cleosides were dissolved in either hot water or 0.02–0.2 N NaOH depending upon solubility. Each compound was tested in two separate experiments, and the results are averaged; Me_2SO served as the positive control and caused 70% differentiation (Table I).

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Registry No. 1, 5536-17-4; 2, 7013-16-3; 3, 24822-51-3; 4, 71203-25-3; 5, 97551-52-5; 6, 97551-53-6; 7, 97551-54-7; 8, 97551-55-8; 9, 69370-82-7; 10, 97590-60-8; 11, 892-49-9; 12, 13153-62-3; 13, 58-61-7; 14, 2946-39-6; 15, 58-63-9; 16, 55627-73-1; 17, 97551-56-9; 18, 13389-16-7; 19, 13389-17-8; 20, 27883-25-6; 21, 574-25-4; 22, 342-69-8; 24, 3001-46-5; 25, 3868-37-9; 26, 21082-30-4; CH_3I, 74-88-4; 8-bromoguanosine, 4016-63-1; 8-(benzyloxy)-guanosine, 3868-36-8.

Synthesis and Some Pharmacological Properties of 18 Potent *O*-Alkyltyrosine-Substituted Antagonists of the Vasopressor Responses to Arginine-Vasopressin†

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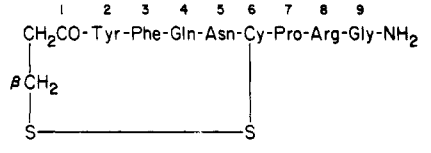
Using the Merrifield solid-phase method, we have synthesized 18 new 2-*O*-alkyltyrosine-substituted analogues (where alkyl = methyl and ethyl) of the arginine-vasopressin (AVP) vasopressor antagonists [1-deaminopenicillamine]-arginine-vasopressin (dPAVP), [1-(β-mercapto-β,β-diethylpropionic acid)]arginine-vasopressin (dEt_2AVP), and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin ($d(CH_2)_5$AVP) and of their 8-D-arginine ($d(R^2)$DAVP) analogues, their 4-valine (dR_2VAVP) analogues, and their 4-valine,8-D-arginine ($d(R_2)$VDAVP) analogues [where R = CH_3 or C_2H_5 and 2R = $(CH_2)_5$]. These analogues were tested for agonistic and antagonistic activities in in vivo rat vasopressor and rat antidiuretic and in vitro rat uterus assay systems. Although many exhibit very low antidiuretic activities, none of the new analogues antagonize antidiuretic responses to AVP. They exhibit no evident pressor activities and are in fact all highly effective antagonists of the vasopressor responses to AVP. They are also potent antagonists of the in vitro oxytocic responses to oxytocin, both in the absence and in the presence of Mg^{2+}. These analogues together with their corresponding antivasopressor pA_2 values are as follows: 1. dPTyr(Et)AVP, 8.40 ± 0.08; 2. dEt_2Tyr(Me)AVP, 8.53 ± 0.06; 3. dEt_2Tyr(Et)AVP, 8.46 ± 0.08; 4. $d(CH_2)_5$Tyr(Et)AVP, 8.47 ± 0.04; 5. dPTyr(Me)DAVP, 8.31 ± 0.08; 6. dPTyr(Et)DAVP, 8.27 ± 0.06; 7. dEt_2Tyr(Me)DAVP, 8.57 ± 0.03; 8. dEt_2Tyr(Et)DAVP, 8.33 ± 0.06; 9. $d(CH_2)_5$Tyr(Me)DAVP, 8.41 ± 0.05; 10. $d(CH_2)_5$Tyr(Et)DAVP, 8.45 ± 0.05; 11. dPTyr(Me)VAVP, 8.36 ± 0.07; 12. dPTyr(Et)VAVP, 8.07 ± 0.13; 13. dEt_2Tyr(Me)VAVP, 8.29 ± 0.08; 14. dEt_2Tyr(Et)VAVP, 8.42 ± 0.06; 15. dPTyr(Me)VDAVP, 7.84 ± 0.06; 16. dPTyr(Et)VDAVP, 8.46 ± 0.03; 17. dEt_2Tyr (Me)VDAVP, 8.35 ± 0.10; 18. dEt_2Tyr (Et)VDAVP, 8.19 ± 0.07. Seven of these analogues are clearly more potent vasopressor antagonists than their respective unalkylated tyrosine-containing parents. In the remaining 11, antagonistic potency was not changed significantly. In no instance did 2-*O*-alkyltyrosine substitution decrease antagonistic potency. With pA_2 values equal to or greater than 8.40, nine of these antagonists (numbers 1–4, 7, 9, 10, 14, and 16) are among the most potent vasopressor antagonists reported to date. They could thus serve as additional valuable pharmacological tools in studies on the roles of AVP in the control of blood pressure in normal and in pathophysiological conditions. These findings may also provide useful clues to the design of more potent and selective antagonists of AVP.

Antagonists of the vasopressor responses to arginine-vasopressin (AVP) that we have previously reported are proving to be valuable pharmacological tools in studies of the role(s) of AVP in the regulation of blood pressure.[1] Over 150 such studies carried out over the past 5 years have been reported. For partial listings of these publications, see ref 2 and 3.

In the design of antagonists of the vasopressor responses to AVP, the incorporation of dialkyl substituents on the β-carbon at position 1 in 1-deaminoarginine-vasopressin (dAVP) and related position-4- and position-8-substituted analogues have been found to be of particular value.[2-6] dAVP[7a] has the following structure:



† Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commision on Biochemical Nomenclature (*J. Biol. Chem.* 1971, *247*, 977). All amino acids are in the L configuration unless otherwise noted. Other abbreviations used: Tyr(Me), *O*-methyltyrosine; Tyr(Et), *O*-ethyltyrosine; AVP, arginine-vasopressin; dAVP, 1-deamino-arginine-vasopressin or [1-(β-mercaptopropionic acid)]arginine-vasopressin; dDAVP, 1-deamino[8-D-arginine]vasopressin; dVAVP, 1-deamino[4-valine]arginine-vasopressin; dVDAVP, 1-deamino[4-valine,8-D-arginine]vasopressin; $dP(d(CH_3)_2)$, 1-deaminopenicillamine or 1-β-mercapto-β,β-dimethylpropionic acid; dEt_2, 1-deaminodiethyl- or 1-β-mercapto-β,β-diethylpropionic acid; $d(CH_2)_5$, 1-deaminocyclopentamethylene or 1-β-mercapto-β,β-cyclopentamethylenepropionic acid; DMF, dimethylformamide; DCCI, dicyclohexylcarbodiimide; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; HOAc, acetic acid; HOBT, *N*-hydroxybenzotriazole; NPE, nitrophenyl ester.

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Thus, the replacement of the hydrogens on the β-carbon at position 1 in dAVP by the penicillamine (P or $(CH_3)_2$), diethyl (Et_2), and the cyclopentamethylene $(CH_2)_5$ groupings gave, respectively, dPAVP, dEt_2AVP, and d-$(CH_2)_5$AVP.[2,6] These three compounds exhibited no vasopressor agonism and are in fact potent antagonists of the vasopressor responses to AVP. The incorporation of these three β,β-dialkyl substitutions at position 1 in 1-deamino[8-D-arginine]vasopressin (dDAVP),[7b] 1-deamino[4-valine]arginine-vasopressin (dVAVP),[7c] and 1-deamino[4-valine,8-D-arginine]vasopressin (dVDAVP)[7d] also resulted in antagonists of the vasopressor responses to AVP.[2] The pharmacological properties of these 12 antagonists have been summarized in ref 2.

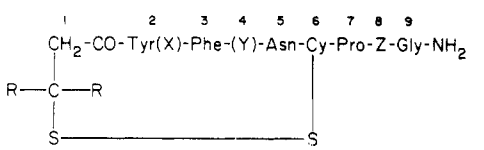
It may be recalled that duVigneaud and co-workers had first utilized these β,β-dialkyl substitutions in 1-deaminooxytocin to obtain potent antagonists of in vitro oxytocic responses to oxytocin and weak antagonists of the vasopressor responses to lysine vasopressin (LVP).[8-10] Also these workers subsequently demonstrated that the incorporation of the diethyl substituent on the β-carbon at position 1 in 1-deamino[lysine]vasopressin (dLVP) gave rise to an antagonist (dEt_2LVP) of the vasopressor responses to LVP.[11]

We had found that the substitution of *O*-methyltyrosine (Tyr(Me)) for tyrosine at position 2 in two of the aforementioned 12 antagonists, i.e. [1-deaminopenicillamine]-arginine-vasopressin (dPAVP) and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin (d$(CH_2)_5$AVP) resulted in two peptides dPTyr(Me)AVP[5] and d$(CH_2)_5$Tyr(Me)AVP[6] that are over twice as potent in inhibiting vasopressor responses to AVP as are their parents, i.e. dPAVP and d$(CH_2)_5$AVP. The antivasopressor potencies of these antagonists were measured by the method of Dyckes et al.[11] and expressed as pA_2 values. pA_2 values as defined by Schild[12] represent the negative logarithms to the base 10 of the average molar concentration of antagonist that will reduce the specific biological response to a given dose of agonist, e.g. 2*x* units, to equal the response obtained with half of this dose, i.e. 1*x* unit of agonist. Thus, dPTyr(Me)AVP and d$(CH_2)_5$Tyr(Me)-AVP had pA_2 values of 7.96 and 8.62, respectively, whereas dPAVP and d$(CH_2)_5$AVP had pA_2 values of 7.45 and 8.35, respectively. Also, the first four antagonists of the antidiuretic responses to AVP were later designed by substituting Tyr(Me) and *O*-ethyltyrosine (Tyr(Et)) for tyrosine at position 2 in [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid),4-valine]arginine-vasopressin (d-$(CH_2)_5$VAVP)[2] and in its 8-D-arginine analogue d-$(CH_2)_5$VDAVP.[4b] The resulting peptides d$(CH_2)_5$Tyr-(alk)VDAVP and d$(CH_2)_5$Tyr(alk)VAVP (where alk = Me or Et) exhibited potent antagonism to the antidiuretic responses to AVP and also to the vasopressor responses to AVP.[13]

On the basis of these findings, we were very curious to learn how the substitution of Tyr(Me) and Tyr(Et) for the tyrosine residue in all the remaining analogues of dAVP, dDAVP, dVAVP, and dVDAVP, which have the P, Et_2, and $(CH_2)_5$ grouping on the β-carbon at position 1,[2] would affect their biological properties. We were particularly intrigued by the possibility that these substitutions might result in (a) analogues with potent antidiuretic antagonism and/or (b) analogues possessing enhanced antivasopressor potencies. We also hoped to obtain further insights to the relative effectiveness of Tyr(Me) and the Tyr(Et) substitutions in modulating the biological activities of their parent free-tyrosine-containing peptides.

On the basis of this rationale, we now report the synthesis and some pharmacological properties of the following 18 new peptides: 1. [1-deaminopenicillamine,2-*O*-ethyltyrosine]arginine-vasopressin (dPTyr(Et)AVP); 2. [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-methyltyrosine]arginine-vasopressin (dEt_2Tyr(Me)AVP); 3. [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-ethyltyrosine]arginine-vasopressin (dEt_2Tyr(Et)AVP); 4. [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-ethyltyrosine]arginine-vasopressin (d$(CH_2)_5$Tyr(Et)AVP); 5. [1-deaminopenicillamine,2-*O*-methyltyrosine,8-D-arginine]vasopressin (dPTyr(Me)DAVP); 6. [1-deaminopenicillamine,2-*O*-ethyltyrosine,8-D-arginine]vasopressin (dPTyr(Et)DAVP); 7. [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-methyltyrosine,8-D-arginine]vasopressin (dEt_2Tyr(Me)DAVP); 8. [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-ethyltyrosine,8-D-arginine]vasopressin (dEt_2Tyr(Et)DAVP); 9. [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-methyltyrosine,8-D-arginine]vasopressin (d$(CH_2)_5$Tyr(Me)DAVP); 10. [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-ethyltyrosine,8-D-arginine]vasopressin (d$(CH_2)_5$Tyr(Et)-DAVP); 11. [1-deaminopenicillamine,2-*O*-methyltyrosine-,4-valine]arginine-vasopressin (dPTyr(Me)VAVP); 12. [1-deaminopenicillamine,2-*O*-ethyltyrosine,4-valine]arginine-vasopressin (dPTyr(Et)VAVP); 13. [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-methyltyrosine,4-valine]-arginine-vasopressin (dEt_2Tyr(Me)VAVP); 14. [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-ethyltyrosine,4-valine]arginine-vasopressin (dEt_2Tyr(Et)VAVP); 15. [1-deaminopenicillamine,2-*O*-methyltyrosine,4-valine,8-D-arginine]vasopressin (dPTyr(Me)VDAVP); 16. [1-deaminopenicillamine,2-*O*-ethyltyrosine,4-valine,8-D-arginine]vasopressin (dPTyr(Et)VDAVP); 17. [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-methyltyrosine-,4-valine,8-D-arginine]vasopressin (dEt_2Tyr(Me)VDAVP); 18. [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-ethyltyrosine,4-valine,8-D-arginine]vasopressin (dEt_2Tyr(Et)-VDAVP).

These analogues have the general structure



where R = CH_3 in dP analogues, R = C_2H_5 in dEt_2 analogues, and 2R = $(CH_2)_5$ in d$(CH_2)_5$ analogues; X = Me

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or Et; Y = Gln or Val; and Z = L or D-Arg.

Peptide Synthesis. The protected peptide precursors required for the synthesis of the 18 new Tyr(alkyl)-containing analogues were prepared by the Merrifield method of solid-phase synthesis,[14] using previously described modifications.[2,4,6,15] Coupling reactions were mediated either by the active ester method[16] or by the DCCI/HOBT method.[17] The *p*-nitrophenyl esters of β-(*S*-benzylthio)-β,β-dimethylpropionic acid,[8] β-(*S*-benzylthio)-β,β-diethylpropionic acid,[9] and β-(*S*-benzylthio)-β,β-cyclopentamethylenepropionic acid[10] were each used in the final coupling steps. The 18 protected acyl octapeptide amides were obtained by ammonolytic cleavage[15,18] from the respective acyl octapeptide resin. Na in NH_3 was used to deblock each protected precursor as previously described,[2,4,6,15,19] and the resulting disulfhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$.[20] The free peptides were desalted and purified by gel filtration[21] on Sephadex G-15 as previously described.[22]

Bioassay Methods. The agonistic and antagonistic potencies of these analogues were measured by previously described methods.[2-6,23] 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 in a medium containing (a) no Mg^{2+} and (b) 0.5 mM Mg^{2+}. The USP posterior pituitary reference standard was used in assays for agonistic and antagonistic activities. Agonistic activities are expressed in units per milligram. Antagonistic potencies were determined and expressed as effective doses and as pA_2 values.[12] The effective dose is defined as the dose (in nanomoles per kilogram) that reduces the response seen from a given dose of agonist (for example 2*x* units) to the response obtained with half this amount, namely from 1*X* 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). Each peptide was administered in two doses, a high dose, which reduced the response to 2*x* units of agonist to less than the response to 1*x* units of agonist, and a low dose, which did not fully reduce the response to that given by 1*x* unit of agonist. The effective dose was estimated by interpolation on a logarithmic scale between the two doses of antagonist. The values for effective doses in the tables represent means ±SE's of a minimum of four such estimates for each analogue. Means were compared by using Student's t-test, and differences were considered significant if $p < 0.05$.

Results and Discussion

The antivasopressor potencies and antidiuretic activities of the 18 new analogues together with those of the aforementioned six previously reported[5,6,13] related *O*-alkyltyrosine-substituted analogues are given in Table I. Their antioxytocic potencies are given in Table II. The antivasopressor and antidiuretic activities of each of the corresponding unalkylated peptides[2] are given in brackets in Table I. None of the new peptides are vasopressor agonists. In fact, they all exhibit potent antagonism to the vasopressor responses to AVP. All of the new peptides exhibit relatively weak antidiuretic agonistic activities, and none of them antagonize the renal tubular responses to AVP. In trying to assess the effects of O-methylation and O-ethylation of the tyrosine residue on antivasopressor potencies and on antidiuretc activities in the entire series of 24 peptides, it is instructive to compare the properties of all of the peptides, especially those that are new, i.e. numbers 1–18, with those of their respective unalkylted tyrosine-containing parents, e.g. to compare the properties of dPTyr(Et)AVP with those of dPAVP, etc. Also, comparison of the properties of each of the Tyr(Et)- and Tyr(Me)-containing analogues in each pair will allow an assessment of the relative effects of the Tyr(Me) and Tyr(Et) substitutions on antivasopressor potencies and on antidiuretic activities.

Effects of O-Alkylation on Antivasopressor Potencies. Comparison of the antivasopressor potencies of the alkylated compounds with those of their respective unalkylated parents (Table I) shows that O-alkylation of tyrosine with *either* *O*–Me or *O*-Et resulted in significant enhancements of antivasopressor antagonistic potencies in 8 out of 12 instances. Neither Tyr(Me) nor Tyr(Et) analogues of dEt_2AVP, $d(CH_2)_5DAVP$, dEt_2VAVP, and dEt_2VDAVP differed significantly in antivasopressor activity from their nonalkylated parents. In one case, the Tyr(Et) analogue showed substantially enhanced activity, but its Tyr(Me) counterpart did not. Thus, dPTyr(Me)-VDAVP, with a $pA_2 = 7.80$, is virtually equipotent with dPVDAVP ($pA_2 = 7.82$), whereas, with a $pA_2 = 8.46$, the corresponding Tyr(Et) analogue is almost 4 times more potent than either analogue. Thus, it is very clear that the effects of tyrosine O-alkylation (*O*-Me/*O*-Et) on the antivasopressor potencies of AVP antagonists are not consistent and depend heavily on the substitutions at other positions.

Relative Effects of Tyr(Me) vs. Tyr(Et) on Vasopressor Antagonism. When the antivasopressor potencies of the 12 pairs of Tyr(Me)/Tyr(Et)-containing analogues in Table I are compared, no clear-cut picture of relative effectiveness emerges. In two pairs, the Tyr-(Et)-containing analogue is clearly more potent, whereas in two other pairs the Tyr(Me)-containing member is clearly more potent; the Tyr(Me) and Tyr(Et) analogues in the remaining eight pairs do not differ significantly. With pA_2 values of 8.40 and 8.46, respectively, dPTyr-(Et)AVP and dPTyr(Et)VDAVP are clearly more potent than dPTyr(Me)AVP and dPTyr(Me)VDAVP, which have pA_2 values of 7.96 and 7.80, respectively. In two other instances, the Tyr(Me)-containing analogue is significantly more potent than the Tyr(Et)-containing analogue. Thus, $d(CH_2)_5$Tyr(Me)AVP ($pA_2 = 8.62$) is more potent than $d(CH_2)_5$Tyr(Et)AVP ($pA_2 = 8.47$), and dEt_2Tyr(Me)DAVP ($pA_2 = 8.57$) is almost twice as potent as dEt_2Tyr(Et)-DAVP ($pA_2 = 8.33$). These findings point to the difficulty of making predictions as to the relative effectiveness of

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Table I. Effects of Tyr(Me)/Tyr(Et) Substitutions on the Antivasopressor and Antidiuretic Potencies of 24 (18 new[i] and 6 previously published) 1-β,β-Dialkyl-Substituted Antagonists of the Vasopressor Responses to AVP

		antivasopressor act.		
no.	antagonist	eff dose (ED),[a] nmol/kg	pA_2[b,c]	antidiuretic act., units/mg
	dPTyr(Me)AVP[d]	0.80 ± 0.07	7.96 ± 0.05* [7.45][g]	3.5 ± 0.5 [42][h]
1	dPTyr(Et)AVP	0.35 ± 0.06	8.40 ± 0.08*	0.59 ± 0.11
2	dEt_2Tyr(Me)AVP	0.20 ± 0.03	8.53 ± 0.06 [8.36]	0.066 ± 0.004 [0.38]
3	dEt_2Tyr(Et)AVP	0.23 ± 0.02	8.46 ± 0.08	0.035 ± 0.007
	$d(CH_2)_2$Tyr(Me)AVP[e]	0.16 ± 0.01	8.62 ± 0.03* [8.35]	0.31 ± 0.05 [0.033]
4	$d(CH_2)_5$Tyr(Et)AVP	0.23 ± 0.02	8.47 ± 0.09	0.079 ± 0.004
5	dPTyr(Me)DAVP	0.44 ± 0.08	8.31 ± 0.08* [7.45]	0.46 ± 0.08 [5.8]
6	dPTyr(Et)DAVP	0.37 ± 0.05	8.27 ± 0.06*	0.23 ± 0.02
7	dEt_2Tyr(Me)DAVP	0.18 ± 0.01	8.57 ± 0.03* [7.96]	0.019 ± 0.003 [0.067]
8	dEt_2Tyr(Et)DAVP	0.32 ± 0.04	8.33 ± 0.06*	0.0003
9	$d(CH_2)_2$Tyr(Me)DAVP	0.36 ± 0.04	8.41 ± 0.05 [8.52]	0.23 ± 0.11 [0.31]
10	$d(CH_2)_5$Tyr(Et)DAVP	0.24 ± 0.03	8.45 ± 0.05	0.076 ± 0.007
11	dPTyr(Me)VAVP	0.37 ± 0.5	8.36 ± 0.07* [7.92]	2.9 ± 0.7 [312]
12	dPTyr(Et)VAVP	0.78 ± 0.21	8.07 ± 0.13	0.62 ± 0.09
13	dEt_2Tyr(Me)VAVP	0.45 ± 0.08	8.29 ± 0.08 [8.29]	0.24 ± 0.01 [1.50]
14	dEt_2Tyr(Et)VAVP	0.33 ± 0.05	8.42 ± 0.06	0.10 ± 0.03
	$d(CH_2)_5$Tyr(Me)VAVP[f]	0.29 ± 0.06	8.32 ± 0.08* [7.97]	antagonist [pA_2 = 7.35] [0.32]
	$d(CH_2)_5$Tyr(Et)VAVP[f]	0.49 ± 0.11	8.16 ± 0.09	antagonist [pA_2 = 7.57]
15	dPTyr(Me)VDAVP	1.1 ± 0.2	7.84 ± 0.06 [7.82]	3.2 ± 0.4 [123]
16	dPTyr(Et)VDAVP	0.29 ± 0.02	8.46 ± 0.03*	~0.2
17	dEt_2Tyr(Me)VDAVP	0.32 ± 0.07	8.35 ± 0.10 [8.18]	0.11 ± 0.01 [0.71]
18	dEt_2Tyr(Et)VDAVP	0.45 ± 0.07	8.19 ± 0.07	0.07 ± 0.01
	$d(CH_2)_2$Tyr(Me)VDAVP[f]	0.28 ± 0.05	8.44 ± 0.07* [7.68]	antagonist [pA_2 = 6.68] [0.10]
	$d(CH_2)_2$Tyr(Et)VDAVP[f]	0.34 ± 0.04	8.31 ± 0.05*	antagonist [pA_2 = 7.10]

[a] The effective dose is defined as the dose (in nanomoles per kilogram) that reduces the response to $2x$ units of agonist to equal the response to $1x$ unit. [b] Estimated in vivo pA_2 values represent the negative logarithms of the effective doses divided by the estimated volume of distribution (67 mL/kg). [c] Means ± SE. [d] From Bankowski et al.[5] [e] From Kruszynski et al.[6] [f] From Sawyer et al. and Manning et al.[13] [g] pA_2 values for each of the corresponding unalkylated tyrosine-containing antagonists reported in ref 2 and 4–6. [h] Antidiuretic activities (units/mg) for each of the corresponding unalkylated tyrosine containing antagonists reported in ref 2 and 4–6. [i] The abbreviations of the 18 new peptides and their full names: 1. dPTyr(Et)AVP, [1-deaminopenicillamine,2-*O*-ethyltyrosine]arginine-vasopressin; 2. dEt_2Tyr(Me)AVP, [1-β-mercapto-β,β-diethylpropionic acid),2-*O*-methyltyrosine]arginine-vasopressin; 3. dEt_2Tyr(Et)AVP, [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-ethyltyrosine]arginine-vasopressin; 4. $d(CH_2)_5$Tyr(Et)AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-ethyltyrosine]arginine-vasopressin; 5. dPTyr(Me)DAVP, [1-deaminopenicillinamine,2-*O*-methyltyrosine,8-D-arginine]vasopressin; 6. dPTyr(Et)DAVP, [1-deaminopenicillamine,2-*O*-ethyltyrosine,8-D-arginine]vasopressin; 7. dEt_2Tyr(Me)DAVP, [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-methyltyrosine,8-D-arginine]vasopressin; 8. dEt_2Tyr(Et)DAVP, [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-ethyltyrosine,8-D-arginine]vasopressin; 9. $d(CH_2)_2$Tyr(Me)DAVP, [1-(β-mercapto-β,β-cyclopentadmethylenepropionic acid),2-*O*-methyltyrosine,8-D-arginine]vasopressin; 10. $d(CH_2)_5$Tyr(Et)DAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-ethyltyrosine,8-D-arginine]vasopressin; 11. dPTyr(Me)VAVP, [1-deaminopenicillamine,2-*O*-methyltyrosine,4-valine]arginine-vasopressin; 12. dPTyr(Et)-VAVP, [1-deaminopenicillamine,2-*O*-ethyltyrosine,4-valine]arginine-vasopressin; 13. dEt_2Tyr(Me)VAVP,[1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-methyltyrosine,4-valine]arginine-vasopressin; 14. dEt_2Tyr(Et)VAVP,[1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-ethyltyrosine,4-valine]arginine-vasopressin; 15. dPTyr(Me)VDAVP,[1-deaminopenicillamine,2-*O*-methyltyrosine,4-valine-8-D-arginine]vasopressin; 16. dPTyr(Et)VDAVP, [1-deaminopenicillamine,2-*O*-ethyltyrosine,4-valine,8-D-arginine]vasopressin; 17. dEt_2Tyr(Me)VDAVP, [1-(β-mercapto-β,β-diethylpropionic acid),2-*O*-methyltyrosine,4-valine,8-D-arginine]vasopressin; 18. dEt_2Tyr(Et)VDAVP,[1-(β-mercapto-β,-β-diethylpropionic acid),2-*O*-ethyltyrosine,4-valine,8-D-arginine]vasopressin. The abbreviations and the full names of the six previously reported peptides: dPTyr(Me)AVP, [1-deaminopenicillamine,2-*O*-methyltyrosine]arginine-vasopressin; $d(CH_2)_5$Tyr(MeAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin, $d(CH_2)_5$Tyr(Me)VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-*O*-methyltyrosine,4-valine]arginine-vasopressin; $d(CH_2)_2$Tyr(Et)VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-ethyltyrosine,4-valine]arginine-vasopressin; $d(CH_2)_5$Tyr(Me)VDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-methyltyrosine,4-valine,8-D-arginine]vasopressin; $d(CH_2)_5$Tyr(Et)VDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-ethyltyrosine,4-valine,8-D-arginine]vasopressin. Asterisk indicates that antagonistic potency is significantly enhanced relative to unalkylated tyrosine analogue.

Tyr(Me) or Tyr(Et) substitutions in bringing about enhancements in antivasopressor potencies based on a small series of analogues. These data are evidence that the relative effects of Tyr(Me) and Tyr(Et) substitutions are entirely sequence dependent. Nine of the new antagonists have antivasopressor pA_2 values equal to or greater than 8.40. These are thus among the most potent vasopressor antagonists reported to date. Their potencies compare very favorably with those of $d(CH_2)_5$Tyr(Me)AVP (pA_2 = 8.62)[6] and $d(CH_2)_5$DAVP (pA_2 = 8.52),[2] our two most potent previously reported antagonists.

Effects of O-Alkylation on Antidiuretic Properties. Comparisons of the antidiuretic activities of all the *O*-alkyltyrosine-substituted peptides with those of their respective parents[2] (Table I) show clearly that the effects of O-alkylation were higly consistent. Alkylation of tyrosine resulted in drastic reductions in antidiuretic activities in all cases. Also the relative effects of O-alkylation exhibit a consistent pattern. In all pairs, the Tyr(Et) derivative exhibits a greater reduction in antidiuretic activity than the corresponding Tyr(Me)-containing derivative. Also, as previously reported,[13] O-alkylation of the tyrosine residues in some of these analogues resulted in antagonists of the antidiuretic responses to AVP. The reduction in antidiuretic activities was most striking for the four deaminopenicillamine (dP) derivatives. dPAVP itself has 42 units/mg[5], the Tyr(Me) analogue has only 3.5 units/mg, and the Tyr(Et) analogue has only 0.59 unit/mg. dPDAVP has 5.8 units/mg,[2] the Tyr(Me) analogue has only 0.46 unit/mg, and the Tyr(Et) analogue has only 0.23 unit/mg. dPVAVP[2] has 312 units/mg, the Tyr(Me) analogue has only 2.9 units/mg, and the Tyr(Et) analogue has only 0.62 unit/mg. Finally, dPVDAVP has 123 units/mg,[4] the Tyr(Me) analogue has only 3.2 units/mg, and the Tyr(Et) analogue has only ~0.2 unit/mg. Thus, O-alkylation of the tyrosine residue in each of the 12 previously reported antagonists has resulted in significant increases in antivasopressor potencies in 12 of the resulting Tyr(Me)- and

Table II. In Vitro Antioxytocic Potencies of Analogues 1–18 and of Related Analogues

	antagonist[c]	antioxytocic pA_2[a] no Mg^{2+}	antioxytocic pA_2[a] 0.5 mM Mg^{2+}
	dPTyr(Me)AVP	7.61 ± 0.14 [6.93][b]	7.24 ± 0.10 [5.93][b]
1	dPTyr(Et)AVP	8.12 ± 0.12	7.35 ± 0.10
2	dEt_2Tyr(Me)AVP	7.96 ± 0.10 [7.30]	8.19 ± 0.07 [7.16]
3	dEt_2Tyr(Et)AVP	8.43 ± 0.07	8.00 ± 0.06
	$d(CH_2)_5$Tyr(Me)AVP	8.13 ± 0.12 [7.32]	7.24 ± 0.07 [7.27]
4	$d(CH_2)_5$Tyr(Et)AVP	8.12 ± 0.12	7.71 ± 0.10
5	dPTyr(Me)DAVP	7.77 ± 0.11 [7.87]	7.36 ± 0.07 [6.71]
6	dPTyr(Et)DAVP	7.85 ± 0.08	7.41 ± 0.06
7	dEt_2Tyr(Me)DAVP	7.72 ± 0.08 [6.95]	8.10 ± 0.04 [6.70]
8	dEt_2Tyr(Et)DAVP	7.15 ± 0.09	7.54 ± 0.07
9	$d(CH_2)_5$Tyr(Me)-DAVP	7.55 ± 0.09 [6.97]	7.81 ± 0.08 [6.59]
10	$d(CH_2)_2$Tyr(Et)-DAVP	7.55 ± 0.05	7.35 ± 0.04
11	dPTyr(Me)VAVP	8.43 ± 0.14 [8.09]	7.90 ± 0.09 [7.72]
12	dPTyr(Et)VAVP	8.64 ± 0.13	7.66 ± 0.09
13	dEt_2Tyr(Me)VAVP	8.59 ± 0.04 [7.32]	7.93 ± 0.04 [7.65]
14	dEt_2Tyr(Et)VAVP	8.13 ± 0.07	7.43 ± 0.13
	$d(CH_2)_5$Tyr(Me)-VAVP	8.36 ± 0.13 [7.34]	7.87 ± 0.11 [7.31]
	$d(CH_2)_5$Tyr(Et)-VAVP	7.88 ± 0.10	7.53 ± 0.07
15	dPTyr(Me)VDAVP	7.50 ± 0.31 [7.23]	7.67 ± 0.11 [7.12]
16	dPTyr(Et)VDAVP	7.82 ± 0.08	7.57 ± 0.10
17	dEt_2Tyr(Me)VDAVP	7.73 ± 0.06 [7.29]	7.93 ± 0.09 [7.21]
18	dEt_2Tyr(Et)VDAVP	7.98 ± 0.03	7.89 ± 0.03
	$d(CH_2)_5$Tyr(Me)-VDAVP	7.93 ± 0.10 [6.63]	7.69 ± 0.09 [6.23]
	$d(CH_2)_5$Tyr(Et)-VDAVP	8.02 ± 0.06	7.06 ± 0.04

[a] In vitro pA_2 were calculated as described in Bankowski et al.[3b] Means ± SE. [b] pA_2 values for each of the corresponding unalkylated tyrosine-containing peptides reported in ref 2 and 4–6. [c] Abbreviations are the same as in Table I.

Tyr(Et)-containing analogues while at the same time bringing about drastic reductions in antidiuretic activities in all 24.

Structural Requirements for Antidiuretic Antagonism. None of the 18 new O-alkylated analogues are antagonists of the antidiuretic responses to AVP. Thus, in the series of 24 analogues in Table I, the critical structural requirements for antidiuretic antagonism involve a combination of the following modifications in dAVP or dDAVP: the cyclopentamethylene ($(CH_2)_5$) group at position 1, a Tyr(Me) or Tyr(Et) substituent at position 2, and a valine residue at position 4. More detailed studies on other antagonists modified at positions 2, 4, 8, and 9 have been reported elsewhere.[24]

Effects of Tyr(Me)/Tyr(Et) Substitutions on in Vitro Antioxytocic Potencies. The antioxytocic potencies in vitro in the absence and in the presence of 0.5 mM Mg^{2+} together with those of related *O*-alkyltyrosine--substituted analogues are given in Table II. The potencies of each of the corresponding unalkylated analogues are given in brackets. Comparisons of the potencies of the unalkylated and alkylated analogues show that alkylation leads to a marked enhancement of antagonistic potency, both in the absence and in the presence of Mg^{2+} in almost every case. These findings are consistent with those obtained in these and in other laboratories, which have shown that Tyr(alkyl) substitutions generally result in enhanced antioxytocic potencies.[23,25] In the series presented here, there appears to be no clear-cut pattern to the relative effectiveness of the Tyr(Me) and Tyr(Et) substituents in bringing about enhancements in antioxytocic potencies. In some instances, e.g. dPTyr(Me)AVP (pA_2 = 7.61 no Mg^{2+}) and dPTyr(Et)AVP (pA_2 = 8.12 no Mg^{2+}), the Tyr(Et) substitutent is clearly more effective, while in others, e.g. $d(CH_2)_5$Tyr(Me)VAVP [pA_2 = 8.36 no Mg^{2+}] and $d(CH_2)_5$Tyr(Et)VAVP [pA_2 = 7.88 no Mg^{2+}], the Tyr(Me) analogue is more potent.

Conclusion

We have reported the synthesis on some pharmacological properties of a series of 18 new Tyr(Me)- and Tyr(Et)-substituted antagonists of the vasopressor responses to AVP. Alkylation of the tyrosine, either Tyr(Me) or Tyr(Et), has effected (a) increases in antivasopressor potency in 8 out of 12 instances, (b) increases in antioxytocic potency in vitro (in the absence and in the presence of Mg^{2+}) in almost all cases, and (c) drastic reductions in antidiuretic activities in all cases. In two instances, i.e. $d(CH_2)_5$VAVP, and $d(CH_2)_5$VDAVP, Tyr O-alkylations resulted in antagonists of the antidiuretic responses to AVP.[13] With pA_2 values equal to or greater than 8.40, a number of these antagonists, dPTyr(Et)AVP (pA_2 = 8.40), dEt_2Tyr(Me)AVP (pA_2 = 8.53), dEt_2Tyr(Et)AVP (pA_2 = 8.46), $d(CH_2)_5$Tyr(Et) AVP (pA_2 = 8.47), $d(CH_2)_5$Tyr(Me)DAVP (pA_2 = 8.41), $d(CH_2)_5$Tyr(Et)DAVP (pA_2 = 8.45), dEt_2Tyr(Et)DAVP (pA_2 = 8.42), and dPTyr(Et)-VDAVP (pA_2 = 8.46), are among the most potent AVP vasopressor antagonists reported to date. They could thus serve as additional valuable pharmacological tools in studies on the role(s) of AVP in cardiovascular regulation and in studies on AVP receptor subtypes. Furthermore, the findings reported here provide new insights to the design of potent and selective receptor antagonists of the vasopressor and antidiuretic responses to AVP. Thus, for example, studies on the effects of D-Tyr(Me) and D-Tyr(Et) substituents in an extensive series of analogues such as that presented here is now well warranted.

Experimental Section

The protected peptide intermediates I–XVIII (Table III) were synthesized by the solid-phase method[14] by previously described procedures.[2,4,5,15] Chloromethylated resin (Bio-Rad Bio-Beads SX-1) was esterified with Boc-Gly to an incorporation of 0.5-0.65 mmol/g by the cesium salt method.[26] Amino acid derivatives, including Boc-Tyr(Me) and Boc-Tyr(Et), were supplied by Bachem, Inc. The *p*-nitrophenyl esters of β-(*S*-benzylthio)-β,β-dimethylpropionic acid,[8] β-(*S*-benzylthio)-β,β-diethylpropionic acid,[9] and β-(*S*-benzylthio)-β,β-cyclopentamethylenepropionic[10] acid were synthesized by published procedures.[16] Triethylamine (TEA) and *N*-methylmorpholine (NMM) were distilled from ninhydrin. 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, Brinkmann Silplate). The following solvent systems were used: (A) butan-1-ol–acetic acid–water (4:1:5 v/v, upper phase); (B) chloroform–methanol (7:3 v/v); (C) butan-1-ol–acetic

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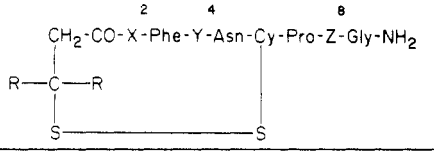
Table III. Physicochemical Properties of Protected Peptides: β-(Benzylthio)-β,β-dialkylpropionyl-X-Phe-Y-Asn-Cys(Bzl)-Pro-Z-Gly-NH_2

no.	R_2	X	Y	Z	formula	yield,[a,b] %	mp, °C	$[\alpha]^{25}_D$ (c 1; DMF)	R_f A	R_f B
I	$(CH_3)_2$	Tyr(Et)	Gln	Arg(Tos)	$C_{71}H_{92}N_{14}O_{14}S_3$	79.9	208–211	−44.9	0.39	0.46
II	$(C_2H_5)_2$	Tyr(Me)	Gln	Arg(Tos)	$C_{72}H_{94}N_{14}O_{14}S_3$	57.1	188–191	−42.6	0.43	0.45
III	$(C_2H_5)_2$	Tyr(Et)	Gln	Arg(Tos)	$C_{73}H_{96}N_{14}O_{14}S_3$	85.7	188–191	−42.6	0.43	0.45
IV	$(CH_2)_5$	Tyr(Et)	Gln	Arg(Tos)	$C_{79}H_{96}N_{14}O_{14}S_3$	74.7	203–205	−46.5	0.39	0.47
V	$(CH_3)_2$	Tyr(Me)	Gln	D-Arg(Tos)	$C_{70}H_{90}N_{14}O_{14}S_3$	72.8	195–196	−29.7	0.44	0.53
VI	$(CH_3)_2$	Tyr(Et)	Gln	D-Arg(Tos)	$C_{71}H_{92}N_{14}O_{14}S_3$	78.4	190–191	−24.6	0.78	0.44
VII	$(C_2H_5)_2$	Tyr(Me)	Gln	D-Arg(Tos)	$C_{72}H_{94}N_{14}O_{14}S_3$	85.1	187–188	−28.5	0.45	0.38
VIII	$(C_2H_5)_2$	Tyr(Et)	Gln	D-Arg(Tos)	$C_{73}H_{96}N_{14}O_{14}S_3$	78.3	188–189	−29.3	0.47	0.38
IX	$(CH_2)_5$	Tyr(Me)	Gln	D-Arg(Tos)	$C_{73}H_{94}N_{14}O_{14}S_3$	70.2	191–192	−31.5	0.45	0.58
X	$(CH_2)_5$	Tyr(Et)	Gln	D-Arg(Tos)	$C_{74}H_{96}N_{14}O_{14}S_3$	71.9	187–188	−32.4	0.58	0.62
XI	$(CH_3)_2$	Tyr(Me)	Val	Arg(Tos)	$C_{70}H_{91}N_{13}O_{13}S_3$	86.7	217–219	−41.5	0.52	0.70
XII	$(CH_3)_2$	Tyr(Et)	Val	Arg(Tos)	$C_{71}H_{93}N_{13}O_{13}S_3$	67.3	220–222	−42.1	0.56	0.83
XIII	$(C_2H_5)_2$	Tyr(Me)	Val	Arg(Tos)	$C_{72}H_{95}N_{13}O_{13}S_3$	92.7	208–209	−40.4	0.50	0.67
XIV	$(C_2H_5)_2$	Tyr(Et)	Val	Arg(Tos)	$C_{73}H_{97}N_{13}O_{13}S_3$	71.5	208–210	−38.9	0.54	0.70
XV	$(CH_3)_2$	Tyr(Me)	Val	D-Arg(Tos)	$C_{70}H_{91}N_{13}O_{13}S_3$	86.4	222–223	−26.5	0.74	0.81
XVI	$(CH_3)_2$	Tyr(Et)	Val	D-Arg(Tos)	$C_{71}H_{93}N_{13}O_{13}S_3$	83.8	225–226	−21.3	0.60	0.77
XVII	$(C_2H_5)_2$	Tyr(Me)	Val	D-Arg(Tos)	$C_{72}H_{95}N_{13}O_{13}S_3$	88.7	222–223	−27.5	0.55	0.65
XVIII	$(C_2H_5)_2$	Tyr(Et)	Val	D-Arg(Tos)	$C_{73}H_{97}N_{13}O_{13}S_3$	93.1	223–224	−29.7	0.52	0.64

[a] Yields were calculated on the basis of the glycine content of the starting resin. [b] All the protected peptides gave the expected amino acid ratios after hydrolysis ±3%.[27]

Table IV. Physicochemical Properties of Antagonists 1–18



no.	struct	R_2	X^2	Y^4	Z^8	wt, mg	yield,[f,g] %	$[\alpha]^{25}_D$	R_f A	R_f C
1	dPTyr(Et)AVP	$(CH_3)_2$	Tyr(Et)	Gln	Arg	59.7	46.1	−62.2[a]	0.12	0.32
2	dEt_2Tyr(Me)AVP	$(C_2H_5)_5$	Tyr(Me)	Gln	Arg	84	64.0	−73.5[b]	0.25	0.50
3	dEt_2Tyr(Et)AVP	$(C_2H_5)_2$	Tyr(Et)	Gln	Arg	70	73.0	−74.9[b]	0.25	0.50
4	$d(CH_2)_5$Tyr(Et)AVP	$(CH_2)_5$	Tyr(Et)	Gln	Arg	55	39.5	−57.4[e]	0.21	0.32
5	dPTyr(Me)DAVP	$(CH_3)_2$	Tyr(Me)	Gln	D-Arg	104	77.0	−38.9[e]	0.23	0.49
6	dPTyr(Et)DAVP	$(CH_3)_2$	Tyr(Et)	Gln	D-Arg	66.6	49.7	−26.0[b]	0.19	0.38
7	dEt_2Tyr(Me)DAVP	$(C_2H_5)_2$	Tyr(Me)	Gln	D-Arg	74	63.1	−37.8[b]	0.25	0.47
8	dEt_2Tyr(Et)DAVP	$(C_2H_5)_2$	Tyr(Et)	Gln	D-Arg	63	59.4	−37.9[b]	0.22	0.28
9	$d(CH_2)_5$Tyr(Me)DAVP	$(CH_2)_5$	Tyr(Me)	Gln	D-Arg	73.5	55.5	−50.6[a]	0.31	0.39
10	$d(CH_2)_2$Tyr(Et)DAVP	$(CH_2)_5$	Tyr(Et)	Gln	D-Arg	97	69.4	−49.7[b]	0.21	0.45
11	dPTyr(Me)VAVP	$(CH_3)_2$	Tyr(Me)	Val	Arg	82	65.1	−99.7[a]	0.24	0.44
12	dPTyr(Et)VAVP	$(CH_3)_2$	Tyr(Et)	Val	Arg	82.5	58.6	−97.1[b]	0.24	0.57
13	dEt_2Tyr(Me)VAVP	$(C_2H_5)_2$	Tyr(Me)	Val	Arg	44	38.2	−88.1[b]	0.28	0.47
14	dEt_2Tyr(Et)VAVP	$(C_2H_5)_2$	Tyr(Et)	Val	Arg	36.5	28.3	−97.4[b]	0.32	0.54
15	dPTyr(Me)VDAVP	$(CH_3)_2$	Tyr(Me)	Val	D-Arg	37.0	43.5	−23.0[c]	0.34	0.47
16	dPTyr(Et)VDAVP	$(CH_3)_2$	Tyr(Et)	Val	D-Arg	69	53.7	−32.3[a]	0.25	0.44
17	dEt_2Tyr(Me)VDAVP	$(C_2H_5)_2$	Tyr(Me)	Val	D-Arg	57	41.3	−41.5[d]	0.25	0.44
18	dEt_2Tyr(Et)VDAVP	$(C_2H_5)_2$	Tyr(Et)	Val	D-Arg	31	29.8	−41.5[d]	0.26	0.46

[a] c 0.5, 1 N AcOH. [b] c 0.3, 1 N AcOH. [c] c 1.0, 1 N AcOH. [d] c 0.25, 1 N AcOH. [e] c 0.4, 1 N AcOH. [f] Yields are based on the amount of protected peptide used in the reduction–reoxidation step in each case. [g] All the free peptides gave the expected amino acid analysis ratios after hydrolysis ±3%.

acid–water–pyridine (15:3:3:10, v/v). Loads of 10–50 μg were applied and chromatograms were a minimum length of 10 cm. Iodine vapor and the chloroplatinate reagent were used for detection. For amino acid analysis,[27] peptides (~0.8 mg) were hydrolyzed with constant-boiling hydrochloric acid (500 μL) containing phenol (10 μL) in evacuated and sealed ampules for 18 h at 120 °C. The analyses were performed on a Model 121 M Beckman automatic amino acid analyzer. Molar ratios were referred to Gly = 1.00. The cysteine content of the free peptides was estimated as the Cy(SO_3H) to Gly ratio from analyses following performic oxidation.[28] All peptides (protected and free) gave the expected amino acid ratios ±3%. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. 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.

β-(*S*-Benzylthio)-β,β-diethylpropionyl-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (Pr XVIII). Boc-Gly resin (1.56 g, 1 mmol) was converted to protected acyl octapeptidyl resin in eight cycles of solid-phase peptide synthesis using as the carboxy component Boc-D-Arg(Tos), Boc-Pro, Boc-Cys(Bzl), Boc-Asn-*p*-nitrophenyl ester (with HOBT as additive[17b]), Boc-Val, Boc-Phe, Boc-Tyr(Et), and finally *p*-nitrophenyl β-(*S*-benzylthio)-β,β-diethylpropionate (with HOBT as additive[17b]), respectively. The protected acyl octapeptide was cleaved by ammonolysis.[15] The crude product was extracted with hot DMF and, after removal of the resin, precipitated by the addition of hot water. The precipitate was collected, dried in vacuo, and reprecipitated from DMF–ethanol to give the required protected peptide amide (XVIII) [1.358 g, 93.1% (based on the Gly content of the starting resin)]. The physiochemical properties of this and the remaining protected peptides I–XVII, which were prepared in essentially the same manner, are given in Table III.

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(28) Moore, S. *J. Biol. Chem.* **1963**, *238*, 235.

[1-(β-Mercapto-β,β-diethylpropionic acid),2-O-ethyltyrosine,4-valine,8-D-arginine]vasopressin (dEt$_2$Tyr(Et)-VDAVP, 18). A solution of the protected nonapeptide amide XVIII (0.135 g, 0.092 mmol) in sodium-dried and redistilled ammonia (400 mL) was treated at the boiling point and with stirring with sodium[19] from a stick of 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, 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 6.5. An excess of a solution of potassium ferricyanide (0.01 M, 16 mL)[20] was added gradually with stirring. The yellow solution was stirred for a further 10 min and for 10 min with anion-exchange resin (Bio Rad AG-3, Cl^- form, 10-g damp weight). The suspension was slowly filtered through a bed of resin (50-g damp weight). The bed was washed with aqueous acetic acid (0.2%, 200 mL), and the combined filtrate and washings were lyophylized. The resulting powder (1.45 g) was desalted on a Sephadex G-15 column (110 × 2.7 cm), eluting with aqueous acetic acid (50%)[22] with a flow rate 5 mL/h. The eluate was fractioned and monitored for absorbance of 280 nm. The fractions comprising the major peak were pooled and lyophylized, and the residue (100 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 4 mL/h.[22] The peptide was eluted in a single peak (absorbance 280 nm). Lyophylization of the pertinent fractions yielded the vasopressin analogue (18) [31 mg, 29.8% (based on the amount of protected peptide used in the reduction–reoxidation procedure)]. The physiochemical properties of this and of the remaining free peptides 1–17, which were prepared in the same way as for 18, are given in Table IV. Their pharmacological properties are presented in Tables I and II.

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Hydroxy Derivatives of Tamoxifen

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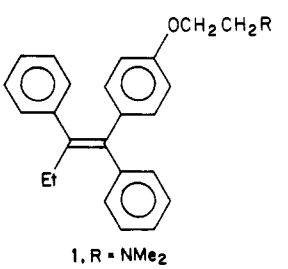
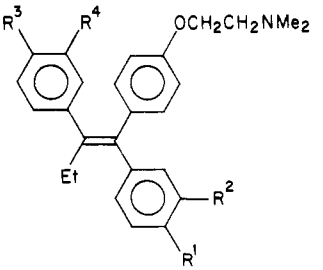
In the exploration of the structural features that affect the RBA (binding affinity for the estrogen receptor of rat uterus relative to that of estradiol) in the tamoxifen [*trans*-(*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene] series, several derivatives variously substituted in the 1-phenyl group have been synthesized. [In the tamoxifen series, the descriptors *E* and *Z*, which define the configuration of the geometrical isomers and depend on the location and nature of substituents in the aromatic moieties and the ethyl group, may vary, although the relative configuration (cis or trans) does not. In order to avoid confusion the terms cis and trans will be used in this paper to refer to the relative positions of the 4-[2-(dimethylamino)ethoxy]phenyl and ethyl (or hydroxyethyl, hydroxypropyl, or bromo) substituents attached to the ethene moiety.] The final stage of each synthesis involved acid-catalyzed dehydration of a tertiary alcohol, and, in contrast to the known 3- and 4-hydroxy derivatives which were obtained as near-equimolar cis,trans mixtures, only the trans forms of the 2-hydroxy, 2-methyl, 2,4-dihydroxy, and 4-hydroxy-2-methyl derivatives were obtained. Also, in contrast to the trans forms of the 3- and 4-hydroxy derivatives, which are readily equilibrated to cis,trans mixtures, the trans 2-hydroxy derivative could not be isomerized. Tamoxifen and 2-methyltamoxifen had similar RBA's (~1% of that of E_2), but that of 2-hydroxytamoxifen was much lower (0.1%). Introduction of a second hydroxyl group (2,4-dihydroxy derivative) enhanced the RBA, and for the 4-hydroxy-2-methyl derivative, the RBA and growth inhibitory activity against the MCF-7 mammary tumor cell line in vitro were high and comparable to those of 4-hydroxytamoxifen, a metabolite of the parent drug. Tamoxifen derivatives hydroxylated at positions 3 or 4 of the 1-butene moiety and the 5-hydroxy-1-pentene analogue were also synthesized, but they had very low RBA values.

Tamoxifen [1, *trans*-(*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene] is a triphenylethylene derivative presently in clinical use[1] for the treatment of hormone-dependent disseminated breast cancer and is thought to act[2] mainly by competing with estradiol for its protein receptor (ER). The relative binding affinity (RBA) of 1 for the ER of rat uterus is ~1% of that of estradiol. Of the human plasma metabolites of 1 so far identified, the *N*-desmethyl[3] (2, major metabolite), *N*-oxide[4] (3), and hydroxyethyl[5] (4) derivatives have RBA values comparable to that of the parent drug, whereas 4-hydroxytamoxifen [5, *trans*-(*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-2-phenyl-1-butene, minor metabolite[3,6]] has an RBA value that is comparable to, and possibly greater than, that of estradiol. The high RBA of 4-hydroxytamoxifen (5) has raised the



1, R = NMe_2
2, R = NHMe
3, R = N(→O)Me_2
4, R = H



5, R^1 = OH, R^2 = R^3 = R^4 = H
6, R^1 = R^3 = R^4 = H, R^2 = OH
7, R^1 = R^2 = R^3 = H, R^4 = OH
8, R^1 = R^2 = R^4 = H, R^3 = OH

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