SYNTHESIS OF [12-^{3}H]-(±)-CALANOLIDE A

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SUMMARY

[12-^{3}H]-(±)-Calanolide A (**9**) was synthesized in five steps from readily available phloroglucinol (**1**). Stereoselective Luche reduction of trans-ketone **8** with cerium(III) chloride and sodium borotritide in methanol gave 338 μCi of **9** with a specific activity of 63.0 mCi/mmol.

Key Words: Calanolide A, HIV-1, reverse transcriptase inhibitor, sodium borotritide

INTRODUCTION

Calanolide A has recently been shown to be a human immunodeficiency virus-1 (HIV-1) reverse transcriptase (RT) inhibitor which possesses a mechanism of action differing from that of typical nucleosidal-based HIV-1 RT inhibitors.[1] Synthesis of radiolabeled Calanolide A would be useful for pharmacological evaluations of its' inhibitory properties. Herein is described the synthesis of [12-^{3}H]-(±)-Calanolide A.

RESULTS AND DISCUSSION

Synthesis of [12-^{3}H]-(±)-Calanolide A was adapted from a previous synthesis of (±)-Calanolide A[2] as shown in Chart 1. Pechmann reaction[3] of phloroglucinol (**1**) with ethyl butyrylacetate in trifluoromethanesulfonic acid gave coumarin **2** in 96% yield. Friedel-Crafts acylation of **2** with tigloyl chloride (**3**)[4] and aluminum chloride in nitromethane and carbon disulfide afforded vinyl ketone **4**.[2] Base catalyzed ring closure of **4** with potassium carbonate in refluxing 2-butanone gave a 1:1 mixture (^{1}H NMR) of *cis*- and *trans*-**5** in 79% yield. Formation of the 2,2-dimethylchromene ring was achieved by reaction of *cis*- and *trans*-**5** with 3-chloro-3-methyl-1-butyne **6**[5], n-butylammonium

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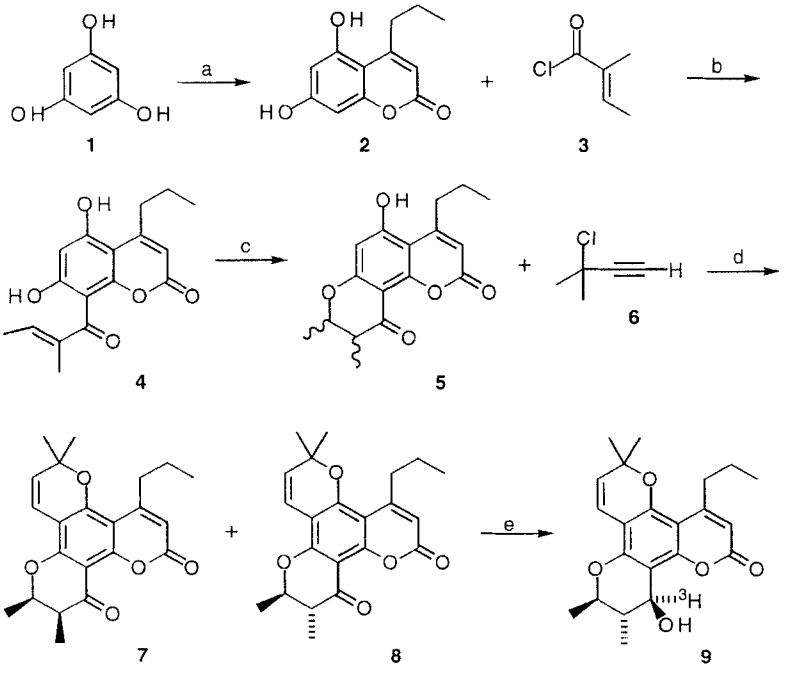
iodide, potassium carbonate, and zinc chloride in 2-butanone and dimethylformamide to give ketones **7** and **8** in a 67% combined yield; trans ketone **8** was isolated from the mixture in 47% yield by column chromatography. Stereoselective Luche reduction[6] of **8** with cerium(III) chloride and sodium borotritide in methanol followed by isolation and purification by preparative TLC and HPLC gave 338 μCi (14% radiochemical yield[7]) of [12-^{3}H]-(±)-Calanolide A (**9**) with a specific activity of 63.0 mCi/mmol.[8] The position of the label is inferred from the structure of the product and the expected stereo- and regiochemistry of the labelling reaction.

Chart 1



a) n-$C_3H_7COCH_2CO_2Et$, CF_3SO_3H
b) $AlCl_3$, $PhNO_2$, CS_2
c) K_2CO_3, $CH_3CH_2COCH_3$
d) K_2CO_3, n-Bu_4NI; then $ZnCl_2$
e) $CeCl_3$, [^{3}H]-$NaBH_4$, MeOH

EXPERIMENTAL[9]

Reagent grade chemicals were purchased from commercial suppliers and used without further purification. Column chromatography was performed with Merck Kieselgel 60 (230-400 mesh ASTM) silica. Radioactive samples were counted on a Packard Tricarb 4000 liquid scintillation counter. HPLC was performed with a Waters Associates Model 6000A dual pump system, a Model U6k septumless injector, and a IN/US Systems, Inc. Model 1B β-RAM Flow Through Radioactivity Monitor.

5,7-Dihydroxy-4-propyl-2*H*-1-benzopyran-2-one (2)[2]

Trifluoromethanesulfonic acid (7.4 mL, 84 mmol) was added over 30 min to a 0 °C solution of phloroglucinol (**1**) (5.0 g, 40.0 mmol) and ethyl butyrylacetate (6.5 mL, 42.0 mmol). The reaction was stirred for 20 h at 20 °C, then ice (50 g) and H_2O (60 mL) were added with vigorous stirring. The yellow solid was collected by filtration and the crude material recrystallized from 80% EtOH-H_2O to afford **2** (8.27 g, 96% yield).

Tigloyl chloride (3)[4]

Tiglic acid (8.0 g, 80 mmol) and PCl_3 (3.8 mL, 40 mmol) were refluxed for 2 h. The reaction was cooled, the upper layer separated from the lower layer, and then distilled (1-2 Torr, 25 °C) to afford **3** (5.74 g, 61% yield).

5,7-Dihydroxy-8-(2-methyl-2-buten-1-onyl)-4-propyl-2*H*-1-benzopyran-2-one (4)[2]

A solution of **3** (3.0 g, 25 mmol) in CS_2 (2.0 mL) was added to a suspension of **2** (5.0 g, 23 mmol) and $AlCl_3$ (1.0 M in nitrobenzene; 96 mL, 96 mmol) in CS_2 (25 mL). The reaction was stirred for 18 h at 75 °C, then poured into a mixture of ice (50 g) and 1 M HCl (200 mL). The solution was extracted with 95:5 $CHCl_3$-MeOH (3 x 100 mL), the organic extracts dried over Na_2SO_4, and the solvents removed *in vacuo.* Column chromatography (19:1 CH_2Cl_2-MeOH) gave a dark red solid which was triturated with EtOAc to afford **4** (1.5 g, 22% yield).

(*cis, trans*)-2,3-Dihydro-2,3-dimethyl-9-hydroxy-8-propyl-4*H*,6*H*-benzo[1,2-b:3,4-b']dipyran-4,6-dione (5)[2]

A solution of **4** (1.5 g, 5 mmol) and K_2CO_3 (2.1 g, 15 mmol) in 2-butanone (20 mL) was refluxed for 2 h. The reaction was cooled, acidified with 1 N HCl (75 mL), and extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried over

Na_2SO_4, then the solvents removed *in vacuo* to afford a 1:1 (^{1}H NMR) mixture of *cis*- and *trans*-**5** (1.2 g, 79% yield).

3-Chloro-3-methyl-1-butyne (6)[5]

2-Methyl-3-butyn-2-ol (24 mL, 250 mmol) was added to a 0 °C solution of $CaCl_2$ (14 g, 130 mmol), CuCl (10 g, 100 mmol), Cu bronze powder (0.1 g, 16 mmol) in concentrated HCl (108 mL, 1.3 mol). The reaction was stirred 1 h at 0 to 5 °C, then the upper layer was separated, washed with cold concentrated HCl (2 x 25 mL) and water (3 x 25 mL), and dried over anhydrous K_2CO_3 to afford **6** (15.0 g, 59% yield).

(*cis*, *trans*)-4-Propyl-10,11-dihydro-6,6,10,11-tetramethyl-2*H*,6*H*,12*H*-benzo-[1,2-b:3,4-b':5,6-b'']tripyran-2,12-dione (7 and 8)[2]

A solution of a 1:1 mixture of *cis*- and *trans*-**5** (1.2 g, 4 mmol), K_2CO_3 (1.3 g, 10 mmol), 3-chloro-3-methyl-1-butyne (**6**) (2.0 g, 20 mmol), and n-Bu_4NI (1.5 g, 40 mmol) in 2-butanone (50 mL) and DMF (5 mL) was stirred for 1 h at 60 °C, then cooled and $ZnCl_2$ (1.0 M in Et_2O; 5.3 mL, 5.2 mmol) added. The reaction was stirred for 16 h at 70 °C, then cooled, quenched with saturated aqueous NH_4Cl, and extracted with EtOAc (3 x 100 mL). The organic extracts were washed with brine, dried over Na_2SO_4, and the solvents removed *in vacuo*. The crude product was chromatographed (2:3 EtOAc-hexane) to afford **8** (0.67 g, 47%) and **7** (0.28 g, 20%).

[12-^{3}H]-(±)-Calanolide A (9)

Cerium(III) chloride (13 mg, 0.053 mmol) was added to a solution of **8** (14 mg, 0.038 mmol) in MeOH (2.50 mL). The solution was cooled to 0 °C, then a 1.80 mL aliquot was withdrawn and added to [^{3}H]-$NaBH_4$ (5.0 mCi, 360 mCi/mmol). The reaction was stirred for 5 min, quenched with H_2O, and extracted with Et_2O. The extracts were combined and the solvents removed *in vacuo*. The crude product was dissolved in CH_2Cl_2 and purified by preparative TLC (Merck Kieselgel 60 F_{254}, 20 cm x 20 cm x 0.25 mm; 2:1 EtOAc-hexane). The desired product was removed from the plate, washed with a 1:1 mixture of CH_2Cl_2-EtOH, and the solvents were removed *in vacuo*. The product was further purified by preparative HPLC (Waters RCM 8 x10, Radial-Pak 8NVC18 insert, 50:30:20 MeOH-H_2O-CH_3CN, 3.0 mL/min) to give 338 μCi (14% radiochemical yield) of **9** (1.63 mg, 16% chemical yield) with a specific activity of 63.0 mCi/mmol.[10] Analysis of **9** by HPLC with radioactivity detection (Dupont Zorbax

ODS, 4.6 x 250 mm column, 70:20:10 MeOH-H_2O-CH_3CN, 1.0 mL/min) gave the following results: t_R 9.57 min (1.1%, unknown), t_R 11:12 min (0.1%, unknown), t_R 13.03 min (0.2%, unknown), t_R 14.43 min (93.2%, **9**), t_R 16:00 min (1.8%, costatolide, the 12-OH isomer of **9**). The remaining 3.6% radioactivity was attributed to general decomposition during the chromatographic process and was confirmed by collecting the peak due to **9** and re-injecting. The product was stabile to storage at -80 °C in toluene solution.

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7. The low yield is due to the considerable loss of material experienced during the purification process.
8. The specific activity is about 70% of the theoretical specific activity of 90 mCi/mmole. The lower than theoretical specific activity may be due to a tritium isotope effect.
9. All compounds gave spectral and physical data consistent with the proposed structures.
10. The specific activity is the average of three separate determinations by weighing and liquid scintillation counting.