SYNTHESIS OF ^{14}C- AND ^{2}H-LABELED 1,3-DIHYDRO-3,3-DIMETHYL-5-(1,4,5,6-TETRAHYDRO-6-OXO-3-PYRIDAZINYL)-2H-INDOL-2-ONE (LY195115), AN ORALLY EFFECTIVE POSITIVE INOTROPE.

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SUMMARY

We have synthesized ^{14}C- and ^{2}H-labeled 1,3-dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2H-indol-2-one (LY195115), an extremely potent, orally-effective cardiotonic with inotropic and vasodilator activities. The ^{14}C-label was introduced in the antepenultimate step by reaction of a β-chloroketone precursor with $Na^{14}CN$; acid-catalyzed hydrolysis and cyclization with hydrazine provided the tetrahydropyridazinone bearing the ^{14}C-label in the oxo-carbon. 1,3-Dihydro-3,3-di(methyl-d_3)-2H-indol-2-one was prepared by exhaustive methylation of 1-acetyl-1,3-dihydro-2H-indol-2-one with sodium hydride and iodomethane-d_3, followed by removal of the nitrogen protecting group. This labeled material was converted in two steps to [$^{2}H_6$]-LY195115.

Key Words: Carbon-14, Deuterium, LY195115, positive inotrope, cardiotonic

INTRODUCTION

The discovery and development of orally effective cardiotonics with combined inotropic and vasodilator activities for the treatment of congestive heart failure has been the focus of an enormous research effort during the past decade.[1,2] A large number of these dual-activity cardiotonics, including milrinone,[3,4] LY175326,[5-7] piroximone,[8] and CI-914,[9,10] are being clinically evaluated.

The hypotensive and antithrombotic activities of the

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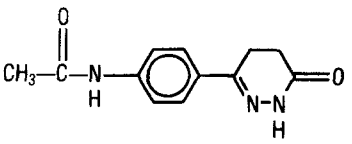
0362-4803/86/040343-12$06.00

Received August 19, 1985

3-phenyltetrahydropyridazin-6-ones have been investigated for almost two decades.[11] Extensive SAR studies demonstrated that compounds bearing a 4-acetamido substituent such as 1 (N-[4-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)phenyl]acetamide) were optimal for inhibiting platelet aggregation and reducing blood pressure in rodents.[12-14]



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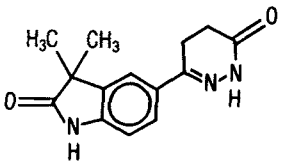


2, LY195115

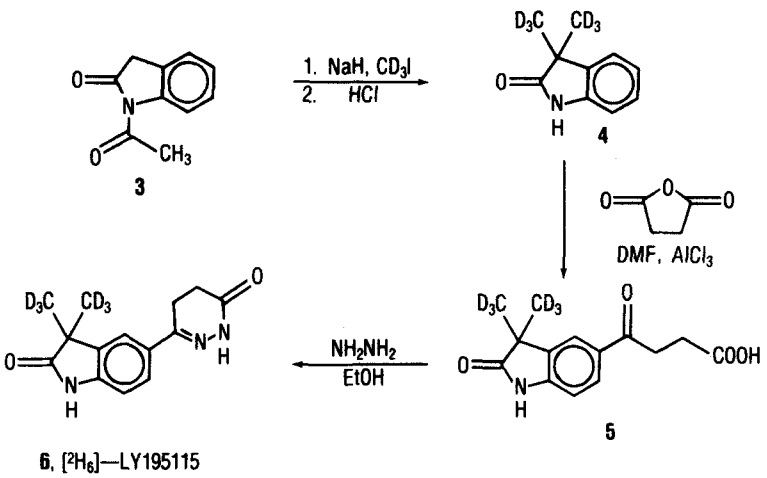
We recently discovered that the most salient pharmacology of this class of compounds in higher mammals is a positive inotropic effect, rather than an antithrombotic or antihypertensive effect. We then prepared a series of bicyclic analogs of 1. The optimal compound of the series, 2 (LY195115), was found to be an extremely potent inotrope; oral administration of only 25 μg/kg to conscious dogs increased contractility by 50%. Moreover, the increased contractility was still evident 24 hours post administration.[15] Because of its promising pharmacological profile and lack of toxicity in extensive animal studies, LY195115 is being developed for the chronic management of heart failure.[15,16]

This report describes the synthesis of ^{14}C- and ^{2}H-labeled LY195115 to enable studies on the metabolism and disposition of this promising cardiotonic.

RESULTS AND DISCUSSION

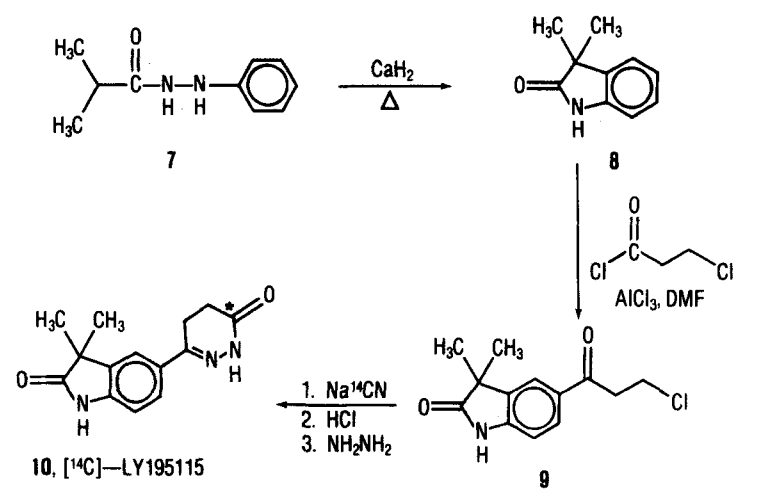
We chose to perdeuterate the geminal dimethyl substituents of LY195115 since this would allow introduction of 6 equivalent deuteria in a metabolically stable site. The five-step preparation of [$^{2}H_6$]-LY195115 (6) is depicted in Scheme 1. The nitrogen of 1,3-dihydro-2H-indol-2-one was protected by reaction with acetic

Scheme 1: Synthesis of ^{2}H-Labeled LY195115



3 4 5 6, [$^{2}H_6$]—LY195115

Scheme 2: Synthesis of ^{14}C-Labeled LY195115



7 8 9 10, [^{14}C]—LY195115

* Denotes ^{14}C-label

anhydride to form 1-acetyl-1,3-dihydro-2H-indol-2-one (3) in 84% yield. Exhaustive methylation of 3 with iodomethane-d_3 and sodium hydride (64.4% yield), followed by acid-catalyzed removal of the protecting group (86.4% yield), provided the di(methyl-d_3) analog 4 (>99 atom % D by mass spectral analysis). After this work was completed, we became aware of some indolone dianion chemistry developed by Kende[17] which would obviate the need for using the nitrogen protection-deprotection sequence.

The tetrahydropyridazinone moiety was then appended to the labeled indol-2-one 4 using standard synthetic procedures.

Friedel-Crafts acylation of 4 with succinic anhydride in an aluminum chloride/DMF melt[14] provided the 4-oxobutanoic acid 5 in 78% yield. Cyclization of 5 with hydrazine in refluxing ethanol led to the precipitation of pure [$^{2}H_{6}$]-LY195115 (6) in 85% yield.

We chose to radiolabel the pyridazinone oxo-carbon since we could find no literature precedent for metabolic cleavage of pyridazinone rings, and this carbon could be introduced late in the synthetic sequence. Scheme 2 depicts our five-step synthesis of [^{14}C]-LY195115 (10). The unlabeled dimethylindolone 8 was prepared in 98% yield by rearrangement[18] of 2-methylpropanoic acid, 2-phenylhydrazide. Instead of the two-step method for construction of the tetrahydropyridazinone ring (vide supra), we employed a four-step sequence which allowed introduction of the label in the antepenultimate step using readily available Na^{14}CN. The indolone 8 was treated with 3-chloropropanoic acid in the presence of aluminum chloride/DMF to provide the 3-chloroketone 9 (75% yield). This material was reacted with ^{14}C-labeled sodium cyanide, and the resulting nitrile was hydrolyzed to the carboxylic acid. The overall yield for these 2 steps was 43%. Finally, hydrazine cyclization provided [^{14}C]-LY195115 (10) in 61% yield after recrystallization from DMF/water. The radiochemical purity of this material was 99.4% and the specific activity was 3.13 mCi/mmol.

CONCLUSIONS

In this paper we have detailed the efficient preparation of ^{14}C- and ^{2}H-labeled LY195115, a cardiotonic with combined inotropic and vasodilator properties that is being developed for the chronic management of congestive heart failure. [$^{2}H_{6}$]-LY195115 (6), in conjunction with quantitative mass spectrometric assay procedures involving the reverse stable isotope dilution approach, can be employed in studying the pharmacokinetics and absolute bioavailability of LY195115.[19] The

methodology we devised for the synthesis of [^{14}C]-LY195115 (10) allowed the introduction of the radiolabel in the antepenultimate step. After introduction of the label, only one purification was necessary to obtain material of 99.4% radiochemical purity. This labeling methodology should prove useful for the synthesis of other radiolabeled pyridazines and partially reduced pyridazines, the fundamental structural elements of a number of biologically active substances.[20]

The isotopomers described herein have permitted a variety of biochemical, metabolic, and pharmacokinetic studies of LY195115.[21] These experiments will be reported in due course.

EXPERIMENTAL

Methods

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are not corrected. Proton magnetic resonance (^{1}H-NMR) spectra were taken on a Bruker WM270 spectrometer. Chemical shifts are reported in ppm downfield from a tetramethylsilane internal standard (δ scale). The ^{1}H-NMR data are presented in the form: (solvent in which spectra were taken), δ value of signal (peak multiplicity, integrated number of protons, coupling constant (if any), and assignment). Routine mass spectra were recorded from a Varian MAT CH-5 spectrometer, at the ionization voltage expressed in parentheses. Only the peaks of high relative intensity or of diagnostic importance are presented in the form: m/e (intensity relative to base peak). High resolution exact mass determinations were recorded from a VG Analytical VG-ZAB3F spectrometer. Microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories.

Except where noted, a standard procedure was used for product isolation. This involved quenching by addition to water, filtration or exhaustive extraction with a solvent (washing of extract with aqueous solutions, on occasion), drying over an

anhydrous salt, and evaporation of solvent under reduced pressure. Particular solvents, aqueous washes (if needed), and drying agents are mentioned in parentheses after the phrase "product isolation."

Radiochemical purity was measured by autoradiography employing E. Merck Silica Gel 60 F-254 TLC plates and Kodak X-ray film BB-5, and by HPLC performed on a Waters 6000A chromatograph using a 4.5 mm x 25 cm Alltech Spherisorb S-5-005 ODS column eluted with 1% ammonium acetate in 80% H_2O/20% THF (w/v) at 2500 psi and at a flow rate of 1 ml/min; the detector was a Waters 440 operated at 315nm. For the HPLC determinations, equal fractions from the column were collected in vials containing PCS scintillation fluid (Amersham), and the radioactivity was measured in a Packard Model 3375 Liquid Scintillation Spectrometer.

Syntheses

1-Acetyl-1,3-dihydro-2H-indol-2-one (3) - A mixture of 1,3-dihydro-2H-indol-2-one (89.59 g, 673 mmol) and acetic anhydride (69.4 mL, 740 mmol) was heated to and maintained at reflux overnight. The homogeneous reaction was cooled to room temperature. Recrystallization of the resulting solid from ethyl acetate provided 99.1 g (84.2%) of 3 as white crystals: mp 127.5-128°C (lit.[22] mp 126°C); ^{1}H-NMR ($CDCl_3$) δ 2.69 (s, 3H, $-COCH_3$), 3.72 (s, 2H,$-CH_2-$), 7.16-8.22 (m, 4H, ArH); mass spectrum (70 eV) m/e (rel intensity) 175 (28, M^+), 133 (100).

Anal. Calcd for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00.

Found: C, 68.36; H, 5.02; N, 7.97.

1-Acetyl-1,3-dihydro-3,3-di(methyl-d_3)-2H-indol-2-one - A solution of 1-acetyl-1,3-dihydro-2H-indol-2-one (40.2 g, 230 mmol) in 500 mL DMF was added over a 1 hour period to a mixture of sodium hydride (18.9 g of a 60% dispersion in oil, 471 mmole; oil removed by hexane trituration) in 100 mL DMF at 0°C. Thirty minutes following the cessation of hydrogen evolution,

iodomethane-d_3 (75 g, 517 mmol, >99 atom % D, Aldrich) was added in a dropwise fashion and then the reaction was allowed to warm to room temperature. After stirring for 2.5 hours at room temperature, product isolation (water, ethyl acetate, water, brine, Na_2SO_4) yielded 39 g of material. Recrystallization from ethyl acetate/hexane provided 30.9 g (64.4%) of product as white crystals: mp 114-115°C; ^{1}H-NMR ($CDCl_3$) δ 2.72 (s, 3H,-$COCH_3$), 7.18-7.36 (m, 3H, ArH), 8.26 (d, 1H, ArH); mass spectrum (70eV) m/e (rel intensity) 209 (19, M^+), 167 (100), 149 (86).

Anal. Mol wt calcd for $C_{12}H_7D_6NO_2$: 209.1323. Found: 209.1325.

1,3-Dihydro-3,3-di(methyl-d_3)-2H-indol-2-one (4) - A mixture of 1-acetyl-1,3-dihydro-3,3-di(methyl-d_3)-2H-indol-2-one (27.24 g, 130 mmol) and 6N hydrochloric acid (300 mL) was refluxed 1 hour. The reaction was cooled to room temperature, diluted with water, and cooled to 0°C. The resulting precipate was filtered and dried *in vacuo* to yield 18.83 g (86.4%) of 4 as a white powder: mp 137°C; ^{1}H-NMR ($CDCl_3$) δ 6.92-7.16 (m, 4H, ArH); mass spectrum (70 eV) m/e (rel intensity) 167 (98, M^+) 149 (100).

Anal. Mol wt calcd for $C_{10}H_5D_6NO$: 168.1295 (FAB, $M^+ + H$). Found: 168.1289.

2,3-Dihydro-3,3-di(methyl-d_3)-γ,2-dioxo-1H-indole-5-butanoic acid (5) - Dimethylformamide (24.6 mL, 312 mmol) was added in a dropwise fashion to anhydrous aluminum chloride (149.0 g, 1.12 mmol), and the exothermic reaction was then allowed to cool to room temperature.[14] An intimate mixture of succinic anhydride (11.2 g, 112 mmol) and 4 (18.65 g, 112 mmol) was slowly added to the $AlCl_3$/DMF melt. The reaction mixture was stirred 1 hour at room temperature, and then 1.5 hours at 70°C. The reaction was slowly poured onto ice and 100 mL of concentrated hydrochloric acid was added. After cooling to 0°C, the precipitate was filtered and recrystallized from DMF/water to afford 23.33 g (78.3%) of

product as a light yellow powder: mp 225-226°C; ^{1}H-NMR (DMSO-d_6) δ 2.55 (t, 2H, -CH_2COOH), 3.20 (t, 2H, -CH_2COAr), 6.94 (d, 1H, ArH ortho to N), 7.86 (d, 1H, ArH at C-6), 7.90 (s, 1H, ArH at C-4); mass spectrum (70 eV) m/e (rel intensity) 267 (63, M^+), 194 (100).

Anal. Mol wt calcd for $C_{14}H_9D_6NO_4$: 268:1456 (FAB, M^+ + H). Found: 268.1468.

1,3-Dihydro-3,3-di(methyl-d_3)-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2H-indol-2-one ([$^2H_6$]-LY195115, (6) - A mixture of 5 (19.1 g, 72 mmol) and 85% hydrazine hydrate (9.3 mL, 157 mmol) in 500 mL absolute ethanol was refluxed 3 hours and then the homogeneous reaction was slowly cooled to 0°C. The resulting precipitate was filtered, washed with ethanol and dried in vacuo to afford 15.92 g (84.6%) of product as a pale yellow powder: mp >300°C; ^{1}H-NMR (DMSO-d_6) δ 2.42 (t, 2H, -CH_2CONH), 2.92 (t, 2H, -CH_2C=N), 6.85 (d, 1H, ArH ortho to NH), 7.56 (d, 1H, ArH at C-6), 7.70 (s, 1H, ArH at C-4); mass spectrum (70 eV) m/e (rel intensity) 263 (100, M^+), 245 (47).

Anal. Mol wt calcd for $C_{14}H_9D_6N_3O_2$: 264.1619 (FAB, M^+ + H). Found: 264.1620.

2-Methylpropanoic acid, 2-phenylhydrazide (7) - A solution of isobutyryl chloride (104.8 mL, 1 mole) in 100 mL DMF was added in a dropwise fashion to a solution of phenyl hydrazine (98.4 mL, 1 mole) and pyridine (88.9 mL, 1.1 mole) in 200 mL DMF at 0°C. The reaction was then allowed to warm to room temperature and stirred overnight. Product isolation (1N hydrochloric acid, ethyl acetate, 1N hydrochloric acid, water, brine, $MgSO_4$) and recrystallization from ethyl acetate provided 133 g (74.7%) of product: mp 130-135°C (lit. [23] 139-140°C); ^{1}H-NMR ($CDCl_3$) δ 1.22 (d, 6H, -CH_3), 2.46 (m, 1H, CH), 6.80-7.26 (m, 5H, ArH); mass spectrum (70 eV) m/e (rel intensity) 178 (32, M^+), 108 (100).

Anal. Calcd for $C_{10}H_{14}N_2O$: C, 67.39; H, 7.92; N, 15.72. Found: C, 67.30; H, 7.66; N, 15.55.

1-3-Dihydro-3,3-dimethyl-2H-indol-2-one (8) - A mixture of 7 (96.42 g, 542 mmol) and calcium hydride (34.15, 813 mmol) was slowly heated.[18] At ca. 190°C, a vigorous, exothermic reaction ensued, accompanied with the evolution of ammonia. The reaction was cautiously heated to 230°C and this temperature was maintained for 0.5 hours. The reaction was cooled to room temperature and a solution of 120 mL water in 300 mL methanol was added in a dropwise fashion. After gas evolution ceased, the mixture was acidified to pH 1 with concentrated hydrochloric acid. The mixture was warmed for 0.5 hour on a steam bath, taken to pH 5 with 5N sodium hydroxide, and cooled to 0°C overnight. The product was filtered and dried to afford 85.7 g (98%) of homogeneous material. Recrystallization from THF/hexane provided 71.6 g (82%) of product: mp 147-151°C (lit.[24] mp 150°C); ^{1}H-NMR ($CDCl_3$) δ 1.41 (s, 6H, $-CH_3$), 6.94-7.22 (m, 4H, ArH); mass spectrum (70 eV) m/e (rel intensity) 161 (68, M^+), 146 (100).

Anal. Calcd for $C_{10}H_{11}NO$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.27; H, 6.93; N, 8.59.

5-(3-Chloro-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2H-indol-2-one (9) - Dimethylformamide (18.0 mL, 230 mmol) was added in a dropwise fashion to anhydrous aluminum chloride (109.0 g, 820 mmol) to form a melt.[14] After the exothermic reaction had cooled to 30°C, 8 (13.2 g, 82 mmol) was added in portions. 3-Chloropropionyl chloride (7.83 mL, 82 mmol) was then added in a dropwise fashion and the resulting mixture was stirred one hour at room temperature. The reaction mixture was slowly added to 500 mL ice and then 100 mL concentrated hydrochloric acid was added. The mixture was cooled to 0°C and the precipitate was collected by filtration. Recrystallization from acetone afforded 15.46 g (75%) of 9 as a light yellow powder: mp 190°C dec.; ^{1}H-NMR ($CDCl_3$) δ 1.46 (s, 6H, $-CH_3$), 3.45 (t, 2H, $-CH_2Cl$), 3.95 (t, 2H, $-CH_2CO$), 7.02 (d, 1H, ArH ortho to NH), 7.86 (s, 1H, ArH at C-4), 7.89

(d, 1H, ArH at C-6); mass spectrum (70 eV) m/e (rel intensity) 251 (40, M^+), 215 (53), 188 (100).

Anal. Calcd for $C_{13}H_{14}ClNO_2$: C, 62.03; H, 5.61; N, 5.56. Found: C, 62.32; H, 5.88; N, 5.52.

1,3-Dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl-6-^{14}C)-2H-indol-2-one ([^{14}C]-LY195115, 10) - A mixture of the chloroketone 9 (1006 mg, 4.0 mmol), ^{14}C-labeled sodium cyanide (Pathfinder Lab; 20.4 mg; 0.402 mmol; 19.90 mCi; sp. act. = 49.8 mCi/mmol), unlabeled sodium cyanide (176 mg, 3.598 mmol), and 4 mL DMSO was stirred at room temperature overnight. TLC analysis indicated only starting material. Water (2 mL) was added to dissolve the sodium cyanide, and after 2 hours TLC analysis indicated the homogeneous reaction was complete. Product isolation (water, ethyl acetate, water, brine, $MgSO_4$) afforded 1.037 g of the ^{14}C-labeled nitrile. TLC analysis (silica gel, ethyl acetate/hexane (1/1)) indicated primarily nitrile (R_f = 0.21) with only a trace of starting material (R_f = 0.06).

A mixture of crude nitrile (1.037 g), 10 mL of 6N hydrochloric acid, and 10 mL methanol was refluxed 1 hour. The methanol was distilled from the reaction and replaced with 10 mL of 6N hydrochloric acid. The reaction was refluxed for an additional 2 hours, cooled to room temperature, and the precipitate was filtered. This product was dissolved in 30 mL of warm methanol, treated with charcoal, filtered through celite, and then the solvent was removed in vacuo. The residue was triturated with warm ethyl acetate and dried in vacuo at 45°C overnight to afford 0.453 g (1.72 mmol; 43% for 2 steps) of ^{14}C-labeled carboxylic acid. TLC analysis (methylene chloride/methanol; 9/1; R_f = 0.22) indicated the product was homogeneous.

A solution of the carboxylic acid (0.453 g, 1.72 mmol) and hydrazine hydrate (3 mL of an 85% solution) in 8 mL ethanol was refluxed for 3 hours. The heterogeneous reaction was cooled to

room temperature and the precipitated product was filtered. Recrystallization from DMF/water (4/1) afforded 0.270 g (1.051 mmol, 61%) of product. The specific activity was 3.13 mCi/mmol. Radiochemical purity as assessed by TLC (methylene chloride/methanol; 9/1; R_f = 0.38) or HPLC was 99.4 or 98.7%, respectively.

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