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Synthesis of [3-^{3}H]20(*S*)-protopanaxadiol

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[3-^{3}H]20(*S*)-Protopanaxadiol (PPD) was prepared via selective reduction of PPD dioxide with sodium borotritide in *N,N*-dimethyl acetamide. A radiochemical yield of 22% was obtained. The radiochemical purity of the final product was 99% and the ratio of C-3/C-12 tritium labeling was 97:3.

Keywords: 20(*S*)-protopanaxadiol; regioselective tritium-reductions of carbonyl functions; radiosynthesis

Introduction

20(*S*)-Protopanaxadiol (PPD, Figure 1) is one of the main aglycones of ginsenosides in *Panax ginseng* and *Panax notoginseng*.[1,2] This compound markedly increases the levels of norepinephrine and 5-hydroxytryptamine (5-HT) in the brain of rats with depression symptoms, and significantly enhances the tremor effect of 5-HT.[3] Currently, PPD is in clinical development as an antidepressant drug candidate. Consequently, radiolabeled PPD is required for animal and human absorption, distribution, metabolism, and excretion studies (ADME).

Wu *et al.* reported the synthesis of tritium-labeled PPD by the reduction of PPD dioxide with sodium borotritide.[4] The reduction of PPD dioxide formed four diastereomers. However, these researchers did not discuss the stereoselective formation and the chiral separation of these radioactive isomers. They claimed that the tritium label occurred at two different sites, C-3 and C-12 position, but did not provide supporting experimental data to confirm or to report the exact ratio of C-3/C-12 tritium labeling. The radiochemical yield reported was very low ($<2\%$).[4] As part of our research program on the ADME properties of PPD, it is important to develop a high yield stereospecific synthetic method to yield tritium-labeled PPD.

Results and discussion

Tritium labeled compounds offer some advantages over their ^{14}C counterparts, primarily because of their relative ease of preparation. However, the position of the ^{3}H label might not be specific.[5] Schemes 1 and 2 show the synthetic route of [3, 12-^{3}H]PPD by Wu *et al.* The major drawback of this synthesis strategy is that methanol was used as the reaction solvent,[4] leading to the decomposition of NaB^3H_4, and the potential of tritium/hydrogen exchange.

The modified [3-^{3}H]PPD synthesis method used in the present study is illustrated in Scheme 3. *N,N*-Dimethyl acetylamide (DMA) was used as the reaction solvent in place of methanol. This modification avoided the decomposition of NaB^3H_4, and increased the radiochemical yield from $<2\%$ to 22%.

With DMA as the reaction solvent, the rate of reduction of **2** with slightly less than equimolar sodium borotritide at 35°C was slower than that in methanol at ambient temperature.[4] A separate reduction step for **2** was performed with equimolar $NaBH_4$, as shown in Scheme 3. The product was analyzed by MS and ^{1}H NMR. MS (ESI): *m/z* 441.4 $[M+H-H_2O]^+$. The ^{1}H NMR data for the product were consistent with those of unlabeled **3** reported in the literature.[6] This suggested that **2** was selectively reduced by $NaBH_4$ at the 3-carbonyl position, as this position was more reactive than the 12-carbonyl position.[6] The radiochromatogram of the reaction mixture (30 h) also showed that **3** was the main radiolabeled product. The **3** and [3,12-^{3}H]PPD accounted for 75 and 5% of the total radioactivity, respectively, based to their peak areas. The ratio of C-3/C-12 tritium labeling was 97:3. Without purification the labeled reaction product was submitted to a subsequent reduction operation using excess unlabeled sodium borohydride. Running the reaction at an increased temperature of 55°C gave **1b**. This did not change the ratio of C-3/C-12 tritium. Furthermore, the unlabeled **3** was further reduced by $NaBH_4$ as shown in Scheme 4. The product was analyzed by MS and ^{1}H NMR. MS (ESI): *m/z* 443.4 $[M+H-H_2O]^+$. The ^{1}H NMR data for the product were consistent with those of PPD in the literature.[7]

The reduction of **2** with sodium borotritide formed four diasteromers. However, PPD, the major product, accounted for 76% the four diasteromers, because the reduction of keto steroids with hydrides, such as $NaBH_4$ and $LiAlH_4$, generally favor equatorial over axial alcohols.[8] By HPLC purification, the four

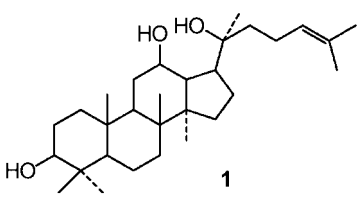

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Figure 1. Structure of PPD.

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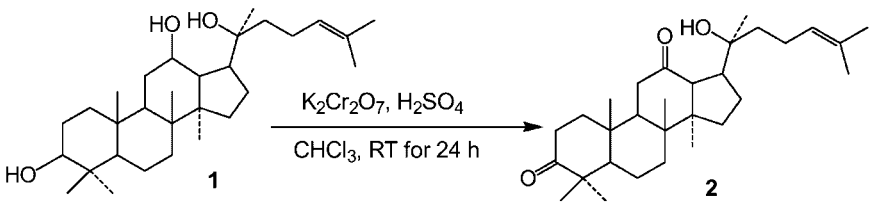
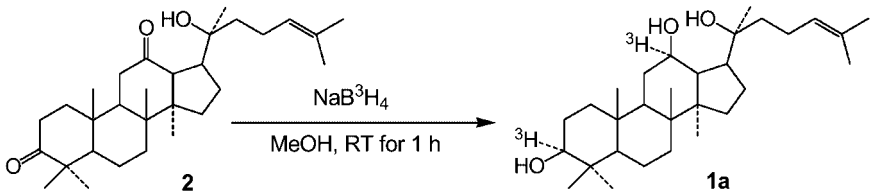
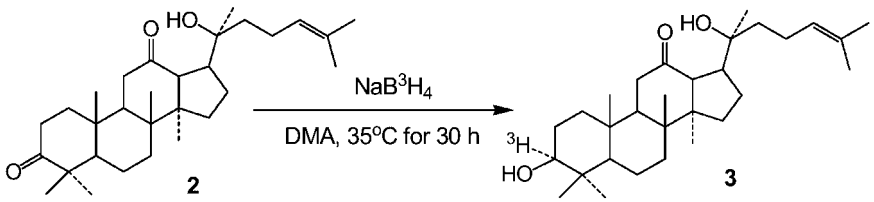


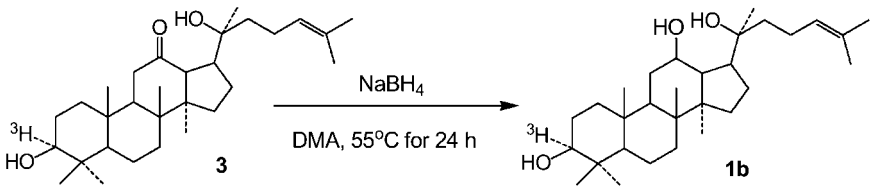
Scheme 1. Synthesis of **2**.



Scheme 2. Synthesis of **1a** reported by Wu *et al.*[4]



Scheme 3. Synthesis of **3**.



Scheme 4. Synthesis of **1b**.

diasteromers were completely separated and only [3-^{3}H]PPD was collected.

The purified product was concentrated under a stream of nitrogen, rather than lyophilization, to avoid the autoradiolysis of the tritium labeled compound. A long-term stability study indicated that the product was stable for four months when stored in absolute ethanol (7.04 mCi/mL) at −20°C. The metabolic stability of [3-^{3}H]PPD was examined following administration of a single 160 μCi/100 mg/kg oral dose of [3-^{3}H]PPD to Sprague–Dawley rats. Radioactivity profiling of plasma, urine, and fecal extracts by HPLC with a dynamic on-line radio flow detector showed that no tritiated water was formed. Total radioactivity was also measured in plasma and urine before and after drying. The results of the study showed no loss of label due to either metabolism or to chemical exchange.[9]

Experimental

Materials

1 was provided by Shanghai Innovative Research Center of Traditional Chinese Medicine (Shanghai, China). Sodium borotritide (0.1 Ci with a specific activity of 8.2 Ci/mmol) was purchased from PerkinElmer Inc. (Boston, USA). DMA (chemically pure grade) was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China) and distilled to remove traces of water. All other chemicals were obtained from commercial sources as chemically pure grade or higher and were used without further purification. HPLC was performed on an Agilent 1100 HPLC system equipped with a variable wavelength ultraviolet detector (Agilent Technologies, Santa Clara, USA) and a dynamic on-line radio flow detector (AIM Research Company, Hockessin, USA). ^{1}H-NMR spectra were recorded on a 300 MHz NMR spectrometer (Varian, Palo Alto, USA). MS was determined on an Agilent 6330 LC/MSD Trap XCT ultra (Agilent Technologies, Waldbronn, Germany). Radioactivity was determined on an LS 6500 liquid scintillation counter (Beckman Coulter Inc., Fullerton, USA).

(14R)-Dodecahydro - 17 - ((S) -2-hydroxy-6-methylhept-5-en-2-yl)-4,4,8,10,14 - pentamethyl -2H-cyclopenta[a]phenanthrene-3,12(4H,14H)-dione (**2**): A solution of $K_2Cr_2O_7$ (250 mg, 0.85 mmol) and H_2SO_4 (100 μL, 1.88 mmol) in water (3 mL) was placed into a 10-mL pear-shaped flask. A solution of **1** (50 mg, 0.11 mmol) in $CHCl_3$ (2 mL) was added dropwise with stirring. The resulting mixture was then stirred for 24 h at room temperature until **1** was consumed. At this point, the crude product was partitioned between trichloromethane and water. The organic phase was

washed twice with water, evaporated to dryness by rotary evaporation and then further purified by silica gel flash chromatography eluted with ethyl acetate/petroleum ether (10:1, v/v) to yield **2** as a white powder (34 mg) with 68% yield. MS (ESI): *m/z* 439.3 $[M+H-H_2O]^+$ and the ^1H NMR data of the compound were consistent with those of **2** in the literature.[10]

[3-^{3}H](14R) - Tetradecahydro -3-hydroxy-17-((S)-2-hydroxy-6-methylhept-5-en-2-yl)-4,4,8,10,14-pentamethyl-2H-cyclopenta[a]phenanthren-12(14H)-one (**3**): A solution of **2** (6.9 mg, 0.015 mmol) in DMA (1 mL) was placed in a 10-mL pear-shaped flask. NaB^3H_4 (0.51 mg, 0.012 mmol) was added under nitrogen. The mixture was stirred at 35°C for 30 h in the dark. A portion of the reaction mixture (15 μL) was taken for radiochromatography analysis and the rest of the mixture was used for the next reaction without further purification.

Analytical HPLC conditions

Column: Zorbax SB-C_{18}, 150 mm × 4.6 mm i.d., 5 μm; solvent: acetonitrile/5 mM ammonium acetate (90:10, v/v); flow: 0.6 mL/min; T_R = 11.04 min for **3** and T_R = 17.46 min for [3,12-^{3}H]PPD.

[3-^{3}H](14R)-Hexadecahydro-17-((S)-2-hydroxy-6-methylhept-5-en-2-yl)-4,4,8,10,14-pentamethyl-1H-cyclopenta[a]phenanthrene-3,12-diol (**1b**): $NaBH_4$ (7.4 mg, 0.196 mmol) was added to the crude **3** and the mixture was stirred at 55°C for 24 h to complete the reaction. The resulting solution was treated with water (5 mL) and stirred for 10 min until no gas evolution was noticeable, then extracted three times with ethyl acetate. The combined extracts were concentrated under a stream of nitrogen, and then reconstituted in methanol for HPLC purification.

Preparative HPLC conditions

Column: Zorbax SB-C_{18}, 150 mm × 4.6 mm i.d., 5 μm; solvent: acetonitrile/water (80:20, v/v); flow: 1.0 mL/min; UV = 203 nm. T_R = 11.7 min for PPD and T_R = 10.3, 15.0, and 15.8 min for its other diastereomers. Only the PPD fraction was collected.

Fractions containing pure product were pooled and concentrated under a stream of nitrogen. The final labeled product was reconstituted in ethanol to afford 22.1 mCi of **1b** (22% yield) with a radiochemical purity of 99%. Specific radioactivity was measured as 2.92 Ci/mmol by HPLC and liquid scintillation counter. The final product was stored at 7.04 mCi/mL in absolute ethanol at −20°C.

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