

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	antioxycotic 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 pA₂ 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 ^d Unpublished data of W. H. Sawyer and M. Manning.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	antioxycotic 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 Mg²⁺ and in a medium containing 0.5 mM Mg²⁺. Agonistic activities are expressed in units/mg and antagonistic activities as pA₂ values. The pA₂ 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 antioxycotic 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 antioxycotic 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, CHCl₃-methanol-acetic acid-water (64:30:2:4); C, CHCl₃-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-Temp²¹ capillary melting point apparatus.

Ac-Cys-Tyr(Me)-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH₂·CH₃CO₂H (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 I₂ in 80% aqueous HOAc. After the solution stirred for an additional 45 min, the excess I₂ was decolorized with 1 N Na₂SO₃. 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-

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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-NH₂-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% KHSO₄ 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: [α]_D²⁵ -30° (c 1, HOAc). Anal. (C₄₉H₈₉N₁₅O₁₃S₂·CH₃CO₂H·6H₂O) 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; NH₃, 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-N₃ 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 MgSO₄. 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; [α]_D²⁷ -25° (c 0.5, MeOH). Anal. (C₂₁H₂₂Cl₃NO₅) 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·H₂O, 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 (CH₃)₂NCH₂CH₂NH₂ 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. (C₆₀H₇₀N₈O₁₂S·2.5H₂O) C, H, N, S.

Ac-Cys(Trt)-ONSu (5). A solution of 37.9 g (93.5 mmol) of Ac-Cys(Trt)-OH²³ 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*-hydroxy-succinimide 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; [α]_D²⁵ +2.0° (c 1, HOAc), +7.3° (c 1, CHCl₃). Anal. (C₂₈H₂₆N₂O₆S) 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; [α]_D²⁵ -15.5° (c 1, MeOH). Anal. (C₅₅H₆₂N₈O₁₉S·1.25HCl·3H₂O) 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; [α]_D²⁵ -18.5° (c 1, MeOH). Anal. (C₇₉H₈₃N₉O₁₂S₂·3.5H₂O) 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.
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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 benzo-hydroxamic 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 = CH₃) with nitrosobenzene² or various postulated *C*-nitroso reactive

intermediates³⁻⁷ yields 1,2-oxazines (**2**) which hydrolyze in acid to 14-(hydroxyamino)codeinone derivatives.^{2-4,6}

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