acetone (6 ml) and 2,2-dimethoxypropane (2 ml) was cooled in an ice bath and 70% perchloric acid (3 drops) was added. The mixture was stirred at 0° for 2 h and then was neutralized with 2 N aqueous KOH. Insoluble material was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in MeOH; the solution was filtered and evaporated to dryness. Crystallization of the product from EtOAc gave 140 mg (61%) of **4** with mp 163–165°: NMR (Me_2SO-d_6) δ 1.33 (s, 3, CH_3), 1.53 (s, 3, CH_3), 6.24 (s, 1, H-1') 7.68 (br, s, 1, NH), 7.80 (br, s, 1, NH), 8.81 (s, 1, H-5). Anal. ($C_{11}H_{16}N_4O_4S$) C, H, N, S.

Acknowledgment. We thank Marie Therese Campbell for excellent technical assistance and L. B. Allen, J. H. Huffman, and R. W. Sidwell for the antiviral data. We also thank Professor Roy L. Whistler, Jon P. Miller, and David G. Streeter for helpful discussions relative to this work.

References and Notes

(1) J. T. Witkowski, R. K. Robins, R. W. Sidwell, and L. N. Simon, *J. Med. Chem.*, **15**, 1150 (1972).
(2) R. W. Sidwell, J. H. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, and R. K. Robins, *Science*, **177**, 705 (1972).
(3) J. H. Huffman, R. W. Sidwell, G. P. Khare, J. T. Witkowski, L. B. Allen, and R. K. Robins, *Antimicrob. Agents Chemother.*, **3**, 235 (1973).
(4) R. W. Sidwell, L. B. Allen, G. P. Khare, J. H. Huffman, J. T. Witkowski, L. N. Simon, and R. K. Robins, *Antimicrob. Agents Chemother.*, **3**, 242 (1973).
(5) G. P. Khare, R. W. Sidwell, J. T. Witkowski, L. N. Simon, and R. K. Robins, *Antimicrob. Agents Chemother.*, **3**, 517 (1973).
(6) R. W. Sidwell, G. P. Khare, L. B. Allen, J. H. Huffman, J. T. Witkowski, L. N. Simon, and R. K. Robins, *Chemotherapy*, **21**, 205 (1975).
(7) Y. Togo, *Antimicrob. Agents Chemother.*, **4**, 641 (1973).
(8) F. E. Durr, H. F. Lindh, and M. Forbes, *Antimicrob. Agents Chemother.*, **7**, 582 (1975).
(9) E. DeClercq, M. Luczak, J. C. Reepmeyer, K. L. Kirk, and L. A. Cohen, *Life Sci.*, **17**, 187 (1975).
(10) D. G. Streeter, J. T. Witkowski, G. P. Khare, R. W. Sidwell, R. J. Bauer, R. K. Robins, and L. N. Simon, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 1174 (1973).
(11) L. N. Simon, R. W. Sidwell, G. P. Khare, D. G. Streeter, J. P. Miller, J. T. Witkowski, J. H. Huffman, and R. K. Robins in "Virus Research", C. F. Fox and W. S. Robinson, Ed., Academic Press, New York, N.Y., 1973, pp 415–436.
(12) J. T. Witkowski, R. K. Robins, G. P. Khare, and R. W. Sidwell, *J. Med. Chem.*, **16**, 935 (1973).
(13) S. R. Naik, J. T. Witkowski, and R. K. Robins, *J. Heterocycl. Chem.*, **11**, 57 (1974).
(14) J. T. Witkowski, M. Fuertes, P. D. Cook, and R. K. Robins, *J. Carbohydr., Nucleosides, Nucleotides*, **2**, 1 (1975).
(15) G. Alonso, M. T. Garcia-Lopez, G. Garcia-Muñoz, R. Madroñero, and M. Rico, *J. Heterocycl. Chem.*, **7**, 1269 (1970).
(16) F. A. Lehmkuhl, J. T. Witkowski, and R. K. Robins, *J. Heterocycl. Chem.*, **9**, 1195 (1972).
(17) A. Bloch in "Drug Design", Vol. IV, E. J. Ariens, Ed., Academic Press, New York, N.Y., 1973, Chapter 8, p 328, and references cited therein.
(18) N. Ototani and R. L. Whistler, *J. Med. Chem.*, **17**, 535 (1974).
(19) M. Bobek, A. Bloch, R. Parthasarathy, and R. L. Whistler, *J. Med. Chem.*, **18**, 784 (1975).
(20) T. Sato, T. Shimidate, and Y. Ishido, *Nippon Kagaku Zasshi*, **81**, 1440, 1442 (1960).
(21) M. Bobek and R. L. Whistler, *Methods Carbohydr. Chem.*, **6**, 292 (1972).
(22) R. U. Lemieux and D. R. Lineback, *Annu. Rev. Biochem.*, **32**, 155 (1963).
(23) J.-L. Imbach, *Ann. N.Y. Acad. Sci.*, **255**, 177 (1975).
(24) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971).
(25) D. G. Streeter et al., unpublished results.
(26) D. G. Streeter, L. N. Simon, R. K. Robins, and J. P. Miller, *Biochemistry*, **13**, 4543 (1974).

Synthesis and Some Pharmacological Properties of Deamino[4-threonine,8-D-arginine]vasopressin and Deamino[8-D-arginine]vasopressin, Highly Potent and Specific Antidiuretic Peptides, and [8-D-Arginine]vasopressin and Deamino-arginine-vasopressin

Maurice Manning,* Lajos Balaspiri,[1] Jacqueline Moehring,

Department of Biochemistry, Medical College of Ohio, Toledo, Ohio 43614

Jaya Haldar, and Wilbur H. Sawyer

Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.
Received December 10, 1975

Deamino[4-threonine,8-D-arginine]vasopressin (dTDAVP), deamino[8-D-arginine]vasopressin (dDAVP), [8-D-arginine]vasopressin (DAVP), and deamino-arginine-vasopressin (dAVP) were synthesized by the solid-phase method and tested for their biological activities. dTDAVP has an antidiuretic potency of 793 ± 95 units/mg and undetectable vasopressor activity, <0.02 unit/mg. The antidiuretic–pressor (A/P) ratio of dTDAVP is greater than 39 000. dDAVP has an antidiuretic potency of 1200 ± 126 units/mg and a vasopressor potency of 0.39 ± 0.02; its A/P ratio is thus 3000. DAVP has an antidiuretic potency of 253 ± 44 units/mg, a vasopressor potency of 1.1 ± 0.04 units/mg, and an A/P ratio of 240. The A/P ratios of dDAVP and DAVP are much higher than those originally reported. dAVP has an antidiuretic potency of 1745 ± 385 units/mg, a vasopressor potency of 346 ± 13, and an A/P ratio of 5; values are in general agreement with those in the literature. Threonine substitution has thus brought about a significant enhancement in antidiuretic specificity, a finding entirely consistent with earlier observations that enhancement of lipophilicity at position 4 alone or in combination in arginine-vasopressin can lead to enhanced antidiuretic specificity.

An investigation of the structural changes in arginine-vasopressin (AVP) which modulate antidiuretic activity and specificity led to the synthesis of deamino-[4-valine,8-D-arginine]vasopressin (dVDAVP),[2a] a peptide possessing high antidiuretic activity, specificity, and increased duration of action. In a subsequent follow-up study[2b] it was shown that enhanced antidiuretic–pressor (A/P) specificity is governed chiefly by 8-D-arginine substitution. Enhanced lipophilicity at position 4 and deamination at position 1 also contribute but to much lesser degrees. We wished to explore further the effects of enhanced lipophilicity at position 4 in combination with

Table I. Biological Activities of Arginine-vasopressin (AVP), [8-D-Arginine]vasopressin (DAVP), Deamino-arginine-vasopressin (dAVP), Deamino[8-D-arginine]vasopressin (dDAVP), Deamino[4-threonine]arginine-vasopressin (dTAVP), Deamino[4-threonine,8-D-arginine]vasopressin (dTDAVP), and Deamino[4-valine,8-D-arginine]vasopressin (dVDAVP)

	Biological activities, units/mg + SE				
	Rat uterus (no Mg^{2+})	Rat uterus (with Mg^{2+})	Rat antidiuresis	Rat vasopressor	Rat antidiuretic/-vasopressor
AVP[a]	13.9 ± 0.5	25.5 ± 0.6	323 ± 16	369 ± 6	1
DAVP[b]	1.07 ± 0.04	1.02 ± 0.08	257 ± 35	1.1 ± 0.03	240
DAVP[c]	0.4		114	4.1	30
dAVP[b]	63 ± 4		1745 ± 385	346 ± 13	5
dAVP[d]	47 ± 2	66 ± 3	1390 ± 140	370 ± 20	4.0
dDAVP[b]	~1.5	~2.9	1200 ± 126	0.39 ± 0.02	3 000
dDAVP[e]	~6.6	~3.9	955 ± 95	0.47 ± 0.02	2 000
dDAVP[c]	5.1		870	11	80
dTAVP[f]	10.2 ± 0.5	19.7 ± 1.8	758 ± 50	30 ± 1	25
dTDAVP[b]	1.02 ± 0.14	0.98 ± 0.35	793 ± 95	<0.02[h]	>39 000
dVDAVP[g]	~8	~2	1230 ± 170	<0.01[h]	>123 000

[a] Values are those reported by Manning et al.[20] [b] All assays were done by the authors and except where indicated the values represent units/mg of lyophilized peptides synthesized by the authors. [c] Values reported by Zaoral et al.[4] [d] 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.[6] The remaining activities were calculated on that basis. [e] Values obtained by assays on a solution kindly supplied by Dr. J. Mulder, Ferring AB Malmo, and reported in ref 2a. [f] Manning et al.[3] [g] Manning et al.[2] [h] Inhibit the vasopressor response to AVP.

8-D-arginine substitution and deamination at position 1 on antidiuretic specificity by incorporating an amino acid which is more lipophilic than glutamine but less so than valine. We had found earlier that threonine substitution at position 4 in deamino-arginine-vasopressin (dAVP) brought about a sixfold enhancement of antidiuretic specificity in the resulting compound, deamino[4-threonine]arginine-vasopressin (dTAVP).[3] This compares favorably with the threefold enhancement of antidiuretic specificity brought about by substituting valine for glutamine in dAVP[2b] (Table I). In light of these findings, and those obtained for dVDAVP,[2a] it appeared worthwhile to determine the effects of a threonine/glutamine interchange in dDAVP on A/P specificity and to compare the properties of the resulting compound deamino[4-threonine,8-D-arginine]vasopressin (dTDAVP) with those of dTAVP and dVDAVP. We thus report the synthesis and some pharmacological properties of dTDAVP.

During the course of our studies on the effects of D-arginine substitution on the antidiuretic specificity of vasopressin analogues, we noted a discrepancy between the previously reported values for the antidiuretic specificities of [8-D-arginine]vasopressin (DAVP)[4] and of dDAVP[5] and those that we obtained for the DAVP and dDAVP samples we synthesized and for a sample of dDAVP received from another source. The low values for the antidiuretic specificities of DAVP and dDAVP given in these earlier reports[4,5] have tended to obscure the profound effects of 8-D-arginine substitution on antidiuretic specificity of vasopressin analogues. We thus report the synthesis and pharmacological properties of DAVP and dDAVP. We also report the first solid-phase synthesis of the highly potent dAVP. Its properties are also compared with those previously reported.[6] All four peptides were synthesized by the Merrifield method,[7,8] as adapted for the synthesis of oxytocin,[9] [4-threonine]oxytocin,[10] and dVDAVP[2a] as described in the Experimental Section. Their pharmacological properties were evaluated by methods previously described.[2b,11,12]

Results and Discussion

The pharmacological properties of DAVP, dDAVP, dAVP, and dTDAVP are presented in Table I. The properties of dAVP reported here are in essential agreement with those previously reported.[6] In comparing the properties of dTDAVP with those of dTAVP and dDAVP it can be seen that the effects of threonine substitution in the 4 position of dDAVP are analogous to its effects when substituted in dAVP.[3] In both instances antidiuretic and vasopressor activities were decreased but to differing degrees, resulting in enhanced antidiuretic specificity. Threonine substitution in dDAVP virtually eliminated pressor potency. At high doses (5 μg per rat) dTDAVP is a weak antagonist of the vasopressor effects of subsequently injected AVP, a property it shares with dVDAVP.[2b] Thus dTDAVP possesses an A/P ratio of >39 000, similar to that reported for dVDAVP. These findings further demonstrate that enhanced lipophilicity at position 4 contributes to enhanced antidiuretic specificity.

The original reports on the properties of DAVP and dDAVP gave A/P ratios of 28[4] and 79,[5] respectively, for these compounds. We have obtained A/P ratios of 240 and 3000 for DAVP and dDAVP, respectively. These values are much more consistent with the A/P values we obtained for dVDAVP,[2a] dTDAVP, and an independently synthesized sample of dDAVP (Table I). These findings further support the view[2b] that D-arginine substitution is by far the most effective single structural change in enhancing A/P specificity of AVP analogues. dTDAVP has also been found to produce protracted antidiuresis[13] in rats, similar to that elicited by dDAVP,[14,15] dVDAVP,[2a,15] and other deamino analogues of arginine-vasopressin.[15] Activity of dTDAVP on the rat uterus was considerably less than that of dTAVP (Table I). This is consistent with the previously observed reduction in oxytocic activity seen when D-arginine was substituted in AVP or dAVP. The oxytocic activities of some D-arginine analogues were highly variable from one assay to the next, and the figures for rat uterus activities of these analogues in Table I are very rought estimates, at best.

Experimental Section

All *tert*-butyloxycarbonylamino acid derivatives were purchased from Bachem, Schwartz BioResearch, and Biosynthetica. The purity of each one was checked by TLC as described in ref 16. *tert*-Butyloxycarbonylglycyl resins were purchased from Schwartz BioResearch. The required protected peptides **1**, **3**, **5**, and **7** were all synthesized with the use of the solid-phase method.[7,8] Manual methods, following procedures previously described[2a,9,10] and with the changes noted below, were used. The individual protected

Table II. Protected Peptides of 8-D-Arginine-vasopressin (1), Deamino-arginine-vasopressin (3), Deamino-8-D-arginine-vasopressin (5) and Deamino[4-threonine]-8-D-arginine-vasopressin (7)

(X)
|
Bzl-S-CH_2-CH-CO-Tyr(Bzl)-Phe(X)-Asn-Cys(Bzl)-Pro(X)-Gly-NH_2
1 2 3 4 5 6 7 8 9

No.	Substituents in positions 1	4	8	Formula[a]	Mp, °C[b]	$[\alpha]^T_D$,[d] deg	Yield,[c] on resin, %	Yield[c] on ammonolysis, %
1	NH-Z	Gln	D-Arg(Tos)	$C_{82}H_{97}N_{15}O_{16}S_3$	216-217	-26.5	99.6	38
3	H	Gln	L-Arg(Tos)	$C_{74}H_{90}N_{14}O_{14}S_3 \cdot H_2O$	211-214	-33.4	61	28
5	H	Gln	D-Arg(Tos)	$C_{74}H_{90}N_{14}O_{14}S_3 \cdot H_2O$	206-207.5	-22.95	97.8	35
7	H	Thr(Bzl)	D-Arg(Tos)	$C_{80}H_{96}N_{13}O_{14}S_3$	217-220	-6.0	33	16

[a] Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. The analytical results were within ±0.4% of the theoretical values; all compounds were analyzed for C, H, and N. [b] Melting points were taken in an open capillary and are uncorrected. [c] Yields are based on the initial glycine incorporation on the resin. [d] In DMF (c 1.0); T = 21, 21.5, 23, 22°, respectively.

Table III. 8-D-Arginine-vasopressin (DAVP, 2), Deamino-arginine-vasopressin (dAVP, 4), Deamino-8-D-arginine-vasopressin (dDAVP, 6), and Deaminol[4-threonine]-8-D-arginine-vasopressin (dTDAVP, 8)

(X)
|
S-CH_2-CH-CO-Tyr-Phe(X)-Asn-Cys-Pro(X)-Gly-NH_2
1 2 3 4 5 6 7 8 9

Peptide	Substituents in positions 1	4	8	$[\alpha]^T_D$[a]	R_f[b]	Yield from protected nonapeptide, %	Yield overall, %[c]
DAVP	NH_2	Gln	D-Arg	+2.0[d]	0.10	87.0	33.0
dAVP	H	Gln	L-Arg	-98.8[e]	0.18	63.0	12.0
dDAVP	H	Gln	D-Arg	-79.8[f]	0.21	77.0	27.0
dTDAVP	H	Thr	D-Arg	-84.6	0.24	51.0	8.0

[a] In 1 M acetic acid (c 0.5); T = 22, 20.5, 21, and 20.5°, respectively. [b] Samples run on silica gel H plates in the upper phase of the solvent system n-BuOH-AcOH-H_2O (4:1:5 v/v, ascending). Electrophoresis in two pyridine acetate buffers of pH 3.5 and 6.5 gave only one spot with the platinum and ninhydrin reagents in all cases. [c] Based on the initial glycine incorporation on the resin. [d] Lit.[4] $[\alpha]^{25}_D$ -23.0° (c 0.4, 1 M acetic acid). [e] Lit.[6] $[\alpha]^{23}_D$ -103 ± 2° (c 1.3, 0.1 M acetic acid). [f] Lit.[5] $[\alpha]^{25}_D$ -82.5 ± 2° (c 0.2, 1 M acetic acid).

intermediates were deblocked with sodium in liquid ammonia[17] and following cyclization with ferricyanide[18] the desired peptides 2, 4, 6, and 8 were purified by gel filtration on Sephadex G-15.[19] The specific details of the syntheses of the protected and free peptides are given below. Their physical characteristics and yields are presented in Tables II and III. For quantitative amino acid analyses samples were hydrolyzed with constant boiling HCl in evacuated sealed ampules at 110° for 18 h and were analyzed by Arro Laboratories, Inc., Joliet, Ill. 60434. All optical rotations were measured on a Bellingham Stanley Ltd., Model A polarimeter, Type P1.

Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (1). *tert*-Butyloxycarbonylglycyl resin (2.16 g, 0.79 mmol of glycine) was treated with the appropriate *tert*-butyloxycarbonylamino acids and reagents, in an eight-cycle procedure as described for oxytocin[9] and [4-threonine]oxytocin,[10] to give the protected nonapeptide resin, wt 2.7 g. Ammonolytic cleavage of the protected nonapeptide resin (2.67 g) was carried out as described for oxytocin. The protected peptide was extracted with DMF and MeOH, and the solvents were removed in vacuo. The semisolid residue was dissolved in DMF (20 ml) and precipitated by the addition of H_2O (0.1 ml) to give 1 as an amorphous white powder: wt 400 mg; mp 200–202°. This was recrystallized twice from acetic acid–absolute ethanol: wt 228 mg (Table II). Amino acid analysis gave Asp, 0.94; Glu, 1.00; Phe, 0.88; Gly, 1.00; Bzl-Cys, 1.90; Arg, 1.00; Tyr, 0.53; Pro, 1.00; NH_3, 2.95.

8-D-Arginine-vasopressin (DAVP, 2). The protected nonapeptide 1 (100 mg) was deblocked by the sodium–liquid ammonia procedure as used in the syntheses of oxytocin[17] and [4-threonine]oxytocin.[10] Reoxidation in aqueous solution at pH 6.5 was effected with the use of potassium ferricyanide[18] with the modifications previously described.[2a] The lyophilized product was purified by gel filtration on Sephadex G-15 in a two-step procedure using 50% AcOH and 0.2 N AcOH, respectively, for elution in each step.[2a,19] 8-D-Arginine-vasopressin obtained as a fluffy white powder, wt 57.5 mg, was shown to be homogeneous by thin-layer chromatography and paper electrophoresis at different pH's as described for [4-threonine]oxytocin[10] (Table III). Amino acid analysis gave Asp, 0.88; Glu, 0.98; Gly, 0.95; Pro, 0.97; Cys, 1.92; Phe, 1.00; Tyr, 0.95; Arg, 0.99; NH_3, 2.90.

***S*-Bzl-β-mercaptopropionyl-Tyr(Bzl)-Phe-Glu-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (3).** *tert*-Butyloxycarbonylglycyl resin (3.00 g, 1.25 mmol of glycine) was treated in an eight-cycle procedure as described for the synthesis of dVDAVP except that Boc-Arg(Tos) and Boc-Gln-nitrophenyl ester were used in the first and sixth incorporation steps, respectively, to give the protected peptidyl resin, 3.54 g. Ammonolytic cleavage of the protected octapeptide resin (3.00 g) was carried out as described earlier[2a,9] and the protected peptide was extracted with DMF and MeOH. Solvents were removed in vacuo and the residue was purified by trituration with 95% EtOH (25 ml), washed on filter with 95% EtOH (3 × 15 ml) and diethyl ether (3 × 15 ml), and dried in vacuo over P_2O_5 to give 1 as an amorphous white powder: wt 284 mg; mp 205–210°. This was recrystallized from acetic acid–absolute ethanol: wt 240 mg (Table II). Amino acid analysis gave Asp, 0.96; Gly, 1.00; Bzl-Cys, 0.97; Phe, 0.98; Tyr, 0.56; Pro, 0.98; Arg, 1.18; Glu, 1.03, NH_3, 3.04.

Deamino-arginine-vasopressin (dAVP, 4). The protected octapeptide 3 (150 mg) was deblocked, reoxidized, deionized, and purified as for 1: wt 77.1 mg (Table III). Amino acid analysis gave Asp, 1.00; Glu, 1.03, Gly, 1.00; Pro, 1.00; Phe, 0.95; Tyr, 0.95; Arg, 1.00; NH_3, 3.32. In addition, cystine (0.19) and the mixed disulfide of cysteine and β-mercaptopropionic acid (0.76) were present.

***S*-Bzl-β-mercaptopropionyl-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (5).** *tert*-Butyloxycarbonylglycyl resin (1.84 g, 0.68 mmol of glycine) was treated as described for the synthesis of 3 except that Boc-D-Arg(Tos) was used in the first incorporation step. Upon completion of the eight-cycle procedure the protected octapeptide resin was collected and dried in vacuo over P_2O_5: wt 2.2 g. Ammonolytic cleavage of the protected octapeptide resin (2.2 g) followed by extraction with DMF (80 ml) and MeOH (50 ml) and evaporation of the solvents gave 5 as a white amorphous powder: wt 400 mg; mp 195–198°.

This was recrystallized twice from glacial acetic acid–absolute ethanol: wt 154 mg (Table II). Amino acid analysis gave Asp, 1.00; Pro, 1.18; Gly, 1.00; Phe, 1.08; Tyr, 1.10; Bzl-Cys, 0.96; Arg, 1.00; Glu, 1.09; NH_3, 1.80.

Deamino-8-D-arginine-vasopressin (dDAVP, 6). The protected octapeptide **5** (100 mg) was reduced, reoxidized, deionized, lyophilized, and purified as for 1 above: wt 55 mg. It was shown to be homogeneous by thin-layer chromatography and paper electrophoresis at two different pH's (Table II). Amino acid analysis gave Asp, 0.97; Glu, 0.98; Pro, 0.94; Gly, 0.95; Phe, 1.00; Tyr, 0.97; Arg, 0.96; NH_3, 2.20; cystine, 0.42; mixed disulfide of cysteine and β-mercaptopropionic acid, 0.48.

***S*-Bzl-β-mercaptopropionyl-Tyr(Bzl)-Phe-Thr(Bzl)-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (7).** *tert*-Butyloxycarbonylglycyl resin (2.74 g, 0.68 mmol of glycine) was treated as described for the synthesis of **3** except that Boc-D-Arg(Tos) and Boc-Thr(Bzl) were used in the first and sixth incorporation steps, respectively. Upon completion of the eight-cycle procedure the protected octapeptide resin was collected and dried in vacuo over P_2O_5: wt 3.06 g. Ammonolytic cleavage of the protected octapeptide resin (2.99 g) followed by extraction with warm (50%) DMF (50 ml) and MeOH (40 ml), evaporation of the solvents, drying, and trituration with 95% ethanol (20 ml) and ether (40 ml) gave **7**: wt 244 mg; mp 207–211°. This was recrystallized from glacial acetic acid–95% ethanol: wt 151 mg (Table II). Amino acid analysis gave Asp, 0.97; Thr, 0.93; Gly, 1.00; Arg, 0.97; Pro, 1.06; Tyr, 0.78; Phe, 0.97; Bzl-Cys, 0.92; NH_3, 2.34.

Deamino[4-threonine]-8-D-arginine-vasopressin (dTDAVP, 8). The protected octapeptide **7** (100 mg) was deblocked, reoxidized, deionized, lyophilized, and purified as for 1: wt 34.1 mg (Table III). It was shown to be homogeneous by the usual methods. Amino acid analysis gave Asp, 0.98; Arg, 0.95; Thr, 0.92; Gly, 0.94; Phe, 0.96; Tyr, 0.90; Pro, 1.00; NH_3, 1.5, cystine, 0.32, mixed disulfide of cysteine and β-mercaptopropionic acid, 0.70.

Acknowledgment. This work was supported in part by research grants from the National Institute of Child Health and Human Development (No. H 606351), the National Institute of Arthritis and Metabolic Diseases (No. AM 01940), the National Heart and Lung Institute (No. HL 12738), and the National Science Foundation (No. BM 571-01238). We wish to thank Dr. John Lowbridge for helpful discussion and Miss Cindy Stanley for invaluable assistance in the preparation of the manuscript.

References and Notes

(1) Visiting investigator from Jozsef Attila University of Szeged, Department of Organic Chemistry, Szeged, Hungary.
(2) (a) M. Manning, L. Balaspiri, M. Acosta, and W. H. Sawyer, *J. Med. Chem.*, **16**, 975 (1973); (b) W. H. Sawyer, M. Acosta, L. Balaspiri, J. Judd, and M. Manning, *Endocrinology*, **94**, 1106 (1974).
(3) M. Manning, E. J. Coy, M. Acosta, and W. H. Sawyer, *J. Med. Chem.*, **16**, 836 (1973).
(4) M. Zaoral, J. Kolc, and F. Sorm, *Collect. Czech. Chem. Commun.*, **32**, 1242 (1967).
(5) M. Zaoral, J. Kolc, and F. Sorm, *Collect. Czech. Chem. Commun.*, **32**, 1250 (1967).
(6) R. L. Huguenin and R. A. Boissommas, *Helv. Chim. Acta*, **49**, 695 (1966).
(7) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).
(8) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).
(9) M. Manning, *J. Am. Chem. Soc.*, **90**, 1348 (1968).
(10) M. Manning, E. Coy, and W. H. Sawyer, *Biochemistry*, **9**, 3925 (1970).
(11) W. H. Sawyer, *Endocrinology*, **63**, 694 (1958).
(12) W. H. Sawyer in "The Pituitary Gland", Vol. 3, G. W. Harris and B. T. Donovan, Ed., Butterworths, London, 1966, p 288.
(13) W. H. Sawyer and M. Manning, unpublished results.
(14) K. Vavra, A. Machova, V. Holocek, J. H. Cort, M. Zaoral, and F. Sorm, *Lancet*, 1, 948 (1968).
(15) W. H. Sawyer, M. Acosta, and M. Manning, *Endocrinology*, **95**, 140 (1974).
(16) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969, p 60.
(17) V. du Vigneaud, C. Ressler, J. M. Swan, P. G. Katsoyannis, C. W. Roberts, and S. Gordon, *J. Am. Chem. Soc.*, **75**, 4879 (1953).
(18) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Biol. Chem.*, **237**, 1563 (1962).
(19) M. Manning, T. C. Wuu, and J. W. M. Baxter, *J. Chromatogr.*, **38**, 396 (1968).
(20) M. Manning, E. J. Coy, W. H. Sawyer, and M. Acosta, *J. Med. Chem.*, **16**, 463 (1973).

Antimalarials. 3. 1,2,4-Triazines

Louis C. March, Gurdip S. Bajwa, Jessie Lee, Khizar Wasti, and Madeleine M. Joullié*

Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19174. *Received July 16, 1975*

The syntheses of a number of substituted 1,2,4-triazines as potential antimalarials are described. The structural requirements for antimalarial activity are discussed with reference to the substituents of a phenyl group in the 6 position and amino groups at the 3 and 5 positions. Of the compounds tested, **2**, **5**, and **7** produced cures in mice infected with *Plasmodium berghei*. Compounds **2** [3,5-diamino-6-(4-trifluoromethylphenyl)-1,2,4-triazine], **3**, **5**, **8**, **12**, and **37** produced cures in chicks infected with *Plasmodium gallinaceum*.

3,5-Diamino-1,2,4-triazines have been reported to exhibit antimalarial activity.[1] 3,5-Diamino-6-(4'-chlorophenyl)-1,2,4-triazine (**1**) was also found to be a competitive antagonist of folic acid and folinic acid in the growth of *Lactobacillus casei*, thus confirming the suspected relationship between folic acid antagonists and antimalarial activity.[2] We, therefore, undertook the synthesis of several 6-substituted 3,5-diamino-1,2,4-triazines to examine the effect of structural changes on the antimalarial activity of this system.

Chemistry. The route employed for the synthesis of 6-aryl- and 6-thienyl-3,5-diamino-1,2,4-triazines **2**–**10** was previously used in the preparation of related 1,2,4-triazines.[1,3] This synthetic scheme involves the reaction of an acylnitrile with aminoguanidine bicarbonate in the presence of 8 N nitric acid and dimethyl sulfoxide. The acylnitrile aminohydrazones, obtained as nitric acid salts **2a**–**10a**, were cyclized with alcoholic potassium hydroxide to the desired 1,2,4-triazines **2**–**10**.

Some structural modifications of active 3,5-diamino-1,2,4-triazines were attempted. The reaction of **4** and **5** with *N*,*N*-dimethylformamide dimethyl acetal in dimethylformamide gave **11** and **12**. The reaction of **5** with phenyl isocyanate in refluxing 1,2-dimethoxyethane and **9** with *p*-toluenesulfonyl isocyanate in refluxing pyridine gave the corresponding monoureido derivatives **13** and **14**,