N-Acetyl-[2-(O-methyl)tyrosine]arginine-vasopressin, an Interesting Antagonist of the Vasopressor Response to Vasopressin

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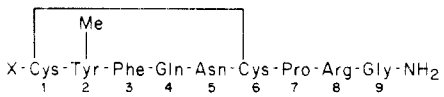
The synthesis of *N*-acetyl-[2-(*O*-methyl)tyrosine]arginine-vasopressin [Ac-Tyr(Me)AVP] was undertaken utilizing a combination of the stepwise active ester and fragment condensation methods. Ac-Tyr(Me)AVP is an antagonist of the vasopressor response to vasopressin ($pA_2 = 7.18 \pm 0.08$), devoid of vasopressor agonist activity, and has an antidiuretic potency of 0.026 ± 0.002 unit/mg, a 15 000-fold decrease over the antidiuretic activity of [2-(*O*-methyl)tyrosine]arginine-vasopressin. The analogue is also an antagonist of the in vitro uterotonic activity of oxytocin with pA_2 values of 7.29 ± 0.08 in the absence of Mg^{2+} and 6.73 ± 0.14 in 0.5 mM Mg^{2+}. This result of N^{α}-acetylation of Tyr(Me)AVP parallels similar results in the oxytocin series and suggests that this substitution should be considered in the design of potential antagonists of the antidiuretic response to vasopressin.

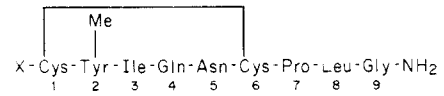
Recently, Bankowski et al.[1] reported on the synthesis and properties of [2-(*O*-methyl)tyrosine]arginine-vasopressin [Tyr(Me)AVP; **1a**] and found that is had antidi-



1a, X = H
b, X = CH_3CO

uretic activity comparable to that of the parent arginine-vasopressin (AVP). It was a weak vasopressor agonist without evident antagonistic activity. In addition, Tyr(Me)AVP has antioxytocic activity in isolated rat uterus assays similar to that of [1-deaminopenicillamine,4-threonine]oxytocin[2] and [1-β-mercapto-β,β-pentamethylenepropionic acid]oxytocin,[3] which were two of the most potent antagonists of the uterotonic activity of oxytocin reported at that time. The analogous substitution of a 2-(*O*-methyl)tyrosine in lysine-vasopressin (LVP) to form [2-(*O*-methyl)tyrosine]lysine-vasopressin [Tyr(Me)-LVP] reduced antidiuretic activity to about one-third that of LVP.[1] Although Tyr(Me)LVP had weak vasopressor agonist activity, it could also inhibit vasopressor responses to LVP in rats and the oxytocic response to oxytocin by rat uteri in vitro.[4] [2-(*O*-Methyl)tyrosine]oxytocin [Tyr(Me)OT] was found to have mixed agonistic and antagonistic activities on the rat uterus in vitro[5] and to have weak antivasopressor activity.[6]

Even more dramatic inhibitory properties have been produced in the oxytocin series by acylating the free amino group at position 1 of Tyr(Me)OT (**2a–d**). Analogue **2a**,

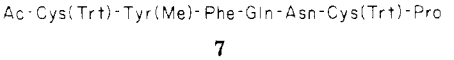


2a, X = CH_3CO **2c**, X = Gly
b, X = NH_2CO d, X = Sar

for example, is about four to seven times as active an inhibitor of oxytocin in the isolated rat uterus as Tyr(Me)OT,[7,8] with **2b**, **2c**, and **2d**[9–11] having somewhat less activity than **2a**.

We now report on the synthesis of *N*-acetyl-[2-(*O*-methyl)tyrosine]arginine-vasopressin [Ac-Tyr(Me)AVP; **1b**] and some of its biological properties.

Chemistry. Compound **1b** was synthesized by a combination of the stepwise active ester and fragment condensation methods, the scheme and details of which have been reported previously for the synthesis of AVP.[12] Boc-Tyr(Me)-OCP (**3**) was coupled with H-Phe-Gln-Asn-Cys(Trt)-Pro[12] in 87% yield to form Boc-Tyr(Me)-Phe-Gln-Asn-Cys(Trt)-Pro (**4**), which in turn was treated with HCl/dioxane to remove the Boc[13] group and then coupled with Ac-Cys(Trt)-ONSu (**5**) to obtain heptapeptide **7**. The



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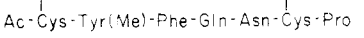
trityl protecting groups on sulfur were removed and the intramolecular disulfide bridge formed with I_2 in 80% aqueous acetic acid to give peptide **8**. The remaining dipeptide unit, Arg-Gly-NH_2, was attached to partially purified **8** by means of the hydroxysuccinimide ester



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(–ONSu) generated in situ. The guanidyl group of arginine was protected as a picrate. Compound **1b** was purified by means of countercurrent distribution (CCD).

Bioassays. The methods utilized for determining the agonistic and antagonistic activities of **1b** have been described previously by Manning et al.[14] Assays on the

(1) K. Bankowski, M. Manning, J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, **21**, 850 (1978).
(2) M. Manning, J. Lowbridge, J. Seto, J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, **21**, 179 (1978).
(3) J. J. Nestor, Jr., M. F. Ferger, and V. du Vigneaud, *J. Med. Chem.*, **18**, 284 (1975).
(4) M. Zaoral, E. Kasafirek, J. Rudinger, and F. Sorm, *Collect. Czech. Chem. Commun.*, **30**, 1869 (1965).
(5) I. Krejci, I. Polacek, and J. Rudinger, *Br. J. Pharmacol.*, **30**, 506 (1967).
(6) H. B. Law and V. du Vigneaud, *J. Am. Chem. Soc.*, **82**, 4579 (1960).
(7) K. Jost and F. Sorm, *Collect. Czech. Chem. Commun.*, **36**, 297 (1971).
(8) I. Krejci, B. Kupkova, T. Barth, and K. Jost, *Physiol. Bohemoslov.*, **22**, 315 (1973).
(9) A. Chimiak, K. Eisler, K. Jost, and J. Rudinger, *Collect. Czech. Chem. Commun.*, **33**, 2918 (1968).
(10) G. W. Bisset and B. J. Clark, *Nature (London)*, **218**, 197 (1968).
(11) G. W. Bisset, B. J. Clark, I. Krejci, I. Polacek, and J. Rudinger, *Br. J. Pharmacol.*, **40**, 342 (1970).
(12) D. A. Jones, R. A. Mikulec, and R. H. Mazur, *J. Org. Chem.*, **38**, 2865 (1973).
(13) Abbreviations according to IUPAC–IUB recommendations: *Pure Appl. Chem.*, **40**, 315 (1974). In addition, OCP = 2,4,5-trichlorophenoxy, DCU = dicyclohexylurea, and DCC = dicyclohexylcarbodiimide.
(14) M. Manning, J. Lowbridge, C. T. Stier, Jr., J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, **20**, 1228 (1977).

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Table I. Agonist and Antagonist Activities of *N*-Acetyl-[2-(*O*-methyl)tyrosine]arginine-vasopressin [Ac-Tyr(Me)AVP] and [2-(*O*-Methyl)tyrosine]arginine-vasopressin [Tyr(Me)AVP]

peptide	vasopressor, units/mg	antidiuretic, units/mg	antivasopressor pA_2[a]	antioxytocic pA_2[a]	
				no Mg^{2+}	0.5 mM Mg^{2+}
Tyr(Me)AVP	9.7 ± 0.5[b]	386 ± 36[b]		7.44 ± 0.12 (18)[b,c]	6.34 ± 0.19 (9)[d]
Ac-Tyr(Me)AVP		0.026 ± 0.002	7.18 ± 0.08 (8)[c]	7.29 ± 0.08 (13)	6.73 ± 0.14 (17)

[a] pA_2 values obtained as described in ref 14. [b] Reference 1. [c] The numbers in parentheses denote the number of assays, and the values are means plus or minus standard errors. [d] Unpublished data of W. H. Sawyer and M. Manning.

Table II. Biological Activities of Vasopressin and Oxytocin Analogues Substituted with *O*-Methyltyrosine in Position 2 and with Various *N*-Acyl Groups

peptide[a]	antivasopressor pA_2 or % of pressor response	antioxytocic pA_2	antidiuretic, units/mg
OT			4.0 ± 0.8[b]
Tyr(Me)OT	weak	mixed[c]	0.01[d]
Ac-Tyr(Me)OT	inhibn[e]	7.58[e]	
Carb-Tyr(Me)OT	inhibn[e]	7.22[e]	
Gly-Tyr(Me)OT	(50)[e]	6.85[e]	
Sar-Tyr(Me)OT	(100)[e]	6.52[e]	
AVP			323 ± 16[f]
Tyr(Me)AVP		7.44 ± 0.12[g]	386 ± 36[g]
Ac-Tyr(Me)AVP[g]	7.18 ± 0.08[h]	7.29 ± 0.08[h]	0.026 ± 0.002[h]
LVP			212 ± 13[g]
Tyr(Me)LVP	mixed[i]	inhibitor[j]	79 ± 11[j]

[a] The abbreviations for the vasopressin and oxytocin analogues follow the system of Sawyer et al.[17] Pertinent examples are: Tyr(Me)OT, [2-(*O*-methyl)tyrosine]oxytocin; Carb-Tyr(Me)OT, [*N*-carbamoyl,2-(*O*-methyl)tyrosine]oxytocin; AVP, 8-arginine-vasopressin; LVP, 8-lysine-vasopressin. [b] Values of Manning et al., ref 16. [c] Showed both agonistic and antagonistic activities; see ref 5. [d] Reference 6. [e] Reference 18. [f] Values of Manning et al., ref 19. [g] Reference 1. [h] This work. [i] Krejci et al., *Br. J. Pharmacol.*, **30**, 497 (1967). [j] Reference 4.

isolated rat uterus were performed in a medium containing no Mg^{2+} and in a medium containing 0.5 mM Mg^{2+}. Agonistic activities are expressed in units/mg and antagonistic activities as pA_2 values. The pA_2 is defined as the negative logarithm (base 10) of the average molar concentration of an antagonist which will make the biological response to $2x$ units of agonist the same as the response to x units of agonist in the absence of antagonist.[15] The USP Posterior Pituitary Reference Standard was used in all assays.

Results and Discussion

Some of the pharmacological properties of Ac-Tyr(Me)AVP can be found in Table I, along with a comparison of the values recently obtained for Tyr(Me)AVP. Acetylation of the free amino group at position 1 of Tyr(Me)AVP reduces the antidiuretic potency approximately 15 000 times and gives the molecule reasonable antivasopressor activity without significantly changing its antioxytocic activity.

A comparison of some of the biological activities of acylated [2-(*O*-methyl)tyrosine]oxytocin and -vasopressin analogues with their free amino counterparts can be found in Table II. The antioxytocic activity of **1b** is very similar to that found for Ac-Tyr(Me)OT.

In the case of Ac-Tyr(Me)AVP, N^{α}-acetylation had dramatic effects on the agonistic activities of Tyr(Me)AVP. Antidiuretic activity was decreased about 15 000 times, and vasopressor agonistic activity was abolished; Ac-Tyr(Me)AVP is an effective antagonist of vasopressor responses. Thus, N^{α}-acetylation, in combination with changes elsewhere in the VP molecule known to contribute to antagonistic activities, may be useful in the design of more potent antivasopressor analogues. The striking result of N^{α}-acetylation of Tyr(Me)AVP on antidiuretic activity suggests that this substitution should be seriously considered in the design of potential antagonists of the antidiuretic response.

Experimental Section

Thin-layer chromatograms (TLC) were performed on 20-cm Merck silica gel G-60 precoated glass plates in the following systems: A, 1-butanol–acetic acid–water (7:1:2); B, $CHCl_3$–methanol–acetic acid–water (64:30:2:4); C, $CHCl_3$–methanol (95:5). Approximately 50 μg of compound was spotted, and spots were revealed with *tert*-butyl hypochlorite, followed by starch (1%)–KI (1%).[20] The countercurrent distribution (CCD) system was 1-butanol–acetic acid–water (4:1:5). Optical rotations were obtained with a Perkin-Elmer Model 141 polarimeter and amino acid analysis on a Beckman Model 121 automatic amino acid analyzer. Melting points were obtained on a Mel-Temp[21] capillary melting point apparatus.

Ac-Cys-Tyr(Me)-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2·CH_3CO_2H (1b). A solution of 4.06 g (2.75 mmol) of **7** in 650 mL of 80% aqueous HOAc was added dropwise at 25 °C at 30 min to a rapidly stirred solution of 668 mL of 4.73×10^{-3} M I_2 in 80% aqueous HOAc. After the solution stirred for an additional 45 min, the excess I_2 was decolorized with 1 N Na_2SO_3. In order to neutralize the HI formed during the reaction, 6.5 mL of 1 M NaOH was added and the solvent was evaporated at reduced pressure. The gum remaining was triturated with cold methanol, and the inorganic salts were removed by filtration. After evaporation of the methanol under reduced pressure, cold ether was added and the resulting solid partially purified by CCD ($K = 2.6$), to give 1.07 g (~42% crude yield): TLC R_f (A) 0.38, plus slow-moving impurities.

The cyclized heptapeptide (**8**) thus obtained (1.15 mmol) was stirred for 1.75 h with 152 mg (1.32 mmol) of *N*-hydroxysuccin-

(15) H. O. Schild, *Br. J. Pharmacol.*, **2**, 189 (1947).
(16) M. Manning, E. J. Coy, and W. H. Sawyer, *Experientia*, **27**, 1372 (1971).
(17) W. H. Sawyer, M. Acosta, L. Balaspiri, J. Judd, and M. Manning, *Endocrinology*, **94**, 1106 (1974).
(18) M. Krojidlo, T. Barth, L. Servitova, K. Dobrovsky, K. Jost, and F. Sorm, *Collect. Czech. Chem. Commun.*, **40**, 2708 (1975).
(19) M. Manning, L. Balaspiri, J. Moehring, J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, **19**, 842 (1976).
(20) R. H. Mazur, B. W. Ellis, and P. S. Cammarata, *J. Biol. Chem.*, **237**, 1619 (1962).
(21) The melting points of the peptides were undefined and therefore not reported.

imide and 272 mg (1.32 mmol) of DCC in 4.5 mL of DMF. The reaction mixture was cooled, the DCU was removed by filtration, and 882 mg (1.32 mmol) of Arg-Gly-NH_2·dipicrate and 0.15 mL (1.35 mmol) of *N*-methylmorpholine were added. After standing at 25 °C for 6 days, the reaction mixture was added to 100 mL of cold, 5% $KHSO_4$ to give a gummy precipitate. The yellow aqueous layer was decanted and the gum dried. In order to obtain pure **1b**, extensive losses were taken. After CCD (240 transfers), tubes 100–130 containing product homogeneous by TLC [R_f (A) 0.16] were pooled and the solvent was evaporated. During CCD, picric acid was exchanged for HOAc on the guanidyl group of Arg. The addition of ether gave 39.2 mg (2.6% yield from H-Phe-Gln-Asn-Cys(Trt)-Pro·HCl) of **1b** as a fluffy powder: $[\alpha]^{25}_D$ −30° (*c* 1, HOAc). Anal. ($C_{49}H_{69}N_{15}O_{13}S_2 \cdot CH_3CO_2H \cdot 6H_2O$) C, H, N. Amino acid analysis: $^1/_2$-Cys, 1.92; Tyr, 0.37;[22] Phe, 1.00; Glu, 1.01; Asp, 1.01; Pro, 0.95; Arg, 1.07; Gly, 1.04; NH_3, 3.3.

Boc-Tyr(Me)-OCP (3). To a suspension of 19.4 g (99.0 mmol) of Tyr(Me) in 100 mL of 50% aqueous dioxane, 25 mL of 4 N NaOH was added in portions until solution was complete. Then, maintaining a constant pH of 10.5, 15.7 g (110 mmol) of Boc-N_3 was added, and the reaction was stirred at 25 °C for 6 h. At this point, the reaction mixture was extracted with ether to remove excess azide and acidified to pH 3 with 125 mL of 2 M citric acid, whereupon the product formed as a yellow oil. The mixture was extracted twice with 100-mL portions of EtOAc. The EtOAc solution was washed twice with water and then with 5 M NaCl and dried over anhydrous $MgSO_4$. The solvent was evaporated at reduced pressure to leave an approximately quantitative yield of product as a gum: TLC R_f (A) 0.78.

A solution of the oily Boc-Tyr(Me)-OH and 20.7 g (105 mmol) of 2,4,5-trichlorophenol in 150 mL of EtOAc was cooled to 4 °C and then a solution of 21.6 g (105 mmol) of DCC in 50 mL of EtOAc was added. After the reaction mixture stirred in an ice bath for 3 h, the DCU was removed by filtration and the EtOAc evaporated to leave a tan solid. Crystallization from ethanol yielded 27.4 g (58.4%) of active ester: TLC R_f (C) 0.85; mp 111–112 °C; $[\alpha]^{27}_D$ −25° (*c* 0.5, MeOH). Anal. ($C_{21}H_{22}Cl_3NO_5$) C, H, N, Cl.

Boc-Tyr(Me)-Phe-Gln-Asn-Cys(Trt)-Pro (4). A solution of 5.55 g (6.14 mmol) of H-Phe-Gln-Asn-Cys(Trt)-Pro·HCl·H_2O, 3.21 g (6.75 mmol) of **3**, and 1.4 mL (12.6 mmol) of *N*-methylmorpholine in 32 mL of DMF was stirred at 25 °C for 48 h, after which 0.2 mL (1.8 mmol) of $(CH_3)_2NCH_2CH_2NH_2$ was added to decompose excess active ester. The cooled (4 °C) reaction mixture was added to 300 mL of cold 1 N HCl to give a precipitate, which was filtered, washed with water, and dried in vacuo at 50 °C. The 2,4,5-trichlorophenol was removed by trituration with ether several times and the product dried again in vacuo for 3 h: yield 86.6%; TLC R_f (A) 0.70. Anal. ($C_{60}H_{70}N_8O_{12}S \cdot 2.5H_2O$) C, H, N, S.

Ac-Cys(Trt)-ONSu (5). A solution of 37.9 g (93.5 mmol) of Ac-Cys(Trt)-OH[23] and 10.1 mL of *N*-methylmorpholine in 450 mL of EtOAc was cooled to −15 °C. Maintaining this temperature, 12.2 g (92.5 mmol) of isobutyl chloroformate was added with vigorous stirring. After 30 s, 11.5 g (100 mmol) of *N*-hydroxysuccinimide was added, the mixture was filtered, and the filtrate was evaporated to a gum. Extraction of the gum with ether removed the active ester from byproducts, and the ether was evaporated in air to give 34.5 g (73.4% yield) of **5**: mp 184–186 °C; TLC R_f (A) 0.91; $[\alpha]^{25}_D$ +2.0° (*c* 1, HOAc), +7.3° (*c* 1, $CHCl_3$). Anal. ($C_{28}H_{26}N_2O_5S$) C, H, N, S.

H-Tyr(Me)-Phe-Gln-Asn-Cys(Trt)-Pro·HCl (6). To a solution of 15.8 g (14.1 mmol) of **4** in 50 mL of glacial acetic acid was added 25 mL of 5.93 N HCl/dioxane. After the solution was left standing at 25 °C for 15 min, the solvent and excess HCl were removed in vacuo at 40 °C. The product was solidified by trituration with ether, filtered, and dried in vacuo (4 h, 65 °C) to give a quantitative yield of **6** containing 1.25 mol of bound HCl: TLC R_f (B) 0.53; $[\alpha]^{25}_D$ −15.5° (*c* 1, MeOH). Anal. ($C_{55}H_{62}N_8O_{19}S \cdot 1.25HCl \cdot 3H_2O$) C, H, N, S, Cl.

Ac-Cys(Trt)-Tyr(Me)-Phe-Gln-Asn-Cys(Trt)-Pro (7). This compound was prepared in an identical manner to that of **4** from 3.70 g (3.31 mmol) of **6** and 1.91 g (3.81 mmol) of **5**. After purification by CCD (400 transfers, K = 9), a 62.0% yield was obtained: TLC R_f (B) 0.86; $[\alpha]^{25}_D$ −18.5° (*c* 1, MeOH). Anal. ($C_{79}H_{83}N_9O_{12}S_2 \cdot 3.5H_2O$) C, H, N, S.

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(22) Amino acid analyses were originally performed by Worthington Biochemical Corp., Freehold, N.J., without correction for loss of Tyr due to incomplete hydrolysis of Tyr(Me). A recent amino acid analysis of **1b**, correcting for Tyr loss, gave: Phe, 1.00; Tyr, 0.99; Gly, 0.99; Pro, 1.01; Glu, 1.03; Asp, 0.97; Arg, 1.00; $^1/_2$-Cys, 1.63.

(23) Kwang-Yuen Zee-Cheng and C. C. Cheng, *J. Med. Chem.*, 13, 414 (1970).

14-(Arylhydroxyamino)codeinones and Derivatives as Analgetics and Antagonists

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Diels–Alder reaction of thebaine (**1**) and *N*-(cyclopropylmethyl)northebaine with nitrosobenzene and *p*-fluoronitrosobenzene gave adducts [6,14-*exo*-(phenyloxyamino)codeine 6-methyl ether and derivatives, **2a–d**] which yielded 14-(phenylhydroxyamino)codeinone and derivatives (**3a–d**) on acid hydrolysis. Rearrangement of **3** (NaOMe) afforded 5,14-*exo*-(phenyloxyamino)thebainone and derivatives (**4a–d**); reduction of **3** led to 14-(phenylamino)dihydrocodeinone and derivatives (**5a–d**). Thebaine also reacted with 1-halo-1-nitrosocyclohexane (halo = Cl, Br) and with benzohydroxamic acid under oxidizing conditions to give 14-(hydroxyamino)codeinone (**6**). All compounds, **3–6**, were evaluated as analgetics and antagonists by the tail-flick, writhing, and Straub tail assays: compounds of types **3–5** were analgetics (*N*-Me), one-third to one-tenth as potent as morphine, or antagonists [*N*-(cyclopropylmethyl)], 50 to 100 times less potent than naloxone; **6** behaved as an antagonist in the tail-flick test but as an agonist in the other assays. Opiate receptor binding studies indicate that compounds of type **3** may be useful as opiate spin-label precursors.

The Diels–Alder reaction of thebaine (**1**, R = CH_3) with nitrosobenzene[2] or various postulated *C*-nitroso reactive intermediates[3–7] yields 1,2-oxazines (**2**) which hydrolyze in acid to 14-(hydroxyamino)codeinone derivatives.[2–4,6]

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(2) K. W. Bentley, P. Horsewood, G. W. Kirby, and S. Singh, *J. Chem. Soc. D*, 1411 (1969).

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