Potent and Selective Antagonists of the Antidiuretic Responses to Arginine-vasopressin Based on Modifications of [1-(β-Mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-valine]arginine-vasopressin at Position 4†

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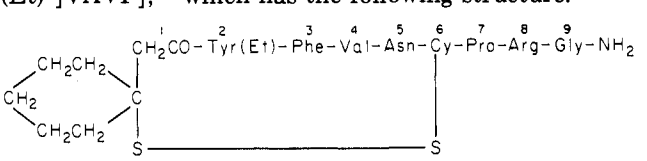
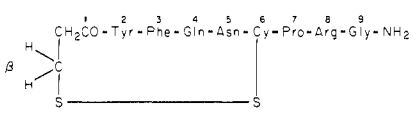
As part of a program in which we are attempting (a) to obtain more potent and/or more selective antagonists of the antidiuretic responses to arginine-vasopressin (AVP) and (b) to delineate the structural features at positions 1–9 required for antidiuretic antagonism, we have synthesized 13 new analogues of the antidiuretic antagonist [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-valine]arginine-vasopressin [d$(CH_2)_5$[D-Ile^2]VAVP] in which the valine residue at position 4 has been replaced by the L-amino acids Abu, Ile, Thr, Ala, Ser, Nva, Gln, Leu, Lys, Cha, Asn, Orn, and Phe and two new analogues of the antidiuretic antagonist [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-phenylalanine,4-valine]arginine-vasopressin [d$(CH_2)_5$[D-Phe^2]VAVP] with the Val^4 residue replaced by Ser and Orn. These analogues are 1, d$(CH_2)_5$[D-Ile^2,Abu^4]AVP; **2**, d$(CH_2)_5$[D-Ile^2,Ile^4]AVP; **3**, d$(CH_2)_5$[D-Ile^2,Thr^4]AVP; **4**, d$(CH_2)_5$[D-Ile^2,Ala^4]AVP; **5**, d$(CH_2)_5$[D-Ile^2,Ser^4]AVP; **6**, d$(CH_2)_5$[D-Ile^2,Nva^4]AVP; **7**, d$(CH_2)_5$[D-Ile^2]AVP; **8**, d$(CH_2)_5$[D-Ile^2,Leu^4]AVP; **9**, d$(CH_2)_5$[D-Ile^2,Lys^4]AVP; **10**, d$(CH_2)_5$[D-Ile^2,Cha^4]AVP; **11**, d$(CH_2)_5$[D-Ile^2,Asn^4]AVP; **12**, d$(CH_2)_5$[D-Ile^2,Orn^4]AVP; **13**, d$(CH_2)_5$[D-Ile^2,Phe^4]AVP; **14**, d$(CH_2)_5$[D-Phe^2,Ser^4]AVP; and **15**, d$(CH_2)_5$[D-Phe^2,Orn^4]AVP. The protected peptide precursors for these peptides were prepared by the solid-phase method, followed by ammonolytic cleavage. The free peptides 1–15 were obtained by deblocking with Na in NH_3, oxidation of the resultant disulfhydryl compounds with dilute $K_3[Fe(CN)_6]$, and purification on Sephadex G-15 in a two-step procedure with 50% HOAc and 0.2 M HOAc as eluants. Analogues 1–15 were tested in rats for agonistic and antagonistic activities by antidiuretic, vasopressor, and oxytocic assays. Analogues 1–4 exhibit no evident antidiuretic agonistic activity. All analogues, with the exception of the Phe^4-containing analogue, are antidiuretic and vasopressor antagonists. Their antiantidiuretic pA_2 values are as follows: 1, 8.22 ± 0.05; **2**, 8.04 ± 0.10; **3**, 7.91 ± 0.09; **4**, 7.76 ± 0.12; **5**, 7.26 ± 0.10; **6**, 7.01 ± 0.09; **7**, 6.96 ± 0.12; **8**, 6.80 ± 0.07; **9**, 6.76 ± 0.05; **10**, 6.64 ± 0.05; 11, 6.51 ± 0.11; **12**, 6.46 ± 0.01; **13**, mixed agonist/antagonist; 14, 7.33 ± 0.11; **15**, 6.5 ± 0.03. All analogues antagonize the in vitro oxytocic responses to oxytocin. Analogues 1–**3**, **6**, **7**, and 14 also antagonize oxytocic responses to oxytocin in vivo. Three of these analogues (1, **2**, and 4) are the most selective antidiuretic/vasopressor antagonists yet reported. With an antidiuretic effective dose of 0.41 ± 0.05 nmol/kg and a pA_2 value of 8.22 ± 0.05, d-$(CH_2)_5$[D-Ile^2,Abu^4]AVP is one of the most potent antidiuretic antagonists yet reported. The data on these analogues provide useful clues for the design of more potent and selective antidiuretic antagonists. Some of the analogues reported here are of value as pharmacological probes for studies of AVP-induced water retention states and also hold promise as therapeutic agents for the treatment of such conditions in humans.

As part of a program in which we are endeavoring to increase the (a) potency, (b) selectivity, and (c) duration of action of our recently reported antagonists of the antidiuretic responses to endogenous and exogenous AVP[1-5] and to delineate the structural features required for antidiuretic antagonism,[6] we have systematically modified the most potent member of the original series, i.e., [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyltyrosine,4-valine]arginine-vasopressin [d$(CH_2)_5$[Tyr(Et)^2]VAVP],[1,2] which has the following structure:



Replacement of the Tyr(Et) residue at position 2 by D-Tyr(Et) led to a substantial enhancement in antiantidiuretic potency.[3] It was subsequently shown that replacement of the Tyr(Et) residue by a series of D-amino acids, e.g., D-Tyr, D-Phe, D-Ile, D-Leu, and D-Val, led to

† Symbols and abbreviations are in accordance with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 977). All amino acids are in the L configuration unless otherwise noted. Other abbreviations used are as follows: Abu, α-aminobutyric acid; Cha, cyclohexylalanine; Nva, norvaline; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; HOAc, acetic acid; HOBT, *N*-hydroxybenzotriazole; NPE, nitrophenyl ester; AVP, arginine-vasopressin; VAVP, [4-valine]arginine-vasopressin; d, 1-deamino; d$(CH_2)_5$, 1-deaminopentamethylene. The d$(CH_2)_5$ abbreviation, rather than the more cumbersome 1-β-Mpa(β-$(CH_2)_5$)[12] abbreviation, is used as the designation for the [1-(β-mercapto-β,β-pentamethylenepropionic acid] residue at position 1 in analogues of [1-deamino]arginine-vasopressin (dAVP) and [1-deamino]oxytocin. dAVP has the following structure:



Thus, the d$(CH_2)_5$ abbreviation as employed in this and in many previous publications from these[1-6] and numerous other laboratories (see, for example, ref 7–9 cited in: Manning, M.; Lammek, B.; Kruszynski, M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1982**, *25*, 408) specifies that the two hydrogens on the β-carbon of the β-mercaptopropionic acid (β-Mpa) residue at position 1 in [1-deamino]arginine-vasopressin and analogues are replaced by a pentamethylene [$(CH_2)_5$] substituent.

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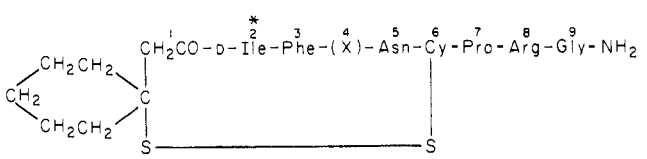
0022-2623/84/1827-0423$01.50/0

either retention or enhancement of antiantidiuretic potency and in some cases remarkable enhancements of antiantidiuretic/antivasodepressor selectivity.[3,4] The two most potent compounds to emerge to date from the study of position 2 are [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-phenylalanine,4-valine]arginine-vasopressin [d$(CH_2)_5$[D-Phe^2]VAVP][4] and [1-(β-mercapto-β,β-pentamethylenepropionic acid,2-D-isoleucine,4-valine]arginine vasopressin [d$(CH_2)_5$[D-Ile^2]VAVP].[4] We selected both of these compounds as lead compounds for the study of further modifications at position 4. We recently reported the synthesis and some pharmacological properties of a series of 14 analogues of d$(CH_2)_5$[D-Phe^2]VAVP with the Val residue at position 4 replaced by the following L-amino acids and glycine: Ile, Abu, Thr, Ala, Gln, Lys, Cha, Nle, Nva, Phe, Leu, Gly, Tyr, and Pro.[5] The first four of these analogues exhibited potent antidiuretic antagonism. Apart from the Pro^4 analogue, the remaining analogues all exhibited definite but weaker antidiuretic antagonism. We now report an analogous series of analogues of d$(CH_2)_5$[D-Ile^2]VAVP with the valine residue at position 4 replaced by the following 13 L-amino acids: (1) Abu, (2) Ile, (3) Thr, (4) Ala, (5) Ser, (6) Nva, (7) Gln, (8) Leu, (9) Lys, (10) Cha, (11) Asn, (12) Orn, (13) Phe. We also report two additional analogues of d$(CH_2)_5$[D-Phe^2]VAVP, with Ser and Orn replacing Val at position 4.

The 15 new analogues are as follows: **1**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-(α-aminobutyric acid)]arginine-vasopressin (d-$(CH_2)_5$[D-Ile^2,Abu^4]AVP); **2** [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-isoleucine]arginine-vasopressin [d$(CH_2)_5$[D-Ile^2,Ile^4]AVP]; **3**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-threonine]arginine-vasopressin [d$(CH_2)_5$[D-Ile^2,Thr^4]AVP]; **4**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-alanine]arginine-vasopressin [d$(CH_2)_5$[D-Ile^2,Ala^4]AVP]; **5**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-serine]-arginine-vasopressin [d$(CH_2)_5$[D-Ile^2,Ser^4]AVP]; **6**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-norvaline]arginine-vasopressin [d$(CH_2)_5$[D-Ile^2,Nva^4]AVP]; **7**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine]arginine-vasopressin [d-$(CH_2)_5$[D-Ile^2]AVP]; **8**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-leucine]arginine-vasopressin [d$(CH_2)_5$[D-Ile^2,Leu^4]AVP]; **9**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-lysine]arginine-vasopressin [d$(CH_2)_5$[D-Ile^2,Lys^4]AVP]; **10**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-cyclohexylalanine]arginine-vasopressin [d$(CH_2)_5$[D-Ile^2,Cha^4]AVP]; **11**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-asparagine]arginine-vasopressin [d$(CH_2)_5$-[Ile^2,Asn^4]AVP]; **12**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-ornithine]arginine-vasopressin [d$(CH_2)_5$[D-Ile^2,Orn^4]AVP]; **13**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-phenylalanine]arginine-vasopressin [d$(CH_2)_5$[D-Ile^2,Phe^4]AVP]; **14**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-phenylalanine,4-serine]arginine-vasopressin [d$(CH_2)_5$[D-Phe^2,Ser^4]AVP]; **15**, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-phenylalanine,4-ornithine]arginine-vasopressin [d$(CH_2)_5$[D-Phe^2,Orn^4]-AVP]. These 15 new analogues have the following general structure:



We now present the synthesis and some pharmacological properties of these 15 analogues.

Peptide Synthesis. The protected peptide precursors required for the synthesis of the 13 analogues of d-$(CH_2)_5$[Ile^2]VAVP and the 2 analogues of d$(CH_2)_5$[D-Phe^2]VAVP, all modified at position 4, were prepared by the solid-phase method of peptide synthesis,[7,8] with modifications as previously described.[9-11] β-(Benzylthio)-β,-β-pentamethylenepropionic acid[12] or its *p*-nitrophenyl ester was used in each final coupling step. All active ester[13,14] couplings were facilitated by the addition of 1-hydroxybenzotriazole (HOBT).[15] The dicyclohexylcarbodiimide (DCC)[16] couplings were carried out without the use of this additive. The 15 protected peptides were obtained as amides by ammonolytic cleavage[9,17] from the respective acyl octapeptide resins. Na in NH_3[18] was used to deblock each protected precursor by a yield-enhancing modification of the standard workup procedure as previously described.[2] Oxidative cyclization of the deblocked disulfhydryl compounds was effected with dilute potassium ferricyanide.[19] The analogues were desalted and purified by gel filtration on Sephadex G-15 as previously described.[20]

Bioassay Methods. The agonistic and antagonistic potencies of these analogues were measured by previously described methods.[1,2,11,21-23] These included antidiuretic assays in rats under ethanol anesthesia, intravenous vasopressor assays in phenoxybenzamine-treated rats under urethane anesthesia, and oxytocic assays on isolated rat uteri and rat uteri in situ. The USP posterior pituitary reference standard was used in assays for agonistic and antagonistic activities, except for the rat uterus in situ. Synthetic oxytocin (Syntocinon, Sandoz) was used as the agonist in these assays. Agonistic activities are expressed in units per milligram. Antagonistic potencies were determined and expressed as "Effective doses" and as pA_2 values.[25] The effective dose is defined as the dose (in nanomoles per kilogram) that reduces the response seen from $2x$ units of agonist to the response with $1x$ unit of agonist. Estimated in vivo "pA_2" values represent the negative logarithms of the effective doses divided by the estimated volume of distribution (67 mL/kg). Inhibition

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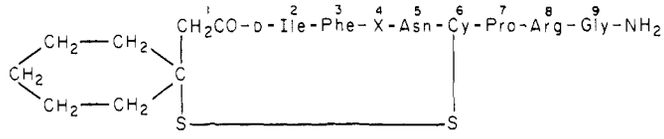
Table I. Antiantidiuretic and Antivasopressor Potencies of Arginine-vasopressin (AVP) Antagonists Based on Modifications of $d(CH_2)_5$[D-Ile^2,Val^4]AVP and of $d(CH_2)_5$[D-Phe^2,Val^4]AVP at Position 4



$d(CH_2)_5$[D-Ile^2,X^4]AVP

no.	peptide[i]	antiantidiuretic potency: ED, nmol/kg	antiantidiuretic potency: pA_2[b,c]	antivasopressor potency: ED, nmol/kg	antivasopressor potency: pA_2[b,c]	ED ratio[d]
1	$d(CH_2)_5$[D-Ile^2,Abu^4]AVP	0.41 ± 0.05[g]	8.22 ± 0.05 (4) [7.96][e]	12 ± 1.3	6.73 ± 0.04 (4) [7.70][e]	29
2	$d(CH_2)_5$[D-Ile^2,Ile^4]AVP	0.67 ± 0.15[g]	8.04 ± 0.10 (4) [8.24]	26 ± 3	6.42 ± 0.06 (4) [7.86]	39
*	$d(CH_2)_5$[D-Ile^2,Val^4]AVP[f]	0.70 ± 0.08[g]	7.98 ± 0.05 (4) [8.07]	8.2 ± 1.4	6.94 ± 0.08 (5) [8.06]	12
3	$d(CH_2)_5$[D-Ile^2,Thr^4]AVP	0.88 ± 0.18[g]	7.91 ± 0.09 (4) [7.62]	10.5 ± 1.2	6.83 ± 0.05 (8) [7.38]	12
4	$d(CH_2)_5$[D-Ile^2,Ala^4]AVP	1.7 ± 0.5[g]	7.76 ± 0.12 (10) [7.52]	66 ± 5	6.03 ± 0.03 (4) [7.55]	39
5	$d(CH_2)_5$[D-Ile^2,Ser^4]AVP	4.6 ± 1.0	7.26 ± 0.10 (10) [7.33][h]	45 ± 11	6.21 ± 0.11 (4) [7.63][h]	10
6	$d(CH_2)_5$[D-Ile^2,Nva^4]AVP	7.0 ± 1.5	7.01 ± 0.09 (4) [6.99]	8.2 ± 2.2	6.96 ± 0.12 (4) [7.73]	1.2
7	$d(CH_2)_5$[D-Ile^2]AVP	8.3 ± 2.7	6.96 ± 0.12 (4) [7.21]	1.15 ± 0.26	7.79 ± 0.09 (4) [8.35]	0.14
8	$d(CH_2)_5$[D-Ile^2,Leu^4]AVP	11 ± 2	6.80 ± 0.07 (4) [6.07]	12.4 ± 2.4	6.75 ± 0.08 (4) [7.70]	1.1
9	$d(CH_2)_5$[D-Ile^2,Lys^4]AVP	11.9 ± 1.4	6.76 ± 0.05 (5) [7.22]	mixed agonist/antagonist	[6.93]	
10	$d(CH_2)_5$[D-Ile^2,Cha^4]AVP	15.6 ± 1.7	6.64 ± 0.05 (5) [7.19]	19.1 ± 2.38	6.56 ± 0.06 (4) [6.93]	1.2
11	$d(CH_2)_5$[D-Ile^2,Asn^4]AVP	25 ± 5	6.51 ± 0.11 (7)	3.9 ± 0.8	7.26 ± 0.09 (4)	0.16
12	$d(CH_2)_5$[D-Ile^2,Orn^4]AVP	49 ± 4	6.46 ± 0.01 (7) [6.50]	11.9 ± 1.8	6.76 ± 0.06 (4) [7.57][h]	0.24
13	$d(CH_2)_5$[D-Ile^2,Phe^4]AVP	mixed agonist/antagonist	[6.07]	mixed agonist/antagonist	[6.45]	
14	$d(CH_2)_5$[D-Phe^2,Ser^4]AVP	3.9 ± 0.8	7.33 ± 0.11 (9)	1.6 ± 0.2	7.63 ± 0.07 (4)	0.41
15	$d(CH_2)_5$[D-Phe^2,Orn^4]AVP	21.3 ± 1.5	6.5 ± 0.03 (4)	1.9 ± 0.4	7.57 ± 0.08 (4)	0.09

[a] The effective dose (ED) is defined as the dose (in nanomoles per kilogram) that reduces the response seen with $2x$ units of agonist to equal the response seen with $1x$ unit of agonist administered before antagonist. [b] Estimated in vivo pA_2 values represent the negative logarithms of the "effective dose" divided by the estimated volume of distribution (6.7 mL/kg). [c] Means ± SE; number of assays in parentheses. [d] ED ratio = antivasopressor ED/antiantidiuretic ED. [e] pA_2 values for each of the corresponding D-Phe^2 antagonists reported in ref 5 and here (14 and 15). [f] From Manning et al.[4] [g] No evident agonist activity. [h] This publication. [i] The abbreviations and their full names are as follows: $d(CH_2)_5$[D-Ile^2,Abu^4]AVP, [1-[1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-(α-aminobutyric acid)]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Ile^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-isoleucine]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Val^4]AVPP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-valine]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Thr^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-threonine]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Ala^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-alanine]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Ser^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-serine]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Nva^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucin,4-norvaline]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Leu^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-leucine]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Lys^4]AVP [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-lysine]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Cha^4]AVP; [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-cyclohexylalanine]arginine-vasopressin; $d(CH_2)_5$[Ile^2,Asn^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-asparagine]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Nva^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-norvaline]arginine-vasopressin; $d(CH_2)_5$[D-Ile^2,Phe^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-phenylalanine]arginine-vasopressin; $d(CH_2)_5$[D-Phe^2,Ser^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-phenylalanine,4-serine]arginine-vasopressin; $d(CH_2)_5$[D-Phe^2,Orn^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-phenylalanine,4-ornithine]arginine-vasopressin.

of antidiuretic responses was tested by injecting the standard 20 min after injecting the antagonist, to allow for recovery from the initial antidiuretic responses to some of the antagonists. For those analogues showing no agonistic activity (compounds 1–4), the standard could be injected 10 min after the antagonist. Each peptide was administered in two doses, a high dose, which reduced the response to $2x$ units of agonist to less than the response to $1x$ units of agonist, and a low dose, which did not fully reduce the response to that given by $1x$ unit of agonist. In each case, the effective dose was obtained by interpolation on a logarithmic scale between the two doses of antagonist.[25]

Results and Discussion

The antiantidiuretic and antivasopressor potencies of the 13 $d(CH_2)_5$[D-Ile^2,X^4]AVP analogues, where X = Abu, Ile, Thr, Ala, Ser, Nva, Gln, Leu, Lys, Cha, Asn, Orn, Phe, and the two $d(CH_2)_5$[D-Phe^2,X^4]AVP analogues, where X = Ser and Orn, together with their antivasopressor/antiantidiuretic "effective dose" ratios, are presented in Table I. The antagonistic pA_2s for the corresponding d-$(CH_2)_5$[D-Phe^2,X^4]AVP analogues are denoted in brackets. The in vitro antioxytocic potencies for all analogues and the in vivo antioxytocic potencies and antioxytocic/antiantidiuretic ED ratios for some are presented in Table II. With the exception of the Phe^4 analogue, which is a mixed agonist/antagonist, all of the remaining 14 new analogues antagonize the antidiuretic and vascular responses to AVP (Table I). A number of these, i.e., 1–4, are quite potent and selective antidiuretic antagonists. With an antiantidiuretic effective dose of 0.41 nmol/kg and pA_2 = 8.22, $d(CH_2)_5$[D-Ile^2,Abu^4]AVP is the most potent of the entire series. It appears to be more potent than its parent analogue, $d(CH_2)_5$D-Ile^{2}VAVP (pA_2 = 7.98)[4] and is, in fact, equal in antiantidiuretic potency to the most potent antagonist previously reported, $d(CH_2)_5$[D-Phe^2,Ile^4]AVP (pA_2 = 8.24).[5] The four most potent antagonists (analogues 1–4) are devoid of any evident antidiuretic agonism. Three of these analogues (1, 2, and 4) exhibit significant enhancements of antiantidiuretic/antivasopressor selectivites

Table II. In Vitro and Some In Vivo Antioxytocic Potencies of AVP Antagonists Modified at Position 4

		antioxytocic activities (rat uterus)				
		in vitro		in vivo		
no.	peptide[g]	no Mg^{2+}, pA_2[b,c]	0.5 mM Mg^{2+}, pA_2[b,c]	ED, nmol/kg	pA_2	ED ratio[d]
1	$d(CH_2)_5$[D-Ile^2,Abu^4]AVP	7.49 ± 0.08 (7)	7.03 ± 0.10 (10)	19 ± 4	6.65 ± 0.12 [7.52]	45
2	$d(CH_2)_5$[D-Ile^2,Ile^4]AVP	7.47 ± 0.05 (8)	7.82 ± 0.04 (9)	9.4 ± 2.8	6.90 ± 0.11 (4) [7.11][e]	14
	$d(CH_2)_5$[D-Ile^2,Val^4]AVP[f]	7.98 ± 0.10 (10)	7.53 ± 0.07 (8)	44 ± 10	6.21 ± 0.09 (4) [6.92]	63
3	$d(CH_2)_5$[D-Ile^2,Thr^4]AVP	8.10 ± 0.05 (6)	7.69 ± 0.06 (7)	9.5 ± 1.8	6.87 ± 0.08 (4) [7.35]	11
4	$d(CH_2)_5$[D-Ile^2,Ala^4]AVP	7.50 ± 0.05 (6)	7.47 ± 0.09 (7)	57 ± 16	6.14 ± 0.12 (5) [6.91]	34
5	$d(CH_2)_5$[D-Ile^2,Ser^4]AVP	7.28 ± 0.08 (16)	6.82 ± 0.06 (7)			
6	$d(CH_2)_5$[D-Ile^2,Nva^4]AVP	7.83 ± 0.11 (7)	7.80 ± 0.11 (9)	22 ± 5	6.53 ± 0.09 (6)	3
7	$d(CH_2)_5$[D-Ile^2]AVP	7.62 ± 0.07 (7)	7.81 ± 0.07 (8)	15.9 ± 0.58	6.63 ± 0.02 (4) [7.25]	2
8	$d(CH_2)_5$[D-Ile^2,Leu^4]AVP	7.84 ± 0.11 (7)	7.57 ± 0.06 (9)			
9	$d(CH_2)_5$[D-Ile^2,Lys^4]AVP	7.10 ± 0.05 (6)	6.60 ± 0.07 (9)			
10	$d(CH_2)_5$[D-Ile^2,Cha^4]AVP	7.44 ± 0.03 (8)	7.09 ± 0.05 (8)			
11	$d(CH_2)_5$[D-Ile^2,Asn^4]AVP	7.93 ± 0.05 (7)	7.40 ± 0.03 (8)			
12	$d(CH_2)_5$[D-Ile^2,Orn^4]AVP	7.57 ± 0.08 (6)	6.71 ± 0.09 (8)			
13	$d(CH_2)_5$[D-Ile^2,Phe^4]AVP	7.13 ± 0.07 (6)	6.92 ± 0.07 (6)			
14	$d(CH_2)_5$[D-Phe^2,Ser^4]AVP	7.64 ± 0.07 (8)	7.83 ± 0.08 (8)	13 ± 2	6.70 ± 0.06 (4)	3
15	$d(CH_2)_5$[D-Phe^2,Orn^4]AVP	7.73 ± 0.06 (7)	7.70 ± 0.06 (10)			

[a-c,e,f] See corresponding footnotes in Table I. [d] ED ratio = antioxytocic ED/antiantidiuretic ED. [g] Abbreviations are the same as in Table I.

relative to $d(CH_2)_5$[D-Ile^2]VAVP. The data also permits a direct comparison of the effects of a D-Ile/D-Phe interchange at the 2-position in 12 new $d(CH_2)_5[Y^2,X^4]$AVP analogues, where Y = D-Phe, D-Ile; X = Abu, Ile, Thr, Ala, Ser, Nva, Gln, Leu, Lys, Cha, Orn, Phe.

Relative Effects of D-Ile^2 and D-Phe^2 on Antidiuretic Antagonism. The pattern of antiantidiuretic potencies previously described[5] for a series of 4-substituted analogues of $d(CH_2)_5$D-Phe^{2}VAVP is, with a few minor exceptions, followed here. The same amino acids are the top five in both cases, except the order for the first three varies. Thus, the order of potencies for the D-Phe^2 analogues was Ile > Val > Abu > Thr > Ala. Whereas for the D-Ile^2 analogues described here, the order is Abu > Ile > Val > Thr > Ala.

The Ser^4, Nva^4, and Orn^4 analogues are almost equipotent in both series. Four of the $d(CH_2)_5$[D-Ile^2,X^4]AVP analogues, i.e., those containing Abu^4, Thr^4, Ala^4, and Leu^4, are somewhat more potent than the corresponding $d(CH_2)_5$[D-Phe^2,X^4]AVP analogues. Five of the latter series, i.e., those containing Gln^4, Lys^4, Cha^4, and Phe^4, appear to be more potent than the corresponding member of the $d(CH_2)_5$[D-Ile^2,X^4]AVP series.

Aliphaticity and β-Branching at Position 4 in d-$(CH_2)_5$[D-Ile^2,X^4]AVP Analogues Are Critical for Antidiuretic Antagonism. The five most potent $d(CH_2)_5$-[D-Ile^2,X^4]AVP antagonists, i.e., with pA_2s greater than 7.5, have Abu, Ile, Val, Thr, and Ala at position 4. All are aliphatic amino acids, and three have substituents on the β-carbon. By contrast, the Phe^4 analogue is a mixed agonist/antagonist. Comparisons of the effective doses of the Ile^4/Leu^4 and the Val^4/Nva^4 pairs show clearly that the β-branched analogues are over 10-fold more potent than those containing their straight-chained isomers. The fourfold enhancement in antidiuretic antagonism of the Lys^4 analogue relative to the Orn^4 analogues and the threefold enhancements of (a) the Gln^4 analogue relative to the Asn^4 analogue and (b) the Ala^4 analogue relative to the Ser^4 analogue are consistent with the view that lipophilicity at position 4 also plays a role in endowing a peptide with optimal antagonistic properties. The presence of a positively charged residue at position 4 is reasonably well tolerated, although for the Lys^4 analogue (**9**) not as well as for its D-Phe^2-containing counterpart.[5]

Effects of Position-4 Modifications on Antivasopressor Potencies. Although all of the $d(CH_2)_5$[D-Ile^2,X^4]AVP analogues, with the exception of the Lys^4- and Phe^4-containing ones, exhibit antagonism to the vascular effects of AVP, only one, the analogue with Gln^4, which has an antivasopressor pA_2 equal to 7.79, could be considered reasonably potent. Thus, the substitution of a D-Ile^2 residue at position 2 in combination with a variety of substitutions at position 4 in $d(CH_2)_5$[Tyr(Et^2)]VAVP have led to drastic reductions of antivasopressor potency.

Enhancement of Antiantidiuretic/Antivasopressor Selectivity. Because of their substantially reduced antivasopressor potencies relative to their antiantidiuretic potencies, five of these new analogs, i.e., those with Abu, Ile, Thr, Ala, and Ser at position 4, exhibit significant enhancements in antiantidiuretic/antivasopressor selectivity. With antiantidiuretic/antivasopressor effective dose ratios of 39, 39, and 29, respectively, the $d(CH_2)_5$[D-Ile^2,Ile^4]AVP, $d(CH_2)_5$[D-Ile^2,Ala^4]AVP, and $d(CH_2)_5$[D-Ile^2,Abu^4]AVP analogues are the most selective of the entire series and, indeed, are the most selective antidiuretic/vasopressor antagonists reported to date.

Relative Effects of D-Ile^2/D-Phe^2 on Antivasopressor Potencies. The replacement of D-Phe by D-Ile at position 2 in the analogue series $d(CH_2)_5$[D-Phe^2,X^4]AVP, where X = Abu, Ile, Thr, Ala, Ser, Nva, Gln, Leu, Lys, and Cha, brought about a dramatic drop in antivasopressor potency in almost every case (Table I). Thus, $d(CH_2)_5$-[D-Ile^2,Ile^4]AVP and $d(CH_2)_5$[D-Ile^2,Ala^4]AVP have only $^1/_{25}$th of the antivasopressor potency of their D-Phe^2 counterparts, respectively.

Effects of Position-4 Substituents in $d(CH_2)_5$[D-Ile^2,X^4]AVP Analogues on in Vitro and in Vivo Antioxytocic Potencies. All of these peptides inhibit in vitro responses to oxytocin on the rat uterus. Seven of the in vitro antagonists were also tested in vivo. These are: $d(CH_2)_5$[D-Ile^2,Abu^4]AVP, $d(CH_2)_5$[D-Ile^2,Ile^4]AVP, $d(CH_2)_5$[D-Ile^2,Thr^4]AVP, $d(CH_2)_5$[D-Ile^2,Ala^4]AVP, $d(CH_2)_5$[D-Ile^2,Nva^4]AVP, $d(CH_2)_5$[D-Ile^2]AVP, and $d(CH_2)_5$[D-Phe^2,Ser^4]AVP. They all have only moderate antioxytocic potency in vivo. The D-Ile^2-containing antagonists exhibit enhanced antiantidiuretic/antioxytocic selectivity relative to the corresponding $d(CH_2)_5$[D-Phe^2,X^4]AVP analogues.

Conclusion

We have shown that the substitution of the Val^4 residue in the potent and selective antidiuretic/vasopressor antagonist $d(CH_2)_5$[D-Ile^2]VAVP[4] by a series of 13 different

amino acids leads to retention of antidiuretic antagonism in a majority of the resulting analogues. This is consistent with our recent findings for a series of similar analogues of the potent antidiuretic/vasopressor antagonist d-$(CH_2)_5$[D-Phe^2]VAVP.[5] Four of these new antagonists exhibit no evident antidiuretic agonism. These are d-$(CH_2)_5$[D-Ile^2,Abu^4]AVP, d$(CH_2)_5$[D-Ile^2,Ile^4]AVP, d-$(CH_2)_5$[D-Ile^2,Thr^4]AVP, and d$(CH_2)_5$[D-Ile^2,Ala^4]AVP. Furthermore, with antiantidiuretic/antivasopressor effective dose ratios of 29, 39, 12, and 39, respectively, three of these are the most selective antidiuretic antagonists reported to date. d$(CH_2)_5$[D-Ile^2,Ala^4]AVP with an antiantidiuretic/antioxytocic effective dose ratio of 119 is the most selective antidiuretic/oxytocic antagonist yet reported. With an antiantidiuretic pA_2 of 8.22, d$(CH_2)_5$[D-Ile^2,Abu^4]AVP is the most potent antidiuretic antagonist of the entire series and appears equipotent with d-$(CH_2)_5$[D-Phe^2,Ile^4]AVP, the most potent antidiuretic antagonist previously reported.[5] The data presented here combined with that previously reported from these laboratories[1-6] provide valuable clues for the design of more potent and more selective antidiuretic antagonists. A number of the antidiuretic antagonists reported here, in particular d$(CH_2)_5$[D-Ile^2,Abu^4]AVP, d$(CH_2)_5$[D-Ile^2,Ile^4]-AVP, d$(CH_2)_5$[D-Ile^2,Ile^4]AVP, d$(CH_2)_5$[D-Ile^2,Thr^4]AVP, and d$(CH_2)_5$[D-Ile^2,Ala^4]AVP, could (a) be of value in pharmacological, physiological, and pathophysiological studies on the role(s) of AVP in water retention[6,26] and (b) be of potential clinical value for the treatment of AVP-induced water retention states in humans.[27]

Experimental Section

The protected peptide intermediates I–XV (Table III) were synthesized by the solid-phase method by previously described procedures.[7-11] Chloromethylated resin (Chemalog, 1% cross-linked S-DVB, 200–400 mesh, 0.75–1.00 mequiv/g) was esterified with Boc-Gly to an incorporation of 0.5 mmol/g by the cesium salt method.[28] Amino acid derivatives were supplied by Bachem Inc. or Chemalog Inc. β-(Benzylthio)-β,β-pentamethylenepropionic acid[12] and its *p*-nitrophenyl ester[12] were synthesized, the former by a recently published improved procedure.[29] Dimethylformamide (DMF) was distilled under reduced pressure. Other solvents and reagents were analytical grade. Thin-layer chromatography (TLC) was on silica gel (0.25 mm, Brinkmann Silpate). The following solvent systems were used: (A) butan-1-ol–acetic acid–water (4:1:1, v/v); (B) butan-1-ol–acetic acid–water (4:1:5, v/v, upper phase); (C) butan-1-ol–acetic acid–water–pyridine (15:3:3:10, v/v); (D) chloroform–methanol (7:3 v/v). Loads of 10–50 μg were applied, and chromatograms were a minimum length of 10 cm. Iodine vapor was used for detection. For amino acid analysis,[30] peptides (~0.7 mg) were hydrolyzed with constant-boiling hydrochloric acid (400 μL) containing phenol (10 μL) in evacuated and sealed ampules for 18 h at 118 °C. The analyses were performed on a Model 121M Beckman automatic amino acid analyzer. Molar ratios were referred to Gly = 1.00. The cysteine content of the free peptides was estimated as $^1/_2$-cystine. Elemental analyses were performed by Integral Microanalytical Laboratories, Inc., Raleigh, NC. The analytical results for elements indicated by their symbols were within 0.4% of theoretical values. Optical rotations were measured with a Rudolph polarimeter Model 80.

Boc-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (A). The protected pentapeptide resin A (27.15 g, 10 mM) was prepared from 20 g (10 mM) of Boc-Gly-resin by solid-phase peptide synthesis methodology,[7-11] i.e., four cycles of deprotection with 1 M

Table III. Physiochemical Properties of the Protected Peptides: β-(Benzylthio)-β,β-pentamethylenepropionyl-Y-Phe-X-Asn-Cys(Bzl)-Pro-Arg(Tos)-GlyNH$_2$ (I-XV)[a]

no.	Y	X	formula	wt, mg	yield, %	mp, °C	$[\alpha]^{25}_D$, deg	R_f A	R_f B	R_f C	amino acid analyses ratios Phe	Gly	Ile	Asp	Cys(Bzl)	Pro	Arg	NH_3	X
I	D-Ile	Abu	$C_{68}H_{93}N_{13}O_{12}S_2$	425	77.0	218–220	−18.1[c]	0.49	0.84	0.87	1.02	1.00	1.00	1.10	1.02	1.00	1.03	2.01	1.00
II	D-Ile	Ile	$C_{70}H_{97}N_{13}O_{12}S_3$	132	23.5	223–225	−31.6[c]	0.50	0.84	0.78	0.94	1.00	0.89	0.97	0.90	0.96	0.97	2.03	0.89
III	D-Ile	Thr(Bzl)	$C_{75}H_{99}N_{13}O_{13}S_3 \cdot 2H_2O$	362	61.2	180–183	−15.7[b]	0.57	0.85	0.74	0.98	1.00	1.01	1.10	1.13	0.98	1.18	1.89	0.89
IV	D-Ile	Ala	$C_{67}H_{91}N_{13}O_{12}S_3 \cdot 2H_2O$	479	70.3	209–210.5	−23.6[b]	0.56	0.84	0.76	0.96	1.00	0.94	0.98	0.83	0.96	1.01	1.83	0.98
V	D-Ile	Ser(Bzl)	$C_{74}H_{97}N_{13}O_{13}S_3 \cdot 2H_2O$	470	60.1	199–201	−23.6[b]	0.62	0.86	0.76	1.03	1.00	1.01	1.05	0.83	1.05	1.04	1.82	0.93
VI	D-Ile	Nva	$C_{69}H_{95}N_{13}O_{12}S_3 \cdot 2H_2O$	505	72.4	227–230	−25.9[b]	0.76	0.86	0.72	0.97	1.00	0.90	1.00	0.84	0.96	1.00	1.88	1.18
VII	D-Ile	Gln	$C_{69}H_{94}N_{14}O_{13}S_3 \cdot 2H_2O$	366	64.3	196–197	−27.0[d]	0.30	0.76	0.68	0.99	1.00	1.02	1.11	1.09	0.98	1.05	2.67	1.07
VIII	D-Ile	Leu	$C_{70}H_{97}N_{13}O_{12}S_3 \cdot 2H_2O$	590	84.5	220–222	−29.0[d]	0.43	0.85	0.82	0.99	1.00	0.98	1.01	0.98	1.02	1.01	2.03	0.97
IX	D-Ile	Lys(Tos)	$C_{77}H_{106}N_{14}O_{14}S_4 \cdot 2H_2O$	678	85.8	179–180	−22.9[b]	0.74	0.83	0.92	0.99	1.00	0.95	1.00	0.88	1.02	1.01	1.96	0.98
X	D-Ile	Cha	$C_{73}H_{100}N_{13}O_{12}S_3 \cdot 2H_2O$	500	77.0	202–205	−20.9[b]	0.63	0.87	0.80	0.98	1.00	0.88	1.01	0.97	0.98	1.05	1.88	0.90
XI	D-Ile	Asn	$C_{68}H_{92}N_{14}O_{13}S_3 \cdot 2H_2O$	490	69.6	208–210	−45.0[b]	0.75	0.82	0.49	0.95	1.00	0.92	0.98	0.96	1.01	1.03	1.99	0.98
XII	D-Ile	Orn(Tos)	$C_{76}H_{102}N_{14}O_{14}S_4 \cdot 2H_2O$	262	34.5	196–198	−21.8[b]	0.61	0.89	0.77	0.98	1.00	0.96	1.00	0.85	0.99	0.98	1.82	0.95
XIII	D-Ile	Phe	$C_{73}H_{95}N_{13}O_{12}S_3 \cdot 2H_2O$	592	82.1	215–216.5	−31.9[b]	0.80	0.82	0.79	0.98	1.00	0.94	1.00	0.92	0.97	1.03	1.87	0.98
XIV	D-Phe	Ser(Bzl)	$C_{77}H_{55}N_{13}O_{13}S_3 \cdot 2H_2O$	272	36.3	181–182	−14.8[b]	0.74	0.85	0.67	1.99	1.00		1.01	0.98	1.41	0.98	1.97	0.95
XV	D-Phe	Orn(Tos)	$C_{79}H_{100}N_{14}O_{14}S_4 \cdot 2H_2O$	632	77.4	173–176	−15.8[b]	0.77	0.89	0.74	2.08	1.00		1.05	0.87	1.05	1.00	2.02	1.04

[a] Abbreviations are the same as in Table I. [b] *c* 1, DMF. [c] *c* 0.8, DMF. [d] *c* 0.5, DMF.

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HCl/HOAc, neutralization with 10% Et_3N in $MeCl_2$, and couplings (mediated by DCC) of Boc-Arg(Tos), Boc-Pro, Boc-Cys(Bzl), and finally Boc-Asn NPE (with HOBT as additive), respectively.

β-(Benzylthio)-β,β-pentamethylenepropionyl-D-Ile-Phe-Abu-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (I). The pentapeptidyl resin (A) (1.1 g, 0.4 mmol) was converted to protected acyl octapeptidyl resin in four cycles of solid-phase peptide synthesis using as the carboxy component Boc-L-Abu, Boc-L-Phe, Boc-D-Phe, and finally *p*-nitrophenyl β-(benzylthio)-β,β-pentamethylenepropionate[12] (with HOBT as additive),[15] respectively. The protected acyl octapeptide was cleaved by ammonolysis.[9,17] The crude product was extracted with hot (60 °C) DMF and, following removal of the resin, precipitated by the addition of water. The precipitate was collected, dried, taken up in hot DMF, reprecipitated with ethanol–ethyl ether, and then dried over P_2O_5/NaOH to give the required protected peptide amide I (Table III).

β-(Benzylthio)-β,β-pentamethylenepropionyl-D-Ile-Phe-X-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (II–XIII) [X = Ile (II), Thr(Bzl) (III), Ala (IV), Ser(Bzl) (V), Nva (VI), Gln (VII), Leu (VIII), Lys(Tos) (IX), Cha (X), Asn (XI), Orn(Tos) (XII), Phe (XIII)]. Each of the protected peptides II–XIII was synthesized from the pentapeptidyl resin (A) (1.1 g, 0.4 mmol) in the same manner as for I, except that in the first coupling, Boc-L-Abu was replaced by the Boc derivative of X in each case. (For VII, Boc-Gln was coupled as the *p*-nitrophenyl ester derivative, and the subsequent deprotection was carried out with TFA as previously described.[9]) Also, in some cases β-(benzylthio)-β,β-pentamethylenepropionic acid[12] was used in the final coupling with DCC. The physiochemical properties of all of the protected peptides I–XIII are given in Table III.

β-(Benzylthio)-β,β-pentamethylenepropionyl-D-Phe-Phe-X-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (XIV–XV) [X = Ser(Bzl) (XIV), Orn(Tos) (XV)]. Both of the protected peptides XIV and XV were synthesized from the pentapeptidyl resin (A) (1.1 g, 0.4 mmol) in the same manner as for I, except that in the first coupling Boc-L-Ile was replaced by the Boc derivative of X in each case and in the third coupling the Boc-D-Ile was replaced by Boc-D-Phe. The physiochemical properties of both of the protected peptides XIV and XV are given in Table III.

[1-(β-Mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-(α-aminobutyric acid)]arginine-vasopressin [d(CH_2)$_5$[D-Ile^2,Abu^4]AVP] (1). The protected acyl octapeptide amide I (120 mg, 0.083 mmol) was dissolved in dry ammonia (500 mL) redistilled from sodium. The solution was treated at the boiling point and with stirring with sodium[18] from a stick of sodium contained in a small-bore glass tube until a light-blue color persisted in the solution for ~30 s. Dry acetic acid (0.4 mL) was added to discharge the color. The ammonia was evaporated, and nitrogen was passed through the flask. After 5 min, the residue was dissolved in degassed aqueous acetic acid (20%, 50 mL) and quickly poured into ice-cold water (~1500 mL).[2] The pH was adjusted to ~7.0 with concentrated ammonium hydroxide. Following the neutralization, an excess of a solution of potassium ferricyanide (0.01 M, 15 mL)[19] was added gradually with stirring. The yellow solution was stirred for an additional 20 min and for 10 min with anion-exchange resin (Bio-Rad AG-3, Cl^- form, 40 g damp weight). The suspension was slowly filtered through a bed of resin (40 g damp weight). The bed was washed with water (200 mL), and the combined filtrate and washings were lyophilized. The resulting powder (2.51 g) was desalted on a Sephadex G-15 column (110 × 2.7 cm), eluting with aqueous acetic acid (50%)[20] with a flow rate of 5 mL/h. The eluate was fractioned and monitored for absorbance at 254 nm. The fractions comprising the major peak were checked by TLC (A), pooled, and lyophilized, and the residue was further subjected to gel filtration on a Sephadex G-15 column (100 × 1.5 cm), eluting with aqueous acetic acid (0.2 M)[20] with a flow rate of 4 mL/h. The peptide was eluted in a single peak (absorbance 254 nm). Lyophilization of the pertinent fractions gave the vasopressin analogue 1 as a white powder (Table IV).

[1-(β-Mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-amino acid X]arginine-vasopressin [d(CH_2)$_5$D-Ile^2,X^{4}AVP] (2–13) [X = Ile (2), Thr (3), Ala (4), Ser (5), Nva (6), Gln (7), Leu (8), Lys (9), Cha (10), Asn (11), Orn (12),

Table IV. Physiochemical Properties of d(CH_2)$_5$Y^{2}X^{4}AVP Antagonists (1–15)

no.	peptide	Y^2	X^4	wt, mg	yield, %	$[\alpha]^{25}_D$, deg	R_f A	R_f B	R_f C	amino acid analyses ratios Ile	Phe	Asp	$^1/_2$-Cys	Pro	Arg	Gly	NH_3	X^4
1	d(CH_2)$_5$[D-Ile^{2}Abu^4]AVP	D-Ile	Abu	56.0	61.7	−100[b]	0.25	0.30	0.47	0.96	1.07	1.13	1.10	0.98	1.10	1.00	1.87	0.99
2	d(CH_2)$_5$[D-Ile^{2}Ile^4]AVP	D-Ile	Ile	42.0	50.2	−94.7[c]	0.29	0.32	0.51	0.91	1.00	1.07	0.86	1.03	1.04	1.00	1.87	0.91
3	d(CH_2)$_5$[D-Ile^{2}Thr^4]AVP	D-Ile	Thr	54.6	63.6	−139.6[d]	0.20	0.29	0.44	0.88	1.02	1.04	1.04	0.93	0.96	1.00	2.01	0.89
4	d(CH_2)$_5$[D-Ile^{2}Ala^4]AVP	D-Ile	Ala	26.6	29.4	−136.0[d]	0.36	0.29	0.44	0.88	0.99	0.98	0.85	1.03	0.99	1.00	1.93	1.00
5	d(CH_2)$_5$[D-Ile^{2}Ser^4]AVP	D-Ile	Ser	28.7	34.6	−124.7[d]	0.27	0.28	0.42	0.94	0.88	1.02	0.89	1.04	0.95	1.00	1.88	0.86
6	d(CH_2)$_5$[D-Ile^{2}Nva^4]AVP	D-Ile	Nva	28.5	31.3	−74.0[e]	0.37	0.34	0.49	0.90	1.00	1.02	0.92	1.09	1.00	1.00	1.87	0.94
7	d(CH_2)$_5$[D-Ile^2]AVP	D-Ile	Gln	62.8	68.8	−114.0[d]	0.12	0.31	0.35	1.00	1.12	1.13	1.12	0.97	1.13	1.00	1.95	1.07
8	d(CH_2)$_5$[D-Ile^{2}Leu^4]AVP	D-Ile	Leu	37.2	40.8	−102.3[d]	0.30	0.33	0.50	0.88	0.99	1.01	0.99	1.06	1.06	1.00	1.97	1.02
9	d(CH_2)$_5$[D-Ile^{2}Lys^4]AVP	D-Ile	Lys	26.5	33.0	−88.3[d]	0.03	0.23	0.20	0.94	0.98	0.99	0.99	1.03	0.95	1.00	1.81	0.98
10	d(CH_2)$_5$[D-Ile^{2}Cha^4]AVP	D-Ile	Cha	15.0	16.7	−97.2[d]	0.42	0.36	0.51	0.95	0.98	1.02	1.08	1.05	0.97	1.00	1.83	1.10
11	d(CH_2)$_5$[D-Ile^{2}Asn^4]AVP	D-Ile	Asn	25.0	27.4	−95.8[d]	0.22	0.28	0.35	0.95	0.96	1.00	1.04	1.05	0.97	1.00	1.98	1.00
12	d(CH_2)$_5$[D-Ile^{2}Orn^4]AVP	D-Ile	Orn	29.0	35.2	−107.6[d]	0.04	0.25	0.20	0.92	1.00	1.00	0.99	1.05	0.99	1.00	1.90	1.11
13	d(CH_2)$_5$[D-Ile^{2}Phe^4]AVP	D-Ile	Phe	22.0	31.8	−109[d]	0.38	0.36	0.49	0.95	0.98	1.02	0.99	1.07	0.97	1.00	1.84	0.98
14	d(CH_2)$_5$[D-Phe^{2}Ser^4]AVP	D-Phe	Ser	29	33.7	−124.4[d]	0.25	0.30	0.42		1.97	1.01	0.89	0.88	0.99	1.00	2.00	0.88
15	d(CH_2)$_5$[D-Phe^{2}Orn^4]AVP	D-Phe	Orn	23	27.7	−112.3[d]	0.03	0.27	0.2		1.96	1.04	0.95	1.04	0.97	1.00	1.88	1.01

[a] Abbreviations are the same as in Table I. [b] *C* 0.6. [c] *C* 0.4, 1 N AcOH. [d] *C* 0.3, 1 N AcOH. [e] *C* 0.3, 50% AcOH.

Phe (13)]. Each of these cyclized free peptides (2–13) was obtained from their respective protected precursors II–XIII by the procedure followed in obtaining the free peptide 1 from the protected intermediate I. (For some of the more insoluble peptides, it was necessary to use up to 100 mL of 50% aqueous acetic acid to dissolve the residue following evaporation of NH_3.) Their physiochemical properties are given in Table IV. The pharmacological properties of peptides 1–13 are presented in Tables I and II.

[1-(β-Mercapto-β,β-pentamethylenepropionic acid)-2-D-phenylalanine,4-amino acid X]arginine-vasopressin [d-$(CH_2)_5$D-Phe^2,X^4,AVP] (14 and 15) [X = Ser (14), Orn (15)]. Both of these cyclized free peptides (14 and 15) were obtained from their respective protected precursors XIV and XV by the procedure followed in obtaining the free peptide 1 from the protected intermediate I. Their physiochemical properties are given in Table IV. The pharmacological properties of both peptides are presented in Tables I and II.

Acknowledgment. This work was supported in part by research grants from the National Institute of General Medical Sciences (GM-25280), the National Institute of Arthritis, Metabolism and Digestive Diseases (AM-01940), and the National Heart, Lung and Blood Institute (HL-12738). The authors thank Dr. Roger Roeske, Indiana University School of Medicine, Indianapolis, IN, for the amino acid analyses and Beverly Cifelli for expert assistance in the preparation of the manuscript.

Registry No. 1, 88686-52-6; 2, 88686-53-7; 3, 88686-54-8; 4, 88686-55-9; 5, 88686-56-0; 6, 88686-57-1; 7, 88686-58-2; 8, 88703-08-6; 9, 88686-59-3; 10, 88686-60-6; 11, 88686-61-7; 12, 88686-62-8; 13, 88686-63-9; 14, 88686-64-0; 15, 88686-65-1; I, 88686-66-2; II, 88686-67-3; III, 88686-68-4; IV, 88703-09-7; V, 88686-69-5; VI, 88703-10-0; VII, 88686-70-8; VIII, 88686-71-9; IX, 88686-72-0; X, 88686-73-1; XI, 88686-74-2; XII, 88686-75-3; XIII, 88686-76-4; XIV, 88686-77-5; XV, 88686-78-6; Boc-Arg(Tos), 13836-37-8; Boc-Pro, 15761-39-4; Boc-Cys(Bzl), 5068-28-0; Boc-Asn NPE, 4587-33-1; Boc-L-Abu, 34306-42-8; Boc-L-Phe, 13734-34-4; Boc-D-Phe, 18942-49-9; *p*-nitrophenyl β-(benzylmercapto)-β,β-cyclopentamethylenepropionate, 55154-81-9; oxytocin, 50-56-6.

Inhibitors of Inosinic Acid Dehydrogenase. 2-Substituted Inosinic Acids

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A series of 2-substituted inosine monophosphate (IMP) and inosine derivatives were synthesized and tested for inhibitory activity against IMP dehydrogenase from *Escherichia coli*. All of the IMP analogues that possessed electron-withdrawing substituents on the phenyl ring of a benzylthio group placed at the 2-position of IMP showed strong inhibition, which was competitive with IMP. No evidence of hydrophobic interactions of the 2-substituent with the enzyme was observed.

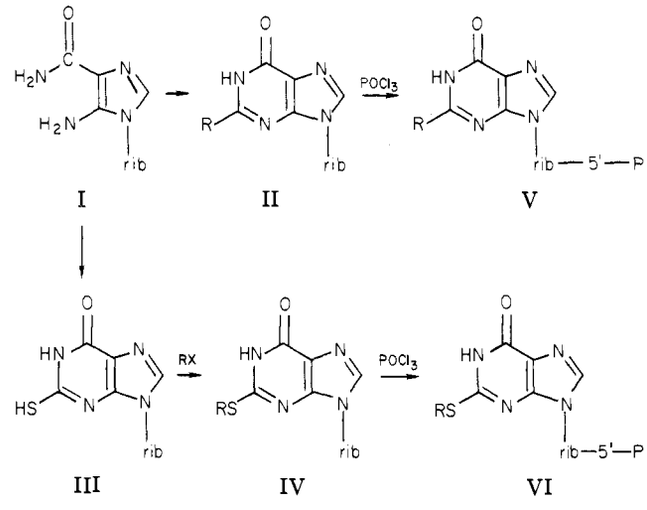
In a previous paper,[1] we discussed the importance of inosinic acid dehydrogenase (IMP dehydrogenase, EC 1.2.1.14) to rapidly growing cells. Most compounds that inhibit IMP dehydrogenase have anticancer activity. We presented some results on inhibition of that enzyme by a series of 8-substituted purine nucleotides.[1] In particular, we found that there is an apparent electron-rich binding site near the IMP site that can bind electron-deficient phenyl substituents on the 8-position of IMP or AMP. We now report the effect of various substituents at the 2-position of IMP on the ability of these compounds to inhibit IMP dehydrogenase, as well as the inhibition of this enzyme by the corresponding 2-substituted inosines.

Results

Synthesis. All of the nucleosides and nucleotides in this report were prepared from 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICA riboside, I). Those inosines with alkyl or aryl 2-substituents (II) were prepared by sodium ethoxide catalyzed ring closure of I with the appropriate ester.[2,3] When this method was used in an attempt to prepare nitro-substituted 2-aryl or aralkyl analogues, however, only intractable mixtures were obtained. Treatment of 2-mercaptoinosine[3] (III) with the appropriate benzyl halide gave a series of 2-[(substituted-benzyl)thio]inosines (IV). These methods are summarized in Scheme I, and the physical properties of all new nucleosides are given in Table I.

All of the inosine analogues were converted to the corresponding IMP analogues (V and VI) by phosphorylation

Scheme I



I II V

III IV VI

in phosphoryl chloride/trimethyl phosphate. The nucleotides were purified either by preparative HPLC or by chromatography on boric acid gel. Physical properties of all the new IMP analogues are given in Table II. Substantial difficulty was encountered in obtaining correct combustion analyses (especially nitrogen) on the nucleo-

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0022-2623/84/1827-0429$01.50/0