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(16) For a quantitative treatment of the photosensitized isomerization of trienes, see R. S. H. Liu and Y. Butt, *J. Amer. Chem. Soc.*, **93**, 1532 (1971); R. S. H. Liu, *Pure Appl. Chem., Suppl.*, **1**, 335 (1971).
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(18) Proton nmr spectra were obtained on a Varian HA-100 spectrometer and chemical shifts are reported in δ units (parts per million) relative to TMS (δ 0) as an internal standard in $CDCl_3$ or benzene-d_6 or an external standard in H_2O or D_2O. The carbon-13 nmr spectra were determined in dioxane at 25.15 MHz on a modified Varian HA-100 spectrometer equipped with a Varian V3530 sweep unit, and chemical shifts are reported in δ units (parts per million) relative to TMS (δ 0); methine, methylene, and methyl carbons were identified by single frequency off-resonance decoupling where the decoupler was tuned to the exact frequency for irradiation of the dioxane protons and appeared as close-spaced doublets, triplets, and quartets, respectively; see M. Tanabe, T. Hamasaki, D. Thomas, and L. Johnson, *J. Amer. Chem. Soc.*, **93**, 273 (1971). Ultraviolet spectra were recorded on a Cary 14 double-beam recording spectrophotometer. Infrared spectra were taken on a Beckman IR-10 spectrophotometer. Mass spectra were determined on a Hitachi Perkin-Elmer RMU-6D single-focusing mass spectrometer operating at 70 eV. Circular dichroism curves were recorded in n-pentane at room temperature on a Cary 61 spectropolarimeter. Optical rotations were determined on a ETL-NPL (Ericsson Telephone Unlimited) automatic polarimeter.
(19) H. Morrison, *J. Chem. Educ.*, **44**, 161 (1967).
(20) Multiplet collapses to an AB quartet ($J = -20$ Hz) when irradiated at 5.6 ppm.
(21) Only a carbon-13 nmr spectrum of a 1:1 mixture of **16** and **17** was obtained: δ 14.5 (2 CH_3), 21.8 (2 CH_2), 26.3 (CH_2), 30.7 (CH_2), 116.4 (CH_2), 117.1 (CH_2), 126.4 (CH), 127.1 (CH), 128.9 (2 CH), 130.8 (CH), 131.2 (CH), 131.9 (CH), 132.5 (CH), 132.9 (CH), 133.9 (3 CH), 137.7 (2 CH).
(22) A. S. Narula and S. Dev, *Tetrahedron Lett.*, 3459 (1969).
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Synthesis of Oxytocin and Related Diastereomers Deuterated in the Half-Cystine Positions. Comparison of Solid-Phase and Solution Methods[1,2]

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Four derivatives of the neurohypophysial hormone oxytocin deuterated at the α and β positions of the two half-cystine residues have been synthesized. The substituted amino acid Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine was used to prepare [1-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin and [6-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin. The diastereomeric mixtures were separated and purified by partition chromatography and gel filtration to give [1-hemi-L-[α,β,β-$^2H_3$]cystine]oxytocin, [1-hemi-D-[α,β,β-$^2H_3$]cystine]oxytocin, [6-hemi-L-[α,β,β-$^2H_3$]cystine]oxytocin, and [6-hemi-D-[α,β,β-$^2H_3$]cystine]oxytocin. The former two compounds were prepared by both solid-phase and solution techniques of peptide chemistry, and the two methods were compared in the synthesis of these derivatives. The solid-phase method was considerably faster and gave better overall yields, while the solution method permitted a slightly more conservative use of deuterated amino acid. It was found that much shorter deprotection and coupling times and much smaller excesses of amino acid were compatible with the solid-phase methodology.

The preparation of amino acids, polypeptides, peptide hormones, and proteins specifically labeled in *nonexchangeable* positions by deuterium is of considerable utility for various chemical, biological, and physical studies. For example, partially deuterated derivatives have been utilized in studies of protein structure, functions, and folding.[3-7] The microdynamical behavior of the neurohypophysial peptide hormones in solution[8,9] and the interaction of these hormones with their biological carrier proteins the neurophysins have been studied utilizing the deuterium label.[9] Deuterated derivatives have also proven very useful for the unambiguous assignment of proton[10-14] and carbon-13[15,16] resonances in nuclear magnetic resonance spectroscopy studies of peptides,[10-12,15,16] peptide hormones,[13-16] and related compounds. Since many of the physical[11-16,17-19] properties and biological activities[20-22] of these compounds are not significantly affected by the perturbation of deuteration, it appears likely that partially deuterated amino acids, peptides, and proteins will find increased utility.

We report here the total synthesis of four partially deuterated derivatives of the posterior pituitary hormone, oxytocin (**1**) (Figure 1) in which the protons on the α and β carbons of the half-cystine residues (positions 1 and 6, Figure 1) have been replaced by deuterons. Since both the hemi-D-cystine and hemi-L-cystine isomers of each of the half-cystine residues were desired, we synthesized [1-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin (**2**) and [6-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin (**3**) and separated the diastereomeric pairs by partition chromatography on Sephadex G-25.[23,24] The [1-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin was synthesized by solid-phase peptide synthesis (SPPS) using the standard chloromethylated resin,[25,26] and by solution methods. In view of the current interest in labeled peptides and the desirability of fast, efficient synthetic routes to these and other peptides, these synthetic studies offered a convenient comparison of the two techniques. The [6-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin was synthesized by solid-phase methods only. Of special interest was the use of SPPS in cases where the usual large excesses[25,26] of amino acid used in coupling (two to four times stoichiometric) could be avoided and the valuable deuterated amino acids could be conserved.

The solid-phase synthesis of [6-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin (**3**) was carried out on a semiautomated device with the methodology shown in Table I. One coupling with a 2.5-fold excess of amino acid and DCC was used for each of the amino acids except for the deuterated cysteine.

The Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine (**4**) was only the fourth residue in this synthesis. Its coupling was varied somewhat in that two couplings were employed using

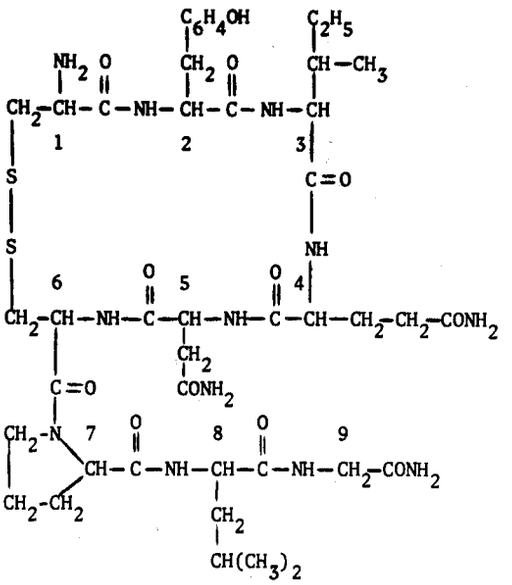

Figure 1. The structure of oxytocin. The numbers indicate the position of the amino acid residues.

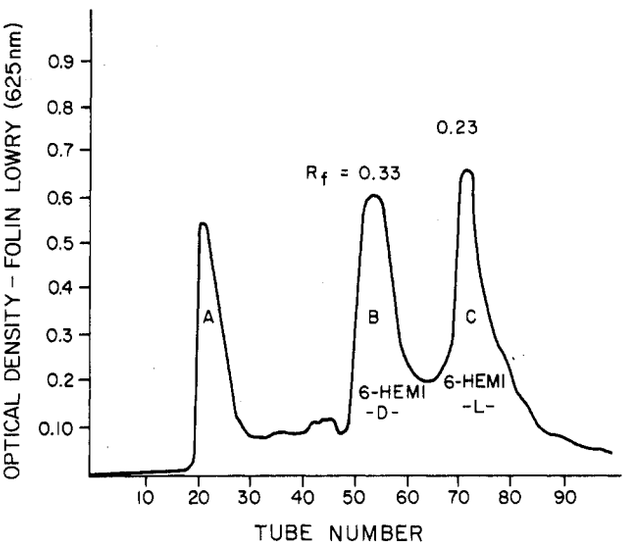

Figure 2. Partition chromatography of [6-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin on Sephadex G-25 (100–200 mesh) using the solvent system 1-butanol–3.5% aqueous acetic acid in 1.5% pyridine (1:1). Peak B is [6-hemi-D-[α,β,β-$^2H_3$]cystine]oxytocin, peak C is [6-hemi-L-[α,β,β-$^2H_3$]cystine]oxytocin, and peak A is by-products from the crude reaction mixture.

respectively 1.1 and 0.4 equiv each of amino acid and DCC. The ninhydrin test method[27] was used to detect completion of reaction after each coupling and was negative except for two cases: the first deuterated cysteine coupling was only slightly positive (~2% free amine) and negative after a second coupling. In the case of the seventh residue, Ile, a slightly positive ninhydrin test was obtained (~1% free amine), but complete coupling was obtained after a second coupling as judged by the ninhydrin test. The steric hindrance provided by the Ile residue is believed to be the cause of the sometimes troublesome coupling which is observed with this amino acid.

After removal of the N-terminal Boc group, the partially protected nonapeptide amide H-Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-DL-[α,β,β-$^2H_3$]Cys(Bzl)-Pro-Leu-Gly-NH_2 (**10**) was obtained by ammonolysis of the corresponding peptide resin ester.

A portion of the nonapeptide was treated with sodium and liquid ammonia[28] and then oxidized with 0.1 *N* $K_3Fe(CN)_6$[29] to [6-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin. The isomers were separated by partition chromatography on Sephadex G-25 using the solvent system 1-butanol–3.5% HOAc in 1.5% aqueous pyridine (1:1). A typical separation is shown in Figure 2. The first peak can probably be attributed to "dimers" of oxytocin[30] and other by-products; the peak at R_f 0.33 represents [6-hemi-D-[α,β,β-$^2H_3$]cystine]oxytocin (**3a**) while the peak at R_f 0.23 is the partially deuterated oxytocin **3b** itself (oxytocin lit. R_f 0.24).[24] Owing to the greater instability of the D isomer in solution, the fractions were separated in such a manner as to give reasonably pure D isomer and the fractions corresponding to the L-isomer peak (as well as some D) were then partition chromatographed again in the same system. The fractions corresponding to the purified products were lyophilized to dryness and the white powders were subjected to a final gel filtration on Sephadex G-25, using 0.2 *N* HOAc as eluting solvent.

The solid-phase synthesis of [1-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin (**2**) was carried out on an automated Vega Series 95 Synthesizer, a solid-state version of our automated instrument.[31] The procedures used are shown in Table II.

Again, reactions were monitored using the ninhydrin test method[27] and indicated complete coupling. In this synthesis the removal of the N^{α} protecting group and the coupling reaction times were shortened considerably. Two 7-min treatments with 40% TFA in CH_2Cl_2 (2% anisole) were used for deprotection and two 20-min coupling reactions with 1.5 equiv of the protected amino acid and DCC were used. In agreement with the findings of Corley, *et al.*,[32] no problems were encountered in utilizing a faster methodology.

Table I
Solid-Phase Methodology Used in Synthesis of [6-Hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin

	Normal Dcc coupling			Nitrophenyl ester coupling (Asn and Gln)		
Step	Solvent or reagent	Duration, min	No. of times	Solvent or reagent	Duration, min (hr)	No. of times
1	CH_2Cl_2	2	3	CH_2Cl_2	2	3
2	TFA–CH_2Cl_2–anisole (50:48:2)	25	1	TFA–CH_2Cl_2–anisole (50:48:2)	25	1
3	CH_2Cl_2	2	5	CH_2Cl_2	2	5
4	DIEA–CH_2Cl_2 (7:93)	6	2	DIEA–CH_2Cl_2 (7:93)	6	2
5	CH_2Cl_2	2	4	CH_2Cl_2	2	3
6	Amino acid[a]–CH_2Cl_2 (2.5 equiv)		1	DMF	2	3
7	DCC in CH_2Cl_2 (2.5 equiv)	90	1	Amino acid[a] in DMF (4 equiv)	(6)	1
8	CH_2Cl_2	2	3	Addn of DIEA (1 equiv)	(2)	1
9	100% EtOH	2	3	DMF	2	3

[a] N^{α}-Boc amino acids were employed throughout; Tyr and Cys were *O*-benzyl and *S*-benzyl protected, respectively.

Table II
Solid-Phase Methodology Used in Synthesis of [1-Hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin

	Normal Dcc coupling			Nitrophenyl ester coupling (Asn and Gln)		
Step	Solvent or reagent	Duration, min	No. of times	Solvent or reagent	Duration, min (hr)	No. of times
1	CH_2Cl_2	1	4	CH_2Cl_2	1	4
2	TFA–CH_2Cl_2–anisole (40:58:2)	7	2	TFA–CH_2Cl_2–anisole (40:58:2)	7	2
3	CH_2Cl_2	1	3	CH_2Cl_2	1	3
4	DIEA–CH_2Cl_2 (10:90)	2	2	DIEA–CH_2Cl_2 (10:90)	2	2
5	CH_2Cl_2	1	3	CH_2Cl_2	1	3
6	Amino acid[a]–CH_2Cl_2 (1.5 equiv)		1	DMF	1	3
7	DCC in CH_2Cl_2 (1.5 equiv)	20	1	Amino acid[a] in DMF (4 equiv)	(6)	1
8	CH_2Cl_2	1	2	Addn of DIEA (1 equiv)	(2)	1
9	100% EtOH	1	2	DMF	1	3
10	CH_2Cl_2	1	3			
11	Amino acid[a]–CH_2Cl_2 (1.5 equiv)		1			
12	DCC in CH_2Cl_2 (1.5 equiv)	20	1			
13	CH_2Cl_2	1	2	CH_2Cl_2	1	2
14	100% EtOH	1	2	100% EtOH	1	2

[a] N^{α}-Boc amino acids were employed throughout; Tyr and Cys were *O*-benzyl and *S*-benzyl protected, respectively.

A modification of the sequence was employed for the addition of the N-terminal amino acid, Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine (**4**). In this case no excess of amino acid was used for the first coupling in order to preserve deuterated material. After the initial coupling, the ninhydrin test indicated about 98% coupling; a second coupling with 0.25 equiv each of Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine and DCC (2-hr duration) resulted in complete coupling.

At the completion of the solid-phase synthesis, the N-terminal Boc protecting group was removed by carrying out steps 1–5 (Table II). The partially protected nonapeptide was cleaved from the resin by ammonolysis to give the protected nonapeptide **8**.

The solution synthesis of [1-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin (Scheme I) involved the coupling of Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cystine (**4**) with the octapeptide Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (**5**). The coupling was carried out using 1.1 equiv of **4** and DCC and a 2.2-fold excess of 1-hydroxybenzotriazole.[33] The N-terminal Boc group was removed by TFA, and the material was then deprotected and oxidized in a similar manner to the solid-phase-derived compounds. Yields of **2a** and **2b** from the corresponding nonapeptides were comparable using the solution- and solid-phase-synthesized precursors. The products from the various syntheses were judged pure by various criteria including single spots and identical behavior with the authentic protio compounds on tlc using at least two solvent systems, carbon-13 and proton nmr, amino acid analysis, optical rotation, and a four-point assay for oxytocic activity for the compounds.

For a total synthesis, the solid-phase method is clearly the most rapid route to the desired nonapeptide precursor to oxytocin. The main advantage of the solution synthesis is that it permits a slightly more conservative use of the valuable deuterated amino acid precursor **4**, at least when this residue is at or near the amino terminal. In general we have found that the solid-phase method requires at least a 20% greater expenditure of labeled amino acid than the solution synthesis. However, it should be noted that the excess of deuterated protected cysteine used in our SPPS methodology is considerably smaller than normally found[25,26,34,35] in most literature solid-phase syntheses. Other workers[36,37] have also used reduced amounts of reagent in SPPS to incorporate labeled residues into peptides.

We also made a comparison of the solid-phase and solution methods in terms of yield of oxytocin. Calculated yields of the nonapeptide hormone precursor using the stepwise nitrophenyl ester couplings are about 45%;[38] in comparison, averages of our own preparations and literature syntheses[39] of nonapeptide using SPPS are about 60%.[40] The yield of oxytocin from protected nonapeptide prepared by either method were comparable (see Experimental Section and ref 38). In preparing deuterated derivatives of oxytocin and its analogs, the position of deuteri-

Scheme I
Solution Synthesis of [1-Hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin (2)

Cbz-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2

↓ HBr–HOAc

Boc-*S*-benzyl-DL-[α, β, β-$^2H_3$]cysteine + Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2

↓ *N*-hydroxybenzotriazole (2 equiv), dicyclohexylcarbodiimide, DMF

Boc-DL-[α, β, β-$^2H_3$]Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2

↓ 1. TFA–anisole; 2. Na/NH_3; 3. $K_3Fe(CN)_6$

[1-hemi-DL-[α, β, β-$^2H_3$]cystine]oxytocin

um in the peptide chain would be a primary concern in deciding whether to employ solution or solid-phase methods. However, our results indicate that either method can be used in achieving reasonably quick and efficient syntheses of deuterated hormones and hormone analogs, and these methods are of course equally applicable to the inclusion of other labels, including ^{14}C, ^{13}C, and ^{15}N.

Experimental Section

Thin layer chromatography (tlc) was done on silica gel G plates in the following solvent systems: (A) 1-butanol–acetic acid–water (4:1:5); (B) 1-butanol–acetic acid–pyridine–water (15:3:10:12). Capillary melting points were determined on a Thomas-Hoover melting point apparatus and are corrected. Amino acid analyses were carried out by the method of Spackman, Stein, and Moore[41] on a Beckman 120C amino acid analyzer after hydrolysis in 6 *N* HCl.

Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine (4). A 1.59-g (7.4 mmol) portion of *S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine[42] was added to 10 ml of peroxide-free dioxane and 10 ml of deionized water. The pH was adjusted to 10 with 4 *N* NaOH and 1.5 ml (11 mmol) of *tert*-butyl azidoformate was added. The pH was maintained at 9.6 for 24 hr with 4 *N* NaOH. The solution was then filtered and washed with 25 ml of ethyl ether. The aqueous phase was acidified to pH 3 using solid citric acid and extracted with 25 ml of ethyl acetate. The aqueous phase was saturated with sodium chloride and then extracted with two 25-ml portions of ethyl acetate.

The organic phase was washed with 50 ml of deionized water and then dried over sodium sulfate. The sodium sulfate was removed by filtration and washed with three 15-ml portions of ethyl acetate, and the washes and filtrate were combined. Solvent was removed by rotary evaporation, leaving a white solid. Recrystallization from ethyl acetate–hexane gave small needles, mp 111–111.3°, 1.85 g (80% of theory). Purity was established by comparison with authentic Boc-*S*-benzylcysteine and an nmr spectrum established that deuterium retention at the α and β positions was ≥95%.

Anal. Calcd for $C_{15}H_{18}D_3NO_4S$: C, 57.84; H, 6.92; N, 4.44. Found: C, 57.59; H, 6.69; N, 4.24.

Solid-Phase Synthesis of *S*-Benzyl-DL-[α,β,β-$^2H_3$]cysteinyl-*O*-benzyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinylprolylleucylglycyl Resin (7). The solid-phase synthesis of the title protected nonapeptide resin was performed on the Vega Model 95 Synthesizer, an automated machine similar to that described by Hruby, *et al.*[31] The support used was a chloromethylated polystyrene resin (crosslinked with 2% divinylbenzene, Cl = 1.9 mmol/g) which had been treated with Boc-glycine for 48 hr in ethanol[26] to achieve a glycine substitution of 0.50 mmol/g as measured by the modified[43] aldimine test.[44] The reaction was run on a 1.25-mmol scale (2.5 g of resin). The cycles of deprotection, neutralization, and coupling were carried out for the introduction of each new residue in the peptide as described in Table II. All washes and reactions were carried out with 25-ml portions. The procedure was varied during the couplings of the asparagine and glutamine nitrophenyl esters as shown in Table II.

The procedure was again varied for the last step, the addition of Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine. In steps 6 and 7 (Table II) only 1 equiv (0.392 g, 1.25 mmol) of the amino acid was used with a similar amount of dicyclohexylcarbodiimide (DCC). After the coupling step and three washing sequences (steps 8–10) a ninhydrin test[27] was employed, and showed incomplete coupling to a limited extent (about 98% coupling was indicated). A second coupling using 100 mg (0.32 mmol) of Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine and 0.32 mmol of DCC for 2 hr was run. After the appropriate washes (steps 1, 13, and 14), the ninhydrin test indicated complete coupling (steps 13, 14, and 1).

Following the last coupling, the terminal Boc group was removed (steps 1–5). The resin was filtered, dried, and found to have increased in weight by 1.2 g. After correcting for the weight of Boc-glycine on the resin, a weight gain of 79% of theory was calculated.

[1-Hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin (2) from 7 and Separation of the Diastereomers. The peptide DL-[α,β,β-$^2H_3$]Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly resin (7) was cleaved from the preceding resin (3.70 g) by ammonolysis. The resin peptide was dissolved in 125 ml of anhydrous methanol which had been saturated with ammonia (freshly distilled from sodium) at −5°. The mixture was stirred for 72 hr in a tightly sealed 250-ml flask. Excess ammonia and methanol were then removed *in vacuo*, the peptide was extracted into 60 ml of warm (55°) dimethylformamide (DMF) for 4 hr and the resin was filtered off. The DMF solution volume was reduced to 30 ml and addition of water yielded a voluminous white precipitate which was filtered and dried overnight *in vacuo* to give 1.06 g (69%) of protected nonapeptide DL-[α,β,β-$^2H_3$]Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (8), mp 212–216°.

The protecting groups were removed by treatment of 320 mg (0.25 mmol) of 8 with sodium metal[28] in freshly distilled ammonia until a blue color persisted for 30 sec. Excess sodium was destroyed with acetic acid, and ammonia was removed by evaporation followed by lyophilization. The white powder was dissolved in 600 ml of 0.1% aqueous acetic acid, the pH was adjusted to 8.5 with 3 *N* ammonium hydroxide, and the sulfhydryl groups were oxidized with 50 ml of 0.1 *N* $K_3Fe(CN)_6$ solution.[29] After 30 min the pH was lowered to 5 with 6 *N* acetic acid and the solution was treated with 4 ml (settled volume) of Rexyn 203 (Cl^- form). The mixture was stirred for 20 min and the resin was removed by filtration and washed with three portions of 10% aqueous acetic acid. The filtrate and washings were combined and lyophilized to dryness. The product [1-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin (2) was separated into its diastereomers and purified using the partition chromatography system described below.

[1-Hemi-D-cystine]oxytocin has been separated from [1-hemi-L-cystine]oxytocin by partition chromatography using the solvent system 1-butanol–benzene–pyridine–0.1% aqueous acetic acid (6:2:1:9).[24] However, in our hands, this system gave variable results, usually yielding R_f values considerably lower than those reported,[24,45] and its use was abandoned in favor of the equally useful system 1-butanol–3.5% aqueous acetic acid in 1.5% pyridine (1:1) for the separation of the deuterated derivatives.

The crude product [1-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin was dissolved in 3 ml of the upper phase and 2 ml of the lower phase of the solvent system 1-butanol–3.5% aqueous acetic acid in 1.5% pyridine (1:1) and applied to a Sephadex G-25 (block polymerizate, 100–200 mesh) column, 2.85 × 62 cm, previously equilibrated with lower and upper phases. Ninety 5.8-ml fractions were collected. Analysis by the Folin–Lowry method[46] showed three peaks, including the by-product peak and two other peaks at R_f 0.34 and 0.25. From the known R_f values of the protio derivatives in this system (0.35 and 0.24),[24] these peaks could be assigned to [1-hemi-D-[α,β,β-$^2H_3$]cystine]oxytocin (**2a**) and [1-hemi-L-[α,β,β-$^2H_3$]cystine]oxytocin (**2b**). The oxytocin peak (R_f 0.23) was isolated to give uncontaminated product (60 mg) while the 1-hemi-D peak (R_f 0.34), which included some oxytocin, was repurified by partition chromatography to give the D isomer **2a**. Both **2a** and **2b** were then separately purified by gel filtration chromatography on Sephadex G-25 (200–270 mesh) using 0.2 *N* acetic acid as eluent solvent. Final purified yields were 76 mg of **2a** and 57 mg of **2b**.

Each of the isomers gave single spots of uniform shape on tlc in the solvent systems A and B, identical with those of authentic protio analogs of **2a**[47] and **2b**.[38] The oxytocin derivative **2b** exhibited proton[13,14] and carbon-13[16] spectra identical with those of authentic oxytocin except for the absence of peaks corresponding to the 1-hemicystine α and β protons and carbons, respectively. Bioassay of **2a** and **2b** for milk-ejecting and oxytocic activity gave results comparable to those of the protio analogs. A sample of each compound was hydrolyzed for 36 hr at 110°. Amino acid analysis gave the following molar ratios: for [1-hemi-L-[α,β,β-$^2H_3$]cystine]oxytocin, aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.06; glycine, 1.0; half-cystine, 2.0; isoleucine, 1.0; leucine, 1.1; tyrosine, 0.88; for [1-hemi-D-[α,β,β-$^2H_3$]cystine]oxytocin, aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.0; glycine, 1.0; half-cystine, 2.0; isoleucine, 1.0; leucine, 1.0; tyrosine, 0.85.

Solution Synthesis of 2 and Separation of the Diastereomers. The synthesis of DL-[α,β,β-$^2H_3$]Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 was also carried out *via* solution techniques by the coupling of the octapeptide Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2[38] with Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cystine (4). The protected octapeptide Z-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (350 mg, 0.286 mmol) was dissolved in 5 ml of anhydrous acetic acid and 5 ml of 5.3 *N* HBr in acetic acid and the solution was stirred for 1 hr. Addition of 300 ml of anhydrous ether yielded a white precipitate. The solid was filtered off, washed with three 35-ml portions of ether, and dried *in vacuo* over KOH. The deprotected octapeptide salt was used in the coupling step without further purification. A solution of 90 mg (0.315 mmol) of Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine and 84 mg (0.63 mmol) of 1-hydroxybenzotriazole[33] in 5 ml of DMF was cooled to 0°. Then 65 mg (0.315 mmol) of DCC was added, and the solution was stirred at −5° for 1 hr and 1 hr at room tempera-

ture. The octapeptide salt was dissolved in 5 ml of DMF, neutralized to pH 7.0 with *N*-methylmorpholine, and added to the active ester solution with two 2-ml DMF washes. The reaction mixture was stirred for 12 hr at room temperature. Addition of 300 ml of anhydrous ethanol gave a fluffy white precipitate which was filtered and washed with three 30-ml portions of ethanol, three 10-ml portions of 50% aqueous ethanol, and three 30-ml portions of ether. The white powder was dried *in vacuo* over KOH, yielding 285 mg (70% of theory) of Boc-DL-[α,β,β-$^2H_3$]Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (**6**), mp 216–221°.

The protected nonapeptide **6** was stirred in a flask containing 5 ml of anisole and 7 ml of trifluoroacetic acid (TFA) for 30 min. Ether (200 ml) was added and a white precipitate was filtered and washed with three 30-ml portions of ether. The white powder was dried overnight *in vacuo* over KOH, yielding 275 mg (95%) of the deprotected nonapeptide (trifluoroacetate salt). The salt was treated with sodium in liquid ammonia to remove remaining protecting groups, oxidized with $K_3Fe(CN)_6$, and purified as described in the preceding section. Final yields of **2a** and **2b** from the nonapeptide were comparable to those obtained in the previous example. The compounds were identical in all respects with those obtained by solid-phase methods.

Solid-Phase Synthesis of *S*-Benzylcysteinyl-*O*-benzyltyrosylisoleucylglutaminylasparaginyl-*S*-benzyl-DL-[α,β,β-$^2H_3$]cysteinylprolylleucylglycyl Resin (9). The solid-phase synthesis of the title protected nonapeptide resin intermediate was performed in a similar manner to that of **7**. The quantity of Boc-glycine substituted resin used was 4 g with an amino acid content of 0.36 mmol/g (1.44 mmol). As in the synthesis of **7**, the reaction time for the active ester couplings (*p*-nitrophenyl *tert*-butyloxycarbonylasparaginate and p-nitrophenyl *tert*-butyloxycarbonylglutaminate) was 6 hr. In an alternate step, 8 (see Table I), 1 equiv (1.44 mmol) of diisopropylethylamine in 5 ml of DMF was added to the nitrophenyl ester and the mixture was shaken for an additional 2 hr.

Most of the other couplings were carried out using a single coupling step of 90-min duration and a 2.5-fold excess of amino acid and dicyclohexylcarbodiimide. Negative ninhydrin reactions were noted after each of the couplings except for the following two cases. (1) The coupling of Boc-*S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine (**4**) to Pro-Leu-Gly resin involved a 1.1-fold excess of the deuterated amino acid (and 1.1 equiv of DCC) and a reaction time of 180 min. A ninhydrin test indicated slightly incomplete (~98%) coupling. An additional 0.4 equiv of **4** and DCC were added in 35 ml of methylene chloride and the coupling was repeated for 120 min. At the completion of the wash steps, the ninhydrin test indicated complete coupling. (2) A single 90-min coupling with Boc-isoleucine and DCC (2.5-fold excess) likewise gave a slightly positive ninhydrin test. The coupling was repeated with an additional 1.25-fold excess of reagents for 90 min to give complete coupling.

[6-Hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin (3) from 9 and Separation of the Diastereomers. The ammonolysis of **9** was carried out in an identical manner with cleavage of **7** (*vide supra*) and yielded 1.02 g (55% based on original glycine substitution) of the protected nonapeptide Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-DL-[α,β,β-$^2H_3$]Cys(Bzl)-Pro-Leu-Gly-NH_2 (**10**), mp 216–221°. Deprotection with sodium and liquid ammonia of 330 mg (0.25 mmol) of nonapeptide **10** was followed by oxidation with $K_3Fe(CN)_6$ as described previously. The solution was treated with Rexyn 203 (Cl^- cycle), filtered, and lyophilized to dryness.

Preliminary studies using oxytocin and [6-hemi-D-cystine]oxytocin[48] showed that the two compounds could be readily separated using the solvent system 1-butanol–3.5% aqueous acetic acid containing 1.5% pyridine (1:1). Identical R_f values were obtained for both the protio and deuterio compounds.

The crude [6-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin was dissolved in 3 ml of the upper phase and 2 ml of the lower phase of the above solvent system, and applied to a Sephadex G-25 column, 2.85 × 62 cm, previously equilibrated with lower and upper phases of the solvent system. The column was eluted with the upper phase and 100 5.8-ml fractions were collected. Analysis by the Folin–Lowry method showed three peaks, including the by-product peak and two other peaks at R_f 0.33 and 0.23 (Figure 2). From the R_f values for the protio compounds and by comparison with the usual R_f of oxytocin in this system (0.24), these peaks could be assigned to [6-hemi-D-[α,β,β-$^2H_3$]cystine]oxytocin (**3a**) and [6-hemi-L-[α,β,β-$^2H_3$]cystine]oxytocin (**3b**), respectively. Each of the peaks was collected separately, lyophilized to dryness, and subjected to gel filtration chromatography on Sephadex G-25 (200–270 mesh) using 0.2 *N* acetic acid. Final purified yields were 53 mg of **3a** and 70 mg of **3b**.

Each of the isomers gave single spots of uniform shape in the solvent systems A and B, and at identical R_f values with those of the protio analogs, and gave identical optical rotations as the protio analogs. The oxytocin derivative **3b** exhibited proton[13,14] and carbon-13[16] spectra identical with those of authentic oxytocin except for the absence of peaks corresponding to the 6-hemicystine α and β protons and carbons, respectively. Bioassays of **3a** and **3b** for milk-ejecting and oxytocic activity gave results comparable to those of the protio analogs. A sample of each compound was hydrolyzed for 36 hr at 110°. Amino acid analysis gave the following molar ratios: for [6-hemi-L-[α,β,β-$^2H_3$]cystine]oxytocin, aspartic acid, 1.0; glutamic acid, 1.1; proline, 1.0; glycine, 1.1; half-cystine, 2.0; isoleucine, 1.0; leucine, 1.0; tyrosine, 0.80; for [6-hemi-D-[α,β,β-$^2H_3$]cystine]oxytocin, aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.0; glycine, 0.92; half-cystine, 1.75; isoleucine, 1.0; leucine, 1.0; and tyrosine, 0.85.

Bioassay Methods. Oxytocic assays were performed on isolated uteri from rats in natural estrus according to the method of Holton,[49] as modified by Munsick,[50] with the use of magnesium-free Van Dyke–Hastings solution as the bathing fluid. Milk-ejecting activities were determined using mouse mammary tissue *in vitro*.[51,52] [1-Hemi-L-[α,β,β-$^2H_3$]cystine]oxytocin and [6-hemi-L-[α,β,β-$^2H_3$]cystine]oxytocin had the same potencies as authentic oxytocin, and [1-hemi-D-[α,β,β-$^2H_3$]cystine]oxytocin and [6-hemi-D-[α,β,β-$^2H_3$]cystine]oxytocin showed the reduced potencies as found in authentic [1-hemi-D-cystine]oxytocin[24] and [6-hemi-D-cystine]oxytocin,[47] respectively, in these assay systems.

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Registry No.—2, 51548-05-1; **2a,** 51548-06-2; **2b,** 51493-96-0; **3,** 51548-07-3; **3a,** 51548-08-4; **3b,** 51493-97-1; **4,** 51493-98-2; **6,** 51493-99-3; **7,** 51494-00-9; **8,** 51494-01-0; **9,** 51494-02-1; **10,** 51494-03-2; *S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine, 51494-04-3; *tert*-butyl azidoformate, 1070-19-5; dicyclohexylcarbodiimide, 538-75-0; Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, 51494-05-4; Z-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, 39005-18-0; *p*-nitrophenyl *tert*-butyloxycarbonylasparaginate, 4587-33-1; *p*-nitrophenyl *tert*-butyloxycarbonylglutaminate, 15387-45-8.

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 are used. Other abbreviations include DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; TFA, trifluoroacetic acid.
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A Synthesis of Racemic Ipomeamarone and Epiipomeamarone

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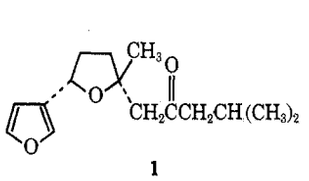
A synthesis of the sesquiterpene ipomeamarone (1) is described. Reaction of the anion of dimethyl 2-oxo-4-methylpentanephosphonate with 1-acetoxy-1-(3-furyl)-4-pentanone (6b) followed by hydrolysis produced 1 as well as epiipomeamarone (2). Kinetic and equilibrium mixtures from the cyclization reaction contained 1 and 2 in approximately equal amounts. The epimers were separated by high-pressure liquid chromatography and characterized.

The presence of the sesquiterpene ipomeamarone (1)[2] in mold-damaged sweet potatoes is well known.[3,4] The enantiomer of ipomeamarone and an epimer (2) have been found in *Myoporum deserti*.[5-7] As part of an investigation of other toxic metabolites found in moldy sweet potatoes, a convenient synthetic source of ipomeamarone was required.



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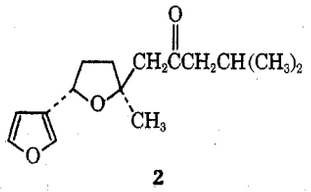
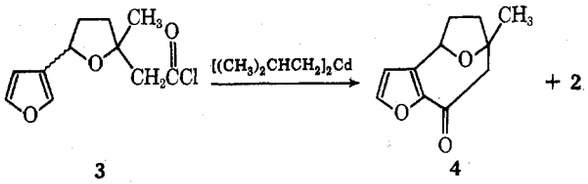


2

Kubota has described a synthesis in which a key step was addition of diisobutylcadmium to acid chloride 3.[4] The reaction gave a 13% yield of material identified as epiipomeamarone (2) and apparently no ipomeamarone. The main product was the tricyclic ketone 4. Treatment of 2 with potassium acetate and acetic anhydride gave so-called acetylisoipomeamarone, saponification of which gave material identical with that produced when natural



3 4

ipomeamarone was treated in the same manner. Acetylisoipomeamarone was later shown to be a mixture of cis and trans isomers **5a** and **5b**.[7a] Saponification of **5a,b** was originally thought to give only the cis-substituted tetrahydrofuran derivative **1**,[8] but a recent report indicates that the reaction actually gives a mixture of **1** and **2**.[7] Presumably both arise from **5a**; **5b** must initially isomerize to **5a** before Michael addition can occur. However, it is not clear whether **1** and **2** are formed directly from **5a** or whether one is the kinetic product and the other arises by subsequent equilibration. Regardless of the stereochemical