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Research Article

Synthesis of tritium-labeled puerarin—a potential antidipsotropic agent

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Abstract: Puerarin **1** (8-β-D-Glucopyranosyl-4′-7-dihydroxyisoflavone, NPI-031G) is the major isoflavone C-glycoside isolated from *Pueraria lobata*, a traditional Chinese medicine widely used for the treatment of alcohol intoxication. In order to understand the mode of action of puerarin in the reward pathway of the central nervous system and to study its bioavailability and pharmacokinetics, we developed a synthetic route for the preparation of tritium-labeled puerarin. The key intermediate **4** was obtained by trimethylsilyl protection of all hydroxyl groups followed by selective deprotection. The corresponding aldehyde **5** was obtained through the subsequent oxidation of the primary alcohol. Standard NaB[^{3}H]$_4$ reduction and hydrolysis produced the tritium-labeled puerarin **6**. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: tritium-labeled puerarin; synthesis; isoflavone C-glycosides

Introduction

Recent studies showed that puerarin reduces craving for alcohol drinking and postulated that the antidipsotropic effect is probably not mediated by conditioned taste aversion. It may involve some steps in the reward pathway of the central nervous system.[1,2] Puerarin was also reported to have inhibitory activity against platelet aggregation *in vitro* and *in vivo*,[3] and has hypoglycemic and coronary artery blood flow increasing activity.[4]

Our preliminary study showed that puerarin may be a weak BZD receptor antagonist or an antagonist targeted at the 5-HT$_{2c}$ receptor.[5] However, the mechanism for the suppression of alcohol drinking is not fully understood. In order to investigate how puerarin is involved in the reward pathway of the central nervous system and to study its bioavailability and pharmacokinetics, it is necessary to synthesize tritium-labeled puerarin.

Results and Discussion

Our initial attempt was to oxidize the primary 6′-OH of the glucose moiety under mild Swern oxidation or Parikh–Doering oxidation conditions. Unfortunately, direct oxidation was unsuccessful. As shown in Scheme 1, puerarin was first treated with 8 equivalents of *N*-trimethylsilylacetamide at 175°C for 15 min to give the pentatrimethylsilylated compound **2** in 86% yield.[6] Compound **2** was unstable on silica gel and decomposed to compound **3**. Selective deprotection of the TMS ether of compound **2** with a solution of K_2CO_3 in anhydrous methanol afforded compound **4** in 43% yield.[7,8] The appearance of a triplet at δ 0.88 ($J = 9$ Hz) in ^{1}H NMR spectrum and the disappearance of the triplet when D_2O was added confirmed that the primary TMS ether was cleaved. Parikh–Doering oxidation of **4** produced the corresponding aldehyde **5** in 12% yield. Subsequent reduction and hydrolysis provided puerarin **1**. Synthetic puerarin **1** was identical to the natural puerarin as shown by comparing their ^{1}H and ^{13}C NMR spectra. Since trimethylsilyl-protected puerarin **2** was unstable, an attempt was made to protect as acetates. The primary hydroxyl group of the glucose moiety was selectively tritylated with triphenylmethyl chloride in pyridine to give the trityl compound in 95% yield.[9] Standard acetylation employing acetic anhydride[10] and detritylation with chlorotrimethylsilane[11] in the presence of NaI in acetonitrile at 0°C produced the desired acetate in 85% yield. Unfortunately, subsequent oxidation under a variety of conditions failed to produce the corresponding aldehyde.

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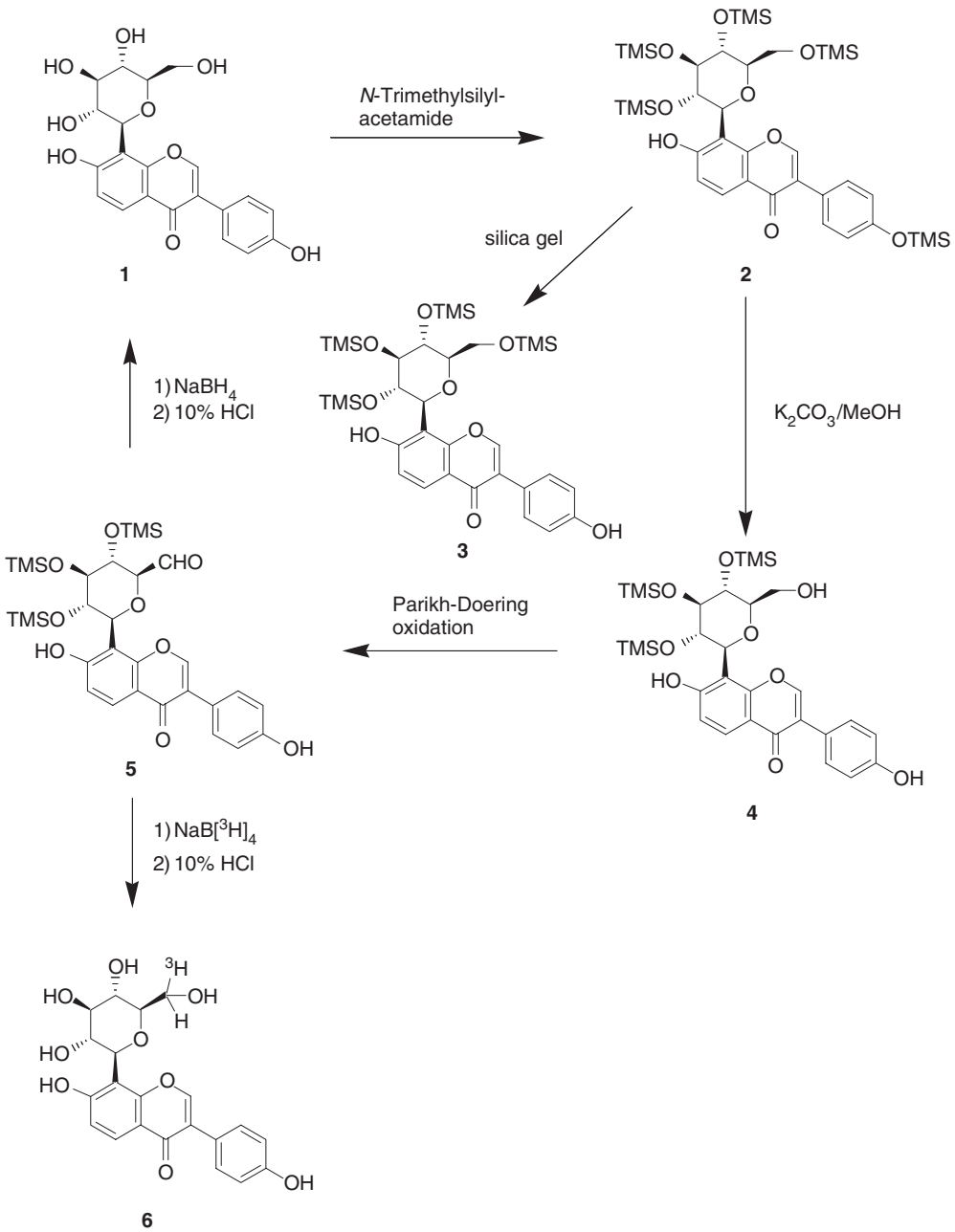

Scheme 1

Following the synthetic method outlined in Scheme 1, the key intermediate **5** was reduced with $NaB[^3H]_4$ and the TMS groups were removed with 10% aqueous HCl solution. After purification by preparative thin layer chromatography, tritium-labeled puerarin **6** was obtained in 75% yield. Tritium-labeled puerarin **6** (0.1 mg) was analyzed by high-performance liquid chromatography (HPLC). The retention time and the specific activity measurement by scintillation counter confirmed that the final product was tritium-labeled puerarin.

Experimental

General

Tritium-labeled sodium borohydride was obtained from American Radiolabeled Chemical Inc. All other chemicals were commercial reagents. TLC analysis with different solvent systems was carried out using precoated-TLC silica gel plates (Merck, 60F254). Preparative purification of the labeled puerarin was carried out using EM science PLC (M13895-7). Column

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chromatography was run on a silica gel 60 Å (Merck 230–400 mesh). The HPLC was performed with waters HPLC system (Waters 1525 binary HPLC pump and Waters dual absorbance detector) at room temperature and the wavelength for UV detection was 254 nm. YMC HPLC column (YMC-Pack, ODS-A, 150 × 4.6 mm ID, S-5 μm, 12 nm) was used. ^{1}H, ^{13}C NMR spectra were recorded on Varian NMR-300 spectrometers using the hydrogenated residue of the deuterated solvents ($CDCl_3$, DMSO-d_6) and TMS as internal standards. Radioactivity was measured by Multi-purpose Scintillation Counter (Beckman) and counter efficiency is 50% with negligible background radiation. The total radioactivity (mCi) is calculated by the formula (mCi = DPM × dilution factor/2.22 × 10^9) and the specific activity is calculated as (mCi/mmol) = mCi × molecular weight (mg/mmol)/sample weight (mg).

Puerarin (1)

Compound **5** (13.3 mg, 0.021 mmol) was dissolved in 0.6 ml THF, then the solution of 1.2 mg (0.032 mmol) $NaBH_4$ in 1.5 ml ethanol was added dropwise at 0°C. Stirred at 0°C for 10 min, then 0.2 ml 10% HCl was added. Stirred at this temperature for 10 min and then another 5 min at room temperature. The solvent was evaporated under a slow stream of nitrogen. The residue was purified by preparative TLC ($CHCl_3$:MeOH = 9:1) to give 6.6 mg of white solid product (m.p. 187–189°C; 75% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.19 (3H, m), 3.66 (1 H, d, J = 10.5 Hz), 3.97 (1 H, brs), 4.51 (1 H, brs), 4.78 (2 H, m), 4.96 (2 H, m), 6.75 (2 H, d, J = 8.4 Hz), 6.94 (1 H, d, J = 9.00 Hz), 7.34 (2 H, d, J = 8.4 Hz), 7.89 (1 H, d, J = 9.00 Hz), 8.30 (1 H, s), 9.50 (1 H, s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 61.50, 70.60, 70.83, 73.48, 78.82, 81.90, 112.71, 115.07, 116.94, 122.59, 123.16, 126.37, 130.13, 152.76, 156.50, 157.22, 161.15, 175.03.

7-Hydroxy-3-(4-trimethylsilanyloxy-phenyl)-8-(3,4,5-tris-trimethylsilanyloxy-6-trimethylsilanyloxy-methyl-tetrahydro-pyran-2-yl)-chromen-4-one (2)

Puerarin (0.26 g, 0.6 mmol) and *N*-trimethylsilylacetamide (0.69 g, 5.2 mmol) were mixed and stirred at 175°C for 15 min. Most of the by-product acetamine was pumped off. The residue was dissolved in ethyl acetate (7 ml). Hexane (14 ml) was added and the precipitate (acetamine) was filtrated off. Solvent was evaporated and the residue was purified by flash column chromatography (very short column, ethyl acetate) to afford compound **2** (0.42 g, 86%) as a white solid. M.p. 161–163°C (dec). ^{1}H NMR ($CDCl_3$): δ –0.39 (s, 9 H), 0.13 (s, 9 H), 0.19 (s, 9 H), 0.20 (s, 9 H), 0.27 (s, 9 H), 3.46 (d, J = 9.0 Hz, 1 H), 3.56 (dd, J = 8.4, 9.0 Hz, 1 H), 3.65 (dd, J = 8.7, 9.3 Hz, 1 H), 3.76 (m, 3 H), 5.15 (d, J = 9.0 Hz), 6.88 (d, J = 7.5 Hz, 2 H), 6.94 (d, J = 9.3 Hz, 1 H), 7.43 (d, J = 7.8 Hz, 2 H), 7.94 (s, 1 H), 8.14 (d, J = 9.3 Hz), 8.90 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ –0.74, –0.11, 0.24, 0.94, 1.30, 60.00, 70.04, 73.40, 76.13, 78.60, 80.62, 111.13, 116.82, 117.76, 120.06, 124.44, 124.98, 127.64, 130.15, 151.62, 155.22, 155.28, 161.57, 175.85.

Compound 2 was unstable on silica gel to give 7-hydroxy-3-(4-hydroxy-phenyl)-8-(3,4,5-tris-trimethylsilanyloxy-6-trimethyl-silanyloxy-methyl-tetrahydro-pyran-2-yl)-chromen-4-one (3)

White solid. M.p.168–172°C (dec). ^{1}H NMR ($CDCl_3$): δ –0.37 (s, 9 H), 0.15 (s, 9 H), 0.21 (s, 9 H), 0.22 (s, 9 H), 3.49 (d, J = 9.3 Hz, 1 H), 3.58 (dd, J = 8.7, 8.4 Hz, 1 H), 3.67 (dd, J = 8.7, 9.3 Hz, 1 H), 3.79 (m, 3 H), 5.17 (d, J = 8.7 Hz), 6.83 (d, J = 7.5 Hz, 2 H), 6.98 (d, J = 9.0 Hz, 1 H), 7.34 (d, J = 7.8 Hz, 2 H), 7.94 (s, 1 H), 8.17 (d, J = 9.3 Hz), 8.95 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ –0.74, –0.11, 0.94, 1.29, 60.05, 70.06, 73.40, 76.12, 78.56, 80.62, 111.16, 115.76, 117.01, 117.58, 123.50, 124.74, 127.64, 130.31, 151.88, 155.37, 156.24, 161.74, 176.47.

7-Hydroxy-8-(6-hydroxymethyl-3,4,5-tris-trimethylsilanyloxy-tetrahydro-pyran-2-yl)-3-(4-hydroxy-phenyl)-chromen-4-one (4)

To a solution of compound **2** (7.51 g, 9.7 mmol) in MeOH (200 ml) at 0°C, K_2CO_3 (1.33 g, 9.6 mmol) was added. After being stirred at 0°C for 4 h, the MeOH solution was quickly passed through a short silica-gel column. Solvent was evaporated in a rotary evaporator. The residue was further purified by flash column chromatography (ethyl acetate:hexane = 3:7 until low polarity by-products elute out, then the ratio is changed to 4:6) to afford compound **4** (2.61 g, 43%) as a white solid. M.p.175–178°C (dec). ^{1}H NMR ($CDCl_3$): δ –0.36(s, 9 H), 0.21(s, 9 H), 0.23(s, 9 H), 0.88 (t, J = 9 Hz, 1 H), 3.73 (m, 6 H), 5.20 (d, J = 9.3 Hz), 6.87 (d, J = 8.7 Hz, 2 H), 6.97 (d, J = 9.0 Hz, 1 H), 7.40 (d, J = 8.7 Hz, 2 H), 7.95 (s, 1 H), 8.18 (d, J = 8.7 Hz, 1 H), 8.80 (broad, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ –0.11, 0.89, 1.25, 61.57, 71.07, 73.58, 76.47, 78.63, 81.45, 110.96, 115.75, 116.78, 117.61, 122.93, 124.77, 127.73, 130.24, 152.02, 155.34, 156.56, 161.26, 176.55.

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6-[7-Hydroxy-3-(4-hydroxy-phenyl)-4-oxo-4*H*-chromen-8-yl]-3,4,5-tris-trimethyl-silanyloxy-tetrahydro-pyran-2-carbaldehyde (5)

To a solution of compound **4** (2.61 g, 4.12 mmol) in CH_2Cl_2 (50 ml) at room temperature, methyl sulfoxide (3.22 g, 41.2 mmol), triethylamine (2.08 g, 20.6 mmol) and sulfur trioxide pyridine complex (2.62 g, 16.5 mmol) were added in sequence. The resulting mixture was stirred at room temperature for 2 h. Methylene chloride was evaporated and the residue was purified by flash column chromatography (ethyl acetate : hexane = 3 : 7 until low polarity by-products elute out, then the ratio is changed to 4:6) to afford aldehyde **5** (0.30 g, 12%) as a white solid. M.p.164–166°C (dec). ^{1}H NMR ($CDCl_3$): δ –0.32 (s, 9 H), 0.20 (s, 9 H), 0.22 (s, 9 H), 3.39 (m, 2 H), 3.91 (t, J = 6.2 Hz, 1 H), 4.30 (d, J = 6.3 Hz, 1 H), 5.42 (d, J = 7.8 Hz, 1 H), 6.86 (d, J = 7.2 Hz, 2 H), 7.03 (d, J = 9.0 Hz, 1 H), 7.27 (s, 1 H), 7.38 (d, J = 6.9 Hz, 2 H), 7.94 (s, 1 H), 8.21 (d, J = 8.7 Hz, 1 H), 8.61 (broad, 1 H), 9.72 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ –0.21, 0.54, 0.75, 72.40, 74.31, 74.84, 84.00, 110.81, 115.66, 116.89, 117.74, 123.62, 124.80, 128.02, 130.34, 151.75, 155.16, 156.09, 161.09, 176.22, 197.46.

Tritium-labeled puerarin (6)

Ethanol (10 ml) was added to $NaB[^3H]_4$ (~5 mCi, 450 mCi/mmol) and unlabeled $NaBH_4$ (0.38 mg, 0.01 mmol) in a small vial. The slurry solution was cooled down to 0°C. Aldehyde **5** (40.6 mg, 0.064 mmol) in THF (3 ml) was added all at one time. The resulting solution was stirred at 0°C for 10 min. TLC indicated that the reaction was complete. Aqueous HCl (10%, 0.7 ml) was added and the solution was stirred for 15 min. The solvent was evaporated under a slow stream of nitrogen. The residue was purified by preparative TLC ($CHCl_3$:MeOH = 9 : 1) to give the tritium-labeled puerarin **6** (20.1 mg, 75%, 3.8 mCi, 79 mCi/mmol) as a white solid. HPLC analysis (MeOH:H_2O:HOAc = 70 : 29.85 : 0.15, flow rate = 1 ml/min) revealed that the retention time (6.68 min) of the tritium-labeled puerarin was identical to the cold puerarin. Radioactivity measurement confirmed that the tritium-labeled puerarin fraction contained the right amount of the radiolabeled material.

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