SYNTHESIS OF HIGH SPECIFIC ACTIVITY CARBON-14 LABELED PD-113,926 AND CI-930, POTENTIAL NEW CARDIOTONIC AGENTS.

Sheryl J. Hays, James L. Hicks and C. C. Huang
Warner-Lambert/Parke-Davis Pharmaceutical Research
Ann Arbor, Michigan 48103

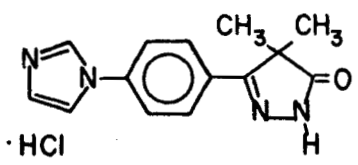
SUMMARY

Double-labeled PD-113,926 and CI-930 were synthesized from [^{14}C]MeI and [^{14}C]$BaCO_3$ respectively in high specific activity (>90 mCi/mmol). The synthesis of CI-930 employed the intermediate 2-butenenitrile-3,4-$^{14}C_2$ which was synthesized by a phase transfer Wittig procedure. This Wittig procedure is fast, produces moderate to good yields of olefin and accommodates the use of aqueous solutions of aldehydes.

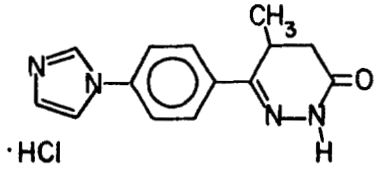
Keywords: Carbon-14, 2-Butenenitrile-3,4-$^{14}C_2$, PD-113,926, CI-930, Cardiotonic

INTRODUCTION

2,4-Dihydro-5-[4-(1H-imidazol-1-yl)phenyl]-4,4-dimethyl-3H-pyrazol-3-one monohydrochloride (PD-113,926) and 4,5-dihydro-6-[4-1H-imidazol-1-yl)phenyl]-5-methyl-3(2H)-pyridazinone monohydrochloride (CI-930) are potent cardiotonic agents.[1]



PD-113,926



CI-930

Due to the small dosage requirements of these drugs, it was necessary to make high specific activity (>90 mCi/mmol), metabolically-stable carbon-14 labeled drugs for pharmacokinetic and metabolic studies. The synthesis of these double-labeled compounds is described herein.[2]

0362-4803/87/101185-09$05.00

Received November 3, 1986

RESULTS AND DISCUSSION

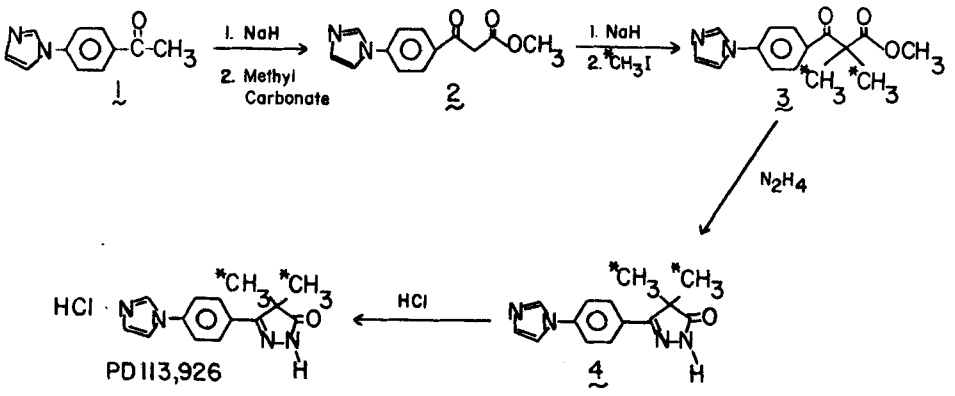
PD-113,926 was synthesized in four steps as outlined in Scheme 1. The reaction sequence of Morrison[3] was adapted for the synthesis of double-labeled carbon-14 material. A modification was made in the pyridazinone ring formation. In the literature procedure, 1.1 equivalents of hydrazine hydrate were refluxed with 3 in ethanol. When this method was attempted, the reaction was sluggish and substantial quantities of side products formed. By using five equivalents of hydrazine hydrate, the product was formed at room temperature in less than 45 minutes. The resulting product was quite clean when compared to the first method.

The unlabeled starting material 2 was made by treating commercially available 1-[4-(1H-imidazol-1-yl)phenyl]ethanone (1) with dimethyl carbonate in the presence of NaH to yield 4-(1H-imidazol-1-yl)-β-oxobenzenepropanoic acid methyl ester (2). The dianion of 2 was made with sodium hydride and allowed to react with [^{14}C]iodomethane to produce the double alkylated carbon-14 product 3. The crude 3 was mixed with hydrazine hydrate as described above to give the free base, 2,4-dihydro-5-[4-(1H-imidazol-1-yl)phenyl]-4,4-di(methyl-$^{14}C_2$)-3H-pyrazol-3-one (4). The hydrochloride salt was formed with excess HCl in ethanol to give PD-113,926 in an overall radiochemical yield of 53% and with a specific activity of 92.4 mCi/mmol.

CI-930 was synthesized in nine steps as shown in Scheme 2. A key reaction in this sequence employs a phase transfer Wittig[4] reaction to produce double-labeled 2-butenenitrile (7) in 72% yield. Unlike the conventional Wittig reaction, this procedure accommodates the use of an aqueous solution of an aldehyde. This is particularly advantageous for radiochemical synthesis since formaldehyde and acetaldehyde can be purchased exclusively in aqueous media.

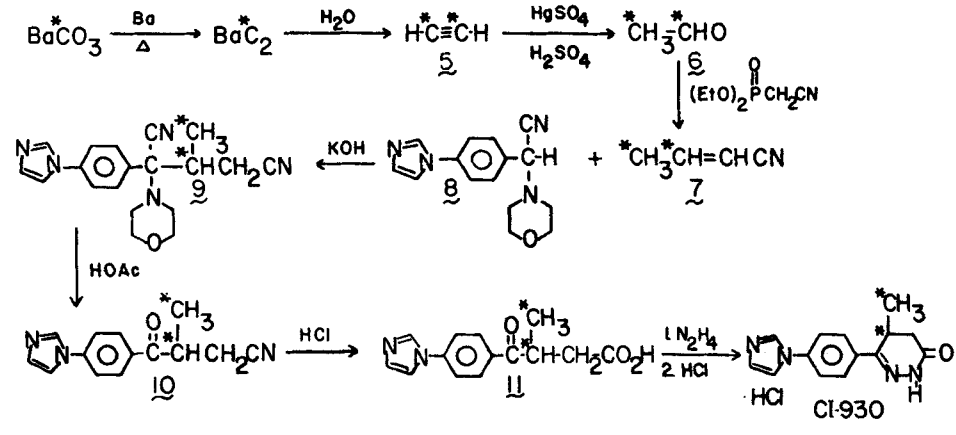
Carbon-14 labeled barium carbonate was mixed with barium filings in an inert atmosphere and fused at high temperature to form barium carbide. Treatment of the carbide with water according to the method of Cox and Warne[5] produced a 92% yield of acetylene-$^{14}C_2$ (5). Modification of the procedure of Cramer and Kistiakowsky[6] was used to synthesize acetaldehyde-$^{14}C_2$ (6). Labeled acetylene 5

Scheme 1



was heated with mercuric sulfate in aqueous sulfuric acid at 95°C for 25 minutes. Aqueous acetaldehyde-$^{14}C_2$ (6) was isolated by distillation in a 92% radiochemical yield. The labeled acetaldehyde 6 and diethylcyanomethylphosphonate were combined to produce 2-butenenitrile-3,4-$^{14}C_2$ (7) using the phase transfer conditions. Addition of the alkene 7 to α-[4-(1H-imidazol-1-yl)-phenyl]-4-morpholineacetonitrile (8) in the presence of potassium hydroxide resulted in an 87% yield of Michael product 9. Hydrolysis of 9 with aqueous acetic acid produced the ketone 10 in a 95% yield. The ketonitrile 10 was further hydrolyzed in 20% hydrochloric acid to yield the carboxylic acid 11 in 60% yield. The acid was then treated with hydrazine at pH 5 to form the pyridazinone ring. After

Scheme 2



preparative HPLC on silica gel, the free base of CI-930 was isolated in a 68% yield. Conversion to the hydrochloride salt proceeded quantitatively. The overall yield for the nine step synthesis was 20.5%. [^{14}C]CI-930 (2) was greater than 97% radiochemically pure and had a specific activity of 106.8 mCi/mmol.

EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined on a Varian XL-200 (200 MHz) spectrophotometer. Chemical shifts were reported in δ units downfield from tetramethylsilane. Mass spectra were recorded on a Finnigan Series 4000 GC-MS. Liquid scintillation counting was performed with a Packard Tricarb 4530 liquid scintillation counter using Mallinckrodt Handifluor liquid scintillation cocktail. [^{14}C]$BaCO_3$ was purchased from Pathfinder Laboratories, St. Louis, Mo. [^{14}C]MeI was purchased from New England Nuclear, Boston, Massachusetts. 4-Fluorobenzaldehyde, 1-[4-(1H-imidazol-1-yl)phenyl]ethanone, and diethylcyanomethylphosphonate were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Thin layer chromatography plates were analyzed for radiochemical purity using a Berthold LB 2832 automatic TLC-analyzer. Silica gel plates (0.25 mm) were purchased from E. Merck. Reversed phase LKC-18F plates (0.20 mm) were purchased from Whatman. High pressure liquid chromatography (HPLC) was performed using a Spectra Physics SP8700 solvent delivery system, Kratos Spectroflow 773 variable wavelength UV detector, Hewlett Packard 3390A integrator and Packard Tricarb RAM 7500 or Trace radioactivity monitor. Gas chromatographic analysis was performed using a Hewlett Packard 5790 instrument with FID.

3-[4-(1H-Imidazol-1-yl)-β-oxobenzenepropanoic acid methyl ester (2). To NaH (4.06 g, 101 mmol, 60% oil dispersion) in THF (30 mL) under N_2 was added 1-[4-(1H-imidazol-1-yl)phenyl]ethanone (1) (18.6 g, 100 mmol) in THF (95 mL) and DMF (30 mL) over 4 h at 25°C. After an additional 30 min, dimethyl carbonate (23 mL, 256 mmol) was added over 30 min. The reaction was refluxed for 15 h, cooled, and filtered. The solid was washed with THF, suspended in cold water and the pH

adjusted to 6.5 with conc. HCl. The mixture was cooled in an ice bath and then filtered. The solid was washed with ice water and dried in a vacuum oven at 40°C to give 18.04 g (74% crude yield) of 1. A portion was recrystallized from CH_2Cl_2/Et_2O: mp 111-112°C, ^1H NMR ($CDCl_3$) δ 7.23-8.09 (m, 7H), 4.01 (s, 2H), 3.75 (s, 3H); IR (KBr) 1742, 1679, 1606, 1523, 1311, 1221 cm^{-1}; MS (EI), m/e (rel. intensity) 244 (M^+, 58), 211 (16), 172 (13), 171 (100), 143 (18), 116 (17), 89 (12).

4-(1H-Imidazol-1-yl)α,α-di(methyl-$^{14}C_2$)-β-oxobenzenepropanoic acid methyl ester (3). To NaH (40 mg, 0.5 mmol, 60% oil dispersion) in DMF (2 mL) was added 1 (122 mg, 0.5 mmol) in DMF (2 mL) at 0°C under N_2 over 30 min. The reaction mixture was warmed to 25°C, stirred for 2 h, placed on a vacuum manifold, degassed, frozen and evacuated. [^{14}C]MeI (50 mCi @ 50 mCi/mmol, 1.0 mmol) was vaccum transferred to the dianion mixture, warmed to 25°C, brought to atmospheric pressure with N_2 and stirred for 17 h. The solvent was removed by static vacuum transfer. Water (1 mL) was added and the mixture extracted with EtOAc (4 x 2.5 mL). The EtOAc was evaporated in vacuo to recover 37 mCi (74% radiochemical yield) of crude 2: TLC: Radiochemical purity 97%, R_f=0.32, silica gel, CH_2Cl_2:MeOH (19:1), same as authentic unlabeled standard.

2,4-Dihydro-5-[4-(1H-imidazol-1-yl)phenyl]-4,4-(dimethyl-$^{14}C_2$)-3H-pyrazol-3-one (4). Crude 3 was dissolved in absolute EtOH (1 mL) and hydrazine hydrate (100 uL, 2.0 mmol) was added. After 45 min, the solvent was removed in vacuo at 50°C. The residual oil was purified by flash chromatography (silica gel, eluted with 5% MeOH in CH_2Cl_2) to give a white solid. Recrystallization from EtOH gave 90 mg (96% yield) of 3: mp 173-174°C; TLC: Radiochemical purity 100%, R_f=0.55, silica gel, p-dioxane:MeOH (4:1); HPLC: Radiochemical purity >99%, t_R=5.3 min; Alltech C18, 5u, 4.6 x 150 mm; MeOH/H_2O (1:1); flow 1.0 mL/min, UV @ 254 nm.

2,4-Dihydro-5-[4-(1H-imidazol-1-yl)phenyl]-4,4-di(methyl-$^{14}C_2$)-3H-pyrazol-3-one monohydrochloride, PD 113,926. The free base 4 was dissolved in warm EtOH (3 mL) and treated with HCl in EtOH (8.0 mL @ 0.048 M). After cooling, the solvent was

evaporated in vacuo. The white solid was dried for 18 h at 25°C using a high vacuum to give 78.3 mg (76% yield) of 4: Specific activity 92.4 mCi/mmol. TLC: Radiochemical purity >99%; System A, R_f=0.37, silica gel, CH_2Cl_2:MeOH (9:1); System B, R_f=0.54, silica gel, p-dioxane:MeOH (4:1); System C, R_f=0.63, C18, MeOH:H_2O (4:1); HPLC: Radiochemical purity >99.5%, t_R=5.6 min (conditions the same as described above); ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.73 (s, 1H), 9.67 (s, 1H), 8.30 (s, 1H), 8.03 (d, 2H, J=6 Hz), 7.88 (s, 1H), 7.86 (d, 2H, J=6 Hz), 1.38 (s, 6H); IR (KBr) 3110 (broad), 1750, 1550, 1460, 1348, 1060, 850, cm^{-1}. Anal. Calcd. for $C_{14}H_{14}N_4O$·HCl: C, 57.85; H, 4.82; N, 19.27. Found: C, 56.82, H, 5.00; N, 18.98.

α-[4-(1H-Imidazol-1-yl)phenyl]-4-morpholineacetonitrile (8). To p-dioxane (60 mL) was added 4-(1H-imidazol-1-yl)benzaldehyde (6.2 g, 36 mmol), p-toluenesulphonic acid monohydrate (6.8 g, 36 mmol) and morpholine (15.7 g, 180 mmol) and refluxed under argon for 30 min. After cooling, KCN (2.3 g, 36 mmol) in H_2O (25 mL) was added to the reaction. The reaction was refluxed for 2.5 h and cooled. The reaction was made alkaline to pH 10 with a 15% Na_2CO_3. The solution was extracted with CH_2Cl_2 (3 x 75 mL). The CH_2Cl_2 layer was washed with 5% $NaHSO_3$ (50 mL), 10% $NaHCO_3$ (50 mL) and H_2O (50 mL). The CH_2Cl_2 layer was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was recrystallized from THF/hexane to produce a pale yellow solid (8.2 g, 84.9% yield) (mp 141-142°C). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.87 (s, 1H), 7.66 (d, 2H), 7.43 (d, 2H), 7.23 (m, 2H), 3.73 (m, 4H), 2.60 (t, 4H). IR (KBr): 3120, 1487, and 1453 cm^{-1}. Anal. Calcd. for $C_{15}H_{16}N_4O$: C, 67.14; H, 6.01; N, 20.88. Found: C, 67.08; H, 6.00; N, 20.90.

Acetaldehyde-$^{14}C_2$ (6). Acetylene-$^{14}C_2$ (5) was made from $BaCO_3$ (139 mCi, 55.9 mCi/mmol) as described in the literature.[5] The acetylene 5 (129 mCi, 1.15 mmol) was transferred via the vacuum manifold into a 25 mL round bottom flask. The reaction was run as described previously[6] with the following modifications. $HgSO_4$ (0.2 g) was dissolved 10% H_2SO_4 (10 mL) and injected via a syringe into the flask containing the condensed acetylene. The reaction was

heated at 95°C for 25 min. The reaction was distilled using an intermittant N_2 stream to collect an aqueous solution of acetaldehyde-$^{14}C_2$ (6) (118 mCi, 91.5% radiochemical yield).

2-Butenenitrile-3,4-$^{14}C_2$ (7). To a 2-neck round bottom flask fitted with a stoppered water-cooled condenser, dropping funnel and stirring bar was added CH_2Cl_2 (10 mL), 50% NaOH (10 mL), 1,10-phenanthroline (36 mg in 1 mL CH_2Cl_2), diethylcyanomethylphosphonate (0.49 mL, 531 mg, 3 mmol) and tetrabutylammonium iodide (78 mg). The aqueous solution of acetaldehyde-$^{14}C_2$ (6) (92.7 mCi) was added dropwise over 15 min. The reaction was exothermic and care was taken to avoid refluxing temperatures. The reaction was stirred for 30 min and the solution was transferred to a separatory funnel. Enough H_2O and CH_2Cl_2 were added to produce two easily separable layers. The H_2O layer was rinsed several times with CH_2Cl_2. The CH_2Cl_2 layer was dried (Na_2SO_4) and concentrated using a short path distillation apparatus. CH_2Cl_2 (15mL) was collected and THF (5 mL) was added to the distillation flask. Fraction 2 contained CH_2Cl_2 and THF (6 mL total). The remaining contents of the distillation flask were then static vacuum distilled to produce a clear solution which was analyzed by capillary GC. CH_2Cl_2 t_R=1.05 min. (15.4%); THF t_R=1.56 min. (83.1%); 2-butenenitrile (E and Z) t_R=1.90 and 1.99 min (1.5%); DB 5 capillary column (30 x 0.025 mm, J & W); 35°C; column flow, 1.54 mL/min of H_2 at a linear velocity of 2.94 m/min. The ^{14}C-labeled 2-butenenitrile 7 (67.2 mCi, 72.5% radiochemical yield) was used in the next reaction without further purification.

2-[4-(1H-Imidazol-1-yl)phenyl]-3-(methyl-^{14}C)-2-(4-morpholinyl)pentanedinitrile-3-^{14}C (9). α-[4-(1H-Imidazol-1-yl)phenyl]-4-morpholineacetonitrile (8) (434 mg, 1.62 mmol) was suspended in THF (1 mL) and 30% methanolic KOH (140 uL). The suspension turned bright yellow and was stirred for 10 min. The ^{14}C-labeled 2-butenenitrile (7) (20.2 mCi) was added in ~12 mL of solvent and the solution was refluxed for 24 h. The reaction was cooled and the solvent was removed in vacuo. The residue was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was placed on a

Florisil column (20 x 2.5 cm) eluted initially with CH_2Cl_2 then a gradient of 1-3% MeOH. The labeled product 9 (17.5 mCi, 86.6% radiochemical yield) coeluted with the unlabeled starting material. TLC: Radiochemical purity >99%, R_f=0.58, silica gel CH_2Cl_2:MeOH (19:1). The mixture of labeled product 9 and unlabeled starting material 8 was used in the next reaction without further purification.

4-(1H-Imidazol-1-yl)-β-(methyl-^{14}C)-γ-oxobenzenebutanenitrile-β-^{14}C (10). A mixture of 8 and 9 were heated at 100°C in 75% HOAc (3 mL) for 3 h. The HOAc was removed under reduced pressure and the residue was partitioned between 10% $NaHCO_3$ and CH_2Cl_2. The CH_2Cl_2 layer was washed with 5% $NaHSO_3$ (30 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The crude material from the CH_2Cl_2 layer (85 mg) was purified using a BondElut (2.8 mL) silica column eluted initially with CH_2Cl_2 followed by increasing MeOH up to 10%. The purified material (12.9 mCi, 73.6% radiochemical yield) was a pale yellow oil. TLC, radiochemical purity >99%, R_f=0.42, silica gel, CH_2Cl_2/MeOH (9:1).

4-(1H-Imidazol-1-yl)-β-(methyl-^{14}C)-γ-oxobenzenebutanoic acid-β-^{14}C (11). The ^{14}C-labeled nitrile 10 (12.9 mCi) was dissolved in 20% HCl (1.8 mL) in a Wheaton 2 mL V-vial. The vial was heated between 90-100°C for 21 h. The reaction was cooled and poured into Na_2CO_3 (10 mL) and extracted with CH_2Cl_2. The H_2O was made alkaline to pH 10 with Na_2CO_3 and extracted with CH_2Cl_2. The aqueous layer was made acidic to pH 4-5 with glacial acetic acid and the H_2O was removed in vacuo. Absolute EtOH was added to the solid salts and product 11. The solid salts were filtered and the keto acid 11 (7.6 mCi, 59.6% radiochemical yield) was retained in the ethanolic filtrate. The ethanol solution of 11 was used without further purification in the next reaction. TLC: Radiochemical purity >93%, R_f= 0.14, silica gel, CH_2Cl_2/MeOH (9:1).

4,5-Dihydro-6-[4-(1H-imidazol-1-yl)phenyl]-5-(methyl-^{14}C)-3(2H)-pyridazinone-5-^{14}C monohydrochloride (CI-930). The ^{14}C-labeled keto acid 11 (6.9 mCi) in ethanol (20 mL) and hydrazine monohydrate (50 uL) were refluxed for 3 hours. The ethanol was removed in vacuo and the solid residue was partitioned between 5%

Na_2CO_3 and CH_2Cl_2. The crude residue (40 mg) was purified by preparative HPLC on a Whatman Spherisorb 5 μ silica column (10 mm x 25 cm) at a flow rate of 4.0 mL/min, UV 280 nm. Ethanolic HCl was added to the purified product CI-930 and the ethanol was removed in vacuo. The final compound (13.9 mg, 4.7 mCi, 68.3% radiochemical yield) was formulated in 13.4 mL H_2O. Specific activity by UV spectroscopy, 106.8 mCi/mmol. TLC: Radiochemical purity >97%: System A, R_f=0.40 silica gel CH_2Cl_2/MeOH (9:1); system B, R_f=0.48 silica gel dioxane/MeOH (4:1) system C, reversed phase MeOH/H_2O (4:1). HPLC: Radiochemical purity >97%, t_R=5.1 min.; Alltech C18 column (10 μ, 150 x 4.6 mm; acetonitrile/ammonium phosphate buffer (0.05 M, pH 7.0, 40:60); flow rate 1.0 mL/min., UV @ 280 nm. The NMR spectrum of the free base was identical to that obtained with an authentic sample. ^1H NMR (200 MHz, $CDCl_3$) δ 7.90 (m, 3H), 7.46 (m, 2H), 7.29 (m 2H), 3.38 (m, 1H), 2.75 (dd, 1H) 2.52 (dd, 1H), 1.26 (d, 3H).

ACKNOWLEDGMENTS

The authors thank Dr. I. Sircar and Mr. G. Bobowski for helpful discussion concerning this project, and the Analytical and Physical Chemistry Section of Warner-Lambert/Parke-Davis Pharmaceutical Research Division for assistance in obtaining analytical and spectral data.

REFERENCES

1. Bristol J. A., Sircar I, Moos W. H.,Evans D. B. and Weishaar R. E.-J. Med. Chem. 27 1101 (1984).

2. This work was presented in part at the Second International Symposium on the Synthesis and Applications of Isotopically Labeled Compounds, Sept. 3-6, 1985, Kansas City, Missouri. Muccino, R. R. (ed.), Synthesis and Applications of Isotopically Labeled Compounds, Amsterdam, 1986, p 313-314.

3. Morrison G. C.-U. S. Patent 4,526,982 (1985).

4. Piechucki C.-Synthesis 12 869 (1974).

5. Cox J. D. and Warne R. J.-J. Chem. Soc. 1893 (1951).

6. Cramer R. D. and Kistiakowsky G. B.-J. Biol. Chem. 137 549 (1951).