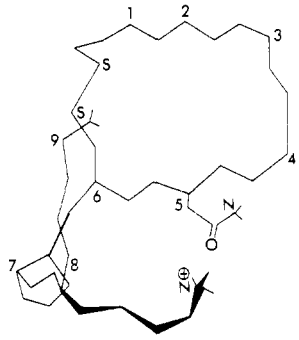

Figure 1. Schematic representation of the biologically active conformation of vasopressin showing the elements (i.e., the side-chain carboxamide of Asn[5] and the side-chain amino group of Lys[8]) responsible for the activation of the antidiuretic receptor. Numbers indicate residue positions.

[5-serine]lysine-vasopressin,[5] analogues in which the carboxyamide has been deleted or replaced by a hydrophilic hydroxyl moiety, respectively.

For oxytocin, Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2, the carboxamide of the 5-asparaginyl residue has also been named as an active element in the biologically active model of the hormone at the uterotonic receptor.[3,6] In our investigations into the role of the primary carboxamide group of the asparagine side chain of oxytocin in its uterotonic activity, [5-aspartic acid]oxytocin[7] and [5-(N^4,N^4-dimethylasparagine)]oxytocin[8] have been synthesized. These analogues, in which the hydrophilic $-NH_2$ portion of the primary carboxamide was replaced by a hydrophilic −OH or in which the hydrogen atoms of the $-NH_2$ were replaced by methyl groups, were the first 5-position neurohypophyseal hormone analogues possessing significant biological activity. As such, they suggested that the N^4-amide protons of the asparaginyl side chain are not as essential as may be the carbonyl moiety for the manifestation of uterotonic activity.

In order to determine whether similar requirements can be ascribed to the primary carboxamide group of the asparagine side chain of vasopressin for its antidiuretic activity, [5-(N^4,N^4-dimethylasparagine),8-lysine]vasopressin has been synthesized and studied biologically. The protected nonapeptide, Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asp[N-$(CH_3)_2$]-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2, was prepared in solution by stepwise elongation, beginning with Z-Cys-(Bzl)-Pro-Lys(Tos)-Gly-NH_2[9] using dicyclohexylcarbodiimide mediated with 1-hydroxybenzotriazole,[10] *p*-nitrophenyl[11] or *N*-hydroxysuccinimide[12] esters (see Table I). The protecting groups were removed by sodium in liquid ammonia,[13] and the disulfhydryl intermediate was converted to the cyclic disulfide by oxidation with 1,2-diiodoethane.[14] The hormone analogue was purified by gel filtration on Sephadex G-15,[15] partition chromatography on Sephadex G-25,[16] and a final gel filtration on Sephadex G-25.

[5-(N^4,N^4-Dimethylasparagine),8-lysine]vasopressin was found to possess a potency of 5.5 ± 0.3 units/mg in the rat antidiuretic assay;[17] the comparable value for lysine-vasopressin is 203 ± 7. In addition, it possesses 2.55 ± 0.05, 0.39 ± 0.03, and less than 0.05 units/mg in the rat pressor,[18] avian vasodepressor,[19] and in vitro rat uterotonic[20] assays, respectively. With approximately 3% of the antidiuretic potency of the parent hormone, this analogue becomes the first 5-position neurohypophyseal hormone analogue to retain significant potency in this assay.

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However, a more definitive conclusion about the effect of dimethylation of this proposed active element must await in vitro antidiuretic studies, in which the analogue can be compared with lysine-vasopressin both in binding to and in its ability to maximally stimulate the mammalian renal receptor.

Experimental Section

General procedures are given in the preceding paper on oxytocin and lysine-vasopressin analogues with N^5,N^5-dialkylglutamine in the 4 position.[21] The following systems (all by volume) were used for TLC: (A) 1-BuOH–HOAc–H_2O (4:1:5, upper phase); (B) 1-BuOH–HOAc–pyridine–H_2O (15:3:10:12); (C) 1-BuOH–pyridine HOAc–H_2O (15:10:3:6); (D) EtOAc–pyridine–HOAc–H_2O (5:5:1:3).

Stepwise Syntheses. Boc-Asp[N$(CH_3)_2$]-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (1) is an example. A sample of Z-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2[9] (0.78 g, 1.0 mmol) was treated in a solution of 10 mL of 2 N HBr for 1 h at room temperature. The hydrobromide salt was precipitated with Et_2O, filtered off, and washed with Et_2O. The salt was dissolved in DMF (3 mL), neutralized with *N*-methylmorpholine, and coupled with Boc-Asp[N$(CH_3)_2$]-OH[22] (390 mg, 1.5 mmol) preactivated[23] with 1-hydroxybenzotriazole (297 mg, 1.94 mmol) and DCC (309 mg, 1.5 mmol) in DMF (4 ml). After 0.5 h at 0 °C and 3 h at room temperature, DCU was filtered off, and the solvent was evaporated in vacuo. The remaining oily residue was triturated with 5% $NaHCO_3$, 10% citric acid, and water. Finally, the oily residue was dried over P_2O_5 and solidified from EtOAc–Et_2O (1:1, v/v). For compounds having NH_2-terminal Boc protection, this group was removed during a 1-h treatment with CF_3CO_2H. Coupling reactions were also effected by use of the appropriate active esters given in Table I. Compound **2** was purified by chromatography on silica gel using the system $CHCl_3$–CH_3OH (75:25, v/v) as the eluent. The yields, physical properties, and other analytical data are given in Table I.

[5-(N^4,N^4-Dimethylasparagine)]lysine-vasopressin (6). Compound **5** (64.5 mg, 39 μmol) was treated with sufficient sodium in anhydrous liquid ammonia until the blue color of dissolved sodium lasted 3 min without further addition. The excess sodium was discharged with a drop of HOAc and the ammonia was evaporated with a stream of N_2. The residue was dissolved in MeOH–H_2O (45:50 mL). ICH_2CH_2I (11 mg, 30 μmol) in MeOH (5 mL) was added, and the disappearance of sulfhydryl groups was monitored by the Ellman method.[24] After 15 min, HOAc (5 mL) was added and the solvents were evaporated to about 1 mL. HOAc (1 mL) was added and the solution was applied to a 2.1 × 110 cm column of Sephadex G-15 (fine) which had been equilibrated with 50% HOAc. The column was eluted with 50% HOAc at 9 mL/h and collected in 2.1-mL fractions. Peptide material was detected by monitoring the eluate at 280 nm, and fractions comprising the peak area (max at 158 mL) were pooled, evaporated, and finally lyophilized from H_2O to give 33 mg. The product was dissolved in 2 mL of the upper phase and 0.5 mL of the lower phase of the system 1-BuOH–EtOH–pyridine–HOAc–H_2O (4:1:1:0.4:6.4, v/v) and applied to a 2.1 × 52 cm column of Sephadex G-25 (block polymerizate, 100–200 mesh) which had been equilibrated with both phases of the solvent system. The column was eluted with the upper phase of the system at 14 mL/h and collected in 3.8-mL fractions. Peptide material was detected by the method of Lowry;[25] fractions comprising the major peak (max at R_f 0.21) were pooled and evaporated, and the product was lyophilized from H_2O to give 23 mg. This material was subjected to a final gel filtration on a 2.8 × 68 cm column of Sephadex G-25 (block polymerizate, 200–270 mesh) using 0.2 M HOAc to give after lyophilization 20.6 mg (44% as diacetate): $[\alpha]^{27}_D$ –64° (c 0.5, 1 M HOAc); TLC R_f (C) 0.32, R_f (D) 0.60. Amino acid analysis: Cys(O_3H), 1.92; Asp, 0.97; Gln, 1.00; Pro, 0.98; Gly, 1.00; Tyr, 0.93; Phe, 0.96; Lys, 0.96; NH_3, 2.17.

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