IR ($CHCl_3$) 1740 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (t, 3, CH_2CH_3), 1.02 (s, 3, C_1-CH_3), 1.07 (s, 3, C_1-CH_3), 1.20 (s, 3, C_{10}-CH_3), 4.13 (t, 2, OCH_2), 4.54 (m, 1, C_6-H), 4.75 (m, 1, C_2-H), and 6.58 (m, 2, olefinic). Anal. ($C_{18}H_{28}O_5$) C, H.

2-[((Cholesteryloxy)carbonyl)oxy]-1,1,10-trimethyl-6,9-epidioxy-Δ^7-octalin (17b). The sample of **17b** was prepared in a manner analogous to that described above for **17a**. Thus, 3.36 g (0.015 mol) of **16** in 5 mL of pyridine was treated with cholesteryl chloroformate (9.0 g, 30 mmol) to give, after purificiation by column chromatography and crystallization from CH_2Cl_2–hexane, 3.08 g of **17b**: mp 175–176 °C; IR ($CHCl_3$) 1740 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.67 (s, 3, $C_{18'}$-CH_3), 0.86 (d, 6, $C_{26'}$-CH_3 and $C_{27'}$-CH_3), 0.91 (d, 3, $C_{20'}$-CH_3), 1.00 (s, 3, C_1-CH_3), 1.01 (s, 3, C_1-CH_3), 1.07 (s, C_{10}-CH_3), 1.19 (s, 3, $C_{19'}$-CH_3), 4.53 (m, 1, $C_{3'}$-H), 4.90 (dd, 1, C_2-H), 5.38 (m, 1, C_6-H), and 6.55–6.69 (m, 3, olefinic). Anal. ($C_{41}H_{64}O_5$) C, H.

2-(Benzoyloxy)-1,1,10-trimethyl-6,9-epidioxydecalin (18a). To a flask containing a stirred mixture of 50 mL of CH_2Cl_2 and 50 mL of MeOH cooled in an ice–NaCl bath (~-10 °C) were added 14a (2.3 g, 0.007 mol) and potassium azodicarboxylate (27.3 g, 0.14 mol). Acetic acid (8.4 g) in 50 mL of CH_2Cl_2 was added dropwise over a period of 45 min and stirred overnight while the temperature was allowed to rise to 23–25 °C. The solid residue was removed and washed with CH_2Cl_2. The filtrate and washings were combined and evaporated to dryness to give 2.31 g of product. Recrystallization from CH_2Cl_2–pentane gave 1.94 g (84%) of **18a**: mp 146–148 °C; ^1H NMR ($CDCl_3$) δ 1.05 (s, 3, C_1-CH_3), 1.18 (s, 3, C_1-CH_3), 1.29 (s, 3, C_{10}-CH_3), 4.12 (m, 1, HCOO), 5.14 (m, 1, HCO), and 7.39–8.03 (m, 5, aromatics). Anal. ($C_{20}H_{26}O_4$) C, H.

2-[((Butyloxy)carbonyl)oxy]-1,1,10-trimethyl-6,9-epidioxydecalin (18b). The reduction of the olefinic analogue **17a** was carried out as described for the benzoyloxy analogue **14a**. Thus, to a stirred suspension of **17a** (3.24 g, 0.010 mol) and potassium azodicarboxylate (39.1 g, 0.2 mol) in 120 mL of a mixture of 50% CH_2Cl_2 and MeOH at 5 to 0 °C was added acetic acid (12.0 g, 0.2 mol) in 50 mL of CH_2Cl_2 over a period of 3.5 h. The stirred reaction mixture was allowed to warm to 23–25 °C and remained in that temperature range overnight. Enough water was added to the mixture to dissolve all of the solids. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The combined extracts were washed with NaCl solution and dried ($MgSO_4$). Evaporation of the solvent gave 3.16 g (97%) of a waxy substance, which solidified under vacuum. Recrystallization from hexanes gave 2.60 g (80%) of the target compound **18b**: mp 46–48 °C; ^1H NMR ($CDCl_3$) δ 0.93 (t, 3, CH_2CH_3), 1.03 (s, 6, C_1-CH_3), 1.23 (s, 3, C_{10}-CH_3), 4.11 (m, 3, OCH_2 and C_6-H), and 4.74 (dd, 1, C_2-H). Anal. ($C_{18}H_{30}O_5$) C, H.

2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin (18c). The reaction was carried out as described for **14a**. Thus, 2.95 g (0.011 mol) of **14b** was reduced with 42.0 g (0.22 mol) of potassium azodicarboxylate and 13.2 g (0.22 mol) of acetic acid. After workup, the residue was recrystallized from CH_2Cl_2–hexanes to give 2.21 g (74%) of **18c**: mp 131–133 °C; ^1H NMR ($CDCl_3$) δ 0.97 (s, 3, C_1-CH_3), 1.02 (s, 3, C_1-CH_3), 1.24 (s, 3, C_{10}-CH_3), 2.02 (s, 3, $COCH_3$), 4.1 (m, 1, HCOO), and 4.89 (dd, 1, C_2-H). Anal. ($C_{15}H_{24}O_4$) C, H.

2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin (18d). Compound **18d** was prepared as described for **18a–c**. Thus, **16** (2.8 g, 0.0125 mol) with potassium azodicarboxylate (46.8 g, 0.24 mol) and acetic acid (14.4 g, 0.24 mol) gave, after recrystallization from CH_2Cl_2–pentane, 2.51 g (87%) of **18d**: mp 148–149 °C; ^1H NMR ($CDCl_3$) δ 0.95 (s, 3, C_1-CH_3), 1.08 (s, 3, C_1-CH_3), 1.22 (s, 3, C_{10}-CH_3), 3.68 (dd, 1, CHOH), and 4.08–4.18 (m, 1, HCOO). Anal. ($C_{13}H_{22}O_3$) C, H.

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Registry No. **4**, 108511-73-5; **6**, 57069-88-2; **7** (isomer 1), 108511-74-6; **7** (isomer 2), 108590-48-3; **8**, 826-56-2; **9**, 4668-61-5; **10**, 91975-35-8; **11a**, 102454-53-5; **11b**, 108511-75-7; **12a**, 108511-75-7; **12a**, 108511-76-8; **12b**, 108511-77-9; **13a**, 94759-83-8; **13b**, 101100-51-0; **14a**, 108511-78-0; **14b**, 108511-79-1; **15**, 100532-28-3; **16**, 108511-80-4; **17a**, 108511-81-5; **17b**, 108511-82-6; **18a**, 108511-83-7; **18b**, 108533-24-0; **18c**, 108511-84-8; **18d**, 108511-85-9; MeI, 74-88-4; C_6H_5COCl, 98-88-4; $ClCO_2C_4H_9$, 592-34-7; cholesteryl chloroformate, 7144-08-3.

Synthesis and Biological Activities of Arginine-vasopressin Analogues with 4-Hydroxyproline in Position 7[†,‡]

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Three arginine-vasopressin (AVP) analogues in which the proline residue in position 7 was substituted with 4-hydroxyproline were synthesized by solid-phase techniques, and their biological activities were evaluated by antidiuretic, pressor, and uterotonic bioassays. The [7-*trans*-4-hydroxy-L-proline]AVP, the 1-desamino[7-*trans*-4-hydroxy-L-proline]AVP, and the 1-desamino[7-*cis*-4-hydroxy-L-proline]AVP analogues showed a high antidiuretic and strikingly high uterine activity, a sharp decrease in pressor activity, and a better antidiuretic and uterine to pressor selectivity than the parent compound, arginine-vasopressin. The uterine activities are the highest so far assayed in AVP analogues with replacements in position 7.

Analogues of the neurophypophyseal hormone arginine-vasopressin that have substitutions for proline in position 7 (the first residue in the tripeptide -Pro-Arg-GlyNH$_2$ "tail" portion of this molecule) are of considerable interest for structure–activity studies. The proline residue is important in positioning the "tail" portion of vasopressin correctly in relation to the "ring" portion of this molecule and thus is very important for biological activity. All replacements of the naturally occurring proline that have been made to date drastically alter the activity and/or specificity of vasopressin. Some 7-substituted analogues, such as [7-glycine]AVP and [7-leucine]AVP[1,2] have de-

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† Abbreviations follow the recommendations of IUPAC–IUB Commission on Biochemical Nomenclature (*Int. J. Pept. Protein Res.* 1984, *24*, 9). Additional abbreviations are as follows: MeBzl, methylbenzyl; *N*-MeAla, *N*-methylalanine; Hyp, 4-hydroxyproline; AcOH, acetic acid; 1-BuOH, 1-butanol; *i*-PrOH, 2-propanol; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; HOBt, 1-hydroxybenzotriazole; DIPEA, diisopropylethylamine; AVP, arginine–vasopressin; d, 1-desamino; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography.

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Table I. Biological Activities of 7-Hydroxyproline-Substituted and Other 7-Substituted Arginine-vasopressin Analogues

	biological act. in units/mg			
			uterus in vitro	
analogue	antidiuretic	pressor	no Mg^{2+}	Mg^{2+}
[7-*trans*-4-Hyp]AVP	712	4	73	268
d[7-*trans*-4-Hyp]AVP	780	1	225	285
d[7-*cis*-4-Hyp]AVP	614	8	63	176
[7-3,4-didehydroproline]AVP[a]	1260	255	41	
d[7-3,4-didehydroproline]-AVP[a]	4134	240	69	
AVP[b]	323	369	12	
dAVP[c]	1745	346	28	
[7-*N*-methylalanine]AVP[d]	343	11	3	4
d[7-*N*-methylalanine]AVP[d]	113	1	2	8
[7-sarcosine]AVP[d]	188	4	9	36
d[7-sarcosine]AVP[d]	580	1	38	49
[7-leucine]AVP[e]	19	–	–	–
[7-glycine]AVP[f]	0.01	0.1		

[a] Ref 3. [b] Ref 26. [c] Ref 27. [d] Ref 4. [e] Ref 2. [f] Ref 1.

creased biological activities. Other 7-substituted analogues, such as [7-3,4-didehydroproline]AVP[3], [7-sarcosine]AVP[4], and [7-*N*-methylalanine]AVP,[4] exhibit activities or selectivity greater than those of the parent compound. [7-3,4-Didehydroproline]AVP, an analogue that retains the conformational restraint imposed by an intact pyrrolidine ring in position 7 and in addition has enhanced electronegativity in that position, is highly potent but only moderately selective compared to vasopressin. Analogues without the conformational restraint imposed by the pyrrolidine ring in position 7 ([Gly^7]AVP and [Leu^7]AVP) have low activity. Analogues in which a part of the conformational constraint of the pyrrolidine ring has been restored by putting a methyl group on the amide nitrogen in position 7 ([Sar^7]AVP and [*N*-MeAla^7]AVP) show moderately high but very selective antidiuretic activity. To continue investigations into the structural and electronic features in position 7 that determine activity and specificity, we synthesized three analogues that contained hydroxyproline in position 7. This amino acid retains the pyrrolidine ring and consequent conformational restraint of the naturally occurring peptide but in addition has electronegatively on the pyrrolidine ring, like didehydroproline. It is, therefore, a good choice for testing generalizations that might be hypothesized from the activities of existing 7-substituted analogues. We report here the synthesis and biological activities of [7-*trans*-4-hydroxy-L-proline]AVP, 1-desamino[7-*trans*-4-hydroxy-L-proline]AVP, and 1-desamino[7-*cis*-4-hydroxy-L-proline]AVP.

Bioassays. Antidiuretic activities were assayed on water-loaded, ethanol-anesthetized rats according to the procedures of Sawyer.[5] Pressor activities were assayed on urethane-anesthetized, phenoxybenzamine-treated rats as described by Dekanski.[6] Uterine activity in vitro was assayed on the isolated rat uterus in solutions with 0.5 mM Mg^{2+} or no Mg^{2+}.[7]

Peptide Synthesis. Analogues were prepared by stepwise solid-phase synthesis[8,9] on a benzhydrylamine hydrochloride (BHA) resin[10] by using a Vega 96 automatic peptide synthesizer (see Experimental Section for details).

Results and Discussion

Table I compares the biological activities of the three hydroxyproline compounds to those of other relevant analogues. All three analogues have high antidiuretic activity like [7-3,4-didehydrohydroxyproline]AVP but also have selectivity like the analogues that have methyl groups on the amino nitrogen in position 7. Antidiuretic activities are greater than that of vasopressin, pressor activities are sharply decreased, and uterine activities are strikingly higher than that of the parent compound. The *cis*-hydroxyproline enantiomer is less active than the trans but not strikingly so. Although the deaminated hydroxyproline analogues have high antidiuretic activity, the antidiuretic effects of hydroxyproline substitution and deamination are not additive.

Proline, because of the conformational constraints of the pyrrolidine ring, is of particular importance in many peptide hormones as well as in vasopressin for their receptor interactions.[11,12] In a proposed model for a biologically active conformation of vasopressin,[13] proline is a corner residue in the β-turn between positions 7 and 8 of this molecule and is therefore very exposed and available for interaction with the receptor. Proline also plays an important role in the spatial orientation of the "tail" tripeptide in vasopressin, with respect to the cyclic moiety. The orientation of the "tail" with respect to the "ring" is thought to be important for specificity as well as for activity. Substituting hydroxyproline for proline produced analogues with high antidiuretic activity. One can assume that hydroxyproline with its potential hydrogen bond and greater electronegativity provides better binding and a better steric fit to this receptor. Conformational changes of the peptide backbone are probably not involved.

Low pressor activity of the hydroxyproline analogues is in agreement with the observation (from the results for many synthetic analogues) that pressor receptors are highly intolerant of changes in position 7 or in any other portion of the "tail" component of vasopressin. An intact and unsubstituted proline ring seems to be required in position 7. The 7-*cis*-hydroxyproline analogue is somewhat less active than the trans. As the only difference between the two analogues is the configuration of the OH group, it is probably this configuration that either forms a less "suitable" hydrogen bond to the receptor or introduces a degree of steric hindrance to binding.

The high uterine activity exhibited by the hydroxyproline analogues is the most surprising result of this substitution. In contrast to [7-hydroxyproline]AVP, [7-hydroxyproline]oxytocin[14] has been reported to be inactive in the rat uterus. Conformational studies show the "tail" portion of oxytocin to be more closely positioned over the "ring" than the "tail" of vasopressin, but this observation

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in no way explains the contrasting uterine activities of [7-hydroxyproline]AVP and [7-hydroxyproline]oxytocin.

Synthetic modifications combining the very active and specific 7-hydroxyproline analogues with additional changes in other positions of the vasopressin molecule may be of great interest in structure–activity studies and in the design of active, selective, and perhaps long-acting analogues for pharmacological purposes.

Experimental Section

Thin-layer chromatography (loads 10–50 μg; chromatogram lengths 10–130 mm) was performed on silica gel GF (Analtech, Newark, De). Spots were detected by chlorination followed by treatment with 1% aqueous KI–starch solution. The following solvent systems (v/v) were used: (A) 1-BuOH–pyridine–AcOH–water (15:10:3:6); (B) ethyl acetate–AcOH–pyridine–water (5:1:5:3); (C) 1-BuOH–pyridine–AcOH–water (15:10:3:12); (D) 1-BuOH–AcOH–water (4:1:1); (E) methanol–ethyl acetate (4:1). Optical rotations were measured with a Carl Zeiss polarimeter (±0.001°). For amino acid analysis the analogues were hydrolyzed for 22 h at 110° C with 6 N HCl containing 0.3% phenol in sealed, evacuated tubes and were then analyzed on a Beckman 6300 high-performance amino acid analyzer. Hydroxyproline elutes in the same position as aspartic acid when the standard buffer (pH 3.2) for amino acid analysis is used. In order to detect hydroxyproline, therefore, and calculate its ratio, the buffer for the amino acid analysis was changed to pH 2.8.

Reverse-phase isocratic HPLC analysis was on a Vydac C-18 RP column for peptides and proteins (1 cm × 25 cm) in an Altex-Beckman instrument (322MPLC) connected with a Hitachi 100-40 multiple-wavelength UV detector and a Hewlett-Packard No. 3380A integrator. The solvent mixtures were 6% 2-propanol in 0.1% TFA for the amino compound and 8% 2-propanol in 0.1% TFA for the desamino compounds, with a flow rate of 4 mL/min at A_{215}, 0.1 AUFS. Peptides were synthesized by stepwise soldphase Merrifield techniques.[8,9] The N^{α} and side-protected amino acids used, except glycine, were all in the L configuration and were purchased from Bachem (Torrance, CA) or from Peninsula Laboratories (San Carlos, CA). All solvents and reagents were of analytical grade or were purified according to procedures recommended for solid-phase peptide synthesis.[15]

The side-chain protections were N^G-tosyl (arginine), *O*-benzyl (*trans*-4-hydroxy-L-proline), and *S*-4-methylbenzyl (L-cysteine and β-mercaptopropionic acid).[16] Boc-L-tyrosine was used unprotected. Boc-*cis*-hydroxyproline was prepared from *cis*-hydroxyproline[17] that was acylated with di-*tert*-butyl dicarbonate. It showed the following analytical data: mp 142–144 °C; R_f 0.58 (D), 0.69 (E); NMR (CD_3COCD_3) δ 1.5 (9 H, *t*-Bu), 2.1–2.4 (2 H, CH_2), 3.3–3.5 (2 H, CH_2), 4.2 (1 H, CH); yield = 60%.

Boc-*cis*-hydroxyproline was used in the solid-phase synthesis without hydroxyl protection, as reported in other syntheses of peptides containing hydroxyproline.[18,19] No indication of any side reaction could be detected.

Cys(MeBzl)-Tyr-Phe-Gln-Asn-Cys(MeBzl)-Hyp(Bzl)-Arg(Tos)-Gly-resin and β-Mercaptopropionyl(MeBzl)-Tyr-Phe-Gln-Asn-Cys(MeBzl)-Hyp(Bzl)-Arg(Tos)-Gly-resin. Syntheses were performed on a Vega 96 automatic peptide synthesizer using BHA resin prepared from polystyrene 1% divinylbenzene[10] (Beckman Chemicals). A well-established synthetic protocol for vasopressin analogues has been utilized in the present work.[16] Briefly, 1 g of 1% cross-linked BHA resin (0.51 mequiv of amine content/g of resin) was subjected to the following deprotection, neutralization, and coupling steps and washings for every synthetic cycle for each amino acid.

Step 1. Deprotection: CH_2Cl_2/30 mL, 4 × 1 min; 50% TFA–CH_2Cl_2–2% anisole/30 mL, 1 × 2 min; 50% TFA–CH_2Cl_2–2% anisole/30 mL, 1 × 30 min; CH_2Cl_2/30 mL, 4 × 1 min.

Step 2. Neutralization: 5% DIPEA–CH_2Cl_2/25 mL, 3 × 2 min; CH_2Cl_2/30 mL, 4 × 1 min; *i*-PrOH/25 mL, 4 × 1 min; CH_2Cl_2/30 mL, 4 × 1 min or DMF/30 mL, 4 × 1 min for HOBt–DCC couplings.

Step 3. Coupling: Boc-AA–DCC/15 mL CH_2Cl_2 as symmetrical anhydride 1 × 15 min or Boc-AA–HOBt–DMF/15 mL, DCC–CH_2Cl_2/10 mL, 1 × 180 min; DMF/30 mL, 4 × 1 min; CH_2Cl_2/30 mL, 4 × 1 min; 1-PrOH/25 mL, 4 × 1 min; CH_2Cl_2/30 mL, 4 × 1 min.

For the incorporation of glycine, the synthesis started at step 2 and coupling was with a 5-fold excess of Boc-glycine and a 5-fold excess of DCC. The same coupling procedure was used for Boc-tyrosine.

The coupling reaction for Boc-N^G-tosylarginine, Boc-asparagine, Boc-glutamine, Boc-O-benzylhydroxyproline, and Boc-*cis*-4-hydroxyproline utilized a 4-fold molar excess of protected amino acids, HOBt in DMF, and DCC in CH_2Cl_2 preactivated for 10 min after each addition at 0 °C.[20,21] Boc-phenylalanine and Boc-*S*-(4-methylbenzyl)cysteine were coupled as their symmetrical anhydrides,[22] followed for Boc-phenylalanine by a repeated coupling with HOBt–DCC. The completeness of the acylation reaction was checked by the ninhydrin test.[23]

[Hyp⁷]AVP and Desamino[Hyp⁷]AVP. The dried polymer carrier (1.2g) was cleaved from the peptide with hydrogen fluoride–*p*-cresol (9:1) for 1 h at 0 °C and washed with ether, and the free peptides were extracted from the resin with 0.2 N acetic acid. The acetic acid solution was diluted with degassed methanol–water (2:1) to a volume of 600 mL and adjusted to pH 8 with ammonia, and the sulfhydryl bonds were oxidized with diiodomethane in methanol[24] (1 equiv based on the amine content/gram of starting resin) to form the cyclic disulfide. The oxidation was monitored by the Ellman Test,[25] and after 10 min, the solution was acidified and reduced in vacuo to a small volume.

For purification, the peptides were subjected to desalting chromatography on Sephadex G-15 in 50% acetic acid and partition chromatography in the solvent system 1-BuOH–ethanol–aqueous 3.5% AcOH containing 1.5% pyridine (4:1:5 by volume) as detailed previously.[16]

Final purification was performed on Sephadex G-15 in 0.2 N AcOH. Yields were between 160 and 175 mg (36–40%) based on the initial 0.51 mequiv of amine groups/g of resin.

The peptides were characterized by amino acid analyses, optical rotation, TLC in solvent systems A, B, and C, and HPLC. They showed the following analytical data.

[Hyp⁷]AVP: TLC R_f 0.30 (A), 0.75 (B), 0.52 (C); $[\alpha]^{25}$ −139° (*c* 0.1, 1 N AcOH); amino acid analysis Asp 1.03, Gln 1.03, Gly 1.04, Cys 1.88, Tyr 0.93, Phe 0.99, Arg 1.01, Hyp 1.08; HPLC t_R (min) 14.8, k' = 2.8.

d[Hyp⁷]AVP: TLC R_f 0.34 (A), 0.67 (B), 0.52 (C); $[\alpha]^{25}$ −69.3 (*c* 0.15, 1 N AcOH); amino acid analysis Asp 0.93, Gln 1.08, Gly 1.06, Cys 1.30, Tyr 0.96, Phe 0.96, Arg 1.02, Hyp 1.04; HPLC t_R (min) 13.5, k' = 2.8.

d[*cis*-Hyp⁷]AVP: TLC R^f 0.41 (A), 0.78 (B), 0.57 (C); $[\alpha]^{25}$ −54.09° (*c* 0.1, 1 N AcOH); amino acid analysis Asp 1.08, Gln 1.05, Gly 1.09, Cys 1.19, Tyr 0.92, Phe 0.91, Arg 0.94; HPLC t_R (min) 11.5, k' = 2.28.

When the carboxyl group and the hydroxyl group at position

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4 of hydroxyproline are in the cis configuration, this amino acid partially forms a lactone upon treatment with acids. The existence of this lactone was detected as a cathodic band on paper electrophoresis (pyridine–acetic acid–water, 10:1:89 v/v/v) at pH 6.5, a position where the neutral hydroxyproline does not migrate. *cis*-Hydroxyproline as an amino acid was observed clearly as a mixed peak with aspartic acid in the amino acid analysis at pH 3.2 and 2.8.

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Registry No. [7-*trans*-4-Hyp]AVP, 108666-16-6; d[7-*trans*-4-Hyp]AVP, 108666-17-7; d[7-*cis*-4-Hyp]AVP, 108739-48-6; H-*cis*-Hyp-OH, 618-27-9; BOC-*cis*-Hyp-OH, 87691-27-8; BOC-Gly-OH, 4530-20-5; BOC-Tyr-OH, 3978-80-1; BOC-Arg(N^G-tosyl)-OH, 13836-37-8; BOC-Asn-OH, 7536-55-2; BOC-Gln-OH, 13726-85-7; BOC-Hyp(*O*-benzyl)-OH, 54631-81-1; (BOC-Phe)$_2$O, 33294-54-1; [BOC-Cys(MeBzl)]$_2$O, 85097-53-6.

Structure–Activity Studies on the C-Terminal Hexapeptide of Substance P with Modifications at the Glutaminyl and Methioninyl Residues

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Analogues of [Orn[6]]-SP_{6-11} have been synthesized in which the SCH_3 group of the Met[11] side chain is replaced by other functional groups, such as $(CH_2)_2NH_2$, COOH, $CONH_2$, and COOR, which have basic, acidic, or neutral character and which may act as either H-bonding donors or H-bonding acceptors. These analogues were tested in guinea pig ileum and rat colon muscularis mucosae, in vitro. Substitution of Lys, Gln, or Glu at position 11 caused a marked reduction in biological activity in both tissues. In contrast, the glutamate benzyl ester analogue had only slightly reduced activity in the guinea pig ileum and an increased (4.7 times) activity in the rat colon. It is concluded that charged groups in the side chain at position 11 of SP_{6-11} reduce the biological activity of SP hexapeptide.

The C-terminal hexapeptide amide of substance P (SP_{6-11}, H-Gln-Phe-Phe-Gly-Leu-Met-NH_2) is the minimal peptide fragment that retains substantial SP-like agonist activity in most pharmacological tests.[1] This applies to gastrointestinal smooth muscle[2,3] and salivary secretion[4] and to the hypotensive effects of this group of peptides.[5] In neuronal preparations (rat spinal cord and rat superior cervical ganglion, in vitro), the C-terminal hepta- and hexapeptides are somewhat more active than the parent undecapeptide.[6,7] However, the N-terminal residues should not be considered redundant since they may well contribute to the potency of the compound in some test systems, while in others, there is an absolute requirement for the N-terminal basic residues.[8,9] In view of the importance of the C-terminal hexapeptide for biological activity, this sequence should provide a basis for examining some aspects of the structure–activity relationship for tachykinin agonists.

In previous studies, it has been shown that the methionine residue is one of the most important elements for activity of SP and related peptides.[1,10] Two key elements of this amino acid, the α-carboxamide group and the side chain, appear to be important in determining the active conformation of the molecule. In the present work, we investigated the nature of the side chain of methionine at position 11 and its contribution to the biological activity of SP_{6-11}. In our investigation, we used as a model peptide the analogue [Orn[6]]-SP_{6-11}, which is almost equipotent with the parent hexapeptide in smooth muscle preparations, has high solubility, and avoids problems associated with formation of the pyroglutaminyl derivative from the Gln[11] residue at position 6. We designed analogues in which the SCH_3 group of methionine was replaced by acidic, basic, or neutral groups that can also act as either H-bonding donors or H-bonding acceptors.

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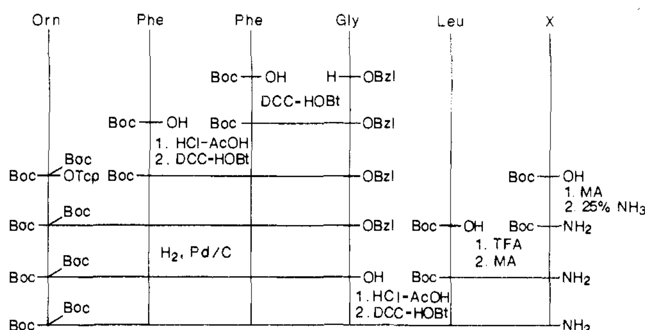
Scheme I. Synthesis of the C-Terminal Hexapeptide of SP Analogues[a]



[a] X = Lys(Z), Glu(OBzl), Gln.

The synthesized analogues were tested in two smooth muscle preparations (guinea pig ileum and rat colon muscularis mucosae, in vitro) representative of the proposed "SP-P" and "SP-E" subtypes of tachykinin recep-

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