during 20 min with a solution of $NaIO_4$ (2.35 g, 11.0 mmol) in H_2O (25 mL). The resulting solution was stirred under N_2 for 1 h and was then treated with NaOH (11.8 mL of 1 N) to produce pH 6. The solid that formed was collected by centrifugation, washed with H_2O (three times with 50-mL portions by centrifugation), and dried to give **27**: mp 290 °C dec; 66% yield (1.46 g); homogeneous by TLC; ^1H NMR (CF_3CO_2D) δ 5.6 (s, 2, CH_2CHO), 8.6 (s, 1, C_8 H), 9.7 (br s, 1, CH_2CHO). Anal. ($C_7H_7N_5O_2 \cdot 1.5H_2O$) C, H, N.

Ethyl 7-Guanine-4-crotonate (28). A solution of 5.00 mmol each of (ethoxycarbonylmethyl)triphenylphosphonium bromide (2.15 g) and NaOMe (270 mg) in MeOH (25 mL) was stirred at 25 °C under N_2 for 1 h. The MeOH was removed by evaporation in vacuo, and the residue was suspended in Me_2SO (10 mL). The stirred mixture was treated dropwise with a solution of **27**·1.5H_2O (424 mg, 2.00 mmol) in Me_2SO (40 mL), and the solution that formed was kept at 25 °C for 3 h before the solvent was removed by evaporation in vacuo. The residue was stirred with boiling C_6H_6 (50 mL), collected, and washed on the funnel with warm C_6H_6 followed by several portions of warm hexane [to remove $(C_6H_5)_3PO$]. The solid was then washed with H_2O and recrystallized from EtOH–H_2O (7:3, v/v) to give pure **28**, mp 280 °C dec, in 50% yield (264 mg): MS *m/e* 263 (M^+), 217 (M^+ - EtOH), 190 (M^+ - CO_2Et). Anal. ($C_{11}H_{13}N_5O_3$) C, H, N.

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[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine]arginine-vasopressin and [1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin, Two Highly Potent Antagonists of the Vasopressor Response to Arginine-vasopressin

Marian Kruszynski, Bernard Lammek, Maurice Manning,*

Department of Biochemistry, Medical College of Ohio, Toledo, Ohio 43699

Janny Seto, Jaya Haldar, and Wilbur H. Sawyer

Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, New York 10032.
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As part of our studies on the design and synthesis of antagonists of the vasopressor response to arginine-vasopressin (AVP), we have synthesized [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine]arginine-vasopressin [**1**, d$(CH_2)_5$Tyr(Me)AVP] (in duplicate) and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin [**2**, d$(CH_2)_5$AVP]. The required protected intermediate for **1** was synthesized by (a) a combination of solid-phase synthesis and an 8 + 1 coupling in solution and (b) entirely by solid-phase synthesis. The required protected precursor for **2** was synthesized entirely by solid-phase methods. **1** and **2** were tested for agonistic and antagonistic activities in rat vasopressor, rat antidiuretic, and rat uterus assay systems. d-$(CH_2)_5$Tyr(Me)AVP exhibits a surprisingly potent and prolonged antivasopressor effect. It has an antivasopressor pA_2 of 8.62 ± 0.03 and an antidiuretic potency of 0.31 ± 0.07 unit/mg. Material from the second synthesis of d$(CH_2)_5$Tyr(Me)AVP has a pA_2 value of 8.67 ± 0.02. d$(CH_2)_5$AVP also exhibited a potent, although less prolonged, antivasopressor effect. It has an antivasopressor pA_2 of 8.35 ± 0.09 and an antidiuretic potency of only 0.033 ± 0.005 unit/mg. These are the two most potent vasopressor antagonists reported to date. Both analogues also antagonize the actions of oxytocin on the rat uterus (a) in the absence of Mg^{2+}, (b) in the presence of 0.5 mM Mg^{2+}, and (c) in situ. They exhibit, respectively, the following pA_2 values in each of the assay systems a–c: (1) a, 8.13 ± 0.12; b, 7.24 ± 0.07; c, 6.62 ± 0.07; (2) a, 8.15 ± 0.20; b, 7.19 ± 0.08; c, 6.79 ± 0.19. With their potent ability to antagonize the vasopressor effects of AVP, d$(CH_2)_5$Tyr(Me)AVP and d$(CH_2)_5$AVP should be valuable additions to our previously reported antagonists for use as pharmacological tools with which to probe the possible role(s) of AVP in cardiovascular regulation in normal and pathophysicological states.

In attempting to design an antagonist of the antidiuretic response to arginine-vasopressin (AVP), we synthesized [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin (dPVDAVP)[1] and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$VDAVP].[2] These analogues were designed by replacing the two hydrogens on the β carbon at position 1 of the highly active and selective antidiuretic peptide [1-deamino,4-valine,8-D-arginine]vasopressin (dVDAVP)[3] with two methyl groups and a cyclopentamethylene group, respectively. These substituents had previously been shown to convert the highly potent oxytocic agonist [1-deamino]oxytocin[4] (dOT) into the potent antagonists of the oxytocic response to oxytocin, [1-deaminopenicillamine]oxytocin (dPOT)[5] and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]oxytocin (d$(CH_2)_5$OT).[6] Neither dPVDAVP nor d$(CH_2)_5$VDAVP were antagonists of the antidiuretic response to AVP, although they possessed $^1/_{10}$ and only $^1/_{10\,000}$ the antidiuretic activity of

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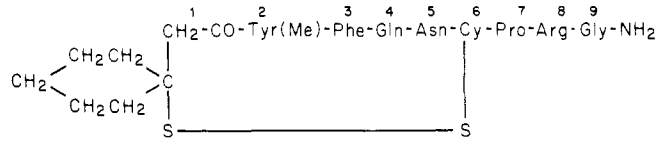
dVDAVP, respectively. They were, however, found to be potent antagonists of the vasopressor response to AVP. Their antagonistic potencies were estimated following the convention of Dyckes et al.[7a] and expressed as pA_2 values. PA_2 values as defined by Schild[8] represent the negative logarithms to the base 10 of the average molar concentrations of antagonists which will reduce the specific biological response to $2x$ units of an agonist to the level of response to x units of the agonist. dPVDAVP and d-$(CH_2)_5$VDAVP had antivasopressor pA_2 values of 7.82[1] and 7.68,[2] respectively. Thus, it appeared that the penicillamine-type substitution, i.e., β,β-dimethyl, was more effective than the β,β-cyclopentamethylene substitution at position 1 in producing a slightly more potent antivasopressor antagonist. This observation was entirely consistent with data for the relative antivasopressor potencies of dPOT and d$(CH_2)_5$OT.[6] Furthermore dPVDAVP and d$(CH_2)_5$VDAVP were clearly more potent than previously reported vasopressor antagonists.[7] Dyckes et al. had shown that substitution of a β,β-diethyl grouping at position 1 in [1-deamino]lysine-vasopressin[7a] and in [1-deamino,4-leucine]lysine-vasopressin[7b] gave antagonists of the vasopressor responses to lysine-vasopressin with pA_2 values of 7.15 and 6.91, respectively. Both dPVDAVP and d-$(CH_2)_5$VDAVP have found use in a variety of studies on the role of AVP in cardiovascular regulation. The findings from three such studies have been reported.[9,10]

The discovery of these two vasopressor antagonists, dPVDAVP and d$(CH_2)_5$VDAVP, sparked our interest in exploring the effects of the β,β-dimethyl and the β,β-cyclopentamethylene substitutions at position 1 in other analogues of AVP. We were particularly intrigued by the possibility that by combining one of these substitutions with *O*-methyltyrosine[11] substitution at position 2 of the highly active antidiuretic and vasopressor agonist [1-deamino]arginine-vasopressin (dAVP)[12] we might obtain an even more potent and selective antivasopressor peptide than dPVDAVP or d$(CH_2)_5$VDAVP. Because the penicillamine substitution at position 1 appeared to produce more antivasopressor potency than the β,β-cyclopentamethylene group, we decided to examine first the antivasopressor effects of the penicillamine substitution alone and in combination with *O*-methyltyrosine substitution at position 2 in dAVP. We recently reported the synthesis and some pharmacological properties of the two peptides designed in this fashion, i.e., [1-deaminopenicillamine]-arginine-vasopressin (dPAVP) and [1-deaminopenicillamine,2-(*O*-methyl)tyrosine]arginine-vasopressin [dPTyr(Me)AVP].[13] With an antivasopressor pA_2 of 7.45, dPAVP was found to be less potent than either dPVDAVP or d$(CH_2)_5$VDAVP. However, dPTyr(Me)AVP has an antivasopressor pA_2 of 7.96 and was the most potent antivasopressor peptide yet reported. Thus, the combination of the penicillamine and the *O*-methyltyrosine substitutions had brought about a threefold enhancement in antivasopressor potency relative to dPAVP.

As a continuation of this approach, we wished to explore the effects on antivasopressor potency of combining the β,β-cyclopentamethylene and *O*-methyltyrosine substitutions in dAVP. The peptide designed according to this rationale, i.e., [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine]arginine-vasopressin [d$(CH_2)_5$Tyr(Me)AVP], has the following structure:



Because d$(CH_2)_5$Tyr(Me)AVP was found to possess a surprising and striking enhancement in antivasopressor potency relative to dPTyr(Me)AVP,[13] it was resynthesized. The antivasopressor potencies of both preparations were found to be identical and are presented here.

We subsequently wished to determine the effects on antivasopressor potency of the β,β-cyclopentamethylene substitution alone in dAVP. We synthesized [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin [d$(CH_2)_5$AVP]. Its structure is identical with that shown above, except that the tyrosine is unmethylated. It was also found to possess a surprising and striking enhancement in antivasopressor potency relative to dPAVP and is, in fact, almost equipotent with d-$(CH_2)_5$Tyr(Me)AVP. We now report the synthesis and some chemical and pharmacological properties of these two highly potent and selective antivasopressor peptides, i.e., d$(CH_2)_5$Tyr(Me)AVP (synthesized in duplicate) and d-$(CH_2)_5$AVP.

Peptide Synthesis. The protected peptide precursors required for the synthesis of both peptides were prepared by the solid-phase method of peptide synthesis.[13-18] In the first synthesis of d$(CH_2)_5$Tyr(Me)AVP, the octapeptide derivative Boc-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg-(Tos)-Gly-NH_2[13] was coupled, following removal of the N-terminal protecting group, with *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate.[6] The active ester coupling[19,20] was facilitated by *N*-hydroxybenzotriazole (HOBT)[21] to yield the immediate protected precursor of d$(CH_2)_5$Tyr(Me)AVP. For the second synthesis of d$(CH_2)_5$Tyr(Me)AVP and for the synthesis of d$(CH_2)_5$AVP, the required immediate protected precursors were synthesized entirely on their respective resins. Each precursor was deblocked with Na in NH_3[22] as previously described,[3,16] and the resulting disulfhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$[4]. The analogues were desalted and purified by gel filtration on Sephadex G-15.[23]

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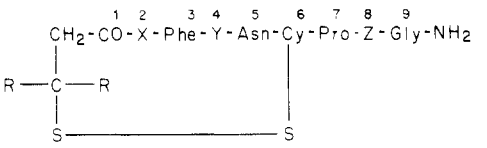
Table I. Pharmacological Properties of [1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin [d(CH_2)$_5$AVP] and [1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine]arginine-vasopressin [d(CH_2)$_5$Tyr(Me)AVP]

peptide	antivasopressor pA_2[a]	antidiuretic act., units/mg	antioxytocic (in vitro) pA[a]		antioxytocic (in vivo) pA_2[a]
			no Mg^{2+}	0.5 mM Mg^{2+}	
d(CH_2)$_5$AVP	8.35 ± 0.09 (6)	0.033 ± 0.005	8.15 ± 0.20	7.19 ± 0.08 (8)	6.79 ± 0.19 (6)
d(CH_2)$_5$Tyr(Me)AVP	(A) 8.62 ± 0.03 (5)	0.31 ± 0.07			6.62 ± 0.07 (7)
	(B) 8.67 ± 0.02 (6)		8.13 ± 0.12 (4)	7.24 ± 0.07 (4)	

[a] pA_2 values were obtained as described in ref 1, 24, and 25 by the method of Schild (ref 8) 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.

Table II. Pharmacological Effects of β,β-Dialkyl Substituents at Position 1 in Some Analogues of 1-Deaminoarginine-vasopressin (dAVP)



peptide[m]	R_2	X	Y	Z	antivasopressor pA_2	antidiuretic act. units/mg	anti-oxytocic pA_2 (no Mg^{2+})
dAVP[a]	H_2	Tyr	Gln	Arg	agonist[b]	1745 ± 385	agonist[c]
dPAVP[d]	(CH_3)$_2$	Tyr	Gln	Arg	7.45 ± 0.11	42 ± 3	6.93 ± 0.10
d(CH_2)$_5$AVP[e]	(CH_2)$_5$	Tyr	Gln	Arg	8.35 ± 0.09	0.033 ± 0.005	8.15 ± 0.20
dVDAVP[f]	H_2	Tyr	Val	D-Arg	7.03 ± 0.11	1230 ± 170	agonist[g]
dPVDAVP[h]	(CH_3)$_2$	Tyr	Val	D-Arg	7.82 ± 0.05	123 ± 22	7.23 ± 0.04
d(CH_2)$_5$VDAVP[j]	(CH_2)$_5$	Tyr	Val	D-Arg	7.68 ± 0.05	0.10 ± 0.02	6.62 ± 0.07
dTyr(Me)AVP[k]	H_2	Tyr(Me)	Gln	Arg	agonist[l]	830 ± 70	7.41 ± 0.18
dPTyr(Me)AVP[d]	(CH_3)$_2$	Tyr(Me)	Gln	Arg	7.96 ± 0.05	3.5 ± 0.5	7.61 ± 0.14
d(CH_2)$_5$Tyr(Me)AVP[e]	(CH_2)$_5$	Tyr(Me)	Gln	Arg	8.62 ± 0.03	0.31 ± 0.07	8.13 ± 0.12

[a] From Manning et al.[12b] [b] 346 units/mg. [c] 47 units/mg. [d] From Bankowski et al.[13] [e] This publication. [f] From Manning et al.[1,3] [g] ~8 units/mg. [h] From Manning et al.[1] [j] From Lowbridge et al.[2] [k] W. H. Sawyer and M. Manning, unpublished results. [l] 4 units/mg. [m] The abbreviations for arginine vasopressin (AVP) analogues follow the system previously suggested.[33] The abbreviations and their full names are as follows: dAVP, [1-deamino]arginine-vasopressin; dPAVP, [1-deaminopenicillamine]arginine-vasopressin; d(CH_2)$_5$AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin; dVDAVP, [1-deamino,4-valine,8-D-arginine]vasopressin; dPVDAVP, [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin; d(CH_2)$_5$VDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin; dTyr(Me)AVP, [1-deamino,2-(*O*-methyl)tyrosine]arginine-vasopressin; dPTyr(Me)AVP, [1-deaminopenicillamine,2-(*O*-methyl)tyrosine]arginine-vasopressin; d(CH_2)$_5$Tyr(Me)AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine]arginine-vasopressin.

Bioassay Methods. The agonistic and antagonistic properties of these analogues were investigated by previously described methods.[1,13,24,25] These included (1) intravenous antidiuretic assays in rats under ethanol anesthesia, (2) vasopressor assays in phenoxybenzamine-treated rats under urethane anesthesia, and (3) assays on the rat uterus in vitro in a medium containing (a) no Mg^{2+} and (b) 0.5 mM Mg^{2+} and (4) on the rat uterus in situ. Agonistic activities are expressed in units per milligram. Antagonistic activities as indicated above are expressed as pA_2 values. The duration of antivasopressor antagonism by d(CH_2)$_5$Tyr(Me)AVP was estimated by methods described under Experimental Section.

Results and Discussion

Some pharmacological properties of d(CH_2)$_5$Tyr(Me)-AVP and d(CH_2)$_5$AVP are presented in Table I. Comparisons of some of these properties with those of related peptides are given in Table II.

With antivasopressor pA_2 values of 8.62 and 8.35, respectively, d(CH_2)$_5$Tyr(Me)AVP and d(CH_2)$_5$AVP are the most potent antagonists of the vasopressor response to AVP yet reported. This enhancement of antivasopressor potency coupled with their very low antidiuretic activities (0.31 unit/mg and 0.033 unit, respectively) gives them a degree of specificity hitherto encountered only in d-(CH_2)$_5$VDAVP.[2] In addition, d(CH_2)$_5$Tyr(Me)AVP exhibited a protracted antivasopressor effect greater than

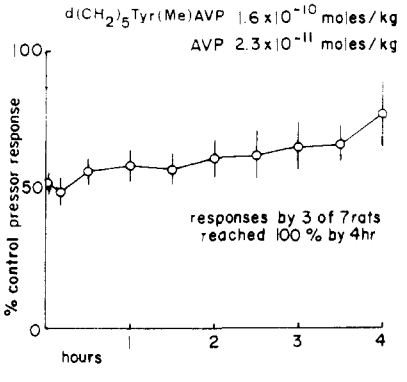


Figure 1. Prolonged inhibition of vasopressor responses by rats to AVP by d(CH_2)$_5$Tyr(Me)AVP. The antagonist was injected in a dose of 184 ng/kg at time 0, and AVP was injected at the intervals shown in a dose of approximately 25 ng/kg. Mean responses plus or minus standard errors (n = 7) are expressed as percent of the responses to the same dose of AVP prior to injection of antagonist.

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any we have previously observed (Figure 1). Antagonism of vasopressor responses by similar doses of $d(CH_2)_5AVP$ usually lasted for only about 30 min. Both peptides exhibit strikingly high antioxytocic potencies in vitro in the absence of Mg^{2+}. These potencies are diminished in the presence of Mg^{2+} and in vivo. Nevertheless, these potencies are in the same range as potencies reported from these laboratories for some potent oxytocic antagonists.[24,25] For example, $d(CH_2)_5OT$[6] has an antioxytocic pA_2 in vivo of 6.65 ± 0.06,[24] which does not differ significantly from that for $d(CH_2)_5AVP$ (6.79 ± 0.19) reported here.

In this series of AVP analogues, substitution of a β,β-cyclopentamethylene group for a β,β-dimethyl group clearly enhances antivasopressor activities and decreases antidiuretic potency (Table II). Comparing the properties of dPAVP and $d(CH_2)_5AVP$ one sees that substitution of a β,β-cyclopentamethylene for a β,β-dimethyl group leads to an eightfold enhancement of antivasopressor activity and a 1250-fold reduction in antidiuretic activity. When dPTyr(Me)AVP and $d(CH_2)_5$Tyr(Me)AVP are compared, this substitution produces a fourfold increase in antivasopressor potency and a tenfold reduction in antidiuretic activity. Thus, in both instances this substitution increased antivasopressor potency and specificity, at least in terms of the ratio of antivasopressor to antidiuretic activities. The substitution of a β,β-cyclopentamethylene[2] for a β,β-dimethyl group in dPVDAVP,[1] however, did not increase antivasopressor potency, although it did markedly decrease antidiuretic activity. We have no logical explanation for these differing effects. These findings point out the need for caution in extrapolating the effects of structural changes from one peptide to another in the design of analogues. The substitution of *O*-methyltyrosine in $d(CH_2)_5AVP$ to give $d(CH_2)_5$Tyr(Me)AVP brought about almost a twofold enhancement in antivasopressor potency. This is consistent with the effect of the same substitution in dPAVP.[13]

With their high antivasopressor potencies and specificity, $d(CH_2)_5AVP$ and $d(CH_2)_5$Tyr(Me)AVP promise to be of value as pharmacological tools for investigating the possible roles of AVP in cardiovascular regulation in normal and pathophysiological states.

Experimental Section

The procedure of "solid phase" synthesis conformed to that published.[14-18] Chloromethylated resin (Bio-Rad Bio-Beads SX-1) was esterified[26] with Boc-Gly to an incorporation of 0.47 and ~0.64 mmol/g. Amino acid derivatives, including Boc-Tyr(Me)[13,24] (R_f (A) 0.7; R_f (B) 0.8), were supplied by Bachem Inc. Triethylamine (TEA) and *N*-methylmorpholine (NMM) were distilled from ninhydrin. The acetic acid used for the HCl–acetic acid cleavage reagent 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, chloroform–methanol (7:3, v/v); E, butan-1-ol–acetic acid–water (4:1:5, v/v, upper phase); 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 and iodine vapor were used for detection. For amino acid analysis,[27] peptides (~0.5 mg) were hydrolyzed with constant-boiling hydrochloric acid (400 μL) in evacuated and sealed ampules for 18 h at 110 °C. 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 symbols were within ±0.4% of theoretical values. Optical rotations were measured with a Bellingham Stanley, Ltd., Model A polarimeter, type p1.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (I). Preparation a, by a Combination of Solid-Phase and Solution Methods. Boc-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2[13,28] (319 mg, 0.26 mmol) was dissolved in TFA (6.5 mL) and stirred at room temperature for 40 min. Cold ether (20 mL) was added, and the precipitated material was filtered and washed with ether (5 × 10 mL). The product was dried in vacuo over sodium hydroxide pellets. This material (318.5 mg) was dissolved in DMF (0.8 mL), and *N*-methylmorpholine (10 μL) was added to give a solution of pH 7–8 to moist pH paper.[20] Then this neutralized solution was stirred at room temperature for 30 min. A solution of *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate[6] (445 mg, 1.155 mmol, in 0.4 mL of DMF) was added. The reaction mixture was stirred at room temperature. After the reaction mixture was stirred for 72 h, TLC (in system D) showed that the reaction mixture still contained a trace of the free octapeptide amide. *N*-Hydroxybenzotriazole[21] monohydrate (39.3 mg, 0.26 mmol) was added. Coupling was complete within 5 h. The precipitate was filtered, then washed with cold ethyl acetate (4 × 10 mL), and dried in vacuo. The crude product (339 mg) was twice reprecipitated from DMF–methanol to give the acyl peptide amide Ia (2.5.2 mg, 77.3%): mp 209–211 °C; $[\alpha]^{24}_D$ −43.6° (*c* 0.5, DMF); TLC R_f (E) 0.45, R_f (F) 0.63. Anal. ($C_{73}H_{94}O_{14}N_{14}S_3$) C, H, N. Amino acid analysis:[27,29] Tyr, 0.80; Phe, 1.01; Glu, 1.04; Asp, 1.02; Cys(Bzl), 0.98; Pro, 1.06; Arg, 1.01; Gly, 1.00; NH_3, 2.91.

I. Preparation b, by Total Synthesis on Resin. Boc-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin[30] (1.11 g, 0.4 mmol) was converted to the acyl octapeptide resin (1.167 g, weight gain 57 mg, 97.6% of theory) in one cycle of deprotection, neutralization, and coupling with *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate.[6] The resin was ammonlyzed[16] and the product extracted with dimethylformamide (DMF). The solvent was evaporated in vacuo, and the residue

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(28) Prepared using solid-phase methodology from Boc-Gly-resin (0.47 mmol/g). The octapeptide derivative gave satisfactory elemental analysis and was indistinguishable by TLC from a previous preparation.[13] Amino acid analysis:[27] Tyr, 1.02; Phe, 1.03; Glu, 1.01; Asp, 1.03; Cys(Bzl), 0.98; Pro, 1.02; Arg, 0.97; Gly, 1.00; NH_3, 2.97.
(29) We found[13,24,28] that hydrolysis 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 were found in this and in previous studies[11,13,24] to result in a non-quantitative recovery of tyrosine due to the incompleteness of demethylation.[11] This demethylation was reported to have been avoided by hydrolysis with sulfuric acid.[11] In our laboratory, we found[13,24] that this method of hydrolysis (i.e., 1.8 M H_2SO_4, 24 h, 130 °C) offered no advantage over constant-boiling hydrochloric acid, so we decided to use the later reagent for hydrolysis of all of the *O*-methyltyrosine-containing protected and free peptides Ia, Ib, IIIa, and IIIb. These were all derived from the Boc octapeptide intermediate[28] or from its resin precursor. The Boc octapeptide has been shown in this[28] and in a previous report[13] to have a Tyr/Gly ratio of 1.02:1.00 following hydrolysis by hydrochloric acid. Thus, the Tyr/Gly ratio of ~0.8 for peptides Ia, Ib, IIIa, and IIIb is presumably due to the aforementioned incomplete demethylation.
(30) Boc-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (2.3 g, 0.9 mmol) was prepared from 1.41 g (0.9 mmol) of Boc-Gly-resin (~0.64 mmol/g) using solid-phase methodology and divided into two parts; 1.022 g (0.4 mmol) of of Boc-heptapeptide resin was used to obtain 1.092 g (0.4 mmol) of Boc-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin; 1.278 g (0.5 mmol) was used to obtain 1.404 g (0.5 mmol) of Boc-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin.

was precipitated by addition of water. The crude product (410 mg) was twice reprecipitated from DMF–ethanol to give the acyl octapeptide Ib (302 mg, 50.7% based upon initial glycine content of the resin): mp 206–208 °C dec; TLC R_f (E) 0.45, R_f (F) 0.63; $[\alpha]^{24}_D$ −43.1° (c 1, DMF). Anal. ($C_{73}H_{94}N_{14}O_{14}S_3$) C, H, N. Amino acid analysis:[27,29] Tyr, 0.79; Phe, 1.01; Glu, 1.03; Asp, 1.04; Cys(Bzl), 0.97; Pro, 1.03; Arg, 0.99; Gly, 1.00; NH_3, 2.95.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (II). Boc-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)Gly-resin[30] (1.46 g, 0.5 mmol) was converted to the acyl octapeptide resin (1.53 g, weight gain 70 mg, 95.9% of theory) in one cycle of deprotection, neutralization, and coupling with *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate.[6] The resin was ammonolyzed[16] and the product extracted with dimethylformamide (DMF). The solvent was evaporated in vacuo, and the residue was precipitated by addition of water. The crude product (723 mg) was reprecipitated from DMF–ethanol and DMF–2% aqueous AcOH (488 mg; 62.4% based upon initial Gly content on the resin): mp 183–185 °C; TLC R_f (E) 0.38, R_f (D) 0.41; $[\alpha]^{23}_D$ −32.9° (c 1, DMF). Anal. ($C_{79}H_{98}N_{14}O_{14}S_3$) C, H, N. Amino acid analysis:[27] Tyr, 0.97; Phe, 1.02; Glu, 1.05; Asp, 1.01; Cys(Bzl), 0.98; Pro, 1.04; Arg, 0.98; Gly, 1.00; NH_3, 2.95.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine]arginine-vasopressin (IIIa). A solution of the protected nonapeptide amide Ia (170 mg, 0.114 mmol) in sodium-dried and redistilled ammonia (400 mL) was treated at the boiling point and with stirring with sodium[22] 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 (0.4 mL) was added to discharge the color. The solution was evaporated and the residue was dissolved in aqueous acetic acid (0.2%; 800 mL), and this solution was treated with 2 M ammonium hydroxide solution to give a solution of pH 7.5. An excess of a solution of potassium ferricyanide (0.01 M, 11.4 mL)[4] was added gradually with stirring. The yellow solution was stirred for 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%; 300 mL),[3] and the combined filtrate and washings were lyophilized. The resulting powder (1386 mg) was desalted on a Sephadex G-15 column (110 × 2.7 cm) eluting with aqueous acetic acid (50%)[23] with a flow rate 4 mL/h. The eluate was fractioned and monitored for absorbance of 280 mm. The fractions comprising the major peak were pooled and lyophilized, and the residue (55.5 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) with a flow rate of 2.5 mL/h.[23] The peptide was eluted in a single peak (absorbance 280 mm). Lyophilization of the pertinent fractions yielded the vasopressin analogue (49 mg, 37.3%): TLC R_f (E) 0.19, R_f (F) 0.30; $[\alpha]^{22}_D$ −59.6° (c 0.19, 1 M AcOH). Amino acid analysis:[27,29] Tyr, 0.81; Phe, 1.01; Glu, 1.04; Asp, 0.98; Pro, 1.04; Arg, 0.95; Gly, 1.00; NH_3, 3.10. Analysis following performic acid oxidation prior to hydrolysis[31] gave a $Cys(O_3H)$–Gly ratio of 1.03:1.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine]arginine-vasopressin (IIIb). Treatment of the acyl octapeptide Ib (160 mg, 0.107 mmol) as detailed for IIIa yielded the analogue IIIb (64 mg, 51.7%), indistinguishable from preparation IIIa by TLC: $[\alpha]^{23}_D$ −59.1° (c 0.5, 1 M AcOH). Amino acid analysis:[27,29] Tyr, 0.80; Phe, 1.02; Glu, 1.02; Asp, 0.98; Pro, 1.03; Arg, 0.96; Gly, 1.00; NH_3, 3.05. Analysis following performic acid oxidation prior to hydrolysis[31] gave a $Cys(O_3H)$–Gly ratio of 1.02:1.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin (IV). Treatment of the acyl octapeptide II (173 mg, 0.111 mmol) as detailed above for IIIa yielded the analogue IV (66 mg, 52.5%): TLC R_f (E) 0.19, R_f (F) 0.43; $[\alpha]^{23}_D$ −58.7° (c 0.5, 1 M AcOH). Amino acid analysis:[27] Tyr, 0.96; Phe, 0.98; Glu, 1.01; Asp, 1.01; Pro, 1.05; Gly, 1.00; NH_3, 2.95. Analysis following performic acid oxidation prior to hydrolysis[31] gave a $Cys(O_3H)$–Gly ratio of 1.01:1.00.

Measurement of Duration of Antivasopressor Action of $d(CH_2)_5Tyr(Me)AVP$ (Figure 1). Rats were prepared as for routine vasopressor assays[32] with urethane anesthesia and phenoxybenzamine. After repeated vasopressor responses to intravenous injections of 2 milliunits of the USP posterior pituitary reference standard were found to remain stable, the antagonist, $d(CH_2)_5Tyr(Me)AVP$, was injected in the "effective dose" corresponding to the previously estimated pA_2. The 2 milliunit dose of standard was injected 1, 10, and 30 min later and each 30 min thereafter over a total period of 4 h. This dose of standard is equivalent in vasopressor activity to 5 ng of AVP or 25 ng/kg.

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Synthesis of Syn and Anti Isomers of 6-[[(Hydroxyimino)phenyl]methyl]-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazol-2-amine. Inhibitors of Rhinovirus Multiplication

J. H. Wikel,* C. J. Paget, D. C. DeLong, J. D. Nelson, C. Y. E. Wu, J. W. Paschal, A. Dinner, R. J. Templeton, M. O. Chaney, N. D. Jones, and J. W. Chamberlin

The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206. *Received October 1, 1979*

The synthesis and antirhinovirus activity of syn and anti isomers of 6-[[(hydroxyimino)phenyl]methyl]-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazol-2-amine (4 and 5) are reported. The structural assignments of 4 and 5 are based upon ^{13}C NMR spectra of both isomers and also X-ray analysis of 5. The anti-isomer 5 was more potent than the syn-isomer 4 when compared as an inhibitor of rhinovirus multiplication in vitro. Both isomers inhibited multiplication of 15 different serotypes of rhinovirus.

Early reports by Thompson[1] and Brown[2] of the inhibition of virus multiplication by benzimidazole stimulated the synthesis and evaluation of a wide variety of benzimidazole analogues. Many of these compounds had no

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