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Synthesis of Specific Deuterium Labeled Tyrosine and Phenylalanine Derivatives and Their Use in the Total Synthesis of [8-Arginine]vasopressin Derivatives: The Separation of Diastereomeric [8-Arginine]vasopressin Derivatives by Partition Chromatography^{1,2}

Diane M. Yamamoto,³ Donald A. Upson,⁴ David K. Linn, and Victor J. Hruby*

Contribution from the Department of Chemistry, University of Arizona, Tucson, Arizona 85721. Received August 23, 1976

Abstract: Derivatives of tyrosine specifically deuterated at the α carbon ($[\alpha\text{-}^2\text{H}_1]$ tyrosine) and at both the α and β carbons ($[\alpha,\beta,\beta\text{-}^2\text{H}_3]$ tyrosine) and a derivative of phenylalanine specifically deuterated at the α carbon ($[\alpha\text{-}^2\text{H}_1]$ phenylalanine) have been synthesized in high yield. These labeled compounds have been resolved enzymatically, and the enantiomers and racemates have been converted to *N-tert*-butyloxycarbonyl derivatives. The deuterium labels were not exchanged under the conditions of the syntheses. The protected derivatives as well as specifically deuterated derivatives of *S*-benzylcysteine and of glycine were used to prepare specifically deuterated analogues of [8-arginine]vasopressin using solid phase peptide procedures. The use of improved synthetic procedures resulted in considerable improvements in the yields of [8-arginine]vasopressin compared with previous reports. In addition, new solvent systems for partition chromatography purification of [8-arginine]vasopressin on Sephadex were developed which allowed a facile one-step separation of diastereomers of [8-arginine]vasopressin containing a racemic amino acid at either the 1-hemicystine or the 2-tyrosine positions of the hormone. The following specifically deuterated hormone derivatives were synthesized: [9- $[\alpha,\alpha\text{-}^2\text{H}_2]$ glycinamide,8-arginine]vasopressin (**15**), [1-hemi- $[\alpha\text{-}^2\text{H}_1]$ cystine,8-arginine]vasopressin (**21**), [1-hemi $[\beta,\beta\text{-}^2\text{H}_2]$ cystine,8-arginine]vasopressin (**17**), [2- $[\alpha\text{-}^2\text{H}_1]$ tyrosine,8-arginine]vasopressin (**19a**), [2- $[\alpha,\beta,\beta\text{-}^2\text{H}_3]$ tyrosine,8-arginine]vasopressin (**20**), [3- $[\alpha\text{-}^2\text{H}_1]$ phenylalanine,8-arginine]vasopressin (**18a**), [1-hemi-D- $[\alpha\text{-}^2\text{H}_1]$ cystine,8-arginine]vasopressin (**16**), [2-D- $[\alpha\text{-}^2\text{H}_1]$ tyrosine,8-arginine]vasopressin (**19b**), [1-hemi-D-cystine,3- $[\alpha\text{-}^2\text{H}_1]$ phenylalanine,8-arginine]vasopressin (**18b**).

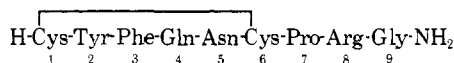
The use of fully deuterated and specifically deuterated amino acids, peptides, and proteins for a variety of physical-chemical⁵⁻¹⁴ and biological¹⁵⁻¹⁷ studies has become increasingly prevalent during the past several years. However, the limited availability of these compounds is still a serious limitation to their use. The principal source of deuterated amino acids has been proteins obtained from algae grown in D₂O solutions.^{6a,b,c} In general, the amino acids obtained from these sources are perdeuterated in all nonexchangeable positions. Furthermore a number of amino acids are not obtained or are obtained in small quantities. In addition, for many purposes it is necessary to have available specific partially deuterated derivatives. For the latter purposes, simple synthetic methods in which the appropriate deuterium label is retained throughout to give the desired labeled derivative are needed. Some examples of the latter have appeared in the literature¹⁵⁻¹⁹ but much remains to be done.

In this paper we report simple, high-yield syntheses of $[\alpha\text{-}^2\text{H}_1]$ tyrosine, $[\alpha,\beta,\beta\text{-}^2\text{H}_3]$ tyrosine, and $[\alpha\text{-}^2\text{H}_1]$ phenylalanine, resolution of the labeled enantiomers, and incorporation of these derivatives and specific deuterium labeled *S*-benzylcysteine and glycine into [8-arginine]vasopressin. In addition,

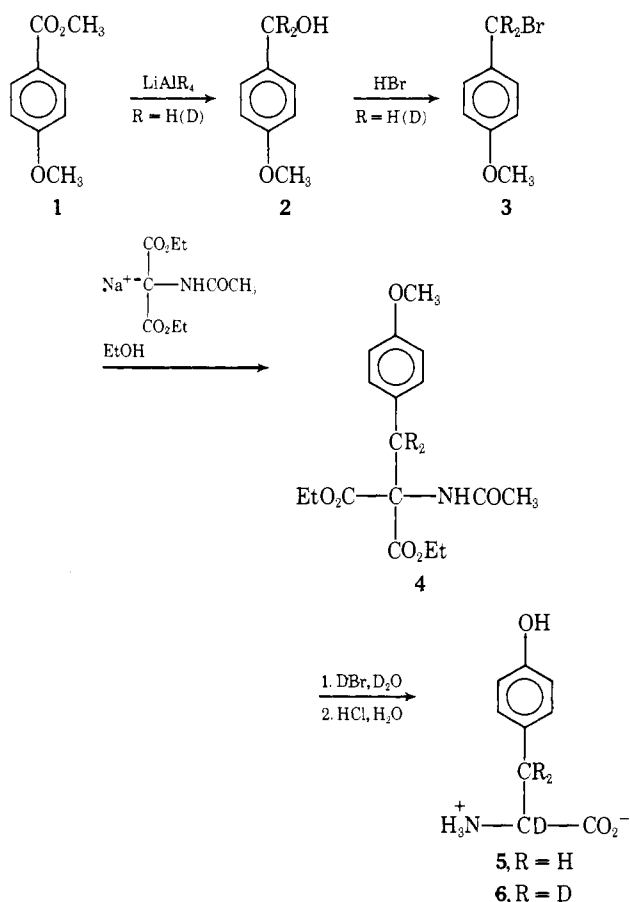
we report the development of a solvent system for partition chromatography which permits separation of [8-arginine]vasopressin (AVP) diastereomers by partition chromatography on Sephadex and the use of recent improvements in synthetic peptide chemistry which substantially improve the overall yields of AVP from those previously reported in the literature.

The syntheses of DL- $[\alpha\text{-}^2\text{H}_1]$ tyrosine (**5**) and DL- $[\alpha,\beta,\beta\text{-}^2\text{H}_3]$ tyrosine (**6**) were accomplished by the procedures outlined in Scheme I. To obtain **5**, methyl *p*-anisate (**1**) was treated with lithium aluminum hydride,²⁰ and the alcohol **2** was converted to **3** with hydrobromic acid. The benzyl bromide **3** was reacted with sodium diethyl acetamidomalonate in ethanol to give the condensation product **4** which was hydrolyzed with deuterium bromide to give DL- $[\alpha\text{-}^2\text{H}_1]$ tyrosine (**5**). To obtain **6**, lithium aluminum deuteride was used in the first step of the scheme. The overall yield of **5** and **6** from **1** was about 65%.

The deuterated DL-tyrosine derivatives **5** and **6** were readily resolved into their enantiomers without loss of label by *N*-trifluoroacetylation,^{21a} followed by reaction with carboxypeptidase A-DFP.^{21b} The resulting deuterated L-amino acids (or the D- or DL-amino acids) could be readily converted to *N-tert*-butyloxycarbonyl (*N*-Boc) derivatives using the general method employing *tert*-butyl azidoformate.²² No detectable loss of label occurred during either the resolution or the preparation of the *N*-Boc derivatives.



Scheme 1



DL- $[\alpha\text{-}^2\text{H}_1]$ Phenylalanine (**11**) was prepared by hydrolysis of diethyl 2-benzyl-2-acetamidomalonate²³ (**10**) in 11 M DCl in D_2O . The compound was resolved by N acetylation, followed by reaction with hog renal acylase.²⁴ The L enantiomer was converted to the *N*-Boc derivative with *tert*-butyl azidoformate.²² All of the deuterated amino acids or *N*-Boc derivatives were deuterio substituted to >95% at the desired position(s) in the amino acids as determined by nuclear magnetic resonance spectral analysis and/or by elemental deuterium analysis.

The syntheses of the partially deuterated [8-arginine]vasopressin (AVP) derivatives were accomplished using the solid phase method²⁵ modified to maximize the yield of the derivatives. The syntheses were carried out using an automated Vega Model 95 synthesizer, a solid state version of our automated instrument,²⁶ or a semiautomated instrument constructed in our laboratory. The method generally used for coupling each amino acid residue to the growing peptide chain is shown in Table I. The major exception to these procedures was in the coupling of the specifically deuterated amino acids Boc-*S*-benzyl-D $[\alpha\text{-}^2\text{H}_1]$ cysteine,^{19d} Boc-*S*-benzyl $[\beta,\beta\text{-}^2\text{H}_2]$ cysteine,^{19d} Boc $[\alpha\text{-}^2\text{H}_1]$ phenylalanine, Boc $[\alpha\text{-}^2\text{H}_1]$ tyrosine, Boc-DL- $[\alpha\text{-}^2\text{H}_1]$ tyrosine, and Boc $[\alpha,\beta,\beta\text{-}^2\text{H}_3]$ tyrosine, in which either a single 90-min coupling with a two-fold excess of the amino acid was used or two 20-min couplings using a total of 1.5–2 equiv of amino acid were used. Each coupling was monitored by the ninhydrin test.²⁷ Boc $[\alpha,\alpha\text{-}^2\text{H}_2]$ glycinate-resin was prepared as previously reported.^{8g}

Previous solid phase syntheses of AVP have been reported,^{28–30} but in all cases the overall yields were low, with the most recent³⁰ and best yield synthesis proceeding in about 11% overall yield.³¹ We have significantly improved the overall yield by the following changes in the syntheses: (a) use of the 3,4-dimethylbenzyl group for protection of sulfhydryl groups (this group has been found to be very stable to the trifluo-

roacetic acid often used in solid phase synthesis,³² but easily removed by sodium in liquid ammonia³³); (b) use of 1-hydroxybenzotriazole^{19d,34} to catalyze the nitrophenyl ester coupling of Boc-Asn-ONP and Boc-Gln-ONP to the growing peptide chain (the reaction is completed in 3–6 h instead of the 8–15 h required without catalyst); (c) use of lower concentrations of trifluoroacetic acid solution³⁵ for removing the *N* α -Boc protecting groups than is often used (using radio-labeled peptides, we have found³⁶ that peptide loss from the resin during solid phase peptide synthesis is significantly reduced under the conditions used here and the *N* α -Boc group is removed); (d) use of subequivalent amounts of DCC relative to Boc-amino acid during DCC coupling to suppress inactivation of the acylating species by excess DCC;³⁷ (e) use of a nitrogen atmosphere before and during the sulfhydryl oxidation step³⁸ to suppress dimer and oligomer formation. The average overall yield, based on starting Boc-glycinate-resin, for the syntheses of the purified specifically labeled [8-arginine]vasopressin compounds using all of these improvements was about 30%. The best yield was 43% for preparation of [1-hemi $[\alpha\text{-}^2\text{H}_1]$ cystine,8-arginine]vasopressin (**21**); the lowest yield was 26% for the preparation of [2- $[\alpha\text{-}^2\text{H}_1]$ tyrosine,8-arginine]vasopressin (**19a**). The other AVP derivatives prepared were the following: [9- $[\alpha,\alpha\text{-}^2\text{H}_2]$ glycinamide,8-arginine]vasopressin (**15**), [1-hemi $[\beta,\beta\text{-}^2\text{H}_2]$ cystine,8-arginine]vasopressin (**17**), [1-hemi-D- $[\alpha\text{-}^2\text{H}_1]$ cystine,8-arginine]vasopressin (**16**), [3- $[\alpha\text{-}^2\text{H}_1]$ phenylalanine,8-arginine]vasopressin (**18a**), [1-hemi-D-cystine,3- $[\alpha\text{-}^2\text{H}_1]$ phenylalanine,8-arginine]vasopressin (**18b**), [2-D- $[\alpha\text{-}^2\text{H}_1]$ tyrosine,8-arginine]vasopressin (**19b**), and [2- $[\alpha,\beta,\beta\text{-}^2\text{H}_3]$ tyrosine,8-arginine]vasopressin (**20**).

The purification of the vasopressin derivatives was accomplished using partition chromatography³⁹ on Sephadex G-25.⁴⁰ The solvent system generally used for purification of labeled AVP derivatives containing only L-amino acids (R_f 0.23) was 1-butanol-ethanol-pyridine-0.1 N acetic acid (4:1:1:7). However, we desired to prepare diastereomeric [8-arginine]vasopressin derivatives containing D amino acid residues in various positions in the molecule and also wished to use labeled DL-amino acids in the synthesis of vasopressin and to separate the diastereomeric peptides after the synthesis. The solvent system mentioned above gave only partial separation of [1-hemi-D- $[\alpha\text{-}^2\text{H}_1]$ cystine,8-arginine]vasopressin (**16**, R_f 0.34) from side products (probably dimers) formed during the reaction, though a good separation from the all L diastereomer (R_f 0.23) was indicated by the differences in the R_f values (see Experimental Section). Therefore we looked for a solvent system that would allow a clean separation of the diastereomeric peptides, as well as a separation of the D-amino acid-containing diastereomer from side products.

Eight different solvent systems were tested (Table II), and the *K* values (partition coefficients) determined for **16** in each of the solvent systems. The expected R_f value of the peptide in each solvent system was estimated from the relation

$$R_f = 1/[1 + V_s/V_H(1/K)]$$

where *K* is the ratio of the amount of peptide material found in the organic layer to that found in the aqueous layer, V_H is the hold-up volume (the eluent volume required for the solvent front to emerge from the column), and V_s is the volume of the stationary phase in the column.⁴¹ The results are shown in Table II.

Solvent system 1 is that used previously as discussed above, and the calculated R_f (0.33 to 0.28) is in good agreement with that found (R_f 0.34, see Experimental Section), suggesting that the other calculated values for the various solvent systems tested should be good indicators of the R_f value obtained on partition chromatography. Since a gel filtration effect in which

Table I

Step	Normal DCC coupling			Nitrophenyl ester coupling (Asn and Gln)		
	Solvent or reagent	Duration, min	No. of times	Solvent or reagent	Duration, min	No. of times
1	CH ₂ Cl ₂	1	4	CH ₂ Cl ₂	1	4
2	TFA-CH ₂ Cl ₂ -anisole 25:73:2	2	1	TFA-CH ₂ Cl ₂ -anisole 25:73:2	2	1
3	TFA-CH ₂ Cl ₂ -anisole 25:73:2	20	1	TFA-CH ₂ Cl ₂ -anisole 25:73:2	20	1
4	CH ₂ Cl ₂	1	3	CH ₂ Cl ₂	1	3
5	DIEA-CH ₂ Cl ₂ 10:90	2	2	DIEA-CH ₂ Cl ₂ 10:90	2	2
6	CH ₂ Cl ₂	1	4	CH ₂ Cl ₂	1	4
7	Boc-amino acid in CH ₂ Cl ₂ (1.5 equiv)		1	DMF	1	5
8	DCC (1.2 equiv) in CH ₂ Cl ₂	20	1	Boc-amino acid-ONP in DMF (4 equiv) (4 equiv HOBT)	1 180-300	5 1
9	CH ₂ Cl ₂	1	2			
10	EtOH	1	2			
11	CH ₂ Cl ₂	1	2			
12	Boc-amino acid (1.5 equiv) in CH ₂ Cl ₂ (1.5 equiv)		1			
13	DCC (1.2 equiv) in CH ₂ Cl ₂	20	1			
14	CH ₂ Cl ₂	1	2	DMF	1	3
15	EtOH	1	3	EtOH	1	3

Table II. Partition Coefficients of [1-Hemi-D-[α -²H₁]cystine,8-arginine]vasopressin in Several Solvent Systems

System no.	Solvent system (vol/vol ratios) ^a	Aqueous phase pH
1	BuOH:EtOH:Pyr:0.1 N HOAc (4:1:1:7)	6.0
2	BuOH:H ₂ O(3.5% HOAc in 1.5% Pyr) (1:1)	4.5
3	BuOH:EtOH:H ₂ O(3.5% HOAc in 1.5% Pyr) (4:1:5)	4.6
4	BuOH:EtOH:C ₆ H ₆ :Pyr:0.1 N HOAc (4:1:1:1:7)	5.8
5	BuOH:C ₆ H ₆ :H ₂ O(3.5% HOAc in 1.5% Pyr) (3:1:4)	4.5
6	<i>i</i> -BuOH:H ₂ O(3.5% HOAc in 1.5% Pyr) (1:1)	4.2
7	<i>i</i> -BuOH:EtOH:H ₂ O(3.5% HOAc in 1.5% Pyr) (5:2:7)	4.5
8	<i>i</i> -BuOH:EtOH:H ₂ O(NH ₄ ⁺ CH ₃ COO ⁻) (5:2:7)	9.0

	<i>K</i> , organic/aqueous	Calcd <i>R_f</i> range
1	0.755	0.33-0.28
2	0.214	0.12-0.09
3	0.421	0.22-0.17
4	0.288	0.16-0.12
5	0.083	0.05-0.04
6	0.125	0.08-0.06
7	0.484	0.24-0.19
8	0.683	0.32-0.25

^a Solvent abbreviations are: BuOH, 1-butanol; EtOH, ethanol; Pyr, pyridine; *i*-BuOH, 2-methylpropanol.

larger molecules are less retarded than are smaller molecules is probably also operative (along with the liquid-liquid partitioning effect) in partition chromatography on Sephadex G-25, it seemed reasonable to assume that a solvent system which retarded AVP would retard dimers and higher oligomers to a lesser extent. Such a system is precisely what is needed to purify the diastereomer containing the D amino acid residue. On the other hand, it is not desirable to have an excessively low *R_f* value since diffusion would become significant and the degree of resolution would be reduced. In addition, since we wished

to be able to separate diastereomeric AVP mixtures, and the all-L diastereomer was expected to have a smaller *R_f* than the diastereomer containing the D amino acid residue in analogy with system 1, this also argued against choice of a system with a low *R_f*. When all these factors were considered, solvent systems 3 and 7 of Table II seemed most promising. Solvent system 3 was chosen and found to effect a clean separation of **16** (*R_f* 0.17) from dimers and other by-products. When a preparation of the corresponding all-L diastereomer **21** was purified using solvent system 3, the peptide eluted at *R_f* 0.12-0.11, with virtually no overlap in the area in which the *R_f* 0.17 peak for the D-amino acid-containing diastereomer had been found. Thus solvent system 3 appeared to be capable of resolving diastereomeric mixtures of [1-hemi-DL-cystine,8-arginine]vasopressin.

To test this capability, [1-hemi-DL-cystine,3-L-[α -²H₁]phenylalanine,8-arginine]vasopressin (**18**) was synthesized and purified by partition chromatography using solvent system 3. Analysis of the eluate revealed a clean separation of the diastereomers (Figure 1) from each other and from dimers and other by-products of the synthesis (see Experimental Section). We were also able to use the solvent system 3 to separate [2-[α -²H₁]tyrosine,8-arginine]vasopressin (**19a**) (*R_f* 0.12) and [2-D-[α -²H₁]tyrosine,8-arginine]vasopressin (**19b**) (*R_f* 0.19) from a diastereomeric mixture of the two compounds. This solvent system should prove to be especially useful in preparing various derivatives (¹⁴C, ¹³C, ²H, etc.) of AVP by synthesis without the need to resolve precious labeled enantiomeric amino acids before incorporating them into the growing chain. This is also of considerable importance since the D-amino acid-containing diastereomer often has interesting physical and biological properties of its own.

Experimental Section

Capillary melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on silica gel G plates using the following solvent systems: (A) 1-butanol-acetic acid-water (4:1:5, upper phase only); (B) 1-butanol-acetic acid-pyridine-water (15:3:10:12); (C) 1-pentanol-pyridine-water (35:35:30); (D) ethyl acetate-pyridine-acetic acid-water (5:5:1:3). Optical rotation values were measured at the mercury green line (547 nm) using a Zeiss Old 4 polarimeter. Elemental analyses were performed by Spang Microanalytical Laboratory or Heterocyclic Chemical Corp., and deuterium analyses

were performed by Joseph Nemeth, Urbana, Illinois. Amino acid analyses were obtained by the method of Spackman, Stein, and Moore⁴² on a Beckman 120C amino acid analyzer after hydrolysis in 6 N HCl for 22 h. Nuclear-magnetic resonance (NMR) spectra were obtained using a Varian T-60 NMR spectrometer or a Bruker WH-90 NMR spectrometer. *N*-Boc-*S*-benzyl[α -²H₁]cysteine, *N*-Boc-*S*-benzyl-D-[α -²H₁]cysteine, and *N*-Boc-*S*-benzyl[β , β -²H₁]cysteine were prepared according to the method of Upson and Hruby.^{19d} *N*-Boc[α , α -²H₂]glycine was prepared as previously reported.^{8g} *N*-Boc protected amino acids and amino acid derivatives were purchased from Fox Chemical Co. and from Biosynthetica or were prepared by published procedures except as discussed below.

***p*-Methoxy[α , α -²H₂]benzyl Alcohol (2).** Following the procedure of Achenbach and Konig,²⁰ 72 g (0.43 mol) of methyl *p*-anisate and 10.0 g (0.24 mol) of lithium aluminum deuteride (Merck Sharp and Dohme, 99% D) gave 58.9 g (97%) of the title compound **2**: bp 120–122 °C (10 mm Hg) [lit.²⁰ bp 137.5 °C (17 mm Hg)]; NMR (neat) δ 3.5 (s, 3 H), 4.35 (absent), 4.9 (s, 1 H), 6.9 (d of d, 4 H).

***p*-Methoxy[α , α -²H₂]benzyl Bromide (3).** The title compound was prepared from 58.9 g (0.42 mol) of *p*-methoxy[α , α -²H₂]benzyl alcohol according to published procedures²⁰ to give 72.7 g (85%) of **3**. The compound was immediately used for the preparation of the malonate derivative **4**.

Diethyl 2-Acetamido-2-*p*-methoxy[α , α -²H₂]benzylmalonate (4b). To a sodium ethoxide solution prepared from 1.32 g of sodium and 160 ml of absolute ethanol was added 13.6 g (63 mmol) of diethyl acetamidomalonnate. The solution was cooled in an ice bath, and 11.7 g (58 mmol) of **3** was slowly dripped in. After stirring for 5 h, 600 ml of water was added to precipitate the product which was filtered and dried to give 16.3 g (83%) of the title compound **4b** as white shiny crystals: mp 94.5–95.5 °C; NMR (CDCl₃) δ 1.3 (t, 6 H), 2.0 (s, 3 H), 3.6 (absent), 3.8 (s, 3 H), 4.3 (q, 4 H), 6.8 (d of d, 4 H).

Diethyl 2-Acetamido-2-*p*-methoxybenzylmalonate (4a). The title compound was prepared from 45.5 g (0.21 mol) of diethyl acetamidomalonnate and 39.25 g (0.20 mol) of *p*-methoxybenzyl bromide in the same manner as for **4b** to give 56.4 g (86%) of **4a**: mp 96–98 °C; NMR (CDCl₃) δ 1.25 (t, 6 H), 2.0 (s, 3 H), 3.6 (s, 2 H), 3.8 (s, 3 H), 4.25 (q, 4 H), 6.8 (d of d, 4 H).

DL-[α -²H₁]Tyrosine (5). To 75 ml of deuterium oxide-*d*₂ (99.8% D, Stohler Isotope Chemicals) cooled in an ice bath was added 22.5 ml of practical grade phosphorus tribromide.¹⁸ The solution was allowed to warm to room temperature and stirring was continued until the solution was homogeneous. To the DBr solution was added 14.18 g of diethyl 2-acetamido-2-*p*-methoxybenzylmalonate (**4a**), and the solution was refluxed for 2 h. The solution was concentrated in vacuo, the residue was taken up in water, and the solution was decolorized with Norite and filtered through Celite. The pH was adjusted to 5 with concentrated ammonium hydroxide, the mixture was cooled, and the produce was filtered off and dried. The NMR spectrum of this compound showed deuteration in the positions ortho to the methoxyl group. Back exchange was achieved by taking the product up in 150 ml of 2 N HCl and refluxing for 37 h. The solution was concentrated in vacuo; a sample from the residue showed the expected A₂B₂ splitting pattern in the NMR (D₂O). The residue was slurried in water and the pH adjusted to 6 with ammonium hydroxide. After cooling in the refrigerator, the flocculent white precipitate was filtered and dried to afford 7.2 g (94%) of DL-[α -²H₂]tyrosine (**5**): NMR (CF₃CO₂H) δ 3.3 (d of d, 2 H), 4.3–4.8 (α -CH, peak absent), 7.0 (d of d, 4 H); deuterium analysis indicated 98.5% deuteration (calcd, 9.09 atom %; found, 8.96 atom %).

Resolution of DL-[α -²H]Tyrosine (5). DL-[α -²H₁]Tyrosine (**5**) (11.5 g, 63 mmol) was dissolved in 60 ml of trifluoroacetic acid. To the solution cooled in an ice bath was added 35 ml of anhydrous ether and 15 ml of trifluoroacetic anhydride (distilled from P₂O₅). After stirring for 2 h the solution was concentrated in vacuo. Water (2.5 ml) was added to the residue which was then boiled for 5 min on a steam bath to give a clear solution which was concentrated in vacuo. The residue was slurried in ether, and the unreacted trifluoroacetate salt of tyrosine (200 mg) was removed by filtration. The filtrate was concentrated in vacuo, and crystallization from ether/toluene afforded 15.25 g (88%) of *N*-trifluoroacetyl-DL-[α -²H₁]tyrosine (**7**) as fine, white needles, mp 188–190 °C (lit.⁴³ mp protio derivative, 192.5–193.5 °C). A sample of **7** (15 g, 54 mmol) was taken up in 500 ml of water, the pH was adjusted to 8.5 with 2 N LiOH, and the solution was stirred at 37 °C. Carboxypeptidase A-DFP (63 mg, 45 U/mg) was added. After 24 h the pH was adjusted to 5.5 with glacial acetic acid and

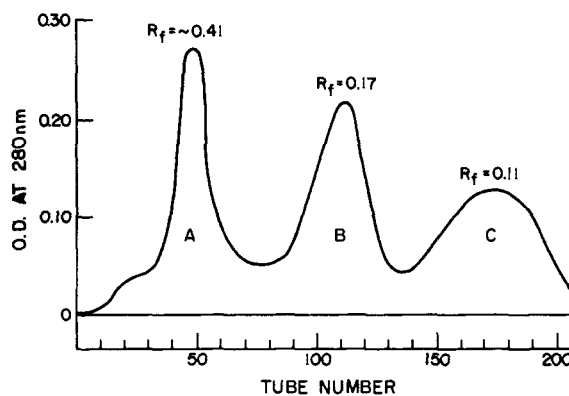


Figure 1. Partition chromatography of [1-hemi-DL-cystine,3-[α -²H₁]phenylalanine,8-arginine]vasopressin (**18**) on Sephadex G-25 using the solvent system 1-butanol-ethanol-H₂O (containing 3.5% HOAc and 1.5% pyridine) (4:1:5). C is [3-[α -²H₁]phenylalanine,8-arginine]vasopressin (**18a**), B is [1-hemi-D-cystine,3-[α -²H₁]phenylalanine,8-arginine]vasopressin (**18b**), and A is dimers, polymers, and other by-products of reaction.

cooled in the refrigerator. The collected precipitate, a mixture of L-[α -²H₁]tyrosine (**5a**) and enzyme, was taken up in 1 N HCl. The solution was treated with Norite, filtered through Celite, adjusted to pH 5 with concentrated NH₄OH, cooled, and filtered. The Norite treatment was repeated to afford 4.27 g (87%) of [α -²H₁]tyrosine: [α]²¹₅₄₇ -11.3° (c 2.04, 1 N HCl); TLC in solvent systems A and B showed single spots identical with authentic L-tyrosine. The aqueous mother liquors containing the *N*-trifluoroacetyl-D-[α -²H₁]tyrosine (**7b**) was adjusted to pH 3 with 2 N HCl and then concentrated in vacuo. The residue was dissolved in ether, and toluene was added until the solution was slightly cloudy. The crystals of *N*-trifluoroacetyl-D-[α -²H₁]tyrosine (**7b**) (4.54 g, 60%) were filtered off and dried in vacuo. The trifluoroacetyl group was removed by refluxing for 2 h in 50 ml of 3 N HCl. The solution was decolorized with Norite, filtered through Celite, adjusted to pH 5 with NH₄OH, cooled, filtered, and the D-[α -²H₁]tyrosine (**5b**) was dried to afford 2.80 g (94%): [α]²¹₅₄₇ +11.4° (c 2.04, 1 N HCl); TLC in solvent systems A and B showed single spots with *R_f* values identical with authentic tyrosine.

DL-[α , β , β -²H₃]Tyrosine (6). To 40 ml of D₂O cooled in an ice bath was added 12 ml of phosphorus tribromide. The mixture was stirred for 1 h and 7.72 g (22.8 mmol) of diethyl 2-acetamido-2-*p*-methoxy[α , α -²H₂]benzylmalonate (**4b**) was added. The solution was refluxed for 1.5 h and concentrated in vacuo. The residue was slurried in water and the mixture adjusted to pH 6 using concentrated ammonium hydroxide. After cooling in the refrigerator, the white solid was filtered off. In order to back exchange the 3',5' aromatic deuteriums, the precipitate was taken up in 75 ml of 2 N HCl and refluxed for 36 h. The product was concentrated in vacuo, slurried in water, decolorized with Norite, and adjusted to pH 6 with NH₄OH. After cooling the product was filtered off and dried to afford 3.81 g (92%) of DL-[α , β , β -²H₃]tyrosine (**6**). Deuterium analysis indicated 94% deuteration (calcd, 27.27 atom %; found, 25.60 atom %).

Resolution of DL-[α , β , β -²H₃]Tyrosine (6). The resolution of the title compound was carried out in a manner identical with that for **5**. From 18.1 g of **6** there was obtained 6.44 g of the L isomer **6a** and 6.0 g of the D isomer **6b**. The compounds had identical properties with the corresponding isomers from **5**.

***N*-Boc-DL-[α -²H₁]tyrosine (8).** DL-[α -²H₁]Tyrosine (**5**) (2.0 g, 1.1 mmol) was slurried in 22 ml of peroxide-free dioxane and 22 ml of H₂O. *tert*-Butyl azidoformate (1.77 g, 1.25 mmol) was added, and the pH was kept at 10.0 using 4.0 N NaOH (Radiometer autotitrator).²² After 36 h the unreacted tyrosine was filtered off, and the filtrate was washed with two 75-ml portions of ether. The aqueous phase was adjusted to pH 3.1 with citric acid and then extracted with three 75-ml portions of ether and 75 ml of ethyl acetate. The combined organic phases were dried over anhydrous sodium sulfate, concentrated in vacuo, and the residue crystallized from a small amount of ethyl acetate to give 2.74 g (86%) of *N*-Boc-DL-[α -²H₁]tyrosine (**8**): mp 126–129 °C (lit.⁴⁴ mp 136–138 °C, lit.²² mp 96–98 °C); TLC in solvent systems A and B gave single spots at an *R_f* identical with authentic *N*-Boc-tyrosine (Fox Chemical).

The *tert*-butyloxycarbonyl derivatives of the other deuterated tyrosine derivatives **5a**, **5b**, **6a**, **6b**, and **6** were prepared in a similar manner to give respectively **8a**, **8b**, **9a**, **9b**, and **9**.

DL- $[\alpha\text{-}^2\text{H}_1]$ Phenylalanine (11). A solution of 6.14 g (0.02 mol) of diethyl 2-benzyl-2-acetamidomalonate²³ (**10**) in 11 M DCl in D₂O (made by adding 13 ml of freshly distilled SOCl₂ dropwise to 31.3 ml of cold D₂O) was heated at reflux for 6 h. The mixture was cooled, and the solvents were removed by rotary evaporation in vacuo. The residue was dissolved in 10 ml of H₂O and neutralized with concentrated NH₄OH. The mixture was cooled at 5 °C overnight, the precipitate filtered off, and the product dried to give 2.76 g (83.5%) of **11**: mp 250–255 °C (lit.⁴⁵ mp 271–273 °C for protio analogue); NMR (CF₃CO₂H) δ 6.90 (s, 5 H), 4.1–4.5 (α -CH, undetectable), 3.00 (q, 2 H).

$[\alpha\text{-}^2\text{H}_1]$ Phenylalanine (11a). DL- $[\alpha\text{-}^2\text{H}_1]$ Phenylalanine (2.5 g, 15 mmol) was refluxed for 2 min with a mixture of 29 ml of acetic anhydride and 4.2 ml of D₂O. The solvents were removed, and the residue was recrystallized from ethyl acetate to give 2.67 g (86%) of *N*-acetyl-DL- $[\alpha\text{-}^2\text{H}_1]$ phenylalanine (**12**), mp 142.5–144 °C (lit.⁴⁶ mp for protio derivative, 146 °C). A 2.50-g (12 mmol) portion of **12** was slurried in 87 ml of H₂O, and the pH was adjusted to 7.1 with 2 N LiOH. The final volume was adjusted to 120 ml by addition of H₂O, 65 mg of acylase I (hog kidney, Calbiochem) was added, and the mixture was stirred 2 days at 38 °C. Another 38 mg of enzyme was added, and the mixture was stirred 2 days more. The mixture was cooled at 20 °C and the pH lowered to 5.0 with HOAc. The precipitate was filtered off, and the solution was concentrated to about 10 ml by rotary evaporation in vacuo. The solution was cooled at 5 °C overnight and the precipitate was filtered off. The filtrate was concentrated to about 6 ml, chilled overnight at 5 °C, and the precipitate filtered off. The combined crops of **11a** weighed 0.40 g (40%): mp 240–245 °C; TLC in solvent system A, single spot identical with authentic L-phenylalanine; NMR (CF₃CO₂H) δ 6.90 (s, 5 H), 4.1–4.5 (α -CH, undetectable), 3.00 (q, 2 H).

***N*-Boc $[\alpha\text{-}^2\text{H}_1]$ Phenylalanine (13)**. The title compound was prepared using the method of Schnabel,²² using 0.40 g (2.4 mmol) of **11a** and 0.50 g (3.5 mmol) of *tert*-butyl azidoformate. There was obtained 0.46 g (83%) of **13**: $[\alpha]^{25}_{547} +28.8^\circ$ (*c* 0.92, EtOH), (authentic *N*-Boc-phenylalanine) $[\alpha]^{25}_{547} +30.3^\circ$ (*c* 1.03, EtOH); NMR (CDCl₃) δ 11.35 (s, 1 H), 7.40 (s, 5 H), 5.30 (s, broad, 1 H), 4.5–4.9 (α -CH, undetectable), 3.25 (q, 2 H), 1.55 (s, 9 H).

Solid Phase Synthesis of Cys(DMB)-Tyr(Bzl)-Phe-Gln-Asn-Cys(DMB)-Pro-Arg(Tos) $[\alpha\text{-}^2\text{H}_1]$ Gly-NH₂ (14). The solid phase synthesis was carried out on a Vega Series 95 automated synthesizer, a machine similar to that described by Hruby et al.²⁶ using 4.3 g of Boc $[\alpha\text{-}^2\text{H}_1]$ glycinate-resin [prepared from polystyrene resin (1% cross-linked with divinylbenzene and chloromethylated to an extent of 1.07 mmol/g) and Boc $[\alpha\text{-}^2\text{H}_1]$ glycine as described previously^{8b}] which was substituted with the labeled amino acid to an extent of 0.23 mmol/g of resin. Removal of the *N*-Boc protecting group, neutralization of the peptide resin salt, and addition of the next amino acid residue followed the program listed in Table I except that the coupling steps were condensed to a single 90-min coupling with a threefold excess of protected amino acid and DCC. For the active ester couplings of asparagine and glutamine, an equivalent molar amount of 1-hydroxybenzotriazole was added to the reaction mixture as a catalyst. The ninhydrin test²⁷ indicated that coupling was complete after 3.5 h and 5 h for asparagine and glutamine derivatives, respectively. *N*-*tert*-Butyloxycarbonyl protection was used throughout. The 3,4-dimethylbenzyl group^{32,33} was used to protect the sulfhydryl groups of cysteine; the tyrosine hydroxyl group was benzyl protected; the guanidyl group of arginine was tosyl protected. After each amino acid residue was coupled, the synthesis was monitored for completion of coupling by use of the ninhydrin test.²⁷ A negative test (>99.4% reaction) was indicated at each step. The total volume of solvent or solution used at each washing or reaction was 30 ml. At the completion of the synthesis the N-terminal Boc protecting group was removed by repeating steps 1–5 of Table I, and the resin was dried in vacuo.

The protected peptide was cleaved from the resin by stirring in 100 ml of freshly prepared anhydrous methanol saturated at –5 °C with anhydrous ammonia (freshly distilled from sodium). The flask was wired shut and stirred at room temperature for 3 days in a desiccator. The solvents were removed by rotary evaporation in vacuo, and the peptide extracted from the resin with two 50-ml portions of DMF at 40 °C. The DMF solution was evaporated down to about 15 ml in vacuo, ether was added, the precipitate was filtered off, and the

product was reprecipitated from acetic acid–ethanol and a small amount of H₂O to give 600 mg (60%) of **14** as white powder: mp 204–207 °C dec; $[\alpha]^{24}_{547} -40.4^\circ$ (*c* 0.55, DMF). Anal. Calcd for C₇₈H₉₇D₂O₁₄N₁₅S₃H₂O: C, 59.04; H, 6.41; N, 13.16. Found: C, 59.26; H, 6.56; N, 13.13.

$[\alpha\text{-}^2\text{H}_1]$ Glycinamide,8-arginine]vasopressin (15). A sample of 200 mg (0.15 mmol) of the protected nonapeptide **14** was dissolved in 100 ml of anhydrous ammonia (freshly distilled from sodium) and treated with a sodium stick until a blue color persisted for 30–45 s. The ammonia was removed by evaporation under a slow stream of nitrogen³⁸ and the last 10 ml by lyophilization. The white powder was dissolved in 300 ml of deaerated 0.1% aqueous HOAc under N₂. The pH was adjusted to 8.5 with 3 N NH₄OH, and the deprotected peptide was oxidized by stirring with 50 ml of 0.01 N K₃Fe(CN)₆⁴⁷ for 30 min. The pH was readjusted to about 4 with 10% aqueous HOAc, and the ferro- and excess ferricyanide ions were removed by addition of Rexyn 203 (Cl[–] form). After 20 min, the resin was removed by filtration and washed with three 25-ml portions of 20% aqueous HOAc. About 50 ml of 1-butanol was added to the combined aqueous solutions, and the solution was concentrated at 30 °C to about 175 ml by rotary evaporation. The solution was lyophilized, and the powder was dissolved in 4 ml of the upper phase and 2 ml of the lower phase of the solvent system 1-butanol–ethanol–pyridine–0.1 N HOAc (4:1:1:7) and subjected to partition chromatography on a 2.8 × 60 cm column of Sephadex G-25 (block polymerizate, 100–200 mesh) which had been equilibrated with the upper and lower phases according to the method of Yamashiro.³⁹ The fractions corresponding to the product (*R_f* 0.23) were pooled and isolated and further purified by gel filtration⁴⁰ on Sephadex G-25 using 0.2 N HOAc as the eluent solvent. The fractions corresponding to the major peak were pooled and lyophilized to give **15** as a white powder, 55 mg (35%): $[\alpha]^{25}_{547} -23.4^\circ$ (*c* 0.31, 0.1 N HOAc) (lit.²⁹ $[\alpha]^{22}_{\text{D}} -22^\circ$ (*c* 0.22, 1 N HOAc). Anal. Calcd for C₄₆H₆₃D₂O₁₂N₁₅S₂H₂O·1.5 C₂H₄O₂: C, 49.27; H, 6.17; N, 17.59. Found: C, 49.13; H, 6.06; N, 17.64. The peptide gave single spots on TLC in the solvent systems A (*R_f* 0.09), B (*R_f* 0.58), and D (*R_f* 0.70) identical with authentic AVP. Detection of peptides on TLC was by UV, ninhydrin, iodine vapors, and fluorescamine. Amino acid analysis gave the following molar ratios: Asp, 0.94; Arg, 1.0; Glu, 1.0; Gly, 0.92; half-Cys, 2.1; Pro, 1.1; Tyr, 1.0; Phe, 1.0. The milk-ejecting activity⁴⁸ of **15** was 110 ± 22 U/mg, identical with that of authentic AVP prepared by the method of Meienhofer et al.²⁹

[1-Hemi-D- $[\alpha\text{-}^2\text{H}_1]$ cysteine,8-arginine]vasopressin (16). Solid phase synthesis of the protected nonapeptide was accomplished using 3.23 g of *N*-Boc-glycinate-resin which had a substitution level of 0.32 mmol/g as determined by the modified⁴⁹ aldimine test.⁵⁰ The coupling of the amino acids to the growing peptide was done as outlined in Table I using 25 ml of solvent or reagent solution, except for the addition of Boc-S-benzyl-D- $[\alpha\text{-}^2\text{H}_1]$ cysteine.^{19d} In this case, the first coupling was done with 0.44 g (1.41 mmol) of the amino acid and 0.26 g (1.26 mmole) of DCC, and the second coupling employed 0.22 g of amino acid and 0.13 g of DCC. The hydroxyl group of tyrosine was not protected; all other side chain groups were protected as before. Following removal of the terminal Boc protecting group, the final peptide-resin weight was 4.42 g. The peptide-resin was ammonolyzed and extracted into DMF, and the peptide was dissolved in acetic acid and precipitated with ethanol as described above. There was obtained 840 mg of D- $[\alpha\text{-}^2\text{H}_1]$ Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(DMB)-Pro-Arg-(Tos)-Gly-NH₂, mp 178–179 °C. Addition of ether to the filtrate precipitated an additional 440 mg. A 365 mg (0.30 mmol) portion of the first crop was deprotected with sodium in liquid NH₃ and oxidized with K₃Fe(CN)₆ in the same manner given above. Purification of the crude product by partition chromatography using the solvent system 1-butanol–ethanol–pyridine–0.1 N HOAc (4:1:1:7) was inadequate. Folin–Lowry analysis⁵¹ revealed a large peak at *R_f* 0.34, which represented the desired peptide, but there was significant contamination with by-products (dimers, higher oligomers) as shown by TLC in solvent systems A, B, and D.

To find a more adequate partition system with which to purify the title compound from by-products of the reaction, eight solvent systems (see Table II) were tested. Approximately 0.3 ± 0.1 mg of the impure peptide was shaken for 1 min on a vortex mixer with 0.5 ml of upper and 0.5 ml of lower phase of each of the eight solvent systems. The layers were allowed to equilibrate for 2 h and then 0.25 ml of each layer were withdrawn and transferred to clean test tubes. The solvents were removed in vacuo. Folin–Lowry tests were performed on the residues, and the optical density values were read at 625 nm. Calculations

lation of the partition coefficient K (see above) for each system allowed prediction of approximate R_f values. The solvent system 1-butanol-ethanol-water (3.5% HOAc in 1.5% pyridine) (4:1:5) gave a partition coefficient of 0.421 from which an expected R_f range of 0.22–0.17 was calculated.⁴¹ The crude peptide was dissolved in 3 ml of upper phase and 2 ml of lower phase of this solvent system and applied to a Sephadex G-25 (block polymerizate, 100–200 mesh) column, 2.85 × 60 cm, previously equilibrated with lower and upper phases. One hundred and sixty 3.8-ml fractions were collected. Analysis by the Folin-Lowry method showed a large peak at R_f 0.17, corresponding to the desired peptide. The dimer and other by-products were spread out over the range R_f 0.45 to 0.22. Further purification of the R_f 0.17 peak by gel filtration chromatography gave the product **16** as a white powder, yield 101 mg. The compound gave a single, uniform spot on TLC in solvent systems A (R_f 0.06), B (R_f 0.53), and D (R_f 0.69). The peptides was detected by UV, ninhydrin, iodine vapors, and fluorescamine. Its optical rotation was $[\alpha]^{21}_{547} -71.9^\circ$ (c 0.524, 1 N HOAc). Amino acid analysis gave the following molar ratios: Arg, 1.0; Asp, 1.0; Glu, 1.0; Pro, 1.1; Gly, 1.0; half-Cys, 1.9; Tyr, 0.90; Phe, 1.0. Anal. Calcd for $C_{46}H_{64}DO_{12}N_{15}S_2 \cdot 2C_2H_4O_2 \cdot H_2O$: C, 49.09; H, 6.26; N, 17.18. Found: C, 48.85; H, 5.84; N, 17.55. The D diastereomer **16** had $\frac{1}{15}$ the potency or authentic AVP in the milk-ejecting assay.

[1-Hemi[β,β - 2H_2]cystine,8-arginine]vasopressin (17). The protected nonapeptide precursor of the title compound, [β,β - 2H_2]Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(DMB)-Pro-Arg(Tos)-Gly-NH₂, was prepared by solid phase synthesis as used in the preparation of **16**. After treatment with Na in liquid NH₃ and oxidation as before, purification of the crude product was effected with partition chromatography using the solvent system 1-butanol-ethanol-water (3.5% HOAc in 1.5% pyridine) (4:1:5) using the same methodology as for **16**. The product was obtained as a large symmetrical peak at R_f 0.12. The lyophilized powder was further purified by gel filtration using 0.2 N HOAc as the eluent solvent. The product (**17**) was isolated as a white powder, yield 97 mg; $[\alpha]^{21}_{547} -23.7^\circ$ (c 0.511, 1 N HOAc). The compound gave a single spot on TLC in solvent systems A, B, and D identical with authentic [8-arginine]vasopressin. Amino acid analysis gave the following molar ratios: Arg, 1.0; Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; half-Cys, 2.0; Tyr, 0.91; Phe, 1.0. The compound exhibited a proton-decoupled carbon-13 NMR spectrum identical with authentic [8-arginine]vasopressin except that the carbon-13 peak due to the β carbon of the 1-hemicystine residue was absent.

[1-Hemi-DL-cystine,3-[α - 2H_1]phenylalanine,8-arginine]vasopressin (18) and Separation of the Diastereomers. The protected nonapeptide was made on a 1 mmol scale starting with 3.04 g of Boc-glycinate-resin (substituted at the level of 0.34 mmol/g). The synthesis was carried out in the usual way but with the following changes. Arginine was introduced with tosyl protection on the guanido nitrogen as usual but with the *tert*-amyloxycarbonyl group (Aoc) protecting the α -nitrogen. Both cysteine sulphydryl groups were benzyl protected. Boc-L-[α - 2H_1]phenylalanine was coupled in two stages using 0.85 mmol in each stage along with 0.80 mmol of DCC. The second coupling was shown to be complete after 40 min by the ninhydrin test.

The final peptide-resin weight was 4.20 g. The peptide-resin was ammonolyzed and extracted into DMF, and the peptide was dissolved in acetic acid and precipitated with ethanol as described above. There was obtained 1.15 g (80%) of DL-Cys(Bzl)-Tyr-[α - 2H_1]Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH₂, mp 145–151 °C. A 365-mg portion of the nonapeptide was deprotected and oxidized in the usual manner. The crude product was purified by partition chromatography using the solvent system 1-butanol:ethanol:water (3.5% HOAc in 1.5% pyridine) (4:1:5). Following a 40-ml column forerun, 210 4.2-ml fractions were collected. Analysis of the fractions by direct UV absorbance readings at 280 nm revealed three peaks, a broad by-product peak centered at R_f 0.41, a peak at R_f 0.17 representing the 1-hemi-D-diastereomer **18b**, and a peak at R_f 0.11 representing the 1-hemi-L-diastereomer **18a**. The fractions corresponding to each of the product peaks were separately pooled and lyophilized. The highly purified peptides were each further purified by gel filtration chromatography. There was obtained 63 mg of [3-[α - 2H_1]phenylalanine,8-arginine]vasopressin (**18**); $[\alpha]^{25}_{547} -24.9^\circ$ (c 0.578, 1 N HOAc). Amino acid analysis gave the following molar ratios: Arg, 1.0; Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; half-Cys, 2.0; Tyr, 0.90; Phe, 1.0. There was obtained 46 mg of [1-hemi-D-cystine, 3-[α - 2H_1]phenylalanine,8-arginine]vasopressin (**18b**); $[\alpha]^{25}_{547} -68^\circ$ (c 0.541, 1 N HOAc). Amino acid analysis gave the following molar ratios: Arg,

1.0; Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; half-Cys, 2.0; Tyr, 0.90; Phe, 1.0. Both compounds gave single, uniform spots on TLC in solvent systems A, B, and D identical with authentic [8-arginine]vasopressin and [1-hemi-D-cystine,8-arginine]vasopressin, respectively.

[2-DL-[α - 2H_1]Tyrosine,8-arginine]vasopressin (19) and Separation of the Diastereomers. The synthesis of the protected nonapeptide of the title compound, Cys(DMB)-DL-[α - 2H_1]Tyr-Phe-Gln-Asn-Cys(DMB)-Pro-Arg(Tos)-Gly-NH₂ was carried out by solid phase procedures similar to those used in the synthesis of **14** and outlined in Table I. The *N*^α-Boc-DL-[α - 2H_1]tyrosine was coupled to the growing peptide chain utilizing a single 90-min coupling step with 2 equiv each of the protected amino acid and DCC. The ninhydrin test²⁷ indicated that coupling was complete at that time. The protected nonapeptide was cleaved from the resin in the usual manner and was obtained as a white powder (75%), mp 201–206 °C. The protected nonapeptide was converted to crude **19** in the usual manner. Purification and separation of the diastereomers was accomplished in the same manner as the separation of **18a** and **18b** by partition chromatography in the solvent system 1-butanol-ethanol-water (3.5% HOAc in 1.5% pyridine) (4:1:5). As was the case for **18a** and **18b**, the diastereomers distinctly separated from each other with the 2-D-tyrosine diastereomer **19b** at R_f 0.19 and the 2-L-tyrosine diastereomer (**19a**) at R_f 0.12. The fractions corresponding to each of the product peaks were separately isolated and each were further purified by gel filtration on Sephadex G-25 with 0.2 N HOAc as eluent solvent. Starting from 370 mg (0.25 mmol) of the protected nonapeptide there was obtained 55 mg of [2-L-[α - 2H_1]tyrosine,8-arginine]vasopressin (**19a**); $[\alpha]^{21}_{547} -23.0^\circ$ (c 0.510, 1 N HOAc). Thin-layer chromatography in solvent systems A, B, and D gave single uniform spots identical with authentic AVP. Amino acid analysis gave the following molar ratios: Arg, 1.0; Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; half-Cys, 1.9; Tyr, 0.90; Phe, 1.0. The compound had milk-ejecting activities identical with authentic [8-arginine]vasopressin. There was obtained 50 mg of [2-D-[α - 2H_1]tyrosine,8-arginine]vasopressin (**19b**); $[\alpha]^{21}_{547} -64.3^\circ$ (c 0.50, 1 N HOAc). Thin-layer chromatography in solvent systems A and D gave single uniform spots. Amino acid analysis gave the following molar ratios: Arg, 0.92; Asp, 1.0; Glu, 0.93; Pro, 1.0; Gly, 1.0; half-Cys, 1.9; Tyr, 1.0; Phe, 1.1.

[2-[α - 2H_1]Tyrosine,8-arginine]vasopressin (19a). The title compound was prepared in the same manner as the DL-tyrosine derivative **19** using the solid phase procedures on a 1.5 mmol scale except that the resolved protected amino acid *N*-Boc[α - 2H_1]tyrosine (**8a**) was used for addition to the growing peptide chain. There was obtained 1.54 g (71%) of the protected nonapeptide, Cys(DMB)-[α - 2H_1]Tyr-Phe-Gln-Asn-Cys(DMB)-Pro-Arg(Tos)-Gly-NH₂, mp 201–206 °C. A 370-mg portion of the protected nonapeptide was deprotected and oxidized in the usual manner and after purification gave 101 mg of the title compound **19a**. The compound gave single uniform spots on TLC in the solvent systems A, B, and D identical with **19a** from the previous synthesis and to authentic AVP; $[\alpha]^{21}_{547} -24.0^\circ$ (c 0.53, 1 N HOAc). Amino acid analysis gave the following molar ratios: Arg, 0.93; Asp, 1.0; Glu, 1.1; Pro, 1.0; Gly, 1.0; half-Cys, 2.0; Tyr, 0.86; Phe, 1.0.

[2-[α,β,β - 2H_3]Tyrosine,8-arginine]vasopressin (20). The protected nonapeptide precursor of **20**, Cys(DMB)-[α,β,β - 2H_3]Tyr-Phe-Gln-Asn-Cys(DMB)-Pro-Arg(Tos)-Gly-NH₂, was prepared by the solid phase method as before on a 1.5 mmol scale except that *N*^α-Boc[α,β,β - 2H_3]tyrosine (**9a**) was used for addition of the deuterated tyrosine to the growing peptide chain. Conversion of 0.25 mmol of the protected nonapeptide to crude **20** was done as before, and the product was purified using partition chromatography in the solvent system 1-butanol-ethanol-pyridine-0.1 N acetic acid (4:1:1:7) followed by gel filtration on Sephadex G-25. The product was obtained as a white powder, yield 105 mg; $[\alpha]^{25}_{547} -25.6^\circ$ (c 0.51, 1 N HOAc). Thin-layer chromatography in solvent systems A, B, and D gave single uniform spots identical with authentic AVP. Amino acid analysis gave the following molar ratios: Arg, 1.0; Asp, 1.0; Glu, 0.93; Pro, 1.1; Gly, 1.0; half-Cys, 1.8; Tyr, 0.87; Phe, 1.0.

[1-Hemi[α - 2H_1]cystine,8-arginine]vasopressin (21). The protected nonapeptide precursor of the title compound, [α - 2H_1]Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(DMB)-Pro-Arg(Tos)-Gly-NH₂, was prepared by the same solid phase methodology as used in the synthesis of the nonapeptide precursor to **16** (vide supra). On a 1 mmol synthesis there was obtained 1.10 g (77%) of the protected nonapeptide. A 365-mg (0.25 mmol) portion of the nonapeptide was converted to the crude product in the usual manner and then subjected to purification by

partition chromatography using the solvent system 1-butanol-ethanol-pyridine-0.1 N HOAc (4:1:1:7). The product (R_f 0.22) was obtained as a lyophilized powder and further purified by gel filtration on Sephadex G-25 using 0.2 N HOAc as eluent solvent. The title compound was obtained as a white powder, yield 152 mg; $[\alpha]_D^{21}$ $_{547}$ -23.3° (c 0.22, 1 N HOAc). Analysis on TLC in solvent systems A, B, and D gave single spots identical with authentic AVP. Amino acid analysis gave the following molar ratios: Arg, 1.1; Asp, 0.92; Glu, 1.0; Pro, 1.1; Gly, 1.0; half-Cys, 1.9; Tyr, 0.90; Phe, 1.0. The compound exhibited a proton-decoupled carbon-13 NMR spectrum identical with authentic AVP except that the peak due to the α carbon of the half-cystine-1 residue was absent. The compound had the same milk-ejecting activity as authentic AVP.

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References and Notes

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- (2) All amino acids except glycine are of the L configuration unless otherwise noted. Standard abbreviations for amino acids, protecting groups, and peptides as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [*J. Biol. Chem.*, **247**, 977 (1972)] are used. Other abbreviations include: DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; TFA, trifluoroacetic acid; DMB, 3,4-dimethylbenzyl; HOBT, 1-hydroxybenzotriazole; DMF, dimethylformamide; AVP, [8-arginine] vasopressin; HOAc, acetic acid.
- (3) Recipient of Smith Kline and French Postdoctoral Fellowship, 1973-1975.
- (4) Recipient of a Lubrizol Foundation Scholarship.
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