Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 7 October 2007, Revised 19 November 2007, Accepted 30 November 2007 Published online 17 January 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1493

Synthesis of 6β-([1-^{14}C]propoxy)celangulin V

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Chinese bittersweet, *Celastrus angulatus* Max., is a widely distributed plant and is used as a traditional insecticide in China. Celangulin V (CA-V), one of the main insecticidal ingredients from the plant, has special insecticidal mechanism. A synthesized compound, 6β-propoxycelangulin V, exhibits more remarkable insecticidal activity against insect pests. In this study, the synthesis of a labeled version of a CA-V analog, 6β-([1-^{14}C]propoxy)celangulin V, was accomplished from 1-[1-^{14}C]propanol and CA-V in two steps with the chemical and radiochemical purities of 98.7 and 99.9%, respectively. The labeled compound can be used as a radiotracer to study the mechanism of action of CA-V analogs.

Keywords: synthesis; 6β-([1-^{14}C]propoxy)celangulin V; celangulin V; insecticide

Introduction

Chinese bittersweet, *Celastrus angulatus* Max., a widely distributed insecticidal plant in China, produces various β-dihydroagarofuran sesquiterpene ployol esters.[1–7] The plant has been well documented as a botanical insecticide due to its effectiveness against insect pests.[8] The insecticidal compound, 1α,2α-diacetoxy-9α-benzoyloxy-4β,6β-dihydroxy-8β,15-bis(isobutanoyloxy)-β-dihydroagarofuran, also known as celangulin V (**1**) (CA-V, Figure 1), is one of the main insecticidal ingredients with the β-dihydroagarofuran sesquiterpene skeleton isolated from Chinese bittersweet.[9] A novel compound 6β-propoxycelangulin V (**2**) (PCA-V, Figure 1), which has 5–10 times higher insecticidal activity than CA-V (**1**) against the 3rd instar larvae of *Mythimna separata* (armyworm), was synthesized from CA-V (**1**). CA-V (**1**) acts on the insect digestive system and damages the larval midgut of *M. separata*.[8] There are receptors of CA-V (**1**) in midgut epithelia of the target insects,[10] but the receptors and insecticidal mechanism of CA-V analogs are still poorly understood.[8] A radiotracer of CA-V analogs is required to support the ongoing study for elucidating the receptor interactions and insecticidal mechanism of the commercialized pesticides. In this paper, the synthesis of 6β-([1-^{14}C]propoxy)celangulin V (**6**) ([^{14}C]-PCA-V, Scheme 1) is described.

Results and discussion

The radiolabeled compound, [^{14}C]-PCA-V (**6**), was synthesized from 1-[1-^{14}C]propanol (**4**) and CA-V (**1**) in two steps and purified by flash chromatography and preparative HPLC. The data from HPLC-MS (ESI), HRMS, ^{1}H NMR, ^{13}C NMR analysis and retention time on HPLC of [^{14}C]-PCA-V (**6**) were consistent with those of the standard sample of PCA-V (**2**). The overall chemical and radiochemical yields were 8% and 4%, respectively. The chemical purity was 98.7% and the radiochemical purity was 99.9%. The specific activity was 16.65 MBq/mmol. This compound can be used as a radiotracer in studies on the receptors and insecticidal mechanism of CA-V analogs.

In radiosynthesis, the labeling atoms must be always located in the skeleton or stable positions of the labeled molecules, so that the labeling atoms may not escape from the labeled molecules in the subsequent tracing experiments. CA-V (**1**), a natural product with the skeleton of β-dihydroagarofuran sesquiterpene, is difficult to synthesize.[11] So far, isolation of CA-V (**1**) from Chinese bittersweet is still a practicable approach to obtaining CA-V (**1**) and preparation of a labeled version of CA-V analogs had been a bottleneck in understanding the receptors and mechanism of action for these compounds.[8] We had attempted to synthesize [6,6-^{3}H$_2$]celangulin V, a tritium-labeled version of CA-V (**1**), through the reduction of 6-carbonylcelangulin V (**3**, Figure 1) with [^{3}H$_4$]NaBH$_4$. CA-V (**1**) was successfully oxidized to 6-carbonylcelangulin V (**3**) with Jones regent,[12] but 6-carbonylcelangulin V (**3**) failed to be reduced to CA-V (**1**) according to the method of the reduction of mortonol A, a natural product with β-dihydroagarofuran sesquiterpene skeleton.[13] PCA-V (**2**), a novel compound synthesized from CA-V (**1**), has demonstrated higher insecticidal activity against insect pests and the 6-propoxyl group in this molecule links to β-dihydroagarofuran sesquiterpene skeleton with an ether bond that is relatively stable. Therefore, labeling the molecule with carbon-14 in 6-propoxyl group is reasonable and practical. Carbon-14 from 1-[1-^{14}C]propanol can be incorporated into PCA-V (**2**) via anhydrous iodination and Williamson reactions.

In the optimization of procedures for the preparation of [^{14}C]-PCA-V (**6**), we observed that the dryness of 1-[1-^{14}C]iodopropane

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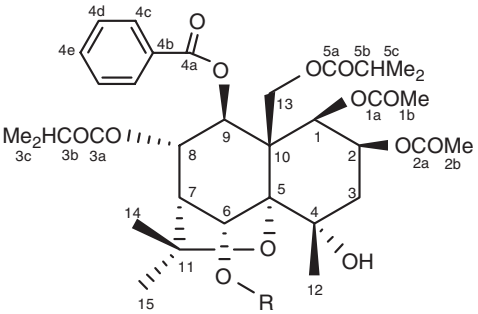
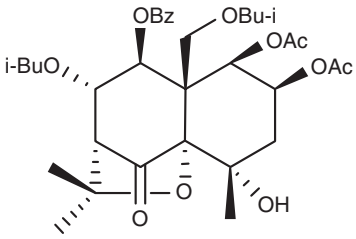
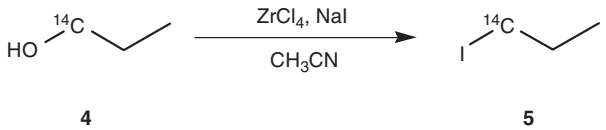


1. R = H; 2. R = Pr

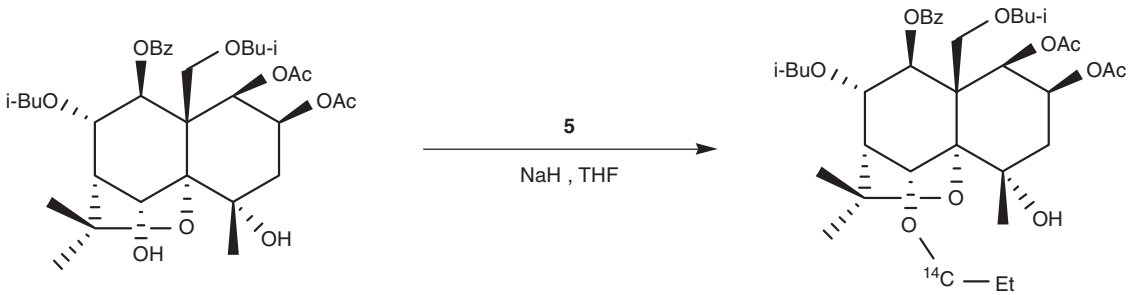


3

Figure 1. Structures of celangulin V (**1**), 6β-propoxycelangulin V (**2**) and 6-carbonylcelangulin V (**3**).



4 **5**



1 **6**

Scheme 1

(**5**), the prescription and the selectivity of the reaction were the main factors that affect the reaction yield. It was essential to remove the trace amount of water in 1-[1-^{14}C]iodopropane (**5**) by CaH_2 before the reaction. Otherwise the starting material CA-V (**1**) and the desired product [^{14}C]-PCA-V (**6**) in tetrahydrofuran would decompose in the process of reaction, which reduced the reaction yield and complicated the workup. In the cold reaction, the yield of PCA-V (**2**) increased to 19% when more 1-iodopropane (50 equiv.) was added to the solution of dry CA-V (**1**) in anhydrous tetrahydrofuran. In the radiosynthesis, 2 equiv. of 1-[1-^{14}C]iodopropane (**5**) was needed, balancing between the utilization of 1-[1-^{14}C]iodopropane (**5**) and the yield of [^{14}C]-PCA-V (**6**). The reaction had poor selectivity for the complicated structure of CA-V (**1**) and yielded some byproducts. Therefore, purification by preparative HPLC was required and the loss in the process was another reason for the relatively low overall yield.

Experimental

General

Radiochemical 1-[1-^{14}C]propanol in anhydrous acetonitrile (2.50 mL, specific activity 1.00 mCi/mmol, radioactive concentration 1.00 mCi/mL) was purchased from the American Radiolabeled Chemicals Inc. (USA). Anhydrous NaI was obtained from Sigma-Aldrich (USA). Anhydrous $ZrCl_4$ was obtained from Acros Organics (Belgium). Standard samples of CA-V and PCA-V (>99%) were provided by the Institute of Pesticide Science, Northwest University of Agriculture and Forestry in Shaanxi Province, China. The melting points were taken on SGW X-4 microscope melting point instrument without calibration (Shanghai precision instruments Co., China). Scintillation cocktail was prepared as following prescription: PPO (7 g) and POPOP (0.5 g) dissolved in the mixture of xylene (650 mL) and 2-methoxyethanol (350 mL). Methanol for HPLC was of chromatographic grade from SK Chemicals (Korea) and ultrapure water (18.2 MΩ/cm, 25°C) was prepared on Milli-Q academic instrument (Millipore, France). The other reagents were of analytical grade or higher and used without purification. The root bark of Chinese bittersweet was collected in Mazhao Village, Zhouzhi County, Shaanxi Province, China, in October 2005, and authenticated by Prof. Mu Xiao-Qian in College of Life Science, Northwest University of Agriculture and Forestry in Shaanxi Province, China. ^{14}C and ^{3}H NMR spectra were measured on Bruker DRX400/500 spectrometers (Bruker, Switzerland). MS and HRMS spectra were recorded on a Brucker Daltonics Apex II

high-resolution MS spectrometer (Bruker Daltonics, USA) and Ionspec 4.7 Tesla Ultima FT-MS (IonSpec, USA), respectively. HPLC-MS and GC-MS (EI) were recorded on an Agilent 1100 LC/MSD SL system (Agilent Technologies, USA) and Quest Trace GC2000/Trace MS spectrometer (Thermo Electron, USA), respectively. GC analysis was carried out with an Agilent 6890N GC system with HP-5 column (30 m × 0.32 mm × 0.25 μm, Agilent Technologies). HPLC analysis was performed on a Waters 996 series system (Water Co., USA) consisting of automated gradient pump, photodiode array detector with Inertsil ODS-3 analytical column (4.6 mm × 250 mm, GL Science Co., Japan) and Hypersil C_{18} preparative column (10 mm × 300 mm, Dalian Elite Co., China). TLC was performed with GF_{254} plates and radioactive TLC plates were scanned on Fujifilm BAS-1800II laser-based fluorescence and radioisotope imaging system (Fuji Co., Japan). Radioactivity was measured on WinSpectral-1414 liquid scintillation spectrometer (Wallac, Finland).

Extraction and separation of CA-V (1)

The dried and ground root bark (8 kg) of *C. angulatus* was extracted with benzene under reflux for 3 h. The extracted material was re-extracted with benzene twice. The combined extracts were concentrated to afford a brown extract (about 380 g). The extract was chromatographed on a D101 absorbing resin column packed with 2.2 kg resin using methanol/water (6:4) as eluting solvents to give 72 fractions (each 500 mL). Fractions 33–52 (19 g) were subjected to silica gel chromatography (200–300 mesh, ethyl acetate/petroleum ether, 1:4, v/v) to give 90 fractions (each 100 mL). Sub-fractions 30–48 were combined and subjected to silica gel chromatography to afford CA-V (**1**) (about 2.15 g). m.p. 197–199°C. HPLC quantitative analysis (MeOH/H_2O, 65:35) demonstrated its chemical purity to exceed 98%.[9]

HPLC-MS (ESI): 685.0$[M+Na]^+$, 681$[M+H+H_2O]^+$, 680$[M+NH_4]^+$, 646$[M+1+H-H_2O]^+$, 645$[M+H-H_2O]^+$. HRMS (EI) for $C_{37}H_{52}O_{13}$ Calcd: 662.2939, Found: 662.2941. ^{1}H NMR ($CDCl_3$, 500 MHz)δ: 5.47(d, H-1), 5.38(dd, H-2), 1.99(dd, H-3), 2.10(m, H-3), 5.21(s, H-6), 2.57(d, H-7), 5.60(dd, H-8), 6.06(d, H-9), 4.86(d, H-12), 4.65(d, H-12), 1.77(s, H-13), 1.72(s, H-14), 1.60(s, H-15), 3.13(brs, C_4-OH), 5.21(brs, C_6-OH), 1.55(s, H-1b), 2.10(s, H-2b), 2.36(sept, H-3b), 0.94(d, H-3c), 0.90(d, H-3c), 7.86(m, H-4c), 7.41(m, H-4d), 7.52(m, H-4e), 2.84(sept, H-5b), 1.34(d, H-5c), 1.35(d, H-5c). ^{13}C NMR ($CDCl_3$, 400 MHz)δ: 75.08(C-1), 67.38(C-2), 41.26(C-3), 72.17(C-4), 91.54(C-5), 76.98(C-6), 53.64(C-7), 73.80(C-8), 75.38(C-9), 50.66(C-10), 84.57(C-11), 61.75(C-12), 24.19(C-13), 30.10(C-14), 26.36(C-15), 169.53(C-1a), 20.47(C-1b), 169.42(C-2a), 21.09(C-2b), 175.79(C-3a), 34.13(C-3b), 18.65(C-3c), 18.49(C-3c), 165.69(C-4a), 129.48(C-4b), 129.41(C-4c), 128.63(C-4d), 133.42(C-4e), 176.71(C-5a), 34.36(C-5b), 19.16 (C-5c), 19.06 (C-5c). The structure was assigned as CA-V (**1**) based on these spectra data and compared with the data reported.[3]

1-[1-^{14}C]Iodopropane (5)

To the mixture of anhydrous sodium iodide (563.0 mg, 3.75 mmol) and anhydrous zirconium tetrachloride (350.1 mg, 1.50 mmol) in anhydrous acetonitrile (5 mL) was added 1-[1-^{14}C]propanol (**4**) (2.50 mCi, 1.00 mCi/mmol) in anhydrous acetonitrile (2.50 mL) under argon. The reaction mixture was stirred at room temperature for 70 min.[14] The progress of reaction was monitored by GC. The resulting solution was diluted with water (10 mL) and extracted with ethyl ether. The combined organic layers were washed with sodium thiosulfate solution (10%) and water, dried over anhydrous $MgSO_4$ and then evaporated *in vacuo* to afford 1-[1-^{14}C]iodopropane (**5**) (274.3 mg, 65%). GC-MS (EI, 70 eV): 170$[M]^+$. GC-MS conditions: HP-5 column (30 m × 0.32 mm × 0.25 μm), injection (200°C), EI (200°C), flow rate 1.0 mL/min, scan range 35–200. Column temperature rose from 40 to 200°C (15°C/min). The retention time was 3.48 min

6β-([1-^{14}C]Propoxy)celangulin V (6)

To the stirred mixture of CaH_2 (200 mg, 4.76 mmol), anhydrous 1-iodopropane (275.7 mg, 1.62 mmol) and anhydrous tetrahydrofuran (4 mL) under argon were added 1-[1-^{14}C]iodopropane (**5**) (274.3 mg, 1.61 mmol) and the mixture was refluxed for 20 min, allowed to cool to room temperature to give the solution of anhydrous 1-[1-^{14}C]iodopropane (**5**) in tetrahydrofuran. The solution was added dropwise to the stirred suspension of anhydrous CA-V (**1**) (1080 mg, 1.63 mmol) and NaH (54 mg, 2.25 mmol) in anhydrous tetrahydrofuran (4 mL) under argon. Stirring was continued at room temperature for 90 min. The reaction mixture was poured into saturated NH_4Cl solution (60 mL) and extracted with ethyl acetate. The combined organic layers were washed with saturated brine, dried over $MgSO_4$ and then concentrated *in vacuo*. The residue was chromatographed on a silica gel flash column (acetonitrile/petroleum ether, 1:3, v/v), and then preparative HPLC to afford [^{14}C]-PCA-V (**6**) (138.4 mg, 12%). HPLC conditions: methanol/water 70:30, flow rate 2.00 mL/min, injection volume 200 μL, detection wavelength 229 nm. The eluted liquid between 20.12 and 30.15 min was collected and evaporated to afford [^{14}C]-PCA-V (**6**).

MS (MALDI) *m/z*: 727.3$[M+Na]^+$. HRMS(MALDI/DHB) Calcd. for $C_{37}H_{52}O_{13}Na$ 727.3300, found 727.3298; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.58(d, H-1), 5.43(dd, H-2), 2.08(dd, H-3), 2.31(m, H-3), 4.94(s, H-6), 2.63(d, H-7), 5.01(dd, H-8), 6.10(d, H-9), 4.97(d, H-12), 4.69(d, H-12), 1.70(s, H-13), 1.73 (s, H-14), 1.68 (s, H-15), 3.98(brs, C_4-OH), 1.54(s, H-1b), 2.08(s, H-2b), 2.37(sept, H-3b), 0.94(d, H-3c), 0.89(d, H-3c), 7.85(m, H-4c), 7.41 (m, H-4d), 7.54(m, H-4e), 2.84(sept, H-5b), 1.38(d, H-5c), 1.36(d, H-5c), 3.66(m, H-1 in propoxy group), 3.38(m, H-1 in propoxy group), 1.87(m, H-2 in propoxy group), 1.01(m, H-3 in propoxy group). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 75.60(C-1), 67.95(C-2), 39.05(C-3), 70.06(C-4), 91.52(C-5), 84.92 (C-6), 49.06(C-7), 74.36(C-8), 75.63(C-9), 50.08(C-10), 84.92(C-11), 62.20(C-12), 25.90(C-13), 27.65(C-14), 29.20(C-15), 169.49(C-1a), 20.48(C-1b), 169.38(C-2a), 21.12(C-2b), 176.19(C-3a), 34.11(C-3b), 18.61(C-3c), 18.42(C-3c), 165.47(C-4a), 129.53(C-4b), 129.42 (C-4c), 128.59(C-4d), 133.32(C-4e), 176.24(C-5a), 34.48(C-5b), 19.27 (C-5c), 18.96 (C-5c), 83.76(C-1 in propoxy group), 22.90(C-2 in propoxy group), 10.95(C-3 in propoxy group). These data matched with those of PCA-V (**2**) standard sample.

The chemical and radiochemical purities of 6β-([1-^{14}C]propoxy)celangulin V (6)

The chemical purity of [^{14}C]-PCA-V (**6**) was 98.7%, as determined by HPLC. The radiochemical purity was 99.9%, measured with thin layer chromatography-isotope imagining analysis method (R_f 0.61, eluted with acetone/petroleum ether 1:3; R_f 0.52, eluted with acetone/petroleum ether 1:2) and HPLC-LSC method that we

had described.[15] HPLC condition: gradient control (min/%A): 0/70, 15/88, 25/100, 30/100 (A was acetonitrile/%0.1 AcOH and B was ultrapure water/%0.1 AcOH); injection volume 20 μL; temperature 25°C. The retention time of [^{14}C]-PCA-V (**6**) was 14.97 min

Acknowledgements

The authors gratefully acknowledge Prof. Jay Gan, Department of Environmental Science, University of California, Riverside, for the initial review of this paper and the financial support from Mayor State Basic Research and Development Program of China (2003CB114404) and the National Natural Science Foundation of China (30130130).

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