For studies with $Pr(fod)_3$, each experiment was run with three or four concentrations of shift reagent by addition of successive volumes of a shift reagent solution (c 0.25 M) to 1.0 mL of a substrate solution (c 0.5 M) up to a mole ratio [$Pr(fod)_3$]/[substrate] of 0.4.

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Synthesis of [3′,5′-$^{13}C_2$]Tyrosine and Its Use in the Synthesis of Specifically Labeled Tyrosine Analogues of Oxytocin and Arginine-Vasopressin and Their 2-D-Tyrosine Diastereoisomers[1,2]

Venkatappa Viswanatha and Victor J. Hruby*

Departments of Chemistry and Biochemistry, University of Arizona, Tucson, Arizona 85721

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DL-[3′,5′-$^{13}C_2$]Tyrosine (91% ^{13}C enriched) was synthesized by a ten-step synthetic scheme in an overall yield of 22% (86+% per step) using [1,3-$^{13}C_2$]acetone as the source of label. The enantiomers were resolved by enzymatic methods, and the labeled DL amino acid or purified enantiomers readily converted to the N^{α}-Boc acids suitable for peptide synthesis. Boc-DL-[3′,5′-$^{13}C_2$]tyrosine was used for the total synthesis of the specifically labeled peptide hormone derivatives [2-DL-[3′,5′-$^{13}C_2$]tyrosine]oxytocin and [2-DL-[3′,5′-$^{13}C_2$]tyrosine,8-arginine]vasopressin by solid phase methods. The diastereoisomers were separated from each other by partition chromatography on Sephadex G-25 followed by gel filtration to give the following specifically labeled hormone derivatives: [2-[3′,5′-$^{13}C_2$]tyrosine]oxytocin, [2-D-[3′,5′-$^{13}C_2$]tyrosine]oxytocin, [2-[3′,5′-$^{13}C_2$]tyrosine,8-arginine]vasopressin, and [2-D-[3′,5′-$^{13}C_2$]tyrosine,8-arginine]vasopressin. The milk ejecting activities were determined.

Recently it has been found that ^{13}C-enriched amino acids, peptides, and proteins, enriched at specific carbon atoms, can be used for a variety of chemical–physical and biological studies related to structure, dynamics, metabolism, etc.[3-10] The use of high enrichment (85–95%) at a specific position in the molecule will generally ensure that the labeled carbon can be unambiguously identified above the natural abundance (1.1%) using nuclear magnetic resonance (NMR) spectroscopy or other physical methods. Some common amino acids can be obtained from hydrolysis of proteins obtained from microorganisms which are grown on ^{13}C-enriched carbonate or other ^{13}C-enriched sources,[11,12] but others are not obtained or are obtained only in small quantities. In addition, the amino acids so obtained usually are uniformly labeled. This is acceptable for some applications, and indeed a number of physical–chemical studies, especially using ^{13}C NMR, have appeared.[13-15] Unfortunately, uniformly labeled amino acids, whether free or as part of a peptide, give very complicated ^{13}C NMR spectra with the resonance line of each carbon greatly reduced in intensity due to splitting by adjacent and further removed ^{13}C nuclei. While in-

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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; DMB, 3,4-dimethylbenzyl; NMR, nuclear magnetic resonance; TFA, trifluoroacetic acid; HPLC, high performance (pressure) liquid chromatography; AVP, arginine-vasopressin.

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terpretation of the spectra of the amino acids is possible, attempts to use these in more complicated applications involving peptides and protein[15] can lead to considerable difficulties in interpretations.[4] It is therefore desirable to have available specific ^{13}C-labeled derivatives of the amino acids with a high level of ^{13}C enrichment which can be used for preparation of peptides and proteins by total synthesis or by biochemical methods.

We have been studying the interactions of the specifically labeled peptide hormones oxytocin

(H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2)
1 2 3 4 5 6 7 8 9

[8-arginine]vasopressin (AVP)

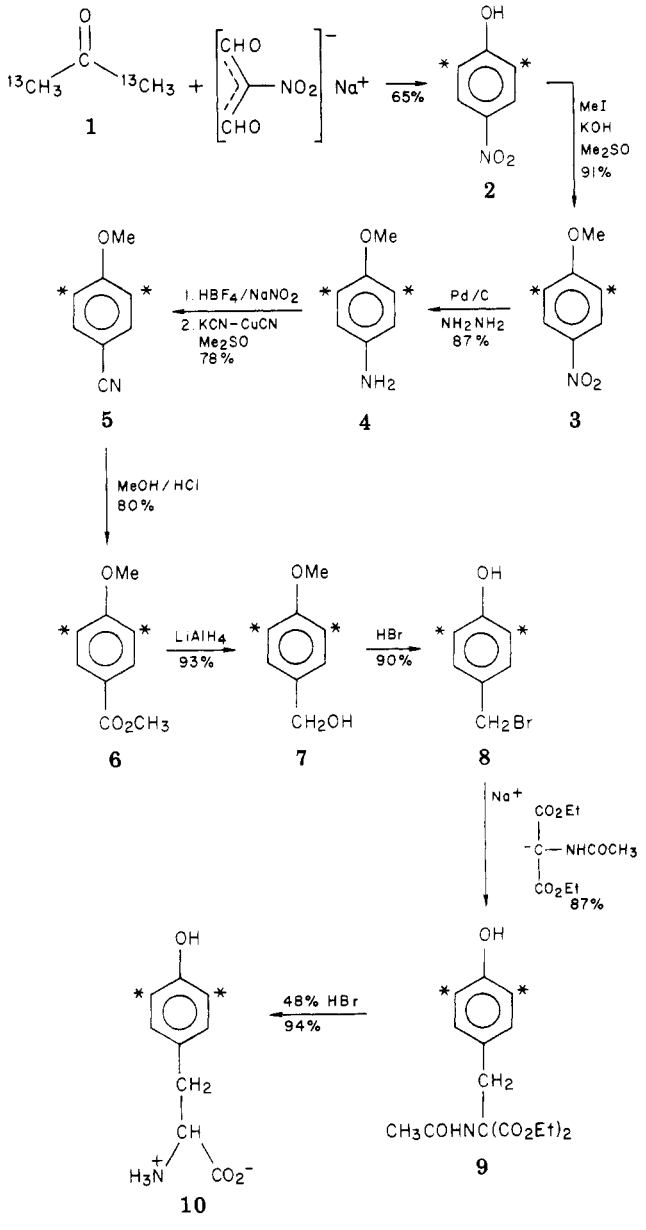
(H-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2)
1 2 3 4 5 6 7 8 9

and their derivatives with their natural carrier proteins, the neurophysins, by ^{13}C NMR.[3,4] The peptide hormones used in these studies are made by total synthesis using specifically labeled amino acids. In this connection we have begun a program to develop simple synthetic methods in which the highly enriched ^{13}C label is introduced into the desired position only[3,4,16] and can then be used for incorporation into the peptide. Most of the specifically labeled amino acid derivatives available thus far are specifically labeled in the C′ (α-carboxyl) or C_α carbon of the amino acid. However, for a number of biophysical, metabolic, and other studies it would be useful to have aromatic amino acids specifically labeled in the aromatic nucleus. In the present case we needed ^{13}C labeling in the aromatic ring of tyrosine in order to get a clear picture of the interaction at this moiety in the hormone peptide–protein complex.

In this paper we report a simple, ten-step synthesis of DL-[3′,5′-$^{13}C_2$]tyrosine (90% ^{13}C enriched), its incorporation into oxytocin and arginine-vasopressin, the separation of the diastereoisomeric peptides, and the resolution of the amino acid.

[1,3-$^{13}C_2$]Acetone[1] (**1**) was condensed with sodium nitromalonaldehyde monohydrate[17] (see Scheme I) by a slight modification of methods previously reported[18,19] to give *p*-nitro[2,6-$^{13}C_2$]phenol (**2**). Careful examination of the ^1H NMR spectra showed that ^{13}C enrichment at the 3′ and 5′ carbon atoms was about 90%. The nitrophenol **2** was smoothly converted to *p*-nitro[2,6-^{13}C]anisole (**3**), and the nitro group was then reduced in high yield with hydrazine hydrate and Pd on carbon to *p*-[2,6-$^{13}C_2$]anisidine (**4**). Highly purified **3** (see Experimental Section) must be used in the reduction to obtain the yields reported here. The attempted conversion of unlabeled *p*-anisidine to *p*-cyanoanisole by Sandmeyer reaction using a $NiCl_2$ and KCN mixture[18] under a variety of conditions gave the desired product in only 40–50% yield. However, the two-step conversion of **4** to **5** was achieved in 78% overall yield by first preparing (*p*-methoxyphenyl)diazonium fluoborate[20] and then treating this product with a solution of copper(I) cyanide and potassium cyanide in dimethyl sulfoxide, a modification of a previously reported procedure.[21] In this regard, it was noticed that the mixture of

Scheme I



potassium cyanide and copper cyanide easily formed a solution in dimethyl sulfoxide, while sodium cyanide and copper cyanide[21] which was also examined did not, and gave a correspondingly lower yield. In their paper, Kobayashi et al.[21] also reported that the sodium cyanide–copper cyanide–dimethyl sulfoxide procedure gave small amounts of biphenyls. However, we did not observe any biphenyl formation in our procedure. *p*-Cyano[2,6-$^{13}C_2$]anisole (**5**) was then smoothly converted to methyl *p*-[2,6-$^{13}C_2$]anisate (**6**) with methanolic hydrochloric acid. The ester **6** was then converted to DL-[3′,5′-$^{13}C_2$]tyrosine (**10**), using methods essentially identical with those previously developed to prepare specific deuterium labeled tyrosine[22] and DL-[2-^{13}C]tyrosine[23] (see Experimental Section). The overall yield of **10** was 22% (86+% per step).

The specifically labeled tyrosine **10** was then resolved via its trifluoroacetyl derivative using carboxypeptidase A-PMSF as previously reported.[22,23] The labeled DL-

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tyrosine derivative or the resolved amino acid was readily converted to the N^{α}-*tert*-butyloxycarbonyl (N^{α}-Boc) derivatives using the Boc-ON reagent.[24]

The specifically labeled hormones, [2-[3′,5′-$^{13}C_2$]tyrosine]oxytocin, [2-[3′,5′-$^{13}C_2$]tyrosine,8-arginine]vasopressin, and the corresponding 2-D-[3′,5′-$^{13}C_2$]tyrosine diastereoisomeric hormone analogues were prepared by the solid phase methods[22,25-28] (see Experimental Section for details). The diastereoisomeric peptide hormone derivatives were purified and separated from one another by partition chromatography on Sephadex G-25 as previously reported.[22,23] Final purification in all cases was accomplished by gel filtration on Sephadex G-25.

A preparative separation and purification and/or a check on the analytical purity of these compounds can also be accomplished by high pressure liquid chromatography (HPLC) using reverse phase systems. All of the ^{13}C-labeled oxytocin[29,30] and AVP derivatives obtained were found to be greater than 99.5% pure and were not detectably contaminated by another diastereoisomer.

[2-[3′,5′-$^{13}C_2$]Tyrosine]oxytocin had milk ejecting[31] activity identical with that of authentic oxytocin. Interestingly, [2-D-[3′,5′-$^{13}C_2$]tyrosine]oxytocin had a milk ejecting activity[31] essentially identical with native oxytocin. However, the oxytocic (uterine contracting) activity of the 2-D-tyrosine diastereoisomer was greatly reduced, being about 1/250 the activity of the native hormone, identical with that previously found[32,33] for [2-D-tyrosine]oxytocin. Similarly, [2-[3′,5′-$^{13}C_2$]tyrosine,8-arginine]vasopressin was found to possess milk ejecting activities identical with the native hormone, arginine-vasopressin.

Finally, the ^{13}C NMR spectra of DL-[3′,5′-$^{13}C_2$]tyrosine and of [2-[3′,5′-$^{13}C_2$]tyrosine]oxytocin, [2-D-[3′,5′-$^{13}C_2$]-tyrosine]oxytocin, [2-[3′,5′-$^{13}C_2$]tyrosine,8-arginine]vasopressin, and [2-D-[3′,5′-$^{13}C_2$]vasopressin were taken. In all cases, sharp (<3 Hz) single resonances were observed for the labeled carbon atoms indicating that the 3′ and 5′ carbon atoms in tyrosine and in the peptide hormones have the same chemical shift or are different but are in fast exchange on the NMR time scale.

Experimental Section

Thin-layer chromatography (TLC) was done on silica gel G plates using the following solvent system: (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 (7:7:6); (D) ethyl acetate–pyridine–acetic acid–water (5:5:1:3). The N-terminal free peptides were detected on the TLC plates using ultraviolet light, iodine vapors, ninhydrin, and fluorescamine. N^{α} protected amino acids and peptides were first treated with aqueous 6 N hydrochloric acid, heated at 100 °C for 10 min, and then detected as before. Capillary melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Literature melting points given in parentheses are for the non-^{13}C-labeled compounds. All organic solutions were dried over anhydrous sodium sulfate before rotary evaporation. Optical rotation values were measured at the mercury green line (546 nm) using a Zeiss Old 4 polarimeter or at the mercury green line or sodium D line on a Perkin-Elmer 241-MC polarimeter. Nuclear magnetic resonance (NMR) spectra were obtained using a Varian T-60 spectrometer or a Bruker WH-90 FT NMR spectrometer. Elemental analysis were performed by Chemalytics, Inc. Amino acid analyses were obtained by the method of Spackman, Stein, and Moore[34] on a Beckman 120C amino acid analyzer after hydrolysis in 6 HCl for 22–24 h at 110 °C. Partition chromatography[35] purification of oxytocin and arginine-vasopressin derivatives was performed on Sephadex G-25 (block polymerisate). Purification of solvents to remove metals and other contaminants was performed as previously described.[36] N^{α}-Boc protected amino acids and amino acid derivatives were purchased from Vega-Fox Biochemical Co. or from Biosynthetica or were prepared by published procedures except as discussed below. Before use, all amino acids were checked for purity by melting point, by thin-layer chromatography in solvent systems A, B, and C, and by the ninhydrin test of Kaiser et al.[37] Following partition chromatography, detection of peptides in eluents was made using UV spectroscopy (280 or 260 nm). The desired peptide fractions were isolated by addition of deionized water to the organic solvents, followed by rotary evaporation in vacuo at 25–30 °C and lyophilization of the aqueous solution. The high-pressure liquid chromatography was performed by methods similar to those used previously.[16,29,30]

***p*-Nitro[2,6-$^{13}C_2$]phenol (2).** To a mixture of [1,3-$^{13}C_2$]acetone (4.85 g) and sodium nitromalonaldehyde[17] (7.0 g) in 700 mL of cold water was added 15.6 mL of 25% NaOH. The mixture was stored in a refrigerator at 5 °C for 5 days and then at room temperature for 10 days. Then CO_2 was slowly bubbled into the red solution for 3 days. The mixture was extracted with ether (6 × 150 mL). The combined ether solution was washed with water (3 × 50 mL), the organic phase was dried, and the solvent was stripped off in vacuo at 25 °C to give 7.5 g (65%) of **2**: mp 106–110 °C (lit.[18,19] mp 108–109 °C); NMR ($CDCl_3$) δ 5.50 (m, 1 H), 7.9–8.4 (m, 4 H).

***p*-Nitro[2,6-$^{13}C_2$]anisole (3).** A mixture of the labeled *p*-nitrophenol **2** (8.9 g), potassium hydroxide (15.8 g), and methyl iodide (17.2 mL) in dimethyl sulfoxide (70 mL) was stirred for 2 h. The mixture was diluted with 300 mL of ice-cold water and extracted with ether (4 × 50 mL). The organic solution was washed with water (2 × 50 mL), and the solvent was removed to give 9.28 g of **3**, mp 50–51 °C (lit.[38] mp 54 °C). Small amounts of a colored impurity were removed by chromatography of the product through a column of silica gel using methylene chloride–petroleum ether (30–60 °C) (1:1) as eluent to give 8.9 g (91%) of **3**: NMR ($CDCl_3$) δ 3.89 (s, 3 H), 5.57 (m, 1 H), 8.0–8.4 (m, 3 H).

***p*-[2,6-$^{13}C_2$]Anisidine (4).** To a warm solution of the labeled *p*-nitroanisole **3** (12.0 g) in 160 mL of 95% ethanol at 60 °C containing 280 mg of 10% Pd/C was added dropwise 20 mL of 85% hydrazine hydrate. The reaction became very vigorous at the end of the addition. After the vigorous reaction had subsided, the addition was continued, and then the mixture was heated at reflux for 2 h. The Pd/C catalyst was filtered off, and the organic solution was evaporated to dryness on a rotary evaporator in vacuo. The residue was taken up in 100 mL of ether, and the organic solution was washed with water (2 × 30 mL). The solvent was removed by rotary evaporation to give 9.35 g (87%) of **4** as a white crystalline solid: mp 57–59 °C (lit.[38] mp 57 °C); NMR ($CDCl_3$) δ 3.38 (s, 2 H), 3.73 (s, 3 H), 5.44 (m, 1 H), 6.59 (bd, 2 H), 7.95 (m, 1 H).

***p*-Cyano[2,6-$^{13}C_2$]anisole (5).** To a stirred solution of 5.47 g of **4** in 22 mL of water and 22 mL of fluoboric acid was added

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a solution of 3.1 g of sodium nitrite in 6 mL of water at 10–13 °C over a 10-min period. The solution was cooled to −5 °C, and the precipitate was filtered off. The precipitate was washed with 5 mL of ice-cold dilute fluoboric acid, 6 mL of ice-cold methanol, and 50 mL of ether and then dried in vacuo to give 8.56 g (87%) of the diazofluoborate salt.

A mixture of 18 g of potassium cyanide, 21.5 g of copper(I) cyanide, and 130 mL of dimethyl sulfoxide was stirred for about 30 min until a viscous solution was obtained. The mixture was cooled to 14 °C in an ice-water bath, and a solution of fluoborate salt in 65 mL of dimethyl sulfoxide was added dropwise over a 45-min period maintaining the temperature at 14 °C. The ice bath was replaced by a water bath at 22 °C, and the mixture was stirred for an additional 45 min. The red solution was poured into 500 mL of ice-water. The precipitate was filtered off and washed several times with ether to dissolve the organic product. The aqueous mother liquor was extracted with ether (3 × 100 mL). The combined organic phase was washed three times with 50 mL of water, and the solvent was removed to give 4.6 g (90%) of **5**: mp 56–58 °C (lit.[18] mp 58 °C); NMR ($CDCl_3$) δ 3.85 (s, 3 H), 5.57 (m, 1 H), 7.47 (bd, 2 H), and 8.25 (m, 1 H).

Methyl *p*-[3,5-$^{13}C_2$]Anisate (6). The labeled nitrile **5** was added to 100 mL of a solution of methanol saturated with dry HCl and stirred overnight. Air was bubbled through the red solution for 1 h, and the solvent was removed by rotary evaporation in vacuo. The residue was dissolved in 30 mL of methanol and diluted with 50 mL of water, and a small amount of precipitate was filtered off. After the mixture was left standing for 2 days, the precipitate was filtered off and dried. This was dissolved in 50 mL of ether and filtered, and the filtrate was evaporated to dryness to give 4.6 g (86%) of **6**: mp 48–49 °C (lit.[38] mp 49 °C); NMR ($CDCl_3$) δ 3.78 (s, 3 H), 3.81 (s, 3 H), 5.44 (m, 1 H), 7.7–8 (m, 3 H).

***p*-Methoxy[3,5-$^{13}C_2$]benzyl Alcohol (7).** The above ester **6** (4.53 g) was treated with 1.2 g of lithium aluminum hydride in 100 mL of anhydrous ether and worked up in the usual manner to give 3.5 g (93%) of the alcohol **7** as a colorless oil: NMR ($CDCl_3$) δ 3.30 (bs, 1 H), 3.65 (s, 3 H), 4.39 (bs, 2 H), 5.39 (m, 1 H), 7.11 (bd, 2 H), 8.03 (m, 1 H).

***p*-Hydroxy[3,5-$^{13}C_2$]benzyl Bromide (8).** A solution of 3.3 g of the labeled alcohol **7** was taken up in 90 mL of benzene and 20 mL of benzene was distilled off. The solution was cooled in an ice-water bath and dry HBr was bubbled into the solution for 1 h and 40 min. The solution was stirred an additional 2 h and then worked up in the usual manner to give 4.3 g (90%) of **8**: bp 125–126 °C (15 mm); NMR ($CDCl_3$) δ 3.72 (s, 3 H), 4.45 (s, 2 H), 5.43 (m, 1 H), 7.17 (bd, 2 H), and 8.05 (m, 1 H). The product was immediately used for the preparation of **9** (see below).

Diethyl 2-Acetamido-2-(*p*-methoxy[3′,5′-$^{13}C_2$]benzyl)-malonate (9). To 45 mL of absolute ethanol containing sodium ethoxide (from 0.53 g of sodium) was added 4.98 g of ethyl acetamidomalonate. The solution was cooled in an ice-water bath and 4.3 g of **8** was added. After the solution was stirred for 5 h, 120 mL of water was added, and the product was filtered off and dried in vacuo to give 6.7 g (87%) of **9**: mp 96–97 °C (lit.[22] mp 94.5–95.5 °C); NMR ($CDCl_3$) δ 1.26 (t, 6 H), 2.0 (s, 3 H), 3.55 (s, 2 H), 3.72 (s, 3 H), 4.18 (q, 4 H), 5.40 (m, 1 H), 6.57 (bs, 1 H), 6.87 (bd, 2 H), 8.06 (m, 1 H).

DL-[3′,5′-$^{13}C_2$]Tyrosine (10). The above diester **9** (5.95 g) was refluxed in 60 mL of 48% aqueous HBr for 4 h. The solution was filtered and evaporated to dryness in vacuo. The residue was dissolved in 35 mL of water, and the pH was adjusted to 7 using concentrated NH_4OH. The precipitate was filtered off and dried in vacuo to give 3.0 g (94%) of **10**. TLC in solvent systems A and B gave single uniform spots at an R_f identical with that of authentic unlabeled L-tyrosine.

N^{α}-Boc-DL-[3′,5′-$^{13}C_2$]Tyrosine Hemihydrate (11). A mixture of 1.5 g of DL-[3′,5′-$^{13}C_2$]tyrosine (**10**), 2 mL of purified triethylamine, and 2.2 g of Boc-ON (Aldrich Chemical Co., Milwaukee, Wis.) in 14 mL of aqueous acetone (1:4 water–acetone, v/v) was stirred at 45 °C for 1.5 h. Then an additional 0.6 mL of triethylamine in 10 mL of 10% aqueous acetone was added, and the mixture was stirred for another 2 h. The reaction mixture was filtered to give 70 mg of unreacted labeled tyrosine, and the precipitate was washed with 5 mL of water. The combined filtrate was evaporated to dryness in vacuo. The residue was dissolved in 50 mL of water, and the aqueous solution was extracted with ethyl acetate (3 × 20 mL) and ether (2 × 20 mL). The aqueous phase was filtered, and then the pH was adjusted to 3 with citric acid. The precipitate was filtered off, washed with water, and then recrystallized from ethyl acetate–hexane to give 1.7 g (77%) of **11** as white powder: mp 113–114 °C; NMR ($CDCl_3$) δ 1.44 (s, 9 H), 2.79–3.08 (m, 2 H) 4.05–4.45 (m, 1 H), 5.38 (m, 1 H), 5.58–5.87 (bd, 1 H), 6.94 (bd, 2 H), 7.93 (m, 1 H); TLC in solvent system A, B, and C gave single uniform spots at an R_f identical with that of the authentic unlabeled N^{α}-Boc-tyrosine. Anal. Calcd for $C_{12}{}^{13}C_2H_{19}NO_2{}^{\cdot 1}/_2H_2O$: C, 58.21; H, 6.84; N, 4.29. Found: C, 58.66; H, 7.05; N, 4.29.

Resolution of DL-[3′,5′-$^{13}C_2$]Tyrosine (11a, 11b). DL-[3′,5′-$^{13}C_2$]Tyrosine (1.72 g) was converted to 2.54 g (97%) of *N*-trifluoroacetyl-DL-[3′,5′-^{13}C]tyrosine, mp 180–183 °C. This was resolved using carboxypeptidase A PMSF as described elsewhere.[22] There was obtained 0.71 g (86%) of [3′,5′-$^{13}C_2$]tyrosine: $[\alpha]^{24}_{546}$ −10.0° (*c* 2.0, 1 N HCl) [lit.[39] $[\alpha]^{25}_{D}$ −10.00° (*c* 2.00, 5 N HCl)]; TLC in solvent systems A and B showed single spots identical with those of authentic L-tyrosine. There was also obtained 0.54 g (65%) of D-[3′,5′-$^{13}C_2$]tyrosine: $[\alpha]^{24}_{546}$ +10.1° (*c* 2.0, 1 N HCl); TLC in solvent systems A and B showed single spots with R_f values identical with those of authentic tyrosine.

[2-DL-[3′,5′-$^{13}C_2$]Tyrosine,8-arginine]vasopressin and Separation of the Diastereoisomers. The synthesis of the protected nonapeptide precursor to the title compounds, H-Cys(DMB)-DL-[3′,5′-$^{13}C_2$]Tyr-Phe-Gln-Asn-Cys(DMB)-Pro-Arg(Tos)-Gly-NH_2, was carried out by solid-phase procedures[22] using 2.2 g of Boc-glycinate-O-resin which had a substitution level of 0.40 mmol of glycine/g of resin. All coupling reactions and other solid-phase procedures were done as previously shown for arginine-vasopressin derivatives,[22] except that N^{α}-Boc-DL-[3′,5′-$^{13}C_2$]tyrosine was coupled to the growing peptide chain utilizing a single overnight coupling step with 1.2 mmol each of the amino acid and dicyclohexylcarbodiimide (DCC). The peptide was cleaved from the resin by ammonolysis to give 700 mg of the protected nonapeptide (mp 195–200 °C), and a 350-mg portion was deprotected with Na in liquid ammonia followed by oxidation in the usual manner.[22] The diastereoisomers were separated and purified by partition chromatography on Sephadex G-25 using the solvent system 1-butanol–ethanol–water (containing 3.5% HOAc and 1.5% pyridine) (4:1:5) to give the D-Tyr^2-containing and all L-labeled diastereoisomers (R_f 0.19 and 0.12, respectively) followed by gel filtration on Sephadex G-25 using 20% HOAc as eluent solvent. There was obtained 57 mg of [2-D-[3′,5′-$^{13}C_2$]-tyrosine,8-arginine]vasopressin as a white powder. Amino acid analysis gave the following molar ratios: Arg, 1.00; Asp, 1.07; Glu, 1.09; Pro, 0.97; Gly, 0.98; half-Cys, 1.81; Tyr, 0.87; Phe, 1.01. TLC in solvent systems A, B, and D gave single uniform spots with R_f values the same as those of authentic [2-D-tyrosine]AVP.[22,32] On HPLC, the product gave a single uniform peak widely separated from authentic AVP. There was also obtained 37 mg of [2-[3′,5′-$^{13}C_2$]tyrosine,8-arginine]vasopressin as a white powder. Amino acid analysis gave the following molar ratios: Arg, 1.00; Asp, 1.01; Glu, 1.00; Pro, 1.08; Gly, 1.02; half-Cys, 1.91; Tyr, 0.88; Phe, 1.02. HPLC gave a single uniform peak identical with that of authentic AVP.

Milk ejecting activities were determined,[31] and [2-[3′,5′-$^{13}C_2$]tyrosine,8-arginine]vasopressin had 100 U/mg of activity identical with that of authentic AVP. [2-D-[3′,5′-$^{13}C_2$]Tyrosine,8-arginine]vasopressin was slightly less active than the former one.

[2-DL-[3′,5′-$^{13}C_2$]Tyrosine]oxytocin and Separation of the Diastereoisomers. The synthesis of the protected nonapeptide of the title compound, namely H-Cys(DMB)-DL-[3′,5′-$^{13}C_2$]-Tyr-Ile-Gln-Asn-Cys(DMB)-Pro-Leu-Gly-NH_2, was carried out by the solid-phase method using 2.2 g of Boc-glycinate-O-resin which had a substitution level of 0.40 mmol of glycine/g of resin. Coupling reactions and other solid-phase procedures followed methods previously used in the synthesis of other diastereoisomeric oxytocin peptides.[27,32] N^{α}-Boc-DL-[3′,5′-$^{13}C_2$]tyrosine was coupled to the growing peptide chain using two 12-h couplings, the first with 1.5 mmol each of the protected amino acid and DCC

(39) "Handbook of Biochemistry and Molecular Biology", Vol. 1, 3rd ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1976.

and the second using 0.5 mmol of each of the reagents. Once the synthesis was completed, the protected nonapeptide from above was obtained in the usual manner[27,32] to give 650 mg of the nonapeptide. A 320-mg (0.25 mmol) sample was treated with Na in liquid ammonia, oxidized to the disulfide products, and then purified by partition chromatography on Sephadex G-25 using 1-butanol–H_2O (containing 3.5% HOAc in 1.5% pyridine) (1:1) to give the purified, specifically labeled all-L- and D-Tyr[2] diastereoisomers with R_f values of 0.23 and 0.41, respectively. Each diastereoisomer was further purified by gel filtration on Sephadex G-25 using 20% aqueous HOAc as eluent solvent. There was obtained 71 mg of [2-D-[3′,5′-$^{13}C_2$]tyrosine]oxytocin as a white powder. Amino acid analysis gave the following molar ratios: Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; half-Cys, 1.8; Ile, 1.0; Leu, 1.0; Tyr, 0.90. TLC in solvent systems A, B, and C gave single uniform spots, identical with those of authentic [2-D-tyrosine]oxytocin.[32] HPLC gave a single uniform spot in the position previously reported[29,30] and no observable (<0.5%) all-L diastereoisomer. There also was obtained 60 mg of [2-[3′,5′-$^{13}C_2$]tyrosine]oxytocin as a white powder. Amino acid analysis gave the following molar ratios: Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; half-Cys, 1.9; Ile, 1.0; Leu, 1.0; Tyr, 0.93. TLC in solvent systems A, B, and C gave single uniform spots with R_f values identical with those of authentic oxytocin. HPLC gave a single uniform peak identical with that of oxytocin[29,30] with no trace (<0.5%) of the D diastereoisomer. We have previously shown the 2-diastereoisomers to be separated by over 20 min under the HPLC conditions used here.[29,30]

Milk ejecting activities were determined[31] for each diastereoisomer and [2-[3′,5′-$^{13}C_2$]tyrosine]oxytocin had about 450 U/mg of activity, identical with that of authentic oxytocin.[31] Interestingly, [2-D-[3′,5′-$^{13}C_2$]tyrosine]oxytocin had nearly 400 U/mg of activity, identical with that of authentic [D-Tyr[2]]oxytocin.

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Registry No. **1**, 7217-25-6; **2**, 70479-88-8; **3**, 70479-89-9; **4**, 70479-90-2; **5**, 70479-91-3; **6**, 70479-92-4; **7**, 70479-93-5; **8**, 70479-94-6; **9**, 70479-95-7; **10**, 70479-96-8; **11**, 70479-97-9; **11a**, 70479-98-0; **11b**, 70479-99-1; sodium nitromalonaldehyde, 34461-00-2; [(3,5-$^{13}C_2$)-*p*-methoxyphenyl]diazonium fluoborate, 70480-01-2; diethyl acetamidomalonate, 1068-90-2; [2-DL-[3′,5′-$^{13}C_2$]tyrosine 8-arginine]vasopressin, 70480-02-3; H-Cys(DMB)-DL-[3′,5′-$^{13}C_2$]Tyr-Phe-Gln-Asn-Cys(DMB)-Pro-Arg(Tos)-Gly-NH_2, 70513-46-1; [2-D-[3′,5′-$^{13}C_2$]tyrosine, 8-arginine]vasopressin, 70480-03-4; [2-[3′,5′-$^{13}C_2$]tyrosine, 8-arginine]vasopressin, 70480-04-5; 2-DL-[3′,5′-$^{13}C_2$]tyrosine]oxytocin, 70480-05-6; H-Cys(DMB)-DL-[3′,5′-$^{13}C_2$]Tyr-Ile-Gln-Asn-Cys-(DMB)-Pro-Leu-Gly-NH_2, 70513-47-2; [2-D-[3′,5′-$^{13}C_2$]tyrosine]-oxytocin, 70480-07-8; [2-[3′,5′-$^{13}C_2$]tyrosine]oxytocin, 70480-06-7; *N*-trifluoroacetyl-DL-[3′,5′-$^{13}C_2$]tyrosine, 70479-87-7.

Acid- and Base-Catalyzed Isomerization of *cis*-1,2-Diarylacrylonitriles

Emanuele Maccarone,* Antonino Mamo, Giuseppe Scarlata, and Michele Torre

Istituto Dipartimentale di Chimica e Chimica Industriale, Università di Catania, 95125 Catania, Italy

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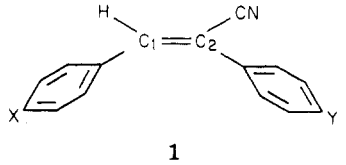
The isomerization kinetics of *cis*-1,2-*p*-substituted-diphenylacrylonitriles and *cis*-1-aryl-2-phenylacrylonitriles (Ar = 2-furyl, 2-thienyl, 2-selenophenyl) have been studied in a solution of decahydronaphthalene with methanesulfonic acid and potassium *tert*-butoxide as catalysts. The acidic isomerization is slower than the basic one and is characterized by highly positive entropy changes. The substituents produce similar effects in both reactions. The general scheme of the isomerization includes (i) nucleophilic addition of the catalyst to the double bond, (ii) free rotation around the formed single bond, and (iii) elimination of the catalyst from the trans rotamer. In the acid isomerization, the heterocycle-containing acrylonitriles react faster than the phenyl derivative owing to the greater inductive effects and the lesser steric congestion of heterocycles. A satisfactory correlation of the reactivities with a combination of polar and steric effects is observed by an appropriate two-parameter equation. In the base-catalyzed isomerization a correlation with mesomeric constants of heterocycles is found.

In previous work we have studied the isomerization kinetics of *cis*-1,2-diarylethylenes[1] and *cis*-1,2-diarylacrylonitriles,[2] in decahydronaphthalene with selenium as catalyst. It has been ascertained that the cis–trans conversion proceeds through a stepwise radical mechanism initiated by the paramagnetic biatomic selenium.[2]

Our interest is now devoted to the study of this isomerization process by other catalysts in order to measure kinetic effects and suggest plausible reaction schemes. Thus, in this paper we report the rate constants and the activation parameters for the acid- and base-catalyzed isomerizations of *cis*-1,2-*p*-substituted-diphenylacrylonitriles (1) in decahydronaphthalene.



1

X = OCH_3, CH_3, H, Cl, NO_2; Y = H
X = H; Y = OCH_3, CH_3, Cl, NO_2

Several cis-substituted ethylenes, for example, stilbenes, unsaturated acids, chalcones, and so on, have been isomerized in aqueous or organic solutions of acids[3–5] and

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