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Novel Approach for Synthesis of (±)-Calanolide A and Its Anti-HIV Activity

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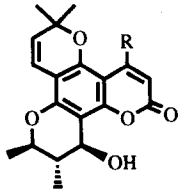
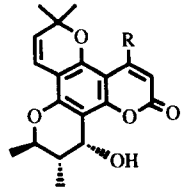
Abstract: *Anti-HIV agent (±)-calanolide A (1) has been synthesized. The key intermediate, chromene 5, was synthesized by the sequence of Pechmann reaction, acylation and chromenylation by 4,4-dimethoxy-2-methylbutan-2-ol. The anti-HIV activity for synthetic (±)-1 has been determined and compared with the natural product.*

Since HIV was identified as the etiological cause of AIDS a decade ago, chemotherapy of AIDS has been one of the most challenging scientific projects. Four antiviral nucleoside agents, AZT, ddC, ddI and d4T, have been approved for clinical use as drugs for the treatment of AIDS. Although these drugs can extend the life of AIDS patients, none of them are capable of curing the disease. Yet, use of these drugs is limited by the associated side effects. More importantly, long-term treatment of patients with these drugs has led to the emergence of drug-resistant HIV strains;[1-3] primary infections have also been found with AZT-resistant HIV strains.[4] Due to the alarming pandemic of AIDS, the need for other promising AIDS drug candidates having improved selectivity and activity against HIV is extremely urgent.[5-7]

A group of coumarin derivatives, isolated from several tropical plants of the genus *Calophyllum*, have recently been identified as HIV-1-specific nonnucleoside inhibitors, among which calanolide A (**1**) and inophyllum B (**2**) are the most potent compounds in the reported series.[8,9] These compounds may belong to a pharmacological class different from that of the previously known nonnucleoside HIV-1 reverse transcriptase inhibitory drugs and represent a novel anti-HIV chemotype for drug development, because they are active not only against the AZT-resistant strain of HIV-1 but also against virus strains resistant to some other nonnucleoside inhibitors such as TIBO, pyridinone and nevirapine.[10-12]



1, Calanolide A (R = n-Pr)
2, Inophyllum B (R = Ph)



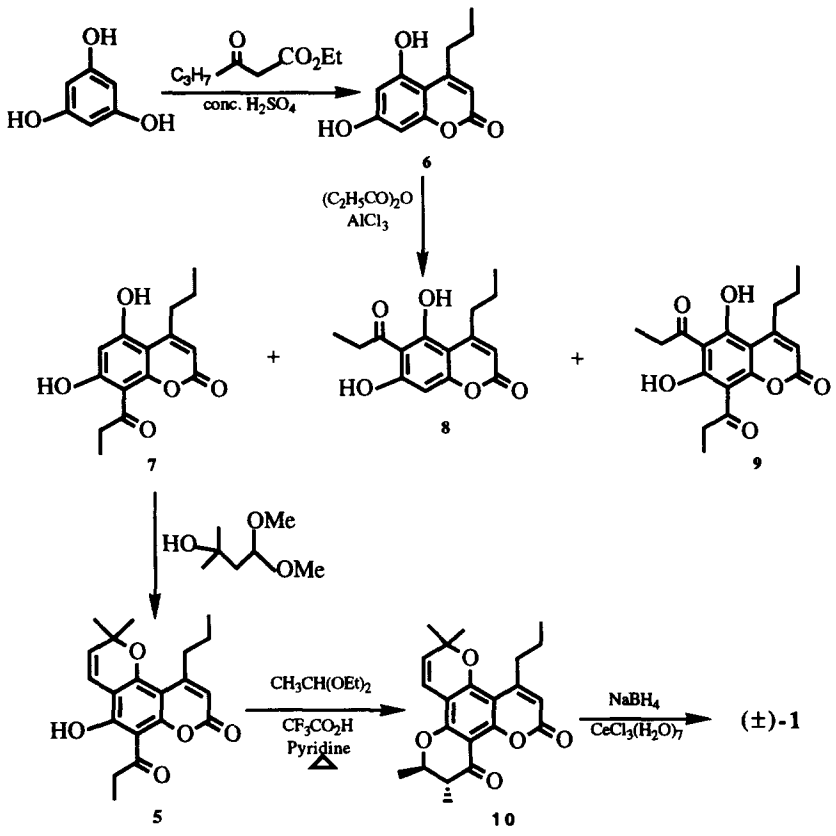
3, Calanolide B (R = n-Pr)
4, Inophyllum P (R = Ph)

However, supplies of the compound from plant material are extremely limited and difficult to obtain, since the only natural source of calanolide A was destroyed and other nearby members of the same species did not contain the same material. Further pharmacological investigation of this class of compounds, as well as related analogues, is dependent upon the ready availability of significant amounts of material. Obviously, a practical total synthesis of calanolide A is of great importance. The recent results reported on the synthesis of (±)-calanolide A and its related derivatives[13-15] prompted us to communicate our synthetic approach and anti-HIV activity of (±)-calanolide A.

The key intermediate in our approach is chromene **5**, which could be synthesized by the sequence depicted in **Scheme I**. Thus, Pechmann reaction[16] on phloroglucinol with ethyl butyrylacetate in the presence of concentrated sulfuric acid afforded 5,7-dihydroxy-4-propylcoumarin (**6**) quantitatively. Product yield and purity were dependent on the amount of sulfuric acid used; 1 mL of H_2SO_4 per gram of ethyl butyrylacetate led to optimal results in a hundred-gram scale reaction. When polyphosphoric acid was used, a 50% yield of coumarin **6** was obtained. Acylation of coumarin **6** with propionyl chloride in the presence of $AlCl_3$ led to a mixture of 8-position acylated coumarin **7** along with 6-acylated **8** and bis-acylated **9** in a ratio of 1:1:1 in 50% yield. The desired 8-acylated product **7**[17] was separated from **8** and **9** by silica gel chromatography. Structural assignments of the 8 or 6-acyl derivatives were made by comparison of UV spectra with analogues.[18] Improved selectivity and yield of the 8-acylated **7** (30% yield) could be achieved by replacing propionyl chloride with propionic anhydride. A route developed for synthesis of *Mammea* coumarins was initially attempted for preparation of compound **7**,[18] but it proved too awkward and low-yielding. The chromene ring is then introduced upon treatment of the 8-acylated coumarin **7** with 4,4-dimethoxy-2-methylbutan-2-ol,[18,19] providing the chromene **5**.[20]

Ring closure of chromene **5** to form chromanone **10** using acetaldehyde in the presence of a base such as K_2CO_3 or LDA was unsuccessful. However, treatment of chromene **5** with acetaldehyde diethyl acetal in the presence of trifluoroacetic acid and pyridine formed racemic chromanone **10** with the desired stereochemical arrangement, which was characterized by spectroscopic data[21] and compared with literature precedent[13] (**Scheme I**). The yield was moderate (30-40% yield). Reduction of ketone (±)-**10** with $NaBH_4$ afforded the hydroxy epimers. Luche reduction[22] of ketone (±)-**10** using $NaBH_4/CeCl_3$ at -10°C was highly selective in affording (±)-calanolide A (**1**) in 90% yield with 90% purity, while at room temperature the selectivity for calanolide A over calanolide B was only 60:40. After being further purified by preparative HPLC, the final product, with initial m.p. 52-54°C (Lit.[13] 56-58°C) which increased to 101-103°C after being thoroughly dried, was fully characterized[23] and the data were in agreement with the reported natural and synthetic product.[8,13]

The purified (±)-**1** has been tested for anti-HIV activity and the EC_{50} value was determined to be approximately 0.14 μM in the *in vitro* XTT assay, which is about 4 times less active than the naturally occurring (+)-calanolide A (**1**) (EC_{50} = 0.03 μM) when it was used as the positive control drug.



Scheme I

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17. Compound **7**: m.p. 244-246°C (corr); ^{1}H NMR (DMSO-d_6): 0.96 (3H, t, J=7.3 Hz, CH_3), 1.10 (3H, t, J=7.2 Hz, CH_3), 1.60 (2H, m, CH_2), 2.88 (2H, t, J=7.7 Hz, CH_2), 3.04 (2H, q, J=7.2 Hz, CH_2), 5.95 (1H, s, H_3), 6.31 (1H, s, H_6), 11.07 (1H, s, OH), 11.50 (1H, s, OH); UV (MeOH) nm: 219, 288, 316; MS (EI): 277 (6.6, M+1), 276 (9.0, M^+), 247 (100, M-C_2H_5); Anal. calc. for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84; Found: C, 64.92; H, 5.83.
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20. Compound **5**: m.p. 96-98°C; ^{1}H NMR (DMSO-d_6): δ 1.00 (3H, t, J=7.3 Hz, CH_3), 1.11 (3H, t, J=7.1 Hz, CH_3), 1.49 (6H, s, 2 CH_3), 1.59 (2H, sextet, J=7.8 Hz, CH_2), 2.88 (2H, t, J=7.7 Hz, CH_2), 3.19 (2H, q, J=7.0 Hz, CH_2), 5.79 (1H, d, J=10.1 Hz, H_7), 6.12 (1H, s, H_3), 6.62 (1H, d, J=10.1 Hz, H_8); MS (EI): 343 (5.7, M+1), 342 (22.5, M^+), 327 (100, M-CH_3); Anal. calc. for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48; Found: C, 70.45; H, 6.92.
21. Chromanone **10:** m.p. 176-177°C (Lit.[13] 130-132°C); ^{1}H NMR ($CDCl_3$): 1.02 (3H, t, J=7.5 Hz, CH_3), 1.21 (3H, d, J=6.8 Hz, CH_3), 1.51 (3H, d, J=7.0 Hz, CH_3), 1.55 (6H, 2s, 2 CH_3), 1.63 (2H, sextet, J=7.0 Hz, CH_2), 2.55 (1H, dq, J=6.9 Hz, J=11.0 Hz, H_{11}), 2.88 (2H, t, J=7.6 Hz, CH_2), 4.28 (1H, dq, J=6.3 Hz, J=11.0 Hz, H_{10}), 5.60 (1H, d, J=9.9 Hz, H_7), 6.04 (1H, s, H_3), 6.65 (1H, d, J=11.8Hz, H_8); MS (CI): 369(100, M+1); Anal. calc. for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57; Found: C, 71.71; H, 6.70.
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23. (±)-(**1**): m.p. 52-54°C, which increased to 101-103°C after it was thoroughly dried (Lit.[13] 56-58°C). ^{1}H NMR ($CDCl_3$): 1.03 (3H, t, J=7.3 Hz, CH_3), 1.15 (3H, d, J=6.8 Hz, CH_3), 1.46 (3H, d, J=6.8 Hz, CH_3), 1.47 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.66 (2H, m, CH_2), 1.93 (1H, m, H_{11}), 2.89 (2H, m, CH_2), 3.52 (1H, broad-s, OH), 3.93 (1H, m, H_{10}), 4.72 (1H, d, J=7.8 Hz, H_{12}), 5.54 (1H, d, J=10.0 Hz, H_7), 5.94 (1H, s, H_3), 6.62(1H, d, J=9.9 Hz, H_8); MS (CI): 371 (75.4, M+1), 370 (16.1, M^+), 353 (100, M-OH); Anal. calc. for $C_{22}H_{26}O_5$: C, 71.33; H, 7.07; Found: C, 71.63; H, 7.21.

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