Synthesis of ^{13}C, ^{14}C and $^{2}H^{13}C$ Labeled Adrenoceptor Antagonists: 6-Chloro-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine Hydrochloride and its N-Desmethyl Analog

A.J. VILLANI,[a] F. ETZKORN,[b] G.A. ROTERT[b] and J.R. HEYS[a*]

[a]Research and Development Division, Smith Kline & French Laboratories, Box 1539, King of Prussia, PA 19406

[b]Chemsyn Science Laboratories, Lenexa, KS 66215

SUMMARY

The compound 6-chloro-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine (SK&F 86466) was prepared in phenyl-U-^{14}C and phenyl-$^{13}C_6$ labeled forms in a six step sequence beginning with the appropriately labeled benzenes. In addition, the N-desmethyl analog of the carbon-14 labeled isotopomer and the N-methyl-$^{2}H_3$ derivative of the carbon-13 isotopomer were prepared via the N-(2,2,2-trichloroethyl) carbamates by hydrolysis and lithium aluminum deuteride reduction, respectively.

Keywords: (^{13}C)Benzazepine, [^{14}C]benzazepine, (^{2}H,^{13}C)benzazepine, ($^{13}C_6$)benzene, [U-^{14}C]benzene.

INTRODUCTION

6-Chloro-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine (SK&F 86466, 1) is a selective α_2-adrenoceptor antagonist which is under development as a cardiovascular agent(1). Isotopically labeled samples of this and related compounds were required for use in metabolism, pharmacokinetic and bioavailability studies. The compound labeled with carbon-14 adjacent to the amine function in the azepine ring was previously prepared in these (SK&F) laboratories(2), using a synthetic method similar to that published(1) for the unlabeled compound, proceeding through the key intermediate (2-chlorophenyl)[1-^{14}C]acetic acid. However, the complex metabolism of the compound made interpretation of experimental results with this isotopomer uncertain. Moreover, this synthetic method could not readily be used to prepare a stable isotope labeled analog suitable for use in isotope dilution mass

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Present address: Department of Chemistry, University of California, Berkeley, CA 94720

Present address: Abbott Laboratories, Abbott Park, IL 60064

*To whom correspondence should be addressed

0362–4803/88/121339–09$05.00
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Received January 27, 1988
Revised April 8, 1988

spectrometric quantitation studies(3), because only one heavy isotope is incorporated. This paper describes the use of alternative synthetic methodology to prepare the desired compounds labeled with ^{13}C or ^{14}C in the benzene ring.

RESULTS AND DISCUSSION

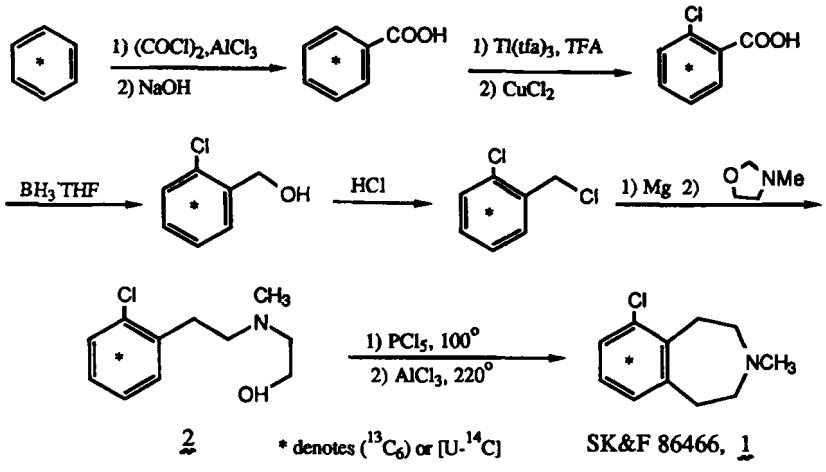
In the original synthesis of SK&F 86466(1), the cyclization of (unlabeled) 2 to 1 gave high yields, so it was advantageous to utilize this reaction for the syntheses of phenyl ring-labeled SK&F 86466. To prepare 2 in the required labeled form, the readily available starting material [U-^{14}C]benzene or ($^{13}C_6$)benzene was converted to benzoic acid by the method of Sokol(4) (Scheme I). The ortho position was then selectively functionalized by thallation with thallium tris-trifluoroacetate by the method of Taylor(5), then the thallium was displaced by chlorine using cupric chloride in dioxane solution(6). The resulting o-chlorobenzoic acid was converted in two steps to o-chlorobenzyl chloride.

In 1945 Senkus reported(7) the reaction of Grignard reagents with N-alkyl oxazolidines to produce N,N-disubstituted ethanolamines:

In unlabeled reactions(8) it was found that o-chlorophenylmethylmagnesium chloride reacted with N-methyloxazolidine to provide unlabeled 2 in high yields. Applied to both isotopically labeled o-chlorobenzyl chlorides, the reaction was similarly successful. Chlorination and cyclization of the labeled samples of 2 thus obtained was then carried out by a recently developed one-pot procedure(9). Thus, as summarized in Scheme I, 15.7 mCi of [phenyl-U-^{14}C]1 at 19.8 mCi/mmole was prepared in 5.1% overall radiochemical yield from [U-^{14}C]benzene, and 1.73 g of (phenyl-$^{13}C_6$)1 at 98.5 mol% $^{13}C_6$ from ($^{13}C_6$)benzene in 7.7% overall yield.

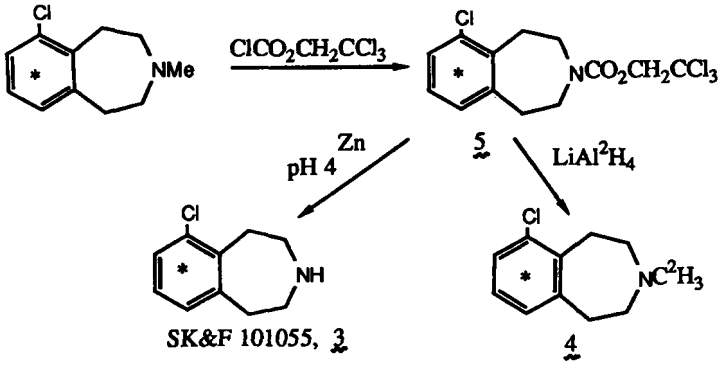
The N-desmethyl analog of SK&F 86466 (SK&F 101055, 3) is a major metabolite of SK&F 86466(10) which also possesses adrenoceptor antagonist activity. It was prepared in carbon-14 labeled form in 48% chemical yield by demethylation of [phenyl-U-^{14}C]1 via the 2,2,2-trichloroethyl carbamate using the method of Montzka, et al.(11) as shown in Scheme II. In addition, [phenyl-$^{13}C_6$, N-methyl-$^{2}H_3$]SK&F 86466.HCl (4) was prepared in 72% yield for use as a mass spectral internal standard by lithium aluminum deuteride reduction of the carbamate prepared similarly from a portion of carbon-13 labeled SK&F 86466.

SCHEME I



2

SCHEME II



EXPERIMENTAL

Materials and Equipment

($^{13}C_6$)Benzene was purchased from Cambridge Isotope Laboratories, Cambridge, MA, and [U-^{14}C]benzene was prepared from barium [^{14}C]carbonate by Chemsyn Science Laboratories. Clin-Elut™ tubes were purchased from Analytichem International, Harbor City, CA. Thin layer chromatographic analyses were carried out on Merck Silica Gel 60F254 plates, and thin layer radiochromatograms were run on a Bioscan TLC Radioscanner or a Berthold LB 2832 Linear Analyzer. ^{1}HNMR spectra were acquired using a Varian EM 360 NMR spectrometer; the spectra of all compounds were consistent with their structures. HPLC analyses were carried

out using a Kontron Model 414 pump equipped with a Rheodyne 7125 injector; UV detection was carried out with a Kontron Model 730LC detector and radioactivity detection with a Radiomatic Flo-One instrument equipped with a 500 μL flow cell and Flo-Scint II scintillation cocktail. All HPLC retention times and TLC R_f's of labeled compounds matched those of authentic samples of the respective unlabeled compounds. GC-EIMS was carried out on a Finnigan 4500 mass spectrometer using DB-5 and DB-17 columns.

[phenyl-U-^{14}C]Benzoic Acid

Oxalyl chloride (1.5 mL, 17 mmol) was added dropwise to a stirred, ice-cooled suspension of aluminum chloride (2.25 g, 16.9 mmol) in carbon disulfide (10 mL) under argon. The mixture was allowed to warm slowly to room temperature over 2.5 hours. [phenyl-U-^{14}C]Benzene (370 mCi, 14 mmol) in carbon disulfide (2.0 mL) was added dropwise over 5 minutes to the suspension, then the mixture was heated at reflux for 3 hours. The reaction was cooled to room temperature and pipetted onto a mixture of ice (30 g) and concentrated HCl (5 mL) and the combination stirred 15 minutes. The layers were separated, the aqueous layer was extracted with chloroform (3 x 10 mL), and the combined organic phases were dried and filtered by passage through a #1003 Clin-Elut™ tube. The solvents were removed in vacuo to give a brown solid. This was treated with 2.5 M aqueous NaOH (20 mL), and the mixture was heated with stirring at 80°C for 16 hours. The reaction was cooled to room temperature, washed with ether, acidified to pH 2 with 6 M HCl, then extracted with ether (3 x 5 mL). The combined extracts were in turn extracted with 2.5 M NaOH (3 x 12 mL), and the combined aqueous phases acidified to pH 2 as before. The resulting white precipitate was extracted into ethyl acetate and the solution dried over Na_2SO_4. Evaporation of solvent in vacuo resulted in 1.125 g (66%) [phenyl-U-^{14}C]benzoic acid (245 mCi, 26.6 mCi/mmol).

2-Chloro[phenyl-U-^{14}C]benzoic Acid

[phenyl-U-^{14}C]Benzoic acid (349 mCi. 11.3 mmol) was dissolved in trifluoroacetic acid (8.0 mL, freshly distilled from P_2O_5) and the solution was added to a stirred solution of thallium trifluoroacetate (6.90 g, 12.7 mmol) in trifluoroacetic acid (7.5 mL) under argon. The mixture was heated at 65°C for 2 hours, then cooled to room temperature. The white precipitate was collected by filtration and dried in a vacuum desiccator over NaOH (0.1 mm, 1.5 hour) to give 2.10 g of an off-white powder. This was dissolved in dry dioxane (20 mL) and added to a suspension of cupric chloride dihydrate (3.04 g, 17.8 mmol) in dioxane (30 mL), held at 80°. The mixture was heated at reflux for 5 hours, then cooled to room temperature. The precipitate was removed by filtration, and the filtrate was concentrated in vacuo to 5 mL. The product was precipitated by the addition of water (25 mL), and extracted into ether (3 x 25 mL), and the combined extracts dried over Na_2SO_4, and evaporated in vacuo. The residue was dissolved in 2.5 M NaOH (5 mL), the solution was filtered, and the filtrate was acidified to pH 2 with 6 M HCl. The white precipitate was extracted into ethyl acetate (50 mL) to give a solution of 2-chloro[phenyl-U-^{14}C]benzoic acid (77.4 mCi).

The residue obtained on evaporation of the filtrate from the thallation was subjected to chromatography on a 3 x 32 cm column of silica gel 60 eluted with a gradient of 0 - 1% acetic acid in 3:1 hexane:acetone to give 165 mCi recovered [phenyl-U-^{14}C]benzoic acid. This was resubjected to the above described thallation-chlorination sequence to provide an additional 22 mCi quantity of 2-chloro[phenyl-U-^{14}C]benzoic acid. The combined product lots (99 mCi, 28.4% yield) showed a 98% purity by TLC analysis (silica gel; hexane:acetone:acetic acid, 35:15:1).

2-Chloro[phenyl-U-^{14}C]benzyl Alcohol

A solution of 1 M borane/THF complex (9.0 mL) was added dropwise to an ice-cooled solution of 2-chloro[phenyl-U-^{14}C]benzoic acid (99 mCi, 4.55 mmol) in dry THF (8.5 mL). The cooling bath was removed and the mixture was stirred for 8 hours, then diluted with ether (160 mL). Saturated aqueous K_2CO_3 (13 mL) was added slowly, and the resulting mixture was stirred at room temperature for 16 hours. The layers were separated and the aqueous layer was extracted with ether (3 x 10 mL). The combined ether layers were washed with water (5 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* to give 2-chloro[phenyl-U-^{14}C]benzyl alcohol as a white solid (637 mg, 99.2 mCi, 100%) of 98% radiochemical purity by TLC (silica gel; hexane:acetone, 4:1).

1-Chloro-2-chloromethyl[U-^{14}C]benzene

An 85.5 mCi (3.8 mmol) portion of 2-chloro[phenyl-U-^{14}C]benzyl alcohol was combined with 5 mL of concentrated hydrochloric acid and the mixture heated at 90°C for 2 hours. The solution was cooled to room temperature and extracted with ether. The extract was dried over $MgSO_4$, filtered and concentrated to an oil by distillation at 1 atmosphere through a 13 cm x 2.5 cm Vigreux column. TLC analysis (silical gel; hexane:ethyl acetate, 49:1) revealed a radiochemical purity of >95%.

N-Methyl-N-[2-(2-chloro[U-^{14}C]phenyl)ethyl]-2-hydroxyethylamine (2)

N-Methyloxazolidine was prepared by refluxing a mixture of 0.5 g (0.05 mol) of 2-(methylamino)ethanol and 1.5 g (0.05 mol) paraformaldehyde in 25 ml of toluene for 1.5 hours while removing water continuously by means of a Dean-Stark trap. The resulting solution was distilled at 1 atmosphere through a 13 cm x 2.5 cm Vigreux column, and three fractions were collected. Fraction #1 had a concentration of N-methyloxazolidine of 3.28 mmol/mL, as determined by ^{1}HNMR analysis.

The 1-chloro-2-chloromethyl[U-^{14}C]benzene from the last step was dissolved in ether (5 mL) and magnesium metal turnings (99.6 mg, 4.1 mmol) added. The mixture was carefully degassed through three freeze-thaw cycles and stirring over oxygen-free argon. Grignard formation was initiated with a trace of 1,2-dibromoethane and the mixture was heated for 2 hours. The resulting solution was cooled to room temperature, and a 1.24 mL portion of the N-

methyloxazolidine solution (4.1 mmol) was added dropwise. The reaction mixture was stirred for 15 hours, then treated with a saturated solution of ammonium chloride (10 mL). The mixture was stirred for 3 hours, then diluted with more saturated ammonium chloride (20 mL) and extracted with ether (80 mL). The organic extract was dried over magnesium sulfate and concentrated in vacuo to an oil (535 mg, 63 mCi, 74%). HPLC analysis (μBondapak C_{18}; 0.25 M triethylammoniumphosphate buffer, pH 2.5:water:acetonitrile, 6:14:5) revealed a radiochemical purity of 94%.

6-Chloro-2,3,4,5-tetrahydro-3-methyl-[phenyl-U-^{14}C]1H-3-benzazepine hydrochloride (1)

The product of the last step (535 mg, 2.5 mmol) was dissolved in 2 mL of trichlorobenzene. Phosphorous pentachloride (261 mg, 1.25 mmol) was added, and the mixture was heated to 100°C for 1 hour. Aluminum chloride (1 g, 7.5 mmol) was added in one portion, and the temperature was raised to 215-225°C over approximately 16 minutes then maintained for 6 hours. The mixture was allowed to cool to room temperature then was treated with 3 M HCl (5 mL). The aqueous acidic layer was then made basic with 50% sodium hydroxide (25 mL), and the liberated oil extracted with ether. The solvent was removed in vacuo, and the oily residue distilled under vacuum. The fraction collected at 60°C/0.03 mm was dissolved in toluene and acidified with gaseous HCl. The toluene was removed in vacuo, leaving 308 mg of crude product. The material was recrystallized from ethyl acetate:methanol (3:1) to give 88 mg (7.5 mCi, 19.8 mCi/mmol) 6-chloro-2,3,4,5-tetrahydro-3-methyl[phenyl-U-^{14}C]-1H-3-benzazepine hydrochloride. HPLC analysis (vide supra) showed a 95% radiochemical purity. HPLC purification of the mother liquor was carried out using a Dynamax C_{18} column (25 cm x 21.8 mm I.D.), and mobile phase as above. Selected eluate fractions were lyophilized, and the product was recovered by dissolution in water, basification and extraction with ether. The ether extracts were dried ($MgSO_4$) and evaporated. The residues were taken up in toluene, gaseous HCl was bubbled through, and the precipitated products filtered and dried in vacuo. This provided an additional 9.2 mCi of product in three batches with purities ranging from 95% to 99%. Total yield: 16.7 mCi (26.5%)

(phenyl-$^{13}C_6$)Benzoic Acid

($^{13}C_6$)Benzene (10.0g, 119 mmol) was reacted in a manner exactly analogous to the carbon-14 labeled compound. The product was isolated by filtration of the alkaline reaction mixture and addition of the filtrate to 6 M HCl (100 mL). The white precipitate was collected by filtration, washed with cold water and dried in vacuo over P_2O_5 for 14 hours to give 13.5 g (86%) (phenyl-$^{13}C_6$)benzoic acid.

2-Chloro(phenyl-$^{13}C_6$)benzoic Acid

(phenyl-$^{13}C_6$)Benzoic acid (11.0 g, 85.9 mmol) was thallated in the same way as the ^{14}C-isotopomer, except that the reaction was run for 46 hours. The thallated intermediate was isolated by filtration to give 18.23 g as an off-white powder, m.p. 240-50 dec. This solid was

chlorinated and worked up as before to give 5.80 g (41.5%) 2-chloro(phenyl-$^{13}C_6$)benzoic acid. Unlike the corresponding reaction with [phenyl-U-^{14}C]benzoic acid, no unreacted starting material was isolated.

2-Chloro(phenyl-$^{13}C_6$)benzyl Alcohol

A 6.77 g (41.6 mmol) portion of 2-chloro(phenyl-$^{13}C_6$)benzoic acid was reduced in the same way as the ^{14}C-isotopomer, to give after chromatography 3.85 g (62%) 2-chloro(phenyl-$^{13}C_6$)benzyl alcohol.

1-Chloro-2-chloromethyl($^{13}C_6$)benzene

2-Chloro(phenyl-$^{13}C_6$)benzyl alcohol (3.85 g, 25.93 mmol) was stirred at reflux with 50 mL concentrated HCl for 3.5 hours then worked up by extraction into n-pentane. Distillation (37-45°C, 0.1 mm) gave 3.78 g (87%) 1-chloro-2-chloromethyl($^{13}C_6$)benzene as a colorless oil.

N-Methyl-N-[2-(2-chloro($^{13}C_6$)phenyl)ethyl]-2-hydroxyethylamine (2)

1-Chloro-2-chloromethyl($^{13}C_6$)benzene (3.5 g, 22 mmol) was converted to the Grignard reagent and reacted with N-methyloxazolidine in the same manner as the carbon-14 isotopomer. Workup provided 4.6 g (97%) of the product as an oil of 95.5 area% purity by HPLC (uBondapak C_{18}; 0.25 M triethylammonium phosphate buffer, pH 2.5:water:acetonitrile, 6:14:5, UV detection at 215 nm).

6-Chloro-2,3,4,5-tetrahydro-3-methyl-(phenyl-$^{13}C_6$)1H-3-benzazepine hydrochloride (1)

The product of the previous step was dissolved in 25 mL of trichlorobenzene, phosphorous pentachloride (2.3 g, 11 mmol) was added, and the mixture was heated to 100° for 1 hour. Aluminum chloride (8.4 g, 63 mmol) was added in one portion, and the mixture was heated to 200-205° for 5 hours while argon was bubbled through the reaction mixture. Workup in the same way as the ^{14}C-isotopomer and distillation (62°, 0.07 mm) yielded 1.9 g of the benzazepine as a clear oil. This was converted to the hydrochloride and recrystallized as before to give 1.73 g (35%) of ($^{13}C_6$)1. The purity of the product was determined by capillary GC (free base) to be >99% (DB-5 and DB-17 columns), and the isotopic composition by GC-EIMS was 98.5 mole% $^{13}C_6$.

6-Chloro-2,3,4,5-tetrahydro-[phenyl-U-^{14}C]1H-3-benzazepine hydrochloride (3)

A 75 mg (7.5 mCi, 0.32 mmol) portion of [phenyl-U-^{14}C]1 was combined with 42 mg (0.18 mmol) of unlabeled 1 and converted to the free base by ether/aqueous sodium hydroxide partition. A solution of the free base was heated at reflux in 5 mL toluene with 530 mg (2.5 mmol) of 2,2,2-trichloroethyl chloroformate for 6 hours. Removal of solvent and excess chloroformate by evaporation in vacuo gave the [^{14}C]carbamate 5 as a semisolid having a

radiochemical purity of 98% by TLC (silica gel; cyclohexane:toluene:acetonitrile:concentrated aqueous ammonia, 40:40:20:0.5, R_f 0.85).

The [^{14}C]5 was dissolved in a mixture of 2 mL tetrahydrofuran and 0.6 mL 1 M potassium phosphate buffer (pH 4) and treated with 327 mg activated zinc dust with stirring at room temperature for 5 hours. The resulting mixture was filtered, and the filtrate was acidified by treatment with gaseous HCl then evaporated in vacuo. An aqueous solution of the residue was extracted with ether, basified with 50% aqueous NaOH, and extracted again with ether. The latter extract was evaporated in vacuo, and the residue was dissolved in toluene and treated with gaseous HCl to precipitate the desmethyl benzazepine as its hydrochloride salt. The salt was recrystallized from n-butanol to yield 52 mg (3.6 mCi, 48%) [phenyl-U-^{14}C]3 hydrochloride. The radiochemical purity was >98% by TLC (silica gel; ethyl acetate:methanol:conc. ammonia, 10:1:1, R_f = 0.3), and 97.4% by HPLC (Waters μBondapak C_{18}, 0.25N triethylammonium phosphate (pH 2.5):water:acetonitrile, 30:70:25, 1.0 mL/min).

6-Chloro-2,3,4,5-tetrahydro-3-($^2H_3$)methyl-(phenyl-$^{13}C_6$)1H-3-benzazepine hydrochloride (4)

A mixture of 117 mg (0.6 mmol) (phenyl-$^{13}C_6$)1, 354 mg (1.67 mmol) 2,2,2-trichloroethyl chloroformate, and 2.3 mg (0.017 mmol) potassium carbonate in 3 mL toluene was refluxed for 17 hours. The resulting solution was washed with water then saturated $NaHCO_3$, dried and the solvent removed in vacuo. The residue of (phenyl-$^{13}C_6$)5 was triturated with cold hexane then reduced with 100 mg (2.4 mmol) $LiAl^2H_4$ in ether at room temperature for 26 hours. Workup by addition of 10 N NaOH and filtration was followed by drying and evaporating the solvent. The residue was dissolved in toluene, the solution was treated with gaseous HCl, and the precipitated solid isolated by filtration and dried to provide 42 mg (72%) 4 of 95.5% purity by HPLC.

ACKNOWLEDGMENTS

The authors thank Dr. W.C.M.C. Kokke and Mr. C.R. Newsome of SK&F Radiochemistry for analyses of the ^{14}C-labeled compounds, and Dr. K. Straub and Ms. P. Levandoski of the SK&F Department of Drug Metabolism for mass spectra of ^{13}C-labeled compounds. Other analyses of (phenyl-$^{13}C_6$)1 were carried out by the Analytical, Physical and Structural Chemistry Department of SK&F. Drs. C. Kowalski and L. Motyka provided helpful discussions of the chemistry.

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