SYNTHESIS OF CARBON-14 LABELED CLOMIPHENE

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SUMMARY

Clomiphene, 2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy]-N,N-diethylethanamine, an antiestrogen, is a nonsteroidal triphenylethylene derivative. For the successful synthesis of carbon-14 labeled clomiphene it is essential to have a free radical inhibitor in the reaction medium.

Key Words: Carbon-14 labeled clomiphene, free radical inhibitor, [α-^{14}C]benzyl chloride, Grignard reaction.

INTRODUCTION

Clomiphene, 2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy]-N,N-diethylethanamine, is a nonsteroidal triphenylethylene derivative generally considered to be an anti-estrogen (1). In the form of the citrate salt (Clomid®, Merrell), clomiphene is currently used to induce ovulation in anovulatory women (2). In order to investigate the pharmacokinetics, metabolism, and distribution of this material, ^{14}C-labeled clomiphene was synthesized.

DISCUSSION

The synthetic procedure employed is a modification of the general method developed by Palopoli *et al.* (3-5) and is presented under Scheme 1. *p*-Hydroxybenzophenone (1) was converted to the diethylaminoethyloxy derivative 2 by treatment with sodium hydride in THF, followed by reaction with *N,N*-diethyl-

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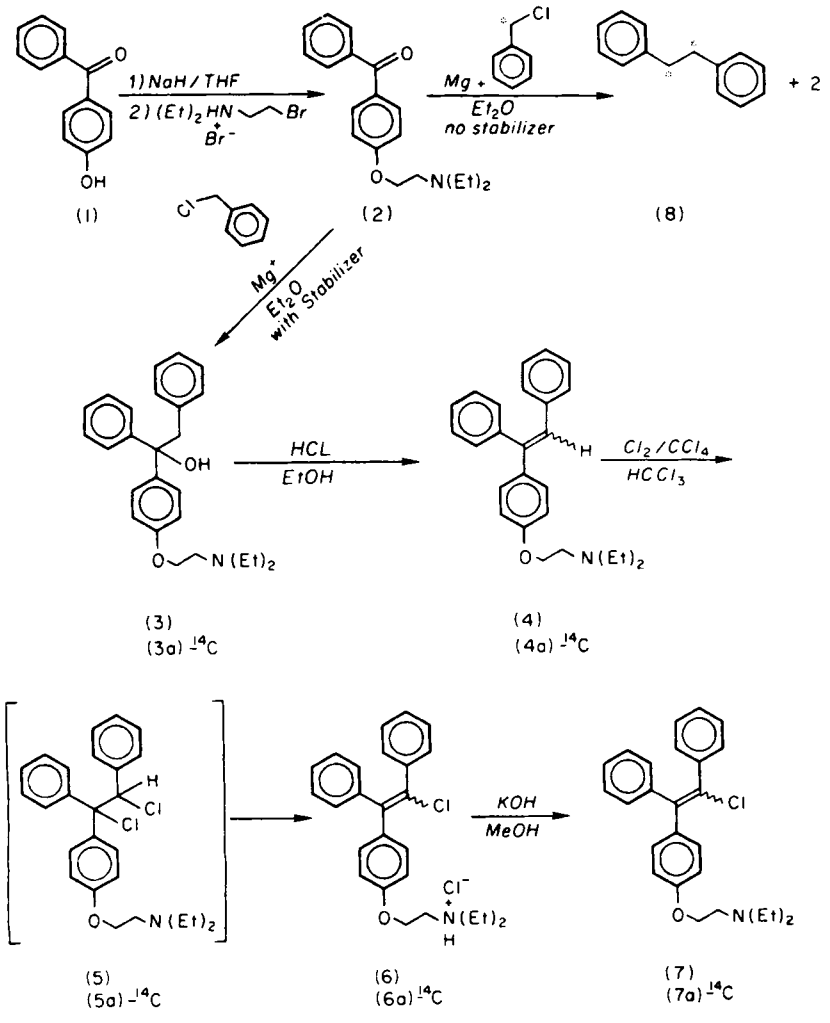
0362-4803/85/030245–11$01.10

Received July 17, 1984
Revised August 23, 1984

Scheme 1



aminoethylbromide hydrobromide. Reaction of 2 with benzylmagnesium chloride in anhydrous ether (Mallinckrodt, reagent grade) gave the triphenylethanol adduct 3. Dehydration of 3 was achieved with ethanolic hydrochloric acid at reflux for three hours to give a mixture of the cis and trans isomers of the triphenylethylene compound salt 4. Reaction of 4 in chloroform with carbon tetrachloride saturated with chlorine at room temperature for two hours followed by reflux for two hours gave clomiphene hydrochloride (6), presumably through elimination of

hydrochloric acid by the intermediate dichloro adduct 5. Treatment of 6 with methanolic potassium hydroxide gave the free base, clomiphene (7).

All reaction conditions were optimized using unlabeled benzyl chloride and the overall yield of 7 from 2 amounted to 60%.

For the synthesis of the labeled compound 7a, the Grignard reagent, initially prepared from [α-^{14}C]benzyl chloride (47 mCi/mmol) and magnesium in sodium-dried ether, on reaction with 2, gave a major product later identified by NMR to be [α,α'-$^{14}C_2$]bibenzyl (8) formed from free radical dimerization of the labeled benzyl chloride. Extensive analysis of this reaction utilizing unlabeled benzyl chloride indicated the primary difference between the labeled and unlabeled Grignard reactions lies in the absence or presence of a free radical inhibitor. The unlabeled reaction was carried out in reagent grade ether containing 0.0001% butylated hydroxytoluene as a stabilizer. In addition, the unlabeled benzyl chloride used contained 0.25% propylene oxide as a stabilizer. In the ^{14}C-labeled reaction, the reagent grade ether was further dried over sodium prior to the reaction, and the [α-^{14}C]benzyl chloride contained no stabilizer. Repetition of the Grignard reaction utilizing unlabeled benzyl chloride and sodium-dried ether resulted in some bibenzyl by-product (~10-20%).

The Grignard reaction with labeled benzyl chloride was then modified by diluting the [α-^{14}C]benzyl chloride with unlabeled benzyl chloride containing propylene oxide stabilizer, by a factor of 3.6 and avoiding the use of sodium-dried ether. This procedure considerably improved the yield of the desired product 3a. Dehydration of 3a proceeded without any difficulty. Chlorination of 4a gave as the major radioactive product, a compound later identified by U.V. spectroscopy and the method of energy dispersive X-ray fluorescence (6) to be the dichloro adduct 5a.

Allen et al. (4) reported a similar result in the chlorination of a related triphenylethylene compound and noted that elimination of hydrogen chloride could

be forced by heating the hydrochloride salt of the amine at 140-150°C for 20 minutes. Using this procedure, compound 5a was successfully converted to the hydrochloride salt 6a. Treatment of 6a with methanolic potassium hydroxide gave the title compound 7a. The high performance liquid chromatographic analysis of ^{14}C-labeled clomiphene to which carrier clomiphene has been added is shown in Figure 1.

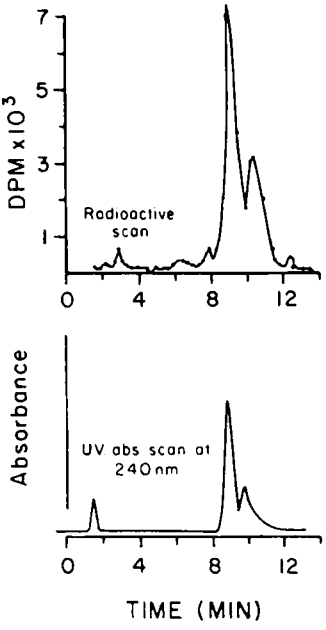


Fig. 1. HPLC analysis of a ^{14}C-labeled clomiphene to which carrier clomiphene has been added on a μPorasil column using heptane:dichloromethane (3:7) containing 0.05% butylamine at a flow rate of 2 ml/min.

EXPERIMENTAL

Most chemicals and solvents were analytical grade and were used without further purification. Some reagents and solvents, such as tetrahydrofuran and triethylamine, were purified according to standard laboratory procedures. [α-^{14}C]Benzyl chloride (46 mCi, 47 mCi/mmol) was purchased from Amersham Corporation, Arlington Heights, Illinois and was used without further purification. All organic extracts were dried over anhydrous sodium sulfate, unless otherwise specified, and evaporated in vacuo.

Purity and identity of new compounds were established by normal spectral (IR, UV, NMR, MS) and analytical (TLC, HPLC, chemical analysis) techniques. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. Ultraviolet spectra were measured in methanol solution using a Varian Cary 210 spectrophotometer. Proton NMR spectra were obtained with Varian EM-390 spectrometer. Mass spectra were recorded on a Finnigan quadrupole mass spectrometer. "Dry" column chromatography was performed on Woelm silica in a nylon column as described by Loev and Goodman (7). Flash chromatography was carried out on Merck grade 60 silica in a J. T. Baker column as described by W. C. Still (8). TLC analyses of unlabeled compounds were done on silica gel GF (Analtech) glass plates (2.5 x 10 cm with 250 μM layer and prescored). TLC analyses of ^{14}C-labeled material were carried out on silica gel GHLF (Analtech) glass plates (5.0 x 20 cm with 250 μM layer) and were monitored by an Atomic Accessories Model RSC-363 radiochromatographic scanner. HPLC analysis of ^{14}C-labeled material was carried out on Waters Associates, Inc. HPLC equipment (Model 6000A pump) employing a normal phase column (μPorasil, 10 μm, 30 cm x 3.9 mm, Waters Associates) and monitored by Laboratory Data Control Spectromonitor III. Radioactivity was determined with a Beckman LS 7500 scintillation counter. Microanalyses were performed by Midwest Microlab, Ltd., Indianapolis, Indiana.

p-[2-(N,N-Diethylamino)ethyloxy]benzophenone (2)

To washed sodium hydride (10 g, 50% suspension, 208 mmol) suspended in dry tetrahydrofuran (500 ml) under dry nitrogen, was added p-hydroxybenzophenone (20 g, 101 mmol) in tetrahydrofuran (250 ml). After the evolution of hydrogen subsided, the reaction mixture was cooled at 0°C and treated dropwise with 2-(N,N-diethylamino)ethylbromide hydrobromide (26.6 g, 102 mmol) in tetrahydrofuran (250 ml). The reaction was then stirred at 0°C for 1 hr and at room temperature overnight. The mixture was subsequently poured into water and extracted with ethyl acetate. The organic extract was washed with brine and

dried. Concentration in vacuo gave 27.5 g of a dark brown oil indicated by NMR to be the crude ether 2. Purification was achieved by "dry" column chromatography (hexane:ethyl acetate: diisopropylamine; 90:8:2) to give 25 g of pure 2. NMR: δ ($CDCl_3$) 1.08(t, J = 7 Hz, 6H, Me), 2.67(t, J = 7 Hz, 4H, N-$\underline{CH_2}CH_3$), 2.9(t, J = 6 Hz, -N-$\underline{CH_2}CH_2O$), 4.13(t, J = 6 Hz, 2H, -$CH_2\underline{CH_2}$-O-), 6.9-8.9(m, 9H, aromatic H); MS: m/e = 297 (M^+); Analysis: Calc'd for $C_{14}H_{23}NO_2$: C, 76.74; H, 7.80; N, 4.71. Found: C, 76.59; H, 7.68; N, 4.94.

1-{4-[2-(N,N-Diethylamino)ethyloxy]phenyl}-1,2-diphenylethanol (3)

Under a nitrogen atmosphere, dry ethylene dibromide (10 µl) was added to flame-dried magnesium (61 mg, 2.51 mmol) in anhydrous ether (5 ml). Evidence of reaction (turbidity, ether reflux, evolution of ethylene) indicated a sufficiently anhydrous condition. Benzyl chloride (0.26 ml, 2.1 mmol) was added and the mixture was stirred at room temperature until the reaction subsided. The Grignard solution was then cooled to 0°C, diluted with dry toluene (5 ml) and treated dropwise with compound 2 (0.6 g, 2 mmol). The reaction mixture was then brought to room temperature and stirred for 1 hour. At the end of this time the reaction was quenched with a saturated solution of ammonium chloride and extracted with ether. The organic extracts were combined, washed with brine, and dried. Concentration in vacuo followed by crystallization from ether:hexane gave 0.6 g of pure 3: mp 95-96°C; NMR: δ ($CDCl_3$) 1.03(t, J = 7.2 Hz, Me), 2.36(br.s., 1H, COH), 2.62(q, J = 7.2 Hz, 2H, N$\underline{CH_2}CH_3$), 2.85(t, J = 6.6 Hz, 2H, -OCH$_2\underline{CH_2}$NO, 3.59(s, 2H, CH_2), 4.02(t, J = 6.6 Hz, 2H, -$\underline{OCH_2}CH_2$N-), 6.68-7.50(m, 14H, aromatic); MS: m/e = 389 (M^+).

1-{4-[2-(N,N,-Diethylamino)ethyloxy]phenyl}-1,2-diphenylethylene (4)

The Grignard adduct (3, 0.6 g, 1.54 mmol) was dissolved in de-oxygenated absolute ethanol (10 ml) in which concentrated hydrochloric acid (0.5 ml) was added. The mixture was stirred and refluxed under an atmosphere of nitrogen for 3 hours. The mixture was then cooled to room temperature, diluted with water,

basified with 2N sodium hydroxide solution and extracted with ethyl acetate. The organic layer was washed with brine and dried. Concentration in vacuo gave 0.48 g of residue indicated by TLC (hexane:ethyl acetate:diisopropylamine; 90:8:2) to consist of a mixture of the cis and trans isomers of the free base (4). NMR: δ ($CDCl_3$) 1.05(t, J = 7.1 Hz, 6H, Me), 2.63(q, J = 7.1 Hz, 4H, $N\underline{CH_2}CH_3$), 2.87(t, J = 6.3 Hz, 2H, $O\underline{CH_2}CH_2N$-), 4.40(t, J = 6.1 Hz, 2H, $-CH_2\underline{CH_2}N$-), 6.77-7.88(m, 15H, aromatic).

1-{4-[2-(N,N-Diethylamino)ethyloxy]phenyl}-1,2-diphenyl-2-chloroethanol (7)

To a solution of the hydrochloride salt of compound 4 (0.5 g, 1.23 mmol) in dry chloroform (15 ml) was added over a period of 2 hr, 10 ml of a carbon tetrachloride solution containing approximately 120 mg (1.7 mmol) chlorine. The mixture was then stirred at room temperature for 1 hr and at reflux for 2 hours. The mixture was then cooled, diluted with water, basified with 2N sodium hydroxide solution, and extracted with ethyl acetate. The organic extracts were combined and washed with brine and dried. Concentration in vacuo gave 0.45 g of residue indicated by TLC to consist of a mixture of the cis and trans isomers of the free base 7. NMR: δ ($CDCl_3$) 0.86-1.21(m, 6H, Me), 2.5(q, J = 7.2 Hz, 4H, $-N\underline{CH_2}CH_3$), 2.67(t, J = 6.0 Hz, 2H, $O\underline{CH_2}CH_2N$), 3.75-4.2(m, 2H, $OCH_2\underline{CH_2}N$), 6.5-7.5(m, 14H, aromatic).

Clomiphene dihydrogen citrate (9)

The free base (7, 0.45 g, 1.1 mmol) in hot butanone (5 ml) was treated with citric acid (0.25 g, 1.3 mmol) in methanol (2 ml). Upon cooling, 0.6 g of clomiphene citrate crystallized out, mp 117-119°C [Lit. (1) 116-118°C] NMR: δ (CF_3COOH) 1.40(m, 6H, Me), 3.23(s, 4H, citrate CH_2), 3.3-3.8(m, 4H, $N\underline{CH_2}CH_3$), 4.33(m, 2H, OCH_2), 6.6-7.5(m, 14H, aromatic).

^{14}C-Labeled clomiphene (7a)

Magnesium (0.036 g, 1.5 mmol) was flame-dried under anhydrous nitrogen and diethyl ether (4 ml, containing 0.0001% butylated hydroxy toluene inhibitor) was

added from a freshly opened can. [α-^{14}C]Benzyl chloride (0.275 mmol, 47 mCi/mmol) was diluted with unlabeled benzyl chloride (0.08 ml, 0.695 mmol, containing 0.25% propylene oxide inhibitor) to give a compound with specific activity of 13 mCi/mmol. It was then added to the magnesium in ether and the reaction mixture stirred at room temperature for 30 min, until the magnesium metal dissolved. At the end of this time the reaction mixture was diluted with anhydrous benzene (4 ml) followed by dropwise treatment with p-[2-(N,N-diethylamino)ethyloxy]benzophenone (2, 0.30 g, 1.01 mmol). The reaction mixture was then stirred at room temperature for an additional 1 hr, cooled to 0°C in an ice bath, and quenched with 10 ml of a saturated ammonium chloride solution. The mixture was then poured into water and extracted three times with ethyl acetate. The organic phases were combined and washed with brine. After drying, the solution was concentrated in vacuo.

A TLC radiochromatographic scan of the reaction product (ether:hexane: triethylamine; 50:45:5) exhibited a major radioactive peak identical in R_f to that of 3.

The product from the above Grignard reaction was dissolved in absolute ethanol and de-oxygenated by bubbling dry nitrogen. Concentrated hydrochloric acid (38%, 0.5 ml) was added and the mixture was refluxed under nitrogen for 2 hours. At the end of this time, the mixture was cooled to room temperature and concentrated under a stream of dry nitrogen at reduced pressure to give 0.8 g of a yellow oil. A small portion of this residue was dissolved in methanol (~1 ml) and treated with 1N potassium hydroxide (~0.5 ml). A TLC radiochromatographic scan of an ethyl acetate extraction of this mixture, using an ether:hexane:triethylamine (50:45:5) solvent system, indicated a major radioactive product identical in R_f to that of the unlabeled triphenylethylene derivative (4).

A solution of the above reaction product (0.8 g), in dry chloroform (3 ml), was de-oxygenated by bubbling dry nitrogen through the mixture for 30 minutes.

Chlorine (0.85 mmol) in dry carbon tetrachloride (5 ml) was then added dropwise at room temperature over a period of 3 hours. After the addition was complete, the reaction mixture was stirred at room temperature for an additional 1 hr followed by reflux for 2 hours. The mixture was then cooled to room temperature and concentrated under a stream of dry nitrogen at reduced pressure. A small portion of the residue was dissolved in methanol (~1 ml) and converted to the free base by treatment with 1N potassium hydroxide (3 ml). A TLC radiochromatographic scan (ether:hexane:triethylamine; 50:45:5) of an ethyl acetate extract of the free base indicated the presence of two radioactive products, the first minor product, being of similar R_f to the previously noted Grignard by-product 8, and the second major product identical in R_f (0.4) to that of unlabeled 7.

The total product, without separating the minor impurity, from the chlorination reaction was then dissolved in methanol (3 ml) and treated with 1N potassium hydroxide (3 ml). This mixture was then poured into water (~5 ml) and the free base was extracted with ether. After concentration *in vacuo* the residue was taken up in butanone (2 ml) and treated dropwise with a solution of citric acid (0.5 g, 2.6 mmol) in methanol (2 ml). Upon cooling, 0.5 g of a crystalline solid precipitated out. Concentration of the mother liquors gave 0.1 g residue. A small portion of the crystalline material was converted to the free base by treatment with methanolic potassium hydroxide. A TLC radiochromatographic scan (ether:hexane:triethylamine; 50:45:5) of this material indicated a single radioactive product identical in R_f (0.4) to that of unlabeled clomiphene. A similar scan obtained for the mother liquors indicated the same product plus an additional, less polar radioactive product similar in R_f to the previously observed Grignard by-product 8. Several attempts were made to determine the specific activity of the crystalline product. Despite repeated crystallizations from butanone, a consistent value could not be obtained with the value varying from 4 to 9 mCi/mmol.

The purity of this product was then examined by HPLC using a μPorasil silica column using different solvent systems. With ethanol as the solvent,

results identical to those obtained by TLC were observed. However, with acetonitrile containing 0.1% ammonium hydroxide, four radioactive peaks were observed for the free base derived from the crystalline material, five peaks for the product from the mother liquors.

The crystalline material was then treated with methanolic potassium hydroxide to obtain the free base and separated into several products by "flash" chromatography on silica gel using acetonitrile containing 0.5% ammonium hydroxide. The mother liquors were similarly treated. Each of these products was analyzed for the presence of chlorine by the method of energy dispersive X-ray fluorescence spectroscopy, and those products giving a positive result were combined. Analysis of this combined material by mass spectroscopy gave an isotopic cluster at m/e 440, 442, and 444 with intensities in the range for two chlorine atoms. The ultraviolet spectrum of this material indicated only a λ_{max} at 285 nm and no absorbance at 240 nm. This observation coupled with the mass spectral data are consistent with the dichloro intermediate 5a.

The dichloro intermediate (5a, 0.024 g) was dissolved in methanol and treated dropwise with concentrated hydrochloric acid (38%, 0.5 ml) to obtain the hydrochloride salt. The reaction mixture was then concentrated *in vacuo*, the residue suspended in xylene (10 ml), and heated at reflux for 2 hr. After concentration *in vacuo* under a stream of dry nitrogen, a u.v. scan of the residue indicated a strong absorbance at 240 nm. The residue was then dissolved in methanol (2 ml) and treated dropwise with 1N potassium hydroxide (2 ml). This mixture was poured into water and extracted with ethyl acetate. Removal of the solvent gave 0.016 g of an oil indicated by mass spectroscopy to consist of a mixture of both unlabeled 7 and labeled 7a (isotopic cluster m/e = 405, 407, and 409). Analysis by h.p.l.c. using a μPorasil column and a solvent system consisting of heptane:dichloromethane (3:7) containing 0.05% butylamine indicated the product to consist of carbon-14 labeled clomiphene. The specific activity of the product was determined to be 10.5 mCi/mmol.

ACKNOWLEDGEMENTS

This investigation was supported by Contract No. 222-80-2024(C) from the National Center for Toxicological Research, Jefferson, Arkansas.

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