Notes

Structure−Activity Modifications of the HIV-1 Inhibitors (+)-Calanolide A and (−)-Calanolide B[1]

Deborah L. Galinis,[2] Richard W. Fuller, Tawnya C. McKee,* John H. Cardellina II, Robert J. Gulakowski, James B. McMahon, and Michael R. Boyd*

Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment, Diagnosis and Centers, National Cancer Institute, Building 1052, Room 121, Frederick, Maryland 21702-1201

Received April 12, 1996[⊗]

The $\Delta^{7,8}$ olefinic linkages within (+)-calanolide A (**1**) and (−)-calanolide B (**2**) were catalytically reduced to determine impact on the anti-HIV activity of the parent compounds. In addition, a series of structure modifications of the C-12 hydroxyl group in (−)-calanolide B was made to investigate the importance of that substituent to the HIV-1 inhibitory activity of these coumarins. A total of 14 analogs were isolated or prepared and compared to (+)-calanolide A and (−)-calanolide B in the NCI primary anti-HIV assay. While none of the compounds showed activity superior to the two unmodified leads, some structure−activity requirements were apparent from the relative anti-HIV potencies of the various analogs.

Introduction

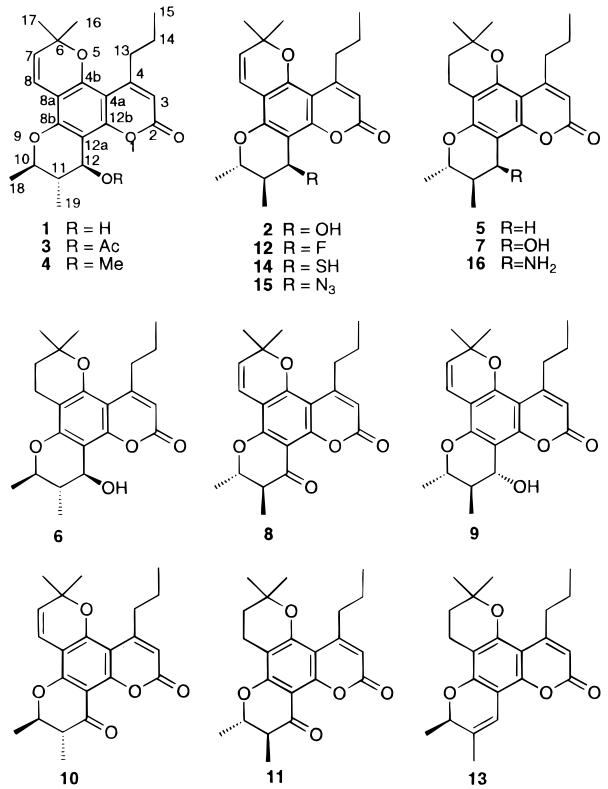
(+)-Calanolide A (**1**, Chart 1), isolated from the tropical rainforest tree *Calophyllym lanigerum* var. *austrocoriaceum*,[3] represents a novel subclass of HIV-1 specific reverse transcriptase inhibitor.[4] It provides comparable cytoprotection to several host cell lines against three laboratory and eight clinical strains of HIV-1.[4d] Recent data from this laboratory point to a complex mechanism involving two binding sites on HIV-1 RT.[4e] During efforts to identify a sufficient source of **1** for preclinical drug development, (−)-calanolide B (**2**) was obtained in relatively high yield (≥15%) from latex of *C. teysmannii* var. *inophylloide*.[5] (−)-Calanolide B (**2**) is active against HIV-1 with a potency similar to that of **1**. The immediate availability of significant quantities of **2** provided us with the opportunity to explore some structure−activity relationships of these molecules.

Our efforts focused on two sites within the calanolide structure motif: the $\Delta^{7,8}$ olefin and the C-12 hydroxyl group. Reduction of the $\Delta^{7,8}$ olefin would permit evaluation of its impact on *in vivo* toxicity and potential access to radiolabeled analogs for use in preclinical development studies. Our interest in the relationship of C-12 functionality and stereochemistry with HIV-inhibitory activity stemmed from prior observations that the 12-acetate (**3**) and 12-methoxyl (**4**) derivatives of (+)-calanolide A were weakly active and inactive, respectively,[3] and that (+)-calanolide A and (−)-calanolide B were potent inhibitors of HIV-1, while their respective enantiomers were devoid of AIDS-antiviral activity.[6]

Results

Chemistry. Initial efforts to reduce the $\Delta^{7,8}$-olefin in **1** utilizing Pd/C yielded only 12-deoxy-7,8-dihydrocalanolide A (**5**), the result of hydrogenolysis of the benzylic alcohol. However, reduction of **1** and **2** with NH_4OH-poisoned PtO_2 gave (+)-7,8-dihydrocalanolide A (**6**) and (−)-7,8-dihydrocalanolide B (**7**), respectively.

Chart 1



Turning to the C-12 functionality, we first sought to invert the stereochemistry at C-12 in (−)-calanolide B to obtain the enantiomer of natural (+)-calanolide A. We used the oxidation−reduction approach, since this would also provide the C-12 ketone for biological evaluation. Jones oxidation afforded the best yields (up to 70%) of (−)-12-oxocalanolide B (**8**). Reduction of **8** with $NaBH_4$ in the presence of $CeCl_3$ gave a 73% diastereomeric excess of (−)-calanolide A (**9**) in a mixture with

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1996.

S0022-2623(96)00282-8
This article not subject to U.S. Copyright. Published 1996 by the American Chemical Society

2. A similar approach was used in the final step of a recently reported synthesis of racemic calanolide A.[8] The Jones oxidation was also used to produce the ketones of (+)-calanolide A **(10)**, and (−)-7,8-dihydrocalanolide B **(11)** for comparative testing.

The 12-fluoro analog **12** was prepared by reacting (−)-calanolide B directly with DAST. Complete ^{1}H-NMR analysis of the product revealed that the H-12 resonance had shifted downfield to δ 5.53 and had $J_{HF} = 51$ Hz and established that the reaction had proceeded with retention of stereochemistry. Purification of **12** was problematic, due to degradation on exposure to silica gel and/or $CDCl_3$. Rapid vacuum liquid chromatography (VLC) on cyano-bonded phase provided adequate quantities of **12** for complete characterization, but incubation of **12** in cell culture media at 37 °C for 24 h gave complete conversion to (−)-calanolide B (**2**). Therefore, **12** could not be independently evaluated in the available anti-HIV assay. The silica gel and $CDCl_3$-promoted decomposition of **12** gave a new product, calanolene (**13**), by elimination of HF.

Nucleophilic displacement of the derived triflate ester was used for the preparation of the azide and thiol analogs. The triflate was generated using standard conditions (Tf_2O/pyr, −78 °C) and was added rapidly by cannula to the nucleophile in THF at −78 °C. NaSH was used to obtain the 12-thio derivative **14**. The reaction of the triflate with NaN_3 provided 12-azidocalanolide B (**15**) in 61% yield. NMR analyses of **14** and **15** revealed a 3.5 Hz coupling constant for H-12, indicating retention of stereochemistry.

We had planned to prepare the 12-amino analog of (−)-calanolide B from the azide, but attempts at selective reduction of the azido group in the presence of the $\Delta^{7,8}$-olefin with Ph_3P, $CrCl_2$, and $SnCl_2$ all failed. Overreduction (or decomposition) was a problem in reducing the azide (**15**) with the poisoned PtO_2 catalyst; therefore, the reduction was stopped prior to completion, thus diminishing yields of 12-amino-7,8-dihydrocalanolide B, **16**.

Biology. Using the XTT assay[9] for cell viability, we ranked compounds **1**−**16** with respect to their protection of human lymphoblastoid CEM-SS cells from the cytopathic effects of HIV-1 (RF strain). In those experiments, the three dihydro derivatives (**6, 7**, and **16**) and the two ketones **8** and **10** were generally comparable in potency to one another and to **1** and **2**. The azide (**15**) was somewhat less potent than the latter group, and the thiol (**14**) was considerably less potent than **15**. The acetate of calanolide A (**3**) had only marginal activity,[3] while 12-methoxycalanolide A (**4**), the 12-deoxy calanolide A derivative **5**, and the alkene **13** were all inactive against HIV-1. (−)-Calanolide A (**9**) was also devoid of HIV inhibitory activity. These results are summarized in Table 1.

Table 1. HIV-Inhibitory Activities of (+)-Calanolide A (**1**), (−)-Calanolide B (**2**), and Derivatives in the NCI Primary Anti-HIV Screening Assay

compd	EC_{50} (μM)[a]	IC_{50} (μM)[a]	TI[b]
1	0.18 ± 0.01	7.3 ± 1.8	40
2	0.2 ± 0.1	5.9 ± 1.9	31
6	0.2 ± 0.1	5.2 ± 0.4	23
7	0.2 ± 0.02	8.2 ± 1.8	41
8	0.4 ± 0.1	8.5 ± 4.2	24
9	*c*	*c*	*c*
10	0.15 ± 0.02	9.3 ± 0.6	62
11	0.37 ± 0.6	7.9 ± 0.6	21
12	*d*	*d*	*d*
13	*c*	*c*	*c*
14	8.4 ± 1.1	>150[e]	*c*
15	1.6 ± 0.1	5.9 ± 2.2	4
16	0.6 ± 0.1	5.8 ± 0.6	10

[a] Averages of quadruplicate determinations. [b] *In vitro* therapeutic indices (IC_{50}/EC_{50}). [c] Not measurable. [d] Not independently evaluated; not stable under assay conditions; rapidly converts to active compound **2** under assay conditions.

Discussion

The inactivity of **5** and **13** indicated that a heteroatom is required at C-12 for activity. The relative potency of the ketones **(8** and **10)** and azide (**15**) strongly suggested that the C-12 functionality acts primarily as a hydrogen bond acceptor, while the decreasing potency in the series **8** > **15** > **3** indicated that there is a fairly stringent spatial limitation and/or stereoelectronic requirement around C-12. The decreased potency of the thiol (**14**) relative to the amino derivative (**16**) was also consistent with this conclusion. The inactivity of the (−) enantiomer of calanolide A[6] indicated a critical stereochemistry requirement, despite the potency of the derived ketone (**8**), i.e., the oxygen substituent must lie in the plane of the aromatic system or possess the *S* configuration. Given the rather similar potencies of (+)-calanolide A and (−)-calanolide B, the stereochemistry at C-11 relative to C-12 must underlie an essential conformation of the dihydropyran ring. Reduction of the $\Delta^{7,8}$ double bond conferred only a slight reduction in potency.

In summary, we have prepared a concise set of analogs of (−)-calanolide B which revealed some interesting structure−activity requirements in the molecule. None of the derivatives was superior in potency to the underivatized natural products (+)-calanolide A or (−)-calanolide B. Racemic calanolide A is now accessible by synthesis,[8,10] but the inactivity of the unnatural isomer indicates that a large-scale stereospecific synthesis of the natural isomer would be desirable.[11] In the meantime, the presently more abundant[5] natural (−)-calanolide B represents a feasible alternative for further preclinical research and development.

Experimental Section

General. NMR spectra were acquired with a Varian VXR 500 spectrometer, using $CDCl_3$ as solvent and internal standard. All triflate displacement reactions were carried out in oven-dried glassware under a positive flow of argon. THF and CH_2Cl_2 were distilled from CaH_2 and stored over 4 Å molecular sieves under argon. Salts were dried over P_2O_5 at 56 °C prior to use. All other commercial reagents were used as received.

(+)-7,8-Dihydrocalanolide A (**6**). Newly purchased PtO_2 was exposed to ammonia vapors by setting an open vial containing PtO_2 in a covered beaker containing concentrated NH_4OH for 2 days. Traces of excess NH_4OH were removed from the catalyst under vacuum (24 h). Racemic synthetic calanolide A (40 mg, 0.11 mmol) was reduced in 10 mL of MeOH with H_2 and 22 mg (0.1 mmol) of poisoned PtO_2 for 60 min under positive atmospheric pressure (balloon). The crude racemic mixture was separated into enantiomers by chiral HPLC (Regis Whelk-O, 1 × 25 cm, hexane−iPrOH, 9:1, 6 mL/min), monitored at 270 nm, to yield (+)-7,8-dihydrocalanolide A (**6**) 17.5 mg (42.7%) and (−)-7,8-dihydrocalanolide A 19.0 mg (46.3%); **16** $[\alpha]_D$ +88° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.29 (s, 1H, H-3), 4.74 (d, J = 7.5 Hz, 1H, H-12), 3.91 (dq, J = 9.5, 6.3 Hz, 1H, H-10), 2.88 (m, 2H, H-13), 2.62 (m, 2H, H-8), 1.90 (m, 1H, H-11), 1.77 (m, 2H, H-7), 1.61 (m, 2H, H-14), 1.44

(d, J = 6.3 Hz, 1H, H-18), 1.39 (s, 3H, H-16 or H-17), 1.35 (s, 3H, H-16 or H-17), 1.14 (d, J = 7.0 Hz, 3H, H-19), 1.01 (t, J = 7.0 Hz, 2H, H-15); ^{13}C NMR ($CDCl_3$) δ 160.8 (C-2), 159.5 (C-4), 155.6 (C-8b), 153.6 (C-12b), 151.9 (C-4b), 109.9 (C-3), 105.3, 105.2 (C-8a, C-12a), 103.9 (C-4a), 76.9 (C-6), 75.7 (C-10), 67.3 (C-12), 40.5 (C-11), 39.2 (C-13), 31.4 (C-8), 27.1 (C-16 or C-17), 26.1 (C-16 or C-17), 23.4 (C-14), 19.0 (C-7), 16.9 (C-7), 15.1 (C-19), 13.9 (C-15); HRFABMS m/z 373.1995 (calcd for $C_{22}H_{29}O_5$, 373.2015).

(−)-7,8-Dihydrocalanolide B (7). (−)-Calanolide B (500 mg, 1.35 mmol) was reduced in 10 mL of MeOH with H_2 and 32 mg (0.15 mmol) of poisoned PtO_2 for 1 h. Catalyst was removed by filtration, and the crude reaction mixture was purified by HPLC on Rainin Dynamax silica (1 × 25cm, eluted with 3:2 hexane−EtOAc) to yield 346 mg of **7** (69%): $[\alpha]_D$ −28° (c = 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.92 (s, 1H, H-3), 4.99 (d, J = 3.0 Hz, 1H, H-12), 4.24 (dq, J = 6.5, 4.5 Hz, 1H, H-10), 2.88 (m, 2H, H-13), 2.63 (q, J = 6.8 Hz, 2H, H-8), 1.77 (t, J = 6.8 Hz, 2H, H-7), 1.73 (m, 1H, H-11), 1.62 (m, 1H, H-14), 1.42 (d, J = 6.5 Hz, 3H, H-18), 1.37 (s, 6H, H-16,17), 1.14 (d, J = 7.0 Hz, 3H, H-19), 1.01 (t, J = 9.0 Hz, 3H, H-15); ^{13}C NMR ($CDCl_3$) δ 161.5 (C-2), 159.3 (C-4), 155.6 (C-8b), 153.1 (12b), 152.1 (C-4b), 109.9 (C-3), 105.2 (C-8a), 104.9 (12a), 103.4 (4a), 75.8 (C-6), 72.8 (C-10), 61.8 (C-12), 39.0 (C-11), 38.3 (C-13), 31.5 (C-8), 26.6 (C-16), 26.5 (C-17), 23.4 (C-14), 18.9 (C-18), 16.9 (C-7), 13.9 (C-15), 12.5 (C-19); HREIMS 372.1919 (calcd for $C_{22}H_{28}O_5$, 372.1936)

(−)-12-Oxocalanolide B (8). (−)-Calanolide B (20 mg, 0.05 mmol) was dissolved in 5 mL acetone and stirred at 0 °C. Jones reagent (100 μL) was added slowly, and the solution was stirred for 10 min. After 10 min, an additional 100 μL Jones reagent was slowly added and the solution was stirred at 0 °C for 40 min. The reaction mixture was diluted with 15 mL of H_2O and extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was washed with saturated $NaHCO_3$ and then dried over anhydrous $MgSO_4$ and filtered. The crude product was purified by HPLC (Rainin Dynamax silica, 2.1 × 25 cm), using hexane−EtOAc (13:7, 20 mL/min) to give 12.4 mg of (−)-12-oxocalanolide B (61% yield): $[\alpha]_D$ −55° (c = 1.27, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.59 (d, J = 9.8 Hz, 1H, H-8), 5.98 (s, 1H, H-3), 5.55 (d, J = 9.8 Hz, 1H, H-7), 4.25 (dq, J = 11.2, 6.9 Hz, 1H, H-10), 2.83 (dt, J = 7.8, 3.0 Hz, 2H, H-13, H-13′), 2.50 (dq, J = 11.2, 6.8 Hz, 1H, H-11), 1.59 (sextet, J = 7.8 Hz, 2H, H-14, H-14′), 1.50 (s, 3H, H-16), 1.49 (d, J = 6.9 Hz, 3H, H-18), 1.47 (s, 3H, H-17), 1.16 (d, J = 6.8 Hz, 3H, H-19), 0.97 (t, J = 7.3 Hz, 3H, H-15); ^{13}C NMR ($CDCl_3$) δ 189.9 (C-12), 159.6 (C-2), 159.0 (C-4), 157.0, 155.9, 155.4 (C-4b, C-8b, C-12b), 126.9 (C-7), 115.8 (C-8), 112.0 (C-3), 105.4, 104.4 (C-8a, C-12a), 103.5 (C-4a), 79.5 (C-10), 79.2 (C-6), 47.2 (C-11), 38.7 (C-13), 28.3 (C-16), 27.9 (C-17), 23.1 (C-14), 19.6 (C-18), 13.9 (C-15), 10.4 (C-19); HRElMS m/z 368.1591 (calcd for $C_{22}H_{24}O_5$, 368.1624).

(−)-Calanolide A (9). A solution of (−)-12-oxocalanolide B (**8**) (18.4 mg, 0.05 mmol) in EtOH (1 mL) was added to a slurry of $NaBH_4$ (5.0 mg, 0.13 mmol) and $CeCl_3{\cdot}(H_2O)_7$ (18.4 mg, 0.05 mmol) in EtOH (4 mL). The resulting mixture was stirred at 25 °C for 0.5 h. Water (2 mL) was added to the reaction mixture, which was then extracted with EtOAc (3 × 5 mL). The combined organic layers were concentrated and purified by silica HPLC, using the same conditions reported above, to give (−)-calanolide B (**2**) (2.6 mg, 13%) and the enantiomer of natural calanolide A (**9**) (16.3 mg, 87%): $[\alpha]_D$ −68° (c = 1.36, $CHCl_3$); HREIMS m/z 370.1770 (calcd for $C_{22}H_{26}O_6$, 370.1780); ^{1}H and ^{13}C NMR spectra were identical to those reported for natural (+)-calanolide A.[3]

(+)-12-Oxocalanolide A (10). (±)-Calanolide A (40 mg, 0.11 mmol) was oxidized using two 100 μL portions of Jones reagent as described above. The crude reaction mixture was resolved by chiral HPLC (Regis Whelk-O, 1 × 25 cm, hexane−iPrOH, 9:1) to yield 2.0 mg of **10** and 2.1 mg of its enantiomer (**8**) (10% overall yield).

10: $[\alpha]_D$ +56° (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.62 (d, J = 9.5 Hz, 1H, H-8), 6.02 (s, 1H, H-3), 5.57 (d, J = 9.5 Hz, 1H, H-7), 4.27 (dq, J = 11.5, 6.3 Hz, 1H, H-10), 2.85 (dt, J = 7.3, 2.5 Hz, 2H, H-13, H-13′), 2.52 (m, 1H, H-11), 1.61 (sextet, J = 7.3 Hz, 2H, H-14, H-14′), 1.53 (s, 3H, H-16), 1.51 (d, J = 6.3 Hz, 3H, H-18), 1.49 (s, 3H, H-17), 1.21 (d, J = 7.0 Hz, 3H, H-19), 1.02 (t, J = 7.0 Hz, 3H, H-15); ^{13}C NMR ($CDCl_3$) δ 189.9 (C-12), 159.1 (C-2), 157.0 (C-4, C-4b), 155.9 (C-8b), 155.5 (C-12b), 127.0 (C-7), 115.8 (C-8), 112.1 (C-3), 105.4 (C-8a), 104.5 (C-12a), 103.5 (C-4a), 79.6 (C-10), 79.2 (C-6), 47.3 (C-11), 38.8 (C-13), 28.3, 28.0 (C-16, C-17), 23.2 (C-14), 19.6 (C-18), 13.9 (C-15), 10.5 (C-19); HRFABMS m/z 369.1698 (calcd for $C_{22}H_{29}O_5$, 369.1702).

(−)-7,8-Dihydro-12-oxocalanolide B (11). (−)-7,8-Dihydrocalanolide B (40 mg, 0.11 mmol) was oxidized, as described above, to yield 26.8 mg of **11** (68%): $[\alpha]_D$ −58° (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.02 (s, 1H, H-3), 4.27 (dq, J = 6.5, 5.0 Hz, 1H, H-10), 2.87 (dd, J = 8.0, 7.5 Hz, 2H, H-13), 2.68 (m, 2H, H-8), 2.52 (m, 1H, H-11), 1.84 (ddd, J = 13.5, 7.0, 6.5 Hz, 2H, H-7), 1.60 (sextet, J = 8.0 Hz, 2H, H-14), 1.52 (d, J = 6.5 Hz, 3H, H-18), 1.43 (s, 3H, H-16 or H-17), 1.41 (s, 3H, H-16 or H-17), 1.21 (d, J = 7.0 Hz, 3H, H-15), 1.08 (t, J = 7.5 Hz, 3H, H-19); ^{13}C NMR ($CDCl_3$) δ 190.1 (C-12), 161.9 (C-2), 160.1 (C-4), 157.6 (C-8b), 156.7 (C-12b), 154.5 (C-4b), 111.8 (C-3), 104.7 (C-8a) 104.4 (C-12a), 102.8 (C-4a), 79.3 (C-6), 77.2 (C-10), 47.1 (C-11), 39.2 (C-13), 31.1 (C-8) 26.9, 26.3 (C-16, C-17), 23.3 (C-14), 19.6 (C-18), 16.7 (C-7), 13.8 (C-15), 10.5 (C-19); HRFABMS m/z 371.1827 (calcd for $C_{22}H_{27}O_5$, 371.1858).

(−)-12-Fluorocalanolide B (12). (−)-Calanolide B (37.8 mg, 0.1 mmol) in CH_2Cl_2 (1 mL) was slowly added to a solution of DAST (8.5 mg, 0.05 mmol) and powdered, oven-dried Na_2CO_3 in CH_2Cl_2 (4 mL) at −78 °C and allowed to warm slowly to room temperature. After 1 h, the reaction mixture was extracted with water and the organic layer was subjected to vacuum liquid chromatography on cyano-bonded phase, eluting with an EtOAc−hexane gradient (5−30%), to give 12-fluorocalanolide B (**12**) (10 mg, 27%): $[\alpha]_D$ −98° (c = 0.015, C_6H_6); ^{1}H NMR (C_6D_6) δ 6.67 (d, J = 9.8 Hz, 1H, H-8), 5.82 (s, 1H, H-3), 5.53 (dd, J = 2.4, 51.3 Hz, 1H, H-12), 5.09 (d, J = 10.2 Hz, 1H, H-7), 3.96, (m, 1H, H-10), 2.51 (m, 3H, H-11, H-13, H-13′), 1.35 (sextet, J = 7.3 Hz, 2H, H-14, H-14′), 1.12 (s, 6H, H-16, H-17), 1.02 (d, J = 6.3 Hz, 3H, H-18), 0.79 (t, J = 7.3 Hz, 3H, H-15), 0.77 (d, J = 6.8 Hz, 3H, H-19); ^{13}C NMR (C_6D_6) δ 159.3 (C-2), 156.7 (C-4), 155.5 (C-12b), 153.7 (C-8b), 152.7 (C-4b), 126.3 (C-7), 116.8 (C-8), 111.9 (C-3), 105.7 (C-8a), 103.9 (C-4a), 102.6 (d, $J_{C,F}$ = 20 Hz, C-12a), 81.8 (d, $J_{C,F}$ = 173 Hz, C-12), 77.6 (C-6), 73.2 (C-10), 38.5 (C-13), 37.9 (d, $J_{C,F}$ = 20 Hz, C-11), 27.5 (C-16 and C-17), 23.3 (C-14), 18.6 (C-18), 14.1 (C-15), 12.1 (C-19); HREIMS m/z 372.1738 (calcd for $C_{22}H_{25}FO_4$, 372.1737).

12-Thiocalanolide B (14). A solution of (−)-calanolide B (75 mg, 0.2 mmol) and pyridine (12 μL) in CH_2Cl_2 (2 mL) was added slowly (dropwise) to a solution of triflic anhydride (50 μL, 0.3 mmol) in CH_2Cl_2 (2 mL) at −78 °C. After 1 h, the reaction mixture was transferred via cannula to a flask containing excess NaSH in THF (2 mL) at −78 °C. After slowly warming overnight, the reaction mixture was extracted with water (3 mL) and the organic layer concentrated. Vacuum-liquid chromatography on silica, using an EtOAc−hexane gradient (5−30%), followed by HPLC on a column (2.1 × 25 cm), eluting with hexane−EtOAc (7:3) at 15 mL/min, gave calanolene (**13**) (20.1 mg, 29%) and 12-thiocalanolide B (**14**) (14.5 mg, 19%).

13: $[\alpha]_D$ +28.7° (c = 0.28, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 6.61 (s, 1H, H-12), 6.59 (d, J = 10.2 Hz, 1H, H-8), 5.90 (s, 1H, H-3), 5.43 (d, J = 10.2 Hz, 1H, H-7), 4.86 (q, J = 6.3 Hz, 1H, H-10), 2.85 (m, 2H, H-13, H-13′), 1.82 (br s, 3H, H-19), 1.62 (sextet, J = 7.3 Hz, 2H, H-14, H-14′), 1.46 (s, 3H, H-16), 1.44 (s, 3H, H-17), 1.36 (d, J = 6.8 Hz, 3H, H-18), 1.00 (t, J = 7.3 Hz, 3H, H-15); ^{13}C NMR ($CDCl_3$) δ 161.0 (C-2), 158.4 (C-4), 150.7, 150.0 (C-8b, C-12b), 149.7 (C-4b), 132.2 (C-12), 127.2 (C-7), 116.4 (C-8), 112.0 (C-11), 110.8 (C-3), 106.4 (C-12a), 103.8 (C-4a and C-8a), 77.7 (C-6), 76.0 (C-10), 38.6 (C-13), 28.0 (C-16), 27.6 (C-17), 23.2 (C-14), 19.3 (C-18 and C-19), 14.0 (C-15); HREIMS m/z 352.1674 (calcd for $C_{22}H_{24}O_4$, 352.1675).

14: $[\alpha]_D$ +203.4° (c = 0.71, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.55 (d, J = 9.7 Hz, 1H, H-8), 5.91 (s, 1H, H-3), 5.45 (d, J = 9.7 Hz, 1H, H-7), 4.99 (d, J = 3.4 Hz, 1H, H-12), 4.56 (dq, J = 10.2, 6.8 Hz, 1H, H-10), 2.85 (dd, J = 7.8, 7.8 Hz, 2H, H-13, H-13′), 2.08 (m, 1H, H-11), 1.62 (sexet, J = 7.3 Hz, 2H, H-14, H-14′), 1.46 (d, J = 6.8 Hz, 1H, SH), 1.44 (s, 3H, H-16), 1.42 (d, J = 6.8 Hz, 3H, H-19), 1.40 (s, 3H, H-17), 1.38 (d, J = 7.3 z, 3H,

H-18), 1.00 (t, $J = 7.3$ Hz, 3H, H-15); ^{13}C NMR ($CDCl_3$) δ 160.7 (C-2), 158.2 (C-4), 152.4 (C-12b), 152.3 (C-8b), 150.7 (C-4b), 126.5 (C-7), 116.7 (C-8), 110.4 (C-3), 106.0 (C-8a), 105.9 (C-12a), 103.4 (C-4a), 77.4 (C-6), 74.3 (C-10), 43.5 (C-12), 39.2 (C-11), 38.6 (C-13), 27.7 (C-17), 27.6 (C-16), 23.2 (C-14), 19.1 (C-18), 15.1 (C-19), 14.0 (C-15); HREIMS *m/z* 386.1530 (calcd for $C_{22}H_{26}O_4S$, 386.1552).

12-Azidocalanolide B (15). Generation of the triflate, displacement with NaN_3, and purification as described above resulted in the formation of calanolene (**13**) (9.8 mg, 28%) and 12-azidocalanolide B (**15**) (24.2 mg, 61%). **15**: $[\alpha]_D$ +105° (*c* = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.58 (d, $J = 9.8$ Hz, 1H, H-8), 5.94 (s, 3H, H-3), 5.51 (d, $J = 9.8$ Hz, 1H, H-7), 4.95 (d, $J = 3.4$ Hz, 1H, H-12), 4.12 (dq, $J = 10.7$, 6.4 Hz, 1H, H-10), 2.87 (dd, $J = 6.4$, 8.9 Hz, 2H, H-13, H-13′), 1.80 (m, 1H, H-11), 1.64 (sextet, $J = 7.3$ Hz, 2H, H-14, H-14′), 1.48 (s, 3H, H-16), 1.46 (s, 3H, H-17), 1.38 (d, $J = 6.4$ Hz, 3H, H-18), 1.10 (d, $J = 6.8$ Hz, 3H, H-19), 1.01 (t, $J = 7.3$ Hz, 3H, H-15); ^{13}C NMR ($CDCl_3$) δ 160.1 (C-2), 158.2 (C-4), 153.6 (C-8b), 152.8 (C-12b), 151.8 (C-4b), 126.9 (C-7), 116.2 (C-8), 110.7 (C-3), 106.0 (C-12a), 103.4 (C-8a), 102.0 (C-4a), 77.8 (C-6), 73.4 (C-10), 55.3 (C-12), 38.5 (C-11), 37.1 (C-13), 27.8 (C-16 and C-17), 23.1 (C-14), 18.8 (C-18), 14.0 (C-15), 13.5 (C-19); HRMS *m/z* 395.1836 (calcd for $C_{22}H_{25}N_3O_4$, 395.1845).

12-Amino-7,8-dihydrocalanolide B (16). Poisoned platinum oxide catalyst (PtO_2, 13.0 mg, 0.06 mmol) was added to (−)-12-azidocalanolide B (**15**, 23.4 mg, 0.06 mmol) in EtOAc (5 mL). The mixture was stirred under a hydrogen atmosphere for 20 min and then filtered rapidly through a 20 μm filter. Concentration of the EtOAc solution yielded a mixture of starting material and desired product. HPLC purification on an amino-bonded phase column (Rainin, 1 × 25 cm), eluting with hexane−EtOAc (17:3) at 4 mL/min and monitoring at 290 nm, gave 12-amino-7,8-dihydrocalanolide B (**16**, 6.4 mg, 28%): $[\alpha]_D$ +101.2° (*c* = 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.94 (s, 1H, H-3), 5.01 (d, $J = 2.9$ Hz, 1H, H-12), 4.13 (dq, $J = 10.7$, 6.3 Hz, 1H, H-10), 2.88 (dt, $J = 2.9$, 8.3 Hz, 2H, H-8), 2.63 (m, 2H, H-13, H-13′), 1.82 (m, 1H, H-11), 1.78 (t, $J = 6.3$ Hz, 2H, H-7), 1.63 (m, 4H, H-14, 12-NH_2), 1.40 (d, $J = 6.3$ Hz, 3H, H-18), 1.38 (s, 3H, H-16), 1.39 (s, 3H, H-17), 1.12 (d, $J = 6.8$ Hz, 3H, H-19), 1.02 (t, $J = 7.3$ Hz, 3H, H-15); ^{13}C NMR ($CDCl_3$) δ 160.6 (C-2), 158.8 (C-4), 155.3 (C-8b), 152.8, 152.6 (C-4b and C-12b), 110.5 (C-3), 104.9 (C-12a), 103.3 (C-4a), 100.8 (C-8a), 75.9 (C-6), 73.3 (C-10), 55.5 (C-12), 39.0 (C-13), 37.2 (C-11), 31.4 (C-8), 26.7 (C-17 and C-18), 23.3 (C-14), 18.9 (C-18), 16.9 (C-7), 13.9 (C-15), 13.5 (C-19); HREIMS *m/z* 371.2083 (calcd for $C_{22}H_{29}NO_4$, 371.2097).

Acknowledgment. We thank A. Kozikowsi and D. Torok for helpful suggestions, L. Pannell and G. Gray for mass spectral analyses, G. Cragg for coordinating the supply of *C. teysmannii* latex, and M. Serit, H. Bokesch, and B. Krepps for technical assistance.

References

(1) Part 30 in the series HIV-Inhibitory Natural Products. For part 29, see: McKee, T. C.; Fuller, R. W.; Covington, C. D.; Cardellina, J. H., II; Gulakowski, R. J.; Krepps, B. L.; McMahon, J. B.; Boyd, M. R. New pyranocoumarins isolated from *Calophyllum lanigerum* and *Calophyllum teysmannii*. *J. Nat. Prod.* **1996**, *59*, 754−758.

(2) Current address: American Cyanamid Co., Agricultural Products Research Division, P.O. Box 400, Princeton, NJ 08543-0400.

(3) Kashman, Y.; Gustafson, K. R.; Fuller, R. W.; Cardellina, J. H., II; McMahon, J. B.; Currens, M. J.; Buckheit, R. W., Jr.; Hughes, S. H.; Cragg, G. M.; Boyd, M. R. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*. *J. Med. Chem.* **1992**, *35*, 2735−2743.

(4) (a) Hizi, A.; Tal, R.; Shaharabany, M.; Currens, M. J.; Boyd, M. R.; Hughes, S. H.; McMahon, J. B. Specific inhibition of the reverse transcriptase of human immunodeficiency virus type 1 and the chimeric enzymes of human immunodeficiency virus type 1 and type 2 by nonnucleoside inhibitors. *Antimicrob. Agents Chemother.* **1993**, *37*, 1037−1042. (b) Boyer, P. L.; Currens, M. J.; McMahon, J. B.; Boyd, M. R.; Hughes, S. H. Analysis of nonnucleoside drug-resistant variants of human immunodeficiency virus type 1 reverse transcriptase. *J. Virol.* **1993**, *67*, 2412−2420. (c) Buckheit, R. W., Jr.; Fliakas-Boltz, V.; Decker, W. D.; Roberson, J. L.; Pyle, C. A.; White, E. L.; Bowden, B. J.; McMahon, J. B.; Boyd, M. R.; Bader, J. P.; Nickell, D. G.; Barth, H.; Antonucci, T. K. Biological and biochemical anti-HIV activity of the benzothiadiazine class of nonnucleoside reverse transcriptase inhibitors. *Antiviral Res.* **1994**, *25*, 43−56. (d) Currens, M. J.; Gulakowski, R. J.; Mariner, J. M.; Moran, R. A.; Buckheit, R. W., Jr.; Gustafson, K. R.; McMahon, J. B.; Boyd, M. R. Antiviral activity and mechanism of action of calanolide A against the human immunodeficiency virus. *J. Pharmacol. Exp. Ther.*, in press. (e) Currens, M. J.; Mariner, J. M.; McMahon, J. B.; Boyd, M. R. Kinetic analysis of inhibition of HIV-1 reverse transcriptase by calanolide A. *J. Pharmacol. Exp. Ther.*, in press.

(5) Fuller, R. W.; Bokesch, H. R.; Gustafson, K. R.; McKee, T. C.; Cardellina, J. H., II; McMahon, J. B.; Cragg, G. M.; Soejarto, D. D.; Boyd, M. R. HIV-Inhibitory coumarins from latex of the tropical rainforest tree *Calophyllum teysmannii* var. *inophylloide*. *BioMed. Chem. Lett.* **1994**, *4*, 1961−2964.

(6) Cardellina, J. H., II; Bokesch, H. R.; McKee, T. C.; Boyd, M. R. Resolution and comparative anti-HIV evaluation of the enantiomers of calanolides A and B. *BioMed. Chem. Lett.* **1995**, *5*, 1011−1014.

(7) Gemal, A. L.; Luche, J.-L. Lanthanoids in organic synthesis. 6. The reduction of α-enones by sodium borohydride in the presence of lanthanoid chlorides: synthetic and mechanistic aspects. *J. Am. Chem. Soc.* **1983**, *103*, 5454−5459.

(8) Chenera, B.; West, M. L.; Finkelstein, J. A.; Dreyer, G. B. Total synthesis of (+)-calanolide A, a non-nucleoside inhibitor of HIV-1 reverse transcriptase. *J. Org. Chem.* **1993**, *58*, 5605−5606.

(9) Gulakowski, R. J.; McMahon, J. B.; Staley, P. G.; Moran R. A.; Boyd, M. R. A semiautomated multiparameter approach for anti-HIV drug screening. *J. Virol. Methods* **1991**, *33*, 87−100.

(10) (a) Kucherenko, A.; Flavin, M. T.; Boulanger, W. A.; Khilevich, A.; Shone, R. L.; Rizzo, J. D.; Sheinkman, A. K.; Xu, Z-Q. Novel approach for synthesis of (±)-calanolide A and its anti-HIV activity. *Tetrahedron Lett.* **1995**, *36*, 5475−5478. (b)Palmer, C. J.; Josephs, J. L. Synthesis of *Calophyllum* coumarins. *Tetrahedron Lett.* **1994**, *35*, 5363−5366.

(11) Since the completion of this work, several approaches to chiral syntheses of the calanolides have been published: (a) Rama Rao, A. V.; Gaitonde, A. S.; Prakash, K. R. C.; Prahlada Rao, S. A concise synthesis of chiral 2-methyl chroman-4-ones: stereoselective build-up of the chromanol moiety of anti-HIV agent, calanolide A. *Tetrahedron Lett.* **1994**, *35*, 6347−6450. (b) Deshpande, P. P.; Baker, D. C. A simple approach to the synthesis of the chiral substituted chroman ring of *Calophyllum* coumarins. *Synthesis* **1995**, 630−632. (c) Deshpande, P. P.; Tagliaferri, F.; Victory, S. F.; Yan, S.; Baker, D. C. Synthesis of optically active calanolide A and B. *J. Org. Chem.* **1995**, *60*, 2964−2965.

JM9602827