Synthesis of ^{14}C-Labeled Levamisole and ^{13}C-Labeled Tetramisole

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SUMMARY

The syntheses of ^{14}C-ring labeled levamisole ([-]-2,3,5,6-tetrahydro-6-phenyl[^{14}C-UL]imidazo[2,1-*b*]thiazole) from acetophenone-ring-UL-^{14}C in 5 steps plus resolution with a 7.5% overall yield, and $^{13}C_6$-ring labeled tetramisole ([±]-2,3,5,6-tetrahydro-6-phenyl[$^{13}C_6$]imidazo[2,1-*b*]thiazole) from benzene-$^{13}C_6$ in 6 steps with a 9.0% overall yield are described.

Key words: [^{14}C]levamisole, [^{13}C]tetramisole, [-]-2,3,5,6-tetrahydro-6-phenyl[^{14}C-UL]imidazo[2,1-*b*]thiazole, [±]-2,3,5,6-tetrahydro-6-phenyl[$^{13}C_6$]imidazo[2,1-*b*]-thiazole

INTRODUCTION

Levamisole has been used extensively as an anthelmintic in animals, as an anthelmintic and immunostimulant in humans, and as an adjuvant agent with 5-fluorouracil in treatment of human colon cancer (1-3). Graziani and DeMartin reported pharmacokinetics studies with thiazolidine-^{14}C labeled levamisole and outlined its synthesis (4); however, synthesis procedures were not presented. Phenyl ring labeled levamisole was needed to investigate the possibility of extensive metabolism and complete removal of the heterocyclic rings. Carbon-13 labeled tetramisole was required for possible residue analyses by isotope ratio mass spectrometry. We report here the synthesis of phenyl-^{14}C labeled levamisole and phenyl-$^{13}C_6$ labeled tetramisole as outlined in Scheme 1.

EXPERIMENTAL

Benzene-$^{13}C_6$, 99% was purchased from Cambridge Isotope Laboratories, Woburn, MA. Acetophenone[ring-^{14}C] was purchased from Chemsyn Science Laboratories, Lenexa, KS. Levamisole hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO.

Electron impact (EI) and fast atom bombardment (FAB) mass spectra were obtained with a Fisons Auto Spec spectrometer (glycerol as a matrix for the FAB process). Nuclear magnetic resonance spectra were obtained with a Bruker AM-400 spectrometer in CD_3OD with tetramethylsilane (TMS) as a reference. Proton NMR spectra were obtained in 5 mm tubes or in 1.8 mm melting point tubes placed inside conventional 5 mm tubes (approximately 30 µg samples were used). Mass and NMR spectra were used for structural and purity determinations. Chiral analyses were done on a Cyclobond I SN column (Advanced Separation Technologies, Inc., Whippany, NJ) using a solvent system of 20% methanol and 80% triethylammonium acetate buffer (1%, adjusted to pH 4.5) at a flowrate of 1 ml/min. Analyses done on the free base at 254 nm yielded two peaks with retention times of 8.03 and 10.38 min for tetramisole. The peak at 10.38 min corresponded to levamisole. Collecting and analyzing

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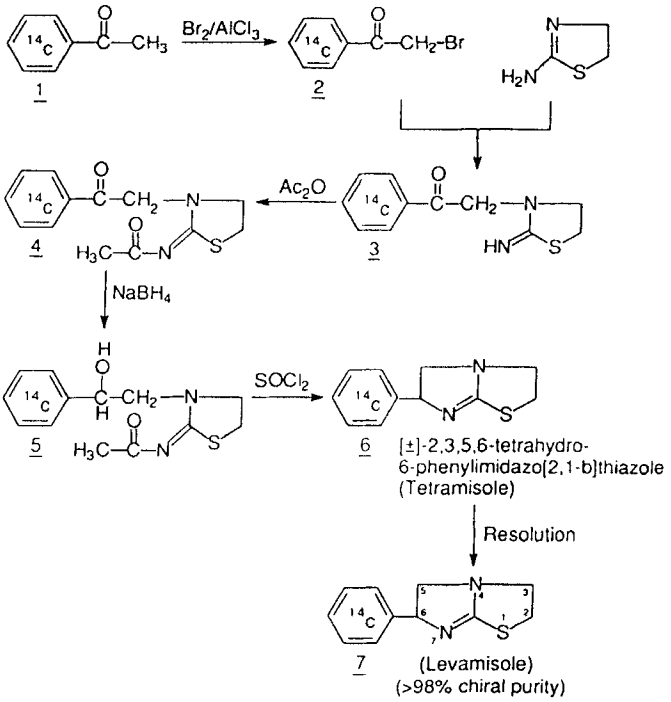
Scheme 1



by liquid scintillation counting of the two peaks (a small amount of unlabeled tetramisole was added to aid in collection of dextramisole) showed that greater than 98% of the ^{14}C activity was in the levamisole peak of the purified material.

Acetophenone (1). Benzene-$^{13}C_6$, 99%, 1.5 g (17.8 mmol) was dissolved in 12 ml of carbon disulfide. The solution was stirred while 5.1 g (38.2 mmol) of aluminum chloride was added. To this solution was added 1.8 ml (19 mmol) of acetic anhydride over 45 min. The brown reaction mixture was stirred an additional 1.5 h at room temperature before most of the carbon disulfide was removed by evaporation and the residue was poured onto ice and 4 N HCl. The product was extracted with methylene chloride, washed with $NaHCO_3$ solution, then with water. The extracts were dried over $MgSO_4$, the solvents were removed and the residue distilled at 20 Torr to yield 2.1 g (91.3%) of 1-^{13}C.

Phenacyl Bromide (2). An ampoule containing 106 mg (47.5 mCi) of acetophenone[ring-^{14}C] was cooled with Dry Ice, and a solution of 1.10 g (9.1 mmol) of unlabeled acetophenone in 3 ml of ether was introduced concurrently with the breaking of the breakseal with a glass rod. The solution was transferred to a 100 ml flask, and 10 mg of aluminum chloride was added. A few drops of bromine were added, and the reaction was stirred at room temperature until the bromine color disappeared. The reaction mixture was then cooled with an ice bath, and a total of 0.55 ml (21.3 mmol) of bromine was added dropwise over 3 min. After the addition was completed, ether and hydrogen bromide were removed with a stream of nitrogen until a solid formed. The solid was dissolved in 10 ml of acetonitrile and the remaining hydrogen bromide was neutralized by dropwise addition of saturated sodium carbonate (until carbon dioxide evolution ceased). The product was used without purification (yield was estimated at >90% based on the yield of 3).

The reaction of 1.2 g (9.5 mmol) of acetophenone [ring-$^{13}C_6$] was conducted as above. The product was used without purification.

2-Imino-3-(phenacyl)thiazolidine Hydrobromide (3). A solution of 1.02 g (10.6 mmol) of 2-amino-2-thiazoline in 25 ml of acetonitrile was added dropwise over 15 min to the acetonitrile solution of 2 (cooling with ice water was initiated when solid began to precipitate). After the addition was completed, the reaction mixture was refluxed for 2 h, then cooled and filtered to yield 2.46 g, 41 mCi of 3-^{14}C (86.7% from 1).

Reaction of 1.02 g (10 mmol) of 2-amino-2-thiazoline with the crude ^{13}C-phenacylbromide yielded 2.02 g (66%) of 3 (ring-$^{13}C_6$).

2-(Acetylimino)-3-(phenacyl)thiazolidine (4). To a solution of 2.46 g (9.4 mmol), 41 mCi of 3 and 3 ml of pyridine in 25 ml of chloroform was added 3.4 ml (36 mmol) of acetic anhydride over 5 min. The reaction mixture was refluxed until it became homogeneous (1.7 h). The chloroform, pyridine, and acetic anhydride were removed at reduced pressure, and the yellow residue was dissolved in 35 ml of methylene chloride and 10 ml of 3 N NH_4OH. The organic layer was separated and washed first with 3 N NH_4OH, then water. The solvent was removed with a stream of nitrogen and the product was recrystallized from 20 ml of toluene to yield 1.34 g, 25.5 mCi of 4-^{14}C (62%). ^1H NMR (CD_3OD) δ 8.03, (dt, J=7.4, 1.3 Hz), 7.66 (tt, J=7.4, 1.3 Hz), 7.54 (td, J=7.4, 1.3 Hz), 5.21 (s), 3.81 (t, J=7.8 Hz), 3.25 (t, J=7.8 Hz). MS(EI) m/z (relative intensity), 262(88), 247(85), 234(35), 220(62), 203(38), 157(79), 143(84), 115(100), 105(93); (+FAB) m/z (relative intensity) 263(83), 247(15), 221(100), 203(17).

The reaction of 1.96 g (6.4 mmol) of 3-$^{13}C_6$, 2.1 ml of pyridine and 2.4 ml of acetic anhydride in 25 ml of chloroform, as described above, yielded 850 mg of 4-$^{13}C_6$ (48% yield). ^1H NMR (CD_3OD) δ 8.03, 7.66, 7.54 (complex multiplets, $^1J_H \approx 168$ Hz, longer range coupling constants could not be determined because of inadequate resolution), 3.81 (t, J=7.8 Hz), 3.26 (t, J=7.8 Hz). MS(EI) m/z(relative intensity), 268(89), 253(86), 240(34), 226(58), 209(38), 157(80), 143(84), 115(100), 111(93).

2-(Acetylimino)-3-(ß-hydroxyphenethyl)thiazolidine (5). Compound 4, 1.34 g, 25.5 mCi, was dissolved in 40 ml methanol and the solution was cooled in an ice bath while 108 mg of $NaBH_4$ was added in small portions with stirring. After the addition was completed the reaction mixture was stirred at room temperature for 4 h. Most of the methanol was evaporated with a stream of nitrogen, and the residue was extracted with water and methylene chloride. The organic layer was dried over Na_2SO_4. The solvent was removed and the residue recrystallized from toluene to yield 13.66 mCi of 5. The solvents were removed from the mother liquors and the residue was chromatographed on alumina. Methylene chloride eluted impurities and ethyl acetate eluted an additional 5.24 mCi of product. A total yield 18.9 mCi (74.1%) of 5-^{14}C was obtained. ^1H NMR, see figure 1.

Reaction of 850 mg of 4-$^{13}C_6$ with 70 mg of $NaBH_4$ in 20 ml of methanol yielded 580 mg of 5-$^{13}C_6$ (67.5% yield). ^1H NMR, see figure 1.

2,3,5,6-Tetrahydro-6-phenylimidazo(2,1-b)thiazole Hydrochloride (6) (Tetramisole). Thionyl chloride, 0.60 ml, was added to a solution of 18.9 mCi of 5 in 30 ml dry chloroform and the reaction mixture was stirred at room temperature for 1 h, then 25 ml of a 10% Na_2CO_3 solution was added and the reaction mixture was refluxed for 1 h. The reaction mixture was cooled, the organic layer was separated, and the aqueous layer was extracted with methylene chloride. The combined extracts were dried over anhydrous Na_2CO_3, and the solvents were removed with a stream of nitrogen. The resulting oil was dissolved in isopropanol and an excess of methanolic hydrogen chloride was added. The solvents were removed and the resulting solid was recrystallized from ethanol/ether to yield 514 mg, 10.07 mCi of 6, 53% from 5, sp. act. 4.72 mCi/mmol.

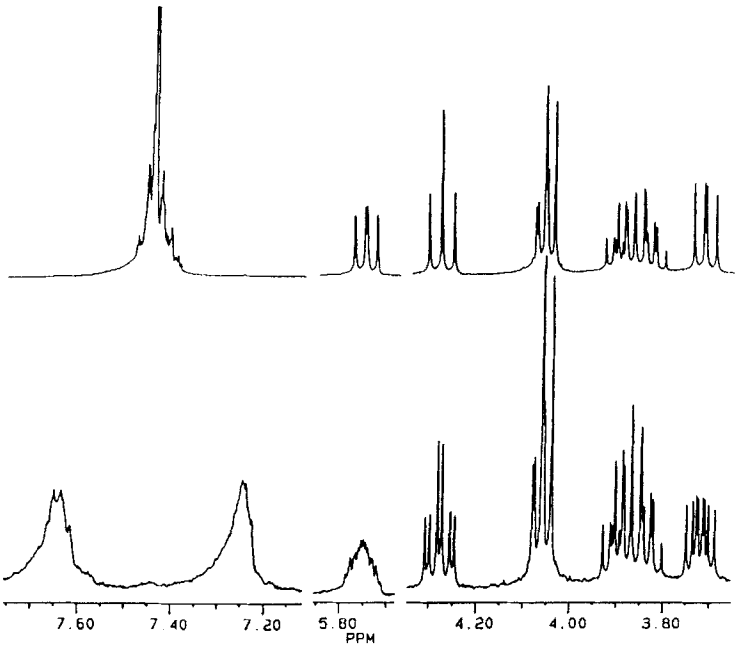


Figure 1. Proton NMR (CD_3OD): (upper) 2-Acetylimino)-3-(ß-hydroxyphenethyl)thiazolidine (5); (lower) $^{13}C_6$-phenyl analogue. Peaks due to TMS, deuteromethanol and water are not shown.

Reaction of 580 mg of 5-$^{13}C_6$ with 157 µl of thionyl chloride in 15 ml of chloroform as described above yielded 205 mg (46%) of 6-$^{13}C_6$ hydrochloride. ^1H NMR, see figure 2. MS(EI) m/z(relative intensity), 210(100), 182(12), 154(81), 127(50), 101(47).

Levamisole (7). Tetramisole (6), 514 mg, 10.07 mCi, was converted to the free base and extracted with chloroform. The chloroform extracts were dried over Na_2CO_3. The solution was concentrated to 10 ml, and 495.8 mg of (1R)-(-)-10-camphorsulfonic acid (*l*-camphorsulfonic acid) and 300 mg of *l*-(-)-6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole *l*-camphorsulfonate (levamisole *l*-camphorsulfonate) were added. The mixture was warmed to

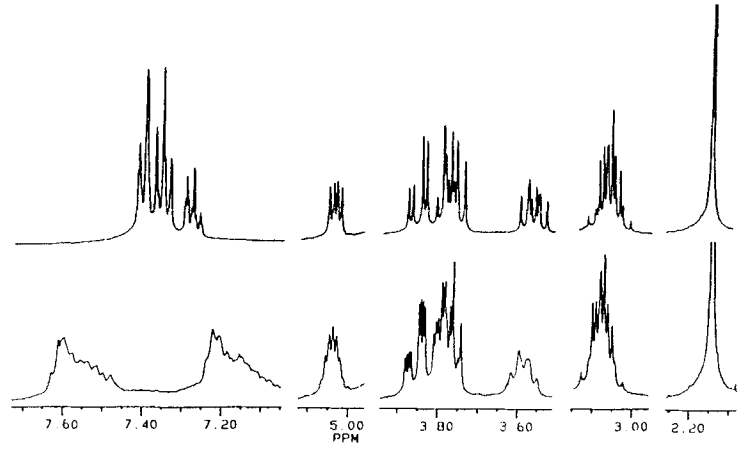


Figure 2. Proton NMR (CD_3OD): (upper) levamisole (7); (lower) $^{13}C_6$-phenyl tetramsiole. Peaks due to TMS, deuteromethanol and water are not shown.

dissolve all the components, 4 ml of hexane was added and the solution seeded with levamisole *l*-camphorsulfonate. On cooling, 3.54 mCi of product crystallized having a chiral purity of 98.8% *l* isomer as established by HPLC on the Cyclobond I SN column. This material was converted to the free base, extracted with methylene chloride, and then converted to the hydrochloride salt with no loss of activity. ^1H NMR, see figure 2. MS(EI) m/z(relative intensity), 204(100), 176(12), 148(84), 127(31), 101(51); (+FAB) m/z(relative intensity) 205(100).

Racemization. The material remaining from the resolution of tetramisole (ca 750 mg, 6.5 mCi, *d/l* ratio approximately 2:1) was dissolved in 5 ml of dry DMSO, and 400 mg of NaH (500 mg of 80% NaH/mineral oil dispersion; washed with dry ether to remove most of the mineral oil) was added. The progress of the racemization was followed by HPLC with the Cyclobond I SN column. No reaction took place when the suspension was stirred at room temperature. A 1:1 *d/l* ratio resulted when the reaction mixture was warmed until all material was in solution; however more than 30% of the material was something other than tetramisole. Conversion to the *l*-camphorsulfonate, and addition of 400 mg of levamisole *l*-camphorsulfonate resulted in 240 µCi of product, 99.2% *l*. The mother liquors were converted to the free base, then to the hydrochloride salt which was purified by recrystallization from ethanol/ether. Conversion to the *l*-camphorsulfonate and addition of another 300 mg of levamisole *l*-camphorsulfonate resulted in isolation of 380 µCi of product, 97.8% *l*. Total yield 620 µCi.

DISCUSSION

The synthesis of ring labeled (^{14}C and $^{13}C_6$) tetramisole was based on the work of Raeymaekers et al. (5) as outlined in scheme 1. Trial reactions with unlabeled reagents were repeated under varied conditions until consistent yields were obtained. Unfortunately some of the reactions with isotopically labeled products gave lower yields than in the trial reactions. We have no explanations for the variations in yields. Graziani and DeMartin reported the use of thiazolidine-^{14}C labeled levamisole and outlined its synthesis; however, no experimental details or yields were reported for the synthetic steps or for the resolution process. The preparation of ^{14}C-ring labeled levamisole reported here consists of 5 synthetic steps plus resolution by recrystallization of the *l*-camphorsulfonic acid salt resulting in an overall yield of 7.5%.

The resolution of tetramisole was based on the work of Bullock et al. (6,7). We found that the "*d-d*" (acid-base respectively) and the "*l-l*" salts crystallized more readily than either the "*d-l*" or the "*l-d*" salts. We therefore chose to resolve tetramisole with *l*-camphorsulfonic acid. Occasionally resolution could be accomplished by seeding with the "*l-l*" salt; however, adding significant quantities (10-40%) of the "*l-l*" salt was the only reliable approach to effecting resolution.

Racemization of the material recovered from the mother liquors of the resolution procedure yielded less than 10% of suitable product, primarily because of the difficulties encountered in removing byproducts. Furthermore, isotopic enrichment was decreased by the addition of unlabeled levamisole-*l*-camphorsulfonate. This is unacceptable for high specific activity experiments, and for isotope ratio experiments as with the $^{13}C_6$ labeled analog. As an alterative method of resolution, we have demonstrated that most of the *d*-isomer of tetramisole can be removed as the *d*-camphorsulfonate via the methodology described in the Experimental section for the *l*-camphorsulfonate. Conversion of the mother liquors (enriched in the *l*-isomer) to the free base, then to the *l*-camphorsulfonate, yielded, after two recrystallizations from $CHCl_3$/hexane, levamisole of >99% chiral purity (66% yield).

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