Synthesis of Carbon-14 Labeled Cilobamine Mesylate

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SUMMARY

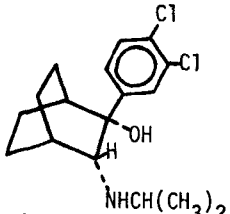
Cilobamine mesylate, cis-2-(3,4-dichlorophenyl-UL^{14}C)-3-[(1-methylethyl)-amino]bicyclo[2.2.2]octan-2-ol methanesulfonate 1, a new antidepressant, was synthesized in 15% yield from the Grignard reaction of 1-bromo-3,4-dichloro-benzene-UL^{14}C and 3-[(1-methylethyl)amino]bicyclo[2.2.2]octan-2-one, 5. The bicycloketoamine 5 was prepared in three steps from bicyclo[2.2.2]oct-2-ene.

Key Words: cilobamine-^{14}C mesylate, carbon 14, epoxy bicyclo[2.2.2]octane, epoxide ring opening.

INTRODUCTION

Cilobamine mesylate, 1, is under development in these laboratories, as an antidepressant. An attempt by previous workers (1) to prepare ^{14}C labeled cilobamine via the large-scale process resulted in material of very low purity which showed little hope of being cleaned-up sufficiently for use.

A synthetic route was needed in which the labeled 3,4-dichlorophenyl ring could be added in the last step. In the most straightforward manner, this could be accomplished by a phenyl Grignard addition to aminoketone 5. It was



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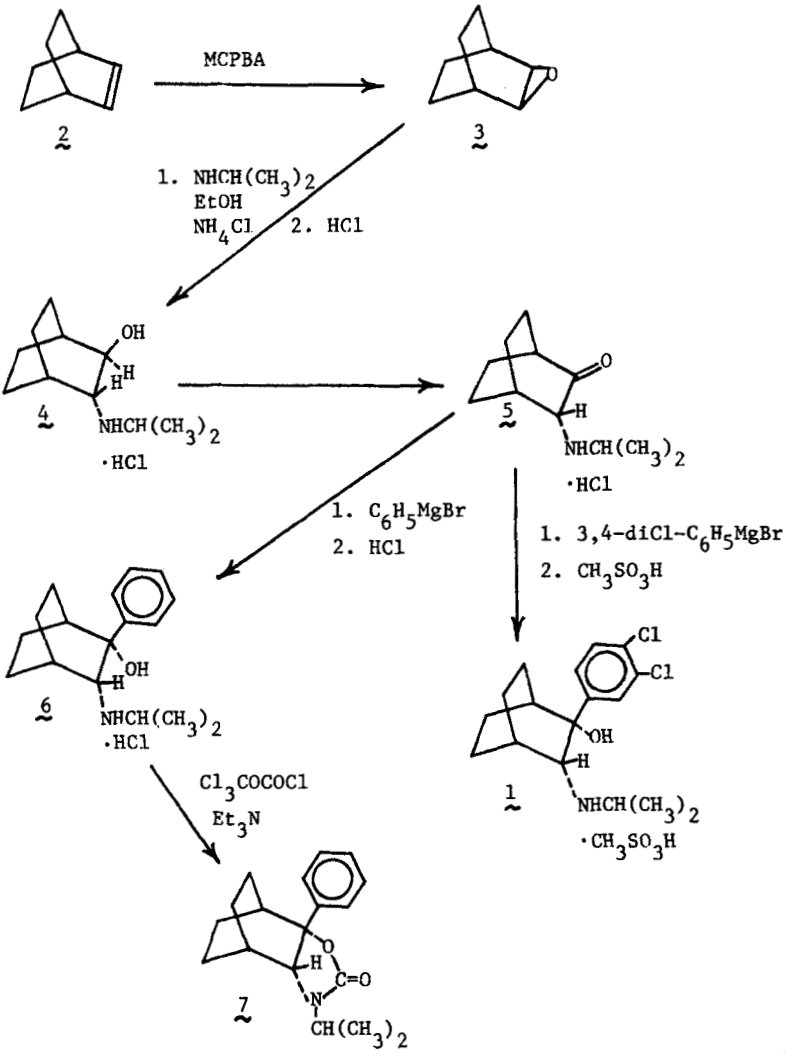
expected that the stereochemical requirement of a cis-amino alcohol relationship would be realized by the steric bulk of the isopropyl group allowing regioselective attack by the Grignard reagent only from the opposite side.

Another encouragement to attempt this route was the observation by Hibbert (2) that Grignard reagents do not attack the proton of secondary amines at temperatures below 70°. Thus, the necessity of blocking the amine would be eliminated if the Grignard reaction could be conducted in ether.

SCHEME



DISCUSSION

Bicyclo[2.2.2]oct-2-ene 2 was readily converted to epoxide 3 (3) with m-chloroperbenzoic acid. However, as might be expected, isopropylamine did not readily open the epoxide. The use of triethylaluminum by the method of Overman and Flippin (4) gave only a complex mixture. Even the use of excess isopropylamine in a sealed tube at 150° (5) failed to give more than a trace of product. Only temperatures of 200-210° for 60 hours would give usable yields of 4. Unreacted epoxide was the only other component and this could be removed easily by sublimation.

The non-sublimable residue, upon treatment with ethereal HCl, gave the amino alcohol hydrochloride 4 in 27% yield. Alternatively, direct treatment of the reaction residue with ethereal HCl gave the insoluble salt directly and unreacted epoxide was converted to the ether soluble chlorohydrin.

Oxidation of 4 with Jones reagent in glacial acetic and work-up using the chromium (III) solubilizing procedure of Mueller and Di Pardo (6) gave a 75% yield of the aminoketone hydrochloride 5.

Treatment of 5 free base with phenyl magnesium bromide in ether gave a single phenyl substituted cis-aminoalcohol 6 in 61% yield. No evidence of the trans isomer was observed by TLC.

To verify that the hydroxyl and isopropylamine groups have a cis relationship, the free base of 6 was reacted with trichloromethylchloroformate in methylene chloride to give a 63% yield of 7. The infrared spectrum showed no NH or OH bands, but did show a carbonyl band at 1720-1700 cm^{-1}. In the mass spectrum, M^+/e=285 confirmed the molecular weight of 7.

The reaction of unlabeled 3,4-dichlorophenylmagnesium bromide with 5 free base gave cilobamine 1 free base which was converted to the mesylate salt in a total yield of 27%. Again only a single product was isolated.

The product was recrystallized readily from acetonitrile and the infrared spectrum of the crystalline salt was identical with the spectrum derived from an authentic sample of cilobamine. The mass spectrum confirmed the molecular weight of 327.

3,4-Dichlorobromobenzene-UL^{14}C was refluxed with magnesium turnings in ether to form the Grignard reagent to which ketoamine 5 free base was added. Upon work-up, methanesulfonic acid in acetonitrile was added and the resulting salt was recrystallized. A total of 62 mg (14.6%) of crystalline product was obtained. The material gave a single peak and a single spot when mixed with authentic cilobamine on HPLC and TLC analyses respectively with no significant impurities. The mass spectrum confirmed the structure and showed a molecular weight of 327.

The material was determined to be 99.5% radiochemically pure with a specific activity of 12.0 ± 0.8 mCi/mmole.

EXPERIMENTAL

Infrared spectra were obtained using a Perkin-Elmer 1310 infrared spectrophotometer. Mass spectra were obtained from a Finnigan model 4023 mass spectrometer equipped with an Incos Series 2000 data system. High Performance Liquid Chromatography was conducted using a Waters 6000 A solvent delivery system with refractive index detector and a LC 55 (UV detector @ 210 nm). All thin layer chromatographs were run on E. Merck TLC plates pre-coated with

Silica Gel 60 F-254 (0.25 mm) and were eluted with 20% EtOH in $CHCl_3$. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected.

Preparation of 2,3-epoxybicyclo[2.2.2]octane, 3

A solution of 15.9 g (0.092 mmole) of m-chloroperbenzoic acid in 200 mL of CH_2Cl_2 was added dropwise to a solution of 10 g (0.092 mmole) of bicyclo-[2.2.2]oct-2-ene (Columbia Organic Chemical, Co.) in 50 mL of CH_2Cl_2. The reaction was chilled to suppress a weak exotherm. After stirring at room temperature for 4 hr, the solution was filtered from the precipitated m-chlorobenzoic acid. The filtrate was washed once with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated at 40°C on a rotary evaporator. The white solid residue was sublimed at 125° and 20 mm Hg to give 8.19 g (72%) of 2,3-epoxybicyclo[2.2.2]oct-2-ene, mp sublimes at 188-90°C, (lit 190-190.3°C) (3).

Synthesis of trans-3[(1-methylethyl)amino]bicyclo[2.2.2]octane-2-ol hydrochloride, 4

Epoxide 3 (7 g, 0.056 mmole) was dissolved in 25 mL of EtOH and 20 mL of isopropylamine, a trace of NH_4Cl was added and the mixture was sealed in a glass tube. The tube was heated at 200-210°C for 60 hr. After cooling, the tube was opened and the clear pale yellow solution was evaporated to leave an oily residue. The oil was dissolved in 50 mL of ether and excess ethereal HCl was added. The fine white precipitate was collected on a filter and washed with ether. Dried on the filter, the solid weighed 3.32 g (27%). By TLC, the material was essentially pure. The compound was recrystallized readily from isopropanol to give mp 231-232°C. IR (Nujol) 3300 (OH), 2800-2400 (NH_2^+) cm^{-1}; NMR (D_2O) 1.35 (d, 3, J=12Hz, iPr), 1.55 (broad, 10), 2.5-4.0 (m, 3), 3.5 (q, 1, J=12Hz, iPrCH) δ; M^+/e=183.

Anal: Calc'd for $C_{11}H_{21}NO$ HCl: C, 60.12; H, 10.09; N, 6.37. Found: C, 60.31; H, 10.24; N, 6.40.

Alternatively, the evaporation residue could be sublimed carefully at 80° and most of the unreacted epoxide could be recovered. The sublimation residue was then converted to the hydrochloride as above.

Oxidation of 4 to 3-[(1-methylethyl)amino]-bicyclo[2.2.2]octan-2-one hydrochloride, 5

A stirred suspension of 1.7 g of 4 (HCl salt) in 15 mL of HOAc was treated dropwise with 3.8 mL of Jones reagent (2.06 mmole/mL). A solid precipitate formed but dissolved when addition was completed. The dark green solution was stirred at room temperature for 0.5 hr. To this was then added 2.7 mL of iPrOH, 45 mL H_2O, 6.9 g of sodium citrate and 3.5 g of amalgamated zinc under a N_2 atmosphere. Stirring was continued for 10 min, the reaction was made alkaline with 5N NaOH, and extracted 4 times into ether. The ether was washed once with saturated NaCl, dried (Na_2SO_4) and evaporated. To the residue, 5 mL of saturated ethereal HCl was added and evaporated. The white solid residue was recrystallized from 20 mL of CH_3CN to give 1.27 g (75%), mp 213-6°C. This compound was very unstable as the free base and could only be maintained in that state for a few hours before decomposition occurred. IR (Nujol) 2900-2400 (NH_2^+), 1760 (C=O) cm^{-1}; NMR (D_2O), 1.35 (d of d, 6, J=12Hz,iPr) 3.7 (M, 1, J=12Hz, Me_2CH) δ; M^+/e=181.

Anal: Calc'd for $C_{11}H_{19}NO$ HCl: C, 60.68; H, 9.26; N, 6.43. Found: C, 60.65; H, 9.11; N, 6.64.

Synthesis of cis-2-phenyl-3-[(1-methylethyl)amino]-bicyclo[2.2.2]octan-2-ol hydrochloride, 6

A solution of 88.6 mg (0.56 mmole) of bromobenzene in 2 mL of ether was heated to reflux with a crystal of iodine and 27.4 mg of Mg metal turnings. The

iodine color disappeared and the solution turned cloudy. The solution was refluxed for 1 hr and the free base which was isolated from 108.5 mg (0.5 mmole) of 5 was added to the reaction in ether. A flocculent precipitate formed in the solution. The mixture was refluxed for 3 hr, then cooled and poured into saturated NH_4Cl and the ether layer was separated. The aqueous layer was washed 2 times with ether and the combined ether layers were washed once with H_2O, dried (Na_2SO_4) and evaporated. The residue was taken up in 20 mL of ether and excess ethereal HCl was added. Cooled, the mixture was filtered and the white product dried on the filter. Recrystallization from MeOH/ether gave 6, mp 249-50°C. IR (Nujol) 3280 (OH), 3100-2600 (NH_2^+) cm^{-1}; NMR (D_2O) 1.2 (d, 6, iPr) 7.4 (m, 5, phenyl) δ; M^+/e=259.

Anal: Calc'd for $C_{17}H_{25}NO$ HCl: C, 69.01; H, 8.86; N, 4.73. Found: C, 68.81; H, 8.73; N, 4.44.

Conversion of 6 to cis-hexahydro-3-(1-methylethyl)-7a-phenyl-4,7-ethano-benzoxazol-2(3H)-one 7

A solution of 20.0 mg of 6 in CH_2Cl_2 was shaken with excess aqueous NaOH. The aqueous layer was washed twice with CH_2Cl_2 and the combined CH_2Cl_2 layers were washed once with H_2O. After drying (Na_2SO_4) and evaporation, the residue was dissolved in 2 mL of ether. To this was added 5 drops of Et_3N and 3 drops of trichloromethylchloroformate. A thick white precipitate formed. An additional 2 drops of trichloromethylchloroformate were added and after a few minutes, the mixture was poured into H_2O. The ether was dried (Na_2SO_4), filtered and evaporated to give a crystalline residue. Recrystallization from CH_2Cl_2/hexane gave 12.3 mg (63%) of white crystals, mp 135-137°C. IR (Nujol) 1710 (broad C=O) (no NH or OH bonds) cm^{-1}; NMR ($CDCl_3$) 1.25 (d, 6, J=12Hz, iPr) 1.2-2.5 (m, 10, ring H's) 4.7-4.2 (m, 2) 7.4 (m, 5, phenyl); M^+/e=285.

LABELED SYNTHESIS

Preparation of cis-2-(3,4-dichlorophenyl-UL-^{14}C)-[(1-methylethyl)amino]bicyclo-[2.2.2]octan-2-ol methanesulfonate, Cilobamine-^{14}C mesylate

Three pieces of Mg (excess, turnings freshly broken) and 5 mL of anhydrous ether were place in a dry flask under a N_2 atmosphere. The 1-bromo-3,4-

dichlorobenzene-UL^{14}C (0.881 mmoles, 10 mCi, 11.3 mCi/mmole, 98.33% pure, Pathfinder Laboratories, Inc.) dissolved in 0.5 mL of ether, was added to the mixture and was washed in with three 1 mL portions of ether. Several small iodine crystals were added to produce a red-brown color. The mixture was heated to reflux with vigorous stirring. HPLC analysis showed the Grignard formation to be virtually complete after 2 1/2 hr. The iodine color had faded. Ketoamine 5 free base was prepared by dissolving the HCl salt in 3 mL of 0.1 N NaOH, extracting 3X with CH_2Cl_2, drying (Na_2SO_4) and evaporating. The residue was dissolved in 2 mL of ether and was added to the reaction. After refluxing for 90 min the reaction was stirred at room temperature overnight. The reaction was quenched with 10 mL of saturated NH_4Cl solution. The aqueous layer was removed and the ether was washed with 3 mL of H_2O, dried (Na_2SO_4), and filtered. The resulting clear yellow solution was concentrated under a slow stream of N_2. Methanesulfonic acid (1 mmol in 2 mL CH_3CN) was added prior to complete dryness and the solution was heated to reflux. Cooled, the solution was diluted with 10 mL of ether and refluxed for 1 hr. The solution was concentrated to about 1.5 mL and crystals formed. Refrigerated overnight, the crystals were collected on a fritted glass filter, washed twice with 0.5 mL portions of cold CH_3CN and dried *in vacuo*. A yield of 62 mg (1.66 mCi, 14.6%) was obtained. The product was shown to be 99.5% pure by HPLC. The mass spectrum and tlc analyses confirmed the identity and purity of the labeled compound.

Specific Activity Determination of ^{14}C Labeled Cilobamine Mesylate

The specific activity was determined by weighing 1.86 ± 0.03 mg of labeled cilobamine into a 10 mL volumetric flask. Diluted to 10 mL with methanol, four 100 µL aliquots were counted on a Packard Prias Tri-carb using external standard ratio method. The specific activity was found to be 12.0 ± 0.8 mCi/mmole or 28.4 µCi/mg.

RESULTS

A total of 1.66 mCi (0.061 GBq) of cilobamine-^{14}C mesylate weighing 62 mg and having a specific activity of 12.0 ± 0.8 mCi/mmole (0.44 GBq/mmole) was obtained in 1 step from 3,4-dichlorobromobenzene-UL ^{14}C. The yield was 14.6% after one recrystallization and the product was >99.5% by HPLC analysis.

Structural confirmation of the labeled product was done by comparative HPLC, TLC, and mass spectral analysis.

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