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Synthesis of *Z*- and *E*-[2,3-$^2H_2$] and [2,3-$^3H_2$]-1,1-Dichloro-2,3-diphenylcyclopropane (^{2}H- and ^{3}H-Analog II and its *trans* Isomer)

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SUMMARY

^2H and ^3H labeled Z- and E-1,1-dichloro-2,3-diphenylcyclopropane (1 and 2) were synthesized starting from NaB^2H_4 and NaB^3H_4 reduction of benzil. The resulting glycols were transformed to the 1,2-labeled Z- and E-stilbenes by thermolysis of their cyclic thionocarbonates in trimethylphosphite. The stilbenes were reacted with phase transfer-generated dichlorocarbene to form the title compounds. The dideuterio isomers were separated by fractional crystallization in yields of 60 and 48 %. Each was greater than 99 % geometrically and 98 % isotopically pure. The ditritio isomers were separated by C-18 HPLC. The radiochemical yields, on a molar basis using benzil as the limiting reagent, were 42 % and 23 %, each with specific activity of 88.5 mCi/mmol and radiochemical purity of > 95%.

Key words: 1,1-dichloro-2,3-diphenylcyclopropane, deuterium, tritium, Analog II

INTRODUCTION

Z-1,1-Dichloro-2,3-diphenylcyclopropane (**1**), also known as Analog II (1), is a novel anti-breast cancer agent, effective *in vivo* against carcinogen-induced and transplantable rat mammary tumors (2,3) as well as *in vitro* against estrogen-dependent and -independent human breast cancer cells grown in culture (4,5). The mechanism(s) of antiproliferative and cytotoxic action shown by **1** is (are) unknown. We are currently studying the metabolism and macromolecule binding of this agent both *in vitro* and *in vivo*, and our studies required both high specific activity radiolabeled **1** for biodistribution/radiotracer experiments and stable isotope heavy-atom labeled **1** for use as an internal standard in mass spectrometric determinations, and as a probe for metabolic reaction mechanisms. This communication describes the synthesis of high specific activity [2,3-$^3H_2$]-labeled and high isotopic purity [2,3-$^2H_2$]-labeled **1**. The synthetic procedures utilized also yielded appreciable amounts of the labeled versions of the biologically inactive *E* isomer **2**.

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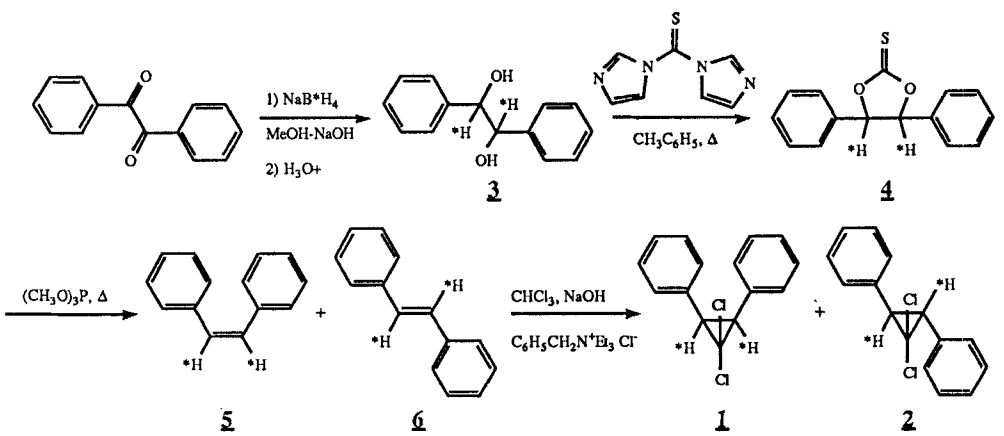
DISCUSSION

For use in preliminary biodistribution studies, ^3H-labeled **1** has previously been prepared by New England Nuclear by the method of catalytic exchange in base with ^3H_2O. The yield and highest specific activity obtained from this method are both low (< 50 % via radiodegradation and specific activity of ≤ 4.4 mCi/mmol) (6). This method requires handling a large amount of radioactivity in the form of ^3H_2O, and it is not known at present if **1** is specifically or generally labeled by this method, although we presume it is the latter. The low specific activity and unknown site(s) of labeling by this method were unsuitable for our present studies, and prompted us to design a synthesis which accomodated our requirements for both tritiated and deuterated **1**.

Synthesis of the dideuterio compound was attempted first, in order to determine if the synthetic procedures would be applicable to the tritium labeled compound, and to determine if the presence of heavy atoms would alter the physical characteristics (e.g., chromatographic behavior) of the labeled versions of **1** to any discernable extent. Benzil was reduced with 1.2 molar equivalents of NaB^2H_4 and the resulting boronate complex was decomposed with aqueous HCl to yield [1,2-$^2H_2$]-1,2-diphenylglycol **3** in quantitative yield. The glycol was transformed to a 1:1 mixture of the *Z*- and *E*-stilbenes **5** and **6** in 60% yield via the Corey-Winter olefin synthesis (7): **3** was converted to its cyclic thionocarbonate **4** by reaction with one molar equivalent of the diimidazole derivative of thiophosgene in boiling toluene, then deoxygenated in boiling trimethylphosphite. The dideuterio stilbenes were purified as a mixture by flash SiO_2 chromatography, then subjected to phase transfer dichlorocyclopropanation using $CHCl_3$, 33% NaOH, and benzyltriethylammonium chloride as the catalyst (1,8). The reaction mixture was extracted and subjected to flash SiO_2 chromatography to purify the cyclopropanes as a 1:1 mixture. The *Z* isomer **[2,3-$^2H_2$]-1** was isolated by fractional crystallization with petroleum ether containing a trace of absolute EtOH, then recystallized from petroleum ether (60 % yield from **5**). The *E* isomer **[2,3-$^2H_2$]-2** was obtained from the mother liquor by recrystallization from absolute EtOH (48 % yield from **6**). Capillary GC-MS and proton NMR analyses indicated that each was > 99% pure geometrically, and that the isotopic purity at C-2 and C-3 was ≥ 98 % ^2H.

The synthesis of the ditritio compounds was performed using the same synthetic route. Use of a 1.2:1 molar ratio of NaB^3H_4 to benzil did not yield complete conversion to the glycol in our hands.



Therefore, a four-fold molar excess of NaB^3H_4 (177 mCi/mmol) was used and the reactions were carried out to the stilbene stage ([2,3-$^3H_2$]-**5** and [2,3-$^3H_2$]-**6**) without purification of the intermediates. The ditritiated stilbenes were partially purified by SiO_2 chromatography, then subjected to phase transfer dichlorocyclopropanation. [2,3-$^3H_2$]-**1** and [2,3-di-$^3H_2$]-**2** were separated and purified by semi-preparative C-18 HPLC (Figure 1). The radiochemical yield was 65% (based on the limiting reagent, benzil). The specific activity of each product was 88.5 mCi/mmol. The final ratio of [2,3-$^3H_2$]-**1** -to- [2,3-$^3H_2$]-**2** being 9:5. Re-analysis by HPLC and scintillation spectrometry of the fractions showed each cyclopropane to be of > 95% radiochemical purity.

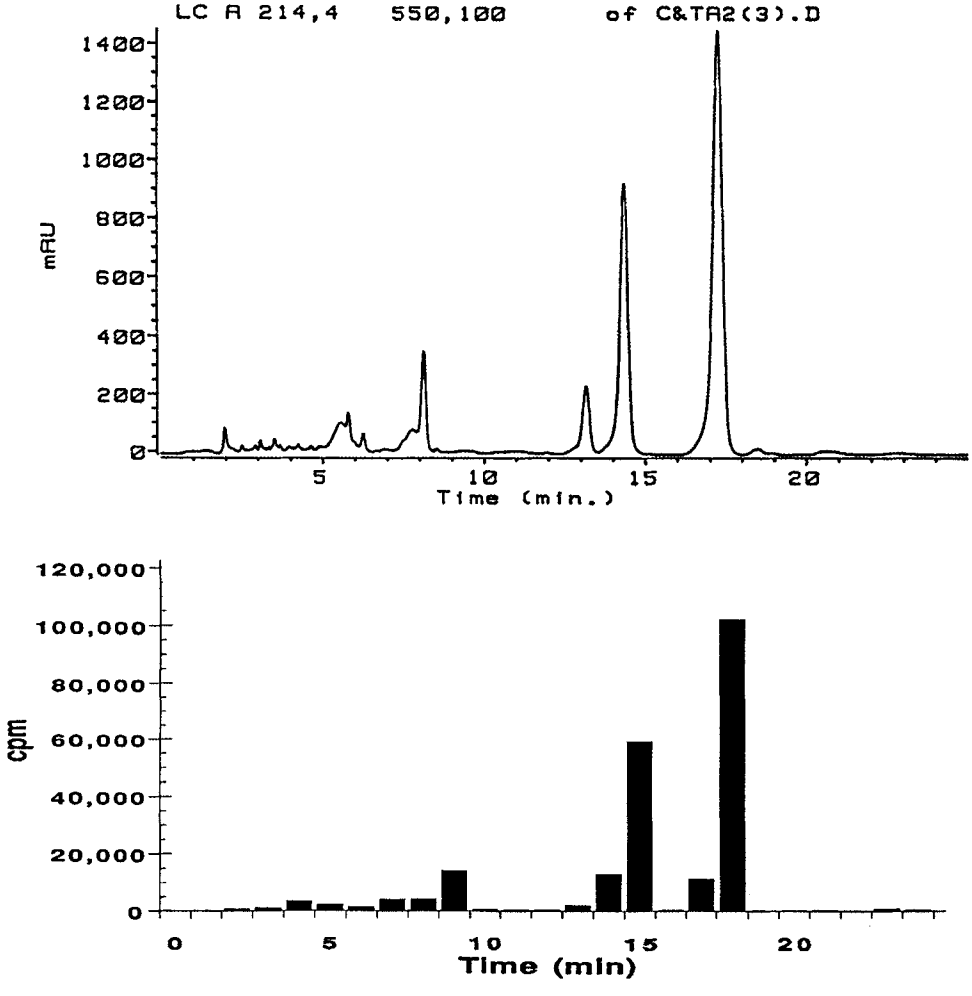


Figure 1. Upper: Representative UV (214 nm) detected chromatogram of the reversed phase C-18 HPLC purification of [2,3-$^3H_2$]-2 (retention time 14.4 min) and [2,3-$^3H_2$]-1 (retention time 17.2 min). Lower: Radiochromatogram of the one min fractions collected from the upper trace. The analyte eluting at 13.3 min is residual trans*-stilbene [1,2-$^3H_2$]-6, while the identity of the analyte eluting at 8 min is unknown.*

EXPERIMENTAL

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Silica gel was of Merck flash grade 9385, 230-400 mesh (Aldrich). ^{1}H-NMR spectra were recorded at ambient temperature on JEOL FX-80 and Varian XL-200 spectrometers in $CDCl_3$. Spectra are reported in parts per million downfield from internal $(CH_3)_4Si$. Capillary column gas chromatography-mass spectrometry was performed on a Hewlett Packard 5971 mass selective detector interfaced to a 5890 Series II GC equipped with a 12 m HP-1 (methylsilicone, 0.2 mm i.d., 0.33 μm film thickness) fused silica capillary column (Hewlett Packard). The carrier gas was high purity He (8 psi column pressure). Mass spectra were determined in electron ionization (70 eV) mode. High performance liquid chromatography (HPLC) was performed on a Hewlett Packard 1090 LC equipped with a 1040 diode array detector and a 300 Chemstation. Compounds were separated on a 7.8 x 300 mm Waters Prep Nova-Pak HR C18 60Å 6 μm reverse-phase column (Millipore) using a He-sparged mobile phase of 7:3 CH_3CN-H_2O. NaB^3H_4 was purchased from New England Nuclear. Other synthetic reagents, chromatographic materials, and solvents were purchased from Aldrich Chemical Co. and were of the highest available purity. Unlabeled **1** and **2** were prepared as previously described (1).

1,2-diphenyl-1,2-ethanediol [1,2-$^2H_2$] ([1,2-$^2H_2$]-3). Benzil (2.1 g, 10 mmol) was dissolved in 30 mL CH_3OH, stirred, and treated portion-wise with NaB^2H_4 (≥ 98 % ^{2}H isotopic purity, 485 mg, 12 mmol) over a 2 h period. The mixture was stirred an additional 1 h at room temperature, then poured onto 100 mL of 10 mM HCl and stirred an additional 0.5 h. The solution was adjusted to pH 8 with NaOH, then extracted with EtOAc (4 x 75 mL). The organic layers were combined, washed with H_2O, dried ($MgSO_4$), filtered, and concentrated on a rotary evaporator to give the product as a white solid (2.16 g, 100 % yield) which, when analyzed by GC-MS, appeared to be 100% pure, and was used without further purification or characterization. EI-MS, *m/z* (%): 108 (100, $(C_6H_5CDOH)^+$), 80 (90), 78 (42), 51 (11).

1,2-Diphenyl-1,2-ethanepyrothionocarbonate [1,2-$^2H_2$] ([1,2-$^2H_2$]-4). Dideuterio compound **[1,2-$^2H_2$]-3** (2.16 g, 10 mmol) was dissolved in 90 mL toluene containing 1,1'-thiocarbonyldiimidazole (90 % purity, 1.98 g, ca. 10 mmol) and heated to reflux with stirring for 2 h. The solvent was removed on a rotary evaporator and the resulting brownish solid was purified by flash SiO_2 chromatography (2:1 CH_2Cl_2-petroleum ether) to give **[1,2-$^2H_2$]-4** as a foul-smelling, white, crystalline solid (2.5 g, 97 % yield). A valid melting point could not be obtained due to the hygroscopic and thermolabile nature of the product. EI-MS, *m/z* (%): 258 (7, $M^{+\cdot}$), 180 (11), 168 (100). ^{1}H-NMR, δ: 7.2-7.07 (m, 6H), 7.03-6.88 (m, 4H).

Z- and *E*-Stilbene [1,2-di-$^2H_2$] ([1,2-$^2H_2$]-5 and 1,2-$^2H_2$]-6). The dideuterio thionopyrocarbonate **[1,2-$^2H_2$]-4** (2.5 g, 9.7 mmol) was dissolved in 100 mL of $(CH_3O)_3P$ (stench!) and heated to reflux with stirring for 90 h. The solution was cooled and the solvent was removed on a rotary evaporator. The resulting brown oil was purified by flash SiO_2 chromatography (petroleum ether followed by 9:1 petroleum ether-CH_2Cl_2) to give a 1:1 mixture of the products (1.73 g, 96 % yield) as a straw oil. EI-MS (the mass spectra of **[1,2-$^2H_2$]-5** and **[1,2-$^2H_2$]-6** were equivalent), *m/z* (%): 182 (100, $M^{+\cdot}$), 167 (28), 153 (8), 90 (12), 77 (8), 51 (5).

Z- and *E*-1,1-Dichloro-2,3-diphenylcyclopropane [2,3-$^2H_2$] ([2,3-$^2H_2$]-1 and [2,3-$^2H_2$]-2). The mixture of **[1,2-$^2H_2$]-5** and **[1,2-$^2H_2$]-6** (1.73 g, mmol) was dissolved in 30 mL of $CHCl_3$ along with benzyltriethylammonium chloride (10 mg, 44 μmol). The solution was stirred and treated drop-wise with chilled 33% aqueous NaOH (25 mL). The resulting two phase mixture was rapidly stirred at room temperature for 96 h, diluted with 100 mL H_2O, and extracted with CH_2Cl_2 (4 x 100 mL). The organic layers were combined, washed with brine (100 mL) and H_2O (100 mL), dried ($MgSO_4$), filtered, and concentrated on a rotary evaporator to give a tan oil. The oil was purified to a straw-colored, oily mixture of the products by flash SiO_2 chromatography (9:1 petroleum ether-CH_2Cl_2), then fractionally crystallized with ca. 99:1 petroleum ether-EtOH to give **[2,3-$^2H_2$]-1** as clear needles, which were recrystallized from petroleum ether to yield a white solid, mp: 57-59 °C. The mother liquor was fractionated with the same solvents three times (753 mg, 60 % yield from the *Z*-stilbene). EI-MS, *m/z* (%): 268 (1.5, $^{37}Cl^{37}Cl$ -M+·), 266 (5, $^{35}Cl^{37}Cl$-M+·), 264 (8.5, $^{35}Cl^{35}Cl$-M+·), 231 (28), 229 (60), 193 (66), 165 (13), 153 (35), 151 (100). ^{1}H-NMR, δ: 7.255-7.2 (m, Ar*H*, 6H), 7.055-7.0 (m, Ar*H*, 4H), 3.296 (s, residual cyclopropyl ^1H, 0.008 H). The final mother liquor was treated with EtOH to yield **[2,3-$^2H_2$]-2** as a white solid, mp: 41-43 °C (602 mg, 48 % yield from the *E*-stilbene). EI-MS, *m/z* (%): 268 (1, $^{37}Cl^{37}Cl$-M+·), 266 (3.5, $^{35}Cl^{37}Cl$-M+·), 264 (5, $^{35}Cl^{35}Cl$-M+·), 231 (20), 229 (58), 193 (50), 153 (38), 151 (100). ^{1}H-NMR, δ: 7.36 (br s, Ar*H*, 10H), 3.225 (s, residual cyclopropyl ^1H, 0.08 H).

Z- and *E*-1,1-Dichloro-2,3-diphenylcyclopropane [2,3-$^3H_2$] ([2,3-$^3H_2$]-1 and [2,3-$^3H_2$]-2). Benzil (1.4 mg, 7 μmol) was dissolved in 1mL of CH_3OH, a 1:1 mixture of NaB^3H_4 (13.9 μmol, 5 mCi) and NaB^1H_4 (13.9 μmol) in 100 μL of 0.1 N NaOH was added, and the solution was stirred at room temperature for 24 h. The mixture was adjusted to approximately pH 2.0 with 1 N HCl and the solution was evaporated to dryness *in vacuo*. The residue was dissolved in 2.0 mL of toluene and 5.0 mg of 1,1'-thiocarbonyldiimidazole was added. This mixture was gently refluxed for 2 h and evaporated to dryness *in vacuo* . To this residue, $(CH_3O)_3P$ (2 mL) was added and the mixture was heated to reflux and stirred under Ar for 30 h. The $(CH_3O)_3P$ was removed *in vacuo* and the yellow, oily residue was applied to a 5 mm i.d. x 50 mm SiO_2 column. The stilbenes were partially purified by eluting the column by gravity flow with a gradient of petroleum ether to CH_2Cl_2 to $CHCl_3$. The combined fractions were concentrated *in vacuo* and the residue containing the 1,2-ditritiated stilbenes was used in the next step without further characterization. The 1,2-ditritiated stilbenes were dissolved in $CHCl_3$ (1.75 mL) containing benzyltriethylammonium chloride (1.5 mg). To this mixture, 35% aqueous NaOH (2.5 mL) was added dropwise while stirring and the reaction was allowed to proceed for 24 h at room temperature. To the resulting two-phase mixture, CH_2Cl_2 (10 mL) was added. The organic layer was separated, washed with H_2O (10 x 10 mL) to remove the catalyst and traces of NaOH, evaporated to dryness, and dissolved in approximately 300 μL of 7:3 CH_3CN-H_2O. C-18 HPLC analysis of this mixture indicated the presence of desired compounds in a *Z*-to-*E* ratio of 9:5. The two compounds were purified on a semi-preparative column, using *ca.* 10% of the crude reaction product for each HPLC separation. Each identified tritiated product co-eluted with its unlabeled counterpart. The overall chemical and radiochemical yield of

[2,3-$^3H_2$]-1 (based on the limiting reagent, benzil) was 42% and the yield of **[2,3-$^3H_2$]-2** was 23%. The specific activity of each cyclopropane was determined to be 88.5 mCi/mmol by scintillation spectrometry. Analysis of each isolated compound by HPLC and scintillation spectrometry showed each to be > 95 % radiochemically pure.

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