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Design of Potent Antagonists of the Vasopressor Response to Arginine-vasopressin

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As part of a program in which we are attempting to design and synthesize antagonists of the vasopressor response to arginine-vasopressin (AVP), [1-deaminopenicillamine]arginine-vasopressin (dPAVP), [2-(*O*-methyl)tyrosine]-arginine-vasopressin [Tyr(Me)AVP], and [1-deaminopenicillamine,2-(*O*-methyl)tyrosine]arginine-vasopressin [dPTyr(Me)AVP] were synthesized by the solid-phase method and assayed for vasopressor, antidiuretic, and oxytocic activities. Tyr(Me)AVP has a vasopressor potency of 9.7 ± 0.5 units/mg and an antidiuretic potency of 386 ± 36 units/mg. These values are 2.5 and 120%, respectively, of the corresponding potencies of AVP. The analogue is an antagonist of the in vitro response to oxytocin (pA_2 = 7.44 ± 0.12). dPAVP has an antivasopressor pA_2 of 7.45 ± 0.11. Its antidiuretic potency is 42.2 ± 2 units/mg, 2.5% that of its parent, 1-[deamino]arginine-vasopressin (dAVP). It is an antagonist of the in vitro response to oxytocin (pA_2 value = 6.93 ± 0.10). dPTyr(Me)AVP has an antivasopressor pA_2 of 7.96 ± 0.05 and an antidiuretic potency of 3.5 ± 0.5 units/mg. It is also an antagonist of the in vitro oxytocic response to oxytocin (pA_2 value = 7.61 ± 0.14). It is thus one of the most potent vasopressor antagonists reported to date.

We recently described the synthesis and properties of [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin (dPVDAVP).[1] This peptide was designed to explore the possibility that the substitution of two methyl groups for the two hydrogens on the β-carbon at position 1 of [1-deamino,4-valine,8-D-arginine]vasopressin (dVDAVP) might convert this potent antidiuretic agonist into an antagonist of the antidiuretic response. A similar substitution in oxytocin[2] and in deamino-oxytocin[2] had converted these oxytocic agonists into potent antagonists of the in vitro oxytocic response. Furthermore, [1-deaminopenicillamine]oxytocin was shown to be a weak antagonist of the vasopressor response to lysine-vasopressin (LVP).[3] Although not an antagonist of the antidiuretic response, dPVDAVP was found to be one of the most potent antagonists of the vasopressor response to arginine-vasopressin (AVP) yet reported.[1]

The high antivasopressor potency of dPVDAVP encouraged us to undertake a systematic investigation of those structural changes in AVP which would facilitate the design of analogues of AVP possessing potent and selective antivasopressor properties. The approach we have followed is based essentially on that utilized in the design of dVDAVP[4] and of [4-threonine,7-glycine]oxytocin,[5] peptides which exhibit highly potent and selective antidiuretic and oxytocic activities, respectively. These peptides were designed by combining in each one those structural changes which individually in analogues of AVP and oxytocin enhanced antidiuretic and oxytocic selectivity relative to AVP and oxytocin, respectively. Our objective then was (a) to determine which structural changes in AVP would individually produce analogues possessing reduced or antagonistic vasopressor properties and (b) to combine these in one molecule in the hope that their effects would be additive and thereby produce an antagonist with greater antivasopressor potency than the individual parent analogues. In this report we present the synthesis and some pharmacological properties of three analogues designed in this fashion: [1-deaminopenicillamine]arginine-vasopressin (dPAVP), [2-(*O*-methyl)tyrosine]arginine-vasopressin [Tyr(Me)AVP], and [1-deaminopenicillamine,2-(*O*-methyl)tyrosine]arginine-vasopressin [dPTyr(Me)AVP]. The rationale for selecting these three to start with was as follows.

(a) Penicillamine Substitution. The penicillamine residue has been substituted in oxytocin,[2] in deamino-oxytocin,[2] and in dVDAVP[1] but it has not been substituted in AVP, in LVP, or in their deamino derivatives. In dVDAVP, as we have seen, it led to a dramatic and selective increase in antivasopressor properties. As a starting point for these studies, therefore, it seemed highly appropriate to determine its effects on the properties of the highly active antidiuretic agonist, [1-deamino]arginine-vasopressin (dAVP).[6,7] dPAVP was chosen for synthesis on this basis.

(b) *O*-Methyltyrosine Substitution. Methylation of the phenolic hydroxyl group of the tyrosine residue at position 2 in oxytocin, besides leading to antioxytocic properties, also gave rise to weak antivasopressor activity in the resultant [2-(*O*-methyl)tyrosine]oxytocin.[8-11] This same substitution in LVP led to a drastic reduction in vasopressor potency [from 258 units/mg in LVP to 2.4 units/mg in [2-(*O*-methyl)tyrosine]lysine-vasopressin[12-14]] coupled with marked tachyphylaxis of the vasopressor response to the analogue and inhibition of the response to vasopressin.[15] We were thus curious to determine the effects of the *O*-methyltyrosine substitution on the vasopressor properties of AVP. Tyr(Me)AVP was selected for synthesis on this basis.

(c) Combination of Penicillamine Substitution and 2-*O*-Methyltyrosine Substitution. No analogue of oxytocin or of vasopressin containing the combination of these two changes has been reported. We were intrigued by the possibility that their effects might be additive. If so, we would produce a peptide with even greater antivasopressor potency than either dPAVP or Tyr(Me)AVP.

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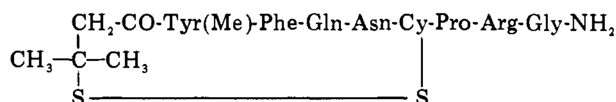
Table I. Pharmacological Properties of [1-Deaminopenicillamine]arginine-vasopressin (dPAVP), [2-(*O*-Methyl)tyrosine]arginine-vasopressin [Tyr(Me)AVP], and [1-Deaminopenicillamine,2-(*O*-Methyl)tyrosine]arginine-vasopressin [dPTyr(Me)AVP]

peptide	antivasopressor pA_2[a]	pressor act., units/mg	antidiuretic act., units/mg	antioxytocic pA_2[a]
dPAVP	7.45 ± 0.11 (8)	anti	42 ± 3	6.93 ± 0.10 (14)
Tyr(Me)AVP		9.7 ± 0.5	386 ± 36	7.44 ± 0.12 (18)
dPTyr(Me)AVP	7.96 ± 0.05 (14)	anti	3.5 ± 0.5	7.61 ± 0.14 (14)

[a] pA_2 values were obtained as described in Manning et al.[1] by the method of Schild[26] by calculating a pA_2 for each assay group; the figures presented are the means ± SE of these. The numbers in parentheses indicate the number of assays.

We thus decided to incorporate both of these changes in dAVP to give dPTyr(Me)AVP. It has the following structure.



We now describe the synthesis and some chemical and pharmacological properties of these three analogues designed according to the above rationale.

Peptide Synthesis. Solid-phase peptide synthesis[16-20] was employed to prepare protected octapeptide amide derivatives of dPAVP and dPTyr(Me)AVP. Removal of the N-terminal protecting group from the protected octapeptides and coupling with β-(*S*-benzylmercapto)-β,β-dimethylpropionic acid[2] by the dicyclohexylcarbodiimide (DCCI)–*N*-hydroxybenzotriazole (HOBt) preactivation method[21,22] yielded the immediate precursors of dPAVP and of dPTyr(Me)AVP. In the case of Tyr(Me)AVP, the protected nonapeptide was synthesized entirely on the resin. Deblocking of all three intermediates was effected with Na in liquid NH_3[23] as previously described[4,18] and the resulting disulfhydryl compounds were subjected to oxidative cyclization with $K_3[Fe(CN)_6]$.[24] The analogues were purified by gel filtration on Sephadex G-15.[25]

Bioassay Methods. Agonistic and antagonistic properties of these analogues were estimated by previously described methods.[1] These included intravenous antidiuretic assays in rats under ethanol anesthesia and vasopressor assays in phenoxybenzamine-treated rats under urethane anesthesia and assays on the isolated rat uterus suspended in a medium containing no Mg^{2+}. Agonistic activities are expressed in units per milligram. Antagonistic activities are expressed as pA_2 values.[26] pA_2 values have been defined as the negative logarithm to the base 10 of the average molar concentration of an antagonist which will reduce the biological response to $2x$ units of agonist to equal the response given by x units of agonist in the absence of antagonist.

Results and Discussion

Some of the pharmacological properties of dPAVP, Tyr(Me)AVP, and dPTyr(Me)AVP are summarized in Table I. Comparisons with some of the previously reported properties of related peptides are presented in Table II. In looking first at their vasopressor properties it can be seen that dPAVP is a relatively potent antivasopressor peptide and that Tyr(Me)AVP, although not an antagonist of the vasopressor response, has greatly reduced pressor activity, i.e., less than $^1/_{30}$th that of AVP. Of real significance is the finding that dPTyr(Me)AVP is a highly potent antivasopressor agent with a pA_2 value of 7.96 ± 0.05. Thus the combination of penicillamine and *O*-methyltyrosine substitutions has resulted in a threefold enhancement of antivasopressor potency and a tenfold reduction of antidiuretic potency of dPTyr(Me)AVP as compared to dPAVP. It is one of the most potent antivasopressor peptides reported to date (Table II).

Table II. Biological Properties of Some Neurohypophysial Peptide Analogues Containing (a) 1-Penicillamine Substitution and (b) *O*-Methyltyrosine Substitution at Position 2 and Related Peptides

peptide[l]	antivasopressor pA_2	vasopressor act., units/mg	antidiuretic act., units/mg
dAVP[a]		346 ± 13	1745 ± 385
dPAVP[b]	7.45 ± 0.11	anti	42 ± 3
dVDAVP[c]	7.03 ± 0.11	anti	1230 ± 170[k]
dPVDAVP[c]	7.82 ± 0.05	anti	123 ± 22
dOT[d]		1.44 ± 0.06	19
dPOT[e]	6.27	anti	
AVP[a]		369 ± 6	323 ± 16
Tyr(Me)AVP[b]		9.7 ± 0.5	386 ± 36
LVP[f]		285 ± 5	212 ± 13
Tyr(Me)LVP[g]		2.4 ± 0.7	79 ± 11
OT[h]		4.3 ± 0.12	4.0 ± 0.8
Tyr(Me)OT[i]		anti	0.01
dPTyr(Me)AVP[b]	7.96 ± 0.05	anti	3.5 ± 0.5
$d(CH_2)_5$VDAVP[j]	7.68 ± 0.05	anti	0.10 ± 0.02

[a] From Manning et al.[7] [b] This publication. [c] From Manning et al.[1] [d] From Hope et al.[24] [e] From Nestor et al.[3] [f] Values are those for a sample synthesized by the solid-phase method (W. H. Sawyer, J. Lowbridge, and M. Manning, unpublished results). [g] From Zaoral et al.[13] [h] Values reported by Manning et al.[36] [i] From Law et al.[8] [j] From Lowbridge et al.[37] [k] From Manning et al.[4] [l] The abbreviations for arginine-vasopressin (AVP) and for oxytocin (OT) analogues follow the system previously suggested.[38] The abbreviations and their full names are as follows: dAVP, [1-deamino]arginine-vasopressin; dPAVP, [1-deaminopenicillamine]arginine-vasopressin; dVDAVP, [1-deamino,4-valine,8-D-arginine]vasopressin; dPVDAVP, [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin; dOT, [1-deamino]oxytocin; dPOT, [1-deaminopenicillamine]oxytocin; Tyr(Me)AVP, [2-(*O*-methyl)tyrosine]-arginine-vasopressin; LVP, lysine-vasopressin; Tyr(Me)-LVP, [2-(*O*-methyl)tyrosine]lysine-vasopressin; Tyr(Me)-OT, [2-(*O*-methyl)tyrosine]oxytocin; dPTyr(Me)AVP, [1-deaminopenicillamine,2-(*O*-methyl)tyrosine]arginine-vasopressin; $d(CH_2)_5$VDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin.

The effects of these substitutions alone and in combination on antidiuretic activities are also of interest. These effects do not follow a consistent pattern either in the direction or in the degree of change (Table II). It can be seen that the penicillamine substitution in the highly potent antidiuretic agonist dAVP greatly diminished its antidiuretic activity (Table II). The reduction in antidiuretic activity is much larger than that obtained on substitution of the penicillamine residue in dVDAVP (Table II), i.e., a $^1/_{40}$th as compared to a $^1/_{10}$th reduction. The contrasting effects of *O*-methyltyrosine substitution on the antidiuretic activities of the analogues in Table II are puzzling. Thus, *O*-methyltyrosine substitution reduced the antidiuretic potency of oxytocin, LVP, and dPAVP but not that of AVP; in fact, it may have enhanced it.

The substitution of penicillamine in dAVP and the substitution of *O*-methyltyrosine in AVP produced ana-

logues that inhibit responses by the isolated rat uterus to oxytocin (Table I). dPAVP has an antioxytocic pA_2 similar to those reported for [1-deaminopenicillamine]oxytocin, 6.94[27] and 7.14.[28] Tyr(Me)AVP actually has a pA_2 value comparable to those reported for [1-(β-mercapto-β,β-pentamethylenepropionic acid)]oxytocin (7.43)[3] and [1-deaminopenicillamine,4-threonine]oxytocin (7.46),[28] two of the most potent antagonists of this response yet reported. The combination of penicillamine and *O*-methyltyrosine substitutions in dAVP to give dPTyr(Me)AVP did not further enhance antagonistic potency to a statistically significant degree. The striking effects of these substitutions alone and in combination on activities on the rat uterus and on vasopressor and antidiuretic assays indicate that the appropriate choice of structural modifications of AVP or oxytocin could provide even more specific antagonists of vasopressor and oxytocic responses.

Experimental Section

The procedure of solid-phase peptide synthesis conformed to that published[16-20] with the exception that the chloroform washes were omitted. Chloromethylated resin (Bio-Rad Bio-Beads SX-1) was esterified[29] with Boc-Gly to an incorporation of ~0.46 mmol/g. Amino acid derivatives including Boc-Tyr(Me)[30] were supplied by Bachem Inc., Beckman Bioproducts Division, and Schwartz/Mann Division of Becton, Dickinson and Co. Triethylamine (TEA) and *N*-methylmorpholine (NMM) were distilled from ninhydrin. Trifluoroacetic acid (TFA) was distilled from P_2O_5. The acetic acid used for the HCl–acetic acid cleavage reagent and for washings bracketing the TFA cleavage following glutamine incorporation[18,31] was heated under reflux with boron triacetate and distilled from the reagent. Dimethylformamide (DMF) was distilled under reduced pressure immediately prior to its use. Methanol was dried with magnesium methoxide and distilled. Other solvents and reagents were of analytical grade. Thin-layer chromatography (TLC) was on silica gel (0.25 mm, Brinkman Silplate). The following solvent systems were used: A, cyclohexane–chloroform–acetic acid (2:8:1 v/v); B, propan-1-ol–ammonia (34%) (2:1 v/v); C, ethanol (95%)–ammonia (34%) (3:1 v/v); D, butan-1-ol–acetic acid–water (4:1:5 v/v, upper phase); E, chloroform–methanol (7:3 v/v); F, butan-1-ol–acetic acid–water–pyridine (15:3:3:10 v/v). Loads of 10–50 μg were applied and chromatograms were of minimum length 10 cm. The chloroplatinate reagent, ninhydrin, chlorine–potassium iodide–tolidine, and iodine vapor were used for detection. For amino acid analysis[32] peptides (~0.5 mg) were hydrolyzed with constant boiling hydrochloric acid (400 μL) containing phenol (20 μL) in evacuated and sealed ampules for 18 h at 110 °C. Products containing the *O*-methyltyrosine residue were also hydrolyzed with sulfuric acid (1.8 M, 400 μL) for 24 h at 130 °C.[33] The analyses were performed using a Beckman Automatic Amino Acid Analyzer Model 121. Molar ratios were referred to Gly = 1.00. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. The analytical results for the elements indicated by their symbol were within ±0.4% of theoretical values. Optical rotations were measured with a Bellingham Stanley, Ltd., Model A polarimeter, type pl.

Boc-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (I). Boc-Gly-resin (20.0 g, 9.2 mmol, of Gly) was subjected to six cycles of deprotection, neutralization, and coupling to yield the protected heptapeptidyl resin (27.42 g, weight gain 7.42 g).

Boc-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (II). A single cycle of solid-phase peptide synthesis with Boc-Tyr(Bzl) as the carboxy component converted heptapeptidyl resin I (6.85 g) to the *tert*-butyloxycarbonyloctapeptidyl resin II (7.15 g).

Boc-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (III). The heptapeptidyl resin I (6.85 g) yielded the *tert*-butyloxycarbonyloctapeptidyl resin III (7.22) in one cycle of solid-phase peptide synthesis with Boc-Tyr(Me) as the carboxy component.

Z-Cys(Bzl)-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg-(Tos)-Gly-resin (IV). The octapeptide resin III (3.30 g) yielded the Z-nonapeptidyl resin IV (3.45 g) in one cycle of solid-phase peptide synthesis with Z-Cys(Bzl) as the carboxy component.

Boc-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (V). The protected octapeptidyl resin II (3.55 g) was ammonolyzed[18] and the product extracted with warm DMF. The solvent was evaporated in vacuo and the residue twice reprecipitated from DMF–H_2O and DMF–methanol (1.03 g). This was triturated with hot EtOH (3 × 20 mL, 2 × 10 mL) to give the protected octapeptide amide as a white powder (450 mg; 28.4% based upon initial Gly content on the resin): mp 197–200 °C; R_f^D 0.52, R_f^E 0.55; $[\alpha]^{27}_D$ -32.2° (*c* 0.8, DMF). Anal. ($C_{69}H_{88}N_{14}O_{15}S_2 \cdot H_2O$) C, H, N. Amino acid analysis[32] gave Tyr, 0.95; Phe, 0.96, Glu, 1.05; Asp, 1.03; Cys(Bzl), 1.07; Pro, 0.94; Arg, 1.00; Gly, 1.00; NH_3, 3.20.

Boc-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (VI). The protected octapeptide resin III (3.41 g) was ammonolyzed[18] and the product extracted with warm DMF. The product was precipitated by addition of H_2O, dried, washed with hot EtOH (2 × 30 mL), and reprecipitated from DMF–methanol to give the pure product as a white powder (671.1 mg; 47% based on initial Gly content on the resin): mp 203–208 °C; $[\alpha]^{26}_D$ -29.5° (*c* 1, DMF); R_f^D 0.58, R_f^E 0.34. Anal. ($C_{63}H_{84}N_{14}O_{15}S_2 \cdot 2H_2O$) C, H, N. Amino acid analysis[32,33] gave Tyr, 1.03; Phe, 0.96; Glu, 1.07; Asp, 1.01; Cys(Bzl), 0.95; Pro, 1.00; Arg, 0.91; Gly, 1.00; NH_3, 3.24.

Z-Cys(Bzl)-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg-(Tos)-Gly-NH_2 (VII). The protected nonapeptide resin IV (3.44 g) was ammonolyzed[18] and the product extracted with warm DMF and precipitated by addition of water. The crude product (1.23 g) was twice reprecipitated from DMF–methanol and finally crystallized from glacial acetic acid (698.0 mg; 42.7% based upon initial Gly content on the resin): mp 229.5–230 °C; R_f^D 0.57, R_f^E 0.83, $[\alpha]^{26}_D$ -43.5° (*c* 1, DMF). Anal. ($C_{76}H_{93}N_{15}O_{16}S_3 \cdot H_2O$) C, H, N. Amino acid analysis[32,33] gave Tyr, 0.74; Phe, 0.95; Glu, 1.00; Asp, 1.02; Cys(Bzl), 2.09; Pro, 0.98; Arg, 1.07; Gly, 1.00; NH_3, 3.20.

***S*-Benzyl-β-mercapto-β,β-dimethylpropionyl-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (VIII).** The *tert*-butyloxycarbonyloctapeptide amide V (141 mg, 0.1 mmol) was dissolved in TFA (2 mL) and left to stand at room temperature for 30 min. Cold ether was added and the precipitated material centrifuged and washed with ether (3 × 15 mL) by successive centrifugations and decantations. The product was dried in vacuo over sodium hydroxide pellets. This material was dissolved in DMF (1.5 mL), and *N*-methylmorpholine (12 μL) was added to give a solution of pH >7 to moist pH paper.[34] A solution of *S*-benzyl-β-mercapto-β,β-dimethylpropionic acid[2] (44.9 mg, 0.2 mmol) and *N*-hydroxybenzotriazole monohydrate[21] (45.9 mg, 0.3 mmol) in DMF (0.5 mL) was cooled in ice and treated with a solution of DCCI (4.12 mg, 0.2 mmol) in DMF (2 mL). This mixture was left to stand at room temperature for 1 h with stirring. The precipitated dicyclohexylurea was centrifuged and the supernatant was added to the neutralized solution of the octapeptide derivative. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated and the residue reprecipitated twice from DMF–EtOH and DMF–2% aqueous AcOH. The precipitate was treated with boiling MeOH (4 × 15 mL); the insoluble material was removed by successive centrifugation. The combined supernatants were evaporated in vacuo and the product was finally reprecipitated from DMF–ether to give the acylpeptide amide VIII (87.9 mg, 57.8%): mp 187–188 °C; R_f^D 0.44, R_f^E 0.82; $[\alpha]^{25}_D$ -46.4° (*c* 0.5, DMF). Anal. ($C_{76}H_{94}N_{14}S \cdot H_2O$) C, H, N. Amino acid analysis[32] gave Tyr, 0.97; Phe, 1.03; Glu, 0.90; Asp, 1.05; Cys(Bzl), 0.97; Pro, 1.07; Arg, 1.01; Gly, 1.00; NH_3, 2.93.

***S*-Benzyl-β-mercapto-β,β-dimethylpropionyl-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (IX).** The *tert*-butyloxycarbonyloctapeptide amide VI (214.5 mg, 0.15 mmol) when deprotected and coupled with *S*-benzyl-β-mercapto-β,β-dimethylpropionic acid[2] in the manner described above yielded the acyloctapeptide amide IX (132 mg, 61%): mp 200–203 °C; R_f^D 0.55, R_f^E 0.45. Anal. ($C_{70}H_{90}N_{14}O_{14}S_3 \cdot H_2O$) C, H, N. Amino acid analysis[32,33] gave Tyr, 0.73; Phe, 0.96; Glu, 1.07; Asp, 1.08; Pro, 0.95; Arg, 0.94; Cys(Bzl), 0.90; NH_3, 3.40.

[2-(*O*-Methyl)tyrosine]-8-arginine-vasopressin [Tyr(Me)AVP, X]. A solution of the protected nonapeptide amide VII (150 mg) in sodium-dried and redistilled ammonia (300 mL) was treated at the boiling point and with stirring with sodium from a stick of the metal contained in a small bore glass tube until

a light blue color persisted in the solution for 30 s. Dry glacial acetic acid (0.5 mL) was added to discharge the color. The solution was evaporated and the residue was dissolved in aqueous acetic acid (0.2%, 750 mL), and this solution was treated with 2 M ammonium hydroxide solution to give a solution of pH 7. An excess of a solution of potassium ferricyanide (0.01 M, 6 mL) was added gradually with stirring. The yellow solution was stirred a further 90 min and for 1 h with anion-exchange resin (Bio-Rad AG-3, Cl^- form, 10-g damp weight). The suspension was slowly filtered through a bed of resin (80-g damp weight). The bed was washed with aqueous acetic acid (0.2%, 500 mL) and the combined filtrate and washings were lyophilized. The resulting powder (927 mg) was desalted on a Sephadex G-15 column (110 × 2.7 cm) eluting with aqueous acetic acid (50%)[25] with a flow rate of 4 mL/h. The eluate was fractionated and monitored for absorbance at 280 nm. The fractions comprising the major peak were pooled and lyophilized and the residue (83.8 mg) was further subjected to gel filtration on a Sephadex G-15 column (100 × 1.5 cm) eluting with aqueous acetic acid (0.2 M)[25] with a flow rate of 3 mL/h. The peptide was eluted in a single, symmetrical peak (absorbance 280 nm). Lyophilization of the pertinent fractions yielded the vasopressin analogue (59 mg, 56.2%): R_f^D 0.10, R_f^F 0.22; $[\alpha]^{26}_D$ −6.4° (*c* 0.5, 1 M AcOH). Amino acid analysis[32,33] gave Tyr, 0.70; Phe, 0.96; Glu, 1.03; Asp, 1.01; Cys, 1.94; Pro, 0.98; Arg, 0.94; Gly, 1.00; NH_3, 3.33.

[1-Deaminopenicillamine]-8-arginine-vasopressin (dPAVP, XI). The peptide intermediate VIII (100 mg) was reduced by sodium in liquid ammonia, reoxidized, deionized, and purified as for X (35 mg, 48.6%): R_f^D 0.16, R_f^E 0.37; $[\alpha]^{25}_D$ −52.0° (*c* 0.5, 1 M AcOH). Amino acid analysis[32] gave Tyr, 1.07; Phe, 1.03, Glu, 1.07; Asp, 1.00; Pro, 1.07; Arg, 1.15; Gly, 1.00; NH_3, 3.17; $^1/_2$Cys, trace. Analysis following performic acid oxidation[35] gave a $Cy(SO_3H)$–Gly ratio of 1.15:1.00.

[1-Deaminopenicillamine,2-(*O*-methyl)tyrosine]-8-arginine-vasopressin [dPTyr(Me)AVP, XII]. The analogue XII was prepared from the intermediate IX (100 mg) in the manner detailed above: yield 35.5 g (46.4%); R_f^D 0.20, R_f^F 0.30; $[\alpha]^{25}_D$ −45.6° (*c* 0.5, 1 M AcOH). Amino acid analysis[32,33] gave Tyr, 0.72; Phe, 0.93; Glu, 1.03, Asp, 0.96; Pro, 0.96; Arg, 1.00; Gly, 1.00; NH_3, 3.20; $^1/_2$Cys, 0.31. A peak superimposed upon the artifact caused by the buffer change pH 3.25 to 4.25 was presumed due to the mixed disulfide of β-mercapto-β,β-dimethylpropionic acid and cysteine. Analysis following performic acid oxidation[35] gave a $Cy(SO_3H)$–Gly ratio of 1.10:1.00.

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(30) On TLC Boc-Tyr(Me) (Bachem 9245) traveled as a single spot (R_f^A 0.70, R_f^B 0.81) detected by ninhydrin following exposure to HCl vapor, well resolved from Boc-Tyr (R_f^A 0.24, R_f^B 0.50). Treatment of Boc-Tyr(Me) with TFA, or with HCl–acetic acid followed by evaporation of the acids, yielded the same cleavage product with both acid treatments which gave a single ninhydrin-positive spot on TLC (R_f^B 0.54, R_f^C 0.69) resolved from Tyr (R_f^B 0.40, R_f^C 0.59).
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(33) Hydrolysis of *O*-methyltyrosine and of protected peptides which have *O*-methyltyrosine at the N-terminal position with hydrochloric acid in the usual way gave a quantitative recovery of tyrosine. However, hydrolysis of protected and free peptides which have *O*-methyltyrosine in an internal position was found in this and in a previous study[8] to result in a nonquantitative recovery of tyrosine due to the incompleteness of demethylation.[8] This demethylation was reported to have been avoided by hydrolysis with sulfuric acid.[8] We found that our *O*-methyltyrosine-containing intermediate peptides and the Tyr(Me)AVP analogues did not undergo complete hydrolysis under these conditions (i.e., 1.8 M H_2SO_4, 24 h, 130 °C). It should be noted, however, that the *O*-methyltyrosine containing Boc octapeptide VI gave a Tyr/Gly ratio of 1.03:1 when hydrolyzed by hydrochloric acid. Since the *O*-methyltyrosine containing protected and free peptides VI, IX, X, and XII were all derived from the Boc octapeptide intermediate or from its resin precursor III, their Tyr/Gly ratios of ~0.7 are presumably due to incomplete demethylation.[8] Moreover these ratios are in agreement with the Tyr/Gly ratio of 0.7 obtained on hydrolysis of 2-(*O*-methyl)tyrosine-oxytocin.[8]
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