resulting hot solution was filtered. After standing overnight at ambient temperature, the filtrate gave 4.5 g (56% yield) of the title compound, mp 101–102°. *Anal.* ($C_{26}H_{32}N_2O_2$) C, H.

3',4"-Dicyano-1,5-diphenoxypentane (26). To an ethanolic sodium ethoxide solution prepared by dissolving 0.23 g (10 mg-atoms) of Na in 50 ml of absolute ethanol was added 1.19 g (10 mmol) of 3-cyanophenol and 2.52 g (10 mmol) of 5,4'-cyanophenoxylamyl bromide. The mixture was allowed to stir at reflux for 24 hr. The resulting hot solution was filtered. After standing at ambient temperature overnight, the filtrate gave 1.8 g (59% yield) of the title compound, mp 82–84°. *Anal.* ($C_{19}H_{18}N_2O_2$) C, H.

4',4"-Diamidino-1,12-diphenoxydodecane Dihydrochloride (6). A solution of 0.8 g (1.96 mmol) of **39**, in 3 ml of absolute ethanol and 70 ml of benzene, was bubbled with HCl gas at 0° for 15 min. The resulting solution was kept at 4° for 7 days. At the end of the period, the solvent was removed under reduced pressure. To the solid residue was added 30 ml of 0.97 *N* NH_3 in ethanol. The mixture was kept at 50–60° for 2 hr and at ambient temperature overnight. Any precipitate formed was filtered off. Anhydrous ether was then added to this clear filtrate until complete precipitation was attained. The precipitate was recrystallized from dilute HCl, collected, and dried under reduced pressure (0.05 mm) at 60° for 2 hr to give 0.6 g (60% yield) of the title compound, mp 228–229° dec. *Anal.* ($C_{26}H_{38}N_4O_2 \cdot 2HCl$) C, H, N.

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Solid Phase Synthesis of [1-Deamino,4-valine]-8-D-arginine-vasopressin (DVDAVP), a Highly Potent and Specific Antidiuretic Agent Possessing Protracted Effects

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In further exploring the selective enhancement of antidiuretic activity exhibitied by certain [4-threonine]-substituted arginine-vasopressin analogs, we have synthesized [1-deamino,4-valine]-8-D-arginine-vasopressin (DVDAVP) by the Merrifield method with an overall yield of 45%. DVDAVP has an antidiuretic potency of 1230 ± 170 units/mg when assayed by intravenous injection into ethanol-anesthetized rats, about four times that of arginine-vasopressin (AVP). Its antidiuretic effect in conscious diabetes insipidus rats is also greatly prolonged when compared to AVP. It has undetectable vasopressor activity, <0.01 unit/mg or less than $^1/_{40,000}$ that of AVP. The antidiuretic/pressor ratio (A/P) of DVDAVP is thus greater than 125,000, a value higher than that of any other peptide known to date. Its oxytocic potency on the rat uterus is about half the potency of AVP. Most of the undesired side effects of AVP derive from its effects on vascular and visceral smooth muscles. Thus, DVDAVP with its high, specific, and protected antidiuretic properties may offer some advantages over lysine- or arginine-vasopressin in the treatment of hypothalamic diabetes insipidus. In addition, studies on this and related peptides may be helpful in further characterizing the receptors that mediate and the enzymes that terminate antidiuretic responses.

During the course of an investigation of the phylogeny of the neurohypophysial hormones, we synthesized [4-threonine]oxytocin.[1,2] This peptide was shown to be a highly potent and specific oxytocic agent. In subsequent studies we found that substitution of threonine for glutamine in the basic neurohypophysial peptides[3] and in their 1-deamino analogs[4] gave rise to peptides possessing specific and in some instances highly potent antidiuretic properties. These 4-threonine-substituted basic peptides all exhibited varying degrees of enhancement of the antidiuretic/pressor ratio (A/P) which in arginine-vasopressin (AVP) has a value of ~1. These later findings were analogous to those previously observed for other basic peptide analogs in other laboratories.[5-11] It seemed worthwhile therefore to further explore this interesting phenomenon of enhanced antidiuretic/pressor selectivity in the hope of uncovering clues to the design of peptides with even greater selectivity than had heretofor been encountered.

Analysis of our own findings[3,4] and those of others[5-11] revealed how a number of different structural alterations in the arginine-vasopressin (AVP) molecule, individually or in combination, could bring about antidiuretic/pressor selectivity in the resulting AVP analogs. AVP has the following structure in which the numbers indicate the positions of the individual amino acid residues. The individual structural alterations of AVP which bring about enhancement of antidiuretic/pressor (A/P) selectivity in the resulting peptides

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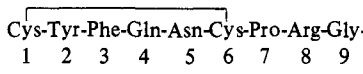
Table I. Biological Activities of Arginine-vasopressin, [1-Deamino]-8-L-arginine-vasopressin, [1-Deamino]-8-D-arginine-vasopressin (DDAVP), and [1-Deamino,4-valine]-8-D-arginine-vasopressin (DVDAVP)

	Biological activities, units/mg				
	Rat uterus				
	No Mg^{2+}	0.5 m*M* Mg^{2+}	Rat antidiuresis	Rat vasopressor	Ratio: antidiuretic/ vasopressor
Arginine-vasopressin[a]	15 ± 1	31 ± 2	332 ± 20	376 ± 6	0.9
[1-Deamino]-8-L-arginine-vasopressin[b]	47 ± 2	66 ± 3	1390 ± 140	370 ± 20	3.8
DDAVP[c]	5.1		870	11	79
DDAVP[d]	~14[e]	~3	955 ± 95	0.47 ± 0.02	2,000
DVDAVP	~8[e]	~2	1230 ± 170	<0.01[f]	>125,000

[a] Assays done on a preparation of arginine-vasopressin prepared by solid phase synthesis but not previously reported. [b] Assays done on a solution kindly supplied by Dr. B. Berde of Sandoz Ltd., Basel. The absolute vasopressor activity of the peptide was assumed to be that originally reported by Huguenin and Boissonnas.[5] The remaining activities were calculated on that basis. [c] Values reported by Zaoral, *et al.*[8] [d] Assays done on a solution kindly supplied by Dr. J. Mulder of Ferring AB, Malmo. The weaker vasopressor response of DDAVP reported here has been confirmed (Dr. J. Mulder, personal communication). [e] Absolute oxytocic (rat uterus) activities for the D-arginine-containing analogs cannot be reported since these analogs have highly variable activities when assayed on uteri from different rats. The figures shown are simple means of several values obtained from different uteri. [f] In high doses a weak inhibitor of the vasopressor response to arginine-vasopressin.



are (A) removal of the amino group from the one position, as in [1-deamino]-8-arginine-vasopressin,[5] A/P, 3.8; (B) replacement of tyrosine at position 2 by phenylalanine, as in [2-phenylalanine]-8-arginine-vasopressin,[6] A/P, 2.9; (C) enhancement of lipophilicity at position 4, *e.g.*, substitution of glutamine by threonine or α-aminobutyric acid giving [4-threonine]-8-arginine-vasopressin[3] (A/P, 2.2) and [4-α-aminobutyric acid]-8-arginine-vasopressin[10] (A/P, 20), respectively; and (D) substitution of D for L-arginine at position 8, as in 8-D-arginine-vasopressin[7] (A/P, 28). Peptides containing some combinations of any two of these four changes have also been found to exhibit antidiuretic/pressor ratios which are much larger than those brought about by any one of the changes (A-D) alone. Thus, [1-deamino,2-phenylalanine]-8-arginine-vasopressin[5] which combines factors A and B was found to have an antidiuretic/pressor ratio of 27. The combination of factors A and C, *i.e.*, removal of the amino group at position 1 coupled with increasing the lipophilic character of the amino acid residue at position 4 also further enhances antidiuretic/pressor selectivity. Thus, [1-deamino,4-threonine]-8-arginine-vasopressin[4] and [1-deamino,4-α-aminobutyric acid]-8-arginine-vasopressin[11] have A/P values of 30 and 95, respectively. In studies[8,9] which have had the most direct bearing on the present report, it had been shown that [1-deamino]-8-D-arginine-vasopressin (DDAVP), a peptide combining changes A and D, was shown to have an A/P value of 79.

The question immediately arising from these considerations was, would a combination of three of these structural alterations of the AVP molecule give a peptide possessing even greater enhancement of antidiuretic/pressor selectivity? To answer this very intriguing question we decided to incorporate a third structural change into the [1-deamino]-8-D-arginine-vasopressin[8] (DDAVP) molecule. We reasoned that if the effects of the structural alterations were indeed additive, then the substitution of glutamine at position 4 in DDAVP by a more lipophilic amino acid (*i.e.*, structural change C) should give rise to a peptide possessing even greater antidiuretic/pressor selectivity than DDAVP possesses. To replace glutamine in the 4 position of DDAVP we selected valine for its highly lipophilic side chain and because it had not heretofore been substituted for glutamine in a vasopressin analog.

We now wish to report the synthesis and pharmacological properties of the peptide designed according to the above rationale, *i.e.*, [1-deamino,4-valine]-8-D-arginine-vasopressin (DVDAVP). This compound incorporates three of the above four structural features A-D, *i.e.*, A, C, and D. It was synthesized by the adaptation of the Merrifield method [12,13] which was used for the synthesis of oxytocin[14] as described in the Experimental Section. Its pharmacological properties were evaluated by methods previously described[15,16] and the duration of its antidiuretic action was estimated by methods described in the Experimental Section.

Results and Discussion

The pharmacological data on [1-deamino,4-valine]-8-D-arginine-vasopressin (DVDAVP) as presented in Table I reveal a striking enhancement of antidiuretic activity coupled with the virtual elimination of the vasopressor response giving rise to an unprecedented antidiuretic/pressor ratio of >125,000. Two further interesting points are (a) its very low oxytocic potency, particularly in the presence of Mg^{2+}, and (b) that DVDAVP on subcutaneous injection into rats with hypothalamic diabetes insipidus elicits a protracted antidiuretic action as compared to that of arginine-vasopressin (Figure 1). In this respect it resembles [1-deamino]-8-D-arginine vasopressin (DDAVP[9]) and [1-deamino,4-α-aminobutyric acid]-AVP.[11] A more detailed investigation of the protracted effects of DVDAVP is currently being carried out and will be presented elsewhere.

It is of interest to further compare the properties of [1-deamino,4-valine]-8-D-arginine-vasopressin (DVDAVP)

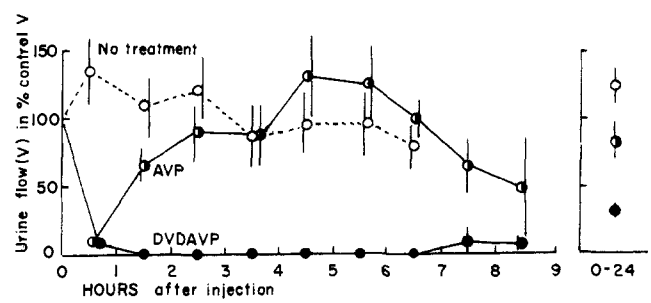


Figure 1. Duration of antidiuresis of [1-deamino,4-valine]-8-D-arginine-vasopressin (DVDAVP) compared to that of arginine-vasopressin (AVP): DVDAVP, 10 antidiuretic mU (8 ng)/100 g body wt *vs.* AVP, 10 mU (30 ng)/100 g, subcutaneous; DI(Brattleboro) rats; mean control, V = 3.4 ml/100 g per hr. The AVP was administered as U.S.P. Reference Standard.

with those of [1-deamino]-8-D-arginine-vasopressin (DDAVP). In this connection it should first be noted that the data for DDAVP reported for the first time here (Table I), which show that it does have a much higher A/P ratio than that reported earlier,[8] were obtained only after the present study on DVDAVP was initiated. The discrepancy between the earlier values[8] for the vasopressor activity of [1-deamino]-8-D-arginine-vasopressin (DDAVP) and those reported here would appear to indicate that the earlier preparation contained some [1-deamino]-8-L-arginine-vasopressin which, as can be seen from Table I, has considerable vasopressor potency. In comparing the properties of DVDAVP with those of DDAVP, it can be seen that the spectra of activities are very similar. In reference to the structural differences between these two molecules, it is now clear that enhancement of lipophilicity at position 4 of DDAVP has, by further increasing the antidiuretic potency and by virtually eliminating the vasopressor response, given rise to a peptide (DVDAVP) possessing an even greater selective enhancement of the antidiuretic/pressor ratio.

In response to the question posed earlier on the additive effects of the structural changes A-D, it is now clear that the arginine-vasopressin molecule can be altered in three different positions giving rise to a peptide (DVDAVP) possessing unprecedented antidiuretic/pressor selectivity. In the light of these findings, therefore, the study of arginine-vasopressin analogs possessing other combinations of the above structural alterations A-D should provide clues to a deeper understanding of all of the factors governing antidiuretic/pressor selectivity.

With its high specific and protracted antidiuretic activity coupled with its virtual lack of effects on vascular and visceral smooth muscles, [1-deamino,4-valine]-8-D-arginine-vasopressin (DVDAVP) is a very attractive peptide for possible use in the clinical treatment of hypothalamic diabetes insipidus. In addition, studies on this and related peptides may be helpful in further characterizing the receptors that mediate and the enzymes that terminate antidiuretic responses.

Experimental Section‡

S-(Bzl)-β-mercaptopropionyl-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (1). *tert*-Butyloxycarbonylglycyl resin (purchased from Schwarz Bioresearch) (4 g, 1.54 mmol) was treated as described for the synthesis of [4-threonine]-oxytocin.[1] Eight cycles of deprotection, neutralization, and coupling were carried out on successive days with the following amino acid derivatives: Boc-D-Arg(Tos), Boc-Pro, Boc-Cys(Bzl), Boc-Asn, Boc-Val, Boc-Phe, and Boc-Tyr(Bzl), *S*-benzyl-β-mercaptopropionic acid[18,19] being incorporated in the final step. All coupling reactions to form peptide bonds were mediated by dicyclohexylcarbodiimide[20] in methylene chloride, except in the case of L-asparagine, and *S*-benzyl-β-mercaptopropionic acid which were allowed to react as the nitrophenyl ester derivatives[19,21] in dimethylformamide (DMF). Also, the Boc-D-Arg(Tos) coupling was carried out in DMF. Ion exchange bound material was removed in the usual manner.[1]

At the conclusion of the synthesis, the protected peptide resin was washed out of the reaction vessel with methylene chloride, methanol, and ether, collected on a filter, and dried *in vacuo*, wt 6.23 g. The weight gain of 2.23 g (1.5 mmol) at this stage indicated a 97.6% incorporation of protected peptide based on the initial glycine content (1.54 mmol) in the resin.

Ammonolytic cleavage of the protected peptide resin (3.06 g) was carried out as described earlier.[1,14] The cleaved peptide was extracted with DMF (5 × 35 ml) and MeOH (2 × 35 ml). Removal of the solvents *in vacuo* on a rotary evaporator, followed by trituration with 95% ethanol and ether and drying *in vacuo* over P_2O_5, gave the required protected octapeptide amide intermediate as a white amorphus powder: wt 760 mg (0.51 mmol); mp 223-225°. This was recrystallized twice from acetic acid-absolute ethanol: wt 582 mg; mp 232-234°; $[\alpha]^{24.5}D$ −17.15 (*c* 1, DMF). *Anal.* Calcd for $C_{74}H_{90}N_{13}O_{14}S_3$: C, 59.98; H, 6.12; N, 12.28. Found: C, 59.93; H, 6.12; N, 12.19. Amino acid analysis[22] gave Asp, 1.12; Pro, 1.10; Gly, 1.00; Phe, 1.00; Tyr, 0.84; Bzl-Cys, 0.81; Val, 0.97; Arg, 0.94; and NH_3, 2.5. When subjected to thin-layer chromatography in BAW the 1-β-mercaptopropionyl-protected octapeptide amide gave a single spot, R_f 0.58. The yield of the purified protected peptide amide from the ammonolytic cleavage, trituration, and recrystallization was 69.5% of the amount expected, based on the weight gain on the resin. The yield based on the amount of glycine originally esterified to the resin was 67.5%.

[1-Deamino,4-valine]-8-D-arginine-vasopressin. The protected octapeptide **1** (150 mg) was deblocked by the sodium-liquid ammonia[23] procedure used in the original synthesis of oxytocin[24] with the modifications previously described.[1,19] Reoxidation in aqueous solution (~600 ml) at pH 6.8 was effected with the use of 0.011 *M* potassium ferricyanide[1,19] (18 ml). After 15 min 10 g of AG 3-x4 resin (chloride form) was added and stirring was continued for 30 min to remove ferrocyanide and excess ferricyanide ions. The suspension was filtered through a bed of AG 3-x4 resin (chloride form) (60 g wet weight) and washed through with ~600 ml of 0.2% acetic acid. (The use of a large volume for washing the AG 3-x4 resin at this stage represents an important modification of earlier published procedures on the synthesis of oxytocin and vasopressin peptides from these and other laboratories. In earlier publications we have used only ~100-200 ml of 0.2% AcOH for washing the resin. The superior yields obtained in this and in other syntheses have resulted from thorough washing of the AG 3-x4 resin.) The washings and filtrate were combined. The resulting solution (~1200 ml) was lyophilized to give 1.4 g of crude product consisting of the required peptide, dimer, and inorganic salts. The lyophilized product was purified by gel filtration on Sephadex G-15 by the previously described[25] two-step procedure involving sequential elution with 50% AcOH and 0.2 *N* AcOH, on two separate columns, as modified for the purification of [1-deamino,4-threonine]-oxytocin.[26] In this instance, a slower flow rate and smaller fractions were employed to effect a very good separation of dimer and monomer materials in the first step. Thus, in the elution of the Sephadex G-15 (2.7 × 110 cm) column with 50% AcOH, 133 fractions were collected at a rate of 7.4 ml/hr with the following fraction sizes: tubes 1-24, 4.1 ml; tubes 25-114, 2.1 ml; tubes 115-133, 4.1 ml. A plot of the uv absorbance values at 280 nm showed the presence of the usual two peaks corresponding to dimer and monomer, very well separated with maxima at tubes 63 and 80, respectively, and clearly separated from salt (tubes 113-133). The contents of the second peak (tubes 72-87) were pooled, diluted with 10 vol of water, and lyophilized, wt 80 mg. To remove contaminating G-15 particles which had been dissolved out during the first step, the second step, entailing elution with 0.2 *N* AcOH on Sephadex G-15 (1.2 × 110 cm), was carried out. Sixty fractions were collected at a flow rate of 8.8 ml/hr with the following fraction sizes: tubes 1-14, 4.1 ml; tubes 15-60, 2.1 ml. The required peptide emerged as a single peak with a maximum at tube 36 but with a pronounced tail at tubes 38-45. Examination of the contents of these latter tubes indicated the presence of only one substance identical with that present in the main peak. Thus, the tailing was probably due to the presence of arginine which caused the peptide to stick more than usual to the gel. The contents of the peak (tubes 34-37) and tail (tubes 38-44) were pooled separately and lyophilized to give 42 and 28 mg, respectively. These were subsequently shown to be identical in all respects and were combined to give [1-deamino,4-valine]-8-D-arginine-vasopressin as a fluffy white powder, wt 70 mg. This repsents a yield of 66.5% in the reduction, reoxidation, and purification steps from the protected octapeptide and an overall yield

‡ All *tert*-butyloxycarbonylamino acid derivatives were purchased from Bachem Ltd. The purity of each one was checked by tlc as described in ref 17. Tlc examination of the protected and free peptides was carried out in *n*-BuOH–AcOH–H_2O (BAW) (4:1:5) and electrophoretic examination of the free peptide was carried out in two pyridine acetate buffers of pH 3.5 (A) and 6.5 (B) as previously described[1] with the platinum and/or Sakaguchi reagents being used for detection. Melting points were taken in an open capillary in a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith laboratories, Knoxville, Tenn. For quantitative amino acid analyses samples were hydrolyzed with constant boiling HCl in evacuated sealed ampoules at 110° for 18 hr and analyzed on a Beckman/Spinco amino acid analyzer Model 121C according to the method of Spackman, *et al.*[22] All optical rotations were measured on a Bellingham Stanley Ltd. Model A polarimeter, Type P1.

of 45% based on the initial glycine incorporation on the resin (this yield is higher than any previously obtained in these laboratories and, as mentioned above, results from thorough washings of the AG 3-x4 resin): $[\alpha]^{22.0}D$ −78.0 (*c* 0.5, 1 *N* AcOH). This material was shown to be homogeneous by tlc, R_f 0.22 (BAW), and by paper electrophoresis. Only one spot in the direction of the cathode was observed in each of the buffer systems A and B. Amino acid analysis[22] gave Asp, 1.02; Arg, 1.05; Gly, 1.00; Phe, 0.98; Pro, 1.01; Tyr, 0.93; Val, 1.02; NH_3, 2.29. In addition, cysteine (0.41) and the mixed disulfide of cysteine and β-mercaptopropionic acid (0.59) were present.

Measurement of Duration of Antidiuretic Action (Figure 1). The duration of the antidiuretic action of DVDAVP was also tested in conscious rats of the Brattleboro strain, homozygous for the hereditary hypothalamic diabetes insipidus trait, by a method adapted from that of Kincl, *et al.*[27] DVDAVP and AVP were injected subcutaneously and spontaneous urine output was measured hourly thereafter. Six female diabetes insipidus rats weighing 185–200 g were used in a simple crossover design. The average rate of urine output for the 24 hr preceding injection in each rat was considered the control rate for that experiment.

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Synthetic Penicillins. Heterocyclic Analogs of Ampicillin. Structure–Activity Relationships

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A number of glycines containing heterocyclic rings, DL-α-thienyl, DL-α-thiazolyl, and DL-α-isothiazolyl and their mono- or dimethyl derivatives, have been prepared as precursors of the title compounds. The two main preparative routes involved reduction of the glyoxalic acid oximes (or their esters) and application of the Baumgarten reaction to the cyanomethyl derivatives. The new penicillins have been prepared from these amino acids *via* a mixed anhydride, and their antibacterial activities against several microorganisms have been determined *in vitro*. Among these compounds, the unsubstituted series showed high, broad-spectrum antibacterial activity similar to that of ampicillin. Particularly, the penicillins derived from 4-thiazolylglycines are a new class of highly active antibacterials. Structure–activity relationships are discussed.

In an effort to develop semisynthetic penicillins having high broad-spectrum antibacterial activity, a number of penicillins containing a heterocyclic ring in the side chain have been synthesized (*e.g.*, cloxacillin).† However, little information was reported on the heterocyclic analogs of 6-[(*R*)-α-aminophenylacetamido)]penicillanic acid (ampicillin), except for a few examples such as 6-[(*R*)-α-amino-3- (or 2-) thienylacetamido]penicillanic acid[2,3] and 6-(α-amino-4-isothiazolylacetamido)penicillanic acid, reported by Raap[4] after completion of this work; they are comparable to ampicillin in activity.

We therefore undertook an extensive synthetic program in order to study the effect of the aryl group on the antibac-

† For recent reviews on penicillins and related compounds see ref 1.