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SYNTHESIS OF DUAL ^{14}C-LABELED (+)-CALANOLIDE A, A NATURALLY OCCURRING ANTI-HIV AGENT

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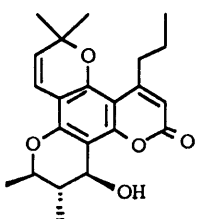
SUMMARY

[10,18-^{14}C]-(+)-Calanolide A [(+)-**6**] was synthesized in four steps from chromeno-coumarin **2**. A Ti-mediated aldol reaction of **2** with [1,2-^{14}C]-acetaldehyde stereoselectively produced the desired *syn* diastereomer (±)-**3**, with carbons at the 13 and 14 positions being ^{14}C-labeled. Intermediate (+)-**3** was isolated by lipase-catalyzed kinetic resolution and cyclized under Mitsunobu conditions to afford (+)-*trans*-2,3-dimethyl chroman-4-one, (+)-**5**. Luche reduction on (+)-**5** in EtOH/THF at -78 °C led to the formation of dual ^{14}C-labeled (+)-**6** with a specific activity of 49.25 mCi/mmol. The overall radiochemical yield was 4.3% based on the starting ^{14}C-acetaldehyde.

Key Words: (+)-calanolide A, HIV-1, nonnucleoside reverse transcriptase inhibitor, [1,2-^{14}C]-acetaldehyde

INTRODUCTION

A naturally occurring anti-HIV agent, (+)-calanolide A [(+)-**1**], originally isolated from the rainforest tree *Calaphyllum lanigerum*,[1] may represent a second generation of HIV-1-specific



(+)-Calanolide A [(+)-**1**]

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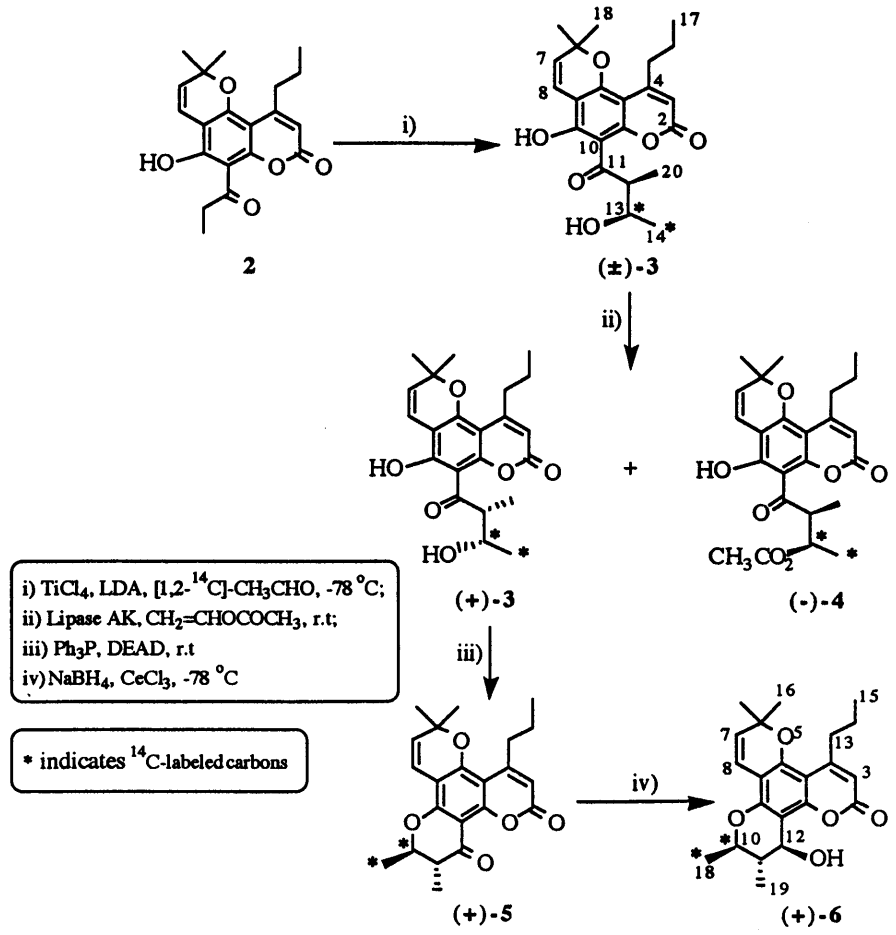
nonnucleoside RT inhibitors and a novel chemotype for anti-HIV drug development.[1-7] For further pharmacological characterization of this compound, chemically stable radiolabeled product of high chemical and radiochemical purity was required. During preparation of the manuscript, a paper describing the synthesis of [12-^{3}H]-(±)-calanolide A was published.[8] Herein we would like to report the synthesis of a dual ^{14}C-labeled enantiopure (+)-calanolide A.

RESULTS AND DISCUSSION

The previously reported synthetic approach was employed for the synthesis of [10,18-^{14}C]-(+)-calanolide A (**Scheme 1**).[9] Thus, the starting chromeno-coumarin **2**, prepared from commercially available phloroglucinol in a 3-step process,[5,10] was subjected to a titanium-mediated aldol reaction with [1,2-^{14}C]-acetaldehyde to stereoselectively afford the dual ^{14}C-labeled *syn* isomer, (±)-**3**, without detection of the corresponding *anti* isomer by HPLC.[9] Lipase-catalyzed kinetic resolution of (±)-**3** in the presence of vinyl acetate led to enantioselective acetylation of (-)-**3**, with the desired enantiomer (+)-**3** left intact. Intermediate (+)-**3** was purified by silica gel column chromatography. The enantiomerically pure (+)-**3** was then cyclized to ^{14}C-labeled *trans*-chromanone (+)-**5** under Mitsunobu conditions.[9,11]

It has been reported that stereoselective Luche[12] reduction of *trans*-chromanone **5** would favor the formation of calanolide A over the other diastereomer, calanolide B.[5,8-11] It was also noted that such stereoselection was temperature dependent, increasing in the selection of calanolide A with decreasing reaction temperature.[10] At lower temperature, however, the rate of reduction was slower, due in part to the limited solubility of *trans*-chromanone **5** in EtOH.[5] It was found during this study that the addition of THF as a co-solvent increased the solubility of *trans*-chromanone (+)-**5** and enabled the Luche reduction to be performed at -78 °C. Under the modified conditions, Luche reduction of (+)-**5** provided [10,18-^{14}C]-(+)-calanolide A, (+)-**6**, with minimal formation of calanolide B, which was removed by crystallization from 20% Et_2O in hexane. The final purification by silica gel column chromatography afforded (+)-**6** with a specific activity of 49.25 mCi/mmol as determined by weight assay. The radiochemical purity of the final product was found to be 97.4%, as determined by normal phase HPLC (Zorbax, 30% ethyl acetate in hexane), and the enantiomeric purity was 98% as determined by chiral HPLC (Chiralpak AS, 4.04% isopropanol in hexane). The overall radiochemical yield was 4.3% based on the starting ^{14}C-acetaldehyde.



Scheme 1

EXPERIMENTAL[13]

All of the chemical reagents and anhydrous solvents were purchased from commercial suppliers and were used as received. Lipase AK was purchased from Amano (Troy, VA). Column chromatography was performed using silica gel 60 Å (200 - 400 mesh from Aldrich). HPLC was performed using a Waters Associates M6000 pump system and Lambda-Max Model 480 UV detector, as well as an NEN in-house model radioactivity detector built using Canberra components. Radioactive samples were counted on a Beckman LS 6000TA counter.

[13,14-^{14}C]-(±)-6,6-Dimethyl-9-hydroxy-10-[12(S^*)-methyl-13(R^*)-hydroxybutyro]-4-propyl-2*H*, 6*H*-benzo[1,2-b:3,4-b']dipyran-2-one [(±)-3]

To a stirred solution of chromene **2** (1.4 g, 4.0 mmol) in dry CH_2Cl_2 (20 mL) at -40 °C under N_2 was added a 1.0 M solution of $TiCl_4$ in CH_2Cl_2 (12 mL, 12.0 mmol). The mixture was then cooled to -78 °C, followed by the slow addition of a 2.0 M solution of LDA in heptane/THF/ethylbenzene (4.4 mL, 8.8 mmol). After stirring 30 min. at -78 °C, [1,2-^{14}C)]-acetaldehyde (1.1 g, 24 mmol) with a specific activity of 58.9 mCi/mmol was added. The reaction mixture was stirred at -78 °C for 2 h, whereupon a saturated NH_4Cl solution (20 mL) was added. Water (20 mL) was added to dissolve the oily solid and the mixture was stirred for 20 min. The layers were separated and the aqueous solution was extracted with ethyl acetate (3 x 30 mL). The combined extracts were washed with brine (30 mL) and dried over Na_2SO_4. After removal of solvents *in vacuo*, the crude product obtained was purified by silica gel column chromatography, eluting with 20% ethyl acetate in hexane, to afford (±)-**3** (1.1 g, 41% yield).

[13,14-^{14}C]-(+)-6,6-Dimethyl-9-hydroxy-10-[12(*S*)-methyl-13(*R*)-hydroxybutyro]-4-propyl-2*H*, 6*H*-benzo[1,2-b:3,4-b']dipyran-2-one [(+)-3]

Into a stirred solution of (±)-**3** (1.1 g, 2.76 mmol) in *tert*-butyl methyl ether (20 mL) at ambient temperature under N_2 were added, successively, vinyl acetate (1.0 mL, 10.86 mmol), 4 Å molecular sieves (0.6 g), and Lipase AK (1.1 g). The resulting mixture was vigorously stirred at ambient temperature and the reaction was monitored using chiral HPLC (Chiralpak AS, 4.04% isopropanol in hexane). After 96 h, all the (-)-**3** was converted into the ester (-)-**4**. The reaction mixture was then filtered through celite and the residue was washed with ethyl acetate (5 mL). The crude product obtained from evaporation was subjected to silica gel column chromatography, eluting with an incontinuous gradient of 5-40% of ethyl acetate in hexane, to afford (+)-**3** (363 mg, 33% yield).

[10,18-^{14}C]-(+)-10(*R*),11(*R*)-*trans*-Dihydro-6,6,10,11-tetramethyl-4-propyl-2*H*, 6*H*,12*H*-benzo-[1,2-b:3,4-b':5,6-b"]tripyran-2,12-dione [(+)-5]

Into a stirred solution of (+)-**3** (0.46 g, 1.2 mmol) in THF (12 mL) were added triphenylphosphine (0.442 g, 1.68 mmol) and diethyl azodicarboxylate (DEAD, 0.28 mL, 1.8

mmol). The resulting reddish solution was stirred at ambient temperature under N_2 for 3 h, after which the reaction mixture was quenched with a saturated aqueous NH_4Cl solution (20 mL) and extracted with ethyl acetate (3 x 40 mL). The combined extracts were washed with brine (50 mL) and dried over Na_2SO_4. The crude product obtained by evaporation was purified by column chromatography on silica gel, eluting with an incontinous gradient of 10-40% of ethyl acetate in hexane, to afford (+)-**5** (253 mg, 63% yield).

[10,18-^{14}C]-(+)-Calanolide A [(+)-6]

To a stirred solution of (+)-**5** (290 mg, 0.79 mmol) in EtOH/THF (1:1, 16 mL) was added $CeCl_3 \cdot 7H_2O$ (596 mg, 1.6 mmol). The mixture was stirred for 1 h at ambient temperature under N_2 and then cooled to -78 °C. After the temperature was equilibrated to -78 °C (ca. 30 min.), $NaBH_4$ (30 mg, 0.8 mmol) was added and stirred at the same temperature for 2 h, whereupon the reaction was quenched with a saturated NH_4Cl solution (20 mL) and extracted with ethyl acetate (3 x 30 mL). The combined extracts were washed with water (20 mL) and brine (20 mL). After being dried over Na_2SO_4, the solvents were removed under reduced pressure. The residue was crystallized from 10% Et_2O in hexane. The mother liquors were collected and purified by column chromatography on silica gel, eluting with 20% ethyl acetate in hexane, to afford (+)-**6**[13] (150 mg, 51% yield) with a specific activity of 49.25 mCi/mmol as determined by weight assay. The radiochemical purity was found to be 97.4%, as determined by normal phase HPLC (Zorbax, 30% ethyl acetate in hexane), and the enantiomeric purity was 98% as determined by chiral HPLC (Chiralpak AS, 4.04% isopropanol in hexane).

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13. All the compounds were analyzed by HPLC and co-eluted with cold standards. The final product (+)-**6** was structurally confirmed by ^{1}H NMR.

