[1-(L-2-Hydroxy-3-mercaptopropanoic acid)] Analogues of Arginine-vasopressin, [8-D-Arginine]vasopressin, and [4-Valine,8-D-arginine]vasopressin

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[1-(L-2-Hydroxy-3-mercaptopropanic acid)]arginine-vasopressin (hydroxy-AVP), [1-(L-2-hydroxy-3-mercaptopropanoic acid),8-D-arginine]vasopressin (hydroxy-DAVP), and [1-(L-2-hydroxy-3-mercaptopropanoic acid),4-valine,8-D-arginine]vasopressin (hydroxy-VDAVP) were synthesized by a combination of the solid-phase and solution methods of peptide synthesis. Protected octapeptides synthesized by the solid-phase method were further acylated by 1 + 8 couplings in solution to furnish the key intermediates. Hydroxy-AVP has antidiuretic potency of 470 units/mg and activity in the rat vasopressor assay of 550 units/mg, representing a small enhancement of activity over that of arginine-vasopressin (AVP) in each case. Hydroxy-DAVP and hydroxy-VDAVP have essentially the same high antidiuretic activity (900 units/mg) and very low vasopressor potencies (0.9 and <0.02 units/mg, respectively). Hydroxy-AVP, hydroxy-DAVP, and hydroxy-VDAVP thus have antidiuretic–pressor selectivity (A/P) of 1, 1000, and >45 000, respectively. These data are compared with those of other vasopressin analogues. Hydroxy-VDAVP is a highly specific antidiuretic peptide and may be useful in pharmacological studies of antidiuresis.

The replacement of the N-terminal amino group of oxytocin by a hydroxy group, effected by the substitution of a residue of L-2-hydroxy-3-mercaptopropanoic acid for the half-cystine residue at position 1, yielded an analogue (hydroxy-oxytocin) with interesting pharmacological properties. Its oxytocic, antidiuretic, and pressor potencies are enhanced some two- to threefold over those of oxytocin.[1-3]

The hydroxy–amino group interchange at position 1 thus had an effect upon the characteristic spectrum of biological activities of oxytocin similar in many respects to that brought about by the deletion of the amino group. Deamino-oxytocin[4-6] also exhibits enhanced oxytocic and antidiuretic potencies relative to oxytocin. It is, however, a weaker pressor agent than oxytocin. It may also be recalled that deaminoarginine-vasopressin (dAVP)[27] exhibits a fivefold enhancement of antidiuretic activity and antidiuretic–pressor selectivity (A/P) relative to AVP (A/P ~1.0).[7,8]

It was of interest therefore to determine the effect of hydroxy substitution at position 1 in AVP. By analogy with the effects of hydroxy substitution in oxytocin and with the effects of deamination on the properties of both oxytocin and AVP, we were intrigued by the possibility that hydroxy-AVP might exhibit greatly enhanced antidiuretic and pressor potencies relative to AVP. We thus report the synthesis and some pharmacological properties of [1-(L-2-hydroxy-3-mercaptopropanoic acid)]arginine-vasopressin (hydroxy-AVP).[28]

The substitutions of D-arginine at position 8 and valine at position 4 alone and in combination in AVP and in dAVP produced peptides which possess greatly enhanced A/P ratios relative to AVP. Thus [8-D-arginine]vasopressin (DAVP)[8] has an A/P ratio of about 240; deamino[8-D-arginine]vasopressin (dDAVP)[8] has an A/P ratio of about 3000; [4-valine,8-D-arginine]vasopressin (VDAVP)[10] has an A/P ratio of 17 650; and deamino[4-valine,8-D-arginine]vasopressin (dVDAVP)[11] has an A/P ratio which is infinite. The latter three compounds also exhibit greatly enhanced antidiuretic activity relative to AVP. Substitutions of 4-valine and 8-D-arginine brought about their profound effects on A/P selectivity largely by drastically reducing pressor potencies.

Hydroxy substitution in oxytocin increased pressor potency. It was anticipated that in AVP it would have a similar effect. It was thus considered very worthwhile to determine whether the aforementioned D-arginine and 4-valine substitutions in hydroxy-AVP might lead to selective diminishment of pressor potency and thus lead to peptides possessing enhanced antidiuretic activity and enhanced A/P specificities relative to AVP. We thus also report the syntheses and some pharmacological properties of the hydroxy analogues of DAVP and VDAVP.

Solid-phase peptide synthesis[12-14] was employed to prepare protected octapeptide amides of the desired sequences. Removal of the N-terminal protecting group from these products and coupling with *S*-benzyl-L-2-hydroxy-3-mercaptopropanoic acid[1] yielded the immediate precursors of the vasopressin analogues. The disulfide-bridged cyclic peptides were generated and purified by methods previously described,[14,15] and their pharmacological properties were evaluated by reported methods.[10,16,17]

Results and Discussion

The oxytocic, antidiuretic, and pressor potencies of hydroxy-AVP, hydroxy-DAVP, and hydroxy-VDAVP are shown in Table I. Data for AVP and other analogues are included for comparison.

A two- to fourfold enhancement of oxytocic activity as a result of the hydroxy–amino group interchange is shown by all three hydroxy analogues reported here. This is consistent with data previously reported for the effect of the interchange upon oxytocic activity in oxytocin,[1-3] [4-threonine]oxytocin,[3] [4-threonine,7-glycine]oxytocin,[18] and in lysine-vasopressin.[9]

Hydroxy-AVP exhibited only slightly higher activity than AVP in both the antidiuretic and pressor assay systems. The degree of enhancement is essentially the same in each case. There is thus no dissocation of the characteristic vasopressin activities. The enhancement of antidiuretic activity observed in hydroxy-AVP is small compared to the fivefold enhancement brought about by deamination.[7,8] Thus the presence of either an amino group or a hydroxy group at the α-carbon of residue 1 of AVP causes a diminished antidiuretic response relative to that of dAVP, where this position is occupied by hydrogen.

Specificity for the antidiuretic effect is shown by hydroxy-DAVP, the change in position 8 of the amino acid sequence bringing about a dramatic reduction in pressor potency from 550 to 1 unit mg^{-1}. This effect was observed previously with DAVP,[8] dDAVP,[8] and dVDAVP,[11] which showed pressor activities of 1, 0.4, and <0.01 units mg^{-1}, respectively. It should be noted, however, that hydroxy-DAVP has twice the antidiuretic activity of hy-

Table I. Biological Activities (Units mg^{-1} ± Standard Error) of AVP and Analogues

Peptide	Rat uterus in vitro: No Mg^{2+}	Rat uterus in vitro: 0.5 mM Mg^{2+}	Rat antidiuretic (A)	Rat vasopressor (P)	A/P
Hydroxy-AVP[a]	31 ± 3	96 ± 9	467 ± 44	549 ± 16	0.9
Hydroxy-DAVP[a]	4.3 ± 0.7	2.6 ± 0.6	886 ± 105	0.86 ± 0.03	1 030
Hydroxy-VDAVP[a]	1.1 ± 0.1	2.8 ± 0.2	892 ± 42	<0.02[f]	>45 000
AVP[b]	16 ± 1	57 ± 9	330 ± 23	382 ± 5	0.9
DAVP[c]	1.07 ± 0.04	1.02 ± 0.08	257 ± 35	1.08 ± 0.03	238
VDAVP[d]	0.60 ± 0.06	0.90 ± 0.10	653 ± 51	0.037 ± 0.002	17 650
dAVP[c]	54 ± 3	167 ± 12	1745 ± 385	346 ± 13	5
dDAVP[c]	1.5	2.9	1200 ± 126	0.39 ± 0.02	3 030
dVDAVP[e]	8	2	1230 ± 170	<0.01[f]	>123 000

[a] Present communication. [b] Previously unpublished assays of recently synthesized AVP. [c] Values reported by Manning et al.[8] with the exception of oxytocic assay data for dAVP previously unpublished. [d] Values reported by Sawyer et al.[10] with the exception of oxytocic assay data previously unpublished. [e] Values reported by Manning et al.[11] [f] Weak antagonists of the vasopressor response to AVP.

Table II. Properties of Peptide Intermediates, X-Tyr(Bzl)-Phe-Y-Asn-Cys(Bzl)-Pro-Z-Gly-NH_2

Compd	X	Y	Z	Mp, °C[a]	$[\alpha]^{24}_D$, deg (c)[b]	R_f[c] in solvent system: A	B	C
I	Boc	Gln	L-Arg(Tos)	194–196	−38 (1.03)	0.56	0.43	0.62
III	Boc	Gln	D-Arg(Tos)	198–199	−26.5 (1.05)	0.64	0.48	0.71
IV	Boc	Val	D-Arg(Tos)	214–216	−30 (0.76)	0.58	0.43	0.64
V	HO·CH(CH_2·S·Bzl)·CO	Gln	L-Arg(Tos)	183–184	−29.5 (1.16)	0.56	0.43	
VI	HO·CH(CH_2·S·Bzl)·CO	Gln	D-Arg(Tos)	198–199	−11.5 (1.05)	0.43	0.33	0.69
VII	HO·CH(CH_2·S·Bzl)·CO	Val	D-Arg(Tos)	218–221	−10.5 (1.14)	0.56	0.50	0.84

[a] Melting points taken in open capillaries, with a Thomas-Hoover apparatus, and are uncorrected. [b] Optical rotations measured with a Bellingham Stanley, Ltd., Model A polarimeter; DMF as solvent. [c] Thin-layer chromatography on silica gel (0.25 mm, Brinkman silplate).

droxy-AVP, an effect not found in the other cases noted above, where antidiuretic activity was either decreased (cf. AVP–DAVP, dAVP–dDAVP) or remained essentially unchanged (dVAVP has antidiuretic activity of 1150 ± 110 units mg^{-1}; cf. dVDAVP). In this respect, then, the effect of [D-Arg[8]] is dependent upon the nature of the residue occupying position 1.

Substitution of valine in the 4 position of hydroxy-DAVP led to a further increase of antidiuretic specificity; hydroxy-VDAVP showing antidiuretic potency equal to that of hydroxy-DAVP but markedly less activity in the pressor assay. As was found with dVDAVP,[11] hydroxy-VDAVP elicited a depressor response at high dosage levels and antagonized the pressor response to AVP.

The high specific antidiuretic activity of hydroxy-VDAVP makes it an attractive peptide for possible use in studies of receptors that mediate antidiuretic responses. The investigation of the duration of action of the peptide is also warranted since such information may be helpful in characterizing enzymes which may terminate antidiuretic responses.

Experimental Section

The procedure of "solid-phase" peptide synthesis conformed to that published[12–14] with the exception that the chloroform washes were omitted and a pyridine hydrochloride treatment[19] followed by neutralization was included after the final coupling step. Chloromethylated resin (Bio-Rad Bio-Beads SX-1) was esterified[20] with Boc-Gly to an incorporation of 0.25 mmol g^{-1}. Amino acid derivatives were supplied by Bachem Inc., Fox Chemical Co., Biosynthetika, and Schwarz Bioresearch Inc. Triethylamine and *N*-methylmorpholine (NMM) were distilled from ninhydrin and trifluoroacetic acid from P_2O_5. The acetic acid used for the hydrogen chloride–acetic acid cleavage reagent and for washings bracketing the trifluoroacetic acid cleavage following glutamine incorporation[21,22] 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 by the ascending technique on silica gel (0.25 mm, Brinkman silplate) or on cellulose (0.10 mm, Brinkman celplate). Solvent systems: A, butan-1-ol–acetic acid–water (4:1:5 v/v, upper phase); B, butan-1-ol–water (3.5% in acetic acid, 1.5% in pyridine) (1:1 v/v, upper phase); C, chloroform–methanol (45:55 v/v); D, ethyl acetate–pyridine–acetic acid–water (5:5:1:3 v/v). Loads of 10–50 μg were applied and chromatograms were of minimum length, 10 cm. Chloroplatinate reagent, ninhydrin, iodine vapor, and chlorine–potassium iodide–tolidine were used for detection. For amino acid analysis samples (ca. 0.5 mg) were hydrolyzed with constant boiling hydrochloric acid (400 μL) containing phenol (1%), in evacuated and sealed ampules, for 18 h at 110 °C. The analyses were performed using a Beckman automatic amino acid analyzer Model 121. Ratios were referred to Gly = 1.00. Elemental analyses were performed by Galbraith Laboratories, Inc. Analytical results, indicated by the elemental symbols, were within ±0.4% of theoretical values.

Boc-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (I). Boc-Gly-resin (8 g, 2 mmol of Gly) was subjected to seven cycles of deprotection, neutralization, and coupling to yield a protected octapeptidyl resin (10.3 g, weight gain 2.3 g, 86% of theory). The resin was ammonolyzed in methanol.[14] Following evaporation of the methanol the product was extracted with warm DMF and precipitated with water. The crude material was reprecipitated from DMF–water and finally from DMF–ether to yield the protected octapeptide amide (2.1 g, 74% based upon initial Gly content of the resin, Table II). Amino acid analysis gave Tyr, 0.97; Phe, 1.01; Glu, 1.02; Asp, 1.02; Cys(Bzl), 1.04; Pro, 1.05; Arg, 1.06; Gly, 1.00; NH_3, 3.2. Anal. ($C_{69}H_{88}N_{14}O_{15}S_2$) C, H, N.

Boc-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-Resin (II). Boc-Gly-resin (12 g, 3 mmol of Gly) was subjected to four cycles of deprotection, neutralization, and coupling to yield the protected pentapeptidyl resin (14.1 g, weight gain 2.1 g, 97% of theory). Amino acid analysis gave Asp, 0.98; Cys(Bzl), 0.94; Pro, 1.03; Arg, 1.06, 1.00; NH_3, 1.04.

Boc-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (III). The foregoing pentapeptidyl resin (4.7 g) was converted to *tert*-butyloxycarbonyloctapeptidyl resin (5.2 g, weight gain 0.5 g, 97% based upon initial Gly content of the resin) from which the protected octapeptide amide (930 mg, 73% based upon

initial Gly content of the resin, Table II) was isolated as detailed in the preparation of its 8-L-Arg analogue, above, and reprecipitated from DMF–water and from DMF–methanol–ether. Amino acid analysis gave Tyr, 0.96; Phe, 1.01; Glu, 1.00; Asp, 1.00; Cys(Bzl), 1.03; Pro, 1.03; Arg, 1.01; Gly, 1.00; NH_3, 3.58. Anal. ($C_{69}H_{88}N_{14}O_{15}S_2$) H, N; C: calcd, 58.45; found, 57.90.

Boc-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (IV). *tert*-Butyloxycarbonyloctapeptidyl resin (5.1 g), derived from *tert*-butyloxycarbonylpentapeptidyl resin II (4.7 g), was ammonolyzed[14] and the protected octapeptide amide product (1 g, 76% based upon initial Gly content of the resin, Table II) isolated, as were the peptide derivatives I and III above, and reprecipitated from DMF–methanol–ether. Amino acid analysis gave Tyr, 1.06; Phe, 1.00; Val, 1.05; Asp, 0.99; Cys(Bzl), 1.05; Pro, 1.04; Arg, 1.00; Gly, 1.00; NH_3, 2.75. Anal. ($C_{69}H_{89}N_{13}O_{14}S_2$) C, H, N.

***S*-Benzyl-L-2-hydroxy-3-mercaptopropanoyl-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (V).** The *tert*-butyloxycarbonyloctapeptide I (284 mg, 0.2 mmol) was dissolved in TFA (4 mL) and the solution set aside for 1 h. Cold ether (20 mL) was added and the precipitated product washed with ether (five times, aliquot 10 mL) by successive centrifugation and decantation, the precipitate being suspended in each wash by vortex mixing, and finally dried in vacuo. This material was dissolved in DMF (1 mL) and NMM (10 μL) added gradually to give a solution of pH ca. 7 to moist pH paper. A solution of *S*-benzyl-L-2-hydroxy-3-mercaptopropanoic acid[1] (47 mg, 0.22 mmol) and *N*-hydroxybenzotriazole monohydrate[23] (34 mg, 0.22 mmol) in DMF (0.4 mL) was added, and the mixture, chilled in ice, was treated with a solution of dicyclohexylcarbodiimide (45 mg, 0.22 mmol) in DMF (0.2 mL). Ice cooling was maintained for 1 h, with occasional vortex mixing. Then the reaction mixture was set aside for 3 h at room temperature. The atmosphere within the reaction vessel maintained a slightly alkaline reaction to moist pH paper during this period.[25] Complete consumption of the ninhydrin positive starting material with the formation of a product giving no color with the reagent was demonstrated by TLC (both materials detected by the chloroplatinate reagent). Ethyl acetate (20 mL) was added and the precipitated material washed with ethyl acetate–ethanol (1:1 v/v, four times, aliquot 10 mL), ethyl acetate (twice, aliquot 10 mL), and ether (twice, aliquot 10 mL) by successive centrifugation and decantation, the precipitate being well suspended in each wash. The crude product was reprecipitated from DMF–aqueous acetic acid (2%) and from DMF–ether to give the acyloctapeptide amide (230 mg, 77%, Table II). Amino acid analysis gave Tyr, 0.84; Phe, 1.01; Glu, 1.00; Asp, 1.04; Cys(Bzl), 1.04; Pro, 1.02; Arg, 1.02; Gly, 1.00; NH_3, 3.08. Anal. ($C_{74}H_{90}N_{14}O_{15}S_3$) C, H, N.

***S*-Benzyl-L-2-hydroxy-3-mercaptopropanoyl-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (VI).** The peptide derivative III (284 mg, 0.2 mmol) was treated as above to yield acyloctapeptide amide VI (150 mg, 50%, Table II). Amino acid analysis gave Tyr, 0.91; Phe, 1.03; Glu, 1.00; Asp, 1.01; Cys(Bzl), 1.00; Pro, 1.03; Arg, 1.00; Gly, 1.00; NH_3, 3.00. Anal. ($C_{74}H_{90}N_{14}O_{15}S_3$) C, H, N.

***S*-Benzyl-L-2-hydroxy-3-mercaptopropanoyl-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg-Gly-NH_2 (VII).** The acyloctapeptide VII (215 mg, 73%, Table II) was prepared from the intermediate IV (278 mg, 0.2 mmol) by the procedure detailed for the preparation of its analogues V and VI. Amino acid analysis gave Tyr, 0.93; Phe, 1.02, Val, 1.03; Asp, 1.04; Cys(Bzl), 1.06; Pro, 1.01; Arg, 1.02; Gly, 1.00; NH_3, 2.10. Anal. ($C_{74}H_{91}N_{13}O_{14}S_3$) C, H, N.

[1-(L-2-Hydroxy-3-mercaptopropanoic acid),8-arginine]vasopressin (VIII). A solution of the peptide intermediate V (100 mg, 0.066 mmol) in sodium-dried and redistilled ammonia (ca. 300 mL) was treated at the boiling point and with stirring with sodium[15] from a stick of the metal contained in a small bore glass tube[4,5,14] until a light blue color persisted in the solution for 20 s. The color was discharged by the dropwise addition of dry glacial acetic acid. The solution was evaporated and the residue taken up in aqueous acetic acid (0.2%, 750 mL). Following the addition of aqueous ammonia (2 *M*) to give a solution of pH ~7.5, an excess of potassium ferricyamide (0.01 M, 15 mL) was added gradually, with stirring. The yellow solution (pH ~6.5) was stirred for 5 min with anion-exchange resin (Bio-Rad AG 3-X4A, chloride form, ~10 g damp weight) and filtered through a bed of the resin (~80 g damp weight). The bed was washed with aqueous acetic acid (0.2%, 200 mL), and the combined filtrate and washings were lyophilized. The resulting powder was partially desalted on Sephadex G-15 (column 110 × 2.7 cm)[25] eluting with aqueous acetic acid (50%) with flow rate ~6.5 mL h^{-1}. The eluate was monitored for absorbance at 280 nm and fractionated. Breakthrough of salt occurred before elution of the major peak of peptide material was complete. The fractions comprising this latter portion of the peak were pooled and lyophilized, and the residue was desalted on Sephadex G-10 (column 100 × 1.5 cm) eluting with aqueous acetic acid (50%). The two samples of salt-free peptide thus obtained were separately subjected to gel filtration with Sephadex G-15 (column 100 × 1.5 cm) eluting with aqueous acetic acid (0.2 M) with flow rate ~5 mL h^{-1}. A single symmetrical peak was observed (absorbance 280 nm) in each case. The vasopressin analogue was isolated by lyophilization of the pertinent fractions and dried in vacuo over P_2O_5. The two portions obtained (total 34 mg, ~47%) were identical in all respects (Table III). Amino acid analysis gave Tyr, 1.06; Phe, 1.04; Glu, 1.02; Asp, 1.04, $^1/_2$Cys, 0.44; Pro, 1.03; Arg, 1.07; Gly, 1.00; NH_3, 2.96; mixed disulfide of cysteine and L-2-hydroxy-3-mercaptopropanoic acid,[26] ~0.55. Analysis following performic acid oxidation gave a Cys(O_3H)–Gly ratio of 0.96:1.00. Anal. ($C_{46}H_{64}N_{14}O_{12}S_2 \cdot 2C$-$H_3CO_2H \cdot 5H_2O$) C, H, N.

[1-(L-2-Hydroxy-3-mercaptopropanoic acid),8-D-arginine]vasopressin (IX). The peptide derivative VI (100 mg, 0.066 mmol) was reduced by sodium in liquid ammonia and reoxidized with potassium ferricyanide[4,5,14,15] as detailed above in the preparation of compound VIII. Desalting of the product was achieved in a single pass on Sephadex G-15, eluting with aqueous acetic acid (50%), and purification was completed by gel filtration with Sephadex G-15 eluting with aqueous acetic acid (0.2 M)[25] to yield the [D-Arg[8]]vasopressin analogue IX (40 mg, ~56%, Table III). Amino acid analysis gave Tyr, 1.00; Phe, 0.98; Glu, 1.01; Asp, 1.04; $^1/_2$Cys, 0.42; Pro, 0.98; Arg, 0.98; Gly, 1.00; NH_3, 3.30; mixed disulfide of cysteine and L-2-hydroxy-3-mercaptopropanoic acid,[26] ~0.40. Analysis following performic acid oxidation gave a Cys(O_3H)–Gly ratio of 1.01:1.00. Anal. ($C_{46}H_{64}N_{14}O_{12}S_2 \cdot 2C$-$H_3CO_2H \cdot 4H_2O$) C, H, N.

[1-(L-2-Hydroxy-3-mercaptopropanoic acid),4-valine,8-D-arginine]vasopressin (X). The valine containing analogue X (37 mg, ~52%, Table III) was prepared from the intermediate VII (100 mg, 0.067 mmol) as described above for the preparation of compound IX. Amino acid analysis gave Tyr, 1.02; Phe, 0.99; Val, 1.00; Asp, 1.02; $^1/_2$Cys, 0.40; Pro, 0.99; Arg, 1.04; Gly, 1.00; NH_3, 2.15; mixed disulfide of cysteine and L-2-hydroxy-3-mercaptopropanoic acid,[26] ~0.40. Analysis following performic acid oxidation gave a Cys(O_3H)–Gly ratio of 1.03:1.00. Anal. ($C_{46}H_{65}N_{13}O_{11}S_2 \cdot 2CH_3CO_2H \cdot 5H_2O$) C, H, N.

Table III. Properties of Hydroxy-AVP, Hydroxy-DAVP, and Hydroxy-VDAVP

Compd	$[\alpha]^{23}D$, deg (*c*)[a]	R_f[b] in solvent system A	B	D	M_{Arg}[c]
Hydroxy-AVP (VIII)	−60 (0.50)	0.45	0.22	0.68	0.33
Hydroxy-DAVP (IX)	−47 (0.53)	0.45	0.22	0.68	0.38
Hydroxy-VDAVP (X)	−47 (0.53)	0.68	0.48	0.85	0.41

[a] 1 M AcOH as solvent. [b] Thin-layer chromatography on cellulose (0.10 mm, Brinkman celplate). [c] Electrophoretic mobility (*M*) relative to that of Arg in the direction of the cathode (pyridium acetate buffer, pH 3.5, 400 V, 2.5 h). Single bands detected in each case (chloroplatinate, chlorine–potassium iodide–tolidine).

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(27) The system of abbreviation for arginine-vasopressin (AVP) analogues is as previously suggested:[10] lower case "d" to indicate deamination of the N-terminal half-cystine, capital "D" to signify the presence of [D-Arg^8], capital "V" for [Val^4]. Where unspecified, optically active amino acids are of the L configuration.
(28) Following completion of these studies the synthesis and some pharmacological properties of [1-(L-2-hydroxy-3-mercaptopropanoic acid)]lysine-vasopressin were reported.[9]

Potential Inhibitors of Collagen Biosynthesis. 4,4-Difluoro-L-proline and 4,4-Dimethyl-DL-proline and Their Activation by Prolyl-tRNA Ligase

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The therapeutic need for selective inhibitors of collagen biosynthesis and the fact that the enzymatic conversion of peptidylproline to peptidyl-4-hydroxyproline occurs almost exclusively in collagen prompted the synthesis of proline analogues bearing geminal blocking substituents at C-4, viz., 4,4-difluoro-L-proline (1) and 4,4-dimethyl-DL-proline (2). The diketopiperazine (7) of 4-hydroxy-L-proline was oxidized with Me_2SO–DCC to the corresponding diketone and the latter fluorinated with SF_4–HF to give the key intermediate, 2,2,7,7-tetrafluorooctahydro-5*H*,10*H*-dipyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-5,10-dione (9). Acid hydrolysis of 9 afforded 1. 2 was prepared by reductive cyclization of α-(β-cyanoethyl)isobutyraldehyde to 5,5-dimethyl-2-piperidone (13) which was converted in two steps to 3-chloro-5,5-dimethyl-2-piperidone (15). Base-catalyzed rearrangement of 15 gave 2. The basic dissociation constant of 2 was similar to that of L-proline, while the pK_a of 1 was lower by ~3.5 units. The ability of these proline analogues to be incorporated into procollagen protein was assayed by their ability to stimulate the proline-dependent ATP–PP_i exchange reaction in the presence of prolyl-tRNA ligase. The difluoro analogue 1 stimulated exchange but at a K_m (2.34×10^{-3} M) 100 times greater than that for L-proline itself. The dimethyl analogue 2 did not stimulate exchange but was a competitive inhibitor with an apparent K_i of 1.5×10^{-2} M.

The growing implication of abnormal collagen production in the development of a variety of pathologic conditions[1] including tumor growth[2] suggests the need for selective inhibitors of collagen biosynthesis. Two unique features of collagen which may serve to enhance the selectivity of such inhibition are (a) its low metabolic turnover rate compared to other proteins and (b) the presence of 4-hydroxy-L-proline residues in its peptide structure. The biosynthesis of the latter involves the hydroxylation of the proline moieties which have been incorporated into a collagen precursor, procollagen, by an α-keto glutarate dependent mixed function oxidase, viz., peptidylproline hydroxylase (PPH, E.C. 1.14.11.2; proline 2-oxoglutarate dioxygenase[3]). The exclusiveness of this hydroxylation step suggests that collagen synthesis should be amenable to biochemical manipulation by synthetic analogues of proline wherein the position of hydroxylation is blocked. A number of proline analogues have been reported to be incorporated into proteins in place of L-proline, viz., L-azetidine-2-carboxylic acid,[4,5] 3,4-dehydro-L-proline,[6] thiazolidine-4-carboxylic acid,[7] *cis*-4-hydroxy-L-proline,[8] *cis*-4-fluoro-L-proline,[4,5,9] and *trans*-4-fluoro-L-proline.[9] Microbial studies of the *cis*-4-fluoro-L-proline incorporated into the actinomycin molecule by *S. antibioticus* indicate that it is converted to 4-keto-L-proline.[10] Whether this cis isomer is also converted to 4-keto-L-proline in a mammalian system has yet to be determined.

Although the isomeric 4-monofluoroprolines have been studied extensively, the use of the corresponding geminally disubstituted analogues as inhibitors of collagen synthesis has not been previously explored. 4,4-Difluoro-L-proline (1) and 4,4-dimethyl-DL-proline (2), the title compounds, represent such proline analogues wherein the position of hydroxylation is blocked by geminal fluoro or methyl substituents. 4,4-Difluoro-L-proline (1) on incorporation